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Cover: An *Abronia graminea* from Mendoza, Mexico.
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Variation of amphibian and reptile composition in forest fragments of Veracruz highlands, Mexico

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Abstract

Variation of amphibian and reptile composition in forest fragments of Veracruz highlands, Mexico. The central mountain region of Veracruz is one of the richest areas of herpetofauna in Mexico. The steep topographic gradient of this region is associated with climatic gradients that enable the occurrence of highly diverse herpetofauna communities. We tested differences among habitats to herpetofauna in an urban area, pine forest, oak forest, and riparian forest of municipality Camerino Z. Mendoza, Veracruz, Mexico. We conducted two-day monthly surveys between 2015 and 2017 in 69 sites from fragments of an urban area, riparian forest, *Quercus* forest, and *Quercus-Pinus* forest and tested for differences in herpetofauna species among these fragments. We found a total of 11 amphibian and 33 reptile species, and a high composition dissimilarity and species replacement among the sites. Our results amplified significantly the species-records within Camerino Z. Mendoza, and showed a great variation of amphibian and reptile composition among sites, highlighting the current role of these forests as a reservoir for herpetofauna and their importance for future conservation strategies in the region.

Keywords: Alfa diversity, beta diversity, herpetofauna, mountain region, richness.

Resumen

Variación de la composición de anfibios y reptiles en fragmentos de bosque del altiplano de Veracruz, México. La región montañosa del centro de Veracruz es una de las regiones con mayor riqueza herpetofaunística de México. El gradiente topográfico de esta región está asociado con diferencias climáticas que permiten la presencia de comunidades herpetofaunísticas muy diversas.

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Analizamos las diferencias en la diversidad herpetofaunística entre un área urbana, bosque de pino, bosque de roble y bosque de ribera del municipio Camerino Z. Mendoza, Veracruz, México. Realizamos censos mensuales de dos días entre 2015 y 2017 en 69 sitios de fragmentos de un área urbana, bosque ribereño, bosque de *Quercus* y bosque de *Quercus-Pinus* y analizamos las diferencias de especies de herpetofauna entre estos fragmentos. Encontramos un total de 11 especies de anfibios y 33 de reptiles, y una alta disimilitud en la composición y el reemplazo de especies entre los sitios. Nuestros resultados aumentaron los registros de especies dentro de Camerino Z. Mendoza, y mostraron una gran variación en la composición de anfibios y reptiles entre sitios, destacando el papel actual de estos bosques como reservorio de herpetofauna y su importancia para futuras estrategias de conservación en la región.

Palabras clave: diversidad alfa, diversidad beta, herpetofauna, región montañosa, riqueza.

Resumo

Variação na composição de anfíbios e répteis em fragmentos de floresta das terras altas de Veracruz, México. A região montanhosa de Veracruz central é uma das regiões herpetofaunísticas mais ricas do México. O gradiente topográfico dessa região está associado a diferenças climáticas que permitem a presença de comunidades herpetofaunísticas muito diversas. Analisamos as diferenças na diversidade da herpetofauna entre uma área urbana, uma floresta de pinheiros, uma floresta de carvalhos e uma mata ciliar no município de Camerino Z. Mendoza, Veracruz, México. Entre 2015 e 2017, realizamos censos mensais de dois dias em 69 fragmentos de uma área urbana, uma floresta ribeirinha, uma floresta de *Quercus* e uma floresta de Quercus-Pinus e analisamos diferenças em espécies entre estes fragmentos. Encontramos um total de 11 espécies de anfíbios e 33 espécies de répteis, e uma grande disparidade na composição e substituição de espécies entre locais. Nossos resultados aumentaram os registos de espécies dentro do município Camerino Z. Mendoza e mostraram uma grande variação na composição de anfíbios e répteis entre sítios, destacando o papel atual dessas florestas como reservatório de herpetofauna e a\ sua importância para futuras estratégias de conservação da região.

Palavras-chave: diversidade alfa, diversidade beta, herpetofauna, região montanhosa, riqueza.

Introduction

Amphibian and reptile diversity are threatened by land use change and deforestation in several regions around the world (IUCN 2021). For example, urbanization replaces natural habitats with urban infrastructure, which decreases ecological niches for species (McDonnell and Pickett 1993, Hamer and McDonnell 2008), reducing the diversity and abundance of native species, as well as favors invasive fauna (Pickett *et al.* 2001, McKinney 2006). Currently, 41% of amphibians and 19% of reptiles worldwide are in a risk category by the IUCN and land change use and deforestation are among the principal causes (IUCN 2021).

Mexico has a variety of ecosystems that hold the second and seventh largest species richness of reptiles and amphibians in the world, respectively (Flores-Villela and Garcia-Vázquez 2014, Parra-Olea *et al.* 2014, AmphibiaWeb 2021). Most of this diversity is in Veracruz, which is the third state with the greatest herpetofauna diversity of Mexico (Guzmán-Guzmán 2011). This state hosts 25% (220 spp.) and 27% (103 spp.) of reptile and amphibian species of the country, respectively. Unfortunately, the transformation of forest habitats into agricultural areas or grazing such as coffee plantations, sugarcane crops and urban areas are threatening the herpetofauna habitats (Murrieta-Galindo *et al.* 2013a,b, Rivera-Hernandez 2015).

The central mountain region of Veracruz is considered one of the richest areas of herpetofauna in the state, harboring approximately 191 species of amphibians and reptiles (Almaraz-Vidal and Cerón de la Luz 2016). This region is located in eastern Mexico and is part of the mountain system where the eastern end of Trans-Mexican Volcanic Belt and the Sierra Madre Oriental meet. The extremely steep topographic gradient of this region, ranging from sea level to more than 5500 m a.s.l., is associated with climatic gradients that enable the occurrence of highly diverse communities of herpetofauna (Almaraz-Vidal and Cerón de la Luz 2016) in alpine grasslands, *Pinus* forest, *Abies* forest, *Quercus* forest, xeric shrublands, riparian vegetation, cloud forests, evergreen tropical forest and deciduous tropical forest (Rivera-Hernández *et al.* 2019).

Camerino Z. Mendoza municipality is in central Veracruz highlands from 1300 to 2380 m a.s.l., and harbored different habitats including pine forest, oak forest, and riparian forest. This municipality is within the Cañón del Río Blanco National Park, and includes 12 villages with different demographic growth and proximity to the forests (CONANP 2021). The municipality also belongs to a great industrial region with several effects on natural habitats such as water pollution and overexploitation of natural resources (Rivera-Hernández 2015). The region where Camerino Z. Mendoza is located has been previously studied (Ochoa-Ochoa and Flores-Villela 2006, 2011, Almaraz-Vidal and Cerón de la Luz 2016, Contreras-Calvario *et al.* 2019), finding high herpetofauna richness as well as differences in species richness and composition among altitude forests. For example, Almaraz-Vidal and Cerón de la Luz (2016) found in the region 191 species of amphibians and reptiles, of which only 50% of them are similar between two altitudinal regions of the Pico de Orizaba volcano. Despite the region where Camerino Z. Mendoza is located has been previously studied, to the best of our knowledge, none of these studies have documented the diversity of this municipality along the different environments.

In this study we provide field sampling and records documentation of herpetofauna from Camerino Z. Mendoza, Veracruz, Mexico. Given that urban areas and their closeness to forests may influence amphibian richness, we hypothesized that forests affected by big human populations would show less richness than forests with low or null human impact. To test this, we examined differences in herpetofauna species among areas with different environments (riparian forest, oak forest, pine-oak forest, and an urban area) and different degrees of disturbance due to urbanization. Our results highlight the current role of fragment forests as a reservoir for herpetofauna that could be used for future conservation strategies.

Materials and Methods

Study Area

The Camerino Z. Mendoza municipality is located in the highlands region of central Veracruz (18°45'–18°49' N, 97°08'–97°12' W) (Figure 1). The region has an annual average precipitation of 900–1600 mm, a tropical wet climate with the lowest temperature at 14°C in winter and highest at 20°C in the summer season (INEGI 2009, CEIEG 2019). Camerino Z. Mendoza and surrounding areas comprise an admixture of habitats with significantly different forest structure and tree composition, including riparian forest, *Quercus* forest, *Quercus-Pinus* forest, and urban areas (Rivera-Hernández 2015) (Figure 1). Additionally, the municipality host four villages with different degree of urbanization: Mendoza City with 45,003 inhabitants and near a riparian forest, La Cuesta with 2,095 inhabitants and surrounded by oak forest, and Necoxtla with 2,890 inhabitants and surrounded by a pine forest (CEIEG 2019). According to the different environments and degree of urbanization that Camerino Z. Mendoza have, we compared the amphibian and

reptile richness among: a riparian forest (RF) near to Mendoza City dominated by *Taxodium mucronatum* Ten. (= *Taxodium huegelii* hort. ex P.Lawson and C.Lawson) (18°47'44"–18°48'15" N, 07°10'57"–97°10'36" W; 1302–1330 m a.s.l.); an oak forest (QF) near to La Cuesta dominated by *Quercus* (18°47'43"–18°47'19" N, 97°10'43" –97°09'22" W; 1350–2000 m a.s.l.); a pine forest (PF) near to Necoxtla dominated by *Pinus patula* Schltdl. and Cham., *Quercus* sp., and *Cupressus* sp. reforestation (18°47'22"–18°45'51" N, 97°09' 21"–97°09'12" W; 2000–2350 m a.s.l.); and the urban area of Mendoza City (UA) dominated by non-native species such as *Ficus benjamina* L., *Livistona chinensis* (Jacq.) R. Br. ex Mart., *Jacaranda mimosifolia* D. Don, *Araucaria heterophylla* (Salisb.) Franco, and *Eucalyptus globulus* Labill. (18°47'35"–18°48'39" N, 97°11' 46"–97°10'29" W; 1310–1390 m a.s.l.) (Figure 1).

Data Collection

We conducted two-day monthly surveys between 2015 and 2017 in four environments: urban area (UA, 12 sites), riparian forest (RF, 13 sites), *Quercus* forest (QF, 33 sites), and *Quercus-Pinus* forest (QF, 11 sites) (Figure 1). Amphibians and reptiles were actively searched during the day in all microhabitats that might be occupied

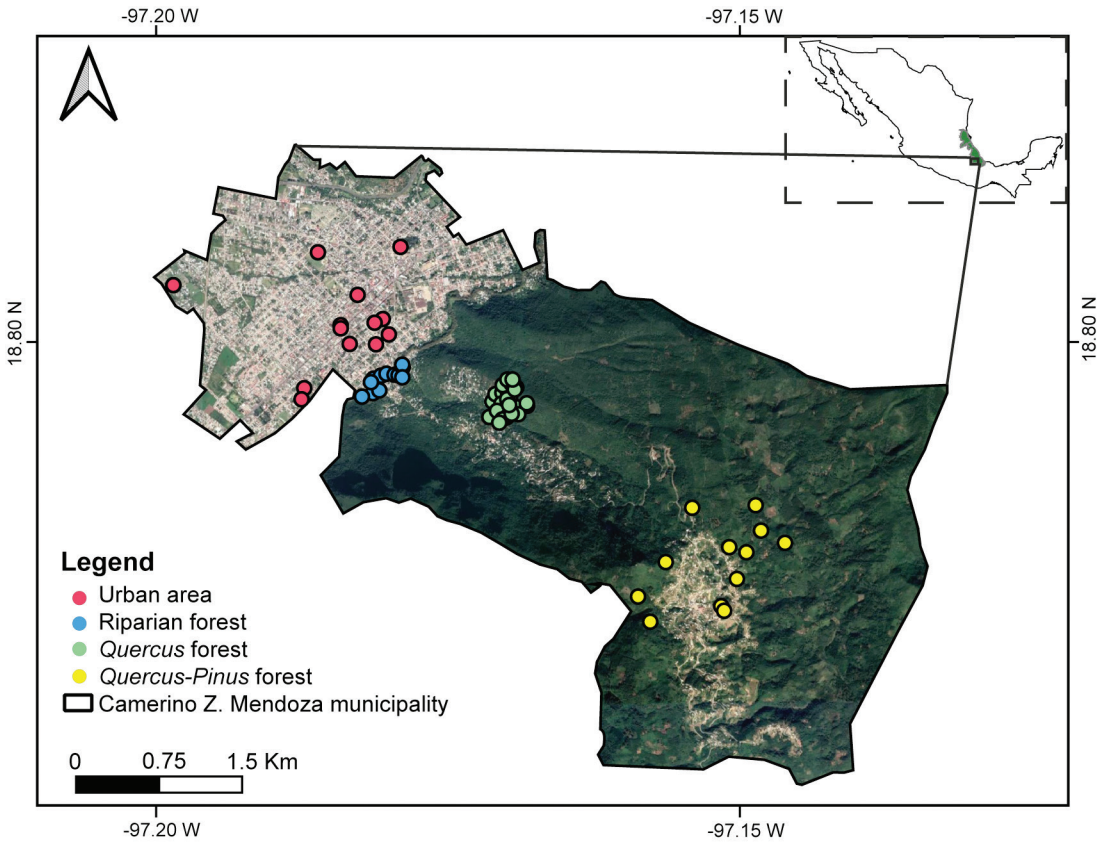

Figure 1. Map of the sites sampled in the urban area, riparian forest, *Quercus* forest and *Quercus-Pinus* forest of Camerino Z. Mendoza municipality.

by amphibians or reptiles. All individuals were collected by hand when encountered for identification, several species were photographed, and all individuals were returned to the collection site. Opportunistically surveys between 2015 and September 2020 also were added to our register.

In addition to our field surveys, we reviewed species reported from this municipality in the VertNet database (http:// portal.vertnet.org/ search), the Global Biodiversity Information Facility database (GBIF https://www.gbif.org/), and the Sistema Nacional de Información sobre Biodiversidad (SNIB) database to which contain records from scientific collections collected in Mendoza municipality.

Data Analysis

We analyzed the compositional differences among sites through species replacement and species richness differences applying the method proposed by Carvalho *et al.* (2012). Briefly, the compositional differences were measured at each two sites from the addition of species replacement and species richness differences (Appendix I). The species richness was defined as the number of species found during the study and in electronic databases. Differences in species richness between sites were tested with Kruskal-Wallis test the function 'kruskal.test' in R (R Core Team 2019). Replacement was defined as the substitution of *n* species in a given site by *N* species in another site. Finally, we obtained an overall measure of compositional dissimilarity among sites using the Jaccard dissimilarity index. To visualize richness and beta diversity, plots were carried using the ggplot2 package (Wickham 2016) in R (R Core Team 2019). Additionally, we determined which species are in a category of risk according to the Red List of Threatened Species (IUCN 2021) and the Mexican law (SEMARNAT 2010), to identify which environments host more species at risk which need conservation strategies.

Results

The total species for Mendoza was composed of four orders, 16 families, 36 genera and 44 species (33 reptiles and 11 amphibians) (Table 1, Figures 2 and 3). Seven amphibian species and 16 reptile species are endemic to Mexico (Table 1, Appendix II). Additionally, four species were found in our search of the HerpNet, GBIF and SNIB databases (Appendix II). One database species, *Scincella gemmingeri* (COPE, 1864), was not recorded during our sampling (Appendix II).

The number of species differed between sites, ranging from 13 in the urban area to 25 in *Quercus* forest (Figure 4A), but it was not statistically significant (Kruskal Wallis test, $\chi^2 = 3$, $df = 3$, $p = 0.3916$). The overall compositional dissimilarity among sites was 0.86 on average, ranging from 0.96 to 0.74. Replacement among sites was 0.64 on average, ranging from 0.87 to 0.39. The difference in species richness was 0.23 on average, ranging from 0.44 to 0.05 (Figure 4B, Appendix III).

According to the IUCN Red List (2021), 32 species were listed as Least Concern (LC), two as Endangered (EN), two as Near Threatened (NT), one as Vulnerable (VU), and one as Critically Endangered (CR). Meanwhile, according to the Mexican Law (NOM-059-SEMARNAT-2010), six species are Subject to Special Protection (Pr), and five are Threatened (A) (Table 1).

Discussion

Our results show that Mendoza municipality has a great variation of amphibian and reptile composition among sites of riparian forest, oak forest, pine-oak forest and urban area, offering suitable conditions for maintaining a portion of amphibian and reptile species of the region. The 44 amphibian and reptile species found in this study amplified significantly the species-records within Mendoza municipality. This highlights the current role of these forests as a reservoir for

Table 1. List of amphibians and reptiles found in Camerino Z. Mendoza. Sites where species were detected: UA, urban area; RF, riparian forest; QF, *Quercus* forest; QPF, *Quercus-Pinus* forest.

Taxon	UA	RF	QF	QPF	IUCN status	NOM-059	Endemic to Mexico
AMPHIBIANS							
Bufonidae							
Incilius valliceps (Wiegmann, 1833)	X	X			LC	-	
Craugastoridae							
Craugastor mexicanus (Brocchi, 1877)			X	X	LC	-	X
Craugastor pygmaeus (Taylor, 1937)		X		X	VU	-	
Eleutherodactylidae							
Eleutherodactylus cystignathoides (Cope, 1877)	X				LC	-	-
Eleutherodactylus nitidus (Peters, 1870)			X		LC	-	X
Hylidae							
Rheohyla miotympanum (Cope, 1863)		X	X		NT	-	X
Smilisca baudinii (Duméril and Bibron, 1841)	X				LC	-	
Plethodontidae							
Bolitoglossa platydactyla (Gray, 1831)	X				NT	Pr	X
Isthmura gigantea (Taylor, 1939)				X	CR	-	X
Pseudoeurycea firscheini Shannon and Werler, 1955			X	X	EN	Pr	X
Ranidae							
Lithobates spectabilis (Hillis and Frost, 1985)	X	X	X		LC	-	X
REPTILES							
Anguidae							
Abronia graminea (Cope, 1864)			X	X	EN	A	X
Gerrhonotus ophiurus Cope, 1867			X		LC	-	X
Colubridae							
Conopsis lineata (Kennicott, 1859)			X	X	LC	-	X
Drymarchon melanurus (Duméril, Bibron, and Duméril, 1854)		X	X		LC	-	
Ficimia olivacea Gray, 1849			X		-	-	X
Lampropeltis polyzona Cope, 1860		X	X		LC	A	X
Mastigodryas melanolomus (Cope, 1868)			X		LC	-	
Ninia diademata Baird and Girard, 1853	X	X	X		LC	-	
Pituophis deppei (Duméril, 1853)	X				LC	A	X

Table 1. *Continued.*

Taxon	UA	RF	QF	QPF	IUCN status	NOM-059	Endemic to Mexico
Pliocercus elapoides Cope, 1860	X	X	X		LC	-	
Stenorrhina degenhardtii (Berthold, 1846)			X		LC	-	
Thamnophis conanti Rossman and Burbrink, 2005				X	-	-	X
Dactyloidae							
Anolis sericeus Hallowell, 1856			X		-	-	
Anolis laeviventris (Wiegmann, 1834)			X		-	-	
Anolis tropidonotus Peters, 1863			X		-	-	
Dipsadidae							
Coniophanes fissidens (Günther, 1858)			X		LC	-	
Chersodromus liebmanni Reinhardt, 1861			X		LC	Pr	X
Leptodeira annulata (Linnaeus, 1758)			X		LC	-	
Leptodeira polysticta (Günther, 1895)		X				-	
Elapidae							
Micrurus diastema (Duméril, Bibron, and Duméril, 1854)		X			LC	Pr	
Gekkonidae							
Hemidactylus frenatus Duméril and Bibron, 1836	X				LC	-	
Kinosternidae							
Kinosternon integrum Le Conte, 1854		X			LC	Pr	X
Phrynosomatidae							
Sceloporus formosus Wiegmann, 1834				X	LC	-	X
Sceloporus grammicus Wiegmann, 1828				X	LC	Pr	
Sceloporus mucronatus Cope, 1885				X	LC	-	X
Sceloporus variabilis Wiegmann, 1834	X	X	X		LC	-	
Scincidae							
Plestiodon brevirostris (Günther, 1860)				X	LC	-	X
Scincella gemmingeri (Cope, 1864)	X				LC	-	X
Typhlopidae							
Amerotyphlops tenuis (Salvin, 1860)	X				LC	-	-
Viperidae							
Crotalus ravus Cope, 1865			X		LC	A	X
Crotalus triseriatus Wagler, 1830			X		LC	-	X
Metlapilcoatlus nummifer (Rüppell, 1845)			X		LC	A	X

Figure 2. Some amphibians found in Mendoza municipality. (**A**) *Pseudoeurycea firscheini*, (**B**) *Isthmura gigantea*, (**C**) *Bolitoglossa platydactyla*, and (**D**) *Eleutherodactylus nitidus*. Photos: JLCJ (A), RAV (B-D)

herpetofauna and their importance for future conservation strategies.

The variation in species richness and species composition among sites suggest that each one offers particular conditions as a product of differences in environments and forest traits. Species richness tends to increase from urban areas to *Quercus* forest, reaching the highest number of species in this site, and then decreasing in the highest elevation site in the *Quercus-Pine* forest. However, *Quercus* forest is one of the most types of vegetation perturbed along Veracruz, and *Quercus-Pine* has changes in land use and forest species extraction (Castillo-Campos *et al.* 2011). Therefore, the species richness in these areas could be threatened.

The high composition dissimilarity and species replacement among the sites suggest that they are a function of landscape heterogeneity, differences in elevation, and forest management (Meza-Parral and Pineda 2015, Almaraz Vidal and Cerón de la Luz 2016, Aldape-López and Santos-Moreno 2016, Illescas-Aparicio *et al.* 2016). For example, 29 species are only in one type of vegetation or altitude, and 15 of them are in the *Quercus* forest. Although the *Quercus* forest was the environment with more access sites which could have increased the possibility of finding species, this environment has the best forest management in the region, including selective firewood collection such as selection of small branches to leave large logs in the area that may be micro-habitats to herpetofauna (Aldape-López and Santos-Moreno 2016, Illescas-Aparicio *et al.* 2016). Other environments such as *Quercus-Pine* forest have a worse forest management without log size selection and

Figure 3. Some reptiles found in Mendoza municipality. (**A**) *Abronia graminea*, (**B**) *Plestiodon brevirostris*, (**C**) *Stenorrhina degenhartdii*, and (**D**) *Metlapilcoatlus nummifer*. Photos: ACC (A), RAV (B–C), JLCJ (D).

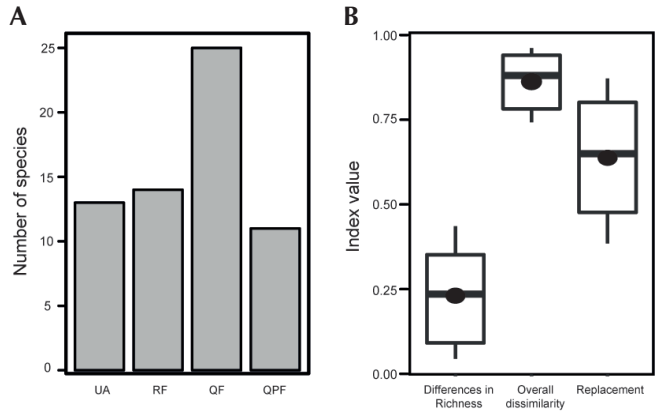

Figure 4. Number of species per site and compositional dissimilarity values between pairs of sites. (**A**) Herpetofauna richness in four sites of Mendoza municipality. (**B**) Overall dissimilarity (βcc), replacement (β-3), and differences in species richness (βrich) are shown separately. Boxes represent 25 and 75 percentile, the horizontal line is the median, red point the mean, and whiskers are maximum and minimum values. Legend: UA, urban area, (RF) riparian forest, (QF) *Quercus* forest, (QPF) *Quercus-Pinus* forest.

deforestation to grow corn. Thus, the good practices in forest management may be maintaining several habitats for herpetofauna species.

