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CAMILA INES ZORNOSA TORRES

ACOUSITC PARAMETERS AND CHYTRID INFECTION VARIATION IN ANURANS ALONG AN ELEVATIONAL GRADIENT IN THE CAPARAÓ NATIONAL PARK

VARIAÇÃO DOS PARÂMETROS ACÚSTICOS E DE INFECÇÃO PELO FUNGO QUITRÍDIO EM ANUROS NUM TRANSECTO ELEVACIONAL NO PARQUE NACIONAL DO CAPARAÓVARIAÇÃO DOS PARÂMETROS ACÚSTICOS E DE INFECÇÃO PELO FUNGO QUITRÍDIO EM ANUROS NUM TRANSECTO ELEVACIONAL NO PARQUE NACIONAL DO CAPARAÓ

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Orientador: Prof. Dr. Luís Felipe de Toledo Ramos Pereira

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> *"Nada na vida deve ser temido, somente compreendido.*
> *Agora é a hora de compreender mais para temer menos"*
> Marie Curie

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Viva a ciência!

RESUMO

Ao longo dos gradientes de elevação a temperatura, umidade, e estrutura da vegetação atuam como fatores limitantes para anuros, já que interagem com componentes da sua ecologia e fisiologia. Estas interações com parâmetros da história natural dos anuros levam a variações na estrutura das assembleias de uma região. A composição das assembleias locais, é determinante na divisão do espaço acústico, esperando a partilha deste espaço para garantir a comunicação efetiva entre os indivíduos e as espécies, especialmente no contexto reprodutivo. Além de influenciar no espaço acústico, a estrutura das assembleias locais e a elevação também influenciam parâmetros epidemiológicos, como no caso do fungo *Batrachochytrium dendrobatidis* (Bd), um patógeno aquático que ataca regiões queratinizadas do corpo dos anfíbios, tendo efeitos letais, subletais e consequências indiretas destes nos indivíduos infectados. Uma das consequências da infecção do Bd é a alteração das características do canto de anúncio dos seus hospedeiros. A hipótese Hamilton e Zuk sugere que machos infestados por parasitas apresentam características sexuais secundarias menos atraentes às fêmeas, levando a escolha de machos saudáveis na hora de acasalar. Mas o contrário também seria esperado, já que esta pode ser a última chance de reprodução favorecendo ambos, o patógeno e o hospedeiro infectado. Considerando o anteriormente citado, o presente estudo caracterizou as comunidades de anuros do Parque Nacional Caparaó (PNC), avaliando a influência da elevação e riqueza nas taxas de infecção pelo Bd, a estrutura do espaço acústico de 10 assembleias de anuros, e o efeito do Bd nos cantos de anúncio de sete espécies de anuros. Além disso, realizamos uma lista de espécies atualizada do PNC e arredores, adicionando 40 espécies à lista disponível no plano de manejo e colocando-o entre as 10 localidades com maior riqueza de anuros da Mata Atlântica. Também, registramos o Bd ao longo de cerca de 2000 m de gradiente de elevação, o maior já amostrado na Mata Atlântica e observamos que existe uma relação negativa entre a elevação e a carga de infecção do Bd, mas não há relação entre a elevação e a prevalência do quitrídio. Ao avaliar a estrutura acústica das assembleias encontramos que estas são compostas por espécies próximas filogeneticamente e suas características acústicas têm uma estrutura dispersa, sugerindo uma partilha do espaço acústico. No entanto, só encontramos relação da riqueza e da similaridade filogenética com parâmetros temporais do canto, indicando uma possível partilha temporal nestas assembleias. Por outro lado, a infeção pelo Bd alterou pelo menos um dos parâmetros avaliados em todas as espécies analisadas, sendo que elas responderam de maneiras diferentes à infecção. Corroborando assim tanto a hipótese de Hamilton e Zuk, quanto uma hipótese alternativa que chamamos de “hipótese dos zumbis sensuais”, na qual propomos que indivíduos infectados pelo Bd são mais atrativos às fêmeas como estratégia reprodutiva que beneficiaria tanto o patógeno quanto o indivíduo infectado. O presente trabalho traz contribuições inéditas para ciência sobre o impacto do Bd em características fundamentais da reprodução dos anuros e sobre a estrutura do espaço acústico em assembleias altamente diversas.

ABSTRACT

Temperature, humidity, rainfall, and vegetation structure act as limiting factors for anurans along elevational gradients, because they interact directly with their ecology and physiology. The interactions with anurans natural history led to variation in assemblage's structure. The composition of local assemblages is fundamental in the division of the acoustic space, expecting a partitioning of this space that guarantee an effective communication among individuals, especially when it is under a reproductive context. Also, assemblages structure may influence epidemiological factors, as is the case of the chytrid fungus *Batrachochutrium dendrobatidis* (Bd), an aquatic pathogen that attacks keratinized regions of the amphibians body, leading to lethal and sublethal effects, and indirect consequences of these effects on infected hosts. A consequence of Bd infection is the alteration of advertisement call parameters. The Hamilton and Zuk hypothesis states that males infested by parasites show secondary sexual characteristics less attractive to females, thus females select healthy males for mating. Contrary outcomes are also expected, as a strategy to favor the pathogen and the infected individual. Considering the stated before, the present study characterized anuran assemblages from the Caparaó National Park (CNP) and surrounding areas, evaluating the influence of elevation and species richness in Bd infection rates, the structure of the acoustic space of 10 anuran assemblages and the effect of Bd on advertisement calls of seven anuran species. We also developed an update species list from the CNP, adding 40 species to the previous species list, placing this area among the 10 richest localities in the Atlantic Forest. We registered Bd along a 2000 m elevational gradient, the highest gradient sampled in the Atlantic Forest, and observed a negative correlation between elevation and Bd load, but not with prevalence. When assessing the acoustic structure of anuran assemblages, we found that these were composed by phylogenetically closer species and had a dispersed acoustic structure, suggesting acoustic partitioning. However, we only found influence of species richness and phylogenetic relatedness in temporal parameters of the advertisement call, indicating a possible temporal partitioning. Conversely, the infection of Bd altered at least one call parameter in all the species analyzed, and each species responded differently to Bd infection. In agreement with the Hamilton and Zuk hypothesis and with an alternative one that we named “sexy zombies hypothesis”, in which we suggest that Bd infected males are more attractive to females as this may be their last chance of reproducing favoring both hosts and pathogen. The present study provides new contributions to science about the impact of Bd on fundamental anuran reproductive characteristics and about acoustic space structure of highly diverse assemblages.

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Introdução geral

Na natureza, diversos são os fatores limitantes da distribuição das espécies. Estes podem ser físicos, como temperatura, luminosidade, umidade relativa e pluviosidade, ou bióticos, como competição, predação, disponibilidade de recursos, parasitas e patógenos (Cox & Moore 2009). Ao longo de gradientes de elevação podem-se observar variações de muitos destes fatores. Por exemplo, na medida em que se aumenta a elevação há diminuição da pressão atmosférica, redução da temperatura (com implicação na umidade relativa do ambiente), e aumento na radiação solar (Körner 2007; Cox & Moore 2009). Além disso, o solo tende a se tornar cada vez menos arenoso com o aumento da elevação, até mesmo chegando ao ponto de apresentar rochas expostas no topo das montanhas, impactando diretamente a vegetação que cresce sobre o solo e por tanto alterando os padrões estruturais do ambiente (Safford 1999). Estas variações limitam a distribuição dos animais ao longo dos gradientes de elevação (Cruz & Feio 2007; Cox & Moore 2009).

Neste contexto, os anfíbios são excelentes modelos de estudo, pois são altamente sensíveis às mudanças ambientais, principalmente devido a sua pele permeável, sua alta dependência da água e padrão fisiológico ectotérmico (Duellman & Trueb 1994; Wells 2007). Dos fatores ambientais que mudam ao longo de gradientes de elevação, a temperatura, pluviosidade, e umidade são os que mais afetam os anuros (Morrison & Hero 2003; Wells 2007). A baixa temperatura associada às altas elevações restringe o tempo de disponibilidade para a acumulação de recursos ou energia, resultando em uma diminuição do período reprodutivo e do número de desovas por cada período (Morrison & Hero 2003). A baixa umidade relativa nestas áreas gera mudanças comportamentais em espécies de anfíbios que não apresentam adaptações à perda de água, como buscar abrigo em ambientes mais úmidos (Navas 2002; Wells 2007). Por outro lado, a variação na temperatura e pluviosidade afeta diretamente atividades reprodutivas como o canto de anúncio, e oviposição; também afeta a abundância e distribuição das presas, o que pode ter influencias no crescimento e sobrevivência dos anuros (Wells 2007). Desta forma, a maioria dos anuros selecionam microhábitats com maior umidade e temperatura constante, buscando abrigo em baixo da serrapilheira, pedras, troncos, em bromélias, e tocas de outros animais, e vivem principalmente próximos a corpos d'água (Navas 2002; Wells 2007).

Estas implicações dos fatores físicos na biologia dos anuros resultam em variações na estrutura das assembleias ao longo de gradientes de elevação. Assim, nos topos das montanhas há um menor número de espécies de anfíbios comparado com as áreas mais baixas (Navas 2002). Entretanto, as espécies presentes nos topos seriam mais abundantes já que estão adaptadas fisiologicamente às condições locais e possuem (nestas condições) menos competidores e predadores, implicando em maior sucesso reprodutivo (Duellman & Trueb 1994; Navas 2002). Devido a estas limitações ambientais, os locais com características favoráveis para a sobrevivência dos anuros podem levar a assembleias com alta abundância, principalmente em regiões tropicais, pois ambientes com estruturas similares alocam espécies com requisitos ecológicos parecidos (Toft & Duellman 1979; Duarte et al. 2019). As necessidades ecológicas específicas de cada espécie lhe permitem sobreviver e se reproduzir em dito ambiente, e fazem parte do conhecido nicho ecológico (Hutchinson 1957).

A coexistência de várias espécies em um mesmo local leva à elevada competição por recursos, muitas vezes prejudicando a subsistência de alguns competidores. A hipótese de partilha de nicho estabelece que para as espécies coexistirem, elas deverão ocupar nichos diferentes, evitando assim a competição por um recurso (Begon et al. 2006). No caso dos anuros, a partilha do nicho pode ser espacial (segregando espécies que habitam fitofisionomias abertas e fechadas, ou corpos d'água lênticos e lóticos), ou temporal (espécies diurnas ou noturnas, ativas unicamente durante a estação chuvosa ou com reprodução prolongada) (Toft & Duellman 1979; Gutiérrez-Lamus et al. 2004; Brasileiro et al. 2005; Folt & Reider 2013).

Além dessas dimensões (espaço e tempo), assembleias de anuros devem partilhar o espaço acústico (Hödl 1977; Drewry & Rand 1983; Krause 1983). O espaço acústico é um espaço multivariado que pode ser descrito em três dimensões principais: frequência, amplitude e tempo e é intimamente relacionado com o nicho acústico, que simplesmente é uma porção do espaço acústico no qual ocorre uma comunicação efetiva (Seur & Farina 2015; de Araújo et al. 2020). Neste sentido, a hipótese de partilha de nicho acústico sugere que as estratégias que evitem interferência dos sinais acústicos e assegurem uma comunicação efetiva seriam selecionados ao longo da evolução das espécies (Hödl 1977; Drewry & Rand 1983; Krause 1983). Desta maneira, as estratégias mais utilizadas pelos indivíduos para partilhar o nicho acústico envolvem tanto o espaço ecológico quanto o acústico, como por exemplo, à segregação espacial (uso de sítios de canto específicos), temporal (atividade vocal em determinada hora da noite, canto em antifonia) e a utilização de diferentes faixas de frequência ou taxas de vocalização (Littlejohn 1977; Garcia-Rutledge & Narins 2001; Pombal 2010; Sinsch et al. 2012).

Da mesma forma que os efeitos da elevação afetam os anfíbios eles também oferecem condições adequadas ou inadequadas para o desenvolvimento dos seus patógenos (Leal et al. 2018). Neste sentido, um dos principais patógenos é o fungo *Batrachochytrium dendrobatidis* (Bd), reportado como causador de vários declínios de populações ao longo do mundo (Lips et al. 2006; Vredenburg et al. 2010; Hoffman et al. 2010; James et al. 2015; Carvalho et al. 2017; Scheele et al. 2019). O Bd é um fungo aquático que coloniza as células queratinizadas da epiderme dos anfíbios afetando a troca de gases, água e eletrólitos pela pele (Berger et al. 2005; Voyles et al. 2007; Voyles et al. 2009; Van Rooij et al. 2015). A epidemiologia do Bd é dependente da temperatura, sendo que este apresenta crescimento ótimo entre 17 e 25 ºC, abaixo de 10 ºC apresenta crescimento mais lento, e acima de 28 ºC o crescimento cessa (Piotrowski et al. 2004; Van Rooij et al. 2015). Assim, na região tropical espera-se maior infecção em elevações altas, dado que as condições de temperatura e umidade nestas áreas seriam ideais para o desenvolvimento e propagação do patógeno (Pounds et al. 2006; Brem & Lips 2008; Gründler et al. 2012; Sapsford et al. 2013; Lambertini et al. 2021). Além disso, a diminuição na riqueza e o aumento na abundância de anuros em zonas elevadas permitiria um efeito de amplificação da doença (Becker & Zamudio 2011; Lambertini et al. 2021), podendo afetar populações de espécies endêmicas destas áreas.

Diversos estudos relacionaram o efeito da elevação na infecção do Bd em gradientes amplos e estreitos de elevação, e os resultados foram variáveis, mostrando que o Bd responde de maneiras diferentes em cada localidade (Brem & Lips 2008; Kriger & Hero 2008; Catenazzi et al. 2010; Kilburn et al. 2010; Gründler et al., 2012; Sapsford et al. 2013; Acevedo et al. 2016; Lambertini et al. 2016; Lambertini et al. 2021). Por exemplo, em um gradiente amplo (1200 – 3700 m) foi detectada uma correlação negativa da infecção do Bd com a elevação, mostrando que altas elevações sobrepassam as temperaturas de crescimento ótimo do patógeno (Catenazzi et al. 2011). No entanto, em um gradiente estreito no Panamá (200 – 700 m) foi observada uma correlação positiva entre a prevalência do Bd e a elevação (Brem & Lips 2008). Na Mata Atlântica, já foi encontrada correlação positiva entre elevação e infecção do Bd (Gründler et al. 2012; Lambertini et al. 2021) e outro estudo não encontrou correlação entre estas variáveis (Lambertini et al. 2016). No entanto, os três estudos foram executados em elevações entre zero e 1600 m acima do nível do mar, o que pode estar influenciando os resultados obtidos.

O Bd, causa disfunções fisiológicas nos seus hospedeiros (hiperplasia, hiperceratose e desequilíbrio eletrolítico) levando-os muitas vezes à morte por parada cardíaca (Berger et al. 2005; Voyles et al. 2007; Voyles et al. 2009). Além destes efeitos letais, também apresenta efeitos subletais, como perda de peso e desequilíbrio nas funções cardíacas e epidérmicas (Cheatsazan et al. 2013; Bovo et al. 2016; Salla et al. 2018). Indivíduos infectados podem apresentar efeitos indiretos da infecção relacionados principalmente com o seu comportamento, como letargia, diminuição das habilidades de nado e salto, reduzida eficiência de forrageio e esforço reprodutivo (Chatfield et al. 2013; Hanlon et al. 2015; An & Waldman 2016; Kelleher et al. 2021). Indivíduos infectados pelo Bd devem realocar reservas de energia para combater a infecção por meio da resposta imune, reduzindo assim a energia destinada a funções vitais como crescimento e reprodução. Contudo, efeitos do Bd na reprodução tem sido pouco explorados e se associam principalmente à atividade de canto, tendo resultados contraditórios (Roznik et al. 2015; An and Waldman 2016; Greenspan et al. 2016; Kelleher et al. 2021).

A variação dos displays reprodutivos em indivíduos infestados/infectados por parasitas/patógenos foi proposta por Hamilton & Zuk (1982) e nomeada de modelo de seleção sexual mediada por parasitas (Hamilton & Zuk 1982) ou hipótese Hamilton & Zuk (HHZ) (Kirkpatrick 1986; Balenge & Zuk 2014). Este modelo assume que indivíduos infestados por parasitas apresentam características sexuais secundárias menos chamativas do que indivíduos saudáveis, assim as fêmeas selecionariam machos não infestados para acasalar (Hamilton & Zuk 1982; Møller 1990; Hamilton & Poulin 1997; Balenge & Zuk 2014; Macedo & Manica 2019). Como no caso de *Boana prasina*, em que machos infestados por helmintos apresentaram taxas de canto reduzidas ao ser comparados com machos saudáveis, tornando-os menos atrativos às fêmeas (Madelaire et al. 2013). Em contraste, machos de *Dryophytes japonicus* e *Pseudophryne pengillegi* infectados pelo Bd apresentam cantos mais longos, maior taxa de canto e são ativos vocalmente durante mais tempo do que machos saudáveis, sugerindo uma maior atratividade às fêmeas (An and Waldman 2016; Kelleher et al. 2021). Estes resultados contraditórios indicam que as respostas à infestação/infecção por parasitas e patógenos são espécie-dependentes e faz-se necessário mais estudos para entender como podem estar afetando o fitness reprodutivo dos anuros.

Considerando todos os fatores apresentados acima, selecionamos o Parque Nacional do Caparaó (PNC) como local para estudar o efeito da elevação nas assembleias de anuros e seu principal patógeno, o Bd. Este local foi selecionado já que apresenta o maior gradiente de elevação da Mata Atlântica (600 – 2800 m) e sendo uma unidade de conservação apresenta condições ideias para o estudo dos anfíbios. Ao longo deste trabalho foi realizada uma lista de espécies de anuros do PNC e arredores, na qual adicionamos 40 espécies à lista que constava no plano de manejo do parque, colocando-o entre as 10 localidades com maior riqueza de anfíbios da Mata Atlântica (Zornosa-Torres et al. 2020). Entre as espécies registradas destacam-se duas espécies endêmicas (*Cycloramphus bandeirensis* e *Physalaemus araxa*) e pelo menos seis espécies novas para a ciência (*Aplastodiscus* sp., *Brachycephalus* sp., *Ischnocnema* spp. (três espécies), e *Physalaemus araxa*) (Zornosa-Torres et al. 2020).

Além disso, no segundo capítulo avaliamos as taxas de infecção do Bd ao longo de cerca de 2000 m de gradiente de elevação, o maior gradiente já amostrado na Mata Atlântica. Neste capítulo testamos se a elevação e a riqueza de espécies influenciam as taxas de infecção do Bd. Recompilamos estudos que analisaram a relação entre taxas de infecção de Bd e elevação, e determinamos se existia ou não uma faixa mínima a ser amostrada para encontrar efeitos da elevação nas taxas de infecção. Registramos o Bd ao longo de todo o gradiente de elevação (698 – 2656 m acima do nível do mar), detectamos uma relação negativa entre carga de infecção e elevação, mas não houve relação entre a elevação com a prevalência do Bd (Zornosa-Torres et al. 2021). Com base nos 10 estudos que analisaram efeitos da elevação no Bd em regiões tropicais, não encontramos uma faixa mínima de elevação que tenha que ser amostrada para encontrar efeitos desta relação. Os capítulos 1 e 2 já estão publicados em revistas revisadas por pares.

No terceiro capítulo determinamos a estrutura do espaço acústico de 10 assembleias de anuros no PNC e testamos se essa estrutura era influenciada pela riqueza, abundância e relações filogenéticas das assembleias. Neste capítulo encontramos que as espécies que compõem as assembleias avaliadas são filogeneticamente próximas, pois observamos uma estrutura agregada das distâncias filogenéticas. No entanto, as características acústicas dos cantos de anúncio destas espécies mostraram uma estrutura dispersa, sugerindo assim uma partilha do espaço acústico. Quando examinamos as relações entre riqueza e filogenia com as características espectrais do canto não encontramos relação, mas houve relação destas propriedades ecológicas com as características temporais do canto, sendo uma relação positiva com a riqueza e uma relação negativa com as distâncias filogenéticas. Isto sugere uma possível partilha na dimensão temporal do espaço acústico para evitar a sobreposição dos cantos, o que pode comprometer a comunicação efetiva nas assembleias. Não encontramos efeito da abundância dos indivíduos nas variáveis estudadas.

Para concluir este trabalho, examinamos se parâmetros temporais e espectrais (duração, taxa de canto, e frequência dominante) do canto de anúncio de sete espécies de anuros brasileiros são influenciadas pela incidência e carga de infecção do Bd, testando assim, a hipótese de Hamilton e Zuk (HHZ). Os resultados obtidos mostram que as respostas à infecção variam dependendo da espécie, mas pelo menos um parâmetro do canto foi afetado pela presença do Bd. Machos saudáveis de *Boana albopunctata, B. pardalis* e *Phyllomedusa burmeisteri* emitem cantos mais longos, sendo assim mais atrativos às fêmeas e corroborando a HHZ. Entretanto, machos infectados de *Dendropsophus minutus* e *Scinax alter* apresentaram cantos mais longos e com maior taxa de canto, tornando-os potencialmente mais atrativos às fêmeas, corroborando a hipótese de investimento terminal.

A pesquisa realizada ao longo deste doutorado e resumida aqui nestes quatro capítulos trouxe contribuições para ciência e a conservação de anfíbios. A lista de espécies e as informações publicadas no primeiro e segundo capítulo permitirão o aprimoramento do plano de manejo do PNC, ajudando entender a distribuição das espécies e do patógeno mais letal dos anfíbios ao longo de gradientes de elevação e em locais diversos e com alto grau de endemismo, assim como os potenciais prejuízos à reprodução das espécies e seu nicho acústico. Futuros estudos ainda são necessários principalmente nas relações entre o Bd e os parâmetros do canto, avaliando respostas das mudanças causadas pelo Bd e a seleção das fêmeas de machos infectados ou não infectados pelo fungo.

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CAPÍTULO 1

ANURANS OF THE CAPARAÓ NATIONAL PARK AND SURROUNDINGS, SOUTHEAST BRAZIL

Phantasmarana apuana
Foto: Luís Felipe Toledo

> *"Qual é a utilidade de fazer todo esse trabalho*
>
> *se não nos divertimos com isso?"*
>
> Rosalind Franklin

Anurans of the Caparaó National Park and surroundings, southeast Brazil

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Abstract

The Atlantic Forest (AF) is one of the biodiversity hotspots of the world, and the most fragmented biome of Brazil. This biome includes different phytophysiognomies, as riparian, slope, cloudy forests, and grasslands. Such complexity, allied to huge latitudinal and high elevational range, provides diverse habitats and conditions for amphibian speciation. As a result, there are over 600 amphibian species known to occur in the AF. Within this biome the Caparaó National Park (CNP) is relevant, as it includes the highest peak of the biome, the *Pico da Bandeira* at almost 3,000 m above sea level, as well as different phytophysiognomies as rocky fields and humid forests. In spite of that, its amphibian fauna is still poorly described. We inventoried amphibians at the CNP and surrounding areas from 2016 to 2018 and recorded 47 anuran species, of which two are locally endemic and at least six have not been described yet. Additionally, we compiled data from previous surveys (2004 to 2008) and secondary data from scientific collections. All together, we registered a total of 61 anuran species from 12 families for the CNP and surroundings, placing this area among the 10 amphibian richest sites in the AF. Some of these species are represented by only one or two collected specimens and have not been registered in the CNP since the 1980’s, such as *Thoropa lutzi* and *Hylodes vanzolinii*. These species could be examples of population declines or even past local extinctions, highlighting the need of further sampling efforts in that highly biodiverse site.

Keywords: Amphibia, frogs, toads, Atlantic Forest, species composition, species lists.

Resumo

A Mata Atlântica (MA) é considerada um dos "*hotspots*" de biodiversidade do mundo, sendo o bioma mais degradado no Brasil. Este bioma compreende muitas fitofisionomias, como floresta ombrófila, semidecidual, e campos de altitude. Esta complexidade, aliada à enorme variação latitudinal e altitudinal, disponibiliza habitats e condições diversas à especiação dos anfíbios. Como resultado, atualmente são conhecidas mais de 600 espécies de anfíbios que ocorrem na MA. Dentro deste bioma o Parque Nacional do Caparaó (PNC) é relevante, pois inclui o ponto mais alto da Mata Atlântica, o Pico da Bandeira com cerca de 3000 m de elevação, assim como diferentes fitofisionomias, como campos rupestres e florestas úmidas. Apesar disso, sua fauna de anfíbios é ainda pobremente descrita. Realizamos inventários de anfíbios no PNC e entorno de 2016 a 2018 e registramos 47 espécies de anuros, das quais duas são endêmicas e pelo menos seis ainda não foram descritas. Adicionalmente, compilamos dados de coletas anteriores (2004 a 2008) e dados secundários de coleções científicas. Ao juntar todos os dados registramos um total de 61 espécies de anuros de 12 famílias para o PNC e arredores, colocando esta área entre as 10 áreas mais ricas em anfíbios da MA. Algumas destas espécies estão representadas apenas por um ou dois exemplares em coleções e não foram registradas no PNC desde a década de 1980, como *Thoropa lutzi* e *Hylodes vanzolinii*. Estas espécies podem ser exemplos de declínios populacionais ou mesmo de extinções locais, destacando a necessidade de maiores esforços amostrais neste local altamente biodiverso.

Palavras chave: Amphibia, composição de espécies, lista de espécies, Mata Atlântica, rãs, sapos.

Introduction

The Atlantic Forest (AF) is one of the most degraded biomes of Brazil, with less than 16% of its original extension, due to severe anthropic pressure over the past couple centuries (Ribeiro et al. 2011, Rossa-Feres et al. 2018). This biome is characterized by having different phytophysiognomies as evergreen, deciduous and semi-deciduous forest, mangroves, swamps, dunes and high-altitude grasslands (Ribeiro et al. 2011), high latitudinal variation (almost from the equator line, down to subtropical regions), and high topographic complexity (Morellato & Haddad 2000, Toledo et al. 2014). Due to the high number of endemic species and the huge habitat loss it has been considered one of the 35 biodiversity hotspots of the world (Mittermeier et al. 2011), and therefore, conservation of this biome is a priority.

The AF amphibians are also diverse and represented by more than 600 species, of which about 75% are endemic, and several species yet to describe (Haddad et al. 2013, Rossa-Feres et al. 2018). In the AF, humidity and vegetation cover are determinants of amphibian species diversity, which are related to altitudinal variation (Vasconcelos et al. 2014). Some of the AF mountains are traditional centers of endemism, due to their elevation, climatic variation and biogeographic history (Guarnizo & Cannatella 2013, Vasconcelos et al. 2014).

The mountain region of the Caparaó National Park (CNP) in the border of the states of Minas Gerais and Espírito Santo, southeastern Brazil, includes the highest peak of the Atlantic Forest, reaching almost 3,000 m above sea level. The area is covered by different phytophysiognomies (e.g., riparian, slope and cloudy forests; grasslands), and houses a diverse amphibian biota (Ferreira et al. 2015), including micro endemic species, as *Cycloramphus bandeirensis* (Heyer 1983). In spite of the potential relevance of the area for amphibian diversity, the single amphibian species list available was based on a 20 days survey and reported only 21 anuran species (Ferreira et al. 2015).

Here, we present an improved list of the anuran species of the Caparaó National Park and surroundings based on a period of almost seven years of fieldwork in the region and accessing information from scientific collections, including sound recordings and vouchered specimens.

Material and Methods

Study site

We searched for amphibians in the Caparaó National Park (CNP) and surroundings (Figure 1A). The Caparaó National Park is located between the states of Minas Gerais and Espírito Santo, southeastern Brazil, and belongs to the massif of Caparaó, which is part of the north region of the Serra da Mantiqueira (a mountain range) in the Atlantic Forest. Seasons in the CNP region are divided in a rainy summer (from November to March) and a wet winter (from April to October). The CNP has a huge elevational variation, from 859 to 2,892 m above sea level (a.s.l.), and the predominant phytophysiognomies are ombrophilous rainforests and high-altitude grasslands (above 1,900 m a.s.l.) (Machado et al. 2016).

Field and scientific collections samplings

We conducted field surveys from 2016 to 2018 in the Caparaó National Park and surroundings. We sampled 62 sample sites/units (SUs) (Figure 1, Table S1), mainly during rainy season (see above), totaling 63 sampling days, as follows: 29 January to 03 February 2016, 27 July to 01 August 2016, 19–25 October 2016, 02–12 December 2016, 06–15 January 2017, 05–12 October 2017, 06–12 December 2017, 16–25 January 2018 and 22 November to 04 December 2018.

During these surveys we actively and acoustically searched for amphibians in possible breeding sites (Figure 2) inside and outside the park, both in its East and West slopes. These slopes were delimited by the downstream direction of the rivers. We also search in specific localities where rare species were registered in the past. Adult anurans were collected, anesthetized, and killed with lidocaine 5%, fixed in 10% formalin, and stored in 70% ethanol. Tadpoles were fixed and stored in 7% formalin. For all individuals we collected muscle tissue samples before fixing the specimens and stored at 100% ethanol. Voucher specimens were deposited at the Museu de Zoologia "Prof. Adão José Cardoso" (ZUEC), Universidade Estadual de Campinas (Unicamp), according to the ICMBio (#58836-1) permit, SISGen (#A8A7729) protocol, and ethics committee (#4827-1/2018) approval. Anuran calls were recorded using digital recorders (TASCAM DR-40, Marantz PMD 661, Sony PCM-M10, Zoom H4n, and Zoom H4n Pro) and were deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV), ZUEC, Universidade Estadual de Campinas (Unicamp). Based on these samples we estimated diversity indexes (ACE, ICE, Chao 1, Chao 2, Jackknife 1, Jackknife 2, and Bootstrap) and constructed a sample-based accumulation curve with 1,000 randomizations using the software EstimateS 9.1.0 (Colwell 2013). We decided to show only the Jackknife 1 index as it is the best fitting index and the most used diversity index in species list studies.

Additionally, we combined this dataset with data from previous surveys carried out from 2004 to 2008, and with data from specimens deposited in the following Brazilian scientific collections: Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, São Paulo; Centro de coleções taxonômicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais; Coleção Célio F. B. Haddad (CFBH), Universidade Estadual Paulista "Júlio de Mesquita Filho", Rio Claro, São Paulo; Museu Nacional (MNRJ), Rio de Janeiro, Rio de Janeiro; and sound recordings from the Fonoteca Neotropical Jacques Vielliard (FNJV), ZUEC, Universidade Estadual de Campinas, Campinas, São Paulo.

Taxonomic nomenclature follows Frost (2020). Species identification was done by comparisons with previously collected individuals in scientific collections (Appendix I), species descriptions, sound recordings from individuals and additional DNA analyses for the ones we needed extra identification (e.g., tadpoles, juveniles or species complexes). For the molecular analyses we extracted total DNA and amplified a fragment of 16S ribosomal DNA, following methods in Lyra et al. (2017). Purified fragments were sent to Macrogen, Republic of Korea for sequencing. We then used the blast tool (BLAST+ application, Camacho et al. 2009) to assign individuals to species. We assigned a species identification if similarity between sequences was higher than 98%. The newly generated sequences were submitted to GenBank (accession numbers: MN450227–MN450262).

