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IMPACTOS DE PARQUES EÓLICOS EM COMUNIDADES DE ANUROS NA CAATINGA, NORDESTE DO BRASIL

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Dissertação apresentada ao Programa de Pós-graduação em Ecologia – PPGE da Universidade Federal Rural de Pernambuco – UFRPE, como pré-requisito parcial para obtenção do título de Mestre em Ecologia.

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EPÍGRAFE

Eu vou te levar
Onde canta o sabiá
Onde a lua nos espia
Com olhar de menina
Com cheiro do mato
O vento vindo das colinas
Nossa cama é a grama
Pra fazer amor menina

Sou caboclo do sertão
Só tenho amor no coração
Pra oferecer
A natureza é minha casa,
Vida viver
Tudo pra eu e ocê

Lá tem um riacho para a gente se banhar
Pega peixe, nadar junto
Até vadiar
Quando for de noite, nós
Acende, nosso amor
Faz fogueira, não tem frio,
Pois sou seu cobertor

Sou caboclo do sertão
Só tenho amor no coração…

ONDE CANTA O SABIÁ
(Composição de Rita de Cássia).

DEDICATÓRIA

Dedico esse trabalho a todos que fazem do mundo um lugar melhor para se viver...

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Aprovada em 27 de fevereiro de 2020

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Sumário

Resumo: Ativades humanas são reconhecidamente responsáveis pelo declínio da biodiversidade. Recentemente, a implantação de parques eólicos têm contribuído com o agravamento de problemas ambientais e pouco se sabe dos efeitos da poluição sonora gerada por turbinas eólicas na Caatinga. Portanto, a presente dissertação visou analisar os impactos do ruído de parques eólicos no comportamento acústico de uma comunidade de anfíbios anuros dentro do bioma Caatinga, Nordeste do Brasil. A pesquisa deu origem a dois manuscritos. No primeiro, intitulado "Impactos de parques eólicos na diversidade de anuros em floresta tropical sazonalmente seca, Nordeste do Brasil", foram avaliados os efeitos do ruído de parques eólicos, da pluviosidade e do tempo nos padrões de diversidade de anuros. Nossos resultados revelaram que o ruído não apresentou relação negativa significativa na estrutura das comunidades, por outro lado, a pluviosidade ao longo do tempo influenciaram fortemente os padrões de abundância e riqueza e diversidade de anuros. E no segundo, intitulado "Efeitos do ruído de parques eólicos no comportamento de canto de anuros na Caatinga, Nordeste do Brasil", foram analisados os efeitos do ruído dos parques eólicos no comportamento de canto de três espécies de anuros. Nossos resultados mostraram que o ruído dos parques eólicos afetou o comportamento de canto das três espécies e amplitude de frequência, frequência dominante e amplitude do canto decresceram significativamente com aumento do ruído. Esse trabalho é pioneiro ao analisar os impactos do ruído de turbinas eólicas sobre comunidades de anuros no Brasil. Estudos futuros com anuros e outros animais dependentes de comunicação acústica ajudarão na compreensão dos impactos da energia eólica na Caatinga e outros ambientes terrestres. Assim, o conhecimento dos efeitos do ruído de parques eólicos pode ser uma importante ferramenta para estudos ecológicos, comportamentais e ações conservacionistas.

Palavras-chave: Canto de anúncio; Parques eólicos; Ruído antropogênico; Sapos.

Abstract: Human activities are known to be responsible for the decline of biodiversity. Recently, the implementation of wind farms has contributed to the worsening of environmental problems and little is known about the effects of noise pollution generated by wind turbines in the Caatinga. Therefore, this dissertation aims to analyze the impact of noise from wind farms in anuran communities in Caatinga, Northeast Brazil. A search gave rise to two manuscripts. In the first, entitled “Impacts of wind farms on the diversity of anurans in a seasonally dry tropical forest, Northeastern Brazil”, the effects of noise from wind farms, rainfall and weather on anuran diversity patterns were evaluated. Our results revealed that the noise did not present a significant negative relationship in the structure of the communities, on the other hand, the rainfall over time strongly influenced the patterns of abundance and richness and diversity of frogs. And in the second, entitled “Effects of noise from wind farms on the behavior of anuran calling in the Caatinga, Northeastern Brazil”, the effects of noise from wind farms on the calling behavior of three species of anurans were analyzed. Our results showed that the noise from wind farms affected the calling behavior of the three species and the frequency amplitude, dominant frequency and call amplitude decreased significantly with increased noise. This work is a pioneer in analyzing the impacts of wind turbine noise on anuran communities in Brazil. Future studies with anurans and other animals dependent on acoustic communication will help in understanding the impacts of wind energy in the Caatinga and other terrestrial environments. Thus, knowledge of the effects of noise from wind farms can be an important tool for ecological, behavioral studies and conservationist actions.

Keywords: Advertisement call; Wind farms; Anthropogenic noise; Frogs.

1. INTRODUÇÃO GERAL

1.1 Diversidade de anuros da Caatinga

A Caatinga é uma floresta sazonalmente seca exclusiva do Brasil, que durante muito tempo teve sua fauna considerada subconjunto dos biomas Cerrado e Mata Atlântica, pobre em espécies e endemismos (GARDA *et al.*, 2018), com aproximadamente 833.000 km² é a principal cobertura vegetal do nordeste brasileiro (FERNANDES *et al.*, 2019). Em Pernambuco, esse bioma estende-se por cerca de 83% do estado (HUECK, 1972), e aproximadamente 11% do território nacional, segundo dados do Ministério de Meio Ambiente (MMA, 2020). A Caatinga apresenta clima semiárido do tipo Aw (ALVARES *et al.*, 2013), e regido por condições adversas como elevadas temperaturas, altos índices de radiação solar e evapotranspiração, estação chuvosa com precipitações esparsas no espaço e no tempo (AB` SABER, 1977; PRADO, 2003). Essas condições impõem adaptações comportamentais, reprodutivas e fisiológicas aos animais, sobretudo para anuros, que dependem da água para reprodução e sobrevivência (WELLS, 1977; VIEIRA *et al.*, 2009; SCHALK *et al.*, 2015).

Com precipitações irregularidade e altas temperaturas ao longo do ano, a riqueza de anuros da Caatinga é surpreendente (GARDA *et al.*, 2017). Embora a quantidade de estudos tenha aumentado, pesquisas com anuros da Caatinga têm focado basicamente na distribuição de espécies (CAMARDELLI; NAPOLE, 2012; GARDA *et al.*, 2017), e ecologia de populações e comunidades (ARZABE *et al.*, 1998; VIEIRA *et al.*, 2007; XAVIER; NAPOLI, 2011; FERREIRA-SILVA *et al.*, 2016). Porém, ainda pouco se conhece da ecologia acústica ou comportamental e informações ecológicas sobre anuros desse bioma ainda são incipientes (GARDA *et al.*, 2017).

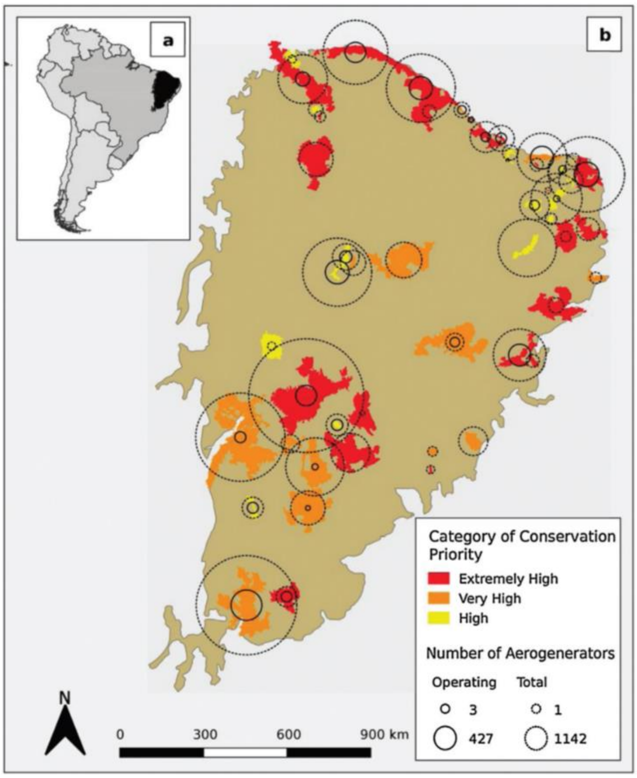
1.2 Energia eólica na Caatinga

Historicamente, impactos ambientais como desflorestamentos, queimadas e desertificação têm modificado as paisagens naturais da Caatinga, e recentemente, a implantação de parques eólicos têm contribuído com o agravamento desses problemas (BERNARD *et al.*, 2014; NERI *et al.*, 2019). Considerada uma matriz de energia competitiva, renovável e confiável (DAI *et al.*, 2015; ARAÚJO; MOURA, 2017;

IQBAL *et al*., 2019), a energia eólica é uma tecnologia apontada como uma das opções globais para redução das emissões de gases do efeito estufa, permitindo os países avançar em direção a uma economia de baixo carbono (GASPARATOS *et al*., 2017).

Com uma capacidade instalada de 15.4 GW, o Brasil é o oitavo colocado no *ranking* mundial de geração de energia eólica, segundo dados da Associação Brasileira de Energia Eólica (ABEEólica, 2018), e embora a Caatinga represente apenas 11% do território brasileiro, representa a área com maior potencial para geração de energia eólica do país. Mas as áreas destinadas para implantação de parques eólicos nesse bioma se sobrepoem às áreas prioritárias para conservação de sua biodiversidade (Figura 1) (NERI *et al*., 2019). Essa situação é bastante preocupante, pois das 6313 turbinas eólicas em funcionamento no Brasil em 2018, 4.925 delas (78%), se encontravam na Caatinga, além disso, é prevista a instalação de mais 14.696, das quais (84.6%), serão instaladas nos domínios da Caatinga, representando um incremento de 253% (NERI *et al*., 2019).

Figura 1 - Mapa da América do Sul (a) destacando a Caaatinga (b) mostrando as áreas prioritárias para conservação (CPA) sem proteção legal agrupados por nível de prioridade (as áreas são coloridas em amarelo, laranja e vermelho). O tamanho dos círculos contínuos representam o total de aerogeradores em operação, enquanto os císculos pontilhados representam as projeções para instalção de aoregeradores. Fonte: (NERI *et al*., 2019).



1.3 Impactos de parques eólicos sobre a diversidade

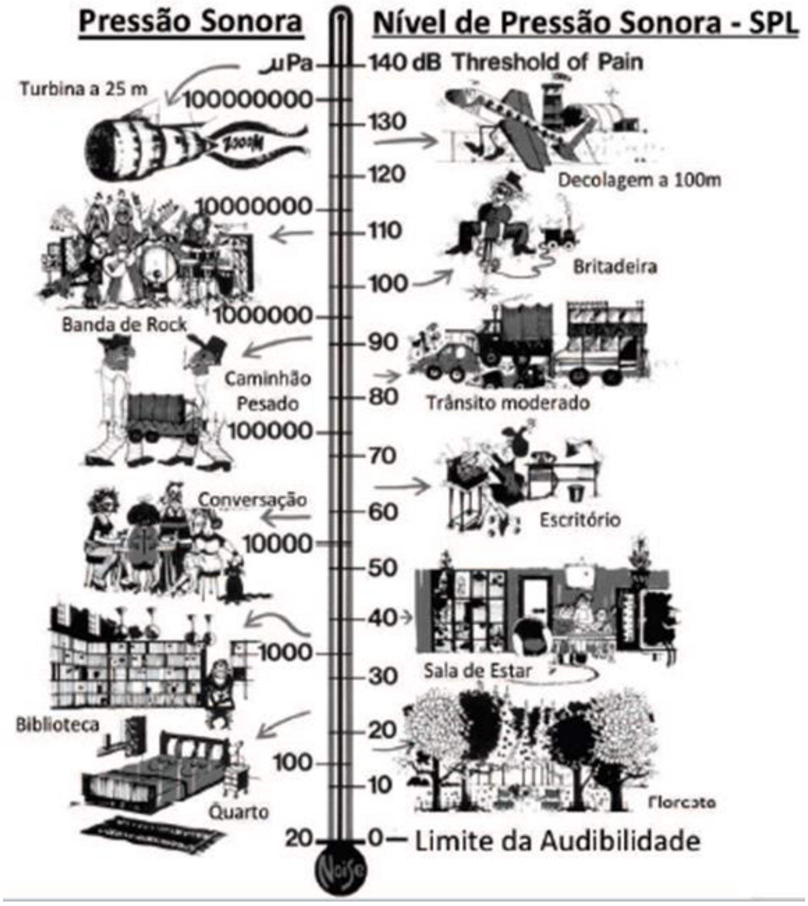
Mesmo havendo pontos positivos, a implantação de parques eólicos também causa impactos ambientais (IQBAL *et al*., 2019; NERI *et al*., 2019). Dentre esses impactos ambientais estão a supressão da vegetação nativa, impermeabilização do solo, redução da disponibilidade de habitats, abertura de novas estradas e linhas de transmissão (DAI *et al*., 2015; ARAÚJO; MOURA, 2017). Embora os impactos ambientais da instalação de parques eólicos sejam relativamente bem conhecidos, as implicações da poluição sonora gerada pelo seu funcionamento ainda são pouco investigadas e requerem mais atenção (DAI *et al*., 2015; JONES *et al*., 2015).

O ruído gerado pelos parques eólicos é resultado do funcionamento das turbinas eólicas e pode ser classificado em três tipos. O ruído mecânico é gerado a partir do funcionamneto das engrenagens internas, o ruído estrutural é produzido pela vibração da própria estrutura e o ruído aerodinâmico é resultado do contato das hélices com o ar (MAIA, 2010; LIMA, 2015). Devido os riscos do ruído de parques eólicos para a saúde humana, muito países adotaram rigorosas normas técnicas, como a 61400-11/2002, da Comissão Eletrotécnica Internacional – IEC e a NBR 10151-10/2000, da Associação Brasileira de Normas Técnicas – ABNT (LIMA, 2015). De acordo com a NBR 10151-10, no Brasil, os níveis de ruído em decibéis (dB) permitidos em sítios e fazendas são de 40 dB durante o dia, e 35 dB durante a noite. No entanto, os níveis de ruído junto a parques eólicos podem ser muito superiores ao permitido (LIMA, 2015).

1.4 Conceitos de acústica

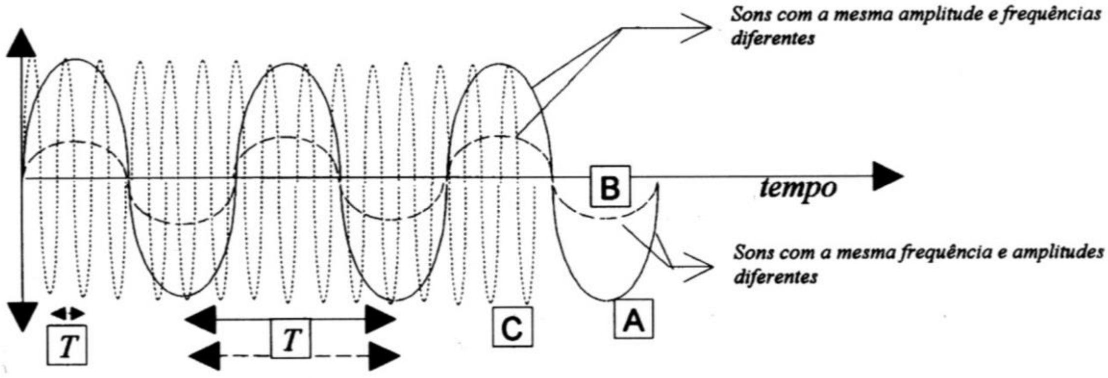
Os ruídos são vibrações sonoras presentes no ambiente (KINSLER, 2000), caso o resultado dessa vibração sonora (processada no cérebro) for agradável ou apresentar algum significado, será classificado como som, caso não haja significado ou não seja agradável, será classificado como ruído (MAIA, 2010). O decibel (dB) é a unidade que expressa a medida do nível de pressão sonora, no entanto, ele não é uma unidade de medida absoluta, mas a razão entre a quantidade medida em determinada área e um valor de referência (MAIA, 2010; LIMA, 2015) (Figura 2). A amplitude de um som equivale ao nível de pressão sonora SPL (do inglês *Sound Pressure Level*), que é uma propriedade do som em uma dada localizacão e é dependente de vários fatores como características da fonte, condições atmosféricas e absorcão do som, e pode ser mensurada diretamente por meio de um microfone (KINSLER, 2000).

Figura 2 – Níveis de pressão sonora (SPL) expressos em decibéis (dB) (SYSTEMS, 2015).



Além disso, todo som apresenta uma faixa de frequência e amplitude que lhes são características (KINSLER, 2000). A frequência representa a taxa de ocorrência das flutuações completas de pressão por segundo e sua unidade de medida é o Hertz (Hz) (LIMA, 2015). A frequência pode ser grave, média ou aguda. A faixa de frequência audível ao ouvido humano encontra-se entre 20 Hz (limiar da audição) e 20 kHz (limiar da dor), assim, sons de frequências inferiores a 20 Hz são chamados infrassons e sons com frequência superior a 20 kHz são denominados ultrassons (KINSLER, 2000). Na natureza, geralmente os sons são emitidos em diferentes frequências e raramente são puros (PEDERSEM; WAYE, 2002) (Figura 3).

Figura 3 – A e B são sons com mesmas frequências, mas amplitudes diferentes; A e C são sons com a mesma amplitude mas frequências diferentes; B e C são sons com amplitudes e frequências diferentes. Fonte: (CARVALHO, 2009).



1.5 Comunicação acústica

A comunicação acústica em vertebrados tem evoluído por cerca de 150 milhões de anos e é amplamente utilizada na escolha de parceiros, sendo crucial para seleção sexual, reconhecimento de espécies e no processo de especiação (WILKINS *et al.*, 2013; CHEN; WIENS, 2020). A comunicação animal ocorre em ambientes que afetam as propriedades do sinal acústico de modo que a estrutura dos sons que chegam aos receptores são versões alteradas dos padrões originais produzidos pelos emissores (RÖHR; JUNCÁ, 2013; VELÁSQUEZ *et al.*, 2018).

A hipótese da adaptação acústica defende que para superar obstáculos ambientais, sinais acústicos devem possuir características ideais para sua transmissão no ambiente (MORTON, 1975). Assim, animais que se comunicam à longas distâncias têm seus sinais acústicos menos atenuados e degradados, embora a vegetação também exerça influência (EY; FISCHER, 2009). Além disso, espécies com ampla distribuição também podem apresentar cantos com características distintas, nesses casos, indivíduos nativos de um dado local têm seus cantos melhor transmitidos e menos degradados que cantos de indivíduos estrangeiros (VELÁSQUEZ *et al.*, 2018).

Adicionalmente, as características do canto podem variar de acordo com a estrutura do habitat onde o canto é emitido, por exemplo na água ou empoleirado em abustos (RÖHR; JUNCÁ, 2013; CAMURUGI *et al.*, 2015). Outros importantes atributos que influenciam os parâmetros do canto são a massa corporal e a temperatura, que interferem na emissão e transmissão do sinal acústico (CAMURUGI *et al.*, 2015; ZIEGLER *et al.*, 2015). Outro ponto chave é a introdução de espécies exóticas que podem competir com espécies nativas por faixas de frequência (MEDEIROS *et al.*, 2016; TENNESSEN *et al.*, 2016), e o aumento do ruído antropogênico em áreas naturais que reconhecidamente, alteraram o nicho acústico desses animais (SHANNON *et al*., 2016). O nicho acústico de uma espécie compreende o microhabitat utilizado para o canto, a duração do canto e a estrutura acústica do canto de anúncio (SINSCH *et al*., 2012). Portanto, os ruídos antropogênicos podem dificultar a comunicação das espécies nativas através de um fenômeno conhecido por mascaramento acústico (BRUMM; SLABBEKOORN, 2005), o qual pode se sobrepor às faixas de frequências utilizadas pelas espécies dependentes da vocalização (GERHARDT; RUBER, 2002).

A vocalização é uma característica conspícua em anuros e constitui sua forma predominante de comunicação (WELLS; SCHWARTZ, 2006). Portanto, a análise de seu repertório vocal pode fornecer informações valiosas para compreensão de seu comportamento (SCHWARTZ; BEE, 2013; HÖBEL, 2014). O canto de anúncio é mais estudado e possui como principais funções a atração de fêmeas para a reprodução e a manutenção de territórios entre os machos, atuando na organização social da população (WELLS, 1977; WELLS; SCHWARTZ, 2006). No entanto, apesar da importância da comunicação acústica entre anuros, ainda não é suficientemente conhecida a eficiência desta atividade em cenários acústicos adversos (BEE, SWANSON, 2007), como acontece na presença do ruído antropogênico (HALFWERK *et al*., 2019). Assim, os incrementos de ruído em ambientes naturais têm chamado atenção pelos seus efeitos no comportamento de diversos grupos animais (SHANNON *et al*., 2016).

1.6 Efeitos do ruído antropogênico sobre a biodiversidade

O ruído antropogênico tem sido apontado como importante fator na crise da biodiversidade global (BRUMM; SLABBEKOORN, 2005; SHANNON *et al*., 2016). Dada sua importância de sinais acústicos em tetrápodas como anfíbios, répteis, aves e mamíferos (CHEN; WIENS, 2020), estudos têm investigado os efeitos do ruído antropogênico na comunicação acústica (GEHRARDT; RUBER, 2002; BRUMM; SLABBEKOORN 2005; HALFWERK *et al*., 2019). Esses ruídos são emitidos por diversas atividades humanas como operação de máquinas (FRANCIS *et al*., 2009), trânsito de carros e motocicletas (PARRIS *et al*., 2009; CAORSI *et al*., 2017), pousos e decolagens de aviões (SUN; NARINS 2005; CRUGER; DU PREEZ 2016).

O ruído antropogêncio pode alterar a emissão e recepção de sinais acústicos em diversos grupos animais (BRUMM; SLABBEKOORN, 2005; HALFWERK *et al*., 2019), como em insetos (RÖMER, 2013), peixes (LADICH, 2013), anfíbios (SUN;

NARINS, 2005; SCHWARTZ; BEE, 2013; CAORSI *et al*., 2017), aves (FRANCIS *et al*., 2009), répteis (BRUMM; ZOLLINGER, 2017) e mamíferos (SLABBEKOORN *et al*., 2018). No caso dos anuros, estudos experimentais mostraram que o ruído antropogênico pode interferir em nível populacional no comportamento de migração e reprodução (TENNESSEN *et al*., 2014), no fitness reprodutivo (SUN; NARINS, 2005), na dinâmica populacional (KAISER; HAMMERS, 2009) e na redução da imunidade (TROÏANOWSKI *et al*., 2017).

Portanto, devido a lacunas no conhecimento esse projeto avaliou a influência do ruído de parques eólicos sobre comunidades de anuros em área de Caatinga do nordeste do Brasil. Para tanto, buscou-se responder as seguintes perguntas: O ruído de parques eólicos é capaz de infuenciar padrões de abundância e riqueza de espécies em anuros? Com relação ao canto de anúncio, que *displays* comportamentais foram produzidos por machos de três espécies em resposta ao ruído dos parques eólicos?

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Impacts of wind farms on anuran diversity in a seasonally dry tropical forest, Northeastern Brazil

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Abstract: Human activities are known to be responsible for the decline in biodiversity. Through a field experiment, we evaluated the influence of wind turbine noise on an anuran community in the Caatinga area of northeastern Brazil. We selected eighteen temporary ponds along two wind farms and recorded the environmental noise in each of them, establishing three classes of noise in each wind farm. Finally, we carry out monthly searches for frogs alternately in the puddles in each wind farm for two consecutive nights. Altogether, we counted 4216 individuals of twenty anuran species. Our results indicate that the noise from wind farms did not have a significant effect on the structure of frog communities. On the other hand, rainfall and weather over the months strongly influenced species abundance, richness and diversity, as expected for the Caatinga, a biome in semi-arid regions where the availability of water during the short rainy season strongly influences the reproductive patterns of anurans. Although our data analysis has not shown a correlation between the noise levels of wind farms and the structure of anuran communities, some studies have shown that anthropogenic noise alone can alter the behavior, physiology and abundance in several animal species. Future work should isolate noise from other variables to more accurately assess the real impacts of wind farms on communities of frogs and other animals in the face of the expansion of the wind sector in Brazil.

Keywords: Caatinga; Diversity; Anthropogenic noise; Frogs; Wind turbines

1. Introduction

The Caatinga is part of the seasonally dry tropical forest biome, occurring only in Brazil (Prado, 2003), and for a long time its fauna was mistakenly considered as a subset of the Cerrado and Atlantic Forest biomes, poor in species and endemism (Garda et al ., 2018). The Caatinga has a semi-arid climate, which imposes adverse conditions such as high temperatures, high levels of solar radiation and evapotranspiration, a rainy season with sparse precipitation, with long periods of drought (Ab 'Saber, 1977; Prado, 2003).

These conditions impose behavioral, reproductive and physiological adaptations on organisms, especially for anurans, which depend on water for reproduction and survival (Schalk et al., 2015; Wells, 1977). Therefore, the availability of water in temporary ponds is crucial for the reproduction of anurans in the Caatinga (Borges-Nojosa and Santos, 2005; Leite Filho et al., 2015; Vieira et al., 2007), and as in other semi-arid ecosystems, rainfall is an important factor in all aspects of these animals lives (Bentley, 1966; Vieira et al., 2009; Wells, 1977). And because the rainy season in this biome is concentrated in a short period, rainfall and weather become important predictors of patterns of abundance, richness and diversity of frogs (Arzabe, 1999; Arzabe et al., 1998; Vieira et al., 2009, 2007).

Historically, environmental impacts such as deforestation, burning and desertification have modified the natural landscapes of the Caatinga (Fonseca et al., 2017; Prado, 2003), and recently, the implementation of wind farms has contributed to the worsening of these problems (Bernard et al., 2014; Neri et al., 2019). Considered a competitive, renewable and reliable energy matrix (Araújo and De Moura, 2017; Dai et al., 2015), wind energy is a technology identified as one of the global options for reducing greenhouse gas emissions, allowing countries move towards a low carbon economy (Gasparatos et al., 2017).

However, like any large enterprise, the implementation of wind farms causes environmental impacts (Neri et al., 2019). Among these impacts are the suppression of native vegetation, waterproofing of the soil, reduced availability of habitats, opening of new roads and transmission lines (Araújo and De Moura, 2017; Dai et al., 2015). Although the environmental impacts of installing wind farms are known, the implications of noise pollution generated by their operation are still poorly investigated and require more attention (Dai et al., 2015; Jones et al., 2015). Although the number of surveys has grown with the expansion of the wind sector, studies on the impact of wind farms have basically focused on populations of birds and bats (Bernard et al., 2014; Thaxter et al., 2017).

Anthropogenic noise is associated with human activities that cause disturbances and some approaches have been developed to isolate noise from other variables, including studies that assess areas with noise and quiet areas (eg Francis et al., 2012, 2009; McClure et al. , 2013; Rabin et al., 2006) When isolated from other variables, studies suggested that noise can directly alter the behavior in several animals (DeRuiter et al., 2013; Karp and Root, 2009). Studies with birds indicate that noises ≥45 dB can alter calling, reduce abundance and richness and increase levels of stress; with noises between 52 and 68 dB terrestrial mammals increase stress levels and have reproductive decreases; noises > 60 dB interfere in the calling of frogs; and noise> 80 dB, affects the foraging of bats (Shannon et al., 2015).

Although there are no studies on the impact of noise from wind farms on anuran communities, previous studies have shown that anthropogenic noise can cause deterrence and interfere with migration and reproduction behavior (Tennessen et al., 2014), in reproductive fitness (Sun and Narins, 2005), population dynamics (Kaiser and Hammers, 2009), color change and reduced immunity due to increased levels of corticosterone (Troïanowski et al., 2017). Even though there is no consensus, it is usually assumed that noise above 60 dB would be potentially impacting for anurans (see discussion in Shannon et al., 2015).

Thus, this study analyzed the influence of noise from wind farms on the patterns of abundance and richness of frogs in temporary pools in the Caatinga. Due to the ability of anthropogenic noise to alter behavioral and physiological aspects of anurans (eg Sun and Narins, 2005; Tennessen et al., 2014; Troïanowski et al., 2017), we tested the hypothesis that noisy temporary ponds would have less abundance, species richness and diversity.

2. Material and Methods

2.1. Study area

The study took place in two horizontal onshore wind farms located in the municipalities of Caetés and Paranatama, Pernambuco State, Brazil (8° 48'21.29 "S 36° 44'17.10" W, 883 m. A. L.) (Figure 1). The local climate is tropical semi-arid Aw (Alvares et al., 2013), with an average temperature of 20.5 ºC and an average annual rainfall of 755 mm, presenting the rainy season from April to August, although sporadic rainfall may occur throughout the year (Climate Date, 2020). The vegetation is typical of Caatinga, characterized by shrub, tree-shrub formations and man-modified landscapes (Oliveira et al., 2017).

Wind farm 1 has fifteen wind turbines with a power of 13,600 KW and an area of approximately 726 hectares. Wind farm 2 has eighteen turbines with a power of

28,900 KW and an area of approximately 567 hectares. In both wind farms, the wind turbines are of the GE Energy 1.7-100 model, with a power of 1,700 KW with winds at 10m/s, these turbines, 96 meters high, are composed of three propellers, each 52 meters in length (https://www.thewindpower.net/turbine_es_593_ge-energy_1.7-100.php).

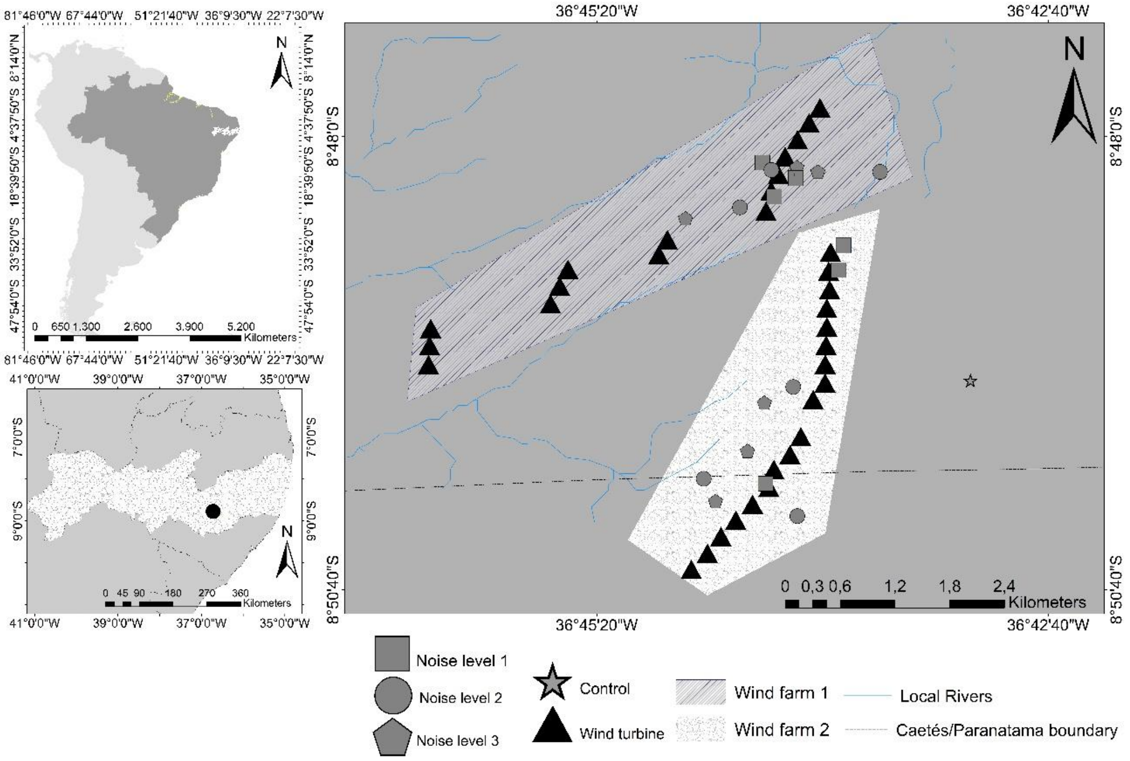

Figure 1. Study area with location of wind farms, and arrangement of temporary ponds in the municipalities of Caetés and Paranatama, Pernambuco State, Brazil. The control is represented by a star, the wind turbines are represented by triangles, the Class of noise (1) is represented by squares, the Class of noise (2) is represented by circles and the Class of noise (3) is represented by pentagons.

2.2. Noise characterization

In each wind farm, nine temporary ponds were selected to record environmental noise. To avoid bias in the results, the selected ponds had similar environmental characteristics such as an average diameter of 42m² (range 22 - 65), type and density of vegetation. Several factors contribute to the propagation of noise such as distance from the source, wind direction and absorption by environmental structures (e.g. soil, rocks and plants) and due to the noise of wind turbines being emitted at low frequencies, a reduction of 20 dB is expected each kilometer away from the source of the noise (Alberts, 2005).

Based on this information, a pond was defined as a control with environmental characteristics similar to the others and two kilometers distant upstream of the wind direction (Figure 1). To measure the noise intensity (dB) of the wind farms in the nineteen temporary ponds, we used a decibel meter (Instrutemp ITDEC 4000, precision of 0.1dB). Then, the noise was recorded with a YOGA HT81 unidirectional microphone coupled to a Tascam DR40 digital recorder, configured at 44.1 kHz and 16-bit resolution. In order to attenuate the interference of the geophony and the zoophony during the recordings, the microphone was positioned manually towards the wind turbines forming an angle of 45º in relation to the ground.

To establish a noise gradient in each wind farm, the variation in noise recorded in temporary puddles was calculated from the formula (Δ = Rf - Ri where, Rf represents the maximum noise value and Ri represents the minimum noise value in each wind farm). Then, the value of Δ was divided into three equal parts and each of these resulting parts was assigned to a noise class (Table 1).

Wind Farmer1			Wind Farmer2		
Noise classes (dB)	Range (dB)	Average (dB)	Noise classes (dB)	Range (dB)	Average (dB)
1	117.5 - 128.3	122.9	1	114.6 - 127.4	121
2	106.8 - 117.5	112.1	2	101.8 - 114.6	108.2

3	96 - 106.8	101.4	3	89 - 101.8	95.4

Table 1. Classes of environmental noise from temporary ponds distributed in the two wind farms in the municipalities of Caetés and Paranatama, Pernambuco State, Brazil. For both wind farms, each class is represented by three temporary pools and their values are independent (not standardized). The table shows the variation and the average noise per class expressed in decibels (dB).

2.3. Collection of data on abundance, richness and diversity

For data collection, active searches were carried out monthly from October 2018 to September 2019 in the nineteen temporary pools, recording the count of individuals of each species of frog per puddle. The searches were carried out by a same researcher and took place alternately in each wind farm for two consecutive nights, from 18:00 to 22:00 hours, totaling 96 hours of sampling effort. As testimony material, an individual of each species was collected and euthanized through topical application of 2% lidocaine pomade. After fixing, the specimens were deposited in the Herpetological and Paleoherpetological Collection of the Federal Rural University of Pernambuco, Recife, Brazil.

