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Cover: Normally colored and albinotic hatchlings of *Natrix tessellata* from Slovakia.

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Predicting distributions of rare species: the case of the false coral snake *Rhinobothryum bovallii* (Serpentes: Colubridae)

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Abstract

Predicting distributions of rare species: the case of the false coral snake *Rhinobothryum bovallii* (Serpentes: Colubridae). Typically, the lack of enough high-quality occurrence data makes it difficult to define the geographic distribution of rare species. However, species distribution models provide a powerful tool for biodiversity management, including efforts to predict the distributions of rare species. Herein, new and historical data are used to model the distribution of the False Tree Coral snake, *Rhinobothryum bovallii.* The prediction map reveals a disjunct distribution for this species, from the Central American Isthmus to the northwestern portion of South America, with the species occupying lowlands and premontane forests below about 1500 m elevation. We identified 491,516 km^2 of suitable habitat for *R. bovallii* (minimum training presence threshold of 0.424) and 59,353 km^2 of core habitat, with concentrations in three relatively isolated core areas (10-percentile training presence threshold of 0.396), as follow: (1) a "northern core" along the Pacific and Caribbean coasts of Panama; (2) a "central core" in the Middle Magdalena Valley in Colombia; and (3) a "southern core" in the Ecuadorian Chocó. The occurrence of this species has a strong positive association with low precipitation seasonality, high precipitation in the warmest quarter, and low variability in annual temperature. Xeric and semiarid areas are unsuitable for this species and may pose environmental barriers limiting its distributional range. These results may lead to the discovery of additional populations of *R. bovallii,* identify priority survey areas, and by determining the extent of its natural habitat promote effective conservation strategies.

Keywords: core habitat areas, disjunct distribution, marginal habitat, species distribution models.

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Resumo

Prevendo distribuições de espécies raras: o caso da falsa cobra coral *Rhinobothryum bovallii* (Serpentes: Colubridae). Normalmente, a falta de dados de ocorrência de alta qualidade suficientes torna difícil definir a distribuição geográfica de espécies raras. No entanto, os modelos de distribuição de espécies fornecem uma ferramenta poderosa para a gestão da biodiversidade, incluindo esforços para prever a distribuição de espécies raras. Aqui, dados novos e históricos são usados para modelar a distribuição da serpente *Rhinobothryum bovallii*. O mapa de previsão revela uma distribuição disjunta para essa espécie, do Istmo da América Central à porção noroeste da América do Sul, com a espécie ocupando terras baixas e florestas pré-montanas abaixo de cerca de 1.500 m de altitude. Identificamos 491.516 km^2 de habitat adequado para *R. bovallii* (limite de corte de presença mínima de treino de 0,424) e 59.353 km^2 de habitat central, com concentrações em três áreas centrais relativamente isoladas (limite de corte de 10-percentil de presença de treino de 0,396), como segue: (1) um "núcleo norte" ao longo das costas do Pacífico e do Caribe do Panamá; (2) um "núcleo central" no Vale do Médio Magdalena, na Colômbia; e (3) um "núcleo sul" no Chocó equatoriano. A ocorrência dessa espécie tem forte associação positiva com a baixa sazonalidade da precipitação, alta precipitação no trimestre mais quente e baixa variabilidade na temperatura anual. Áreas xéricas e semiáridas são inadequadas para essa espécie e podem representar barreiras ambientais que limitam sua faixa de distribuição. Esses resultados podem levar à descoberta de populações adicionais de *R. bovallii*, identificar áreas prioritárias de levantamento e, ao determinar a extensão de seu habitat natural, promover estratégias de conservação eficazes.

Palavras-chave: áreas centrais de habitat, distribuição disjunta, habitat marginal, modelos de distribuição de espécies.

Introduction

A complex interaction of biotic (e.g., source-sink dynamics, habitat heterogeneity, competition, and mutualism), abiotic (e.g., climate, hydrologic and topographic changes), and evolutionary (e.g., speciation rates, extinction rates, and niche conservatism) processes have been suggested to play a key role in shaping Neotropical biodiversity (Van der Hammen and Hooghiemstra 2000, Antonelli and Sanmartín 2011, Antonelli *et al.* 2018). The Neotropical region (i.e., tropical America) is the most species-rich region on Earth and houses nearly a third of the world's biodiversity hotspots (Mittermeier *et al.* 2004). The Chocó Biogeographic Region (located within the Tumbes-Chocó-Magdalena hotspot, formerly called the Chocó-Darién-Western Ecuador hotspot) harbors an exceptional species richness and endemism in a relatively small area (187,400 km^2, Mittermeier *et al.* 2004) less than ~ 3% of the area of the Amazon Basin. For reptiles, especially snakes, the Chocó and Amazonian ecoregions have a close biogeographic affinity at the suprageneric-level, sharing about 55% of genera and 66% of families (data analyzed from Lynch *et al.* 2016). This fact suggests that these ecoregions had an ancient common history before the Andean uplift (between 2.7–5 Ma; Gregory-Wodzicki 2000), which may have resulted in allopatric speciation when populations of lowland species became isolated on either side of the cordillera (Hernández-Camacho *et al.* 1992, Smith *et al.* 2014, Winterton *et al.* 2014).

The two species of *Rhinobothryum* Wagler, 1830 may provide the clearest example of allopatric distributions among Neotropical snakes. The distributions of *Rhinobothryum bovalli* (Andersson, 1916) and *Rhinobothryum lentiginosum* (Scopoli, 1785) suggest the separation of a once-continuous range into two geographically isolated, or allopatric, parts (Rojas-Morales 2012). *Rhinobothryum bovalli*

occurs in lowlands from southern Honduras to Costa Rica and Panama in Central America, and from western Colombia to northwestern Ecuador and northeastern Venezuela in South America (Savage 2002, Rojas-Morales 2012, McCranie 2015, Rojas-Runjaic and Infante-Rivero 2018, Turcios-Casco *et al.* 2018, Martínez-Fonseca *et al.* 2019). In contrast, *R. lentiginosum* occurs in lowlands along the Amazon Basin in South America, with records from Colombia, Ecuador, Peru, Bolivia, Paraguay, Brazil, French Guiana, Suriname, Guyana, and Venezuela (Peters and Orejas-Miranda 1970, Martins and Oliveira 1998, Miranda *et al.* 2009, Wallach *et al.* 2014, Gomes de Arruda *et al.* 2015). The geographic distributions of these species differ substantially; nevertheless, the limits of their ranges are uncertain. This is especially true of *R. bovallii,* a snake that is rarely seen and that apparently has disjunct distributions within its geographical range (Savage 2002, Wilson and McCranie 2003, Martínez-Fonseca *et al.* 2019).

Methods for estimating the distribution of a species based on fundamental ecological niche theory allow us to: (1) model the distribution of poorly known species (e.g., Raxworthy *et al.* 2003, Meza-Joya *et al.* 2018); (2) predict the potential geographic range of invasive species (e.g., Rödder and Lötters 2010, Urbina-Cardona and Castro 2010); (3) develop conservation action plans (e.g., Araújo *et al.* 2019, Botero-Delgadillo *et al.* 2012); (4) predict the effects of climate change on species distributions (e.g., Ochoa-Ochoa *et al.* 2012, Velásquez-Tibatá *et al.* 2013); (5) develop vector-borne disease spread and risk models (e.g., Peterson 2006, Fatima *et al.* 2016); and (5) identify historic refugia for biodiversity (e.g., Waltari *et al.* 2007, Vega *et al.* 2010). Species distribution models (SDMs) use predictor variables and species occurrences (either presence-only or presence-absence) to estimate objects in geographic space, referring to the actual distribution of a given species (Peterson and Soberón 2012). However, predicting the distribution of rarer species is challenging because the factors that contribute to rarity (e.g., range size, habitat specificity, and local density) are poorly known for most taxa (Rabinowitz 1981, Karl *et al.* 2002, Franklin *et al.* 2009).

Rare species are highly sensitive to both natural and human-related disturbances, which increases their vulnerability, and therefore, their risk of extinction (Davies *et al.* 2004). Here we study the distribution of *R. bovallii,* a species that is rarely encountered and that apparently has a naturally disjunct distribution from lowest Central America to northwest South America (Savage 2002, Rojas-Morales 2012, McCranie 2015, Martínez-Fonseca *et al.* 2019). The rarity of this species has been attributed to sampling bias, low encounter rates, and the difficulty of sampling arboreal habitats (Arredondo *et al.* 2017, Rojas-Runjaic and Infante-Rivero 2018). Our primary objective was to update the distributional range of *R. bovallii* using reliable historical, as well as new records in conjunction with predictive species distribution models. We also assess the effect of predictor variables that have a major influence in shaping the current environmental niche of this species. Last, we identified areas where the predicted habitat suitability differs in order to identify core and marginal habitats for conservation purposes. Our results underscore the utility of predictive models in exploring the distribution of elusive and rare species.

Materials and Methods

Species Data

We compiled locality information for *Rhinobothryum bovalli* from museum specimens, well-supported reports from published literature, and records from our field surveys (collection permits 164-2014, 1166-2014 by Corporación Autónoma Regional de Caldas-Corpocaldas, and 0047-2015 by Autoridad Nacional de Licencias Ambientales-ANLA). We obtained additional data from the Global Biodiversity Information Facility (http://www.gbif.org), the VertNet data

portal (http://www.vertnet.org), and SiB Colombia (http://www.sibcolombia.net) accessed on 20 October 2019. Our analyses only include well-supported records from these electronic databases because they are prone to contain inaccuracies (Nogueira *et al.* 2011, Zaher *et al.* 2011). The accuracy of an occurrence record was considered reliable if: (1) it was published by a reliable source (e.g., herpetological collection or museum); (2) it was supported by a voucher specimen; and (3) its taxonomic diagnose was confirmed by a curator or previous studies. We also included photographic records from http://www.inaturalist.org because this snake is very conspicuous and easily recognized by photographs. Each record available was checked to identify uncertainty and mistakes in the geographic information. We retained localities with geographic coordinates assigned by other authors based on collection locality data (Appendix I). Although there is uncertainty associated with these coordinates, we expect them to fall near or in the correct pixel of the environmental data (pixel size is 30 arc-seconds or about 1 km^2). We excluded records with incomplete, inconsistent, or imprecise geographic information.

Environmental Data

To explore predictors that are likely to affect the distributions of species, we used climatic data from 19 WorldClim variables and elevation (Hijmans *et al.* 2005) at a 30 arc-seconds spatial resolution (ca. 1 km^2). We restricted the selection of environmental data from "background" pixels to a region that matched areas from which of *Rhinobothryum bovalli* is known to occur and thus, might be representative of suitable environmental conditions for the species (Anderson and Raza 2010, Peterson *et al.* 2011, Galante *et al.* 2018). We used a rectangle encompassing a four-degree buffer (ca. 450 linear km) around the most extreme locality at each cardinal direction. This selection method seems appropriate because it excludes large areas that are environmentally suitable, but where the species is likely unable to disperse—because of the presence of insurmountable physical or physiological barriers to dispersal—and/or is not known to occur (i.e., geographic areas where the species has not been reported or collected historically or inventory pseudo-absences).

Sampling Bias

To reduce the likely effects of spatial autocorrelation in our initial occurrence dataset ($N = 103$ unique localities), we used SDMtoolbox to filter localities keeping the maximum number of occurrences that were at least 20 km apart (Brown *et al.* 2017). This distance threshold was selected because *Rhinobothryum bovalli* is distributed mainly in lowlands, which are expected to have low levels of topographic heterogeneity. This procedure produced a maximum number of 65 points of occurrence. To address possible sensitivity of this procedure in resulting models (Galante *et al.* 2018), we first ran a series of starting models with the unfiltered dataset and then with the spatially filtered one. To build these models, we used the recommended feature classes to be appropriate for our sample sizes—all feature classes for the unfiltered dataset and linear plus quadratic plus hinge for the filtered one (Phillips and Dudík 2008). For each feature class combination, we used a narrow range of regularization multiplier values close to the default value—i.e., 0.5, 1 (default value), 1.5, and 2. Regularization was employed to reduce the number of variables selected for inclusion in the resulting models (Phillips and Dudík 2008, Elith *et al.* 2011). We used the occurrence dataset from the best-ranked model (i.e., unfiltered dataset) to build the suite of final candidate models.

Variable Reduction

To ensure the quality of the final suite of habitat suitability models and to reduce potential over-parameterization, we followed

the process outlined by Warren *et al.* (2014). For this, we calculated the contribution scores (permutation importance and percentage contribution) for each variable in our suite of starting models (see above). To obtain alternate estimates of which variables are most important in the model, we also ran a jackknife test. Then, we calculated the spatial correlations (Pearson coefficient) between variables using the software ENMTools version 1.4.4 (Warren *et al.* 2010). We used contribution scores in conjunction with the scores from correlations to select the final set of environmental variables. First, we eliminated variables with low contribution scores (< 5%) in the starting model. Then, we deleted variables that were highly correlated ($|r| > 0.85$) following Elith *et al.* (2010), keeping the variables with the highest contribution scores. We extracted from the best-ranked model the reduced set of variables to build the suite of final candidate models.

Species Distribution Modelling

We generated SDMs using MaxEnt version 3.4.1, a presence-background algorithm that integrates environmental data with georeferenced occurrence records to model the habitat suitability of a given species (Phillips *et al.* 2006). Although many methods are available for modeling distributions, MaxEnt has high predictive accuracy for a wide range of species in diverse regions, even when available information is incomplete and the sample size is small (e.g., Pearson *et al.* 2007, Anderson and Raza 2010, Chunco *et al.* 2013, Ramos *et al.* 2018). We used recommended default values for convergence threshold (10^{-5}), maximum number of iterations (500), maximum number of background points (10^4), and default prevalence of the species (0.5). Last, we selected the logistic output format, which yields continuous values ranging from 0 to 1 that indicate the probability of suitable environmental conditions for the species (Phillips and Dudík 2008). We built final candidate models randomly excluding 30% of the observation records to use as test data, with 100 bootstrapped replicates.

Model Calibration

Final candidate models were generated using multiple combinations of distinct values of regularization multipliers and feature classes to identify the parameter settings that lead to best-fitting models. These models were built using unfiltered occurrence (Table 1) and withheld environmental data (Table 2). We created a suite of models by allowing increasing complexity of the feature classes employed, taking into account our sample size (i.e., 103 occurrence points; see Phillips and Dudík 2008, Elith *et al.* 2011): hinge (H), linear plus quadratic (LQ), linear plus quadratic plus hinge (LQH), and all features (default setting). For each feature class combination, we built models using the same range of regularization multiplier values as in starting models (i.e., 0.5, 1, 1.5, and 2).

Table 1. Summary of evaluation statistics of the starting models for *Rhinobothryum bovallii*. Results are provided for two model-selection techniques (AUC and OR) for two datasets (unfiltered and filtered localities) using recommended features classes (all features and linear plus quadratic plus hinge, respectively). Omission rates (OR) based on the minimum training presence threshold.

Feature	Regularization	OR	AUC
Unfiltered (103 occurrences)	0.5	0.037	0.954
	1	0.013	0.941
	1.5	0.023	0.933
	2	0.031	0.912
Filtered (65 occurrences)	0.5	0.085	0.962
	1	0.057	0.933
	1.5	0.061	0.921
	2	0.029	0.893

Table 2. Percentage of contribution and permutation importance of the most important variables used to build final species distribution models for *Rhinobothryum bovallii*. The four variables with the highest contributions are presented in bold.

Variable	Code	Permutation importance	Percent contribution
Precipitation seasonality	BIO15	**25**	**16**
Precipitation of warmest quarter	BIO18	**7.4**	**14.7**
Elevation	-	**10.6**	**14.1**
Precipitation of driest quarter	BIO17	**6.9**	2
Temperature annual range	BIO7	5.1	**13.4**

Model Selection

We compared all the models generated by using the scores of two quantitative measures: (1) area under a receiver operating characteristic curve (AUC; threshold-independent), and (2) omission rate by applying the minimum training presence threshold (OR; threshold-dependent). Specifically, the AUC gives a relative measure of the overall discriminatory ability of a model (Peterson *et al.* 2011), whereas omission rates indicate whether a model is overfitted to the calibration data (Galante *et al.* 2018). By using this threshold rule, we expect 0% test omission; thus, values above zero indicate overfitting (Shcheglovitova and Anderson 2013). Hence, we used OR as the primary criterion for selecting optimal combinations of feature classes and regularization multiplier (Shcheglovitova and Anderson 2013). We first identified the models that displayed the lowest average OR, and then, from that subset of models, we chose the one with the highest average AUC score.

Binary Model and Core Areas

Once we selected the best-ranked model, its logistic output was transformed into a binary prediction model for the suitable habitat of the species (i.e., a presence/absence map) by applying the minimum training presence threshold value for the training occurrence data obtained by MaxEnt. This threshold rule was chosen because it has a straightforward ecological interpretation by identifying pixels predicted to be at least as suitable as those from which the species has been recorded (Pearson *et al.* 2007). Then, we evaluated the final binary model by visual examination based on our knowledge of the natural history and geographic distribution of *Rhinobothryum bovalli*. To identify core habitats, we follow the approach proposed by Molloy *et al.* (2014). We converted predictions of the best-ranked model by applying the threshold cut-off training value for the 10-percentile training presence threshold. This approach seems rational because the threshold rule excludes all regions having suitability values lower than those for the lowest 10% of occurrence records (Radosavljevic and Anderson 2014) and leads to a smaller geographical prediction of high-quality habitats in environmental terms. Following this approach, areas with environmental values containing 90% of occurrence records were designated as core habitats (i.e., pixels of the model in which test occurrence points had a high probability of falling and where the species is most likely to sustain viable populations), whereas the remaining areas correspond to marginal habitats (i.e., pixels of the model representing a marginal part of the fundamental niche of the species in which survival and reproduction are thought to be poor because of the limiting environmental conditions).

Results

Models Performance

In the starting models, the AUC values were slightly better for the models with the unfiltered dataset than those for models with filtered one (except for models calibrated to 0.5 regularization value). As expected, starting models of both datasets (spatially unfiltered and filtered) had OR values above the expected 0% for the minimum training presence threshold. However, starting models calibrated using the unfiltered dataset and all feature classes [as recommended by Phillips and Dudík (2008) for sample sizes > 80 occurrence points] produced higher average evaluation scores (mean AUC = 0.935 ± 0.018) and lower average omission rates (0.026 ± 0.010) across regularization multipliers. Although the used of filtered occurrence results in the model with the highest AUC score (AUC = 0.962), its OR was six times greater (OR = 0.085) than the model with the lowest one (OR = 0.013), indicating greater overfitting in the first. The model performance measures (AUC and OR) of the suites of starting models are given in Table 1.

Observed average AUCs for our final models varied, with lower regularization multipliers leading to higher AUCs within a given feature class (Figure 1A). Default features (i.e., all features) displayed the highest average AUC values across regularization multipliers (mean AUC = 0.930 ± 0.019), followed by LQH (mean AUC = 0.922 ± 0.021), H (mean AUC = 0.920 ± 0.018), and LQ features (mean AUC = 0.863 ± 0.009; Figure 1B). The highest AUC value was observed for default features at the lowest regularization multiplier (AUC = 0.950 ± 0.007). However, the difference between this value and that for LQH and H features at the same regularization multiplier (i.e., 0.5) was minimal (0.004 for LQH and 0.006 for H features; Figure 1A).

Model calibration showed great differences in average ORs among feature classes, with LQH and LQ features obtaining the lowest OR values at low regularization multipliers and then dropping in performance at high regularization multiplier values. In contrast, default and H features showed the opposite pattern (Figure 1C). Linear plus quadratic plus hinge features displayed the lowest average OR values across regularization multipliers (mean OR = 0.012 ± 0.009), followed by LH (mean AUC = 0.014 ± 0.008), default (mean AUC = 0.035 ± 0.010), and H features (mean AUC = 0.038 ± 0.010; Figure 1D). The lowest OR value was observed for the LQH feature at the lowest regularization multiplier (OR = 0.000), which fix to the theoretical expectation of zero omission of evaluation localities using the minimum training presence threshold (Figure 1B).

Based on the criteria employed, the default settings (i.e., all feature classes and regularization multiplier of 1) were not optimal for our dataset. We identified the LQH feature class and the lowest examined regularization multiplier (i.e., 0.5) as the optimal combination for our datasets. Given the sequential nature of the selection criteria used here, we did not necessarily select the combination with the highest evaluation AUC as optimal. However, the difference between the AUC of the optimal combination (i.e., LQH features and regularization multiplier of 0.5) and that of the combination with the highest AUC (i.e., default features and regularization multiplier of 0.5) was negligible (AUC difference of 0.004). The model performance measures (AUC and OR) of the suites of final models are given in Figure 1.

Variable Contribution and Response Curves

The analysis of variable contributions reveals that precipitation seasonality, precipitation of warmest quarter, elevation, precipitation of driest quarter, and temperature annual range, significantly affect the distribution of *Rhinobothryum bovalli* (Table 2). Based on both metrics (i.e., permutation importance and percent contribution), precipitation seasonality has the greatest influence on the distribution of this

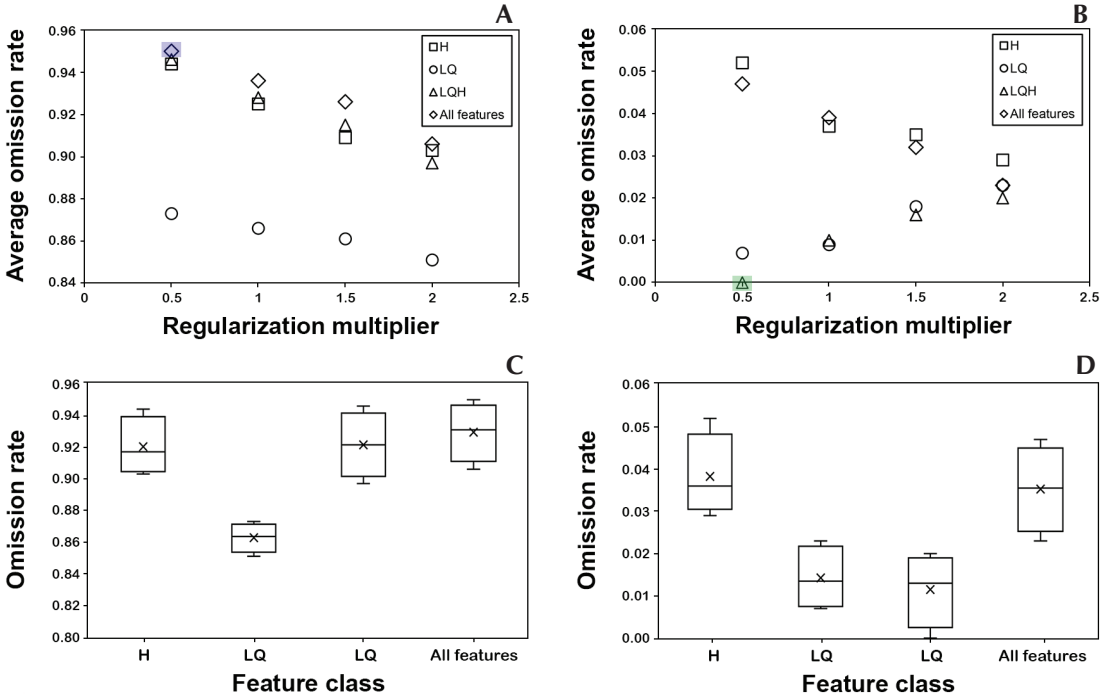

Figure 1. Evaluation statistics resulting from calibration of Maxent models for *Rhinobothryum bovallii.* Top panels show the test Area Under the Curve (AUC) scores (**A**) and the omission rates (OR) at minimum training presence threshold (**B**). Bottom panels show the statistics (i.e., median, first and third quartiles, minimum, and maximum) for AUC (**C**) and ORs (**D**) values compared across regularization multipliers for each feature class. In each panel, the optimal model is highlighted. Model statistics are shown as feature classes (H = hinge, LQ = linear plus quadratic, LQH = linear plus quadratic plus hinge, and all features) increasing in regularization multiplier (i.e., 0.5, 1, 1.5, and 2).

species (25% and 16%, respectively). The jackknife test of variable importance shows that the environmental variable with the highest gain when used in isolation is temperature annual range—therefore contains the most useful information by itself, whereas precipitation seasonality decreases the gain the most when it is omitted—and thus contains information not present in any other variable (Figure 2). Response curves show that suitability was positively linked with low values of precipitation seasonality (< 30%). Suitability was also maximized around 500 mm of precipitation in the warmest quarter and a temperature annual range of about 12°C. As expected, presence probability is highest at low elevations and decreases at higher altitudes (> 1000 m a.s.l.).

Species Distribution Range

To generate SDMs, we compiled 103 reliable locality records for *Rhinobothryum bovalli* (84 historical and 19 new). Known records of this species range from about 0° to 15° N latitude, with the archaeological site "Ciudad Blanca" in Honduras and the Caique River in Ecuador, the northernmost and southernmost known localities, respectively (Pazmiño-Otamendi 2017, Turcios-

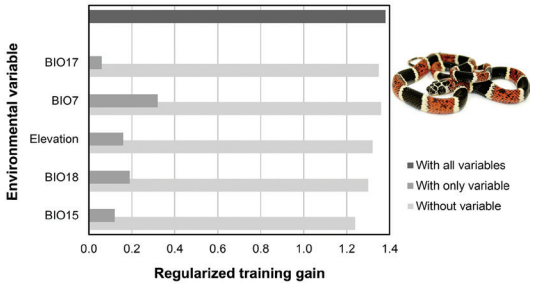

Figure 2. Jackknife test of variable importance showing regularized training gain of individual predictor variables important in the development of final models for *Rhinobothryum bovallii*.

Casco *et al.* 2018). Records come from 12 of the ecoregions proposed by Dinerstein *et al.* (2017): Central American Atlantic moist forests, Talamancan montane forests, Isthmian-Atlantic moist forests, Isthmian-Pacific moist forests, Chocó-Darién moist forests, Western Ecuador moist forests, Cauca Valley montane forests, Magdalena Valley montane forests, Magdalena-Urabá moist forests, Magdalena Valley dry forests, Sinú Valley dry forests, and Cordillera Oriental montane forests. Review of digital photographs of a specimen of *R. bovallii* from Caquetá department (Solano municipality) on the Amazon versant of Colombia (IAvH-R 6453, IAvH-R 6454 in Martínez-Fonseca *et al.* 2019) confirm that it is *R. lentiginosum*. Examination of a specimen from the same locality deposited at Universidad de la Amazonia in Colombia (UAM-R uncatalogued) supports this determination. Specimens of *R. bovallii* from Suriname and Guyana (see http://vertnet.org) probably correspond to misidentifications of the congeneric species *R. lentiginosum* (see also Martínez-Fonseca *et al.* 2019). Based on geographic coordinates of locality records (Appendix I), the altitudinal range for *R. bovallii* ranges from sea level (QCAZ-R 4652) to 1535 m (KU 75749). The updated distribution of *R. bovallii* is summarized in Figure 3A (For additional details, see Appendix I).

The logistic output of the best-ranked model identified several regions of high prediction for *R. bovalli* in most parts of the Caribbean coast of southern Nicaragua, Costa Rica, and Panama; tenuously connected areas of the Chocó region in Panama, Colombia, and Ecuador; Middle Magdalena Valley in Colombia; and isolated portions of the Caribbean regions in Colombia and Venezuela. Furthermore, the model identified small, isolated areas of high suitability in regions from which there are no records for the species. These areas include the eastern slope of the Cordillera Oriental, the upper basin of the Magdalena River, and the Catatumbo Basin in Colombia, as well as the Machango, Misoa, and Rio Viejo basins in Venezuela, all of which drain to the east of the Maracaibo Lake. South of this area, the model predicts the potential presence of *R. bovalli* in the foothills of the Merida Cordillera (Figure 3B). According to the binary map generated using the cut-off training value for the minimum training presence threshold (i.e., 0.424), we estimated an extent of occurrence of 491,516 km^2 for the species (Figure 4A). After applying the cut-off value for the 10-percentile training presence threshold (i.e., 0.396) we identify three main core habitat areas, summing approximately 59,353 km^2 (Figure 4B): a "northern core" along the Pacific and Caribbean coasts of Panama (~ 31,529 km^2); a "central core" in the Middle Magdalena Valley in Colombia (~ 20,939 km^2); and a "southern core" in the Ecuadorian Chocó (~ 6,885 km^2).

Discussion

Models Performance

The starting models using unfiltered versus filtered datasets yielded substantially higher OR values when the minimum training presence threshold was applied to both datasets. However, the unfiltered dataset leads to models with higher AUC scores and lower ORs across regularization multipliers. Use of the unfiltered dataset led to models with areas of higher prediction

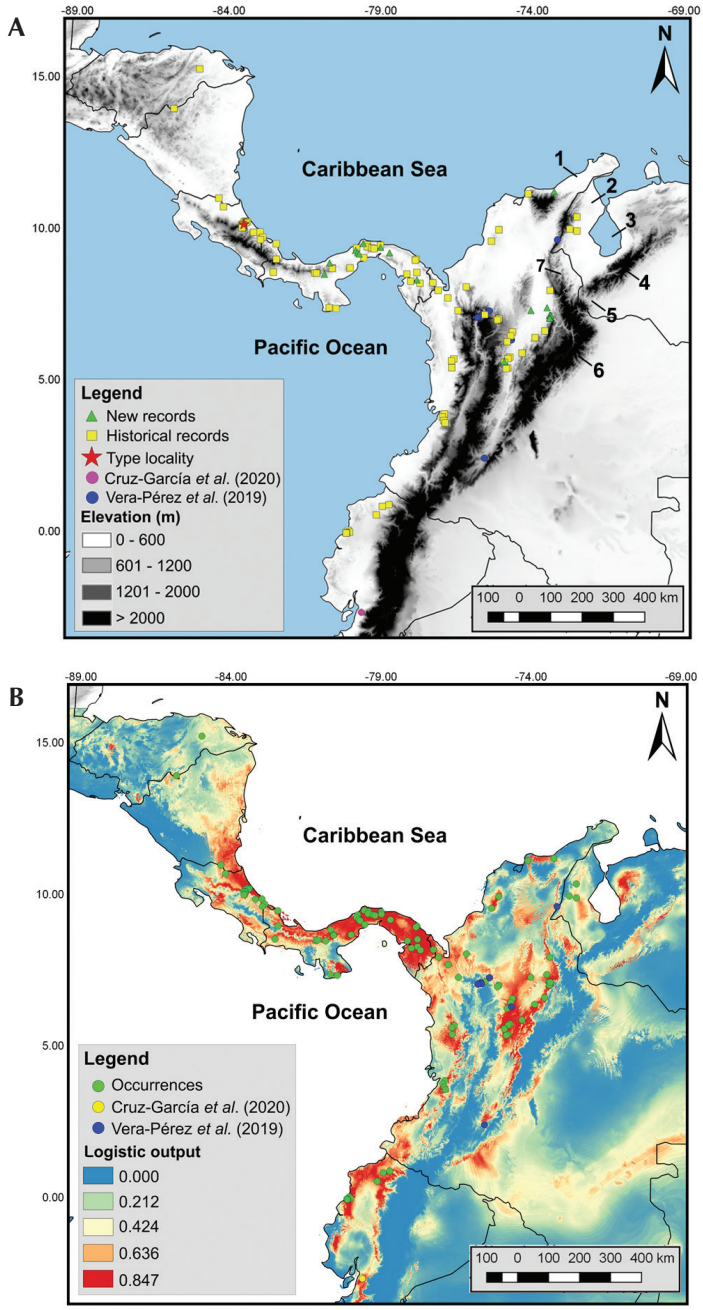

Figure 3. Updated distributional map (**A**) and logistic output for the best-ranked model (**B**) for *Rhinobothryum bovallii*. Map numbers indicate: 1 = Guajira-Barranquilla xeric scrub ecoregion (Colombia), 2 = Paraguaná xeric scrubs ecoregion (Venezuela), 3 = Maracaibo Lake (Venezuela), 4 = Cordillera de Mérida (Venezuela), 5 = Táchira depression (Venezuela), 6 = Cordillera Oriental (Colombia), 7 = Catatumbo basin (Colombia). Geodetic datum = WGS-84.

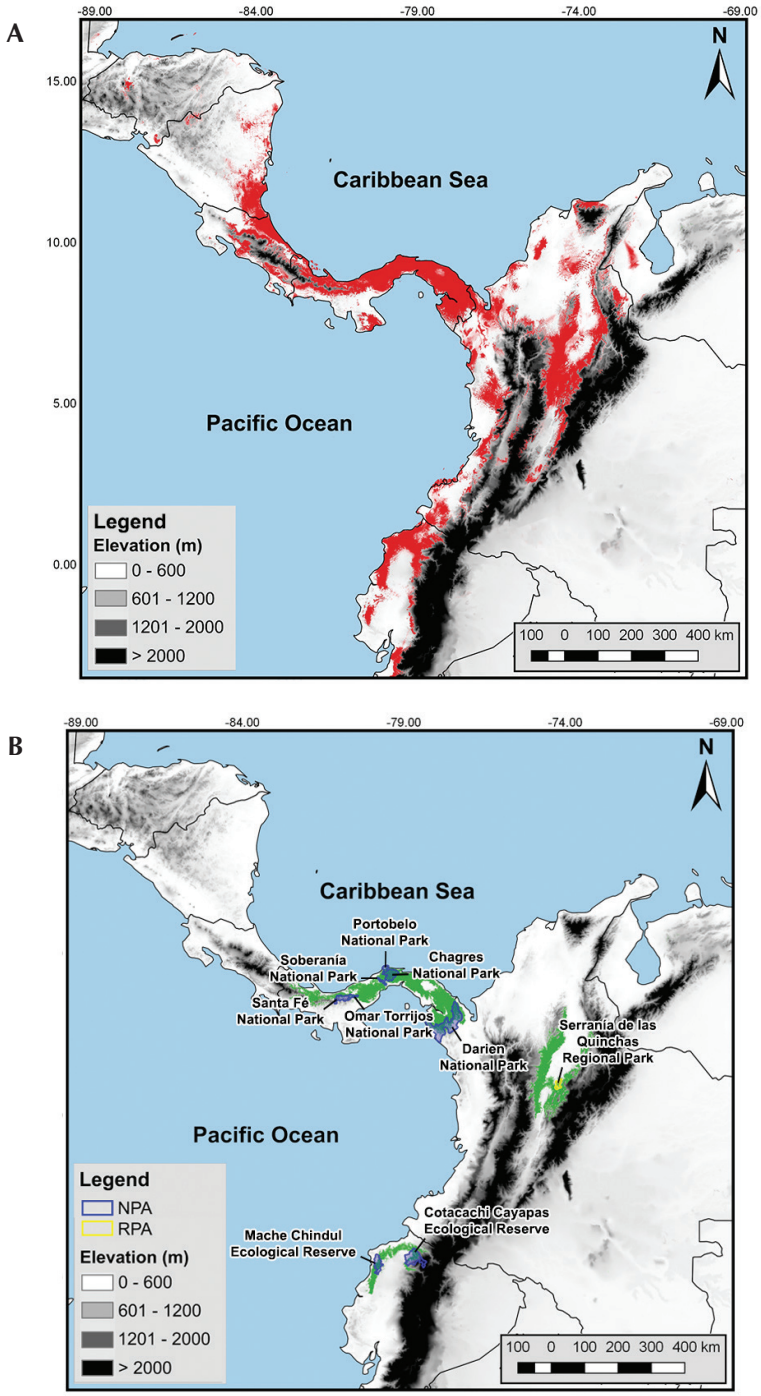

Figure 4. Binary distribution map (**A**) and main core habitat areas (**B**) for *Rhinobothryum bovallii*. NPA = National protected areas. RPA = Regional protected areas. Geodetic datum = WGS-84.

concentrated around known records, suggesting low sampling bias, as well as more realistic geographic predictions, in contrast to the filtered dataset. Although spatially filtered data is most likely to match the assumption of unbiased sampling (Galante *et al.* 2018), it is probable that the rarity of *Rhinobothryum bovalli* throughout its range may contribute to reduced sampling bias in our unfiltered dataset, and as a consequence, to best geographic predictions. Although the unfiltered dataset used here seems to be minimally affected by bias problems, it is critical to conduct filtering analysis to assess whether the resulting models are affected by spatial sampling bias (Radosavljevic and Anderson 2014, Boria *et al.* 2014, Galante *et al.* 2018).

In the model calibration, lower regularization multipliers lead to higher AUC values within a given feature class. Although OR scores were variable across the feature classes, low regularization multipliers (0.5) were necessary to reduce overfitting to the lowest levels (Figure 1). Although the highest point in AUC occurs when default features (i.e., all features) are used, ORs indicate much better performance with LQH features. The fact that optimal performance corresponds to more complex sets of feature classes supports the findings of previous studies (e.g., Shcheglovitova and Anderson 2013, Galante *et al.* 2018, Ramos *et al.* 2018). Notably, these studies examined species with few occurrence records (< 23 localities), whereas *R. bovalli* has 103 occurrence points. Thus, our finding that overfitting decreases at regularization multipliers lower than default when used with more complex sets of feature classes contrasts with the results from other studies using both small (< 23 localities) and large (> 208 localities) datasets (e.g., Shcheglovitova and Anderson 2013, Radosavljevic and Anderson 2014, Ramos *et al.* 2018). While low regularization values tend to result in overly complex models (Radosavljevic and Anderson 2014), it is probable that the removal of highly correlated variables in our study provides additional protection against overfitting that leads to low regularization scores and an increase in performance.

Variable Contribution and Response Curves

The predicted habitat suitability for *Rhinobothryum bovalli* in environmental space is represented by low variability of monthly precipitation values during the year (i.e., precipitation seasonality), high values of precipitation during the warmest three months of the year (i.e., precipitation in the warmest quarter), and low variability in annual temperature (i.e., temperature annual range). In general, these results show that climatic habitat suitability for this species is highest in most equatorial regions and decreases toward more northerly and southern latitudes. Areas with high habitat suitability coincide largely with tropical moist forests, which are characterized by high levels of rainfall and low variability in annual temperature (Rangel-Ch and Arellano-P 2004). Chocó-Darién moist forests, Northern Andean montane forest, and Isthmian-Atlantic moist forests are the best-represented ecoregions in the distribution of this species. In addition, our model produced low habitat suitability scores for higher elevations (i.e., above ~ 1000 m a.s.l.) in the Andes of northern South America and the mountain ranges of Central America (Figure 3B). The effect of elevation on species distributions is realized by changes in ecological variables, such as temperature, rainfall, and solar radiation (Elith and Leathwick 2009) that determine vegetation structure and habitat quality (Pokharel *et al.* 2016). Investigation of the point at which environmental variables diverge to distinguish core versus marginal habitats may result in a better understanding of conditions that favor adaptation to lower-quality habitats.

Species Distribution Range

The geographic information presented here indicates that the distribution of *Rhinobothryum bovalli* is a disjunct within its range between the

Central American Isthmus and the northwestern portion of South America, where the snake occupies lowlands and premontane forests below ~ 1500 m elevation (Figure 3A; Appendix I). The most eastern reported localities for *R. bovalli* lie on the eastern flank of the Serranía del Perijá (or Sierra de Perijá) in Venezuela (Figure 3A; Rojas-Runjaic and Infante-Rivero 2018). Although the species has been reported to occur in moist forest on the western slope of the Cordillera Oriental, inter-Andean Valleys, and Sierra Nevada de Santa Marta in Colombia (Figure 3A), its presence in the Venezuelan Sierra de Perijá is unanticipated because there is no known basimontane moist corridor along which the species could disperse. However, *R. bovalli* might have colonized the area during the Pliocene (~ 3.30 Mya) prior to an intense global glaciation that occurred after the major known Andean uplift (2000–3000 m a.s.l.) at the end of this period (Lazala 2007). Validation of this hypothesis would require a dated phylogeny for snakes that includes several populations of both species of *Rhinobothryum.*

Our distribution model includes small areas of high probability of occurrence east of the Andes. Included are the eastern slope of the Cordillera Oriental in Colombia (with the Catatumbo Basin), as well as areas around the Maracaibo Lake and the foothills of the Cordillera de Mérida in Venezuela (Figure 4A). Given that the potential habitat for *R. bovalli* is characterized by low seasonal precipitation, high precipitation in the warmest quarter, and low variability in annual temperature (Table 2, Figure 2), it is logical to suppose that arid and semiarid conditions would restrict the distribution of this snake. Therefore, xeric areas in the Guajira-Barranquilla and Paraguaná xeric scrubs ecoregions (Dinerstein *et al.* 2017) and semiarid areas of the Táchira Depression (La Marca 1997) may represent environmental barriers limiting the distribution of this species south Cordillera de Mérida. Unexplored or under-sampled areas must be surveyed to generate more robust SDMs for *R. bovalli.*

Two studies reporting new locality records for *R. bovalli* were published recently—including the first record from the Colombian slope of the Serranía del Perijá (Vera-Pérez *et al.* 2019) and the second from Ecuador (Cruz-García *et al.* 2020) (Figure 3A, Appendix I). These appeared coincident with the submission of this manuscript for publication; thus, neither was included in our species distribution modeling. However, these records coincide with areas of high habitat suitability predicted by our models, especially the noteworthy records from the upper Magdalena Valley (Huila Department) and Serranía del Perijá (Cesar Department) in Colombia, and the southernmost record from Guayas province in Ecuador (Figure 3B). This suggests that our models have a high predictive performance and underscores the importance of this methodological approach for making predictions about the distribution of elusive and rare species (Raxworthy *et al.* 2003, Pearson *et al.* 2007, Anderson and Raza 2010, Chunco *et al.* 2013). Last, it is important to note that other factors not incorporated in our models (e.g., land-cover change and interspecies interactions) also may limit the presence of this species in otherwise suitable areas.

Historical Biogeography

The phylogenetic relationships of Neotropical colubrids are unresolved. However, recent studies suggest that *Rhinobothryum* may be closely related to Nearctic colubrids such as *Drymarchon, Drymoluber, Mastigodryas,* and *Palusophis* (Montingelli *et al.* 2019, Zaher *et al.* 2019), with the divergence between *Rhinobothryum* and the clade formed by *Drymoluber, Mastigodryas,* and *Palusophis* occurring in the middle Miocene, at ~ 16 Mya (Zaher *et al.* 2019). These results support Duellman's proposal (1990) that the *Rhinobothryum* clade is old and originated in the north long before the lineage arrived in South America shortly after the final closure of the Isthmus of Panama during the Pliocene, at ~ 3.5–4.5 Mya. The allopatric ranges of current species

of *Rhinobothryum* are separated by major Andean mountain ranges, thereby suggesting an ancestral vicariance event (Rojas-Morales 2012). Our models indicate small areas of high habitat suitability for *R. bovalli* in the southeastern foothills of the Cordillera Oriental in Colombia (Figure 4A). This region represents an Andes-Amazon-Orinoquia transition area, where biotic elements of these three large units converge (Hernández-Camacho and Sánchez 1992). Although this area contains pixels of high environmental suitability, it is unlikely that *R. bovalli* occurs there because environmental barriers would limit the distribution of the species south Cordillera de Mérida (discussed above).

