ISSN 1519-1397

PHYLLOMEDUSA

Journal of Herpetology

Volume 20 Number 1 June 2021

PHYLLOMEDUSA
Journal of Herpetology

ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
Printed in Brazil in June 2021

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Cover: A male *Lithobates megapoda* from Lake Chapala, Mexico.
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ISSN 1519-1397 (print)
ISSN 2316-9079 (online)

PHYLLOMEDUSA

Journal of Herpetology

VOLUME 20 - NUMBER 1
JANUARY–JUNE 2021

Phyllomedusa

IS PUBLISHED BY **UNIVERSIDADE DE SÃO PAULO,**
ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ"

PIRACICABA ESALQ FEALQ BIANNUAL

CREDENCIAMENTO E APOIO FINANCEIRO:
PROGRAMA DE APOIO ÀS PUBLICAÇÕES CIENTÍFICAS PERIÓDICAS DA USP
COMITÊ CIENTÍFICO

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
Financial support
(Process 441298/2019-2)

Phyllomedusa	Piracicaba	v.20	n.1	pp. 1–136	Jan–Jun 2021

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Journal of Herpetology

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Phyllomedusa: Journal of Herpetology—Vol. 5, No. 1, 2006—Piracicaba, SP, Brazil: Departamento de Ciências Biológicas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo.

v.; il

Vol. 1 (2002) to Vol. 3 (2004) published by Melopsittacus Publicações Científicas, Belo Horizonte, MG, Brazil.

Vol. 1 (2002) to Vol. 5 (2006) Phyllomedusa: Journal of Neotropical Herpetology

Biannual
Articles and abstracts in English; additional abstracts in Portuguese, Spanish, French, Italian, or German are optional.

ISSN 1519-1397 (print) ISSN 2316-9079 (online)
1. Herpetology CDU - 598

Phyllomedusa 20(1):3–13, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p3-13

Evolutionary dynamics shape two passive defensive mechanisms in Neotropical snake radiations

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Abstract

Evolutionary dynamics shape two passive defensive mechanisms in Neotropical snake radiations. We mapped the distribution of two defensive behaviors (balling and head hiding) of Neotropical snakes to evaluate its distribution in distinct phylogenetic groups. Balling behavior was observed in 58 out of the 167 examined species across seven families. Head hiding was observed in a total of 100 species across nine families. From the high prevalence of balling behavior in basal groups of snakes, such as Anomalepididae, Boidae, Leptotyphlopidae, Tropidophiidae, and Typhlopidae, and the low prevalence among species of recent diversification radiations, such as Elapidae and Viperidae, we suggest that this behavior evolved in basal groups and persisted in some derived taxa. Balling was not observed in association with other defensive strategies, while head hiding can occur in combination with caudal elevation, caudal vibration, and body flattening. Therefore, head hiding, in contrast to balling behavior, presents itself as putatively more flexible, as it should allow for an escalated degree of defensive displays.

Keywords: Balling, defensive behavior, evolution, head hiding, Neotropical snakes, predation.

Resumo

Dinâmicas evolutivas moldam dois mecanismos de defesa passivos em radiações de serpentes neotropicais. Neste estudo, mapeamos a distribuição de dois mecanismos defensivos (embolar e esconder a cabeça) em serpentes neotropicais, visando observar sua distribuição em relação a diferentes grupos filogenéticos. O comportamento de embolar foi observado em 58 das 167 espécies

Received 02 October 2020
Accepted 16 April 2021
Distributed June 2021

analisadas, estando presente em sete famílias. Esconder a cabeça foi observado em 100 espécies distribuídas em nove famílias. O predomínio do comportamento de embolar em grupos basais de serpentes, como Anomalepididae, Boidae, Leptotyphlopidae, Tropidophiidae e Typhlopidae, e sua pouca incidência em espécies pertencentes a famílias mais recentes, como Elapidae e Viperidae, nos levam a sugerir que esse comportamento originou-se em grupos basais, persistindo em grupos derivados. O comportamento de embolar não foi observado em associação com outras estratégias defensivas, enquanto que esconder a cabeça ocorreu em combinação com elevação de cauda, vibração de cauda e achatamento do corpo. Desse modo, esconder a cabeça apresenta-se como putativamente mais flexível do que embolar, uma vez que permitiria a exibição escalonada de outros comportamentos defensivos.

Palavras-chave: comportamento defensivo, esconder a cabeça, embolar, evolução, predação, serpentes neotropicais.

Introduction

Avoiding predation is a fundamental challenge to living organisms (Dawkins and Krebs 1979, Greene 1988, Ruxton *et al.* 2004). Hence, they have evolved a wide range of defensive strategies to reduce predation risk. Understanding and describing different defensive mechanisms may provide insights on evolutionary trends of predator/prey relationships among taxa and defensive character selection pressures (Greene 1983). Squamates have diverse anti-predator mechanisms, including cryptic coloration, mimicry, aposematism, and various behavioral displays in order to avoid predation (Greene 1973, Tozetti *et al.* 2009, Lewis and Lewis 2010, Pough *et al.* 2016). Two of the most iconic defensive behaviors in snakes involve balling and head hiding. Balling consists of rolling the body with the head hidden, forming a sphere (Bustard 1969, Mitchell 1978; Figure 1). In addition to protecting the head, balling has been considered to be especially effective for snakes inside burrows (Mitchell 1978). Head hiding is the ability of concealing the head under the snake's own body, ultimately confounding the predator or avoiding predation (Tozetti *et al.* 2009). These behaviors may provide snakes with important generalized protection against consumption, since snakes are potential prey for predatory mammals, birds, other reptiles (including snakes), large amphibians, and even invertebrates (Greene 1997).

Although defensive snake tactics are apparently well documented (Greene 1988), relationships between defensive tactics and their evolutionary dynamics have not yet been tested under a phylogenetic framework, leaving an important gap as to why and how these tactics have evolved in snakes. Both behaviors (balling and head hiding) also seem to be phylogenetically widespread among snakes, and available information is largely scattered (Bustard 1969). In order to improve our knowledge on snake defensive behaviors, we present a set of original data on balling and head hiding behaviors, with special focus on Brazilian species (several of which are widely distributed in the Neotropical region), while also providing comments on their phylogenetic relationships.

Materials and Methods

Our dataset was obtained during 33 years of observations made in field trips in Brazil from 1985 to 2018. We also compiled some personal information kindly provided by Brazilian herpetologists (see Acknowledgments section). We defined balling as the behavior when the snake forms a ball with its body when approached by the researcher or during handling (Mitchell 1978), and head hiding when the animal conceals its head with other parts of the body when touched or handled (Tozetti *et al.* 2009). We compiled data from 167 snake species of 10 families, representing about 42% of total of

Figure 1. Defensive behavior displayed by distinct snake groups: *Liotyphlops ternetzii* (**A**), *Siagonodon brasiliensis* (**B**), *Tropidophis paucisquamis* (**C**), *Epicrates assisi* (**D**), *Dipsas bucephala* (**E**), *Siphlophis worontzowi* (**F**), *Philodryas olfersii* (**G**), *Erythrolamprus atraventer* (**H**), *Bothrops fonsecai* (**I**), and *Micrurus corallinus* (**J**). Photograph credits: Daniel Loebmann (A, B, E, G), Fernanda Stender (C), Paula H. Valdujo (D), Luís Felipe Toledo (F, J), and Julián Faivovich (H).

Brazilian snake richness (Costa and Bérnils 2018). We included in our analysis 114 species that exhibited balling and/or head hidden behaviors. Taxonomy adopted follows Costa and Bérnils (2018).

For ancestral likelihood reconstruction of head hiding and balling behaviors, we used the *rotl* package (Michonneau *et al.* 2016) to incorporate our list of species (N = 114) into an existing phylogenetic tree file of snake species provided as supplementary material from Figueroa *et al.* (2016) using the *castor* package (Louca and Louca 2017) for the tree subset. The resulting matches for the existing species in the phylogeny included 80 species. We used the *phylotools* package (Revell 2012) for the Ancestral Character Estimation (ACE) and mapping maximum likelihood visualizations using the two behaviors as independent binary datasets (i.e., 1 = species exhibits the behavior; 0 = species did not exhibit the behavior) at 95% confidence. While we used this method for ancestral representation, the original dataset of 114 species was used to quantitatively report the overall distribution of behaviors in our study species.

Results

Ancestral reconstruction of head hiding and balling behavior showed an inverse relationship trend in species (Figure 2, inset ACE graph). Head-hiding behavior appears to be widespread with 88% of species exhibiting the behavior, and ancient in snake lineages according to its presence throughout the root state. Contrastingly, only 51% of species exhibited balling behavior and, as the reconstruction shows, is a relatively recent development throughout the clades that is somewhat unevenly distributed. However, the vast majority of snakes that exhibited head hiding behavior did not exhibit balling behavior with few exceptions in each clade.

Based on the Brazilian snakes' species list (Costa and Bérnils 2018), the most well represented families in our study were Boidae, Dipsadidae, Tropidophiidae, and Viperidae (Table 1). Balling was observed in 58 out of the 167 examined species and distributed in seven families (Table 1, Figure 1). This behavior was observed in all species of Anomalepididae, Leptotyphlopidae, Tropidophiidae, and Typhlopidae. We did not observe balling behavior in Colubridae, Elapidae, and Aniliidae (Tables 1 and 2).

Head hiding was observed in a total of 100 species distributed into nine families (Tables 1 and 2; Figure 1), accounting for 58.9% of the species examined and among 26% of the Brazilian species. This behavior was absent in Leptotyphlopidae, but present in all Elapidae, Tropidophiidae, Typhlopidae, and Aniliidae species examined, in addition to 87.5% of Boidae and 64.6% of Dipsadidae species. This behavior was also recorded in 18.2% of Colubridae, 50% of Anomalepididae, and 47.6% of Viperidae species (Tables 1 and 2).

Discussion

From the observation of high percentage of balling in the basal families Anomalepididae, Boidae, Leptotyphlopidae, Tropidophiidae, and Typhlopidae it appears to be ancestral (Greene 1997). Also, this behavior is much rarer in more recently derived families, such as Elapidae and Viperidae. Instead, Elapidae and Viperidae have evolved defensive mechanisms largely based on the ability to deliver venomous strikes. It is possible that head hiding was positively selected over balling behavior in these families, as this might allow the species to strike more actively. Therefore, we suggest that balling was present in an ancestral and was lost several times across the phylogenetic tree, especially among the derived venomous taxa. Still, further observations under natural conditions are warranted to evaluate if this relatively rare behavior is not present in cryptic or poorly known viperid and elapid taxa.

Alternatively, balling behavior may be related to snake morphology and anatomy. This behavior requires great body contraction and it

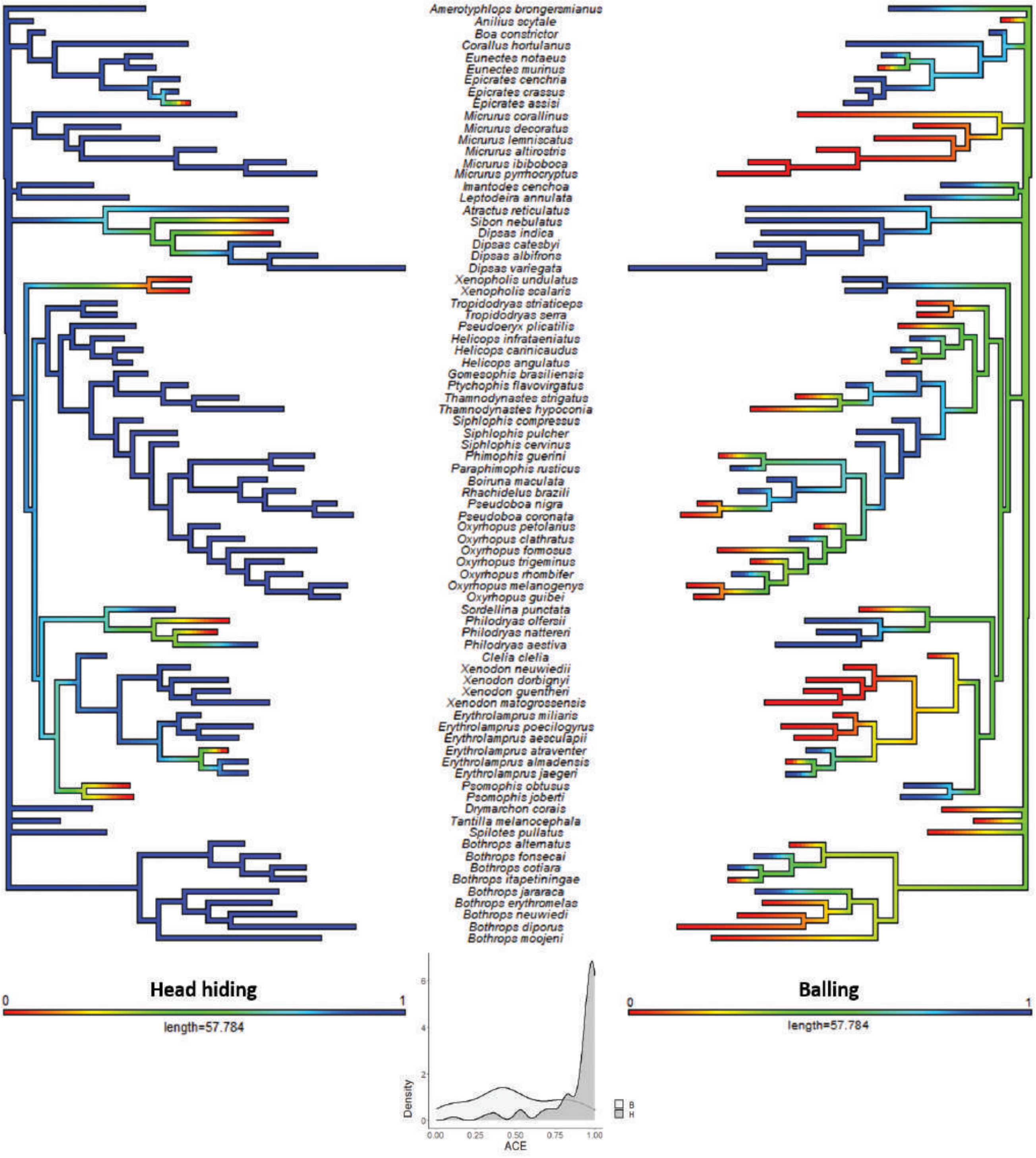

Figure 2. Ancestral state reconstruction of Neotropical snake species (*N* = 80) exhibiting contrasting differences of head hiding and balling defensive behaviors. Density of ancestral character estimates provided for distribution comparisons of the two behaviors.

Table 1. Balling behavior and head hiding behaviors in snake families, the percentage and number of species tested in each family. For the Brazilian list category values are presented as percentage of studied species (total species in Brazil/species tested). For the defensive behaviors, values are presented as the percentage of species that displayed the behavior (species tested/species that displayed the behavior).

Family	Brazilian list	Balling behavior	Head hiding
Aniliidae	100% (1/1)	0% (1/0)	100% (1/1)
Anomalepididae	28.6% (7/2)	100% (2/2)	50% (2/1)
Boidae	66.7% (12/8)	87.5% (8/7)	87.5% (8/7)
Colubridae	64.7% (34/22)	0% (22/0)	18.2% (22/4)
Dipsadidae	41.1% (241/99)	43.4% (99/43)	68.7% (99/68)
Elapidae	25.9% (27/7)	0% (7/0)	100% (7/7)
Leptotyphlopidae	7.1% (14/1)	100% (1/1)	0% (1/0)
Tropidophiidae	100% (1/1)	100% (1/1)	100% (1/1)
Typhlopidae	16.7% (6/1)	100% (1/1)	100% (1/1)
Viperidae	75% (28/21)	14.3% (21/3)	47.6% (21/10)
Total	43.9% (371/163)	30.7% (163/50)	58.9% (163/96)

would easier for stronger species (such as the constrictors) or those with small scales, which allow more flexibility. This is in agreement with our findings. For example, boids such as *Python regius* (Shaw, 1802) and *Calabaria reinhardtii* (Schlegel, 1851) (LFT pers. obs.) in the Neotropical (Table 2) and African regions are both thick-bodied snakes with smooth scales that display balling behavior. However, because both boids and tropidophiids are basal families where most members have thick bodies and smooth scales, the relative importance of these two traits to balling behavior is difficult to separate.

Considering the last review presented by Greene (1988), it is imperative to affirm that defensive behaviors of snakes are poorly documented and widely scattered in the literature, in need of a wide review, although this was not the scope of our study. A comprehensive review about balling and head hiding behaviors would complement our results, providing a broader dataset, which will allow a deeper understanding of the occurrence of defensive strategies among snakes and tracing accurately their evolution. However, negative results regarding the display of a behavior do not guarantee it is absent in the species, or other higher taxonomic levels (such as genus, tribe or family). Some snakes exhibit both behaviors, but the distribution of the occurrence of the displays among snake families is variable.

Mitchell (1978) hypothesized that balling is displayed by snakes inside holes or burrows, as it would be difficult to remove a ball from an opening that was only large enough to allow a snake to enter. However, this does not seem to be the case of Neotropical snakes. All individuals were not in holes when displaying this behavior and many are arboreal species that do not occupy burrows. Most of our suggestions still need experimentation and corroboration, but they may provide a framework for future studies on defensive strategies, contributing to a better understanding of animal evolution.

Table 2. List of species observed in the present study that displayed the balling behavior or head hiding behavior.

Species	**Balling behavior**	**Head hiding**
Aniliidae		
Anilius scytale (Linnaeus, 1758)		X
Anomalepididae		
Liotyphlops beui (Amaral, 1924)	X	X
Liotyphlops ternetzii (Boulenger, 1896)	X	
Boidae		
Boa constrictor Linnaeus, 1758	X	X
Corallus caninus (Linnaeus, 1758)	X	X
Corallus hortulana (Linnaeus, 1758)	X	X
Epicrates assisi Machado, 1945	X	X
Epicrates cenchria (Linnaeus, 1758)	X	X
Epicrates crassus Cope, 1862	X	X
Eunectes murinus (Linnaeus, 1758)		X
Eunectes notaeus Cope, 1862	X	X
Colubridae		
Drymarchon corais (Boie, 1827)		X
Simophis rhinostoma (Schlegel, 1837)		X
Spilotes pullatus (Linnaeus, 1758)		X
Tantilla melanocephala (Linnaeus, 1758)		X
Dipsadidae		
Atractus pantostictus Fernandes and Puorto, 1993	X	
Atractus paraguayensis Werner, 1924		X
Atractus reticulatus (Boulenger, 1885)	X	X
Atractus snethlageae Cunha and Nascimento, 1983		X
Boiruna maculata (Boulenger, 1896)	X	X
Clelia clelia (Daudin, 1803)		X
Clelia plumbea (Wied-Neuwied, 1820)	X	X
Dipsas albifrons (Sauvage, 1884)	X	X
Dipsas alternans (Fischer, 1885)	X	X
Dipsas bucephala (Shaw, 1802)	X	X
Dipsas catesbyi (Sentzen, 1796)	X	X
Dipsas indica Laurenti, 1768	X	
Dipsas mikanii (Schlegel, 1837)	X	X
Dipsas neuwiedi (Ihering, 1911)	X	X

Table 2. *Continued.*

Species	**Balling behavior**	**Head hiding**
Dipsas variegata (Duméril, Bibrón, and Duméril, 1854)	X	X
Dipsas petersi Hoge and Romano-Hoge, 1975	X	X
Dipsas turgida Cope, 1868		X
Dipsas ventrimaculata (Boulenger, 1885)	X	X
Echinanthera cephalostriata Di-Bernardo, 1996		X
Echinanthera cyanopleura (Cope, 1885)		X
Erythrolamprus aesculapii (Linnaeus, 1758)		X
Gomesophis brasiliensis (Gomes, 1918)	X	X
Helicops angulatus (Linnaeus, 1758)		X
Helicops carinicaudus (Wied-Neuwied, 1824)	X	X
Helicops infrataeniatus Jan, 1865	X	X
Helicops leopardinus (Schlegel, 1837)	X	X
Helicops polylepis Günther, 1861		X
Imantodes cenchoa (Linnaeus, 1758)	X	X
Leptodeira annulata (Linnaeus, 1758)	X	X
Erythrolamprus almadensis (Wagler, 1824)		X
Erythrolamprus atraventer (Dixon and Thomas, 1985)	X	
Erythrolamprus frenatus (Werner, 1909)	X	X
Erythrolamprus jaegeri (Günther, 1858)	X	X
Erythrolamprus miliaris (Linnaeus, 1758)		X
Erythrolamprus poecilogyrus (Wied-Neuwied, 1824)		X
Erythrolamprus reginae (Linnaeus, 1758)		X
Mussurana bicolor (Peracca, 1904)		X
Mussurana montana Franco, Marques, and Puorto, 1997		X
Mussurana quimi Franco, Marques, and Puorto, 1997		X
Oxyrhopus clathratus Duméril, Bibrón, and Duméril, 1854	X	X
Oxyrhopus formosus (Wied-Neuwied, 1820)		X
Oxyrhopus guibei Hoge and Romano, 1977		X
Oxyrhopus melanogenys (Tschudi, 1845)		X
Oxyrhopus petolarius (Linnaeus, 1758)		X
Oxyrhopus rhombifer Duméril, Bibrón, and Duméril, 1854	X	X
Oxyrhopus trigeminus Duméril, Bibrón, and Duméril, 1854		X
Paraphimophis rusticus (Cope, 1878)	X	X
Philodryas aestiva Duméril, Bibrón, and Duméril, 1854	X	X
Philodryas nattereri (Steindachner, 1870)	X	

Table 2. *Continued.*

Species	**Balling behavior**	**Head hiding**
Philodryas olfersii (Lichtenstein, 1823)	X	
Phimophis guerini (Duméril, Bibrón, and Duméril, 1854)		X
Pseudoboa coronata Schneider, 1801		X
Pseudoboa haasi (Boettger, 1905)	X	X
Pseudoboa nigra (Duméril, Bibrón, and Duméril, 1854)		X
Pseudoeryx plicatilis (Linnaeus, 1758)		X
Psomophis joberti (Sauvage, 1884)	X	
Psomophis obtusus (Cope, 1863)	X	
Psomophis sp.		X
Ptychophis flavovirgatus Gomes, 1915	X	X
Rhachidelus brazili Boulenger, 1908	X	X
Sibon nebulatus (Linnaeus, 1758)	X	
Siphlophis cervinus (Laurenti, 1768)	X	X
Siphlophis compressus (Daudin, 1803)	X	X
Siphlophis pulcher (Raddi, 1820)	X	X
Siphlophis worontzowi (Prado, 1940)	X	
Sordellina punctata (Peters, 1880)		X
Taeniophallus bilineatus (Fischer, 1885)	X	X
Taeniophallus occipitalis (Jan, 1863)		X
Thamnodynastes hypoconia (Cope, 1860)		X
Thamnodynastes strigatus (Günther, 1858)		X
Tomodon dorsatus Duméril, Bibrón, and Duméril, 1854	X	X
Tropidodryas serra (Schlegel, 1837)		X
Tropidodryas striaticeps (Cope, 1870)		X
Xenodon dorbignyi (Bibrón, 1854)		X
Xenodon guentheri Boulenger, 1894		X
Xenodon matogrossensis (Scrocchi and Cruz, 1993)		X
Xenodon neuwiedii Günther, 1863		X
Xenopholis scalaris (Wucherer, 1861)	X	
Xenopholis undulatus (Jensen, 1900)	X	
Elapidae		
Micrurus altirostris (Cope, 1860)		X
Micrurus corallinus (Merrem, 1820)		X
Micrurus decoratus (Jan, 1858)		X
Micrurus ibiboboca (Merrem, 1820)		X

Table 2. *Continued.*

Species	**Balling behavior**	**Head hiding**
Micrurus lemniscatus (Linnaeus, 1758)		X
Micrurus pyrrhocryptus (Cope, 1862)		X
Micrurus tricolor (Hoge, 1956)		X
Leptotyphlopidae		
Trilepida brasiliensis (Laurent, 1949)	X	
Tropidophiidae		
Tropidophis paucisquamis (Müller, 1901)	X	X
Typhlopidae		
Amerotyphlops brongersmianus (Vanzolini, 1976)	X	X
Viperidae		
Bothrops bilineatus (Wied-Neuwied, 1821)		X
Bothrops diporus Cope, 1862		X
Bothrops erythromelas Amaral, 1923		X
Bothrops itapetiningae (Boulenger, 1907)		X
Bothrops neuwiedi Wagler, 1824		X
Bothrops jararaca (Wied-Neuwied, 1824)	X	X
Bothrops moojeni Hoge, 1966		X
Bothrops alternatus Duméril, Bibrón, and Duméril, 1854		X
Bothrops cotiara (Gomes, 1913)	X	X
Bothrops fonsecai Hoge and Belluomini, 1959	X	X

Acknowledgments

We are deeply indebted to Editor Jaime Bertoluci, Associate Editor J. P. Lawrence, and two anonymous reviewers for their corrections, comments, and suggestions to our manuscript. We thank Fausto E. Barbo, Ivan Sazima, Otávio A. V. Marques, Paula H. Valdujo, and Paulo A. Hartmann for having kindly provided unpublished information, and Fernanda Stender, Paula H. Valdujo and Julián Faivovich for photographs. FAPESP and CNPq provided grants and fellowships to LFT; CAPES, CNPq (310859/2020-4), and FAPERGS provided grants to AMT, DL and OME-N. Daniel Loebmann thanks CNPq for a research grant (310859/2020-4).

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Editor: *J. P. Lawrence*

Phyllomedusa 20(1):15–25, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p15-25

Sexual dimorphism in the South American water snake *Helicops polylepis* (Serpentes: Dipsadidae)

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Abstract

Sexual dimorphism in the South American water snake *Helicops polylepis* (Serpentes: Dipsadidae). Sexual selection, fecundity selection and ecological divergence have been the main explanations proposed for the origin and maintenance of sexual dimorphism. In this study we provide evidence of sexual dimorphism in the South American aquatic snake *Helicops polylepis*, which is mainly determined by body and head sizes. Males have longer tails and more subcaudal scales, and females have larger body and head and more ventral scales. The sexual dimorphism observed in different morphological characters of *H. polylepis* occurs in other species of xenodontine snakes and is interpreted as a consequence of sexual selection pressures. Data on growth rates associated with prey availability and female size-related offspring size are necessary to refine our analyzes and test specific hypotheses about the ecological and evolutionary bases of sexual dimorphism in *H. polylepis*.

Keywords: Fecundity, Hydropsini, sexual selection, Squamata.

Resumo

Dimorfismo sexual na serpente aquática sul-americana *Helicops polylepis* (Serpentes: Dipsadidae). A seleção sexual, a seleção de fecundidade e a divergência ecológica têm sido as principais explicações propostas para a origem e a manutenção do dimorfismo sexual. Neste estudo, fornecemos evidências de dimorfismo sexual na serpente aquática sul-americana *Helicops polylepis*, que é determinado principalmente pelo tamanho do corpo e da cabeça. Os machos apresentam caudas mais longas e maior número de escamas subcaudais, e as fêmeas apresentam maior corpo e cabeça e maior

Received 05 February 2020
Accepted 07 June 2021
Distributed June 2021

número de escamas ventrais. O dimorfismo sexual observado em diferentes caracteres morfológicos de *H. polylepis* ocorre em outras espécies de serpentes xenodontíneas e é interpretado como consequência de pressões de seleção sexual. Dados sobre as taxas de crescimento associadas à disponibilidade de presas e tamanho da prole relacionado ao tamanho da fêmea são necessários para refinar nossas análises e testar hipóteses específicas sobre as bases ecológicas e evolutivas do dimorfismo sexual em *H. polylepis*.

Palavras-chave: fecundidade, Hydropsini, seleção sexual, Squamata.

Introduction

Sex-related differences in body morphology have evolved in many invertebrates and vertebrates, and efforts to understand the evolution of sexual dimorphism have mainly focused on adaptive and physiological hypotheses (Andersson 1994). The main hypotheses proposed to explain the evolution of sexual dimorphism are sexual selection (e.g., male ritual combat), fertility selection and ecological divergence (Shine 1989, 1993, 1994, Shetty and Shine 2002). Most studies reporting sexual dimorphism in snakes were based on external morphological characters such as body size (Crews *et al.* 1984, Shine 1988, Mesquita *et al.* 2010), head size (Shine and Crews 1988, Shine 1991, King *et al.* 1999, Luiselli *et al.* 2002, Giraudo *et al.* 2014), tail size (Shine *et al.* 1999), body color (Shine 1993), and shape, size and texture of scales (Avolio 2006). However, sexual dimorphism may be also related to venom composition (Furtado *et al.* 2006) and morphology of cloacal glands (Thorpe 1989, Kissner *et al.* 1998). Sex-related differences in these characters are attributed to the reproductive success of the species, in males related to sexual competition, in females to energy storage (Bonnet *et al.* 1998); competition and niche partitioning (Shine 1991, Luiselli *et al.* 2002), and to evolutionary pressure in relation to the use of the environment (Shine 1989, Mesquita *et al.* 2010).

The extent to which males exhibit larger adult body size than females indicates that body size correlates with the intensity of male-male competition (intrassexual selection) or female choice for mates (intersexual selection; Shine 1986). Combat between males is common in snakes where males are larger than females, since reproductive success is size-related, generally providing greater mating opportunities for larger-bodied males (Anderson and Vitt 1990, Almeida-Santos and Salomão 2002, Marques *et al.* 2009). However, in those species for which females are larger than males, larger females may have a reproductive advantage, since larger females can produce more offspring per breeding season (e.g., Shine 1993, 1994, Balestrin and Di-Bernardo 2005, Pizzato *et al.* 2007, Mesquita *et al.* 2010).

The genus *Helicops* clusters with *Hydrops* and *Pseudoeryx* to form the monophyletic tribe Hydropsini, endemic to South America (Zaher *et al.* 2009). Sexual dimorphism has been found in the genus [e.g., *Helicops infrataeniatus* Jan, 1865, *H. leopardinus* (Schlegel, 1837)], usually as larger tail size in males and larger body and head sizes in females (Aguiar and Di-Bernardo 2005, Ávila *et al.* 2006). *Helicops polylepis* Günther, 1861 is a viviparous aquatic nocturnal species which feeds mainly on fish and occasionally on amphibians (Teixeira *et al.* 2017). The species inhabits forest streams and riverbanks, generally using roots and riparian vegetation as shelter and foraging sites (Silva Jr. 1993, Santos-Jr. and Ribeiro 2005). No information on sexual dimorphism has been reported for this species. Herein we present data on sexual dimorphism in meristic and morphometric characters measured in *H. polylepis,* to determine sexual dimorphism in body size and shape.

Materials and Methods

Data Collection

We examined 186 specimens of *Helicops polylepis* from 11 herpetological collections (Appendix I). Specimens originated from Brazil and Colombia, including most of the Amazon Basin and the northwestern Brazilian Cerrado, from Acre (09°01'30" S, 70°48'64" W) to the Gurupi River (01°50'06" S, 46°21'11" W) along the west-east axis, and from Chaves (00°12'06” S, 49°48'01" W) to Chapada dos Guimarães (Manso River, 15°01'36" S, 55°47'59" W) along the north-south axis (Figure 1).

We used scale counts to quantify sexual dimorphism based on meristic characters. Subcaudal scale counts followed Peters (1964), and ventral scale counts followed Dowling (1951). In addition, we analyzed seven morphometric variables: SVL, snout–vent length (measured from the anterior margin of the rostral scale to the cloacal opening); TL, tail length (measured from the cloacal opening to the tip of the tail); HL, head length (measured from the anterior margin of the rostral scale to the quadro-mandibular joint); HH, head height (greatest height, on the posterior portion of the head); HW, head width (measured at the widest part of the posterior portion of the head, using distal region of the mandible as the reference point); SL, snout length (measured from the anterior margin of the rostral scale to the anterior part of the ocular orbit); EMD, eye–mouth distance (measured from the lower edge of the eye socket to the mouth opening). We used

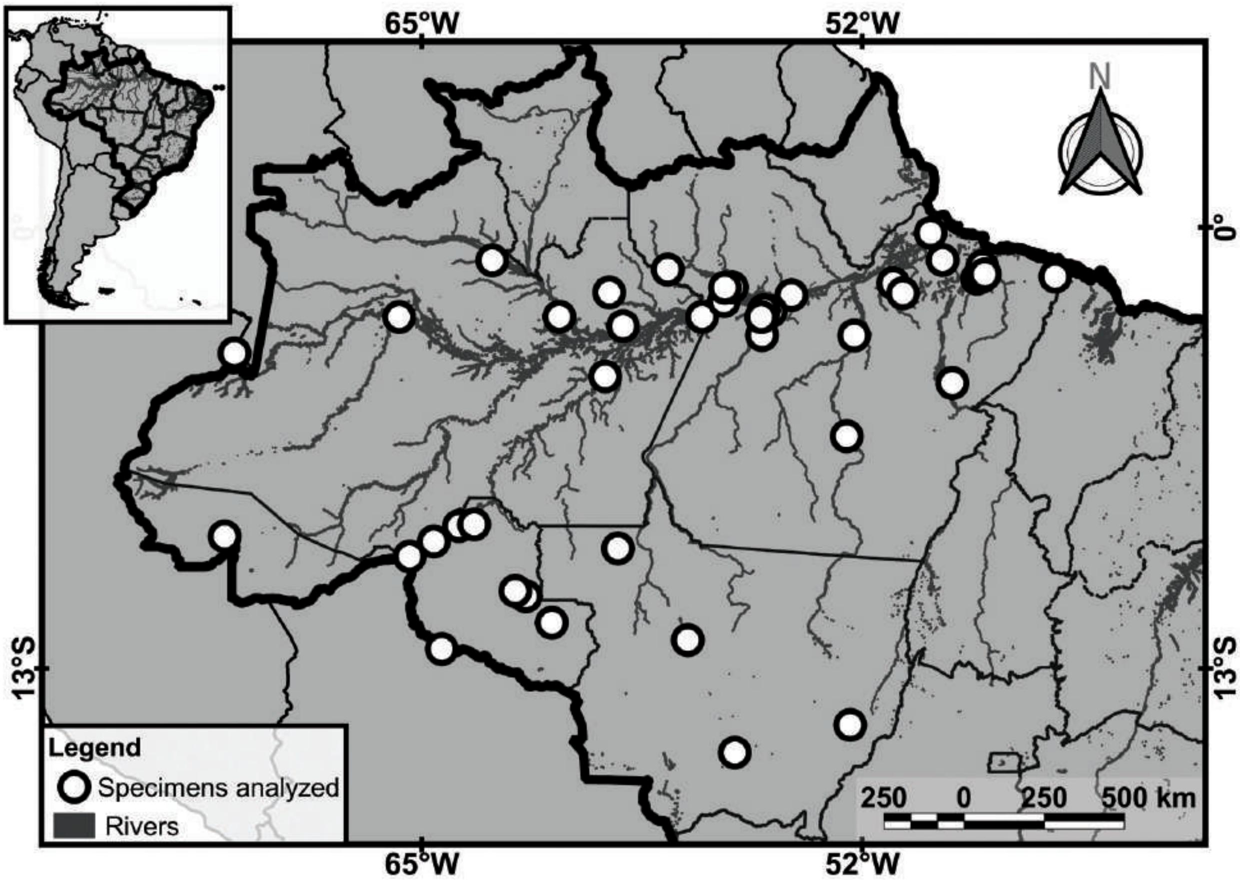

Figure 1. Geographic distribution of the *Helicops polylepis* specimens analyzed for sexual dimorphism (black circles).

digital calipers (accuracy 0.01 mm) to measure all variables, except SVL and TL, which were measured with a ruler to the nearest millimeter.

Sex of the specimens was determined by direct examination of the gonads. Males with convoluted deferent ducts were considered mature (Shine 1988), as were females with follicles ≥ 5 mm (Shine 1988). The smallest mature male measured 328 mm SVL and the smallest mature female 486 mm SVL. Based on this criterion, the number of adult males in our sample was 66, and adult females were 32.

Data Analysis

We tested sexual dimorphism based only on sexually mature specimens, except for the number of ventral and subcaudal scales, which were measured for all specimens. To reduce the effects of size on the morphometric variables, we transformed each variable using the formula $Z = Y_i\ (SVL_0/SVL_i)^b$, where Z represents transformed value of the variable Y (variable affected by SVL), Y_i is the individual value of the variable, SVL_0 is the mean of the SVL, SVL_i is the individual value of the SVL, and b represents the slope of the linear regression between logY and logSVL (Lleonart *et al.* 2000). We verified the effectiveness of canceling the size effect after the transformations of the variables the analysis (Student's t-test) of the slope resulting from the regression of the SVL $\log_{10}$-transformed by the variables transformed by the formula presented above (Villamil *et al.* 2017). All transformed variables were not correlated with SVL ($p > 0.05$) and the slope of linear regression between these variables was statistically indistinguishable from zero.

We used ANOVA or Wilcoxon Mann-Whitney models to test sexual dimorphism in SVL (original data), TL (transformed as above), and number of ventral and subcaudal scales. Assumptions of normality and homoscedasticity were evaluated using Kolmogorov-Smirnov's and Levene's tests, respectively (Zar 2010). We assessed variation in body shape and the contribution of each morphometric variable to the overall sexual dimorphism using Principal Component Analysis (PCA). We excluded SVL and TL from the PCA. TL was removed because of the large number of specimens with mutilated tails ($N = 29$, 30% of the adult specimens). We also removed specimens with missing data from the sample, because it is not possible to calculate pairwise Euclidean distances on incomplete datasets (final sample = 63 males and 30 females). The statistical support for the PCA was performed with an analysis of variance (ANOVA) using the scores of the first principal component of each sex.

We performed the univariate analysis using R 3.3.3 (R Development Core Team 2011) and the multivariate analysis using PAST 3.07 (Hammer *et al.* 2001). We assumed $\alpha = 0.05$ to decide by accepting or rejecting null hypotheses for all tests.

Results

Descriptive statistics of morphometric and meristic variables are shown in Table 1. The first principle component captured 65% of the variation observed in the PCA, and the second component captured 24% (accumulated variance = 89%) (Table 2). The transformed variables with the greatest contributions to component 1 and component 2 were HL and HW (Table 2). The two-dimensional projection of the first two principle components showed levels of overlap between sexes (Figure 2A); however, significant differences in component 1 scores between males and females of *H. polylepis* were observed (ANOVA: $F_{1,\ 91} = 26.47$; $p = 0.0001$; Figure 2B).

In the univariate analysis, significant differences were observed between the sexes for all variables tested (Table 1). Males showed higher values than females for subcaudal scales and TL, and females showed higher values for ventral scales, SVL, EMD, SL, HL, HW, and HH (Figure 3).

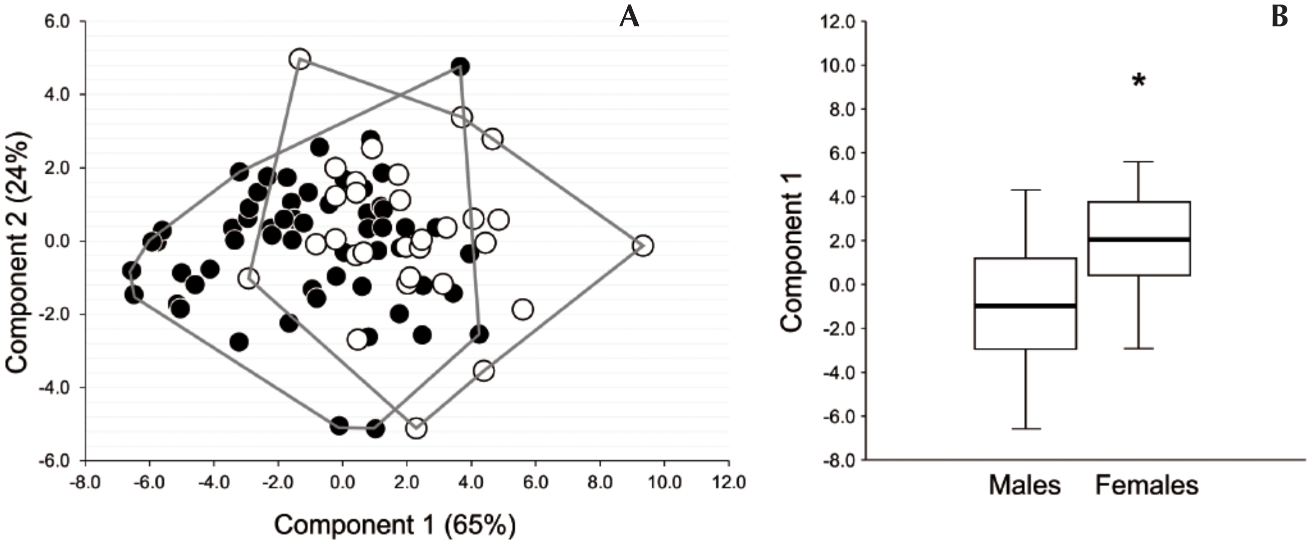

Figure 2. (**A**) Two-dimensional projection on morphometric variables of *Helicops polylepis* represented by two principal components from a PCA (black circles = males; white circles = females). (**B**) Males and females principal component 1 scores. Boxplot shows mean (solid bar), interquartile range (box) and minimum and maximum values (solid terminal bars). Asterisks represent outliers.

Discussion

We found that females of *Helicops polylepis* have larger body size than males, consistent with most viviparous snakes (Fitch 1981, Shine 1994). The same pattern has been found for two congeners: *H. infrataeniatus* (Aguiar and Di-Bernardo 2005) and *H. leopardinus* (Ávila *et al.* 2006). According to Shine (1978), females have larger body sizes than males in nearly 60% of snake species, probably due to selection for increased fertility, enabling larger females to produce and store greater numbers of eggs or embryos (Darwin 1981, Shine 1994, Olsson *et al.* 2002, Pizzatto and Marques 2006). Furthermore, female snakes often delay sexual maturity to maximize fecundity and reproductive success mediated by increased body fat accumulation (Brown and Weatherhead 1999, King 1999). In the analyzed sample, the smallest mature female of *H. polylepis* (SVL 486 mm) was 158 mm longer than the smallest mature male (SVL 328 mm), corroborating the hypothesis of late maturity for females.