Our results showed that 15 species have wide habitat distribution in more than one type of vegetation. This suggests that the high diversity of Mendoza Municipality is not only in one type of vegetation and is distributed over a wide range of elevations. Previous studies in Veracruz also have found high levels of dissimilarity and replacement in the composition of herpetofauna related to differences in elevation and the degree of heterogeneity (Wake *et al.* 1992, Pineda and Halffter 2004, Murrieta-Galindo *et al.* 2013b, Meza-Parral and Pineda 2015, Almaraz-Vidal and Cerón de la Luz 2016). These results indicate that to preserve the herpetofauna of this region, the forest should be protected over a wide range of elevations.

We found 37 species in some category of risk according to the IUCN and the Mexican law (NOM-059). Despite Mendoza municipality being partially within the Rio Blanco National Park, the *Quercus-Pine* forest is not within this protected area (CONANP 2017). Additionally, anthropogenic activities such as the pollution of Río Blanco river, forest fires, charcoal production, and irregular human settlements are increasing and threatening the species in the region inside and outside the protected area (Rivera-Hernandez 2015). Future studies should incorporate monitoring studies that allow us to know the current state of the populations to incorporate conservation strategies.

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Appendix I. *Formula of compositional dissimilarity indexes.*

Index	Formula	Description
Jaccard dissimilarity index	$\beta_{cc} = (b+c)/(a+b+c)$	a is the number of species in both sites, b is the number of species occurring only in the first site, and c is the number of species occurring only in the second site. min(b,c) is the minimum number of exclusive species in one of the two sites
Species replacement	$\beta_{-3} = 2^* \min(b,c)/(a+b+c)$	
Species richness differences	$\beta_{pich} = \lvert b-c \rvert / (a+b+c)$	

Appendix II. *Checklist of amphibians and reptiles of Mendoza Municipality, Veracruz, Mexico, in scientific collections. Legend to Institutions: 1. UMMZ University of Michigan, Museum of Zoology; 2. IBUNAM Instituto de Biología, Universidad Nacional Autónoma de México; 3. ENCB-IPN Laboratorio de Cordados Terrestres, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional; 4. Computarización de las colecciones de vertebrados terrestres de la Escuela Nacional de Ciencias Biológicas, IPN - Fases 2 y 3; 5. Colección Herpetológica de la Estación de Biología Tropical Los Tuxtlas, Veracruz.*

Species	Longitude	Latitude	IUCN category	Endemic to Mexico	Catalog number	Institution	Source
Stenorrhina degenhardtii mexicana (Steindachner, 1867)	-97.192	18.808	LC	-	119277	1	SNIB
Scincella gemmingeri (Cope, 1864)	-97.179	18.804	LC	X	18844	2	SNIB
Sceloporus mucronatus (Cope, 1885)	-97.179	18.805	LC	X	18803	2	SNIB
Bolitoglossa platydactyla (Gray, 1831)	-97.181	18.804	NT	X	7364	3	SNIB
Bolitoglossa platydactyla (Gray, 1831)	-97.181	18.803	NT	X	7364	4	GBIF
Scincella gemmingeri (Cope, 1864)	-97.179	18.804	LC	X	18844	5	GBIF
Sceloporus mucronatus (Cope, 1885)	-97.179	18.805	LC	X	18803	5	GBIF

Appendix III. *Compositional dissimilarity values between pairs of study sites according to Carvalho* et al. *(2012).*

A. Total dissimilarity values.

	UA	RF	QF
RF	0.75	-	
QF	0.88235	0.74194	-
QPF	0.95652	0.95834	0.875

B. Replacement values.

	UA	RF	QF
RF	0.7	-	
QF	0.6	0.3871	-
QPF	0.86957	0.83333	0.4375

C. Difference in species richness values.

	UA	RF	QF
RF	0.05	-	
QF	0.35294	0.35484	-
QPF	0.08696	0.125	0.4375

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Morphological variation and new distributional records of *Rhadinella dysmica* (Serpentes: Dipsadidae), with comparisons with other dark-colored congeners

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Abstract

Morphological variation and new distributional records of *Rhadinella dysmica* (Serpentes: Dipsadidae), with comparisons with other dark-colored congeners. Small, secretive snakes comprise an important part of the herpetofauna of the Neotropics and yet most species are known from a handful of specimens due to their habits and relatively inaccessible localities. The Mexican endemic *Rhadinella dysmica* is the westernmost species of the genus and was described based on a single adult female. Herein we provide information on new specimens, including their morphological variation and hemipenial structure, expand the known geographic range for the species, and comment on the morphological similarities of the "dark-colored" species of the genus.

Keywords: Endemic species, extended diagnosis, Guerrero, hemipenial morphology, snakes.

Resumo

Variação morfológica e novos registos de distribuição de *Rhadinella dysmica* (Serpentes: Dipsadidae), com comparações com outros congêneres de coloração escura. Serpentes de pequeno porte e com hábitos secretivos constituem uma parte importante da herpetofauna dos Neotrópicos, mas a maioria das espécies é conhecida a partir de poucos exemplares devido a seus hábitos e localidades

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relativamente inacessíveis. A serpente endémica mexicana *Rhadinella dysmica* é a espécie mais ocidental do género, tendo sido descrita com base em uma única fêmea adulta. Fornecemos aqui informações sobre novos espécimes, incluindo a sua variação morfológica e da estrutura do hemipênis, expandimos a distribuição geográfica conhecida para a espécie e discutimos as similaridades morfológicas das espécies "de coloração escura" do gênero.

Palavras-chave: diagnose estendida, espécie endêmica, Guerrero, morfologia hemipeniana, serpentes.

Introduction

Small, secretive snakes from the Neotropics have habits that limit their study, and knowledge on their biology remains fragmentary in many cases. Low population densities, secretive life styles, small geographic ranges, and specialized microhabitats have been proposed as the main limiting factors in studying them (Myers 2003). The genus *Rhadinella* contains a group of 20 species of small, slender snakes (Campillo *et al.* 2016, McCranie 2017, Ariano-Sánchez and Campbell 2018), collectively ranging from Guerrero and Veracruz, Mexico, southwards to western Panama (Myers 1974, Campillo *et al.* 2016). Members of the genus tend to be highly secretive and some species remain known only from their holotypes (Campbell 2015, Campillo *et al.* 2016, Ariano-Sánchez and Campbell 2018). *Rhadinella dysmica* Campillo, Dávila-Galavíz, Flores-Villela and Campbell, 2016 is the westernmost member of the genus and was described from a single female specimen from near Cueva Tepozonales in central Guerrero, Mexico (Campillo *et al.* 2016). Recent fieldwork by personnel from the Universidad Autónoma de Guerrero and Universidad Nacional Autónoma de México has led to the collection of new specimens of this species, including the first known males. Here we illustrate the hemipenis, report on the variation of this species, and compare it with other dark-colored congeners [*R. donaji* Campbell, 2015, *R. dysmica, R. pilonaorum* (Stuart, 1954), *R. schistosa* Smith, 1941, *R. posadasi* (Slevin, 1936), and *R. xerophila* Ariano-Sánchez and Campbell, 2018].

Materials and Methods

We examined specimens in the herpetological collections of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (ENCB) and the Museo de Zoología "Alfonso L. Herrera, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC). One specimen from the collection of the Laboratorio Integral de Fauna Silvestre at the Universidad Autónoma de Guerrero was examined, and subsequently deposited at MZFC-UNAM. Two live specimens were collected by the authors in June 2018 and later deposited at MZFC. Another adult snake collected by a field team on June 2020 was offered to us for examination, but this specimen was lost during transportation. However, digital photographs of this specimen are available for study.

All measurements were taken with dial calipers and rounded to the nearest 0.1 mm, except for snout–vent length (SVL) and tail length, which were measured with a metal ruler and rounded to the nearest millimeter. Ventral scales were counted according to Dowling (1951), and segmental counts are as defined by Savage and Lahanas (1991). Hemipenial preparations were made following the procedures of Myers and Cadle (2003) and Zaher and Prudente (2003), as modified by Smith and Ferrari-Castro (2008). Hemipenial terminology follows Dowling and Savage (1960) and Myers and Campbell (1981). Maxillary dentition was observed in situ by making a longitudinal incision between the supralabials and the maxillary arch, removing tissues and counting teeth and empty sockets.

Results

Four specimens, two males and two females, plus photos of a fifth specimen, were examined (Table 1).

Expanded Definition Based on Additional Material

A small dipsadid snake (166–265 mm SVL), with a long tail (33.1% of SVL in males, 33.7% in females); head slightly distinct from neck; snout moderately elongated, broadly rounded in dorsal profile; rostral not extending between internasals; cephalic plates arranged in regular colubrid fashion; internasals paired, prefrontals paired, broad median frontal, supraoculars paired, narrow, and parietals elongate, paired; nuchals 6–7; nasal divided; postnasal contacting prefrontal, loreal and supralabials 1–2; loreal single, broader than high, more than half length of eye; preocular one, subpreocular absent; postocular one; temporal scales 1+2, anterior temporal above supralabials 5–6; supralabials 7/7, 3–4 entering the orbit; eye small, with a round pupil; infralabials 9/9, first pair in broad contact with the posterior of mental, 1–4 in contact with anterior pair of chinshields; chinshields in two pairs, longer than wide, anterior pair slightly longer than posterior pair; posterior pair of chinshields separated for posterior part of their length by two gulars; mental groove present; dorsal scales smooth, in 17-17-17 rows, apical pits usually absent but present at midbody in one adult male (MZFC 31981); ventrals 155–172 (155–165 in males, 169–172 in females); cloacal plate divided; subcaudals paired, 105–108 (105 in one juvenile male, 108 in one juvenile female; Table 1); segmental counts 263–274 (274 in one male, 263 in one female); maxillary teeth 10–12, increasing progressively in size posteriorly. Variation in selected morphological characters is summarized in Table 1.

Table 1. Selected morphological characters and locality information on the currently known specimens of *Rhadinella dysmica*.

Specimen	Sex	SVL (cm)	Tail Length (cm)	Supralabials	Infralabials	Dorsal scale rows	Ventrals	Subcaudals	Locality	Coordinates
ENCB 18951	♀	256	85, incomplete	7/7	7/8	17-17-17	172	63, incomplete	Cueva de Tepozonales, Cuajilotla, 20 km S Mochitlán	17.2853° N, 99.3662° W
MZFC 31981	♂	221	30, incomplete	7/7	7/8	17-17-17	165	25, incomplete	Tecoantepec, municipality of Tecoanapa	16.99841° N, 99.24917° W
MZFC 35581	♀	193	97	7/7	9/9	17-17-17	169	105	Las Humedades, Sierra de Tecpan	17.50997° N, 100.70355° W
MZFC 35583	♂	111	55	7/7	9/9	17-17-17	155	108	Las Humedades, Sierra de Tecpan	17.50997° N, 100.70355° W
Uncatalogued	-	-	-	-	-	-	-	-	East Rio Santiago, Sierra de Atoyac	17.25639° N, 100.31095° W

Coloration

In most specimens dorsal surfaces of the head, body, and tail are dark gray. In some specimens subtle darker stripes are visible against the background (Figure 1), but these became obscured with preservation. A pale nuchal collar encompasses 3–3½ dorsal scale lengths laterally and narrows to two scales middorsally, occasionally dark pigment is present at the narrowest point. The nuchal scales bordering the parietals are creamy white or white with yellow margins; the collar encompasses the borders of the parietals, secondary temporals, the uppermost part of the primary temporals and fades towards the venter. Pale markings are present on a few supralabials (frequently in 5–7), but in the holotype these markings are present on all supralabials. The mentals have dark brown markings; the infralabials have irregular dark brown vertical streaks; the venter is creamy white with scattered brown markings; the subcaudals are dingy white with dark brown borders.

Coloration of the new specimens is mostly in agreement with the holotype, except for a specimen from Río Santiago that exhibits dark brown dorsal surfaces of the body, tail and head, and an orange-pink pale nuchal collar that encompasses not only the posterior of the

Figure 1. Coloration variation in *Rhadinella dysmica*. (**A–B**) Specimens from Ejido Las Humedades, Sierra de Tecpan (MZFC 35583 and 35581, respectively). (**C**) holotype from near Cuajilotla exhibiting dark striping in life (ENCB 18951). (**D**) specimen from east of Río Santiago, Sierra de Atoyac (not vouchered). All localities are in Guerrero, Mexico.

head, but also the anterior part of the snout, including the rostral, internasals and distal margins of the prefrontrals (Figure 1). This specimen was lost, so further comparisons of this "morphology" require the procurement of additional material.

Hemipenis

Both hemipenes of MZFC 31981 were everted at the time of preservation and the left organ was removed and prepared with colored petroleum jelly (Figure 2). The everted organ is slightly recurved, and the capitulum represents about one-third of the organ length. The retractor muscle reaches subcaudal 20. The organ is very slightly bilobated, unicapitate, and spinose, with a centrolineal sulcus spermaticus bifurcating at about the middle of the organ and bellow the edge of the capitulum. On the sulcate side, the lower third of the organ is naked with two large basal hooks; the second third of the organ bears three rows of spines; and the uppermost third of the organ is covered by papillate calyces. These calyces cover the entire capitulum and an adjacent region of near the bifurcation of the sulcus spermaticus. On the asulcate side the rows of spines terminate near the center at the level of the second third of the organ; two large spines are present on the middle of the organ; the capitulum ornamentation is essentially the same as for the sulcate side (Figure 2). There are three conspicuous naked pockets on the hemipenis: one on the basal portion on one side of the hemipenis; one as an enlarged calyx at the lower edge of the capitulum on the asulcate and lateral view; and a large nude, concave space bellow the capitulum on the asulcate side, surrounded by numerous spines and a basal hook on the lowermost part (Figure 2).

Figure 2. Hemipenis of the specimen MZFC 31981 in sulcate, lateral and asulcate view. Scale bar = 1 mm.

Distribution, Habitat and Conservation

As currently understood, the distribution of *Rhadinella dysmica* is restricted to central Guerrero at elevations from 430–725 m a.s.l., on the windward side of the Sierra Madre del Sur of Guerrero. Specifically, from the Sierra de Tecpan south-eastwards to the southern slopes of the Sierra de Mochitlán and the hills north of Tecoanapa near the Río Omitlán (Figure 3). All the above localities, including the type locality, are covered by tropical deciduous forest and those from the Sierra de Atoyac and Sierra de Tecpan are covered by tropical semi-evergreen forest.

The new specimens were obtained from Tecoantepec, 430 m a.s.l., municipality of Tecoanapa (MZFC 31981); east of Río Santiago, 725 m a.s.l., municipality of Atoyac de Álvarez (not vouchered); and Las Humedades, 675 m a.s.l. municipality of Tecpan de Galeana (MZFC 35581, 35583; Table 1), extending the known distribution 34 km SW, 105.6 and 150.8 km W, respectively, from the type locality.

The specimens from Las Humedades (MZFC 35581, 35583) were obtained during the rainy season on 19 June 2018 under rotting logs. The specimen from Rio Santiago (not vouchered) was found crossing a paved road at night after a moderate rain on 10 June 2020. The specimen from Tecoantepec (MZFC 31981) was found inactive under a rock during a rain on 04 October 2008. Campillo *et al.* (2016) reported finding the holotype near the entrance of a cave crawling on the ground on 17 July 2014. Similar to many small semifossorial snakes, it is possible that *R. dysmica* has a wider and more or less continuous

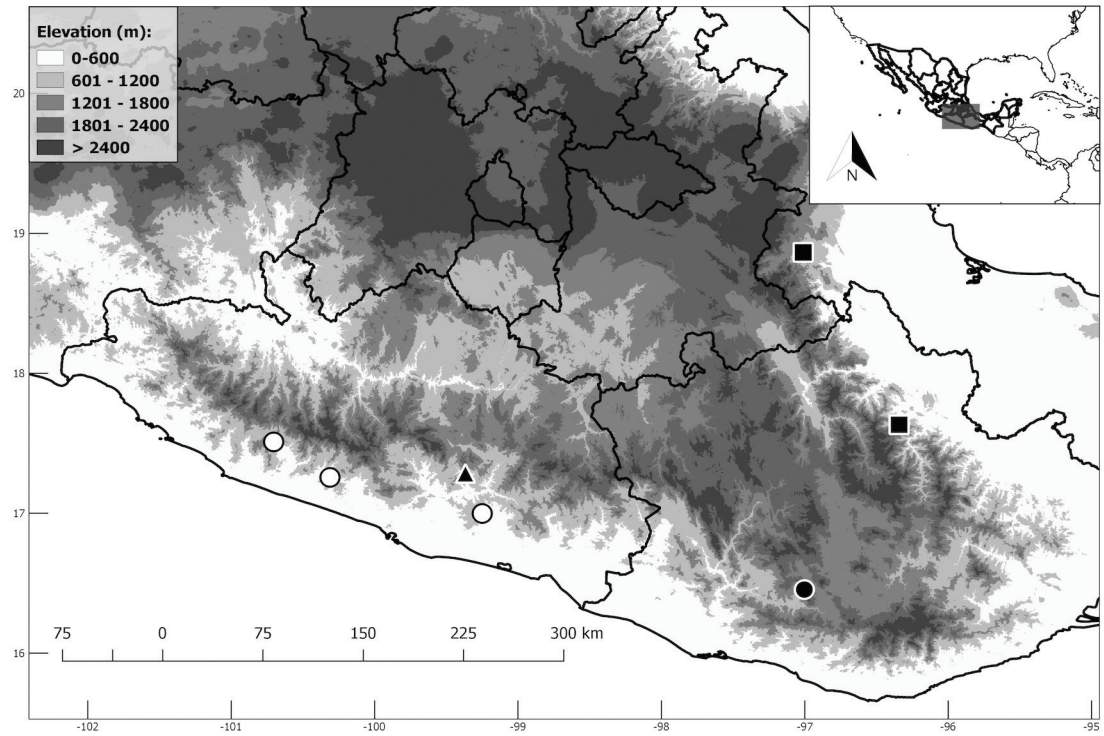

Figure 3. Geographic distribution of the species of *Rhadinella* west of the Isthmus of Tehuantepec. *Rhadinella donaji:* closed circle; *R. dysmica:* open circles, triangle represents type locality; *R. schistosa:* closed squares.

distribution at moderate elevations of the Sierra Madre del Sur, but remains undetected due to its cryptic habits.

Despite the new specimens slightly increase the geographic distribution of *R. dysmica,* it is still considered restricted to Guerrero. Information on the natural history of the species is still limited, so we consider that the risk assessment proposed by Palacios-Aguilar and Flores-Villela (2018) using the system of Environmental Vulnerability Score (EVS) is appropriate, considering this species highly vulnerable (EVS, 6 + 8 + 2 = 16), based mainly on its restricted ecological and geographical distribution.

Discussion

The new material reported herein provide a wider understanding of the morphological variation of *R. dysmica.* However, most species of *Rhadinella* are very similar in traditional diagnostic characters (see Campbell 2015, Campillo *et al.* 2016, and Ariano-Sánchez and Campbell 2018, for comparative tables). Most scutellation characters overlap between the dark-colored species, except for the relatively low number of subcaudals in *R. schistosa.*

Most of the diagnostic characters in the genus previously reported rely on color pattern. However, the color variation present in *R. dysmica* suggests that these characteristics of color pattern may not be as diagnostic as previously thought. Problems with assessing color pattern has been reported in other Dipsadidae taxa, for example in *Apostolepis* (Entiauspe-Neto *et al.* 2020), *Atractus* (Meneses-Pelayo and Passos 2019), *Coniophanes* (Palacios-Aguilar and Flores-Villela 2020), *Geophis* (Pavón-Vázquez *et al.* 2011) and hence, taxonomic decisions based solely on color patterns should be interpreted with caution. New species of *Rhadinella* have been described recently based on morphological differences, including color pattern (Campbell 2015, McCranie 2017). The color variation of *R. dysmica* might suggest that more than a single taxon is represented. However, we consider this unlikely because all specimens were found along a more-or-less continuous belt of tropical forests below 1000 m a.s.l., where no major geographic barriers exist. Rather, we consider that this is another case of color polymorphism, a feature that can vary individually, ontogenetically, geographically, and possibly sexually, in response to various selective pressures (e.g., Cox and Davis-Rabosky 2013).

Hemipenial features such as the bilobation, capitation, presence of basal pockets and spinulate calyces at least on one portion of the border of the capitulum have been reported previously on other members of the genus (Myers 1974, Holm and Cruz-Díaz 1994). However, the presence of a nude pocket on the capitulum and the asulcate side have been reported previously only on *R. godmani* (Günther, 1865) and *R. lachrymans* (Cope, 1870) (Myers 1974).

Most species of *Rhadinella* inhabit mesic habitats such as rain, cloud, and pine-oak forests (Campbell 2015), but recent explorations in Central America have discovered populations inhabiting seasonally dry forests (Ariano-Sánchez and Campbell 2018). *Rhadinella dysmica* seems to be present through an almost continuous belt of tropical deciduous and tropical semievergreen forests along the windward slope of the Sierra Madre del Sur of Guerrero.

The dark-colored species of *Rhadinella* might form a monophyletic supraspecific group of six species (*R. donaji, R. dysmica, R. pilonaorum, R. posadasi, R. schistosa,* and *R. xerophila*), that can be defined by the absence of a subpreocular scale; dorsal scale rows17-17-17; ventral scales 136–172; hemipenes bilobed (or, if single, with a divided retractor muscle); spinulate calyces on the edge of the capitulum; dorsal coloration dark gray, obscuring a hint of black longitudinal stripes on the body; and a white or cream collar. This group is distributed from Guerrero and Veracruz, Mexico, southwards to western El Salvador. An examination of larger samples for other morphological characters besides scutellation (hemipenes, maxillary dentition, osteology), in addition to assessment of molecular data would further elucidae the phylogenetic relationships of these under-studied Neotropical snakes.

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Comparative vulnerability of *Indosylvirana temporalis* and *Clinotarsus curtipes* (Anura: Ranidae) tadpoles to water scorpions: importance of refugia and swimming speed in predator avoidance

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Abstract

Comparative vulnerability of *Indosylvirana temporalis* and *Clinotarsus curtipes* (Anura: Ranidae) tadpoles to water scorpions: importance of refugia and swimming speed in predator avoidance. The comparative vulnerability of two co-existing tadpole species (*Indosylvirana temporalis* and *Clinotarsus curtipes*) to their common predator, water scorpions (*Laccotrephes* sp.; Hemiptera: Nepidae), and the importance of refugia in predator avoidance were studied in the laboratory. In a total of 60 experimental trials, 10 tadpoles each of *I. temporalis* and *C. curtipes* of comparable body sizes were exposed to water scorpions (starved for 48 h). Thirty trials included refugia while 30 did not. The results of this study showed that in both the absence and the presence of refugia *C. curtipes* tadpoles fell prey to water scorpions more frequently than *I. temporalis* tadpoles. A main difference between the two species is the speed of swimming; V_{max} of *C. curtipes* (24.73 cm/s) tadpoles is lower than that of *I. temporalis* (30.78 cm/s) tadpoles. This is likely to be the reason why more *C. curtipes* tadpoles were preyed upon than were *I. temporalis* tadpoles. Predation risk of tadpoles of both species was affected significantly by the presence of refuge sites. The vulnerability of both tadpole species was lower where refuge sites were available. The present study clearly shows that *I. temporalis* tadpoles avoid predation by water scorpions more effectively than do *C. curtipes* tadpoles.