Because rainfall exerts a strong influence on all life stages of anurans, especially in semi-arid environments (Schalk et al., 2015; Vieira et al., 2009), rainfall over the twelve months was recorded to assess its relationship with patterns of abundance, wealth and diversity of frogs. These pluviometric data were accessed on the website of the Instituto Agronômico de Pernambuco, which provides daily rainfall data from the headquarters of the municipality of Caetés, 15 km from the study area (http://www.ipa.br/indice_pluv.php#calendario_indices).

2.4. Data analysis

The analysis of the diversity of the anuran community was based on the Hill numbers of orders q = 0, 1 and 2 which are associated, respectively, with species richness, Shannon diversity and Simpson diversity (Gotelli and Chao, 2013). The interpolation-extrapolation method based on the sample coverage proposed by Chao and Jost (2012) was used to obtain estimates of Hill numbers comparable between sample situations, adopting a sample coverage of 83.37%. Hill's number estimates were obtained only for sample situations that included at least five individuals and two species, following the guidance of Chao and Jost (2012).

The Hill number estimates of orders q = 0, 1 and 2 were interpreted in terms of the effective number of species (Gotelli and Chao, 2013) and were modeled using the Multivariate Regression Model with normally distributed additive errors (Díaz-García et al., 2003). The model's design matrix included the independent variables (1) Noise Level (dB), (2) Time (in months) and (3) Precipitation (mm). The variables Time and Precipitation were included in the model using cubic splines with degrees of freedom (df) respectively equal to df = 3 and df = 4.

To quantify the significance of including the independent Noise Level variable in the model, the Likelihood Ratio Test (LRT) was used for generalized hypotheses presented in Seber (2004). The asymptotic distribution of the test statistic and also its exact distribution for some particular cases can be found in Anderson (2003). The Wald test statistic, usually presented in results from regression models with univariate response, was also used to test the significance of the regression coefficient associated with the Noise Level for each Hill number in isolation. Similarly, the LRT and Wald tests were performed for the other independent variables.

The quality of fit of the Multivariate Regression Models was accessed based on the statistical theory described in Díaz-García et al. (2003) and in Russo et al. (2009), including graphs of studentized residues against the index of observations and values predicted by the model and also a graph of Mahalanobis distances.

All statistical analyzes were performed using software R version 3.6.1 (R Core Team, 2019) based on the computational implementation of the methods proposed in (Díaz-García et al., 2003) and Seber (2003), in addition to the features available in statistical packages iNEXT (Hsieh et al., 2016) and ggplot2 (Wickham, 2009).

3. Results

Basic information about the characteristics of the data obtained throughout the study is shown in Tables 2 and 3. We estimate that 100% of the anuran species in the communities were sampled, a total of twenty species, of which fourteen were considered rare and six dominant species (Table 3). Rainfall and Time over the months had a strong influence on the wealth and diversity of frogs (Tables 4 and 5, results). The distribution of rainfall over time can be followed graphically in Figure 2 (b) and (c).

Statistics	Value
Sample size (N)	4216
Number of observed species (S)	20
Coverage estimate for entire dataset	100%

Cut-off point for determining abundant species [N/S]	210
Number of observed individuals for rare group	1156
Number of observed species for rare group	14
Estimate of the sample coverage for rare group	100%
Number of observed individuals for abundant group	3060
Number of observed species for abundant group	6

Table 2: Characteristics of the data sample, including estimates of total sampling coverage and for rare species. N = total of individuals observed, S = total of species observed and [N/S] = smallest integer closest to the N/S ratio. Species with an abundance greater than [N / S] were considered dominant, while species with an abundance less than or equal to [N/S] were considered rare.

Species	Total Abundance	Number of Sample Where Ocurred	Dominant Species
Trachycephalus atlas	2	2	
Pipa carvalhoi	4	4	
Dendropsophus soaresi	11	3	
Ceratophrys joazeirensis	31	10	
Corythomantis greeningi	38	14	
Proceratophrys cristiceps	68	18	
Dematonotus muelleri	69	14	
Physalaemus cuvieri	78	17	
Dendropsophus branneri	86	19	
Dendropsophus oliveirai	114	23	
Leptodactylus troglodytes	117	33	
Physalaemus albifrons	142	36	
Odontophrynus carvalhoi	194	31	
Rhinella jimi	202	40	
Physalaemus cicada	306	35	X
Rhinella granulosa	437	35	X
Pithecopus nordestinus	490	52	X
Scinax pachycrus	564	51	X
Pleurodema diplolister	630	42	X
Scinax x-signatus	633	55	X

Table 3: Sampling abundance and frequency of occurrence for each of the twenty species observed. Dominant species are indicated with “X” and correspond to species with an abundance greater than 210 individuals.

The relationship between the Noise Level and the Effective Number of Species for each order of Hill numbers (q = 0, 1, 2) is represented in the scatter plots (a) of Figure 2, each accompanied by a smoothing curve LOWESS that shows the relationship between the variables. In general, there was no clear relationship between the Noise Level and the Effective Number of Species for any order of Hill numbers, with the LOWESS curves approximately horizontal in all three cases. For the other independent variables (rainfall and wether), a non-linear relationship with the Effective Number of Species was observed (Figure 2B and C). Similar results were obtained for the relationship between abundance and Noise Level, Rainfall and Time (Supplementary Material, Figure 1).

A formal test to assess the significance of the relationship between the Noise Level and the Effective Number of Species is presented in Table 4. The Noise Level was not considered significant to explain the variability observed in the Effective Number of Species (LRT with *p-value* = 0.523). In this sense, the cubic splines for the independent variables Rainfall and Wether were significant with LRTs presenting *p-values* below 0.01 (Table 4). The estimates obtained for the parameters of the multivariate regression model are shown in Table 5. For the noise level, the regression coefficients were not considered significantly different from zero by the Wald tests, with a *p-value* greater than 0.05 in all cases (Table 5).

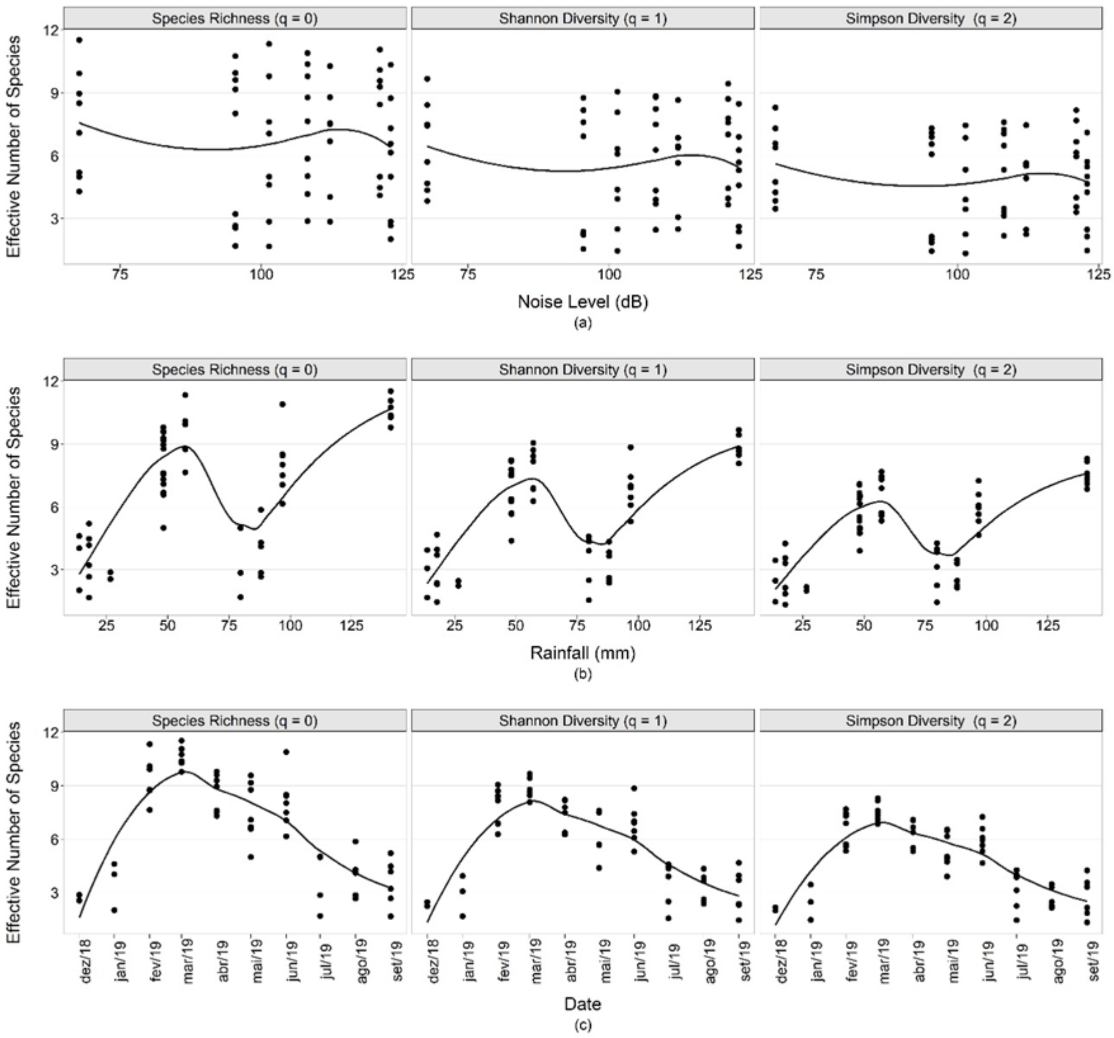

Figure 2: Scatter plots for the Effective Number of Species (calculated based on Hill numbers of order 0, 1 and 2) against the variables (a) Noise Level in dB, (b) Precipitation in mm and (c) Time in months. Hill numbers of order 0, 1 and 2 correspond, respectively, to Richness, Shannon Diversity and Simpson Diversity. Each scatter plot is accompanied by a LOESS smoothing curve to indicate the shape of the relationship between variables.

H_0	Statistics	P-value	Distribution Under H_0
B = O (Omnibus Test)	248.069	0.000	Chi-Square (df = 9)
Intercept = O	1.567	0.208	F (df_1 = 3, df_2 = 56)
Noise Level = O	0.756	0.523	F (df_1 = 3, df_2 = 56)
Rainfall = O	102.723	0.000	Chi-Square (df = 9)
Time = O	29.201	0.001	Chi-Square (df = 9)

Table 4: Result of the hypothesis tests and statistical significance associated with the independent variables that make up the estimated multivariate regression model. The quantity B indicates the matrix of regression coefficients of the estimated model; The quantity O indicates a null matrix with adequate dimensions for testing the corresponding H0 hypothesis. (df, df1 and df2), correspond to the degrees of freedom of the probability distribution of the test statistic under the null hypothesis H0.

Regression Parameter	Estimate	Std. Error	T	P-value
Species Richness (q = 0)				
Intercept	-1.533	0.956	-1.604	0.115
Noise Level	-0.012	0.009	-1.312	0.195
Rainfall (degree = 1)	7.924	1.820	4.355	0.000
Rainfall (degree = 2)	-5.829	2.274	-2.563	0.013
Rainfall (degree = 3)	13.670	2.787	4.905	0.000
Rainfall (degree = 4)	3.537	0.966	3.661	0.001
Time (degree = 1)	21.663	2.499	8.668	0.000
Time (degree = 2)	-5.218	1.811	-2.881	0.006
Time (degree = 3)	2.917	0.939	3.107	0.003
Shannon Diversity (q = 1)				
Intercept	-1.263	0.777	-1.625	0.111
Noise Level	-0.011	0.008	-1.445	0.155
Rainfall (degree = 1)	6.744	1.480	4.558	0.000
Rainfall (degree = 2)	-5.198	1.849	-2.811	0.007
Rainfall (degree = 3)	11.529	2.266	5.087	0.000
Rainfall (degree = 4)	2.975	0.786	3.787	0.000
Time (degree = 1)	17.758	2.032	8.737	0.000
Time (degree = 2)	-3.903	1.473	-2.650	0.011
Time (degree = 3)	2.486	0.763	3.257	0.002
Simpson Diversity (q = 2)				
Intercept	-1.014	0.663	-1.530	0.133
Noise Level	-0.010	0.006	-1.508	0.138
Rainfall (degree = 1)	5.767	1.262	4.569	0.000
Rainfall (degree = 2)	-4.504	1.577	-2.856	0.006
Rainfall (degree = 3)	9.807	1.933	5.073	0.000

Rainfall (degree = 4)	2.553	0.670	3.810	0.000
Time (degree = 1)	14.874	1.734	8.580	0.000
Time (degree = 2)	-3.108	1.256	-2.474	0.017
Time (degree = 3)	2.169	0.651	3.332	0.002

Table 5: Estimates for the regression coefficients of the multivariate model defined in the Methods Section. Column T corresponds to Wald's statistic for the regression coefficient test β under the null hypothesis H0: β = 0, the test p-value is indicated in the *P-value* column. Std. Error = Standard Error.

The quality of the fit of the multivariate regression model was studied from the residual graphs (a) and (b) and the Mahalanobis distance graph (c) in Figure 3. The residues showed adequate behavior indicating that there were no serious deviations from the assumptions required by the type of model estimated. Less than 10% of Mahalanobis distances were above the 90% quantile of the Chi-Square distribution with 3 degrees of freedom.

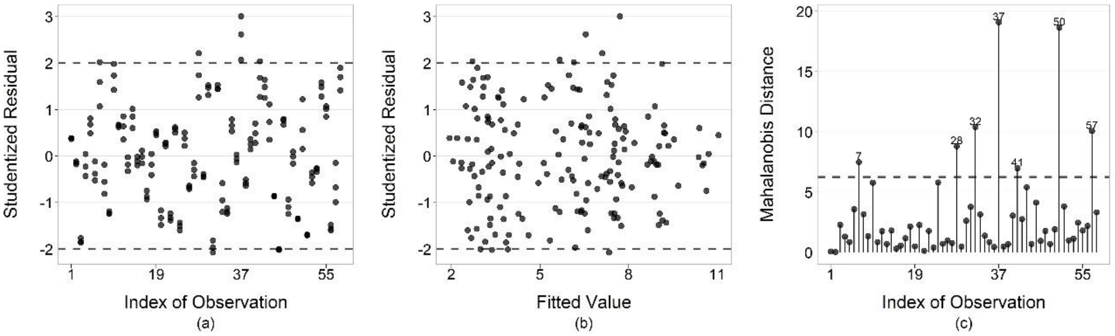

Figure 3: Diagnostic graphs of the estimated multivariate regression model. (a) Graph of studentized residues against the index of observations, (b) Graph of studentized residues against values predicted by the model and (c) Graph for the Mahalanobis distance. Cut-off point of the Mahalanobis distance graph equal to the 90% quantile of the Chi-Square distribution with 3 degrees of freedom.

4. Discussion

4.1 Influence of anthropogenic noise on frog diversity

We investigated the effects of noise from wind farms on the structure of anuran communities in the Caatinga and found no significant relationship between noise and patterns of abundance, richness and diversity of species. On the other hand, studies suggest that different sources of anthropogenic noise (e.g. vehicles, motorcycles, airplanes, wind turbines and oil extraction machines), can alter the behavior and physiology of several animal species (Brumm, 2010; Brumm and Slabbekoorn, 2005; Gerhardt and Huber, 2002).

The effects of noise on terrestrial animals are quite variable and different behavioral responses are reported for each species (Shannon et al., 2015). They can occur since alteration in the process of metamorphosis in crabs (Pine et al., 2012), alteration in the squirrel anti-predator behavior (Rabin et al., 2006), increased stress levels, predation of eggs and chicks (Karp and Root, 2009) and reduction of bird abundance and richness (Francis et al., 2009; McClure et al., 2013), in addition to the reduction of seed dispersing animals (Francis et al., 2012).

In anurans, noise triggers a series of responses that include interference with migration, decreased reproduction and reduced immunity (Sun and Narins, 2005; Kaiser and Hammers, 2009; Tennessen et al., 2014; Troïanowski et al., 2017). Despite the effects of anthropogenic noise on the abundance of anurans (Grace and Noss, 2018), more tolerant species may benefit from the absence of sensitive species and expand their distribution, this would explain in part, the adaptation of birds and anurans in noisy areas (Francis et al., 2009; Francis et al., 2012; Halfwerk et al., 2019). However, this hypothesis must be tested and requires further studies.

4.2 Influence of rainfall and weather on anuran diversity

In this study, when analyzing the influence of rainfall and weather over the months, we found that these variables have a strong relationship in the patterns of abundance, richness and diversity of frogs (Arzabe, 1999; Arzabe et al., 1998; Ferreira-Silva et al ., 2016; Vieira et al., 2007; Xavier and Napoli, 2011). Therefore, in semiarid environments such as the Caatinga, the availability of water during the short rainy season strongly influences the reproductive patterns of anurans (Bentley, 1966; Schalk et al., 2015; Vieira et al., 2009; Wells, 1977). Our data revealed abundance peaks between February and April (Figure 1, Supplementary Material), a period that coincides with the highest rainfall in the Caatinga (Arzabe, 1999; Borges-Nojosa and Santos, 2005; Ferreira-Silva et al., 2016; Vieira et al., 2007).

The seasonal variation in the abundance of species shown here seems to reflect the type of reproduction, whether explosive or prolonged *sensu* Wells (1977), as well as the reproductive mode of anurans in the Caatinga *sensu* Vieira et al. (2009). In this sense, species of explosive reproduction with peaks of abundance from December to February, commonly deposit their eggs directly in water or in floating foam nests that protect from solar radiation and feed embryos (e.g. *Physalaemus albifrons*, *P. cicada*, *P. cuvieri* and *Pleurodema diplolister*) (Arzabe, 1999; Arzabe et al., 1998; Borges-Nojosa and Santos, 2005; Vieira et al., 2009, 2007). Other species appear to allocate resources early in the rainy season and reproduce later with abundance peaks from February to April (e.g. *Dendropsophus branneri*, *D. Oliveirai* and *Pithecopus nordestinus*). During this period, environmental conditions are more favorable and can use marginal vegetation and macrophytes for cutting, egg deposition and exotrophic development of tadpoles (Arzabe et al., 1998; Vieira et al., 2009, 2007).

These reproductive characteristics seem to reflect the environmental conditions available during the short rainy season in the Caatinga, such as the unpredictability of rainfall in space and time (Ab ’Saber, 1977; Prado, 2003). In addition, these reproductive patterns are influenced by altitude, hydroperiod duration and rainfall regime (Arzabe, 1999; Arzabe et al., 1998; Borges-Nojosa and Santos, 2005; Oliveira et al., 2017; Vieira et al., 2007; Xavier and Napole, 2011). Therefore, due to the strong correlation of the variables Rainfall and weather in the life cycle of anurans in semiarid ecosystems (Bentley, 1966; Schalk et al., 2015; Vieira et al., 2009; Wells, 1977), isolating the effects of noise from wind farms in the structure of these communities becomes quite complex.

However, this assessment is highly necessary in view of the expansion of the wind sector in Brazil and world (Dai et al., 2015; Jones et al., 2015). Although investigation using a noise exposure gradient (e.g. this study) is more efficient than quiet *versus* noisy treatments (Shannon et al., 2015), there are other methods that can help in assessing the impacts of wind farms on populations and communities, such as assessing species abundance via acoustic monitoring (Grace and Noss, 2018), the marking and recapture technique (Keehn et al., 2019), demographic and behavioral analysis of populations (Francis et al., 2009; Keehn et al., 2019; McClure et al., 2013), or a combination of these approaches.

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Supplementary Material

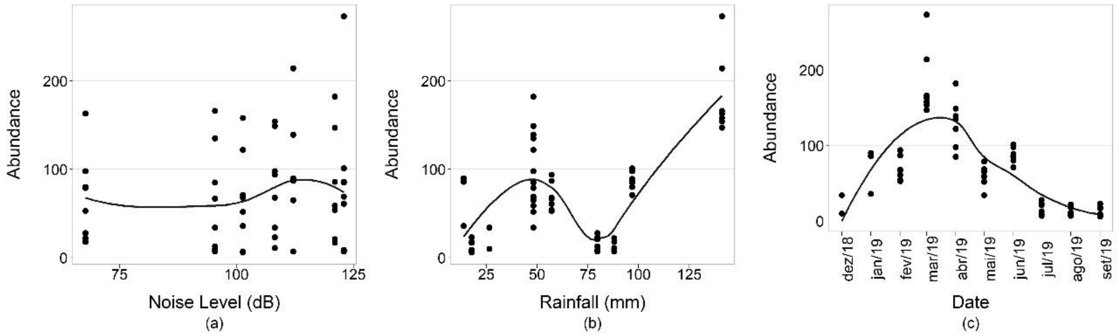

Figure 1: Scatter plots for Sample Abundance and variables (a) Noise Level, (b) Rainfall and (c) Weather. Each scatter plot is accompanied by a LOESS smoothing curve to indicate the shape of the relationship between the variables.

H_0	Statistics	P-value	Distribution Under H_0
B x D = O (Omnibus Test)	73.441	0.000	F ($df_1 = 3$, $df_2 = 56$)
Intercept x D = O	1.555	0.210	F ($df_1 = 3$, $df_2 = 56$)
Noise Level x D = O	0.412	0.745	F ($df_1 = 3$, $df_2 = 56$)
Rainfall x D = O	19.681	0.000	F ($df_1 = 3$, $df_2 = 56$)
Time x D = O	3.560	0.003	F ($df_1 = 3$, $df_2 = 56$)

$$D = \begin{bmatrix} 1 & -1 & 0 & \dots & 0 \\ 0 & 1 & -1 & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & 1 & -1 \end{bmatrix}$$

Table 1: Result of the hypothesis tests for the difference in the regression coefficients between the three response variables that make up the estimated multivariate regression model. B indicates the matrix of estimates of the regression coefficients of the estimated model; The indicates a null matrix with adequate dimensions for testing the corresponding H0 hypothesis; D indicates the matrix of contrasts to compare regression coefficients between pairs of dimensions of the model response. df, df1 and df2 correspond to the degrees of freedom of the probability distribution of the test statistic under the null hypothesis H0.

	Species Richness (q = 0)	Shannon Diversity (q = 1)	Simpson Diversity (q = 2)
Species Richness (q = 0)	1.000		
Shannon Diversity (q = 1)	0.979	1.000	
Simpson Diversity (q = 2)	0.933	0.986	1.000

Table 2: Estimation of the correlation matrix between the variables that make up the response of the estimated multivariate regression model.

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Effects of wind farm noise on frog calling behavior in Caatinga, Northeastern Brazil

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Abstract: Several human activities have contributed to the global biodiversity crisis, including noise pollution. This study analyzed the adaptation of the acoustic parameters of the advertisement call of three neotropical anurans exposed to noise from wind farms in the Caatinga, Northeast Brazil. We recorded the environmental noise in similar temporary ponds distributed in two wind farms and established a noise gradient. We recorded the advertisement call of individuals of the three species along the noise gradient and subjected the data to statistical analysis to model the mean and variability of the spectral and temporal variables of the anuran call under the different noise levels. We found a negative linear relationship between the noise levels and the calling parameters of the three species, with the calling intensity, frequency amplitude and dominant frequency decreasing significantly in noisy areas. The effects of anthropogenic noise on the spectral parameters of anuran calling are quite variable and different displays are used, such as adjusting the amplitude or frequencies of the call. As with other anthropogenic activities, we bring evidence that the noise of wind turbines affects the behavior of anuran calling and this is reflected in the assessment of the environmental impacts of wind energy and in the conservation of these animals in the Caatinga and other terrestrial environments in Brazil.

Keywords: Bioindicators; Seasonally dry tropical forest; Anthropogenic noise; Frogs; Wind turbines

INTRODUCTION

In the environment, acoustic signals are transmitted between transmitters and receivers and provide a range of information such as body size and reproductive fitness, being crucial as a strategy for reproduction and survival (Ey and Fisher 2009; Chen and Wiens 2020). These signals are emitted in environments that affect the propagation and properties of the signal so that the structure of the signals that reach the receivers are altered versions of the originals produced by the emitters (Röhr and Juncá 2013; Velásquez et al. 2018). Therefore, every environment has its own characteristics for transmitting and distributing sound (Ziegler et al. 2011). Habitats in open areas are characterized by low vegetation density and greater air turbulence, which promote more variable conditions for sound propagation, while habitats in closed areas, due to the reverberation and absorption of surfaces, promote more constant acoustic conditions (Morton 1975; Shy and Morton 1986; Schneider et al. 2008).

Therefore, to overcome environmental obstacles, acoustic signals must have ideal characteristics for their transmission, “Acoustic Adaptation Hypothesis” (Morton 1975), which aims to optimize their propagation in the environment (Rothstein and Fleischer 1987; Slabbekoorn and Smith 2002; Brumm and Slabbekoorn 2005). This hypothesis was tested on vertebrates and corroborated in studies with birds (Morton 1975; Shy and Morton 1986), frogs (Ryan et al. 1990; Velásquez et al. 2018) and mammals (Sugiura et al. 2006; Ey and Fisher 2009).

Although animals such as insects and frogs are naturally used to natural noises (Gerhardt and Huber 2002), noise increases represent a new stimulus that changes the acoustic conditions of the habitat (Bastos et al. 2011; Halfwerk et al. 2019). Several studies have shown that anthropogenic noise causes acoustic masking, interfering with the emission and reception of signals in several animal groups, such as insects (Römer

2013), fish (Ladich 2013), amphibians (Sun and Narins 2005; Kaiser and Hamers 2009), birds (Francis et al. 2009; 2012), reptiles (Brumm and Zollinger 2017) and mammals (Slabbekoorn et al. 2018). The negative effects of noise reflect from changes in individual behaviors to changes in the structure of populations and communities (Brumm and Slabbekoorn 2005; Shannon et al. 2015).

The use of acoustic signals is a conspicuous characteristic in anurans and constitutes its predominant form of communication, acting in the social organization of populations (Wells 1977; Wells and Schwartz 2006). However, despite the importance of acoustic communication in anurans, the efficiency of this activity in adverse acoustic scenarios is not sufficiently known (Bee and Swanson 2007). Therefore, due to increases in noise in natural areas, such as the rapid expansion of the wind sector in Brazil, the effects of noise from wind farms on the anuran calling behavior need to be further investigated (Dai et al. 2015; Thaxter et al. 2017).

The production of electricity from wind energy is characterized as a competitive, reliable and renewable technology (Dai et al. 2015; Araújo and De Moura 2017). This technology is identified as one of the global alternatives for reducing greenhouse gas emissions and, in this context, it can help countries to move towards a low carbon economy (Gasparatos et al. 2017). However, like any large enterprise, its implementation is associated with the suppression of vegetation, exposure and soil erosion and the opening of new roads and transmission lines (Dai et al. 2015; Araújo and De Moura 2017). However, the environmental problems resulting from the implementation of wind farms are more significant when installed along the route of migratory birds and semiarid ecosystems (Dai et al. 2015).

The Caatinga is located predominantly in the northeastern region of Brazil, comprising only 11% of the national territory, however, comprising the region of greatest interest for the expansion of wind farms in the country (Bernard et al. 2014; Neri et al. 2019). Areas with wind farms installed or with potential for their installation overlap with the relevant or extremely relevant areas for biodiversity conservation in the Caatinga (Neri et al. 2019). This biome has an estimated power generation capacity of 75 GW (Agência Brasileira de Energia Elétrica 2008), and represents 70% of the total priority areas for wind power generation in Brazil (Bernard et al. 2014).

Knowing the implications of anthropogenic noise in the acoustic communication of several animal groups (Francis et al. 2009; 2012), including anurans (Sun and Narins 2005; Bee and Swanson 2007), this study evaluated the adaptation of the acoustic parameters of the advertisement call of the three species of anurans exposed to noise from wind farms in the Caatinga area in northeastern Brazil. Since in anurans the acoustic signals must have ideal attributes for their propagation in the environment (Ryan et al. 1990; Ey and Fisher 2009; Velásquez et al. 2018), we hypothesized that the three species used as biological models would adapt their acoustic parameters and that would shift spectral characters from the call in response to the noise of wind farms.

MATERIAL AND METHODS

Study area

The study was realized in two “Onshore” wind farms located in the municipalities of Caetés and Paranatama, Pernambuco State, Brazil (Figure 1). The local climate is semi-arid tropical Aw (Alvares et al. 2013), the average temperature is 20.5 ºC and the average altitude is 883m, the average annual rainfall is 755 mm with the rainy season going from April to August, although they may sporadic rainfall occurs throughout the year (Climate Date 2020). The vegetation is typical of Caatinga and characterized by distinct physiognomies (Oliveira et al. 2017).

Wind farm 1 has fifteen wind turbines with a power of 13,600 KW and an area of approximately 726 hectares. Wind farm 2 has eighteen wind turbines with a power of 28,900 KW and an area of approximately 567 hectares. In both wind farms, the wind turbines are of the GE Energy 1.7-100 model, with a power of 1,700 KW with winds at 10m/s, these turbines, 96 meters high, are composed of three helix, each with 52 meters long (Wind Energy Market Intelligence https://www.thewindpower.net/turbine_es_593_ge-energy_1.7-100.php).

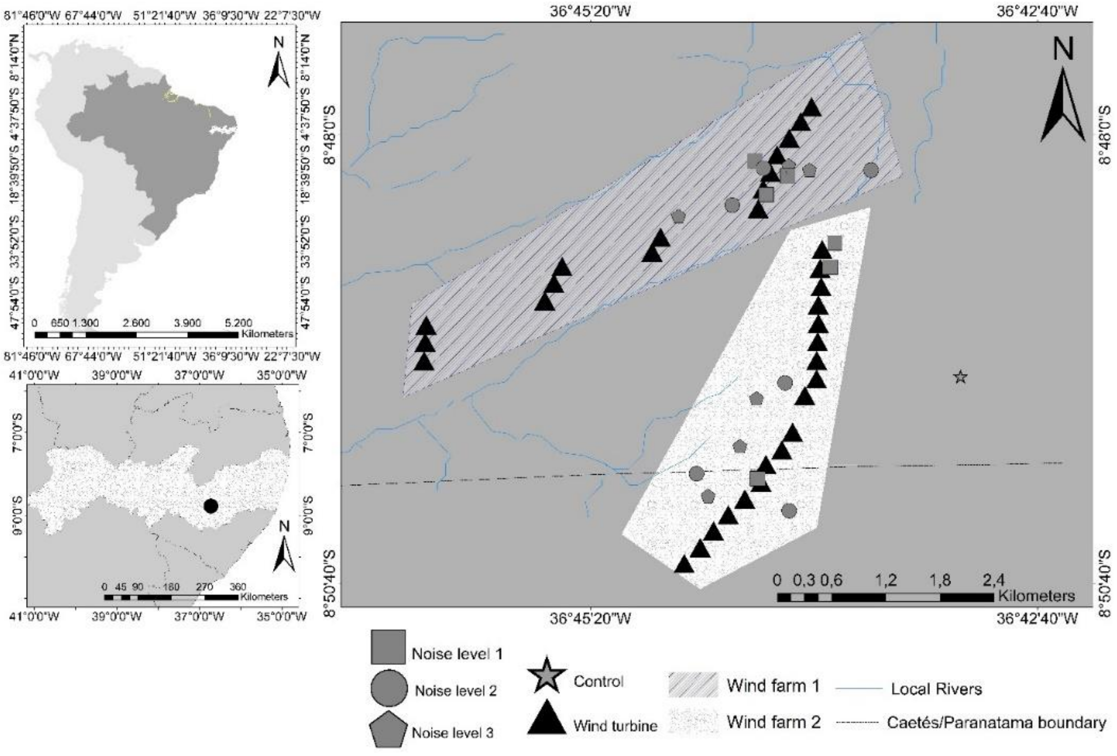

Figure 1

Study area with location of wind farms and provision of artificial temporary puddles in the municipalities of Caetés and Paranatama, Pernambuco, Brazil. Wind farm 1 has fifteen wind turbines and an area of 726 hectares and wind farm 2 has eighteen turbines and 567 hectares. Control is represented by a star, wind turbines are represented by triangles, Class of noise 1 is represented by squares, Class of noise 2 is represented by circles and Class of noise 3 is represented by pentagons.

Environmental noise

In each wind farm, nine temporary pools were selected to record environmental noise. To avoid bias in the results, the selected puddles had similar environmental characteristics such as an average diameter of $42m^2$ (range 22 - 65), type and density of vegetation. Several factors contribute to the propagation of noise, such as the distance from the source, wind direction and absorption by environmental structures (eg soil, rocks and plants) and due to the noise of wind turbines being emitted at low frequencies, a reduction of 20 dB is expected every kilometer away from the source of the noise (Alberts, 2005).

Based on this information, a puddle was defined as a control with environmental characteristics similar to the others and two kilometers distant upstream of the wind direction (Figure 1). To measure the noise intensity (dB) of the wind farms in the nineteen temporary pools, we used a decibel meter (Instrutemp ITDEC 4000, precision of 0.1dB). Then, the noise was recorded with a YOGA HT81 unidirectional microphone coupled to a Tascam DR40 digital recorder, configured at 44.1 kHz and 16-bit resolution. In order to attenuate the interference of the geophony and the zoophony during the recordings, the microphone was positioned manually towards the wind turbines forming an angle of 45º in relation to the ground.

To establish a noise gradient in each wind farm, the variation in noise recorded in temporary puddles was calculated from the formula ($\Delta = Rf - Ri$ where, Rf represents the maximum noise value and Ri represents the minimum noise value in each wind farm). Then, the value of Δ was divided into three equal parts and each resulting part was assigned to a noise class (Table 1).

Table 1

Classes of environmental noise from temporary artificial ponds distributed in the two wind farms in the municipalities of Caetés and Paranatama, Pernambuco State, Brazil. For both wind farms, each class is represented by three temporary pools and their values are independent (not standardized). The table shows the variation and the average noise per class expressed in decibels (dB).

Wind Farmer1			Wind Farmer2		
Noise classes (dB)	Range (dB)	Average (dB)	Noise classes (dB)	Range (dB)	Average (dB)
1	117.5 - 128.3	122.9	1	114.6 - 127.4	121
2	106.8 - 117.5	112.1	2	101.8 - 114.6	108.2
3	96 - 106.8	101.4	3	89 - 101.8	95.4

Focal species

We selected three species of Neotropical anurans with different acoustic profiles and that could easily be found in the nineteen temporary ponds. A summary of the acoustic profile of each species is shown in Table 2.

Table 2

Body size (Snout-vent length - SVL) and traits of the advertisement call (call duration, call repetition rate and dominant frequency) of the three species.

Species	Snout-vent Length (SVL)	Call duration (s)	Call Repetition Rate (call/min)	Dominant frequency (Hz)	Reference

Physalaemus cicada Bokermann, 1966	27.5mm	0.025	1500	3046.9	(Hepp and Pombal 2020)
Scinax Pachycrus (Miranda-Ribeiro, 1937)	30mm	0.3	37	1830	(Carneiro et al. 2004)
Scinax x-signatus (Spix, 1824)	35mm	0.2	8.6	1250	(Rivero 1969; Novaes and Zina 2016)

For recording the advertisement call, we use a YOGA HT81 unidirectional microphone was used, positioned 50 cm from the animals in calling activity, coupled to a Tascam DR40 digital recorder, configured at 44.1 kHz and 16-bit resolution. After recording the song, we measured the air temperature and humidity with an ISBB-804 digital thermohygrometer.

