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Cover: A group of *Crocodylus acutus* from Tárcoles River, Central Pacific, Costa Rica
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An index to assess the level of vulnerability to crocodiles in coastal communities

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Abstract

An index to assess the level of vulnerability to crocodiles in coastal communities. Human-wildlife negative interactions are a recurring phenomenon worldwide, originating from the shared habitats and resources between both. In several coastal communities, negative interactions occur due to the presence of the American Crocodile (*Crocodylus acutus*). We have developed an index to assess the level of vulnerability of communities to this reptile. The construction of this index is based on the Approximate Sustainability Index developed by Gutiérrez-Espeleta in 1994. The Index of Vulnerability (IVU) is built upon several indicators across four parameters: social, biological-environmental, institutional, and spatial. These indicators are assessed using a performance scale and interpretation. The IVU assigns values to the vulnerability condition, which are presented in a color scale corresponding to defined intervals. For each indicator, reference categories and rating scales are represented with traffic light colors and numerical ratings. The IVU value obtained for a community can be visualized with a map and a corresponding figure, including a table of values for the assessed parameters.

Keywords: American Crocodile, Biological parameter, *Crocodylus acutus,* Institutional parameter, Social parameter, Spatial parameter, Wildlife.

Resumen

Un índice para evaluar el nivel de vulnerabilidad a cocodrilos en comunidades costeras. Las interacciones negativas entre seres humanos y animales silvestres es un fenómeno recurrente a nivel mundial y que se origina desde que ambos comparten los mismos hábitats y recursos. En varias comunidades costeras existen interacciones negativas dada la presencia del cocodrilo americano (*Crocodylus acutus*). Construimos un índice para evaluar el grado de vulnerabilidad de las comunidades ante este reptil. La construcción de este índice está basada en el Índice Aproximado de Sostenibilidad generado por Gutiérrez-Espeleta en 1994. El índice de vulnerabilidad (IVU) se basa

en varios indicadores de cuatro parámetros: social, biológico-ambiental, institucional y espacial, con una escala de desempeño e interpretación de estos. El IVU genera valores de la condición de vulnerabilidad que se representan en una escala de colores según intervalos definidos. Para cada indicador se anotan las categorías de referencia y las escalas de calificación con colores tipo semáforo con una valoración numérica. El valor del IVU obtenido para una comunidad se puede representar de manera gráfica con un mapa en una figura que incluye un cuadro de los valores para los parámetros evaluados.

Palabras clave: Cocrodilo americano, *Crocodylus acutus,* Parámetro biológico, Parámetro espacial, Parámetro institucional, Parámetro social, Vida silvestre.

Resumo
Um índice para avaliar o nível de vulnerabilidade a crocodilos em comunidades costeiras. As interações negativas entre humanos e animais selvagens são um fenômeno recorrente em todo o mundo, originado dos habitats e recursos compartilhados entre ambos. Em várias comunidades costeiras, as interações negativas ocorrem devido à presença do crocodilo americano (*Crocodylus acutus*). Desenvolvemos um índice para avaliar o nível de vulnerabilidade das comunidades a esse réptil. A construção desse índice baseia-se no Índice de Sustentabilidade Aproximada desenvolvido por Gutiérrez-Espeleta em 1994. O Índice de Vulnerabilidade (IVU) é construído com base em vários indicadores em quatro parâmetros: social, biológico-ambiental, institucional e espacial. Esses indicadores são avaliados por meio de uma escala de desempenho e interpretação. O IVU atribui valores à condição de vulnerabilidade, que são apresentados em uma escala de cores correspondente a intervalos definidos. Para cada indicador, as categorias de referência e as escalas de classificação são representadas com cores de semáforo e classificações numéricas. O valor da IVU obtido para uma comunidade pode ser visualizado em um mapa e em uma figura correspondente, incluindo uma tabela de valores para os parâmetros avaliados.

Palavras-chave: Crocodilo americano, *Crocodylus acutus,* Parâmetro biológico, Parâmetro espacial, Parâmetro institucional, Parâmetro social, Vida silvestre.

Introduction

Negative interactions between humans and wildlife are increasingly common, widespread, and significant among the challenges faced in conservation. These conflicts hinder coexistence and adversely affect both wildlife and human well-being (Solano-Gómez and Mora 2023). These conflicts create a barrier to achieving sustainable biodiversity conservation and community development (Solano-Gómez and Mora 2023).

In numerous areas where humans coexist with wild animals, both intentional and unintentional negative interactions between them are becoming increasingly problematic (Matanzima *et al.* 2022). The scope of this phenomenon is not limited to a specific geographic region or climatic condition; it occurs in all regions where human populations and wildlife share habitats and resources (Márquez and Goldstein 2014). This is a two-way problem: it arises when the needs and behaviors of wildlife negatively impact human life, and also when goals pursued by humans negatively impact wildlife needs (García-Grajales and Buenrostro-Silva 2015). Due to increasing human populations and the reduction of natural habitats, human-wildlife conflict has emerged as a "wicked problem": these conflicts are becoming more frequent, severe, and widespread, presenting extremely challenging obstacles to resolution (Sillero-Zubiri *et al.* 2023).

Negative interactions between humans and wildlife have consequences across various

dimensions. Economically, they impact agriculture, livestock, and infrastructure. Socially, they result in threats, injuries, and even fatalities for humans and their domestic animals. Additionally, these interactions contribute to a negative perception of wildlife in society as a whole (García-Grajales and Buenrostro-Silva 2015). In response to these situations, the capture, aggression, and sacrifice of wild animals are becoming more frequent as emotional reactions from humans (Lamarque *et al*. 2009).

The escalation in the frequency and severity of negative interactions involving large predators is a direct outcome of human encroachment into wildlife habitats (Lamarque *et al*. 2009). Consequently, the management of wildlife populations entangled in conflicts poses multiple challenges related to conservation, perceptions of nature, animal welfare, and the economics of natural resources. Therefore, strategies aimed at addressing these conflicts need to take all these factors into consideration (Sillero-Zubiri *et al*. 2023).

Crocodilians are frequently involved in negative interactions with humans throughout their worldwide distribution (González-Desales *et al*. 2021). In the context of the American Crocodile, *Crocodylus acutus* (Cuvier, 1807), attacks on humans have been attributed to a combination of anthropic and biological factors. A crucial factor is the expansion of human settlements and activities, both productive and recreational, within crocodile habitats (Garel *et al*. 2005). On the contrary, it seems that at least some populations of the American Crocodile have experienced a recovery (Rainwater *et al*. 2022).

Wild populations of crocodilians were profoundly affected by hunting across their distribution areas (Casas-Andreu and Guzmán Arroyo 1970, Álvarez del Toro 1974). At the outset of the 1970s, the American Crocodile faced significant threats from hunting and habitat destruction (Thorbjarnarson 1989, Savage 2002). Nevertheless, due to conservation endeavors, including reproductive and reintroduction programs executed in various countries, population recovery

has been achieved (Webb *et al*. 2001, Thorbjarnarson *et al*. 2006, Sánchez-Herrera *et al*. 2011). However, the American Crocodile confronts several threats, primarily habitat loss and degradation, alongside challenges posed by fishing nets, illegal hunting, and hybridization with sympatric species, mainly with Morelet´s Crocodile, *Crocodylus moreletii* (Duméril and Bibron, 1851) (Rainwater *et al*. 2022). The apparent success in recovery has led to an upsurge in crocodile populations and, consequently, interactions with humans, often evoking a negative response in society by associating them with dangerous species (Caldicott *et al*. 2005).

Conservation efforts are poised to fail if they do not address the fundamental causes of biodiversity loss, which often involves understanding human behaviors and the underlying attitudes driving them (Than *et al*. 2022). Human-crocodile interactions are recognized as a complex issue and quantifying them has proven highly challenging due to the absence of effective strategies to manage them (García-Grajales 2013). These interactions necessitate a comprehensive analysis of all the involved components, encompassing an evaluation of the vulnerability level of coastal communities to crocodile presence (García-Grajales 2013). To assist in achieving this objective, we have developed a vulnerability index that integrates the examination of indicators within the social, biological-environmental, institutional, and spatial dimensions.

The creation of a vulnerability index to address adverse human-crocodile interactions holds paramount importance, as it could not only save lives but also contribute to the promotion of crocodile conservation. This paper aims to introduce this technical tool that facilitates the determination of the extent of vulnerability within coastal communities in the presence of the American Crocodile.

The vulnerability index relies on four parameters: social, biological-environmental, institutional, and spatial. These parameters are assessed through indicators tailored to the

requirements and anticipated outcomes of each category. In this paper, we present and examine the indicators for each of these four parameters, along with their corresponding scales and values, in order to establish the level of vulnerability of a specific community to crocodile interactions.

Materials and Methods

To formulate our Index of Vulnerability (IVU), we drew upon the theoretical and practical underpinnings of the Approximate Sustainability Index (ASI; Gutiérrez-Espeleta 1994). The ASI introduces the assessment of four parameters aligning with the dimensions of sustainable development (social, environmental, economic, and institutional). In relation to each of the IVU parameters, we identified a set of indicators that we scrutinized utilizing a performance or interpretive scale that indicates the level of risk associated with crocodile encounters (Table 1). To calculate the ultimate value of each parameter, we employed the following equation:

Equation 1: $C_{kt} = \frac{1}{4 \times I_{kt}} \left\{ \sum_{i=1}^{I_{kt}} V_{ikt} \right\} + \frac{1}{2},$

where: C_{kt} = Score of parameter k in year t, I_{kt} = Number of indicators that estimate the parameter k in year t, V_{ikt} = Value (-2, 2) of the i-th indicator that estimates k for year t.

Once the value of each of the parameters has been calculated, we estimate the IVU with the following equation:

Equation 2: IVU = $\dfrac{\sum_{k=1}^{4} C_{kt} I_{kt}}{\sum_{k=1}^{4} I_{kt}}$

The IVU value ranges from zero (0) to one (1), with 0 representing the lowest vulnerability and 1 indicating the highest. We defined five vulnerability levels, each aligned with a twentieth (20th) percentile. In order to create a visual representation that conveys the degree of vulnerability, each of these levels is linked to a color scale resembling that of a traffic light (Table 2).

Table 1. Performance and interpretation scale of the indicators used to estimate an Index of Vulnerability (IVU). Source: Adapted from Gutiérrez-Espeleta (1994).

Risk assessment	Value
Very low	-2
Low	-1
Medium	0
High	1
Very high	2

Table 2. Vulnerability condition and respective color scale, according to each interval of the Vulnerability Index (IVU).

Interval	Vulnerability condition
0.00–0.20	Little vulnerable
0.21–0.40	Something vulnerable
0.41–0.60	Moderately vulnerable
0.61–0.80	Vulnerable
0.81–1.00	Highly vulnerable

Results

Indicators of the Social Parameter

1. *Percentage of water bodies visited.*—The frequency of visitation is determined by analyzing the number of people visiting each of the water bodies near the community. The suggested time for this parameter is one year, during which respondents enumerate how many bodies of water they visited in that time period. We consider that more than a year is not advisable, as it is more likely that people may confuse their activities from earlier dates. This indicator operates under the assumption that as the number of visited sites increases, the likelihood of encountering crocodiles also rises. The existing water bodies in the community area should be

counted, and it should be determined which ones are frequented by people. Risk levels are measured based on overall visitation percentages, and corresponding risk categories are assigned accordingly: 0–5% of water bodies visited indicate a very low risk, with 5.1–10% of water bodies visited, the risk is low. This increases to medium when 10.1–30% of water bodies are visited, and to high when 30.1–70% of water bodies are visited. If 70.1–100% of water bodies are visited, the risk is very high (Table 3), all referring to visitation within a year.

2. *Percentage of population engaged in activities within crocodile habitat.*—Risky activities encompass all actions carried out by both residents and visitors within the habitat of crocodiles that pose a significant potential for incidents. It is evident that an increased exposure to danger through such activities corresponds to a higher likelihood of incidents occurring. The identification of risky activities is based on the criteria established by Sandoval-Hernández *et al*. (2017). The risk assessment is categorized into three levels: high, medium, and low. The high level pertains to activities directly conducted in the water. The medium level applies to activities undertaken at the margins or shores of water bodies, or on the water using boats or machinery. The low level covers activities carried out at a safe distance from water bodies. Counts of the number of people involved in each identified risky activity during the last year must be conducted to estimate the percentage and assign the corresponding risk level (Table 3).

3. *Frequency of risky activities conducted within the crocodile habitat.*—The frequency of risky activities corresponds to the regularity with which high-risk actions are performed by people within the crocodile habitat (Table 3). We rely on the high-risk activities delineated in the Social Indicator

#2 "Percentage of population engaged in activities within crocodile habitat" that encompass all activities related to resource utilization, recreation, and work. This indicator serves as a complementary element to Indicator #2, as an increased frequency of risky activities directly correlates with a higher likelihood of incidents. The risk assessment is categorized into three levels: high, medium, and low. Only one visit during the last year indicates a very low risk, a semiannual visit equals a low risk, a monthly visit signifies a medium risk, weekly visits carry a high risk, and daily visits mean a very high risk (Table 3), all during the last year.

4. *Perception of risk of suffering a crocodile attack.*—The perception of risk corresponds to the residents who recognize the potential for a crocodile attack while participating in activities within the species' habitat. For example, if only 0–20% of the inhabitants perceive the risk, the vulnerability is considered very high (Table 3). This indicator complements with indicator #5 "Level of knowledge of the habitants about basic aspects of crocodile biology", that is aim to gauge how well people are informed about those crocodile-related aspects that put them at risk of incidents and how to avert them. It is crucial for individuals to be able to identify a crocodile and grasp five fundamental aspects of crocodile biology. A very low level signifies knowledge in only one or none of these aspects, while a very high level corresponds to understanding all five aspects (Table 3). Interviews should be conducted within the local community, with a representative sample of the population, to assess the community's risk perception in the study area.

5. *Level of knowledge of the habitants about basic aspects of crocodile biology.*—To assess the residents' level of knowledge

Table 3. Selected indicators to evaluate the social parameter of the Vulnerability Index (IVU). For each indicator, the reference categories and the rating, coloring and numerical assessment scales are noted.

Social			
Indicators	**Categories**	**Score**	**Value**
(1) Percentage of water bodies visited	0–5% water bodies visited	Very low	-2
	5.1–10% water bodies visited	Low	-1
	10.1–30% water bodies visited	Medium	0
	30.1–70% water bodies visited	High	1
	70.1–100% water bodies visited	Very high	2
(2) Percentage of population engaged in activities within crocodile habitat	0–5% of risky activities	Very low	-2
	5.1–10% of risky activities	Low	-1
	10.1–30% of risky activities	Medium	0
	30.1–70% of risky activities	High	1
	70.1–100% of risky activities	Very high	2
(3) Frequency of risky activities conducted within the crocodile habitat	Very low (annual)	Very low	-2
	Low (semiannual)	Low	-1
	Medium (monthly)	Medium	0
	High (weekly)	High	1
	Very high (daily)	Very high	2
(4) Perception of risk of suffering a crocodile attack	Very high (80.1–100%)	Very low	-2
	High (60.1–80%)	Low	-1
	Medium (40.1–60%)	Medium	0
	Low (20.1–40%)	High	1
	Very low (0–20%)	Very high	2
(5) Level of knowledge of the habitants about basic aspects of crocodile biology	Very high (80.1–100% correct answers)	Very low	-2
	High (60.1–80% correct answers)	Low	-1
	Medium (40.1–60% correct answers)	Medium	0
	Low (20.1–40% correct answers)	High	1
	Very low (0–20% correct answers)	Very high	2
(6) Percent of residents taking measures to prevent incidents with crocodiles	80.1–100% residents take action	Very low	-2
	60.1–80% residents take action	Low	-1
	40.1–60% residents take action	Medium	0
	20.1–40% residents take action	High	1
	0–20% residents take action	Very high	2
(7) Percent of residents consent to participate in environmental education processes	Very high (80.1–100% of people agree)	Very low	-2
	High (60.1–80% of people agree)	Low	-1
	Medium (40.1–60% of people agree)	Medium	0
	Low (20.1–40% of people agree)	High	1
	Very low (0–20% of people agree)	Very high	2

concerning fundamental aspects of crocodile natural history, biology, and habitat, the following elements should be evaluated: (1) ability to identify a crocodile, (2) familiarity with crocodile habitat locations, (3) awareness of crocodile dietary habits, (4) understanding of courtship and nesting sites and behaviors (crocodiles tend to be more aggressive during the reproductive season), and (5) Understanding of the causes of attacks on humans. Interviews should be conducted within the local community, involving a representative sample of the population, to assess the residents' understanding of basic aspects of crocodile biology. To evaluate residents' knowledge in each of these aspects, correct and incorrect responses are tallied in each case. The primary objective here is to determine how well individuals are informed about the specific aspects of crocodile behavior that put them at risk of incidents and how to prevent them. It is essential for people to be able to recognize a crocodile and comprehend these five fundamental aspects of crocodile biology. A very low level indicates knowledge in only one or none of these aspects, while a very high level corresponds to understanding all five aspects (Table 3).

6. *Percent of residents taking measures to prevent incidents with crocodiles.*—Preventive measures refer to actions taken by residents to reduce the risk of incidents with crocodiles while engaging in activities near or in the water bodies close to the community. In the Costa Rican context, the measures recommended by the National Crocodile Commission, a part of the National System of Conservation Areas (SINAC), serve as a reference. Interviews should be conducted within the local community, involving a representative sample of the population, to estimate the percentage of people taking preventive measures to avoid incidents with crocodiles (Table 3).

7. *Percent of residents consent to participate in environmental education processes.*—Consent to participate in environmental education processes indicates the interest or willingness expressed by residents to engage in educational and informational activities related to crocodiles. Interviews should be conducted within the local community, involving a representative sample of the population, to estimate the percentage of community members consenting to participate in environmental education processes (Table 3).

Indicators of the Biological-Environmental Parameter

1. *Presence of crocodiles in the habitat.*—To quantify the presence of crocodiles in waterbodies near the community, the percentage of water bodies occupied by crocodiles or another feasible method should be calculated during the implementation of the tool. A straightforward approach to establish the percentage is by determining the ratio of the number of waterbodies where crocodiles are observed to the total number of waterbodies sampled, and then multiplying by 100:

$$\text{Percentage of water bodies occupied by crocodiles} = \frac{\text{Number of water bodies where crocodiles were sighted}}{\text{Number of water bodies sampled}} \times 100$$

The percentage of waterbodies occupied by crocodiles is categorized from very low (0–5% of waterbodies occupied), to very high (70.1–100% of waterbodies occupied) (Table 4).

2. *Index of crocodile number per kilometer.*—This is determined by the number of individuals recorded per linear kilometer along the waterbody's edge. One potential technique for measurement is using encounter rates (crocodiles/km of survey route), a method widely employed (e.g., Sasa and

Table 4. Selected indicators to evaluate the biological-environmental parameter of the Vulnerability Index (IVU). For each indicator, the reference categories and the rating, coloring and numerical assessment scales are noted.

Biological-Environmental			
Indicators	**Categories**	**Score**	**Value**
(1) Presence of crocodiles in the habitat	Very low (0–5% waterbodies occupied)	**Very low**	-2
	Low (5.1–10% waterbodies occupied)	**Low**	-1
	Medium (10.1–30% waterbodies occupied)	**Medium**	0
	High (30.1–70% waterbodies occupied)	**High**	1
	Very high (70.1–100% waterbodies occupied)	**Very high**	2
(2) Index of crocodile number per kilometer	None (0 crocodiles/km)	**Very low**	-2
	Low (1–10 crocodiles/km)	**Low**	-1
	Medium (de 10–20 crocodiles/km)	**Medium**	0
	High (de 20–40 crocodiles/km)	**High**	1
	Very high (> 40 crocodiles/km)	**Very high**	2
(3) Percentage of adult crocodiles present in waterbodies nearby the community	0–5%	**Very low**	-2
	5.1–10%	**Low**	-1
	10.1–15%	**Medium**	0
	15.1–20%	**High**	1
	More than 20.1%	**Very high**	2
(4) Distribution of crocodiles in the habitat	Grouped-localized	**Very low**	-2
	Singles-random	**Low**	-1
	Grouped-random	**Medium**	0
	Singles-uniform	**High**	1
	Grouped-uniform	**Very high**	2
(5) Reproductive season: copulation, nesting and hatchling	No activity	**Very low**	-2
	Post season	**Low**	-1
	Beginning of season	**Medium**	0
	Peak of copulations	**High**	1
	Copulation and hatching	**Very high**	2
(6) Percent of properties having domestic animals near crocodile habitat	0–5%	**Very low**	-2
	5.1–10%	**Low**	-1
	10.1–30%	**Medium**	0
	30.1–70%	**High**	1
	70.1–100%	**Very high**	2

Chaves 1992, Sánchez *et al.* 1996, Charruau *et al.* 2005, Hernández-Hurtado *et al.* 2011). Chabreck (1966) and Charruau *et al.* (2005) utilized a nocturnal visual counting method, identifying the animals by their eye reflection using lamps. Crocodiles are counted along one edge, and then during a second sampling, they are counted along the opposite edge, preventing the duplication of individual counts. The level of risk associated with crocodiles recorded per linear kilometer is determined based on previously recorded values in several coastal communities of the country (e.g., Sasa and Chaves 1992, Sánchez *et al.* 1996) (Table 4).

3. *Percentage of adult crocodiles present in waterbodies nearby the community.*—The determination of crocodile sizes can be achieved through various methods. One commonly used approach involves estimating the distance from the tip of the snout at the level of the nostrils to the midpoint of the eyes (Cedeño-Vázquez *et al.* 2006). The obtained value can be multiplied by 7 to estimate the approximate total length (TL) of the crocodile (J. Bolaños, pers. comm.). However, the method for estimating TL varies among researchers. García-Grajales and Buenrostro-Silva (2021) noted, based on various sources, that in practice, a well-trained observer can estimate the length from the tip of the snout to the anterior corner of the eyes, and this is multiplied by 10 to obtain an approximation of the TL. The estimated TL forms the basis for establishing size and age categories, ranging from neonates (TL < 30 cm) to adults (TL > 180 cm) (Charruau *et al.* 2005, Platt and Thorbjarnarson 2000). The percentage of adult crocodiles (> 180 cm) is calculated by dividing the number of adults by the total number of individuals and multiplying by 100. The risk assessment is categorized into five levels or percentages of adult crocodiles present in waterbodies near the community.

If only 0 to 5% of the individuals are adults, the risk is very low. However, if the percentage of adults is more than 20.1%, the risk is very high (Table 4).

4. *Distribution of crocodiles in the habitat.*—Crocodile distribution refers to the extent of clustering and dispersion of individuals within their habitat. Various categories can be defined in this context, ranging from grouped and localized to individual and random, where individuals do not form clusters and are distributed randomly. According to the methods for crocodile censuses described earlier, during searches, each crocodile is counted as being alone, in scattered groups, or in clusters. Fatal attacks have occurred both by solitary animals, mostly, and by animals close to each other, at least in the Tárcoles River in Costa Rica. However, we hypothesize that when crocodiles are clustered, the likelihood of one of them attacking a person is higher (Table 4). Two known facts by the authors, the first being the attack on a person in 2014 in the Tárcoles River, and the second a dead coati thrown into the Tárcoles River in 2017, attest to this behavior. In natural conditions, crocodiles position themselves in areas frequented by potential preys. Due to the reduction of water bodies caused by droughts, animals approaching to drink water have a higher probability of being attacked. We have learned of several cases reported by Tárcoles River residents of crocodiles attacking cows due to this behavior.

5. *Reproductive season: copulation, nesting and hatchling.*—This indicator highlights behaviors associated with crocodile reproduction. The reproductive season includes courtship, defense of high-quality sites, nesting, and parental care of the offspring. The presence of offspring (less than 50 cm in length) indicates the presence of reproductive females and males in the

area. The reproductive phases included in this indicator are: copulation, the union of a pair during mating, and dorsal rubbing; nesting, where the female constructs a nest or lays eggs; defense, where the animal protects the nest or young. It is crucial to determine when crocodiles engage in these behaviors in the study area, as they are much more aggressive during these phases (Cupul-Magaña *et al.* 2010). During this period, female Morelet's crocodile is very aggressive (González-Ramón and López-Luna 2018). Many females protect their nests and are potentially dangerous. When this is the case, the nest is very close or visible (González-Ramón and López-Luna 2018). The risk assessment is categorized into five categories from very low risk when there is not any activity of the reproduction parameters included (copulation, nesting and hatchling), low risk at post season, medium risk at the beginning of the season, high risk during the copulation peak and very high risk during the time of copulation and hatchling of young (Table 4).

6. *Percent of properties having domestic animals near crocodile habitat.*— The percentage of properties with domestic animals (i.e., pets, farm animals, and cultivated species) within 100 m of water bodies where crocodiles inhabit is considered. This information should be gathered through surveys of the population regarding the ownership of domestic animals. This indicator highlights the fact that crocodiles are opportunistic animals that feed on a wide variety of prey. Therefore, the presence of domestic animals and cultivated species (such as fish and shrimp) near their habitat can represent an easily accessible source of food. In this context, Bolaños (2012) documented the presence of crocodiles in tilapia farming ponds. Only free-ranging domestic animals or animals confined in tanks, in the case of cultivated animals, are included. If all properties (100%) have domestic animals, the risk of crocodile attraction is very high, corresponding to a high score (Table 4).

Indicators Within the Institutional Parameter

The institutional parameter includes policies, plans, programs, and protocols developed and implemented by the institutions in charge or competent in planning, development, and control of wildlife

1. *Crocodile management plan.*—A management plan is a tool containing a description of the species' biological aspects, spatial and ecological characterization of the habitat, analysis of population status, determination of population spatial distribution, and assessment of habitat utilization and health. All of this information is crucial for species management and conservation, as well as for developing strategies to foster harmonious coexistence with human populations. Collaboration among researchers and responsible authorities such as the local wildlife agency is needed to obtain information on the percentage of the management plan that has been implemented at the time of the IVU application. For example, if 41% to 60% of the management plan has been executed, the score to tally is medium with a value of 0 for the index (Table 5).

2. *Environmental education program.*—This indicator should encompass several essential elements to contribute to the harmonious coexistence between humans and wildlife (Marchini and Luciano 2009). Within the framework of this parameter, the presence and level of implementation of an environmental education program (EEP) in the study area should be assessed as execution categories (Table 5). To evaluate the efficacy of an EEP, an analysis of the intended goals and objectives is conducted. This assessment

Table 5. Selected indicators to evaluate the institutional parameter of the Vulnerability Index (IVU). For each indicator, the reference categories and the rating, coloring and numerical assessment scales are noted.

Institutional			
Indicators	**Categories**	**Score**	**Value**
(1) Crocodile Management Plan	running between 81–100%	Very low	-2
	running between 61–80%	Low	-1
	running between 41–60%	Medium	0
	running between 21–40%	High	1
	running between 0–20%	Very high	2
(2) Environmental Education Program	running between 81–100%	Very low	-2
	running between 61–80%	Low	-1
	running between 41–60%	Medium	0
	running between 21–40%	High	1
	running between 0–20%	Very high	2
(3) Training of officers in charge of handling dangerous situations	81–100% of staff trained	Very low	-2
	61–80% of staff trained	Low	-1
	41–60% of staff trained	Medium	0
	21–40% of staff trained	High	1
	0–20% of staff trained	Very high	2
(4) Policies for the management of crocodile conflicts	Applied in the last year	Very low	-2
	Applied 1–2 years ago	Low	-1
	Applied 3–4 years ago	Medium	0
	Applied 4–5 years ago	High	1
	Applied more than 6 years ago	Very high	2
(5) Informative workshops for officials on crocodile issues	Applied in the last year	Very low	-2
	Applied 1–2 years ago	Low	-1
	Applied 3–4 years ago	Medium	0
	Applied 4–5 years ago	High	1
	Applied more than 6 years ago	Very high	2
(6) Information sources for prevention of crocodile incidents	Abundant adequate and up-to-date sources	Very low	-2
	Some adequate and up-to-date sources	Low	-1
	Very few adequate and up-to-date sources	Medium	0
	Unsuitable or outdated sources	High	1
	There are no sources of information	Very high	2
(7) Inventory of the crocodile population in the main waterbodies	Less than 1 year of elaboration	Very low	-2
	1–2 years of elaboration	Low	-1
	3–4 years of elaboration	Medium	0
	5–6 years of elaboration	High	1
	More than 6 years of elaboration	Very high	2

includes an examination of the strategies and activities required to attain these goals and objectives, undertaken by the individuals responsible for their implementation, along with the budget and sources of funding. Additionally, the EEP should incorporate achievement indicators and mechanisms for assessing the program's impact. Collaboration among researchers and responsible authorities such as the local wildlife agency is needed to obtain information on the percentage of the EEP that has been implemented at the time of IVU application. For example, if there is no EEP or only 0% to 20% of it has been executed, the score is very high (Table 5).

3. *Training of officials in charge of handling dangerous situations.*—This indicator covers a crucial aspect to ascertain the existence and currency of a dedicated ongoing training program, aimed at keeping these officials well-versed in the latest wildlife capture and management techniques (Table 5). This training program should encompass information on the requisite procedures, techniques, and equipment essential for the adept and secure management of wildlife. To gauge the effectiveness of the program, metrics such as the count of trained personnel and the tally of addressed situations can be used. Collaboration among researchers and the personnel responsible for managing dangerous situations is needed to obtain information on how many of these officials have been trained in handling such situations. For example, if between 80% and 100% of the officials have been trained, the score is very low (Table 5).

4. *Policies for the management of crocodile conflicts.*—The generation and implemen-tation of tangible measures by government agencies through responsible institutions are assessed, aiming to foster the conservation and management of both the species and its habitats (Table 5). Collaborative work among

researchers and authorities responsible for managing conflicts with crocodiles is necessary to obtain information on when these policies were applied. If the policies were applied within the last year, the score is very low (Table 5). Conversely, if the policies were applied more than six years ago, the score is very high (Table 5).

5. *Informative workshops for officials on crocodile issues.*—This indicator involves the implementation of informative workshops for responsible officials, serving as a mechanism for gathering insights on the progression of human-crocodile interactions. Furthermore, its purpose is to foster the formulation of suggestions and initiatives that can be integrated into forthcoming policies and the crocodile management plan. Collaboration among researchers and personnel responsible, such as the local wildlife agency, is necessary to obtain information about informational workshops for officials regarding crocodile-related matters. If the workshops were conducted within the last year, the score is very low (Table 5). Conversely, if the workshops were conducted more than six years ago, the score is very high (Table 5).

6. *Information sources for the prevention of crocodile incidents.*—This indicator pertains to the number, placement, and condition of information sources concerning preventive measures that residents need to adopt to avert encounters with crocodiles in their communities (Table 5). Information sources include posters, brochures, radio announcements, television segments, and informational workshops. The currency, quality, and accessibility of this information to all community members are evaluated. To assess this indicator, relevant information about the availability of crocodile incident prevention resources in the community needs to be gathered. If there are no information sources, the score is very high (Table 5).

7. *Inventory of the crocodile population in the main waterbodies.*—This information needs to be systematized within a database, accessible online and available free of charge, ensuring its accessibility for public institutions, non-governmental organizations, or any entity requiring it. Collaboration among researchers and responsible authorities, such as the local wildlife agency, is necessary to obtain information about the timing of crocodile population inventories in the community water bodies. If the last inventory was conducted within the last year, the score is very low (Table 5). Conversely, if the last inventory was conducted more than six years ago, the score is very high (Table 5).

Indicators of the Spatial Parameter

Apparently, the sites where crocodile attacks occur have very particular conditions (Guido-Patiño 2015). In this regard, variables such as altitude, distance from the community to water bodies, the presence of flood-prone areas, human population density, and the density of drainage networks have been used to determine areas at higher risk of crocodile attacks on humans in Mexico (Guido-Patiño 2015) as well as in Costa Rica (Sandoval *et al*. 2019). The spatial parameter involves evaluating the geographical and geomorphological characteristics that impact the existence of potential habitats and, consequently, the occurrence of crocodiles within a specific area or locality (Table 6).

1. *Proximity of the community to crocodile habitats.*—This indicator operates under the assumption that the nearer a community is to the crocodile habitat, the higher the likelihood that residents will engage in activities within it (Table 6). The proximity of the community is defined as the linear distance from the community boundary to the edge of the nearest natural water body where crocodile presence has been documented. If the distance to the water bodies is large, there is

less likelihood that people will visit them, and therefore, there is a lower risk of accidents. Various software tools or other digital resources can be employed to calculate this distance. For example, some GPS devices with an uncertainty of less than 5 m are available. This measurement encompasses the banks of rivers, streams, other water bodies, and potential flood-prone areas influenced by heavy rains and tides. During such times, crocodiles tend to move into these areas, effectively extending their habitat. This indicator correlates with Spatial Indicator #3, "Presence of flooding areas," and establishes a link between social and spatial aspects. As an example, we constructed categories of proximities with their respective scores and values, with the highest score and value for a distance from 0 to 100 m between the community boundaries and the nearest water edge (Table 6).

