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PROPAGAÇÃO E RECONHECIMENTO ESPECÍFICO DO CANTO DE ANÚNCIO DE ANUROS: NO TEMPO E ESPAÇO

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Manaus, Amazonas

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Sinopse:

Os estudos publicados sobre a hipótese de adaptação acústica em anuros são compilados e analisados, destacando os principais problemas no teste da hipótese e propondo soluções. Efeitos da vegetação, morfologia e filogenia são testados em uma hipótese macroevolutiva, aplicada à família Hylidae. Testa-se ainda, o reconhecimento específico e populacional de machos de duas populações de *Allobates femoralis* e uma de *Allobates hodli* a cantos coespecíficos e heteroespecíficos que apresentam grande variação geográfica.

Palavras-chave: Evolução, bioacústica, herpetologia

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RESUMO

Neste estudo, avalia-se a influência da estrutura da vegetação sobre a propagação do canto de anúncio e evolução da comunicação acústica em anuros. No primeiro capítulo faz-se uma revisão da literatura sobre os efeitos da vegetação na transmissão do canto de anúncio, abordando diretamente, ou não, a Hipótese de Adaptação Acústica. Os estudos encontrados foram analisados em detalhe e sugere-se o incremento do desenho experimental ao aumentar o número de unidades amostrais, levar em consideração os efeitos filogenéticos e do tamanho corporal e usar uma medida quantitativa para a estrutura da vegetação. No segundo capítulo, testa-se a influência da vegetação, do ruído de fundo e da morfologia na evolução do canto de anúncio, utilizando-se a família Hylidae como modelo de estudo. Foram comparados os resultados obtidos pela estatística tradicional, que não incorpora a informação filogenética nas análises, e diferentes análises do método comparativo, realizadas sobre duas hipóteses filogenéticas. Todos os resultados apontaram um modelo adaptativo de evolução, sugerindo seleção estabilizadora ou forte evolução direcionada, com um ou dois picos adaptativos, como bons modelos para a evolução dos cantos de anúncio em função das variáveis ambientais testadas. O deslocamento da frequência dominante em ambientes ruidosos não foi encontrado. A evolução em frequência dominante está relacionada com a evolução do tamanho corporal. Pressões locais podem promover variação geográfica no canto de anúncio e reconhecimento do canto entre as populações de uma mesma espécie ou entre espécies filogeneticamente próximas. No terceiro capítulo, testa-se a capacidade de machos de *Allobates femoralis* e *Allobates hodli* em reconhecer cantos coespecíficos e heteroespecíficos, com grande variação geográfica em número de notas. Realizaram-se experimentos de playback-cruzados entre as populações simpátricas de *A. femoralis* (quatro notas) e *A. hodli* (duas notas) e playbacks a outra população de *A. femoralis* (quatro notas), que recebeu estímulos de populações alopátricas de *A. hodli* (duas notas), *A. femoralis* (três notas) e *A. myersi* (seis notas). Os machos de *A. femoralis* das duas populações (simpátrica e alopátrica) e de *A. hodli* responderam de maneira similar a todos os estímulos apresentados, indicando que as taxas de evolução do canto de anúncio e da percepção do canto são distintas, uma vez que o comportamento agressivo dos machos não acompanhou a variação geográfica nos cantos.

Advertisement call propagation and species recognition in anurans: in time and space