Data analysis

The environmental noise recorded in the nineteen temporary ponds and the acoustic parameters of the advertisement call of the three species were analyzed with Raven Pro 1.5 for Windows from the Cornell Lab of Ornithology (Bioacustic Research Program 2012). All configurations followed Raven's "standard".

For statistical analysis, the six variables were divided into two groups: (i) spectral variables, composed of Call Amplitude (Hz), Dominant Frequency (Hz) and Frequency Amplitute (Hz), and (ii) temporal variables, composed of Call Duration (seconds), Call Pulses (pulses/call) and Call Repeat Rate (call per minute). The calling variables (spectral and temporal), were log-transformed and for each group of variables a Multivariate Linear Regression Model was estimated with additive errors normally distributed (Díaz-García et al. 2003).

The design matrix, identical in both models, included an intercept and the independent variables Noise Level (dB), Species (P. cicada, S. pachycrus and S. x-signatus), Air Temperature (°C) and Air Relative humidity (%). For the independent variable Species, with nominal measurement level, the following dummy codifications were defined: P. cicada = (1, 0), S. pachycrus = (0, 1), S. x-signatus = (-1, - 1). Thus, in both models the intercept represents a general mean vector, which is defined as the arithmetic mean of the mean vectors for each species of anuran.

To quantify the significance of including the independent Noise Level variable in both models associated with the groups of response variables (i) and (ii), the Likelihood Ratio Test (LRT) presented in Díaz-García et al. (2003). The asymptotic distribution of the test statistic and also its exact distribution for some particular cases can be found in Anderson (2003). The Wald test statistic, usually presented in results from regression models with univariate response, was also used to assess the significance of the regression coefficient associated with the Noise Level for each acoustic variable in isolation. Similarly, the LRT and Wald tests were performed for the other independent variables.

The fit quality of the Multivariate Regression Models was accessed based on Díaz-García et al. (2003) and in Russo et al. (2009), including graphs of studentized residues against the index of observations and values predicted by the models and also graphics of Mahalanobis distances. All statistical analyzes were performed using software R version 3.6.1 (R Core Team 2019) based on the computational implementation of the methods proposed in (Díaz-García et al. 2003).

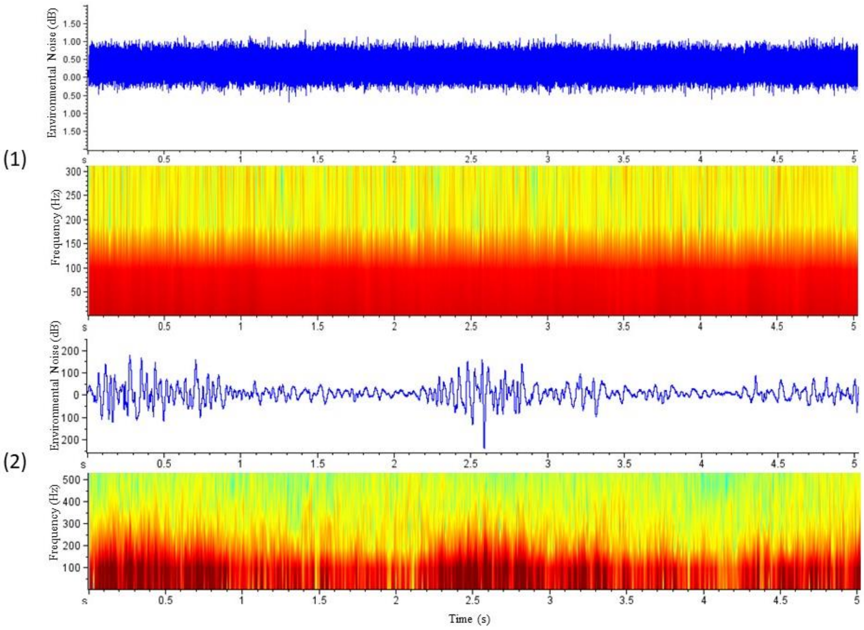

Figure 2

Characterization of environmental noise: Control (1) and noisy area (2). In the control, the average peak noise was 67 dB (67.2 - 69.2) and in the noisy area the average peak noise was 114 dB (110.5 - 120.2). In images 1 and 2, the oscillograms are shown above and the spectograms below.

RESULTS

The calls of 182 specimens were analyzed, *Physalaemus cicada* (N = 42), *Scinax Pachycrus* (N = 70) and *Scinax x-signatus* (N = 70), and we found that the noise from wind farms negatively affected the spectral variables of the calling (Tables 3 and 4). In the scatter plots (Figure 3), it is possible to see that the slope of the regression lines for the spectral variables was negative (showed decreases), while remaining horizontal (stable) for the temporal variables. The partial effects associated with the other independent variables inserted in the multivariate regression models are presented in the Supplementary Material Section (Figures 2 and 3). The descriptive measures, mean, standard deviation, maximum and minimum of the six acoustic variables are gathered in Table 1 of the Supplementary Material Section, and were calculous without differentiating the three species of anurans included in the sample.

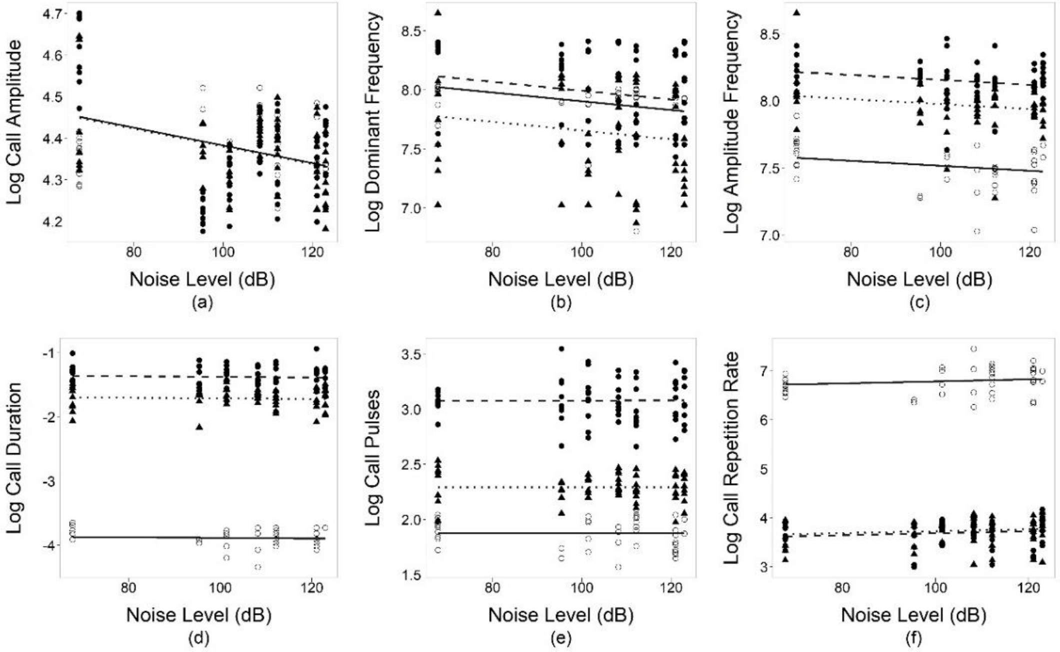

Figure 3

Partial noise effect (dB) of wind farms on variables dependent on models (i) and (ii) (see Methods). Graphs (a), (b) and (c) refer to model 1. Where, (a) is logarithm of the call amplitude (dB), (b) logarithm of the dominant frequency (Hz) and (c) logarithm of the amplitude frequency (Hz). Graphs (d), (e) and (f) refer to model 2. Where, (d) is logarithm of the call duration (s), (e) logarithm of the call pulses and (f) logarithm of the call repetition rate. The three species are differentiated in the graphs as follows: *P. cicada* = open circle, *S. pachychrus* = filled circle and *S. x-signatus* = filled triangle. The regression lines are differentiated for each species as follows: *P. cicada* = solid line, *S. pachychrus* = dashed line and *S. x-signatus* = dotted line.

In the regression model (i) (spectral variables), the independent variables Species and Air Relative Humidity were statistically significant (see Tables 3 and 4). Similarly, for the regression model (ii) (temporal variables), the independent variables

Species, Air Temperature and Air Relative Humidity were significant (see Tables 5 and 6). To identify which spectral variables were affected by noise, we performed Wald tests (Table 4). From the hypothesis tests associated with the parameters of the regression model, they showed that the increase in noise levels negatively influenced the means of the three spectral variables (Table 3). On the other hand, the results of the LRT and the Wald test both led to the same conclusion, that the noise was not able to explain significantly variations in the temporal variables (Tables 5 and 6).

Table 3

Results of generalized hypothesis tests for the model parameters associated with the group of spectral variables, logarithm of the call amplitude, logarithm of the dominant frequency and logarithm of the amplitude frequency. column t indicates the value of the test statistic (see Methods section) and the Dist column. Under H0 indicates the distribution of T under the restrictions of the null hypothesis given in column H0. B represents the matrix of regression parameters of the model, and O indicates a null matrix of appropriate dimensions for the hypothesis test defined in H0. df, df1 and df2 correspond to the degrees of freedom of the probability distribution of the test statistic under the null hypothesis H0.

H_0	T	P-value	Dist. Under H_0
B = O (Omnibus Test)	1632.033	0.000	Chi-Square ($df = 9$)
Intercept = O	302863.946	0.000	F ($df_1 = 3$, $df_2 = 168$)
Noise Level = O	11.479	0.000	F ($df_1 = 3$, $df_2 = 168$)
Species = O	58.277	0.000	F ($df_1 = 3$, $df_2 = 168$)
Relative Humidity = O	4.819	0.003	F ($df_1 = 3$, $df_2 = 168$)
Air Temperature = O	0.503	0.681	F ($df_1 = 3$, $df_2 = 168$)

Table 4

Estimates for the regression coefficients of model (i), associated with the dependent variables logarithm of the call amplitude, logarithm of the dominant frequency and logarithm of the amplitude frequency (see Methods). Column T presents the Wald test statistic for the null hypothesis that the regression coefficient is equal to zero against the alternative hypothesis that the regression coefficient is different from zero. P-value associated with the Wald test is shown in the P-value column. Std. Error = Standard Error.

Regression Parameter	Estimate	Std. Error	T	P-value
Log Call Amplitude (Hz)				
Intercept (Global Mean)	4.373	0.007	585.507	0.000
Noise Level	-0.002	0.000	-4.743	0.000
Global Mean – Mean of *P. cicada*	0.001	0.012	0.085	0.932
Global Mean – Mean of *S. pachycrus*	0.001	0.010	0.092	0.927
Relative Humidity	0.004	0.001	3.146	0.002
Air Temperature	0.009	0.008	1.165	0.246
Log Dominant Frequency (Hz)				
Intercept (Global Mean)	7.835	0.026	300.394	0.000
Noise Level	-0.004	0.002	-2.375	0.019
Global Mean – Mean of *P. cicada*	0.053	0.042	1.249	0.214
Global Mean – Mean of *S. pachycrus*	0.143	0.036	4.005	0.000
Relative Humidity	0.010	0.004	2.580	0.011
Air Temperature	0.016	0.026	0.620	0.536
Log Amplitude Frequency (Hz)				
Intercept (Global Mean)	7.875	0.012	631.040	0.000
Noise Level	-0.002	0.001	-2.480	0.014
Global Mean – Mean of *P. cicada*	-0.367	0.020	-18.227	0.000
Global Mean – Mean of *S. pachycrus*	0.274	0.017	16.049	0.000
Relative Humidity	0.001	0.002	0.730	0.466
Air Temperature	-0.003	0.013	-0.239	0.812

Table 5

Results of the tests of generalized hypotheses for the parameters of the model (ii), of the dependent variables logarithm of the call duration, logarithm of call pulses, logarithm of the call repetition rate (see Methods). Column T indicates the value of the test statistic and column Dist. Under H0 indicates the distribution of T under the restrictions of the null hypothesis given in column H0. B represents the matrix of regression parameters of model 1 and O indicates a null matrix of appropriate dimensions for the hypothesis test defined in H0. df, df1 and df2 correspond to the degrees of freedom of the probability distribution of the test statistic under the null hypothesis H0.

H_0	T	P-value	T Dist. Under H_0
B = O (Omnibus Test)	1928.816	0.000	Chi-Square (*df* = 9)

Intercept = O	110261.461	0.000	F (df_1 = 3, df_2 = 168)
Noise Level = O	1.132	0.338	F (df_1 = 3, df_2 = 168)
Species = O	1292.662	0.000	F (df_1 = 3, df_2 = 168)
Relative Humidity = O	4.698	0.004	F (df_1 = 3, df_2 = 168)
Air Temperature = O	2.721	0.046	F (df_1 = 3, df_2 = 168)

Table 6

Estimates of the regression coefficients of the model (ii), for the dependent variables logarithm of the call duration, logarithm of the call pulses and logarithm of the call repetition rate. Column T presents the Wald test statistic for the null hypothesis that the regression coefficient is equal to zero against the alternative hypothesis that the regression coefficient is different from zero. P-value associated with the Wald test is shown in the P-value column. Std. Error = Standard Error.

Regression Parameter	Estimate	Std. Error	T	P-value
Log Call Duration (s)				
Intercept (Global Mean)	-2.327	0.012	-194.401	0.000
Noise Level	0.000	0.001	-0.628	0.531
Global Mean – Mean of *P. cicada*	-1.562	0.019	-80.759	0.000
Global Mean – Mean of *S. pachycrus*	0.949	0.016	57.945	0.000
Relative Humidity	-0.006	0.002	-3.317	0.001
Air Temperature	-0.033	0.012	-2.714	0.007
Log Call Pulses (pulses/call)				
Intercept (Global Mean)	2.414	0.012	202.292	0.000
Noise Level	0.000	0.001	0.006	0.995

Global Mean – Mean of *P. cicada*	-0.541	0.019	-28.053	0.000
Global Mean – Mean of *S. pachycrus*	0.663	0.016	40.618	0.000
Relative Humidity	-0.005	0.002	-2.542	0.012
Air Temperature	-0.018	0.012	-1.510	0.133
Log Call Repetition Rate (calls/min)				
Intercept (Global Mean)	4.739	0.020	231.818	0.000
Noise Level	0.002	0.001	1.642	0.103
Global Mean – Mean of *P. cicada*	2.050	0.033	62.067	0.000
Global Mean – Mean of *S. pachycrus*	-1.046	0.028	-37.409	0.000
Relative Humidity	0.004	0.003	1.382	0.169
Air Temperature	0.008	0.021	0.385	0.700

The adequacy of the estimated models to the observed data is summarized through regression diagnosis graphs in Figure 4 of the Supplementary Material Section. Most graphs did not indicate severe deviations from the assumptions imposed by the Multivariate Regression Model with normal additive errors. Even so, the graphs (b) and (e) of studentized residues against predicted values seem to suggest the need for a better investigation of the models, especially the model associated with spectral variables. In Tables 2 and 3 of the Supplementary Material Section, the estimated correlation matrices for the dependent variables of both multivariate regression models are presented.

DISCUSSION

We analyzed the influence of noise from wind farms in the advertisement call of three neotropical anurans and through regression models, we found evidence that noise affects calling behavior. The increase in noise along a gradient caused decreases in calling intensity, frequency amplitude and the dominant frequency call of the three studied species, supporting the idea of the potential impact of anthropogenic noise on animals that use acoustic communication (Brumm and Slabbekoorn, 2005; Brumm 2010; Shannon et al. 2016). Thus, we present evidence of an important source of noise and its potential consequences on anuran communication (Sun and Narins 2005; Bee and Swanson 2007; Cunnington and Fahrig 2010; Kruger and Du Preez 2016; Caorsi et al. 2017).

Effects of noise from wind farms on the spectral parameters of calling

In fact, the effects of anthropogenic noise on the acoustic parameters of anuran calling are quite variable. The shift in spectral parameters of the call in response to noise from wind farms observed here is similar to that observed for other sources of anthropogenic noise such as vehicles, motorcycles and airplanes (Bee and Swanson 2007; Kaiser and Hammers 2009; Kruger and Du Preez 2016). Thus, the adjustment of amplitude (Shen and Xu 2016; Leon et al. 2019) and call frequencies are commonly behavioral displays used by anurans in noisy areas (Sun and Narins 2005; Parris et al. 2009; Cunnington and Fahrig 2010; Kruger and Du Preez 2016).

The emission of calls with low dominant frequencies in response to noise from wind farms can have consequences in the reproduction of frogs. First, the temporary ponds in the present study are located in open areas, characterized by low vegetation density that causes greater variability in the propagation of the calls compared to forest habitats (Morton 1975; Shy and Morton 1986; Schneider et al. 2008; Ey and Fisher 2009). In addition, the emission of calls with low dominant frequencies is less efficient in open habitats than in forest habitats, where they are more efficient (Shy, E. Morton 1986; Ey and Fisher 2009; Röhr et al. 2016).

Important in the co-specific recognition of species, the dominant frequency is an important acoustic parameter involved in the reproduction of frogs (Gerhardt and Huber 2002; Parris et al. 2009; Röhr et al. 2016). Females of many species of anurans show preference for males that emit calls with low dominant frequencies, since it reflects their reproductive fitness (Kime et al. 2004). In addition, this acoustic parameter is essential to assess the size of opponents, avoid physical combat and maintain uniform spatial distribution (Bastos et al. 2011).

In this sense, acoustic masking can alter the ability of females to distinguish the origin of the signal emitted by males and thus, reduce the chances of alerting other males and attracting co-specific females (Bee and Swanson 2007; Lengagne 2008; Barber et al. 2010). The fact that frogs emitted lower noises in noisy areas may constitute a trade-off between reducing audibility and maintaining the attractiveness of mates (Ryan 1988; Parris et al. 2009). In addition, species affected by noise from wind farms, by reducing the calling frequencies, can compete with the others for frequency bands, generating interspecific competition (Gerhardt 1994; Gerhardt and Huber 2002).

In the present study, the species presented calls with less intensity in noisy areas. The modulation of the amplitude of the song is strategy to make the signal more flexible in environments in the presence of anthropogenic noise and to reduce acoustic masking. Some studies have reported increased calling intensity in species of the families

Hylidae, Microhylidae and Ranidae exposed to different sources of anthropogenic noise (Sun and Narins 2005; Cunnington and Fahrig 2010; Vargas-Salinas et al. 2014; Caorsi et al. 2017). However, our results showed a reduction in calling intensity, corroborating with other studies (Shen and Xu 2016; Leon et al. 2019).

The fact that the noise from wind farms is correlated with decreases in the calling frequencies of the three species of anurans studied, contradicts the hypothesis that species that vocalize at high frequencies and have a small spectral overlap with background noise (such as *Physalaemus cicada* 3046.9 Hz, see Hepp and Pombal 2020), would be less affected by anthropogenic noise (Cunnington and Fahrig 2010; Caorsi et al. 2017). In addition to the direct effects, anthropogenic noise can have indirect effects in this community, influencing the calling activity of heterospecific males who did not have their vocalization analyzed in this study, as already reported for other communities (Sun and Narins 2005; Kaiser and Hammers 2009; Kruger and Du Preez 2016).

Although there is evidence that anthropogenic noise can reduce the abundance of birds (McClure et al. 2013) and anurans (Grace and Noss 2018), we find several sympatric species of anurans reproducing successfully in temporary ponds, suggesting that anurans do not seem to avoid noisy areas to reproduce (Sun and Narins 2005; Parris et al. 2009). Although there is a consensus that plasticity in anuran calling allows communication in an adverse acoustic scenario (Bee and Swanson 2007; Halfwerk et al. 2019), in some species these changes in frequency ranges can take up to two decades (Parris et al 2009). This suggests that changes in anuran calling behavior could be instantaneous and fast, or gradual and slow (Halfwerk et al. 2019).

Knowing that the populations of the three species studied were exposed to similar environmental conditions in all temporary ponds and that their calling behavior is innate and unlearned (as in birds), the change in frequency presented by the three species may represent an evolutionary adaptation to wind farm noise (Ryan et al. 1990; Ey and Fisher 2009; Velásquez et al. 2018). However, several generations would be necessary and would depend on the pressure of the selection of low frequency calls (Parris et al. 2009), unlike short-term changes, where animals show plasticity to external acoustic stimuli (Bee and Swanson 2007; Cunnington and Fahrig 2010).

Wind farms and frogs conservation in Caatinga

Caatinga is one of the seasonally dry forests with the greatest biodiversity on the planet (De Albuquerque et al. 2012; da Silva et al. 2017; Fernandes et al. 2019), despite its importance, only 2% of its area is fully protected in Conservation Units (Fonseca et al. 2017). In 2018, of the 6313 wind turbines in operation in Brazil, (78%) were in the Caatinga and another 14,696 are expected to be installed (Neri et al. 2019). This fact is worrying because the impacts of the installation of wind farms are more significant in semi-arid ecosystems, since their ability to recover is very slow (Dai et al. 2015; Thaxter et al. 2017). Although it represents a promising line of research, noisy anthropogenic activities such as wind power generation, it has still received little attention (e.g. Rabin et al. 2006; Francis et al. 2009; Francis et al. 2012).

CONCLUSION

Our results confirmed the hypothesis that anurans exposed to anthropogenic noise alter their acoustic signals to improve their propagation in the environment and thus maintain their role in the social organization of populations, defense of territory and conquest of reproductive partners (Wells and Schwartz 2006). This study is pioneering in analyzing the effects of noise from wind farms on the behavior of anuran calling in the Caatinga and future studies may show additional evidence necessary to confirm our results in view of the growth of wind farms in this biome (Neri et al. 2019).

In fact, many factors can influence the variation of acoustic parameters of anuran calling, such as evolutionary stochastic processes (Erdtmann and Amézquita 2009), divergent selective pressures in different habitats or even a combination of these two processes (Gerhardt 1994). In addition, individual intrinsic variables (not considered here in the regression model), such as body size (mass and SLV), also influence the acoustic parameters of anuran calling (Wells and Schwartz 2006; Ziegler et al. 2011; Röhr et al. 2016), and therefore should be considered in future studies.

Finally, we highlight that actions to mitigate anthropogenic noise in terrestrial ecosystems should bet on technological innovations such as wind turbine helix, car engines and quieter airplanes (McGregor et al. 2013; Shannon et al. 2015), since they would be more efficient than noise barriers (e.g. on highways) (Lengagne 2008). In addition, mitigation methods should coincide with biologically sensitive periods of the target species, such as in the breeding season (e.g. anurans) or during seasonal movement (e.g. bird migration) (Shannon et al. 2015).

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SUPPLEMENTARY MATERIAL

Table 1: Descriptive measures for the acoustic variables CA = Call Amplitude, DF = Dominant Frequency, AF = Frequency Amplitude, CD = Call Duration, CP = Call Pulses and CRR = Call Repetition Rate. Std. Dev = Standard Deviation.

	CA	DF	AF	CD	CP	CRR
Mean	79.743	2710.832	2879.366	0.170	13.952	265.141
Std. Dev.	8.727	948.100	785.609	0.098	7.341	404.152
Maximum	110.037	5706.522	5739.130	0.391	34.700	1708.000
Minimum	65.100	899.351	1125.665	0.013	4.800	20.000

Figure 1: Box-plot graphs showing the sample distribution of variables with logarithm of: (a) Call Amplitude (dB), (b) Dominant Frequency (Hz), (c) Amplitude Frequency (Hz), (d) Call Duration (s), (e) Call Pulses (f) Call Repetition Rate (calls/min) for each of the three species included in the experiment: *P. cicada*, *S. pachycrus* and *S. x-signatus*.

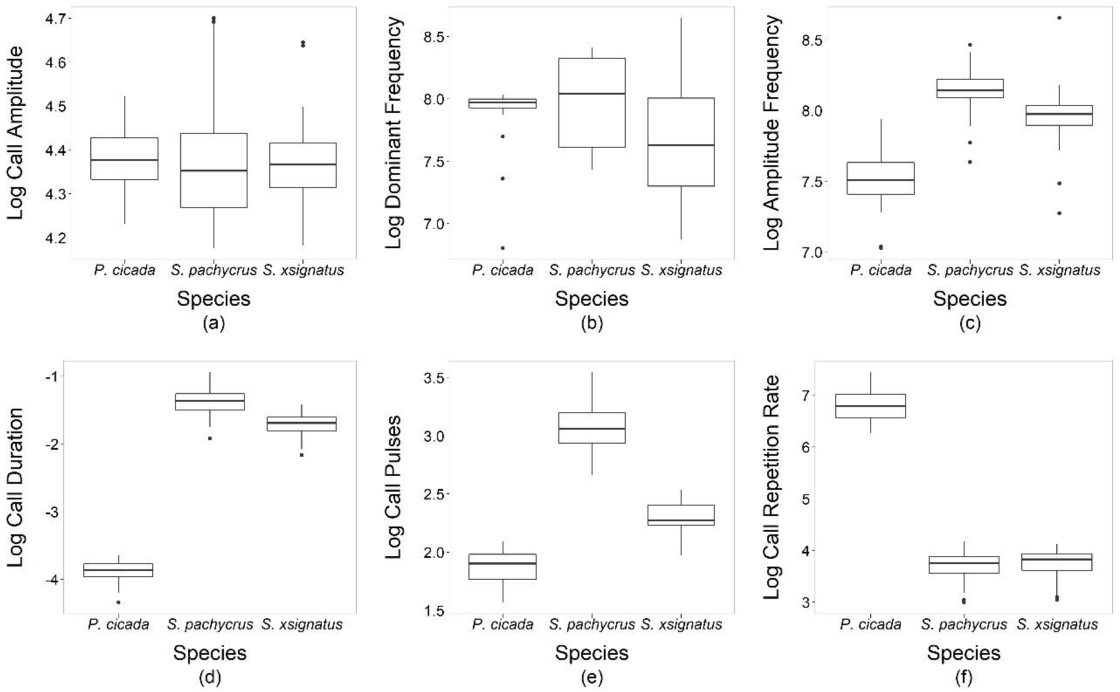

Figure 2: Partial effect of Air Temperature (°C) on the dependent variables of models (i) and (ii) (see Methods). Graphs (a), (b) and (c) referring to model (i), with logarithm of: (a) call amplitude (dB), (b) dominant frequency (Hz) and (c) amplitude frequency (Hz). Graphs (d), (e) and (f) for model (ii), with logarithm of: (d) call duration (s), (e) call pulses and (f) call repetition rate (calls/min). Observed data for the three anuran species are differentiated in the graphs as follows: *P. cicada* = open circle, *Scinax pachychrus* = filled circle and *S. x-signatus* = filled triangle. Regression lines are differentiated by anuran species as follows: P. cicada = solid line, *S. pachychrus* = dashed line and *S. x-signatus* = dotted line.

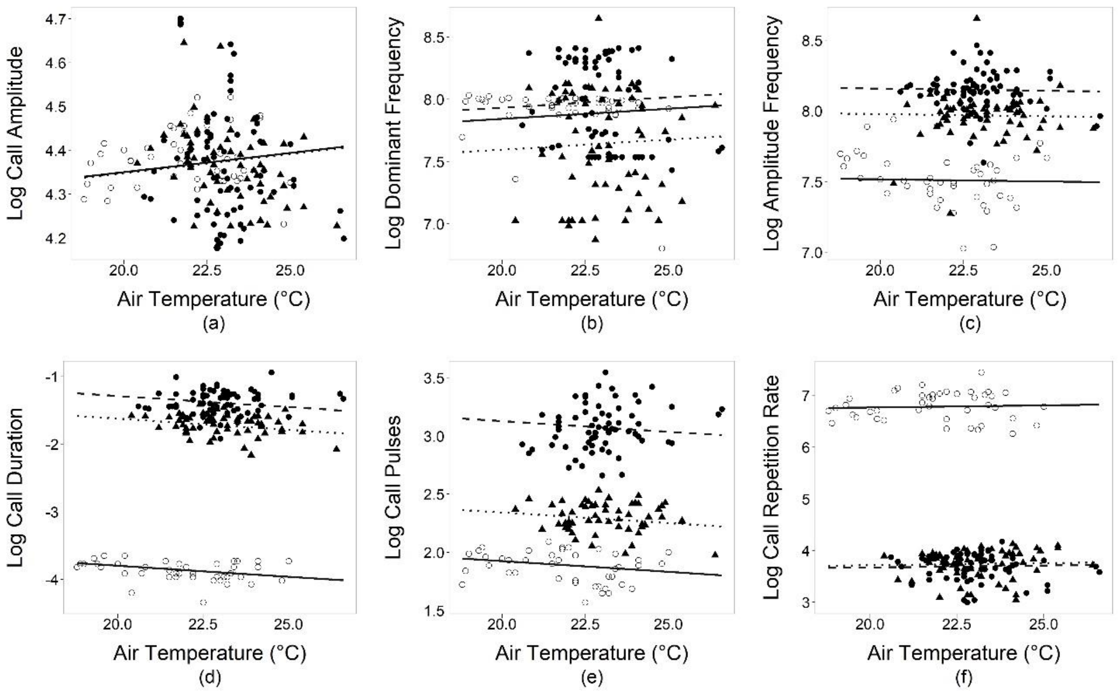

Figure 3: Partial effect of Air Relative humidity (%) on the dependent variables of models (i) and (ii) (see Methods). Graphs (a), (b) and (c) referring to model (i), with logarithm of: (a) the call amplitude (dB), (b) dominant frequency (Hz) and (c) amplitude frequency (Hz). Graphs (d), (e) and (f) referring to model (ii), with logarithm of: (d) call duration (s), (e) call pulses and (f) call repetition rate (calls/min). Observed data for the three anuran species are differentiated in the graphs as follows: *P. cicada* = open circle, *Scinax pachychrus* = filled circle and *S. x-signatus* = filled triangle. Regression lines are differentiated by anuran species as follows: *P. cicada* = solid line, *S. pachychrus* = dashed line and *S. x-signatus* = dotted line.

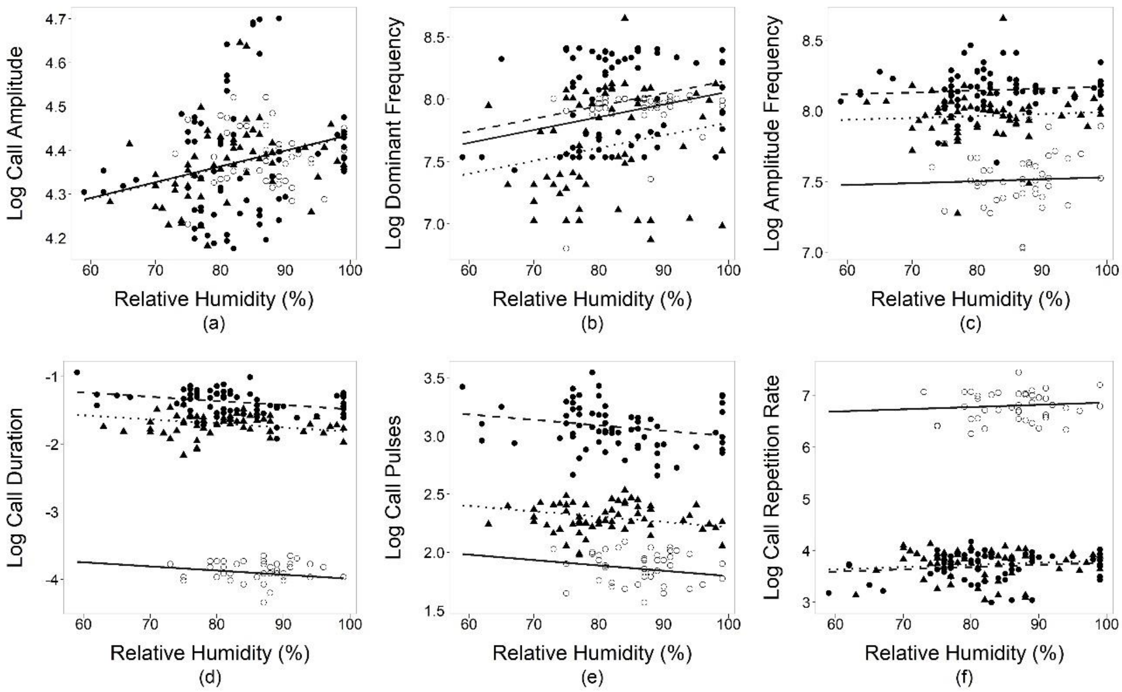

Figure 4: Diagnostic graphs of regression models (i) and (2). Graphs (a), (b) and (c) referring to model (i) adjusted for dependent variables with logarithm of the call amplitude, logarithm of the dominant frequency and logarithm of the amplitude frequency. Graphs (d), (e) and (f) referring to model (ii) adjusted for dependent variables with logarithm of the call duration, logarithm of the call pulses and logarithm of the call repetition rate. (a) and (d) graph of studentized residues against the sample's observations index; (b) and (e) graphs of studentized residues against adjusted values; (c) and (f) graphs of the Mahalanobis distance for each observation in the sample. Cut-off point for the Mahalanobis distance based on the 90% quantile of the Chi-Square distribution with 3 degrees of freedom.

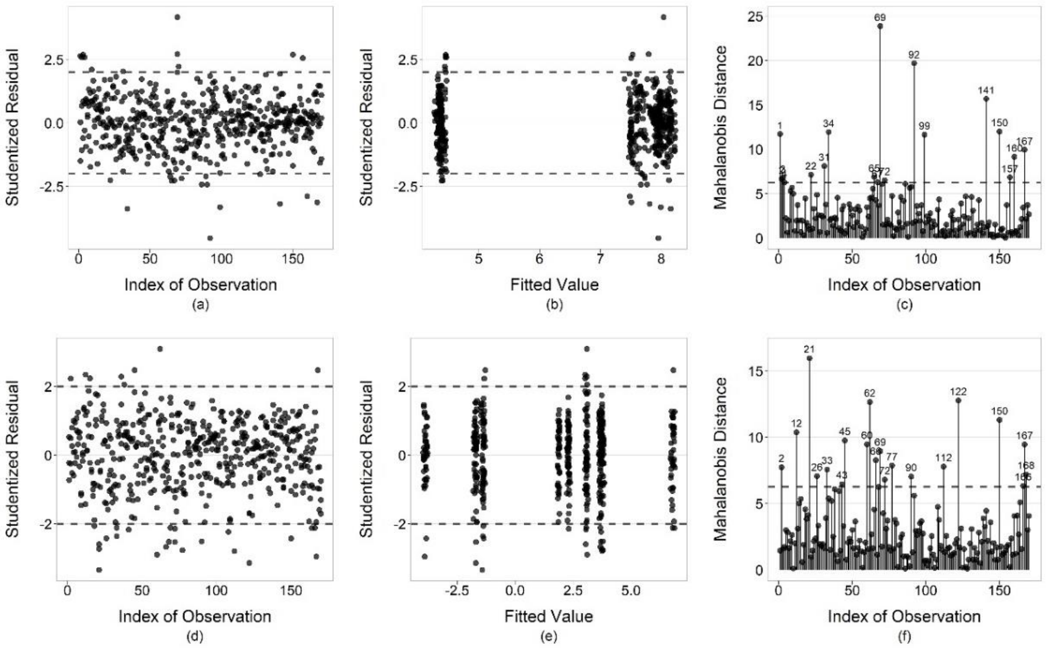

Table 2: Matrix of correlations between the dependent variables of model 1. CA = Call Amplitude, DF = Dominant Frequency and AF = Amplitude Frequency.