2. *Altitude.*—The altitude must be assessed or measured for each specific situation. For instance, the crocodile habitat in the Costa Rican Central Pacific is associated with altitudes below 700 m above sea level (Sandoval *et al*. 2019). The closer the altitude is to this threshold, the lower the risk associated with crocodile interactions (Table 6). To determine the altitude, a Digital Elevation Model (DEM) can be created using contour lines from the country's official base map. In Costa Rica, for example, cartographic sheets (recommended scale of at least 1:50,000) from the National Geographic Institute (IGN) database can be utilized, along with the vector layer of contour lines. Subsequently, an interpolation process is executed, resulting in the DEM using the ArcGIS "interpolation/topo to raster" tool. This method, recommended by ESRI, is specialized for generating digital elevation models (Chavarría-Trejos 2019, Sandoval *et al*. 2019). In cases where greater precision in the value is needed, field verifications of

Table 6. Selected indicators to evaluate the spatial parameter of the Vulnerability Index (IVU). For each indicator, the reference categories and the rating, coloring and numerical assessment scales are noted.

Spatial			
Indicators	**Categories**	**Score**	**Value**
(1) Proximity of the community to crocodile habitats	> 400 m	Very low	-2
	301–400 m	Low	-1
	201–300 m	Medium	0
	101–200 m	High	1
	0–100 m	Very high	2
(2) Altitude	> 300 m a.s.l.	Very low	-2
	151–300 m a.s.l.	Low	-1
	101–150 m a.s.l.	Medium	0
	51–100 m a.s.l.	High	1
	0–50 m a.s.l.	Very high	2
(3) Density of the drainage network	0–0.64	Very low	-2
	0.65–1.28	Low	-1
	1.29–1.92	Medium	0
	1.93–2.56	High	1
	2.57–3.20	Very high	2
(4) Percentage of flooding areas	0–5% of flooding area	Very low	-2
	5.1–10% of flooding area	Low	-1
	10.1–30% of flooding area	Medium	0
	30.1–70% of flooding area	High	1
	70.1–100% of flooding area	Very high	2
(5) Percentage of crocodile habitat reduction	0–1% habitat reduction	Very low	-2
	1–2% habitat reduction	Low	-1
	2–3% habitat reduction	Medium	0
	3–5% habitat reduction	High	1
	> 5% habitat reduction	Very high	2

altitude could be conducted using a GPS, and the obtained values could be interpolated in a GIS. The risk assessment is categorized into five altitude categories, with a very low risk at above 300 m a.s.l. because there are very few crocodiles at this altitude, and very high

risk at 0–50 m a.s.l. given that most attacks occur here (Table 6).

3. *Density of the drainage network.*— Concerning the drainage network, areas with the highest concentrations of channels are

linked to optimal habitat conditions for the American crocodile (Sandoval *et al*. 2019). All potential water bodies, whether temporary or permanent, should be considered, and we recommend on-site verifications. Once the Digital Elevation Model (DEM) has been created, (see indicator #2 "Altitude"), the drainage network was derived by Sandoval *et al*. (2019) using the ArcGIS Hydrology tool, which is used in watershed delineation to ensure that river and stream channels match the DEM and prevent the displacement of existing river layers. With the generation of the hydrological network in the study area, Sandoval *et al*. (2019) applied the density/ Kernel Density tool to estimate the river density per square kilometer, facilitating the integration of variables later on. Sandoval *et al*. (2019) classified each variable into three categories (high, medium, and low) using equal interval classification. However, we used the reference values presented by Sandoval (2017) to establish the five categories included in table 6. With the drainage density variable classified and delimited per km², Sandoval (2017) reclassified it into three categories: low, medium, and high. We took the minimum and maximum values from that classification and divided that range into five categories, such that the category with the lowest value (0.64) is associated with a very low risk, and the highest (3.20) is associated with a very high risk (Table 6).

4. *Percentage of flooding areas.*—The indicator assesses the proportion of flooding areas within the study site (Table 6). Flooding areas arise from prolonged rainfall in flat terrains, facilitating the presence and movement of the American crocodile (Cupul-Magaña 2012). Regions highly susceptible to flooding pose a very high risk (Table 6). For instance, flooding areas within a specific region in Costa Rica can be extracted from the National Emergency Commission's

(CNE) database. This indicator is interconnected with social indicator #1, "Percentage of population living near crocodile habitat," and is based on the information and methodologies outlined by Sandoval *et al*. (2019). Coastal areas, such as those on the Costa Rican Central Pacific region, are prone to flooding due to both rainfall and tidal effects, something we have experienced many times in the field. It usually happens that many water bodies become interconnected due to flooding, allowing crocodiles to move toward and into communities. Because of this, the risk categories for this indicator are not proportionally similar. Instead, an area with only 5% flooding area still presents a risk. As a result, for the purposes of the scores assigned, we assess 5% as a very low risk, between 5.1% and 10% as low risk, but we consider anything between 10.1% and 30% to be at least a medium risk (Table 6). Between 30.1% and 100% of flooding area, we divide into high and very high risk to complete the five proposed categories for all indicators (Table 6).

5. *Percentage of crocodile habitat reduction.*— This pertains to the reduction in the American Crocodile's habitat caused by human activities like the expansion of agricultural and livestock activities as well as increasing human settlements. We hypothesize that a greater rate of crocodile habitat loss corresponds to a higher risk of incidents. We propose to evaluate the alteration in crocodile habitat that has occurred over the last five years. All indicators should have a time scale as relevant as possible, although in some cases, there may need to be differences due to the nature of each indicator. For this particular indicator, we recommend evaluating changes over the last five years because it is not advisable to extend it much beyond the period of one or two years of other indicators. Additionally, it is important

to consider that land use changes in coastal areas have accelerated significantly in recent years, especially toward urbanization, putting more people at risk each year. An additional aspect to consider is the response time of the respective authorities regarding decision-making, such as adopting or implementing corrective measures for land use changes. Extended periods, for example, 10 years or more, would have a very negative impact, as corrective measures, for instance, wouldn't be taken with the required immediacy. As an example, 2 to 3 % of habitat reduction in the last five years would corresponds to a Medium score (Table 6).

Degree of Vulnerability of the Communities

The vulnerability level, and consequently the IVU value obtained for a specific community, can be visualized with a map in a figure including a table of values for the assessed parameters and indicators with the corresponding color scale. Figure 1 provides an illustration of such map and an IVU value generated to depict the vulnerability degree of a coastal community to the presence of crocodiles, modified from one presented by Chavarría-Trejos (2019).

Discussion

Our vulnerability index presented here should be modified in some aspects according to each specific case. Some of the weaknesses that we can currently point out mainly relate to the fact that certain indicators require specific technical knowledge for their estimation. The primary case is some of the indicators in the spatial parameter, as these involve having knowledge and skills in the use of Geographic Information Systems (GIS). However, even other aspects, including some very basic ones, require some degree or level of prior training. For example, a fundamental aspect is the estimation of the body size of crocodiles, which even involves training the personnel, including, for instance, practices

with animals in captivity (J. Bolaños, pers. comm.). It is also essential to take the necessary sampling precautions to ensure the representativeness of different sectors of the community being worked with and include them in interviews, especially in large communities, so that their results are valid. Additionally, since the IVU provides a single overall result of the processing of information obtained from the four parameters, it must be ensured that the information is obtained within a reasonable time frame, so that the information is temporally comparable.

Despite these weaknesses, our vulnerability index can be employed to design targeted mitigation strategies aimed at reducing negative human-crocodile interactions. This vulnerability index relies on four parameters: social, biological-environmental, institutional, and spatial. Indicators are used to measure these parameters according to their respective needs and expected outcomes. We present and discuss the indicators for each parameter, along with their scales and values, to determine the level of vulnerability of a given community to crocodiles.

In broad terms, the tool we developed must effectively assess the contribution of each of the four parameters to the index's vulnerability evaluation. This ensures the indicators are consistent in their theoretical foundation, methodological design, and the sensitivity of their interpretation scales, offering comprehensive information to measure the risk of attacks. However, it is essential to adjust the indicators and even the parameters to fit the specific region, conditions, and needs of local communities. Primary aspects for adjustment include reevaluating the rating scale of indicators to suit each unique case, such as updating them in line with changes in community population and specific ecological conditions over time. Generally, indicators should be updated based on the ecological, social, and temporal dynamics of each case being analyzed.

As an example, we had included a seventh indicator for the biological-environmental
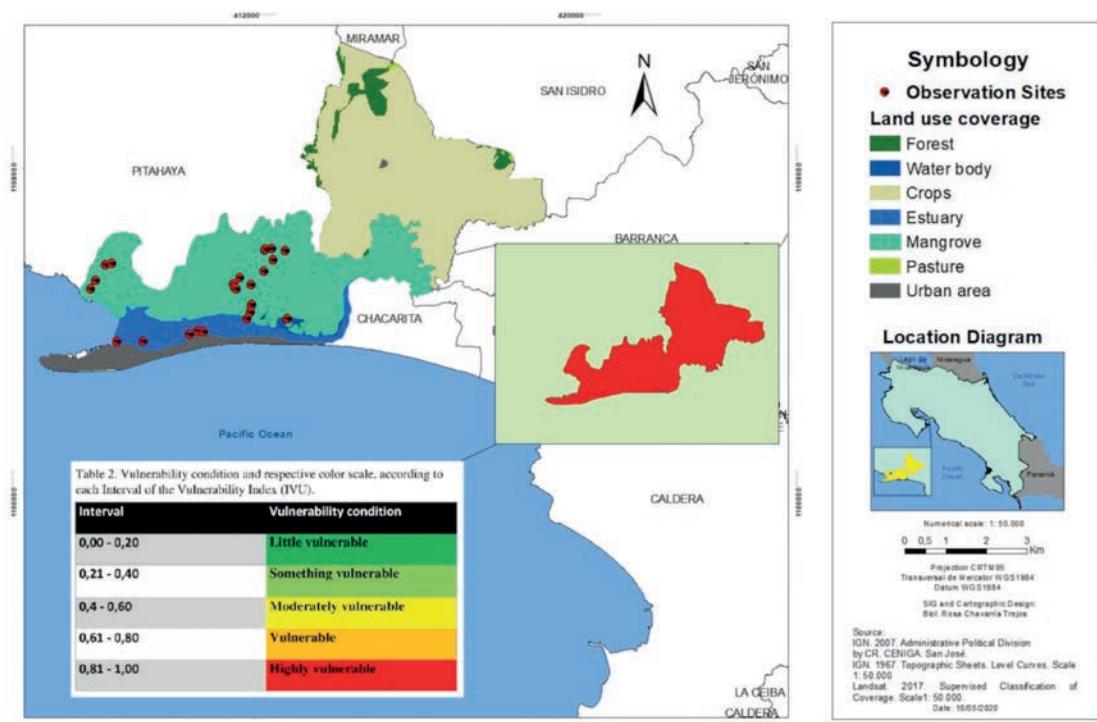
Figure 1. An example of how the degree of vulnerability in a community to the presence of the American Crocodile (*Crocodylus acutus*) can be mapped. The box should contain the values of the Vulnerability Index (IVU) and the social, biological-environmental, institutional and spatial parameters. Modified from Chavarría-Trejos (2019).

parameter: Incidents with crocodiles (fatal and non-fatal) reported in the community. However, after several considerations, including the fact that the percentages of reports may not necessarily be similar in different communities, we decided to remove this indicator and instead we suggest to correlate this value with the index calculation at the community that has been evaluated as a way to test or calibrate the IVU.

In the social parameter, the analysis of social indicators enables an understanding of the role of individuals residing in the area in generating negative interactions between humans and crocodiles. This is accomplished by evaluating the quantity and frequency of hazardous activities conducted within the crocodile's habitat, along

with the implementation of measures to prevent attacks, among other six indicators (Table 3).

The analysis of the biological parameter includes aspects related to the biology of the crocodile such as its presence, abundance, percentage of adults, distribution and behaviors on site (Table 4). On the other hand, policies, plans, programs, and protocols are evaluated by the institutional parameter and the actions of planning, development, and control of wildlife encompass environmental policies related to crocodile issues, management plans for the species, environmental education programs, training protocols for officials, and information campaigns to prevent crocodile attacks. However, institutional parameter includes indicators that

may not be available at some communities or regions, or may be very different among communities.

One key indicator within the institutional parameter is a crocodile management plan, a tool that must have been developed with technical and scientific rigor, guided by current legislation, and possessing financial and operational viability (Castañeda *et al*. 2012). If a crocodile management plan is not available, references can be utilized to identify the essential components that such a plan should encompass. For instance, the standard management plan for the conservation and sustainable use of the Morelet´s Crocodile in Mexico (Balderas *et al*. 2014) can serve as a valuable example.

Similar to a management plan, an environmental education program (EEP) is useful because it must serve as an educational tool that fosters education for sustainable development and promotes the care of the natural environment and biodiversity (Avendaño 2012). An EEP should encompass objectives such as generating fundamental knowledge about the species' biology, behaviors, and habitat, as well as raising awareness about the causes and consequences of negative interactions between crocodiles and humans. It should involve planning actions that encourage sustainable coexistence between people and wildlife.

As the fourth indicator of the institutional parameter, collaboration between governmental entities plays a pivotal role in formulating and implementing public policies that encompass the comprehensive handling of crocodile-related matters. This effort should harmonize with and advance the social, economic, and cultural progress of coastal communities. Officials from public entities responsible for wildlife have the duty of addressing situations involving wild animals. To effectively discharge this role, a comprehensive management protocol should be in place, encompassing requisite methodologies, techniques, and equipment to promptly and securely handle any wildlife-related incidents.

The percentage of personnel trained gives scores and values to the IVU following the determined categories (Table 5).

In addition, the indicator "Informative Workshops for Officials on Crocodile Issues" serves the purpose of fostering the formulation of suggestions and initiatives that can be integrated into forthcoming policies and the crocodile management plan. Accompanying this, the indicator regarding information sources for the prevention of crocodile incidents pertains to the number, placement, and condition of information sources concerning preventive measures that residents need to adopt to avoid encounters with crocodiles in their communities (Table 5).

Fauna population inventories are a widely employed tool for conducting temporal and spatial analyses of wildlife (Ministerio del Ambiente de Perú 2015). For crocodiles, these inventories should encompass current data concerning the population size, sex ratio, size distribution, and geographic distribution of the animals in the main waterbodies within the region (Bolaños *et al*. 1996, 2019, Sánchez *et al*. 1996, Sánchez 2001, Escobedo and González 2006). Consequently, the inventory's validity is crucial as an indicator for the IVU (Table 5).

Data for the spatial parameter are acquired through Geographic Information Systems (GIS) in conjunction with thorough on-site validation. All this information is key when evaluating the degree of risk of interactions between humans and crocodiles. For example, in coastal regions, the presence of rivers, streams, estuaries, and lagoons—both permanent and temporary—is crucial for the species' establishment (Ross 1998).

Initially, we included an indicator in the social parameter regarding the number of people living at a specific distance from the edge of the nearest waterbodies where the presence of crocodiles has been recorded. This was based on the fact that higher population density increases the likelihood of an incident involving a

crocodile. However, this social indicator requires human demographic information that is not always available or easy to obtain. As a social indicator the information is based on human behavior, which is more difficult to predict or measure. Additionally, even if there are few people living nearby the crocodile's habitat, but incidents still occur, it means that the key point is that ultimately the threat comes from the crocodile, and the proximity of people to their habitat is crucial.

The Vulnerability Index can be a tool capable of quantifying the level of risk of suffering crocodile attacks to which the inhabitants of coastal communities are exposed. However, it is crucial to validate the IVU through the evaluation of at least four experts, with one representing each of the IVU components: biological-environmental, social, institutional, and spatial.

The indicators within each parameter have been proposed to provide enough information to satisfactorily establish the risk level of a community. However, adjustments can be made in the scales, especially in the social aspects that, by their nature, are more complex to rate. To strengthen the scope of the IVU, additional indicators can be incorporated to evaluate, for example, physical factors of the habitat besides changes in land use such as urban growth models, identification of vulnerable ecosystems, fragmentation indices, and loss of ecological continuity, among others. This would make it possible to better relate changes in the crocodile population parameters with modifications of its habitat.

Frequent interactions between humans and wild animals often lead to adverse consequences, and conflicting perspectives on managing these interactions can give rise to conflicts (Matanzima *et al*. 2022). The incidence of human-wildlife conflicts is projected to increase in the near future, demanding greater attention (Mora and Solano-Gómez 2022).

Although the American Crocodile is protected under Costa Rica's Biodiversity and Protected Area Laws (e.g., La Gaceta 2005), the government has not yet integrated local people's attitudes into conservation and management strategies. Studies have shown that people can possess high levels of knowledge about the local environment, crocodiles, and their habitats (Than *et al*. 2022). However, attitudes towards wildlife are spatially heterogeneous, influenced by cultural and demographic contexts (Than *et al*. 2022).

Nevertheless, sound management decisions based on science and public participation can lead to better conflict resolution. Several essential steps are required, including increasing environmental education, greater involvement of higher authorities and institutions, and the implementation of innovative tools, such as the vulnerability index proposed here.

Acknowledgments

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The role of modified teeth in the function of prolonged bites in *Hierophis viridiflavus* (Serpentes: Colubridae)

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Abstract

The role of modified teeth in the function of prolonged bites in *Hierophis viridiflavus* (Serpentes: Colubridae). Analysis of the maxillary, palatine, pterygoid, and dentary bones of the Western Whipsnake, *Hierophis viridiflavus carbonarius,* revealed the presence of grooves and ridges in the teeth on the four dentiferous bones. Enlarged and modified rear teeth were found on the posterior maxillaries, separated by alveolar diastema and aligned differently from the anterior maxillary teeth. In both live and dissected specimens, Duvernoy's gland, associated with the production of toxins, surrounds the rear maxillary teeth, which deliver the secretions produced by the gland. These characters, plus the infliction of prolonged bites, facilitate the subduing of prey. The morphology of the palatomaxillary arch places *H. viridiflavus* in the group of opisthoglyphous colubroids, whose modified fangs facilitate the inoculation of secretions, considered a "primitive form of venom." Other species of large sympatric colubroids were also examined, and some analogous structures were observed.

Keywords: Duvernoy's glands, Modified fangs, Opisthoglyphous, Western Whipsnake.

Resumo

O papel dos dentes modificados em mordidas prolongadas de *Hierophis viridiflavus* (Serpentes: Colubridae). A análise dos ossos maxilares, palatinos, pterigóides e dentários de *Hierophis viridiflavus carbonarius* revelou a presença de sulcos e cristas nos dentes dos quatro ossos dentíferos. Dentes posteriores ampliados e modificados foram encontrados nos maxilares posteriores, separados por diástemas alveolares e alinhados de forma diferente dos dentes maxilares anteriores. Tanto nos espécimes vivos como nos dissecados, a glândula de Duvernoy, associada à produção de toxinas, circunda os dentes maxilares posteriores, que liberam as secreções produzidas pela glândula. Essas características, além da infliçção de mordidas prolongadas, facilitam a dominação da presa. A morfologia do arco palatomaxilar coloca *H. viridiflavus* no grupo dos colubróides opistóglifos, cujas presas modificadas facilitam a inoculação de secreções, consideradas uma "forma primitiva de veneno". Outras espécies de grandes colubróides simpátricos também foram examinadas, e algumas estruturas análogas foram observadas.

Palavras-chave: Glândula de Duvernoy, Opistoglifodonte, Presas modificadas, Serpentes.

Introduction

When we find ourselves in front of a snake, the first and most common need is to establish whether it is venomous. The Western Whipsnake, *Hierophis viridiflavus* (Lacépède, 1789), the subject of this study, is a colubrid species that is widespread in Italy and southern France, in addition to adjacent countries including Switzerland, Croatia, and Spain (Kreiner 2007). It occurs in Germany as an introduced allochthonous species (Paterna 2023). To date, this snake is considered a non-venomous colubrid (Sindaco *et al.* 2006, Kreiner 2007, Di Nicola *et al.* 2021) or aglyphous, meaning that it does not possess a venom fang model similar to those of opisthoglyphous colubrids or real venom glands. The opisthoglyphous dentition is characterized by the presence of enlarged and modified rear maxillary teeth (Weinstein *et al.* 2011) and postocular glands called Duvernoy's glands, which produce venom in several species (Rodriguez-Robles 1994, Lumsden 2004). Other authors have stated that the Western Whipsnake is equipped with such glands, and the toxicity of this species has been demonstrated (Phisalix and Caius 1916). Cases in which adult humans reported clinical complications after the bite of this snake have been reported (Weinstein *et al.* 2011, Dutto *et al.* 2015).

Following observations of live specimens of *H. viridiflavus carbonarius* in the field, as well as observing the presence in the palatomaxillary arch of a gland located near the posterior maxillary tooth, I performed dissections and microscopy of the jaws of deceased specimens and other sympatric colubroid species for comparison.

Materials and Methods

The samples used in the osteological investigation came from three adult males and two females of *Hierophis viridiflavus carbonarius* that were found dead on the road in the Abruzzo region, Italy, in spring and summer 2023. An adult male *Zamenis longissimus* (Laurenti, 1768) and a subadult female *Natrix helvetica* (Lacépède, 1789), roadkills from the same region, were used for comparison. Bones of the specimens were prepared at OPHIS Museo Paleontologico e Centro Erpetologico (Teramo, Italy) using surgical tools and sodium hypochlorite. From each specimen the two maxillary bones, the two palatines, the two pterygoids, and the two dentaries were examined. Shed teeth from captive bred *Elaphe quatuorlineata* Lacépède, 1789, originally from Apulia, Italy, were collected from live specimens at OPHIS. Microphotographs of the cranial bones and teeth were taken using a stereomicroscope Nikon SMZ1500 together with a Digital Sight DS-2Mv camera at the Faculty of Veterinary Medicine of the University of Teramo.

Live specimens of *H. viridiflavus, Z. longissimus, E. quatuorlineata, Hemorrhois hippocrepis* (Linnaeus, 1758), and *N. helvetica,* part of the OPHIS collection, were used for in vivo anatomical comparisons. Photographic material of the latter was obtained with a Sony α6000 digital camera, while photos of the cranial bones of *H. viridiflavus* were obtained with a Nikon Coolpix P510.

Results

Microscopy of the Dentiferous Bones

In prepared samples of *Hierophis viridiflavus carbonarius,* minor sulci and crests were found in the teeth of all four dentiferous bones. The maxillary teeth have a depression on the lingual surface that is longitudinally incised by a thin groove (Figure 1A), while the mid-posterior teeth have a deeper canal-like fossa posteriorly (Figure 1B). Pterygoid teeth have a basal fossa and a distal ridge in the labial wall (Figure 1C). Palatine teeth present a slight depression labially located as the nutritive alveolar foramina and a thin ridge on the lingual surface. Additional isolated furrows are present in the teeth of the maxilla and the pterygoid (Figure 1D). The most

anterior teeth in the dentaries feature a small fossa close to the edge of the crown and a distal second fossa (Figure 1E). The posterior teeth of the dentary also feature slight lingual depressions and a mesial cutting edge.

The most posterior teeth of the maxillae are distinguishable, even by eye, as longer and thicker than all other teeth (Racca *et al.* 2020). The alveoli of the two posterior teeth are adjacent to each other but separated by a diastema from the anterior one, for a length slightly shorter than that of an alveolus corresponding to the ectopterygoid process (Figure 1F). Here the maxillary arch presents a deviation of about 20° labially, positioning the enlarged teeth off-axis from the anterior teeth, which are uniformly placed with the same interdental space to the rostral extremity of the maxilla. The anterior maxillary teeth, and the teeth present in the other three dentiferous bones, show a lingulolabially compressed base and an anteroposteriorly backwards-bent crown that gives the entire tooth a shark fin-shaped silhouette. This laterally compressed shape is also observable in the alveoli of the maxilla, while the two last separated alveoli display a circular alveolar margin (Figure 1F).

These two posterior teeth differ from the rest in the morphology of the crown. Mesially a sulcus/canal is present, delimited by two ridges running along the entire length of the tooth from the base of the crown to its apex (Figure 2). Labially, in the basal half of the tooth, a triangular fossa is delimited by two prominent ridges, converging in a "V" shape, which reach the crown's tip. A second longitudinal fossa is located labiodistally after the posterior one of the two ridges that border the basal fossa. In the distal half of the teeth, the intervals between the mesial sulcus and the fossae highlight the four ridges that converge at the apex of the crown, giving the tooth a star-shaped section in lingual view (Figure 2). Rugosities occur on the projected surface of the central ridges in the basal half. In the two rear maxillary teeth, the basal portion in contact with the bone appears more compact, with a more circular section, widening like a bulb before resuming the "sharp" shape. At the base of the last maxillary tooth, a small portion of the root is visible. Here the nutritional foramen assumes the shape of an inverted teardrop, where the angled lower end culminates directly in the crown, which is consequently indented. A slight longitudinal groove twice as long as the nutritional foramen originates from this notch. This condition is absent in the other maxillary teeth, in which the nutritional foramen is usually circular in shape and located at the base of the root.

Dissection

The head of a roadkilled adult male *Hierophis viridiflavus carbonarius* was dissected; scales and skin were removed dorsally and laterally. In lateral view Duvernoy's gland is visible at the posterior end of the maxilla, anteriorly reaching and surrounding the two rear maxillary teeth (Figure 3). The posteriormost maxillary tooth emerges from the gland at the apex of the crown, visible in both lateral and ventral views. Also in lateral view, dorsoposteriorly in contact with Duvernoy's gland, is the Harderian gland, delimited ventroanteriorly by the ectopterygoid and the postocular (Figure 3).

In Vivo *Observations*

During field studies (Paterna 2015, unpubl. data) and while observing captive specimens of *Hierophis viridiflavus carbonarius,* two reddish to purplish glands at the posterior ends of the maxillae were observed at the level of the sixth supralabial scale (Figure 4A). These glands correspond to the position of Duvernoy's glands observed in the dissected skull in both lateral and ventral view. Such glands are visible in vivo in both adult and juvenile specimens. The glands are easily distinguished from the surrounding mucosa by color variation. The tip of the rear maxillary tooth emerges from the cuff of tissue and can be further uncovered by moving the
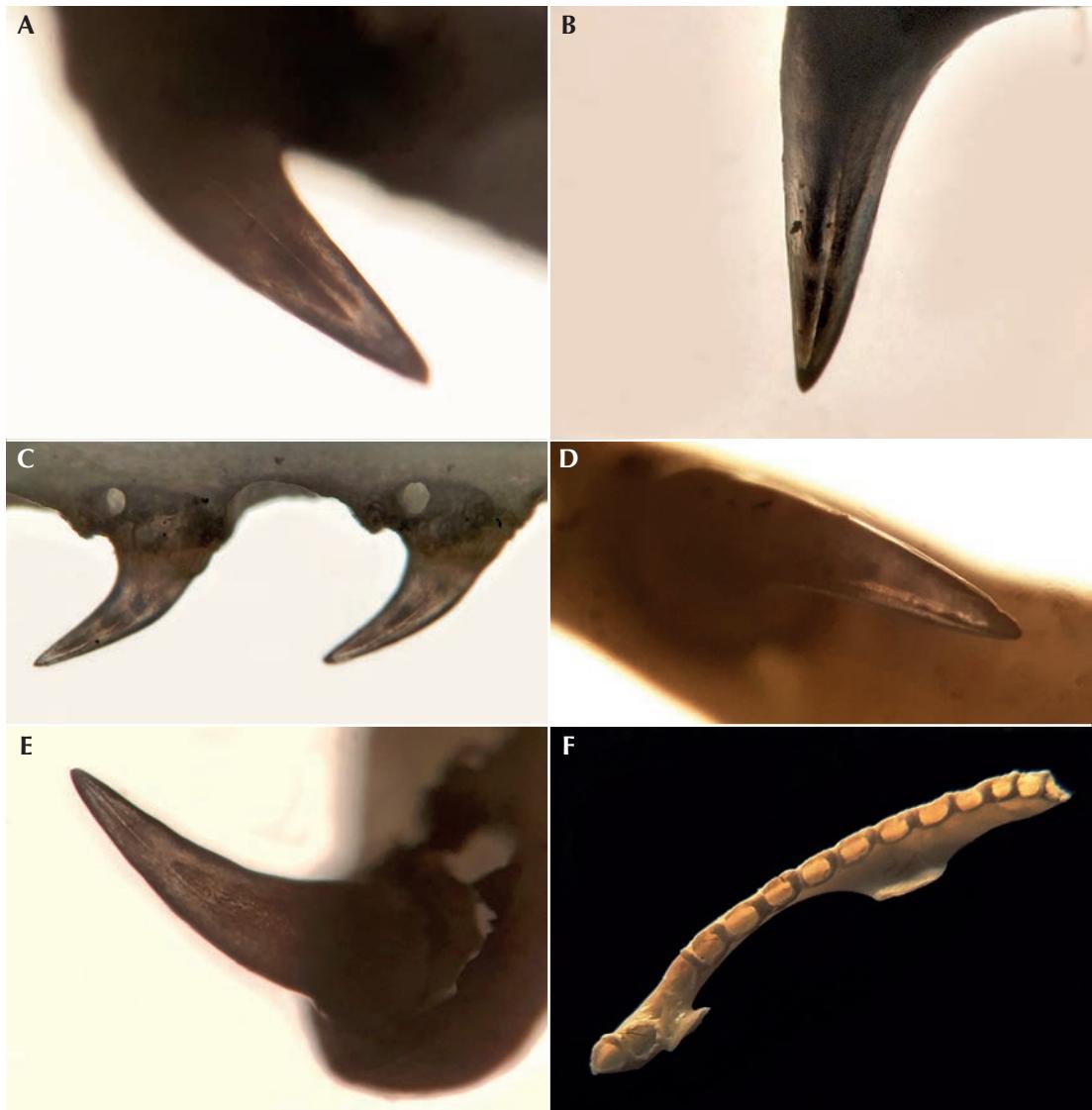
Figure 1. Stereomicroscope pictures of the groove details in the teeth of the dentiferous bones in specimens of *Hierophis viridiflavus carbonarius* from Abruzzo, Italy. (**A**) Adult female, right maxillary tooth in lingual view. (**B**) Adult male, maxillary tooth in distal view. (**C**) Adult female, right pterygoid teeth in lingual view. (**D**) Detail of the lingual groove in the right maxillary tooth of an adult female. (**E**) Adult female, anterior right dentary tooth in lingual view. (**F**) Photo of the toothless right maxilla in an adult male in ventral view.

mucosa rostrodorsally. Anterolaterally to this, corresponding with the maxillary deviation occurring between the line of the two rear and the anterior teeth, it is possible to distinguish a pocket, which is more easily identifiable in younger specimens.

In the other species examined (*Zamenis longissimus, Elaphe quatuorlineata, Hemorrhois*

Figure 2. Stereomicroscope pictures in varied contrast of a left rear maxillary tooth in mesiolingual view from an adult male of *Hierophis viridiflavus carbonarius* from Abruzzo, Italy.

hippocrepis, and *Natrix helvetica*), the above characters were found only in *H. hippocrepis* (Figure 4B). As in the case of *H. viridiflavus,* these characters were more readily observed in juveniles. Photographs of *H. viridiflavus, H. hippocrepis,* and *Z. longissimus* (Figure 4C) demonstrate the presence or absence of the gland.

Distinctive Features in the Dentition of the Species

More or less obvious furrows and ridges are found in the dentiferous bones of *Zamenis longissimus* and *Natrix helvetica* (Figure 5A–F). Enlarged maxillary teeth have been found in *N. helvetica,* in which the rear maxillary teeth share the "blade tooth" morphology. The posteriormost tooth, saber-shaped, exhibits a distal carina and a smaller mesial one (Figure 5A). The other maxillary teeth also feature slight keels but are more tapered and undulated (Figure 5C). In *Z. longissimus,* the anterior maxillary teeth are larger and longer than the posterior teeth. In the maxillae of these two species, the alveoli and consequentially the teeth, are aligned without any relevant diastema, unlike *Hierophis viridiflavus. Elaphe quatuorlineata* exhibits long maxillary teeth (Figure 5G, H), with the posteriormost featuring a mesial longitudinal sulcus in the distal half of the crown (Figure 5G).
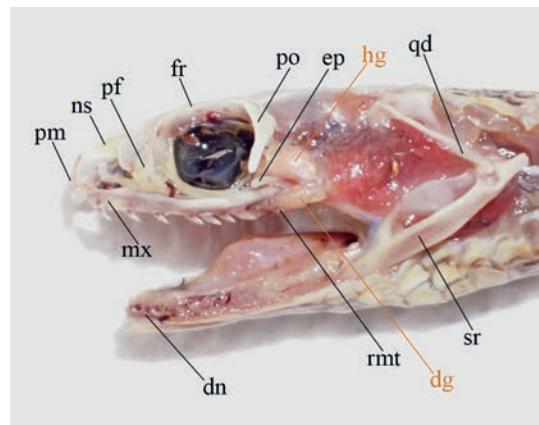
Figure 3. Dissected skull of an adult male *Hierophis viridiflavus carbonarius* from Abruzzo, Italy. Abbreviations: dg, Duvernoy's gland; dn, dentary; ep, ectopterygoid; fr, frontal; hg, Harderian gland; mx, maxilla; ns, nasal; pf, prefrontal; pm, premaxilla; po, postocular; qd, quadrate; rmt, rear maxillary tooth; sr, surangular.