ABSTRACT

This study evaluates the influence of the vegetation structure on the advertisement call propagation and the evolution of acoustic communication in anurans. The first chapter reviews the literature on the effects of vegetation in the advertisement-call transmission, addressing directly or not, the Acoustic Adaptation Hypothesis. The studies found were analyzed in detail and, the importance of improving the experimental design by increasing the number of sampling units, controlling for phylogeny and body size and, using a quantitative measure of the vegetation structure was highlighted. The second chapter tests the influence of vegetation, background noise and, morphology in the evolution of the advertisement calls, with the Hylidae family as the study model. We compared the results obtained by traditional statistical, which does not incorporate information on phylogeny into the analyses, and different analyses of the comparative method, performed on two phylogenetic hypotheses. All the results pointed to an adaptive model of evolution, suggesting stabilizing selection or strong directional evolution, with one or two adaptive peaks, as good models for the advertisement call evolution considering the tested environmental traits. Displacement in dominant frequency in noisy environments was not found. The evolution in dominant frequency is related to the evolution in body size. Local pressures can promote geographic variation in advertisement calls and in call recognition among populations of the same species or between phylogenetically related species. Therefore, the third chapter tests the ability of *Allobates femoralis* and *Allobates hodli* males to recognize conspecifics and heteroespecíficos calls, with wide geographical variation in number of notes. Cross-playback experiments were performed between sympatric populations of *A. femoralis* (four-note calls) and *A. hodli* (two-note calls) and, playback experiments were performed in another population of *A. femoralis* (four-note calls), where were broadcasted stimuli of allopatric populations of *A. hodli* (two-note calls), *A. femoralis* (three-note calls) and *A. myersi* (six-note calls). Males of *A. femoralis* from both populations (sympatric and allopatric) and *A. hodli* showed similar phonotatic response to all stimuli, indicating that evolutionary rates of advertisement call design and call perception are different, since the male's aggressive behavior did not follow the geographical variation in calls.

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INTRODUÇÃO GERAL

Sinais acústicos constituem o principal meio de comunicação em anfíbios anuros. Entre os tipos de vocalizações emitidos o canto de anúncio é o mais conhecido, sendo usado para reconhecimento específico, defesa do território, atração de fêmeas e localização do macho emissor por outros machos e/ou fêmeas (Duellman e Trueb, 1994). A importância da comunicação na diversificação recente das espécies (Mayr, 1976; Panhuis *et al.*, 2001; Streelman e Danley, 2003) está no fato de que a comunicação acústica implica no reconhecimento e discriminação entre cantos de heteroespecíficos e coespecíficos, o que leva à manutenção de acasalamentos coespecíficos (Mayr, 1976; Littlejohn, 1988; Wilczynski e Ryan, 1999), reforçando o processo de especiação (Mayr, 1976; Gerhardt e Huber, 2002; Streelman e Danley 2003).

A evolução dos sinais de anúncio é frequentemente atribuída ao reconhecimento específico (Pfening, 1998; Candolin, 2003); seleção sexual (Panhuis *et al.*, 2001; Carson, 2003; Boul *et al.*, 2007), *e.g.* pela existência de preferências sensoriais (Ryan e Rand, 1993); pressões ambientais, *e.g.* ruído ambiental (Gerhardt e Klump, 1988; Gerhardt, 1994; Wollerman e Wiley, 2002); adaptação ao hábitat (McCracken e Sheldon, 1997; Seddon, 2005; Ord e Martins, 2006); pressão de predação (*e.g.* Tuttle e Ryan, 1981) ou seleção por parasitas (*e.g.* Bernal *et al.*, 2006); e mudanças estocásticas, por deriva genética e mutações (Panhuis *et al.*, 2001; Gerhardt e Huber, 2002), *e.g.* evolução pleiotrópica seguindo mudanças morfológicas ou fisiológicas (*e.g.* Cocroft e Ryan, 1995; Podos, 2001; Seddon, 2005). Além disso, a manutenção de certa semelhança comportamental entre espécies diferentes é esperada simplesmente devido às relações filogenéticas compartilhadas entre as mesmas (Ryan, 1986; Wilczynski e Ryan, 1999).