	CA	DF	AF
CA	1.000		
DF	0.201	1.000	
AF	-0.194	0.211	1.000

Table 3: Matrix of correlations between the dependent variables of model (ii). CD = Call Duration, CP = Call Pulses and CRR = Call Repetition Rate.

	CD	CP	CRR
CD	1.000		

CP	0.753	1.000	
CRR	0.103	0.240	1.000

ANEXOS

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A new species of *Dendropsophus* Fitzinger, 1843 (Anura: Hylidae) from the Pernambuco Endemism Center, Northeastern Brazil

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Short title: **A new species of *Dendropsophus* from Northeastern Brazil**

Abstract: We describe a new species of the *Dendropsophus decipiens* Clade, morphologically most resembling *D. haddadi* but genetically more closely related to *D. oliveirai* and likely endemic from Atlantic Forest biome, northeastern Brazil. The new species can be distinguished from all species of the *D. decipiens* Clade based on the combination of morphological features, advertisement call and phylogenetic position based on mitochondrial DNA gene sequences. The phylogenetic position of the species of the *D. decipiens* Clade is provided for the first time. Although distributed within the Pernambuco forest refuge, Pleistocene climatic oscillations had no effect on the cladogenesis of the new species as its origin dates back to the Miocene. The new taxa represent the fifth species of the *D. decipiens* Clade, which likely harbors more undescribed taxa, corroborating the view that Neotropical species richness is fairly underestimated.

INTRODUCTION

The genus *Dendropsophus* Fitzinger, 1843 currently comprise 108 species broadly distributed across Neotropical rainforests and open areas from Southern Mexico to northern Argentina and Uruguay, east of Andes [1]. Based on morphological and molecular data, nine species groups are currently recognized within this genus: *D. columbianus, D. garagoensis, D. labialis, D. leucophyllatus, D. marmoratus, D. microcephalus, D. minimus, D. minutus* and *D. parviceps* [2]. The *D. microcephalus* Group is the most speciose encompassing approximately 40 species arranged in the *D. decipiens*, *D. microcephalus* and *D. rubicundulus* Clades, although a rigorous test is still needed to confirm their monophyly [2].

The *D. decipiens* Clade currently comprises four species: *D. berthalutzae* (Bokermann, 1962), *D. decipiens* (Lutz, 1925), *D. haddadi* (Bastos and Pombal, 1996) and *D. oliveirai* (Bokermann, 1963), which exhibits as putative synapomorphies the behavior of egg laying in leaves overhanging water and tadpoles with an oral disc bearing a posterior row of marginal papillae on [2,3]. Species of the *D. decipiens* Clade are distributed along eastern Brazil and mostly associated with the Atlantic Forest Biome [1], except for *D. oliveirai* which is also found in ecotonal areas of Caatinga (a seasonally dry tropical forest) and Atlantic Forest biomes in northeastern Brazil from Bahia to Rio Grande do Norte states [1,4,5]. More specifically, *D. berthalutzae* occurs along a narrow Atlantic Forest zone in southeastern Brazil from Paraná to Espírito Santo state, *D. decipiens* occurs across São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo states and *D. haddadi* occurs from the Espírito Santo to Pernambuco states [1].

Because of high rates of endemism and alarming levels of habitat loss, the Atlantic Forest biome is among the world`s top priorities for biodiversity conservation [6]. Although the process of habitat loss occurred along the entire coastal region, it was more striking along northeastern Brazilian coast [7]. The Atlantic Forest also represents an important biogeographic component for studies focusing on anuran diversification. For instance, the historical climatic stability and subsequent persistence of forest refuges during Quaternary glacial cycles Carnaval and Moritz model [8], predicts genetic structure and phylogeographic endemism of organisms associated with this biome. Along the northern portion of the Atlantic Forest, these refuges comprise the Central Corridor in Bahia State and the Pernambuco Endemism Center, from Alagoas State to southern Paraiba State [8,9]. Moreover, the São Francisco River flows between these two refuge zones, which act as a barrier to gene flow for some taxa distributed in northeastern Brazil [10,11], especially for small to medium sized species with high fidelity and low vagility such observed among anurans. Despite some controversies regarding the generalization of the Carnaval and Moritz model [15,16], the historical dynamic process of forest expansion/retraction triggered by Pleistocene climate oscillations partly explain the high species richness of the Atlantic Forest biome in comparison to other South American biomes [17].

Although there are records for *D. haddadi* from the Bahia refuge zones [18,19] and northwards to the São Francisco River, in Pernambuco Endemism Center [20–23], there are no studies that evaluated the genetic diversity and/or acoustic identify of these populations, which were identified based solely on external phenotypic traits. The high levels of morphological similarity and intraspecific variation hinders the advance of taxonomic resolutions among *Dendropsophus* species [24,25], making the use of different lines of evidence highly desirable to elucidate patterns of cryptic diversity within the genus.

Herein, we combine morphological, acoustic and molecular data to describe a new species of *Dendropsophus*, morphologically similar to *D. haddadi*, *D. decipiens*, and *D. oliveirai*, representing the fifth species of *D. decipiens* Clade likely endemic to the

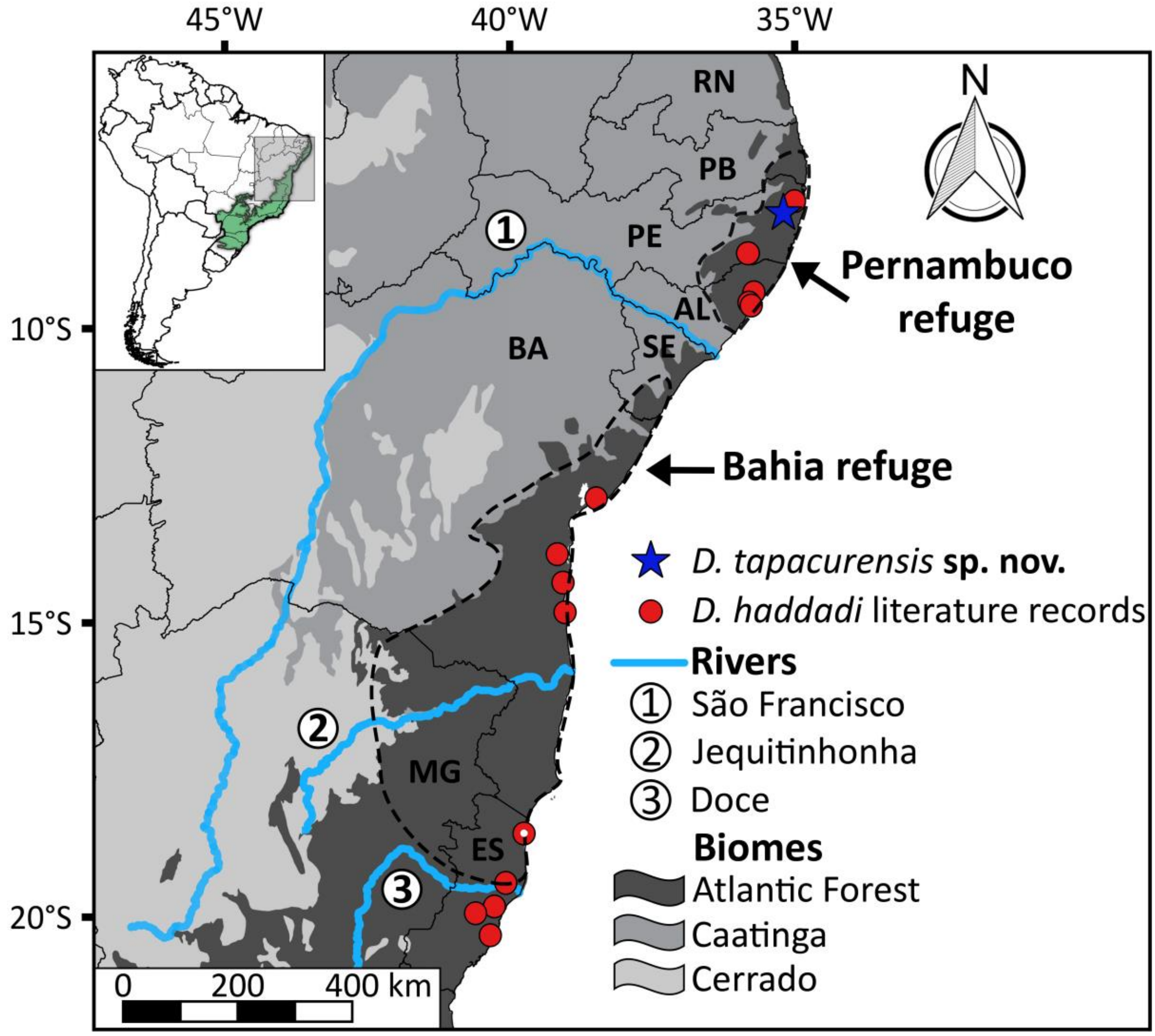
Atlantic Forest biome, and the first with type locality at the Pernambuco Endemism Center, which represents one of the most endangered Atlantic Forest remnants in Brazil [6,7].

MATERIAL AND METHODS

Study area and reference material

We conducted field work on July 2018 at two conservation units: the Buchada Forest (100 ha) (8°2'26.13"S, 35°12'0.43"W; 122 m above sea level [a.s.l.], DATUM WGS84) and the Camocim Forest (200 ha) (8°1'59.75"S, 35°12'3.79"W; 131 m a.s.l., DATUM WGS84). These two conservation units compose the Tapacurá Ecological Station, a protection unit administered by the Universidade Federal Rural de Pernambuco, located at São Lourenço da Mata municipality, Pernambuco State, Brazil (Fig. 1). The area is characterized by semi deciduous seasonal Atlantic Forest fragments [26], the climate is monsoon type (Am) according to classification by [27], and the rainy season occurs from February to September with average annual precipitation of 1.900 mm [28].

Fig. 1. Geographic distribution of the new species and literature data on the occurrence of *Dendropsophus haddadi* (white dot denotes the type locality). Dashed lines represent an approximation of forest refuge areas as predicted by Carnaval and Moritz climatic models [8]. Brazilian State acronyms = AL: Alagoas, BA: Bahia, ES: Espírito Santo, MG: Minas Gerais, PB: Paraíba, PE: Pernambuco, RN: Rio Grande do Norte, and SE: Sergipe.



We collected a total of 21 adult specimens in the studied area (permit IBAMA/RAN 087/07). Specimens were euthanized with 5% lidocaine, fixed in 10% formaldehyde, preserved in 70% ethanol and deposited at Coleção Herpetológica e Paleoherpetológica of the Universidade Federal Rural de Pernambuco, Recife, Brazil (CHP-UFRPE 5697–5717). Locality data is given in Table 1 and Fig 1. For comparisons, we evaluated specimens of *D. berthalutzae* from Paranapiacaba, São Paulo State (topotypes), *D. decipiens* from Duas Barras (distant 125 km from type locality), Rio de Janeiro State, *D. haddadi* from Santa Teresa (paratopotypes) and Sooretama (distant 65 km from type locality), Espírito Santo State, and *D. oliveirai* from Maracás, Bahia State (topotypes), all of which are housed at Collection of frogs (AAG-UFU) at Universidade Federal de Uberlândia, Uberlândia municipality, Minas Gerais State (S1 Appendix). Other specimens examined by us are listed in Appendix S1. Institutional abbreviations followed [29]. Tissue samples were made available by the tissue sample collection of the Laboratório de Anfíbios e Répteis da Universidade Federal do Rio Grande do Norte (CLAR–UFRN) or previously acquired by some of us.

Table I. Locality data for specimens of the new species and *Dendropsophus haddadi* available in literature.

Species	**Municipality**	**State**	**Latitude**	**Longitude**	**Re**
Dendropsophus sp. nov.	São Lourenço da Mata	PE	-8.04	-35.20	**Th**
D. haddadi	Igarassú	PE	-35.00	-7.83	
D. haddadi	Lagoa dos Gatos	PE	-8.72	-35.85	
D. haddadi	Maceió	AL	-35.72	-9.38	
D. haddadi	Maceió	AL	-35.80	-9.56	
D. haddadi	Maceió	AL	-35.76	-9.61	
D. haddadi	Igrapiúma	BA	-39.17	-13.83	
D. haddadi	Ilhéus	BA	-39.03	-14.82	
D. haddadi	Itacaré	BA	-39.07	-14.32	
D. haddadi	Mata de São João	BA	-38.48	-12.88	
D. haddadi	Conceição da Barra	ES	-39.75	-18.58	
D. haddadi	Linhares	ES	-40.07	-19.42	
D. haddadi	Aracruz	ES	-40.27	-19.82	
D. haddadi	Santa Teresa	ES	-40.60	-19.94	

D. haddadi	Vitória	ES	-40.34	-20.31

Morphometry

We measured specimens using a Digimess digital caliper (to the nearest 0.1mm). Eight measurements followed [35] terminology: snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), tympanum diameter (TD), eye-nostril distance (END), foot length (FL), and shank length (SL). For the other two characters, we follow [36]: hand length (HAL) and thigh length (THL). Webbing formula followed [37].

Acoustics

We recorded advertisement calls with a TASCAM DR40 digital recorder set at 44.1 kHz and resolution of 16 bits, coupled to a Yoga HT81 directional microphone. Measurements were analyzed using Raven Pro 1.5 for Windows from The Cornell Lab of Ornitology [38]; spectrogram settings were Hann, window size = 1024 samples, 3 dB bandwidth = 270 Hz, Overlap = 85%, hop size = 0.792, DFT size = 1024 samples, and grid spacing = 46.9 Hz. All other settings followed the ‘default’ of Raven. Sound figures were obtained in the Seewave package v. 1.5.9 [39], on the R platform version 3.6.1 [40]; Seewave settings were Hanning window, 256 points resolution (FFT), and 85% of overlap. Call terminology follows [41], using a note-centered approach. The recording files were deposited at the Sonoteca Coaxar of the Coleção Herpetológica e Paleoherpetológica of the Universidade Federal Rural de Pernambuco, Recife, Brazil (SCLEHP 18–28; see SII Appendix).

Molecular analysis

We assembled a total of 19 tissue samples comprising topotypical and paratopotypical representatives of *Dendropsophus oliveirai* and *D. haddadi*, respectively, also including five paratopotypes of the new species. We extracted genomic DNA from liver tissues and amplified the mitochondrial H-strand transcription unit 1 (H1; which include segments of the *12S* and *16S* ribosomal genes, and the intervening valine-*tRNA*) using primers and Polymerase Chain Reactions (PCRs) protocols provided by [2]. Total DNA was extracted from tissue samples using Kasvi's Mini Spin DNA Extraction Kit following the protocols described in the kit manual, except for the addition of QIAGEN's Tissue Lysis Buffer in the first DNA extraction step. PCR products were then purified using Invitrogen's PureLink ™ Genomic DNA Mini Kit following the protocol described in the kit without any modification. Purified PCR products were sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit. We assembled a complete H1 segment (~2400 base pairs [bp]) for three samples, while the remaining 16 samples had at least the final *16S* segment (ca. 550pb; primers 16Sar–br from [42]) sequenced. We checked sequencing quality and edited chromatograms in the program Geneious v1.8.7 [43].

To infer the phylogenetic relationships of species in the *Dendropsophus decipiens* Clade, we created a final H1 alignment dataset for 87 specimens (our 19 sequenced individuals plus GenBank sequences) that had the complete H1 segment available in GenBank, encompassing individuals from all species groups or phenetic clades proposed for the genus [2,44,45]. As outgroups, we selected 21 terminals including species of genus *Lysapsus*, *Pseudis*, *Scarthyla*, *Scinax*, *Sphaenorhynchus*, and *Xenohyla*. We aligned all sequences using MAFFT algorithm [46] default configuration also implemented in Geneious v1.8.7 [43]. Some regions along the H1 segment (e.g., hypervariable regions) are difficult to align because of the high amounts of gaps generated along them and, in most cases, the resolution of alignment homology is not straightforward and maybe not replicable. Therefore, we filtered our alignment in GBLOCK v. 0.91b [47] to remove all poorly aligned regions from our final matrix. This approach yielded a final H1 dataset of 2084 bp containing segments of 12S and 16S ribosomal and the intervening valine-tRNA mitochondrial genes. Shorter sequences were included in the trimmed H1 alignment after GBLOCK procedure.

A major question of this work is to acknowledge if populations previously assigned to *Dendropsophus haddadi* occurring along Pernambuco refuge are reciprocally monophyletic to paratopotypical populations from Espírito Santo and if genetic diversification among these populations was driven by Pleistocene climatic oscillations (see Fig 1). For such purpose, we estimated phylogenetic relationships along with divergence time for *Dendropsophus* species under Bayesian approach using BEAST v.1.10.4 [48] implementing the GTR+I+G substitution model as suggested by the Akaike Information Criterion [49] in jModeltest version 2.1.6 [50]. To calibrate the molecular clock, we used a normal prior distribution (mean = 7.35 x 10^{-3} substitutions/site/million year; SD = 1.0 x 10^{-3}) on the substitution rate of the mtDNA (*12S* and *16S*) following recent estimates for *D. minutus* [51], which implemented geological constraints as calibration points. We analyzed the dataset under a relaxed uncorrelated lognormal clock [52] allowing branches to have independent substitution rates along with a birth-death speciation model as tree prior. We run BEAST analyses for 50 million generations sampling every 5000 generations, discarding the initial 25% as burn-in. We assessed convergence (ESS>200) with Tracer v1.7, generated the maximum credibility tree (MCC) with TreeAnnotator v1.10.4 (https://beast.community/treeannotator), and drew all phylogenetic trees using FigTree v1.4.2 [53]. We considered Bayesian PP < 0.95 as significant [48].

Finally, we computed between-group mean distances using [54], corrected p-distances with MEGA v6.0.6 [55]. Considering that we recovered some genetically structured and/or paraphyletic lineages (see results), we employed the Automated Barcoding Gap Discovery (ABGD) method [56] to objectively delimit genetic clusters used to calculate between-group distances. Because this method does not accept gaps in the alignment (or assume that gaps count as additional mutational steps), we used a pruned 375 bp of the final 16S segment to calculate genetic distances between species in the *Dendropsophus decipiens* Clade, also comparing with *D. bromeliaceus* and *Xenohyla truncata* sequences. For the ABGD analysis, we set priors for minimum and maximum intraspecific divergence as 0.01 and 0.1, respectively, and relative gap width was set as 1.5. Remaining configurations were set as default. We selected the most constantly recovered groupings in the recursive partitions, as proposed by [56]. GenBank accession numbers for all sequences used by us are given in SIII Appendix.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts contained have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn: lsid: zoobank.org: pub: xxxxxxx. The electronic edition of this work was published in a journal with an ISSN has been archived and is available from the following digital repositories: PubMed Central and LOCKSS.

Species description

Dendropsophus sp. nov.

(Fig 2, 3, 4 and 5)

Fig 2. Holotype of *Dendropsophus* sp. n. (CHP-UFRPE 5710). (A) dorsal and (B) ventral views of body.

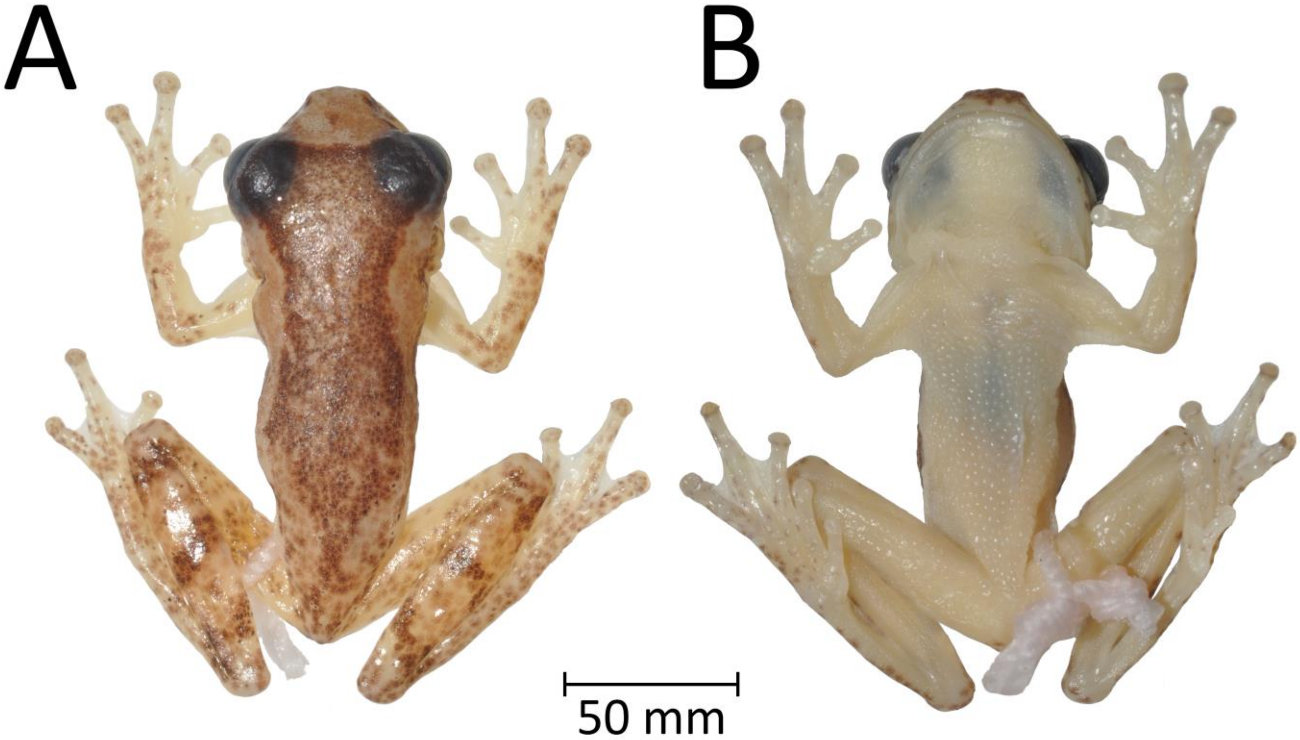


Fig 3. Holotype of *Dendropsophus* sp. n. (CHP-UFRPE 5710). (A) Dorsal and (B) profile views of head. Views of (C) hand and (D) foot. View of female CHP-UFRPE 5704 (E) hand and (F) foot, evidencing the presence of pointed discs.

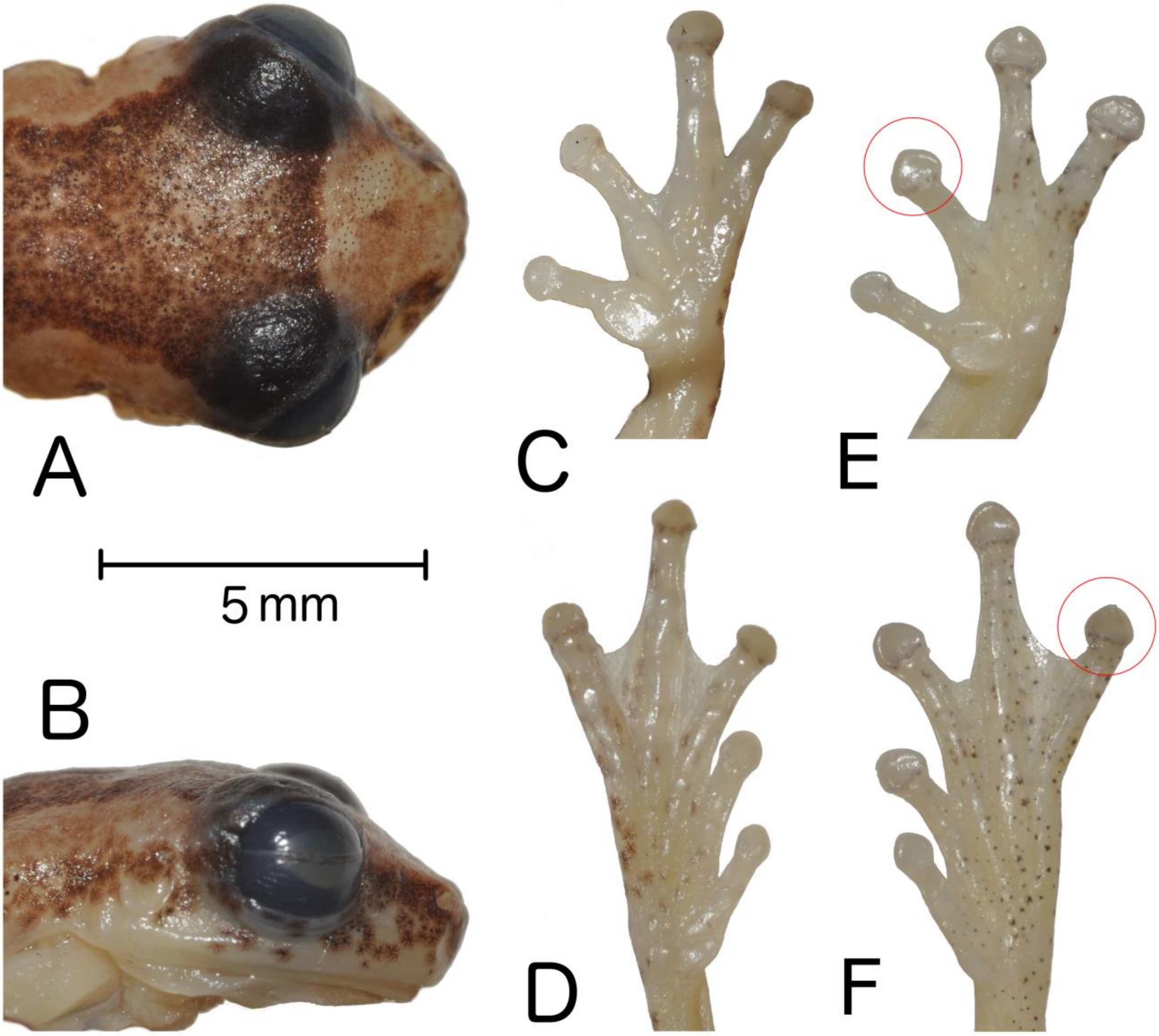


Fig 4. Specimens of *Dendropsophus* sp. n. depicting differences in pigmentation patterns and size. Females (A) CHP-UFRPE 5713 and (B) CHP-UFRPE 5704; and males (C) CHP-UFRPE 5703 and (D) CHP-UFRPE 5714.

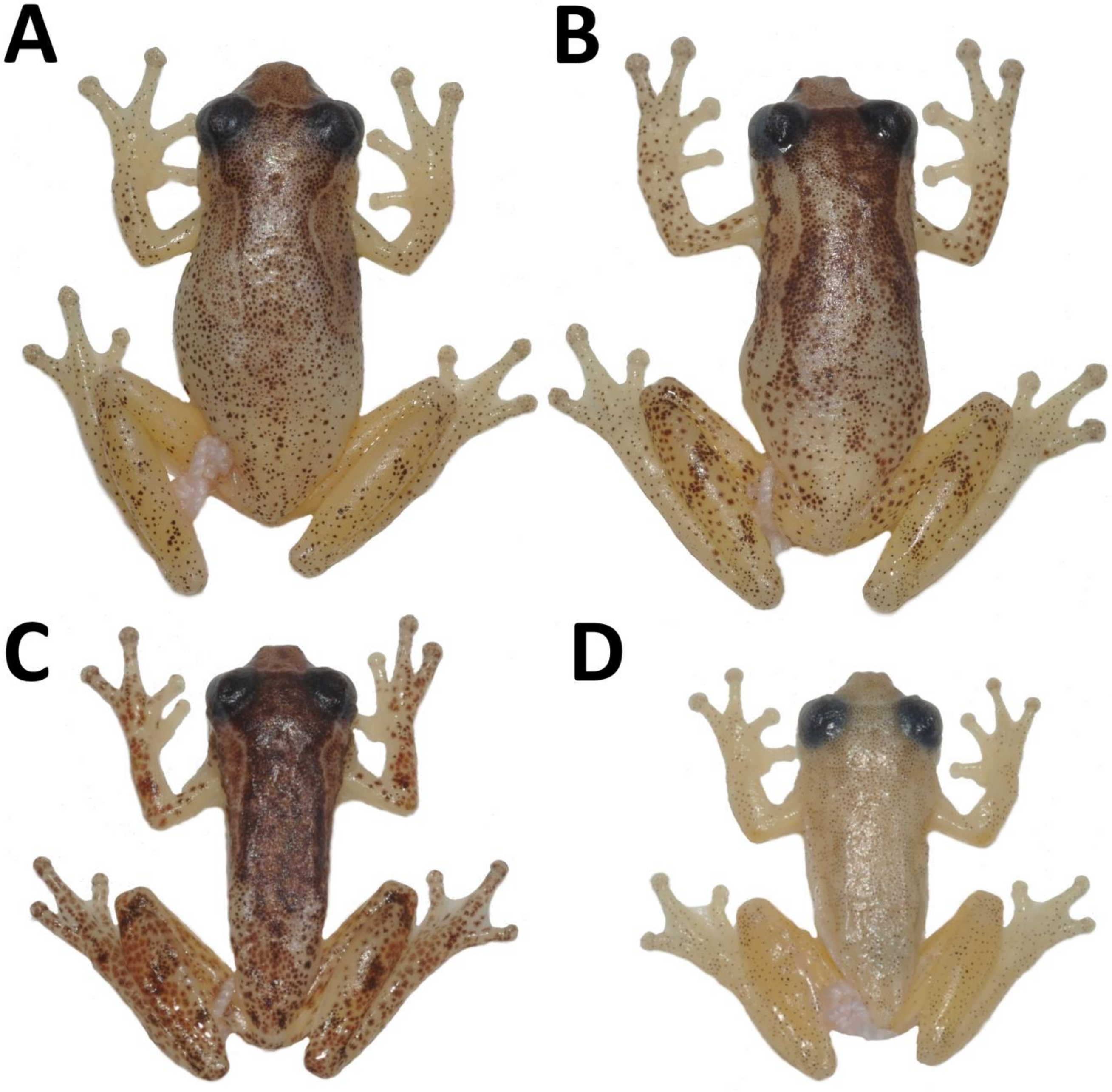


Fig 5. Live specimens of *Dendropsophus* sp. n. from type locality. (A) adult male; (B) adult female; (C) amplectant pair; (D) egg mass deposited on leaves overhanging at standing water.

Holotype

Adult Male (CHP-UFRPE5709), collected in July 13, 2018, by Rogério F. de Oliveira and Felipe de M. Magalhães in the Estação Ecológica do Tapacurá, Municipality of São Lourenço da Mata, Pernambuco State, Brazil (8°2'26.13"S, 35°12'0.43"W; 122 m a.s.l., DATUM WGS84).

Paratypes

Eighteen adult males CHP-UFRPE 5697–5702, CHP-UFRPE 5704–08, CHP-UFRPE 5710–11, CHP-UFRPE 5713–17 and two egg-bearing females CHP-UFRPE 5703 and CHP-UFRPE 5712, collected along with the holotype.

Diagnosis

We attributed the new species to the genus *Dendropsophus* and specifically to the *D. decipiens* Clade based on our phylogenetic analysis results (see below). In addition, the new species closely resemble the other species of the Clade (specially *D. haddadi*) and lay eggs on leaves overhanging temporary ponds (Fig 5D), a feature that is considered a putative synapomorphy of the *D. decipiens* Clade [2].

Dendropsophus sp. n. can be distinguished from congeners by the combination of the following features: (1) small size, adult males 15.5–17.6 mm SVL (mean 16.4 mm) and adult females 19.5–20.7 mm SVL (mean 20.1 mm); (2) head wider than long; (3) vocal sac single, subgular, and light yellow (in life); (4) the presence of transversal bars on the shanks; (5) dorsolateral region delimited by an irregular light brown strip that extends from the posterior region of the eye towards the inguinal region; (6) presence of a triangular-shaped mark in the loreal region with the same coloration of the dorsolateral strip; (7) advertisement call with only one type of note; (8) pulse repetition rate ranging from 143 to 368 pulses/s; and (9) dominant frequency ranging from 5578–6422 Hz.

Holotype description

Adult male. Proportions of body parts in relation to SVL (16.5 mm): head length 0.31; head width 0.36, eye diameter 0.17, tympanum diameter 0.06, hand 0.32, foot 0.4, femur 0.45, tibia 0.51. Head wider than long; snout truncated, discretetly mucronate in dorsal view and rounded in lateral view; snout tip (mucronate condition) perceivable in lateral view at the level of nostrils; canthus rostralis rounded, slightly curved; loreal region slightly concave; nostrils slightly protuberant, directed dorsolaterally; interorbital area flat; eyes large and protuberant; pupil horizontally elliptical; lower eyelid mostly transparent, its free border pigmented as the upper eyelid; supratympanic fold barely visible, not extending beyond tympanum; tympanum distinct, circular, annulus barely defined dorsally; choanae oval; vomerine teeth small, only perceptible when probed; tongue cordiform, notched behind, posterior ¼ free from mouth floor; vocal slits well defined; vocal sac developed, single, subgular, extending over chest; forearm slender than arm; axillary membrane reaching half arm; out margin of forearm smooth; fingers bearing circular discs, albeit disc of finger IV slightly pointed; relative length of fingers I < II <IV < III; second subarticular tubercles well-defined, shallow, round on fingers I, II III and cordiform on IV; inner metacarpal tubercle flat, shallow, fused to the adjacent subarticular; outer metacarpal tubercle indistinct; nuptial pads covering the prepolical area, perceived as discrete asperities; webbing formula ItraceII2–3III21/2–2+IV; hind limb long and slender; no tarsal fold; no calcar ornament; toe discs I, II, III and V circular, IV discretely pointed; relative lengths of toes I < II < IV < III < V; subarticular tubercles round and shallow; inner metatarsal tubercle shallow, flat, elliptical, fused to the adjacent subarticular; outer metatarsal tubercle indistinct; foot webbing formula I2–2 1/2II1 1/2–2 3/4III1 1/2–2 1/3IV2 1/3 –1 V; skin on dorsum, head, dorsal surfaces of forearms and thighs, flanks and groin smooth; skin on belly and ventral surfaces of thighs granular; cloacal opening directed posteriorly, covered by a dorsal sheath (vellum) from above; cloacal ornamentation absent.

Holotype coloration in preservative

The general body coloration is pale yellow. Dorsal region dark brown colored, extending from the interorbital region towards the inguinal region. The dorsal colored area is irregular, with a narrowed area at shoulder girdle region, followed by an enlargement in the mid region of the body and an abrupt narrowing towards sacral region. The dorsolateral region is delimited by an irregular lighter brown (ocher) strip that extends from the posterior region of the eye to the inguinal region. The narrowing and enlargement of dorsal coloration is a reflex of the enlargement and narrowing of the dorsolateral strip, which is superiorly delimited by the dorsal colored area and inferiorly by a non-homogeneous dark brown line. The loreal region has the same color of dorsolateral strip and exhibit a triangular shaped mark delimited by a dark brown of dorsum in the interorbital region and by a darker brown line in the canthus rostralis. Arm, forearm, thigh and feet pale yellow. Dorsal surfaces of tibia with three dark brown bars. Ventral region homogeneously light cream colored.