Discussion

Hierophis viridiflavus is known to bite if handled, and its bite is prolonged with repeated chewing-like movements of the jaws. Bites from this species may produce temporary neurotoxic symptoms in humans (Weinstern *et al.* 2011,

Figure 4. Details of Duvernoy's gland and rear maxillary teeth in the palatomaxillary arch of (**A**) an adult female *Hierophis viridiflavus carbonarius,* and (**B**) a young male *Hemorrhois hippocrepis.* (**C**) Palatomaxillary arch of an adult male *Zamenis longissimus.* Abbreviations: g, cuff of gland; p, pocket; rf, rear fang.

Dutto *et al.* 2015) and severe neurotoxic and hemotoxic symptoms leading to death in small mammals (Phisalix 1922). The teeth of *Hierophis viridiflavus carbonarius* exhibit ridges and grooves both of which confer a better grip during the bite (Oliveira *et al.* 2016) and transmit mixed oral secretions to the penetrated tissues.

Young and Kardong (1996) examined the teeth of the four dentiferous bones of 661 snake species including 739 colubrid specimens. Although species names and numbers of individuals of each species were not provided, some information may be obtained from this study. Furrowed teeth were present in the anterior maxilla in 0.5% of the individuals examined and in the posterior maxilla in 1% of the specimens. In 1% the furrowed teeth were in the palatine, in 2% the pterygoid, and in 5% the dentary. Only three opisthoglyphous species were named: *Ahaetulla prasina preocularis* (Taylor, 1922), *Boiga cyanea* (Duméril, Bibron and Duméril, 1854), and *Rhachidelus brazili* Boulenger, 1908, in which furrows were reported in the anterior half of the maxilla. Of the 36 specimens of colubrids examined that exhibited furrowed teeth, these teeth occurred in both the palatine and the pterygoid in 5 (14%) of the specimens (Young and Kardong 1996). *Hierophis viridiflavus* exhibited furrowed teeth in all four dentiferous bones, and according to Young and

Kardong (1996) this condition is present in very few colubrid or colubroid species.

The heterodonty found within the maxillary teeth and the morphology of the maxillary bone place *H. viridiflavus* within the opisthoglyphous snakes. Not only does this species have separated and enlarged posterior maxillary teeth but also these teeth are characterized by the presence of prominent grooves and ridges. Elongate maxillary posterior teeth, together with well-defined Duvernoy's glands, represent a pre-adaptation to the subduing of prey, from which venom glands evolved for rapid killing (Kardong 1982). Other studies consider Duvernoy's gland not different from the venom glands present in elapids and viperids (Weinstein and Kardong 1994, Fry *et al.* 2008). Contrary to the venom glands present in solenoglyphous and proteroglyphous snakes, the duct of Duvernoy's gland is not directly channeled into the teeth, but rather leads into a defined space or cuff around one or more teeth (Zalisko and Kardong 1992, Kardong and Lavin-Murcio 1993); in *H. viridiflavus* these are the two posteriormost maxillary teeth. In the dissected specimen, these enlarged teeth are completely surrounded by Duvernoy's gland, leaving only the apex of the crown visible. When a prey animal is bitten, the mucous membrane comes into direct contact with the surface of the tegument, which, following the compression
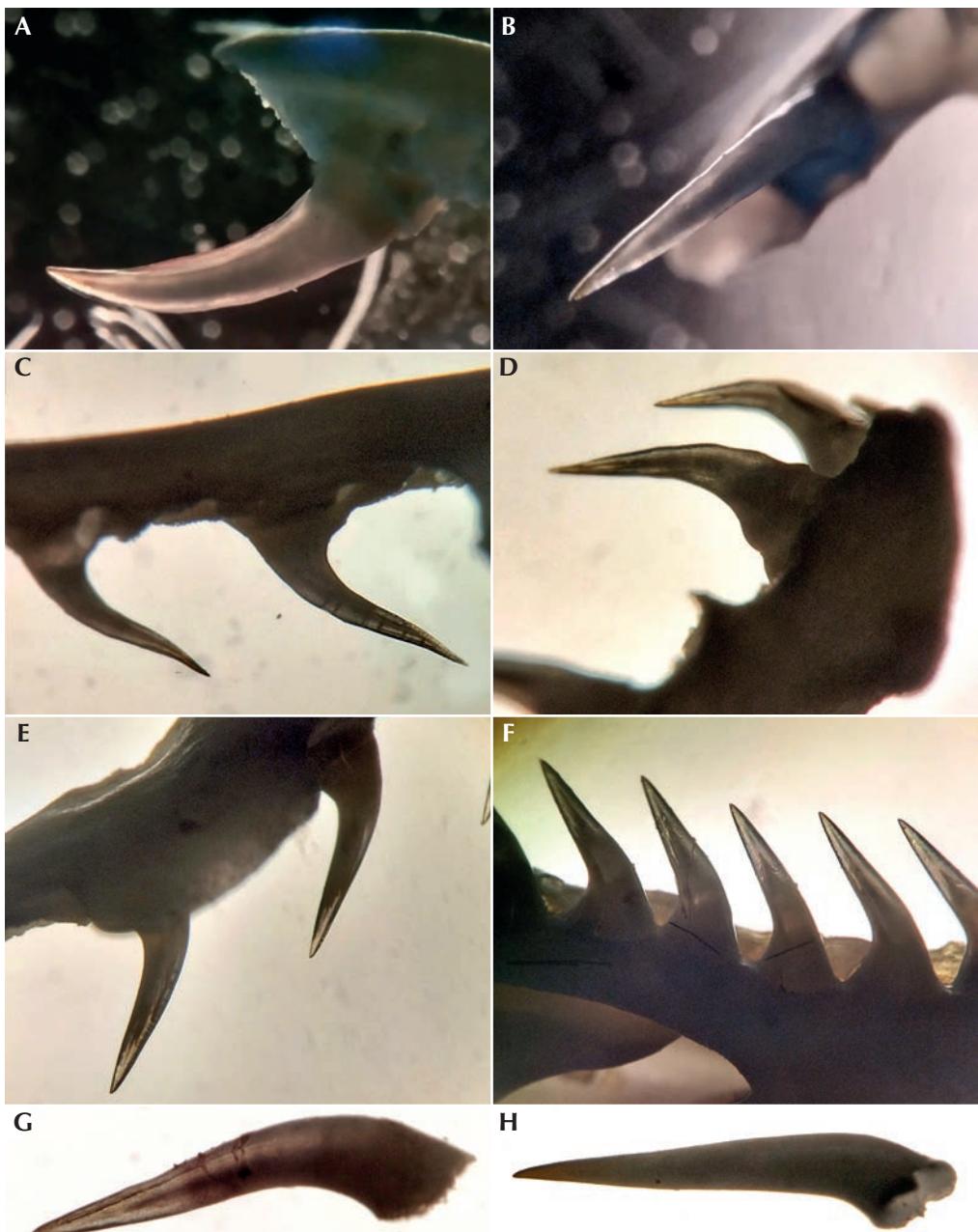
Figure 5. Stereomicroscope pictures of the dentiferous bones of the colubrid species investigated. (**A**) *Natrix helvetica lanzai,* rear maxillary tooth in lingual view. (**B**) *Natrix helvetica lanzai,* rear maxillary tooth in mesial view. (**C**) *Natrix helvetica lanzai,* maxillary teeth in lingual view. (**D**) *Natrix helvetica lanzai,* left dentary in lingual view. (**E**) *Zamenis longissimus,* left maxillary teeth in lingual view. (**F**) *Zamenis longissimus,* left dentary teeth in lingual view. (**G**) *Elaphe quatuorlineata quatuorlineata,* rear maxillary tooth in mesial view. (**H**) *Elaphe quatuorlineata quatuorlineata,* maxillary tooth in distal view.

generated by the bite, causes the secretion of Duvernoy's glands to be released directly on the wound. The pressure of the bite unsheaths the rear maxillary teeth from the mucosa, and the secretion enters the bite along the ridges and grooves of the teeth. The effectiveness of the bite is augmented by the grooves present in other teeth and by the masticating action of the prolonged bite. In addition, the pockets located labially to the enlarged maxillary teeth may accumulate the secretions of Duvernoy's glands, as in the American water snake *Nerodia sipedon* (Linnaeus, 1758) (Ranayhossaini 2010). Substantial amounts of secretions inside the mouth of *H. viridiflavus* were observed, especially in adult specimens.

The above constitute mechanisms that maximize the amount of secretion in a "low pressure" system (Taub 1967, Kardong and Lavin-Murcio 1993, Weinsten *et al.* 2013), lacking muscular insertions in the venom glands typical of snakes with anterior venom fangs. The bite and neurotoxicity of the secretion may play an important role in predation by *H. viridiflavus,* which takes a wide variety of prey (Filippi *et al.* 2003, Mondino *et al.* 2022) despite that it cannot be considered a "constrictor" snake.

Dentition in Other Species

Enlarged and modified rear maxillary teeth were found in *Natrix helvetica lanzai.* Although different from the teeth of *Hierophis viridiflavus,* its fangs resemble those observed in several opisthoglyphous colubrids (Weinstein *et al.* 2011). *Natrix helvetica* (*Natrix natrix sensu lato*), along with *H. viridiflavus,* has been considered an aglyphous ophid (Sindaco *et al.* 2006, Kreiner 2007, Di Nicola *et al.* 2021) even though the clinical consequences of its bite in humans has been documented (Gardner-Thorpe 1967, Satora 2004, GläßerTrobisch and Trobisch 2008).

Analogous glands to those surrounding the rear maxillary teeth of *H. viridiflavus* have been observed in the palatomaxillary arch of *Hemorrhois hippocrepis.* Cases of mild local

effects following the bite of the congeneric *Hemorrhois algirus* and *Hemorrhois nummifer* are present in literature (Mamonov 1977, Malik 1995, Weinstein *et al.* 2011, Kazemi *et al.* 2023).

Conclusion

Several unsuspected characters in the upper jaws of *Hierophis viridiflavus* that are involved in the inoculation of salivary secretions, especially those produced by Duvernoy's glands, were found. These morphologies are linked to the predisposition of this species to inflict prolonged, "chewing" bites, a widespread and distinctive behavior of this snake among the Italian ophidian fauna. The morphology of the maxillary bone places this species within the opisthoglyphous snakes, equipped with modified fangs apposite for the transmission of secretions that can be considered a "primitive form of venom." The presence of grooves on most teeth suggests that *H. viridiflavus* should be considered polyglyphous, rather than aglyphous. Grooves were observed in the four dentiferous bones of other European species, although it is uncommon within colubroids. Among the Italian fauna, the opisthoglyphous species occur in limited northern border areas and small islands in the south (Sindaco *et al.* 2006), making *H. viridiflavus* an exception within the large "aglyphous" colubrids on the mainland.

Similar inoculation systems were observed in the large European colubroids *Natrix helvetica* and *Hemorrhois hippocrepis;* the presence of Duvernoy's glands and neurotoxic secretions have previously been documented in both genera (Phisalix 1922, Ovadia 1984, Jackson 2003, Weinstein *et al.* 2011).

Although changing the status of *H. viridiflavus* from harmless to humans is not recommended, special attention should be given not only to this species, but to the entire Palearctic whipsnake/racer complex (*sensu* Nagy *et al.* 2004) and the genus *Natrix.* All these species possess morphology capable of delivering toxic bites.

Acknowledgments

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A chance encounter in central Texas yields insights on the ecology of aestivating *Siren nettingi* (Caudata: Sirenidae)

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Abstract

A chance encounter in central Texas yields insights on the ecology of aestivating *Siren nettingi* (Caudata: Sirenidae). *Siren* spp. are often dominant vertebrates in the wetlands they occupy and are known to estivate when such wetlands dry up. Practical considerations limit *in-situ* observations of estivating individuals. On 12 October 2021, we incidentally discovered an estivating aggregate of *Siren nettingi* in Bastrop County, Texas, USA. These salamanders were excavated from compact, rocky soil adjacent to a caliche road, at depths that ranged between ~0.2 to 1.5 m. The dominant vegetation at this site included *Ulmus crassifolia, Persicaria* sp., and various grass species. We recovered 140 individuals of which seven were salvaged and 133 were captured live. We measured 115 of these for snout–vent length (SVL) and observed the aggregate was predominated by juveniles. We estimated an estivation density of 2.33 sirens/m^2 that is comparable to densities estimated for non-estivating populations. However, in-lieu of monitoring that was in place for this study, we expect a mass mortality event would have likely occurred. We therefore suggest that roadway construction in preferred habitat be considered as a threat to siren populations.

Keywords: Amphibian, Conservation, Dormancy, Dynamic habitat, Roadways, Wetland.

Resumo

Encontro casual na região central do Texas fornece informações sobre a ecologia da estivação de *Siren nettingi* (Caudata: Sirenidae). *Siren* spp. costumam ser vertebrados dominantes nas áreas úmidas que ocupam e são conhecidas por estivar quando essas áreas úmidas secam. Considerações práticas limitam as observações *in situ* de indivíduos em estivação. Em 12 de outubro de 2021, descobrimos por acaso um agregado em estivação de *Siren nettingi* no condado de Bastrop, Texas, Estados Unidos. Essas salamandras foram escavadas em solo compacto e rochoso adjacente a uma estrada de caliche, em profundidades que variavam entre ~0,2 e 1,5 m. A vegetação dominante nesse local incluía *Ulmus crassifolia*, *Persicaria* sp. e várias espécies de gramíneas. Recuperamos 140

indivíduos, dos quais sete foram resgatados e 133 foram capturados vivos. Medimos 115 deles quanto ao comprimento rostro-cloacal (SVL) e observamos que o agregado era dominado por jovens. Estimamos uma densidade de estivação de 2,33 indivíduos/m² que é comparável às densidades estimadas para populações sem estivação. No entanto, como não houve monitoramento para esse estudo, provavelmente tenha ocorrido um evento de mortalidade em massa. Portanto, sugerimos que a construção de estradas no habitat preferido seja considerada uma ameaça às populações dessas salamandras.

Palavras-chave: Anfíbios, Conservação, Dinâmica de habitat, Dormência, Estradas, Pântano.

Introduction

Amphibians inhabiting environments that experience seasonal episodes of drought often burrow into the soil and enter into a state of dormancy when faced with the dual challenge of no food and no standing water in such habitats. This adaptive tactic includes formation of a cocoon around the body to mitigate desiccation and a reduced metabolic rate to increase the duration of survival on endogenous body stores (Secor and Lignot 2009).

Sirens serve as a useful example of amphibians that inhabit dynamic wetlands. These aquatic salamanders possess gills, have lidless eyes, and compressed tails with fin blades. Additionally, pelvic girdles and associated hindlimbs are absent (Martof 1974). Body measurements and proportions, coloration and patterns of the body, and the number of costal grooves, each corresponding to single vertebrae and associated trunk muscles, are used to distinguish among species (Powell *et al.* 2016, 2019, Fedler *et al.* 2023). Given that sirens possess few physical attributes for diagnosis among species and that body coloration and patterns can vary among individuals within a species and from the same locality, questions regarding *Siren* phylogeny largely remain unresolved. Greater Sirens (*Siren lacertina* Österdam, 1766) and Lesser Sirens (*Siren intermedia* Barnes, 1826) are among species reported within this taxonomic group. *Siren lacertina* are known to range from Virginia south to Florida and west to Southwestern Alabama (Petranka 2010), while *S. intermedia* occurs in the Coastal Plain from southeastern North Carolina to southern Florida and westward in the Gulf states to the lower Rio Grande Valley and adjacent Mexico as well as northward in the Mississippi River drainage through Illinois, Indiana, and southwestern Michigan (Martof 1973, Fedler *et al.* 2023). More recently, morphological and genetic data have been used to describe the Leopard or Reticulated Siren (*Siren reticulata* Graham, Kline, Steen, and Kelehear, 2018) from southern Alabama and the Florida panhandle (Graham *et al.* 2018) and the Seepage Siren (*Siren sphagnicola* Fedler, Enge, and Moler, 2023) from the Florida parishes of Louisiana to the western Florida panhandle (Fedler *et al.* 2023). Further, Goin (1942) characterized the Western Lesser Siren (*Siren intermedia nettingi* Goin, 1942) as distinct from *S. intermedia intermedia* and *S. lacertina* based on the presence of light spots on the sides and venter and the number of costal grooves (Fedler *et al.* 2023). We follow Fedler *et al.* (2023) in our usage of *Siren nettingi* for the study species.

Notwithstanding the taxonomic uncertainty surrounding these salamanders, prior studies have yielded insights on *Siren* ecology. Sirens are known to occupy both stationary and moving bodies of water often being the dominant vertebrate in wetland communities (Frese *et al.* 2003, Secor and Lignot 2010). The latter is particularly true for *S. intermedia* that is reportedly quick to colonize and become a

dominant secondary consumer in newly formed ponds (Gehlbach and Kennedy 1978). These salamanders can attain a high standing crop biomass of up to 72 g/m^2, with such productivity attributed to high fecundity and rapid growth rates to sexual maturity (Gehlbach and Kennedy 1978, Frese *et al.* 2003). Further, like other sirens, *S. intermedia* also estivates in temporarily dry ponds to facilitate its dominance (Gehlbach and Kennedy 1978, Luhring and Holdo 2015). The estivation strategy consists of burrowing in mud or existing crayfish burrows, forming a cocoon of dried mucus to mitigate desiccation, and reducing metabolic rate to rely on fat stores during the duration of estivation (Gehlbach *et al.* 1973).

Although the propensity to estivate under adverse conditions is known for *S. intermedia,* there are practical limitations for *in-situ* observations with current knowledge on estivation behavior known from laboratory studies (Gehlbach *et al.* 1973). Here, we provide details on a chance discovery of estivating *S. nettingi* in Bastrop County, Texas, USA that yielded insights on habitat use, size distribution, and estivation density.

Materials and Methods

On 12 October 2021, the installation of a roadway culvert was begun in Bastrop County (30°07'51.5" N, 97°07'56.1" W; Figure 1). The construction site occurred within occupied habitat for the federally endangered Houston Toad [*Bufo* (= *Anaxyrus*) *houstonensis*], requiring that the culvert installation using heavy machinery be monitored to prevent toad mortality and minimize potential disturbance to the habitat adjacent to the roadway. During this time, we encountered an aestivating population of *S. nettingi,* which was initially discovered after lifting a cedar elm (*Ulmus crassifolia* Nutt.) tree that had fallen along the roadside. We diagnosed these as *S. nettingii* by counting the number of costal grooves that were not touching the limbs (Fedler *et al.* 2023). We observed several

estivating individuals within the associated matrix of roots and soil. Other dominant vegetation within the construction area included *Persicaria* sp., and various grass species. The roadway and adjacent roadsides were dry, with only limited vegetation that would have indicated its prior impoundment. Continued excavation revealed additional estivating individuals. We searched the substrate manually to minimize harm and enable capture of all *S. nettingi* that were excavated. Monitoring continued until adequate substrate had been excavated to allow for culvert installation.

At the time of excavation, dry conditions precluded a release site. Thus, all extracted individuals were kept indoors at an ambient temperature of 24°C within tubs filled with purified drinking water. Individuals were restrained within snake restraining tubes of an appropriate size and measured for snout–vent length (SVL) and total length (TL). Following a rain event on 14 October 2021, release of individuals was enabled on 15 October 2021 into an adjacent ephemeral creek within the same drainage system as the ongoing construction project. That creek flowed downstream into the wetland complex proximal to where the sirens were discovered (Figure 1). Salvaged individuals were fixed in 10% buffered formalin, stored in 70% ethanol, and deposited at the Amphibian and Reptile Diversity Research Center at the University of Texas at Arlington (UTA A-66394–66397) and the Texas State University Herpetofauna Teaching Collection. We generated histograms of SVL measurements to provide a size distribution of the estivating population.

Results

We found *S. nettingi* in soils directly adjacent to and touching the caliche road at depths ranging between ~0.2 to 1.5 m. These *S. nettingi* were ~20 m from an ephemeral creek that contained some water and ~100 m from a proximal wetland. We did not find any *S. nettingi* while digging in the extremely compacted soils

Figure 1. Map inset shows the state of Texas, USA with Bastrop County highlighted (in green). Base map shows the excavation site (orange polygon) for culvert installation where an estivating aggregate of *Siren nettingi* was discovered on 12 October 2021. *Siren nettingi* were only found in an area of approximately 60 m² on the northeast side of the road. Captured sirens were released on 15 October 2021 into an ephemeral stream (release site) leading downslope toward the same wetland complex from which the siren originated.

that made up the roadbed itself. We captured 133 live *S. nettingi* and salvaged an additional seven that had been killed during the roadway construction and culvert installation process. We searched a total area of approximately 300 m² (Figure 1) but only found *S. nettingi* in an area of approximately 60 m² on the northeast side of the road. Given the total number of *S. nettingi* captured and salvaged, as well as the area searched, we estimate aestivation density to be 2.33 sirens/m².

Siren nettingi showed activity (i.e., were moving) on excavation and subsequent handling. Mean (± 1SE) SVL was 101.85 ± 81.96 mm (*N* =115; Figure 2). Of the 115 individuals measured,

93 (81%) were < 100 mm in SVL. All sirens observed at the time of excavation showed a nub like gill ramus and no filaments (Figure 3), however we observed the reappearance of gill filaments following 40 hours of inundation in water.

Discussion

Despite the abundance with which *Siren* occur in the habitats they occupy; particular aspects of their biology remain poorly known. Most studies of these salamanders are limited by practical considerations to sampling non-estivating individuals using passive trapping
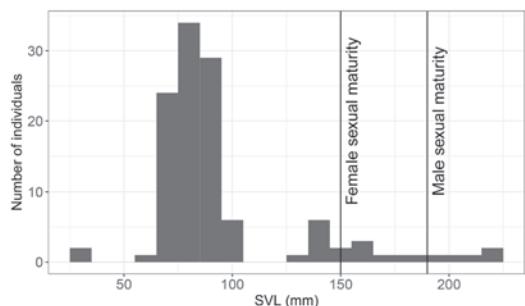
Figure 2. Size distribution (snout–vent length) of estivating *Siren nettingi* captured at a roadway construction site in Bastrop County, Texas, USA on 12 and 13 October 2021. We measured 115 individuals for SVL and observed our sample was comprised of predominantly juveniles. Thresholds lengths for sexual maturity are from Davis and Knapp (1953).

Figure 3. *Siren nettingi* restrained in a snake restraining tube for snout–vent length measurement. Following excavation, siren had atrophied gills with a nub like gill ramus and no gill filaments. We observed reappearance of gill filaments following 40 hours of inundation in water.

methods (Luhring *et al.* 2016). In particular, *in-situ* observations of estivating sirens are sparse since searching for estivating individuals poses the risk of physical harm from excavation (Aresco and Gunzburger 2004). Our chance encounter of an estivating aggregate enabled us to provide insights on estivation habitat characteristics, density, and size distribution.

Based on specimens collected in central Texas, male and female *S. nettingi* are estimated to attain sexual maturity at 190 and 150 mm SVL respectively (Davis and Knapp 1953). Similarly, in Arkansas, USA, the smallest female *S. intermedia* possessing yolked ovarian follicles measured 165 mm SVL (Trauth *et al.* 1990). Therefore, *S. nettingi* excavated during our study were predominantly juveniles. Frese *et al.* (2003) reported that 39% of the *S. intermedia* population sampled during their study comprised of juveniles. The proportion of juveniles in our sample was more than twice as large, although we are unsure of factors that explain the size/age distribution that we observed. Non-aestivating populations of *S. intermedia* occur at high densities, ranging from 1.1 to 2.17 sirens/m² (Gehlbach and Kennedy 1978, Frese *et al.* 2003).

We report a comparable density for estivating *S. nettingi*. To our knowledge, this study represents the first report on such *in-situ* observations of estivating *S. nettingi*.

Given the high density at which we observed sirens estivating immediately adjacent to this roadway, roadway construction conducted proximal to wetland habitat may pose a serious risk to siren populations. In our instance, culvert installation in lieu of monitoring would have resulted in desiccation and physical injury and therein mortality of over 100 sirens. In examining organic sediment removed from lake beds in Florida, Aresco and Gunzburger (2004) reported that large aquatic salamanders (*Siren* spp., *Amphiuma means* Garden, 1821) were among the most abundant herpetofauna encountered. They reported that mortality from sediment removal operations in these wetlands was likely skewed to taxa with limited dispersal abilities and which relied on dried lake sediments for estivation (Aresco and Gunzburger 2004). Further, Cagle and Smith (1939) observed an aggregate of 100 *S. intermedia* in a cement culvert. This was considered a hibernating aggregate with the culvert offering 'ready access

to either pond' (Cagle and Smith 1939). Such temporarily occupied culverts could enable connectivity among wetland habitats. However, the process of culvert installation or replacement should consider means to mitigate mortality risks to the species. In regions where *Siren* spp. are imperiled, we recommend oversight of excavation activities in or proximal to wetland habitat. We emphasize here that the detection of these animals was also the first scientific documentation of the taxon in Bastrop County in Texas (Bohannon *et al.* 2022). We demonstrate that monitoring or oversight during construction minimized mortality, although with unavoidable losses still occurring.

Distribution assessments for herpetofauna in Texas continue to show gaps in such updated distributions (Dixon 2013, Bassett 2023). We suggest further surveys be conducted on the western edge of the currently known distribution to address such distributional gaps.

Acknowledgments

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Relative susceptibility of tadpoles of *Uperodon taprobanicus* (Anura: Microhylidae) and *Duttaphrynus melanostictus* (Anura: Bufonidae) to predacious *Hoplobatrachus tigerinus* (Anura: Dicroglossidae) tadpoles: significance of refugia and swimming speed in predator avoidance

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Abstract

Relative susceptibility of tadpoles of *Uperodon taprobanicus* (Anura: Microhylidae) and *Duttaphrynus melanostictus* (Anura: Bufonidae) to predacious *Hoplobatrachus tigerinus* (Anura: Dicroglossidae) tadpoles: significance of refugia and swimming speed in predator avoidance. The relative susceptibility of two closely associated herbivorous tadpole species (*Uperodon taprobanicus* and *Duttaphrynus melanostictus*) to their natural carnivorous predatory tadpole, *Hoplobatrachus tigerinus* and the significance of refugia in predator avoidance was studied in the laboratory. In a total of 50 trials, 10 tadpoles each of *U. taprobanicus* and *D. melanostictus* of comparable sizes were exposed to starved *H. tigerinus*. Twenty-five trials included refugia while 25 did not. The results of this study showed that in both the presence and absence of refugia, *D. melanostictus* tadpoles fell prey to *H. tigerinus* more frequently than *U. taprobanicus* tadpoles. A key difference between the two prey species is the speed of swimming; V_{max} of *D. melanostictus* (13.58 cm/s) tadpoles is significantly lower than that of *U. taprobanicus* (24.89 cm/s) tadpoles. This is likely to be the main reason why more *D. melanostictus* tadpoles were preyed upon than were *U. taprobanicus* tadpoles. It is important to note that the V_{max} of the predator (60.21 cm/s) is much greater than those of the two prey species. However, predation risk of both prey tadpole species was affected significantly by the presence of refugia. The susceptibility of both prey tadpole species was lower where refugia were available. The present study clearly demonstrates that the more efficient avoidance of predation by *U. taprobanicus* tadpoles could be due to better use of refugia and their faster rate of movement.

Keywords: Antipredator behavior, Anuran larvae, Ephemeral ponds, Mortality, Predation threat, Prey-predator interactions, Refuge use.

Resumo

Suscetibilidade relativa dos girinos de *Uperodon taprobanicus* (Anura: Microhylidae) e *Duttaphrynus melanostictus* (Anura: Bufonidae) aos girinos predadores de *Hoplobatrachus tigerinus* (Anura: Dicroglossidae): importância dos refúgios e da velocidade de natação para evitar o predador. A suscetibilidade relativa de duas espécies de girinos herbívoros intimamente associados (*Uperodon taprobanicus* e *Duttaphrynus melanostictus*) ao seu girino predador carnívoro natural, *Hoplobatrachus tigerinus,* e a importância do refúgio na fuga do predador foram estudadas em laboratório. Em um total de 50 testes, 10 girinos de *U. taprobanicus* e 10 girinos de *D. melanostictus* de tamanhos comparáveis foram expostos a girinos de *H. tigerinus* famintos. Vinte e cinco testes incluíram refúgios, enquanto 25 não incluíram. Os resultados desse estudo mostraram que, tanto na presença como na ausência de refúgios, os girinos de *D. melanostictus* foram predados com mais frequência do que os girinos de *U. taprobanicus.* Uma diferença importante entre as duas espécies de presas é a velocidade de natação; a *Vmax* dos girinos de *D. melanostictus* (13,58 cm/s) é significativamente menor do que a dos girinos de *U. taprobanicus* (24,89 cm/s). É provável que esse seja o principal motivo pelo qual mais girinos de *D. melanostictus* foram predados em relação aos girinos de *U. taprobanicus.* É importante observar que a *Vmax* do predador (60,21 cm/s), é muito maior do que a das duas espécies de presas. No entanto, o risco de predação de ambas as espécies de girinos foi afetado significativamente pela presença de refúgios. A suscetibilidade de ambas as espécies de girinos foi menor quando havia refúgios disponíveis. O presente estudo demonstra claramente que a evasão mais eficiente da predação pelos girinos de *U. taprobanicus* pode ser devida ao melhor uso dos refúgios e à sua taxa de movimento mais rápida.

Palavras-chave: Ameaça de predação, Comportamento anti-predador, Girinos, Interações predador-presa, Lagoas temporárias, Mortalidade, Uso de abrigos.

Introduction

The interaction between predator and prey is an evolutionary arms race in which early detection by either party is often the key to their success (Ferrari *et al.* 2010). Predation leads certainly to the elimination of prey individuals from an ecological system, which can have major impacts on the population dynamics of prey organisms. Therefore, for any prey organism it is important to assess predation risk accurately and develop necessary antipredator defense strategies in order to optimize its survival and fitness (Lima and Dill 1990). Most of the anurans opportunistically breed in temporary water bodies and their larvae live in such waters until metamorphosis (Newman 1992, Saidapur 2001). In such aquatic systems, larval anurans commonly face threats from pond desiccation, crowding, limited food resources, and more importantly from predators. As a consequence,

they have evolved a variety of defense strategies (Loman 1999, Lardner 2000, Benard 2004, Mogali *et al.* 2011, 2017). The most common antipredator defense strategies of anuran tadpoles observed to perceived predation threat include increased activity or high swimming speed in order to run away from predators (Hews 1988, Van Buskirk and McCollum 2000), reduction in activity levels to avoid detection or also reduce the encounter rate with predators, especially ambush predators (Schmidt and Amezquita 2001, Saidapur *et al.* 2009, Mogali *et al.* 2011, Hossie *et al.* 2017), aggregation (Spieler and Linsenmair 1999) and increased use of refuge sites (Hossie and Murray 2010, 2011, Mogali *et al.* 2019, 2022) depending upon species. Because they exist in aquatic environments, anuran larvae mostly use chemical signals to assess predation threats since visual information may be obscured in water that is turbid or densely vegetated (Kiesecker *et al.* 1996, Mogali 2018).

In and around the city of Dharwad, Karnataka state of Southern India, many anuran species including the present study species, the Asian common toad, *Duttaphrynus melanostictus* (Schneider, 1799) (family: Bufonidae) and the Indian painted frog, *Uperodon taprobanicus* (Parker 1934) (family: Microhylidae) reproduce in rain-filled ephemeral water bodies formed during the South-West monsoon (Saidapur 2001, Mogali *et al.* 2017). The tadpoles of *D. melanostictus* and *U. taprobanicus* are mainly bottom dwellers and thrive on detritus and algal matter. The visibility is generally low in these ephemeral water bodies due to shadows from vegetation, turbid water and the benthic area that is naturally covered by leaf litter and detritus (our personal observation). These water bodies are also home to several types of invertebrate and vertebrate predators, including the carnivorous tadpoles of the Indian bullfrog, *Hoplobatrachus tigerinus* (Daudin, 1802) (family: Dicroglossidae). The tadpoles of *H. tigerinus* are voracious predators that hunt actively and detect their prey including tadpoles by means of both visual and chemical senses. All three study species used in the present experiment have conservation status Least Concern according to the IUCN Red List (Van Dijk *et al.* 2004, Padhye *et al.* 2008, Inger *et al.* 2016). During our regular field visits, we noticed that herbivorous tadpoles of *D. melanostictus* and *U. taprobanicus* are preyed upon by carnivorous tadpoles of *H. tigerinus.* Most studies of the tadpole prey-predator interactions studies have focused mainly on aquatic insects, fishes, or salamanders as predators (e.g., Chivers and Mirza 2001, Mathis 2003, Mogali *et al.* 2020). So far there seems to be a paucity of research showing the influence of carnivorous tadpoles on the behavioral responses of herbivorous tadpoles.

