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GEOGRAPHIC VARIATION IN NEOTROPICAL BATS: ECO-EVOLUTIONARY PROCESSES BEYOND ACOUSTIC DIVERSIFICATION AND COMMUNITY ASSEMBLY PATTERNS

PORTO ALEGRE

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Tese apresentada ao Programa de Pós Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutora em Biologia Animal.

Área de concentração: Biologia e Comportamento Animal – Ecologia Animal

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Universidade Federal do Rio Grande do Sul
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Universidad de Costa Rica

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"O planeta todo transborda com o vigor de uma ressonância tão completa e expansiva quanto delicada e equilibrada. Todos os lugares, com suas vastas populações animais e vegetais, se transformam em salas de concerto. Em cada um desses recintos, há uma orquestra única que executa uma sinfonia sem igual, na qual cada espécie toca sua parte na partitura. É uma obra-prima de alta sofisticação, composta pela natureza."

Bernie Krause
A Grande Orquestra Da Natureza

A Ita,
porque mi primera sinfonía
la escuché en tu jardín.

obrigada
MJ
BiMaLab
Amora
Cíntia
João
PPGBAN
Nuno
Bernal
Bugre Lucena
Eu
Tío
SBEQ
Pelos treinos
CNPq
Juanka
Pelas gravações
UFRGS
Tuni
Macaco
Patico y Pa
Pelas saídas de campo
Pelo apoio
Amigøs brasileirøs
Pela música
Morcegos
Pelos cafés
Por fazer parte desta viagem

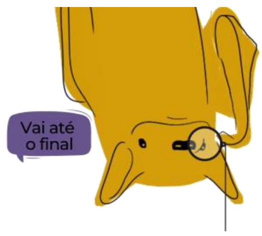
Sumário

Resumo

Investiguei a variação geográfica nos chamados de ecolocalização de morcegos neotropicais não filostomídeos em relação a fatores climáticos e ecológicos. Testei as hipóteses Sensory Drive e Jame´s rule para determinar se a componente de frequência constante dos chamados de ecolocalização do morcego pescador *Noctilio leporinus* ao longo da sua distribuição geográfica é influenciada pelo clima, o tamanho corporal e a relação com a filogenia da espécie (Capítulo II); estudei a variação acústica dos chamados de ecolocalização dos grupos de espécies *Pteronotus fulvus* x *P. davyi*, e *P. psilotis* x *P. personatus* nas zonas de contacto na América Central tentando elucidar diferenças acústicas associadas à distribuição geográfica teórica das espécies e a existência de distintos grupos fônicos (Capítulo III); e finalmente, descrevi os padrões espaço-temporais e espectrais de diversas comunidades acústicas de morcegos do Cerrado e do Pantanal, em relação a diferenças microclimáticas e tipo de habitat (Capítulo IV). Em paralelo, desenvolvi e divulguei diversos materiais gráficos de comunicação da ciência (Material de divulgação) (Capítulo VI). De acordo com a hipótese Sensory Drive, as frequências dos chamados de ecolocalização das espécies ao longo da sua distribuição variam em resposta a diferentes condições de atenuação atmosférica. De acordo com a Jame´s rule, para uma única espécie, o tamanho do corpo correlaciona-se com as condições de umidade e temperatura. Assim, investiguei a influência de fatores climáticos (umidade e temperatura) e do tamanho do corpo na variação geográfica dos chamados de ecolocalização de *N. leporinus* em um gradiente de umidade e temperatura ao longo da sua distribuição geográfica. Encontrei diferenças significativas na porção de frequência constante dos chamados de ecolocalização da espécie explicada principalmente pela umidade e pelo tamanho do corpo, apoiando parcialmente as duas hipóteses. A extensão em que as frequências mudam devido à variação do clima ou variação do tamanho do corpo mediada pelo clima difere entre as subespécies (tamanhos), sugerindo que tanto a seleção ecológica quanto a história filogenética desempenham um papel importante na divergência acústica da espécie. Para avaliar a variação acústica dos chamados de ecolocalização de grupos de espécies do gênero *Pteronotus* na América Central realizei um agrupamento hierárquico de k-means sobre componentes principais (HCPC) usando amostras acústicas do México, Honduras, El Salvador, Nicarágua e Costa Rica. Avaliei se essas diferenças acústicas estavam relacionadas com a distância e a localização geográficas. Encontrei evidências de simpatria para três grupos fônicos dentro de cada complexo de espécies, sem uma correspondência clara com a distribuição conhecida das espécies, sendo que as mudanças de frequência dos chamados de ecolocalização seguem um padrão semelhante à variação geográfica das espécies no tamanho do corpo. Concluo que estudos futuros na América Central deverão incluir a captura de espécimenes, marcação e gravação acústica individual para ajudar na resolução do dilema de distribuição levantado aqui. Finalmente, investiguei como a informação acústica (complexidade acústica) das primeiras duas horas de atividade dos morcegos após o pôr do sol muda em resposta à temperatura do ar, umidade relativa e tipo de habitat em comunidades de morcegos amostradas no Cerrado e Pantanal brasileiros. Encontrei padrões espectrais e temporais idiossincráticos nas diversas localidades amostradas no Cerrado e no Pantanal e um efeito significativo e positivo da temperatura na quantidade de informação acústica. Estudos anteriores sugerem que a temperatura é um fator que influencia a atividade de forrageio dos morcegos insetívoros, além da intensidade dos chamados de ecolocalização por meio do efeito da atenuação atmosférica do som. Estudos futuros são necessários para avaliar os efeitos das condições climáticas e da atividade dos morcegos na quantidade de informações capturadas pelos índices acústicos.

Palavras chave: assinatura acústica, atenuação atmosférica, identificação acústica, variação acústica, grupos fônicos, *Noctilio leporinus*, *Pteronotus davyi*, *Pteronotus fulvus*, *Pteronotus personatus*, *Pteronotus psilotis*.

Material de divulgação

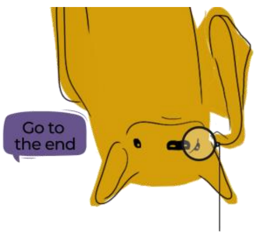


Abstract

I investigated the geographic variation in the echolocation calls of non-phylostomid neotropical bats in relation to climatic and ecological factors. I tested the Sensory Drive Hypothesis and the Jame's rule to determine whether the constant frequency of the echolocation calls of the fishing bat *Noctilio leporinus* along its geographic distribution is influenced by climate, body size and the relationship with the species' phylogeny (Chapter II); I studied the acoustic variation of echolocation calls of the species groups *Pteronotus fulvus* x *P. davyi*, and *P. psilotis* x *P. personatus* in the contact zones in Central America, trying to elucidate acoustic differences associated with the theoretical geographic distribution of the species and the existence of distinct phonic groups (Chapter III); and finally, I describe the spatiotemporal and spectral patterns of various acoustic bat communities from the Cerrado and Pantanal, in relation to microclimatic differences and habitat type (Chapter IV). In parallel, I develop and disseminate various graphic materials for science communication (Material de divulgação) (Chapter VI). According to the Sensory Drive hypothesis, the frequency of echolocation calls along the distribution of a species varies in response to different conditions of atmospheric attenuation. According to James' rule, for a single species, body size correlates with humidity and temperature conditions. Then, I investigated the influence of climatic factors (humidity and temperature) and body size on the geographic variation of the echolocation calls of *N. leporinus* across a gradient of humidity and temperature conditions along with its geographic distribution. I found significant differences in the constant frequency portion of species echolocation calls explained mainly by humidity and body size, partially supporting both hypotheses. The extent to which frequencies change due to climate variation or climate-mediated variation in body size differs between subspecies (sizes), suggesting that both ecological selection and phylogenetic history play an essential role in the acoustic divergence of the species. To assess the acoustic variation of echolocation calls of the genus *Pteronotu's* species groups in Central America, I performed a hierarchical clustering of k-means on principal components (HCPC) using acoustic recordings from Mexico, Honduras, El Salvador, Nicaragua and Costa Rica. I assessed whether the acoustic differences were related to geographic distance and geographic location. I found evidence of sympatry for three phonic groups within each species complex, without a clear correspondence with the known species distribution. The frequency changes of echolocation calls follow a similar pattern to the body size geographic variation of species. I conclude that further studies in Central America should include specimen capture, tagging, and individual acoustic recording to help resolve the distribution dilemma raised here. Finally, I investigated how the acoustic information (acoustic complexity) during the first two hours of bat activity changes in response to air temperature, relative humidity, and habitat type. I found idiosyncratic spectral and temporal patterns in the different sampled locations in the Cerrado and Pantanal and a significant and positive effect of temperature on the amount of acoustic information. Previous studies suggest that temperature is a key factor influencing the foraging activity of insectivorous bats, and the intensity of echolocation calls through the effect of sound atmospheric attenuation. Yet, future studies are needed to assess the impact of weather conditions and bat activity on the amount of information captured by acoustic indices.

Key words: acoustic signature, acoustic identification, acoustic variation, atmospheric attenuation, *Noctilio leporinus*, phonic groups, *Pteronotus davyi*, *Pteronotus fulvus*, *Pteronotus personatus*, *Pteronotus psilotis*.

Science communication material (Material de divulgação)



Capítulo I

Introdução geral

Os animais percebem e respondem a informações sobre o seu ambiente através de diversos mecanismos sensoriais, envolvendo a produção e a recepção de sinais. Esses sinais evoluem, sendo um componente da adaptação do animal ao seu ambiente sujeito a seleção; portanto, o estudo da comunicação animal pode ser usado como uma ferramenta para elucidar princípios evolutivos gerais (Bradbury & Behrenkamp 1998). Por exemplo, a variação geográfica nas características sensoriais é informativa para testar o papel do ambiente na diferenciação de características e populações (Odendaal et al. 2014). Aliás, o estudo em conjunto dos sinais de comunicação com as características morfológicas pode ajudar na classificação taxonômica das espécies (Bradbury & Behrenkamp 1998).

Os sinais de comunicação, sejam visuais, olfativos ou acústicos são particularmente influenciados pelas condições climáticas (Endler 1992, Mutumi et al. 2016). Em especial, o clima pode ser importante na evolução dos sistemas de sinalização acústica através do seu efeito na absorção atmosférica do som, onde a energia é redistribuída como calor ou perdida (Griffin 1971, Bass et al. 1984). A taxa de absorção do som está diretamente relacionada com a umidade e a temperatura do ar e com a frequência do som, sendo os sons de alta frequência, como os chamados de ecolocalização dos morcegos, mais atenuados na atmosfera quando comparados com sons de baixa frequência, como os sons audíveis para humanos e os infrassons (Lawrence & Simmons 1982). Portanto, diferenças de umidade e temperatura ao longo da distribuição geográfica de uma espécie de morcego ecolocalizador podem selecionar diferentes frequências de ecolocalização de modo a que a atenuação atmosférica devido a esses fatores climáticos seja minimizada (Mutumi et al. 2016).

Os morcegos são um grupo altamente diverso, incluindo mais de 1400 espécies (Simmons & Cirranello 2020); apresentam altas taxas de diversificação, ocupam habitats muito diferentes por todo o globo e desempenham papéis ecológicos e econômicos importantes (Medellín et al. 2000, Simmons & Conway 2003, Jones et al. 2009). Na região Neotropical coexistem nove famílias de morcegos e, com a exceção da maioria dos Phyllostomidae, as famílias restantes - Emballonuridae, Furipteridae, Molossidae, Mormoopidae, Natalidae, Noctilionidae, Thyropteridae e Vespertillionidae - utilizam a ecolocalização como principal sentido de navegação e orientação espacial, detecção e obtenção de presas (Kalko & Schnitzler 1998, Schnitzler & Kalko 2001, Fenton 2003, Denzinger & Schnitzler 2013). Essas famílias apresentam uma grande diversidade fenotípica, incluindo características morfológicas (por exemplo, tamanho do corpo) e comportamentais (por exemplo, ecolocalização). Tais características tornam os morcegos modelos ideais para investigar padrões de variação ecológica.

A variação geográfica nos chamados de ecolocalização dos morcegos é influenciada pelas diferenças ambientais, incluindo fatores climáticos como temperatura e umidade que afetam a propagação do som, pela filogenia e fatores associados ao nicho ecológico, como as diferenças no uso e seleção de habitat e no comportamento de forrageio (Siemers & Schnitzler 2004, Jones & Holderied 2007, Jiang et al. 2015). O estudo de fatores ecológicos e evolutivos na variação dos chamados de ecolocalização pode ajudar a elucidar como a história evolutiva e as condições ambientais atuais interagem para promover diferenças populacionais em espécies de morcegos neotropicais. Logo, compreender como as espécies se adaptam e respondem às características ambientais pode subsidiar ações de conservação adequadas. No entanto, estudos sobre variação geográfica de chamados de ecolocalização de morcegos Neotropicais são escassos.

Nesta tese procuro investigar a variação geográfica nos chamados de ecolocalização de morcegos neotropicais não-filostomídeos em relação às diferenças induzidas por fatores climáticos e ecológicos. No primeiro capítulo, investigo os padrões intra-específicos de variação acústica nos chamados de ecolocalização do morcego pescador *Noctilio leporinus* ao longo da sua distribuição geográfica, tentando entender os fatores climáticos subjacentes (temperatura e umidade) e o tamanho corporal na variação da frequência e a relação com a filogenia da espécie. Para isso testo duas hipóteses: a hipótese de condução sensorial e a regra de James. No segundo capítulo, descrevo os padrões de variação nos chamados de ecolocalização de dois grupos de espécies-irmãs: o primeiro incluindo *Pteronotus fulvus* e *P. davyi*, e o segundo *P. psilotis* e *P. personatus*, tentando elucidar acusticamente a identidade taxonômica nas áreas de possível simpatria na América Central. No terceiro capítulo, descrevo os padrões espaciotemporais e espectrais de distintas comunidades de morcegos do Pantanal e do Cerrado, investigando os efeitos do microclima e do habitat na variação acústica.

Finalmente, concluo a tese apresentando o material de divulgação desenvolvido ao longo desses anos de doutorado com o intuito de comunicar ciência e, em particular, características biológicas e ecológicas dos morcegos, e os serviços que prestam aos ecossistemas, de forma lúdica, esteticamente atraente e simples para dentro e fora da academia.

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Estrutura da Tese

Esta tese encontra-se organizada em seis capítulos: uma introdução geral, três capítulos no formato de artigos científicos, um capítulo de conclusões gerais, e um capítulo final apresentando materiais de divulgação científica desenvolvidos ao longo do período do doutorado. Os artigos encontram-se formatados de acordo com as regras de formatação dos periódicos respectivos a que foram ou serão submetidos; para facilitar a leitura, figuras e tabelas são apresentadas ao longo dos textos.

Capítulo II

Submetido a: Behavioral Ecology and Sociobiology

What drives echolocation call variation in greater bulldog bats *Noctilio leporinus* (Linnaeus, 1758)?

Adriana Arias-Aguilar, Bernal Rodríguez-Herrera & Maria João Ramos Pereira

What drives echolocation call variation in greater bulldog bats *Noctilio leporinus* (Linnaeus, 1758)?

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Abstract

Climate is a crucial factor for the evolution of bat echolocation calls, mostly due to its effect on atmospheric sound absorption. According to the Sensory Drive hypothesis species' echolocation call frequencies across their distribution range will vary in response to different conditions of atmospheric attenuation. Besides, variation in call frequency may be the result of climate-mediated variation in body size. According to James´s Rule, for a single species, body-size will be correlated with humidity and temperature conditions. The Neotropical fishing bat *Noctilio leporinus* is a perfect model to test these hypotheses because it occurs across a wide gradient of humidity and temperature conditions across its geographic range. Here, we investigated the influence of climatic factors (humidity and temperature) and body size on geographic variation in the echolocation calls of the greater bulldog bat, *Noctilio leporinus*, from North, Central and South America. We found significant differences in the constant frequency portion of the echolocation calls of the species explained by both climatic conditions, mainly humidity, and by body size, partially supporting the two hypotheses. The extent to which frequencies change due to climate variation or climate-mediated body size variation differs between subspecies, suggesting that both ecological selection and phylogenetic history play an important role in the acoustic divergence of the species.

Significance Statement

Echolocation calls of bats are highly affected by atmospheric sound absorption. Variation in frequency traits of the echolocation calls of a species can be correlated with differences in climatic conditions across its geographic range. Using a large acoustic data set covering almost the whole distribution of the greater bulldog bat, *Noctilio leporinus*, we investigated the effects of humidity, temperature and body size on the constant frequency portion of their echolocation calls. By testing the Sensory Drive Hypothesis and the James´ Rule we conclude that acoustic variation of the species is a result of ecological adaptation, body size and phylogenetic history. This study expands our knowledge on geographic acoustic variation of Neotropical bats.

Key-words: acoustic variation, atmospheric attenuation, geographic variation, James' Rule, Sensory Drive

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Availability of data and material: The data used for this study are available from the corresponding author on request.

Authors' contributions: AAA and MJRP conceived the study. AAA collected the data. AAA and MJRP analyzed the data and wrote the manuscript. BRH provided advice on the acquisition and interpretation of data. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

Code availability (software application or custom code): Not applicable

Introduction

Geographic variation in sensory traits is informative in testing the role of environment in trait and population differentiation (Odendaal et al. 2014). In particular, climate may be important in the evolution of acoustic signaling systems through its effect on atmospheric sound absorption (Griffin 1971; Bass et al. 1984; Endler 1992). High-frequency sounds, such as bat echolocation calls, are attenuated in the atmosphere at a higher rate than low-frequency sounds and the rate at which sound absorption occurs is directly related to both air humidity and frequency (Lawrence and Simmons 1982). Therefore, differences in humidity and temperature across the geographic range of an echolocating bat species may select for different echolocation frequencies so that atmospheric attenuation due to these climatic factors is minimized (Mutumi et al. 2016).

Variation in bat echolocation calls also results from direct and indirect effects of the phylogenetic history, morphology and the associated factors regarding the ecological niche, such as habitat and foraging behavior (Siemers and Schnitzler 2004; Jones and Holderied 2007, Luo et al. 2019), so both evolutionary and ecological factors need to be considered when determining how evolutionary history and contemporary environment interact to promote the population differences in the acoustic trait of interest. This approach provides insights into the nature of the interaction, especially when environmental differences may affect the evolution of geographic variation in bat echolocation calls (Jiang et al. 2015).

The Sensory Drive Hypothesis proposes that lineage diversification may be driven by environmentally-mediated differences in communication signals (Endler 1992). It suggests that climate drives the evolution of acoustic signals through its effects on atmospheric sound absorption, so call frequency would be a response to climate-induced differences in atmospheric attenuation (Snell-Rood 2012; Mutumi et al. 2016). Atmospheric attenuation results from a complex interaction between air humidity, temperature and sound frequency (Hartley 1989; Lawrence and Simmons 1982). This hypothesis predicts that the sound absorption experienced by a species (across their range or habitat), should be correlated with its signal characteristics (Snell-Rood 2012). In particular, frequency characteristics of sounds should vary with sound absorption, given that absorption decreases with frequency decrease (Bass et al. 1984). So, increases in atmospheric attenuation select for calls of lower frequency as an adaptive rather than stochastic response of acoustic signals to environmental variables (Mutumi et al. 2016).

Besides, divergence in call frequency may be the result of climate-mediated variation in body size and the inverse correlation with echolocation frequency of bats (Jones 1999). Reformulating Bergmann's Rule, James (1970), using several bird genera, showed a tendency within species for geographical variation in mean body size related to a combination of climatic variables, including temperature and moisture (Blackburn, Gaston and Loder 1999). Termed as James´s Rule, it considers that for a single species, body-size will generally increase with decreasing humidity and temperature, so smaller animals will occur in hot humid environments than in cooler, dryer ones, while larger animals will occur in cool, dry areas (James 1970).

The greater bulldog bat, *Noctilio leporinus* (Noctilionidae, Chiroptera) is one of the few bat species that feeds on fish. It ranges from Mexico to northern Argentina and occurs near streams, coastal habitats, major river basins or other moist places (Barques et al. 2008). Based on morphological characters, Davis (1973) proposed three geographic areas of differentiation and recognized three subspecies: *Noctilio leporinus mastivus* with mean forearm length of 85.2 mm, occurring at the lowlands of México, Central America, the West Indies, and northern South America; *N. leporinus leporinus*, the smallest of the subspecies, with forearm length averaging less than 80.0 mm, occurring in the Guianas and the Amazon Basin of Brazil, Ecuador and Peru; and *N. leporinus rufescens*, the largest of the subspecies, with forearm length averaging 88.2 mm, occurring in Bolivia, Argentina, Paraguay, and southern Brazil. In recent phylogeographic studies, two monophyletic lineages were identified partially corresponding with the subspecies *mastivus* and combined *leporinus* and *rufescens* geographic ranges (Pavan et al. 2013; Khan et al. 2014). Those lineages, thus, present a possibility to test whether geographic variation in their calls exists and, if so, whether this variation can be explained by differences in humidity and temperature across the geographic range of the species.

Echolocating bats produce a wide variety of sonar pulses and communication calls that can be broadly grouped as frequency modulated (FM) or constant frequency (CF) signals or in a combination of both

(Metzner and Müller 2016). *Noctilio leporinus*, particularly, uses CF/FM calls to locate fish on the water surface (Altringham 2011). Variation in the echolocation calls of *N. leporinus* along its geographic distribution has never been studied. A recent revision on the main parameters used for echolocation call description revealed the existence of acoustic variability between specimens recorded in México, Central and South America and the Caribbean (e.g. the frequency of maximum energy – FME – varies between 31.03 and 65.00 kHz) (Arias-Aguilar et al. 2018).

Here our main objective is to characterize the intraspecific acoustic variation and to determine patterns of acoustic divergence along the geographic distribution of *N. leporinus*. Specifically, we aim to determine, by evaluating the acoustic variability within the species, the existence of a geographic structure associated with local conditions of temperature and humidity. Because of the inverse correlation with frequency, we also considered the influence of body size on the geographic variation of the echolocation calls. Following, we intend to describe those patterns of acoustic divergence between the subspecies proposed by Davis (1973) and correlate them with the lineage phylogeny. As acoustic and morphological traits involved in sound production are not independent of phylogeny (Collen 2012), and considering that the species is genetically homogeneous, with the South American populations closely related (Pavan et al. 2013; Khan et al. 2014), a certain call structure from a shared common ancestor should be retained (Russo et al. 2017).

Following the Sensory Drive Hypothesis, we expect the constant frequency component of the search calls of *N. leporinus* to be climate-driven. Populations inhabiting warmer and more humid sites should exhibit lower frequencies due to higher atmospheric attenuation than populations inhabiting cooler and dryer sites, which may experience less atmospheric attenuation and so, frequencies should be higher. Contrarily, under the James's Rule, it is the body-size variation that should be climate-driven. So, under this hypothesis the prediction is that bats occurring in warm, humid areas should be smaller than bats inhabiting cool, dry areas. In this case, we would expect for the constant frequency component of the search calls to decline as body size increases. Here, we use the aforementioned Davis (1973) subspecies classification as proxy for body size.

The results of this study should shed light on how bats can adapt their echolocation behavior to local climatic conditions, and also advance our knowledge on how climate shapes the evolution of geographic variation in bat echolocation calls. This study also underlines the importance of acoustic monitoring as tool for ecological studies particularly, in this case, the study of phenotypic plasticity in bats.

Material and methods

Acoustic behavior of the greater bulldog bat

The greater bulldog bat, *N. leporinus* is classified as a trawling bat as it forages for prey drifting on water surfaces (Denzinger et al. 2016). Based on the signals that are emitted when bats are searching for prey, the calls of *Noctilio* can be categorized as pure constant frequency (CF) signals (i.e. pure tones with no bandwidth) and CF signals usually terminated with a broadband sweep (FM component), which enhances localization performance (Schnitzler et al. 1994; Kalko et al. 1998; Schnitzler et al. 2003). Search pulses are often emitted in pairs or triplets, with the first of a pair or middle pulse in a triplet being mostly CF, while the second (or first and third) has an abbreviated CF component that terminates in a FM sweep (Schnitzler et al. 1994). Signal design is probably mainly adapted for detection of minor disturbances on smooth water surfaces (Surlykke and Kalko 2008). According to the distance to water surfaces, the hunting strategies of *N. leporinus* include low and high search flights where the bat emits two to four echolocation signals always containing at least one CF pulse and one CF-FM pulse, where the CF portion of both pulses is similar and maintained within a range of about 400 Hz (Schnitzler et al. 1994). The frequency of the CF pulses changes within a range of 2-4 kHz (Schnitzler et al. 1994).

Acoustic data

We collated echolocation calls for *N. leporinus* through a combination of fieldwork and donated material along a latitudinal gradient ranging from 22°N to 30°S (Fig. 1). Combined, we obtained sound recordings from bat populations of Mexico, Costa Rica, Panama, Guadeloupe, Martinique, French Guiana and Brazil, in a total of 17 localities. Sound recordings were obtained mainly from passive acoustic monitoring of free-flying bats, except for the Mexican recordings, which were obtained from hand-released bats (see ESM 1 for recording site details).

We used Avisoft SAS Lab Pro software, Version 5.2.13 for sound analysis of search-phase echolocation calls. We used only sequences with good signal-to-noise ratio. Spectrograms were generated with a Flat top window at 1024 FFT, 100% frame size and 98.43% overlap.

We classified search-phase calls as CF and FM types, following Schnitzler et al. (1994). In general, both CF and FM calls showed an initial ascending modulated element followed by a constant portion (hereafter, FF: flat frequency), ending with a descending modulated element. In CF calls the FF is longer (mean=8.2 ms, min=5.3 ms, max=10.2 ms) than in FM calls (mean=5.0 ms, min=3.2 ms, max=6.7 ms) and the terminal element is narrowly modulated in CF and broadly modulated in FM calls.

Call parameters were measured with automatic parameter measurements using three thresholds and manually supervised. Start and End of the calls were defined at -24dB (start) and -12 dB (end) relative to the peak frequency of the sound signal. Similarly to the approach of Jung et al. (2014), we obtained automatic measurements of the peak frequency in regular intervals of 0.1 ms within the signal (thus each interval corresponds to an observation) to build representative curves of CF and FM call types for each locality. We defined as initial and terminal elements the modulated portions of signals showing a difference >0.1 kHz in frequency between frequency intervals and flat frequency (FF) as the constant portion of each signal with a variation <0.1 kHz between frequency intervals.

Climate data

To describe the atmospheric conditions of each locality we obtained mean values of temperature, relative humidity and atmospheric pressure from the NASA Geospatial Interactive Online Visualization and Analysis Infrastructure (Giovanni) system (Acker and Leptoukh 2007) (http://giovanni.gsfc.nasa.gov/giovanni/). Time-averaged maps of 2-meter air temperature (°C) (monthly average over 2000-2020, resolution of 0.5x0.625 deg, [MERRA-2 Model M2IMNXA v5.12.4]), relative humidity at surface (%) (monthly average over 2002-2020, resolution 1 deg, Daytime/Ascending, AIRS-only, [AIRS AIRS3STM v006]) and surface pressure (hPa) (monthly average over 2000-2020, resolution 0.5x0.625 deg. [MERRA-2 Model M2TMNXSLV v5.12.4]) were downloaded as GeoTIFF (.tif) files. Metadata was extracted in R using the rgdal and raster packages. Then, using those values we calculated absolute humidity for each locality. Additionally, we calculated the atmospheric absorption experienced by the mean FF for CF and FM calls of each locality, using the online calculator by the National Physical Laboratory (2018).

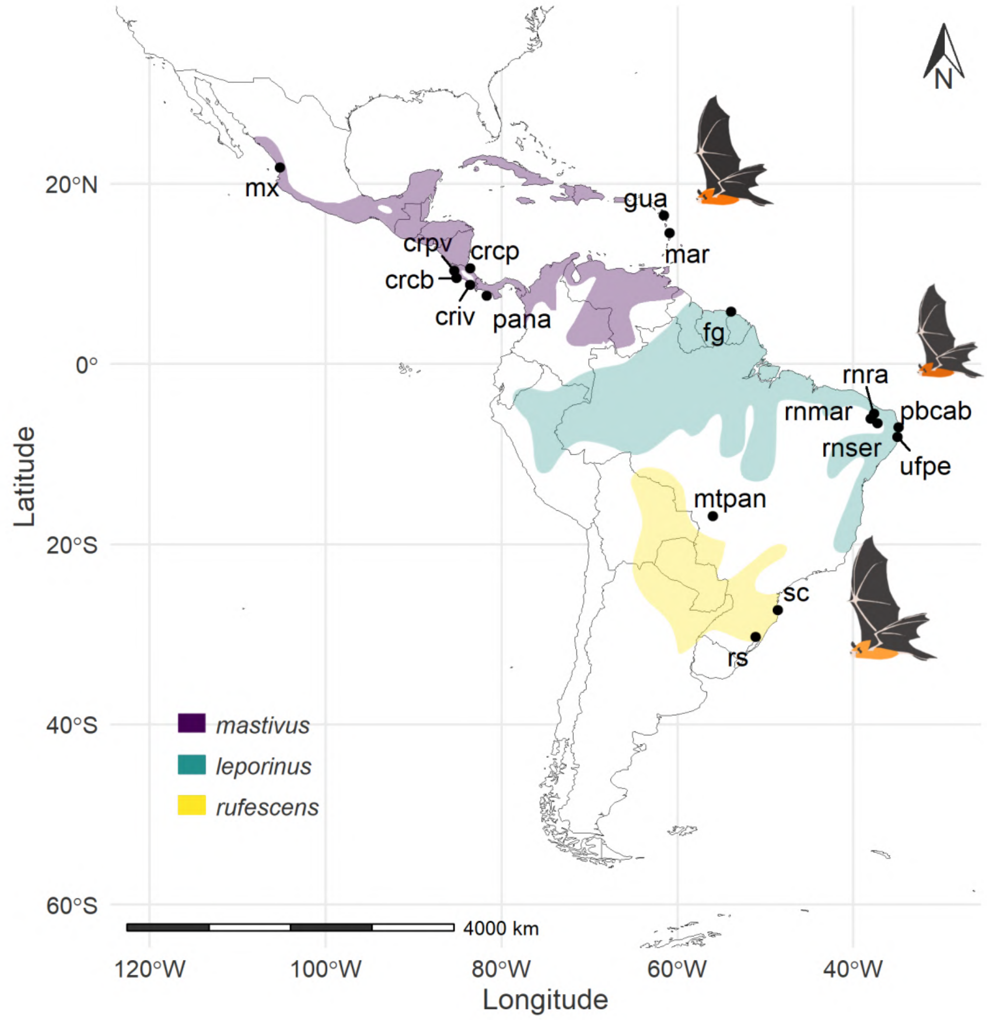

Figure 1. Map showing the Neotropical region and in circles the sampled localities for the greater bulldog bat, *Noctilio leporinus*. Colored areas represent subspecies geographic distribution according to Davis (1973): purple – *mastivus*; blue – *leporinus*; yellow – *rufescens*. Localities: mx: Mexico; crpv: Costa Rica – Palo Verde; crcb: Costa Rica – Cabo Blanco; criv: Costa Rica – Isla Violín; crcp: Costa Rica – Caño Palma; pana: Panamá; gua: Guadeloupe; mar: Martinique; fg: French Guiana; rnmar: Rio Grande do Norte, BR – Martins; Rio Grande do Norte, BR – Seridó; Rio Grande do Norte, BR – Rio Apodi; pbcab: Paraíba, BR – Cabedelo; ufpe: Pernambuco, BR; sc: Santa Catarina, BR; rs: Rio Grande do Sul, BR

Statistical analysis

To test for acoustic variation among localities we performed Kruskal-Wallis and Dunn´s comparison tests (Zar 1999). Data from CF calls and FM calls were tested separately as FF differed significantly between call types (Kruskal-Wallis test: H=293.87, p-value <0.001). To assess patterns of call variability within localities we calculated coefficients of variation (CV=SDx100/mean) from the means and standard deviation of FF for CF and FM calls based on all observations for each locality.

To assess if differences in FF means for CF and FM calls were related to the geographic distance, we calculated a dissimilarity matrix of acoustic distances using FF differences (in hertz) between populations and a geographic distance matrix from the latitude and longitude of each locality. Then, we compared the acoustic and geographic distance matrices using a Mantel test based on the Spearman's rank correlation with 9999 permutations with Bonferroni correction, using the R package vegan (Oksanen et al. 2017). To test the association between the FF and the geographic location we conducted linear regressions of FF means against longitude and latitude of each locality.