Keywords: Defensive behavior, Hemiptera, *Laccotrephes* sp., Nepidae, predator-prey relationships, refuge sites, tadpoles.

Resumo

Vulnerabilidade comparativa dos girinos *Indosylvirana temporalis* e *Clinotarsus curtipes* (Anura: Ranidae) aos escorpiões da água: importância de abrigos e da velocidade de natação na evitação de predadores. A vulnerabilidade comparativa de duas espécies de girinos coexistentes (*Hylarana temporalis* e *Clinotarsus curtipes*) ao seu predador comum, o escorpião-d'água (*Laccotrephes* sp.; Hemiptera: Nepidae), e a importância dos abrigos na evitação de predadores foram estudados em laboratório. Em um total de 60 ensaios experimentais, 10 girinos de de cada

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espécie, tamanhos de corpo comparáveis, foram expostos a escorpiões-d'água (mantidos sem alimento por 48 horas). Trinta experimentos incluíram abrigos, enquanto outros 30 não o fizeram. Os resultados deste estudo mostraram que, tanto na ausência como na presença de abrigos, os girinos de *C. curtipes* foram predados mais frequentemente do que os girinos de *I. temporalis*. Uma diferença principal entre as duas espécies foi a velocidade de natação; a V_{max} dos girinos de *C. curtipes* (24,73 cm/s) foi menor que a dos girinos de *I. temporalis* (30,78 cm/s). Provavelmente foi esse o motivo pelo qual mais girinos de *C. curtipes* foram predados em relação *I. temporalis*. O risco de predação de girinos de ambas as espécies foi significativamente influenciado pela presença de locais de refúgio. A vulnerabilidade de ambas as espécies foi menor onde havia locais de abrigo disponíveis. O presente estudo mostra claramente que os girinos de *I. temporalis* evitam mais eficazmente a predação por escorpiões-d'água do que os girinos de *C. curtipes.*

Palavras-chave: comportamento defensivo, girinos, Hemiptera, *Laccotrephes* sp., locais de abrigo, Nepidae, relações predador-presa.

Introduction

In nature, predation is a major selective force acting on prey that forces the evolution of strategies for assessment of predation threat and the development of antipredator defense strategies in order to optimize survival and fitness (Lima and Dill 1990). In aquatic environments, tadpoles of most anurans face varying levels of predation threat and therefore evolve a variety of defense strategies. Antipredator strategies of anuran tadpoles observed in earlier studies include increased activity or high swimming speed in order to run away from predators (Hews 1988, Van Buskirk and McCollum 2000), reduction in activity levels to avoid detection (Kiesecker *et al.* 1996, Schmidt and Amézquita 2001, Saidapur *et al.* 2009, Mogali *et al.* 2011, 2012, 2020a), aggregation (Spieler and Linsenmair 1999) and increased use of refuge sites (Stauffer and Semlitsch 1993, Nystrom and Abjornsson 2000, Hossie and Murray 2010, Mogali *et al.* 2019) depending upon species. Because they exist in aquatic environments, anuran tadpoles mainly use chemical signals to assess predation threats since visual information may be obscured in water that is turbid or densely vegetated (Kiesecker *et al.* 1996, Mogali 2018).

The tadpoles of *Indosylvirana temporalis* (Günther, 1864) and *Clinotarsus curtipes* (Jerdon, 1853) co-exist along gently flowing streams and in isolated pockets of water along sides of streams during the post-monsoon season in the South-Western Ghats of India (Hiragond and Saidapur 2001, Mogali *et al.* 2012, 2016). They are mainly bottom dwellers and thrive on detritus and algal matter (Hiragond and Saidapur 2001). Visibility is low in these water bodies due to shadows from vegetation, and the benthic area that is naturally covered by leaf litter and detritus (Mogali *et al.* 2019). These water bodies are home to several types of predatory invertebrates including water scorpions, *Laccotrephes* sp. (Hemiptera: Nepidae). Water scorpions are considered ambush/sit-and-wait, non-gap-limited predators with excellent vision. In nature, they are well camouflaged in the vegetation or detritus, and ambush unsuspecting prey including tadpoles of *I. temporalis* and *C. curtipes* with a quick grasping action of the forelegs (Mogali *et al.* 2020b).

Earlier studies from our laboratory show that, under predation threat by water scorpions, the availability of refuge sites minimizes the larval mortality of both predator-naïve and predator-experienced *I. temporalis* (Mogali *et al.* 2019) and *C. curtipes* (unpubl. data). They also show that predator-experienced tadpoles of both species use refuge sites more effectively and survive better than predator-naïve tadpoles.

In natural environments, we noticed that

tadpoles of both species co-exist and use similar hiding places when needed, hence it is very important to know about the comparative vulnerability of tadpoles to their common predator, water scorpions. Hence, the present study was designed to determine the comparative vulnerability of wild-caught tadpoles (predator-experienced) of *I. temporalis* and *C. curtipes* of comparable body size at early stages of development (Gosner stages 25–27) to free hunting water scorpions both in the presence and the absence of refuge sites. We hypothesized that there should be a difference in the vulnerability between two anuran tadpole species.

Materials and Methods

Tadpoles of *Hylarana temporalis* (Gosner stages 27–28; N = ~ 800) and *Clinotarsus curtipes* (Gosner stage 25; N = ~ 800) were collected from a stream in the Western Ghats near Anmod village (15.43088° N, 74.37360° E), Karnataka State, India in November and brought to the laboratory. Tadpoles of each species were placed separately in glass aquaria (90 × 30 × 15 cm) containing 25 L of aged tap water and used as a stock. Tadpoles of both species are herbivores and were fed boiled spinach to sustain growth and development. The water scorpions (*Laccotrephes* sp.; predators; N = 70) were collected from the same location that the tadpoles were obtained and were reared individually in plastic tubs (14 cm diameter and 7 cm deep) with 500 mL of aged tap water to avoid cannibalism. Prior to the commencement of the experiment, predators were daily fed equally with both prey species (3 *I. temporalis* + 3 *C. curtipes* tadpoles; Gosner stage 25) for at least four days.

Experiment 1: Comparative Vulnerability of Prey Species

This experiment was designed to determine the comparative vulnerability of *I. temporalis* and *C. curtipes* tadpoles to predatory water scorpions and the importance of refugia in predator avoidance. We carried out a total of sixty experimental trials over a week period. Ten trials were conducted per day, in ten separate experimental tubs each containing one of two treatments. Each trial started at 07:00 AM and ended at 07:00 the next day. In each trial ten tadpoles each of *I. temporalis* (Gosner stages 27–28; 21.12 ± 0.23 mm in total length; 5.37 ± 0.14 mm in width and weight 58.00 ± 3.42 mg; mean ± SD across all trials) and *C. curtipes* (Gosner stage 25; 21.10 ± 0.24 mm in total length; 5.35 ± 0.16 mm in width and weight 57.80 ± 3.50 mg; mean ± SD) of comparable body sizes were released in a plastic tub (32 cm diameter and 14 cm deep) containing 3 L of aged tap water. They were allowed to acclimate for 30 min. Then one water scorpion (61.50 ± 3.49 mm in total length, 10.18 ± 0.24 mm in width and weighing 629.0 ± 13.15 mg; mean ± SD across all trials) starved for 48 h was introduced into the tub. After 24 h the number of surviving *I. temporalis* and *C. curtipes* tadpoles was recorded to compute the number of tadpoles of each species lost due to predation.

In thirty trials (five per day over six days) the tubs containing the tadpoles and predators provided no refugia for the tadpoles. In a second thirty trials, carried out five per day over the same six days, the tubs contained structural refuges made using water soaked (two days) leaves of *Aporosa lindleyana* (dry mass 15 ± 1.6 g; mean ± SD) chopped into ~ 1 cm^2 pieces. These were spread at the bottom of the testing tubs to serve as shelters/ refuge sites. Predation risk was studied as described above. The test tubs in all trials were washed thoroughly before each trial. Both tadpole species were well fed with boiled spinach before trials. However, during the trial hours they were not provided any food. All experimental trials were carried out at room temperature (25°C). Relative vulnerability of *I. temporalis* and *C. curtipes* tadpoles to predation in each experiment was tested using Mann-Whitney *U*-tests (SPSS software ver. 16.0).

Experiment 2: Burst Swimming Speed of Prey Tadpoles

The vulnerability of the prey species (*I. temporalis* and *C. curtipes* tadpoles) to predation by water scorpion differed significantly (Experiment 1). It was thus of interest to know the differences in the swimming speeds between the prey species. To determine V_{max}, a single test tadpole (either *I. temporalis* or *C. curtipes*) of comparable body size (see expt. 1) was placed in a plastic tub (20 cm diameter and 10 cm deep) filled with aged tap water to a depth of 2.5 cm and left undisturbed for 5 min to adjust to new conditions. A handycam (Sony, DCR-SR300/E) was positioned above the tub to record activity in the entire tub. The handycam was connected to a computer with the Ethovision Video Tracking System (Noldus Information Technology, The Netherlands) to track the movements of the test tadpole. After 5 min of acclimation, the test tadpole (either *I. temporalis* or *C. curtipes*) was chased continuously for 1 min by prodding the tail base with a delicate wire as described by Van Buskirk and McCollum (2000). The movement of the tadpole was tracked to determine the V_{max}. A total of 25 trials were carried out for both tadpole species with a new test tadpole of each species every time. Both tadpole species were well fed with boiled spinach before trials. The V_{max} of two tadpole species was compared by Mann-Whitney *U*-test (SPSS software ver. 16.0).

Results

Experiment 1: Comparative Vulnerability of Prey Species

Either in the absence or presence of refuge sites significantly higher numbers of *C. curtipes* tadpoles fell prey to water scorpions as compared to *I. temporalis* tadpoles (Table 1). Predation risk of tadpoles of both species was affected significantly by the presence of refuge sites. The vulnerability of both tadpole species was lower where refuge sites were available (Table 1).

Experiment 2: Burst Swimming Speed of Prey Tadpoles

There was a significant difference in the swimming speed between tadpoles of the two

Table 1. Number of prey tadpoles (mean ± SE) of *Indosylvirana temporalis* and *Clinotarsus curtipes* consumed by the predator, *Laccotrephes* sp., in a 24 h trial period (N = 30 trials. #Mann-Whitney U-test; *indicates significant difference between two treatments.

Treatment	Tadpoles consumed		U and p values#
	Indosylvirana temporalis	*Clinotarsus curtipes*	
Without refuge sites	2.60 ± 0.23	4.03 ± 0.40	$U = 268.0$, $p < 0.05$*
With refuge sites	1.43 ± 0.21	2.53 ± 0.27	$U = 256.0$, $p < 0.05$*
U and p values#	$U = 229.5$, $p < 0.05$*	$U = 265.5$, $p < 0.05$*	

Table 2. Burst swimming speed (V_{max}; mean ± SE) of *Indosylvirana temporalis* and *Clinotarsus curtipes* tadpoles (N = 25 trials). #Mann-Whitney U test; *indicates significant difference between two species.

Species	V_{max} (cm/s)
Indosylvirana temporalis	30.78 ± 1.24
Clinotarsus curtipes	24.73 ± 1.01
U and p values#	$U = 147.50$, $p < 0.05$*

species. The *I. temporalis* tadpoles exhibited a greater V_{max} (30.78 cm/s) than that of the *C. curtipes* (24.73 cm/s) tadpoles (Table 2).

Discussion

In aquatic environments, most prey organisms including larval anurans live under great predation pressure. This results in the evolution of defense means to escape from predation and promote survival (Schmidt and Amézquita 2001, Relyea 2007). The results of this study showed that in both the absence and the presence of refuge sites, *C. curtipes* tadpoles fell prey to water scorpions more easily than *I. temporalis* tadpoles. A main difference between the two species is the speed of swimming; the V_{max} of *C. curtipes* tadpoles is lower than that of *I. temporalis* tadpoles. Hence, *C. curtipes* tadpoles are more vulnerable to capture by predators than are *I. temporalis* tadpoles. Our results conform to those of earlier studies (Van Buskirk and McCollum 2000, Dayton *et al.* 2005). It is generally believed that refuge sites reduce predation risk (Nystrom and Abjornsson 2000, Mogali *et al.* 2019). In the present study the vulnerability of both tadpole species was lower where refuge sites were available.

The results of the present study show that *C. curtipes* tadpoles are more vulnerable to predators than those of *I. temporalis.* The present study on comparative vulnerability of tadpoles of *I. temporalis* and *C. curtipes* was conducted only at early larval stages of development (Gosner stages 25-27). The vulnerability of the two species may not be the same throughout the larval period because the tadpoles of *I. temporalis* complete its larval period within 3–4 months (Hiragond and Saidapur 1999, Saidapur 2001, Mogali *et al.* 2016) but those of *C. curtipes* grow to larger body size over very long larval periods i.e., from six months to one year (Saidapur 2001). Further studies comparing the species throughout development are therefore needed. The finding of the present study clearly shows that at early stages of development, *I. temporalis* tadpoles have developed better predator avoidance behavior than that of *C. curtipes* tadpoles.

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High prevalence of anomalies in *Nyctimantis brunoi* (Anura: Hylidae) from a restinga protected area in southeastern Brazil

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Abstract

High prevalence of anomalies in *Nyctimantis brunoi* (Anura: Hylidae) from a restinga protected area in southeastern Brazil. In the present study we monitored a population of *Nyctimantis brunoi,* a species commonly found in restingas of southeastern Brazil. Field activities were carried out in the Parque Nacional da Restinga de Jurubatiba (PNRJ), a protected area located in the northern portion of the state of Rio de Janeiro. Specimens were sampled through a complete species inventory. We analyzed 218 individuals, 32 (14.7%) of which have anomalies. Additionally, a subsample of 15 specimens were radiographed to verify the occurrence of skeletal anomalies not externally detectable and to verify if the classification of anomalies attributed by means of external examination are detectable in the osteological structure of the specimen. There are 12 types of anomalies recognized in this population, three of them only detectable through internal investigation (radiography). We verified that most of anomalies externally detectable were correctly classified when compared to the osteological morphology of the radiographed specimens. Thus, in this investigation, the study of external malformations was capable to detect 60% of the types of anomalies. We conclude that further ecotoxicological and epidemiological studies of the population of *N. brunoi* in the PNRJ are necessary to establish the origins of anomalies in this species.

Keywords: Amphibia, bioindicators, Casque-headed treefrogs, contaminants, morphological abnormalities, skeletal deformities.

Resumo

Alta prevalência de anomalias em *Nyctimantis brunoi* (Anura: Hylidae) de uma área protegida de restinga do sudeste do Brasil. No presente estudo, nós monitoramos uma população de *Nyctimantis brunoi*, espécie comumente encontrada em restingas do sudeste do Brasil. As atividades de campo foram realizadas no Parque Nacional da Restinga de Jurubatiba (PNRJ), uma unidade de conservação federal localizada na porção norte do estado do Rio de Janeiro. Os espécimes foram

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amostrados por meio de inventário completo de espécies. Foram analisados 218 indivíduos, dos quais 32 (14,7%) apresentaram algum tipo de anomalia. Além disso, uma subamostra de 15 espécimes foi radiografada para verificar a ocorrência de anomalias esqueléticas não detectáveis externamente e para verificar se as classificações das anomalias atribuídas por meio do exame externo são detectáveis na estrutura osteológica dos espécimes. Foram encontrados 12 tipos de anomalias nesta população, sendo três delas detectáveis apenas através de imagens de radiografia. Verificamos que a maioria das anomalias detectáveis externamente foram corretamente classificadas quando comparadas à morfologia osteológica dos espécimes radiografados. Assim, nesta investigação, o estudo das malformações externas foi capaz de detectar 60% dos tipos de anomalias. Concluímos que mais estudos ecotoxicológicos e epidemiológicos da população de *N. brunoi* no PNRJ são necessários para estabelecer a origem das anomalias nessa espécie.

Palavras-chave: Amphibia, anomalias morfológicas, bioindicadores, deformidades esqueléticas, pererecas-de-capacete, contaminantes.

Introduction

The global declines of amphibians and the increasingly common records of species with abnormal features have promoted concern among researchers in the current century (Meteyer *et al*. 2000, Roelants *et al*. 2007, Hayes *et al*. 2010, Green *et al*. 2020). Morphological abnormalities in anurans have been relatively well reported to several populations worldwide (Meteyer *et al*. 2000, Schoff *et al*. 2003, Thigpen *et al*. 2014, Monroy-Vilchis *et al*. 2015, Rebouças *et al*. 2019). One of the most frequently reported abnormalities in amphibians is the occurrence of external malformations, mainly in the hindlimbs and fingers (Mann *et al.* 2009). Although some of these malformations are associated to natural conditions (Stuart *et al*. 2004, Ballengée and Sessions 2009, Lunde and Johnson 2012), many others have unknown causes and may be related to several factors, mostly related to recent human-caused environmental changes, such as contamination/alteration of soil and water (see Ankley *et al.* 2004, Lanno 2008). One of the main causes of amphibian population losses, habitat destruction, does not seem to explain declines occurring in undisturbed areas (Marco *et al*. 1999). An apparently suitable habitat for the stability of amphibian populations may be considerably altered, for instance, by chemical contaminants that permeate lakes, ponds and streams (Marco *et al*. 1999). It is known that anurans exposed to large amounts of chemicals (e.g., pesticides and fertilizers) and/or solid, liquid and suspension residues may show abnormalities, which are increasingly frequent (Miles and Pfeuffer 1997, Marco *et al*. 1999, Shivaramaiah *et al*. 2005, Moreira *et al*. 2012, Guerra and Aráoz 2016, Gonçalves *et al*. 2017, Araújo *et al*. 2020). Anurans have a relevant function as biological indicators of environmental conditions (Almeida *et al.* 2019), as they have permeable skin, unshelled eggs and often have an aquatic larval stage before metamorphosing into a terrestrial adult (Blaustein and Kiesecker 2002, Blaustein and Johnson 2003, Simon *et al.* 2011, Aguillón-Gutiérrez and Ramírez-Bautista 2018). Therefore, they are exposed to aquatic, atmospheric and soil stressors (Almeida *et al.* 2019).

Nyctimantis Boulenger, 1882 comprises seven species of Casque-headed treefrogs distributed in the Amazonian and Atlantic rainforests (Blotto *et al.* 2020). *Nyctimantis brunoi* (Miranda-Ribeiro, 1920) is the most well-known species of the genus, with several studies related to ecological issues (e.g., Trueb 1970, Andrade and Abe 1997, Teixeira *et al*. 2002, Mesquita *et al*. 2004, Wogel *et al*. 2006, Jared *et al*. 2015, Carmo and Woitovicz-Cardoso 2018, Murta-Fonseca *et al*. 2020). As in other species of the genus, *N. brunoi* has a heavily ossified skull, with cranial crests, ridges, and flanges (Trueb 1970). Such anatomical attributes were suggested as evolutionary

adaptations to habitats with low water potential (Trueb 1970) and associated to phragmotic (e.g., using the head to plug burrows) and other defensive behaviors (Pimenta *et al*. 2009, Jared *et al*. 2015). The species is endemic to Atlantic Forest morphoclimatic domains, occurring from the state of São Paulo to the state of Bahia (Frost 2021), and is relatively common in sandy coastal environments, being one of the anuran species most frequently recorded in this ecosystem in southeastern Brazil (Rocha *et al.* 2008). Although the taxon is found mainly associated with bromeliads (see Teixeira *et al*. 2002, Mesquita *et al*. 2004), it uses temporary swamps and periodically flooded areas for breeding and spawning during the rainy season (Freire *et al*. 2019).

In the present study we reported the morphological abnormalities found in *N. brunoi* from Parque Nacional da Restinga de Jurubatiba, a sandy coastal environment in southeastern Brazil. Additionally, we verified if the classification of malformations externally visible are detectable in the osteological structure. We also discussed possible causes for these malformations that must be further investigated and emphasize the importance of developing plans for protecting the natural habitats of this species and other possibly threatened anurans.

Materials and Methods

Study Area and Fieldwork

We collected individuals of *N. brunoi* from August 2013 to June 2019 by means of complete species inventory (Scott and Norman 2001). We also included in our analysis specimens from PNRJ collected since 1999 (not included on the map) and deposited in the Amphibian Collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). In sandy coastal environments from southeastern Brazil, *N. brunoi* is one of the most common anuran species (Teixeira *et al*. 2002), being also abundant in the present study area (Carmo *et al*. 2019). Therefore, we choose the species as a model to assess for the first time the malformation rate in this type of habitat.

Data Collection and Analysis

We categorized adults, juveniles, males, and females based on Mesquita *et al*. (2004). These categorizations were made through direct observation of gonads, vocal slits, nuptial pads and snout–vent length (SVL). Morphological abnormalities detected externally were photographed in a Leica M205C stereoscope coupled to a DFC 450 camera. Additionally, a subsample of 15 specimens were radiographed to verify if the classification of externally visible malformations are detectable in the osteological structure, and to verify the occurrence of skeletal anomalies not externally noticeable. This subsample was randomly constituted, since it was not possible to radiograph all malformation individuals. As a criterion, if an anomaly was detected more than once in the same individual, it was quantified as a single case, rather than being quantified as the number of times it was detected. As all contingency tables showed values of expected frequencies greater than five, we performed chi-square tests (see Gotelli and Ellison 2011) to examine the prevalence of anomalies between juveniles and adults, males and females, and to verify if the prevalence of anomalies found in this study was similar to the threshold of 5% of naturally expected malformation proposed by Lunde and Johnson (2012). For all tests performed, a significance level of 0.05 was adopted.

Voucher specimens were collected, anesthetized and killed with lidocaine 2%, fixed in formaldehyde 10%, subsequently preserved in 70% ethanol [usual techniques described by McDiarmid (1994)], and deposited in the amphibian collection of MNRJ. We follow the guide to malformations of frogs and toads proposed by Meteyer (2000), Zaks (2008), Vershinin (2015), and Henle *et al*. (2017) with adaptations (for details, see Table 1).