In natural environments, we noticed many similarities between tadpoles of *D. melanostictus* and *U. taprobanicus*. Hence, it is very important to know about the relative susceptibility of tadpoles to their common predator, *H. tigerinus.*

The present study was designed to determine the relative susceptibility of wild-caught tadpoles of *D. melanostictus* and *U. taprobanicus* of comparable body size at early stages of development (Gosner stages 26–27) to the free moving active predator, *H. tigerinus,* both in the presence and the absence of refuge sites. In the present study, we primarily hypothesized that the presence of refuge sites (leaf-litter) could reduce the vulnerability of both species and we secondarily hypothesized that there should be a difference in vulnerability between two prey tadpole species. Thus, the outcome of this study will provide some novel information in the field of behavioral ecology of anuran tadpoles with special reference to prey-predator interactions.

Materials and Methods

Tadpoles of *Uperodon taprobanicus* (Gosner stages 26–27; *N* = ~600; Gosner 1960) and *Duttaphrynus melanostictus* (Gosner stages 26–27; *N* = ~600) were collected from temporary ponds in and around (within 0.5 km distance) the Karnatak University Campus (latitude 15.440407° N, longitude 74.985246° E, elevation 750 m a.s.l.), Dharwad, Karnataka state, India. Soon after collection, they were brought to the laboratory. Tadpoles of each species were placed separately in glass aquaria (90 × 30 × 15 cm) containing 25 L of aged tap water and used as a stock. Tadpoles of both species are herbivores and were fed boiled spinach to sustain growth and development. The tadpoles of *Hoplobatrachus tigerinus* (Gosner stages 32–33; *N* = ~80; predators) were also collected from the temporary ponds in the Karnatak University campus. They were reared individually in plastic tubs (14 cm diameter and 7 cm deep) with 500 mL of aged tap water to avoid cannibalism. Prior to the commencement of the experiment, each predator tadpole was fed daily equally with both prey species (3 *U. taprobanicus* + 3 *D. melanostictus* tadpoles; Gosner stages 26–27) for at least three days.

Experiment 1: Relative Susceptibility of Prey Species

This experiment was designed to determine the relative susceptibility of *U. taprobanicus* and *D. melanostictus* tadpoles to the predator *H. tigerinus* and the significance of refugia in predator avoidance. We carried out a total of fifty experimental trials in a five day period. Ten trials were conducted per day, in ten separate experimental tubs each containing one of two treatments. Each trial started at 7:00 am and ended at 7:00 am the next day. In each trial, ten tadpoles each of *U. taprobanicus* (Gosner stages 26–27; 16.30 ± 0.25 mm in total length; mean ± SE; *N* = 100) and *D. melanostictus* (Gosner stages 26–27; 16.32 ± 0.28 mm in total length; mean ± SE; *N* = 100) of comparable body sizes were released in a plastic tub (32 cm diameter and 14 cm deep) containing 3 L of aged tap water. They were allowed to acclimate for 30 min. Then one *H. tigerinus* tadpole (Gosner stages 32–33; 37.45 ± 0.35 mm in total length; mean ± SE; *N* = 25) starved for 48 h was introduced into the tub. After 24 h the number of surviving *U. taprobanicus* and *D. melanostictus* tadpoles was recorded to compute the number of tadpoles of each species lost due to predation.

In twenty-five trials (five per day over five days) the tubs containing the tadpoles and predators provided no refugia for the prey tadpoles. In a second twenty-five trials, carried out five per day over the same five days, the tubs contained structural refugia made using water soaked (2 days) leaves of *Eucalyptus* (dry mass 15 ± 0.4 g; mean ± SE) chopped into ~1 cm² pieces. These were spread at the bottom of the testing tubs to serve as shelters. Predation risk was studied as described above. The experimental tubs in all trials were cleaned before each trial. The experimental tubs were placed on a flat surface in a room temperature (25°C). The positions of the experimental tubs were randomized daily to avoid possible effects of position. The daily water temperature of various tubs (with refugia and without refugia) fluctuated between 23–24°C. All experimental trials were carried out under natural photoperiod (12 h light: 12 h dark). Both prey tadpole species were well fed with boiled spinach before the experimental trials. However, during the trial period they were not provided any food. All test tadpoles used in the experiment were healthy. Data were analyzed using mixed model ANOVA where the effects of experimental containers were included as random effects, and the effects of prey species identity and refuge access and their interaction were included as fixed effects. Relative susceptibility of *U. taprobanicus* and *D. melanostictus* tadpoles to predation in each experiment was tested using Independent samples t test (SPSS software ver. 16.0).

Experiment 2: Burst Swimming Speed of Prey and Predator Tadpoles

Experiment 1 showed that susceptibility of the prey species (*D. melanostictus* and *U. taprobanicus* tadpoles) to predation by *H. tigerinus* tadpoles differed significantly. Hence, it was of interest to know the differences in the swimming speeds of prey species and also the predator species. To determine V_{max}, a single test tadpole of one of the three species (either prey, *D. melanostictus* or *U. taprobanicus* or predator, *H. tigerinus*) was placed in a plastic tub (20 cm diameter and 10 cm deep) filled with aged tap water to a depth of 2.5 cm and left undisturbed for 5 min to adjust to new conditions. A handycam (Sony, DCR-SR300/E) was positioned above the plastic tub to record activity in the entire tub. The handycam was connected to a computer with the Ethovision Video Tracking System (Noldus Information Technology, The Netherlands) to track the movements of the test tadpole. After 5 min of acclimation, the test tadpole was chased continuously for 1 min by prodding the tail base with a delicate wire as described by Van Buskirk and McCollum (2000). The movement of the tadpole was tracked to

determine the V_{max}. A total of 25 trials were carried out for each tadpole species with a new healthy test tadpole of each species every time. All test tadpole were well fed before trials. The obtained V_{max} of tadpole species were compared by one-way ANOVA followed by Tukey HSD post-hoc test (SPSS software ver. 16.0).

Results

Experiment 1: Relative Susceptibility of Prey Species

Mixed model ANOVA showed significant main effect of species ($p < 0.01$, Table 1) and refuge availability ($p < 0.01$, Table 1) but not of their interaction ($p = 0.268$, Table 1).

Both in the absence ($t_{48} = -11.415$, $p < 0.01$) and presence ($t_{48} = -10.415$, $p < 0.01$) of refugia significantly more *D. melanostictus* than *U. taprobanicus* tadpoles fell prey to *H. tigerinus*

tadpoles (Table 2). Predation threat to tadpoles of both species was affected significantly by the presence of refugia. The susceptibility of both tadpole species (*U. taprobanicus*: $t_{48} = 7.250$, $p < 0.01$; *D. melanostictus*: $t_{48} = 7.071$, $p < 0.01$) to predation was low where refugia were available (Table 2).

Experiment 2: Burst Swimming Speed of Prey and Predator Tadpoles

There was a significant difference in the swimming speed among tadpole species ($F_{2, 72} = 4243.0$, $p < 0.01$; Figure 1). The predacious *H. tigerinus* tadpoles exhibited a significantly greater ($p < 0.01$) V_{max} (60.21 cm/s; Figure 1) than the prey tadpole species. The *U. taprobanicus* tadpoles exhibited a significantly higher ($p < 0.01$) V_{max} (24.89 cm/s; Figure 1) than that of *D. melanostictus* tadpoles (13.58 cm/s; Figure 1).

Table 1. Results of mixed model ANOVA for species and refuge sites and their interactions. The response variable is the mean number of prey tadpoles (*Uperodon taprobanicus* and *Duttaphrynus melanostictus*) lost due to predation by *Hoplobatrachus tigerinus* tadpoles. *Indicates significant differences.

Source	df	MS	F	p
Species	1	94.090	238.706	< 0.01*
Refuge sites	1	39.090	100.693	< 0.01*
Species × refuge sites	1	0.490	1.243	0.268

Table 2. Number of prey tadpoles (mean ± SE) of *Uperodon taprobanicus* (Gosner stages 26–27) and *Duttaphrynus melanostictus* (Gosner stages 26–27) consumed by the predator, *Hoplobatrachus tigerinus* (Gosner stages 32–33) in a 24 h trial period (*N* = 25 trials per treatment). #Independent samples t test; *indicates significant difference between two treatments.

Treatment	Tadpoles consumed		t and p values#
	U. taprobanicus	*D. melanostictus*	
Without refuge sites	2.76 ± 0.11	4.84 ± 0.15	$t_{48} = -11.415$, $p < 0.01$*
With refuge sites	1.64 ± 0.12	3.44 ± 0.13	$t_{48} = -10.415$, $p < 0.01$*
t and p values#	$t_{48} = 7.250$, $p < 0.01$*	$t_{48} = 7.071$, $p < 0.01$*	
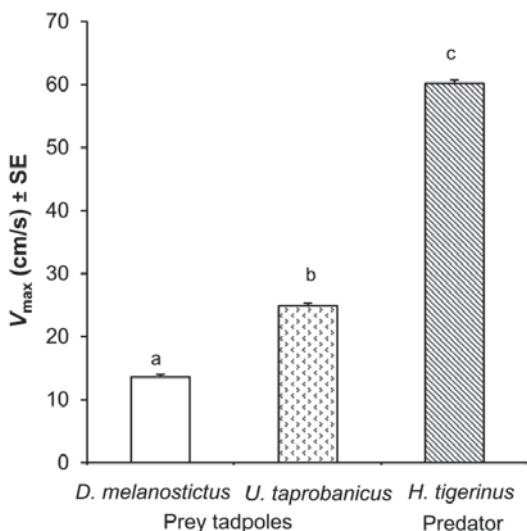
Figure 1. Shows burst swimming speed (V_{max}) of prey (*Duttaphrynus melanostictus, Uperodon taprobanicus*) and predator (*Hoplobatrachus tigerinus*) tadpoles (*N* = 25 trials for each species). Data represents mean ± SE and analyzed by one-way ANOVA followed by Tukey HSD post-hoc test. Dissimilar letters above the bars indicate significant difference between the groups.

Discussion

In aquatic environments, most prey organisms including anuran tadpoles live under great predation pressure. This results in the evolution of defense means to escape from predation and promote survival (Schmidt and Amezquita 2001, Relyea 2007). The results of this study showed that species and refuge site act independently and do not interact hence they independently affect the larval survival following their encounter with the predator, *H. tigerinus*. Both in the absence and the presence of refugia, *D. melanostictus* tadpoles fell prey to *H. tigerinus* more easily than *U. taprobanicus* tadpoles. A main difference between the two prey species is the speed of swimming; the V_{max} of *D. melanostictus* tadpoles (13.58 cm/s) is lower than that of *U. taprobanicus* tadpoles

(24.89 cm/s). Hence, *D. melanostictus* tadpoles are more susceptible to capture by predators than are *U. taprobanicus* tadpoles. Alternatively, it is also possible that better spatial avoidance by *U. taprobanicus* tadpoles or a preference of *H. tigerinus* to consume *U. taprobanicus* tadpoles over *D. melanostictus*. Our results conform to those of earlier studies (Van Buskirk and McCollum 2000, Dayton *et al.* 2005, Mogali *et al.* 2021). From the results of the present study it is clear that the V_{max} of predator, *H. tigerinus* tadpoles (60.21 cm/s) is much higher than both prey species hence it could capture both prey tadpole species easily. Irrespective of its high V_{max}, why do predator tadpoles preferably capture more *D. melanostictus* tadpoles than *U. taprobanicus* tadpoles? The answer might be predator put less effort to capture its prey (the one with low V_{max} i.e., *D. melanostictus*) and thus predator might conserve its energy for its growth and development. Alternatively, it could also be that capturing the slower prey is a good strategy to maximize the predator's energy intake rate (e.g., following the predations from optimal foraging theory; Werner and Hall 1974). It is generally believed that refugia reduce predation risk (Nystrom and Abjornsson 2000, Hossie and Murray 2010, 2011, Mogali *et al.* 2019, 2022). In the present study, we randomly observed the experimental tubs only during the day time, and we eye-witnessed that, basically both prey tadpole species used refuge sites when available as a consequence in the present study, in general, the susceptibility of both tadpole species was lower where refuge sites were available. Also, we have seen that *U. taprobanicus* tadpoles used more refuge sites or spent more time in refugia than that of *D. melanostictus* tadpoles. The position of the rearing tubs was randomized and changed daily to rule out position effects, if any.

In conclusion, the present study showed that *D. melanostictus* tadpoles are more susceptible to predators than those of *U. taprobanicus*. The present study on relative susceptibility of tadpoles of *U. taprobanicus* and *D. melanostictus* was conducted only at early larval stages of

development (Gosner stages 26–27). The susceptibility of the two species may change over time. Further studies comparing the species throughout development are therefore needed. The finding of the present study clearly shows that at early stages of development, *U. taprobanicus* tadpoles have developed better predator avoidance behavior than that of *D. melanostictus* tadpoles.

Acknowledgments

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Comparative histology of the vocal sac in three species of hylid frogs with comments on its functional correlates

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Abstract

Comparative histology of the vocal sac in three species of hylid frogs with comments on its functional correlates. The vocal sacs of frogs are elastic structures responsible for the circulation of air during vocalization, amplifying the sounds produced by these animals during multimodal communication. Vocal sacs present a wide array of morphologies among species and may be single, paired, or absent in adult males. Most studies on vocal sacs in Anura deal with their external morphology, and not with their internal structure, which has been explored in only a handful of species. The aim of this study was to assess vocal sac structure in three hylid species, *Dendropsophus haddadi, D. elegans,* and *Scinax fuscovarius,* using histological techniques. These species differ greatly in the degree of development and histological properties of the gular skin and submandibular musculature. In particular, elastic fibers are abundant in the thick *m. interhyoideus* and the relatively tight gular skin of *S. fuscovarius.* In contrast, in both species of *Dendropsophus* (although more evident in *D. elegans*), the *m. interhyoideus* is extremely thin and expanded, with a negligible number of elastic fibers that appear as a loose, pleated sheet when deflated. We analyzed videos of calling males of the three species and their close relatives, which show two different patterns of inflation/deflation. These patterns are strongly correlated with the histological properties of the vocal sac wall. The three species have different vocal sac shapes and rely differently on elasticity for vocal sac function.

Keywords: Animal histology, Anura, Elastic fibers, Morphology, Vocal slits, Vocalization.

Resumo

Histologia comparativa do saco vocal de três espécies de hilídeos com comentários sobre suas correlações funcionais. Os sacos vocais dos anuros são estruturas elásticas responsáveis pela circulação do ar durante a vocalização, amplificando os sons produzidos por esses animais durante a comunicação multimodal. Os sacos vocais apresentam uma ampla gama de morfologias entre as espécies, podendo ser únicos, pareados ou ausentes em machos adultos. A maioria dos estudos sobre sacos vocais em Anura trata de sua morfologia externa, não incluindo dados sobre sua estrutura interna, que foi explorada em apenas algumas espécies. O objetivo deste estudo foi avaliar a estrutura do saco vocal de três espécies de hilídeos, *Dendropsophus haddadi, D. elegans* e *Scinax fuscovarius,* por meio de técnicas histológicas. Essas espécies diferem muito no grau de desenvolvimento e nas propriedades histológicas da pele gular e da musculatura submandibular. Em particular, as fibras elásticas são abundantes no músculo interioidal espesso e pele gular relativamente esticada de *S. fuscovarius.* Ao contrário, em ambas as espécies de *Dendropsophus* (embora mais evidente em *D. elegans*), o músculo interioidal é extremamente fino e expandido, com conteúdo não-significativo de fibras elásticas, aparecendo como uma folha solta e pregueada quando desinflado. Analisamos vídeos de vocalizações de machos das três espécies e parentes próximos, que mostram dois padrões bem diferentes de inflação/deflação. Além disso, estes estão fortemente correlacionados com as propriedades histológicas da parede do saco vocal, uma vez que as três espécies têm diferentes formatos de saco vocal e dependem diferentemente da elasticidade para a função do saco vocal.

Palavras-chave: Anura, Fendas vocais, Fibras elásticas, Histologia animal, Morfologia, Vocalização.

Introduction

Vocalization is an important feature in frog biology and plays a key role in reproduction, defense of territory, and conspecific recognition (Wells 2007). Vocal sacs are structures present only in adult males of most (but not all) species of frogs and toads and are key elements of their multimodal communication in both intra- and intersexual interactions (Boulenger 1882, Liu 1935, Wells 2007, Starnberger *et al.* 2014). Their extraordinary elasticity is often evident with the naked eye because they are capable of receiving enormous volumes of air and recovering their original shape at great speed. The function of this elasticity was only recently suggested by Ryan (1985), who reasoned that the vocal sac could store the strain energy of the air column during vocalization and recycle it for inflation of the lungs. During vocalization, the mouth and nostrils remain closed and the air stored in the lungs is cycled through the larynx to the oral cavity into the vocal sacs, and then back into the lungs (Gans 1973, Dudley and Rand 1991). In the absence of a diaphragm or rib cage, the lungs in anurans are filled by a mechanism called the buccal pump, which consists of actively pushing air from the oral cavity by depression and compression of the floor of the mouth (Gans 1973). If males vocalize with their mouths open, the air dissipates into the atmosphere, forcing them to repeat the cycle of lung filling, which is costly in both time and energy (Butcher *et al.* 1982, Prestwich 1994). Calling activity in frogs is among the most energetically expensive activities in ectotherm vertebrates (Taigen and Wells 1985). Storing air within an elastic cavity greatly reduces the energetic costs of vocalization because the column of air and the associated strain energy are passively reused (Dudley and Rand 1991).

This hypothesis was supported by the finding of a layer of abundant elastic fibers in the structures associated with vocalization (Jaramillo *et al.* 1997). The elastic fibers are abundant in the trunk muscles responsible for forcing air into the buccal cavity and, in turn, the vocal sac and the lungs, which expand enormously during the

process. This discovery expanded the traditional concept that vocalization was based on muscular action. Since then, studies aimed at characterizing this system have been scarce (Savitsky *et al.* 2000, 2002, Targino *et al.* 2019, Elias-Costa *et al.* 2021), and the taxonomic distribution of the abundance of elastic fibers among different species and their spatial organization are unknown.

Anatomically, vocal sacs consist of three elements: the gular skin, the superficial submandibular musculature composed of the *m. intermandibularis* and *m. interhyoideus,* and an internal mucosa derived from the evagination of the buccal floor (Noble 1931, Inger and Greenberg 1956, Tyler 1971, 1974). Several studies described the anatomy of submandibular muscles and vocal sac structures in a wide variety of anuran families (Duellman 1956, 2001, Inger 1956, Liem 1970, Trueb 1971, Trueb and Tyler 1974, Tyler 1971, 1972, 1974, Drewes 1984, Burton 1998, Faivovich *et al.* 2011, Elias-Costa and Faivovich 2019, Elias-Costa *et al.* 2021). Only a few of these studies included histological techniques to assess the diversity in tissue composition (Jaramillo *et al.* 1997, Elias-Costa *et al.* 2017, 2021, Targino *et al.* 2019, Moura *et al.* 2021). In particular, Targino *et al.* (2019) compared the histological properties of the vocal sac wall in several microhylids and studied their evolution in the family, including the functional implications of the differences in the content of elastic fibers among species.

Hylidae, composed of 1050 described species to date, is the most diverse family of anurans (Frost 2023). Vocal sac structure in this family has been studied by several authors (e.g., Liu 1935, Duellman 2001, Tyler 1971, 1974, Faivovich 2002, Faivovich *et al.* 2011, Elias-Costa *et al.* 2021, Moura *et al.* 2021, Araujo-Vieira *et al.* 2023). Despite these extensive surveys, the histology of the vocal sac wall remains mostly unexplored. The two papers that referred to histological properties of the vocal sac wall did not discuss differences among species in light of vocalization dynamics (Elias-Costa *et al.* 2021, Moura *et al.* 2021).

The present study provides novel insights into the comparative histology of the vocal sac of three species of hylids. We considered differences among species in a morphofunctional context to hypothesize about the possible implications of the observed patterns in the dynamics of inflation of the vocal sac.

Materials and Methods

Taxon Sampling

We studied eleven specimens of three species of Hylidae found in Brazil: *Dendropsophus elegans* (Wied-Neuwied, 1824) (CFBH 36849*, 36851, and 36853), *Dendropsophus haddadi* (Bastos and Pombal, 1996) (CFBH 33203*, 36825, 36829, and 36832), and *Scinax fuscovarius* (Lutz, 1925) (CFBH 19629*, 40349, 40351, and 40354). All specimens were adult males with well-developed vocal sacs. Specimens indicated with an asterisk were only examined externally to assess gross morphology. Collection acronyms follow Sabaj *et al.* (2022).

Histological Procedures

Samples were obtained from collection specimens fixed in formalin and stored in 70% ethanol. The entire buccal floor, tongue, associated musculature, and the gular skin were separated from the mandible using a scalpel and submitted to standard histological processing. Samples were embedded in histological paraffin and cut at 6 μm-thick sections with a Microm HM340E semi-automatic microtome. Sections were stained with a standard Hematoxylin-Eosin stain for general observation of the tissuesand nitric orcein in order to reveal the presence of elastic fibers (Humason 1972, Bancroft and Gamble 2008). Inspection of the slides was performed using light microscopy; images were recorded with an Olympus BX61 microscope and cellSens Dimension software.

Qualitative Assessments of Vocal Sac Inflation Dynamics

To hypothesize if the histological differences observed among species have a functional correlation, we qualitatively studied videos of calling males of the three species. We observed inflation of the vocal sac during vocalization, paying attention to how the column of air is moved between the lungs and the vocal sac, and whether resting volumes of air in the vocal sac remained in between calls. To provide a wider context for our discussion, we studied videos of other closely related species (sources listed in Appendix 1).

Results

Comparative Histology

From observations and analyses of histological sections, we identified and described the structures present in the vocal sacs of the three species of hylids. Anatomically, their structure resembles that described for most anuran species: an internal mucosa derived from the buccal floor is externally enveloped by a thin lamina of muscle fibers, the *m. intermandibularis,* anteriorly, and the *m. interhyoideus,* posteriorly. This muscle layer is ventrally covered by the gular skin, and separated by a space, the submandibular and pectoral lymphatic sacs. In this region of the body, this space is divided by a thin membrane of connective tissue, the postmandibular lymphatic septum, which connects the *m. interhyoideus* and the gular skin. Elastic fibers are dispersed throughout the extension of the vocal sac wall in the three species. They form a mesh mostly concentrated around the external layer of muscle fibers and the internal layer of the dermis, but with scattered elastic fibers interspersed inside all tissues. The relative degree of development, abundance, and distribution of elastic fibers differed among the three species.

In *Scinax fuscovarius,* the gular skin of fixed specimens is relatively tight with only a few large folds (Figure 1). The vocal sac mucosa occupies the space underlying the posterior half of the *m. intermandibularis* and the entire *m. interhyoideus.* This muscle is thick and scarcely folded. The anterior third of the *m. interhyoideus* is similar in thickness as the *m. intermandibularis,* and the limit between them cannot be easily seen in parasagittal sections. Large numbers of elastic fibers were identified both in the *m. interhyoideus* and the vocal sac internal mucosa. They form trabeculae intertwined among muscle fibers and bundles concentrated in the exterior layer of the *m. interhyoideus* and the internal layer of the dermis. The *m. interhyoideus* only contacts the gular skin through the well-developed postmandibular lymphatic septum. In its most posterior portion, the vocal sac mucosa is extremely folded showing an irregular pattern on the internal surface of the vocal sac. This excess tissue suggests that the vocal sac is highly distensible during vocalization but contracted when at rest.

Dendropsophus elegans (Figure 2) shows a different morphofunctional pattern. The *mm. intermandibularis* and *interhyoideus* are extremely thin and expanded. In a relaxed position, they are loose and highly folded. Most of the vocal sac cavity is supported by the *m. interhyoideus,* which contacts the gular skin mostly through the postmandibular lymphatic septum but also through several smaller, punctual bridges of connective tissue. The vocal sac is less elastic than in *S. fuscovarius,* a condition evident by the loose and pleated aspect of the muscle, which does not revert to its original position, the smaller amount of elastic fibers in histological sections (only a very thin layer in the external layer of the *m. interhyoideus* and the internal layer of the dermis), and the smooth aspect of the internal mucosa, which lacks excess tissue.

Dendropsophus haddadi (Figure 3) shows an intermediate condition between the two other species. The *m. interhyoideus* is very thin and
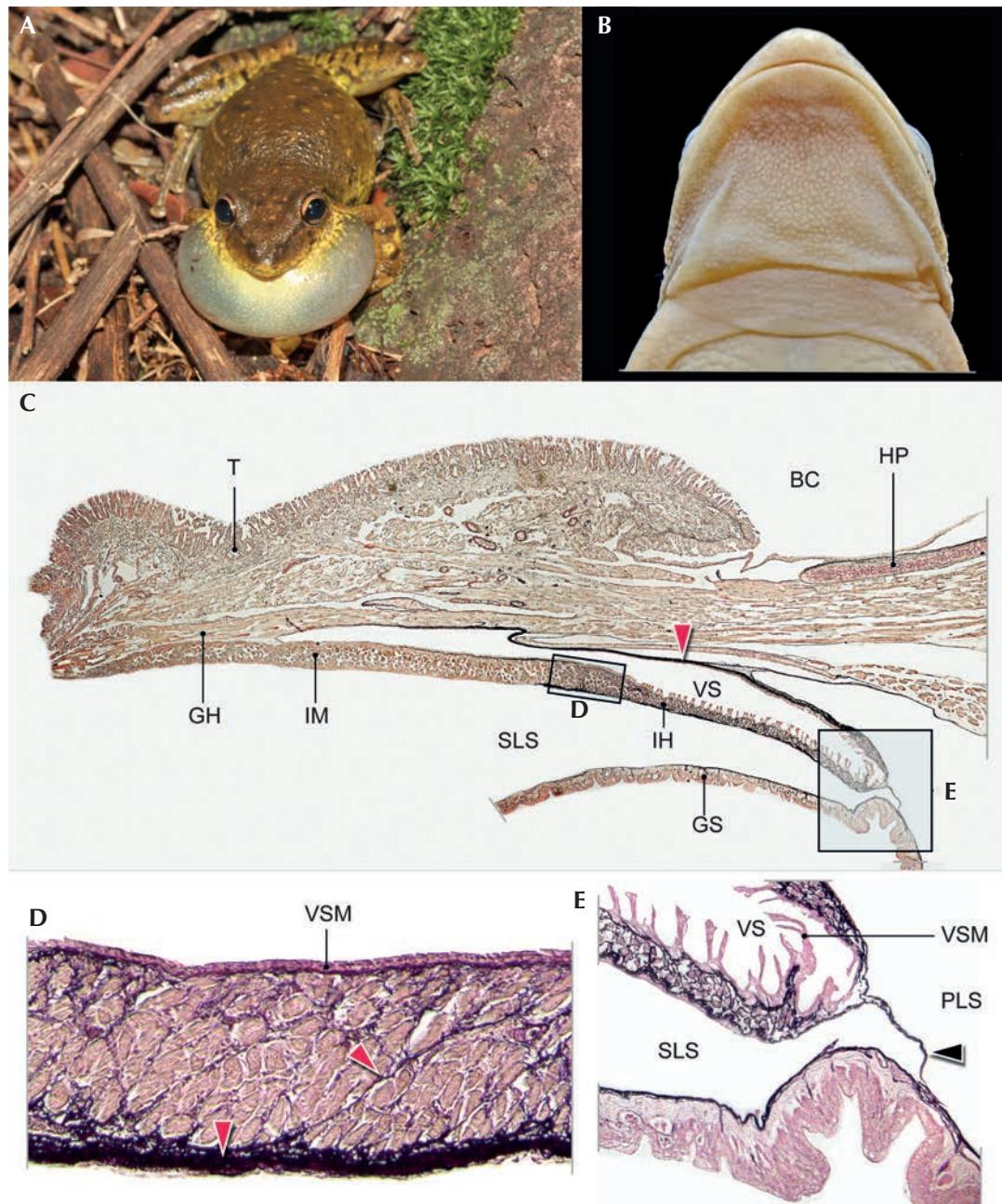
Figure 1. Vocal sac of *Scinax fuscovarius.* (**A**) Vocalizing male. (**B**) Ventral view of fixed specimen (CFBH 19629). (**C**) Sagittal section (40×) of vocal sac stained with nitric orcein (elastic fibers in black). (**D–E**) Details (400×) of regions indicated in (C). BC: buccal cavity, HP: hyoid plate, IH: *m. interhyoideus,* IM: *m. intermandibularis*, SLS: submandibular lymphatic sac, PLS: pectoral lymphatic sac, GS: gular skin, GH: *mm. geniohyoidei*, T: tongue, VSM: vocal sac mucosa, black arrow: postmandibular lymphatic septum, red arrow: bundles of elastic fibers. Photo (A) by Diego Baldo.
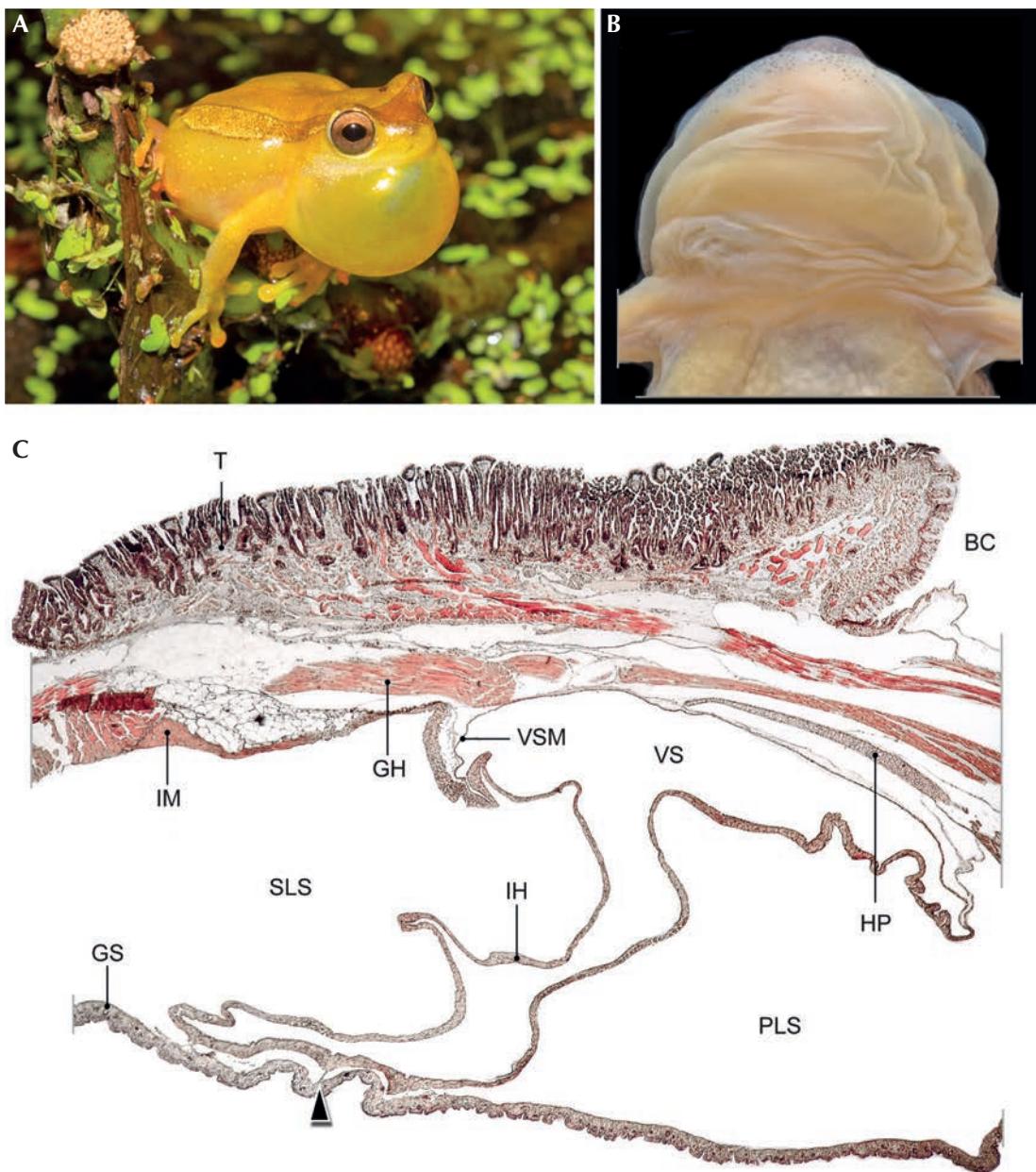
Figure 2. Vocal sac of *Dendropsophus elegans*. (**A**) Vocalizing male. (**B**) Ventral view of fixed specimen (CFBH 36849). (**C**) Sagittal section (40×) of vocal sac stained with Masson's trichrome. BC: buccal cavity, GH: *mm. geniohyoidei,* GS: gular skin, HP: hyoid plate, IH: *m. interhyoideus*, IM: *m. intermandibularis*, PLS: pectoral lymphatic sac, SLS: submandibular lymphatic sac, T: tongue, VS: vocal sac cavity, VSM: vocal sac mucosa, black arrow: postmandibular lymphatic septum. Photo (A) by Ricardo Marques.
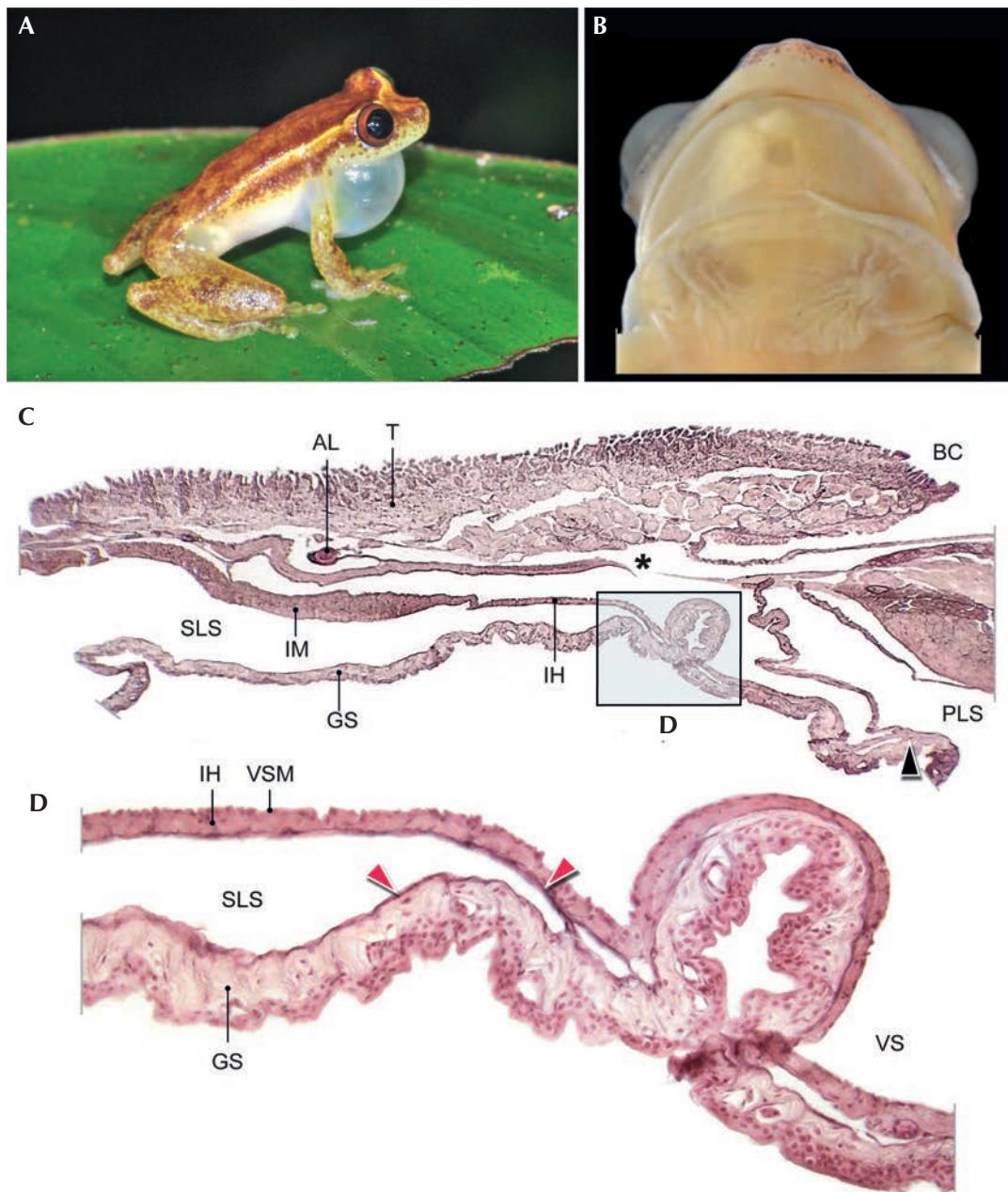
Figure 3. Vocal sac of *Dendropsophus haddadi*. (**A**) Vocalizing male. (**B**) Ventral view of fixed specimen (CFBH 19629). (**C**) Parasagittal section (40×) of vocal sac stained with nitric orcein (elastic fibers in dark red). (**D**) Details (400×) of region indicated in (C). AL: anterolateral process of hyoid (hyale), BC: buccal cavity, HP: hyoid plate, IH: *m. interhyoideus*, IM: *m. intermandibularis*, SLS: submandibular lymphatic sac, PLS: pectoral lymphatic sac, GS: gular skin, GH: *mm. geniohyoidei*, T: tongue, VSM: vocal sac mucosa, asterisk: vocal slit, black arrow: postmandibular lymphatic septum, red arrows: bundles of elastic fibers. Photo (A) by Peter Janzen.