To account for potential multicollinearity amongst climatic variables we performed principal components analysis (PCA) on temperature, relative humidity and absolute humidity using the package FactoMineR (Lê et al. 2008) and then extracted the principal component scores used in subsequent models.

To test the effects of climate (Sensory Drive Hypothesis) and body size (James´s Rule) on flat frequency variation we used linear mixed-effects models (LME) with all observations of FF from CF and FM calls as response variables, the first and the second principal components from the PCA on environmental variables (PC1 and PC2), as well as Davis (1973) subspecies classification (proxy for body size) as fixed factors, and site and file nested within site as random effects. These random effects were considered to account for the autocorrelation design as a result of the multiple sampling from a single location (random effect for site) and variation associated with the recording equipment – ultrasound detector, sampling frequency, recording system – (random effect for file nested within site). We used ANOVA to determine which predictor variables significantly contributed to the variation in

FF in both the CF and the FM calls. Inspection of residuals showed a close approximation to a normal distribution and no evidence for violation of homogenous variance assumption (ESM 2). LME were fitted using the R package lmerTest (Kuznetsova et al. 2017).

Moreover, to asses for climatic differences between the three groups of localities corresponding to different body sizes – each of the subspecies defined by Davis (1973) – we tested for differences in temperature, relative humidity, absolute humidity and atmospheric absorption of CF and FM calls using linear models. Multiple comparisons were done using the R package multcomp (Hothorn et al. 2008). All our analyses were conducted in RStudio version 1.2.5033.

Results

Geographic variation in flat frequencies

We analyzed the FF of 1,740 CF calls from 201 sequences, corresponding to 146,175 observations, and 2,243 FM calls from 213 sequences, corresponding to 107,417 observations, from 17 localities where *N. leporinus* occurs along its distributional range (Table 1, ESM 3). Maximum variation in mean FF across localities was approximately 6 kHz. For all samples the mean FF of CF calls ranged from 53.18 (SD=0.68, CV=1.27) to 59.41 kHz (SD=0.71, CV=1.20) and the mean FF of FM calls ranged from 53.77 (SD= 0.82, CV=1.53) to 59.96 kHz (SD=0.74, CV=1.24) (Fig. 2). The FF for the two call types differed significantly between localities (CF calls: H=120620, df=16, p-value<0.001; FM calls: H=77845, df=16, p-value <0.001; Fig. 2). Besides, Dunn´s multiple comparisons test revealed significant geographic variation between localities except for the CF calls of French Guiana vs. Martins, RN, Brazil (rnmar) and Martinique vs. Mato Grosso, Brazil (mtpan) vs. Santa Catarina, Brazil (p>0.05); for the FM calls there was no significant differences between Cabo Blanco, Costa Rica (crcb) vs. Panama (pana); Isla Violín, Costa Rica (criv) vs. Mexico (mx); Cabedelo, PB, Brazil (pbcab) vs. Pernambuco, Brazil (ufpe); and Martins, RN, Brazil (rnmar) vs. Seridó, RN, Brazil (all p>0.05). Highest call variability within localities was found in French Guiana (CF calls CV=2.62 and FM calls CV=2.72). Additional data exploration (ESM 4) showed two frequency peaks of average flat frequency of the echolocation calls recorded from this locality. The lowest variability for CF calls was found in Santa Catarina, Brazil (CV=0.96) and, for FM calls, in Cabo Blanco, Costa Rica (CV=0.89).

Flat frequency variation of CF and FM calls was significant and positively associated with geographic distances between localities (Mantel test CF calls: $r=0.46$, $p<0.001$; Mantel test FM calls: $r= 0.41$, $p<0.05$; Fig. 3), meaning that the more distant the populations, the more distinct the frequencies. Regression analyses showed only significant and negative correlation between FF and longitude, for both CF and FM calls (CF calls: $r^2= 0.61$, $p<0.001$; FM calls: $r^2= 0.56$, $p<0.001$), exhibiting a tendency towards frequency increase eastward (Fig. 3).

Table 1. Coordinates, subspecies, sample size (N: number of observations; calls; sequences), flat frequency (FF: mean±SD) and coefficient of variation (CV) of constant frequency (CF) and frequency modulated (FM) echolocation calls of *Noctilio leporinus* for each sampled locality. Localities: mx: Mexico; crpv: Costa Rica - Palo Verde; crcb: Costa Rica - Cabo Blanco; criv: Costa Rica - Isla Violín; crcp: Costa Rica - Caño Palma; pana: Panamá; gua: Guadeloupe; mar: Martinique; fg: French Guiana; rnmar: Rio Grande do Norte, BR - Martins; Rio Grande do Norte, BR - Seridó; Rio Grande do Norte, BR - Rio Apodi; pbcab: Paraíba, BR - Cabedelo; ufpe: Pernambuco, BR; sc: Santa Catarina, BR; rs: Rio Grande do Sul

				CF calls			FM calls		
Locality	Latitude	Longitude	Subsp.	N	FF (kHz)	CV (%)	N	FF (kHz)	CV (%)
mx	21.805822	-105.20401	*mastivus*	10323; 146; 14	53.18±0.68	1.27	16640; 422; 17	54.31±1.00	1.84
crpv	10.35048	-85.33192	*mastivus*	10325; 132; 27	56.02±0.62	1.11	14352; 268; 37	56.22±0.83	1.47
crcb	9.578161	-85.135472	*mastivus*	8454; 91; 6	55.15±0.88	1.6	6371; 116; 7	55.68±0.5	0.89
criv	8.791549	-83.624201	*mastivus*	20979; 226; 29	53.98±1.11	2.05	15283; 256; 27	54.32±0.98	1.80
crcp	10.633651	-83.543355	*mastivus*	4445; 60; 7	54.41±0.74	1.37	1200; 25; 7	55.26±0.64	1.16
pana	7.524141	-81.676925	*mastivus*	12046; 138; 13	55.04±0.95	1.72	11791; 237; 14	55.72±1.02	1.83
gua	16.430933	-61.535653	*mastivus*	3906; 62; 5	53.61±0.78	1.45	3839; 83; 9	53.77±0.82	1.53
mar	14.54232	-60.85721	*mastivus*	3957; 61; 6	55.85±1.26	2.26	3686; 74; 7	56.66±1.13	2.00
fg	5.747073	-53.937355	*leporinus*	19377; 206; 15	58.57±1.53	2.62	5507; 145; 15	58.46±1.59	2.72
rnmar	-6.070933	-37.884588	*leporinus*	24290; 258; 25	58.4±0.8	1.38	7870; 185; 25	59.1±0.79	1.34
rnra	-5.592972	-37.686667	*leporinus*	5124; 72; 11	58.6±0.79	1.35	2641; 65; 9	58.71±0.72	1.23
rnser	-6.579375	-37.255567	*leporinus*	2106; 26; 3	58.82±0.75	1.27	1424; 26; 3	59.32±0.67	1.13
ufpe	-8.051716	-34.949883	*leporinus*	2002; 26; 6	59.09±0.95	1.61	1813; 42; 6	59.96±0.74	1.24
pbcab	-7.063692	-34.856622	*leporinus*	3036; 58; 7	59.41±0.71	1.2	2731; 87; 7	59.77±0.66	1.11
mtpan	-16.89095	-55.98764	*rufescens*	5219; 63; 10	55.75±0.68	1.22	4332; 79; 10	55.94±0.65	1.17
sc	-27.31033	-48.566778	*rufescens*	6356; 70; 9	55.78±0.53	0.96	6088; 96; 7	55.78±0.75	1.34
rs	-30.23576	-51.102697	*rufescens*	4230; 45; 8	54.54±0.68	1.25	1849; 37; 6	55.16±0.77	1.40

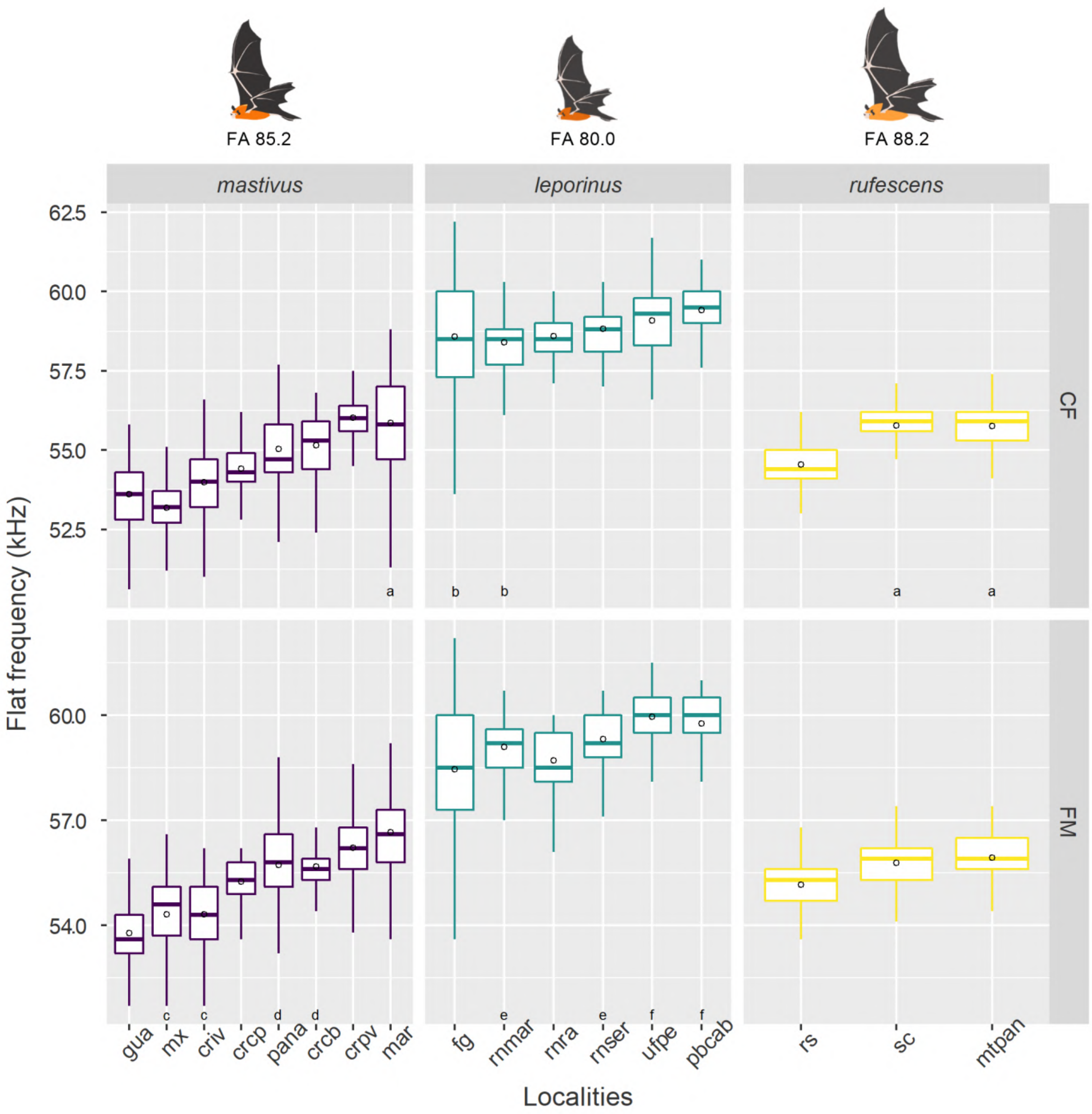

Figure 2. Flat frequency of constant frequency (CF) and frequency modulated (FM) search-phase echolocation calls of *Noctilio leporinus* grouped by subspecies – *mastivus*, *leporinus*, and *rufescens*. Boxplots show median (dark line), mean (circle), lower and upper quartile (box base and top), min and max values (vertical lines). The same letters represent non-significant differences between localities (Dunn´s test p>0.05). Localities: mx: Mexico; crpv: Costa Rica - Palo Verde; crcb: Costa Rica - Cabo Blanco; criv: Costa Rica - Isla Violín; crcp: Costa Rica - Caño Palma; pana: Panamá; gua: Guadeloupe; mar: Martinique; fg: French Guiana; rnmar: Rio Grande do Norte, BR - Martins; Rio Grande do Norte, BR - Seridó; Rio Grande do Norte, BR - Rio Apodi; pbcab: Paraíba, BR - Cabedelo; ufpe: Pernambuco, BR; sc: Santa Catarina, BR; rs: Rio Grande do Sul. FA: forearm length

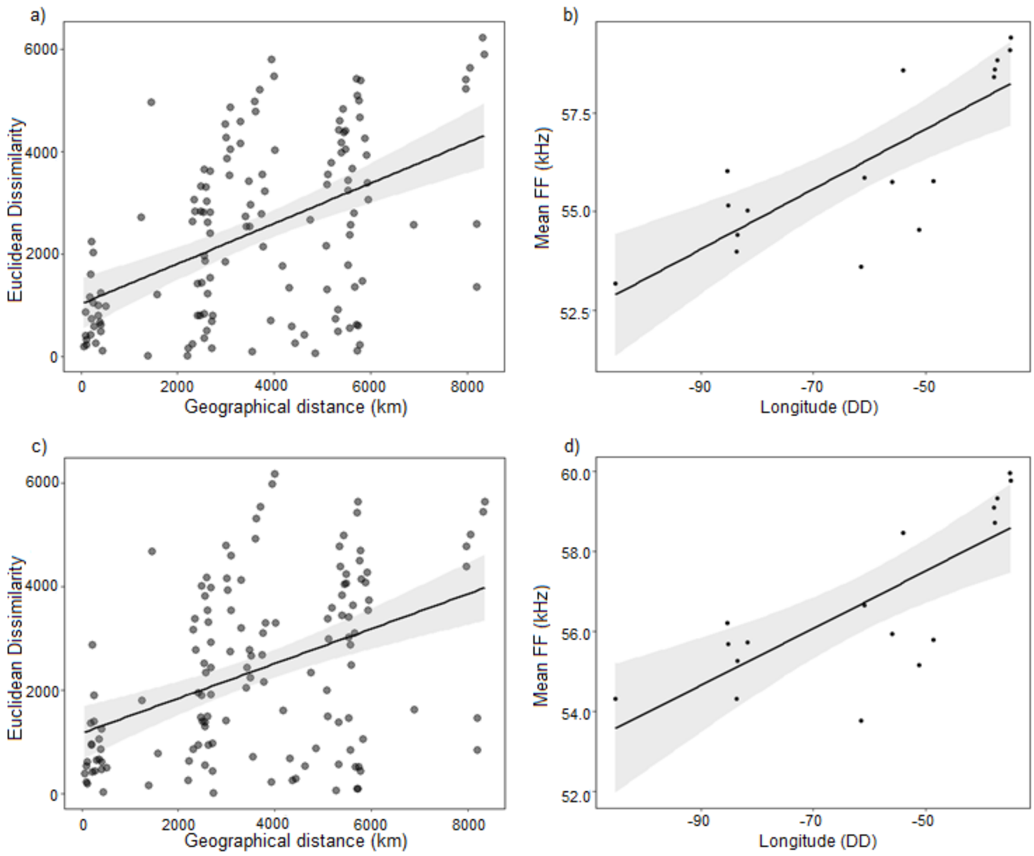

Figure 3. Relationships of flat frequency differences of constant frequency (CF) and frequency modulated (FM) echolocation calls of *Noctilio leporinus* with geographic distance (a: CF calls, c: FM calls) and linear regressions between mean flat frequency and longitude (b: CF calls, d: FM calls). The grey-shaded areas represent 95% confidence intervals

Call variation explained by climate and body size

The PCA yielded three principal component loadings (PC1, PC2 and PC3). PC1, on which absolute humidity and relative humidity loaded highest, accounted for 65.1% of the variation between localities while PC2, on which temperature loaded the highest, explained 34.8% of the variation (Fig. 4). In total, these two components, accounted for 99.9% of the variation. PC3 accounted for <0.1% proportion of variance and was thus omitted from further analyses.

After controlling the effects of locality and variability associated with recording equipment, the LME model for CF calls showed that FF varied in response to environmental conditions mainly associated to humidity (PC1, $F_{12.235}$=7.80, $p<0.05$) and body size (CF calls: $F_{11.952}$=54.37; $p<0.001$). For FM calls, the LME model indicated that only body size influence the FF variation (FM calls: $F_{12.401}$= 44.16; $p<0.001$). For both call types the smallest of the subspecies, *leporinus* showed higher frequencies than *mastivus* and *rufescens* (intermediate and larger sizes, respectively) (Table 2).

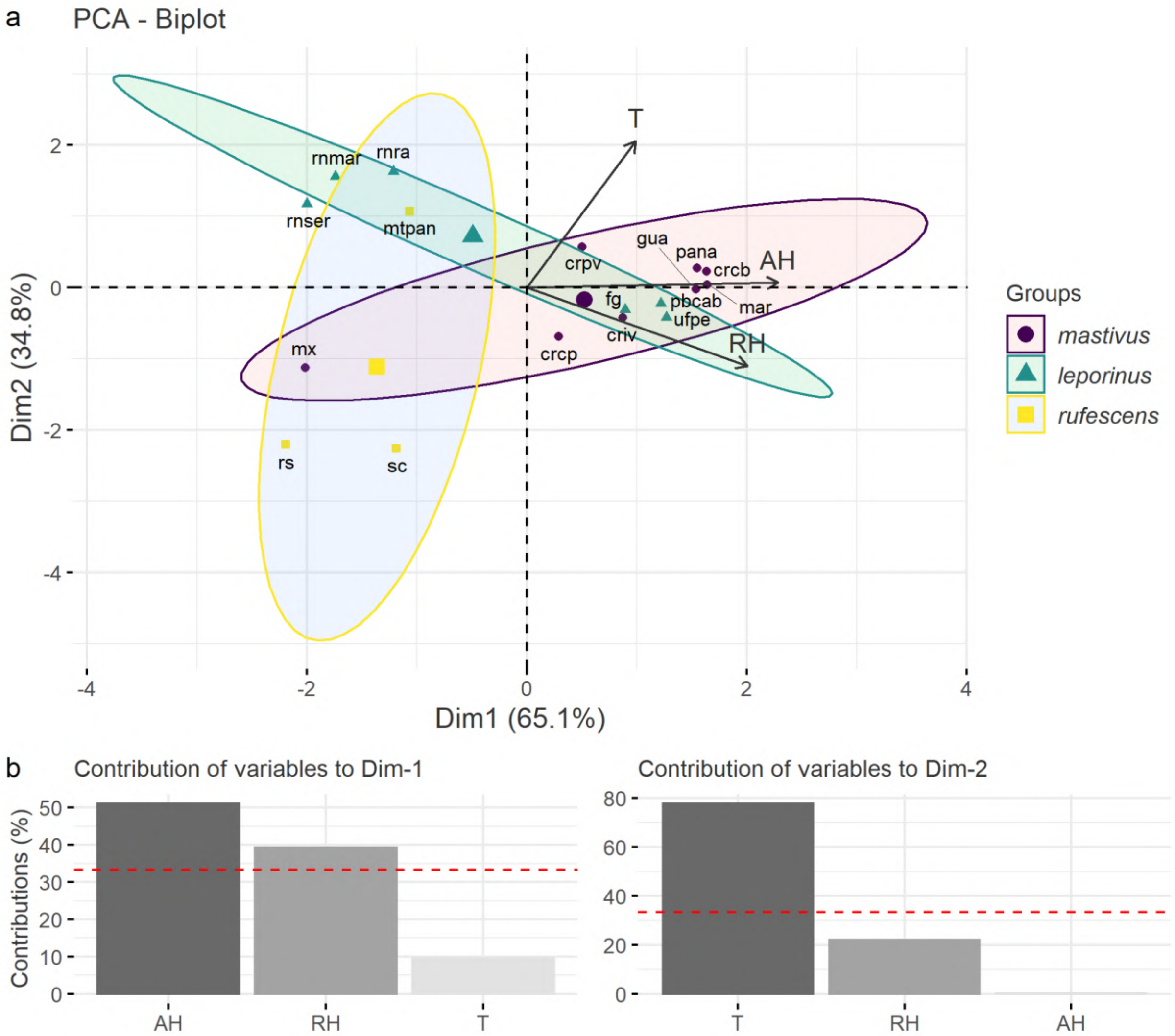

Figure 4. Variation in environmental conditions (T: temperature, AH: absolute humidity, RH: relative humidity) based on principal component analysis across acoustic sampling localities of *Noctilio leporinus* (a) PCA, biplot showing the localities grouped by subspecies and (b) contribution of each environmental variable to dimension-1 and dimension-2. Dim1 and Dim2 correspond to PC1 and PC2 respectively. Localities: mx: Mexico; crpv: Costa Rica - Palo Verde; crcb: Costa Rica - Cabo Blanco; criv: Costa Rica - Isla Violín; crcp: Costa Rica - Caño Palma; pana: Panamá; gua: Guadeloupe; mar: Martinique; fg: French Guiana; rnmar: Rio Grande do Norte, BR - Martins; Rio Grande do Norte, BR - Seridó; Rio Grande do Norte, BR - Rio Apodi; pbcab: Paraíba, BR - Cabedelo; ufpe: Pernambuco, BR; sc: Santa Catarina, BR; rs: Rio Grande do Sul

Table 2. Summary statistics for the linear mixed-effects model (LME) fitted by REML on the flat frequency (FF) of constant frequency (CF) and frequency modulated (FM) echolocation calls of *Noctilio leporinus*

	Effects	Variable	Estimate	SE	DF	t-value	p-value
CF calls	Fixed	(Intercept)	58.7344	0.2952	12.501	198.935	<0.001
		PC1	0.3902	0.1397	12.235	2.792	<0.05
		PC2	0.2706	0.1768	11.7934	1.531	0.15
		mastivus vs. *leporinus*	-4.2017	0.403	12.0966	-10.426	<0.001
		rufescens vs. *leporinus*	-2.4067	0.5965	12.035	-4.035	<0.01
	Random		Variance	SD			
		file:site	0.5645	0.7513			
		site	0.3850	0.6205			
		Residual	0.3913	0.6256			
FM calls	Fixed	Variable	Estimate	SE	DF	t-value	p-value
		(Intercept)	59.065	0.3214	13.1521	183.777	<0.001
		PC1	0.252	0.151	12.4878	1.668	0.12
		PC2	0.189	0.1926	12.4598	0.982	0.34
		mastivus vs. *leporinus*	-4.0752	0.4363	12.4675	-9.341	<0.001
		rufescens vs. *leporinus*	-2.8711	0.6481	12.5785	-4.43	<0.001
	Random		Variance	SD			
		file:site	0.7272	0.8528			
		site	0.4475	0.6689			
		Residual	0.3736	0.6112			

PC: principal component; SE: standard error; DF: degrees of freedom

Geographic variation in climate variables among subspecies localities

The GLM revealed a significant difference in absolute humidity for the localities where the subspecies *rufescences* occurs, which were dryer than the localities of occurrence of *mastivus* (estimate=-5.65; z=-2.65; p<0.05). Also, the temperature for the localities of occurrence of *rufescens* were in average 4.54 C° cooler than the localities of occurrence of *leporinus* (z=-2.85; p<0.05), and 3.74 C° cooler than the localities of occurrence of *mastivus* (z=-2.46; p<0.05) (Fig. 5). The atmospheric attenuation experienced by CF and FM calls was significantly lower among the localities of occurrence of *mastivus* (CF calls: estimate=-0.29; z=-5.73; FM calls: estimate=-0.28; z=-5.59; p<0.001) and *rufescens* (CF calls: estimate=-0.25; z=-3.82; FM calls: estimate=-0.26; z=-4.02; p<0.001) when compared to those localities where *leporinus* occurs (Fig. 5).

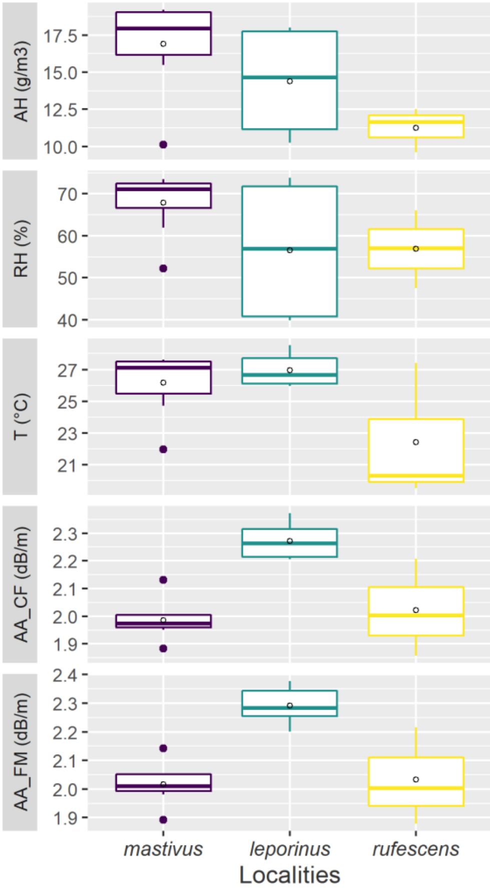

Figure 5. Temperature (T), absolute humidity (AH) and relative humidity (RH) of sampled localities and atmospheric attenuation (AA) experienced by constant frequency (CF) and frequency modulated (FM) calls of the three subspecies of *Noctilio leporinus* – *mastivus*, *leporinus*, and *rufescens*. Boxplots show median (dark line), mean (circle), lower and upper quartile (box base and top), min and max values (vertical lines)

Discussion

We investigated the acoustic variation in the constant frequency portion (flat frequency – FF) of the search-phase echolocation calls of *N. leporinus* across its distribution range, testing the Sensory Drive Hypothesis and the James´s rule. We found significant differences in the FF of the species across its distribution range, with a tendency of frequency increase eastward; FF variation was explained by differences in climatic conditions, mainly associated with humidity and also with body size. These results indicate that the variation in *N. leporinus* echolocation calls is likely the result of ecological selection, partially supporting the two hypotheses. The extent to which the FF changes as a result of climate-driven frequencies or climate-mediated variation in body size differs between subspecies, suggesting the role of both ecological selection and phylogenetic history on the intraspecific pattern of acoustic divergence.

Patterns of geographic variation in FF

Average geographic variation in the constant portion of both the CF and the FM search-phase echolocation calls of *N. leporinus* was of approximately 6 kHz. This value is about half of the previously reported variation in the constant frequency component for the species, which was reported to range from 50 to 61 kHz (Suthers 1965; Schnitzler et al. 1994; O´Farrel, Miller and Gannon 1999; Surlykke and Kalko 2008; Barataud et al. 2013, 2015; Briones-Salas et al. 2013; Rivera-Parra and Burneo 2013; López-Baucells et al. 2016; Zamora-Gutierrez et al. 2016; Mancina et al. 2017). It should be noted that we considered that maximum/high frequency values as part of the CF portion. As our models revealed, part of the acoustic variation in FF not explained by the chosen predictors was associated with the sampling method (indicated by the random effect), so the greater variability reported in the literature is probably related with different sampling conditions (e.g. equipment technology) and, eventually, the component associated with sound analyses (e.g. software and chosen parameters).

We observed significant pairwise differences in the echolocation calls between the majority of the 17 localities sampled and found that geographic variation in FF showed significant spatial structuring by longitude. Similar to our results, Jiang et al. (2010) found a negative correlation between geographic variation in echolocation calls of *Hipposideros larvatus* and longitude, and surmised that the effect of longitude on resting frequency variation is probably related to local climate conditions, including temperature and humidity. In our case, the relationship between flat frequency and longitude may be influenced by the driest conditions of the Northeastern region of Brazil, particularly the localities in the Caatinga biome (Rio Grande do Norte). As those localities correspond to *leporinus*, the smallest of the subspecies, we cannot state that the correlation between longitude and FF results by the isolated effects of actual climate conditions, by body-size mediated frequency or their combination. Moreover, the findings of Mutumi et al. (2016) and Jacobs et al. (2017) on the variation of echolocation calls of *Rhinolophus clivosus* suggest that besides climate, stochastic or deterministic factors (e.g. drift and/or selection) may also exert an influence on frequency. Indeed, for example, the role of cultural drift on bat call divergence has been suggested when there is evident correlation between geographic acoustic variation and geographic distance (Yoshino et al. 2008; Chen et al. 2009; Jiang et al. 2010).

Call variation: climate and body size effects

Our results show humidity (PC1) playing a main role in the environmental variation between localities. When all localities were analyzed together, the influence exerted mostly by absolute humidity indicated a small frequency variation of approximately 400 Hz on the constant frequency portion of the CF calls. This may reflect the flexibility of bats to constantly adjust their echolocation calls in response to even slight changes in temperature and relative humidity during a single night (Jacobs et al. 2017).

Each *N. leporinus* subspecies is characterized by external diagnostic features. The smallest, *leporinus*, differs substantially in forearm length from the intermediate and largest, *mastivus* and *rufescens*, respectively, but the two largest subspecies differ only slightly from each other (Davis 1973). When comparing the localities where the different subspecies occur, we observe that the highest atmospheric attenuation values occur in localities where *leporinus* is present; in these localities we find the highest temperatures, highly variable average humidity conditions, and the highest frequency calls emitted by the species. Meanwhile the lowest (and more similar) frequencies emitted by *mastivus* and *rufescens*, suffer low atmospheric attenuation under clearly distinct humidity conditions. Our results support the hypothesis that the geographic variation in the echolocation calls of *N. leporinus* is constrained by body size and that the most notable effect of the increase in the atmospheric attenuation is a pronounced increase in call frequency (Griffin 1971). Still, the effect of absolute humidity on the atmospheric attenuation cannot be neglected (Lawrence and Simmons 1982). If high frequencies for bats in humid environments imply a detrimental effect on ecological performance (Guillén et al. 2000), bats will echolocate at lower frequencies in more humid conditions (Heller and v. Helversen 1989; Guillen et al. 2000; Snell-Rod 2012) and will adjust their calls to the variation in sound absorption between seasons (Snell-Rod 2012).

Sensory Drive or James's rule: what drives echolocation call variation in greater bulldog bats?

Ecological selection appears to be the process largely responsible for the acoustic divergence found in the search-phase echolocation calls of *N. leporinus*. By testing the acoustic differences between the subspecies we are assuming that they correspond to different populations. Under the Wilkins et al. (2012) framework, we observe that the variation of the flat frequency between populations covaries with ecological divergence (body size) and with environmental features (humidity, atmospheric absorption), which certainly imply changes on ecological performance (e.g. prey capture). In fact, higher atmospheric attenuation is mainly a result of an increase in frequency (Lawrence and Simmons 1982) mediated by body size, but with humidity also having a significant effect (Griffin 1971). Even when scaling relationships are influenced by phylogeny, acting indirectly on morphological traits involved in sound production (Luo et al. 2019), body size allows predicting, to some extent, some characteristics of the echolocation calls, contributing to intraspecific acoustic variation (Jones et al. 2000). Besides, sound absorption and climate have relatively minor, but significant, effects on the evolution of signals (Snell-Rod 2012). In this sense, the observed frequencies and, necessarily, the atmospheric attenuation experienced by the echolocation calls of the subspecies *mastivus* correlates with sound transmission properties of the habitat, rather than with body size, as seems to occur in the *leporinus* and the *rufescens* subspecies. These results match the Sensory Drive Hypothesis and the James's rule predictions, respectively. Following the Sensory Drive, the flat frequency of the echolocation calls of *N. leporinus* is predominantly climate driven in the lineage corresponding to the subspecies *mastivus*, and, as proposed by the James's rule, the frequency variation in the constant portion of the echolocation calls of the lineage corresponding to the subspecies *leporinus* and *rufescens* seems to be primary the result of climate-mediated variation in body size.

Probably the origin of Noctilionidae, like many other existing Neotropical clades, goes back to the Neogene, with the clade reaching the current diversity under the dominant climatic instability of the Quaternary (Rull 2011). At a taxonomic family level, acoustic variation in bat echolocation calls is widely explained by differences in body size and phylogenetic relationships (Luo et al. 2109). From the inferred phylogenetic history of *N. leporinus* (Khan et al. 2014; Pavan et al. 2013) we may conclude that its echolocation calls evolved by ecological selection, so that the divergence in body size predicts acoustic divergence (Wilkins et al. 2012). Considering the genetic-based lineages proposed by Khan et al. (2014) we hypothesize that, under an ancestral and potentially different ecological scenario, evolutionary conservatism in *N. leporinus* has been a key factor limiting the available variation for acoustic signal divergence. Such conservatism seems to occur through a constraint enforced by body size, imposing physiological limits to the frequency range; then, under a contemporary scenario, habitat features via sensory drive constraint the available acoustic variation for ecological selection (Wilkins et al. 2012).