Results

We registered 47 species of anurans, from 11 families: Brachycephalidae (7 spp.), Bufonidae (2 spp.), Centrolenidae (2 spp.), Craugastoridae (1 sp.), Cycloramphidae (2 spp.), Hylidae (18 spp.), Hylodidae (1 sp.), Leptodactylidae (9 spp.), Microhylidae (2 spp.), Odontophrynidae (1 sp.), and Phyllomedusidae (2 spp.). Two of the species are endemic for the CNP [*Cycloramphus bandeirensis* and *Physalaemus* sp. (aff. *maculiventris*)] and at least six of those species [*Aplastodiscus* sp. (possible *Aplastodiscus* sp. 6 of Berneck et al. 2016), *Physalaemus* sp. (aff. *maculiventris*), *Ischnocnema* spp. (three species), and *Brachycephalus* sp.] have not been described yet. From these not described species, *Physalaemus* sp. (aff. *maculiventris*) is abundant in the mountaintop but has extremely restricted distribution. On the other hand, the other five species [*Aplastodiscus* sp., *Brachycephalus* sp., and *Ischnocnema* spp. (three species)] are less abundant and were registered in forested microhabitats.

Gathering with the information from our previous surveys and secondary data from scientific collections, we compiled a list of 61 anuran species comprised in 12 families (Table 1; Figure 3; Appendix I), placing the Caparaó National Park (CPN) among the 10 amphibian richest sites in the AF (Table 2). The Jackknife 1 index estimated that we registered 93% of the species during the most recent two years of fieldwork (Figure 1B; Table S2). However, these species corresponded to about 75% of the total species richness we were able to list. Species richness differed between both East and West slopes and among altitudinal ranges (Figure 1A, C). The site with the highest species richness was in the East side from 600 to 900 m, where we registered 34 species (Figure 1C). Although higher altitudes have less species, it is where the endemic ones were found, *Cycloramphus bandeirensis* and *Physalaemus* sp. (aff. *maculiventris*).

Two species present in scientific collections (*Hylodes vanzolinii* and *Thoropa lutzi*) were not sampled in the study area in recent times, even after directed efforts in their specific microhabitats. As an example, *H. vanzolinii* is known only by the holotype and one paratype, both collected in 1980 (Heyer 1982), and *T. lutzi*, which was collected in the CNP only in November 1980 and has never been registered again.

Discussion

This is a first comprehensive list of anurans for the Caparaó National Park (CNP) and surroundings. This update adds 40 anuran species to the list used for the park management plan (21 species) and place the CNP among the 10 amphibian richest sites in the Atlantic Forest (Table 2). Considering the size of the CNP and the other areas in the AF, the CNP is the 4th richest site, even we did not have access to all the areas of the park. Many of the species that were registered outside the limits of the CNP may also occur inside its boundaries. The CNP shares 40 species with the municipality of Santa Teresa (Ferreira et al. 2019) and 33 species with the Serra do Brigadeiro State Park (SBST) (Moura et al. 2012). The shared species are mainly abundant and widely distributed species, such as *Rhinella crucifer*, *Boana albomarginata*, *B. albopunctata*, *B. faber*, *B. pardalis*, *B. polytaenia*, *B. semilineata*, and *Dendropsophus minutus*. Resemblance in their amphibian species composition could be due the similar biogeographic origins, as these three localities belongs to the northern sector of the Mantiqueira mountain range (Gontijo-Pascutti et al. 2012), and also could be due to the proximity (140 km and 74 km straight-line distance, respectively), environmental and elevational similarities (Moura et al. 2012, Ferreira et al. 2019). Additionally, rare species, such as *Hylodes babax*, has been rediscovered in the past 10 years outside the CNP boundaries at Serra do Brigadeiro State Park and at Uaimií State Forest, both in the state of Minas Gerais, near the type locality (CNP) where the last record was in 2008 with a single specimen (Pirani et al. 2010, Verdade et. al. 2011).

The two endemic species found in this area are restricted to high-altitude grasslands (above 2,000 m). *Physalaemus* sp. (aff. *maculiventris*) is an abundant species with restricted distribution to altitude lagoons inside the CNP (Lagoa da sombra and Três lagoas) and has explosive reproduction. *Cycloramphus bandeirensis* can be found in high-altitude habitats at CNP and has a characteristic semiterrestrial reproductive mode (Verdade et al. 2019). High-altitude grasslands in the CNP undergo extreme temperature oscillations that could impact local amphibian populations (Veríssimo et al. 2012). For example, the minimum temperature recorded in July 2017 was -14 °C (Oliveira 2017) and there are recent reports of fires in CNP (G1 Minas 2019), which leads to local high temperatures and habitat loss, as observed in other high-altitude localities (Aximoff 2011). The undescribed species *Ischnocnema* spp. (three species), *Brachycephalus* sp. and *Aplastodiscus* sp. are less abundant, and although they inhabit decidual forests, a common environment in this area, we do not have specific information of their distribution range.

The lack of records of *Hylodes vanzolinii* and *Thoropa lutzi* in the Caparaó National Park could be related with local population declines or extinctions (see Verdade et al. 2011 for review of potential factors leading to amphibian declines in Brazil). If that is the case, a plausible cause would be the chytrid fungus, which spiked in the AF during the 1980's and was related to several amphibian extinctions in that decade (see Carvalho et al. 2017). We therefore indicate the need of higher sampling efforts and specific searches for those species. Furthermore, as this area has some species that were not described yet, it is even possible that some other not described species have vanished before our knowledge of their existence.

The publication of species lists is mandatory for the understanding of the regional biodiversity and is the basis for local wildlife conservation efforts. In this case, the current survey highlights one of the most amphibian diverse regions in the Atlantic Forest and reveals several species that should be described in the following years.

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Table 1. Anuran species list from the Caparaó National Park (CNP) and surrounding areas. An asterisk * represents species that has the CNP as type locality; a hashtag ♯ indicates an undescribed species; a cross † indicates a species that is not collected since the 1980s in the CNP. Slopes are west (W) and east (E). Data is classified as secondary (S), collected/primary (C), sound recorded (R) and identification confirmed by molecular analyses (M). Sample sites: Table S1.

Species	Altitude	Slope	Data	Sample site
Brachycephalidae				
Brachycephalus sp. ♯	-	-	S	-
Ischnocnema izecksohni (Caramaschi & Kisteumacher, 1989)	851-1350	W, E	C, S	17, 34, 38, 41, 47, 49, 50
Ischnocnema sp. (aff. *guentheri* / CS3) ♯	1000	E	C, S	27
Ischnocnema feioi Taucce, Canedo & Haddad, 2018	1200	W	C, S	38
Ischnocnema abdita Canedo & Pimenta, 2010	1006	E	C, R, S	32
Ischnocnema sp. (aff. *lactea*) ♯	-	-	S	-
Ischnocnema sp. (gr. *parva*) ♯	1000	E	C, S	27
Ischnocnema parva (Girard, 1853)	1000	E	C, S	27
Ischnocnema verrucosa (Reinhardt & Lütken, 1862)	-	-	S	-
Ischnocnema octavioi (Bokermann, 1965)	1215	W	C	54
Bufonidae				
Dendrophryniscus carvalhoi Izecksohn, 1994	-	-	S	-
Rhinella crucifer (Wied-Neuwied, 1821)	758-1591	W, E	C, R, S	2, 11, 13, 17, 20, 22-24, 36, 38, 41, 43, 53
Rhinella granulosa (Spix, 1824)	843	E	C, R	18
Centrolenidae				
Vitreorana eurygnatha (Lutz, 1925)	1572	W	C, R, S	55
Vitreorana uranoscopa (Müller, 1924)	1000-1200	W, E	C, R, S	27, 41

Craugastoridae				
Haddadus binotatus (Spix, 1824)	990-1307	W, E	C, R, S	18, 33, 38, 41, 45, 47, 50, 52
Cycloramphidae				
Cycloramphus bandeirensis Heyer, 1983 *	2405	E	C, R, S	60
Thoropa lutzi Cochran, 1938†	-	-	S	-
Thoropa miliaris (Spix, 1824)	829-1259	W, E	C, S	13, 33, 38
Zachaenus carvalhoi Izecksohn, 1983	-	-	S	-
Hemiphractidae				
Fritziana ohausi (Wandolleck, 1907)	-	-	S	-
Hylidae				
Aplastodiscus arildae (Cruz & Peixoto, 1987)	-	-	S	-
Aplastodiscus cavicola (Cruz & Peixoto, 1985)	851	E	C, R	17
Aplastodiscus sp. ♯	-	-	S	-
Boana albopunctata (Spix, 1824)	796-1005	W, E	C, R, S	4, 12, 19, 21, 22, 31
Boana albomarginata (Spix, 1824)	758-922	E	C, R, S	2, 3, 10, 15
Boana crepitans (Wied-Neuwied, 1824)	796-826	E	C, R, S	3, 4, 10
Boana faber (Wied-Neuwied, 1821)	806-1282	W, E	C, R, S	4, 11, 12, 17, 18, 22, 37, 38, 42, 45, 46, 49, 50
Boana pardalis (Spix, 1824)	781-1259	W, E	C, R, S	4, 5, 9, 13, 17, 21, 26, 31, 34, 38, 44
Boana polytaenia (Cope, 1870)	815-1746	W, E	C, R, S	4, 12, 13, 22, 47, 49, 57
Boana semilineata (Spix, 1824)	698	E	C, R	1
Bokermannohyla caramaschii (Napoli, 2005)	815-1200	W, E	C, S	9, 18, 27, 34, 37, 40, 41, 50,

				54
Dendropsophus elegans (Wied-Neuwied, 1824)	758-922	E	C, R, S	2, 4, 10, 12, 13, 15, 17, 20, 22,
Dendropsophus branneri (Cochran, 1948)	-	-	S	-
Dendropsophus haddadi (Bastos & Pombal, 1996)	806-843	E	C	16
Dendropsophus pseudomeridianus (Cruz, Caramaschi & Dias, 2000)	698-922	E	C, R, S	1, 2, 10, 22
Dendropsophus minutus (Peters, 1872)	758-1215	W, E	C, R, S	2, 4, 12, 13, 15, 17, 20, 36, 49
Itapotihyla langsdorffii (Duméril & Bibron, 1841)	-	-	S	-
Ololygon tripui (Lourenço, Nascimento & Pires, 2010)	1179-1872	W	C, R, S	35, 38 - 41, 48, 51, 58, 59
Scinax alter (Lutz, 1973)	698-1282	W, E	C, R	1, 2, 6, 12, 13, 15, 16, 20, 22, 23, 27, 34, 36, 49
Scinax eurydice (Bokermann, 1968)	758-922	E	C, R, S	2, 10, 12, 16, 18, 22
Scinax cf. *hayii* (Barbour, 1909)	872-990	E	C, R, S	19
Scinax fuscovarius (Lutz, 1925)	796-886	E	C, R, S	2, 3, 16, 18, 20, 21
Hylodidae				
Hylodes babax Heyer, 1982	-	-	S	-
Hylodes vanzolinii Heyer, 1982 * †	-	-	S	-
Phantasmarana apuana Pombal, Prado & Canedo, 2003	1200	W	C, S	38
Leptodactylidae				
Pseudopaludicola restinga Cardozo, Baldo, Pupin, Gasparini & Haddad, 2018	796-843	E	C, R	3

Physalaemus cuvieri Fitzinger, 1826	796-1005	W, E	C, R	3, 10, 21, 25, 31
Physalaemus feioi Cassini, Cruz & Caramaschi, 2010	824-886	E	C, R	12, 20
Physalaemus sp. (aff. *maculiventris*) * ♯	2425-2656	W	C, R, S	61, 62
Adenomera thomei (Almeida & Ângulo, 2006)	-	E	NC	-
Leptodactylus fuscus (Schneider, 1799)	698-1005	W, E	C, R, S	1, 3, 8, 22, 24, 31
Leptodactylus mystacinus (Burmeister, 1861)	758-933	W, E	C, S	18
Leptodactylus spixi Heyer, 1983	796-1040	E	C, S	3, 4, 35
Leptodactylus latrans (Steffen, 1815)	696-1005	W, E	C, S	1, 7, 12, 22, 28-31
Microhylidae				
Chiasmocleis sp.	-	E	R	-
Elachistocleis cf. *cesarii* (Miranda-Ribeiro, 1920)	820	E	C, R	10
Myersiella microps (Duméril & Bibron, 1841)	-	-	S	-
Odontophrynidae				
Proceratophrys boiei (Wied-Neuwied, 1824)	986-1294	W, E	C, R, S	35, 41, 47, 49
Phyllomedusidae				
Phyllomedusa burmeisteri Boulenger, 1882	820-886	E	C, R, S	10, 12, 15, 17, 18, 20
Pithecopus rohdei (Mertens, 1926)	758	E	C	2

Table 2. Amphibian most rich localities in the Atlantic Forest. Time sampled (†) in a site refers to time reported in the literature and does not include historical samplings that can be retrieved in museum records. One asterisk (*) refer that values were rounded to integer numbers. Two asterisks (**) symbolize that the area used was the area of the Tijuca National Park. Three asterisks (***) refer to an area unable to calculate. Brazilian states: (BA) Bahia, (ES) Espírito Santo, (MG) Minas Gerais, (RJ) Rio de Janeiro, (SP) São Paulo. RPPN, Reserva Particular do Patrimônio Natural (Private Reserve of Narutal Heritage).

Locality	**Species richness**	**Time sampled†**	**Area (km^2)***	**Species richness/ km^2**	**Reference**
Municipality of Santa Teresa (ES)	108	≈ 10 years	683	0.16	Ferreira et al.(2019); Moura et al. (2012)
RPPN Serra Bonita (BA)	80	88 days	20	4.00	Dias et al. (2014)
Reserva Ecológica de Guapiaçu (RJ)	73	≈ 7 years	13	5.62	Almeida-Gomes et al. (2014)
Maciço da Tijuca (RJ)	69	-	40**	1.73	Vacine et al. (2018)
Reserva Biológica de Paranapiacaba and surroundings (SP)	69	-	***	-	Verdade et al. (2009)
Estação Biológica da Boracéia (SP)	67	≈ 5 years	165	0.41	Heyer et al. (1990)
Parque Estadual da Serra do Mar - Núcleos Curucutu, Santa Virgínia and São Sebastião (SP)	65	81 days	558	0.12	Silva et al. (2017)
Parque Estadual Carlos Botelho (SP)	65	76 days	378	0.17	Forlani et al. (2010)

Caparaó (MG, ES)	**61**	**63 days**	**318**	**0.19**	**Present study**
Parque Estadual Turístico do Alto Ribeira (SP)	60	15 days	357	0.17	Araujo et al. (2010)

Figure Legends

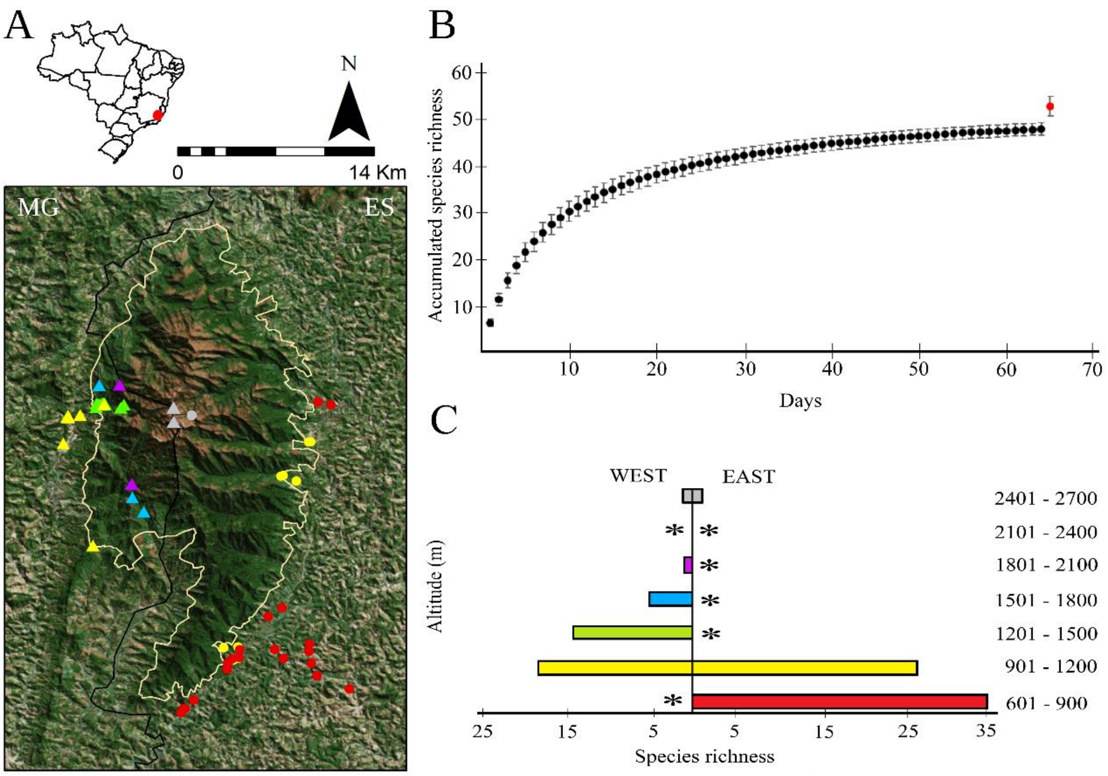
Figure 1. (A) Distribution of anuran species in the Caparaó National Park (CNP): yellow line determines the limits of the CNP, triangles correspond to registers in the west slope, dots in the east slope, and colors correspond to altitudinal ranges; black line represents the boundaries between states of Minas Gerais (MG) and Espírito Santo (ES). (B) Accumulated species richness during 63 days of survey; red dot corresponds to the jackknife 1 estimator for species richness. (C) Number of species registered in each altitudinal range; asterisks indicate areas not sampled. Geographical coordinates of all sample units are in Table S1. Diversity index values (ACE, ICE, Chao 1, Chao 2. Jackknife 1, Jackknife 2, Bootstrap) are in Table S2.

Figure 2. Anuran breeding sites at the Caparaó National Park (CNP) and surrounding areas. (A) swamp in forest areas, (B) swamp in open areas, (C) altitude lagoons, (D) artificial lagoons, (E) rivers and streams, (F) deciduous forest.

Figure 3. Anuran species found in the Caparaó National Park (CNP) and surrounding areas (SVL, snout-vent length, in millimeters). (A) *Ischnocnema izecksohni* (ZUEC 24371, SVL = 28.1); (B) *I. feioi* (not vouchered); (C) *Ischnocnema* sp. (gr. *parva*) (ZUEC 24365, SVL = 17.2); (D) *I. parva* (ZUEC 24352, SVL = 17.9); (E) *I. verrucosa* (UFMG 17230); (F) *Dendrophryniscus carvalhoi* (not vouchered); (G) *Rhinella crucifer* (not vouchered); (H) *R. granulosa* (ZUEC 23631, SVL = 50.3; ZUEC 23632, SVL = 62.2); (I) *Vitreorana eurygnatha* (ZUEC 24380, SVL = 16.8); (J) *V. uranoscopa* (ZUEC 24376, SVL = 25.2); (K) *Haddadus binotatus* (not vouchered); (L) *Cycloramphus bandeirensis* (not vouchered); (M) *Thoropa miliaris* (not vouchered); (N) *Zachaenus carvalhoi* (not vouchered); (O) *Fritziana ohausi* (UFMG 17263); (P) *Aplastodiscus arildae* (UFMG 17259); (Q) *A. cavicola* (ZUEC 24368, SVL = 32.0); (R) *Boana albomarginata* (not vouchered); (S) *B. albopunctata* (ZUEC 23597, SVL = 48.5); (T) *B. crepitans* (ZUEC 24578, SVL = 55.1); (U) *B. faber* (ZUEC 23582, SVL

= 77.9); (V) *B. pardalis* (not vouchered); (W) *B. polytaenia* (ZUEC 23617, SVL = 26.4); (X) *B. semilineata* (ZUEC 24364, SVL = 43.0); (Y) *Bokermannohyla caramaschii* (ZUEC 24351, SVL = 64.4); (Z) *Dendropsophus elegans* (ZUEC 23616, SVL = 25.7); (A1) *D. haddadi* (ZUEC 23629, SVL = 17.3); (B1) *D. pseudomeridianus* (not vouchered); (C1) *D. minutus* (not vouchered); (D1) *Ololygon tripui* (ZUEC 23628, SVL = 28.4); (E1) *Scinax alter* (not vouchered); (F1) *S. eurydice* (not vouchered); (G1) *Scinax* cf. *hayii* (ZUEC 23598, SVL = 34.4); (H1) *S. fuscovarius* (not vouchered); (I1) *Hylodes babax* (UFMG 17258); (J1) *H. babax* ventral view (UFMG 17258); (K1) *Phantasmarana apuana* (ZUEC 23497); (L1) *Pseudopaludicola restinga* (not vouchered); (M1) *Physalaemus cuvieri* (ZUEC 24566, SVL = 28.7); (N1) *P. feioi* (ZUEC 24557, SVL = 20.3); (O1) *Physalaemus* sp. (aff. *maculiventris*) (not vouchered); (P1) *Leptodactylus mystacinus* (ZUEC 24573, SVL = 53.1); (Q1) *L. spixi* (ZUEC 23627, SVL = 43.9); (R1) *L. latrans* (not vouchered); (S1) *Elachistocleis* cf. *cesarii* (ZUEC 24579, SVL = 30.5); (T1) *Proceratophrys boiei* (ZUEC 23621, SVL = 51.2); (U1) *Phyllomedusa burmeisteri* (not vouchered); (V1) *Pithecopus rohdei* (ZUEC 24570, SVL = 37.4). Photographs K, R, S, T, F1, M1, N1, P1, S1, U1, and V1 by Victor Fávaro Augusto.

Figure 1.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Accumulated species richness
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60
70
Days
C
WEST
EAST
Altitude (m)
2401 - 2700
2101 - 2400
1801 - 2100
1501 - 1800
1201 - 1500
901 - 1200
601 - 900
*
25
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15
25
35
Species richness

Figure 2.

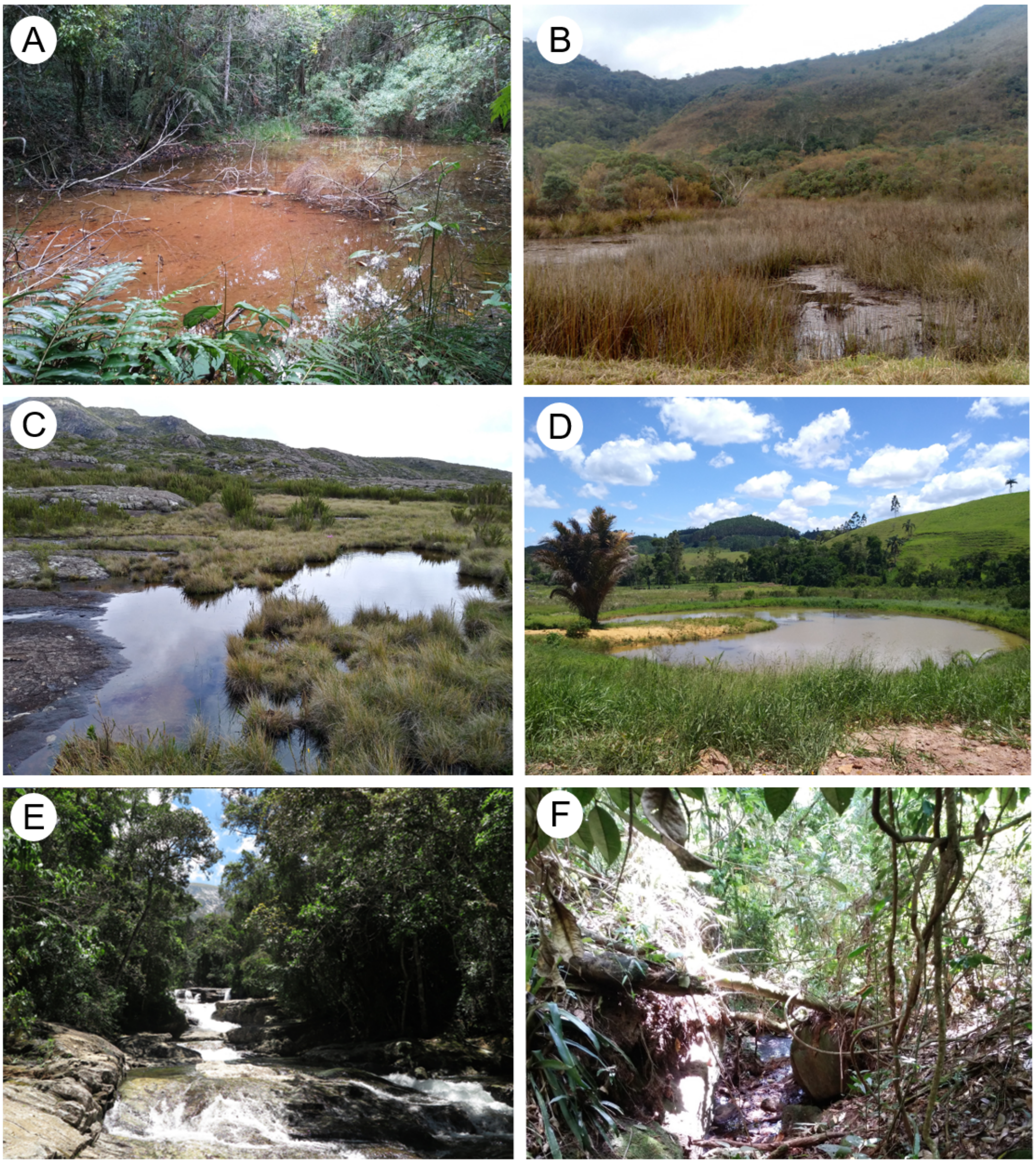
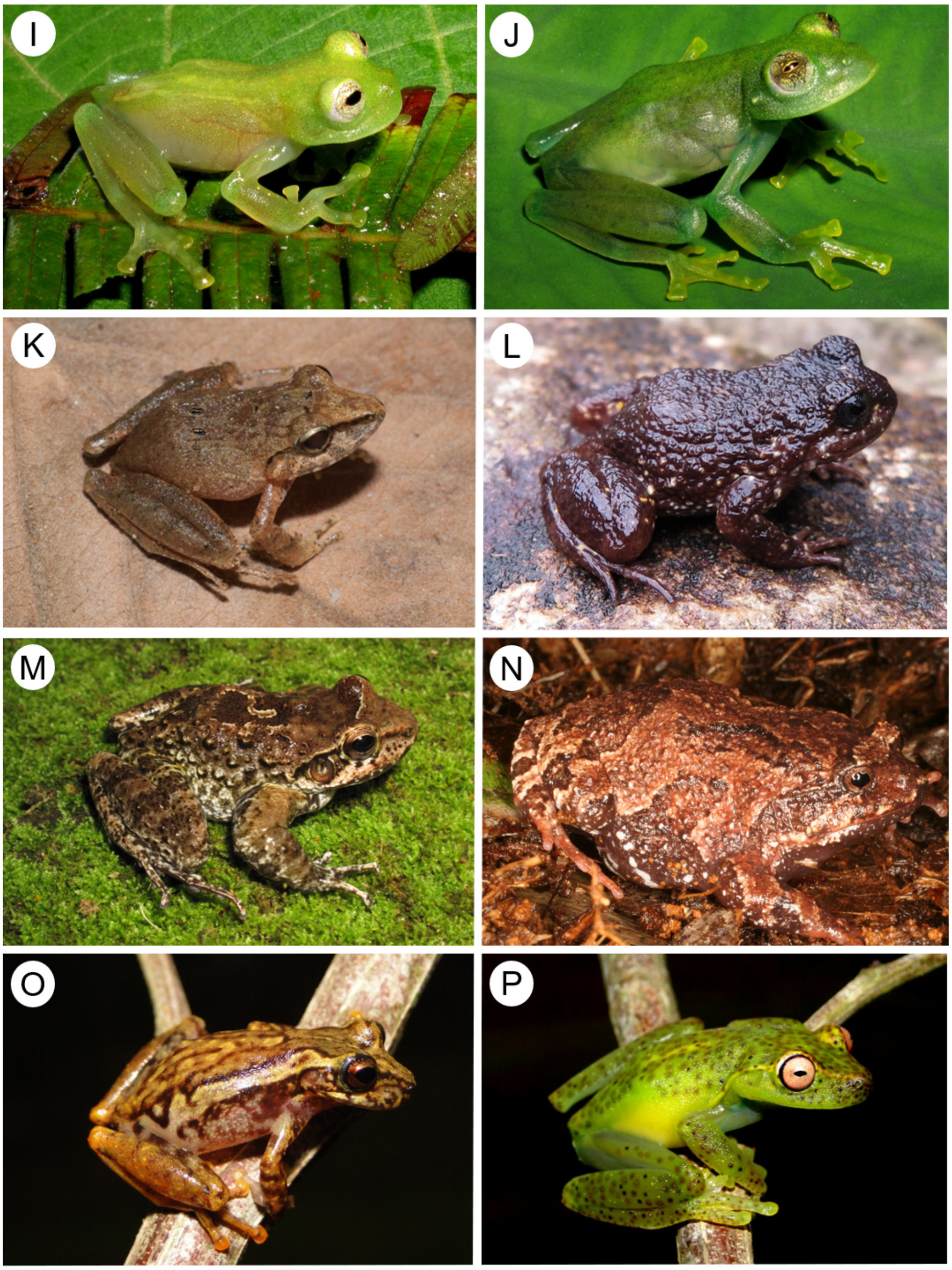