Variation in morphology and color

Measurements of the type series in Table 2. Finger disc of toe IV can be circular in some males. Alternative webbing formulae includes: I 2–2 II 11/2–21/2 III 2+–2+ IV and I 2–2+ II 11/2 – 21/3 III 11/2 – 21/3 IV 2+ – 11/2 V. Females are larger than males (Fig 4). Morphology and color pattern are most of times concordant with the holotype, however, the degree of dorsal pigmentation varies greatly (Fig 4) from mostly dark brown to pale yellow with scattered dark spots (individual melanophores). The lighter dorsolateral strip almost always discernible (usually not homogeneously pigmented). The dorsolateral strip may be regular, without any enlargement or narrowing (as depicted in Fig 5B). Dorsum and limbs coloration varying from light brown to dark brown. The transversal bars on tibia posterior surface vary in size and thickness being more (Fig 5B) or less evident (Fig 5A and C); this feature may disappear in preserved specimens. Some specimens exhibit light colored spots below eyes (Fig 5A–C). In living specimens, a light brown line outlines the eyes superiorly. Both females have better-defined pointed digit discs than males (Fig 3E and F).

Table 2. Measurement (in mm) for adult males and females of *Dendropsophus* sp. n. type series. Values are presented as mean ± SD (range). *n* = total number of measured individuals.

Morphometric variables	**Holotype (Male)**	**Male (*n*=19)**	**Female (*n*=2)**
Snout-vent length	16.32	16.4 ± 0.6 (15.5–17.6)	20.1 ± 0.6 (19.5–20.7)
Head length	5.25	4.9 ± 0.5 (3.9–5.6)	5.8 ± 0.8 (5.1–6.6)
Head width	5.85	5.9 ± 0.3 (5.5–6.3)	6.8 ± 0.01 (6.8–6.8)
Eye diameter	2.41	2.5 ± 0.1 (2.4–2.8)	2.7 ± 0.06 (2.6–2.7)
Tympanum diameter	1.05	1 ± 0.03 (1.0–1.1)	1.2 ± 0.02 (1.2–1.2)
Eye-nostril distance	1.45	1.4 ± 0.02 (0.8–1.9)	1.6 ± 0.06 (1.5–1.6)
Hand length	5.07	5.4 ± 0.3 (4.8–5.8)	6.5 ± 0.4 (6.1–6.8)
Foot length	7.32	6.7 ± 0.6 (5.5–8.0)	8.8 ± 0.03 (8.5–9.0)
Thigh length	7.68	7.5 ± 0.5	9.5 ± 0.4

		(6.5–8.3)	(8.9–9.6)
Shank length	8.02	8.5 ± 0.5 (7.0–8.9)	10.5 ± 0.3 (10.3–10.7)

Comparisons with other species

The new species most resembles species of the *D. microcephalus* Group (as defined in [2]) in general morphology, call traits and color pattern. Although not reciprocally monophyletic (see results), we compared *Dendropsophus* sp. n. with species in the *D. microcephalus* Group and, more specifically, to species in the *D. decipiens* Clade. Morphometric comparisons in the *D. decipiens* Clade are shown in Table 3.

Table 3. Measurement (in mm) for adult males of species in the *Dendropsophus decipiens* Clade: *D. berthalutzae* topotypes (Paranapiacaba/São Paulo), *D. decipiens* (Duas Barras/Rio de Janeiro), *D. haddadi* paratopotypes (Santa Teresa and Sooretama/Espírito Santo), *D. oliveirai* topotypes (Maracás/Bahia) and *Dendropsophus* sp. nov. type series (São Lourenço da Mata/Pernambuco).

Variables	***D. berthalutzae*** **(*n*=6)**	***D. decipiens*** **(*n*=4)**	***D. haddadi*** **(*n*=8)**	***D. oliveirai*** **(*n*=6)**	***Dendropsophus*** sp. nov. **(*n*=19)**
Snout-vent length	20.4 ± 0.5 (20.0–21.2)	17.4 ± 0.6 (16.7–18.4)	17.5 ± 0.6 (16.8–18.4)	14.3 ± 0.7 (13.6–15.5)	16.4 ± 0.6 (15.5–17.6)
Head length	7.1 ± 0.2 (6.8–7.5)	5.9 ± 0.1 (5.8–6.0)	7.1 ± 0.4 (6.6–7.6)	5.5 ± 0.3 (5.2–6.0)	4.9 ± 0.5 (3.9–5.6)
Head width	6.5 ± 0.03 (6.0–7.0)	5.2 ± 0.2 (5.0–5.5)	6.0 ± 0.2 (5.6–6.2)	4.0 ± 0.3 (4.7–5.5)	5.9 ± 0.3 (5.4–6.3)

Eye diameter	2.6 ± 0.02	2.3 ± 0.2	2.5 ± 0.2	2.0 ± 0.1	2.5 ± 0.1
	(2.3–2.9)	(2.1–2.4)	(2.2–2.7)	(2.0–2.1)	(2.4–2.8)
Tympanum diameter	1.6 ± 1.1	0.8 ± 0.0	0.9 ± 0.2	0.9 ± 0.1	1 ± 0.03
	(0.8–1.8)	(0.7–0.8)	(0.6–1.1)	(0.7–1.0)	(1.0–1.1)
Eye-nostril distance	1.8 ± 0.3	1.7 ± 0.1	1.9 ± 0.1	1.5 ± 0.3	1.4 ± 0.02
	(1.4–2.1)	(1.6–1.9)	(1.8–2.0)	(1.1–1.9)	(0.8–1.9)
Hand length	6.2 ± 0.02	5.3 ± 0.3	6.1 ± 0.5	4.8 ± 0.3	5.4 ± 0.3
	(6.0–6.7)	(5.0–5.7)	(5.4–7.0)	(4.3–5.0)	(4.8–5.8)
Foot length	9.2 ± 0.3	7.8 ± 0.3	8.5 ± 0.5	6.6 ± 0.4	6.7 ± 0.6
	(8.7–9.5)	(7.2–7.8)	(7.8–9.3)	(6.0–7.0)	(5.5–8.0)
Thigh length	10.0 ± 0.4	9.2 ± 0.4	9.8 ± 0.4	7.8 ± 0.2	7.5 ± 0.5
	(9.2–10.2)	(8.6–9.8)	(9.0–10.5)	(7.5–8.0)	(6.5–8.3)
Shank length	10.3 ± 0.2	9.6 ± 0.2	10.1 ± 0.4	7.2 ± 1.2	8.5 ± 0.5
	(10.1–10.5)	(9.5–10.0)	(9.5–10.8)	(5.5–8.6)	(7.0–8.9)

Dendropsophus sp. n. (SVL = 15.5–17.6 mm), differs from *D. berthalutzae* (SVL = 20.0–21.2 mm) by being smaller, by the absence of a X-shaped mark on dorsum (present in *D. berthalutzae*), by the presence of the dorsolateral strip that extends from the posterior region of the eye to the inguinal region, and colored loreal region (absent in *D. berthalutzae*). From *D. decipiens* (16.7–18.4 mm) by being slightly smaller (although values overlap), and by its slender body shape. From *D. haddadi* (16.8–18.4 mm) by being slightly smaller (although values overlap) and by its slender body shape. From *D. oliveirai*, by the presence of light brown dorsolateral strip instead of white strip as in *D. oliveirai*. In general, specimens of *D. oliveirai* exhibit a darker brown coloration on dorsum; specimens of *Dendropsophus* sp. n. are bigger than *D. oliveirai* (13.6–15.5 mm).

The new species differs from *D. anataliasiasi* (Bokermann, 1972), *D. araguaya* (Napoli and Caramaschi, 1998), *D. cachimbo* (Napoli and Caramaschi, 1999), *D. cerradensis* (Napoli and Caramaschi, 1998), *D. cruzi* (Pombal and Bastos, 1998), *D. elianeae* (Napoli and Caramaschi, 2000), *D. jimi* (Napoli and Caramaschi, 1999), *D. juliani* (Moravec et al. 2006), *D. rhea* (Napoli and Caramaschi, 1999), *D. rubicundulus* (Reinhardt and Lütken, 1862), *D. tritaeniatus* (Bokermann, 1965), *D. rozenmani* Jansen, Santana, Teixeira, and Köhler, 2019, *D. microcephalus* (Cope, 1886), *D. minusculus* (Rivero, 1971), *D. sanborni* (Schmidt, 1944), *D. walfordi* (Bokermann, 1962), *D. meridianus* (Lutz, 1954), *D. ozzyi* (Orrico et al., 2014), *D. shiwiarum* Ortega-Andrade and Ron 2013, and *D. riveroi* (Cochran and Goin, 1970) by the presence of the light brown strip present on the dorsum lateral limits of the body that extends from the interorbital region towards the inguinal region and same colored loreal region. From *D. bromeliaceus* Ferreira, Faivovich, Beard, and Pombal, 2015 by the absence of a cream mid-dorsal stripe from mid dorsum to cloaca, and by not rely on bromeliads for reproduction.

From *D. bipuncatus* (Spix, 1824), *D. studerae* (Carvalho-e-Silva et al., 2003), *D. branneri* (Cochran, 1948), *D. werneri* (Cochran, 1952), *D. reichlei* (Moravec et al., 2008), and *D. gaucheri* (Lescure and Martin, 2000) by the absence of one or numerous spots under the eyes. Differs from *D. joannae* (Köhler and Lötters, 2001), *D. julianae* (Moravec et al., 2006), and *D. coffeus* (Köhler et al., 2005) by the absence of dorsal tubercles. From *D. mathiassoni* (Cochran and Goin, 1970) by the absence of lymphatic sacs visible throughout the skin. From *D. gryllatus* (Duellman, 1973) (SVL = 22.6–25.5 mm), *D. sartori* (Smith, 1951) (SVL = 22.2–25.0 mm), and *D. robertmertensi* (Taylor, 1937) (SVL = 23.5–26.6 mm) by its smaller SVL (15.5–17.6 mm in *Dendropsophus* sp. n.). Differs from *D. phlebodes* (Stejneger, 1906), *D. rhodopelus* (Günther, 1858), and *D. nanus* (Boulenger, 1889) by having a single noted call [57–60]. Differs from *D. pseudomeridianus* (Cruz et al.,

2000) by having calls emitted mainly in sequences, isolated notes in *D. pseudomeridianus* [61]. The new species (39-110 ms) differs from *D. tintinnabulum* (Melin, 1941) (10–21 ms) by its longer note duration and greater pulse number (2–7 in *Dendropsophus* sp.n. and 2–4 in *D. tintinnabulum*) [60]. From *D. haddadi* by its lower (4312–4875 Hz) dominant frequency (5.578–6.422 Hz in *D. haddadi*) [19]. From *D. oliveirai* (56–161 pulses/s) by its higher pulse rate (143–368 pulses/s) [62]. From *D. decipiens* (4770–5230 Hz) by its higher dominant frequency (5578–6422 Hz).

Advertisement call

The advertisement call of *Dendropsophus* sp. n. (Fig 6) is composed of one type of pulsed note emitted in series of 3–9 notes, or rarely isolated (*n* = 6 males; Table 4). The first note of a series may has between-pulses interval (Fig 6B), and the last pulse of each note is longer than those preceding. Notes lasting from 39–110 ms (mean: 62 ms; SD = 6.4; *n* = 83). Notes with 9–29 pulses (mean: 18.0; SD = 1.2; *n* = 83), pulse duration varies from 2 to 7 ms (mean 3.4 ms; SD = 0.3; *n* = 96), and pulse repetition ratefrom 143–368 pulses per second (mean 291; SD = 19.9; *n* = 83). Call group rate varies from 2.9 to 5.0 notes per second (mean 3.8; SD = 0.4; *n* = 29). Internote interval in the series varies from 146–364 ms (mean 201 ms; SD = 26.5; *n* = 67). Dominant frequency varies from 5.578–6.422 Hz (mean 5.876 Hz; SD = 222.4; *n* = 83).

Fig 6. Advertisement call of *Dendropsophus* sp. n. (A) waveform of a call series with five notes (two seconds section), the note outlined is detailed in the (B, spectrogram) and (C, waveform; sound file: SCLEHP22) Air 24.5ºC and humidity 72%.

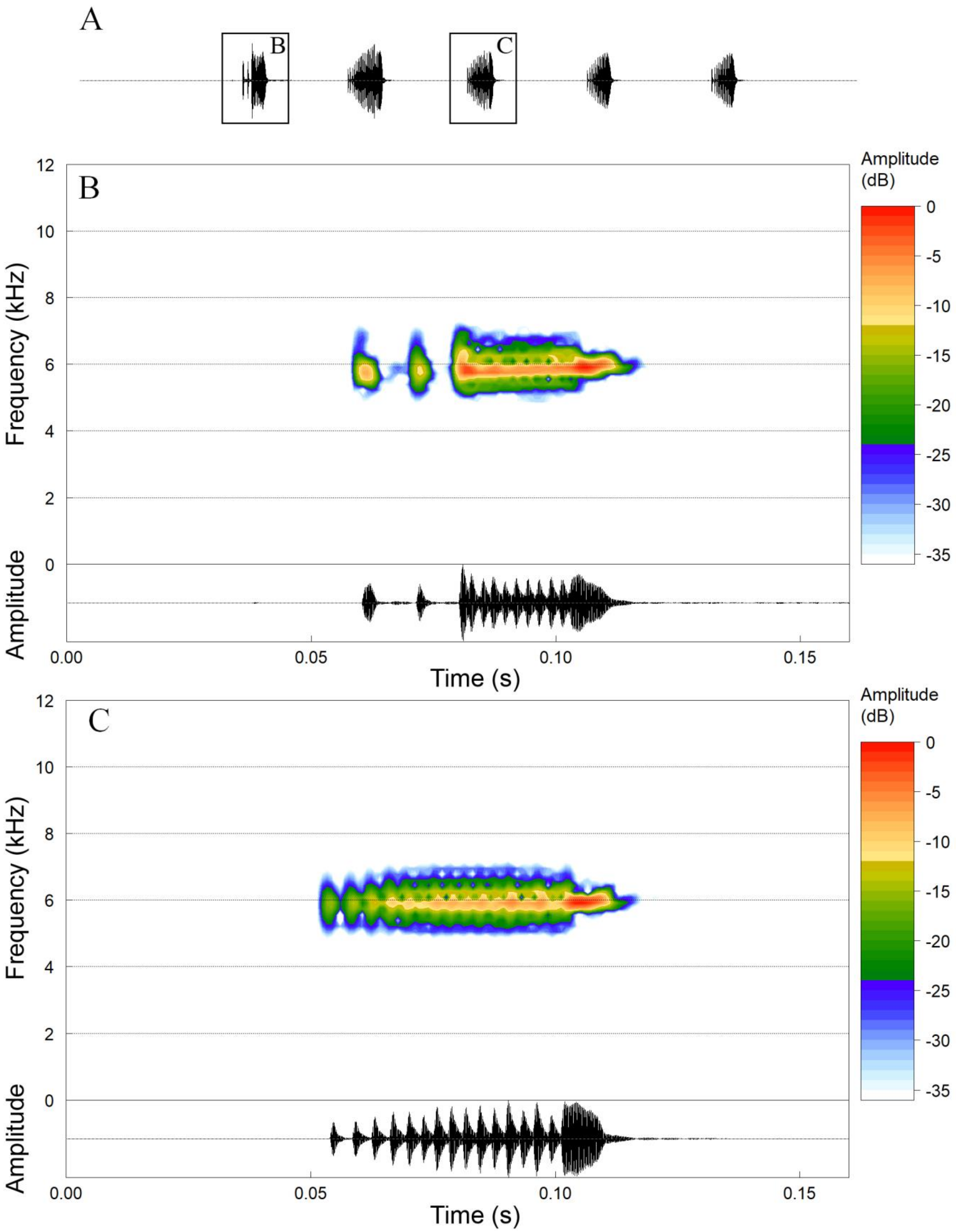

A
B
C
B
12
10
8
6
4
2
0
Frequency (kHz)
Amplitude
0.00
0.05
0.10
0.15
Time (s)
Amplitude (dB)
0
-5
-10
-15
-20
-25
-30
-35
C
12
10
8
6
4
2
0
Frequency (kHz)
Amplitude
0.00
0.05
0.10
0.15
Time (s)
Amplitude (dB)
0
-5
-10
-15
-20
-25
-30
-35

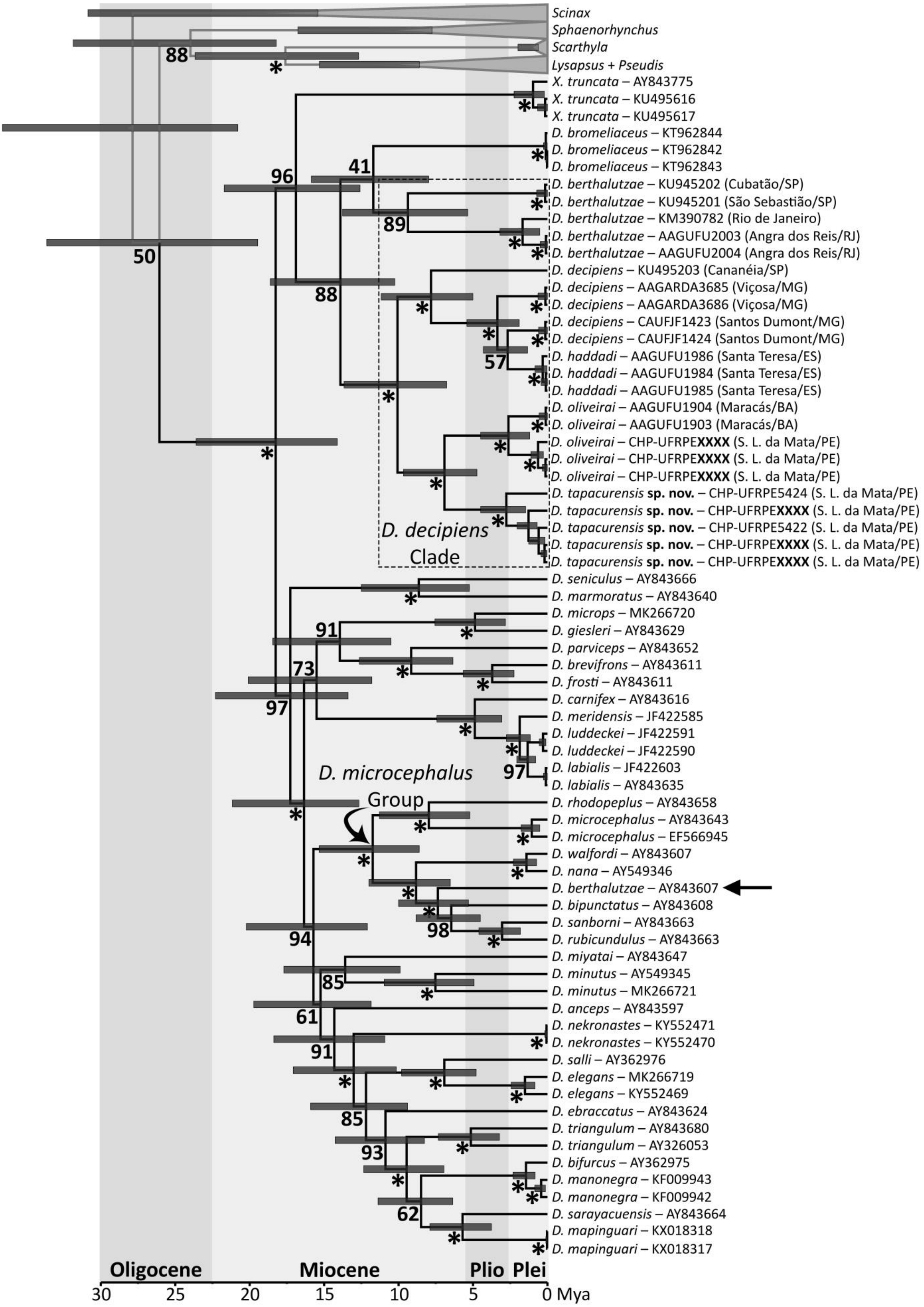
Table 4. Advertisement calls features for species of the *Dendropsophus decipiens* Clade. *Type B of notes
n = total number of analyzed notes.

Species	Call type	Notes per call	Pulses per notes	Note duration (ms)	Pulse rate	Dominant freque (kHz)
D. berthalutzae (*n*=139)	complex call	2–9*	2–6	10–96	#	3881–4294
D. berthalutzae (*n*=32)	complex call	1–2	2–12	10–46	109–343	4315–4765
D. decipiens (*n*=16)	simple call	4–11	#	20–130	#	4770–5230
D. haddadi (*n*=86)	simple call	1–3	1–8	4–59	60–421	4312–4875
D. oliveirai (*n*=28)	simple call	1	5–14	62–155	56–161	5685–6869
Dendropsophus sp. n. (*n*=83)	simple call	3–9	9–29	39–110	143–368	5578–6422

Phylogenetics relationships

Unexpectedly, *Dendropsophus* was recovered as non-monophyletic, and two major clades were recognized (Fig 7): one major group encompasses all *Dendropsophus* species group (but *D. decipiens* Clade; pp = 0.97) and another one clusters *Xenohyla truncata*, in the *D. decipiens* Clade and *D. bromeliaceus* with significant support in the Bayesian analysis (pp = 0.96). The *D. decipiens* Clade [2] was also recovered as non-monophyletic with two deeply divergent lineages assigned to *D. berthalutzae* forming a clade with *D. bromeliaceus* (with non-significant node support; pp = 0.41), which instead are sister to the remaining species in the *D. decipiens* Clade (pp = 0.88). The phylogenetic placement of *Dendropsophus* sp. n. within the *D. decipiens* Clade as the sister taxon of *D. oliveirai* was recovered with significant node support (pp = 1.0). Despite being morphologically more similar to *Dendropsophus* sp. n., paratopotypical sequences of *D. haddadi* were recovered imbedded within three genetically structured *D. decipiens* lineages, also with significant node support (pp = 1.0), rendering *D. decipiens* paraphyletic.

Fig 7. Time-calibrated phylogenetic tree of *Dendropsophus* as estimated by a Bayesian inference (BEAST) from the 2084 base pairs *H1* dataset. The topology is the maximum clade credibility tree from BEAST. Posterior probabilities are given near the nodes. Asterisks indicate significant support (=1.0 or 100). Dark bars along nodes indicate the 95% highest posterior density (confidence interval) on divergence time estimates. Time scale abbreviations = Plei: Pleistocene; Plio: Pliocene. Arrow highlight the position of a misidentified sample previously referred as a member of the *D. decipiens* clade.


Scinax
Sphaenorhynchus
Scarthyla
Lysapsus + Pseudis
88
*
X. truncata – AY843775
X. truncata – KU495616
X. truncata – KU495617
*
D. bromeliaceus – KT962844
D. bromeliaceus – KT962842
D. bromeliaceus – KT962843
*
96
41
D. berthalutzae – KU945202 (Cubatão/SP)
D. berthalutzae – KU945201 (São Sebastião/SP)
D. berthalutzae – KM390782 (Rio de Janeiro)
D. berthalutzae – AAGUFU2003 (Angra dos Reis/RJ)
D. berthalutzae – AAGUFU2004 (Angra dos Reis/RJ)
89
50
88
D. decipiens – KU495203 (Cananéia/SP)
D. decipiens – AAGARDA3685 (Viçosa/MG)
D. decipiens – AAGARDA3686 (Viçosa/MG)
D. decipiens – CAUFJF1423 (Santos Dumont/MG)
D. decipiens – CAUFJF1424 (Santos Dumont/MG)
57
D. haddadi – AAGUFU1986 (Santa Teresa/ES)
D. haddadi – AAGUFU1984 (Santa Teresa/ES)
D. haddadi – AAGUFU1985 (Santa Teresa/ES)
D. oliveirai – AAGUFU1904 (Maracás/BA)
D. oliveirai – AAGUFU1903 (Maracás/BA)
D. oliveirai – CHP-UFRPE**XXXX** (S. L. da Mata/PE)
D. oliveirai – CHP-UFRPE**XXXX** (S. L. da Mata/PE)
D. oliveirai – CHP-UFRPE**XXXX** (S. L. da Mata/PE)
D. tapacurensis **sp. nov.** – CHP-UFRPE5424 (S. L. da Mata/PE)
D. tapacurensis **sp. nov.** – CHP-UFRPE**XXXX** (S. L. da Mata/PE)
D. tapacurensis **sp. nov.** – CHP-UFRPE5422 (S. L. da Mata/PE)
D. tapacurensis **sp. nov.** – CHP-UFRPE**XXXX** (S. L. da Mata/PE)
D. tapacurensis **sp. nov.** – CHP-UFRPE**XXXX** (S. L. da Mata/PE)
D. decipiens Clade
D. seniculus – AY843666
D. marmoratus – AY843640
D. microps – MK266720
D. giesleri – AY843629
91
D. parviceps – AY843652
73
D. brevifrons – AY843611
D. frosti – AY843611
97
D. carnifex – AY843616
D. meridensis – JF422585
D. luddeckei – JF422591
D. luddeckei – JF422590
97
D. labialis – JF422603
D. labialis – AY843635
D. microcephalus Group
D. rhodopeplus – AY843658
D. microcephalus – AY843643
D. microcephalus – EF566945
D. walfordi – AY843607
D. nana – AY549346
D. berthalutzae – AY843607
D. bipunctatus – AY843608
98
D. sanborni – AY843663
D. rubicundulus – AY843663
94
D. miyatai – AY843647
85
D. minutus – AY549345
D. minutus – MK266721
61
D. anceps – AY843597
91
D. nekronastes – KY552471
D. nekronastes – KY552470
D. salli – AY362976
D. elegans – MK266719
D. elegans – KY552469
85
D. ebraccatus – AY843624
93
D. triangulum – AY843680
D. triangulum – AY326053
D. bifurcus – AY362975
D. manonegra – KF009943
D. manonegra – KF009942
62
D. sarayacuensis – AY843664
D. mapinguari – KX018318
D. mapinguari – KX018317
Oligocene
Miocene
Plio
Plei
30
25
20
15
10
5
0 Mya

Considering divergence time estimates, most cladogenesis events within the *Dendropsophus decipiens* Clade (including the clade formed by *D. berthalutzae* + *D. bromeliaceus*) took place in the Neogene (a geologic period spanning from 23 Ma to 2.5 Ma; [65]. Specifically, *Dendropsophus* sp. n. shared a most recent common ancestor (MRCA) with *D. oliveirai* at 6.9 Ma (95% highest posterior density [HPD] = 4.7–9.6 Ma) and the *D. decipiens* Clade (not considering *D. berthalutzae* + *D. bromeliaceus*) originated at approximately 10 Ma (95% HPD = 6.8–13.7 Ma). Additionally, our results support that the morphologically similar Atlantic Forest species within the species *D. decipiens* Clade (*D. haddadi* and *Dendropsophus* sp. n.) do not share a direct MRCA and neither the intensification of climatic oscillations in the Pleistocene or a vicariant effect caused by the São Francisco river course change (around 400 kya; [66] played a major role on the diversification of the new species (see Fig 7), therefore rejecting our initial assumptions.

The ABGD analysis identified a total of 11 genetic clusters, and also recovered all sequences assigned to *Dendropsophus* sp. n. in a single cluster. The average pairwise genetic distances for a 375 bp 16S segment between *Dendropsophus* sp. n. and remaining species in the *D. decipiens* Clade is very high (Table 5), especially if compared to the threshold of 3% proposed for Neotropical anurans [67]. For instance, if compared to all lineages/species in the *D. decipiens* Clade, *Dendropsophus* sp. n. exhibit at least 10% of genetic distance (e.g., between the topotypical *D. oliveirai* lineage from Maracás, Bahia State and a *D. decipiens* lineage from Santos Dumont, Minas Gerais State; Table 5).

Table 5. Tamura-Nei corrected pairwise distances (average p-values) in the *Dendropsophus decipiens* Cla
bp of the *16S* rDNA gene. The symbol '#' indicates that the intraspecific divergence was not estimated in
available. Groups are the ones delimited by the ABGD analysis. *Dendropsophus decipiens* populations fro

municipalities are represented by MG and MG2, respectively. Genetic distances between the new species a

are highlighted in bold.

		1	2	3	4	5	6	7	8	9
1	*Dendropsophus* sp. n.									
2	*D. berthalutzae* (RJ)	**0.16**								
3	*D. berthalutzae* (SP)	**0.18**	0.12							
4	*D. bromeliaceus*	**0.17**	0.13	0.14						
5	*D. decipiens* (SP)	**0.12**	0.17	0.16	0.17					
6	*D. decipiens* (MG)	**0.11**	0.14	0.16	0.14	0.09				
7	*D. decipiens* (MG2)	**0.10**	0.14	0.16	0.15	0.10	0.03			
8	*D. haddadi*	**0.13**	0.18	0.18	0.18	0.12	0.05	0.04		
9	*D. oliveirai* (PE)	**0.11**	0.17	0.17	0.17	0.08	0.12	0.10	0.14	
10	*D. oliveirai* (BA)	**0.10**	0.18	0.17	0.15	0.09	0.11	0.09	0.13	0.0
11	*Xenohyla truncata*	**0.14**	0.14	0.15	0.15	0.14	0.15	0.16	0.18	0.1

Geographic distribution

Dendropsophus sp. n. is only known from the type locality, municipality of São Lourenço da Mata, Pernambuco State, Brazil (Fig 1).

Natural History

We found specimens of *Dendropsophus* sp. n. in calling activity on shrubs and marginal vegetation of temporary sandy bottomed ponds 15 to 100 cm deep (Fig 8). Males were commonly observed perched on leaves and branches at 10 to 150 cm from the ground. The species exhibits a prolonged breeding activity as calling males were heard during the entire rainy season (from April to August). Males usually start to call around 18:00 h and remain active until 23:00 h. The new species was found sympatrically with *Boana albomarginata*, *B. raniceps*, *Agalychnis granulosa*, *Scinax eurydice*, *S. auratus, S. pachycrus*, *Sphaenorhynchus prasinus*, *Leptodactylus natalensis*, *L. macrosternum*, *L. vastus*, *Physalaemus cuvieri* and *Dermatonotus mulleri*. Moreover, *Dendropsophus* sp. n. occurs syntopically with five other species of the genus *Dendropsophus*: *D. branneri*, *D. elegans*, *D. soaresi*, *D. minutus* and *D. oliveirai*.

Etymology

Due to the requirements of the International Zoological Nomenclature Committee, this item is reserved only for publication in a specialized scientific journal.

Fig 8. Temporary ponds where we found the new species in calling activity. (A) temporary pond within an Atlantic Forest strictly protected area; (B) temporary pond outside protected area amidst a sugar cane landscape.

Remarks

Dendropsophus sp. n. was only registered at type locality, however, there are several records attributed to *D. haddadi* in areas located northwards of the São Francisco River, within the Pernambuco refuge and/or geographically close to its type locality (Fig 1, Table 1). Because *Dendropsophus* sp. n. and *D. haddadi* can be considered morphologically cryptic species, it is likely that records of *D. haddadi* along areas northwards of São Francisco River might actually represent *Dendropsophus* sp. n., because the identification of these populations was based solely on external morphology. Therefore, the identity of these populations should be further investigated including acoustic and molecular evidence to better evaluate the new species potential occurrence area and conservation status.

Discussion

In opposite to previous molecular, morphologic and cytogenetic evidences [2,68–70], we recovered the genus *Dendropsophus* as non-monophyletic while such studies [45,71–73] always recovered *Xenohyla truncata* as the sister clade of *Dendropsophus* [except for 74]. *Xenohyla* was raised as a genus apart from all other hylid frogs based on its distinctive morphology [75], further supported by the existence of putative synapomorphic characters within Hylidae [2] and differences in chromosome number (2n=26) [69] in comparison to the 30-chromosome *Dendropsophus* species [32,69,76]. Accordingly, members of the *D. decipiens* Clade also exhibit 2n=30 [77,78], hindering a clear understanding of the phylogenetic proximity we found.

A plausible explanation for such disagreements between our topology and these previously mentioned researches lies on differences of taxa sampling, considering that this is the first work to actually sample members of the *D. decipiens* Clade in a phylogenetic framework. For instance, all previously mentioned phylogenetic approaches did not include individuals from the *D. decipiens* Clade, although an individual referred to as *D. berthalutzae* (GenBank access #AY843607) is available. We sequenced two samples of *D. berthalutzae*, and included three other sequences available in GenBank that had never been analyzed under a phylogenetic approach, confirming that the sample AY843607 (identified as *D. berthalutzae* on GenBank database) is distantly related to species in the *D. decipiens* Clade, which instead clusters closely to species in the *D. microcephalus* Group (see Fig 7). This indicates tissue sample contamination or non-correspondence between samples and genetic vouchers.

Moreover, this is the first time that phylogenetic position of members in the *D. decipiens* Clade is given, precluding further comparisons between our work and all available phylogenetic hypothesis to date. However, based on molecular evidence, it is clear that the *D. decipiens* Clade should not belong to the *D. microcephalus* Group [2], which was also proposed by [79] based on differences in larval external morphologic features of species in the *D. decipiens* Clade compared to those of *D. microcephalus*+*D. rubicundulus* Clades. Despite that, our phylogenetic hypothesis is overall congruent to those previously mentioned, except for the unexpected phylogenetic position of *Xenohyla*. Because our objective lies solely on the phylogenetic position of the new taxa, future molecular systematic studies should reassess the position of the *D. decipiens* Clade within *Dendropsophus* under a comprehensive taxa and gene (including nuclear) sample.

Although the intensification of climatic oscillations during the Pleistocene with subsequent formation of forest refuges played an important role for the genetic diversification of species associated with the Atlantic Forest biome [12,17], many studies have been revealing that Atlantic Forest frog species diverged very early in the geologic time scale, around the Eocene/Oligocene [70,82,83] and exhibit many old histories of vicariant diversification, mainly during the Miocene [84]. Accordingly, *Dendropsophus* sp. n. shared a MRCA with its sister species (*D. oliveirai*) during the Miocene (around 6 Ma; Fig 7) but the broad confidence interval on divergence time estimates hinders us to infer the potential isolation mechanism that promoted this cladogenesis event, which is certainly older than predicts the Carnaval and Moritz model [8]. Therefore, the evolutionary history of *Dendropsophus* sp. n. was shaped by complex factors that are beyond forest fragmentation caused by Pleistocene climatic oscillations or vicariant speciation due to isolation by river.

Within the *Dendropsophus decipiens* Clade, *Dendropsophus* sp. n., morphologically most resembles *D. decipiens*, *D. haddadi* and *D. oliveirai*. The intraspecific variation of coloration patterns and body shape hampers discriminating species based solely on external morphology, specially based on a series of few individuals and/or long-time preserved ones. Apart from the *D. decipiens* Clade, the presence of dorsolateral strip that extends from the posterior region of the eye to the inguinal region and colored loreal region distinguishes the *Dendropsophus* sp. n. from all species of *D. microcephalus* Group but *D. tintinnabulum* [85] (Fig 1B), *D. gryllatus* [35] (Fig 1A), and *D. bromeliaceus* [80] (Fig 3B – C). The presence of discreetly pointed discs on finger (as in *Dendropsophus* sp. n.) is present in a few species of *D. microcephalus* Group, being reported only for *D. shiwiarum* and *D. ozzyi* [86,87].