Social selection may also influence frequency variation within and between populations and, combined with environmental selection, can lead to species diversification between habitats with distinct patterns of air humidity, and can also produce assortative mating (Guillén et al. 2000). Social selection studies considering different Neotropical biomes should thus follow to fully understand how all these processes interact. Acting together, they can result in speciation with dispersal barriers or new adaptive zones available between populations (Guillén et al. 2000; Boughman 2002).

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Supplementary materials

ESM 1 Audio file discrimination per locality, including information on recording equipment and parameters, call type, main author and collaborators

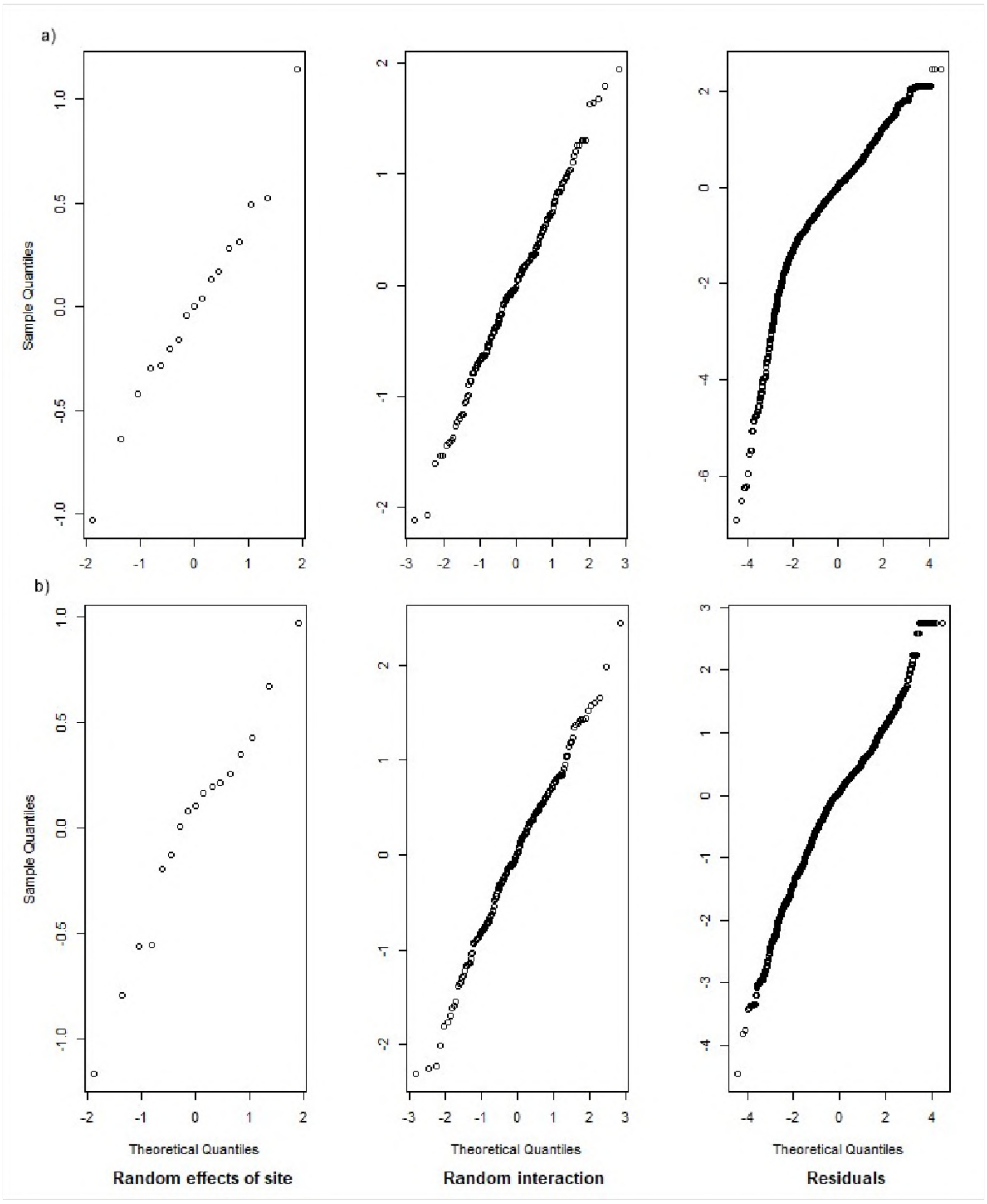
Locality	Locality name	File name	Detector	Sampling Frequency	Recording mode	Call type	Author	Collaboration	Data (Month/Year)
crcb	Cabo Blanco, Costa Rica	CB_20-III-17_M00595	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcb	Cabo Blanco, Costa Rica	CB_20-III-17_M00619	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcb	Cabo Blanco, Costa Rica	CB_20-III-17_M00659	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcb	Cabo Blanco, Costa Rica	CB_20-III-17_M00660	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcb	Cabo Blanco, Costa Rica	CB_20-III-17_M00818	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcb	Cabo Blanco, Costa Rica	CB_20-III-17_M00939	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcb	Cabo Blanco, Costa Rica	CB_M01493	d500x	300	rt	ff	A. Arias-Aguilar	RNACB	III/2017
crcp	Caño Palma, Costa Rica	CP_5CA2B500	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA2B780	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA2BB18	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA2E458	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA325A8	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA332A0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA42D68	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
crcp	Caño Palma, Costa Rica	CP_5CA44500	audiomoth	192	rt	ff	A. Arias-Aguilar	EBCP	IV/2019
criv	Isla Violín, Costa Rica	IV_5C68ADB0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68AE78	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68AEF0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68AF40	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68AF68	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68B0F8	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68B2B0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C68B580	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6909E0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C690A08	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C690D78	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6914A8	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019

criv	Isla Violín, Costa Rica	IV_5C6919A8	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C691E08	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6921F0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C692768	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C692920	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C692C18	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C692EC0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6937A8	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C693848	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C693A50	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C693D98	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6A2BE0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6A7050	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6A8A40	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6A8BA8	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6A8FE0	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
criv	Isla Violín, Costa Rica	IV_5C6A9008	audiomoth	384	rt	ff	A. Arias-Aguilar	A. Vicente	II/2019
crpv	Palo Verde, Costa Rica	PV_5CA80528	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA80550	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA81428	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA81608	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA81860	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA826E8	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA82A08	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA82BE8	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA83048	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA834D0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA837C8	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA838E0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA83A20	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA83B38	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA83B88	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA84060	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA843D0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019

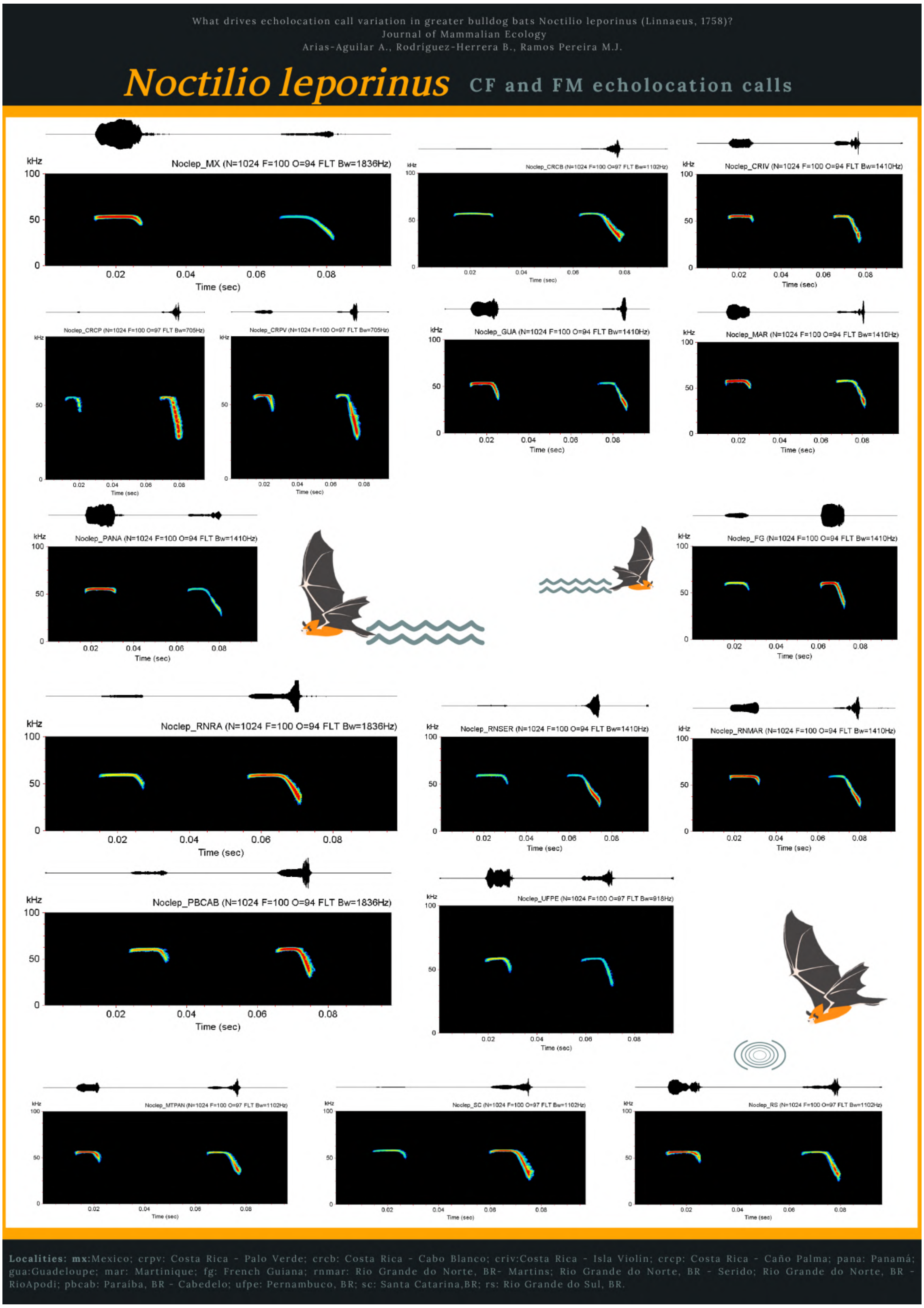
crpv	Palo Verde, Costa Rica	PV_5CA845B0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA84A10	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA84DD0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA84F10	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA85050	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA85258	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA85E38	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA871E8	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA87D50	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA87DA0	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA88098	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA94578	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA96418	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA96530	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA98560	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA9B738	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA9B8C8	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA9BA80	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA9BF08	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_5CA9C160	audiomoth	192	rt	ff	A. Arias-Aguilar	EBPV	IV/2019
crpv	Palo Verde, Costa Rica	PV_VBSR18_20181207_222024	sm2	384	rt	ff	J. Ramírez-Fernández		XII/2018
crpv	Palo Verde, Costa Rica	PV_VBSR18_20181208_043035	sm2	384	rt	ff	J. Ramírez-Fernández		XII/2018
crpv	Palo Verde, Costa Rica	PV_VBSR18_20181208_225000	sm2	384	rt	ff	J. Ramírez-Fernández		XII/2018
fg	Guiana Francesa	Guy_M00028_Simili_29.10.2011-22h53	d240?	38.4x10	te	ff	M. Barataud		X-XI/2011
fg	Guiana Francesa	Guy_M00072_Mana_30.10.2011-19h45	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h27_M109	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h30_M110	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h30_M111	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h34_M113	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h36_M114	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h42_M117	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h46_M119a	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_18h54_M122	d240?	38.4x10	te	ff	M. Barataud		XI/2009
fg	Guiana Francesa	Kaw_07nov09_21h22_M129	d240?	38.4x10	te	ff	M. Barataud		XI/2009

rnser	ESEC do Seridó, RN	LAJ-1_20180402_184000	sm3	384	rt	ff	J. C. Vargas-Mena	Projeto Morcegos Potiguares	IV/2018
rnser	ESEC do Seridó, RN	LAJ-1_20180402_184414	sm3	384	rt	ff	J. C. Vargas-Mena	Projeto Morcegos Potiguares	IV/2018
rnser	ESEC do Seridó, RN	SBEQ_38_CA_RN	sm2	192	rt	ff	F. Hintze	SBEQ	
rs	Lami e Itapuã, RS, Brazil	It_M001309	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000023	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000049	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000053	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000057	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000058	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000160	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
rs	Lami e Itapuã, RS, Brazil	La_M000261	d500x	300	rt	ff	A. Arias-Aguilar		VII-X/2018
sc	Praia do Marcelo, SC, Brazil	SC_M001037	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001059	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001070	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001074	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001076	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001078	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001079	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001135	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001137	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
sc	Praia do Marcelo, SC, Brazil	SC_M001142	d500x	300	rt	ff	A. Arias-Aguilar		II/2019
ufpe	UFPE, PE, Brazil	PE_2015_Oct_27_195524	dodotronic	250	rt	ff	F. Hintze		X/2015
ufpe	UFPE, PE, Brazil	PE_ccb5	dodotronic	250	rt	ff	F. Hintze		X/2015
ufpe	UFPE, PE, Brazil	PE_ccb8	dodotronic	250	rt	ff	F. Hintze		X/2015
ufpe	UFPE, PE, Brazil	PE_Nov_26_203721	dodotronic	250	rt	ff	F. Hintze		XI/2015
ufpe	UFPE, PE, Brazil	PE_Oct_27_195524.6363	dodotronic	250	rt	ff	F. Hintze		X/2015
ufpe	UFPE, PE, Brazil	PE_Oct_27_195524.9888	dodotronic	250	rt	ff	F. Hintze		X/2015

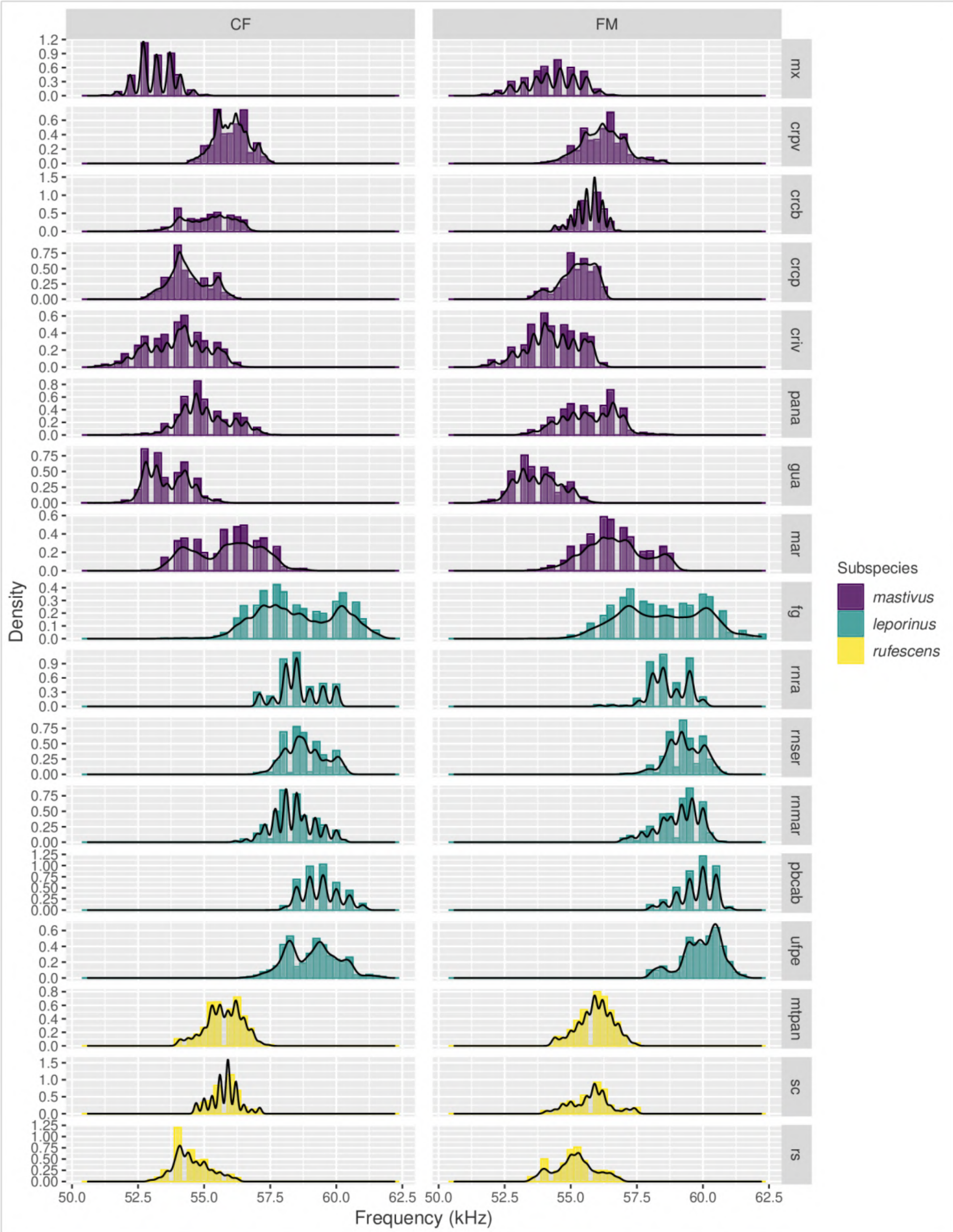
Abbreviations:
rt: real-time; te: time expansion; ff: free-flying; hr: hand release
RNACB: Reserva Natural Absoluta Cabo Blanco; EBCP: Estación Biológica Caño Palma; EBPV: Estación Biológica Palo Verde;
SIMMA: Sistema Mexicano de Monitoreo Acústico; SBEQ: Sociedade Brasileira para o Estudo dos Quirópteros



ESM 2 Model validation graphs showing a closely aproximation of residuals to a normal distribution and no violation of homogeneity of variance assumption. a) graphs for constant frequency (CF) calls; b) graphs for frequency modulated (FM) calls



ESM 3_CF and FM echolocation calls



ESM 4_Flat frequency histograms

Capítulo III

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Acoustic clue: bringing echolocation call data into the distribution dilemma of *Pteronotus* (Chiroptera: Mormoopidae) complexes in Central America

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Acoustic clue: bringing echolocation call data into the distribution dilemma of *Pteronotus* (Chiroptera: Mormoopidae) complexes in Central America

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Short running title: Acoustic variation in Central American *Pteronotus*

Abstract

In Central America, the distributional limits and the contact zones of some *Pteronotus* species like the naked-backed bats and the lesser mustached bats are unclear. To elucidate the distributional dilemma of the species-groups *P. fulvus* x *P. davyi* and *P. psilotis* x *P. personatus* in Central America we study the acoustic variation of their echolocation calls along the range of possible contact zones and the existence of distinct phonic groups. We performed a Hierarchical k-means clustering on principal components (HCPC) using acoustic samples from Mexico, Honduras, El Salvador, Nicaragua, and Costa Rica to describe the global acoustic diversity, possibly overlooking differences between species groups. We assessed if those acoustic differences were related to the geographic distance and geographic location. We found evidence of sympatry for three phonic groups within each species complex, without a clear correspondence with species known distribution. The frequency changes of their echolocation calls seem to follow a similar pattern to the species geographic variation in body size. Future studies in Central America should include an integrative sampling of individually captured, tagged, and recorded bats to help in the resolution of the distribution dilemma raised here.

Keywords: acoustic identification - HCPC - phonic groups - *Pteronotus davyi* - *Pteronotus fulvus* - *Pteronotus personatus* - *Pteronotus psilotis*

Introduction

The Mormoopidae are a Neotropical bat family consisting of two genera of living species, *Mormoops* (known as ghost-faced bats) and *Pteronotus* (known as mustached bats and naked-backed bats). Mormoopids have a wide geographical distribution ranging from southern United States, into Central America, the Caribbean to central Brazil and from west Andes to Peru (Koopman, 1993). Species of the family inhabit tropical rainforest, semi-arid and arid environments below 3000 m (Emmons, 1997; Smith, 1972; Patton & Gardner, 2007). All species are insectivorous, gregarious, and obligatory cave-dwellers (Koopman, 1993; Simmons & Conway, 2001).

Multiple lines of evidence, morphological, morphometric, ecological, acoustic, and molecular (e.g. Smith, 1972; Simmons & Conway, 2001; Dávalos, 2006; Mancina *et al*., 2012; Clare *et al*., 2013; Pavan & Marroig, 2016; 2017) have allowed a better understanding of species delimitation and evolutionary history within this family, particularly for the genus *Pteronotus*. The most recent phylogenetic hypothesis recognizes the high diversity of this genus, and its subdivision into three subgenera and four clades (Pavan & Marroig, 2016).

In general, *Pteronotus* species from distinct species complexes overlap in terms of their geographic distribution, while species within the same clade (species-group) show separate distributions (Pavan & Marroig, 2016). However, in Central America, for some species-groups like the naked-backed bats and the lesser mustached bats, with supposedly allopatric distribution, the distributional limits and the contact zones are unclear (Smith, 1972; Pavan & Marroig, 2016; 2017).

The recognized distribution of the Thomas´s naked-backed bat *Pteronotus fulvus* (Thomas, 1892) and the Davy's naked-backed bat *Pteronotus davyi* Gray, 1838 primary follows the subspecies geographic range proposed by Smith (1972) with *P. fulvus* ranging from southern Mexico to eastern Honduras and El Salvador; and *P. davyi* from Nicaragua to northern South America (Pavan & Marroig, 2016; Pavan, 2019). Based on morphological geographic variation, allopatric populations may be easily distinguished on the base of cranial and external size, with *P. fulvus* smaller than *P. davyi*, gradually increasing in size southward (Smith, 1972). Smith (1972) proposed a narrow intergradation zone in northern Nicaragua and eastern Honduras and El Salvador. However, the lack of molecular data for populations of *P. davyi* on its northern distribution limit inhibits determining its exact geographic limits (Pavan & Marroig, 2016; 2017). Unpublished molecular data (AC Pavan, personal comm.) suggest the occurrence of *P. fulvus* and *P. davyi* in sympatry in Costa Rica, which may correspond to a secondary contact zone.

Similarly, the recognized distribution of the Wagner´s lesser mustached bat *Pteronotus psilotis* Wagner, 1843 and the Dobson´s lesser mustached bat *Pteronotus personatus* Dobson, 1878 is based on the subspecies geographic range proposed by Smith (1972), with *P. psilotis* occurring from southern Mexico to eastern Honduras and El Salvador; and *P. personatus* from western Costa Rica to South America. Considering cranial and external size *P. psilotis* is smaller than *P. personatus*, gradually increasing in size southward, with a probable intergradation zone in southeastern Honduras and eastern El Salvador (Smith, 1972).

The high levels of genetic differentiation and the significant morphometric variation of the lesser mustached bats throughout their distribution indicate that this clade is a species complex with at least five lineages corresponding to a new undescribed subgenus (Pavan & Marroig, 2016; Zárate-Martínez *et al*., 2018). Nevertheless, the phylogenetic status of Central American *P. personatus* populations has not been assessed and the northern limits of its distribution are unclear, probably extending to Nicaragua or Costa Rica (Pavan, 2019). Pavan & Marroig (2017) resumed the findings of Smith (1972) indicating a possible contact zone located in Nicaragua and Costa Rica.

In general, the echolocation calls of mormoopids show low levels of interspecific variation (Fenton, 1994; Ibáñez *et al*., 1999; Macías & Mora, 2003). Particularly, the echolocation calls of the aforementioned *Pteronotus* species-groups share similar design, with multiple harmonics, initial and terminal components of constant frequency (CF) linked by a frequency modulated

(FM) component (Griffin & Novick, 1955; Novick & Vaisnys,1964; O'Farrell & Miller, 1997; Ibáñez *et al.*, 1999; Macías & Mora, 2003). Acoustic identification relies on species-specific frequencies of the CF segment of the second harmonic (Macías *et al.*, 2006), which is less susceptible to frequency changes during distinct behavioral circumstances (Ibáñez *et al.*, 1999). Acoustic information of those species is scarce and available mainly from a few localities in Mexico (e.g. Morelos, Oaxaca, Yucatán, Veracruz), Central America (e.g. Belize, Costa Rica, Panama) and the Caribbean (Puerto Rico, French Guiana, Guadeloupe) (for a review on this topic see Arias-Aguilar *et al*., 2018).

Because the areas of sympatry are uncertain, and in the absence of an ideal acoustic integrative sampling (including individual acoustic, morphological and molecular information), species identification from common passive acoustic monitoring in those areas may be ambiguous. So, there is a need to identify and describe acoustic groups before assigning them to a specific species. The study of acoustic variation along the range of possible contact zones may contribute to elucidate the distributional dilemma of those species.

Here, we aim to study the acoustic variation of the species-groups *P. fulvus* x *P. davyi* and *P. psilotis* x *P. personatus* in Central America within their possible contact zones. Specifically, we aim to investigate the existence of distinct phonic groups and their correspondence with the known or estimated distribution of the different species. We hypothesize that different phonic groups will occur within the species intergradation zone and we expect that the acoustic variation will follow a similar pattern to the morphological geographic variation proposed by Smith (1972).

Material and methods

Acoustic data

Through our own recordings and donations, we compiled echolocation calls from species of the genus *Pteronotus* from Mexico, Honduras, El Salvador, Nicaragua, and Costa Rica, corresponding to populations within the recognized distribution of *P. fulvus*, *P. davyi* (Fig. 1), *P. psilotis*, and *P. personatus* (Fig. 2) (Table S1). Recordings were made on free-flying bats. Since the recordings were made with different bat detectors and different sampling frequencies, to maintain the same frequency resolution for all files (188 Hz), we resampled those recordings with a different sampling rate to 192 kHz before the acoustic analysis.

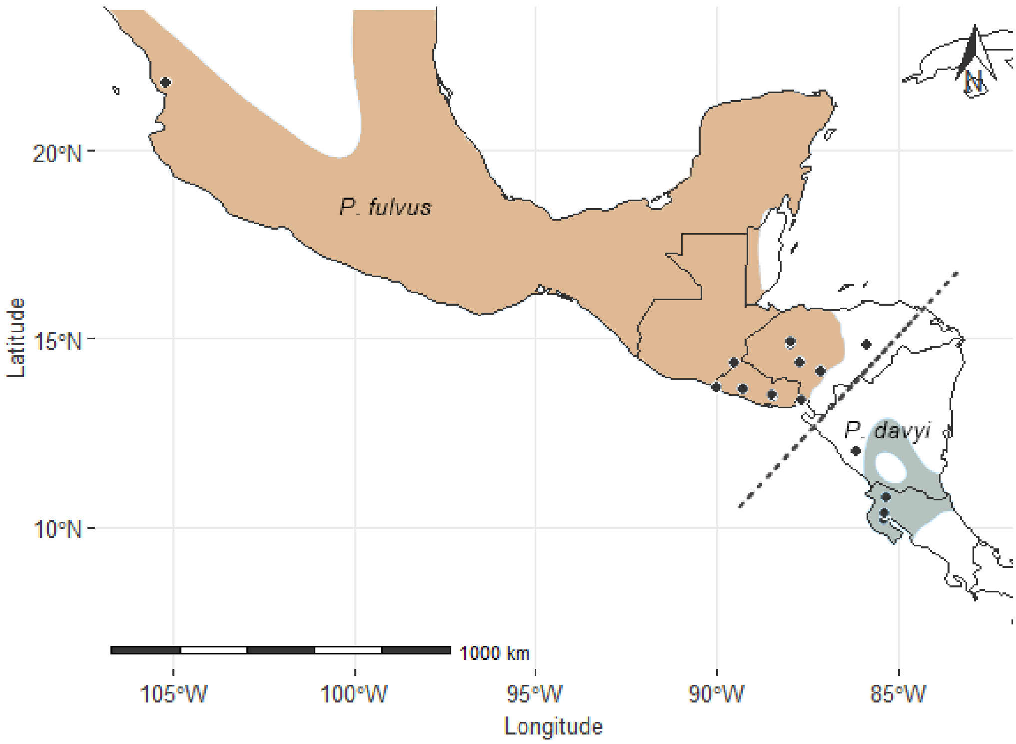

Figure 1. Map showing the localities of acoustic monitoring (black dots) with shaded areas representing part of the distribution of *Pteronotus fulvus* (in brown) and *P. davyi* (in grey) (modified from Pavan, 2019). The dotted line indicates the area of species intergradation proposed by Smith (1972).

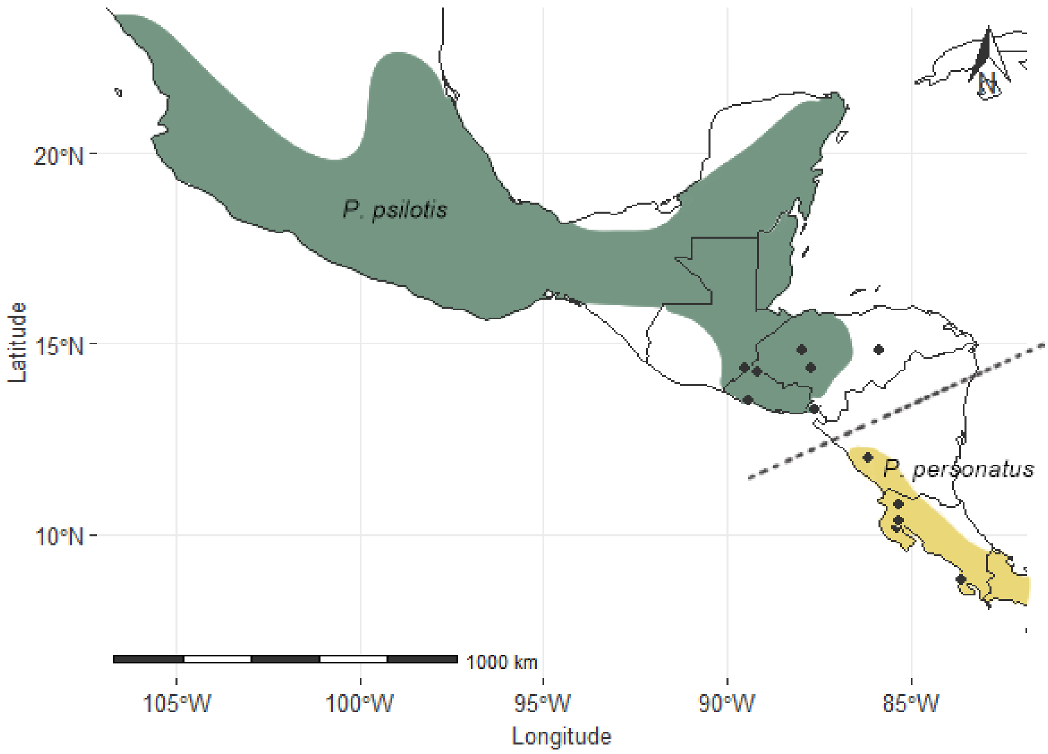

Figure 2. Map showing the localities of acoustic monitoring (black dots) with shaded areas representing part of the distribution of *Pteronotus psilotis* (in green) and *P. personatus* (in yellow) (modified from Pavan, 2019). The dotted line indicates the area of species intergradation proposed by Smith (1972).

Acoustic analysis

Spectrograms were generated with 1024 point fast Fourier transformation (FFT), FlatTop window, 100% frame size and 98.43% overlap. The echolocation call parameters were measured from the second harmonic of each pulse using Avisoft SAS Lab Pro software, Version 5.2.13. Frequencies under and above the second harmonic were filtered. When possible, 10 pulses (minimum 5) were measured for each file including consecutive and non-consecutive calls. Only search phase calls with no overlap or overloading and clearly distinguished from the background (signal-to-noise ratio >20 dB) were measured.

We used the automatic parameter measurements tool for computing the peak frequency at the start and end of the element (call); the lowest peak frequency; the highest peak frequency; the mean peak frequency; and the maximum amplitude of each element. Hereinafter: fstart, fend, fmin; fmax; fmean; and fme, respectively. We obtained the derived parameters bandwidth (difference between fmax and fmin); sumentire' (frequency change from the start to the end); and meanentire' (average frequency slope of the element expressed in kHz/ms). The unit of all temporal measurements was seconds, and the unit of all frequency measurements was Hz.

We measured the duration of each element from start to end. Additionally, we measured the duration of the initial and terminal CF components, with a frequency change threshold of 2000 Hz.