Figure 3.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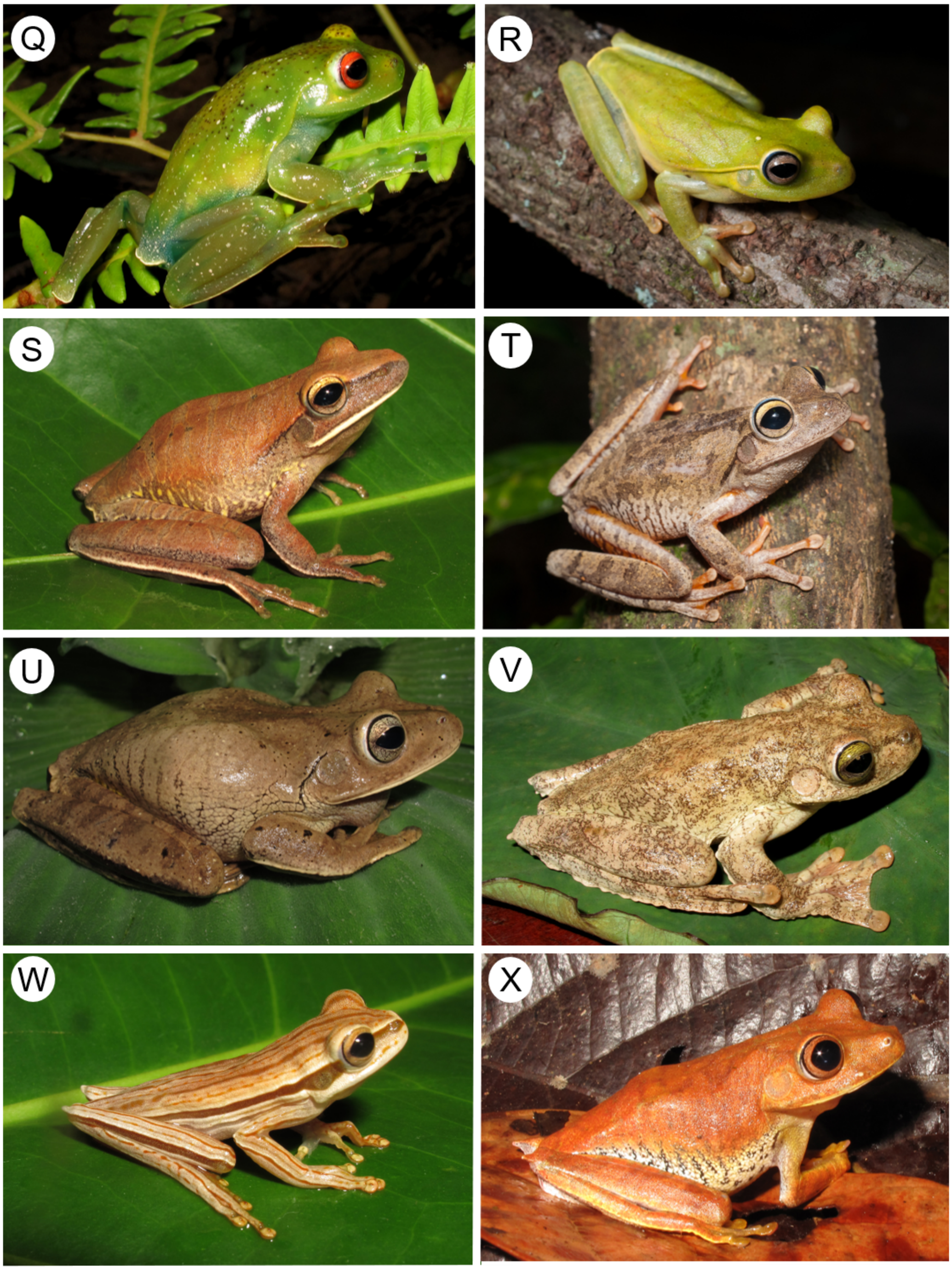
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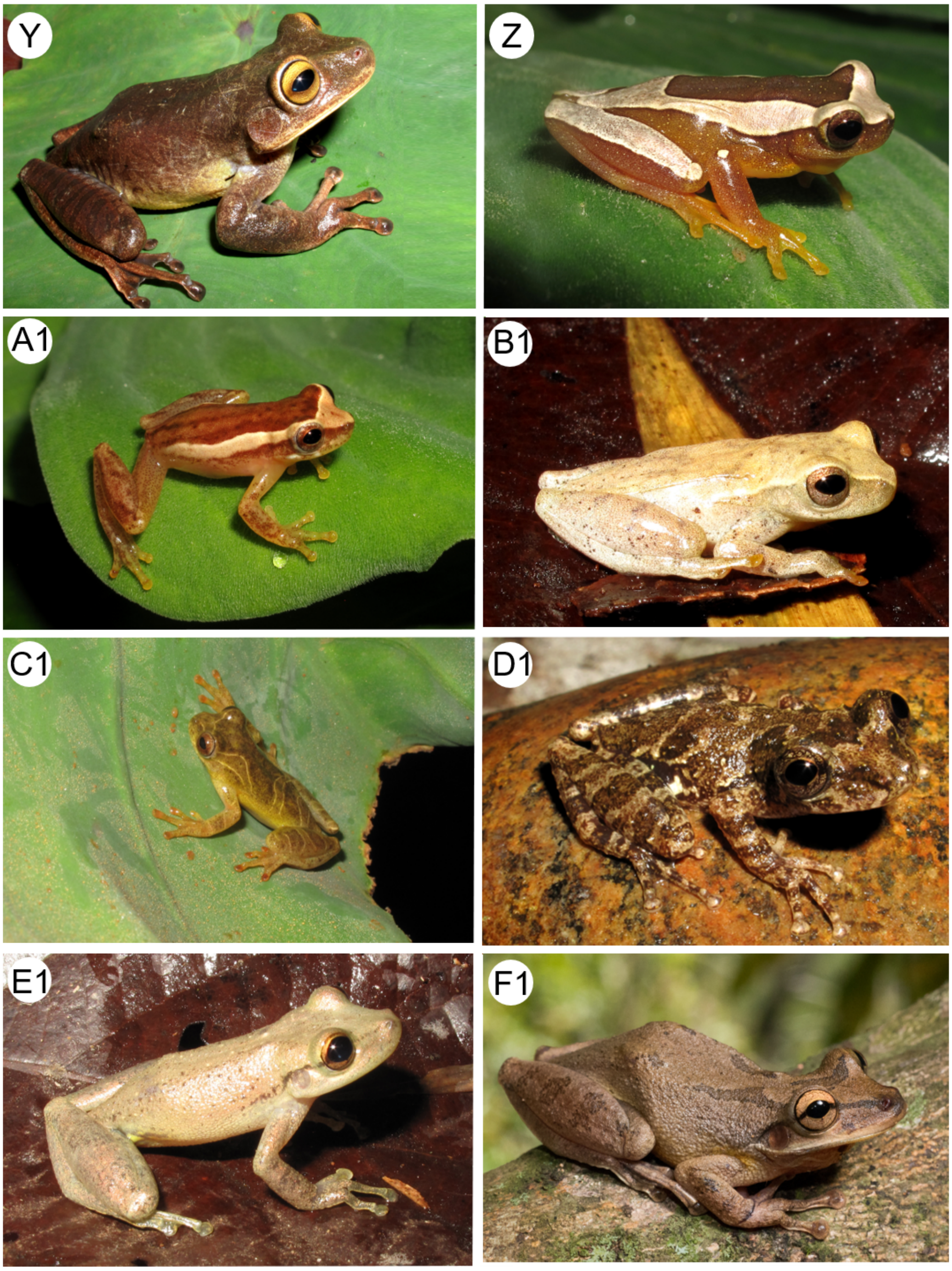
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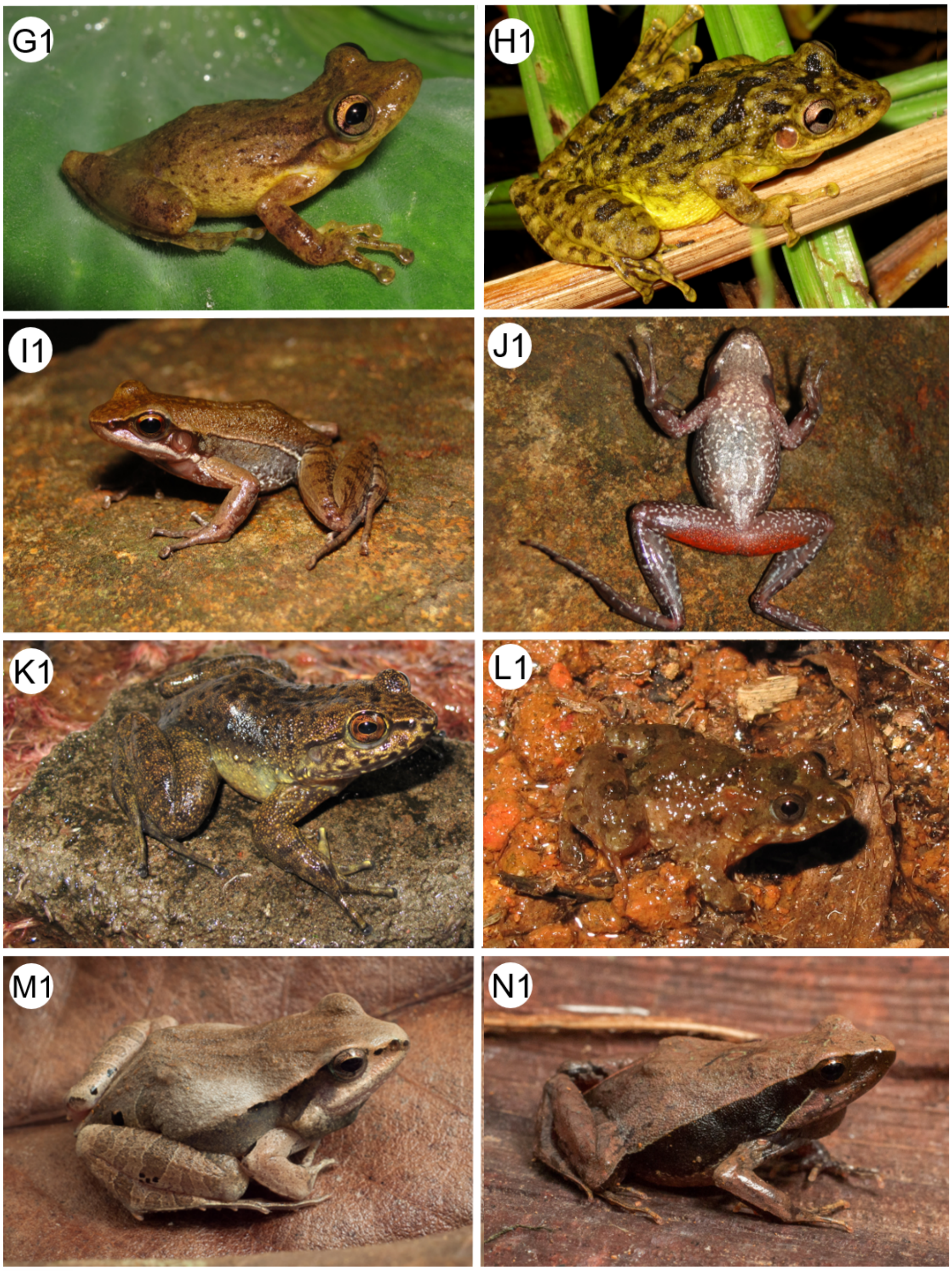
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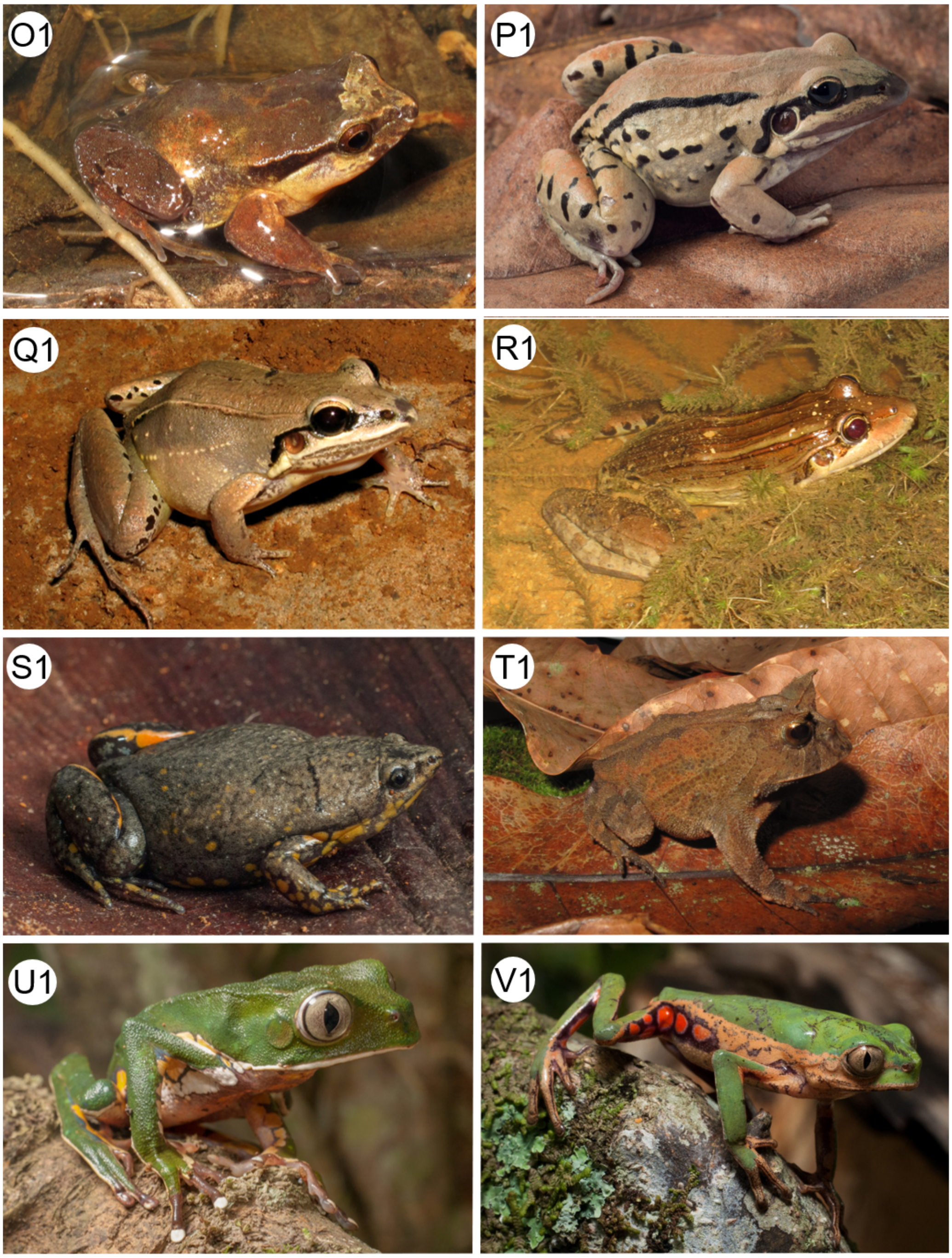
O1
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APPENDIX I. Specimens or sound recordings examined and vouchered.

***Brachycephalus* sp.:** MZUSP 135709–10, 139152–4.

***Ischnocnema izecksohni*:** CFBH 40977–80; FNJV 32236–7, 33251– 5; UFMG 17196–8, 17203, 17206, 17215–6, 17220–1; MZUSP 135690–3; ZUEC 22560–1, 22936, 23488, 23490, 23494–6, 24371.

***Ischnocnema* sp. (aff. *guentheri*):** CFBH 35167, TLFT 3164; ZUEC 24366.

***Ischnocnema feioi*:** UFMG 17078; ZUEC 23483, 23489.

***Ischnocnema abdita*:** FNJV 33210–1; MZUSP 139162, 139165; TLFT 2831; ZUEC 23635.

***Ischnocnema* sp. (aff. *lactea*):** UFMG 17214, 17244.

***Ischnocnema parva*:** MZUSP 135694–5, 139184; ZUEC 24352.

***Ischnocnema* sp. (aff. *parva*):** MZUSP 135696; 139160; TLFT 3163; UFMG 17201–2, 17210–2, 17218, 17226, 17236; UFMGT 4823, 4829, 4849, 4862, 4864, 4879, 4881, 4888; ZUEC 24365.

***Ischnocnema verrucosa*:** UFMG 17230–3, 17246.

***Ischnocnema octavioi*:** TLFT 2835; ZUEC 23639.

***Haddadus binotatus*:** FNJV 32238, 33230; MZUSP 135699–708; UFMG 17199–200, 17204, 17208, 17219; ZUEC 22558–9, 22945, 23482, 23484–7, 23492–3, 23636, 23640, 24353–6, 24358.

***Dendrophryniscus carvalhoi*:** MZUSP 135662–4.

***Rhinella crucifer*:** FNJV 36674, 36682, 40108, 40113, 40162, 40168, 40173; MZUSP 135650–2; TLFT 2304, 2313; UFMG 17222; ZUEC 22567, 22935, 22937, 22942, 22944, 23581.

***Rhinella granulosa*:** ZUEC 23631–2.

***Vitreorana eurygnatha*:** FNJV 36644–6, 36678; MZUSP 139159; ZUEC 24377–80.

***Vitreorana uranoscopa*:** FNJV 33294–5; MZUSP 135659, 139158; UFMG 17213, 17227; ZUEC 23576, 24376.

***Cycloramphus bandeirensis*:** FNJV 34622–3, 34649–50; MZUSP 135668–78; ZUEC 24101–05.

***Thoropa miliaris*:** MNRJ 42088–9; MZUSP 135683–6, 135711, 139136–9, UFMG 17207, 17253–4; ZUEC 23584–8, 23623, 23633–4, 24357.

***Thoropa lutzi*:** MZUSP 57954, 58596.

***Zachaenus carvalhoi*:** MZUSP 139102, 140431–2.

***Fritziana ohausi*:** MZUSP 57924, 139161; UFMG 17263; UFMGT 4828.

Aplastodiscus arildae: UFMG 17259–61; UFMGT 4863, 4872, 4889.

Aplastodiscus cavicola: FNJV 36620, 36659, 36671; TLFT 3165–7, 3213; ZUEC 24367–70.

Boana albopunctata: FNJV 33206, 36577, 36579–80, 36582–3, 36599–600, 36621, 36653, 36684, 40090, 40094, 40200, 40235; ZUEC 23577, 23597, 23656, 24342, 24565.

Boana albomarginata: FNJV 36629, 36647–8, 40068, 40073, 40150–6, 40160, 40170, 40172, 40175, 40184, 40189–90; ZUEC 24393–7.

Boana crepitans: FNJV 33207, 40178, 40182; TLFT 2784, 2786, 3192; ZUEC 23590–3, 24390, 24577–8.

Boana faber: FNJV 32390, 32392, 36649, 36679, 40072; ZUEC 22930–1, 22933, 22946, 23491, 23582–3, 23630, 24556.

Boana pardalis: FNJV 36611, 36634–5, 36642–3, 40088, 40091, 40095–6, 40098–9; TLFT 2298, 2788–9, 2817, 3007, 3141–7, 3157, 3168–71, 3204; ZUEC 22929, 23481, 23594–5, 23622, 24116, 24343–50, 24359, 24372–5, 24408, 24558–64.

Boana polytaenia: CFBH 35168–72; FNJV 40219, 40221–4, 40227, 40229–31, 40236; MNRJ 42028–31, 50407–8; MZUSP 57939–40, 135660–1, 139150; UFMG 17223, 17238; ZUEC 23600, 23617–8, 24382, 24215–7, 24409.

Boana semilineata: ZUEC 24364.

Bokermannohyla caramaschii: MNRJ 42035–6; MZUSP 57925, 135687–9; UFMG 17217, 17245, 17247, 17252; ZUEC 22927, 23402, 23589, 23659, 24351.

Dendropsophus elegans: FNJV 36586–36587, 36589, 36593, 36601–2, 36605–10, 36612, 36624, 36655–7, 40071, 40074–7, 40080, 40084–6, 40133, 40137, 40142, 40163–4, 40171, 40185, 40191, 40207, 40215, 40226, 40237; UFMG 17243; ZUEC 23599, 23614–6.

Dendropsophus haddadi: FNJV 40203; TLFT 2825, 4172, 4183; ZUEC 23629, 24580.

Dendropsophus pseudomeridianus: CFBH 35175; FNJV 36603–36604, 36613–36614, 36628, 36636–36639, 36651, 36670, 36681, 40102, 40104, 40106–40107, 40109, 40111, 40114–40117, 40119, 40124–40131, 40181; ZUEC 23604, 24339–24340, 24360–24362.

Dendropsophus minutus: CFBH 35176; FNJV 32391, 34626–7, 34630, 34651, 34653, 36581, 40070, 40083, 40112, 40120–1, 40139, 40144, 40209–10, 40232–4; MZUSP 135665–6, 139155–7; UFMG 17239–42; ZUEC 23605–13, 23657, 24106–8.

Itapotihyla langsdorffii: CFBH 35173.

Ololygon tripui: CFBH 42850–1; FNJV 32389, 32395, 32898, 32902, 32904; MNRJ 42032–4, 78121; MZUSP 57942, 135667, 139178–80; ZUEC 22926, 22928, 22934, 23357, 23398–401, 23403–4, 23418–9, 23628, 23658, 24112, 24176, 24402, 24406–7.

Scinax alter: FNJV 34652, 34654, 36591, 36595, 36597–8, 36676, 40067, 40078–9, 40081–2, 40101, 40103, 40105, 40110, 40118, 40141, 40157–9, 40161, 40165–7, 40174, 40194–5, 40197–9, 40201, 40204–6, 40208, 40213–4, 40216, 40218, 40220, 40228; ZUEC 23601–3, 24109–11, 24113–5, 24177, 24195–7.

Scinax eurydice**:** FNJV 40122–3, 40149, 40179–80, 40193, 40196, 40202, 40211–2, 40225; MZUSP 139181; TLFT 2821; ZUEC 24569, 24574–6.

Scinax* cf. *hayii**:** FNJV 33218–20; MZUSP 57943–4; UFMG 17205, 17224–5, 17228–9, 17256–7; ZUEC 23598, 23626.

Scinax fuscovarius**:** FNJV 36623, 36677; UFMG 17264; TLFT 2819, 2820, 3191, 4185; ZUEC 23624–5, 24389.

Hylodes babax**:** MZUSP 57949; UFMG 17258.

Hylodes vanzolinii**:** MZUSP 57950, 52923.

Phantasmarana apuana**:** MNRJ 42027; MZUSP 139166–77; ZUEC 22566, 22947, 23205–6, 23392–6, 23497, 23641.

Pseudopaludicola restinga**:** FNJV 33214–5, 40148; TLFT 2824, 3211; ZUEC 24192, 24384–7, 24398–401.

Physalaemus cuvieri**:** FNJV 36575–6, 36578, 36633, 36652, 36654, 36660, 36683, 40089, 40092–3, 40097, 40188; ZUEC 23637, 24388, 24566.

Physalaemus feioi**:** FNJV 36596, 40087; ZUEC 24218, 24557.

***Physalaemus* sp. (aff. *maculiventris*):** FNJV 34638–48, 34655–6; UFMG 17250–1; ZUEC 24094–100, 24117–20, 24403–5, 24428.

Leptodactylus fuscus**:** CFBH 35174; FNJV 33212, 36584, 36626, 36650, 40176; MZUSP 135657–8; UFMG 17262; ZUEC 22941, 22943, 24341.

Leptodactylus mystacinus**:** FNJV 40146; ZUEC 22557, 24572–3.

Leptodactylus spixi**:** FNJV; UFMG 17268; ZUEC 23596, 23627, 24391–2.

Leptodactylus latrans**:** ZUEC 23578–80.

Proceratophrys boiei**:** FNJV 33213, 33266–8; MZUSP 135653–6, 139144–8; UFMG 17209, 17255; ZUEC 23575, 23621, 24410–2.

Phyllomedusa burmeisteri**:** FNJV 36588, 36669, 40069, 40134–6, 40140, 40143, 40145, 40147, 40183, 40186–7; MZUSP 139149; UFMG 17248; ZUEC 23619–20.

***Chiasmocleis* sp.:** FNJV 33204–5.

Elachistocleis* cf. *cesarii**:** FNJV 36658, 40177, 40192; ZUEC 24579.

Myersiella microps**:** MZUSP 133850–1.

Table S1. Sample sites/units (SUs) used in the anuran survey from Caparaó National Park and surrounding areas. Geographical coordinates in degrees, minutes, and seconds, based on the WGS84 datum. Elevation in meters above sea level. Environment types: swamp in forest areas (SFA), swamp in open areas (SOA), altitude lagoons (ALL), artificial lagoons (ARL), rivers and streams (RS), and deciduous forest (DF).

SUs	Geographic coordinates	Elevation (m)	Environment	Date
1	20° 35' 55.1" S; 41° 42' 37.8" W	698	SOA	18/1/2018
2	20° 36' 22.8" S; 41° 47' 51.4" W	758	SOA	26-28/11/2018
3	20° 33' 13.9" S; 41° 43' 58.2" W	796	SOA	22/1/2018
4	20° 34' 39.3" S; 41° 42' 54.9" W	806	SOA	8/12/2016
5	20° 34' 56.3" S; 41° 42' 55.6" W	806	SOA	8/12/2016
6	20° 35' 25.5" S; 41° 42' 50.7" W	806	SOA	8/12/2016
7	20° 36' 26.4" S; 41° 41' 23.2" W	806	SOA	8/12/2016
8	20° 35' 11.2" S; 41° 45' 37.9" W	808	SOA	18/1/2018
9	20° 35' 15.2" S; 41° 45' 54.3" W	815	SOA	17/1/2018
10	20° 36' 52.4" S; 41° 47' 22.4" W	820	ARL	29/11/2018
11	20° 35' 14" S; 41° 43' 55.4" W	823	ARL	11/12/2017
12	20° 35' 24.2" S; 41° 46' 3.1" W	824	SOA	17/1/2018, 24/11/2018, 4/12/2018
13	20° 34' 52.2" S; 41° 44' 15.7" W	829	SOA	8/12/2016, 11/12/2017, 18/1/2018
14	20° 33' 34.1" S; 41° 44' 30.8" W	843	SOA	10/12/2016
15	20° 37' 12.7" S; 41° 47' 45.9" W	843	SOA	23/11/2018
16	20° 37' 12.9" S; 41° 47' 41.5" W	843	SOA	3/12/2018
17	20° 35' 39.3" S; 41° 46' 5.2" W	851	ARL	19-20/1/2018
18	20° 25' 5.4" S; 41° 42' 35.7" W	872	SOA	17/1/2018
19	20° 25' 12.3" S; 41° 42' 5.9" W	872	RS	8/12/2016
20	20° 34' 53.4" S;41° 45' 36.4" W	886	ARL	10-12/12/2017, 27/11/2018
21	20° 35' 18" S; 41° 45' 51" W	920	SOA	25/11/2018
22	20° 35' 13.5" S; 41° 45' 39.9" W	922	ARL	16-18/1/2018, 2/12/2018
23	20° 34' 46.5" S; 41° 45' 40.1" W	923	SOA	10/12/2017, 16-19/1/2018
24	20° 26' 43.9" S; 41° 55' 23.2" W	933	SOA	1/2/2016
25	20° 26' 39.9" S; 41° 42' 52.8" W	983	SOA	9/12/2016
26	20° 28' 12.8" S; 41° 43' 25.9" W	986	SOA	9/12/2016
27	20° 34' 48.4" S; 41° 46' 13" W	1000	DF	17-21/1/2018
28	20° 25' 41.3" S; 41° 52' 13.6" W	1002	SOA	1/2/2016
29	20° 25' 41.4" S; 41° 52' 13.4" W	1003	SOA	1/2/2016

30	20° 25' 41.5" S; 41° 52' 12.8" W	1004	SOA	1/2/2016
31	20° 25' 38.2" S; 41° 52' 12.2" W	1005	SOA	7-9/12/2017, 23/1/2018
32	20° 26' 40.5" S; 41° 42' 56.5" W	1006	DF	11/12/2016
33	20° 28' 1.2" S; 41° 44' 0.9" W	1022	DF	18/1/2018
34	20° 25' 11.2" S; 41° 50' 57.2" W	1022	DF	8/10/2017, 8/12/2017
35	20° 27' 59.8" S; 41° 43' 56.2" W	1062	SOA	9/12/2016
36	20° 25' 36" S; 41° 51' 44.5" W	1090	ARL	7/12/2017
37	20° 30' 46" S; 41° 51' 15" W	1166	DF	10/10/2017
38	20° 25' 11.5" S; 41° 50' 48.7" W	1184	RS	27-30/07/2016, 1/8/2016, 19-25/10/2016, 7/10/2017, 23-25/1/2018
39	20° 25' 11" S; 41° 50' 49.5" W	1200	RS	1/8/2016
40	20° 25' 10" S; 41° 50' 47" W	1200	RS	25/1/2018
41	20° 25' 11.5" S; 41° 50' 48.7" W	1215	SFA	2/12/2016, 6-15/1/2017
42	20° 25' 18.7" S; 41° 50' 10.3" W	1227	DF	31/1/2016
43	20° 25' 11.5" S; 41° 50' 3.4" W	1238	DF	1/2/2016
44	20° 25' 9" S; 41° 50' 53.1" W	1244	DF	31/1/2016
45	20° 25' 17.1" S; 41° 51' 7.1" W	1244	DF	31/1/2016
46	20° 25' 10.9" S; 41° 50' 57.2" W	1266	DF	31/1/2016
47	20° 25' 8.8" S; 41° 50' 52.7" W	1275	DF	1/2/2016, 8/1/2017
48	20° 25' 9.1" S; 41° 50' 48" W	1275	RS	31/1/2016
49	20° 25' 12.6" S; 41° 50' 56.9" W	1282	SFA	7-8/10/2017, 9-10/12/2017, 25/1/2018
50	20° 25' 9.4" S; 41° 51' 6.5" W	1285	DF	8/12/2017
51	20° 25' 10" S; 41° 50' 47" W	1302	RS	31/1/2016
52	20° 25' 9.8" S; 41° 51' 2.6" W	1307	SOA	3/2/2016
53	20° 25' 8.7" S; 41° 50' 48.2" W	1312	DF	1/2/2016
54	20° 25' 8.5" S; 41° 50' 51.1" W	1321	DF	30/1/2016
55	20° 29' 25.8" S; 41° 49' 17.5" W	1572	RS	21/1/2018
56	20° 24' 25.5" S; 41° 50' 59.7" W	1591	DF	2/2/2016
57	20° 28' 51.5" S; 41° 49' 43.3" W	1746	SOA	21/1/2018
58	20° 28' 19.7" S; 41° 49' 44.9" W	1829	RS	12/10/2017
59	20° 24' 24.5" S; 41° 50' 13.3" W	1872	DF	29/1/2016
60	20° 25' 37.5" S; 41° 47' 27" W	2405	RS	6/10/2017
61	20° 25' 51.5" S; 41° 48' 9.4" W	2539	ALL	5/10/2017, 6/12/2017

62	20° 25' 18.4" S; 41° 48' 9" W	2551	ALL	5-10/10/2017, 25/1/2018

Table S2. Diversity index values estimated for the amphibian assemblage of the Caparaó National Park.

Index	**Mean ± SD**
ACE	50.38 ± 0
ICE	50.44 ± 0
Chao 1	49.25 ± 1.73
Chao 2	49.23 ± 1.71
Jackknife 1	52.92 ± 2.13
Jackknife 2	51.09 ± 0
Bootstrap	51.03 ± 0

Informações adicionais:

O texto acima reflete exatamente o artigo publicado, exceto por atualização taxonômica de alguns taxa. Após a publicação do artigo foi descrita a espécie *Thoropa bryomantys*, a qual não foi registrada na nossa amostragem, mas ocorre no PNC. Também foi nos indicado a possível presença de duas espécies de *Ischnocnema* (*I. oea* e *I. garciai*) que no nosso trabalho registramos como *I. feioi*, mas estudos moleculares são necessários para corroborar esta informação. Finalmente, a espécie aqui listada como *Physalaemus* sp. (aff. *maculiventris*) foi descrita como *Physalaemus araxa* como uma consequência deste inventário e o artigo foi publicado este ano no *European Journal of Taxonomy*.