Finally, we highlight the importance of the Atlantic Forest biome for biodiversity conservation, a fact associated with high endemism levels (~ 8,000 species) and habitat reduction, considering that approximately 11 to 16% of the original cover is still remaining [88]. We also emphasize the importance of UC`s along this Biome, highlighting the Pernambuco Endemism Center, due to its high diversity and potential for the discovery of new species [89–91]. In addition, it should be noted as aggravating that this area consists of small and highly fragmented Atlantic Forest remnants and therefore considered the most threatened in Brazil [6,7,88].

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List of Appendix

S1 Appendix. Additional material examined for comparisons.

Dendropsophus anataliasiasi: type series: MZUSP 74204 (Ex. WCAB 45272); MZUSP-73790 (Ex. WCAB-45373); MZUSP 73788–73789 (Ex. WCAB 45257–45258); TOCANTINS: Brejinho de Nazaré (topotypes): AAG-UFU 0926–0939; Formoso do Araguaia: ZUEC 10158; ZUEC 10170–10171; ZUEC 10177–10178; ZUEC 101201; Nova Olinda: MNRJ 73004; MNRJ 66823.

Dendropsophus araguaya: type series: MZUSP 66803, MNRJ 17240–14241, MZUSP66796–66802, MZUSP 66719–66721, MNRJ 66796–66802; topotypes: AAG-UFU 1726–1724, MZUSP 152371–152380.

Dendroposphus berthalutzae: topotypes: AAG-UFU 5031–5038, AAG-UFU 0257–0259; SÃO PAULO: Ubatuba: AAG-UFU 4425–4426, Salesópolis: AAG-UFU 5410.

Dendropsophus bipunctatus: BAHIA: Ilhéus: AAG-UFU 0240; RIO DE JANEIRO: Macaé: AAG-UFU 0538–0540, Duas Barras: AAG-UFU 0702–0705, Cachoeiras de Macacu: AAG-UFU 6227; ESPÍRITO SANTO: Santa Teresa: AAG-UFU 6158–6160.

Dendropsophus branneri: BAHIA: Ilhéus: AAG-UFU 0241–0244, Prado: AAG-UFU 5971–5973; ESPÍRITO SANTO: Santa Teresa: AAG-UFU 6161–6163.

Dendropsophus cachimbo: type series: MNRJ 17298–17299; MZUSP 21910–21918; MZUSP 21920–21924; PARÁ: Novo Progresso: AAG-UFU 1489–1504; AAG-UFU 1506–1514 (topotypes); CHUNB 13098–13108; CHUNB 34445–34469; CHUNB 40178–40195.

Dendropsophus cerradensis: type series: MZUSP 06733, MNRJ 17293, ZUEC 06734–06737; MATO GROSSO DO SUL: Ribas do Rio Pardo: AAG-UFU 1709–1721.

Dendropsophus cruzi: type series: MNRJ 21791–21795; GOIÁS: Silvânia: AAG-UFU 1787–1792, AAG-UFU 5094–5097, Pirenópolis: AAG-UFU 0335–0339, Uruaçu: AAG-UFU 1009, AAG-UFU 1408, Alto Paraíso: AAG-UFU 1331, Minaçu: AAG-UFU 5053, Niquelândia: 5070; MATO GROSSO: Pontal do Araguaia: AAG-UFU 0218–0227, AAG-UFU 1087–1088, Alto Garças: AAG-UFU 5715–5717; MINAS GERAIS: Ituiutaba: AAG-UFU 0343–0348, AAG-UFU 0644–0645, Araporã: AAG-UFU 0657–0658, Limeira do Oeste: AAG-UFU 1755–1757; TOCANTINS: Brejinho de Nazaré: AAG-UFU 0917–0920, Mateiros: AAG-UFU 1987–1995, Palmas: AAG-UFU 2754–2758, AAG-UFU 3277–3292, MARANHÃO: Carolina: AAG-UFU 2840–2845.

Dendropsophus decipiens: RIO DE JANEIRO: Duas Barras: AAG-UFU 0706–0709.

Dendropsophus elianeae: MINAS GERAIS: Uberlândia: AAG-UFU 1891–1894, AAG-UFU 2294–2296, Sacramento: AAG-UFU 0895–0897, Perdizes: AAG-UFU 1030–1032; MATO GROSSO DO SUL: Bela vista (topotypes): AAG-UFU 0128–0142, Ribas do Rio Pardo: AAG-UFU 0160–0174, AAG-UFU 0655–0656.

Dendropsophus haddadi: ESPÍRITO SANTO: Santa Teresa: AAG-UFU 6170–6179, Sooretama: AAG-UFU 6203–6205.

Dendroposphus jimi: type series: MZUSP 21980, MNRJ 2198, MNRJ 21983–21990, MNRJ 21982, MINAS GERAIS: Araporã: AAG-UFU 0653–0654, Delta: AAG-UFU 0559–0562, Parque Nacional Grande Sertão Veredas: AAG-UFU 1907–1910, Uberlândia: AAG-UFU 2318–2327.

Dendropsophus meridianus: RIO DE JANEIRO: Manguinhos (topotypes): MNRJ 20761–20775; Cachoeiras de Macacu: AAG-UFU 6216–6225.

Dendropsophus aff. *microcephalus*: RONDÔNIA: Espigão d'Oeste: AAG-UFU 5798–5797.

Dendropsophus nanus: ARGENTINA: Resistencia (topotypes): UNNEC 12429–39; BRASIL: MINAS GERAIS: Araguari: AAG-UFU 0550–0552, Araporã: AAG-UFU 1825–1827, MATO GROSSO DO SUL: Bela vista: AAG-UFU 0159; TOCANTINS: AAG-UFU 0921–0925; MATO GROSSO: Cáceres: AAG-UFU 5255–5258; GOIÁS: Guarani de Goiás: AAG-UFU 1962; RONDÔNIA: Vilhena: AAG-UFU 5268, AAG-UFU 5362–5364.

Dendropsophus oliveirai: type series: MZUSP 7359, MZUSP 74146-74148; BAHIA: Maracás: 5673-5679.

Dendropsophus ozzyi: type series: MZUSP 154084–154088.

Dendropsophus pseudomeridianus: RIO DE JANEIRO: Macaé: AAG-UFU 0548–0549, AAG-UFU 758–760, MINAS GERAIS: AAG-UFU 1103.

Dendropsophus rhea: type series: MZUSP 9104, MZUSP 30983–30984, MZUSP 14450–14471, MZUSP 9105–9113; SÃO PAULO: Pirassununga: topotypes: AAG-UFU 1089–1097.

Dendropsophus rodhopeplus: EQUADOR: Santa Cecília: MZUSP 55812–55821, BRASIL: Rondônia: Itapuã do Oeste: AAG-UFU 5816–5819.

Dendropsophus rubicundulus: MINAS GERAIS: Lagoa Santa (topotypes): AAG-UFU 0021–0022, Parque Nacional da Serra do Cipó: AAG-UFU 0030–0044, Vargem Bonita: AAG-UFU 0605, São Gotardo: AAG-UFU 1749–1754, Buritis: AAG-UFU 1754–1768, Curvelo: AAG-UFU 0306, Juatuba AAG-UFU 0327, Parque Nacional Grande Sertão Veredas: AAG-UFU 1895-1906; Paracatu: AAG-UFU 0647–0650; MATO GROSSO: Cuiabá: 1447–1461; MARANHÃO: Carolina AAG-UFU 2833–2839; GOIÁS: Guarani de Goiás: AAG-UFU 1953–1961; Silvânia AAG-UFU 1785–1786, Uruaçu: AAG-UFU 1003–1008, Teresina de Goiás: AAG-UFU 1355–1379, Padre Bernardo: AAG-UFU 1526–1535; TOCANTINS: Mateiros: AAG-UFU 2149, 2160–2162; Paranã: AAG-UFU 3293–3305; BAHIA: São Desiderio AAG-UFU 5434–5436.

Dendropsophus sanborni: URUGUAI: MALDONADO: Maldonado: CH-UR 17632–17636, ZUEC 10073–10077; SAN JOSÉ: Ciudad del Plata: MZUSP 77997–98, MZUSP 78000, MZUSP 78002, MZUSP 78006–78009, MZUSP 78001–78012; CERRO LARGO: Plácido Rosas: MZUSP 7761–7764; BRASIL: SÃO PAULO: Botucatu: AAG-UFU 1704–1708, Águas da Prata 162 AAG-UFU 3526–3527, Itatiba: AAG-UFU 4431; MINAS GERAIS: Poços de Caldas: AAG-UFU 4680–4681, AAG-UFU 0001–0002.

Dendropsophus tritaeniatus: MATO GROSSO: Chapada dos Guimarães: AAG-UFU 1467–1474.

Dendropsophus walfordi: type series: MZUSP 73652 (ex WCAB 8436), MZUSP 74019–74041, MZUSP 74424, MZUSP 73653; RONDÔNIA: Costa Marques (topotypes) AAG-UFU 5306–5327, MZUSP 129903–130122, Itapuã do Oeste: AAG-UFU 5820–5829; Abunã: MZUSP 104496–104503; ACRE: Rio Branco: AAG-UFU 5886–5894, Feijó: AAG-UFU 5894, Cruzeiro do Sul: AAG-UFU 5903–5906, AMAZONAS: Borba: MZUSP 51199–51212.

Dendropsophus tintinnabulum: AMAZONAS: São Gabriel da Cachoeira: AAG-UFU 3854–3857, 3882–3884; Tarauacá: MZUSP: 188212–188213.

SII Appendix. Sound recordings and associated information.

Sound file	**Species**	**Voucher**	**Locality (State)**	**Air temperature/humidity**
SCLEHP18	*Dendropsophus* sp. n.	Unvouchered	São Lourenço da Mata (PE)	23.8ºC/73%
SCLEHP19	*Dendropsophus* sp. n.	CHP_UFRPE5697	São Lourenço da Mata (PE)	23.8ºC/73%
SCLEHP20	*Dendropsophus* sp. n.	Unvouchered	São Lourenço da Mata (PE)	23.9ºC /74%
SCLEHP21	*Dendropsophus* sp. n.	CHP_UFRPE5704	São Lourenço da Mata (PE)	23.9Cº /74%
SCLEHP22	*Dendropsophus* sp. n.	CHP_UFRPE5705	São Lourenço da Mata (PE)	24.5Cº/72%
SCLEHP23	*Dendropsophus* sp. n.	CHP_UFRPE5706	São Lourenço da Mata (PE)	24.8ºC/78%
SCLEHP24	*Dendropsophus* sp. n.	CHP_UFRPE5707	São Lourenço da Mata (PE)	22.9ºC/85%
SCLEHP25	*Dendropsophus* sp. n.	CHP_UFRPE5708	São Lourenço da Mata (PE)	23.8ºC/73%
SCLEHP26	*Dendropsophus* sp. n.	CHP_UFRPE5709	São Lourenço da Mata (PE)	22.9ºC/79%
SCLEHP27	*Dendropsophus* sp. n.	CHP_UFRPE5710	São Lourenço da Mata (PE)	23.5ºC/76%
SCLEHP28	*Dendropsophus* sp. n.	CHP_UFRPE5711	São Lourenço da Mata (PE)	23.9ºC/74%

SIII Appendix. GenBank information of genetic samples used herein. NA: Not applicable; TBS: to be subr

#	Species	Voucher	Locality	**GenBan nu**
				rRNA-12S
Newly sequenced samples from *D. decipiens* Clade				
1	*Dendropsophus* sp. n.	CHP-UFRPE5700	São Lourenço da Mata/PE	NA
2	*Dendropsophus* sp. n.	CHP-UFRPE5701	São Lourenço da Mata/PE	NA
3	*Dendropsophus* sp. n.	CHP-UFRPE-5706	São Lourenço da Mata/PE	NA
4	*Dendropsophus* sp. n.	CHP-UFRPE-5707	São Lourenço da Mata/PE	**TBS**
5	*Dendropsophus* sp. n.	CHP-UFRPE-5708	São Lourenço da Mata/PE	NA
6	*Dendropsophus berthalutzae*	AAGUFU2003	Angra dos Reis/RJ	NA
7	*Dendropsophus berthalutzae*	AAGUFU2004	Angra dos Reis/RJ	NA
8	*Dendropsophus decipiens*	AAGARDA3685	Viçosa/MG	NA
9	*Dendropsophus decipiens*	AAGARDA3686	Viçosa/MG	NA
10	*Dendropsophus decipiens*	CAUFJF1423	Santos Dumont/MG	NA
11	*Dendropsophus decipiens*	CAUFJF1424	Santos Dumont/MG	NA
12	*Dendropsophus haddadi*	AAGUFU1984	Santa Teresa/ES	NA
13	*Dendropsophus haddadi*	AAGUFU1985	Santa Teresa/ES	NA
14	*Dendropsophus haddadi*	AAGUFU1986	Santa Teresa/ES	NA
15	*Dendropsophus oliveirai*	AAGUFU1903	Maracás/BA	NA
16	*Dendropsophus oliveirai*	AAGUFU1904	Maracás/BA	NA
17	*Dendropsophus oliveirai*	CHP-UFRPE-5504	São Lourenço da Mata/PE	**TBS**
18	*Dendropsophus oliveirai*	CHP-UFRPE-5505	São Lourenço da Mata/PE	**TBS**
19	*Dendropsophus oliveirai*	CHP-UFRPE-5506	São Lourenço da Mata/PE	**TBS**
GenBank samples from *D. decipiens* Clade				

20	*Dendropsophus berthalutzae*	NA	Rio de Janeiro	NA
21	*Dendropsophus berthalutzae*	CFBHT02256	São Sebastião/SP	NA
22	*Dendropsophus berthalutzae*	CFBHT05532	Cubatão/SP	NA
23	*Dendropsophus decipiens*	CFBHT07254	Cananéia/SP	NA
Misidentified samples				
24	*Dendropsophus berthalutzae**	CFBH5418	Duque de Caxias/RJ	AY843607
Remaining GenBank samples included in molecular analysis				
25	*Dendropsophus anceps*	NA	NA	AY843597
26	*Dendropsophus bifurcus*	NA	NA	AY362975
27	*Dendropsophus bipunctatus*	NA	NA	AY843608
28	*Dendropsophus brevifrons*	NA	NA	AY843611
29	*Dendropsophus bromeliaceus*	NA	NA	KT962842
30	*Dendropsophus bromeliaceus*	NA	NA	KT962843
31	*Dendropsophus bromeliaceus*	NA	NA	KT962844
32	*Dendropsophus carnifex*	NA	NA	AY843616
33	*Dendropsophus ebraccatus*	NA	NA	AY843624
34	*Dendropsophus elegans*	NA	NA	KY552469
35	*Dendropsophus elegans*	NA	NA	MK266719
36	*Dendropsophus frosti*	NA	NA	JQ088283
37	*Dendropsophus giesleri*	NA	NA	AY843629
38	*Dendropsophus labialis*	NA	NA	AY843635
39	*Dendropsophus labialis*	NA	NA	JF422603
40	*Dendropsophus luddeckei*	NA	NA	JF422590
41	*Dendropsophus luddeckei*	NA	NA	JF422591
42	*Dendropsophus manonegra*	NA	NA	KF009942

43	*Dendropsophus manonegra*	NA	NA	KF009943
44	*Dendropsophus mapinguari*	NA	NA	KX018317
45	*Dendropsophus mapinguari*	NA	NA	KX018318
46	*Dendropsophus marmoratus*	NA	NA	AY843640
47	*Dendropsophus meridensis*	NA	NA	JF422585
48	*Dendropsophus microcephalus*	NA	NA	AY843643
49	*Dendropsophus microcephalus*	NA	NA	EF566945
50	*Dendropsophus microps*	NA	NA	MK266720
51	*Dendropsophus minutus*	NA	NA	MK266721
52	*Dendropsophus miyatai*	NA	NA	AY843647
53	*Dendropsophus nekronastes*	NA	NA	KY552470
54	*Dendropsophus nekronastes*	NA	NA	KY552471
55	*Dendropsophus parviceps*	NA	NA	AY843652
56	*Dendropsophus rhodopeplus*	NA	NA	AY843658
57	*Dendropsophus rubicundulus*	NA	NA	AY843661
58	*Dendropsophus salli*	NA	NA	AY362976
59	*Dendropsophus sanborni*	NA	NA	AY843663
60	*Dendropsophus sarayacuensis*	NA	NA	AY843664
61	*Dendropsophus seniculus*	NA	NA	AY843666
62	*Dendropsophus triangulum*	NA	NA	AY326053
63	*Dendropsophus triangulum*	NA	NA	AY843680
64	*Dendropsophus walfordi*	NA	NA	AY843683
65	*Dendropsophus minutus*	NA	NA	AY549345
66	*Dendropsophus nanus*	NA	NA	AY549346
67	*Xenohyla truncate*	NA	NA	AY843775

68	*Xenohyla truncate*	NA	NA	NA
69	*Xenohyla truncate*	NA	NA	NA
70	*Lysapsus caraya*	NA	NA	EF152999
71	*Lysapsus laevis*	NA	NA	EF152998
72	*Lysapsus limellum*	NA	NA	EF153000
73	*Pseudis bolbodactyla*	NA	NA	EF153005
74	*Pseudis boliviana*	NA	NA	MK293751
75	*Pseudis cardosoi*	NA	NA	EF152997
76	*Pseudis minuta*	NA	NA	EF152996
77	*Pseudis paradoxa*	NA	NA	EF153008
78	*Pseudis fusca*	NA	NA	EF153003
79	*Pseudis Tocantins*	NA	NA	EF153004
80	*Scarthyla goinorum*	NA	NA	AY843752
81	*Scarthyla ostinodactyla*	NA	NA	AY326035
82	*Scinax alter*	NA	NA	MK266759
83	*Scinax fuscovarius*	NA	NA	MK266760
84	*Scinax vsignatus*	NA	NA	MK266761
85	*Sphaenorhynchus dorisae*	NA	NA	AY843766
86	*Sphaenorhynchus lacteus*	NA	NA	AY549367
87	*Sphaenorhynchus prasinus*	NA	NA	MK266754

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ORIGINAL ARTICLE

Seasonal influence on foraging activity of scorpion species (Arachnida: Scorpiones) in a seasonal tropical dry forest remnant in Brazil

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ABSTRACT

In Brazil, the Caatinga ecoregion is the only biome that lies completely within the country, covering approximately 11% of the national territory. This biome exhibits a strong climatic seasonality that may affect the activity of its fauna. We aimed to assess the effects of seasonality on scorpion foraging activity in an area of hypoxerophytic Caatinga, located in the municipality of Caetés, Pernambuco. Scorpions were collected through active nocturnal searching using ultraviolet flashlights during the rainy (May, June, and July) and dry (September, October, and November) seasons in 2017. A total of 96 specimens were collected, belonging to the Buthidae and Bothriuridae families, comprising seven species, *Ananteris* sp., *Tityus pusillus, T. stigmurus, T. neglectus, Jaguajir rochae, Bothriurus asper*, and *B. rochai*. No differences in foraging activity (rainy season, n = 47; dry season, n = 49) were found between rainy and dry seasons. These findings indicate that the studied area has a high scorpion richness, emphasizing the importance of conserving the area, as these animals are sensitive to habitat changes. In addition, the lack of variation between seasons found in this work may reflect the ecotonal nature of hypoxerophytic Caatinga.

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Community ecology; Caatinga; Buthidae; Bothriuridae

Introduction

The seasonal tropical dry forests and woodlands (STDFWs) comprise a global biome with an annual rainfall of less than 1800 mm per year with 5–6 months of low rainfall (<100 mm) occurring in frost-free regions (Murphy & Lugo 1986; Gentry 1995; Pennington et al. 2009; Queiroz et al. 2018). This biome comprises an area of about 2,700,000 km^2 in the New World with 67% being found on the South American continent (Queiroz et al. 2018). According to Queiroz et al. (2018), most of the STDFWs in South America (45%) are represented by Caatinga vegetation. This vegetation is found entirely in Brazilian territory; it occupies an area of approximately 800,000 km^2, equivalent to 11% of the country's total area (MMA 2011). More than half of the Caatinga area (ca. 64%) has undergone chronic disturbance, particularly because of the unsustainable exploitation of native wood and conversion of the vegetation to pastures and plantations (e.g. Brasil 2014; Ribeiro et al. 2015; Ribeiro-Neto et al. 2016; Leal et al. 2018). In addition to these pressures, 94% of the Caatinga area is under increased risk of desertification due to the increasing global temperatures (Vieira et al. 2015). Among Brazilian ecosystems, the Caatinga is one of the most vulnerable areas to this rapidly growing threat (Oyama & Nobre 2003; Lima et al. 2011). Despite these threats, Caatinga vegetation sustains an impressive number of species (e.g. 3150 plants, 386 fishes, and 548 birds), with an endemism rate ranging between 6% for mammals to 53% for fishes (Silva et al. 2017).

However, the arthropod biota of Caatinga are little known despite the recent efforts of researchers to investigate this fauna (e.g. Bravo & Calor 2014; Bravo 2017). These efforts resulting in many species described or expanded in their distribution, although there are still large unsampled areas in Caatinga (e.g. Bravo & Calor 2014; Santos-da-Santos-da-Silva et al. 2017; Carvalho & Botero-Trujillo 2019). This is particularly true for an important taxon of arthropod predators: scorpions (Porto et al. 2014). These arachnids are ecologically important in arid and semi-arid habitats, and they have the potential to strongly affect community dynamics and structure, especially among arthropods (Polis 1990, 1993; Polis & Yamashita 1991). Ecological studies on scorpions at a study site in Caatinga reported two to six species, with the foraging activity of these arachnids increasing during the rainy season (Araújo et al. 2010; Carmo et al. 2013; Lira et al. 2019b). These authors suggested that the increase in the foraging activity of scorpions is related

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to the increase in the abundance of arthropod prey species. In ecosystems with a clear distinction between rainy and dry seasons, such as Caatinga, climatic conditions can exert a strong influence on arthropod populations (e.g. Moura et al. 2006; Vasconcellos et al. 2010; Nobre et al. 2012). However, the responses of these arthropods to climate may vary according to the studied taxa and their habitat characteristics (Vasconcellos et al. 2010; Creão-Duarte et al. 2016; Lira et al. 2018). This variation in taxon response may provide a continuous supply of prey for generalist predators such as scorpions. Thus, in this study, we analyzed scorpion foraging activity in a Caatinga fragment during the rainy and dry seasons. We tested the hypothesis that scorpion foraging activity is affected by seasonality, predicting that foraging will be greater in the rainy season than in the dry season.

Material and methods

Study area

This study was performed in a preserved hypoxerophytic Caatinga vegetation remnant of 6.30 ha in Vale do São José located in Caetés municipality (8°46′31,96"S; 36° 43′5,19"W; 860 m.a.s.l.), Pernambuco state, Brazil (Figure 1). The area is characterized by a mean annual temperature of 20.5°C and an annual rainfall of 755 mm, with the climate considered semiarid tropical (Aw) according to

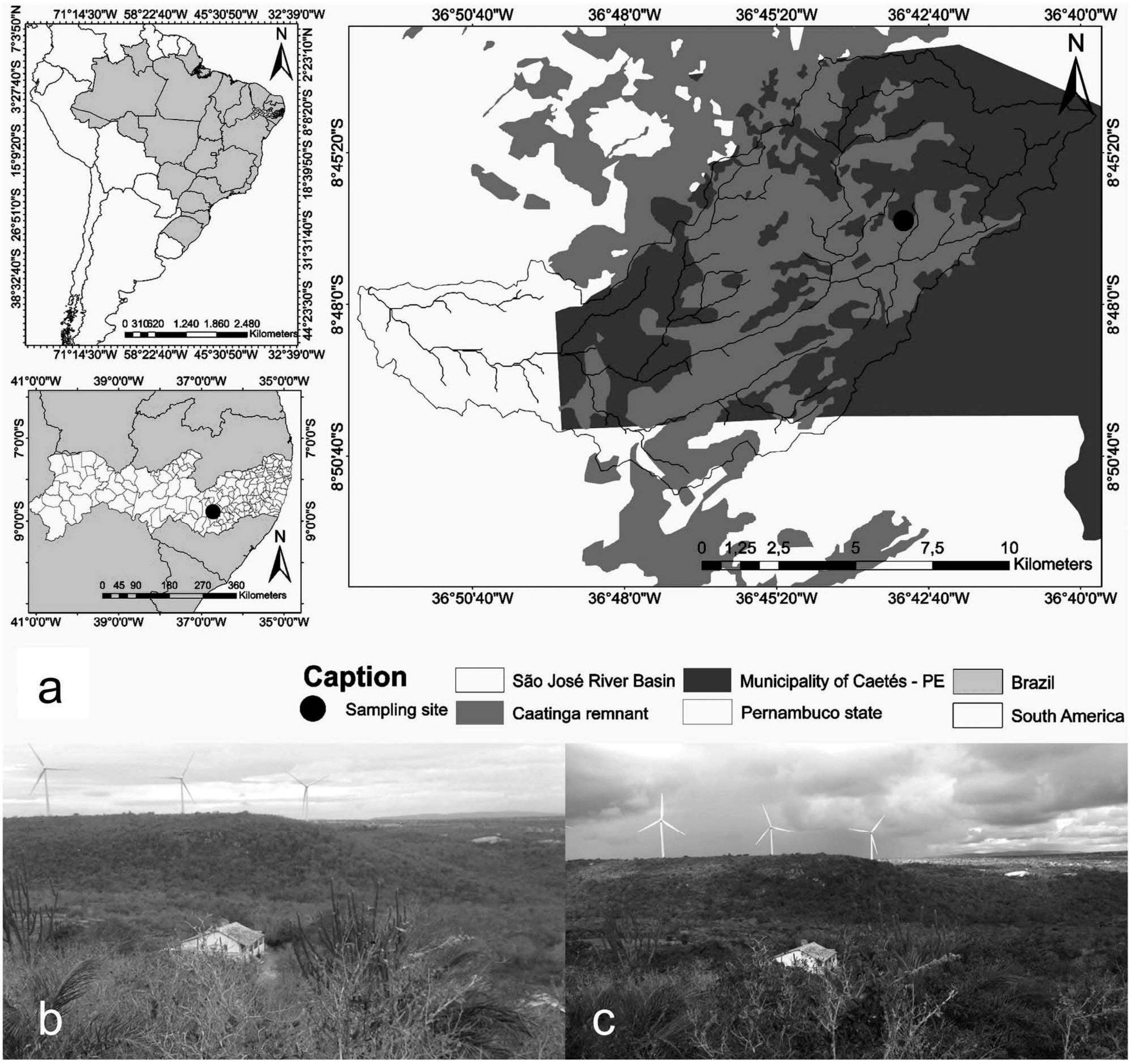

Figure 1. Location of Vale do São José, Caetés municipality, Brazil (A) and vegetation of the study area during dry (B) and rainy seasons (C).

the Köppen classification (Climate data 2019). Vegetation in the studied site is dominated by shrub-arboreal individuals belonging to families Euphorbiaceae, Anacardiaceae, Fabaceae, Bromeliaceae, and Cactaceae.

Scorpion sampling

Field expeditions were performed during the rainy season (May, June, and July 2017, with 157.2 ± 59.9 mm rainfall) and the dry season (September, October, and November 2017, with 27.5 ± 21.7 mm rainfall) (APAC 2019). Scorpions were collected by four researchers via active searching using ultraviolet flashlights between 20:00 and 00:00 h during two nights per month. This total effort represented 24 h per season. All observed scorpions were captured manually using forceps, stored in vials with 70% ethanol, and identified according to the report by Lourenço (2002) and Esposito et al. (2017). Voucher specimens were deposited at the Arachnological Collection of the Universidade Federal de Pernambuco, Brazil.

Data analysis

Scorpion richness was determined using a collector's curve, which considered the number of species observed versus the number of individuals collected over the 6 months of sampling. The species accumulation curve was generated using 999 randomizations run in EstimateS v.8.2 software (Colwell 2009). To evaluate differences in scorpion foraging activity between dry and rainy seasons, we performed non-metric multidimensional scaling (NMDS) based on a Bray–Curtis similarity matrix with 1000 randomizations. To assess the contribution of each species (%) between each season, we performed a similarity percentage analysis (SIMPER) based on a Bray–Curtis similarity matrix with 1000 randomizations. Statistical significance was tested using analysis of similarity (ANOSIM). Scorpion richness in both dry and rainy seasons was evaluated by a Shannon–Wiener index. All analyses were performed using PAST 3.18 (Hammer et al. 2001). We also calculated the species dominance in each season as follows: $D\% = (i/t) \times 100$, where i is the abundance of species A and t is the total abundance. In this way, scorpion species were classified as eudominant ($D > 10\%$), dominant ($5\% < D \leq 10\%$), subdominant ($2\% < D \leq 5\%$), recessive ($1\% < D \leq 2\%$), or rare ($D < 1\%$) (Palissa et al. 1979).

Results

The species accumulation curve indicated that the sampling effort in this study was sufficient to capture the 97% diversity of scorpions (Figure 2). Scorpion fauna was represented by seven species (Figure 3), belonging to the families Buthidae and Bothriuridae, in a total of 96 collected scorpions (Table 1).

Buthid scorpions were predominant during the rainy and dry seasons, corresponding to 70% of scorpions sampled and comprising five species from the three genera: *Jaguajir, Tityus*, and *Ananteris*. Two species were largely predominant in both seasons: *Jaguajir rochae* corresponding to 33.3% ($n = 32$), followed by *Tityus pusillus* comprising 25% ($n = 24$) of the whole sample. Other buthid species were represented by *T. stigmurus* ($n = 6$; 6.2%), *T. neglectus* ($n = 3$; 3.1%), and *Ananteris* sp. ($n = 3$; 3.1%). The remainder of the sample (30%) was represented by the bothriurid species *Bothriurus rochai* ($n = 22$; 22.8%) and *B. asper* ($n = 6$; 6.2%) (Table 1). According to SIMPER, *T. pusillus, J. rochae,* and *B. rochai* contributed together with 74.5% of scorpion assemblage (Table 2). Overall foraging activity was similar (NMDS: stress value = 0.04; ANOSIM: $R = 0.037$; $p = 0.300$) between the rainy ($n = 47$) and dry seasons ($n = 49$) (Figure 4). Despite this, the dominance of *B. asper, T. neglectus*, and *T. stigmurus* changed according to season (Table 1). The other species were classified as eudominant (*B. rochai, J. rochae*, and *T. pusillus*) and subdominant (*Ananteris* sp.) in both seasons (Table 1).

The same number of species that was found in each season ($n = 6$) shows a similar Shannon–Wiener index (dry = 1.60; rainy = 1.46). However, two species were recorded in only one season: *B. asper* in the dry season and *T. neglectus* in the rainy season (Table 1).

Discussion

Our study assessed how seasonality influenced scorpion foraging activity in Caatinga hypoxerophytic vegetation. Contrary to our hypothesis, the foraging activity was not significantly different between the rainy and dry seasons.

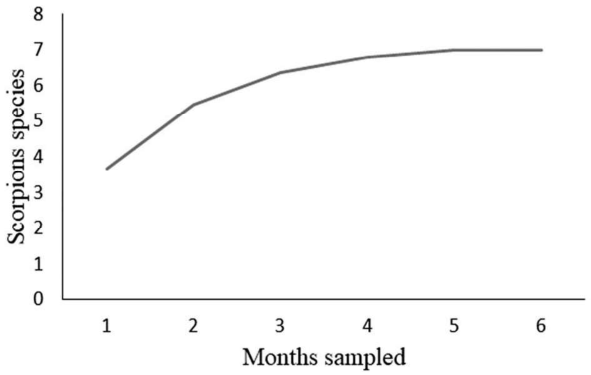

Figure 2. Species accumulation curve for the 6 months of scorpion collection in Caatinga vegetation in Vale do São José, Caetés municipality, Brazil.

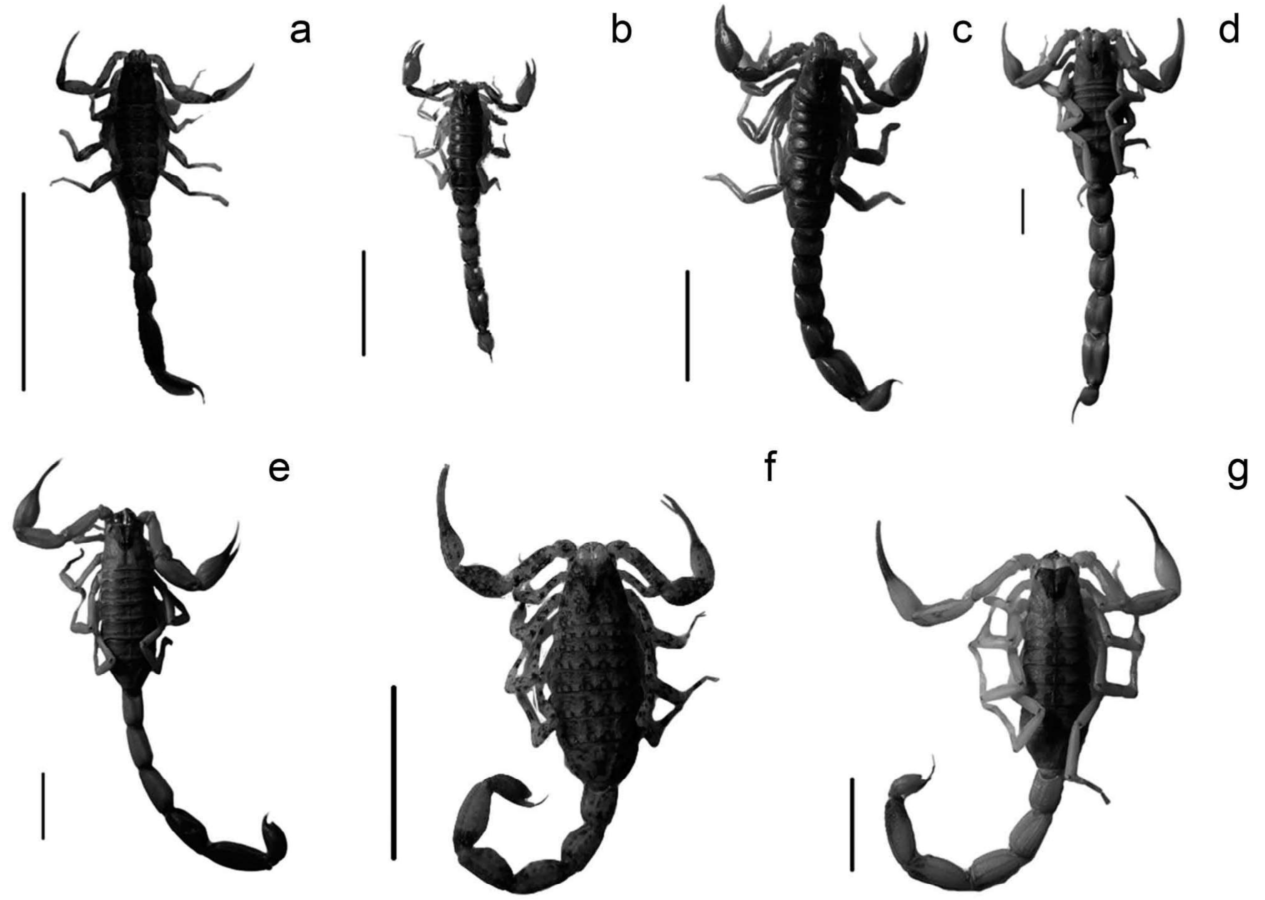

Figure 3. Scorpion species sampled in Caatinga vegetation in Vale do São José, Caetés municipality, Brazil. *Ananteris* sp. (A), *Bothriurus asper* (B), *Bothriurus rochai* (C), *Jaguajir rochae* (D), *Tityus neglectus* (E), *Tityus pusillus* (F), and *Tityus stigmurus* (G). Scale bar 1 cm. Photos by Hugo Neves.