Sexual dimorphism is a consequence of several forces, such as fertility selection and sexual selection, which act on the sexes and favor larger size in many organisms (Blanckenhorn 2005). The small slender body size of *H. polylepis* males may indicate the absence of ritual fighting between males. Sexual selection favors maturation earlier and at a smaller size in males than in females, thereby allowing greater likelihood of finding mature females (Pizzatto and Marques 2006). Early maturation may increase reproductive production due to the development of chemosensory senses, high mobility, and reduced costs of mating dispersal and courtship of females (Madsen and Shine 1994). Additionally, sex-related body size in aquatic snakes may affect the ability to follow reproductive chemical clues, because larger females may produce greater amounts of pheromone, which reduces the effects of dilution in aquatic habitats (Shine 1993). This is apparently a phylogenetically conservative character, because no species in which males are larger than females has been found in Hydropsini snakes.

Table 1. Summary of the meristic and morphometric variables analyzed in males and females of *Helicops polylepis*. Sexual dimorphism was tested for each variable by analysis of variance ANOVA = *F* (*) or Wilcoxon-Mann-Whitney test = *W* (**), depending on the normality or homoscedasticity of each variable. The letter Z indicates transformed variables following the method proposed by Lleonart *et al.* (2000). Dashes indicate that the tests were performed on transformed (not raw) data.

Variables	**Males**			**Females**			**Statistics**	
	N	Range	Mean ± SD	*N*	Range	Mean ± SD	*F or W*	*p*
Ventral scales	93	110-139	126.0 ± 3.9	92	118-139	128.0 ± 4.1	2816**	< 0.001
Subcaudal scales	77	71-110	92.0 ± 9.1	71	71-102	78.0 ± 6.5	710**	< 0.001
Snout–vent length (mm)	66	328-675	480.0 ± 75.8	32	486-914	638.0 ± 103.3	73.9*	< 0.001
Eye–mouth distance (mm)	64	1.4-3.3	2.2 ± 0.4	31	1.8-4.3	3.3 ± 0.7	-	-
Z eye–mouth distance	64	1.85-3.21	2.4 ± 0.3	31	1.78-3.43	2.7 ± 0.4	12.1*	< 0.001
Head height (mm)	65	5.5-12.6	9.0 ± 1.6	31	9.4-21.3	12.7 ± 2.4	-	-
Z head height	65	6.73-13.17	10.0 ± 1.3	31	8.13-14.35	10.7 ± 1.5	6.5*	0.012
Head length (mm)	66	15.4-29.6	22.0 ± 2.8	31	22.6-39.1	30.2 ± 4.0	-	-
Z head length	66	17.80-30.46	24.0 ± 2.4	31	22.25-30.60	26.5 ± 1.9	417**	< 0.001
Head width (mm)	65	8.6-21.6	13.8 ± 2.5	31	13.6-27.1	20.9 ± 3.9	-	-
Z head width	65	12.25-20.88	15.5 ± 2.2	31	11.42-22.47	17.4 ± 2.4	549**	< 0.001
Snout length (mm)	66	3.2-6.2	4.7 ± 0.7	32	4.4-8.2	6.2 ± 0.9	-	-
Z snout length	66	4.22-6.35	5.1 ± 0.5	32	3.78-6.30	5.3 ± 0.5	779**	0.036
Tail length (mm)	48	202-380	283.0 ± 47.9	18	228-350	283.5 ± 37.1	-	-
Z tail length	48	237.09-372.79	297.5 ± 33.5	18	226.55-318.19	258.8 ± 23.2	143**	< 0.001

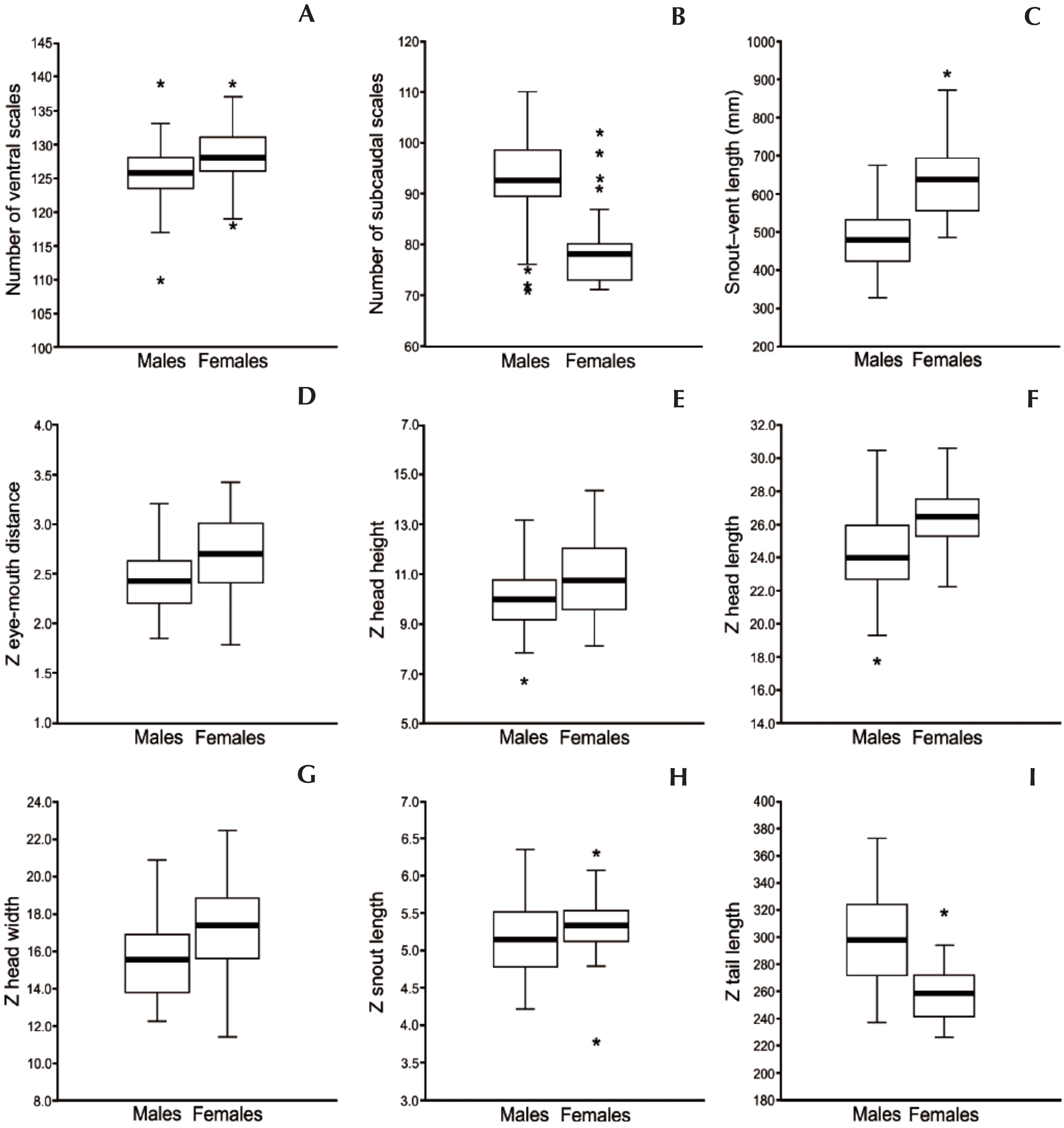

Figure 3. Comparison of meristic and morphometric variables between males and females of *Helicops polylepis.* Boxplot shows mean (solid bar), interquartile range (box) and minimum and maximum values (solid terminal bars). Asterisks represent outliers. (**A**) Number of ventral scales, (**B**) number of subcaudal scales, (**C**) snout-vent length, (**D**) Z eye-mouth distance, (**E**) Z head height, (**F**) head length, (**G**) Z head width, (**H**) Z snout length, (**I**) Z tail length.

Table 2. Contribution of each variable to the principal components 1 and 2 with the main morphometric characters (bold) that contributed to the variation observed in the Principal Component Analysis.

Variables	Component 1	Component 2
Eye-mouth distance	0.6394	0.1204
Head height	0.5066	0.3448
Head length	**0.8756**	**0.4517**
Head width	**0.8240**	**-0.5662**
Snout length	0.4546	0.1947
Eigenvalue	8.99661	3.27995
% variance	65	24

The levels of sexual dimorphism we found for tail length is consistent with literature for many snake species (Shine *et al.* 1999), including representatives of the genus *Helicops* (Aguiar and Di-Bernardo 2005, Santos-Jr. and Ribeiro 2005, Ávila *et al.* 2006). Longer tails in male snakes may be related to the presence of the hemipenis and the associated retractile muscles located at the base of the tail (King *et al.* 1999). Sexual dimorphism in tail length may also be related to reproductive efficiency, since males with larger tails have advantages over smaller tailed males in ritualized mating displays (Pizzatto *et al.* 2007). We do not have data to test this hypothesis for *H. polylepis*, and ritual combat is very unlikely. We also found sexual dimorphism in head size, which may allow predation on prey of different sizes (Camilleri and Shine 1990) and provide more energy for reproductive investment (Shine 1994). This is not necessarily strictly based on food limitation or between-sex competition but may be related to independent adaptation of each sex to increase the efficiency of foraging and habitat use (Shine 1986). Sexual dimorphism in head size influences the shape and size of structures directly associated with feeding, which are usually larger in the sex with the larger head (Camilleri and Shine 1990).

The levels of sexual dimorphism detected in this study suggest sexual selection as a major process causing morphological divergence between sexes. This finding is supported by the fact that sexual dimorphism has been found in *H. polylepis* neonates (Santos-Jr. and Ribeiro 2005), which shows that morphological differences between sexes are more likely to be innate than acquired over life by environmental pressure. However, additional data on growth rates associated with prey availability and female size-related offspring size are necessary to refine our analyzes and test specific hypotheses about the ecological and evolutionary bases of sexual dimorphism in *H. polylepis*.

Acknowledgments

We thank all the curators who allowed us to visit their collections and to access and borrow specimens: Martha Calderón (ICN), Andrés R. Acosta-Galvis (IAvH), Ana Prudente (MPEG), Paulo Passos (MNRJ), Felipe Franco Curcio (UFMT), Giuseppe Puorto (IBSP), Moisés Barbosa (UFAC), Fernanda Werneck (INPA-AM), Hipócrates Chalkidis (FIT) and Emil Hernández (UFPA-Altamira). Adrian Barnett helped with the English. We thank Jaime Bertoluci, Ross MacCulloch, and the anonymous reviewers for comments and suggestions. I.C.M.C thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the master's study grant. CAPES provided a postdoctoral fellowship to RF (PNPD).

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Editor: Ross D. MacCulloch

Appendix I. *Specimens analyzed. We analyzed 186 specimens of* Helicops polylepis *from the following collections (acronyms are those used by the institutions): Centro de Estudos e Pesquisas Biológicas da Universidade Católica de Goiás, Goiânia, Brazil (CEPB), Instituto Butantan, São Paulo, Brazil (IBSP); Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA); Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG); Universidade Federal do Mato Grosso, Cuiabá, Brazil (UFMT-R); Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Laboratório de Zoologia de Altamira da Universidade Federal do Pará, Altamira, Brazil (LZATM), Universidade Federal do Oeste do Pará, Santarém, Brazil (UFOPA-H); Linha de Pesquisa em Herpetologia da Amazônia das Faculdades Integradas do Tapajós, Santarém, Brazil (LPHA); Universidade Nacional da Colômbia, Bogotá, Colombia (ICN), Instituto Alexander Von Humboldt, Villa de Leyva, Boyacá, Colombia (IAvH-R).*

BRAZIL: ACRE: CCBN 74, CCBN 75, CCBN 273, CCBN 352, MPEG 20516. **MANAUS**, *Amazonas*, *Alto Rio Solimões:* MNRJ 641. *Barcelos*: INPA-H 25360. *Borba*: MNRJ 1559. *Lindóia:* MPEG 23514, MPEG 23513. *Parintins:* MNRJ 13333. *Novo Airão:* IBSP 80595, IBSP 80596, IBSP 80597, IBSP 80.598, IBSP 80599, IBSP 80600, IBSP 80601, IBSP 80602, IBSP 80603, IBSP 80604, IBSP 80605, IBSP 80619, IBSP 80620, IBSP 80621, IBSP 80622, IBSP 80623, IBSP 80624, IBSP 80625, IBSP 80626, IBSP 80627, IBSP 80628, IBSP 80629, IBSP 80630, IBSP 80631, IBSP 80632, IBSP 80633, IBSP 80634, MPEG 24374. *Usina Hidrelétrica de Balbina:* CEPB-565. **MATO GROSSO:** UFMT-R 215. *Chapada dos Guimarães:* UFMT-R 506, UFMT-R 507, UFMT-R 1218, UFMT-R 1219. *Colniza, Estação Ecológica do Rio Roosevelt:* UFMT-R 6683; UFMT-R7796. *Príncipe da Beira:* UFMT-R 9285, UFMT-R 9292. *Nova Xavantina*: UFMT-R 7768. **PARÁ**, *Altamira:* LZATM 61, LZATM 121, LZATM 860. *Baia do Souzel - Baixo Rio Xingu*: MPEG 16715. *Barcarena:* MPEG 17680, MPEG 17729, MPEG 20480, MPEG 21856. *Belém:* MPEG 828, MPEG 16258, MPEG 18523, MPEG 18572, MPEG 22295. *Belterra, Comunidade de Porto Novo:* IBSP 86122, LPHA 360, LPHA 641, LPHA 1477, LPHA 1556, LPHA 1557, LPHA 2149, LPHA 2150, LPHA 2172, LPHA 2173, LPHA 2174, LPHA 2251, LPHA 2436, LPHA 2438, LPHA 2439, LPHA 2440, LPHA 2441, LPHA 2444, LPHA 2445, LPHA 2447 LPHA 2448, LPHA 2449, LPHA 2450, LPHA 2451, LPHA 2452, LPHA 2453, LPHA 2454, LPHA 2457, LPHA 2459, LPHA 2437, LPHA 2446, LPHA 2455, LPHA 2456, LPHA 2458, LPHA 2584, LPHA 2587, LPHA 2588, LPHA 2589, LPHA 2591, LPHA 2592, LPHA 2594, LPHA 2595, LPHA 2596, LPHA 2597, LPHA 2598, LPHA 2599, LPHA 2610, LPHA 2611 LPHA 2612 LPHA 2613, LPHA 2614, LPHA 2615, LPHA 2618, UFOPA-H 141, UFOPA-H 142, UFOPA-H 143, UFOPA-H 144,_UFOPA-H 859, UFOPA-H 861, UFOPA-H 872, UFOPA-H 873, UFOPA-H 874, UFOPA-H 875. *Chaves:* MPEG 12427, MPEG 14793, MPEG 21118. *Colônia Nova:* MPEG 1851, MPEG 2943, MPEG 8604, MPEG 8616, MPEG 15082, MPEG 15104, MPEG 15108, MPEG 20952, MPEG 21188. *Faro:* MPEG 18160 MPEG 21179. *Ilha de Mosqueiro*: MPEG 1215, MPEG 16440. *Ilha de Marajó*: MPEG 8871. *Jacundá:* MPEG 18353. *Juruti:* MPEG 21189, MPEG 21190, MPEG 22671, MPEG 23250, MPEG 23284, MPEG 24210. *Melgaço:* MPEG 15078, MPEG 18472, MPEG 18535, MPEG 21855, MPEG 21857, MPEG 22140, MPEG 23249. *Monte Alegre:* MPEG 21511, UFOPA-H 387. *Oriximiná:* MNRJ 7789, MNRJ 17940. *Oriximiná, Lago de Sapuacá*: UFMT-R 9732, UFMT-R 10241 UFMT-R 10246. *Portel*: MPEG 15105, MPEG 23248. *Santarém:* UFOPA-H 387, UFOPA-H 1036, LPHA 2586, LPHA 2590. **RONDÔNIA**, *Porto Velho*: INPA-H 10330, INPA-H 12427, INPA-H 14793, MPEG 26058. *Distrito de Abunã:* MPEG 16754 MPEG 16337, MPEG 21125. *Ji-Paraná* MPEG 18359. *Ouro Preto do Oeste:* MNRJ 9034. *Pimenta Bueno,* UFMT-R 8194. *Usina Hidrelétrica Jirau:* MPEG 22672. *Usina Hidrelétrica de Samuel:* CEPB-981.

COLOMBIA: *Letícia:* ICN-R 8524, ICN-R 8579, IAvH-R 2267, IAvH-R 2296, IAvH-R 2297, IAvH-R 3079.

Phyllomedusa 20(1):27–35, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p27-35

Phylogenetic position of the glassfrog *“Cochranella” megista* (Anura: Centrolenidae) and first records for Ecuador

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Abstract

Phylogenetic position of *“Cochranella” megista* (Anura: Centrolenidae) and first records for Ecuador. *“Cochranella” megista* is an Endangered and rarely encountered species of glass frog that, until now, had been only registered in the Colombian Andes. Here we report this species for the first time in Ecuador, expanding its known distribution ca. 530 km south of its original range. Additionally, we include *C. megista* in a molecular phylogeny for the first time and unambiguously place the species in the genus *Nymphargus,* resulting in a new combination. Habitat in both countries is fragmented and is threatened by mining concessions and agriculture.

Keywords: Amphibians, conservation, Greater Andean Glassfrog, *Nymphargus,* Río Manduriacu Reserve, threatened species.

Resumo

Posição filogenética de *“Cochranella” megista* (Anura: Centrolenidae) e primeiros registos para o Equador. *“Cochranella”* megista é uma perereca-de-vidro ameaçada e raramente encontrada, que até o momento só havia sido registrada nos Andes colombianos. Relatamos aqui a presença dessa espécie pela primeira vez no Equador, expandindo sua distribuição conhecida para cerca de 530 km ao sul de sua área de distribuição original. Adicionalmente, incluímos *C. megista* em uma filogenia molecular pela primeira vez e alocamos inequivocamente a espécie no gênero *Nymphargus*, resultando em uma nova combinação. O habitat em ambos os países é fragmentado e está ameaçado pelas concessões mineiras e pela agricultura.

Palavras-chave: anfíbios, conservação, espécie ameaçada, *Nymphargus*, perereca-de-vidro-andina-grande, Reserva do Rio Manduriacu.

Received 09 February 2021
Accepted 24 May 2021
Distributed June 2021

Introduction

The highest diversity of glassfrogs (Centrolenidae) is concentrated in the northern Andes, with 83 species (Guayasamin *et al.* 2020). Still, new records (e.g., Culebras *et al.* 2020) and descriptions of new species (Guayasamin *et al.* 2019a, b, 2020) increase our knowledge about their diversity and biogeography nearly every year. Under this context, prolonged fieldwork programs often result in the discovery of species that are rare, have low detectability, or inhabit microhabitats that are difficult to access by herpetologists. Such efforts are particularly important in Andean forests, where glassfrog diversity is high and many species are poorly known, and generally restricted by more narrow breadths of suitable habitat than lowland species (Guayasamin *et al.* 2020). One such species is *"Cochranella" megista*, a relatively large glassfrog currently known only from the Pacific slopes of the Colombian Andes, from the Departments of Antioquia, Chocó, Risaralda, and Valle del Cauca at elevations of 1,400–2,100 m a.s.l. (Rivero 1985, Acosta-Galvis 2000, Rada and Guayasamin 2008, IUCN SSC Amphibian Specialist Group 2017). Little is known about this taxon with only 12 observations reported since the type specimen and Neotype (MHUA 5851; Rada and Guayasamin 2008) were collected in 1980 (Rivero 1985). A photo record of *C. megista* on iNaturalist (2012) corresponds to a field observation made during the collection effort that yielded the ICN specimens in the 1990s (M. Rada pers. comm.), from which Bernal and Lynch (2008) cite 11 museum specimens (ICN 17242-44, 27763-8, 27718, 28796). Only one additional observation was recorded after the ICN specimens were collected, which was a single observation from the type locality of Parque Nacional Orquideas, Colombia (IUCN SSC Specialist Group 2017). Moreover, in the most recent taxonomic review of glassfrog systematics (Guayasamin *et al.* 2009), *"Cochranella" megista* was considered of uncertain generic placement within Centrolenidae because its unusual combination of morphological traits (i.e., presence of webbing between Fingers III and IV, absence of humeral spines) and the lack, at that moment, of complementary molecular data.

Here we report the first records of *C. megista* from Ecuador, representing a substantial range extension and only the second set of observations in over 20 years. Additionally, we include, for the first time, DNA sequences of *C. megista* into a phylogenetic analysis, thereby improving our understanding of the evolutionary relationships of the species. This new record increases the number of glassfrogs in Ecuador to 61 species (updated from Guayasamin *et al.* 2020), and is the ninth glassfrog species known to occur at the relatively small Río Manduriacu Reserve (Maynard *et al.* 2020).

Materials and Methods

Study Sites

Fieldwork was primarily carried out at the Río Manduriacu Reserve (RMR), a protected area managed by Fundación EcoMinga (https://ecomingafoundation.wordpress.com/about/), located on the Pacific Andean slopes in western Imbabura, Ecuador (see Lynch *et al.* 2014, Guayasamin *et al.* 2019b, Maynard *et al.* 2020; Figure 1). Opportunistic surveys were also carried out at Séptimo Paraíso (0.0293 S, 78.7652 W; 1,550 m a.s.l.), a locality 40 km from RMR, near the town of Mindo, in Pichincha Province. Habitat at RMR and Séptimo Paraíso consists of primary and mature secondary lower montane and cloud forest habitat. The habitat surveyed for *C. megista* consists of primary cloud forest and is located along a mountain crest at elevations 1,800–2,000 m a.s.l.

Sampling time frames at RMR are outlined in Guayasamin *et al.* (2019b) and Maynard *et al.* (2020); however, sampling concentrated within cloud forest habitat occurred from 01 to 06 March 2019 (RJM, SK, SJT, JC, José Maria Loaiza, Rolando Peña, Fabricio Narvaez, and

Figure 1. Geographic distribution of *Nymphargus megistus* in Colombia and Ecuador: **A**, type locality (red dot), published in the original description by Rivero (1985), Parque Nacional Natural "Las Orquídeas", Antioquia Department; **B**, on border of the Chocó and Valle del Cauca Departments; **C**, Municipality of Pueblo Rico, Risaralda Department; **D**, Municipality of Carmen de Atrato, Chocó Department; **E**, Municipality of Sipí, Chocó Department; **F**, new record, Río Manduriacu Reserve, Imbabura Province, (yellow dot); **G**, new record, near Mindo, Pichincha Province, (yellow dot). Map constructed using Google Earth Pro (7.3.3.7699).

two assistants) and 02 to 09 December 2019 (RJM, SJT, JC, José Maria Loaiza, Rolando Peña, and one assistant). Visual encounter surveys in RMR were conducted along transects of various lengths within primary cloud forest, and along five narrow streams (i.e., 1–3 m wide) between 19:00 and 02:00 h. General area searches were conducted when vegetation was too dense for transects of the stream. Data collection included the following: relative humidity, ambient temperature, date, time of observation, geographic coordinates, sex, age class, behavior (if any), snout–vent length, perch height, and perch diameter (when applicable). Climate data were collected using a Kestrel 3500 Weather Meter, geographic coordinates with a Garmin GPSmap 62s handheld unit (WGS84 datum), and SVL with dial calipers.

Collected specimens were euthanized using benzocaine and were fixed and preserved in 70% EtOH. Muscle and liver samples were preserved in 96% EtOH. Specimens were deposited at the Museo de Zoología of the Universidad San Francisco de Quito (ZSFQ) under permits No. 018-2017-IC-FAU-DNB/MAE and No. MAE-DNB-CM-2018-0105 by the Ministerio del Ambiente del Ecuador. The identification of preserved specimens as "*Cochranella*" *megista* was based on the following diagnostic traits: large body size (SVL in adults > 30 mm), green dorsum with black spots, lack of humeral spines, and moderate webbing between Fingers

III and IV (Rada and Guayasamin 2008); no other glassfrog from Pacific slopes of the Andes of Ecuador and Colombia has the aforementioned combination of traits. The study was carried out in accordance with the guidelines for use of live amphibians and reptiles in field and lab research (Beaupre *et al.* 2004), compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR).

Terminology

For taxonomic nomenclature in glassfrogs, we follow the proposal by Guayasamin *et al.* (2009); note that quotation marks around a genus name indicate that the placement of a species in that genus is uncertain. For general terminology and descriptions of morphological characters we follow the proposals by Lynch and Duellman (1973), Cisneros-Heredia and McDiarmid (2007), and Guayasamin *et al.* (2009, 2020). Webbing formulae follow the method of Savage and Heyer (1967), as modified by Guayasamin *et al.* (2006).

Molecular Sequences and Phylogenetic Analysis

Genetic sequences for a fragment of the mitochondrial gene 16S of *"Cochranella" megista* were generated at the Laboratorio de Biología Evolutiva, Universidad San Francisco de Quito, following Guayasamin *et al.* (2008). The phylogeny was inferred with the Maximum likelihood (ML) criterion using the taxon and gene sampling described in Guayasamin *et al.* (2019a), which includes all 12 genera recognized within the centrolenid family (Guayasamin *et al.* 2009). ML trees were estimated using GARLI 0.951 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006). GARLI uses a genetic algorithm that finds the tree topology, branch lengths, and model parameters that maximize lnL simultaneously (Zwickl 2006). Default values were used for other GARLI settings, as per recommendations of the developer (Zwickl 2006). Bootstrap support was assessed via 1,000 pseudoreplicates under the same settings used in tree search. Genetic distances (uncorrected *p*) between the new species and its closest relatives were calculated using PAUP v.4.0a (Swofford 2002).

Results

***Nymphargus megistus* (Rivero, 1985)**

Figures 1, 2
Centrolenella megista Rivero, 1985
Cochranella megista Ruiz-Carranza and Lynch, 1991
"Cochranella" megista Guayasamin *et al.*, 2009
Nymphargus megistus, **new combination**

Common name.—We propose the common name of "Greater Andean glassfrog" for *Nymphargus megistus,* following the criteria defined by Coloma and Guayasamin (2020). The Greek word *megistos* means "largest" or "greatest". In Spanish the common name is "rana de cristal andina grande".

Identification.—Nymphargus megistus can be distinguished from all other species of *Nymphargus* by exhibiting the following combination of features: large size (SVL in adult males = 30.8–37.4 mm SVL; in adult females = 36.9–40.0 mm), green dorsum with black spots, moderate webbing between Fingers III and IV of the hand, absence of humeral spines in adult males, and lacking iridophores on the gastrointestinal peritoneum (Rada and Guayasamin 2008). *Nymphargus megistus* is an atypical species of the genus *Nymphargus*, where all species have a reduced webbing between Finger III and IV. In contrast, the hand webbing in *N. megistus* is more extensive: III 2—(1 1/2–1 3/4) IV. No additional variation or sexual dimorphism has been observed.

Evolutionary relationships and generic placement.—Our phylogenetic results (Figure 3) clearly indicate that the species is part of the genus *Nymphargus*. Note that, as explained in the methods section, our taxon sampling included taxa from all the currently recognized genera in Centrolenidae. Thus, we formally place *Centrolenella megista* Rivero 1985 in the genus *Nymphargus* sensu Guayasamin *et al.* (2009). *Nymphargus megistus* and *N. grandisonae* are recovered as sister taxa; the genetic distance between these two species is 2.0%.

New records.—ECUADOR: one adult male, SVL: 30.8 mm; Ecuador, Imbabura Province, Río Manduriacu Reserve; 00°20.217' N, 78°51.445' W, 1,857 m a.s.l.; 21:14 h, 01 March 2019; Ross Maynard, Jaime Culebras, and Scott Trageser; primary cloud forest, perched on broad-leaf stalk 2 cm diameter, 1 m high, 15.6°C, 95% relative humidity, light rain occurred earlier in the day; ZSFQ 4071 (field number 2019-013). ECUADOR: one adult female, SVL: 38.9 mm; Ecuador, Imbabura Province, Río Manduriacu Reserve; 00°20.206' N, 78°51.452' W, 1,850 m a.s.l.; 20:19 h, 07 December 2019; Ross Maynard, Scott Trageser, and Leslie Rochefort; primary cloud forest, perched on leaf, 60 cm high, 14.8°C, 100% relative humidity, 5 mph wind, found during heavy rain; ZSFQ 3924 (field number SCA 1600). ECUADOR: one adult

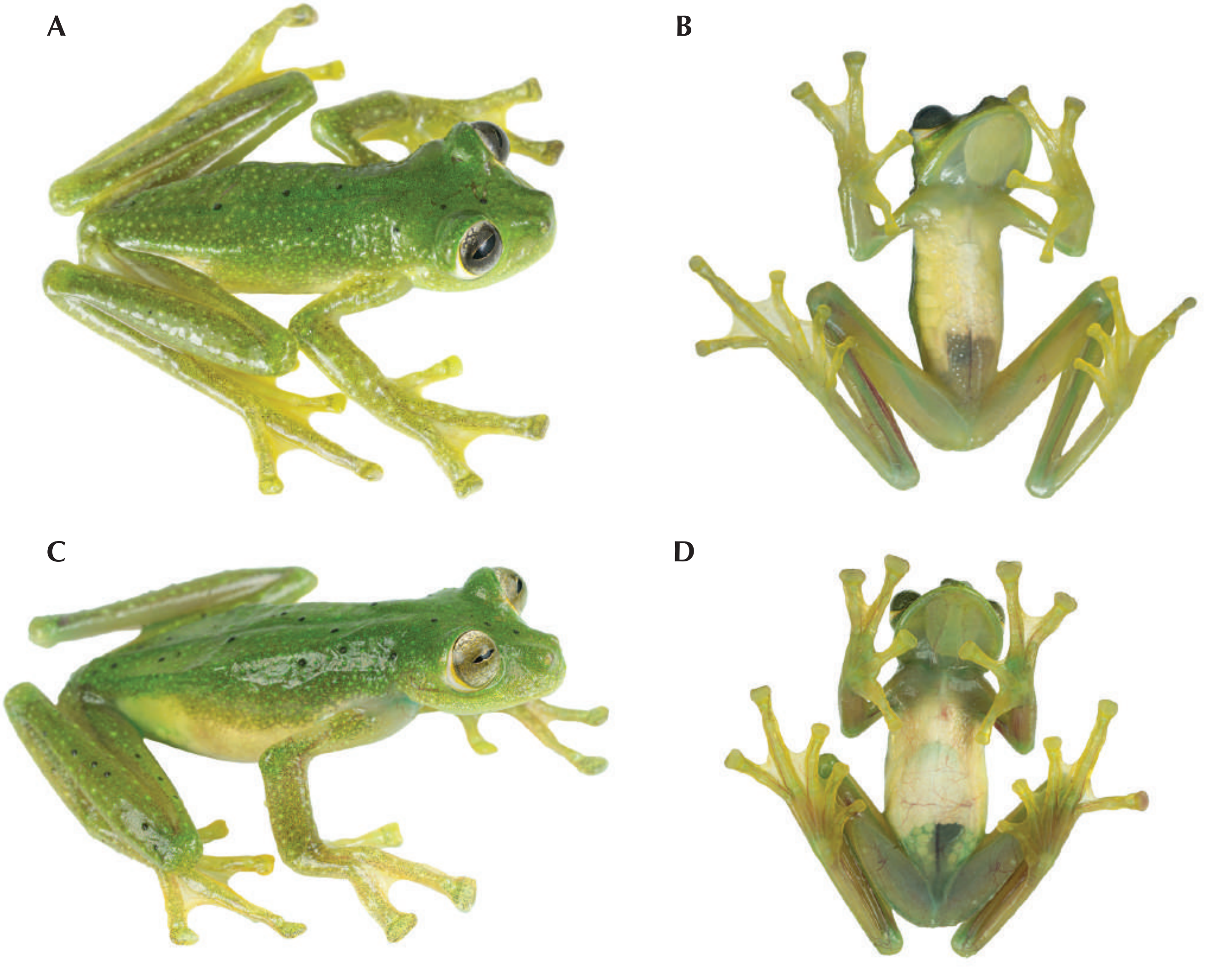

Figure 2. *Nymphargus megistus.* (**A**) Adult ♂, ZSFQ-4071, SVL = 30.8 mm, dorsal view. (**B**) Adult ♂, ZSFQ-4071, SVL = 30.8 mm, ventral view. (**C**) Adult ♀, ZSFQ-3924, dorsal view, SVL = 38.9 mm. (**D**) Adult ♀, ZSFQ-3924, ventral view, SVL = 38.9 mm.

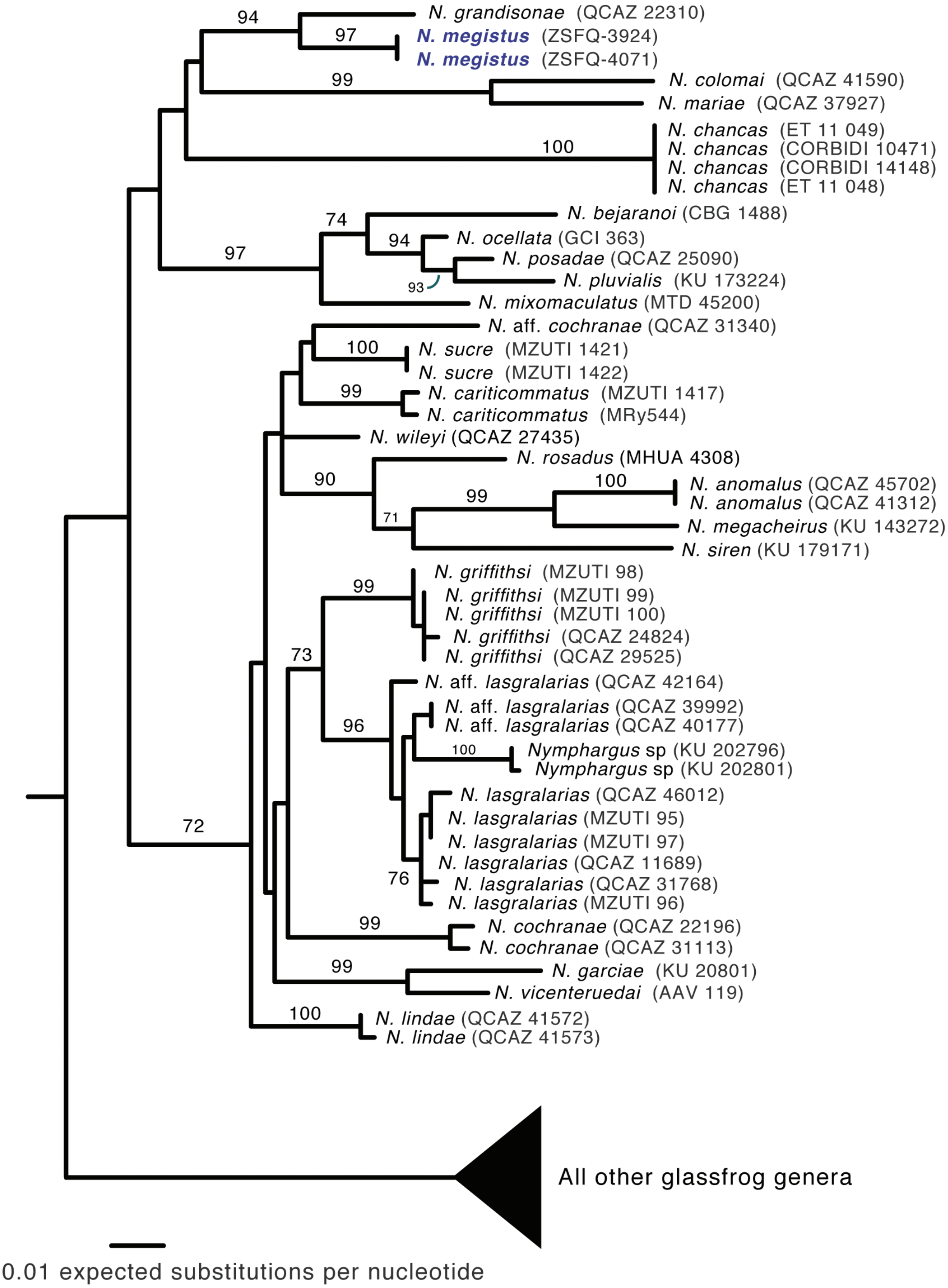

Figure 3. Phylogenetic placement of *Nymphargus megistus* inferred under Maximum Likelihood using the mitochondrial gene 16S. Genbank accession numbers for *N. megistus* are as follow: MZ314501 (ZSFQ 4071) and MZ314502 (ZSFQ 3924).

male, SVL: 35.9 mm; Ecuador, Pichincha Province, Séptimo Paraíso, nearby the town of Mindo; 00°1.758' S, 78°45.912' W, 1,550 m a.s.l.; 22:00 h, 11 February 2020; Amanda Quezada and Frank Pichardo; primary cloud forest, perched on leaf 20 cm high, found during a cloudy night, about 2 meters from creek; TH-822.

Discussion

The two records we report of *Nymphargus megistus* from the Río Manduriacu Reserve and the one individual from near Mindo represent the first observations of the species in Ecuador, extending its known distribution ca. 530 km south from the nearest record in the southern Chocó Department of Colombia (Figure 2). Although a substantial range extension, it is not unexpected; a number of anuran species are known to have similar distributions along the western slope of the Cordillera Occidental of Colombia and northern Ecuador, including other glassfrog species that have been reported from the Río Manduriacu Reserve (i.e., *Centrolene peristicta, Espadarana prosoblepon, Nymphargus balionotus, and N. grandisonae;* Maynard *et al.* 2020). Despite the seemingly low detectability of *N. megistus* at the localities in which it has been observed, the absence of records between the Ecuadorian localities and those from Colombia demonstrate the need for additional survey efforts within this region.

Our observations of *Nymphargus megistus* at RMR were made nine months apart (March and December 2019) in primary cloud forest near the upper reaches of RMR just below the ridgeline. Interestingly, both individuals were observed ca. 175 m from the nearest stream, and within 25 m of one another. Similar observations are recorded in the field notes of Maria Cristina Ardila-Robayo from August 1987 reporting three individuals (ICN 17242–4) found in mature forest, away from bodies of water, and 0.5–2 m above the ground. Rada and Guayasamin (2008) suggest, based off these observations, that during non-breeding periods the adults migrate towards the interior of the forest away from streams. During early December and early March, our surveys of four streams at the upper reaches of RMR did not yield observations of *N. megistus* or their advertisement calls. Although this suggests a lack of breeding activity, the observation of the gravid female in December 2019 indicates that reproduction in this species likely does occur during this time frame, despite the fact that we did not observe this. In fact, call data for this species has yet to be recorded and described; however, males were recorded in the notes by either Maria Cristina Ardila or Pedro M. Ruiz (M. Rada pers. comm.) to have been vocalizing at an unknown date on streamside branches in Araceae plants 1.5–1.8 m above the ground (Rada and Guayasamin 2008). The incidental observation near Mindo of a male near a stream in mid-February does correspond to potential rainy season breeding activity, though it was not observed calling. Considering the proximity of our RMR observations away from a stream, and at different times of year, it is possible this area serves as an important migration corridor.

The IUCN currently recognizes *N. megistus* as Endangered (IUCN SSC Amphibian Specialist Group 2017). Although our two records from RMR extend the range of *N. megistus* considerably, it is unknown if the Ecuadorian populations are disjunct from those in Colombia. Results from the Colombia Red List Assessment Workshop (2016) indicate that the majority of the population resides in small subpopulations with no genetic exchange. Furthermore, severe fragmentation may already exist along specific corridors at elevations necessary for this species to disperse, as Andean cloud forests in Colombia are disproportionately excluded from regional and national protected area networks (Forero-Medina and Joppa 2010). Additionally, in light of increasing mining activity in Ecuador (Roy *et al.* 2018) and immediate threats facing RMR (Guayasamin *et al.* 2019b, Maynard 2020), additional surveying of the vast area between the

Colombian and Ecuadorian occurrences is necessary to better understand its distribution, natural history, and conservation status.

Acknowledgments

The authors thank Carolina Reyes-Puig and Diego F. Cisneros-Heredia (ZSFQ) for assistance in organizing the logistics and museum accession of related fieldwork at RMR. We are grateful to Fundación EcoMinga for their continued partnership and efforts to protect and manage RMR, and to José María Loaiza, Rolando Peña, Marcelo Lesano, Jimmy Álvarez, José Vieira, and Leslie Rochefort for their invaluable help during portions of the field work. We are also grateful to the Villamizar family, Socio Bosque Program, IUCN Netherlands, The Dutch National Postcode Lottery, World Land Trust, Fundación Cóndor Andino and other donors for the continued funding of the reserve and the purchase of additional properties to protect. We are especially grateful to the Santa Rosa de Manduriacu community for their openness to our research, providing access to private property, logistical assistance, and their incredible hospitality. RJM and ST are especially grateful for a generous donation provided by Stephanie Fogel to The Biodiversity Group, which helped fund a portion of the fieldwork that led to data presented herein. Genetic sequences were generated at the Laboratorio de Biología Evolutiva at Universidad San Francisco de Quito, with the help of Nathalia Valencia and Daniela Franco. We also thank the Ministerio de Ambiente y Agua for granting all required research permits. JMG's research is supported by USFQ (Collaboration Grant 11164, 16871; COCIBA grants: 5467, 5521, 16808; Programa Inédita, SENESCYT "Respuestas a la crisis de biodiversidad: La descripción de especies como herramienta de conservación"). We are also grateful to the reviewers for their time, effort, and knowledge during the peer review process.

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Editor: Vanessa K. Verdade

Phyllomedusa 20(1):37–52, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p37-52

Diet, reproductive biology, and ecological interactions of *Fritziana goeldii* (Anura: Hemiphractidae)

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Abstract

Diet, reproductive biology, and ecological interactions of *Fritziana goeldii* (Anura: Hemiphractidae). Aspects of the diet, reproduction, and interactions with other taxa are reported for the egg-brooding tree frog, *Fritziana goeldii*, an endemic phytotelma-dwelling species from southeastern Brazil. A total of 56 frogs (48 males; 8 females) was collected between December 2018 and November 2019. A total of 218 dietary items, representing at least 28 taxa was recovered. Hymenoptera and Coleoptera were the most abundant, mainly early in the year (January–April), with Formicidae being the most represented group. Parasitic interactions with ostracods and helminths were observed in 12 and 13 frogs, respectively, with most of these organisms being found in the intestines of *F. goeldii*. *Fritziana goeldii* is sexually dimorphic, with females ($\bar{x}$ = 36.0 mm) being larger than males ($\bar{x}$ = 28.3 mm). All females collected had oocytes, and two also had eggs attached to the dorsum. No relation was found between the female's size and size and number of oocytes. The reproduction of *F. goeldii* seems to be prolonged, with males calling throughout the year.