expanded in a relaxed position, but to a lesser degree than *D. elegans,* indicating a relatively smaller vocal sac. Unlike the two previous species, the vocal sac does not extend anteriorly, but is restricted to the throat and pectoral regions. This condition is evident in ventral view, where only the skin of the posterior half of the gular region is expanded and pleated. Likewise, as observed in the sagittal sections, the *m. intermandibularis* is considerably thicker than the *m. interhyoideus,* which likely accounts for the smaller expansion of the former during vocalization. Because expansion of the vocal sac follows the direction of less resistance, inflation occurs posteriorly. The number of elastic fibers is similar to that of *D. elegans* and considerably smaller than that of *S. fuscovarius,* with very few fibers in the external layer of the *m. interhyoideus* and the internal layer of the dermis.

Qualitative Description of Vocal Sac Inflation

When analyzing videos of vocalizing males of the three species (obtained from public, online sources; see Appendix I), we observed different dynamics of vocal sac inflation. In *S. fuscovarius,* males completely inflate and deflate their vocal sacs in each cycle of sound emission (Pattern A). Deflation is almost automatic, since the air rapidly exits the vocal sac into the lungs after each burst. The vocal sac is almost completely deflated after each cycle, and air is mostly stored in the lungs. This pattern was also observed in several other species of *Scinax* (see Appendix I), although not in *S. fuscomarginatus* (Lutz, 1925) and *S. madeirae* (Bokermann, 1964). In contrast, in both species of *Dendropsophus,* the vocal sac remains almost fully inflated even in between calls (Pattern B). The amount of air used for sound production seems to be relatively small compared to the volume stored in the lungs and the vocal sac. Deflation is subtle and relatively slow. This pattern was observed in all *Dendropsophus* studied as well as in other hylids (Appendix I).

Discussion

Vocal sacs in anurans show an astounding morphological diversity, which is even greater if the internal structure is considered. This variation occurs in shape, size, color, and diverse modifications of the gular skin (Wells 2007, Köhler *et al.* 2017). This morphological diversity has been extensively surveyed both in taxonomic and systematic studies (e.g., Boulenger 1882, Liem 1980, Drewes 1984, Duellman 2001, Faivovich 2002), as well as in contributions specifically exploring this structure (e.g., Liu 1935, Tyler 1971, 1972, 1974, Tyler and Duellman 1995). Only a handful of papers have been dedicated to the histology of the vocal sac wall and its functional implications in different species (Jaramillo *et al.* 1997, Elias-Costa *et al.* 2017, Targino *et al.* 2019, Moura *et al.* 2021).

In the present study, we explored the fine structure of the vocal sac wall in three species of hylids. We identified several features in *S. fuscovarius* that suggest that males of this species rely on elasticity for vocal sac function. These features include the large amount of highly concentrated elastic fibers, forming bundles and networks present in extensions of the *m. interhyoideus* and the gular skin, the folded surface of the internal mucosa, and the relatively tight aspect of the muscles and gular skin in fixed specimens. This arrangement implies a much more energetically efficient process, since the air and strain energy used in sound production are passively recycled and redirected to the lungs to be used in the next vocalization (Dudley and Rand 1991, Jaramillo *et al.* 1997).

In both species of *Dendropsophus,* although more clearly in *D. elegans,* the vocal sac wall is loose and pleated when deflated. Elastic fibers were found in smaller numbers and were infrequent in the tissues of the gular region. The *m. interhyoideus* is extremely thin and very loose in the gular region, both in live and fixed specimens. These two species differ in the relative size and position of their vocal sacs, which are larger and occupy the entire gular

region in *D. elegans,* and smaller and restricted to the throat in *D. haddadi.* These elements suggest that these species do not depend as strongly on elasticity for vocal sac function.

By qualitative evaluation of videos of calling males of these three species, it is evident that they manage the column of air differently during vocalization. While the vocal sac in *S. fuscovarius* is rapidly deflated after each note, instantly refilling the lungs, the vocal sac in both species of *Dendropsophus* remains partially inflated in between calls. Most likely this arrangement is derived from the differing content of elastic fibers, which greatly affects the relationship of the *m. interhyoideus* and the gular skin to internal air pressure (Dudley and Rand 1991). As suggested by studies in the Túngara frog, *Engystomops pustulosus* (Cope, 1864), the abundance of elastic fibers observed in the vocal sac wall are most likely correlated with their abundance in the lungs and the trunk muscles, which receive the force of the column from the vocal sac (Jaramillo *et al.* 1997).

These two patterns of vocal sac inflation/ deflation may represent two extremes of a continuum. Some species are hard to assign to one pattern or another since the volume of the vocal sac greatly varies during vocalization, but a considerable volume of air is retained after sound emission [e.g., *Dendropsophus minutus* (Peters, 1872), *Scinax squalirostris* (Lutz, 1925)]. Future studies using quantitative methodologies will prove useful in fully understanding this phenomenon. In the species we studied, a clear correlation exists between the inflation dynamics and the histology of the vocal sac wall, a pattern that can be easily extrapolated to all frogs and toads.

Conclusions

Anura currently contains more than 7600 described species; however, the histology of the vocal sac is only known for a handful of them. Herein, we reported the fine structure of the vocal sac and compared species with diverse shapes and functional patterns. We hope this contribution will inspire further studies that quantify the content of elastic fibers and a statistically supported comparison with acoustic parameters of the vocalization.

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Appendix I. *Sources of online videos of vocalizing males.*

<u>Pattern A:</u>
Studied species:
Scinax fuscovarius: https://youtu.be/ndhVNhEvuqM, https://youtu.be/HxifDT3KKoo

<u>Other closely-related species:</u>
Aplastodiscus eugenioi: https://youtu.be/jean8fJCz00
Dendropsophus seniculus: https://youtu.be/g3NDSM6-0BU
Scinax duartei: https://youtu.be/CQ84iu_CYz8
Scinax elaeochroa: https://youtube.com/shorts/c5Ghawc0lX4?feature=share
Scinax eurydice: https://youtu.be/ZtwfqsCOL54
Scinax fuscovarius: https://youtu.be/ndhVNhEvuqM, https://youtu.be/HxifDT3KKoo
Scinax garbei: https://youtu.be/riAWAc9K1fE
Scinax granulatus: https://youtu.be/ZxrllNOPNRE
Scinax nasicus: https://youtu.be/CMt0eiPgrfA
Scinax nebulosus: https://youtu.be/IN4sW150QkQ
Scinax perereca: https://youtube.com/shorts/pEZvsl6J4Wg?feature=share
Scinax ruber: https://youtu.be/TIeKpSg42zo
Scinax squalirostris: https://youtu.be/xAFn8m4eWoc
Scinax sugillatus: https://youtu.be/Jky7jd-2c4g
Scinax tymbamirim: https://youtu.be/LuoXxgP6nY4

<u>Pattern B:</u>
Studied species:
Dendropsophus elegans: https://youtu.be/E-npc9AQdGc
Dendropsophus haddadi: https://youtu.be/UMkSAoQNiDo

<u>Other closely-related species:</u>
Boana pulchella: https://youtu.be/iz51sqVszfw
Dendropsophus acreanus: https://youtu.be/ZxZeKEsf4eM
Dendropsophus ebraccatus: https://youtu.be/fB4ElgW2tNA
Dendropsophus jimi: https://youtu.be/PeFWnguisjQ
Dendropsophus leali: https://youtu.be/M4D-NUS6__k
Dendropsophus microps: https://youtu.be/3vjOrVr0Z1E
Dendropsophus nanus: https://youtu.be/VoFt_7blF7w
Dendropsophus rubicundulus: https://youtu.be/od469uVL9U8
Dendropsophus sarayacuensis: https://youtu.be/_3Tk95S16C4
Julianus uruguayus: https://youtu.be/xt4SKlXsQc4
Ololygon berthae: https://youtu.be/n0FALJsXpJA
Ololygon perpusillus: https://youtu.be/oMGNWW93v30
Scinax fuscomarginatus: https://youtu.be/WFvCg1g1_Vo
Scinax madeirae: https://youtu.be/ZNdPddxUv3g
Sphaenorhynchus caramaschii: https://youtu.be/zR43uQI3FTY

<u>Species not assigned to any pattern (mixed):</u>
Dendropsophus minutus: https://youtu.be/CwIqeujoeQM
Scinax squalirostris: https://youtu.be/xAFn8m4eWoc

Diet of *Engystomops pustulosus* (Anura: Leptodactylidae) from Colombia and current knowledge of its dietary ecology

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Abstract

Diet of *Engystomops pustulosus* (Anura: Leptodactylidae) from Colombia and current knowledge of its dietary ecology. We investigated the diet of *Engystomops pustulosus* from a population in the Middle Magdalena River valley, including an evaluation of the effect of body and head size on prey number and volume. We present the current state of knowledge of the diet of *E. pustulosus* from published information in addition to our data. We found a total of 400 prey items representing two phyla, Arthropoda and Mollusca; seven orders and nine families were detected. Arthropods, mainly insects, were the most frequent prey in the diet. Among arthropods, Acari and Isoptera were numerically dominant. We did not observe effects of body and head size on prey number and volume. The published literature of the diet of *E. pustulosus* included 66 prey items, among which Isoptera (termites), Acari, and Formicidae were the most common groups, suggesting dietary specialization. Prey items consumed by *E. pustulosus* varied among different localities; Blattodea, Orthoptera, and Thysanoptera were unique at certain localities. Further study of prey availability and diets associated with land-use changes across major geographic localities will contribute to a better understanding of the predator-prey interactions in these anthropogenic environments.

Keywords: Ecosystem, Feeding habits, Frog, Predator interaction, Trophic specialist.

Resumen

Dieta de *Engystomops pustulosus* (Anura: Leptodactylidae) de Colombia y conocimiento actual de su ecología alimentaria. Investigamos la dieta de *Engystomops pustulosus* de una población del valle medio del río Magdalena, incluyendo una evaluación del efecto del tamaño del cuerpo y de la cabeza sobre el número y volumen de presas. Presentamos el estado actual de conocimiento sobre la dieta de *E. pustulosus* a partir de información publicada más nuestros datos. Encontramos un total de 400 presas representando dos phyla, Arthropoda y Mollusca; siete órdenes y nueve familias de invertebrados. Los artrópodos, principalmente insectos, fueron las presas más frecuentes en la dieta. Entre los artrópodos, Acari e Isoptera fueron numéricamente dominantes. No se observaron efectos del tamaño del cuerpo y la cabeza sobre el número y volumen de presas. La literatura publicada sobre la dieta de *E. pustulosus* incluyó 66 taxones presas, entre los que Isoptera, Acari y Formicidae fueron los grupos más comunes, lo que sugiere una especialización en la dieta. Las presas consumidas por *E. pustulosus* varían entre localidades; Blattodea, Orthoptera y Thysanoptera, son únicos en algunas localidades. Estudios más detallados de la disponibilidad de presas y dietas asociadas a los cambios en el uso del suelo en mas localidades geográficas contribuirá a una mejor comprensión de las interacciones depredador-presa en estos entornos antropogénicos.

Palabras clave: Ecosistema, Especialización trófica, Hábitos alimentarios, Interacción depredadora, Rana.

Resumo

Dieta de *Engystomops pustulosus* (Anura: Leptodactylidae) da Colômbia e conhecimento atual de sua ecologia alimentar. Investigamos a dieta de *Engystomops pustulosus* de uma população do vale médio do rio Magdalena, incluindo uma avaliação do efeito do tamanho do corpo e da cabeça sobre o número e o volume das presas. Apresentamos o estado atual do conhecimento sobre a dieta de *E. pustulosus* com base em informações publicadas e em nossos próprios dados. Encontramos um total de 400 itens alimentares representando os filos Arthropoda e Mollusca; sete ordens e nove famílias de invertebrados. Artrópodes, principalmente insetos, foram os itens mais frequentes na dieta. Entre os artrópodes, Acari e Isoptera foram numericamente dominantes. Não foram observados efeitos do tamanho do corpo e da cabeça sobre o número e o volume de presas. A literatura publicada sobre a dieta de *E. pustulosus* incluiu 66 táxons, entre os quais Isoptera, Acari e Formicidae foram os grupos mais comuns, sugerindo especialização na dieta. Os itens consumidos variaram entre as localidades; Blattodea, Orthoptera e Thysanoptera foram exclusivos de algumas localidades. Estudos mais detalhados da disponibilidade de presas e dietas associadas a mudanças no uso do solo em mais localidades geográficas contribuirão para uma melhor compreensão das interações predador-presa nesses ambientes antropogênicos.

Palavras-chave: Ecossistema, Especialização trófica, Hábitos alimentares, Interações predador-presa, Rã.

Introduction

Understanding trophic interactions is essential to disentangling community assembly processes and ecosystem functioning (Ings *et al.* 2009, Ryser *et al.* 2021). The study of trophic interactions has traditionally focused on the use of species diet proxies (Morales-Castilla *et al.* 2015, Laigle *et al.* 2018), primarily because diet preferences affect ecosystem energy flow and influence inter- and intraspecific interactions (Cloyed and Eason 2017). Anurans are a key vertebrate group used to study trophic interactions given their role in ecosystem energy transformation (Colón-Gaud *et al.* 2009) and the high consumption of invertebrates (Solé and Rödder 2010, Vitt and Caldwell 2014). As such, the study of diet in these vertebrates is essential to understanding

ecosystem functioning and stability (Connelly *et al.* 2008, Whiles *et al.* 2013).

Anuran diet composition may vary across space (Maneyro and da Rosa 2004, Miranda *et al.* 2006), time, and species functional traits (Vitt and Caldwell 2014, Atencia *et al.* 2020). Spatial conditions, for instance, have strong effects on the abundance and diversity of invertebrates (e.g., Eisenhauer *et al.* 2011, López-Bedoya *et al.* 2021, 2022a). These changes among invertebrate communities may in turn impact prey composition of anurans (Agudelo-Cantero *et al.* 2015, Moroti *et al.* 2021). Likewise, functional traits such as body size (snout–vent length; SVL) or mouth morphology (mouth width; MW) act as limiting factors on the size of prey that anurans can ingest, thus affecting prey composition (Parmelee 1999, Moroti *et al.* 2021).

Engystomops pustulosus (Cope, 1864) is a species widely distributed in lowlands (0-1300 m) in northern South America (Colombia, Guyana and Venezuela; Köhler 2011, Cole *et al.* 2013, Ospina-L and Bedoya-Cañón 2018, Barrio-Amorós *et al.* 2019). Despite its relatively wide geographic distribution, its diet has been rarely studied except in a few localities from Colombia and Venezuela (e.g., González-Duran *et al.* 2012, Viña-Albornoz *et al.* 2020, Blanco-Torres *et al.* 2021a). The main goals of our study are threefold: (i) to describe diet composition of a previously studied Colombian population; (ii) to test the relationship between snout-vent length (SVL) and maximum width of the mouth (MW) and prey number and prey volume; and (iii) to examine the current state of knowledge of the diet of *E. pustulosus* considering all extant information.

Materials and Methods

Study Area

The study area was the San Pedro farm (5.559946, -74.860329; 470 m a.s.l.; municipality of Victoria, Caldas department, Colombia). The San Pedro farm is located in the Middle Magdalena River valley, an area with a mean precipitation of 5500 mm/year, distributed in two rainy seasons (April–May and September–December), with an average temperature of 25°C (Cardona *et al.* 2010). The landscape consists of degraded primary forest, cattle pastures, and cocoa or annual crop plantations.

Sampling Design and Laboratory Work

We sampled the population of *E. pustulosus* using visual encounter surveys (see Doan 2003) in ponds associated with cattle pastures for three consecutive days (19–21 April 2016), between 18:00 and 23:00 h. We captured frogs manually and transferred them to the field station within a maximum of two hours after capture to minimize bias associated with digestion (Parmelee 1999). The frogs were sacrificed using Xylocaine and fixed in 10% formaldehyde (Gutierrez-Cárdenas *et al.* 2016). Snout–vent length (SVL) and mouth width (MW) were measured for each specimen with a digital caliper to the nearest 0.1 mm. Specimens were preserved in 75% ethanol and transported to the zoology laboratory of the Universidad de Caldas.

Stomach contents were identified for each individual using a stereomicroscope; small prey such as Acari and Formicidae were identified using a microscope. We identified prey to the lowest possible taxonomic level using general keys for invertebrates (Triplehorn and Johnson 2005, Adis 2002) and specific keys for Coleoptera (Arnett Jr. and Thomas 2000), Hymenoptera (Fernández 2003, Fernández *et al.* 2015, Ješovnik and Schultz 2017, Fernández *et al.* 2019, Pérez-Pedraza and Fernández 2019), and Acari (Balogh and Balogh 1988, 1990, Badejo 2002).

Stomach Contents Analysis

Prey consumption of *E. pustulosus* was quantified as the number of prey items (N_i), volume of prey items (V_i), and frequency of occurrence (O_i) of each prey taxon (*see*

Gutiérrez-Cárdenas *et al.* 2016). Each individual prey item was measured for length (L) and width (W) using a stereomicroscope coupled with digital measuring to the nearest 0.1 mm. These measurements were employed to estimate the prey volume (mm³) using the formula for a prolate spheroid [$V_i = 4/3\pi \, (L/2) \, (W/2)^2$] (Dunham 1983).

We compared the relative proportion of each prey category using the index of relative importance (IRI) following Pinkas *et al.* (1971): $\%IRI = (\%O_i) \, (\%N_i + \%V_i)$. The latter was done because any measurements used alone are biased toward large-sized or sporadically abundant prey (Pinkas *et al.* 1971, Manicom and Schwarzkopf 2011). Finally, we evaluated the correlation between functional traits and prey of *E. pustulosus*. We tested for correlations between SVL and MW to prey number and prey volume using the Spearman Rank Correlation (r_s) on log-transformed data in the R program (R Core Team 2022).

Current State of Knowledge of the Diet of *E. pustulosus*

To describe the diet of *E. pustulosus*, we included published articles from the personal database of the last author (PDAGC), in addition to articles identified in the recent revision of the Neotropical herpetofauna (*see* Urbina-Cardona *et al.* 2023). When multiple studies were published from the same dietary dataset (e.g., Blanco-Torres *et al.* 2020, 2021a, b), we included only one study to avoid duplication of data (i.e., the study showing more detailed information). For each study we extracted the following variables: (i) country, (ii) year of publication, (iii) habitat cover, and (iv) prey items consumed by *E. pustulosus*.

Results

We captured 58 individuals of *E. pustulosus* [52 males and six females; mean SVL, 27.4 ± 1.59 mm (range 24.37–30.94 mm); MW

of 7.56 ± 0.63 mm (6.25–8.87 mm)]. We used only the 63.8% ($N = 37$) of individuals that contained prey items.

A total of 400 prey items were distributed in two phyla, Arthropoda and Mollusca, which included seven orders and nine families, primarily insects (Table 1). Acari (mites; $\%N_i = 48.75$) and Isoptera (termites; $\%N_i = 41$) were the numerically dominant prey taxa. Volumetrically, Isoptera ($\%V_i = 86.2$) and Formicidae ($\%V_i = 6.49$) were the dominant prey items. Isoptera ($\%O_i = 51.35$) and Formicidae (ants; $\%O_i = 45.95$) were the most frequently consumed prey items. Isoptera (IRI = 3009.67) and Acari (IRI = 1394.44) were the taxa with the highest IRI values in the diet of *E. pustulosus*. We did not find significant correlations between prey number ($r_s = -0.035$, $p = 0.838$; $N = 37$) and prey volume ($r_s = 0.032$, $p = 0.852$; $N = 37$) with SVL. Likewise, no correlations were found for prey number ($r_s = 0.202$, $p = 0.230$; $N = 37$) and prey volume ($r_s = 0.030$, $p = 0.857$; $N = 37$) with MW.

Seven studies of the diet of *E. pustulosus* includes our present work (Table 2). These studies were made during the last two decades and have focused on the composition of prey assemblages consumed by the species in cattle pastures. Among these studies, 66 prey items were consumed by *E. pustulosus,* with Isoptera, Formicidae, and Acari as the dominant prey taxa in terms of prey number (Table 3). Different prey items occurred in the stomach contents among geographic localities: some invertebrate prey items were unique to a single geographic locality. For example, Blattodea (cockroaches) was found only in stomach contents in a locality from Venezuela, and other prey items such as Collembola, Coleoptera, Diptera, Hemiptera, and Orthoptera were found in other geographic localities (Table 3).

Discussion

We investigated the diet composition of a previously studied Colombian population of *E.*

Table 1. Prey composition of the diet of *Engystomops pustulosus* from Victoria municipality (Caldas, Colombia). Abbreviations: *N*, number of prey items; V, prey volume (in mm³); O, frequency of occurrence; IRI, index of relative importance (only for prey taxa in which it was possible to calculate the volume).

Prey taxa	N (%)	V (%)	O (%)	IRI
ARTHROPODA				
Arachnida				
Araneae	1 (0.25)	0.52 (0.04)	1 (2.70)	0.77
Acari (Oribatida)				
Ceratozetidae	2 (0.50)	0.66 (0.04)	2 (5.41)	2.94
Trhypochthoniidae				
Archegozetes sp.	193 (48.25)	47.78 (3.24)	10 (27.03)	1391.5
Diplopoda				
Polydesmida				
Fuhrmanodesmidae				
Fuhrmannodesmus sp.	1 (0.25)	1.57 (0.11)	1 (2.70)	0.96
Insecta				
Coleoptera				
Chrysomelidae	1 (0.25)	10.47 (0.71)	1 (2.70)	2.59
Staphylinidae				
Paederinae	1 (0.25)	18.85 (1.28)	1 (2.70)	4.13
Euconnus sp.	3 (0.75)	17.28 (1.17)	2 (5.41)	10.38
Hymenoptera				
Figitidae	1 (0.25)	2.36 (0.16)	1 (2.70)	1.11
Formicidae				
Myrmicinae	6 (1.50)	46.29 (3.13)	5 (13.51)	62.63
Atta cephalotes	1 (0.25)	1.18 (0.08)	1 (2.70)	0.89
Cyphomyrmex sp.	2 (0.50)	2.82 (0.19)	1 (2.70)	1.87
Myrmicocrypta sp.	1 (0.25)	1.05 (0.07)	1 (2.70)	0.87
Pheidole sp. 1	8 (2.00)	6.28 (0.43)	3 (8.11)	19.67
Pheidole sp. 2	6 (1.50)	14.66 (0.99)	2 (5.41)	13.47
Sericomyrmex amabilis	3 (0.75)	10.60 (0.72)	1 (2.70)	3.97
Solenopsis sp.	1 (0.25)	3.53 (0.24)	1 (2.70)	1.32
Strumigenys grytava	1 (0.25)	0.52 (0.04)	1 (2.70)	0.77
Strumigenys marginiventris	1 (0.25)	7.33 (0.50)	1 (2.70)	2.02
Wasmannia auropunctata	2 (0.50)	1.77 (0.12)	2 (5.41)	3.35
Isoptera				
Kalotermitidae	71 (17.75)	1144.33 (77.48)	8 (21.62)	2059.11
Termitidae	93 (23.25)	128.83 (8.72)	11 (29.73)	950.56
MOLLUSCA				
Pulmonata	1 (0.25)	8.38 (0.57)	1 (2.70)	2.21
TOTAL	**400**	**1476.86**		

Table 2. Literature on the diet of *Engystomops pustulosus*. Details on country, publication year, habitat cover, study type, taxonomy of prey items, and relevant prey items in terms of prey number (*N*). The order of the items in the table is according to prey number (*N*).

Country	Year	Habitat cover	Relevant prey items (*N*)	Reference
Colombia	2012	Pasture lands	Isoptera, Formicidae, Acari	González-Duran *et al.* 2012
Colombia	2013	Pasture lands	*Digitonthophagus gazella*	Blanco-Torres *et al.* 2013
Venezuela	2019	No data	Isoptera, Formicidae, Lepidoptera, Araneae	Cañizales 2019
Colombia	2020	Pasture lands	Isoptera, Acari, Formicidae	Atencia *et al.* 2020
Venezuela	2020	Agricultural and pasture lands	Isoptera, Acari, Formicidae	Viña-Albornoz *et al.* 2020
Colombia	2021	Agricultural and pasture lands	Isoptera, Formicidae	Blanco-Torres *et al.* 2021a
Colombia	2022	Pasture lands	Acari, Isoptera, Formicidae	This study

pustulosus from the Middle Magdalena River valley and evaluated the relationship between anuran morphometric measurements and prey size and volume. We found 37 individuals with prey items and 21 individuals with empty stomachs. This result could be due to the beginning of the breeding season (Hirai and Matsui 2000), during which males spend more time calling and searching for females and consequently less time foraging for food. In this context, *Engystomops* and congeners breed from April to December (Ryan *et al.* 1983), and we captured the males in our study during April.

Our data indicated that *E. pustulosus* consumes arthropods as do most anurans (Parmelee 1999, Narvaez and Ron 2013, Womack and Bell 2020), with the exception of a few species that have been reported to prey on vertebrates such as birds, rodents, and other amphibian species (Santos *et al.* 2004, Caicedo-Martínez *et al.* 2021). This pattern is not surprising because invertebrates are an abundant resource in almost all ecosystems and represent an advantageous food source because of the low energetic costs associated with their capture and consumption (Taigen and Pough 1983, Biavati *et al.* 2004, Vitt and Caldwell 2014, Pacheco *et al.* 2017).

The greater occurrence of Isoptera, Acari, and Formicidae found in the studied population of *E. pustulosus* was similar to the diet of the species throughout its range (Gonzalez-Duran *et al.* 2012, Blanco-Torres *et al.* 2021a, b). This species is terrestrial and can be found in open habitats including pastures (Ospina-L and Bedoya-Cañón 2018), and termites, ants, and mites typically occur in large numbers in these habitats. In this sense, *Engystomops* and the closely related genus *Physalaemus* (Leptodactylidae; Lourenço *et al.* 2015) can be classified as specialists of small prey such as termites or ants (Duellman 1978, Vitt and Caldwell 1994, Parmelee 1999, Narvaez and Ron 2013, Almeida *et al.* 2019). The lack of correlation between morphometric measurements and prey number or prey volume found in this study may be explained by the higher proportion of termites, ants, and mites even though morphologically they could consume larger prey (Guzman and Salazar 2012).

Table 3. Data (%N) on prey types and total prey consumed by *Engystomops pustulosus* obtained from the published literature and the present work. Sources: (1) González-Duran *et al.* 2012; (2) Cañizales 2019; (3a) Atencia *et al.* 2020; locality: Santa Inés; (3b) Atencia *et al.* 2020; locality: Coloso; (3c) Atencia *et al.* 2020; locality: El Roble; (4) Viña-Albornoz *et al.* 2020; (5) Blanco-Torres *et al.* 2021a; (6) this study. Undetermined prey items (und.) are noted when higher taxonomic resolution was not available. "X" represents the presence of prey in the stomach contents.