Os cantos de anúncio são compostos por características espectrais e temporais, que podem responder diferentemente às pressões evolutivas (*e.g.*, Cocroft e Ryan, 1995; McCraken e Sheldon, 1997; Erdtmann e Amézquita, 2009). Geralmente, características espectrais apresentam baixa variabilidade intra e interindividual, sendo conhecidas como características estáticas. Estas participam do processo de reconhecimento específico. As características temporais, ou dinâmicas, apresentam alta variabilidade intra e interindividual, estando associadas ao processo de escolha sexual (Gerhardt, 1991). A seleção sexual pode promover rápidas mudanças evolutivas, levando à especiação (Panhuis *et al.,* 2001), como proposto para as populações do sapo amazônico, *Physalaemus petersii,* com base em experimentos de *playback* em campo, dados genéticos e simulações em computador (Boul *et al.*, 2007).

Características ambientais podem representar uma grande força seletiva em sinais acústicos usados para a comunicação a longa distância, pois estes são particularmente afetados pelas características ambientais ao longo do seu caminho de propagação, tais como: a temperatura do ar, a estrutura da vegetação e o ruído de fundo. A estrutura da vegetação pode aumentar a atenuação (diminuição da amplitude do sinal em função da distância) e a degradação (diminuição da integridade do canto, pela perda de definição nas características temporais e amplitude (Barker, 2008) do sinal acústico, diminuindo a distância de transmissão acústica e a precisão do sinal. As características temporais e espectrais dos sinais acústicos são afetadas de maneiras diferentes pelas condições ambientais, por exemplo, frequências mais altas atenuam mais rapidamente do que as frequências mais baixas em qualquer tipo de ambiente (Morton, 1975; Gerhardt e Huber, 2002; Barker, 2008). Na Hipótese de Adaptação Acústica (HAA), a seleção sobre o ambiente direciona a seleção sobre o sinal acústico (Morton, 1975). A HAA é comumente testada através de experimentos de *playback* em campo, com o intuito de quantificar a atenuação e a degradação do sinal. Esses experimentos são executados no hábitat onde a espécie vive e em um hábitat contrastante, geralmente, floresta *vs*. área aberta. A HAA prevê que os sinais acústicos em áreas abertas terão duração mais curta, maior taxa de repetição, modulação de frequência, maior frequência mínima, maior frequência máxima, maior frequência dominante e maior amplitude de frequências em comparação com sinais acústicos emitidos em áreas densamente vegetadas (por exemplo, uma floresta) (Morton, 1975).

A adaptação acústica foi encontrada em uma variedade de espécies de aves, algumas de mamíferos e grilos e, em menor número, em anuros. Apesar da aceitação geral da HAA para os cantos de aves, Boncoraglio e Saino (2007), utilizando uma meta-análise de 26 trabalhos, encontraram uma fraca influência do ambiente (áreas de floresta *vs*. áreas abertas) sobre as características do canto de aves, sugerindo que outras pressões evolutivas podem ter um importante papel na estrutura e evolução do canto das aves, como o reconhecimento específico (*e.g.*, Seddon, 2005) e morfologia do bico (*e.g.*, Podos, 2001). Ey e Fisher (2009) analisaram 42 artigos sobre a HAA em aves e apenas três em anuros, incluindo aqueles examinados por Boncoraglio e Saino (2007), os autores sugerem que os resultados sobre a HAA são, em geral, ambíguos, e que a resposta à pressão ambiental pode estar variando em uma escala fina que, geralmente, não é testada. Apenas dois estudos de campo corroboram a

HAA em anuros (Ryan *et al.*, 1990; Bosch e De La Riva, 2004). Plasticidade acústica em resposta às características ambientais foi encontrada duas vezes em anuros (Lardner e Lakim, 2002; Ziegler *et al.*, 2011). No entanto, a pressão ambiental na evolução do canto de anúncio de anuros nunca foi testada.