Statistical analysis

We used two different datasets, one including *Pteronotus fulvus* and *Pteronotus davyi*, hereinafter referred as complex *fulvus*/*davyi* (containing 250 files and a total of 2330 echolocation calls) and one for *Pteronotus psilotis* and *Pteronotus personatus*, hereinafter referred as complex *psilotis/personatus* (containing 123 files and a total of 1040 echolocation calls). Each file summarized the mean values of the acoustic parameters for all the calls of the individual within that file. We only selected recording with calls undoubtedly belonging to the same individual, although the same individual may have been recorded in different files. Nonetheless, for the sake of simplicity we will refer to the data regarding the mean values of the acoustic parameters obtained in each file as an individual acoustic sample.

The following analyses were performed for each dataset. To reduce multicollinear variables in the analysis, the maximal information coefficient (MIC) was performed with the R package ‘minerva’ (Albanese *et al*., 2013). All variables with correlations >0.7 were discarded. The following parameters were kept for the analyses: call duration, duration of CF terminal component, fmin, fmax, fme, fmean, slope, fchange and bandwidth. Bandwith was only used for *fulvus*/*davyi* because bandwidth MIC for the other pair was above 0.7.

Hierarchical k-means clustering on principal components (HCPC)

To describe the global acoustic diversity, possibly overlooking differences between species groups, we first did a mixed Principal Component Analysis (PCA) as pre-processing step to the hierarchical clustering analysis (HCA). We included as active quantitative variables in the PCA: call duration, fmin, fmax, fme and fmean; as quantitative supplementary variables: duration of terminal CF component, slope, fchange and bandwidth; and as qualitative supplementary variables: locality and site. We then performed a Hierarchical k-means clustering on principal components (HCPC) considering the two main dimensions retained from PCA. HCPC delineates clusters of individuals (acoustic samples) with similar characteristics. For the hierarchical tree we used Ward's criterion and Euclidean distance and built it without any pre-specified number of clusters. Tree partitioning was consolidated by the centroid-based algorithm k-means partitioning. We successively combined the samples into clusters, minimizing the within-cluster variation and maximizing the between-cluster variation. HCPC was performed using the package FactoMineR (Lê, Josse & Husson, 2008).

Geographic variation

To assess if differences in the frequency parameters with a major contribution to cluster separation (fmean, fmin, fmax) were related to the geographic distance, we calculated a dissimilarity matrix of acoustic distances using frequency differences (in hertz) between localities and a geographic distance matrix using the physical distance (Haversine distances) between localities. Then, we compared the acoustic and geographic distance matrices using a Mantel test based on Pearson correlation with 9999 permutations, using the R packages vegan (Oksanen *et al.*, 2016) and geosphere (Hijmans, 2019). To test the association between frequency parameters and the geographic location we conducted linear regressions of fmean, fmin and fmax means against longitude and latitude of each locality. All analyses were conducted using R software, version 3.6.3 (R Foundation for Statistical Computing, 2016).

Results

Complex fulvus/davyi

The PCA was applied to a matrix of 250 acoustic samples characterized by 11 variables (Fig. S1). In the decomposition of the total inertia, the first two principal components (PC1 and PC2) accounted for 87.57% of the total data variance. Therefore, the variability of the data was well reflected in the first projection plane and was used to interpret the data for the next classification step. The main characteristics of this first dimension are summarized in Table 1. Note that the f.mean, the site Mexico, and the localities Metapan (El Salvador), Montecito (El Salvador) and PNLT (Honduras) are highly correlated with dimension 1 (respective correlation of 0.94, 0.98, 0.9, 0.95, 0.98).

Table 1. PCA results for the acoustic datasets of *fulvus*/*davyi* and *psilotis/personatus*.

Dataset	Principal component	Eigenvalue	Variance (%)	Cumulative (%)
fulvus/davyi	PC 1	3.482	69.649	69.649
	PC 2	0.896	17.925	87.574
psilotis/personatus	PC 1	3.370	67.397	67.397
	PC 2	0.922	18.441	85.838

Within this dimension, only samples from Mexico are clearly separated from those of other sites (Fig. S1A). Besides, there is no clear separation when the acoustic samples are classified accordingly to the actual species distribution (Fig. S1B).

The HCPC returned a set of three clusters, grouping samples broadly similar to each other. Each can be positioned within the cluster it belongs to, on a factor map to visualize individual positions in relation to Dim1 and Dim2 of the PCA (Fig. 3A). The cluster analysis was performed first according to variables and then according to individuals (acoustic samples). The main variables that better described the partitioning of the clusters were f.mean, f.min and f.max (Eta2= 0.73; 0.71; 0.69; $p<0.0001$) (Fig. 3B, Table S2).

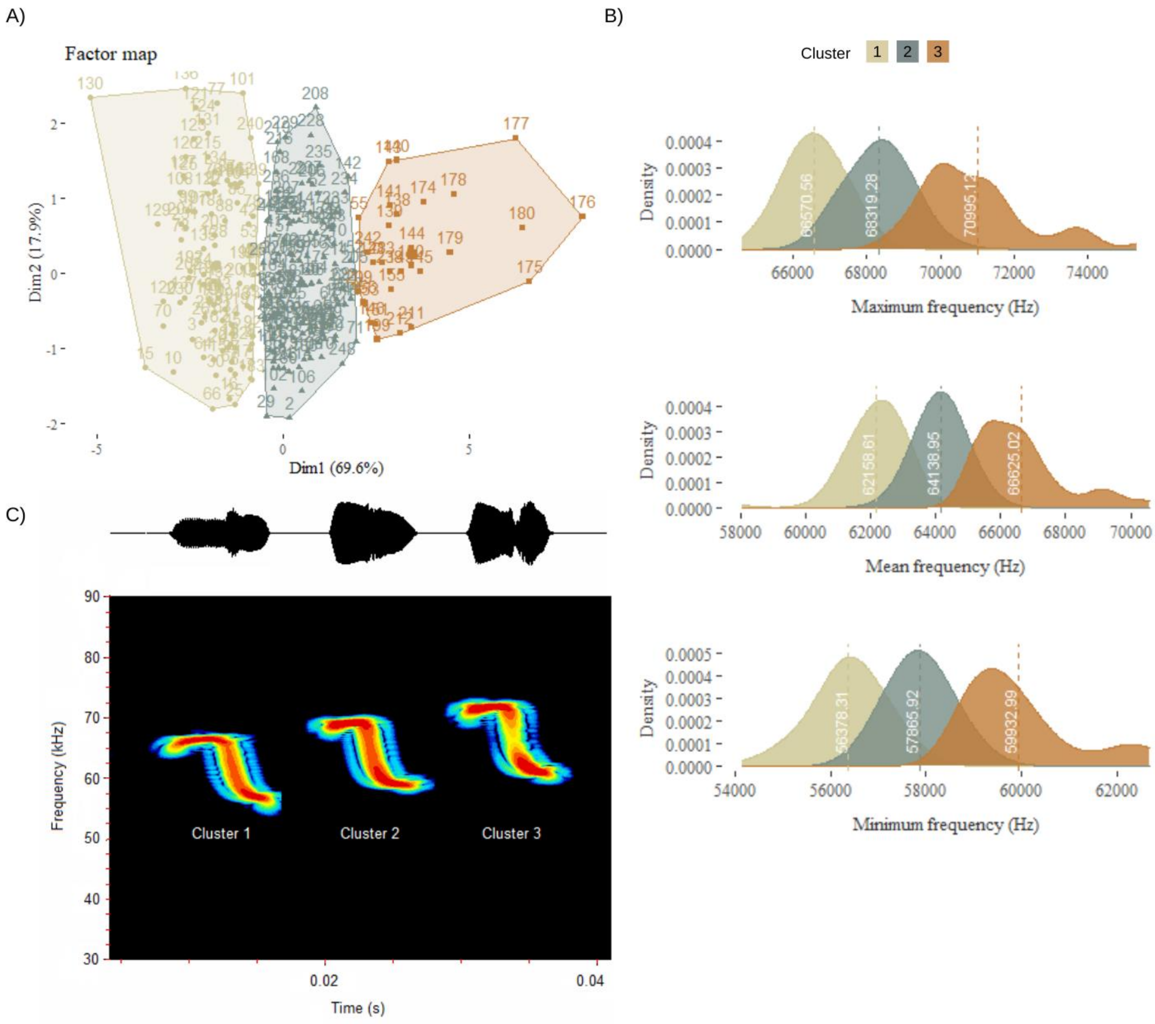

Figure 3. Characterization of the echolocation calls of the complex *fulvus/davyi* according to the: A) results of the hierarchical clustering on principal components; B) density plots of the main frequency variables characterizing the three phonic groups (Cluster 1-3); and C) spectrograms of representative echolocation calls (paragons) for each cluster of the complex *fulvus/davyi*.

The Cluster 1 is made of individuals sharing low values for the variables f.mean, f.min and f.max, meaning that calls within this cluster have significantly lower frequencies than the overall files. Besides, samples here have significant lower coordinate's values in dimension 1 than overall samples (Table S3). All samples from the localities PV_M3 (Costa Rica) and Catacamas (Honduras) belong to this cluster. And around 47.1% and 8.9% of samples from Costa Rica and El Salvador, respectively, belong to this cluster (Table S4).

The Cluster 2 is made of individuals sharing high values for the variable f.mean and low values for the variables call duration and duration of CF end section, meaning that acoustic samples within this cluster have significantly higher frequencies and shortest duration than the overall samples. Also, they show significant lower coordinate's values in dimension 1 and higher coordinate's values in dimension 2 than the overall samples (Table S3). All samples from El Tigre (El Salvador), 90.9 % of those from La Naturaleza (Honduras) and 89.7% of those from PV_M2 (Costa Rica) belong to this cluster (Table S4).

The Cluster 3 is characterized by high values for the variables f.max, f.min and f.mean, meaning that calls within this cluster have significantly higher frequencies. Besides, acoustic samples here have significant higher coordinate's values in dimension 1 (Table S3). All samples from Mexico and 26.7% of those from El Salvador belong to this cluster while 80.0% and 70.0% of samples from Cueva Viejo (Honduras) and Montecito (El Salvador) belong to this cluster (Table S4).

Clustering also involves the identification of paragons, which are the individuals whose coordinates are closest to the barycenter of each group. Accordingly, the profile of this sample best characterizes the cluster to which it belongs. The paragons are the acoustic samples 96 from Palo Verde, Costa Rica (Cluster 1), 217 from El Tigre, El Salvador (Cluster 2) and 144 from Cueva Viejo, Honduras (Cluster 3). Then their profile defines typical acoustic variables of each cluster (Fig. 3C). Samples most distant from other clusters are: 130 from Catacamas, Honduras (Cluster 1), 208 from Alegría, El Salvador (Cluster 2), and 176 from Santiago de Ixcuintla, Mexico (Cluster 3), representing the more specific calls in each cluster.

Geographic variation

Mean, minimum and maximum frequency variation was significant and positively associated with geographic distances between localities (Mantel test for fmean: $r= 0.68$, $p<0.05$; fmin: $r= 0.72$, $p<0.01$; fmax: $r=0.70$, $p<0.01$; Fig. 4), meaning that the more distant the populations, the more distinct the frequencies. Regression analyses showed significant correlations between all the frequency parameters with longitude (fmean: $r^2=0.53$; fmin: $r^2=0.57$; fmax: $r^2=0.57$; $p<0.001$) and latitude (fmean: $r^2=0.34$; fmin: $r^2=0.43$; fmax: $r^2=0.45$; $p<0.01$), exhibiting a tendency towards frequency increase westward and northward (Fig. 5, Fig. S2). Nevertheless, when we excluded Mexico (statistically considered as an outlier) from the analysis, the significance of the test result is lost.

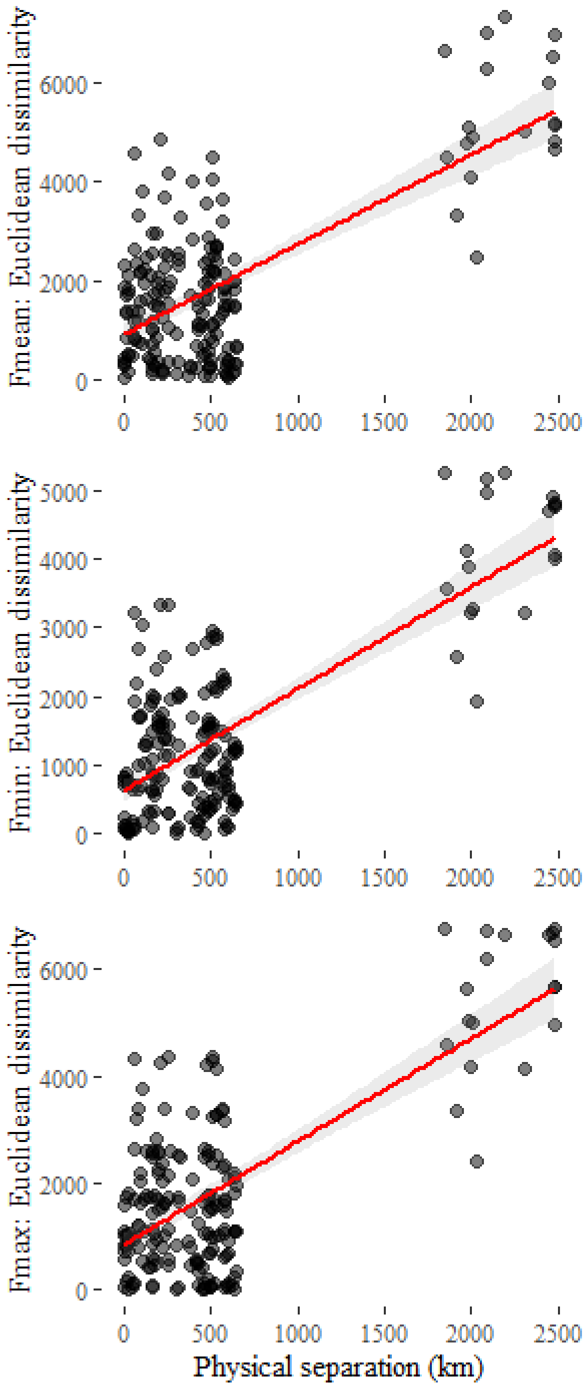

Figure 4. Relationships between frequency parameters for the echolocation calls of the complex *fulvus/davyi* and geographic distance.

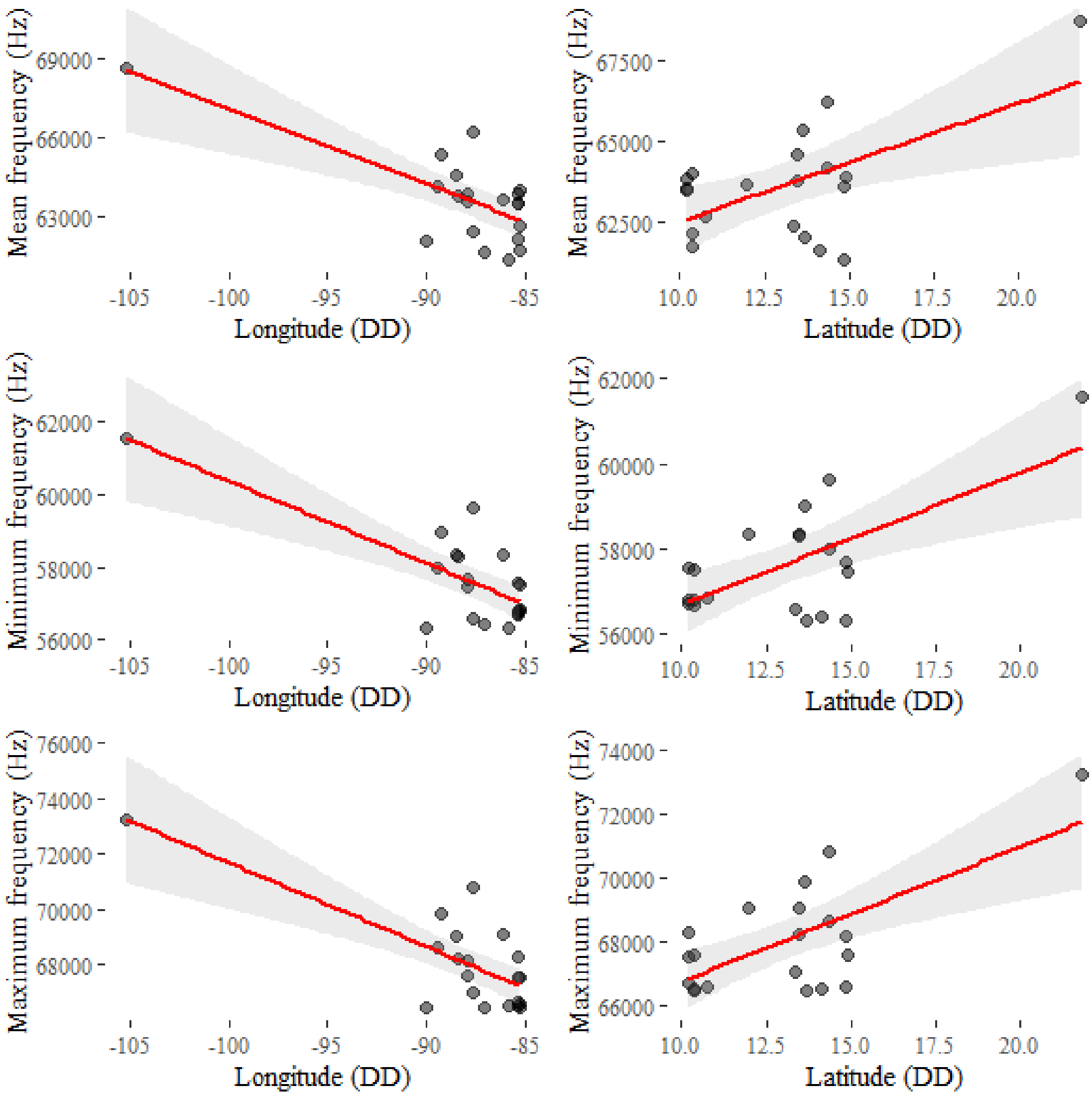

Figure 5. Relationships between frequency parameters for the echolocation calls of the complex *fulvus/davyi* with longitude and latitude.

Complex psilotis/personatus

The PCA was conducted on a matrix of 123 acoustic samples characterized by 10 variables (Fig. S3). In the decomposition of the total inertia, the first two principal components (PC1 and PC2) accounted for 85.84% of the total data variance. Therefore, the variability of the data was well reflected in the first projection plane, and was used to interpret the data for the next classification step. The main characteristics of this first plane are summarized in Table 1. Note that the f.mean, the sites El Salvador and Honduras, and the locality El Flor (El Salvador) are highly correlated with dimension 1 (>0.90). Within this dimension, there was no clear separation of individuals (acoustic samples) according to site or species (Fig. S3A and B).

The HCPC returned a set of three clusters, grouping acoustic samples that are broadly similar to each other. Each sample can be positioned within the cluster it belongs to, on a factor map to visualize individual positions in relation to Dim1 and Dim2 of the PCA (Fig. 6A). The cluster analysis was performed first according to variables and then according to individuals (acoustic samples). The main variables that better describe the partitioning of the clusters are f.mean, f.max and f.min (Eta2= 0.80; 0.73; 0.71; $p<0.0001$) (Fig. 6B, Table S5).

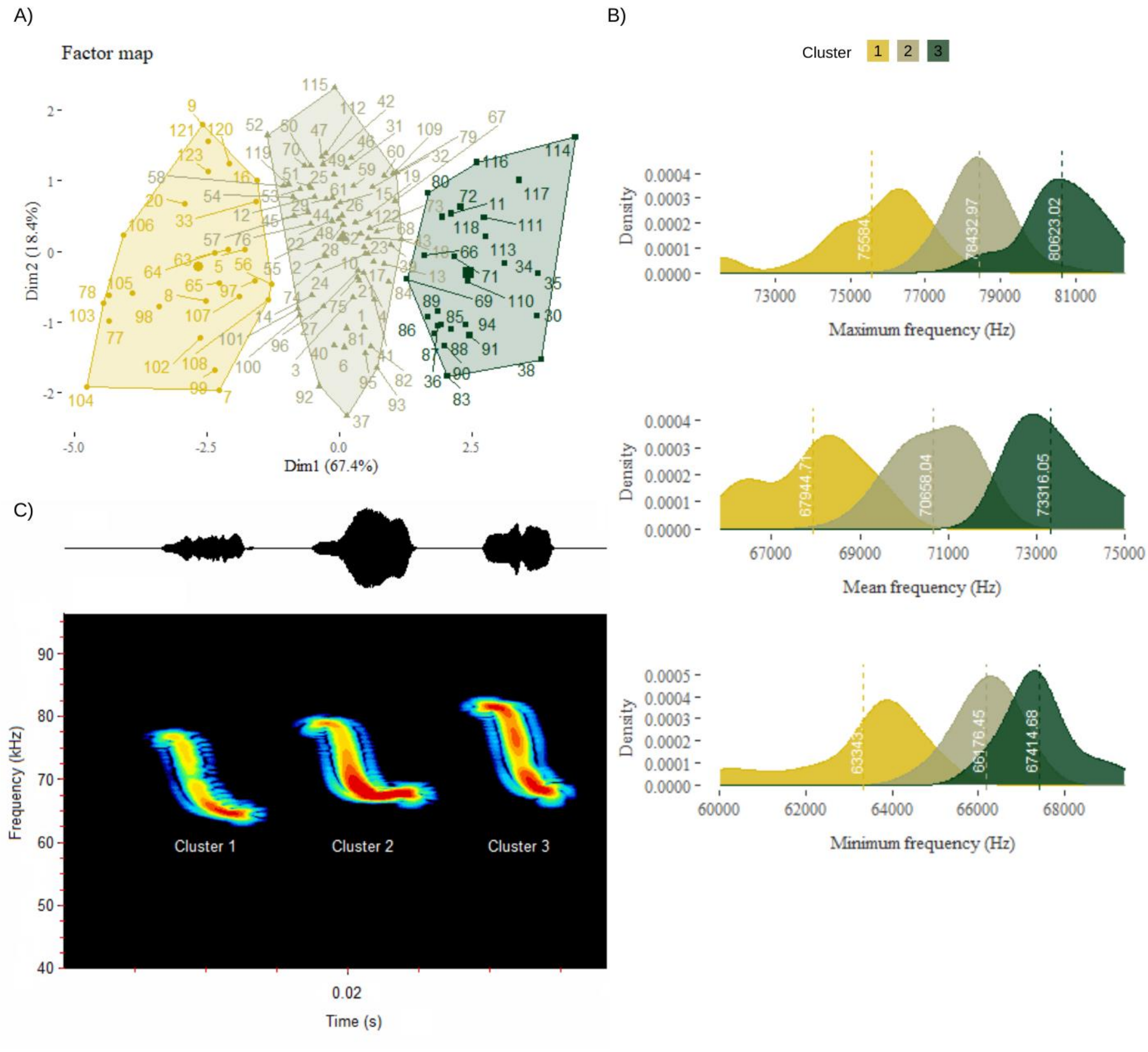

Figure 6. Characterization of the echolocation calls of the complex *psilotis/personatus* according to the: A) results of the hierarchical clustering on principal components; B) density plots of the main frequency variables characterizing the three phonic groups (Cluster 1-3); and C) spectrograms of representative echolocation calls (paragons) for each cluster of the complex *psilotis/personatus*.

The Cluster 1 is composed by individuals sharing low values for the variables f.min, f.max and f.mean, meaning that calls within this cluster have significantly lower frequencies (Table S6). Approximately 85.7% of samples from El Salvador including all of those El Flor and around 66.7% of those from Metapan belong to this cluster (Table S7).

The Cluster 2 composed by individuals sharing high values for the variable duration of the terminal CF component, and lower values for slope and f.change meaning that calls within this cluster have significantly longer CF component at the end of the call and more modulation (Table S6). Approximately 78.1% of samples from Costa Rica and 40.0% of those from Honduras are included in this cluster (Table S7).

The Cluster 3 is characterized by high values for the variables f.mean, f.max and f.me, meaning that calls within this cluster have significantly higher frequencies (Table S6). Approximately 52.5% of samples from Honduras, including 70.0% and 64.3% of those from Catacamas and Golfo de Fonseca, respectively, belong to this cluster. Also, 83.3% of samples from Palo Verde M2 are within this cluster (Table S7).

The paragons identified are the samples 64 from Amapala, Honduras (Cluster 1), 28 from Palo Verde M3, Costa Rica (Cluster 2) and 110 from Catacamas, Honduras (Cluster 3). Then their profile defines typical acoustic variables for each cluster (Fig. 6C). Samples most distant from other clusters are the files 110 from El Flor, El Salvador (Cluster 1), 115 from Catacamas, Honduras (Cluster 2), and 114 from Catacamas, Honduras (Cluster 3), representing the more specific calls in each cluster.

Geographic variation

The Mantel test did not show any significant association between the main frequency parameters with major contribution for the description of the clusters and the geographic distances. Regression analyses showed only a significant correlation between minimum frequency and longitude (r^2=0.27; $p<0.05$), exhibiting a tendency towards frequency increase eastward (Fig. 7, Fig. S4).

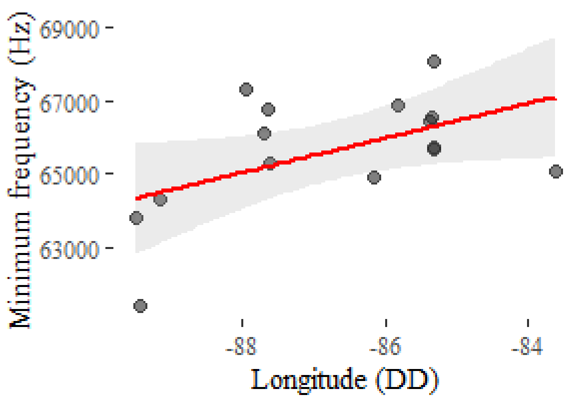

Figure 7. Relationship between minimum frequency of the echolocation calls of the complex *psilotis/personatus* with longitude.

Discussion

We provide the first insights into the acoustic variation of sister species *Pteronotus fulvus* x *P. davyi* and *P. psilotis* x *P. personatus* within their possible contact zones in Central America. Further, for the two species complexes we found evidence of sympatry for the three phonic groups within each complex, mainly separated by frequency parameters and without a clear correspondence with each of the species known or estimated distribution (Fig. S5-6). However, the frequency changes of the echolocation calls for both species complexes seem to follow a similar pattern to the species geographic variation in body size.

For the complex *fulvus/davyi* the three phonic groups were found to coexist in El Salvador, Honduras, Nicaragua and Costa Rica probably suggesting that the narrow intergradation zone proposed by Smith (1972) in the northern border of Nicaragua is actually wider and possibly those species, supposedly allopatric (Pavan, 2019), occur in sympatry at least in some parts of this region. Besides, the lack of a clear frequency separation between the species of the complex may reflect body size similarities in the Central American localities, as was noted by Smith (1972) for populations of *P. fulvus* (larger individuals from the southern part of their distribution) and *P. davyi*, (smaller individuals in the northern part of their distribution) in northern Nicaragua, and eastern Honduras and El Salvador.

Although, statistically the data from Mexico may be seen as an outlier, it seems to bring relevant biological information. There is an acoustic information gap between the Central American localities and Mexico that needs to be filled, but there is morphological evidence suggesting geographic variation along the sampled sites, so a similar acoustic trend is to be expected. Indeed, the geographic variation in the echolocation call parameters and the trend towards frequency increase at higher longitudes and latitudes mirrors the geographic variation in body size found by Smith (1972) and the frequency scaling with body size known to occur in bats' echolocation calls (Jones, 1999). Because of the physical properties of sound production, even small differences in body size can result in different frequencies (Lin *et al*., 2014). Recently, Méndez-Rodríguez *et al*. (2021) examined size variation and hybridization process between *P. fulvus* and *P. gymnonotus* from Mexico to Costa Rica and found individuals with intermediate forearm size between both species, corroborating the correlation with the latitudinal gradient. While Méndez-Rodríguez *et al*. (2021) identified the individuals from Nicaragua and Costa Rica as *P. fulvus*, including the intermediate forms based on nuclear genes and microsatellites, the geographic delimitation of the lineages *P. fulvus* and *P. davyi* identified by Pavan & Marroig (2016) and Clare *et al*. (2011) in Central America remains unclear. Further comprehensive sampling is required (individual capture and recording) to corroborate body size-frequency trends and to verify species correspondence with the phonic groups.

Similarly, the three phonic groups found for the complex *psilotis/personatus* did not follow the species distribution and occurred in sympatry in most of the sampling localities. The acoustic differences between the phonic groups and their occurrence in part of the intergradation zone proposed by Smith (1972) could also mirror the size overlap found by the author between the species in this area.

On the other hand, Central America is recognized as the origin of *P. personatus* (*sensu lato*), with a basal clade from Guatemala, and two diversification routes, one towards Mexico and another towards South America (Pavan & Marroig, 2017; Zárate-Martínez *et al*., 2018). Can the acoustic variation of the phonic groups and the trend of increasing frequency to the east reflect the distribution patterns of the species? A comprehensive sampling coupling bioacoustics and genetic evidence is crucial to check the correspondence between the phonic groups and the distinct lineages occurring in Central America.

For the two species complexes we found significant but slight acoustic variation (<6 kHz) between the sympatric phonic groups. Similarly, López-Baucells *et al*., (2017) found small differences in the frequency of maximum energy (FME) between sympatric populations of *Pteronotus rubiginosus* and *P. alitonus*, unlikely related to prey size detection or resource partitioning. Kingston *et al*. (2001) pointed out that acoustic divergences below 10 kHz in sympatric populations is not enough for significant resource partitioning and more likely a result of local adaptation and restrictive social interactions leading to selection for non-interference in acoustic signals between populations. Indeed, they suggest that ecological segregation may be achieved by differences in the use of microhabitat. Concerning *P. fulvus vs* P. *davyi* and *P. psilotis vs P. personatus* in Central America, morphological, molecular, dietary, and fine-scale habitat use analyses of individually captured, tagged, and recorded bats will undoubtedly be a valuable aim for future studies focusing on the resolution of the distribution dilemma raised here.

Future sampling locations in Central America should include the remnants of the Central American dry forest, an ecoregion listed as globally threatened (Janzen, 1988; Gillespie *et al*., 2000;) and areas of aerobic caves. Special attention should be paid to sampling in Nicaragua and Costa Rica, as they represent the major discrepancy zone of the distribution dilemma. We suggest as priority localities to be sampled the Areas of Importance for the Conservation of Bats - AICOMs (RELCOM) Masaya Volcano National Park and Barra Honda National Park, because they house large colonies of syntopic bats of the genus *Pteronotus* in their caves (Leiva, 2012; Girón-Galván LE, 2020; Medina *et al*. 2020). Finally, we would like to highlight the importance and the necessity of local bat acoustic libraries to support the acoustic identification of these and other species widely distributed across the Neotropical region.

Conclusion

This study examines the acoustic variation of the species-groups *P. fulvus* x *P. davyi* and *P. psilotis* x *P. personatus* within their possible contact zones in Central America. For both, our acoustic analysis revealed the existence of three phonic groups without a clear correspondence with the known distribution of the species but with a frequency variation that mirrors geographical variation in body size. The paucity of integrative information, including morphological, molecular and ecological analyzes, requires further study, especially in Nicaragua and Costa Rica, the apparent key point of the distribution dilemma. Until then, we recommend reporting the Fmean, Fmax, and Fmin of echolocation calls obtained from free-flying species recordings and keep them as species complexes in the lack of other integrative information.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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Supplementary materials: tables

Table S1. Acoustic sampling localities of *Pteronotus davyi*, *P. fulvus*, *P. psilotis* and *P.personatus* with the number of sequences and echolocation calls from each locality.

Site	Locality	Longitude	Latitude	Nº of sequences *P. fulvus/davyi*	Nº of calls *P. fulvus/davyi*	Nº of sequences *P. psilotis/personatus*	Nº of calls *P. psilotis/personatus*
Mexico	Santiago de Ixcuintla	-105.204	21.806	7	67	–	–
El Salvador	Barra de Santiago	-90.008	13.701	3	21	–	–
	Metapan	-89.504	14.369	7	70	6	51
	Zonte El Flor	-89.429	13.495	–	–	7	64
	Montecito	-89.285	13.653	10	91	–	–
	El Refugio	-89.165	14.288	–	–	1	10
	Alegría	-88.497	13.494	12	104	–	–
	El Tigre	-88.424	13.477	13	124	–	–
Honduras	Yojoa	-87.966	14.909	8	69	–	–
	La Naturaleza	-87.958	14.842	11	100	2	20
	Cueva del Viejo	-87.703	14.338	10	90	5	48
	Golfo de Fonseca	-87.645	13.356	5	34	14	129
	Amapala	-87.627	13.291	–	–	9	68
	Parque Nacional La Tigra	-87.142	14.147	5	27	–	–
	Catacamas	-85.839	14.827	11	99	10	86
Nicaragua	Volcán Masaya	-86.158	11.989	10	88	5	26
Costa Rica	Palo Verde_M3	-85.366	10.342	24	240	35	286
	Palo Verde_M2	-85.332	10.350	30	300	16	130
	Barra Honda_M1_int	-85.359	10.176	34	340	2	20
	Barra Honda_M2_tap	-85.356	10.176	30	300	–	–
	Barra Honda_M1_ph	-85.354	10.176	8	80	–	–
	Santa Rosa	-85.336	10.760	12	86	2	24
	Isla Violín	-83.624	8.792	–	–	9	78
			Total	250	2330	123	1040

Table S2. Correlation ratio between the cluster variable and the quantitative variables statistically significant (*p*<.0001). Acoustic dataset *fulvus*/*davyi*.