It is improbable that an area of sympatry exists between the two species of *Rhinobothryum,* owing to the presence of environmental and topographical barriers limiting their distributions. Pliocene uplift events (i.e., final uplift of the Northern Andes and Huancabamba Depression) may have promoted the divergence between *R. bovallii* and *R. lentiginosum* by isolating eastern from western populations of the genus (Rojas-Morales 2012). The most western known locality for *R. lentiginosum* in Colombia is Araracuara (Solano municipality) at the Caquetá River (IAvH-R 6453–54)—this site is about 400 km (airline) from the Cordillera Oriental in Colombia. This distance suggests that there may be additional dispersal barriers, such as Amazonian rivers that are thought to have played a key role as barriers to dispersal and prompting diversification and speciation events (Pomara *et al.* 2014). Moreover, expansion and retraction of dry tropical forest and grassland habitats in the Amazon and Orinoco basins since the Last Glacial Maximum (Anhuf *et al.* 2006) also might promote diversification.

Implications for Conservation

Species conservation often must be prioritized to achieve the best outcomes from the resources invested (Hughey *et al.* 2003). Core areas are a priority for the conservation of *Rhinobothryum bovalli,* because these areas possess a unique combination of environmental features that is likely to ensure the survival and maintenance of viable populations through time. Relative to the predicted range of *R. bovalli* (i.e., 491,516 km^2), the sum of its core areas is minimal (59,353 km^2), and the cores are fragmented and relatively isolated from one another (Figure 4B). These areas largely coincide with lowland (below ~ 500 m a.s.l.) tropical moist forests, which represent primary habitat for this species. Core areas in Panama and Ecuador coincide with more extensive and least-fragmented forest, and they also possess an important number of national protected areas with strict categories of management (Category I and II *sensu* Dudley 2008)—viz., six National Parks in Panama and two Ecological Reserves in Ecuador. In contrast, the core area in Colombia coincides with areas suffering extensive deforestation primarily resulting from commercial agriculture, cattle ranching, hydroelectric generation, and extractive industry (Garzón and Gutiérrez 2013, Fagua *et al.* 2019); only a regional protected area for strict conservation exists in this region—Serranía de Las Quinchas Regional Natural Park (Figure 4B).

As for many other species, the range of *R. bovalli* comprises a few, large core habitats surrounded by numerous, smaller marginal ones. However, these peripheral populations differ from those inhabiting core areas because often they are patchily distributed, less dense, more isolated, and subject to limited resources and greater environmental variability, all of which contributes to their risk of extinction (Eckert *et al.* 2008, Hardie and Hutchings 2010, Peterman *et al.* 2013). We do not favor the conservation of marginal habitats at the expense of core ones, but we do think it is crucial to determine which marginal habitats are likely to be most important for conservation purposes, and protect them before they are lost (e.g., those inhabited by populations with high evolutionary potential or/and acting as links between existing metapopulations). From a conservation perspective, key areas that can ensure the long-term viability of

populations (e.g., priority habitats within core areas) and connectivity (e.g., marginal "stepping stone" areas connecting metapopulations) should be protected.

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Appendix I. *Confirmed locality records for* Rhinobothryum bovallii. *Records are ordered in a latitudinal gradient. Institutional codes for museum collections follow those of Sabaj-Perez (2016), except for Circulo Herpetológico de Panamá, Panama (CH), Colección Herpetológica of the Museo de Historia Natural at Universidad de Caldas, Colombia (MHN-UCa), Colección Zoológica Dr. Eustorgio Méndez del Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama (COZEM), Museo de Historia Natural de la Universidad del Cauca, Colombia (MHNUC), University of Kansas Biodiversity Institute Herpetological Collection, USA (KU), University of Texas at Arlington Digital Collection, USA (UTADC). Elevations (m a.s.l.) in bold were estimated from geographic coordinates. Geographic coordinates are provided in decimal degrees (as used to build the models) in the WGS-84 datum. *Type locality, coordinates from Martínez-Fonseca* et al. *(2019).* [1]*Coordinates by M. A. Turcios-Casco.* [2]*Coordinates from Martínez-Fonseca* et al. *(2019).* [3]*Coordinates from Helgen* et al. *(2013).* [4]*Coordinates from Cadle (2012).* [5]*Coordinates estimated or corrected based on the locality provided by the source.* [6]*Locality corrected based on the coordinates provided by the source.* [7]*Coordinates from the online catalog of the herpetological collection where the specimen voucher is deposited.*

Locality	Record type	Latitude	Longitude	Elevation (m a.s.l.)	Source
Honduras					
Gracias a Dios, The Lost City of the Monkey God	UVS-V-01080	15.245244	-84.965346	204	Turcios-Casco *et al.* 2018
El Paraíso, Trojes, near Arenales[1]	LACM 20488	13.934110	-85.812119	**467**	Campbell and Howell 1965
Nicaragua					
Río San Juan, Reserva de la Biósfera Río San Juán, Refugio Bartola	UMMZ 247120	10.970686	-84.333780	65	Martínez-Fonseca *et al.* 2019
Costa Rica					
Alajuela, Pital, Laguna Lagarto	Photograph	10.687000	-84.180900	**38**	iNaturalist 2648512
Limón, Siquirres, near El Carmen[2]	Distribution map	10.210000	-83.460000	**20**	Savage 2002
Limón, Siquirres, near Encanto[2]	Distribution map	10.190000	-83.390000	**10**	Savage 2002
Limón, Siquirres, near El Peje de Cairo[2]	Distribution map	10.170000	-83.510000	**23**	Savage 2002
Limón, Siquirres, near La Lucha[2]	Distribution map	10.160000	-83.440000	**19**	Savage 2002
Limón, Siquirres, near La Florida[2]	Distribution map	10.100000	-83.580000	**266**	Savage 2002
Limón, Siquirres*	GNM 1221	10.100000	-83.510000	**81**	Andersson 1916
Limón, Siquirres, near El Coco	Photograph	10.090437	-83.541017	**228**	iNaturalist 1562800
Limón, Siquirres, near Guayacán[2]	Photograph	10.040000	-83.530000	**565**	Savage 2002
Limón, Siquirres, Pacuare River	NCSM 84544	9.998700	-83.542400	**243**	Norton 2018
Limón, Siquirres, 3 km NE Tres Equis	LACM 154272	9.990613	-83.570140	**500**	Feeney 2018
Limón, Valle de La Estrella, near Bananito Sur[2]	Distribution map	9.850000	-82.970000	**20**	Savage 2002
Limón, Banano River Basin[2]	Distribution map	9.840000	-83.190000	**1025**	Savage 2002
Limón, Talamanca, near Bribrí[2]	Distribution map	9.680000	-82.880000	**162**	Savage 2002
Limón, Talamanca, near Shiroles[2]	Distribution map	9.600000	-82.960000	**188**	Savage 2002
Panama					
Colón, Portobelo, Cerro Bruja	LACM-R 2380-84	9.471734	-79.567519	946	This study
Bocas del Toro, Changuinola, La Gloria[2]	Reported	9.460000	-82.440000	**9**	Lotzkat 2014
Panamá, Parque Nacional Chagres[2]	Distribution map	9.430000	-79.430000	**350**	Ray and Ruback 2015

Appendix I. *Continued.*

Locality	Record type	Latitude	Longitude	Elevation (m a.s.l.)	Source
Guna Yala, Llano Carti, Burbayar[2]	Photograph	9.420000	-79.000000	**54**	McConnell 2014
Panamá, Parque Nacional Chagres[2]	MCZ R-50220	9.360000	-79.440000	**496**	Ray and Ruback 2015
Guna Yala, Narganá, footpath Nusagandi	CH 08759	9.344417	-78.996390	335	This study
Guna Yala, Cerro Brewster, Parque Nacional Chagres	CH 06095	9.321585	-79.282010	872	EMBL-EBI 2019
Colón, Boyd-Roosevelt Highway, near Rita River	UMMZ 147770	9.320000	-79.790000	**151**	Martínez-Fonseca *et al.* 2019
Panamá, Chepo, Zahinas, Cocobolo Nature Reserve[2]	Photograph	9.300000	-79.210000	**444**	Leenders 2017
Colón, 19 Km NW Gamboa, Canal Zone (navy Pipeline Road)	COZEM-REP 0372	9.242696	-79.822077	**61**	This study
Colón, Pipeline Road	CH 07631	9.170808	-79.753484	136	This study
Panamá, Road to Tortí	CH 10437	9.160108	-78.698850	90	This study
Colón, Gamboa, Canal Zone	COZEM-REP 0371	9.125603	-79.704555	**156**	This study
Panamá, Panamá city, Canal Zone[2]	MCZ R-45404	9.000000	-79.550000	**63**	Morris 2018
Bocas del Toro, Changuinola River basin[2]	Reported	8.950000	-82.439000	**950**	Lotzkat 2014
Guna Yala, San Blás, Sasardi camp[3]	KU 112463–64	8.950000	-77.817000	**41**	Brown 2018
Guna Yala, San Blás, Summit camp[4]	KU 112462	8.916000	-77.850000	**361**	Brown 2018
Colón, Donoso, Petaquiya stream	Photograph	8.826253	-80.678855	180	This study
Coclé, La Pintada, El Copé, near Omar Torrijos National Park	CH 05938	8.662691	-80.590435	712	EMBL-EBI 2019
Panamá Oeste, near Altos del María[5]	Observation	8.662151	-80.003867	276	Ray and Ruback 2015
Coclé, La Pintada, El Copé, near Omar Torrijos National Park	Photograph	8.644611	-80.589944	**926**	iNaturalist 1309822
Chiriquí, Boquerón, Bágala, Cerro Colorado, Escopeta camp	USNM 297732	8.518700	-82.537100	**900**	USNM
Darién, Ucurganti River, ca 7 km above mouth[2]	KU 112461	8.510000	-77.800000	**46**	Brown 2018
Veraguas, Rasca River, above El Paredón[6]	SMF 91577	8.491000	-81.169400	775	Lotzkat 2014
Veraguas, Santa Fé, Cerro Tute	SMF 90022	8.488140	-81.109840	1116	Carrizo 2010
Darien, Río Tuira at Río Mono[2]	KU 112460	8.460000	-78.130000	**96**	Brown 2018
Veraguas, Calobre, La Laguna	COZEM-REP 0370	8.455487	-80.851749	**651**	This study
Darién, Pinogana, Reserva Forestal Canglón	CH 00535	8.266388	-77.796801	17	This study
Darién, Serranía del Bagre, Reserva Chepigana de Darién[2]	Reported	8.220000	-78.000000	**20**	Martínez-Fonseca *et al.* 2019
Darién, Pinogana, Yaviza	MCZ R-38236	8.152900	-77.697300	10	Morris 2018

Appendix I. *Continued.*

Locality	Record type	Latitude	Longitude	Elevation (m a.s.l.)	Source
Darién, Tacarcuna[2]	KU 75749	8.170000	-77.270000	**1535**	Brown 2018
Veraguas, Mariato, Cerro Hoya National Park	UTADC 8654	7.345690	-80.691330	533	Flores *et al.* 2016
Los Santos, Tonosí, Ave María, Guanico River	KU 107839	7.316700	-80.466700	**104**	Brown 2018
Colombia					
La Guajira, Dibulla, Pozo Azul, Santa Rita de la Sierra	Specimen	11.193333	-73.267000	124	This study
Magdalena, Santa Marta, Minca, Arimaca	Photograph	11.115820	-74.124465	**843**	iNaturalist 16216599
Bolivar, San Juán Nepomuceno, Miraflores	Photograph	9.925884	-75.093211	167	Barreto-Martínez and Rojas-Morales 2020
Cesar, La Jagua de Ibirico, La Victoria de San Isidro	Observation	9.595222	-73.158861	623	Vera-Pérez *et al.* 2019
Sucre, Colosó, El Sereno Alto, El Sereno stream	IAvH-R-5486	9.542939	-75.340000	300	Borja-Acosta 2017
Córdoba, Tierralta, Palmira, La Plumilla	ICN 053929	8.033056	-76.170556	**162**	Rojas-Morales 2012
Chocó, Unguía, Peye	UTCH 1172	7.920430	-77.096770	40	Rengifo and Pino 2018
Cesar, San Alberto, Miramar[5]	ICN 054087a	7.917869	-73.401721	715	Rojas-Morales 2012
Antioquia, Chigorodó, near Turbo	USNM 154026	7.670000	-76.780000	**10**	Martínez-Fonseca *et al.* 2019
Santander, Sabana de Torres, Reserva Cabildo Verde	Photograph	7.344955	-73.500397	168	This study
Antioquia, Mutatá	MHUA-R 14276	7.247222	-76.439167	140	Rojas-Morales 2012
Bolívar, Cantagallo, Caguí	Photograph	7.252528	-74.033500	98	This study
Antioquia, Valdivia, La Habana	Observation	7.238667	-75.403556	642	Vera-Pérez *et al.* 2019
Antioquia, Briceño, between Ticuita and Capitan stream[7]	MHUA-R 14703	7.112200	-75.554200	**1350**	Rojas-Morales 2012
Santander, Girón, Sogamoso	Specimen	7.095583	-73.382694	563	This study
Santander, Betulia, La Putana	Photograph	7.094102	-73.416165	398	This study
Santander, Girón, Sogamoso	Photograph	7.090167	-73.384389	457	This study
Antioquia, Toledo, La Cascarela	Observation	7.085917	-75.694333	381	Vera-Pérez *et al.* 2019
Santander, Girón, Sogamoso, Las Cruces	UIS-R 2776	7.083218	-73.383432	323	Ramírez-Pinilla and Meneses-Pelayo 2020
Antioquia, Toledo, El Valle	MHNUC-He-0713	7.041222	-75.670694	523	Vera-Pérez *et al.* 2019

Appendix I. *Continued.*

Locality	Record type	Latitude	Longitude	Elevation (m a.s.l.)	Source
Antioquia, Sabanalarga, Orobajo	Observation	7.025000	-75.793194	394	Vera-Pérez *et al.* 2019
Santander, Betulia, Sogamoso, Corintios	Photograph	7.000917	-73.393676	349	This study
Santander, Betulia, Sogamoso, Corintios	Photograph	6.996707	-73.416801	761	This study
Antioquia, Anorí, El Retiro[7]	MHUA-R 14559	6.985000	-75.089722	**871**	Rojas-Morales 2012
Antioquia, Anorí, Dam wall at Porce III dam	Photograph	6.938611	-75.140219	**633**	iNaturalist 41131
Antioquia, Amalfi, María Teresa	Photograph	6.924417	-75.142528	657	Vera-Pérez *et al.* 2019
Santander, El Carmen de Chucurí, Riosucio	IAvH-ACX566	6.577139	-73.573111	760	Acosta-Galvis and Borja-Acosta 2018
Antioquia, Maceo, Las Brisas, Santa Barbara farm[7]	MHUA-R 14583	6.546900	-74.643600	**577**	Rojas-Morales 2012
Antioquia, Puerto Berrío, Hermilda	Photograph	6.411665	-74.701710	**687**	iNaturalist 8360221
Santander, Landazuri[7]	ICN 054086	6.358611	-73.899444	**202**	Rojas-Morales 2012
Antioquia, Puerto Nare, Canteras	Observation	6.278500	-74.675908	214	Vera-Pérez et al. 2019
Antioquia, San Carlos, El Jordán, Juanes, Casino Viejo	ISAGEN 46-2978	6.219139	-74.817139	**717**	Martínez-Fonseca *et al.* 2019
Santander, Sucre, La Tipa	UIS-R 4278	5.956132	-74.065343	**564**	Ramírez-Pinilla and Meneses-Pelayo 2020
Boyacá, Puerto Boyacá, Las Pavas, Puerto Romero[7]	ICN 054085	5.843056	-74.326667	**359**	Rojas-Morales 2012
Antioquia, La Miel River[2]	IAvH-R-5880–81	5.710000	-74.730000	**169**	Borja-Acosta 2017
Caldas, Norcasia, Quiebra Roque, Reserva Río Manso	Observation	5.664167	-74.785833	280	Rojas-Morales 2012
Chocó, Atrato, San Martín de Purré	UTCH 0299	5.649520	-76.579380	80	Rengifo and Pino 2018
Chocó, Atrato, Samurindó	UTCH 0339	5.587500	-76.654167	36	Rengifo and Pino 2018
Caldas, Norcasia, road to Berlín (Samaná)	MHN-UC 0306	5.572690	-74.904020	648	This study
Caldas, Norcasia, Puerto Norcasia, Amaní dam	MHN-UC 0238	5.564766	-74.907133	550	This study
Caldas, Samaná, Cañaveral[7]	MHUA-R 14142	5.537028	-74.908694	**623**	Rojas-Morales 2012
Chocó, Unión Panamericana, Salero	UTCH 1240, 1244	5.360278	-76.645833	115	Rengifo and Pino 2018

Appendix I. *Continued.*

Locality	Record type	Latitude	Longitude	Elevation (m a.s.l.)	Source
Caldas, La Dorada, Guarinocito, Purnio	Photograph	5.355227	-74.798606	**305**	Flórez-Jaramillo and Barona-Cortés 2016
Caldas, Victoria, El Llano[7]	MHUA-R 14785	5.329388	-74.849305	289	Rojas-Morales 2012
Valle del Cauca, Buenaventura, San Cipriano	Observation	3.834167	-76.890556	115	Rojas-Morales 2012
Valle del Cauca, Buenaventura, Guamía	UVC-13662, 13685	3.731667	-76.958333	**41**	Rojas-Morales 2012
Valle del Cauca, Buenaventura, Bajo Anchicayá	UVC-9591	3.613741	-76.910950	536	Salinas and Valencia-Aguilar 2018
Valle del Cauca, Buenaventura, Bajo Anchicayá dam	UVC-5266	3.533263	-76.867508	702	Salinas and Valencia-Aguilar 2018
Huila, Gigante, La Honda	MHNUC-He-0693	2.369611	-75.571722	725	Vera-Pérez *et al.* 2019
Venezuela					
Zulia, Rosario de Perijá, Puerto Nuevo, Cogollo River	MBLUZ R-486	10.346944	-72.516111	350	Rojas-Runjaic and Infante-Rivero 2018
Zulia, Kasmera, Yasa River basin, Sierra de Perijá	MBLUZ R-182	9.943333	-72.749167	270	Pons 1965
Zulia, Ipika, Tokuko River, Sierra de Périjá	MHNLS 20940	9.881667	-72.516111	560	Rojas-Runjaic and Infante-Rivero 2018
Ecuador					
Esmeraldas, Eloy Alfaro, Playa de Oro, Reserva Cotacachi Cayapas	QCAZ-R 10703	0.828464	-78.722010	**567**	Pazmiño-Otamendi 2019
Esmeraldas, Zapallo Grande	QCAZ-R 1595	0.773994	-78.936000	**487**	Pazmiño-Otamendi 2019
Esmeraldas, Eloy Alfaro, Telembí, Reserva Tesoro Escondido	QCAZ-R 15012	0.493760	-79.136080	675	Pazmiño-Otamendi 2019
Manabí, Cuaque River, 10 min from Pedernales, Canoa way	QCAZ-R 8962	-0.021260	-80.068930	**25**	Pazmiño-Otamendi 2019
Manabí, 15 km Pedernales, Jama way	QCAZ-R 5757	-0.078821	-80.021630	36	Pazmiño-Otamendi 2019
Manabí, Jama, Reserva Ecológica Jama-Coaque	QCAZ-R 4652	-0.090978	-80.147190	**3**	Pazmiño-Otamendi 2019
Manabí, Jama, Reserva Ecológica Jama-Coaque	QCAZ-R 5889	-0.109405	-80.117529	392	Pazmiño-Otamendi 2019
Manabí, Jama, Reserva Ecológica Jama-Coaque	No voucher	-0.110156	-80.128183	278	Steinke 2016
Guayas, Naranjal, Molleturo-Mollepungo, Cerro de Hayas	ZSFQ 4081	-2.730800	-79.628600	110	Cruz-García *et al.* 2020

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Albinism in *Natrix tessellata* (Serpentes: Natricidae)

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Abstract

Albinism in *Natrix tessellata* (Serpentes: Natricidae). The Dice Snake, *Natrix tessellata,* varies in color and pattern, and has several color morphs. Except for melanism, color aberrations are quite rare in the species. Of the two published records of albinism in *N. tessellata,* the first, from the mid–19th century in Italy is dubious; the second is from Israel. Herein, albinism in juvenile *N. tessellata* from Slovakia is documented, and the occurrence of albinism in all species of European snakes is discussed.

Keywords: Central Europe, color aberration, Dice Snake, European snakes, Natricinae.

Resumo

Albinismo em *Natrix tessellata* (Serpentes: Natricidae). *Natrix tessellata* varia em cor e padrão e tem várias formas de cores. Exceto pelo melanismo, as aberrações cromáticas são muito raras na espécie. Dos dois registros de albinismo publicados para essa espécie, o primeiro, de meados do século 19 na Itália, é duvidoso; o segundo é de Israel. Documentamos aqui o albinismo em jovens de *N. tessellata* da Eslováquia e examinamos o contexto de albinismo observado entre outras serpentes da Europa.

Palavras-chave: aberração cromática, albinismo, Europa Central, Natricinae, serpentes européias.

Introduction

Natrix tessellata (Laurenti, 1768) comprises nine mitochondrial evolutionary lineages that have a rather uniform morphology (Mebert 2011) across the wide range of the species (Guicking *et al.* 2009). It is one of the most common semiaquatic snake species in the western Palearctic (Gruschwitz *et al.* 1999) belonging to the family Natricidae (Zaher *et al.* 2019); it inhabits watercourses with mostly rocky shores (Conelli *et al.* 2011) and feeds mainly on fish or amphibians (Frotzler *et al.* 2011, Storm 2018). The species is known for its color and pattern variation in natural populations (Sterijovski *et al.* 2011), including different color morphs (e.g., uniformly brown or yellowish), and except for melanism, color aberrations are rare (e.g., In den Bosch *et al.* 1998, Sterijovski *et al.* 2011, Tuniyev *et al.* 2011).

To date, only two albinotic individuals of *Natrix tessellata* have been reported—one from Italy and one from Israel. The albinotic juvenile snake from Kfer Netter, Israel, was found alive; other than a photograph, no further information

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was provided by Werner (2016: 268). The snake from Italy was found by Riccardo Magnani on 09 August 1879 at Cantone delle Tre Miglia, near Pavia in northern Italy. It is a male with a snout–vent length of 44.5 cm and tail length of 11.5 cm (total length, 55 cm). The specimen has two preocular, three postocular, and eight supralabial scales (Razzetti in litt. 2018). Pirotta (1879) described it to be pinkish in color with two darker spots on the scales on the top of the head, and slight brownish shades on ventral and subcaudal scales. However, leucism cannot be excluded in this case as the original author Mr. Magnani placed the specimen in alcohol, believing it a venomous species. After a few hours in alcohol, Pirotta (1879) could not recognize the color of the iris; this led Pirotta (1879) to consider the specimen as either leucistic or albinotic. The snake is deposited in the zoological collection of the museum in the Natural History Museum in Pavia, under voucher number CR0373 (Figure 1).

The first record of albinism in *N. tessellata* from Slovakia reported here is important. Although albinism occurs in all vertebrates, it is rare in wild populations, especially in adults (Ferri and Bettiga 1992). The condition is a genetic anomaly caused by lack of activity of the enzyme tyrosinase, which is responsible for formation of melanin (Creel *et al.* 1974), resulting in the absence of melanin in the skin, the iris, and the choroid (Spadola and Di Toro 2007, Krecsák 2008). Estimates of the occurrence of albinism in the wild vary from 1:10,000 to 1:30,000 (Bechtel 1995).

Materials and Methods

On 16 July 2018 at about 18:00 h, an anonymous person informed MO of a clutch of eggs of *Natrix tessellata* from Šaca, Slovakia (48°38'38.4" N, 21°09'46.8" E; 252 m a.s.l.), where the species is known to occur (Lác and Lechovič 1964). The clutch of 18 eggs was

Figure 1. A potentially albinotic individual of *Natrix tessellata* found in Pavia in 1879 and deposited in the Natural History Museum in Pavia, Italy (voucher no. CR0373).

between wooden boards to be used for construction. Three mold-infested, unfertilized eggs were removed from the clutch, and the rest of the eggs were placed in an incubator. During incubation (moist vermiculite; 26°C; 60–70% humidity), two eggs started to rot and were removed on 20 and 23 July 2018. On 11 August 2018, 13 hatchlings, eight normally colored and five albinos, hatched after 26 days inside the incubator. The incubation period for *N. tessellata* eggs for Central Europe is 41–72 days (Velenský *et al.* 2011). All individuals are kept in plastic boxes with pine bark. Each box also contains a large water bowl, a shelter, and a branch of appropriate size. Photoperiod and heating are provided by heat lamps with temperatures reaching up to 28°C in a basking spot and room temperature in other parts of the enclosure. Individuals are fed separately every 7–10 days on aquarium fish provided by local breeders. The eggs were collected and maintained in captivity under permit number 10783/2018-6.3 from the Ministry of Environment of the Slovak Republic—Directorate for Nature, Biodiversity and Landscape Protection. Blood samples were taken from all individuals and each has its voucher number (DJ8522–8534), deposited in the collection of the Department of Zoology, Comenius University in Bratislava.

Morphometric and meristic data for all snakes were recorded 1 wk after the snakes hatched. The following measurements were taken by SP with a digital caliper to the nearest 0.1 mm: head length (HL), head width (HW), mouth length (ML). Snout–vent length (SVL), tail length (TL) and total length (TotL = SVL + TL) were measured using a tailor´s tape measure. The following meristic characters were recorded: preocular (PREOC), postocular (POSTOC), supralabial (SUPL), sublabial (SUBL), ventral (VENT), subcaudal scales (SUBC) and dorsal scale row (DORS), following the methods of Moravec (2015). We also documented possible anomalies in scalation. All individuals were photographed 30 days after hatching from the dorsal and ventral sides and close up photographs of the head from upper, bottom and lateral side were taken. Detailed photos of five albino individuals are presented in Appendix I. Six individuals [two albinotic (DJ8524, DJ8526) and four normally colored (DJ8530–8532, DJ8534)], died independently within 5 mo after hatching. One year after hatching, the remaining living individuals were measured (Table 1). We compared all metric and meristic characters with results of Laňka (1973) and Opoldusová (2008) and with our own data for *N. tessellata* from Slovakia. At the time of this writing (April 2020), three albinotic and four normal individuals are being kept in captivity.

To compare rarity of albinism in other European species of snakes, we reviewed and summarized published and unpublished (observations from field herpetologists supported by a photo; Appendix II) data about albinotic snakes (Table 2).

Results

Albinotic individuals from Šaca, Slovakia, have unpigmented, pinkish skin, and reddish eyes lacking pigmentation (Figure 2; Appendix I). Darker pinkish spots are present on cephalic scales, mainly on the parietal, frontal and supraocular scales. The inverted V-pattern behind the head is not as visible as it is in normally colored individuals, but darker spots occur on a few scales in that region. There is a ventral pattern of alternating yellowish and pinkish spots in each albino snake (Figure 2).

At hatching, all juvenile *N. tessellata*—both albinotic and normally colored—had relatively similar SVL and head measurements (HL, HW, and ML), but TL differed sexually. Mean SVL of newly hatched juveniles was 23.27 cm (range 21.40–24.00; SD ± 0.63). Similarly, mean TL was 6.16 cm (range 5.40–6.80; SD ± 0.46). Of the13 hatchlings, seven were males and six females; a total of five was albinotic—four males and one female. All metric and meristic data are summarized in Table 1. There were no anomalies in the numbers of PREOC and POSTOC scales.

Table 1. Basic metric (cm) and meristic characteristics recorded from all hatchling *Natrix tessellata* from one clutch. Metric data from one-year-old snakes shown in italics. SVL = snout–vent length; TL = tail length; TotL = total length; HL = head length; HW = head width; ML = mouth length; PREOC = preocular; POSTOC = postocular; SUPL = supralabial; SUBL = sublabial; VENT ventral = SUBC = subcaudal scales; DORS = dorsal scale row.

No.	Sex	SVL	TL	TotL	HL	HW	ML	PREOC	POSTOC	SUPL	SUBL	VENT	SUBC	DORS	Ventral coloration
8522	M	23.2 *28.1*	6.4 *7.8*	29.6 *35.9*	1.3 *1.4*	0.6 *0.7*	1.0 *1.1*	2/2	3/3	7/7	8/8	174	71	19	Many yellow spots on pinkish base
8523	F	23.3 *31.3*	5.7 *8.1*	29.0 *39.4*	1.4 *1.7*	0.6 *0.7*	1.0 *1.2*	2/2	3/3	8/9	9 + 1/9	168	60	19	As above
8524	M	24.0	6.3	30.3	1.4	0.6	1.0	2/2	3/3	8/8	8/8	175	72	19	Few yellow spots on pinkish base
8525	M	23.1 *29.1*	6.2 *8.2*	29.3 *37.3*	1.3 *1.4*	0.6 *0.7*	1.0 *1.1*	3/2	3/3	8/8	9/8	178	72	19	Many white-yellow spots on pinkish base
8526	M	23.9	6.5	30.4	1.3	0.6	0.9	2/2	3/3	8/8	9/8	176	69	19	white-yellow spots on pinkish base
8527	F	23.3 *27.4*	5.7 *7.2*	29.0 *34.6*	1.3 *1.5*	0.6 *0.7*	1.0 *1.1*	2/2	3/3	8/7	9/8	168 + 3	61	19	White-yellow spots on brown base
8528	F	23.3 *28.3*	5.4 *6.7*	28.7 *35.0*	1.3 *1.4*	0.6 *0.6*	1.0 *1.0*	2/2	3/3	8/8	9/8	170	60	19	white spots on brown base
8529	M	23.4 *32.0*	6.8 *9.2*	30.2 *41.2*	1.2 *1.5*	0.6 *0.7*	1.0 *1.1*	2/2	3/4	8/7	8/8	175	69	19	white-yellow spots on brown base
8530	M	23.3	6.5	29.8	1.3	0.6	0.9	2/2	3/3	7/7	8 + 1/9	176	68	19	As above
8531	M	23.2	6.4	29.6	1.2	0.6	1.0	2/2	3/3	7/7	8/8	174 + 2	70	19	As above
8532	F	21.4	5.4	26.8	1.2	0.6	1.0	2/2	3/4	6/7	8/9	169 + 4	58	19	Yellow spots on brown base
8533	F	23.7 *32.4*	6.3 *8.6*	30.0 *41*	1.3 *1.6*	0.6 *0.8*	1.0 *1.3*	2/2	3/3	8/7	8/9	171 + 3	62	19	white-yellow spots on brown base
8534	F	23.4	6.5	29.9	1.2	0.5	0.9	2/2	3/3	8/8	8/8	178	71	19	As above

Table 2. List of published and unpublished data recording albinotic species of snakes in Europe; *true albinism not verified. Unpublished observations (in litt. or pers. comm.) are supplemented by photos and presented in Appendix II.

Species	*N*	Country	References
Coronella austriaca	1	Austria	Happ 1994
	2	Czech Republic	Rehák 1992, B. Trapp (in litt.)
	1	Italy	Pirotta 1879
	1	Netherlands	Lenders 2008
	1	Slovakia	D. Revaj (pers. comm.)
Coronella girondica	1	France	Geniez and Grillet 1989
	1	Spain	Martínez-Silvestre *et al.* 2009
Dolichophis jugularis	1	Cyprus	Baier *et al.* 2013
Elaphe sauromates	2	Bulgaria	Petzold 1975, Jablonski *et al.* 2019
Hierophis viridiflavus	1	Italy	Scali 1992
Malpolon monspessulanus	1	Spain	Martínez-Silvestre and Soler 2018
Natrix helvetica	2	France	Baudin 2003, Varanguin 2012
	1	U.K.	Boulenger 1913
Natrix maura	5	Spain	Pérez and Collado 1975, Herrador and Pulido 2006, Alaminos and López 2011, L. Gonzáles García (in litt.), E.R. Ara (in litt.)
Natrix natrix	1	Austria	Sackl and Putz 2002
	1	Czech Republic	Musilová *et al.* 2006
	1	Germany	M. Bollhorn (in litt.)
	1	Poland	T. Krajča (pers. comm.)
*Natrix tessellata**	1	Italy	Pirotta 1879
Natrix tessellata	5	Slovakia	This study
Vipera ammodytes	1	Italy	Krecsák 2008
Vipera aspis	3	France	Bruno 1985, Naulleau 1997, Guiller 2007
Vipera berus	1	Bulgaria	Stojanov 2014
	2	Finland	Vainio 1931, Krecsák 2008
	1	Germany	Buchner 1917
	2	Sweden	Edelstam 1971, Krecsák 2008
	1	Slovakia	Gezova *et al.* 2018
	4	U.K.	Leighton 1901, Harris 1936, Krecsák 2008
Zamenis longissimus	3	Austria	Erber 1879, Sochurek 1955, Esterbauer 2014
	1	Bosnia & Herzegovina	Ćurić 2019
	1	Italy	Ferri and Bettiga 1992
	1	Serbia	Radovanović 1941
	8	Slovakia	Balthasar 1935, Gabzdil 2003, Musilová *et al.* 2006, Gezova *et al.* 2018, S. Kľúčiková-Píšová (in litt.), D. Revaj (pers. comm.), Ľ. Magyariová and Š. Bánovský (in litt.)
	1	Slovenia	Krofel 2004
	1	Switzerland	Bruno and Maugeri 1990
Zamenis scalaris	4	Spain	Rollinat 1934, Lesparre 2001, Manjón 2011, D. Lerena and R.C. Zárate (in litt.)

Figure 2. Comparison of normally colored and albinotic hatchlings of *Natrix tessellata* from Šaca, Košice, Slovakia: frontal (**A**), dorsal (**B**), and ventral (**C**) views.

However, the following anomalies in scalation were observed in 11 of the 13 individuals: fused SUPL in DJ8522; fused SUBL in DJ8533; inserted SUBL in DJ8523 and DJ8530; fused SUBC in DJ8522–8524, DJ8526, DJ8529, and DJ8531; inserted VENT in DJ8527 and DJ8531–8533; and split VENT in DJ8528 (numbers listed in Table 1).

We found records of a total of 65 truly albinotic snakes representing four families and eight genera in Europe (Table 2); this total includes the five juveniles from Šaca, Slovakia, as a new record for Europe. The total excludes the dubious record of *N. tessellata* (CR0373) from Pavia, Italy, as well as the albinotic Israelic snake (Werner 2016). Among the albinotic snakes recorded in Europe, the genus *Zamenis* contains the most albinos (20 individuals, representing ~ 33% of the total; the five individuals from Šaca were excluded from this calculation because they were found as eggs and hatched in captivity), which were found in eight countries. Only one albino snake is recorded for each of the genera *Dolichophis* (Cyprus), *Hierophis* (Italy), and *Malpolon* (Spain). Spain has the greatest number of albinotic snakes; a total of 11 individuals of four species is known—*Coronella girondica* (Daudin, 1803), *Malpolon monspessulanus* (Hermann, 1804), *Natrix maura* (Linnaeus, 1758), and *Zamenis scalaris* (Schinz, 1822). Each of the following countries has only one record of an albino snake: the Netherlands, Bosnia and Herzegovina, Cyprus, Poland, Serbia, Slovenia, and Switzerland. In the genus *Natrix,* albino individuals occur in almost all species [viz., *N. natrix* (Linnaeus, 1758), *N. helvetica* (Lacépède, 1789), *N. maura,* and *N. tessellata*] with the exception of *N. astreptophora* (Seoane, 1885). Four albino *N. natrix* were found in each of four countries (Austria, Czech Republic, Germany, and Poland), three *N. helvetica* in two countries (France and the United Kingdom), and five *N. maura* in one country (Spain; Table 2). Six truly albinotic *N. tessellata* individuals occur in two countries—one in Israel and five described here from Slovakia (excluding the dubious record from Italy).

Discussion

Albinism in snakes is considered to be a rare, autosomal recessive mutation with an expected ratio of normally colored to albinotics of 3:1 if mating two heterozygotes (Clay 1935, Bechtel and Bechtel 1985). When mating heterozygotes and homozygotes, the probability of albinotic juveniles is approximately 50% (Bechtel and Bechtel 1985). When two homozygotes mate then 100% of offspring will be of the albino phenotype. However, without information on the coloration of unhatched *N. tessellata,* we can only estimate the genotype of the parents; most likely one was a heterozygote and the other a recessive homozygote. The frequency of recessive alleles is greater in small, fragmented populations (Laikre 1999) and that could be the case in this study. The population in eastern Slovakia is at the northern edge of the range of the species (Gezova and Jablonski 2018) and one might assume that populations occurring near the range limits have lower densities and effective population sizes (Vucetich and Waite 2003). The occurrence and survival of albinotic individuals also may be related to abiotic environmental features such as elevation and temperature (Kehas *et al.* 2005). Moreover, environmental pollution (Kolenda *et al.* 2017) that may occur in an urban area, such as the one where the egg clutch was found, may be correlated abnormal scutelation in reptiles, but the relationship has not been well studied (Mulder 1995, Cruz-da-Silva *et al.* 2018 and literature therein). However, anomalous scalation in *N. tessellata* is not unusual in central Europe (e.g., Laňka 1978, Rehák 1989, Baruš *et al.* 1992).

Abnormally colored species of snakes may be subject to higher predation pressure by visually oriented predators, and thus, be negatively affected by the lack of pigmentation because they are more easily visible. In addition, reproductive fitness of albino snakes is reduced (Krecsák 2008, McCardle 2012). The lack of pigment in the eyes adversely affects vision; thus, it is more difficult for albino snakes to find suitable prey, avoid danger, and perceive visual signals during mating season (Miller 2005, Krecsák 2008). These facts may partially account for the fact that more albino juveniles were encountered in the wild than adults (e.g., Alaminos and López 2011, Gezova *et al.* 2018, Martínez-Silvestre and Soler 2018). Nevertheless, albinotic adults of several genera of European snakes, including the genus *Natrix,* have been observed in the wild (e.g., Sackl and Putz 2002, Krecsák 2008, Alaminos and López 2011, Bruni 2017, Jablonski *et al.* 2019). Krecsák (2008) posits that there are more aberrant European Viperinae snakes in northern regions than in southern regions; however, we observed the opposite in our review in which the most albinos occurred in central Europe and the Iberian Peninsula with the greatest number of albinos recorded in the genera *Zamenis, Vipera,* and *Natrix.* We suggest that there is no clear pattern in albinism in European snakes and that albinotic individuals seem to be distributed randomly in the natural populations. However, ecological, and environmental factors need to be investigated more thoroughly.

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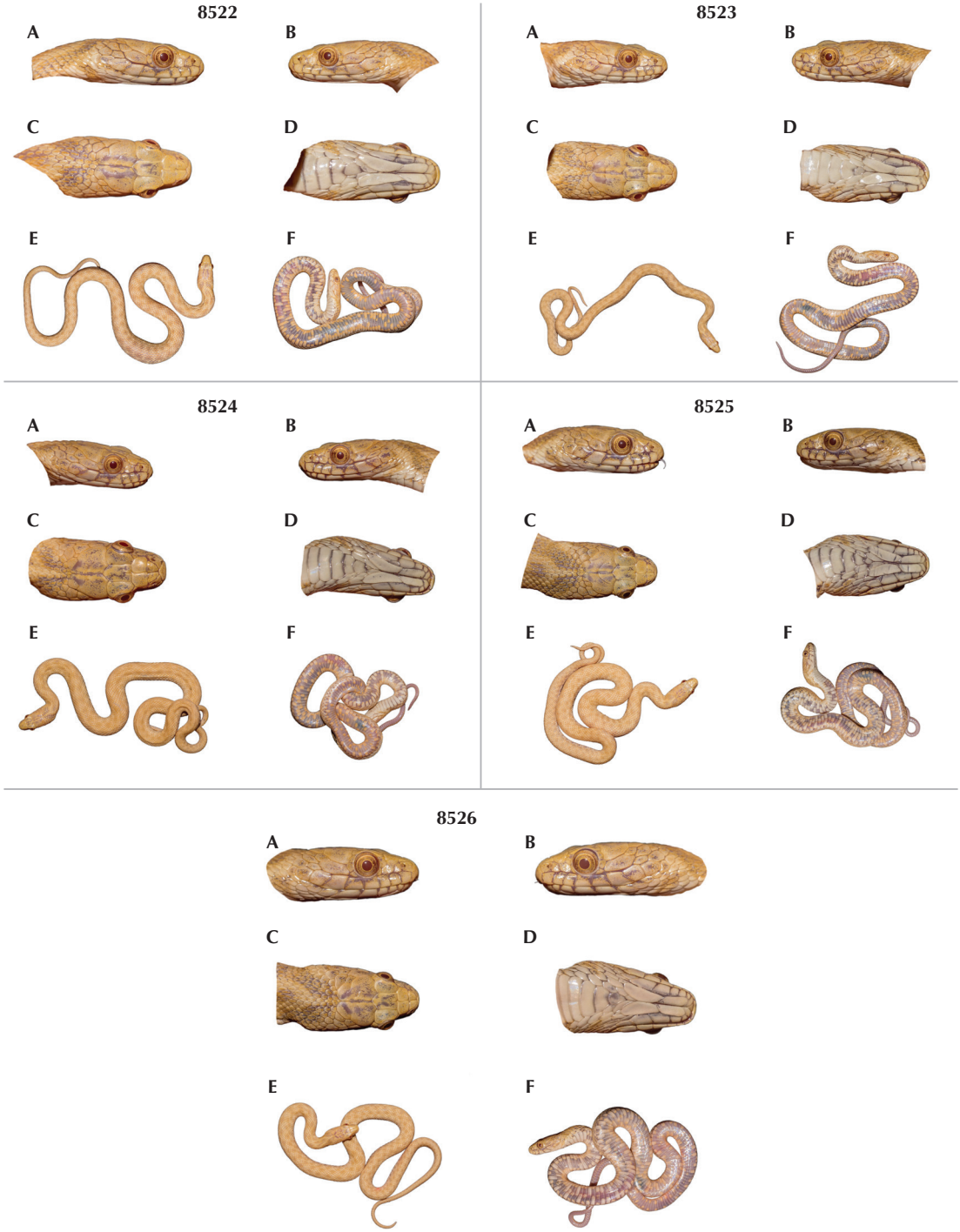
Appendix I. *Albinotic individuals No. 8522–8526 from* Šaca, *Košice. Head in lateral (**A, B**), dorsal (**C**) and ventral (**D**) views. Dorsal (**E**) and ventral (**F**) views of body. Numbers of individuals written below correspond to the numbers listed in Table 1.*



Appendix II. *Previously unpublished photographs of albinotic snakes from Europe.* (***A***) Coronella austriaca, *Czech Republic (B. Trapp);* (***B***) C. austriaca, *Slovakia (D. Revaj);* (***C***) Natrix maura, *Spain (L. Gonzáles García);* (***D***) N. maura, *Spain (E.R. Ara);* (***E***) Natrix natrix, *Germany (M. Bollhorn);* (***F***) N. natrix, *Poland (T. Krajča);* (***G***) Zamenis longissimus, *Slovakia (S. Kľúčiková-Píšová);* (***H***) Z. longissimus, *Slovakia (L. Magyariová and Š. Bánovský);* (***I***) Z. longissimus, *Slovakia (D. Revaj);* (***J***) Zamenis scalaris, *Spain (D. Lerena and R.C. Zárate).*

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Trophic ecology and morphology of *Anolis bartschi* (Squamata: Dactyloidae) in Viñales National Park, Cuba

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Abstract

Trophic ecology and morphology of *Anolis bartschi* (Squamata: Dactyloidae) in Parque Nacional Viñales, Cuba. Little is known about the trophic ecology of most anoles of Cuba. Morphology is directly related to ecological functions in lizards, such as feeding strategies, interspecific competition or energetic demands linked to reproduction. *Anolis bartschi* is a regionally endemic species, restricted to karstic hills of western Cuba. Here, we offer new insights into the trophic ecology of this species, and its relation to head morphology. We captured 131 adults; males were larger than females in size and head width. Most of them had prey in their stomachs. Males consumed more prey than females, but the latter consumed larger prey. Prey overlap within sexes was detected in the dry season, but trophic segregation occurred in the rainy season. Hymenoptera was the most frequently consumed prey in both sexes. In addition, females ate Blattodea and Coleoptera, and males consumed more Diptera. We suggest that this lizard prefers sedentary rather than mobile prey. According to our dataset and field observations, *A. bartschi* is a bimodal forager lizard, but research on temporal (daily and annual) variation in diet is recommended for a proper forage classification of this lizard.