O ruído de fundo constitui uma grande pressão sobre a comunicação acústica, especialmente para animais que vivem em hábitats ruidosos, como cachoeiras. Em um ambiente onde o ruído de fundo é constante pode haver o desenvolvimento de novos sistemas de comunicação, tais como a sinalização visual (para uma revisão, ver Hödl e Amézquita, 2001) e o uso de ultrassons (Feng *et al.*, 2006). Algumas espécies de anuros que são encontradas em cachoeiras produzem cantos de anúncio com frequências mais elevadas do que a frequência do ruído produzido pela cachoeira, independentemente de seu tamanho corporal (Preininger *et al.*, 2007). Cantos coespecíficos e heteroespecíficos também constituem ruído de fundo, interferindo na produção e detecção do canto de anúncio (*e.g.*, Wollerman e Wiley, 2002; Wong *et al.*, 2009). Sons antropogênicos, como aqueles produzidos por uma estrada, podem interferir nas características do canto de anúncio (Hoskin e Goosem, 2010) ou no espaço acústico ativo (Bee e Swanson, 2007).

Morfologia é uma restrição para a produção de sinais acústicos. Nos anuros a relação entre o tamanho corporal e a frequência dominante é comum (*e.g.*, Zimmerman, 1983; Ryan, 1988; Gerhardt, 1991), sendo que machos maiores produzem cantos com frequências mais baixas. Enquanto a evolução das características espectrais pode estar relacionada à evolução de características morfológicas, a evolução de características temporais pode ser limitada pela evolução de características comportamentais e fisiológicas, como a estridulação e a capacidade expiratória (Ryan e Kime, 2003). Cocroft e Ryan (1995) sugerem que as características do canto de anúncio relacionadas com características morfológicas são evolutivamente mais conservativas do que aquelas relacionadas às características comportamentais ou fisiológicas. Zimmermann (1983) ao estudar os cantos de anúncio e tamanho corporal de 56 espécies de anuros amazônicos, de áreas abertas e florestais, encontrou que as espécies de áreas abertas apresentavam tamanho corporal menor do que as espécies florestais e que este estava inversamente relacionado à frequência dominante do canto. Os resultados de Zimmermann (1983) concordam com as predições da HAA, pois em áreas de florestas as frequências altas degradam mais rapidamente do que as frequências baixas (Morton, 1975), porém a autora ressalta a influência do tamanho corporal e da filogenia na diversificação dos cantos das espécies estudadas.

Porém, algumas variações nas características do canto podem ser resultantes de processos estocásticos, como a deriva genética aleatória ou evolução direcional flutuante, com a direção da seleção variando aleatoriamente sobre a filogenia (Ord e Martins, 2006). Métodos filogenéticos comparativos (PCMs, sigla para o termo em inglês Phylogenetic Comparative Methods) foram desenvolvidos para considerar a não independência das unidades amostrais (ou seja, as espécies) nas análises estatísticas. Incorporar a filogenia nas análises nos permite compreender melhor os padrões ecológicos, a evolução de características fisiológicas e comportamentais e testar hipóteses utilizando uma matriz de dados interespecífica.

A variação geográfica em cantos de anúncio entre populações alopátricas ou espécies-irmãs pode surgir como resultado de deriva genética ou devido a pressões seletivas exclusivas para a área geográfica ocupada pela população (Littlejohn, 1988; Coyne e Orr, 2004). A ocorrência de espécies relacionadas em simpatria pode facilitar a divergência entre os cantos de anúncio e das preferências intra e interespecíficas (Gerhardt, 1999; Lemmon, 2009), favorecendo as características relacionadas com o reconhecimento específico ao invés daquelas relacionadas com qualidade sexual, *i.e.*, por deslocamento de caractere.