Prey taxa	Sources of data on prey categories							
	1	2	3a	3b	3c	4	5	6
ARTHROPODA								
Arachnida								
Acari und.	9.6					0.51	9.68	
Ixodida								
Argasidae						0.11		
Trombidiformes und.			10.67	23.29	5.16	29.48		
Trombidiidae						0.15		
Oribatida								
Ceratozetidae								0.5
Trhypochthoniidae								48.25
Araneae und.		X					0.65	0.25
Theridiidae						0.04		
Pseudoscorpiones und.						0.07	0.08	
Chilopoda	0.4							
Diplopoda und.	0.1					0.15		
Polydesmida								
Fuhrmanodesmidae								0.25
Crustacea								
Isopoda						0.84	0.98	
Insecta								
Collembola und							0.08	
Dycirtomidae	1.5							
Isotomidae						0.7		
Sminthuridae						0.92		
Blattodea								
Ectobiidae						0.04		
Coleoptera und.							0.9	
Anobiidae						0.04		
Carabidae						0.04		
Chrysomelidae	0.1							0.25
Coccinellidae						0.04		
Curculionidae			0.56			0.04		
Dytiscidae			0.56	0.24				
Elateridae						0.26		
Lampyridae						0.07		
Mycetophagydae	0.2							
Nitidulidae	0.1							

Table 3. *Continued.*

Prey taxa	Sources of data on prey categories							
	1	2	3a	3b	3c	4	5	6
Passalidae						0.11		
Scarabaeidae	0.1		0.56					
Silvanidae	0.2							
Staphylinidae	0.5		1.12			0.52		1
Tenebrionidae			1.12	0.24				
Trogossitidae	0.1							
Diptera und.							0.16	
Ceratopogonidae						0.47		
Chironomidae	0.1							
Dolichopodidae						0.04		
Drosophilidae	0.6							
Ephydridae						0.08		
Micropezidae	0.4							
Phoridae						0.04		
Psychodidae	1							
Sphaeroceridae	6					0.07		
Stratiomydae						0.04		
Tephritidae						0.07		
Hemiptera und.							0.24	
Cicadellidae	0.1							
Fulgoridae	0.2							
Miridae						0.07		
Tingidae						0.07		
Hymenoptera und.						0.04	16.48	
Diapriidae	0.1							
Figitidae	0.1							0.25
Formicidae	18.8	X	39.89	0.71	1.07	9.94		8
Isoptera und.		X					70.3	
Kalotermitidae								17.75
Termitidae	59.4		43.82	75.06	94.72	54.09		23.25
Lepidoptera		X				0.08		
Orthoptera und.							0.16	
Psocoptera								
Psocidae			0.56			0.04		
Thysanoptera								
Thripidae	0.1							
MOLLUSCA und.	0.1					0.51	0.08	
Pulmonata								0.25
Physidae			0.56	0.24				
Total prey items in each study	**1061**		**178**	**426**	**833**	**2727**	**1219**	**400**

Despite the dominance of termites, ants, and mites in the stomach contents across geographic localities, we found a shift in the taxonomic identity of other prey items. We found that 66 prey items were consumed among different studies of the diet of *E. pustulosus*. Unique prey items were found in specific localities (Atencia *et al.* 2020, Viña-Albornoz *et al.* 2020). Changes in consumption of prey items showed that the diet of *E. pustulosus* can have plasticity across different localities (Atencia *et al.* 2020), possibly due to differences in spatial and temporal variables in prey availability (Agudelo-Cantero *et al.* 2015). For example, rainy seasons could affect the humidity of topsoil layers, a determining factor for the abundance and presence of mites. Rainy periods may also affect the production of vegetation and foliage consumed by many species of termites and ants. This study included different years and seasons (i.e., rainy or dry seasons), and geographical localities with changes in habitat covers (*e.g.*, more or less disturbance in cattle pastures or agricultural development). These parameters clearly affect the presence of different prey items. To our knowledge, no information about prey availability is available to corroborate this assumption.

We encourage further assessment of major geographic localities to better understand the composition of prey consumed by this anuran species. It is also key to study prey availability (*e.g.*, del Rio-García *et al.* 2014) and dietary variation in response to spatial and temporal changes (*e.g.*, Blanco-Torres *et al.* 2021a, López-Bedoya *et al.* 2022b). The distribution and composition of invertebrate communities differ between natural and anthropic ecosystems, and between rainy and dry periods (*e.g.*, Ospina-Bautista *et al.* 2022). Given the importance of predator-prey interactions for stability and energy flow in different natural and anthropogenic ecosystems, further insight on broader knowledge gaps (*e.g.*, land-use change and prey availability) may aid understanding predator-prey interactions in anthropogenic environments (*i.e.*, plantations, pastures; Konopik *et al.* 2014, Moskowitz *et al.* 2020).

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Reproductive ecology and natural history of *Kinosternon herrerai* (Testudines: Kinosternidae) at the center of its distribution

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Abstract

Reproductive ecology and natural history of *Kinosternon herrerai* (Testudines: Kinosternidae) at the center of its distribution. Mexico harbors 10 endemic species of mud turtles of genus *Kinosternon,* but natural history information is lacking for most of them. Herein we describe some generalities of the reproductive ecology and natural history of one population of the Mexican endemic, Herrera's mud turtle, *K. herrerai* from Hidalgo, Mexico. Females and males were similar in body mass and carapace length. Additionally, larger and heavier turtles moved more than smaller and lighter ones. Clutch size varied from one to six eggs, with an average of 3.4 ± 1.7, and egg laying occurred at environmental temperatures between 15 and 21°C ($\bar{x}$ = 18.6°C). Total nesting time (from selection of nesting site to oviposition) lasted ca. 37 min, and the incubation period was 75 days *in situ.* These results enhance our understanding of natural history aspects of *Kinosternon* mud turtles, which are necessary to carry out conservation actions to preserve all of its populations.

Keywords: Clutch size, Hidalgo, Movement rate, Mud Turtles, Reproduction.

Resumen

Ecología reproductiva e historia natural de *Kinosternon herrerai* (Testudines: Kinosternidae) en el centro de su distribución. México alberga 10 especies endémicas de tortugas de barro del género *Kinosternon*, pero hace falta información de historia natural para la mayoría de ellas. Aquí describimos algunas generalidades de la ecología reproductiva e historia natural de una población de la tortuga Casquito de Herrera, endémica mexicana, *K. herrerai* en Hidalgo, México. Las hembras y los machos fueron similares en masa corporal y longitud del carapacho, además, las tortugas más

grandes y pesadas se movieron más que aquellas más pequeñas y livianas. El tamaño de la puesta varió de uno a seis huevos, con un promedio de 3.4 ± 1.7, y la puesta de huevos ocurrió a temperaturas ambientales entre 15 and 21°C ($\bar{x}$ = 18.6°C). El tiempo total de anidamiento (desde la selección del nido hasta la ovoposición) duró ca. 37 min y el periodo de incubación fue de 75 días *in situ*. Estos resultados mejoran nuestra comprensión de los aspectos de historia natural de las tortugas de barro *Kinosternon*, que son necesarios para llevar a cabo acciones de conservación para preservar todas sus poblaciones.

Palabras clave: Hidalgo, Reproducción, Tamaño de puesta, Tasa de movimiento, Tortugas de barro.

Resumo

Ecologia reprodutiva e história natural de *Kinosternon herrerai* (Testudines: Kinosternidae) no centro de sua distribuição. O México abriga 10 espécies endêmicas de tartarugas-de-lama do gênero *Kinosternon*, mas faltam informações sobre a história natural da maioria delas. Aqui, descrevemos algumas generalidades da ecologia reprodutiva e da história natural de uma população de Hidalgo da tartaruga-de-lama-de-herrera, *K. herrerai*, endêmica do México. As fêmeas e os machos eram semelhantes em termos de massa corporal e comprimento da carapaça. Além disso, as tartarugas maiores e mais pesadas se movimentavam mais do que as menores e mais leves. O tamanho da ninhada variou de um a seis ovos, com uma média de 3,4 ± 1,7, e a postura dos ovos ocorreu em temperaturas ambientais entre 15 e 21°C ($\bar{x}$ = 18,6°C). O tempo total de nidificação (desde a seleção do local de nidificação até a ovipostura) durou cerca de 37 min, e o período de incubação foi de 75 dias *in situ*. Esses resultados aumentam nossa compreensão dos aspectos da história natural das espécies de *Kinosternon*, que são necessários para realizar ações de conservação para preservar todas as suas populações.

Palavras-chave: Hidalgo, Reprodução, Tamanho da ninhada, Tartarugas-de-lama, Taxa de movimento.

Introduction

Mud turtles of the genus *Kinosternon* have their greatest taxonomic diversity and endemism in Mexico; however, there are important information gaps in the understanding of their natural history and ecology (Pritchard and Trebbau 1984, Macip-Ríos *et al.* 2009, 2015, Legler and Vogt 2013). In Mexico this genus is represented by 17 species (Iverson 1991a, López-Luna *et al.* 2018, Loc-Barragán *et al.* 2020, TTWG 2021, Berriozabal-Islas *et al.* 2023, Ramírez-Bautista *et al.* 2023) and nine subspecies (Iverson 1985, Legler and Vogt 2013, Berriozabal-Islas *et al.* 2023). *Kinosternon* species inhabit humid, arid, and dry tropical environments from the United States of America to Argentina (Legler and Vogt 2013). All species are aquatic, but many spend significant amounts of time in terrestrial habitats.

Most *Kinosternon* species are omnivorous, feeding on insects, fish, carrion, and plant matter (Legler and Vogt 2013). Within the genus, studies of systematics, distribution, biogeography (Iverson 1985, Legler and Vogt 2013), demography (Iverson 1991b, Macip-Ríos *et al.* 2011), and conservation (Macip-Ríos *et al.* 2015, Berriozabal-Islas *et al.* 2020, 2023) predominate. Of the 17 species found in Mexico, 10 (59%) are endemic (Berriozabal *et al.* 2023, Ramírez-Bautista *et al.* 2023). Population ecology has only been studied in some of these endemics including *Kinosternon abaxillare* Baur, 1925 (Reyes-Grajales *et al.* 2021), *K. alamose* Berry and Legler, 1980 (Iverson 1989), *K. chimalhuaca* Berry, Seidel, and Iverson, 1997 (Butterfield *et al.* 2020), *K. creaseri* Hartweg, 1934 (Macip-Ríos *et al.* 2018), *K. oaxacae* Berry and Iverson, 1980 (Vázquez-Gómez *et al.* 2015, 2016), and *K. vogti* López-Luna, Cupul-Magaña, Escobedo-Galván,

González-Hernández, Centenero-Alcalá, Rangel-Mendoza, Ramírez-Ramírez, and Cazares-Hernández, 2018 (Rosales-Martínez *et al.* 2022). Behavior and reproduction have been extensively studied only in *K. integrum* (Iverson 1999, Macip-Ríos *et al.* 2009, 2011). Thus, basic biological information is lacking for most species, including the widely distributed Mexican endemic, Herrera's mud turtle, *Kinosternon herrerai* Stejneger, 1925.

Herrera's mud turtle, *Kinosternon herrerai* (Figure 1) is considered to be aquatic (Legler and Vogt 2013, Berlant and Stayton 2017). The reduced plastron size in this species (Figure 1B) suggests that it is an inhabitant of more permanent water bodies (Berry 1977, but see Iverson 1991c). It occurs in the eastern states of Tamaulipas, Veracruz, San Luis Potosí, Hidalgo, and Puebla (Figure 2; TTWG 2021), with records of introduced individuals in the state of Mexico (Legler and Vogt 2013). Data from northern and southern populations (Legler and Vogt 2013) have shown that individuals are both diurnal and nocturnal (Aguirre-León and Aquino-Cruz 2004), they reproduce (courtship and copulation) underwater (Carr and Mast 1988) during the dry season (July and August) and produce multiple clutches from two to four eggs (Carr and Mast 1988). Although descriptive data of Iverson (1991c) might indicate that *K. herrerai* has male-biased sexual size dimorphism, detailed studies of populations at the extremes of its distribution have shown differing patterns. In northern populations, males are larger than females, and both tend to be more frugivorous (Carr and Mast 1988), while in southern populations, sexes are similar in body size, but males are heavier than females, and both tend to be more carnivorous (Aguirre-León and Aquino-Cruz 2004). Populations in the central part of the distribution have not been extensively studied, probably because of rarity and small population sizes. Therefore, additional information about populations in this region would be a valuable contribution to the knowledge of this widely distributed *Kinosternon* species and to the generation of national and international conservation efforts. This study describes some general aspects of the ecology (local movements, sex ratio, reproductive period, clutch size) of one population of *K. herrerai* from Hidalgo, Mexico.

Figure 1. (**A**) Adult female *Kinosternon herrerai* from the municipality of Acatlán, Hidalgo, Mexico (photo by authors). (**B**) A plastral view of adult male from Veracruz, Mexico (photo by John Iverson).

Materials and Methods

The study was carried out in three ponds (Santa Rosa, El Transformador, and Totoapita Canutillo), separated from each other between 300 m to 400 m a.s.l., within the municipality of Acatlán, Hidalgo, Mexico (Figure 2). The study area is located at 2120 m a.s.l., where the main vegetation type is xeric scrubland (Pavón and Mesa-Sánchez 2009). The three ponds were from 0.20 m to 1.5 m depth and 35 m long by 13 m wide, and they were permanent water bodied whose level fluctuated depending on the seasonality of precipitation and the irrigation system of nearby field crops.

We sampled three days each month for one year (from October 2008–October 2009), using the same sampling effort (one person for each site). Each sampling period was from 07:00 to 11:00 h and from 17:00 to 19:00 h. The sampling design was developed based on the species' activity cycle (Carr and Mast 1988, Aguirre-León and Aquino-Cruz 2004, Legler and Vogt 2013). We visually located and hand captured each turtle and recorded the following data: date, carapace length (CL in mm, taken to the nearest 0.01 mm using a digital caliper, measured parallel to the mid-plastral plane; Iverson and Lewis 2018), and body mass (BM in g, measured with an electronic portable scale). Measurements
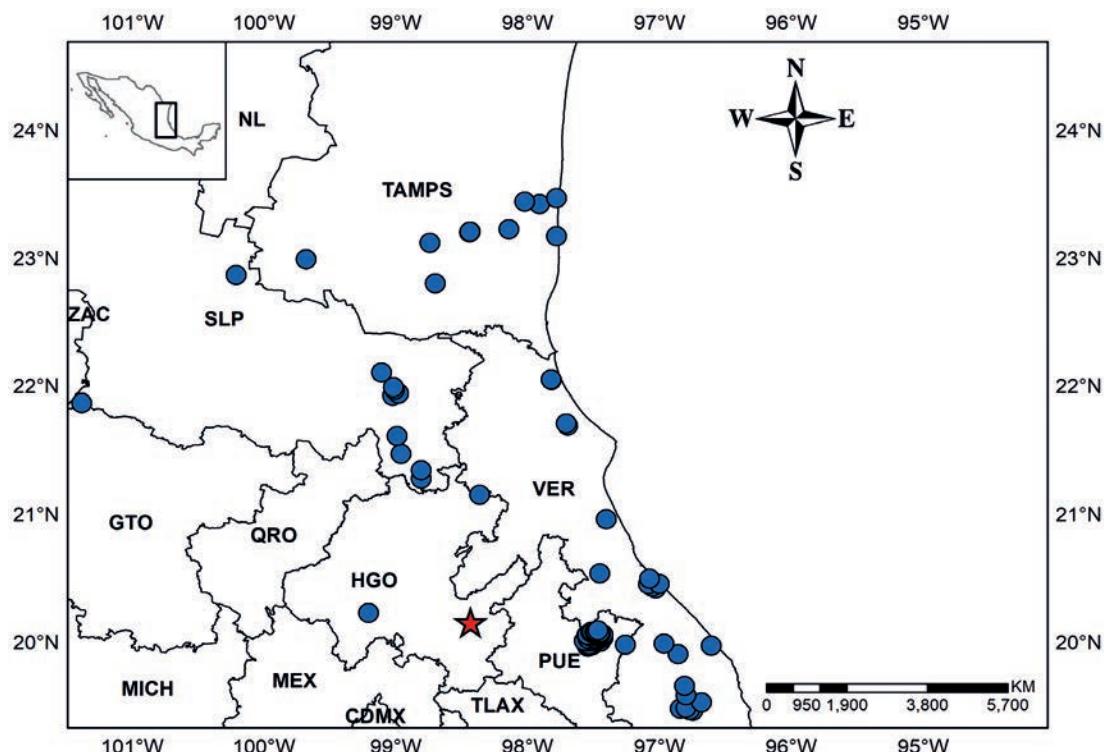
Figure 2. The known distribution range of *Kinosternon herrerai* in Mexico. The blue dots are records obtained from GBIF (2023) datasets, which consider specimens recorded by governmental national institutions and those deposited in national and international scientific collections. The red star is the municipality of Acatlán, where the three studied ponds are located. Mexican states shown are Nuevo León (NL), Tamaulipas (TAMPS), Zacatecas (ZAC), San Luis Potosí (SLP), Guanajuato (GTO), Querétaro (QRO), Hidalgo (HGO), Michoacán (MICH), Estado de México (MEX), Ciudad de México (CDMX), Tlaxcala (TLAX), Puebla (PUE), and Veracruz (VER).

are reported as mean ± 1 SE. The distance between exact capture and recapture sites (measured with a tape measure in m) was used to calculate the movement rate of each turtle. Sex and age class were also determined for each turtle. Sex was determined by secondary sexual characters such as the elongated tail, concave plastron, and proportionally larger head for males, compared with shorter tails, a flat plastron, and smaller head for females. For both sexes, age class was assigned as hatchling (16.2–21.0 mm CL), juvenile (52.0–110.6 mm CL), and adult (111.5–140.4 mm CL) following Legler and Vogt (2013). Turtles were marked using indelible ink. We painted a number (sequential) on the carapace of each individual; the number was permanent enough to last for at least one year of study. The reproductive period was determined by observations of specific events, including courtship and mating, egg laying, and the first detection of hatchlings in the studied ponds (Carr and Mast 1988). When oviposition was observed, we measured the environmental temperature of the nest (at ground level) using a Miller-Weber rapid-registering thermometer (± 0.2°C).

To determine whether the sex ratio was biased, we carried out a chi-square analysis. We performed Mann-Whitney U tests to evaluate intersexual differences in CL and BM. A Kruskal-Wallis test was carried out to compare female CL and BM among ponds. Spearman correlations were calculated to test for a relationship between CL and BM with movement rate. To evaluate whether movement rate differed among ponds, we used a one-way analysis of variance. We considered results significant at $p \leq 0.05$. Statistical analyses were performed in Statistica 10.0 (Statsoft Inc).

Results

The total of 40 different individual turtles were captured in the three ponds a total of 24 times. This number included 27 adults, seven juveniles, and six hatchlings. For two ponds with

adequate sample size, the sex ratio was 1:2.5 female-biased ($\chi^2 = 4.9$, $p = 0.03$). Females and males were similar in BM and CL both within and among ponds (Table 1). When data were pooled, female BM was 438.4 ± 14.6 g (range 340–530) and CL was 126.4 ± 2.2 mm (range 112–140) ($N = 17$); for males BM was 426.5 ± 16.1 g (range 345–503) and CL was 126.9 ± 2.24 mm (range 113–140) ($N = 10$). No differences were detected between the sexes for BM ($Z = 0.33$, $p = 0.74$) or CL ($Z = -0.18$, $p = 0.86$).

Based on 24 recaptures and 40 total captures, movement rate was similar among the three ponds ($F_{2, 61} = 0.61$, $p = 0.85$, $N = 64$) when considering all individuals of both sexes; however, movement rate was positively correlated with CL (Figure 3A) and BM (Figure 3B). Hence, larger and heavier turtles moved more than smaller and lighter turtles. No turtles were observed to move between ponds during our one-year study period.

The reproductive period occurred from early spring (March) to early fall (October). During this time, we observed mature females ($N = 5$) initiate intrasexual fighting behavior (female-female) using their bodies, forelimb claws, and heads. Once the fighting ended, a nearby male pursued, subdued, and copulated with the winning female. Within the study period (October 2008 to May 2009) we found five clutches, three around the Santa Rosa pond, and two nearby Totoapita Canutillo pond. Clutch size varied from one to six eggs, with an average of 3.4 ± 1.7. Egg laying occurred in nests composed of moist soil (mud), and wet grass and weeds, where environmental temperatures varied between 15 and 21°C ($\bar{x} = 18.6$ °C). Total nesting time (from selection of nesting site to oviposition) lasted ca. 37 min and the incubation period was 75 days *in situ*.

Discussion

The results of this study increase our knowledge of the natural history of Herrera's mud turtle, *Kinosternon herrerai* (Legler and
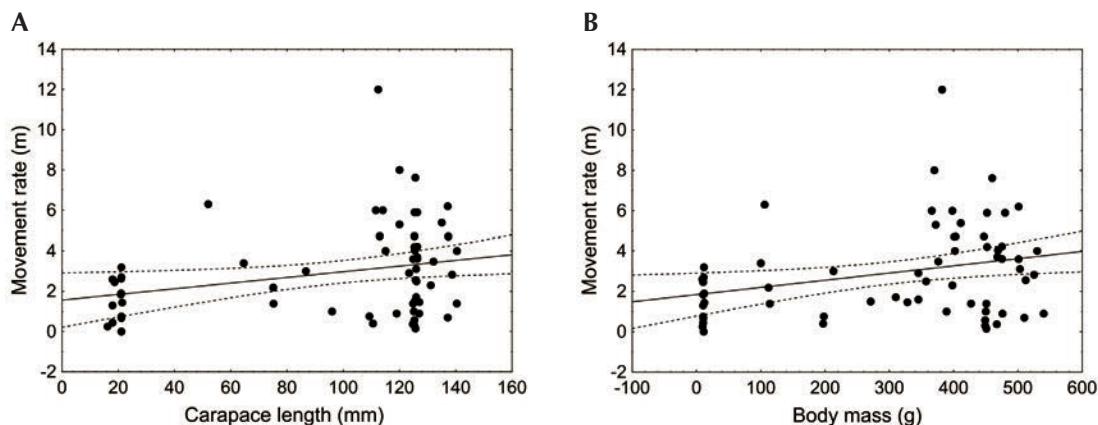
Figure 3. Relationship between carapace length (**A**; r = 0.25, *p* = 0.04, *N* = 64) and body mass (**B**; r = 0.27, *p* = 0.03, *N* = 64) with movement rate of *Kinosternon herrerai* from southeastern Hidalgo, Mexico. Dashed lines represent 95% confidence intervals.

Vogt 2013). Prior to oviposition (March), the population in our study area (considering all three ponds together) consisted of only 10 adult males, 17 adult females, seven juveniles, and six hatchlings. This is a small population size compared with other studies of Mexican turtles (Macip-Ríos *et al.* 2011, Vázquez-Gómez *et al.* 2016, Reyes-Grajales *et al.* 2021). We attribute this result in part to the lack of trapping, since mud turtles are generally collected using baited hoop traps; however, we were seeing the same few individuals multiple times suggesting that the number of turtles in each pond is quite small (mean recapture rate: 60%).

Our results provide evidence of the typical female-biased sex ratio of kinosternids (Vázquez-Gómez *et al.* 2016, Reyes-Grajales *et al.* 2021, De la Cruz-Merlo *et al.* 2022). In general, the sex ratio could be explained by an equilibrium of population size through fecundity (Stearns 1992) and by regulation of nest environmental temperature during embryonic development (Pough *et al.* 2001, Macip-Ríos *et al.* 2009). Considering population structure, the number of individuals found in the three ponds indicates a reasonably stable population, as occurs in other *Kinosternon* species (Macip-Ríos *et al.* 2011) but further study is needed to support this conclusion.

In this study, nests experienced a range of environmental temperatures between 15°C to 21°C, which are related to the production of female-biased clutches (Ewert *et al.* 2004). We did not find intersexual differences in body size (CL) and body mass, which is a pattern more similar to southern populations of *K. herrerai* (Aguirre-León and Aquino-Cruz 2004), but contrast with that found in northern populations of this species (Carr and Mast 1988). In most species of the genus *Kinosternon,* males are larger than females (Berry and Shine 1980, Iverson 1999, Macip-Ríos *et al.* 2009, Ceballos and Iverson 2014), most likely because they defend territories against other males (Macip-Ríos *et al.* 2009). However, there is evidence of species with female biased sexual size dimorphism (Iverson 1985, De la Cruz-Merlo *et al.* 2022). The results found here could be explained by little or no competition among males for resources (i.e., space, food, and mates) along with competition among females for mates; or they could be due to the effect of small sample size. A closely related taxon, *K. creaseri* did not show differences in body size between males and females (Macip-Ríos *et al.* 2018).

In this population of *K. herrerai,* the reproductive activity period for both sexes is

Table 1. Carapace length (CL, in mm) and body mass (BM, in g) of females and males of *Kinosternon herrerai* from each sampled pond in Hidalgo, Mexico. Mann-Whitney U test parameters are shown for within sexes and among ponds (males only) comparisons. Kruskal-Wallis test parameters are also shown for comparisons of females among ponds.

Trait / Sex	Santa Rosa Female	Santa Rosa Male	El Transformador Female	El Transformador Male	Totoapita Canutillo Female	Totoapita Canutillo Male	Kruskal-Wallis H	Kruskal-Wallis P	U Mann-Whitney Z	U Mann-Whitney P
	N = 4	N = 7	N = 9	N = 3	N = 4	-				
CL	131.1 ± 3.4 (125.1–138.7)	127.4 ± 3.3 (113.0–140.4)	126.3 ± 3.0 (112.5–140.4)	126 ± 0.5 (125.3–127.0)	122.0 ± 5.9 (111.5–137.1)	-	1.96	0.38	0.00	1
	Z = 0.66, p = 0.51		Z = 0.0, p = 1.0							
BM	474.5 ± 26.8 (411.0–525.0)	411.0 ± 20.4 (345.0–503.0)	423.6 ± 20.2 (340–530)	462.7 ± 7.1 (452–476)	435.8 ± 32.8 (366.0–510.0)	-	2.2	0.33	1.60	0.11
	Z = 1.61, p = 0.11		Z = -1.11, p = 0.27							

long (March-October). During this time females might produce up to three (maybe more) clutches of a small number of eggs (3.4 ± 1.7), similar to the reported clutch size for other populations of the same species (3.7 eggs; Legler and Vogt 2013) and another congeneric species *K. integrum* (4 eggs; Iverson 1999). This reproductive period is longer compared to the other species such as *K. integrum,* (July–October; Macip-Ríos *et al.* 2009), *K. oaxacae* (Vázquez-Gómez *et al.* 2015), and *K. hirtipes* (Wagler, 1830) (De la Cruz-Merlo *et al.* 2022). In general, turtles of the genus *Kinosternon* have small clutch sizes, which are related to body size (Macip-Ríos *et al.* 2017, Heston *et al.* 2022).

Reproductive behavior (i.e., courtship, nesting, and oviposition) occurred in and/or on the shore of the ponds, where movement rate is determined by size (CL and mass) rather than sex, with larger individuals moving longer distances, and more frequently than smaller individuals. Although we did not observe movement almong ponds, given the proximity of the ponds in the study area, we consider them part of the same population. Movement rate within the ponds could be related to searching for mates and food, but thermal ecology also could have a role in movement rate and patterns of turtles (Parlin *et al.* 2017).

Most species of the genus *Kinosternon* require a specific type of water source (e.g., ponds, streams, or lakes) to live and reproduce. *K. herrerai* appears to be more aquatic than some congeners and therefore less likely to move between suitable aquatic habitats. Unfortunately, turtles from this population inhabit degraded ponds, which are potentially polluted with heavy metals and pesticides. Hence, conservation efforts should include rehabilitation of the aquatic habitats and protection of these and other ponds. Given that *K. herrerai* is a Mexican endemic species, it might be subject to trade in the illegal specialty reptile trade. The small numbers of turtles in our study ponds could easily be decimated by illegal trapping. The species has an international and national

conservation status of Near Threatened (NT; van Dijk *et al.* 2007) and is subject to Special Protection (Pr; SEMARNAT 2010). Conservation actions should be taken to preserve all populations, especially those near their altitudinal limit as in the present study.

Acknowledgments

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SHORT COMMUNICATION

Ocular abnormalities in two sympatric salamanders (Caudata: Plethodontidae) in a pine-oak forest of La Malinche National Park, Mexico

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Abnormalities are permanent structural defects caused by errors in the morphogenesis of organisms due to genetic factors (flawed genes), epigenetic factors (e.g., contamination, poor nutrition), and physical trauma (Lannoo 2008, Sánchez-Domene *et al.* 2018, Ferreira *et al.* 2019). Abnormalities have been described in various structures (e.g., the spine, limbs, toes and fingers, head, jaw, skin color, and eyes), and each one arises during different periods of development; for example, in the embryonic period, prior to or during metamorphosis (Sánchez-Domene *et al.* 2018).

In amphibians, most cases of malformations have been primarily documented in anurans (Lannoo 2008, Ferreira *et al.* 2019, Bosch *et al.* 2021, Venerozo-Tlazalo *et al.* 2022); nevertheless, this could be related to the fact that salamanders and caecilians exhibit more cryptic habits that make them less detectable (Davic and Welsh Jr. 2004, Gower *et al.* 2004, Müller *et al.* 2020, Cante-Bazán 2022). In Mexico, the documentation of malformations in salamanders is scarce in comparison to anurans (Cruz-Pérez *et al.* 2009, Soto-Rojas *et al.* 2017, Venerozo-Tlazalo et al. 2022), and the malformations most frequently observed are associated with frog limbs (Aguillón-Gutiérrez and Ramírez-Bautista 2015, Monroy-Vilchis *et al.* 2015, Domínguez-Moreno *et al.* 2018, Carmona-Zamora *et al.* 2020, Reyes-Servín and Díaz-García 2023). Ocular abnormalities have been recorded in salamanders in only two studies (Díaz-García *et al.* 2019, Venerozo-Tlazalo *et al.* 2022). We present the first records of abnormalities in two species of salamanders that inhabit in sympatry in a temperate forest of Mexico.

Aquiloeurycea cephalica (Cope, 1865) and *Pseudoeurycea leprosa* (Cope, 1869) are plethodontid salamanders endemic to Mexico. They are distributed in mountainous regions of the Transmexican Volcanic Belt, Sierra Madre del Sur, and the Gulf of Mexico. In addition, the species share their distribution in the state of México, México City, Veracruz, and Hidalgo, and coexist in sympatry in the La Malinche mountain, located between the states of Puebla and Tlaxcala (Ramírez-Bautista and Arizmendi 2004, Díaz de la Vega-Pérez *et al.* 2019, Frost 2023). They are considered by the International Union for Conservation of Nature in the category of least concern (IUCN 2016, 2020); nevertheless, both species are listed as threatened in accordance with national laws (Norma Oficial Mexicana-059-SEMARNAT-2010; DOF 2019).

During the sampling to determine the population size of *P. leprosa,* on 06 August 2020, we collected an *A. cephalica* hatchling that presented a case of proptosis. In this case, the right eye was notably swollen and protruding from its orbital socket, possibly due to trauma (Figure 1A). On the other hand, on the 07 August 2020, we recorded another hatchling of *P. leprosa* without the presence of the right eye and with the orbital socket reduced and completely covered by tissue, indicating anophthalmia (Figure 1B). We found a total of 153 individuals of *P. leprosa* and just one presented an abnormality; in the case of *A. cephalica,* we did not record the number of individuals, and the encounter with the individual with malformation was fortuitous. We found both organisms actively moving in leaf litter within a pine-oak forest located in La Malinche mountain, Mexico (19°17'34.7" N, 98°02'28.8" W) and released them after examination.

Abnormalities in amphibians have been the subject of growing concern. Some of the causes that give rise them include contamination by heavy metals and pesticides, fungal infections (*Batrachochytrium dendrobatidis* Longcore, Pessier, and D. K. Nichols, 1999 and *Batrachochytrium salamandrivorans* A. Martel,

Figure 1. (**A**) Lateral view of the ocular malformation observed in *Aquiloeurycea cephalica*. (**B**) Dorsolateral view of the case of anophthalmia in *Pseudoeurycea leprosa*. La Malinche National Park, Mexico. The arrow indicates the malformation in the individual.

M. Blooi, F. Bossuyt, F. Pasmans, 2013), and parasitism, such as that caused by flukes of the *Ribeiroia* genus (Lannoo 2008, Aguillón-Gutiérrez and Ramírez-Bautista 2015, Monroy-Vilchis *et al.* 2015, Silva 2022). However, although it has been suggested that ocular abnormalities are linked to teratogenic agents, such as UVB radiation, nickel contamination, and hybridization (Rengel *et al.* 1994, Ouellet 2000), the causes have not been explored as widely as those related to bone structure and limbs (Ouellet 2000). Additionally, there is the difficulty of identifying the potential agents responsible for the abnormalities in the field. In this regard, some authors suggest that this type of abnormalities could arise due to natural mutation rates (Sánchez-Domene *et al.* 2018) or as a result of failed predation attempts (Ferreira *et al.* 2019).

Although we could not determine the causes the observed abnormalities, these observations represent the first report of ocular anomalies in the two sympatric species of the family Plethodontidae inhabiting a temperate forest in Mexico. Furthermore, they constitute the first record of anomalies in salamanders for the state of Puebla, and are significant as they exemplify the repercussions of disturbances impacting the health of ecosystems for Mexican amphibians, particularly plethodontids. They have the potential to guide future investigations in this region aimed at determining the presence of these and other abnormalities, as well as uncovering their underlying causes. This, in turn, will contribute to a better understanding of the physiological and morphological health of salamanders and amphibians in general.

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SHORT COMMUNICATION

New records of phoresy of *Elpidium* (Ostracoda: Limnocytheridae) by anurans in the Brazilian Atlantic Forest

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One of the most common types of commensalism is phoresy (Houck and O'Connor 1991). Phoresy occurs when one organism, the phoront, attaches itself to another organism, the host, to be dispersed to a new habitat (Houck and O'Connor 1991, Bartlow and Agosta 2021). This strategy is commonly used by species with reduced size and restricted dispersal abilities that inhabit ephemeral and isolated habitats (Binns 1982, Bartlow and Agosta 2021) such as those formed in bromeliads.