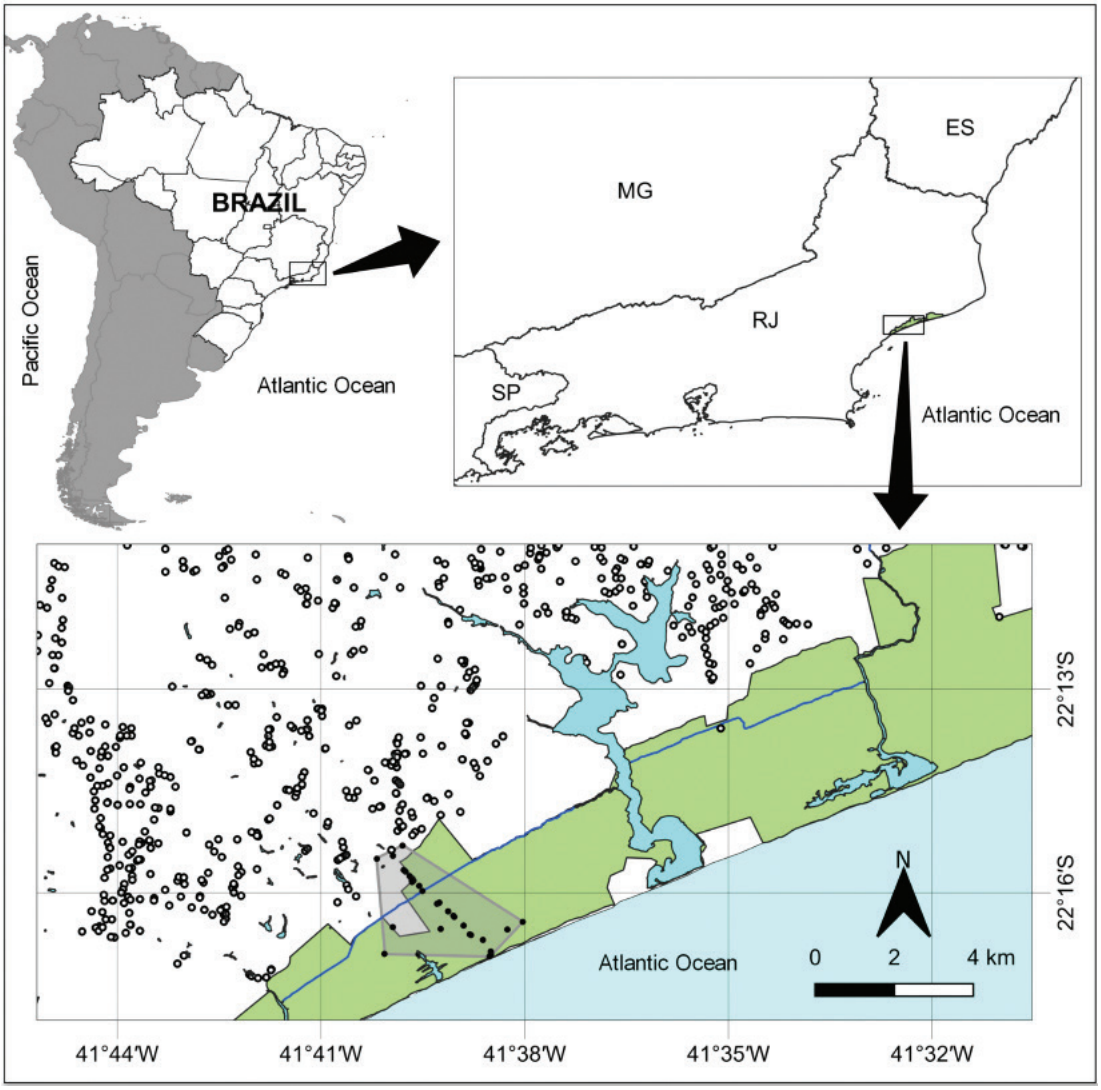

Figure 1. Map of the study area. Green: Parque Nacional da Restinga de Jurubatiba area. Black dots: surveyed start points; gray area around black dots: sample area extrapolated by wrap convex; dark blue line: Campos-Macaé Channel; open circles: farms in the municipalities of Carapebus and Quissamã (IBGE 2017a), and buildings of agricultural, farming, vegetal extraction and/or fishing activities (IBGE 2018). States of São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ) and Espírito Santo (ES).

Results

We analysed 218 individuals of *N. brunoi*, being 142 adults (45 males and 97 females) and 73 juveniles (29 young males, 39 young females and five with sex undetermined). It was not possible to determine sex and age for three individuals in the sample.

We recorded a total of 32 malformed specimens (14.7% of our sample): extra-

numerical tubercle, absence of subarticular tubercle, abnormal adhesive disc, brachydactyly, microdactyly, ectrodactyly, polyphalangia, phalanx rotation, syndactyly, scoliosis, tarsalia abnormal and urostyle torsion (Table 1). Ten of the 32 malformed specimens (4.6% of our sample; 31.3% of the malformed specimens) were affected by more than one type of anomaly (maximum of five), totaling a number of 48 malformation cases of *N. brunoi* analyzed).

All external anomalies affected the locomotors appendages, specifically the digits (45 cases of external anomalies out of 48 cases of anomalies detected = 93.8%) (Figure 2). Among the 12 recorded types of anomalies, brachydactyly (45.8%; $N = 22$ cases), microdactyly (16.7%; $N = 8$ cases) and abnormal adhesive disc (14.6%; $N = 7$ cases) were the most frequent. From the 12 types of morphological abnormalities recognized in this population, three were only detectable through internal investigation (radiography) and affected the appendicular skeleton (tarsalia abnormal) (Figure 2B) and the axial skeleton (vertebral column and urostyle) (Figure 3). Additionally, one radiographed specimen (MNRJ 66384) had an enlargement on the right tibia-fibula (Figure 3B), resembling a tumor. Since it was not possible to verify if this enlargement was really a tumor or a consolidated fracture without a histological analysis, we did not include this observation as an anomaly. For the 15 radiographed specimens (six young and nine adults), we registered 21 cases of malformations: three revealed only in the radiographs and 18 externally detected prior to radiography. From the 18 externally detectable cases of malformations, 13 were correctly classified when compared to the osteological morphology of the specimens on the radiographs; two were incorrectly classified; and three could not be confirmed, since the portion affected by the anomaly was not exposed on the radiograph. The 21 cases of malformations registered for the 15 radiographed specimens corresponded to six types of malformations, three of which were only detectable in the radiographs and three that were externally detectable. Thus, the study of external malformations alone would contribute to the detection and correct classification of 60% of the cases of anomalies and 50% of the types of anomalies we found in our subsample of *N. brunoi*.

Discriminating our sample by life stage, we detected malformations in 21 (14.8%) of the 142 adults analyzed and in 11 (15.1%) of the 73 juveniles. Phalanx rotation, polyphalangia, urostyle torsion, and scoliosis were found only in juveniles. Ectrodactyly, syndactyly, abnormal subarticular tubercles, and tarsalia abnormal were found only in adults. There is no difference in the prevalence of anomalies between adults and juveniles ($\chi^2 = 0.93$; $p = 0.34$), then we analyzed juveniles and adults altogether for the comparison between sexes. We detected malformations in 11 (14.9%) of the 74 males analyzed and in 20 (14.7%) of the 136 females. Phalanx rotation, ectrodactyly, urostyle torsion and tarsalia abnormal were found only in males, and abnormal subarticular tubercles, polyphalangy, syndactyly and scoliosis were found only in females. There is no difference in the prevalence of anomalies between sexes ($\chi^2 = 0.85$; $p = 0.36$). Thus, we considered the prevalence of the population as a whole and compared it to the threshold of 5% of naturally expected malformation (Lunde and Johnson 2012). In the population of *N. brunoi* of PNRJ, 14.7% of the analyzed specimens have some anomaly, a prevalence that is far beyond what is naturally expected ($\chi^2 = 42.99$; $p < 0.01$).

Discussion

Our results demonstrate that the prevalence of anomalies in *Nyctimantis brunoi* from PNRJ is significantly higher than the expected natural rate of 5% for amphibians (Lunde and Johnson 2012). *Nyctimantis brunoi* has indirect development, depending on aquatic environments to reproduce. Their eggs and exotrophic larvae are found in lentic waters (reproductive mode 1 *sensu* Haddad

Table 1. Description and frequency of anomalies (total of 48 anomalies in 32 malformed specimens) recorded in a sample of 218 specimens of *Nyctimantis brunoi* from Parque Nacional da Restinga de Jurubatiba, southeastern Brazil.

Type of Abnormality	Description	Frequency (%)	References
Digits			
Brachydactyly	Normal number of metacarpal and metatarsal bones, however, the number of phalanges is reduced	45.8	Meteyer 2000
Microdactyly	Short digit due to reduction in phalanx size (s)	16.7	Zaks 2008
Ectrodactyly	Absent digit, including metacarpal and metatarsal bones, in addition to the absence of phalanges	4.2	Meteyer 2000
Polyphalangy	double phalanx	4.2	Meteyer 2000
Abnormal adhesive disc	Abnormally shaped adhesive discs, for example, rounded or narrow and / or reduced in size	14.6	New proposal
Presence of supernumerary tubercles	Extra tubercles on the ventral face of the joint between phalanges	2.1	New proposal
Phalanx rotation	Distal phalanges articulate to proximal phalanges rotated in relation to the longitudinal axis of the digit.	2.1	Meteyer 2000
Syndactyly	Fused digits	2.1	Meteyer 2000
Absence of subarticular tubercle	Tubercle of the ventral face of the joint between phalanges is absent	2.1	New proposal
Limbs			
Scoliosis	Torsion of vertebral column, generating lateral deviation (either to the right or left) from its normally straight orientation.	2.1	Meteyer 2000
Tarsalia abnormal	The tarsal bones of the foot are fused or missing or additional bones are present	2.1	Henle *et al.* 2017
Urostyle torsion	Urostyle bent, deviated from its normally straight orientation.	2.1	Vershinin 2015

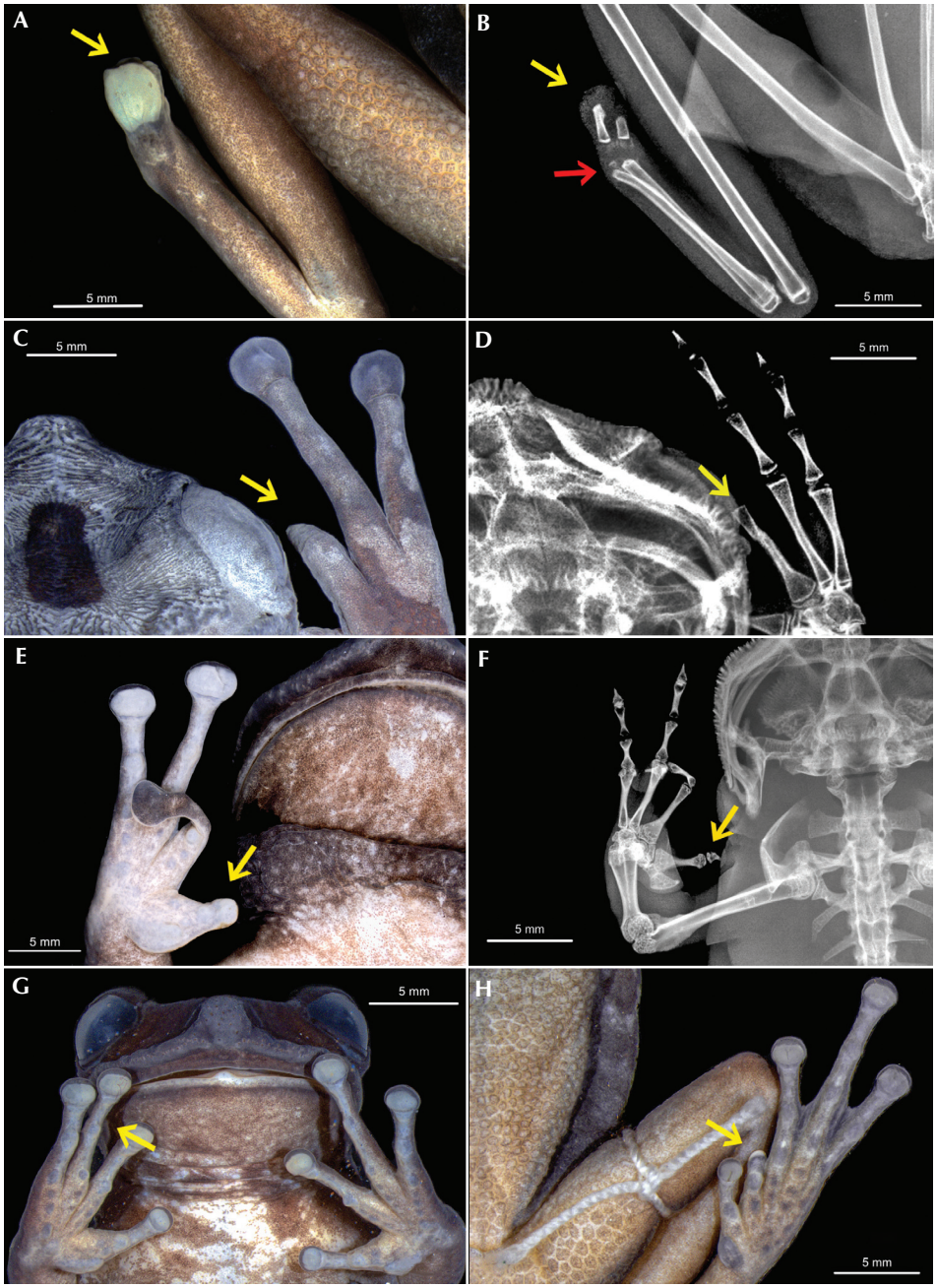

Figure 2. Anomalies detected with the naked eye and their respective radiographs (except for "G" and "H" images). (**A–B**) Adult male with abnormal adhesive disc, ectrodactyly (yellow arrow) and tarsalia abnormal (red arrow) in right hindlimb (MNRJ 88014). (**C–D**) Adult male with brachydactyly and microdactyly in right forelimb (yellow arrow) (MNRJ 92815). (**E–F**) Adult male with brachydactyly in right forelimb (yellow arrow) (MNRJ 89418). (**G–H**) Adult female with brachydactyly in right forelimb and in left hindlimb (yellow arrow) (MNRJ 92604).

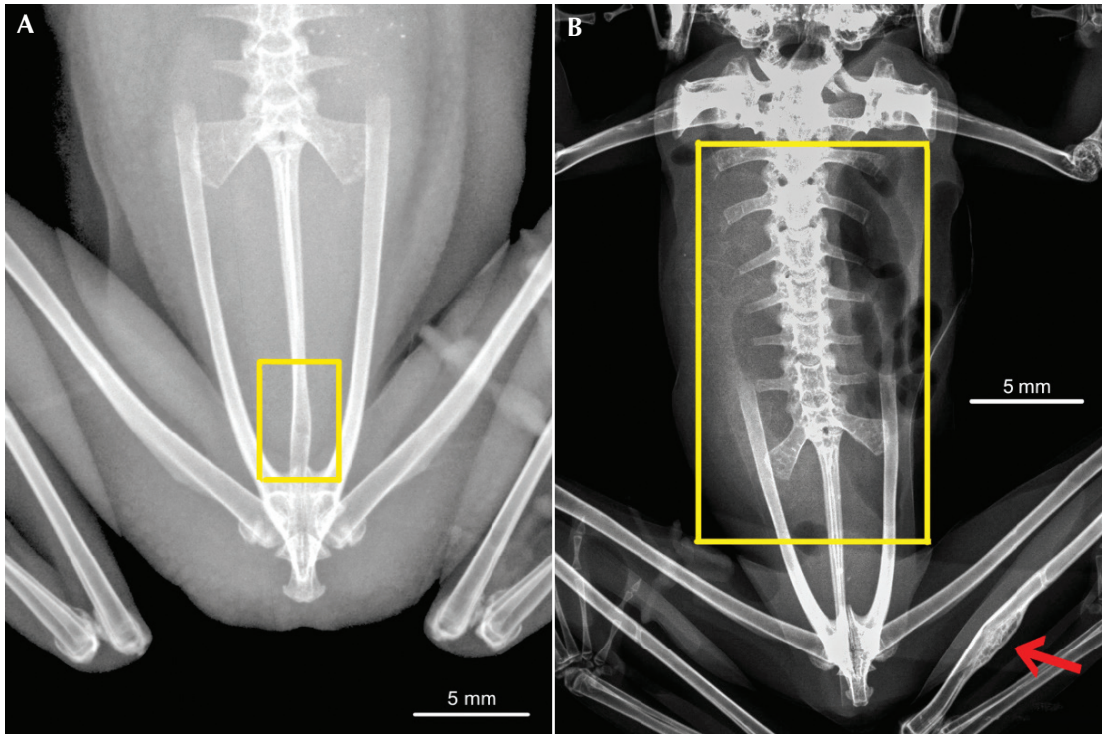

Figure 3. Anomalies in the axial skeleton detected via radiography. (**A**) Young male with urostyle torsion (MNRJ 92586); (**B**) Juvenile female with scoliosis (MNRJ 66384) and enlargement on the right tibia-fibula (red arrow).

and Prado 2005) in temporary puddles (Wogel *et al*. 2006). These characteristics can influence the exposure to factors that trigger anomalies, as well as their prevalence in the species (Johnson *et al*. 2010, Laurentino *et al*. 2016). Among the recorded specimens with anomalies, the most frequent type was the absence and/or reduction of the extremities of the limbs (brachydactyly, microdactyly and abnormal adhesive disc). Apparently, anomalies related to absence and/or reduction of segments are common in anurans, since it has been well reported in many other studies (e.g., Meteyer *et al*. 2000, Fayzulin *et al.* 2018, Ascoli-Morrete *et al*. 2019, Ramírez-Jaramillo 2019, Rebouças *et al*. 2019, Pedroso-Santos *et al*. 2020, Santana *et al*. 2020).

Different factors may be related to the occurrence of anomalies in amphibians and, according to previous studies, it is possible to correlate certain types of anomalies to potential causal factors. Carmona-Zamora *et al*. (2020) suggested that records of brachydactyly and ectrodactyly in individuals of *Rheohyla miotympanum* (Cope, 1963), a species that also has records of parasitoidism by flies (Vázquez-Corzas *et al*. 2018), could be related to bioaccumulation of organochlorine pesticides (see Valdespino *et al*. 2015). Anomalies in the autopodia (e.g., brachydactyly and ectrodactyly) have also been detected in individuals with parasitic infection by nematodes, trematodes, and a high incidence of pesticides and heavy metal residues in body tissues (Linzey *et al*. 2003). In addition, reductions in the autopodia were also detected in individuals sampled in highly industrialized regions, with rates of

anomalies being higher in species associated with water bodies (Flyaks and Borkin 2004). Besides the brachydactyly and ectrodactyly, other anomalies recorded here have been detected in individuals from agricultural areas in other studies, such as polyphalangia and syndactyly (Ouellet *et al*. 1997, Peltzer *et al*. 2011, Moreira *et al*. 2012, Agostini *et al*. 2013, Ascoli-Morrete *et al*. 2019, Ferrante and Fearnside 2020), which may be related to the exposure to chemical contaminants. A recessive and semi-lethal mutation denominated M_5, which affects the tadpoles of *Xenopus laevis* (Daudin, 1802), has been shown to be involved in the appearance of some anomalies (Droin and Fischberg 1980), including brachydactyly, syndactyly and ectrodactyly, which were also recorded in our sample. The PNRJ is surrounded by small farms of livestock and crops and the study site is located at the municipality of Carapebus (Figure 1). According to the federal census, this municipality has 549 farms, of which only 20 used pesticides and more than 350 applied fertilizers (IBGE 2017b). Since this data are auto declaratory and not restricted to the neighbouring areas of the PNRJ, we do not have data to relate the occurrence of pesticides and fertilizers with the high prevalence of abnormalities observed in the population of *Nyctimantis brunoi*.

Sub-lethal predation can also explain anomalies involving the absence and/or reduction of limbs and limb segments (Ballengée and Sessions 2009). Such anomalies may represent normal regenerative responses to the injuries caused by predation attempts (Ballengée and Sessions 2009), due to the regenerative capacity of the amphibians (Kollros 1984). The greater the stage of development in anurans, the greater the possibilities of incomplete regeneration due to the ontogenetic decline in regenerative capacity (Ballengée and Sessions 2009). Based on field observations and available literature, macroinvertebrates such as Hirudinea, Arachnida, Coleoptera (Dytiscidae), Odonata, and Hemiptera, are the main sub-lethal predators of the anuran larvae (França and Callisto 2007, Gambale *et al*. 2014), and a great variety of them occur in the PNRJ. Temporary water bodies harbor smaller abundance of predators than permanent water bodies (Santos *et al.* 2007). Although *N. brunoi* uses mostly temporary ponds for reproduction, we observed individuals in reproductive activity in semi-permanent lentic water bodies in the PNRJ. Our data is not conclusive about the occurrence of sub-lethal predation on early stages in *N. brunoi* as a cause of anomalies. However, the similar prevalence of anomalies between juveniles and adults may indicate that there is no anomaly caused by sub-lethal predation in adult specimens. So, if there is sub-lethal predation on *N. brunoi,* it occurs in the early stages of life.

In addition to the potential causes aforementioned, UV-B radiation, viral infections, infection caused by the trematodes *Ribeiroia ondatrae* (Price, 1931) Price, 1942, *Acanthostomum burminis* (Bhalerao, 1926) Bhalerao, 1936, and *Strigea robusta* (Szidat, 1928), and parasitic copepod invasion [*Lernaea cyprinacea* (Linnaeus, 1758)] are also associated with developing of limb malformations in amphibians (Stocum 2000, Blaustein and Johnson 2003, Johnson *et al*. 2004, Burton *et al*. 2008, Rajakaruna *et al*. 2008, Kupferberg *et al*. 2009, Svinin *et al*. 2020).

Anomalies in the limbs can affect species of arboreal habit more severely than species of terrestrial or semi-aquatic habits (Agostini *et al.* 2013). *Nyctimantis brunoi* belongs to the Hylidae family (Blotto *et al.* 2020), which is known to encompass arboreal species, which spend most of their time perched (Almendáriz *et al.* 2014). As hylid frogs depend heavily on limbs and digits to climb, malformed individuals may have their activities related to arboreal habit compromised. Despite being potentially negative, the anomalies occur at a similar prevalence between juveniles and adults, indicating they are probably not affecting survival.

The expected natural rate of anomalies (5%) adopted was proposed based only on studies

from temperate amphibian populations (Lunde and Johnson 2012) and maybe not apply properly to Neotropical amphibians. However, the high prevalence of anomalies found at PNRJ (almost three times higher than the threshold naturally expected) brings a warning sign that something could be negatively impacting this population of *N. brunoi.* Therefore, we encourage more studies on abnormalities in Neotropical amphibians, which may shed light on the relevance of the 5% threshold in populations other than those of temperate environments.

Although we have no evidences on the causes of the high prevalence of anomalies observed in the *N. brunoi* from PNRJ, we must consider chemical pollution. These insights are useful for a better understanding of the potential causal factors that should be investigated. The PNRJ is crossed by the Canal Campos-Macaé (Figure 1), an artificial channel that receives effluents and agrochemicals from various urban and agricultural regions along its route outside the park (ICMBio 2020b). Indirectly, the Canal Campos-Macaé can act as a carrier of pollutants and residual substances from agricultural activities into the park, since it crosses several agricultural regions (Silva *et al*. 2012). In addition, chemical compounds from agricultural areas close to the limits of the PNRJ can be transported through leaching and surface carrying, tending to result in contamination of groundwater and favoring contamination of surface water, respectively (Spadotto *et al.* 2004). Transport through volatilization and loss to neighboring areas by drift can also occur with some pesticides (Spadotto *et al*. 2004). Thus, amphibians that inhabit close to agricultural areas are subject to exposure to different concentrations of chemical pollutants and the effects of such substances in the wildlife are not yet fully understood (Mann *et al*. 2009, Gonçalves *et al*. 2019). Thereby, interactions between anurans and environmental stressors can affect species at the population level and, although a single stressor may not be sufficient to generate damage, multiple stressors can be extremely severe, since amphibians are susceptible to exposure to various abiotic agents (Blaustein and Kiesecker 2002).