Variable	Eta2
f.mean	0.727
f.min	0.706
f.max	0.686
f.me	0.479
slope	0.283
call_dur	0.203
bandwidth	0.173
f.change	0.167
cf.end_dur	0.108

Table S3. Cluster description by the quantitative variables. Acoustic dataset *fulvus*/*davyi*.

Quantitative Var	Mean in category	SD in category	Overall mean	Overall SD	v.test	p.value
Cluster 1						
slope	-1.37	0.23	-1.51	0.24	7.06	0.000
call_dur	0.007	0.001	0.006	0.001	6.83	0.000
cf.end_dur	0.003	0.001	0.002	0.001	5.14	0.000
f.change	-9274.84	538.82	-9464.30	657.18	3.47	0.001
bandwidth	10192.25	501.70	10430.32	631.99	-4.54	0.000
f.me	60057.57	2021.25	62609.62	3387.76	-9.07	0.000
f.max	66570.56	764.65	67996.85	1655.82	-10.37	0.000
f.min	56378.31	710.26	57566.53	1323.65	-10.81	0.000
f.mean	62158.61	850.02	63708.51	1658.53	-11.25	0.000
Dim.1	-1.816	0.773	0.000	1.866	-11.716	0.000
Cluster 2						
f.mean	64138.95	716.58	63708.51	1658.53	4.19	0.000
f.min	57865.92	590.42	57566.53	1323.65	3.66	0.000
f.me	63300.49	2706.90	62609.62	3387.76	3.30	0.001
f.max	68319.28	837.05	67996.85	1655.82	3.15	0.002
slope	-1.55	0.17	-1.51	0.24	-2.68	0.007
cf.end_dur	0.002	0.001	0.002	0.001	-3.66	0.000
call_dur	0.006	0.001	0.006	0.001	-4.12	0.000
Dim.1	0.493	0.660	0.000	1.866	4.271	0.000
Dim.2	-0.138	0.856	0.000	0.947	-2.356	0.018
Cluster 3						
f.max	70995.12	1547.12	67996.85	1655.82	10.55	0.000
f.min	59932.99	1119.74	57566.53	1323.65	10.42	0.000
f.mean	66625.02	1361.17	63708.51	1658.53	10.25	0.000
f.me	67488.19	2457.53	62609.62	3387.76	8.39	0.000
bandwidth	11062.13	694.39	10430.32	631.99	5.83	0.000
cf.end_dur	0.002	0.0003	0.002	0.001	-2.00	0.045
call_dur	0.006	0.001	0.006	0.001	-3.81	0.000
f.change	-10163.61	658.37	-9464.30	657.18	-6.20	0.000
slope	-1.76	0.21	-1.51	0.24	-6.35	0.000
Dim.1	3.464	1.476	0.000	1.866	10.818	0.000

Table S4. Cluster description by the qualitative variables. Acoustic dataset *fulvus*/*davyi*.

Qualitative Var	Cla/Mod	Mod/Cla	Global	v.test	p.value
Cluster 1					
locality=PV_M3	100.00	26.09	9.6	6.92	0.000
locality=CA	100.00	11.96	4.4	4.39	0.000
site=CR	47.10	70.65	55.2	3.76	0.000
locality=PNLT	100.00	5.43	2	2.73	0.006
locality=BH_M2_tap	56.67	18.48	12	2.32	0.020
locality=LN	9.09	1.09	4.4	-1.96	0.050
locality=SA	0.00	0.00	2.8	-2.07	0.038
site=MX	0.00	0.00	2.8	-2.07	0.038
locality=Alegría	8.33	1.09	4.8	-2.13	0.033
locality=Montecito	0.00	0.00	4	-2.61	0.009
locality=CV	0.00	0.00	4	-2.61	0.009
locality=El Tigre	0.00	0.00	5.2	-3.07	0.002
locality=PV_M2	10.34	3.26	11.6	-3.30	0.001
site=ES	8.89	4.35	18	-4.56	0.000
Cluster 2					
locality=PV_M2	89.66	20.31	11.6	4.57	0.000
locality=El Tigre	100.00	10.16	5.2	3.84	0.000
locality=LN	90.91	7.81	4.4	2.71	0.007
locality=BH_M1_int	67.65	17.97	13.6	2.04	0.041
locality=YO	87.50	5.47	3.2	2.03	0.043
locality=PNLT	0.00	0.00	2	-2.22	0.027
locality=SA	0.00	0.00	2.8	-2.75	0.006
site=MX	0.00	0.00	2.8	-2.75	0.006
locality=CA	0.00	0.00	4.4	-3.62	0.000
locality=PV_M3	0.00	0.00	9.6	-5.74	0.000
Cluster3					
locality=SA	100.00	23.33	2.8	5.22	0.000
site=MX	100.00	23.33	2.8	5.22	0.000
locality=CV	80.00	26.67	4	4.97	0.000
locality=Montecito	70.00	23.33	4	4.29	0.000
site=ES	26.67	40.00	18	3.02	0.003
locality=Alegría	33.33	13.33	4.8	1.96	0.050
locality=PV_M3	0.00	0.00	9.6	-2.06	0.040
locality=PV_M2	0.00	0.00	11.6	-2.34	0.019
locality=BH_M2_tap	0.00	0.00	12	-2.39	0.017
site=CR	0.72	3.33	55.2	-6.40	0.000

Table S5. Correlation ratio between the cluster variable and the quantitative variables statistically significant (p<.0001). Acoustic dataset *psilotis/personatus*.

Variable	Eta2
f.mean	0.797
f.max	0.729
f.min	0.706
f.me	0.488
slope	0.301
cf.end_dur	0.256
call_dur	0.204
f.change	0.163

Table S6. Cluster description by the quantitative variables. Acoustic dataset *psilotis/personatus*.
*SD: standard deviation

Quantitative var	Mean in category	SD* in category	Overall mean	Overall SD	v.test	p.value
Cluster 1						
slope	-1.86	0.28	-1.99	0.31	2.37	0.018
call_dur	0.006	0.001	0.006	0.001	2.22	0.027
f.me	64910.15	1693.14	67246.84	2426.53	-5.51	0.000
f.mean	67944.71	1106.40	70667.96	1974.56	-7.89	0.000
f.max	75584.33	1515.19	78311.56	1943.44	-8.02	0.000
f.min	63343.40	1413.05	65849.40	1651.46	-8.68	0.000
Dim.1	-2.68	1.02	-1.9E-14	1.84	-8.33	0.000
Cluster 2						
cf.end_dur	0.003	0.001	0.003	0.001	3.85	0.000
slope	-1.91	0.27	-1.99	0.31	3.10	0.002
f.change	-11816.14	753.68	-12003.52	936.23	2.54	0.011
f.min	66176.45	654.84	65849.40	1651.46	2.51	0.012
call_dur	0.006	0.001	0.006	0.001	2.27	0.023
Dim.2	0.20	0.92	-6.0E-15	0.96	2.62	0.009
Cluster 3						
f.mean	73316.05	815.68	70667.96	1974.56	7.86	0.000
f.max	80623.02	985.85	78311.56	1943.44	6.97	0.000
f.me	70027.73	2920.15	67246.84	2426.53	6.71	0.000
f.min	67414.68	784.32	65849.40	1651.46	5.55	0.000
f.change	-12715.79	888.80	-12003.52	936.23	-4.46	0.000
call_dur	0.01	0.00	0.01	0.00	-4.91	0.000
cf.end_dur	0.00	0.00	0.00	0.00	-5.56	0.000
slope	-2.31	0.22	-1.99	0.31	-6.04	0.000
Dim.1	2.44	0.78	-1.9E-14	1.84	7.78	0.000

Table S7. Cluster description by the qualitative variables. Acoustic dataset *psilotis/personatus*.

Qualitative Var	v.test	p.value	Cla/Mod	Mod/Cla	Global
Cluster 1					
site=ES	5.46	0.000	85.71	46.15	11.38
locality=El Flor	4.43	0.000	100.00	26.92	5.69
locality=Metapan	2.34	0.019	66.67	15.38	4.88
locality=Fonseca	-2.18	0.029	0.00	0.00	11.38
site=CR	-2.40	0.016	12.50	30.77	52.03
site=HN	-2.64	0.008	7.50	11.54	32.52
locality=PV_M3	-2.75	0.006	5.71	7.69	28.46
Cluster 2					
locality=PV_M3	5.58	0.000	94.29	47.14	28.46
site=CR	4.95	0.000	78.13	71.43	52.03
site=HN	-2.58	0.010	40.00	22.86	32.52
locality=El Flor	-3.07	0.002	0.00	0.00	5.69
site=ES	-3.35	0.001	14.29	2.86	11.38
Cluster 3					
site=HN	5.45	0.000	52.50	77.78	32.52
locality=Fonseca	3.57	0.000	64.29	33.33	11.38
locality=UNA	3.29	0.001	70.00	25.93	8.13
locality=PV_M2	3.10	0.002	83.33	18.52	4.88
site=ES	-2.24	0.025	0.00	0.00	11.38
site=CR	-3.49	0.000	9.38	22.22	52.03
locality=PV_M3	-4.17	0.000	0.00	0.00	28.46

Supplementary materials: figures

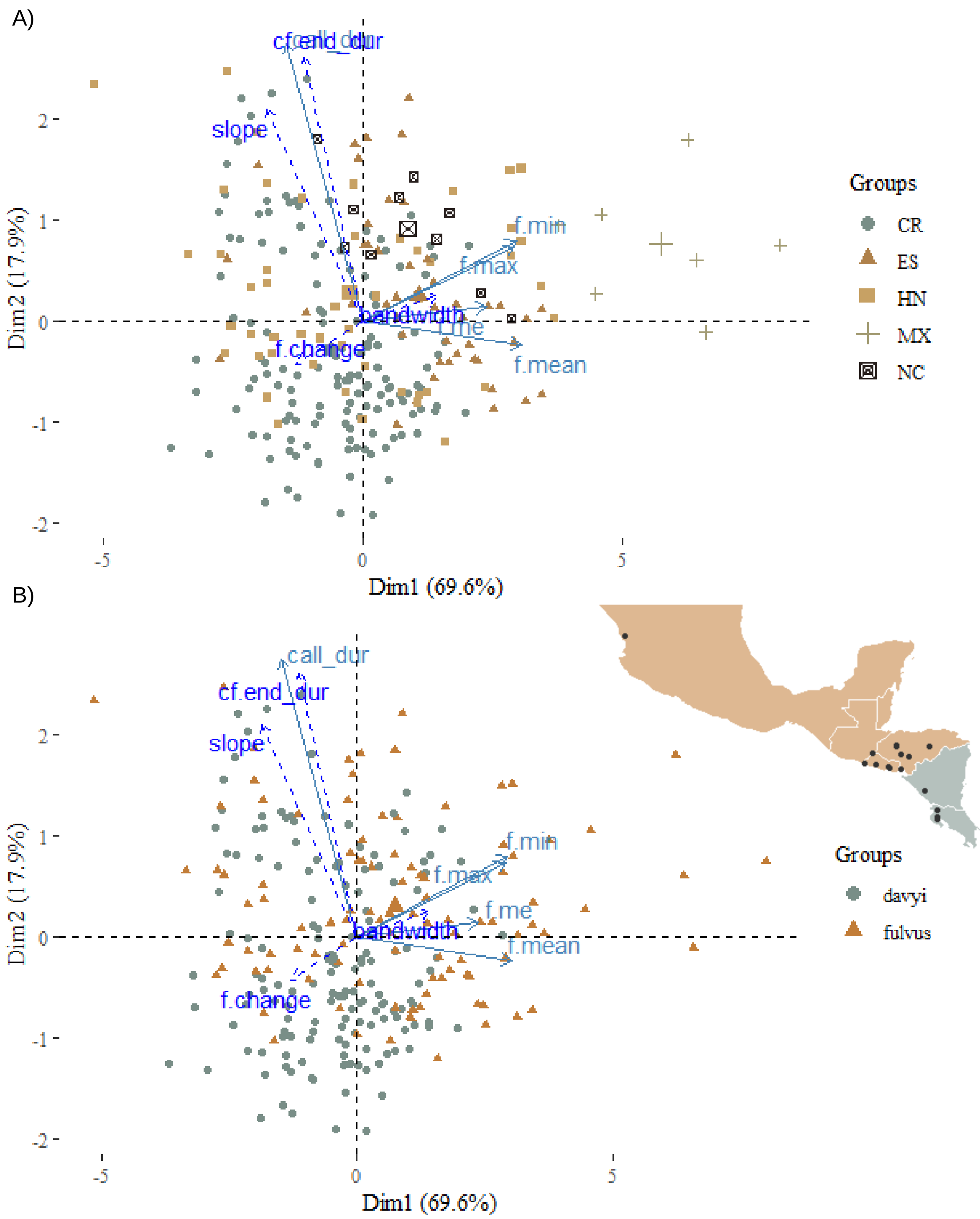

Figure S1. PCA - Biplot of the complex *fulvus*/*davyi* acoustic datasets showing the individuals (acoustic samples) colored by group: A) site and B) species, with a map of species distribution and sampling localities (black dots). Active variables in light blue: call duration (call_dur), minimum frequency (f.min), maximum frequency (f.max), mean frequency (f.mean), frequency of maximum energy (fme); and supplementary variables in navy blue: duration of CF component, slope, frequency change and bandwidth.

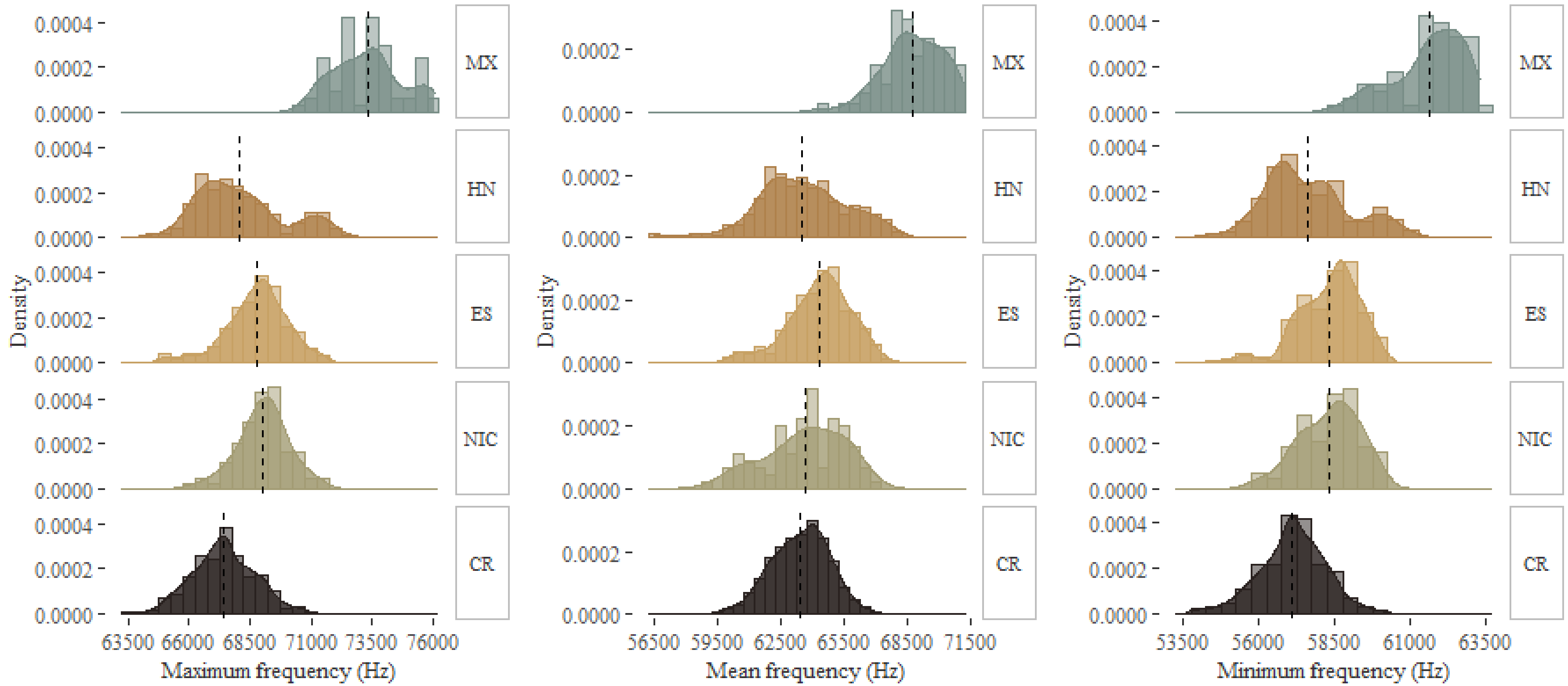

Figure S2. Histograms and density plots of main frequency parameters recorded for naked-backed bats (complex *fulvus*/*davyi*) from Mexico (MX), Honduras (HN), El Salvador (ES), Nicaragua (NIC), and Costa Rica (CR).Dotted lines indicate average frequency values per site.

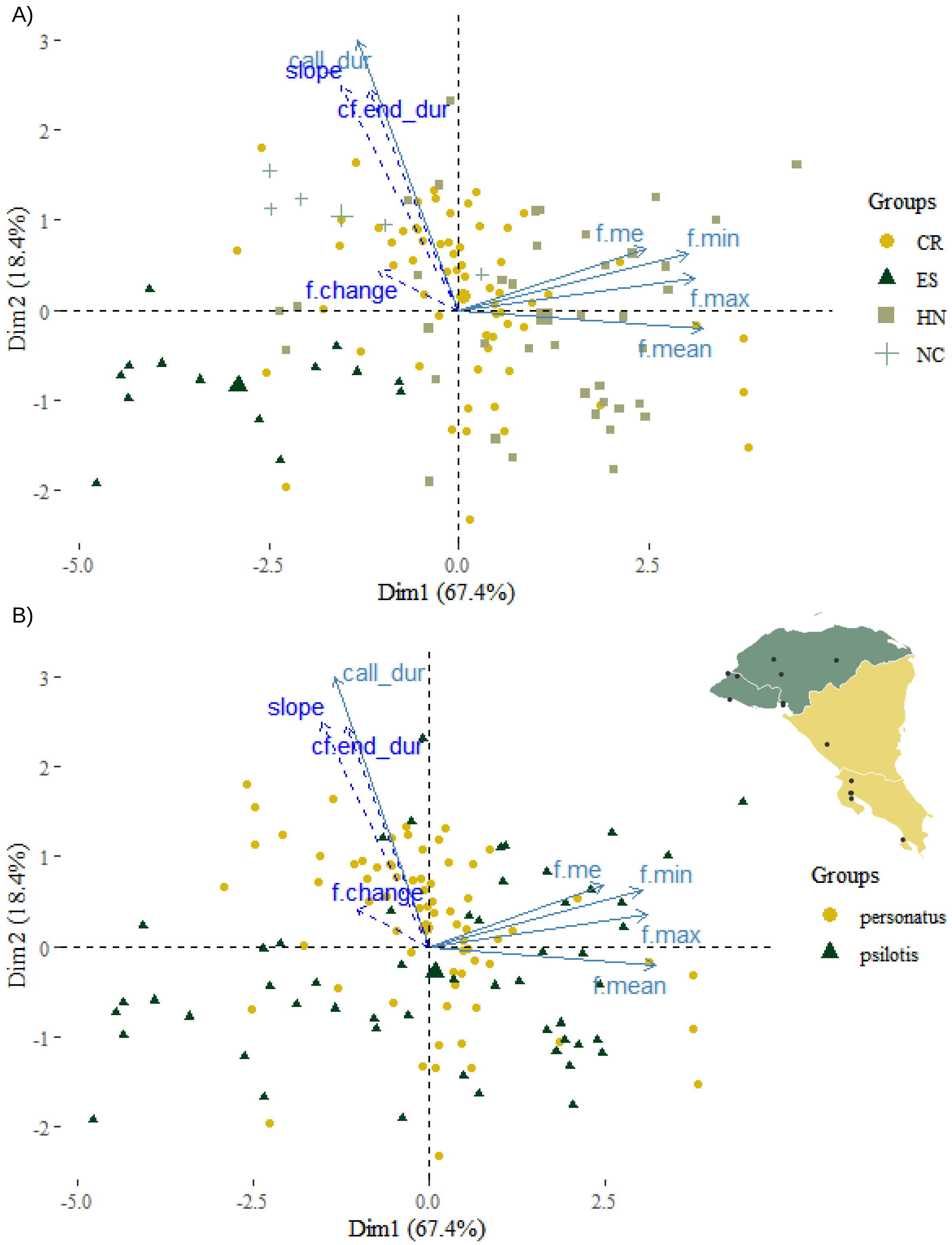

Figure S3. PCA - Biplot of the complex *psilotis/personatus* acoustic datasets showing the individuals (acoustic samples) colored by group: A) site and B) species, whit a map of species distribution and sampling localities (black dots). Active variables in light blue: call duration (call_dur), minimum frequency (f.min), maximum frequency (f.max), mean frequency (f.mean), frequency of maximum energy (fme); and supplementary variables in navy blue: duration of CF component, slope and frequency change.

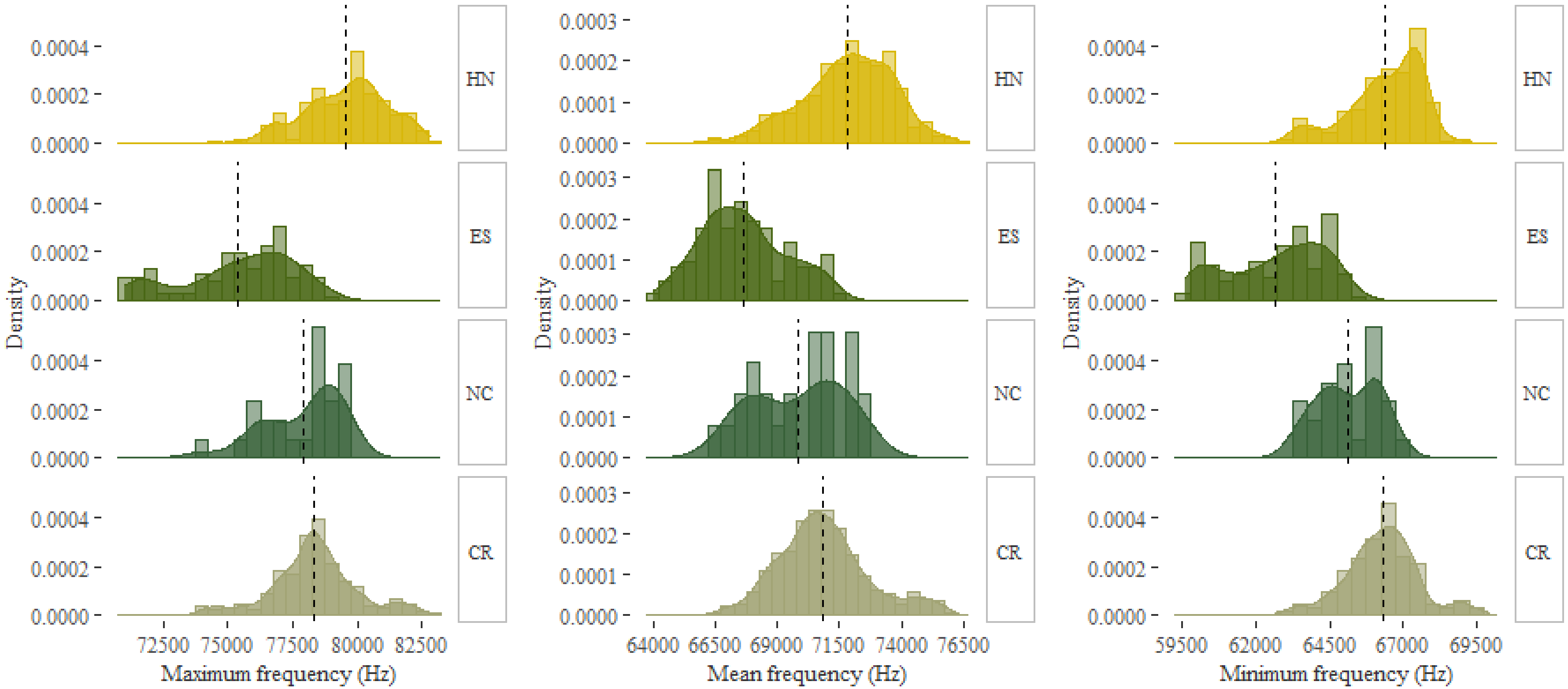

Figure S4. Histograms and density plots of main frequency parameters recorded for lesser mustached bats (complex *psilotis*/*personatus*) from Honduras (HN), El Salvador (ES), Nicaragua (NIC), and Costa Rica (CR). Dotted lines indicate average frequency values per site.

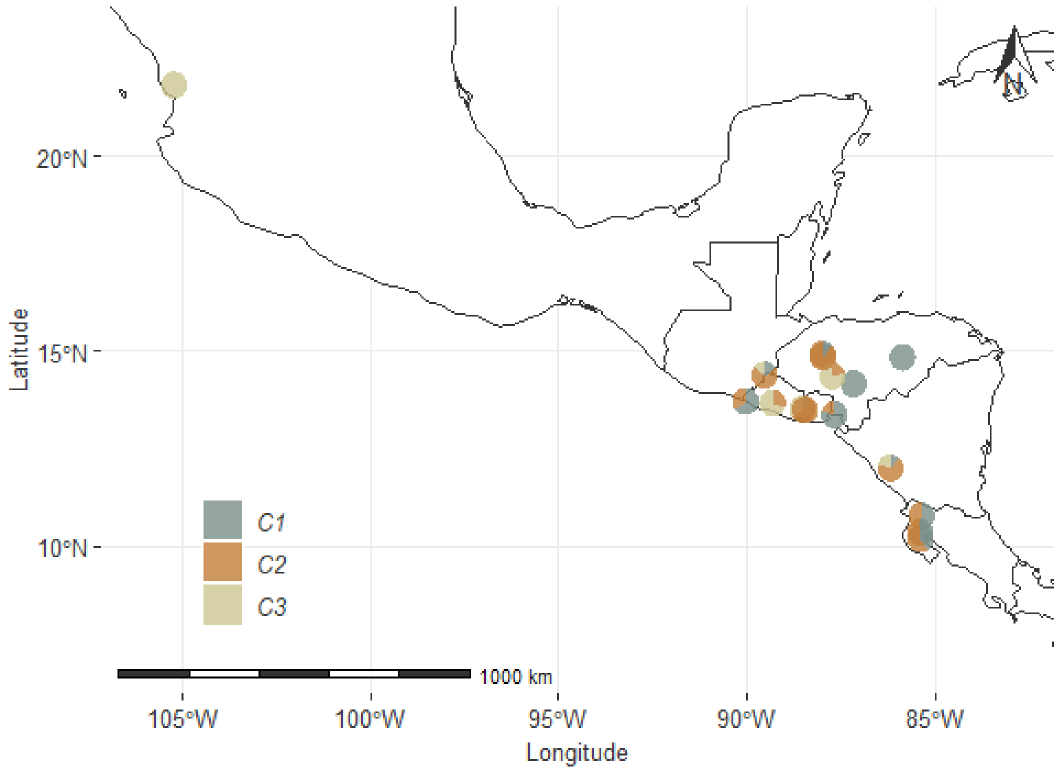

Figure S5. Map showing the localities of acoustic monitoring of the complex *fulvus/davyi* with per-locality pie-charts of the proportional number of acoustic samples of the three phonic groups (clusters: C1, C2, C3).

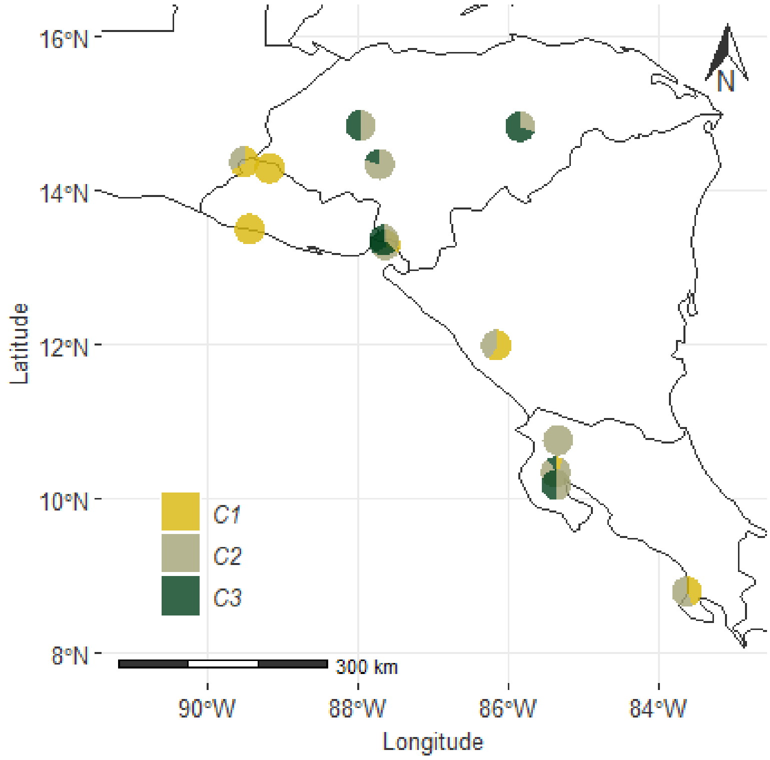

Figure S6. Map showing the localities of acoustic monitoring of the complex *psilotis/personatus* with per-locality pie-charts of the proportional number of acoustic samples of the three phonic groups (clusters: C1, C2, C3).

Capítulo IV

A ser submetido a: Bioacoustics - the International Journal of Animal Sound and its Recording

Tune in high: an ecoacoustics approach for comparing Neotropical bat communities

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Tune in high: an ecoacoustics approach for comparing Neotropical bat communities

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Abstract

Under a traditional bioacoustics approach and depending on the objectives of each study, the activity and identity of bat species are usually recorded. Ecoacoustics as a new analytical approximation to studying bat ecology and behaviour offers the possibility of investigate the relationship between bat sounds and the physical properties of the environment. Here, using the Acoustic Complexity Index – ACItf – we describe the spatiotemporal and spectral characteristics of distinct and highly diverse Neotropical bat communities within different habitat types and microclimates from two major Brazilian domains, the Cerrado and the Pantanal. We investigate how their acoustic information (from the first two hours after sunset) changes in response to air temperature, relative humidity, and habitat type. We found idiosyncratic spectral and temporal patterns among sites for both the Cerrado and the Pantanal and a significant and positive effect of temperature on the amount of acoustic information. Previous studies showed that temperature is a key factor for the foraging activity of insectivorous bats and has an important effect on echolocation call intensity through the effect of sound attenuation. Further studies are necessary to evaluate the effects of weather conditions and bat activity on the amount of information captured by acoustic indices.

Keywords: acoustic signature; bat activity; Cerrado; Pantanal

Introduction

The majority of the over 1400 species of bats (Chiroptera, Mammalia) present a variety of echolocation calls and echolocation behaviors. Monitoring and analyzing these sounds allows answering several ecological questions. In the traditional bioacoustics approach, bat activity is registered, and individual species may be identified depending on the knowledge accumulated thus far on species-specific acoustic parameters, and on the aims of each study. In the Neotropical region, and particularly in Brazil, several studies based on acoustic monitoring have been developed in the recent years, describing echolocation calls for taxonomic identification (Falcão et al. 2015, Arias-Aguilar et al. 2018) and investigating aerial insectivorous bat richness and distribution (Silva and Bernard 2017, Hintze et al. 2019, Hintze et al. 2021), habitat use (Marques et al. 2015; Appel et al. 2017, Torrent et al. 2018, Amaral et al. 2020), geographical variation (López-Baucells et al. 2018), and vocal plasticity (Oliveira et al. 2018).

