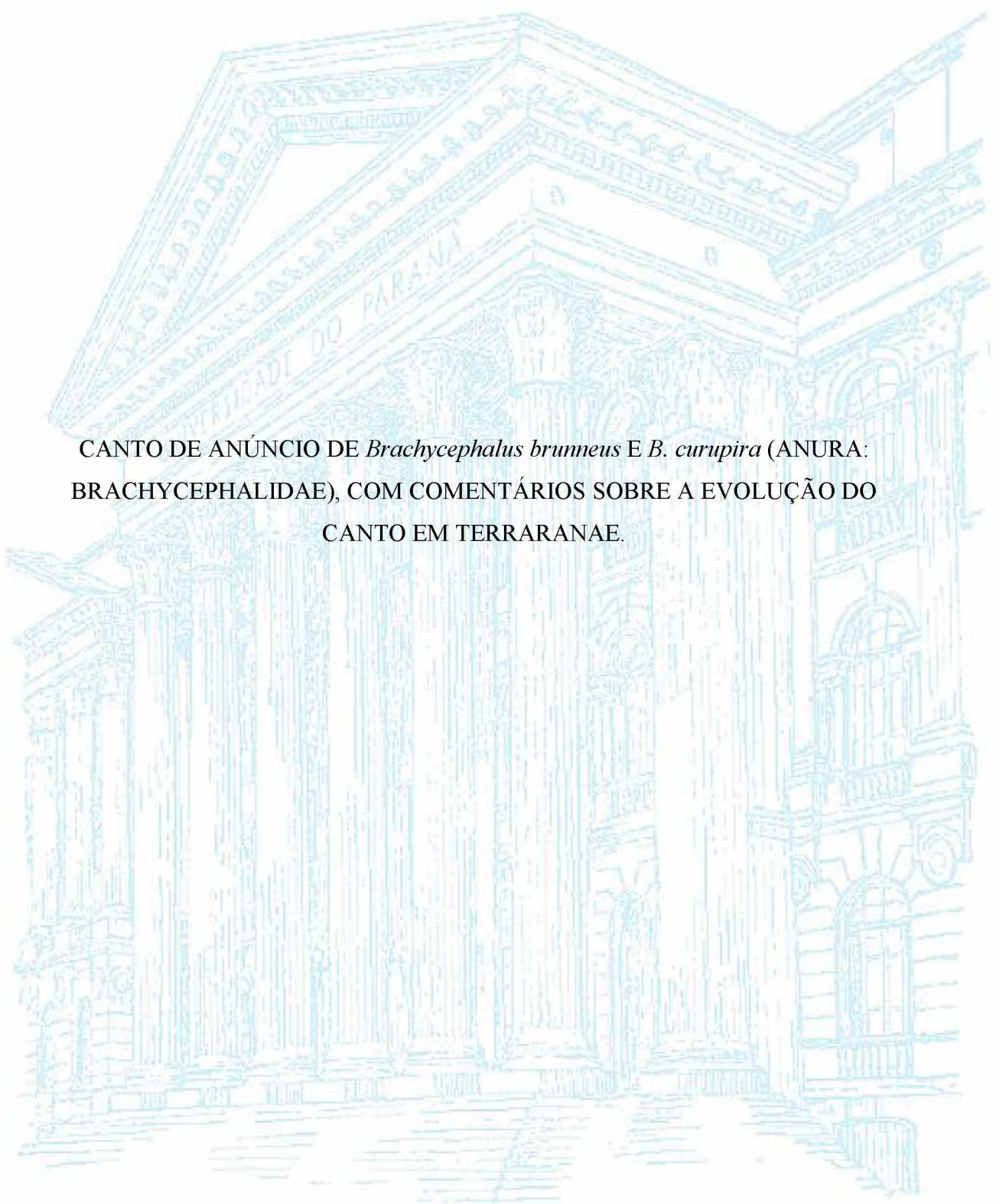


UNIVERSIDADE FEDERAL DO PARANÁ

ANDRÉ EDUARDO CONFETTI



CANTO DE ANÚNCIO DE *Brachycephalus brunneus* E *B. curupira* (ANURA: BRACHYCEPHALIDAE), COM COMENTÁRIOS SOBRE A EVOLUÇÃO DO CANTO EM TERRARANAE.

CURITIBA  
2018

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BRACHYCEPHALIDAE), COM COMENTÁRIOS SOBRE A EVOLUÇÃO DO  
CANTO EM TERRARANAE.

Dissertação apresentada ao Programa de Pós-Graduação  
em Zoologia do Departamento de Zoologia, Setor de  
Ciências Biológicas da Universidade Federal do Paraná  
como requisito parcial para a obtenção do título de Mestre  
em Zoologia.

**Orientador:** Prof. Dr. Marcio Roberto Pie

**Co-orientador:** Dr. Marcos Ricardo Bornschein

Curitiba  
2018

Universidade Federal do Paraná. Sistema de Bibliotecas.  
Biblioteca de Ciências Biológicas.  
(Telma Terezinha Stresser de Assis –CRB/9-944)

Confetti, André Eduardo

Canto de anúncio de *Brachycephalus brunneus* e *B. Curupira* (Anura: Brachycephalidae), com comentários sobre a evolução do canto em Terraranae./ André Eduardo Confetti. – Curitiba, 2018.  
54 p. : il. ; 30cm.

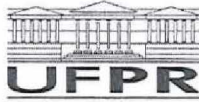
Orientador: Marcio Roberto Pie

Co-orientador: Marcos Ricardo Bornschein

Dissertação (Mestrado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Zoologia.

1. Anuro. 2. Alometria. 3. Canto. I. Título. II. Pie, Marcio Roberto. III. Bornschein, Marcos Ricardo. IV. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Zoologia.

CDD (20. ed.) 597.8



MINISTÉRIO DA EDUCAÇÃO  
SETOR SETOR DE CIÊNCIAS BIOLÓGICAS  
UNIVERSIDADE FEDERAL DO PARANÁ  
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO  
PROGRAMA DE PÓS-GRADUAÇÃO ZOOLOGIA

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ZOOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ANDRÉ EDUARDO CONFETTI** intitulada: **Canto de anúncio de *Brachycephalus brunneus* e *B. curupira* (Anura Brachycephalidae), com comentários sobre a evolução do canto em *Terraranae***, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua Aprovação no rito de defesa.

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## AGRADECIMENTOS

Gostaria primeiramente de agradecer a minha família, meus pais e irmão, por todo o apoio e incentivo que me deram para estudar cada vez mais. Não é fácil sair de casa, e se não fosse o apoio deles talvez não teria chegado onde cheguei. Não poderia deixar de agradecer minha família sem agradecer o “tio Zé” e a “tia Célia”, que assim como meus pais, sempre estiveram do meu lado fazendo o máximo pela minha formação;

Gostaria também de agradecer a minha segunda família, Izaquel e Elbe, que me acolheram em sua casa durante meu mestrado, fazendo o possível para que eu me sentisse na minha própria casa;

Não poderia deixar de agradecer também meu Orientador, o Prof. Dr. Marcio R. Pie, que aceitou me orientar, e, foi além do que é um orientador, foi um grande amigo que eu recebi, uma inspiração. De coração, muito obrigado Marcio;

Outra pessoa especial que não poderia estar de fora dessa lista é minha melhor amiga e namorada Bruna, que durante esses dois anos esteve sempre do meu lado, me apoiando e me levantando nos momentos mais difíceis, sem você eu não teria conseguido;

Também não poderia deixar de agradecer a melhor equipe de campo, meu coorientador, Dr. Marcos R. Bornschein; o Dr. Luis F. Ribeiro, que posso considerar meu outro coorientador durante essa trajetória; e o Júnior, que sem sombra de dúvidas foi graças ao incentivo (as vezes até demais) dele que eu consegui subir os morros que subi. Vocês, junto com o Marcio, são os melhores “sapólogos” com quem eu poderia trabalhar;

Gostaria de agradecer também toda a galera do Pielab, André, Daniel, Cristian, Raquel, e novamente, Junior. Graças a vocês o trabalho se tornou um local divertido, vocês são os melhores *labmates* que alguém poderia ter. Não posso deixar de agradecer a “leva antiga” do Pielab, Andreas, Patrícia e Ricardo, que me acolheram logo no início. Cabe também um agradecimento especial aos agregados do lab, a Amanda e o Alê, obrigado pelas conversas e boas risadas;

Outro laboratório que me acolheu nas mais diferentes ocasiões, foi o Laboratório de farmacologia do trato gastrointestinal e dor, em especial a Prof. Dra. Maria Fernanda, Dani e Jorge; e as “IC’s” Beatriz e Luiza, obrigado por tudo;

Quero agradecer também aos meus colegas de disciplina, turmas 2016/2 e 3 2017/1, que tornaram as disciplinas muito mais agradáveis, mas em especial a Joyce e a Sara, que tornaram aquilo que parecia impossível em algo impossível, porém com muito mais graça, obrigado por tudo;

Também gostaria de agradecer a toda a galera da herpeto aqui do departamento, André Luiz, Caio, Lucas, Michelle, Pedro, Peterson, e também Prof. Dr. Mauricio Moura, que sempre estiveram à disposição para sanar qualquer “herpetodúvida”.

Meu mais sincero obrigado também para toda a galera aqui do departamento, os amigos de corredor, em especial ao Pedro, que diretamente ou indiretamente me ajudaram no desenvolvimento (ou na procrastinação) da dissertação;

Há de ser agradecido também todo o povo do futebol de quinta, que embora tenha conhecido recentemente, foi de grande importância nessa reta final. Sem vocês eu talvez teria “surtado” muito mais;

Embora, não tenha conhecido vocês pessoalmente (ainda), estiveram comigo nesses dois anos de forma online, muito obrigado Dani, Matheus Dorigo, Pedro Jr. e Shutz. Vocês são a prova de que podemos ter amigos mesmo à distância;

Por fim, não poderia deixar de agradecer a quem diretamente ou indiretamente tenham me ajudado a escrever, ou nos momentos de lazer. Muito obrigado Metallica, Powerwolf e Turisas, por terem feito músicas tão boas. Gostaria também de agradecer à equipe da Blizzard, por terem feito essa maravilha chamada World of Warcraft, que em muitos momentos foi o responsável por tirar o estresse do mestrado.

Que a força esteja com vocês;  
Pela Aliança!