CAPÍTULO 2

AMPHIBIAN CHYTRID INFECTIONS ALONG THE HIGHEST ELEVATIONAL GRADIENT OF THE ATLANTIC FOREST

Paisagem PNC
Foto: Camila Zornosa Torres

> *"Enquanto a mulher estiver só,*
> *será sempre o ser frágil a mercê das circunstâncias.*
> *Quando se unirem, elas tornar-se-ão uma grande força"*
> Bertha Lutz

Amphibian chytrid infections along the highest elevational gradient of the Atlantic Forest

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Abstract

Environmental variation along elevational gradients shapes conditions for pathogen development, which influences diseases outcomes. Chytridiomycosis is a non-vectored disease caused by the aquatic fungus *Batrachochytrium dendrobatidis* (Bd) and is responsible for massive declines of amphibian populations all over the world. Several biotic and abiotic factors are known to influence Bd infection dynamics in amphibians, including temperature and host species richness. Here, we quantified Bd prevalence and load along an elevational gradient in the Caparaó National Park (CNP) and tested for associations of Bd infections with elevation, temperature, and species richness. We hypothesized that Bd infections would increase as local species richness decreases with elevation. We detected Bd along all the elevational gradient, and found a negative association between infection load and elevation. We did not detect significant associations between infection prevalence and elevation. Our findings are consistent with other wide elevational gradient studies, but are contrary to two other studies performed in the Atlantic Forest. We did not find the minimum elevational range that should be sampled to detect the influence of elevation on Bd variation. Our study represents the widest elevational gradient that has been sampled in Brazil, and contributes to a better understanding of Bd distribution and dynamics in natural systems.

Keywords: *Batrachochytrium dendrobatidis*, Elevation, Species Richness, Temperature, Tropical Forest.

1. INTRODUCTION

Ecological processes vary along elevational gradients (Safford 1999, Körner 2007) due to the influence of abiotic factors including temperature, luminosity, relative humidity and pluviosity, and biotic factors including species richness, competition, predation, parasites, pathogens, resource availability and partitioning (Cox & Moore 2009). The variation of these factors across a gradient may limit species distribution in a given region, as well as directly influence host-pathogen dynamic interactions (Cruz & Feio 2007, Cox & Moore 2009). Along elevational gradients, for example, as elevation increases, solar radiation increases, but atmospheric pressure, temperature and relative humidity decreases (Körner 2007). Such environmental variation influences disease outcomes by shaping suitable or unsuitable conditions for the pathogen development (Leal et al. 2018). For example, low Lyme disease prevalence at higher elevations can be explained by the effect of elevation (via temperature) on the abundance of tick vectors (Jouda et al. 2004, Gilbert 2010). Environmental factors may also directly influence the occurrence of pathogens responsible for non-vectored diseases, such as the influenza virus, which increases during the rainy season in the tropics and during the dry season in temperate climates, as a result of the effects of humidity on the persistence of the virus (in droplets; Peci et al. 2019).

Chytridiomycosis, a disease caused by the aquatic fungus *Batrachochytrium dendrobatidis* (hereafter Bd), is a non-vectored disease, and has been associated with massive amphibian declines globally (Lips et al. 2006, Vredenburg et al. 2010, James et al. 2015, Carvalho et al. 2017, Scheele et al. 2019). Within this disease system, several biotic and abiotic factors influence Bd infection dynamics in wild amphibians, including temperature, rainfall, vegetation density, amphibian abundance, species richness and natural history (Ron 2005, Rödder et al. 2008, Vredenburg et al. 2010, Becker & Zamudio 2011, Gründler et al. 2012, Becker et al. 2014, Ruggeri et al. 2015, Lambertini et al. 2017, Mesquita et al. 2017).

A number of previous studies have detected associations between elevation and Bd infections prevalence and load (Brem & Lips 2008, Catenazzi et al. 2010, Gründler et al. 2012, Sapsford et al. 2013, Acevedo et al. 2016a). This relationship can be explained by lower temperatures at higher elevations, when these temperatures reflect the optimal thermal conditions for Bd development (Piotrowski et al. 2004, Voyles et al. 2017). However, this pattern may vary under different conditions and study scales. For example, across a narrow range of elevation (200 – 700 m above sea level; a.s.l.) in Brazil, Bd infection prevalence and load were not associated to elevation (Lambertini et al. 2016). On the other hand, across a different narrow range elevational gradient (80 – 760 m a.s.l.) in Panama, Bd infection prevalence was positively associated with elevation (Brem & Lips 2008). In contrast, across a wider elevational gradient (1200 – 3700 m a.s.l.) in Peru, a negative association was detected between Bd infection prevalence and elevation (Catenazzi et al. 2010). The suggested mechanism for this pattern was that the temperatures of the highest elevations exceeded the optimal temperature range for Bd (Catenazzi et al. 2010).

Besides climatic conditions, biotic factors also influence Bd infection prevalence and load. Host species richness can influence both Bd prevalence and load due to amplification or dilution effects (Becker & Zamudio 2011, Searle et al. 2011, Becker et al. 2014). Even though both effects may occur concomitantly in the wild (Luis et al. 2018), they may vary according to spatial scale: in general the dilution effect is expected to be detectable at small scales and the amplification effect at large scales (Halliday & Rohr 2019). Therefore, several factors (i.e. environmental conditions, host richness, host species) are known to influence chytrid-amphibian dynamics, and additional studies are needed for a complete and clear picture of these interactions.

In this study, we assessed the variation in Bd infection prevalence and load over a ~2000 m elevational gradient in the highest peak of the Brazilian Atlantic Forest. The

Brazilian Atlantic Forest is severely impacted by historical chytridiomycosis outbreaks and synchronous amphibian population extinctions (Carvalho et al. 2017). We tested whether elevation and host species richness explain Bd infections along the elevational gradient. We hypothesized that as local amphibian richness decreases with elevation (Zornosa-Torres et al. 2020) Bd infections would increase with elevation, due to dilution effects at lower elevations. We also summarized previous studies that analyzed the association between Bd and elevation, to determine whether there is a minimum elevational range that is necessary to detect effects of elevation on Bd infection dynamics.

2. METHODS

2.1 Study site and data collection

We sampled anurans in the Caparaó National Park (CNP) and surrounding area along an elevational gradient, from 698 to 2656 m above sea level (a.s.l.). The CNP is located along the border of the states of Minas Gerais and Espírito Santo in southeastern Brazil, and is part of the north mountain region of the Brazilian Atlantic Forest Serra da Mantiqueira. The CNP varies in elevation from 859 to 2892 m a.s.l., and the predominant habitats include ombrophilous rainforests (dominant across the area), and high-elevation grasslands above 1900 m (Machado et al. 2016). The anuran fauna is composed of 61 species. Species richness decreases with elevation from 34 species in the lowlands to 2 species at the peak of the mountain (Zornosa-Torres et al. 2020).

In an effort to sample the highest species diversity for each collecting site, individual amphibians were sampled using active search during the breeding seasons of 2016 (February, August, October, and December), 2017 and 2018 (January, October, and December), totaling 63 sampling days. Frogs were individually collected using clean plastic collection bags, in which they were stored until the time of swabbing. At the time of capture, we measured environmental temperature using a thermohydrometer and a HOBO-MX2301 datalogger. The factor richness was considered as the number of species sampled in each site.

In total, 474 amphibians were sampled in 34 sampling sites across the CNP elevational gradient, and represent 11 families, 20 genera, and 47 species (Table S1). We aimed to sample the greatest possible amphibian diversity by selecting different types of amphibian habitats, such as swamps, streams, rivers, temporal ponds, wetlands, lagoons, forest with leaf litter, and open areas. A map of the sites distribution is presented in Figure S1. We did not find amphibian habitats between 1601 – 2400 m a.s.l.

2.2 Bd prevalence and infection load

We swabbed the skin of each collected individual following established protocols (Hyatt et al. 2007, Lambertini et al. 2013). Each swab was placed in a dry and sterile 1.5 ml cryovial and stored at -20 °C until diagnostic analyses were conducted. For detection and quantification of Bd we first extracted DNA from swabs using 50 µl of PrepMan ULTRA® (Life Technologies) and then proceeded with quantification of infection loads for each sample using a TaqMan® qPCR Assay (Life Technologies) (Boyle et al. 2004), with modifications described in Kriger et al. 2006 and Lambertini et al. 2013. We used the strain CLFT 159 as a quantitative standard for our qPCR reactions, serially diluted from 10^3 to 10^{-1} zoospore genomic equivalents (g.e.). This strain was isolated from a tadpole individual (*Hylodes cardosoi*; state of Paraná, Brazil), and belongs to the *Global Pandemic Lineage* (Jenkinson et al. 2016). Our quantitative standards from 10^3 to 10 g.e. were run in duplicate; the standards 1 and 10^{-1} g.e. in quadruplicate, and the negative control (nuclease-free water) in duplicate. The samples were in run singlicate. We considered positive samples for Bd when at least one g.e.

was detected (Kriger et al. 2007) and rounded infection load values to integer numbers (Santos et al. 2020).

2.3 Statistical analyses of Field Data

To compare the effect of elevation and species richness on Bd infection prevalence and load, we fit a generalized linear model (GLM) and a linear model (LM) to our data in R (vrs. [3.4.4] (R Core Team 2018)), using Bd infection prevalence and positive infection load (log-transformed) as respective response variables, and elevation and species richness as predictor variables. Before fitting the models, we checked the fulfillment of the assumptions using Shapiro-Will test and log-transform Bd infection load data. Normality of the residuals was also tested by QQ-plot and visual parameters. To run the model for prevalence we used the *lm* function and for Bd infection load we used the *glm* function, with Gamma error distribution (as the data was not normal) and inverse link function. Both models were fit using the 'R stats' package. The significance of the predicted variables was calculated using the function *Anova* of the package 'car'.

To test for relationships between Bd infection prevalence and load with temperature along the elevational gradient, we established three different elevational ranges (600 – 1100; 1101 – 1600; 2400 – 2700 m a.s.l.) using the relative differences of mean environmental temperature of one of the localities at each elevational range (0; -0.09; -9.86 °C). Prior to the analysis we tested the normality of our data using a Shapiro-Wilk test, and data was not normal. We then performed the non-parametric Kruskal-Wallis test and a post-hoc *Dunn test* to detect where the differences were, using the package 'PMCMRplus' and a Holm-adjusted *P* value.

2.4 Literature Review

A systematic review was performed of published papers that have explored Bd infections along elevational gradients. To this end, Google Scholar archives were searched using the keywords: *Batrachochytrium dendrobatidis*, altitude, elevation, and altitudinal gradients.

To compare against previous studies, we fit a binomial logistic regression using the relationship between elevation and Bd in each study as the response variable coded as 0 (no association observed) or 1 (association observed), and the range of the sampled elevational gradient as the predictor variable (maximum elevation sampled - minimum elevation sampled) using the function *glm*, with Binomial error distribution and logit link function in R (vrs. [3.4.4] (R Core Team 2018)).

3. RESULTS

3.1 Bd infection prevalence

We detected Bd throughout the elevational gradient, in 70% of the sampling sites, and the prevalence of infected individuals varied from 10% to 66.7% throughout the study area (Table 1).

The LM analysis indicated that the variation in Bd infection prevalence between sites was not explained either by elevation nor by species richness ($F_{[1, 21]} = 1.88$; $P = 0.18$; $F_{[1, 21]} = 2.40$; $P = 0.13$, respectively; Table S2). Bd infection prevalence tended to be higher in areas with lower temperatures and higher elevations, but these data could not be statistically tested because of the small sample size from areas with lower temperature (Fig. 1A).

3.2 Bd infection load

We detected a mean infection load across the transect of 955 g.e. (range: 1– 55,095 g.e.), with the highest infection load rate in the elevational range of 600 – 1100 m a.s.l. in an individual hylid treefrog (Tables 1 & 2). There was a positive relationship between Bd infection load and temperature, with lower Bd infection loads in areas with lower temperatures and higher Bd infection loads in areas with higher temperatures ($X^2 = 23.71$; $P < 0.0001$; Fig.1B). Our GLM analysis indicated that the differences in Bd infection loads between sampling sites were explained by elevation but not by species richness ($X^2 = 24.12$; $P < 0.0001$; Fig.1C; Table S2), with higher Bd infection load in low elevation sites (600 – 1100 m a.s.l.) than in high elevation sites (above 1600 m) ($P < 0.0005$).

3.3 Elevational gradient range

We found ten studies that explored Bd infection along elevational gradients in different parts of the world (Table S3). While only four studies evaluated Bd load in the sampled sites, all ten studies evaluated Bd prevalence along the sampled elevational range. Therefore, the logistic regression was fit only with results for Bd prevalence. No minimal elevational range was detected for the ability to observe an association between elevation and Bd prevalence (binomial logistic regression, $z = 1.03$; $P = 0.30$).

4. DISCUSSION

Previous studies stated that at higher elevations Bd infection prevalence and load should be higher, as a consequence of temperature decrease (Ron 2005, Rödder et al. 2008). However, we observed a decrease of Bd infection load as elevation increased. Both patterns (increase or decrease of infection load with the elevation) are dependent on context, as depending on geographic region, different elevational gradients may contain optimal temperatures for the pathogen growth at upper or lower ends of the range. In the tropics, suboptimal temperatures for Bd are most likely to be found at sea level elevations, or also in high elevations (e.g., above 1500 m a.s.l.); whereas optimal temperatures are likely to be found in intermediate elevations. High elevation sites in the CNP (above 1600 m) presented a mean temperature of 10 °C during a summer night, which is a suboptimal temperature for Bd growth (Piotrowski et al. 2004, Neely et al. 2020). Other studies have shown that extreme temperatures (relative to the optimal Bd growth temperature range) reduce infection intensity or slow pathogen growth (Piotrowski et al. 2004, Becker & Zamudio 2011). Consequently, lower Bd infection loads could be observed in localities with extreme temperatures, as we detected above 1600 m, where altitudinal grasslands were predominant, presenting open areas with high incidence of wind and low vegetation cover, contributing with low extreme temperatures at night and high sun incidence during the day.

Associations between Bd infection prevalence and load with elevational gradients have been analyzed by numerous past studies (Brem & Lips 2008, Kriger & Hero 2008, Catenazzi et al. 2010, Kilburn et al. 2010, Gründler et al. 2012, Sapsford et al. 2013, Acevedo et al. 2016a,b, Lambertini et al. 2016), and different results have been found due to the elevational gradient range and the maximum elevation sampled (Table S2). Wide range elevational gradient studies in Colombia (200 – 3600 m a.s.l) and Peru (1200 – 3700 m a.s.l), have detected a negative relationship between Bd prevalence and elevation (Catenazzi et al. 2010, Acevedo et al. 2016a), in our wide range elevational gradient we did not find significant relationship between prevalence and elevation. Other studies made on both wide (45 – 1215 m a.s.l; 2000 – 3200 m a.s.l) and narrow (90 – 885 m a.s.l; 200 – 700 m a.s.l) elevational gradients also found no associations between Bd prevalence and elevation (Kriger & Hero

2008, Kilburn et al. 2010, Acevedo et al. 2016b, Lambertini et al. 2016) reinforcing the importance of evaluate different factors to explain Bd infection prevalence along elevational gradients.

Few studies evaluated associations between infection load and elevation (Kriger & Hero 2008, Catenazzi et al. 2010, Lambertini et al. 2016, Lambertini et al. 2021), our findings on Bd infection load are contrary to these studies, as they found no association (Kriger & Hero 2008, Catenazzi et al. 2010, Lambertini et al. 2016) or positive association with elevation (Lambertini et al. 2021), but only one of these studies was performed along a wide range elevational gradient (Catenazzi et al. 2010). Even among studies performed in the Atlantic Forest, there have been positive relationships (Gründler et al. 2012; Lambertini et al. 2021) or no relationship (Lambertini et al. 2016) detected between elevation and Bd infection prevalence or load. Clearly there is not a unidirectional pattern of this association. Other factors, such as community composition (Becker & Zamudio 2011), forest cover (Becker et al. 2016), and temperature variation (Ruggeri et al. 2015) may influence the existence and the direction of these associations.

In the CNP we detected a negative association between elevation and Bd load and no association between elevation and Bd prevalence, although prevalence in higher elevations was slightly higher than in lower elevations. These possible opposite patterns have previously been observed in the Atlantic Forest, and were suggested to be mediated by the scale of both prevalence and load (Gründler et al. 2012). Infection prevalence is a characteristic of the population or the community, while infection load is a characteristic of the individual; because of that, different processes (environmental, demographic, and those intrinsic to the individual host) control infection dynamics and can result in different outcomes (Gründler et al. 2012). We also hypothesize that these different outcomes on Bd load and prevalence association with elevation could be explained by the few breeding sites available for amphibians above 1600 m in the CNP. In high altitude areas of the CNP, the most available breeding habitats for amphibians were lotic (streams and rivers). The few availability of lentic habitats (as ponds and lakes) is directly related to reduced amphibian species richness in the mountaintop (only two species occur in areas above 2400 m) (Zornosa-Torres et al. 2020), which may explain why host species richness did not predict Bd infections in our study. The dilution effect on Bd infections has already been detected in small scale studies (Searle et al. 2011, Becker et al. 2014), while amplification effect has been detected in large scale studies (Becker and Zamudio 2011, Lambertini et al. 2021), but the influence of amphibian species richness on Bd infections is context-dependent, and the fact that we did not detect any associations between these variables could be explained by the types of habitats we sampled (most lotic habitats).

Although we observed that there is not a minimum elevational range that has to be sampled to detect associations between elevation and Bd infections, we suggest that there must be a turning point along elevational gradients where Bd infection prevalence and load could be positively or negatively associated to elevation due to variation of temperature, vegetation cover, light incidence, and habitat availability throughout the gradient. However, further studies are needed to support this hypothesis. We recommend that future studies explore factors other than elevation that could explain Bd dynamics along elevational gradients, such as temperature, host diversity, and vegetation cover.

Our sampling represents the widest elevational gradient ever sampled in Brazil. Wide elevational gradients provide an excellent opportunity to evaluate which biotic and abiotic factors influence Bd infection in host amphibians. The development of further studies might increase our ability to predict Bd distribution and dynamics in natural systems more effectively. This will be critical to future conservation actions in vulnerable amphibian communities.

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Table 1. Amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) prevalence and load in each elevational range. Temperature difference is the difference between one site and another in each elevation range. a.s.l. = above sea level; n = number of sampled individuals.

Elevational range (m a.s.l.)	**Temperature difference (ºC)**	**n**	**Prevalence (%)**	**Load (g.e.)**
600 - 1100	0	356	32.30	1198.18
1101 - 1600	-0.09	97	22.68	29.30
2400 - 2700	-9.86	21	38.10	2.04

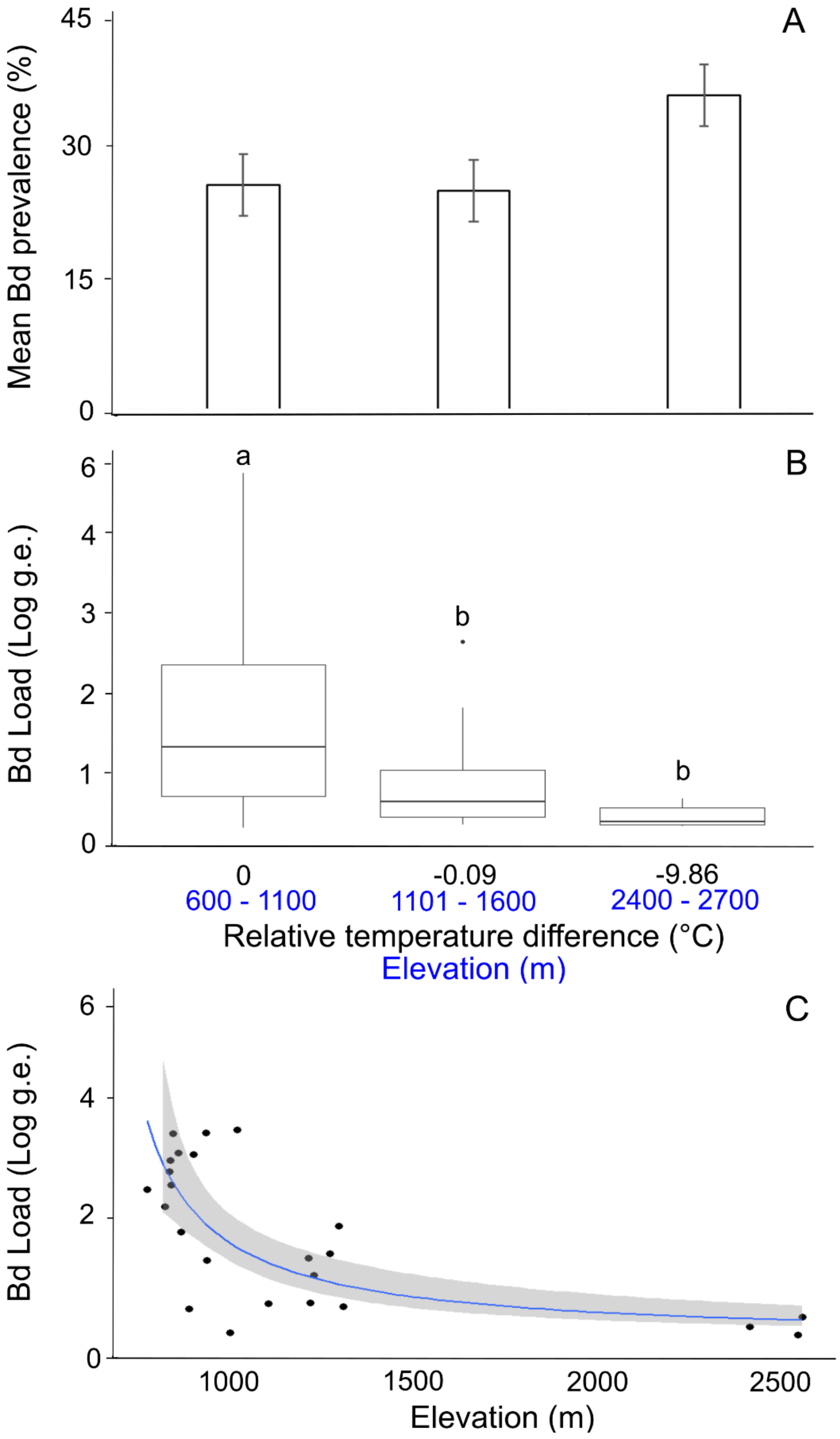
Table 2. Anuran families sampled along an elevation gradient in the Caparaó National Park, showing the number of individuals sampled in each family (n), the elevational range, municipality, *Batrachochytrium dendrobatidis* prevalence and infection load (presented as mean ± SD (range)).

Family	**n**	**Elevational range (m)**	**Municipality**	**Prevalence (%)**	**Load (g.e.)**
Brachycephalidae	29	600 - 1100; 1101 - 1600	Alto Caparaó, Divino de São Lourenço, Dores do Rio Preto, Ibitirama	17.24	63.25 ± 106.54 (2 – 250)
Bufonidae	19	600 - 1100; 1101 - 1600	Alto Caparaó, Divino de São Lourenço, Dores do Rio Preto, Ibitirama, Mundo Novo	21.05	2.76 ± 1.26 (1– 4)
Centrolenidae	6	600 - 1100; 1101 - 1600	Alto Caparaó, Divino de São Lourenço, Dores do Rio Preto	16.67	1.83 ± 0 (2 – 2)
Craugastoridae	19	600 - 1100; 1101 - 1600	Alto Caparaó, Ibitirama	15.79	4.22 ± 2.91 (2 – 7)
Cycloramphidae	24	600 - 1100; 1101 - 1600; 2400 - 2700	Alto Caparaó, Divino de São Lourenço, Ibitirama	25.00	7.63 ± 10.99 (1 – 30)
Hylidae	300	600 - 1100; 1101 - 1600	Alto Caparaó, Divino de São Lourenço, Dores do Rio Preto, Ibitirama, Mundo Novo	32.67	1338.80 ± 6269.14 (1 – 55095)
Hylodidae	2	1101 - 1600	Alto Caparaó	50.00	63.42 ± 0 (63 – 63)

Leptodactylidae	52	600 - 1100; 2400 - 2700	Alto Caparaó, Divino de São Lourenço, Dores do Rio Preto, Ibitirama, Mundo Novo	30.77	11.82 ± 14.93 (1– 44)
Microhylidae	1	600 - 1100	Dores do Rio Preto	100.00	195.83 ± 0 (196 – 196)
Odontophrynidae	4	600 - 1100; 1101 - 1600	Alto Caparaó, Ibitirama	50.00	3.42 ± 0.09 (3 – 3)
Phyllomedusidae	18	600 - 1100	Divino de São Lourenço, Dores do Rio Preto, Ibitirama, Mundo Novo	44.44	800.81 ± 1025.62 (12 – 2943)

Figure legend

Figure 1. *Batrachochytrium dendrobatidis* (Bd) prevalence (A) and load (B) in three different temperatures along the altitudinal gradient. In A and B temperature is given as the difference between one site and another in each elevation range, vertical lines represent error bars. Relationship between *Bd* infection load and elevation, give by the generalized linear model (blue line) and confidence interval (grey shadow) (C).


A
Mean Bd prevalence (%)
45
30
15
0
B
Bd Load (Log g.e.)
6
4
3
2
1
0
a
b
b
0
600 - 1100
-0.09
1101 - 1600
-9.86
2400 - 2700
Relative temperature difference (°C)
Elevation (m)
C
Bd Load (Log g.e.)
6
4
2
0
1000
1500
2000
2500
Elevation (m)

Supplementary material

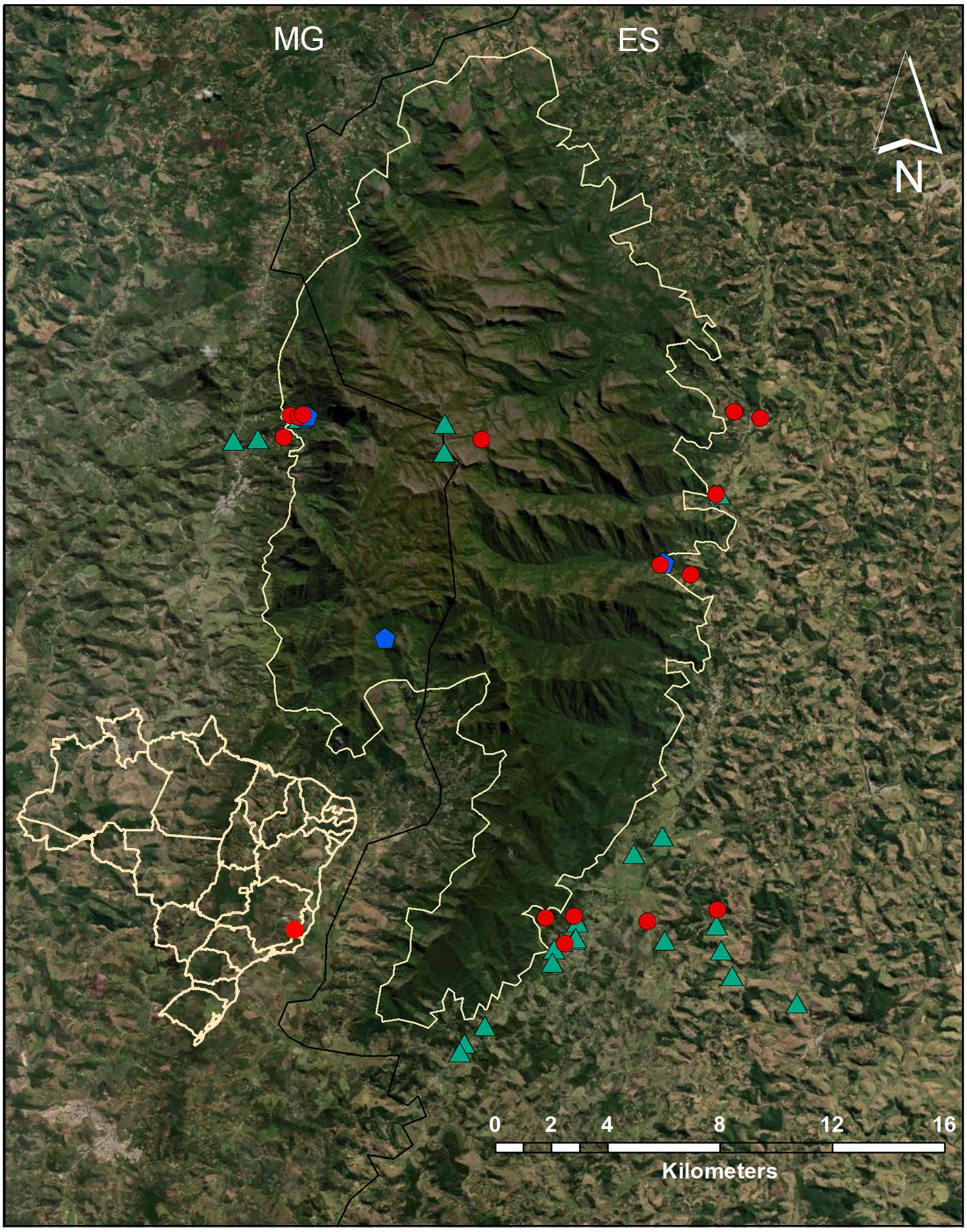
Table S2. Results of LM (prevalence) and GLM (load) fitted models. GLM results, intercept and slope are given in the inverse link function.

Model	**Variables**	**Intercept**	**Slope**	**t value**	***P* value**
Linear model (LM)	Elevation	66.027	-0.012	-1.370	0.185
	Richness	66.027	-2.488	-1.551	0.136
Generalized linear model (GLM)	Elevation	-1.104	0.002	3.989	0.000
	Richness	-1.104	0.018	0.797	0.434

Table S3. Published and unpublished studies that evaluated *Batrachochytrium dendrobatidis* prevalence along altitudinal gradients. Minimum and maximum elevation refers to the lowest and the highest elevation sampled in each study. Elevational range refers to the total elevation sampled during each study (i.e., the difference between the maximum and minimum elevation). ↑ represents increasing values. ↓ represents decreasing values.