Here, we suggest that *N. brunoi* is a relevant bioindicator species for studies of environmental biomonitoring in sandy coastal environments, since it is a species commonly found in this ecosystem and uses aquatic environments for oviposition. In conclusion, ecotoxicological and epidemiological studies of *N. brunoi* from the Parque Nacional da Restinga de Jurubatiba are necessary to establish the causes of abnormalities in this population. It is essential to expand the knowledge about the several factors that can influence the environmental quality of a region, especially concerning protected areas and how they can be affected by urban or agricultural surrounding areas. The monitoring of these areas is essential to recognize external factors which can affect the biodiversity, and to develop mitigation measures to reduce the impacts.

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SHORT COMMUNICATION

Prey dismemberment in the feeding behavior of the Australian skinks *Lampropholis delicata* and *L. guichenoti* (Squamata: Scincidae)

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Keywords: Communal feeding, feeding behavior, food ingestion, *Laxta granicollis*, lizards, predation.

Palavras-chave: alimentação comunal, comportamento alimentar, ingestão de alimento, lagartos, *Laxta granicollis,* predação.

Lizards primarily ingest prey whole (Brown *et al.* 2003); however, there are some exceptions where prey bodies are dismembered prior to consumption. The most common examples are carcass feeding by Komodo dragons, *Varanus komodoensis* Ouwens, 1912, (Moreno *et al.* 2008, D'Amore *et al.* 2011) and other monitor lizards (Uyeda *et al.* 2013, Fitzsimons and Thomas 2016), which enable these animals' diets to extend to larger prey species. In addition, there are also field observations of large skinks scavenging morsels from carcasses (O'Brien *et al.* 2007) and laboratory observations of the Broad-headed skink, *Plestiodon laticeps* (Schneider, 1801), dismembering invertebrates and mice (Cooper 1981). To the best of our knowledge, these reports appear to be the only published accounts of prey dismemberment by skinks.

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Skinks are commonly observed lizards in much of Australia and other continents. The Delicate skink *Lampropholis delicata* (De Vis, 1988) and Garden skink *L. guichenoti* (Duméril and Bibron, 1839) occur across broad expanses of eastern and southeastern Australia (Wilson and Swan 2021). Both are well-suited to settled areas and readily encountered in suburban gardens (Howard *et al.* 2003, Shea 2010, Wilson 2012), with *L. guichenoti* preferring open-structured microhabitats with ground litter and *L. delicata* preferring more moist and shade microhabitats (Wilson and Swan 2021). Their environmental adaptability is demonstrated in *L. delicata* colonizing new regions, including Lord Howe Island, New Zealand and Hawaii (Baker 1979, Chapple *et al.* 2015, 2016). Both *L. delicata* and *L. guichenoti* are generalist feeders that prey on a wide range of invertebrates, particularly insects and spiders (Crome 1981, Lunney *et al.* 1989, Resasco *et al.* 2018); however, there is also evidence that certain prey items are preferred over others (Martin 2015).

We report three observations of *Lampropholis delicata* and *L. guichenoti* preying on, and dismembering, bark cockroaches *Laxta granicollis* (Saussure, 1862), a Blaberidae of southeastern Australia usually found beneath logs and bark (Roth 1992). These observations occurred in suburban gardens in Sydney, New South Wales, Australia. In all instances, *L. granicollis* were encountered by skinks above ground without any ground debris being removed or disturbed.

On 29 October 2020 at approximately 13:00 h, we observed one *Lampropholis delicata* approach a *Laxta granicollis* that was partially emerged from a layer of woody mulch. The *L. delicata* bit the *L. granicollis* when it moved and dragged it onto the surface of the mulch. It proceeded to latch its mouth onto the lateral rim of the abdomen of the *L. granicollis* and perform rapid headshakes. This was repeated up to 15 times, a few times resulting in the *L. granicollis* being released and flipped on its back. The time between headshakes appeared to be associated with whether the *L. granicollis* was released during headshakes, and its response. Headshakes were performed closer together when the *L. delicata* maintained its grasp during the headshake and the *L. granicollis* was moving or the *L. granicollis* was released and started to move away, while there were generally longer periods between headshakes (5–10 s) when the *L. granicollis* was still within grasp but not moving or released and flipped on its back (thus unable to move away). The *L. delicata* then latched onto one of the limbs of the *L. granicollis* while it was flipped on its back and removed the limb with a headshake, which was swallowed. The *L. delicata* then repeated this behavior, taking a further four limbs before losing interest and retreating.

On 16 February 2021 at approximately 17:00 h, we observed two *Lampropholis delicata* drawn to a *Laxta granicollis* partially emerged from soil. Similar to the first observation, one of the *L. delicata* pulled it clear of the soil. Both *L. delicata* proceeded to perform bite-headshake maneuvers on the lateral rim of the head and abdomen of the *L. granicollis*. Both *L. delicata* undertook these maneuvers with rapid succession over a period of approximately 2 min, pausing briefly only when a section of abdomen had been detached that was small enough to swallow. Often one *L. delicata* was latched onto the *L. granicollis* while the other *L. delicata* performed the headshake. They also detached limbs like the *L. delicata* in the first observation. After approximately 1 min, a *L. guichenoti* also joined in this behavior. Between the three skinks, we estimate eight instances where headshakes resulted in a portion of the *L. granicollis* being dismembered and consumed, mostly limbs and portions of the lateral rim of the abdomen. After approximately 2 min, all three skinks lost interest and retreated.

On 07 September 2021 at approximately 09:00 h, we observed one *Lampropholis delicata* approach a *Laxta granicollis* that had emerged from a layer of woody mulch. This *L. delicata* also performed the bite-headshake maneuvers repeatedly for approximately 2 min with few pauses lasting more than 2 s unless it had dismembered a portion of the *L. granicollis* to consume. During this period, the *L. delicata* dismembered three portions of the abdomen and two limbs. A *L. guichenoti* joined in performing the bite-headshake maneuvers, during which the two skinks were often latched onto the *L. granicollis* while the other skink performed a headshake. Within approximately 1 min, the *L. guichenoti* had dismembered and consumed the head of the *L. granicollis*. It then latched onto the rim of the thorax and carried away the *L. granicollis*, which prompted the *L. delicata* to give chase. Both skinks and the *L. granicollis* disappeared from our view.

To the best of our knowledge, we are not aware of any other published accounts of these skink species dismembering prey prior to consumption. However, based on us incidentally observing three instances of this behavior within a 12-month period, these are potentially common occurrences for these species, and

may also extend to other skink species. In two of our observations, *Laxta granicollis* were dismembered whilst grasped by more than one skink, during which skinks managed to break off portions of the abdomen and head. By comparison, the first observation involved only a lone *Lampropholis delicata*, and the food obtained by this skink was limited to the limbs of the *L. granicollis*. This suggests that skinks benefit from engaging in bite-headshake maneuvers in tandem with other individuals, in which skinks provide leverage for each other's attempt to dismember the prey item. This communal feeding behavior has been reported in other lizards (Black 2012), as well as other reptiles such as crocodilians (Pérez-Higareda *et al.* 1989, Platt *et al.* 2007). Feeding interactions between multiple individuals of *L. delicata* and *L. guichenoti* are not unusual, with both species known to hunt in the presence of conspecifics (Martin 2015, M. Mo and E. Mo, pers. obs.), which is foreseeable considering the abundance of both species at numerous locations such as our observation sites. Despite potential benefits from communal feeding, skinks were also observed pursuing each other when an individual had latched onto food (similar to observations by Martin 2015), suggesting a degree of rivalry between individuals rather than communal feeding being strictly cooperative in nature.

Both *Lampropholis delicata* and *L. guichenoti* locate prey by a combination of active foraging and sit-and-wait methods (Rawlinson 1971, Lunney *et al.* 1989). Both species were abundant throughout the sites we made observations, such that we could not determine whether the skinks happened upon *Laxta granicollis* by active foraging or the skinks had been situated nearby in sit-and-wait positions. Notably, in the first two observations, the *L. granicollis* sustained injuries but were not killed during the skinks' feeding; though, their fates afterwards were not observed. It could be possible that skinks may from time to time obtain food in this manner without killing their prey.

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SHORT COMMUNICATION

Mating behavior of *Anolis punctatus* (Squamata: Dactyloidae) in the Brazilian Amazonia

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Lizards perform a broad behavioral repertoire during their courtship and mating events that allows recognition of the opposite sex, males to assess female receptivity, and females to choose a mate. These behaviors ultimately maximize mating success (Tokarz 1995, 2007, Simon 2011). Studying such behavior is particularly interesting in diurnal, highly visually oriented and territorial lizards, such as those of the genus *Anolis* Daudin, 1802 (Jenssen 1978, Bull 2000, Losos 2009, Reedy *et al.* 2017). During courtship events, anole lizards emit a diverse array of visual displays, including head bobbing, push-ups, tail lifting, throat dewlap extension, and/or changing color (Losos 2009, Simon 2011, Driessens *et al.* 2014, Steffen and Guyer 2014, Beltrán *et al.* 2016). Most of these visual displays are stereotypic and emitted in other social contexts, such as during territorial interactions (Jenssen 1978, Losos 2009, Reedy *et al.* 2017, Horr 2019). Regarding the behaviors performed during mating events, males of anole lizards most commonly bite the nape of females, grasp them, and consummate mating by inserting one of their hemipenes (Losos 2009).

The duration of lizard courtship and mating events is highly variable and influenced by a trade-off between the benefits of reproduction and exposure to a greater predation risk (Lima and Dill 1990, Cooper 1999, Simon 2007, Gerhardt 2014). Mating events of anoles follow this pattern and can vary in duration among species from less than one second to more than one hour, but events of several minutes are more commonly reported (Losos 2009, Alfonso *et al.* 2014, Beltrán *et al.* 2016). Mating duration can also differ among breeding pairs of a single anole species, increasing throughout the breeding season (Losos 2009) or in response to the presence of a predator or an observer (Beltrán *et al.* 2016). Nevertheless, observing and gathering behavioral data to improve knowledge of these

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events can be particularly challenging, especially considering species that usually mate perched on trees, such as anoles. As a result, much of the knowledge associated with reproductive events of these lizards comes from observations of laboratory experiments or captive animals (e.g., Stamps 1975, Lima and Souza 2006, Pandav *et al.* 2007, 2010, Driessens *et al.* 2014).

During recent fieldwork in Amazonian Brazil, JAO observed a complete reproductive event of the Amazon Green Anole (*Anolis punctatus* Daudin, 1802). This anole is a thermoconforming species, mainly arboreal, and distributed in both Amazonia and the Atlantic Forest (Ávila-Pires 1995, Vitt *et al.* 2003). Its mating behavior was briefly described based on a casual observation by Silva-Neto *et al.* (2019) in central Amazonia (as *Anolis philopunctatus* Ávila-Pires, 1995, currently synonymized with *A. punctatus*). To our knowledge, a complete description of its reproductive behavior and mating duration remain unknown. Herein we describe this reproductive event and compare it to the known behavior for this species and its congeners.

Individual behaviors were sampled through focal animal and all-occurrence sampling methods (Altmann 1974, Lehner 1996). To avoid interfering with the emitted behavioral signals, the observer remained silent and at least 3 m from the individuals during the entire reproductive event. The reproductive event was recorded using photos and video footage with the aid of a digital camera (Canon t3i; Tokyo, Japan) and a telephoto lens (Canon EF 70-300 mm USM; Tokyo, Japan). Individuals were sexed by analyzing sexually dimorphic characteristics of the external morphology. Males of *A. punctatus* have larger body size, more elongated snouts, bright orange-colored throat dewlaps, and thicker tail bases (hemipenial pouches) (Ávila-Pires 1995, Vitt *et al.* 2003). Females have thinner bodies and tail bases, shorter snouts, and no dewlaps (Ávila-Pires 1995, Vitt *et al.* 2003).

This observation occurred on 08 September 2020 at the peak of the regional dry season along one of the trails of the Cristalino Lodge, which is part of the Cristalino Private Natural Heritage Reserve. This ecotourism lodge is located on the western bank of the Cristalino River in southern Amazonia (Alta Floresta, Mato Grosso state, Brazil; 09°35'51" S, 55°55'53" W, datum WGS 84). The regional climate in this locality is hot and humid with a pronounced seasonality. The annual means of temperature and rainfall reach 26°C and 1,950 mm, respectively (Alvares *et al.* 2013). Most of the regional landscape is dominated by *terra firme* forest that is not susceptible to the seasonal flooding of the Cristalino River. *Anolis punctatus* typically is found in this habitat (Ávila-Pires 1995, Vitt *et al.* 2003). The air temperature was around 24°C during the observation, which lasted approximately two hours.

At 09:00 h, two adult male *A. punctatus* were spotted at an observation station within the forest. These males were perched on distinct trees about 10 m apart, where they adopted survey postures, observing their surroundings by laterally moving their heads, and walking around for short distances. Sporadically, these males used visual displays during territorial interactions, such as flexing the anterior body by doing push-ups and alternating body color between greenish and brownish tones. At 09:45 h, one of these males approached a tree trunk with a diameter of about 15 cm, showing clear signs of agitation by slightly extending its dewlap (Figure 1A), head bobbing, and acquiring a stronger brown color. This male jumped on a nearby tree, disappearing from the field of vision of the observer. When going around the tree to see the lizard, the observer noticed that the male was then mating with a female (Figure 1B), suggesting that the previous behaviors were visual displays emitted in a courtship context. Because the female was out of the initial field of vision, the observer did not obtain information on the displays emitted by her prior to mating or on the behaviors that triggered the observed mating position. When the breeding pair was spotted, the male was curving its body upon the female, immobilizing

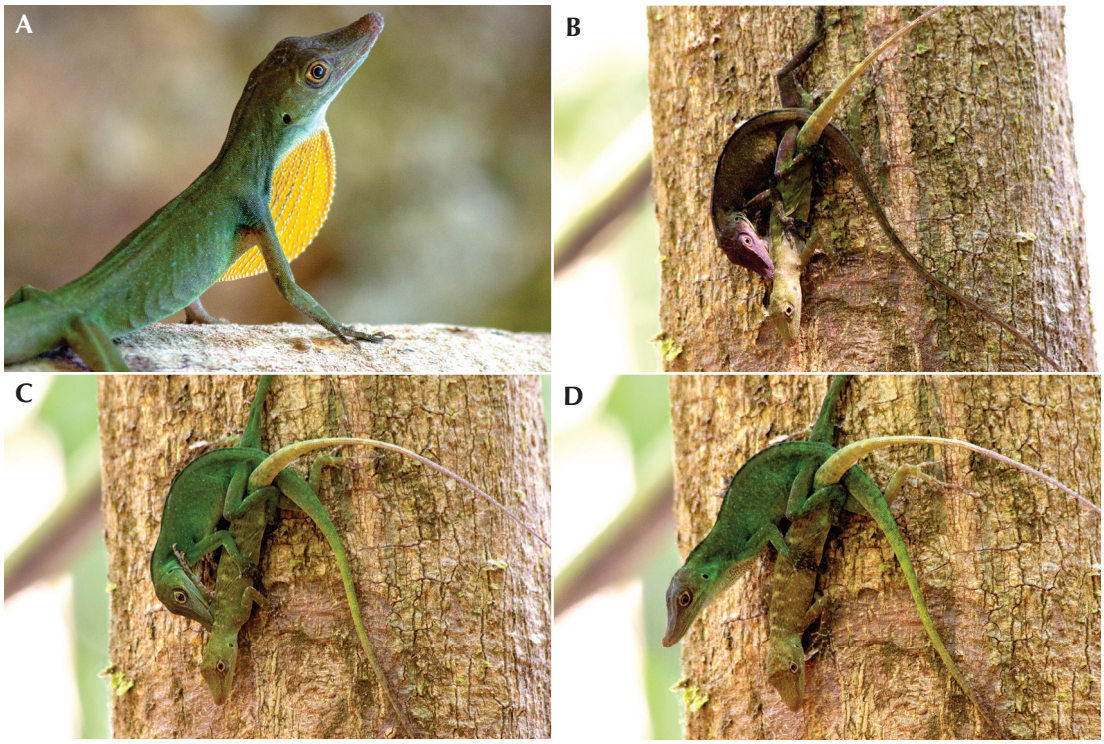

Figure 1. Mating behavior of *Anolis punctatus,* recorded in southern Brazilian Amazonia (Mato Grosso state, Alta Floresta, RPPNs Cristalino). (**A**) Male extending its dewlap. (**B**) Mating, with male immobilizing the female and acquiring an intense and uniform brown color. (**C–D**) Near the end of the mating, female trying to disengage, and male acquiring a bright green color and later stopping biting her neck.

her using a firm bite at the nape (right side), the left arm holding her at the midbody, and the left leg around her inguinal region (Figure 1B). The right limbs of the male were propped on the trunk, giving him support. Assuming this position, the male was most likely inserting its left hemipenis into the female. Both individuals had their heads toward the ground (Figure 1B).

Once in this position, the individuals remained almost immobile during the entire mating event, with some exceptions listed below. We noticed subtle movements of the female's tail and the base of the male's tail at least 19 times throughout the mating event, suggesting insertion of the hemipenis and insemination. After about 36 min, the female became agitated and displayed random movements, apparently trying to disengage herself from the male on three occasions. The male continued to immobilize her through the same previously described strategies (Figure 1C). After 71 min (near the end of the mating event) the female made a new attempt to disengage, and the male stopped biting her nape (Figure 1D). After stopping the bite, the male immediately extended its dewlap and performed rapid head bobbing. Approximately 7 min later, the female shook her body again, and the male repeated the head bobbing. A few seconds later, the female made a new attempt to disengage herself, slightly moving in a short distance and dragging the lower body region of the male, as he continued immobilizing her in the inguinal

region with his left leg. After 6 min, the male began to disengage himself from the female, apparently retracting most of the hemipenis after the female lifted the tail. Both individuals moved separately a short distance, the male toward the canopy, and the female toward the ground, thus ending the 84-min mating event at 11:09 h. After disengaging, the male remained with the tail slightly raised, rubbing the cloaca toward the substrate while completing the retraction of the hemipenis. Both individuals remained immobile until the end of the observation period (87 min).

During the mating event, the overall color of both individuals varied. The male started the mating with a brownish green color and acquired a considerably intense and uniform brown color over time (Figure 1B). During the female's attempts to disengage, the male abruptly changed to a greenish color, returning to intense brown when she became passive. After about 60 min the male, still immobilizing the female, acquired a bright green color typical of the species, and remained that way until they disengaged (Figure 1C, D). The color of the female remained relatively stable throughout the mating event, ranging from light brown at the beginning to brownish green at the end (Figure 1B–D).

Although we did not observe the entire courtship event, behaviors recorded from the breeding pair of *A. punctatus* prior to the mating were congruent with those displayed by most anole species (i.e., male head bobbing, push-ups, and extending dewlaps; Rodrigues 1988, Losos 2009, Simon 2011, Cook *et al.* 2013, Beltrán *et al.* 2016). According to the literature, the behavior and positioning of individuals during mating, as well as the chosen substrate, may vary among anole species (Losos 2009). The positioning of the breeding pair of *A. punctatus* and the chosen substrate were almost entirely consistent with that previously observed *in-situ* for this species in central Amazonia (Silva-Neto *et al.* 2019). The single notable divergence was the direction of the heads of the individuals: toward the ground in this observation and toward the canopy in the report of Silva-Neto *et al.* (2019). Compared to congeneric species, the observed mating position of *A. punctatus* resembled that reported for *Anolis smallwoodi* Schwartz, 1964 (Alfonso *et al.* 2014) and *Anolis notopholis* Boulenger, 1896 (Beltrán *et al.* 2016), although the latter has only been recorded mating on the ground.

The observed behavior of several disengagement attempts by the female *A. punctatus* was not reported for this species by Silva-Neto *et al.* (2019), but it was reported for the congeneric sympatric anole *Anolis fuscoauratus* D'Orbigny, 1837 (Rodrigues 1988). Rodrigues (1988) suggested that this behavior could derive from female dissatisfaction with exposure during mating and substrate choice (a tree trunk), as she remained immobile in a subsequent mating attempt, with the breeding pair sheltered under a palm leaf. Alfonso *et al.* (2014) also reported abrupt movements of females of *A. smallwoodi* during mating events but attributed them as a response to hemipenial insertion (copulatory phase). This idea was supported by the fact that they did not observe abrupt movements during the rest of the mating events (Alfonso *et al.* 2014). Considering these reports for congeneric species, it is impossible to determine whether the "disengagement behavior" observed here for the female *A. punctatus* was triggered by discomfort with mating conditions such as the chosen substrate, a reaction to hemipenial insertion and insemination, or even some additional factor.

Changing color from hormonal control is a common feature of several anole species and occurs in a stereotypic manner in many social contexts, such as exploration, territorialism, escape, aggressiveness, and stress (Jenssen *et al.* 1995, Greenberg 2002, Horr 2019). Color changes during mating events have not been widely reported for these lizards, except for *A. notopholis* (Beltrán *et al.* 2016). In fact, Silva-Neto *et al.* (2019) did not report color change for mating individuals of *A. punctatus,* but it is possible that such behavior went unnoticed during the short period they observed the mating event. Our observation corroborates those of

Beltrán *et al.* (2016) by showing that for *A. punctatus,* mating is an additional social interaction that may lead to a color change in anoles. We hypothesize that the change in a male's color to an intense brown may be a result of the combined effect of his excitement during the hemipenial insertion and insemination once it progressively intensified during mating, and a strategy to reduce his conspicuousness through disruptive camouflage at the most vulnerable time of mating (Boyer and Swierk 2017). Because color change is a stereotypic behavior for anoles, we cannot discard the hypothesis that this change may have been triggered by alternative social stimuli.

Mating events of anole species usually is of long duration, ranging from ca. 10–50 min (Losos 2009, Beltrán *et al.* 2016) to up to 64 min for *A. smallwoodi* (Alfonso *et al.* 2014), potentially exposing the breeding pairs to greater predation risk. The long mating duration recorded here for *A. punctatus* (84 min) is noteworthy as it is considerably above this range. Since Silva-Neto *et al.* (2019) observed a mating event of *A. punctatus* for only ca. 20 min, it is impossible to draw a parallel between our observations regarding mating duration. Nevertheless, our combined evidence supports the suggestion that long mating events are common for *A. punctatus,* and that they range from at least about 21–84 min. The long mating duration in our observation may be a result of different environmental conditions throughout the natural reproductive seasonality of the species (Losos 2009), or even a disturbance effect triggered by the presence of a nearby observer, as suggested by Beltrán *et al.* (2016). Only further studies, ideally isolating most of the extrinsic factors and involving various breeding pairs, can elucidate whether the mating duration reported here is part of the typical behavioral repertoire of *A. punctatus.*

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SHORT COMMUNICATION

Notes on the mating behavior of *Kentropyx altamazonica* (Squamata: Teiidae): first evidence of courtship display for the genus

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Keywords: Brazilian Amazonia, copulation, Cocha Whiptail, Neotropics, natural history, reproduction.

Palavras-chave: Amazônia brasileira, calango-verde, cópula, história natural, Neotrópicos, reprodução.

A wide behavioral repertoire is known for the courtship and mating events of lizards, and studying such behaviors is a key step toward understanding their reproductive strategies (Tokarz 1995, Losos 2009). As part of this behavioral repertoire, many lizards exhibit visual displays to improve recognition by the opposite sex during courtship, allowing the male to access female reproductive readiness and receptivity, and the female to choose her mate (Tokarz 1995, Losos 2009). Nevertheless, mating strategies vary depending on lifestyles of the species (Bull 2000, Zaldívar-Rae and Drummond 2007). In the case of non-territorial lizards (e.g., the family Teiidae Gray, 1827), males often actively search for females and accompany them before and after mating (mate-guarding behavior) (Bull 2000). By doing this, they can reduce access of other males to the female, mate multiple times, or stimulate the female, thereby increasing the chance of fertilization (Beecher and Beecher 1979, Zaldívar-Rae and Drummond 2007, Sales and Freire 2021).