Comunicação acústica em *Allobates femoralis*

A rã pan-amazônica *A. femoralis* (Boulenger, 1884) é amplamente distribuída em florestas de terra-firme da Amazônia. Trata-se de uma espécie diurna onde os machos defendem um território multi-propósito (Roithmair, 1992; Ringler *et al*., 2009). Estudos anteriores encontraram machos de *A. femoralis* defendendo territórios por até 90 dias em uma população da Amazônia peruana (Roithmair, 1992), e por mais de 79 dias em uma população na Guiana Francesa (Ringler *et al.,* 2009). Variação geográfica no número de notas que compõem o canto de anúncio é conhecida para *A. femoralis* (Hödl *et al*., 2004; Amézquita *et al*., 2005; Amézquita *et al.*, 2006). Cantos com uma nota são encontrados no Parque Nacional Yasuní, Equador (Read, 2000), e ao longo do Rio Juruá, Brasil (P.I. Simões e A.P. Lima, dados não publicados); cantos com três notas são conhecidos para a Estação Panguana, no Peru (Hödl *et al*., 2004; Amézquita *et al*., 2006). Cantos com quatro notas são geograficamente difundidos, sendo descritos para as localidades na região central da Amazônia brasileira, bacia do Rio Madeira, Colômbia e Guiana Francesa (Hödl *et al*., 2004;

Amézquita *et al.*, 2006; Simões *et al.*, 2008; Amézquita *et al.*, 2009). Uma espécie relacionada, *Allobates myersi* (Pyburn, 1981), tem sua distribuição conhecida restrita à Amazônia colombiana (Lötters *et al.*, 2007; Frost, 2011), recentemente foi encontrado na cidade de São Gabriel da Cachoeira no alto Rio Negro, Amazonas, Brasil (Simões e Lima, 2011). Seu canto de anúncio é muito semelhante ao de *A. femoralis*, mas é composto por um número médio de seis notas (Simões e Lima, 2011). A população com duas notas anteriormente referida como pertencente à *A. femoralis*, recentemente recebeu o status de espécie (*Allobates hodli* Simões, Lima e Farias, 2010), é distribuída na margem esquerda do alto Rio Madeira, Acre, Brasil (Simões *et al.*, 2010). Na margem esquerda do alto curso do Rio Madeira, uma população de *A. hodli*, duas notas, encontra uma população de *A. femoralis*, quatro notas, em uma estreita zona de contato (Simões *et al.*, 2008; Simões *et al.*, 2010).

A emissão de cantos coespecíficos acima de 68 dB (re 20 TPa) promove o comportamento de *phonotaxis* em machos de *A. femoralis*, que consiste em cessar a atividade de vocalização, orientar a cabeça e o corpo e, em seguida, saltar até aproximar-se do alto-falante (Narins *et al.*, 2003). O mecanismo de reconhecimento do canto em *A. femoralis* foi estudado em experimentos de campo utilizando estímulos sintéticos apresentados a machos da espécie. O uso de estímulos sintéticos permite modificar uma característica acústica por vez e desta forma testar diferentes hipóteses. Já foram analisados, por exemplo, o número de notas e frequência dominante do canto (Amézquita *et al.*, 2005), variações na modulação da frequência das notas (Hödl *et al.*, 2004) e a duração do intervalo entre as notas (Göd *et al.*, 2007). A probabilidade de resposta de machos de *A. femoralis* correspondeu ao valor da frequência dominante para uma população colombiana (quatro notas), mas também combinava com a faixa de variação de frequência do canto de anúncio de heteroespecíficos, indicando que a frequência dominante sozinha não foi suficiente para que os machos pudessem discriminar entre os cantos de coespecíficos e heteroespecíficos (Amézquita *et al.*, 2005). A mudança da modulação ascendente típica do canto de anúncio de *A. femoralis* a uma modulação descendente ou a um canto não modulado não promove diferenças nos padrões de resposta de machos de *A. femoralis* (Hödl *et al.*, 2004). Machos de *A. femoralis* podem reconhecer cantos de anúncio com intervalo de silêncio entre as notas variando até 60% do valor médio da população (Göd *et al.*, 2007). Estudos anteriores não foram capazes de apontar uma característica acústica responsável pelo reconhecimento específico, porém demonstraram que nem todas as características são relevantes para o reconhecimento. Provavelmente, não haja uma única característica acústica responsável pelo reconhecimento específico, sendo esse alcançado pela interação ou soma de diferentes características (Gerhardt e Huber, 2002; Göd e*t al.*, 2007).