Reference	Minimum elevation (m)	Maximum elevation (m)	Elevational range (m)	Country	Relationships
Acevedo et al. 2016a	200	3600	3400	Colombia	↑Altitude ↓Bd prevalence
Acevedo et al. 2016b	2000	3200	1200	Colombia	No relationship
Brem & Lips 2008	80	880	800	Panamá	↑Altitude ↑Bd prevalence
Catenazzi et al. 2010	1200	3700	2500	Peru	↑Altitude ↓Bd prevalence
Gründler et al. 2012	0	1600	1600	Brazil	↑Altitude ↑Bd prevalence
Kilburn et al. 2010	45	1215	1170	Panamá	No relationship
Kriger et al. 2010	90	885	795	Australia	No relationship
Lambertini et al. 2016	200	700	500	Brazil	No relationship
Lambertini et al. 2021	10	1155	1145	Brazil	↑Altitude ↑Bd prevalence
Sapsford et al. 2013	18	718	700	Australia	↑Altitude ↑Bd prevalence
Present study	**698**	**2656**	**1958**	**Brazil**	**No relationship**

Figure S1. Sample sites distribution in the Caparaó National Park (CNP) and surroundings: yellow line represents the limits of the CNP, red circles symbolize terrestrial habitats; green triangles symbolize lentic habitats; blue hexagons symbolize lotic habitats. Black line represents the boundaries between states of Minas Gerais (MG) and Espírito Santo (ES).



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CAPÍTULO 3

TEMPORAL CALL TRAITS SHAPE ACOUSTIC SPACE PARTITION AMONG NEOTROPICAL FROGS ASSEMBLAGES

Scinax eurydice
Foto: Victor Fávaro

"Que nada nos defina, que nada nos sujeite.
Que a liberdade seja nossa própria substância, já que viver é ser livre"
Simone de Beauvior

Temporal call traits shape acoustic space partition among Neotropical frogs assemblages

******A ser submetido para Bioacoustics

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Abstract

Different tropical anuran species congregate in the same breeding sites, leading to abundant frog choruses that compose a complex acoustic space. Under this conditions, acoustic masking may take place, impairing individuals communication. Thus, it is expected that sympatric individuals and species use strategies to avoid the overlap of their acoustic signals. Therefore, examining the structure of 10 anuran assemblages in the Caparaó National Park, Brazil, we tested if acoustic space tended to overlap (aggregated structure) or partition (dispersed structure) by full-null model comparisons using acoustic distances. We also determined the influences of phylogenetic similarities and species richness in the acoustic space and characterized it with species occurrence and individuals abundance approaches. We found that on average, phylogenetic distances had an aggregated structure (phylogenetically closer species), while spectral and temporal acoustic distances were dispersed suggesting acoustic partitioning. However, when we examined the relationship between species richness and phylogenetic distances with acoustic parameters, we found that temporal distances and species richness were positively related, but phylogenetic and temporal distances were negatively related. Thus, the assemblages were composed of closely related species with more different temporal parameters than expected by chance. Representing the acoustic structure of communities weighted by individuals abundance was no different than using species occurrence. Acoustic masking is more likely when both temporal and spectral components of the acoustic signals overlap. The observed patterns suggest that in assemblages with higher interspecific competition potential (richer assemblages), the dispersed temporal parameters may be a strategy of temporal acoustic partitioning, but no indicators of spectral partitioning were observed. Our findings are relevant to comprehend the influence of ecological and historical factors in the organization of tropical anurans acoustic space under the acoustic niche partitioning hypothesis.

Introduction

Anuran species are typically found using similar reproductive environments, such as ponds, swamps, and streams (Wells 2007). Sharing these environments may lead to crowdy assemblages, mainly in tropical regions that are also characterized by having diverse and complex acoustic spaces (Duarte et al. 2019; Ulloa et al. 2019). Inhabiting crowded and acoustic complex environments may impair acoustic communication, causing mismating (Höbel & Gerdhardt 2003), hybridization (Oliveira et al 1991; Haddad et al. 1994; Vargas-Salinas & Amézquita 2013), and acoustic masking (Gerdhardt & Klump 1988; Both & Grant 2012; Medeiros et al. 2017) in syntopic species.

Masking acoustic signals is generally observed in dense and loud choruses, where males may modulate their calls in order to overcome the possible masking and attract females (Gerdhardt & Klump 1988; Both & Grant 2012; Medeiros et al. 2017). Modulations can be increasing calling amplitude (Gerdhardt & Klump 1988), increasing/decreasing dominant frequency and call duration (Both & Grant 2012; Medeiros et al. 2017). Usually, these strategies are used to avoid acoustic masking and consequently mismating and hybridization (Blair 1964), but some species have shown different outcomes. Brazilian treefrogs (*Boana bischoffi*, *B. leptolineata* and *Dendropsophus minutus*) decreased their dominant frequency in response to low frequency advertisement calls of an invasive and native species, increasing spectral overlap (Medeiros et al. 2017). However, modifications of acoustic parameters involve high energy costs, and may lead to false cues of male conditions, affecting mating and consequently reducing the species fitness (Gerdhardt 1991; Both & Grant 2012).

Anuran advertisement calls are mainly used for females attraction, and carries valuable information such as males specific identity, health condition, size and localization (Toledo et al. 2015a; Köhler et al. 2017). Thus, the effective transmission of this type of signals is mandatory to establish communication between signalers and receivers.

Consequently, it is expected that individuals use strategies to avoid acoustic overlap favoring effective communication, especially in dense tropical habitats where this drawback may be common. The collection of heterospecific calls can be described as an acoustic space with n-dimensions representing acoustic features, such as temporal and spectral ones (Feynman et al. 1963; de Araújo et al. 2020). Additionally, the acoustic niche is defined by the portion of the acoustic space in which an effective communication occurs (Seur & Farina 2015; de Araújo et al. 2020) and is where acoustic overlap may take place. The acoustic niche partitioning hypothesis predicts that strategies that minimize acoustic interference and ensure effective communication may be selected along species evolution (Hödl 1977; Drewry & Rand 1983; Krause 1983). Under this hypothesis and considering interspecific competition, the strategies used by individuals to avoid acoustic niche overlap could be spatial (e.g. calling microhabitats), temporal (e.g. calling activity during the night, duration of calls or calls in antiphony), or spectral (e.g. calling in different frequency bands) (Garcia-Rutledge & Narins 2001; Sinsch et al. 2012).

Several studies have evoked acoustic partitioning to explain the differences in one or more dimensions of the acoustic space in anuran assemblages (Hödl 1977; Drewry & Rand 1983; Garcia-Rutledge & Narins 2001; Chek et al. 2003; Pombal 2010; Sinsch et al. 2012; Villanueva-Rivera 2014; Bignotte-Giró et al. 2019; Duarte et al. 2019). Some of them observed acoustic dispersion only in the temporal dimension (Drewry & Rand 1983; Pombal 2010; Duarte et al. 2019). Nevertheless, for acoustic masking to occur it is necessary to be both temporal and spectral overlap of the signals, leading to an inefficient information transmission (de Araújo et al. 2020). Consequently, temporal partitioning is effective to avoid acoustic overlap, as it may occur in different scales, such as seasonal, diuturnal, and instantaneous (minutes-seconds) time scales (Garcia-Rutledge & Narins 2001), and temporal traits are more variable (generally dynamic) than spectral ones (generally static) (Gerhardt 1991; Köhler et al. 2017).

Despite many studies have evaluated different strategies to avoid acoustic niche overlap in anuran assemblages (Hödl 1977; Drewry & Rand 1983; Garcia-Rutledge & Narins 2001; Chek et al. 2003; Pombal 2010; Sinsch et al. 2012; Villanueva-Rivera 2014; Bignotte-Giró et al. 2019; Duarte et al. 2019; Guerra et al. 2020; Sugai et al. 2021), few considered ecological features, such as species richness and assemblage composition, which may also influence the acoustic space partitioning (Duarte et al. 2019; Ulloa et al. 2019; Sugai et al. 2021). Tropical anuran assemblages showed acoustic overlap when the number of calling species was high, as the species richness influenced positively males calling emission rate in a breeding arena (Duarte et al. 2019; Ulloa et al. 2019).

Therefore, considering the highly diverse tropical anuran assemblages and how the occupancy and partitioning of the acoustic space can affect communication effectiveness, we determined the structure of the acoustic space in anuran assemblages, exploring the hypotheses that the acoustic space is influenced by: i) the anuran species richness, ii) abundance, and iii) the phylogenetic similarity of the anuran assemblage. To accomplish that, we sampled 10 anuran assemblages in southeastern Brazil and used acoustic and phylogenetic distances to determine the acoustic space structure. We also fitted GLMMs to assess the influence of phylogenetic similarities and species richness on acoustic space. We expected that, in agreement with the acoustic niche partitioning hypothesis, anuran assemblages would present dispersed structure in all the evaluated dimensions (temporal and spectral) as an influence of species richness (negative influence), abundance (negative influence), and phylogenetic relatedness (positive influence).

Methods

Study site and audio recordings

To characterize the acoustic space of anurans and monitor their temporal activity, we sampled 10 assemblages at the Caparaó National Park (CNP) and surrounding areas (Table S1), mainly during the rainy season between December and March of 2016 and 2018, comprising the entire breeding period of anuran assemblages at the study site (Zornosa-Torres et al. 2020). The CNP is located on the state border between Minas Gerais and Espírito Santo, in southeast Brazil, in the Atlantic Forest biome. These localities were either natural temporal wetlands, artificial or natural swamps, and artificial or natural ponds in open and forested areas (Table S1). Sites were between 600 and 1300 m a.s.l (Table S1), corresponding to ombrophilous Atlantic forest, the predominant phytophysiognomy in the CNP (Machado et al. 2016).

To characterize species acoustic parameters, we sampled as many individuals per species as it was possible, recording calling males from about 70 cm with digital recorders (TASCAM DR-40, Marantz PMD 661, Sony PCM-M10, Zoom H4n, and Zoom H4n Pro) and their built-in microphones. The 212 recordings made lasted in average 2.2 ± 0.9 min. For species with less than four or no recordings, we searched for their advertisement calls in the recordings registered with autonomous units (also placed in the same breeding sites) and selected those containing vocalizations with high signal-to-noise ratio to complete information about their acoustical parameters (Table S2). Prior to analyses we standardized all recordings to mono, 24 kHz of sample rate and 16 bits of audio depth; and normalized to an amplitude of -1.0 dB with Adobe Audition 2020. We measured seven acoustic parameters of each call on Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019): dominant frequency (measured with the peak frequency function), frequency 5%, frequency 95%, band width 90%, center frequency, call duration, and center time. We calculated Pearson correlations for all acoustic parameters and selected four uncorrelated parameters (dominant frequency, band width 90%, call duration and center time; $r \leq 0.41$). Spectrograms were generated using Hann window type, 30 % brightness, 76 % contrast, windows size and DFT (Discrete Fourier Transformation) of 1024 samples, 70 % time grid overlap and a 3dB filter bandwidth of 33.7 Hz in Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019). Spectral parameters were measured from the spectrogram and temporal parameters were measured from oscillograms. We calculated mean and standard deviation of the four uncorrelated acoustic parameters for each species using manual and autonomous recordings. We checked mean and standard deviation variables correlations using Pearson correlation and selected mean values of the previous stated acoustic parameters ($r \leq 0.37$).

Recordings were deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV), Museu de Diversidade Biológica (MDBio), Universidade Estadual de Campinas (Unicamp) (Table S2). Sampling permits were provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBio #58836-1), Sistema Nacional de Gestão do Patrimônio Genético (SISGen #A45E77D), and animal use ethics committee of Unicamp (CEUA #4827-1/2018). Individuals were not collected for this study, but taxonomic identities were confirmed and based on Zornosa-Torres et al. (2020).

Acoustic monitoring

In addition to manual recordings, we monitored the same 10 assemblages from around 19h to 03h, during one to six nights (Table 1), along the anuran reproductive seasons (December to March), using autonomous recording units (Wildlife Acoustics SM3) and their two built-in microphones, positioned in the border of the water body, with a sample rate of 24 kHz, audio depth of 16 bit and microphone gain of 24 dB set in both microphones. Sampled sites were on average 1.3 km apart from each other, except for two of them (sample sites 2 and 4), which had two recorders in the same large swamp, but separated by approximately 60 m, therefore considered as different assemblages (thus, we had 8 sampled breeding sites, but 10 assemblages). Autonomous recorders were configured to register one minute per hour from 18h to 3h (GMT-3) matching a higher amphibian vocal activity at this period. Autonomous recordings were assessed to identify calling species using aural and visual recognition in Raven Pro spectrograms.

We calculated the relative abundance of calling individuals using the amphibian calling index proposed by the North American amphibian monitoring program (NAAMP) (Weir & Mossman 2005) in 1 minute recording made each hour. This index rates the acoustic activity from zero to three as follow: (0) no activity recorded, no frogs were calling; (1) individual calls can be individualized, calls do not overlap; (2) distinguishable calls, there is overlap of some of the anuran calls; (3) overlapped, constant and continuous calls or choruses (Weir & Mossman 2005). We used visual (spectrogram) and aural approaches to calculate the amphibian calling index in autonomous recordings from the 10 monitored assemblages.

Acoustic and phylogenetic spaces

The acoustic space of hourly assemblages was represented for each single acoustic parameter separately. Using the mean value for each species we first, standardizing acoustic and phylogenetic values applying the method *standardize* (zero mean and unit standard deviation) of the function *decostand* and calculated Euclidean pairwise distances between all species using the *vegan* package (Oksanen et al. 2019). To characterize phylogenetic similarity among syntopic species (those in the same breeding habitat), we used a time calibrated tree (Pyron & Weins 2011) pruned to the species found in the sampled sites. For species that were not present in the phylogenetic tree, we searched for synonymies (Frost 2021) or for the closest sister taxa (based on recent phylogenies) and inserted as a polytomy in the tip. Namely, *Dendropsophus haddadi* was added as polytomy in the *D. decipiens* tip (Orrico et al. 2021), *Physalaemus feioi* and *Physalaemus araxa* were added as polytomies in the *P. cuvieri* tip (Leal et al. 2021), and *Scinax alter* and *S. eurydice* as polytomies in the *S. ruber* tip (Frost 2021). We then calculated patristic distances among all species. For these analyses, we used the R packages *ape*, *geiger*, and *adephylo* (Jombart & Dray 2008, Pennell et al. 2014, Paradis & Schliep 2019).

To assess the structure of anuran acoustic assemblages and based on the pairwise matrices of acoustic parameters and phylogenetic relatedness, we calculated a measure of dispersion of the assemblages by: i) assessing the set of co-signaling species from each hour within each site (hereafter signaling assemblages), ii) for each species in the signaling assemblages we determined the closest distance from the co-signaling species for each acoustic parameter and phylogenetic distance (i.e., nearest distance), iii) we calculated the mean value per signaling assemblage of the nearest distances (mean nearest taxon distance, MNTD) for acoustic parameters and phylogenetic distances, and iv) calculated the standardized effect size (SES) of the MNTD of each acoustic parameter (hereafter acoustic similarity) and phylogenetic distances (hereafter phylogenetic similarity) (Webb et al. 2002, Cadotte et al. 2012). The MNTD is the mean of the shortest phylogenetic distance calculated for each species to its closest co-signaling relative for each signaling assemblage (Cadotte et al. 2012). This approach by itself does not provide the magnitude of the effect of interest, to contour this, we calculated the SES that enabled comparisons between assemblages (Botta-Dukát 2018). The SES was calculated by i) randomizing species names in the acoustic and phylogenetic pairwise distance matrices, ii) calculating a null MNTD, and iii) repeating the previous steps 1000 times. With the null distributions of MNTD from each signaling assemblage, we computed the observed MNTD minus the mean of the corresponding null distribution of MNTD and divided by standard deviation of the null distribution, for each acoustic parameter and phylogenetic distance (Swenson & Weiser 2014). Positive SES MNTD values indicate more dispersed acoustic and phylogenetic spaces, while negative SES MNTD indicate more aggregated spaces (Swenson & Weiser 2014). Finally, we calculated species richness as the total number of species in each signaling assemblages. We used the package *picante* in R (Kembel et al. 2010).

Steps i to iv were also calculated to the abundance-weighted acoustic and phylogenetic distances, using an adapted mean nearest distances to be weighted by each species relative abundance on signaling assemblages (see Cadotte et al. 2012). As ecological requirements and acoustic attributes are often conserved across the phylogeny, we assumed that competition would be higher (similar acoustic parameters) when more similar species (closest phylogenetic distances) co-occur in assemblages (Toledo et al. 2015b).

Statistical analyses

To explore the influence of phylogenetic similarities and species richness on the acoustic space structure of signaling assemblages, we fitted linear mixed models (Appendix 1) on the standardized effect size of the mean nearest neighbor distance (SES MNTD) for each acoustic parameter considering signaling assemblages, representing both occurrence (presence/absence) and abundances (weighted by the amphibian calling index) using the function *lmer* of the package *lme4* (Bates et al. 2015) in R (R core team 2020). Each model was fitted on the SES MNTD of a single acoustic parameter, including the phylogenetic similarity of each assemblage and species richness as fixed factor, and monitored day nested within site as random factors. We tested model significancy with full-null model comparison using likelihood ratio tests. Heteroscedasticity and normal distribution of model residuals were checked with visual inspection of Q-Q plots and residuals plotted against fitted values. Correlation of the variables was tested with the Variance Inflation Factor and no correlations were found (VIF < 1.1). For significant models ($P < 0.05$) we calculated confidence intervals using 1000 bootstrap iterations with the function *confint.merMod* of the package *lme4* (Bates et al. 2015).

Results

We registered 23 species in the sampled assemblages (Table 1) corresponding to about a third of the CNP species richness (61 species; Zornosa-Torres et al. 2020). Species richness per sampled site varied from 7 to 13 species [10 ± 1.73 (mean ± SD)], and per signaling assemblages varied from 0 to 7 species (3.14 ± 1.54). We were able to obtain directional and manual recordings and measurements of 212 individuals, corresponding to 87 % (n = 20) of such species (Table 2). The most representative species in the sampled communities was *Dendropsophus minutus*, which was present in all assemblages, followed by *Boana faber*, *Scinax alter* and *S. eurydice*, that were present in 7 of the 8 sampled sites (Table 1).

The assemblages general acoustic space showed a tendency toward positive SES MNTD values for dominant frequency (0.70 ± 0.64 kHz), BW 90% (0.91 ± 1.00 kHz), call duration (0.37 ± 0.86 s) and center time (0.51 ± 0.75 s) (Table 3). This was also observed for species occurrence and individual abundance-weighted data (Figure 1; Table 3), indicating a tendency toward dispersion (dispersed acoustic space structure), considering that spectral parameters had higher mean values (≥ 0.69) and temporal parameters had lower mean values (≤ 0.51) (Table 3). Assemblages also had more similar species than expected by chance (-0.53 ± 1.02; Table 3), explained by the negative phylogenetic similarity values (tendency to aggregated phylogenetic structure). Thus, acoustic parameters presented a more disperse distribution (positive SES MNTD) while phylogenetic similarity more aggregated (negative SES MNTD) than expected by chance.

When checked if acoustic space of anuran assemblages was influenced by phylogenetic relatedness and species richness, we observed that the acoustic MNTD were generally positively influenced by phylogenetic distances (with exception of call duration that showed a weak negative correlation; Figure 2), and negatively related with species richness (Figure 2). Nonetheless, when we controlled the bias of MNTD by calculating the SES MNTD (acoustical and phylogenetical similarities), we found that the general results varied between MNTD and SES MNTD (Figure 2). When we assessed the variability of the spectral acoustic space (i.e., distribution of spectral parameters, represented by SES MNTD) considering the ecological properties of assemblages (i.e., phylogenetic structure and species richness) we found that the distribution of spectral parameters in assemblages was not influenced by phylogenetic similarities or species richness (Table 4). Conversely, temporal parameters similarities were influenced by phylogenetic similarities and species richness, for both species occurrence and individuals abundance-weighted data (Figure 2; Table 4), which means that assemblages composed by phylogenetically similar species have more dispersed temporal acoustic space. Because of these differences between MNTD and SES MNTD results, it is important to consider both methods to analyze the data, as they showed contradictory outcomes.

Models were significant only for temporal parameter’s similarities ($P < 0.05$; Table 4), with mean distribution of call duration negatively correlated to phylogenetic similarities, and mean distribution of center time positively correlated to species richness in both occurrence and abundance-weighted data (Figure 2; Table 5). Therefore, assemblages composed by phylogenetically closer species had more different distributions of temporal parameters, while assemblages with higher species richness had less similar distribution if temporal parameters. As observed, occurrence and abundance-weighted outcomes were similar (Table 4; 5), therefore species composition in the signaling assemblages is relevant for acoustic space competition, independently of the abundance of those species.

Discussion

Acoustic partitioning was detected in several sound producing-animals, such as insects, fishes, frogs and birds (Garcia-Rutledge & Narins 2001; Sueur 2002; Henry & Wells 2010; Sinsch et al. 2012; Villanueva-Rivera 2014; Krishnan & Tamma 2016; Bignotte-Giró et al. 2019; Chitnis et al. 2020; Wilson et al. 2020), but little has been explored about the ecological assemblages properties that may regulate such partitioning (Aide et al. 2017; Robert et al. 2018; Duarte et al. 2019; Ulloa et al. 2019; Krishnan 2019; Sugai et al. 2021). Here we assessed the acoustic space structure of anuran assemblages, considering phylogenetic similarities and species richness from each signaling assemblage. We found that the phylogenetic relatedness of the studied assemblages has an aggregate structure, which suggests that closer phylogenetically related species compose anuran assemblages in the CNP and surrounding areas. This result was expected because the sampled points were located in the same region, had similar structure (i.e., lentic environments) and resources (i.e., microhabitats, surrounding vegetation) and because of that comprise species with similar reproductive requirements (Ulloa et al. 2019), thus phylogenetically closer.

On the other hand, acoustic attributes showed a dispersed structure suggesting that advertisement calls of the species composing the assemblages differed from each other, consistent with expectations from the acoustic niche partitioning hypothesis (Hödl 1977; Drewry & Rand 1983; Krause 1983). However, outcomes are contradictory depending on the evaluated dimension of acoustic space, some studies have not detected spectral dispersion in anuran assemblages due to the conservative signal carried by advertisement call frequencies and the tuned frog audition sensible to species-specific dominant frequency (Drewry & Rand

1983; Pombal 2010; Chek et al. 2013; Sugai et al. 2021). However, others, including the present study, have detected spectral partitioning and related this to advantages in individual and species recognition, avoiding signals overlap (Hödl 1977; Garcia-Rutledge & Narins 2001; Sinsch et al. 2012; Villanueva-Rivera 2014; Bignotte-Giró et al. 2019). Furthermore, temporal partitioning, as detected here, has also been observed in several studies, as temporal patterns are important in anurans mate choice and must be communicated clearly (without overlap) to ensure success (Hödl 1977; Drewry & Rand 1983; Pombal 2010; Köhler et al. 2017; Bignotte-Giró et al. 2019; Duarte et al. 2019). Nevertheless, for acoustic masking to occur it is necessary to be both temporal and spectral overlap, causing inefficient information transmission (de Araújo et al. 2020). Temporal partitioning is effective and probably more common in anuran assemblages, as temporal parameters contain individual information related to male's fitness *status* whereas spectral characteristics may be less variable and more conserved in anurans (Garcia-Rutledge & Narins 2001; Köhler et al. 2017).

When assessing the ecological attributes that may be influencing acoustic parameters distribution, we observed that spectral parameters were not influenced by phylogenetic relatedness or species richness, and temporal parameters were influenced by both ecological attributes. Under this competition scenario, ecological properties (i.e., phylogenetic relatedness and species richness) are not leading to a spectral dispersed pattern of the signaling assemblages acoustic space. Conversely, species inhabiting assemblages with high phylogenetic similarities presented advertisement calls with more different distribution of temporal parameters. Commonly, when we think of closely related species, we assume that call attributes may be similar, due to proximities in anatomical characteristics (e.g., body size and anatomy), leading to similar vocal apparatus (Narins & Smith 1986; McClelland et al. 1996; Suthers et al. 2006). This assumption may be valid for aggressive calls (such as territorial calls) but does not always apply for advertisement calls (Freitas & Toledo 2021).

Advertisement calls are responsible for mate attraction and serve as a pre-zygotic barrier as it leads to species recognition (Toledo et al. 2015a; Köhler et al. 2017), because of that acoustic trace from this anuran call is species-specific and have been selected over evolution in a way to optimize signal transmission and avoid mismating (Freitas & Toledo 2021).

We also observed that in assemblages with higher species richness, the temporal distribution of calls was less similar from each other, independently of the abundance. It is expected that in richer and more abundant assemblages there is also a higher diversity of acoustic signals being emitted concomitantly, thus more chance of acoustic overlap (Aide et al. 2017). Hence, the relationship between richness and temporal parameters distribution of our results could be considered a strategy of temporal acoustic partitioning in assemblages with highly competitive potential (with more and phylogenetically closer species). Few studies have assessed species richness as an explanatory variable to acoustic niche partitioning, however the results are consistent even if the evaluated assemblages are from different taxonomical groups (e.g., birds, anurans and insects) (Aide et al. 2017; Robert et al. 2018; Duarte et al. 2019). These studies agreed that species richness has a positive influence on acoustic space occupancy (Aide et al. 2017; Robert et al. 2018; Duarte et al. 2019), as we have also seen in our signaling assemblages for temporal parameters distribution. Therefore, in assemblages with high species richness is important to avoid acoustic overlap, and anuran species of the CNP assemblages may be using temporal parameters partitioning to accomplish that, independently of individuals abundance. Also, other aspects of species ecology as spatial occupancy (e.g., heigh and calling microhabitats) and differentiated hourly activity may be being segregated and are important to be considered in future studies.

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Table 1. Recorded and total species richness in sampled sites on the Caparaó National Park and surrounding areas. Asterisks indicates the sites where 2 autonomous recorders were placed; numbers corresponded to the number of recorded individuals from each species at each site; and an x indicates that the species was registered but not recorded at that site.

Species	**Site 1**	**Site 2***	**Site 3**	**Site 4***	**Site 5**	**Site 6**	**Site 7**	**Site 8**
Aplastodiscus cavicola			3					
Boana albomarginata				11		1	3	
Boana albopunctata	5	2				1		
Boana faber		3	1	1	x	2	1	1
Boana pardalis	x		2					1
Boana polytaenia		12			x	x		
Dendropsophus elegans		4	x	4		13	8	5
Dendropsophus haddadi							1	
Dendropsophus minutus	x	3	1	5	4	x	4	4
Dendropsophus pseudomeridianus				18		2		
Ischnocnema izecksohni			x		x			
Leptodactylus fuscus	1					x		
Leptodactylus latrans	x	x	x	3		1		
Physalaemus araxa					1			
Physalaemus cuvieri	7	3				1		
Physalaemus feioi		3						1
Proceratophrys boiei					x			
Phyllomedusa burmeisteri		1	x				x	8
Pithecopus rohdei				x				
Rhinella crucifer			1	4		x	x	x
Scinax alter	1	2		12	2	5	11	5
Scinax eurydice	x	2	x	4		2	2	x
Scinax fuscovarius				1			x	2
Monitored time (days)	2	4	1	5	3	6	4	5

Recorded richness	4	10	5	10	3	9	7	8
Total richness	8	11	10	11	7	13	10	10

Table 2. Acoustic parameters measured from sampled species. Data is given as: number of males / number of calls; mean ± SD (min – max).