Ecoacoustics goes beyond, by offering the possibility of studying, at various temporal and spatial scales, the relationship between biological sounds and the physical properties of the environment, like landscape composition and configuration, and climate (Farina et al. 2011, Farina 2014, Farina 2019). So, ecoacoustics provides new tools to study bat ecology and behavior by the means of the analysis of their sounds in specific environmental conditions.

The concept of acoustic communities, introduced by Farina and James (2016), can thus be applied for the study of bat ecology, understanding a bat acoustic community as a temporary aggregation of species producing sound that exchanges acoustic information in the (human) audible range (<20 kHz) and at higher (ultrasonic) frequencies (>20 kHz). An acoustic community varies in space and time (Farina and James 2016) and has a specific acoustic signature dependent on the species assemblage in a specific habitat (Bormpoudakis et al. 2013). An acoustic signature is the equivalent of a biological code (Barbieri 2015) and can be defined as a fingerprint resulting from the distribution of the frequency categories of the sounds emitted by the species of an acoustic community (Farina and James 2016). This fingerprint is species and community specific (Farina and Pieretti 2014a; Malavasi et al. 2014).

Here, our main objective is to explore the use of the ecoacoustics approach to describe the spatiotemporal and spectral characteristics, i.e., the acoustic signature, of distinct and highly diverse Neotropical bat communities. Specifically, by using the Acoustic Complexity Index – ACItf –, we aim to describe the acoustic patterns that emerge during the first hours of activity of bat communities within different habitat types and microclimates from two major Brazilian domains, the Cerrado – the world's most diverse savanna –, and the Pantanal – the world's largest alluvial floodplain. We hypothesize that acoustic complexity/information varies with the microclimatic conditions and also between habitats, and we predict an increase in acoustic information with increasing temperature and relative humidity, as well as highest values of complexity within the more heterogeneous habitats.

Materials and methods

Study area

The Brazilian Cerrado: the world's most diverse savanna

The Cerrado is one of the world's biodiversity hotspots (Myers et al. 2000) and the second-largest Brazilian domain with an extension of 2.000.000 km² (MapBiomas 2020). It is composed of a mosaic of vegetation forming a structural gradient from grasslands to forested habitats (Oliveira and Ratter 2002, Silva and Bates 2002, Ribeiro and Walter 2008). It is characterized by wet tropical weather (Aw type) (Alvarez et al. 2013) with a savanna subtype, with dry winter and maximum rain during the summer (Macena et al. 2008). In general, the region presents well-defined rainy and dry seasons. A high diversity of bats is found within this biome with at least 118 species of the181 species known to occur in Brazil (Aguiar and Zorteá 2008, Aguiar et al. 2016, Garbino et al. 2020).

The Brazilian Pantanal: the world's largest alluvial floodplain

The Pantanal is the largest alluvial plain on Earth, occupying 150.000 km² (MapBiomas 2020). It is characterized by a mosaic of vegetation influenced by other phytogeographic domains like the Cerrado, Chaco, Amazon, and Atlantic Forest. The Pantanal is characterized by wet tropical weather (Aw type) with dry winters and rainy summers (Hasenack et al. 2003). It shows a pronounced seasonality with the alternation of inundation and severe drought (Mittermeier et al. 2003, Nunes da Cunha and Junk 2004). At least 65 species of bats occur in this domain (Fischer et al. 2018).

Sampling localities

We acoustically sampled eleven sites in the Cerrado, within the municipality of Mambaí, Goiás (14°29′46″S 46°06′22″W), and eleven sites in the Pantanal, within the municipality of Barão de Melgaço, Mato Grosso (16°54'30.8"S 55°53'47.0"W) (Fig. 1A). The acoustic monitoring was done during the dry season for eleven days in July 2015 in the Cerrado, and for eleven days between September and October 2015 in the Pantanal. We selected the sites as to represent the widest diversity of vegetation cover in each region. The recordings were collected using two Pettersson Bat Detector D500x. We fixed the recorders at ~2 m height on a tree trunk, pointing the microphone to the above open space at an angle of 45°. We programmed the recorders to operate at a sampling rate of 384 kHz and a resolution of 16 bits, producing files of 5 s each 15 s from sunset to midnight.

The acoustic material used for this study was previously collected within a wider bat bioacoustics project, where we identified bat recordings to the lowest taxonomic level possible (Tab. S1). For this study, we used all recordings (with and without bats) from the first two hours after sunset. This period accounts for the highest nocturnal activity peak of many insectivorous bats in the Neotropic (Bernard 2002).

Habitat characterization

We classified the habitat type around each bat detector according to the dominant vegetation cover. For the Cerrado we identified the following classes: i) savanna: well-preserved savanna-like formations (e.g. Cerrado *sensu stricto*); ii) modified savanna: savannas where the original vegetation cover has been changed due to agricultural or cattle-raising related practices; and iii) forest: including gallery forest and seasonal semideciduous forest (Fig. 1B i-iii). For the Pantanal we identified the following classes: i) river: sampling sites at the edge of rivers and within riparian vegetation; ii) cambarazal: dense formations of the tree *Vochysia divergens* (cambará); and field of murundus: open-like earthmound grasslands, locally known as 'campos de murundus'; these are small circular elevations, approximately one meter high and four to six meters in diameter, possibly representing incipient dunes, usually occurring at sites with the presence of water in the soil (Fig. 1B iv-vi).

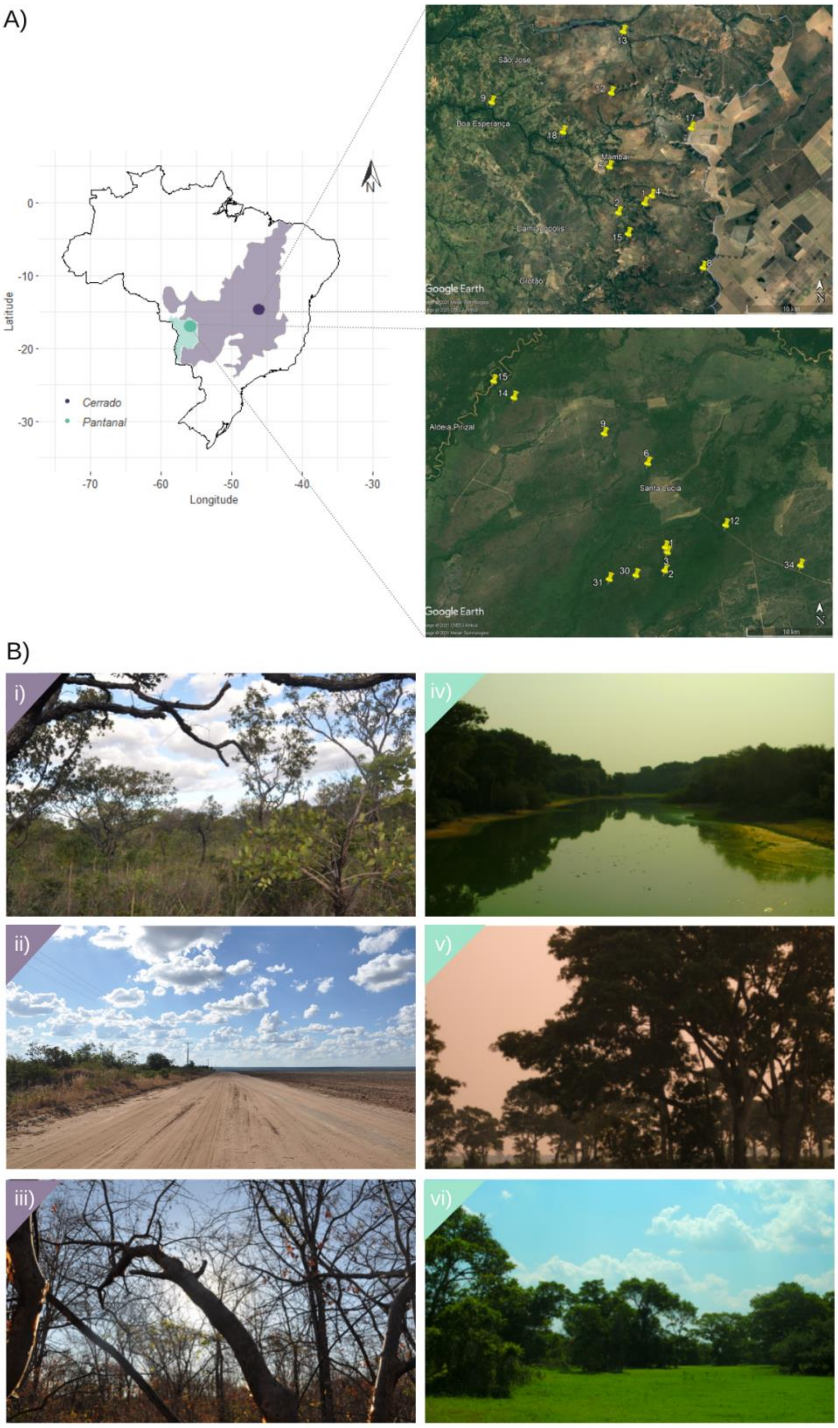

Figure 1. A) Location of the sampling sites (yellow pins) in the Brazilian Cerrado (light purple) and Pantanal (light green). B) Overall aspect of the habitat classes samples: in the Cerrado i) savanna, ii) modified savanna, iii) forest (gallery forest); in the Pantanal iv) river, v) cambarazal, vi) field of murundus.

Sound analyses

The Acoustic Complexity Index – ACI – is used to calculate the amount of acoustic information or complexity present in an acoustic file and is formed by two indices ACItf and ACIft (Pieretti et al. 2011, Farina et al. 2016, 2018). ACItf measures the acoustic information in each frequency band across a temporal aggregation interval, while ACIft measures the acoustic information in each temporal frame across the frequency bands (Farina et al. 2021). We used ACItf to investigate the temporal and spectral characteristics of bat acoustic activities. We processed a total of 3863 (561 with bats) acoustic files for the Cerrado and 3491 (1381 with bats) acoustic files for the Pantanal. The ACItf values were obtained by processing the recordings (WAV files) using SoundscapeMeter (Farina et al. 2012), a plug-in application to the WaveSurfer software (Sjölander and Beskow 2000, Sjölander 2002). We adopted a filter of 5000mV^2/Hz to eliminate most of the background noise across the entire spectrogram. To calculate the ACI, we set the following parameters: FFT 512 points, Hamming window, lowest frequency 12 kHz, highest frequency 120 kHz, and clumping of 1 s. The successive computation produced 184 frequency bins of 585.9375 Hz and 2815 elements of 0.021337 s. The selected frequency range (from 12 to 120 kHz) is intended to include the vast majority of the echolocation calls emitted by the aerial insectivorous bats known to occur in the sampled areas.

Microclimate data

We obtained air temperature and relative humidity information for the 22 sampled locations using a datalogger coupled to the bat detector. Data were taken every minute, concurrently with the acoustic sampling period.

Statistical analysis

To tested if the mean acoustic information varies between habitat types within each domain, we applied a one-way ANOVA, using ACItf aggregated by the total sampling period length (two hours) per site as response variable, and habitat type as factor, followed by a Tukey's post-hoc test (Dytham 1999).

To investigate, at a finer temporal resolution, how acoustic information (acoustic complexity) of bat communities changes in response to air temperature, relative humidity, and habitat type we ran linear mixed models assuming a normal distribution, with the average of ACItf per minute for each locality as the response variable. We fit LMM using the Restricted Maximum Likelihood (REML) with habitat type and domain (Cerrado/Pantanal) as factors, air temperature and the percentage of relative humidity per minute (previously scaled) as predictors, and site as random effect. For this we used the lmer function from the lme4 R package (Bates et al. 2015; R Core Team 2020).

We evaluated the performance of the models using the Akaike's Information Criterion (AIC), considering as equally well-adjusted models with $\Delta AIC<2$. For all the models, we evaluated the assumptions using validation graphs, including residuals *vs* fitted values and Q–Q plots.

Results

General patterns of bat acoustic communities

The sampled areas in the Pantanal (all localities plotted together) averaged the highest ACItf values. Most of the acoustic information/complexity in Pantanal was registered below 25 kHz, with some peaks around 32, 42, and 75 kHz (Fig. 2A); the ACItf presented high values mostly during the first hour after sunset (Fig. 2C). The average ACItf values in the Cerrado (all localities plotted together) slowly decreased from 12 to 40 kHz with a slight peak around 42 kHz (Fig. 2A); the ACItf showed higher values during the first ten minutes of recording, remaining quite stable during the following sampled period (Fig. 2C).

For both the Cerrado and the Pantanal, the spectral (acoustic signature) and temporal patterns were idiosyncratic among sites (Fig. S1-2).

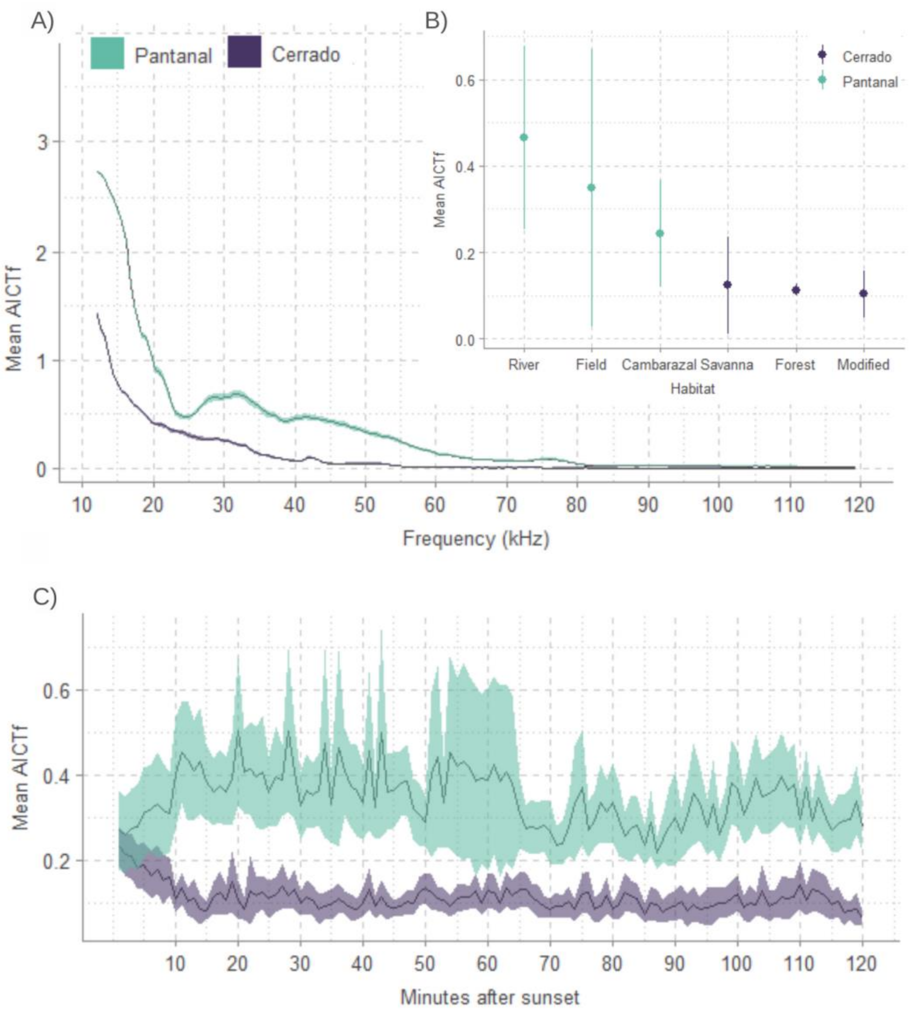

Figure 2. Spectral and temporal characterization of bat acoustic communities sampled in the Cerrado (purple) and the Pantanal (green): A) mean frequency distribution of the ACItf with all sites plotted together in each domain; B) mean (circle) and 95% confidence intervals (whiskers) for the ACItf in each sampled habitat class; C) temporal trend of acoustic complexity for the Cerrado and the Pantanal – lines represent the mean ACItf and the shaded area the 95% confidence interval resulting from the sampling of the first two hours after sunset at the eleven recording sites in each domain.

Acoustic information variability

When aggregating ACItf for the whole of the 2-hour sampling period we found no significant differences between habitat classes for either the Cerrado or the Pantanal (Fig. 2B, S3).

Among the set of possible models describing the relationship between the amount of acoustic information, habitat type, and microclimate (Tab. S2), the best adjusted, carrying 96% of the cumulative model weight, included only ambient temperature as predictor (Tab. 1), with acoustic information (ACItf /minute values) linearly increasing with rising temperatures (Fig. 3).

Table 1. Results of the linear mixed model on mean ACItf per minute, including sampling site as random effect

		Variance	Std. Dev.	N° obs.	Groups	
Random effects	(Intercept)	0.029	0.170	2544	22	
	Residual	0.0407	0.202			
		Estimate	StD. Error	df	T value	Pr (>|t|)
Fixed effects	Intercept	0.235	0.037	20.788	6.418	0.001
	Temperature	0.096	0.018	374.113	5.409	0.001

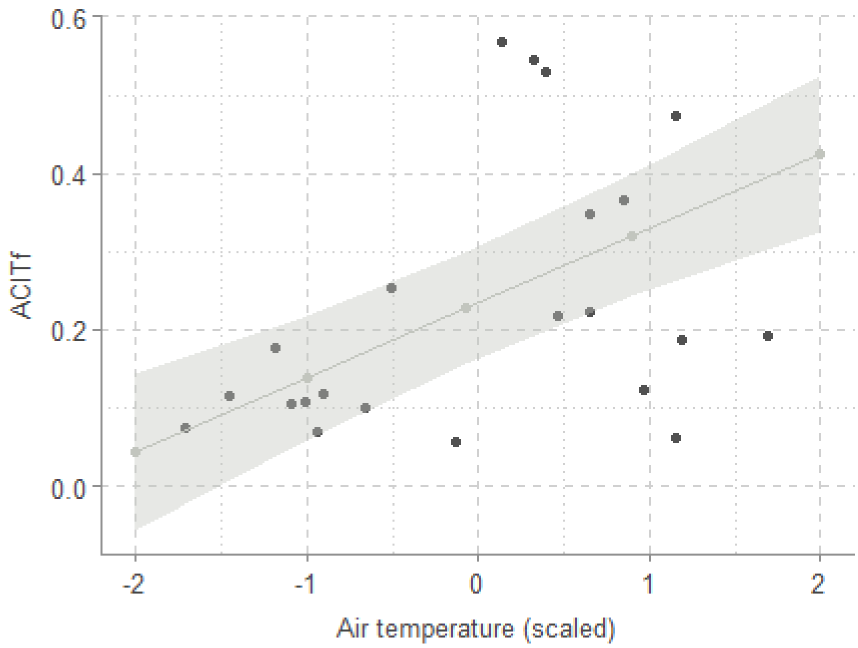

Figure 3. Predicted values for the Acoustic Complexity Index/minute for temperature according to the linear mixed model. Dots represent the mean observed values for each site. The line represents the fitted model and the gray shadow represents the 95% model confidence interval.

Discussion

Here, we describe for the first time the bat community acoustic temporal and spectral features in different habitat classes and microclimates for two of the major Brazilian domains, the Cerrado and the Pantanal.

Even when we did not assess the species-specific acoustic signatures and ACI do not discriminate between species, it still allows the discrimination of sound into different frequency components (Farina et al. 2012, Farina and Pieretti 2014b). By knowing the acoustic characteristics, like the frequency of maximum energy (FME), of echolocation calls of the bat species occurring in the area, we may get a general notion of their contribution to the observed acoustic patterns.

For example, the acoustic signature when all the Pantanal sites are plotted together shows a peak that rises from 25 kHz to 38 kHz and may be influenced by the calls of species like *Molossus currentium* (FME between 30 and 35 kHz), *M. molossus* (FME between 34 and 45 kHz), *Promops nasutus* (FME ~35 kHz) and species of the *Eptesicus* (FME between 30 and 40 kHz). Between 50 and 60 kHz we find the harmonics with maximum energy for most of the species of the *Myotis*, *Molossops temminckii* (FME between 50 and 55 kHz), and the fishing bat, *Noctilio leporinus* (FME ~55 kHz). The shallow peak at 75 kHz matches with the FME of *Noctilio albiventris*. The general acoustic signature for the Cerrado sites when plotted together is subtle, but the shallow peak at ~42 kHz may include the *Peropteryx macrotis* and *P. trinitatis* with FME around 37-39 kHz and 42-44 kHz, respectively. In the two domains, ACIft values for frequencies below 18 kHz may correspond to the echolocation calls of several large-bodied aerial insectivores, specifically species of the Molossidae, including the genera *Eumops*, *Nyctinomops*, and *Cynomops* (Arias-Aguilar et al. 2018).

However, it is important to highlight that the ACI is obtained not only with the main acoustic frequency parameters used for taxonomic identification or activity measurements (following a bioacoustics approach), but it also takes into account the acoustic information that species jointly produce during their activity (Farina and Pieretti 2014b). Then, the ACI captures the acoustic information contained in different harmonics, and the echolocation sequences as whole for the species assemblage at a given time.

A more detailed analysis, when aggregating ACItf per minute, showed a significant and positive effect of temperature on the amount of acoustic information. Indeed, this is no surprise, as several studies have already shown that echolocation calls of bats vary with temperature over different temporal scales, like seasons or nights (Snell-Rood 2012, Chaverri and Quiros 2017), and that temperature is key for the foraging activity of insectivorous bats in temperate (e.g. Hayes 1997, Vaughan et al. 1997, Ciechanowski et al. 2007, Müller et al. 2012, Bender and Hartman 2015) and tropical assemblages (Meyer et al. 2004, Appel et al. 2019, Arias-Aguilar et al. 2019).

Moreover, changes in weather conditions might lead to changes in call intensity emitted by bats to improve the detectability of their sounds and the corresponding echoes in response to increasing sound attenuation (Surlykke and Kalko 2008 Hage et al. 2013, Luo et al. 2015, Lu et al. 2020). This reduction in sound amplitude (atmospheric attenuation) as the sound travels through the air is determined mainly by the frequency of the sound, besides air temperature, relative humidity, and pressure (Attenborough 2007), and involves a complex non-linear relationship (Griffin, 1971; Attenborough 2007, Stilz and Schnitzler 2012, Luo et al. 2014, Goertlitz 2018).

Still, further studies are necessary to evaluate the effects of bat call intensity and modulation, bat acoustic activity and species richness, as well the effects of weather conditions on the amount of information captured by acoustic indices.

Caveats and potential future application of ecoacoustics

The most significant limitations of our study were the number of sampling sites per habitat type and the 2-hour sampling period that are certainly insufficient at capturing the whole bat species assemblage and the temporal acoustic variation present in each study site and habitat type.

Further analyses based on an ecoacoustics sampling scheme (Pieretti et al. 2015) and analytical guidelines (Bradfer-Lawrence et al. 2019) considering the ecological characteristics of bats are hence required. Our work should thus be seen as a very first approach attempting to characterize bat communities and exploring the possibilities of ecoacoustics towards the study of tropical bat ecology through sound.

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Supplementary materials

Table S1. List of bat species and sonotypes recorded during the first two hours after sunset at different habitat types in the Brazilian Cerrado (Mambaí, GO) and Pantanal (Barão de Melgaço, MT). Cerrado habitat types: S) savanna, M) modified savanna, and F) forest. Pantanal habitat types: R) river, C) cambarazal, and F) field (campos de murundu).

Family	Species	Cerrado	Pantanal
Emballonuridae	*Diclidurus ingens*	M	
	Peropteryx macrotis	S-F-M	
	Peropteryx trinitatis	S-F	
	Rhynchonycteris naso		R
	Saccopteryx bilineata		C-R
	Saccopteryx leptura	F	
Vespertilionidae	*Rhogeessa hussoni*	M	
	Eptesicus chiriquinus		C-R
	Eptesicus furinalis	S-F-M	C-F-R
	Myotis cf *simus*		C-F-R
	Myotis nigricans	S-F-M	
Molossidae	*Molossus currentium*		C-F-R
	Molossus molossus	S-F-M	C-F-R
	Molossus rufus		C-F-R
	Molossops temminckii	S-M	C-F-R
	Promops nasutus	S	R
	Tadarida brasiliensis		R
Mormoopidae	*Pteronotus gymnonotus*	S-F	R
	Pteronotus rubiginosus	S-F-M	
Noctilionidae	*Noctilio albiventris*		C-F-R
	Noctilio leporinus		C-R
Phyllostomidae	*Lonchorhina aurita*	F	
Sonotypes	*Eumops/Cynomops/Nyctinomops*	S-F-M	C-F-R
	Molossus spp	F	
	Myotis riparius/M. nigricans		C-F-R
	Phyllostomidae	S-M	

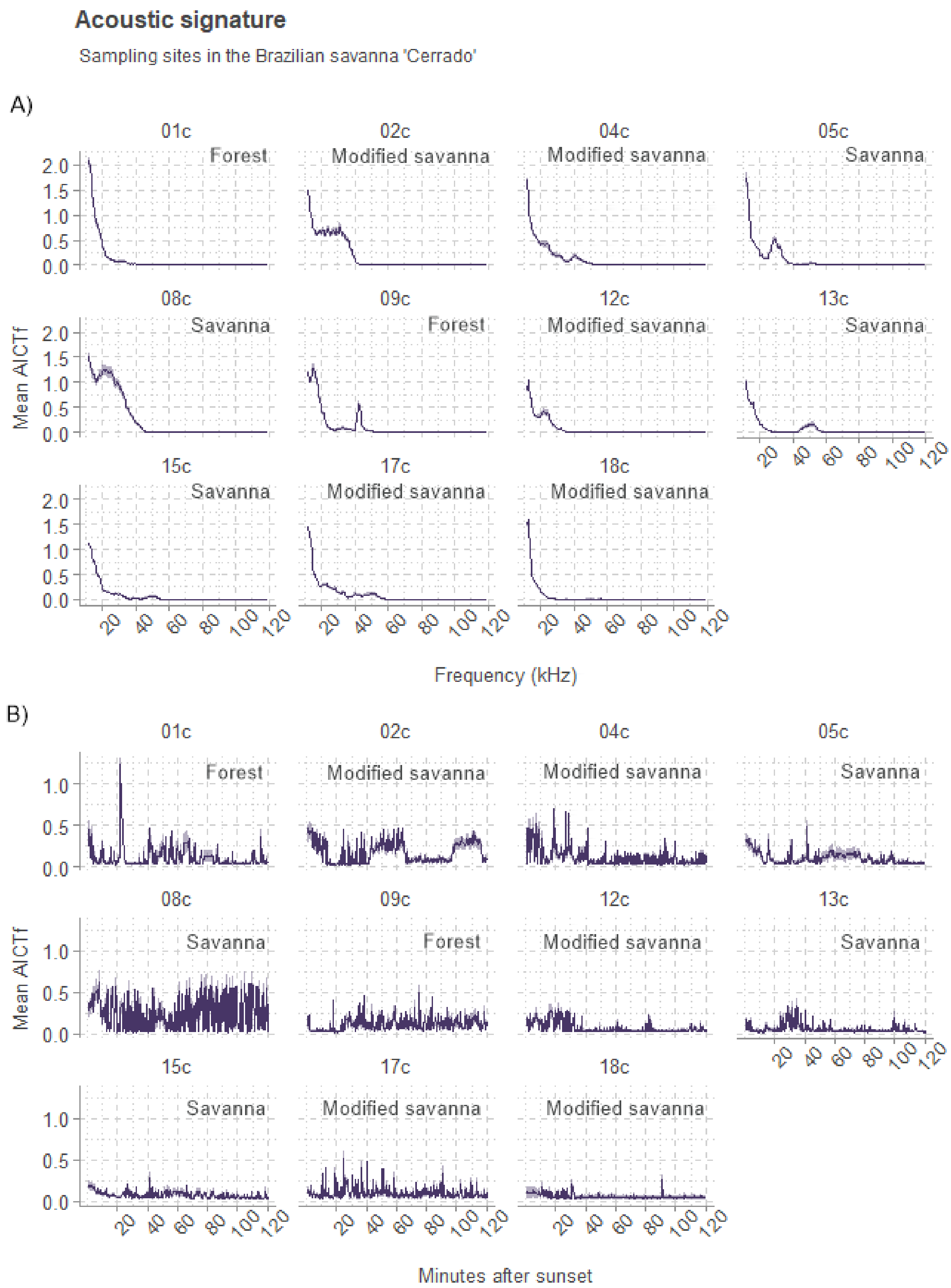


Figure S1. Spectral (A) and temporal (B) characterization of bat acoustic communities from the Cerrado. The purple line represents the mean pattern and the 95% confidence intervals (shaded area) resulting from sampling on the first two hours after sunser at each recording site.

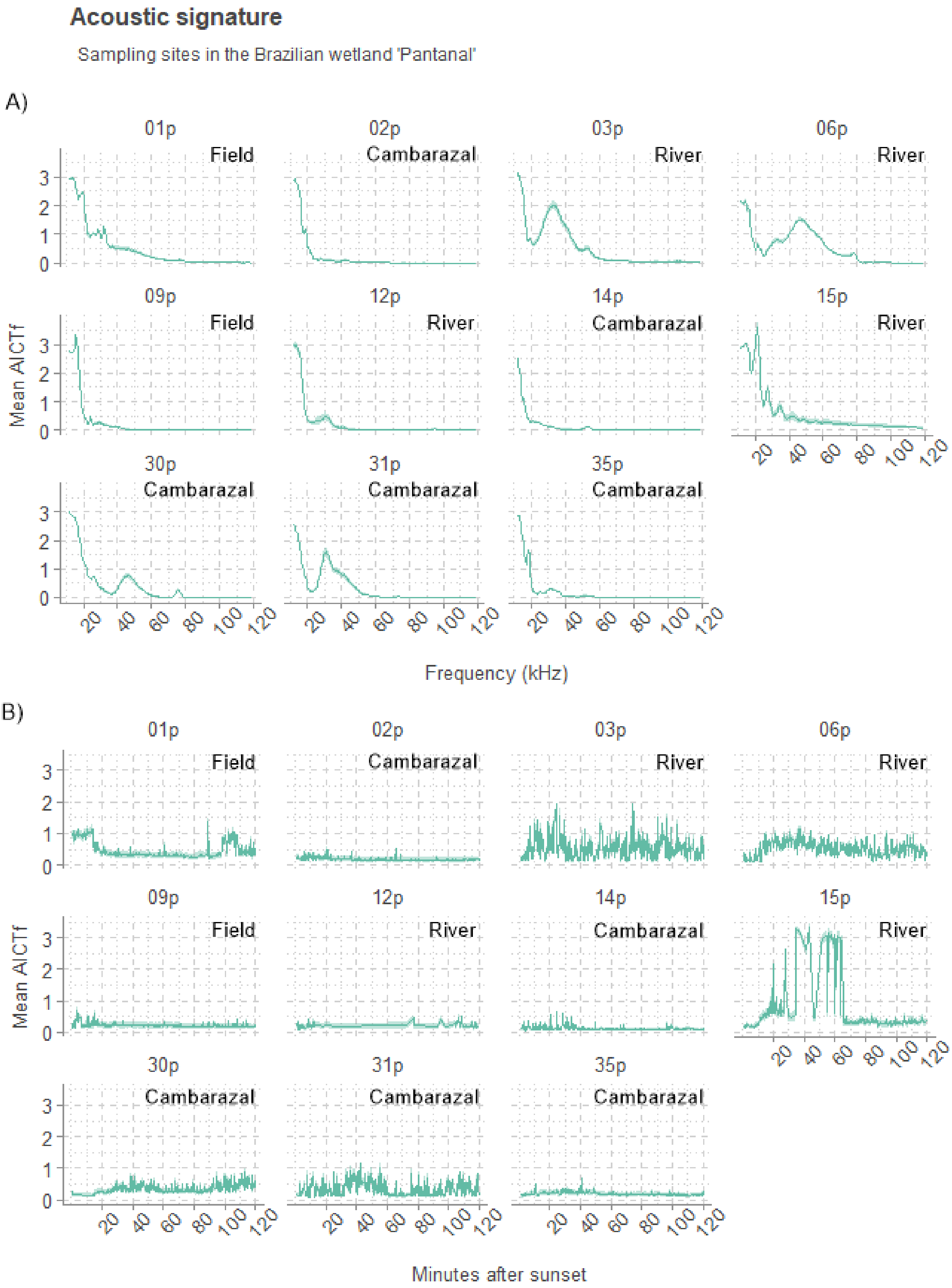


Figure S2. Spectral (A) and temporal (B) characterization of bat acoustic communities from the Pantanal. The green line represents the mean pattern and the 95% confidence intervals (shaded area) resulting from sampling on the first two hours after sunser at each recording site.