*“ Há uma teoria que indica que sempre que qualquer um descobrir exatamente o que, para que e porque o universo está aqui, o mesmo desaparecerá e será substituído imediatamente por algo ainda mais bizarro e inexplicável... Há uma outra teoria que indica que isto já aconteceu. ”*

- Douglas Adams

## RESUMO

A comunicação acústica desempenha um papel importante na biologia dos animais, sendo usada tanto para a atração de parceiro sexual quanto para a defesa de território. Tendo em vista seu importante papel, a vocalização dos anuros sofre influência direta de pressões seletivas, sendo causadas pelo meio ou pela morfologia dos animais. Com a importância da comunicação destacada, o presente trabalho foi dividido em duas partes, sendo a primeira a descrição do canto de anúncio de *Brachycephalus brunneus* e de duas populações de *B. curupira*. *Brachycephalus brunneus* e *B. curupira* apresentam um canto de anúncio pulsado, sem modulação, com alta taxa de repetição e de alta frequência. Ambas as espécies possuem o canto muito semelhante, sendo que a maior diferença está na taxa de repetição das notas, onde *B. curupira* apresenta maior repetição do que *B. brunneus*, e na estrutura do canto, onde *B. brunneus* em raras ocasiões emite notas isoladas em sua vocalização de anúncio. Na segunda parte, foi abordada a evolução do canto em Terraranae (Anura). Terraranae é um grupo de anuros que possuem desenvolvimento direto e que compreendem animais muito pequenos, como os animais do gênero *Brachycephalus*, até animais maiores como *Eleutherodactylus zeus*. Com isso foi testado o efeito que o tamanho do corpo dos animais exerce sobre a frequência dominante, onde foi encontrado uma relação proporcionalmente inversa. Além dessa relação foi encontrado sinal filogenético entre esses parâmetros utilizando métodos filogenéticos comparativos, mostrando que a relação evolutiva entre o canto e o tamanho dos animais do grupo Terraranae, mostrando assim que essa influência se dá ao longo da história evolutiva dos anuros. Por fim, podemos frisar a importância de métodos comparativos para estudar evolução de organismos biológicos.

**Palavras-chave:** *Brachycephalus*; Canto de Anúncio; Alometria; Frequência dominante; Tamanho corporal.



## ABSTRACT

Acoustic communication plays an important role in animals biology, being used both for the attraction of sexual partner and for the defense of territory. Considering its important role, the vocalization of the anurans has direct influence by selective pressures, being caused by the environment or by the morphology of the animals. With the importance of the communication, the present work was divided in two parts, the first being the description of the advertisement call of *Brachycephalus brunneus* and two populations of *B. curupira*. *Brachycephalus brunneus* and *B. curupira* have a pulsed non-modulated call, with high repetition rate and high frequency. Both species present a very similar call, with the greatest difference being in the note rate, where *B. curupira* presents a higher repetition than *B. brunneus*, and in the call structure, where *B. brunneus* rarely emits isolated notes in his advertisement call. In the second part, it was discussed the evolution of call in Terraranae (Anura). Terraranae is an anura group that have a direct development and comprise very small frogs, such as the animals of the genus *Brachycephalus*, to larger animals like *Eleutherodactylus zeus*. With this, the effect that the body size of the animals exerts on the dominant frequency was tested, where a proportionally inverse relation are founded. In addition to this relation, a phylogenetic signal was found among these parameters using phylogenetic comparative techniques, showing that the evolutionary relationship between the call and the body size of the Terraranae frogs, thus showing that this influence occurs throughout the evolutionary history of the anurans. Lastly, we can emphasize the importance of comparative methods to study the evolution of biological organisms.

**Keywords:** *Brachycephalus*; Advertisement call; Allometry; Dominant frequency; Body size.

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## LISTA DE ABREVIATURAS E SIGLAS

dB; Decibéis

H0; Hipótese nula

H1; Hipótese alternativa

PIC; *Phylogenetic Independent Contrast*

PGLS; *Phylogenetic Generalized Least Squares*

SVL; *Snout Vent Length*

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## 1. INTRODUÇÃO

A comunicação tem um papel importante na seleção de parceiros reprodutivos em espécie de animais, onde indivíduos de ambos os sexos repassam informações um para o outro permitindo seu reconhecimento e assim sua aptidão reprodutiva (Amézquita *et al.* 2006, Castellano *et al.* 2002). A seleção de parceiro por meio da comunicação ainda implica na evolução de estruturas de recepção e emissão, tanto de quem envia quanto de quem recebe o sinal (Höbel & Gerhardt 2003).

Alguns animais se adaptaram para unir as diversas formas de comunicação, como é o caso dos anuros do gênero *Hylodes*, onde há uma junção de comunicação acústica e visual (De Sá *et al.* 2016). O caso de *Hylodes* é uma adaptação ao ambiente ao qual o animal está inserido. Animais em ambiente ruidoso tendem a alterar as frequências dos campos a fim de evitar que haja perda de sinal acústico por conta do ruído. (Brumm & Slabbekoorn 2005). Por exemplo, *Crossodactylus schmidti*, tende a alterar a estrutura espectral do canto em ambientes com ruído, além de aumentarem a amplitude (dB) do canto sob pressão sonora elevada (Caldart *et al.* 2016).

O repertório acústico dos anuros é bastante amplo, e pode ser dividido em três categorias: (1) Reprodutivo (canto de anúncio, canto de corte, canto de amplexo, canto de liberação pós amplexo, canto de liberação pós oviposição, canto de chuva), (2) Agressivo (canto territorial, canto de encontro, canto de luta, canto de deslocamento), (3) Defensivo (grito de alarme, grito de agonia e grito de aviso) (Toledo *et al.* 2015).

Há muitos trabalhos na literatura citando a importância do canto em anuros, podendo inclusive influenciar na morfologia dos animais. Em *Engystomops pustulosus*, o canto exerceu uma forte pressão de seleção promovendo o surgimento de uma massa fibrosa na laringe do animal, que possibilita a emissão de uma nota atrativa para as fêmeas (Ryan & Guerra 2014; Ryan & Rand 1999). Acredita-se que a maior diversidade de anuros se encontra na região neotropical, devido principalmente às características fitogeográficas dos neotrópicos, e principalmente, a grande plasticidade de distribuição dos anuros (Wiens 2002)

A região neotropical, que se estende desde o sul do México até o sul da América do Sul, abriga a maior parte da biodiversidade de espécies animais e vegetais do globo, incluindo uma alta taxa de endemismo. (Olson *et al.* 2001). Os neotrópicos abrangem duas grandes áreas de alta biodiversidade, as florestas tropicais da América Central e as florestas tropicais da América do Sul, sendo que destacam-se dessas áreas as Florestas da Mesoamérica, Cerrado brasileiro e Floresta Atlântica, considerados *Hotspots*. De acordo com a classificação de Myers e colaboradores (2000) *Hotspots* são locais de rica biodiversidade, com um elevado grau de endemismo e ameaça. Dado esse grau de endemismo, a Região neotropical abriga um peculiar grupo de anuros de desenvolvimento direto, o clado Terraranae (Hedges *et al.* 2008) que é composto de 1103 espécies (FROST 2018).

*Brachycephalus* é um gênero de anuros da família Brachycephalidae, que faz parte de Terraranae. É um gênero extremamente peculiar, possui tamanho corpóreo reduzido, no qual algumas espécies apresentam distribuição micro endêmica em regiões montanhosas da mata atlântica brasileira, e estendendo desde o sul da Bahia, ao norte de Santa Catarina (Bornschein *et al.* 2016). O gênero compreende um total de 35 espécies descritas (Frost, 2018). Ribeiro e colaboradores (2015) dividiram o gênero em três grupos taxonômicos de acordo com suas características morfológicas, sendo eles os grupos *B. ephippium*, *B. didactylus* e *B. pernix*, restrito a região mais ao sul da distribuição (estados do Paraná e Santa Catarina). Os *Brachycephalus* do grupo *B. pernix* tem a característica principal de apresentar corpo bufoniforme e não possuírem coossificação dérmica (Bornschein *et al.* 2016). O grupo *B. pernix* compreende as seguintes espécies: *Brachycephalus actaeus*, *B. auroguttatus*, *B. boticario*, *B. brunneus*, *B. ferruginus*, *B. coloratus*, *B. curupira*, *B. fuscolineatus*, *B. izecksohni*, *B. leopardus*, *B. mariaeterezae*, *B. olivaceus*, *B. pernix*, *B. pombali*, *B. tridactylus*, e *B. verrucosus* (Ribeiro *et al.* 2015).

Dessa maneira, a presente dissertação foi dividida em dois capítulos, sendo o primeiro intitulado *Advertisement call of Brachycephalus brunneus and curupira (Anura: Brachycephalidae)*, onde foi feita a descrição do canto de anúncio de *B. brunneus* e *B. curupira*. O primeiro capítulo já está em formato de submissão de acordo com as normas da revista Zootaxa. O capítulo dois por sua vez irá abordar a evolução do canto de anúncio dentro do grupo de anuros

Terraranae, que é composto por anuros que tem como característica o desenvolvimento embrionário direto, e irá se intitular *Evolução do canto de anúncio em Terraranae*.



## 2. ARTIGO CIENTÍFICO 1

Artigo científico submetido para publicação na revista Zootaxa

### 2.1 The advertisement calls of *Brachycephalus brunneus* and *B. curupira* (Anura: Brachycephalidae)

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**Abstract**

*Brachycephalus* (Anura: Brachycephalidae) is a genus of miniaturized frogs endemic to the Atlantic Forest of Brazil, with many of its species showing microendemic distributions in montane and cloud forests. Little is known about their biology, including their acoustic communication. In this study we use a note-centered approach to describe the advertisement calls of two *Brachycephalus* species — *B. brunneus* and *B. curupira* — recorded at their respective type localities, as well as a newly discovered population of *B. curupira*. The calls of both species lasted between 0.5 and 3.3 min and were divided into isolated notes and note groups, with a note rate between 10 and 91 notes per minute. Each note showed high dominant frequencies (10-91 kHz) and was composed of 1-4 pulses. There was broad overlap in the calls of both species with respect to their acoustic parameters (e.g. call duration, frequency, number of pulses), despite subtle differences between populations of *B. curupira*. On the other hand, there were differences between species in note rates and intervals, as well as a relative scarcity of isolated notes in *B. brunneus*. Such limited interspecific differences in their calls is not unexpected, however, given that the overall lack of sympatry between *Brachycephalus* species of the *B. pernix* species group provides little opportunity for selection favoring prezygotic isolation mechanisms. On the other hand, our results underscore the utility of a note-centered approach to uncover important interspecific differences in advertisement calls within *Brachycephalus* that could be overlooked in a call-centered approach.