During courtship events, lizards often exhibit visual displays that are phylogenetically conserved (Losos 2009). Considering teiid lizards, such visual displays include the male performing circular movements around the female, push-ups, gular expansions, head movements, and/or cloacal rubbing (Carpenter 1962, Quesnel 1979, Ribeiro *et al.* 2011, Costa *et al.* 2013, Sales and Freire 2021). The behavioral repertoire of lizards can be stereotypic, and several visual displays used during courtship are emitted in different contexts of social interaction, such as territoriality and aggressiveness (Barlow and Stamps 1973, Ribeiro *et al.* 2011).

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Although lizards employ a wide variety of behavioral strategies in social interactions, the challenges of observing individuals of many species in natural habitats make it particularly difficult to gather behavioral data, especially for occasional events such as courtship and mating (Costa *et al.* 2013). As a result, much of the knowledge associated with these events comes from laboratory experiments and observations of captive animals (e.g., Stamps 1975, Greenberg 1977, Grassman *et al.* 1991, Lima and Sousa 2006, Pandav *et al.* 2007, 2010), while observations in natural habitats are scarce and rarely scientifically reported (e.g., Mahrdt 1976, Censky 1995, Zaldívar-Rae and Drummond 2007, Sales and Freire 2021).

During recent fieldwork in Brazilian Amazonia, JAO observed a courtship and mating event for the teiid lizard *Kentropyx altamazonica* (Cope, 1875). This diurnal and heliothermic lizard has terrestrial and sub-arboreal habits, and is endemic to Amazonia, mostly occupying its western region (Ávila-Pires 1995, Vitt *et al.* 2001). To the best of our knowledge, courtship and mating behaviors of *K. altamazonica* remain unreported in the literature. In order to expand knowledge of social interactions of this species, we describe this field observation and compare it with behavioral events reported for closely related species.

The observation occurred on 9 June 2018 (i.e., at the beginning of the regional dry season) within the limits of the protected area Reserva de Desenvolvimento Sustentável Mamirauá, on the northern bank of the Solimões River (Uarini, Amazonas state, Brazil; 03°03'47" S, 64°50'56" W, datum WGS 84). The climate in this region has a fairly uniform seasonality and is mostly hot and humid, with mean annual temperatures of 26°C and annual rainfall of 2900 mm (Alvares *et al.* 2013). This protected area is mainly composed of periodically flooded forests (*várzea* forests), subjected to the annual flood pulse of the Solimões River. Such flooded forests correspond to preferred habitats occupied by *K. altamazonica* (Ávila-Pires 1995, Vitt *et al.* 2001). Individuals of *K. altamazonica* were observed exposed on stilts of a floating lodge dedicated to ecotourism (Uakari Lodge), located on a river channel connecting the Solimões River to the Japurá River. Despite low solar incidence during the observation of the reproductive event, the air temperature during the day was high.

Individual behaviors were sampled using focal animal and all-occurrence sampling methods (Altmann 1974, Lehner 1996). The observer remained silent and at least ca. 3 m from the individuals to avoid interfering in the behavioral signals. The reproductive event was recorded using photos and video footage, with the aid of a digital camera (Canon t3i; Tokyo, Japan) and a telephoto lens (Canon EF 70-300 mm USM; Tokyo, Japan). We made a video compiling non-continuous footage freely available online (https://youtu.be/QQE634vIIps). During the observation, other people passed by at a distance of about 5 m from the individuals (apparently without interfering in the behavioral signals), but most of the time only the single observer was present. We could not confirm the sex of individuals by direct inspection, but we confirmed the sex by analyzing sexually dimorphic characteristics of the external morphology. Males of *K. altamazonica* have distinctly larger heads, hypertrophied jaw muscles, and thicker tail bases (hemipenial pouches), whereas females have comparatively smaller heads and considerably more robust abdomens (Vitt *et al.* 2001, Costa *et al*. 2013). In addition, teiid males usually have bright colors during reproductive periods (Baird *et al*. 2003), which was evident in the observed male by the presence of several bluish spots on the lateral sides of the body, and a brighter dorsum.

At 15:18 h, the two individuals of *K. altamazonica* were observed performing a series of repetitive behaviors composed of diverse body displays (interpreted as part of the courtship behavior given the later mating). During this series, the male moved in circles around the female, displaying an inflated gular region (Figure 1A–D). After short pulsed movements,

the male repeatedly raised and lowered the head (heading behavior), as well as the entire anterior body (push-up behavior) (Figure 1A–D). Periodically, the male performed inspection licks (tongue flicking) on the substrate and, sometimes, on the female's tail. The female, who remained in a central position relative to the male, rotated on its own axis and repeated body movements similar to those exhibited by the male, but with a slightly arched neck while slowly and periodically shaking the arms (wave behavior). In addition, the female also kept its mouth open, threatening to bite (Figure 1A–D) during most of the behavioral series, and occasionally slightly arched the base of its tail.

After about 90 s maintaining this behavioral series, a first attempt of mating occurred, starting with the advancement of the female toward the male, directing a bite to his neck. Quickly, the male displayed the same movement in reverse, briefly biting the female's neck, and positioning himself upon the female. This attempt did not result in effective mating due to a rapid movement by the female. A second attempt occurred a few seconds later, triggered by a new advance by the female toward the male, but again, this behavior did not result in effective mating. The individuals continued to exhibit this same repetitive behavioral series until mating finally occurred on the third attempt at 15:25 h, totaling 7 min of courtship. In this last attempt, the male rapidly jumped toward the female, and the female bit the male's neck in response (Figure 1E, F). The male quickly spun onto the female's right side and she loosened the bite. With a quick bite and a rotating movement of both individuals in body contact, the male positioned himself upon the female for mating, without further biting her (Figure 1G, H). Holding the female around the midbody with its arms, the male then attached the posterior region of his body to the right side of the female's tail base, possibly consummating the mating with an insertion of its left hemipenis. Unfortunately, behaviors emitted after this could not be monitored because of time constraints.

Our casual report of the courtship and mating behavior of *K. altamazonica* demonstrates that such events may include a set of visual displays as part of the behavioral repertoire of this species. Our observations differ considerably from that reported for the congeneric species *Kentropyx calcarata* (Spix, 1825) by the presence of a courtship behavioral repertoire (not recorded for *K. calcarata;* Costa *et al*. 2013). Although this difference potentially suggests real and relevant interspecific variation, visual displays prior to the mating of *K. calcarata* may simply not have been observed, as suggested by the authors (Costa *et al*. 2013). Our observations indicate that *K. calcarata* may exhibit a more complex courtship repertoire, but whose discovery depends on new observations.

The circular movement behavior performed by the male *K. altamazonica* around the female during the courtship has been reported as part of the behavioral repertoire of other teiids, such as *Aspidocelis costatus* (Cope, 1878) (Zaldívar-Rae and Drummond 2007) and *Ameiva tobagana* Cope, 1879 (Quesnel 1979). During consensual reproductive events of these species, mating was consummated without the male chasing or biting the female, who exhibited a passive and permissive behavior (Quesnel 1979). Females are also generally described as passive during consensual courtships and mating of other teiids (Alfonso and Torres 2012, Costa *et al*. 2013, Quesnel 1979, Sales and Freire 2016). Interestingly, our observations of visual displays emitted by the female *K. altamazonica* (great agitation, mouth opening, repetitive push-ups, and biting attempts) are also exhibited in a stereotypic manner in aggressive contexts by other lizards (Ribeiro *et al.* 2011). Because more complex courtship behaviors are unknown for the genus *Kentropyx* Spix, 1825, it is difficult to affirm whether the signals emitted by this female represent standard courtship behaviors of this species or are indicative of a forced mating event, which usually occurs in other lizards (Lima and Sousa 2006, Gogliath *et al.* 2010, Sales and Freire 2021). A consensual mating is

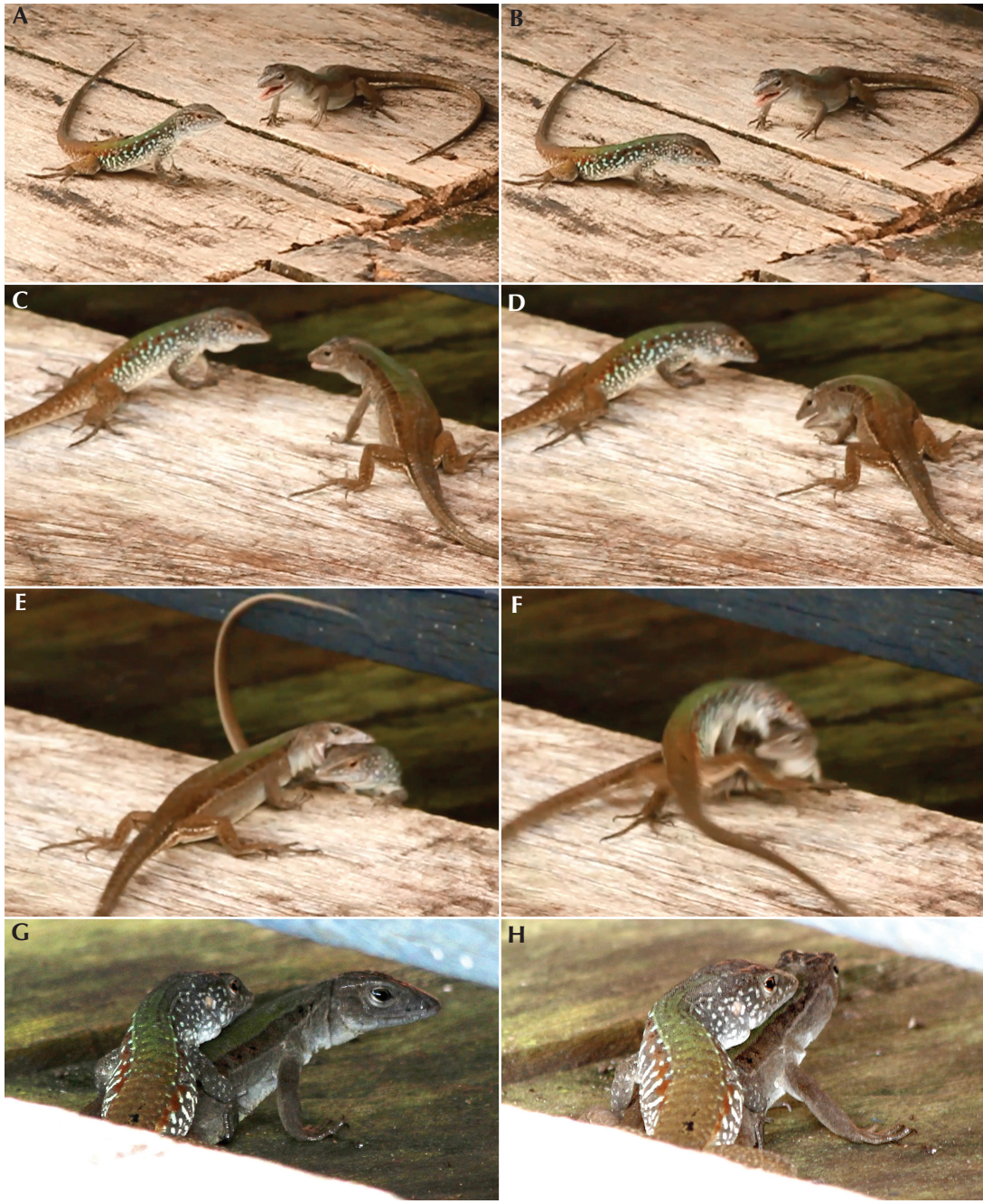

Figure 1. Courtship and mating behavior of *Kentropyx altamazonica* recorded in western Brazilian Amazonia. (**A–D**) Distinct behaviors emitted during the courtship series, with the male (on the left) circling the female, flexing the trunk and head, and keeping the gular region inflated, while the female rotated on its own axis replicating such movements, but also opening its mouth and waving its arms. (**E–F**) Pre-mating body contact, starting with a female's bite on the male neck (**E**) and then reversing positions (**F**). (**G–H**) Mating.

the most plausible hypothesis in this case, given the prior occurrence of a courtship event and the absence of continuous immobilization of the female with bites (Zaldívar-Rae and Drummond 2007, Sales and Freire 2021). Most likely visual displays exhibited by the female *K. altamazonica* in our observation are part of the courtship behavioral repertoire of the species.

Regarding the mating behavior, our observation for *K. altamazonica* was similar to that reported for the congeneric *K. calcarata*, with the male moving onto the female without biting her neck during the intromission and ejaculation phase (Costa *et al.* 2013). This mating posture has been reported for other teiids, including *A. tobagana* and *Pholidoscelis plei* (Duméril and Bibron, 1839) (Quesnel 1979, Censky 1995). Another major type of mating posture known for teiids is that in which the male bites the female's trunk and acquires an arched (ring-shaped) posture during the ejaculation phase ("doughnut posture" *sensu* Crews 1987). Such a posture has been reported for *Ameivula ocellifera* (Spix, 1825) (Sales and Freire 2021), *Pholidoscelis auberi* (Coctaeu, 1838) (Alfonso and Torres 2012) and species of the genus *Aspidoscelis* Fitzinger, 1843 (Carpenter 1962, Mahrdt 1976, Crews 1987, Anderson and Vitt 1990). The drivers of such interspecific variation in teiid mating behaviors are poorly understood. A correlation of this variation with the species' body size is likely because the "doughnut posture" seems more commonly employed by smaller-bodied teiids (e.g., Crews 1987, Sales and Freire 2021). Our casual observation for a single breeding pair of *K. altamazonica* precludes broad comparisons but provides relevant evidence for further studies dealing with the generation and maintenance of this behavioral variation.

Because of time constraints, we could not determine the post-mating behavior emitted by the breeding pair of *K. altamazonica*. Mate-guarding behavior following the mating event is expected for this species, considering that it is recurrent in teiid reproductive events (Anderson and Vitt 1990, Zaldívar-Rae and Drummond 2007, Ribeiro *et al.* 2011, Sales and Freire 2016, 2021) and provides several benefits (see Beecher and Beecher 1979, Anderson and Vitt 1990, Bull 2000, Zaldívar-Rae and Drummond 2007). Accumulated observations of teiid reproductive behavior has increasingly revealed a wider range of repertoires, and different reproductive strategies have been observed even within a single species (Sales and Freire 2016, 2021). This is the case for *A. ocellifera*, in which consensual mating (i.e., after courtship) may or may not be followed by mate-guarding behavior, and forced mating is also frequent (Sales and Freire 2016, 2021). Further studies are needed to properly assess the range of reproductive strategies and post-mating behaviors of *K. altamazonica*.

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Short Communication

New records of predators of *Ameivula ocellifera* (Squamata: Teiidae), with an emphasis on predation by birds

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Palavras-chave: dieta, ecologia, interações predador presa, lagarto, história natural.

Predation is one of the most important characteristics related to animal evolution (Krebs and Davies 1996, Quinn and Cresswell 2004). It is considered to be one of the determining processes in the natural structure of communities, where prey and predators use chemosensory, visual, and acoustic cues, among others, to detect each other's presence and to assess the associated risks involved (Pianka 1975, Connell 1978, Kinderman *et al*. 2009, Mathot *et al*. 2009).

Ameivula ocellifera (Spix, 1825) is a widely distributed lizard species found in the Diagonal of Open and Dry Areas in South America, occurring in Brazil, Argentina, and Bolivia. Individuals inhabit areas of sandy soils and high temperatures with shrubby–herbaceous vegetation and forest edges where leaf litter provides foraging sites (Mesquita and Colli 2003, Dias and Rocha 2004, 2007, Menezes *et al.* 2011, Cabrera 2012, Sales *et al.* 2012, Jose *et al.* 2014, Souza *et al.* 2014). This non-territorial species is an active forager and a heliothermal thermoregulator. It is sexually dimorphic in body size and shape; males exceed 100 mm and females are less than 80 mm in CRC (Anderson and Vitt 1990, Pianka and Vitt 2003, Sales *et al.* 2012). Predators of these lizards include a diverse range of animals, including invertebrates (Ribeiro *et al.* 2011, Moura *et al.* 2015), lizards (Sales *et al.* 2010, Gogliath *et al.*

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2010), snakes (Vitt and Vangilder 1983, Bocchiglieri and Mendonça 2009, Mesquita *et al.* 2013, Oliveira *et al.* 2018, Coelho *et al.* 2019), mammals (Olmos 1993) and some birds (Morais and Pinho 2007, Almeida *et al.* 2013, Vieira *et al.* 2018, Lisboa *et al.* 2020) (Table 1).

Table 1. List of species reported as predators of *Ameivula ocellifera* (*recorded in the field).

Predator categories	Species	Source
BIRDS		
Ardeidae	*Ardea alba* Linnaeus, 1758	Almeida *et al.* 2013*
Strigidae	*Glaucidium brasilianum* (Gmelin, 1788)	Vieira *et al.* 2018*
Bucconidae	*Nystalus maculatus* (Gmelin, 1788)	Lisboa *et al.* 2020
	Nystalus chacuru (Vieillot, 1816)	Gurgel and Quintas-Filho 2013
Accipitridae	*Rupornis magnirostris* (Gmelin, 1788)	Morais and Pinho 2007
	Gampsonyx swainsonii Vigors, 1825	This study*
Falconidae	*Falco femoralis* Temminck, 1822	This study*
Tyrannidae	*Machetornis rixosa* (Vieillot, 1819)	This study*
LIZARDS		
Teiidae	*Ameivula ocellifera* (Spix, 1825)	Sales *et al.* 2010
	Ameiva ameiva (Linnaeus, 1758)	Gogliath *et al.* 2010
Tropiduridae	*Tropidurus itambere* Rodrigues, 1987	Faria and Araujo 2004
	Tropidurus hispidus (Spix, 1825)	Costa *et al.* 2010, Zanchi *et al.* 2012*
	Tropidurus torquatus (Wied-Neuwied, 1820)	Kokubum and Lemos 2004*
SNAKES		
Dipsadidae	*Phimophis guerini* (Duméril, Bibron, and Duméril, 1854)	Bocchiglieri and Mendonça 2009
	Oxyrhopus trigeminus Duméril, Bibron, and Duméril, 1854	Vitt and Vangilder 1983, Mesquita *et al.* 2013, Coelho *et al.* 2019, This study*
	Philodryas nattereri (Steindachner, 1870)	Vitt and Vangilder 1983, França *et al.* 2008, Mesquita *et al.* 2011
Viperidae	*Bothrops erythromelas* Amaral, 1923	Oliveira *et al.* 2018
MAMMALS		
Felidae	*Herpailurus yagouaroundi* (É. Geoffroy Saint-Hilaire, 1803)	Olmos 1993
INVERTEBRATES		
Scolopendridae	*Scolopendra* sp.	Moura *et al.* 2015
Formicidae	*Dinoponera quadriceps* Kempf, 1971	Ribeiro *et al.* 2011

The behavioral records presented herein confirm various predators of *A. ocellifera,* in addition to providing detailed descriptions of the sequence and duration of the observed behavioral events.

The first predation event was recorded by EFM on 29 February 2020, at 09:20 h, in a rural area (06°35'38" S, 37°37'26" W) located in the municipality of Paulista, state of Paraíba, northeastern Brazil. A collared falcon, *Falco femoralis* (Temminck, 1822) (Falconidae), captured a small individual of *A. ocellifera.* During the observation, the time of capture could not be determined. The bird was spotted in flight, already carrying the lizard. It then perched on a fence stake, at which time it was photographed (Figure 1A). In an attempt to approach the falcon to take a more detailed photo, the bird flew away.

The second predation event was recorded by JDS, on 27 June 2020, at 10:28 h in a rural area (07°30'58" S, 37°17'10" W) located in the municipality of Brejinho, state of Pernambuco, northeastern Brazil. An adult *Oxyrhopus trigeminus* (Duméril, Bibron and Duméril, 1854) (Dipsadinae, Colubridae) (sex not determined; total length around 400 mm) was observed in rocky soil, where it was in the process of swallowing head first an *A. ocellifera* (total length around 150 mm) (Figure 1B). After 86 s, the snake left with the lizard in its mouth and stopped in a more sheltered location among leaves and rocks, where it finished ingesting the lizard after 5 min, taking 2 min to ingest the body and 3 min to ingest the tail.

The third predation event was recorded by CJSB on 03 July 2020 at 14:10 h and was observed in a rural area (07°22'40" S, 37°11'24" W), located in the municipality of Itapetim, state of Pernambuco, northeastern Brazil. A *Gampsonyx swainsonii* (Vigors, 1825) hawk (Accipitridae) was observed as it captured a small individual of *A. ocellifera.* At the time, the lizard was still struggling, but it was quickly subdued by the bird with pecks to the neck and head region (Figure 1C). The entire process of subjugation and prey ingestion (documented in photographs) lasted 30 min, starting at 14:11 h and ending at 14:41 h. During the ingestion of prey, the predator discarded some internal organs (probably the stomach), but avidly ate the intestines, as well as the other internal and external structures. After the predation event, the bird cleaned a small piece of meat from its beak on several branches before flying away.

The fourth predation event was recorded by JVAM on 24 January 2021 at 08:28 h in an area of Caatinga converted into pasture at the rural property of Sítio Castelo dos Montes (06°42'03" S, 36°56'45"W), located in the municipality of Ouro Branco, state of Rio Grande do Norte, Northeastern Brazil. A juvenile *A. ocellifera* was captured by a cattle tyrant *Machetornis rixosa* (Vieillot, 1819) (Tyrannidae) while foraging inside a bush. The bird initially took the lizard to a branch off the ground, where it subdued it by holding the lizard's head with its beak and hitting the lizard's body against the branch. The bird then took the lizard to the ground, where it ripped off the tail and parts of the limbs (Figure 1D), repeating the same movement of holding and hitting it. After that, another *M. rixosa* tried to steal the prey and both flew away. It was not possible to observe if they ingested the entire lizard. The process was fast and lasted seconds after the lizard was beaten and became immobile.

Top predators can act as structuring agents and biodiversity indicators in some ecosystems or regions, but not in others. The most common pattern is the effectiveness of the top predator; however, secondary predators appear depending on the species and context (Sergio *et al.* 2008, Estes *et al.* 2011, Pokharel 2020). Thus, our records corroborate Sergio *et al.* (2008) because birds of prey are globally distributed predators and have great potential for structuring biological communities (Sergio *et al.* 2008).

An extensive literature addresses how predators choose their prey (Pokharel 2020). Falconiformes employ various hunting strategies, many of which are associated with catching prey at high speeds. On the other hand, *M. rixosa* has an essentially insectivorous diet, and only one record in the literature describes vertebrate predation. The two predation events we observed

Figure 1. Predation of *Ameivula ocellifera* by (**A**) *Falco femoralis,* (**B**) *Oxyrhopus trigeminus,* (**C**) *Gampsonyx swainsonii,* and (**D**) *Machetornis rixosa.*

may be opportunistic (Martins *et al.* 2015).

Several records of saurophagy by the snake *O. trigeminus* are based on an analysis of stomach contents (Vitt and Vangilder 1983, Rocha *et al.* 2005, França *et al.* 2008, Alencar *et al.* 2012, Mesquita *et al.* 2013, Coelho *et al.* 2019). Only one study describes observations in nature (Mikalauskas *et al.* 2017). Although the lizard *A. ocellifera* is mentioned in the literature as prey of this snake, this is the first record describing the predation event.