Table S2. Results of all fitted linear mixed models on mean ACItf by minute, including sampling site as random effect.

Model predictors	AICc	Random effects	Name	Variance	Std.Dev.			Significance
t	-813.6		(Intercept)	0.029	0.17			
			Residual	0.0407	0.202			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.235	0.037	20.788	6.418	2.4E-06	p < 0.001
		t	0.096	0.018	374.113	5.409	1.1E-07	p < 0.001
h+t	-806.94	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.0275	0.0159			
			Residual	0.0407	0.0217			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.235	0.036	19.295	6.589	2.4E-06	p < 0.001
		h	0.024	0.022	49.397	1.079	0.286	
		t	0.108	0.021	52.421	5.076	5.2E-06	p < 0.001
h+t+biome	-803.68	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.027	0.163			
			Residual	0.041	0.202			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.170	0.072	53.487	2.348	0.023	p < 0.05
		h	-0.006	0.037	348.252	-0.156	0.876	
		t	0.080	0.035	398.280	2.262	0.024	p < 0.05
		biomePantanal	0.130	0.127	86.661	1.025	0.308	
biome	-800.03	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.0188	0.137			
			Residual	0.0411	0.203			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.113	0.042	19.927	2.698	0.014	p < 0.05
		biomePantanal	0.244	0.059	19.978	4.134	0.001	p < 0.001
h+t+habitat	-795.26	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.019	0.138			
			Residual	0.041	0.202			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.134	0.086	35.794	1.558	0.128	
		h	0.009	0.036	218.275	0.245	0.807	
		t	0.094	0.035	294.877	2.701	0.007	p < 0.01
		field	0.141	0.117	14.805	1.208	0.246	
		forest	0.075	0.161	35.841	0.464	0.646	
		mod_sav	0.061	0.147	55.301	0.414	0.680	
		river	0.325	0.095	15.658	3.403	0.004	p < 0.01
		savanna	0.047	0.139	42.733	0.336	0.738	
Null model	-792.92	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.034	0.183			
			Residual	0.041	0.203			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.234	0.039	21.012	5.975	6.2E-06	p < 0.001
h	-792.76	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.051	0.226			
			Residual	0.041	0.202			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.234	0.048	17.177	4.845	0.000	p < 0.001
		h	-0.060	0.019	601.771	-3.09	0.002	p < 0.01
habitat	-789.68	Random effects	Name	Variance	Std.Dev.			
			(Intercept)	0.014	0.117			
			Residual	0.041	0.203			
		Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)	
		(Intercept)	0.242	0.053	15.647	4.559	0.000	p < 0.001
		field	0.105	0.099	15.635	1.052	0.309	
		forest	-0.130	0.099	15.635	-1.305	0.211	
		mod_sav	-0.138	0.075	15.639	-1.836	0.085	p < 0.1
		river	0.264	0.080	15.793	3.305	0.005	p < 0.01
		savanna	-0.119	0.080	15.638	-1.495	0.155	

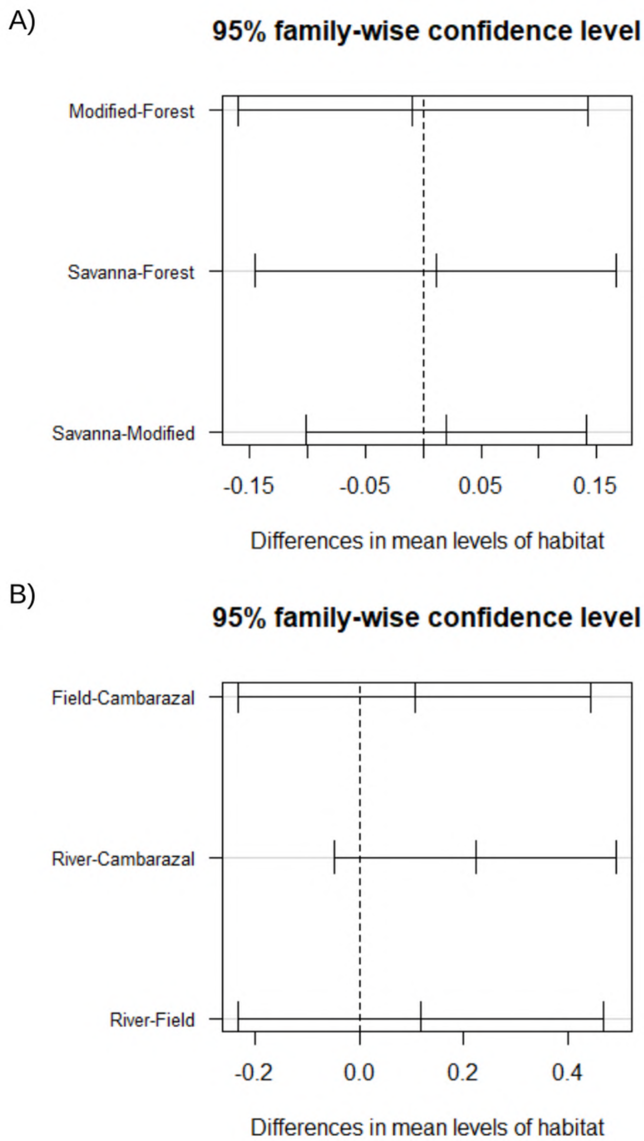


Figure S3. Tukey HSD plot showing multiple comparisons between habitat classes within A) the Pantanal and B) the Cerrado. Lines represent the mean difference for each pair of group and 95% confidence intervals.

Capítulo V

Conclusões gerais

Os resultados alcançados nesta tese ampliam o nosso conhecimento sobre a variação acústica geográfica dos morcegos neotropicais. As minhas análises incluíram distintos níveis desde a variação acústica intraespecífica, variação acústica entre grupos de espécies até a variação acústica das comunidades. Novas possibilidades surgem para o estudo da ecologia tropical de morcegos através do som.

Mostrei que a variação nas características de frequência dos chamados de ecolocalização de uma espécie podem correlacionar-se com diferenças nas condições climáticas ao longo da sua distribuição geográfica. Usando um grande conjunto de dados acústicos cobrindo quase toda a distribuição do morcego pescador, *Noctilio leporinus*, concluo que a variação acústica da espécie é resultado de adaptação ecológica, do tamanho do corpo e da história filogenética, corroborando parcialmente as hipóteses testadas de condução sensorial e a regra de James.

Mostrei que a variação acústica dos grupos de espécies *P. fulvus* x *P. davyi* e *P. psilotis* x *P. personatus* dentro de suas possíveis zonas de contato na América Central é representada pela existência de três grupos fônicos sem uma correspondência clara com a distribuição conhecida das espécies, mas com uma variação de frequência que reflete a variação geográfica no tamanho do corpo. Concluo que para resolver o dilema de distribuição das espécies se precisa de informações integrativas, incluindo análises morfológicas, moleculares e ecológicas. Até então, identificações acústicas de chamadas de ecolocalização obtidas a partir de registros de espécies em vôo livre devem considerar a possível existência em simpatria das espécies nas zonas de contacto.

Finalmente, mostrei que a variação acústica das comunidades de morcegos pode ser estudada com as ferramentas de análise que a Ecoacústica fornece, como o Índice de Complexidade Acústica, indo além do uso das características acústicas para a identificação taxonômica das espécies.

O nosso conhecimento acústico sobre as espécies de morcegos neotropicais tem aumentando exponencialmente, assim como as possibilidades de acesso a equipamento de monitoramento acústico e as ferramentas de análise que o enfoque tradicional bioacústico e as novas possibilidades da ecoacústica oferecem. Ainda há muito a ser aprendido sobre como os fatores climáticos e ecológicos, tanto históricos como contemporâneos influenciam as características acústicas dos chamados de ecolocalização. Para entender essas interações e os mecanismos por trás é preciso estudar a variação acústica tanto na escala local, como regional como na área total da distribuição de uma determinada espécie.

Capítulo VI

Materiais de divulgação

Adriana Arias-Aguilar

MATERIAL DE DIVULGAÇÃO

Bird and Mammal
Evolution, Systematics and Ecology Lab

SBEQ

bimalab.ufrgs

sbeq_morcegos

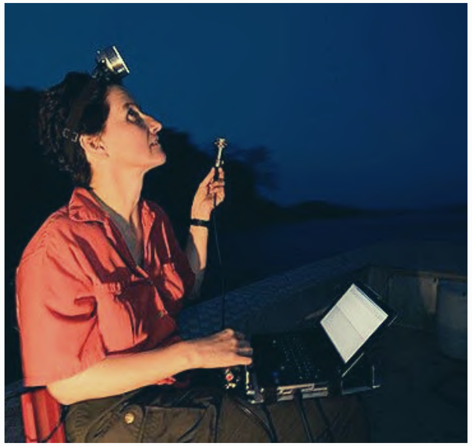
@NeoBatSounds

aranh_art

Ciência por elas

CONHECENDO OS MORCEGOS

MULHERES EXEMPLARES QUE NOS INSPIRAM



Alemanha

PROF. DR. ELISABETH KALKO

University of Ulm-STRI
Fisiologia, Comportamento, Ecologia, História Natural e Evolução
Iniciou e liderou projetos sobre ecologia,bioacústica, biodiversidade e zoonoses em todos os continentes

Venezuela

PROF. DR. SHARLENE SANTANA

Santana Lab-University of Washington
Biomecânica, Ecologia, Evolução e Sistemática
O foco da sua pesquisa é entender a dinâmica evolutiva entre comportamento, morfologia e função

Brasil

PROF. DR. LUDMILLA AGUIAR

Lab. de Biologia e Conservação de Morcegos-UnB
Biologia, Ecologia, Serviços ambientais, Bioacústica e Conservação
Fundadora RELCOM-SBEQ
Reconhecida com o Prêmio Spallanzani 2017

EVENTO: UFRGS PORTAS ABERTAS

PESQUISADORAS DO BIMALAB-UFRGS

Portugal

PROF. DR. MARIA JOÃO RAMOS PEREIRA

Coordenadora do BiMaLab
Ecologia, filogeografia, genética populacional e modelagem ecológica
Suas pesquisas em morcegos focam sobretudo aspectos ecológicos e evolutivos da diversidade de espécies na região neotropical

Costa Rica

MS. ADRIANA ARIAS-AGUILAR

Estudante de doutorado no PPG Biologia Animal
Ecologia, bioacústica, biogeografia e história natural
Sua pesquisa avaliará a variação acústica de espécies neotropicais ao longo de sua distribuição e a sua relação com o clima

Brasil

BSC. ISADORA BRAUNER LOBATO

Estudante de mestrado no PPG Ecologia
Ecologia e conservação
Tem interesse particular em ecologia populacional de morcegos, com foco em espécies sinantrópicas, ou seja, aquelas que ocupam preferencialmente ambientes urbanos

Brasil

BSC. CÍNTIA DA COSTA

Estudante de mestrado no PPG Ecologia
Ecologia, biogeografia, evolução e conservação
Sua pesquisa avaliará a conectividade da paisagem do Pampa para morcegos através de modelos de ocupação baseados em detecção acústica

EVENTO: UFRGS PORTAS ABERTAS

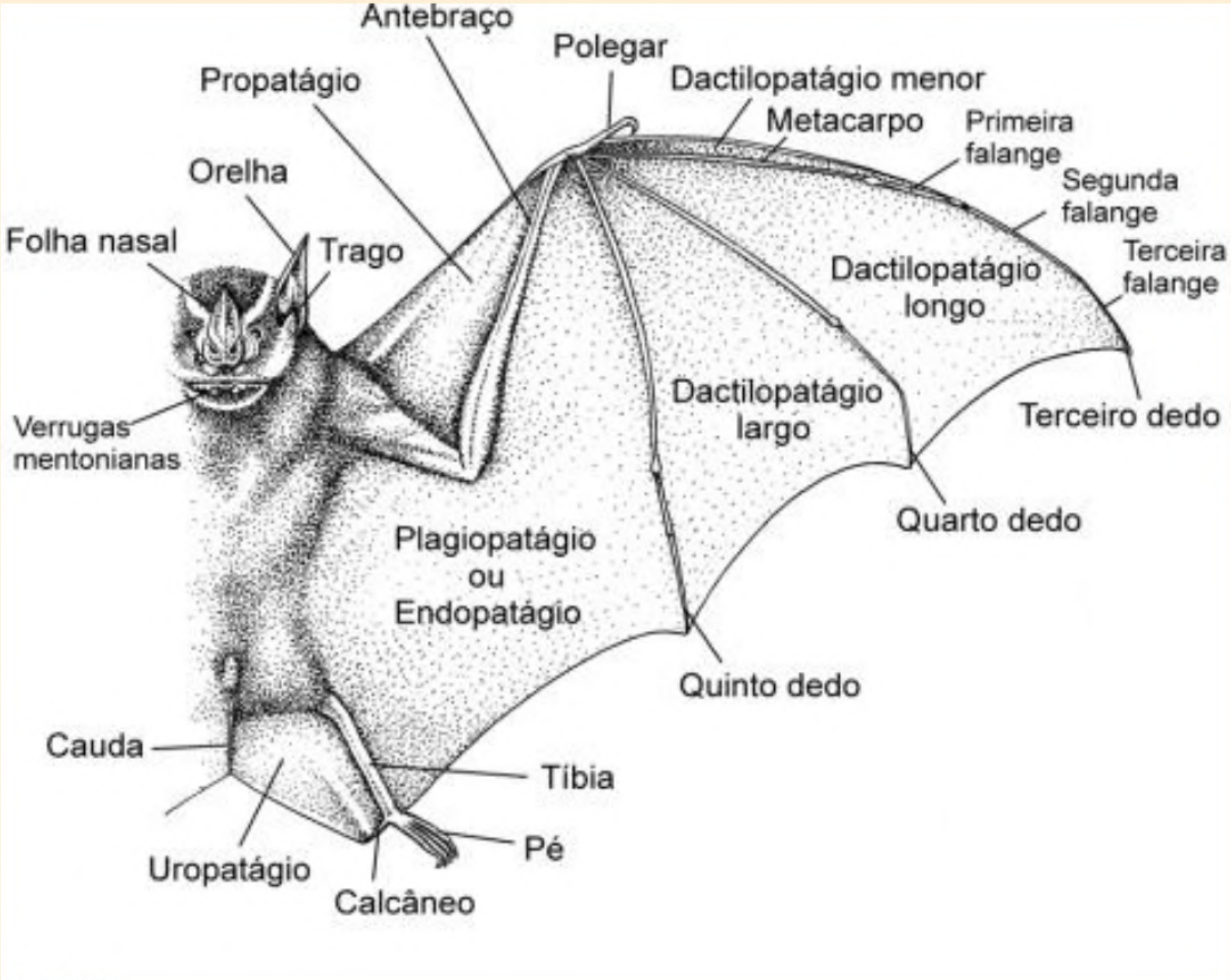
WORKSHOP BIMALAB: IDENTIFICAÇÃO E TAXIDERMIA DE MORCEGOS

ELABORADO POR:
ADRIANA ARIAS-AGUILAR

Morcegos Neotropicais

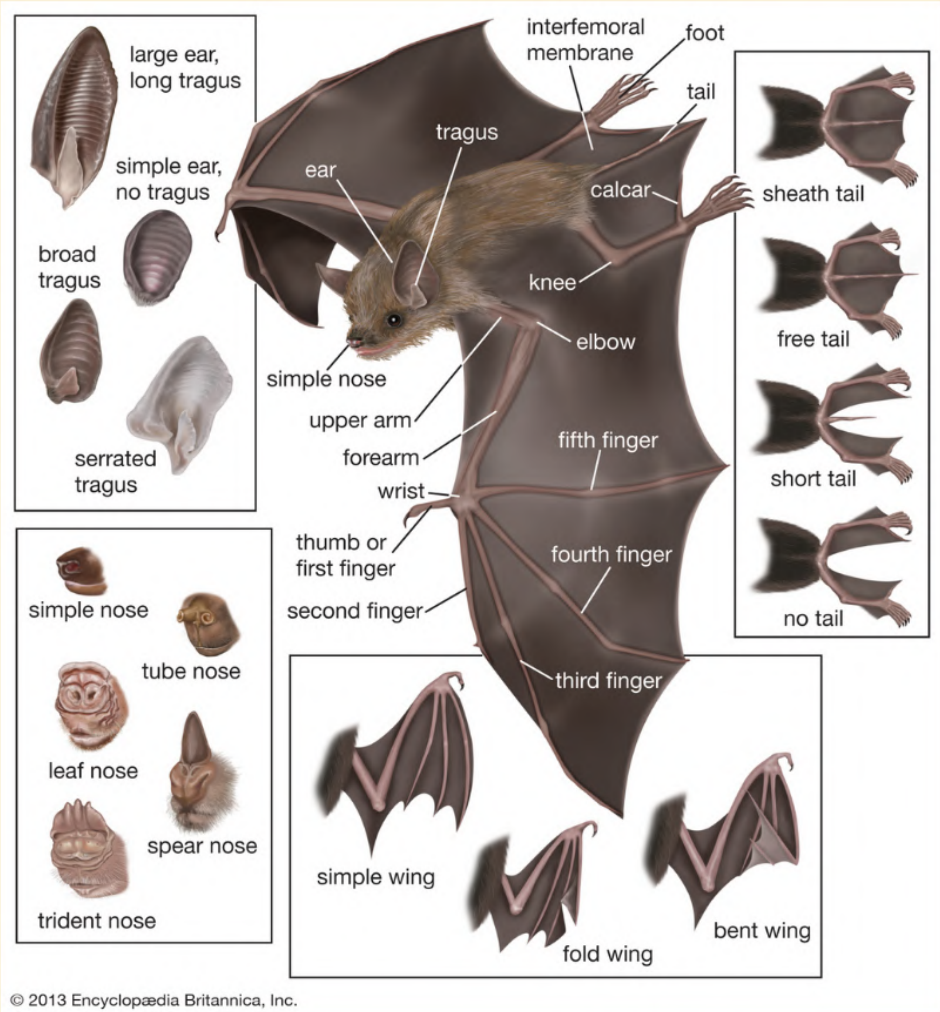
Noções básicas para a sua identificação morfológica

Bird and Mammal
Evolution, Systematics and Ecology Lab



Reis et al. (2007)

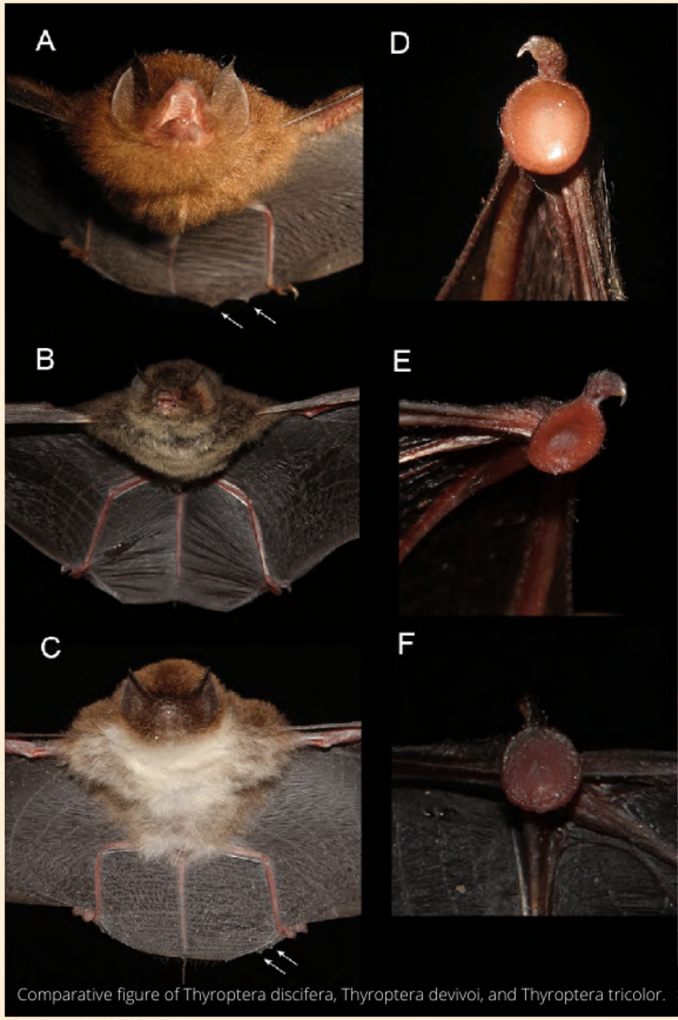
Caracteres morfológicos



Caracteres morfológicos utilizados na identificação

- Orelha e trago: forma e comprimento
- Antebraço: comprimento, pelos
- Cauda: livre ou dentro, comprimento
- Uropatágio: forma e comprimento, orla
- Calcâneo
- Inserção das asas: tornozelo ou pê

THYROPTERIDAE



Comparative figure of Thyroptera discifera, Thyroptera devivoi, and Thyroptera tricolor.

Check List 13(4):355–361

jeb204024 doi: 10.1242/jeb.204024

Discos adesivos na base do polegar e pê

FURIPTERIDAE

Polegar reduzido

NATALIDAE

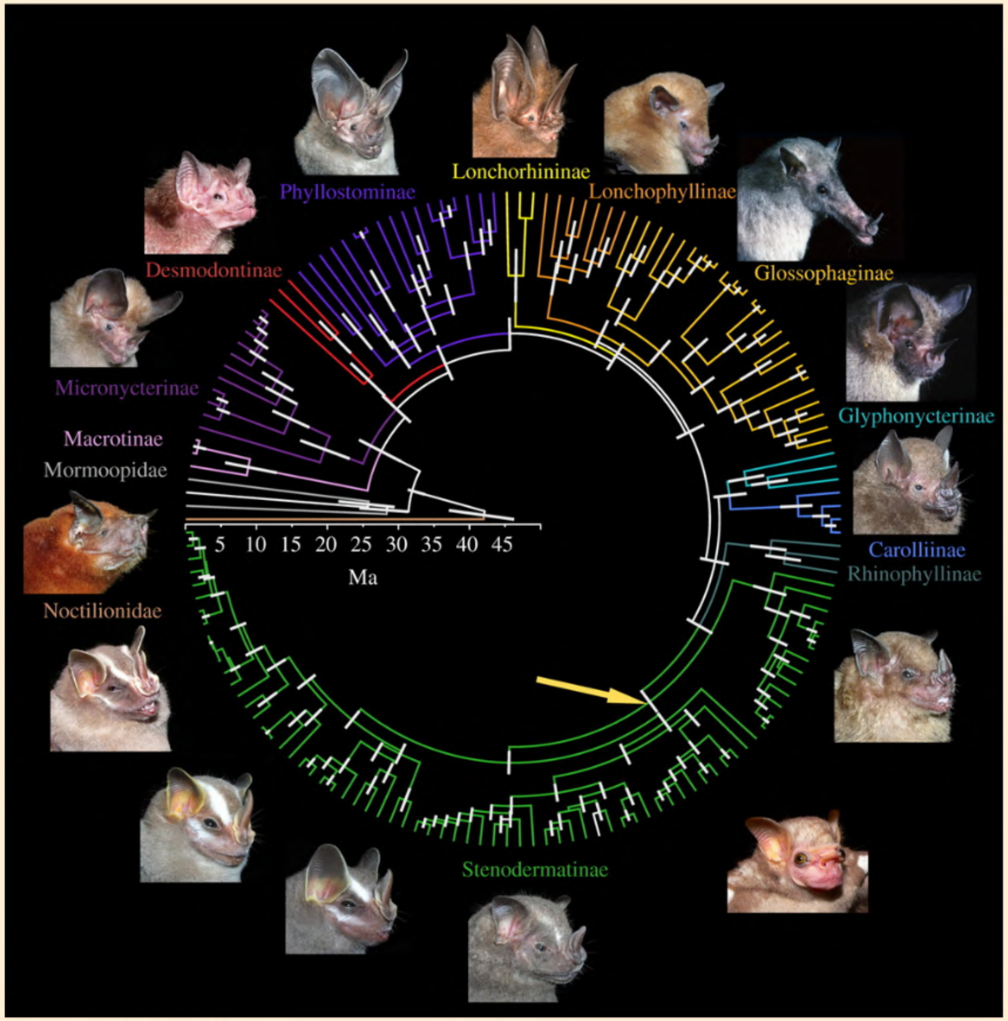
Fig. S9: Natalus macrourus (UFPB7102). Note as orelhas em forma de funil e o lábio inferior liso.
Mastozoologia Neotropical 24(2)

Orelhas de funil

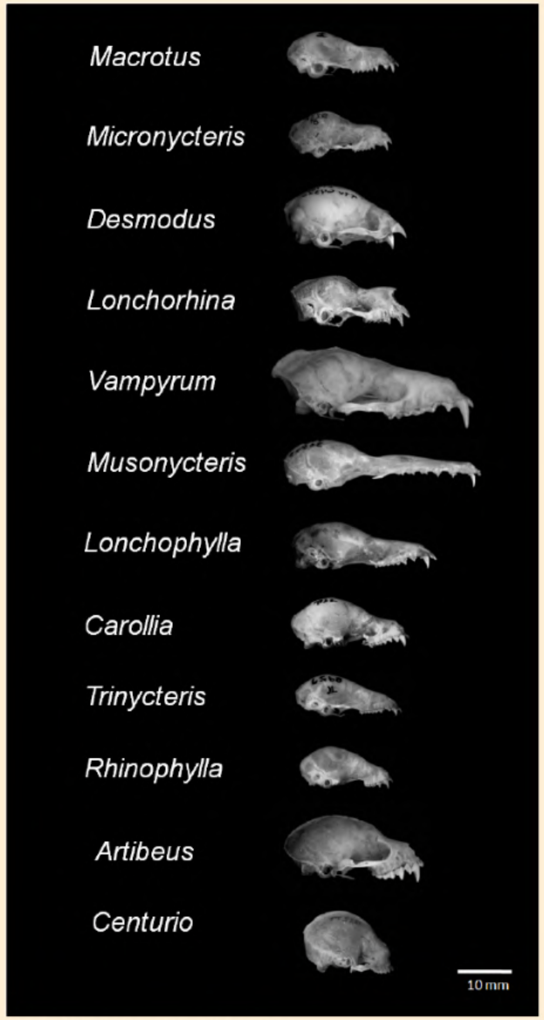
EMBALLONURIDAE

- Sacos glandulares no propatágio ou uropatágio ou sem
- Listras
- Tamanho do antebraço

PHYLLOSTOMIDAE



https://doi.org/10.1098/rspb.2011.2005



DOI: 10.1017/CBO9781139045599.012

Folha nasal

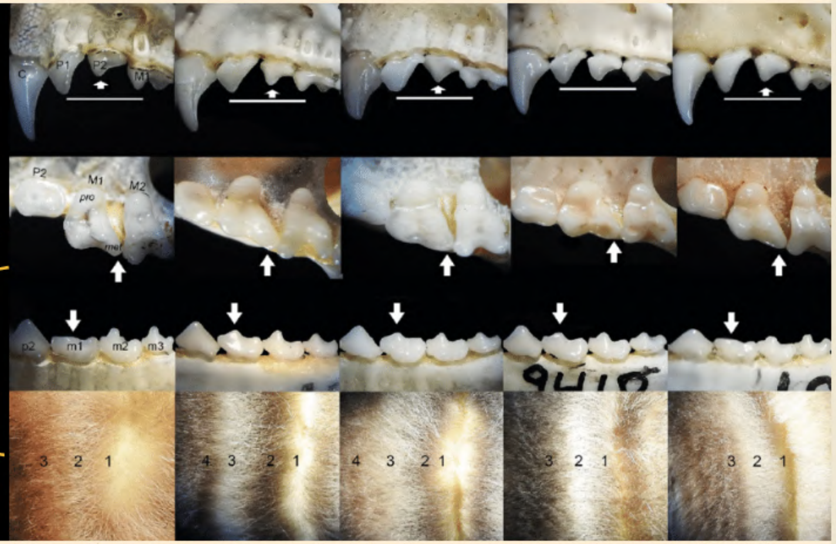
DESMODONTINAE

Joel Sartore

A) vampiro-comum (Desmodus rotundus), B) vampiro-de-asas-brancas (Diaemus youngi), C) vampiro-de-pernas-peludas (Diphylla ecaudata).
Isbn: 978-85-67788-01-2

- **Polegar desenvolvido**
- **Incisivos superiores tipo daga**

CAROLLIINAE



Comparación de caracteres de las especies de Carollia ordenadas de izquierda a derecha: C. benkeithi (MUSM 24583), C. brevicauda (MUSM 41642), C. manu (MUSM 11786), C. perspicillata (MUSM 9418) y Carollia sp. Revista Peruana de Biologia 24(4):363

- **Queixo em forma de "V" com verruga central grande**
- **Pelos dorsais com "bandas"**
- **Antebraço e incisivos inferiores**

GLOSSOPHAGINAE

- **Focinho estreito e estendido**
- **Orelhas pequenas**
- **Folha nasal pequena e triangular**
- **Lábio inferior com sulco profundo**

PHYLLOSTOMINAE

- **Orelhas e folha nasal desenvolvidas**
- **Saliências no queixo**
- **Número de incisivos**
- **Tamanho do antebraço**
- **União das orelhas**

STENODERMATINAE

- **Cauda ausente**
- **Listras faciais/dorsal**
- **Uropatágio reduzido**
- **Focinho curto**
- **Tamanho do antebraço**
- **Incisivos**

NOCTILIONIDAE

- Lábio leporino
- Pelo curto e cheiroso 🐟
- Listra dorsal
- Tamanho do antebraço e pês

MORMOOPIDAE

Fig. S7: Pteronotus gymnonotus (UFPB7109). Note as asas ligadas na região medial do dorso.
Mastozoologia Neotropical 24(2)

- **Lábio com rugas ou dobras**
- **Asas unidas no corpo ou dorso**
- **Tamanho do antebraço**

MOLOSSIDAE

- **Cauda livre**
- **Assas estreitas e compridas**
- **Comprimento do antebraço**
- **Forma e tamanho do trago**
- **Caracteres dentários**

VESPERTILIONIDAE

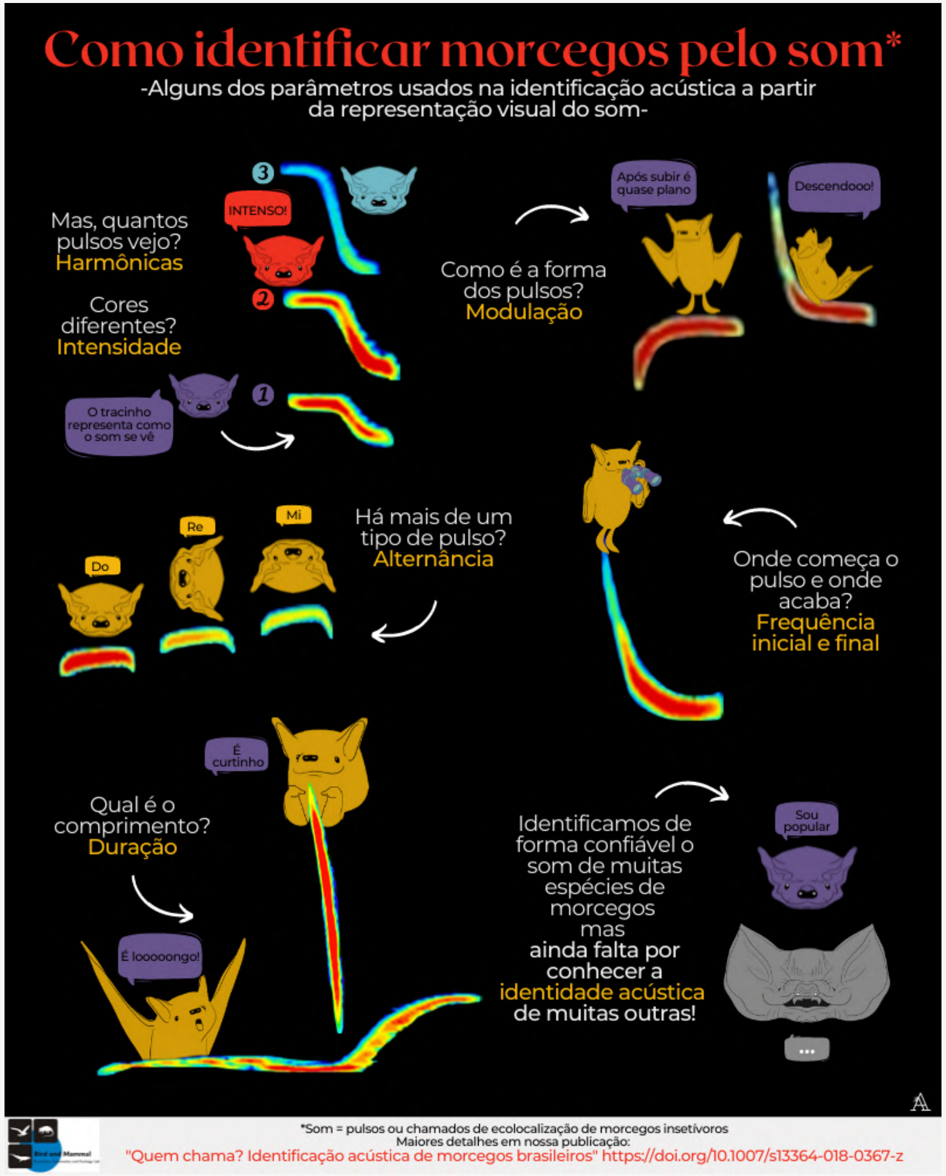
- **Uropatágio em "V"**
- **Tamanho do antebraço**
- **Caracteres dentários e craniais**
- **Tamanho e forma do trago**
- **Forma do focinho**
- **Inserção do plagiopatágio**

Fig. 22.—Vampire Bat.