Species	Dominant frequency (kHz)	Bandwidth 90% (kHz)	Call duration (s)	Center time (s)	Frequency 5% (kHz)	Frequency 95% (kHz)	Center frequency (kHz)
Brachycephalidae							
Ischnocnema izecksohni (2 / 9)	2.31 ± 0.08 (2.23 – 2.51)	1.9 ± 0.07 (1.78 – 1.99)	1.05 ± 0.12 (0.83 – 1.19)	97.41 ± 60 (11.48 – 193.21)	1.8 ± 0.03 (1.76 – 1.85)	3.7 ± 0.07 (3.61 – 3.82)	2.31 ± 0.03 (2.27 – 2.37)
Bufonidae							
Rhinella crucifer (5 / 29)	1 ± 0.1 (0.82 – 1.17)	0.35 ± 0.13 (0.19 – 0.66)	2.8 ± 1.14 (0.78 – 6.13)	26.7 ± 17.32 (2.8 – 64.41)	0.81 ± 0.07 (0.73 – 0.96)	1.16 ± 0.12 (1.01 – 1.38)	1 ± 0.08 (0.91 – 1.15)
Hylidae							
Aplastodiscus cavicola (3 / 103)	0.65 ± 0.02 (0.59 – 0.68)	0.14 ± 0.02 (0.07 – 0.19)	0.31 ± 0.11 (0.18 – 0.5)	61.04 ± 39.92 (1.74 – 159.2)	0.57 ± 0.01 (0.56 – 0.63)	0.71 ± 0.02 (0.63 – 0.77)	0.63 ± 0.01 (0.61 – 0.68)
Boana albomarginata (15 / 806)	1.94 ± 0.5 (1.13 – 2.48)	0.95 ± 0.46 (0.14 – 1.43)	0.2 ± 0.2 (0.09 – 1.23)	62.61 ± 32.44 (4.47 – 135.31)	1.21 ± 0.27 (1.03 – 2.23)	2.16 ± 0.45 (1.24 – 2.63)	1.94 ± 0.49 (1.15 – 2.46)
Boana albopunctata (8 / 98)	1.46 ± 0.63 (0.54 – 2.23)	1.09 ± 0.38 (0.15 – 1.83)	143.84 ± 564.96 (0.12 – 2460.94)	130.3 ± 250.29 (1.13 – 1148.44)	0.93 ± 0.34 (0.49 – 2.09)	2.13 ± 0.66 (1.22 – 4.56)	1.49 ± 0.63 (0 – 2.18)

Boana faber (9 / 240)	0.86 ± 0.47 (0.35 – 3.35)	1.02 ± 0.22 (0.63 – 2.81)	0.1 ± 0.03 (0.03 – 0.17)	274.96 ± 851.25 (0.65 – 3119.79)	0.39 ± 0.19 (0.33 – 3.19)	1.41 ± 0.29 (1.08 – 4.1)	0.87 ± 0.4 (0.38 – 3.59)
Boana pardalis (3 / 56)	0.86 ± 0.29 (0.54 – 1.55)	0.84 ± 0.11 (0.75 – 1.15)	0.13 ± 0.02 (0.08 – 0.18)	53.49 ± 30.07 (0.62 – 113.42)	0.56 ± 0.09 (0.49 – 0.82)	1.4 ± 0.2 (1.24 – 1.83)	0.9 ± 0.21 (0.66 – 1.31)
Boana polytaenia (12 / 264)	6.19 ± 0.26 (5.51 – 7.01)	0.93 ± 1.06 (0.21 – 6.14)	0.09 ± 0.05 (0.04 – 0.3)	90.31 ± 57.27 (0.6 – 266.55)	5.85 ± 0.25 (5.23 – 6.35)	6.77 ± 1 (5.91 – 11.58)	6.18 ± 0.25 (5.51 – 6.61)
Dendropsophus haddadi (1 / 8)	5.15 ± 0.05 (5.09 – 5.23)	0.86 ± 0.04 (0.82 – 0.94)	0.07 ± 0.01 (0.06 – 0.09)	13.46 ± 9.9 (3.03 – 25.39)	4.76 ± 0.01 (4.73 – 4.78)	5.61 ± 0.05 (5.55 – 5.7)	5.13 ± 0.03 (5.11 – 5.18)
Dendropsophus elegans (34 / 706)	3.62 ± 0.29 (1.78 – 4.2)	1.08 ± 0.78 (0.01 – 5.39)	63.19 ± 501.54 (0.08 – 4195.31)	211.42 ± 618.41 (1.2 – 3304.69)	3.11 ± 0.23 (2.46 – 3.82)	4.07 ± 0.27 (3.52 – 5.72)	3.59 ± 0.49 (0 – 4.13)
Dendropsophus minutus (21 / 282)	5.66 ± 1.03 (1.99 – 7.13)	3.16 ± 1.3 (0.05 – 6.54)	0.69 ± 3.16 (0.05 – 53.08)	169.3 ± 326.41 (0.26 – 1343.44)	3.39 ± 0.9 (1.95 – 5.98)	6.56 ± 0.82 (1.99 – 9.56)	5.94 ± 0.45 (1.97 – 6.75)
Dendropsophus pseudomeridianus (20 / 654)	4.99 ± 0.25 (4.5 – 6.82)	0.76 ± 0.35 (0.21 – 4.64)	0.03 ± 0.01 (0.02 – 0.07)	27.39 ± 18.1 (1.03 – 92.81)	4.62 ± 0.23 (4.03 – 6.49)	5.38 ± 0.38 (4.78 – 9.19)	5 ± 0.23 (4.52 – 6.84)
Scinax alter (38 / 827)	2.39 ± 1.04 (0.96 – 4.99)	2.61 ± 0.62 (0.77 – 4.92)	0.36 ± 0.42 (0.1 – 11.73)	127.26 ± 354.47 (1.16 – 2835.17)	1.36 ± 0.13 (0.4 – 2.13)	3.88 ± 0.47 (1.17 – 5.06)	2.89 ± 0.59 (0.87 – 4.78)

Scinax eurydice (10 / 387)	1.54 ± 0.77 (0.73 – 2.53)	1.98 ± 0.24 (1.55 – 3.02)	0.14 ± 0.02 (0.08 – 0.2)	30.54 ± 20.24 (0.69 – 90.82)	0.74 ± 0.02 (0.7 – 0.84)	2.72 ± 0.24 (2.27 – 3.77)	1.85 ± 0.46 (0.8 – 2.39)
Scinax fuscovarius (3 / 101)	0.84 ± 0.08 (0.73 – 1.29)	1.16 ± 0.58 (0.21 – 1.9)	0.21 ± 0.02 (0.16 – 0.25)	87.84 ± 45.25 (9.08 – 172.52)	0.76 ± 0.02 (0.68 – 0.8)	1.93 ± 0.59 (0.98 – 2.67)	0.9 ± 0.04 (0.82 – 1.05)
Leptodactylidae							
Leptodactylus fuscus (1 / 31)	2.19 ± 0.06 (2.09 – 2.3)	1.18 ± 0.11 (0.91 – 1.29)	0.35 ± 0.02 (0.32 – 0.38)	26.37 ± 14.77 (1.94 – 50.52)	1.12 ± 0.12 (1.01 – 1.38)	2.3 ± 0.02 (2.23 – 2.32)	2.14 ± 0.03 (2.04 – 2.18)
Leptodactylus latrans (4 / 59)	0.33 ± 0.05 (0.23 – 0.42)	0.14 ± 0.02 (0.12 – 0.19)	0.16 ± 0.02 (0.12 – 0.2)	30.58 ± 15.38 (0.66 – 59.75)	0.23 ± 0.02 (0.19 – 0.28)	0.37 ± 0.04 (0.33 – 0.45)	0.32 ± 0.05 (0.26 – 0.4)
Physalaemus cuvieri (11 / 369)	0.81 ± 0.22 (0.47 – 1.2)	0.71 ± 0.12 (0.49 – 1.03)	0.17 ± 0.03 (0.11 – 0.37)	105.72 ± 271.54 (0.98 – 1241.98)	0.51 ± 0.06 (0.42 – 0.68)	1.22 ± 0.1 (1.03 – 1.52)	0.73 ± 0.13 (0.49 – 1.13)
Physalaemus feioi (4 / 19)	2.75 ± 0.05 (2.65 – 2.84)	0.66 ± 0.15 (0.49 – 0.94)	3.56 ± 1.34 (1.64 – 5.86)	33.07 ± 22.87 (3.76 – 75.97)	2.48 ± 0.15 (2.3 – 2.67)	3.14 ± 0.06 (3.05 – 3.28)	2.75 ± 0.05 (2.65 – 2.84)
Physalaemus araxa (1 / 21)	1.6 ± 0.02 (1.57 – 1.66)	1.34 ± 0.07 (1.17 – 1.45)	0.14 ± 0.01 (0.09 – 0.16)	60.71 ± 6.16 (51.79 – 70.15)	1.09 ± 0.06 (1.01 – 1.27)	2.43 ± 0.02 (2.39 – 2.46)	1.6 ± 0.02 (1.57 – 1.62)

Phyllomedusidae							
Phyllomedusa burmeisteri (9 / 49)	1.06 ± 0.25 (0.56 – 1.52)	0.74 ± 0.19 (0.45 – 1.27)	0.21 ± 0.08 (0.1 – 0.38)	75.3 ± 43.14 (5.61 – 161.99)	0.81 ± 0.12 (0.52 – 1.01)	1.55 ± 0.24 (1.15 – 2.27)	1.05 ± 0.19 (0.61 – 1.41)

Table 3. Acoustic and phylogenetic similarities presented in SES MNTD values.

Parameter	**Occurrence**	**Abundance**	**General**
Dominant Frequency	0.70 ± 0.64 (-1.42 – 2.10)	0.69 ± 0.63 (-1.42 – 2.11)	0.70 ± 0.64 (-1.42 – 2.11)
BW 90%	0.90 ± 1.00 (-1.57 – 2.76)	0.92 ± 1.00 (-1.57 – 2.74)	0.91 ± 1.00 (-1.57 – 2.76)
Call duration	0.35 ± 0.86 (-0.91 – 3.10)	0.39 ± 0.87 (-0.90 – 3.21)	0.37 ± 0.86 (-0.91 – 3.21)
Center time	0.50 ± 0.75 (-1.00 – 2.81)	0.51 ± 0.76 (-0.96 – 2.73)	0.51 ± 0.75 (-1.00 – 2.81)
Phylogenetic distance	-0.51 ± 1.01 (-2.82 – 1.51)	-0.56 ± 1.02 (-2.69 – 1.48)	-0.53 ± 1.02 (-2.82 – 1.51)

Table 4. GLMM fitted models for each acoustic parameter, representing both occurrence (presence/absence) and abundances (weighted by the amphibian calling index). Bold model factors indicate significant results when compared observed and null models.

	Acoustic Parameter	**Models**	**AIC**	**BIC**	**Deviance**	**ChiSq**	**Df**	***P* value**	**R2m**	**R2c**	**VIF**
Occurrence	Dominant Frequency	Observed	551.7	570.1	541.7	6	2	0.05	0.02	0.19	1
		Null	553.7	564.7	547.7						
	BW 90%	Observed	780.1	798.5	770.1	3.9	2	0.1			1
		Null	780.1	791.1	774.1						
	Call duration	**Observed**	**442.9**	**461.1**	**432.9**	**51.4**	**2**	**< 0.001**	**0.12**	**0.61**	**1.1**
		Null	490.3	501.2	484.3						
	Center time	**Observed**	**456**	**473.91**	**445.99**	**11.454**	**2**	**0.003**	**0.05**	**0.3**	**1.1**
		Null	463.45	474.2	457.45						
Abundance-weighted	Dominant Frequency	Observed	546.6	565	536.6	2.5	2	0.292			1
		Null	545.1	556.1	539.1						
	BW 90%	Observed	777.7	796.1	767.7	2.8	2	0.243			1
		Null	776.5	787.6	770.5						
	Call duration	**Observed**	**467**	**485.2**	**457**	**44.4**	**2**	**< 0.001**	**0.11**	**0.59**	**1.1**
		Null	507.4	518.3	501.4						
	Center time	**Observed**	**454.53**	**472.47**	**444.5**	**12.237**	**2**	**0.002**	**0.05**	**0.3**	**1.1**
		Null	462.8	474	456.77						

Table 5. GLMM coefficients and confidence intervals for significant models for each acoustic parameter, representing both species occurrence (presence/absence) and individual abundances (weighted by the amphibian calling index). Bold factors indicate the ones that influenced acoustic parameters

			Std error	**t-value**	**Estimate**	**2.5% CI**	**97.5% CI**
Occurrence	Dominant frequency	Intercept	0.6	0.13	4.75	0.36	0.84
		Mean nearest phylogenetic distance	0.08	0.04	2.2	0.01	0.15
		Species richness	0.04	0.03	1.18	-0.02	0.1
	BW 90%	Intercept	1.1	0.21	5.26		
		Mean nearest phylogenetic distance	0.09	0.06	1.67		
		Species richness	-0.06	0.05	-1.16		
	Call duration	Intercept	0.19	0.13	1.41	-0.09	0.44
		Mean nearest phylogenetic distance	**-0.24**	**0.03**	**-7.53**	**-0.31**	**-0.18**
		Species richness	-0.01	0.03	-0.29	-0.07	0.05
	Center time	Intercept	-0.02	0.13	-0.13	-0.28	0.23
		Mean nearest phylogenetic distance	-0.05	0.04	-1.3	-0.12	0.03
		Species richness	**0.11**	**0.03**	**3.37**	**0.04**	**0.17**
Abundance-weighted	Dominant frequency	Intercept	0.6	0.13	4.75		
		Mean nearest phylogenetic distance	0.08	0.04	2.2		
		Species richness	0.04	0.03	1.18		
	BW 90%	Intercept	1.1	0.21	5.26		
		Mean nearest phylogenetic distance	0.09	0.06	1.67		
		Species richness	-0.06	0.05	-1.16		
	Call duration	Intercept	0.3	0.14	2.24	0.04	0.56
		Mean nearest phylogenetic distance	**-0.24**	**0.03**	**-6.92**	**-0.31**	**-0.17**
		Species richness	-0.03	0.03	-1.03	-0.09	0.02

Center time	Intercept	0	0.13	-0.01	-0.26	0.25
	Mean nearest phylogenetic distance	-0.05	0.04	-1.33	-0.12	0.03
	Species richness	**0.1**	**0.03**	**3.17**	**0.04**	**0.17**

Figure 1. Standardized effect sizes of mean nearest taxon distance (SES MNTD) of each acoustic parameter and phylogenetic relatedness, with and without abundance weighted data.

Figure 2. Relationships between the mean nearest taxon distance (MNTD) and SES MNTD of acoustic parameters (dominant frequency (A), BW 90% (B), call duration (C) and center time (D)) with phylogenetic similarities and species richness as predictors.

Figure 3. Generalized linear mixed models (GLMM) fitted on SES MNTD of acoustic parameters statistically significant related with phylogenetic relatedness and species richness.

Figure 1

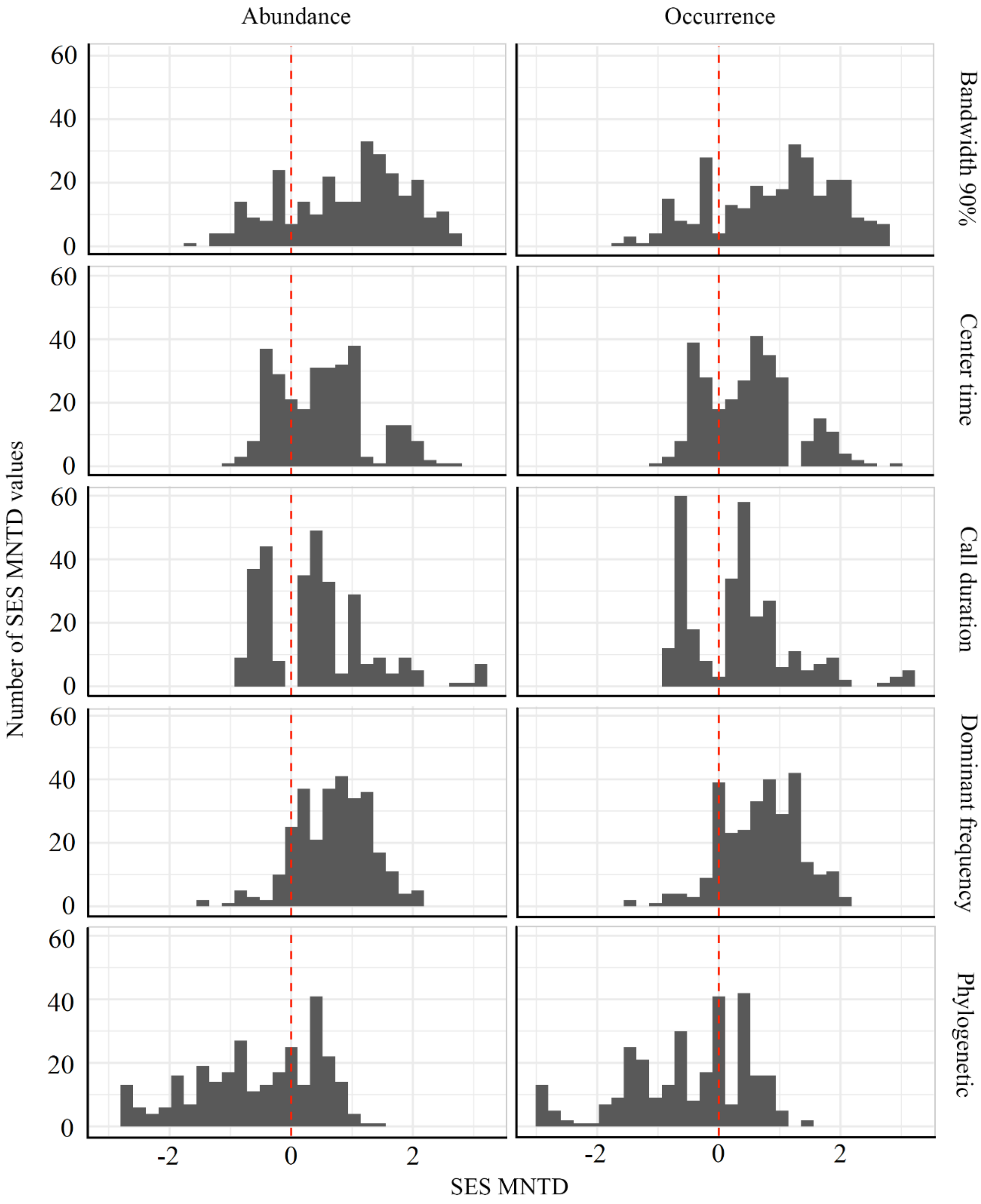

Abundance
Occurrence
Bandwidth 90%
Center time
Call duration
Dominant frequency
Phylogenetic
Number of SES MNTD values
60
40
20
0
-2
0
2
SES MNTD

Figure 2

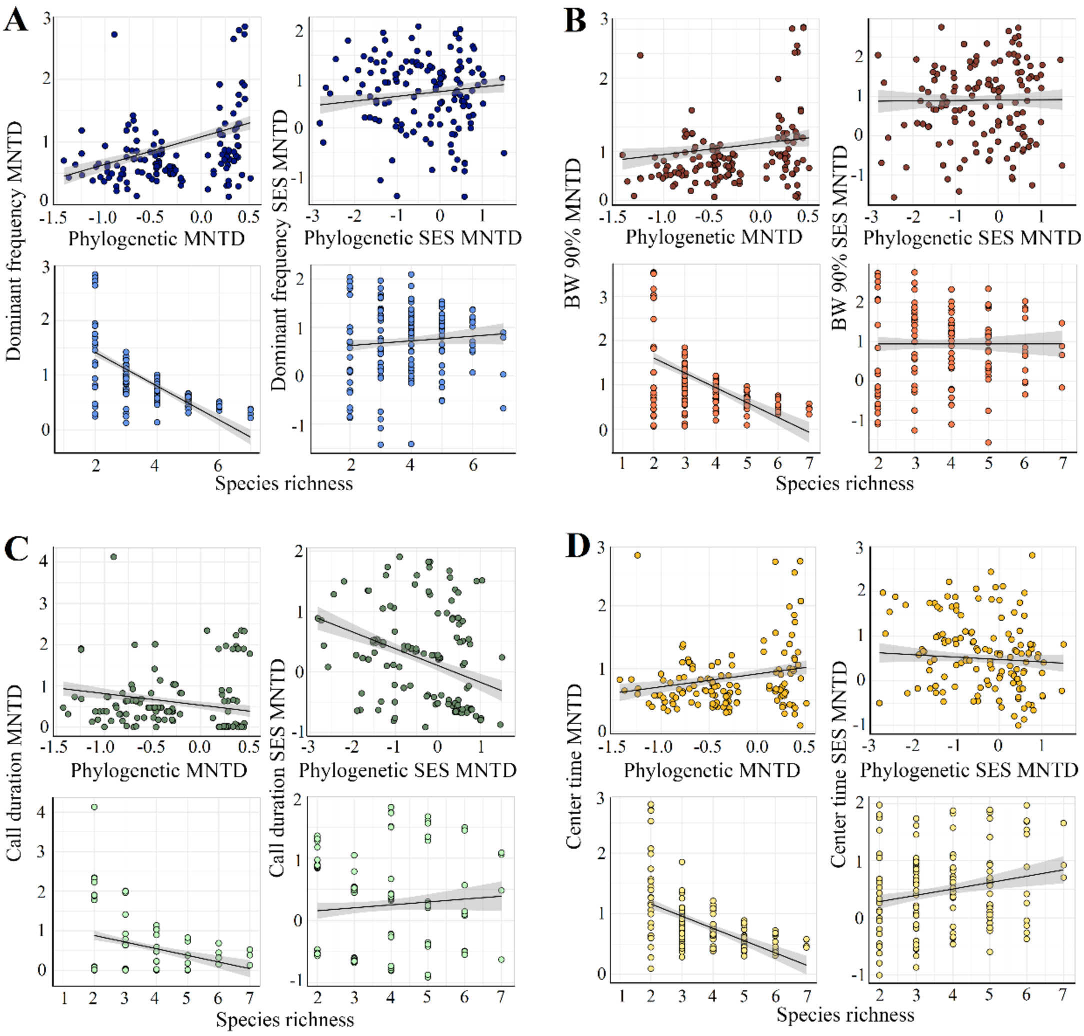
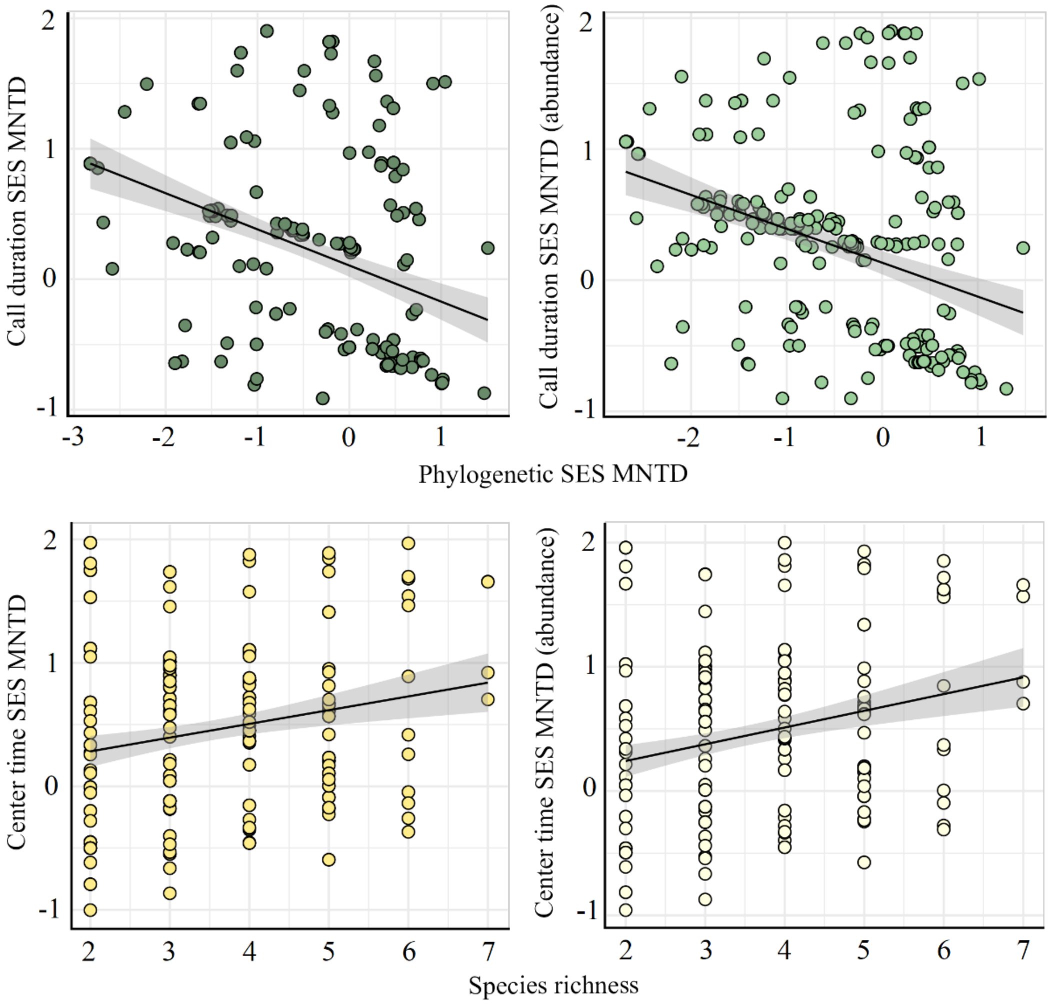

A
Dominant frequency MNTD
Dominant frequency SES MNTD
Phylogenctic MNTD
Phylogenetic SES MNTD
Species richness
B
BW 90% MNTD
BW 90% SES MNTD
Phylogenetic MNTD
Phylogenetic SES MNTD
Species richness
C
Call duration MNTD
Call duration SES MNTD
Phylogenctic MNTD
Phylogenetic SES MNTD
Species richness
D
Center time MNTD
Center time SES MNTD
Phylogenctic MNTD
Phylogenetic SES MNTD
Species richness

Figure 3


Call duration SES MNTD
Call duration SES MNTD (abundance)
2
1
0
-1
-3
-2
-1
0
1
Phylogenetic SES MNTD
Center time SES MNTD
Center time SES MNTD (abundance)
2
3
4
5
6
7
Species richness

Supplementary material

Appendix 1. GLMM Models to determine the influence of phylogenetic relatedness and species richness in the acoustic space of anuran assemblages

Occurrence SES MNDT (acoustic parameter) ~ phylogenetic SES MNDT + species richness + random (monitored day nested within site)

Abundance-weighted SES MNDT (acoustic parameter) ~ phylogenetic SES MNDT + species richness + random (monitored day nested within site)

Table S1. Sampled sites location and habitats characteristics. Brazilian states: MG = Minas Gerais, ES = Espírito Santo.

Sample site	Municipality	State	Elevation (m)	Coordinates	Habitat
1	Alto Caparaó	MG	1005	20°25´38.2” S 41°52´12.2” W	Natural, open area, wetland
2	Divino de São Lourenço	ES	824	20°35'24.2" S 41°46'03.1" W	Natural, open area, swamp
3	Divino de São Lourenço	ES	851	20°35'39.3" S 41°46'05.2" W	Artificial, open area, lagoon
4	Mundo Novo	ES	758	20°37'22.8" S 41°47'51.4" W	Artificial, open area, lagoon
5	Alto Caparaó	MG	1282	20°25´12.6” S 41°50´56.9” W	Natural, forest, lagoon
6	Divino de São Lourenço	ES	922	20°35'13.5" S 41°45'40" W	Artificial, open area, lagoon
7	Dores do Rio Preto	ES	843	20°37´12.7” S 41°47´46” W	Artificial, open area, swamp
8	Divino de São Lourenço	ES	886	20° 34' 53.4" S 41° 45' 36.4" W	Artificial, open area, lagoon

Table S2. Species list showing the sample site and voucher numbers (deposited at FNJV collection) of the analyzed recordings. A dash (-) indicates that no recordings were captured or analyzed for that species in that specific sampling site.

Species	Sample site	Manual recordings	Autonomous recordings
Brachycephalidae			
Ischnocnema izecksohni	5	32236, 32237	-
Bufonidae			
Rhinella crucifer	3	36682	-
	4	40108, 40113, 40162, 40168	-
Hylidae			
Aplastodiscus cavicola	3	36620, 36659, 36671	-
Boana albomarginata	4	40150 - 40155, 40160, 40170, 40172, 40175	-
	6	36629	-
	7	40068, 40073, 49349	-
Boana albopunctata	1	36577, 36580, 36582, 36583	-
	2	40235	48871
	6	40200	-
Boana faber	2	36679	48469, 48627
	3	-	49285
	4	-	46676
	6	36649	47891
	7	40072	-
	8	-	49688
Boana pardalis	3	36642, 36643	-

	8	-	49896
Boana polytaenia	2	40219, 40221-40224, 40227, 40229-40231, 40236	48795, 48846
Dendropsophus elegans	2	40226, 40237	48469, 48529
	4	40163, 40164, 40171	46914
	6	36601, 36602, 36605-36610, 36655, 36656, 36657	47893, 47891
	7	40074, 40076, 40080, 40084, 40085, 40086, 40207, 40215	-
	8	40133, 40137, 40142	49750, 49791
Dendropsophus haddadi	7	40203	-
Dendropsophus minutus	2	40232 - 40234	-
	3	-	49287
	4	40112, 40120, 40121	46675, 46688
	5	-	47795, 47807, 47813, 47881
	7	40083, 40209, 40210	49398
	8	40139, 40144	49761, 49799
Dendropsophus pseudomeridianus	4	40102, 40104, 40106, 40107, 40109, 40111, 40114, 40115, 40117, 40119, 40124-40131	-
	6	36603, 36604	-
Scinax alter	1	-	48178
	2	40220, 40228	-

	4	40101, 40103, 40105, 40110, 40118, 40157, 40159, 40161, 40165-40167	46688
	5	-	47813, 47880
	6	40194, 40195, 40197-40199	-
	7	40067, 40078, 40081, 40082, 40205, 40206, 40208, 40213, 40214, 40216, 40218	-
	8	36595, 36597, 36598, 40141	49795
Scinax eurydice	2	40225	48541
	4	40122, 40123, 40149	46675
	6	40196, 40202	-
	7	40211, 40112	-
Scinax fuscovarius	4	40169	-
	8	40132	49844
Leptodactylidae			
Leptodactylus fuscus	1	36584	-
Leptodactylus latrans	4	-	46701, 46675, 47589
	6	-	47921
Physalaemus cuvieri	1	36575, 36633, 36652, 36654, 36683	48175, 48185
	2	-	48743, 48810, 48871
	6	-	47925
Physalaemus feioi	2	40087	48798, 48871

	8	36596	-
Physalaemus araxa	5	-	47875
Phyllomedusidae			
Phyllomedusa burmeisteri	2	36669	-
	8	40134-40136, 40138, 40140, 40143, 40145	49791

CAPÍTULO 4

COULD THE CHYTRID FUNGUS TURN FROGS INTO SEXY ZOMBIES?

Boana crepitans
Foto: Victor Fávaro

“Falar de igualdade entre mulheres e homes

é falar pela vida daquelas que não puderam ainda se defender da violência”

Marielle Franco

Could the chytrid fungus turn frogs into sexy zombies?

**A ser submetido para Behavioral Ecology

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Abstract

The parasite-mediated sexual selection model, or the Hamilton-Zuk hypothesis (HZH), predicts a negative relationship between parasite burden and extravagance of secondary sex characters, such as brighter colorations or more attractive vocalizations. Therefore, less infested or infected males will be those selected by females improving their fitness. On the other hand, it is also possible that infested/infected individuals would display more attractive signals in an attempt to mate, as that may be their last chance of reproducing. We termed this as the 'sexy zombies hypothesis' (SZH). Therefore, we tested these opposing hypotheses with the chytrid-amphibian (pathogen-host) model. Specifically, we hypothesized that the infection by the chytrid fungus (Bd) leads to anuran advertisement call modifications. If these modified calls are less attractive when males are infected, it would be in agreement with the HZH. Alternatively, if infected males call in a more attractive fashion, it would corroborate the opposing SZH. Based on recordings of seven Neotropical treefrogs, we found that Bd infection influenced in at least one call parameter of all analyzed species, but the responses varied among them. Infected males of *Dendropsophus minutus* and *Scinax alter* had longer and more frequent calls, making them possibly more attractive to females than uninfected males, corroborating SZH. Instead, uninfected males of *Boana albopunctata, B. pardalis* and *Phyllomedusa burmeisteri* emitted longer calls, corroborating the HZH. Thus, we showed that the effect of Bd in secondary sex characters of anurans is species-dependent (as previous literature also suggests), and that such variation may also have consequences to anuran communication, reproductive fitness, and consequent long-term population maintenance in the wild. This may be another indirect effect of Bd infections in frogs, helping to explain slow amphibian populations declines across the globe.

Keywords: Anura, *Batrachochytrium dendrobatidis*, advertisement call, calling behavior, mate attraction, parasite-mediated sexual selection model, Hamilton-Zuk hypothesis, sexy zombies hypothesis, disease sublethal effects.

Introduction

Parasites or pathogens cause multiple damage to their hosts, having both sublethal and lethal consequences. In addition to the host responses aimed to contour the infestation/infection, changes in host reproductive behavior attributed to parasite manipulation have also been documented in a variety of taxa and considered as sublethal effects of the infection/infestation (Poulin 1995; Polak & Starmer 1998; Adamo 1999; Abbot & Dill 2001; Moore 2002; Thomas et al. 2005; Lefèvre et al. 2008; Cooley et al. 2018). These behavioral changes may benefit parasite transmission (parasite-mediated changes) or host reproduction (host-mediated changes) and are adaptive for both parasites and hosts, respectively (Klein 2003).

For example, parasite-mediated behavioral changes were observed in the entomophthorelean fungus *Massospora cicadina*, which altered male cicada's courtship behavior, making males in the first stage of infection (stage I) to mimic receptive female's behaviors known as wing-flicks (Cooley et al. 2018). Uninfected males were as attracted to these novel wing-flicks (made by infected males) as they were to female's wing-flicks, favoring the fungus transmission by copulation between infected and uninfected males (Cooley et al. 2018). In contrast, host-mediated behavioral responses to infestation/infection may reduce parasite/pathogen load by reducing the contact rates between infested/infected individuals, as reported for the western mosquitofish (*Gambusia affinis*), in which males prefer to mate with non-parasitized females (Klein 2003; Deaton 2009).