Keywords: Egg-brooding tree frog, food habits, ostracods, parasitism, phoresy, South America.

Resumo

Dieta, biologia reprodutiva e interações ecológicas de *Fritziana goeldii* (Anura: Hemiphractidae). Aspectos da dieta, reprodução e interações com outros táxons foram reportados para a perereca marsupial *Fritziana goeldii*, uma espécie bromelígena endêmica da região sudeste do Brasil. Um total de 56 indivíduos (48 machos; 8 fêmeas) foram coletados entre dezembro de 2018 e novembro de 2019. Um total de 218 itens alimentares, representando pelo menos 28 táxons, foram identificados. Hymenoptera e Coleoptera foram os táxons mais abundantes, principalmente nos primeiros meses do

Received 25 February 2021
Accepted 19 May 2021
Distributed June 2021

ano (janeiro-abril), com Formicidae sendo o grupo mais representativo. Interações com ostrácodes e helmintos foram observadas em 12 e 13 indivíduos, respectivamente, com a maior parte desses organismos sendo encontrados no intestino dos espécimes de *F. goeldii*. *Fritziana goeldii* apresenta dimorfismo sexual, com as fêmeas ($\bar{x}$ = 36.0 mm) sendo maiores que os machos ($\bar{x}$ = 28.3 mm). Todas as fêmeas coletadas apresentavam ovócitos, e duas também tinham ovos aderidos no dorso. Nenhuma relação foi encontrada entre o tamanho das fêmeas e o tamanho e número de ovócitos. A reprodução de *F. goeldii* aparenta ser do tipo prolongada, com machos vocalizando em todos os meses ao longo do ano.

Palavras-chave: América do Sul, foresia, hábitos alimentares, ostrácodes, parasitismo, perereca-marsupial.

Introduction

Several studies have sought to understand the interspecific relationships of anurans by studying natural history (Kehr and Hamann 2003, Freitas *et al.* 2008). Knowledge of foraging modes, ecological interactions, and diet composition is a vital part of a species' natural history, and helps us to understand the intra- and interspecific relationships of the frogs. (Anderson and Mathis 1999, Teixeira and Vrcibradic 2003, Mendonça *et al.* 2020).

Most adult anurans can be described as generalist and opportunistic predators, consuming mainly arthropods (Solé and Rödder 2009). Plant material and fruits also are often found in their diet, usually consumed accidentally along with prey, with only a few studies showing an active intake of plants by frogs (e.g., Das 1996, Silva and Britto-Pereira 2006). Prey quantity and quality can change according to seasonality and the type of environment in which the individuals are found (Duellman and Trueb 1994). The feeding strategies of frogs also may vary, with species actively searching for prey or using the sit-and-wait strategy (Toft 1980, Vaz-Silva *et al.* 2005).

Another kind of interaction is phoresy, an interaction like commensalism in which one individual acts as a host or vector and the other as the phoretic, adhering to the host's body to be transported to another location, leading to its dispersal (Houck and O'Connor 1991, Sabagh *et al.* 2012). In amphibians, phoresy is mainly reported for species related to phytotelmic environments, such as bromeliads, where the accumulation of water and debris provides a microhabitat for many species of invertebrates and vertebrates (Oliveira *et al.* 1994, Kitching 2000, Lopez *et al.* 2005, Araújo *et al.* 2019). Whether using bromeliads exclusively for refuge and feeding (bromelicolous species) or to carry out their entire life cycle (bromeligenous species), amphibians are suitable hosts for the phoretic invertebrate fauna present in the phytotelmic community, such as micro-crustaceans, annelids, and ciliates (Lopez *et al.* 2002, Sabagh *et al.* 2011, Moroti *et al.* 2019).

Parasitism is a frequent interaction in nature. In addition to causing diseases, parasites regulate host populations by influencing their rates of mortality and birth, nutrition, and growth, stabilizing the food chains, and structuring animal communities (Minchella and Scott 1991, Tavares *et al.* 2016). The relationship between parasite and host also may elucidate aspects (e.g., habitat, feeding pattern, and main predators) of the parasitized species' biology and habits (Minchella and Scott 1991, Leivas *et al.* 2018, Goldberg and Bursey 2019). Studies addressing the actual diversity of parasitic species and their potential hosts are necessary to understand how these interactions arose and what the consequences are for the parasitized individual (Campião *et al.* 2015); only a few studies have explored these interactions in amphibian species (e.g., Campião *et al.* 2014, 2015, 2016).

The reproductive biology of species is a significant part of its natural history (Haddad and Prado 2005). Anurans have 41 described reproductive modes, with neotropical frogs having the greatest diversity (Pombal and Haddad 2005, Malagoli *et al.* 2021). A reproductive strategy is defined as the combination of morphological and physiological attributes that act together to produce an optimum number of offspring in specific environmental conditions (Duellman and Trueb 1994). Reproductive biology includes the reproductive temporal pattern—i.e., prolonged or explosive—the time of day when the males call, the calling site, and sexual dimorphism (Haddad 1991).

Hemiphractidae occurs in tropical regions of Central and South America and currently comprises six genera in two subfamilies (Castroviejo-Fisher *et al.* 2015, del Pino 2018, Walker *et al.* 2018, Frost 2020). The genus *Fritziana* belongs to the subfamily Hemiphractinae, which currently is composed of seven endemic species of the Brazilian Atlantic Forest (Folly *et al.* 2018, Frost 2020). *Fritziana goeldii* is endemic to southeastern Brazil and includes three population lineages; its range encompasses the lowlands and slopes of the mountains to elevations of 2,200 m a.s.l. in the states of São Paulo and Rio de Janeiro (Walker *et al.* 2018, Frost 2020). These frogs are nocturnal and can be found among bromeliad leaves or in crevices of tree bark, and like other members of the family, the females carry eggs on their backs until the tadpoles hatch—hence they are called egg-brooding tree frogs (Weygoldt and Carvalho-e-Silva 1991, Castroviejo-Fisher *et al.* 2015, Walker *et al.* 2018).

Although *Fritziana goeldii* is the best-known species of the genus (Walker *et al.* 2016); however, little is known about its natural history other than egg development (Duellman and Gray 1983, Weygoldt and Carvalho-e-Silva 1991) and tadpole feeding behavior (Weygoldt 1989). According to the IUCN Red List (IUCN 2021), the species is categorized as Least Concern, with a stable population. However, Walker *et al.* (2018) recognized three lineages within *F. goeldii*, calling attention to the possibility of cryptic diversity and suggesting changing the species status to Data Deficient.

We investigated the natural history of a population of *Fritziana goeldii* from the state of Rio de Janeiro in southeastern Brazil, focusing on the diet and ecological interactions of the species and its reproductive biology. Our goals were to: (1) describe the composition of the diet of *F. goeldii*; (2) identify variations in the composition of the diet through the year; (3) describe the ecological interactions of the frog; (4) ascertain whether the numbers and sizes of oocytes are correlated with female body size; and (5) determine whether there is sexual dimorphism in body size.

Materials and Methods

Sampling

The study was carried out at the Parque Nacional da Tijuca (PNT) (23º35'15.89" S, 43º 28'58.59" W) in the state of Rio de Janeiro in southeastern Brazil. This fragment of Atlantic Forest is divided into three sections (ICMBio 2020): Serra da Carioca, Pedra Bonita/Pedra da Gávea, and Floresta da Tijuca; the latter was our study site, which was selected based on prevalence of bromeliads, the most common habitat for this species.

The frogs were identified as *Fritziana goeldii* by their possession of interorbital triangle with a bilobed blotch, dark brown half-moon-shaped spot in the sacral region, and median transverse dark brown stripes on thighs, shanks, and feet (Figure 1; Folly *et al.* 2014, Walker *et al.* 2018). They are likely part of the *F. goeldii* lineage from the coastal regions of Rio de Janeiro (Go-II lineage in Walker *et al.* 2018).

The fieldwork was conducted once a month from December 2018 to November 2019, always starting around 17:30/18 h and lasting around 4 hr. During the first 2 months (2018 December and 2019 January), the individuals were collected

Figure 1. Individuals of *F. goeldii* found at Parque Nacional da Tijuca, Rio de Janeiro state, southeastern Brazil. (**A**–**C**) Individuals found on bromeliad leaves; (**D**) individual in a bromeliad base; (**E**) a calling male; (**F**) a female with eggs on her back. Photos: Luis Felipe Peixoto (A), Isabela C. Rocha (B), Lucas O. Passos (C), Andressa M. Bezerra (D), Isabela C. Rocha (E), and Ariel Wendt (F).

under a permit that allowed up to 20 individuals per year (ICMBio#65470-1). The individuals collected in the subsequent months were under a second license (ICMBio#69339-1), which limited the sample to as many as 48 individuals per year. Because of this limitation, in the first 2 mo, we collected up to 10 frogs/month, and in the subsequent months, we collected only the first five individuals encountered (whenever possible), although other individuals may have been encountered.

We actively searched for the frogs. The species of bromeliads were identified following Sabagh *et al.* (2017). The frogs were collected by hand, euthanized with 5% lidocaine, and fixed in 10% formaldehyde, thereby inhibiting digestion of their gastrointestinal contents. After 72 hr, the specimens were preserved in 70% ethanol. All procedures followed the practice guidelines of Conselho Nacional de Controle de Experimentação Animal - CONCEA. Voucher specimens were deposited at the amphibian collection of Laboratório de Anfíbios e Répteis, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (ZUFRJ).

Diet

Gastrointestinal tracts of the frogs were removed, and the stomach and intestine contents were analyzed separately under a Leica stereoscopic microscope. Because several studies use methods such as stomach flushing to evaluate species diet, relying exclusively upon stomach contents, we wanted to investigate whether the inclusion of intestinal content be useful to find food items not encountered in the frog’s stomach.

Food items were identified to the lowest possible taxonomic level with keys mentioned below. When possible, the length and width of the food items were measured to estimate their volumes based on the ellipsoid volume formula: $V = (4\pi/3)(L/2)(W/2)^2$, where V is the prey volume, L its length, and W its width. We also calculated the Index of Relative Importance (IRI) of the food items with the following formula: $IRI = (PO)(PI + PV)$, where PO is the percentage of occurrence (= frequency), PI is the percentage of individuals (= absolute number), and PV is the percentage of the volume of each measured item (Solé and Rödder 2009). When it was impossible to calculate the volume of a food item owing to the advanced stage of fragmentation or digestion, only the absolute number and frequency were calculated (Solé and Rödder 2009). Given that there is no record of active plant intake by *Fritziana goeldii,* stomachs and intestines containing only plant material were classified as empty.

The expected taxon richness in *Fritiziana goeldii*’s diet was calculated based on an extrapolation sampling curve using the iNEXT package in R environment (Hsieh *et al.* 2020, R Core Team 2020). To run this analysis, we used a matrix of sampling-units incidence, with 95% confidence interval. We performed the extrapolation sampling curve with the food items that were identified to the most specific level possible as the operational taxonomic units (UTOs), if there was no overlap between taxa, totaling 28 UTOs.

Non-insect arthropods were identified with Brusca *et al.* (2016), and hexapods were identified with the key to orders and images in Rafael *et al.* (2012) and Triplehorn *et al.* (2005). To compare the frequency of taxa of food items during the study, the number of frogs having a particular organism in the stomach and/or intestine was divided by the number of frogs collected in that month. Data were standardized by using the highest taxonomic level—in this case, order—except for the class Chilopoda, which was not identified at a lower taxonomic level. To visualize the taxa frequency through time we generated a heat map using ggplot2, hrbrthemes, tibble and reshape packages (Wickham 2007, 2016, Müller and Wickham 2020, Rudis 2020) in R environment (R Core Team 2020).

Reproductive Biology

The frog's sex was determined by examining the gonads (Figure 2) and by the presence of vocal slits in preserved males. The snout–vent length (SVL) of each individual was measured with digital calipers (to the nearest 0.1 mm). The eggs and oocytes of females were counted and measured with the aid of a Leica stereomicroscope under a 0.63 or 1.0 magnification.

We performed an Analysis of Variance (ANOVA) to test for sexual dimorphism in body size. Spearman's correlation coefficient was used to determine whether the sizes and numbers of oocytes were correlated with female body size, because the data were not normally distributed according to results of the Shapiro-Wilk Normality Test. All analyses were conducted in the R environment (R Core Team 2020). We considered a $p < 0.05$ significant for the ANOVA and Spearman's correlation coefficient. Only the number of oocytes was included in the correlation test because all females had oocytes, but only two had eggs. Results are shown in a boxplot and linear regression, respectively, both generated using the ggplot2 package in R environment (Wickham 2016, R Core Team 2020). Individuals from the ZUFRJ amphibian collection collected in previous expeditions at the same location (PNT) from 2016 and 2018 were also included in the analysis to increase the ANOVA accuracy (Appendix I).

To determine whether *Fritziana goeldii* reproductive activity is prolonged (i.e., all year) or seasonal, we observed whether males were calling (as a proxy of mating activity) in the sites visited in each survey.

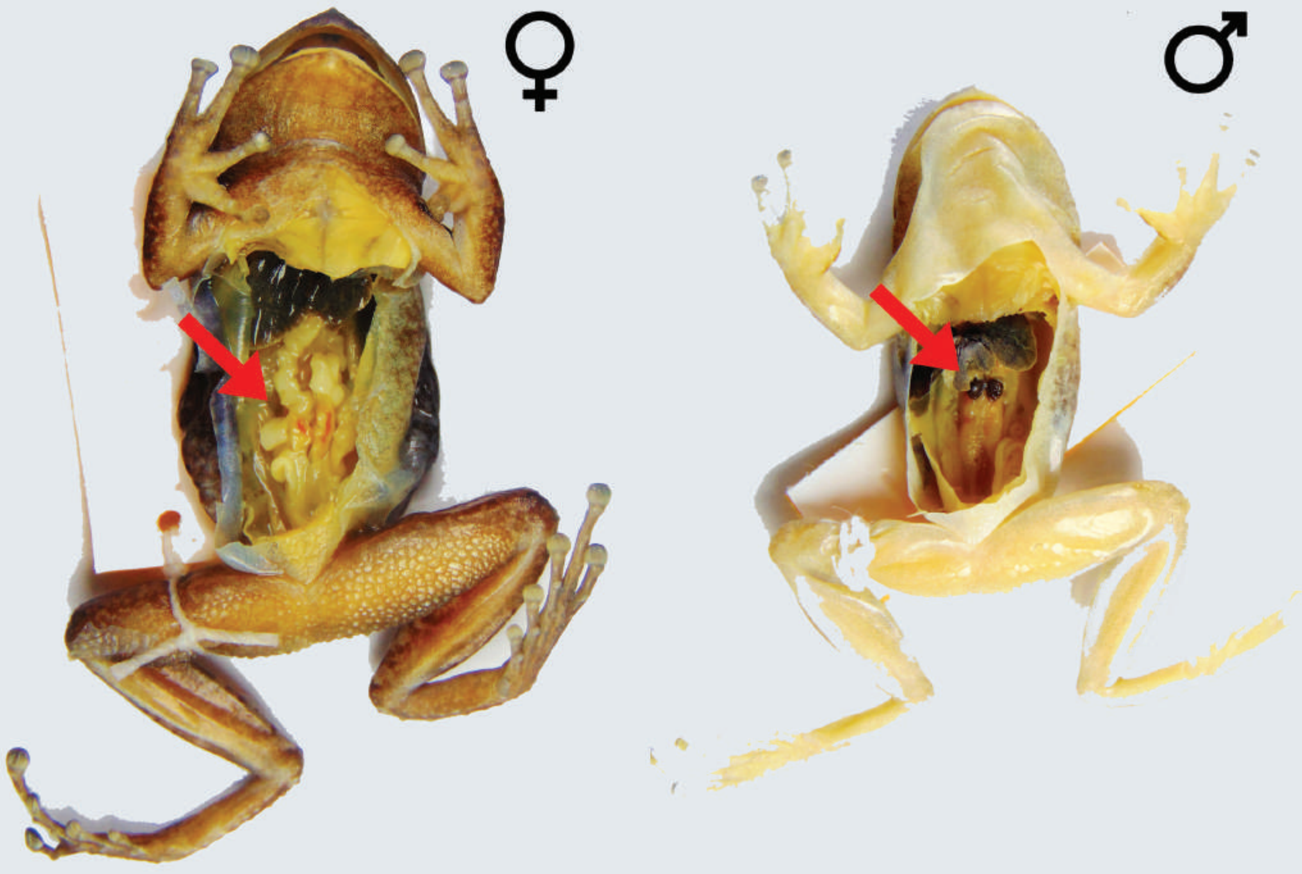

Figure 2. Female (F16792) and male (F16598) gonads (arrows) of *Fritziana goeldii* from Floresta da Tijuca southeastern Brazil.

Results

Diet

We collected 56 specimens of *Fritziana goeldii* during the 12 surveys (Appendix); 48 are males, with SVLs ranging from 22.3–32.7 mm ($\bar{x}$ = 28.3 ± 1.8 mm) and 8 females with SVLs ranging from 33.7–39.5 mm ($\bar{x}$ = 36.0 ± 3.2 mm). Of the 56 gastrointestinal tracts examined, 53 (94.6%) had identifiable material in at least one of the organs (i.e., stomach and/or intestine); 6 individuals had food only in the stomach (11.3%), 18 only in the intestine (33.9%), and 29 in both organs (54.7%). We identified a total of 218 food items, representing at least 28 taxa belonging to 13 orders and four subphyla, with most identified at the familial level (Table 1).

Among the food items, Hymenoptera was the most frequent and had the highest index of relative importance (IRI), with Formicidae being the most representative group. Coleoptera had the second highest frequency and IRI, followed by Araneae and Blattodea, respectively (Table 1, Figure 3). Formicidae and Coleoptera were more frequent than the other taxa from January–April and Araneae in April and June (Figure 3). Other food items appeared in low frequency and were restricted to just 1 mo; examples are Odonata in May, Mantodea in August, and Chilopoda in April (Figure 3).

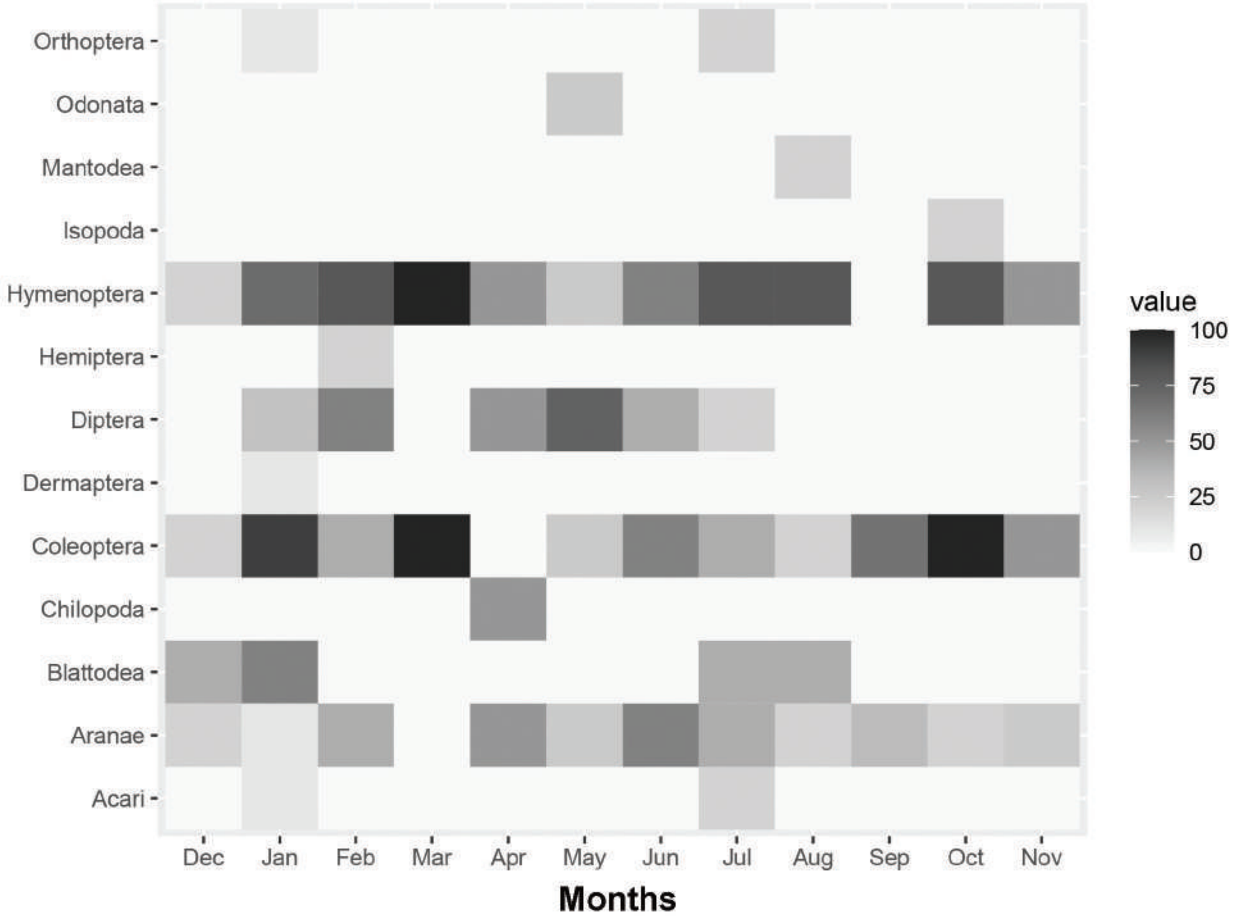

Figure 3. Heat map showing variation in the frequency of occurrence of each taxon per month. Dark grey indicates a higher frequency of the taxon in each month.

Table 1. Food items found with their respective values in absolute number (N), relative volume (V), frequency of occurrence (F) and relative importance index (IRI).

	N(%)	V(%)	F(%)	IRI
CHELICERATA				
Acari	2(0.91)	-	2(3.57)	-
Aranaeae	21(9.63)	249.49(68.15)	18(32.14)	509.84
Araneidae	3(1.37)	168.87(46.13)	3(5.35)	254.14
Corinnidae	2(0.91)	71.02(19.4)	1(1.78)	35.15
Salticidae	1(0.45)	-	1(1.78)	-
HEXAPODA				
Blattodea	13(5.96)	67.33(18.38)	11(19.64)	300.75
Isoptera	4(1.83)	9.28(2.53)	2(3.57)	15.58
Termitidae	-	-	1(1.78)	-
Coleoptera	19(28.56)	98.75(26.97)	27(48.2)	713.8
Polyphaga	15(6.88)	90.5(24.7)	11(19.64)	619.11
Staphylinoidea	2(0.91)	-	2(3.57)	-
Curculionidae	-	-	1(1.78)	-
Elateridae	1(0.45)	21.7(7.6)	1(1.78)	11.33
Scarabeidae	-	-	3(5.35)	-
Scirtidae	1(0.45)	-	1(1.78)	-
Tenebrionidae	6(2.75)	68.8(18.79)	6(10.7)	230.69
Lagriinae	1(0.45)	-	1(1.78)	-
Carabidae	-	-	3(5.35)	-
Dermaptera	1(0.45)	-	1(1.78)	-
Diptera	-	-	11(19.64)	-
Brachycera	-	-	5(8.92)	-
Chironomidae	-	-	3(5.35)	-
Tipulomorpha	-	-	7(12.5)	-
Tipulidae	-	-	2(3.57)	-
Limoniidae	-	-	2(3.57)	-
Hemiptera	-	-	1(1.78)	-
Reduviidae	-	-	1(1.78)	-
Hymenoptera	56(25.69)	21.92(5.97)	50(89.27)	1329.69
Ichneumonoidea	2(0.91)	6.62(1.8)	2(3.57)	9.7
Formicidae	53(24.31)	15.3(4.17)	43(76.78)	1319.99
Apoidea	1(0.45)	-	1(1.78)	-
Mantodea	-	-	1(1.78)	-

Table 1. *Continued.*

	N(%)	V(%)	F(%)	IRI
Thespidae	-	-	1(1.78)	-
Odonata	1(0.45)	8.39(2.29)	1(1.78)	4.88
Orthoptera	1(0.45)	-	3(5.35)	-
Gryllidae	-	-	2(3.57)	-
Trigonidiidae	1(0.45)	-	1(1.78)	-
CRUSTACEA				
Oniscidea	1(0.45)	0.92(0.25)	1(1.78)	1.24
MYRIAPODA				
Myriapoda	2(0.91)	11.05(3.01)	3(5.35)	20.9
Chilopoda	2(0.91)	11.05(3.01)	2(3.57)	13.9
Newportiidae	1(0.45)	11.05(3.01)	1(1.78)	6.17

The extrapolation sampling curve estimated 40 taxa as potentially part of the diet of *Fritziana goeldii* with the confidence interval varying from 29–53 taxa (Figure 4).

Ecological Interactions

Most frogs were found in the following species of bromeliads—*Aechmaea nudicaulis, Quesnelia marmorata* and *Vriesea bituminosa*.

Ostracods of the genus *Elpidium* were found in 12 frogs. Two had ostracods either inside their intestine or adherent to their skin externally; eight had ostracods only in the intestines and two had *Elpidium* in both their intestines and stomach. Most *Elpidium* found in the gastrointestinal tracts were intact and varied from 1–12 ostracods in the intestine and from 1 or 2 in the stomach.

We collected 13 *Fritziana goeldii* with helminths, which were identified as Nematoda. One frog was captured with a nematode under the skin of its head and another had a nematode in its eye. However, most helminths were found in the intestines of the frogs; only one frog had nematodes both in the stomach and intestines. In one individual, the nematodes were present inside the body cavity, but outside the gastrointestinal tract.

Reproductive Biology

The difference between the body size of females and males was highly significant ($p < 0.001$), with no overlap between the values (Figure 5). All females were collected from bromeliads and had oocytes in the oviducts; two also had eggs on their backs (Figure 1F). The number of oocytes ranged from 10–19 ($\bar{x} = 15.2 \pm 1.4$, $N = 8$) per female and the number of eggs ranged from 12–20 ($\bar{x} = 16.0 \pm 5.7$, $N = 2$). The sizes of oocytes ranged from 0.8–4.3 mm ($\bar{x} = 2.7 \pm 1.2$ mm, $N = 107$) and the sizes of eggs from 3.6–4.9 mm ($\bar{x} = 4.2 \pm 0.3$ mm, $N = 23$). Sizes and numbers of oocytes were not significantly correlated with the female size ($p = 0.27$ and $p = 0.27$, respectively) (Figure 6).

We heard and saw males calling from bromeliad leaves every month of the study (Figure 1). Even when the individuals were found at lower densities, it was possible to hear males calling from farther away and/or at greater heights.

Discussion

Walker *et al.* (2018) found three lineages within *Fritziana goeldii*—one from the state of São Paulo and two from Rio de Janeiro. Although there is no specimens from the Parque Nacional da Tijuca included in the analysis of Walker *et al.* (2018), we believe that the subpopulation sampled in our study belongs to the lineage from the coastal regions of Rio de Janeiro (Go-II) based on the PNT location. Based on the number of individuals found (sampled and seen) during this study and the fact that the PNT is a National Conservation Unit, we think that this subpopulation is Least Concern and Stable according to the IUCN criteria (IUCN 2021).

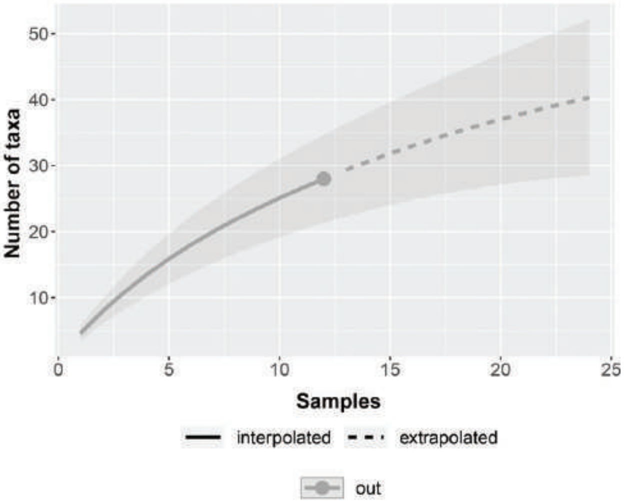

Figure 4. Accumulation curve of taxa present in the diet of specimens of *Fritziana goeldii* collected at Parque Nacional da Tijuca, southeastern Brazil.

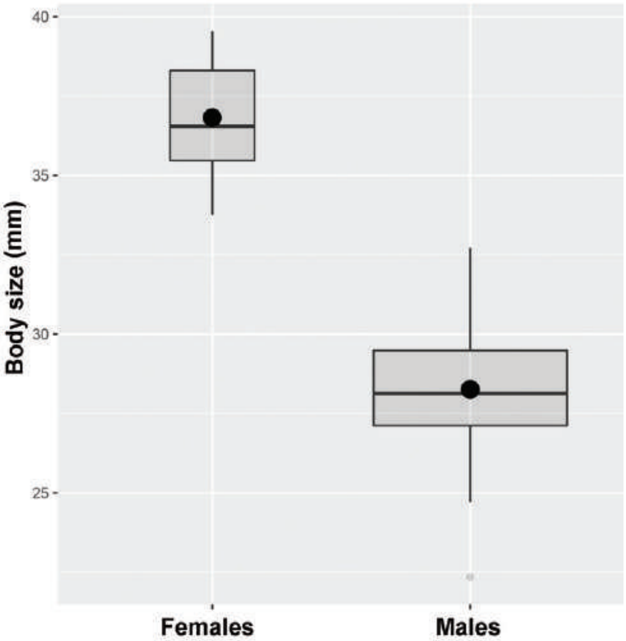

Figure 5. Difference in body size between females (F) and males (M). Black circles indicate the mean and bold lines the median.

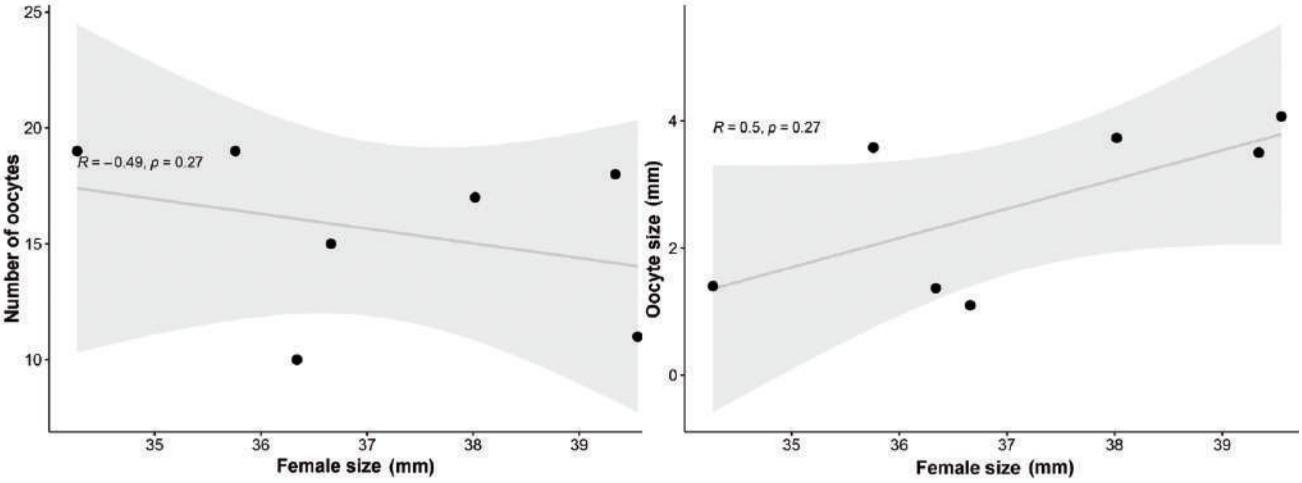

Figure 6. Linear regression between the number of oocytes, oocyte size and female size. Both correlations were not significant ($p > 0.05$). Trend lines in light grey, and confidence interval in shades of grey.

Diet

We observed that the most prevalent food items in the diet of *Fritziana goeldii* are specimens with more resistant body structures—i.e., beetles and arachnids. This is explained by the accelerated digestion process that the items go through when they reach the stomach. More delicate parts and sensitive tissues are digested faster, leaving in the intestine more rigid structures such as elytra, heads, sclerites and legs (Solé *et al.* 2005). Consequently, prey with soft bodies tend to be underestimated (Solé and Rödder 2009).

Anurans with an active foraging strategy tend to feed on small prey that are less agile and live in groups; these prey items have a body rich in chitin but low in energy content. Active foragers usually have a greater number of food items per stomach/intestine than do sit-and-wait predators (Toft 1981, Solé and Rödder 2009). The diversity of food items in the diet of *Fritziana goeldii* is mainly composed of ants, beetles, and arachnids, indicating that the species is a foraging generalist (sensu Weygoldt and Carvalho-e-Silva 1991). Arthropods, such as spiders, ants and beetles, are highly abundant in the environment, with beetles having the greatest diversity within Insecta and therefore a typical food item for neotropical anurans (Teixeira *et al.* 2009, Martins *et al.* 2010, Almeida *et al.* 2019, Moser *et al.* 2019). Representatives of these taxa were present almost every month in the diet of *Fritziana goeldii* and had the highest IRI values (Table 1, Figure 3).

As expected, the inspection of both the stomach and intestine yielded many more taxa of food items, because some (e.g., Odonata and Hemiptera) were found only in the intestine. Ostracods were mainly observed in the intestine; this probably reflects the resistance of their body parts to digestion, such as the head and elytra of Odonata and Hemiptera, respectively, and the outer carapace of Ostracoda (made of chitin and calcium carbonate).

Most diet studies usually identify prey to the ordinal level (e.g., Solé and Rödder 2009, Batista *et al.* 2011, Oliveira *et al.* 2017, Pacheco *et al.* 2017); probably this is because the food items are found at an advanced stage of digestion. We were able to identify most (78.5%) prey at the familial level. This result contributes not only to knowledge of the diet of *Fritziana goeldii*, as well as the faunal diversity of the frogs' habitat. Thus, studies that aim to understand how environmental disturbances could affect the availability of prey, and consequently, the population of *F. goeldii* and other anurans in that location (Kittel and Solé 2015) are enabled.

Hymenoptera and Coleoptera were the most frequent taxa in the diet between January and March and between July and October. Araneae is more frequent between April and July. A higher frequency in the first months may have been influenced by the fact that in January, we collected 10 *Fritziana goeldii,* instead of five in the other months; thus, the greater number of food items obtained probably reflects the frequency of the observed taxa.

The species accumulation curve probably is not stabilized owing to the need of a greater sampling effort, both in the numbers of days and individuals. In addition, we know that the advanced digestion of food items hinders the process of identifying prey and may underestimate the absolute richness of species. A larger sample would also enable the identification of more items to the most specific level possible (Solé and Rödder 2009).

Abiotic factors, such as the seasonal availability of prey in the environment, also can influence the diet pattern of anurans (Toft 1980, Freitas *et al.* 2008). Because several taxa were found in the diet of *Fritziana goeldii* throughout the year, its feeding strategy appears to be generalist and opportunistic. However, we could not properly evaluate the selectivity index because we did not sample the proportion of prey items available at the study site (Simon and Toft 1991).

Ecological Interactions

Phoresy has been observed between ostracods of the genus *Elpidium* and several anuran species, including the egg-brooding tree frog *Fritziana goeldii* (Lopez *et al.* 2005, Sabagh *et al.* 2011, Sabagh and Rocha 2014, Araújo *et al.* 2019, Moroti *et al.* 2019). *Fritziana goeldii* was the first anuran species for which adult ingestion of ostracods was reported (Guarabyra *et al.* 2020). It is still unknown whether these microcrustaceans in the gastrointestinal tract could be explained by accidental or intentional ingestion (i.e., as part of their diet); thus, we chose not to include the ostracods found in the gastrointestinal tract of *F. goeldii* as food items. Further studies on the fecal content of *F. goeldii* are necessary to confirm whether there is dispersion through the passage of the digestive tract.

This study is the first record of interaction between nematodes and *Fritziana goeldii.* Because all nematodes occurred either under the skin or organs of *F. goeldii,* we think that the nematodes were parasitic. The study of parasites in amphibians is uncommon, and more frequent for temperate-zone species (Aguiar *et al.* 2014). In tropical regions, most interactions between nematodes and frogs reports the nematodes parasitizing gastrointestinal tracts of frogs (Tavares *et al.* 2016). Recently published works probably underestimate the diversity of parasites because they are difficult to identify and require the use of diagnostic tools other than external morphology (e.g., molecular analysis, phylogenetics, and bioassays approaches; Tavares *et al.* (2016).

Reproductive Biology

The few females collected may reflect their habit of foraging in the leaf litter when they are active, as observed by Weygoldt and Carvalho-e-Silva (1991). Females with eggs on their backs are in the first phase of egg maturation in which the eggs are yellow as is characteristic of yolk (Figure 1F). During this phase, the females remain inside bromeliads until the eggs mature and the embryos begin to develop, and the eggs become darker in color (Weygoldt and Carvalho-e-Silva 1991). The females that we found that did not have eggs on their backs had well-formed oocytes, indicating that they could be in the bromeliads to mate, attracted by a calling male.

In contrast to females, males are naturally more exposed because they perch on the leaves of bromeliads and call to attract females and search for food. We only captured the first five or ten that we first encountered; it is possible that we found males more easily than females, leading to a greater number of males collected.

Given the limited number of *Fritziana goeldii* that were allowed to collect, no statistical analyses were carried out involving individuals per month. Furthermore, seasonality is not marked in southeastern Brazil (Sant'Anna Neto 2005). Nevertheless, environmental factors such as temperature, rainfall and humidity vary daily, and the parameters recorded on any one day may not represent the entire month.

The dimorphism in male and female body sizes in *Fritziana goeldii* was expected, given that about 90% of anuran species exhibit sexual dimorphism in body size (Shine 1979). Many factors can influence this difference in size and most of them have been addressed extensively in the literature. Examples include larger females producing larger eggs and spawning, leading to greater fertility (Crump 1974), and small males being able to expend their "extra" energy with vocalization and territorial defense (Woolbright 1983, Freitas *et al.* 2008).

The number of eggs we found in *Fritziana* goeldii is consistant with the numbers reported in the studies of Weygoldt and Carvalho-e-Silva (1991) and Duellman and Gray (1983). The wide variation found in the sizes of the oocytes reflects the stage of development of these in each female. As the accumulation of yolk increases, so does the size of the oocytes (Weygoldt 1989). Weygoldt and Carvalho-e-Silva (1991) also commented that the size and number of eggs in

F. goeldii seem to vary and that the same female can produce a different number of eggs in each reproductive encounter.

We found no significant relationship between the size of the female and the size and number of oocytes, contrary to the findings in studies of other species (e.g., Praderio and Robinson 1990, Prado *et al.* 2000, Rodrigues *et al.* 2003, Han and Fu 2013). This may have resulted from the high variability of our sample as reported by Weygoldt and Carvalho-e-Silva (1991), or by the low number of females sampled.

Conclusions

The diet of *Fritiziana goeldii* is that of a generalist that consumes a wide variety of prey, especially hymenopterans, coleopterans, and arachnids. Although the species accumulation curve is not stabilized owing to small sample sizes, it was possible to identify at the familial level many food items, thereby characterizing for the first time the diet of this frog. Interactions with phoretic and parasitic individuals were confirmed, but further studies are needed to identify the species of parasites present and understand what leads to these interactions and the consequences for both individuals of *F. goeldii* and the organisms that interact with this species. The reproduction of *F. goeldii* seems to be prolonged, with males singing all year. Male and female *F. goeldii* are sexually dimorphic in size, but no relationship between the size of the female and the sizes and numbers of oocytes was found. Future studies that seek to understand the relationship between abiotic and biotic factors and the reproductive period of *F. goeldii* are critical to supplement the limited knowledge about the natural history of this endemic species in southeastern Brazil.

Acknowledgments

We are grateful to Jorge Luiz Nessimian, Leandro Lourenço Dumas and André Wanderley do Prado for their help and support. We thank Ana M.P.T. Carvalho-e-Silva, Clarissa Canedo, Daniel Fernandes, Fábio Hepp, Leandro Sabagh, and Manuella Folly for their useful comments to improve the manuscript. We thank the anonymous reviewer for the comments and suggestions to improve the manuscript, and to the editors Jaime Bertoluci, J. Roger Downie and Linda Trueb for their comments and English corrections. AMB thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the financial support. BG thanks CAPES for the financial support and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship. This work was carried out with the license numbers #69339-1 and #65470-1.

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Editor: J. Roger Downie

Appendix I. *Specimens collected and deposited in the amphibian collection of Laboratório de Anfíbios e Répteis, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (ZUFRJ).*

***Fritziana goeldii*, Parque Nacional da Tijuca:** ZUFRJ 16774, 16791, 16773, 16786, 16775, 16777, 16597, 16787, 16593, 16602, 16659, 16794, 16785, 16797, 16822, 16823, 16772, 16600, 16796, 16599, 16778, 16655, 16594, 16780, 16793, 16782, 16792, 16658, 16776, 16824, 16598, 16788, 16783, 16591, 16656, 16789, 16601, 16595, 16795, 16790, 16784, 16590, 16589, 16781, 16592, 16596, 16821, 16657, 16779, 16529, 16603, 51, 52, 53, 54, 55, 15831, 15830, 16552, 16553, 15942, 16403, 16551, 16406, 15943, 16554, 15944, 16540, 16550, 16070, 16530, 16405, 15832, 16555, 16531**.**

Phyllomedusa 20(1):53–66, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p53-66

Breeding site attendance and breeding success in *Phyllomedusa trinitatis* (Anura: Phyllomedusidae)

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Abstract

Breeding site attendance and breeding success in *Phyllomedusa trinitatis* (Anura: Phyllomedusidae). Using a natural marker, we documented breeding site attendance patterns by males and females of the Trinidad Leaf Frog, *Phyllomedusa trinitatis*. We followed attendance at a cluster of three isolated ponds over 53 and 56 consecutive nights in 2016 and 2019 respectively. Most females attended only once, but for those that attended more than once we calculated an inter-nesting interval (mean 27.6 days, $N = 7$). Males showed high pond fidelity, but some did attend at two of the ponds, always with a strong preference for one of them. Males showed three attendance patterns. A few attended on multiple consecutive nights (maximum, 19 nights); more were sporadic (one attended seven times over 46 nights with gaps of 15 and 19 days in the sequence); some attended only once (2016: 12, 2019: 15), but most were found to be present on multiple nights (2016: 38, 2019: 32). Our analysis suggested that these latter frogs were either newly recruited individuals or had been predated during the study. Our data show that rainfall has some influence on attendance. We found no relationship between male body condition and attendance pattern. In addition, there was no evidence that a particular male attendance pattern was optimal for breeding success.