## 1. Introduction

*Brachycephalus* Fitzinger, 1826 is a fascinating frog genus characterized by miniaturized body size (snout-vent length around 8-18 mm) and the microendemic distribution of many of its species (Pie *et al.* 2013, Bornschein *et al.* 2016). Little is known about the biology of most *Brachycephalus* species, including their acoustic communication. Advertisement calls have been described for members of all three *Brachycephalus* species groups (*sensu* Ribeiro *et al.* 2015), namely *B. ephippium* (Pombal *et al.* 1994, Araújo *et al.* 2012, Condez *et al.* 2014, Guimarães *et al.* 2017), *B. didactylus* (Verdade *et al.* 2008, Condez *et al.* 2016), and *B. pernix* groups (Wistuba 1998, Garey *et al.* 2012, Bornschein *et al.* 2018a,b, Monteiro *et al.* 2018a, b), but the calls of most species remains unknown.

Given the lack of sympatry between the vast majority of species within species groups, one would expect that interspecific call differences within species groups of *Brachycephalus* should be relatively subtle, which indeed seems to be the case (Bornschein *et al.* 2018a). This is particularly true for species of the *B. pernix* species group, which tend to share similar calls in terms of their frequency parameters (see Table 3 in Bornschein *et al.* 2018a). However, a recent study on the call of *B. albolineatus* Bornschein, Ribeiro, Blackburn, Stanley & Pie, 2016 demonstrated that the pattern of intervals between notes and note groups (in a note-centered approach *sensu* Köhler *et al.* [2017]) provided important insight that was unnoticed in previous descriptions within the *B. pernix* species group, which tended to use a call-centered approach (i.e. considering each note as the complete call) and thus did not explicitly measured intervals between notes, nor recognized the existence of note groups (Bornschein *et al.* 2018). More studies in a note-centered approach might therefore prove to be revealing in uncovering interesting call variation between species that could otherwise be overlooked.

In this study, we describe the advertisement calls of two species of the *B. pernix* species group, namely *B. brunneus* Ribeiro, Alves, Haddad & Reis, 2005 and *B. curupira* Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017 using a note-centered approach, based on recordings at their type localities. In addition, over the course of our field work in Morro do Canal, state of Paraná, we were able to uncover a new population of *B. curupira*, which gave us the opportunity to also assess intraspecific variation in their advertisement calls.

## 2. Materials and methods

We recorded one advertisement call per individual of a total of 14 individuals of *Brachycephalus brunneus* from Pico Caratuva (25°14'33"S, 48°50'04"W) on January 24<sup>th</sup>, 2017 and one advertisement call per individual of a total of 16 individuals of *B. curupira* from Serra do Salto (25°42'07"S, 49°03'44"W) on several occasions between November 15<sup>th</sup>, 2012 and January 28<sup>th</sup>, 2016. These sites correspond to the type localities of each species, which are in the state of Paraná, southern Brazil (Ribeiro *et al.* 2005, 2017). We did not collect voucher specimens, given that these are relatively small and vulnerable populations and that several specimens from exactly those locations were obtained for their descriptions. We also obtained one advertisement call record per individual of a total of 10 individuals of a population of *Brachycephalus curupira* from Morro do Canal, state of Paraná (25°30'55"S, 48°58'56"W, see Bornschein *et al.* [2016]) on January 27<sup>th</sup>, 2017. Individuals of this population match the diagnostic characters of *B. curupira*, including an overall brown coloration and the presence of a golden iris that are the main diagnostic characters of the species (Ribeiro *et al.* 2017). We did not collect vouchers of calling individuals from this location, but representative specimens from *B.*

*curupira* from Morro do Canal have been deposited at Museu de História Natural do Capão da Imbuia (MHNCI) in Curitiba, state of Paraná.

We used a Sony PCM-M10 digital recorder coupled to an external Sennheiser ME 66/K6 shotgun microphone and Tascam DR44-WL digital recorder coupled to an external Sennheiser ME 67/K6 shotgun microphone. All recordings were obtained in wave format. Air temperature and relative humidity during recordings were obtained using a digital thermohygrometer with internal sensor to measure the air temperature and relative humidity and an external sensor to measure the temperature of the leaf litter. Air temperature during recordings at Pico Caratua ranged between 16.7-20.9 °C, leaf litter temperature ranged between 15.8-17.4 °C, and air relative humidity varied between 87-88%. At Serra do Salto, air temperature during recordings ranged between 18.6-22.6 °C, leaf litter temperature ranged between 17.5-21.3 °C, and relative humidity varied between 64-100%. At Morro do Canal, air temperature during recordings ranged between 16.2-17.1 °C, leaf litter temperature was 16.5 °C throughout all recordings, and air relative humidity varied between 93-100%. Given the difficulty in the access to these locations, it was difficult to standardize a time of the day for recordings, but all of the analyzed calls were carried out from 09:00 to 18:00h. Recordings were deposited in the MHNCI collection. We analyzed 10-20 notes per call using a sampling frequency rate of 44.1 kHz and 16 and 24-bit resolution in the mono pattern. Bioacoustic analyses were carried out using Raven Pro 1.4 (Bioacoustics Research Program 2011), whereas spectrogram and oscillogram plots were generated using the package SEEWAVE v. 2.0.5 (Sueur *et al.* 2008) in R 3.2.2 (R Development Core Team, 2017). Spectrograms were obtained using a Fast Fourier Transform of 256 points, overlap 50%, and Hamming window type. We used the color scheme of Standard Gamma II in Raven Pro. The brightness and contrast of the spectrogram were set at 80 for brightness and 50 for contrast 24-bit notes and 50

for brightness and 50 for contrast on 16-bit notes so as to make their color palette similar to one another and facilitate their comparison.

We estimated a total of 17 acoustic parameters (units between brackets): 1) call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of the call including only note groups (s); 4) note rate (notes per minute, with time being counted from the first note to immediately before the last note of the call); 5) number of notes per call; 6) number of isolated notes per call; 7) number of note groups per call; 8) note duration of isolated notes (s); 9) duration of note groups (s); 10) number of pulses per isolated notes; 11) number of pulses in each note within note group; 12) inter-note interval in isolated notes (s), defined as the time from the end of one isolated note to the beginning of the next note isolated note; 13) inter-note group interval (s), defined as the time from the end of one note group to the beginning of the next note group; 14) inter-note interval within note groups (s), defined as the time from the end of the first note to the beginning of the next note of the same note group; 15) note dominant frequency (kHz); 16) highest frequency (kHz); and 17) lowest frequency (kHz). The definition of the advertisement call and definitions of acoustic parameters follow Köhler *et al.* (2017), in which we use a note-centered approach (i.e. defining the entire coherent unit of sound as a call), and the modification of some parameters as in Bornschein *et al.* (2018). We estimated the mean  $\pm$  1 standard deviation for all the parameters.

### 3. Results

Measurements of the acoustic characteristics of advertisement calls of *Brachycephalus* according to their corresponding populations are provided in Table 1. The advertisement calls of *B. brunneus* (Fig. 1A) and *B. curupira* (Fig. 1B) shared the same overall structure of a succession of isolated, pulsated notes, followed by note groups

of 2-4 notes (with a few cases of note groups with 5 notes in *B. curupira*). The notes that make up note groups are similar to those emitted in isolation at the beginning of the call (Tables 1, 2). However, isolated notes were rare in *B. brunneus* and only occurred in four of the recorded calls (two at the beginning of the calls and two in-between note groups, accounting for 4 of all 220 recorded notes), such that the duration of the calls including only note groups was nearly identical to the duration of their complete calls (Table 1).

There was broad overlap among calls of *Brachycephalus brunneus* and *B. curupira* parameters related to call/note duration, frequencies, number of pulses, notes, and note groups (Table 1). On the other hand, note rate was 92.3% faster in *B. curupira* in relation to *B. brunneus* (Table 1). Interestingly, the difference between the calls of each species is associated with a faster emission of note groups while maintaining comparable inter-note intervals within note groups. This difference in overall structure of the calls, together with the relative rarity of isolated notes in *B. brunneus* (only 1.8 % of all recorded notes) in relation to their frequency in *B. curupira* (6.4 % of all recorded notes), represents the main contrast between the advertisement call of those species.

There was broad overlap between the ranges of estimated spectral acoustic parameters between the population of *B. curupira* from Morro do Canal and Serra do Salto, despite some variation in average values (Table 1). However, the population of Morro do Canal tended aspects of the higher *tempo* of the call structure in relation to the population from Serra do Salto, such as similar inter-note group intervals and inter-note interval within note groups.

#### **4. Discussion**

In this study we adopted a note-centered approach (*sensu* Köhler *et al.* 2017) to the description of advertisement calls of *Brachycephalus*. As argued elsewhere (Bornschein

*et al.* 2018), we believe that using a note-centered approach facilitates comparisons with calls of congeners, as well as underscores the considerable differences in call structure among species groups. Moreover, the most obvious differences between the calls of *B. brunneus* and *B. curupira* such as variation in note rates could easily be overlooked in a call-centered approach, as commonly used in in the *B. pernix* species group. Unfortunately, interspecific temporal variation between notes is poorly known in species of *Brachycephalus* in general, and in the *B. pernix* group in particular, but the available evidence is highly suggestive that they might represent important bioacoustic characteristics. For instance, *B. tridactylus* does not emit note groups (Bornschein *et al.* 2018), as opposed to the note groups found in *B. albolineatus* Bornschein, Ribeiro, Blackburn, Stanley & Pie, 2016 (Bornschein *et al.* 2018) and *B. mirissimus* Pie, Ribeiro, Confetti, Nadaline, and Bornschein, 2018 (Pie *et al.* 2018). The number of notes per note groups might also vary, from 2 notes per note groups in *B. albolineatus* and *B. mirissimus* to 4-5 notes per note group in *B. brunneus* and *B. curupira*. As new *Brachycephalus* calls are described within a note-centered approach, the temporal structure of these calls might provide valuable information to delimit species within this species group. The advertisement call of *B. pernix* species group, with exception of *B. tridactylus*, shows the same characteristic that have their call composed by isolated notes and note groups. The *B. pernix* species group has similar values of dominant frequency of the call, with exception of *B. tridactylus*, which has lower dominant frequency. Most of the differences between the advertisement call of *B. pernix* species group is on the temporal variation of the call. This variation is evidenced when we use the note-centered approach.