Table 1. Foraging activity (in %) and dominance of scorpions found during the rainy and dry seasons in Caatinga vegetation in Vale do São José, Caetés municipality, Brazil.

Scorpion species	Rainy season	Dominance	Dry season	Dominance	Total (*n*)
Bothriuridae					
Bothriurus asper Pocock, 1893	0	Rare	100	Eudominant	6
Bothriurus rochai Mello-Leitão, 1932	63.6	Eudominant	36.4	Eudominant	22
Buthidae					
Ananteris sp. Thorell, 1891	25	Subdominant	75	Subdominant	4
Jaguajir rochae (Borelli, 1910)	46.8	Eudominant	53.2	Eudominant	32
Tityus neglectus Mello-Leitão, 1932	100	Dominant	0	Rare	3
Tityus pusillus Pocock, 1893	50	Eudominant	50	Eudominant	24
Tityus stigmurus (Thorell, 1876)	33.3	Subdominant	66.67	Dominant	6

Table 2. Summary of similarity percentage analysis (SIMPER) on scorpion assemblage during dry and rainy seasons in Caatinga vegetation in Vale do São José, Caetés municipality, Brazil.

Scorpion species	Contribution (%)	Cumulative (%)	Mean dry	Mean rainy
Tityus pusillus Pocock, 1893	26.33	26.33	4	4
Jaguajir rochae (Borelli, 1910)	25.07	51.41	5	5.67
Bothriurus rochai Mello-Leitão, 1932	23.05	74.46	4.67	2.67
Bothriurus asper Pocock, 1893	11.55	86.01	0	2
Tityus neglectus Mello-Leitão, 1932	5.94	91.95	1	0
Tityus stigmurus (Thorell, 1876)	5.34	97.29	0.66	1.33
Ananteris sp. Thorell, 1891	2.71	100	0.33	0.66

Previous studies performed in South American semi-arid regions showed that scorpion foraging activity increases during the rainy season (Araújo et al. 2010; Carmo et al. 2013; Toscano-Gadea 2013; Schwerdt et al. 2016). These authors reported that the increases in scorpion foraging activity during the rainy season occur because potential prey species become widely available. In dry forests such as Caatinga, the rainy season brings renewed plant growth,

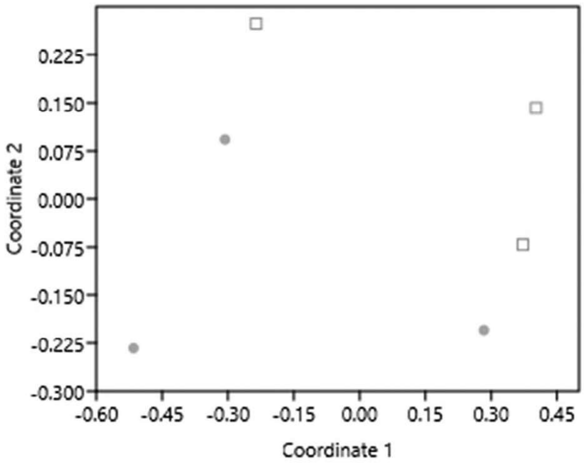

Figure 4. Non-metric multidimensional scaling (NMDS) ordination using a Bray–Curtis similarity matrix of scorpion foraging activity in dry (square) and rainy (circle) seasons in Caatinga vegetation in Vale do São José, Caetés municipality, Brazil.

accelerated decomposition of leaf litter that accumulated during the dry season, and increased movement of insects that were restricted to moister sites during the dry season (Wolda 1988; Whitford 1996). However, in our study, despite these landscape changes caused by precipitation, the foraging activity of scorpions did not differ between seasons. Carmo et al. (2013) analyzed the effects of seasonality on scorpion assemblages in two climatically different Caatinga areas: hyperxerophytic and hypoxerophytic. These authors only found an increase in scorpion foraging activity during the rainy season in hyperxerophytic vegetation. The hypoxerophytic Caatinga comprises an ecotonal zone between coastal Atlantic Forest and hyperxerophytic Caatinga; thus, it has an intermediate climate (Andrade-Lima 2007). This climate may mitigate the effects of severe drought experienced by hyperxerophytic Caatinga and enable the provision of food resources throughout the year for generalist predators, such as scorpions.

The scorpion species richness recorded here suggests that the Vale do São José area is one of the richest Caatinga fragments studied with regard to these arachnids. In 15 Caatinga fragments previously analyzed in this northeast Brazilian region, the number of species found only ranged from two to six per site (Araújo et al. 2010; Carmo et al. 2013; Lira et al. 2019b). In a similar way, the species richness found here is higher than richness reported to a neighbor biome, the Atlantic forest, where the species number ranges from two to five (Dias et al. 2006; Lira & Albuquerque 2014; Lira et al. 2018, 2019b). The elevated species richness described in our study area may be associated with the environmental gradients present in Vale do São José. This area possesses a high vegetation diversity; therefore, the high scorpion richness observed corroborates the results of several studies that suggest that scorpion richness is positively associated with habitat complexity (Smith 1995; Druce et al. 2007; Lira et al. 2016, 2018). Assemblages of these arachnids are composed of microhabitat-specialist species because scorpions may be aggressive with heterospecifics (Polis 1990; Lira et al. 2018). In addition to higher microhabitat availability, the elevated scorpion richness may also be related to the ecotonal nature of hypoxerophytic Caatinga, in the Vale do São José area, as we found species typical to the Atlantic Forest, such as *T. pusillus* and *A. mauryi*, and species typical for Caatinga, such as *J. rochae* and *B. rochai* (Lira et al. 2019a). Our data corroborate with findings by Lira et al. (2019a) that evaluated the scorpion diversity along an Atlantic forest-Caatinga gradient. These authors found a higher diversity in an ecotonal zone than the extremes of gradient.

Differences found in species composition and dominance between seasons may be related to the biological traits of species. For example, *B. asper* is a small (21–40 mm) fossorial species that shows niche overlaps with *B. rochai*, a mid-sized (29–46 mm) scorpion (Lourenço 2002), and was only found during the dry season. Body size has been recognized as an important factor in interactions between scorpion species, with larger-sized species acting as predators of juveniles or smaller-sized species (e.g. Polis & McCormick 1987; Moreno-González & Hazzi 2012; Lira et al. 2017). In this manner, many small species evolved behaviors to avoid predation in the presence of large species (Polis & McCormick 1987; Dionisio-da-Silva et al. 2019). Another example as the biological traits of species may play a key role in scorpion composition and dominance is the presence of *T. neglectus* during only the rainy season. The increment in foraging activity of this species may be related to the reproductive season. Climatic conditions during the rainy season may be favorable to reproductive dispersion by males. Many scorpion species found in Caatinga vegetation increased their foraging activity during this season (Araújo et al. 2010; Lira et al. 2018).

In summary, our study shows no differences in scorpion foraging activity between seasons in a Caatinga vegetation area. These results suggest that scorpions may be active throughout the year in hypoxerophytic Caatinga. In addition, the high species richness found in our sampled area reinforces the importance of this area for biodiversity conservation, as pointed out by Tabarelli and Silva (2003). These authors suggest that Caatinga vegetation in Caetés municipality is important to conserve; thus, they suggest the creation of conservation areas. Therefore, our findings provide motivation for future studies aimed at conserving this area.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Predation of *Dendropsophus branneri* (Cochran, 1948) (Anura: Hylidae) by wandering spiders (Araneae: Ctenidae) in an Atlantic Forest remnant

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Anurans are considered important to ecosystems because they are predators as well as prey of many vertebrates and invertebrates (Duellman and Trueb, 1994; Haddad et al., 2013). Spiders are among the most important invertebrate predators of anurans (Toledo, 2005; Wells, 2007). Although field observations have shown that spiders frequently prey on anurans (e.g., Prammuk and Alamillo, 2002; Rodrigues and Arruda, 2007; Santana et al., 2009), these reports are still insufficiently documented in many tropical forests. Atlantic Forest communities are home to 625 anuran species and have endemism rates ranging from 65 to 87% (Haddad and Prado, 2005; Haddad et al., 2013; Rossa-Feres et al., 2017). Most instances of spider predation have been recorded from the southern part of the Atlantic Forest (e.g., Barbo et al., 2009; Maffei et al., 2014; Amaral et al., 2015). This is particularly true for hylid frogs of the genus *Dendropsophus* Fitzinger, 1843 (Baracho et al., 2014; Sena and Solé, 2019). *Dendropsophus* are small frogs that are widely distributed across Neotropical forests and are commonly found in marginal vegetation (Gondim et al., 2013; Frost, 2020). Spiders are an important component of the *Dendropsophus* diet; however, this relationship is size-dependent, with large frogs being spider predators (e.g., Abegg et al., 2014; Bovo et al., 2014; Castro et al., 2016), and small frogs being spider prey. Observations of spider predation provide important information to understand predator-prey interaction networks (Toledo et al., 2007; Wells, 2007). We herein report a case of *Dendropsophus* predation by a spider in the Atlantic Forest. In addition, we provide a compiled list of published records of *Dendropsophus* predation by spiders in the Neotropical region.

Predation was observed during nocturnal (20:00–22:00 h) fieldwork in July 2019 at Estação Ecológica do Tapacurá (8.03334°S, 35.21667°W; WGS 84), Municipality of São Lourenço da Mata, Pernambuco State, northeast Brazil. The reserve has a total area of 400 ha, composed of the semi-deciduous seasonal Atlantic Forest (Moura et al., 2012; Moura, 2019). We recorded a spider with its chelicerae inserted into the dorsal surface of a dead frog in the marginal vegetation (ca. 50 cm above ground) of a temporary pond. Even after the spider was captured and killed, it did not release its prey (Fig. 1). The list of published records of predation in the Neotropical region was compiled by searching the keywords "*Dendropsophus*", "spider", and "predation" in Google Scholar.

Figure 1. Predation of *Dendropsophus branneri* by a *Ctenus* sp. juvenile in July 2019 at Estação Ecológica do Tapacurá, Pernambuco State, northeast Brazil. Photo by Rogério Oliveira.

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Once found, the spider fled without releasing its prey. Both animals were collected, and the spider was sent to spider taxonomist Leonardo Carvalho (Universidade Federal do Piauí – UFPI) for identification. Because of the absence of diagnostic features and the difficulty in identifying juveniles, the spider was classified to the genus level as *Ctenus* Walckenaer, 1805. The frog was identified as *Dendropsophus branneri* (Cochran, 1948), with characteristic white blotches below the eyes. *Dendropsophus branneri* is a small hylid anuran widely distributed along the Brazilian coast, from the states of Maranhão to Rio de Janeiro (Frost, 2020). Voucher material was deposited in the Arachnological collection of UFPI and the Herpetological collection of Universidade Federal Rural de Pernambuco.

We compiled 30 predation records for 17 *Dendropsophus* species by spiders from the families Araneidae, Ctenidae, Lycosidae, Nephilidae, Pisauridae, and Trechaleidae (Table 1). The most common arachnid predators belonged to the family Ctenidae (Table 1). Our report is the first record of *Ctenus* spiders preying on *D. branneri*. This species, like other frog species,

Table 1. Records of predation of *Dendropsophus* tree frogs by spiders in the Neotropical region.

Anuran species	Spider (Family)	Reference
D. branneri	*Thaumasia* sp. (Pisauridae)	Baracho et al. (2014)
	Nephila clavipes (Nephilidae)	Souza et al. (2019)
	Ctenus sp. (Ctenidae)	This study
D. brevifrons	*Ancylometes rufus* (Ctenidae)	Pinto and Campos-Costa (2017)
D. ebraccatus	*Cuppienius coccineus* (Trechaleidae)	Szelistowski (1985)
	Ctenidae	Donnelly and Guyer (1994)
D. elegans	*Ancylometes* sp. (Ctenidae)	Serafim et al. (2007)
	Phoneutria nigriventer (Ctenidae)	Santana et al. (2009)
D. haddadi	*Parawixia kochi* (Araneidae)	Sena and Solé (2019)
D. kamagarini	*Phoneutria* sp. (Ctenidae)	von May et al. (2019)
D. leali	*Ancylometes* sp. (Ctenidae)	von May et al. (2019)
D. leucophyllatus	*Ancylomet*es sp. (Ctenidae)	Jansen and Schulze (2008)
D. melanargyreus	*Ancylomet*es *rufus* (Ctenidae)	Moura and Azevedo (2011)
	Ancylometes concolor (Ctenidae)	Fadel et al. (2019)
D. microcephalus	*Cupiennius salei* (Trechaleidae)	Ríos-Rodas et al. (2016)
D. micros	*Thaumasia velox* (Pisauridae)	Bovo et al. (2014)
D. minutus	*Dolomedes* sp. (Pisauridae)	Bastos et al. (1994)
	*Ancylomet*es *rufus* (Ctenidae)	Bernarde et al. (1999)
	*Ancylomet*es *rufus* (Ctenidae)	Menin et al. (2005)
	Dolomedes sp. (Pisauridae)	Pombal jr (2007)
	Ancylometes concolor (Ctenidae)	Bocchiglieri et al. (2010)
	Aglaoctenus oblongus (Ctenidae)	Abegg et al. (2014)
	Thaumasia velox (Pisauridae)	Bovo et al. (2014)
	Thaumasia sp. (Pisauridae)	Moura et al. (2019)
	Parawixia sp. (Araneidae)	Moura et al. (2019)
D. nanus	*Thaumasia* sp. (Pisauridae)	Prammuk and Alamillo (2002)
D. pseudomeridianus	*Hogna* sp. (Lycosidae)	Folly et al. (2014)
D. sanborni	*Diapontia* cf. *uruguayensis* (Lycosidae)	Del-Grande and Moura (1997)
D. sarayacuensis	*Ancylomet*es *rufus* (Ctenidae)	Rodrigues and Arruda (2007)
	Ancylometes sp. (Ctenidae)	von May et al. (2019)
D. werneri	Lycosidae	Oliveira et al. (2010)

aggregates in temporary and permanent ponds in open areas during the breeding season, where frogs can be found under vegetation (Haddad and Prado, 2005). *Ctenus* spiders are nocturnal predators that typically forage on leaf litter, actively searching for prey and capturing them after a quick attack (Griswold et al., 2005; Rego et al., 2005). Aggregations of *D. branneri* and their nocturnal activity may facilitate encounters with spiders of the genus *Ctenus*. Thus, small frogs may be an important component of the diet of Ctenidae spiders during the rainy season, when temporary ponds abound.

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Influence of the edge-core gradient on the scorpion assemblage (Arachnida, Scorpiones) in a Brazilian Atlantic forest

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ORIGINAL RESEARCH ARTICLE

Influence of the edge-core gradient on the scorpion assemblage (Arachnida, Scorpiones) in a Brazilian Atlantic forest

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Abstract

Habitat structure plays a major role in the distribution of species in a given area. Edge-core gradients may be useful for examining the effects of habitat modifications, as they represent changes in habitat structure at a small spatial scale. Here, we analyzed how scorpion assemblages were affected by variations in habitat structure along an edge-core gradient in a Brazilian Atlantic forest fragment. Scorpions were collected during 20 months (May 2007 – December 2008) in 112 pitfall traps, arranged from the fragment edge to 500 m inside the forest interior. All pitfall traps were checked in each two days with all scorpions found being collected. We also measured eight environmental variables along the edge-core gradient: canopy cover, leaf litter depth, diameter at breast height (DBH), plus the densities of fallen logs, stones, trees, shrubs, and bromeliads. We collected a total of 767 scorpions, all within the Buthidae family, and five species were identified (*Ananteris mauryi*, *Tityus brazilae*, *T. neglectus*, *T. pusillus*, and *T. stigmurus*). The abundance of these arachnids was positively influenced by habitat structure, with more individuals occurring in areas with trees having higher DBH values. However, species richness and composition were not affected by any of the environmental variables. Our results suggest that, at small spatial scales, scorpion assemblage (i.e. abundance) is sensitive to changes in habitat structure. Here, we have highlighted the importance of habitat structure for the maintenance of scorpion assemblages in their habitats.

Keywords Biodiversity · Spatial distribution · Edge effect · Neotropical forest

Introduction

Habitat fragmentation is largely recognized as the primary factor responsible for the current decline in biodiversity (Haddad et al. 2015; Newbold et al. 2015). In addition to habitat loss *per se*, the formation of new edges has disturbing effects on the plant and animal composition along the forest, increasing extinction rates of many species and the replacement of natural vegetation (Haddad et al. 2015; Pfeifer et al. 2017). However, there are differing opinions about how fragmentation truly affects biodiversity (e.g. Fletcher et al. 2018; Fahrig et al. 2019). The biodiversity and productivity are influenced differently depending on patch size, edge effects, and the habitats surrounding forest matrix (Flinn et al. 2005; Fletcher et al. 2018; Fahrig et al. 2019). Knowledge about fragmentation, especially edge effects, is essential in regard to global warming because several forests are affected by fragmentation, for instance, 9% of Atlantic forests are within 1 km of forest edges (Ribeiro et al. 2009; Haddad et al. 2015).

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This is alarming because temperatures in tropical forests are affected by edge effects (Arroyo-Rodríguez et al. 2017).

The microclimates in forest edges often differ from core, because the edges are transition zones between the areas surrounding forest. Such conditions make forest edges more vulnerable to strong winds, higher light levels, higher air temperature, and lower humidity levels than forest interiors (e.g. Davies-Colley et al. 2000; Pinto et al. 2010; Magnago et al. 2017). These differences act as primary factors influencing the characteristics of forest structure; resulting in increased litterfall, higher tree mortality rates, and more canopy gaps at the forest edges (Laurance et al. 2002). These factors lead to higher accumulation and lower decomposition of leaf litter in forest edges, which in turn constrains seed survival and germination and the reduction of biomass in forest edges (Bruna 1999; Scariot 2000; Laurance et al. 2000, 2002; Crockatt and Bebber 2015).

Areas near the forest edge have a lower abundance of secondary succession trees; however, there is a higher abundance of short-lived pioneer trees comprising a lower canopy with a high rate of leaf litter replacement (Oosterhoon and Kappelle 2000; Laurance et al. 2002). In addition, the mean tree height and basal area are lower at forest edges because their microclimate conditions negatively affect the larger and older trees (Magnago et al. 2017). Haddad et al. (2015) found that reduced fragment size and increased forest edge caused a loss of large and old trees at forest edges, followed by a shift to more pioneer trees, which had subsequent impacts on the community composition of animals (Laurance et al. 2000; Pfeifer et al. 2017). Thus, conditions near forest edges are often similar to those of disturbed habitats (Pardini 2004) and may favor invasion by species that are typically found in disturbed habitats. Therefore, the effects of forest edges on biodiversity gradually changes from the edge to the interior, with forest-specialist species thriving better near the forest core (Oosterhoon and Kappelle 2000; Eggleton et al. 2002; Carpio et al. 2009).

Variations in the abiotic and biotic factors found along an edge-core gradient may depend on the magnitude of depth-of-edge influence (Harper et al. 2005). In forest ecosystems, the intensity of an edge effect ranges from a few meters to 250 m; although some studies point out that edge effects may go as far as 1 km (e.g. Laurance et al. 2007; Ewers and Didham 2008; Gonzalez et al. 2010). Different species respond differently to the edges; even those inhabiting the same forest edge have distinctive affinities to their features (e.g. Altamirano et al. 2016; Fonseca et al. 2017; González et al. 2017; Salomão et al. 2018). González et al. (2017) found higher hexapod richness and abundance at the edge of a Chaco Serrano forest, which may result from the simultaneous presence of forest and matrix species. In contrast, edaphic beetles in a Brazilian Atlantic forest had higher abundance and species richness in a sampling site 100 m inside a forest (Salomão et al. 2018). Thus, understanding how changes in invertebrate assemblages occur across a forest edge-core gradient may be essential to our understanding of how habitat alteration influences the establishment of arthropod populations and their contribution to forest ecosystems. Invertebrates are also important to the diet of several animal groups, further highlighting the need to study their distribution across habitats (e.g. Maneyro et al. 2004; Mamou et al. 2016; Leal et al. 2018).

Among arthropods, groups with a limited dispersion capacity and a strong relationship with habitat structure (Due and Polis 1986; Foord et al. 2015; Lira et al. 2018a, b) may be affected more by habitat alterations found in an edge-core gradient. The local scorpion assemblage typically comprises microhabitat-specialist species (Polis 1990; Lira et al. 2018a, b), with abundance and richness being affected by changes in habitat structure (Druce et al. 2007; Lira et al. 2016, 2019a, b). A previous study performed in a Brazilian Atlantic Forest fragment revealed a differential response to the edge effect in litter-dwelling scorpions: the abundance of *Tityus pusillus* Pocock, 1893 was significantly reduced, while *Ananteris mauryi* Lourenço, 1982 was not affected by an edge-core gradient (Dionisio-da-Silva et al. 2018). The authors suggest that microhabitat environmental factors in the forest fragment influenced the abundance of leaf litter scorpions in the Brazilian Atlantic Forest. However, no studies have been conducted to investigate how edge effects influence scorpion assemblage. Here, we analyzed the species richness, abundance, and taxonomic composition of the scorpion assemblage in relation to habitat structure in an edge-core gradient in a fragment of the northeastern Brazilian Atlantic Forest. The Brazilian Atlantic forest is largely fragmented and has only 12% of its original extent due to continuous anthropogenic disturbances, such as logging and agricultural activities (Ribeiro et al. 2009). The remnants are mostly composed of secondary-growth forest fragments of less than 100 ha and have an high perimeter-to-area ratio. Almost half of all Brazilian Atlantic rainforests are located within a transition zone 90 to 100 m from the forest edge (Ranta et al. 1998; Ewers and Didham 2007; Metzger et al. 2009; Ribeiro et al. 2009). We tested the hypothesis that the scorpion assemblage is negatively affected by the edge and predicted that species richness and abundance are higher in the forest core, which is less disturbed.

Materials and methods

Study area

The Tapacurá Ecological Station (TES) (Fig. 1a) is composed of three fragments: Mata do Camucim (200 ha), Mata do Alto da Buchada (100 ha), and Mata do Toró (100 ha), which are located in the São Lourenço da Mata municipality (8º 2’ 12’’ S, 35º 11’ 41’’ W), Pernambuco state, in northeastern Brazil.

The TES fragments are surrounded by a sugar cane monoculture. Mata do Alto da Buchada and Mata do Camucim are classified as semideciduous seasonal Atlantic forest, and Mata do Toró is an ombrophilous Atlantic forest (Moura et al. 2012, Moura 2019). The vegetation is dominated by tree families such as Anacardiaceae, Lecythidaceae, Melastomataceae, Annonaceae, Apocynaceae, Sapotaceae, Celastraceae, Lauraceae, Burseraceae, and Fabaceae (Ferraz et al. 2012). The area is characterized by an annual rainfall of 1,900 mm, with a rainy season between May and August (Moura et al. 2012).

Scorpion sampling

Scorpions were sampled montly for 20 months (May 2007 to December 2008) in the Mata do Camucim and Mata do Alto da Buchada fragments (Fig. 1a). Animals were collected by pitfall traps, using a guide fence as described by Gibbons and Semlitsch (1981). Pitfalls traps consisted of cylindrical plastic containers arranged in a 'Y' configuration; with each sampling plot consisting of a central trap (diameter 50 cm) and three peripherical traps (diameter 30 cm) (Fig. 1b). Pitfall traps were installed at the center of each plot (20 m × 20 m) and checked every two days. To assess the edge effect, plots were arranged within four, 500 m long transects along an edge-core gradient, with plots being 80 m apart. There were seven plots per transect (10, 90, 170, 250, 330, 410, and 490 m from the edge). Within each forest fragment, two transects were separated by 500 m. The edge of the forest fragments used in this study faced Tapacurá Lake. A total of 112 pitfalls traps (28 "Y" sampling plots) were used. All collected scorpions were stored in 70% ethanol and *a posteriori* identification was performed according to Lourenço (2002). Voucher specimens were deposited in either the Aracnological collection of Universidade Federal de Pernambuco, or the Entomological collection of Universidade Federal Rural de Pernambuco.

Environmental variables

Habitat structure along the edge-core gradient was bimonthly analyzed in each 20 m × 20 m plot described above. We recorded eight environmental variables: canopy cover, leaf litter depth, diameter at breast height (DBH), and the densities of fallen logs, stones, trees, shrubs, and bromeliads. Canopy cover was measured through a five 0.25 m^2 squares PVC frame, whose internal area was divided by nylon wires spaced at 2.5 cm intervals. The internal area of the frame was filled with 100 identical parcels, which we used to estimate (as a percentage) the open (unshaded) or covered (shaded) area of the canopy. Leaf litter depth was recorded as a point measurement in all corners and in a central point of each 20 m × 20 m plot with a Marberg caliper (precision of 0.05 mm). Tree diameter was measured at breast height (DBH) using a millimeter tape.

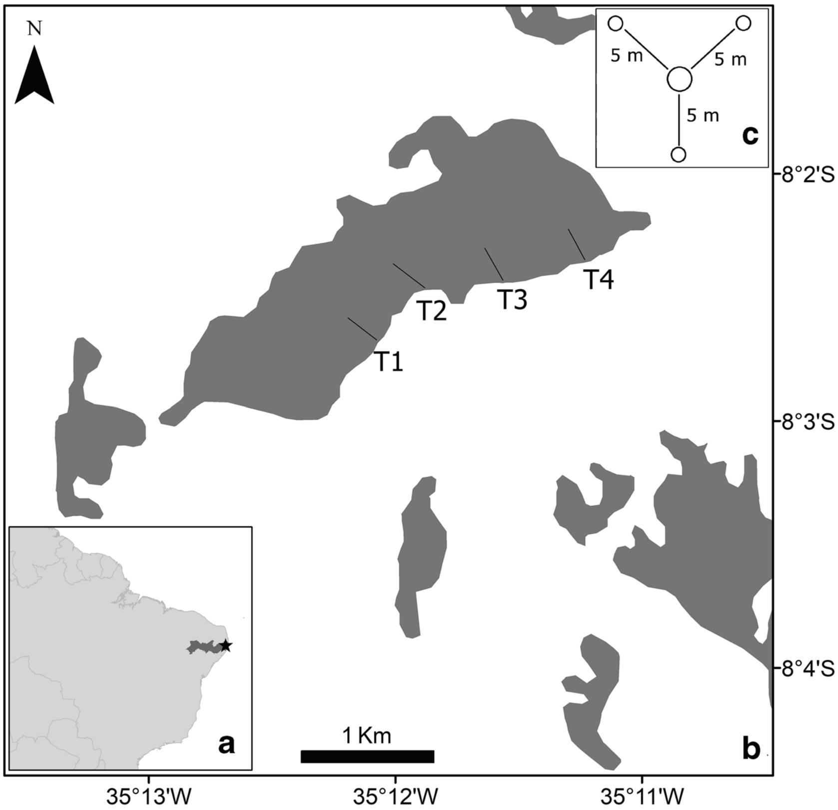

Fig. 1 Map of South America (**a**) showing the sampling transects (T1-4) (**b**) and a scheme of trap design (**c**) along an edge-core gradient in a Brazilian Atlantic forest fragment

Fallen logs, stones, trees, shrubs, grasses, and bromeliad densities (units per m²) were recorded by direct counting in each 20 m × 20 m plot.

Data analyses

The richness of the scorpion assemblage was assessed by a collector curve considering the number and abundance of species observed in each sampling plot. The species accumulation curve was generated by 9,999 randomizations using the R software version 3.2.0. (R Core Team 2015).

The degree of collinearity between environmental variables was determined through the variance inflation factor (VIF) (Eisenlohr 2014). A VIF < 10 indicates an absence of data multicollinearity in the linear model analysis (Zuur et al. 2010). To characterize the edge-core gradient, the relationships between environmental variables and edge distance was assessed with a Pearson correlation. Scorpion richness and abundance along the edge-core gradient were evaluated by fitting by generalized linear models (GLM) with a negative binomial distribution for abundance and Poisson distribution for species richness. We used DBH, leaf litter depth, and tree density as independent variables and included edge distance as a cofactor in our models. In the model, independent variables were reduced based on the Akaike Information Criteria (AIC). The variables excluded in the most parsimonious models were considered to be not related to the variance in diversity metrics. The significance of the dependent variables was tested using a likelihood ratio test between the full and the reduced model. All of procedures were performed using the lmtest package (Zuur et al. 2009; Hothorn et al. 2018). The relationships between the most abundant species and the environmental variables were verified by normal multivariate regressions and by simple linear regressions for each species in R software.

Results

Five of the eight environmental variables were excluded due to multicollinearity (Table S1), and we considered in our analysis the following variables: DBH, leaf litter depth, and tree density.

Sampling resulted in five species and 864 individual scorpions collected throughout the study period, all of them classified within the Buthidae family, *Tityus pusillus*, *T. stigmurus* (Thorell, 1876), and *Ananteris mauryi* were the most abundant scorpion species, representing 99.4% of the collected specimens. *Tityus pusillus* and *A. mauryi* were widespread inside the Atlantic forest fragment (found across the edge-core gradient), while *T. stigmurus* was found mostly within 90 m of the edge (Table 1). The other two species, *T. brazilae* Lourenço and Eickstedt, 1984 and *T. neglectus* Mello-Leitão, 1932, along the gradient without a clear pattern in their distributions. Overall, local diversity of scorpions was sampled by the method used in this study according to accumulation curve (Fig. 2).

We found no correlations between edge distance and the DBH of trees, leaf litter depth, or tree density, which indicates a similar structure along the gradients analyzed (Table 2). Only, leaf litter depth and DBH were retained by the best model for scorpion abundance. Although leaf litter depth did not affect scorpion abundance (X^2 = 11.33; d.f. = 1,5; p = 0.06), DBH had a significant (X^2 = 7.32; d.f. = 1,4; p = 0.04) positive relationship with abundance (Fig. 3). However, DBH did not affect species richness (X^2 = 1.04; d.f. = 1,5; p = 0.81). Leaf litter depth and tree density were not retained by the best model for explaining scorpion species richness. *Tityus pusillus* and *T. stigmurus* were the most abundant species and represented 92% of the overall scorpion assemblage abundance (69.56 and 22.45%, respectively). Thus, the effects of the environmental factors on both species were analyzed separately. For these species, there was no relationship between DBH

Table 1 Scorpion abundance and richness along an edge-core gradient in two Brazilian Atlantic forest fragments

Edge distance (m)	Scorpion species						
	Ananteris mauryi	*Tityus pusillus*	*Tityus stigmurus*	*Tityus brazilae*	*Tityus neglectus*	Total	Richness
10	1	51	52	-	-	104	3
90	2	29	38	1	-	70	4
170	2	122	6	1	-	131	4
250	25	113	-	2	-	140	3
330	4	97	-	-	-	101	2
410	7	110	1	1	-	119	4
490	22	79	-	-	1	102	3
Total	63	601	97	5	1	767	5

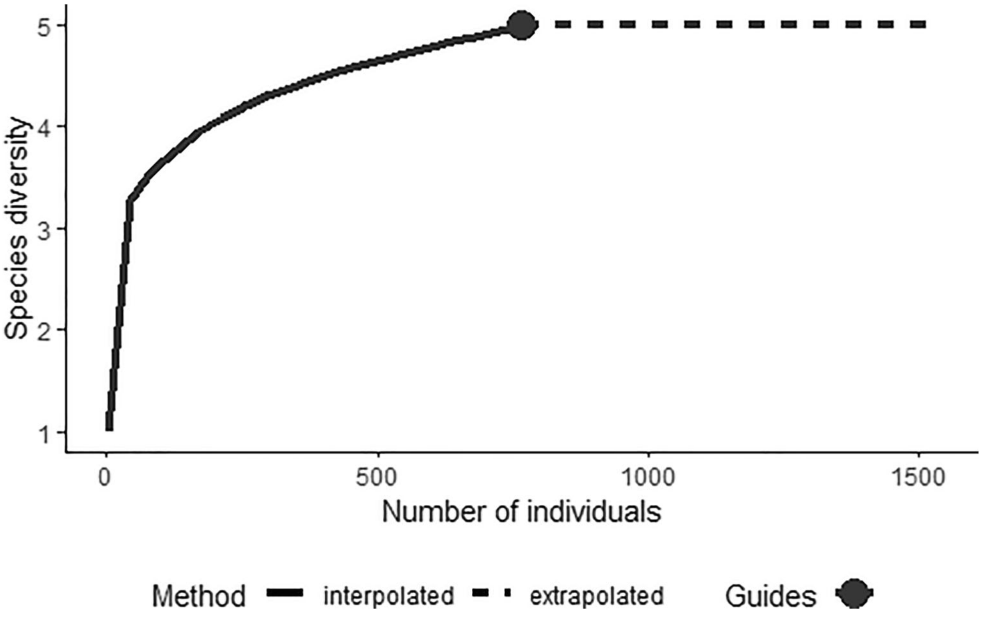

Fig. 2 Scorpion species accumulation curves along an edge-core gradient in a Brazilian Atlantic forest fragment

of trees, leaf litter depth, or tree density and relative abundance (*T. pusillus*: $F_{3,3} = 6.01$; $p = 0.08$; $R^2 = 0.50$; *T. stigmurus*: $F_{3,3} = 3.57$; $p = 0.16$; $R^2 = 0.28$).

Discussion

In this study, we evaluated how habitat structure influences the scorpion assemblage along an edge-core gradient in a Brazilian Atlantic forest fragment. We found comparable DBH, leaf litter depth, and tree density along the edge-core gradients, indicating similar habitat structures. DBH was positively correlated with scorpion abundance but was not related to species richness or composition. Areas with higher DBH values indicate the presence of older-age trees, suggesting a state of dynamic equilibrium (Pessoa and Araujo 2014). This state of dynamic equilibrium may be reinforced by the distribution of *T. pusillus* along the edge-core fragment, as previous studies reported that this scorpion species is sensitive to habitat change (Lira et al. 2015, 2019a, b; Dionisio-da-Silva et al. 2018). Dionisio-da-Silva et al. (2018) investigated the edge effects in *T. pusillus* abundance and found a lower abundance in an anthropogenic edge than in the forest core. In addition, this species exhibited lower abundance in monocultures than in neighboring forest fragments (Lira et al. 2019a, b). In contrast, our results indicates that this species possess a widespread distribution along edge-core gradient in a fragment analyzed. Edges created by natural processes (mainly by succession), and edges repeatedly disturbed by anthropogenic activities (forestry, agriculture, urbanization), have different structural and functional characteristics, and different influences on species assemblages (Strayer et al. 2003). For example, Fonseca et al. (2017) analyzed the spatial distribution of *Amblyomma* spp. ticks in an Atlantic forest fragment and found that the ticks were more abundant in agropastoral landscapes than in a natural edge. Forest edges maintained by natural processes had significantly higher species richness than their interiors and differed from edges created by anthropogenic actions (e.g. monoculture). This scenario of dynamic equilibrium may favor generalist predators like scorpions, thereby supporting a balanced abundance along an edge-core gradient found in our study.