Keywords: conservation, diet, food niche, lizards, niche overlap, protected area, West Indies.

Resumen

Ecología trófica y morfología de *Anolis bartschi* (Squamata: Dactyloidae) en el Parque Nacional Viñales, Cuba. Se conoce poco sobre la dieta de los anolinos cubanos. La morfología en lagartos está directamente relacionada con funciones ecológicas, como estrategias de forrajeo, competencias interespecíficas, o demanda energética ligada a la reproducción. *Anolis bartschi* es un endémico regional, restringido a las alturas cársicas del occidente de Cuba. Aquí ofrecemos datos nuevos sobre la ecología trófica de esta especie y su relación con la morfología de su cabeza. Se capturaron 131

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individuos adultos, donde los machos poseen mayor tamaño del cuerpo y de la cabeza que las hembras. La mayoría de los individuos tenía presas en el estómago. Los machos consumirán más presas que las hembras, pero de menor tamaño que estas. Se detectó solapamiento trófico en época de seca, pero hubo segregamiento trófico en lluvia. Hymenoptera fue la presa más consumida por ambos sexos. Además, las hembras consumieron más Blattodea y Coleoptera, mientras los machos más Diptera. Esta especie prefiere presas sedentarias en lugar de móviles. Sugerimos que esta especie prefiere presas sedentarias a presas móviles. De acuerdo a nuestros datos y las observaciones de campo, *A. bartschi* es un forrajeador bimodal, aunque se recomiendan futuras investigaciones sobre la variación temporal (diaria y anual) en la dieta de esta especie para una apropiada clasificación sobre su actividad forrajeadora.

Palabras claves: Caribe, conservación, dieta, lagarto, solapamiento trófico.

Resumo

Ecologia trófica e morfologia de *Anolis bartschi* (Squamata: Dactyloidae) no Parque Nacional Viñales, Cuba. Pouco se sabe sobre a ecologia trófica da maioria dos anoles de Cuba. Nos lagartos, a morfologia está diretamente relacionada com funções ecológicas, tais como estratégias de forrageio, competição interespecífica ou demandas energéticas ligadas à reprodução. *Anolis bartschi* é uma espécie endêmica regional, restrita a colinas cársticas do oeste de Cuba. Apresentamos aquí novos dados sobre a ecologia trófica dessa espécie e sua relação com a morfologia da cabeça. Capturamos 131 adultos; os machos eram maiores do que as fêmeas em tamanho na largura da cabeça. A maioria dos indivíduos apresentou presas nos estômagos. Os machos consumiram mais presas do que as fêmeas, mas estas últimas consumiram presas maiores. Na estação seca, detectamos uma sobreposição de presas entre os sexos, mas na estação chuvosa ocorreu segregação trófica. Hymenoptera foi a categoria de presas mais frequentemente consumida em ambos os sexos. Além disso, as fêmeas consumiram Blattodea e Coleoptera, e os machos, mais Diptera. Sugerimos que esse lagarto prefere presas sedentárias em vez de presas móveis. De acordo com nosso conjunto de dados e observações de campo, *A. bartschi* é um lagarto forrageador bimodal, mas recomenda-se a investigação sobre a variação temporal (diária e anual) da dieta para uma classificação adequada de sua estratégia de forrageio.

Palavras-chave: área protegida, conservação, dieta, Índias Ocidentais, lagartos, nicho trófico, sobreposição de nicho.

Introduction

In ecology, the knowledge of diet and trophic interactions are essential for understanding aspects of the natural history, population dynamics, assessment of energy flow, and food webs in the communities (Anderson 1991, Solé and Rödder 2010). Dietary information can aid in elucidating predator-prey interactions, especially in endangered species (Solé and Rödder 2010), and this knowledge may lead to the development of management and conservation programs (Anderson 1991). Morphological traits may be directly related to ecological functions in lizards, such as feeding strategies (e.g. sit-and-wait or active foragers), interspecific competition (Astudillo *et al*. 2015), or energetic demands (Andrews and Asato 1977), because differences in lizard size may lead to differences in resource intake (Pianka 1986, Herrel *et al*. 2004). In trophic ecology, morphology of the head is essential to understand the diet preferences of lizards (Schoener and Gorman 1968, Gvozdik and Boukal 1998, Beuttner and Koch 2019) because lizards with broader and higher heads are capable of producing stronger bite forces (McBrayer and Corbin 2007, Miles *et al*. 2007, Losos 2009,

Campos 2016) and are thus capable of feeding on harder prey (Herrel *et al*. 2001a, b, Verwaijen *et al*. 2002, Herrel 2007, Huyghe *et al*. 2009, Losos 2009).

In Cuba, 64 species of lizards belong to the family Dactyloidae, all within the genus *Anolis* (Torres *et al*. 2017). The diet of most anoline lizards in western Cuba is poorly known. Martínez (1998) analyzed the diet of anoline lizard communities in western Cuba, and Pérez and Rodríguez (2009) and Rodríguez (2010) offered new data on the diet of two related species, *A. quadriocellifer* and *A. homolechis,* respectively. Most published records of the diet of *Anolis* are fortuitous observations (Valido 2006, Cajigas *et al*. 2018, Armas and Iturriaga 2019, Iturriaga and Armas 2019). Such studies suggested that these lizards mostly consume hymenopterans (especially ants) (Martínez 1998, Pérez and Rodríguez 2009, Rodríguez 2010), coleopterans (Pérez and Rodríguez 2009), and heteropterans (Rodríguez 2010), independent of ecomorph or body size.

Anolis bartschi (Cochran, 1928) is a regional endemic species, restricted to cliffs of karstic hills (*mogotes*) of Pinar del Río Province. It is known from 49 localities in Sierra de los Órganos (León *et al.* 2019) located at the western part of Cordillera de Guaniguianico, in Cuba. This species is associated with rock walls and reproduces at the entrances of caves (Novo and Estrada 1986, Henderson and Powell 2009). Due to its particular morphology (e.g. extremely long hindlimbs) and ecology (e.g. rock-cave dweller) (Losos 2009), *A. bartschi* is not included in any of the traditionally recognized ecomorph categories (Poe 2005, Losos 2009). In addition, this species has not been assigned to any IUCN threat category (Torres López *et al.* 2017). In terms of trophic ecology, Rodríguez and Martínez (1992) studied a single population at San Vicente, Viñales National Park, and offered the first data on the diet of this species. They found that it feeds mostly on ants, snails, beetles, dipterans, roaches, arthropod larvae, and plant material (Rodríguez and Martínez 1992). No further research on the trophic ecology of this species has been conducted for more than 27 years following this diet analysis. In this paper, we offer new data on the trophic ecology of *Anolis bartschi* from two populations in Viñales National Park, Cuba. Because differences in head morphology could determine prey preferences in lizards (Losos 2009), we analyzed the head morphology of *A. bartschi* to determine whether this species exhibits different feeding behavior in the two localities and between the sexes.

Materials and Methods

Fieldwork

We visited two karstic hills (*mogotes*) in Viñales National Park: Mogote El Capón (22°38'208" N, 83°41'448" W; WGS 84, ~139 m a.s.l.), and San Vicente (22°40'123" N, 83°42'115" W; WGS 84, ~131 m a.s.l.). Each area was visited once in March and April (dry season), and in June and August (wet season) of 2019. We performed 100 m-transect surveys to capture lizards in each area. In order to avoid bias due to niche characteristics, both transects were selected to be homogeneous in soil characteristics, altitude, and vegetation type. The forests of these *mogotes* are mesophyllic semi-deciduous distributed in strips at the base of the mogotes (Luis 2000). The typical vegetation is composed of *Oxandra lanceolata* (Sw.) Baill. (Annonaceae), *Pachira emarginata* A. Rich. (Malvaceae), *Spathelia brittonii* P. Wilson (Rutaceae), and large trees, such as *Ficus* spp. (Moraceae) (Luis 2000).

Data Analysis

To obtain diet information we used the stomach flushing method following Legler and Sullivan (1979). Snout–vent length (SVL) and head width (HW; measured at the junctions of the mandible) were measured on each individual with a caliper to the nearest 0.02 mm. The

specimens were sexed *in situ* and released at the site of capture. Stomach content samples were placed in 20 cm^3 containers with 75% ethanol immediately after extraction and identified in the laboratory under a dissecting microscope to the lowest possible taxonomic level (Espinosa and Ortea 2009, Mancina and Cruz 2017).

We determined the prey volume (mm^3) following the ellipsoid formula $V = 4/3\pi\ (L/2)\ (W/2)^2$ (Magnusson *et al.* 2003). In addition, we report the total number of consumed items (*N*) and calculated the percent ($N_{\%}$); the frequency of occurrence (*F*, number of stomachs in which a given prey category was found); and the percent ($F_{\%}$) and the volume (V) and its percent ($V_{\%}$) for each prey category. The Index of Relative Importance is calculated for every prey category by $IRI = (N_{\%} + V_{\%} + F_{\%})/3$ (Biavati *et al.* 2004). We use the Levins index (*B*) (Krebs 1989) to calculate the trophic niche breadth: $B = 1/(\Sigma Pi^2)$, where Pi = fraction of items in the prey category *i*; range from 1 to *N*. Values of B vary from 1 (no diversity, exclusive use of a single prey category, specialist) to *N* (highest diversity, prey items in the majority of categories, generalist). In addition, we calculated niche overlap using Pianka's overlap index (*POI* hereafter) (Pianka 1973), which varies from 0 (no overlap) to 1 (complete overlap). We compared male-female food niche breadth and niche overlap between dry and wet season.

To define the foraging strategy of *Anolis bartschi*, the consumed prey was categorized as either "sedentary" or "mobile" following Beuttner and Koch (2019). We classified as sedentary the prey that moves slowly and/or only covers short distances during movements (e.g. slugs, ants, diplopods, Lepidoptera larvae), and mobile as prey that moves fast enough to escape from capture and/or can cover great distances (over 3 m from predator) (e.g. bees, dipterans, crickets, chilopods, roaches).

To demonstrate if the data were homogenous, we applied the Kolmogorov-Smirnov normality test and the Levene variance homogeneity test. Because the data are normally distributed, T-tests and one factor ANOVAs were used to determine if significant differences exist for (1) the total prey consumed by males and females in each area; (2) the total amount of prey items consumed in each study area; and (3) the SVL and head width of the lizards in the two study areas. We also use the Pearson correlation coefficient for niche overlap between males and females. A simple linear regression is made to test the tendencies and correlations between SVL/HW, and HW/prey length.

Results

Morphological Analysis

There were 131 adults of *Anolis bartschi* captured, 66 males and 65 females. Females are smaller (range; mean ± SD) (57.3–69.68 mm; 63.2 ± 4.92, $N = 46$) in SVL than males (69.92–81.28 mm; 73.37 ± 5.01, $N = 56$) ($p = 0.000$; $F = 0.590$; $N = 102$). No differences in size were found when comparing males and females together from both localities ($p = 0.730$; $N = 102$). Comparison of the SVL of males between areas showed no significant difference ($p = 0.718$; $F = 2.092$; El Capón: 73.42 ± 5.08 mm, $N = 32$; San Vicente: 78.41 ± 2.01 mm, $N = 24$). Differences in SVL were found among females ($p = 0.001$; $F = 2.558$; El Capón: 67.94 ± 1.65 mm, $N = 27$; San Vicente: 61.88 ± 2.97 mm, $N = 19$). In all individuals, HW was significantly wider in males than in females ($p = 0.020$; $F = 5.606$; $N = 87$), and a positive correlation was found between SVL and HW (Figure 1).

Diet Analysis

A total of 102 individuals of *Anolis bartschi* had prey in the stomachs (77.9 % of all captured individuals), whereas 22.1% had empty stomachs ($N = 29$). We found 19 prey categories in the stomachs, plus ripe fruits, reptile skin molt (Table 1), plant material (other than ripe fruits),

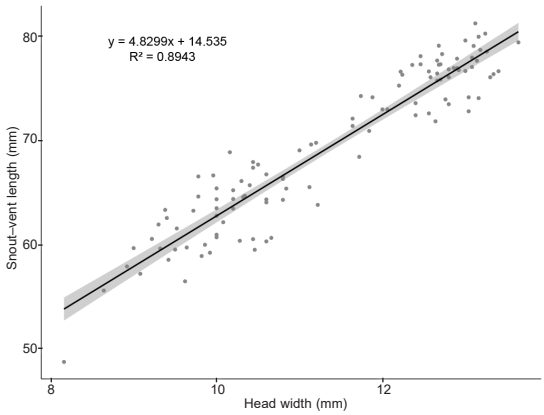

Figure 1. Correlation between snout–vent length and head width in adults of *Anolis bartschi* (*N* = 112) from both study areas.

and dirt/stones. Were found 346 prey items in the stomachs of *A. bartschi*, with a rate of 3.39 prey items/stomach (*N* = 102). All females together had consumed 184 prey items (3.41 prey items/stomach, *N* = 54), and had a large volume of prey (*V* = 6152.58, total $V_{\%}$ = 58.7). Males had consumed 162 prey items together (3.38 prey items/stomach, *N* = 48), with a smaller volume of prey items than females (*V* = 4328.62, total $V_{\%}$ = 41.3) (Table 1).

We found that *A. bartschi* consumed more prey items during the dry season (206 total items consumed, with a rate of 4.38 prey/stomach; *N* = 47) than during the wet season (146 total items consumed, with 2.65 items/stomach; *N* = 55) (p = 0.025; *F* = 14.585) (Table 2).

With regard to the prey niche breadth, males consumed more prey categories in both seasons (Levin index, dry *B* = 2.74, wet *B* = 2.53) than females (dry *B* = 2.53, wet *B* = 1.56). No significant differences were observed in niche overlap (Pearson index, $p < 0.05$) between sexes in both seasons. In the dry season males and females shared almost the same food resources (*POI* = 0.942), whereas we observed some segregation in food resource exploitation (*POI* = 0.877) in the wet season.

Stomach Contents

In *Anolis bartschi,* the most abundant prey consumed was Hymenoptera, detected in 63.73% of samples, followed by Coleoptera (25.49%), Arachnida and Gastropoda (with 20.59% each). Within Hymenoptera, Formicidae was found in 42.5% of the stomach samples. We especially note the presence of large numbers of invasive fire ants [*Wasmannia auropunctata* (Roger, 1863)], with a mean of 1.16 individuals per stomach (Table 1). Except for the large consumption of hymenopterans by both sexes, differences in prey preference were detected: females consumed mostly Coleoptera and Blattodea, whereas males consumed more Diptera and Gastropoda (Table 1). All Lepidoptera found in the stomachs were still in the larval stage. We were able to identify the genus or species of some taxa consumed: bee (*Apis mellifera* Linnaeus, 1758; Hymenoptera, Apidae), amblypygid (*Phrynus* sp.; Phrynidae), cockroaches (*Eurycotis cribosa* Rehn and Hebard, 1927 and *Periplaneta americana* Linnaeus, 1758; Blattodea), cave cricket [*Otteius thoracicus* (Saussure, 1874); Orthoptera], earwig [*Euborellia annulipes* Lucas, 1847); Dermaptera], land snails (*Jeanneretia* sp., *Zachrysia* sp., Gastropoda), and land slug (*Leidyula* sp., Gastropoda). In addition, we observed fruits in the stomach of 14 lizards (13.7% of the sampled lizards), but only *Ficus* sp. could be identified, which was observed in four stomach samples.

Foraging Strategy

In general, *A. bartschi* preferred sedentary over moving prey (Table 2). In this study 87 items ($N_{\%}$ = 26.0%) were assigned to moving prey, with a volume of *V* = 1587.26 mm³ ($V_{\%}$ = 15.2%), which were found in 71 stomachs ($F_{\%}$ = 35.6 %). In contrast, 247 items were sedentary prey ($N_{\%}$ = 43.4%), with a volume of *V* = 8887.79 mm³ ($V_{\%}$ = 84.9%), which were found in 129 stomachs ($F_{\%}$ = 65.0%). We also observed that the *IRI* of sedentary prey (149.21) is 2.9 times higher than the *IRI* of mobile prey (51.31). We found higher values in

Table 1. Prey consumed by *Anolis bartschi*. For each category the number (*N*), the percent by number ($N_{\%}$), the frequency (*F*), the percent by frequency ($F_{\%}$), the volume (V; mm^3), and the percent ($V_{\%}$) is given. The Index of Relative Importance (*IRI*) of each prey category is given for males, and females separately. The volume and *IRI* of ripe fruits and skin molt could not be calculated because no entire item was obtained.

Groups	**Males (*N* = 48)**				**Females (*N* = 54)**			
	F (%)	*N* (%)	*V* (%)	*IRI*	*F* (%)	*N* (%)	*V* (%)	*IRI*
Diplopoda	2 (2.25)	2 (1.18)	79.98 (1.85)	1.76	4 (3.64)	4 (2.44)	197.0 (3.2)	3.09
Chilopoda	2 (2.25)	2 (1.18)	115.23 (2.66)	2.03	0	0	0	0
Scutigeromorpha	1 (1.12)	1 (0.59)	138.71 (3.2)	1.64	0	0	0	0
Orthoptera	2 (2.25)	2 (1.18)	305.32 (7.05)	3.49	3 (2.73)	3 (1.83)	472.59 (7.68)	4.08
Blattodea	5 (5.62)	5 (2.96)	198.0 (4.57)	4.38	9 (8.18)	9 (5.49)	381.17 (6.2)	6.62
Coleoptera	9 (10.11)	9 (5.33)	6.9 (0.16)	5.2	17 (15.45)	18 (10.98)	14.87 (0.24)	8.89
Hexapoda (unidentified)	1 (1.12)	1 (0.59)	1.54 (0.04)	0.58	0	0	0	0
Hymenoptera (non Formicidae)	13 (14.61)	16 (9.47)	3006.47 (69.46)	31.18	13 (11.81)	19 (11.59)	4431.2 (72.02)	31.81
Formicidae	22 (24.72)	87 (51.48)	15.16 (0.35)	25.52	17 (15.45)	58 (35.37)	14.3 (0.23)	17.02
Hemiptera	0	0	0	0	5 (4.55)	6 (3.66)	97.46 (1.58)	3.26
Dermaptera	0	0	0	0	1 (0.91)	1 (0.61)	35.51 (0.58)	0.7
Lepidoptera (larvae)	4 (4.49)	4 (2.37)	271.9 (6.28)	4.38	5 (4.55)	5 (3.05)	312.8 (5.08)	4.23
Diptera	10 (11.24)	22 (13.02)	16.95 (0.39)	8.22	8 (7.27)	11 (6.71)	15.03 (0.24)	4.74
Aranae	4 (4.49)	4 (2.37)	67.0 (1.55)	2.8	8 (7.27)	8 (4.88)	72.65 (1.18)	4.44
Amblypygi	3 (3.37)	3 (1.78)	22.41 (0.52)	1.89	3 (2.73)	3 (1.83)	19.98 (0.32)	1.63
Opiliones	1 (1.12)	1 (0.59)	3.01 (0.07)	0.59	2 (1.82)	2 (1.22)	5.73 (0.09)	1.04
Isopoda	0	0	0	0	4 (3.64)	6 (3.66)	3.62 (0.06)	2.45
Gastropoda	10 (11.24)	10 (5.92)	80.1 (1.85)	6.34	11 (10.0)	11 (6.71)	78.67 (1.28)	6.0
Ripe fruits	5 (5.62)	5 (5.92)	–	–	9 (8.18)	9 (5.49)	–	–
Skin molt	3 (3.37)	3 (1.78)	–	–	2 (1.82)	2 (1.22)	–	–

sedentary prey among males and females compared with mobile prey items (Table 2). Although sedentary prey items were preferred by both sexes, females consumed slightly more mobile prey than males, which ate larger numbers of sedentary prey items than females (Table 2).

Discussion

Herein we report the largest SVL recorded for this species, with a maximum SVL of 81.28 mm in males, and 69.68 mm in females. Rodríguez and Martínez (1992) categorized *Anolis bartschi* as opportunistic because they take advantage of prey availability despite seasonality. Most *A. bartschi* had prey in the stomach, and a high prey/stomach rate (3.39) was detected. Differences in prey type may reflect intersexual differences in size, microhabitat use, or energetic demands (Losos 2009). Andrews and Asato (1977) found that higher consumption of prey by females could be due to energetic demands; females of *Anolis limifrons* Cope, 1862 had a two-fold energy expenditure producing eggs during reproduction, compared to sperm production in males (Andrews and Asato 1977). In our study, the head width was not directly related to prey size: females consumed slightly fewer and larger prey than males. *Anolis bartschi* consumed more food during the dry season than during the rainy season, possibly because in the dry season they gather energy from a variety of prey items to prepare themselves for reproduction. In the rainy season, when more prey is available, they eat fewer prey items and prey categories because they can choose their prey and/or spend more time reproducing. We suggest that the consumption of larger prey by females of *A. bartschi* is directly related to energy gain before March and April, during May to September, and after reproduction (October to February) (Novo and Estrada 1986).

Similarities in niche breadth of males and females were observed during the dry season, but in the rainy season males consumed a significantly higher diversity of prey than females (B = 2.78, and B = 1.56 respectively). In the dry season, both males and females exploited all resources available, and some competition in type of prey may occur, but in the rainy season, when invertebrates are abundant, males seemed to consume a higher diversity of prey categories, and females were more selective. In addition, overlap was detected in type of prey consumed by males and females in the dry season, but less overlap occurred in the rainy season, even though no significant difference was observed. Rodríguez and Martínez (1992) found similar results, suggesting that this difference is due to higher prey availability in the rainy season, and no competition is needed for food resources,

Table 2. Moving (MP) and sedentary (SP) prey consumed by *Anolis bartschi* by sex. Shown are the number of items (*N*) and percentage (%), the frequency of occurrence (*F*) and percentage (%), the volume (V) and percentage (%), and the *IRI*. The number of MP and SP categories represents the number of prey categories assigned to MP and SP.

	MP (7)		**SP (12)**	
	Male	Female	Male	Female
F (%)	31 (34.83)	40 (36.36)	59 (66.29)	70 (63.64)
N (%)	43 (25.44)	44 (26.83)	127 (75.15)	120 (73.17)
V (%)	683.62 (15.79)	903.64 (14.69)	3638.85 (84.06)	5248.94 (85.31)
IRI	25.35	25.96	75.16	74.04

whereas in the dry season less prey availability may lead to competition for food resources. Estrada and Novo (1986, 1987) also observed overlap in space, substrate and temperature, but this species was found to have lower overlap in perch height (Estrada and Novo 1986, LYGP unpubl. data), which may influence spatial segregation during foraging, as suggested by Rodríguez and Martínez (1992).

Stomach Contents

Ant consumption in anoles is common in Cuban species (Rodríguez and Martínez 1992, 1994, 1996). Ants are clumped species that are easier to catch, and the higher individual numbers compensate for the diminutive size (Rodríguez 2010), even for a medium-large lizard such as *Anolis bartschi*. This species mainly consumed Hymenoptera, especially ants (Rodríguez and Martínez 1992, this study). We found a large number of non-ant Hymenoptera (mostly bees), which occupy most of volume in *A. bartschi* stomachs (Table 1); followed by ants, but, as expected, ants had the greatest $N_{\%}$ (80.6%) in both sexes. We report for the first time the presence of the introduced fire ant (*Wasmannia auropunctata*) consumed intentionally ($F_{\%}$ = 18.8, $N_{\%}$ = 42.3) for any Cuban reptile, and consequently for *A. bartschi*. This fire ant is invasive in Cuba, and no evaluation of damage to human and/or native fauna has been studied. Rodríguez-Cabrera *et al.* (2020) reported fire ants in the stomach of the snake *Tropidophis maculatus* (Bibron, 1840), but due to the low number of ants, this consumption seemed to have been accidental. The intentional consumption of this ant was detected in frogs (García-Padrón and Borrego *in press*, LYGP unpubl. data). This observation may be a natural response to invasion and may point to this lizard as another natural control to this invasive species, even though the quantity of fire ants found in stomachs of *A. bartschi* is small.

Besides Hymenoptera, males consumed in general the largest number of dipterans and mollusks (25% each), and beetles (22.5%), while females consumed mostly beetles (48.6%), arachnids (37.1%), mollusks (31.4%), and roaches (25.7%). It seems that another mechanism of females to avoid trophic overlap (besides temporal segregation) is to consume more categories and larger prey than males. Fruit consumption is known in lizards (Schoener 1968, Herrel *et al.* 2004, Vitt and Caldwell 2013). We observed ripe fruits in the stomach (N = 14) of *A. bartschi*, most of them consumed in the rainy season (71.43%); of which we could only identify the genus *Ficus* sp., observed in four stomachs. This observation agrees with Rodríguez and Martínez (1992) who found that a greater amount of fruit was consumed by this species in the rainy season. Females consumed more fruit in the rainy (80%, N = 8) than in the dry season (25%, N = 1). On the contrary, males eat slightly more fruit in the dry (75%, N = 3) than in the rainy season (20%, N = 2). We believe that the dirt/stones and (non-fruit) plant material found in the stomachs were consumed accidentally while hunting. Skin molt in the stomach content is common in samples of *A. bartschi* (Rodríguez and Martínez 1992, this study), and Rodríguez and Martínez (1992) disavowed the possibility of saurophagy or cannibalism. We believe that skin molt belongs to the same individual who consumed it.

Foraging Mode

Anolis bartschi consumed more sedentary than mobile prey items. During the study, we observed *A. bartschi* consuming preferentially clumped, colonial (e.g. ants) or sedentary prey (e.g. Lepidoptera larvae), rather than mobile prey (e.g. crickets, roaches, dipterans). In general, males consumed more sedentary prey than females, but it seems as if this preference changed seasonally: in the dry season, males consumed more sedentary (73.7%) and less mobile prey (26.3%) than females (64.9% and 35.1% respectively). On the contrary, in the rainy season females consumed more "sedentary"

prey (65.6%) and less "mobile" prey (34.4%) than males (55.6%, and 44.4% respectively). The consumption of sedentary prey can be advantageous for this lizard, as it enables low energy cost during hunting. Active foragers usually consume sedentary prey, have streamlined body shapes, long and narrow heads (Vitt and Caldwell 2013), and can be selective in food choice (Bergallo and Rocha 1994), resulting in low food diversity. Sit-and-wait predators feed on a large variety of prey types, are territorial, and use visual signaling for reproduction (Vitt and Caldwell 2013). According to our dataset and field observation, *A. bartschi* is a bimodal forager: it feeds on sedentary prey, even though this depends on sex and seasonality; consumes a great variety of prey types; has a streamlined body shape with a long and narrow head; is territorial; and uses visual signals for reproduction. Even so, research on diet preferences linked to reproduction and ecology is recommended for a proper forage classification of this lizard.

Urbanization through tourism, agriculture and clear cutting of forests for lumber production around Viñales National Park are the main ecological problems in our study areas. These disturbances could affect not just the forest dynamics, but also the composition of the associated invertebrate fauna and consequently its predator-prey dynamics (Kattan and Álvarez-López 1996). That is why we consider that modification and/or destruction of the habitat may determine the future of this local endemic and ecologically restricted lizard. A fast response to this growing problem must be given by the authorities. Studies on behavior, reproductive ecology, population dynamics, and continuing diet research are needed to clarify aspects of trophic ecology yet unanswered [e.g. trophic dynamics between age classes, potential disturbance on the lizards and their food (the invertebrate community) by habitat fragmentation], and that may contribute to the conservation and management plan for this local endemic species.

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Natural history of the marsupial frog *Gastrotheca albolineata* (Anura: Hemiphractidae) in lowland Brazilian Atlantic Forest

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Abstract

Natural history of the marsupial frog *Gastrotheca albolineata* (Anura: Hemiphractidae) in lowland Brazilian Atlantic Forest. *Gastrotheca albolineata* is a marsupial frog endemic to the Atlantic Forest in southeastern Brazil. It remains poorly studied in nature and is uncommon in herpetological collections. We studied the natural history of *G. albolineata* during a four-year period (2015 to 2019), in Ubatuba, São Paulo state, Brazil, at its southernmost distribution. Our results show that *G. albolineata* is arboreal, perches from low to medium heights, and breeds during the dry season without chorus aggregation. Calling activity occurs during the day but is more intense during the first half of the night. We used dorsal body markings to identify individuals. Six individuals were recaptured during the study, indicating site fidelity during the active season. The defensive repertory of *G. albolineata* contains seven different behaviors, including a high-pitched distress call. Egg development in the female's dorsal pouch took at least 87 days, and fully formed froglets were born with a snout–vent length of 16 mm. Our data substantially add to the knowledge of the natural history of Brazilian marsupial frogs and can be helpful to delineate conservation strategies for elusive species such as *G. albolineata.*

Keywords: behavior, breeding, defense, larval development, natural marks, site fidelity.

Resumo

História natural da perereca marsupial *Gastrotheca albolineata* (Anura: Hemiphractidae) na Mata Atlântica brasileira de baixada. *Gastrotheca albolineata* é uma perereca marsupial endêmica da Mata Atlântica do sudeste do Brasil. Essa espécie permanece pouco estudada na natureza e é incomum em coleções herpetológicas. Estudamos a história natural de *G. albolineata* durante um período de quatro anos (2015 a 2019), em Ubatuba, estado de São Paulo, Brasil, em sua distribuição mais austral. Nossos resultados mostram que *G. albolineata* é uma espécie arborícola, que se empoleira em alturas baixo-médias e se reproduz durante a estação seca sem agregação de coro. A

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atividade de canto ocorre durante todo o dia, mas é mais intensa na primeira metade da noite. Utilizamos marcações dorsais naturais como identificação individual e recapturamos seis indivíduos durante o estudo, o que indicou fidelidade de sítio durante a temporada de atividade. O repertório defensivo de *G. albolineata* contém sete comportamentos diferentes, incluindo um canto de socorro estridente. O desenvolvimento do ovo na bolsa dorsal da fêmea levou pelo menos 87 dias, e os filhotes totalmente formados nasceram com 16 mm de comprimento rostro-cloacal. Nossos dados aumentam substancialmente o conhecimento da história natural das pererecas marsupiais brasileiras e podem ser úteis para delinear estratégias de conservação para espécies como *G. albolineata*.

Palavras-chave: comportamento, desenvolvimento larval, fidelidade de sítio, marcas naturais, reprodução.

Introduction

The most remarkable characteristic of frogs in the family Hemiphractidae is their capacity to brood eggs on their dorsa (Del Pino and Escobar 1981, Warne and Catenazzi 2016, Del Pino 2018). The so-called marsupial frogs have attracted scientific interest and have been studied in recent years (review in Duellman 2015). However, little scientific effort has been directed to the study of the natural history of marsupial frogs, even though many species are endangered (Duellman 2015).

Gastrotheca (Fitzinger, 1843) is the most diverse genus in Hemiphractidae, with 75 recognized species (Frost 2020). In this genus, eggs are carried by the female on the dorsum or in a dorsal pouch, with direct development into froglets (Castroviejo-Fisher *et al.* 2015), a reproductive mode classified as Mode 37 *sensu* Haddad and Prado (2005). *Gastrotheca* is well represented in the Andean highlands and in the mountain and lowland forests of Central and South America, with one species in the Amazon basin (Castroviejo-Fisher *et al.* 2015, Duellman 2015). In Brazil, species of *Gastrotheca* occur mostly in the Atlantic Forest domain, where two distinct phylogenetic groups are recognized: that of *G. fissipes* (Boulenger, 1888) and that of *G. microdiscus* (Andersson, 1910) (Blackburn and Duellman 2013, Monteiro *et al.* 2019).

Gastrotheca albolineata (Lutz and Lutz, 1939) is presently included in the *G. microdiscus* group (Monteiro *et al.* 2019), and its distribution encompasses Atlantic Forest in the states of Espírito Santo, Rio de Janeiro, and extreme northeast of São Paulo (Pontes *et al.* 2012). Like most Atlantic Forest *Gastrotheca* species, *G. albolineata* is mainly arboreal (Pontes *et al.* 2012). Some information is available in literature, including the advertisement call (Izecksohn and Carvalho-e-Silva 2008); defensive behavior (Muscat and Rotenberg 2016); breeding activity during the dry season (Pontes *et al.* 2012); and egg number and size (Caramaschi and Rodrigues 2007). Demographic data, seasonal variation in breeding activity, egg development, parturition, and newly hatched froglet size for *G. albolineata* remain unknown.

Gastrotheca albolineata is ranked as Least Concern in the IUCN Red List of Threatened species (Carvalho-e-Silva and Telles 2004), but it is poorly represented in herpetological collections and rarely seen in nature (Pontes *et al.* 2012). Although not endangered, populations are decreasing (Carvalho-e-Silva and Telles 2004). Natural history studies are essential to evaluate its local conservation status (Pontes *et al.* 2012) and to promote effective amphibian conservation (Michaels *et al.* 2014). Herein, we present new data on the natural history of a population of *G. albolineata* monitored for four

years at its southernmost distribution (Ubatuba, São Paulo state, southeastern Brazil) and provide information on temporal and habitat use, defensive behavior, and breeding biology.

Materials and Methods

Study Area and Monitoring Data

We conducted the study from August 2015 to October 2019 (~ 960 days, or 51 months) at the Projeto Dacnis private reserve in Ubatuba, São Paulo state, southeastern Brazil (23°27'46" S 45°07'58"W; WGS-84, 34 m a.s.l.). The Projeto Dacnis reserve covers 1.36 km^2 of mostly secondary lowland Atlantic Forest, with some primary forest patches. We used the average climate data, including mean, maximum and minimum temperature and rainfall, for the municipality of Ubatuba (Köppen 2020) to validate seasonal activity. The classification of Ubatuba's climate is Af, with significant rainfall throughout the year (Köppen 2020), but it is similar to Cfa in terms of hydric availability (Rolim *et al.* 2007). We defined the dry season from May to September, at which time rainfall abruptly ceases, and the rainy season from October to March.

We monitored the study area five days a week throughout the study period, with a total sampling effort of 3500 person-hours, both diurnal and nocturnal. We searched for the frogs visually and acoustically in forest strata up to 10 m in height, and we made visual and sound recordings depending on the height at which the frog perched. When we found an individual of *G. albolineata,* we recorded its location with a GPS receiver (Garmin eTrex 22×) and the height of the frog's perch from the ground with a digital laser distance meter (Bosch GLM20). When in hand, we determined the sex based on size, presence of pouch, and vocal sac. Individuals ≤ 30 mm were juveniles. In addition, we quantified the number of frogs/person-hour for each month to estimate capture rate. Results are presented as mean ± standard deviation.

Habitat Use

To understand the distribution of *G. albolineata* in the study area, we organized a Kernel Density Estimate map in QGis 3.12.1 software. This map took into account the capture density, showing the "hot spots" where we found the species more frequently. For every capture, we classified the phytocenosis type (Table 1) and calculated the observation frequency for each phytocenosis.

Non-Invasive Mark-Recapture

We photographed all individuals of *G. albolineata* with digital cameras (Canon 600D, 70D and 7D Mark II). We took pictures of the frogs' dorsa and used the pattern of whitish lines and dark spots as identification marks (e.g. Lama *et al.* 2011, Caorsi *et al.* 2012). We counted only adults in the census to avoid possible ontogenetic changes in the natural markings of juvenile frogs (Bardier *et al.* 2020). We visually identified each photographed individual, adding it to the image database and assigning it a number (for example, ID01_date). We employed this mark-recapture technique coupled with the location to check the distance between two findings of the same individual.

We defined the breeding season as the period when we heard males vocalizing and found females carrying eggs (see Duellman 2015).

Defensive Behavior

We induced defensive behavior in 14 individuals of *G. albolineata.* One of us grasped the frog by the hind limbs (Toledo and Haddad 2009) and, after careful restraint, applied gentle pressure on its back, taking care not to harm the frog. We filmed each behavior with a digital camera (Canon 600D). We did the test only once per frog. After the test, we released each individual at its place of capture. We followed Toledo *et al.* (2011) to classify defensive behaviors. We collected one individual as a voucher (id21) and deposited it in the UNESP-Litoral herpetological collection (HCLP-A276).

Table 1. Classification of the phytocenoses found in the study area in the municipality of Ubatuba, São Paulo state, Brazil.

	Forest	Understory density and height	Canopy	Elevation (m a.s.l.)
Type 1	Alluvial Ombrophilous Dense Forest	low density, up to 4 m	up to 25 m	20
Type 2	Submontane Ombrophilous Forest	medium density, up to 3 m	up to 25 m	35
Type 3	Forest border exposed to anthropic action	shrubs up to 3 m	absent	24
Type 4	Alluvial Ombrophilous Dense Forest	low density, up to 4 m	up to 15 m	20
Type 5	Alluvial Ombrophilous Dense Forest	low density, up to 4 m	up to 8 m	11
Type 6	Submontane Ombrophilous Forest	low density, up to 4 m	up to 25 m	93

Egg Development, Parturition, and Newly Hatched Froglet Data

We collected one egg-brooding female (Id20) and kept her in captivity for 90 days to record egg development, parturition behavior, and froglet morphology and behavior. After that, we released her and the froglets at the place of capture. We kept the female housed in a room at Projeto Dacnis in Ubatuba in a well-ventilated plastic box (80 × 40 × 50 cm) with topsoil, a variety of perches, a shelter, and a water bowl. We fed her every two days with house crickets and cockroaches. We did not control air temperature, which varied according to external air temperature, and maintained humidity by misting the box once a day. We took measurements of her eggs every five days and recorded her behavior using a web camera installed in the enclosure. We measured the embryos' snout–vent length (SVL) during their development, as in Del Pino and Escobar (1981).

Results

Monitoring Data and Habitat Use

We captured a total of 25 individuals, including 20 adult males, two egg-brooding females, and three unsexed juveniles. We caught most frogs during the dry season, especially in July and August (0.05 and 0.04 frogs/person-hour respectively). December yielded 0.0071 frogs/person-hour, October 0.0057 frogs/person-hour, January 0.0035 frogs/person-hour, and September 0.0028 frogs/person-hour. No frogs were caught from February to June, and in November (Figure 1). The number of frogs varied across years: six records in 2015, 14 in 2016, one in 2017, five in 2018, and nine in 2019. Calling activity peaked in July (N = 30) and August (N = 28) in all survey years, but we also heard a few isolated calls during the rainy season (October to March). Vocalization of *G. albolineata* was loud, heard from distances greater than 10 m, and sometimes with an interval of over two hours between calls. During the calling activity peak, we recorded a maximum of 33 calling males in one night, with no formation of aggregations or choruses. We also heard males during the day, mostly in the morning and late afternoon, but also infrequently at midday. Curiously, we observed more vocal activity during windy days in all years.

During the 51 survey months, we saw *G. albolineata* 34 times, totaling 25 individuals (Figure 2A). We observed *G. albolineata* individuals perched on vegetation at an average height of 127 ± 59 cm (N = 34; Figure 2B). Sound recordings allowed us to estimate that

individuals perched as high as 10 m. We found all juveniles of *G. albolineata* during the rainy season (2015, 2017, and 2018); two of them were perched on the same *Heliconia rostrata* Ruiz & Pav. plant (both at the same height, 200 cm). We found the third juvenile during the dry season, perched at a height of 120 cm. We observed *G. albolineata* most frequently in phytocenosis types IV (44%) and II (32%) (for a description of the phytocenoses, see Table 1). Phytocenosis type IV also yielded the most recaptures (75%). Capture rates were lower in phytocenoses types V (9%), I and III (6% each), and VI (3%) (Figure 2C).

Non-Invasive Mark-Recapture

Throughout the four years, we recaptured six individuals of *G. albolineata.* All were males, and three were recaptured twice (Figure 3; Table 2). All recaptures were in the same phytocenosis as the first capture. Almost all recaptures occurred during the same season and in close proximity to the original place of capture (< 2 m). The greatest distance between capture and recapture during the same breeding season was 20 m (Id01). We found this individual one year later only 2 m from its original place of capture. Frog Id02 was the only one found at a greater distance (100 m) from one breeding season to the next.

Defensive Behavior

All 14 frogs we tested displayed at least one defensive strategy. Five of the seven defensive behaviors displayed had not been previously recorded for *G. albolineata:* cloacal discharge, active escape, mouth gaping, distress call, and hiding (Figure 4). In order of frequency, the tactics we recorded were: cloacal discharge (N = 14, or 100% of frogs); inflating the body (N = 13, 93%); active escape (N = 6, 42.8%); thanatosis (N = 3, 21.4%); mouth gaping (N = 3, 21.4%); distress call (N = 3, 21.4%); and hiding (N = 1, 7.14%).