The variation on reproductive displays caused by parasites infestation was hypothesized by Hamilton and Zuk (1982) for North American passerines and named as the parasite-mediated sexual selection model (Hamilton & Zuk 1982), or later as the Hamilton-Zuk hypothesis (HZH) (Kirkpatrick 1986; Balenge & Zuk 2014). This model assumes co-adaptive cycles between parasites and their hosts, with parasites acting as selective agents for more resistant males and inducing extravagant secondary characters (Hamilton & Zuk 1982; Møller 1990; Hamilton & Poulin 1997; Balenge & Zuk 2014; Macedo & Manica 2019). Individuals with lower parasite infestation load may exhibit more extravagant characters (as brighter plumage or more ornamented phenotypes) and better body condition (Borgia 1986; Zuk et al. 1990; Costa & Macedo 2005; Quiñónez et al. 2011). Consequently, females will prefer and select healthier or better-quality males (Hamilton & Zuk 1982; Macedo & Manica 2019). For example, males of the treefrog *Boana prasina* that presented higher helminth load had lower call rates when compared with healthier males, making them less attractive to females (Madelaire et al. 2013). Blue-black grassquits (*Volatina jacarina*) infected with coccidian oocyst exhibited a negative association of blue-black nuptial plumage cover and frequency of leaps display with oocyst counting (Costa & Macedo 2005). Also, females' mice were able to detect *Trypanosoma cruzi* infestation in male's urine, and visited more frequently urine samples of uninfected males, which predicted females mating preferences (Quiñónez et al. 2011).

Alternatively to the HZH, hosts can respond to parasites infestation by increasing their reproductive performance (e.g., Polak & Starmer 1998; Adamo 1999; Branelly et al. 2021). For example, in Sonoran desert fruit-flies (*Drosophila nigrospiracula*) infested with *Macrocheles subbadius* mites, the infestation load was positively related with males' reproductive effort (Polak & Starmer 1998). Also, females of the house cricket (*Acheta domesticus*) laid more eggs when infected by the bacteria *Serratia marcescens* (Adamo 1999). In some cases, host response to parasite infestation or pathogen infection can also benefit the parasitic organism, as it leads to an increased contact rate between hosts, that would facilitate transmission (Klein 2003; Beltran-Bech & Richard 2014). In agreement, Wistar and Long-Evan female rats spent more time sniffing the odor sample of males infested by *Trypanosoma gondii* (Dass et al. 2011). Such behavior is related to an increase of the attractiveness of infected males, enhancing the chance for parasite to infest new hosts as *T. gondii* is sexually and vertically transmitted in rats (Dass et al. 2011).

The amphibian-chytrid fungus *Batrachochytrium dendrobatidis* (hereafter Bd), has been causing severe impacts on amphibian populations worldwide, and is one of the main causes of well-documented global declines (Hoffman et al. 2010; Scheele et al. 2019). Bd causes physiological dysfunctions by attacking epidermal keratinized cells, such as irregular skin sloughing, hyperplasia and hyperkeratosis, and eventually electrolyte imbalance and asystolic cardiac arrest (Berger et al. 1998; Voyles et al. 2007; Voyles et al. 2009). Additionally, sublethal effects of Bd infection have been described, such as weight loss (Cheatsazan et al. 2013), heart bradycardia, lower ventricular mass, reduced heart contraction force, lower cardiac pumping capacity (Salla et al. 2018), increased skin resistance associated with decrease of evaporative water loss (Bovo et al. 2016), increased melanomacrophage cell area, liver leukocyte infiltration and severe reduction in glycogen stores due to energetic imbalance (Salla et al. 2020). Bd-infected individuals (Bd^+) may also undergo indirect effects, mainly related to their behavior, such as alterations of jumping ability, foraging efficiency and reproductive effort (Chatfield et al. 2013; Hanlon et al. 2015; An & Waldman 2016; Brannelly et al. 2021; Kelleher et al. 2021). Some studies have also suggested that Bd^+ individuals are more likely and easier to be captured when compared with healthy (Bd^-) individuals (Murray et al. 2009; Brannelly et al. 2015; Brannelly et al. 2018).

Bd-induced effects on reproductive behavior are particularly relevant as they might alter individual and species fitness, and in turn impact long-term population survival and community dynamics (Bovo et al. 2016). Infected individuals may allocate energy reserves to fight against infection (immune responses), draining energy from other vital functions such as growth and reproduction. As immune responses, calling behavior is also energetically expensive as well as crucial in several social contexts (Ryan 1988; Pough et al. 1992;

Prestwich 1994; Wells 2001; Toledo et al. 2015). In anurans, the advertisement call is the most common vocalization and plays a key role in sexual selection, acting as a pre-zygotic barrier and conveying information of the emitter's condition and features (Toledo et al. 2015; Köhler et al. 2017). In this sense, changes in acoustic parameters of the advertisement call might hinder mate choice and affect individual fitness. Despite anuran calling activity is a well-studied behavior, little is known about the impact of pathogens and parasites on this key life-history trait.

Several studies have accessed the effects of both abiotic (e.g. temperature, rainfall, relative humidity) and biotic factors (e.g. body size, individual condition) on temporal and spectral parameters of anuran advertisement calls (Lingnau & Bastos 2007; Pombal 2010; Ospina et al. 2013; Villanueva-Rivera 2014; Bignotte-Giró et al. 2019; Pérez-Granados et al. 2019). However, few studies have assessed changes on acoustic parameters caused by parasites infection, and they found mixed evidence (Madelaire et al. 2013; Roznik et al. 2015; An & Waldman 2016; Greenspan et al. 2016; Kelleher et al. 2021). Studies have shown that three anuran species (*Ranoidea rheocola, Dryophytes japonicus* and *Pseudophryne pengillegi*) increased their calling rate, call duration, or nightly probability of calling when infected with Bd (Roznik et al 2015; An & Waldman 2016; Kelleher et al. 2021), suggesting that such behavioral response may increase infected males mate attraction, and consequently the pathogen reproductive success and the maintenance of Bd in those amphibian populations (Kelleher et al. 2021). However, the opposite response was observed in males of the treefrog *Boana prasina*, which had lower call rates than healthier males when presenting higher parasites load (helminths), making them less attractive to females (Madelaire et al. 2013). Moreover, there was not influence of Bd infection on *Ranoidea rheocola* advertisement calls, indicating that such response may be species-dependent (Greenspan et al. 2016).

Here we tested if anuran advertisement calls were influenced by Bd presence and infection load. We sampled seven anuran species of different families in order to assess several calling structures and evaluate spectral and temporal acoustic features of infected and uninfected males. We hypothesized that Bd modifies the acoustic parameters of the anurans advertisement calls, making infected males less (in agreement with the HZH) or more attractive to females than healthier males, depending on the species. If the advertisement calls are more attractive, we suggest an opposing hypothesis to the HZH, hereby termed as ‘sexy zombies hypothesis’ (SZH), as that this attractiveness might be a response to a lethal disease, and could imply in the last chance of reproducing by the infected male. Our results contribute to a better understanding on how Bd infection influence anuran acoustic parameters and as a consequence individuals reproduction and fitness.

Methods

Study site

We searched for anuran males in 16 water bodies at the Caparaó National Park (CNP) and surrounding areas, between December 2016 and December 2018. The CNP is located on the state border between Minas Gerais and Espírito Santo, in southeast Brazil, and belongs to the massif of Caparaó (northern mountain region of the Serra da Mantiqueira) in the Brazilian Atlantic Forest. The study sites correspond to temporal wetlands, permanent or temporal swamps, and artificial or natural lagoons. Together with other characteristic phytophysiognomies of this biome, the area presents a predominance of ombrophilous rainforests and high altitude grasslands (above 1,900 m a.s.l.) (Machado et al. 2016). Fieldwork was mainly conducted during the rainy season, comprising the entire breeding season of anuran communities at the study area, composed by at least 61 species (Zornosa-

Torres et al. 2020). Bd has already been detected in anurans sampled in all elevations across the CNP (Zornosa-Torres et al. 2021).

Audio recordings

We recorded the calling activity of focal males from ca. 70 cm using digital recorders (TASCAM DR-40, Marantz PMD 661, Sony PCM-M10, Zoom H4n, and Zoom H4n Pro) and their built-in microphones during a mean period of 79 s (8 – 268 s). After each recording (WAV format, sample rate of 48 kHz and 24 bit depth), we measured air temperature and relative humidity at the calling point of each male using a thermohydrometer and a HOBO-MX2301 datalogger (Onset Inc., Bourne, MA, USA). Anuran calls were deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV), Museu de Diversidade Biológica (MDBio), Universidade Estadual de Campinas (Unicamp) (Table S1).

Batrachochytrium dendrobatidis sample and detection

Focal individuals were captured and stored in separate plastic bags to avoid pathogen cross contamination. We then swabbed hosts' skin for sampling mycotic material, following established protocols (Hyatt et al. 2007, Lambertini et al. 2013). For detection and quantification of Bd, we extracted DNA from individual swabs using PrepMan ULTRA® (Thermo Scientific), and quantified infection intensities for each individual using a TaqMan® qPCR Assay (Thermo Scientific) (Boyle et al. 2004, Kriger et al. 2006, Lambertini et al. 2013). We used the strain CLFT 159 (Global Pandemic Lineage / GPL; Jenkinson et al. 2016) as a quantitative standard, serially diluted from 10^3 to 10^{-1} zoospore genomic equivalent (g.e.) for the qPCR reactions, and considered positive samples (Bd^+) with at least one Bd g.e. (Kriger et al. 2007). Voucher specimens were deposited at the MDBio, Unicamp, according to the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio #58836-1) permit, Sistema Nacional de Gestão do Patrimônio Genético (SISGen #A45E77D) protocol, and ethics committee (CEUA #4827-1/2018) approval. Males that were not collected, were released in their calling location after the swab procedure.

Acoustic parameters and data analyses

Prior to the acoustic analyses, we standardized all recordings to mono and equal sample rate (48 kHz) and audio depth (24 bit). Recorded calls were individually peak normalized to an amplitude of -1.0 dB with Adobe Audition 2020. We then measured three acoustic parameters of each call on Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019): call duration (ms), call rate (number of calls per minute) and dominant frequency (kHz). Temporal parameters were measured from oscillograms, whereas dominant frequency was measured from spectrograms (Peak Frequency function) generated using Hann window type, 50 % brightness, 76 % contrast, FFT (Fast Fourier Transformation) of 1024 points, 70 % time grid overlap and a 3dB filter bandwidth of 67.4 Hz. Call rate was calculated as the number of calls emitted by each calling male divided by the total recorded time analyzed.

To test for the influence of Bd presence on acoustic parameters of anuran advertisement calls, we fitted general linear mixed-effects models (GLMM) using the *lmer* function from the ‘lme4’ package (Bates et al. 2015). For each acoustic parameter, we calculated a single GLMM, using mean values per individual as the response variable, species and Bd presence (0 = uninfected, Bd^-; 1 = infected, Bd^+) as fixed factors, and population from which the individuals were recorded as random factor. The interaction term between Bd presence and species was added to test for differences in the effect of Bd among species. Additionally, we included air temperature and relative humidity as covariates to account for the effect of weather conditions on anuran calls. These last variables were previously z-transformed to improve model convergence and interpretation of coefficients.

We also tested collinearity between variables (temperature, relative humidity, Bd presence) using the VIF parameter and no collinearity issues were found (VIF < 1.25). The normal distribution of the model residuals was tested using QQ-plot and other visual parameters. We included the random slope as long as it did not compromise the convergence of the model. The interaction between Bd presence and species was tested by comparing models with and without the interaction term. When no interaction was observed, the inference of the models was established by full-null model comparisons (i.e. with and without fixed factors). All these comparisons were performed using the function *anova* of the 'stats' package (R Core Team 2018).

To test if Bd infection load influenced acoustic parameters, we fitted a linear regression for the most abundant species (more than 11 individuals with at least 35% of Bd^+ males) (*Dendropsophus minutus*, *Scinax alter* and *Phyllomedusa burmeisteri*), using the *lm* function from the 'stats' package (R Core Team 2018). We used the mean calculated values for the acoustical parameters as response variables and the Bd infection load (Bd^+ log-transformed) as the predicted variable. All statistical analyses were performed using the software R v. 3.6.1 (R Core Team 2018).

Results

We analyzed a total of 1436 calls from 82 males of seven anuran species in two families (Table 1). Bd prevalence varied from 23 – 64 % between species, and 41 % of the recorded males were Bd^+ (Table 1; Table S1). Infected males of all studied species showed differences in at least one acoustic parameter of their advertisement calls comparing to uninfected males. Such differences were species-specific and varied on acoustic parameter, direction, and effect size.

As shown by the full-null comparison, model predictors influenced dominant frequency of the advertisement calls of the focal males ($X^2 = 91.81$; df = 7; $P < 0.0001$). Specifically, this acoustic trait was affected by species ($P < 0.0001$; df = 6) and Bd presence ($P < 0.005$; df = 1), but not by temperature or relative humidity ($P > 0.1$; df = 1; Table 2). The advertisement calls of infected males of *Boana albopunctata, Dendropsophus minutus, Scinax alter* and *Phyllomedusa burmeisteri* had higher dominant frequency than the advertisement calls of uninfected males, while no differences were observed in other species (Figure 1A).

Call duration was also influenced by model predictors, as observed by the full-null comparison ($X^2 = 14.01$; df = 6; $P < 0.005$). Differences in call duration among individuals was determined by the interaction between species and Bd presence ($P < 0.005$; df = 6; Table 2), indicating that Bd affected call duration differently across species. *Dendropsophus minutus* and *S. alter* had longer advertisement calls when infected, whereas *B. albopunctata, B. pardalis* and *P. burmeisteri* exhibited shorter advertisement calls in response to Bd infection (Figure 1B) and other two species showed no variation in call duration (Figure 1B). Temperature and relative humidity did not influence this acoustic parameter ($P > 0.1$; df = 1; Table 2).

The interaction between species and Bd presence also determined the call rate of focal individuals ($X^2 = 18.66$; df = 6; $P < 0.005$). Infected males from six out of seven species showed differences in their call rate, when comparing to uninfected males. Most species exhibited higher call rates in response to Bd infection, while only *B. pardalis* had lower call rates when infected (Figure 1C). Relative humidity ($P < 0.05$; df = 1), but not temperature ($P > 0.1$; df = 1; Table 2), also affected this temporal acoustic trait.

Bd infection load positively influenced call duration in *D. minutus* ($P < 0.05$; $r^2 = 0.73$; Figure 1D), but not the other abundant species ($P > 0.1$). Furthermore, dominant frequency and call rate were not affected by Bd infection load ($P > 0.1$).

Discussion

Although abiotic factors have been shown to influence anurans call traits (Lingnau & Bastos 2007; Ziegler et al. 2011; Llusia et al. 2013; Ospina et al. 2013; Pérez-Granados et al. 2019), biotic factors as pathogens or parasites have not attracted much attention and previous studies reported contradictory results (Madelaire et al. 2013; Rosnik et al. 2015; An & Waldman 2016; Greenspan et al. 2016; Kellerher et al. 2021). Here, we showed that temporal and spectral parameters of the advertisement calls of seven neotropical treefrog species were influenced by Bd presence. Our findings of a pathogen influencing behavioral displays related to reproduction are in agreement with both the Hamilton-Zuk hypothesis (HZH: Hamilton & Zuk 1982; Møller 1990; Hamilton & Poulin 1997; Balenge & Zuk 2014; Macedo & Manica 2019) and the hereby proposed as sexy zombies hypothesis (SZH), where infected individuals are more attractive to uninfected or infected mates.

The HZH foresees uninfected males exhibiting more extravagant displays or secondary sexual characteristics than infected males, and therefore females would prefer uninfected males (Hamilton & Zuk 1982; Møller 1990; Hamilton & Poulin 1997; Balenge & Zuk 2014; Macedo & Manica 2019). As observed in infected Blue-black grassquits (*Voltina jacarina*) exhibiting higher percentage of nuptial plumage (Costa & Macedo 2005) and females' mice preferences on uninfected males (Quiñónez et al. 2011). In anurans, females generally prefer males with lower frequency, longer, more frequent, and rapidly repeated calls (Gerhardt & Huber 2002). By the assumptions made by the HZH, males that display these traits should be healthier (Hamilton & Zuk 1982; Balenge & Zuk 2014). As observed in the present study only for call duration of *Boana albopunctata, B. pardalis* and *P. burmeisteri,* and for call rate of *B. pardalis*, uninfected males of these species presented longer and more frequent calls, respectively. This pattern was also observed in males of *B. prasina* with lower helminths load presenting higher call rates (Madelaire et al. 2013). Additionally, uninfected and infected males of *B. polytaenia* and *D. elegans* had similar dominant frequency and call duration, and males of *P. burmeisteri* had similar call rates. These findings were also reported for males of *Ranoidea rheocola* and *Dryophytes japonicus*, in which either all acoustic parameters or the dominant frequency were not influenced by Bd infections, respectively (An & Waldman 2016; Greenspan et al. 2016).

In contrast to the HZH, we found that infected males of *Dendropsophus minutus* and *S. alter* had longer and more frequent calls, making them possibly more attractive to females as suggested by Gerhardt & Huber (2002). These patterns are similar to that reported for *D. japonicus* (another Hylidae species), where Bd^+ males presented longer calls and higher pulse rates (An & Waldman 2016) and for *Pseudophryne pengillegi* infected males, that emitted advertisement calls with higher call rates in comparison to healthy males (Kelleher et al. 2021). In these cases, we can state the sexy zombie's hypothesis (SZH), in which both the infected male and the pathogen may immediately benefit from the extravagant calling display, as these males would attract more mates for reproduction and Bd may be directly spreading by the contact of the pair during amplexus (Morell 2016). However, the offspring of the infected males may inherit the susceptibility trait from their parents leading to a decrease in the future population fitness and success caused by Bd infection (Hill 1999; Schurr 2007; Kelleher et al. 2021).

A relevant finding is that the influence of Bd presence on acoustic parameters vary within different species. Depending on the importance of each parameter for mate choice, females may select infected or uninfected males. For example, infected males of *D. minutus* presented higher pitched, longer, and more frequent calls when compared to uninfected males. If dominant frequency is the main trait related to individual discrimination (Morais et al. 2012), and this assumption is also valid for mate choice, females would prefer uninfected males, which displayed lower dominant frequencies. However, if temporal parameters are those preferably selected by females (Haddad & Cardoso 1992), they would pair with infected males, and in the case of *D. minutus* males with higher infection loads, similar to what was reported for *D. japonicus* and *P. pengillegi* (An & Waldman 2016; Kelleher et al. 2021). These results reinforce the importance of evaluating different species, and also testing females' acoustic preferences by playback experiments for a better understanding on how Bd or other parasitic infections can affect anuran communication and reproductive fitness.

The different responses of species acoustic behavior to Bd presence may also be explained by the differences on host's response to the infection, as Bd development on infected hosts depends on several factors, such as timing of host exposure to the pathogen, different host species, pathogen virulence, environmental variation, and host body condition (Rollins-Smith & Woodhams 2012; Greenberg et al. 2017). It is known that Bd infection varies spatially in the Caparaó National Park (Zornosa-Torres et al. 2021) and it can also be affected by species behavioral ecology, individual life history stage, temperature, community richness, phylogeny and individual immunity system (Bancroft et al. 2011; Rollins-Smith & Woodhams 2012; Greenberg et al. 2017; Mesquita et al. 2017; Neely et al. 2020).

The analyzed species here belongs to the Hylidae and Phyllomedysidae families, these families share several natural history and biological traits, such as climbing ability (from shrubs to trees canopy; Wells 2007) and reproductive modes (exotrophic larvae developed in lentic water bodies; Duellman et al. 2016; Nunes-de-Almeida et al. 2021). Species from the Hylidae family generally present higher Bd infection rates comparable with other anuran families due to their association with water (Valencia-Aguilar et al. 2015, Lambertini et al. 2021), but individual species vary in response to Bd infections (Becker et al. 2014). For example, individuals of *D. minutus* had high survival rates when infected by Bd (Mesquita et al. 2017) and presented lower infection rates comparing to individuals of *S. hayii* and *B.*

bandeirantes (Becker et al. 2014), even belonging to the same family and sharing natural history characteristics.

Fighting against Bd infection is an energetically expensive process (van Rooij et al. 2015; Rollins-Smith & Woodhams 2012), as well as calling behavior (Wells 2001; Prestwich 1994; Pough et al. 1992; Ryan 1988). Therefore, it is possible that infected males of different species may vary on energy investment depending on their response to infection, allocating it to increase their immune response against Bd (in agreement with HZH) or to increase acoustic parameters (in agreement with SZH). Based on that, we can suggest that infected males that increase their calling attractiveness may have higher survival rates and may invest more energy on calling behavior than on immune response against Bd infection. As observed here for call rate and duration of *D. minutus*, which as stated before it is known to have high survival rate when infected by Bd (Mesquita et al. 2017).

As we showed here, species presented different responses to Bd infection, therefore variation on their acoustic parameters due to infection influences. Few studies have focus on explore this influence (Rosnik et al. 2015; An & Waldman 2016; Greenspan et al. 2016; Kellerher et al. 2021) and it is important as it may be the beginning of investigating the indirect impacts that Bd may have on amphibians reproductive fitness. Further studies focusing on better understanding the mechanisms of Bd infections influence in different host species are essential. Therefore, we suggest future infection experiments using different species to test the "Sexy Zombies Hypothesis" in order to explore possible consequences of Bd infection on anurans reproduction and host fitness.

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Table 1. Number of focal males, advertisement calls recorded and *Batrachochytrium dendrobatidis* (Bd) presence for each anuran species sampled at the Caparaó National Park and surroundings. Bd presence corresponds to the number of infected individuals / total sampled individuals.

Species	**N. of males**	**N. of calls**	**Bd presence**
Hylidae			
Boana albopunctata	6	70	3/6 (50.0%)
Boana pardalis	9	199	3/9 (33.3%)
Boana polytaenia	9	196	4/9 (44.4%)
Dendropsophus elegans	13	262	3/13 (23.1%)
Dendropsophus minutus	11	139	6/11 (54.5%)
Scinax alter	23	470	8/23 (34.8%)
Phyllomedusidae			
Phyllomedusa burmeisteri	11	44	7/11 (63.6%)

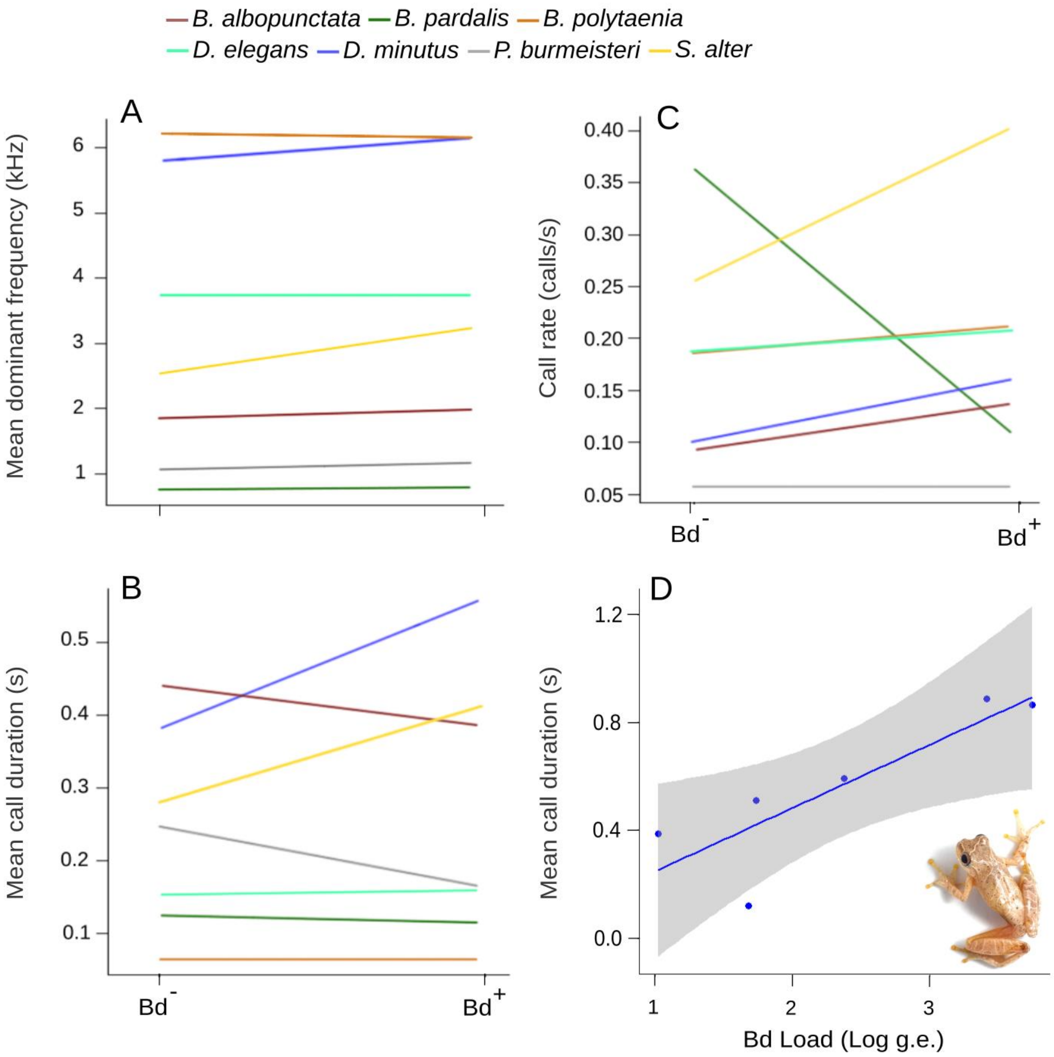
Table 2. GLMM on the effect of *Batrachochytrium dendrobatidis* infection on acoustic parameters of anuran advertisement calls. Predictors having significant influences on acoustic parameters are in bold.

Response	Fixed factors	Df	AIC	LRT	Pr (Chi)
Mean dominant frequency	Temperature	1	1248.0	1.392	0.238
	Relative Humidity	1	1246.7	0.084	0.772
	Bd presence	**1**	**1254.6**	**7.996**	**0.005**
	Species	**6**	**1323.6**	**87.034**	**< 0.001**
Mean call duration	Temperature	1	314.23	0.0677	0.795
	Relative Humidity	1	314.69	0.5311	0.466
	Bd presence * Species	**6**	**323.17**	**19.014**	**0.004**
Call rate	Temperature	1	-89.792	0.8327	0.362
	Relative Humidity	**1**	**-85.677**	**4.9467**	**0.026**
	Bd presence * Species	**6**	**-81.958**	**18.6659**	**0.005**

Figures

Figure 1. Interaction plot showing each advertisement call parameter [dominant frequency (A), call duration (B), and call rate (C)] and the results for uninfected (Bd^-) and infected (Bd^+) males. Linear regression for Bd infection load and mean call duration of males of *Dendropsophus minutus* (D).

Figure 1


B. albopunctata
B. pardalis
B. polytaenia
D. elegans
D. minutus
P. burmeisteri
S. alter
A
Mean dominant frequency (kHz)
1
2
3
4
5
6
B
Mean call duration (s)
0.1
0.2
0.3
0.4
0.5
Bd-
Bd+
C
Call rate (calls/s)
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Bd-
Bd+
D
Mean call duration (s)
0.0
0.4
0.8
1.2
1
2
3
Bd Load (Log g.e.)

Supplementary material

Table S1. Anuran calling males sampled in the Caparaó National Park and surrounding areas, with *Batrachochytrium dendrobatidis* presence (0 = uninfected, 1 = infected), load and the measured acoustic parameters (mean of dominant frequency, mean of call duration, and call rate).

FNJV Vaucher	Species	Bd presence	Bd load (g.e.)	Mean dominant frequency (kHz)	Mean call duration (ms)	Call rate (calls/ms)
36580	*Boana albopunctata*	0	0	1.572	504.8	62.5
36582	*Boana albopunctata*	0	0	2.109	388.2	61.07
40200	*Boana albopunctata*	0	0	1.865	429.4	154.55
36577	*Boana albopunctata*	1	7.7	2.1	394.3	227.41
36583	*Boana albopunctata*	1	6.2	1.994	397.3	99.16
40090	*Boana albopunctata*	1	137.5	1.861	366.2	86.19
36611	*Boana pardalis*	0	0	0.924	103.2	70.68
36634	*Boana pardalis*	0	0	0.672	92.6	183.87
36635	*Boana pardalis*	0	0	0.918	138.5	470.34
36643	*Boana pardalis*	0	0	0.697	127.9	350.74
40088	*Boana pardalis*	0	0	0.603	170.9	542.5
40091	*Boana pardalis*	0	0	0.653	117	565.32
36642	*Boana pardalis*	1	1.1	1.259	94.5	187.5
40096	*Boana pardalis*	1	20.3	0.6	108.9	55.67
40098	*Boana pardalis*	1	33	0.598	136.9	87.63
40219	*Boana polytaenia*	0	0	6.144	58.8	48.56
40221	*Boana polytaenia*	0	0	5.953	68.4	293.58
40222	*Boana polytaenia*	0	0	6.416	60.9	366.08
40229	*Boana polytaenia*	0	0	6.344	63.4	73.62
40230	*Boana polytaenia*	0	0	6.383	66.1	145.73
40223	*Boana polytaenia*	1	3.4	6.041	68.9	129.94

40224	*Boana polytaenia*	1	9	6.162	67	308.17
40227	*Boana polytaenia*	1	138.4	6.7	64.8	199.82
40231	*Boana polytaenia*	1	1265	6.384	56.1	209.25
40076	*Dendropsophus elegans*	0	0	3.659	164.9	128.67
40084	*Dendropsophus elegans*	0	0	3.516	157	377.64
40133	*Dendropsophus elegans*	0	0	3.469	143.9	140.93
40137	*Dendropsophus elegans*	0	0	3.703	128.8	100.25
40142	*Dendropsophus elegans*	0	0	3.778	172.4	337.02
40163	*Dendropsophus elegans*	0	0	3.912	141.4	89.69
40164	*Dendropsophus elegans*	0	0	3.867	162.9	82.19
40171	*Dendropsophus elegans*	0	0	3.996	169.2	225.2
40185	*Dendropsophus elegans*	0	0	3.823	113.8	106.14
40226	*Dendropsophus elegans*	0	0	3.537	164.8	290.91
40074	*Dendropsophus elegans*	1	132.1	3.999	152.7	184.11
40080	*Dendropsophus elegans*	1	4379.2	3.681	157.2	238.46
40207	*Dendropsophus elegans*	1	24.8	3.553	169.8	205.74
40083	*Dendropsophus minutus*	0	0	6.063	1302	434
40144	*Dendropsophus minutus*	0	0	4.889	119.9	138.52
40232	*Dendropsophus minutus*	0	0	6.141	187.1	121.58
40233	*Dendropsophus minutus*	0	0	6.061	216.1	106.02
40234	*Dendropsophus minutus*	0	0	5.813	120.7	53.26
40112	*Dendropsophus minutus*	1	46.5	6.445	120.6	88.42
40120	*Dendropsophus minutus*	1	235.7	6.319	591.2	71.52
40121	*Dendropsophus minutus*	1	9.5	5.898	386.4	177.78
40139	*Dendropsophus minutus*	1	53.1	5.862	509.6	234.2
40209	*Dendropsophus minutus*	1	5488.9	6.42	864.9	239.22
40210	*Dendropsophus minutus*	1	2572.1	6.026	887.9	151.5
40067	*Scinax alter*	0	0	2.766	275.4	141.09

40081	*Scinax alter*	0	0	2.399	256	95.93
40082	*Scinax alter*	0	0	2.441	238.1	45.59
40105	*Scinax alter*	0	0	2.338	385.3	435.71
40141	*Scinax alter*	0	0	1.547	235.9	130.28
40157	*Scinax alter*	0	0	3.272	309.4	260.02
40165	*Scinax alter*	0	0.5	3.279	267.4	254.33
40166	*Scinax alter*	0	0	3.312	248.4	276.79
40167	*Scinax alter*	0	0.8	2.232	248.4	218.31
40197	*Scinax alter*	0	0	3.381	388.8	606.59
40205	*Scinax alter*	0	0	2.531	227.5	200.58
40206	*Scinax alter*	0	0	1.869	263.1	232.65
40208	*Scinax alter*	0	29.9	2.833	244.1	166.86
40216	*Scinax alter*	0	0	2.567	317	270.51
40218	*Scinax alter*	0	0	1.441	266.5	473.74
40078	*Scinax alter*	1	56.6	3.489	255.3	190.36
40101	*Scinax alter*	1	158.5	2.26	523.8	433.04
40103	*Scinax alter*	1	380.8	3.6	421.5	331.1
40118	*Scinax alter*	1	1214.2	4.01	346.8	111.16
40159	*Scinax alter*	1	296.1	1.5	309.3	842.18
40161	*Scinax alter*	1	3.5	3.805	470.7	391.73
40194	*Scinax alter*	1	17.1	3.708	472.2	468.42
40195	*Scinax alter*	1	1.2	3.609	456.7	441.7
36669	*Phyllomedusa burmeisteri*	0	0	1.429	273.8	46.17
40134	*Phyllomedusa burmeisteri*	0	0	0.875	212.2	35.55
40135	*Phyllomedusa burmeisteri*	0	0	1.036	299.1	94.88
40145	*Phyllomedusa burmeisteri*	0	0	0.975	203.2	57.83
40136	*Phyllomedusa burmeisteri*	1	11.6	1.055	119.4	62.69
40138	*Phyllomedusa burmeisteri*	1	346	1.031	235.4	34.93

40140	*Phyllomedusa burmeisteri*	1	29.9	1.137	137.5	34.32
40143	*Phyllomedusa burmeisteri*	1	621.9	1.299	204.9	49.95
40183	*Phyllomedusa burmeisteri*	1	21.5	1.228	133.7	71.65
40186	*Phyllomedusa burmeisteri*	1	1643.9	1.163	208.3	68.88
40187	*Phyllomedusa burmeisteri*	1	2943	1.219	115.9	70.97

Considerações finais

A Mata Atlântica é considerada um *hotspot* de biodiversidade, já que apresenta uma alta diversidade de organismos e alto grau de endemismo. Neste bioma ainda existem locais pouco conhecidos, que constituem uma fonte ótima para novas descobertas. O Parque Nacional do Caparaó (PNC) é um destes locais, que devido a sua localização e grande variação de elevação, apresenta diferentes fitofisionomias que abrigam uma enorme diversidade de plantas e animais.