Bromeliads (Bromeliaceae) are nearly endemic to the Neotropical region (Benzing 1990, Ulloa Ulloa *et al.* 2017) and possess complex foliar structures with overlapping leaves that collect rainwater and form phytotelmata (Zotz and Thomas 1999). Phytotelmata are aquatic micro-ecosystems formed in plant structures, sustaining microenvironments suitable

for the occurrence of specialized aquatic communities (Kaehler *et al.* 2005). Among bromeliad inhabitants, ostracods of the genus *Elpidium* Müller, 1880 are aquatic microcrustaceans that live almost exclusively in this environment (Müller 1880, Pereira *et al.* 2023). Because they do not have structures for terrestrial locomotion, they are not capable of colonizing new bromeliads by themselves (Müller 1880). Instead, they rely on a passive dispersal mode (Kneitel 2018), attaching themselves to larger animals that use bromeliads. Confirmed records as host organisms exist only for amphibians and reptiles (Müller 1880, Binns 1982, Seidel 1989, Lopez *et al.* 1999).

The occurrence of *Elpidium* ostracods in Brazil has been confirmed for several localities in the south, southeast, and northeast regions, all within the Atlantic Forest (Pinto and Purper 1970, Lantyer-Silva *et al.* 2016, Malfatti *et al.* 2022, Pereira *et al.* 2022, 2023). In other localities, ostracods identified only to class have been reported from bromeliads (Mestre *et al.* 2001). Even though these specimens were not identified to genus, they likely correspond to *Elpidium,* because no records of other ostracods in the phytotelms of bromeliads have been reported (Lopez *et al.* 2009). Although the distribution of *Elpidium* in Brazil has been confirmed only in the Atlantic Forest, the genus could potentially occur in bromeliads and other phytotelms in other biomes (Müller 1880, Pinto and Jocqué 2013).

The many anurans that inhabit bromeliads in the Atlantic Forest can be divided into two categories: bromelicolous and bromeligenous (Peixoto 1995). Bromelicolous species do not reproduce in bromeliads, whereas bromeligenous species utilize bromeliads for reproduction (Peixoto 1995). Many species of anurans are known to transport *Elpidium* (Lopez *et al.* 2005, Colombo *et al.* 2008, Sabagh *et al.* 2011, 2014, Lantyer-Silva *et al.* 2016, Araújo *et al.* 2019, 2020, Moroti *et al.* 2019, Guarabyra *et al.* 2021).

This type of phoresy has been recorded in the south (Colombo *et al.* 2008), southeast (Lopez *et*

al. 1999), and northeast (Lantyer-Silva *et al.* 2016) regions of Brazil. Despite this broad geographic area, the records are from few localities with large gaps in-between. It is likely that the interaction also occurs within these gaps and remains unnoticed because of the small size of *Elpidium* and the lack of research on these ostracods, even though the phoresy can be easily visualized in the field. So far, 21 species of anurans have been listed as dispersal hosts in the review of Moroti *et al.* (2019); one additional species was added to the list by Araújo *et al.* (2020).

Herein we report new records of the phoresy of *Elpidium* by anurans, including new species as dispersal hosts and new localities of occurrence. We provide a review of the relationship and an updated version of the list compiled by Moroti *et al.* (2019). Additionally, we map the geographical distribution of this phoresy, and highlight areas that lack records of this relationship.

Our new records were found during careful inspections of bromeliads around anuran vocalization sites; further, we examined whether anurans in these areas had ostracods adhered to them. Most of our observations occurred in Reserva Biológica Estadual Mata Paludosa, municipality of Itati, state of Rio Grande do Sul, Brazil, a protected area at the southern limit of the Atlantic forest. We extensively sampled this reserve from 2015 to 2022 as part of an amphibian monitoring project. We also sampled bromeliads from 2005 to 2022 at other localities throughout the Atlantic forest. Nomenclature for amphibian species follows Frost (2023). Ostracods were identified only to the generic level because diversity of the genus is understudied; most likely several undescribed and endemic species occur throughout the study area (Pereira *et al.* 2023). Characteristics used to identify *Elpidium* included having a larger width than height and a flat ventral surface; in addition, this genus is the only one currently associated with bromeliads (Pereira *et al.* 2022). To create a distribution map, we combined our records with

those from the literature to visualize the spatial pattern of phoretic records in the Neotropical region.

We found 19 new anuran species as dispersal hosts of *Elpidium* sp., including the families Hylidae (15 spp.), Centrolenidae (2), and Bufonidae (2) (Figure 1; Table 1). The new records are from 10 Brazilian localities, seven in the south and three in the southeast regions (Figure 2; Table 1). Ten of the dispersal hosts were recorded in Reserva Biológica Estadual Mata Paludosa: *Boana bischoffi, B. guentheri, Dendropsophus microps, Itapotihyla langsdorffii, Ololygon catharinae, O. rizibilis, Phyllomedusa distincta, S. perereca, S. tymbamirim,* and *Trachycephalus mesophaeus*. With the exception of *S. perereca* and *S. tymbamirim,* which were found in other localities, these interactions were found exclusively at this locality.

Four other anuran species carrying *Elpidium* were recorded at Parque Estadual da Serra do Mar, state of São Paulo, Brazil: *Bokermannohyla astartea, B. circumdata, Scinax hayii,* and *Dendrophryniscus imitator*. In all other localities only one species was found as a dispersal host (Table 1). In Reserva Biológica Estadual Mata Paludosa we also found *Fritziana mitus* carrying ostracods, a species first reported as a dispersal host in the state of São Paulo (Moroti *et al.* 2019). In Reserva Particular do Patrimônio Natural Caruara, state of Rio de Janeiro, Brazil, we found *Nyctimantis brunoi* carrying ostracods, a new locality north of its previous records (Lopez *et al.* 1999, 2005). Most of the records are from adult anurans, although some juveniles of *Dendropsophus microps* were recorded as dispersal hosts.

We report the first record for the family Centrolenidae and for the genera *Bokermannohyla, Itapotihyla,* and *Trachycephalus.* In addition, we report the first records for the states of Santa Catarina, the farthest inland at approximately 180 km from the coast, and Espírito Santo, the locality with the highest altitude (1600 m a.s.l.). We also report the first non-adult amphibian as a dispersal host.

With the addition of our records, Brazil has 40 anuran species known as phoretic hosts, 10 of which are bromeligenous, and 30 bromelicolous. They are from 23 localities, with the majority of records close to coastal regions of the Atlantic Forest (Figure 2). Even with the addition of our records, the distribution map of this phoresy shows large geographical gaps (Figure 2). These gaps likely represent a lack of sampling rather than a non-occurrence of the relationship. In the southeast, several gaps are within "restinga," an ecoregion with abundant bromeliads where several amphibian communities have been studied (Schineider and Teixeira 2001, Oliveira and Rocha 2015, Martins *et al.* 2019). The coast of the Santa Catarina State, likewise, still lacks phoretic records, even though several individuals of *Elpidium* were sampled and described for the region (Pinto and Purper 1970). The largest sampling gap is in northeastern Brazil, with phoretic records in only two localities, despite several anurans sampled from bromeliads throughout the region (Gondim-Silva *et al.* 2016, Dubeux *et al.* 2020).

The greatest diversity of anurans as dispersal hosts in the Atlantic Forest was found in Reserva Biológica Estadual Mata Paludosa, where 11 anuran species carry *Elpidium.* Bromeliads are abundant at this locality, and 14 of 18 treefrog species in this area use these plants. A few other localities have been searched for phoretic *Elpidium*, resulting in finding between five and 10 species of anurans as hosts (Lopez *et al.* 2005, Sabagh and Rocha 2014, Araújo *et al.* 2020). The large number of records from this locality may be related to our intense sampling efforts and to a larger number of anurans that use bromeliads in this particular area. In any case, a detailed comparative study would be necessary to draw further conclusions. The other new localities presented here, despite having fewer current records, are likely to have other dispersal hosts if sampling efforts are increased.

Bromelicolous anurans, despite having a facultative association with bromeliads, form the majority of hosts for *Elpidium* dispersion.
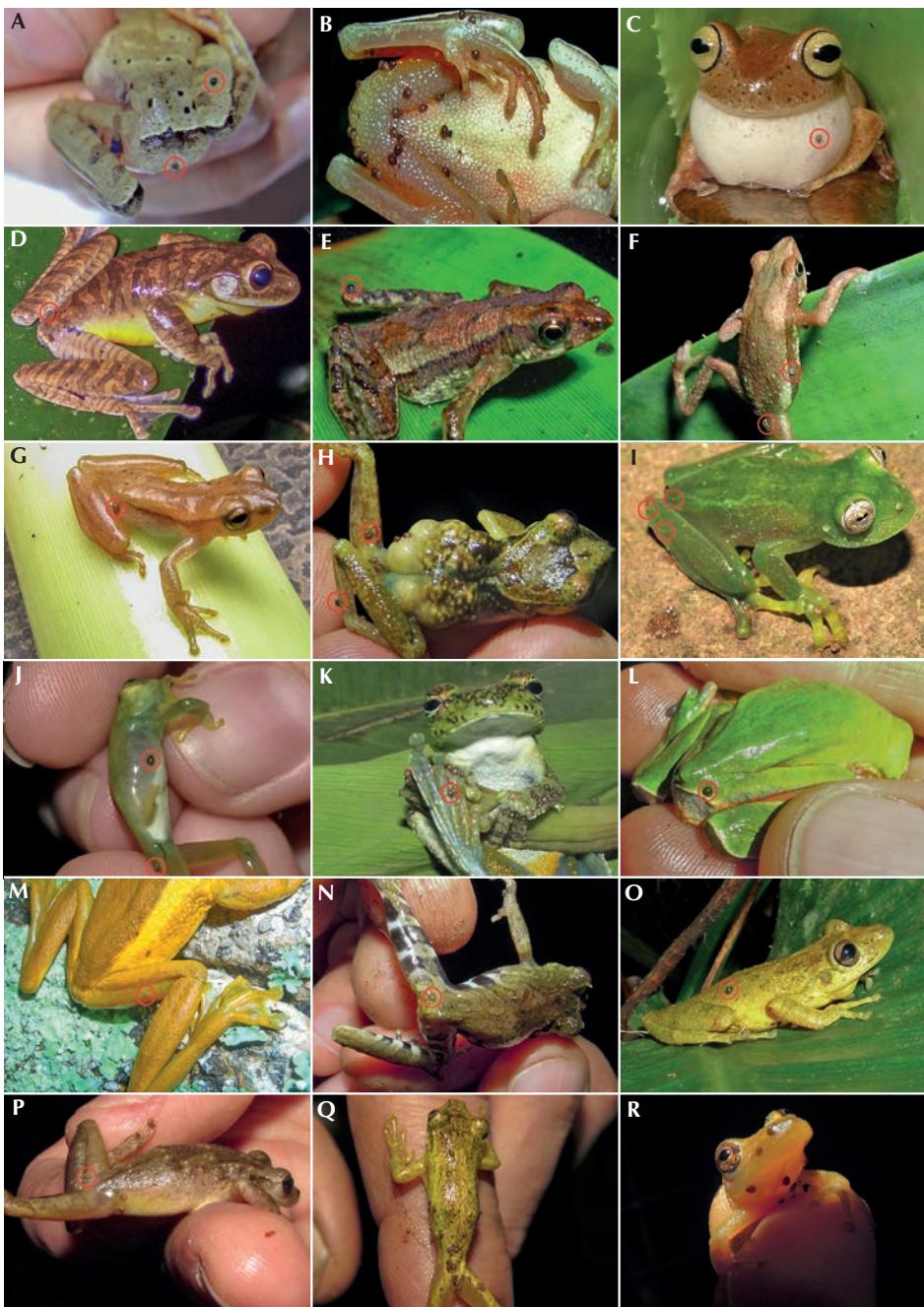
Figure 1. New records of anurans as dispersal hosts of *Elpidium*. (**A**) *Boana bischoffi*, (**B**) *Boana guentheri*, (**C**) *Boker-mannohyla astartea*, (**D**) *Bokermannohyla circumdata*, (**E**) *Dendrophryniscus imitator,* (**F**) *Dendrophryniscus krausae*, (**G**) *Dendropsophus sanborni*, (**H**) *Fritziana mitus*, (**I**) *Vitreorana uranoscopa*, (**J**) *Vitreorana eurygnatha*, (**K**) *Itapotihyla langsdorffii*, (**L**) *Phyllomedusa distincta*, (**M**) *Trachycephalus mesophaeus*, (**N**) *Ololygon catharinae*, (**O**) *Scinax hayii*, (**P**) *Scinax perereca,* (**Q**) *Ololygon rizibilis*, (**R**) *Scinax tymbamirim.*
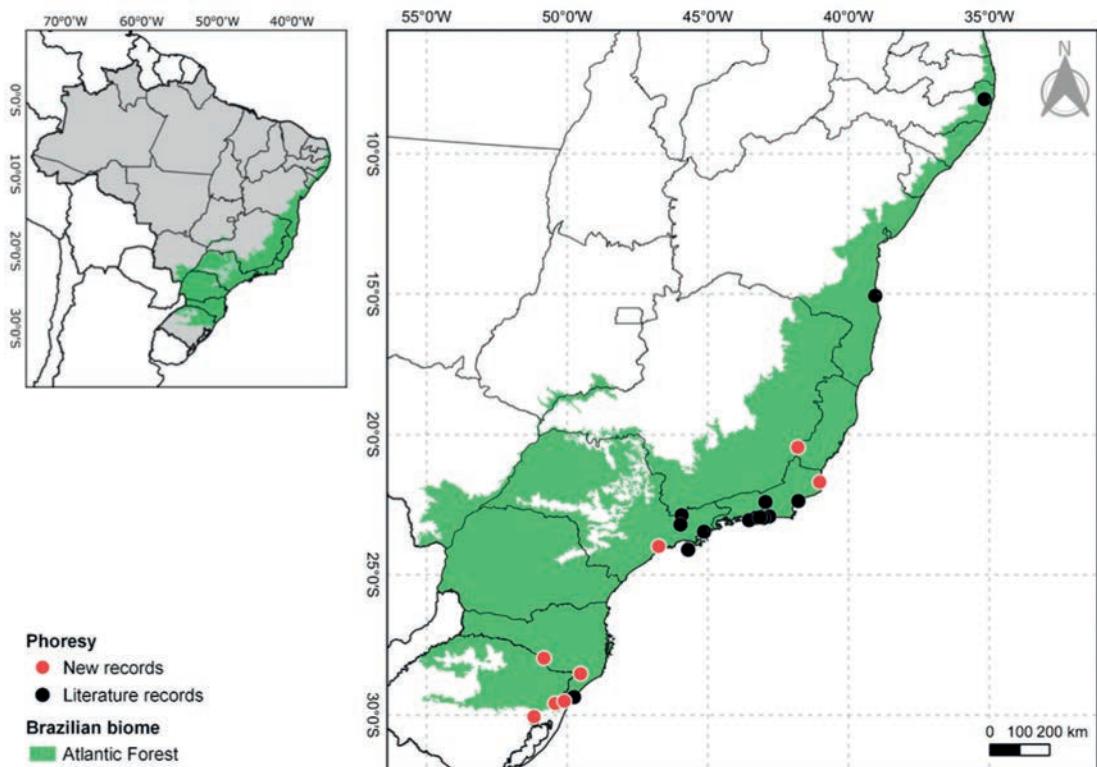
Figure 2. Distribution map of the phoresy between anurans and *Elpidium*. In black, records obtained from the literature, and in red, localities of records added by this work. The area of the Brazilian Atlantic Forest is shown in green. Even though the southernmost record appears not to be within the limits of the Atlantic Forest, the locality contains vegetation remnants related to the Atlantic Forest.

Bromelicolous frogs may move around more than bromeligenous species and provide more opportunities for dispersion of *Elpidium*. Differences in dispersal potential for *Elpidium* also exist among sexes within species, such as in *B. astartea,* in which males remain at particular bromeliads but females move among bromeliads (Malagoli *et al.* 2021). Our observations revealed that resident males had fewer attached ostracods than females. The diversity of bromelicolous species recorded as phoretic hosts may occur because they are relatively more abundant, widespread, and better studied.

The skin of many amphibians has glands that produce efficient chemical defenses (Daly *et al.*

2005, Jeckel *et al.* 2015). The proximity of *Elpidium* with some of these anurans suggests that these microcrustaceans might, on some level, avoid or be resistant to the wide diversity of chemicals secreted by anurans. We highlight *Elpidium* adhesion to *T. mesophaeus* and *P. distincta,* species well-known for their skin toxicity. The genus *Phyllomedusa* has skin components that, in mammals, can induce physiopathological alterations (Conceição *et al.* 2007) and lead to sedation and catalepsy (Toledo and Jared 1995).

The phoresy between anurans and *Elpidium* is, for now, restricted to the Atlantic Forest at several Brazilian localities, frequently those with

Table 1. Compilation of all the phoretic records between anurans and *Elpidium*, including the new records added by this work. Legend to Brazilian states: BA = Bahia, ES = Espírito Santo, PE = Pernambuco, SP = São Paulo, RJ = Rio de Janeiro, RS = Rio Grande do Sul, SC = Santa Catarina.

Taxa	Relation with bromeliads	Locality	State	Coordinates	Elevation (m a.s.l.)	References
Bufonidae						
Dendrophryniscus brevipollicatus Jiménez de la Espada, 1870	Bromeligenous	Projeto Dacnis, Ubatuba	SP	23°27'45" S, 45°07'58" W	37	Moroti *et al.* 2019
Dendrophryniscus imitator (Miranda-Ribeiro, 1920)	Bromeligenous	Núcleo Curucutu, Parque Estadual da Serra do Mar	SP	23°59'1.88" S, 46°44'8.40" W	795	This work
Dendrophryniscus krausae Cruz and Fusinatto, 2008	Bromeligenous	Reserva Biológica da Serra Geral	RS	29°35' S, 50°10' W	600	This work
Centrolenidae						
Vitreorana eurygnatha (Lutz, 1925)	Bromelicolous	Parque Nacional do Caparaó	ES	20°26'53" S, 41°48'02" W	1900	This work
Vitreorana uranoscopa (Müller, 1924)	Bromelicolous	Cascata do Chuvisqueiro, Riozinho	RS	29°34'54.90" S, 50°25'34.20" W	130	This work
Cycloramphidae						
Thoropa miliaris (Spix, 1824)	Bromelicolous	Costão de Itacoatiara, Parque Estadual Serra da Tiririca	RJ	22°58' S, 43°01' W	145	Sabagh and Rocha 2014
		MoNa Morro da Urca e Pão de Açúcar, Rio de Janeiro	RJ	22°57' S, 43°09' W	-	Sabagh and Rocha 2014
Hemiphractidae						
Fritziana goeldii (Boulenger, 1895)	Bromeligenous	Parque Nacional da Serra dos Órgãos	RJ	22°24' S, 42°57' W	963	Lopez *et al.* 2005
		Floresta da Tijuca, Parque Nacional da Tijuca	RJ	23°35'15.89" S, 43°28'58.59" W	-	Guarabyra *et al.* 2021
Fritziana mitus Walker, Wachlevski, Nogueira da Costa, Nogueira-Costa, Garcia, and Haddad, 2018	Bromeligenous	Projeto Dacnis, Ubatuba	SP	23°27'45" S, 45°07'58" W	37	Moroti *et al.* 2019
		Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Hylidae						
Aplastodiscus arildae (Cruz and Peixoto, 1987)	Bromelicolous	Parque Nacional da Serra dos Órgãos	RJ	22°24' S, 42°57' W	963	Lopez *et al.* 2005
Boana albomarginata (Spix, 1824)	Bromelicolous	Grumari, Rio de Janeiro	RJ	23°03' S, 43°32' W	10	Sabagh *et al.* 2011
Boana bischoffi (Boulenger, 1887)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work

Table 1. *Continued.*

Taxa	Relation with bromeliads	Locality	State	Coordinates	Elevation (m a.s.l.)	References
Boana guentheri (Boulenger, 1886)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Boana semilineata (Spix, 1824)	Bromelicolous	Parque Nacional de Jurubatiba, Macaé	RJ	22°22' S, 41°47' W	9	Lopez *et al.* 2005
Bokermannohyla astartea (Bokermann, 1967)	Bromeligenous	Núcleo Curucutu, Parque Estadual da Serra do Mar	SP	23°59'8.29" S, 46°44'37.11" W	800	This work
Bokermannohyla circumdata (Cope, 1871)	Bromelicolous	Núcleo Curucutu, Parque Estadual da Serra do Mar	SP	23°59'53.60" S, 46°44'47.09" W	830	This work
Dendropsophus decipiens (Lutz, 1925)	Bromelicolous	Alto da Buchada, São Lourenço da Mata	PE	08°03' S, 35°10' W	200	Araújo *et al.* 2019
Dendropsophus microps (Peters, 1872)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Dendropsophus minutus (Peters, 1872)	Bromelicolous	Parque Estadual de Itapeva, Torres	RS	29°21'20" S, 49°45'19" W	7	This work
Dendropsophus sanborni (Schmidt, 1944)	Bromelicolous	Florestal Gateados, Campo Belo do Sul	SC	27°58'2.19" S, 50°49'22.66" W	960	This work
Itapotihyla langsdorffii (Duméril and Bibron, 1841)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Nyctimantis arapapa (Pimenta, Napoli, and Haddad, 2009)	Bromeligenous	Reserva Natural Boa União, Ilhéus	BA	15°03'59" S, 39°03'00" W	95	Lantyer-Silva *et al.* 2016
Nyctimantis brunoi (Miranda-Ribeiro, 1920)	Bromelicolous	Barra de Maricá, Rio de Janeiro	RJ	22°55' S, 42°49' W	6	Lopez *et al.* 1999, 2005
		Parque Nacional de Jurubatiba, Macaé	RJ	22°22' S, 41°47' W	9	Lopez *et al.* 2005
		Reserva Particular do Patrimônio Natural Caruara, São João da Barra	RJ	21°41'13.60" S, 41°1'28.29" W	0	This work
Ololygon catharinae (Boulenger. 1888)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Ololygon rizibilis (Bokermann, 1964)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Phyllomedusa distincta Lutz, 1950	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Scinax alcatraz (Lutz, 1973)	Bromeligenous	Ilha dos Alcatrazes, São Sebastião	SP	24°06'18" S, 45°41'50" W	134	Moroti *et al.* 2019
Scinax auratus (Wied-Neuwied, 1821)	Bromelicolous	Alto da Buchada, São Lourenço da Mata	PE	08°03' S, 35°10' W	200	Araújo *et al.* 2019, 2020

Table 1. *Continued.*

Taxa	Relation with bromeliads	Locality	State	Coordinates	Elevation (m a.s.l.)	References
Scinax crospedospilus (Lutz, 1925)	Bromelicolous	Projeto Dacnis, São Francisco Xavier	SP	22°52'27" S, 45°55'50" W	884	Moroti *et al.* 2019
		Universidade do Vale do Paraíba, São José dos Campos	SP	23°12'30" S, 45°58'12" W	591	Moroti *et al.* 2019
Scinax cuspidatus (Lutz, 1925)	Bromelicolous	Costão de Itacoatiara, Parque Estadual Serra da Tiririca	RJ	22°58' S, 43°01' W	145	Sabagh and Rocha 2014
Scinax hayii (Barbour, 1909)	Bromelicolous	Núcleo Curucutu, Parque Estadual da Serra do Mar	SP	23°59'57.48" S, 46°44'14.90" W	750	This work
Scinax littoreus (Peixoto, 1988)	Bromeligenous	Costão de Itacoatiara, Parque Estadual Serra da Tiririca	RJ	22°58' S, 43°01' W	145	Sabagh *et al.* 2011, Sabagh and Rocha 2014
Scinax pachycrus (Miranda-Ribeiro, 1937)	Bromelicolous	Alto da Buchada, São Lourenço da Mata	PE	08°03' S, 35°10' W	200	Araújo *et al.* 2019, 2020
Scinax perereca Pombal, Haddad, and Kasahara, 1995	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
		Treviso	SC	28°31'20" S, 49°31'16" W	276	This work
Scinax perpusillus (Lutz and Lutz, 1939)	Bromeligenous	MoNa Morro da Urca e Pão de Açúcar, Rio de Janeiro	RJ	22°57' S, 43°09' W	-	Sabagh *et al.* 2011, Sabagh and Rocha 2014
Scinax tymbamirim Nunes, Kwet, and Pombal, 2012	Bromelicolous	Jardim Botânico de Porto Alegre	RS	30°03'7.05" S, 51°10'36.29" W	48	This work
		Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
		Treviso	SC	28°31'20" S, 49°31'16" W	276	This work
Scinax x-signatus (Spix, 1824)	Bromelicolous	Alto da Buchada, São Lourenço da Mata	PE	08°03' S, 35°10' W	200	Araújo *et al.* 2019, 2020
Sphaenorhynchus caramaschii Toledo, Garcia, Lingnau, and Haddad, 2007	Bromelicolous	Parque Estadual de Itapeva, Torres	RS	29°21'20" S, 49°45'19" W	33	Colombo *et al.* 2008
Trachycephalus mesophaeus (Hensel, 1867)	Bromelicolous	Reserva Biológica Estadual Mata Paludosa, Itati	RS	29°30' S, 50°06' W	250	This work
Xenohyla truncata (Izecksohn, 1959)	Bromelicolous	Barra de Maricá, Rio de Janeiro	RJ	22°55' S, 42°49' W	6	Lopez *et al.* 1999, 2005
Strabomantidae						
Pristimantis ramagii (Boulenger, 1888)	Bromelicolous	Alto da Buchada, São Lourenço da Mata	PE	08°03' S, 35°10' W	200	Araújo *et al.* 2020

abundant bromeliads. Other phytotelmata, such as in the family Eriocaulaceae, also have records of *Elpidium* occurring in them (Pereira *et al.* 2023). We suggest that expanding the study of geographical regions and dispersal hosts can provide additional information about this complex relationship.

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SHORT COMMUNICATION

Notes on facultative use of bat-modified "leaf tents" by *Agalychnis* Red-eyed treefrogs (Anura: Hylidae)

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Oviposition on terrestrial leaves has evolved in multiple lineages of anuran amphibians that portray a wide array of strategies to breed on land (Duellman and Trueb 1986, Wells 2007). In particular, the subfamily Phyllomedusinae (Hylidae; taxonomy following Faivovich *et al.* 2018) is a Neotropical clade that includes typically green treefrogs with vertical pupils commonly known as leaf frogs, which are generally associated to vegetation overhanging lentic environments, where they perch, call, rest, and lay eggs (Duellman 1970, Faivovich *et al.* 2010). Oviposition sites include tree trunks, logs, stems, vines, roots, but more predominantly, leaves (Duellman 1970).

Phyllomedusines show remarkable adaptations that enhance the survivorship of embryos when using leaves as oviposition sites (Pyburn 1970, 1980, Cruz 1990, Warkentin 2000). For instance, parents of *Phyllomedusa* Wagler, 1830 and *Phasmahyla* Cruz, 1991 use their hind limbs to fold leaves around the egg clutches to construct a purse-like, protective "nest" composed of one or more curled up leaves that protect egg clutches (Faivovich *et al.* 2010). Alternatively, females of *Cruziohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler, 2005 and some *Agalychnis* Cope, 1864 spend some time submerged underwater to fill their bladders before oviposition occurs to subsequently hydrate the egg jelly capsules, which are commonly laid in open leaves (Pyburn 1970).

Duellman (1970) noted that *Agalychnis callidryas* (Cope, 1862) seldom used particular kinds of folded leaves (not constructed by the frogs) to lay eggs but without providing further detail on the plant structures. In the context of these observations, here we report on findings of *Agalychnis* species using bat-modified "leaf tents," which are folded leaf structures constructed by a group of tropical bats of the family Phyllostomidae that modify leaves as shelters (Rodríguez-Herrera *et al.* 2018). Tent production is a relatively poorly documented phenomenon in which bats select leaves of certain plants to build a shelter using their teeth, feet, and thumbs to break or cut some leaf fibers

(Rodríguez-Herrera *et al.* 2018). The leaves fold down resembling tent-like structures with a variety of architectural designs that offer multiple benefits for bats such as relative permanency, microclimate stability, and reduced risks of predation (Rodríguez-Herrera *et al.* 2008, 2016, Villalobos-Chaves *et al.* 2013). We discuss the implications of this frog-bat interaction with respect to *A. callidryas* and *A. spurrelli* Boulenger, 1913 in terms of diurnal retreats and oviposition sites.

We conducted fieldwork between October 2018 and April 2023 as part of a research project assessing the ecological networks of tent-making bats along the northern edge of the Fila Matama in the Cordillera de Talamanca, Costa Rican Central Caribbean (09°55'21" N, 83°10'2" W, 200–800 m a.s.l.). We conducted more than one hundred days of observations in six years of sampling in an area of approximately 50 km², and we registered a total of 130 leaf-tents, which were altogether checked on at least 350 occasions. Field surveys included, but were not exclusive of, the surroundings of artificial breeding ponds used for anuran conservation purposes within the private reserve Veragua Rainforest (see Salazar-Zúñiga *et al.* 2019).

On 08 March 2019 at ca. 08:24 h, we observed an adult *Agalychnis spurrelli* sleeping inside a tent built by *Ectophylla alba* (Allen, 1892) in a *Heliconia trichocarpa* G. S. Daniels and F. G. Stiles leaf at a height of ca. 2 m and located 20 m from the closest pond (Figure 1A–C). On 29 September 2019 at ca. 11:44 h, we observed an adult *Agalychnis callidryas* resting inside of a tent built by *Vampyressa thyone*
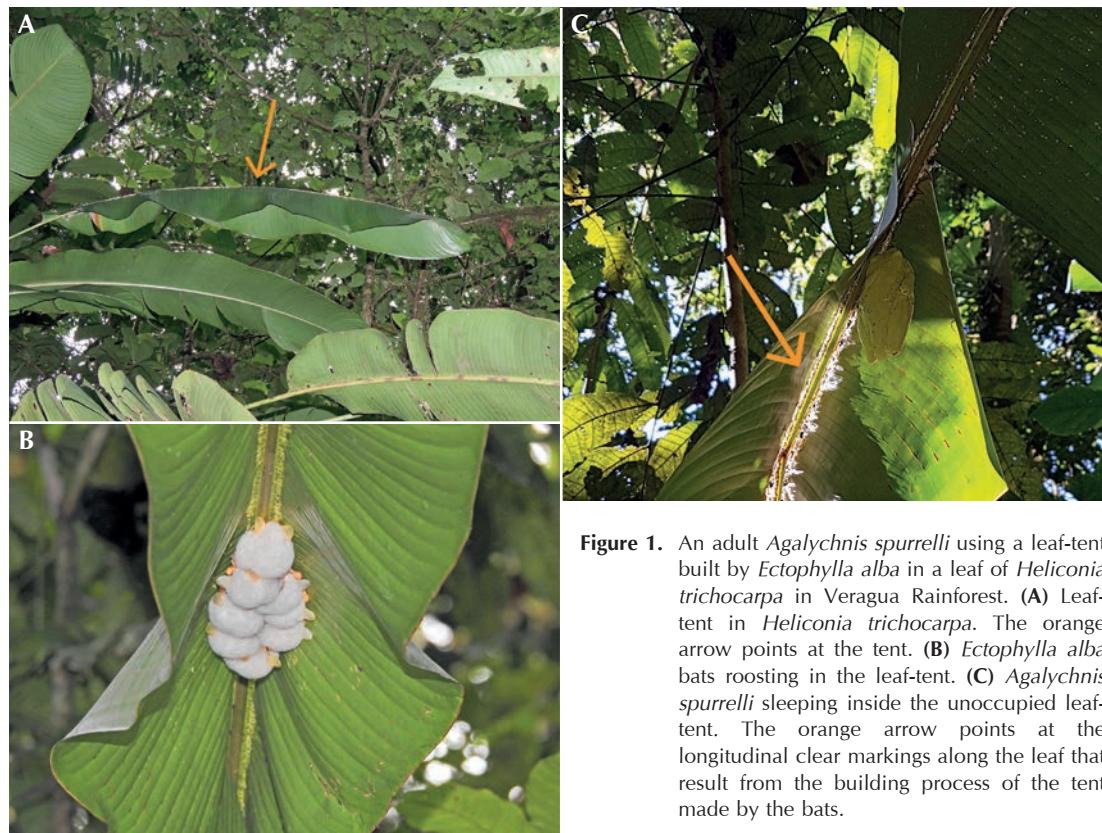
Figure 1. An adult *Agalychnis spurrelli* using a leaf-tent built by *Ectophylla alba* in a leaf of *Heliconia trichocarpa* in Veragua Rainforest. **(A)** Leaf-tent in *Heliconia trichocarpa*. The orange arrow points at the tent. **(B)** *Ectophylla alba* bats roosting in the leaf-tent. **(C)** *Agalychnis spurrelli* sleeping inside the unoccupied leaf-tent. The orange arrow points at the longitudinal clear markings along the leaf that result from the building process of the tent made by the bats.
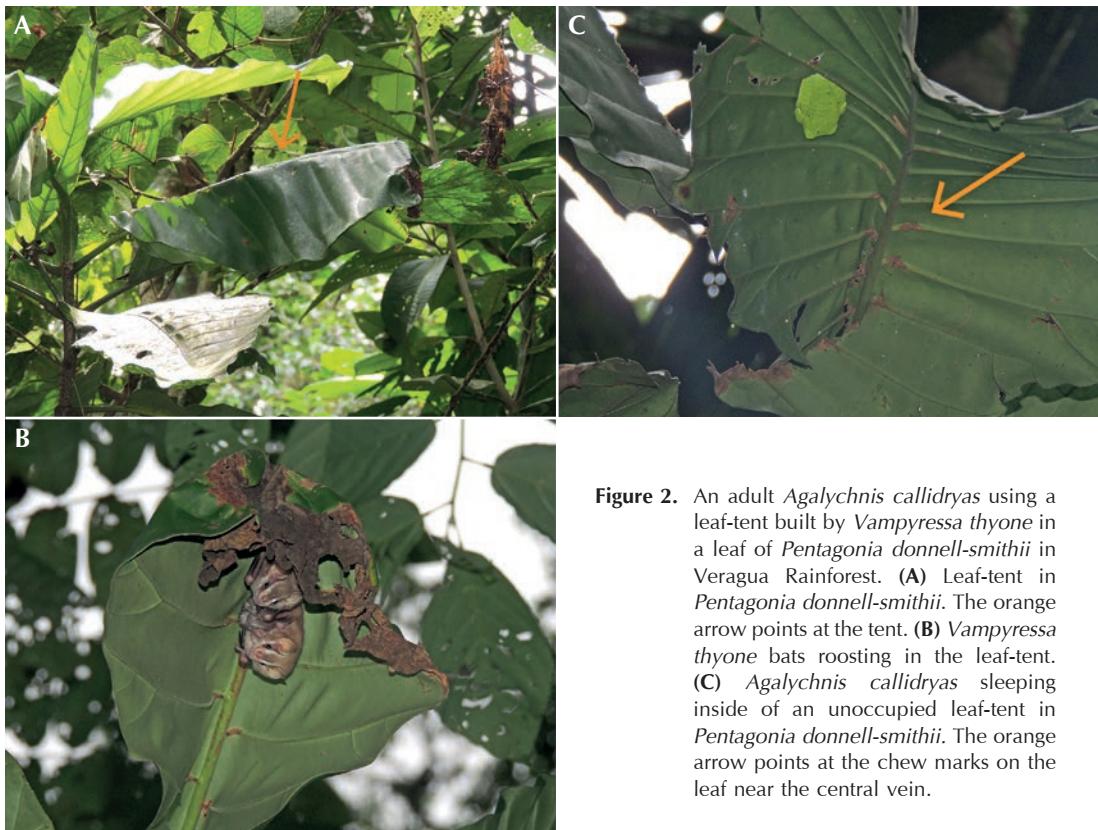
Figure 2. An adult *Agalychnis callidryas* using a leaf-tent built by *Vampyressa thyone* in a leaf of *Pentagonia donnell-smithii* in Veragua Rainforest. **(A)** Leaf-tent in *Pentagonia donnell-smithii*. The orange arrow points at the tent. **(B)** *Vampyressa thyone* bats roosting in the leaf-tent. **(C)** *Agalychnis callidryas* sleeping inside of an unoccupied leaf-tent in *Pentagonia donnell-smithii*. The orange arrow points at the chew marks on the leaf near the central vein.

Thomas, 1909 in a *Pentagonia donnell-smithii* (Standl.) Standl. leaf at a height of ca. 3 m and located ca. 50 m from the closest pond (Figure 2A–C). On 26 February 2020 at ca. 12:04 h, we observed another adult *A. callidryas* resting inside of a second tent built by *E. alba* in a *H. trichocarpa* leaf at a height of ca. 1.5 m and located 30 m from the closest pond. On 15 April 2023 at ca. 09:38 h, we observed an egg clutch of *A. callidryas* in an early developmental stage according to Gosner (1960). The egg clutch was found inside of a third tent built by *E. alba* in a *H. trichocarpa* leaf at a height of ca. 1.5 m, and it was located above a breeding pond (Figure 3A–C). In the eggs of *A. callidryas,* the yolk is pale green in early hatchings and then turns yellowish throughout the development of the eggs, which are evenly distributed within a mass of clear jelly (Duellman 1970; Figure 3B). We monitored the egg clutch on a daily basis as of our initial observation and found it depredated after seven days by an unknown predator (Figure 3C). All tents were unoccupied by bats at the moment of our observations.

Information on interactions between frogs and bats is biased towards predatory events by several bat species (see review in Jacobs and Bastian 2016), with the most notable example being the frog-eating *Trachops cirrhosus* (Spix, 1823), which specializes in echolocating the calls of *Engystomops pustulosus* (Cope, 1864) to prey on calling individuals by eavesdropping on their vocalizations (Tuttle and Ryan 1981). Bats have also been reported as diet items of big-size

Figure 3. An egg clutch of *Agalychnis callidryas* in a leaf-tent built by *Ectophylla alba* in a leaf of a *Heliconia trichocarpa* plant located above an artificial breeding-pond used for anuran conservation purposes. **(A)** Bat-modified leaf-tent. The orange arrow points at the tent. **(B)** Egg clutch inside of an unoccupied leaf-tent. **(C)** Depredated egg clutch.

treefrogs [e.g., *Trachycephalus typhonius* (Linnaeus, 1758) in Strüssmann and Sazima 1991] although these events are seldom documented. Our findings depict a novel interaction between frogs and bats in the form of a potential commensal relationship considering that frogs likely benefit from the shelter built by bats (see below). However, an interesting aspect to consider is the observation that the leaf-tents were unoccupied during our study. This raises the question of whether this can be unequivocally considered a commensal relationship. It is plausible that the bats had abandoned the tents at the point of our observations, challenging the notion of an ongoing commensal association.

Both bat species reported in this study are frugivorous and should not represent a predation risk on *Agalychnis* treefrogs. Nonetheless, more research is needed to better interpret the observed bat-frog interactions in this context.

Several frog species have been reported to co-habit with other animals in shelters (not build by the frogs), including burrows (Roznik and Johnson 2009, Simioni *et al*. 2014), termite mounds (Simioni *et al*. 2014), and cracks and crevices in dry soil (Nunes and Costa 2011). However, our findings are the first to document anurans using leaf-made shelters. Leaf tents are usually observed at the understory of the forest and can be functional for several days, weeks or

months (Rodríguez-Herrera *et al*. 2007). Documentation on other organisms taking advantage of bat leaf-tents is scant and restricted to only a handful taxa (e.g., wasps in Timm and Clauson 1990, monkeys in Boinski and Timm 1985). Our observations show that *Agalychnis* species rarely use leaf-tents as we only documented frogs in 1% of our observations and in 3 % of the sampled leaf-tents. However, if available, we presume that bat-modified folded leaves may offer protection to arboreal frogs from adverse environmental conditions during daytime when they retreat (e.g. direct sunlight; see Blaustein and Kiesecker 2002). Although both *Agalychnis callidryas* and *A. spurrelli* spend a considerable amount of time in the canopy of the forest during the day (Duellman 1970), adults may also seek diurnal retreat sites on the understory vegetation after oviposition occurs in the early morning (pers. obs.).

Using bat-tents for oviposition purposes could also preclude the embryos from being detected by certain predators and it could in turn offer appropriate conditions of humidity and temperature on the inside of the tent for the development of the egg clutches (Duellman and Trueb 1986). Yet, this hypothesis remains to be tested pending on a more comprehensive experimental assessment. As shown by our observation, laying eggs inside of already folded leaves does not hinder predation over recently laid clutches. We speculate that the egg mass could have been eaten by a snake given that the entire clutch was consumed (see Warkentin 1995). Frog-eating snakes such as *Leptodeira septentrionalis* (Kennicott, 1859) are commonly observed at the study area eating egg clutches and adults of *A. callidryas* on vegetation above water bodies (see also Pyburn 1963, Wells 2007).

Bat-modified leaf tents may play a more complex role than being only oviposition and resting sites for treefrogs, especially when accounting for the ecology of arboreal anurans that are being protected through the use of artificial ponds for conservation purposes (Salazar-Zúñiga *et al*. 2019). Besides presumably

providing frogs and their eggs with a stable environment and keeping them hidden from visual predators, we suspect that since tent-roosting bats are frugivorous, the feces and leftovers of fruits and seeds released during the feeding process could alternatively provide anuran larvae with nutrients in pond-like environments (Gautam *et al*. 2020).

Our speculations regarding the deliberate decision-making process by the frogs in selecting leaf-tents as resting or oviposition sites may indeed be subject to interpretation. It is plausible that the frogs, being arboreal in nature, simply encounter these modified leaf structures incidentally rather than actively seeking them out for specific purposes. Unfortunately, we do not have explicit data on the occurrence of frogs on non-tent leaves, which could provide valuable insights into whether their use of leaf-tents is intentional or coincidental. To better understand the nature of this interaction, we propose that future experiments be conducted, possibly involving the creation of artificial leaf-tents, to assess whether *Agalychnis* frogs actively choose these structures or if their presence is a result of random encounters in their arboreal environment. Controlled experiments could provide valuable insights into the decision-making process of the frogs and help clarify the extent to which this behavior is intentional. While our observations provide a unique insight into a novel interaction between frogs and bat-modified leaf tents, we acknowledge the need for caution in interpreting these behaviors as entirely deliberate. We encourage future studies to assess more deeply the interactions between leaf-tent making bats and treefrogs in the Neotropics.

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Dodd, C. K. Jr. 2023. **Frogs of the United States and Canada.** Second Edition. Johns Hopkins University Press, Baltimore, Maryland, USA (www.press.jhu.edu). xxxiv + 954 pp.

ISBN 9781421444918; hardback; US $199.00.

The second edition of the award-winning *Frogs of the United States and Canada* is a remarkable compilation of thousands of research articles, books, agency reports, dissertations, and other sources that have been published on the frogs of these two countries. At almost a thousand pages, it remains the most complete work ever written about frogs from these two countries. This book is an essential starting place to gain a comprehensive understanding of the 106 frog species (and the eight introduced species) that occur north of the United States–Mexican border.

The 23-page introduction to the book is an excellent overview of nearly all aspects of the biology of frogs. It begins with a basic description of frog morphology, including line drawings that show the major characteristics of the basic frog body plan. Detailed drawings of the hind feet describe differences among frogs, toads, and hylids (treefrogs), as well as the keratinous spades used for digging by spadefoots (*Scaphiopus, Spea*). Other illustrations depict frogs in axillary amplexus and the common types of eggs (masses or clumps, surface films, and strings). A section on anuran evolution describes early evolution from the time that vertebrates left the water to more recent discoveries of morphological changes leading to modern frogs. Details of eggs and egg masses, skin and its functions, tadpole morphology, behavior, the use of pheromones, vocalizations, and habitats provide an overview of these amazing animals. A section on anuran conservation reviews amphibian declines and their possible causes, including diseases, habitat loss, especially wetlands, and the consequences of exposure to many pesticides and toxins produced by the ever-expanding human population.
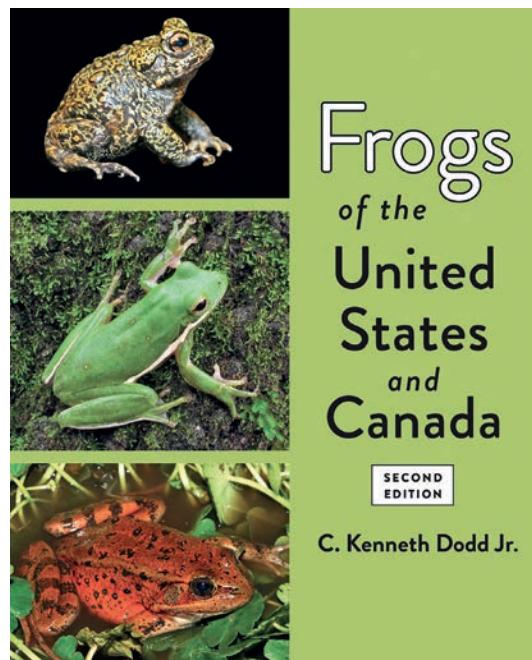
A wonderful section in the introduction covers frogs as recurring subjects in human culture. Color photographs and illustrations include items from Dodd's collection as well as from various museums. The role of frogs in mythology of indigenous peoples is detailed, and several examples are given from Marty Crump's excellent book on this topic (Crump 2015). The number of examples of the use of frogs, toads, and tadpoles in all sorts of objects such as stamps, coins, jewelry, and as mascots for companies (i.e., Toad Hollow Vineyards) depict the various ways that different cultures have interacted with frogs. Three websites are given in this section for those interested in further information; however, I was unable to access two of them. The website given to access information about the relationship of frogs with the peoples of the Tlingit Nation in Alaska did not work, but I found relevant information by going to www.akherpsociety and following links therein, and also by going to Kiks.adi, a site given in the book. On the latter site, while not obvious, clicking on the black-and-white frog

totem photograph on this page leads to a site with colored photographs and the history and locations of other frog totems. The website listing the various uses of and stories about frogs in other indigenous cultures was also no longer extant, but searching for frog legends and mythology of individual tribes revealed numerous websites with extensive information on these topics.

The section "Measurements, Precision, and Generalizations" in the introduction will be useful for anyone conducting research on frogs. Dodd notes that his reading of thousands of research papers led to the realization that researchers have been imprecise in their use of terminology, often leading to confusion about various topics. For example, calling and breeding (oviposition) are different; male frogs of a particular species may call for months, but oviposition may occur only sporadically or infrequently. Breeding season may be assumed to occur continuously if calls are heard in different seasons, when in fact, some species at the same locality have distinct breeding periods in different seasons (Caldwell 1986). Other concepts that can be confused include clutch size/fecundity and time to sexual maturity/larval duration. Dodd cautions researchers to be aware of imprecise terminology. Other misconceptions arise when generalizations made at one location are assumed to be the same as in populations in other regions. A host of variables may affect what populations of the same species do at different latitudes. The classic example is the American bullfrog, *Lithobates catesbeianus*, which has large tadpoles that take 2–3 years to metamorphose in northern latitudes but may only require one year to transform in southern localities.

Other sections in the introduction include one on etymology, which gives the derivation of the generic names of the 23 genera represented in the book. For those seeking additional reading or reference material, an extensive list of sources includes 25 books geared to specific topics related to frogs, such as amphibian declines, conservation, habitat management, and tadpole identification and biology. Various internet sites, state atlases, some of which are online, and sources for frog calls are provided. Abbreviations used throughout the book are listed.

The major part of the book is composed of species accounts of the 106 native species and the eight introduced species in the United States and Canada. Each account typically includes 18 sections, in addition to other sections that are relevant to specific species. For example, a fascinating section on the commercial use of the American Bullfrog, *Lithobates catesbeianus,* explains how huge numbers of bullfrogs have been introduced all over the world for frog farming and the massive monetary incentives involved. Each section in the species accounts summarizes what is currently known about the topic and cites extensive relevant literature, in addition to pointing out areas where nothing is known about a particular topic. The sections in the accounts include the following: **Nomenclature:** Recent molecular analyses have resulted in much debate and confusion about which generic names should be applied to various lineages and how phylogenetic lineages should be named. Generally, the book follows the online website *Amphibian Species of the World* maintained by the American Museum of Natural History. Common names for all species are given in English (Crother 2017), in French for species in Canada (Green 2012), and in Hawaiian for the introduced species in Hawai'i (McKeown 1996). **Etymology:** In addition to the etymology of genera included in the introduction (see above), the etymology of specific names is included in the accounts. **Identification:** Dodd notes that the book is not intended to be a field guide (and at a weight of over 5 pounds, the book would not be suitable for carrying in a backpack). However, in addition to a key to the genera of the frogs in the U.S. and Canada provided in the introduction, the verbal descriptions, photographs, and range maps given in each species account can be relied on to identify adults of most species. As a further aid,

field guides and internet atlases are available for many states in the U.S. Dodd correctly points out that identification of tadpoles is an "art form," so no attempt is made to describe them in detail, i.e., listing number of tooth rows or other characters of the oral disc, for example. The excellent tadpole photographs in each account, coupled with the range and season will aid in tadpole identification. Altig and McDiarmid (2015) have written a comprehensive handbook with keys to eggs and tadpoles of the United States and Canada. **Distribution:** Maps have been updated based on the primary literature and the latest field guides. Each account presents extensive references regarding distribution. **Fossil Record:** Although little information is available in general for most frog species, the known information is summarized. **Systematics and Geographic Variation:** Evolutionary relationships are given, typically including the clade or species group for each species. Differences among populations, hybridization, color morphs, and other relevant topics are covered. **Adult Habitat; Terrestrial and Aquatic Ecology; Breeding Sites or Oviposition Sites:** These sections describe what is known about the macroenvironment, microenvironment, and the characteristics of the breeding sites. Oviposition sites are described for species that deposit eggs on land. **Calling Activity and Mate Selection:** Aspects of calls, timing of arrival at breeding sites by males and females, reproductive success related to call rates and male size, and many other topics are covered in detail. **Reproduction:** Frog species differ widely in the timing and length of the breeding season, weather patterns that initiate breeding, clutch size, whether eggs are deposited as small packets, large masses, strings, etc., size at hatching, and many other aspects of reproduction. Dodd notes that detailed information is lacking on nearly all these variables for most species. Long-term natural history studies at multiple locations on all species are encouraged. **Larval Ecology:** Length of larval period, size at metamorphosis, the influence of food availability on growth, and

tadpole response to predators are addressed. **Diet:** Information about both adult and tadpole diets is given. Diets of most adult frogs are composed of various invertebrates, especially insects, whereas most, but not all, tadpoles graze on algae and detritus. **Predation and Defense:** An extensive literature on these topics shows that primary predators of many frogs are other vertebrates, especially birds and snakes, whereas eggs and tadpoles are typically preyed on by fish and aquatic invertebrates. A variety of escape behaviors are used by frogs, including diving or burrowing in mud, distasteful skin secretions, and defensive calls. Defense mechanisms used by tadpoles against predaceous fish or aquatic invertebrates include crypsis, timing of breeding, for example, during cold weather when fewer aquatic insects are active, noxious secretions, and formation of large schools. **Population Biology:** Research on aspects of population biology varies widely, from estimates of population size, whether individuals are annuals or long-lived, growth rates, timing of sexual maturity, and sex ratios. **Community Ecology:** Competition among species, habitat preferences in overlapping species, competitive advantages of larvae of one species causing decline of another species, and mesocosm experiments with larvae are among the topics discussed. **Diseases, Parasites, and Malformations:** Frogs are susceptible to bacterial, viral, and fungal infections, as well as infections caused by other protozoans and invertebrates. Chytridiomycosis (Bd) is discussed in detail because of its role in frog declines around the world. The history of the disease is mentioned in one account, noting that the fungus was found in the Southern Leopard Frog, *Lithobates sphenocephalus,* in Illinois in 1888. Nematodes, trematodes, and cestodes are other organisms that cause malformations in adult frogs and tadpoles. **Susceptibility to Potential Stressors:** Frogs are exposed to a variety of stressors, including numerous pesticides and other chemicals, fertilizers such as nitrates and nitrites, UV radiation, and pH. Metals such as cadmium,

mercury, lead, and arsenic are also present in the environment where frogs live and breed. In some cases, toxins from introduced plants can be harmful to tadpoles. Some species have been examined extensively, typically because of their large ranges and population size, whereas little or nothing is known about other species. **Status and Conservation:** Many variables affect the status of frogs throughout their ranges, including the above-mentioned stressors, the effects of development, habitat destruction, roads that are hazardous during breeding migrations, and even nonnative plants. In one case, the Northern Leopard Frog, *Lithobates pipens,* is thought to have disappeared from a large portion of its northern range because fisheries departments introduced predaceous fish for sport fishing into farm ponds and other types of fishless habitats used by frogs. Individual states and other agencies list the status of frogs in their areas; researchers and others should check these listings before planning to work with any frog species.

Reading through the accounts reveals how much is known for certain widespread species, and how little is known for other species, which is generally but not always correlated with a limited range size in the latter. For example, 25 pages in the book are devoted to the American Toad, *Anaxyrus americanus,* 27 pages are devoted to the widespread American Bullfrog, and 15 pages and 12 pages, respectively, to the morphologically identical Cope's Gray Treefrog, *Dryophytes chrysoscelis,* and the Gray Treefrog, *Dryophytes versicolor.* In contrast, the three species of *Eleutherodactylus,* which primarily occur in southern parts of Texas, have about two pages devoted to each species. A primary outcome of having so much information summarized in one place is that this great source of knowledge leads one to realize that further research is needed in nearly every area. For those who are interested in particular topics, i.e., predation, feeding, reproduction, conservation, etc., reading through those sections in sequence throughout the book provides an excellent overview of the literature and suggests numerous

ideas for further research. Even though the book is specifically about species in the U.S. and Canada, the numerous topics addressed in this book apply to frogs in any part of the world and thus will be a valuable resource for researchers everywhere.

Each species account is liberally sprinkled with excellent color photographs taken by the author as well as many other herpetologists. Every account has a well-designed line drawing depicting the range. Of course, frog populations may occur unevenly based on how suitable habitat is distributed. Readers may want to consult other websites for details of distribution, for example iNaturalist, which shows point locations for frogs photographed and identified by this community of researchers and naturalists. The photographs for each account in the book include at least one, and sometime 3 or 4, photographs of adult frogs, individual or sometimes aggregations of tadpoles, and typical breeding habitats. In addition, line drawings of the heads of 11 of the 25 species of toads allow easy comparisons of the postorbital and cranial crests and parotoids, characters frequently used for identification in these species. Many accounts have photographs of eggs, egg masses, or egg strings (toads), and some show amplexing pairs or adult color morphs. The unusual behavior of foot-flagging is depicted in the account of the Blanchard's Cricket Frog, *Acris blanchardi.* While the book is beautifully illustrated, if I had to choose an additional illustration or two to include, phylogenetic trees depicting the relationships of the 106 species would be a valuable addition given the recent proliferation of molecular studies.

The literature alone takes up 156 pages (approximately 16%) of the book. Over 8500 citations include nearly all publications about U.S. and Canadian frogs. References extend from 1664 to 2021, when the book went into publication. Most references are from the late 1900s and 2000s, but numerous new references have been added to the literature since the publication of the first edition. Although I did

not count the number of new refences in all 156 pages of this edition, a rough estimate is that at least 12–14% of the references are new since the first edition was published ten years ago. Despite the extensive amount of knowledge this literature represents, the author notes in at least two places his concern about how little we still know about frogs. Because our emphasis has shifted from natural history to genetics, meta-analyses, and experimental studies, all of which generate research funding, we still have much to learn about the basic biology of frogs. In the account on the Northern Leopard Frog, *Lithobates sphenocephalus,* for example, which is one of the most extensive accounts, we are deficient in knowledge of its longevity, sex ratios, population size and class structure, clutch size variation, and other aspects of its biology. I am certain that this situation prevails for most species. Other researchers have lamented the lack of natural history data for both amphibians and reptiles (Greene 1986, Vitt 2013, and see *Amphibian Species of the World,* Curator's blog, 30 November 2018).

I highly recommend reading the Preface to the first edition (reprinted in the second edition) and the Preface in this edition. Both give insights into Dodd's motivation for writing this book and aspects of his early life that led to the study of biology. Many of us would agree with his statement that nothing is more fascinating than the evolution of life. Dodd also mentions being drawn to the silence of nature, with which I would agree, but I would also point out that nothing is more thrilling than the raucous sound of a frog chorus. In addition, I greatly appreciate the author's frank assessment of his values and feelings about the present state of the world. His concern about whether we humans will begin to understand our interconnectedness with the biodiversity, the ecosystems, and the climate of the world before it is too late is a concern we should all share.

Marion Lovene Griffey's beautiful and moving poem "In the Eyes of a Toad," graced the frontal matter of the book. Given the propensity of humans to engage in all manner of creative activities, I am certain many herpetologists and others have been moved to write about, sculpt, or paint frogs and toads because of their beautiful eyes, colorful patterns, and unique forms. Thinking of poetry reminded me of a delightful poem about toads written by the late Charles C. Carpenter (Caldwell and Vitt 2004). For those interested in reading more poetry inspired by frogs and toads, I highly recommend Jill Carpenter's anthology of 86 poems and short prose featuring amphibians (Carpenter 1998; out of print but hopefully will continue to be available on Amazon).

In summary, the wealth of information synthesized for each of the 106 native frog species in the U.S. and Canada is overwhelming. It is safe to say that Dodd has met and surpassed his goals for writing this book. The book will be an essential reference for anyone interested in frogs for many years into the future. The book should be a mandatory addition to school, university, and public libraries. Not only professional herpetologists, but also beginning graduate students in herpetology and many related fields, resource managers, naturalists with an interest in biodiversity and conservation, and those who are just fascinated with frogs and toads will find this book indispensable.

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Author Index - Volume 22 (2023)

Subject Index - Volume 22 (2023)

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General Information. *Phyllomedusa* publishes articles dealing with the entire field of herpetology. The journal also maintains sections for Short Communications and Book Reviews. Manuscripts are considered on the conditions that they: (1) have not been published elsewhere; (2) are not under consideration for publication, in whole or in part, in another journal or book; and (3) are submitted by the authors in the format and style of *Phyllomedusa* and in accordance with the specifications included in the Instructions to Authors. Manuscripts should be submitted as a single Microsoft Word document via e-mail. High-quality color images are accepted. Manuscripts must be written in English with appropriate abstracts in alternate languages. If English is not your primary language, arrange to have your manuscript reviewed for English usage before you submit it. Direct any questions about manuscript submission to the primary editor. Publication in *Phyllomedusa,* including color images, is free of charge.

Scope. Manuscripts must contain significant new findings of fundamental and general herpetological interest. Surveys and taxonomic descriptions are published only if there is sufficient new biological information or taxonomic revision to render the paper of general herpetological interest. Lower priority is accorded confirmatory studies, investigations primarily of localized interest, range extensions, technique papers with narrow application, descriptions of phenomena based on insufficient data, and descriptive work that is not placed in a significant context. Manuscripts should include a clear statement of the purpose of the study or the hypothesis that was tested.

Peer Review. At least two referees, an Associate Editor, and the Editor will review each manuscript that is deemed to fall within the scope of *Phyllomedusa.* Authors will be notified of the status of their manuscript within 90 days. Revised manuscripts accepted for publication will be edited for English usage and syntax prior to final acceptance for publication.

Manuscript Style and Format. Use the active voice when possible; thus, you should write "I/we studied the frog," rather than "The frog was studied by me/us" (passive voice). Use American spelling and punctuation. Double space the entire manuscript, including references, tables, table captions, and legends for illustrations. Use Times New Roman 12-point font, and set up document with margins of at least 2.54 cm (1 in.) on each side. Do not justify the text; it should be left aligned and ragged right. Number manuscript pages consecutively and lines continuously, **followging the arrangement and format outlined below exactly.**

- **Title:** Bold-faced caps and lower-case Roman; sentence capped, left aligned; use colons to separate ranked taxonomic names.
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- **Abstract:** Should not exceed 350 words (including lead title) and one paragraph and only is included in regular articles. Alternate-language abstracts may be included, but these must match the content of the English abstract. See example:

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Title of paper in bold-faced Roman. Content of abstract follows in light-faced Roman; left alignment.

- **Keywords:** Light-faced Roman; separate words with commas; capitalize only proper nouns; include descriptors not contained in the title in alphabetical order.
- **Body of Article:** The text of the article will include the following parts indicated by **primary headings in bold-faced Roman aligned to the left (except for References, which should be centered).**

Introduction
Materials and Methods
Results
Discussion
Acknowledgments
References

Secondary headings within major sections are title-capped, italics aligned left. **Tertiary headings** follow a paragraph indentation; they are sentence capped, and set in italics. Tertiary headers are followed by a point and an em-dash. Follow example:

Material and Methods [Primary header]

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*Selection of site.—*This is a Tertiary, or third-level, heading. Note that it is indented and lacks a hard return. The heading is followed by a point or period and a long (em-dash).

- **Body of Short Communication or Book Review:** These shorter articles do not include the primary headings Introduction, Materials and Methods, Results, and Discussion. "Acknowledgments" is treated as a third-level, or tertiary header.
- **Tables:** Number tables consecutively with Arabic numbers. Refer to tables in text as Table 1, Tables 2 and 3, and Tables 2–5. Exceedingly long tables should be placed in appendices. Table captions should be placed above the table. Horizontal rules may be used in the table header and at the foot of the table. No rules (horizontal or vertical) should appear in the body of a table. Consult Vol. 20 (2) of *Phyllomedusa* for proper format of table captions and contents.
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- **Vector graphics:** *Maps, graphs, and line drawings* should be prepared with an illustration program such as Adobe Illustrator, CorelDRAW, or Deneba Canvas. Graphs and maps generated in other programs (e.g., Sigma Plot, Excel) can be imported into these illustration programs and manipulated (or used as a template to produce a new drawing) to produce an acceptable figure at the size intended for publication. Similarly, drawings executed by hand, should be scanned (300–600 dpi) and imported into an illustration program in which they can be sized and labeled for publication. Follow the instructions for labeling provided above, along with the following guidelines for illustrations at column and page widths.
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Editorial conventions.

- **Taxonomy.** All generic and specific names must appear in italics. At the first mention of a species in any paragraph, provide its complete binomial name; in subsequent references to the same species, the generic name may be abbreviated. The first citation of a species must include the authority and date, but the authority does not have to be cited in the References. Hierarchical taxa are separated with colons (e.g., Anura: Leptodactylidae). New taxonomic names should not appear in the Abstract or Keywords.
- **Dashes.** There are three kinds of dashes. Short dashes (-) are used as hyphens. En-dashes (–) are used to denote ranges (e.g., 5–10, May–September) and the minus sign in mathematics. Em-dashes (—) are used in Tertiary Headings, and frequently as a substitute for parentheses and colons. There should be no space on either side of any of these dashes.
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- **Citations.** Authorities are cited in text as follows. Single: (Caballero 1944); double: (Bursey and Goldberg 2006); three or more (Goldberg *et al.* 2002). Note use of "and" and italics for "et al." Multiple text citations should be listed in chronological order and separated by commas—thus: (Crump 1974, Duellman 1978a–c, 1980, Duellman and Trueb 1986). Two or more publications by the same author should be cited in the following pattern: (Vanzolini 1991, 1992) or Cadle (1984a, b, 1985).

- **References.** All publications cited in the text (except taxonomic authorities) must be included in the References in alphabetical order. "Gray literature" (e.g., technical reports, theses, dissertations that have limited distribution or are difficult to identify and acquire) should be avoided. Follow the formats shown below.

  - ✓ **Normal journal articles:**

  Vanzolini, P. E. 1993. A new species of turtle, genus *Trachemys,* from the state of Maranhão, Brazil (Testudines, Emydidae). *Revista Brasileira de Biologia 55:* 111–125.

  - ✓ **Two authors in a journal series:**

  Zamudio, K. R. and H. W. Greene. 1997. Phylogeography of the bushmaster (*Lachesis muta:* Viperidae): implications for Neotropical biogeography, systematics, and conservation. *Biological Journal of the Linnean Society 62:* 421–442.

  - ✓ **More than two authors in a journal series:**

  Hero, J.-M., W. E. Magnusson, C. F. D. Rocha, and C. P. Catterall. 2001. Antipredator defenses influence the distribution of amphibian prey species in the central Amazon rain forest. *Biotropica 33:* 131–141.

  - ✓ **Chapter in an edited volume:**

  Hedges, S. B. 1999. Distribution patterns of amphibians in the West Indies. Pp. 211–254 *in* W. E. Duellman (ed.), *Patterns of Distribution of Amphibians. A Global Perpective.* Baltimore and London. The Johns Hopkins University Press.

  - ✓ **Unpublished thesis or dissertation:**

  Verdade, V. K. 2001. Revisão das espécies de *Colostethus* Cope, 1866 da Mata Atlântica (Anura, Dendrobatidae). Unpublished M.Sc. Dissertation. Universidade de São Paulo, Brazil.

  - ✓ **Book:**

  McDiarmid R. W. and R. Altig (eds.). 1999. *Tadpoles. The Biology of Anuran Larvae.* Chicago and London. The University of Chicago Press. 633 pp.

  - ✓ **Material from the World Wide Web:**

  Frost, D. R. (ed.). 2010. Amphibian Species of the World: an Online Reference. Version 5.4 (8 April 2010). Electronic Database accessible at http: // research.amnh.org/vz/herpetology/amphibia/American Museum of Natural History, New York, USA. Captured on 22 August 2010.

  - ✓ **Software:**

  Maddison, W. P. and D. R. Madison. 2010. Mesquite. A Modular System for Evolutionary Analysis. Version 2.73. URL: http://mesquiteproject.org

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Editor-in-Chief

Contents

Volume 22   Number 2

July–December 2023