The species richness observed in our work ($n = 05$) is higher than the species richness described in other Atlantic forest studies ($n = 02–6$) (Dias et al. 2006; Lira et al. 2016, 2018a, b, 2019a, b). In general, the scorpion assemblage is composed by species with a very narrow habitat specificity, due to the aggressive behavior between different scorpion species (Polis 1990; Lira et al. 2018a, b). Thus, complex habitats reflect greater microhabitat availability, which is associated with higher species richness (Smith 1995; Druce et al. 2007; Lira et al. 2016). Lira et al. (2018a, b analyzed the differences in scorpion fauna in mature and secondary-growth Atlantic forest fragments and found no difference in scorpion richness between the two forest types. This suggests that after 65 years of natural regeneration, the secondary forest possesses a habitat structure that is similar to a mature forest. Similar results were described in the Chaco ecoregion of Argentina (an arid ecosystem with vegetation characterized by dry xerophilous woodland), where there were no differences in scorpion richness between an area with 15 years of secondary forest growth and an old-growth forest (Nime et al. 2014).

Scorpion composition was similar along the edge-core gradient. Two of the five species (*T. pusillus* and *A. mauryi*)

Table 2 Environmental variables along an edge-core gradient in a Brazilian Atlantic forest, with a summary of the Pearson's correlation coefficients

Variables	Edge distance (m)							R
	10	90	170	250	330	410	490	
DBH	33.35 ± 9.10	38.25 ± 7.09	61.25 ± 56.91	40.80 ± 8.26	61.30 ± 47.89	42.50 ± 19.83	44.57 ± 5.14	0.36
Tree density	42.25 ± 14.43	31.25 ± 6.23	24.50 ± 12.23	26.25 ± 4.99	28.72 ± 16.84	31.17 ± 12.47	36.72 ± 16.27	0.05
Leaf litter depth	2.50 ± 0.57	2.43 ± 0.47	2.37 ± 0.50	2.75 ± 0.03	2.43 ± 0.47	2.56 ± 0.47	2.37 ± 0.50	0.04

exhibited widespread distributions in all plots inside the fragment. However, *T. stigmurus* individuals were mostly found within 90 m of the forest edge. This is a synanthropic scorpion, adapted to more disturbed environments such as that found in the outer areas of the edge-core gradient. This species also exhibits a higher degree of ecological plasticity, as it can be found in seasonal tropical dry forests (Caatinga) and in Atlantic forest remnants (Lira et al. 2016, 2019a, b). Our findings suggest that *T. stigmurus* is more capable of using to use forest edge habitats. In contrast, *T. neglectus* was only found in the fragment core. However, this is a bromeliaceous species (Lourenço and Eickstedt 1988; Santos et al. 2003) and our sampling technique (pitfall trap) may not be an effective method to sample. Scorpions are usually sedentary animals that don't stray too far from their shelter (Polis 1990). Pitfall trap is widely method used to collect scorpions (e.g. Dias et al. 2006; Araujo et al. 2010; Nime et al. 2013; Lira et al. 2018a). However for a less abundant and microhabitat specialist species collection such as *T. neglectus* (Lira et al. 2018a, b) may be indicated active sampling method. Sissom et al. (1990) suggests that active sampling methods (e.g. active search) generate better results than passive sampling methods (e.g. pitfall traps), because scorpions are collected directly in their shelters.

Our long-term and intensive study collected a relatively small abundance of scorpions when compared to previous studies of Atlantic forest scorpions (e.g. Lira et al. 2016, 2019a). However, the scorpion abundance in our study is larger than that of previous studies that used pitfall traps to collect these animals (Dias et al. 2006; Araujo et al. 2010; Carmo et al. 2013; Nime et al. 2014). For example, 104 scorpions were sampled during 24 months in Caatinga (Brazilian tropical seasonal dry forest) (Araujo et al. 2010). According to Araujo et al. (2010) Nime et al. (2013), scorpion sampling by pitfall traps depend upon the behavior of each species, with wanderer or fossorial species being more easily caught by traps than sedentary species. In fact, our samples were dominated by the soil-dwelling species *A. mauryi, T. pusillus*, and *T. stigmurus*, corroborating finding of previous studies. In summary, we analyzed the correlations between environmental variables and the assemblage of scorpions along an edge-core gradient in a Brazilian Atlantic forest fragment. Forest

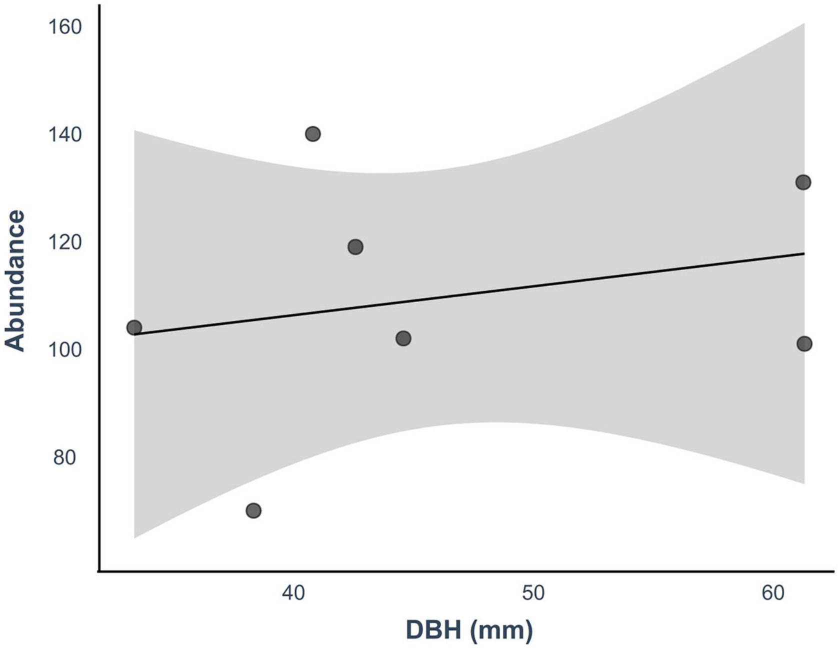

Fig. 3 Relationship between scorpion abundance and diameter at breast height (DBH) along an edge-core gradient in a Brazilian Atlantic forest fragment

edges can present a barrier along anthropogenic-natural habitats, and have gradual effects on biodiversity from the forest edge to the interior (Oosterhoon and Kappelle 2000; Eggleton et al. 2002; Carpio et al. 2009). We found that scorpion abundance was influenced by habitat structure along an edge-core gradient; however, species richness was not affected. Our results also suggest that forest edges created by natural successional events do not influence scorpion assemblages differently from anthropogenic edges; however, this hypothesis needs to be tested. Finally, our results should be interpreted with caution, since a long duration of our sampling may have affected the scorpion abundance and the environmental variables measured.

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Compliance with ethical standards

The field collection was complied with Brazilian law. Voucher specimens were deposited in the scientific collections of the Universidade Federal de Pernambuco and Universidade Federal Rural de Pernambuco following standard procedures, and there are no conflicts of interest (financial and non-financial).

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ABELHAS (HYMENOPTERA: ANTHOPHILA) EM UMA ÁREA DE CAATINGA NO ESTADO DE PERNAMBUCO

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RESUMO - As abelhas são insetos que ocorrem em quase todas as regiões do planeta. Grande parte delas costuma nidificar em ocos de árvores, vivas ou mortas (moirões de cercas, vigas, estacas), entretanto, algumas espécies também nidificam no solo, utilizando ninhos abandonados ou ativos de outros animais sociais. O presente trabalho teve como objetivo realizar um levantamento das espécies de abelhas e dos substratos utilizados por elas para nidificação na região da bacia hidrográfica do riacho São José, em Caetés - PE. Foram registradas 20 espécies de abelhas ocorrendo na área do Vale do São José, sendo elas: *Frieseomelitta doederleini* (Friese, 1900), *Frieseomelitta varia* (Lepeletier, 1836), *Apis mellifera scutellata* Lepeletier 1836, *Dialictus opacus* (Moure, 1940), *Trigona spinipes* (Fabricius, 1793). Como substratos arbóreos utilizados por essas abelhas foram registradas sete espécies botânicas, sendo elas: *Parapiptadenia zehntneri* (Harms) M.P. Lima and H.C. Lima, *Mimosa tenuiflora* (Willd.), *Ruprechtia laxiflora* Meisn, *Brasiliopuntia brasiliensis* (Willd.) A. Berger, *Erythrina velutina* Willd, *Senegalia polyphylla* (DC.) Britton & Rose e *Ziziphus joazeiro* Mart. O presente artigo constitui-se no primeiro registro de espécies de abelhas e de substratos na área estudada, contribuindo, assim, para possíveis estudos de conservação de espécies locais, e para o conhecimento da interação entre a fauna e flora nativa da Caatinga.

Palavras – chave: Brasil: Conservação: Insetos: Espécies: Insetos: Substratos arbóreos

BEES (HYMENOPTERA: ANTHOPHILA) IN A CAATINGA AREA IN THE STATE OF PERNAMBUCO

ABSTRACT - Bees are insects that occur in almost all regions of the planet. Many of them usually nest in hollows of trees, live or dead (fence posts, beams, stakes), however, a large portion of species also nest in the soil, using abandoned or active nests of other social animals. The present work had as objective to survey of the species of bees and the substrates used by them for nesting in the region of the hydrographic basin of the São José stream, in Caetés - PE. In order to accomplishment this work, field observation were performed, monthly, in the period of 22 months, from September 2016 to July 2018, in the dry season and in the rainy season. Twenty species of bees occurred in the São José Valley area, namely: *Apis mellifera scutellata* Lepeletier 1836, *Dialictus opacus* (Moure, 1940), *Frieseomelitta doederleini* (Friese, 1900), *Frieseomelitta varia* (Lepeletier, 1836), *Trigona spinipes* (Fabricius, 1793). As tree substrates used by these bees, twenty six botanical species were recorded, namely: *Brasiliopuntia brasiliensis* (Willd.) A. Berger, *Erythrina velutina* Willd, *Mimosa tenuiflora* (Willd.), *Parapiptadenia zehntneri* (Harms) MP Lima and HC Lima, *Ruprechtia laxiflora* Meisn, *Senegalia polyphylla* (DC.) Britton & Rose and *Ziziphus joazeiro* Mart. This article constitutes the first record of bee species and substrates in the studied area, thus contributing to possible conservation studies of local species, and to the knowledge of the interaction between the native fauna and flora of the Caatinga.

Keywords: Brazil: Conservation: Insects: Species: Tree substrates

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INTRODUÇÃO

As abelhas (Anthophila) compõem um dos grupos mais diversos da ordem Hymenoptera, sendo composto por mais de 20.000 espécies descritas para todo o mundo (ITIS, 2018), estas reunidas em 481 gêneros e 56 tribos, onde o Brasil se destaca por uma fauna igualmente diversa, com cerca de 1.700 espécies já catalogadas, até 2007 (Moure*et al*. 2007).

A família Apidae é a mais diversa, contendo cerca de 5800 espécies (distribuídas em 205 gêneros e 52 tribos), sendo a única que contem abelhas verdadeiramente sociais (FREITAS, 2003). Dentre os Apidae corbiculados, podemos destacar a tribo Meliponini, a qual é composta pelas abelhas "sem ferrão" (com ferrão atrofiado, vestigial, tendo perdido a capacidade de ferroar), representada por cerca de 480 espécies distribuídas pelas regiões tropicais e subtropicais do mundo (cerca de 420 delas ocorrendo na região Neotropical), estas agrupadas em 53 gêneros (PEREIRA, 2006; OLIVEIRA, 2013; PALUMBO, 2015; ITIS 2018). Ainda sobre Meliponini, a fauna Brasileira é composta por cerca de 250 espécies válidas, agrupadas em 29 gêneros (PEDRO, 2014).

Estudos têm mostrado que os insetos são os principais polinizadores da flora do planeta, especialmente as abelhas, borboletas, mariposas, besouros, moscas, vespas e formigas, com destaque para as abelhas, consideradas o grupo de polinizadores mais importante, tanto pela sua morfologia, quanto pela sua biologia e comportamento (FREITAS; SILVA, 2015). Apesar de sua grande diversidade e importância ecológica, as abelhas, principalmente as nativas, têm diminuído em grande velocidade, em termos de abundância, devido principalmente, grande influência negativa do homem sobre os ambientes onde elas habitam (SOUZA *et al.,* 2009).

De acordo com Steffan-Dewenter *et al.* (2006), dentre as principais causas da diminuição das populações nativas de polinizadores estão, o impacto do desmatamento, fragmentação de habitats, introdução de espécies exóticas e práticas agrícolas irracionais. Esta última, por sua vez, é suspeita de ser a causa da baixa produção de frutos e sementes em muitas plantas agrícolas (PEREIRA, 2015).

Em termos de biodiversidade, o bioma Caatinga tem sido um dos biomas mais subamostrados. Ganem (2017) aponta que um dos principais problemas relacionados ao bioma é a carência de informação, incluídos dados geográficos, de vegetação e diversidade faunística. Santos *et al.,* (2011) demonstraram que este é o bioma brasileiro com menor esforço de pesquisa e geração de conhecimento, com um número muito pequeno de artigos publicados em revistas internacionais abrangendo o bioma. Tal fato implica também no conhecimento que se têm referente à fauna apícola local, onde ainda há desconhecimento sobre as espécies de abelhas e dos substratos utilizados para nidificação por elas, o que dificulta a mudança desse quadro e impossibilita a elaboração de práticas de manejo e conservação desses animais.

A primeira síntese do conhecimento sobre invertebrados da Caatinga foi elaborada por Brandão e Yamamoto (2000), a partir de dados obtidos por consulta bibliográfica, consultas a coleções zoológicas e a especialistas nos diferentes grupos (BRANDÃO; YAMAMOTO, 2000). Em relação às abelhas, pode-se citar como trabalhos pioneiros o desenvolvido por Ducke (1911) no qual foram coletados vários exemplares de plantas e abelhas no estado do Ceará e os produzidos por Zanella (2000, 2003) que apresentaram listas de espécies pertencentes ao domínio da Caatinga.

Algumas obras tratam sobre espécies de abelhas e das plantas utilizadas para forrageamento por esses animais na Caatinga, dentre elas podemos citar: Aguiar (2003), Aguiar *et al.,* (2003), Zanella e Martins (2003), Souza (2005), Teixeira (2007), Rodarte et al. (2008), Pigozzo e Viana (2010), Silva *et al.* (2012) e Maia-Silva (2012). No entanto, para a região da Caatinga estudos que tenha como objetivo amostrar fauna apícola ainda são escassos, tendo como exemplo os produzidos por Aguiar (2003), Aguiar *et al.,* (2003), Zanella e Martins (2003), Souza (2005), Bezerra (2010) Silva *et al*., (2014), especialmente quando levamos em consideração a variedade de fitofisionomias do bioma Caatinga.

Mediante esse cenário de escassez de trabalhos que visem amostrar a fauna de abelhas, bem como os substratos utilizados por essas abelhas para nidificação, toda ação que possa contribuir efetivamente para a diminuição dessa lacuna do conhecimento da Caatinga deve ser incentivada. Nesse contexto, o presente estudo teve como objetivo geral realizar um levantamento das espécies de abelhas e dos substratos utilizados por elas para nidificação numa bacia hidrográfica na caatinga, considerando tanto substratos arbóreos ou não.

MATERIAL E MÉTODOS

Área de estudo

A microbacia hidrográfica do riacho São José (8° 46’20.38” S 36° 40’56.05” O) é parte integrante da bacia hidrográfica do rio Ipanema, tendo cerca de 12.500 hectares (ha). Abrangendo a zona rural de quatro municípios do estado de Pernambuco na transição entre as fitoregiões Agreste e Sertão. São estes municípios: Caetés, Paranatama, Pedra e Venturosa. A área é composta por várias fitofisionomias, com áreas florestais remanescentes, onde em alguns pontos há influência de Brejos de Altitude, florestas semideciduais, florestas pluviais estacionais, florestas secas, carrascos e áreas antropófitas distintas. Há ainda ampla gama de ecossistemas rupestres onde são dominantes as famílias Bromeliaceae, Cactaceae e Euphorbiaceae (VIEIRA *et al*., 2017).

Nas áreas de entorno aos locais de coleta há presença de campo destinado a pastagem de gado, plantação de palma (*Opuntia fícus indica* (L.) Mill., e diversos impactos como queima e retirada de vegetação, determinando antropização na área de estudo.

Procedimentos em campo

Para a realização deste trabalho, foram realizadas incursões para observação em campo, mensalmente, no período de 22 meses, de setembro de 2016 a julho de 2018, na estação seca e na estação chuvosa, com início às 08:00 h e término às 14:00 h, totalizando 180 h de esforço amostral.

Para a coleta dos espécimes de abelhas foram utilizados dois métodos: a instalação de armadilhas coloridas de água (ARCAs) (SANTANA; OLIVEIRA 2010), em pontos pré-determinados na área, e busca ativa nas flores com auxílio de rede entomológica, onde era percorrido um percurso.

Uso de ARCAs – conhecidas também como *Pantraps* (nome no idioma Inglês), consistem de recipientes coloridos contendo uma solução de água e detergente. Eles foram instalados pela manhã, no solo, próximo à vegetação aberta, alternando-se as cores dos pratos, e foram retirados no dia seguinte, após 24 h. Foram instaladas ARCAs nas cores Azul (n=5), Vermelho (n=5) e Branco (n=5), de forma intercalada, totalizando 15 ARCAs por ponto de coleta. As instalações das *ARCAs* procederam-se em dois pontos ao longo da área de estudo: em um ponto controle que era fixo ao longo das coletas, ou seja, todos os meses havia instalação de armadilhas nesse ponto, o outro ponto variava ao longo dos meses, buscando uma maior abrangência da área de estudo.

Censo em flores com rede entomológica: consiste na captura e observação de abelhas sobre as flores com o auxílio de redes entomológicas. As coletas foram realizadas por um coletor, durante dois dias consecutivos, no mesmo momento de instalação e retirada das ARCAs, quando foram também percorridos um transecto para a captura e observação de abelhas nas flores. O transecto percorrido variou durante todo o estudo, para que houvesse uma maior abrangência da área.

Para observação dos substratos utilizados pelos Meliponini para nidificação, foram percorridas rotas em áreas com diferentes fitofisionomias, objetivando locais com maiores chances de se encontrar ninhos. Todas as rotas foram georreferenciadas com auxílio de aparelhos GPS. Ao encontrar um tipo de substrato em campo, foram feitas as observações para a constatação da presença do ninho, bem como, a identificação do substrato. Para identificação e confirmação das espécies arbóreas encontradas foram utilizados os seguintes trabalhos: Gomes *et al.*, (2011), Maia (2004), Maia-Silva *et al.*, (2012), Vieira (2017).

Logo após, anotou-se o número de ninhos encontrados naquele substrato. Também foram realizados registros fotográficos dos ninhos, além da caracterização do contexto ambiental do mesmo. Os dados referentes às idas a campo, como: localização, distância percorrida, mapa de área percorrida, foram analisados com auxílio do aplicativo Android Velocímetro. Os dados referentes aos exemplares observados e coletados foram analisados, apenas em nível de porcentagem.

Procedimento em laboratório

As abelhas coletadas foram colocadas em recipientes contendo álcool 70%, sendo posteriormente levadas ao laboratório de Zoologia da Universidade de Pernambuco – UPE *Campus* Garanhuns, onde se encontra a coleção Didática de Zoologia.

A identificação dos exemplares foi realizada pela Prof.ª Drª Favízia Freitas de Oliveira, taxonomista de abelhas e coordenadora do Laboratório de Bionomia, Biogeografia e Sistemática de Insetos (BIOSIS), localizado no Instituto de Biologia da Universidade Federal da Bahia (UFBA), campus de Ondina em Salvador, tendo sido os exemplares depositados no acervo entomológico de referência do BIOSIS, unidade associada ao Museu de História Natural da UFBA (MHNBA), para estudos mais aprofundados.

RESULTADOS E DISCUSSÃO

No presente estudo foi coletado um total de 91 exemplares de abelhas, distribuídos em 3 famílias, 11 tribos, contabilizando 20 espécies amostradas (Tabela 1).

Tabela 1 – Checklist de espécies de abelhas existentes no vale do riacho São José.

FAMÍLIA	**SUBFAMÍLIA**	**TRIBO**	**ESPÉCIE**
ANDRENIDAE	Andreninae	Protandrenini	*Anthrenoides rodrigoi* Urban, 2005
			*Psaenythia*sp.1
		Calliopsini	*Callonychium* (*Callonychium*) *brasiliense* (Ducke, 1907)
			*Acamptopoeum*sp.1
APIDAE	Apinae	Apini	*Apis mellifera scutellata* Lepeletier, 1836
		Centridini	*Centris* (*Paracentris*) *hyptidis* Ducke, 1908
		Emphorini	*Diadasina riparia* (Ducke, 1907)

			Melitomella murihirta (Cockerell, 1912)
			Melitoma segmetaria (Fabricius, 1804)
		Exomalopsini	*Exomalopsis* (*Exomalopsis*) *analis* Spinola, 1853
		Meliponini	*Frieseomelitta varia* (Lepeletier, 1836)
			Frieseomelitta meadewaldoi (Cockerell, 1915)
			Trigona spinipes (Fabricius, 1793)
		Xylocopini	*Xylocopa* (*Neoxylocopa*) *ordinaria* Smith, 1874
HALICTIDAE	Halictinae	Halictini	*Dialictus opacus* (Moure, 1940)
		Augochlorini	*Pseudaugochlora pandora* (Smith, 1853)
			Pseudaugochlora sp.1
			*Augochlora*sp.1
			*Augochlora*sp.2
		Rophitini	*Ceblurgus longipalpis* Urban e Moure, 1993

Dentre os táxons identificados, a família mais representativa foi Apidae com 10 espécies (50 %) seguida por Halictidae com seis espécies (30%). A família com o menor número de espécies foi a Andrenidae com quatro espécies (20%), tendo sido, assim, amostrados exemplares de apenas três das cinco famílias de abelhas pertencentes à fauna brasileira. Considerando a biodiversidade de abelhas no estado de Pernambuco, um estudo realizado por Milet-pinheiro e Schlindwein (2008) em Chã-Grande, Agreste Pernambucano, registrou 79 espécies distribuídas nas cinco famílias que ocorrem no território brasileiro. Portanto, este estudo apresentou uma riqueza baixa de espécies, se compararmos com o estudo citado que foi realizado na mesma região e estado.

Se ainda compararmos com estudos realizados em bioma Caatinga como um todo, como em Viana (1999) que registrou 31 espécies distribuídas nas cinco famílias, Zanella (2003) 100 espécies distribuídas em quatro famílias, Aguiar e Zanella (2005) 60 espécies distribuídas em quatro famílias, Batata-Filho (2007) 49 espécies distribuídas nas cinco famílias, o status de baixa riqueza apresentado neste estudo permanece. Todos os estudos citados foram realizados no período de um ano, com exceção de Batata-Filho (2007) que perdurou por dois anos, Viana (1999), porém houve coletas em apenas quatro meses deste ano e Aguiar e Zanella (2005) um ano e dois meses.

A baixa riqueza de abelhas encontrada neste estudo pode estar relacionada ao horário de coleta no qual a rede entomológica utilizada, visto que todos os estudos acima citados seguiram a metodologia proposta por Sakagami (1967) onde a coleta ocorre das 8h às 18h, tal metodologia é nomeada pelo autor como método de varredura. No entanto, no presente estudo as coletas foram realizadas, como já explicitado, das 8h às 14h, fato que pode explicar o baixo número de espécies coletadas com a rede. Soma-se a isso, a baixa habilidade e experiência dos coletores com o uso da rede entomológica.

Em relação às abelhas sociais da Tribo Meliponini, foram registradas três espécies apenas: *Frieseomelitta varia* (Lepeletier); *Frieseomelitta meadewaldoi* (Cockerell,); *Trigona spinipes* (Fabricius,). De forma similar, Milet-pinheiro e Schlindwein (2008) registraram apenas duas espécies, uma delas em comum com o presente estudo, *T. spinipes*. Locatelli e Machado (2001) em região de Brejo de Altitude com pouco impacto humano, em Pernambuco, catalogaram cinco espécies de Meliponini. O baixo número de espécies de meliponíneos encontrados na área de estudo evidencia a antropização característica da área, assim como constatado também em Milet-pinheiro e Schlindwein (2008), onde há destruição de substratos arbóreos utilizados para nidificação dessas abelhas e práticas realizadas por meleiros, que, segundo Pereira (2006) são pessoas hábeis em encontrar o ninho de abelhas em árvores, fazendo a retirada predatória do mel para a venda ou consumo próprio, terminando, assim, por destruir a colônia. Zanella (2003) ainda relacionou tal antropização ao baixo número de Meliponini. Tal relação se torna mais evidente, se observarmos que o estudo realizado por Locatelli e Machado (2001) foi em área com pouco impacto humano, culminou por registrar cinco espécies de Meliponini. Soma-se às discussões acerca do baixo número de Meliponini registrado na área de estudo, a baixa habilidade, experiência dos coletores e a menor faixa de horário em que as coletas foram realizadas, além destas terem sido finalizadas no horário de maior temperatura.

Referentes aos substratos de nidificação, durante todo o trabalho foram contabilizados 77 ninhos em cinco diferentes tipos de substratos (Tabela 2). Dentre os substratos amostrados, dois apresentaram maiores números de ninhos: arbóreo (árvore viva) (64,9 %) e os troncos e mourões de cerca (árvore morta) (28,5%) (Tabela 2). Nos substratos arbóreos foram contabilizados 50 ninhos em 26 espécies da flora local (tabela 3). Não foram identificadas as espécies de abelhas encontradas nos ninhos, com exceção da espécie *T. spinipes,* que possui um ninho de fácil identificação.

Tais resultados são superiores aos levantamentos de substratos realizados por Martins (2004) onde foram registradas 12 espécies arbóreas, Marinho *et al.*, (2002) com seis espécies e Souza *et al.*, (2005) com 10 espécies, todos em bioma Caatinga e este último realizado no *Campus* Ondina da UFBA (Universidade Federal da Bahia). Martins (2004) constatou em seus experimentos, múltiplos ninhos de abelhas em *Poincianella pyramidalis* (tul.) L.P.Quiroz e *Schinopsis brasiliensis*. No presente estudo, além de terem sido constatados múltiplos ninhos em *P. pyramidalis,* outras espécies botânicas apresentaram tal característica, sendo elas: *Spondias tuberosa* Arruda, *Commiphora leptophloeos* (Mart.) J.B.Gillett. e *Anadenanthera colubrina* var.cebil (Griseb.) Altshu. “Este fato está relacionado com a maior possibilidade de formação de ocos em árvores dessas espécies, uma vez que elas apresentam densidade inferior a 0,90 g.cm-3” (SOUZA, 2009, p.4). Segundo este mesmo autor, essa característica pode favorecer a formação de ocos, que são utilizados pelas abelhas para nidificação.

Tabela 2. Tipos de substratos e números de ninhos encontrados na área de estudo.

SUBSTRATO	**QUANTIDADE DE NINHOS (%)**
ARBÓREOS	65%
TRONCOS E MOURÕES	29%
SOLO	3%
ROCHA	1,5%
NINHOS ABANDONADOS POR OUTROS ANIMAIS (**CUPINS**)	1,5%

No entanto, segundo Martins (2004) não se pode ainda, supor uma preferência da fauna apícola por tais espécies arbóreas, uma vez que, a distribuição de ninhos em espécies vegetais da Caatinga e a disponibilidade de cavidades ainda não são conhecidas. O que se pode observar, segundo este autor, é a utilização de um número pequeno de árvores utilizadas para nidificação, ou seja, concentração de ninhos em poucas espécies, visto que a disponibilidade de cavidades depende de fatores naturais e antrópicos, como presença da espécie *A. mellifera* e o desmatamento.

Se comparado aos levantamentos citados, o presente trabalho apresenta uma quantidade considerada de espécies vegetais. Isto pode ser explicado pela a grande abundância de ninhos de *T. spinipes* na área de estudo, nidificando em 16 das 25 espécies botânicas, como *Erythrina velutina* Willd., *Senegalia polyphylla* (DC.) Britton & Rose *C. leptophloeos, S. tuberosa, A. colubrina, Parapiptadenia zehntneri* (Harms) M.P. Lima & Lima, *Ruprechtia laxiflora* Meissn, *Ziziphus joazeiro* Mart., *Jatropha mollissima* (Pohl) Baill, *Ceiba glaziovii* (Kuntze) K.Schum, laranjinha, *Pilosocereus* sp., *Brasiliopuntia brasiliensis* (Willd.) A. Berger, *Cedrela odorata* L., *Termillia catappa* L. e *Prosopis juliflora* (Sw) DC L.

O grande número de ninhos encontrados em troncos de árvores vivas evidencia a dependência das espécies de Meliponini levantadas na área de estudo por esse tipo de substrato, como já constatado por Pereira (2006), sendo que a destruição de espécies arbóreas da Caatinga se configura como uma das maiores causas de impacto sobre as populações dessas abelhas nesse bioma (Thiago, 2010).

No vale do São José, foram observados novos registros de Meliponini nidificando em espécies arbóreas ainda não mencionadas na literatura consultada para esse tipo de interação (CARVALHO; MARCHINI, 1999; AGUIAR, 2003; AGUIAR, *et al.*, 2003; LORENZON *et al.*, 2003; MARTINS, 2004; BIESMEIJER *et al.*, 2005; SOUZA *et al.*, 2005; TEIXEIRA *et al.*, 2007; RODARTE *et al.*, 2008; SOUZA, 2009; PIGOZZO; VIANA 2010; SANTANA; OLIVEIRA, 2010); sendo elas: *Sapium argutum* (Mull.Arg.) Huber (burra leiteira), *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *leiostachya* (L.) Wild (pau-ferro), *Piptadenia viridiflora* (Kunth) Benth. (jiquiri), *Parapiptadenia zehntneri* (Harms) M.P. Lima & Lima (angico- monjola), *Brasiliopuntia brasiliensis* (Willd.) A.Berger(cacto-mamão), *Erythrina velutina* Willd. (mulungu), *Senegalia polyphylla* (DC.) Britton & Rose (carcará).

O grande número de espécies arbóreas usadas para nidificação dessas abelhas pode ser explicado pela grande diversidade florística da área (OLIVEIRA *et al.*, 2014; Vieira *et al.*, 2017). No entanto, Souza (2009) afirma que as abelhas nativas se adaptam a nidificar em espécies exóticas devido a diminuição da flora nativa, onde nidificavam originalmente. Assim como observado em Souza *et al.*, (2005) e Souza (2009), foi também observada na área de estudo uma adaptação no hábito de nidificação das abelhas sem ferrão, passando a utilizar a *Mangifera indica* L.(mangueira), *Terminalia catappa* L. (castanhola) e *Prosopis juliflora* (Sw) DC L. (algaroba) como substratos de nidificação.

A divergência entre o baixo número de espécies de Meliponíneos coletados neste estudo e o número expressivo de substratos arbóreos identificados, deve-se à elevada abundância de *T. spinipes* nos substratos, uma vez que na maioria deles somente essa espécie ocorreu.

TABELA 3. Espécies arbóreas usadas para nidificação na área de estudo.

FAMÍLIA	ESPÉCIE	NOME POPULAR	STATUS
ANACARDIACEAE	*Myracrodruon urundeuva (*Allemão) Engl	Aroeira	Nativa
	Schinopsis brasiliensis Engl.	Braúna	Nativa
	Spondias tuberosa Arruda	Imbuzeiro/ Umbuzeiro	Endêmico da Caatinga
	Mangifera indica L.	Mangueira	Exótica
APOCYNACEAE	*Aspidosperma pyrifolium* Mart	Pereiro	Endêmico da Caatinga
BIGNONIACEAE	*Handroanthus impetiginosus* Mattos	Pau d'arco-roxo	Nativo
BOMBACACEAE	*Ceiba glaziovii* (Kuntze) K. Schum.	Barriguda	Endêmico da Caatinga
BURSERACEAE	*Commiphora leptophloeos* (Mart.) J.B.Gillett.	Imburana/ Umburana	Nativo
CACTACEAE	*Pilosocereus gounellei* Weber.	Alastrado	Endêmico da Caatinga
	Pilosocereus sp.	Facheiro	Nativo
	Brasiliopuntia brasiliensis (Willd.) A. Berger	Cacto-mamão	Nativo

COMBRETACEAE	*Terminalia catappa* L.	Castanhola	Exótica
EUPHORBIACEAE	*Sapiu margutum* (Mull.Arg.) Huber	Burra-leiteira	Nativa
	Jatropha mollissima (Pohl) Baill	Pinhão-bravo	Endêmica da Caatinga
FABACEAE – CAESALPINIACEAE	*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *leiostachya* (L.) Wild	Pau-ferro	Nativa
	Poincianella pyramidalis (tul.) L.P.Quiroz	Catingueira	Endêmica da Caatinga
FABACEAE – MIMOSACEAE	*Mimosa tenuiflora* (Willd.) Poir	Jurema-preta	Nativa
	Anadenanthera colubrina var. cebil (Griseb.) Altshul	Angico	Nativa
	Parapiptadenia zehntneri (Harms) M.P. Lima & Lima	Angico-monjola	Nativa
	Senegalia polyphylla (DC.) Britton & Rose	Carcará	Nativa
	Piptadenia viridiflora (Kunth) Benth.	Jiquiri	Nativa
	Prosopis juliflora (Sw) DC L.	Algaroba	Exótica
FABACEAE – PAPILONIACEAE	*Erythrina velutina* Willd.	Mulungu	Nativa
MELIACEAE	*Cedrela odorata* L.	Cedro	Nativa
POLYGONACEAE	*Ruprechtia laxiflora* Meissn.	Cachão/caixão	Nativa
RHAMNACEAE	*Ziziphus joazeiro* Mart.	Juazeiro	Endêmica da Caatinga
INDETERMINADO	sp. 1	Laranjinha	Nativa

Embora no presente artigo não tenha sido possível a coleta para identificação das abelhas cujos ninhos foram contabilizados, os dados sobre as espécies botânicas importantes para a nidificação das abelhas na região são bastante importantes, especialmente visando à criação de planos de manejo da apifauna local, bem como da conservação de abelhas na região.

CONCLUSÕES

O presente estudo torna-se relevante, uma vez que apresenta o primeiro levantamento da fauna de abelhas na área de estudo. Além disso, traz uma melhor compreensão dos substratos utilizados para nidificação por abelhas em uma área do semiárido nordestino, encurtando assim, o caminho para novas estratégias de manejo, preservação e conservação desses animais. No entanto, são necessários estudos mais aprofundados sobre essa fauna levantada, com novos inventários e estudos de caráter comportamentais e ecológicos, para que o máximo de informações seja levantado, podendo num futuro subsidiar ações políticas de conservação e preservação na área estudada.

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