LOGOS SBEQ:
SECRETARIAS REGIONAIS

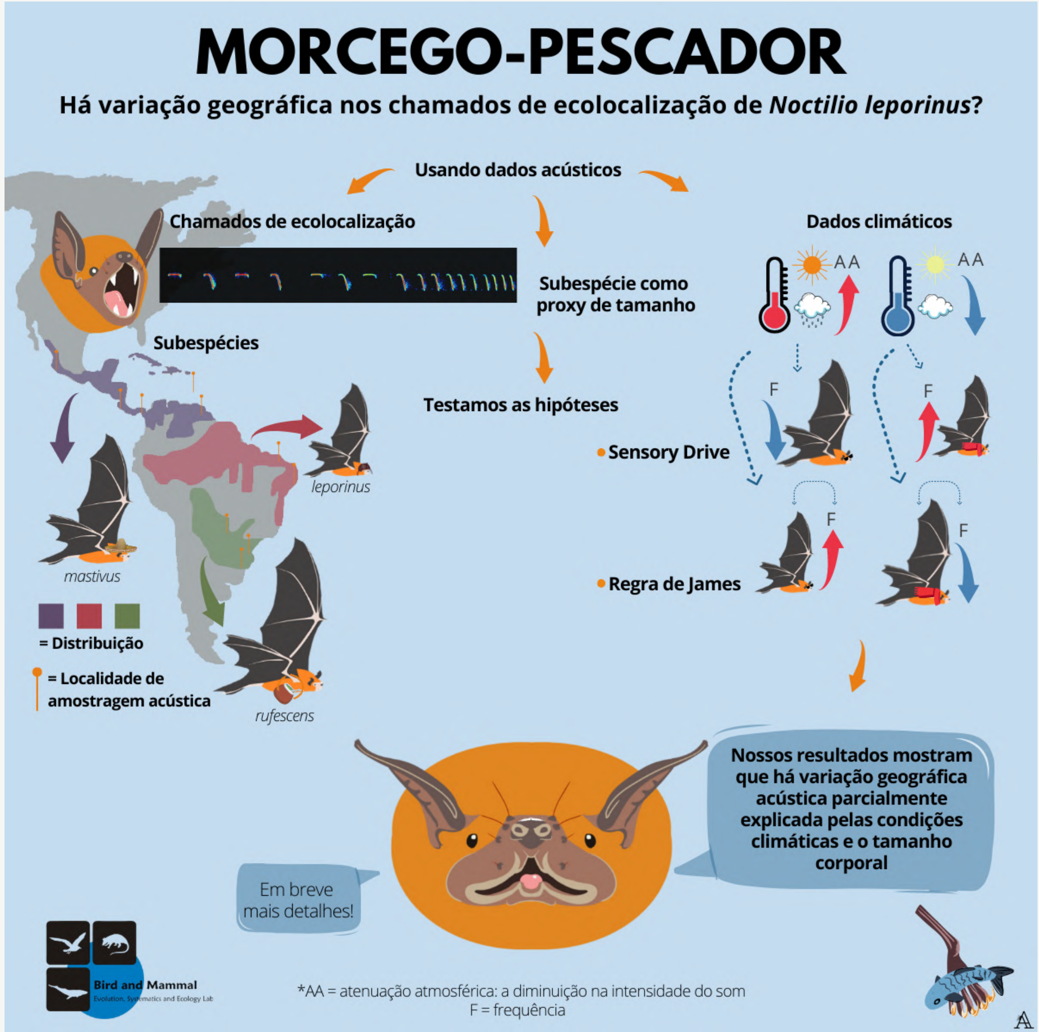
DIA NACIONAL DA AMAZÔNIA

DIA NACIONAL DO CERRADO



DIVULGAÇÃO DE PESQUISA

DIVULGAÇÃO DE PESQUISA



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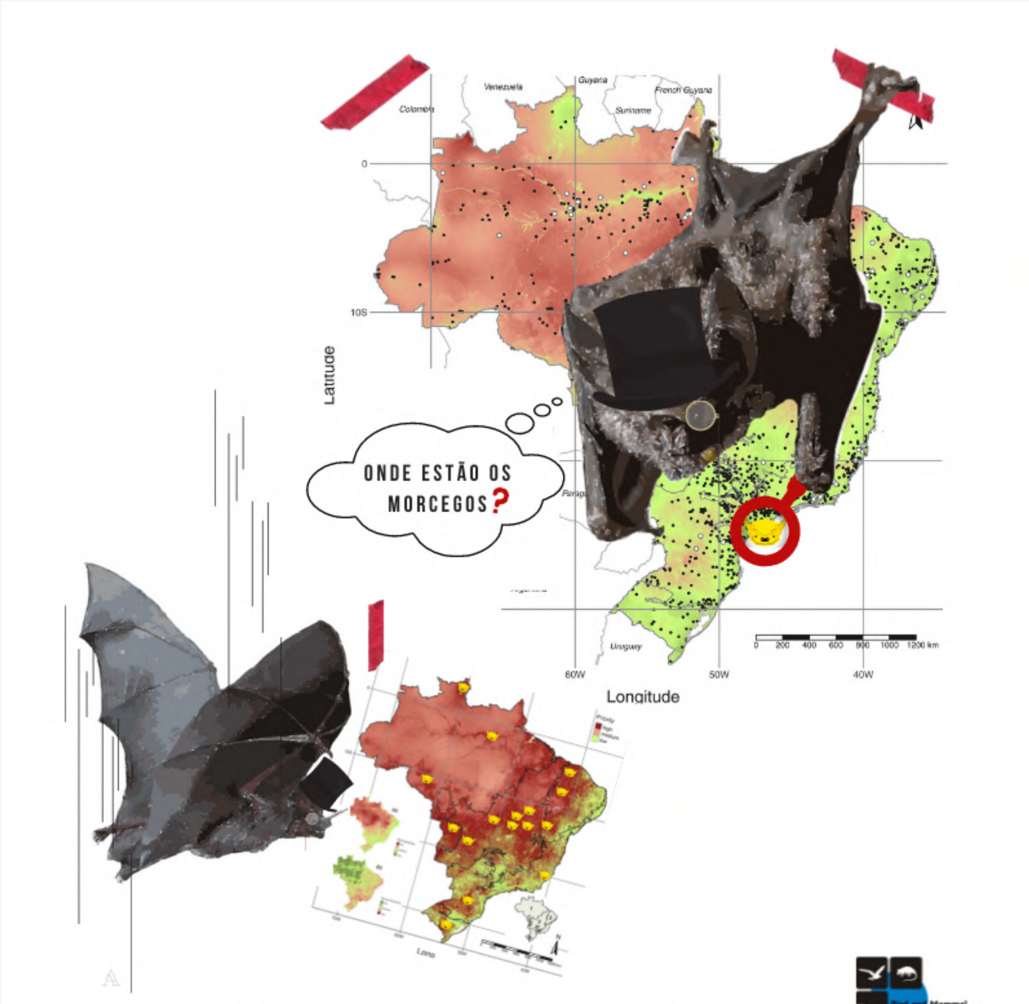
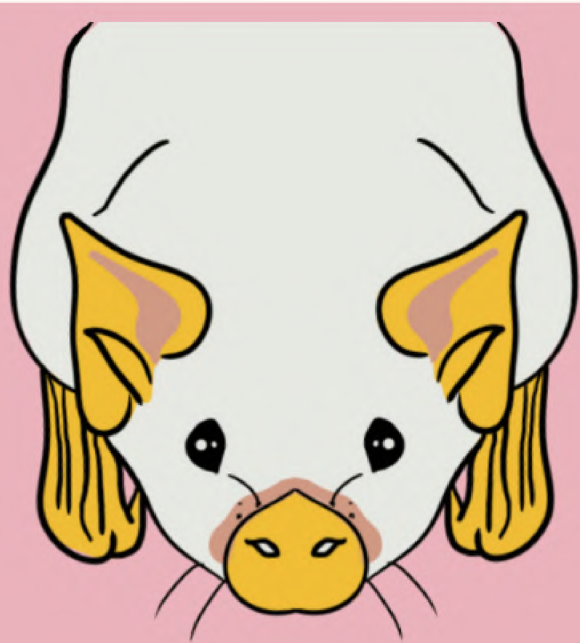


Fonte: Where are the bats? An environmental complementarity analysis in a megadiverse country. doi: 10.1111/ddi.13137

DIVULGAÇÃO DE PESQUISA

Esse morcego é...

DIA INTERNACIONAL DOS MORCEGOS



Lindo?


Feio?

Bird and Mammal

SBEQ


Assustador?

Por quê?

Sabes o que é...

Lindo?

É a polinização das flores!

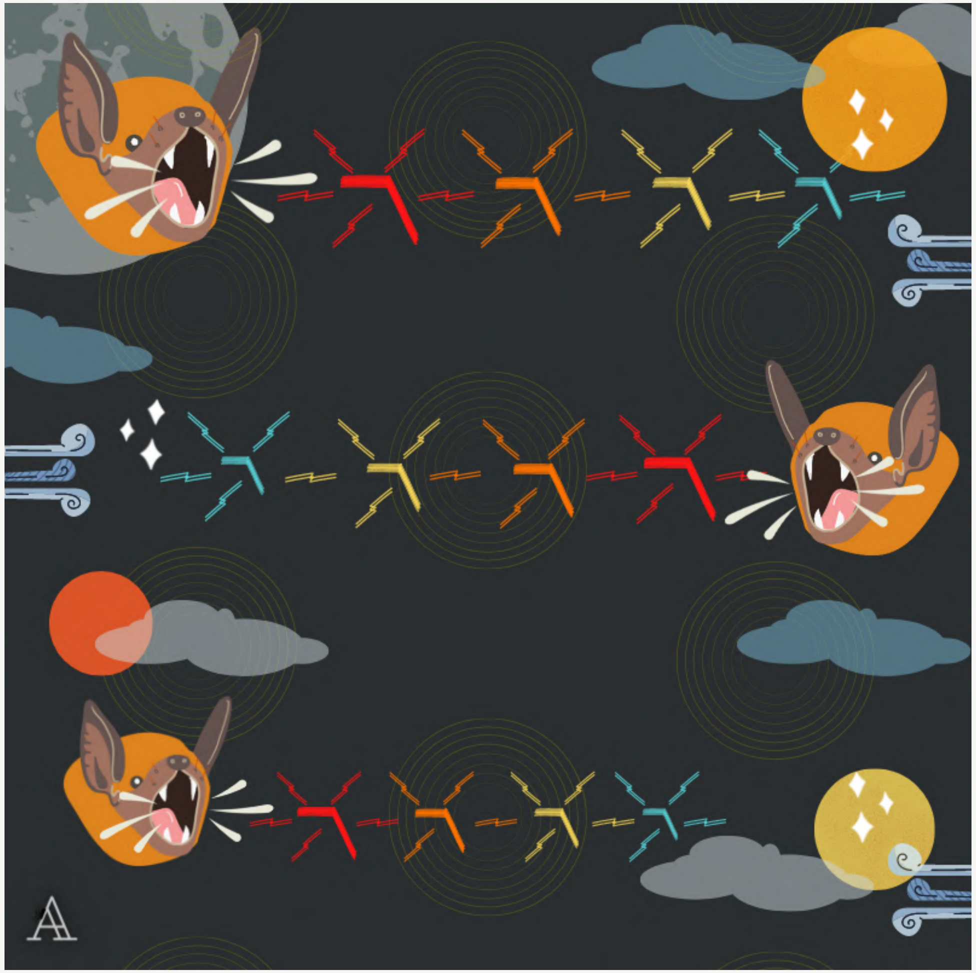

Feio?
É que a tequila acabe!


Perigosos?
São os agrotóxicos!
SBEQ

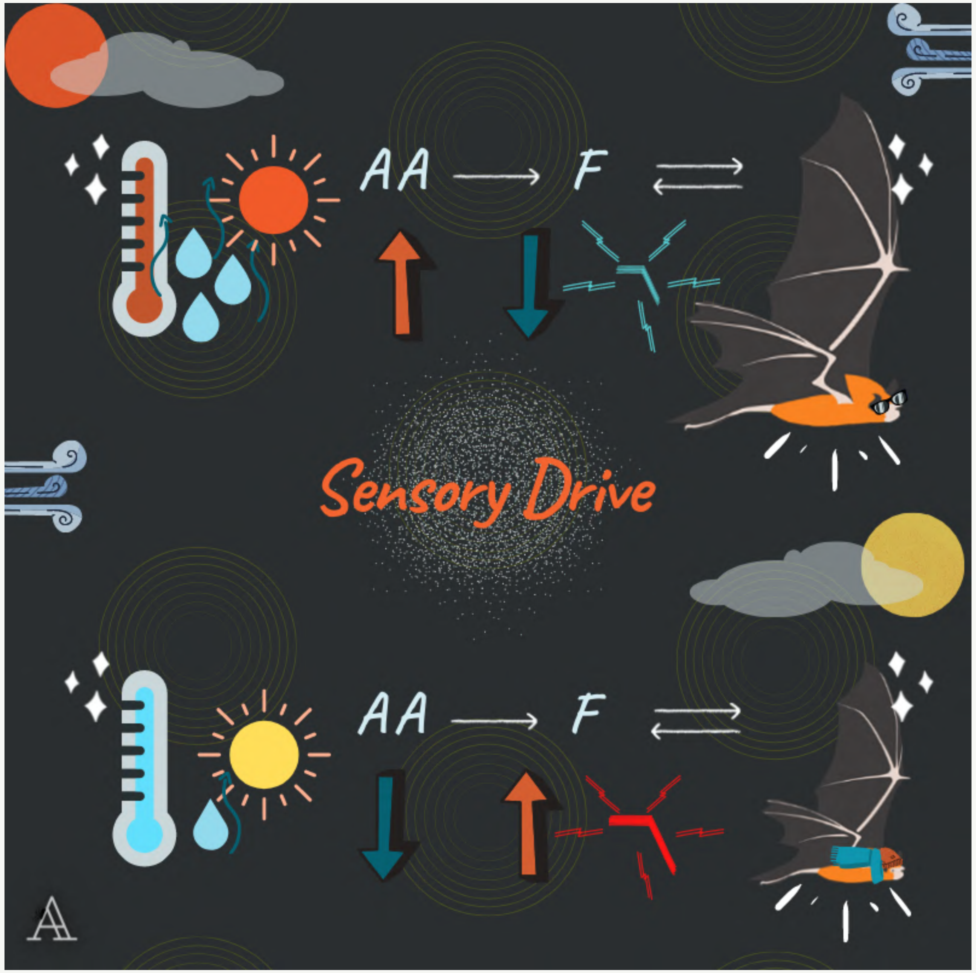

Assustador?
É viver sem florestas!

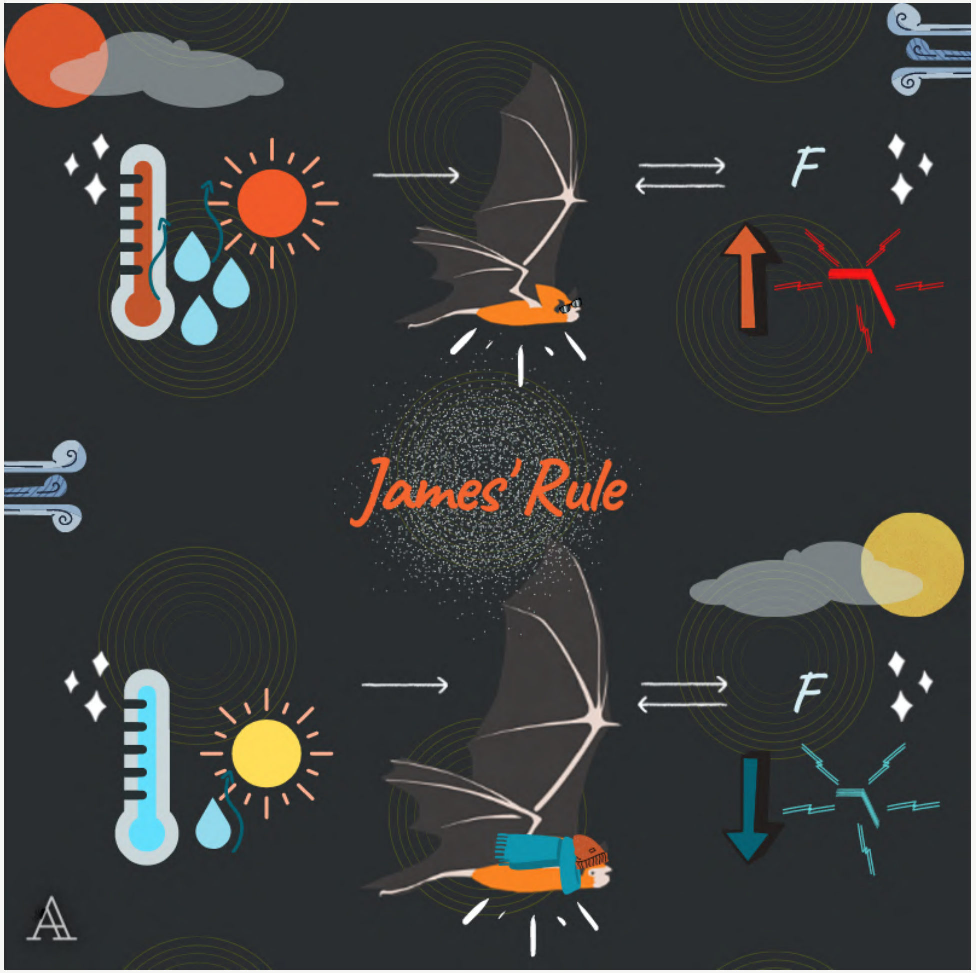
Bird and Mammal
Evolution, Systematics and Ecology Lab

SBEQ
SOCIEDADE BRASILEIRA PARA
O ESTUDO DE QUIRÓPTEROS



I CONFERÊNCIA SBEQ NO TWITTER
SEGUNDO LUGAR PRÊMIO ELIZABETH KALKO


AA → F ⇄
Sensory Drive
AA → F ⇄

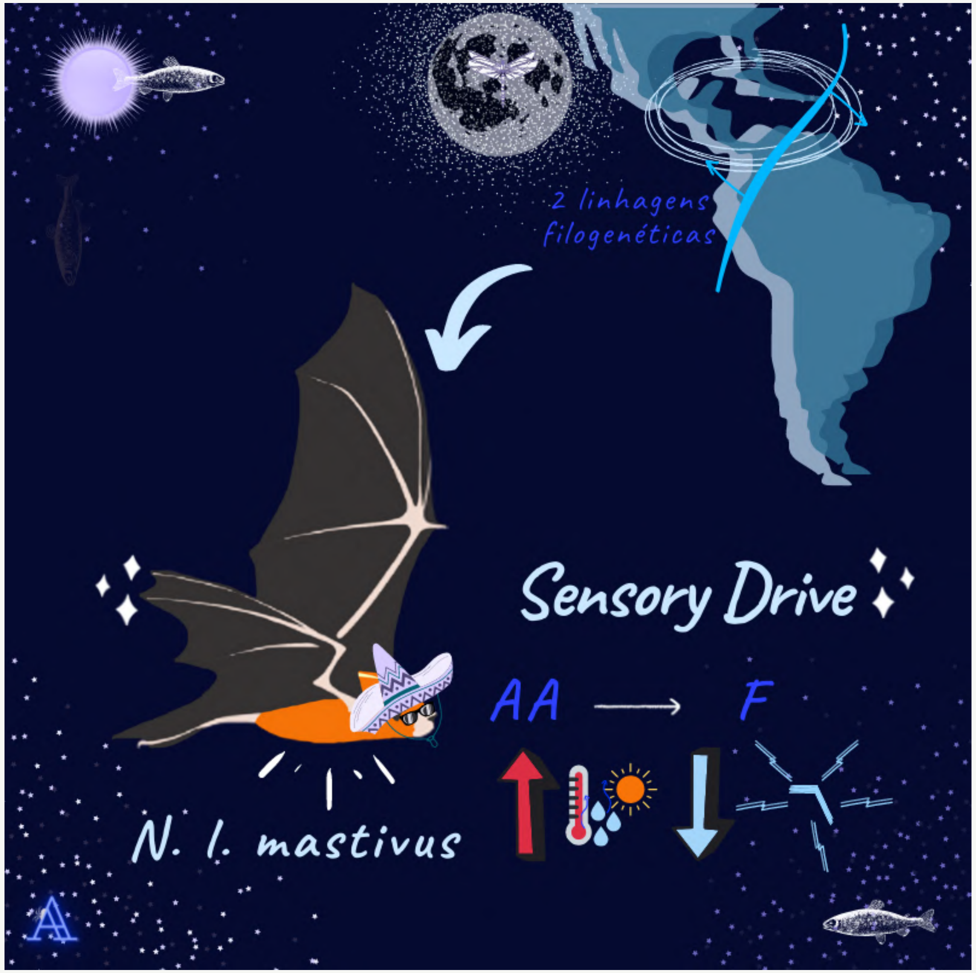

⇄ F
James' Rule
⇄ F

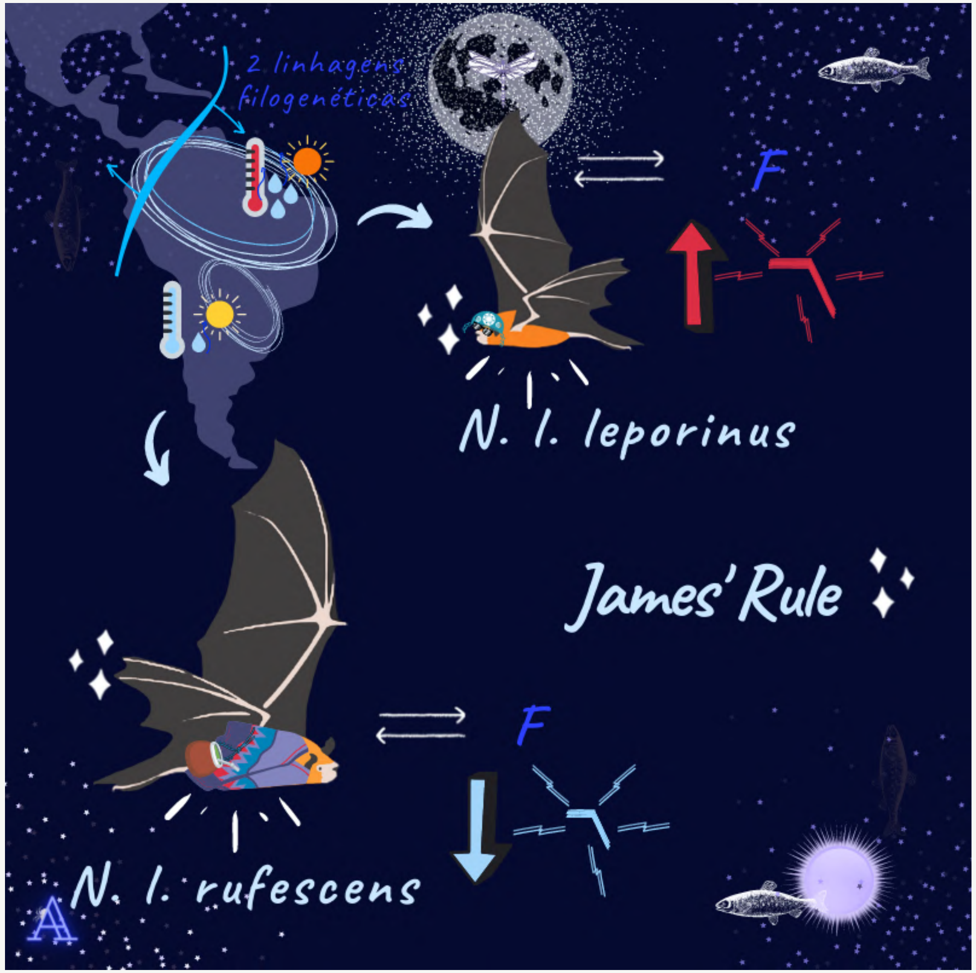
O morcego pescador

Neotropical Bat Bioac... · Oct 29, 2020

[2/3] #CMBT1 #EcoInt #BioA

Usando um amplo conjunto de dados acústicos, climáticos ☀️ 💧 e considerando o tamanho e a filogenia, testamos as hipóteses SD e JR … nos perguntamos: 🤔 Há variação acústica 🌎 nos 📢 de ecolocalização do 🦇🎣 Noctilio leporinus?





Neotropical Bat Bioac... · Oct 29, 2020

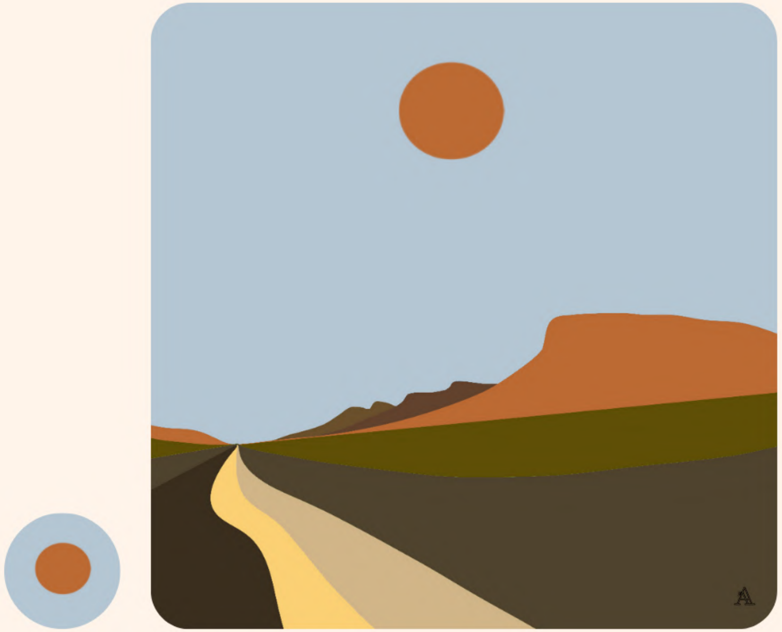
[3/3] #CMBT1 #EcoInt #BioA

Nossos resultados mostram que há variação geográfica 🌎 nos 📢 de ecolocalização do🦇🎣 parcialmente explicada pelas condições climáticas ☀️💧 e o tamanho corporal! SD✔️ JR✔️

Em breve mais detalhes😉💜🦇 @BiMaLab1

DIA MUNDIAL DA ÁGUA

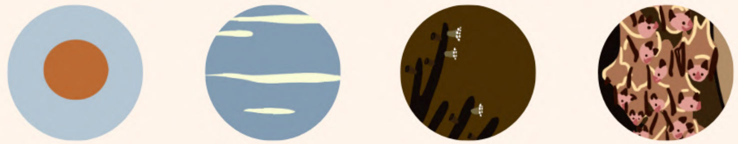
DIA NACIONAL DA CAATINGA



Com as cores da terra
a Caatinga se veste
a seca a chuva encerra
e aquece o Nordeste

De cores se enfeita
quando a terra umedece
o calor ainda à espreita
a Caatinga resplandece

A Caatinga noturna
suas cores deixa ver
morcegos na caverna
e cactos florescer.



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DIA INTERNACIONAL PARA A APRECIAÇÃO DOS MORCEGOS

DIA LATINO-AMERICANO DOS MORCEGOS

SEMANA DE DIVULGAÇÃO:
DIA MUNDIAL DOS ANIMAIS

e

CORROMPEM

a **destruição** e **sobreexploração** dos recursos marinhos diminuem em uma taxa acelerada as populações de cetáceos

Bird and Mammal
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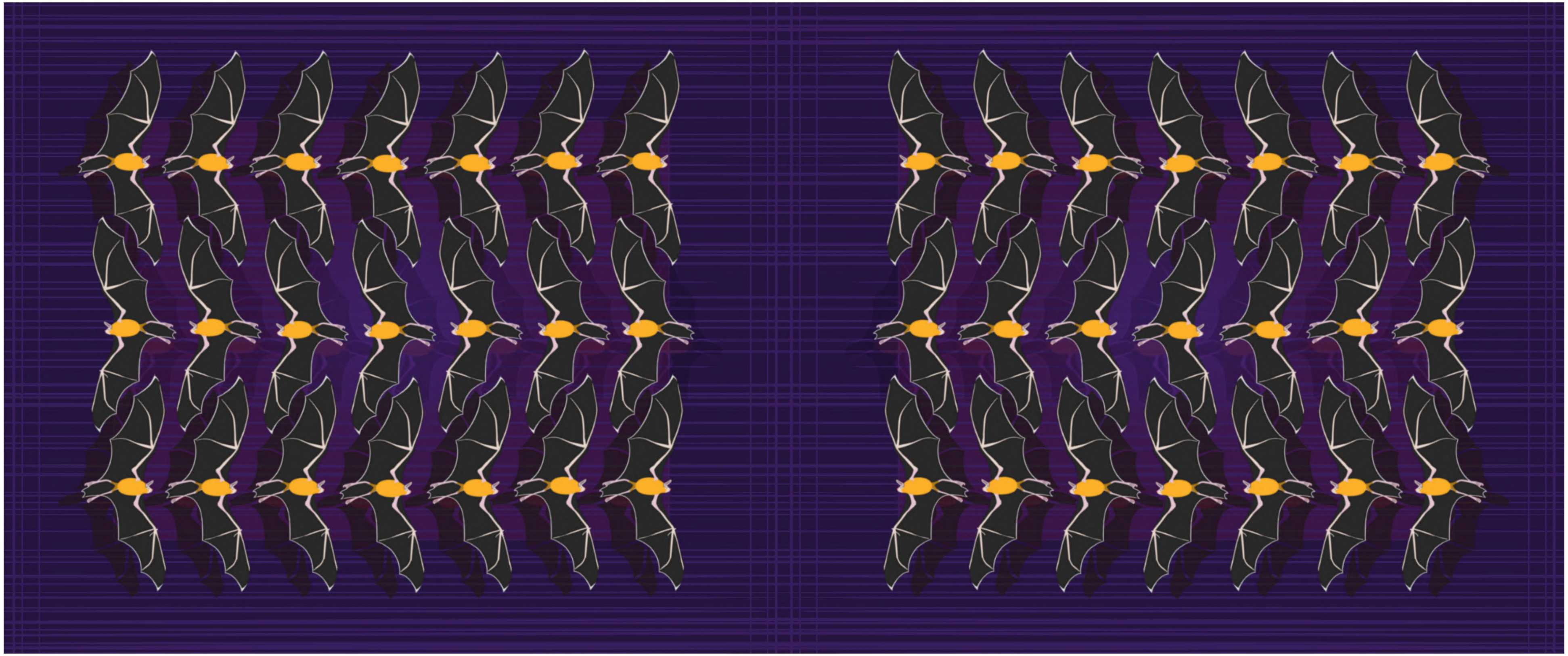
a
VIDA
no DIA MUNDIAL DOS ANIMAIS repensemos,
ajamos com respeito e responsabilidade
Bird and Mammal

SEMANA DE DIVULGAÇÃO

SEMANA DE DIVULGAÇÃO:
MÊS PARA A APRECIAÇÃO DOS MORCEGOS



@NeoBatSounds

aranh_art