No presente estudo analisamos taxocenoses de anuros ao longo do maior gradiente de elevação da Mata Atlântica. Como resultado de quase quatro anos de amostragem registramos mais de 60 espécies no PNC e arredores, adicionando 40 espécies à lista previamente disponível no plano de manejo e colocando-o entre os 10 locais com maior riqueza de anuros da Mata Atlântica. Durante este período de coleta foi possível amostrar 93% das espécies do local, entre estas espécies endêmicas, como *Physalaemus araxa* e *Cycloramphus bandeirensis*, e algumas que ainda necessitam descrição (como duas espécies de *Ischnocnema* coletadas).

Além disso registramos o patógeno mais letal dos anfíbios (*Batrachochytrium dendrobatidis*; Bd) ao longo dos cerca de 2000 m de elevação amostrados. Identificamos que ao longo deste gradiente existe uma relação negativa entre a elevação e a carga de infecção, mas não encontramos relação com a prevalência. Nossos resultados são similares a outros estudos realizados em gradientes de elevação amplos e estreitos, sugerindo que não há um mínimo de elevação que deve ser amostrado para encontrar relações entre taxas de infecção do Bd e elevação.

Também determinamos a estrutura acústica de 10 taxocenoses de anuros e descobrimos que estas são compostas por espécies filogeneticamente próximas e que apresentam uma estrutura do espaço acústico dispersa, sugerindo uma possível partilha do espaço acústico. Avaliamos se a estrutura das características espectrais e temporais do canto estava influenciada pelas relações filogenéticas, a riqueza da comunidade ou a abundância das espécies registradas. Unicamente encontramos influência da riqueza e das relações filogenéticas nos parâmetros temporais do canto, o que indica que em taxocenoses com alto potencial competitivo (mais ricas e próximas filogeneticamente) pode estar ocorrendo uma partilha temporal do espaço acústico, mas não uma partilha espectral.

Ademais avaliamos o efeito do Bd nas características espectrais e temporais do canto de sete espécies de anuros. As sete espécies respondem de maneiras diferentes à infecção pelo Bd, mas todas tiveram pelo menos um parâmetro do canto modificado. Com isso observamos que machos saudáveis de algumas espécies seriam mais atrativos às fêmeas, corroborando a hipótese de Hamilton e Zuk (HHZ). No entanto, outras espécies apresentaram resultados contrários, nas quais machos infectados pelo Bd seriam mais atrativos às fêmeas. Neste caso sugerimos a hipótese que chamamos hipótese dos zumbis sensuais (HZS), já que provavelmente o patógeno estaria influenciando os cantos atrativos dos machos para assim conseguir se dispersar mais por meio do contato dos indivíduos no amplexo.

Ao longo deste doutorado realizamos contribuições à ciência que ajudaram a compreender melhor os padrões de distribuição do Bd ao longo de gradientes de elevação amplos, a estrutura do espaço acústicos das taxocenoses de anuros e os efeitos da infecção pelo Bd nos cantos das espécies. Futuros estudos ainda são necessários, principalmente para a melhor compreensão de como as mudanças nos cantos dos machos infectados podem interferir na seleção sexual, testando experimentalmente as hipóteses HHZ e HZS. De igual maneira seria muito interessante avaliar a eficiência da comunicação acústica em taxocenoses que usam a partilha de uma única dimensão do canto (temporal ou espectral) como estratégia para evitar o mascaramento dos sinais acústicos.

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ANEXOS

Anexo 1. Certificado Comitê de ética para amostragem e manipulação de animais

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Variação dos parâmetros acústicos e de infecção pelo fungo quitrídio em anuros num transecto altitudinal no Parque Nacional do Caparaó**, registrada com o nº **4827-1/2018**, sob a responsabilidade de **Prof. Dr. Luís Felipe de Toledo Ramos Pereira e Camila Inés Zornosa Torres**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal** (**CONCEA**), **tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP**, em **25 de abril de 2018**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	**01/05/2018-31/12/2020**
Vigência da autorização para manipulação animal:	**01/05/2018-31/12/2020**
Espécie / linhagem/ raça:	**Anfíbio / Espécies nativas de anuros**
No. de animais:	**500**
Idade/Peso:	**01 ano / 200g**
Sexo:	**400 machos / 100 fêmeas**
Origem:	**Parque Nacional do Caparaó**
Biotério onde serão mantidos os animais:	**LaDiVert, DBA/IB/UNICAMP**

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao **IBAMA**, **SISBIO** ou **CIBio** e é **restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, 25 de abril de 2018.

Prof. Dr. Wagner José Fávaro
Coordenador

Fátima Alonso
Secretária Executiva

IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

Anexo 2. Declaração de Direitos Autorais

DECLARAÇÃO

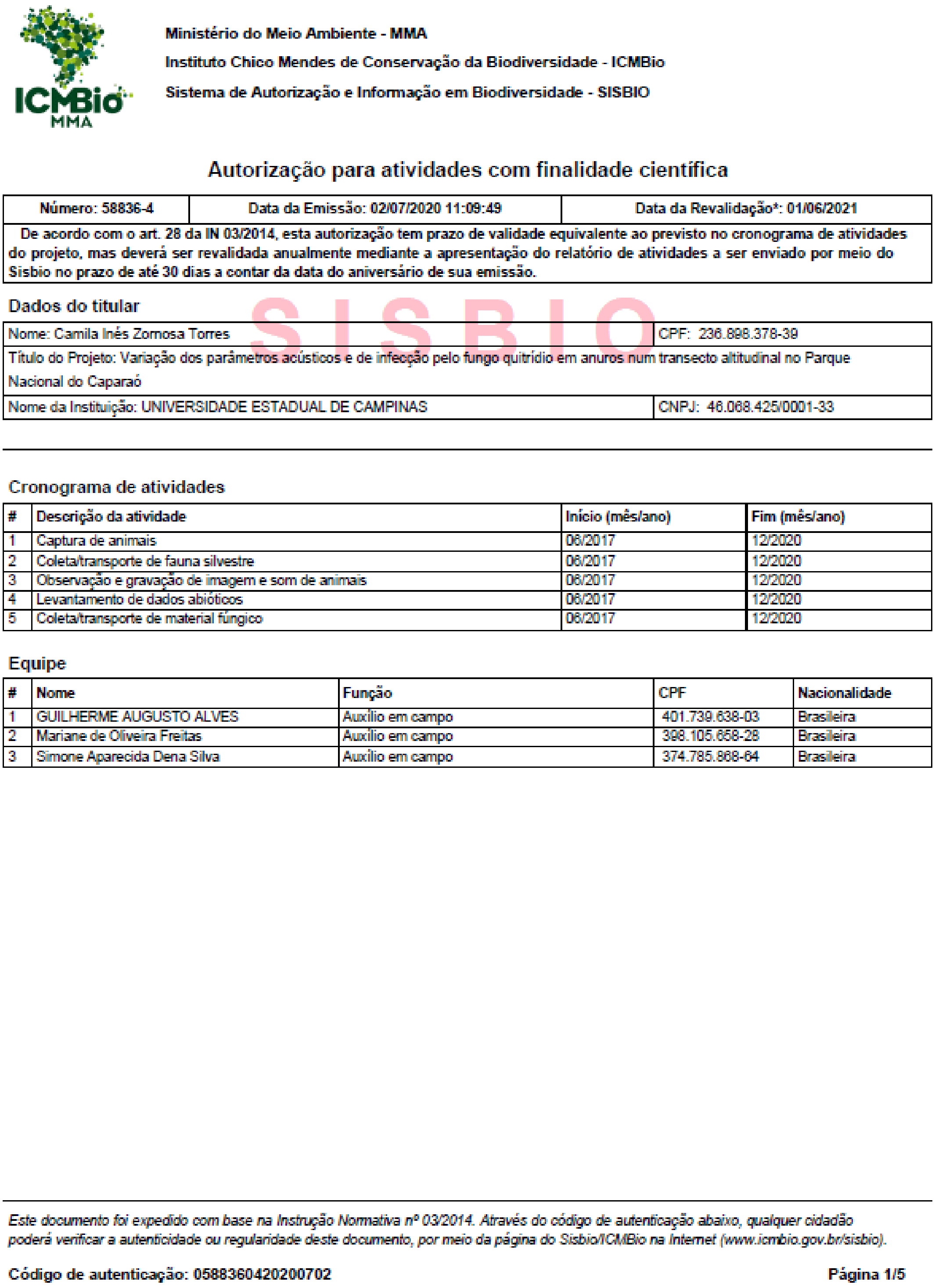
As cópias de artigos de minha autoria ou de minha coautoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Tese de Doutorado, intitulada "**Variação dos parâmetros acústicos e de infecção pelo fungo quitrídio em anuros num transecto elevacional no Parque Nacional do Caparaó**", não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 10 de novembro de 2021

Assinatura: ______________________________
Nome da Autora: **Camila Ines Zornosa Torres**
RNE n.° V996846-U

Assinatura: ______________________________
Nome do Orientador:**Prof. Dr. Luís Felipe de Toledo Ramos Pereira**
RG n.° 28.465.361-5

Anexo 3. Licença de coleta de anfíbios para o Parque Nacional do Caparaó



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Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 58836-4	**Data da Emissão: 02/07/2020 11:09:49**	**Data da Revalidação*: 01/06/2021**

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Dados do titular

Nome: Camila Inés Zornosa Torres	CPF: 236.898.378-39
Título do Projeto: Variação dos parâmetros acústicos e de infecção pelo fungo quitrídio em anuros num transecto altitudinal no Parque Nacional do Caparaó	
Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Captura de animais	06/2017	12/2020
2	Coleta/transporte de fauna silvestre	06/2017	12/2020
3	Observação e gravação de imagem e som de animais	06/2017	12/2020
4	Levantamento de dados abióticos	06/2017	12/2020
5	Coleta/transporte de material fúngico	06/2017	12/2020

Equipe

#	Nome	Função	CPF	Nacionalidade
1	GUILHERME AUGUSTO ALVES	Auxílio em campo	401.739.638-03	Brasileira
2	Mariane de Oliveira Freitas	Auxílio em campo	398.105.658-28	Brasileira
3	Simone Aparecida Dena Silva	Auxílio em campo	374.785.868-64	Brasileira

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Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	O pesquisador somente poderá realizar atividade de campo após o término do estado de emergência devido à COVID-19, assim declarado por ato da autoridade competente.
3	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
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5	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
6	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
7	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
8	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.
9	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.

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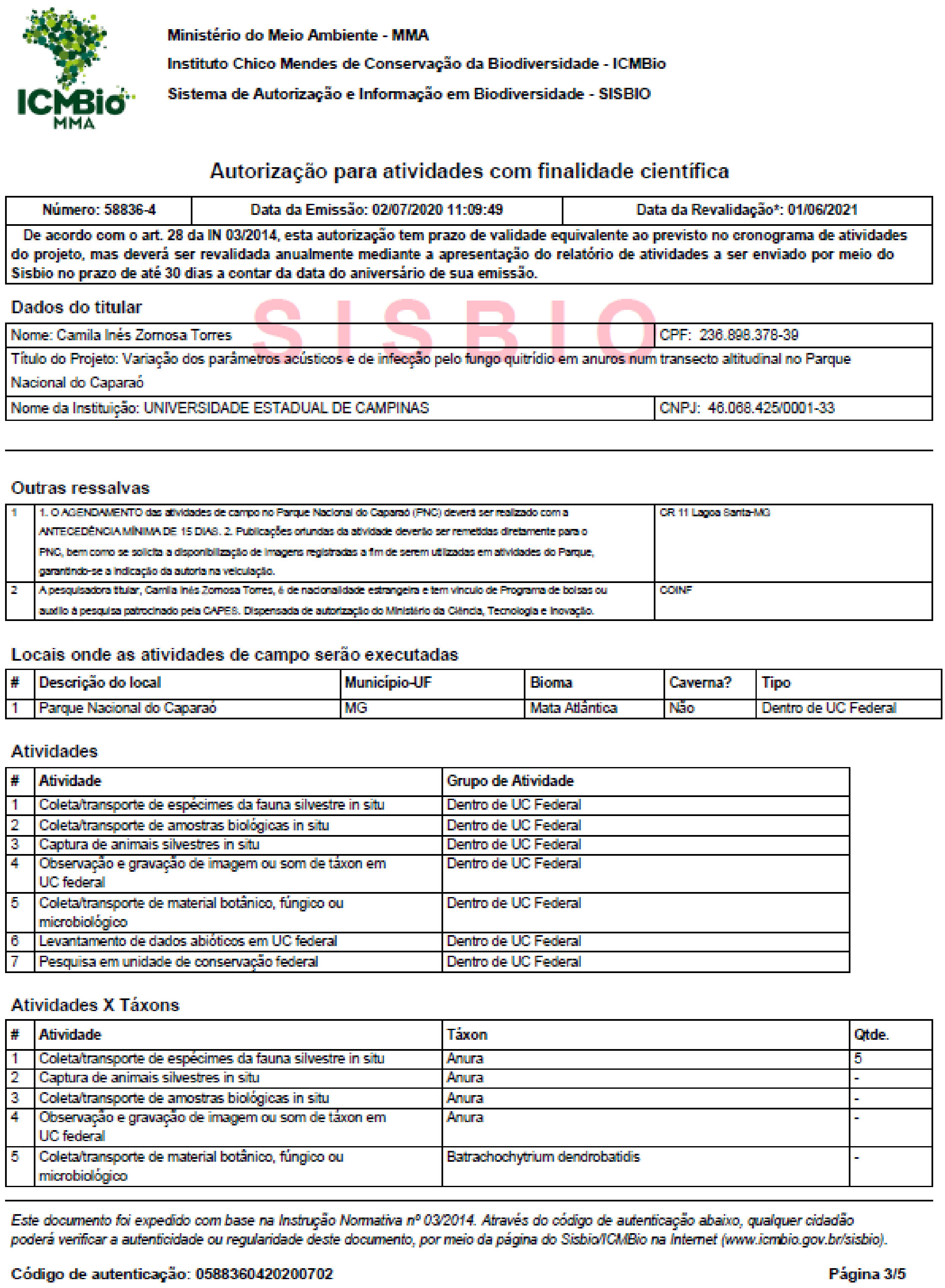

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Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Outras ressalvas

1	1. O AGENDAMENTO das atividades de campo no Parque Nacional do Caparaó (PNC) deverá ser realizado com a ANTECEDÊNCIA MÍNIMA DE 15 DIAS. 2. Publicações oriundas da atividade deverão ser remetidas diretamente para o PNC, bem como se solicita a disponibilização de imagens registradas a fim de serem utilizadas em atividades do Parque, garantindo-se a indicação da autoria na veiculação.	CR 11 Lagoa Santa-MG
2	A pesquisadora titular, Camila Inés Zornosa Torres, é de nacionalidade estrangeira e tem vínculo de Programa de bolsas ou auxílio à pesquisa patrocinado pela CAPES. Dispensada de autorização do Ministério da Ciência, Tecnologia e Inovação.	COINF

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Parque Nacional do Caparaó	MG	Mata Atlântica	Não	Dentro de UC Federal

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de espécimes da fauna silvestre in situ	Dentro de UC Federal
2	Coleta/transporte de amostras biológicas in situ	Dentro de UC Federal
3	Captura de animais silvestres in situ	Dentro de UC Federal
4	Observação e gravação de imagem ou som de táxon em UC federal	Dentro de UC Federal
5	Coleta/transporte de material botânico, fúngico ou microbiológico	Dentro de UC Federal
6	Levantamento de dados abióticos em UC federal	Dentro de UC Federal
7	Pesquisa em unidade de conservação federal	Dentro de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de espécimes da fauna silvestre in situ	Anura	5
2	Captura de animais silvestres in situ	Anura	-
3	Coleta/transporte de amostras biológicas in situ	Anura	-
4	Observação e gravação de imagem ou som de táxon em UC federal	Anura	-
5	Coleta/transporte de material botânico, fúngico ou microbiológico	Batrachochytrium dendrobatidis	-

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Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Anfíbios)	Animal encontrado morto ou partes (carcaça)/osso/pele
2	Amostras biológicas (Fungos)	Outras amostras biológicas
3	Método de captura/coleta (Anfíbios)	Captura manual, Bioacústica, Peneira
4	Método de captura/coleta (Fungos)	Outros métodos de captura/coleta(Swab)

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE ESTADUAL DE CAMPINAS	Coleção

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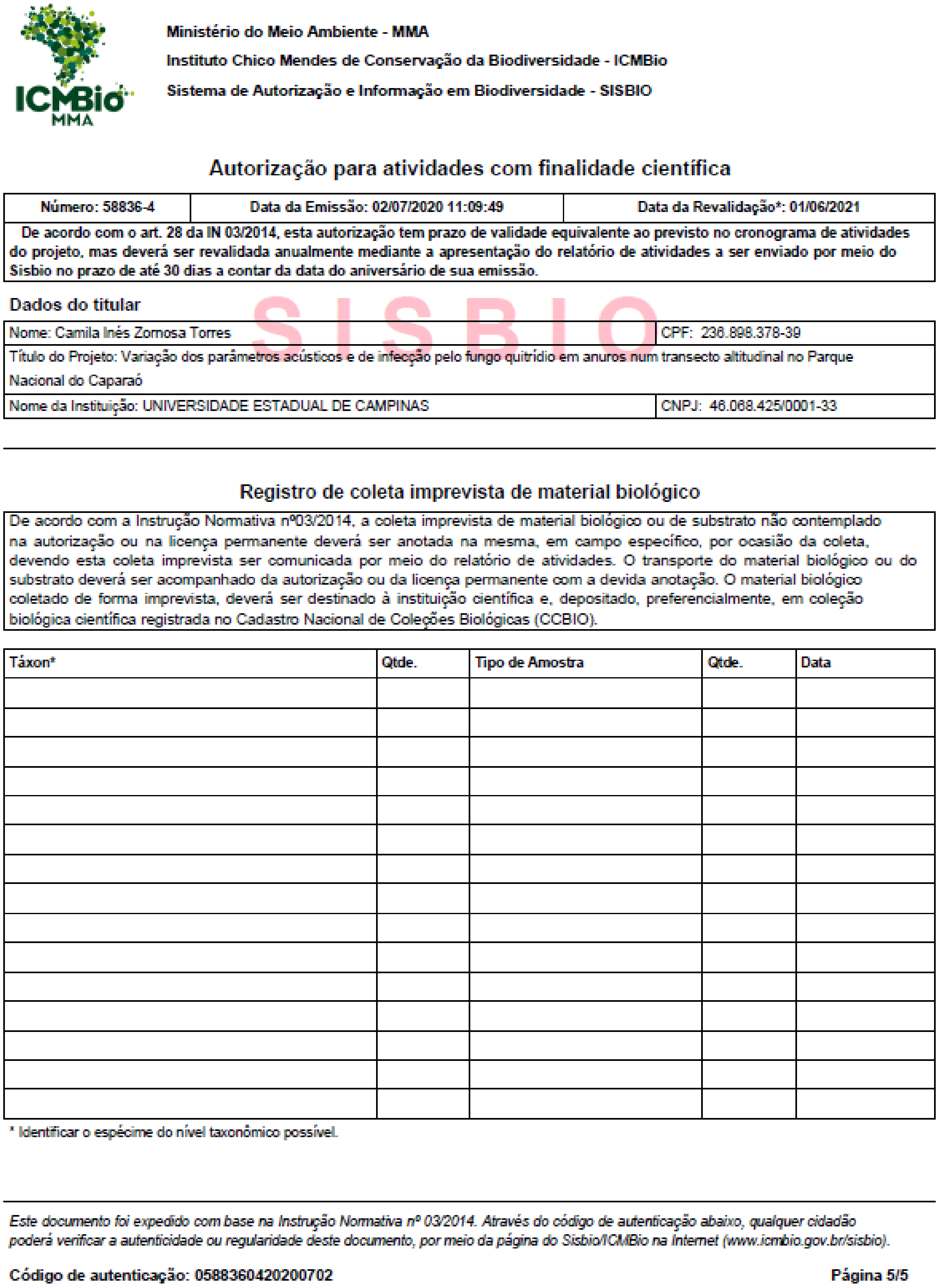

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Número: 58836-4	**Data da Emissão: 02/07/2020 11:09:49**	**Data da Revalidação*: 01/06/2021**

De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: Camila Inés Zornosa Torres	CPF: 236.898.378-39
Título do Projeto: Variação dos parâmetros acústicos e de infecção pelo fungo quitrídio em anuros num transecto altitudinal no Parque Nacional do Caparaó	
Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

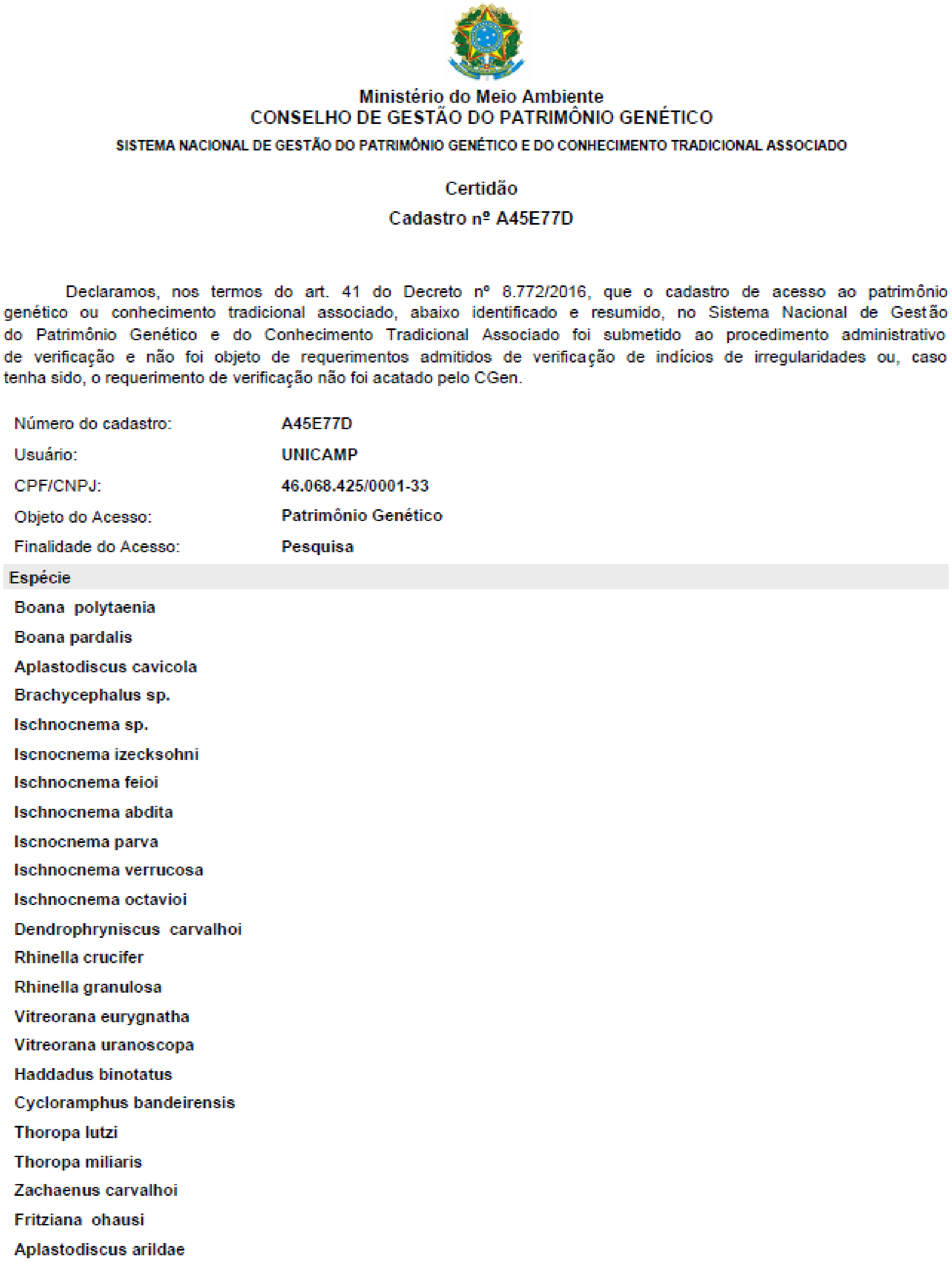
Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0588360420200702 **Página 5/5**

Anexo 4. Certidão do Conselho de Gestão do Patrimônio Gentético – SISGen



Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Certidão
Cadastro nº A45E77D

Declaramos, nos termos do art. 41 do Decreto nº 8.772/2016, que o cadastro de acesso ao patrimônio genético ou conhecimento tradicional associado, abaixo identificado e resumido, no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado foi submetido ao procedimento administrativo de verificação e não foi objeto de requerimentos admitidos de verificação de indícios de irregularidades ou, caso tenha sido, o requerimento de verificação não foi acatado pelo CGen.

Número do cadastro:	**A45E77D**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

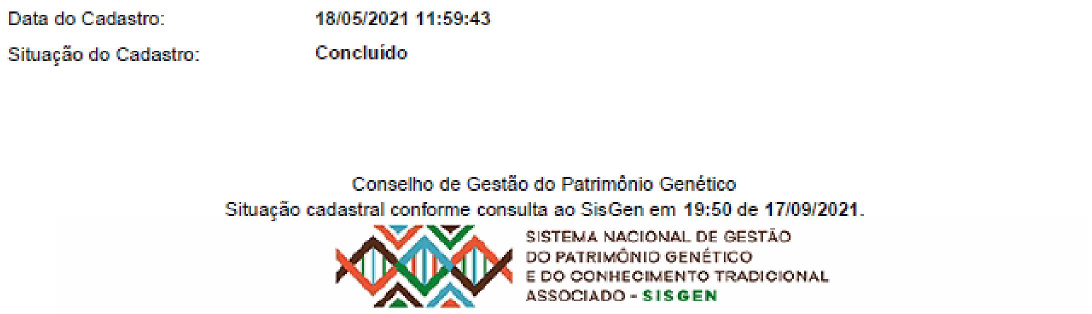
Espécie
Boana polytaenia
Boana pardalis
Aplastodiscus cavicola
Brachycephalus sp.
Ischnocnema sp.
Iscnocnema izecksohni
Ischnocnema feioi
Ischnocnema abdita
Iscnocnema parva
Ischnocnema verrucosa
Ischnocnema octavioi
Dendrophryniscus carvalhoi
Rhinella crucifer
Rhinella granulosa
Vitreorana eurygnatha
Vitreorana uranoscopa
Haddadus binotatus
Cycloramphus bandeirensis
Thoropa lutzi
Thoropa miliaris
Zachaenus carvalhoi
Fritziana ohausi
Aplastodiscus arildae

Aplastodiscus sp.
Boana albopunctata
Boana albomarginata
Boana crepitans
Boana faber
Boana semilineata
Bokermannohyla caramaschii
Dendropsophus elegans
Dendropsophus branneri
Dendropsophus haddadi
Dendropsophus pseudomeridianus
Dendropsophus minutus
Itapotihyla langsdorffii
Ololygon tripui
Scinax alter
Scinax eurydice
Scinax cf. hayii
Scinax fuscovarius
Hylodes babax
Hylodes vanzolinii
Megaelosia apuana
Pseudopaludicola restinga
Physalaemus cuvieri
Physalaemus feioi
Physalaemus sp.
Adenomera thomei
Leptodactylus fuscus
Leptodactylus mystacinus
Leptodactylus spixi
Leptodactylus latrans
Chiasmocleis sp.
Elachistocleis cf. cesarii
Myersiella microps
Proceratophrys boiei
Phyllomedusa burmeisteri
Pithecopus rohdei
Batrachochytrium dendrobatidis

Título da Atividade: Variação dos parâmetros acústicos e de infecção pelo fungo quitrídio em anuros num transecto altitudinal no Parque Nacional do Caparaó

Equipe

CAMILA INES ZORNOSA TORRES	UNICAMP



Data do Cadastro: **18/05/2021 11:59:43**

Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **19:50** de **17/09/2021**.

SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN