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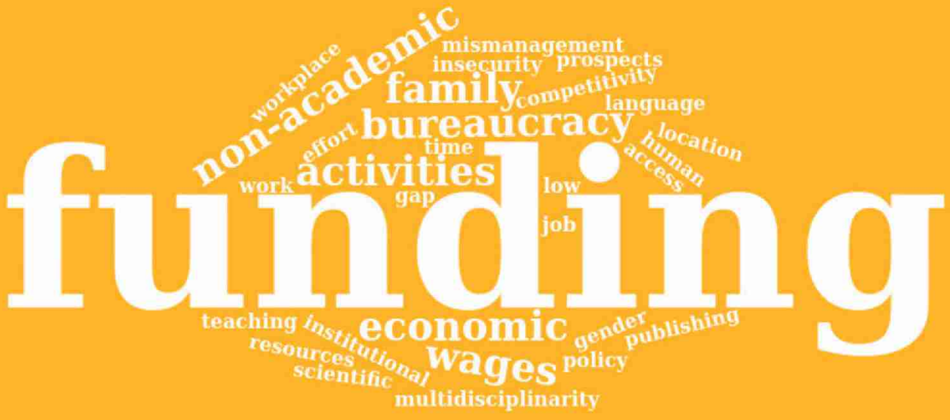

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Gender inequities in herpetology: the case of the Argentine community

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ABSTRACT

Women in scientific fields have achieved meaningful gains in terms of participation, and they even reached gender parity in Argentina. However, in spite of several documented attempts to close the gender gap, inequalities still remain in different academic areas. Considering that disciplines have developed under different historical contexts and institutional settings, it is relevant to describe gender equality indicators for them. This could also lead us to a better understanding of the mechanisms modelling global patterns of gender bias. Here we present a detailed analysis on gender proportion in different roles that researchers perform in the Argentine herpetological community. We gathered data on gender composition of the director boards and active members of the Argentine Association of Herpetology, and the organizing committees and participants of the Argentine Congress of Herpetology. We also performed a survey on gender topics to the attendants to the 20th Argentine Congress of Herpetology. Our main results pointed out a low number of women in the highest positions of the community hierarchy, such as chair positions in the AHA and conferences and symposia in congress. This leads to a lesser visibility of women in contrast to men, and is a potential reason for the low number of female herpetologists as role models. Furthermore, we detected low numbers of female herpetologists with long academic paths, depicting a major drop out of women along their careers. Gender bias in science has a strong hierarchical component and this pattern was recovered in different scientific activities within herpetology. In this sense, directed actions and targeted policies are required to guarantee the access of women to power positions and for the promotion or retention for senior female researchers. Identifying the weakest points in terms of gender equality, and the areas where gender biases are historically established is necessary to build a more egalitarian community.

Key Words: Gender Gap; Science; Female Representation; AHA; Congress Participation.

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RESUMEN

Las mujeres han logrado avances significativos en cuanto a su participación en el campo científico y en Argentina incluso han alcanzado la paridad de género. Sin embargo, a pesar de varios intentos documentados de cerrar la brecha de género, las desigualdades aún persisten en varias áreas académicas. Considerando que distintas disciplinas se han desarrollado bajo diferentes contextos históricos y escenarios institucionales, resulta relevante describir los indicadores de equidad para las mismas. Esto además puede conducir a una mejor comprensión de los mecanismos que modelan los patrones globales de sesgo de género. En el presente trabajo presentamos un análisis detallado sobre la proporción de género en diferentes roles que desempeñan los/las investigadores/as en la comunidad herpetológica argentina. Recogimos datos sobre la composición por género de las comisiones y los miembros activos de la Asociación Herpetológica Argentina, y de los comités organizadores y los participantes del Congreso Argentino de Herpetología. Además, realizamos una encuesta sobre temas de género a los asistentes al XX Congreso Argentino de Herpetología. Nuestros principales resultados señalaron un bajo número de mujeres en los puestos de mayor jerarquía dentro de la comunidad, tales como presidencias en la AHA y participación en conferencias y simposios en congresos. Esto conduce a una menor visibilidad de las mujeres en comparación con los científicos varones y es una posible razón del número comparativamente bajo de herpetólogas como mentoras o modelos a seguir. Además, detectamos un bajo número de herpetólogas con trayectorias de larga duración, lo que indicaría una importante deserción a lo largo de sus carreras. El sesgo de género en ciencia presenta un fuerte componente jerárquico y este patrón se recuperó en diferentes actividades científicas dentro del campo de la herpetología. En este sentido, se requieren acciones dirigidas y políticas focalizadas para garantizar el acceso de las mujeres a puestos de toma de decisiones/mayor exposición y para la promoción o retención de investigadoras senior. Identificar los puntos más débiles en términos de igualdad de género y las áreas donde los prejuicios de género están históricamente establecidos es necesario para construir una comunidad más igualitaria.

Palabras claves: Brecha de Género; Ciencia; Representación Femenina; AHA; Participación en Congresos.

Introduction

In recent years, a process of self-evaluation related to the gender gap has been triggered within the scientific community (de Kleijn *et al.*, 2020; Huang *et al.*, 2020). Thus, to measure gender inequities, several indicators have been defined and implemented at different scales (O'Brien *et al.*, 2019; Huang *et al.*, 2020). Various studies assessed gender gap in terms of disparities in authorship, productivity, citations, or access to funding in almost all disciplines and countries (e.g., Hill *et al.*, 2010; Larivière *et al.*, 2013; Shen, 2013; Holman *et al.*, 2018; de Kleijn *et al.*, 2020; Huang *et al.*, 2020). However, gender inequity is a multidimensional problem rooted in a historical gender imbalance that impacts the success rate of women in academia, therefore, any single indicator is often not capable of including all its relevant dimensions (Astegiano *et al.*, 2019; O'Brien *et al.*, 2019). This highlights the need of evaluating gender bias in an integrative way and considering the multiple roles that researchers develop.

One important aspect of the scientific career is the visibility of the researchers within their discipline or respective community (Martin, 2014). Conference attendance, presentations, and plenary talks are relevant instances for researchers to achieve visibility, which lately influence their perceived quality and peer-recognition (Schroeder *et al.*, 2013; Jones *et al.*, 2014). In addition, scientific societies play a large role in visibilization of their members, providing opportunities for networking both formally and informally (Potvin *et al.*, 2018). As a consequence, scientific societies and all the events or activities promoted by them may have the capacity to prompt women in their careers, as well as to promote changes throughout science to achieve gender equality.

Female participation in science has increased considerably during the last decades, and Argentina has been recently pointed out as one of the countries that reached overall gender parity (de Kleijn *et al.*, 2020). As such, women represent 61% of the total tenure researchers, and 53% considering only Life Sciences (https://cifras.conicet.gov.ar/publica/) in the main research institution of the country (CONICET). In spite of this rising number of women, a recent big data analysis including the career path length of researchers across the world showed a widening gender gap for certain indicators, such as productivity and impact (Huang *et al.*, 2020). Studies like this emphasize the relevance of weighing the imbalanced access to opportunities faced by women, even from early career and varying according to geographic region or discipline (Ceci *et al.*, 2014; Maas *et al.*, 2020). In this sense, a disaggregated analysis of indicators and activities for specific scientific disciplines in Argentina is relevant since it could reveal a growing gender gap therein.

In Herpetology, particularly, gender disparities have been indicated regarding female participation in different academic roles, in authorship positions, and organizing and speaking in symposia or conferences (Parenti and Wake, 2016; Sardelis and Drew, 2016; Salerno *et al.*, 2019; Rock *et al.*, 2021). Recently, Grosso *et al.* (2021) found a pattern of male preferential connections (male homophily) in herpetological publications that marginalizes women, both at regional and global scales. In addition, a low number of women in the highest hierarchies in academia, fewer women than men publishing papers, and a lack of incentives for women were pointed out in the Brazilian herpetological community (Carnaval, 2016; Benício and Fonseca, 2019; Werneck *et al.*, 2019; Slobodian *et al.*, 2021).

The Argentine Herpetological Association (AHA) is the oldest herpetological society in South America, holding annual meetings (Argentine Congress of Herpetology) since 1983, and publishing its own journal (Cuadernos de Herpetología) for 36 years. Up to now, there is only one study about gender bias in the dynamics of the publication process in the Argentine herpetological community (Grosso *et al.*, 2021). Therefore, the aim of this work is to evaluate the gender proportion at complementary academic roles that researchers develop, such as participation in the AHA and the Argentine Congress of Herpetology. In addition, we tested the hypothesis that under an unbiased gender scenario, the proportion of women in top hierarchical or high exposure positions reflects the observed proportion of female members of the AHA with long career paths. Finally, we performed a survey to characterize the general perception of the Argentine herpetological community on gender topics and to detect other barriers excluded by the quantitative data. The analyses presented herein are necessary to identify the weakest points in terms of gender equality, to establish which are the areas where gender biases are historically established, and to propose actions that may help to close the gender gap.

Materials and methods

We built a gender-database for director boards of the AHA, its active members, the organizing committees of the Argentine Congress of Herpetology, and the congress participants. The gender of the researchers (assigned as male/female) was identified using their first names and following the free database of Gender Checker (https://genderchecker.com). Alternatively, gender was determined by visual inspection of ResearchGate profiles or images found in Google when the author's name was ambiguous. The gender approach used here was binary, and those individuals that could not be categorized as male or female were excluded from the analyses. Considering that these were just a few cases, this removal did not affect the overall sample-size nor the relevance of the conclusions. We are aware that the author's self-perceived gender could mismatch with the gender assigned here, and that the binary approach excludes other identities that may be present in the community. However, we were unable to consider additional identities due to analytical and operational limitations.

We gathered information about the gender composition of the director boards of the AHA by hierarchies (*i.e.*, chair and board members) during the 1982–2021 period. We also recorded the gender of the active members of the association during the 2019–2021 period, and calculated the length of their academic paths as years from her/his first published paper up to 2021. The list of members was collected from http://aha.org.ar/socios-activos/ and the year of the publication of their papers was collected from CONICET webpage, ResearchGate or Google Scholar (full datasets are available in Supplementary Table S1).

The gender composition of the Argentine herpetological community through time was estimated using the gender proportion of attendants to the congress spanning the 1999–2019 period. We also evaluated gender participation in the Argentine Congress of Herpetology from 1999 to 2019. We compiled data of organizing committees and of authors in the different types of presentations (dataset available in Supplementary Table S2). The authors were grouped into two main categories: regular exhibitors and invited speakers. Regular exhibitors are those presenting short oral communications and posters. Invited speakers are peer-selected, commonly funded by the congress organizers, and their presentations comprise main conferences, conferences of young herpetologists and symposia. These are usually long-lasting lectures, carried out at the prime-time slot of the congresses. The total time of exposition for main conferences was calculated for male and female speakers (considering 45 minutes each talk). Data of the attendants/authors gender and the composition of the organizing committees were obtained from the books of abstracts of each congress.

We aimed to evaluate if the observed number of women in hierarchical/high exposure positions through years was the expected by chance, or if, on the contrary, existed an underlying process shaping the gender distribution. In order to do this, we performed 1.000.000 simulations of random gender assignment to chair members in the AHA and to speakers in main conferences, calculated the proportion of women in these positions under this neutral gender scenario, and then compared them with the observed values. These simulations were performed in R (version 3.6.3, R Core Team 2020), and using as baseline the observed number of active members of the AHA with long academic paths (*i.e.*, ≥ 15 years). Finally, we performed a survey among the attendants to the 20th Argentine Congress of Herpetology in 2019 (dataset available in Supplementary Table S3). We asked them to respond to a six-point questionnaire about self-gender biases, caregiving tasks, and career challenges. The questions were: A) K. Smith recently published a paper. Which do you suppose is the first name of this author, Kevin or Karen?, B) Give the full name of three authors that have influenced your career, C) Do you have/had to take care of other people? e.g. children, elder parents, D) If you do, how does/did affect/ed your professional life?, E) Do you agree that maternity/paternity leave in Argentina is enough?, and F) Write a short paragraph that summarizes the biggest obstacles in your career.

All supplementary files were shared as datasets uploaded to figshare repository (https://doi.org/10.6084/m9.figshare.16530618).

Results

The analyzed data showed only one woman as chair member of the AHA, throughout 38 years (1982–2020 period). After the election of authorities in 2020 a change was observed and both chair positions were occupied by women (raising up the percentage of women to 7.5% for the whole period; Fig. 1A). Until 1990, female participation on the director boards was lower than 40% but since 1991 the female proportion has increased, resulting in more than half of the board occupied by women at the present (54%; Fig. 1A).

During the 2019–2021 period, women represented 52% of the active members of the AHA. When analyzing the length of members' academic paths, women were the majority of the researchers with short careers paths (*i.e.*, 0 to 14 years), but men were more represented among long career paths (*i.e.*, ≥ 15 years; Fig. 1B).

Our results showed that in the last 20 years of the Argentine Congress of Herpetology women constituted on average 52% of the organizing committees and 45% of the authors attending the event (Fig. 1C–E). Although the overall gender participation of authors was balanced over the years, differences were noticeable when comparing the gender proportion of invited speakers and regular exhibitors (Fig. 1F–G). Of the 724 invited speakers, 38% were women (Fig. 1F). For main conferences, 35 women and 86 men were invited, summing up 26.25 h and 64.50 h of exposition, respectively. For young herpetologists' conferences, 13 women and 9 men participated during the nine years in which this event was held. For symposia, 581 authors participated, 38% of them were women, and female participation was higher than male only in two years (Fig. 1F). Opposite to what occurred in the category of invited speakers, in regular presentations female participation climbed to 46%, and they even were majority in some years (Fig. 1G).

The baseline of researchers used to perform the simulations was composed by 40% of female herpetologists. The simulations found that the probability to obtain the observed number of women as chair members of the AHA (observed proportion: 1 woman out of 46) was 0.001%, while the probability of being invited as a speaker in main conferences (observed proportion: 35 women out of 121) was

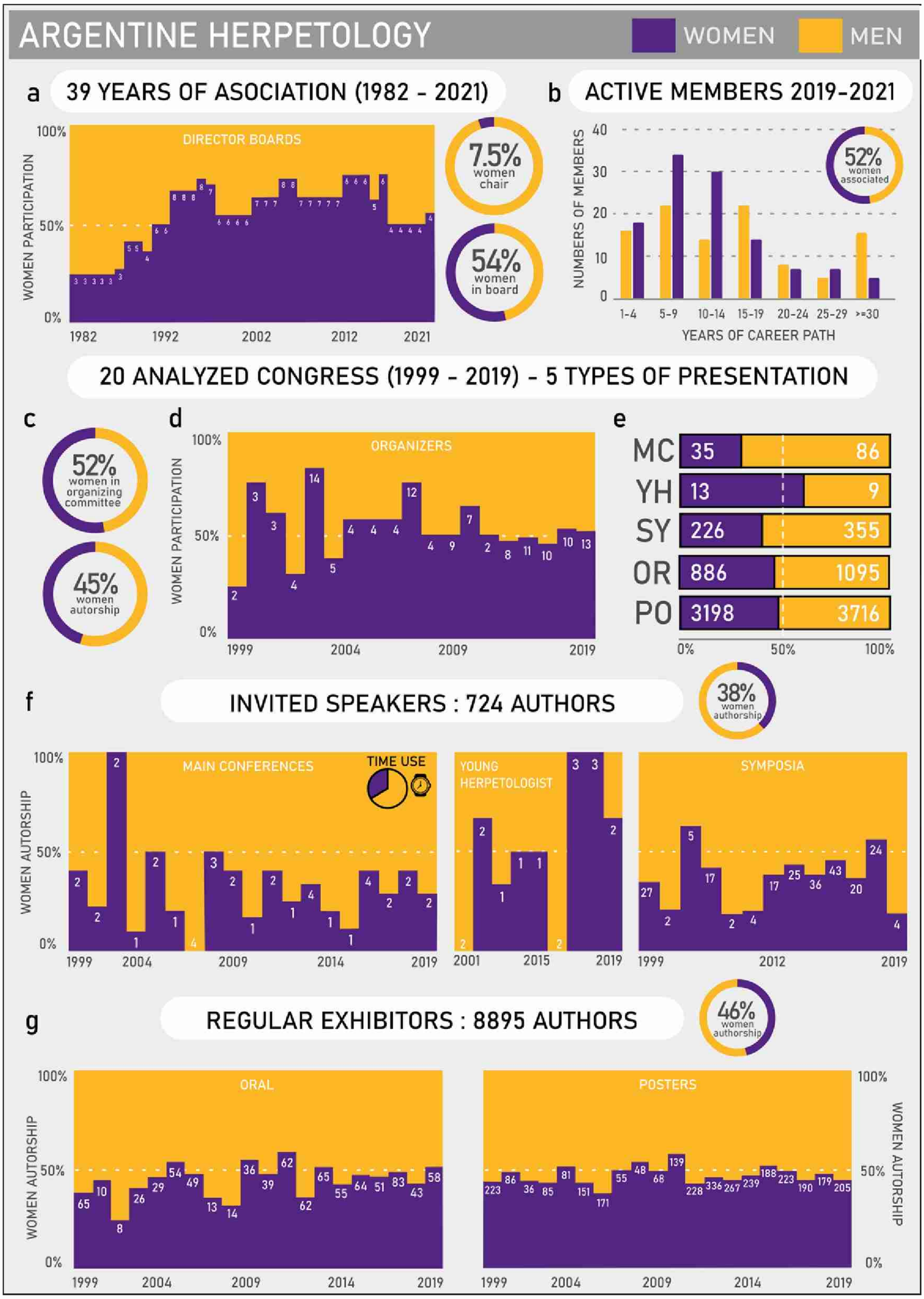

Figure 1. Gender distribution in Argentine Herpetology. (a) Director boards during the 39 years of the Argentine Herpetological Association (AHA). (b) Career path lengths of active members of the AHA during 2019–2021 period. (c–g) Gender participation in the Argentine Congress of Herpetology from 1999 to 2019. (c–e) Organizing committees of the congresses and overall participation among the five different types of presentations. (f) Percentages of participation of invited speakers. (g) Percentages of participation of regular exhibitors. White numbers on the histograms represent absolute values of women. Abbreviations: MC = main conferences; YH = young herpetologists conference; SY = symposia; OR = oral presentations; PO = posters.

0.007% (see Supplementary Information Fig. S1).

The survey was answered by 84 congress attendees of different academic status, from which 68% were women (Fig. 2). When asked to complete the first name of an author given the initial letter of the name, most of the surveyed thought in a man (87.5%; Fig. 2A). The same occurred when the surveyed attendants were asked to mention the three most influential researchers in their careers, the majority named a man as a reference (68%; Fig. 2 B). The survey also revealed that 47% of women researchers were involved in caregiving tasks and that most of them felt overwhelmed when balancing work and life duties (Fig. 2 C–D). Most participants (94%) answered that leaves for childbirth in Argentina are too short, especially for fathers (46%; currently two days after childbirth; Fig. 2E). Men and women differed when recognizing the biggest obstacles in their careers (Fig. 3). Women's answers pointed primarily to the family burden since the words that popped out mostly were "children" and "family", while money-related issues like "resources", "economy", and "funding" were in second place (Fig. 3A). In contrast, men's most significant concerns were related to money, since the word that popped out mostly was "funding", and items non-related to academic activities like "family" or "bureaucracy" appeared in second place (Fig. 3B).

Discussion

According to our results, women in Argentine herpetology have almost equal representation as men in several academic roles—as board members of the association, members of the organizing committees, and authors in congresses. However, gender disparity is evidenced when analyzing women participation in top hierarchical/high exposure positions—as chair members of the association, and speakers at main conferences and congress symposia. Moreover, through the performed simulations we showed that about 99% of the simulated cases resulted in a better scenario for women than portrayed by the real data. In other words, this depicted a significantly lower representation of women in those positions than expected in an unbiased gender scenario.

An increasing number of women have been incorporated in scientific disciplines during the last decade (de Kleijn *et al.*, 2020; Huang *et al.*, 2020). Nevertheless, Potvin *et al.* (2018) pointed out that women fulfilling leadership roles represent only 9% within zoological societies in South America. This gender mismatch at the hierarchical levels reveals a widespread phenomenon known as "glass ceiling", which was described as the intangible barriers—like peer-recognition, unequal family burden, gender stereotypes, among others—that prevent the promotion of highly qualified women to power positions (Akpinar-Sposito, 2013). In Argentina, the AHA has incorporated two women as chair members during the election of authorities in 2020, raising the proportion of women in this academic role. Further participation of women in decision making positions has also been reported recently for some Brazilian professional associations, since they had incorporated women as presidents, directorships and board members (Slobodian *et al.*, 2021). The increase in the representation of women in power positions is meaningful in a historical gender-unbalanced scientific landscape (Astegiano *et al.*, 2019), but considering that research is constructed in a collaborative fashion, the hierarchical structure of academia should be under debate as well.

We found two contrasting distribution patterns among the active members of the AHA. There are more women than men among researchers with short career paths; on the contrary, men are the majority among researchers with long career paths (Fig. 1B). This occurs in spite of the recruitment of female herpetologists has been constant and similar to that of men since 1999. Thus, low proportions of women with long academic paths do not seem to be the result of low recruitment in the past, but the consequence of the "leaky pipeline". This phenomenon describes the higher dropout rate of women compared to men´s at all stages of their academic trajectory (Alper, 1993; Goulden *et al.*, 2011; Shaw and Stanton, 2012), and was documented in several scientific fields (e.g., Luckenbill-Edds, 2002; Holmes *et al.*, 2008; Martin, 2012; Valentova *et al.*, 2017; Jadidi *et al.*, 2018; Huang *et al.*, 2020).

Multiple factors may lead women to leave research in sciences, but according to the surveyed information, caregiving tasks and motherhood could be the most significant within the herpetological community. These results are reinforced by previous studies showing that women are more prone than men to resign labor activities in favor of domestic life (e.g., Holmes *et al.*, 2008; McGuire *et al.*, 2012; Cerrato and Cifre, 2018; Lione, 2018), and that motherhood seems to be a breakthrough event in

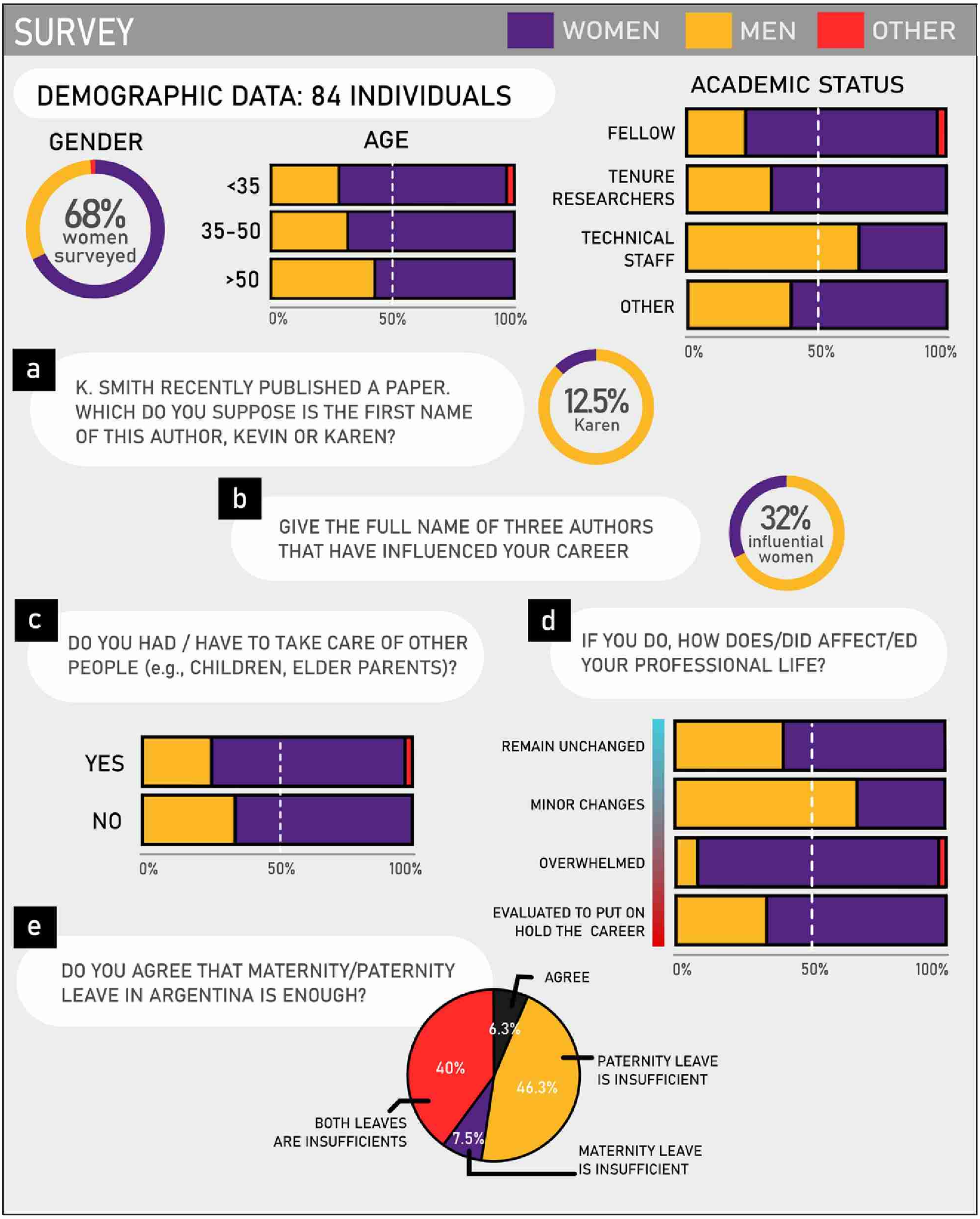

Figure 2. Summary of the survey answered by 84 attendees of the 20th Argentine Congress of Herpetology in 2019. (a–e) Questions with their summarized responses. Color codes in (a), (b) and (e) correspond to the gender referred in the answer.

women's academic careers (e.g., Kyvik and Teigen, 1996; Stack, 2004; Fox, 2005; Hunter and Leahey, 2010; Ceci and Williams, 2011; Cech and Blair-Loy, 2019). In the Argentine academic field—a population with high levels of education and a strong presence of paid work—women spend more time than

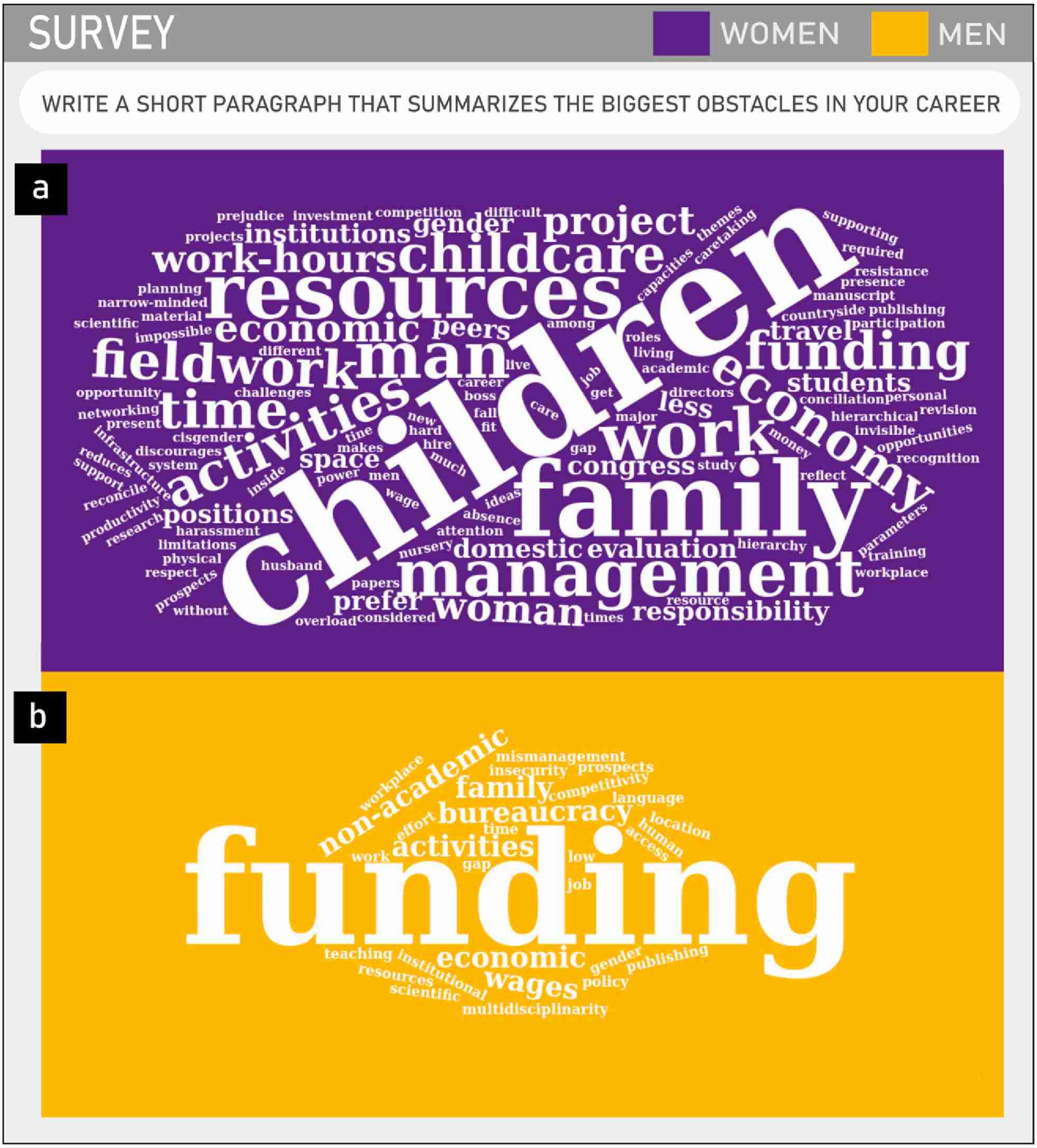

Figure 3. Word cloud constructed from gender-differentiated responses of the survey released by 84 participants of the 20th Argentine Congress of Herpetology in 2019.

men in domestic labors and caregiving tasks (Lione, 2018; Andreozzi *et al.*, 2019). However, such choices might be constrained by gender stereotypes of the society where female researchers are educated and develop their scientific careers (Ceci and Williams, 2011).

The performed survey also showed that most herpetologists thought of a man when asked to name influencer scientists in their fields (Fig. 2), highlighting the low number of women constituting role models. This is possibly related to the homophilic dynamics and the consequent marginalization of women described for the publication process in Herpetology (Grosso *et al.*, 2021). Also, this may be an outcome of the overall low proportion of women occupying high exposure positions in this field. In this regard, we found a significant low number of women as invited speakers in the Argentine Congress of Herpetology (Fig. 1F), which leads to a lesser visibility and may influence their peer-recognition. Our results agree with previous analyses of gender participation in congresses for other biological disciplines, where women were significantly underrepresented among invited speakers (Schroeder *et al.*, 2013; Kalejta and Palmenberg, 2017) or had reduced exposure compared to men (Isbell *et al.*, 2012; Jones *et al.*, 2014). When analyzing the causes of these biases, the imbalanced gender proportion of the organizers has been pointed as responsible for the low numbers of invited women (Isbell *et al.*, 2012; Casadevall and Handelsman, 2014; Sardelis and Drew, 2016; Débarre *et al.*, 2018). This was not the case in the organizing committees of the Argentine herpetological congresses, where women's proportions were high (Fig. 1C). Thus, further studies are needed to elucidate the factors driving gender bias in speaker selection.

Achieving gender parity within the scientific community, does not ensure that women will "break the glass ceiling" and will occupy high exposure and decision-making positions, currently occupied by their male counterparts (McGuire *et al.*, 2012; Shaw and Stanton, 2012; Holman *et al.*, 2018). Directed actions and targeted policies are needed to guarantee promotion and retention of female herpetologists, particularly of those with more than 15 years of career path length (Holman *et al.*, 2018; Huang *et al.*, 2020). Accordingly, since 2016 CONICET has given funding and institutional endorsement to those meetings reaching gender parity, in order to prompt visibility of female scientists and improve access of young women to role models and mentors. In this sense, more women in power positions not only encourages equity, but also enriches professional relationships and leads to a higher quality of science (Woolley *et al.*, 2010; Campbell *et al.*, 2013; Nielsen *et al.*, 2018).

We are aware that deep systemic and intrinsic changes are required and, in the words of Harding (1996), the transformation of the very foundations of science and of the culture that gives its value are needed. Nevertheless, questioning the practices that perpetuate gender inequities is a first step necessary to build a more egalitarian scientific system.

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Trabajo

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Filling some of the gap of ecological information's of a Brazilian Vulnerable lizard (*Tropidurus hygomi*)

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ABSTRACT
We studied a population of *Tropidurus hygomi* in an area of geographic disjunction located in Brazil's Northeast, investigating how individuals use the microhabitats, what is their daily activity in different seasons, how does growth and mass gain occur and what is the sex ratio, besides analyzing the population dynamics and the recruitment period. We collected data patch between January and December 2008 from 6:00 to 18:00 h combining active search techniques, pitfall traps and markings on animals. We observed lizards mainly on sunny days, motionless on the sand near bushes that formed sun and shade mosaic. Sit-and-wait foraging and preference for adequate light supply are conservative behaviors for *Tropidurus*. The sand allows to quickly achieve optimal thermal conditions. The lizards presented extended activities, with some seasonal differences, but with a more uniformity compared to congeners in other biomes demonstrating influence of local characteristics. We observe sexual dimorphism with larger and heavier males. Distinctions in growth were noticeable between males, females and juveniles (historical influence) but not for mass gain. Sex ratio was displaced in favor of males. This is not common in the genus but could be advantageous for females in choosing better partners. There were fluctuations in population growth, probably related to climate seasonality and mortality in the juvenile phases. *Tropidurus hygomi* recruitment begins in the rainy season and continues until the beginning of the dry season, unlike other congeners, suggesting the action of ecological effects. We hope that our results will assist in the development of conservation strategies for the populations of *T. hygomi* that live on the coast (continuous distribution), besides to guiding the execution of new studies.

Key Words: Iguania; Lizards; Relictual Distribution; Sergipe; Serra de Itabaiana National Park.

RESUMO
Nós estudamos uma população de *Tropidurus hygomi* em uma área de disjunção geográfica localizada no Nordeste brasileiro investigando como os indivíduos utilizam os microhabitats, como é a atividade diária nas diferentes estações do ano, de que maneira ocorre o crescimento e o ganho de massa e qual a razão sexual, além de analisar a dinâmica populacional e o período de recrutamento. Coletamos dados entre janeiro e dezembro de 2008 das 6:00 às 18:00 h, combinando técnicas de busca ativa, armadilhas de queda e marcações nos animais. Observamos lagartos principalmente em dias ensolarados, imóveis próximos a arbustos na areia em mosaico de sol e sombra. O tipo de forrageio senta-e-espera e a preferência por suprimento adequado de luz são comportamentos conservativos para *Tropidurus*. A areia permite atingir rapidamente a condição térmica ótima. Os lagartos apresentaram atividades prolongadas, com algumas diferenças sazonais, mas com padrão mais uniforme em comparação com congêneres de outros biomas, demonstrando influência de características locais. Observamos dimorfismo sexual,

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com machos maiores e mais pesados. As distinções no crescimento foram perceptíveis entre machos, fêmeas e juvenis (influência histórica), mas não para o ganho de massa. A razão sexual foi deslocada em favor dos machos. Isso não é comum no gênero, mas pode ser vantajoso para as fêmeas na escolha de melhores parceiros. Ocorreram flutuações no crescimento populacional, provavelmente relacionadas à sazonalidade climática e mortalidade nas fases juvenis. O recrutamento de *Tropidurus hygomi* começa no período chuvoso e adentra o início da estação seca, diferente de outros congêneres, sugerindo ação de efeitos ecológicos. Esperamos que nossos resultados auxiliem no desenvolvimento de estratégias de conservação para as populações de *T. hygomi* que vivem no litoral (distribuição contínua), além de nortear a execução de novos estudos.

Key words: Iguania; Lagartos; Distribuição Relictual; Sergipe; Parque Nacional Serra de Itabaiana.

Introduction

Lizards have been widely used as model organisms in ecological research because they are easily observed in the environment and taxonomically well understood (Rocha, 1994; Sites Jr. *et al.*, 2011). Besides being ideal for the execution of these studies, it is important to understand that the comprehension of the factors that operate on the lizard's biology can be extended to other organisms in the constant search for ecological patterns (Vitt and Pianka, 1994; Domingos *et al.*, 2017).

Among some of the most explored ecological models, the genus *Tropidurus* stands out for its representativeness in open formations in South America (Rodrigues, 1987; Carvalho *et al.*, 2013). As with other lineages of lizards, there is an agreement that phylogeny (or historical factors) and recent interactions (or ecological factors) act together in determining characteristics of the tropidurid life history (Vitt, 1993; Kohlsdorf *et al.*, 2001; Gonçalves-Sousa *et al.*, 2019). Thus, characters related to activity and preference for certain times of day or even breeding period, for example, usually vary in different biomes and seasonal regimes (Van Sluys, 1992; Caldas *et al.*, 2015; Albuquerque *et al.*, 2018). On the other hand, thermoregulation behavior, sit-and-wait foraging, preferential dietary items and heliophilia suggest the action of a niche phylogenetic conservatism (Ribeiro and Freire, 2010; Gomes *et al.*, 2015; Pelegrin *et al.*, 2017).

Tropidurus hygomi is part of the *torquatus* group, to which species such as *Tropidurus hispidus*, *Tropidurus itambere*, *Tropidurus oreadicus* and *Tropidurus torquatus* are also allocated, and are relatively common in certain areas of Brazil (Frost *et al.*, 2001). Some aspects of the biology of *T. hygomi* have been studied in recent years; as far as habitat is concerned, we know that it prefers areas close to palm groves and bromeliads (Martins *et al.*, 2010). In addition, the species is often observed in sandy microhabitats where litter and shrubs predominate, besides to having an extended activity throughout the day (Vargens *et al.*, 2008). As for thermal preferences, body temperature regulation occurs from the heat provided by sandy substrates or by air (Vargens *et al.*, 2008; Martins *et al.*, 2018). Other studies indicate that males tend to be larger and/or more robust than females (Vanzolini and Gomes, 1979; Vasconcelos *et al.*, 2019).

Although there are important published data on *T. hygomi*, all those previously mentioned were obtained from populations of coastal areas, which are usually abundant but have a restricted distribution, occurring in a range of approximately 400 km from the north of Bahia to the north of Sergipe (Vanzolini and Gomes, 1979; Martins *et al.*, 2010; ICMBIO, 2018). The Serra de Itabaina National Park (PNSI) corresponds to an area of geographical disjunction, about 40 km from the coastal area (Carvalho *et al.*, 2005; Rodrigues, 2005). In this location, this lizard is found associated with the so-called "white sands" (Vicente *et al.*, 1997; Carvalho *et al.*, 2005). The species may have successfully established itself in this region because it is part of an ecological group whose occurrence is currently limited to sandy soils (*Tropidurus cocorobensis, Tropidurus psammonastes* and *T. hygomi*), presenting relictual characteristics of distribution (Rodrigues *et al.*, 1988; Carvalho *et al.*, 2013). Lima-Silva *et al.* (2019) conducted the only study on *T. hygomi* at PNSI, reporting that adults and juveniles do not differ in the use of microhabitats and that recruitment is continuous, with juveniles found throughout every month of the year. However, there is still a lack of information on the local population related to other factors of its dynamics, such as sex ratio over the months of the year, an understanding of population growth, how seasonality influences the daily activity, the use of microhabitats for thermoregulation and escape, or data related to intraspecific variations.

We hope that the present study will fill in the gaps pointed out for *T. hygomi* in the PNSI. The species is listed as vulnerable throughout its occurrence in the Brazilian territory, mainly because the coastal strip where it is normally found suffers strong anthropic pressure (Martins *et al.*, 2010; MMA, 2014). Besides providing information that can directly assist in the management of the PNSI, one of the priority areas for conservation in the state of Sergipe (MMA, 2002), we can also generate parameters that can guide measures to be implemented in the preservation of coastal populations. We will also understand even better which characteristics in the life history of *Tropidurus* are conservative and which are subject to local variations by comparing our data with other studies. Thus, we seek to answer the following questions: 1 – How does *T. hygomi* use microhabitats from its area of occurrence in the PNSI? 2 – Is there a difference in the behavior of this lizard between the dry and rainy seasons? 3 – What is the growth and mass gain between females and males throughout the year and what is the sex ratio of the species? 4 – What is the variations observed in the population dynamics of *T. hygomi* and at what time of the year does recruitment occur?

Materials and methods

Study area

The Serra de Itabaiana National Park (PNSI; 10°40' S, 37°25' W; Fig. 1) is of 8,024.79 ha and is located between the municipalities of Itabaiana, Areia Branca, Itaporanga D'Ajuda, Campo do Brito, Malhador and Riachuelo and including the Comprida, Cajueiro and Itabaiana mountains, the last being the largest of all at an altitude of 650 m, and where the data collection area is located (Carvalho and Vilar, 2005). The region has a remarkable anthropic influence and relief covered by hills that characterize the Atlantic Forest's morphoclimatic domain (Ab'Saber, 1967).

According Vicente *et al.* (1997), the habitat of interest for this work is composed of irregularly distributed open formations with white sandy soil, which may or may not be covered by vegetation. In regions with vegetation, there may be greater abundance of creeping or shrubby plants, whether clustered or sparse. The vegetation cover consists predominantly of grasses: cyperaceae, cactaceae, velloziacea, and lichen (Vicente *et al.*, 1997; 2005). Although some characteristic species of Cerrado and Restinga occur in the area, the most correct way to classify this phytophysiognomy is associated with the rupestrian fields (Dantas and Ribeiro, 2010).

Data Collection

We collected data between January and December 2008 from 06:00 to 18:00 h. The time spent in the field was two consecutive days every two weeks, totaling a sampling effort of 52 d or approximately 624 h. The work area was the white sand spot of approximately 12,793 m^2 (10°44'56.63" S, 37°20'26.42" W) near the base of the Institute Chico Mendes de Conservação da Biodiversidade (ICMBIO). Due to methodological adjustments and adherence to the collection license, the animals only started to have mass and snout-vent length (SVL) registered in March. Therefore, there was a period of 1 y (January to December) sampling of the spatial and temporal niche (visual sampling, not dependent on sex determination) and a period of 10 months (March to December) available for aspects that involved the biometrics of the lizards. This explains any differences in the sample sizes used in the analyses.

The main methodology used was the "active search" for individuals (Crump and Scott, 1994), where the whole area was inspected carefully following the trails already in place. For catches, nylon loops attached to telescopic fishing rods (2.5 m) were adopted (Santana *et al.*, 2014; Caldas *et al.*, 2015). In addition, 20 pitfall traps were also used (Cechin and Martins, 2000), made of 5-L plastic buckets buried at

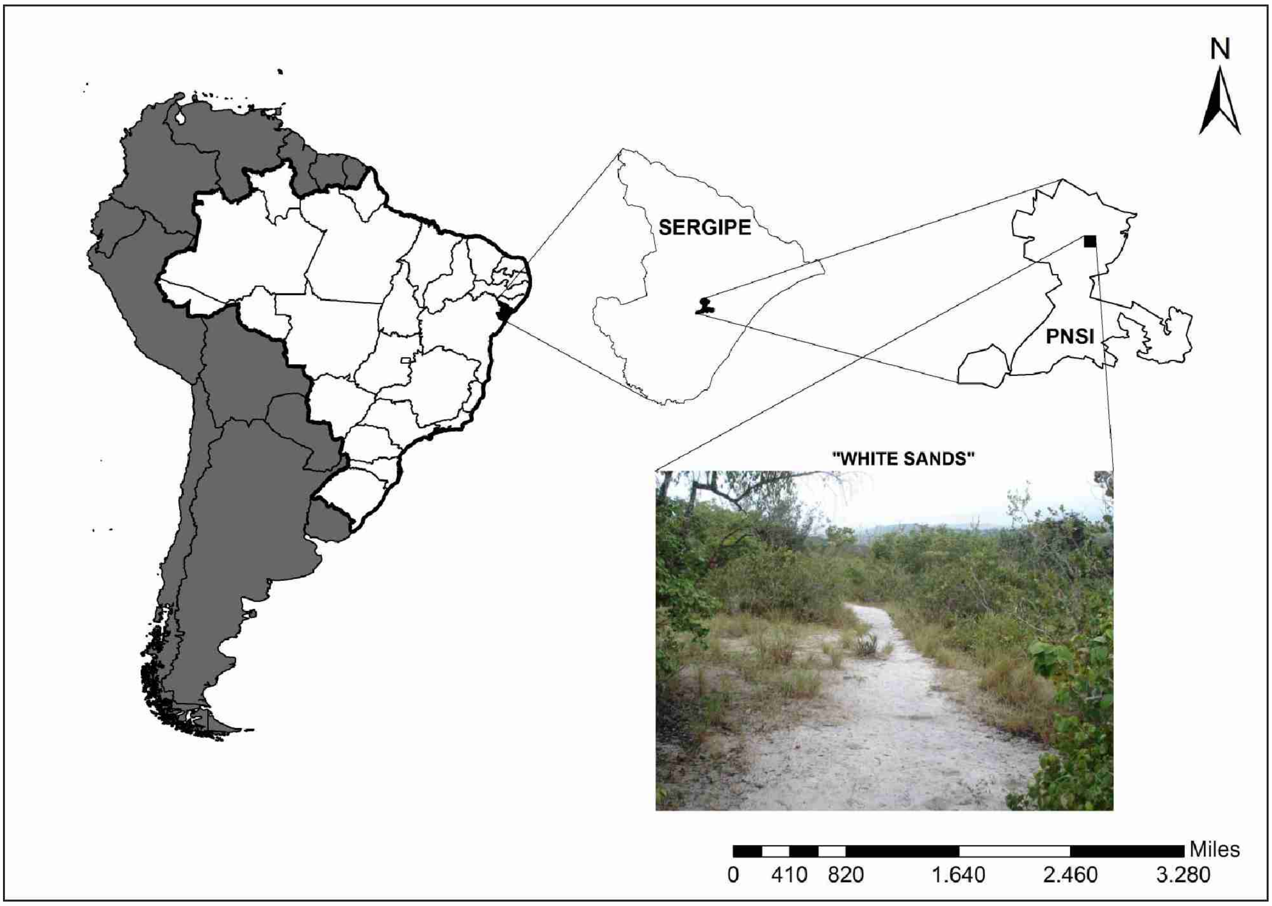

Figure 1. Map of South America showing Brazil (thick line) and Sergipe (black inset). Right side shows Serra de Itabaina National Park – PNSI and the "white sands" area.

ground level. The buckets were distributed in lines parallel to the main trail in the area (10 m away) and positioned 5 m apart. They were inspected regularly at the end of each one-hour interval throughout the day and the animals captured during this time period were released. This allowed us to use these data in the analysis of the species activity period.

For each observed lizard, regardless of capture or recapture, we recorded the following information: microhabitat and activity (1 – during the first sighting; and 2 – after the observer approach), date, time of day, weather conditions, exposure of the lizard to sunlight and perch height (m). Animals captured in pitfall traps did not contribute to these data categories. This also explains any differences in the sample sizes used in the analyses. During each observation we also used a thermometer (Minipa®) to record the temperatures of the substrate, the air, and the air 1 cm from the soil.

All animals captured or recaptured, regardless of the method adopted, had their mass measured using Pesola® type scales (1.0 g and 0.5 g accuracy) and the SVL measured using a ruler (1 mm). The SVL was chosen as a measurement parameter to investigate differences in morphometry because the collection permit only provided for catches and the large number of animals and the need to register them generated considerable time demands.

The manipulated lizards received two markings: 1 – Permanent, made by "toe clipping" (systematic cutting of the toes); and 2 – Temporary, made with non-toxic paint (see Santana *et al.*, 2014; Caldas *et al.*, 2015), so that the animals could be identified at a distance and not be recaptured unnecessarily. Even so, these visualizations could be included in the records referring to the spatial and temporal niche. We distinguished adults (males and females) from juveniles by the presence or absence of secondary sexual characteristics, which correspond to patches in the thigh and flap region exclusive to reproductive males and already known for the genus *Tropidurus* (Pinto *et al.*, 2005; Bruinjé *et al.*, 2018). At the same time, we defined a minimum SVL range (4.8 cm) with the presence of such characteristics, based on the captures and/or recaptures that were processed during the months. Individuals who reached this range but did not have the spots were considered females. Organisms below this morphometric limit were treated as juveniles (non-reproductive). The lizards that were captured and identified as female within the established threshold for sexual distinction did not show any spots when recaptured in subsequent months, which supported our method. All captured animals were released in the same spot as the first sighting. Specimens obtained from the traps were also marked, measured, weighed and released near the capture site. All procedures previously described (including toe clipping) were authorized by collection license **#10504-1**, issued by SISBIO (Sistema de Autorização e Informação em Biodiversidade).

To classify the rainy and dry seasons, we considered the monthly rainfall averaged over a 10-y period for the PNSI. May, June and July were considered months comprising the rainy season, as they have the highest monthly rainfall (White and Ribeiro, 2011); the others were considered as belonging to the dry season.

Data Analysis

First, before performing the statistical procedures we checked the normality of the data by the Shapiro–Wilk test to determine the type of analysis to be adopted subsequently. We calculated the niche breadth (B) spatial (substrates) and temporal (activity hours) using Simpson's diversity index (Simpson, 1949)

$$B = \frac{1}{\sum_{i=1}^{n} p_i^2}$$

where p is the ratio of substrate category or activity time class used, i and n correspond to the number of microhabitat categories (10 in total) or time categories (12 in total). The value of B ranges from *1* (exclusive use of one of the substrate categories or schedules) to n (homogeneous use of all categories). We took account of the abundance of individuals every hour of the day (total of 12 h) in the dry and rainy seasons. This allowed us to investigate possible seasonal variations in activity periods using the T-test. The intention was to know whether differences in the abundance of individuals observed per hour (dependent variable) were influenced by the different seasons, dry and rainy (independent variable).

We investigated whether there were possible differences in SVL and mass of adult individuals (sexual dimorphism) by analysis of variance (ANOVA) and analysis of covariance (ANCOVA), respectively. In the second case we adopted SVL as covariate in order to reduce the influence of size on the results as well as the chances of misinterpretation. The SVL and mass data were first log-transformed (base 10) in order to meet the normality assumptions and reduce possible scale effects that could interfere with the analyses. In this case, we used data referring to the first capture, avoiding including the same individual more than once in the analysis.

We obtained growth and mass gain rates from differences in SVL or lizard masses captured in successive months divided by the number of days elapsed. To reduce deviations due to measurement errors and seasonality effects, we used only data taken at intervals of 25 to 40 days between capture and recapture (Van Sluys, 1998). The values of growth rates and mass gain were compared between males, females and juveniles by Kruskal–Wallis test and, in the case of growth, a Dunn Test was performed subsequently. Each individual contributed only once to this analysis, regardless of being recaptured several times.

The population dynamics of *T. hygomi* was calculated through capturing and recapturing individuals. For this we used the proportions of adult (male and female) and juvenile specimens observed each month. The intrinsic population growth rate (r) was calculated using the formula:

$$r = Ln\mathrm{N}_2 - Ln\mathrm{N}_1$$

where N_2 represents the population density in month "*i*" and N_1 represents the population density in the month prior to "*i*". We estimated the intrinsic mean growth rate ($\bar{r}$) by the arithmetic mean of the intrinsic population growth rates obtained for each month of the study (Caughley, 1980). As this analysis depended on the data obtained in the previous month, they are presented as from April. To analyze the age structure of the population, we divided the lizards into 10 size classes with 0.49-cm intervals between each (2.30–7.0 cm). The monthly proportions of each size class were then used for the estimate.

To investigate possible variations in sex ratios during the study period, we estimated monthly values of these rates for adult individuals in this population. The mechanism of sexual determination for *T. hygomi* is of type XX:XY (Kasahara *et al.*, 1996), the chances of birth of males and females being equal, in other words, 1:1. Based on this, we used the Chi-square test to analyze whether there were differences between estimated sex ratios and how they behaved relative to that expected (1:1).

All data were stored in Excel spreadsheets and statistical analyses performed in the R program for Windows (R Development Core Team, 2018). The significance level adopted for the tests was 5%.

Results

Microhabitat and activity period

We obtained 1,915 records of characteristics related to the *T. hygomi* space niche, taking into account views (1,460) and animals captured (290) and recaptured (165) by fishing rod (i.e., excluding pitfall traps). Most of the time *T. hygomi* was found motionless (78.33%), at the borders of shrub formations (38.96%) and exposed white sand soil areas (31.49%). As the observer approached, the specimens usually ran (76.29%), taking refuge preferably inside the bushes (41.04%) or at the base of bromeliads (25.90%) (Fig. 2). The spatial niche widths for both types of microhabitat were similar ($B_1 = 3.58$ and $B_2 = 3.51$). *Tropidurus hygomi* also used the vertical space, positioning themselves at heights not exceeding 39 cm (94.65%; Fig. 2). The animals were observed mainly on sunny days (80.85%), in places that formed sun and shade mosaics (63.10%; Fig. 2).

We obtained 2,012 records of characteristics related to the temporal niche of *T. hygomi*, taking into account views (1,460) and animals captured (290) and recaptured (165) by fishing rod and captured (66) and recaptured (31) by pitfall traps. Variations were observed in relation to the activity periods recorded during the dry season and the rainy season ($t = -11.364$, df = 11; $P < 0.0001$; $n_{dry} = 1,398$; $n_{wet} = 614$). Although *T. hygomi* maintained activity from 06:00 to 18:00 h throughout the year, the distribution was more uniform during the dry season compared to the rainy season ($B_{dry} = 11.53$; $B_{wet} = 10.45$; Fig. 3). In general, the ambient temperatures (substrate, air and air at 1 cm) were lower during the rainy season. It was at this station that we recorded a smaller number of individuals in the early and late hours of the day, especially in the interval between 17:00 and 18:00 h, where there were almost no lizards in activity (Fig. 3). The time between 09:00 and 11:59 h was also used by a larger number of animals in the rainy season, contrary to that observed in the dry season (Fig. 3).

Biometrics, Growth and Mass Gain

We observed signs of SVL-related sexual dimorphism in adults of *T. hygomi*, considering individuals from captures only ($F = 269.3$; df = 1; $P < 0.0001$), with larger males [mean SVL = 6.0 ± (standard deviation, SD) 0.4 cm, n = 83; range, 4.8–7.0 cm] when compared to females [mean SVL = 5.2 ± 0.2 cm, n = 51; range, 4.8–5.5 cm]; the mean for juveniles was calculated regardless of future sexual condition (mean SVL = 3.8 ± 0.7 cm, n = 198; range, 2.3–4.7 cm). The smallest male with the secondary sexual characteristics in early formation had an SVL of 4.8 cm and the largest (with fully established marks) of 7.0 cm.

The covariate (SVL) had no influence on the mass of the individuals ($F = 0.4330$; df = 1; $P = 0.51$); however, it was observed that the "sex" factor directly affected lizard mass ($F = 106.145$; df =1; $P < 0.0001$). Males had higher masses [mean mass = 9.6 ± 1.8 g, n = 83; range, 5.0–13.0 g] than females [mean mass = 5.1 ± 0.7 g, *n* = 51; range, 4.0–6.5 g]. The mean for juveniles (regardless of sex) was lower than that of adults [mean mass = 2.7 ± 0.9 g, *n* = 198; range, 0.5–4.0 g].

We observed differences in growth rates of males, females and juveniles of *T. hygomi* ($H = 7.5660$; df = 2; $P = 0.02$) the largest being verified among males and juveniles ($P < 0.05$). Males had a mean growth rate of 0.002 cm/d, females 0.004 cm/d and juveniles 0.007 cm/d. However, we did not observe distinctions in mass gain ($H = 0.6268$; df = 2; $P = 0.73$). Males had a mass gain rate of 0.009 g/d, females 0.010 g/d and juveniles 0.014 g/d.

Sex ratio and population dynamics

To evaluate these parameters, we considered the total number of catches and recaptures (fishing rods and pitfall traps) that we performed between March and December 2008. Thus, 332 lizards were captured and tagged: 83 males (25%), 51 females (15%) and 198 juveniles (60%). Of these, 189 individuals (57%) were recaptured at least once: 74 males (39%), 42

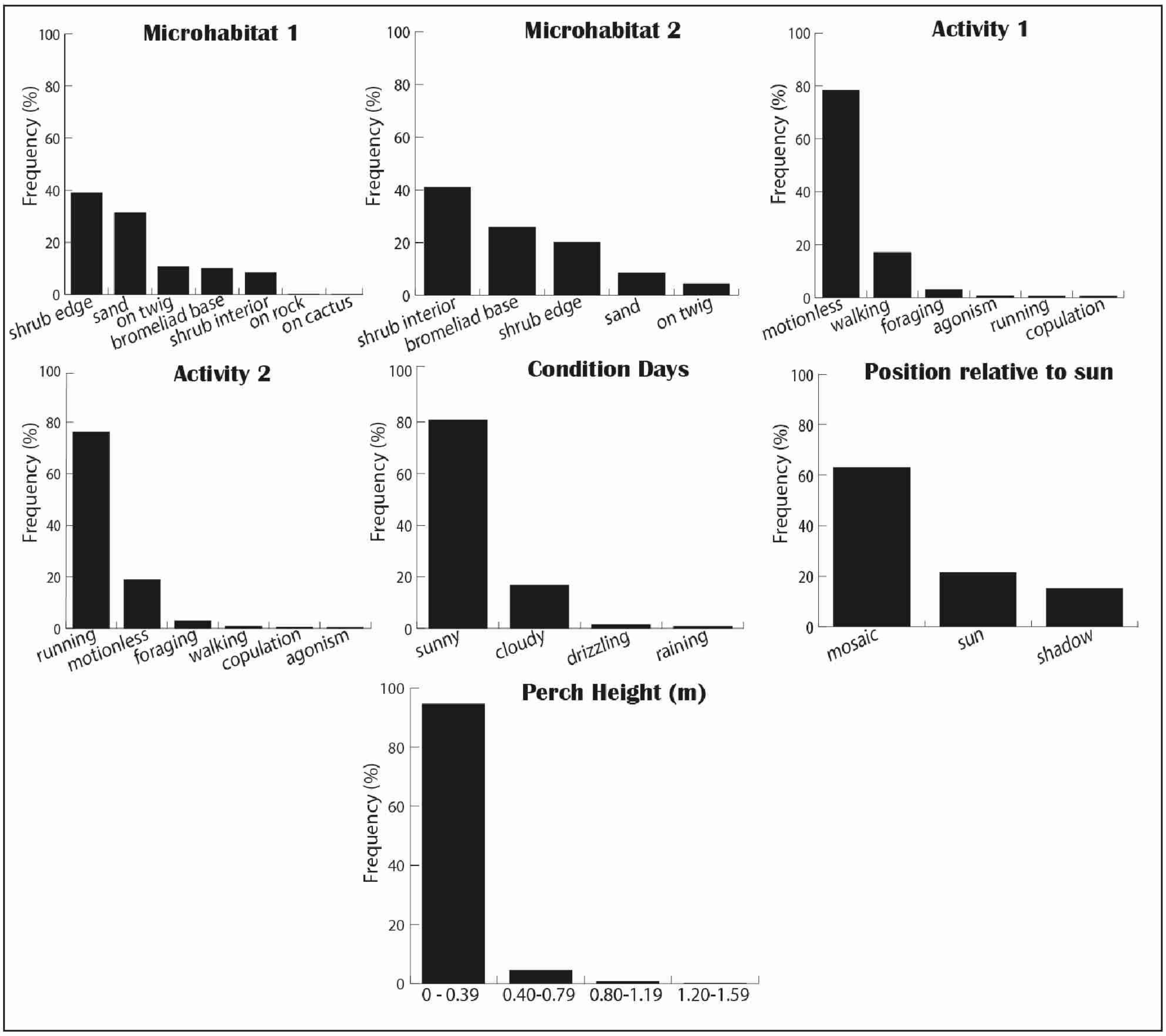

Figure 2. Relative frequencies of observations of *T. hygomi* related to space niche and thermoregulation activity.

females (22%) and 73 juveniles (39%). A summary of the sex ratios for adult individuals between March and December (total) and for each month (monthly) is shown in Table 1. Most monthly results differed significantly from the 1:1 ratio. In general, there was a shift in favor of males (6 mo), which exceeded the number of months that there was a difference in favor of females (3 mo). The highest rates for males occurred in April (3:1), besides August, where we registered no females (Table 1). This male predominance is also perceived in the total sex ratio, considering the abundance of captures and recaptures of males and females accumulated over the months (2:1; Table 1).

We observed variations in intrinsic population growth rates between the months sampled (Fig. 4). Positive values correspond to the months in which the population increased and negative values indicate population declines. During April, June, September, October and December, positive values for population growth rates were recorded (Fig. 4). In May, July, August and November, the values were negative (Fig. 4). The intrinsic mean population growth rate was 0.0039.

In the first three months the population size remained more or less constant (Fig. 4). Soon after, there was a large decline in population with the lowest growth value observed in August. From June to September the lowest values of SVL were found in the population: in the first, the population growth rate showed a slight increase in relation to the previous months, and in July following there

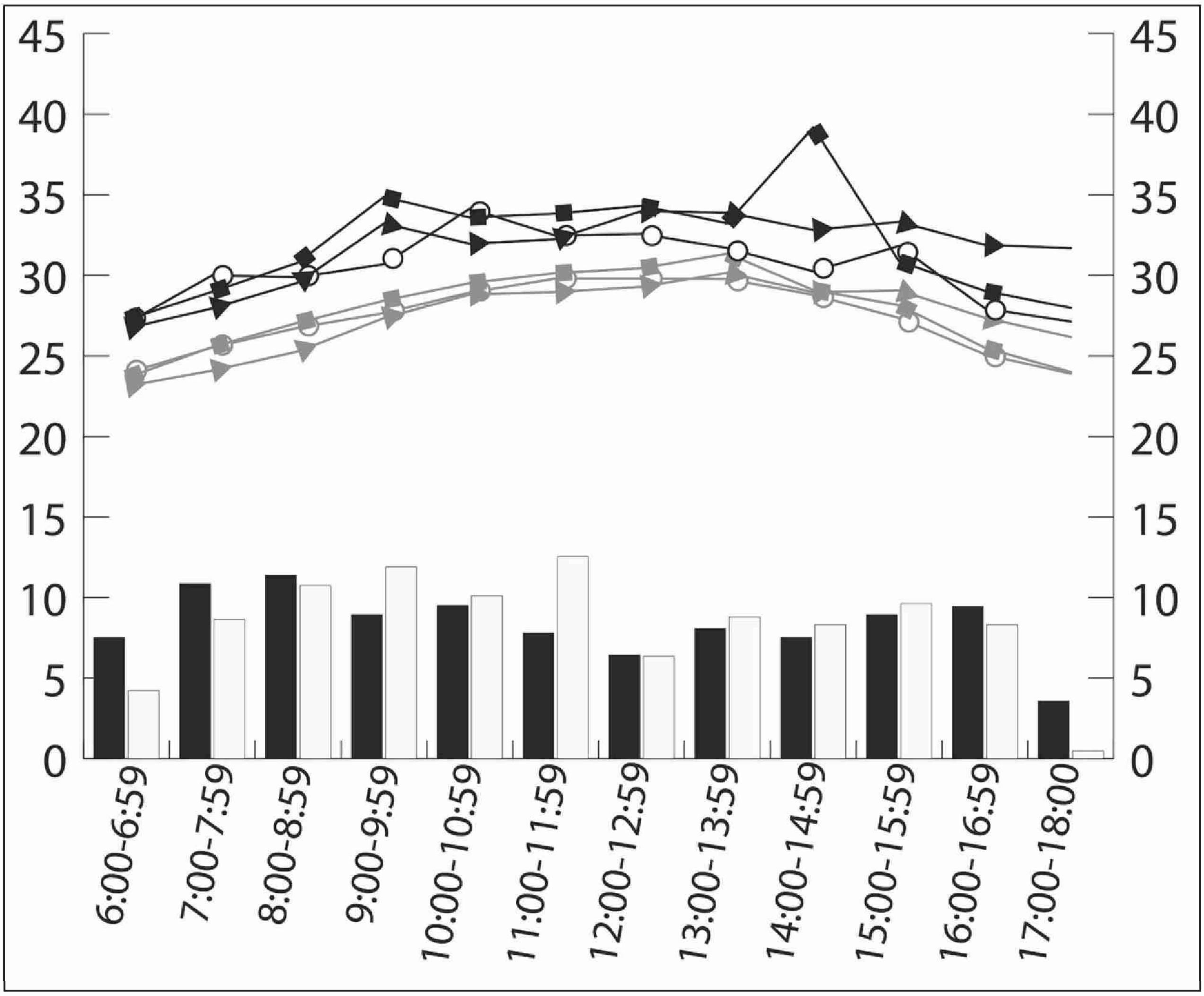

Figure 3. Relative frequency of activity of T. hygomi in the dry and rainy seasons. Black bars indicate the relative frequency of lizards in the dry season and grey in the rainy. The black and grey symbols in the lines correspond to the same seasons and mean: ► = substrate temperature, ▪ = air temperature at 1 cm, o = air temperature.

was a remarkable decline in the same (Fig. 4). In September, there was a large population increase, this being the month with the highest intrinsic value obtained (Fig. 4).

We found an increase in number of juveniles in the population from July to December (July = 71%, August = 71%, September = 71%, October = 81%, November = 75%, December = 59%). In the other months, the number of adults (males and females) surpassed that of juveniles (March = 78%, April = 86%, May = 73%, June = 61%). We noticed variations in the age structure population of *T. hygomi* during the study months, with predominance of individuals with SVL over 4.79 cm (males and females) from March to May (Fig. 5). Among males, larger individuals (above 6.29 cm) were abundant in the months of March, September, November and December and absent in July and August (Fig. 5). Females basically maintained two size categories, but larger females (5.30–5.79 cm) occur in less quantity throughout the year. The first three months of sampling were characterized by a population of juveniles close to maturity and adults, possibly from the previous breeding season in 2007. This year we did not monitor the population, but our prediction can be corroborated by analyzing what occurred from November and December 2008, where the intermediate classes (3.80–4.79 cm) grow progressively. From June to Setember the lowest SVL values were found for juveniles (Fig. 5). These values should be the closest to those observed at hatching and show us that recruitment is adjusted to these months.

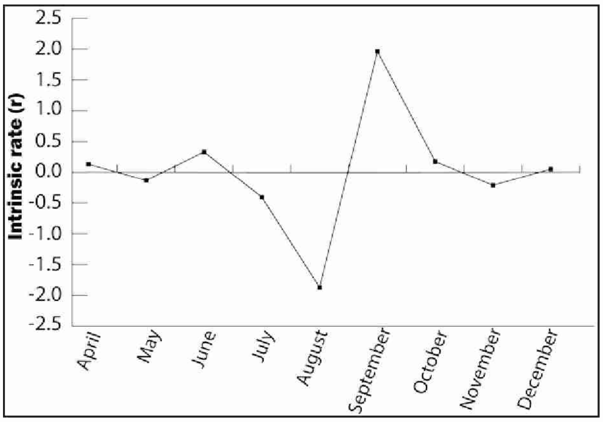

Figure 4. Intrinsic rate of *T. hygomi* population growth during the months sampled.

In July, smaller juveniles (2.30–3.79 cm) exceeded larger juveniles (3.80–4.79 cm) and in August they become the majority in their age range (Fig. 5). This result may indicate the establishment of juveniles hatched in the previous months in the population of *T. hygomi*, as they persisted and became larger.

Discussion

Microhabitat and activity period

Individuals of *T. hygomi* were usually sighted occupying the edges of shrubs and the exposed soil of the sandy formation they inhabit, usually on substrates/perches not exceeding 39 cm in height. This is a typically psamophilous species, as has already been verified in other localities that integrate its occurrence area in northeast Brazil (Vanzolini and Gomes, 1979; Vargens *et al.*, 2008; Martins *et al.*, 2010). The choice of such microhabitats may be related to the shorter time spent in thermoregulation (Teixeira-Filho *et al.*, 1996; Maia-Carneiro and Rocha, 2020). In sandy environments, where there is a high incidence of sunlight, both the soil and the first few centimeters of the air layer heat up quickly, allowing a shorter exposure time of the lizards and rapid warming of their bodies. Behavioral similarities of this type have already been described for psamophilous species such as *T. psammonastes* (Arraial Paulista – BA; Rodrigues *et al.*, 1988) and *T. hygomi* (Jandaíra – BA and Pirambu – SE; Martins *et al.*, 2018), as well as *T. torquatus*, which may occasionally occupy sandy soils (Barra de Maricá – RJ; Teixeira-Filho *et al.*, 1996). On the other hand, the preference for the base of the shrub over the exposed soil may be related to the supply of shade, which has already been registered for *T. hygomi* in the PNSI (Lima-Silva *et al.*, 2019). As sandy substrates heat up quickly, areas of greater vegetation cover can offer less drastic temperatures at warmer times of day, allowing individuals to extend their area and activity time (Grover, 1996).

The shading provided by vegetation also helps us understand the sun exposure behavior shown by the species. There was a predominance in places with sun and shade mosaic, a choice that can help lizards avoid overheating of their bodies in contact with the sand, thus reducing the possibility of physiological maladjustment and death (Vitt and Carvalho, 1995; Teixeira-Filho *et al.*, 1996). The intrinsic thermoregulation needs of *T. hygomi* may also be related to what has been observed: *T. hispidus*, for example, has a lower mean body temperature compared to other species of the genus (Vitt, 1995; Ribeiro and Freire, 2010) and belongs to the same group (*torquatus*) as *T. hygomi*. If the latter shows the same peculiarity, it would not have problems in thermoregulation environments with higher incidence of shadow because the optimal thermal conditions would be quickly achieved. Still, *T. hygomi* showed a clear preference for sunny days, a pattern already recorded in the northeast Restingas (Vargens *et al.*, 2008; Martins *et al.*, 2010; 2018) and which is common for heliophilic organisms of open areas and typical of the genus *Tropidurus* (Vitt, 1993; Maia-Carneiro and Rocha, 2020), indicating that there is a phylogenetic conservatism determining this ecological trait (Wiens and Graham, 2005; Losos, 2008).

The lizards were motionless most of the time and fled quickly when the observer approached. This is widespread behavior in sit-and-wait foragers and is well understood within the genus *Tropidurus* (Santana *et al.*, 2014; Gomes *et al.*, 2015; Pelegrin *et al.*, 2017). This more sedentary pattern responds to a clear phylogenetic influence and is conservative within the Iguania clade, which encompasses Tropiduridae, with little or no influence from local environmental characteristics, and may determine other aspects of the biology of these animals (e.g., mobile prey consumption, coloration cryptic, etc.) (Perry, 1999; 2007). The escape of *T. hygomi* into shrubbery and bromeliad base may occur because they act as obstacles and make access difficult for predators. Martin and López (1995) showed that the *Psammodromus algirus* lizard tolerated the observer's approach less and developed larger escapes in the spring, when the bushes had no leaves for

Table 1. Summary of sex ratios of *T. hygomi* from Serra de Itabaiana National Park - SE and Chi-square test.

Month	Males	Females	Sex ratios (approximately)	X^2	*P*	*n*
March	0.67	0.33	2:1	10.89	0.0010	18
April	0.78	0.22	3:1	30.25	< 0.0001	55
May	0.67	0.33	2:1	10.89	0.0010	42
June	0.67	0.33	2:1	10.89	0.0010	49
July	0.67	0.33	2:1	10.89	0.0010	15
August	1.00	0.00	-	98.01	< 0.0001	2
September	0.65	0.35	2:1	8.41	0.0037	17
October	0.31	0.69	1:2	13.69	0.0002	13
November	0.29	0.71	1:2	16.81	< 0.0001	14
December	0.40	0.60	1:2	3.61	0.0574	25
Total	0.63	0.37	2:1	6.25	0.0124	250

it to hide. Thus, there is evidence that vegetation may be important for the success of *T. hygomi* in finding refuges to ensure its survival.

When analyzing the temporal niche of *T. hygomi*, we noticed that there was extended activity in the dry and rainy seasons. Long active periods have been described for other species of the genus *Tropidurus* (Hatano *et al.*, 2001; Van Sluys *et al.*, 2004; Gomes *et al.*, 2015). However, we observed seasonal variation in the daily activity of *T. hygomi* in the PNSI. The main differences are noticeable in the behavior of the species during the first and last hours of the day, which are most explored in the dry season. Moreover, in the rainy season activities intensify even closer to noon when compared to the dry season. Regarding the other times, there is a certain uniformity throughout the year. The seasonality we recorded do not follow the dichotomy found in areas of Brazil that have most rigorous variations. In the southeast, for example, *T. itambere* and *T. torquatus* tend to have well-defined activity periods, unimodal in the cold (dry) season and bimodal in the warmer (rainy) season (Van Sluys, 1992; Teixeira-Filho *et al.*, 1996). In the PNSI, *T. semitaeniatus* presents similarity to *T. hygomi* when the conditions of the warmer (drier) and colder (rainy) seasons are compared (Caldas *et al.*, 2015). This higher uniformity observed in the PNSI matches the regularity of the climate in the northeast region, which has high temperatures throughout the year, coupled with the low thermal amplitude (Ferreira and Mello, 2005). Even though lizards of the genus *Tropidurus* have extended activity throughout the day, it is noticeable that this factor is subject to environmental variations in each region. Individuals of *T. hygomi* followed this rule allowing us to conclude that their activity period is clearly influenced by ecological factors (Vitt and Zani, 1998).

Biometrics, growth and mass gain

We found that sexual dimorphism exists in *T. hygomi* when we analyzed the SVL values and mass obtained for males and females. Males had a mean SVL of 6.0 cm and 9.6 g, while females had 5.2 cm SVL and 5.1 g. Differences in measurements related to body size, mass and sometimes shape or factors such as coloration are common to the genus *Tropidurus*, occurring in the most varied phylogenetic groups (Vanzolini and Gomes, 1979; Perez-Mellado and de la Riva, 1993; Ortiz *et al.*, 2014; Bruinjé *et al.*, 2018). Territory defense, sexual selection, lower energy costs in gamete production and differential use of prey may justify the larger size of adult males. In contrast, females have a high cost in gamete production and do not defend territories (Faria and Araujo, 2004; Pinto *et al.*, 2005; Meira *et al.*, 2007). Regarding the mass, possible morphological restrictions imposed by the size of the mouth may contribute to the different

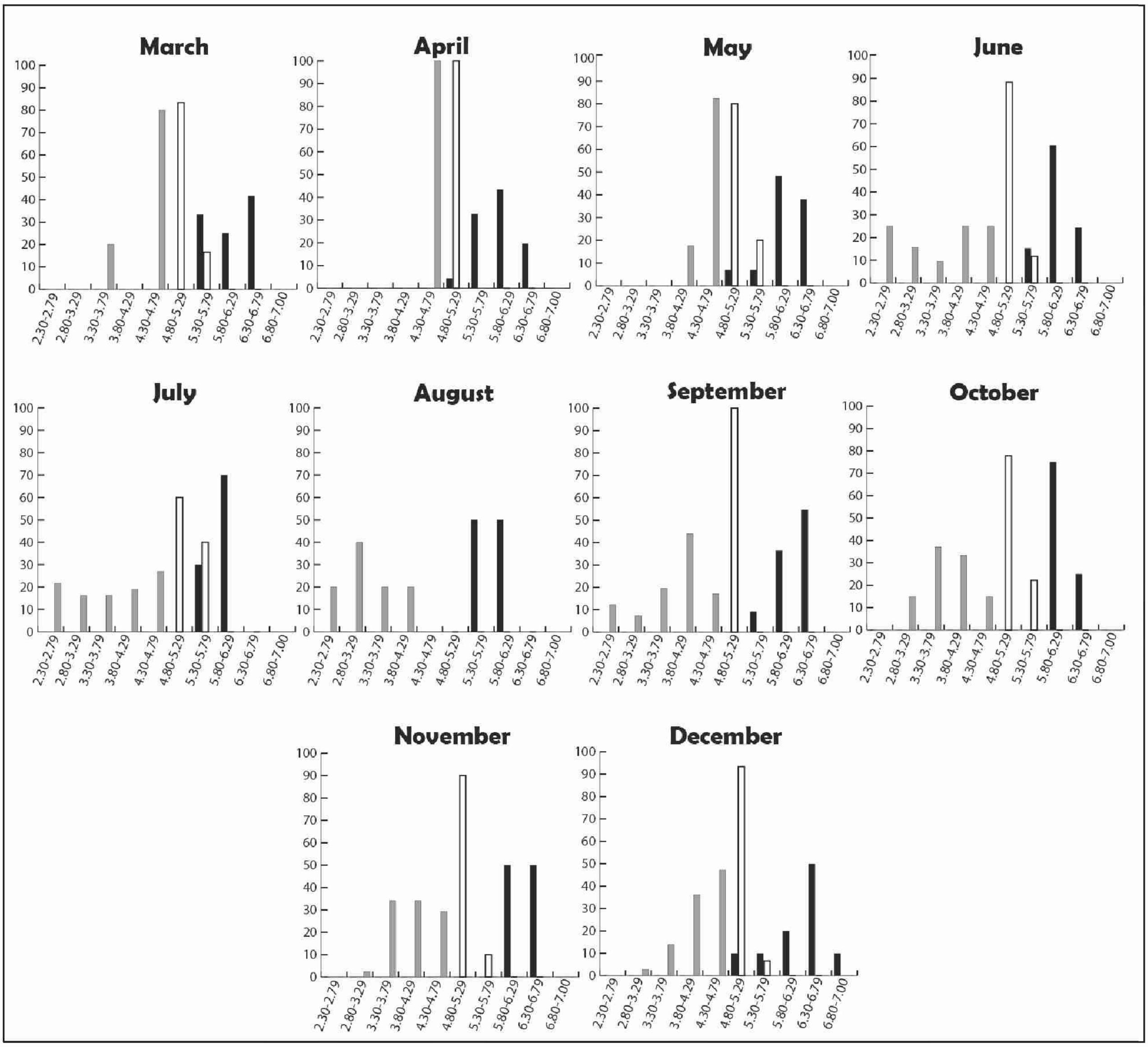

Figure 5. Monthly age distribution (March to December) of *T. hygomi* population. X-axis: SVL measured in cm; Y-axis: relative frequency. Males = black bars, Females = unfilled bars, Juveniles = grey bars.

use of food resources, justifying sexual variations. In small lizards, the dimensions of the mouthpiece limit the size of food items consumed or the forms (long or larger prey) that are more or less easily eaten (Perez-Mellado and de la Riva, 1993; Gomes *et al.*, 2015). Thus, males of larger sizes would have the possibility of ingesting larger and more energetic prey or even different from those consumed by females or juveniles (Van Sluys, 1993a).

Juvenile and female growth rates outnumbered that of males over the months with the first group having a remarkably higher mean value than the others. This rapid increase in size may be crucial for the survival of juvenile lizards, which naturally correspond to the life cycle phase most impacted by predation, likely to occur from a variety of vertebrate or invertebrate organisms (Bauer, 1990; Siqueira and Rocha, 2008). As for adults, differences in growth between females and males have been recorded in *T. itambere* (Van Sluys, 1998) and *T. oreadicus* (Meira *et al.*, 2007) with the first group showing higher rates at the beginning sexual maturity, whether corroborated by statistical methods or by indirect inferences based on observations. Our results could be supported by explanations from these authors: females would initially grow faster in order to reach the reproductive phase; upon reaching it, they would slow their growth and reallocate energy to reproduction. In turn, males throughout life would continue to grow, getting larger than females (Van Sluys, 1998; Meira *et al.*, 2007). We believe that our 10-mo monitoring may have covered this period when females invest more in growth.

We did not observe differences regarding the mass gain over the months between the analyzed categories. Although *Tropidurus* females and males usually allocate energy for different purposes as discussed earlier, mass gain may be more subject to fluctuations in resource/food availability. Thus, the expected changes could be obtained if specific and/or more favorable periods of the year were analyzed, which we cannot delimit in the present work. Merker and Nagy (1984) have shown that the lizard *Sceloporus virgatus*, another sit-and-wait forager of the Iguania clade, exhibits variations in mass gain according to the different typical seasons of the western USA: while males lose body mass in spring females produce eggs and keep it. Already by the summer, both sexes show the same increase in mass. These authors observed the animals feeding and measured their metabolic rates, something that we could not do in the present work and which may have influenced the results obtained. Our inferences about growth (SVL), on the other hand, may be free of this condition, even if there are opinions that mass is a direct function of body length (Ricker, 1973; Pauly, 1981). As we found no significant influence of SVL on mass we believe that each factor is distinctly influenced throughout the study period. Even so, we realize that in general the size and mass in Tropiduridae tend to be strongly influenced by historical factors, and some morphological characteristics seem to be well conserved with small variations of each region (Ortiz *et al.*, 2014). The population of *T. hygomi* suited this condition well.

Sex ratio and population dynamics

The sex ratio of *T. hygomi* adults differed from 1:1 in almost every month sampled and in the total value obtained, showing a shift in favor of males in this species. Our results differ from the observed condition for *T. itambere* in southeastern Brazil, which showed a 1:1 sex ratio with deviations for females in some months of the year (Van Sluys, 2000) or that registered for *T. torquatus* in an area of Cerrado in Brasilia, where there was also displacement toward females (Wiederhecker *et al.*, 2003). The higher proportion of females would be in accordance with the typical polygynic behavior of *Tropidurus* species, but would contradict the prevailing system of sexual determination for these animals (XY:XX), as each individual is equally likely to be born with either sex (1:1; Smith, 1989). However, according to Wiederhecker *et al.* (2003), these deviations may result from the absence of some organisms in the sampled areas. The authors believe that in their study younger males were expelled to lower-quality territories by older males, reflecting the larger number of females observed. We believe that the results we have obtained needs further investigation, aiming to answer why the proportions of males are higher in this population of *T. hygomi*. There could be greater exposure of males throughout the day due to the typical territorial behavior of *Tropidurus* (Faria and Araujo, 2004; Bruinjé *et al.*, 2019) and this would have been reflected in more records of this sex during our observations. This displacement could also be favorable at times, allowing females to have a wider range of choices, choosing males with better attributes (e.g., greater body proportions) that could be inherited by their hatchlings.

We registered juveniles in the environment in every month of the year, but their increase in population occurred in a very specific period: in July, the number exceeded that of adults and remained higher until December. Juveniles of *T. hygomi* have already been observed in the PNSI for a full year, and the authors involved in the research suggested that the recruitment of the species would be continuous (Lima-Silva *et al.*, 2019). However, we have evidence to believe that this actually happens between June and Setember, covering the peak of the rainy season and the transition to the dry season (White and Ribeiro, 2011), because it was during this period that we recorded the lowest SVL values, probably the closest to hatching. The rainy season is the season with the greatest availability of food (arthropods) and seems to be the most suitable for different tropidurids to reproduce which occurs in distinct biomes of South America (Perez-Mellado and de la Riva, 1993; Cruz, 1997; Meira *et al.*, 2007). In addition to this detail, it is in the rainy season that the most favorable levels of humidity generally exist so that egg incubation and embryo development can take place without major difficulties (Brown and Shine, 2006). As climate factors are most associated with variations in lizard reproductive strategies (Mesquita and Colli, 2010), we do not rule out the possibility of the results found by Lima-Silva *et al.* (2019) and for us to reflect abiotic particularities of the different sampled periods, which allowed *T. hygomi* to present different adjustments throughout each year.

Although species of the genus *Tropidurus* converge in the recruitment adjustment that usually occurs during the rains, this trait is subject to the seasonal particularities of each biome or region. In the Central Brazilian Cerrado, recruitment of *T. torquatus* begins in the mid-rainy/summer season and extends to the first month of the dry season, covering the period from March to June (Wiederhecker *et al.*, 2002). In Caatinga in Paraíba State, Brazil, juveniles of *T. hispidus* with the lowest SVL values begin to appear in the second month (April) of the rainy/winter season, entering a few months of the dry/summer season (Albuquerque *et al.*, 2018). Finally, in the Brazilian Pampa biome the rains are regular throughout the year and recruitment of *T. catalanensis* (previously = *T. torquatus*) occurs during the period with temperatures more favorable to the development of newborns, from February to August (Arruda *et al.*, 2019). All of these species are from the same group as *T. hygomi*. Comparison between these results shows that lizard reproductive strategies are influenced by local environmental conditions (Santos *et al.*, 2015).

The variations we observed in the population density of *T. hygomi* throughout the year seem to reflect mainly the seasonal reproduction of the species and the increases and decreases of juveniles in the population (Van Sluys, 2000; Wiederhecker *et al.*, 2002; 2003). Reduced values in intrinsic growth rate are associated with population reductions, which may occur after the reproductive season, indicating signs of mortality on the part of hatched organisms (James, 1991; Van Sluys, 1993b). These rates, noticeable within a few months of collection, may have influenced the overall demographic mean, which was low (0.0039). A similar result was recorded by Van Devender (1982) studying *Basiliscus basiliscus* in some areas of Costa Rica. The author argued that there was a high mortality in the early stages and that fluctuations in population size appeared to be natural over the years, corrected later by the high survival rate of adults. However, Vargas-García *et al.* (2019) have shown that different species of *Sceloporus* lizards face declines in population numbers following drastic (cold and dry) seasons in southeastern Mexico because they impair adult recruitment and survival. In turn, Wiederhecker *et al.* (2003) found over three years of study that a high proportion of *T. torquatus* individuals disappeared from their monitored population after the first reproductive season. This information can help us understand the behavior observed for *T. hygomi*: in June the lizards began to hatch and, although not the most abundant age category, increased the population size; in July and August they became more abundant than adults, but the high mortality in the early stages generated a sharp decrease in individuals; finally, in September, the population number peaked and in the following months showed some stability, just as it had at the beginning of the year.

Different studies have shown that species with shorter life cycles, such as those in the family Tropiduridae, maintain stable population size by balancing births, individual persistence and death (Van Sluys, 2000; Wiederhecker *et al.*, 2003). However, some authors seem to agree that these organisms are subject to greater variations in population size over the years compared to long-lived species: a direct consequence of the success or failure of recruitment during the reproductive period (Andrews, 1988; Andrews and Wright, 1994). This seems to be in line with what we found, allowing us to conclude that the overall mean we recorded (low) may be the result of an annual momentary condition.

Conclusion

We hope that the information gathered here may assist in management alternatives in the PNSI, mainly because it is one of the few protected areas that shelters *T. hygomi*. In this way, our work can guide conservation and measures for monitoring of the species in locations outside the PNSI also. It is also necessary to ascertain whether the populations distributed in the coastal strip have their niches and other autecological characteristics similar to those of the disjunction areas or whether the isolation period was sufficient to produce significant changes. Answers to these questions will only be elucidated by the replication of studies along the occurrence range of *T. hygomi*, and our present contribution can help in this regard.

Acknowledgments

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Trabajo

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Composition and natural history of the snake community in a preserved forest in Central Amazonia, Pará, Brazil

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ABSTRACT
We present information about a snake community in a preserved forest in central Amazonia and describe the composition, habitat use, daily activity, diet, and reproduction. The study area is mainly in the municipality of Itaituba in Parque Nacional da Amazônia, state of Pará, Brazil. We set up 26 plots along of a 135 km on a stretch of the BR-230 highway. We used three quantifiable capture methods: Time Constrained Visual Search (TCS), Road Search by Car (RS) and Pitfall Traps with drift fence (PTS). We also recorded all snakes eventually found by the team outside the collection protocol (occasional encounters) and those obtained by other people (third-party encounters). Reproductive (sexual dimorphism, reproductive maturity, recruitment, and fecundity), and feeding biology (diet composition and frequency of food items) are presented. We recorded 61 species from 38 genera and seven families. *Imantodes cenchoa*, *Helicops angulatus*, and *Bothrops atrox* were the most abundant species. Most species have terrestrial habits and nocturnal activity. The most consumed prey was lizards and frogs. Observing the number of specimens distributed monthly, we noted that most specimens with eggs and embryos were recorded in the dry season, while births occurred throughout the year. Compared to some snake communities located south and north of the Amazon River, the snake community of Parque Nacional da Amazônia has high species diversity, resulting from the presence of different lineages as well as different functional groups, which reflect past and present events that have occurred in this region.

Key Words: Amazon rainforest; Diet; Reproduction; Tapajós River; Activity.

Introduction

The complexity of ecological relationships and species richness of snakes distinguish the Neotropical region from other regions of the world (Henderson *et al.*, 1979; Vitt, 1987). Species richness patterns in this region have recently been described, and appear to follow the same trends as other vertebrates, suggesting that similar factors may determine the diversity of ectothermic and endothermic animals (Roll *et al.*, 2017; Guedes *et al.*, 2018). This high diversity of snakes in the neotropics is the result of a complex history of origin, colonization and diversification of distinct clades, which has resulted in irregular diversity patterns (Cadle and Greene, 1993; Head *et al.*, 2009; Fenker *et al.*, 2014; Zaher *et al.*, 2019). High phylogenetic endemism of snakes in this region has been attributed to the combination

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of recent and past events (Harrison, 2013; Thornhill *et al.*, 2017). In particular, topography and certain characteristics related to climate have been invoked to explain the historical and recent diversity of Neotropical snakes (Azevedo *et al.*, 2019). Some studies suggest that the topography acts as a climatic barrier in the distribution of snakes in the Brazilian Atlantic Forest, indicating that the physiological restrictions determine the patterns of biogeographic distribution in this ectothermic group (Moura *et al.*, 2016). Likewise, thermal conditions would better explain the compositional and phylogenetic beta-diversity of snakes in the Atlantic Forest, while factors related to water would better explain the structure of assemblies within the same climatological regime (Moura *et al.*, 2016).

There is considerable heterogeneity among climatically-similar sites in the Amazon basin. Faunal surveys for the southwestern Amazon, for example, reveal lower species diversity than sites in northwestern and central Amazonia (Rabosky *et al.*, 2019). An interesting pattern of variation was observed along a north-south gradient, with localities north of the main Amazon channel showing 30% higher richness and diversity of snakes compared to the lowlands of southwestern Amazonia (Silva and Sites, 1995; Rabosky *et al.*, 2016; Guedes *et al.*, 2018; Roll *et al.*, 2017). However, this condition is not observed when the snake assemblies located in the south (Frota, 2004; Frota *et al.*, 2005; Bernarde and Abe, 2006; Ávila-Pires *et al.*, 2009; Maschio *et al.*, 2009; Prudente *et al.*, 2010; Bernarde *et al.*, 2011; Silva *et al.*, 2011) are compared to those located north of the Amazon River (Avila-Pires, 2005; Lima, 2008; Avila-Pires *et al.*, 2010; Campos *et al.,* 2015; Moraes *et al.*, 2017; Prudente *et al.*, 2020) (see Table and Figure in Appendix). Specifically, comparing intensely sampled areas with similar sampling efforts and protocols, it is observed that sites north of the Amazon River (such as the Serra do Navio region, see Prudente *et al.*, 2020) have fewer snakes than the area southern area of the river (such as the National Forest de Caxiuanã, see Santos-Costa *et al.*, 2015) (see Table and Figure in the Appendix).

Comparing the richness and diversity of different snake communities has been a challenge for herpetologists working in the Amazon. According to Rabosky *et al.* (2019), the difficulty in understanding variations in species richness and turnover in the composition of the snake community can be attributed to three factors: variation of the geographical scale of sampling in published inventories; difficulty in sampling this group of animals; and absence of a standardized protocol for sampling squamate communities from tropical forests.

Using different snake sampling protocols, studies conducted south of the Amazon River revealed that the species use a wide variety of habitats, with variable patterns of seasonal activity, diet and reproduction (Santos-Costa *et al.*, 2006; Maschio *et al.*, 2007; Prudente *et al.*, 2007; Maschio *et al.*, 2009; Nascimento *et al.*, 2013; Santos-Costa *et al.*, 2015). Here, we present the inventory of the snake community at Parque Nacional da Amazônia (PARNA), located in a preserved forest in the central region south of the Amazon River, in Brazil. We provide information on composition, abundance, habitat use, daily activity, diet, and reproduction of the species, and compare our results with those of other snake communities located on both banks of the Amazon River.

Materials and methods

Study area

Parque Nacional da Amazônia (PARNA) (03°50'S, 56°32' W) is situated on the left bank of the Tapajós River, mostly within the municipality of Itaituba, along the middle Tapajós River in southwestern state of Pará, northern Brazil (Fig. 1) (IBDF, 1978; Silva-Forsberg, 2006).

Tapajós, a tributary of the right bank of the Amazon River, is a river of transparent waters approximately 760 km long, that flows into the Amazon River in the municipality of Santarém, Pará. The Tapajos's main tributaries are the Jamanxim, Crepori, Teles Pires and Juruena Rivers. The PARNA covers 1,100,679 hectares, and most of the area consists of dense submontane ombrophilous upland forest (Silva-Forsberg, 2006).

The main access route to the PARNA is the Trans-Amazon Highway (BR-230), an unpaved road that runs through the park for 116 km (from km-51 to km-167), linking the municipalities of Itaituba and Jacareacanga in the state of Pará, Brazil. Elevation in the region reaches up to 245 meters above sea level, with prevailing tropical rainy climate and an average annual temperature of 27°C. Annual rainfall (2009 to 2010) was approximately 2000 mm. Precipitation during the rainy season (December to June) is most intense from February to May, with average monthly rainfall of 245 mm. During the dry season (July and October) precipitation falls as low as 63 mm in the month of August.

We set up a total of 26 plots in dense ombrophilous forest (Fig. 1). We established the plots between km-51 and km-93 of the Trans-Amazon Highway, with the following geographic coordinates: **P 1** (4°28'0.02"S, 56°17'8.78"W); **P 2** (4°28'32.25"S, 56°16'45.03"W); **P 3** (4°29'10.83"S 56°17'0.47"W); **P 4** (4°29'17.83"S, 56°16'41.47"W); **P 5** (4°29'27.40"S, 56°16'10.80"W); **P 6** (4°29'52.70"S, 56°16'14.80"W); **P 7** (4°40'6.70"S, 56°27'22.80" W); **P 8** (4°32'38.65"S, 56°18'6.85"W); **P 9** (4°32'39.00"S, 56°18'34.80"W); **P 10** (4°32'18.40"S, 56°18'50.40"W); **P 11** (4°33'20.82"S, 56°17'56.94"W); **P 12** (4°3P3'21.10" S, 56°18'13.30"W); **P 13** (4°33'45.50"S, 56°18'27.30"W); **P 14** (4°35'45.13" S, 56°19'21.74" W); **P 15** (4°35'52.00"S, 56°19'40.62"W); **P 16** (4°36'9.00"S, 56°19'49.40"W); **P 17** (4°37'14.35"S, 56°19'49.01"W); **P 18** (4°37'37.93"S, 56°23'13.54"W); **P 19**(4°37'12.79"S, 56°23'27.53"W); **P 20** (4°36'59.60"S, 56°23'49.60"W); **P 21** (4°36'50.20"S, 56°24'13.40"W); **P 22** (4°36'38.30" S, 56°24'35.40"W); **P 23** (4°36'30.20"S, 56°24'53.20"W); **P 24** (4°40'18.90" S, 56°27'6.80" W); **P 25** (4°28'57"S, 56°16'21"W); and **P 26** (4°34'30"S, 56°18'42"W).

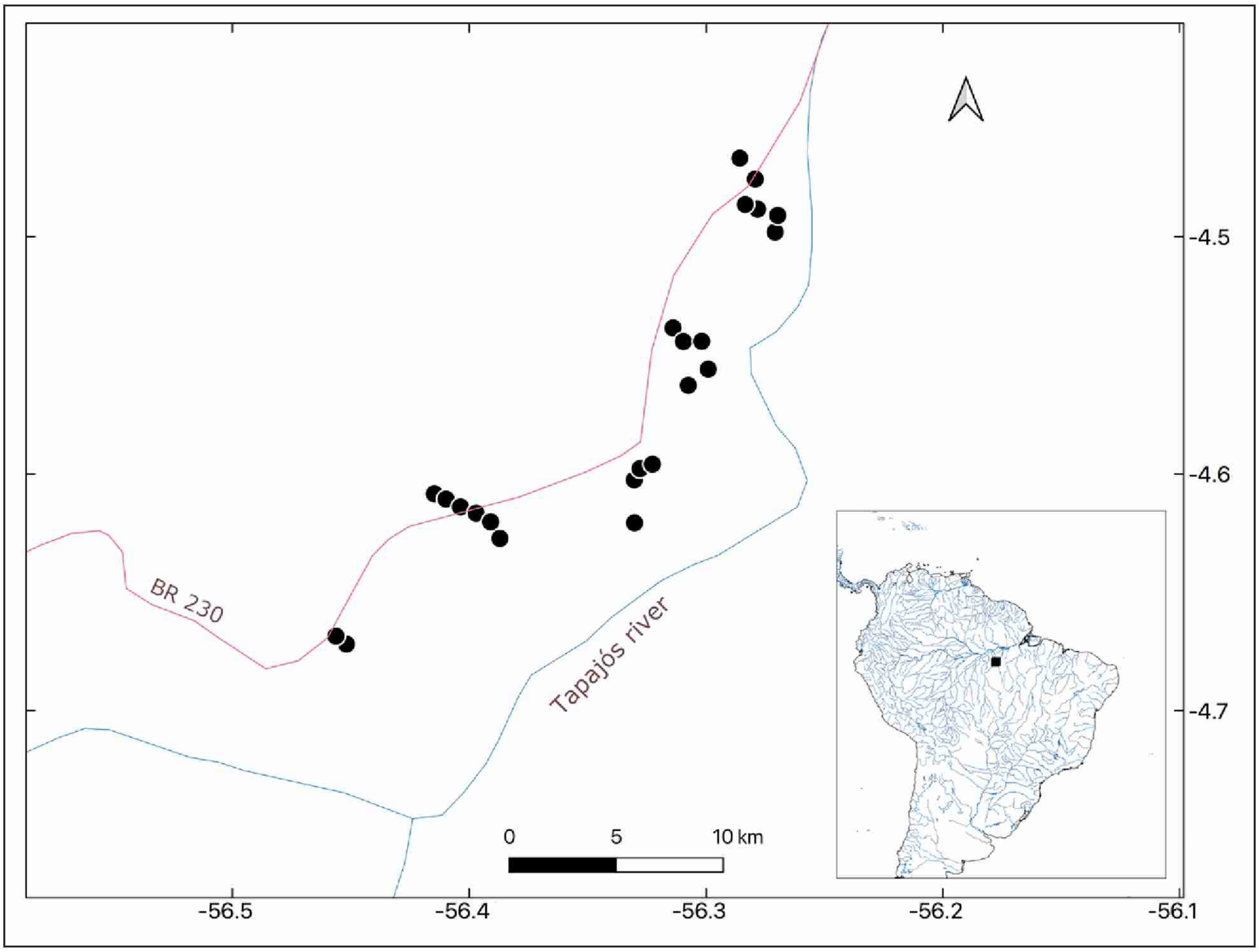

Figure 1. Location of the Parque Nacional da Amazônia, in the city of Itaituba, state of Pará, Brazil. Study area indicated by thered circle.

Each plot had an area of 3,000 m^2 (150m x 20m) spread throughout an area of approximately 200 km^2. We inspected plots 1–24 over six expeditions in 2009 (August, September and November) and 2010 (January, March and July), each lasting 14 consecutive days. We set up the plots 25 and 26 and inspected then in August and November 2011, each for a period of eleven consecutive days.

Field methods

We captured the specimens manually using hooks or herpetological tongs, always by four observers, using three quantifiable capture methods: Time constrained search (TCS), Road Search by Car (RS) and Pitfall Traps with drift fence (PTF). TCS was performed in all plots and consisted of walking through each plot during the day and at night, inspecting all possible shelters and recording the elapsed time (adapted from Martins and Oliveira, 1999) for a total of 1,220 search hours.

We set up four sets of PTF, two in plot 25 and two in plot 26, arranged in rows 200 meters apart (modified from Cechin and Martins, 2000) (Fig. 1). In each row, we used ten 30-liter buckets, five meters apart and joined by a drift fence: a plastic sheet about 70 cm high by 50 m long. The buckets had holes drilled in the bottom to prevent the accumulation of water and we added pieces of styrofoam to avoid drowning in the event of a momentary flood. We buried the drift fence about 15 cm deep and supported it standing with wooden stakes. We installed a total of 40 buckets along 200 m of drift fence, remaining open for 22 days, totaling 880 bucket day. The inspection of PTFs occurred daily.

The RS consisted of displacement by car at a speed of up to 40 km/h along the Trans-Amazon Highway, between km-51 and km-167, to collect live or dead specimens found on the road. We conducted the search during the day, in 2010 (July, August, September, November and December) and 2011 (January, March, April, May and June), without calculating the sample effort. This qualitative method aimed to obtain complementary information about the animals found along the road, when we move between the collection sites.

We consider all snakes found by the team outside the allotted collection regimeas occasional enconters (OE) (Martins and Oliveira, 1999), while was considered third-party encounters (TE) those obtained by other people (Cunha and Nascimento, 1978). Live specimens were euthanized with an overdose of Lidocaine hydrochloride 5%. We deposited the specimens collected and tissue samples in the herpetological collection of Museu Paraense Emílio Goeldi (MPEG) (Appendix – Material analyzed). We used 28 specimens previously collected at PARNA from the MPEG collection to complement the data. We carried out identifications to the species level and, whenever possible, to the subspecies level, to increase the refinement of our results. The taxonomic nomenclature and species identification followed: Starace (1998); Fraga *et al.* (2013); Campbell and Lamar (2004); Graboski *et al.* (2015); Costa and Bérnils (2018); Ascenso *et al.* (2019); Nogueira *et al.* (2019); Uetz and Hosek (2020); and Melo-Sampaio *et al.* (2021).

Data acquisition and analysis

During fieldwork, we collected information about the period of activity (diurnal and nocturnal) and habitat (terrestrial, arboreal, and aquatic). Snakes were considered active when discovered in motion, immobile in thermoregulation activity or still, yet attentive and prepared to escape. Snakes were considered inactive when at rest and/or when they showed little or no immediate reaction when disturbed (Cadle and Green, 1993). For all the specimens analyzed, we measured snout-vent length (SVL) and tail length, with the aid of a graduated ruler (to the nearest 1.0 mm).

Snakes exhibit defensive behavior when they feel intimidated by a predator. At the time of capture, we observed whether the snake showed defensive behavior: preparing to strike, flattening, raising or hiding the head, opening the mouth, flattening the anterior dorsal region of the body, dorsoventral body compression, constricting, pressing with tail spine, shaped body S, cloacal discharge, hide your head, tongue exhibition, escape, stick with the tail, stays still, inflates the body, gular inflation, raise his head, erratic movement, protect the head in the shaped body ball, among others (Martins, 1996; Martins *et al.*, 2008).

To evaluate the diet, a dissection was performed (longitudinal incision on the venter for each specimen) only on specimens collected by TCS and RS. We analyzed and identified the stomach contents to major groups (lizard, bird, rodent, anuran, bird egg, centipede, earthworm,insect larva, mollusk, squamata egg, shrimp, tadpole, and fish).

To describe the reproductive aspects, all snakes collected or from scientific collection of specimens from the study area were dissected. Here, we assume that macroscopic analyzes of the gonads have methodological flaws and that histological studies are more appropriate (See Almeida-Santos *et al.*, 2014). However, the absence of studies using histological is rare for Amazonian snakes (Maschio *et al.*, in press). Therefore, we considered males mature when having a snout-vent length (SVL, mm) equal or superior to the length of the smallest male of its species that had coiled ductus deferens (Shine, 1988). Females were considered mature when eggs or embryos were found in the oviducts, and when they had a flaccidor pleated oviduct or, when information on the size of the follicles in secondary vitellogenesis was not available, when SVL was equal to or superior to that of the smallest female of its species that presented follicles in secondary vitellogenesis (largest diameter>10.0 mm; Shine, 1977, 1988). Specimens were considered newborns or young if they showed fissures or visible umbilical scars, or snout-vent length (SVL in mm) compatible to that presented by young specimens (Santos-Costa *et al.*, 2006). We inferred the oviposition period based on the seasonal distribution of pregnant females, and estimated fecundity (reproductive rate) based on the number of vitellogenic follicles (secondary yolk stage) or egg-bearing oviducts. The reproductive cycles of females were found by analyzing gonads and correlating the maturity stage (mature and immature) by seasons (rainy and dry).

Results

We recorded 61 species belonging to 38 genera and eight families (Aniliidae, Boidae, Colubridae, Dipsadidae, Elapidae, Thyphlopidae, Leptotyphlopidae and Viperidae) in the PARNA (N= 278 specimens; Table 1; Figs 2-5). Eleven other species were recorded in the vicinity of the PARNA (*Apostolepis nigrolineata, Erythrolamprus miliaris amazonicus, Helicops polylepis, Micrurus surinamensis* and *Typhlophis squamosus*) and the region of the middle Tapajós

Table 1. Species of snakes registered for the Parque Nacional da Amazonia, municipality of Itaituba, Pará State, Brazil. Legend: **N** = number of specimens collected in this study. **MPEG** = number of specimens deposited in the herpetological collection of Museu Paraense Emílio Goeldi, no methods, habitat, activity, diet, reproduction and defensive behavior information. **Methods**: TCS = Time constrained search; RS = Road search by car; PTF = Pitfall traps with drift fence; OE = Occasional enconters; and TE = Third-party encounters. **Habitat**: Te = Terrestrial; Arb = Arboreal; Aq = Aquatic. **Activity**: Di = Diurnal; No = Nocturnal; * inactive specimens. **Diet**: Li = Lizard; Bi = Bird; Ro = Rodent; An = Anura; Beg = Bird egg; Ce = Centipede; Ea = Earthworm; Il= Insect larva; Mo =Mollusk; Seg = Squamata egg; Sh = Shrimp; Ta = Tadpole; Fi = Fish. **Reproduction**: reproductive period prescribed in month; Fo2º = Secondary follicles vitellogenic; Eg = Eggs; Emb = Embryos; Ne = Newborns; Yo = Youth. **Defensive behavior**: 1 =flatten the head or triangular head; 2 =bite;3=constriction;4=shaped bodyS; 5 =cloacal discharges; 6= tail display; 7 = coiling; 8 =tail coiling; 9=hide the head; 10=tongue exhibition; 11=escape; 12=stick withthe tail; 13 =stays still; 14 = gape; 15 =inflates the body; 16=gular inflation; 17=raises his head; 18=erratic movement; 19=protect the head in the shaped body ball; 20 =body rotation; 21=hiss; 22 = strike; 23=vibrates its tail; 24 =motionless body in a zig-zag position; and 25 = evert hemipenis.

Family/Species	N	MPEG	Methods (specimens)	Habitat (specimens)	Activity (specimens)	Diet	Reproduction (month)	Defense behavior
ANILIIDAE								
Anilius scytale (Linnaeus, 1758)	1		RS(1)	Te(1)	Di(1)			5, 10, 19
BOIDAE								
Boa constrictor Linnaeus, 1758	5	1	RS(2), TE(3)	Arb(2), Te(3)	Di(5)	Li; Bi	Yo(Jan, Nov); Ne(Feb, May, Jul, Nov)	2, 4, 5
Corallus batesii (Gary, 1860)	5		TCS(4), OE(1)	Arb(5)	No(5)	Bi	Yo(Aug, Nov)	2, 3, 4, 5, 19
Corallus hortulana (Linnaeus, 1758)	4		TCS(3), TE(1)	Arb(4)	No(4)		Yo(Aug)	2, 3, 4, 5, 19
Epicrates cenchria (Linnaeus, 1758)	6		TCS(2), OE(3), TE(1)	Te(6)	No(6)		Ne(Jan, Feb)	3, 17, 19
Eunectes murinus Gray, 1849	3		TCS(2), RS(1)	Aq(2), Te(1)	Di(2), No(1)	Ro	Ne(Mar, Aug); Yo (Jul)	3, 5, 19, 21
COLUBRIDAE								
Chironius fuscus (Linnaeus, 1758)	9		TCS(3), RS(2), OE(3), TE(1)	Te(6), Arb(3)	Di(6), No(3*)	An	Fo2º(Jan)	4, 5, 16, 22, 23, 24
Chironius multiventris Schmidt and Walker, 1943	7		TCS(2), RS(1), TE(4)	Te(5), Arb(2)	Di(5), No(2*)	An		2,4,5,16,17, 22,23,24

Chironius scurrulus (Wagler, 1824)	2		OE(1), TE(1)	Te(2)	Di(2)		Fo2º(Jan)	2, 4, 17, 18, 20
Dendrophidion dendrophis (Schlegel, 1837)	2	1	TCS(1), TE(1)	Te (2)	Di(2)	An	Fo2º(Jan)	15, 17, 23
Drymarchon corais Boie, 1827	1	1	RS(1)	Te(1)	Di(1)	Li	Eg(Aug)	2, 5, 10, 15, 20
Drymoluber dichrous (Peters, 1863)	7	2	TCS(6), RS(1)	Te(3), Arb(4)	Di(3), No(4*)	Li; An	Fo2º(Mar, Jul); Eg(Jan); Yo(Nov)	2, 4, 15, 17, 18, 20
Leptophis ahaetulla (Linnaeus, 1758)	7		TCS(2), RS(2), OE(3)	Arb(7)	Di(7)	An		2, 4, 5, 14,17, 18, 20
Mastigodryas boddaerti (Sentzen, 1796)	8	2	RS(2), OE(1), TE(5)	Te(8)	Di(8)	Li	Eg(Dec, Jan); Yo(jun)	2, 11, 13
Oxybelis aeneus (Wagler, 1824)	8		RS(7), TE(1)	Te(8)	Di(8)	Li	Eg(Jul)	10, 13
Oxybelis fulgidus (Daudin, 1803)	9		TCS(1), RS(4), OE(3), TE(1)	Te(8), Arb(1)	Di(8), No(1*)	Li		2, 13
Phrynonax polylepis (Peters, 1867)	3		RS(2), OE(1)	Te(2), Arb(1)	Di(3)	Beg	Ne(Mar)	2, 16, 17, 18, 22, 24
Rhinobothryum lentiginosum (Scopoli, 1785)		1		Te(1)	No(1)			1, 23
Spilotes pullatus (Linnaeus, 1758)	6		RS(3), TE(3)	Te(6)	Di(6)		Ne(Jan, Nov, Dec)	17
Spilotes sulphureus (Wagler, 1824)	1	1	TCS(1)	Te(1)	Di(1)			3, 15, 16, 17
Tantilla melanocephala (Linnaeus, 1758)	2		TCS(2)	Te(2)	No(2)	Ce		5, 13, 18
DIPSADIDAE								
Atractus boimirim Passos, Prudente & Lynch, 2016	3		TCS(1), TE(1)	Te(3)	Di(3)	Ea	Ne(Jan, Mar); Fo2º(Feb)	18
Atractus snethlageae Cunha & Nascimento, 1983	2		PFT(1)	Te(2)	Di(2)	Ea; Il	Eg(Nov)	10
Dipsas catesbyi (Sentzen, 1796)	1		TCS(1)	Te(1)	No(1)	Mo		9, 20
Dipsas pavonina Schlegel, 1837	2		TCS(2)	Arb(2)	No(2)	Mo		13
Drepanoides anomalus (Jan, 1863)	4	1	TCS(4)	Te(4)	No(4)	Seg		10, 13, 20
Erythrolamprus aesculapii (Linnaeus, 1766)	1	4	RS(1)	Te(1)	Di(1)		Fo2º(Feb)	1, 8, 10, 17, 18, 25
Erythrolamprus oligolepis	3	1	TCS(1), RS(1), TE(1)	Te(3)	Di(3)		Fo2º(Aug, Sep)	10
Erythrolamprus poecilogyrus (Wied, 1824)	2		OE(1), TE(1)	Te(2)	Di(2)	An	Ne(Jul)	10, 18
Erythrolamprus reginae (Linnaeus, 1758)	4	2	OE(4)	Te(4)	Di(3), No(1)	An	Ne(Feb, Mar, Jul)	5, 10, 18

Erythrolamprus taeniogaster (Jan, 1863)	2		TE(2)	Te(2)	Di(2)			10, 18
Erythrolamprus typhlus (Linnaeus, 1758)	4		TCS(1), RS(1), OE(2)	Te(4)	Di(4)	An	Ne(Jul)	1, 5, 10, 13, 17
Helicops angulatus (Linnaeus, 1758)	20	2	TCS(19), OE(1)	Aq(20)	No(20)	An; Ta; Fi	Fo2(Jul); Ne(Jan, Jul, Nov); Yo(Aug)	1, 2, 3, 5, 20
Hydrodynastes bicinctus (Herrmann, 1804)	3		TCS(2), TE(1)	Aq(3)	Di(2),No(1)	Sh	Ne(Jan); Yo(Jun)	2, 10, 20
Hydrops martii (Wagler, 1824)	1		TCS(1)	Aq(1)	No(1)			10
Imantodes cenchoa (Linnaeus, 1758)	30	1	TCS(25), RS(1), OE(4)	Te(1), Arb(29)	No(30)	Li	Fo2º(Jan, Mar, Nov); Eg(Jan, Mar, Nov); Ne(Feb); Yo(Aug, Sep)	5, 10
Imantodes lentiferus (Cope, 1894)	2		TCS(2)	Arb(2)	No(2)			5, 10
Leptodeira annulata (Linnaeus, 1758)	5		TCS(3), RS(2)	Arb(5)	No(5)	An		5, 13
Oxyrhopus formosus (Wied, 1820)	3		OE(3)	Te(3)	No(3)	Li		10, 18
Oxyrhopus melanogenys (Tschudi, 1845)	5		RS(4), TE(1)	Te(4)	No(4)	Li	Ne(Aug)	18
Philodryas argentea (Daudin, 1803)	1		OE(1)	Arb(1)	Di(1)			24
Philodryas viridissima (Linnaeus, 1758)	2	1	OE(1), TE(1)	Te(2)	Di(2)		Yo(Feb)	
Pseudoboa coronata Schneider, 1801	3	1	TCS(2), RS(1)	Te(3)	No(3)	Li	Yo(Aug)	10, 13
Pseudoeryx plicatilis (Linnaeus, 1758)	1		RS(1)	Te(1)	Di(1)			
Siphlophis cervinus (Laurenti, 1768)	1		TCS(1)	Arb(1)	No(1)			1, 19
Siphlophis compressus (Daudin, 1803)	9		TCS(6), RS(1), OE(2)	Arb(9)	No(9)	Li	Fo2º(Aug); Eg(Sep)	4, 5, 10
Taeniophallus brevirostris (Peters, 1863)	2		OE(2)	Te(2)	Di(2)	Li	Eg(Jul); Ne(Aug)	5, 10
Taeniophallus quadriocellatus Santos, Di-Bernardo & Lema, 2008	5	2	TCS(2), RS(1), PTF(1), OE(1)	Te(5)	Di(3), No(1)	Li	Fo2º(Jul); Eg(Oct)	5, 10, 18
Xenodon rhabdocephalus (Wied, 1824)		1		Te(1)	Di(1)			
Xenopholis scalaris (Wucherer, 1861)	3	1	TCS(2), PFT(1), OE(1)	Te(3)	No(3)	An	Fo2º(Aug)	1, 5, 10, 13, 18, 19
ELAPIDAE								
Micrurus hemprichii (Jan, 1858)	1		OE(1)	Te(1)	No(1)			6, 17

Micrurus lemniscatus (Linnaeus, 1758)	2		TCS(1), RS(1)	Te(2)	No(2)		Ne(Jan)	
Micrurus paraensis Cunha & Nascimento, 1973	1		OE(1)	Te(1)	No(1)			2, 6, 9, 10, 18
Micrurus spixii Wagler, 1824	1		TCS(1)	Te(1)	No(1)			6, 9, 10, 18
LEPTOTYPHLOPIDAE								
Trilepida macrolepis (Peters, 1857)	3	1	TCS(3)	Arb(3)	No(3)		Ne(Oct)	10, 18
TYPHLOPIDAE								
Amerotyphlops reticulatus (Linnaeus, 1758)	2		PTF(2)	Te(2)	No(2)			10, 12, 18
VIPERIDAE								
Bothrops atrox (Linnaeus, 1758)	16	1	TCS(6), RS(5), OE(1), TE(4)	Te(14), Arb(2)	Di(4), No(12)	Li; Ro	Emb(Jul); Ne(Dec); Yo(Mar)	2, 5, 7, 22, 10, 13, 23
Bothrops bilineatus (Wied, 1821)	1		TCS(1)	Arb(1)	Di(1)			2, 4, 7, 17, 22
Bothrops brazili Hoge, 1954	1		TE(1)	Te(1)	Di(1)			
Bothrops taeniatus Wagler, 1824	1		RS(1)	Te(1)	No(1)			
Lachesis muta (Linnaeus 1766)	1		TE(1)	Te(1)	No(1)		Ne(Jan)	4, 10
TOTAL	250	28						

river (*Chironius exoletus, Clelia plumbea, Hydrops triangularis, Erythrolamprus breviceps, Sibon nebulatus* and *Xenodon severus*) (Frota, 2004).

Considering the five capture methods, both quantifiable and non-quantifiable, we registered a total of 250 individuals for 61 species. For each method, we record the following numbers and percentage of species: TCS (N= 35; 57.4%), PTF (N= 5; 8.2%), RS (N= 26; 42.6%), OE (N= 23; 37.7%), and TE (N= 22; 36%). Sampling effort using only quantifiable methods showed the following results: 1) TCS captured 117 specimens with a sampling effort of 1,220 hours/observer (616 hours during the dayand604 hours at night), with an average encounter rate of 0.09 snakes per person-hour (one snake very 11 person-hours of search).The encounter rate during the day was 0.04 (one snake every 27 hours), whereas, at night, it was 0.15 (one snake every 6hrs 50min); PTF had a sampling effort of 2,112 bucket-hours (22non-consecutive days)and an encounter rate of 0.0023 snakes per bucket-hour (one snake every 422 open bucket-hours); and RS recorded twenty-six species (N= 50) by traveling 1,624 km of road, with an encounter rate of 0.03 snakes/km (one snake every 32.5 km or every 50 min.

Imantodes cenchoa (N= 30; 12.0%), *Helicops angulatus* (N= 20; 8.0%), and *Bothrops atrox* (N= 16; 6.4%) were the most abundant species, considering all methods used (Table 1).The most common species recorded by TCS was *Imantodes cenchoa* (N= 25), while 10 species were recorded exclusively by this method (*Bothrops bilineatus, Dipsas catesbyi, D.pavonina, Drepanoides anomalus, Hydrops martii, Imantodes lentiferus, Micrurus spixii, Spilotes sulphureus, Siphlophis cervinus*, and *Tantilla melanocephala*). Four species (*Atractus snethlageae, Taeniophallus quadriocellatus, Amerotyphlops reticulatus*, and *Xenopholis scalaris*) were recorded using PTF, and *Amerotyphlops reticulatus* was recorded exclusively by this method. *Oxybelis fulgidus* was the most frequent species recorded by RS (N= 8), followed by *O. aeneus* (N= 7), *Bothrops atrox* (N= 6), and *Spilotes pullatus* (N= 5). Five species (*Anilius scytale, Bothrops taeniatus, Drymarchon corais, Erythrolamprus aesculapii* and *Pseudoeryx plicatilis*) were recorded only by RS.

Among the species recorded in this study at the PARNA, 34 were found exclusively in terrestrial habitat (55.7%), 12 in arboreal habitat (19.7%) and three in aquatic habitat (5.0%). Some species used more than one habitat, including *Boa constrictor, Bothrops atrox, Oxybelis fulgidus, Phrynonax polylepis*, and others found both on the ground and above. We highlight the record of *Trilepida macrolepis*, a

Figure 2. General view in life of snakes from Parque Nacional da Amazônia, Itaituba, Pará, Brazil. A – *Anilius scytale*; B - *Corallus batesii*; C - *Corallus hortulana*; D- *Epicrates cenchria*; E – *Chironius fuscus*; F – *Dendrophidion dendrophis*; G – *Drymarchon corais* (photo by Jerriane Gomes); H– *Drymoluber dichrous* (young).

Figure 3. General view in life of snakes from Parque Nacional da Amazônia, Itaituba, Pará, Brazil. **A** – *Leptophis ahaetulla*; **B** - *Mastigodryas boddaerti*; **C** – *Phrynonax polylepis*; **D**– *Spilotes sulphureus*; **E** – *Spilotes pullatus*; **F** – *Dipsas catesbyi*; **G**– *Dipsas pavonina*;**H** – *Drepanoides anomalus*.

Figure 4. General view in life of snakes from Parque Nacional da Amazônia, Itaituba, Pará, Brazil. **A** - *Erythrolamprus reginae*; **B** - *Erythrolamprus typhlus;* **C** – *Helicops angulatus*; **D**– *Hydrodynastes bicinctus;* **E** - *Hydrops martii;* **F**-*Imantodes cenchoa*; **G** – *Oxyrhopus formosus*; **H** - *Oxyrhopus melanogenys*.

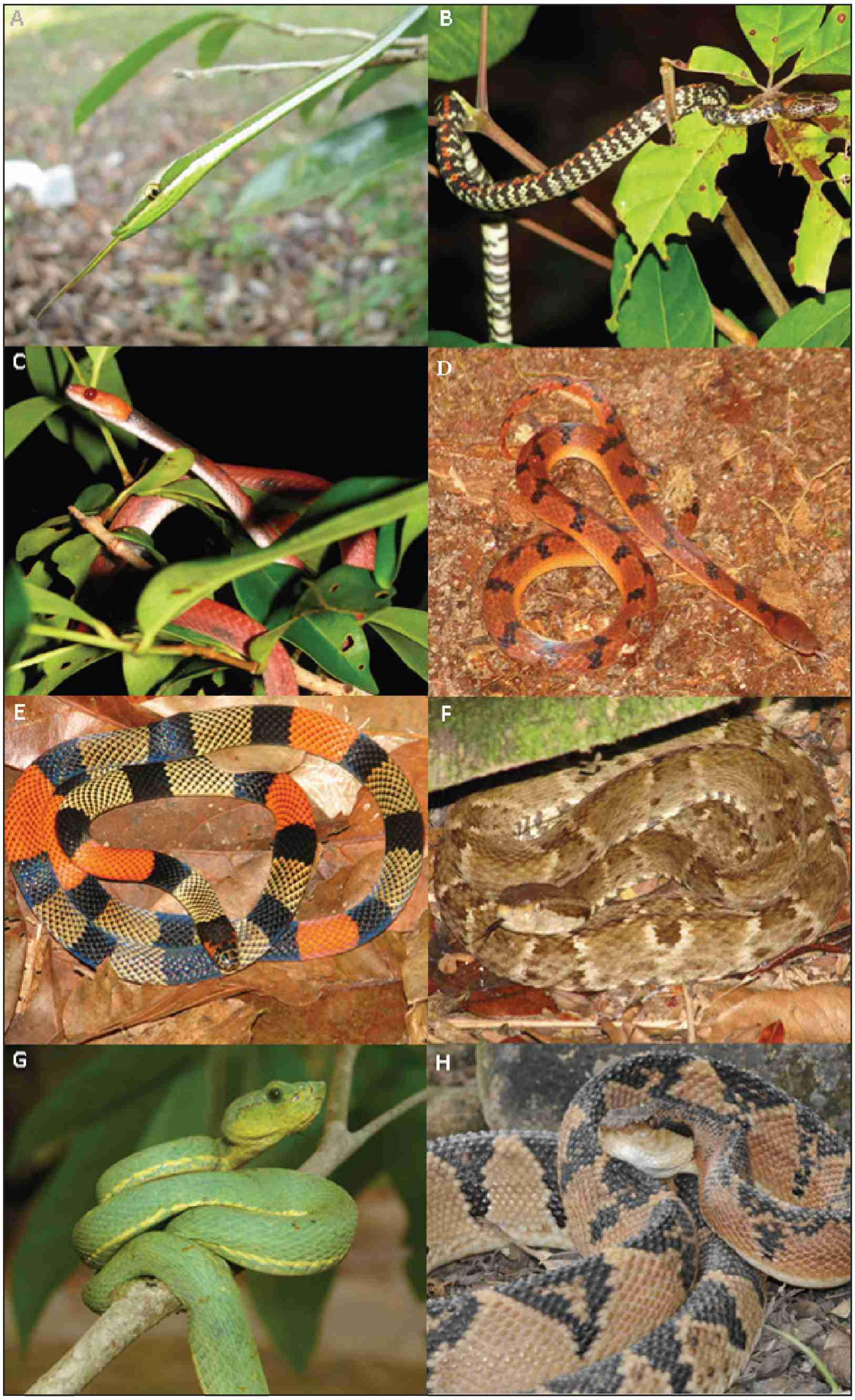

Figure 5. General view in life of snakes from Parque Nacional da Amazônia, Itaituba, Pará, Brazil. **A** – *Philodryas argentea;* **B** - *Siphlophis cervinus*; **C**– *Siphlophis compressus*; **D** - *Xenopholis scalaris;* **E** – *Micrurus spixii;* **F** - *Bothrops atrox*; **G**– *Bothrops bilineatus*; **H –** *Lachesis muta* (photo by Rafael Balestrin).

species considered fossorial, however, in this study we found three isolated specimens in arboreal settings (between 1 and 6 meters high). *Pseudoeryx plicatilis,* an aquatic species, was found dead on the road near a lake in the late afternoon. Two other specimens (not included in this study) were also found road killed during the day near flooded environments.

A total of 46 species were recorded in activity. Most species in this assemblage had nocturnal habits (28 species), followed by diurnal habits (25 species), and a few with both diurnal and nocturnal activities (*Bothrops atrox, Eunectes murinus*, *Erythrolamprus reginae,* and *Taeniophallus quadriocellatus*) (Table 1).

Of the total of 278 specimens analyzed, 87 (31.3%) had identifiable stomach contents (N= 121). The most frequent items consumed were lizards, adult anurans, tadpoles and fish (Table 1). Lizards were consumed exclusively by 11 species of snakes, and anurans by only nine species. Of the 121 items consumed it was possible to identify the direction of ingestion of 81% (N= 98). Prey ingested head-first (71.4%, N= 70) were more frequent than prey ingested tail-first (23.1%, N= 28). Lizards (88.9%, N= 32) and frogs (61.7%, N= 21) were ingested mostly by the anterior body region. Prey ingested from the anterior region of the body ranged from 8.0 to 187.0 mm TL (mean= 44.9 mm), representing 3-46% of the snake SVL. By contrast, prey ingested from the posterior region ranged from 17 to 56 mm TL (mean= 35.3 mm), accounting for 2–14% of the snake SVL.

Reproductive information was obtained for 35 species (n= 239, 148 males and 91 females). Observing the number of specimens distributed monthly (Fig. 6; Table 1), we note that some snakes with eggs and embryos were recorded in the dry season and the beginning of the rainy season, while births occurred throughout the year. We observed vitellogenic secondary follicles (>10 mm) from fifteen species, of which 10 were carrying eggs and one was carrying embryos (Table1).

The escape behavior (movement to get out of a dangerous situation) was the most recorded defensive act (N= 27), followed by cloacal discharge (N= 20) and erratic movements (N= 19). A few defensive behaviors involving intimidation or attack were observed (Table 1).

Discussion

We recorded 72 species in 42 genera and eight families of snakes in the Itaituba region on the middle Tapajós River, with 61 species found inside the PARNA and 11 species in surrounding areas. This represents a surprisingly high diversity, considering that this study area contains 47% (153 species) of species registered for the entire state of Pará (Costa and Bérnils, 2018) and 38% (189 species) of the species registered for the Brazilian Amazonia (Prudente, 2017). Historically, the state of Pará has been intensely sampled and several surveys of snake fauna point to a high diversity. Obviously, there are still regions that are difficult to access that were few samples, reflecting in knowledge gaps. The high diversity of snakes observed in the middle Tapajós region reflects a previously observed pattern for tropical forests, since localities with a predominance of forested vegetation types tend to have relatively higher diversity (Martins and Oliveira, 1999; Maschio *et al.*, 2009; Santos-Costa *et al.*, 2015; Guedes *et al.*, 2014; Rabosky *et al.*, 2019) when compared to open formations in Neotropics (Rocha and Prudente, 2010; Rodrigues and Prudente, 2011). On the other hand, other factors can influence the high diversity of species, for example, the type of matrix that is in contact with the forest, which depending on it can assist in the flow of animals or prevent their passage, such as the presence of urban areas and monocultures (Franklin and Lindenmayer, 2009). In the case of the study area, the great diversity can be explained in part by the structural complexity of the well-preserved continuous forest that forms a mosaic of protected areas, in addition to areas drained by several streams and rivers. However, the presence of a road (Trans-Amazon Highway) crossing PARNA, can negatively affect the local snake community, as shown by the high record of snakes run over on the road.

Choice of survey methods depends on the scope of the study (Doan, 2003). The combination of different collection methods in the same area can provide significant results in a short period of time; allow more robust comparisons between different areas (Cechin and Martins, 2000). The observed complementarity of quantifiable surveys (TCS, RS and PTF) with other methods (OE and TE) confirms the importance of optimizing these practices for surveying the herpetofauna, as noted by Cechin and Martins (2000). Subsequent studies have indicated that the combination of complementary methods is necessary for efficient sampling and that synchronized adoption of multiple techniques in field studies

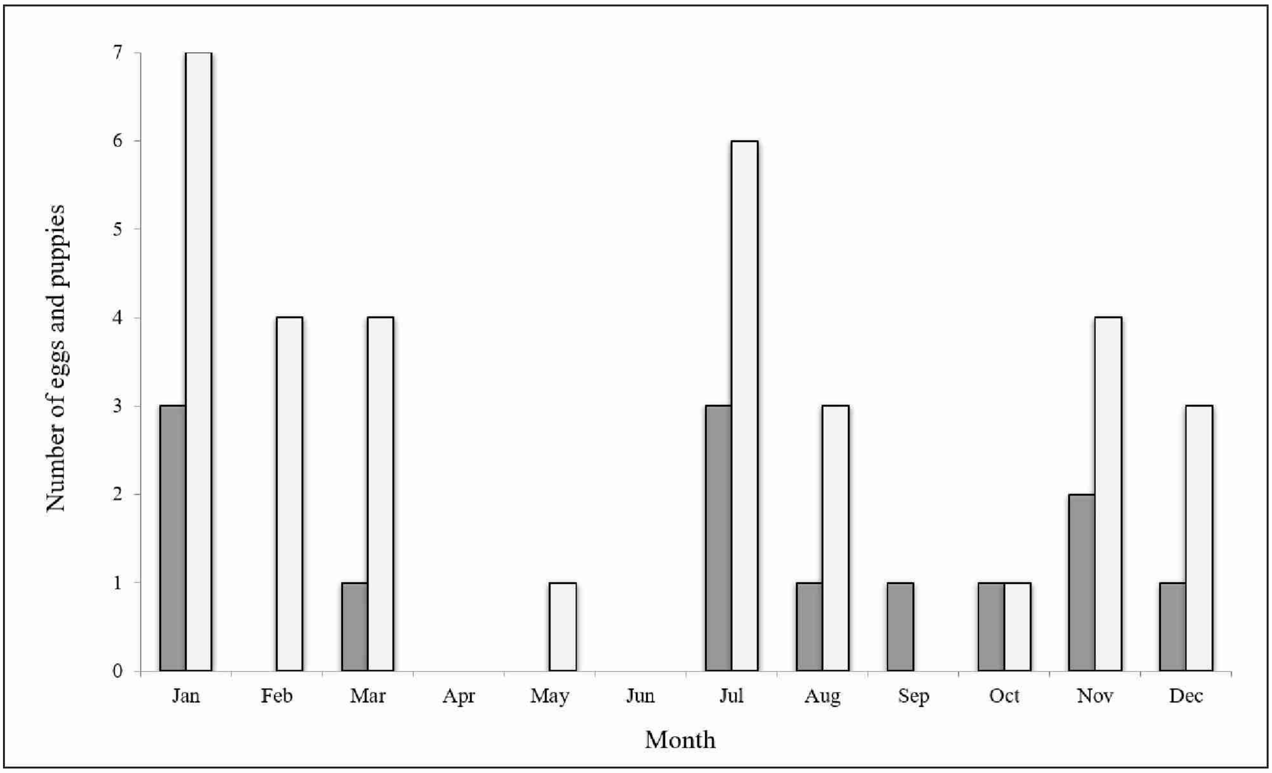

Figure 6. Proportion of eggs (gray bar) recorded in the oviduct of snakes and newborn (white bar) Parque Nacional da Amazônia, Itaituba, Pará, Brazil.

helps to improve sample representation and, thus, the understanding of species distribution and human impacts on herpetofauna in tropical florests (Ribeiro Jr. *et al.*, 2008).

The record of 35 species (11 exclusive) using the PLT is similar to the encounter rates in other snake communities in the Amazon (40 species, Frazão *et al.*, 2020), representing the method that most registers specimens and species. Despite the low efficiency of PTF in this study, this method is considered important in inventories of the herpetofauna because it accesses cryptozoic and fossorial species, which are usually difficult to observe or capture (Maschio *et al.*, 2009; Santos-Costa *et al.*, 2015). The choice of using traps should be evaluated considering the scope of the work, as mentioned by Ribeiro *et al.* (2011). As one of our goals was to obtain a complete list of PARNA snake species, we believe that the use of PTF was important, even if a single species was collected exclusively by this method. The results obtained for RS plus OE and TE indicate that 38 species (n= 88; 35.2% of the total specimens) were present, active or not, on the road. There are several factors that lead to the prevalence of snake road kill, such as foraging (Lima and Obara, 2004), mate seeking during reproductive periods (Marques *et al.*, 2000) and thermoregulation (Ashley and Robinson, 1996; Gokula, 1997). The availability of food along or near highways such as seeds, fruits, herbaceous plants, and so on attracts birds, mammals and other prey items (Lima and Obara, 2004).

Imantodes cenchoa is one of the most frequent species in PARNA, as in other Amazonian assemblages such as in Juruti (Menks, 2012) and Caxiuanã (Maschio *et al.*, 2009; Santos-Costa *et al.*, 2015). The high frequency of this species may be a reflection of the capture method (TCS) and consequently detectability. The ease in locating this species by TCS may be related to its nocturnal activity and foraging strategy in higher vegetation strata (Henderson and Nickerson, 1976; Bartlett and Bartlett, 2003; Sousa *et al.*, 2014). Many snakes are easily detected at night because the color and texture of their scales show up when spotlighting with headlamps (Martins and Oliveira, 1999). This easy detectability of snakes at night may also be related to their slower behavior, which would facilitate capture (Martins and Oliveira, 1999). These results were similar to those obtained by Maschio *et al.* (2009), who found a better efficiency in nocturnal collections. Despite the more efficient sampling of Amazonian snakes at night, we believe that focusing collections only in this period can lead to error or underestimation of the number of species in a given area. Diurnal collecting is also important to identify snakes with different habits, so were commended dividing surveys between both periods.

The snake community from PARNA is characterized by the predominance of terrestrial and nocturnal species, with prevalent richness of Dipsadidae and low richness of Boidae, Elapidae and Viperidae. The differences between the number of terrestrial species recorded in PARNA when compared with assemblages in western (Duellman, 1978) and Central (Martins and Oliveira, 1999) Amazonia may be related to species composition as well as to difficulties in categorizing habitat use for tropical snakes, since a single species may use the habitat in different ways (Cadle and Greene, 1993). In PARNA, it was common to find primarily terrestrial snakes (*Boa constrictor, Bothrops atrox, Chironius fuscus, Chironius multiventris, Drymoluber dichrous* and *Phrynonax polylepis*) resting on the vegetation as well as arboreal species (*Dipsas catesbyi*, *Oxybelis fulgidus* and *O. aeneus*) foraging on the ground. A similar structure was observed in relation to the patterns of spatial distribution of snakes in the regions close to PARNA, with some species exclusively terrestrial and others terrestrial, but using vegetation to sleep and forage (Silva *et al.*, 2011; Santos-Costa *et al.*, 2015). This variation in habitat may be associated with a decreased predation pressure that is less in tropical seasonal environments (Martins, 1993; Bernarde and Abe, 2006; Martins and Oliveira, 1999).

The low number of aquatic species recorded in PARNA when compared to that of other areas in the Amazon clearly reflects the lack of suitable capture methods, in addition to hydrographic and phytophysiognomic characteristics, where Terra Firme forests predominate.

The record of *Trilepida macrolepis*, a fossorial snake, in arboreal environments (between 1 and 6 meters in height), suggests that this species seeks food in termite mounds built on tree trunks in the Amazon, or that this species also seeks vegetation to shelter in rainy periods. Foraging behavior in vegetation was recorded for *Epictia diaplocia*, which was on the trunk of a palm tree ca. 2 m above the ground, trying to enter a termite nest during a light rain (Martins and Oliveira, 1999).

As confirmed in this study, snake diets in Amazonian community are composed mainly oflizards (Duellman, 1978; Martins and Oliveira, 1999; Bernarde and Abe, 2006; Bernarde and Abe, 2010; Santos-Costa *et al.*, 2015). By contrast, anurans are the most consumed food item in the Atlantic Forest (Marques and Sazima, 2004; Hartmann *et al.*, 2009), southern grasslands (Di-Bernardo *et al.*, 2007), Pantanal (Strüssmann and Sazima,1993), Cerrado (França *et al.*, 2008) and the Caatinga (Guedes *et al.*, 2014). These differences probably reflect differences in species composition, since the extra-Amazonian regions harbor a larger proportion of xenodontines that feed on anurans (Cadle and Greene, 1993; Colston *et al.*, 2010).

Regarding reproduction, the results show that some snakes with eggs and embryos were recorded in the dry season and the beginning of the rainy season, while births occurred throughout the year. We observed vitellogenic secondary follicles (>10 mm) in fifteen species, and 10 were carrying eggs and one was carrying embryos. We observed that several species in PARNA have distinct reproductive cycles than those recorded by other authors. For example, previous studies recorded *Dipsas catesbyi, D. neivai* (Alves *et al.*, 2005), *Tantilla melanocephala* (Santos-Costa *et al.*, 2006), *Dendrophidion dendrophis* (Prudente *et al.*, 2007), *Chironius fuscus* (Nascimento *et al.*, 2013) and *Mastigodryas boddaerti* (Siqueira *et al.*, 2013) as having a continuous reproductive cycle. However, some species, such as *Anilius scytale* (Maschio *et al.*, 2007), *Dipsas pavonina* and *Bothrops atrox* (Martins and Oliveira, 1999) have a seasonal reproductive cycle. These differences in the reproductive cycle can also occur between populations of the same species, as in the case of *Imantodes cenchoa*, where some populations exhibit two seasonal peaks of reproductive activity (Sousa *et al.*, 2014), while others reproduce throughout the year (Pizzatto *et al.*, 2008). These studies indicate that in order to study the reproductive cycle of snakes, it is necessary to consider that factors such as geographic distribution, phytophysiognomy, resource availability and season, among others, may interfere in the results.

Snakes have developed many strategies (behavioral and chemical) to prevent attack by predators, and the greater this defense capacity, the higher the chance of surviving an attack (Fraga *et al.*, 2013). The large number of escape behaviors observed in the snakes of PARNA, followed by cloacal discharges, was expected. Most snakes, when exposed to a predator, either escape or discharge their feces or other fetid substances to disrupt the predator and escape (Marques and Sazima, 2003). According to Martins *et al.* (2008), arboreal snakes, as they are more exposed to predators that approach from different directions, displayed more visual defensive tactics than terrestrial ones.

The great diversity of lineages found in the tropical region hinders our understanding of patterns involving the structuring of Amazonian snake communities. Ecological, biogeographical and natural history inventories require large sampling efforts and complementary methodologies in order to identify the largest possible number of species at the local level. In general, the study community is composed of species that mainly prey on frogs and lizards, use the terrestrial and arboreal layers both for foraging and resting, and have a continuous reproductive cycle. Some groups have specific morphological adaptations both in their diet (e.g. *Tantilla*/centipedes, *Dipsas*/mollusks, *Imantodes*/ lizards) and habitat use (e.g. *Corallus, Eunectes, Trilepida*).

Several authors have hypothesized that areas north of the Amazon River have greater diversity than areas to the south (Silva and Sites, 1995; Rabosky *et al.*, 2016; Guedes *et al.*, 2018; Roll *et al.*, 2017). To test this hypothesis, it is necessary to consider different factors such as environmental and physical variables. The gradient of snake species richness from north to south in the western Amazon basin, also observed in other organisms, has been related to environmental factors such as climate and vegetation (Rabosky *et al.*, 2019). This hypothesis has not yet been tested for snake communities in eastern Amazonia, however. We observed a high diversity of snakes recorded in communities with a sampling effort greater than 500 hours (using the active sampling method), regardless of their position in relation to the Amazon River (see in Appendix – Table and Figure). This fact indicates that any effort to test the gradient hypothesis must use comparable methods and sampling effort.

When comparing the number of species among communities in eastern Amazonia, we found that PARNA is among the areas with the highest richness, probably as a result of the presence of different evolutionary lineages, as well as different functional groups in the region. However, the fact that the Park is in the region of the middle Tapajós River, in the endemic area of Tapajós (Ribas *et al.*, 2012) and an area of environmental preservation, does not protect it from deforestation.

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APPENDIX - Material analyzed

Aniliidae: *Anilius scytale*: MPEG 25043. **Boidae**: *Boa constrictor:* MPEG 25049, MPEG 25050, MPEG 25051, MPEG 25052, MPEG 21221, MPEG 25053; *Corallus batesii:* MPEG 25054, MPEG 25055, MPEG 25056, MPEG 25293, MPEG 25294; *Corallus hortulana:* MPEG 25057, MPEG 25058, MPEG 25296, MPEG 25297; *Epicrates cenchria*: MPEG 25061, MPEG 25062, MPEG 25289, MPEG 25290, MPEG 25291, MPEG 25292; *Eunectes murinus:* MPEG 25059, MPEG 25060, MPEG 25295. **Colubridae**: *Apostolepis nigrolineata*: MPEG 27228;*Chironius fuscus:* MPEG 25227, MPEG 25265, MPEG 25266, MPEG 25267, MPEG 25268, MPEG 25279, MPEG 25280, MPEG 25281, MPEG 25306; *Chironius multiventris:* MPEG 25273, MPEG 25274, MPEG 25277, MPEG 25278, MPEG 25303, MPEG 25304, MPEG 25305; *Chironius scurrulus:* MPEG 25275, MPEG 25276; *Dendrophidion dendrophis*: MPEG 21243, MPEG 25093, MPEG 25094; *Drymarchon corais:* MPEG 25160, MPEG 25312; *Drymoluber dichrous:* MPEG 24620, MPEG 25100, MPEG 25101, MPEG 25102, MPEG 25103, MPEG 25104, MPEG 25110, MPEG 25111, MPEG 25112;*Leptophis ahaetulla*: MPEG 25194, MPEG 25195, MPEG 25196, MPEG 25197, MPEG 25198, MPEG 25199, MPEG 25200; *Mastigodryas boddaerti:* MPEG 21237, MPEG 21238, MPEG 25221, MPEG 25222, MPEG 25223, MPEG 25224, MPEG 25225, MPEG 25226, MPEG 25228, MPEG 25229; *Oxybelis aeneus*: MPEG 25113, MPEG 25114, MPEG 25115, MPEG 25116, MPEG 25117, MPEG 25118, MPEG 25119, MPEG 25120; *Oxybelis fulgidus:* MPEG 25121, MPEG 25122, MPEG 25123, MPEG 25124, MPEG 25125, MPEG 25269, MPEG 25270, MPEG 25271, MPEG 25272; *Phrynonax polylepis*: MPEG 25090, MPEG 25091, MPEG 25092; *Rhinobothryum lentiginosum*: MPEG 25232; *Spilotes pullatus*: MPEG 25233, MPEG 25234, MPEG 25235, MPEG 25236, MPEG 25299, MPEG 25300; *Spilotes sulphureus*: MPEG 21139, MPEG 25301; *Tantilla melanocephala*: MPEG 25147, MPEG 25148. **Dipsadidae**: *Atractus boimirim*: MPEG 25259, MPEG 25260, MPEG 21233; *Atractus snethlageae*: MPEG 25149, MPEG 25150; *Dipsas catesbyi*: MPEG 25097; *Dipsas pavonina*: MPEG 25095, MPEG 25096; *Drepanoides anomalus:* MPEG 25130, MPEG 25131, MPEG 25132, MPEG 25133, MPEG 25134; *Erythrolamprus aesculapii:* MPEG 25129, MPEG 21230, MPEG 25231, MPEG 24550, MPEG 26778; *Erythrolamprus oligolepis:* MPEG 25158, MPEG 25315, MPEG 25316, MPEG 25317; *Erythrolamprus poecilogyrus:* MPEG 25098, MPEG 25099; *Erythrolamprus reginae:* MPEG 21245, MPEG 24615, MPEG 25285, MPEG 25286, MPEG 25287, MPEG 25288; *Erythrolamprus taeniogaster*: MPEG 25248, MPEG 25313; *Erythrolamprus typhlus:* MPEG 21242, MPEG 25282, MPEG 25283, MPEG 25284; *Helicops angulatus*: MPEG 24617, MPEG 25201, MPEG 25202, MPEG 25203, MPEG 25204, MPEG 25205, MPEG 25206, MPEG 25206, MPEG 25207, MPEG 25208, MPEG 25209, MPEG 25210, MPEG 25211, MPEG 25212, MPEG 25213, MPEG 25214, MPEG 25215, MPEG 25216, MPEG 25217, MPEG 25218, MPEG 25219, MPEG 25220; *Helicops polylepis*: MPEG 27229;*Hydrodynastes bicinctus bicinctus:* MPEG 25230, MPEG 25231, MPEG 25232; *Hydrops martii*: MPEG 25249; *Imantodes cenchoa:* MPEG 21234, MPEG 21235, MPEG 25165, MPEG 25166, MPEG 25167, MPEG 25168, MPEG 25169, MPEG 25170, MPEG 25171, MPEG 25172, MPEG 25173, MPEG 25174, MPEG 25175, MPEG 25176, MPEG 25177, MPEG 25178, MPEG 25179, MPEG 25180, MPEG 25181, MPEG 25182, MPEG 25183, MPEG 25184, MPEG 25185,

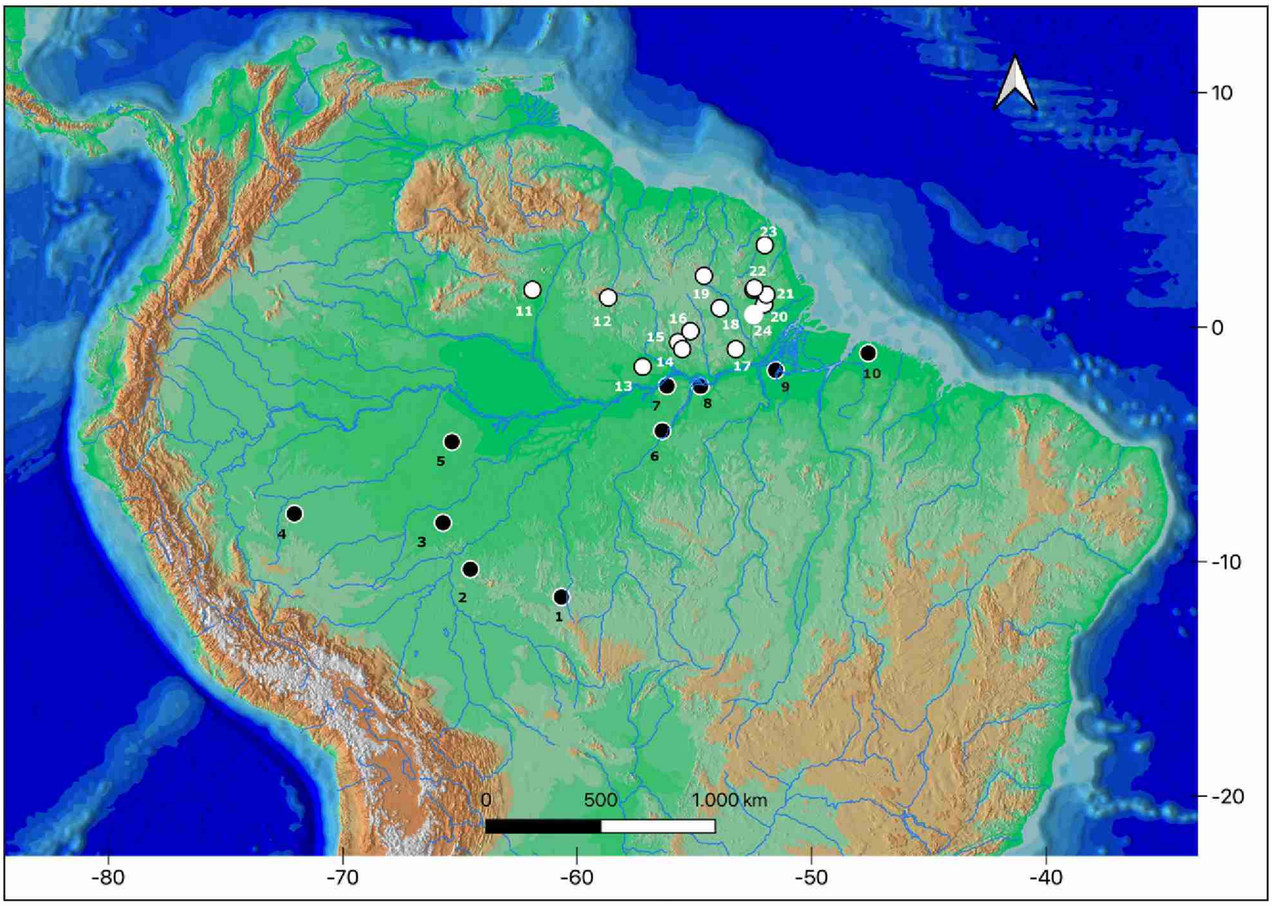
MPEG 25186, MPEG 25187, MPEG 25188, MPEG 25189, MPEG 25190, MPEG 25191, MPEG 25192, MPEG 25193; *Imantodes lentiferus:* MPEG 25238, MPEG 25239; *Leptodeira annulata:* MPEG 25105, MPEG 25106, MPEG 25107, MPEG 25108, MPEG 25109; *Oxyrhopus formosus:* MPEG 25126, MPEG 25127, MPEG 25128; *Oxyrhopus melanogenys:* MPEG 25240, MPEG 25241, MPEG 25242, MPEG 25243, MPEG 25244; *Philodryas argentea:* MPEG 25250; *Philodryas viridissima:* MPEG 21239, MPEG 25246,MPEG 25247; *Pseudoboa coronata:* MPEG 21244, MPEG 25135, MPEG 25136, MPEG 25137; *Pseudoeryx plicatilis:* MPEG 25245; *Siphlophis cervinus:* MPEG 25314; *Siphlophis compressus:* MPEG 25138, MPEG 25139, MPEG 25140, MPEG 25141, MPEG 25142, MPEG 25143, MPEG 25144, MPEG 25145, MPEG 25146; *Taeniophallus brevirostris*: MPEG 25253, MPEG 25255; *Taeniophallus quadriocellatus:* MPEG 25252, MPEG 25254, MPEG 25256, MPEG 25257, MPEG 25258, MPEG 24613, MPEG 24616; *Xenodon rhabdocephalus:*MPEG 21225; *Xenopholis scalaris:* MPEG 25261, MPEG 25262, MPEG 25263, MPEG 25264.**Elapidae**: *Micrurus hemprichii*: MPEG 25063; *Micrurus lemniscatus*: MPEG 25066, MPEG 25068; *Micrurus paraensis*: MPEG 25065; *Micrurus spixii*: MPEG 25064. **Leptotyphlopidae**: *Trilepida macrolepis:* MPEG 24618, MPEG 25046, MPEG 25047, MPEG 25048. **Typhlopidae**: *Amerotyphlops reticulatus*: MPEG 25044, MPEG 25045. **Viperidae**: *Bothrops atrox*: MPEG 25087, MPEG 25071, MPEG 25072, MPEG 25074, MPEG 25076, MPEG 25077, MPEG 25078, MPEG 25298, MPEG 25081, MPEG 25082, MPEG 25083, MPEG 25073, MPEG 25084, MPEG 25085, MPEG 25087, MPEG 25079, MPEG 25080; *Bothrops bilineatus*: MPEG 25070; *Bothrops brazili:* MPEG 25075; *Bothrops taeniatus*: MPEG 25069; *Lachesis muta*: MPEG 25088.

APPENDIX - Table. List of snake communities located south (1-10) and north (11-24) of the Amazon River, used for comparisons. Legends: TCS= Time constrained search; RS= road search by car; PTF= Pitfall traps with drift fences; CTP= Captures by third parties; OE = Occasional enconters; AS= active sampling; and TE = third-party encounters

Site number	Site name	Sampling methods	Number of species	Characteristic of vegetation	References
1	Município de Espigão do Oeste, Rondônia State (11030'S; 60o40'W)	TCS (960 hours), PTF, TE, OE	55	Deforested and transformed into pasture and farming areas	Bernarde and Abe (2006)
2	Parque Estadual de Guajará-Mirim, Rondônia State (10019'S; 64o33'W)	AS	39	Reduced hunting pressure due to its protected status as a state park	Ávila-Pires *et al.* (2009)
3	Rio Ituxi, Amazonas State (8° 20'S; 65°43'W)	AS	32	Consisted of a mix of varzea and terra firme forest.	Ávila-Pires *et al.* (2009)
4	Reserva Extrativista Riozinho da Liberdade, Acre State (7° 57'S; 72°04'W)	TCS (720 hours), PTF (90 days), OE	42	Open Ombrophilous Forest, characterized by the open dossel and the abundance of palm trees, liana or bamboo	Bernarde *et al.* (2011)
5	Província Petrolífera de Urucu, Amazonas State (4°53'S; 65°20'W)	TCS (51 days), RS, PTF, OE	45	Ombrophilous Forest, Terra Firme.	Prudente *et al.* (2010)
6	Município de Itaituba, Pará State (03°50'S, 56°32'W)	TCS (1,220 hours), RS, PTF (22 days), TE, OE	68	Dense submontane ombrophilous forest of low plateau and dense submontane forest of flattened relief and lowmountain ranges	This study
7	Município de Juruti, Pará State (02°09'S; 56°05'W)	TCS (1,160 hours), PTF (94 days), TE, OE	74	Dense ombrophilous forest, with lattosoil type Dystrophic Oxisol	Menks (2012)
8	Município de Belterra (2.5166 S; 54.733 W)	TCS, RS, PTF, TE, OE	53		Frota *et al.* (2005)
9	FLONA de Caxiuanã, Pará State (1o47'S;	TCS (2,639 hours), PTF (132 days), TE, OE	70	Dense ombrophilous forest	Maschio *et al.* (2009)

10	Município de Barcarena, Pará State (1°30’S; 48°37’W)	TCS (336 hours), PTF (37 days), OE	38	Primary Forest (vegetation without significant human disturbance) and degraded areas or areas in early stages of plant regeneration after suffering great human action	Silva *et al.* (2011)
11	Parque Nacional da Serra da Mocidade, Roraima State (01°36'N; 61°54'W)	AS (22 days), PTF (22 days), trammel nets, hook with meat bait, glue traps, shotgun.	12	Dense forests, shrubby vegetation or moss forests, surrounded by a lowland matrix of either tropical forests or savanna ecosystems	Moraes *et al.* (2017)
12	Estação Ecológica do Grão Pará (ESEC Grão Pará – North), Pará State (1°17’N; 58°41’W)	AS (200 hours), PTF (240 days)	16	Terra-firm forest, with steep slopes and creeks	Avila-Pires *et al.* (2010)
13	Floresta Estadual de Faro, Pará State (1°42’ S; 57°12’ W)	AS (144 hours), PTF (192 days)	12	Terra-firme forest (3 trails); flooded forest (1 trail); disturbed areas	Avila-Pires *et al.* (2010)
14	Floresta Estadual Trombetas, Pará State(0°57’ S; 55°31’ W)	AS (118 hours), PTF (237 days)	13	Terra-firme forest with creeks (some areas with many Cecropia); an open area with rock outcrop	Avila-Pires *et al.* (2010)
15	Estação Ecológica do Grão Pará (ESEC Grão Pará – Centre), Pará State (0°37’N; 55°43’W)	AS (260 hours), PTF (240 days)	12	Transition zone at the W margin of a large island of terra-firme forest within a large savanna enclave.	Avila-Pires *et al.* (2010)
16	Estação Ecológica do Grão Pará (ESEC Grão Pará – South), Pará State (0°9’S; 55° 11’W)	AS (170 hours), PTF (208 days)	10	Terra-firme forest with creeks bordered by flooded areas; an extensive lake bordered by palms	Avila-Pires *et al.* (2010)
17	Floresta Estadual Paru, Pará State (0°56’S; 53° 14’W)	AS (96 hours), PTF (208 days)	10	Terra-firme forest with creeks and different degrees of perturbation; (dry) flooded forest along margin of river	Avila-Pires *et al.* (2010)
18	Reserva Biológica de Maicuru, Pará State (0°49’N; 53°55’ W)	AS (220 hours), PTF (240 days)	10	Terra-firme forest and (dry) flooded forest; small beach along margin of river	Avila-Pires *et al.* (2010)
19	Parque Nacional Montanhas do Tumucumaque 2, Amapá State (2º11’36”N; 54º35’15”W)	AS (400 hours), PTF	13	Terra-firme forest	Lima (2008)
20	Parque Nacional Montanhas do Tumucumaque 4, Amapá State (1º23’13”N; 51º55’39”W)	AS (400 hours), PTF	14	Terra-firme forest	Lima (2008)
21	Parque Nacional Montanhas do Tumucumaque 1, Amapá State (1°36’N; 52°29’W)	AS (400 hours), PTF	13	Terra-firme forest	Lima (2008)

22	Parque Nacional Montanhas do Tumucumaque 5, Amapá State (1°50’41”N; 52°44’28”W)	AS (300 hours), PTF	20	Terra-firme forest	Lima (2008)
23	Parque Nacional Montanhas do Tumucumaque 3, Amapá State (3°29’51”N; 52°18’0”W)	AS (400 hours), PTF	11	Terra-firme forest	Lima (2008)
24	Serra do Navio Region, Amapá State (01°00’N; 52°03’W)	AS(784 hours), PTF (98 days), TE, OE	57	Terra-firme forest	Prudente *et al.* (2020)



APPENDIX - Figure. List of snake communities located south (1-10) and north (11-24) of the Amazon River, Brazil (see Table). Legends: 1 - Município de Espigão do Oeste; 2 -Parque Estadual de Guajará-Mirim; 3- Rio Ituxi; 4- Reserva Extrativista Riozinho da Liberdade; 5 - Província Petrolífera de Urucu; 6 - Município de Itaituba; 7 - Município de Juruti; 8 - Município de Belterra; 9 - FLONA de Caxiuanã; 10 - Município de Barcarena; 11 - Parque Nacional da Serra da Mocidade; 12 - Estação Ecológica do Grão Pará (ESEC Grão Pará – North); 13 - Floresta Estadual de Faro; 14 - Floresta Estadual Trombetas; 15 - Estação Ecológica do Grão Pará (ESEC Grão Pará – Centre); 16 - Estação Ecológica do Grão Pará (ESEC Grão Pará – South); 17 - Floresta Estadual Paru; 18 - Reserva Biológica de Maicuru; 19 - Parque Nacional Montanhas do Tumucumaque 2; 20- Parque Nacional Montanhas do Tumucumaque 4; 21 - Parque Nacional Montanhas do Tumucumaque 1; 22- Parque Nacional Montanhas do Tumucumaque 5; 23- Parque Nacional Montanhas do Tumucumaque 3; 24 - Serra do Navio Region.

Trabajo

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Diet of two sympatric species of the *Leptodactylus fuscus* group: *Leptodactylus longirostris* (Boulenger, 1882) and *Leptodactylus mystaceus* (Spix, 1824) in the Brazilian Amazon rainforest

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ABSTRACT

Leptodactylus longirostris and *L. mystaceus* are sympatric species of frogs occurring in the Amazon basin in forest areas. In this study, diet composition, niche overlap and niche breadth were described for the two of species from the north domain of the Brazilian Amazon forest. A total of 68 individuals of *L. longirostris* and 43 individuals of *L. mystaceus* were analyzed. The most important prey for *L. longirostris* was Hymenoptera (Formicidae) and for *L. mystaceus* was Coleoptera. The niche breadth of *L. mystaceus* was wider than the another species. The value of the niche breadth ($B \leq 0.50$) of both sympatric frogs suggests a specialization on ants (Formicidae) and beetles (Coleoptera).

Key Words: Species coexistence; Food items; Leptodactylidae; Feeding overlap.

RESUMEN

Dieta de dos especies simpátricas del grupo *Leptodactylus fuscus*: *Leptodactylus longirostris* (Boulenger, 1882) y *Leptodactylus mystaceus* (Spix, 1824) en la selva amazónica brasileña. *Leptodactylus longirostris* y *L. mystaceus* son especies simpátricas de ranas que se encuentran en la cuenca del Amazonas en áreas forestales. En este estudio, se describió la composición de la dieta, la superposición de nichos y la amplitud de nichos para las dos especies del dominio norte de la selva amazónica brasileña. Se analizaron un total de 68 individuos de *L. longirostris* y 43 individuos de *L. mystaceus*. La presa más importante para *L. longirostris* fue Hymenoptera (Formicidae) y para *L. mystaceus* fue Coleoptera. La amplitud del nicho de *L. mystaceus* era más amplia que la de la otra especie. El valor de la amplitud del nicho ($B \leq 0.50$) de ambas ranas simpátricas sugiere una especialización en hormigas (Formicidae) y coleópteros (Coleoptera).

Palabras claves: Coexistencia de especies; Items-Presa; Leptodactylidae; Superposición de alimentación.

Introduction

Anurans occupy an important position in trophic chains from both terrestrial and aquatic environments, being important consumers of arthropods at adult stages (Duellman and Trueb, 1994). According to Toft (1980) neotropical anurans can be classified in a continuum between specialist predators, that feed on a specific prey, or generalists, that feed on arthropods in general. The trophic guilds and functional traits related to food niche dimension identified in the studies of Toft (1980) and subse-

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quent studies demonstrate that the trophic axis of ecological niche has an important role structuring anuran community (Lima and Magnusson, 1998; Vignoli and Luiselli, 2012; Cloyed and Eason, 2017).

Within anuran communities, the occurrence of ecologically similar species is important to understanding patterns of competition and partition of resource in trophic niche dimension (Leibold and McPeek, 2006; Vogt *et al.*, 2017). Cogeneric species exhibits very similar ecological demands and so may be expected to show intense competition for limiting resources (Holt, 1977). Resource partitioning can reduce competition and promotes the coexistence of congeneric species that, presumably, occupy similar niches. In Neotropical region, due to their high diversity, anuran communities often contain sympatric congeneric species that share similar microhabitats.

The genus *Leptodactylus* Fitzinger, 1826 are one of the most diverse genera in Neotropical region, widely distributed in South America (Frost, 2020). Particularly, *L. fuscus* species group has the great number of species (De Sá *et al.*, 2014), in which the coinciding range of distribution of the different species may lead to sympatric occurrences. Over the upper Amazon Basin region, *Leptodactylus longirostris* and *Leptodactylus fuscus* occurs in sympatry over a portion of their ranges.

Leptodactylus longirostris Boulenger, 1882 is a small to moderate sized frog, snout-vent-length (female 33.3–45.6 mm; male 33.1–44.2 mm, De Sá *et al.*, 2014). This species occurs in the Guiana Shield region and in the Brazilian states of Amazonas, Pará, Roraima and Amapá. It is commonly found occupying open and forested areas (Crombie and Heyer, 1983; De Sá *et al.*, 2014; Dias-Souza *et at.*, 2018). *Leptodactylus mystaceus* (Spix, 1824) has a moderate size (female 44.5–56.1 mm; male 42.4–52.2 mm, De Sá *et al.*, 2014), widely distributed in the Amazon basin throughout Brazil (Affonso *et al.*, 2016). In Guiana Shield region of Amapá state, *L. mystaceus* and *L. longirostris* were found occurring in sympatry in savanna and open forest areas (Lima *et al.*, 2017; Silva e Silva and Costa-Campos, 2018).

Both species share some biological traits, but currently there is no information about diet composition for *L. longirostris* and *L. mystaceus* occurring in sympatry across open forest environments in Eastern Amazon. Understanding trophic interactions is essential for the development of successful conservation strategies at local scale (Young *et al.*, 2001; Wells, 2007). In Neotropics, effects associated with landscape fragmentation cause alteration in trophic interactions, which may contribute to population declines. Herein, the diet composition of two sympatric species *L. longirostris* and *L. mystaceus* in the north domain of the Brazilian Amazon, Guiana Shield region were analyzed to improve its conservation status of these species and provide management tools in these environments. Four questions are made: 1) which prey types was consumed by each species? 2) what was the level of niche overlap and niche breadth between the two frog species? 3) there were relationships between the body size and jaw width with the prey volume? 4) did the diet of *L. longirostris* and *L. mystaceus* is similar to other species of the genus?

Materials and methods

Study area – Sampling was carried out in the Parque Natural Municipal do Cancão, Serra do Navio Municipality (0°54'9.9" N, 52°0'19.9" W), Amapá State, Brazil (Fig. 1). This area comprises 370.26 hectares within the Amazon forest, including vegetation of *terra firme* rainforests, streams, open areas, and treefall gaps (Silva e Silva and Costa-Campos, 2018). It is situated in the monsoon climate (climate symbol "Am") with precipitation values ≥ 60 mm in the driest month, according to the Köppen-Geiger (Peel *et al.*, 2007).

Sampling –Individuals of *L. longirostris* and *L. mystaceus*, were deposited in the Herpetological Collection at the Universidade Federal do Amapá (CECC), in the city of Macapá, Amapá State, Brazil. Individuals

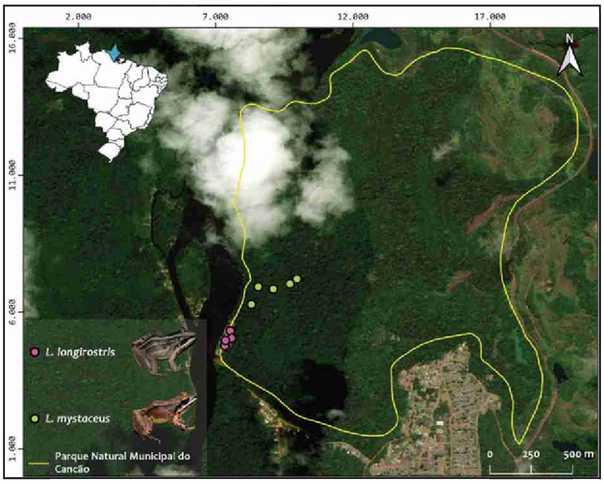

Figure 1. Map of the Parque Natural Municipal do Cancão, Serra do Navio municipality, Amapá State, Brazil, indicating the points where *Leptodactylus longirostris* and *Leptodactylus mystaceus* were collected.

were collected by hand using the visual and auditory search method (Heyer *et al.,* 1994) from October 2017 to March 2018.

Laboratory protocols – Each individual was euthanized with lidocaine overdose, fixed in 10% formalin, and conserved them in 70% alcohol solution, according to the collection license provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio #48102-2). Individuals was dissected for the removal of stomach contents (stomach and intestine considered as a single sample) through a ventral incision and analyzed with the aid of a stereoscopic microscope ZEISS model Stemi 2000-C. The stomach flushing technique (Solé *et al.,* 2005) was not used because the examined anurans were also used for another study, which required euthanasia to obtain samples. The identification of the prey consumed was conducted out at the taxonomic level of order according to the identification key of Rafael et al. (2012).

Analyses – The obtained items were measured as length and width. We estimated the prey volume using the ellipsoid formula $V = 4\pi/3*L/2*(W/2)^2$, where V = volume, L = prey length, and W = prey width (Colli and Zamboni, 1999). The obtained items were measured as length and width (with a caliper to the nearest 0.1 mm). The Index of relative importance (IRI) of each prey category, proposed by Pinkas et al. (1971), following the equation: $IRI = (F\% + N\% + V\%)/3$, where IRI = Index of relative importance; $F\%$ = frequency of occurrence; $N\%$ = numerical frequency; $V\%$ = volumetric frequency of each category of prey item were calculated.

To access the level of specificity of the diet, the trophic niche breadth was calculated using the Levins index (B) as described by Pianka (1986), following the equation: ($B = 1/\Sigma p_j^2$), where B = niche breadth and pj = proportion of item j in the diet. In this case, when the value of B is between 0 and 0.50 the species was considered as specialist, and values between 0.51 and 1.0 was named generalist species.

Trophic Niche Overlap Index of Pianka (*Ojk*) (Pianka, 1974) as follow,

$$\emptyset_{JK} = \sum_{i=1}^{n} p_{ij}\, p_{ik} \Big/ \sqrt{\sum_{i=1}^{n} p_{ij^2} \sum_{i=1}^{n} p_{ik^2}},$$

where *Ojk* is the niche overlap index between the species *j* and *k*; *Pij* and *Pik* are the proportions of prey categories consumed by the species; *j*, *k*, *n* are the total number of resource categories consumed by species *j* and *k*. Index ranged from 0 to 1, in which values equal to zero indicate absence of overlap, and values equal to 1 point out to a complete overlap between species' diets (Krebs, 1999). This analysis was performed using EcoSim Version 7.71 (Entsminger, 2014 free trial http://www.garyentsminger.com/ecosim/index.htm).

To analyze the sampling size and taxonomic richness of prey consumed by the two leptodactylids species rarefaction curves based on the number of specimens and food items were plotted using Estimates 9.1 (Gotelli and Colwell, 2001 free trial http://purl.oclc.org/estimates). Principal Component Analysis (PCA) and One-Way ANOVA analysis to test if diet composition varies between *L. longirostris* and *L. mystaceus* were performed. PCA analysis conducted in the R software version 4.0.3 (R Development Core Team, 2017) using the vegan package (Oksanen *et al.,* 2015).

Snout-vent length (SVL) and jaw width for each frog specimen using a digital caliper (accuracy precision 0.01 mm) were measured. To test if the SVL or jaw width affects prey volume consumed simple linear regression as independent variables was used using prey-items volume as dependent variables (Zar, 1999). The values of prey items were log-transformed ($\log_{10}$) to fit requirements of normality. The analyses were performed Bioestat 5.0 software (Ayres *et al.,* 2007), using $p < 0.05$ as the significance level.

Results

Leptodactylus longirostris diet

Nine prey categories from the stomach contents of 68 *L. longirostris* specimens were determined. From the total, 27 (39.7%) stomachs were empty or composed of unidentifiable preys on advanced stage of decomposition. The three most abundant categories in terms of volume and frequency were Formicidae, Coleoptera and Hemiptera (Table 1). Other prey categories with large volumetric contribution, that had a low frequency in the stomachs, were Lepidoptera (larvae), Blattaria and Araneae. The IRI results showed that the most important preys, in decreasing order, were Formicidae (IRI = 77.05), Coleoptera (IRI = 71.10) and Hemiptera (IRI = 20.68). Results of correlation tests indicated that both jaw width (JW) and SVL were not correlated with variation in largest ingested prey (SVL, $F = 1.328$, $p = 0.259$; JW, $F = 0.847$, $p = 0.630$).

Table 1. Numerical frequency (N%), frequency of occurrence (F%), volume (V%) and Index of relative importance (IRI) in the diet of *Leptodactylus longirostris* and *Leptodactylus mystaceus* from the Serra do Navio municipality, Amapá State, Brazil.

Prey category	***Leptodactylus longirostris***							***Leptodactylus mystaceus***						
	N	**N (%)**	**F**	**F (%)**	**V**	**V (%)**	**IRI**	**N**	**N (%)**	**F**	**F (%)**	**V**	**V (%)**	**IRI**
Araneae	4	5.06	3	7.14	16.58	2.26	12.96	3	21.43	3	25.00	57.30	2.04	16.16
Coleoptera	23	29.11	13	30.95	242.98	33.11	71.10	8	57.14	6	50.00	572.07	20.38	42.51
Hemiptera	10	12.66	3	7.14	19.39	2.64	20.68	---	---	---	---	---	---	---
Hymenoptera	2	2.53	2	4.76	11.63	1.58	7.82	---	---	---	---	---	---	---
Formicidae	27	34.18	13	30.95	262.35	35.75	77.05	---	---	---	---	---	---	---
Ponerineae	1	1.27	1	2.38	5.85	0.80	3.91	---	---	---	---	---	---	---
Solenops sp.	1	1.27	1	2.38	0.65	0.09	3.68		---	---	---	---	---	---
Lepidoptera larvae	2	2.53	2	4.76	84.00	11.45	11.11	---	---	---	---	---	---	---
Orthoptera	---	---	---	---	---	---	---	2	14.29	2	16.67	1954.29	69.63	33.53
Isoptera	4	5.06	2	4.76	15.78	2.15	10.54	---	---	---	---	---	---	---
Blattaria	1	1.27	1	2.38	71.48	9.74	6.89	---	---	---	---	---	---	---
Insecta larvae	4	5.06	1	2.30	3.19	0.43	7.59	---	---	---	---	---	---	---
Annelida	---	---	---	---	---	---	---	1	7.14	1	8.33	222.88	7.94	7.81

Leptodactylus mystaceus diet

The stomach contents from 43 individuals, of which 11 (25.6%) stomachs were empty or contained only unidentifiable preys on advanced stage of decomposition were determined. Prey items into four taxonomic categories were classified. The most abundant prey categories were Coleoptera and Araneae. As for volume the categories with largest representation were Orthoptera and Coleoptera. The IRI revealed a great importance of Coleoptera and Orthoptera. We did not find a correlation between JW and SVL of predator and the volume of the largest prey ingested (SVL, $F = 0.299$, $p = 0.602$; JW, $F = 0.006$, $p = 0.936$).

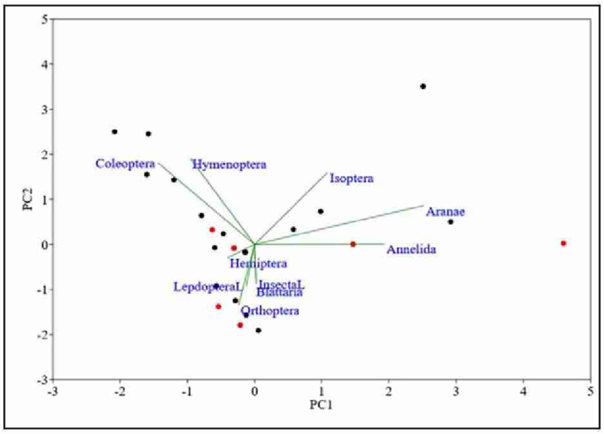

Figure 2. Principal components analysis to graphically represent the differences in the diet of *Leptodactylus longirostris* (black dots) and *Leptodactylus mystaceus* (red dots) from Brazilian Amazon rainforest.

Niche measures

Diet composition did not differentiate between both frogs species ($F = 9.168$, $df = 11$, $p = 0.359$; Fig. 2). Araneae and Coleoptera were common prey consumed by both species. Items consumed exclusively by *L. longirostris* were Blattaria, Hymenoptera (Formicidae), Hemiptera, Lepidoptera (larvae), Isoptera. While Orthoptera and Annelida were items consumed exclusively by *L. mystaceus*. Considering the abundance of prey items in each food category, *L. mystaceus* had a slightly larger niche (Levins standardized niche) $B_{st} = 0.5$ than *L. longirostris* $B_{st} = 0.39$. The trophic niche overlap of Pianka Index was 59% ($Ojk = 0.59$) considering the abundance of prey items in each prey category. The rarefaction curve of taxonomic prey richness did not reach the asymptote for *L. longirostris* and *L. mystaceus*, indicating prey richness is still underestimated (Fig. 3).

Discussion

Diet composition of *L. longirostris* and *L. mystaceus* were similar, indicating a trend through of *L. fuscus* species group to be conservative in feeding behavior (Silva and Rossa-Feres, 2010). The most important prey items were ants (Formicidae) for *L. longirostris* and beetles (Coleoptera) for *L. mystaceus*. Ants and beetles comprise two of the most diverse and abundant arthropod groups in terrestrial ecosystems of

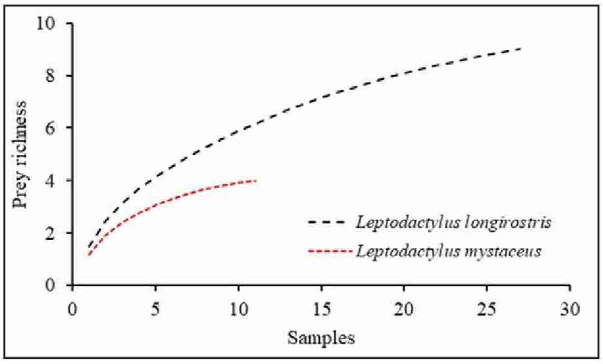

Figure 3. Rarefaction curves of the *Leptodactylus longirostris* and *Leptodactylus mystaceus* based on prey richness to the number of individuals sampled.

Neotropical region (Lofgren, 1986; Hölldobler and Wilson, 1990). Their abundance in leaf litter makes them prey of easy consumption present in the diet of many anurans from the genus *Leptodactylus* (Camera *et al.,* 2014; Baía *et al.,* 2020).

The great number of strongly chitinized preys such as ants and beetles, may be due to the opportunistic feeding behavior adopted by *Leptodactylus* species (Toft, 1981; Solé and Rödder, 2010; Santana *et al.,* 2019). However, this genus also preys on anurans (*L. macrosternum*, Sousa *et al.,* 2016; *L. podicipinus*, Ceron *et al.,* 2018; *L. chaquensis*, Cuestas-Carrillo *et al.,* 2019; *L. mystaceus*, Moreira-Brito *et al.,* 2020), small mammals (Castro *et al.,* 2011; Marques-Pinto *et al.,* 2019), as well as other sedentary invertebrate preys (e. g. larval forms of insects). It would be a reflect of the passive foraging mode of *Leptodactylus* species, which "sit and wait" for prey (Toft, 1980; 1981), eating more uncommon prey that comes in their way.

The niche breadth of *L. mystaceus* was broader than that of *L. longirostris*. One possible explanation may be that its has a diet more equitable with regards to prey distribution even this species consumed a lower number of preys. The niche breadth is narrowed as the abundance is increased toward a few prey categories within the set of prey (Pianka, 1974). This explain the niche breadth of *L. longirostris* is narrower due to the high consume of ants compared to other prey categories.

In addition, another possible explanation is that dietary niche breadth decreases as interspecific competition increase. Competition is caused by limiting ecological resources such as food, which drives to specialization that allows coexistence through segregation of resources explored between species, leading to a narrowing of niche breadth (Gainsbury and Meiri, 2017). It would explain the presence of ants in the diet of *L. longirostris*, as ants are an uncommon food category found in the diet of *Leptodactylus* species (Toft, 1980; Rebouças and Solé, 2015).

The high niche overlap (*Ojk* > 0.50) recorded for *L. longirostris* and *L. mystaceus* reinforced that sympatric and congeneric amphibian species have similar diets (Baía *et al.,* 2020). This variation in diet richness found among these species may be influenced by prey size, mobility, palatability, availability and abundance in the environment (Menin *et al.,* 2005; Almeida *et al.,* 2019). The last may be explain the low number of prey items in the diet of *L. mystaceus*.

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Trabajo

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Breeding success and conservation of *Eretmochelys imbricata* in the Delta do Parnaíba Environmental Protection Area, northeastern Brazil

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ABSTRACT
Sea turtles are one the most threatened group worldwide mainly due to environmental disturbances caused by anthropogenic actions. Herein, we aimed to investigate the breeding success of hawksbill turtles in two beaches of Piauí state, contained within the Delta do Parnaíba Environmental Protection Area. Turtle nests were monitored according to the methodology proposed by Projeto TAMAR-ICMBio. A multi-model inference approach using Akaike's Information Criterion (AICc) obtained through the Generalized linear models (GLM) was used to investigate what are the best predictors of hawksbill turtles' breeding success. We observed that vegetation distance is the main driver of its breeding success (each nest survival rate), reinforcing the needed to preserve the natural vegetation cover existing in coastal environments. With our constant monitoring of nests avoiding predatory attacks, it was allowed the protection of almost four thousand eggs and the consequent liberation of more than three thousand live hatchlings. Therefore, we think that studies, as presented here, are important for the maintenance and conservation of marine turtle populations and, in particular, to subsidize the protection and conservation of hawksbill turtles in the Parnaíba River Delta.

Key Words: Hawksbill turtle; Breeding success; Parnaíba River Delta; Piauí; Sea turtles.

Introduction

Five of the seven species of sea turtles visit the Brazilian coast (Costa and Bérnils, 2018), being both considered threatened species (IUCN, 2021), in which, human-induced mortality is also the main cause to explain decline of sea turtle populations. It includes sea pollution; reproductive habitat destruction; incidental capture; and human hunting (National Research Council, 1990; Mcclenachan *et al.*, 2006; Marcovaldi *et al.*, 2011).

Among these sea turtles, *Eretmochelys imbricata* (Linnaeus, 1766), commonly known as hawksbill turtle, is classified as critically endangered and its population decreases worldwide (Mortimer and Donnelly, 2008; IUCN, 2021), despite being widely distributed in the Brazilian coastal zone (Valls *et a*l., 2011; Brito *et al.*, 2015; Costa and Bérnils, 2018). This species usually tends to nest in or near vegetation, mainly in heterogeneous environments (Diamond, 1976; Kamel and Mrosovsky, 2005), nevertheless, there is some lack of information about what are the best predictors for hawksbill turtles' nests choice and the nests sites in the Brazilian coast. For instance, we believed for a long time, that hawksbill turtle Brazilian spawning areas were distributed from Espirito Santo to Ceará states, with hatchlings occurring in the north-northeast Brazilian coast (Marcovaldi *et*

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al., 2011), however, it was found nests of hawksbill turtles also in the Delta do Parnaíba Environmental Protection Area, Piauí state (Santana *et al.*, 2009).

Studies dealing with the breeding biology of hawksbill turtles date from the seventies (Diamond, 1976), henceforth, several studies have been development worldwide. For instance, Kamel (2013) observed that vegetation cover predicts temperature in nests of the hawksbill sea turtle, for consequence, influencing the offspring sex ratios. At Guadeloupe, French West Indies, it was reported a significant repeatability of nest site choice, suggesting a heritable behavior in hawksbill turtles (Kamel and Mrosovsky, 2005). With respect to Brazilian coast, most studies were focusing on hatching success, mainly concentered in the coastal zone of Bahia, Pernambuco, and Rio Grande do Norte states, northeastern Brazil (e.g., Marcovaldi *et al.*, 1999; Serafini *et al.*, 2009; Santos *et al.*, 2010; Moura *et al.*, 2012), being scant studies in the north-northeast Brazilian coast.

Although in Brazilian beaches the spawning peak of most sea turtle species occurs in spring and summer months, from September to March (Marcovaldi and Marcovaldi, 1999), little is known about the reproductive biology of hawksbill turtles in the coastal zone of Piauí state. Therefore, herein (i) we report key data about the spawning peak and breeding success of *Eretmochelys imbricata*, (ii) investigate what are the best predictors of hawksbill turtles' breeding success in the Delta do Parnaíba Environmental Protection Area, and (iii) discuss the importance of this area for preservation and conservation of the species.

Materials and methods

Study area

The present study was carried out in two beaches from the Delta do Parnaíba Environmental Protection Area (EPA): Praia do Coqueiro (02°54'10" S, 041°33'51" W) and Praia do Arrombado (02°54'10" S, 041°32'50" W), both in Luís Correia municipality, Piauí state, northeastern Brazil (Fig. 1). The EPA Delta do Parnaíba was created by a Federal Decree

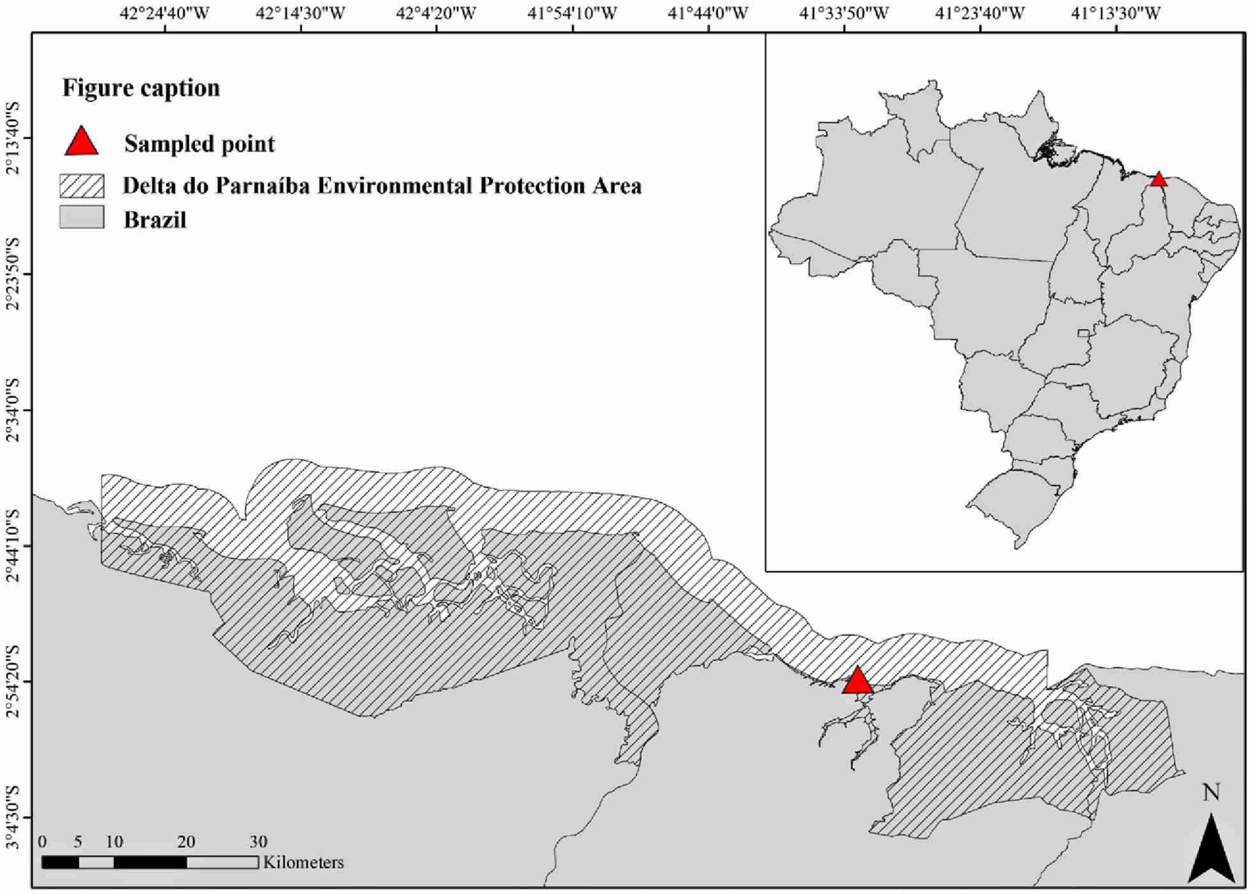

Figure 1. Schematic map of the sampled area in Praia do Coqueiro (LC) and Praia do Arrombado (AT), Delta do Parnaíba Environmental Protection Area (EPADP), Piauí state, northeastern Brazil.

on 28 August 1996 and involves 313.809 ha in three states (Maranhão, Piauí and Ceará) with a perimeter of 460.812 m, including the maritime area (IBAMA, 1998).

Sampling

Sea turtles breeding sampling was taken according to the Projeto TAMAR-ICMBio (Marcovaldi and Marcovaldi, 1999) between summer and autumn months (December 2011 to May 2012). We monitored the hawksbill turtle (Fig. 2) nests along a 12 km straight line between the two sampled beaches during night hours. Nests were localized either according to presence of females or by detecting their crawls. Once a nest was detected it was immediately marked with PVC pipe to facilitate monitoring and isolated with wood stakes to avoid anthropic disturbances (Pritchard and Mortimer, 1999). Monitoring was made periodically every 24 hours since egg-laying to hatching. Once most hatchlings have emerged, we excavated nests within the posterior 24 hours in order to known clutch size by counting eggshells and unhatched eggs (Miller, 1997). A hatchling voucher specimen was deposited in the Zoological Collection of the Universidade Federal do Delta do Parnaíba – UFDpar (Voucher: CZDP (J1) 0003).

Statistical analyzes

To measure the hawksbill turtle breeding success, we used the apparent reproductive success (number of successful clutches/total of clutches), live hatchlings percentage (number of hatchlings that entered into the ocean/clutch size) x 100 (Skutch, 1985), and the Mayfield method (Mayfield, 1975) to obtain the survival probability estimate considering the nests incubation mean period (60 days).

Through the live hatchlings percentage, we calculated the survival rate (SR) of each one of the 27 hawksbill turtle nests, thenceforth, we did a multi-model inference approach using the Akaike's Information Criterion (AICc) (Burnham and Anderson, 2002) to measure the direction and relative importance of the predictor variable (SR) on the response variables (nest distance from the Restinga vegetation, nest distance to ocean, clutch size, and incubation period). The model with the lowest value

Figure 2. Adult female of *Eretmochelys imbricata* (hawksbill turtle) in Delta do Parnaíba Environmental Protection Area (EPADP), Piauí state, northeastern Brazil.

of AICc was considered further adjusted (Burnham and Anderson, 1998). We ran these statistical models as generalized linear models (GLMs) with Poisson error structure and log-link function. In addition, we used the VIF values to test for potential collinearity between our explanatory variables, and we used the Spearman's rank test (rho) to show graphically the most correlated variables.

Statistical analyses were performed in R software (R Core Team, 2021), using Corrplot (Wei and Simko, 2017), MuMIn (Barton, 2009), RColorBrewer (Harrower and Brewer, 2003), USDM (Naimi *et al.*, 2014), and Vegan packages (Oksanen *et al.*, 2016).

Results

We found 27 well-successful clutches of *Eretmochelys imbricata* during the breeding period that began on 24 December 2011 and end on 01 May 2012 with a peak of nests observed in February and March (32% and 39%, respectively). These clutches involve 3.870 eggs that were protected and conduct to let out in ocean 3.148 live hatchlings. Clutch size varied from 97 to 221 eggs-laying in the nests, with a mean of 142.7 eggs/nest (Fig. 3).

The apparent reproductive success and survival probability estimate was 93% and 91.8%, respectively, while the eggs hatching success was 81.3%. The model selection results indicate that nest distance from the Restinga vegetation is the most important predictor for the hawksbill turtle breeding success, which means, nests closest to natural vegetation tend to present a higher nest survival rate. In addition, the clutch size has also an important influence on the predictor variable (Fig. 4, Table 1). No variable from the 5 input variables has collinearity problem (VIF values ranged from 1.087662 to 2.076622).

Discussion

Previous studies recorded a hatching success varying from 58.3% (eleven nests registered/ one breeding season) in Costa Rica (Bjorndal *et al.*, 1985), 65.6% (350 nests registered/ three breeding seasons) in Ipojuca municipality, Pernambuco state (Moura *et al.*, 2012), 73.3% (141 nests registered/ four breeding seasons) on Cousine Island, Seychelles (Hitchins *et al.*, 2004), 78.3% (38 nests registered/ three breeding seasons) in Itacaré and Uruçuca municipalities, Bahia state (Camillo *et al.*, 2009), to 79.8% (41 nests registered/ one breeding season) in João Pessoa and Cabedelo municipalities (Mascarenhas *et al.*, 2003). Indeed, we found a high hatching success percentage (81.3%, 27 nests registered/ one breeding season), but we also suggest more studies should be undertaken in the Parnaíba River Delta aiming to investigate how this hatching success rate might vary according to the nesting seasons.

As important as describing the aspects of spawning peak and breeding success is to investigate what are the best predictors of hawksbill turtles' breeding success in the Delta do Parnaíba Environmental

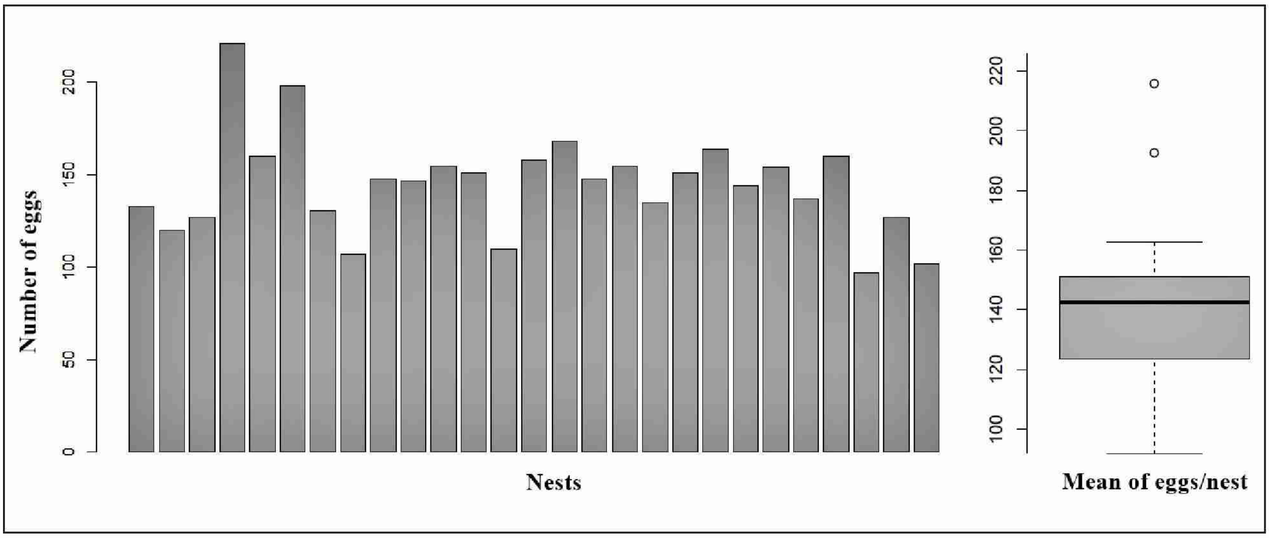

Figure 3. Number of eggs in each one of the 27 nests (left) and mean of eggs/nest (right) of Eretmochelys imbricata in Delta do Parnaíba Environmental Protection Area (EPADP), Piauí state, northeastern Brazil.

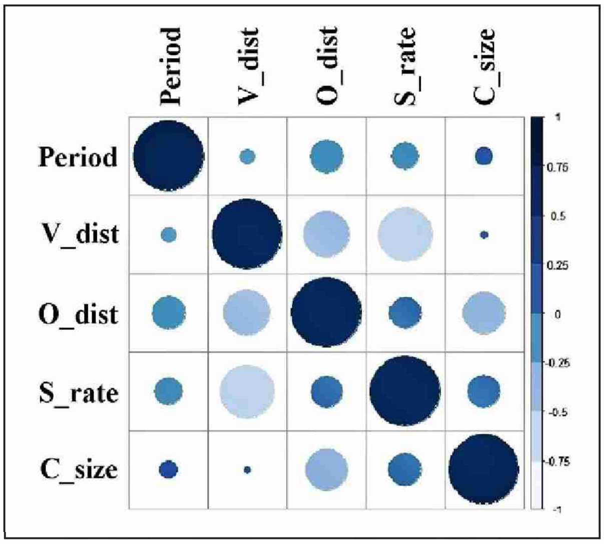

Figure 4. Correlation between the variables nests survival rates (S_rate), incubation period (Period), clutches sizes (C_size), nest distance from the ocean (O_dist), and Restinga vegetation (V_dist). As larger the circles more correlated are the variables and the blue tones variation indicates the positive or negative correlation sign.do Parnaíba Environmental Protection Area (EPADP), Piauí state, northeastern Brazil.

Protection Area. Although hatch success is a complex process, involving multiple environmental and individual variables, it was observed the vegetation is an important component of hawksbill nesting habitat in eastern Caribbean (Ditmer and Stapleton, 2012). Likewise, Horrocks and Scott (1991) found that hatching success of hawksbill nests was higher in the vegetation zone in Barbados, also in Caribbean, and Kamel and Mrosovsky (2005) associated the hatching success with the vegetated zone in Guadeloupe, French West Indies. Our results corroborate these above-cited studies once we observed the most important predictor for the hawksbill turtle nests survival rates, consequently breeding success, was the natural vegetation cover proximity.

In addition, preserving natural vegetation cover at hawksbill nesting beaches is crucial in order to maintain the thermal diversity of nesting sites and, potentially, mitigate the impacts of increasing global temperatures (Kamel, 2013). Therefore, we also underscore the needed to maintain healthy these Restinga environments and to investigate how beaches' environmental characteristics as sand structure, Restinga physiognomies, illumination, and pollution influence the nest choice and survival, and the consequent breeding success of the Sea turtles along the Parnaíba River Delta.

Regarding the clutch size, we observed an average of 142.7 eggs/nest, being similar to the mean fecundity registered in Ipojuca municipality, Pernambuco state (Moura *et al.*, 2012), in Itacaré and Uruçuca municipalities, Bahia state (Camillo *et al.*, 2009), in João Pessoa and Cabedelo municipalities (Mascarenhas *et al.*, 2003), in Guadeloupe, French West Indies (Kamel and Mrosovsky, 2005), and in Costa Rica (Bjorndal *et al.*, 1985). In addition, it is important to point out that clutches size was an important driver for nest survival, consequently the hawksbill turtle breeding success in this area.

Furthermore, nest predation may be considered one the main reasons of failure on the life cycle of sea turtles (Spencer, 2002). Predation of nests depends of environmental nest characteristics and the number of predators in the area (Tinkle *et al.*, 1981). Likewise, nesting site choice has also an important influence on sea turtle reproductive success (Kamel and Mrosovsky, 2005). Indeed, we observed potential nest predators in the study sites but in reason nests were exhaustively monitored predation was avoided, allowing also a high breeding success of *Eretmochelys imbricata.*

We found three nests / km^2 an average value similar to those registered in Porto de Galinhas and Maracaípe beaches, Pernambuco state (Moura *et al.*, 2012), but lower than the reported for other sites along the Brazilian northeastern coastline (Mascarenhas*et al.*, 2003; Camillo *et al.*, 2009; Moura *et al.*, 2012). We think that beach characteristics as coral reefs, artificial light, and high tourist flow, mainly in Praia do Coqueiro, might have inhibited nests choice by hawksbill turtles in the coastal zone of Piauí state. Thus, it should be crucial to preserve beaches into which this threatened turtle choice to nest and the Parnaíba River Delta as an important spawning area for hawksbill turtles.

The environmental monitoring of *Eretmochelys imbricata* in the Delta do Parnaíba Environmental Protection Area, northeastern Brazil provided observation of important biological aspects and describe details of this sea turtle breeding period in the Piauí state coastline, besides underscore and reinforce the importance of the Restinga environments for hawksbill turtles' breeding success. Overall, we protected and let out more than three thousand live hatchlings of hawksbill turtles, thus, our study is important for maintenance and conservation, besides being able to subsidize actions aimed at the protection and conservation of hawksbill turtles in the Parnaíba River Delta.

Table 1. Delta AICc and associated measures from all possible models predicting the nests survival rates of *Eretmochelys imbricata* in Parnaíba River Delta

Model	K	AICc	ΔAICc	Weight	LogLik
V_dist	3	223.3	0.00	0.262	-108.151
C_size + V_dist	4	223.4	0.07	0.253	-106.798
O_dist + V_dist	4	224.2	0.86	0.171	-107.193
Period + V_dist	4	225.7	2.40	0.079	-107.966
C_size + O_dist + V_dist	5	225.8	2.48	0.076	-106.486
C_size + Period + V_dist	5	225.9	2.52	0.074	-106.506
Period + O_dist + V_dist	5	226.3	2.99	0.059	-106.739
C_size + Period + O_dist + V_dist	6	228.3	4.91	0.022	-106.030
C_size	3	234.0	10.66	0.001	-113.484
Null	2	235.1	11.75	0.001	-115.300
C_size + O_dist	4	236.4	13.06	0.000	-113.293
C_size + Period	4	236.6	13.27	0.000	-113.400
Period	3	237.6	14.24	0.000	-115.274
O_dist	3	237.6	14.28	0.000	-115.294
C_size + Period + O_dist	5	239.3	16.00	0.000	-113.244
Period + O_dist	4	240.3	17.00	0.000	-115.262

Abbreviations: incubation period (Period), clutches sizes (C_size), nest distance from the ocean (O_dist), and Restinga vegetation (V_dist).

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Trabajo

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Foraging behavior and diet composition of the gecko *Phyllopezus periosus* (Squamata: Phyllodactylidae) in the Brazilian semiarid Caatinga

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ABSTRACT
In this study, we provide new data about the foraging behavior and feeding ecology of *Phyllopezus periosus*, a large-sized gecko endemic from the semiarid Caatinga, northeastern Brazil, looking to answer the following questions: (1) how is the foraging mode of *P. periosus*? (2) Which types of prey compose its diet? (3) Are lizards selective in diet? (4) Is prey size correlated with lizard body size? The fieldwork consisted of two ten-day excursions during the dry season in the Seridó Ecological Station, state of Rio Grande do Norte. We registered foraging behavior with a voice recorder and determined movement patterns of each focal lizard. For dietary data, we used stomach flushing to obtain consumed prey items. Prey availability was estimated with sets of pitfall traps placed in the surroundings of rocky outcrops where lizards occur. Our results indicate that *P. periosus* is a typical sit-and-wait forager, spending most of the time immobile (proportion time moving: 1.7 ± 1.9%, number of moves per minute: 0.4 ± 0.3) and performing mainly head moves and postural adjustments. The diet was massively composed of beetles, present in 48.4% of stomachs, representing 44.4% of total prey items and 63.5% of total volume. Lizards consumed beetles in a proportion slightly higher than their proportional availability in the environment, suggesting some preference for this prey category. Males and females presented similar body sizes and did not differ in diet composition. Body size was correlated with maximum prey size, but not with minimum prey size, suggesting that lizards in this population add larger prey items to the diet while growing, but at the same time continue consuming smaller ones. In addition, records of predation upon sympatric lizards and tree sap foraging evidence that *P. periosus* has opportunistic feeding habits.

Key Words: Electivity; Gekkota; Prey size; Saurophagy; Sit-and-wait foraging.

Introduction

Lizard diets are influenced by both intrinsic and extrinsic factors (Pianka, 1986). In seasonal environments, lizards often switch food based on availability, and diets tend to be more generalized when food is scarce (Pianka, 1970; Dunham, 1981; Carne and Measey, 2013; Sales and Freire, 2015). Besides extrinsic factors, foraging behavior, body size, sex, and ontogeny are important intrinsic factors that might influence lizard diets (Huey and Pianka, 1981; Perry, 1996; Vitt, 2000; Sales *et al.*, 2012). Moreover, lizards of some squamate clades, such as Gekkota (geckos and flap-footed lizards), have a developed

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chemosensory system that allow discrimination of prey (Cooper, 1995), hence their diets may not include all range of available prey because individuals are able to avoid less profitable or potentially toxic items (Vitt and Pianka, 2005; Lisboa *et al.*, 2012).

The methods of food acquisition in lizards have been historically classified dichotomically as active (or wide) and sit-and-wait (or ambush) foraging (Pianka, 1966; Schoener, 1971). Active foragers move through the habitat searching for prey by sight and by frequently tongue-flicking to collect chemical cues, whereas sit-and-wait foragers usually remain immobile while scanning visually for approaching prey (Huey and Pianka, 1981). From the classic study of Pianka *et al.* (1979), which diffused quantitative parameters to describe foraging (proportion time moving – PTM; moves per minute – MPM), a continuum of foraging strategies have been identified in lizards, from highly sedentary ambushers that remain most of the time immobile to extreme active foragers that spend more than 80% of time moving while searching for prey (Perry, 1999; 2007).

Most geckos are considered sit-and-waits foragers, presenting low levels of PTM and MPM (Cooper *et al.*, 1999; Perry, 1999; Werner and Chou, 2002). However, a fluctuating foraging mode was identified in some species, characterized by relatively long periods of sedentary behavior, alternating with short bouts of very high activity (Werner *et al.*, 1997; 2006; Perry, 1999). This strategy presumably enables these nocturnal visual predators to ambush mobile prey while immobile, without wasting locomotor energy, and occasionally to actively search for sedentary prey, thereby increasing feeding opportunities (Werner *et al.*, 1997).

Phyllopezus periosus Rodrigues, 1986 (Fig. 1) is one of the largest Brazilian geckos (snout-vent length up to 126 mm; Passos *et al.*, 2013), endemic from the oriental portion of the Caatinga region, northeastern Brazil, from Ceará to Sergipe states (Passos *et al.*, 2013; Mesquita *et al.*, 2017). Information on the ecology of this species includes data on thermal ecology, daily activity and microhabitat use (Andrade *et al.*, 2013; Passos *et al.*, 2013; Ragner *et al.*, 2014), parasite infection (Almeida *et al.*, 2008), reproduction (Lima *et al.*, 2011), and some anecdotal observations about diet (Andrade *et al.*, 2016). *Phyllopezus periosus* has nocturnal activity and shows a fidelity for areas with rocky outcrops in the Caatinga, where it stays on rocky surfaces and climbs trees in the vicinity (Andrade *et al.*, 2013; Passos *et al.*, 2013).

In this study, we provide new data about the foraging behavior and feeding ecology of *P. periosus*, looking to answer the following questions: (1) how is the foraging mode of *P. periosus*? (2) Which types of prey compose its diet? (3) Is *P. periosus* selective in diet or does it capture all available prey in the environment? (4) Is prey size correlated with lizard body size? Our initial hypothesis and predictions were: (1) *P. periosus* behaves as a typical sit-and-wait forager, hence low values of PTM and MPM would be detected in the focal observations; (2) *P. periosus* presents a generalist diet directly associated to prey availability, hence a wide range of prey categories would be found in the stomach contents, and a significant correlation would exist between diet composition and prey availability in the environment; (3) lizards undergo an ontogenetic shift in the size of prey because larger lizards are able to capture larger prey while juveniles are gape limited, hence maximum prey size would to be correlated with lizard size; and (4) due to generalist feeding habits, larger lizards tend to capture all available prey regardless of their sizes, hence minimum prey size would not be correlated with lizard size.

Materials and methods

Study site

The Seridó Ecological Station (ESEC Seridó; central point: 06°34'36.2"S, 37°15'20.7"W, datum: WGS84, altitude: 192 m) comprises about 1165 hectares and is located in the municipality of Serra Negra do Norte, Rio Grande do Norte state, northeastern Brazil. The climate is semiarid, hot and dry (Bswh in Köppen classification) and the average temperature ranges from 28° C to 30° C; relative humidity ranges from 30 to 50% during dry months and from 50 to 70% in the rainy season (Varela-Freire, 2002). The ESEC Seridó is located at the "Depressão Sertaneja Setentrional" ecoregion of the Caatinga, and rainfall irregularity is a major feature of this ecoregion, as there is a quite pronounced water deficit for most of the year; the average annual precipitation ranges from 500 to 800 mm (Velloso *et al.*, 2002).

Fieldwork and morphometry

The fieldwork consisted of two ten-day excursions during the dry season in the study site, one in July 2015 and the other in October 2015. During each excursion, we dedicated a total of eight days for collecting dietary data, conducted by two collectors

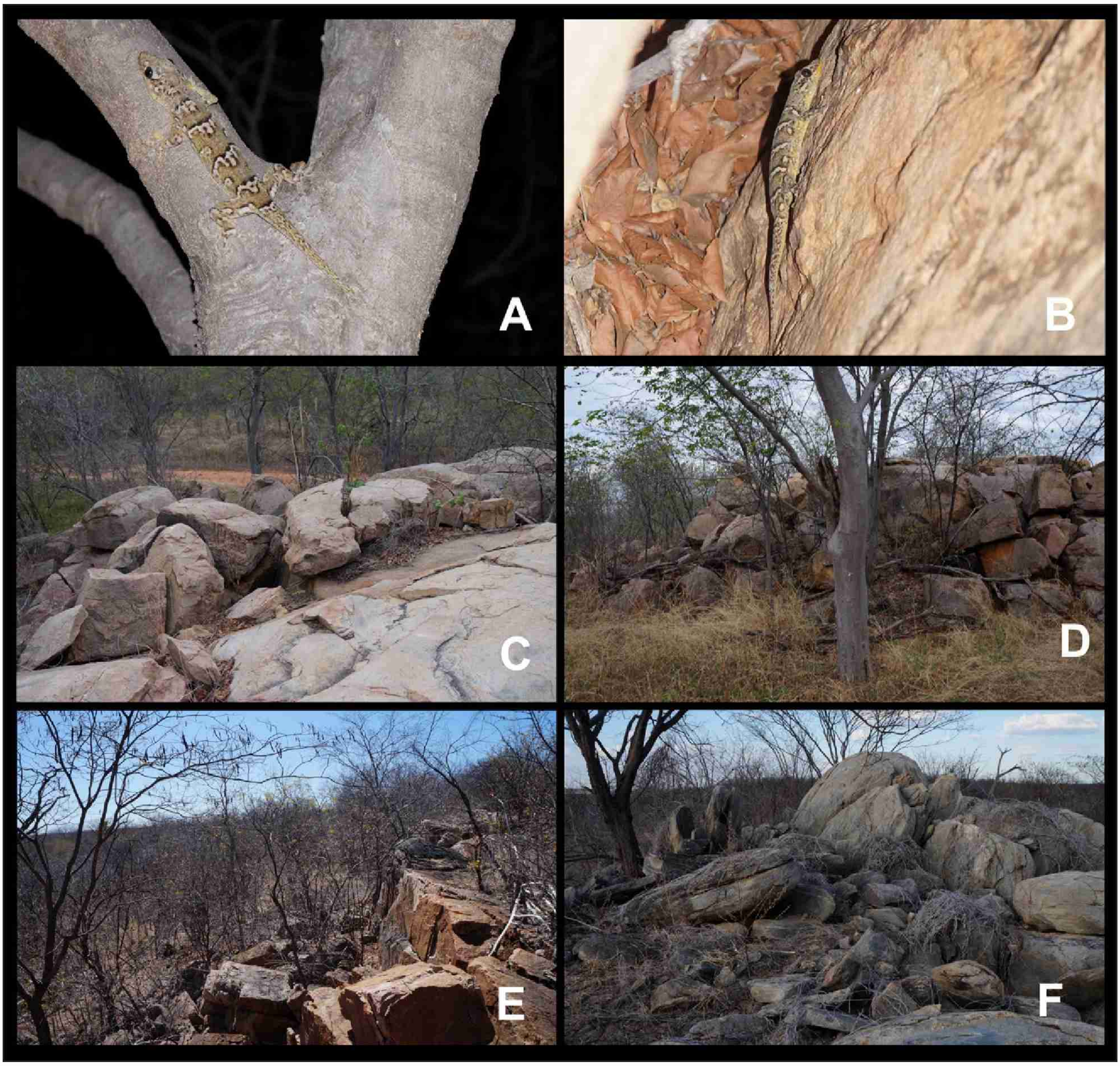

Figure 1. Adult individuals of *Phyllopezus periosus* recorded (A) perched on a tree close to a rocky outcrop and (B) on a boulder of a rocky outcrop, in Seridó Ecological Station, Rio Grande do Norte state, northeastern Brazil. (C-F) General view of rocky outcrops with crevices and shrubs and trees nearby, the habitat of *P. periosus* in the study site. (Photographed by Cristiane Palmeira).

at the same time (CNSP and UG), and two days for collecting behavioral data, conducted by a single observer (CNSP) in an exclusive observational area with 265 square meters (i.e. not surveyed for capturing lizards), hence different lizards were used for evaluation of diet composition and foraging behavior to avoid influence of capture on lizard behavior. The greater sampling effort spent to collect dietary data is justified by the fact that *Phyllopezus periosus* is difficult to capture manually, due to the facility of the lizards to shelter in refuges inside rock crevices.

To locate lizards in the field, we performed haphazard active searches, walking slowly through areas with rocky outcrops and arboreal-shrubby vegetation previously identified, where *P. periosus* occurs (Fig. 1). We alternated sampling times from 1700 to 0000 on one day, from 0000 to 0600 on the second day, and so on, covering the entire nighttime, which corresponds to the activity period of *P. periosus* (Andrade *et al.*, 2013). The sampling effort during the two field campaigns totaled 104 hours for gathering dietary data, and 26 hours for gathering behavioral data. We changed locations every 60 minutes of sampling effort to increase the chances of locating different lizards.

We recorded foraging behavior without marking the lizards before or after focal observations. Thus, to avoid pseudo-replication, we noted with a GPS device the location where each focal lizard was and did not survey it again (Perry, 2007). To collect dietary data, we captured the lizards by hand and took them alive to the research facilities of ESEC Seridó. Then, with the aid of a digital caliper (0.01 mm precision), we took the following measurements: snout-vent length (SVL), from the tip of the snout to the anterior end of cloaca; head length (HL), from the posterior margin of the tympanum to the tip of the snout; head width (HW) at the widest point of the skull; head height (HH) at the maximum height of the skull; and jaw length (JL), from the tip of the snout to the labial commissure. We determined the sex of the animals by injecting a small amount of mineral water with a disposable syringe at the base of the tail near the cloaca; with this procedure, the hemipenis was everted in males. The classification of individuals into adult and juvenile categories was done according to Rodrigues (1986), considering juveniles those individuals with higher number of well-defined dorsal stripes and SVL < 70 mm, and adults those lizards with gold-yellow belly and SVL > 70 mm. We marked the captured lizards with a non-toxic ink to avoid pseudo-replication and released them and in their respective capture locations.

Foraging behavior

To register foraging behavior, when a lizard was located, the observer stopped moving and waited 5 min with the head flashlight on (355 lumens = 1649.74 lux) but not directed to the lizard to reduce the effect of human disturbance. After this habituation time, the observation period was started, with the observer remaining static and always keeping a minimum distance of 3 m from the animal. The flashlight was not aimed at the lizard, but laterally so that the periphery of the beam of light was sufficient to visualize it. If the lizard showed some indication of disturbance by human presence (e.g., increased head bobbing, flee the foraging spot at high speed), the observation was discontinued. Some of the locomotion of the individuals was towards the observer, hence presumably the observer did not affect them (Werner *et al.*, 1997). Only adults were sampled, and since we did not collect focal lizards (observational areas were different from areas used for dietary data), we could not determine their sex. We observed only individuals that showed any sign of foraging behavior, such as changing the direction of the head, tongue-flicking the substrate, turning the whole body to another direction, slowly crawling, and/or "micromoves", which are quick moves of a few seconds, followed by immobility (Werner *et al.*, 1997).

Whenever possible, each lizard was observed for up to 15 min (Perry, 2007); however, observations of ≥ 5 min were included in our sample. We classified the focal animal as either "immobile" or "moving" based on its behavior. Lizards were considered immobile when their behavior did not result in displacement (postural changes, movements of head, limbs or tail, turning the body to another direction), whereas lizards exhibiting translational movements (displacement to a new location; e.g., walks, races, jumps), were considered moving. We noted the beginning and the end of each movement performed by the focal animal using an audio recorder. We adopted the methodology used by Werner *et al.* (1997) and Sales and Freire (2015) in the observations, defining a movement as an outbreak of locomotion separated from others through pauses of >1 sec. That is, when a moving animal stopped, the observer counted one second mentally before considering the movement as finished and defined the focal animal in the "immobile" category. The duration of each movement was calculated by summing the time between the beginning and the end of lizard mobility provided in the audio recordings. After completing the analysis of recordings, we calculated PTM and MPM for each focal lizard.

Diet composition

To obtain stomach contents of captured lizards, we used the stomach-flushing method described by Solé *et al.* (2005), with some adaptations. The flushing was done without any anaesthetizing and with careful handling of the animals. Lizards were held in the midbody, with the forelimbs and head supported by the fingers of the same hand. With the other hand, the mouth was opened with the help of a spatula, and a plastic probe was carefully inserted until just after the esophagus. Then mineral water was flushed into the stomach using a syringe of 20 mL for juveniles and of 60 mL for adults. When the water started to return through the mouth, the lizard was positioned vertically with the mouth down and its abdominal region was massaged to stimulate regurgitation of stomach contents, which were collected in an empty plastic container. This procedure was repeated three times in each lizard to increase the chances of collecting the entire stomach contents. All retrieved food items were labeled and preserved in 70% ethanol. We kept stomach-flushed lizards under observation for 10 hours for survival control after the technique (Legler and Sullivan, 1979).

Stomach contents were spread in petri dishes and examined using a stereomicroscope to identify the ingested food items usually to the taxonomic level of order (Sales and Freire, 2015). Ants (Formicidae) were treated as an exclusive prey category in the order Hymenoptera. Vertebrates (lizards) were identified to the level of species by comparing prey items with voucher specimens of the Herpetology Collection of Rio Grande do Norte Federal University (UFRN-CH). We measured the length and width of each prey item with the help of millimeter-ruled graph paper, and the volume was estimated by the formula for a prolate spheroid: $V = 4/3\ (\text{length}/2)\ (\text{width}/2)^2$ (Dunham, 1983). We determined the frequency of occurrence of each prey category as the number of stomachs containing the prey category *i*, divided by the total number of stomachs. Also, we calculated the numeric and volumetric percentages of each prey category for the pooled stomachs. We calculated the importance index (*I*) for each prey category by the formula: (frequency of occurrence + numerical percentage + volumetric percentage)/3 (Mesquita and Colli, 2003). This index was calculated for males, females, juveniles, and the total sample.

To evaluate prey availability, we placed 12 pitfall traps made up of 500 mL plastic pots (three sets of four pots) buried at ground level, placed about four to five meters away from each other, surrounding the rocky outcrops. Each pitfall remained open during four days in each field excursion, in daytime and nighttime periods. The pitfalls contained 150 mL of 93° ethanol to preserve individuals; 50 g of salt (sodium chloride) to prevent the biological material from disintegrating in the solution; 25 mL of neutral detergent to break the surface tension of the water; and 225 mL of water to mix all the mentioned ingredients (Costa TB, pers. communication). We identified the collected material to the level of order to compare with stomach contents.

For each lizard, we calculated maximum and minimum prey size by considering the items with the largest and smallest volumes in each stomach, respectively. Lizards that ingested fewer than two prey items were excluded from prey-size analyses due to incompatibility of estimating both maximum and minimum prey sizes (Sales *et al.*, 2012).

Statistical analyses

We tested for differences in body size (SVL) between males and females using a Student's t-test. To assess sexual differences in head dimensions (HL, HW, HH, JL), we performed analyses of covariance (ANCOVA), with SVL as the covariate. The degree of qualitative similarity in the diet of adult males and females was examined with the Pianka's Overlap Index – O_{jk} (Pianka, 1973), in which values range from 0 (no similarity) to 1 (complete similarity). The Mann-Whitney U test was applied to verify the existence of sexual differences in diet for the number of food items ingested and the volumetric sum of all prey items inside the stomach. The electivity of prey was calculated using the Ivlev's electivity index (Krebs, 1999): $IEI = (ri - ni)/(ri + ni)$, where ri is the percentage of prey category *i* in diet, and ni is the percentage of prey category *i* in environment. The values of this index range from -1 to +1, with values near +1 indicating preference, values near -1 indicating rejection, and values near 0 indicating consumption in the same proportion of the environment. We used Spearman's correlation to verify the relationship between diet composition and prey availability, using the number of prey items of each category. Linear regressions were performed to test the relationship between lizard size (SVL, HL, HW, HH, JL) and prey size (maximum and minimum prey volume), with all variables $\log_{10}$-transformed to meet the requirements of normality. All statistical analyses were performed using SPSS Statistics 20.0 software for Windows, with α set at 0.05. Before performing all the parametric tests, all variables were tested for normality and homoscedasticity of variances. Throughout the text, the descriptive statistics are represented as a mean ± standard deviation (SD).

Results

Morphometry

We collected 45 specimens of *Phyllopezus periosus*, 18 adult males, 20 adult females and seven juveniles. The mean SVL did not differ between sexes (males: 103.0 ± 12.2 mm, females: 101.2 ± 17.3 mm; $t = 0.360$, $df = 34.1$, $p = 0.721$). HL (males: 25.6 ± 2.4 mm, females: 25.7 ± 3.4 mm; $F_{1,35} = 2.098$, $p = 0.156$), JL (males: 17.9 ± 2.3 mm, females: 17.9 ± 2.3 mm; $F_{1,35} = 1.939$, $p = 0.173$), and HW (males: 18.3

± 1.8 mm, females: 17.9 ± 2.4 mm; $F_{1,35} = 0.325$, p = 0.572) were also similar between sexes, but HH was significantly higher in males (males: 11.3 ± 1.6 mm, females: 10.4 ± 1.9 mm; ANCOVA, $F_{1,35} = 7.160$, p = 0.011).

Foraging behavior

We recorded foraging behavior of 21 adult individuals of *P. periosus*. Total observation time reached 208 min and averaged 10.0 ± 2.0 min (range: 5.9 – 13.0 min) for each individual. Twelve focal lizards (57.1%) were in rock crevices, six lizards (28.6%) were above exposed rocks, and three lizards (14.3%) were on "catingueira" trees (*Cenostigma pyramidale*) in the vicinity of rocky outcrops, perched 2.5 to 4 meters from the ground. Focal lizards usually tongue-flicked the substrate and slowly moved the head sideways or upwards (at an angle of 90°) while immobile, presumably looking for prey by using both visual and chemical cues. They spent most of the time immobile, performing only directional moves with the head or body (i.e. turning the body to another direction). Translational moves were brief and resulted in displacement for short distances (some centimeters). When moving, lizards made jumps (possibly trying to capture some prey), slowly crawled the substrate or made "micromoves". Average PTM was 1.7 ± 1.9% (range: 0–6.5%), and average MPM was 0.4 ± 0.3 (range: 0–1.03). Although the recorded jumps were possibly prey capture attempts, we did not record successful prey capture episodes (i.e. lizard chewing after the capture attempt). Nonetheless, one lizard perched on a "catingueira" was observed feeding on the sap of the tree, moving its tongue slowly to obtain the sap released by a natural crack in the tree bark.

Diet composition

After the stomach-flushing procedure, only six lizards (three females and three males; 13.3% of total sample) did not regurgitate prey items, indicating that they had empty stomachs. Plus, eight individuals (four females and four juveniles; 17.8% of total sample) contained stomach contents in an advanced degree of digestion, making it impossible to identify any food item. In the other lizards (15 males, 13 females and three juveniles), we identified a total of 90 food items distributed in 16 prey categories, mostly arthropods (Table 1). The main prey category consumed by *P. periosus* was Coleoptera, present in 48.4 % of stomachs, representing 44.4% of total prey items, 63.5% of total volume, and with a notably higher importance index compared to other prey categories ($I = 52.1$). Besides invertebrates (arthropods and mollusks) and some plant matter, we also identified two vertebrates in the stomach contents of *P. periosus*: the leaf-toed gecko *Hemidactylus agrius* (several body parts in high degree of digestion, evidencing consumption of the entire lizard) and the lava lizard *Tropidurus semitaeniatus* (only the tail, suggesting partial consumption).

Coleoptera was the predominant prey category in the diet of adult males (N = 15) and adult females (N = 13; Table 1). Both sexes ingested 11 different prey categories, and dietary niche overlap was high, both based on numerical ($O_{jk} = 0.705$) and in volumetric percentages ($O_{jk} = 0.914$). The number of prey items ingested (males: median = 4 items, females: median = 3 items; Mann-Whitney U = 84.5, p = 0.555) and the volumetric sum of all prey items inside the stomach (males: median = 125.6 mm^3; females: median: 287.1 mm^3; Mann-Whitney U = 84.0, p = 0.555) were also statistically similar between sexes. Juveniles (N = 3) presented a quite different diet composition, with consumption of only four prey categories, mainly Blattodea and Pseudoscorpiones (Table 1), but the small sample size does not allow major comparisons with adults.

The most registered taxa in the environment were Formicidae (41.8%), Coleoptera (20.2%), Collembola (9.2%), Diptera (8.6%), Blattodea (6.8%) and Acari (5.1%). The comparison between the diet composition of *P. periosus* and prey availability (Table 2) suggests that Coleoptera was slightly elected (IEI = 0.37) and Formicidae was avoided (IEI = -0.58). Moreover, some prey categories proportionally less consumed by *P. periosus*, such as Scorpiones, Phasmatodea, Mantodea, Gastropoda, Lepidoptera larvae and Vertebrata, showed positive values of electivity (Table 2). Diet composition and prey availability in the environment were not significantly correlated ($r_s = 0.352$, p = 0.071).

The body size of lizards showed a positive association with maximum prey volume ($R^2 = 0.247$, $F_{1,22} = 7.219$, p = 0.013, $y = 2.72x + 3.41$; Fig. 2), but not with minimum prey volume ($R^2 = 0.042$, $F_{1,22} = 0.964$, p = 0.337; Fig. 2). Head and mouth dimensions (HL, HW, HH, JL) also showed positive associations with maximum prey volume (R^2 values from 0.188 to 0.328, p-values < 0.05), but not with minimum prey volume (R^2 values from 0.012 to 0.056, p-values > 0.05).

Table 1. Diet composition of *Phyllopezus periosus* at the Seridó Ecological Station, Rio Grande do Norte, Brazil. F = frequency of occurrence, N = number, V = volume (mm3), I = importance index (T = total sample – N = 31; M = adult males – N = 15; F = adult females – N = 13; J = juveniles – N = 3). "—" indicates no individuals of that prey category were found.

Prey category	F (%)	N (%)	V (%)	IT	IM	IF	IJ
Acari	3 (9.7)	3 (3.3)	0.5 (<0.1)	4.3	6.1	3.4	—
Araneae	3 (9.7)	3 (3.3)	42.7 (0.5)	4.5	6.4	3.5	—
Blattodea	2 (6.5)	6 (6.7)	139.1 (1.7)	4.9	3.8	—	47.7
Coleoptera	15 (48.4)	40 (44.4)	5236.4 (63.5)	52.1	46.1	65.8	—
Formicidae	8 (25.8)	10 (11.1)	183.4 (2.2)	13.0	12.6	16.4	—
Gastropoda	1 (3.2)	1 (1.1)	659.4 (8.0)	4.1	—	8.6	—
Hemiptera	1 (3.2)	1 (1.1)	68.2 (0.8)	1.7	—	3.9	—
Lepidoptera (larvae)	2 (6.5)	3 (3.3)	193.8 (2.4)	4.0	4.8	4.1	—
Mantodea	1 (3.2)	1 (1.1)	26.7 (0.3)	1.6	—	3.6	—
Plant material	3 (9.7)	5 (5.5)	85.4 (1.0)	5.4	11.6	—	—
Orthoptera	5 (16.1)	5 (5.5)	157.6 (1.9)	7.9	13.4	3.6	—
Phasmatodea	1 (3.2)	1 (1.1)	613.9 (7.4)	3.9	8.3	—	—
Pseudoscorpiones	3 (9.7)	3 (3.3)	0.9 (<0.1)	4.3	—	—	43.6
Scorpiones	2 (6.5)	2 (2.2)	103.9 (1.3)	3.3	—	3.9	27.0
Thysanura	4 (12.9)	4 (4.4)	68.4 (0.8)	6.1	6.5	3.5	14.9
Vertebrata (lizards)	2 (6.5)	2 (2.2)	475.7 (5.8)	4.8	10.2	—	—
U.A.R.	6 (19.4)	—	188.1 (2.3)	—	—	—	—

Table 2. Diet composition of *Phyllopezus periosus* at the Seridó Ecological Station, Rio Grande do Norte, Brazil. F = frequency of occurrence, N = number, V = volume (mm3), I = importance index (T = total sample – N = 31; M = adult males – N = 15; F = adult females – N = 13; J = juveniles – N = 3). "—" indicates no individuals of that prey category were found.

Taxon	Diet (%)	Environment (%)	IEI
Acari	3 (3.3)	141 (5.1)	-0.21
Araneae	3 (3.3)	62 (2.2)	0.20
Blattodea	6 (6.7)	189 (6.8)	-0.01
Chilopoda	—	1 (<0.1)	-1
Coleoptera	40 (44.4)	559 (20.2)	0.37
Coleoptera (larvae)	—	2 (<0.1)	-1
Collembola	—	254 (9.2)	-1
Diplopoda	—	1 (<0.1)	-1
Diptera	—	237 (8.6)	-1
Embioptera	—	1 (<0.1)	-1
Hymenoptera Formicidae	10 (11.1)	1156 (41.8)	-0.58
Hymenoptera Chalcidoidea	—	48 (1.7)	-1
Isoptera	—	5 (0.2)	-1
Gastropoda	1 (1.1)	—	1
Hemiptera	1 (1.1)	25 (0.9)	0.10
Isopoda	—	4 (0.1)	-1
Lepidoptera (larvae)	3 (3.3)	2 (<0.1)	0.96
Mantodea	1 (1.1)	—	1
Plant material	5 (5.5)	6 (0.2)	0.93
Orthoptera	5 (5.5)	27 (1.0)	0.69

Phasmatodea	1 (1.1)	—	1.0
Pseudoscorpiones	3 (3.3)	16 (0.6)	0.69
Psocoptera	—	1 (<0.1)	-1
Scorpiones	2 (2.2)	—	1
Thysanura	4 (4.4)	17 (0.6)	0.76
Trichoptera	—	9 (0.3)	-1
Vertebrata (lizards)	2 (2.2)	—	1

Discussion

Our behavioral observations indicate that *Phyllopezus periosus* spends most of the time immobile while foraging (low PTM and MPM values), performing mainly head moves and postural adjustments. Hence, our results suggest a sit-and-wait foraging mode for *P. periosus*, and do not support a fluctuating foraging mode in this species, as suggested for some geckos, characterized by relatively long periods of sedentary behavior, alternating with short bouts of high activity (Werner *et al.*, 1997; 2004). Moreover, a widely accepted correlate of foraging mode is that sit-and-wait foragers will tend to eat larger, more active prey, whereas active foragers will tend to eat sedentary or spatially unpredictable prey (Huey and Pianka, 1981; Perry and Pianka, 1997). The diet composition of *P. periosus*, with predominance of beetles, which are mobile and evasive prey, is also a good indirect evidence of sit-and-wait foraging mode in this lizard.

Werner *et al.* (1997), based on movement pattern data, considered geckos as a group of mixed strategists, using a combination of sit-and-wait and active-foraging tactics. Bauer (2007), based on personal qualitative field observations of approximately 250 species of gekkotans, also argued for a fluctuating foraging behavior among some nocturnal geckos. Moreover, Werner *et al.* (1997; 2004) demonstrated that extending the focal observation period for geckos to 30 min reveals significant variation in movement patterns and, in some cases, suggests active-foraging behavior that would have been missed by observations of short duration. In our study, we did not found evidence of short bouts of high activity in any of focal lizards, but since we adopted a shorter duration of observations, ranging from 6 to 13 min, we do not discard the possibility of occasional episodes of more active foraging behavior in *P. periosus*. This saxicolous nocturnal gecko forages not only on rocks, but also climbs trees near rocky outcrops, so it is possible that these lizards, which are able to use both visual and olfactory cues (Cooper, 1995), may exploit serially different ambush sites, becoming more active when searching for these sites (Bauer, 2007).

Bauer (2007) conducted a literature review of studies on foraging modes of gekkotans and verified that only 9% of species and 44% of genera were investigated. However, most studies that characterized foraging mode of geckos used qualitative anecdotal field observations and/or indirect evidence such as diet composition to categorize species as sit-and-wait or active foragers. In fact, characterization of foraging mode in geckos based on movement pattern data (PTM, MPM or both) is restricted to approximately twenty species (Cooper *et al.*, 1999; Werner *et al.*, 1997; 2004; 2006; Perry, 1999; 2007; Werner and Chou, 2002; Hibbits *et al.*, 2005; Bauer, 2007; Murray *et al.*, 2015), which represents only 1% of gekkotan diversity. To our knowledge, this is the first quantitative study about foraging behavior of a gecko of the family Phyllodactylidae.

The diet of *P. periosus* at ESEC Seridó was predominantly composed of beetles (Coleoptera). Predominant consumption of Coleoptera also occurred, for instance, in the phyllodactylid geckos *Phyllopezus pollicaris* in western Brazil (Albuquerque *et al.*, 2013), *Phyllopezus maranjonensis*, *Phyllodactylus delsolari* and *Phyllodactylus reissii* in northern Peru (Aurich *et al.*, 2011), and *Homonota fasciata* in northwestern Argentina (Cocilio *et al.*, 2016). Although with much less importance, other prey categories were consumed by *P. periosus*, such as Formicidae, Orthoptera, Thysanura, Blattodea, Vertebrata (lizards), plant matter, among others. Moreover, the predation of the lizards *Hemidactylus agrius* and *Tropidurus semitaeniatus* reveals opportunistic feeding behavior in *P. periosus*. These two lizard species share the same habitats (rocky outcrops) and microhabi-

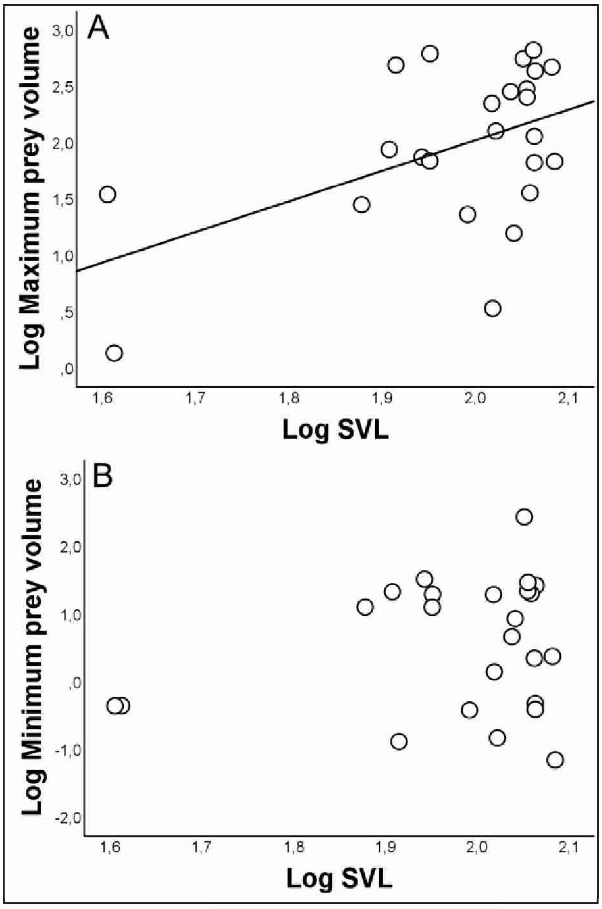

Figure 2. Relationship between snout-vent length – SVL (mm) and prey size (mm3) in *Phyllopezus periosus* at the Seridó Ecological Station, Rio Grande do Norte, Brazil. (A) SVL against maximum prey volume ($y = 2.72x + 3.41$, $p < 0.05$), (B) SVL against minimum prey volume ($p > 0.05$).

tats (rock crevices) with *P. periosus*, so it is likely that occasional encounters between them often result in predation attempts. Saurophagy has been reported for several geckos around the world, including cases of cannibalism (e.g. Bonfiglio *et al.*, 2006; Daza *et al.*, 2009; Blanco *et al.*, 2012). Additionally, it is worth mentioning the consumption of plant matter by *P. periosus*, including some seeds and sap of trees. Besides the consumption of the sap of *Cenostigma pyramidale* ("catingueira") recorded during focal observations, we also casually recorded an individual of *P. periosus* consuming the sap of a "anjico-branco" tree (*Anadenanthera colubrina*; CNSP, pers. Obs.). Besides these two new records of tree sap foraging, Andrade *et al.* (2016) recorded in three different occasions individuals of *P. periosus* eating the sap of the "baraúna" tree, *Schinopsis brasiliensis*.

Males and females of *P. periosus* consumed similar prey types and ingested a similar amount of food items. Sexual differences in diet composition are usually attributable to sexual dimorphism in body and head dimensions that allows a differential consumption of prey between sexes, with the larger sex having the potential to consume larger prey (Schoener, 1967; Preest, 1994). Therefore, the occurrence of dietary differences between sexes in *P. periosus* was not expected, since there were no sexual differences in body size (SVL) and most of head dimensions (HL, HW, JL). Similarly, Rocha and Anjos (2007) did not find sexual differences in the diet of the House gecko *Hemidactylus mabouia*, which also shows no sexual dimorphism in body size, in an outcrop rocky area of southeastern Brazil.

The absence of relationship between the diet composition of *P. periosus* and prey availability in the environment suggests that lizards are, at least to some degree, selective about what they eat, rather than capturing all available prey items. This assumption is supported by the values of the Ivlev's Electivity Index, which indicated preferences for some prey categories and rejection of others. Lizards consumed beetles in a proportion slightly higher than their proportional availability in the environment, suggesting some degree of preference for this prey category. Moreover, some prey categories proportionally less consumed by *P. periosus*, such as Scorpiones, Phasmatodea, Mantodea, Gastropoda, Lepidoptera larvae and Vertebrata, showed positive values of electivity because they were scarce in the environment. On the other hand, the electivity index showed that ants (Formicidae) were avoided, as they were the predominant prey in the environment, but relatively little consumed by *P. periosus*. Gekkotans have a developed nasal chemosensory (Cooper, 1995) and are possibly able to detect and discriminate prey by chemical cues. Therefore, rejection of Formicidae may be associated with chemosensory avoidance, since many ants produce chemical defenses that may be noxious for predators (Vitt and Pianka, 2005).

The consequences of body size on feeding ecology have been studied in diverse taxonomic groups. In some cases, predators exclude smaller prey items from the diet as they grow and add larger items, in such a way that both minimum and maximum prey sizes increase at similar rates (e.g., Costa *et al.*, 2008; Costa, 2009; Sales *et al.*, 2012). In other cases, the predator adds larger items to its diet as it grows but continues to eat small prey; a positive relationship between predator body size and maximum prey size occurs, but minimum prey size remains constant or increases with a much slighter slope (e.g., Scharf *et al.,* 2000; Sales *et al.,* 2011). Finally, the predator may not add larger items to its diet as it grows; in this case, minimum and maximum prey sizes are not correlated with body size (dietary specialists, e.g., Vitt *et al.,* 1997; Colli *et al.,* 2003). Our results support the second scenario for *P. periosus*, as body and head size were positively correlated with maximum prey size, but not with minimum prey size. Despite adding larger prey to their diet, larger lizards continue to consume small prey consistently, leaving minimum prey size constant. A skewness of available prey sizes, with predominance of smaller ones, is a possible explanation for this maintenance of consumption of small prey by larger individuals of *P. periosus.* A similar pattern of predator-prey size relationships was found in the sympatric teiid lizard *Ameiva ameiva* in the same study site (Sales *et al.,* 2011).

Conclusion

We conclude that *Phyllopezus periosus* is a sit-and-wait forager that consumes predominantly beetles at ESEC Seridó. Data indicates that these lizards are selective in food use and avoid some prey types with high abundance in the environment. In addition, predation of sympatric lizards and tree sap foraging are evidence of opportunistic feeding habits by *P. periosus.* Males and females have similar body sizes and do not differ in diet composition. Finally, the positive association between lizard body size and maximum prey size, but absence of relationship with minimum prey size suggests that lizards in this population experience an ontogenetic change in the diet, adding larger prey items while growing, but at the same time still continue consuming smaller ones.

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Declaration of interest statement

There are no conflicts of interest that may affect the content, results or conclusions of the article.

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Trabajo

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Uso do pó fluorescente no estudo de rastreamento de serpentes na Floresta Atlântica, Nordeste, Brasil

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ABSTRACT
There are few studies on snake tracking and this number is even lower when it comes to the Atlantic Rain Forest in Brazil. There are relatively few methodologies for carrying out these studies and most researchers use radio telemetry when possible. In this work we use the fluorescent powder in snakes, glimpsing studies on displacement, occupation and area of, as well as comparing the technique in terms of positive and negative points with other methods. 16 individuals of nine species were monitored in captures that took place bimonthly between 2016 and 2017, in an urban fragment of Atlantic rain forest in the Parque Estadual Dois Irmãos in northeastern Brazil. The tracking resulted in an area covered up to 23 m, in which terrestrial species had their tracks more easily identified, as was the case of *Xenodon rabdocephalus*. The humidity of the air, the heterogeneity of the forested environment seems to be the main obstacles to the loss of trails. The fluorescent powder is an affordable not invasive method that provides details of displacement, however is limited in terms of durability of the traces.

Key Words: Ecology; Tracking; Marking; Herpetofauna.

RESUMO
São poucos os trabalhos sobre rastreamento de serpentes e esse número torna-se ainda mais reduzido quando se trata da Mata Atlântica no Brasil. Existem relativamente poucas metodologias para se realizar esses estudos e grande parte dos pesquisadores recorrem à radiotelemetria quando possível. Neste trabalho utilizamos o pó fluorescente em serpentes, vislumbrando estudos sobre deslocamento, ocupação e área de uso, bem como comparamos a técnica quanto aos pontos positivos e negativos com outros métodos. Foram acompanhados 16 indivíduos de nove espécies, em coletas ocorridas bimestralmente entre 2016 e 2017, em um fragmento urbano de mata atlântica no Parque Estadual de Dois Irmãos, no nordeste do Brasil. O rastreamento resultou em uma área percorrida de até 23 m, na qual as espécies terrestres tiveram seus rastros mais facilmente identificados. A umidade do ar e a heterogeneidade do ambiente florestado parecem ser as principais causas da perda dos rastros em floresta. O pó fluorescente é um método de baixo custo e não invasivo que fornece detalhes do deslocamento das serpentes, todavia é limitado quanto à durabilidade dos rastros.

Palavra Chave: Ecologia; Rastreamento; Marcação; Herpetofauna.

Introdução

Estudos comportamentais em ambiente natural relacionados às serpentes, bem como, metodologias aplicadas para rastreio das mesmas ainda são deficitários (Dorcas *et al.*, 2009; Tozetti *et al.*, 2000) e poucos se propõem em investigar o deslocamento e área de uso desses animais (Bastos *et al.*, 2005; Sma-

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niotto *et al.*, 2020). Dados relacionados a padrões de movimentos e área de uso contribuem para melhor compreensão da ocupação e uso do hábitat, seus limites, tamanho da área em relação à capacidade suporte, aspectos reprodutivos e territorialidade, fornecendo subsídios para manejo e conservação das espécies (Elzinga *et al.*, 2001; Waddell *et al.*, 2016; Rivas, 2015). Metodologias como uso de carretéis com barbantes, rádios transmissores, manejo na captura e recaptura de espécimes marcados em uma área de estudo podem influenciar no dado comportamental do animal e exigir um maior investimento (Furman *et al.*, 2011; Rivas 2005; Smaniotto *et al.*, 2020). Um dos procedimentos considerados mais invasivos em estudos com serpentes são os rádios transmissores (telemetria), aplicados por meio da ingestão forçada do aparelho, preso ao corpo do animal através de adesivos ou por cirurgia para sua implantação sob a pele, podendo ocasionar lesões que afetam o desempenho da atividade natural do animal e, em casos extremos, levar ao óbito (Holtzman *et al.*, 2002; Torzetti *et al.*, 2009). O uso da telemetria forneceu informações interessantes sobre a história natural de espécies como a sucuri-amarela, *Eunectes notaeus* Cope, 1862, mas, apesar das vantagens, falhas do equipamento e perda de transmissores e de indivíduos ocorrem, além de perda frequente de sinal em ambientes fechados ou cobertos (Smaniotto *et al.*, 2020).

O rastreamento através do uso de pó fluorescente é uma técnica recente de baixo custo e subestimada para o estudo do comportamento das rotas ou deslocamento do animal e pouco testada em serpentes (Furman *et al.*, 2011). Desenvolvida inicialmente para ser utilizada no rastreamento de pequenos mamíferos (Leman *et al.*, 1985) e posteriormente utilizada em estudos com anuros (Graeter *et al.*, 2007) e tartarugas (Roe, 2008), o pó fluorescente foi aplicado para rastrear a serpente *Thamnophis sirtalis* (Linnaeus, 1758) em ambiente aberto, no Canadá, sendo útil para localização de tocas e padrões de movimento no ambiente natural (Furman *et al.*, 2011). Esta metodologia pode fornecer um histórico exato do deslocamento do indivíduo (Stark *et al.*, 2000), tornando-se um artifício de grande ajuda para o entendimento da rota ou trajeto do animal reagindo a diferentes obstáculos e substratos de preferência.

No Brasil a riqueza de serpentes encontra-se em torno de 412 espécies (Nogueira *et al.*, 2020), das quais 220 ocorrem na Floresta Atlântica (Marques *et al.*, 2019) e informações sobre a história de vida desses animais em ambiente natural são incipientes, muitas vezes relacionadas à própria dificuldade do registro (Marques, 1998; Santos *et al.*, 2017). Quanto a informações sobre padrões de movimentos podemos citar os trabalhos de Smaniotto *et al.* (2020) com *Eunectes notaeus* e de Tozetti *et al.* (2009) em pesquisa com *Crotalus durissus*. Ambos os estudos utilizaram rádios transmissores em adultos e obtiveram informações de movimentação variando entre 0,1 m e 2,3 ha e 2 m e 191 m, com um total de 218 e 59 dias de rastreamento respectivamente. O presente trabalho teve como objetivo descrever uma experiência com o uso do pó fluorescente em serpentes registradas num fragmento de floresta atlântica no nordeste do Brasil, contribuindo assim com informações sobre padrões de movimento e deslocamento das espécies em ambiente florestado, bem como, avaliar os pontos negativos e positivos nos estudos com serpentes.

Material e método

Área de Estudo

O estudo foi realizado no Parque Estadual de Dois Irmãos (8°7'30"S e 34°52'30"W) uma Unidade de Conservação (ver SEUC, 2009) de floreta ombrófila urbana com uma área total de 1.157,72 ha, localizada na cidade do Recife, estado de Pernambuco (Fig. 1). A região possui clima do tipo As'- tropical chuvoso, quente e úmido com temperatura média de 23ºC (Coutinho *et al.*, 1998). Nessa área foi implantado um módulo do Programa de Pesquisa em Biodiversidade (PPBIO) / Mata Atlântica do Nordeste, que consiste em 10 parcelas de 250 metros, distribuídas por 1 x 5 km. Nesse estudo foram monitoradas quatro parcelas (PE 1 – 500, PE 1 – 1500, PE 1 – 2500 e PE 1 – 4500/ Figura 1), duas em cada área (floresta madura e floresta jovem).

Amostragem de serpentes e teste do pó fluorescente

As coletas de dados foram realizadas a cada dois meses durante o período de agosto/2016 a março/2017, durante dez dias consecutivos em períodos noturnos e diurnos. Inicialmente, foi realizada uma caracterização dos ambientes propícios para registro das serpentes, como grutas, amontoados de rochas, vegetação, colônias de bromélias, troncos caídos. No final de cada uma das quatro parcelas PPBIO foram instaladas armadilhas de interceptação e queda (*pittfal traps*), consistindo em quatro sistemas no formato

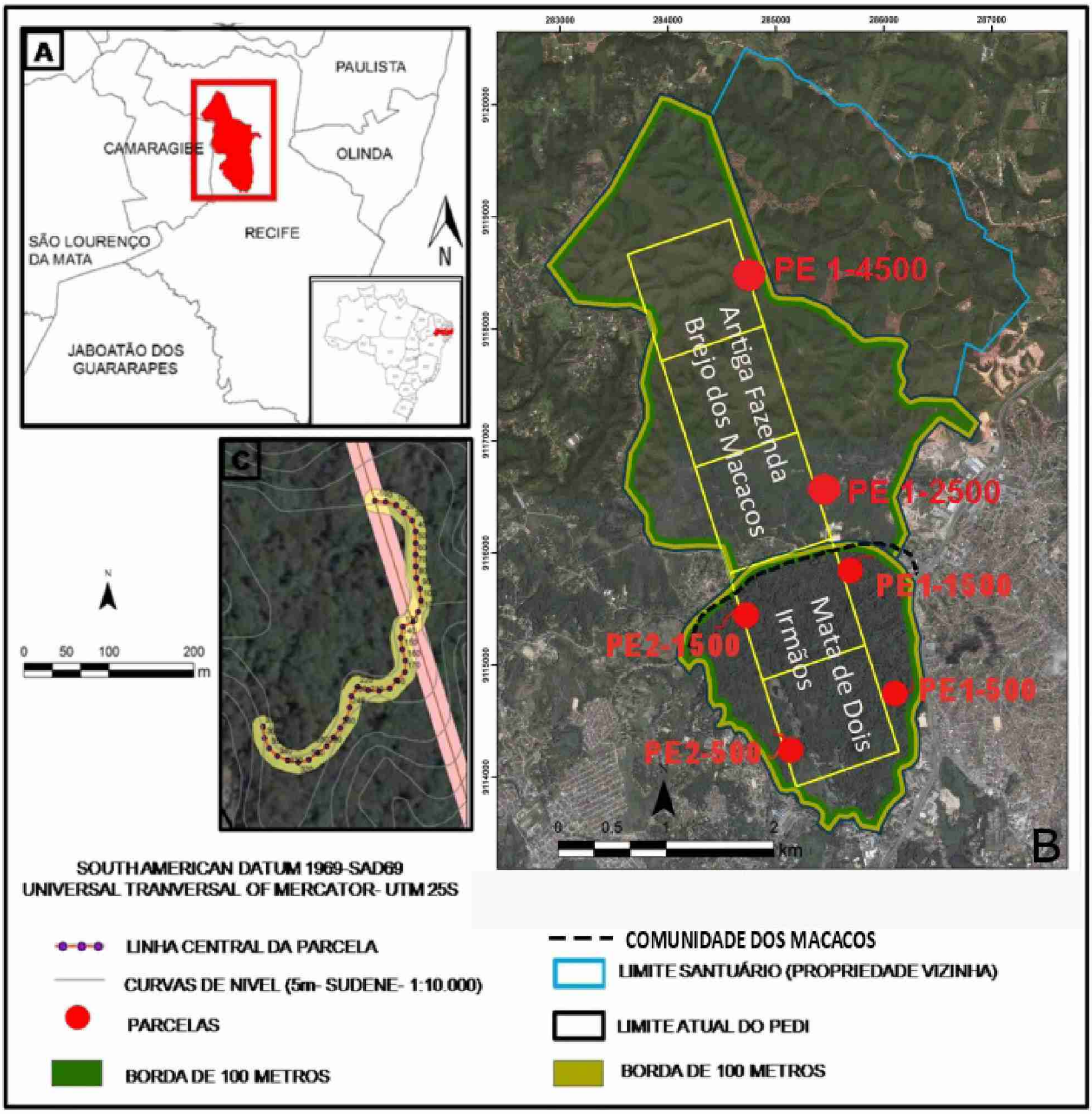


Figura 1. A- Parque *Estadual* de Dois Irmãos, B- Módulo do PPBio instalado na Unidade de Conservação PEDI, C- Parcela de 250 metros do PPBio.

Y por parcela, cada um composto por quatro baldes de 80 L, interligados por lona plástica, com um total de 64 baldes. O esforço amostral total foi de 2560 baldes-dia. Também foram realizadas buscas ativas seguindo a metodologia RAPELD (Magnussum *et al.*, 2005; Fig. 2-A), com um total de 240 horas (n= 2 pessoas), distribuídas entre turnos da manhã, tarde e noite. O esforço amostral não foi igual entre as parcelas devido as questões de segurança (ex. roubo das armadilhas na PE 1 – 2500 e PE 1 – 4500) (Fig. 1).

Dados ecológicos e comportamentais das espécies foram anotados em cadernetas de campo: comportamento, tipo de ambiente, habitat, microhabitat, e altura do substrato, esse último com auxílio de uma trena. Os animais capturados, tanto por busca ativa quanto passiva, foram identificados (seguindo Marques *et al.*, 2019), medidos (trena, régua e/ou paquímetro com precisão de 0,1 mm), pesados (balança Pesola®, precisão de 0,1g), marcados (bioelastômero Alpha TagVI/Master, picote de escamas ventrais e/ou microship Friendchip, esse último para animais com mais de um metro de

Figura 2. A- *Pitfalls* montados na PE 1-500 no Parque Estadual de Dois Irmãos para captura dos animais. C- Lanterna de luz negra e utilização do pó no corpo de *Boa constrictor* no PEDI. E- *Boa constrictor* com pó aplicado, destacando a região próxima aos olhos que não entram em contato com o produto. B- Rastro iluminado por lanterna de luz negra na área florestada do PEDI. D- Realização da busca pelo rastro. F- *Pseudoboa nigra* durante processamento.

comprimento), fotografados, sexados e soltos nos seus respectivos locais de captura (Licença ICMBIO: 11218-1 e autorização da gestão da Unidade de Conservação Estadual). Equipamentos como luvas, pinção e gancho foram utilizados no manejo para captura dos animais, todo o procedimento seguiram as recomendações de Melgarejo-Giménez (2002).

Antes da soltura, os animais tiveram seus ventres, laterais e dorsos cobertos por pó fluorescente, atóxico e biodegradável (Fig. 2-C, E), feito a base de amido (Furman *et al.*, 2011), atentando para que não entrasse em contato com a região da cabeça. Após a soltura, foi realizado o acompanhamento dos rastros deixados pelo animal, com auxílio de uma lanterna de luz negra, até a trilha ser perdida (Fig. 2-B, D). Os rastros encontrados foram marcados com auxílio de pequenas estacas e, em seguida, foi passada uma linha de barbante para obter o formato e a medida da trilha feita pelo animal (adaptado de Furman *et al.*, 2011). O coeficiente de correlação de Spearman (rs), programa BioEstat 5.0, foi utilizado considerando um $p \leq 0,05$ (Ayres *et al.*, 2007) para comparar se houve relação entre o tamanho do animal e o comprimento do rastro.

Busca bibliográfica de outros métodos para a comparação com o pó fluorescente

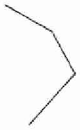
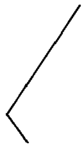
Concomitantemente, uma avaliação dos pontos positivos e negativos dos métodos de rastreamento com serpentes foi realizada através das publicações encontradas na base de dados do Google acadêmico utilizando as seguintes palavras-chave encontradas nos títulos das pesquisas: pó fluorescente; radiotelemetria; serpente; técnica de rastreamento; área de uso; fluorescent powder; radio-telemetry; snake; technique; thread trailing; tracking; home range. Os artigos foram escolhidos por serem mais recentes (entre 10 a 15 anos) e apresentarem uma descrição mais detalhada do método.

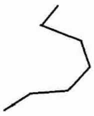
Resultados e Discussão

Um total de 16 espécimes de nove espécies e três famílias, foi marcada e rastreada através de vestígios deixados pelo pó florescente (Tab. 1), sem recapturas. A maior eficiência em termos de registro/captura de serpentes foi através de busca ativa, fato comum em estudos de serpentes (Barbosa, 2018; Marques *et al.*, 2019). Apenas *Xenodon rabdocephalus* foi capturada através de busca passiva (*pitfall*). Todas as serpentes foram registradas e monitoradas na área de floresta madura, em especial na parcela PE-1.500, de forma similar a pesquisas anteriores realizadas no PEDI (Santos *et al.*, 2017; Barbosa, 2018).

As características morfológicas de cada animal, bem como, seu hábito de vida foram fatores relevantes para maior ou menor efetividade na utilização do método. Indivíduos maiores em geral deixaram rastros mais extensos e de fácil registro,

Tabela 1. Serpentes capturadas e seus rastros durante o período de agosto/2016 a fevereiro/2017, no Parque Estadual de Dois Irmãos, Recife-PE.

ESPÉCIES	CRC	TEMPO/ RASTREAMENTO	RASTRO (m)	DESENHO DA-TRILHA	HÁBITO
BOIDAE					
Boa constrictor Linnaeus, 1758 IND. 1	64 cm	3 dias	12 m		Semi- arborícola
IND. 2	84 cm	2 dias	13 m		Semi- arborícola
IND. 3	52 cm	1 dia	6 m		Semi- arborícola
IND. 4	45 cm	1 dia	8 m		Semi- arborícola
Corallus hortulana (Linnaeus, 1758)	147 cm	1 dia	3 m		Arborícola
COLUBRIDAE					
Chironius flavolineatus (Boettger, 1885) IND. 1	71 cm	3 dias	9 m		Semi- arborícola
IND. 2	87 cm	1 dia	2 m		Semi- arborícola
Dendrophidion atlantica Freire, Caramaschi & Gonçalves, 2010	73 cm	1 dia	19 m		terrícola
Leptophis ahaetulla (Linnaeus, 1758) IND. 1	94 cm	1 dia	1 m		Arbóreo
IND. 2	87 cm	1 dia	1 m		Arbóreo

			DIPSADIDAE		
Erythrolamprus viridis (Günther, 1862)	27 cm	2 dias	7 m		terrícola
Philodryas olfersii (Lichtenstein, 1823) IND. 1	97cm	2 dias	21 m		terrícola
IND. 2	75 cm	1 dia	12 m		Terrestre
Xenodon rabdocephalus (Wied, 1824) IND. 1	71 cm	3 dias	23 m		terrícola
IND. 2	75 cm	1 dia	12 m		terrícola
			ELAPIDAE		
Micrurus ibiboboca (Merrem, 1820)	86 cm	1 dia	12 m		Semifossorial

embora sem significância estatística (rs= -1.746; *p*= 0.5178; n= 16). Serpentes terrestres deixaram rastros mais longos (até 23 m) que as arborícolas (menores que 1,5 m), demonstrando a influência do hábito de vida na eficácia do método (Tab.1). Os rastros de *Corallus hortulana, Chironius flavolineatus* (Ind 2) e *Leptophis ahaetulla* (Ind. 1 e Ind. 2 respectivamente) foram difíceis de acompanhar devido às dificuldades em observar as pistas do pó verticalmente no estrato arbustivo e arbóreo da floresta, impossibilitando o desenho esquemático do rastro. *Micrurus ibiboboca*, apresentou um deslocamento de 12 m sobre a serapilheira, o que consideramos uma surpresa tendo em vista seu hábito considerado semifossorial, sendo esperado um menor rastro, uma vez que os indivíduos da espécie vivem em tocas ou sob folhedo (Santos *et al.*, 2017). De forma geral, as serpentes apresentaram rotas lineares, sem muitas curvas acentuadas, apenas se desviando de obstáculos (Tab. 1).

A utilização do pó fluorescente em serpentes florestais foi eficiente, especialmente para o rastreamento dos indivíduos terrestres em trilhas pequenas

(7–23 m) com rastros visíveis até o terceiro dia após a aplicação do pó. A serrapilheira, os estratos da vegetação e a quantidade de substratos como, buracos, tocas, troncos caídos e galhos constituem diferentes obstáculos para registro das trilhas em ambiente de Floresta Atlântica, ocasionando perda do pó e consequentemente falhas nas rotas deixadas no substrato durante o deslocamento das serpentes. Além disso, a umidade e a precipitação podem apagar os rastros, limitando a visualização do pó (Nicolas e Colum 2007; Furman *et al.*, 2011). Em ambiente aberto e mais seco, os rastros deixados por serpentes podem chegar a 200 metros, permitindo o registro dos locais de abrigo como tocas (Furman *et al.*, 2011), podendo ainda fornecer informações quanto à profundidade, com algumas limitações (Waddell *et al.*, 2016). Assim, incentivamos a utilização do pó florescente em ambientes de Cerrado e Caatinga, na tentativa de obter trilhas mais longas e contínuas, particularmente de espécies terrestres.

De modo geral as técnicas para os estudos de movimentação e área de uso encontrados em nossa busca bibliográfica puderam ser divididas em: rastreadores (n = 4) e as captura/recaptura (n = 5) – a qual envolve marcações individuais mais permanente (Tab. 2). Os métodos mais eficientes no que se refere à precisão dos registros de rota são os de maiores custos financeiros, como é o caso da radiotelemetria (Tab. 2), a qual, todavia, necessita de intervenção cirúrgica (Kenward, 2000; Jacob

Tabela 2. Registros de metodologias de rastreamento e captura/marcação utilizadas em estudos com serpentes.

Método de estudo	Pontos positivos	Pontos negativos	Fonte
		Rastreamento	
Barbantes e carretéis amarrados ao animal	Baixo custo e fácil execução	Pode interferir no deslocamento, gerando desvios no comportamento; possibilidade do animal ficar preso.	Waddell *et al.*, 2016; Tozetti *et al.*, 2009
Rádio Telemetria	Alta precisão, principalmente em ambientes abertos	Procedimento invasivo (transmissor inserido no corpo através de fitas adesivas, implante (pequena cirurgia) ou ingestão, além de ter alto custo; são utilizados em animais grande e adultos; comportamento do animal pode ser afetado.	Kenward *et al.*, 2006; Ujvari & Korsós, 2000; Ward, 2013; Tozetti & Martins, 2007; Tozetti *et al.*, 2009; Smaniotto *et al.*, 2020.
Pó fluorescente	Baixo custo e pouco invasivo	Cobre apenas pequenas trilhas de deslocamento. Eficiência atrelada a fatores como substrato, tamanho do animal e umidade do ambiente.	Furman *et al.*, 2011
Isótopos radioativos	Permite coletar informações ecológicas com mais eficiência; rastreamento por um período longo	Alto custo; contaminação do ambiente e outros animais; comportamento do animal pode ser afetado.	Mellor *et al.*, 2004
		Recaptura	
Bioelastômero	Considerável durabilidade e fácil aplicação	É necessária uma detalhada análise do local da marcação para sua identificação, bem como destreza com a seringa em marcas mais elaboradas.	Major *et al.*, 2020
Cauterização	Alta durabilidade, fácil identificação do indivíduo	O procedimento deve ser feito com cuidado para não causar injúrias.	Winne *et al.*, 2006
Corte de Escamas	Simples e de baixo custo.	Durabilidade irregular, pois pode ocorrer regeneração das escamas dificultando a identificação; aplicação pode ser difícil em animais muito pequenos.	Pontes *et al.*, 2009
Microchip	Marcação definitiva e precisa	Custo alto e ainda inviável em animais muito pequenos; raramente pode ocasionar lesões, rejeição ou perda.	Pellett *et al.*, 2013
Fotografia de marcas Naturais	Marcação definitiva, menor custo, imagem compartilhada	Dificuldade para identificação; necessidade de programas de computador para melhor identificação dos indivíduos; mais eficiente em populações pequenas e com padrões de marcas ou desenhos característicos	Bentes *et al.*, 2017

et al., 2003). O avanço tecnológico possibilitou a diminuição do tamanho de transmissores fabricados e técnicas cirúrgicas para implante nos animais (Dorcas *et al.*, 2009) além do uso de linhas e carretéis para rastreamento (Tozetti *et al.*, 2007; Waddell *et al.*, 2016). Porém, ambas metodologias apresentam obstáculos para sua execução e leitura na resposta do resultado, carregar um transmissor mesmo que pequeno pode se tornar uma tarefa que requer muito gasto de energia para um animal rastejante e de pequeno porte (Furman *et al.*, 2011). Além disso, o uso de rastreadores em ambiente de floresta muitas vezes apresenta dificuldade nas transmissões dos sinais de rádio (Smaniotto *et al.*, 2020).

A utilização de linhas e carretéis apesar de ser um método mais acessível e de baixo custo (Waddell *et al.*, 2016), também requer ponderações em relação ao seu uso. Indivíduos que se movem através de vegetação densa, movimentam-se entre galhos, entram em tocas correm o risco de ficarem presos e vulneráveis a predadores, assim como a possibilidade de apresentarem padrões anormais em seu comportamento comprometendo os resultados (Lemckert *et al.*, 2000). No entanto, cabe o pesquisador avaliar os custos e benefícios para cada espécie, evidenciando tamanho, número e indivíduos, comportamento, hábitos e habitats. Ressalta-se que a marcação permanente individual, como, por exemplo, o uso do microchip e/ou bioelastômero, se faz necessário juntamente com o uso de rastreadores para certificar a identidade do indivíduo caso seja recapturado.

Objetivando maior simplicidade, menores riscos e custos a aplicação da utilização de pó colorido florescente para o acompanhamento de serpentes é interessante como um método menos invasivo, de baixo custo e fácil uso (ver Waddell *et al.*, 2016). Esta metodologia fornece o exato padrão de movimentação do animal em sua área de uso (Leman *et al.*, 1985; Hubbs *et al.*,2000; Corbalan e Dibandi, 2009; Dodd, 1992; Stark *et al.*, 2000) e foi pouco utilizada em serpentes (Furman *et al.*, 2011), necessitando mais testes e como sugestão em áreas abertas. De um modo geral o método forneceu informações interessantes em um ambiente de floresta úmida, especialmente para espécies terrícola, mas recomendamos sua adoção em ambientes abertos e secos, em que a possibilidade de sucesso pode ser maior.

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Trabajo

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Reptiles of the Iron Quadrangle: a species richness survey in one of the most human exploited biodiversity hotspots of the world

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ABSTRACT
Currently, more than 10,700 species of reptiles are known to occur worldwide, of these 842 occur in Brazil. The Iron Quadrangle (IQ) is an important region in southeastern Brazil, in the state of Minas Gerais, due to the intense iron mining activity, its rich and unique biodiversity. However, data available for the reptile fauna are scattered in the literature which make the diversity of this group in the IQ remains poorly known. Here we aimed to characterize the reptile fauna of the IQ, representing the first inventory for the area. We found 128 reptile species occurring in the IQ, which corresponds to 49.23% of the reptile fauna for the state of Minas Gerais, and 15.20% of the Brazilian reptile biodiversity. Among these, three species are included in at least one national or international red-list category, four have their type-locality inside the limits of the IQ, seven had not been previously documented for the area, and four have their range extensions to be included in the IQ.

Key Words: Diversity; Conservation; Herpetofauna; Lizards; Snakes.

RESUMO
Atualmente, existem mais de 10.700 espécies de répteis descritas no mundo, das quais 842 ocorrem no Brasil. O Quadrilátero Ferrífero (QF) é uma importante região localizada no estado de Minas Gerais, sudeste do Brasil, devido à intensa atividade de mineração de ferro e à rica e única biodiversidade. No entanto, sua fauna de répteis permanece pouco conhecida. O objetivo deste estudo foi caracterizar a fauna de répteis do QF, representando o primeiro inventário para a área. O presente estudo encontrou 128 espécies de répteis para o QF, o que corresponde a 49,23% da fauna de répteis do estado de Minas Gerais e 15,20% da biodiversidade de répteis brasileiros. Entre essas, três espécies estão incluídas em pelo menos uma categoria de Lista Vermelha nacional ou internacional, quatro possuem localidade tipo no QF e quatro apresentam extensões de ocorrência no QF.

Palavras chave: Diversidade; Conservação; Herpetofauna, Lagartos; Serpentes.

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Introduction

Brazil is one of the richest countries worldwide regarding its reptile fauna. Currently, housing about 795 reptile species, and 47 subspecies, representing 842 taxa are widely distributed throughout the territory (Costa and Bérnils, 2018; Nogueira *et al.*, 2019; Uetz and Hosek, 2019). Among the reptile species known to occur in Brazil, the state of Minas Gerais holds the fourth position in taxon diversity (260 species; Costa and Bérnils, 2018) due to its high environmental heterogeneity, large territorial extension, and the increase in taxonomic studies for this group (Zaher *et al.*, 2009; Rodrigues *et al.*, 2014; Montingelli *et al.*, 2019).

Despite the scientific advances in the knowledge of Minas Gerais' reptile fauna (e.g. São-Pedro and Pires, 2009; Cruz *et al.*, 2014; Tunes *et al.*, 2020) there are several gaps regarding geographical distribution, and ecological knowledge of the species addressed to the Iron Quadrangle region. Thus, the present study aimed at characterizing the fauna of reptiles of the IQ region in the state of Minas Gerais, Brazil, representing the first inventory for the area.

Materials and methods

Area of study

The IQ is located in the south-central region of the state of Minas Gerais, southern Brazil, and encompasses over 7.160 km^2 of 35 municipalities (Fig. 1) (Prado Filho and Souza, 2004; Lobato *et al.*, 2005). It is considered one of the most important mineral provinces of the world, serving as a source of iron, aluminum, gold, and manganese, among others (Spier *et al.*, 2003; Brasil, 2006; Roeser and Roeser, 2010) which account for 80% of the Brazilian iron extraction and 65% of its mining production (Carmo and Campos, 2012), and 26.8% of the state of Minas Gerais total Gross Domestic Product (DEGEO, 2009). According to Diniz *et al.* (2014), the area explored for mining in the IQ in the year of 2011 was approximately 11,967 ha which accounted for almost 1.66% of the IQ area.

The IQ is associated with two main Brazilian biodiversity hotspots, the Cerrado and the Atlantic Forest. Both of these biomes are considered priority areas for global conservation due to their high biodiversity, endemism incidence and elevated level of threat (Mittermeier *et al.*, 1998; Myers *et al.*, 2000). The vegetation of the IQ itself has a montane grassy-shrubby composition, which grows on rocky outcrops, quartzite, sandstone, and ferruginous rocky fields, thus forming a vegetation mosaic (Vincent *et al.*, 2002; Mourão and Stehmann, 2007; Silveira *et al.*, 2016). Although the area of the IQ is of great historical and biological value, it also experiences environmental degradation due to the lack of planning for land occupation, expansion of urban areas, mineral production and agricultural activities (Drummond *et al.*, 2005; Marent *et al.*, 2011). Furthermore, even given the worldwide emphasis on the great economic importance of the region, little is known about its biology and ecology (Jacobi and Carmo, 2008).

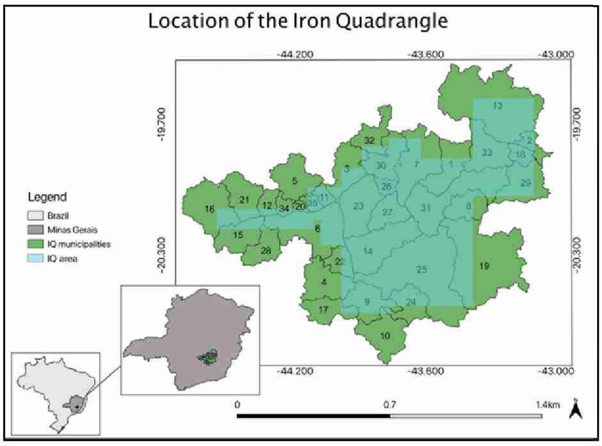

Figure 1. Map showing the Iron Quadrangle area considered in this study formed by 35 municipalities in the state of Minas Gerais, Brazil, which are (in alphabetical order): Barão de Cocais (1), Bela Vista de Minas (2), Belo Horizonte (3), Belo Vale (4), Betim (5), Brumadinho (6), Caeté (7), Catas Altas (8), Congonhas (9), Conselheiro Lafaiete (10), Ibirité (11), Igarapé (12), Itabira (13), Itabirito (14), Itatiaiuçu (15), Itaúna (16), Jeceaba (17), João Monlevade (18), Mariana (19), Mário Campos (20), Mateus Leme (21), Moeda (22), Nova Lima (23), Ouro Branco (24), Ouro Preto (25), Raposos (26), Rio Acima (27), Rio Manso (28), Rio Piracicaba (29), Sabará (30), Santa Bárbara (31), Santa Luzia (32), São Gonçalo do Rio Abaixo (33), São Joaquim de Bicas (34), and Sarzedo (35).

Data acquisition and organization

We gathered the data by examining specimens housed in the following herpetological depositories: Coleção de Répteis do Centro de Coleções Taxonômicas da Universidade Federal Minas Gerais (UFMG), Coleção de Herpetologia do Museu de Ciências Naturais da Pontifícia Universidade Católica de Minas Gerais (MCNR), Coleção de Serpentes da Fundação Ezequiel Dias (FUNED) and Laboratório de Zoologia de Vertebrados da Universidade Federal de Ouro Preto (LZV-UFOP). Additional specimens were also examined from the following scientific collections: Coleção Zoológica setor de

Répteis da Universidade Federal do Mato Grosso (UFMT); Coleção de Répteis do Museu de Ciências Tecnológicas da Pontifícia Universidade Católica do Rio Grande do Sul (MCP - Répteis); Instituto Nacional da Mata Atlântica (MBML); Coleção Herpetológica "Alphonse Richard Hoge" from Instituto Butantan (IBSP), and Coleção de Répteis do Museu de Zoologia da Universidade Estadual de Campinas (ZUEC-REP) (Supplementary Material: https://doi.org/10.5281/zenodo.5256260).

Furthermore, relevant scientific and technical literature (i.e., scientific papers, management plans, environmental technical reports for the IQ) were also surveyed, such as Drummond *et al.* (2007); Bertoluci *et al.* 2009; São-Pedro and Pires (2009); Silveira *et al.* (2010); Passos *et al.* (2012); Linares and Eterovick (2013); PBCM (2013); Cruz *et al.* (2014); Ribeiro-Júnior (2015); VALE S.A. (2015); Costa *et al.* (2016); Martins *et al.* (2019), Nogueira *et al.* (2019) and Tunes *et al.* (2020). We considered all data with at least only one individual of each species being considered for the IQ.

We created a database containing all data gathered through examination of specimens and literature. We organized the data, keeping information about the institution, housed number, locality, and georeferenced coordinates (using SIRGAS2000). Data gathered from literature were also tabulated considering taxonomic classification following Costa and Bérnils (2018).

We identified the species following taxonomic studies (e. g., Zaher *et al.*, 2009; Rodrigues *et al.*, 2014; Pinto and Fernandes, 2017) and some inaccuracies, such as species identified as "aff.", "cf.", "gr." and "sp." were solved through careful examination of the specimens and comparison with original descriptions of the species. Finally, taxa unknown to occur in the IQ region were treated separately and excluded from our final list if they were donated to the scientific collection (came from pet collections) or lacked evidence that the taxon has dispersed into the IQ region.

Maps were created using the Free and Open Source QGIS v.3.10 software for desktop that can be found at: (https://www.qgis.org/en/docs/index.html) with multiple spatial layers from the Instituto Brasileiro de Geografia e Estatística (IBGE).

Results

Through examination of 2,147 specimens housed in scientific collections summed to 1,247 data gathered from literature, we recorded 135 species of reptiles in the IQ. The total of species is representative of 22 families (Dipsadidae = 58 spp; Colubridae = 12 spp; Gymnophthalmidae = 7 spp.). The most representative order was Squamata (119 species) followed by Testudines (four species) and Crocodylia (one species), respectively. From the total of reptile species, 124 species were previously recorded to occur in the IQ (Fig. 2), four have their type locality within the IQ (Fig. 3), four have their range extended to include the IQ (Fig. 4), and seven species and one family (Iguanidae) are not naturally considered to occur in the study area. Thereby, excluding the species that are not naturally found within the IQ, the list presented here is composed of 128 species of reptiles, distributed throughout 21 families (Table 1).

A total of 29 species have a wide geographical distribution occurring in ten or more municipalities as shown in (Table 1), and the municipality with the highest number of species recorded were Belo Horizonte and Ouro Preto (N = 64), followed by Nova Lima (N = 53), Ouro Branco (N = 50) and Brumadinho (N = 46) (Fig. 5).

Discussion

The reptile fauna of the IQ represents 49.23% (N = 128) of the reptile fauna of the state of Minas Gerais (260 species according to Costa and Bérnils, 2018). The reptile diversity can be explained by the geophysical and climatic characteristics of the IQ, which support rich and diverse vegetation distributed among the Cerrado and Atlantic Forest biomes, by the sampling effort of taxonomic groups, and also by the roadside access in the study area (Myers *et al.*, 2000; Drummond *et al.*, 2009; Oliveira *et al.* 2016, 2017).

Species with type localities in the IQ area

Among the 128 species registered in this study, four of them have their type localities within the IQ (Fig. 3): *Caaeteboia amarali* (Weestein, 1930), *Chironius brazili* Hamdan and Fernandes, 2015, *Heterodactylus lundii* Reinhardt and Luetken, 1862, and *Trilepida jani* (Pinto and Fernandes, 2012).

The colubrid snake *C. amarali* inhabits areas of the Atlantic Forest from the state of Bahia in northeast Brazil to the state of São Paulo in southeast Brazil (Passos *et al.*, 2012). The type locality of

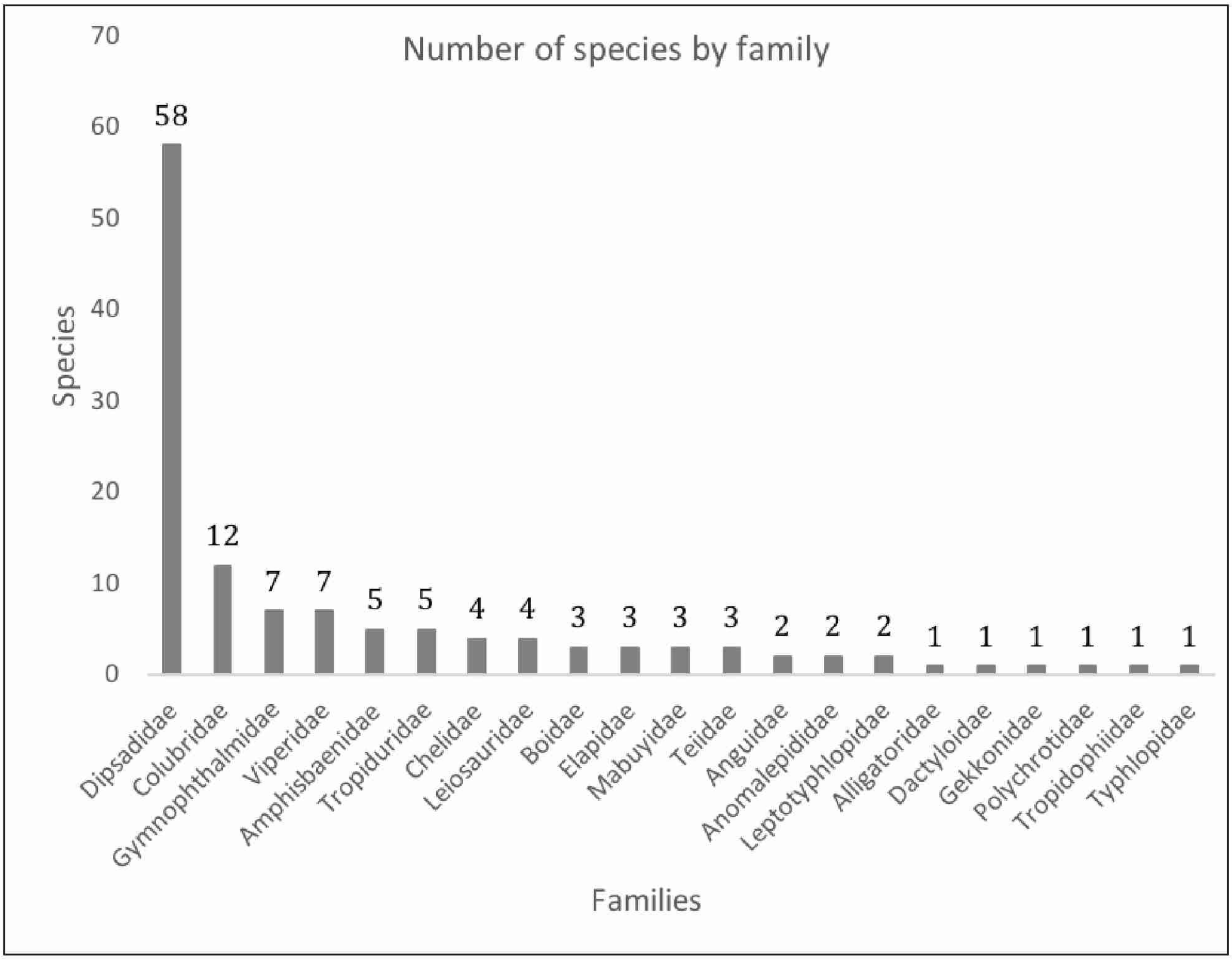

Figure 2. Diversity of reptiles along the 21 families recorded for the Iron Quadrangle region, state of Minas Gerais, Brazil.

the species is the municipality of Belo Horizonte, although there has been some debate due to the gap in its distribution (Passos *et al.*, 2012). It suggests that the isolation of *C. amarali* in Belo Horizonte is due to extensive deforestation and anthropogenic action, especially after the 1930s, when the city started its transformation into an industrial and urban area (Passos *et al.*, 2012).

The colubrid *C. brazili* is found in the Cerrado biome of center-west to south regions of Brazil at altitudes of 700-900 m a. s. l., with type locality being the RPPN Santuário do Caraça in the municipality of Catas Altas (Hamdan and Fernandes, 2015).

Heterodactylus lundii is an endemic lizard of Minas Gerais State, occurring in the Cerrado and Atlantic Forest biomes (Vanzolini, 1982; Fundação Biodiversitas, 2003; Novelli *et al.*, 2011). The type locality for the species is Serra da Piedade in the municipality of Caeté (Reinhardt and Luetken, 1862; Uetz and Hosek, 2019).

Trilepida jani is an endemic blind snake that occurs in *Campos Rupestres* (Rupestrian Fields), a phytophysiognomy of the Cerrado (Silveira *et al.*, 2016; Thomassen *et al.*, 2016), in the state of Minas Gerais (Pinto and Fernandes, 2012). The type locality for the species is Parque Municipal das Mangabeiras in the municipality of Belo Horizonte (Pinto and Fernandes, 2012).

Moreover, due to the lack of a broad knowledge for the IQ reptile biodiversity, we included the following results, in order to elucidate the species previous absence for the region.

New occurrences for the IQ (range extensions)

We identified the first occurrence of four species in which their range are extended for the IQ: *Ameivula cipoensis* Arias, Carvalho, Zaher and Rodrigues, 2014, *Enyalius boulengeri* Etheridge, 1969,

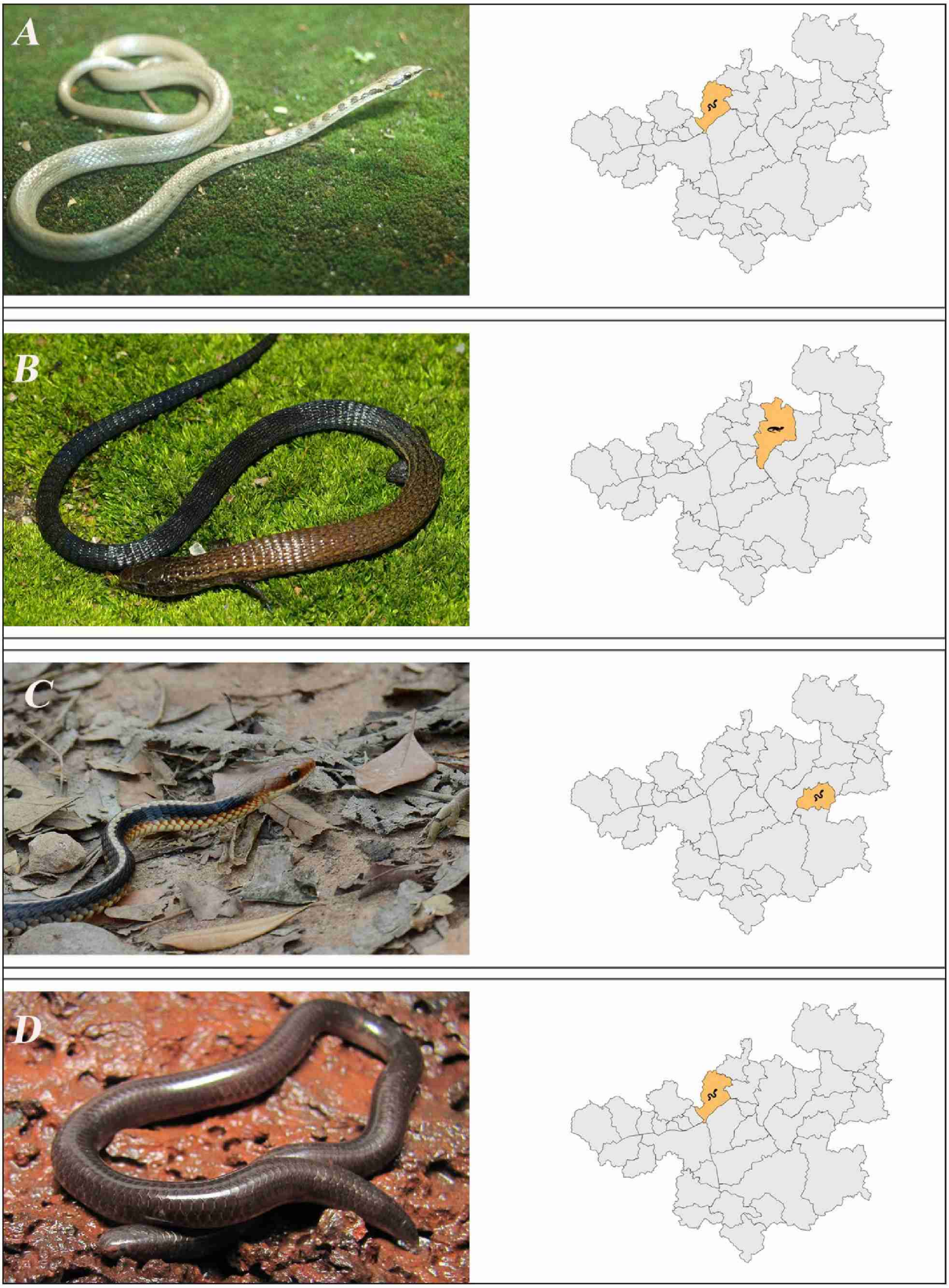

Figure 3. Species with type-localities (orange areas) inside the limits of Iron Quadrangle area. Caaeteboia amarali (A), *Chironius brazili* (B), *Heterodactylus lundii* (C) and *Trilepida jani* (D). Photos by Marcelo Ribeiro Duarte, Mario Jorge Martins, Pedro Henrique Bernardo and Henrique Caldeira Costa, respectively.

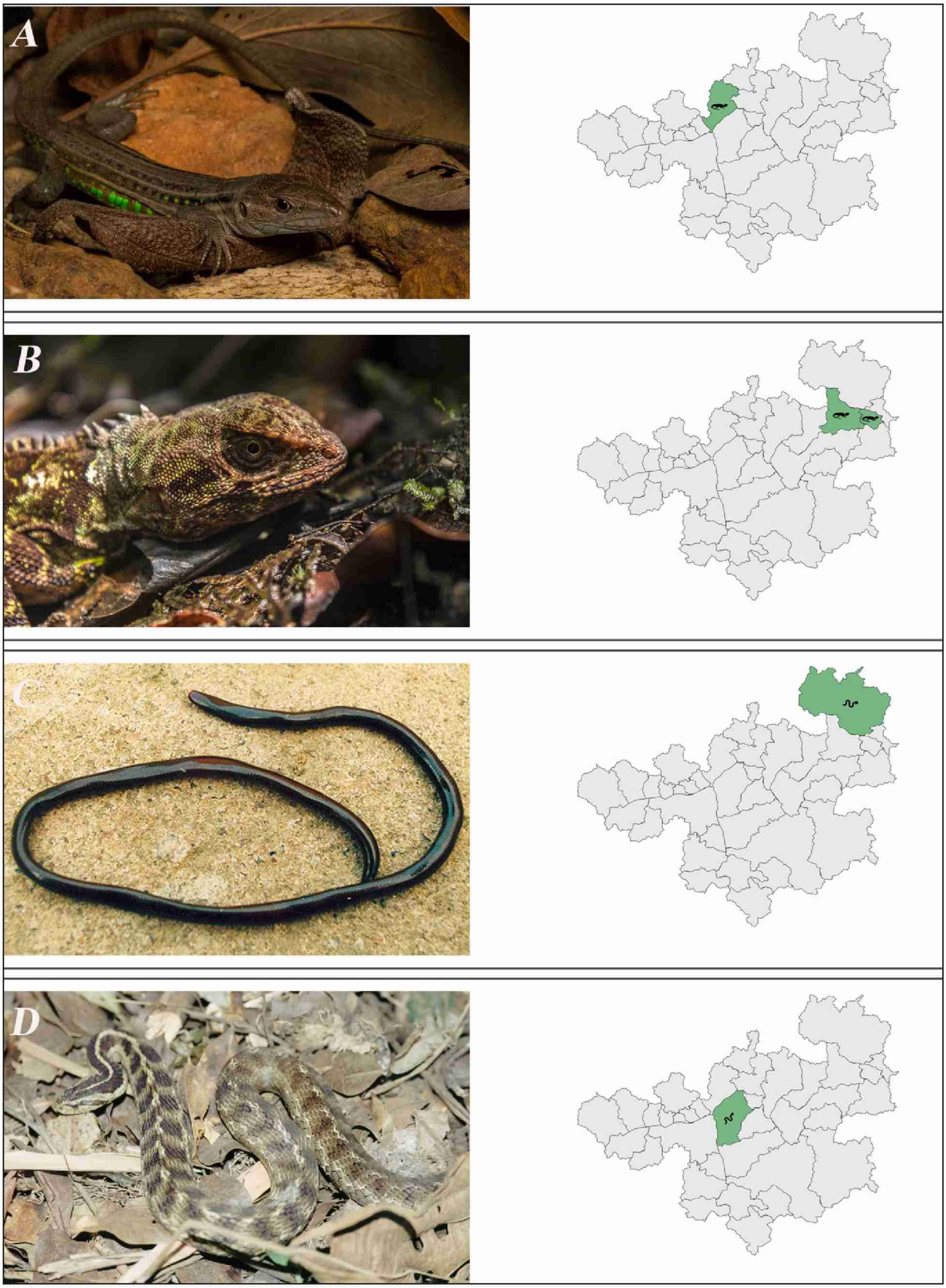

Figure 4. New occurrences (range extensions – green areas) for the Iron Quadrangle, Minas Gerais, Brazil. *Ameivula cipoensis* (A), *Enyalius boulengeri* (B), *Liotyphlops wilderi* (C) and *Tomodon dorsatus* (D). Photos by Pedro Henrique Tunes, João Pedro Kloss-Degen, and Marco Antônio Freitas (C-D) respectively.

Table 1. Species of reptiles recorded to the Iron Quadrangle region. (#) represents a species that is not classified for Brazilian lists, but it is in a category of threat by IUCN (2015). (SC – Scientific Collections) and (L – Literature). In Distribution in the IQ numbers represent the municipalities as follow: Barão de Cocais (1), Bela Vista de Minas (2), Belo Horizonte (3), Belo Vale (4), Betim (5), Brumadinho (6), Caeté (7), Catas Altas (8), Congonhas (9), Conselheiro Lafaiete (10), Ibirité (11), Igarapé (12), Itabira (13), Itabirito (14), Itatiaiuçu (15), Itaúna (16), Jeceaba (17), João Monlevade (18), Mariana (19), Mário Campos (20), Mateus Leme (21), Moeda (22), Nova Lima (23), Ouro Branco (24), Ouro Preto (25), Raposos (26), Rio Acima (27), Rio Manso (28), Rio Piracicaba (29), Sabará (30), Santa Bárbara (31), Santa Luzia (32), São Gonçalo do Rio Abaixo (33), São Joaquim de Bicas (34), and Sarzedo (35). Consulted literature used to identify specimens in each depository: Amaral (1924); Berthold (1859); Boettger (1876); Boie (1827); Boulenger (1885, 1886); Cope (1862, 1869 1884); Costa *et al.* (2016); Curcio *et al.* (2012); Daudin (1802, 1803); Da Silva and Rodrigues (2008); Dixon (1974, 1985); Duméril and Bibron (1835, 1837, 1839); Duméril *et al.* (1854); Fernandes and Puorto (1993); Ferrarezzi *et al.* (2005); Franco *et al.* (1997); Garman (1883); Girard (1857); Gomes (1918); Gunther (1858, 1861, 1863); Hamdan and Fernandes (2015); Hoge (1965); Hoge-Romano (1977); Hutterer (1995); Ihering (1910); Jackson (1978); Jan (1861, 1862, 1863); Jensen (1900); Lacerda (1884); Laurent (1949); Lesson (1830); Lichtenstein and Hinrich (1823); Martins *et al.* (2019); Melo-Sampaio *et al.* (2020); Merrem (1820); Mikan (1820); Moreau de Jonnès (1818); Muller (1923); Peracca (1904); Peters (1863); Pinto and Fernandes (2012, 2017); Prado (1942); Raddi (1820); REinhardt and Lutken (1862); Reuss (1834); Rodrigues (1987); Rodrigues *et al.* (2006, 2014); Roze (1989); Sauvage (1884); Sawaya and Sazima (2003); Schenkel (1901); Schlegel (1837); Schweigger (1812); Spix (1825) and Vanzolini (1976, 1982).

TAXA	DISTRIBUTION IN THE IQ	REGISTER	N. OF ANALYZED SPECIMENS
CROCODYLIA			
ALLIGATORIDAE			
Caiman latirostris (Daudin, 1801)	3, 9, 33	L	1
SQUAMATA			
AMPHISBAENIA			
AMPHISBAENIDAE			
Amphisbaena alba Linnaeus, 1758	1, 3, 6, 7, 13,14, 19, 23, 24, 25, 29, 30 31, 32, 35	SC	36
Amphisbaena dubia Müller, 1924	6	L	1
Amphisbaena vermicularis Wagler in Spix, 1824	3, 6	SC	2
Leposternon infraorbitale (Berthold, 1859)	9	L	1
Leposternon microcephalum Wagler in Spix, 1824	3, 10, 13 18, 24, 25, 31	SC	13
"LIZARDS"			
ANGUIDAE			
Ophiodes fragilis (Raddi, 1826)	1,3,9,10,19,24,25	SC	13
Ophiodes striatus (Spix, 1825)	3,6,14,23,24,,29,30	SC	39
DACTYLOIDAE			
Norops fuscoauratus (D'Orbigny, 1837)	13, 33	SC	2
GEKKONIDAE			
Hemidactylus mabouia (Moreau de Jonnès, 1818)	3,6,7,12,15,24,27,33	SC	12
GYMNOPHTHALMIDAE			
Cercosaura quadrilineata Boettger, 1876	23, 25, 27	SC	3
Cercosaura schreibersii Wiegmann, 1834	23	L	1
Colobosaura modesta (Reinhardt & Luetken, 1862)	6	SC	1
Ecpleopus gaudichaudii Duméril & Bibron, 1839	1,3,8,13,15,19, 24,25,29,30,33,34	SC	65
Heterodactylus imbricatus (Spix, 1825)	1,7,8,9,14,19,23,25,31	SC	30
Heterodactylus lundii Reinhardt & Luetken, 1862	7, 19, 23	SC	3
Rachisaurus brachylepis (Dixon, 1974)	6, 27	SC	2
LEIOSAURIDAE			
Enyalius bilineatus Duméril & Bibron, 1837	3, 6, 7, 8, 9, 10, 12, 13, 14, 15, 19, 22, 23, 24, 25, 26, 29, 30, 33, 34	SC	82
Enyalius boulengeri Etheridge, 1969	18, 33	SC	2

Enyalius perditus Jackson, 1978	7, 24, 25, 27	SC	55
Urostrophus vautieri Duméril & Bibron, 1837	6, 8, 24	SC	4
MABUYIDAE			
Aspronema dorsivittatum (Cope, 1862)	3, 7, 8, 9, 22, 23, 34	SC	10
Copeoglossum nigropunctatum Spix, 1825	23	SC	1
Notomabuya frenata (Cope, 1862)	1, 3, 6, 7, 9, 10, 12, 14, 23, 25, 34	SC	23
POLYCHROTIDAE			
Polychrus acutirostris Spix, 1825	3, 5, 6, 7, 25	SC	8
TEIIDAE			
Ameiva ameiva (Linnaeus, 1758)	1, 3, 6, 7, 12, 13,19, 23, 24, 25, 27, 29, 30, 33	SC	26
Ameivula cipoensis Arias, Carvalho, Zaher & Rodrigues, 2014	3	SC	2
Salvator merianae Duméril & Bibron, 1839	3, 6, 7, 24, 33	SC	6
Tupinambis teguixin (Linnaeus, 1758)	23	SC	1
TROPIDURIDAE			
Tropidurus hispidus (Spix, 1825)	4, 7, 12	SC	4
Tropidurus itambere Rodrigues, 1987	6, 7, 9, 14, 23, 24, 27	SC	16
Tropidurus montanus Rodrigues, 1987	1, 3, 7, 8, 9, 13,14,19, 23, 25, 27, 31	SC	41
Tropidurus oreadicus Rodrigues, 1987	6, 19, 30	SC	3
Tropidurus torquatus (Wied-Neuwied, 1820)	1, 2, 3, 12, 19, 21, 23, 25, 27, 30, 31, 33	SC	43
SERPENTES			
ANOMALEPIDIDAE			
Liotyphlops beui (Amaral, 1924)	23	SC	1
Liotyphlops wilderi (Garman, 1883)	13	SC	1
BOIDAE			
Boa constrictor Linnaeus, 1758	32	SC	1
Corallus hortulanus (Linnaeus, 1758)	3	SC	1
Epicrates crassus Cope, 1862	3, 6, 21, 23	SC	7
COLUBRIDAE			
Chironius bicarinatus (Wied-Neuwied, 1820)	3, 7	SC	2
Chironius brazili Hamdan & Fernandes, 2015	7, 30, 31	SC	3
Chironius exoletus (Linnaeus, 1758)	1, 3, 6, 7, 8, 9, 19, 22, 25	SC	13
Chironius flavolineatus (Jan, 1863)	1, 3, 6, 8, 9,12,14, 19, 22, 23, 24,25, 27, 31	SC	29
Chironius quadricarinatus (Boie, 1827)	5, 6,14,15,19, 23, 25	SC	11
Drymoluber brazili (Gomes, 1918)	3, 12, 19, 30	SC	6
Drymoluber dichrous (Peters, 1863)	9, 25	SC - L	1
Palusophis bifossatus (Raddi, 1820)	3, 5, 6, 18, 19, 35	SC	7
Simophis rhinostoma (Schlegel, 1837)	9, 12, 21, 33	SC	4
Spilotes pullatus (Linnaeus, 1758)	3,5,6,19,23,25,30,31,33	SC	12
Tantilla boipiranga[‡] Sawaya & Sazima, 2003	6, 9, 25, 32	SC	15
Tantilla melanocephala (Linnaeus, 1758)	6	SC	1
DIPSADIDAE			

Apostolepis ammodites Ferrarezzi, Barbo & Albuquerque, 2005	3, 5	SC	2
Apostolepis assimilis (Reinhardt, 1861)	3, 6,11,14,15,16, 22, 23, 24, 25	SC	16
Atractus guentheri (Wucherer, 1861)	3	SC	1
Atractus pantostictus Fernandes & Puorto, 1994	1, 3, 5, 6, 7, 10, 11, 13, 14, 15, 23, 24, 25, 27, 30, 31, 32, 35	SC	71
Atractus reticulatus (Boulenger, 1885)	5	SC	1
Atractus zebrinus (Jan, 1862)	1, 13, 14, 24, 25, 33	SC	14
Boiruna maculata (Boulenger, 1896)	3, 14, 23, 24, 25, 27	SC	7
Boiruna sertaneja (Zaher, 1996)	9	SC	1
Clelia clelia (Daudin, 1803)	33	SC	1
Clelia plumbea (Wied-Neuwied, 1820)	9, 25	SC - L	1
Coronelaps lepidus (Reinhardt, 1861)	25	SC	4
Dipsas albifrons (Sauvage, 1884)	7, 14, 16, 24	SC	5
Dipsas mikanii (Schlegel, 1837)	1,3,5,6,7,9,10,11,13, 14,15,16,19, 23, 24, 25, 27,30,31,32,33,34	SC	82
Dipsas neuwiedi (Ihering, 1911)	3, 7, 8, 9, 19, 23, 24, 32	SC	63
Dipsas ventrimaculata (Boulenger, 1885)	9, 10, 14, 25	SC	4
Echinanthera melanostigma (Wagler in Spix, 1824)	1, 8, 19, 25, 30	SC	16
Elapomorphus quinquelineatus (Raddi, 1820)	1,10, 19, 24,25, 29, 30	SC	12
Erythrolamprus aesculapii (Linnaeus, 1766)	3,4,5,6,7,9,10,11,12,14,19,21,23, 24, 25, 30, 32,34	SC	28
Erythrolamprus almadensis (Wagler in Spix, 1824)	6, 9, 11, 13, 14,19, 22, 23, 25, 31, 32	SC	22
Erythrolamprus cobella (Linnaeus, 1758)	23	SC	1
Erythrolamprus jaegeri (Günther, 1858)	19, 24, 25, 31	SC	8
Erythrolamprus maryellenae (Dixon, 1985)	8, 14, 19, 25, 31	SC	6
Erythrolamprus miliaris (Linnaeus, 1758	3, 6, 16, 19, 21, 23, 24, 31	SC	27
Erythrolamprus poecilogyrus (Wied-Neuwied, 1825)	1,3,5,6,7,9,10,11,14, 16,18,19,21,22,23,24, 25,31,32,33,35	SC	64
Erythrolamprus reginae (Linnaeus, 1758)	3, 5, 6, 14, 23, 32,	SC	7
Erythrolamprus typhlus (Linnaeus, 1758)	3, 18, 24	SC	3
Helicops modestus Günther, 1861	3, 5, 14, 30	SC	5
Helicops nentur Costa, Santana, Leal, Koroiva, Garcia, 2016	3	SC	1
Imantodes cenchoa (Linnaeus, 1758)	1, 3, 6, 9, 13, 14, 19, 23, 24, 27, 30, 31	SC	15
Leptodeira annulata (Linnaeus, 1758)	3, 5, 6, 9, 10, 14, 19, 23, 24, 27, 35	SC	20
Lygophis lineatus (Linnaeus, 1758)	5, 31	SC	2
Lygophis meridionalis (Schenkel, 1901)	3, 25	SC	2
Mussurana bicolor (Peracca, 1904)	9	L	1
Mussurana quimi (Franco, Marques & Puorto, 1997)	25	SC	1
Oxyrhopus clathratus Duméril, Bibron & Duméril, 1854	1, 14, 19, 23, 24, 25, 27, 31	SC	12
Oxyrhopus guibei Hoge & Romano, 1978	3,5,6,7,9,10,11,12, 13,14,17,18,19,21,22, 23, 24,25,27,30, 31,32,35	SC	85
Oxyrhopus petolarius (Reuss, 1834)	23	SC	1
Oxyrhopus rhombifer Duméril, Bibron & Duméril, 1854	6,8,10,14,19,23,24,		

25	SC	77	
Oxyrhopus trigeminus Duméril, Bibron & Duméril, 1854	1, 3, 6, 7, 11, 23, 25, 26, 27, 30, 33	SC	17
Philodryas aestiva (Duméril, Bibron & Duméril, 1854)	14, 19, 25	SC	5
Philodryas olfersii (Liechtenstein, 1823)	1,3,5,6,7,13,14, 19,21,22,23,24, 25,27,32,33,34,35	SC	35
Phimophis guerini (Duméril, Bibron & Duméril, 1854)	12, 32	SC	2
Pseudablabes agassizii (Jan, 1863)	23, 24, 25	SC	4
Pseudablabes patagoniensis (Girard, 1858)	3,14,19,23,24,25,31	SC	57
Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)	6, 9, 22, 24, 25	SC	5
Psomophis joberti (Sauvage, 1884)	23	SC	1
Taeniophallus affinis (Günther, 1858)	3, 6, 7, 24, 33	SC	15
Taeniophallus occipitalis (Jan, 1863)	1, 3, 19, 23, 24, 25	SC	7
Thamnodynastes hypoconia (Cope, 1860)	1, 3, 5, 6, 8, 9, 14, 15, 19, 23, 24, 25, 27	SC	34
Thamnodynastes nattereri (Mikan, 1828)	7, 25, 27, 32, 33	SC	5
Thamnodynastes rutilus (Prado, 1942)	3	SC	1
Thamnodynastes strigatus (Günther, 1858)	8, 31, 33	SC	10
Tomodon dorsatus Duméril, Bibron & Duméril, 1854	23	SC	1
Tropidodryas serra (Schlegel, 1837)	23	SC	1
Tropidodryas striaticeps (Cope, 1869)	1, 3, 5, 6, 7, 8, 9, 11, 13, 14, 18, 19, 23, 25, 27, 31, 33	SC	67
Xenodon merremii (Wagler in Spix, 1824)	3,5,6,7,10,14,19,21,22, 23, 24, 25, 30, 31,33	SC	41
Xenodon neuwiedii Günther, 1863	3, 7, 19, 24,25,31	SC	38
Xenopholis undulatus (Jensen, 1900)	3, 22	SC	2
ELAPIDAE			
Micrurus corallinus (Merrem, 1820)	13, 19	SC	2
Micrurus frontalis (Duméril, Bibron & Duméril, 1854)	1,3,5,6,7,10,13,14,15, 18, 19, 21, 23, 24, 25,30,31,34	SC	57
Micrurus lemniscatus carvalhoi Roze, 1967	3, 6, 25, 31	SC	8
Micrurus lemniscatus lemniscatus (Linnaeus, 1758)	3, 6, 8, 14, 19, 23, 25, 31	SC	15
LEPTOTYPHLOPIDAE			
Trilepida brasiliensis (Laurent, 1949)	3, 7, 14	SC	5
Trilepida jani (Pinto & Fernandes, 2012)	3,7,8,11,14,23,24, 25, 27,30	SC - L	10
TROPIDOPHIIDAE			
Tropidophis preciosus Curcio, Nunes, Argôlo, Skuk & Rodrigues, 2012	7	SC	1
TYPHLOPIDAE			
Amerotyphlops brongersmianus (Vanzolini, 1976)	3	SC	1
VIPERIDAE			
Bothrops alternatus Duméril, Bibron & Duméril, 1854	10, 12, 15, 16,19, 24, 25, 30	SC	33
Bothrops jararaca (Wied-Neuwied, 1824)	1,3,6,7,8,9,10,14, 15,18,19,23,24,25, 27,29, 33	SC	85
Bothrops jararacussu Lacerda, 1884	3, 24	SC	2
Bothrops marmoratus Silva & Rodrigues, 2008	7	SC	1
Bothrops moojeni Hoge, 1966	3, 15, 21,25,34	SC	5

Bothrops neuwiedi Wagler in Spix, 1824	1,3,5,6,7,8,9,10,11,12,14,15,16, 19,20,21,22,23,24,25,27,28,30, 31,34,35	SC	158
Crotalus durissus Linnaeus, 1758	1,3,4,5,6,7,9,10,12, 13,15,16,18,21,22, 24, 25, 28, 30, 32, 33,34,35	SC	60
TESTUDINES			
CHELIDAE			
Acanthochelys spixii (Duméril & Bibron, 1835)	9	L	1
Hydromedusa maximiliani (Mikan, 1820)	8, 10, 19	SC	3
Hydromedusa tectifera (Cope, 1870)	3	SC	1
Phrynops geoffroanus (Schweigger, 1812)	31	SC	1

Liotyphlops wilderi (Garman, 1883), and *Tomodon dorsatus* Duméril, Bibron and Duméril, 1854 (Fig. 4).

Ameivula cipoensis is a small teiid lizard that occurs from the northeastern to the central region of Brazil (Arias *et al*., 2014). The specimens recorded in this study (UFMG 1786, 3247) came from the municipality of Belo Horizonte (-19.9638, -43.9186; -19.9789, -43.9585) located 100 km from its type locality - Serra do Cipó, MG. Morphological examination revealed that the specimens have characters of the morphological diagnosis proposed by Arias *et al.* (2014), such as the presence of three supraocular scales, five supraciliary scales, eight rows of ventral scales and a brown tail.

The lizard *E. boulengeri* inhabits arboreal and semi-arboreal forests in Brazil (Jackson, 1978; Rodrigues *et al*., 2006). After a revision of the genus, which included a voucher from Viçosa, a municipality in the state of Minas Gerais, Rodrigues *et al.* (2014) revalidated the species using molecular data. The specimens (MCNR 4341, UFMG 879, 1053, 1054, 1061, 1070), are from the municipalities of João Monlevade and São Gonçalo do Rio Abaixo (-19.8886, -43.3660), which are 161 km and 195 km from the municipality of Viçosa, respectively.

Liotyphlops wilderi is a blind snake with occurrence in the Atlantic Forest biome in the states of Minas Gerais, Rio de Janeiro and São Paulo (Centeno *et al*., 2010). The specimen analyzed here (UFMG 1807) is from the municipality of Itabira (-19.6184, -43.2266) - 167 km from the type locality the municipality of Alvorada de Minas, state of Minas Gerais.

Tomodon dorsatus is a dipsadid snake distributed in the southern region of Brazil and in neighboring countries. The specimen analyzed here (FUNED 2648) is from the municipality of Nova Lima (-19.9856, -43.8467).

Taxonomic uncertainties and previous unconfirmed records for the IQ

Furthermore, seven of the species registered on our surveys were considered not to naturally occur in the IQ: *Amerotyphlops reticulatus* (Linnaeus, 1758) Typhlopidae, *Amphisbaena prunicolor* (Cope, 1885) Amphisbaenidae, *Bothrops atrox* (Linnaeus, 1758) and *Bothrops leucurus* Wagler, 1824 Viperidae, *Cnemidophorus lemniscatus* (Linnaeus, 1758) Teiidae, *Iguana iguana* (Linnaeus, 1758) Iguanidae, and *Lampropeltis getula* (Linnaeus, 1766) Colubridae. The following explanations elucidate the reasons why those were classified this way.

The blind snake *A. reticulatus* occurs in the central, north and northeast areas of Brazil (Uetz and Hosek, 2019). The individual analyzed here (FUNED 264) is from Serra da Moeda in the municipality of Moeda). It can be distinguished from its sister species *A. brongersmianus*, which occurs in the state of Minas Gerais, by the presence of a white snout and a number of dorsal scales near the tail that are also pigmented white (Hoser, 2012). We believe that this register had its locality of collection accidentally changed in the specimen depositary.

Amphisbaena prunicolor occurs in Argentina, Paraguay and south and southern regions of Brazil - from the states of Rio Grande do Sul to the state of Espírito Santo (Gans, 1966; Perez *et al*., 2012). Although, Gans (1966) recorded the species to the state of Minas Gerais, Brazil, and in our surveys we also found it in a technical report for the municipality of Congonhas in the same state, we decided to follow Perez *et al.* (2012) and Costa and Bérnils (2018) and keep it as a doubtful record for the IQ

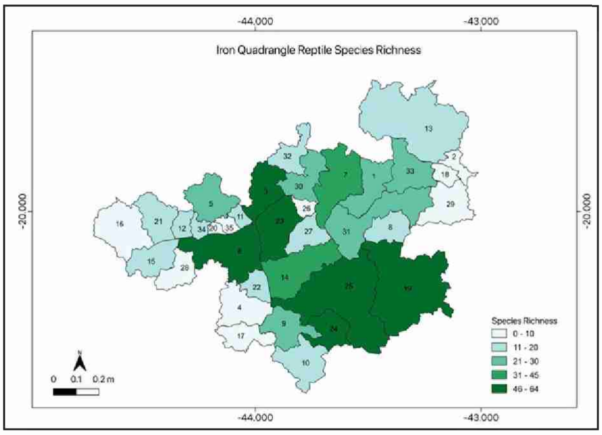

Figure 2. Distribution of reptile species by municipalities in the Iron Quadrangle, Minas Gerais, Brazil. Numbers represent the municipalities in alphabetical order: Barão de Cocais (1), Bela Vista de Minas (2), Belo Horizonte (3), Belo Vale (4), Betim (5), Brumadinho (6), Caeté (7), Catas Altas (8), Congonhas (9), Conselheiro Lafaiete (10), Ibirité (11), Igarapé (12), Itabira (13), Itabirito (14), Itatiaiuçu (15), Itaúna (16), Jeceaba (17), João Monlevade (18), Mariana (19), Mário Campos (20), Mateus Leme (21), Moeda (22), Nova Lima (23), Ouro Branco (24), Ouro Preto (25), Raposos (26), Rio Acima (27), Rio Manso (28), Rio Piracicaba (29), Sabará (30), Santa Bárbara (31), Santa Luzia (32), São Gonçalo do Rio Abaixo (33), São Joaquim de Bicas (34), and Sarzedo (35).

region, once we did not find the voucher-specimens from the state of Minas Gerais.

The viperid snake *B. atrox* occurs in the north and part of the northeast regions of Brazil as well as countries bordering these regions (Martins and Oliveira, 1998; Oliveira and Martins, 2001; Nogueira *et al.*, 2019). The specimen registered in our survey (FUNED 963), was part of the live research herd of the institution located in the municipality of Belo Horizonte. Thus, this locality is not a reliable one for the species (Nogueira *et al.*, 2019).

Bothrops leucurus occurs in the Atlantic Forest along the Brazilian coast from the northeast to the southeast regions (Porto and Teixeira, 1995; Lira-da-Silva *et al.*, 2009; Nogueira *et al.*, 2019), including some municipalities of Minas Gerais (Carvalho Jr. and Nascimento, 2005; Nogueira *et al.*, 2019). The specimen recorded in the present survey was also part of the live research herd housed at FUNED under the voucher (FUNED 1783).

A single individual of the teiid species *C. lemniscatus* (MCNR 3854) was recorded from the Instituto Inhotim (Botanical Garden and Museum of Contemporary Art), located in the region of Brumadinho. The lizard was collected after a flora rescue from the state of Pará (Linares and Eterovick, 2013; Linares, 2016, personal communication).

Nine specimens of *I. iguana* were deposited under the vouchers (UFMG 3231 to 3239), with the locality of Belo Horizonte. These specimens were seized from wildlife trafficking, thus the place of origin is not reliable.

The natural distribution of the snake *L. getula* is restricted to the United States (Connant and Collins, 1998). The species is widely used as a pet both inside and outside the USA (Gibbons *et al.*, 2000; Franke and Telecky, 2001; Sodhi *et al.*, 2004; SCBD, 2010), which may explain its occurrence in the municipality of Belo Horizonte, the specimen here analyzed (FUNED 2755) was captured in a train station.

Municipalities of Minas Gerais with larger areas, as well as those adjacent to the state capital of Belo Horizonte, responsible for many of the studies related to the fauna and flora of the state (FJP, 2003; Oliveira *et al.*, 2017), present the richest areas in reptile species. These also include municipalities part of the IQ, as Belo Horizonte, Ouro Preto, Ouro Branco, Nova Lima, Mariana, Brumadinho and Itabirito (Fig. 5).

Furthermore, in our survey, we found (N = 51) species of snake in the municipality of Ouro Preto and (N = 36) species in Ouro Branco, while in previous studies of the snake fauna of these municipalities Silveira *et al.* (2004) São-Pedro and Pires (2009) demonstrated a discrepant result of species richness where Silveira *et al.* (2004) found almost twice species of snakes in Ouro Preto as the one recorded in Ouro Branco region by São-Pedro and Pires (2009) (N = 51 spp. and N = 28 spp. respectively).

This dissimilarity could partially be explained by differences in effort sample size between the studies, increase of field trips, occasional encounters provoked by habitat loss, especially in the municipality of Ouro Branco, and by the state of conservation of protected areas. Ouro Preto has three conservation units in its surroundings, while Ouro Branco does not have any conservation unit, and suffers from the growing destruction of its natural landscapes due to agricultural and mining activities (São-Pedro and Pires, 2009). Additionally to the reptile biodiversity in the region of Ouro Branco, Cruz *et al.* (2014), found 15 species of lizards in a restricted area of the region (Serra do Ouro Branco), while we registered 14 species for the municipality.

Moreover, we registered 46 reptile species in the municipality of Brumadinho (N = 13 lizards + N = 33 snakes), while Linares and Eterovick (2013) found 33 species of reptiles (12 spp. of lizards, 21 spp. of snakes) in a restricted area of the same municipality. We attribute the difference in the number of snake species in the studies to two factors: the first to the size of the area analyzed, and the second to the new distribution extensions to the area such as the colubrid snake *Tantilla boipiranga* Sawaya and Sazima, 2003 (Tunes *et al.*, 2020).

In contrast to the municipalities with the greatest species records, the municipalities with less than five species of reptiles may represent underestimated data, since these municipalities have mineral and agricultural production, as well as conservation areas, such as environmental parks and natural monuments such as APA Rio Manso, Serra do Gambá, Serra de Jeceaba and Serra de Santa Cruz (JECEABA, 2009; IEF, 2016).

According to the 29 most widespread species of reptiles found in our survey (Table 1), most species of snakes present a mixed of daily activities, displaying behaviors in daylight and in the night shift, as demonstrated by França and Braz (2013), while lizards are exclusively diurnal as demonstrated by Filogonio *et al.* (2010) and Cruz *et al.* (2014), facilitating encounters with some of these species. In our study, we found that Dipsadidae was the most species-rich family in the IQ (Fig. 2; Table 1). That may be because it possesses the most tropical species (>700) (Vidal *et al.*, 2010), and to the rearrangement of the Caenophidia clade and the paraphyletic family Colubridae (Underwood and Kochva, 1993; Zaher, 1999; Zaher *et al.*, 2009). Moreover, the viperidae species *Bothrops neuwiedi* (Wagler, 1824) and *Crotalus durissus* (Linnaeus, 1758) and the Dipsadidae species *Dipsas mikanii* (Schlegel, 1837), *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825) and *Oxyrhopus guibei* (Hoge and Romano, 1978) were distributed among the greatest number of municipalities (n>20) (Table 1). These snakes are highly plastic and can persist in urban environments (MS, 1988, Carvalho and Nogueira, 1998; Marques *et al.*, 2004; SMA, 2013; Nogueira *et al.*, 2019). Their wide distributions, especially that of *C. durissus*, may be related to the conversion of forested areas into open environments due to habitat loss. *Crotalus durissus* is considered an open savannah species, and so the suppression of forest creates favorable open environments with greater food availability from human communities (Salomão *et al.*, 1995; Araújo *et al.*, 2003; Bastos *et al.*, 2005).

Regarding the 128 species of reptiles recorded for the IQ by the present study, three taxa deserve special attention because of their threat levels. Only two species occurring in the IQ are threatened at regional (COPAM, 2010) national (MMA, 2014) and global (IUCN, 2019) levels: *Heterodactylus lundii* Reinhardt and Luetken, 1862 and *Hydromedusa maximiliani* (Mikan, 1820). A third species, *T. boipiranga* is listed as Vulnerable only at global level (IUCN, 2019). One of the species is endemic to Serra da Piedade, part of the Espinhaço mountains range; four species have their type locality to the IQ area, and other four species had their extension ranges included in the Iron Quadrangle, thus reinforcing the need for the implementation of conservation measures and mitigation of anthropogenic impacts in the region.

The freshwater turtle *H. maximiliani* occurs in the Atlantic Forest in the southeast and south of the northeast regions of Brazil (Souza, 2005). The species recorded here is the only one in the IQ with the status of Vulnerable (VU) at regional (COPAM, 2010) and global (IUCN, 2019) levels.

The IQ is an important region of the state of Minas Gerais due to its cultural, biological and archaeological attributes, as well as its large mining reserves. It encompasses an area of transition between two biodiversity hotspots, the Atlantic Rainforest and the Cerrado (Myers *et al.*, 2000), highly threatened by human activity, especially by the mining industry and urban sprawl. These reasons highlight the importance of knowledge related to the region biodiversity, essential for conservation efforts and recovery strategies for the species and the habitats that occur within its boundaries. As stated above, the IQ is inserted in an area which makes it especially important due to its high level of threat and species endemicity, not only the flora but also for other organisms, in addition to the richness of other vertebrate groups (Carmo and Jacobi, 2012). These factors highlight the importance of this study, once it provides the first list of reptile species that occur in the area, as well as to the numerous existing publications for other living groups of fauna and flora of the IQ.

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Nota

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Behavioral aspects during the subjugation of prey by *Micrurus ibiboboca* (Merrem, 1820) (Squamata: Elapidae)

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ABSTRACT

Here we report the first description of subjugation of an *Amphisbaena* cf *vermicularis* by *Micrurus ibiboboca* in free life. The snake was sighted with an *A.* cf *vermicularis* within the mouth then started to prey it on the hole, diverging from other species from the genus *Micrurus,* which starts to ingest the prey completely out of the underground.

Key Words: Diet; Snake; Predation.

Snakes of the genus *Micrurus* and *Leptomicrurus* are commonly known in Brazil as “cobras-corais” or “corais-verdadeiras” due to most of the species possess colored rings around the body (black, red or orangish and white or yellowish) (Bernarde, 2014). Coral snakes possess proteroglyphous fangs, small eyes, and a short tail (Grantsau, 2013), feature upon in which the genus *Micrurus* was named (from the Greek *Mikros* = small and *Oura* = tail; see Hoser, 2012). The genus *Micrurus* is broadly spread throughout South America, registered on different biomes, presenting semi-fossorial behavior, and aquatic in some species (*Micrurus surinamensis* and *M. nattereri*) (Passos and Fernandes, 2005; Silva Jr *et al.*, 2016).

The Brazilian territory hosts around 35 species of Elapidae (among the genera *Micrurus* and *Leptomicrurus*) distributed in different biomes (Silva Jr *et al.* 2016; Nogueira *et al.* 2019). The species *Micrurus ibiboboca* (Merrem, 1820) is broadly distributed on the northeast region of the country and can be found in forests, open fields, and urbanized areas (França *et al.*, 2012). Prey items such as amphibians, lizards, elongated vertebrates and re-ingestion of regurgitated animals were reported for *M. ibiboboca* (Bernarde, 2014; Silva Jr *et al.*, 2016; Barbosa *et al.*, 2019), however, many gaps regarding the subjugation method still remain open, perhaps due to its semi-fossorial behavior, hindering studies in this area. Here we report the first description of subjugation of *Amphisbaena* cf *vermicularis* by *Micrurus ibiboboca*.

On April 15th, 2019 at 01:20 pm on an anthropized area on the *campus* of Universidade Federal Rural de Pernambuco - UFRPE (8°00’51.86”S, 34°57’02.14”W), Recife, Pernambuco, Brazil, an adult male of *M. ibiboboca* was seen with the first third of its body in a hole on the ground (Fig. 1A). The snake was pulling itself out of the hole and then we concluded that it was biting the head of an *A.* cf *vermicularis* (Fig. 1B), which was completely inside the hole, and made lateral movements with its head while holding and pulling the *A.*cf *vermicularis* out of the hole which still showed squirming movements and pulling the body into the hole. At 01:26 pm the *A.*cf *vermicularis* did not present any more movement, and thus, the *M. ibiboboca* began the ingestion process by moving its head laterally

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and upwards, gradually removing the prey from the ground by ingesting it (Fig. 2). All the process (from subjugation to complete ingestion) lasted 1 hour and 59 minutes. Afterward, the snake performed serpentine movements to finish swallowing for 2 minutes and 25 seconds (Fig. 2D). After complete ingestion, the snake fixed its skull bones opening and closing the mouth three times (in yawn movements) for 49 seconds. The whole process lasted 2 hours, 2 minutes and 14 seconds. At the end of the feeding, the coral snake slithered away in the opposite direction of the *A.* cf *vermiculairs* tunnel. The individual was captured and carried to the lab for morphometric analyses (600 mm in snout-vent length, 45 mm in tail length and weighing 31 g), and then it was released in a non-urbanized area.

The presence of the human watching and taking photographs did not change the animal's behavior, as the snake did not stop the feeding nor tried to escape or regurgitate the prey.

Snakes can usually hunt actively or by lurking, as there are species capable of using both strategies (Bernarde *et al.*, 2000; Sazima and Marques, 2007; Turci *et al.*, 2009). The *Micrurus ibiboboca* proved to be an active predator such as *M. albicintus* elucidated by Souza *et al.* (2011), and possibly both species are guided by chemical sensors and olfaction to find their prey (Bernarde, 2012). Although we have not measured the *A.* cf *vermicularis*, the proportion between predator and prey is considered small, with prey being slightly smaller than the predator (Fig. 1). This predator-prey similar body size was reported for other species within the genus (Souza *et al.*, 2011).

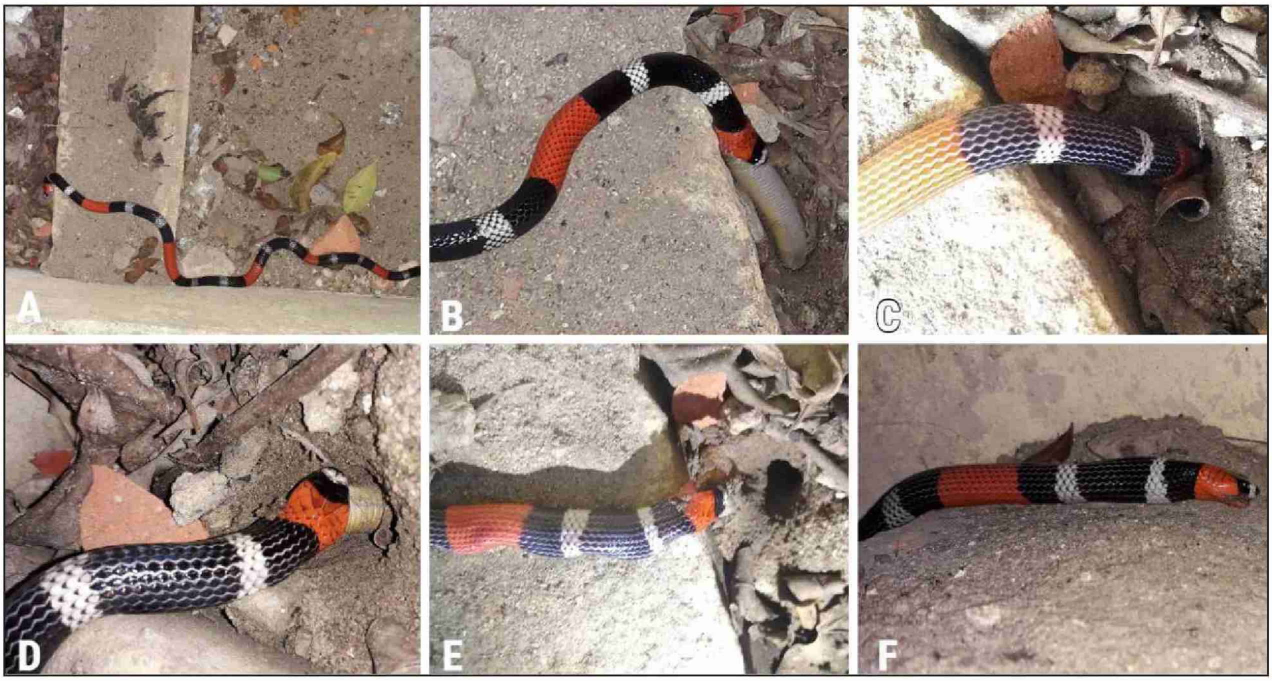

Figure 1. The subjugation of *Amphisbaena* cf *vermicularis* by *Micrurus ibiboboca* on Universidade Federal Rural de Pernambuco. A – *M. ibiboboca* holding the *A.* cf. *vermicularis* on the mouth; B - Beginning of the ingestion; C - Pulling movements from the ground; D - Lateral head movements removing the prey from the hole; E - Complete withdraw with serpentine movements; F - Finishing the ingestion and moving away from the hole.

The *M. ibiboboca* captured and injected venom into prey still underground as *M. albicintus* and *M. coralinus*, which have data in captivity (Serapicos *et al.*, 2002; Souza *et al.*, 2011). However, the *M. ibiboboca* observed here differed from the others species by swallowing while removing prey from the soil, whereas the other species removed the prey altogether before starting the ingestion (Souza *et al.*, 2011). The strategy of envenoming underground can be a method of minimizing injuries to the predator since *Amphisbaena* spp. are able to bite and rotate the body as a defensive tactic (Barbo and Marques, 2003; Albuquerque *et al.*, 2008; Souza *et al.*, 2011). Yawning after ingestion has been described for the

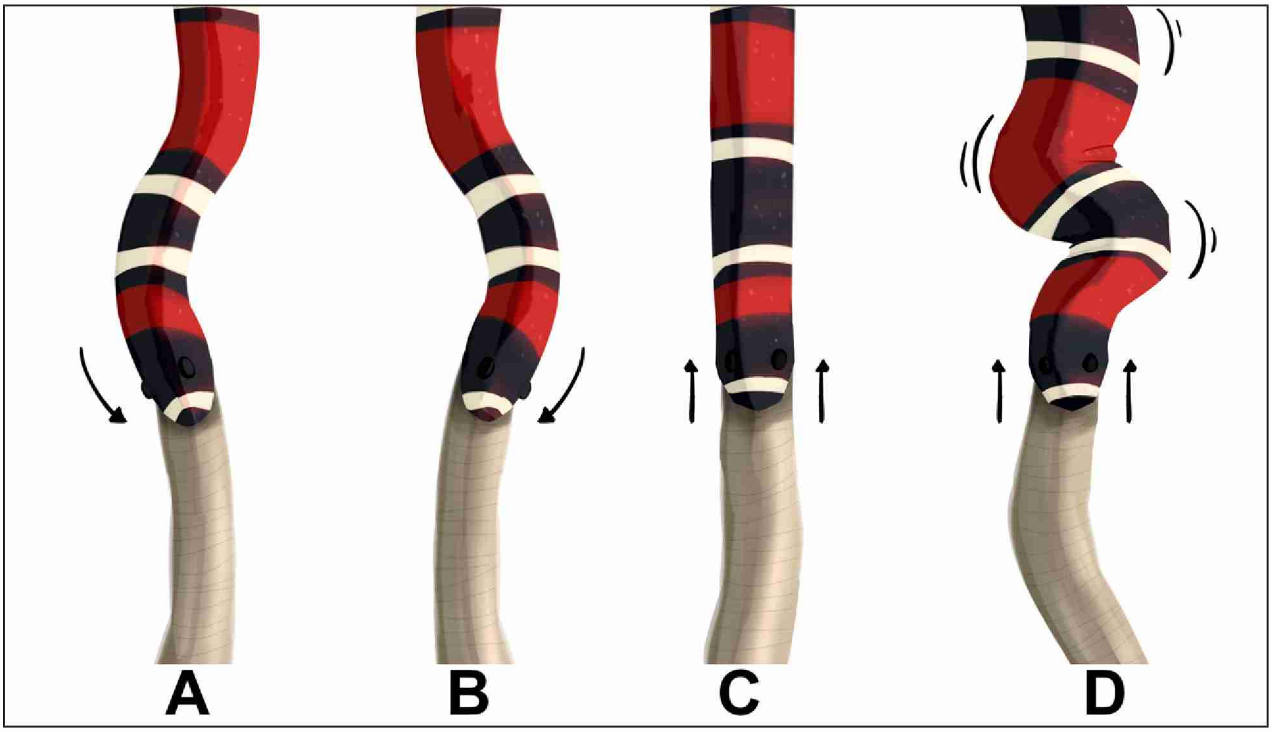

Figure 2. Illustration of head movements used by *Micrurus ibiboboca* during the predation observation. A and B - Head lateral movements; C - Pulling movements to remove the *Amphisbaena* cf *vermicularis* from the hole; D – Serpentine movements were used at the and of ingestion.

Viperidae family. Other authors classify yawns as long or short, as verified in some *Crotallus durissus* individuals (Sueiro *et al.*, 2006). In this study, also according to Sueiro *et al.* (2006), the specimen of *M. ibiboboca* adjusted the skull with long during yawns, in which it performed three wide openings of the mouth and made movements of the glyphs. The strategy of envenoming underground for this and other species of *Micrurus*, reinforces the foraging behavior for the genus. Our report presents *M. ibiboboca* actively hunting, however, we cannot say whether the foraging started on the surface of the ground and then went to the tunnel where *A.* cf *vermicularis* was, similar to the reported of Souza *et al.* (2011), or if it started foraging underground. Thus, we emphasize the need for further studies on the strategies of capture and ingestion seeking a better understanding of the feeding behavior of the species and the whole genus.

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Nota

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Novel behavioral observations of the lizard *Tropidurus hispidus* (Squamata: Tropiduridae) in Northeastern Brazil

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ABSTRACT
In this study, we report additional cannibalism and predation events in *Tropidurus hispidus* and, by reviewing our data with that found in the literature, we quantify the relative importance of vertebrate categories as prey for *T. hispidus*. In addition, we provide an ethogram for events related to territoriality, courting and copulation.

Key Words: Copulation; Diet; Ecology; Natural history; Territoriality.

Studies that highlight issues related to diet, habitat use and reproduction are essential for understanding general patterns and processes of populations and help to define conservation strategies based on the knowledge of species (Sawaya *et al.*, 2008). This information can be obtained through observations in the natural environment (Sawaya *et al.*, 2008; Vasconcelos *et al.*, 2019), as well as in the laboratory (Coelho *et al.*, 2018; Pelegrin, 2019).

Tropidurus hispidus (Spix, 1825) (Tropiduridae) is a medium-sized lizard, considered to be the largest species in the genus, with a wide distribution throughout South America in countries like Venezuela (incl. Cojedes), Isla Margarita, Suriname, French Guiana and Brazil (Ávila-Pires, 1995; Uetz *et al.*, 2020). In Brazil, it is an abundant and generalist species related to use of habitat occurring in different microhabitats in the Caatinga biome being observed mainly on rocks, tree trunks and walls (Rodrigues, 1987; 1988; Ávila-Pires, 1995; Carvalho, 2013). This is a heliophilic and insectivorous lizard (Vitt *et al.*, 1996) that adopts a sit-and-wait foraging strategy (Schoener, 1971) and exhibits territorial behaviors (Ribeiro *et al.*, 2012).

This species has been the subject of several studies, that have addressed aspects of its ecology and natural history (Rodrigues, 1988; Vitt *et al.*, 1996; Teixeira and Giovanelli, 1999; Kolodiuk *et*

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al., 2009; Kolodiuk *et al.*, 2010; Ribeiro *et al.*, 2010; Ribeiro and Freire, 2011; Santana *et al.*, 2011; Santana *et al.*, 2014; Gomes *et al.*, 2015; Lima *et al.*, 2017; Melo *et al.*, 2017; Albuquerque *et al.*, 2018), cytogenetics (Kasahara *et al.*, 1987; Frost *et al.*, 2001; Matos *et al.*, 2016) and parasitism (Almeida *et al.*, 2008; Delfino *et al.*, 2011; Brito et *al.*, 2014), although relatively well studied, there are still many aspects of the natural history of this species that remain unknown, especially behavioral aspects related to territoriality, reproduction and predation on vertebrates. Diet-related data can be easily obtained in the laboratory, however, information on behavioral traits is more difficult to observe in captivity, as even in natural environments aggressive encounters and predation events are rarely reported (Passos *et al.,* 2016). In this study, we report novel records of cannibalism and predation in *T. hispidus*, and by incorporating our data with previous literature; we estimate the importance of each category of vertebrate registered as prey of *T. hispidus*. We also present new observations of territoriality, courtship and copulation displays of this lizard. Finally, we provide an ethogram for events related to territoriality, courtship and copulation.

Most of the events (n= 10) were recorded on a private property, in a rural area, named Sítio Fechado (-7.299166667 S; -37.29777778 W, WSG84; 737 m.a.s.l.; Garmin GPSMAP 64s), an area of Caatinga located in the municipality of Brejinho, state of Pernambuco, Northeastern Brazil. One of the cannibalism events was recorded in an urban area (-7.206388889 S, -36.82361111 W; WSG84; 532 m.a.s.l.; Garmin GPSMAP 64s), in the municipality of Taperoá, in the state of Paraíba, Northeastern Brazil. Two other predation events were also recorded on private properties in Caatinga areas, both in rural areas, one of them named Sítio Clarinha (-7.378333333 S, -37.19027778 W; WSG84; 637 m.a.s.l.; Garmin GPSMAP 64s) and another Sítio Goiana (-7.375555556 S, -37.20638889 W; WSG84; 647 m.a.s.l.; Garmin GPSMAP 64s), both located in the municipality of Itapetim, state of Pernambuco, Northeastern Brazil.

All observations were occasional records and the individuals were not collected. However, all events were photographed, and the images are used here to illustrate the behaviors. Since our study is only descriptive, without the aim of comparing classes of behaviors, individuals or species, we used the *Ad libitum* observation method (Altman, 1974), that does not require systematic sampling, and where all of an individual's behaviors are recorded in an unlimited period of time.

To describe the *T. hispidus* diet, we performed a bibliographic search in the main online electronic libraries: Web of Science, JSTOR, Scielo, Scopus, Semantic Scholar and Google Scholar, using keywords: "*Tropidurus hispidus*" and a combination of "*Tropidurus hispidus*" and "diet" or "prey" or "saurophagy" or "anuran" or "frog" or "bird" or "mammal". Based on the number of registered events we quantified the relative importance of each category for prey (lizards, anurans, birds and mammals).

Diet

Cannibalism

The first cannibalism event was observed on December 31st, 2016, at 17:27 h, in Sítio Fechado, which lasted 4 min. A *T. hispidus* adult was on the cement wall with the head and tail of a juvenile protruding from its mouth (Fig. 1A). After 3 min, the adult lizard shook its head and completed the ingestion of its prey. The second event of cannibalism was observed on June 11st, 2020, at 14:07 h, in an urban area, lasting 1 min and 15 sec. A *T. hispidus* juvenile climbed onto a rock and soon after was captured by a conspecific adult (Fig. 1B). Then the adult lizard escaped with its prey in its mouth and was no longer visible to record further observations.

Predation

The first predation event occurred on April 30th, 2016, at 10:29 h, in Sítio Clarinha, when a *T. hispidus* adult male was observed trying to prey on a small adult rodent (*Mus musculus*). Both individuals were on the ground close to a house, and the mammal was subjugated by the lizard. The lizard moved across on the ground (~100 cm) keeping the mammal inside its mouth, holding it by the head. Then, the predator stopped for 1 min, so the mammal tried to escape and, quickly, the lizard moved the head in direction to the ground, pressing the prey to immobilize it. The lizard kept still for 10 min, and then, climbed a residence wall and stopped again at 130 cm from the floor for 40 sec still holding the mammal by the head in its mouth (Fig 1C). At this moment, the lizard noticed the presence of the observer and directed its head to the ground, lifted the tail and bobbed its head four times. Subsequently, the lizard climbed onto the roof of the residence, and it was no longer possible to follow the interaction. The whole event lasted 15 min.

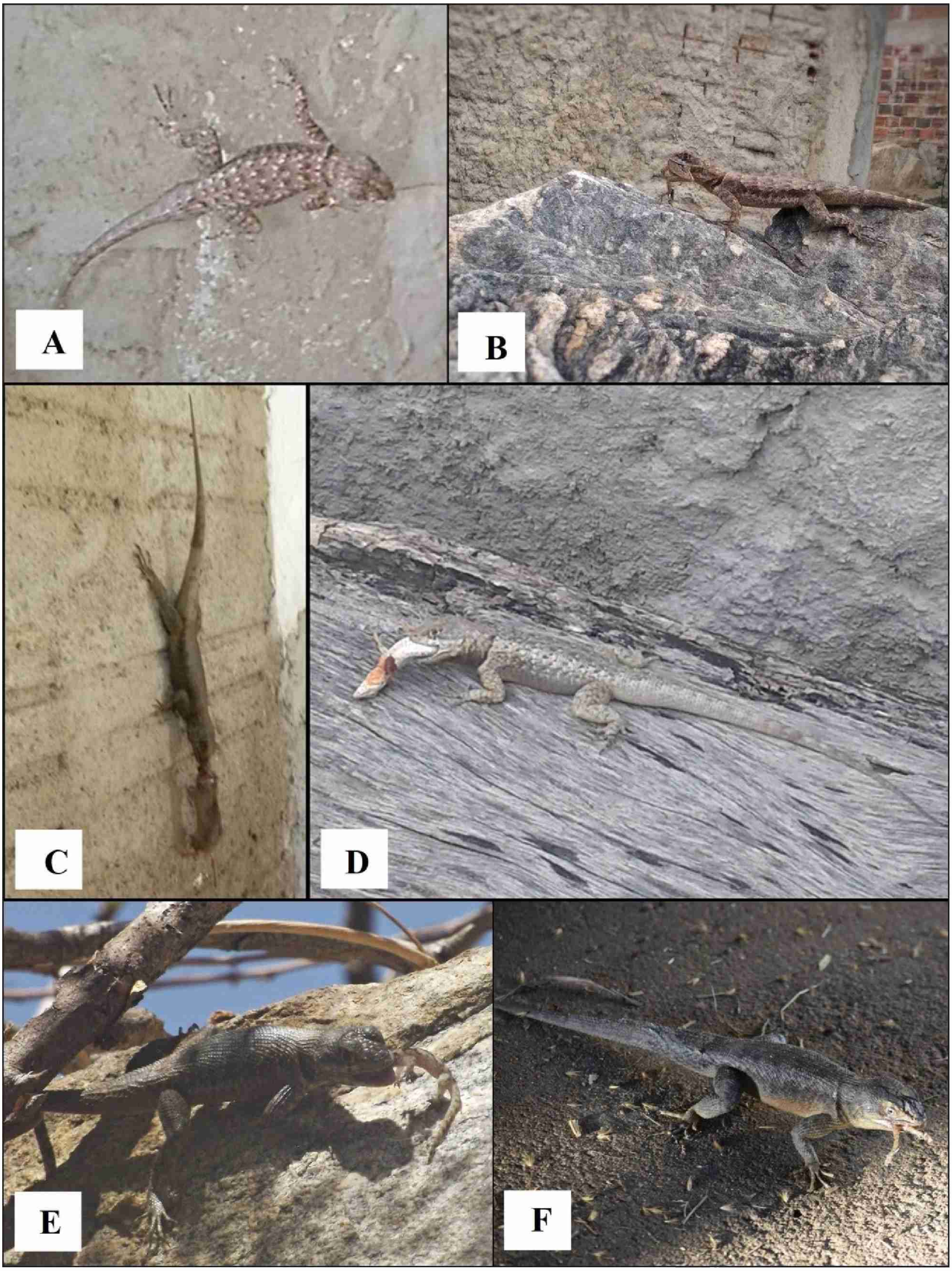

Figure 1. Behavioral events related to the diet of *Tropidurus hispidus,* in Sítio Fechado, Pernambuco, Brazil: A—B- cannibalism; C- predation on *Mus musculus;* D- predation on *Ameivula ocellifera*; E- predation on *Phyllopezus pollicaris*; F- predation on *Scinax x-signatus*

The second predation event was recorded on October 17th, 2017, at 14:22 h, in Sítio Fechado, lasting 30 min. A *T. hispidus* adult was under a tree with a *Ameivula ocellifera* individual in its mouth, having already ingested the tail and part of the limbs. The presence of the observer caused the lizard to move 150 cm, climbed on a trunk and remained still (Fig. 1D), just bobbing its head for 10 min. Later, it moved another 100 cm and climbed onto the wall of a residence, reaching the roof. The individual moved another 75 cm climbing onto the tiles with the prey in its mouth; it then stopped moving and completed the ingestion of the prey in 4 min.

The third predation event occurred on December 29th, 2018, at 11:48 h, in Sítio Goiana, lasting 50 sec. A *T. hispidus* adult was seen chasing a juvenile of the lizard *Phyllopezus pollicaris*, capturing the prey after 5 sec. Soon after, it started eating its prey starting from the head (Fig. 1E) and completing the process in 40 sec.

The fourth predation event was recorded on July 3rd, 2020, at 16:44h, in Sítio Fechado, when a *T. hispidus* adult stood on the wall of a residence and spotted the prey. It quickly reached the ground and captured a tree frog (*Scinax x-signatus*), with only the hindlimbs hanging out of its mouth (Fig. 1F). After 2 min it completed the ingestion of the prey.

According to the literature, invertebrates are the main food items of *T. hispidus* in different biomes (Vitt *et al.,* 1996; Van Sluys *et al.,* 2004; Kolodiuk *et al.,* 2010; Ribeiro and Freire, 2011). Therefore, observations of this species preying on other vertebrates continue to be uncommon. However, the fact that *T. hispidus* preys on vertebrates is not surprising in view of its medium size (68—114 mm; Kolodiuk *et al.,* 2010) and its sit-and-wait foraging strategy which makes the species more opportunistic and less selective (Vitt *et al.*, 1996; Ribeiro and Freire, 2011).

Our records corroborate data already cited in the literature for *T. hispidus* feeding on the amputated tail of a conspecific (Sales *et al.*, 2011), and body parts of a *T. hispidus* juvenile in the stomach contents of an adult (Carneiro *et al.*, 2020). Predation of the lizard *A. ocellifera* has also been reported in the literature (Costa *et al.*, 2010; Zanchi *et al.*, 2012), as well as predation of the lizard *P. pollicaris* (Dubeux *et al.,* 2020), the tree frog *S. x-signatus* (Ribeiro and Freire, 2009; Mendes, 2017), as well as the deadly-fresh rodent *M. musculus* (Virginio *et al.,* 2017).

T. hispidus is a sit-and-wait predator (Rodrigues, 1987), consuming a wide range of prey, including insects, plant parts and small vertebrates (Table 1), which suggests that this species is opportunistic in the trophic aspect. Furthermore, it is also known

Table 1. List of vertebrate prey species reported for *Tropidurus hispidus* in Brazil. * = field record.

Prey categories	Species	Source
ANURANS		
Microhylidae	*Elachistocleis ovalis* (Schneider, 1799)	Vitt *et al.*, 1996
Hylidae	*Scinax x-signatus* (Spix, 1824)	Ribeiro and Freire, 2009; Mendes, 2017*; This study*
BIRDS		
Rhynchocyclidae	*Todirostrum cinereum* (Linnaeus,1766)	Guedes *et al.*, 2017*
Trochilidae	*Eupetomena macroura* (Gmelin, 1788)	Guedes *et al.*, 2017*
	Chlorostilbon lucidus (Shaw, 1812)	Fernandes *et al.*, 2020*
LIZARDS		
Gymnophtalmidae	*Colobosaura modesta* (Reinhardt and Lutken, 1862)	Costa *et al.*, 2010
	Vanzosaura multiscutata (Amaral, 1933)	Oliveira *et al.*, 2020
Tropiduridae	*Tropidurus* sp. (tail*)	Van Sluys *et al.*, 2004
	Tropidurus hispidus (Spix, 1825)	Sales *et al.*, 2011*; Carneiro *et al.*, 2020; This study*
	Tropidurus jaguaribanus (Passos *et al.*, 2011)	Passos *et al.*, 2016*
Gekkonidae	*Hemidactylus mabouia* (Moreau de Jonès, 1818)	Silva *et al.*, 2012*; Pagel *et al.*, 2020*
Teidae	*Ameiva ameiva* (Linnaeus, 1758)	Rodrigues *et al.*, 2015*
	Ameivula ocellifera (Spix, 1825)	Costa *et al.*, 2010; Zanchi *et al.*, 2012*; This study*
Phyllodactylidae	*Gymnodactylus geckoides* (Spix, 1825)	Pergentino *et al.*, 2017*
	Phyllopezus pollicaris (Spix, 1825)	Dubeux *et al.*, 2020*; This study*
Dactyloidae	*Norops auratus* (Daudin, 1802)	Costa-Campos and Souza, 2013
MAMMALS		
Muridae	*Mus musculus* (Linnaeus, 1758)	Virginio *et al.*, 2017* This study*

Table 2. List of predator species reported for *Tropidurus hispidus* in Brazil.

Predator categories	Species	Source
ANURANS		
Leptodactylidae	*Leptodactylus troglodytes* (A. Lutz, 1926)	Alcantara *et al.*, 2014
BIRDS		
Cathartidae	*Cathartes burrovianus* (Cassin, 1845)	Aragão *et al.*, 2020
Accipitridae	*Rupornis magnirostris* (Gmelin, 1788)	De-Carvalho *et al.*, 2011
SNAKES		
Dipsadidae	*Oxyrhopus trigeminus* (Duméril *et al.*, 1854)	Alencar *et al.*, 2012; Mikalauskas *et al.*, 2017
	Philodryas nattereri (Steindachner, 1870)	Vitt and Vangilder, 1983; Mesquita *et al.*, 2011; Menezes *et al.*, 2013
	Boiruna sertaneja (Zaher, 1996)	Vitt and Vangilder, 1983
	Siphlophis leucocephalus (Gunther, 1863)	Maia-Carneiro *et al.*, 2016
Colubridae	*Oxybelis aeneus* (Wagler, 1824)	Mesquita *et al.*, 2012; Sousa *et al.*, 2020; Santana and Teixeira, 2020
Viperidae	*Crotalus durissus ruruima* (Hoge, 1965)	Farias and Primeiro, 2020
LIZARDS		
Teidae	*Salvator merianae* (Duméril and Bibrons, 1839)	Silva *et al.*, 2013
	Ameivula ocellifera (Spix, 1825)	Tavares *et al.*, 2017
Tropiduridae	*Tropidurus hispidus* (Spix, 1825)	Sales *et al.*, 2011; Carneiro *et al.*, 2020; This study*
SPIDERS		
Theraphosidae	*Lasiodora klugi* (C.L. Koch, 1841)	Vieira *et al.*, 2012
MAMMALS		
Callithrichidae	*Callithrix jacchus* (Linnaeus, 1758)	Melo at al., 2018

to be a species consumed by a variety of animal species (Table 2), making this species a significant link in the food chain of its habitat.

Among the categories of vertebrates recorded as prey of *T. hispidus* in the literature and in the present study, lizards are the most frequent item, followed by birds, anurans and mammals (Fig. 2). The lizard species mentioned as prey of *T. hispidus* are abundant, demonstrate diurnal activity and have saxicolous habits, a combination of factors that make them more available in relation to other groups of vertebrates. The only vertebrates found in the diet of the congener *Tropidurus torquatus*, in Restinga environments (Siqueira *et al.*, 2013), were lizards and anurans, with the former being the most frequent category, which may indicate a possible preference for eating lizards or that this is a more accessible item according to the time of year.

Territoriality

Territorial behavior was observed twice (n=2), in Sítio Fechado. The first observation occurred on October 31st, 2018, at 10:28 h, between two *T. hispidus* males, and consisted of a physical confrontation under a tree, which presented a crack on its trunk that sheltered a couple of this species. The resident male, visibly larger, performed head bobbing movements and then attacked the other male (invader), who entered his territory and approached the tree. The fight took place with an altercation between bites on the mouth (Fig. 3A) and head (Fig. 3B), with staring (direct eye contact) and head bobbing movements, in addition to 360° circular movements on the body's own axis (Fig. 3C). This interaction was completed after 1 min and 23 sec, when the invader ran away.

The second observation of territorial behavior occurred on December 12th, 2018, at 11:12 h, lasting 2 min and 03 sec. Two males, apparently of the same size, were observed in a confrontation, in which one male bit the mouth of the other (Fig. 3D). The

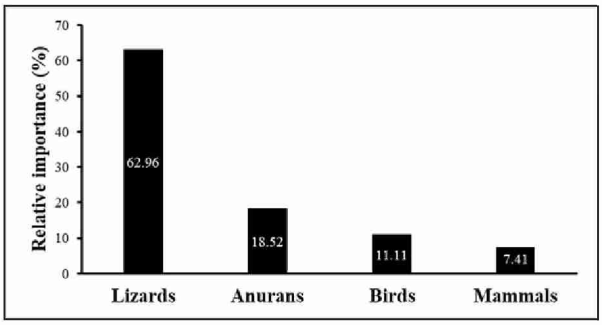

Figure 2. Relative frequency of the vertebrate prey categories that make up the diet of *T. hispidus* in Brazil. * = field record.

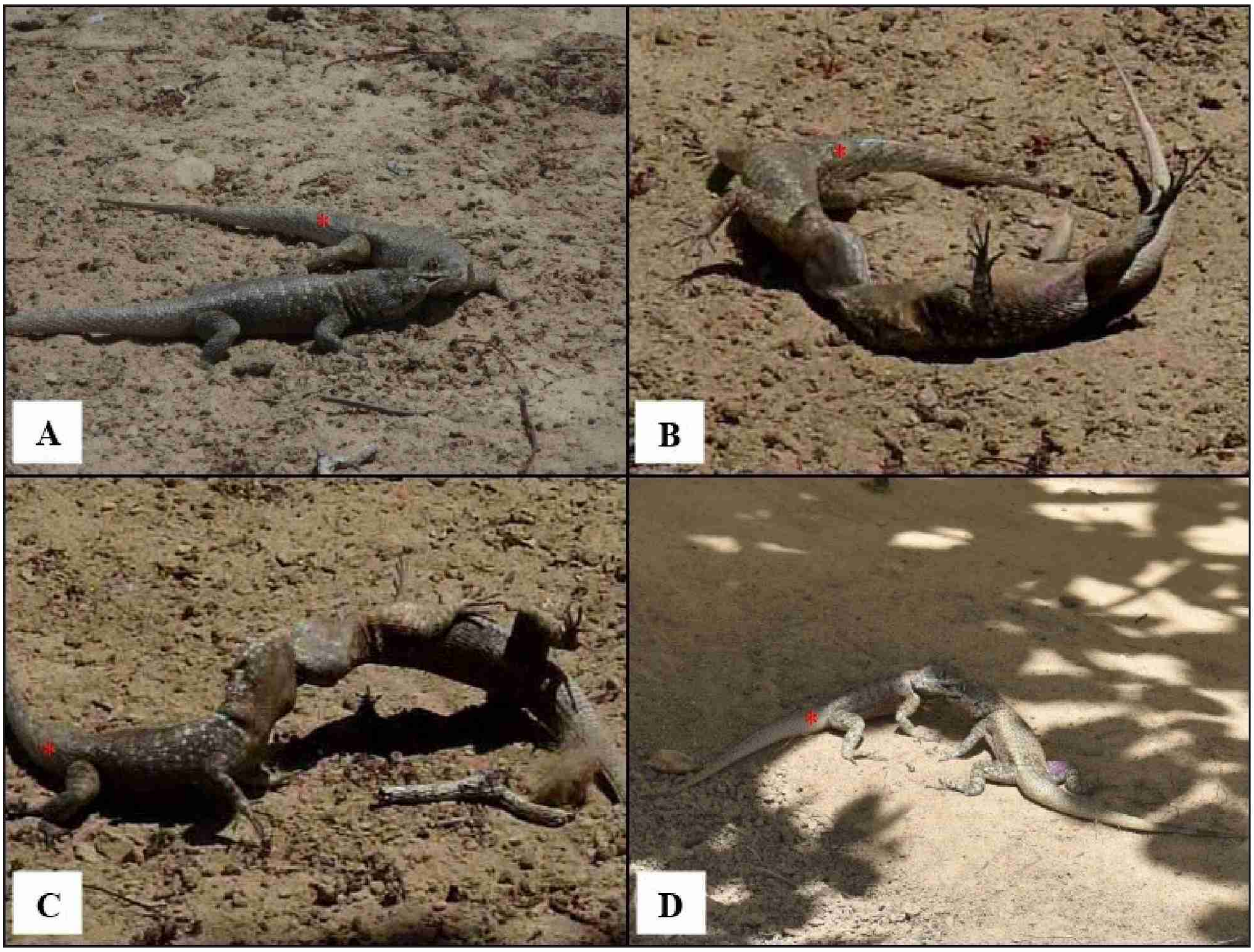

Figure 3. Behavioral events related to territoriality in males of *Tropidurus hispidus*, in Sítio Fechado, Pernambuco, Brazil: **A**- mouth bites; **B**- head bites; **C**- 360° circular movements on the body's own axis; **D**-male biting the mouth of another. Red asterisk = resident male.

presence of the observer caused the lizards to escape in different directions.

Territoriality in lizards may be related to ecological factors, such as the availability of food resources, female defense, breeding sites and/or thermoregulation sites (Ferguson *et al.*, 1983). Lizards such as *T. hispidus* present evident territorial behavior, with aggressive and confrontational modes. These aggressive behaviors performed by males involve chases and bites and are observed, mainly, when an invader male approaches the female or simply when a male enters the territory of others (Carpenter, 1977). Records in the literature corroborate what was recorded here for *T. hispidus*, indicating that territoriality is a striking characteristic of this species, consisting in behaviors with aggressive displays, chases and bites (Carpenter, 1977; Pinto, 1999). However, the combination of biting, holding and the performance of 360° circular movements has not yet been reported.

Courtship and copulation

The courtship and copulation behaviors were observed five (n=5) times, in Sítio Fechado. The first observation was on January 15th, 2019, at 12:09 h, where a *T. hispidus* couple was on stony soil, when the male was observed biting the female's neck (Fig. 4A) and, after 38 sec, it shook its head up and down, bobbed its head and positioned his whole body on the female. Subsequently, the male held his hindlimbs to the pelvic region of the female immobilizing her for copulation, which lasted 25 sec (Fig. 4B). Finally, the male released the female and they separated, moving in opposite directions, with no post-copulation interaction, with this event lasting 3 min.

The second courtship and copulation events were observed on September 24th, 2019, at 11:50 h, with a total duration of 2 min 16 sec. A *T. hispidus* couple stood under the wall of a residence (Fig. 4C),

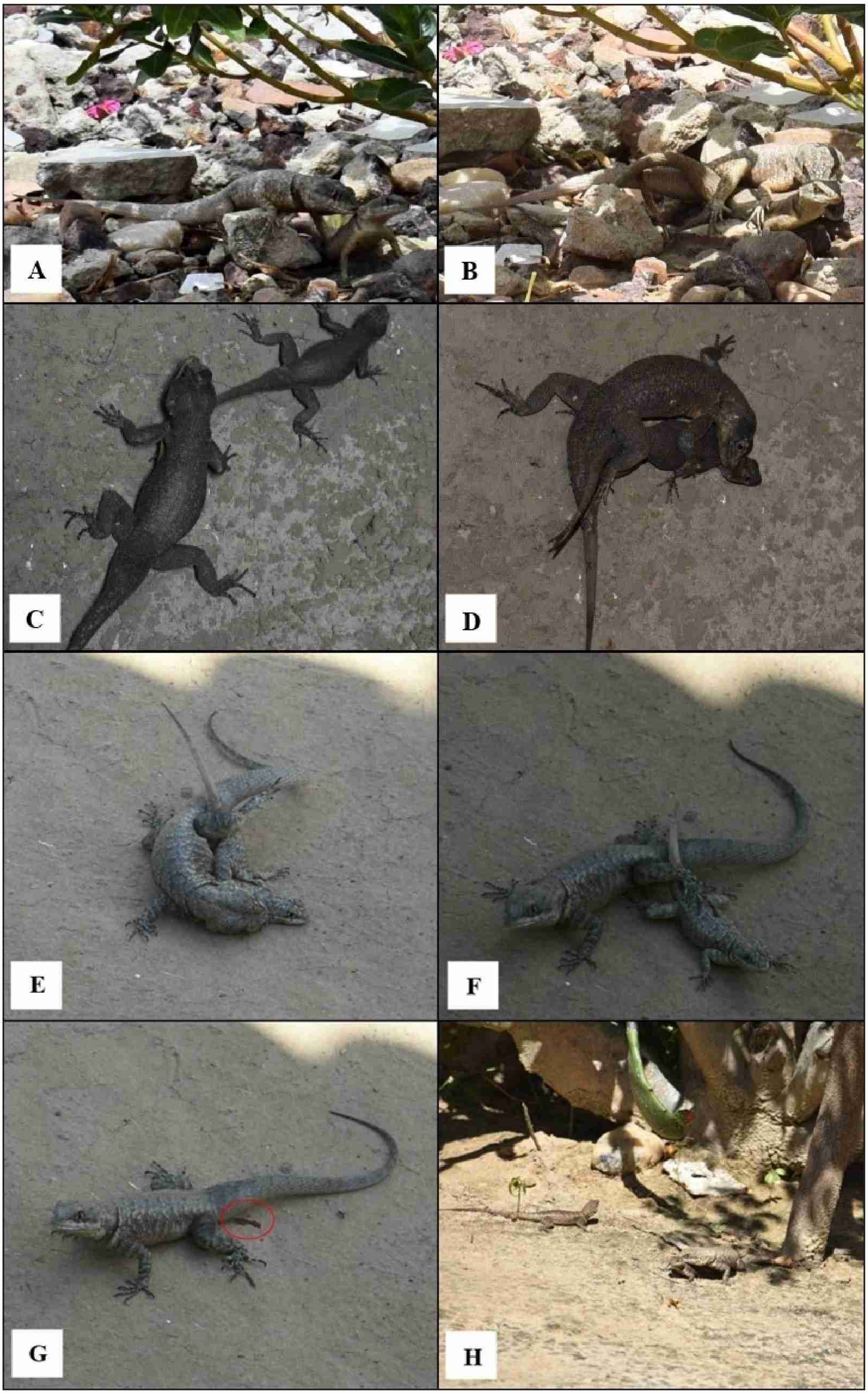

Figure 4. Courtship and copulation behavioral events in *Tropidurus hispidus,* in Sítio Fechado, Pernambuco, Brazil: first event: **A**- male biting female's neck; **B**- copulation; second event: **C**- male approaching female; **D**-copulation; third event: **E**- lifting tail female, male positioning the posterior limb under the pelvic region of the female and beginning of copulation; **F**- end of copulation and distancing to both; **G**- male bleeding to cloaca; **H**- male biting the female's neck with another lizard nearby, but with no interaction.

when the male bit the female near the pelvic region and, after 15 sec, bit her neck and both moved 45 cm. When they stopped, the male positioned its body on the female who raised her tail, bents its body and performed the copulation, lasting 21 sec (Fig. 4D). When the event finished, the female and male stayed close to each other (~10 cm) but with no interaction.

The third courtship and copulation event was observed on July 19th, 2020, at 14:45 h, when a male was biting a female's neck under compacted clay soil. After 46 sec, the female moved about 300 cm with the male on her back, stopped and, after 42 sec, the female lifted her tail and began copulation, which lasted 1 min (Fig. 4E). At the end of the copulation, the male stopped biting the female's neck and both began to drift away (Fig. 4F) bobbing their heads. The entire event lasted 2 min and 47 sec. When the female completely moved away, it was possible to notice the presence of blood coming out of the male's cloaca (Fig. 4G), who still stood for 6 min, doing bobbing its head.

The fourth courtship and copulation event was observed on July 30th, 2020, at 10:46 h, in which a male was biting a female's neck and there was another lizard (sex not determined) near (~20 cm) to the couple (Fig. 4H) but with no interaction. After 1 min 12 sec, the female lifted its tail, the male bent his body and began copulation, which lasted 13 sec. At the end of the copulation event, the male stopped biting the female's neck, the couple was close (~5 cm), both bobbing their heads. The entire event lasted 2 min and 10 sec.

On August 21st, 2020, at 15:08 h, there was a fifth event related to courtship and copulation with a total duration of 3 min and 18 sec. A male was biting a female's neck, so it moved about 400 cm with the male on her back, stopped and, after 23 sec, moved from side to side, then the male let go of the female's neck and continued on her back. Both performed head bobbing and the male licked the female's neck three times (Fig. 5A), she then moved another 80 cm and stopped, the male approached and also licked three times near the female's cloaca (Fig. 5B). After the licks, the male climbed again on the back of the female and bit her neck, tried to copulate, but the female came out and climbed onto the wall of a residence, soon after the male also left.

In the literature there are some studies that describe aspects related to the reproductive behavior of the *Tropidurus* genus. Carpenter (1977) reported that *T. hispidus*, *T. torquatus* and *T. occipitalis* females displayed the typical rejection posture of the Iguanidae female to the approaching or courting males. It has also been reported that *T. hispidus* and *T. torquatus* males present similar performances, however, with aggressive inclinations of the head, in contrast to the flexions with longer dewlap and accentuated lateral compression observed in *T. occipitalis* (Carpenter, 1977). Ávila and Cunha-Avellar (2006) observed in *Tropidurus etheridgei* that the female stood on four legs, arched her back and showed herself to the male while raising her tail. The male, in turn, bit the female's neck twice and the base of her tail once (Ávila and Cunha-Avellar 2006). Vaz-Nunes *et al.* (2008)

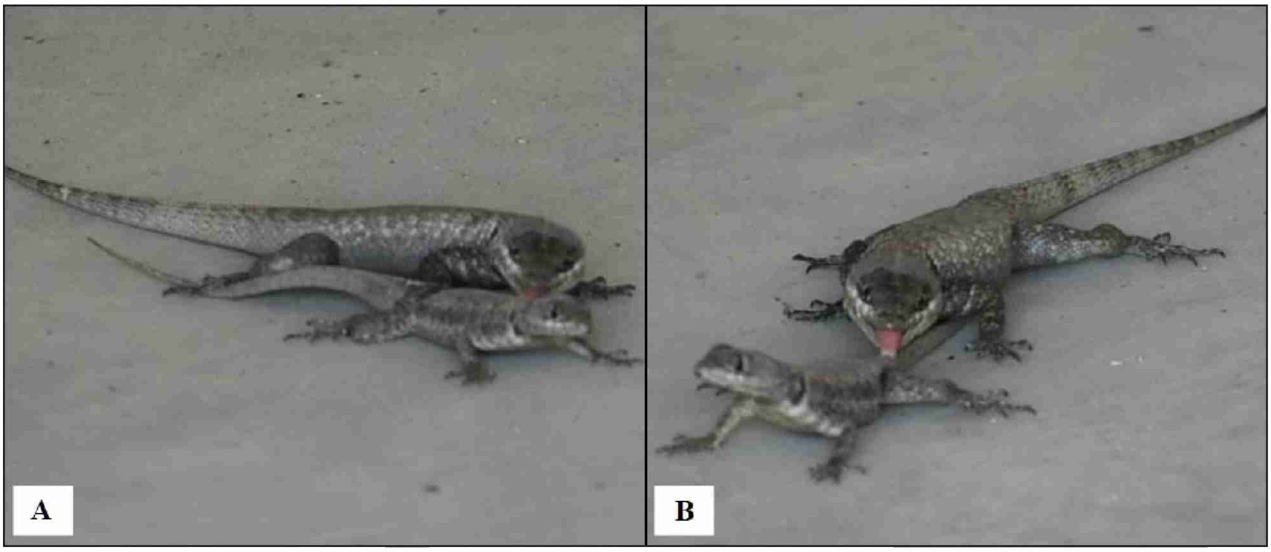

Figure 5. *Tropidurus hispidus* male licking the neck (A) and the cloaca of a female (B), in Sítio Fechado, Pernambuco, Brazil.

reported that in *Tropidurus itambere* the female stands on all four legs, arches the back and raises the tail up while the male approaches performing head movements with the inflated gular region. In addition, the male bit the female's neck and pushed her cloacal region with his nose (Vaz -Nunes *et al.* 2008).

Vasconcelos *et al.* (2013) and Vasconcelos *et al.* (2019) observed that in *Tropidurus hygomi* the male approached the female during locomotion, then the male stopped wagging his tail and shaking his head with its body positioned laterally towards the female. The female then lifted her body off the ground, keeping her limbs firm and lifted her tail, allowing the male to assume the copulation position. After separating from the female, the male moved slowly in circles around the female until she was gone (Vasconcelos *et al.*, 2013; Vasconcelos *et al.*, 2019). Pelegrin (2019) observed that in *Tropidurus spinulosus* the male approaches a female and licks her neck, if she is receptive, she remains immobile and the male bites her on the neck, then the male stays on top while biting the skin of her neck. After a few seconds, the male brings his tail under her tail, placing a hind leg on his back and putting the cloaca in contact. One of the hemipenis is then inserted into the female's cloaca and when the copulation ends, the male releases the female's neck, comes off her back, retracts the hemipenis and leaves. If the female is not receptive, she whips her tail to fend off the male who ends up going away. Comparing our records with what is in the literature, it is evident that most courting and copulation behaviors are common to species of the *Tropidurus* genus.

Displays during reproduction vary widely among lizard species, playing a key role in mating success, because in addition to being specific to each species, it also signals and informs the individual quality of males and females (Miles *et al.*, 2007). Before copulation, male tropidurids perform head bobs and bite females' necks during copulation (Carpenter, 1966; Lima *et al.*, 2017; Vasconcelos *et al.*, 2019). Neck licking behavior has been reported for *T. spinulosus* (Pelegrin, 2019) and cloaca licking behavior in *T. torquatus* (Scandelai, 2005). Licking behavior has been highlighted as a way of recognizing and selecting the appropriate reproductive partner (Schwenk, 1995; Martín and López, 2015; Pelegrin, 2019).

The consummation of copulation occurs when the hemipenis is inserted into the female's cloacae and closes at the time it is removed (Pelegrin, 2019). The duration of this behavior varies in tropidurids. Here, copulation events lasted from 13 sec to 1 min, therefore, providing the longest recorded copulation time for this genus, compared to *T. spinulosus* (40 a 55 sec) (Pelegrin, 2019), *T. itambere* (Vaz-Nunes *et al.*, 2008) and *T. hygomi* (Vasconcelos *et al.*, 2019) (20 sec in both species).

The post-copula bleeding of the male in the third copulation event described here is not represented in literature, especially for the Tropiduridae family. However, this copulation event lasted for the longest amount of time (1 min), and this may have been the reason for the bleeding.

Ethogram

The ethogram or behavioral repertoire is a basic tool for better understanding the biology, ecology and behavior of an animal species, in captive or natural environmental conditions (Altman, 1974; Alcock, 1997; Del-Claro, 2004). This tool consists of a list of behaviors related to a category or several behavioral categories, and may encompass behaviors related to reproduction, rest, defense or locomotion, including behavioral acts and their descriptions (Grier, 1984; Yamamoto and Volpato, 2011). Considering that the events presented here can serve as the basis for future work involving the behavioral ecology of tropidurids, we developed two ethograms for behaviors related to territoriality (Table 3) and courtship and copulation (Table 4).

The observation of behavioral events in nature are fortuitous however, they can provide important information about the life history and trophic relationships of a species (Valdujo *et al.*, 2002; Hartmann *et al.*, 2003; Turci *et al.*, 2009; Guedes *et al.*, 2017). These records are important in confirming diet data obtained from stomach content analysis, as well as providing, in detail, the sequence of behavioral acts, and their duration. There is great difficulty in registering and/or trying to quantify these events in nature, since not all behaviors are common and these could have been easily affected by the presence of the observer. Visual records of these behaviors, although infrequent, provide a detailed view of behavioral acts and supply information about the use of environments, microhabitats, daily activities and feeding behavior (Teixeira and Giovanelli, 1999; Nogueira *et al.*, 2003; Hartmann *et al.*, 2005; Turci *et al.*, 2009).

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Table 3. Ethogram of the behaviors exhibited by *Tropidurus hispidus* during territoriality events in Sítio Fechado, Pernambuco, Brazil.

Behavior	Description
Warning displays	Lizards make direct eye contact while head bobbing. Head bobbing: fast vertical movements with head, carried out to ward off other males from their territory.
Confrontation	Bites on the mouth. Bites on the head. If the confrontation is long, in addition to the bites, the lizard rotates the body on its own axis (360 ° rotation movement) in a clockwise and counter-clockwise direction, causing the other to also rotate.
Flight	After the 360°circular movements one of the lizards (invader) manages to detach and quickly escapes from the other individual (resident).

Table 4. Ethogram of the behaviors exhibited for *Tropidurus hispidus* during courtship and copulation in Sítio Fechado, Pernambuco, Brazil.

Behavior	Description
Bite	The male holds the female biting the neck skin. The male immobilizes the female by placing the forelimbs and posterior limbs on it.
Lift the tail	The female raises the tail, allowing the male to insert the hemipenis into the cloacal opening, initiating copulation. The male releases the female's neck and then removes the hemipenis from her cloaca. Vertical rapid movements with the head performed by males and females before and/or after copulation.
Neck's Licking	The male licks the female's neck.
Cloaca's licking	The males lick the female's cloaca.
Drag the cloaca	The male moves with the cloacal region being pressed against the substrate, right after copulation.
Move away	The male and female slowly move away from each other bobbing their heads.

second cannibalism event. José Henrique thanks the residents of RVS Matas do Siriji for their great support. Juliana Delfino thanks the UFCG for the incentive with a research initiation grant (PIBIC 2019-2020).

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Nota

Cuad. herpetol. 35 (2): 319-322 (*2021*)

Diet of the lizard *Ameiva ameiva* (Linnaeus, 1758) (Teiidae: Squamata) from northwestern Mato Grosso, Brazil

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ABSTRACT

Studies related to the diet of lizard species are important to learn about their biology. This study aimed to identify the diet of the *Ameiva ameiva* species. The knowledge of the diet was performed through the analysis of the stomach contents of individuals collected in an area in the municipality of Juína, Mato Grosso state. The stomach contents of 49 *A. ameiva* individuals were analyzed, and it was possible to observe a diet consisting mainly of individuals of the classes Insecta, Arachnida, and Crustacea. Regarding the Insecta class, the orders with the higher number of individuals were Hymenoptera, Coleoptera, and Hemiptera, respectively, and belonging to 11 families, with Formicidae and Gryllidae being observed, respectively, with higher frequency in feeding. The data obtained from the feed of *A. ameiva* demonstrate the importance of this species in the ecosystem's balance because of the range of prey from which it feeds.

Key Words: Reptiles; Ecology; Amazon forest; Cerrado.

The genus *Ameiva* has 14 described species, *Ameiva aggerecusans* Koch, Venegas, Rödder, Flecks and Böhme, 2013, *Ameiva ameiva* (Linnaeus, 1758), *Ameiva atrigularis* Garman, 1887, *Ameiva bifrontata* Cope, 1862, *Ameiva concolor* Ruthven, 1924, *Ameiva fuliginosa* (Cope, 1892), *Ameiva jacuba* Giugliano, Nogueira, Valdujo, Collevatti and Colli, 2013, *Ameiva nodam* Koch, Venegas, Rödder, Flecks and Böhme, 2013, *Ameiva pantherina* Ugueto and Harvey, 2011, *Ameiva parecis* (Colli, Costa, Garda, Kopp, Mesquita, Péres, Valdujo, Vieira and Wiederhecker, 2003), *Ameiva praesignis* (Baird and Girard, 1852), *Ameiva provitaae* Garcia-Perez, 1995, *Ameiva reticulata* Landauro, García-Bravo and Venegas, 2015, *Ameiva tobagana* Cope, 1879 (Uetz *et al.*, 2021). The *Ameiva ameiva* species is common in degraded areas and is distributed in Colombia, Suriname, Guyana, in some regions of Venezuela, Bolivia, Ecuador, Peru, Paraguay, and Argentina (Uetz *et al.*, 2021). In Brazil it has a wide distribution, having been registered in the states of Acre, Amazonas, Bahia, Paraná, Minas Gerais, Goiás, Pernambuco, Espírito Santo, Ceará, Mato Grosso, Sergipe, Rio de Janeiro, Piauí, Rio Grande do Norte, and São Paulo (Araújo and Santos, 2011; Uetz *et al.*, 2021). Because it is a species that inhabits anthropized areas, it is easily found even in urban areas. According to the feeding behavior, individuals of this species are classified as active foragers; they go looking for their food (Huey and Pianka, 1981). When analyzing individuals' stomach contents, it is possible to observe their prey and thus understand their role in the food chain.

Studies related to diet contribute to determining the trophic niche, being important for all groups of organisms, considering that eating habits

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involve strategies that are the result of extensive evolutionary processes over time (Winemiller *et al.*, 2015). Lizards present different feeding strategies, being classified as sit-and-wait as *Tropidurus torquatus* (Wied-Neuwied,1820) (Rodrigues *et al.*, 1988); ambushed, standing still waiting for prey or as active foragers, looking for their food, as in the case of the *Ameiva ameiva* species (Magnusson *et al.*, 1985). The foraging behavior and the habitat that the species occupy determine the type of food they consume, with consequent community structure (Pianka, 1973; Dufour *et al.*, 2018). Regarding food, most lizards are insectivores, and others also act as primary consumers, consuming fruits and acting as seed dispersers for various plant species, having high importance for the maintenance of ecosystems (Pianka and Vitt., 1988; Martins and Molina, 2008; Winemiller *et al.*, 2015).

This study aimed to identify the diet of the *Ameiva ameiva* species. The individuals were collected in a region with an area of 80.34 hectares, located at the geographic coordinates: 11°26'50" South and 58°43'1" West, in the municipality of Juína, in the northwestern region of the Mato Grosso state (Figure 1). The collected points (Figure 2) were distributed as follows: P01- Geographic coordinates 11°26'50.4" South and 58°43'19.5" West. It is an anthropized area, with the presence of grasses and a high incidence of solar radiation; P02 - Geographic coordinates 11°26'47.9" South and 58°43'27.1" West. It is a fragment of native forest with large trees, dense leaf litter, and shaded places; P03 - Geographic coordinates 11°27'01.2" South and 58°43'14.9" West. Area connected to the riparian forest and also an area of environmental preservation of neighboring properties, presenting dense vegetation, with stretches of grassy-shrub of 3 to 4 meters in height, medium shading and a medium leaf litter layer; P04 - Geographic coordinates 11°26'41.1" South and 58°43'13.8" West. Located on the banks of a stream, with a native forest of seasonal semi-deciduous type, with tall trees from 10-25 m, and a medium leaf litter layer subject to flooding at the height of the rainy season.

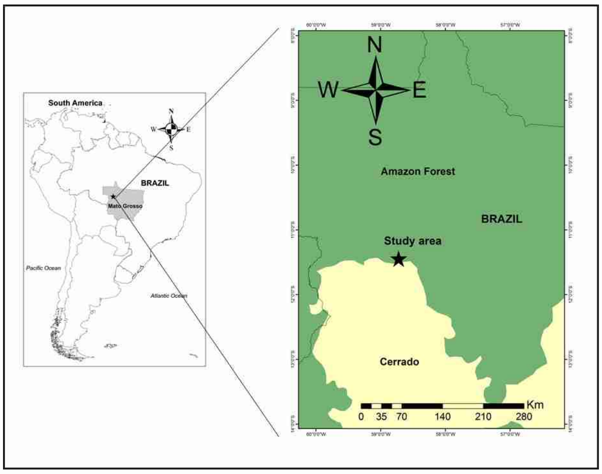

Figure 1. Study area between Cerrado hotspot and Amazon Forest.

Figure 2. The locality of points of the study area. P01, P02, P03, and P04 indicate the points of fieldwork.

Were collected 49 adult individuals of *Ameiva ameiva* and deposited in the Herpetological Collection of IFMT from October 2014 to April 2015 (Coleção Herpetológica do Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso - IFMT – campus Juína). The stomach contents were removed and preserved in 70% alcohol for screening and identification of the main groups used as a food source. The organisms found in the stomach contents were identified to the lowest possible taxonomic level, using Carrano-Moreira (2015) and by entomologists. Food items were analyzed quantitatively.

We analyzed 49 stomach contents belonging to adult individuals of the *Ameiva ameiva* species. Point P03 showed the highest number of individuals analyzed, with 36 individuals, point P01 nine individuals, point P04 four individuals, and point P02 no individuals. In the stomach contents of *A. ameiva* were identified individuals from three taxonomic classes, with 150 individuals belonging to the Insecta, 19 to the Arachnida, and 2 to the Crustacea class (Figure 3). We also registered vegetable material in the stomach of 24 individuals, unidentified eggs

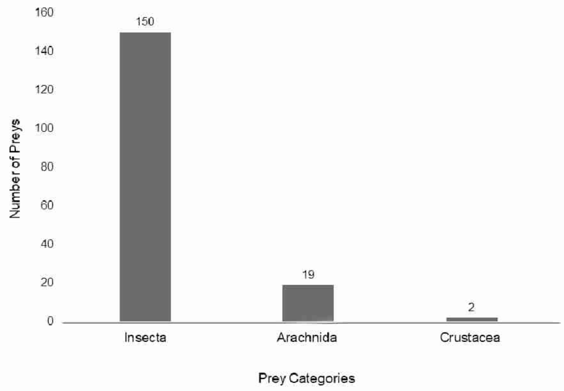

Figure 3. Number of individuals in each class found in the diet of *Ameiva ameiva* (N= 49).

in 6 individuals, and the presence of worms in 5 individuals. The worms found may be parasites of the animal itself and not necessarily ingested as a food source. A more accurate analysis of these worms is necessary to clarify this information. Studies with the diet of species of the genus *Ameiva* in different environments in the Brazil and Venezuela showed that this group's main prey belongs to the classes Arachnida, Insecta, and Gastropoda (González and Prieto, 1997; Sales *et al.*, 2011; Sales, 2013).

For the Insecta class, the order Hymenoptera presented the highest number of individuals. Of the 49 individuals of *A. ameiva* analyzed, 41 fed on species of this order. The Coleoptera order was observed in a significant number of individuals, followed by the order Hemiptera. The order found less number of individuals in the *A. ameiva* diet was Isoptera (Figure 4).

We identified 11 families in the *A. ameiva* diet. Among them, the family Formicidae had the highest frequency (in 17 of the 49 individuals analyzed), followed by Gryllidae with representatives in 11 individuals (Table 1). Silva *et al.* (2003) found data, although less predominant, similar to those observed more frequently in the present study. This fact demonstrates that the group most predated for *A. ameiva* may be related to the offer of each type of prey available in each region. Regarding the number of individuals from each family predated by the *A. ameiva* species, the most present family was Formicidae with 46 different types of prey (i.e. individuals of the Formicidae), showed in 17 analyzed individuals. Therefore, it can be inferred that there is the highest offer of individuals from this family in this region. Also, 14 prey of the Gryllidae family were found in 11 individuals (Table 1). A study on the ecology of *A. ameiva* in the Restinga de Guriri in the municipality of São Mateus, in the Espírito Santo state-Brazil (Silva *et al.*, 2003), analyzed 52 individuals and 18 prey categories were identified, with a predominance of cockroaches, termites and larvae beetles. In this way, it was possible to verify that *A. ameiva* feeds on the same taxonomic groups in different Brazilian regions, even if in different prey predominance. This fact may be related to the higher offer of certain prey in each region.

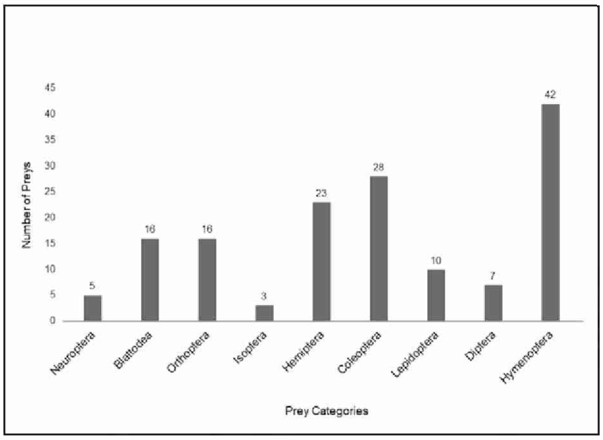

Figure 4. Number of individuals of orders belonging to the Insecta class found in the diet of *Ameiva ameiva* (N= 49).

Our data show that *A. ameiva* species mainly inhabits open areas modified by humans and with thin vegetation (i.e. P01 and P03). The analyzed samples belong to an area of approximately 80 ha. However, the data obtained here can be extended to the region of Juína, considering that the analy-

Table 1. Food items found in the stomach of *Ameiva ameiva* (N= 49). Frequency of prey (total number of prey categories) and Number of prey (total number of individuals) of families belonging to the Insecta class.

Family	Frequency of prey	Number of prey
Chrysopidae	5	5
Gryllidae	11	14
Termitidae	1	2
Cercopidae	3	3
Cicadidae	1	1
Curculionidae	4	4
Scarabaeidae	4	4
Chrysomelidae	1	1
Formicidae	18	46
Vespidae	2	2
Apidae	1	1

zed area has geological formations and vegetation identical to that observed for the region. *Ameiva ameiva* inhabits naturally open and anthropic areas, therefore, this species may influence pest control, as it feeds on species known in the region for damage caused to plantations (e.g. leafhopper and leaf-cutting ant; see Imenes and Ide, 2002). The data obtained demonstrate the importance of this species in the ecosystem's balance because of the range of prey from which it feeds. However, we suggest further studies on the importance of this species in promoting these ecosystem services, fully linked to human well-being.

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Nota

Cuad. herpetol. 35 (2): 325-328 (*2021*)

First report of hemiclitores in female lizards of the genus *Ophiodes* Wagler, 1828 (Squamata: Anguidae)

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ABSTRACT
In some species of Squamata, the hemiclitoris is a structure homologous to the hemipenis, and may be used as a taxonomic character. These structures showed similar morphology in males and females in different species of the group; however, some studies have identified morphological variation among them. In this context, we described for the first time the morphological characteristics of the hemiclitoris and compared them with those observed in the hemipenis of *Ophiodes* cf. *fragilis*. We analysed the hemipenes and hemiclitores of four specimens belonging to the Herpetological Collection of the Laboratory of Reptiles at the Federal University of Juiz de Fora, highlighting external morphology and histological characteristics. We observed that the external morphology varied between the structures, such as number of lobes and ornaments; however, both had similar histological characteristics. These data contribute to a better understanding of the taxonomic character of lizards, helping with the taxonomic elucidation of the genus.

Key Words: Hemipenis: lizards; morphology; taxonomy.

The first study related to hemipenis in lizards was carried out by Cope (1896), describing morphological differences found in this structure. The hemiclitores in Squamata females were registered for the first time by Böhme (1995) and were considered homologous to the hemipenes. The morphology presented in these structures is of great importance in taxonomic studies, presenting characters relevant to the knowledge of phylogenetic relationships (Arnold, 1986a, b; Valdecantos and Lobo, 2015; Quipildor *et al.*, 2018).

The genus *Ophiodes* (Anguidae) encompasses species of lizards that are exclusively Neotropical, distributed in Brazil, Bolivia, Paraguay, Argentina, and Uruguay (Macey *et al.*, 1999; Uetz *et al.*, 2020). It currently consists of six species: *Ophiodes fragilis* (Raddi, 1820); *Ophiodes striatus* (Spix, 1825); *Ophiodes vertebralis* Bocourt, 1881; *Ophiodes intermedius* Boulenger, 1894; *Ophiodes luciae* Cacciali and Scott, 2015; and *Ophiodes enso* Entiauspe-Neto *et al.*, 2017. Additionally, three morphotypes have not been formally described: *Ophiodes* sp. “1”; *Ophiodes* sp. “2” and *Ophiodes* sp. “3” (Borges-Martins, 1998; Pizzato, 2005; Montechiaro *et al.*, 2011; Cacciali and Scott, 2012, 2015; Costa and Bérnils, 2018; Oliveira *et al.*, 2016; Entiauspe-Neto *et al.*, 2017).

Ophiodes cf. *fragilis* (Raddi, 1820) (Squamata, Anguidae, Diploglossinae), popularly known as glass snake, is distributed in Brazil in the Southeast, Central-West, South and Northeast regions of the country (Costa and Bérnils, 2018). Due to the morphological similarities and frequent occurrence

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of sympathy, the genus *Ophiodes* is likely a species complex (Barros and Teixeira, 2007). However, a taxonomic and phylogenetic revision of this group is lacking (Barros and Teixeira, 2007), and several gaps of knowledge on their biological and ecological characteristics indicate that studies are needed on these lizards.

Reproductive morphology studies in species of the genus *Ophiodes* are almost nonexistent. This fact, related to taxonomic impasses, indicates that studies that analyses structures used in taxonomy are extremely important. In this context, our aim was to morphologically describe the hemiclitoris and hemipenis in specimens of *O.* cf. *fragilis* from southeastern Brazil.

The hemiclitoris and hemipenis of four specimens of *O.* cf. *fragilis* (two males and two females) were removed during necropsy for histological processing. The specimens used in this study belong to the Herpetological Collection of the Laboratory of Reptiles at the Federal University of Juiz de Fora (CHUFJF-Répteis) with voucher numbers: 1108, 1644, 1646 and 1648. According to the records, the specimens were fixed in 10% formaldehyde for at least 24 hours, and then transferred to a 70% ethyl alcohol solution.

For the studies of the material, under light microscopy, bilateral anatomical pieces (right and left) were integrally used (without cleavage), dehydrated in increasing concentrations of alcohol, diaphanized in xylol and impregnated / embedded with paraffin. Subsequently, 4 μm thick serial cuts were made (Tolosa *et al.*, 2003). The sections were stained with hematoxylin-eosin (HE). The analysis of the material and the respective photo documentation were performed in an Olympus BX41 microscope with Canon A3100S digital photographic equipment, coupled with an eyepiece micrometer.

The hemiclitores observed in *O.* cf. *fragilis* were unilobular structures, projecting laterally into the cloacal opening (Fig.1a, b, c, d). Sulcus spermaticus were identified in the center-lateral position of the analyzed specimens. The ornamentation was similar in the apical and trunk / basal portions, presenting the same morphotype (Fig. 1b, c): delicate pointed structures, with a rounded apex, which followed along the entire hemiclitoris, and arranged in several layers.

The hemipenial morphology is paired and bifurcated and each of its structures protrudes laterally from the cloacal opening (Fig. 1e, f, g, h). It presented a broad trunk, with a central lineal sulcus spermaticus evident on the dorsal side. The ornamentation was similar at the apex, trunk and base (Fig. 1f): in the apical portion of the lobes, the presence of surrounding vertical lines was observed; in the trunk portion, close to the basal portion, small pointed structures were arranged along the hemipenis, in several layers, up to the basal portion; and in the most basal portion, there was no evident ornamentation, being a smooth tissue structure.

Histological analysis revealed the outer lining of the hemipenis consisting of a thin layer of keratinized epithelial tissue. Underlying the coating, there was a thick layer of loose connective tissue superficially well vascularized. In the central median region of each lobe, a layer of smooth muscle tissue and the retractor magnus muscle of the hemipenis were observed (Fig. 1g, h). The histological analysis of the hemiclitoris showed characteristics similar to those observed for the hemipenis (Fig. 1c, d). However, the keratinized superficial epithelial layer of the hemiclitoris proved to be more delicate when compared to that of the hemipenis.

The hemipenial characteristics of the genus *Ophiodes* have previously been studied. Borges-Martins (1998) (unpublished) analyzed and described the hemipenis, stressing characteristics that were shared among *Ophiodes* species. Nunes *et al.* (2014) described the hemipenial morphology of *Ophiodes fragilis*, highlighting the absence of bony and spiny structures in the analyzed specimens. Both authors described complementary characteristics similar to those observed in the specimens we analyzed. Despite the absence of studies that highlight the hemiclitoris in *Ophiodes*, Borges-Martins (1998) highlighted in his work the possibility of eversion of a structure with characteristics similar to the hemipenis, without ornamentation, in analyzed female specimens of the genus.

Like the hemipenis, the hemiclitoris is considered an apomorphy of Squamata reptiles (Ax, 2003; Valdecantos and Lobo, 2015). These structures may have unique characteristics of the genus or species (Card and Kluge, 1995; Koch *et al.*, 2010; Klaczko *et al.*, 2017) in addition to the morphological variation in ornamentation, size and shape between the sexes (Valdecantos and Lobo, 2015; Quipildor *et al.*, 2018). Among Squamata, the study of the morphological characteristics of the hemipenis are more present in the group of snakes (Arnold, 1986a), revealing that it is more complex and diverse when compared to

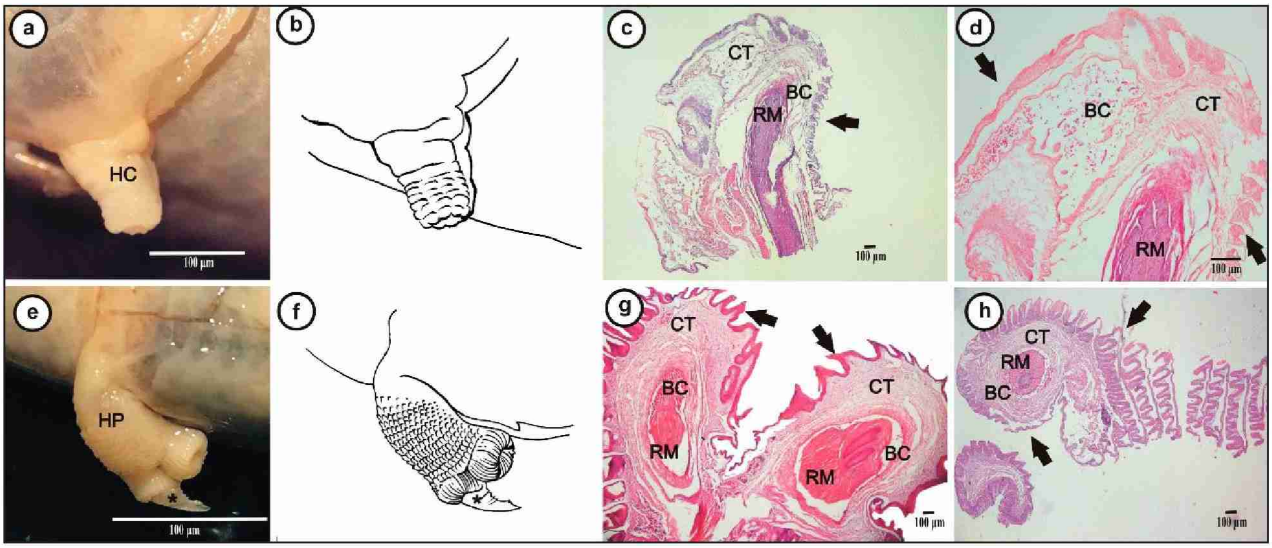

Figure 1. Hemiclitoris (**a, b, c, d**) and hemipenis (**e, f, g, h**) by *Ophiodes cf. fragilis*. (**a, e**) Photograph of the hemiclitoris and hemipenis. (**b, f**) Schematic figure of the hemiclitoris and hemipenis. (**c, d, g, h**) Microphotographs of the hemiclitoris and hemipenis. HP: hemipenis; HC: hemiclitoris; (*): hemipenis projection tissue artifact; CT: connective tissue; RM: retractor muscle of the hemipenis and hemiclitoris; BC: blood cells; Arrow: ornamentation of the hemipenis and hemiclitoris.

that of lizards (Klaczko *et al.*, 2017). Thus, the use of hemipenis / hemiclitoris as a taxonomic character in lizards should be used with caution since it may show morphological variation (as ornamentation and number of lobes) between sexes, as noted in this study, or it may be morphologically conservative in other strains (Gredler *et al.*, 2014).

Studies with hemipenes and hemiclitores mainly describe the variation of external morphology (see Kasperoviczus *et al.*, 2011; Valdecantos and Lobo, 2015) and macroscopic characterization (see Quipildor *et al.*, 2018) of these structures. Thus, information on tissue composition and organization is practically nonexistent. In our study, we explored histological analysis as a way of describing the internal tissue and morphological organization of the hemiclitores and hemipenes of *O.* cf. *fragilis*, highlighting the abundant portion of connective tissue, the smooth musculature that forms the retractor muscle in both structures, and the presence of blood cells in the space that would be filled promoting the eversion of the hemipenis and, possibly, of the hemiclitoris.

We described the external morphological structure of the hemiclitores and highlighted histological characteristics of the hemipenis and hemiclitoris of *O.* cf. *fragilis* for the first time in the literature. We observed that the external morphology (composed of ornaments, size and number of lobes-shape) varied between genders; however, the internal morphology (represented by tissue analysis and the presence of retractor muscle) was similar between the analyzed structures. We emphasize the need for further studies on the morphological characteristics of the hemipenis / hemiclitoris of *Ophiodes* species as an additional way to elucidate the taxonomy of the genus.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest related to this work.

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Diet of *Dendropsophus counani* (Anura: Hylidae) during breeding season in the Eastern Amazonia

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ABSTRACT

Dendropsophus counani is a rare neotropical treefrog that descend from the canopy of the trees to breed. Due to the low rate of encounter, ecology information is insuficient. The objective of this study was described here its diet composition during breeding season from Eastern Amazon. The stomach contents of N = 46 specimens (37 males and 9 females) were analyzed. A total of N = 37 prey items that included into 10 categories were determined for males while two categories for females. Araneae was the most important prey item for both sexes. Niche breadth (*Bsta*) was 0.48, suggesting that *D. counani* is a generalist predator. Diet composition of *D. counani* contribute to the natural history data and it may use in future categorization of neotropical frogs.

Key Words: Treefrogs, Food Items, Generalist Habits, Niche Breadth.

In amphibians, the description of the diet composition and feeding behavior provide important information regarding to natural history and the ecological niche (Sih and Christensen, 2001; Solé and Rödder, 2010; Anderson, 2017). Generally, due to the opportunistic behavior of many species, anurans are usually generalist predators (Duellman and Trueb, 1994) and they are considered fundamental in trophic webs (Wells, 2007) as well as in the biological control on herbivorous insects (Attademo *et al.,* 2005), Despite, there has been a little increase of studies that describe the diet of some neotropical anurans, diet of several other anurans has not yet been investigated.

Dendropsophus counani Fouquet, Orrico, Ernst, Blanc, Martinez, Vacher, Rodrigues, Ouboter, Jairam, and Ron 2015 is an arboreal frog species commonly found in flooded parts of stream and riverbeds of primary and secondary lowland ombrophilous dense forests (Fouquet *et al.,* 2015). It dwells the canopy of trees such as *Minquartia guianensis* Aubl. and *Virola surinamensis* (Rol.) Warb. and is likely to be detectable during the breeding season in the rainiest months of the year, from February to May, when the individuals descend from the canopy to breed in temporary ponds formed after heavy rains (Fouquet *et al.,* 2015). The species is currently unevaluated in the IUCN Red List of Threatened Species (IUCN, 2021).

Ecology data are insufficient mainly due to its recently description and the low rate of encounter. The objective of this study was described the diet composition of *D. counani* from a stream-dweller population of a primary forest, based on individuals captured during the breeding season.

Fieldwork was carried out at the Cancão Mu-

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nicipal Natural Park (0.90275°N, 52.00497°W), in a population of *D. counani* located 10 m at the east margin of the Amapari River and 2.5 km NW of the village of Pedra Preta, Municipality of Serra do Navio, state of Amapá, Brazil (Fig. 1). Frogs were captured at night (18:00–22:00 h) through auditory and visual survey methods established for tailless amphibians (Heyer *et al.*, 1994). Field activities were conducted for three consecutive nights per month in the period of four consecutive months (February to May 2018).

The specimens collected were killed *in situ* with 2% lidocaine hydrochloride to stop digestion, fixed in 10% formalin and stored in 70% ethanol. Collecting permits was provided by Brazilian Institute of Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – license SISBIO number #48102-2). The individuals were sexed by gonad analysis and the presence of colored vocal sac (identifying males). Snout-vent length (SVL) of each individual was measured using a digital caliper (0.01 mm precision) and body mass using a portable scale (0.1g precision). Specimens were dissected through an abdominal longitudinal incision for the removal of their stomach contents, which were labeled and stored in 70% ethanol. Stomach items were examined under a stereomicroscope and identified at the lowest possible taxonomic level following the taxonomic key of Rafael *et al.*, (2012). All specimens are deposited in the herpetological collection of the Universidade Federal do Amapá (collection number range - CECC 1603–1649).

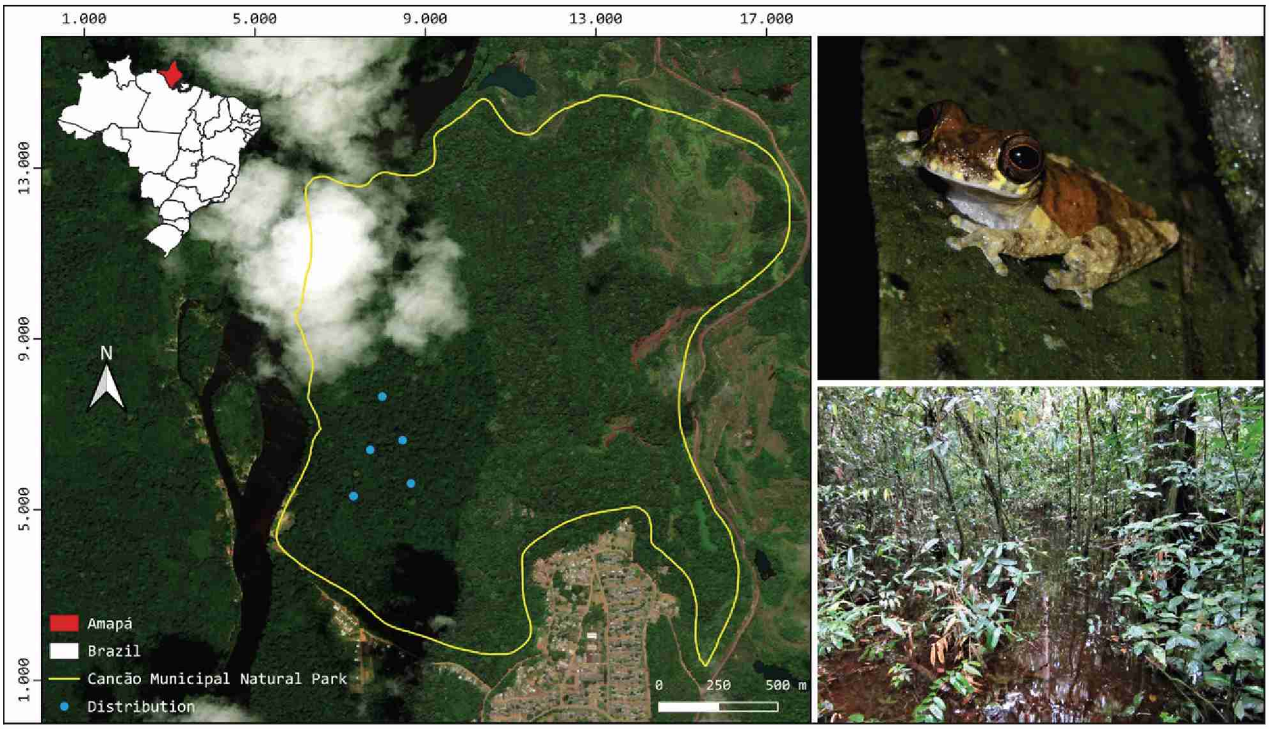

Figure 1. (**A**) Map showing location of Cancão Municipal Natural Park, Municipality of Serra do Navio, state of Amapá, Brazil and occurrence records of *Dendropsophus counani*. (**B**) Male *Dendropsophus counani*. (**C**) primary forest floor after the first rains.

For each individual (males and females separately) and for the total of examined stomachs, the numerical and volumetric percentages of each prey category were calculated. Width (w) and length (l) of each entire prey item (80% of the identified items) were measured to estimate prey volume through the ellipsoid volume formula of Griffiths and Mylotte (1987): $V = (4\pi/3)\,(w/2)^2\,(l/2)$, where l is the length of each prey and w is the width of each prey. The Index of Value Importance (IVI) was calculated to determine the importance of each prey category following Biavati *et al.* (2004): $IVI = (F\% + N\% + V\%)\ /\ 3$, where F% is the mean percentage of prey occurrence, N% is the numerical percentage of prey and V% is the volumetric percentage of prey.

The trophic niche breadth was estimated through Levins (B) index, by using the percentages of number (N%) of prey categories (Pianka ,1986), where *p* is the numerical or volumetric proportion of prey category *i*, and *n* is the number of prey categories, defined by: $B = 1 / \Sigma^{n}_{i=1} pi^{2}$. To allow comparisons, the Standardized Index of Levins (*Bsta*) was calculated using the following equation: $Bsta = (B-1) / (n-1)$, where n represents the number of resources recorded. Values close to zero are attributed to a specialist diet, while values close to one are attributed to a generalist diet. Spearman correlation test was performed to correlate snout-vent length (SVL) and volume of the largest prey for each individual. Lastly, plotted rarefaction curves based on the number of individuals and food items were performed to relate the sampling size and taxonomic richness of prey consumed by species. Statistical tests were performed using BioEstat 5.0 (Ayres *et al.*, 2007) and Ecosim 7.0 (Gotelli and Entsminger, 2001) softwares. The rarefaction curves were performed with Estimates 9.1 (Gotelli and Colwell, 2001). All statistical analysis were performed with a significance level of $\alpha = 0.05$.

The stomach contents of 46 specimens of *D. counani* (37 males, SVL range = 18.43–22.87 mm and 9 females, SVL range = 20.30–25.98 mm) were analyzed. A total of 37 prey items, included into 10 categories, were found in the stomachs of males, while only two prey items, placed into two categories, were found for females (Table 1). Mean number of items per stomach was 1.22 (min = 1, max = 3, SD = 0.50) and 1 (min = 1, max = 1, SD = 0.0) for males and females, respectively. Seven empty stomachs of were found among females (77.7%) while males have more stomachs with at least one identifiable prey item (94.5%) were observed. Araneae was the most important prey items for males (IRI% = 41.2) and females (IRI% = 41.4). For males and females, prey volume was not correlated to SVL (rs = 0.483, $p = 0.103$; rs = 0.190, $p = 0.298$, respectively). The rarefaction curves did not reach the asymptote, indicating that prey richness may increase by increasing sample size (Fig. 2). Niche breadth (*Bsta*) based on prey numbers was 0.48.

Similar of what were described for other treefrogs species *D. counani* consume mainly on arachnids and a variety of insects (Toft, 1980; 1981; Solé and Rödder, 2010). Previous studies on *Dendropsophus* species demonstrated that spiders are also the most important preys found in the diets of *D. sanborni* and *D. nanus* from Argentina (Macale *et al.*, 2008), *D. ebraccatus* and *D. phlebodes* from Costa Rica (Jiménez and Bolaños, 2012), *D. colombianus* from Colombia (Méndez-Narváez *et al.*, 2014), and *D. minutus* from southern Brazil (Leivas *et al.*, 2018). As demonstrated in previous dietary studies with treefrogs (Fonseca-Pérez *et al.*, 2017; Huckembeck *et al.*, 2018; Michelin *et al.*, 2020; Oliveira *et al.*, 2020), *D. counani* follows the same pattern found in most hylid species, exhibiting a non-selective strategy with regards to feeding.

The results of our study demonstrated that *D. counani* feeds mostly on arthropods characterized as soft-chitinized and of high mobility (e.g. Araneae and Orthoptera). However, other chitinized insects

Table 1. Prey categories consumed by *Dendropsophus counani*. N = number of prey in each category; V = total volume of preys (in mm3); F = frequency of occurrence of each prey category; IVI = Index of Value Importance; (%) = percentage values over total.

	Males (n = 37)				**Females (n = 9)**			
Prey item	N (%)	F (%)	V (%)	IVI	N (%)	F (%)	V (%)	IVI
Acari	1(2.86)	1(2.63)	0.032(0.004)	1.83	--	--	--	--
Araneae	14(40.0)	13(34.20)	390.1(49.43)	41.20	1(50.0)	1(25.0)	31.43(49.18)	41.39
Coleoptera	3(8.57)	1(2.63)	79.81(10.11)	7.11	1(50.0)	1(25.0)	32.47(50.82)	41.95
Diptera	2(5.71)	2(5.26)	5.76(0.73)	3.90	---	---	---	---
Hymenoptera	2(5.71)	1(2.63)	37.12(4.70)	4.35	---	---	---	---
Formicidae	4(11.40)	4(10.50)	88.47(11.21)	11.10	---	--	---	---
Isoptera	1(2.86)	1(2.63)	---	---	---	---	---	---
Diptera Larvae	3(8.57)	3(7.89)	41.54(5.26)	7.24	---	--	---	---
Neuroptera	1(2.86)	1(2.63)	10.05(1.27)	2.25	---	---	---	---
Orthoptera	4(11.40)	4(10.50)	136.3(17.27)	13.10	---	---	---	---
Plant Material	---	3	---	---	---	2	---	---

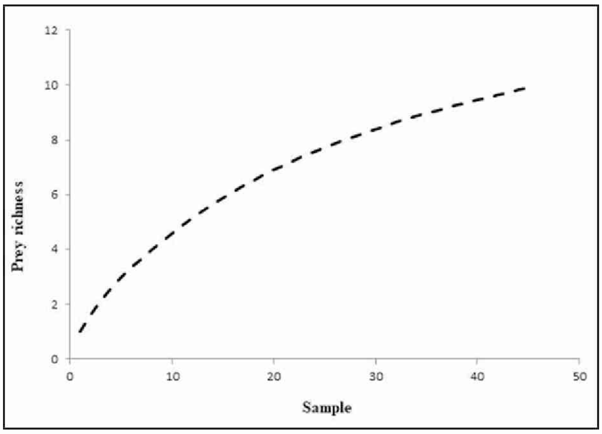

Figure 2. Rarefaction curve based on diet items of *Dendropsophus counani.*

such as ants and beetles had high abundance in its diet. Toft (1980) employed the terms "ant-specialist", "non-ant specialist" and "generalist" to characterize distinct feeding habits adopted by tropical anurans. According to the composition of its diet and the relatively low number of preys per stomach, *D. counani* seems to fit as a generalist feeder, with no category reaching up more than 40% of all consumed prey.

The results indicate that *D. counani* showed similar feeding habits in relation at other species of the genus: *D. colombianus* (Méndez-Narváez *et al.,* 2014); *D. microcephalus* (Fonseca-Pérez *et al.,* 2017); *D. minutus* (Leivas *et al.,* 2018); *D. nanus* and *D. sanborni* (Menin *et al.,* 2005); except for the pattern observed for *D. branneri*, considered a "non-ant specialist" instead of a generalist predator (Castro *et al.,* 2016). In addition, our data showed that the diet of *D. counani* over the seasons may reveal new categories of prey that were not observed in our study, given that our sample rarefaction curve did not reach asymptote. *D. counani* diet composition contributes to a better knowledge of the natural history and may serve as ecological information in categorization status of neotropical frogs.

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Nota

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A Goliath among Davids: An impressive new size record for *Siphonops annulatus* (Mikan, 1920)

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ABSTRACT

Siphonops annulatus (Mikan, 1822) is a neotropical caecilian widely distributed in the Amazon and Atlantic Forests. It is among the best studied caecilians in the Neotropical region, but many aspects of its natural history remain poorly known. Here we report an exceptionally large individual of *S. annulatus* from Rondonia, Brazil. At 720 mm total length, this specimen is much larger than the previously largest reported for the species at 539 mm.

Key Words: Caecillian, Body Size, Maximum Length, Siphonopidae, Siphonops spp

Caecillian amphibians (order Gymnophiona) are distributed mainly across the wet tropics, except for Madagascar, Wallacea and Oceania (Frost, 2021). They are mainly characterized by having limbless, elongated and externally annulated bodies, reduced eyes, and a pair of sensory tentacles (Taylor 1968; Gower & Wilkinson 2005, 2008; Wilkinson 2012). Most species burrow in soil, at least as adults. Due to their mainly fossorial way of life, caecilian amphibians are the least known order of terrestrial vertebrates, having little data sampling, apart from the collection of specimens, although recent data show that some species are much more abundant than was assumed (Gower & Wilkinson 2005; Maciel and Hogmoed, 2011).

The neotropical family Siphonopidae Bonaparte 1850 was recently resurrected, and the group diagnosed based on a combination of character states: oviparity, perforate stapes and inner mandibular teeth (Wilkison et al. 2011). Curently, Siphonopidae includes five genera and 28 species (Maciel et al., 2019; Frost, 2021).

Siphonops annulatus (Mikan, 1822) is one of the first described species and one of the most iconic caecilians. It possibly has the largest distribution of any caecilian species, occurring throughout most of Cis-Andean South America (Maciel and Hoogmoed, 2011; Maciel et al., 2013).

One of us collected a *S. annulatus* (Fig. 1) in the municipality of Itapuã do Oeste (Rondonia state, Brazil), in Floresta Nacional do Jamari - Flona Jamari. The specimen was collected in a pitfall trap (collection permit numbers: SISBIO by the license # 30902-1), fixed in formaline 10%, stored in ethanol 70%, and deposited in the Zoological Collection of the Universidade Federal Rondonia, Porto Velho, state of Rondonia, Brazil (UFRO-H 3214).

This specimen has 90 annuli, and its total length in preservation is 720 mm, as measured a metric tape. This is by far the largest published record of the species after the report by Maciel et al. (2013) of a 539 mm long specimen (Table 1).

The measurement of this specimen is impressive. Maciel et al. (2013) presented new data on the rediscovery of *S. annulatus* in the Brazilian state of Pará, including a new record for the maximum length of the species. Our record, in addition to being only the third of the species for the state of

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Figure 1. Specimen of *Siphonops annulatus* (UFRO-H 3214; 720 mm SVL) in life, from Floresta Nacional do Jamari, municipality of Itapuã do Oeste, Rondônia state, Brazil.

Rondônia (following Taylor, 1968; and Maciel et al., 2013), is the largest individual captured to date and reported for the species and both Siphonopidiae family. These reports prompt the question whether the populations of this species in the Amazon have a greater predisposition to large size than the populations of the Atlantic Forest, or if these data are instead explained by the greater abundance of records for Amazonia.

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Table1. Maximum total length record for *Siphonops annulatus*.

Source	Voucher	Sex	Total length (mm)	Total annuli
Taylor, 1968	s/n		~450	78–98
Taylor, 1968	ANSP 11346		425	91
Maciel et al, 2013	MPEG 32559	Male	539	91
This study	UFRO-H 3214	Male	720	90

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Nota

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First record of *Scinax tropicalia* Novaes-e-Fagundes, Araujo-Vieira, Entiauspe, Roberto, Orrico, Solé, Haddad, and Loebmann, 2021, for the Pernambuco Endemism Center, northeastern Brazil

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ABSTRACT
Scinax tropicalia was recently described for the Atlantic Rainforest of northeastern Brazil, displaying a disjoined geographic distribution in the states of Bahia and Ceará. This report presents the first record of *S. tropicalia* for the Pernambuco Endemism Center, based on morphology, coloration and acoustic data, including new records from the states of Alagoas and Pernambuco, expanding the geographic distribution of the species in more than 800 km north from its type locality.

Key Words: Anura, Atlantic Forest, Geographic distribution, Hylidae.

Recently Araujo-Vieira *et al.* (2020) designated a neotype for *Scinax x-signatus* and redescribed the species, providing genetic, acoustic and morphological data. This major contribution aided in elucidating the taxonomy of other *Scinax ruber* clade populations for the Atlantic Rainforests of northeastern Brazil, previously considered as *S. x-signatus*, but, in fact, comprising a new species described as *Scinax tropicalia* (Novaes-e-Fagundes *et al.*, 2021).

Scinax tropicalia occurs in the Atlantic Rainforest of Bahia, displaying a restricted population at the Serra de Baturité mountain range, in the state of Ceará (Novaes-e-Fagundes *et al.*, 2021). These authors, however, mentioned that the species probably displays a wider distribution along northeastern Atlantic Rainforests. This disjoined distribution could also be an artifact, since no individuals from other states across the distribution gap of the species were examined.

After scrutinizing the morphology, coloration and vocalization of individuals from several localities in the Pernambuco Endemism Center (Prance, 1982; Silva & Castelleti, 2003), in the states of Pernambuco and Alagoas (Table 1), we confirmed the occurrence of *Scinax tropicalia* in this sub-biogeographical region for the first time.

We recorded *Scinax tropicalia* in four distinct Atlantic Forest fragments, namely the Matas de Siriji Wildlife Refuge (RVS-MS), the municipality of São Vicente Ferrer, 600 m a.s.l., the Dois Irmãos State Park (PEDI), the municipality of Camaragibe, 200 m a.s.l., the Frei Caneca Natural Heritage Private

Table 1. New geographic distribution records of *Scinax tropicalia* in the Pernambuco Endemism Center.

State	Municipality	Locality	Latitude	Longitude	Altitude (m)
Alagoas / Pernambuco	Chã Preta (AL), Quebrangulo (AL), Lagoa do Ouro (PE)	Pedra Talhada Biological Reserve	-9.253790°	-36.343100°	470
Pernambuco	Jaqueira	Frei Caneca and Pedra D'Antas Natural Heritage Private Reserves	-8.708220°	-35.854400°	715
	Recife/Camaragibe	Dois Irmãos State Park	-7.979600°	-34.958800°	48
	São Vicente Ferrer	Matas de Siriji RVS	-7.614330°	-35.506500°	536

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Reserve (RPPN-FC) and the Pedra D'Antas Natural Heritage Private (RPPN-PD), both part of the Serra do Urubu complex, in the municipalities of Jaqueira and Lagoa dos Gatos, and the Pedra Talhada Biological Reserve (REBIO-PT), in the state of Alagoas (Fig. 1). The examined individuals were found calling perched in vegetation surrounding pond margins and in streams inside and at the edge of the forest, ranging from 0.30 m to 2 m in height.

The advertisement calls of individuals from PEDI and RVS-MS were recorded with a digital Tascam DR-40 recorder coupled with a unidirectional Sennheiser ME 66 microphone. At the REBio-PT, recordings were obtained using a Marantz PMD 660 recorder with a YOGA EM-9600 microphone, all positioned at approximately 50 cm from the target males. The recorders were set at a 44.1-kHz sampling rate and 16-bit depth. Air temperature and relative humidity at the time of recording were determined using an Incoterm© thermo hygrometer (accuracy ±0.5). We also measured the snout-vent length of the individuals using a digital caliper (0.1 mm). Calls were analyzed using the Raven Pro 1.5 (Bioacoustics Research Program, 2020) software, with DFT = 256, Hanning window, FFT size = 1024 samples, FFT overlap = 90%; and the amplitude spectrum was generated with a FFT size = 2500 samples. The following temporal traits were measured: note duration (s), dominant frequency (Hz), frequency 5% (Hz), frequency 25% (Hz), frequency 75% (Hz), frequency 95% (Hz), bandwidth 90% (Hz), duration 50% (s), duration 90% (s), note interval (s), notes per second, pulse duration (s), pulse per note and pulse sub-units. The call recordings were deposited at the of the Interdisciplinary Amphibian and Reptile Laboratory sound library at the Federal Rural University of Pernambuco (UFRPE-SLIAR290, UFRPE-SLIAR291, UFRPE-SLIAR292, UFRPE-SLIAR293, UFRPE-SLIAR294). The voucher individuals were deposited at the Ceará Federal University (CHUFC-A-9109–13) and Cariri Regional University (URCA-H-5117, 6256–7, 6262, 6540) Herpetological Collections.

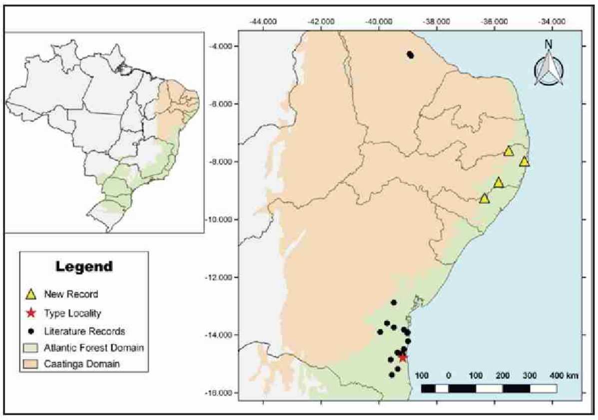

Figure 1. Geographic distribution map of *Scinax tropicalia*, with the new records for the states of Pernambuco and Alagoas (yellow triangle), and literature records for the state of Bahia and Ceará (black dots).

We confirmed *S. tropicalia* identification based on the absence of spicule-shaped papillary epidermal projections on the nuptial pads, absence of pectoral glands in male and coloration pattern of the hidden surfaces of the shanks and tarsi (Novaes-e-Fagundes *et al.*, 2021) (Fig. 2). In addition, the advertisement

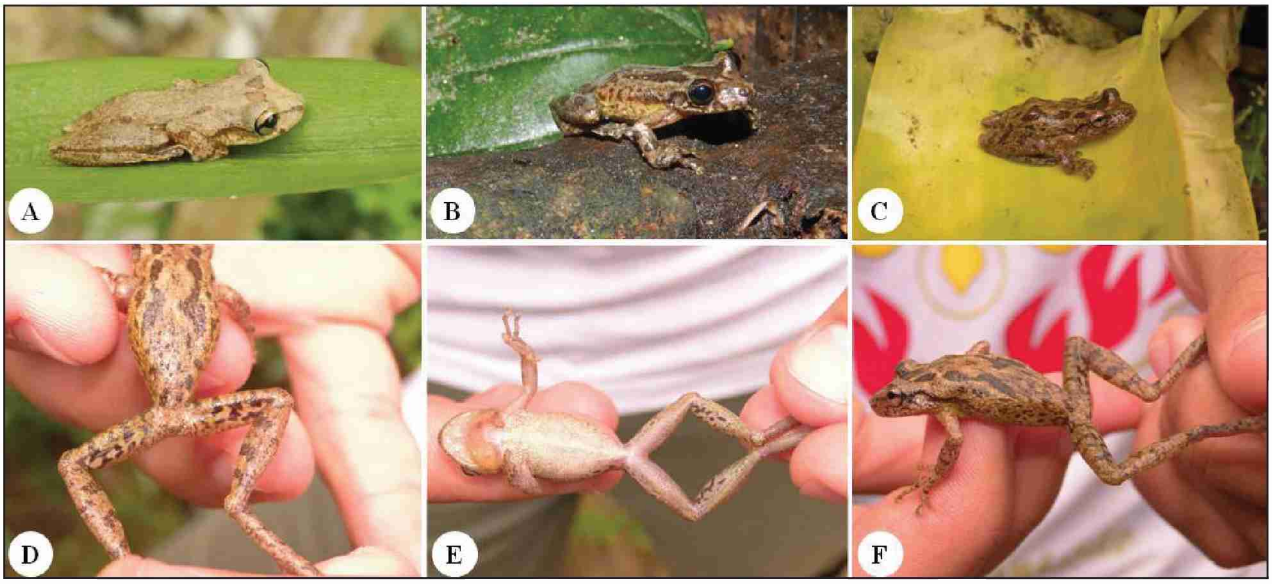

Figure 2. Live individuals of *Scinax tropicalia* from the Pernambuco Endemism Center, a-RVS-MS, b- PEDI, c- RPPN-FC.

calls parameters of Pernambuco and Alagoas populations match the described call for the species (Fig. 3; Table 2).

The advertisement call of the *S. tropicalia* populations from the Pernambuco Endemism Center agree with the call described for the Ceará and Bahia populations (Table 2), composed of a single multipulsed note composed by sub-pulses, without harmonics, mean call duration of 0.164 ± 0.032 s (n = 125), mean intercall interval of 0.502 ± 0.186 s (n = 104), number of pulses per note 11 ± 2 pulses (n = 125), with mean pulse duration of 0.015 ± 0.001 s (n = 125) and peak frequency ranging from 1593–1852 Hz (n = 125) 75% (3162 ± 612.2, n = 125) (Table 2; Fig. 3).

Some differences in the call of individuals from Pedra Talhada (n = 10) were noted, mostly associated to temporal parameters, with higher values than *S. tropicalia* individuals from the other investigated localities, as follows: higher call duration (0.334 ± 0.029 s), Duration 50% (0.122 ± 0.016 s), Duration 90% (0.277 ± 0.024 s) and a higher number of pulses per note (20.4 ± 1.7). Spectrally, most parameters were consistent across all populations, with the exception of individuals from the Pedra Talhada population, which also displayed a slight variation of increased frequency, frequency 75% (3731.2 ± 59.2 Hz); frequency 95% (4218.7 ± 171.1 Hz) and bandwidth 90% (2718.7 ± 171.1 Hz). The temporal and spectral parameters of the advertisement calls presented both dynamic and/or static features (Köhler *et al.*, 2017), which may be the result of morphological nd environmental influences, such as body condition, temperature and relative humidity (Rodríguez *et al.*, 2015; Ziegler *et al.*, 2015).

The populations referred as the *Scinax ruber* clade at the Serra do Urubu mountain range (Roberto *et al.*, 2017) are in fact *Scinax tropicalia*, as this species occurs in sympatry with the congeners *Scinax x-signatus*, *S. eurydice*, *S. nebulosus* and *S. cretatus* in this region. At the Pedra Talhada Biological Reserve, Carvalho-e-Silva *et al.* (2015) and Dubeux *et al.* (2020) only mentioned the occurrence of *Scinax x-signatus*, while our study also indicates the occurrence of *S. tropicalia* in this region, found in ephemeral ponds inside and bordering the forest, whereas *S. x-signatus* occurs mainly in open areas. A similar pattern was detected at the PEDI.

Our new record expands the geographic distribution of the species 889 km north from the type locality of *S. tropicalia* (Ilhéus, in the state of Bahia) and 612 km south of the Serra de Baturité mountain range, in the state of Ceará, the northern distribution record for the species.

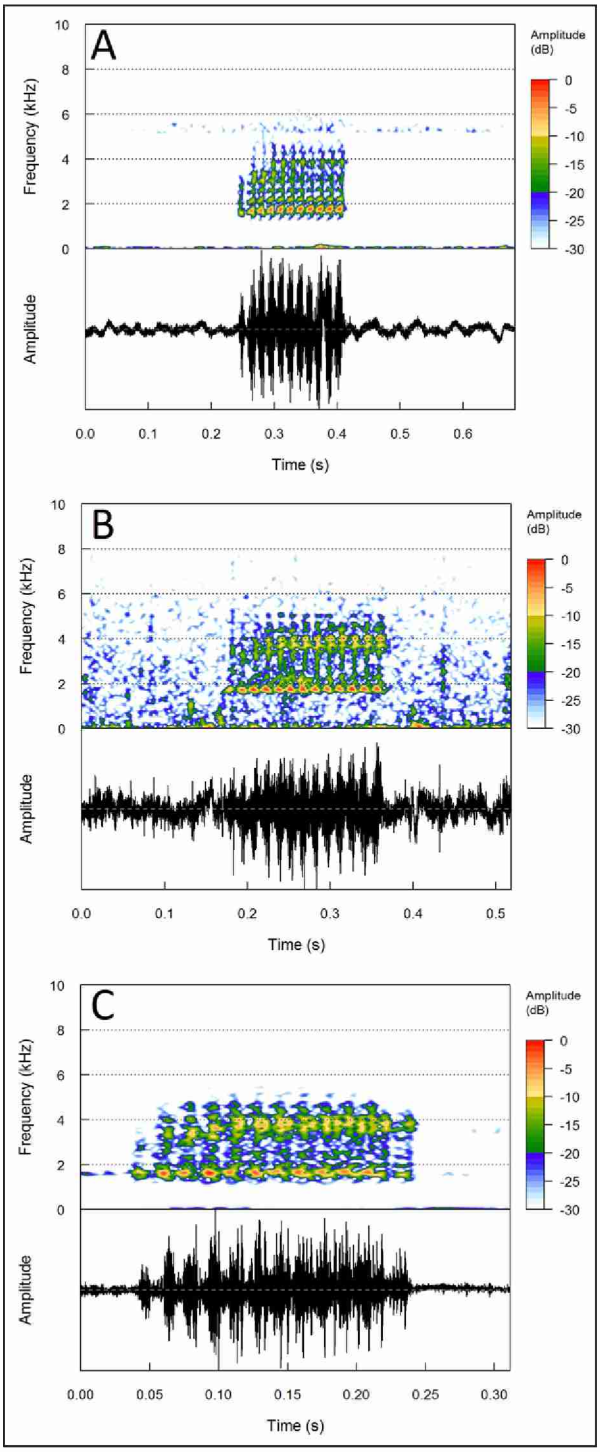

Figure 3. Advertisement call of Scinax tropicalia from Pernambuco endemismo center. Espectrogram and oscilograma: (A) municipality of São Vicente Ferrer, state of Pernambuco (UFRPE-SLIAR291), (B) Parque Estadual Dois Irmão, state of Pernambuco, (UFRPE-SLIAR293) and (C) REBIO Pedra Talhada, state of Alagoas (UFRPE-SLIAR294).

Table 2. New geographic distribution records of *Scinax tropicalia* in the Pernambuco Endemism Center.

Parameters	**RVS-MS**	**PEDI**	**REBio-PT**	**Novaes-e-Fagundes *et al.* (2021)**
Note duration (s)	0.158 ± 0.019 (0.119–0.183) N = 20	0.183 ± 0.02 (0.147–0.211) N=20	0.334 ± 0.029 (0.286–0.375) N=10	0.164 ± 0.032 (0.114–0.310) N=125
Dominant frequency (Hz)	1739.8 ± 35.3 (1722.6–1808.7) N = 20	1757.1 ± 43.2 (1722.6–1808.7) N = 20	1650 ± 48.4 (1593.7–1687.5) N = 10	1708 ± 68.7 (1593–1852) N = 125
Frequency 5% (Hz)	1653.7 ± 35.3 (1636.5–1722.6) N = 20	1662.3 ± 40.4 (1636.5–1722.6) N=20	1527.3 ± 57.5 (1500–1636.5) N=10	1609 ± 73.1 (1507–1766) N = 125
Frequency 25% (Hz)	1736.3 ± 38.6 (1687.5–1808.7) N = 20	1724.2 ± 32.2 (1687.5–1808.7) N = 20	1668.7 ± 39.5 (1593.7–1687.5) N = 10	1703 ± 73.9 (1593–1895) N = 125
Frequency 75% (Hz)	3027.5 ± 623.7 (2239.5–3875.9) N = 20	3057.7 ± 885.6(1808.7–3962.1)N = 20	3731.2 ± 59.2 (3656.2–3843.7) N = 10	3162 ± 612.2 (1766–4565) N = 125
Frequency 95% (Hz)	4086.9 ± 286.9 (3617.6–4651.1) N = 20	4031 ± 149.4 (3703.7–4478.9) N = 20	4218.7 ± 171.1 (4125–4687.5) N = 10	4022 ± 427.6 (3058–5125) N = 125
Bandwith 90% (Hz)	2441.8 ± 296 (1981.1–3014.6) N = 20	2368.6 ± 156.8 (2067.1–2842.3) N = 20	2718.7 ± 171.1 (2625–3187.5) N = 10	2413 ± 378.4 (1550–3359) N = 125
Duration 50% (s)	0.075 ± 0.007 (0.063–0.087) N = 20	0.077 ± 0.01 (0.058–0.092) N = 20	0.122 ± 0.016 (0.096–0.149) N = 10	0.071 ± 0.016 (0.049–0.140) N = 125
Duration 90% (s)	0.098 ± 0.028 (0.064–0.15) N = 20	0.146 ± 0.018 (0.116–0.174) N=20	0.277 ± 0.024 (0.24–0.314) N = 10	0.136 ± 0.028 (0.091–0.261) N = 125
Note interval (s)	0.426 ± 0.239 (0.224–1.3) N = 20	0.286 ± 0.057 (0.2–0.388) N = 20	0.236 ± 0.077 (0.146–0.413) N = 10	0.502 ± 0.186 (0.291–1.336) N = 104
Note /s	1.4 ± 0.502 (1–2) N = 20	2.15 ± 0.366 (2–3) N = 20	1.1 ± 0.316 (1–2) N = 10	1.6 ± 0.34 (0.7–2.3) N = 104
Pulse duration (s)	0.015 ± 0.0002 (0.014–0.015) N = 20	0.014 ± 0.0003 (0.014–0.015) N = 20	0.016 ± 0.0004 (0.016–0.017) N = 10	0.015 ± 0.001 (0.014–0.016) N = 125
Pulse/Note	10.4 ± 1.2 (8–12) N = 20	12.3 ± 1.3 (10–14) N = 20	20.4 ± 1.7 (18–23) N = 10	11 ± 2 (8–20) N = 125
Pulse sub-units	4.6 ± 0.1 (2–5) N = 20	4 ± 0.652 (1–5) N = 20	4.5 ± 0.164 (3–5) N = 10	5 (5) N = 125
SNL (mm)		37 and 39	-	30.8 to 39.7
Mass (g)	-	2.1	-	-
Relative humidity (%)	81 and 74	85	-	79 to 90
Temperature (oC)	22.6 and 26.4	23.3	-	22.7 to 25
Voucher	UFRPE-SLIAR291 and UFRPE-SLIAR290	UFRPE-SLIAR292 and UFRPE-SLIAR293	UFRPE-SLIAR294	FonoZoo 12689–710

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Novedad zoogeográfica

Cuad. herpetol. 35 (2): 343-345 (*2021*)

Presencia de *Oxyrhopus petolarius* (Serpentes: Colubridae) en el Pantanal Paraguayo

Pier Cacciali, Marcela Ferreira Riveros, Alexis Arias, Asterio Ferreira

Guyra Paraguay, Av. Cnel. Carlos Bóveda, Parque Ecológico Capital Verde – Viñas Cué, Asunción, Paraguay.

Localidad– Paraguay, Departamento Alto Paraguay, Distrito de Bahía Negra, Reserva Pantanal Paraguayo, Estación Biológica Los Tres Gigantes (20°04’42.93”S; 58°09’4544”W, 83 m s.n.m., Fig. 1). El 2 de julio de 2020, a las 08:23 h, fue colectado un ejemplar macho de *Oxyrhopus petolarius* en el sendero de un bosque ribereño. El espécimen está depositado en la colección herpetológica del Museo Nacional de Historia Natural del Paraguay (MNHNP 12825).

Comentarios– La especie se encuentra distribuida ampliamente desde el sur de México hasta Paraguay y la Provincia argentina de Misiones (Bailey, 1970; Giraudo, 2001; MacCulloch *et al.*, 2009). En la zona más austral de su distribución está asociada al Bosque Atlántico (Nogueira *et al.*, 2019). En Paraguay, *O. petolarius* es una serpiente poco frecuente de encontrar, con pocos registros en colecciones biológicas y hasta el momento conocida únicamente dentro del Bosque Atlántico (Cabral y Scott, 2014; Cacciali *et al.*, 2016). El nuevo registro proveído aquí, situado dentro del Pantanal Paraguayo, dista a unos 46 km al oeste de Fuerte Coimbra (Corumbá, Mato Grosso do Sul, Brasil), la localidad más cercana conocida (Nogueira *et al.*, 2019), y 714 km oeste-noroeste de los alrededores de Ciudad del Este, localidad más al norte conocida en Paraguay.

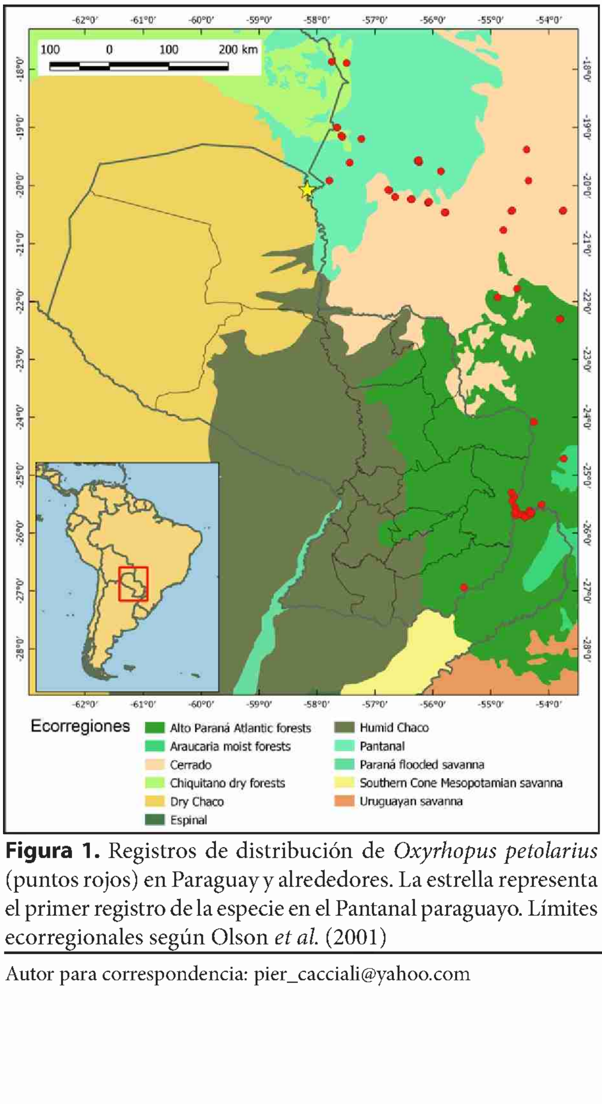

Figura 1. Registros de distribución de *Oxyrhopus petolarius* (puntos rojos) en Paraguay y alrededores. La estrella representa el primer registro de la especie en el Pantanal paraguayo. Límites ecorregionales según Olson *et al.* (2001)

El Pantanal es un área de planicies inundables con una vegetación que presenta elementos con influencia de distintas provincias fitogeográficas tales como Amazonas, Cerrado, Bosque Atlántico y Chaco; debido a lo cual, existen pocos endemismos (Pott y Silva, 2015). En Brasil, *O. petolarius* fue registrada para el Pantanal (Nogueira *et al.*, 2019), la cual contaba con registros únicamente al este del Río Paraguay.

El ejemplar mide 53,4 mm de longitud hocico-cloaca, 18,1 mm de extremidad caudal, escamas dorsales 21-19-17, lisas, ventrales 192, placa anal dividida, subcaudales en 100 pares, loreal 1/1 (datos presentados en izquierda/derecha), preocular 1/1, postoculares 2/2, temporales 2+3+4/2+3+3, supralabiales 8(4–5)/8(4–5), infralabiales 11(01–6)/10(1–6). La coloración es predominantemente anillos anchos gris negruzco, con 17 anillos (incluyendo el nucal) rojos aproximadamente un tercio o menos del tamaño de los anteriores (Fig. 2). En general, los datos de lepidosis coinciden con lo conocido para la especie (Giraudo, 2001), con excepción del conteo de subcaudales (100) que no coincide para lo conocido de ejemplares del Bosque Atlántico (60–69), pero sí con datos de animales de origen amazónico (84–126)

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Figura 2. Ejemplar de *O. petolarius* (MNHNP 12825) registrado en el Pantanal paraguayo. Se observa que el mismo se encuentra en proceso de ecdisis.

(Lynch, 2009; MacCulloch *et al.*, 2009).

Este registro es un aporte más al conocimiento de la fauna en áreas silvestres protegidas del Paraguay. *Oxyrhopus petolarius* es una especie categorizada como En Peligro para Paraguay, con escasos registros en colecciones biológicas, representada hasta el momento en un hábitat que ha perdido gran parte de su cobertura original como lo es el Bosque Atlántico del Alto Paraná y se estima que la extensión de la especie en el país es de menos de 100 km^2 (Martínez *et al.*, 2020). La especie era conocida en tres áreas protegidas en el este de Paraguay (Cacciali *et al.*, 2015). Este registro aumenta a 33 las especies de reptiles protegidas en la Reserva Pantanal Paraguayo (Cacciali *et al.*, 2015).

Agradecimientos

Agradecemos a Carolina Álvarez y Pedro Duarte por la ayuda durante los trabajos de campo, a Guyra Paraguay a través de José L. Cartes, Rodrigo Zárate y Tatiana Galluppi por la logística y el apoyo constante, al World Land Trust y Swire Pacific Offshore por el soporte financiero. A Frederick Bauer por corroborar la identidad del ejemplar, y a Martha Motte y Nicolás Martínez por permitir el acceso a la colección de herpetología del Museo Nacional de Historia Natural del Paraguay. El permiso de colecta N° 111/2019 fue proveído por el Ministerio del Ambiente.

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Novedad zoogeográfica

Cuad. herpetol. 35 (2): 347-348 (*2021*)

First record of *Nectocaecilia petersii* (Boulenger, 1882) (Gymnophiona: Typhlonectidae) for the state of Acre, Brazil

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Locality: Brazil, Acre, Cruzeiro do Sul, Universidade Federal do Acre, 7°33'35" S; 72°43'3.2" W (Fig. 1). Marllus Rafael Negreiros de Almeida, 13/01/2020. UFACF 4398. Coleção de Herpetologia da Universidade Federal do Acre, Campus Floresta (UFACF).

Comments: Typhlonectidae Taylor, 1968 includes 14 species of aquatic and semiaquatic caecilians distributed along the South American hydrographic systems (Taylor, 1968; Maciel *et al.*, 2015). Four species are present in the Amazon Basin, two of them, *Potamotyphlus kaupii* (Berthould, 1859) and *Typhlonectes compressicauda* (Duméril and Bibron, 1841) have long been known by science to be widely distributed (Taylor, 1968; Cintra *et al.*, 2010; Maciel and Hoogmoed, 2011; Oliveira *et al.*, 2012; Alves-Silva *et al.*, 2017). Up to 2011, *Atretochoana eiselti* (Taylor, 1968) was known by only two specimens with no refined collection data, but it was discovered in at least three great Amazonian rivers, which indicates it can be of wide distribution as well (Hoogmoed *et al.*, 2011; Vaz-Silva *et al.*, 2015). Finally, *Nectocaecilia petersii* (Boulenger, 1882) was recently recorded in the Tapajós sub-basin, considerable far from the three localities where it was known before (Fraga *et al.*, 2018).

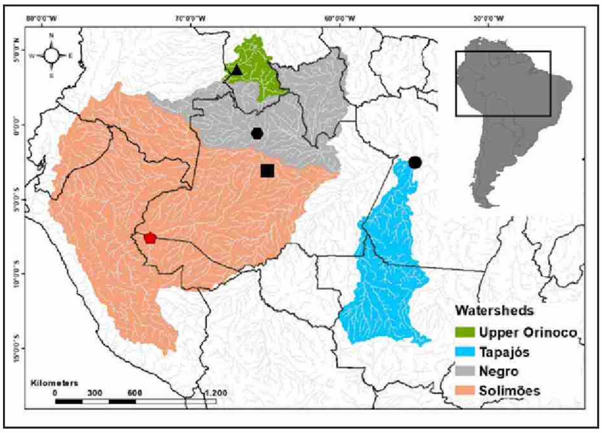

Figure 1. Distribution map of *Nectocaecilia petersii.* Pentagon represents the new locality record in the state of Acre, Brazil. Triangle represents Cerro Yapacana, Venezuela (Gorzula and Señaris, 1998). Hexagon represents Rio Tea, Amazonas, Brazil (Maciel and Hoogmoed, 2011). Square represents Mamirauá, Uarini, Amazonas, Brazil (Maciel and Hoogmoed, 2011). Circle represents Alter do Chão, Pará, Brazil (Fraga *et al.*, 2018).

On 13 January 2020, at 5 PM, Marllus Rafael Negreiros de Almeida found a caecilian in the campus of the Universidade Federal do Acre (7°33'35" S; 72°43'3.2" W), municipality of Cruzeiro do Sul, state of Acre, Brazil. The caecilian was found moving in a muddy soil 6 meters from a small stream, and when approached tried quickly to hide its head below rotten leaves and trunks in the mud. It also released mucus from its skin when handled. The place is 6,4 km from the left margin of the Moa River and 10 km from the left margin of the Juruá River. The specimen (Fig. 2) is an adult male with total length 530 mm and was catalogued as UFAC 4398 in the Herpetological Collection of Universidade Federal do Acre, Campus Floresta, Cruzeiro do Sul. We identified the specimen as Typhlonectidae by its dorsoventrally compressed head, dorsal eyes, and well-marked cloacal disc (Taylor, 1968; Wilkinson and Nussbaum, 1999). Furthermore, the caecilian presents all conditions on external characters to fit within the diagnosis of *Nectocaecilia petersii*: 145 primary grooves, dark primary grooves, cylindrical body, and absence of a "dorsal fin" (Maciel and Hoogmoed, 2011).

Our register was made approximately 1000 km southwestern far from the closest known occurrence of the species, in Uarini, Amazonas, Brazil (Figure 1), and it is the first record of *Nectocaecilia* to the region of the Juruá Sub-basin, and to the state

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Figure 1. Alive specimen of *Nectocaecilia petersii* found in Cruzeiro do Sul, Acre, Brazil. Left, under leaves. Right, in muddy soil.

of Acre, Brazil. This discovery reinforces that *N. petersii* can be widely distributed in Amazonia, probably being just less common than *P. kaupii* and *T. compressicauda*, which are more numerous in scientific collections (Maciel and Hoogmoed, 2011). In fact, elements that drive demographic dynamics in populations of caecilians remain unknown, especially when referring to aquatic species.

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Novedad zoogeográfica

Cuad. herpetol. 35 (2): 349-351 (*2021*)

Distribution extension of *Ceratophrys joazeirensis* Mercadal, 1986 (Anura: Ceratophryidae): second record from the state of Minas Gerais, southeastern Brazil

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Locality – Brazil. State of Minas Gerais, municipality of São João da Ponte, Santa Mônica farm (16°01'26.40"S; 43°42'00"W, 505 m.a.s.l.; Fig. 1). Date: November 15th, 2016. Collected by Maria Clara do Nascimento and Fred Victor de Oliveira, under collection permit IEF 034.012/2016/MG. Voucher: UFMG 19076, deposited in the Amphibian Collection of Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, MG (UFMG).

Comments – The horned-frog genus *Ceratophrys* Wied, 1824 currently comprises eight species from tropical South America, five of which occur in Brazil: *Ceratophrys aurita* (Raddi, 1823), *C. cornuta* (Linnaeus, 1758), *C. cranwelli* Barrio, 1980, *C. ornata* (Bell, 1843), and *C. joazeirensis* Mercadal, 1986 (Segalla *et al.*, 2019; Frost, 2020).

Ceratophrys joazeirensis is the only species of the genus occurring in the semiarid Caatinga ecoregion of northeastern Brazil from where it is considered endemic (Faivovich *et al.*, 2014), although marginally reaching the Cerrado ecoregion (Maciel *et al.*, 2013). The species is known from few localities from Rio Grande do Norte (Vieira *et al.*, 2006; Jorge *et al.*, 2012) southwards to northern Bahia (Mercadal, 1986; Borges-Nojosa & Arzabe, 2005; Vieira *et al.*, 2006; Caramaschi, 2008; Santos *et al.*, 2009; Zaidan & Leite, 2012; Jorge *et al.*, 2012; Valdujo *et al.*, 2012; Roberto *et al.*, 2013; Faivovich *et al.*, 2014; Santana *et al.*, 2014; Almeida *et al.*, 2016), plus a single record in Minas Gerais (Maciel *et al.*, 2013) (Fig. 1). Our record from São João da Ponte is the second from Minas Gerais, filling a gap of 860 km in a straight line between the two southernmost localities where the species was previously recorded: Buritizeiro (Minas Gerais, ca. 190 km from the new record) and Feira de Santana (Bahia, ca. 680 km from

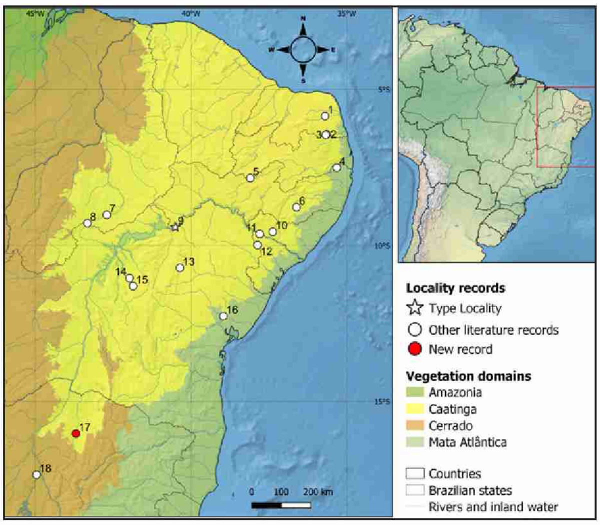

Figure 1. Map showing the known localities of *Ceratophrys joazeirensis*. based on the literature and the new record. 1) Rio Grande do Norte, Santa Maria, Fazenda Tanques [05°51'14.4"S; 35°42'03.6"W] (Jorge *et al.*, 2012); 2) Rio Grande do Norte, Passa e Fica [06°26'16.8"S; 35°38'38.4" W] (Vieira *et al.*, 2006). 3) Paraíba, Araruna, Parque Estadual da Pedra da Boca [06°27'14.4"S; 35°40'48"W] (Borges-Nojosa & Arzabe, 2005; Vieira *et al.*, 2006; Faivovich *et al.*, 2014). 4) Pernambuco, Timbaúba [07°30'18"S; 35°19'04.8"W] (Caramaschi, 2008); 4) Pernambuco, Triunfo, Café do Brejo [07°50'16.8"S; 38°06'07.2"W] (Santos *et al.*, 2009); 6) Pernambuco, Caetés, Vale do Rio São José [08°46'19.2"S; 36°37'19.2"W] (Oliveira *et al.*, 2017). 7) Piauí, São Raimundo Nonato [09°00'57.6"S; 42°41'56.40"W] (Roberto *et al.*, 2013); 8) Piauí, Caracol, Lagoa do Carlinho [09°16'44.4"S; 43°19'48"W] (CRIA, 2020; Mângia com. pess.). 9) Bahia, Juazeiro [09°24'39.6"S; 40°30'25.2"W] (Mercadal, 1986 – type locality). 10) Alagoas, São José da Tapera [09°33'28.8"S; 37°22'51.6"W] (Almeida *et al.*, 2016). 11) Sergipe, Canindé do São Francisco, Xingó PowerPlant [09°37'37.2"S; 37°48'07.2"W] (Santana *et al.*, 2014); 12) Sergipe, Poço Redondo, Serra da Guia [09°58'51.6"S; 37°52'04.8"W] (Santana *et al.*, 2014). 13) Bahia, Pindobaçu, Poço Pelado [10°43'12"S; 40°21'18"W] (Zaidan & Leite, 2012); 14) Bahia, Jussara [11°02'52.8"S; 41°58'15.6"W] (Gama *et al.*, 2020); 15) Bahia, Irecê, Estrada Lapão da Pedreira [11°18'14.4"S; 41°51'21.6"W] (CRIA, 2020); 16) Bahia, Feira de Santana [12°16'01.2"S; 38°58'01.2"W] (Faivovich *et al.*, 2014). 17) Minas Gerais, São João da Ponte, Fazenda Santa Mônica [16°01'26.4"S; 43°42'00"W] (present study); 18) Minas Gerais, Buritizeiro, Fazenda Jatobá [17°21'03.6"S; 44°57'43.2"W] (Maciel *et al.*, 2013; Valdujo *et al.*, 2012).

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the new record) (Fig. 1). It is also the southernmost record of *C. joazeirenzis* within the Caatinga (*sensu* IBGE, 2019).

The voucher specimen (UFMG 19076) was collected at 8:00 p.m., after a rainstorm that lasted from afternoon to early evening. The specimen was on an unpaved road between pastures and a riparian forest fragment at the margins of the Verde Grande river (Fig. 2). On the occasion, other specimens of *Ceratophrys joazeirensis* were calling from the riparian forest fragment, which had many temporary ponds, but none could be sighted/located. Specimens of *C. joazeirensis* usually can be recorded calling from the edges of ephemeral ponds in open areas (Zaidan & Leite, 2012; Maciel *et al.*, 2013; Jorge *et al.*, 2015). Although we heard specimens calling from inside a riparian forest, the site was largely impacted by human activities. This riparian forest is about 20 m wide from the riverbanks to the road, and is composed mostly of small trees, less than 10 m high (Fig. 2).

The collected specimen is a juvenile whose external morphology fits well in the diagnosis *of Ceratophrys joazeirensis*, such as a triangular, medium developed eyelid appendix and the dorsal color pattern with three dorsolateral blotches on each side, resembling a trident (Mercadal, 1986) (Fig. 3). Two weeks after the collection, HCC visited the site but the weather was dry. The ponds have dried up and no horned-frog was heard or spotted, reinforcing the explosive breeding behavior of this elusive species.

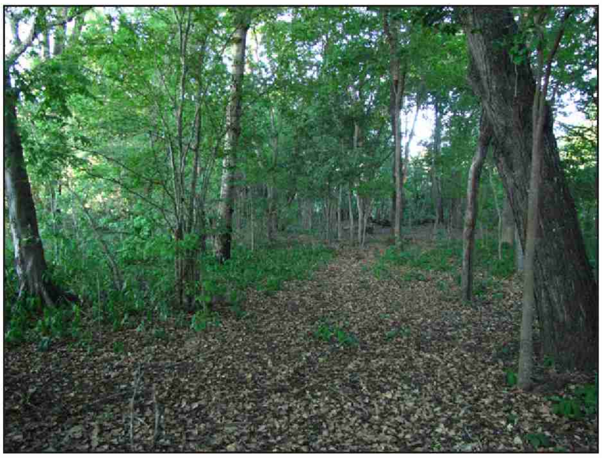

Figure 2. Narrow forest fragment between the margins of Verde Grande river (to the left) and an unpaved road (to the right), where the specimen of *Ceratophrys joazeirensis* (UFMG 19076) was captured.

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Figure 3. Juvenile specimen of *Ceratophrys joazeirensis* (UFMG 19076) in life, showing color pattern characteristic of this species and the odontoids on either side of the mandibular symphysis, characteristic of the genus.

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Novedad zoogeográfica

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Extension of the distribution of *Pantherophis emoryi* (Baird & Girard, 1853) (Squamata: Colubridae) in Mexico

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Localities- Mexico, Veracruz, Municipality of Paso de Ovejas, in the locality of Paso Limón (19.240858°N; 96.509931°W; WGS 84; 195 m a.s.l.; Fig. 1). On 20 May 2018, around 0745 h AMH found a dead adult individual, which presented reddish-brown back of body pattern with dark brown spots, light and dark arrow-shaped markings (V) on top of head; 44 blotches on the body and 18 on the tail; 8/8 supralabial. The individual was run over in the vicinity of a pig farm. We deposited a photographic voucher for the

Figure 1. An adult specimen of *Pantherophis emoryi* (LACM PC 2763-2764) from the municipality of Paso de Ovejas, Veracruz, Mexico.

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specimen in the digital collection of the Natural History Museum of Los Angeles County (LACM PC 2763-2764).

Mexico, Veracruz, Municipality of Paso del Macho, in the locality of Paso Espuela (18.919942°N; 96.542096°W; WGS 84; 230 m a.s.l.; Fig. 2). On 8 July 2020, around 1440 h, VVC, EOB, RPH and JLCJ found a subadult male individual. It had a snout vent length of 721 mm and total length of 830 mm. Back of body pattern light brown with dark brown spots, light and dark arrow-shaped markings (V) on top of head; 47 blotches on the body and 20 on the tail; 27 rows on mid-body; 8/8 supralabial and 11/11 infralabial; 221 ventrals and 69 subcaudals. The individual was found dead on a path, the vegetation corresponds to grassland. We deposited a photographic voucher for the specimen in the digital collection of the Natural History Museum of Los Angeles County (LACM PC 2765-2766).

Figure 2. A subadult specimen of *Pantherophis emoryi* (LACM PC 2765-2766) from the municipality of Paso del Macho, Veracruz, Mexico.

Comments- Great Plains Rat Snake, *Pantherophis emoryi* (Baird & Girard, 1853) (Squamata: Colubridae), it´s a medium-size snake (total length=1530 mm; Heimes, 2016). Its distribution is wide, ranging from southwestern Illinois, Missouri, southern South Dakota and southeastern Colorado in the United States, to the south in Mexico in the states of Aguascalientes, Chihuahua, Coahuila, Durango, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas and Veracruz (Heimes, 2016; Uetz *et al.*, 2021) Recently, Quintero-Díaz *et al.* (2016), provide the first record of *P. emoryi* in the state of Aguascalientes and a list of localities and museum specimens. It inhabits a great variety of environments, in arid and tropical regions; its occurrence seems to be limited to the proximity of permanent bodies of water (Ramírez-Bautista *et al.*, 2014).

The records presented here are the result of opportunistic encounters. We determined the individuals considering the diagnostic characteristics of *P. emoryi* proposed by Ramírez-Bautista *et al.* (2014) and Heimes (2016). A medium sized snake with a dorsal pattern of dark blotches; arrow-shaped light and dark markings on top of the head; There are 28–45 blotches on the body and 11–23 on the tail; 8–9 (rarely 6 or 7) supralabials, 10–13 (rarely 14 or 15) infralabials, 1 loreal, 1 preocular, 2 postocular; midbody scale rows usually 27 but often 29; ventrals 201–236. Dorsal scales are smooth except for the middorsal rows which are very weakly keeled; the ventrals have distinct lateral keels. The determination was verified by Luis Canseco Márquez. In the center of Veracruz, the snakes *Pseudelaphe flavirufa* (Cope, 1867), and *Senticolis triaspis* (Cope, 1866) are distributed, species that have dorsal patterns similar to *P. emoryi*. Nevertheless, we can distinguish *P. emoryi* of *P. flavirufa* in that the former has a lower number of supralabials (6–9 vs 9–10), infralabials (10–13 vs 12–15) and ventrals (201–236 vs 242–269). Same, we can differentiate *P. emoryi* of juveniles of *S. triaspis* in which it presents a lower number of dorsal scales in rows at midbody (27–29 vs 31–39) and a lower number of dorsal blotches on the body and tail (39–68 vs 60–111). The inverted V-shaped spot on the head in *P. emoryi* is absent in *P. flavirufa* and in juveniles of *S. triaspis*.

In the state of Veracruz, *P. emoryi* only had been reported in the northern region (Heimes, 2016; Quintero-Díaz *et al.*, 2016; Uetz *et al.*, 2021): at 17 mi. south of Pánuco (Pérez-Higareda and Smith, 1991), El Higo (Colección Nacional de Anfibios y Reptiles; CNAR: 00929; Flores-Villela, 1998), and 29.3 mi. north of Temporal (Milwaukee Public Museum; MPM: 18387; Flores-Villela, 1998). Additionally, two specimens are deposited in the Florida Museum of Natural History (UF 49401, 49402), in locality San Luis Potosi border, 57 km west of Tampico (Flores-Villela, 1998), nevertheless, also these individuals have been mentioned in platform of VertNet (2021) incorrectly with the verbal description of the locality "Mexico 70, west of Tampico near San Luis Potosi state border". Thus, our records significantly increase the distribution of *P. emoryi*, ~ 362 km to the south in the state of Veracruz and 282 km south of the closest known locality in Yahualica in state of Hidalgo (Fig. 3), particularity Paso Espuela, is the most equatorial in the distribution of the species. Additionally, we report the maximum number of blotches on the body in 47 vs 45 (Heimes, 2016).

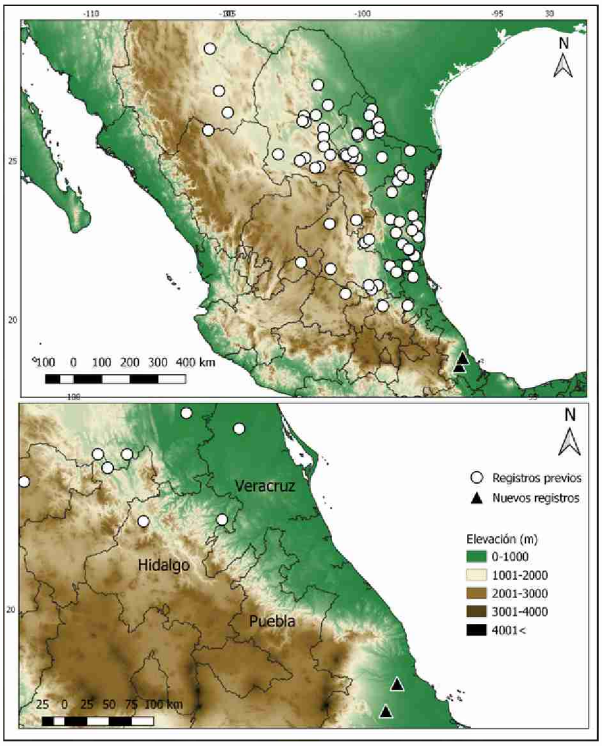

Figure 3. Distribution map of *Pantherophis emoryi* with collecting localities in México. The new localities in black triangle and previous localities in white circles, black lines represent state boundaries. The previous localities were taken of vouchers museum of Pérez-Higareda and Smith (1991), Flores-Villela (1998), Quintero-Diaz *et al.* (2016) and CONABIO (2021).

Some regions of central Veracruz are considered biologically important areas or "hot spots" (Ochoa-Ochoa and Flores-Villela, 2011). These sites have been of great interest to find new species of amphibians and reptiles like *Geophis lorancai* (Canseco-Márquez *et al.*, 2016), *Isthmura corrugata* (Sandoval-Comte *et al.*, 2017), and *Pseudoeurycea granitum* (García-Bañuelos *et al.*, 2020). In addition, in recent years, the report of herpetological novelties has increased (see Solano-Zavaleta *et al.*, 2017; Peralta-Hernández *et al.*, 2020; De La Torre-Loranca *et al.*, 2020; Pineda *et al.*, 2020), in most cases in mountainous areas at elevations above 900 m. Nevertheless, to the best of our knowledge, no work has been carried out on this group in the municipality of Paso de Ovejas and in the municipality of Paso del Macho is scarce and recent (Vásquez-Cruz *et al.*, 2021). We consider this as the main cause of the absence of previous records of *P. emoryi* in the center of Veracruz. Another probable cause is that in nearby regions to our localities *P. emoryi* has been confused with similar species such as *P. flavirufa* and with juveniles of *S. triaspis*.

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Novedad zoogeográfica

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Nuevos registros de distribución para *Dendropsophus rhodopeplus* (Amphibia: Anura: Hylidae) en los departamentos de Caquetá y Putumayo – Colombia

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Localidad.— Colombia, Departamento de Caquetá, municipio de Belén de los Andaquíes, vereda Agua Dulce, Finca las brisas 01° 20’ 11.23” N; 75° 49’ 43.62” W. 26/07/2012, Diego Huseth Ruiz-Valderrama. Los individuos fueron depositados en la colección de herpetología del Museo de Historia Natural de la Universidad de la Amazonia (UAM, Tabla 1, Fig. 1). Colombia, Departamento de Putumayo, municipio de Orito, Vereda Isla de Cartagena 0°26’42.44” N; 77° 5’10.31” W. 26/06/2019, Alejandra Salazar y Vladimir Bernal. Los individuos fueron depositados en la colección del Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt (IAvH, Tabla 1, Fig. 1).

Comentarios.— *Dendropsophus rhodopeplus* es una rana perteneciente a la familia Hylidae, descrita por Günther, 1858, registrándose desde el sur de Colombia a través de Ecuador, Perú, Brasil y el norte de Bolivia a una altura entre 300 a 1700 m.s.n.m comprendiendo zonas de piedemonte y tierras bajas amazónicas (Duellman, 1972, Frost, 2021). Para Colombia se ha reportado en la región amazónica en los departamentos de Amazonas y Caquetá (Ruiz *et al.*, 1996; Lynch, 2005; Medina-Rangel *et al.*, 2019). El objetivo de esta nota es ampliar la distribución conocida de esta especie de rana, registrando dos nuevas localidades sobre el piedemonte andino amazónico en Colombia.

Tabla 1. Registros de distribución para *Dendropsophus rhodopeplus* en la región amazónica de Colombia (UAM-H: Universidad de la Amazonia – Colección Herpetológica; IAvH-Am: Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt – Colección de Anfibios).

Código UAM	Ubicación	Fecha Colecta	Latitud	Longitud	Altura (m s.n.m)	Referencia
UAM-H 1478	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio
UAM-H 1479	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio
UAM-H 1480	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio
UAM-H 1481	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio
UAM-H 1482	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio
UAM-H 1483	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio
UAM-H 1484	Finca Las Brisas, Vereda Agua dulce, Municipio Belén de los Andaquíes, Departamento Caquetá, País Colombia	2016	1.3365	-75.8288	277	Este estudio

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UAM-H-1534	Vereda El Guamo, Municipio Cartagena del Chaira, Departamento de Caquetá, País Colombia	2018	0.2524	-74.3054	180	Medina-Rangel et al., (2019)
UAM-H-1586	Laguna La Culebra Vereda Peñas Rojas, Municipio Solano, Departamento Caquetá, País Colombia	2018	-0.0787	-74.2624	165	Medina-Rangel et al., (2019)
UAM-H-1626	Resguardo Indígena Bajo Aguas Negras, Municipio Solano, Departamento Caquetá, País Colombia	2018	0.0000	-74.6449	170	Medina-Rangel et al., (2019)
UAM-H-1641	Resguardo Indígena Bajo Aguas Negras, Municipio Solano, Departamento Caquetá, País Colombia	2018	0.0000	-74.6449	170	Medina-Rangel et al., (2019)
IAvH-Am-15962	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15963	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15964	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15965	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15966	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15967	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15968	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15969	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15982	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-15995	Vereda Islas de Cartagena, Municipio Orito, Departamento Putumayo, País Colombia	2019	0.4451	-77.0862	503	SiB-Colombia, (2021)
IAvH-Am-4521	Km11, Municipio Leticia, Departamento Amazonas, País Colombia	1987	-4.1261	-69.9446	83	Lynch, (2005)
AvH-Am-4522	Km11, Municipio Leticia, Departamento Amazonas, País Colombia	1987	-4.1261	-69.9446	83	Lynch, (2005)
IAvH-Am-4520	Km11, Municipio Leticia, Departamento Amazonas, País Colombia	1987	-4.1261	-69.9446	83	Lynch, (2005)

Se registran once individuos de *D. rhodopeplus* para el departamento del Caquetá, tomando en cuenta que cuatro de ellos son registrados por Medina-Rangel *et al.*, (2019), en tierras bajas de la Amazonia en los municipios de Solano y Cartagena del Chairá; los siete restantes fueron colectados el 26 de Julio de 2012 por Diego Huseth Ruiz Valderrama, en el municipio de Belén de los Andaquíes hacia el noroccidente del departamento del Caquetá sobre piedemonte andino amazónico (Estrella amarilla – Fig. 1), a una distancia lineal entre 198 a 235 km con respecto a registros previos en el departamento .

Para el departamento de Putumayo se reportan diez individuos colectados el 26 de junio de 2019 por Alejandra Salazar y Vladimir Bernal en el municipio de Orito sobre el piedemonte andino amazónico (Estrella roja – Fig. 1) a una distancia lineal con respecto a el nuevo registro en el departamento del Caquetá de 171 km (Estrella amarilla), y con los anteriores registros una distancia lineal entre 276 km 941 km (Triángulos blancos-Fig. 1). Tomando en cuenta lo anterior se reporta un nuevo registro de distribución

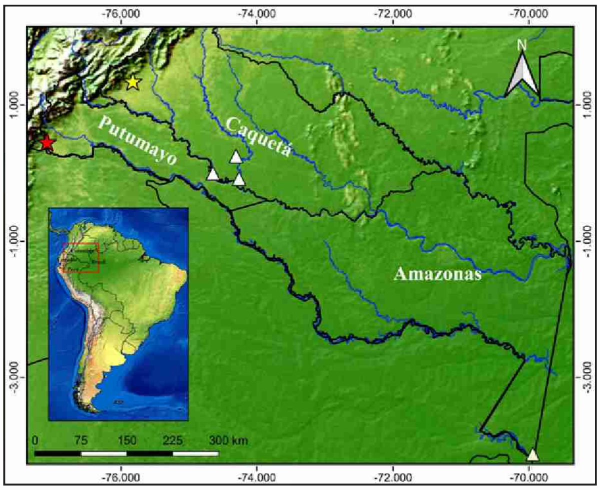

Figura 1. Mapa de distribución de *Dendropsophus rhodopeplus* en la región amazónica y andino amazónica, Colombia (Estrella amarilla y Estrella roja – Nuevos registros. Triángulos blancos – Antiguos registros Lynch, (2005) – Medina-Rangel et al., (2019)).

para el departamento del Caquetá y primer registro para el departamento de Putumayo (Acosta, 2021).

Las características taxonómicas que permiten su identificación según Read y Ron, (2018) en vida: (**1**) Dorso amarillo y liso; (**2**) Vientre amarillo claro y granular; (**3**) Banda dorsolateral café desde el hocico a la ingle; (**4**) Iris gris con banda roja rodeando la pupila (Fig. 2); (**5**) Membrana axilar ausente; (**6**) Las membranas manuales se extienden en los dígitos II, III y IV hasta la tercera parte de la longitud del digito; (**7**) Las membranas pedales se extiende hasta la mitad de la longitud del dígito; (**8**) en estado de preservación el dorso es bronce crema con manchas pequeñas de color marrón; (**9**) Antebrazos, brazos y muslos color crema (Fig. 3A-B).

Es una especie de hábitos nocturnos que suelen encontrarse en bosques primarios, secundarios o incluso en áreas abiertas cerca de cuerpos de aguas permanentes o temporales para su proceso de reproducción (Duellman, 1978; Rodríguez y Duellman, 1994). Estas características de hábitat fueron diferentes en los individuos registrados en este estudio, ya que se encontraban asociados a una zona de bosque intervenido con influencia de un sistema agroforestal de caucho (*Theobroma grandiflorum*) y presencia de cuerpos de agua temporales, indicando la tolerancia de esta especie frente a los cambios antrópicos que se generen.

Figura 2. Vista lateral de ejemplar vivo de *Dendropsophus rhodopeplus*. Colombia, Caquetá, Solano. Foto: D.H. Ruiz-Valderrama, 2018.

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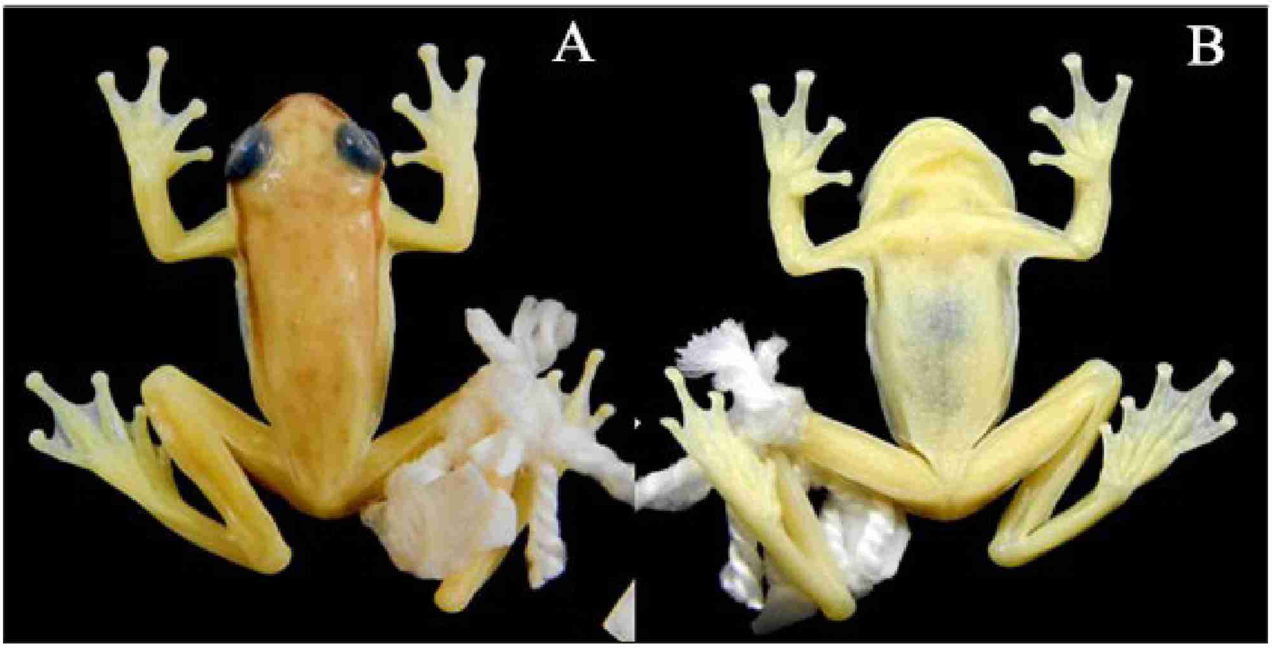

Figura 3. Vista dorsal (A) y ventral (B) de *Dendropsophus rhodopeplus*. LRC 18.9 mm (UAM-H-1478 – DHR007) colectado en Belén de los Andaquíes, departamento de Caquetá, Colombia.

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