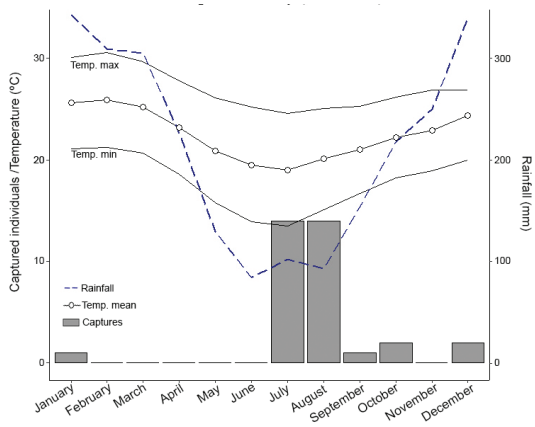

Figure 1. Captures and temporal activity (2015–2019) of *Gastrotheca albolineata* vs. climate data recorded in the municipality of Ubatuba, São Paulo state, Brazil.

Egg Development, Parturition, and Newly Hatched Froglet Data

We found two egg-brooding females during the study. The first was found on 23 July 2016 (Id06) carrying 17 eggs in her dorsal pouch (Figure 5A). We caught the second female on 29 July 2019, with 18 eggs in the pouch. It took 87 days from capture until the last froglet was expelled from the female's pouch (detailed embryo development in Appendix I). In the first 74 days, egg diameter doubled (from 6 to 12 mm) and the proportion of yolk decreased from 80% to none as the embryos grew. On 19 October 2019, at 06:30 h, the first froglet was released from the dorsal pouch after the mother performed a series of jumps. On the next day, at 22:50 h, she began using her foot to push froglets out of the pouch. After a few minutes, she inflated her body and expelled two live froglets (Figure 5B) and seven dead ones. On 21 October 2019, at 02:00 h, two froglets left the mother's pouch by themselves. On 23 October 2019, the internal pouch membrane completely everted with six other froglets and/or embryos still attached to it. Again, the female jumped, inflated her body, and used her foot to

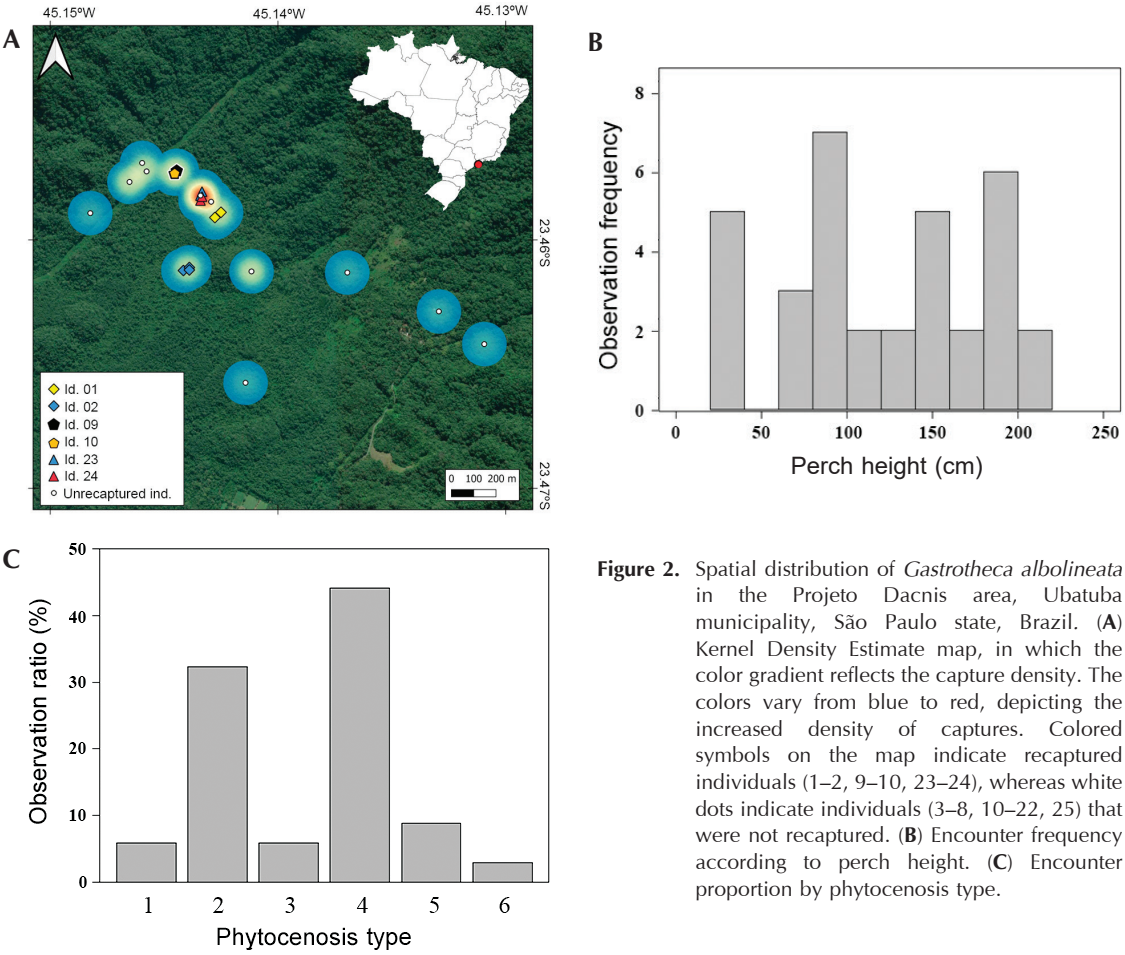

Figure 2. Spatial distribution of *Gastrotheca albolineata* in the Projeto Dacnis area, Ubatuba municipality, São Paulo state, Brazil. (**A**) Kernel Density Estimate map, in which the color gradient reflects the capture density. The colors vary from blue to red, depicting the increased density of captures. Colored symbols on the map indicate recaptured individuals (1–2, 9–10, 23–24), whereas white dots indicate individuals (3–8, 10–22, 25) that were not recaptured. (**B**) Encounter frequency according to perch height. (**C**) Encounter proportion by phytocenosis type.

Table 2. Recaptures of *Gastrotheca albolineata* in the study area in the municipality of Ubatuba, São Paulo state, Brazil. Distance calculated as a straight line between consecutive captures.

ID	Capture	1st Recapture	Distance (m)	2nd Recapture	Distance (m)
01	30/07/2015	06/10/2015	20	29/08/2016	< 2
02	23/08/2015	30/09/2015	< 2	29/08/2016	100
09	23/07/2016	03/08/2016	< 2	30/08/2016	< 2
10	31/07/2016	30/08/2016	< 2	-	-
23	02/08/2019	13/08/2019	< 2	-	-
24	08/08/2019	13/08/2019	< 2	-	-

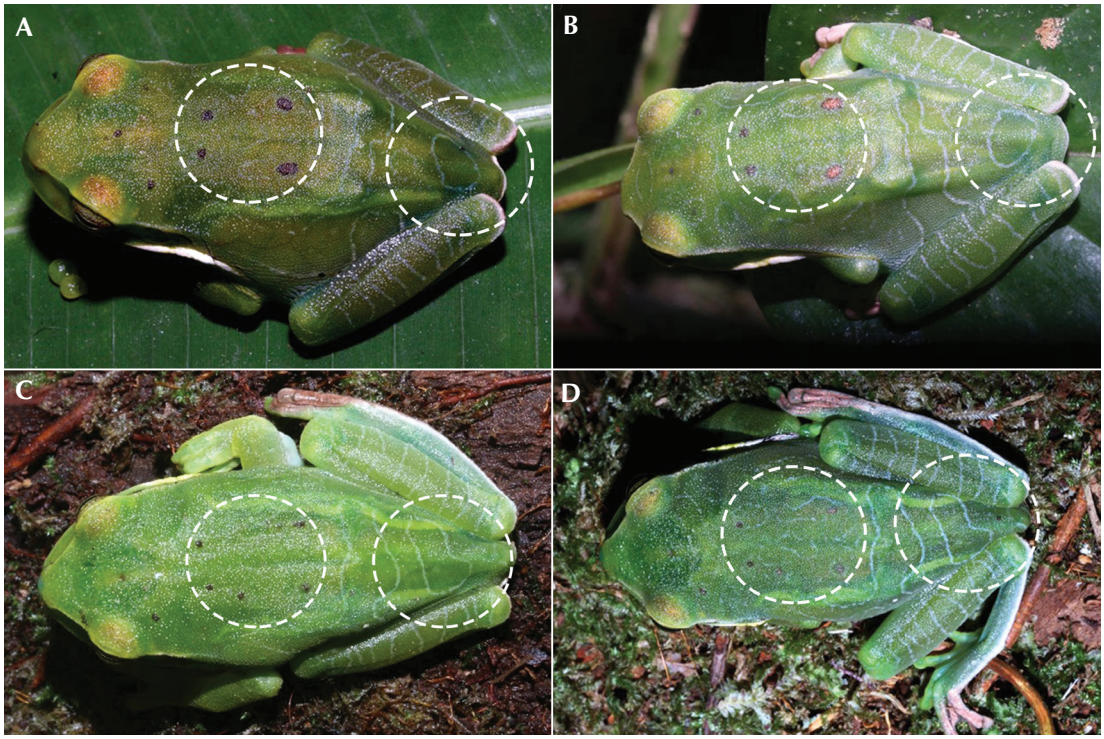

Figure 3. Examples that validate the recapture photo identification of individuals of *Gastrotheca albolineata* in the Projeto Dacnis area, Ubatuba municipality, São Paulo state, Brazil. (**A, B**) Individual 1 recaptured for the first time on 06 October 2015 and again on 29 August 2016; (**C, D**) Individual 2 recaptured on 30 September 2015 and recaptured again on 29 August 2016. Dashed circles indicate natural marks (dark spots and light lines).

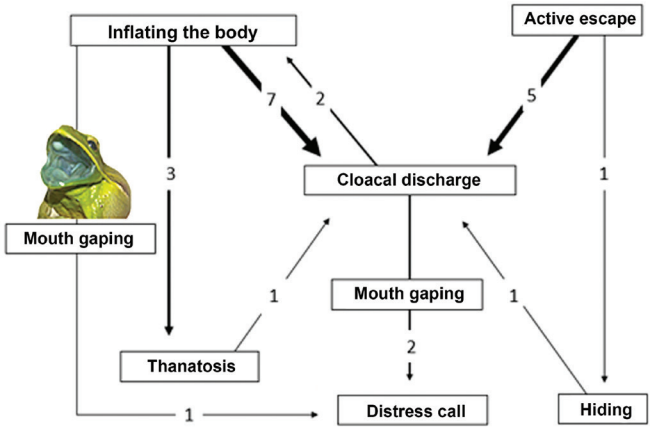

Figure 4. Ethogram of defensive behaviors displayed by *Gastrotheca albolineata.* The arrows and the numbers indicate the frequency of observation and the sequence in which each defensive behavior was displayed. The thicker arrows point out the most common defensive behaviors.

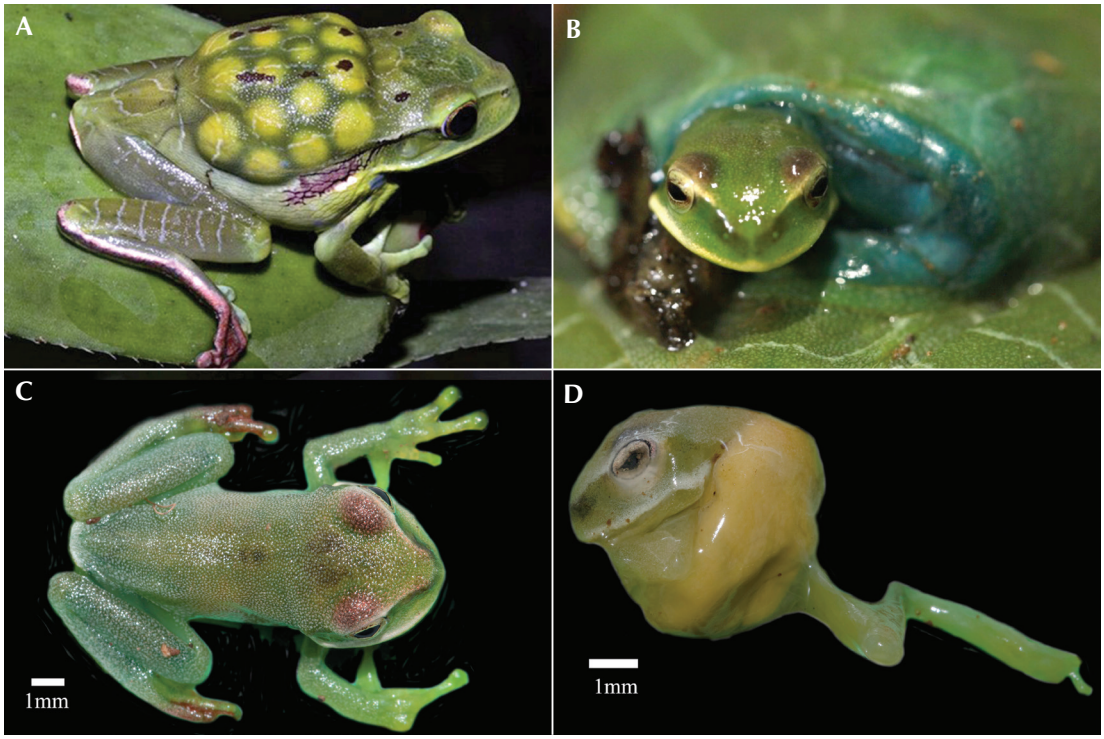

Figure 5. (**A**) *Gastrotheca albolineata* female (Id20) with eggs in her dorsal pouch. (**B**) A froglet emerges from the pouch (note bluish color of internal pouch membrane). (**C**) A newly expelled froglet. (**D**) A dead embryo with incomplete development.

push the rest of the froglets out of the pouch. This last effort resulted in two live froglets (Figure 5C) and four dead embryos (Figure 5D) expelled from the pouch. Live newly released froglets averaged 16 ± 1.68 mm SVL and 0.34 ± 0.05 g, while dead froglets averaged 12 ± 3.22 SVL mm and 0.25 ± 0.08 g.

Discussion

Monitoring Data and Habitat Use

Our data indicate that *G. albolineata* reproduces mainly during the dry season (mostly in July and August, austral winter), which agrees with Pontes *et al.* (2012), who found males calling on trees during the dry season. We recorded no breeding activity outside of the dry season. During our study, several *G. albolineata* males vocalized during cold, windy nights, which matches observations in the escarpments of the municipality of Nova Friburgo, state of Rio de Janeiro, Brazil (Renato Bernils, pers. comm.). We recorded calling activity of *G. albolineata* during the day, but mostly during the first half of the night, the same as observed in other Atlantic Forest species of *Gastrotheca* (Monteiro *et al.* 2019). We often found *G. albolineata* in low to medium understory up to 3–4 m high, in areas with a deep leaf litter layer and abundant bromeliads and lianas, which agrees with a similar habitat description for *G. microdiscus* (Monteiro *et al.* 2019). Several species of *Gastrotheca* are arboreal and seem to

prefer perches 1–4 m high (Catenazzi and Von May 2011, Teixeira Jr. *et al.* 2012, Monteiro *et al.* 2019), which again agrees with our findings.

Non-Invasive Mark-Recapture

Using natural body markings and photography as an individual marking technique is non-invasive, but restricted to species with distinct and identifiable color patterns (e.g., Lama *et al.* 2011, Caorsi *et al.* 2012). Further studies may use photographic identification of *G. albolineata* because each adult individual has a unique combination of lines and dorsal spots that are easily recognizable. Using this technique, we were able to conclude that *G. albolineata* displays site fidelity at least during the breeding season, and that males remain close to the same calling places for long periods. A similar pattern is reported for *Gastrotheca marsupiata* (Duméril and Bibron, 1841), which uses the same places for at least four consecutive weeks (Sinsch and Joermann 1989).

Defensive Behaviors

Inflating the body was the second most frequent defensive behavior observed, and *G. recava* Teixeira, Vechio, Recoder, Carnaval, Strangas, Damasceno, Sena, and Rodrigues, 2012 and *G. megacephala* Izecksohn, Carvalho-e-Silva, and Peixoto, 2009 employ this strategy frequently (Lourenço-de-Moraes *et al.* 2016). Thanatosis is reported only for *G. albolineata* (Muscat and Rotenberg 2016) and *G. megacephala* (Lourenço-de-Moraes *et al.* 2016). We also observed synergistic behaviors in several individuals. The combination of different behaviors can increase the defensive effectiveness against a variety of predators in different contexts (Toledo *et al.* 2011).

Egg Development, Parturition, and Newly Hatched Froglet Data

The direct-development reproductive mode, as observed in *Gastrotheca albolineata,* is linked to a series of physiological modifications (Duellman 2015, Warne and Catenazzi 2016, Del Pino 2018). One of its limitations is how many eggs the female can brood. The number of eggs carried by the two egg-brooding females in our study is close to the 16 eggs previously reported (Caramaschi and Rodrigues 2007) and is similar to those of other direct-development *Gastrotheca* species such as *G. fissipes, G. megacephala,* and *G. recava* (Caramaschi and Rodrigues 2007, Izecksohn *et al.* 2009, Teixeira *et al.* 2012). The number of eggs produced by species of *Gastrotheca* with direct egg development is smaller than in species that rely on indirect egg development. For example, Del Pino and Escobar (1981) report 128 eggs for the indirect egg development *G. riobambae* (Fowler, 1913). The egg diameter is larger than in indirect-development species of *Gastrotheca:* indirect cycle species, 2.3–6.3 mm; direct cycle species, 4.0–13.2 mm (review in Duellman 2015). The eggs of *G. albolineata* have among the largest diameters reported for any species of *Gastrotheca* (Duellman and Köhler 2005, Duellman and Chavés 2010, Gagliardo *et al.* 2010, Del Pino 2018). The behavior of the female pushing the embryos out of the dorsal pouch with her feet has been reported both for species with direct egg development that release their froglets in the water (Del Pino and Escobar 1981) and for species with indirect egg development (Del Pino and Escobar 1981, Duellman and Köhler 2005). A female *G. recava* kept in captivity for a month produced 20 live froglets weighing 0.8 g each (Teixeira *et al.* 2012), which is twice the weight of *G. albolineata* froglets. Duellman and Chavés (2010) induced expulsion of froglets in *G. testudinea* (Jiménez de la Espada, 1870); the froglets had SVLs of 9.4–11.7 mm, but only those over 11 mm were fully developed. Size of newly expelled froglets of *G. albolineata* in our study follows the same pattern as that reported by Duellman and Chavés (2010). Although most frogs are strictly lecithotrophic (including marsupial species with indirect egg development),

matrotrophy was reported in the direct-development species *G. excubitor* Duellman and Fritts, 1972 (Warne and Catenazzi 2016), so it is possible that female brood investment in *G. albolineata* exceeded pre-fecundation yolk production, and that the nutritional condition in captivity affected egg development and expulsion of live froglets.

As a final note, our four-year study substantially adds to the knowledge of natural history of *G. albolineata,* an elusive treefrog endemic to the Atlantic Forest of southeastern Brazil. Natural history oriented studies are fundamental to conservation projects, both of a target species or the biome in which the target species dwells (Michaels *et al.* 2014).

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Appendix I. *Detailed embryo development of* Gastrotheca albolineata *recorded from a captive female in the municipality of Ubatuba, São Paulo state, Brazil.*

Date	Egg diameter (mm)	Embryo morphology
29 July 2019	6	Eggs yellowish-green with no clear embryonic structure
9 August 2019	6	Increase in the number of vessels irrigating the egg. The embryo eyes were visible as two symmetrical dark spots
18 August 2019	7	A thin, dark, median line appeared in the middle of the egg
2 September 2019	7	Yolks decreased in volume, taking up about 80% of the eggs. Head contours visible in some of the embryos
27 September 2019	8	First embryo movements inside the pouch. The yolk took up about 50% of the egg
01 October 2019	10	The yolk occupied barely 20% of the egg, and the embryos were constantly moving
10 October 2019	12	Most of the yolks had disappeared
19 October 2019	-	First froglet ejected from the dorsal pouch
20 October 2019	-	The female expelled two live froglets and seven dead
21 October 2019	-	Two live froglets left the mother's pouch by themselves
23 October 2019	-	The internal pouch membrane completely everted. Two live froglets and four dead embryos were expelled from the pouch

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Multimodal signaling in *Boana albopunctata* (Anura: Hylidae): reading visual and acoustic cues

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Abstract

Multimodal signaling in *Boana albopunctata* (Anura: Hylidae): reading visual and acoustic cues. The acoustic mode of communication is important for anurans, but visual communication is beginning to be considered essential for some species, genera, and families. This study focuses on visual and acoustic signals in *Boana albopunctata* (Hylidae: Cophomantinae) in an attempt to increase our understanding of signaling in this nocturnal Neotropical treefrog. Visual signals were assessed to determine whether they are directed toward conspecific opponents, as has already been observed for some diurnal anurans, or associated with potential morphological asymmetries. Associations between visual and acoustic signals were explored. The results suggest that males may combine visual and acoustic signals (multimodal signaling) synergistically, thereby strengthening the efficiency of information transmission. Thus, in nocturnal anuran species, visual signals may function primarily as an alerting component and multimodal signaling may be a relevant way of communication.

Keywords: alerting function, territoriality, visual signaling.

Resumo

Sinalização multimodal em *Boana albopunctata* (Anura: Hylidae): compreendendo os sinais visuais e acústicos. O modo de comunicação acústico é importante para os anuros, mas a comunicação visual está começando a ser considerada essencial para algumas espécies, gêneros e famílias. Ao investigar sinais visuais e acústicos, o objetivo deste estudo é a melhor compreensão da sinalização em *Boana albopunctata* (Hylidae: Cophomantinae), uma perereca noturna neotropical. Foi verificado se os sinais visuais são direcionados aos oponentes coespecíficos, como já observado para alguns anuros diurnos, ou se são relacionados a potenciais assimetrias morfológicas. Estudou-se também associações entre sinais visuais e acústicos. Esse estudo leva à compreensão de que os machos podem combinar sinergicamente sinais acústicos e visuais (sinalização multimodal), potencializando assim a eficiência da transmissão de informação. Conclui-se que para espécies noturnas de anuros os sinais visuais podem atuar como componentes de alerta e que a sinalização multimodal pode ser um meio relevante de comunicação.

Palavras-chave: função de alerta, sinalização visual, territorialidade.

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Introduction

Acoustic communication is important for intraspecific interactions of anurans and has been widely studied. Calls are of central importance to social organization, such as advertisements for conspecifics and establishment of breeding territory limits (Wells 2010, Brunetti *et al.* 2014, Toledo *et al.* 2015, Chuang *et al.* 2017). Calls also play a fundamental role as prezygotic reproductive isolation mechanisms, thereby affecting the processes of speciation (Blair 1958, Doherty and Gehardt 1983, Gerhardt 1994, Grenat *et al.* 2013). Visual communication also has begun to be recognized an important and even essential component of intraspecific communication in anurans, particularly for diurnal species (Haddad and Giaretta 1999, Hödl and Amézquita 2001, de Sá *et al.* 2016, 2018). Although subject to some restrictions for message transfer (e.g., shorter range and higher light-dependence), visual signals have a lower energy cost than acoustic signals and can be as efficient as any other mode of short-range communication (Alcock 2013). Despite its high dependence on light, visual communication has been observed as a potentially relevant message transfer channel, even among nocturnal anuran species (Aho *et al.* 1988, Cummings *et al.* 2008, Gomez *et al.* 2009).

There may be a diversity of both visual and acoustic signals in some anuran families, and the social context must be considered when attempting to understand what is being communicated (Hödl and Amézquita 2001, Toledo *et al.* 2015). The selection of repertoires and signal use within them may differ according to context, such as courtship, male combat, or territorial maintenance. We know, for example, that males can modify their calls during closer interactions with conspecifics to protect their territories and ensure reproductive success, such as *Acris blanchardi* Harper, 1947 (Hylidae; Wagner Jr. 1992, Burmeister *et al.* 2002), *Bokermannohyla ibitiguara* (Cardoso, 1983) (Hylidae; Nali and Prado 2014), and *Boana goiana* (Lutz, 1968) (Hylidae; Morais *et al.* 2015). Also, recent studies reveal that associations among distinct communication modes are common (e.g., associations between acoustic and visual signals, or between tactile and visual signals) and these may transfer information more accurately. These synergistic associations represent multimodal communication and have been described for different anuran species, such as *Engystomops pustulosus* (Cope, 1864) (Leptodactylidae; Rosenthal *et al.* 2004), *Crossodactylus schmidti* Gallardo, 1961 (Hylodidae; Caldart *et al.* 2014), *Hylodes japi* de Sá, Canedo, Lyra, and Haddad, 2015 (Hylodidae; de Sá *et al.* 2016), and *Hyperolius puncticulatus* (Pfeffer, 1893) (Hyperoliidae; Starnberger *et al.* 2018).

Males of the treefrog genus *Boana* (Hylidae: Cophomantinae) typically possess morphological adaptations for physical confrontations (such as a spine on Finger I and hypertrophied arm muscles; Heyer *et al.* 1990) and exhibit aggressive and territorial behaviors. Indeed, de Sá *et al.* (2014) have already reported aggressiveness and territoriality for males of the nocturnal species *Boana albopunctata* (Spix, 1824). Although advertisement and territorial calls (Heyer *et al.* 1990, De La Riva *et al.* 1997, Kwet *et al.* 2002), and the visual signals—toe trembling, finger trembling, arm lifting, leg kicking, and vocal sac display (Toledo *et al.* 2007, Ramalho 2012, de Sá *et al.* 2014)—already have been described for this species, more detailed hypotheses about variation in the use of repertoires and synergistic associations of different signaling modes have yet to be explored. Much remains to be learned about both visual signaling in nocturnal anurans and multimodal signaling in anurans in general. Intensive efforts to collect data directly in the field and new approaches to explore signaling in anurans are needed to gather previous data from the literature and to improve our current knowledge, with the goal of informing our understanding of intraspecific communication in males of *B. albopunctata* in discrete social contexts.

Based on field studies, we describe the repertoires of visual and acoustic signals, along with the associated contexts of each in *Boana albopunctata.* We specifically tested whether: (1) visual signals are directed toward opponents, as previously observed for diurnal anurans, or if they are associated with potential asymmetric morphologies; and (2) visual and acoustic signals are performed together, demonstrating mutual message reinforcement and an alerting function (previously proposed for other species; Moller and Pomiankowski 1993, Hebets and Papaj 2005, Preininger *et al.* 2009, Grafe *et al.* 2012) and multimodal signaling in nocturnal anurans.

Materials and Methods

Study Site

We performed the study in permanent and temporary flooded portions of the Cantaclaro Ranch (22°19'36" S, 47°42'57" W; datum WGS 84), district of Itapé, municipality of Rio Claro, state of São Paulo in southeastern Brazil. The area is located in an ecotone between Cerrado (savannah vegetation) and Atlantic Forest (semi-deciduous seasonal forest). The current rural landscape contains zones of grazing and *Eucalyptus* plantations. The region has two well-established seasons—one wetter and hotter (rainy season), and the other drier and colder (dry season). The rainy season typically occurs from October–March, whereas the dry season occurs from April–September.

Behavioral Data

All behavioral data were obtained in the field by means of in-situ natural observations during the rainy season in a total of seven expeditions between 15 October 2015 and 10 March 2016. We started to collect data immediately after the sunset (when the males began calling) and stopped when the calling activity subsided or ceased completely. We video-recorded and audio-recorded calls using a Sony Cybershot DSC-W270 video camera (Sony Corporation) with supplementary lighting (using headlamps with red filters, which minimize disturbance to the focal males) and a Marantz PMD-660 audio recorder (Sound United, LLC) equipped with a Sennheiser ME-66 unidirectional microphone (Sennheiser electronic GmbH & Co. KG). The camera and microphone were positioned about 1.3 m from the calling male. We measured air temperature to standardize call recordings (Taigen *et al.* 1996, Guimarães and Bastos 2003, Wells 2010). We recorded and analyzed a total of 460 min of video and audio data (114 video files and 114 audio files).

Repertoire of Visual Signals

To identify the visual signals of *Boana albopunctata,* we randomly selected and observed 30 breeding males. We filmed each individual under natural conditions for 2 min (N = 30 videos) without interfering with their behavior; the context of these recordings is termed "advertisement." Given that males *B. albopunctata* are territorial and remain at the same breeding site for months (de Sá *et al.* 2014), we chose distinct and well-spaced individuals to avoid duplication of frog recordings.

Data during "experimental territorial" contexts were gathered to understand the distinct use of signals (discussed in next section). We recorded all movements performed by resident males by watching all the video files generated and identifying visual signals that have been described in the literature (Hödl and Amézquita 2001, Hartmann *et al.* 2005). As defined in Hödl and Amézquita (2001), the following visual signaling behaviors included those that: (1) provide a visual cue during intraspecific interactions; (2) are redundant, conspicuous, and stereotyped; and (3) might provoke response by the conspecific receiver that will benefit the sender. We built the species repertoire of visual signals by concatenating the tabularized data, including the context in which each signal was performed.

Experimental Analysis of Visual Signals

Some visual signals observed were directed to the right, to the left, or to both sides simultaneously—i.e., bilaterally. To detect whether males direct their visual signals toward an opponent, we conducted a field experiment with the same 30 males used for establishing the repertoire of visual signals. After the first 2 min of video recording under natural conditions, we inserted a conspecific male simulating an intruder in the territory of the resident male, with the "intruder male" being placed about 20 cm from resident male. As an intruder, we chose the first male randomly captured minutes before the experiment. We gently tied a nylon line to the waist of the intruder male to facilitate identification and move him wherever needed. We introduced the intruder in specific positions relative to the resident male: to his left side (N = 28 videos), to his right side (N = 28 videos), and in front of him (N = 28 videos). To minimize interference, we employed a 2-min adaptation period from the introduction of the conspecific male to the beginning of the observations and video recordings of the resident male's behaviors. We observed and recorded each intruder position for 2 min. We followed a random order for the positioning of the introduced conspecific male to avoid any potential bias. Experiments were classified as being in "territorial context."

We analyzed the video files obtained from the experimental trials following the same methods used for the construction of the repertoire of visual signals, with the addition of recording where the intruder was positioned with respect to the resident male. We tested whether resident males chose sides for signals that can be performed by distinct limbs independently, according to the intruder's position. For this we counted the number of times that each signal was performed on both sides simultaneously (frontal signal), only on the right side (right signal), and only on the left side (left signal). The frontal signal is likely detected by the intruder regardless of where the it was positioned, whereas the right signal is likely more detectable by an intruder placed to the right side of the signaler, and the left signal is likely to be detected by placing an intruder to the left of the resident male. We excluded arm lifting, face wiping, and leg kicking in this analysis because they were so rarely recorded. The vocal sac display was excluded because it is not a bilateral signal.

For all statistical analyses here and in the next sections we used BioEstat Version 5 software (Ayres *et al.* 2007). We conducted one-way ANOVA to investigate if there were significant differences between the frequency of visual signals performed on both sides simultaneously, only on the right side, or only on the left side. When we found significant differences, we ran a post-hoc Tukey test to investigate which specific groups differed.

To detect whether males direct their visual signals based on their body morphology, we verified potential morphological asymmetries (Dill 1977). We visited the Célio F. B. Haddad collection (CFBH) in the Departamento de Biodiversidade, Instituto de Biociências of Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Rio Claro, São Paulo state, Brazil, and measured males of *B. albopunctata* from the Cantaclaro Ranch and nearby localities (N = 20), as follow: 9 from Cantaclaro Ranch (CFBH 07438, 04144, 06052, 06673, 06676, 22088, 22090, 39114, and 39123); 2 from Floresta Estadual Edmundo Navarro de Andrade (22°25' S, 47°33' W; datum WGS 84; 20 km NW Cantaclaro Ranch, municipality of Rio Claro, SP, Brazil) (CFBH 22084 and 38517); and 9 from Estação Ecológica de Itirapina (22°13'10" S, 47°53'54" W; datum WGS 84; 22 km SE from ranch Cantaclaro, municipality of Itirapina, SP, Brazil) (CFBH 07059–07063, 38534, 38536, 38540, and 38541). We identified adult males by the presence of sexually dimorphic traits and measured morphological features based on Heyer *et al.* (1990) and Duellman (2001): finger and toe lengths, thigh length, tibia length, tarsus length, and foot length. A Student's t-test was

used to evaluate the presence of morphological asymmetries in male *B. albopunctata.*

Calls Repertoire

We conducted audio recordings simultaneously with the video recordings described above to detect advertisement and agonistic call types of the same sample of 30 males. We analyzed the audio recordings with Raven Pro Version 1.4 software using a sampling frequency rate of 48 kHz and 16-bit resolution in the mono pattern (Center for Conservation Bioacoustics 2011). Concatenating all calls recorded, we distinguished and counted the advertisement and agonistic calls emitted by detecting notes A, B, and T (A and B as advertisement notes, and T as a territorial note; *sensu* Toledo *et al.* 2007); by associating the calls with each behavioral context (i.e., advertisement and territorial), we documented the acoustic repertoire of the species. We conducted a Friedman test to compare the numbers of A, B, and T notes performed in different contexts. All calls selected and analyzed were recorded at similar air temperatures (27–30°C).

Multimodal Signaling

From all the video files analyzed, we assessed potential associations between visual and acoustic signals, and measured the intervals of transition between these two signaling modes when they were associated. Three types of association occurred, as follow: (1) visual signal performed immediately before a call; (2) visual signal performed immediately after a call; and (3) visual signal and call performed simultaneously. If the transition time between two distinct signaling modes was less than five seconds, we considered them to be associated. The vocal sac display was not included in this analysis because the studied males remained with the vocal sac inflated for long periods of time, including periods when they were not vocalizing (configurating a continuous visual signal). Face wiping and leg kicking signals were also not included in the statistical analysis (scarce samples for comparisons).

The total numbers of visual signals and multimodal signals were recorded to assess the relative importance of each. The average time of transition between signaling modes was calculated; the values obtained when visual signals preceded acoustic signals were compared with those obtained when visual signals followed acoustic signals. In the former case, we measured the amount of time from the end of the visual signal to the beginning of the acoustic signal, and in the latter case, we measured the amount of time from the end of the acoustic signal to the beginning of the visual signal. We applied Chi-square tests to verify whether there are significant preferences for performing both signal modes (visual and acoustic) simultaneously or asynchronously. For this test, we excluded data from arm lifting, arm wiping, and leg kicking (scarce samples). We applied the Wilcoxon matched-pairs test to determine whether there was a significant difference between the average transitional times between visual and acoustic modes, and vice-versa. By testing preferences and functions in multimodal signaling, we tested the propositions of mutual message reinforcement and of alerting function (Moller and Pomiankowski 1993, Hebets and Papaj 2005).

Results

Repertoire of Visual Signals

Male *Boana albopunctata* perform a variety of movements. We identified all the visual signals, as well as the primary occurrence of each visual signal relative to its frequency in discrete social contexts (Figure 1; Table 1). Toe trembling, finger trembling, arm lifting, leg lifting, face wiping, and vocal sac display are associated mainly in the context of advertisement, but also occur secondarily in the territorial context; leg kicking has a similar frequency in advertisement and territorial contexts. Vocal sac

Table 1. Repertoire of visual signals for male *Boana albopunctata* (*N* = 30) with a description and the respective contexts of occurrence.

Visual signal	Description	Context
Toe trembling (*N* = 110)	Fast lifting or trembling of one or more toes without a pattern. May be combined with finger trembling (*N* = 49).	Mainly advertisement, with secondary occurrence in territorial context.
Finger trembling (*N* = 81)	Fast lifting or trembling of one or more fingers without a pattern. May be combined with toe trembling (*N* = 49).	Mainly advertisement, with secondary occurrence in territorial context.
Arm lifting (*N* = 19)	Fast and short lifting of an arm, with it not passing above the head. May be combined with leg lifting (*N* = 6).	Mainly advertisement, with secondary occurrence in territorial context.
Leg lifting (*N* = 60)	Fast and short lifting of a leg. It can be completely vertical or laterally oriented (*N* = 3). May be combined with arm lifting (*N* = 6) or face wiping (*N* = 5).	Mainly advertisement, with secondary occurrence in territorial context.
Face wiping (*N* = 7)	Lifting of one arm and setting upon the head. Similar to a head-whipping movement. May precede leg lifting (*N* = 5).	Mainly advertisement, with secondary occurrence in territorial context.
Leg kicking (*N* = 4)	Fast and long movement of one leg. It is a fast stretching with the return of the leg to the initial position.	Territorial and advertisement.
Vocal sac display (*N* = 25)	Vocal sac remains inflated between two calls (*N* = 24) or rarely when the male was not calling (*N* = 1; Figure 2).	Mainly advertisement, with secondary occurrence in territorial context.

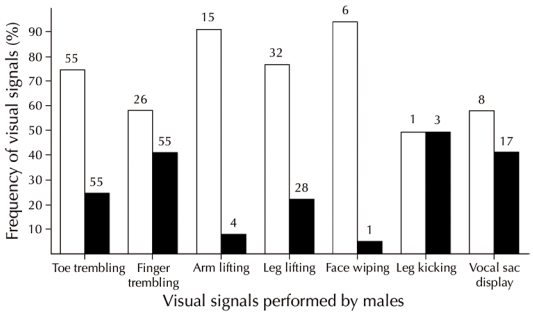

Figure 1. Frequency of visual signals performed by male *Boana albopunctata* in the field without the introduction of a conspecific male (advertisement context; white bars) and with the conspecific male (territorial context; black bars). The total number of records for each signal is indicated at the top of the bars, but frequencies (%) from territorial contexts are divided by three to correct the three times longer recording in that context, and allowing comparison with frequencies from advertisement context.

display (Figure 2) is not associated with any other visual signal. To estimate the main contexts in which each signal occurs, the frequencies of each signal performed in the territorial context were divided by three. Because recordings in the territorial context were three times longer than those in the advertisement context, we divided the territorial frequencies by three to bring the values into parity with one another.

Direction of Visual Signals

On 13 out of 30 occasions, the resident male *Boana albopunctata* became immobile and quiet during the entire period that the conspecific intruder was inside its territory. However, in the other 17 observations, we occasionally observed the resident frog to shift the orientation of his body when the intruder was present. Sometimes

Figure 2. Male *Boana albopunctata* performing the vocal sac display.

the resident male turned its entire body to the side where the intruder was located (N = 4, recorded in 2 males), whereas other times the resident male only turned its head toward the intruder (N = 5, recorded in 3 males). On two occasions, the resident male moved ahead and attacked the intruder. These three movements indicated some agonistic and territorial defense levels by the resident males. Moreover, twice the resident male was observed to jump and attack the intruder, which was obviously aggressive behavior associated with territorial defense. These responses indicate that resident males recognized the presence of the conspecific intruder and were bothered by the invasion of their territories.

We selected three visual signals to analyze intentional direction of visual signals by resident males—viz., toe trembling, finger trembling, and leg lifting. Toe trembling and finger trembling were performed more often with only the right foot or right hand, respectively, regardless of the location of the intruder (Figures 3, 4). Leg lifting was performed more often with only the left leg when the conspecific intruder was absent, but more often with the right leg when the intruder was present; these signals also were independent of the location of the intruder (Figure 5). According to a one-way ANOVA, the side of the frog's body used for the toe- and finger-trembling signals are significantly different {Toe-trembling: [F (2, 9) = 4.979, p = 0.035] and finger trembling [F (2, 9) = 12.679, p = 0.028]}, whereas there is no significant difference for leg lifting [F (2, 9) = 1.653, p = 0.244]. A post-hoc Tukey test revealed significant differences between signals performed with the right side (most frequent) and with both sides simultaneously (least frequent) for toe trembling ($p < 0.05$) and for finger trembling ($p < 0.01$). The similarity of the sizes of all the right and the left fingers, toes, and forelimbs is statistically significant (Table 2).

Calls Repertoire

The calls of male *Boana albopunctata* are simple, having only one note per call (notes A, B, or T), and composite calls with two or more notes per call. We recorded the advertisement notes A and B, and the territorial note T (*sensu* Toledo *et al.* 2007) more frequently in the composite calls than in the simple calls. Some common note combinations for the composite calls were AABT, ABB, ABT, and BBT. When used, note A was always the first note emitted and always emitted in composite calls, whereas note T was always the last. Both notes A and B have similar dominant frequencies, with A having 2.28 kHz ± 0.1 (N = 56; 2.06–2.43 kHz) and B having 2.35 kHz ± 0.1 (N = 33; 2.06–2.43 kHz). Nonetheless, the notes can be distinguished by their durations, with A lasting 0.37 s ± 0.04 (N = 56; 0.26–0.45 s) and B lasting 0.56 s ± 0.12 (N = 33; 0.46–0.94 s). Note that T is clearly differentiated audibly from notes A and B (note T: N = 14; dominant frequency of 1.31 kHz ± 0.6; 0.19–2.25). Also, T has a different duration from note A (note T: 0.53 s ± 0.08; 0.43–0.69 s; N = 14). The number of notes A, B, and T emitted during distinct contexts (advertisement and territorial) is statistically similar [Note A χ^2 (1) = 0.833; p = 0.361; Note B χ^2 (1) = 0.533; p = 0.465; Note T χ^2 (1) = 0.133; p = 0.715]. Our results here confirmed the acoustic repertoire

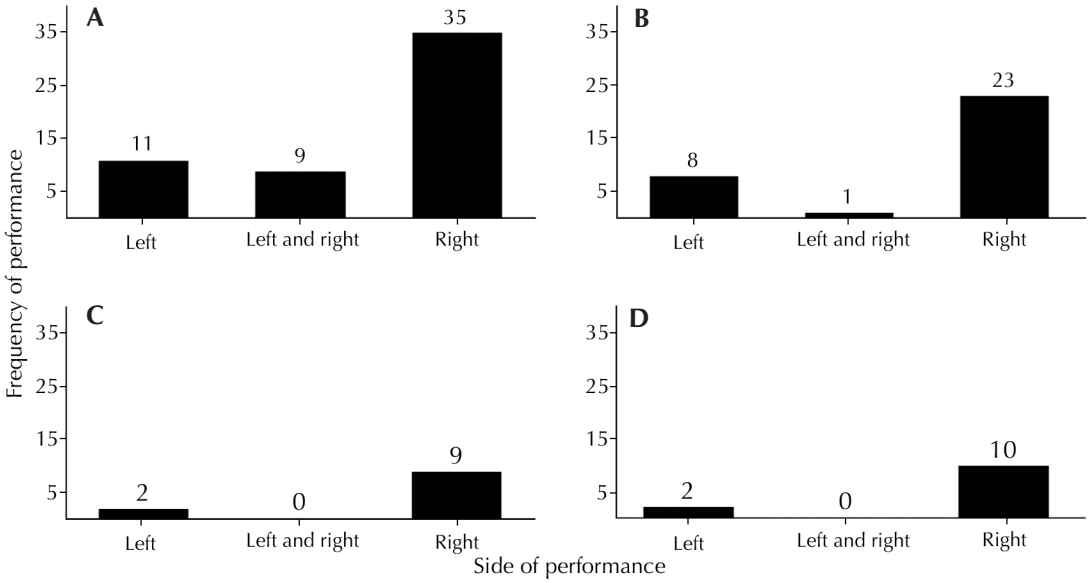

Figure 3. Frequency of performance of the toe-trembling signal according to side by male *Boana albopunctata* in discrete contexts, (**A**) without and with conspecific intruder male (**B**) in front, (**C**) on the left, and (**D**) on the right side of the resident male. Signals were performed by the resident male using the left side, the left and right sides simultaneously, or the right side. The number of records for each direction is at the top of the bars.