Keywords: breeding success, inter-nesting interval, Phyllomedusinae, reproduction, Trinidad Leaf Frogs.

Resumo

Atendimento do local de reprodução e sucesso reprodutivo em *Phyllomedusa trinitatis* (Anura: Phyllomedusidae). Utilizando um marcador natural, documentamos os padrões de atendimento do local de reprodução por machos e fêmeas da rã-folha-de-trinidad, *Phyllomedusa trinitatis.* Acompanhamos o atendimento de um grupo de três lagos isolados ao longo de 53 e 56 noites consecutivas em 2016 e 2019, respectivamente. A maioria das fêmeas atendeu os ninhos apenas uma vez, mas, para as que atenderam mais de uma vez, calculamos o intervalo de tempo entre as nidificações (média de 27,6 dias, $N = 7$). Os machos mostraram grande fidelidade ao lago, mas alguns atenderam em duas lagoas, sempre com uma forte preferência por uma delas. Os machos exibiram três padrões de atendimento. Alguns atenderam em várias noites consecutivas (máximo de 19 noites); outros foram esporádicos (um atendeu sete vezes em 46 noites com intervalos de 15 e 19

Received 01 December 2020
Accepted 14 June 2021
Distributed June 2021

dias na sequência); alguns assistiram apenas uma vez (2016: 12, 2019: 15), mas a maioria esteve presente em várias noites (2016: 38, 2019: 32). Nossa análise sugeriu que esses últimos machos eram indivíduos recém-recrutados ou tinham sido predados durante o estudo. Nossos dados mostram que a precipitação exerce alguma influência sobre o atendimento. Não encontramos qualquer relação entre a condição corporal dos machos e o padrão de atendimento. Além disso, não encontramos evidências de que um determinado padrão de atendimento dos machos fosse ótimo para seu sucesso reprodutivo.

Palavras-chave: intervalo entre nidificações, Phyllomedusinae, rã-folha-de-trinidad, reprodução, sucesso reprodutivo.

Introduction

Reproductive activity in anuran amphibians can be broadly classified into two patterns. In ‘explosive’ breeding, all adults arrive at the breeding site over a short period, undergo a brief period of mate selection usually involving nocturnal calling and competitive interactions by the males, before couples enter amplexus, spawn and then depart, the whole process often lasting just a few days (Wells 1997, Gottsberger and Gruber 2004). In temperate regions, breeding generally occurs only once a year, but in the tropics, it may occur several times throughout the year, depending on the weather conditions, particularly rainfall (Wells 1997, Gottsberger and Gruber 2004, Ulloa *et al.* 2019). Alternatively, breeding can be ‘continuous’ with asynchronous availability of gravid females at the breeding site over many weeks or months (Given 1988, Aichinger 1992, Wells 1997). In this pattern, which is commonly observed in the tropics, males may defend a territory and attract females to mate there (Given 1988, McCauley *et al.* 2000). Exceptionally, females may be territorial, usually in the defence of access to food, but mating still depends on their being ready to breed (Wells 1977, 2007, Meuche *et al.* 2011).

An extended breeding season creates problems for the males. Since they may not be able to predict when gravid females will appear, it might seem best for them to attend the breeding site every night. However, persistent attendance can be costly in several ways: attendance, along with calling behavior, could make an individual conspicuous to predators (Ryan *et al.* 1981, Lucas and Howard 1995); attendance by multiple males could increase the risk of injurious aggressive interactions; and attendance with calling could be energetically costly, and eventually debilitating if accompanied by a reduction in foraging opportunities (Wells 1977, 2007, Ryan *et al.* 1981, Lucas *et al.* 1996). These costs could all reduce the only benefit of nightly attendance, mating opportunity. Therefore, a trade-off may operate, whereby individuals attend often enough to have an increased chance of mating, but not so often as to do so at a high risk (Lucas and Howard 1995).

Phyllomedusa trinitatis Mertens, 1926, also known as the Leaf-nesting Frog and Trinidadian Monkey Frog (Kirton 2014), can be found in Trinidad and Venezuela but is not seen in Tobago (Murphy 1997, Downie *et al.* 2013). A terrestrial frog, its habitats are moist and include lowland forest and its edges and montane rainforest, but it has also been found in ditches located near to secondary forests (Murphy 1997, Kirton 2014). Males and females live in trees and are typically solitary, until the mating season which runs through the wet season (May-December) (Kirton 2014, Gourevitch and Downie 2018). Males perch on leaves or branches near water and call to attract females (Downie *et al.* 2013). In this time the males are territorial and will fight other males if they enter their perching site. Fighting is preceded by leg waving, but if this does not deter the competitor, males will grapple until one falls or is forced to leave the area. When females approach the breeding area, males, sometimes several at the same time, will attempt to mount her. When mating, a female will carry a male on her back to a suitable egg-laying site. *P. trinitatis* constructs its nests over pools of still water, folding leaves around a mass of eggs, which, when hatched, will fall into the pool of water where they will develop until metamorphosis (Downie *et al.* 2013). The female seals the nest with a jelly plug after the male fertilizes the eggs (Downie *et al.* 2013). More generally, male phyllomedusine frogs show high but not complete site fidelity, perhaps due to the unpredictable attendance of females (Wogel *et al*. 2006, Dias *et al*. 2017, Borges *et al*. 2018).

Previous work on phyllomedusine frogs used a variety of techniques to identify individuals including toe clipping (Wogel *et al*. 2005, 2006, Frost 2020), photography of variable thigh or flank markings (Oliveira *et al*. 2012, Borges *et al*. 2018, Frost 2020), and fluorescent implants (Dias *et al*. 2017). Oliveira *et al*. (2012) identified some individuals that attended their site in all three consecutive years of the study. All of these studies made valuable observations, but none followed individuals over a substantial number of consecutive nights.

Here we report on the results of a long running study of a single population of individually identified *P. trinitatis* males and females and their attendance patterns at a breeding site. We hypothesised that male attendance patterns are influenced by external factors, including predation and weather or by individual variations, including site fidelity or body condition. We hoped that by assessing both male and female attendance we could determine if one sex influences the other. We assessed (1) the attendance patterns of gravid females and how they relate to male attendance, (2) the attendance patterns of males in relation to site fidelity, weather, body condition, predation, and recruitment, and (3) male breeding success (including number of matings and number of tadpoles released from nests) in relation to attendance patterns.

Materials and Methods

Study Site and Breeding Behavior

As reported by Gourevitch and Downie (2018), the grounds of the William Beebe Tropical Research Centre (‘Simla’) in the Arima Valley of Trinidad’s Northern Range mountains (10°41'33.2" N, 61°17'22.7" W) contain three sets of concrete ponds, originally built for fish research, but now colonized by several frog species as breeding sites, including *P. trinitatis.* The ponds are distributed as a triangle, with the maximum distance between ponds being 60m. Each of the ponds is surrounded by vegetation, some of which has branches and large leaves overhanging the water. Ponds 1 and 2 (P1 and P2) are 4 × 6 m and 2 × 3 m respectively, are close to the Centre’s buildings and receive some nocturnal illumination; Pond 3 (P3) (3 × 5 m) is downhill a short distance within the forest and is dark at night. All the ponds are located well above the valley floor, and there are no other bodies of standing water in the vicinity. This means that all *P. trinitatis* individuals in the local population must use these ponds for breeding. The ponds are deep and shaded enough that, unless deliberately drained, they hold water all year round.

Phyllomedusa trinitatis breed throughout the rainy season, from late May or early June and continue to the end of the rainy season in December (Kenny 1966). Males call from perches on vegetation from about 20:00 h (sunset is around 18:30h) and remain there for up to 5 hours; calls are quiet and sporadic in this species, unlike the loud continuous calls made by many chorusing frogs. Frogs are located by their call or visually using a head torch: the reflection of torchlight in their eyes helps to find the frogs, which otherwise remain well hidden amongst the leaves.

Once a pair has begun amplexus, they move together to a site suitable for nest building, generally overhanging standing water. Eggs and jelly capsules are deposited in a coherent mass on a leaf, with jelly plugs above and below the eggs. The leaf, often along with other leaves, is folded around the egg mass to enclose it, with generally only narrow openings leading to the jelly plugs above and below. The frogs then leave the 'nest'. Hatching into the water below occurs after about seven days (Downie *et al*. 2013).

Breeding Site Attendance

We made a preliminary survey of attendance patterns over 22 nights from 05 June to 06 July 2015. This was repeated more intensively in 2016 (53 consecutive nights from 14 June to 05 August) and in 2019 (56 consecutive nights from 09 June to 03 August). In 2016, surveys were conducted by a team of four observers, all of whom received training in call recognition and frog handling. A similar team surveyed in 2019. In 2016, surveys beginning at 23:00 h were completed by 01:00 h, at which time observations indicated that frogs began to leave the site. However, observations extended later when pairs in amplexus were found. Ponds were surveyed in the same order each night: P1, then 2, then 3, but it was not possible that year to identify every frog at P3 because of the density and height of the vegetation. Because of this, only P1 and P2 were included when analyzing data from 2016. In 2019, ponds were surveyed in the reverse order, P3 then 2 then 1 and the vegetation was less dense, allowing a more complete record to be made of the frogs at P3. Because there was a focus in 2019 on breeding success, a major effort was made to locate and record all mating pairs and completed nests. This required a more intensive survey strategy: each pond was visited several times over the night from 21:00 h to 03:00 h, to ensure that each frog had been located.

Once located, each frog was removed from its perch and its throat markings, as described by Smith *et al*. (2019), were photographed and measured in the field. Its snout–vent length (SVL) was measured using dial calipers (accurate to 0.1 mm) and its mass measured using an electronic balance (accurate to 0.1 g). In 2016, frogs were held for photography by one observer, with the ventral surface facing upwards to allow it to be photographed by a second observer. In 2019, we found that the frogs would perch on the fingers of an open hand, with their throat markings easily visible for photography. This method appeared to be less stressful for the frogs. Photographs were taken using a flash, to ensure a standardized level of illumination, at as similar an angle as possible to allow comparability of images. Photographs were then stored electronically. Depending on the time of night when observations were completed, identification of individual frogs was made back in the laboratory that night or next morning, using the accumulating database of photographs. It was not always possible to identify frogs because, on occasion, the photographs were too indistinct. In addition, particularly at P3, a few frogs perched in inaccessible places and could not that night be identified. In both years, frog-handling time lasted for always less than one minute. After all records were taken, each frog was returned to its original perch.

In order to assess whether individual frogs exhibited pond and perching site fidelity, a map was made of each pond and the location where each frog was found each night was noted. The maps showed pond length measurements and the positions of landmarks such as plants and rocks; heights above water were measured with a measuring tape to the nearest 1 cm.

Breeding Success

When a pair in amplexus was seen, it was checked every 10 minutes. Once the male had disengaged and moved away, he was captured to record his identification, mass and SVL. The female was observed until she had completed the nest: she was then captured and her biometric and identification data were also recorded. Each nest was flagged with an orange ribbon and left in place for six days. It was then collected and brought to our laboratory where incubation continued at ambient temperature until hatching, with the nest suspended above a plastic tank containing water to a depth of 4 cm. After hatching, the numbers of live tadpoles, dead tadpoles and undeveloped eggs were recorded, and live tadpoles were returned to their pond of origin.

General Observations

Any notable observations, such as aggressive encounters between males, presence of potential predators, and presence of metamorphosing individuals were noted during the general surveys.

Weather Records

In 2016, temperature and relative humidity were recorded each night when surveys began using a digital hygro-thermometer (Xintest HT-IL). Rainfall was assessed qualitatively as zero, low, medium and high. On 'high' rainfall days, the rain lasted for five or more hours, with varying levels of intensity; 'medium' rainfall involved a shower or showers lasting 2–4 hours; 'low' rainfall included showers of 1–2 hours; 'zero' could include a trace of rain from a shower lasting a few minutes only. In 2019, temperature and relative humidity were not measured, but a rainfall gauge was set up in the Centre's garden, in a location unaffected by trees. Rainwater was collected through a 12 cm diameter funnel and measured each morning at 09:00 h, then the volume converted to a daily value in mm.

Data Analysis

Male body condition was calculated by dividing body mass by SVL (Jayson *et al*. 2018). All statistical analyses and model selections were conducted in R Studio version 3.6.1 (R Core Team 2019). A one-way ANOVA was conducted to compare the breeding site attendance over the first and last 20 days of surveying in 2016 and in 2019 respectively. Male body condition was analyzed using a t-test to compare the body condition of males that attended the breeding site for only one day with males that attended for five or more days in 2019. One-way ANOVA's were used to compare the number of frogs in attendance and the amount of rain (mm) that had fallen on the night of surveying, the number of appearances of individual frogs and the number of nests fathered by each frog and, finally, the number of tadpoles hatched from each nest compared to the body condition of the father.

Results

Male and Female Attendance

In 2016, over 53 consecutive nights we identified 43 adult males and 17 adult females. Of these, four males and two females had been encountered among the 54 frogs identified during the pilot study in 2015. In 2019, we identified 47 males and 17 females, with no frogs from the 2016 survey re-appearing. The operational sex ratios (OSR) were 0.40 (2016) and 0.36 (2019) respectively. Numbers and operational sex ratio (ratio of females to males at the breeding site during the study) were similar over the two years, although we might have expected more individuals in 2016 because we surveyed all three ponds that year, rather than just two as in 2019.

In both years, there was considerable variation in the number of nights each male attended, with some appearing once only and others many times (Table 1). In 2016, one frog appeared 36 times, 68% of the surveyed nights; the highest attendance in 2019 was 15 nights (29%). Of the male frogs first encountered in 2015, two were frequent attenders in 2016 (36 and 16 nights) whereas two were seen only two and three times respectively. These 2015 frogs all first appeared during the first week (14 to 18 June) of the 2016 study. A limitation regarding our data is the number of unidentified frogs, 2.8 per night on average in 2016. This was due either to poor photographs or to some frogs being inaccessible. There were also unidentified frogs in 2019, although they were not counted due to the different objectives of each sampling season.

Table 1. Frequencies of breeding site attendance by male and female *Phyllomedusa trinitatis* at Simla over two years. The first column gives the numbers of nights attended, and the 2016 and 2019 columns show the numbers of frogs in the two years that attended for the different numbers of nights.

Number of nights	2016	2019
Males		
1	12	15
2	4	4
3	2	8
4	2	1
5	3	4
6	-	5
7	1	2
8	1	2
9	1	-
10	1	1
11	1	1
12	1	-
13	1	2
14	2	-
15	-	1
16	3	1
17	2	-
20	2	-
23	1	-
27	1	-
30	1	-
36	1	-
Total	**43**	**47**
Females		
1	11	13
2	5	2
3	1	2
Total	**17**	**17**

In 2016, most males were faithful to a particular pond. Of the frogs that attended more than once ($N = 38$), 25 appeared only at P1 and five at P2, with eight frogs appearing at both ponds on different nights (six predominantly at P1; only two mainly at P2). In 2019, of the males which attended more than once ($N = 32$), 22 appeared at only one pond; the other 10 appeared at two ponds, but never at all three, and usually with a strong preference for one of the ponds. For example, one frog appeared 15 times at P3, and once at P1; another, 12 times at P1 and once at P3. Of the frogs that attended at more than one pond, five used P3 and 2; four used P1 and 3; and one used P1 and 2. We did collect data on the perch sites used by male frogs, in order to test whether they showed strong positional preferences, but we have not presented those data here.

The overall data on male attendance frequencies become more revealing when we analyze individual attendance patterns. In both years, similar patterns were seen in the first appearances of individual males, with about half of the frogs first appearing in the first 10 days, and the remainder at some point over the next six weeks (Table 2). Clearly, for late arrivals, the opportunities for multiple attendances during the study period were limited. For example, in 2016, two frogs were first seen on the final night of the study period, and so are reported in Table 1 as appearing only once. However, some frogs were first recorded early in the study period and only attended once (six frogs in the first 10 days in 2016; seven in 2019); this is considered in the Discussion.

The patterns of attendance by those frogs attending more than once were highly variable (Table 3). Some attended every night over a substantial period (the longest was for a 2016 frog; 19 nights). Others interspersed long gaps in their attendance sequences (one 2016 frog had 15 and 20 day gaps in an attendance sequence of seven appearances over 46 nights). Table 3 shows that some frequent attenders were being recorded until near the end of the study period, while others showed a substantial number of absent nights after their final appearances.

Table 2. Proportions (%) of male *Phyllomedusa trinitatis* appearing for the first time at the Simla ponds over the progress of the studies in 2016 and 2019, with study days divided into 10-day periods. 2016, *N* = 43; 2019, *N* = 47 male frogs.

Year	**Study days**					
	1–10	11–20	21–30	31–40	41–50	51–end
2016	57	25	12	3	0	3
2019	49	30	7	7	2	5

Table 3. Attendance patterns for male *Phyllomedusa trinitatis* attending the Simla ponds on 10 or more occasions over the two years.

Frog ID code	**Number of appearances**	**Days from first to last appearance**	**Longest gap**	**Days from last appearance to study end**
2016				
91	30	41	5	12
81	24	49	6	4
95	16	44	12	9
109	15	24	4	29
71	12	48	15	4
94	36	49	5	3
99	12	33	13	18
112	20	51	8	0
56	27	49	9	0
86	15	38	24	1
99	14	22	4	27
91	16	40	13	8
113	17	18	2	30
111	17	46	7	1
71	20	46	5	0
63	12	39	22	4
72	11	27	9	0
2019				
6	10	39	17	16
3	13	37	8	18
1	16	51	9	3
5	11	47	15	3
2	15	31	5	18
4	13	39	8	7

As expected, attendance by individual females was much less frequent than for males (Table 1). Most appeared only once, but in both study years, a few attended two or three times. When a female attended twice many days apart, the interval between appearances ranged from 19 to 42 days (mean ± SD: 27.6 ± 7.0, $N = 7$). In two cases, females appeared two or three times in a short period. In the first, the female appeared on three successive nights (nesting not recorded); in the second, the female appeared twice three nights apart and produced two egg clutches, the first of 323 eggs, the second only 119.

Factors Influencing Male Attendance Patterns

Season Progression.—Table 4 shows changes in male and female attendance over the study period in both years. In 2016, there was a clear decline in the mean nightly attendance by males, when we compare the first 20 days with the final 20 days (ANOVA: $F_{(1,38)}$: 58.05, Pr(<F): > 0.0001), but there was no statistically significant trend in 2019 (ANOVA: $F_{(1,38)}$: 1.76, Pr(<F): 0.19), when nightly attendance was never as high as in the early days in 2016. The number of females declined over the course of the study in both years, while the number of nights with zero females increased with time.

Predation.—We never observed a predation event on any of the frogs, but we did observe potential predators. *Bothrops asper* (Garman, 1883) (Fer-de-lance; Murphy 1997), including large and small individuals, were seen close to all three ponds, active at night on eight occasions in 2016 and on four occasions in 2019. Another unidentified snake was seen moving around the edge of a pond in 2019. Individuals of the large teiid lizard *Tupinambis teguixin* (Linnaeus, 1758), known locally as the Tegu Lizard (Murphy 1997), were also frequently observed at the site in all years**.**

Male Body Condition.—We analyzed body condition in relation to days since first appearance in the six males that attended 10 or more times over the study period in 2019. Body condition ranged between 0.2 and 0.3 g/cm, but no clear pattern emerged; in particular, there was no sign of a decline over time or appearances. Another possible role for body condition could be that males embarking on a series of appearances are in better condition than those electing to appear only once. However, a comparison of initial body condition of the males which appeared five or more times with those which appeared only once in 2019 found no significant difference (t-test: $T_{(27.837)}$: -0.09, $p = 0.92$).

Weather.—In 2016, temperatures at night mostly ranged from 23 to 25°C, very occasionally

Table 4. Mean male and total female *Phyllomedusa trinitatis* attendance numbers at Simla as field seasons progressed (divided into consecutive 10-day periods: total nights = 53 in 2016; 56 in 2019).

Study period (nights)	Mean nightly males		Total females		Nights with zero females	
	2016	2019	2016	2019	2016	2019
1–10	13.9	4.7	10	10	4	4
11–20	13.6	3.3	10	6	5	7
21–30	11.4	4.0	8	5	6	7
31–40	5.1	5.6	0	7	10	7
41–53(56)	6.3	2.3	5	1	9	12
Totals			**33**	**29**	**34**	**37**

reaching 28°C. Relative humidity was always high, usually ranging between 80–100%. Daily rainfall ranged from zero (24 days), to low (9 days), medium (7 days) and high (12 days). There was no statistically significant relationship between any of these variables and the nightly number of males attending**.** In 2019, rainfall ranged from zero or trace (22 days) to 45 mm. Only four days over the study period had rainfall over 20 mm. There was a significant positive relationship (ANOVA: $F_{(1,51)}$: 8.58, Pr(<F): 0.005) between the nightly total of attending frogs (males and females) and rainfall. However, it was noticeable that after the heaviest day of rainfall, only one frog appeared, suggesting that exceptional rainfall may deter frogs, while moderate amounts attract them. There were two sequences of several days (3, 4) without rain, and on both occasions, no frogs attended on the night following such a sequence.

Breeding Success, Competition and Tadpoles

In 2016, 32 cases of amplexus were recorded, but male identity was not known in enough cases to allow for an analysis. In 2019, we were able to relate 19 nests to particular males. Of these, one male had three successes and three had two, and there was a significant positive relationship between appearances at the breeding site and nest number (ANOVA: $F_{(1,29)}$: 7.44, Pr(<F): 0.01). However, if we relate the likelihood of nesting to the number of appearance nights for the male population overall (number of nests made, divided by the number of frogs making those nests multiplied by the number of nights these frogs attended the breeding site), the likelihood of success was as high or higher for frogs which appeared only once as it was for frogs which attended often (Table 5).

Interference in amplexus by additional males was observed both in 2016 and in 2019. In 2019, from 22 amplexus events recorded, nine (41%) were subject to interference by one or more additional males with the second male dislodging the first on at least one occasion. In addition, pseudo-amplexus between groups of two or more males (up to six males) was occasionally seen.

We recorded the number of tadpoles produced in 19 nests (range 54–487) where we knew the identity and body condition of the male. The data suggested a positive relationship between body condition and tadpole number (ANOVA: $F_{(1,17)}$: 4.09, Pr(<F): 0.059). In 2019, *Phyllomedusa trinitatis* breeding had occurred before our arrival on site in early June, since tadpoles were visible in P1 by then. Metamorphic individuals were observed sitting on leaves around the pond by 22 July (Figure 1).

Table 5. Relationship of breeding site attendance by male *Phyllomedusa trinitatis* at Simla to number of nests produced by these frogs (2019).

Appearances (a)	Number of frogs (b)	Number of nests (c)	Nests per attendance night (c/ab)
1	14	3	0.21
2	4	1	0.13
3	8	1	0.04
4–5	5	2	0.08
6	5	2	0.07
7–8	4	4	0.13
10–16	6	6	0.08
15	1	3	0.20

Figure 1. Metamorphosing *Phyllomedusa trinitatis* on a leaf beside a pond. Both images taken at the William Beebe Tropical Research Centre (Simla).

Discussion

There are few previous published studies on the breeding strategies of phyllomedusine frogs, and even fewer that have followed the behavior of identified individuals over extended times. In our study, we used a naturally occurring individually variable throat marking in *P. trinitatis* (Smith *et al*. 2019) to follow the reproductive behavior of individuals in a set of three ponds, all within 60m of each other. We followed frog attendance patterns for 22 nights in a pilot year (2015), then over 52 consecutive nights in 2016 and 56 nights in 2019. A small number of both males and females first identified in 2015 reappeared in 2016, but not in 2019, providing some estimate of longevity.

Female Attendance

Since at least some males attended each pond every night, females could be sure to encounter a potential mate. Considering that we recorded cases of females attending on two nights in succession, it is likely that females exercise some choice, and attend on a second night following a lack of success. The example where a female laid two clutches, the first large and the second smaller, only three days apart, may be a case of a female exercising choice as a reproductive strategy. We also recorded seven females over the two main study years that attended twice with a mean interval of 27.6 days. This provides an estimate of the inter-nesting interval in these frogs, on the assumption that females only attend the breeding site when ready to spawn that night or soon thereafter (we observed spawning in some but not all of these cases). This is a variable that has rarely been reported in phyllomedusines. Kenny (1966) reported a marked female *P. trinitatis* that bred at least three times over a season (June, July and October), but he did not record the inter-nesting intervals. Wogel *et al*. (2006) reported intervals of 26, 32 and 60 days between matings by three female *Pithecopus rohdei* (Mertens, 1926), but since they did not monitor the population every night, we cannot be sure that all these figures represent inter-nesting intervals. The ability of female anurans in continuous mating systems to produce multiple clutches over a breeding season has not often been measured.

The operational sex ratios (OSR) in our study were 0.40 (2016) and 0.36 (2019) respectively. Wogel *et al*. (2006) reported an OSR of 0.48 for *P. rohdei* and Oliveira *et al*. (2012) 0.42 for *Pithecopus megacephalus* (Miranda-Ribeiro, 1926), both similar to our results, with over twice as many males as females. The exceptional result is that of Borges *et al*. (2018) with an OSR of only 0.09 in their study on *Pithecopus ayeaye* Lutz, 1966. In our study, females were absent from the set of ponds on 64% (2016) and 66% (2019) of nights respectively, similar to the proportion (62%) in the study of Wogel *et al*. (2006) on *P. rohdei.*

Male Attendance

Pond fidelity was high, with only a minority of frogs occasionally appearing at a second pond. Attendance frequency by individuals was also highly variable, with a few attending on many nights over the study period, but others only occasionally and some only once. A study by Green *et al.* (1990) tracked the movements of individual Túngara frogs [*Engystomops pustulosus* (Cope, 1864)] over 10 days during the breeding season. They found that some calling occurred every night over the study period, but that individual males varied greatly in their attendance patterns: some called each night over an 11-day period; others called on as few as three nights, a result that agrees with our findings. The results of a feeding experiment suggested that individual attendance patterns were not related to energy constraints. Males tended to join larger choruses, possibly to reduce predation risk, as the likelihood of a predator choosing a given frog would be lower than in a small chorus. Green *et al.* (1990) also found that attendance was influenced by rainfall and temperature.

We found no influence related to energy constraints, in the sense that changes in body condition showed no relationship to attendance. We have no data on calling, but our experience is that *Phyllomedusa trinitatis* calls are quiet and infrequent and sporadic enough not to be useful in locating individuals and would not result in high rates of energy expenditure (JRD pers. obs.). Results on breeding site fidelity from other studies on male phyllomedusine frogs also generally show high but not complete fidelity (Wogel *et al*. 2006, Dias *et al*. 2017, Borges *et al*. 2018), suggesting that the unpredictable attendance of females may lead to variable behavior amongst males. This could be particularly the case where several breeding sites occur over a relatively small area, as at our site at Simla.

In one year, we found a positive correlation between rainfall and attendance, while sequences of days with no rain resulted in a drop in attendance. Neither temperature nor humidity appeared to have any influence, but these are rather stable at the study site, with little variation in nighttime temperature, and humidity always high. Rodrigues *et al*. (2007) found an interesting difference in the relationship between matings and weather in a comparison between *Pithecopus azureus* (Cope, 1862) and *Phyllomedusa sauvagii* Boulenger, 1882. *Pithecopus azureus* were found to mate throughout the rainy season irrespective of actual rainfall, whereas *P. sauvagii* only mated on nights following heavy rain, or the one to two following nights.

When considering factors responsible for the observed attendance patterns, one possibility is that as the breeding season progresses, the number of attending males declines. If this were so, the underlying causes could be predation, or anticipated lack of females, or some factor related to changes in body condition, for instance sperm depletion. Frogs appearing for the first time late during the study seasons, in late July or early August, were most likely recruits to the adult population from previous years. We do not know the time it takes to reach adulthood in this species in the wild, but Smith *et al*. (2019) reported that captive-reared individuals could reach adulthood about one year after metamorphosis. If this is the case in the wild, recruits from the previous year should be entering the adult population from late July onwards. The finding of the first metamorphs from 2019 spawning in mid-July fits with this scenario. Indirect support for this argument can be drawn from the fact that all the frogs identified in 2015 that re-appeared in 2016 did so within the first week of the study. Another explanation could be that after attending a few times individuals then dispersed to other breeding locations, but as there are no other breeding sites located locally and individuals that appeared once or only a few times were not seen in subsequent years (unpubl. data) we believe this explanation is unlikely. A more likely explanation is predation. We never observed predation on

Phyllomedusa trinitatis, but snakes known to prey on frogs (*Bothrops asper;* Farr and Lazcano 2017) and Large Tegu lizards (*Tupinambis teguixin;* Murphy 1997) were commonly encountered in the vicinity of the ponds, and other predators are possible, such as owls and bats: the frog-eating bat *Trachops cirrhosus* (Spix, 1823) occurs in Trinidad, although *P. trinitatis* adults are larger than its usual prey (Gomes and Reid 2015). Ffrench (1991) lists several owl species that occur in Trinidad as preying on frogs. We suggest that predation is the most likely explanation for male frogs that attended once or a few times near the start of the study periods, and never again over several weeks. .

Mating Success and Competition

Several previous studies have demonstrated a relationship between breeding site attendance and male mating success. Ryan (1983) found males attended on average 7.2 nights over a period of 43 days, and the more frequent attenders achieved the most matings. Greer and Wells (1980) followed male glass frog attendance over 30 nights: there was a significant positive relationship between attendance nights and the number of successful matings. Glass frog attendance patterns showed some similarities to our findings on *P. trinitatis:* some individuals attended on up to 10 consecutive nights; others left the area for 1–18 nights before returning to their original territories. Although we found a relationship between attendance and mating success, it was not at all clear that frequent attendance was the best strategy.

We found that amplectant pairs were often interrupted by one or more additional males which either joined in the amplexus or attempted to dislodge the original male. We do not have data on how often these efforts were successful in terms of fertilizing eggs. Presumably, the occurrence of such competition events is related to the number of unmated males in the vicinity when a pair forms. This kind of interference competition has often been reported in other phyllomedusine species (Wogel *et al*. 2005, Oliveira *et al*. 2012, Dias *et al*. 2017), where attempts to displace the original male have been reported as generally unsuccessful; however, we are not aware of any results indicating the relative genetic contributions of initial compared to competitor males in phyllomedusine frogs.

Conclusion and Limitations

Our study takes a deep dive into the behaviors of *Phyllomedusa trinitatis* when attending breeding sites. As our data was collected over two non-consecutive years, this gives us an interesting insight into how repeatable attendance patterns are for the individual and the overall population. We found that females will usually only attend a breeding site once but that males will often attend multiple times and show high pond fidelity, seemingly with a strong preference. We were able to identify three attendance patterns among males: attend for multiple consecutive nights, attend once, attend on multiple non-consecutive nights, but none of these attendance patterns appeared to be optimal for breeding success. We identified that rainfall does influence attendance but that the number of night attending a breeding site does not impact male body condition.

We hope that our results will stimulate further research into *Phyllomedusa* mating patterns, using the non-invasive individual recognition method reported by Smith *et al.* (2019). An avenue not explored within this paper is the link between breeding site attendance and animal personality, a subject area that we believe would lend itself well to this study system, perhaps providing an explanation for the behaviors that we see here. A limitation of our work was the number of frogs we failed to identify, caused by the density of vegetation in some parts of the habitat: however, we do not think that the missing data compromised our conclusions.

Acknowledgments

We thank staff and students on the University of Glasgow's Trinidad Expeditions in 2015, 2016 and 2019 for help with fieldwork, and multiple agencies for assistance from small grants, notably the University of Glasgow's Chancellor's Fund, Glasgow Natural History Society, Denis Curry's Charity, the Thriplow Trust and the Gilchrist Educational Trust. We also thank the Asa Wright Nature Centre for providing accommodation at Simla, and especially curators Ronnie and Rupert for their help. The Trinidad Government's Wildlife Section through Anna Bandoo kindly provided permits for our work in all three years.

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Editor: Ariovaldo A. Giaretta

Phyllomedusa 20(1):67–74, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p67-74

Efficiency of photo identification of inguinal color patterns of *Pithecopus gonzagai* (Anura: Phyllomedusidae) from northeastern Brazil

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Abstract

Efficiency of photo identification of inguinal color patterns of *Pithecopus gonzagai* (Anura: Phyllomedusidae) from northeastern Brazil. Animal monitoring research involving mark-recapture techniques increasingly requires non-invasive methods of individual identification. The photographic identification method (PIM) is an excellent tool for this purpose and has been applied successfully to many taxa. However, the utility of PIM is a function of species-specific features that are judged suitable for a given target-species. Herein, the suitability of inguinal color patterns for photo identification of individuals of *Pithecopus gonzagai* are evaluated by comparing two widely used computer-assisted photographic matching programs (I³S and Wild.ID). Both programs accurately identified more than 70% of individuals in the top 20 potential matching photographs. Wild.ID was slightly better than I³S in matching efficiency and has a faster processing time. Thus, PIM is useful to identify individual *P. gonzagai;* however, before implementing the technique in animal-monitoring studies of other taxa, one must evaluate the suitability PIM for the target species and calibrate the relative efficiency of the software programs in identifying individuals.

Keywords: Anurans, individual identification, mark-recapture method, I³S, Wild.ID.

Resumo

Eficiência da fotoidentificação dos padrões de cores inguinais de *Pithecopus gonzagai* (Anura: Phyllomedusidae) do nordeste do Brasil. A pesquisa de monitoramento de animais envolvendo técnicas de marcação-recaptura requer cada vez mais métodos não invasivos de identificação individual. O método de identificação fotográfica (PIM) é uma excelente ferramenta para esse propósito e tem sido aplicado com sucesso a diversos táxons. No entanto, a utilidade do

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Received 05 February 2020
Accepted 05 May 2021
Distributed June 2021

PIM é em função de características específicas da espécie que são julgadas adequadas para uma determinada espécie-alvo. Aqui, a adequação dos padrões de cores inguinais para identificações fotográficas de indivíduos de *Pithecopus gonzagai* é avaliada comparando dois programas amplamente usados de correspondência fotográfica assistida por computador (I³S e Wild.ID). Ambos os programas identificaram com precisão mais de 70% dos indivíduos nas 20 melhores fotografias possíveis. Wild.ID foi ligeiramente melhor do que I³S em eficiência de correspondência e tem um tempo de processamento mais rápido. Assim, PIM é útil para identificação individual de *P. gonzagai*; entretanto, antes de implementar a técnica em estudos de monitoramento animal em outros táxons, deve-se avaliar a adequação do PIM para as espécies-alvo e calibrar a eficiência relativa dos programas de software na identificação de indivíduos.

Palavras-chave: anuros, identificação individual, método de recaptura de marca, I3S, Wild.ID.

Introduction

Individual recognition is essential to several kinds of biological studies, such as faunal monitoring, population demography, and behavioral ecology. However, to select the most appropriate way to identify individuals of a target species, one must carefully evaluate the benefits and disadvantages of various techniques for the specific system; biological constraints, financial costs, and any inherent ethical issues associated with a given taxon should be considered (Langkilde and Shine 2006, Caorsi *et al.* 2012). For example, despite the widespread usage of toe clipping and subcutaneous implants (elastomer or tags) in anurans (Brow 1997), these methods are invasive and controversial because their use may be harm the frog, especially in arboreal species in which adhesive discs are essential to perch (e.g., Clarke 1972, May 2004, Funk *et al.* 2005).

The Photographic Identification Method (PIM) is a useful, non-invasive marking alternative that can be used to distinguish individuals of species that possess characteristic features or natural markings (Würsig and Würsig 1977, Long and Azmi 2017). Many computer-assisted systems (pattern recognition or photographic matching software) have been developed that enable researchers to process large numbers of photographic images in relatively short timeframes. Thus, due to its biological, logistic, financial and ethical advantages, PIM has been increasingly used in research studies of an array of taxa, such as insects and sea stars (Chim and Tan 2012, Caci *et al.* 2013), sharks and rays (Tienhoven *et al.* 2007, Marshall and Pierce 2012), marine and freshwater teleost fishes (Correia *et al.* 2014, Dala-Corte *et al.* 2016), aquatic and terrestrial mammals (Kniest *et al.* 2010, Bolger *et al.* 2012), and turtles and lizards (Knox *et al.* 2013, Long and Azmi 2017), as well as toads and salamanders (Gamble *et al.* 2008, Caorsi *et al.* 2012).

Among anurans, hylid treefrogs comprise one of the largest families, with more than 750 described species (Frost 2018). Hylidae, Pelodryadidae (ca. 210 sp.), and Phyllomedusidae (ca. 70 sp.) compose the Arboranae lineage, which includes primarily arboreal species (Duellman *et al.* 2016). Many arboranan species have distinctive color patterns on the flanks, groins, or legs; these patterns do not change and thus, are suitable to track individuals in mark-recapture studies (e.g., Kenyon *et al.* 2009, Del Lama *et al.* 2011, Kim *et al.* 2017). We choose the arboreal phyllomedusid *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo, and Bruschi, 2020 [previously included with *P. nordestinus* (Caramaschi, 2006) (Caramaschi 2006, Vilaça *et al.* 2011, Andrade *et al.* 2020)] as a target species because its morphological, ecological, and ethological features make it an excellent model for behavioral studies (Brasileiro *et al.* 2020, 2021). First, like most phyllomedusids, *P. gonzagai* has contrasting and colorful inguinal patterns (Figure 1) which make it highly suitable for individual identification (Oliveira *et al.* 2012). Second, individual frogs are easily habituated to humans, allowing for detailed recording of several stereotyped behaviors (e.g., fighting, Brasileiro *et al.* 2020). To validate the suitability and efficiency of PIM in *P. gonzagai,* we (1) assessed the use of inguinal color patterns in this species as a natural mark for individual identification and (2) evaluated the relative efficiency of two popular photographic image-matching programs for recognition of individual frogs.

Materials and Methods

Fieldwork was carried out in a temporary pond (03°52'4.02" S, 40°22'12.06" W; WGS84; 110 m a.s.l.; 1.058 m² of perimeter) belonging to the Rio Acaraú Basin, in the municipality of Groaíras, state of Ceará, northeastern Brazil. The study site is dominated by herbaceous vegetation (Figure 2) but is surrounded by open phytophysiognomies typical of the Caatinga Domain, including thorny and deciduous shrublands, as well as Carnaúba [*Copernicia prunifera* (Mill.) H.E.Moore] forest (Moro *et al.* 2015). Data were collected from February–June 2017. We captured individual *Pithecopus gonzagai* by active visual and acoustic searches around the margins of ponds where the tree frogs vocalized and spawned from 18:00–00:00 h, for a total of 37 h of sampling effort. The specimens were captured at random throughout the sampling period. For each frog captured/recaptured, one of us (FLA) took standardized photographs of the flanks with the inguinal color-pattern exposed (Figure 1). We released the individuals at their site of capture. Photographs were taken with a Sony DSC HX200V digital camera (f/4.5, 1/160s, ISO-400). All the sampling procedures were performed under authorization of Brazilian environmental agencies (ICMBio license nº 13587).

Figure 1. Inguinal color pattern of *Pithecopus gonzagai* composed of black stripes on an orange background. The region used for individual identification is delimited with a rectangle.

We selected the best photograph of each frog based on the focal quality and light exposure (but including slightly different angles) and cropped the images to encompass only the left inguinal region (Figure 1). The images were then compiled in a catalog to be submitted to matching process as follows. First, we performed a visual identification method (VIM) in which three researchers did a pairwise comparison of each photograph with the entire catalogue without any computer assistance. Second, we performed computer-assisted PIM, submitting the catalogue to two independent photographic image-matching programs, I³S (Tienhoven *et al.* 2007) and Wild.ID (Bolger *et al.* 2012).

The I³S software—Interactive Individual Identification System (available at http://www.reijns.com/i3s/)—delimits key-points of matching in each image, and presents a list of potential matching photographs, from which the user can identify the exact matching; see application in Town *et al.* (2013). The Wild.ID software (available at http://wildid.teamnetwork.org/index.jsp) automatically performs pattern recognition and matching, and also presents a rank of 20 most similar photographs classified according similarity; see application in Dala-Corte *et al.* (2016). The subset of the 20 most likely matches generated by each program was followed by inspection by human observers to evaluate a possible correct match in the lists.