We compiled a list of 20 predators of *A. ocellifera* based on this study and the literature. Snakes ($N = 9$) were the most common predators of *A. ocellifera* (34.6% of predators reported; Table 1), followed by birds ($N = 8$; 30.8%), lizards ($N = 6$; 23.1%), invertebrates ($N = 2$; 7.7%), and mammals ($N = 1$; 3.8%).

Our observations corroborate literature records that show snakes and birds are predators

of *A. ocellifera.* Our observations further indicate predation by three species of birds previously unknown as predators of *A. ocellifera.* Although studies of stomach contents have recorded the snake *O. trigeminus* as a predator of *A. ocellifera,* we provide the first description of the snake's predatory behavior in nature. *Ameivula ocellifera* is consumed by a variety of animals, which makes this species a significant link in the food chain.

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Short Communication

A failed predation attempt by *Chrysopelea paradisi* (Serpentes: Colubridae) on *Hemidactylus frenatus* (Squamata: Gekkonidae) in Borneo, Malaysia

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Keywords: Feigning death, play dead, reptiles, thanatosis, tonic immobility.

Palavras-chave: imobilidade tônica, répteis, simular morte, tanatose.

Predation is fundamental to the lives of animals because it influences key aspects of fitness such as feeding, breeding, and ultimately, mortality (Humphreys and Ruxton 2018). Animals that are preyed upon develop a broad range of defense strategies, one of which is tonic immobility exhibited late in the sequence of a predation event (Humphreys and Ruxton 2018). This strategy has been called thanatosis in the literature and is described as a behavior where the animal is observed feigning death. This behavior is present in several taxonomic groups, including reptiles (Honma *et al.* 2006, Caro 2014). Humphreys and Ruxton (2018) suggested that a better description of this behavior would be tonic immobility (TI), by not assuming a predatory response or underlying mechanisms leading to a predatory response. We agree with the authors, and herein will refer to TI to describe our observations. This behavior is effective against predators that need movement as a cue for further handling of their prey (Toledo *et al.* 2011).

TI is a defensive mechanism considered the last option after the physical contact between the predator and its prey (Humphreys and Ruxton 2018). It has been described in a wide range of taxa, although often anecdotally (Humphreys and Ruxton 2018). It is widespread among vertebrates, observed in mammals, birds, reptiles, amphibians, and fish (Caro 2014, Humphreys and Ruxton 2018). Among lizards, TI has been described for Crotaphytidae (Gluesing 1983), Dibamidae (Torres-Cervantes *et al.* 2004), Gymnophthalmidae (Muscat *et al.* 2016, Machado-Filho *et al.* 2018), Liolaemidae (Rocha 1993, Santos *et al.* 2010), Scincidae (Langkilde *et al.* 2003, Patel *et al.* 2016), Tropiduridae (Galdino and Pereira 2002, Gomes *et al.* 2004,

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Kohlsdorf *et al.* 2004, Bertoluci *et al.* 2006) and Gekkonidae (Parmar 2020, Costa-Anaissi *et al.* 2020). The exhibition of TI, however, may vary among lizards, depending on age, sex, reproductive period, anatomical structures, and other features (Segovia *et al.* 2019).

Reptiles are highly diversified in Borneo, Malaysia, including several species that are widespread throughout southeastern Asia and others that are endemic (Das 2010). The Paradise Flying Snake (*Chrysopelea paradisi* Boie, 1827) and the Spiny-tailed House Gecko (*Hemidactylus frenatus* Duméril and Bibron, 1836) are examples of the first group. *Hemidactylus frenatus* occurs in southeastern Asia and has been introduced to several islands, as well as to Australia, America, and Africa (Das 2010). It is the planet's most widespread lizard, having been spread around the world following human activity (Cornelis *et al.* 2018). This small gecko measures about 67 mm SVL. It has a grayish-brown or dusky brown dorsum, sometimes with darker markings, and a uniformly cream or light beige venter (Das 2010). *Hemidactylus frenatus* is nocturnal (Daniel 2002, Parves and Alam 2015) or, at least, predominantly nocturnal (Neogi and Islam 2017). They generally hide during the day (Parmar and Tank 2019), but some diurnal activity has been observed, including basking and foraging (Nordberg 2019). This diurnal activity explains why *H. frenatus* is preyed upon by several diurnal species, including birds (e.g. Rojas-González and Wakida-Kusunoki 2012, Yannarella and Abarca 2017, Puri and Joshi 2021) and primates (e.g. Mata-Silva *et al.* 2013). *Hemidactylus frenatus* may occur naturally in forested areas, but it is usually associated with human habitations (Parmar and Tank 2019). It is often found inside buildings feeding on insects (Neogi and Islam 2017).

Chrysopelea paradisi is a slender, colorful arboreal colubrid snake, common from lowlands to 1500 m in elevation (Das 2010). The species occurs in Southeastern Asia, including Borneo (Das 2010). This diurnal snake is found in primary and secondary forests, tree-shaded gardens, and old wooden houses (Mačát *et al.* 2016). One unique feature of *C. paradisi,* shared with other species in the genus, is that it is able to glide, moving up to 100 m through the air (Socha 2002, Das 2010, Holden *et al.* 2014).

The diet of this snake consists of lizards, amphibians, small birds, small bats, and small invertebrates (Malkmus *et al.* 2002, Baker and Lim 2012, Chan and D'Rozario 2013, Goh 2019, Maglangit *et al.* 2021), but mainly geckos (Das 2010), including *H. frenatus* (Morgany 2018, Tan and Chapman 2019). In this note, we report a failed predation event by an individual of *C. paradisi* on *H. frenatus* and give some possible explanations for the lizard's escape.

On 26 October 2011 at 14:35 h we observed a *Chrysopelea paradisi* that captured a *Hemidactylus frenatus* (Figure 1). The observation was made approximately 50 m from the headquarters area of Bako National Park in Sarawak, Borneo, Malaysia (01°42'56" N, 110°26'36" E, 11 m a.s.l.). The snake (about 60 cm long) bit the gecko and threw two coils of its body around the anterior torso of the lizard. The snake remained almost stationary, apparently constricting the gecko, for about 2–3 min. Movements of the gecko became erratic and less frequent until they ceased. The snake released the coils, changed position, and moved to the gecko´s head to begin consuming it headfirst. At that time the gecko was not struggling and looked dead, but some seconds after the gecko was freed from the coils, it fled. After that, the snake climbed a nearby wooden pole and left (Figure 2A). The gecko ran to the grass and climbed onto a wooden structure where it perched for several minutes near a hole (Figure 2B). The snake did not follow it. We watched the gecko for more than 10 min until it moved higher up where we could no longer see it. Although this snake is believed to be mildly venomous (Tan *et al.* 2012), the lizard showed no evidence of envenomation.

Tail loss and escape are the most common defensive behaviors reported for lizards (Greene 1988, Autumn and Han 1989, Costa-Anaissi *et*

Figure 1. *Chysopelea paradisi* "constricting" a *Hemidactylus frenatus* in Bako National Park, Borneo, Malaysia.

al. 2020), but the behavior we observed *H. frenatus* perform was tonic immobility. Other observations have been made on *C. paradisi* capturing lizards that defended themselves. A skink, *Lamprolepis smaragdina* (Lesson, 1829), was able to free itself several times, but was caught by the snake with a bite on its leg (Gaulke 1986). A gecko, *Gekko horsfieldii* (Gray, 1827), was bent into a horseshoe shape with its head and tail in the mouth of the snake, and after 5 min in this position, the snake opened its mouth and the gecko escaped (Mačát *et al.* 2016). Our observation is the first report of a lizard performing TI as an escape strategy to avoid predation by *C. paradisi.* The behavior seems common among lizards in the genus *Hemidactylus,* but it has only been reported while the lizard was being handled (Costa-Anaissi *et al.* 2020, Parmar 2020). Our report is the first to show *H. frenatus* performing TI in a predatory context.

Figure 2. (**A**) *Chysopelea paradisi* after it failed to predate a *Hemidactylus frenatus.* (**B**) The gecko escaped and went up a nearby wooden structure. Bako National Park, Borneo, Malaysia.

Tonic immobility (TI) is normally triggered in situations where the prey perceives that death is a likely outcome. The behavior occurs during the final stages of predation and is elicited by a strong and sustained tactile stimuli consistent with having been caught by a predator (Rogers and Simpson 2014). Though widespread, few observations of TI in reptiles from southeastern Asia have been reported; for example, only seven observations have been reported in India (Patel *et al.* 2016). The occurrence of TI in reptiles in general is poorly documented. Additional studies of this behavior and its role in survival of the animal are needed (Patel *et al.* 2016, Costa-Anaissi *et al.* 2020). *Hemidactylus frenatus* is a common gecko in southern Asia, and although this is the first case of TI in a predatory context for this species, it would be a good subject to investigate this behavior at the population level.

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SHORT COMMUNICATION

Repertoire of antipredator mechanisms in the Brazilian toad *Frostius pernambucensis* (Anura: Bufonidae)

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Keywords: Amphibia, body inflation behavior, defensive behavior, Frost's Toad, legs interweaving.

Palavras-chave: Amphibia, comportamento defensivo, comportamento de inflar o corpo, entrelaçamento de pernas, sapo-de-frost.

Anuran amphibians, along with the diversification of species, established themselves in practically all regions of the globe (except Antarctica and most of the oceanic islands (Duellman and Trueb 1994, Frost 2021). These animals, throughout all stages of life, correspond to an important element of the food chain, serving as predators and prey for various vertebrate and invertebrate groups, both in aquatic and terrestrial environments (Duellman and Trueb 1994, Verburg *et al.* 2007). They have developed several morphological, physiological and behavioral adaptations to overcome their many predators (Duellman and Trueb 1994, Verburg *et al.* 2007, Ferreira *et al.* 2019). These defensive strategies can be exhibited singly or in combination, depending on the factors underlying predator and prey interactions (Duellman and Trueb 1994).

Most antipredator mechanisms expressed by anurans can be observed in the species that live in the Atlantic Forest (Haddad *et al.* 2013, Ferreira *et al.* 2019), an ecoregion considered a biodiversity hotspot worldwide (sensu Myers *et al.* 2000). Part of this is due to the high diversity of species, which are currently known more than 600 amphibian species, of which around 80 percent are endemic to this ecoregion (Rossa-Feres *et al.* 2017). With this said, the basic aspects of the natural history of many species remains poorly understood, including defensive repertoires (Ferreira *et al.* 2019).

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Among these species, *Frostius pernambucensis* (Bokermann, 1962) (Figure 1) is a small bufonid toad endemic to the Atlantic Forest of Northeastern Brazil (states of Pernambuco, Alagoas, Sergipe and the northern region of the state of Bahia; Frost 2021). Some defensive behaviors for this species have been listed by Haddad *et al.* (2013) and Ferreira *et al.* (2019) such as mechanisms to avoid detection (camouflage) or to avoid predation (aposematism, body inflation, contraction, death feigning, counterattack, and poisonous secretions). Some of these behaviors are associated with a variety of postures in order to play dead or to break-up the animal's characteristic silhouette promoting the predator's disinterest (Toledo and Haddad 2009, Ferreira *et al.* 2019). Additionally, these movements may maximize the display of the aposematic coloring of the belly and limbs. Here, we update the repertoire of defensive behaviors of *F. pernambucensis,* including one previously unregistered mechanism for the species: leg interweaving, being the first record for a representative of the Bufonidae family.

The records were taken during two field expeditions at the Estação Ecológica de Murici (ESEC de Murici), municipality of Murici, state of Alagoas, Northeastern Brazil (09°13'09.6" S, 35°52'44.2" W; datum WGS84; 562 m a.s.l.). On 20 March 2021 at 17:40 h, we found a *F. pernambucensis* individual in a basal axil of a terrestrial bromeliad of the species *Aechmea leptantha* (Harms) Leme and J.A. Siqueira. After the individual failed to escape capture, it displayed a sequence of defensive behaviors. Initially, we realized that the individual was performing a contraction display. Briefly, the individual remaining immobile, ventral side upwards, with their fore and hind limbs contracted close to the body as to display the yellowish color of the belly (Figure 1A). When placed on a leaf, the individual performed the "leg interweaving" behavior (Figure 1B–C). This behavior consisted of an irregular movement of the hind limbs, where the individual kept their limbs crossed in an "X" shape while remaining ventral side upwards. During the leg interweaving behavior, the individual kept its forelimbs bent, close to its body with its eyes closed, tilting its head backwards. After a few seconds, the individual returned to its original position, perched on the leaf.

The second record was taken on 27 July 2021 at 21:30 h. We found an individual vocalizing on a tree leaf 1.5 m above the ground. We manually relocated the individual from the leaf to a nearby area, to avoid immediate escape after the contact, where we stimulated it with soft touches in order to obtain a defensive response. First, the toad performed death feigning behavior while remaining immobile, with its entire body in contact with the ground. In death feigning, the anuran assumes a posture in which it remains immobile even when touched, appearing to be dead, keeping its fore and/or hind limbs loose, causing the predator to lose interest (Toledo *et al.* 2010). While in this state the individual had notably slow reflexes: for example, when we stretched its hind limb, the animal did not immediately retract it, but instead kept it outstretched for about five seconds (Figure 1E). When we touched it again, the animal returned the limb close to its body. Following this display, the individual performed body inflation behavior every time we approached it, inflating its body and tilting its head forwards (Figure 1F). This behavior occurs when the anuran fills its lungs with air, increasing in size (Toledo *et al.* 2011). This behavior can make it difficult to attack or handle the anuran, and it can also scare off a potential predator (Caro 2014, Ferreira *et al.* 2019). After taking photographic records, the animal was returned to the same location where it was found. None of the individuals were collected.

Contraction and death feigning behaviors are relatively well-documented in bufonid species, mainly from the genus *Rhinella* and may be associated with aposematic coloration, such in *Melanophryniscus* and *Atelopus* genus (Toledo *et al.* 2010, Ferreira *et al.* 2019), as observed in *Frostius pernambucensis.* Both

Figure 1. Defensive behaviors registered for *Frostius pernambucensis*. Individual registered in the first expedition, presenting contracting (**A**), legs interweaving or limbs interweave (**B–C**), and undoing the latter behavior (**D**). Individual registered in the second expedition presenting death feigning behavior (**E**) and body inflation behavior (**F**). Photos: MJMD (A–D) and Marcio Campelo (E–F).

behaviors can be displayed after an approach of a potential predator or after being handled (Toledo *et al.* 2010). However, contraction is mainly associated with toxic species and can be displayed even after subjugation by a predator (Toledo *et al.* 2010). Once swallowed, the contracted anuran protects its vital body parts and produces noxious secretions into the predator's digestive tract, inducing its regurgitation (Sazima 1974, Toledo *et al.* 2010). In *F. pernambucensis,* the handling of the animal induced the display of the contraction behavior. The individual may have interpreted it as being swallowed and contracted its limbs to protect the vital parts. As for death feigning, this behavior was induced by small touches on the toad and may have been performed as an attempt to make the predator lose interest.

With respect to leg interweaving, as far as we know, there has been no mention in the literature of this behavior for *F. pernambucensis* or any other representative of Bufonidae. This defense mechanism is poorly-documented (Ferreira *et al.* 2019) and is known to occur in only six anuran families: Craugastoridae (1 species); Hylidae (3); Hyperoliidae (1); Leptodactylidae (1); Phyllomedusidae (1); and Ranidae (1) (Channing and Howell 2003, Gally *et al.* 2014, Lourenço-de-Moraes *et al.* 2014, Ferreira *et al.* 2019, Rojas-Padilla *et al.* 2019, Souza *et al.* 2020). Distinguishing it from other anuran species, who keep their hind limbs intertwined dorsally and the body turned dorsally, *F. pernambucensis* maintained its body in a ventral position during the leg interweaving display, displaying the yellowish color of its belly. This behavior, associated with aposematic coloration on the ventral region of the body, has only previously been shown in the African hyperoliid tree frog *Hylambates keithae* (Schiøtz, 1975) (Channing and Howell 2003). According to Toledo *et al.* (2011), leg interweaving can occur synergistically with other defense mechanisms such as the display of disruptive and/or aposematic color patterns, or toxic substance secretions. In *F. pernambucensis,* this behavior occurred simultaneously with the contraction of anterior limbs and synergistically exposing its aposematic colors. The presence of toxic secretions was not observed, although this mechanism has been described for the species (Ferreira *et al.* 2019).

Aposematic coloration can aid in predator avoidance, but the flashy color can sometimes have an adverse effect, increasing an individual's detectability in the environment (Hall *et al.* 2013, Ferreira *et al.* 2019, Rößler *et al.* 2019). A way to compensate for this is to have two strategies: camouflage and aposematism, the chosen strategy is dependent on body position (Barnett *et al.* 2017) as in *F. pernambucensis.* In these cases, the conspicuous feature is exposed when the animal moves (Channing and Howell 2003, Hall *et al.* 2013, Rößler *et al.* 2019), such as with the exposed aposematic coloration on the hands and feet of *Atelopus spumarius* Cope, 1871 (Rößler *et al.* 2019) and can be observed, similarly, in *F. pernambucensis.* We question whether the exposure of these colors in *F. pernambucensis* also has the same effect of increasing its detectability for some of its visually-oriented predators while moving, a behavior that exposes these parts, or if these animals benefit from this exposure. Thus, we show that more studies are needed to better investigate and elucidate these issues inherent to the natural history of this species.

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Short Communication

First record of necrophilia in *Smilisca baudinii* (Anura: Hylidae) from Mexico

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Palavras-chave: amplexo, comportamento reprodutivo, hilídeo, necrogamia.

Necrophilia, also known as necrogamy (Bettaso *et al.* 2008), thanatophilia (Patel *et al.* 2016), and Davian behavior (Dickerman 1960), is a form of reproductive behavior in which a living specimen (usually a male) attempts to copulate with a dead conspecific (usually a female). It has been reported in all major extant groups of tetrapods (Caldeira-Costa *et al.* 2010). Among anurans it has been reported in at least 37 species from six families: Ascaphidae (one species), Bombinatoridae (1), Bufonidae (15), Hylidae (8), Leptodactylidae (1), and Ranidae (11). This list suggests that this behavior occurs more often in frogs with explosive reproduction (see Pintanel *et al.* 2021 and Costa-Campos *et al.* 2021 for recent reviews).

Necrophilia generally does not result in successful reproduction and may represent a waste of time and energy for the males (reviewed in Marco and Lizana 2002). However, the case of *Rhinella proboscidea* (Spix, 1824), in which the male can promote the expulsion and fertilization of the oocytes from dead females by compressing their abdominal cavities with his arms, was hypothesized as "functional necrophilia" because it could minimize the losses of both sexes during the explosive reproduction events and favors the strongest and most persistent males (Izzo *et al.* 2012).

Here we provide the first report of necrophilia in the common Mexican tree frog *Smilisca baudinii* (Duméril and Bibron, 1841). This hylid reaches a snout–vent length of 76 mm in males and 90 mm in females, has a wide and flat head indistinct from the body, and is distinguished from other species by having a row of warts along the lower part of the arm (Lee 2000, Tunstall 2021). Its wide geographic range encompasses several biogeographic provinces, ranging from the extreme southcentral United States to southeastern and southwestern Costa Rica (Lee 2000, Savage 2002, Powell *et al.*

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2016). *Smilisca baudinii* usually reproduces between the months of June and October (Duellman 1970) in ephemeral ponds following heavy rains (Malone 2004).

On 08 July 2021 at approximately 07:30 h, in a dry forest setting located in Ejido el Tablón, municipality of El Rosario, Sinaloa, Mexico (23°04'57.84" N, 105°59'25.22" W; 68 m a.s.l.), we found two males in amplexus with a dead female on the shore of an ephemeral grassy pond (Figure 1). One male was found in axillary amplexus, but directed to the flank of the female; while the other male was found amplexing the female´s hind legs. We also noticed that the female was dead and missing the upper part of its snout (for unknown reasons) and that its abdomen contained abundant oocytes although none were expelled.

Necrophilic behavior has been reported previously in hylids with explosive reproduction (Pintanel *et al.* 2021). Thus, it is not surprising that necrophilia occurs in *Smilisca baudinii,* a species with explosive breeding behavior (Donnelly and Guyer 1994). It is also important to mention that interspecific amplexus involving *S. baudinii* and other species has been previously reported (e.g., Streicher *et al.* 2010, Heyborne *et al.* 2018, Vásquez-Cruz *et al.* 2019). These reports of misdirected amplexus (interspecific and necrophilia) are considered maladaptive (Ayres 2010) and suggests little or no ability to recognize viable potential reproductive partners when reproductive pressure is high (Marco and Lizana 2002).

The absence of egg deposition does not preclude functional necrophilia in *Smilisca baudinii* since we did not continue with the observation until amplexus was completed.In addition, because the males were not found in complete interaxillary amplexus, we believe their positions would have hindered the expulsion of the oocytes.

Acknowledgments.—We thank Nefatali Camacho for cataloging the photograph (Figure 1) in the digital collection of Natural History Museum of Los Angeles County.

Figure 1. Two male *Smilisca baudinii* in amplexus with a dead conspecific female along the shore of grassy ephemeral pond, Sinaloa, Mexico (LACMPC 2753).

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Short Communication

Novel breeding habitat, oviposition microhabitat, and parental care in *Bokermannohyla caramaschii* (Anura: Hylidae) in southeastern Brazil

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Keywords: Atlantic Forest, ecological plasticity, egg guarding, reproductive strategy, temporary pool.

Palavras-chave: estratégia reprodutiva, guarda de ovos, Mata Atlântica, plasticidade ecológica, poça temporária.

Selection of novel breeding habitats and oviposition microhabitats are influenced by the plasticity of a species to adapt to habitat changes (Silva and Giaretta 2008, Campos *et al.* 2013). The mountainous region of the Brazil's Atlantic Forest provides a wide range of habitats and microhabitats. The Atlantic Forest has changed from continuous forest to smaller and isolated remnants, requiring anurans to cross over or even reproduce on human-modified habitats (Ferreira *et al.* 2016, Mageski *et al.* 2018).

Breeding habitat and oviposition microhabitat influence parental care in anurans (Storti *et al.* 2019), because parental care influences the survivorship of offspring (Furness and Capellini 2019). Anurans exhibit a variety of parental care strategies, from egg guarding to tadpole feeding (Wells 2007). More complex parental care requires more energy expenditure and physiological and morphological adaptations. Egg and tadpole guarding are present in 6% of the anurans in the world (Furness and Capellini 2019). Parental care is important for species that breed in water bodies with reduced capability for rainwater storage, such as bromeliads, bamboo, and cattle footprints (Gally and Zina 2013, Ferreira *et al.* 2019).

Endemic to the Atlantic Forest, the treefrog *Bokermannohyla caramaschii* (Napoli, 2005) is distributed across mountainous forests above 650 m in the states of Minas Gerais and Espírito Santo, southeastern Brazil (Napoli 2005, Frost 2020). Females lay egg masses in rocky cavities backwater areas of streams (Pezzuti *et al.* 2015). The present study characterizes a new type of

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breeding habitat and oviposition microhabitat, and, in addition, describes parental care in *B. caramaschii.*

The study was carried out in Parque Estadual do Forno Grande (PEFG; 20.311951° S, 41.64949° W, WGS 84; 1200 to 1535 m a.s.l.), municipality of Castelo, in a mountainous region of the state of Espírito Santo, southeastern Brazil. The PEFG has 913 hectares of Dense Montana Ombrophylous Forest with rough terrain that reaches up to 2039 m a.s.l. (Simonelli and Fraga 2007).