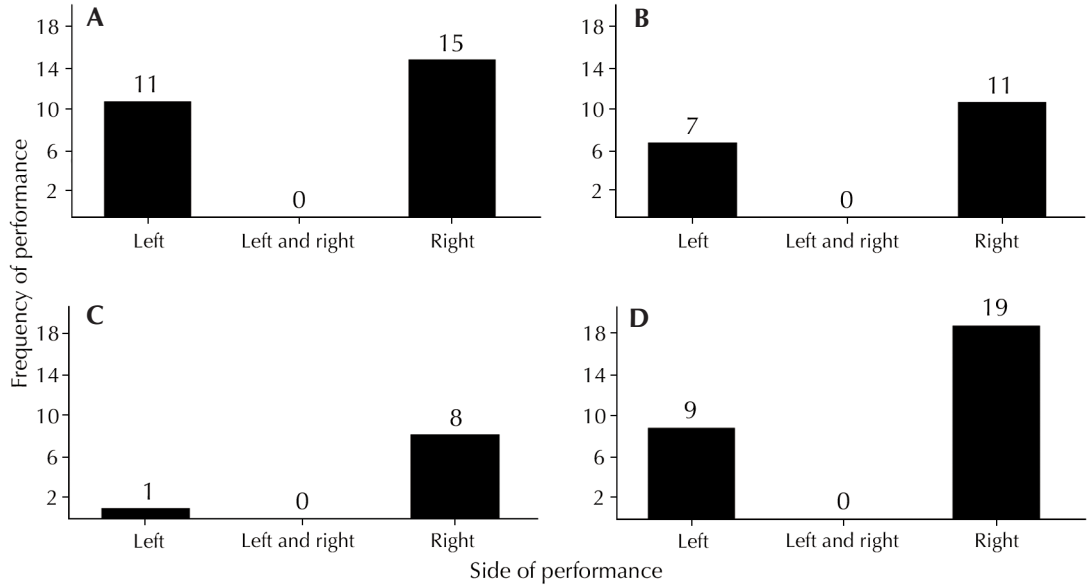

Figure 4. Frequency of performance of the finger-trembling signal according to side by male *Boana albopunctata* in distinct contexts, (**A**) without and with conspecific intruder male (**B**) in front, (**C**) on the left, and (**D**) on the right side of the resident male. Signals were performed by the resident male using the left side, the left and right sides simultaneously, or the right side. The number of records for each direction is at the top of the bars.

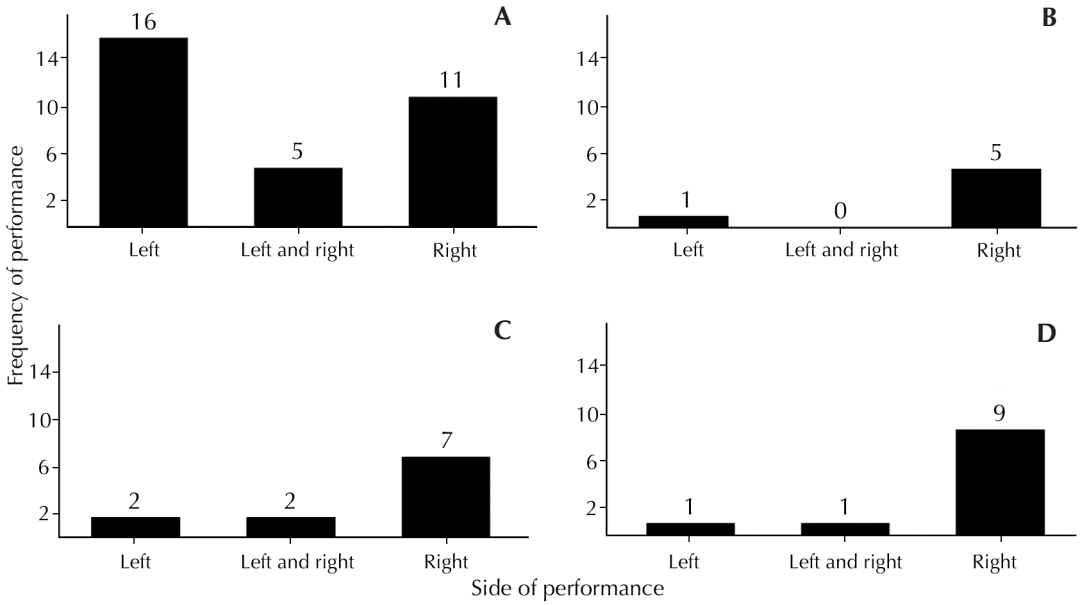

Figure 5. Frequency of performance of the leg lifting signal according to side by male *Boana albopunctata* in distinct contexts, (**A**) without and with conspecific intruder male (**B**) in front, (**C**) on the left, and (**D**) on the right side of the resident male. Signals were performed by the resident male using the left side, the left and right side simultaneously, or the right side. The number of records for each direction is at the top of the bars.

described for the species (Haddad *et al.* 1988, Heyer *et al.* 1990, De la Riva *et al.* 1997, Kwet *et al.* 2002, Bastos *et al.* 2003, Toledo *et al.* 2007).

Multimodal Signaling

There is an association between visual and acoustic signaling modes (visual and acoustic), thereby documenting multimodal signaling. All visual signals that were considered part of the multimodal signaling were performed by resident males immediately prior to calling, immediately after calling, or while in the process of calling. The vocal sac display, as it is defined, was performed when the male was not calling as a pure visual signal (Figure 2); however, by necessity, it was also inflated during call emission, presenting a multimodal signal. The other visual signals recorded (N = 281; Table 1) often were performed in association with acoustic signals (N = 268). Therefore, around 95% of visual signals recorded were observed in association with acoustic signals, indicating a high degree of association between these two signaling modes (Figure 6).

There are statistically significant preferences for performing visual signals such as toe trembling, finger trembling, and leg lifting before or after calls based on Chi-square tests [χ^2 (2, N = 240) = 9.836; p = 0.007]. The interval between both modes together with the number of transitions (visual to acoustic and vice-versa) corroborated the revealed preferences (Table 3). The numbers of visual signals preceding and following calls are about the same (Table 3). For all visual signals that were statistically analyzed, the transition intervals between signaling modes with visual signals preceding calls are significantly shorter than the intervals with visual signals being performed after calls (Figure 7; Table 4).

Table 2. Measurements of fingers, toes, and forelimbs, and Student-t values from morphological asymmetry tests for male *Boana albopunctata* (*N* = 20). Vouchers are from Cantaclaro Ranch and nearby localities and are housed at Célio F. B. Haddad collection (CFBH) in the Departamento de Biodiversidade, Instituto de Biociências of Universidade Estadual Paulista (UNESP), Rio Claro, São Paulo state, Brazil. Separating traits bilaterally, average (mm) SD values for morphological measurements are shown with t- and *p*-values from statistical comparisons. All morphological traits measured are statistically symmetric ($p > 0.05$).

Trait	Left	Right	t	*p*
Finger I	5.65 0.6	5.46 0.6	0.966	0.34
Finger II	6.34 0.6	6.01 0.6	1.587	0.121
Finger III	8.1 0.87	7.83 0.71	1.043	0.304
Finger IV	6.78 0.8	6.22 0.8	1.943	0.06
Toe I	5.08 0.7	5.38 0.8	1.245	0.221
Toe II	6.25 0.7	6.44 0.9	0.716	0.479
Toe III	9 1.2	9 1.2	0.164	0.871
Toe VI	12.8 1.4	12.5 1.6	0.466	0.644
Toe V	8.3 0.8	8.5 1	0.504	0.618
Thigh	26.22 2.1	25.92	0.427	0.672
Tibia	27.28 2.1	27.25	0.052	0.959
Tarsus	16.25 1.2	16.5 1.4	0.535	0.596
Foot	20.3 1.8	19.9 1.8	0.664	0.511

Table 3. The number of transitions of visual signals performed before or after acoustic signals, and the number of visual signals performed simultaneously with acoustic calls. We also show average transition intervals between visual signals and acoustic signals performed by male *Boana albopunctata* (SD; *N* = 30 males).

Visual signal	Visual signal performed before call (*N*)	Visual signal and call performed simultaneously (*N*)	Visual signal performed after call (*N*)	Time from visual signal to call (s)	Time from call to visual signal (s)
Toe trembling	39%	25%	36%	1.69 1.3	2.33 1.2
Finger trembling	41%	21%	38%	1.6 1.3	2.65 1.2
Arm lifting	46%	8%	46%	1.02 0.7	2.36 1.3
Leg lifting	45%	12%	43%	1.42 1	1.83 1.1
Face wiping	46%	0	54%	1.77 1.1	1.78 0.1
Leg kicking	57%	0	43%	1.66 1.2	2.15 0.8

Table 4. Wilcoxon test (two tailed), matching pairs of transitions (s) from visual to acoustic signals and from acoustic to visual signals for male *Boana albopunctata* (*N* = 30 males). All results were statistically significant ($p < 0.05$), indicating that those time intervals are different.

Visual signal	*Z*	*P*	*N* (pairs)
Toe trembling	-2.912	0.004	57
Finger trembling	-1.716	0.001	37
Arm lifting	-2.49	0.013	11
Leg lifting	-2.12	0.034	37

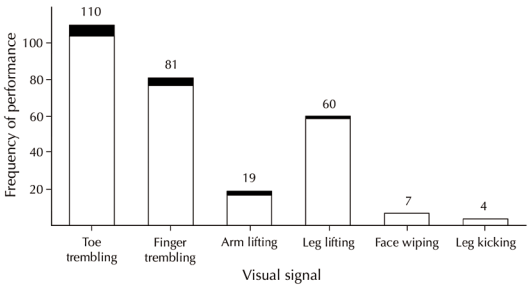

Figure 6. Frequency of visual signals recorded in association with calls for male *Boana albopunctata* (*N* = 30 males). White areas of the bars indicate the incidence of visual signals performed associated with calls and black areas indicate the incidence of visual signals not associated with calls. Numbers at the top of the bars indicate total frequency of performances for each visual signal.

Discussion

Visual Signaling for Boana albopunctata

Our total of seven visual signals constitutes the most complete repertoire of visual signals for male *Boana albopunctata* reported thus far. These include all signals previously recorded for this nocturnal treefrog (Toledo *et al.* 2007, Ramalho 2012, de Sá *et al.* 2014), with the addition of two newly recognized signals (leg lifting and face wiping), thereby improving the understanding of visual signaling by the species. We document that visual signaling by male *B. albopunctata* is more closely associated with advertisement than territorial contexts. Also, the observation that resident male frogs perform visual signals frequently in the absence of a conspecific intruder male further confirms that these stereotyped movements are true visual signals, not only a byproduct of some social excitement from visual detection.

Male *Boana albopunctata* do not direct their visual signals toward their conspecific receivers in territorial contexts. Overall, we found that the male frogs prefer to use their right sides to perform visual signals, regardless of the social context and the location of the intruder male. Our statistical analyses reveal that these preferences of sides are not associated with morphological asymmetries, in contrast from observations of *Pseudacris regilla* (Baird and Girard, 1852) (Hylidae; Dill 1977). As detected in the present study for *B. albopunctata,* preferential use of the right side has also been documented in other nocturnal frog species, such as *Bufo bufo* (Linnaeus, 1758) and *Rhinella marina* (Linnaeus, 1758) (Bufonidae; Bisazza *et al.* 1997, Robins *et al.* 1998). These reports coupled with the present study provide some insights into the evolution of brain lateralization among anurans, indicating that it might have a long evolutionary history, dating back to early tetrapods (Bisazza *et al.* 1997; Robins *et al.* 1998).

Behavioral studies of diurnal anuran species of the genera *Hylodes* and *Micrixalus* have demonstrated that individuals direct their visual signals toward their conspecific receivers (Preininger *et al.* 2013, de Sá *et al.* 2016). Male *Hylodes japi,* for example, perform visual signals by using their double vocal sacs independently, inflating either the right or left vocal sac depending on the location of the target conspecific (de Sá *et al.* 2016, Elias-Costa *et al.* 2017). Similarly, male *Micrixalus saxicola* (Jerdon, 1853) coordinate the use of their

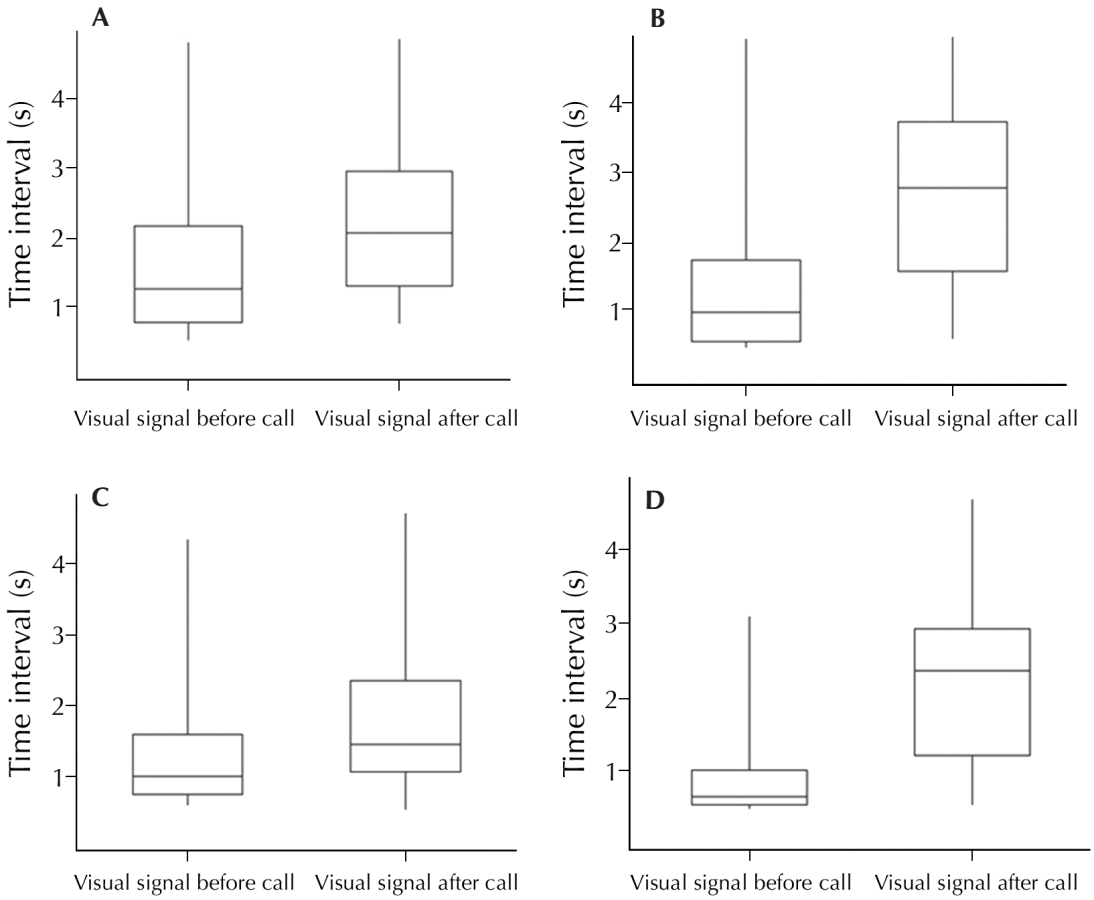

Figure 7. Time interval of transition from visual signals to calls and from calls to visual signals from the visual signals analyzed for resident male *Boana albopunctata* (*N* = 30 males): (**A**) toe trembling (*N* = 57), (**B**) finger trembling (*N* = 37), (**C**) leg lifting (*N* = 37), and (**D**) arm lifting (*N* = 11). Box plots show the median, mean (line in the middle of the box), maximum, and minimum (vertical lines above and below) time interval.

forelimbs toward the position of a conspecific (Preininger *et al.* 2013). Although the directionality of visual signals is unknown in nocturnal species of hylids, these species commonly have relatively rich repertoires of visual signals (as shown here for *B. albopunctata*), suggesting some level of importance for these stereotyped movements during intraspecific communication (Hartmann *et al.* 2005, Toledo *et al.* 2007, Gomez *et al.* 2009). Moreover, visual signals in nocturnal hylids seem to function in attracting females, as reported for *Aplastodiscus eugenioi* (Carvalho-e-Silva and Carvalho-e-Silva, 2005) (Hartmann *et al.* 2004), *Boana curupi* (Garcia, Faivovich, and Haddad, 2007) (Lipinski *et al.* 2012), and *Scinax cardosoi* (Carvalho-e-Silva and Peixoto, 1991; = *Ololygon cardosoi*) (Moroti *et al.* 2017). In addition, movements that produce visual signals also produce seismic signals (Narins 1990), especially

when on high perches, with less stable substrates, such as breeding sites. For example, in the nocturnal frog *Hyperolius puncticulatus,* tapping signals produce vibrations on the substrates, acting in close-range signaling (Starnberger *et al.* 2018). Because daylight is believed to have facilitated the evolution of visual signaling among anurans (Hödl and Amézquita 2001), it is possible that diurnal species may have more accurate and highly developed visual signaling than nocturnal species; however, it does not exclude the possibility that visual communication has evolved in nocturnal anurans. Nocturnal habits do not provide a significant barrier to signal perception among anurans because they can see well in the dark (Kelber *et al.* 2017, Mohun and Davies 2019).

Multimodal Signaling for a Nocturnal Anuran Species

Our results show that associations between visual and acoustic signals are surprisingly frequent in male *Boana albopunctata.* We noted that males tend to perform visual signals immediately before or after emitting calls, rather than performing them while calling. There is less time between visual signals performed before the start of a call than between the end of the call and the start of visual signals performed following it. Frequently, male *B. albopunctata* combine visual and acoustic signals to form multicomponent signals, with visual signals functioning as alerts for the calls. According to Hebets and Papaj (2005), a first signaling mode gets the attention of the receiver for the subsequent and associated signaling mode, with the latter carrying a more important message. This synergistic combination of distinct signaling modes may reinforce the message sent by an emitter to a conspecific (Moller and Pomiankowski 1993).

Previous information on multimodal signaling (visual and acoustic) has been reported for *B. albopunctata* (Toledo *et al.* 2007, Ramalho 2012) and for other hylids with nocturnal habits, such as *Aplastodiscus perviridis* Lutz, 1950 and *B. bischoffi* (Boulenger, 1887) (Toledo *et al.* 2007), *A. eugenioi, B. albomarginata,* and *Scinax eurydice* (Bokermann, 1968) (Hartmann *et al.* 2005), and *Hyla arborea* (Linnaeus, 1758) (Gomez *et al.* 2009). Our findings open a new window of knowledge, better suggesting how visual signals might work for a nocturnal anuran. Visual signals in nocturnal anurans (likely more connected with advertisement contexts) may have a more subtle role, alerting and actually improving communication performed via calls. This possibility reinforces that in nocturnal anurans calls convey the main message during intraspecific communication, but it does not exclude an additional important communication role for visual signaling. As far as we know, we provide the first evidence that visual signals may have an alerting function among anurans (Moller and Pomiankowski 1993, Hebets and Papaj 2005). Similar tests as those conducted here have reported calls as the alerting message for subsequent visual signals among diurnal species of the genus *Staurois* (Grafe and Wanger 2007, Preininger *et al.* 2009, Grafe *et al.* 2012). Therefore, visual and acoustic signals may have distinct roles among diurnal and nocturnal anuran species, and the synergistic combination of visual and acoustic signals might have evolved differently, most likely affected by the presence or absence of daylight (Hödl and Amézquita 2001).

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Observations on metamorphosing tadpoles of *Hyalinobatrachium orientale* (Anura: Centrolenidae)

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Abstract

Observations on metamorphosing tadpoles of *Hyalinobatrachium orientale* (Anura: Centrolenidae). Metamorphosis, when anuran amphibians resorb their tails and remodel their mouthparts and internal organs, is a vulnerable stage in the frog's life history. As larvae metamorphose from tadpoles to adult frogs, they are neither suited to aquatic life nor ready for active terrestrial life. Previous studies have examined the duration of metamorphosis in a range of species, with respect to tadpole size, habitat, and other factors; however, the duration of metamorphosis relative to where it takes place has not been reported in centrolenids. In *Hyalinobatrachium orientale,* metamorphosis takes place on the upper surfaces of the leaves of low understory plants and lasts 3.5–4.0 days, a little longer than expected for the tadpole of this body size. Metamorphs seem to shift their perches from leaf to leaf randomly. There are no significant differences in the temperature or relative humidity of the upper and lower surfaces of leaves in the forest understory; thus, the presence of the metamorphs on the upper surfaces of leaves may provide moisture from the upper story vegetation after rain and protect them from terrestrial predators.

Keywords: Anurans, Tobago Glass Frog, life history, metamorphic duration.

Resumo

Observações sobre girinos de *Hyalinobatrachium orientale* (Anura: Centrolenidae) em metamorfose. A metamorfose, processo em que os anfíbios anuros reabsorvem suas caudas e remodelam suas partes bucais e órgãos internos, é um estágio vulnerável de sua história de vida. Durante a transformação de larvas em adultos, os girinos não est**ão** bem adaptados à vida aquática nem **à** vida terrestre ativa. Estudos anteriores examinaram a duração da metamorfose em diversas espécies com relação ao tamanho do girino, habitat e outros fatores; entretanto, a duração da metamorfose em relação ao local onde esta ocorre não foi relatada pantrolenídeos. Em *Hyalinobatrachium orientale,* a metamorfose ocorre nas superfícies superiores das folhas das plantas de sub-bosque baixo e dura de 3,5 a 4,0 dias, um pouco mais do que o esperado para um girino desse tamanho de corpo. Os indivíduos recém-metamorfoseados parecem mudar seus poleiros de folha para folha aleatoriamente. Não há diferenças significativas na temperatura ou umidade relativa das superfícies superior e inferior das folhas no sub-bosque da floresta; assim, o uso das superfícies superiores das folhas pode fornecer-lhes umidade da vegetação do estrato superior após as chuvas e protegê-los de predadores terrestres.

Palavras-chave: anuros, duração da metamorfose, história natural, pererecas-de-vidro-de-tobago.

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Introduction

The transformation of anuran larvae into froglets during metamorphosis is a crucial stage in the life history of anurans. Because the metamorphs are neither well suited to aquatic life nor ready for life on land, they are vulnerable. For this reason, Wassersug and Sperry (1977) suggested that the period of metamorphosis is likely to be subject to minimizing selection. However, in a survey of 14 taxonomically diverse species, Downie *et al*. (2004) reported that metamorphic duration ranges from 2.0–7.3 days and that it is correlated with body sizes of the tadpoles and ecological factors, such as external temperature. The authors also noted that species that resorb the tail quickly emerge from the water early, whereas species in which the tail is retained longer, usually emerge from the water later. The survey included seven tree frog species from Trinidad, five being early emergers and two late.

There is no information about metamorphic behavior in centrolenid frogs, a diverse lineage comprising more than 160 species (AmphibiaWeb 2020). Hoffmann (2010) described the morphology of 13 species of centrolenid tadpoles. He achieved growth and development in captivity until just before metamorphosis (i.e. Gosner 1960, Stage 41) in two of these species. He noted that it is difficult to study centrolenid development because typically, the larvae live hidden in the stream substrate and they are difficult to maintain in captivity.

The Tobago population of the glass frog *Hyalinobatrachium orientale* is distinct enough from the Venezuelan population to merit subspecies designation as *tobagoense* (Jowers *et al*. 2014). Parental care (Lehtinen and Georgiadis 2012), development to hatching (Nokhbatolfoghahai *et al*. 2015), early tadpole morphology (Downie *et al*. 2015), and early tadpole behavior (Byrne *et al*. 2018) have been described in *Hyalinobatrachium orientale*. Herein, we report observations on metamorphosing individuals found in the field.

Materials and Methods

We surveyed six streams in northeastern Tobago (Figure 1) for *Hyalinobatrachium orientale* from June–August for each of the years 2016–2019. Surveys were conducted by teams of two to five observers who walked up the streams at night, locating glass frogs either visually or acoustically or searched the streams during the day for metamorphs and egg clutches. The tail and snout–vent lengths (SVL) of metamorphs were measured with dial callipers accurate to 0.1 mm. In addition, the distance of the metamorph from the nearest stream was recorded, as well as its height above ground (with a tape measure accurate to 1.0 mm) and location—i.e., on the ground or on a plant (leaf upper or lower side, or stem). To test the hypothesis that metamorphs move further away from streams as they develop, a one way ANOVA (analysis of variance) was conducted to examine the effect of tail length on metamorph height above the stream and metamorph distance from stream. In 2019, we marked the locations of the plants using white paper held by a stone, so that the original position of the metamorphs could be easily relocated on subsequent visits, often 3 or 4 days apart. In 2019, we transferred three metamorphs to our base in Charlotteville, where they were kept as individuals in plastic aquarium tanks containing a substrate of leaves and damp leaf litter. We assessed the rate of tail resorption in these individuals by measuring tail length daily until only a stump remained. The locations of the metamorphs in the tanks were recorded to monitor their movements. After metamorphosing, the frogs were released at their original locations.

In 2016, we measured two microhabitat parameters—viz., temperature and relative humidity. Eight Tiny Tag-2 dataloggers (Gemini, UK) that record temperature (accurate to 0.4°C in the 20–30°C range) and relative humidity (accurate to ± 3.0% at 25°C), were attached with duct tape to the upper and lower

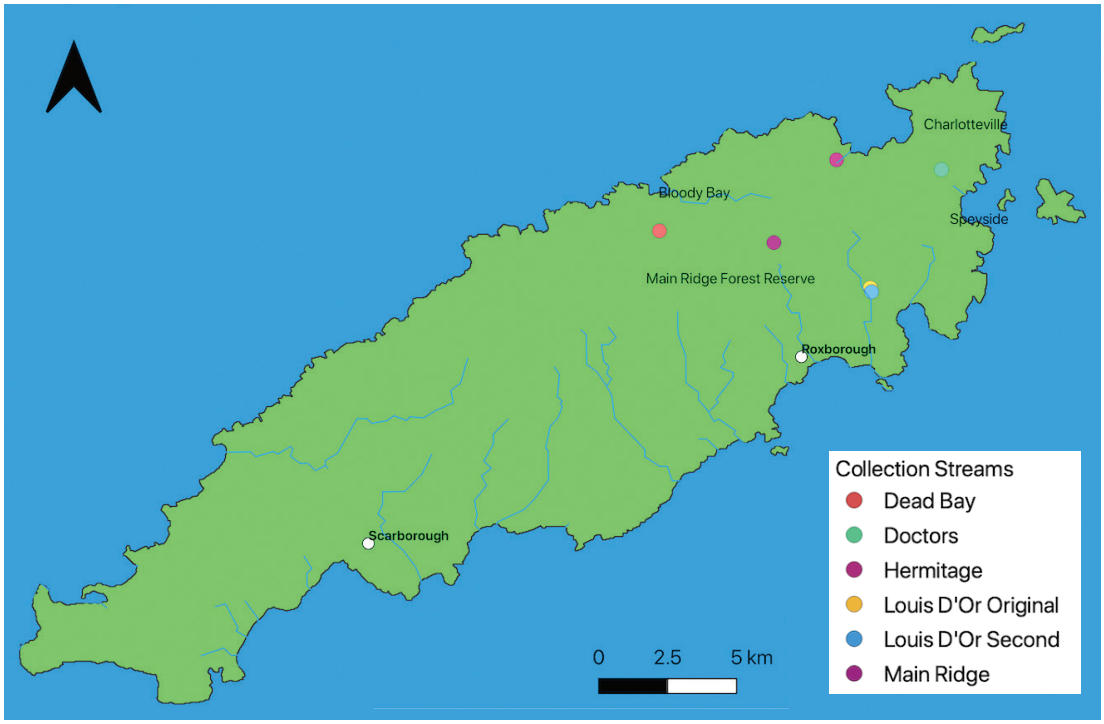

Figure 1. Map of Tobago showing the glass frog localities visited in the northeast. Map created using QGIS 3.12.

leaf surfaces of *Heliconia bihai* along four streams (Dead Bay, Main Ridge, Doctor's, and Hermitage) where glass frogs were found (Figure 1). The data loggers were deployed for 42 consecutive days in June and July and set to record once every six hours (168 recordings by each data logger). The timing of these recordings varied among the different sites, but are grouped, as follow: "Early morning," 01:30–05:30 h; "mid-morning," 07:30–11:30 h; "afternoon," 13:30–17:30 h; and "early night," 20:45–23:45 h. Data were extracted using Tiny Tag Explorer version 4.10. Site altitudes were measured using a Garmin hand-held satellite GPS. A one way ANOVA was conducted to test the effect of leaf side on temperature and humidity. The test was run for each set period of the day. Data analyses were completed using R software.

Results

Adult *Hyalinobatrachium orientale* and egg clutches were found on the undersides of leaves overhanging the streams; the frogs were found perched on leaves less than a meter above the stream to perches several meters high, usually on the leaves of *Heliconia bihai*. This general observation is based on several years of data collection, as follow: 2016, 29 surveys between 19 June and 18 August yielded 9 metamorphs and 156 egg clutches, as well as adults; 2017, 33 surveys between 20 June and 21 August yielded 10 metamorphs and 257 clutches; 2018, 1 survey on 20 June produced 3 metamorphs; and 2019, 24 surveys between 17 June and 25 July yielded 31 metamorphs. A total of 53 metamorphs was found in the 4-year study, and tail lengths were measured of 42 metamorphs in the field, with the

following results: six had the short tails (0–5.0 mm); nine had tails 5.1–10.0 mm long; 17 had tail lengths of 10.1–15.0 mm; and 10 had tails that were 15.1–20 mm long. Although these data seem to reflect a reduction in the proportions of the size classes during the 4 days of metamorphosis, a test of the results compared to the null hypothesis gives chi squared (three degrees of freedom) = 6.12, $p > 0.05$, a non-significant difference.

Metamorphs were found on the upper sides of the leaves of small plants (e.g., shrubs and tree seedlings of several different species); none was found on the ground (Figure 2). The heights of the perches ranged from 15–245 cm, and their distances from the stream from 0 (1 individual on a leaf immediately above a stream) to 300 cm. We hypothesized that initially, metamorphs would utilize the nearest leaf encountered after leaving the water (i.e., low height; short distance from water) and then move higher and farther away as metamorphosis progressed, but we found no relationship between tail length and height on plant or distance from stream (ANOVA, p = 0.13 for height; p = 0.14 for distance; N = 34).

No metamorph was found at the same location 24 hr later. Likewise, we observed that the captive metamorphs were not immobile, moving from their original leaf to new perches and substrates within the container.

The coloration of metamorphic *Hyalinobatrachium orientale* resembles that of adults—i.e., green dorsally and transparent ventrally (Figure 3A, B). Tail resorption in the three captive metamorphs was completed in 4 days (16.3–1.0 mm), 3.5 days (14.9–0.9 mm) and 3.5 days (15.0–0.7 mm), respectively (Figure 2C, D). The digestive tract and other internal organs were most clearly defined in metamorphs with the shortest tails. The three longest tails measured in the field were 19.0, 19.5, and 20.0 mm (54.3, 59.9, and 60.0% of total length, respectively). Mean body length (SVL) at the end of metamorphosis, taken as short tail stump stage (< 2.0 mm) was 10.8 ± 1.3 mm (range 8.9–13.6 mm; N = 9).

Temperature and relative humidity data from *Heliconia* leaves are shown in Table 1. The highest and lowest temperatures recorded were 34.8°C at Hermitage on the upper side of a leaf and 21.5°C at Main Ridge on the upper side of a leaf. The highest and lowest relative humidities recorded were 100% which was recorded at all locations, and 89% at Hermitage on the top side of a leaf. An analysis of variance showed no significant differences between top and lower sides of leaves at any of the locations or at any time of day for both temperature and humidity (ANOVA, $p > 0.4$ for all comparisons). Temperature changed only a little over the daily cycle, with the peak occurring in the afternoon. Relative humidity was always high, with the lowest values in the afternoon. Highland sites were a little cooler than lowland sites, but there was no difference in humidity.

Figure 2. Metamorph of *Hyalinobatrachium orientale* perched on the upper side of a plant leaf.

Discussion

Although our sample size is too small to determine the duration of metamorphosis with accuracy, and we lack data for metamorphs with

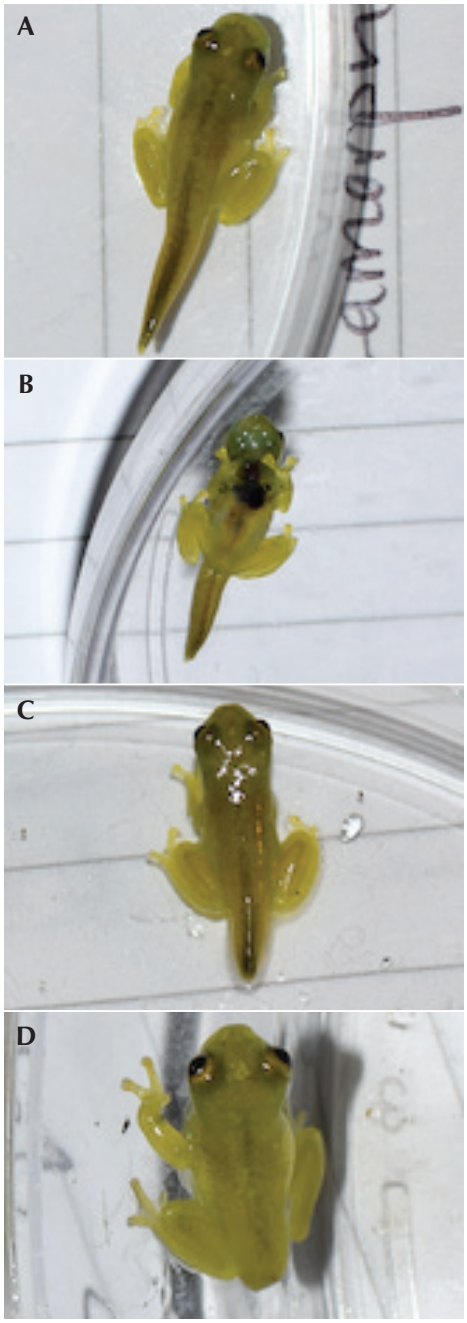

Figure 3. Metamorphs of *Hyalinobatrachium orientale* photographed in captivity. Early-stage metamorph in dorsal (**A**) ventral (**B**) views. Dorsum of mid-stage metamorph (**C**). Dorsum of late-stage metamorph tail stump (**D**).

the longest tails (19–20 mm), our data indicate that metamorphosis lasts slightly more than 3.5 days (3.6 ± 0.2). In their comparison of metamorphosis duration in 14 tadpole species representing five families (Hylidae, Leptodactylidae, Pipidae, Dendrobatidae, and Bufonidae), Downie *et al*. (2004) estimated that metamorphosis was completed in slightly more than three days in tadpoles with a maximum tail length of 20 mm. Thus, *Hyalinobatrachium orientale* is taking a little longer than expected, but we do not know how long forelimb emergence (the start of metamorphosis) takes before we find metamorphs on leaves. Crump (1984) found that hylid metamorphs left the water soon after forelimb emergence, as did Downie *et al*. (2004).

In amphibians, the duration of metamorphic climax has a direct relationship with environmental temperature, accelerating as temperature rises (Walsh *et al.* 2008, Downie *et al.* 2004). Our sample was too small to determine the effect of temperature on the duration of metamorphosis in the captive metamorphs.

Wassersug and Sperry (1977) hypothesized that high predation levels during the vulnerable stage of metamorphosis would lead to short metamorphic durations. Our finding that metamorphs of *Hyalinobatrachium orientale* always perched on the upper sides of leaves suggests that this may be a strategy to avoid ground-dwelling predators. Mating adults and egg clutches always are found on the lower sides of leaves (Nokhbatolfoghahai *et al*. 2015), but these leaves are over water. There are some 10 species of snakes on Tobago (Auguste 2018) that may prey on small frogs and most likely would approach from the ground. We analyzed our data for evidence of predator pressure; if predators are successful, one would expect that metamorphs with the shortest tails would be the least numerous. However, we found no significant difference in the proportions of size classes in the 42 metamorphs. Other potential predators include bats, crabs, and some insects, but well camouflaged, silent and mainly motionless metamorphs may be difficult for predators to locate.

Table 1. Mean temperature and relative-humidity values for upper and lower sides of leaves at different times of day and at different altitudes (Lowland sites: Hermitage, 39 m; Doctor's, 65 m. Highland sites: Main Ridge, 420 m; Dead Bay, 206 m).

Site	Time Period	Temperature (°C)		Humidity (%)	
		Upper	Lower	Upper	Lower
Lowland	Early Morning	25.1 ± 0.7	25.3 ± 0.7	99.2 ± 2.4	99.4 ± 2.3
	Mid-morning	26.8 ± 1.7	26.8 ± 1.7	93.9 ± 7.5	94.0 ± 7.5
	Afternoon	27.9 ± 1.9	28.3 ± 2.1	89.5 ± 8.2	89.4 ± 8.6
	Early night	25.6 ± 0.7	25.7 ± 0.7	97.8 ± 2.8	98.9 ± 2.3
Highland	Early Morning	23.8 ± 1.2	23.9 ± 1.9	98.9 ± 4.5	98.8 ± 4.8
	Mid-morning	24.6 ± 1.1	24.7 ± 1.0	98.3 ± 5.2	98.4 ± 5.2
	Afternoon	26.2 ± 1.8	25.9 ± 1.6	93.4 ± 7.7	94.3 ± 6.4
	Early night	24.2 ± 1.4	24.3 ± 1.4	98.4 ± 4.5	98.5 ± 4.9

The mobility of the metamorphs needs more investigation. Our data do not support the hypothesis that metamorphs would move to higher locations or farther from the stream as metamorphosis progressed. Metamorphs might move to confuse potential predators or perhaps the froglets are searching for moisture to avoid dehydration through cutaneous water loss, which is likely given the high surface area/volume ratio of small frogs compared to larger ones (Levy and Heald 2016).

The variation in numbers of clutches and metamorphs found year to year reflects different objectives of each year's fieldwork. In 2016 and 2017, we focused on centrolenid clutches and their development, but less so in 2018; in 2019, we emphasized collecting data on metamorphs.

We explored whether metamorphs on the upper surfaces of leaves are subject to more water loss than those on lower surfaces. Because the leaves on which the metamorphs typically perched were too small to support the weight of the TinyTag data loggers, temperature and humidity data were recorded from the leaves of *Heliconia,* which have a similar surface texture to those used by metamorphs. *Hyalinobatrachium orientale* typically deposits it egg clutches on leaves of *Heliconia bihai* if it is present (Byrne *et al.* unpubl. data). Relative humidity and temperature readings at the upper and lower surfaces of leaves are similar—doubtless because these understory plants usually are near streams in tropical rain forests, where neither direct sunlight nor direct rainfall usually penetrates. In addition, temperatures varied only slightly across the daily cycle. By perching on the upper surfaces of leaves, metamorphs will be hydrated by water that drips from the upper canopy when it rains.

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Phenology and mortality of embryos in a Colombian population of *Nymphargus grandisonae* (Anura: Centrolenidae)

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Abstract

Phenology and mortality of embryos in a Colombian population of *Nymphargus grandisonae* (Anura: Centrolenidae). Studies of phenology and natural history of anurans provide information useful for its conservation. This is particularly important in current scenarios of emergent diseases, high rates of deforestation, and climate change. We recorded the timing of breeding of the Glass Frog *Nymphargus grandisonae* and its relationship to precipitation and environmental temperature for four years in a population located in the Central Andes of Colombia, South America. In addition, we recorded the causes of mortality in egg clutches of this species. We quantified infestation by fly larvae and its impact on the survival of embryos. We found that the reproductive activity of *N. grandisonae* (number of males calling) was seasonal and occurred in months with highest precipitation. The main cause of embryo mortality in clutches was associated with the infestation by a spittlebug fly of the genus c.f. *Cladochaeta* (Drosophilidae). The survival of embryos in infested clutches was significantly lower than in those clutches not infested by fly larvae. Our results provide baseline data for the monitoring of anuran population dynamics in the Andean region using the glass frog *N. grandisonae* as a model system.

Keywords: Amphibians, Glass Frogs, fly larvae infestation, timing of breeding.

Resumo

Fenologia e mortalidade de embriões em uma população colombiana de *Nymphargus grandisonae* (Anura: Centrolenidae). Estudos de fenologia e história natural dos anuros fornecem informações úteis para sua conservação. Isso é particularmente importante nos cenários atuais de doenças emergentes, altas taxas de desmatamento e mudanças climáticas. Monitoramos a época de reprodução da perereca-de-vidro *Nymphargus grandisonae* (Centrolenidae) e sua relação com a precipitação e a temperatura ambiental durante quatro anos em uma população localizada nos Andes Centrais da Colômbia, América do Sul. Além disso, registramos as causas de mortalidade nas

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desovas dessa espécie. Quantificamos a infestação por larvas de moscas e seus efeitos na sobrevivência dos embriões. Descobrimos que a atividade reprodutiva de *N. grandisonae* (número de machos vocalizando) foi sazonal e ocorreu nos meses de maior precipitação. A principal causa de mortalidade embrionária nas desovas esteve associada à infestação por uma mosca do gênero c.f. *Cladochaeta* (Drosophilidae). A sobrevivência de embriões em desovas infestadas foi significativamente menor do que naquelas não infestadas pelas larvas da mosca. Nossos achados fornecem dados básicos para o monitoramento da dinâmica populacional de anuros na região Andina usando a perereca-de-vidro *N. grandisonae* como modelo de estudo.

Palavras chave: anfíbios, época de reprodução, infestação por larvas de mosca, pererecas-de-vidro.

Introduction

Phenology or the timing of breeding of organisms is influenced by biotic and abiotic factors of the environment (van Shaik *et al.* 1993, Mistlberger and Rusak 2005, Chuine and Régnière 2017). Precipitation, temperature, and photoperiod seem to be the most important abiotic factors affecting timing of breeding in diverse vertebrates. Those factors influence patterns of photosynthesis, growth, and reproduction in plants, which in turn determine the availability of food items that trigger breeding activity in vertebrates (Losos 2009, Lillywhite 2014, Winkler 2016, Strier 2017). On the other hand, biotic factors influencing timing of breeding include predation and competition. For instance, nest predation and interspecific competition might delay reproduction and determine the use of specific places for breeding in birds and mammals (Verhulst and Tinbergen 1991, Samplonius and Both 2017, Strier 2017). Timing of breeding is often less constrained in tropical humid regions than in highly seasonal environments such as those in temperate regions and arid regions (Winkler 2016, Strier 2017, Torres-Cervantes *et al.* 2019). Moreover, timing of breeding can be plastic in many organisms, but such plasticity is constrained by developmental, hormonal, genetic and evolutionary aspects (Sockman *et al.* 2006, Chuine and Régnière 2017).

Amphibian anurans are ectothermic vertebrates with complex life cycles that depend directly on environmental conditions for survival and reproduction (Duellman and Trueb 1994, Wells 2007). Given that anurans are present in almost all areas of the world, species in this clade face diverse environmental conditions, likely leading to high variation in timing of breeding that reflects adaptations to local conditions (Andrade *et al.* 2016, Hernández-Salinas *et al.* 2018, Torres-Cervantes *et al.* 2019, Vargas-Salinas *et al.* 2019). In both temperate and tropical regions, anurans exhibit explosive and prolonged breeding; the former are those species whose breeding events endure only one or few nights, while the latter are those species whose breeding persist several weeks (Wells 1977). Whether an anuran species is a prolonged or explosive breeder is attributed in part to abiotic factors of the environment, especially precipitation and temperature. These factors determine the availability of food that a species can invest in breeding and the availability of resources used directly for breeding (McCauley *et al.* 2000, Wells 2007). Timing of breeding in anurans aids in understanding the outcome of biotic interactions and also provides information for detecting and monitoring potential effects of disturbances and environmental changes (Paton and Crouch III 2002, Lips *et al.* 2005, Llusia *et al.* 2013, Walls *et al.* 2013).

In the Andes of northern South America, hundreds of anuran species have a restricted distribution (Navas 2006, Bernal and Lynch 2008, Vasconcelos *et al.* 2019). Among those species, glass frogs (Centrolenidae) comprise a

clade with more than 155 species that have reached their highest species diversity at intermediate altitudes (Hutter *et al.* 2013a, Frost 2020) where the effects of emergent diseases, deforestation and climate change are expected to be particularly strong (Lips *et al.* 2005, Navas 2002, 2006, Navas *et al.* 2013). Glass frog females deposit their eggs on substrates out of water along streams, and after several days or weeks (e.g. > 20 days), the eggs hatch and embryos fall into the water where they complete development (Cisneros-Heredia and McDiarmid 2007). Because of their oviposition out of water, it is generally assumed that the timing of breeding in glass frogs is associated with seasons of high rainfall; however, some species reproduce during the summer season (Delia *et al.* 2010, Cabanzo-Olarte *et al.* 2013, Ortiz-Ross *et al.* 2020). In addition, timing of breeding can change between populations or even within a single population among years depending on variations in environmental factors (Wells 2007, Dodd *et al*. 2012). This variability in timing of breeding restricts generalizations found in studies of specific taxa and highlights the necessity to quantify phenology in a diversity of species (Duellman and Trueb 1994, Wells 2007, Ortiz-Ross *et al.* 2020).

In addition to phenology, causes and extent of mortality in anurans provide a quantitative basis for possible alterations in the dynamics of anuran populations (Dodd Jr. *et al.* 2012). Anurans are subjected to multiple causes and levels of mortality, which is particularly high in egg clutches and larval stages (Wells 2007, Vitt and Caldwell 2014). Glass frog embryos and other lineages of anurans with oviposition out of water are especially susceptible to desiccation (Juncá 1996, Touchon and Warkentin 2010, Delia *et al.* 2013, 2014, 2019, Salica *et al.* 2017), predation and parasitism by terrestrial vertebrates and invertebrates (Villa 1977, Villa and Townsend 1983, Hayes 1983a, Warkentin 1995, Neckel-Oliveira and Wachlevski 2004). In some species, mortality can be especially high in early developmental stages when egg capsules are not well hydrated or the embryos have not developed enough to escape predators (Warkentin 1995, Delia *et al.* 2017, 2020). Predation and infestation by fly larvae are frequently recorded on egg clutches of glass frogs (McDiarmid 1978, Toledo 2007), but their effect on the survival of embryos has rarely been quantified in detail (Villa 1977, Hawley 2006, Delia *et al.* 2013, Salgado and Guayasamín 2018).

We studied the phenology of the glass frog *Nymphargus grandisonae* (Cochran and Goin, 1970) and the causes and extent of mortality in its egg clutches. We examined the following two questions: (1) Is the timing of breeding of the glass frog *N. grandisonae* related to levels of precipitation and temperature? and (2) Are embryos in clutches infested by fly larvae more prone to mortality than embryos in uninfested clutches? Our results complement available information for reproductive ecology of glass frogs in the Central Andes of Colombia (e.g. Basto-Riascos *et al.* 2017, Arcila-Pérez *et al.* 2017, Ospina-L *et al.* 2017, Rios-Soto *et al.* 2017) and provide baseline data for further monitoring of the population dynamics of anurans in the Andean region of northern South America.

Materials and Methods

Species and Study Area

Nymphargus grandisonae is a nocturnal and arboreal species distributed in Colombia and Ecuador between 1230–3230 m elevation (Frost 2020, Figure 1). Adult males exhibit a body size (snout–vent length, SVL) between 25.1–29.3 mm, while adult females are a bit larger (SVL: 28.9–30.7 mm) (Duellman 1980, Vanegas-Guerrero *et al.* 2014). Males are territorial and call from leaves located between 0.5 and > 4 m height (Hutter *et al.* 2013b). The clutches have between 30–74 eggs enclosed in a jelly rich matrix, and they are attended by the females for a few hours immediately after oviposition (Ospina-Sarria *et al.* 2011, Guevara-

Molina and Vargas-Salinas 2014, Delia *et al.* 2017, 2020).

This study was performed at the Granja Experimental Bengala (04°41'08.8" N, 75°37'02.9" W), located in the municipality of Filandia, Quindío department, Central Andes of Colombia (Figure 1). The original native vegetation in the area is premontane forest; however, at present the landscape is dominated by open areas used for raising cattle and agriculture (Renjifo 2001). The annual temperature in the study area varies between 12°C and 27°C. The precipitation regime is bimodal, and the average annual rainfall is around 2739 mm. Most rainfall occurs between April–May and October–November (Franco and Bravo 2005, Arcila *et al.* 2011).

Methodology

Phenology.—From April 2015 to January 2016 and September 2017 to May 2018, we made 47 fieldtrips, each lasting one or two nights and separated by one or two weeks. Field trips were most frequent in months with higher levels of rainfall because breeding activity of *N. grandisonae* is lower at summer; that is, from January to March and June to August (F. Vargas-Salinas and C. Guevara-Molina, pers. observ.). Visual encounter surveys (Crump and Scott Jr. 1994) were conducted in two linear transects. One transect (200 m long) was established along a stream, and the other transect (400 m long) was established along a path (Figure 1). The sampled stream and path form an "X"; the path crosses the stream and is directed in a semi-parallel way to it in both directions. The longest distance between the stream and a given point on the sampled path was around 20 m. We performed nightly surveys (19:00–00:00 h) to record number of calling males and number of females arriving to breed; diurnal surveys (8:00–11:00 h, 14:00–16:00 h) were performed to look for egg clutches and to monitor survival of embryos. Each nocturnal and diurnal survey consisted of walking the two transects by 2–4 researchers. The total nightly sampling effort in this study was 860 person-hours (April 2015–January 2016: 325 h; September 2017–May 2018: 535 h), while the total diurnal sampling effort was 830 person-hours (April 2015–January 2016: 325 h; September 2017–May 2018: 505 h).

When a frog or an egg clutch was observed for the first time, we recorded the date, time, and type and height of the substrate. The location was marked with a small labeled flag. Body size of frogs (SVL: snout–vent length to the nearest ± 0.01 mm) was measured with a digital caliper; the sex was assigned based on calling behavior, body size, presence of humeral spine (in males) and presence of eggs in the belly (females). Photographs of frogs were used as a non-invasive identification technique because individuals of *N. grandisonae* have distinctive red dots on their back that vary in number, size and distribution. The use of color patterns of individuals is useful for unambiguously distinguishing individuals in many anurans (Donnelly *et al.* 1994, Ferner 2010), and have been successfully used in other studies with glass frogs (e.g. Basto-Riascos *et al.* 2017, Ospina-L. *et al.* 2017, Díaz-Ricaurte *et al.* 2019).

Causes and levels of embryo mortality.—A photograph of the clutches was taken during each diurnal and nocturnal survey for recording and monitoring the number of embryos and their stage of development (*sensu* Gosner 1960, Salazar-Nicholls and del Pino 2015). Because some clutches exhibit infestation by fly larvae (Rojas-Morales and Escobar-Lasso 2013), photographs were also used to document their presence, development and number. For two infested clutches, we placed a small container under the clutch so that the hatched tadpoles fell into it (Hayes 1983b). Those tadpoles were collected and fixed in 96% alcohol. Three clutches infested by fly larvae were covered with an awning to collect metamorphosed flies. These flies were killed and preserved by

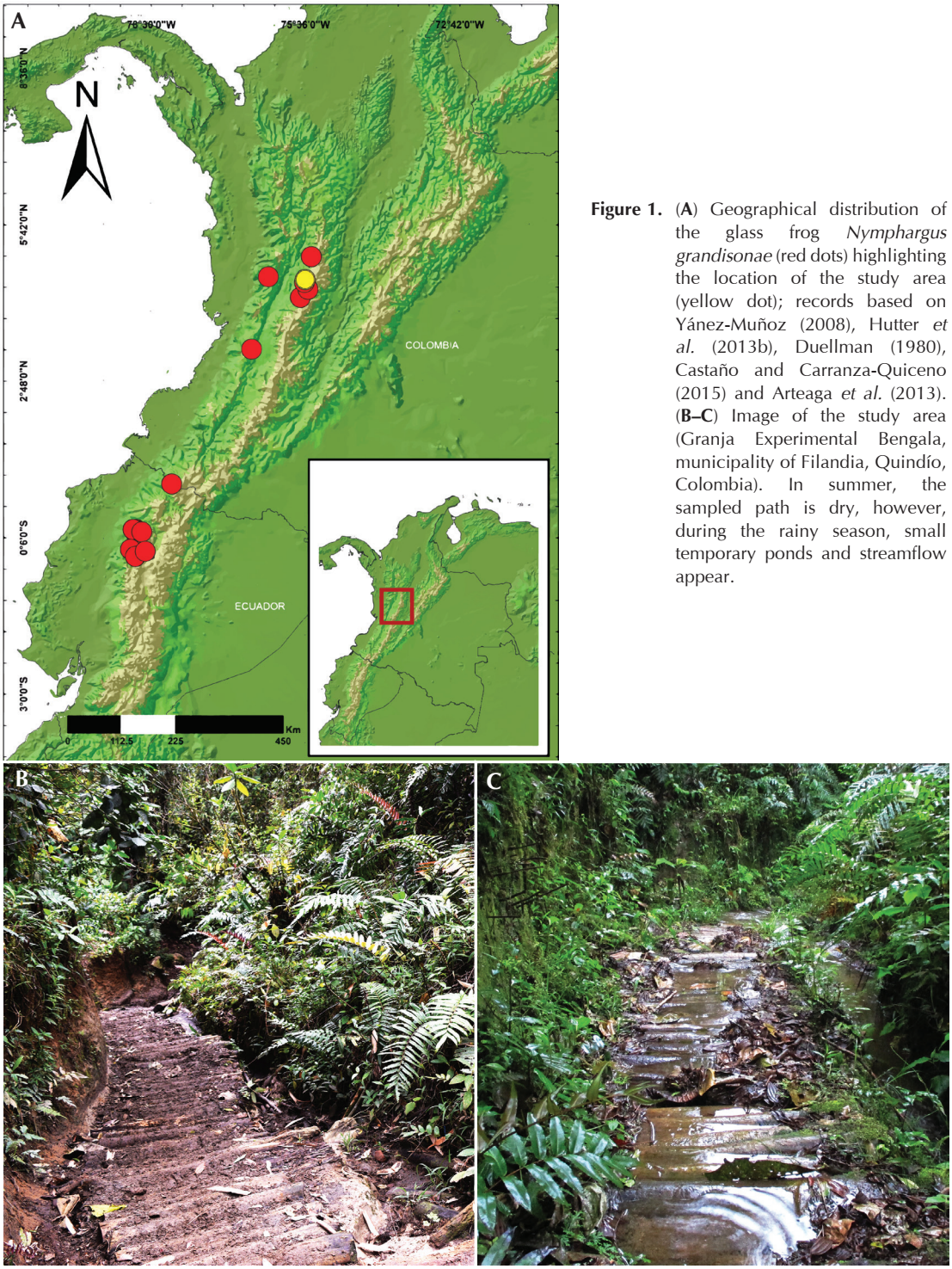

Figure 1. (**A**) Geographical distribution of the glass frog *Nymphargus grandisonae* (red dots) highlighting the location of the study area (yellow dot); records based on Yánez-Muñoz (2008), Hutter *et al.* (2013b), Duellman (1980), Castaño and Carranza-Quiceno (2015) and Arteaga *et al.* (2013). (**B–C**) Image of the study area (Granja Experimental Bengala, municipality of Filandia, Quindío, Colombia). In summer, the sampled path is dry, however, during the rainy season, small temporary ponds and streamflow appear.

immersion in 96% alcohol, and identified based on Grimaldi (1990), and with the help of Deivys M. Alvarez (Ph.D student at University of São Paulo, Brazil).

Data Analysis

Descriptive statistics summarizing the key characteristics of clutches and frogs were recorded in this study and expressed in terms of mean ± standard deviation (SD) and range in parentheses. To test whether larger males were more prone to call more nights than smaller males we used a Spearman correlation (Sr) analysis. To assess the relationship between environmental factors and breeding activity we used four Spearman correlations, two for testing the relationship of temperature with number of males calling and number of clutches laid by females; the other two for testing the relationship between precipitation level with males calling and number of clutches. For these correlations we pooled data of surveys per month; that is, the unit of analysis was the sampled month (N = 19). As independent variables we used the average value of environmental temperature recorded in a given month; for precipitation level we used total rainfall per month. These environmental data were obtained from a weather station located in the study area (data courtesy of the Corporación Autónoma Regional del Quindío, CRQ). As dependent variables we used the average number of calling males recorded in surveys per month, and the number of egg clutches recorded by the first time per month. To examine the effect of fly larvae infestation on the mortality of embryos of *N. grandisonae,* we performed a Kaplan-Meier survival test, applying censorship to those clutches whose cause of mortality occurred for other reasons than fly larvae infestation (Therneau and Grambsch 2000). All the mentioned statistical analyses were made with the software program SPSS v.21 (IBM 2012).

Results

Phenology

We recorded 31 adult males with an average body size of 28.17 mm ± 2.43 (22.09–32.31 mm). Most of males called for a small number of nights (1–3 nights, Figure 2). The body size of males was unrelated to the number of nights they called, although a tendency is apparent (Sr = 0.307, p = 0.093, N = 31 adult males). The mean time elapsed between the first capture and the last recapture of the same male was 130.57 days ± 102.23 (7–284 days, N = 19 males). More calling males were found during months with higher precipitation, compared to months with lower precipitation (Sr = 0.444, p = 0.057, N = 19 months, Figure 3). Temperature was not related to the number of calling males (Sr = -0.191, p = 0.433, N = 19 months). A relationship between number of females in breeding activity (i.e. in amplexus or gravid females) and environmental variables was not possible because of the small number of females recorded in our study: eight adult females with an average body size of 32.14 mm ± 1.94 (29.39–34.32 mm).

We recorded 21 egg clutches on the upper surface of small leaves at 2.02 m height ± 0.57 (0.47–5.00 m). The clutches when first observed consisted of 52.26 eggs ± 12.52 (17–75 eggs). The number of clutches recorded for the first time was unrelated to precipitation levels (Sr = 0.319, p = 0.183, N = 19 months) and temperature (Sr = -0.209, p = 0.390, N = 19 months). In these two analyses we assumed that females laid only one clutch per reproductive season.

Causes and Levels of Embryo Mortality

Thirteen clutches (62% of total clutches located) were infested by spittlebug flies of genus c.f. *Cladochaeta*: Drosophilidae. These 13 infested clutches exhibited a lower embryo survival than the 8 non-infested clutches

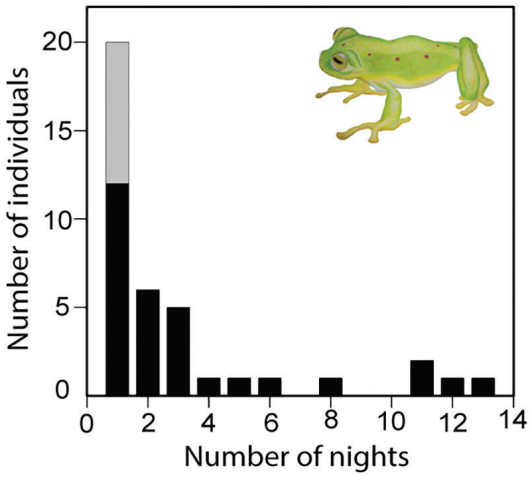

Figure 2. Frequency of breeding activity (number of nights the same frog was recorded) in *Nymphargus grandisonae* at the Estación Experimental Bengala, department of Quindío, Central Andes of Colombia. The black bars correspond to calling males and the gray bar to females. Illustration of the frog by María José Tovar-Gil.

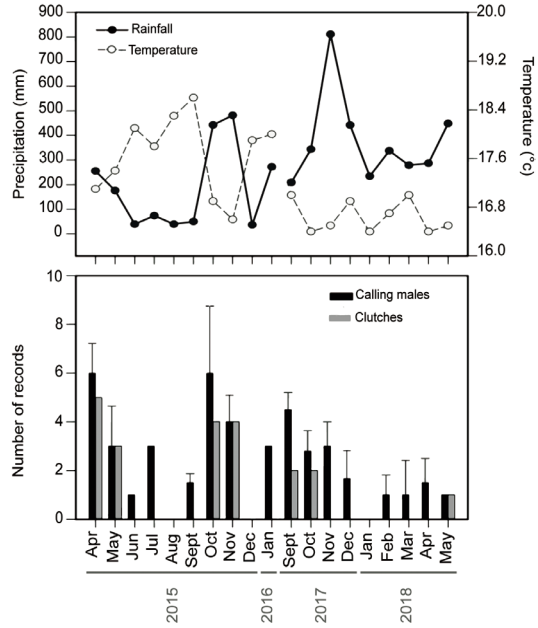

Figure 3. Relationship between precipitation, environmental temperature, and indicators of breeding activity (number of males calling, number of clutches) in the glass frog *N. grandisonae.* Black bars in the lower plot correspond to averages and standard deviations; Gray bars are the sum of egg clutches observed for the first time each month. We did not perform fieldtrips between February 2016 and September 2017.

(Kaplan-Meier Test, $\chi^2 = 5.08$, $p = 0.024$, Figure 4). In one of the two infested clutches in which we implemented the methodology suggested by Hayes (1983b) to catch hatchlings, we recorded 18 embryos of *N. grandisonae*. Those embryos were at development stage 17–18 (*sensu* Gosner 1960, Salazar-Nicholls and del Pino 2015); the fly larvae in this clutch continued their development normally. The container in the second clutch was lost after heavy rainfall. Another cause of mortality in clutches of *N. grandisonae* was observed. Two egg clutches disappeared after heavy rains; one had 28 fly larvae and no embryos of *N. grandisonae,* and the other had 7 fly larvae and 44 embryos of *N. grandisonae* (development stage 19). During our monitoring we did not observe any predation events on the clutches.

After oviposition, a clutch was composed of a single layer of eggs (Figure 5A) and covered by a jelly that began to absorb water from the environment and gain volume (Figure 5B). The first observation of fly larvae infestation was recorded when the embryos were in developmental stage 17–18 (Gosner 1960, Salazar-Nicholls and del Pino 2015). The infestation by fly larvae was evident by the presence of small whitish larvae that spread throughout the jelly covering the eggs (Figure 5C). After some days (5–10), fly larvae grouped in the lower part of the jelly (Figure 5D) where they continued their development until the pupa stage (Figure 5E). Finally, the flies emerged (Figure 5F).

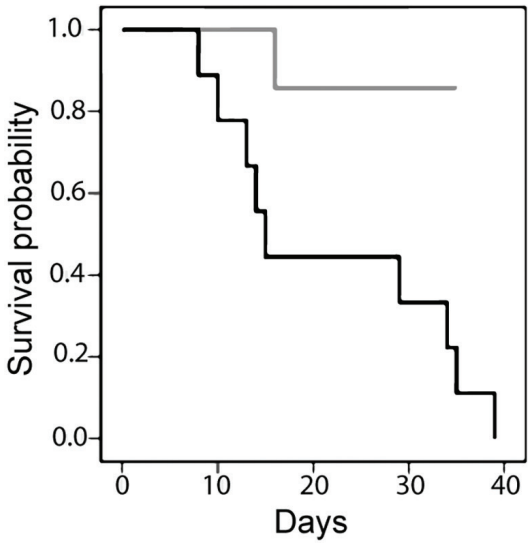

Figure 4. Difference in survival of embryos between infested (black line) and non-infested (grey line) egg clutches of the glass frog *Nymphargus grandisonae.* Infestation of egg clutches by fly larvae of the genus c.f. *Cladochaeta* (Drosophilidae). The X-axis corresponds to the number of days passed from the first observation of the clutch (i.e. it does not necessarily reflect time from oviposition).

Discussion

The timing of breeding of *N. grandisonae* in the study area occurred during months of higher precipitation level. Calling males and the presence of egg clutches were scattered through such months, although with some peaks of male activity at times of highest rainfall. Overall, data suggest that *N. grandisonae* is a seasonal prolonged breeder. Sporadic surveys before and after our sampling periods and the observation of egg clutches throughout the rainy season support this tendency.

Most of the males of *N. gradisonae* called for only few nights (i.e. 1–3 nights). A similar pattern in calling activity of males has been observed in other glass frogs [*Hyalinobatrachium fleischmanni* (Boettger, 1893) (Greer and Wells 1980); *Hyalinobatrachium valerioi* (Dunn, 1931) (Mangold *et al.* 2015); *Espadarana prosoblepon* (Boettger, 1892) (Basto-Riascos *et al.* 2017); *Centrolene savagei* (Ruiz-Carranza and Lynch, 1991) (Ospina-L. *et al.* 2017)] and other anuran families [Hyperoliidae: *Hyperolius marmoratus* Rapp, 1842 (Dyson *et al.* 1998); Dendrobatidae: *Oophaga pumilio* (Schmidt, 1857) (Pröhl 2003); Hylidae: *Hyla arborea* (Linnaeus, 1758) (Friedl and Klump 2005); Leptodactylidae: *Leptodactylus labyrinthicus* (Spix, 1824) (Zina and Haddad 2005)]. This pattern may be the result of trade-offs between costs and benefits of calling (Schaffer 1974, Crenshaw 1966, Dugas *et al.* 2015). It is well stablished that calling is energetically expensive for males in anurans (Pough *et al.* 1992, Wells 2001, Bevier 2016). Calling subjects males to the risk of being easily located by predators and parasites (Tuttle and Ryan 1981, Gerhardt and Huber 2002, Bernal *et al.* 2006). However, increasing the time (number of nights in this study) that a male is calling is often associated with higher mating success in anurans (Greer and Wells 1980, Jacobson 1985, Wells 2007, Mangold *et al.* 2015). Therefore, the number of nights a male calls is influenced by intrinsic physiological and biochemical characteristics and extrinsic local environmental factors (Gerhardt and Huber 2002, Bevier 2016). Further studies will be necessary to establish whether males of *N. grandisonae* calling more nights obtain higher mating opportunities but are more prone to predation than males calling fewer nights.

A positive relationship between rainfall and the number of males calling in *N. grandisonae* has been found in multiple anuran lineages (Dendrobatidae: Pröhl and Hödl 1999; Microhylidae: Prado *et al.* 2005 and Hawley 2006; Hylidae: Muñoz-Guerrero *et al.* 2007; Bufonidae: Ngo and Ngo 2013; Leptodactylidae: Giaretta and Menin 2004; Centrolenidae: Cabanzo-Olarte *et al.* 2013, Noronha and Rodrigues 2018). This relationship has been attributed to the dependence of anuran species on humid environmental conditions and water bodies for oviposition and the subsequent

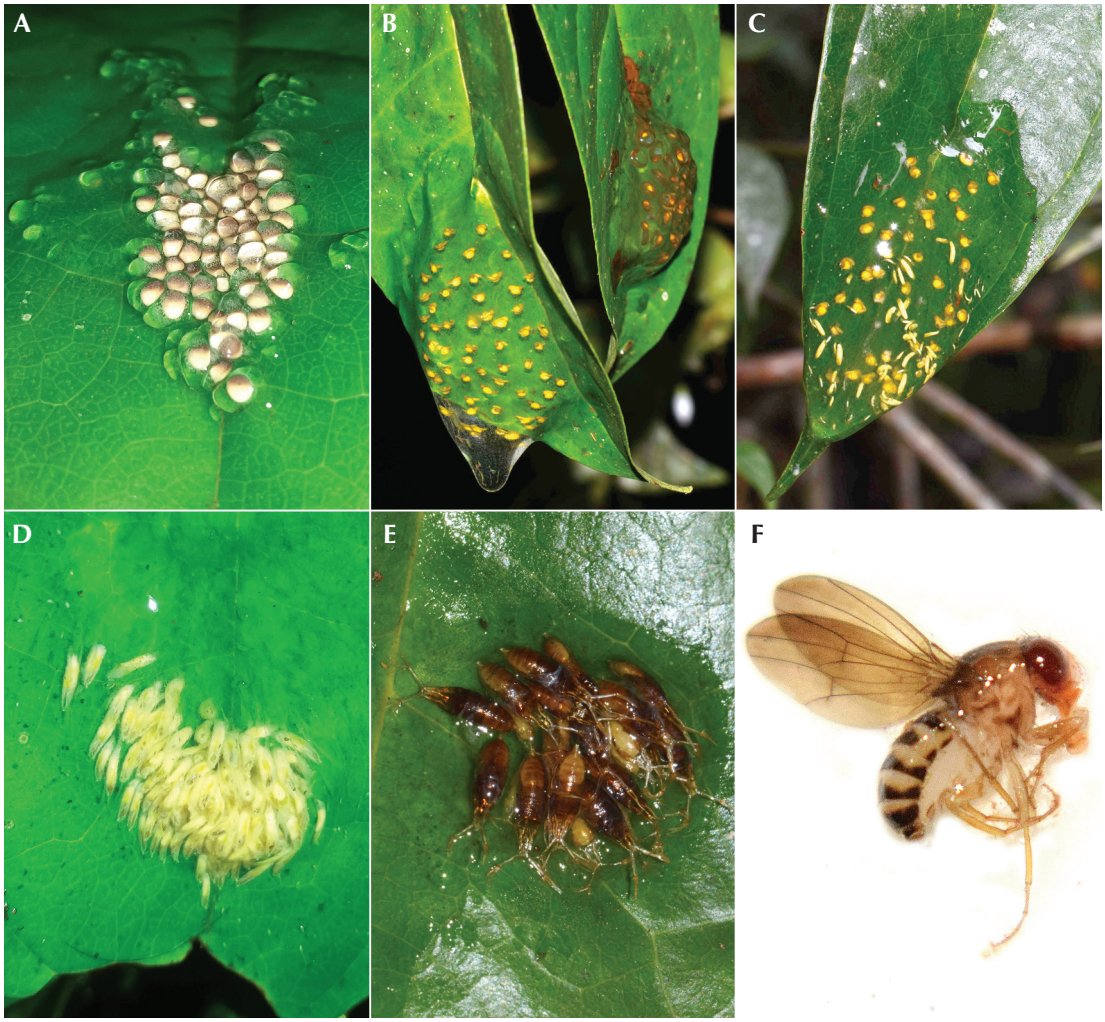

Figure 5. Clutches of the glass frog *Nymphargus grandisonae* and development sequence of the fly larvae of genus c.f. *Cladochaeta:* Drosophilidae. Image of a clutch minutes after oviposition (**A**), and after some days of embryo development (**B**); note that the clutches swell because the volume of the jelly matrix covering eggs increases. Image of an infested clutch by fly larvae in early stages of development; embryos of *N. grandisonae* are still observed (**C**). Clutch when the embryos of *N. grandisonae* are gone and only larvae of the fly remains (**D**). Clutch in advanced stage of fly larvae infestation (**E**). External morphology of the fly after emerging from the pupae (**F**).

development of embryos and tadpoles (Wells 2007). In contrast, we did not find any association between temperature and number of males calling in *N. grandisonae,* despite this factor playing an important role in the reproductive phenology of many anurans (Saenz *et al.* 2006, Steen *et al.* 2013, Green 2016). Breeding activity patterns in tropical anurans is mainly related to rainfall (Crump 1974, Aichinger 1987, Duellman 1995, Gibbs and Breisch 2001, Wells 2007,

Segev *et al.* 2012) unlike anuran species of temperate zones, in which phenological patterns are influenced mainly by temperature (Reading 1998, Silva-Ximenez and Tozetti 2015, Plenderleith *et al.* 2017).

We recaptured 19 of 31 males, but no females were recaptured. This result is frequent in studies of reproductive activity of anurans because males remain at breeding sites longer than females (Duellman and Trueb 1994, Wells 2007, Vitt and Caldwell 2014). Moreover, in several species the females skip reproductive seasons without breeding (Halliday and Tejedo 1995). Males of *N. grandisonae* are highly territorial and engage in physical contests (Hutter *et al.* 2013b), and endurance rivalry is a male-male competition mechanism usually associated with mating success in glass frogs (Greer and Wells 1980, Jacobson 1985, Mangold *et al.* 2015, Basto-Riasco *et al.* 2017, Ospina-L *et al.* 2017). This behavior may be associated with a positive relationship between male body size and the number of nights a male of a *N. grandisonae* was recorded calling (a proxy to endurance rivalry in anurans) and not an artifact of our sampling design (i.e. surveys separated by one or two weeks). Further studies are necessary to corroborate this hypothesis because patterns of timing of breeding in anurans may or may not be associated with male body size (Hernández-Salinas *et al.* 2018, Torres-Cervantes *et al.* 2019, Pompeu *et al.* 2020).

The number of egg clutches could be used as an indicator of the level of reproductive activity of females in anurans (Wells 2007, Vitt and Caldwell 2014). In species with oviposition out of water, oviposition may be associated with high rainfall or with microhabitats that remains wet even in summer (e.g. under roots and fallen trunks, cavities in the ground) (Crump 1974, Wells 2007, Vargas-Salinas *et al.* 2014, Ortiz-Ross *et al.* 2020). In the case of *N. grandisonae,* clutches are laid on leaves and thus, during development, embryos are exposed to environmental conditions that make them dependent on the presence of rainfall and temperature for survival and development (Ospina-Sarria *et al.* 2011, Delia *et al.* 2020). Therefore, we did not expect the absence of a relationship between the number of clutches of *N. grandisonae* and the levels of environmental temperature and precipitation we found. Females of *N. grandisonae* might avoid to depositing eggs near the peak of rainfall because heavy storms could promote clutch mortality, as recorded in this study. However, it is probable that the absence of said relationship is a sampling artifact. This possibility is because numerous clutches of *N. grandisonae* could be unrecorded by us, especially those placed at higher locations. In addition, we did not survey every day and night of the reproductive season, which produced a gap between the date of the clutch record and the night that the females performed oviposition. Further detailed data are required to fully understand the relationship between environmental characteristics and the arrival of *N. grandisonae* females at breeding sites. In any case, the observation of clutches throughout the rainy season is the expected pattern in prolonged breeders (Wells 1977, 2007).

Clutches of *N. grandisonae* infested by fly larvae exhibited lower embryonic survival than non-infested clutches. Egg clutches of glass frogs are preyed upon by diverse vertebrates and invertebrates that have been recorded in our study area (Delia *et al.* 2019, 2020, Ospina-L. *et al.* 2020), but the infestation by fly larvae was the most common cause of mortality in the study population. Similar observations have been seen in another population of *N. grandisonae* (Rojas-Morales and Escobar-Lasso 2013) and diverse anurans with oviposition out of water (Wells 2007). For instance, 74% mortality in egg clutches of *Physalaemus cuvieri* Fitzinger, 1826 (Leptodactylidae) was attributed to predation by fly larvae of *Beckeriella niger* (Williston, 1897) (Menin and Giaretta 2003), and 70–100% of *Hyalinobatrachium fleischmanni* (Centrolenidae) clutches were infested by larvae of *Hirtodrosophila batracida* Grimaldi, 1994 (Villa 1977, 1984). Because fly larvae can cause high levels of mortality in terrestrial and arboreal egg clutches, embryos are expected to have evolved

defensive strategies. Embryos of some anurans have evolved the capacity to evaluate mortality risks and respond accordingly, hatching earlier before reaching an optimal stage of development (Chivers *et al.* 2001, Warkentin and Caldwell 2009, Cohen *et al.* 2016). Early hatching is induced in embryos of glass frogs by predation, dehydration, and fungal infection (Delia *et al.* 2019); thus, it is possible that fly larvae cause similar behavior in *N. grandisonae.* The capture of embryos at stage 17–18 out of the jelly matrix using the technique of Hayes (1983b) in an infested clutch, do not allow us to test alternate hypotheses. Also, will be necessary to corroborate if the fly larvae feed on embryos of *N. grandisonae,* as reported for other anurans (e.g. *Hyalinobatrachium fleischmanni, Agalychnis callidryas* (Cope, 1862), and *A. annae* (Duellman, 1963); Villa 1977, Villa and Townsend 1983, Hughey *et al.* 2011), or are only using the jelly matrix of the clutch for development.

A thick jelly layer in clutches laid out of water appears to be an adaptation that reduces mortality of embryos by desiccation and predation in glass frogs (Delia *et al.* 2020), and possibly in other anuran lineages (Duellman and Trueb 1994, Altig and McDiarmid 2007, Wells 2007). However, it is possibly that in some species, for example *N. grandisonae,* such jelly matrix makes egg clutches especially susceptible to be infested by fly larvae. This hypothesis need to be tested, but some suggestive examples supporting it involve clutches infested by dipterans in anurans whose clutches have a thick jelly layer: *Espadarana andina* (Rivero, 1968) (Cabanzo-Olarte *et al.* 2013), *Agalychnis annae* (Villa and Townsend 1983), *A. callidryas* (Hughey *et al.* 2011), *Hyalinobatrachium fleischmanni* (Villa 1977, Hayes 1991), *H. valerioi* (Vockenhuber *et al.* 2008), *Hyperolius lateralis* Laurent, 1940 (Vonesh 2000), and *Centrolene peristicta* (Lynch and Duellman, 1973) (Salgado and Guayasamín 2018). In addition, two species of glass frogs in the study area (*Centrolene savagei, "Centrolene" quindianum* Ruiz-Carranza and Lynch, 1995) have clutches without or with only a thin jelly layer, respectively, but during our sampling and previous studies, we did not record fly larvae in their clutches (Rios-Soto *et al.* 2017, Vargas-Salinas *et al.* 2017, Ospina-L *et al.* 2017, 2020). In spite of said potential cost associated with infestation by fly larvae, comparative analyses and experimental evidence demonstrate for glass frogs that an egg clutch with a thick jelly layer and reduced parental care by females (as in *N. grandisonae*) exhibit a similar overall survivorship to egg clutches with a reduced jelly layer and extended parental care by males (Delia *et al.* 2020).

Our results show that the glass frog *N. grandisonae* is a prolonged breeder with higher reproductive activity in months with high rainfall. With respect to the causes of mortality in egg clutches, infestation by fly larvae is the factor that reduces the survival of embryos most strongly. More studies are necessary to establish the specificity of the interaction between the fly larvae and the embryos of *N. grandisonae,* and hence, how it would respond to changes in environmental conditions (Marcogliese 2001). Aspects of natural history and the causes of mortality such as those recorded in this study offer baseline data that will provide a better understanding of the ecology, behavior and evolutionary biology of glass frogs, and will aid in the design of conservation and management plans (Clemmons and Buchholz 1997, Paton and Crouch III 2002).

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The relationship between body and substrate color for *Ambystoma altamirani* (Caudata: Ambystomatidae) from the Arroyo los Axolotes, Mexico

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Abstract

The relationship between body and substrate color for *Ambystoma altamirani* (Caudata: Ambystomatidae) from the Arroyo los Axolotes, Mexico. To determine whether the coloration of *Ambystoma altamirani* varies and whether the color is related to the color of the salamander's substrate, *A. altamirani* from the Arroyo los Axolotes, state of México, Mexico, was examined. The study was conducted from February 2018 to January 2019, and the colors classified by comparison with a standard color wheel. The most common base color was olive-green (64%) followed by black (21%). The most common color combinations were olive-green with black markings (44%), solid black (14%), and olive-green with black and yellow markings (11%). Olive-green salamanders were present in every month of the study, whereas the other base colors were found in fewer months, with the greatest diversity of base colors being observed from April to July. Olive-green, black, and "light" *A. altamirani* were found on dark substrates more frequently than expected based on availability compared to intermediate or light substrates. Because there is no evidence for substrate color matching, substrate selection likely is based on other environmental features.

Keywords: adult, color, salamander, stream.

Resumen

Relación entre el color del cuerpo y del sustrato de *Ambystoma altamirani* (Caudata: Ambystomatidae) en el Arroyo los Axolotes, México. Examinamos si la coloración de *Ambystoma altamirani* varía y si ésta está relacionada con el color del sustrato usado por esta salamandra, en la población de Arroyo los Axolotes, Estado de México, México. Estudiamos esta

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población desde febrero 2018 hasta enero 2019, y clasificamos los colores comparando a estos con una rueda de color estándar. El color de fondo de cuerpo más común fue el verde olivo (64%) seguido por el negro (21%). Las combinaciones de colores de cuerpo más comunes fueron el verde olivo con marcas negras (44%), negro sólido (14%), y verde olivo con negro y marcas amarillas (11%). Salamandras verde olivo estuvieron presentes en todos los meses de estudio, mientras que los otros colores base se registraron en menos meses, con la mayor diversidad de colores base siendo observada desde abril hasta julio. Individuos de *A. altamirani* de colores verde olivo, negro y pálido, todos utilizaron sustratos oscuros más frecuentemente que lo esperado con base a la disponibilidad comparado a los sustratos intermedios o pálidos. Debido a que no hubo evidencia de coincidencia con el color del sustrato, esto sugiere que la selección del sustrato se base en otras características ambientales.

Palabras Clave: adulto, arroyo, color, salamandra.

Resumo

Relação entre a cor do corpo e do substrato de *Ambystoma altamirani* (Caudata: Ambystomatidae) em Arroyo los Axolotes, México. Examinamos se a coloração de *Ambystoma altamirani* varia e se está relacionada com a cor do substrato usado por essa salamandra na população de Arroyo los Axolotes, Estado do México, México. Estudamos essa população de fevereiro de 2018 a janeiro de 2019 e classificamos as cores comparando-as com uma roda de cores padrão. A cor de fundo do corpo mais comum foi o verde-oliva (64%) seguido pelo preto (21%). As combinações de cores corporais mais comuns foram verde oliva com manchas pretas (44%), preto sólido (14%) e verde oliva com manchas pretas e amarelas (11%). As salamandras verde-oliva estiveram presentes em todos os meses de estudo, enquanto as restantes cores de base foram registadas em menos meses, sendo a maior diversidade de cores de base observada de abril a julho. Indivíduos de *A. altamirani* verde oliva, pretos e de cores claras usaram substratos escuros com mais frequência do que o esperado com base na disponibilidade em comparação com substratos intermediários ou claros. A falta de evidência de correspondência com a cor do substrato sugere que a seleção do substrato é baseada em outras características ambientais.

Palavras-chave: adulto, coloração, riacho, salamandra.

Introduction

Previous studies have shown that the coloration of adult and larval *Ambystoma* can vary with environmental conditions. In some cases, coloration varies with abiotic conditions, such as darkening at colder temperatures in larval *A. barbouri* Kraus and Petranka, 1989 and *A. texanum* (Matthes, 1855) (Garcia *et al.* 2003) or darkening with exposure to UV radiation in larval *A. gracile* (Baird, 1859) and *A. macrodactylum* (Belden and Blaustein 2002) and larval *A. barbouri* and *A. texanum* (Garcia *et al.* 2004). There also is evidence that *Ambystoma* can change color, or at least their darkness or lightness, to match their substrate or environment (Fernandez and Collins 1988, Belden and Blaustein 2002, Garcia and Sih 2003). Such changes seem to be particularly evident in the presence of predators, when salamanders tend to match their substrate or background, thereby increasing their survivorship (Storfer *et al.* 1999, Garcia *et al.* 2009). In addition to changing color, there is also evidence that *Ambystoma* can select substrates that better match their coloration or choose their habitat based on its color. For example, larval *A. texanum* select the substrate that is most similar to their body color (Garcia and Sih 2003). Substrate color is a significant factor in determining the presence or absence of

A. leorae (Taylor, 1943) in sites along the Río Tonatzin in the state of México (Lemos-Espinal *et al.* 2017), and a previous study found that *Ambystoma altamirani* Dugès, 1895 in the Arroyo los Axolotes used black substrates more than expected and white-yellow sites less than expected (Lemos-Espinal *et al.* 2016).

In this study, we examined the base color and color pattern of *A. altamirani* from the Arroyo los Axolotes, state of México, Mexico (Figure 1). Specifically, we quantified the frequency of different base colors by month, and determined whether base color was associated with substrate color. We expected there to be degree of substrate matching by *A. altamirani,* with lighter salamanders using lighter substrates and darker salamanders using darker substrates.

Figure 1. A subadult *Ambystoma altamirani* from Sierra del Ajusco, México City, Mexico, illustrating the species color pattern. Photo by Eric Centenero-Alcalá.

Materials and Methods

Our study took place in the Llano las Navajas in Arroyo los Axolotes, Mpio. Isidro Fabela, Sierra de las Cruces, state of México (19°32'12.2" N, 99°29'52.7" W; 3479 m a.s.l.). Llano las Navajas is an extensive grassland of approximately 100 ha surrounded by a forest of *Pinus hartwegii* Lindl. The Arroyo los Axolotes runs along the southern edge of this grassland. The Arroyo los Axolotes is a permanent stream; however, between December and April it is limited to a main channel that includes several shallow pools ($\leq$ 3.5 m diameter, $\leq$ 1 m depth) connected by branches of the main channel. In contrast, between May and November, surface water flows in two main channels, which are deeper and wider.

We established 25 permanent sites, each 5 m long and 40 m apart, along the Arroyo los Axolotes using a mobile GPS unit. We visited the Arroyo los Axolotes multiple times per month (range: 1–10) from February 2018 to January 2019. Visits took place between 11:00 and 15:00 h. when we visually searched the permanent sites along the stream for *A. altamirani;* we spent about 10 min per site, and used a herpetological hook along the bottom and cavities at the sides of the stream to induce the salamanders to move so that we could see them. In addition, we searched under rocks and other objects in the stream at each site. We captured salamanders with a net. Adult males were identified by the presence of a bulge on each side of the tail near the cloaca and adult females by the absence of such a bulge (Brandon and Altig 1973). We categorized individuals as larvae if they possessed gills and as adults if they lacked gills. The base color (i.e., the color covering the majority of its body) and the colors of any additional markings or patterns were recorded for each salamander captured, with the colors being identified by comparison with the Comex Color Pantone guide (http://tienda.comex.com.mx/familias-de-color). Colors were assessed in natural light in the field. The color of the substrate where the salamanders were originally observed was recorded in the same way as it was for the salamanders. All salamanders were released at the point of capture.

Chi-square tests were used to determine whether there was a correlation between the most common base colors and substrate colors between males and females, and between larvae and adults. To determine the availability of substrate colors, we visited each site monthly

independent of salamander sampling and classified the available substrate colors irrespective of the presence or absence of a salamander. Each substrate color was identified by comparison with a Comex Color Pantone guide. These values were used to calculate average percent availability of each substrate color during the study, and chi-square tests were used to compare the observed frequency of different base colors on different substrate colors. To facilitate this analysis and avoid small sample sizes for some base and substrate colors, we pooled substrate colors into Dark (black, dark brown, brown; 57.2%), Intermediate (light brown, gray; 28.2%), and Light (yellow and white; 14.5%) and limited the analyses to the two most common base colors (olive-green, black) and pooled the "light" base-colored individuals (gray, pale green, translucid, white, yellow).

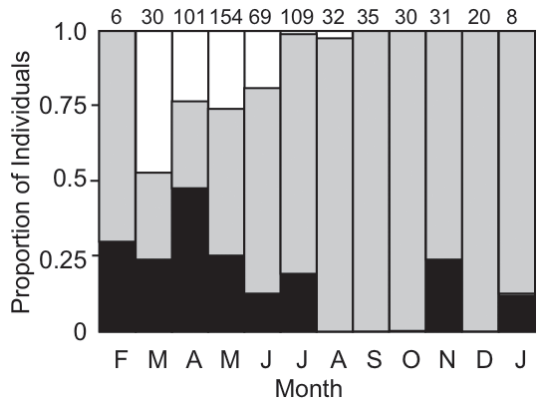

Figure 2. The relative frequency of *Ambystoma altamirani* of the two most common base colors, Black (black portion of bars) and olivegreen (gray portion of bars), from February 2018 to January 2019 in the Arroyo los Axolotes, State of México, Mexico (white portion of bars represents the relative frequency of all other colors combined). Number at top of bar indicates total number of individuals for that month.

Results

The most common base color is olive-green (64%; 397 of 624 observations) followed by black (21%; 132 of 624 observations), with no other base color accounting for > 5% of the observations (Appendix I). The most common color combination is olive-green with black markings (44%; 273 of 624 observations) followed by solid black (14%; 86 of 624 observations) and olive-green with black and yellow markings (11%; 68 of 624 observations) (Appendix II). No other combination accounts for > 5% of the observations (Appendix II).

The abundance of base colors observed in this population of *Ambystoma altamirani* varies during the study period. The most common base color, olive-green, was found throughout the study (Figure 2). The second most common base color, black, was most frequent in April and May, with a few individuals in other months, but mainly from March to July (Figure 2). The other base colors were less common, with the greatest diversity of base colors being observed from April–July (Appendix I).

Males and females do not differ in the frequency of the two most common base colors (black and olive-green; Table 1; $\chi^2_1 = 0.137$, $p = 0.71$). They also do not seem to differ in the less-common base colors, but the sample sizes are too small to analyze the frequencies statistically (Table 1). Males and females do not differ in their use of the most commonly used substrate colors (dark brown, brown, light brown, gray; Table 1; $\chi^2_3 = 0.19$, $p = 0.98$). These two results clearly demonstrate that there is no sexual dimorphism in base color or use of substrate colors in adult *A. altamirani*.

Larval *Ambystoma altamirani* have a higher frequency of black base color and lower frequency of olive-green base color than do adults (Table 1; $\chi^2_1 = 9.32$, $p = 0.0023$). There also are larvae that have some base colors that are rare or not found in adults (e.g., dark gray, green, green yellow, and yellow; Table 1).

Larval and adult *A. altamirani* differ in the frequency with which they use the most common

substrate colors (dark brown, brown, light brown, gray, and white) (Table 2; $\chi^2_4 = 155.9$, $p < 0.0001$). Adults use dark brown and brown substrates slightly more than do larvae. However, adults use white substrates substantially more than larvae do; only two observations of larvae were made on white substrates compared to 177 for adults (Table 2).

Olive-green individuals use dark substrates at a much higher frequency than expected based on availability (Table 3; $\chi^2_2 = 62.81$, $p < 0.0001$). Black individuals primarily are found on dark brown substrates as well, but proportionally fewer individuals are found on other substrate colors and none was found on white substrates. Black individuals used dark substrates at a higher than expected rate compared to the other colored substrates (Table 3; $\chi^2_2 = 39.6$, $p < 0.0001$). Other base colors of salamanders are also found primarily on dark brown—even white and yellow salamanders that were never found on yellow or white substrates. "Light" salamanders used dark substrates at a higher frequency than expected compared to other substrates (Table 3; $\chi^2_2 = 9.57$, $p = 0.0083$).

Discussion

Based on our observations, it is clear that within a single population of *Ambystoma altamirani,* color varies substantially (Appendices I, II); previous studies have documented that color variation in other populations of this species, as well. The base color of larval *A. altamirani* has been described as "yellowish," with "dark dorsal bands" (Brandon and Altig 1973); however, Campbell and Simmons (1962) described a black base color with yellow markings. These authors also described the base color of adult *A. altamirani* as gray with black spots or markings.

Our results suggest that the color of individuals or populations of *A. altamirani* may change during a year. There may also be differences in detectability of different colors at different times of the year. We lack the data necessary to determine which, if either, of these alternatives occurs in the Arroyo los Axolotes. However, these observations do suggest that the *A. altamirani* in the Arroyo los Axolotes may provide a good model system in which to explore the factors driving coloration in populations of

Table 1. The frequency of different base colors of male and female *Ambystoma altamirani* and larval and adult *A. altamirani* in the Arroyo los Axolotes, state of México, Mexico. The identification in parentheses is the color's key number from Comex Color Pantone guide (http://tienda.comex.com/familias-de-color).

Base color	Males	Females	Larvae	Adult
Black (Neg-01)	48	54	68	55
Dark brown (Casob-01)	0	0	1	0
Dark gray (316-07)	3	4	21	1
Dark green (216-07)	1	2	3	0
Gray (316-05)	7	7	2	8
Gray green (316-06)	8	2	3	7
Green (215-06)	2	4	6	0
Green yellow (217-03)	0	0	8	0
Olivegreen (224-07)	138	169	150	229
Pale green (217-01)	0	3	2	0
White (Blco-01)	0	0	1	0
Yellow (AmAle-01)	3	1	19	0

Table 2. The use of different colored substrates by adult male and female *Ambystoma altamirani* and larval and adult *A. altamirani* in the Arroyo los Axolotes, state of México, Mexico. The identification in parentheses is the color's key number from Comex Color Pantone guide (http://tienda.comex.com/familias-de-color). * indicates those colors compared by chi-square tests.

	Black (Neg-01)	Dark brown (Casob-01)	Brown (Chocolate-0013)	Light brown (Pino-0021)	Gray (Cocoa-010	Yellow (Marfil-01)	White (Blco-01)
Males	2	134*	30*	29*	8*	2	5
Females	2	157*	32*	36*	9*	4	3
Larvae	3	184*	30*	58*	21*	4	2*
Adults	0	200*	47*	28*	14*	2	177*

Table 3. Observed and expected frequency of *Ambystoma altamirani* of different base colors using different substrate color categories.

Base color	Frequency	Substrate color category		
		Dark	Intermediate	Light
Black	Observed	110	18	3
	Expected	75	37	19
Olivegreen	Observed	302	73	19
	Expected	227	112	56
"Light"	Observed	34	10	0
	Expected	25	12	6

Ambystoma, especially by gathering data on color in marked individuals over their lifetime.

The differences between larval and adult *A. altamirani* in the frequency and diversity of base colors suggests that there is either selection against certain colors resulting in these colors being less common in adults, or there is an ontogenetic change in coloration in *A. altamirani.* Adult *A. altamirani* seem to be found in or near streams throughout their life (J. A. Lemos-Espinal, pers. observ.), indicating that neither differential migration nor selection in the terrestrial environment is likely in this species. Larvae of both *A. barbouri* and *A. texanum* become lighter as they mature (Garcia *et al.* 2003), suggesting there can be ontogenetic changes in the coloration. A study that follows marked individuals and that tracks their coloration will be required to address these possibilities.

Adults use white substrates more frequently than do larvae and used dark brown and brown substrates slightly more than larvae, thereby suggesting that there is an ontogenetic shift in the color of the substrate used by *A. altamirani.* We cannot determine whether this shift is caused by the color or the composition of the substrate because the color and nature of the substrate are not independent at our study site.

We have only equivocal evidence for substrate matching in *A. altamirani* because both darker and lighter salamanders can be found on darker substrates, but not light substrates.; these results are consistent with those of a more limited study on this population by Lemos-Espinal *et al.* (2016). Thus, it seems likely that *A. altamirani* selects

darker substrates because they provide resources for the salamanders or are correlated with other stream characteristics that are important for them, rather than choosing a substrate color for camouflage. For example, the darker substrates often include mud as the base material, and mud is an important substrate for *A. altamirani* (Lemos-Espinal *et al.* 2016, Villarreal Hernández *et al.* 2020) and other Mexican *Ambystoma*—e.g., *A. leorae* (Lemos-Espinal *et al.* 2017) and *A. rivulare* (Taylor, 1940) (Bille 2009). *Ambystoma altamirani* also seems to avoid gravel or bedrock substrates (Lemos-Espinal *et al.* 2016, Villarreal Hernández *et al.* 2020) that tend to be lighter in color; this may explain the usual absence of salamanders on lighter substrates.

We do not preclude the possibility that base colors may be selected to match available substrate colors because the two most common base colors are black and olive-green and the two most common color combinations are olive-green with black markings and solid black; together, these account for 58% of all observations. The ability to camouflage increases survivorship in the presence of predators (Storfer *et al.* 1999). The color variation may exist, in part, because there are no predatory fish in the Arroyo los Axolotes to select against the more visible color combinations. Although there are predatory snakes, *Thamnophis scaliger* (Jan, 1863), found along the stream (Villareal Hernández *et al.* 2019), it is unclear how they might select prey relative to coloration. A caveat that must be associated with our study is that we only viewed color as perceived by humans, not as perceived by potential predators; see Hantak and Kuchta (2018) for an example involving a terrestrial salamander. Additional studies on color variation in this population are needed to examine how color might change in individuals and how color affects predation risk. It would also be interesting to conduct similar studies on color variation and substrate color use by *A. altamirani* in streams with predatory fish.

The results of this study document the apparent importance of substrate in determining the distribution of *Ambystoma altamirani* in streams, along with ontogenetic variation in substrates that are selected by the salamanders. Although we focused on substrate color, the evidence linking substrate color to the color of an individual salamander is mixed (Lemos-Espinal *et al.* 2016). Substrate selection by these salamanders doubtless is more complex than simple color choice because we know that substrate type (e.g., mud vs. bedrock) seems to be important in determining substrate use in multiple populations of *A. altamirani* (Lemos-Espinal *et al.* 2016, Villarreal Hernández *et al.* 2020). From a conservation standpoint, it seems that conserving darker, muddy substrates may be necessary to maintain populations of this salamander, especially in the larval stage.

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Appendix I. *The frequency of* Ambystoma altamirani *of different base colors in different months from February 2018 to January 2019 in the Arroyo los Axolotes, state of México, Mexico. The identification in parentheses is the color's key number from Comex Color Pantone guide (http://tienda.comex.com/familias-de-color).*

Base color	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Total
Black (Neg-01)	2	7	48	39	9	19	0	0	0	7	0	1	132
Dark brown (Casob-01)	0	0	1	0	0	0	0	0	0	0	0	0	1
Dark gray (316-07)	0	0	5	17	0	0	0	0	0	0	0	0	22
Dark green (216-07)	0	0	0	2	2	0	0	0	0	0	0	0	3
Gray (316-05)	0	1	0	8	5	0	0	0	0	0	0	0	14
Gray green (316-06)	0	0	0	6	4	0	1	0	0	0	0	0	11
Green (215-06)	0	0	4	2	0	0	0	0	0	0	0	0	6
Green yellow (217-03)	0	7	1	0	0	0	0	0	0	0	0	0	8
Olive green (224-07)	4	9	30	72	46	89	31	35	30	24	20	7	397
Pale green (217-01)	0	0	3	1	0	0	0	0	0	0	0	0	4
Translucid (000-27)	0	0	0	1	0	0	0	0	0	0	0	0	1
White (Blco-01)	0	0	0	0	2	1	0	0	0	0	0	0	3
Yellow (AmAle-01)	0	6	9	6	1	0	0	0	0	0	0	0	22

Appendix II. *Summary of the frequency of color combinations (i.e., base color and the color of additional patterns or markings) observed in* Ambystoma altamirani *for the Arroyo los Axolotes, state of México, Mexico. The identification in parentheses is the color's key number from Comex Color Pantone (http://tienda.comex.com/familias-de-color). Percent of observations is given in parentheses.*

Color combination	Number of individuals (%)
Black (Neg-01)	86 (13.8)
Black with green (Neg-01 & 215-06)	1 (0.16)
Black with olive-green (Neg-01 & 224-07)	6 (0.96)
Black with olive-green and yellow (Neg-01 & 224-07 & AmAle-01)	2 (0.32)
Black with transparent parts (Neg-01 & 000-27)	7 (1.1)
Black with transparent parts and olive-green (Neg-01 & 000-27 & 224-07)	1 (0.16)
Black with yellow (Neg-01 & AmAle-01)	28 (4.49)
Black with yellow and white (Neg-01 & AmAle-01 & Blco-01)	1 (0.16)
Dark brown with yellow (Casob-01 & AmAle-01)	1 (0.16)
Dark gray (316-07)	22 (3.53)
Gray (316-05)	1 (0.16)
Gray with black (316-05 & Neg-01)	9 (1.44)
Gray with black and yellow (316-05 & Neg-01 & AmAle-01)	1 (0.16)
Gray with dark green (316-05 & 216-07)	1 (0.16)
Gray with yellow (316-05 & AmAle-01)	1 (0.16)
Gray green with black (316-06 & Neg-01)	12 (1.92)
Dark green with black (216-07 & Neg-01)	3 (0.48)
Green with black (215-06 & Neg-01)	4 (0.64)
Green with yellow and black (215-06 & AmAle-01& Neg-01)	2 (0.32)
Green yellow with black (217-03 & Neg-01)	8 (1.28)
Olive-green (224-07)	20 (3.20)
Olive-green with black (224-07 & Neg-01)	273 (43.8)
Olive-green with black and gray (224-07 & Neg-01 & 316-05)	13 (2.08)
Olive-green with black and white (224-07 & Neg-01 & Blco-01)	6 (0.96)
Olive-green with black and yellow (224-07 & Neg-01 & Blco-01)	68 (10.90)
Olive-green with gray (224-07 & 316-05)	2 (0.32)
Olive-green with yellow (224-07 & AmAle-01)	12 (1.92)
Olive-green with yellow and transparent parts (224-07 & AmAle-01 & 000-27)	3 (0.48)
Pale green with black (217-01 & Neg-01)	4 (0.64)
Translucid (000-27)	1 (0.16)
White with black (Blco-01 & Neg-01)	3 (0.48)
Yellow with black (AmAle-01 & Neg-01)	22 (3.53)

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Short Communication

Notes on the reproduction and thermal biology of *Porthidium ophryomegas* (Serpentes: Viperidae)

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Palabras clave: Honduras, Sabanagrande, Tamagás negro, termorregulación, viviparidad.

Palavras-chave: Honduras, Sabanagrande, termorregulação, víbora-negra, viviparidade.

To maintain a preferred body temperature, ectothermic vertebrates have unique anatomical and physiological characteristics, in addition to environmental requirements (Raske *et al.* 2012). For example, in live-bearing reptiles, the impact of the thermal environment on embryos can be buffered by the thermoregulatory behavior of the gravid female; however, the optimal temperatures for various physiological functions can vary widely (Dawson 1975, Beuchat 1988, Garrick 2008). In addition, the body and environmental temperatures differ, and this difference may affect the behavior of the snakes, as well as their physiology and ecological functionality (Arnold and Bennett 1984, Huey and Kingsolver 1989, Schiereck 1989).

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The Slender Hognose Viper, *Porthidium ophryomegas* (Bocourt, 1868), occurs at low and moderate elevations on the Pacific slope of Central America, from southwestern Guatemala to west-central Costa Rica, and on the Atlantic slope in the interior valleys of Guatemala, as well as in several Honduran coastal localities. Throughout much of Honduras, this species occurs mostly in disjunct populations in open habitats (McCranie 2011). McCranie (2011) reported that *P. ophryomegas* occurs in Lowland Dry Forests, Lowland Arid Forests, Premontane Moist Forests, Premontane Dry Forest, and peripherally in Lowlands Moist Forests in Honduras.

Although sexual dichromatic variation has been reported for this species in Guatemala (Lawson 1997) and Costa Rica (Solórzano *et al.* 1988), based on examination of alcohol-preserved specimens, McCranie (2011) stated

that sexual dichromatic variation does not seem to occur in *Porthidium ophryomegas* of Gracias a Dios in northeastern Honduras. Little information is available on the ecology and behavior of this species in the wild or in captivity; for example, fluctuations in the body temperature of gravid females has not been studied. Here we describe body temperatures of a gravid female maintained in captivity, and provide some data on habitat, reproductive biology, and sizes of newborn *P. ophryomegas.*

A male and a female *Porthidium ophryomegas* were rescued from two private properties located in Sabanagrande, Departament of Francisco Morazán, central Honduras. The female was found on 08 April 2017 at Los Nanzales (13°42'12" N, 87°14'17" W, WGS84 datum; 425 m a.s.l.), and the male on 14 April 2017 at Los Portillos (13°43'49" N, 87°14'32" W, WGS84 datum; 404 m a.s.l.). The day that the snakes were captured, they were taken to the Colección Privada y Centro de Rescate de Fauna Silvestre El Ocotal. The center is authorized and registered by the wildlife department of the ICF (Instituto Nacional de Conservación y Desarrollo Forestal, Áreas Protegidas y Vida Silvestre) under resolution GG-MP-066-2001. We collected external morphometric data according to McCranie (2011). The male [snout–vent length (SVL) = 54.6 cm, tail length (TL) = 7.1 cm, total length (ToL) = 61.7 cm] was smaller than the female (SVL = 62.2 cm, LT = 7.6 cm; ToL = 69.8 cm), and when they arrived at El Ocotal, the female was not gravid. The snakes were encountered in a crop field of *Zea mays* (Poaceae) within a Tropical Dry Forest based on the life zones of Holdridge (1987) composed of pines (*Pinus oocarpa* and *P. maximinoi*) and oaks (*Quercus oleoides*). In Honduras, *P. ophryomegas* occurs at elevations between sea level and 1400 m a.s.l., and the distribution of the species includes the department of Francisco Morazán (McCranie 2011). The Sabanagrande localities are new for this species; however, it has been recorded from cornfields in the department of Santa Bárbara in western Honduras (Espinal *et al*. 2014).

The snakes were housed in a terrarium that was 51 cm high × 76 cm wide × 71 cm long, and contained sand, rocks, and small logs to simulate their natural habitat. The animals were maintained under a 12L/12D photoperiod (Lawson 1997). One 100W reflector bulb was placed 45 cm above the substrate to provide heat (Carretero 2012). The snakes were fed with white mice (*Mus musculus*) every seven days. Fresh water was provided every three days in a 500mL dish that was inserted in the sand. Approximately 1 yr later, we observed the male courting the female on three occasions. As described by Lawson (1997), the male pushed the posterior part of the female's head while attempting to hold her, particularly along her sides.

We adopted the methods of Hare *et al.* (2007) and Carretero (2012) to register body temperatures of the snakes with a thermometer laser (FLUKE 62 MAX with ±1.5°C of reading precision). We pointed the laser at the center of the midbody of the snake, in line with the body axis, at a distance of approximately 25 cm (based on the specifications of the thermometer, this distance measures an area of infrared to 1 cm^2). The temperature reading was stabilized at 10–15 s. We collected the body temperatures at 08:00 h and 17:00 h from May–July 2018 for a period of 35 days before and after parturition (excluding data from 25 June 2018 from statistical analysis). The terrarium temperature was recorded with a standard thermometer that was placed in the left corner inside of terrarium when the body temperatures of the snakes were taken.

Considering the normality of the measurements, we analyzed and compared the mean prepartum and postpartum temperatures of the female with a two-way analysis of variance (ANOVA). One-way ANOVAS were performed to calculate the mean external morphometric measurements of the neonates and were compared to those of Lawson (1997). We used the statistical software R 3.4.2 (R Core Team 2015) for all the analyses.

Our results are based on the analysis of 426 temperature measurements comprising female (71 prepartum temperatures and 71 postpartum temperatures) and male body temperatures, and the terrarium temperatures. The prepartum temperature (29.10–32.30°C) of the female was lower (ANOVA $t_{1,69}= -12.43$, $p < 0.05$) than the postpartum temperature (31.00–35.20°C) (Figure 1), which is consistent with the results of Sanders and Jacob (1981) and Gao *et al*. (2010), who reported that gravid viviparous snakes may have lower body temperatures than non-gravid females.

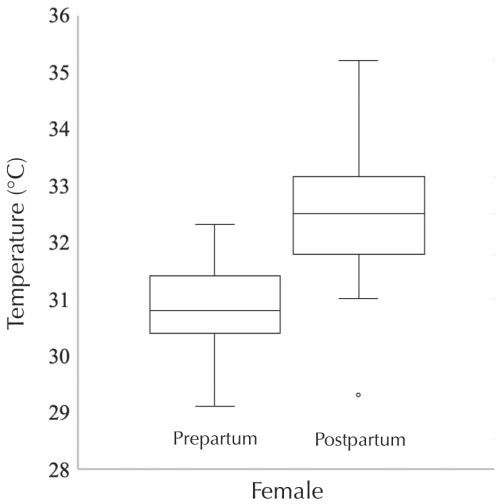

Figure 1. Box plots of the mean prepartum (30.80°C) and postpartum (32.50°C) temperatures of female *Porthidium ophryomegas.*

Birthing (see https://www.dropbox.com/s/7cm7ojuscvxkf9l/birthday.mov?dl=0) occurred on 25 June 2018 from 22:49 h (first newborn) to 23:49 h (last newborn), during which time the temperature of the female was 32.20–32.40ºC; the temperature of the male was 31.70–33.10ºC, and the temperature of the terrarium was 31.30–32.80°C. In total, 14 snakes were born, and at birth all had the same temperature as the female. See Table 1 for comparison of the measurements and body mass of the newborns.

The newborn *Porthidium ophryomegas* did not accept mice until the third week; we did not observe any cannibalism. We offered them Yellow-headed Geckos (*Gonatodes albogularis*), which were not accepted. Juvenile *Porthidium nasutum* have been reported to feed on earthworms, frogs, lizards of the genus *Anolis*, mice, as well as members of their own species (*Álvarez* del Toro 1973, Picado 1976, Porras *et al*. 1981).

Lawson (1997) reported that a captive female *Porthidium ophryomegas* from Guatemala gave birth to 19 live neonates in April. Solórzano (2004) indicated that female snakes in Costa Rica give birth to at least 15 newborn from May–August. McCranie (2011) collected three gravid females from northeastern Honduras in July, but herein we report for the first time the number of neonates and the month in which they were born (June), as well as the first documented records of body temperatures for *P. ophryomegas* during the reproductive season. The Honduran neonates are larger than those reported by Lawson (1997) from Guatemala. The link, if any, between variation in offspring size and offspring fitness in other viviparous snakes with regard to the thermoregulatory behavior of gravid females is unknown (Gao *et al*. 2010).

The body temperatures of gravid *Porthidium ophryomegas* are lower than postpartum temperatures. However, individual body temperatures of thermoregulating reptiles may vary between day and night and seasonally, as well as being affected by snake behavior (Webb and Shine 1998, Seebacher and Franklin 2005).

One reason that temperatures may differ in females is that the higher body temperature of gravid females allows the embryos to adapt more easily to the ambient external temperature. Gao *et al*. (2010) suggested that the thermoregulatory behavior of gravid snakes may be associated with the thermal optimality that they will provide to the embryos. For example, prolonged gestation periods at low body temperatures increases

Table 1. Snout–vent length (SVL), tail length (TL), total length (ToL), body mass and observations of the 14 neonates of *Porthidium ophryomegas* born on 25 June 2018 compared with the data of Lawson (1997). The measurements of this study were taken 2 days and Lawson's data were taken 1 day after birth.

Individuals	SVL (mm)		TL (mm)		ToL (mm)		Body mass (g)	
	Lawson (1997)	This study	Lawson (1997)	This study	Lawson (1997)	This study	Lawson (1997)	This study
1	15.0	23.2	2.4	4.1	17.4	27.3	3.15	5.0
2	15.7	21.5	2.4	3.0	18.1	24.5	4.14	5.0
3	16.8	22.6	2.2	3.2	19.0	25.8	4.31	5.5
4	16.0	22.4	2.1	3.5	18.1	25.9	3.33	5.5
5	16.7	20.5	2.2	2.6	18.9	23.1	3.89	4.5
6	15.3	21.8	2.3	2.6	17.6	24.4	3.4	5.0
7	16.4	21.4	2.0	2.8	18.4	24.2	4.22	5.0
8	16.5	21.9	2.2	2.8	18.7	24.7	4.01	6.0
9	17.1	22.4	2.2	3.2	19.3	25.6	4.43	5.5
10	16.5	23.9	2.5	3.8	19.0	27.7	3.94	5.0
11	15.5	22.6	2.2	3.0	17.7	25.6	3.34	5.5
12	17.4	21.6	2.3	2.9	19.7	24.5	4.45	5.0
13	16.0	21.6	2.3	3.1	18.3	24.7	3.79	5.5
14	16.4	22.0	2.2	2.9	18.6	24.9	3.85	5.0
15	17.2	-	2.4	-	19.6	-	4.36	-
Mean ± SD	**16.3 ± 0.71**	**22.1 ± 0.84**	**2.26 ± 0.13**	**3.11 ± 0.43**	**18.6 ± 0.70**	**25.21 ± 1.22**	**3.91 ± 0.43**	**5.21 ± 0.38**

reproductive costs. Furthermore, females of viviparous snakes may change their thermal preference when they are gravid (Li *et al*. 2009, Gao *et al*. 2010), and they may increase the thermoregulatory behavior for the efficiency of the embryogenesis to provide an optimum thermal environment to the embryos (Sanders and Jacob 1981, Brown and Weatherhead 2000). We recommend continued monitoring of the temperature of gravid female Slender Hognose Vipers during a longer time span and in different seasons, both in captivity and in nature, to confirm that nongravid females have lower temperatures.

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Short Communication

New record and updated distribution map of the rare *Amphisbaena spurrelli* (Amphisbaenia: Amphisbaenidae)

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Keywords: Colombia, geographic distribution, Panama, Wallacean shortfall, worm lizard.

Palavras-chave: anfisbena, Colômbia, distribuição geográfica, Panamá, déficit Wallaceano.

Amphisbaenia is a group of squamate reptiles, popularly known as worm lizards, adapted to a subterranean lifestyle. About 200 extant species are recognized, most from Latin America, especially Brazil (Costa and Garcia 2019, Uetz *et al.* 2019). In Central America, amphisbaenians are known only from Panama, where two taxa occur—viz., *Amphisbaena fuliginosa varia* Laurenti, 1768, also known from northern South America, and *A. spurrelli* Boulenger, 1915, also known from Colombia (Gans 1962a, Vanzolini 2002, Ray *et al.* 2015). The occurrence of a third species, *A. alba* Linnaeus, 1758 in Panama is questionable (Gans 1962b, Köhler 2008, Jaramillo *et al.* 2010).

The description of *Amphisbaena spurrelli* is based on two specimens from Andagoya, Colombia, collected by Herbert George Flaxman Spurrell (1877–1918), a British zoologist and physician (Boulenger 1915, Beolens *et al.* 2011). Subsequently, three additional individuals were collected, and the species was redescribed by Gans (1962a). The first was recorded from Boca de la Raspadura by Burt and Burt (1931). The second was found at the type locality, and the third was found in Panama (Tucuti Branch, Tuira River) (Gans 1962a). More recently, Ibáñez *et al.* (2017) reported the species in Chagres National Park, Panama, but provided no information on voucher specimens.

Amphisbaena spurrelli is distinguished from its congeners by a combination of morphological features, as follow: possession of 218–222 dorsal annuli; 18–20 caudal annuli with the tail tip covered by tuberculate segments; 16–18 dorsal and 16–19 ventral midbody segments; three supra- and three infralabials; one or two postgenial rows; and a postmalar row (Gans 1962a). Morphologically, the species resembles *A. rozei* Lancini, 1963 from Venezuela, which was placed in the synonomy of *A. spurrelli*

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(Donoso-Barros 1968, González-Sponga and Gans 1971). However, *A. spurrelli* and *A. rozei* differ slightly in the range of body annuli and ventral midbody segments, and in the shape of the second infralabial; in *A. spurrelli* the second infralabial is the largest, whereas in *A. rozei* the first infralabial is longer than the second in the mouth-line (Costa *et al.* 2018).

While visiting the reptile collection of the American Museum of Natural History (AMNH), I encountered a specimen of *A. spurrelli* from Panama that was not recorded in the literature. This specimen (AMNH 115649; Figure 1) was collected on 14 August 1976 by Pedro Galindo and Rodolfo Hinds in a flooded forest in the Majecito River of the Bayano River Drainage (ca. 9.123° N, 78.875° W; coordinates found with Google Earth Pro). At the time the worm lizard was collected, the Bayano River was doubtless affected by the filling of a large hydroelectric dam (Finley-Brook and Thomas 2010, AES Panamá 2017).

Unfortunately, the collection data do not specify whether the specimen was collected in a forest that naturally was seasonally flooded or flooded by the Bayano Dam. Despite their fossorial habits, amphisbaenians can swim (Maschio *et al.* 2009, van der Hoek 2018). A small Venezuelan species from the Orinoco Plains (*A. gracilis* Strauch, 1881) is somewhat unusual because it lives in wetlands covered by palms (*Mauritia flexuosa* L.f.), known as 'morichales'; the worm lizards launch themselves in the water to flee from predators (Señaris 2001). A Colombian species (*A. medemi* Gans and Mathers, 1977) inhabits seasonally flooded pastures and scrublands (Gans and Mathers 1977). Thus, the occurrence of *A. spurrelli* in a fooded forest may not be as unlikely as it might seem.

The identity of AMNH 115649 as *A. spurrelli* was confirmed by comparison with three other specimens of this taxon [AMNH 18261, FMNH 130988 (Field Museum), and MCZ 39784 (Museum of Comparative Zoology)], and with photographs of the lectotype [NHMUK (former BMNH) 1915.10.21.9 and the paralectotype (NHMUK 1915.10.21.8) housed at the Natural History Museum, UK, as well as with the data from the species redescription (Gans 1962a). As shown in Table 1, AMNH 115649 clearly is *A. spurrelli,* and the inclusion of this specimen increases the upper range of dorsal body annuli known for this species to 223. The color pattern of AMNH 115649 fits the description by Gans (1962a). Dorsal segments are dark brown with cream margins, giving a reticulate appearance. This pattern is observed in the third to fifth segment below the lateral sulcus; ventrad from this, all segments are cream colored.

Figure 1. *Amphisbaena spurrelli* (AMNH 115649) from the Majecito River (9.123° N, 78.875° W), Panama.

The Majecito River site is located between the existing Panamanian records for *A. spurrelli.* The Majecito site is 55 km southeast of Chagres National Park and 160 km northwest of the Tucuti Branch of the Tuira River and lies in a straight line between these localities that are separated by 210 km (Figure 2, Table 2). The species seems to be a forest dweller, occurring in the moist forests of the Chocó-Darién Ecoregion and the Isthmian Atlantic Ecoregion in the Tropical & Subtropical Moist Broadleaf Forest Biome (Dinerstein *et al.* 2017). The localities where *A. spurrelli* occurs have three different

Table 1. Morphological data for *Amphisbaena spurrelli*. Data from the lectotype (NHMUK 1915.10.21.9) and the paralectotype (NHMUK 1915.10.21.8) are based on photographs and the species redescription (Gans 1962a). All specimens have a tail tip slightly laterally compressed and covered by tuberculate segments. A solidus (/) separates left/right sides. * I consider the scale posterior to the third supralabial (and posterior to the mouth commissure) to be a postsupralabial (Gans 1963a, b), which some authors (Pinna *et al.* 2010) would consider a fourth supralabial. **One could argue that MCZ 39784 does not have a post-malar row, because it is behind the mouth commissure (Gans 1962c, 1964). Discussions regarding the problem of the definition of 'post-malar row' are outside the scope of this work, but the specimen has a row of seven scales posterior to the second row of genials.

Character	**AMNH 115649**	**AMNH 18261**	**FMNH 130988**	**MCZ 39784**	**NHMUK 1915.10.21.8**	**NHMUK 1915.10.21.9**
Body annuli	223	221	222	221	219	218
Lateral annuli	4	4	4	4	4	5
Caudal annuli	19	19	19	20	19	18
Autotomic annulus	7	7	6	Not visible	7?	6?
Lateral sulcus	Yes	Yes	Yes	Yes	Yes	Yes
Dorsal sulcus	No	No	No	No	No	No
Midbody dorsal segments (MDS)	16	16	16	16	18	16–18
Midbody ventral segments (MVS)	18	16–18	16	18	19	18
MDS+MVS	34	32–34	32	34	37	34–36
Precloacal pores	4	4	4	4	4	4
Precloacal segments	6	6	6	6	6	6
Post-cloacal segments	12	12	12	12	12	12
Supralabials	3	3	3	3	3	3
Infralabials	3	3	3	3	3	3
Temporals	1	1	1	1/2	1	1
Post-ocular	1	1	1	1	1	1
Post-supralabial*	1/0	1	1	1	1	0/1
Genials on 1st row	3	2	1	2	3	2
Genials on 2nd row	0	0	4	3	3	0
Post-malars	7	7	9	7**	7	7
Snout–vent length (mm)	260	225	230	207	297	228
Tail length (mm)	21.9	21.0	22.0	19.0	27	22
Head width (mm)	7.0	5.8	6.7	5.2	?	?
SVL/HW	36.9	38.9	34.3	40.1	?	?

types of dominant soil (Figure 2, Table 2)—cambisol ("brown forest soil" formed by the weathering of adjacent mountains), gleysol (typical of some seasonally flooded lowlands), and nitisol (deep well-drained soils in some tropical rainforests) (FAO *et al.* 2012, IUSS Working Group WRB 2015). These soils present medium to fine textures, varying from loam to clay loam and light clay (FAO *et al.* 2012). *Amphisbaena spurrelli* would be expected to occur in medium-textured soils because the species has a small, round head that is not specialized to dig in finer, textured clayic soils (Gans 1968, Navas *et al.* 2004). However, Kazi and Hipsley (2018) found no relationship between soil texture and cranial shape among Caribbean worm lizards, and the occurrence of *A. spurrelli* in clayic soils in Chagres National Park is consistent with this observation. Individuals of this species probably excavate only at shallow depths, as its countershaded color pattern suggests (Gans 1968); this may explain its presence in fine-textured soils. In this type of habitat, the worm lizards may prefer to inhabit rotten logs, as observed for *A. bakeri* Stejneger, 1904 in Puerto Rico (Schwartz and Henderson 1991). More refined studies investigating the relationships between cranial shape and soil type, and between color pattern and the microhabitat occupied are needed to test this hypothesis.

Available evidence indicates that *A. spurrelli* is a lowland species (Table 2), probably adapted to seasonally flooded forests or at least associated with riparian forests. The widely separated locality records probably reflect inadequate sampling owing to the difficulty of finding these fossorial lizards (Pramuk and Alamillo 2003), because the vegetation and soil types are similar between the northernmost and the southernmost records (Figure 2). Thus, the range of *A. spurrelli* could be much greater than currently suggested (Ibáñez *et al.* 2017).

Amphisbaena spurrelli is a species of medium vulnerability in Panama (Jaramillo *et al.* 2010) and globally categorized as Data Deficient (DD) by the IUCN "because the distribution is relatively small but not well-known, and nothing is known of its exposure or sensitivity to any threats or its population trend" (Ibáñez *et al.* 2017). Additionally, Ibáñez *et al.* (2017) stated that the distribution of *A. spurrelli* in Panama "is in need of clarification". The meager knowledge of the distributions of species is one of the main

Table 2. Summary of the locality records for *Amphisbaena spurrelli*. All coordinates and elevations are approximate.

Voucher	Country	Department / Province	Locality	Latitude N (°)	Longitude W (°)	Elevation (m a.s.l.)	Soil type (FAO *et al.* 2012)
NHMUK 1915.10.21.8, 1915.10.21.9; FMNH 130988	Colombia	Chocó	Andagoya, at the junction of the Condoto and San Juan rivers	5.090	76.695	55	Cambisol
AMNH 18261	Colombia	Chocó	Boca de la Raspadura	5.266	76.700	90	Gleysol
MCZ 39784	Panama	Darién	Tucuti branch (= Río Balsas), Tuira River	7.983	77.925	15	Cambisol
AMNH 115649	Panama	Panama	Majecito River, Bayano Drainage	9.123	78.875	70	Nitisol
Unknown	Panama	Panama	Chagres National Park	9.300	79.400	~ 200	Cambisol

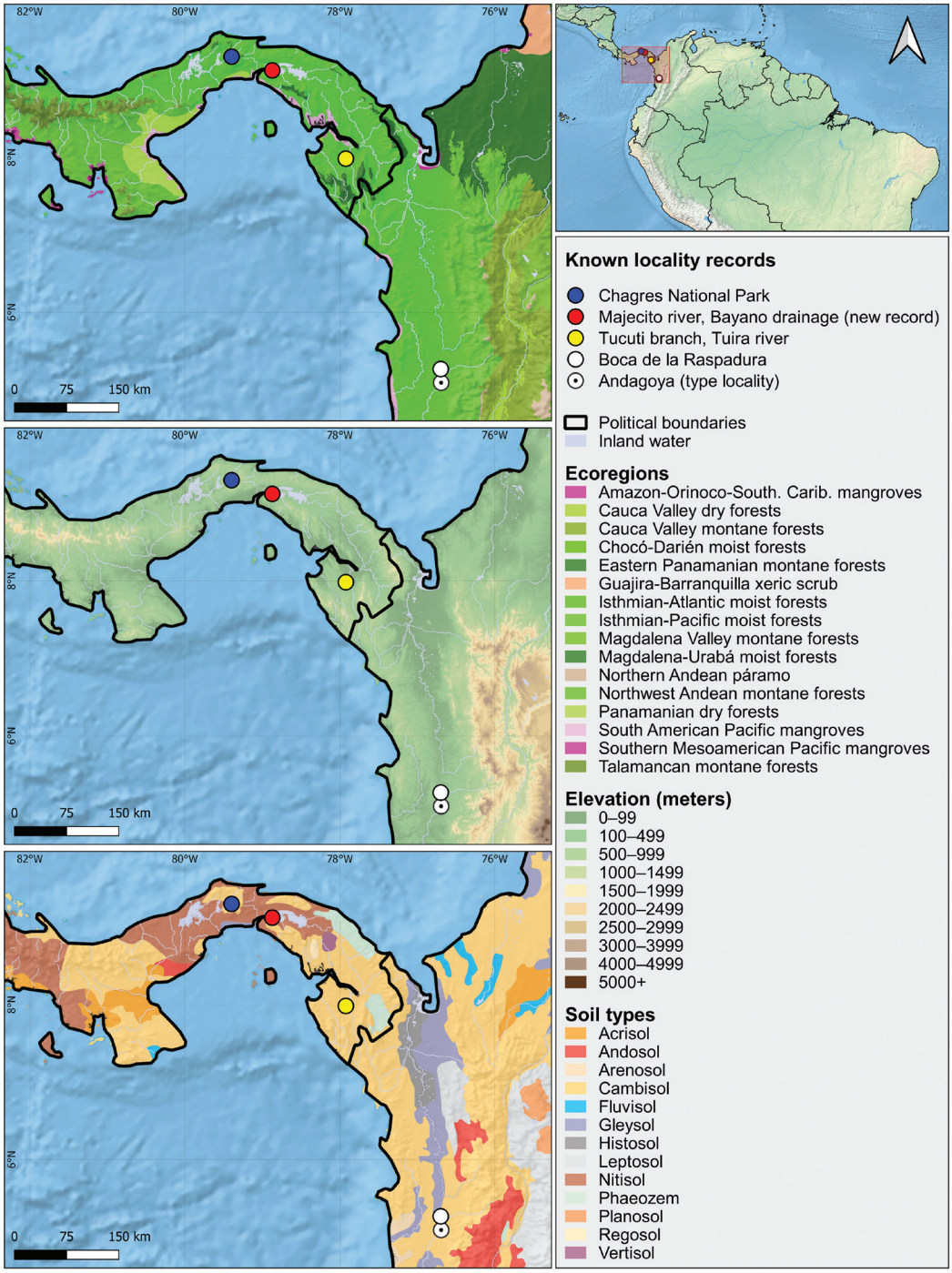

Figure 2. Distribution records of *Amphisbaena spurrelli,* over ecoregions (Dinerstein *et al.* 2017) (top map), elevation (middle map) and soil types (FAO *et al.* 2012) (bottom map).

biodiversity data shortfalls of the 21st century (Hortal *et al.* 2015) and has been responsible for the neglect of reptiles in initiatives of global conservation priorities (Tingley *et al.* 2016). Today, about 21% of reptile species worldwide are considered DD by the IUCN criteria; most of these taxa are fossorial and semi-fossorial groups such as Amphisbaenia that require the attention of researchers (Böhm *et al.* 2013). Because *A. spurrelli* is one such DD species, any new locality record merits publication to ensure better future assessments of its conservation status.