For analytical purposes, we assumed that VIM precisely recovered all the recaptures of

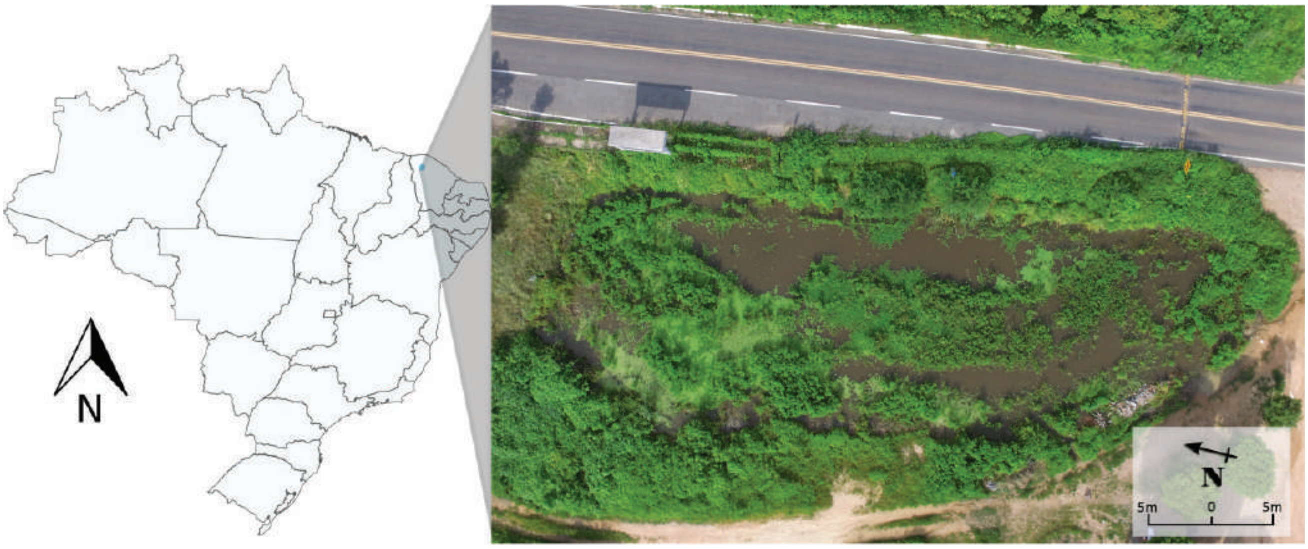

Figure 2. Location of the study site, a temporary pond at the municipality of Groaíras, state of Ceará in northeastern Brazil.

our dataset. To evaluate the efficiency of software tested in the PIM of *Pithecopus gonzagai,* we compared the performance of each program with respect to two criteria: (1) the rate of correct matching (number of correct identification/total number of recaptures by VIM); and (2) how long the image processing took (the average time in seconds to import the photographs from the catalog and to select the area of interest—i.e., the inguinal region). The rate of correct identification was calculated based in the entire catalogue (123 photographs), checking if each target photograph was included among the 20 first potential matching photographs provided by both programs. The duration of image processing was calculated for each program based in average time spent to import and select the inguinal region of 60 images.

Results

We recorded 26 recaptures by VIM; therefor, this total was used as the reference to compare the efficiency of the photographic image-matching programs. The rates of correct matching were quite similar. The Wild.ID (Figure 3) calculated 76.9% (N = 20 recaptures) correctly, and the I^{3}S (Figure 4) calculated 73.1% (N = 19 recaptures) correctly.

Image processing in Wild.ID was much faster than in I^{3}S. The Wild.ID took 105 seconds to import the 60 photographs (average of 1.75 seconds per image), whereas I^{3}S required more than 100 minutes (average of 100.72 seconds per image).

Discussion

Inguinal color patterns of *Pithecopus gonzagai* can be used to identify individual frogs, thereby allowing the implementation of photographic identification methods. Visual identification without computer assistance may be more accurate than some computer-assisted programs (Kim *et al.* 2017); however this technique requires so much time to analyze a large database, that it is practically unfeasible (Markowitz *et al.* 2003, Carter *et al.* 2014). Thus, many authors recommend its use as a supplement to computer-assisted systems of pattern recognition (Gamble *et al.* 2008, Del Lama *et al.* 2011).

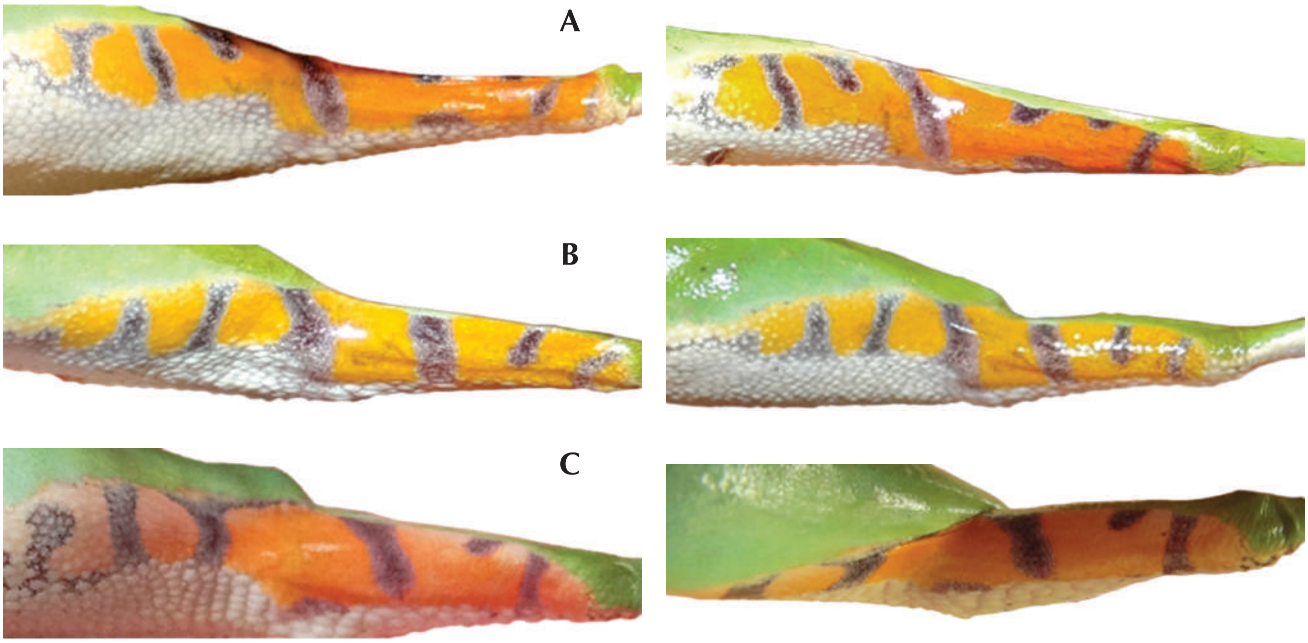

Figure 3. Results of three recapture matches with Wild.ID. Images in the left column correspond to focal images, and those in the right column correspond to the images with a high level of correspondence. Images (**A**) and (**B**) have the best level of correspondence (level 01 of 20), whereas image (**C**) has a relatively low level of correspondence (level 18 of 20), probably a result of the angle of the photograph.

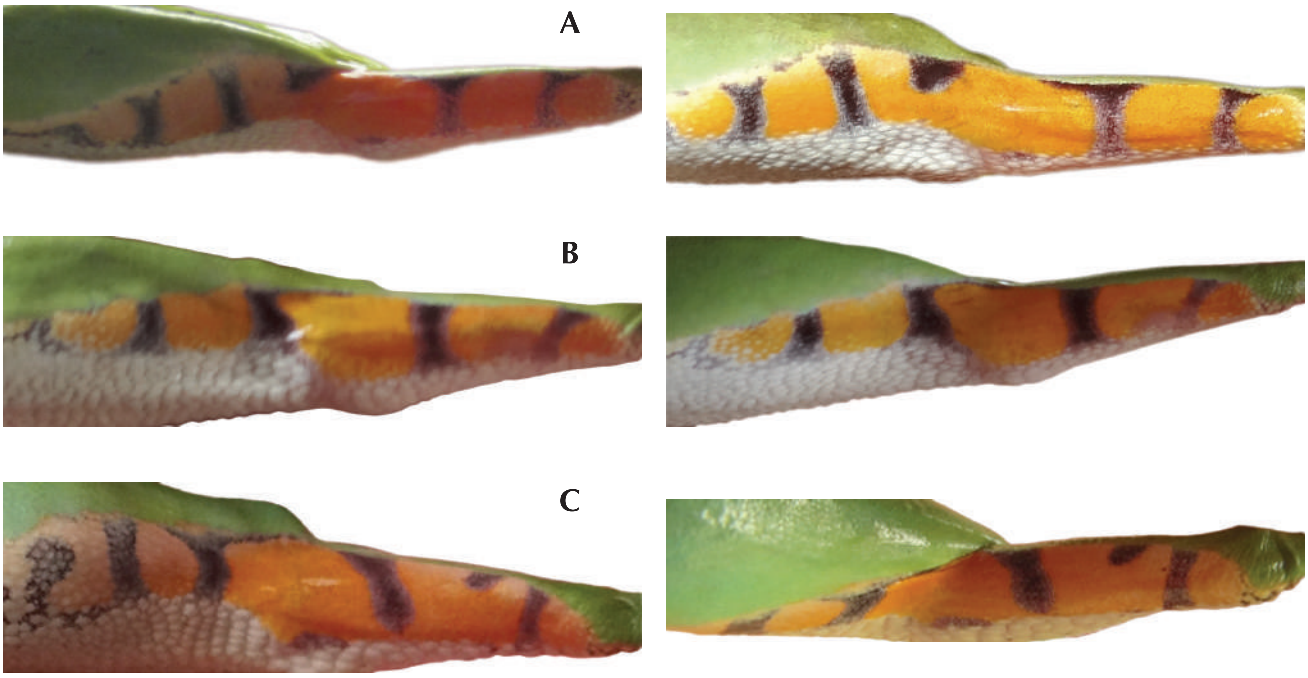

Figure 4. Results of three correspondence recaptures in the I^{3}S software. Images are presented as in Figure 3. Images (**A**) and (**B**) matching the best, whereas image (**C**) had a relatively low level of correspondence, probably due to the difference of angulation.

The two photographic image-matching programs that we tested identified correct recaptures in the 70% range, but with a performance difference of 3.8%. The 70% value is reasonably efficient and and about the same as those reported in other studies of the performance of computer-assisted photographic identification (e.g., Kenyon *et al.* 2009, Beirão *et al.* 2014). Although both programs only performed at the 70% level, the enhanced processing speed is beneficial. Thus, the use of PIM software seems to be a suitable alternative for many scientific purposes (e.g., avoidance of pseudo-replication).

The Wild.ID software is much more efficient than I³S because it imports photographs so much more rapidly than does I³S (6946% slower than in Wild.ID), thereby decreasing the amount of time significantly to analyze the dataset. Several other studies also have commended the processing speed of Wild.ID relative to that of other software (Bendik *et al.* 2013, Halloran *et al.* 2015).

We conclude that both Wild.ID and I³S are suitable to identify individual *Pithecopus gonzagai* by the inguinal color pattern of the thighs. The results reinforce the suitability of photographic identification methods for studies of phyllomedusid frogs (e.g., Oliveira *et al.* 2012, Oliveira 2017). However, it is worth noting that some photographic variables, such as photo angle and exposure, can adversely affect the accuracy of the software performance. We recommend standardizing the photography procedure to eliminate variants that will affect the performance of the software. Additionally, researchers should consider the sizes of their datasets. If the sample is large, then the speed of Wild.ID is advantageous, whereas I³S is adequate for smaller samples.

Acknowledgments

We thank the Instituto Chico Mendes de Conservação da Biodiversidade for the collect permit (license nº 13587). FLA is also grateful to the class 2017.2 of the Comunicação Científica course of the Programa de Pós-Graduação em Ecologia e Conservação, Universidade Federal Rural do Semi-Árido (UFERSA) for their contributions to an early draft of this manuscript. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001 (scholarship).

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Editor: J. P. Lawrence

Phyllomedusa 20(1):75–88, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p75-88

Feeding habits of *Lithobates megapoda* (Anura: Ranidae), a threatened leopard frog used for human consumption, in Lake Chapala, Mexico

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Abstract

Feeding habits of *Lithobates megapoda* (Anura: Ranidae), a threatened leopard frog used for human consumption, in Lake Chapala, Mexico. The Big-footed Leopard Frog (*Lithobates megapoda*) is a threatened and endemic species from western Mexico. This species has aquatic habits and it is distinguished by its large size, particularly by the length of its legs, reason for which it is captured for human consumption. Also, knowledge about its natural history is scarce and incomplete. In this study, we analyzed the composition of the diet of *L. megapoda* on the shore of Lake Chapala, the largest lake in Mexico. A total of 69 adult individuals were collected lifeless in fishing nets, during the rainy season (June-October), of which 48 had stomach contents. A total of 96 prey items were identified, which correspond to 13 prey categories. Fish constituted the most dominant prey category in the diet in terms of number, volume, frequency of occurrence, and relative importance. No significant differences were found in the consumption by prey type (aquatic or terrestrial). However, the aquatic preys had a greater relative importance and were more voluminous than the terrestrial ones. The diversity of prey categories, in terms of prey volume, indicates males may have a higher dietary diversity than females, but we not evaluated possible bias. In addition, a significant effect was found in the interaction of size (SVL) of frogs with the average of prey volume. Females (that are larger than males) consumed prey within a wide volume range and, the larger they are, more voluminous are prey. In this way it is possible that intraspecific competition for trophic resources in the environment is reduced. This study helps us understand the trophic ecology of *L. megapoda*, a frog species that plays an important role in the food web where it lives, as a predator feeding on aquatic and terrestrial organisms.

Keywords: Amphibians, diet, fish, intersexual variation, trophic ecology.

Received 27 March 2020
Accepted 15 June 2021
Distributed June 2021

Resumen

Hábitos alimenticios de *Lithobates megapoda* (Anura: Ranidae), una rana leopardo amenazada utilizada para consumo humano, en el Lago de Chapala, México. La rana patona (*Lithobates megapoda*) es una especie endémica y amenazada del occidente de México. Esta especie es de hábitos acuáticos y se caracteriza por su gran tamaño, particularmente por la longitud de sus ancas, razón por la cual es capturada para consumo humano. Además, el conocimiento sobre su historia natural es escaso e incompleto. En este estudio, se analizó la composición de la dieta de *L. megapoda* en las inmediaciones del Lago de Chapala, el lago más grande de México. Un total de 69 individuos adultos fueron recolectados sin vida en redes de pesca, durante la época de lluvias (junio-octubre), de los cuales 48 tuvieron contenido estomacal. En total se identificaron 96 presas, las cuales corresponden a 13 categorías de presa. Los peces constituyeron la categoría más valiosa en la dieta en cuanto a número, volumen, frecuencia de ocurrencia e importancia relativa. No se encontraron diferencias significativas en el consumo por tipo de presa (acuática o terrestre). Sin embargo, las presas acuáticas tuvieron mayor importancia relativa y fueron más voluminosas que las terrestres. La diversidad de categorías de presa, en términos de volumen de presa, indica que los machos pueden tener una mayor diversidad dietaría que las hembras, pero no evaluamos un posible sesgo. Además, se encontró un efecto significativo en la interacción del tamaño (SVL) de las ranas con el volumen promedio de presa. Las hembras (más grandes que los machos) consumieron presas dentro de un rango de volumen amplio y, entre más grandes son, más voluminosas son las presas. De esta forma es posible que se reduzca la competencia intraespecífica por los recursos tróficos en el ambiente. Este estudio nos permite comprender la ecología trófica de *L. megapoda*, una especie que juega un papel importante en la red alimenticia donde vive, como depredador de organismos acuáticos y terrestres.

Palabras clave: anfibios, dieta, ecología trófica, peces, variación intersexual.

Resumo

Hábitos alimentares de *Lithobates megapoda* (Anura: Ranidae), uma rã-leopardo ameaçada utilizada para consumo humano, no Lago Chapala, México. A rã-leopardo-de-pés-grandes (*Lithobates megapoda*) é uma espécie ameaçada e endêmica do México ocidental. Essa espécie tem hábitos aquáticos e distingue-se pelo seu grande tamanho, particularmente pelo comprimento das patas, razão pela qual é capturada para consumo humano. Além disso, o conhecimento sobre sua história natural é escasso e incompleto. Neste estudo, analisamos a composição da dieta de *L. megapoda* nas margens do Lago Chapala, o maior lago do México. Um total de 69 indivíduos adultos foram coletados mortos em redes de pesca durante a época das chuvas (Junho-Outubro), dos quais 48 apresentravam conteúdo estomacal. Foi identificado um total de 96 itens, o que corresponde a 13 categorias de presas. Os peixes constituíram a categoria de presas mais dominante na dieta em termos de número, volume, frequência de ocorrência e importância relativa. Não foram encontradas diferenças significativas no consumo por tipo de presa (aquática ou terrestre). No entanto, as presas aquáticas apresentaram maior importância relativa e eram mais volumosas que as terrestres. A diversidade de categorias de presas, em termos de volume de presas, indica que os machos podem exibir uma maior diversidade alimentar do que as fêmeas, mas não avaliamos possíveis vieses. Além disso, foi encontrado um efeito significativo na interacção do tamanho (SVL) das rãs com a média do volume de presas. As fêmeas (que são maiores do que os machos) consumiram presas dentro de uma vasta gama de volumes, e quanto maiores, mais volumosas as presas. Dessa forma, é possível que a competição intraespecífica por recursos alimentares no ambiente seja reduzida. Este estudo ajuda-nos a compreender a ecologia trófica de *L. megapoda,* uma espécie de anuro que desempenha um papel importante na teia alimentar do ambiente em que vive, como predador que se alimenta de organismos aquáticos e terrestres.

Palavras-chave: anfíbios, dieta, ecologia trófica, peixes, variação intersexual.

Introduction

Amphibians, particularly anurans, are an important component of the trophic structure of ecosystems because they are abundant, show high biomass (Gibbons *et al*. 2006), and constitute a link between aquatic and terrestrial communities (Stebbins and Cohen 1995, Duré *et al*. 2009), acting both as predators (e.g., Luría-Manzano and Ramírez-Bautista 2017, 2019) and as preys of a wide variety of organisms (e.g., Fulgence *et al.* 2020, Nuñez-Escalante and Garro-Acuña 2021). Therefore, knowing the feeding habits of anurans help us to understand their role on the energy flow and on the interactions of the food webs in which they are involved (Duellman and Trueb 1994, Luría-Manzano and Ramírez-Bautista 2017). Besides ecological importance, dietary aspects of anurans may have implications for conservation. For example, knowledge of dietary diversity is necessary to detect those species with narrow trophic niches, which are potentially vulnerable to changes in the structure of the prey community they consume (Luría-Manzano and Ramírez-Bautista 2019).

Most anurans are considered generalist consumers because they feed on a wide prey range (Duellman and Trueb 1994, Solé and Rödder 2010). However, there is evidence that some of them have a narrow diet, showing a specialized consumption on certain biological groups (Agudelo-Cantero *et al*. 2015, Lopes *et al*. 2017, Araujo-Vieira *et al*. 2018). Differences in the diet composition have been related to different factors, which may be extrinsic, such as the site traits (Bonansea and Vaira 2007), competition (Duellman and Trueb 1994), or seasonality (Berazategui *et al.* 2007), which can offer different food resources, or intrinsic factors, such as ontogenetic changes (Whitfield and Donnelly 2006), reproductive behavior, and sex (Lamb 1984, Maneyro *et al.* 2004), which influence the nutritional requirements of anurans. Both extrinsic and intrinsic factors are related to the use of food resources and the variation in the diet composition between and within species (Lima and Magnusson 2000).

Lithobates megapoda (Taylor, 1942), commonly known as Big-footed Leopard Frog (Liner and Casas-Andreu 2008), is an endemic anuran species to western Mexico in the states of Nayarit, Jalisco, Guanajuato, and Michoacán (Webb 1996). Its large body size, with females reaching a snout–vent length of up to 157 mm (Webb 1996) and males of up to 117 mm (Taylor and Smith 1945), makes this species one of the largest anurans in Mexico. This frog is predominantly aquatic and inhabits and breeds in perennial water sources, in shrubland and pine-oak forest from 823 to 1,520 m a.s.l. (IUCN SSC Amphibian Specialist Group 2020). In addition, this species is threatened mainly due to habitat destruction and degradation, water pollution, droughts due to climate change, introduction of exotic species (IUCN SSC Amphibian Specialist Group 2020), and extraction for human consumption (Barragán-Ramírez *et al.* 2017, IUCN SSC Amphibian Specialist Group 2020). These impacts have led to this frog being currently protected by Mexican law NOM-059-SEMARNAT-2010 under the Special Protection category (Pr) (SEMARNAT 2010) and considered as Near Threatened by the IUCN (IUCN SSC Amphibian Specialist Group 2020). Despite this, basic information about its natural history is scarce.

In this study, we evaluated dietary composition of adult individuals of *L. megapoda* on the shore of Lake Chapala.

Materials and Methods

Study Site

Lake Chapala is the largest and most important lake in Mexico, it is located between the boundaries of the states of Jalisco and Michoacan (20º14' N, 103º00' W, elevation 1,524 m a.s.l., Figure 1). It has a surface area of 1,146 km^2 (28 km from north to south and 79 km from east to west), a maximum storage capacity of

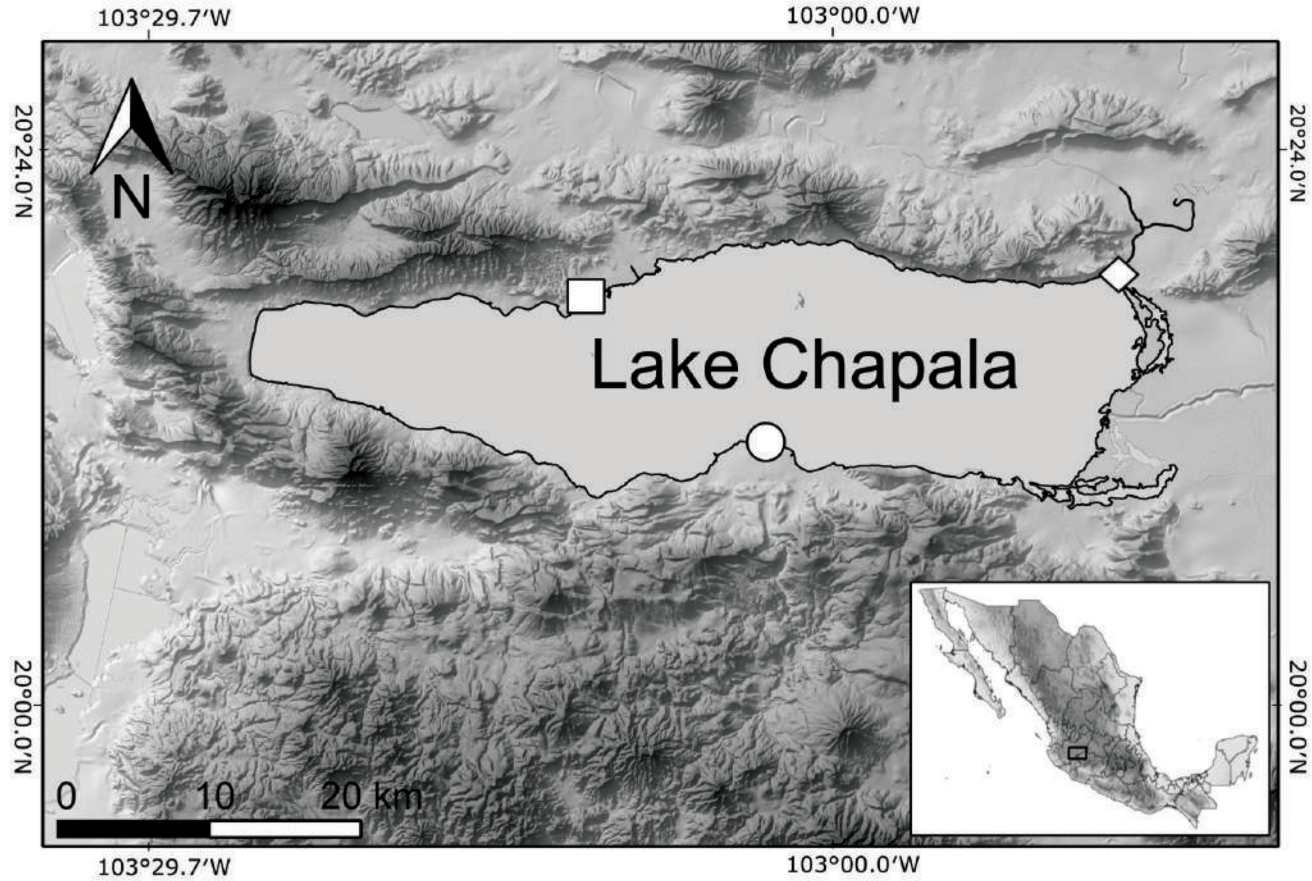

Figure 1. Map showing the sampled areas of Chapala (square), Tizapan El Alto (circle) and Ocotlan (diamond) along the Lake Chapala, state of Jalisco, Mexico.

7,897 m^3 and a maximum of 8 m of depth (CEA Jalisco 2020). Mean annual temperature in the region is 19.9ºC and varies from 9 to 30ºC. Annual rainfall is estimated at 735 mm (Filonov *et al.* 2001), with most rains occurring between June and September.

Data Collection

Sixty-nine adult individuals of *Lithobates megapoda* were examined for the present study. All individuals were found dead in fishing nets during the rainy season (June-October) in different years. Eleven frogs were collected in Ocotlán (20°19'06" N, 102°47'20" W) between September-October 2020; forty-seven frogs collected in the vicinity of Chapala (20°17'38" N, 103°10'40" W) between June and October 2016, and the rest of the individuals were captured between June and September 2015: seven in Tizapán El Alto (20°09'04" N, 103°02' 48" W) and four from Ocotlán (Figure 1). All frogs were collected using a special permit SGPA/DGVS/03444/15 issued by Mexican Ministry of Environment (Secretaría de Medio Ambiente y Recursos Naturales). Specimens were fixed in 10% formalin solution and preserved in 70% alcohol after data collection and deposited in a vertebrate scientific collection at Centro de Estudios en Zoología, Universidad de Guadalajara (CZUG).

Each individual was measured, weighed and its sex was identified. A Mitutoyo® digital caliper was used to record snout–vent length

(SVL) (to the nearest 0.01 mm). Body mass was measured using an Ohaus® digital balance (± 0.1 g of accuracy). Values are presented as mean ± standard deviation (SD). Maturity and sex of individuals were determined either by secondary sexual characters (presence of vocal sacs and nuptial pads in males; absent in females) or by gonadal identification after dissection. We removed the stomach of each individual and the content was placed in a Petri-dish and examined under a stereomicroscope (ZEISS Stemi DV4). Prey items were classified taxonomically to the Order level (prey category) using the keys of Coronado and Márquez (1977) and Triplehorn and Johnson (2005); when possible, items were identified to the genus and/or species level. Only in vertebrate prey, taxonomic Class level is equal to "prey category". Later, we counted and classified each prey according to its habits: aquatic or terrestrial. Prey volume was estimated by fluid displacement to the nearest 0.1 mL (Magnusson *et al.* 2003). Subsequently, the units in mL were converted to cm^3. Stomach contents were preserved in 70% ethanol and stored in the entomological collection at Centro de Estudios en Zoología of the Universidad de Guadalajara (CZUG). Furthermore, those prey items that were too fragmented or that could not be identified due to advanced stage of digestion, were included in the "undetermined remains" category.

Data Analyses

In order to determine the contribution of each prey category, we calculated an index of relative importance using the following formula I = (F% + N% + V%)/3, where F%, N% and V% are the percentages of frequency, number of prey and prey volume, respectively (Biavati *et al.* 2004). Differences in consumption per prey type (aquatic vs. terrestrial) and volume of prey consumed were analyzed with Mann-Whitney U-test. To determine differences in diet composition between the sexes by prey categories in terms of abundance and volume prey, we constructed contingency tables and a chi-square test (χ^2) was applied.

Diet diversity was estimated with the Shannon index (Shannon and Weaver 1949) using the number of items and volume of each prey category as diversity attributes: $H' = -\sum_{i=1}^{S} p_i \ln p_i$, where p_i is the proportion of the resource *i* (prey category) in the diet and *S* is the total number of prey categories. The exponential to Shannon's diversity values was calculated in order to represent the results in terms of effective species number (true diversity), sensu Jost (2006) : ${}^1D = \exp(H')$, where ${}^1D$ represented the dietary diversity observed (${}^1D_{obs}$). Additionally, we estimated dietary diversity (${}^1D_{est}$) with a Jacknife estimator (Zahl 1977) and statistically compared it among sexes by calculating 95% confidence intervals.

The relationship SVL-average of prey volume, SVL-total prey volume, and SVL-prey number were analyzed under a Covariance Analysis structure (ANCOVA) where a Generalized Linear Model (GLM) was used with a Gamma distribution as error and a square-root link function. Volume and prey number were selected as dependent variables and the sex as covariate. All statistical analyses were performed using R software (R Core Team 2017).

Results

Diet Composition

We obtained 69 adult individuals of *Lithobates megapoda*, 40 females and 29 males. Females had a snout–vent length (SVL) of 123.30 ± 18.52 mm and a body mass of 289.0 ± 118.4 g. Males had a SVL of 96.67 ± 9.33 mm and body mass 132.7 ± 35.1 g. Females were larger (Mann-Whitney U = 458, $p < 0.001$) and heavier (Mann-Whitney U = 382, $p < 0.001$) than males, with more than twice the mass of males on average (Table 1).

A total of 48 individuals had stomach content (29 females and 19 males); 42 with at least one identifiable prey item and six with only plant

Table 1. Snout–vent length (SVL), body mass and dietary parameters for females and males of *Lithobates megapoda.*

	Females		Males	
	Mean ± SD (range)	*N*	Mean ± SD (range)	*N*
SVL (mm)	123.30 ± 18.52 (77.10–150.88)	40	96.67 ± 9.33 (80.02–112.76)	29
Body mass (g)	289.0 ± 118.4 (57.0–567.0)	40	132.7 ± 35.1 (65.0–208.0)	29
Number of prey per stomach	2.4 ± 2.0 (1–8)	27	2.1 ± 1.9 (1–8)	15
Prey volume (cm^3)	0.81 ± 1.41 (0.02–7.86)	65	0.19 ± 0.31 (0.01–1.52)	31
Stomach content volume (cm^3)	2.06 ± 2.12 (0.09–7.86)	29	0.57 ± 0.47 (0.03–1.70)	19

material and non-identifiable remains. We identified 96 prey items belonging to 13 prey categories: 11 invertebrates and only two vertebrates (anurans and Actinopterygii fishes) (Table 2). The mean number of prey items per stomach was 2.00 ± 1.95 (range 1–8). We found a range of 0.01 to 7.86 cm^3 of prey volume per stomach, and a range of 0.03 to 7.86 cm^3 of volume of stomach contents. Fish were the most dominant prey category in terms of frequency (50.1%), number (36.5%) and volume (57.3%), which comprised two taxonomic orders: Cyprinodontiformes and Perciformes. Also, this food category was the most important in the diet of *L. megapoda*, $I = 48.0$ (Table 2, Figure 2). Araneae was the second most important prey category ($I = 12.5$) and Hemiptera was the third one ($I = 7.8$). Plant material and undetermined remains were also part of the diet, these were found in six (12.5%) and 25 (52.1%) stomachs and they constitute 2.6% and 14.3% of the total volume ingested, respectively (Table 2).

The consumption by prey type, aquatic and terrestrial, comprised 43.8% and 56.2% of the total number of prey items, respectively (Table 2). Frogs with only identifiable prey items in their stomach ($N = 42$) showed a range of 0–7 terrestrial prey per stomach, followed by a range of 0–5 aquatic prey per stomach, with no differences between the consumption of both prey types (Mann-Whitney U = 875, $p = 0.95$). Regarding the total volume of ingested prey, aquatic preys (76.4%) surpassed the terrestrial ones (23.6%). The volume range of aquatic prey consumed was 0.04–7.86 cm^3 ($N = 42$), while that of the terrestrial prey was 0.01–3.00 cm^3 ($N = 54$). Terrestrial preys were smaller (volumetrically) than the aquatic ones (Mann-Whitney U = 1868, $p < 0.001$). The index of relative importance showed that aquatic preys were more important than terrestrial in the diet of *L. megapoda* (Table 2).

Diet Variation

Analyzing the prey consumption data by sex, we found differences in the prey volume (Mann-Whitney U = 1542, $p = -0.001$) and in the volume of stomach content (Mann-Whitney U = 431, $p = -0.001$). Females consumed bulky prey and showed a greater volume of stomach content than males (Table 1). Also, we found differences in the numerical ($X^2 = 45.95$, df = 12, $p < 0.001$) and volumetric ($X^2 = 60.53$, df = 12, $p < 0.001$) proportions of prey categories consumed between the sexes. Fish (Actinopterygii) were the most important prey category in the diet of females ($I = 52\%$) and males ($I = 25.4\%$) (Table 3). Dietary diversity (${}^1D$) observed and estimated, using the abundance of prey categories, was similar in both sexes (females ${}^1D_{obs} = 6.68$, ${}^1D_{est} = 7.72$; males ${}^1D_{obs} = 6.68$, ${}^1D_{est} = 7.72$). However, when analyzing dietary diversity using prey volume, we found that males show greater diversity (${}^1D_{obs} = 5.23$, ${}^1D_{est} = 12.14$) than females (${}^1D_{obs} = 2.81$, ${}^1D_{est} = 3.22$) (Figure 3).

Table 2. Diet composition of *Lithobates megapoda* (*N* = 48) on the shore of Lake Chapala, Jalisco, Mexico. *N,* Number of individuals; *V,* Prey volume (cm^3); *F,* Frequency of occurrence of each prey item; *I,* Index of relative importance. *Endemic to Lake Chapala basin and the surrounding area; **Introduced to Lake Chapala.

Taxonomic Class/ Prey category	**Prey type**	***N***	***N%***	***F***	***F%***	***V***	***V%***	***I***
INVERTEBRATES								
Arachnida								
Araneae		14	14.6	10	20.8	1.45	2.1	12.5
Lycosidae	T	6	6.3	5	10.4	1.16	1.6	6.1
Tetragnathidae	T	6	6.3	4	8.3	0.26	0.4	5.0
Unidentified	T	2	2.1	2	4.2	0.03	0.0	2.1
Opiliones								
Sclerosomatidae								
Leiobunum sp.	T	1	1.0	1	2.1	0.10	0.1	1.1
Diplopoda								
Polydesmida								
Paradoxosomatidae	T	5	5.2	2	4.2	0.39	0.6	3.3
Hexapoda								
Coleoptera		7	7.3	5	10.4	1.55	2.2	6.6
Chrysomelidae								
Disonycha sp.	T	1	1.0	1	2.1	0.01	<0.1	1.0
Omophoita sp.	T	1	1.0	1	2.1	0.01	<0.1	1.0
Scarabaeidae								
Phyllophaga sp.	T	1	1.0	1	2.1	0.26	0.4	1.2
Xyloryctes sp.	T	2	2.1	2	4.2	1.13	1.6	2.6
Staphylinidae								
Platydracus sp.	T	1	1.0	1	2.1	0.04	0.1	1.1
Unidentified	T	1	1.0	1	2.1	0.10	0.1	1.1
Dermaptera								
Forficulidae								
Doru sp.	T	9	9.4	3	6.3	1.07	1.5	5.7
Hemiptera		6	6.3	6	12.5	3.38	4.8	7.8
Belostomatidae (adult)	A	2	2.1	2	4.2	2.24	3.2	3.1
Belostomatidae (nymph)	A	2	2.1	2	4.2	0.84	1.2	2.5
Notonectidae	A	1	1.0	1	2.1	0.10	0.1	1.1
Unidentified	T	1	1.0	1	2.1	0.20	0.3	1.1
Hymenoptera		3	3.1	3	6.3	0.15	0.2	3.2
Apidae								
Apis mellifera	T	2	2.1	2	4.2	0.12	0.2	2.1
Formicidae								
Atta mexicana	T	1	1.0	1	2.1	0.03	< 0.1	1.1

Table 2. *Continued.*

Taxonomic Class/ Prey category	**Prey type**	***N***	***N%***	***F***	***F%***	***V***	***V%***	***I***
Lepidoptera								
Unidentified (larvae)	T	2	2.1	2	4.2	0.12	0.2	2.1
Odonata		7	7.3	6	12.5	2.22	3.1	7.6
Coenagrionidae								
Argia sp.	T	2	2.1	2	4.2	0.09	0.1	2.1
Libellulidae	T	2	2.1	1	2.1	0.48	0.7	1.6
Unidentified (adult)	T	2	2.1	2	4.2	1.38	2.0	2.7
Unidentified (nymph)	A	1	1.0	1	2.1	0.27	0.4	1.2
Orthoptera								
Unidentified	T	4	4.2	3	6.3	6.76	9.6	6.7
Crustacea								
Isopoda								
Armadillidiidae	T	2	2.1	1	2.1	0.11	0.2	1.4
VERTEBRATES								
Amphibia								
Ranidae								
Lithobates sp. (larvae)	A	1	1.0	1	2.1	0.91	1.3	1.5
Actinopterygii (Fishes)		35	36.5	24	50.1	40.43	57.3	48.0
Cyprinodontiformes		29	30.2	21	43.8	26.32	37.3	37.1
Goodeidae								
*Chapalichthys encaustus**	A	1	1.0	1	2.1	0.07	0.1	1.1
Goodea atripinnis	A	1	1.0	1	2.1	3.95	5.6	2.9
Unidentified	A	3	3.1	3	6.3	4.51	6.4	5.3
Poeciliidae								
Poecilia sp.**	A	7	7.3	6	12.5	12.34	17.5	12.4
Poeciliopsis infans	A	3	3.1	3	6.3	0.38	0.5	3.3
Non-identified	A	14	14.6	9	18.8	5.07	7.2	13.5
Perciformes		6	6.3	3	6.3	14.11	20.0	10.8
Cichlidae								
Oreochromis sp.**	A	5	5.2	2	4.2	12.91	18.3	9.2
Non-identified	A	1	1.0	1	2.1	1.20	1.7	1.6
Aquatic preys (A)	-	42	43.8	29	60.4	44.79	63.5	55.9
Terrestrial preys (T)	-	54	56.2	24	50.0	13.85	19.6	42.0
Plant material	-	-	-	6	12.5	1.86	2.6	-
Undetermined remains	-	-	-	25	52.1	10.07	14.3	-
Totals	**-**	**96**	**100**	**48**	**100**	**70.57**	**100**	**-**

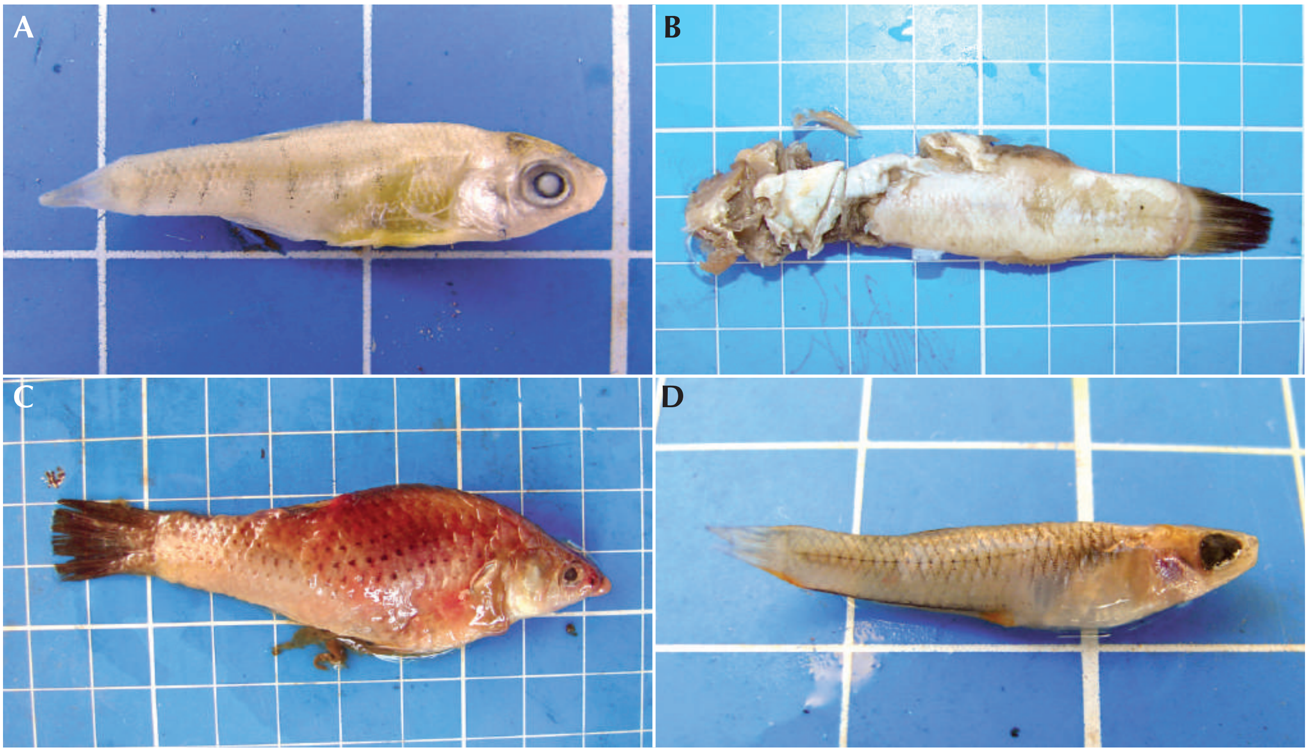

Figure 2. Some fish samples extracted from the stomach contents of *Lithobates megapoda*. Goodeidae: (**A**) *Chapalichthys encaustus*, (**B**) *Goodea atripinnis*. Poecilidae: (**C**) *Poecilia* sp., (**D**) *Poeciliopsis infans*. Scale: each square = 1 cm^2.

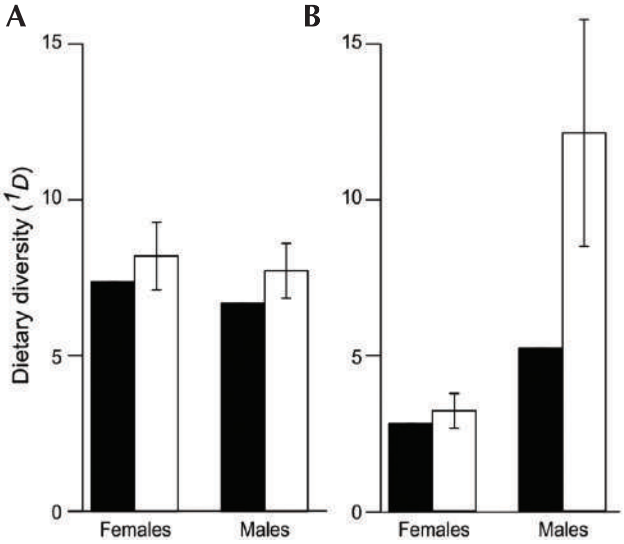

Figure 3. Dietary diversity (^{1}D) with abundance (**A**), and prey volume (**B**) in males and females of *Lithobates megapoda*. Dark bars = observed dietary diversity; white bars = estimated dietary diversity. Error bars are 95% confidence intervals.

We found correlation between the average of prey volume and SVL ($F_{1,40} = 60.009$; $p < 0.001$), which was different between the sexes ($F_{1'38} = 54.1$; $p < 0.001$), with a negative trend in males and a positive trend in females (Figure 4A). We found differences between total prey volume per SVL ($F_{1,46} = 57.61$; $p < 0.001$) and sex ($F_{1,45} = 46.98$; $p < 0.001$), with females presenting larger volumes of prey in their stomach than males of similar size (Figure 4B). No differences were found in the correlation of number of prey and SVL ($F_{1,40} = 52.38$; $p = 0.54$) and sex ($F_{1,39} = 50.77$; $p = 0.2$) (Figure 4C).

Discussion

The results of this study indicate that the diet of *Lithobates megapoda* on the shore of Lake Chapala, during the rainy season, is composed of a wide variety of prey, as occurs with other

Table 3. Intersexual diet composition of *Lithobates megapoda* (N = 48) on the shore of Lake Chapala, Jalisco, Mexico. *N*, Number of individuals; *V*, Total volume of preys (cm^3); *F*, Frequency of occurrence of each prey item; *I*, Index of relative importance.

Prey class/Prey order	**Females (N = 29)**				**Males (N = 19)**			
	N (%)	*FO (%)*	*V (%)*	*I (%)*	*N (%)*	*FO (%)*	*V (%)*	*I (%)*
Arachnida	7 (10.7)	7 (24.1)	0.72 (1.1)	12.0 (10.9)				
Araneae	6 (9.2)	6 (20.7)	0.62 (1.0)	10.3 (9.1)	8 (25.8)	4 (21.1)	0.83 (7.7)	18.2 (20.6)
Opiliones	1 (1.5)	1 (3.4)	0.10 (0.1)	1.7 (1.5)	-	-	-	-
Diplopoda (Polydesmida)	5 (7.7)	2 (6.9)	0.39 (0.7)	5.1 (4.6)	-	-	-	-
Hexapoda	21 (32.4)	12 (41.4)	12.00 (20.0)	31.3 (28.5)	17 (54.8)	11 (57.9)	3.25 (30.0)	47.6 (54.0)
Coleoptera	4 (6.2)	3 (10.3)	1.43 (2.4)	6.3 (5.6)	3 (9.7)	2 (10.5)	0.12 (1.1)	7.1 (7.7)
Dermaptera	4 (6.2)	1 (3.4)	0.96 (1.6)	3.7 (3.3)	5 (16.1)	2 (10.5)	0.11 (1.0)	9.2 (10.0)
Hemiptera	3 (4.6)	3 (10.3)	1.62 (2.7)	5.9 (5.2)	3 (9.7)	3 (15.8)	1.76 (16.2)	13.9 (15.2)
Hymenoptera	2 (3.1)	2 (6.9)	0.12 (0.2)	3.4 (3.0)	1 (3.2)	1 (5.3)	0.03 (0.3)	2.9 (3.2)
Lepidoptera	2 (3.1)	2 (6.9)	0.12 (0.2)	3.4 (3.0)	-	-	-	-
Odonata	3 (4.6)	2 (6.9)	1.52 (2.5)	4.7 (4.1)	4 (12.9)	4 (21.1)	0.7 (6.5)	13.5 (14.7)
Orthoptera	3 (4.6)	2 (6.9)	6.23 (10.4)	7.3 (6.4)	1 (3.2)	1 (5.3)	0.53 (4.9)	4.5 (4.9)
Crustacea (Isopoda)	2 (3.1)	1 (3.4)	0.11 (0.2)	2.2 (2.0)	-	-	-	-
Anura	1 (1.5)	1 (3.4)	0.91 (1.5)	2.1 (1.9)	-	-	-	-
Actinopterygii	29 (44.6)	18 (62.0)	38.68 (64.7)	57.1 (52.0)				
Cyprinodontiformes	23 (35.4)	15 (51.7)	24.57 (41.1)	42.7 (37.7)	6 (19.4)	6 (31.6)	1.75 (16.1)	22.4 (25.4)
Perciformes	6 (9.2)	3 (10.3)	14.11 (23.6)	14.4 (12.7)	-	-	-	-
Plant material	-	4 (13.8)	1.05 (1.8)	-	-	2 (10.5)	0.81 (7.5)	-
Undetermined remains	-	12 (41.4)	5.87 (9.8)	-	-	13 (68.4)	4.2 (38.7)	-
Totals	**65 (100)**	**29 (100)**	**59.73 (100)**	**-**	**31 (100)**	**19 (100)**	**10.84 (100)**	**-**

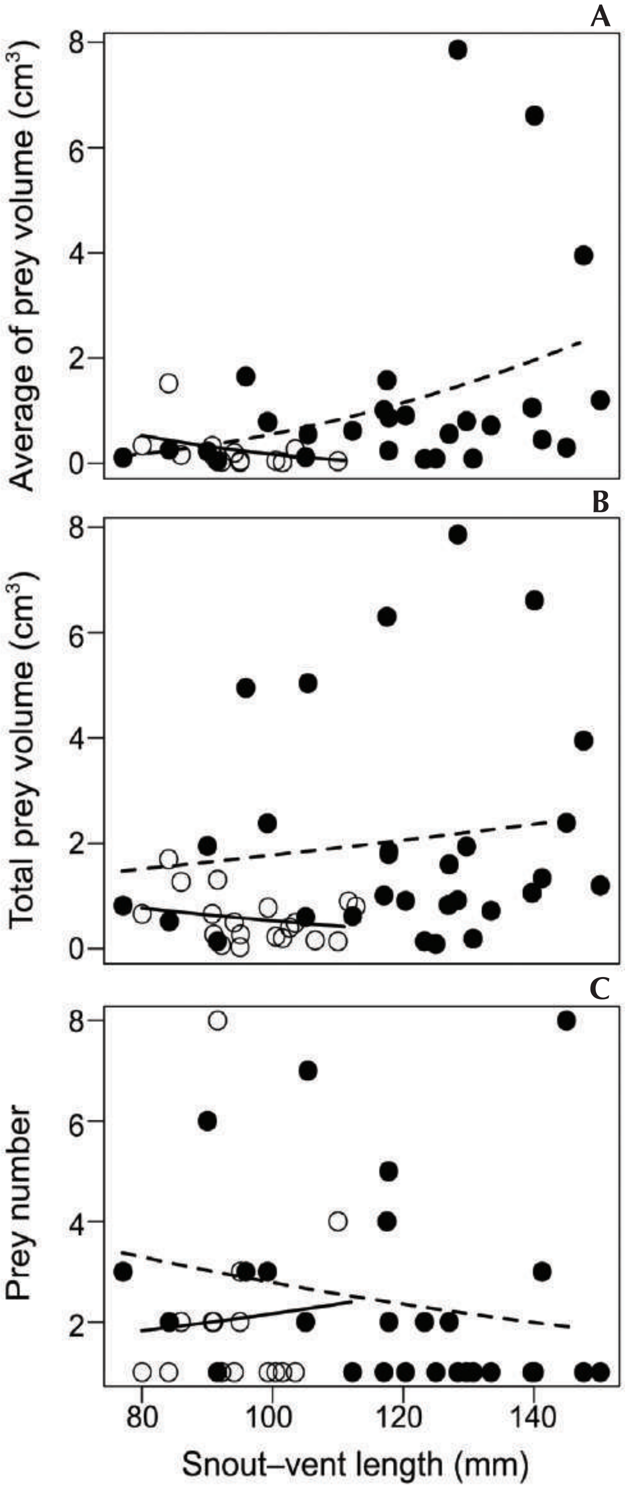

Figure 4. Correlation between snout–vent length (SVL) of adults *Lithobates megapoda* and (**A**) average of prey volume, (**B**) total prey volume, and (**C**) number of prey consumed. Open circles and trend lines represent males and solid circles and dashed lines represent females.

ranids that are considered generalist-opportunistic predators (Hirai and Matsui 1999, Wu *et al.* 2005). However, *L. megapoda* shows a preference for fish consumption, which according to the relative importance index is the most valuable prey category in the diet of this frog. Because its diet includes both aquatic and terrestrial organisms, this frog species represents a link between aquatic and terrestrial communities where it plays an important role in the food web.

Unlike other frogs that can feed on fish, *Lithobates megapoda* shows a high percentage of frequency of fish consumption (~ 50%), which is above that reported in other ranids in Mexico (with less than 5%) as *L. berlandieri* (Baird, 1859) and *L. johni* (Blair, 1965) (Hernández-Austria *et al.* 2019), *L. brownorum* (Sanders, 1973) (Ramírez-Bautista and Lemos-Espinal 2004), *L. vaillanti* (Brocchi, 1877) (Ramirez *et al.* 1998, Ramírez-Bautista and Lemos-Espinal 2004, Luría-Manzano and Ramírez-Bautista 2019), and *L. zweifeli* (Hillis, Frost, and Webb, 1984) (Mendoza-Estrada *et al.* 2008). We need further studies to evaluate the high contribution of fish as food source for *L. megapoda*. It can be related to different factors, such as opportunistic habits (Premo and Atmowidjojo 1987, Hirai and Matsui 1999, Dietl *et al.* 2009), digestibility (Secor *et al.* 2007) promoting greater nutritional contribution (Das 1996), especially during reproductive period due to the energy expenditure involved (Lamb 1984, Ryser 1989), or high availability in the habitat (Parker and Goldstein 2004, López *et al.* 2009).

We recorded at least five species of fishes in the diet of *L. megapoda*, three are native to Lake Chapala: *Chapalichthys encaustus* (Jordan and Snyder, 1899) (endemic to Lake Chapala basin), *Goodea atripinnis* Jordan, 1880, and *Poeciliopsis infans* (Woolman, 1894). The other two are non-native species, *Poecilia* sp. and *Oreochromis* sp., the latter known as “Tilapia” and native to Africa (Miller *et al.* 2009, Moncayo-Estrada *et al.* 2012). All these fishes are frequent in areas with aquatic vegetation (Miller *et al.* 2009), where *L. megapoda* is commonly found. Aquatic prey, which were more valuable than terrestrial prey according to the relative importance index, included along with fish, organisms such as belostomatids, damselfly nymph, and a tadpole, indicating the ability to capture prey underwater, as already suggested for other ranids (Stewart and Sandison 1972). The presence of a congeneric tadpole in one of the stomachs indicates the occurrence of cannibalism, reinforcing the opportunistic behavior of this frog. Similar cases have been widely reported in other ranids (e.g., Mendoza-Estrada *et al.* 2008, Silva *et al.* 2009, Alvarez 2013).

The presence of terrestrial preys in the diet of *L. megapoda* suggests that individuals forage near the lake margins, a behavior also documented to other ranids (e.g., Ramírez-Bautista and Lemos-Espinal 2004). The floating and aquatic vegetation constitute an important habitat for different invertebrates, like spiders and dragonflies (Akamatsu *et al.* 2004), which were important prey in the diet of *L. megapoda* after fish.

Considering the low percentage of occurrence and volume in the stomachs, the ingestion of plant material is probably accidental, as reported for other ranids (Hedeen 1972, Kramek 1972, Mendoza-Estrada *et al.* 2008).

Dietary diversity based on abundance seems similar between sexes, as has happened in other species of anurans (e.g., Hirai and Matsui 1999, Silva *et al.* 2009, Luría-Manzano and Gutiérrez-Mayén 2014), but females seem to consume prey with a wider volume range, usually larger ones when compared to males of similar size. Unfortunately, we did not access prey availability or sex or size variation among the three different collection sites within the Lake Chapala, and the results should be considered cautiously, since sample biases are possible. The effect of sex and size of the frogs may suggest larger intraspecific competition between large mature males and smaller young females. Additionally, foraging behavior of males and females may vary. An interesting future approach would be to compare the diet composition of adults out of the rainy season (which is also the breeding season) to see if differences remain.

Acknowledgments

We thank Agustín Camacho-Rodríguez for its valuable support in the fish identification and the CONACyT for support of the PhD scholarship to J. L. Barragán-Ramírez. We are grateful to the anonymous reviewers for their comments and suggestions to improve the manuscript.

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Editor: Vanessa K. Verdade

Phyllomedusa 20(1):89–92, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p89-92

SHORT COMMUNICATION

Dietary records for *Oxybelis rutherfordi* (Serpentes: Colubridae) from Trinidad and Tobago

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Keywords: diet, island ecology, lizards, predator-prey relationship, Rutherford's vine snake.

Palavras-chave: dieta, ecologia de ilhas, relação predador-presa, serpente-arborícola-de-rutherford.

Snakes feed on a variety of prey (Greene 1983). The diet of the Brown Vine Snake, *Oxybelis aeneus* (Wagler, 1824), is well known; lizards are the most common prey. This species has no apparent taxonomic proclivity in its dietary choices, which suggests that their selection of lizards is random (Mesquita *et al*. 2012, Sousa *et al*. 2020). However, reports on the diet of Rutherford's Vine Snake, *Oxybelis rutherfordi* Jadin, Blair, Orlofske, Jowers, Rivas, Vitt, Ray, Smith, and Murphy, 2020, are limited (Murphy *et al*. 2013) and usually lack the specific identity of the prey (Murphy *et al*. 2018). Herein we provide a list of previously undocumented prey records for *O. rutherfordi* from the Republic of Trinidad and Tobago in the West Indies.

Oxybelis rutherfordi is arboreal and occurs across northern South America, including the islands of Trinidad and Tobago (Jadin *et al*. 2020). Jadin *et al.* (2019) recognized that *O. rutherfordi* is distinct from *O. aeneus* and described the species (Jadin*et al*. 2020). Because previous natural history information for *O. rutherfordi* was combined with *O. aeneus* (Murphy *et al*. 2018), it is appropriate to provide new information for *O. rutherfordi.*

Three separate predation events by *O. rutherfordi* were observed in January and February 2021 involving three lizard species on Trinidad. The encounter with each predation event was fortuitous. All occurred in the afternoon—one on Trinidad and two on different days on the island of Chacachacare, northwest of Trinidad (Figure 1). We observed each event for approximately 1 or 2 min to minimize disturbance of the snake and avoid it regurgitating the prey. Photographs were taken at approximate distances 1–3 m (Figure 2). Lizard prey species were identified using Murphy *et al*. (2018).

All predation events involved adult *Oxybelis rutherfordi.* The first took place in D'Abadie, an urban residential area in north central Trinidad

Received 26 February 2021
Accepted 29 March 2021
Distributed June 2021

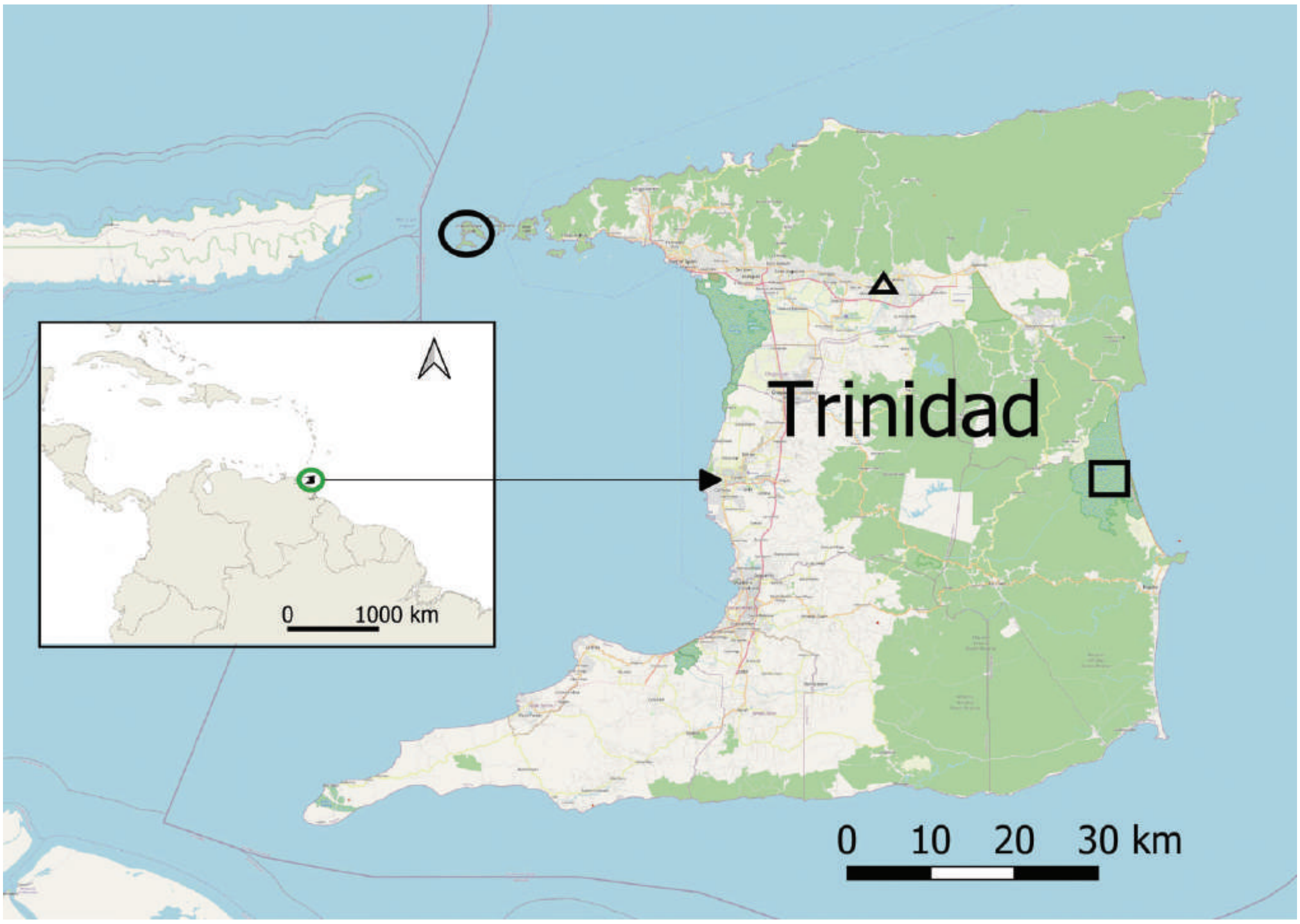

Figure 1. Location of predation events by *Oxybelis rutherfordi* in Trinidad in the Republic of Trinidad and Tobago. Chacachacare Island's location is shown with the oval; D'Abadie's location is indicated by the triangle, and the location of Nariva Swamp by the square. Map created using QGIS (version 3.10.2) with OpenStreetMap feature.

(10.6992 N, -61.3069 W), at 15:25 h on 25 January 2021. The snake preyed on an adult female *Gonatodes vittatus* (Lichtenstein and Martens, 1856) in a potted plant less than 1 m off the ground. The second event occurred at about 13:45 h on 31 January 2021 on Chacachacare Island (10.6844 N, -61.7555 W) in clearings along the forest edge, where we observed *O. rutherfordi* preying on an adult *Gymnophthalmus* sp. on the ground in an area of scrub vegetation 20 m from the coastline. Two species of *Gymnophthalmus* occur on Chacachacare Island (Murphy *et al.* 2018), but without examining the specimen in hand, it is difficult to distinguish between them. The third predation event also took place on Chacachacare Island (10.6831 N, -61.7548 W) in a forest-edge clearing at 14:30 h on 13 February 2021 about 30 m from the location of the second event. The snake was preying on an adult female *Cnemidophorus lemniscatus* (Linnaeus, 1758) on the ground in a partially open canopy area with scrub vegetation. This may have been the same snake as the previous observation given the proximity of the location. Though we did not observe the initial capture of any of the lizard prey, each event took place on the ground or within 1 m above the ground, suggesting that *O. rutherfordi* descended to the ground to capture the

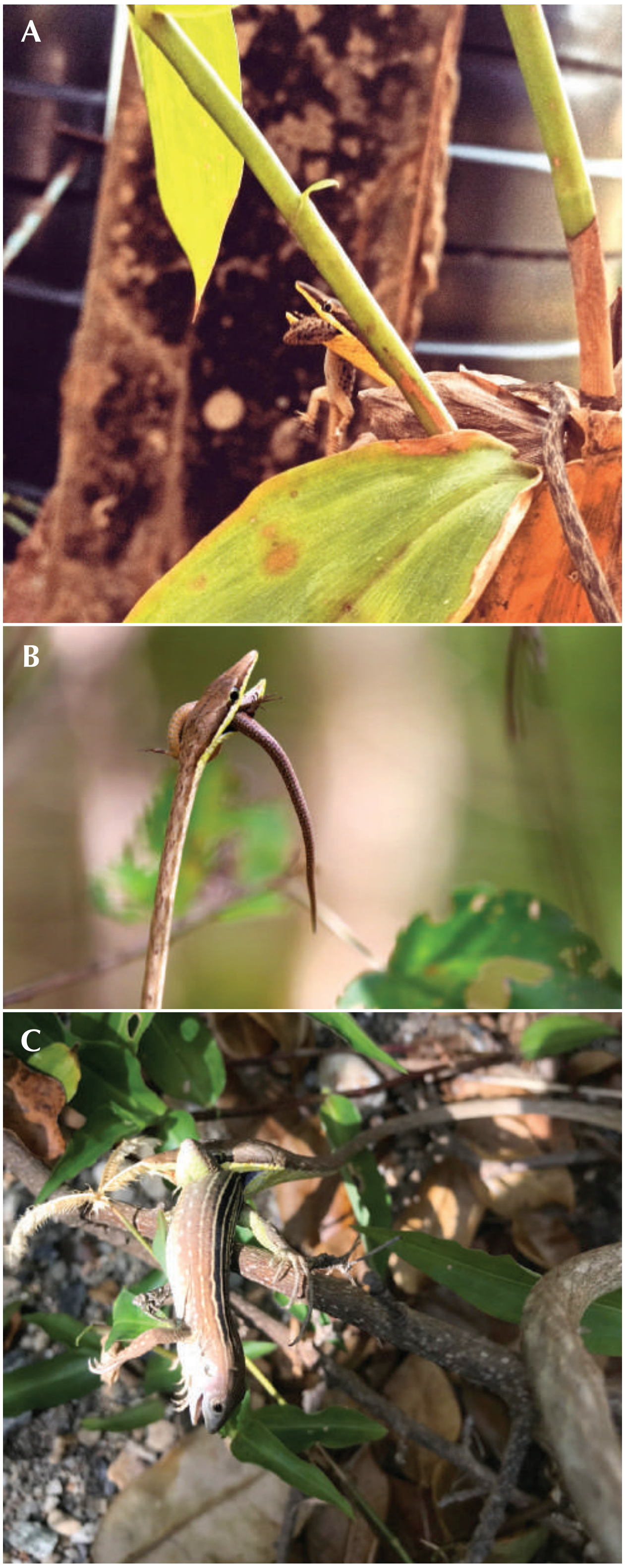

Figure 2. The lizards *Gonatodes vittatus* (female) (**A**), *Gymnophthalmus* sp. (**B**), and *Cnemidophorus lemniscatus* (female) (**C**) being preyed upon by *Oxybelis rutherfordi*. Photos: K. E. (A), J.-M. M. (B), and M.-E. M. (C).

lizards, all of which are terrestrial (Murphy *et al*. 2018).

In addition to these three observations, one of us (RJA) came across a photograph of an adult *Oxybelis rutherfordi* preying on an adult *Anolis planiceps* (Troschel, 1848) in Trinidad. The photographer is unknown; only the general location of the event is known—a forested area in Nariva Swamp, Trinidad, on 27 February 2021 during the day. This predation event is not surprising because the distributions of *O. rutherfordi* and *A. planiceps* overlap (Murphy *et al*. 2018). Based on the four lizard prey species reported here, and a record of predation on *Copeoglossum aurae* Hedges and Conn, 2012 (Murphy *et al*. 2013), *O. rutherfordi* feeds on at least five species of lizards from five families, suggesting that lizards probably are a staple of their diet, resembling the dietary habits of *O. aeneus* (Mesquita *et al*. 2012).

As citizen scientists, our natural history observations contribute to a better understanding of the ecology of *Oxybelis ruthrfordi,* and reinforce the concept that citizen scientists with minimal training can provide useful ecological data (Auguste 2020). We anticipate that additional predation events by *O. rutherfordi* will be observed, given the widespread distribution of the species across the Republic of Trinidad and Tobago.

Acknowledgments.—We thank the Wildlife Section of the Forestry Division of Trinidad and Tobago, Ministry of Agriculture, Land, and Fisheries for issuing a Special Game License research permit to RJA (Permit date: 27 January 2021). We are grateful to the editors and reviewers for their helpful suggestions to improve this report.

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Editor: Ross D. MacCulloch

Phyllomedusa 20(1):93–98, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p93-98

SHORT COMMUNICATION

The South American false coral snake *Erythrolamprus aesculapii* (Serpentes: Dipsadidae) as a possible mimic of *Micrurus averyi* (Serpentes: Elapidae) in Central Amazonia

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Keywords: Anavilhanas National Park, color patterns, coral snake, mimicry.

Palavras-chave: Cobra-coral, mimetismo, padrões de coloração, Parque Nacional de Anavilhanas.

Mimicry among coral snakes is a controversial issue in evolutionary biology (Dunn 1954, Hecht and Marien 1956, Wickler 1968, Gelbach 1972, Greene and McDiarmid 1981, Brodie III and Brodie Jr. 2004). There is some evidence that a coral-snake color pattern might offer some protection against predators such as birds or mammals (Brodie III 1993, Brodie III and Janzen 1995, Pfennig *et al.* 2007, Kikuchi and Pfennig 2010, 2012). However, the assertion that specific coral-snake patterns may confer different levels of protection requires further investigation. Many studies have elucidated the ways in which coral-snake mimicry operates in nature (Kikuchi and Pfennig 2010, Davis-Rabosky *et al.* 2016, França *et al.* 2017, Akcali *et al.* 2019), but the role of the mimicry process that produces this coevolutionary pattern must be explored in more depth. The first step in evaluating mimetic complexes is to describe the color patterns of the different species occurring in a given region.

Color patterns among the species of neotropical coral snakes of the genus *Micrurus* Wagler, 1824 vary significantly with respect to the sequence and width of rings (Savage and Slowinsky 1992). In Amazonia, where many species of *Micrurus* occur, snakes with different color patterns may be sympatric. Thus, there may be a wide array of potential models for other snakes to mimic; most of the potential mimics are members of Dipsadidae Bonaparte, 1838 (Martins and Oliveira 1998, Campbell and Lamar 2004, Almeida *et al.* 2014).

Received 23 September 2019
Accepted 23 April 2021
Distributed June 2021

South American false coral snakes of the genus *Erythrolamprus* Boie, 1826 often are associated with mimicry complexes involving sympatric species of *Micrurus* (Greene and McDiarmid 1981, Marques and Puorto 1991, Starace 1998, Almeida *et al.* 2016), including cases of concrete homotypy (sensu Pasteur 1982) with perfect and imperfect mimics. For instance, the monad-patterned (i.e., one black band/ring separating red bands/rings) specimens of *Erythrolamprus aesculapii* (Linnaeus, 1758) in the Brazilian Atlantic Forest, as well as melanistic *E. guentheri* Garman, 1883 in Peru, seem to represent precise mimics of their respective models, *Micrurus corallinus* (Merrem, 1820) and *M. margaritiferus* Roze, 1967 (Greene and McDiarmid 1981, Marques and Puorto 1991). In contrast, *Erythrolamprus* with a diad pattern (i.e., two black bands/rings separating red band/rings) may be imprecise mimics of triad patterned venomous coral snakes (Marques and Puorto 1991), because the similarities among mimics and models may vary, but the number and combinations of black and white rings never match.

The variations of color pattern in the false coral snake *Erythrolamprus aesculapii aesculapii* (Linnaeus, 1758) (Dipsadidae: Xenodontinae) has been documented in Amazonia with the co-occurrence of several different morphotypes (Starace 1998, Campbell and Lamar 2004, Almeida *et al.* 2016). Such Amazonian populations are remarkably polytypic across the broad distribution of the species, and include specimens with monads, triads and tetrads, as well as different degrees of melanism (Martins and Oliveira 1998, Curcio 2008, Almeida *et al.* 2016) occurring in sympatry different species of *Micrurus.* Presumably this favors the successful establishment of imperfect mimics (Almeida *et al.* 2016, Sherratt and Peet-Paré 2017, Akcali *et al.* 2019).

The Black-headed Coral Snake *Micrurus averyi* Schimdt, 1939 is a moderately slender species (nearly 700 mm in total length) occurring in the central and northern parts of Amazonia, in southern Guyana, southern Suriname, and northern Brazil (Martins and Oliveira 1998, Campbell and Lamar 2004, Silva Jr. *et al.* 2016). It is unique having an almost entirely black head, as well as a conspicuous body pattern combining monads with narrow black and white rings, interspaced by distinctively long red rings (Martins and Oliveira 1998, Campbell and Lamar 2004, Silva Jr. *et al.* 2016).

Herein we report the co-occurrence of specimens of *Erythrolamprus aesculapii aesculapii* with distinct color patterns, one of which is a precise mimic of the syntopic venomous coral snake *M. averyi.* From January to March of 2006, one of us (HAA) surveyed snakes for 45 days in an area of dense, ombrophilous forest in the Anavilhanas National Park (Figure 1) (02°23'41" S, 60°55'14" W; 50 m a.s.l., 350.018 ha), lower Rio Negro, Brazilian Central Amazonia (Hudson 2007).

The methods employed a combination of 22 pitfall traps and 46 funnel traps connected by 300 m of drift fences (Greenberg *et al.* 1994, Cechin and Martins 2000, Enge 2001). Twenty-two snakes of 10 species were collected and included the following: one *Micrurus hemprichii* (Jan, 1858); two *M. averyi* (CHUFJF 000390: total length = 550 mm; CHUFJF000377: total length = 330 mm); and two *Erythrolamprus aesculapii aesculapii* (MNRJ 014183: total length = 920 mm; MNRJ 014185: total length = 800 mm) captured in funnel traps. Vouchers are housed at the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro state (MNRJ), and Universidade Federal de Juiz de Fora, Minas Gerais state (CHUFJF).

The specimens of *Micrurus averyi* (Figures 2, 3A) have the typical color pattern of the species (Campbell and Lamar 2004); the color patterns of the two *Erythrolamprus aesculapii aesculapii* (Figures 2, 3B–C) differ from each other. Both snakes have long red rings, but one of them has black rings arranged in monads (Figures 2, 3B) and the other in diads (Figures 2, 3C). These two individuals differ from one of

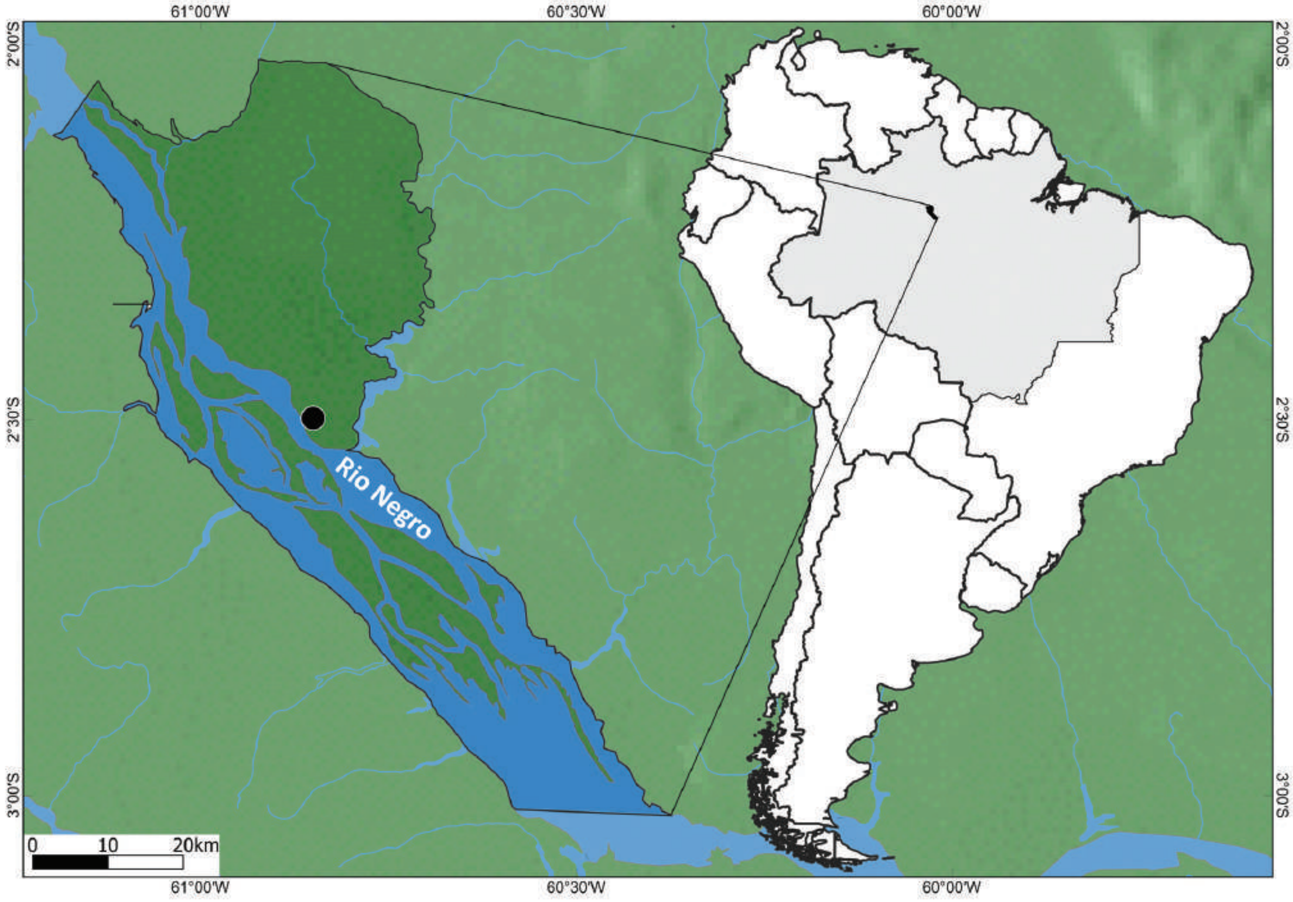

Figure 1. Locality of the sampling site (black circle) of *Erythrolamprus aesculapii* and *Micrurus averyi* at the margin of Rio Negro, Anavilhanas National Park. The gray area on map of South America represents the limits of the Brazilian Legal Amazonia.

most common patterns of *E. aesculapii* found throughout Amazonia (diads usually equal in length or longer than red rings Figures 2, 3D). The monad-patterned specimen (Figures 2, 3B) closely resembles the general coloration of *M. averyi,* not only with respect to dorsal pattern with monads, but also by having an almost entirely black head. The black rings of the tail, usually composed of diads even in monad-patterned specimens of *Erythrolamprus,* tend to fuse dorsally; however, the similarities with the bicolored tail pattern of *M. averyi* are superficial.

The two specimens of *Erythrolamprus aesculapii aesculapii* in our sample promote discussion of instances of precise and imprecise mimicry in cases of concrete homotypy. Concrete homotypy applies to cases in which the level of resemblance between mimics and models allows prompt association of the latter to a particular taxon at the generic level (Pasteur 1982). This is the case of most snakes with a coral-snake pattern, independent of the sequence and length of dorsal rings. In Amazonia, not only *E. aesculapii aesculapii* (Figures 2, 3D), but also most *Atractus latifrons* (Günther, 1868) have populations with diads on the body (Almeida *et al.* 2014, 2016) that differ of all triad- and monad-patterned *Micrurus* with respect of the sequence of rings. Nonetheless, the scattered presence of specimens with tricolor monads

Figure 2. (**A**) *Micrurus averyi* (CHUFJF000377, TL = 330 mm), Anavilhanas. (**B**) *Erythrolamprus aesculapii aesculapii* with larger red ring and monad (MNRJ 014183, TL = 920 mm), Anavilhanas. (**C**) *E. a. aesculapii* with larger red ring and diads (MNRJ 014185, TL = 800 mm), Anavilhanas. (**D**) *E. a. aesculapii* showing narrow red ring and diads, a pattern frequently found in other regions in Amazonia.

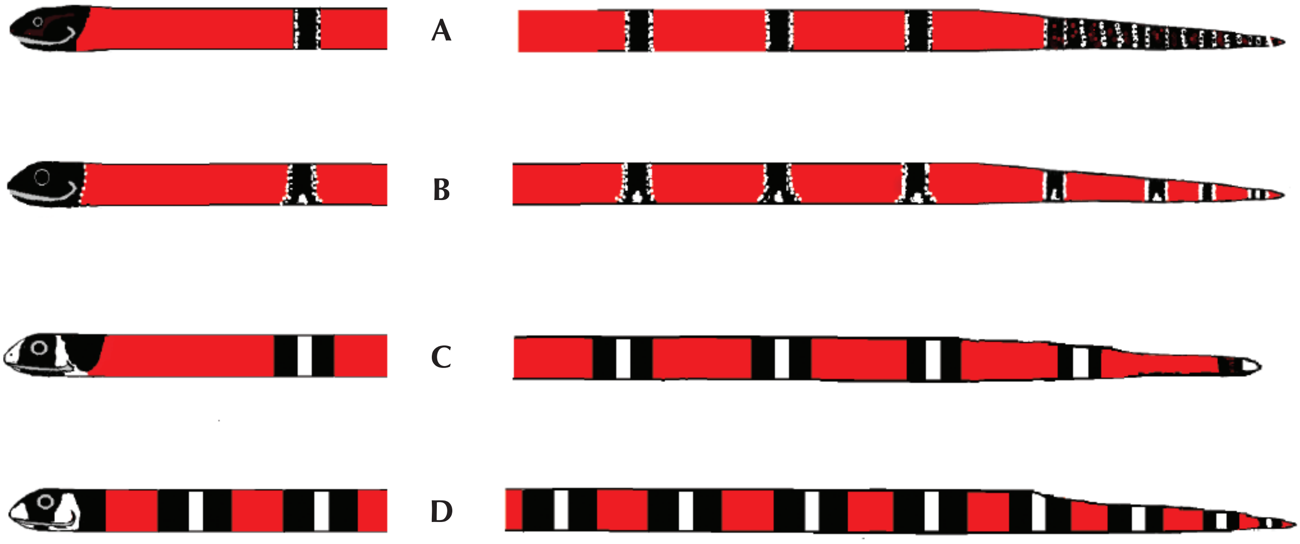

Figure 3. Coloration of the different snakes, showing the different color patterns and arrangement of the rings of the head, body and tail in coral snakes and their possible Batesian mimics. (**A**) *Micrurus averyi* (the model). (**B**) *Erythrolamprus aesculapii aesculapii* (the possible precise mimic). (**C**) *E. a. aesculapii* (the possible imprecise mimic). (**D**) *E. a. aesculapii* (the pattern frequently found in other regions in Amazonia).

(also bicolor in the case of *Atractus*) may reflect instances of perfect mimics involving monad-patterned species of *Micrurus* (e.g., *M. paraensis* Cunha and Nascimento, 1973; *M. averyi*, and *M. albicinctus* Amaral, 1925) (Almeida *et al.* 2016). The sparse records of monad-patterned *E. aesculapii aesculapii* in Amazonia (Almeida *et al.* 2016) do not necessarily coincide with the distribution of *M. averyi;* nevertheless, none of these records includes mimics as perfect as the specimen recorded here.

The existence of presumed mimics that only superficially resemble their potential sympatric models of the genus *Micrurus* is supported by the "multiple models hypothesis" (Edmunds 2000, Sherratt 2002), in which the mimics would benefit from a mosaic of features that may reflect similarities shared with more than one dangerous species. On the other hand, the similarities shared by mimics and models tend to increase when the mimic co-occurs with one single model species, often producing instances of precise mimicry (Akcali *et al.* 2019). Other *Micrurus* have been recorded in the region of the Anavilhanas National Park , e.g., *M. hemprichii, M. lemniscatus* (Linnaeus, 1758), *M. spixii* Wagler, 1824 (Hudson 2007), but *M. averyi* seems to be the mimetic model for the *E. aesculapii aesculapii* described here. The two individuals from Anavilhanas National Park have long red rings, that match the general pattern of *M. averyi,* but one of them has body monads and a mostly black head to accommodate the strict definition of a perfect mimic. Their syntopic occurrence with *M. averyi* suggests that, independent of the general polymorphism of the mimic species, precise mimicry may play a role in the selection of particular phenotypes. Although the precise mimics (monads and long red rings) can be more difficult to distinguish from the model by humans, the imperfect mimic (diads and long red rings) may also provide more effective protection against natural enemies than the usual pattern of *E. aesculapii aesculapii* (diads and short red rings). This is because the proportions of red and black (long red rings, in this case) may be more important than the arrangement of the rings as a sign to identify the highly poisonous model (Kikuchi and Pfennig 2010, França *et al.* 2017). Experimental studies using plasticine models (e.g., Brodie III 1993, Hinman *et al.* 1997, Kikuchi and Pfennig 2010, França *et al.* 2017, Banci *et al*. 2020) would be a useful tool to test such assumptions.

Acknowledgments.—Collection was authorized by Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA) (Collection permit nº 283/2005; Processo IBAMA nº 02002.002414/05.91). BMS and OAVM was supported by Productivity fellowship by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We are grateful to Laurie Vitt who kindly provided one of the photos of *Erytrolamprus aesculapii* (Figure 2D).

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Editor: Jaime Bertoluci

Phyllomedusa 20(1):99–104, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p99-104

Short Communication

Novel cave habitat used by the cryptic lizard *Pinoyscincus abdictus abdictus* (Squamata: Scincidae) on Dinagat Islands, Philippines

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Keywords: Cave, endemic lizard, habitat, trogloxene.

Palavras-chave: caverna, habitat, lagarto endêmico, trogloxeno.

The Philippines supports the highest concentration of endemic terrestrial vertebrates (Heaney 2000, 2007) per unit land area in the world and is intrinsically linked to diverse tropical habitats and a rich geological history (Catibog-Sinha and Heaney 2006, Brown and Diesmos 2009) that included larger landmasses during periods of low sea levels often referred to as Pleistocene aggregate island complexes (PAICs: Brown and Diesmos 2002, Brown *et al.* 2013). A recent comprehensive account of the reptile diversity throughout the entire archipelago included 355 species (Gonzalez *et al.* 2018). The Dinagat island is the third largest island in the Mindanao biogeographic subregion (e.g., Bohol, Leyte, Samar islands), situated just north of northeastern Mindanao (Villanueva 2009, Lillo *et al.* 2019) with approximately 80,212 hectares of land area including 47 islands and islets adjacent to mainland Dinagat (Lillo *et al.* 2019). Topographically, the Dinagat islands are composed of several habitats including

Received 09 February 2021
Accepted 19 May 2021
Distributed June 2021

limestone forest and karst caves spanning from northwestern Dinagat to southwestern part of the island group and shelters numerous endemic reptiles. Cave dwelling reptiles were frequently recorded utilizing different cave microhabitats (e.g., cave walls, crevices, groundwater) for shelter (Sy and Parcon 2014, Nuñeza and Galorio 2015), and as a source of available food (Nuñeza *et al.* 2015). They are not uniformly distributed in the Philippines and have limited dispersal ability (Romero 2009, Culver and Pipan 2013), depending on specific microclimatic features (e.g., temperature, humidity, illuminance) (Lunghi *et al.* 2017) and degree of anthropogenic disturbance on cave habitats. Despite such rich biodiversity and wide array of ecosystems, habitat loss, human overpopulation, high rates of deforestation (Mallari *et al.* 2001, Ong *et al.* 2002, Venturina *et al.* 2020), mining and logging (Lillo *et al.* 2019), illegal hunting, wildlife trade (Gonzalez *et al.* 2018), and persecution of wildlife (i.e. particularly reptiles) for food consumption are the threats to the habitats and associated reptilian communities throughout the Dinagat Islands.

The genus *Pinoyscincus* is a group of scincid lizards that contains both morphologically distinct and cryptic species (Linkem *et al*. 2010, 2011). Earlier studies support the recognition and validity of taxonomic species and subspecies within this group, recognized through sufficient ecological evidence, morphological distinctiveness, distinct biogeographic faunal boundaries and collective information on field surveys (Brown *et al.* 1996, 2000, Ferner *et al.* 2000). Recent taxonomic revision of the genus *Sphenomorphus* resulted in the recognition of the genus *Pinoyscincus* as a distinct group of Philippine skinks (Greer 1974, Linkem *et al.* 2010, 2011). There are eight known species within this genus of which five are reported in Mindanao PAIC and three out five species are partitioned into two subspecies (Linkem *et al.* 2011). This includes the enigmatic *P. abdictus abdictus* (Brown and Alcala 1980) with a co-cryptic subspecies [*P. abdictus aquilonius* (Brown and Alcala 1980)] in Greater Luzon and *P. jagori* *jagori (*Peters, 1864) with a co-cryptic subspecies [P. *jagori grandis* (Taylor, 1922)] in West-Visayas, respectively found in Eastern Mindanao and Dinagat; *Pinoyscincus coxi coxi* (Taylor, 1915) in Central-Western Mindanao with a co-cryptic subspecies [*P. coxi divergens* (Taylor, 1922)] in Southern Luzon and Mindoro; *P. llanosi* (Taylor, 1919) in Samar and Leyte and *P. mindanensis* (Taylor, 1915) in Northern Mindanao (Linkem et al. 2011).

The Mindanao forest skink *P. abdictus abdictus* (Brown and Alcala 1980) was formerly known as *Sphenomorphus abdictus abdictus*; it is a moderately large (SVL range: 81–98 mm) terrestrial skink endemic to Mindanao PAIC subregion (Linkem *et al.* 2010). This scincid lizard is known to occur in the islands of Bohol, Camiguin Sur, Dinagat, and Northeastern Mindanao (Buekema 2011, Nuñeza *et al.* 2015, Sanguila *et al.* 2016, Uetz *et al.* 2020), inhabiting riparian and open forested habitats (Linkem *et al.* 2010) up to 500 m a.s.l. (Uetz *et al.* 2020). Other than this knowledge, its natural history and ecology remains poorly understood.

Herein, we provide a novel cave utilization and unique habitat use of the cryptic Mindanao forest skink *Pinoyscincus abdictus abdictus* found in Hinophopan Cave (10°21'40.8" N, 125°34'00.5" E, WGS 84) at 80 m a.s.l. in a mixed agricultural limestone habitat of Barangay San Juan, Municipality of Loreto in Dinagat Islands. We observed five adults of *P. abdictus abdictus* in the twilight zone (13 m from entrance) and at the entrance zone of a cavern, crawling and actively foraging for food (Figure 1B–C) on cave floors and crevices from 9:00 to 18:00 h during our herpetological survey conducted from 28 January to 4 February 2020. They were observed feeding on ants, crickets and small arthropods in the vicinity of the entrance zone and twilight zone and often basked in the opening of the cave. The vegetation surrounding the cave was mainly composed of limestone outcrops dominated by *Cocos nucfiera* L., *Musa acuminata* L., *Aglaomorpha quercifolia* (L.) J. Sm., *Lygodium circinnatum* Sw., *Phyllanthus ramosii* Quisumb and Merr., and other understory plants. We spent almost 1.3 hours during each visit (three visits; five persons) searching in crevices and cave walls for lizards. We collected a single adult male of moderate size (SVL 83.1 mm, tail length 71.1 mm, weight 13.76 g) in Hinophopan Cave. The dorsum of the individual of *P. abdictus abdictus* had a distinct dark brown pattern with a uniformly ragged, yellow pattern along the rear creating a dorsolateral band from head to tail (less pronounced). The ventral body scales were relatively gray with white fissured lines along the lateral surface of the body. An external tympanum was present and the forelimbs were smaller than the hind limbs. The specimen had large eyes with a unique yellow eye ring coloration and smooth scales on the entire body (Figure 1D).

Lizards were identified using the published accounts of Linkem *et al.* (2011) and Sanguila *et al.* (2016). Voucher specimen was collected and preserved (humanely euthanized with aqueous chloretone, fixed in 10% buffered formalin and subsequently transferred to 70% ethanol) following the standard preservation protocol (Heyer *et al.* 1994, Simmons 2002), and deposited in the Mindanao State University-Iligan Institute of Technology Natural Science Museum (MSU-IIT NSM; collection number NSM 4087).

Utilization of cave habitats by Philippine lizards (e.g., breeding site, foraging, refuging) were also previously reported from different parts of the Philippine archipelago. Records include *Draco spilopterus* (Wiegmann, 1834)

Figure 1. The Mindanao Forest Skink *Pinoyscincus abdictus abdictus* is frequently encountered in limestone habitat and utilized Hinophopan cave system of Loreto Dinagat Island (**A**) to forage for food (**B-D**). Photos by EPM.

and *Gekko mindorensis* (Taylor, 1919) in Cebu and Panay Island (Ferner *et al*. 2000, Supsup *et al.* 2016), *Gekko gigante* (Brown and Alcala, 1978) in Gigantes Island (Bucol *et al.* 2010), *Cyrtodactylus agusanensis* (Taylor, 1915), *Cyrtodactylus annulatus* (Taylor, 1915), *Pinoyscincus jagori jagori* (Peters, 1864), *Sphenomorphus faciatus* (Gray, 1845), *Sphenomorphus variegatus* (Peters, 1867), and *Tropidophorus misaminius* (Stejneger, 1908) in Mindanao Island (Sy and Parcon 2014, Nuñeza *et al.* 2015, Sanguila *et al.* 2016), *Cyrtodactylus philippinicus* (Steindachner, 1867) in Romblon Island Group (Siler *et al.* 2012), and *Gekko gecko* (Linnaeus, 1758) in Siargao Island (Nuñeza and Galorio 2015). *Pinoyscincus abdictus abdictus* have not been reported to use cave habitats before.

Further herpetological surveys and research on Dinagat island group and other areas throughout the Mindanao PAIC are needed to better evaluate the population status and use of cave systems (e.g., seasonal or temporal) and species-habitat association (e.g., niche differentiation, niche overlap) of *P. abdictus abdictus* to other *Pinoyscincus* sympatric species (e.g., *P. jagori jagori* and *P. mindanensis*).

Acknowledgments.—Support for fieldwork was provided by the Department of Science and Technology-Science Education Institute through *Accelerated Science and Technology Human Resource Development Program* (ASTHRDP). We thank the Philippine Department of Environment and Natural Resources through DENR CARAGA Region XIII for the providing research permit (Wildlife Gratuitous Permit No: R13-2019-31). We are grateful to the active support of Provincial Environment and Natural Resource Office (PENRO) of Dinagat Islands, to the local government unit of Loreto through Municipal Environment and Natural Resource Office (MENRO) and Disaster Risk and Reduction Management Office (DRRMO) for providing personal protective equipment and technical assistance on cave surveys, to the Philippine National Police (PNP) of Loreto for transportation and to our enthusiastic field guides Lino M. Olaco and Edilito A. Seda. We also thank Mr. Emerito B. Batara and Ms. Alyana O. Macapayag of Mindanao State University-Iligan Institute of Technology Natural Science Museum for facilitating specimen collection. We extend our appreciation to Benjamin Tapley, Russel Evan L. Venturina and Antonio N. Lorezo II for their comments and suggestions on an earlier draft of this manuscript. We are also grateful for the valuable reviews and constructive criticism provided by the reviewers and associate editor.

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Editor: Claudia Koch

Phyllomedusa 20(1):105–108, 2021

ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p105-108

SHORT COMMUNICATION

Frugivory by *Tropidurus hispidus* (Squamata: Tropiduridae) on the cactus *Pilosocereus pachycladus* in the Brazilian Caatinga

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Keywords: Cactaceae, lizard, mutualism, saurochory.

Palavras-chave: Cactaceae, lagarto, mutualismo, saurocoria.

The lizard *Tropidurus hispidus* (Spix, 1825) occurs in several types of open habitats in South America (Carvalho 2013). It has a continuous distribution, mainly in areas of Caatinga and in coastal areas of northeastern Brazil, where it extends across a large part of the Espinhaço mountain range and occurs in sympatry with several congeners (Carvalho 2013). *Tropidurus hispidus* is omnivorous and consumes small arthropods, vertebrates, and plant material (e.g., leaves, flowers, and fruits) (Vitt 1995, Ribeiro *et al.* 2011). In their generalist diet, plant material has been reported as one of the three most important food items for Caatinga populations (Albuquerque *et al.* 2018); however, data concerning the identity of the plant species consumed are scarce.

Pilosocereus pachycladus subsp. *pernambucoensis* (Ritter) Zappi is an arboreal, columnar cactus that attains a height of 6 m, and is endemic to the Brazilian semiarid region, occurring mainly in rupicolous environments (Zappi *et al.* 2015, Batista *et al.* 2018). It is distributed in the Caatinga ecosystem in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, and Rio Grande do Norte (Zappi *et al.* 2015). Its dehiscent fleshy fruits (3.81 ± 0.45 cm in length and 5.05 ± 0.50 cm in width) are produced at the top of the cactus branch, with purplish pericarp (Figure 1 A, B). The fruits have many small, black seeds embedded in an intense magenta funicular pulp (Abud *et al.* 2010). Typically, this cactus is ornithochoric, as are other *Pilosocereus*

Received 21 August 2020
Accepted 21 December 2020
Distributed June 2021

spp. (Zappi 1994). Frugivory and seed dispersal studies focusing on *Pilosocereus* have recorded birds, lizards, ants, and bats as seed dispersers (Munguía-Rosas *et al.* 2009, Gomes *et al.* 2016, 2017, Santos *et al.* 2019, Vazquéz-Castillo *et al.* 2019).

The Serra do Jatobá is a rocky outcrop located in the municipality of Serra Branca in the state of Paraíba (07°29'49" S, 36°44'32" W); this part of the Caatinga has a seasonal climate and a dry period that extends from May–December. Seven cacti species occur in this area: *Pilosocereus pachycladus* subsp. *pernambucoensis, Xiquexique gounellei* (F.A.C. Weber) Lavor and Calvente (= *P. gounellei*), *P. chrysostele* (Vaupel) Byles and G.D. Rowley, *Cereus jamacaru* D.C., *Melocactus ernestii* Vaupel, *M. zehntneri* (Britton and Rose) Luetzelb., and *Tacinga inamoena* (K. Schum.) N.P. Taylor and Stuppy; all of which produce attractive fleshy fruits consumed by a diversity of animals (Leal *et al.* 2017).

We undertook systematic focal observations of bird-seed dispersers on specimens of *Pilosocereus pachycladus* subsp. *pernambucoensis* fruiting in Serra do Jatobá, and recorded individuals of *Tropidurus hispidus* consuming pulp and seeds for 3–5 minutes around 10:15 h in May 2019 (Figure 1C). The lizard climbed the cactus in seconds, ascending 6 m from the ground to reach the fruit. During the visits, the lizard inserted its head multiple times into the fruit until it reached and removed portions of pulp and seeds. The lizard ingested the seeds entirely without chewing them.

Herpetochory or saurochory on members of the genus *Melocactus* is classified as a positive evolutionary interaction (Guerrero *et al.* 2012). Records of seed dispersal by lizards from cacti usually are associated with globular species *Melocactus.* These plants rarely reach 0.50 m in height (Taylor and Zappi 2004), which facilitates access to fruits and flowers on the cephalium structure by the lizards (Figueira *et al.* 1994, Gomes *et al.* 2014). However, other studies have shown that interactions between lizards and

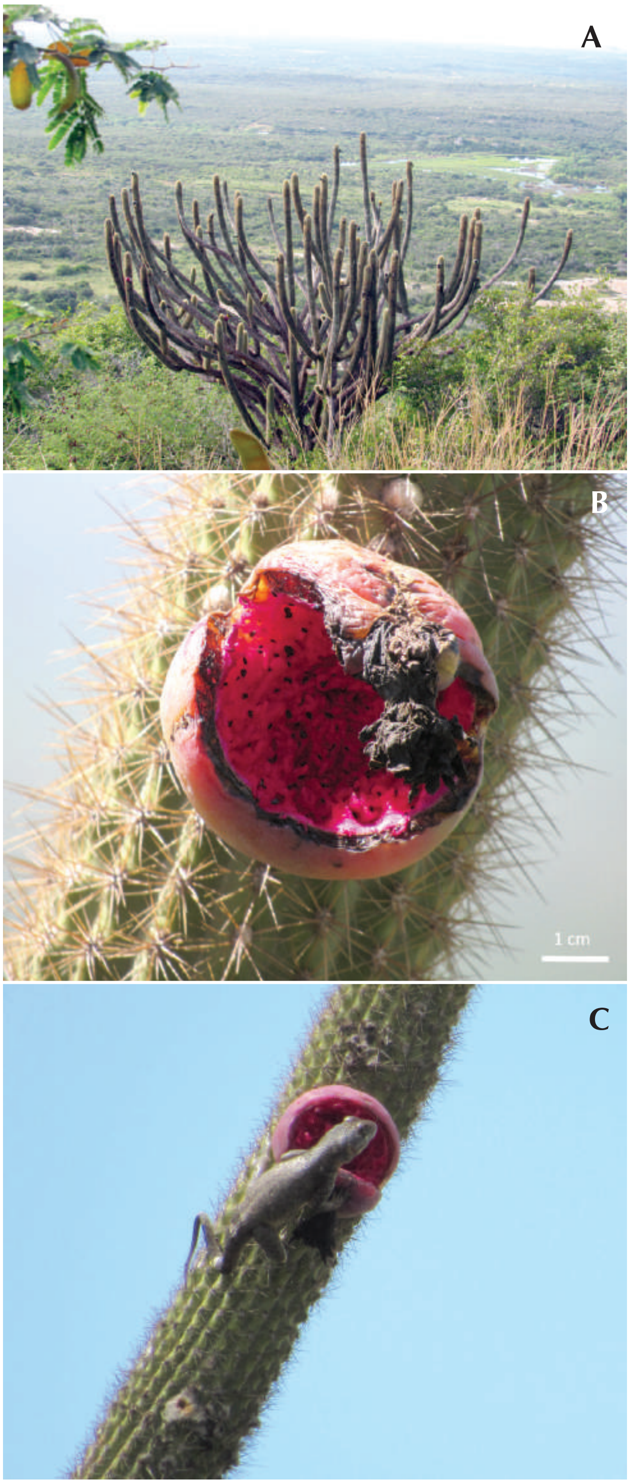

Figure 1. (**A**) *Pilosocereus pachycladus* subsp. *pernambucoensis* in the Serra do Jatobá, in the municipality of Serra Branca, state of Paraíba in northeastern Brazil. (**B**) Fruit showing black seeds embedded in the funicular pulp. (**C**) *Tropidurus hispidus* consuming fruits of *P. pachycladus* subsp. *pernambucoensis.*

cactus are not restricted to globular cacti, and that *Tropidurus* frequently disperses seeds of columnar cacti. For example, in the Caatinga area of the "*Cariri Paraibano*" fruit consumption of *Pilosocereus chrysostele* (6 m high) by *Tropidurus semitaeniatus* (Spix, 1825) and *Pilosocereus gounellei* (4 m high) by *T. semitaeniatus* and *T. hispidus* (Gomes *et al.* 2016, 2017) was recorded, whereas, on rock outcrops in the Atlantic Forest, individuals of *T. torquatus* (Wied-Neuwiedi, 1820) consume fruits of *Coleocephalocereus fluminensis* (Miq.) Backeb. (Koski *et al.* 2018).

Fleshy cactus fruits have a high caloric value, as well as high water content, making them an important food resource for lizards in seasonal environments such as the Caatinga (Fonseca *et al.* 2008, Gomes *et al.* 2014, 2017). Our results report a new and important interaction between lizards and columnar cacti, demonstrating how much these animals expose themselves in their search for resources (e.g., thorn damage, predation risks by predatory birds). Guerrero *et al.* (2012) pointed out that seed dispersal by lizards is considered an infrequent dispersal system for Cactaceae and other angiosperms, specially because studies focusing on other tribes that produce attractive fruits for reptiles within Cactaceae are still missing. The Caatinga biota includes more than 16 species of *Pilosocereus* (Zappi *et al.* 2015) and nine species of *Tropidurus* (Mesquita *et al.* 2017). We think that lizards in the Caatinga disperse columnar cacti seeds frequently and expect new examples to be reported soon for this seasonal ecosystem.

Meiado (2012) found that the presence of the funicular pulp inhibits seed germination for *Pilosocereus gounellei* subsp. *gounellei* and *P. pachycladus* subsp. *pernambucoensis*. Such data reinforce the importance of dispersers in increasing the germination rates of seeds of *Pilosocereus.* In fact, seeds of *P. gounellei* subsp. *gounellei* ingested and defecated by *Tropidurus semitaeniatus* have better germination rates than do undigested seeds (Gomes *et al.* 2016). We collected 30 fecal samples of *T. hispidus* containing intact cactus seeds. To confirm the role of *T. hispidus* as an effective seed disperser of *P. pachycladus* subsp. *pernambucoensis,* studies evaluating the effects of passing seeds through the digestive tract of the lizard are necessary.

Acknowledgments.—We thank Daniela Zappi and Ânderson Brener for identification of cactus and lizard species, respectively, and Maria Doralice Barbosa for permission to work at Serra do Jatobá. Edito Romão and Ellen Moura for providing fieldwork logistics. We also thank Capes for the 6-mo Master's scholarship granted to INSN and CNPq for the PCI-DB fellowship granted VGNG.

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Editor: Jaime Bertoluci

Phyllomedusa 20(1):109–115, 2021

ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p109-115

Short Communication

Reassessing overlooked information about the diagnosis of *Brachycephalus atelopoide* (Anura: Brachycephalidae), a neglected problem for the taxonomy of the genus

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Keywords: *Brachycephalus ephippium* Group, *Brachycephalus pernix* Group, *Brachycephalus tridactylus,* ontogeny.

Palavras-chave: *Brachycephalus tridactylus,* Grupo *Brachycephalus ephippium,* Grupo *Brachycephalus pernix,* ontogenia.

The anuran genus *Brachycephalus* is divided into three phenetic species groups based on body shape and presence/absence of hyperossification (Ribeiro *et al.* 2015), characteristics that are relatively easy to examine in preserved specimens. Pie *et al.* (2018) reported a third feature (the *linea masculinea*) that differentiates these species groups, in addition to body shape and hyperossification. On the basis of these characters, all species of *Brachycephalus* can be assigned to one of the three species groups (e.g., Bornschein *et al.* 2019) except for one—*B. atelopoide* Miranda-Ribeiro, 1920, whose holotype is presumed to be lost (Pombal 2010). In 1920, Miranda-Ribeiro described four varieties of *B. ephippium* (Spix, 1824)—viz., *atelopoide*, *nodoterga*, *garbeana*, and *bufonoides* (Miranda-Ribeiro 1920). At the time, these "varieties" were not recognized as species because some frogs were found on the presumptive type localities that departed from their original description (Miranda-Ribeiro 1920). Seventy years later, the variety *nodoterga* was recognized as a full species of *Brachycephalus* (Heyer *et al.* 1990) when it was found at Estação Biológica de Boracéia, municipality of Salesópolis, state of São Paulo, Brazil.

Pombal (2010) reassessed the taxonomic status of the varieties of *Brachycephalus* described by Miranda-Ribeiro (1920) and

Received 15 July 2020
Accepted 03 December 2020
Distributed June 2021

recognized all of them as valid species. He also confirmed that specimens of *B. garbeanus* Miranda-Ribeiro, 1920 had been collected at the municipality of Nova Friburgo, state of Rio de Janeiro, Brazil, with additional reports of populations of this species having been reported since (e.g., Clemente-Carvalho *et al.* 2011, Dorigo *et al.* 2012). No recent reports exist of known populations of *B. atelopoide* and *B. bufonoides* Miranda-Ribeiro, 1920.

The *atelopoide* variety described by Miranda-Ribeiro (1920) was based on one individual of 30 that "perfectly" matched *B. ephippium* collected at Piquete, on the slope of the Mantiqueira Mountain Range, municipality of Piquete, state of São Paulo, Brazil. The *atelopoide* variety was distinguished by lacking "cephalic coating" and "dorsal shields", and by having "protruding warts over the skin" (Miranda-Ribeiro 1920). *Brachycephalus ephippium* lacks protruding warts and has conspicuous hyperossification plates on the dorsal surfaces of head and body (e.g., Clemente-Carvalho *et al.* 2009); see the plate depicting *B. ephippium* in Miranda-Ribeiro (1920).

Hyperossification associated with a bufoniform body shape is the diagnosis of the *Brachycephalus ephippium* species group (Ribeiro *et al.* 2015), which includes *B. bufonoides*, *B. garbeanus*, and *B. nodoterga* Miranda-Ribeiro, 1920 (Bornschein *et al.* 2016a). However, *B. atelopoide* was not included in any of the three species groups of *Brachycephalus* (*sensu* Ribeiro *et al.* 2015) because presence/absence of hyperossification could not be determined (Bornschein *et al.* 2016a). In addition to the *B. ephippium* group, *Brachycephalus* also includes the *B. pernix* and *B. didactylus* groups that lack hyperossification. Both the *B. pernix* and *B. ephippium* groups have bufoniform bodies that distinguish them from members of the *B. didactylus* group with their leptodactyliform body shape (Ribeiro *et al.* 2015). Miranda-Ribeiro (1920) did not describe the body shape of *B. atelopoide,* but given that he distinguished *B. atelopoide* from a series of specimens identified as *B. ephippium* based on ossification characteristics and skin texture (Miranda-Ribeiro 1920), one would expect that it also had a bufoniform body.

Recent studies have shown that the extent of hyperossification in species of the *Brachycephalus ephippium* group varies intraspecifically and ontogenetically (Clemente-Carvalho *et al.* 2009, 2011, Campos *et al.* 2010, Pombal 2010). Pombal (2010) mentioned that the extent of hyperossification varies individually in *B. garbeanus,* being less well developed in smaller (and presumably more immature) frogs (see also Clemente-Carvalho *et al.* 2009, 2011). Hyperossification also varies ontogenetically in *B. ephippium* (Campos *et al.* 2010, Goutte *et al.* 2019). Thus, we might question the level of developmental maturation of the specimen described as *B. atelopoide,* as well its specific status.

Herein, we revise the diagnosis of *Brachycephalus atelopoide* by reviewing its original description and comparing this taxon with the other varieties that were described by Miranda-Ribeiro (1920), redescribed by Pombal (2010), and referenced in other taxonomic studies. We agree with Ribeiro *et al.* (2015), who suggested that the taxonomic status of *B. atelopoide* should be revisited. The type material of the other varieties described by Miranda-Ribeiro (1920) was not examined because the specimens are in poor condition and because there are excellent photographs available in Pombal (2010). The material we have examined of the other species is listed in Appendix I.

Upon reviewing Miranda-Ribeiro (1920), we noted three features in the description of *Brachycephalus atelopoide* that warrant discussion with respect to the description of *B. nodoterga,* which also was described based on a single individual. First, Miranda-Ribeiro (1920) mentioned that the varieties *nodoterga* and *atelopoide* have the same body shape, but *nodoterga* differs by having more abundant, large, elongate warts, in pairs, that might be ossified [osteoderms?]. This may indicate that *nodoterga* lacked hyperossification (i.e., "cephalic coating" and body "dorsal shields"), as does *atelopoide*. However, in the redescription of *B. nodoterga,* Pombal (2010) reported hyperossification along the vertebrae and a pair of bony dorsal plates. Hyperossification of the trunk and of the center and sides of the head of the type of *B. nodoterga* is evident in Pombal (2010: Fig. 2); see also Figs. 5 and 6 of Clemente-Carvalho *et al.* (2009) and Fig. 6 of Haddad *et al.* (2010). Second, Miranda-Ribeiro (1920) reported that *nodoterga* was 15 mm long and apparently immature, like *atelopoide.* Pombal (2010) reported that the holotype of *nodoterga* was 12.4 mm in snout–vent length (SVL). Finally, Miranda-Ribeiro (1920) commented that *nodoterga* has a larger number of warts, which were themselves larger and more elongate, than did *atelopoide.* However, in the redescription of *B. nodoterga,* Pombal (2010) mentioned only the presence of "sparse protuberances, like warts".

The characteristics of the holotype of *Brachycephalus atelopoide* relative to those of *B. nodoterga* suggest that *B. atelopoide* is a juvenile only about 12 mm long, having few warts on the body and presumably about the same level of hyperossification on the head and back as the holotype of *B. nodoterga.* These features were overlooked by Pombal (2010), as well as in most other studies of *Brachycephalus.* Of the 20 species of *Brachycephalus* species described between 2011 and 2020, most authors disregarded *B. atelopoide.* Thirteen species were described without mentioning *B. atelopoide* in their diagnoses—viz., *B. albolineatus* Bornschein, Ribeiro, Blackburn, Stanley, and Pie, 2016, *B. auroguttatus* Ribeiro, Firkowski, Bornschein, and Pie, 2015, *B. boticario* Pie, Bornschein, Firkowski, Belmonte-Lopes, and Ribeiro, 2015, *B. coloratus* Ribeiro, Blackburn, Stanley, Pie, and Bornschein, 2017, *B. curupira* Ribeiro, Blackburn, Stanley, Pie, and Bornschein, 2017, *B. fuscolineatus* Pie, Bornschein, Firkowski, Belmonte-Lopes, and Ribeiro, 2015, *B. guarani* Clemente-Carvalho, Giaretta, Condez, Haddad, and Reis, 2012, *B. leopardus* Ribeiro, Firkowski, and Pie, 2015, *B. mariaeterezae* Bornschein, Morato, Firkowski, Ribeiro, and Pie, 2015, *B. olivaceus* Bornschein, Morato, Firkowski, Ribeiro, and Pie, 2015, *B. quiririensis* Pie and Ribeiro, 2015, *B. tridactylus* Garey, Lima, Hartmann, and Haddad, 2012, and *B. verrucosus* Ribeiro, Firkowski, Bornschein, and Pie, 2015 (Clemente-Carvalho *et al.* 2012, Garey *et al.* 2012, Pie and Ribeiro 2015, Ribeiro *et al.* 2015, 2017, Bornschein *et al.* 2016b). Of the seven other species descriptions that do mention *B. atelopoide* in their diagnoses, we noted the following: (1) one (*B. mirissimus* Pie, Ribeiro, Confetti, Nadaline, and Bornschein, 2018; Pie *et al.* 2018) lacked a comparison because the holotype *B. atelopoide* is lost; (2) another (*B. pulex* Napoli, Caramaschi, Cruz, and Dias, 2011; Napoli *et al.* 2011) was compared without citing the source of data; (3) *Brachycephalus crispus* Condez, Clemente-Carvalho, Haddad, and Reis, 2014 (Condez *et al.* 2014) was compared using features that differ from those of the original description (Miranda-Ribeiro 1920) and redescription (Pombal 2010) of *B. atelopoide;* (4) the four remaining species descriptions included comparisons with the diagnosis of *B. atelopoide. Brachycephalus margaritatus* Pombal and Izecksohn, 2011 was compared with a holotype of *B. atelopoide* without hyperossification (Pombal and Izecksohn 2011). *Brachycephalus sulfuratus* Condez, Monteiro, Comitti, Garcia, Amaral, and Haddad, 2016 and *B. actaeus* Monteiro, Condez, Garcia, Comitti, Amaral, and Haddad, 2018 were compared to a holotype with an intermediate condition of hyperossification of the skull and skeleton (Condez *et al.* 2016, Monteiro *et al.* 2018) (these studies agreed with our interpretation, although these authors did not justify their conclusions). And last, *B. darkside* Guimarães, Luz, Rocha, and Feio, 2017 was compared with a holotype with osteoderms (Guimarães *et al.* 2017).

If ones assumes that the holotype of *Brachycephalus atelopoide* had an intermediate level of hyperossification, the species resembles a juvenile *B. ephippium.* Campos *et al.* (2010) reported on the levels of hyperossification in the heads and vertebrae of juveniles of *B. ephippium* with SVLs of 8.1 mm, 10.6 mm, 12.8 mm, and 13.6 in SVL in contrast to adults of this species that attain a SVL to 19.7 mm (Pombal 2001). However, *B. ephippium* does not possess large and elongate warts. If *B. atelopoide* had an intermediate amount of hyperossification, it would also resemble adult *B. nodoterga, B. alipioi* Pombal and Gasparini, 2006, *B. crispus, B. guarani, B. pitanga* Alves, Sawaya, Reis, and Haddad, 2009, *B. toby* Haddad, Alves, Clemente-Carvalho, and Reis, 2010, and *B. vertebralis* Pombal, 2001, all of which have lower hyperossification than do adult *B. ephippium* (Campos *et al.* 2010, Haddad *et al.* 2010, Clemente-Carvalho *et al.* 2012, Condez *et al.* 2014, 2020). In having large warts, *B. atelopoide* resembles *B. nodoterga, B. pitanga, B. crispus* (Campos *et al.* 2010, Condez *et al.* 2014, 2020), and especially *B. pitanga,* which has fewer of these warts.

In the absence of the holotype of *Brachycephalus atelopoide,* we cannot exclude the possibility that a species described after *B. atelopoide* may be a synonym of this species. For example, both *B. nodoterga* and *B. pitanga* are similar to *B. atelopoide.* Moreover, recently discovered populations of *B. tridactylus* in São Paulo (Appendix I) bear warts that are similar to those described for *B. atelopoide* on the sides of the body (MRB *et al.*, unpub. data, 2019). The taxonomic problem is further complicated by the equivocal descriptions and analysis of Miranda-Ribeiro (1920), who overlooked the presence of hyperossification in *B. nodoterga* and *B. bufonoides* and mistakenly identified three *B. nodoterga* as *B. ephippium* (Pombal 2010). Flaws in diagnoses of recently described species of *Brachycephalus* with respect to *B. atelopoide* also contribute to the confusion. Perhaps the best working solution is to adopt Cochran's (1955) proposal to place *Brachycephalus atelopoide* in the synonymy of *B. ephippium,* thereby avoiding nomenclatural confusion, according to article #23.9.3 of the International Zoological Nomenclature Code. We hope that in forthcoming taxonomic reviews, the authors will explore the morphological evidence more thoroughly.

Acknowledgments.—This study was partially funded by Fundação Grupo Boticário de Proteção à Natureza, through a project (1149_20191) conducted by Mater Natura - Instituto de Estudos Ambientais. We thank Vanessa K. Verdade and two anonymous reviewers for important comments that improved the manuscript.

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Editor: Vanessa K. Verdade

Appendix I. *Specimens examined. Abbreviations: CFBH = Célio F. B. Haddad collection, Departamento de Zoologia, Universidade Estadual Paulista, campus de Rio Claro, São Paulo state, Brazil; DZUP = Coleção Herpetológica do Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná state, Brazil; MHNCI = Museu de História Natural Capão da Imbuia, Curitiba, Paraná state, Brazil; MNRJ = Museu Nacional, Rio de Janeiro, Rio de Janeiro state, Brazil; MZUSP = Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo state, Brazil; and ZUEC = Museu de História Natural, Universidade Estadual de Campinas, Campinas, São Paulo state, Brazil.*

Brachycephalus actaeus. BRAZIL. SANTA CATARINA: Serra da Tiririca, municipality of Itapopá MHNCI 10832–10834, 11024–11030.

Brachycephalus albolineatus. BRAZIL. SANTA CATARINA: Morro Azul, on the border between the municipalities of Pomerode and Rio dos Cedros MHNCI 10291, 10301, 10846; Morro Boa Vista, boundary of the municipalities of Jaraguá do Sul and Massaranduba MHNCI 10290 (holotype), MHNCI 10295–10300 (paratypes), MHNCI 10293 (juvenile), MHNCI two uncatalogued specimens, MNRJ 90349 (paratype); Morro do Garrafão, municipality of Corupá MHNCI 10836–10842; Morro do Schmidt, municipality of Pomerode MHNCI four uncatalogued specimens.

Brachycephalus alipioi. BRAZIL. ESPÍRITO SANTO: Alto Castelinho, municipality of Vargem Alta MHNCI 10804–10806.

Brachycephalus auroguttatus. BRAZIL. SANTA CATARINA: Pedra da Tartaruga, municipality of Garuva DZUP 375 (holotype), DZUP 373–374, 376–385, 387–389 (all paratypes); trail to Pedra da Tartaruga, municipality of Garuva MHNCI six uncatalogued specimens.

Brachycephalus boticario. BRAZIL. SANTA CATARINA: Morro do Cachorro, boundary of the municipalities of Blumenau, Gaspar, and Luiz Alves DZUP 440 (holotype), DZUP 414, 415, 438, 439, 444, 445, 459 (all paratypes), MHNCI three uncatalogued specimens.

Brachycephalus brunneus. BRAZIL. PARANÁ: Abrigo 1, municipality of Campina Grande do Sul DZUP 517–519; Camapuã, Serra dos Órgãos, boundary of the municipalities of Campina Grande do Sul and Antonina MHNCI 10165–10174; Caratuva, Serra dos Órgãos, municipality of Campina Grande do Sul MHNCI 1919, 1920, 10175–10184, 10733, 10734, MNRJ 40289–40291 (paratypes); Mãe Catira, Serra da Graciosa, municipality of Quatro Barras DZUP 140, 230, MHNCI 10784.

Brachycephalus coloratus. BRAZIL. PARANÁ: Estância Hidroclimática Recreio da Serra, Serra da Baitaca, municipality of Piraquara MHNCI 10273 (holotype), MHNCI 10274–10279 (paratypes), MNRJ 89949, 89950 (paratypes), DZUP 600; Pão de Ló, Serra da Baitaca, municipality of Quatro Barras MHNCI one uncatalogued specimen.

Brachycephalus curupira. BRAZIL. PARANÁ: Morro do Canal, municipality of Piraquara MHNCI 10724–10728; Morro do Vigia, municipality of Piraquara MHNCI 10723; Serra do Salto, Malhada District, municipality of São José dos Pinhais MHNCI 10280 (holotype), MHNCI 10281–10287, 10292 (all paratypes).

Brachycephalus didactylus. BRAZIL. RIO DE JANEIRO: municipality of Engenheiro Paulo de Frontin ZUEC 10825; Sacra Família do Tinguá, municipality of Engenheiro Paulo de Frontin ZUEC 1132, 1133 (topotypes), MZUSP 13613–13620, 64810, 64811, 94621 (topotypes).

Brachycephalus ephippium. BRAZIL. RIO DE JANEIRO: Parque Nacional Serra dos Órgãos MZUSP 104140–104147; Vale de Revolta MCZ A-108655. SÃO PAULO: municipality of Cotia MHNCI 2611–2616.

Brachycephalus ferruginus. BRAZIL. PARANÁ: Olimpo, Serra do Marumbi, municipality of Morretes MHNCI 125, 128 (topotypes), DZUP 562 (topotype), MHNCI five uncatalogued specimens (topotypes).

Brachycephalus fuscolineatus. BRAZIL. SANTA CATARINA: Morro do Baú, municipality of Ilhota DZUP 159 (holotype), DZUP 158, 160, 401–405 (all paratypes), MHNCI three uncatalogued specimens; Morro Braço da Onça, municipality of Luiz Alves MHNCI 10850, 10851.

Brachycephalus hermogenesi. BRAZIL. SÃO PAULO: Picinguaba, Parque Estadual da Serra do Mar, municipality of Ubatuba ZUEC 9715 (holotype), ZUEC 9716–9725 (paratypes); Corcovado, municipality of Ubatuba MHNCI 10823–10825.

Brachycephalus izecksohni. BRAZIL. PARANÁ: Torre da Prata, Serra da Prata, boundary of the municipalities of Morretes, Paranaguá, and Guaratuba CFBH 7381, 7382, 7384 (all paratypes), MHNCI 10835, DZUP 561.

Brachycephalus leopardus. BRAZIL. PARANÁ: Morro dos Perdidos, municipality of Guaratuba DZUP 274–283, MHNCI 10783, MHNCI three uncatalogued specimens; Serra do Araçatuba, municipality of Tijucas do Sul MHNCI 10250 (holotype), MHNCI 10239–10249, 10251, 10252 (all paratypes), MHNCI two uncatalogued specimens, DZUP 520–523.

Brachycephalus mariaeterezae. BRAZIL. SANTA CATARINA: Reserva Particular do Patrimônio Natural Caetezal, top of the Serra Queimada, municipality of Joinville MHNCI 9811 (holotype), DZUP 372, 393–399 (all paratypes).

Brachycephalus mirissimus. BRAZIL. SANTA CATARINA: Morro Santo Anjo, municipality of Massaranduba MHNCI 10793 (holotype), MHNCI 10794–10803 (paratypes), MHNCI two uncatalogued specimens.

Brachycephalus nodoterga. SÃO PAULO: Reserva Biológica Tamboré, municipality of Santana de Parnaíba MZUSP 147711–147716.

Brachycephalus olivaceus. BRAZIL. Santa Catarina: base of the Serra Queimada, municipality of Joinville MHNCI 9813 (holotype), DZUP 371 (paratype); Castelo dos Bugres, municipality of Joinville MHNCI 9814–9818 (paratypes), MHNCI 10 uncatalogued specimens; Morro do Boi, municipality of Corupá MHNCI 10288, 10289; Pico Jurapê, municipality of Joinville MHNCI one uncatalogued specimen.

Brachycephalus pernix. BRAZIL. Paraná: Anhangava, Serra da Baitaca, municipality of Quatro Barras MNRJ 17349 (holotype), CFBH 2597, 2598 (paratypes), MHNCI 1818, 1889, 3000–3004 (all paratypes), MHNCI 1820, ZUEC 9433–9437 (paratypes), MHNCI 9806–9810, 10153–10164.

Brachycephalus pitanga. BRAZIL. São Paulo: rodovia SP-125, municipality of São Luiz do Paraitinga MHNCI 10733, 10734; Trilha do Ipiranga 50 m from the Rio Ipiranga, Núcleo Santa Virgínia, Parque Estadual da Serra do Mar, municipality of São Luiz do Paraitinga MHNCI 10733, 10734, 10821, 10822, 10843 (all topotypes), DZUP 409 (topotype).

Brachycephalus pombali. BRAZIL. Paraná: Morro dos Padres, Serra da Igreja, municipality of Morretes CFBH 8042 (holotype), 8043–8053 (paratypes), DZUP 213–220, 603–613, MHNCI nine uncatalogued specimens.

Brachycephalus quiririensis. BRAZIL. Santa Catarina: Serra do Quiriri, municipality of Campo Alegre MHNCI 10261 (holotype), MHNCI 10260, 10262–10272 (all paratypes), DZUP 531–535.

Brachycephalus sulfuratus. BRAZIL. Paraná: Caratuval, near the Parque Estadual das Lauráceas, municipality of Adrianópolis DZUP 139; Corvo, municipality of Quatro Barras DZUP 150–157; Fazenda Thalia, municipality of Balsa Nova DZUP 221–224; Mananciais da Serra, municipality of Piraquara MHNCI 10302; Recanto das Hortências, municipality of São José dos Pinhais DZUP 463; Salto do Inferno, Rio Capivari, municipality of Bocaiúva do Sul MHNCI 9800. Santa Catarina: Morro do Garrafão, municipality of Corupá MHNCI 10826–10828. São Paulo: base of the Serra Água Limpa, municipality of Apiaí DZUP 362; near Jurupará dam, municipality of Piedade MHNCI 10829–10831.

Brachycephalus toby. BRAZIL. São Paulo: Morro do Corcovado, Parque Estadual da Serra do Mar, municipality of Ubatuba MHNCI 10807–10809 (topotypes).

Brachycephalus tridactylus. BRAZIL. Paraná: Serra do Morato, Reserva Natural Salto Morato, municipality of Guaraqueçaba MHNCI 10185–10189, 10294, 10729, 10730 (topotypes), CFBH 43887–43890 (topotypes). São Paulo: Bairro Rio Vermelho, municipality of Barra do Turvo MHNCI two uncatalogued specimens; Estrada das Conchas, municipality of Barra do Turvo MHNCI one uncatalogued specimen; Fazenda Fronteira, municipality of Barra do Turvo MHNCI seven uncatalogued specimens; Morro do Bisel, Serra do Guaraú, municipality of Cajati DZUP 687; Serra do Pinheiro, municipality of Cajati MHNCI five uncatalogued specimens; Serra Pelada, municipality of Barra do Turvo DZUP 688–691; Torre Embratel, municipality of Cajati MHNCI 10848, 10852, DZUP 676–686.

Brachycephalus verrucosus. BRAZIL. Santa Catarina: Morro da Tromba, municipality of Joinville MHNCI 9819 (holotype), MHNCI 9820 (paratype), DZUP 464–478 (paratypes).

Brachycephalus vertebralis. BRAZIL. Rio de Janeiro/São Paulo: Morro Cuscuzeiro, Núcleo Picinguaba of the Parque Estadual da Serra do Mar and Parque Nacional da Serra da Bocaina, boundary of the municipalities of Parati, Rio de Janeiro state, and Ubatuba, São Paulo state MHNCI 10810–10820.

***Brachycephalus* sp.** BRAZIL. Paraná: Chapeuzinho, boundary of the municipalities of Morretes and Piraquara DZUP 502–504; Pedra Branca do Araraquara, Serra do Araraquara, municipality of Guaratuba DZUP 400; Serra Canasvieiras, boundary of the municipalities of Guaratuba and Morretes MHNCI 10785, DZUP 452, 453. Santa Catarina: Morro da Pedra, municipality of Navegantes MHNCI two uncatalogued specimens; Morro da Prata, municipality of Ilhota MHNCI four uncatalogued specimens; Morro dos Monos; municipality of Luiz Alves MHNCI two uncatalogued specimens.

Phyllomedusa 20(1):117–123, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p117-123

Short Communication

Limb abnormalities in *Peltophryne florentinoi* (Anura: Bufonidae) from Cuba

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Keywords: Cuban toads, Critically Endangered, inbreeding, skeletal anomalies, Zapata Swamp.

Palavras-chave: anomalias esqueléticas, Criticamente Ameaçada, endogamia, Pântano de Zapata, sapos cubanos.

Abnormal individuals in natural populations of amphibians have been reported for more than 400 species from around the World (Henle *et al.* 2017a, Haas *et al.* 2018). Several forms of externally visible abnormalities have been described, according to the types and location (Meteyer *et al.* 2000, Lannoo 2008, Henle *et al.* 2017b). Skeletal duplications, absence and reduction of skeletal elements, and other skeletal abnormalities (bony bridges, rotated limbs, truncated vertebral column, and torsion of the tail or body) are probably the most common in adults. However, many other non-skeletal morphological anomalies, such as duplication of the eyes and the spiraculum, absence of the tympanum, microphthalmy and anophthalmy, edema, tumors and several color abnormalities have been also reported (Henle *et al.* 2017b). Malformed mouthparts have been widely documented in tadpoles of several species (Lannoo 2008, Henle *et al.* 2017a). Recently, malformed adult individuals, tadpoles with abnormal mouthparts and anomalously colored frogs have been documented in Cuba (Alonso Bosch *et al.* 2017, García-Padrón and Alonso Bosch 2017, 2019). According to these authors, such abnormalities may indicate degraded environmental health or genetic disorders.

The Zapata Toad, *Peltophryne florentinoi* (Moreno and Rivalta, 2007), is a Cuban toad restricted to the coastal microphyllous evergreen forest on limestone landscapes, near Playa Girón

Received 02 October 2020
Accepted 06 April 2021
Distributed June 2021

and Guasasa, Zapata Swamp, Mantanzas Province (Moreno and Rivalta 2007, Alonso Bosch and Cobos 2016). Recently, one individual of this species was located in Guajimico Villagein, Cienfuegos Province (Díaz *et al*. 2019). Based on its reduced geographical distribution (Figure 1) and the threat that sea level rise would represent for this species, *P. florentinoi* was categorized as Critically Endangered (CR), according to the criteria of the IUCN (Rivalta 2008). Its habitat is being transformed by small-scale agriculture, the extraction of forest elements and dumping of solid waste (Alonso Bosch and Cobos 2016). Warmer and drier conditions, occasional inundations and saltwater intrusion may represent additional stressors in the near future (Cobos and Alonso Bosch 2018).

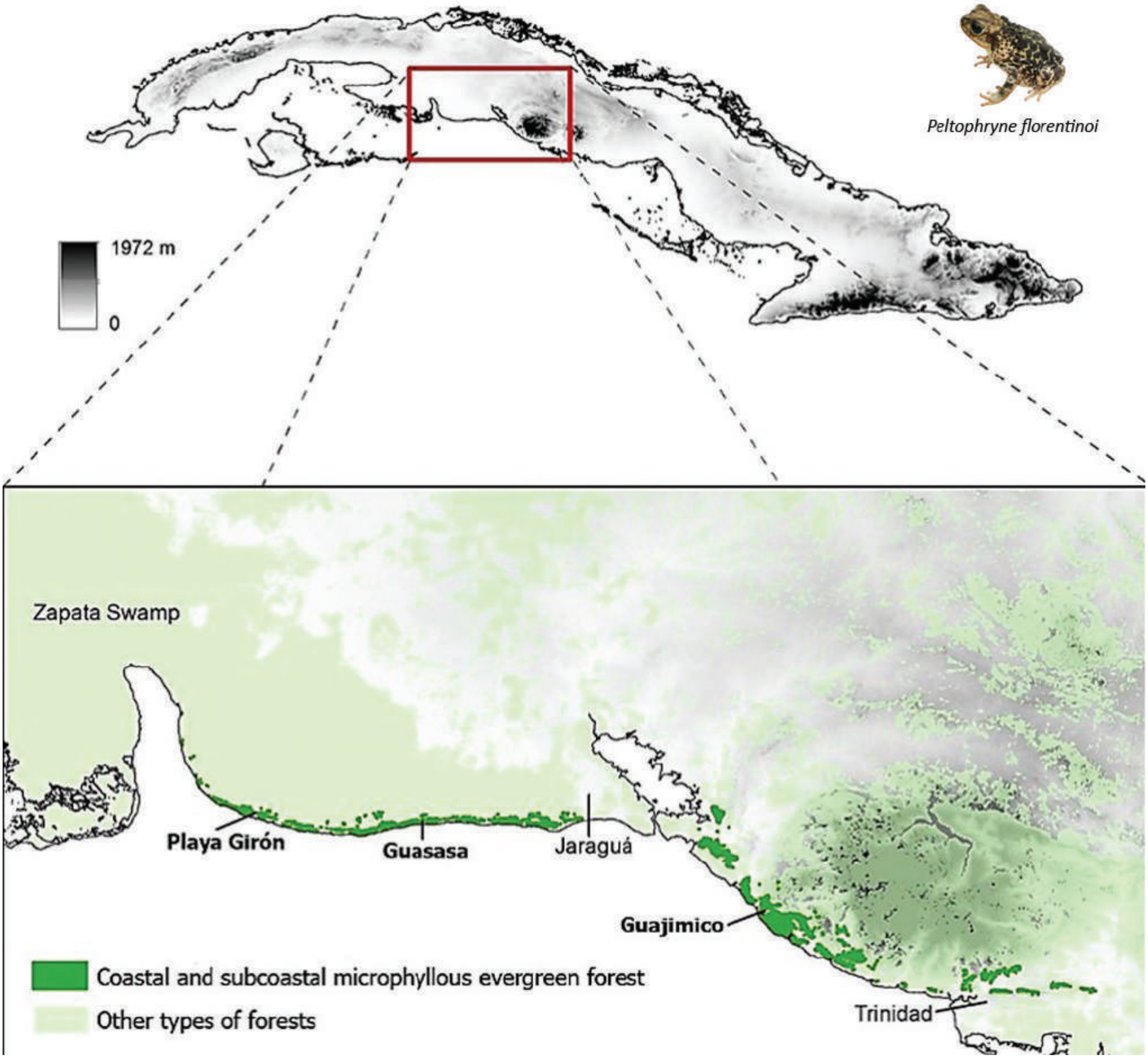

Figure 1. Geographic distribution of *Peltophryne florentinoi* in the Microphyllous Evergreen Forest of the southern coasts of Matanzas and Cienfuegos provinces of Cuba (courtesy of Marlon Cobos). The names of known localities of *P. florentinoi* are highlighted in bold.

During an ongoing amphibian-monitoring initiative focused on the study of the abundance, phenology and habitat quality of *P. florentinoi* and its environment, adult individuals with limb abnormalities were observed. We visited the coastal Microphyllous Evergreen forests surrounding the type locality, Playa Girón (22°4'27.91" N, 81°2'73" W; 7 m a.s.l.) and the vicinity of Guasasa town, 20 km E of the type locality (22°3'28.74" N, 80°47'56.81" W; 4 m a.s.l.). At the beginning (May) and the middle (August) of the rainy season, we visited both areas in 2016 and 2018. We also inspected a third area near the type locality during May–June 2019. Via a combination of visual and acoustic encounter surveys (Crump and Scott 1994, Zimmerman 1994), we inspected forest patches, using headlamps, searching active animals and in breeding sites between 21:00-24:00 h, during three consecutive nights. Each captured individual was marked by a unique combination of color points (using permanent markers: black-blue-red) placed on ante-orbital crests and then released at the site of collection.

We detected externally visible skeletal abnormalities in adult individuals from both localities during the three years of the study. The frequency of abnormalities was slightly similar between localities. We caught 61 adult individuals from Playa Girón, 10 of them (16.4%) exhibited at least one type of abnormality. We identified three abnormal individuals (17.6%) among a sample of 17 animals captured at Guasasa. In total, thirteen adult specimens of both sexes had one or more abnormalities. Fore and hind limb abnormalities of adults were observed. Based on the nomenclature suggested by Henle *et al.* (2017b), we recognized at least six types of abnormalities in the present study (Figure 2). Brachydactyly was the most frequently observed abnormality, but the combination of mixed abnormalities (*N* = 5), usually included brachydactyly too. We observed amely, ectropody and syndactyly only once each. Most limb abnormalities were unilateral (*N* = 1 bilateral); abnormal fore and hind limbs in the same individual were rarely observed (*N* = 1).

Although the relationship between the causative agents and the observed abnormalities in the environment is still poorly understood, physicochemical environmental stress factors, such as ultraviolet radiation and pollution, have been suggested to explain such observations in wild amphibian populations (Blaustein and Johnson 2003, Linzey *et al.* 2003, Henle *et al.* 2017c). On the other hand, biological stress factors such as predation, pathogen infections, hybridization and inbreeding have also been considered among the possible causes (Johnson *et al.* 1999, 2001a, b, Bowerman *et al.* 2010). Some authors have proposed synergistic interactions between some environmental or biological factors may exist, as well (Ouellet 2000, Bancroft *et al*. 2008, Reeves *et al*. 2010, Lunde and Johnson 2012).

The causes for the abnormalities recorded in the Zapata's Toad remain unknown. Even though we should not rule out any of the aforementioned biotic or abiotic factors, some seem unlikely to apply to this system. Small-scale agriculture has been identified as threat to habitat quality of the Zapata's Toad (Alonso Bosch and Cobos 2016), but chemical pesticides or fertilizers are not widely used here. No evidence of predation or parasitic infection have been found. Limb anomalies have been observed in laboratory experiments that evaluated the exposure to UV radiation, yet the overall existing literature indicates that it is an unlikely cause for limb anomalies observed in natural populations (Henle *et al.* 2017c). The phenology of this species (reproductive activity exclusively during the wettest days of the rainy season), and the particular features of its breeding sites, suggest that the adverse effects of UV radiation should be monitor carefully in the future. These ephemeral breeding sites, usually located in areas with poor or no vegetation cover, are particularly vulnerable to the impacts of solar radiation, high temperatures and high rates of evaporation (Cobos and Alonso Bosch 2018).

Figure 2. Some limb abnormalities exhibited by adult *Peltophryne florentinoi* toads from Playa Girón and Guasasa, Zapata Swamp, Matanzas, Cuba. (**A**) Amely in adult male. (**B**) Ectropody in adult female. (**C**) Brachydactyly + syndactyly. (**D**) Both abnormal hindlimbs, schizodactyly is shown on the right side of the photo, with ectromely on the left side. (**E-G**) Brachydactyly. (**H**) Ectromely shown on the left side of the photo. Photos by L. Gómez Castillo.

UV-B radiation is rapidly attenuated in aquatic ecosystems, often within a few centimeters (Diamond *et al.* 2002), but the reproductive activity and larval development of *P. florentinoi* take place exclusively in small and very shallow depressions of karstic soil typical of the region, that temporarily accumulate rainwater (Díaz and Cádiz 2008).

Henle *et al.* (2017c) discard inbreeding as a probable cause of abnormalities in a population of *Bufotes viridis* from southern Germany. These authors considered that inbreeding would lead to the same types of anomalies in all affected individuals, not to the heterogeneous observed. However, the high frequency of malformations detected in insular populations of toads from Brazil, ranging from mouthpart anomalies, limb reduction to loss of eyes, has been associated with genetic structure and inbreeding (Toledo and Ribeiro 2009, Tolledo and Toledo 2015, Bessa-Silva *et al.* 2016, Reboucas *et al.* 2019). The small population of Zapata's Toad is restricted to a few localities from the eastern part of the Zapata region, Matanzas province to the east of Cienfuegos City, Cienfuegos Province, associated with coastal microphyllous evergreen forest with different level of human perturbation. Due to the specific ecological requirements, these toads have a small population and a distribution restricted to perturbed habitats and a limited availability of breeding sites (Alonso Bosch and Cobos 2016, Cobos and Alonso Bosch 2018). Coupling these factors with apparent site fidelity may increase the probability for consanguineous mating, the consequent loss of genetic diversity, and exposure of deleterious recessive mutations. Further studies are needed to understand the causes and consequences of this phenomenon along the distribution of this endemic and highly threatened Cuban toad.

Acknowledgments.—We are indebted to numerous friends, colleagues and students who helped us during field expeditions. Pablo Bouza, Director of Empresa para la Conservación de la Ciénaga de Zapata, Matanzas, Cuba, provided valuable logistical support. This work was supported in part by a grant from the Mohammed bin Zayed Species Conservation Fund, project #152511898, and the institutional project of the Facultad de Biología, Universidad de La Habana: "La caracterización de los sitios de reproducción de *Peltophryne florentinoi:* Una herramienta clave para la conservación de un anfibio endémico y amenazado de la Ciénaga de Zapata, Matanzas, Cuba". Collection permits and Environmental Licenses to carry out fieldwork in Ciénaga de Zapata Biosphere Reserve were kindly authorized by the Centro de Inspección y Control Ambiental (CICA). Andrew J. Crawford offered valuable suggestions and corrections to the language, and helped enhance the quality of the article. Two anonymous reviewers provided constructive criticism.

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Editor: Franco L. Souza

Phyllomedusa 20(1):125–129, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p125-129

OBITUARY

Richard Carl Vogt (1949–2021)

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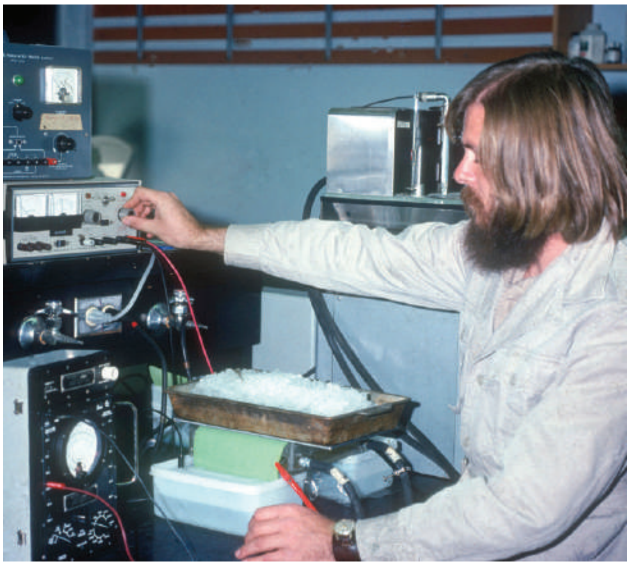
Richard Carl Vogt was born in Madison, Wisconsin, on 6 August 1949 and that may have been the last time anyone used his whole name. To the world, he was just Dick Vogt. He explored the wilds of Wisconsin as a youth, and completed Bachelors (1967-1971) and Masters Degrees (1974) at the University of Wisconsin. That was the background he needed to write his first book, *The Natural History of Amphibians and Reptiles in Wisconsin*, published in 1981, three years after he completed his doctoral thesis entitled *Systematics and Ecology of the False Map Turtle Complex (Graptemys pseudogeographica)*, supervised by William G. Reeder, also at the University of Wisconsin. Although he would continue to dabble in studies of other amphibian and reptilian taxa, and even co-authored a paper describing a new species of pit viper (*Cerrophidion petlalcalensis*), it was during his Ph.D. that he fixated on turtles and from that time on just about everything else was peripheral to his studies of chelonians.


Dick working on temperature-dependent sex determination in Wisconsin. Photo from Dick´s personal collection, photographer unknown.

Dick was awarded a scholarship by the Richard Mellon Foundation to study at the Carnegie Museum of Natural History in Pittsburgh in 1978 and he remained there until 1981 under the mentorship of C. J. (Jack) McCoy. McCoy had a major influence on Dick. Although I never heard him mention his parents, Dick often referred to the influence of his mentor, and they published 11 papers together. He was shocked when McCoy died of a heart attack at age 58 in 1993. During his time at the Carnegie Museum, Dick also collaborated extensively with James J. Bull on studies of sex determination in turtles and they co-authored seven papers, two in collaboration with Jack McCoy. The paper by Bull and Vogt published in Science is one of the most cited papers on temperature-dependent sex determination (Bull and Vogt, 1979).

Dick moved to Mexico in 1981 and was employed as a researcher at the Los Tuxtlas Tropical Biology Station of the Universidad Nacional Autónoma de México, where he was also adjunct professor in the Science Faculty. He remained there until 2000 and, although he co-edited the book *Historia Natural de Los Tuxtlas*, most of his publications in that period related to other areas. I attended the Joint Annual Meeting of the Herpetologist´s League & Society for the Study of Amphibians and Reptiles organized by Dick and G. Casas-Andreu in Veracruz in 1987. Dick invited me to Los Tuxtlas afterward and gave me tips on catching turtles, so I suggested that he visit Manaus, which he did as a visiting professor in 1989. He supervised students from Brazil and published with them on Amazonian turtles throughout the 1990s before finally moving definitively to Manaus in 2000. As a result of these diverse experiences, Dick often mixed English, Spanish and Portuguese in the one sentence, which meant that you sometimes had to concentrate to get the gist of his message.

Dick was employed by the Instituto Nacional de Pesquisas da Amazônia (INPA) as a level III researcher, which gave him freedom to concentrate on his turtle research. The studies of Dick and his students resulted in over 100 papers on aspects of turtle biology ranging from taxonomy to diet and reproduction. However, the study that most gained the attention of the world scientific community was co-authored with Camila Ferrara and Renata Sousa-Lima on turtle vocalizations as the first evidence of post-hatching parental care in chelonians (Ferrara et al. 2012). The concentrations of female *Podocnemis expansa* that remain around nesting beaches for months after egg laying had been noted before, but no-one imagined that those behemoth mothers were waiting for the tiny babies to hatch so that they could call to them and lead them on the annual migrations of hundreds of kilometers. Dick and his collaborators had shown that far from being simple automatons of interest only to dedicated herpetologists, turtles are fascinating creatures with complex behaviors and communication systems. You only have to know how to listen to what they are saying!

Dick had not forgotten Mexico, and he collaborated with John Legler on the definitive book on Mexican turtles, *The Turtles of Mexico: Land and Freshwater Forms*, published in 2013. John died in 2014, taking much of his great store of information on New World and Australian turtles to the grave with him. I remember Dick bringing John to my study site in 2012 and I was worried that John might not be able to make it back up the hill because he used a walking stick with four supports and was obviously frail. I was even more worried when he asked to borrow a diving mask to look for turtles and hobbled over to the stream. I told him that it was almost impossible to find turtles during the day, but he tipped into the water and paddled off. It was less than 10 minutes later that he swam back holding a turtle! Without Dick, I am sure that John Legler´s greatest contribution to the study of Mexican turtles would never have come about and the book is an unusual example of collaboration between two great herpetologists. The Mexican turtle *Kinosternon vogti* was named in Dick´s honor in 2018.

Dick was a Researcher 1A of Brazil´s National Science Foundation (CNPq), which is the highest level attainable, and in 2012 he received a grant from Petrobras, Brazil´s national oil company, to create the Center for Study of Amazonian Chelonians (CEQUA), with a large building and display tanks for turtles in INPA´s "Science Forest", which is a major tourist attraction and educational center in Manaus. In 2014 he won the coveted Behler Turtle Conservation Award. He also won a grant for a National Science Institute, which is the largest scientific financial award given in Brazil. However, the unstable political situation in Brazil in recent years meant that he did not receive all that was promised and he had great difficulty supporting CEQUA in the last few years, especially during the COVID-19 pandemic.

Dick supervised ten undergraduate theses, 37 Masters dissertations and 11 doctoral theses. Many of the students he supervised are now university professors working in critical areas for turtle conservation. Dick´s love of turtles was contagious and I attribute to this the number of students who were willing to devote the great effort needed to study turtles in the Amazon.

Now, 30 years after he first started working in Brazil, it is hard to imagine how the state of art of turtle biology and conservation would have been if he had never come, but it would surely have been much poorer.

Not all was turtles. Dick met and married Oneide soon after he started visiting Manaus. Oneide is as tall and elegant as Dick was squat and turtle-like. They complemented each other in other ways. As with most couples, they had their ups and downs, but Oneide basically looked after Dick from their first outings nightclubbing in Manaus till his last days in hospital. They unsuccessfully tried to open a restaurant to attend to Dick´s love of cooking and Oneide´s interest in the performing arts, they successfully raised two sons of whom Dick was inordinately proud, and Oneide often assisted Dick in field work. Their stories are so intermingled that it is hard for me to remember how Dick was before he met Oneide.

When Dick gave me a copy of his book on Wisconsin amphibians and reptiles many years ago, he wrote in the cover "But wondering what would have happened had one of us been a woman!" I didn´t say so, but I thought at the time that if I had been a woman I probably would have avoided him like the plague because he was prone to making sexist comments that were denigrating to women. He probably would have said that he was treating women as equals and his comments were just words, but words can hurt and sometimes harm. Dick would pay heavily for his callousness.

His private behavior did not change in later years. He continued to make the same comments to men and women friends that he knew would not be offended, but he altered his public discourse. Dick was sick during the Latin American Herpetology Congress in Quito in 2017 and could not present his paper. As the time slot had to be maintained, I suggested to the session coordinator that they present Dick´s slides even though he was not there to explain them. It was only after they agreed that I had second thoughts in case there was sexist material in the slides. However, all the slides were appropriate and the legends explicative, so I and the audience learned much about turtle vocalizations.

In 2018, Dick was nominated for the Herpetologist´s League Distinguished Herpetologist Award. Some members thought, appropriately, that the award should not be given to someone who had made sexist statements at previous meetings. However, they were unable to stop the nomination through the regular channels, so they hatched a nefarious plan. Speakers had to leave their slides with the organizers before their talk, so a researcher who had been deeply offended by Dick at previous meetings modified the slides by putting black boxes over the women´s shorts and tops, giving the impression that they were naked. She did not, however, black out the clothes of the men in the pictures who were also appropriately dressed for work on tropical beaches.

Dick and the audience were surprised when photographs of apparently naked women appeared on the screen, but responsibility did not fall on the shoulders of those who had committed the crime. Adulterating someone else´s photos was unethical and deeply insulting to the dedicated female researchers and volunteers who appeared in the photos, including Dick´s wife. Many of Dick´s women collaborators leapt to his defense and a major state funding agency published the original photos in its website to show that there was nothing unseemly in them. However, the damage was done and the news swept the world that an eminent herpetologist had used inappropriate photographs in his presentation. Dick was devastated.

I last saw Dick in November 2020. We were at a celebration dinner for a student we had co-supervised who had defended his thesis. Dick was worried about COVID-19 and said that he was taking the cocktail of drugs recommended by the Brazilian government as prophylaxis. I told him that those drugs had been proven ineffective against COVID-19 and could cause dangerous side effects for someone, such as

Dick, who was overweight, had previously had a heart attack, had bone defects requiring two hip replacements and a liver that had suffered from many years of heavy drinking. However, he did not stop the treatment and when he was admitted to hospital in January 2021 after a heart attack, the doctors said that he did not have COVID-19, but was suffering from the side effects of the drugs; they could not save him.

Dick Vogt was a character, and I suspect that he sometimes purposefully presented a caricature of himself at public events. Few people who interacted with him were indifferent to him; most loved him, but some detested him. Nevertheless, in the long term, he will not be remembered for the quirks of his personality. He will be remembered as the person who probably contributed more to our knowledge of New World chelonians than any other researcher of his generation. Thank you Dick.

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(**A**) Dick with his first-born son Riandro in Manaus in September 2000. Photo by Bill Magnusson. (**B**) Dick with Walter Hödl at the Joint Herpetology and Ichthyology Meeting in Manaus that Dick organized in 2003. Photo by Rafael Bernhard. (**C**) Dick with a Tucunaré that he plans to convert into a delicious dinner. Photo by Camila Ferrara. (**D**) Oneide, second from the right as part of the team releasing radio tagged *Podocnemis expansa* that would reveal the species´ long-distance migrations. Photo by Dick Vogt. (**E**) Dick signing copies of his book on Amazonian turtles. Photo by Otávio Lima. (**F**) Dick at CEQUA trying to order a *Podocnemis expansa* back to the lake. Photo by Otávio Lima. (**G**) On the day that Dick died, Matheus Moraes photographed this cloud in the form of a turtle that formed over Manaus, a fitting natural tribute to one of the region's greatest naturalists.

Phyllomedusa 20(1):131–133, 2021
© 2021 Universidade de São Paulo - ESALQ
ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v20i1p131-133

OBITUARY

Marcelo Menin (1975–2021)

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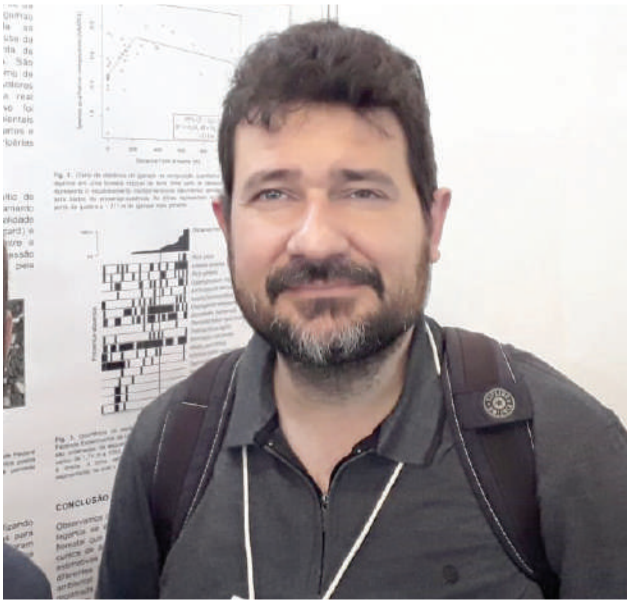
Is it possible to be a pleasant and calm person and also a tireless and competent professional? Know about Marcelo Menin.

Losses are always sad and difficult. In this very complicated period that we are going through with the Brazilian government attacking and discrediting science and minorities, with the uncontrolled pandemic in Brazil, and many other attacks on common sense, on the respect for differences and for the justice, certain qualities such as serenity, resilience, competence, dedication, ability to aggregate people, to work tirelessly, capacity to overcome challenges and limitations without complaining, and keeping a calm persistence, are more than necessary. Sadly, unfortunately, we lost a lot of those at the beginning of this 2021 year with the departure of Marcelo Menin, taken from our coexistence by Covid in Manaus at the age of 45.

Marcelo Menin gathered these and many other qualities together. An internal and common joke among those who knew him was to say: - Talking about Menin's qualities is easy, I want to see someone to finding a flaw! A very special, honest and fair person, with a very kind and pleasant presence, of comprehensive, calm and welcoming personality. A professional of the highest competence, dedicated, extremely organized, assertive, tireless, generous and extremely patient with our limitations, confusions and much slower pace than his. Most importantly, he was always the same person in any situation and with anyone. The pleasant and calm person and the tireless and competent professional were an inseparable fusion, and this represented a lot in terms of support and understanding in difficult times in which all of us, his colleagues, students and advisers have gone through our lives; in terms of encouragement and inspiration in times of discouragement; in terms of showing us the way and the ways of acting correctly and competently, without hurting or belittling anyone!


Marcelo Menin at IX Congresso Brasileiro de Herpetologia, Campinas, 2019.

Professor at the Universidade Federal do Amazonas (UFAM) since 2006, his competence is evidenced in his curriculum, with more than 90 publications including scientific articles, books and book chapters. This number will increase even more, as Marcelo Menin was at the peak of his career, having still many researches in progress in partnership with several colleagues which, as much painful it may be, they will be finalized in his honor and memory.

He was passionate about the Amazon Forest, having greatly contributed to the fill of one of the most important gaps in Brazilian Herpetology knowledge: the lack of information about biology and natural history. Since very young he was an amateur naturalist, maintaining and observing many different animals in his house and making field excursions with his friends in Jaú (his hometown), as used to tell us Gustavo Quevedo Romero (professor at UNICAMP), his friend since the Fundamental School. In line with his passion, Marcelo Menin published several articles with new and important information on habitat use, diet, and anuran behavior from the Central Amazonia, as well as described the tadpoles of 17 species (three of which are redescriptions). He also published in community ecology of anurans, lizards and snakes.

At UFAM he was the Coordinator of the Postgraduate Program in Zoology, of which he was one of the creators. In this Program, he was the advisor of 20 students (master's and doctorate), in addition to having supervised 43 undergraduate students or graduated technicians. Currently, he was supervising two PhD students, three master students, one for scientific initiation and a graduated technician, with a scholarship linked to a project of which he was the coordinator.

In addition, he was the sub-curator of the Paulo Bührnheim Amphibian Collection and the founder of the Tadpoles collection, both of them at UFAM. He obtained CNPq' government financial for four major projects in which he was the coordinator, and also he was an ad-hoc reviewer to CNPq for many times. He yet was a reviewer of scientific articles for more than 40 scientific journals, the vast majority from abroad, and he was a member of more than 80 master's and doctoral judging boards. These and other information about Marcelo Menin's scientific production, research projects and professional life can be accessed at: http: //lattes.cnpq. br / 3661952638867868. Anyhow, this is a small technical summary that does not come close to representing the generous and aggregator professional that was Marcelo Menin. For instance, as one of the first professors to obtain funding from CNPq in his scientific area at UFAM, he sought to develop broad projects which included colleagues who studied other taxonomic groups, in addition of course, to seeking the best possible knowledge about the biodiversity of the Amazon Forest.

Marcelo Menin in the field at Reserva Ducke-Manaus, an area that he walked throughout all many times, and where he developed many of his projects.

Photo: Domingos J. Rodrigues.

A very dedicated professor, responsible and passionate about teaching, he was one of the most beloved professors by students, having received many honors at graduation ceremonies and other events. As a result of his dedication, competence, and productivity as a professor as a researcher, he achieved great recognition in the academic environment, having been an affiliated member of the Brazilian Academy of Sciences and received a CNPq Research Productivity Grant, in addition to several awards, including one from the City Council from Manaus, for the relevant services rendered to the society of the area of Biology, in addition to several honorable mentions to research results presented in scientific events.

Personally, I met Marcelo Menin in 1995, when he started his undergraduate course in Biological Sciences at UNESP-São José do Rio Preto- SP, where I am a professor. His great interest, his seriousness and the evident knowledge that he already had, besides making me feel extremely trusted in him since the first contact, made me accept him as an intern, together with his classmate Tiago J. Izzo (Professor at Universidade Federal de Mato Grosso), despite both of them were still in the 1st year of the course, and the Ecology course I taught was only offered in the 3rd year. The internship program was developed by Tiago and him with such a quality and success that it resulted in the first scientific publication of the two friends. In addition to being an excellent intern, Marcelo was an excellent student in the undergraduate course, maintaining this standard in the Master Course (Ecology and Conservation of Natural Resources, Universidade Federal de Uberlândia, supervised by Ariovaldo A. Giaretta) and along his PhD (Ecology, Instituto Nacional de Pesquisas da Amazônia, supervised by Albertina P. Lima).

In Manaus he met Sumaia Vasconcellos, his wife, an agronomist and PhD in Tropical Forest Sciences-INPA, a spectacular hardworking, generous and competent person, in short, the ideal life partner! With her he gained two stepchildren, Karol and Rômulo, and a daughter, Maria Clara, who is now 9 years old. He was a loving and dedicated husband and a responsible and passionate father for his children.

Marcelo Menin was a great research partner, totally reliable, extremely organized, calm, persevering, competent, cooperative, resolute and generous, assertive and an aggregator. I had the honor of sharing with him the authorship of several articles, books and book chapters, in addition to having some still in progress, which will be finalized in his honor. But more than all of that, or along with all of that, Menin was a great friend, a son of my heart, supportive, trustworthy, and inspiring. Whenever I find myself at an impasse I think: “What would Menin do in this situation?” And I will continue with this habit because he left with us his example of conduct, love and unrestricted respect for science, teaching and to all people.

Marcelo Menin and his family: Sumaia Saldanha de Vasconcelos Menin, his wife; Susan and Rômulo Vasconcelos Valentim, his stepchildren, and Maria Clara, his 9 years old daughter, in 2018 January at Susan’s Architeture Graduation ceremony.

Rest in peace dear friend, your mission has been accomplished with immense success and you will continue to bear fruit here, through his many students, advisers, and professional colleagues, who will certainly take his legacy of dedication, correctness, honesty and passion in doing science and teaching to the future generations of professors and researchers.

INSTRUCTIONS TO AUTHORS

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 Microsoft Word document via e-mail or via surface delivery on a CD. 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.
- **Name(s) of author(s):** Bold-faced caps and lower-case Roman; left aligned; use serial commas. Follow example:
  José Wellington Alves dos Santos[1,2], Roberta Pacheco Damasceno[1,2], and Pedro Luís Bernardo da Rocha[2,3]
- **Institutional affiliation(s):** Light-faced caps and lower-case Roman; left aligned. Follow example:
  [1] Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo. 05508-900, São Paulo, SP, Brazil. E-mail: wly@ib.usp.br.
  [2] Departamento de Zoologia, Instituto de Biologia Universidade Federal da Bahia. 40170-210, Salvador, BA, Brazil. E-mail: peurocha@ufba.br.
  [3] Current address: Department of Ecology and Evolutionary Biology, The University of Kansas, Lawrence. Kansas 66045-7580, USA.
- **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:
  Abstract
  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]
  Study Site [Secondary header]
  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. 9 (1) of *Phyllomedusa* for proper format of table captions and contents.
- **Appendices:** Number appendices consecutively with Roman numerals. Refer to tables in text as Appendix I, Appendices II and III, and Appendices II–V. Appendix captions should be placed above the appendix content. Most appendices should follow the format instructions for tables. Extensive lists of specimens examined should be included as an appendix. Consult Vol. 9 (1) of *Phyllomedusa* for proper format and arrangement of specimens examined.
- **Figure captions or legends:** All figures must be numbered consecutively and their legends or captions formatted in *Phyllomedusa* style (Vol. 9, No. 1). The captions should be listed in order separate from the images. Refer to figures in text as Figure 1, Figures 2 and 3, Figures 2–5, Figure 4A, and Figure 4A, B. “Figure” or “Figures” are always spelled out—even in parentheses. Figures must be cited in order in the text. See specific instructions for preparation of figures.
- **Figures for review:** Embed all figures in order at the end of the Word document as PNG (Portable Network Graphic) files. Identify each with the figure number and a short caption, and **indicate whether the figure is intended for reproduction at column or page width, or as a broadside.**

Preparation of Figures for Publication. All figures should be submitted digitally as TIF files with LZW compression, **separately from the files embedded in the manuscript** for review. Each figure should be submitted at **the exact size** intended for publication. There are three choices: **page width** (34 picas, 145 mm, 5 and 11/16 in.), **column width** (16.5 picas, 70 mm, 2 and 3/4 in.), or **broadside** (193 mm × 145 mm). All illustrations must **allow room for a caption to be printed below the figure,** while conforming to these measurements.

- **Labeling figures:** Labels must be consistent on a figure and among all figures included in the article. **Use a sans serif font** that is common to Windows and Macintosh platforms (e.g., Arial). Subunits of multipart figures must be labeled with capital letters (A, B, C) placed in the **upper, left-hand area** of each unit. The letters **should be about 10 points large** (not to exceed 12 pt); they must be identical in size and typeface on each figure included in the manuscript.

INSTRUCTIONS TO AUTHORS

Labeling within figures (e.g., anatomical parts, legends on axes of graphs, etc.) **should be in the range of 8–9 pt and in a sans serif font, such as** Arial. Scale bars should be labeled with their values on the face of the figure (e.g., 5 mm); **the minimal size of lettering that may be used is 7 points** in a sans serif font for scale bars, longitude and latitude on maps, etc.

- **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.
    - ✓ Sized for publication, lines (strokes) should be between 0.25 and 2 points wide.
    - ✓ Tick marks on graphs should be on the outside of the axis line. Sized for publication, they are between 3 and 5 points in length and 0.25 pt in weight. Longitude and latitude marks should be on the inside of the map border.
    - ✓ All maps must have an appropriate scale in kilometers.
    - ✓ Overlapping symbols and lines must be counter shadowed with white.
    - ✓ Export completed image as a TIF document for submission.
- **Raster graphics:** *Photographs (color and gray-scale [black & white]) and tone (gray-scale) renderings* should be submitted as a RGB document in TIF format sized for publication (described above) at a resolution between 300 and 600 dpi (after reduction/sizing). To label raster images, import them into a vector graphic program, follow the directions above, and export the completed image as a TIF document for submission.

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 (separated by a comma; do not use *et al.*), 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. Authors should cite taxonomic literature they used in taxon determination. Voucher numbers should be included where appropriate (e.g., Results, Material examined).
- **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.
- **Numbers and units.** All measurements are noted in Arabic, unless the number starts a sentence.
    - ✓ Measurements include distances, areas, dimensions, volumes, weights, time (e.g., hours, days, seconds, minutes), temperatures, etc. **Standard SI units are used**—e.g., time: 08:16 h; distances and areas: 7 km, 12.5 mm, 17,840 ha; geographic coordinates: 04°43'23" S; temperature: 24°C. To indicate degrees, use a degree sign (°), not a superscript oh (º). Note that degrees and minutes are straight quotation marks or prime signs; do not use curly quotes.
    - ✓ Use the **double-digit rule** for numbers other than measurements. Numbers less than 10 are spelled out—e.g., "… nine animals were sampled"; numbers of 10 and more are denoted in Arabic—e.g., "… but 10 larvae were collected."
- **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
- **Animal care and permits.** The editorial staff of *Phyllomedusa* subscribes to humane and ethical treatment of all animals; all contributors to the journal must comply with this principle. In addition, all required state and federal permits (IBAMA license for Brazil) must have been obtained and must be cited in the Acknowledgments.
- **Proofs.** The publisher will undertake proofreading, unless specifically advised otherwise by the corresponding author when the contribution is accepted for publication.
- **Reprints.** Authors will receive a PDF of their contribution, and the senior author will receive a hardcopy of the issue of *Phyllomedusa* in which the paper appeared.
- **Submission.** Send manuscripts as Microsoft Word (.doc or .docx) via e-mail to the Editor (phyllomedusa@usp.br).

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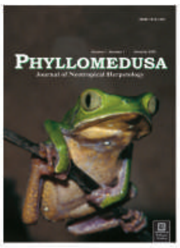
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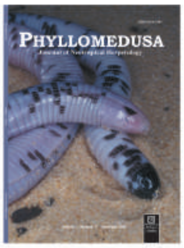
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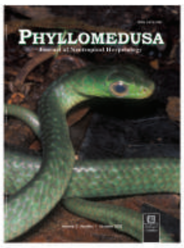
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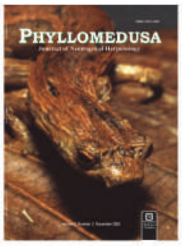
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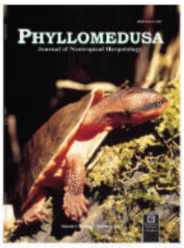
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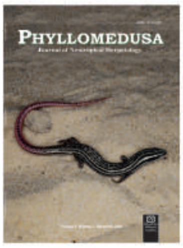
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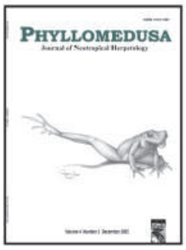
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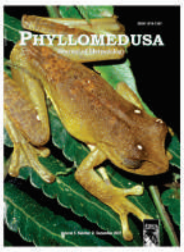
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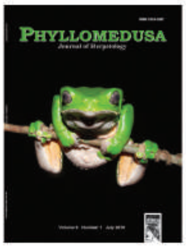
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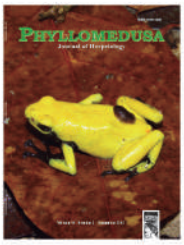
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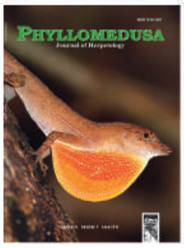
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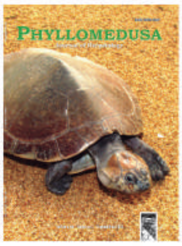
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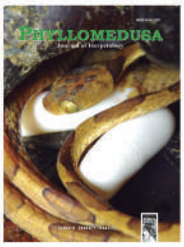
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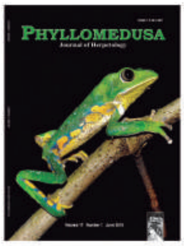
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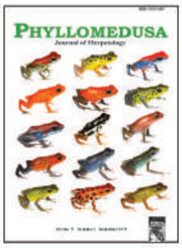
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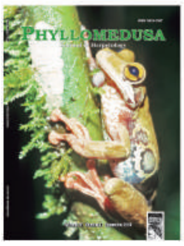
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Contents

Volume 20 Number 1
January–June 2021

Articles

Short Communications

Obituaries

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