We conducted nocturnal sampling in October 2016 using active visual and auditory searches. We measured and characterized the oviposition microhabitat and breeding habitat of *B. caramaschii,* including diameter, depth, and distance to the nearest stream. We collected two specimens (MBML 10618 and MBML 11072; collection permits: IEMA 52838-1; SISBIO 50402) and deposited them at the Museu de Biologia Mello Leitão (MBML) from Instituto Nacional da Mata Atlântica, Santa Teresa, Espírito Santo state, Brazil.

We defined used pools by having spawning eggs and/or tadpoles. Those with eggs or tadpoles were defined as "used pools". We evaluated the differences between used and unused oviposition microhabitats regarding diameter, depth, and distance to the nearest stream by using T-tests. We ran the Shapiro-Test Normality Tests and Two Sample T-tests using the package "vegan" 2.5-6 in the R 3.6.1 (R Core Team 2019).

We found *Bokermannohyla caramaschii* at two sites (Site A and B) at PEFG. Site A had adults, egg masses, and tadpoles in water-filled rocky cavities (usual oviposition microhabitat) along a stream (usual breeding habitat; Figure 1). Site B had adults, egg masses, and tadpoles in pools formed in cattle footprints (novel oviposition microhabitat) near a stream in a pasture (novel breeding habitat; Figure 2).

Site B (2.7 × 3.9 m) had 19 pools, of which nine (47%) pools had eggs and/or tadpoles (used pools) and 10 (53%) pools had no eggs and/or tadpoles (unused pools) of *B. caramaschii.* The used pools were larger (t = -2.10; df = 14.81; p < 0.05) and deeper (t = -3.17; df = 13.23; p < 0.05) than unused pools (Figure 3). The used pools had a mean diameter of 16.2 ± 3.7 cm and a mean depth of 6.4 ± 3.1 cm. The unused pools had a mean diameter of 11.9 ± 4.5 cm and a mean depth 2.6 ± 1.9 cm. However, both used and unused pools were approximately the same distance from the stream (t = 1.21; df = 13.40; p = 0.88). The used pools were 16.6 ± 6.2 cm from the stream and unused pools were 22.1 ± 12.6 cm (Figure 3).

At site A, adults of *B. caramaschii* were near egg masses (i.e., egg guarding; N = 2) and tadpoles (i.e., tadpole guarding; N = 2) in rocky cavities. Also at site A, carcasses of adults (N = 2) were preyed upon near a spawning site (Figure 4). At site B, no adult was observed near the eggs or tadpoles.

This report is the first to describe egg deposition by *B. caramaschii* in cattle footprints in a pasture. In addition, it is the first record of species of *Bokermannohyla* spawning in temporary pools. The congeners *B. napolii* Carvalho, Giaretta, and Magrini, 2012 and *B. izecksohni* (Jim and Caramaschi, 1979) also oviposit in water-filled backwater pools and rocky cavities near streams. *Boana pardalis* (Spix, 1824) and *B. faber* (Wied-Neuwied, 1821) are the most closely related species that use cattle footprints for oviposition (Bokermann 1968, Andrade *et al.* 2017). Our data demonstrate that *B. caramaschii* has the ability to utilize human-modified breeding habitats and oviposition microhabitats.

The selection of deeper and larger pools shows the ability of *B. caramaschii* to influence the survival of the offspring. Deeper and larger pools probably reduce the risk of desiccation because these pools may store water for a longer time compared to small shallow pools. This selection of microhabitats probably increases the chance of reproductive success. Oviposition in temporary water bodies such as cattle footprints and rock pools reduces the risk of predation of offspring compared to permanent water bodies

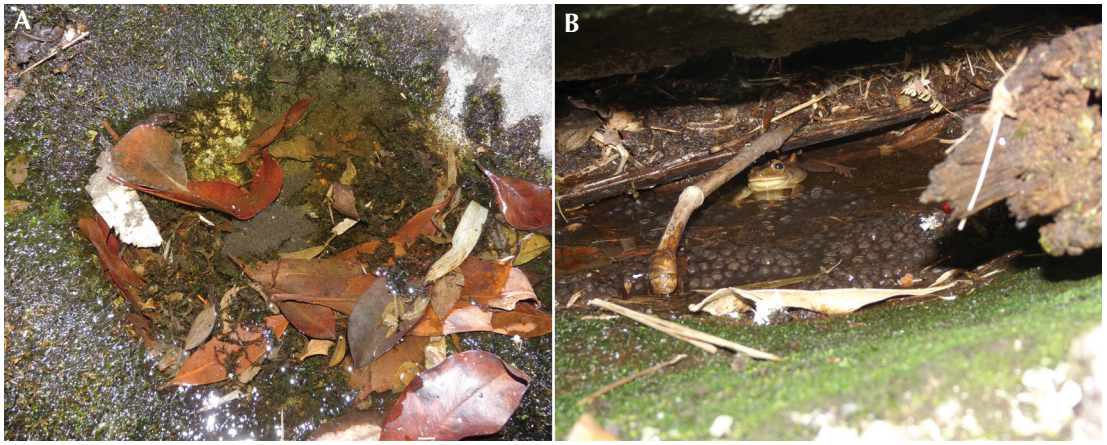

Figure 1. (**A**) Spawning of *Bokermannohyla caramaschii* in a water-filled rocky cavity (usual microhabitat) along a stream (usual habitat) and (**B**) adult guarding eggs and tadpoles (parental care) at Parque Estadual do Forno Grande, state of Espírito Santo, southeastern Brazil. Photos: TSS.

Figure 2. (**A**) Pools formed by cattle footprints (novel microhabitat) in a pasture (novel habitat) and (**B**) close-up of a pool with eggs and tadpoles of *Bokermannohyla caramaschii*. Photos: TSS.

where the density of fish increases predation of larvae (Rieger *et al.* 2004).

We provide the first report on parental care for species of *Bokermannohyla.* Egg guarding is the most common parental care in anurans, possibly because this behavior requires less energy compared to other types of parental care (Wells 2007). Egg guarding decreases the rate of egg predation, thus increasing reproductive success (Furness and Capellini 2019). *Boana faber* oviposits in temporary pools, and also displays egg and tadpole guarding (Martins *et al.* 1998). However, parental care can also increase the risk of predation of the caring parent because the parent remains immobile near the offspring (Clutton-Brock 1991).

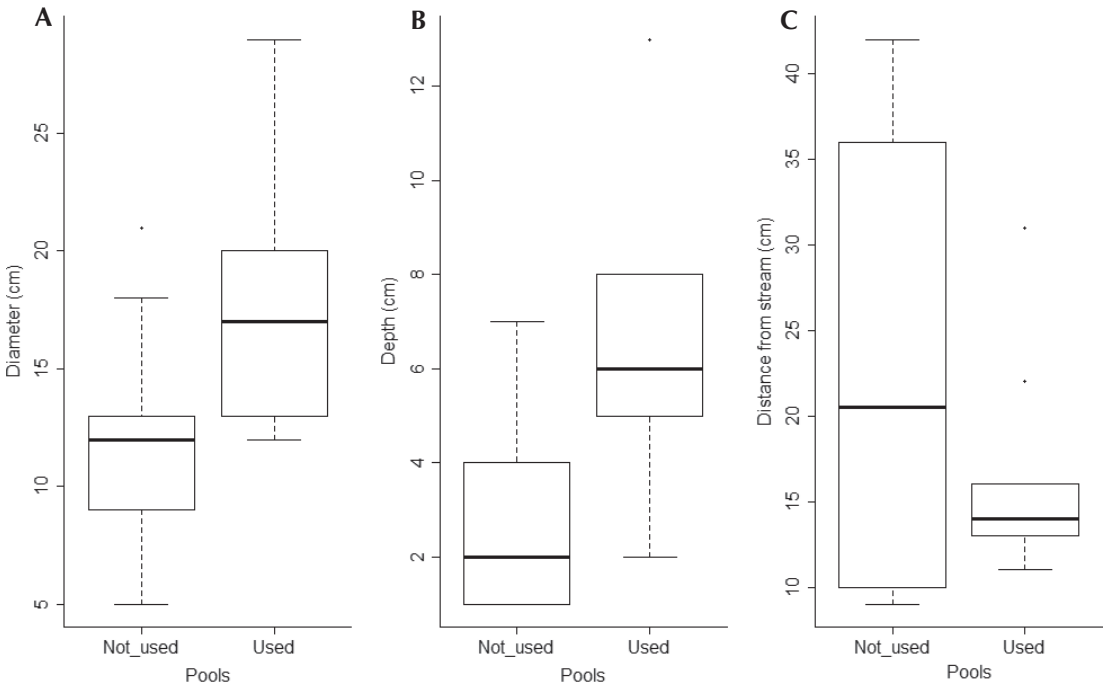

Figure 3. (**A**) Diameter, (**B**) depth and (**C**) distance from stream between non-used and used cattle footprints by eggs and tadpoles of *Bokermannohyla caramaschii*.

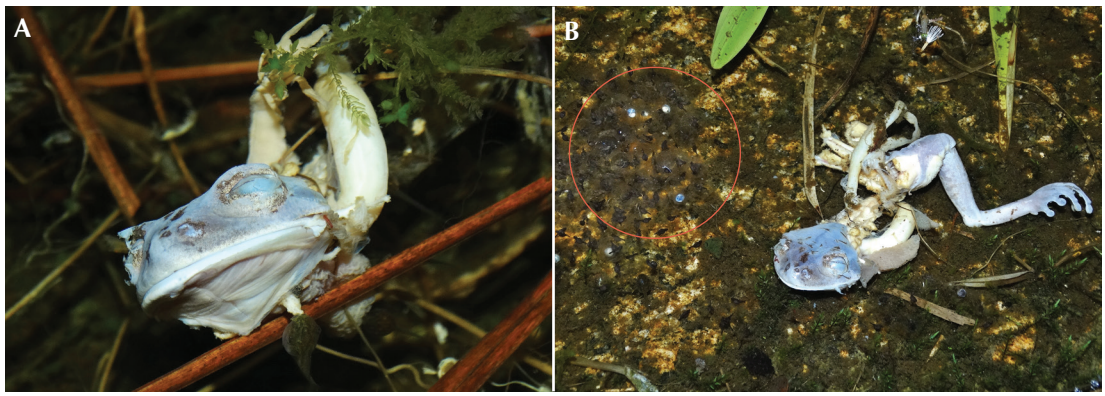

Figure 4. (**A**) Carcass of *Bokermannohyla caramaschii* preyed upon in a water-filled rocky cavity along a stream. (**B**) Carcass near a spawning. Photos: ATM.

Our study contributes to understanding reproductive plasticity of microhabitats and habitats used during egg deposition by *B. caramaschii*. It is not possible to determine the environmental pressure for *B. caramaschii* using a new oviposition microhabitat and habitat. Nevertheless, the use of oviposition microhabitat and habitat does not occur randomly and is probably critical to avoid desiccation of eggs and tadpoles. Egg and tadpole guarding are likely to

increase survival of offspring. We recommend that future studies evaluate the influence of habitat and microhabitat selection on development and survival of eggs and tadpoles of *B. caramaschii.*

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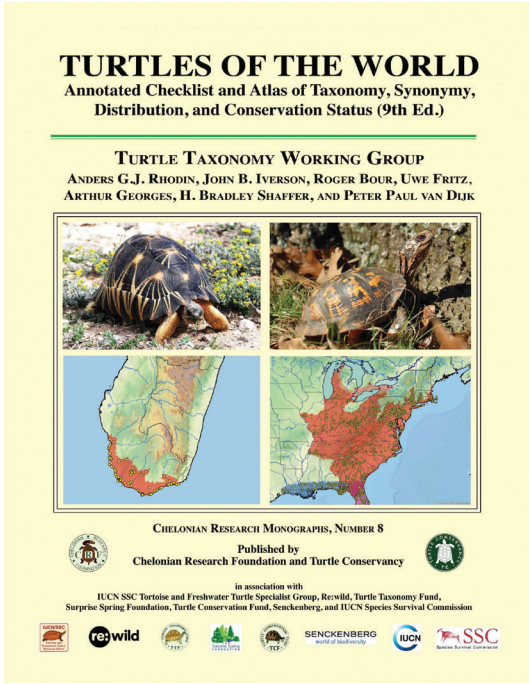
Turtle Taxonomy Working Group (Rhodin, A. G. K., J. B. Iverson, R. Bour, U. Fritz, A. Georges, and H. B. Shaffer). 2021. **Turtles of the World Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status** (9th Ed.). *Chelonian Research Monographs 8:* 1–472. DOI:10.3854/crm.8.checklist.atlas.v9.2021.



Turtles are among the smallest of vertebrate groups in terms of modern diversity, but they are also among the most endangered. More than half, 51% to 56% of taxa are considered threatened or endangered by various government and international agencies. Some 10 modern species group taxa (species or subspecies) out of 486, have become extinct since 1500 CE. Advancement of study and conservation of all members of this group requires that an up-to-date, well organized, and thoroughly researched checklist of nomenclature is easily available to all turtle biologists. The Turtle Taxonomy Working Group (TTWG) has labored for the last four years on a revision of the previous checklist (TTWG 2017). The 9th edition of the work is a detailed and complex volume that easily accessed for free and will greatly facilitate the efforts of all cheloniologists.

The members of the Order Testudines are ancient survivors, with a fossil history spanning back to the Triassic, some 230 million years. They have survived two mass extinctions, one at the end of the Triassic (the great dying), and a second at the end of the Cretaceous (KT boundary). Their survival through periods during which some 80% of all life went extinct is testament to their survival abilities. Their ecological role as mostly omnivorous, thoroughly armored generalists, may have helped, although some are highly specialized. They have diversified many times in their long history and are one of the few living vertebrate groups in which the number of described fossil taxa greatly outnumbers the living ones. Living turtles are clearly the product of hundreds of millions of years of evolution, a treasure trove of living fossils.

The 9th edition of the Turtles of the World Checklist is a well presented, logically ordered volume presenting all the essential baseline information for every species and subspecies of turtle in the world. The Introduction begins with the statistics of the volume, number of taxa, percent at risk etc. Then moves on to a detailed methodology. This methodology explains how this volume was brought together, discussing nomenclatural changes and how they were derived, and new additions to this edition such as type specimens and body size. Included here are the guidelines the authors followed for introducing a taxonomic or nomenclatural change. Further they discuss how the species distributions and maps were developed. All of this is essential to a stable Checklist, one that will be used and followed. By explaining the methodology, they demonstrate the thoroughness and hence authority of the Checklist.

This 9th edition of the Checklist has been dedicated to two giants of turtle research, Peter C. H. Pritchard and Roger Bour, both of whom sadly passed away recently. Their contributions to turtle biology were vast and they were mentors to many current turtle biologists including myself. It is noteworthy that they have been honored together.

The 9th Edition of the TTWG Turtles of the World Annotated Checklist consists of accounts for all species and subspecies of turtles that have existed since 1500 CE with this cutoff deemed modern turtles. Species are treated in taxonomic groups that reveal current understanding of higher turtle relationships. Each account includes, the nomenclatural history, distribution and conservation status of the species. Numerous photographs have been added in this edition, including many important diagnostic views of plastral, carapace and head morphology, of each taxon where possible. There are tables of new taxa added since the 8th edition and names that have been synonymized, and useful discussions of the working groups methodologies and positions on several issues in nomenclature. Other important discussions on genetic pollution were also presented; it is extremely risky to release turtles of unknown origin, even into their home range. Of prime importance is the appendix of annotations (TTWG 2021:356-376), an essential part of any checklist, which adds explanation of every nomenclatural and taxonomic decision made throughout the work. This edition includes annotations from previous editions of the checklist (TTWG 2021:377-407), which are provided for relevant taxa in this volume. It is encouraged for all users of this work to read the annotations, particularly if you disagree with any of the nomenclature in the current volume.

It is important to recognize that this checklist is a synthesis of all available taxonomic and nomenclatural information that carefully conforms to the ICZN Code of nomenclature. The product of this synthesis is the generally accepted nomenclature for the community of biologists that works with and uses the presented taxonomy and nomenclature (Garnett *et al.* 2020). This community, in this case cheloniologists of any discipline, has a say in what is the accepted classification as discussed by Krell (2021). It is therefore prudent to examine this work in terms of the principles laid out in Garnett *et al.* (2020) and their subsequent papers for the development of Global Lists. As such one of the main principles was to aim for community acceptance, although not everyone will agree with every decision, this work does in general present the most widely accepted classification and taxonomy of turtles.

Another point discussed by the Global Species List Working Group (GSLWG) of the International Union of Biological Sciences (IUBS) is that checklists must be transparent. Checklist development is not a black box and it is important to document how specific decisions were made. This is the value of the annotations and the inclusion of a detailed methodology in the checklist reviewed here. It is clear for every taxon how any taxonomic decision has been reached. The dilemma faced by the authors is that it is important to have stability, but same time this must be balanced against timeliness and academic freedom. Hence names are changed judiciously to promote stability, but are changed to maintain currency if the evidence is clear (Garnett *et al.* 2020). In an effort to meet requirements of traceability, all editions of the checklist are available together online, and the annotations from all editions are included in this 9th edition.

One criticism offered about the 8th edition of the checklist from 2017, was that it offered options in highly contentious taxa. This is not the purpose of a checklist and creates instability. As such it is good to see this problem corrected in the 9th edition. A checklist is not a taxonomic or nomenclatural work per se; it is a synthesis of previous works that seeks to provide a stable classification framework. The 8th edition (TTWG, 2017) addressed these issues in annotations but did not present a single name for each taxon. An

example of this is the generic group names for certain American pond turtles, in listed as "*Emys*… or *Actinemys*…" (TTWG 2017:75). This is confusing and can lead to instability. In the 9th edition the authors have taken a better path this set of species in the genus *Actinemys* (TTWG 2021:171) with pointers to the annotations for a discussion of the issues. Stability in taxonomy is a primary purpose of checklists such as this one and this improvement in the 9th edition addresses this important issue.

A particularly useful and desirable feature of the Checklist is the highly detailed maps. These maps are for species level but show both the estimated distribution and the confirmed distribution by the presence of vouchers. These vouchers represent either museum specimens or published locality accounts for the species. For those species with subspecies the map uses color coding of the distribution to differentiate these. The *Testudo graeca* complex is an excellent example of this (TTWG 2017:297). Distribution maps can be utilized by many specialists for a large variety of reasons and are particularly necessary in Taxonomy, Conservation and Management.

Another important discussion point is a strong suggestion to researchers who are considering nomenclatural changes. They are advised to take care to carefully follow the ICZN rule changes for electronic publication (ICZN 2012) and ensure that the journal, if not print on paper, is properly registered with ZooBank and archived accordingly.

The acknowledgements of the 9th edition are large and pay tribute to the many turtle specialists that provided information, distribution information and photographs of the species they specialize in, myself included. I will acknowledge that I was consulted on a number of the species from the family Chelidae. Garnett *et al.* (2020) are clear that this is an important factor in the GSLWG assessments of checklists, as it is important that all people involved in checklist development are given credit for their contributions. This encourages further collaboration, stability and usage of the volume. In the case of this 9th edition, I note specifically that Peter Uetz was acknowledged and this is valuable as he heads the Reptile Database, the preeminent Reptile Checklist of the world of which turtles are of course a part. It is important for stability that these major checklists are in communication. Other checklists and global entities using this checklist include Wikimedia Foundation (Wikipedia and Wikispecies), Catalogue of Life and many Government entities and NGO's in a variety of countries.

I would be remiss not to applaud the authors and publishers for the way this volume is made available. It can be purchased as a hardbound copy for US$49.00 but more importantly can be downloaded in Pdf form for free from the website (https://iucn-tftsg.org/checklist/) as can all previous versions. This makes this important volume available to anyone, particularly students in countries where the prohibitive cost of text books prevents them ever seeing them. Much of the supporting literature can also be obtained in pdf form from https://iucn-tftsg.org/taxonomic-literature-database/.

Without doubt for anyone working on turtles, or thinking about doing so, this is one of the major volumes they will need. The species-specific information is invaluable and the literature cited will give you lists of the pdfs to obtain. Furthermore, the emphasis on careful documentation of taxonomic decision and adherence to the principles of the ICZN Code of nomenclature provide excellent guidance from the thoroughly experienced group of turtle researchers.

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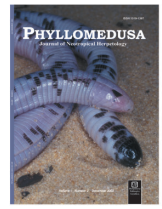
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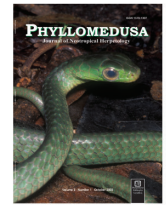
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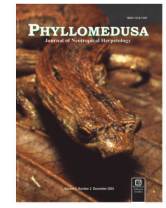
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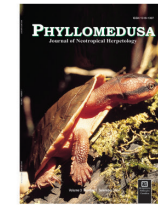
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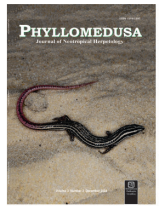
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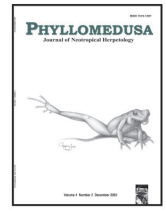
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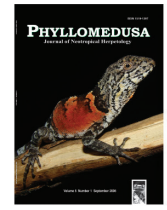
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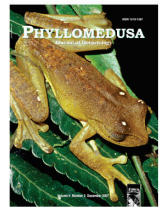
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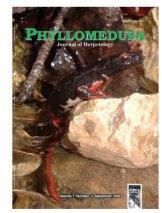
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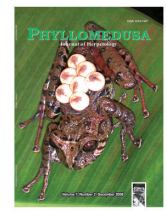
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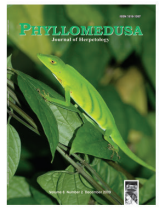
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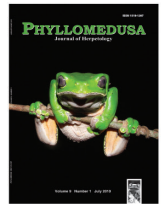
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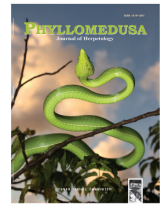
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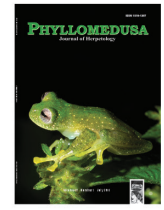
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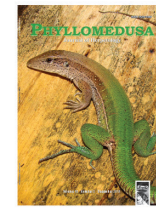
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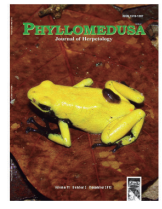
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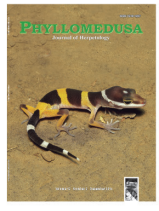
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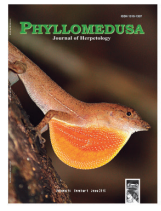
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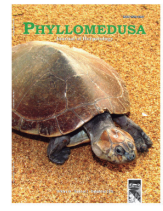
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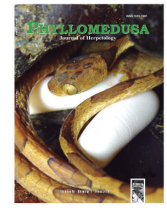
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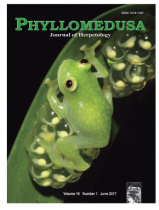
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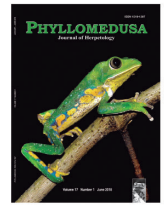
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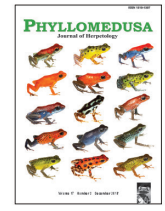
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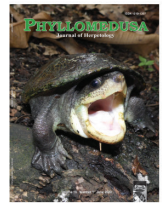
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