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Short Communication

New records of avian and interspecific predation in lizards of the genus *Liolaemus* (Squamata: Liolaemidae)

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Palavras-chave: *Agriornis montanus,* Chile, saurofagia, *Turdus falcklandii.*

Liolaemus is a diverse lizard genus distributed in southern South America, mainly in Argentina and Chile, currently containing 257 species and two subgenera—*Liolaemus* (sensu stricto) and *Eulaemus* (Abdala and Quinteros 2014). Few records of interspecific saurophagy have been reported. Avila and Belver (2000) noted the predation by an adult male *L. koslowsky* Etheridge, 1993 on a hatchling *L. pseudoanomalus* (Cei, 1981). Avila and Morando (2002) documented an adult male *L. petrophilus* Donoso-Barros and Cei, 1971 preying on a hatchling *L. bibronii* (Bell, 1843). Pérez *et al.* (2009) reported the predation by an adult (sex not mentioned) *L. austromendocinus* Cei, 1974 on an adult female *L. bibronii.* Torres-Mura (2013) described the predation by an adult (sex not mentioned) *L. monticola* Müller and Hellmich, 1932 on a juvenile *L. lemniscatus* Gravenhorst, 1838 (incorrectly stated to be the first record of saurophagy on *Liolaemus*).

Likewise, few records of predation by passerine birds on species of *Liolaemus* have been reported. Pérez and Avila (2005) reported an adult Black-bellied Shrike-tyrant [*Agriornis montanus* (D'Orbigny and Lafresnaye, 1837)] capturing and killing a subadult *L. petrophilus.* Santoyo-Brito *et al.* (2014) stated that they "confirmed in the field" that *A. montanus* also preys on neonate *L. leopardinus* Müller and Hellmich, 1932, without more details. Santoyo-Brito *et al.* (2014) reported an adult Rufous-banded Miner [*Geositta rufipennis* (Burmeister, 1860)] capturing and killing a neonate *L. leopardinus.* Bianchini (2014) reported a predation event from a photograph of a Long-tailed Meadowlark [*Leistes loyca* (Molina, 1782)] holding a *L. multimaculatus* (Duméril

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and Bibron, 1837) in its beak. Until these records, *A. montanus* was reported to prey on several small vertebrates (Salvador and Bodrati 2013), and both *G. rufipennis* and *L. loyca* were thought to feed solely on arthropods and plants (Del Hoyo *et al.* 2003).

Herein, we report a new instance of interspecific saurophagy in *Liolaemus* and two new cases of saurophagy by passerine birds, the identities of which were confirmed with Couve *et al.* (2016). The reports are based mainly on anecdotal field observations, which were photographed. We only examined one dead *Liolaemus;* the sexes of the other individuals were determined based on the dorsal color pattern.

On 07 December 2015, we photographed an adult Austral Thrush (*Turdus falcklandii* Quoy and Gaimard, 1824) holding a dead adult female *Liolaemus tenuis* (Duméril and Bibron, 1837) in its beak and beating it several times against the ground (Figure 1A). The observation took place at 16:15 hr near the house of one of the authors (JTP) in Santiago City (33°31' S, 70°45' W, 480 m a.s.l.) in the Metropolitan Region of Chile. The observer (JTP), who was walking down the street, saw the bird with the lizard in its beak; he returned to his home to get a camera to photograph the bird's behavior and returned within 1 min. During the second observation, JTP was located about 5 m away from the bird for about 2 min, when the bird noticed his presence and escaped with its prey. *Turdus falcklandii* occurs in Chile and Argentina, where the birds are found as solitary or in pairs in both in urban and rural areas (Couve *et al.* 2016). The species feeds on annelids and fruits in some seasons (Orellana *et al.* 2014); this is the first record of its predation on a vertebrate. *Liolaemus tenuis* ranges across the Coquimbo and Los Ríos regions (Núñez and Gálvez 2015, Troncoso-Palacios 2019). The lizard was identified as a female based on its gray dorsum bearing short, transverse black stripes and the light green snout that is diagnostic of this species; also, it is the most common species of lizard found in central Chilean cities and has a maximum snout–vent length of 59.0 mm (Pincheira-Donoso and Núñez 2005).

On 30 January 2019, we photographed an adult male *Liolaemus tenuis* preying on a juvenile (unknown sex) *L. lemniscatus* (Figure 1B). The observation took place at 13:12 hr, in the Reserva Nacional Río Clarillo (33°43' S, 70°23' W, 1100 m a.s.l.) in the Metropolitan Region of Chile. The observer (IV) was 4 m away from the lizards for a period of 3 min. Although the predator was kept in sight until half of the prey was swallowed, we neither observed the capture nor the entire consumption of the prey. This is the first known report of saurophagy in *L. tenuis,* the species is thought to feed solely on arthropods (Donoso-Barros 1966, Pincheira-Donoso 2008). *Liolaemus lemniscatus* ranges across the Coquimbo and Los Ríos regions (Núñez and Gálvez 2015, Troncoso-Palacios 2019). Although the prey individual was examined at a distance, its identity was determined based on the absence of a vertebral line, and presence of a yellowish brown occipital band, dark-brown para-vertebral stripe and light yellow dorsolateral stripes (Pincheira-Donoso and Núñez 2005). The geographical ranges of both lizard species overlap; however, the taxa differ in microhabitat use. While *L. tenuis* is a saxicolous and tree-perching lizard, *L. lemniscatus* is a ground lizard (Troncoso-Palacios 2019).

On 02 December 2019, we photographed an adult Black-bellied Shrike-tyrant (*Agriornis montanus*) holding a dead adult male *Liolaemus atacamensis* Müller and Hellmich, 1933 in its beak and beating the lizard several times against the ground (Figure 1C, D). The observation took place at about 10:50–11:30 hr, while we carry out a field study for the mining project Delirio by Santiago Metals (26°45' S, 69°54' W, 1850 m a.s.l.), located 6 km NE of Inca de Oro, Atacama Region, Chile. The saurophagy event was observed (YMA and DH) for 10 min at a distance of 15 m. When the bird noticed our presence, it flew away abandoning its prey, thereby allowing

Figure 1. (**A**) An Austral Thrush, *Turdus falcklandii* preying on an adult female *Liolaemus tenuis*. (**B**) An adult male *Liolaemus tenuis* preying on a *Liolaemus lemniscatus*. (**C, D**) A Black-bellied Shrike-tyrant, *Agriornis montanus,* preying on an adult male *Liolaemus atacamensis*.

us to examine the prey; the lizard was a known, previously marked individual. *Agriornis montanus* is widely distributed in southern South America, and ranges from Colombia to Chile where it is found as solitary individuals or in pairs (Couve *et al.* 2016), and preys on vertebrates such as lizards, nestling birds, eggs, small rodents, and even fishes (Salvador and Bodrati 2013). *Liolaemus atacamensis* is endemic to Chile, inhabiting the regions of Coquimbo and Atacama (Troncoso-Palacios 2014). The maximum snout-vent length (SVL) of this lizard is 67.2 mm (Troncoso-Palacios and Garín 2013). The identity of the lizard described here is based on the presence of a rounded black spot on the shoulder, as well as the presence of a few light-blue dorsal scales (Troncoso-Palacios and Garín 2013).

Because we did not observe the birds capturing and killing the lizards, it is possible that the lizards were dead before the birds picked them up and consumed the dead bodies

(opportunistic feeding behavior). Nevertheless, we think that these observations are predation records for the following reasons. First, in both instances, the dead lizard had autotomized tail, which is a defense mechanism against capture (Jaksic and Fuentes 1980). Second, there is blood spot on the tail of the *Liolaemus atacamensis* and on the snout of the *L. tenuis,* which is indicative of a recent death by predation. Each record of saurophagy reported here, *Agriornis montanus* on *L. atacamensis, Turdus falcklandii* on *L. tenuis,* and *L. tenuis* on *L. lemniscatus,* occurred between species with widely overlapping distributional ranges in Chile; thus, saurophagy may not be uncommon. However, if this is true, it is remarkable that no previous records of saurophagy have been reported in the literature.

Cannibalism has been recorded frequently in *Liolaemus.* Two adult male *L. lutzae* Mertens, 1938 preyed on juveniles (Rocha 1992) and an adult male *L. darwini* (Bell, 1843) preyed on a hatchling (Ripoll and Acosta 2007), as did an adult male *L. rothi* Koslowsky, 1898 (Kozykariski *et al.* 2009) and an adult male *L. zapallarensis* Müller and Hellmich, 1933 (Pincheira-Donoso 2012). Villareal *et al.* (2012) recorded the only known record of cannibalism between captive individuals involving an adult male *L. forsteri* Laurent, 1982 preying on a neonate. Jiménez-Robles and De la Riva (2017) reported an adult female of *L. orientalis* Müller, 1924 preying on a juvenile. Congeneric predation has been hypothesized to explain patterns of special segregation in *L. huacahuasicus* Laurent, 1985 (Halloy and Halloy 1997). Additionally, Álvarez and Zelada (2014) reported an adult (sex unknown) of *L. pictus* (Duméril and Bibron, 1837) preying on a juvenile *Liolaemus* of undetermined species. Last, Pincheira-Donoso (2012) mentioned a case of cannibalism on *L. chiliensis* (Lesson, 1830) that was to be published in the first issue of the journal "Revista de Integración Biológica de Concepción"; however, we cannot confirm this record in publication.

Saurophagy is recorded in the subgenera of *Liolaemus* and occurs in diverse clades. In the *Liolaemus* (sensu stricto), saurophagy is known in (parenthetical subclades fide Morando *et al.* 2003, Troncoso-Palacios *et al.* 2015, Panzera *et al.* 2017) *L. austromendocinus, L. petrophilus* (*petrophilus* group), *L. monticola* (*monticola* group), *L. pictus* (*pictus* group), *L. zapallarensis* (*nigromaculatus* group), and *L. tenuis* (species without close relatives). In the subgenus *Eulaemus,* saurophagy is recorded in (parenthetical subclades fide Abdala and Quinteros 2014, Avila *et al.* 2013) *L. darwini, L. koslowsky* (*darwini* group), *L. fosteri, L. orientalis* (*montanus* group), *L. lutzae* (*wiegmannii* group), and *L. rothi* (*rothi* group). This strongly suggests that in *Liolaemus,* saurophagy may be ancestral behavioral feature.

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SHORT COMMUNICATION

Envenomation records of *Hemidactylus mabouia* (Squamata: Gekkonidae) by *Ctenus rectipes* (Araneae: Ctenidae) in an urban area of northeastern Brazil

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Palavras-chave: aranha, espécie exótica, lagarto, envenenamento, predação.

Hemidactylus mabouia (Moreau-De-Jonnès, 1818), popularly known as Tropical House Gecko, is a nocturnal lizard of African origin that is well adapted to human dwellings (Jesus *et al.* 2001, Short and Petren 2012). This species was introduced in several countries in the Americas a few hundred years ago, probably through slave ships coming from Africa during European colonization (Vanzolini 1968). In Brazil, it currently occurs in anthropogenic environments in all biomes (Amazon Rainforest, Cerrado, Caatinga, Atlantic Forest) and on some coastal islands (Rocha *et al.* 2011). *Hemidactylus mabouia* is a generalist and opportunist regarding its diet, and is known to feed on diverse groups of arthropods, including spiders (Zamprogno and Teixeira 1998, Bonfiglio *et al*. 2006, Rocha and Anjos 2007, Iturriaga and Marrero 2013).

Like the Tropical House Gecko, wandering spiders (Ctenidae) are also nocturnal (Keyserling 1877). Among the 48 genera of ctenids, the genus *Ctenus* Walkenaer, 1805 is the richest, with 212 species widely distributed on all continents except Antarctica (World Spider Catalog 2020). Wandering spiders can be very abundant and are also generalists, preying on diverse taxa of arthropods (Gasnier and Höfer 2001) and occasionally on small vertebrates such

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as amphibians and reptiles (Barbo *et al.* 2009, Oliveira *et al.* 2017, Nyffeler and Altig, 2020). Consequently, they frequently come into contact with this gecko.

Here, we report four cases of probable envenomation of *Hemidactylus mabouia* by *Ctenus rectipes* in an urban area in municipality of Maceió, state of Alagoas, northeastern Brazil.

Observations were made at a residence at Condomínio Chácaras da Lagoa (09°35'01.0" S, 35°47'12.0" W), in the municipality of Maceió, state of Alagoas, Brazil. We collected the geckos and spiders and took them to the Laboratório de Biologia Integrativa, Universidade Federal de Alagoas to be analyzed and photographed. The specimens were incorporated in the Coleção Aracnológica (MUFAL 605–607 - *Ctenus rectipes*) and in the Coleção Herpetológica (MUFAL 15013–15016 - *Hemidactylus mabouia*), both deposited in the Museu de História Natural, Universidade Federal de Alagoas.

We made four observations of probable envenomation of *Hemidactylus mabouia* by *Ctenus rectipes* within an eight-day period. In three encounters, both prey and predator were found dead. On these occasions, the spider was partially inside the gecko's mouth. Three of the four geckos, even dead, were kept above the floor level by adherence of the left hind limb to the substrate.

The first encounter took place on 17 June 2019 at 11:00h (Figure 1A). The *H. mabouia* male (MUFAL 15013, SVL 61.35 mm; Figure 1B) and an immature male *C. rectipes* (MUFAL 605; cephalothorax + abdomen 12.7 mm; Figure 1C) were about 50 cm above floor level. The gecko had its head turned down. The predation attempt and possible envenomation probably occurred the previous night, because both individuals were not in an advanced state of decomposition. The spider's chelicerae were found still inserted into the anterior region of the gecko's tongue, which had signs of necrosis (Figure 1D–E).

The second observation took place on 21 June 2019 at 15:00h. The *H. mabouia* female (MUFAL 15014, SVL 58.12 mm) had a male of *C. rectipes* (MUFAL 606) in her mouth, but both were already decomposing, and were found on the floor behind furniture during house cleaning (Figure 2A). The third record occurred on 24 June 2019. A female of *H. mabouia* (MUFAL 15015, SVL 53.52 mm) had a *C. rectipes* male (MUFAL 607) in its mouth. The animals were found at 18:30h two meters high in a window casing with their heads down in a similar situation as the previously observed animals (Figure 2B). The fourth dead gecko individual, a female (MUFAL 15016, SVL 56.06 mm), was found 1.80 m from the floor level and had no visible spider in its mouth (Figure 2C). However, the gecko had the left hind limb extended and appeared to have been paralyzed when it died.

The Brazilian native gecko *Coleodactylus meridionalis* (Boulenger, 1888) has been preyed on by *Ctenus rectipes* (Oliveira *et al.* 2017) and by *Parabatinga brevipes* (Keyserling, 1891) (Almeida *et al.* 2015), both spiders of the family Ctenidae. The spider *Ctenus medius* Keyserling, 1891 was observed preying on the anuran *Adenomera marmorata* Steindachner, 1867 (Barbo *et al.* 2009). In both these cases, the spider subdued and fed on the vertebrates. In a different context, Ramires and Fraguas (2004) conducted an experiment where they induced encounters between *H. mabouia* and the brown spider *Loxosceles intermedia* (Sicariidae). Success of the predation by the gecko depended on the region of the spider that was contacted. Among 123 observations, 10 geckos successfully ate the spider, although two geckos died due to poisoning when the gecko attempted to eat the spider's cephalothorax, which has dermonecrotic venom.

The genus *Ctenus*, which includes cursorial and nocturnal spiders, produces poison with neurotoxic action (Lucas 2015); some species are synanthropic, such as *C. medius* (Brescovit *et al.* 2011). The habits of spiders of this genus would facilitate their encounter with the nocturnal gecko *Hemidactylus mabouia*. Spiders are frequently part of the diet of *H. mabouia*,

Figure 1. Observations of the envenomation of *Hemidactylus mabouia* (MUFAL 15013) by *Ctenus rectipes* (MUFAL 605). (**A**) Gecko with spider in its mouth 50 cm above floor level. (**B**) Same animals in the ventral position. (**C**) Dorsal and ventral views of spider found during the predation event. (**D**) Detail of the chelicera still inserted in the oral mucosa of the gecko. (**E**) Detail of the necrosis in the anterior tip of the gecko's tongue.

Figure 2. Specimens of *Hemidactylus mabouia* [(**A**) MUFAL 15014, (**B**) MUFAL 15015, (**C**) MUFAL 15016] and *Ctenus rectipes* [(**A**) MUFAL 606, (**B**) MUFAL 607] found dead and in an advanced decomposition stage.

which is a generalist and opportunistic predator of arthropods (Zamprogno and Teixeira 1998, Bonfiglio *et al.* 2006).

Our data suggest that predation of the spider *Ctenus rectipes* by the gecko *Hemidactylus mabouia* may be unsuccessful when the lizard attempts to ingest the spider from the head, because its fast-acting poison can be deadly for the common domestic gecko.

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Short Communication

Predation of anurans by spiders: four cases in Mexican tropical forests

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Keywords: interaction, diet, predator, prey.

Palavras-chave: interação, dieta, predador, presa.

Anurans are components of the trophic networks in ecosystems in which they occur, given their high population densities and being a food source for diverse groups of vertebrates and invertebrates (Wells 2007, Hocking and Babbitt 2014). Spiders are among the most important invertebrate predators on anurans (Menin *et al.* 2005, Toledo 2005). However, observations of predator-prey interactions are scarce, because they are infrequent and are rarely the primary focus of fieldwork that has been designed to document them. Herein, we describe four novel predator-prey interactions involving four spider species and four anuran species in two tropical forests of Mexico.

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We observed two predation events during fieldwork in the Zihuapilapa stream, located in the municipality of San Sebastián Tlacotepec in southeastern Puebla (18°24'17" N, 96°50'43" W; 264 m a.s.l.). At 01:03 h on 07 February 2013, an adult *Ptychohyla zophodes* Campbell and Duellman, 2000 (Hylidae; snout–vent length, SVL = 28.81 mm) was being consumed by a female *Ctenus* sp. (Ctenidae; total length, TL = 28 mm) on a leaf 1.1 m above the stream (Figure 1A). Two days later, at 01:42 h on 09 February 2013, we found an adult *Rheohyla miotympanum* (Cope, 1863) (Hylidae; SVL = 24.53 mm) being consumed by a female *Ctenus* sp. (TL = 19 mm) on a leaf at 98 cm above the ground (Figure 1B).

In the private protected area called Área de Protección y Desarrollo de Ceratozamia, located in the municipality of Ixhuatlán del Sureste in southern Veracruz (18°02'34" N, 94°21'32" W;

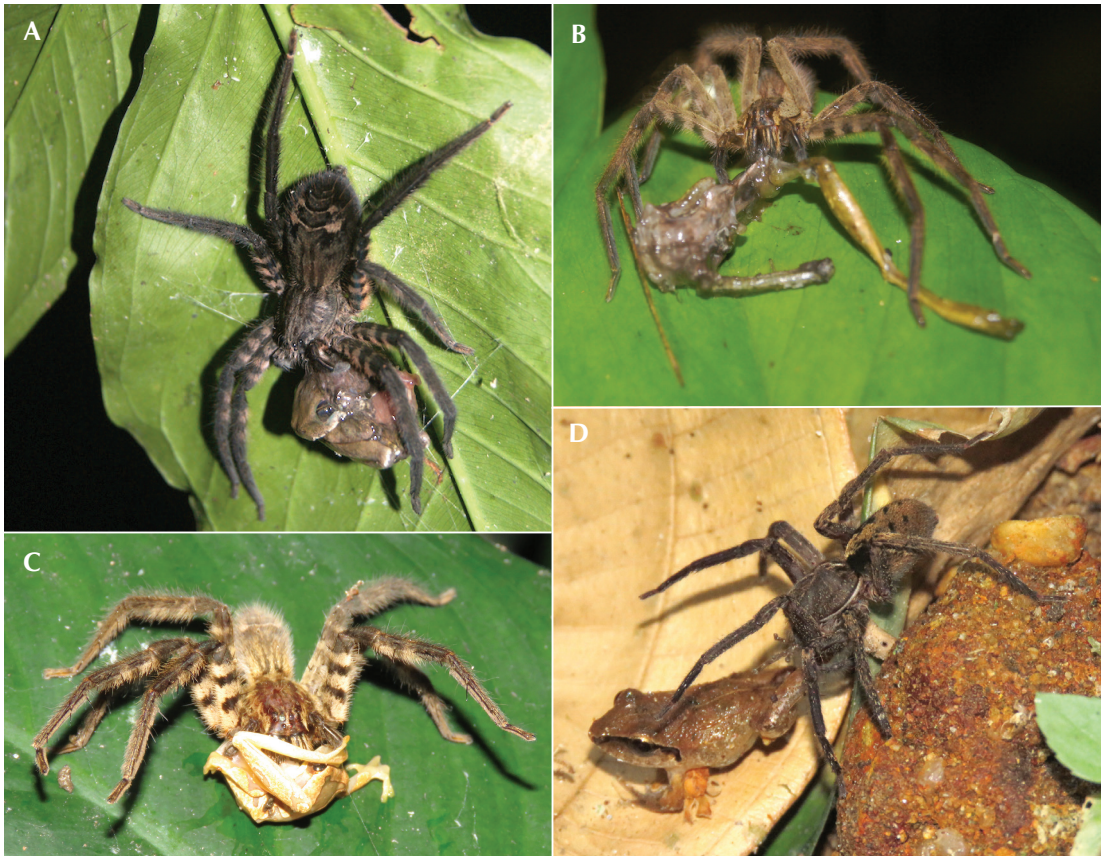

Figure 1. Predator-prey interaction between *Ptychohyla zophodes* and *Ctenus* sp. (**A**), *Rheohyla miotympanum* and *Ctenus* sp. (**B**), *Dendropsophus ebraccatus* and *Cupiennius salei* (**C**), and *Craugastor loki* and *Ctenus* sp. (**D**).

10 m a.s.l.), we encountered two more events of predation of anurans by spiders. On 12 May 2019 at 21:26 h, a *Dendropsophus ebraccatus* (Cope, 1874) (Hylidae; SVL = 21 mm) was being consumed by a female *Cupiennius salei* (Keyserling, 1877) (Trechaleidae; TL = 24 mm), on the leaf of a shrub 1.2 m above the ground (Figure 1C); the frog emitted distress vocalizations during the predation event. On 13 May 2019 at 21:49 h, a *Craugastor loki* (Shannon and Werler, 1955) (Craugastoridae; SVL = 17 mm) was observed being consumed by a male *Ctenus* sp. (TL = 17 mm) in the leaf litter (Figure 1D). The predation events were interrupted in each of the four cases, and the specimens collected for taxonomic determination. The frogs were identified with the keys of Duellman (2001) and Köhler (2011) and the spiders with Ubick´s key (2005). Specimens were not deposited in a scientific collection because of their state of deterioration.

Ctenus sp. is the first known predator of *Ptychohyla zophodes.* In the case of *Rheohyla miotympanum, Ctenus* sp. is the third species of spider known to prey on this anuran; the other species are *Cupiennius salei* (García-Vinalay and Pineda 2017) and *Dolomedes holti* Carico, 1973 (Deluna and Montoya 2017). Other

predators of *R. miotympanum* include hemipterans of the family Belostomatidae—a species of the genus *Abedus* (Pineda 2003) and an unidentified genus and species (Hernández-Salinas *et al.* 2012). The predation of *Dendropsophus ebraccatus* by *C. salei* is the third record of predation on this anuran by spiders; earlier records include *Cupiennius coccineus* Pickard-Cambridge, 1901 (Szelistowski 1985) and a ctenid spider of an unidentified genus (Donnelly and Guyer 1994). Other predators of *D. ebraccatus* include snakes (*Leptodeira* sp.) consuming adults, Odonata larvae (family Aeshnidae) and a hemipteran (family Belostomatidae) preying on tadpoles (Ospina and Touchon 2018), and hymenopterans [*Agelaia centralis* Haliday, 1836; *Agelaia* sp.; *Azteca* sp.; *Polybia rejecta* (Fabricius, 1798)] and dipterans (*Drosophila* sp.) consuming eggs (Toledo 2007, Touchon and Warkentin 2009, Ospina and Touchon 2018). The predation of *Craugastor loki* by *Ctenus* sp. represents the first record of predation by a spider and the second known predator for the species. The only predator previously known is the snake *Leptodeira septentrionalis* (Kennicott, 1859) (Cabrera-Guzmán *et al.* 2009, Platt *et al.* 2016, Nieblas-Camacho 2017).

The observations reported here supplement the various observations of spiders of the genera *Ctenus* and *Cupiennius* consuming anurans in the neotropics (Menin *et al.* 2005, Toledo 2005, 2007, Ervin *et al.* 2007, Barbo *et al.* 2009, Cicchi *et al.* 2010, De-Carvalho *et al.* 2010, Gibbons *et al.* 2010, Vázquez-Cisneros 2011, Amaral *et al.* 2015, Ríos-Rodas *et al.* 2016, De Mira-Mendes *et al.* 2017, Folt and Lapinski 2017, Vázquez-Cruz *et al.* 2017, Abarca *et al.* 2018, Salas *et al.* 2019) and suggest that these spider genera are among the most important predators on postmetamorphic anurans in this region. Further, the observations contribute to the knowledge of the multitrophic interactions of species and their relevance in the functioning of the ecosystem. Spiders may have an impact on anuran populations, but the effect of the impact is not known (Menin *et al.* 2005); therefore, further studies are needed to evaluate these effects.

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SHORT COMMUNICATION

Fruit consumption by *Salvator merianae* (Squamata: Teiidae) in the Brazilian Caatinga

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Keywords: Black-and-white Tegu, Cactaceae, diet, natural history, *Pilosocereus gounellei*, seed dispersal.

Palavras-chave: Cactaceae, dieta, dispersão de sementes, história natural, *Pilosocereus gounellei,* teiú.

Salvator merianae (Duméril and Bibron, 1839) is a native teiid lizard widely distributed in South America, occurring in all Brazilian environments, such as Caatinga dry forest, Cerrado savanna, Pampa grassland, and Atlantic and Amazon rainforest (Van Sluys and Rocha 1999, Kiefer and Sazima 2002, Vieira *et al*. 2018). It is the largest species in the genus, reaching 1.2 m total length and weighing over 4.5 kg (Milstead 1961, Fitzgerald 1992). The Black-and-white Tegu is known to have an omnivorous diet, which includes invertebrates, vertebrates, eggs, and many fruits (Kiefer and Sazima 2002). In fact, in the family Teiidae, frugivorous habits have been reported in 36 species (Valido and Olesen 2007, 2019).

Despite advances in the study of the diet of *S. merianae*, most of the data available for fruit eating in the diet of this lizard come from experiments in which individuals are presented with fruits, through stomach content analyses, or traditional knowledge (Kiefer and Sazima 2002, Castro and Galetti 2004, Silva *et al*. 2014). Recently, passive sampling methods, such as video recording with camera traps, have increased the data about the natural diet and feeding behaviour of *S. merianae* (Luciano *et al*. 2020).

Here we describe new records of *S. merianae* consuming fruits directly from the plant at a Caatinga site. Primary seed dispersal, when seeds are consumed before reaching the ground (Muller-Landau and Hardesty 2005), was unknown in *S. merianae* prior to this study.

We conducted our fieldwork at the Municipality of Lajes, Rio Grande do Norte state, northeastern Brazil (05°47'55" S, 36°14'22" W). The region is characterized by Caatinga vegetation with thorny trees and shrubs, and a cacti physiognomy. The climate is dry semiarid, marked by an extended dry season and erratic and unpredictable rainfall that usually occurs from February to May, when the total amount of precipitation reaches 350 mm (Szilagyi 2007).

We monitored vertebrate frugivory interactions on *Pilosocereus gounellei* (F. A. C.

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Weber) Byles and Rowley (Cactaceae), a shrubby columnar cactus that can reach up to 3 m in height, is covered by thick spines of 150 mm in length, and bears glabrous dehiscent fruits (4 × 6 cm) with a pinkish pericarp and a magenta fleshy pulp with many ($N \leq 4000$) small (1 × 1 mm) seeds (Abud *et al*. 2012). We used camera traps (Bushnell 8 MP Trophy Cam) from September 2018 to July 2019, totaling 621 camera-days, to monitor 17 individual cacti. Cameras were installed close to (2–5 m) and pointing toward cactus fruits. They were programmed to take two pictures followed by a 10-s video in intervals of one minute when triggered. The cameras were set in the field to work 24 h per day to detect frugivory events (Blanco *et al*. 2019).

We recorded *S. merianae* consuming two fruits of *P. gounellei* on two consecutive days, 22 and 23 May 2019, at 10:27 h and 11:04 h, respectively. By comparing the individual's morphology and color patterns on the images, we confirmed that the fruits were consumed by the same individual (Figure 1). At the time the frugivory was recorded, two fruits were available at a height of 0.7 m. The animal stood in a bipedal position and ate the pulp from both fruits, using its hands to improve balance by touching the thick spines (Figure 1; link to video: https://youtu.be/7t5MdJQKs_Y).

Figure 1. *Salvator merianae* consuming fruits of *Pilosocereus gounellei* at a Caatinga site in Lajes municipality, Rio Grande do Norte state, northeastern Brazil.

This report is the first evidence of primary seed dispersal by *S. merianae* as well as the first record of fruit consumption of *P. gounellei* by this large lizard. *Salvator merianae* has been reported to consume fruits from another columnar cactus, *Cereus jamacaru*, at another Caatinga area, although it is not clear if the animals accessed fruits fallen on the ground or in the cactus (Silva *et al.* 2014). This teiid lizard ate fruits of the cactus *Cereus peruvianus* when offered in a controlled experiment but the passage of the seeds through *S. merianae*'s gut did not affect the percentage and time of seed germination (Castro and Galetti 2004). However, it is known that *Pilosocereus* spp. depend on zoochoric vectors to remove chemical barriers present in their fruits and to promote seed germination (Meiado *et al.* 2012).

Despite being a generalist, the diet of *S. merianae* can include up to 50% of plant matter, such as fruits (Kiefer and Sazima 2002). Pulp fruits are easily metabolized because they are low in fiber and protein content, and many are rich in water, such as the fruits of *Pilosocereus* spp. (Nascimento *et al.* 2011). In addition to eating fruit, lizards potentially disperse seeds (Valido and Olesen 2019). *Salvator merianae* differs from other seed-dispersing lizards in the Caatinga, such as *Tropidurus* spp. (Gomes *et al.* 2016), because it is larger in size and weight (Fitzgerald 1992). These lizards search for food throughout their large home ranges ($\geq$ 4 ha) (Vieira 2016). Thus, *S. merianae* potentially disperses seeds for a long distance, considering that it defecates intact seeds (Castro and Galetti 2004, Silva *et al.* 2014).

This study shows that *S. merianae* consumes fresh fruits directly from the plant as part of its natural diet, adding information about the diversity of plant species known to provide food for this lizard. It also increases knowledge about the intimate relationship between the lizards and cacti of the Caatinga (Guerrero *et al.* 2012, Leal *et al.* 2017). Future work should evaluate the role of *S. merianae* as an effective seed disperser of Caatinga plant species. Considering the intense poaching pressure this lizard suffers in Brazilian semiarid ecosystems, this mutualism could be lost before it is understood (Alves *et al.* 2012).

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SHORT COMMUNICATION

Novel predator-prey interactions of *Rhinella major* (Anura: Bufonidae) from an urban area in northern Brazil

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Keywords: amphibians, predation, ontogeny, feeding behavior.

Palavras-chave: anfíbios, predação, ontogenia, comportamento alimentar.

Direct field observations of predator-prey interactions improve our knowledge about species niches and the ways in which vertebrate and invertebrate assemblages are structured, as well as aiding in the conservation of species (Vitt and Caldwell 2009, van den Burg and Miguel 2020). As anurans grow, the degree of capture specialization by type and size of prey changes (Donnelly 1991, Simon and Toft 1991), but the increase in prey size with ontogeny is more than a passive effect of selection for larger prey in some species of leaf-litter frogs (Lima *et al*. 2000). An organism's diet is influenced by its morphology, physiology, and behavior in response to selective pressures and phylogenetic constraints (Toft 1981, Duellman and Trueb 1994, Wells 2007, Solé *et al*. 2009). Anurans have been shown to be flexible predators in trophic chains (Toledo *et al*. 2007, Oliveira *et al*. 2013). Data on anuran's diet inform us about their life history, environmental conditions, and the possible consequences of habitat alterations, in addition to as well as instructing us about the distributions of prey species and the reasons for fluctuations in anuran populations (Lips *et al*. 2005, López *et al*. 2015).

We report observations of feeding behavior of the Granular Toad, *Rhinella major* (Müller and Helmich, 1936), during its breeding season. Included in diet of this toad in an urban area in the municipality of Macapá, state of Amapá, Brazil are the anomalepidid blindsnake, *Typhlophis squamosus* (Schlegel, 1839), the Napo Tropical Bullfrog, *Adenomera hylaedactyla* (Cope, 1868), and the millipede *Asiomorpha coarctata* (De Saussure, 1860), and we provide additional comments on a possible potential prey item for this toad as a supplier of alkaloids.

Rhinella major is a terrestrial species of moderate size (SVL 35.8–72.8 mm in adult males and 33.9–81.1 mm in adult females); the species is a member of the *R. granulosa* Group and is widespread in the Chaco Region of Argentina, Paraguay, and Bolivia, and in open formations along the rivers Madeira, Beni, Amazonas, Tapajos, and Xingu in Brazil

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(Narvaes and Rodrigues 2009, Frost 2020). In Amapá state, the species is abundant in urban and disturbed areas (Pedroso-Santos pers. obs.). Although relatively abundant throughout Amazonia, data on diet of *R. major* are sparse in the literature; however, predatory activity in this species has been reported (Pedroso-Santos *et al*. 2019).

Typhlophis squamosus (Schlegel, 1839) is a small snake no longer than about 30 cm (Fraga *et al*. 2013); it is widely distributed across the Guiana Shield (Hoogmoed 1979) and abundant in the Amazon Basin (Amaral 1978) and in urban areas (Marques *et al*. 2009). In the municipality of Macapá, this species has been observed leaving its fossorial habitats in urban areas at the end of the rainy season. Predation of this species is poorly described in the literature (Sazima and D'Angelo 2011). The leptodactylid *Adenomera hylaedactyla* (Cope, 1868) is a nocturnal frog ranging across Amazonian forests through the Guianas, in Colombia, Venezuela, Ecuador, Peru, Bolivia and Brazil (Frost 2020). According to Kokubum and Sousa (2008), this species usually is associated with primary and secondary forest, forest edges, and savannah, but it can be found in areas of open vegetation, such as stream and river edges.

The predator-prey interactions of *Rhinella major* were observed during its breeding period on 27 October and 17 November 2019 in a auditory survey of amphibians in an urban area in the municipality of Macapá in the state of Amapá (0.0083º S, 51.0953º W; datum: WGS84; 7 m a.s.l.) in the extreme north of Brazil. According to the Köppen-Geiger classification (Alvares *et al*. 2013), the area is characterized by an equatorial climate with annual precipitation of 2850 mm, concentrated between February and May (average ~ 400 mm per month) and average annual temperatures ranging from 27.6–41.0 °C.

Rather than collecting the toads, we photographed them and deposited the images in the Herpetological Collection of the Universidade Federal do Amapá (photographic vouchers CECCAMPOS 0001-0002). The species could be identified confidently by its diagnostic characters (Narvaes and Rodrigues 2009) evident in these photographs and in our field notes. The prey items were identified as *Typhlophis squamosus* applying the diagnosis of Kok and Fuenmayor (2008) and *Adenomera hylaedactyla* following the morphological traits described by Angulo and Icochea (2010).

The first observation occurred at 20:35 h. It took place approximately 10 m from the margin of a small puddle, and lasted about 11 min. One of us observed a female *Rhinella major* with a *Typhlophis squamosus* in its mouth (Figure 1A) on the ground and close to fragments of PVC pipe. The body parts of the blindsnake protruding from the toad's mouth were moving. During the observation, the toad moved only a few times, positioning itself on the ground and positioning the prey in its mouth for consumption. After swallowing the prey, the toad inflated its lungs and blinked its eyes repeatedly, perhaps in response to expanding its gastrointestinal tract to accommodate the prey. At the same place, at about 21:57 h, FPS observed a female *R. major* preying on an *Adenomera hylaedactyla* in the vicinity of conspecific vocalizing males. The prey must have been ingested headfirst because when we observed it, only the posterodorsal portion of the frog's body was visible (Figure 1B). While swallowing the frog, the toad quickly stretched one of its legs and blinked its eyes several times. This successful predation event lasted at about 8 min. In neither predation event was the prey regurgitated.

Bufonidae is one of the most widely distributed anuran families in the world (Sabagh and Carvalho-e-Silva 2008). Members of the family feed on arthropods and vertebrates such as amphibians, snakes, and rodents and bats occasionally; the toads also may ingest plant parts (pieces of leaves) (e.g., Quiroga *et al*. 2009, Silva *et al*. 2010, Chaves *et al*. 2012, Oliveira *et al*. 2017, Pedroso-Santos *et al*. 2019). Although invertebrates of the Class Diplopoda have been reported as prey items for *Rhinella* [e.g., *R. scitula* (Maragno and Souza 2011), *R. icterica*

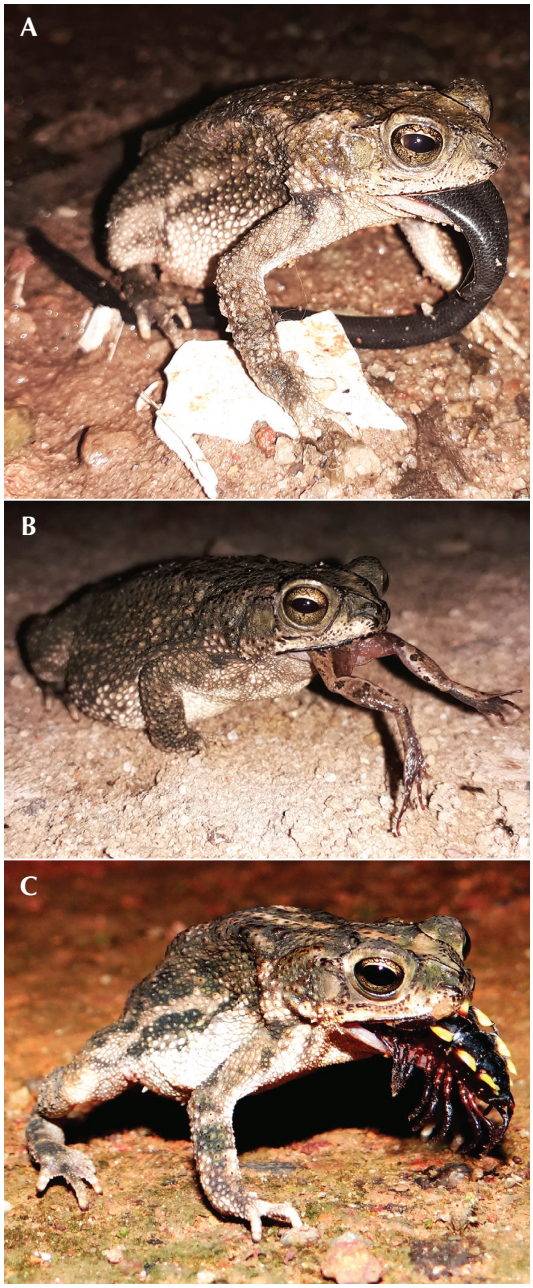

Figure 1. Female *Rhinella major* ingesting **(A)** a blindsnake *Typhlophis squamosus* (CECCAMPOS 0001), **(B)** the leptodactylid frog *Adenomera hylaedactyla* (CECCAMPOS 0002), and (**C**) the millipede *Asiomorpha coarctata*.

(Sabagh and Carvalho-e-Silva 2008), and *R. schneideri* (Batista *et al*. 2011)], the authors do not discuss the importance of these invertebrates in the diets of these toads. Diets composed of ants and mites have been widely cited as a specialization in anurans to facilitate the uptake of alkaloids (Biavati *et al*. 2004, Wells 2007). However, some species of millipedes, including those with aposematic coloration, have toxic substances such as alkaloids and hydrogen cyanide (Roberts and Wink 1998) that also might be used by anurans.

We observed a female *Rhinella major* preying upon a millipede *Asiomorpha coarctata* (De Saussure, 1860) (Diplopoda: Paradoxosomatidae) with aposematic coloration (Figure 1C). We do not know if this invertebrate species has the potential to supply alkaloids to anurans in order to retain these toxins in their skin or in paratoid glands. The millipede was identified following the descriptions of Shelley and Lehtinen (1998) and Decker and Tertilt (2012). This millipede is known to cause epidermal lesions in humans (Haddad *et al*. 2000, Cardoso *et al*. 2003) and is widely distributed in urban areas (Shelley and Lehtinen 1998, 1999).

Rhinella major has been reported to prey on a lizard (*Hemidactylus mabouia)* in an urban area (Pedroso-Santos *et al*. 2019); no other reports of predation events were found. To our knowledge, the only other predator reported for *Typhlophis squamosus* is a bird (*Turdus leucomelas*; Sazima and D'Angelo 2011), also in a disturbed area, and *Adenomera hylaedactyla* is a prey item for snakes and spiders (e.g., Tavares-Pinheiro *et al*. 2019a,b), with few records for anurans. We suggest that in urban areas, *R. major* may forage opportunistically on larger prey, as observed by Pedroso-Santos *et al*. (2019), and that the practice may be more frequent during the toad's breeding period when the energy demand is higher. There may be a relationship between the consumption of larger prey and ontogeny, as well as quality of the composition of its diet (De-Carvalho *et al*. 2008, Sugai *et al*. 2012).

These observations may encourage future studies on the feeding ecology of *Rhinella major* because the potential of *Asiomorpha coarctata* as a prey supplying alkaloids will only be confirmed by additional diet data. We encourage researchers to seek additional information on the trophic ecology of *R. major,* particularly with regard to ontogenetic changes in the toad's diet. This report shows the importance of using opportunistic sampling methods to describe natural history by direct observations in the field and photographic records (Cullen *et al*. 2006).

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