Given that *B. sulfuratus*, of the *B. didactylus* species group, has been recorded in the Atlantic forest of the state of Paraná (Bornschein *et al.* 2016, Condez *et al.* 2016), one could be tempted to assume that some of our recordings could actually belong to that



species, given that species. In particular, because all three species call from underneath the leaf litter, direct observations of calling males is precluded. However, we can safely rule out this possibility given the substantial difference between the calls of *B. sulfuratus* and all species of the *B. pernix* species group whose call has been studied to date, including the regular repetition of five or six high-frequency pulsed notes that allows one to easily recognize their calls in the field.

Although the population of Morro do Canal presents all of the diagnostic characters of *B. curupira*, including the presence of a golden iris that is absent from *B. brunneus* (Ribeiro *et al.* 2017), as well as being consistent with genetic evidence (Pie *et al.* 2017), we found some intriguing differences between them. At this point it is unclear whether these subtle differences represent genuine geographical differences in their advertisement rates or simply variation in arousal state between males in relation to environmental conditions and/or the reproductive season.

### **Acknowledgments**

MRP was supported through a grant from CNPq/MCT (301636/2016-8571334/2008-3). Fieldwork was funded by Fundação Grupo Boticário de Proteção à Natureza, through a project (A0010\_2014) conducted by Mater Natura - Instituto de Estudos Ambientais.

**Figure captions**

**FIGURE 1.** Oscillograms and spectrograms of the advertisement calls of *Brachycephalus brunneus* (A, recorded on January 24<sup>th</sup>, 2017) and *B. curupira* (B, recorded in Serra do Salto on January 22<sup>th</sup>, 2016, and C, recorded in Morro do Canal on January 27<sup>th</sup>, 2017). Oscillograms and spectrograms represent instances of note groups (D, E, F) and individual notes (G, H, I) of the calls immediately above them. A–C were made with 8192 FFT resolution and D–I with 512 FFT resolution.

Figure 1

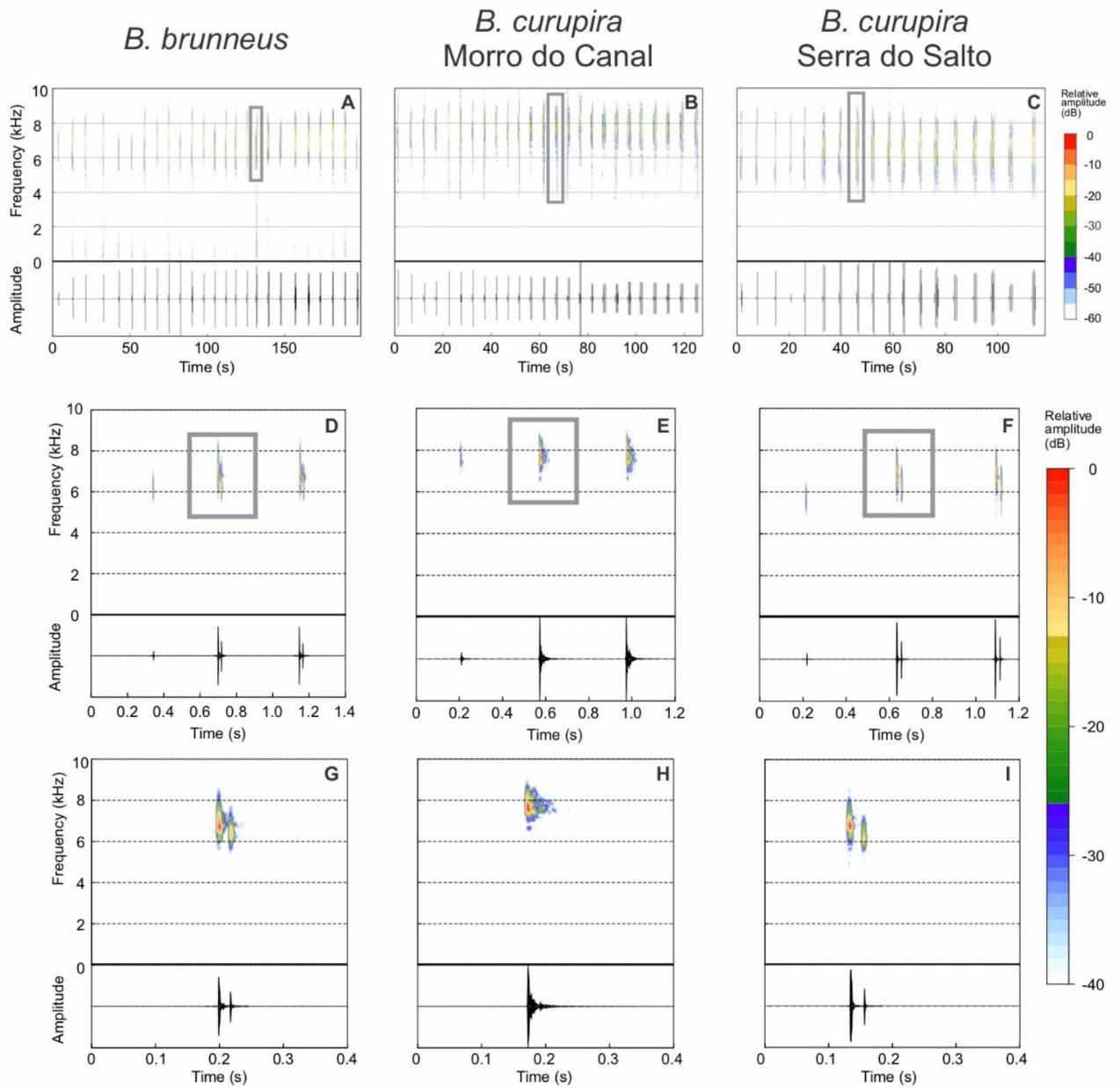


Table 1. Measurements of advertisement calls of the studied *Brachycephalus* populations. Values are provided as mean±SD (range; N).

Measurement	<i>B. brunneus</i>	<i>B. curupira</i> (Serra do Salto)	<i>B. curupira</i> (Morro do Canal)
1. Call duration (s)	123.6±51.4 (30.8-198.9; N=14)	67.2±24.1 (27.5-113.3; N=16)	115.8±27.6 (77.0-158.7; N=10)
2. Duration of the call including only isolated notes (s)	- <sup>1</sup>	8.7±6.6 (2.8-17.3; N=5)	10.5±13.3 (0.02-40.6; N=8)
3. Duration of the call including only note groups (s)	117.8±49.9 (29.9-191.1; N=13)	62.6±26.6 (18.9-113.3; N=16)	102.6±31.5 (57.4-147.5; N=10)
4. Note rate (notes per minute)	20.1±4.5 (10.8-29.8; N=14)	45.9±13.4 (28.1-71.1; N=16)	32.5±2.5 (27.6-35.7; N=10)
5. Number of notes per call	39.4±14.4 (10-63; N=552)	48.1±13.8 (30-83; N=770)	66.8±17.6 (41-91; N=468)
6. Number of isolated notes per call	1.4±0.5 (1-2; N=5)	4.8±2.9 (2-8; N=24)	2.8±2.2 (1-8; N=23)
7. Number of note groups per call	15.4±6.3 (4-26; N=216)	13.8±5.1 (8-24; N=221)	19.5±19.5 (10-28; N=195)
8. Number of pulses per isolated notes	1.5±0.5 (1-2; N=7)	1.0±0.2 (1-2; N=26)	1.1±0.3 (1-2; N=13)
9. Number of pulses in each note group	1.9±0.8 (1-4; N=1052)	1.7±0.5 (1-3; N=1238)	1.3±0.8 (1-2; N=627)
10. Note duration of isolated notes (s)	0.011±0.011 (0.002-0.022; N=4)	0.005±0.003 (0.002-0.010; N=8)	0.02±0.008 (0.008-0.030; N=12)
11. Duration of note groups (s)	0.7±0.3 (0.3-1.4; N=65)	0.8±0.5 (0.2-1.9; N=78)	0.7±0.3 (0.3-1.7; N=52)
12. Inter-note interval in isolated notes (s)	- <sup>1</sup>	2.6±0.3 (2.0-3.1; N=20)	4.2±2.2 (4.8-5.8; N=19)
13. Inter-note group interval (s)	8.3±2.3 (3.7-23.4; N=44)	4.0±1.5 (1.4-6.2; N=81)	4.9±2.2 (4.0-6.0; N=50)
14. Inter-note interval within note groups (s)	0.4±0.04 (0.3-0.5; N=105)	0.3±0.03 (0.2-0.4; N=167)	0.3±0.02 (0.3-0.4; N=87)
15. Note dominant frequency (kHz)	6.6±0.2 (5.8-7.0; N=68)	6.8±0.4 (4.9-7.6; N=325)	7.2±0.3 (6.8-7.5; N=103)
16. Highest frequency (kHz)	8.0±0.4 (7.0-9.0; N=68)	8.0±0.5 (5.9-9.5; N=325)	8.4±0.3 (7.8-8.8; N=103)
17. Lowest frequency (kHz)	5.3±0.4 (4.1-6.1; N=68)	5.2±0.6 (3.1-6.9; N=325)	5.9±0.5 (5.2-6.6; N=103)

<sup>1</sup> There were no two successive isolated notes recorded for the *B. brunneus*.

## Figure List

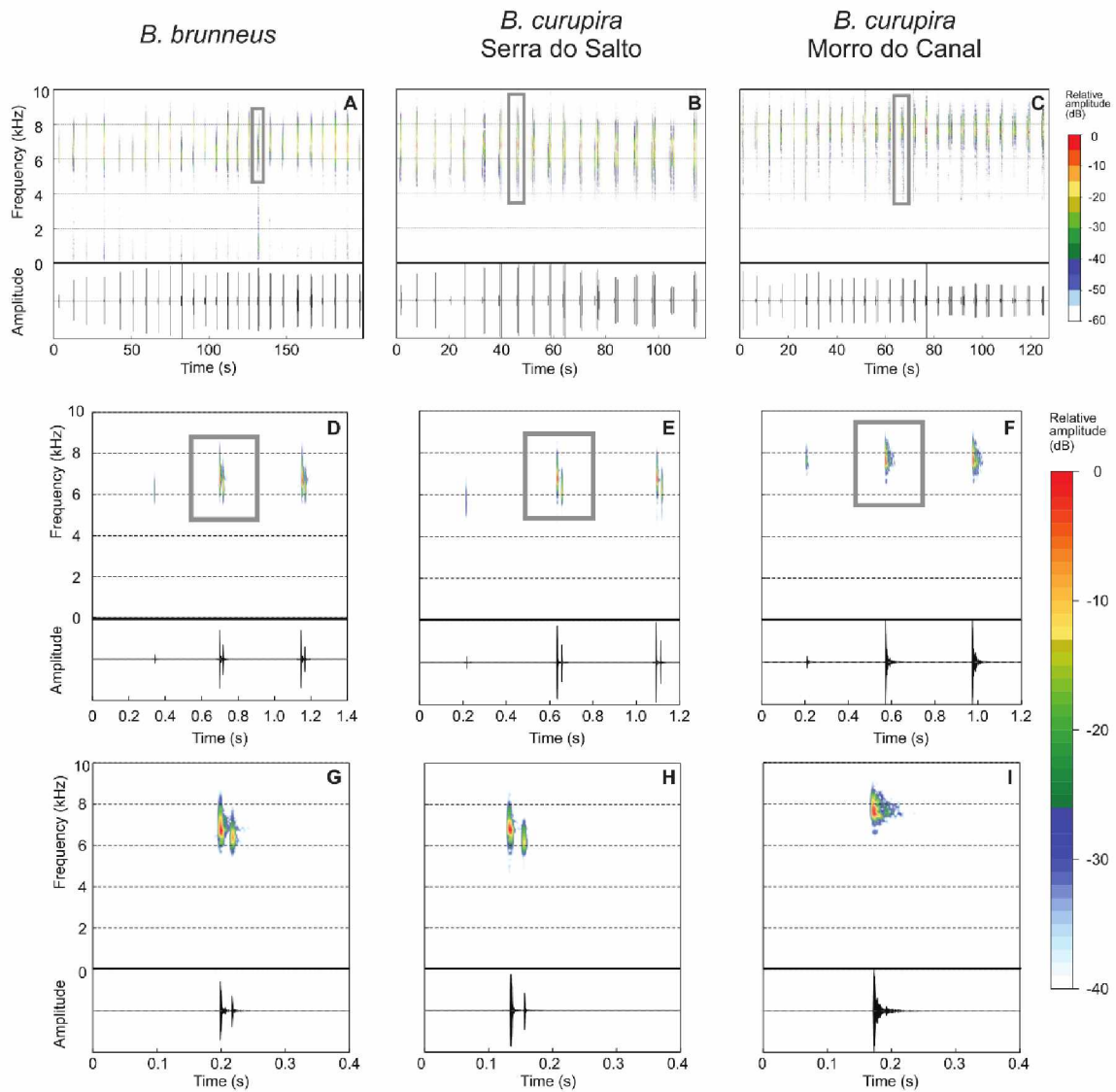


Figure 1. Advertisement calls of *Brachycephalus brunneus* (A, recorded on January 24th), and *B. curupira* (B, recorded in Serra do Salto on January 22th, 2016; and C, recorded in Morro do Canal on January 27th, 2017). Figures below them represent instances of note groups (D, E, F) and individual notes (G, H, I) of the call immediately above them. A–C were made with 8192 FFT resolution; D–I with 512 FFT resolution.

### 3. ARTIGO CIENTÍFICO 2

RESEARCH ARTICLE: PeerJ

#### 3.1 Evolution of Terraranae (Anura) call

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**Abstract**

The communication has an important role in the animal social behavior. For the most animals, the acoustic communication is used to attract reproductive partner, and, because of that, the vocalization of animals may be considered a pre zygotic barrier mechanism. The aim of this work is test the relation that body size exerts in the vocalization of direct development anurans (Anura: Terraranae), and how the vocal characteristics evolved in Terraranae frogs. The data matrix was obtained with search in internet with “advertisement call” and Terraranae genus as keywords. We used phylogenetic comparative analysis to measure the evolutionary rates of advertisement call of Terraranae frogs. We found phylogenetic signal in acoustic parameters, with this we performed an evolutionary rates analysis where we saw that minimum frequency evolve faster than the others acoustic parameters. This work shows too the relationship of the body size on the dominant frequency of the call, when we compared this parameters in a phylogenetic context we find that exist an inverse relation between the body size and the dominant frequency of the call. The use of phylogenetic analysis can help us to fill a gap about the evolutionary history of animals, and this work give us a better understanding over how the advertisement call evolve in Terraranae frogs.

## 1. Introduction

Communication – the transfer of information from emitter to a receptor – plays an important role for animal evolution (Castellano et al., 2002; Amézquita et al., 2006). This is particularly true in anurans, which rely on acoustic communication for territory defense and sexual mate attraction (Toledo et al., 2015). Given this importance, one would expect strong selective pressures on this character, which is among the first mechanisms of pre-zygotic isolation of the anurans. (Sasa et al., 2008). Anuran vocalization consists of the passage of air from the lungs through the vocal chords and vocal sacs (Martin & Gans, 1972; Ryan & Guerra, 2014). Some of the evolutionary constraints on anuran communication have already been identified. For instance, Ryan & Guerra (2014) describe the importance of larynx size on sound production in *Engystomops* (= *Physalaemus*) *pustulosus*, and because of that, this anatomic structure is under direct selective pressure. Another characteristic of the call of *E. pustulosus* is its structure, which is composed by two parts, the *wine* and *chuck calls*, which in turn are directly related to the size of the larynx (Ryan & Guerra, 2014).

With the importance of the vocalization on reproduction, anurans tend to shape their call according to the environment in which they live to avoid acoustic attenuation. (Brumm & Slabbekoorn, 2005). Some studies on *Hylodes* describe different mechanisms of visual communication associated with vocalization to compensate the environment noise (De Sá, Zina & Haddad, 2016). This adaptability of the anuran call in relation to the environment is described in the acoustic adaptation hypothesis, which posits that the anurans tend to change the temporal and spectral parameters of the call according to your habitat and microhabitat (Ey & Fischer, 2009; Goutte et al., 2018). The physical characteristics of the environment tend to mold the vocalization, particularly by the



propagation mechanisms, such as wind or places where the call may reverberate, like rocks and trees (Ey & Fischer, 2009). According to that theory, animals in closed habitats tend to emit calls with: 1) longer duration; 2) lower repetition rates; 3) less frequency modulating elements; 4) low maximum frequency; 5) low minimum frequency; 6) low medium frequency; 7) low dominant frequency; 8) narrow frequency bandwidth; and, may be the opposite to open ambient animals. (Ey & Fischer, 2009). The influence of the open or closed environment on the structure of the anuran call are given as if certain. However, Goutte et al. (2013, 2018) show the importance of the microhabitats to acoustic propagation, being more complex than simply the environment macrostructure.

Besides the environment, another factor that can influence the anuran call is their body size. Many authors have tested a negative relation of the body size on the dominant frequency of the call (Zweifel, 1968; Ryan, 1980; Sullivan, 1982; Ramer, Jenssen & Hurst, 1983; Given, 1987; Wagner, 1989; Gerhardt, 1991; McClelland, Wilczynski & Ryan, 1996; Bee, 2000; Smith & Roberts, 2003; Gingras et al., 2013; Goutte et al., 2016). Based on this hypothesis, the allometric relation between body size and dominant frequency, we chose as the target of the work the anuran group Terraranae. Terraranae is a new world anuran taxonomic group, which has the singular characteristic of presenting direct development during the embryonic stage, and comprise the families: Brachycephalidae; Caugrastoridae; Eleutherodactylidae; Phyzelaphrynae e Strabomantidae (Hedges et al. 2008). At this moment, Terraranae has 1103 described species, about 16% of all anuran species in the world (Frost, 2018). The goals of this work are (1) to test the relationship between body size x dominant frequency (body size-frequency allometry hypothesis) in a phylogenetic context, and (2) to assess whether different bioacoustic parameters of their calls evolve at different rates.

## 2. Methods

We compiled a dataset through a bibliographical search on the Google Scholar platform using as keywords “Advertisement call” and the family names of Terraranae. The dataset was built based on five call parameters, namely minimum frequency (Hz); maximum frequency (Hz); frequency bandwidth (Hz); dominant frequency (Hz), and total call duration (s) (the duration of call was standardized according Köhler et al., 2017). We also obtained the corresponding log-transformed snout-ventral length (SVL) (mm) of each species as a proxy for body size. The SVL values were available from the mean value between male and female sizes. The final dataset included 95 species, with at least one representative from the following genera: *Adelophryne*, *Barycholos*, *Brachycephalus*, *Bryophryne*, *Craugastor*, *Diasporus*, *Eleutherodactylus*, *Haddadus*, *Holoaden*, *Ischnocnema*, *Oreobates*, *Phyzelaphryne*, *Pristimantis*, and *Psychrophrynella*. Some parameters were not available for all species (Supplementary Table S1). The phylogenetic relationships among the studied lineages were based on the phylogeny of Jetz & Pyron (2018).

We estimated the degree of phylogenetic signal in each of the studied variables using Blomberg’s  $K$  (Blomberg; Garland & Ives, 2003) and Pagel’s  $\lambda$  (Pagel, 1999), being  $\lambda$  a scale between the correlations of the species in relation to that expected by Brownian evolution, and  $K$  a ratio between the scale of the variance of the species on the variance of the contrasts, using the PHYTOOLS 0.6-44 package (Revell 2012). Given that most traits showed evidence of significant signal (see below), we tested their relationship with body size using PGLS (Phylogenetic Generalized Least Squares analyses). Finally, we explored patterns of trait evolution using ancestral trait reconstruction by maximum likelihood using the function `contmap` in PHYTOOLS. All analyses were carried out using R 3.4.3 (R Core Team 2018).

### 3. Results

We found significant phylogenetic signal by Blomberg's  $K$  and Pagel's  $\lambda$  for most acoustic parameters (Table 1), except for frequency bandwidth and call duration, which was significant for  $\lambda$  but not for  $K$  ( $p_{\lambda} = 1.0$  vs.  $p_K < 0.001$ ). One possibility for this discrepancy is the large polytomy in the genus *Pristimantis* (see below), which could potentially bias estimates of phylogenetic signal. Indeed, when these analyses were repeated without *Pristimantis*, both tests agree that there is no evidence of phylogenetic signal in this trait ( $p_{\lambda} = 0.501$  vs.  $p_K < 0.102$ , see Table S2). We performed too an evolutionary rates analysis with SVL and the acoustic parameters (dominant frequency, minimum and maximum frequency), and, interestingly, minimum frequency evolved faster than both dominant and maximum frequency (Table 2). Frequency bandwidth and call duration doesn't show phylogenetic signal, because of that we not performed evolutionary rate analysis to this parameters.

PGLS analyses were performed between SVL and the studied acoustic variables. The relationship between body size and dominant frequency and body size and minimum frequency was statistically significant (Fig. 1 & 2, Table 3). This result was corroborated by the ancestral character reconstruction analysis, which showed that body size and dominant frequency showed very similar evolutionary trajectories between lineages (Fig. 3). It is important to note that nearly identical results were obtained after omitting *Prismantis*, with the exception that the relationship between the body size and the maximum frequency show significant result (Table S3).

### 4. Discussion

An inverse relationship was found between body size and dominant and minimum frequency of advertisement calls in Terraranae. Studies on the relationship between body

size and acoustic parameters of frog communication have been explored in the literature for a long time (Bartholomew & Collias, 1962, Ryan, 1980; Sullivan, 1982; Given, 1987; Wagner, 1989; Gingras et al., 2013; Goutte et al., 2016). Indeed, the importance of body size in acoustic communication has been investigated in a variety of animal groups, including birds (Ryan & Brenowitz, 1985; Wiley, 1991; Podos, 2001; Martin et al., 2011), mammals (Fitch, 1997; Rendall et al., 2005; Ey, Pfefferle & Fischer, 2007; Masataka, 2013; Bowling et al., 2017), and frogs (Bartholomew & Collias, 1962, Ryan, 1980; Sullivan, 1982; Given, 1987; Wagner, 1989; Gingras et al., 2013; Goutte et al., 2016). Our work went considerably farther than previous studies in this area in two main respects. First, to the best of our knowledge, our study was the first to test for the relationship between body size and frequency of the call (minimum, maximum, and dominant frequency) using phylogenetic comparative methods (i.e. PGLS), and thus is not plagued with the widely recognized potential biases of considering species as independent data points. This factor have allowed us to demonstrate that the relationship between body size and frequency of advertisement calls in Terraranae is indeed robust.

One possible mechanism for the relationship of body size and acoustic parameters we found might be related to the amount of air passing through the vocal cords and the anatomical cavities of the structures responsible for calling (McClelland, Wilczynski & Ryan, 1996). Alternatively, the differentiation of the anatomical structures may be involved in the evolution of the acoustic traits, as found in *Engystomops pustulosus*, in which the preference of the females was able to select the call, which, consequently, affected the morphology of the larynx and the properties of call of the species (Ryan, Rand & Weigt, 1996; Ryan & Guerra, 2014). Similar instances have been reported for birds, in which the size and opening of the beak affects the sound being produced, as is the case of Darwin's finches (Podos, 2004; Huber & Podos, 2006). Body size itself can

influence the size of the anatomical structures involved in call production (beak, in the case of birds, or larynx and vocal chords / sacs for primates and anurans). This association between body size with dominant frequency can also be influenced by the acoustic preference of females, given that for many anurans, the dominant frequency is an important parameter of sexual selection of the call (Howard & Palmer, 1995; Howard & Young, 1998).

We found significant evidence of phylogenetic signal for spectral parameters of the Terraranae vocalization (maximum frequency, minimum frequency and dominant frequency), which allowed us to estimate their corresponding evolutionary rates. We found a highest rate of evolution of minimum frequency, followed by dominant frequency and maximum frequency. Lower frequencies tend to be selected in closed environments, their propagation in the environment is more effective, due to the physical structure of the call (Ey & Fischer, 2009, Gerhardt, 1994). Prestwich et al. (1989) tested the energy efficiency of call of three species of hyloid frogs, to which they concluded that lower intensity frequencies are more efficient to travel longer distances, maximizing the transmission of information. Low frequencies may be related not only to the increase in the size of the animals, but may be influenced by the increase of the vocal apparatus itself, regardless of the body size of the animals (Fitch & Hauser, 1979). The low frequencies emission is related to the size and mass of the larynx, since the larger the structure, the lower the frequency of vibration (McClelland, Wilczynski & Ryan, 1996).

(DISCUTIR O RESGATE)

## **5. Conclusions**

The dominant frequency of an animal's call is directly influenced by body size. This influence occurs through a proportionally inverse relation, in which larger animals emit

low frequency sounds, whereas smaller animals can vocalize at high frequency, and this relationship occurs gradually over the evolution of the groups. The vocal frequency and body size values of the animals presented a phylogenetic signal, so we can consider that the evolution of the call frequency in the Terraranae advertisement call occurred together with the evolution of the body size of these animals. With this, we can emphasize the importance of evolutionary analysis in the comparison of evolutionary importance characters, as is the case of advertisement call with the use of these techniques, we can expand our view not only on the relation that is exerted, but also on the evolutionary patterns along the line of the study group.

## 7. Figure List

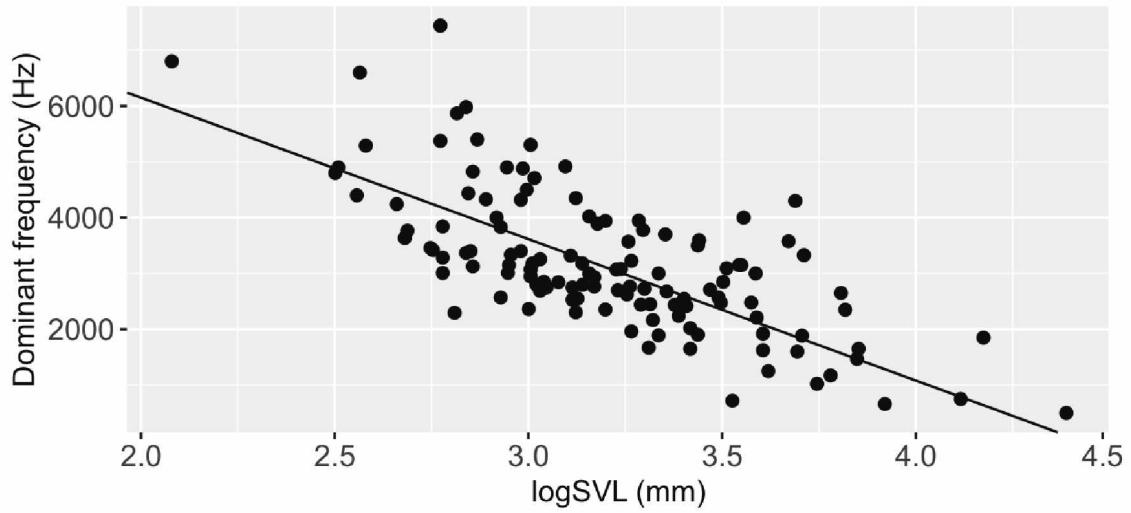


Figure 1. Phylogenetic Generalized Least Squares analysis of the relationship with log of body size (logSVL) between the dominant frequency of the Terraranae anurans.

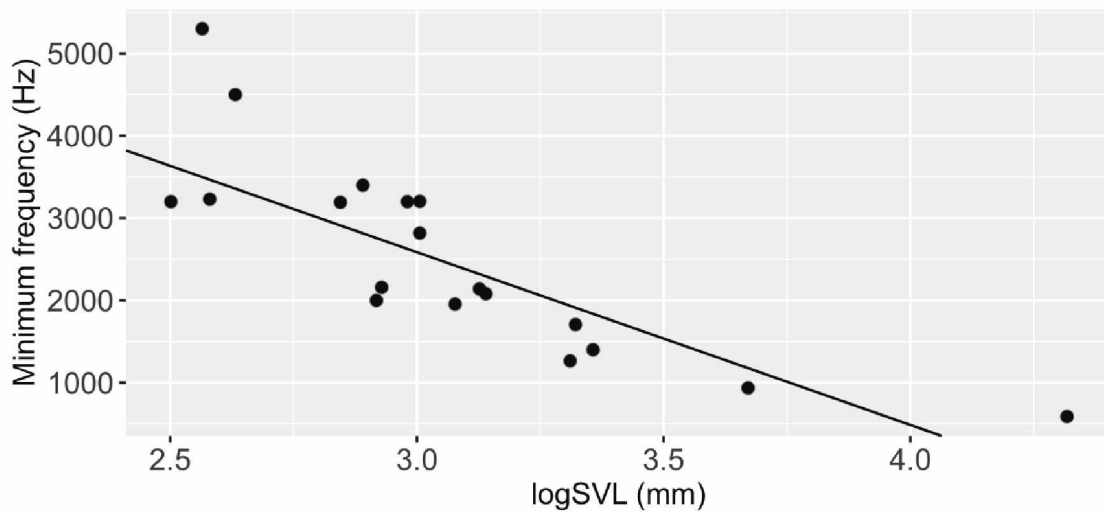


Figure 2. Phylogenetic Generalized Least Squares analysis of the relationship with log of body size (logSVL) between minimum frequency of the Terraranae anurans.

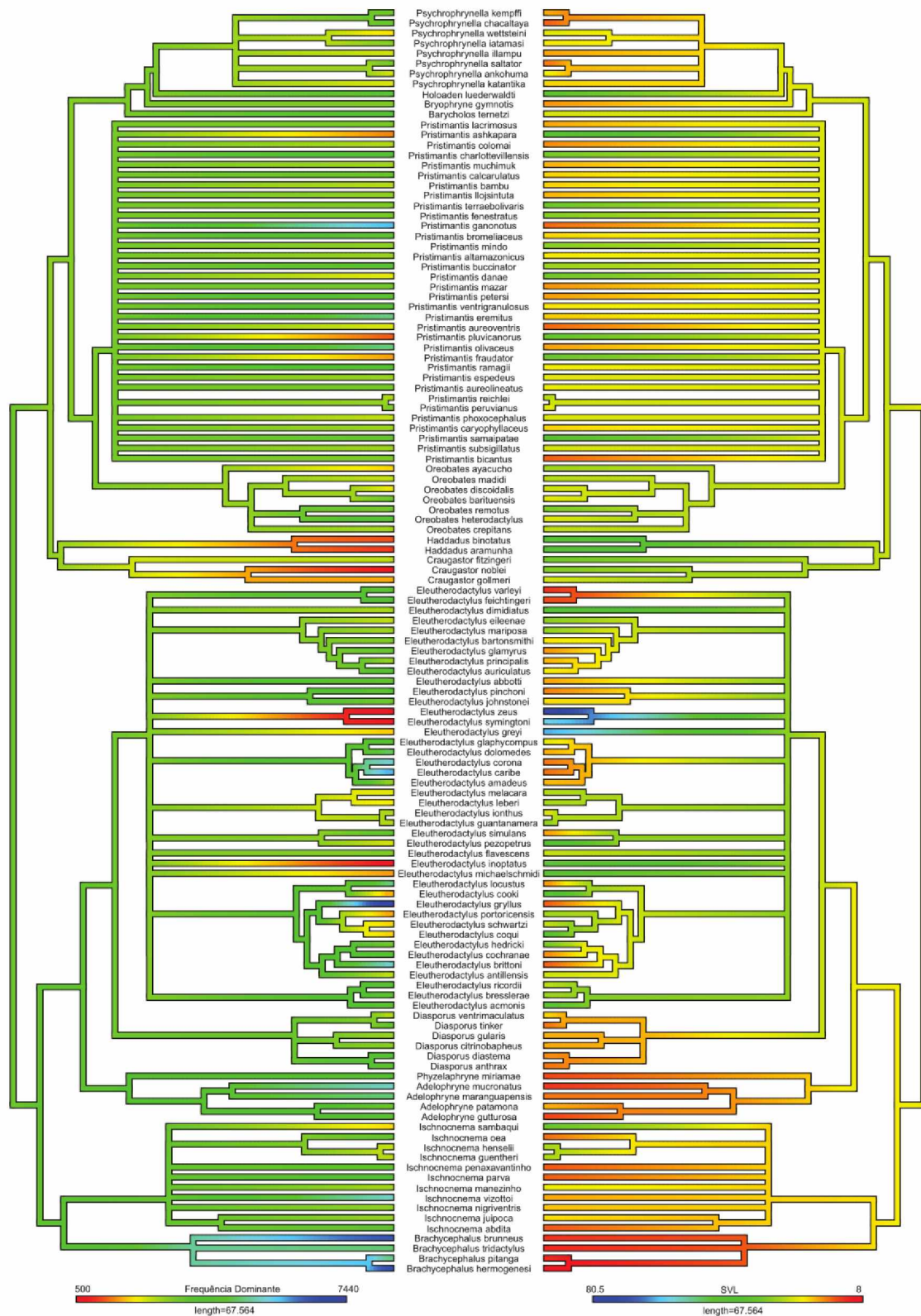


Figure 3. Ancestral state reconstruction analysis. Warm colors represents lower values than colder colors, which in turn, higher values, both for the dominant frequency, and for SVL values. In Terraranae anurans, centered values of both parameters are more common in recent lines. The relationship between body size and dominant frequency tends to change together through the evolution of the clade.



## 8. Table List

Table 1. Values of the phylogenetic signal tests, using the Blomberg's K and Pagel's  $\lambda$ .

	K	p	$\lambda$	logL ( $\lambda$ )	logL( $\lambda=0$ )	p
Body size	0.55	0.001	0.78	-40.03	-54.78	<0.001
Minimum frequency	0.85	0.031	0.88	-239.28	-240.94	0.068
Maximum frequency	0.93	0.029	0.99	-232.58	-233.43	0.190
Dominant frequency	0.44	0.002	0.55	-1063.59	-1068.17	<0.001
Call duration	0.28	0.627	<0.001	-276.77	-276.77	1

Table 2. Values of the evolutionary rates from body size and acoustics parameters.

	sigsq1	log-Likelihood	N
Body size	2.36	-468.33	125
Minimum frequency	44521.65	-232.58	26
Maximum frequency	23514.59	-240.08	28
Dominant frequency	39930.61	-1076.63	125

Table 3. p, t and slope values of the PGLS tests between acoustic parameters and body size.

	Slope	t-value	P-value
SVL x Dominant frequency	-2098.7	-8.49	<<0.001
SVL x Minimum frequency	-48.41	-2.16	0.039
SVL x Maximum frequency	-55.26	-1.78	0.087

## 9. Supplementary Material

Table S1. Raw data used in the analysis. N/A = Not available measures.

Species	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Frequency Bandwidth (Hz)	Dominant Frequency (Hz)	Call Duration (s)	SVL (mm)
<i>Adelophryne gutturosa</i>	N/A	N/A	N/A	4242	1.27	14.3
<i>Adelophryne maranguapensis</i>	N/A	N/A	N/A	4824.3	N/A	17.4
<i>Adelophryne mucronatus</i>	3230	6090	2860	5290	0.029	13.2
<i>Adelophryne patamona</i>	N/A	N/A	N/A	3255	0.63	20.7
<i>Barycholos ternetzi</i>	N/A	N/A	N/A	3777	0.049	27
<i>Brachycephalus brunneus</i>	5300	8000	2700	6600	0.38	13
<i>Brachycephalus ephippium</i>	3400	5300	1900	N/A	0.112	18
<i>Brachycephalus hermogenesi</i>	N/A	N/A	N/A	6800	1.16	8
<i>Brachycephalus pernix</i>	4500	6700	2200	N/A	0.045	13.9
<i>Brachycephalus pitanga</i>	N/A	N/A	N/A	4900	N/A	12.3
<i>Brachycephalus tridactylus</i>	3200	6400	3200	4800	0.11	12.2
<i>Bryophryne gymnotis</i>	N/A	N/A	N/A	3010	0.121	19.05
<i>Craugastor augusti</i>	589	1017	428	N/A	0.43	75
<i>Craugastor fitzingeri</i>	N/A	N/A	N/A	2479.5	2.563	35.7
<i>Craugastor gollmeri</i>	1265	3955	2690	1670	1.734	27.4
<i>Craugastor noblei</i>	N/A	N/A	N/A	719.7	0.148	34
<i>Craugastor raniformis</i>	N/A	N/A	N/A	3237.6	1.301	N/A
<i>Diasporus anthrax</i>	3192.2	N/A	N/A	4437.5	0.06	17.2
<i>Diasporus citrinobapheus</i>	2818	3190	372	2946.7	0.19	20.2
<i>Diasporus diastema</i>	2158.7	N/A	N/A	3831.7	0.12	18.7
<i>Diasporus gularis</i>	N/A	N/A	N/A	2800	0.11	20.5

<i>Diasporus tinker</i>	N/A	N/A
<i>Diasporus ventrimaculatus</i>	2140	2995
<i>Eleutherodactylus abbotti</i>	N/A	N/A
<i>Eleutherodactylus acmonis</i>	N/A	N/A
<i>Eleutherodactylus amadeus</i>	N/A	N/A
<i>Eleutherodactylus antillensis</i>	N/A	N/A
<i>Eleutherodactylus auriculatus</i>	N/A	N/A
<i>Eleutherodactylus bartonsmithi</i>	N/A	N/A
<i>Eleutherodactylus blairhedgesi</i>	N/A	N/A
<i>Eleutherodactylus bresslerae</i>	N/A	N/A
<i>Eleutherodactylus brittoni</i>	N/A	N/A
<i>Eleutherodactylus caribe</i>	N/A	N/A
<i>Eleutherodactylus cochranae</i>	2000	4800
<i>Eleutherodactylus cooki</i>	N/A	N/A
<i>Eleutherodactylus coqui</i>	N/A	N/A
<i>Eleutherodactylus corona</i>	N/A	N/A
<i>Eleutherodactylus dimidiatus</i>	N/A	N/A
<i>Eleutherodactylus dolomedes</i>	N/A	N/A
<i>Eleutherodactylus eileenae</i>	N/A	N/A
<i>Eleutherodactylus eneidae</i>	N/A	N/A
<i>Eleutherodactylus feichtingeri</i>	N/A	N/A
<i>Eleutherodactylus flavescens</i>	N/A	N/A
<i>Eleutherodactylus glamyrus</i>	N/A	N/A
<i>Eleutherodactylus glaphycompus</i>	N/A	N/A

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N/A	3400	0.05	17.3
855	2550	0.07	22.8
N/A	4500	0.344	20
N/A	4300	0.618	40
N/A	2750	N/A	21.05
N/A	2450	N/A	27.5
N/A	3890	0.008	24
N/A	3000	0.526	23.5
N/A	3800	0.557	N/A
N/A	3500	0.215	31.1
N/A	5375	N/A	16
N/A	5980	0.107	17.1
2800	4000	0.18	18.5
N/A	1650	N/A	47.1
N/A	1885	1.95	40.7
N/A	5400	0.056	17.6
N/A	2650	0.012	45
N/A	4710	0.013	20.4
N/A	2475	0.177	33
N/A	3250	N/A	N/A
N/A	3766.6	0.027	14.7
N/A	3000	0.102	28.1
N/A	3334.5	0.09	19.2
N/A	3570	N/A	26

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<i>Eleutherodactylus greyi</i>	N/A	N/A
<i>Eleutherodactylus gryllus</i>	N/A	N/A
<i>Eleutherodactylus</i> <i>guantanamera</i>	N/A	N/A
<i>Eleutherodactylus hedricki</i>	N/A	N/A
<i>Eleutherodactylus inoptatus</i>	N/A	N/A
<i>Eleutherodactylus ionthus</i>	N/A	N/A
<i>Eleutherodactylus johnstonei</i>	N/A	N/A
<i>Eleutherodactylus leberi</i>	N/A	N/A
<i>Eleutherodactylus locustus</i>	N/A	N/A
<i>Eleutherodactylus mariposa</i>	N/A	N/A
<i>Eleutherodactylus melacara</i>	N/A	N/A
<i>Eleutherodactylus</i> <i>michaelschmidi</i>	N/A	N/A
<i>Eleutherodactylus pezopetrus</i>	N/A	N/A
<i>Eleutherodactylus pinarensis</i>	N/A	N/A
<i>Eleutherodactylus pinchoni</i>	N/A	N/A
<i>Eleutherodactylus</i> <i>portoricensis</i>	N/A	N/A
<i>Eleutherodactylus principalis</i>	N/A	N/A
<i>Eleutherodactylus ricordii</i>	N/A	N/A
<i>Eleutherodactylus ronaldi</i>	N/A	N/A
<i>Eleutherodactylus schwartzi</i>	N/A	N/A
<i>Eleutherodactylus simulans</i>	3200	3700
<i>Eleutherodactylus symingtoni</i>	N/A	N/A
<i>Pristimantis terraebolivaris</i>	6000	9000
<i>Eleutherodactylus thomasi</i>	N/A	N/A
<i>Eleutherodactylus varians</i>	N/A	N/A
<i>Eleutherodactylus varleyi</i>	N/A	N/A

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N/A	1850	0.234	65
N/A	7440	26.6	16
N/A	2572	0.507	32.8
N/A	3150	N/A	34.8
N/A	660	0.29	50.4
N/A	2625	0.275	25.9
N/A	3180	0.314	23.08
N/A	2020	0.137	30.5
N/A	4900	N/A	19
N/A	2710	0.054	32.1
N/A	2240	1.44	29.6
N/A	1600	0.533	40.2
N/A	2350	0.691	45.5
N/A	2200	0.147	N/A
N/A	4329	0.145	18
N/A	1650	N/A	30.5
N/A	2800	0.009	20.8
N/A	3700	0.568	28.6
N/A	3770	0.021	N/A
N/A	1890.6	0.638	28.1
500	3400	1.03	19.7
N/A	750	0.4	61.3
3000	3000	0.138	36.1
N/A	3050	0.486	N/A
N/A	2680	0.169	N/A
N/A	4400	0.447	12.9

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<i>Eleutherodactylus zeus</i>	N/A	N/A
<i>Haddadus aramunha</i>	N/A	N/A
<i>Haddadus binotatus</i>	N/A	N/A
<i>Holoaden luederwaldti</i>	936	6244
<i>Ischnocnema abdita</i>	N/A	N/A
<i>Ischnocnema guentheri</i>	N/A	N/A
<i>Ischnocnema henselii</i>	N/A	N/A
<i>Ischnocnema izecksohni</i>	N/A	N/A
<i>Ischnocnema juipoca</i>	2080.2	4049.1
<i>Ischnocnema manezinho</i>	N/A	N/A
<i>Ischnocnema nigriventris</i>	1955	3932
<i>Ischnocnema oea</i>	N/A	N/A
<i>Ischnocnema parva</i>	N/A	N/A
<i>Ischnocnema penaxavantinho</i>	N/A	N/A
<i>Ischnocnema sambaqui</i>	N/A	N/A
<i>Ischnocnema vizottoi</i>	3204	7379
<i>Oreobates ayacucho</i>	N/A	N/A
<i>Oreobates barituensis</i>	N/A	N/A
<i>Oreobates crepitans</i>	1400	4600
<i>Oreobates discoidalis</i>	1706	3100
<i>Oreobates heterodactylus</i>	N/A	N/A
<i>Oreobates madidi</i>	N/A	N/A
<i>Oreobates remotus</i>	N/A	N/A
<i>Phyzelaphryne miriamae</i>	N/A	N/A
<i>Pristimantis altamazonicus</i>	N/A	N/A
<i>Pristimantis ashkapara</i>	1150	1830
<i>Pristimantis aureolineatus</i>	N/A	N/A
<i>Pristimantis aureoventris</i>	N/A	N/A

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N/A	500	1.744	80.5
N/A	1022	0.038	42.3
N/A	1172.8	0.021	43.8
5308	3577	0.032	39.3
N/A	3418.3	0.898	15.7
N/A	2542.8	1.05	30.02
N/A	2416	5.957	30.2
N/A	2222.9	0.0015	N/A
1968.9	2798.4	0.46	23.1
N/A	2700	0.56	25.3
1977	2842	0.379	21.7
N/A	3367.8	6.02	17.1
N/A	3841.3	0.468	16.1
N/A	3455.8	0.07	15.6
N/A	1920	0.592	36.8
4175	5303.5	0.052	20.2
N/A	1902	0.756	31.1
N/A	3226	0.185	26.2
3200	2677.3	0.04	28.7
1394	2165	0.612	27.7
N/A	3946.9	0.176	26.7
N/A	2436.4	0.988	29.3
N/A	3150	0.104	34.6
N/A	3635	0.04	14.6
N/A	2764.9	0.057	23.8
680	1470	0.036	46.9
N/A	3078.4	0.525	25.5
N/A	2295	0.021	16.6

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<i>Pristimantis bambu</i>	N/A	N/A
<i>Pristimantis bicantus</i>	N/A	N/A
<i>Pristimantis bromeliaceus</i>	N/A	N/A
<i>Pristimantis buccinator</i>	N/A	N/A
<i>Pristimantis calcarulatus</i>	N/A	N/A
<i>Pristimantis caryophyllaceus</i>	2320	3340
<i>Pristimantis charlottevillensis</i>	2000	6000
<i>Pristimantis colomai</i>	2137	3093
<i>Pristimantis danae</i>	N/A	N/A
<i>Pristimantis eremitus</i>	4673	5287.3
<i>Pristimantis espedeus</i>	N/A	N/A
<i>Pristimantis fenestratus</i>	N/A	N/A
<i>Pristimantis fraudator</i>	1400	2100
<i>Pristimantis ganonotus</i>	N/A	N/A
<i>Pristimantis lacrimosus</i>	N/A	N/A
<i>Pristimantis llojsintuta</i>	N/A	N/A
<i>Pristimantis mazar</i>	N/A	N/A
<i>Pristimantis mindo</i>	N/A	N/A
<i>Pristimantis muchimuk</i>	2228.3	3310.5
<i>Pristimantis olivaceus</i>	4350	5320
<i>Pristimantis orcus</i>	N/A	N/A
<i>Pristimantis peruvianus</i>	N/A	N/A
<i>Pristimantis petersi</i>	N/A	N/A
<i>Pristimantis phoxocephalus</i>	N/A	N/A
<i>Pristimantis pluvicanorus</i>	N/A	N/A
<i>Pristimantis ramagii</i>	N/A	N/A
<i>Pristimantis reichlei</i>	N/A	N/A
<i>Pristimantis samaipatae</i>	N/A	N/A

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N/A	2530	0.0055	22.5
N/A	3008.5	2.7	16.1
N/A	4350	0.012	22.7
N/A	3595	0.05	31.2
N/A	3318	3.69	22.4
1020	2750	0.04	22.5
4000	4000	0.051	35
956	2570	0.084	18.7
N/A	2210	0.011	36.2
614.3	4916.6	1.1	22.1
N/A	2765.6	0.33	26.1
N/A	3093.1	0.152	33.5
700	1624	0.006	36.8
N/A	5870	0.8	16.7
N/A	3070	0.015	20.2
N/A	2850	0.365	20.9
N/A	3148.9	0.018	19.1
N/A	2848.4	N/A	33.2
1082.2	2691.5	0.041	20.7
970	4880	0.05	19.8
N/A	2048	N/A	N/A
N/A	3070	0.03	25.2
N/A	4320	0.085	19.7
N/A	2441	0.182	26.85
N/A	1250	0.339	37.3
N/A	3941	0.151	24.5
N/A	2501.4	0.03	29.9
N/A	3326.7	0.29	40.9

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<i>Pristimantis subsigillatus</i>	N/A	N/A
<i>Pristimantis ventrigranulosus</i>	N/A	N/A
<i>Pristimantis vilarsi</i>	N/A	N/A
<i>Pristimantis dundeei</i>	N/A	N/A
<i>Psychrophrynella ankohuma</i>	N/A	N/A
<i>Psychrophrynella chacaltaya</i>	N/A	N/A
<i>Psychrophrynella iatamasi</i>	N/A	N/A
<i>Psychrophrynella illampu</i>	N/A	N/A
<i>Psychrophrynella katantika</i>	N/A	N/A
<i>Psychrophrynella kempffi</i>	N/A	N/A
<i>Psychrophrynella saltator</i>	N/A	N/A
<i>Psychrophrynella wettsteini</i>	N/A	N/A

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N/A	2727.5	0.37	27.1
N/A	4021	0.04	23.5
N/A	4036	0.008	N/A
N/A	4005	0.53	N/A
N/A	2354	0.057	24.5
N/A	3284	0.096	16.1
N/A	2934	0.161	23.8
N/A	2363	0.423	20.1
N/A	2304	0.457	22.7
N/A	3180	0.085	20.3
N/A	3126	0.047	17.4
N/A	1962	0.191	26.2

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Table S2. Values of the evolutionary rates from body size and acoustics parameters, without *Pristimantis*.

	K	p	$\lambda$	logL ( $\lambda$ )	logL( $\lambda=0$ )	p
Body size	0.57	0.001	0.90	-343.72	-358.92	<0.001
Minimum frequency	1.04	0.005	0.85	-156.77	-161.05	0.003
Maximum frequency	1.06	0.012	0.99	-149.51	-151.28	0.059
Frequency bandwidth	0.88	0.102	0.99	-145.88	-146.03	0.591
Dominant frequency	0.41	0.001	0.61	-785.91	-792.01	<0.001
Call duration	0.28	0.546	<0.001	-211.24	-211.24	1

Table S3. p, t and slope values of the PGLS tests between acoustic parameters and body size without *Pristimantis*.

	Slope	t-value	P-value
SVL x Dominant frequency	-78.76	-8.33	<<0.001
SVL x Minimum frequency	-46.70	-2.31	0.03
SVL x Maximum frequency	-72.38	-2.51	0.02
SVL x Call duration	-0.04	-1.15	0.25

#### 4. CONSIDERAÇÕES FINAIS

Os animais do grupo *B. pernix* apresentam um canto de anúncio de alta de frequência e ampla taxa de repetição de notas. *Brachycephalus brunneus* e as duas populações analisadas de *B. curupira* não fogem desse padrão, sendo que os cantos de ambas as espécies apresentaram limites grandes de frequência máxima e taxa de repetição elevada. As duas espécies apresentam o canto de anúncio parecido, tanto nos padrões temporais e espectrais, tendo a diferença proeminente apenas na taxa de repetições de notas, sendo que a maior taxa é de *B. curupira*. Embora a maior diferença entre essas espécies se dê em apenas um parâmetro acústico, trabalhos de descrição do canto de espécies de anuros podem ser usados como diagnose de espécies. Se comparadas com outras espécies de *Brachycephalus* do grupo *B. pernix* com o canto descrito, *B. brunneus* e *B. curupira* apresentam um canto bastante diferente, principalmente na parte estrutural dos grupos de notas.

O canto dos anuros exerce forte seleção reprodutiva, com isso é de se esperar que o canto também mude com a linhagem dos animais. O canto pode ser influenciado pelo meio ao qual os anuros vocalizam ou pela sua morfologia. A relação que o tamanho do corpo exerce sobre os aspectos físicos do canto dos anuros foi testada, onde temos clara a relação do corpo dos animais para com a frequência dominante. A alometria tamanho de corpo-frequência dominante foi testada com o grupo Terraranae, e mostrou que há uma relação inversa, onde, quanto maior o animal menor a frequência dominante, e quanto menor o animal, maiores serão as frequências emitidas em seu canto. Foi também encontrado sinal filogenético entre os valores testados na teoria da alometria, mostrando uma forte relação evolutiva da frequência dominante e do tamanho do corpo dos animais. A reconstrução de caracteres ancestrais e a análise de PGLS só reforçaram a relação evolutiva dos parâmetros acústicos com o tamanho do corpo dos animais, mostrando por sua vez que a evolução é mais influente que a própria teoria da alometria nos parâmetros acústicos dos anuros do grupo Terraranae. Com isso, ressalta-se a importância de análises filogenéticas comparativas para os estudos biológicos. Essas análises podem nos mostrar mais sobre a história evolutiva dos grupos e como ela se desenrolou ao longo das linhagens.

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