Ao considerar a variação do número de notas por canto de anúncio (duas a quatros notas), foram encontradas diferenças nas curvas de reconhecimento para os cantos com duas notas, sendo sugerido que esta resposta diferencial possa indicar uma adaptação local à presença de cantos de outras espécies, ou ainda, uma reação a uma série de notas que não é típica da população-alvo (Amézquita *et al*., 2005).

Neste estudo, avalia-se a influência da estrutura da vegetação sobre a propagação do canto de anúncio e evolução da comunicação acústica em anuros através de análises modernas do método comparativo e experimentos de *playback* executados em campo.

OBJETIVOS

Os objetivos gerais de cada capítulo foram os seguintes:

Capítulo 1 – Realizar a revisão bibliográfica sobre os efeitos da vegetação nas características do canto de anúncio dos anuros e sobre como a Hipótese de Adaptação Acústica vem sendo tratada em anuros, propondo maneiras de incrementar o desenho experimental de estudos neste tema;

Capítulo 2 – Avaliar o efeito da vegetação, hábitat e sítio de vocalização, e da morfologia sobre as características do canto de anúncio em uma escala macroevolutiva, utilizando como modelo de estudo a família Hylidae;

Capítulo 3 – Avaliar os padrões de reconhecimento específico e populacional em machos de *Allobates femoralis* e *A. hodli* a cantos coespecíficos e heteroespecíficos, que apresentam uma grande variação geográfica.

CAPÍTULO 1

Erdtmann, L.K. & Lima, A.P. Environmental effects on anuran call design: what we know and what we need to know. Aceito para publicação na *Ethology Ecology & Evolution*.

Environmental effects on anuran call design: what we know and what we need to know

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Long-range acoustic signals are subject to a variety of evolutionary pressures, such as sexual selection, species recognition, body-size constraints, physiological constraints, and natural selection by environmental constraints. Anuran advertisement calls are long-range acoustical signals with two essential functions: to attract females, and to defend a territory against other males. Nevertheless, the environment offers obstacles to sound transmission. The call can be attenuated and degraded, and the surrounding environment might impose a strong constraint on it by means of sound refraction, reflection, and absorption along the transmission path. The Acoustic Adaptation Hypothesis (AAH) predicts that the call could be adapted in order to maximise the transmission distance by minimising the call attenuation and degradation. The predictions of the AAH have been reviewed twice for birds, once for mammals and anurans. This study extends the anuran review, focusing on the environmental effects on anuran call design, and their conformity to the AAH predictions. A small number of studies were found, and the results were conflicting. These studies were carefully analysed, and we report a lack of standardised methodology to test for environment effects. We discuss in detail the diverse methodologies and point out how the question has been treated. We highlight the importance of improving the project design by increasing the sample size, controlling for phylogenetic and body-size effects, and using a quantitative representation of vegetation structure.

KEY WORDS: bioacoustics, acoustic communication, advertisement call, adaptation, Anura.

INTRODUCTION

Acoustic signals are primordial for communication in a variety of animal groups. They may contain information about the informer's identity, physical location, body size, species, and sexual status (GERHARDT & HUBER 2002). However, a communication system is only efficient when the signal emitted by an individual travels through the environment and influences the receiver (BRADBURY & VEHRENCAMP 1998; RUXTON & SCHAEFER 2011). Acoustic signal evolution can be shaped by several selective pressures, e.g., sexual selection, morphology, physiology, phylogeny, predation, parasitism, and environment (ENDLER 1992; FORREST 1994). These selective pressures could act in isolation or integrated with each other, sometimes even in opposite directions; for instance, when the signal production attracts both mates and predators (RYAN 1986). The local environment, i.e. described by the environmental characteristics around the calling site or along the call propagation path, may represent a strong selective force on acoustic signals, because they are particularly affected by the environmental characteristics of their propagation path, such as temperature, vegetation structure, and background noise (FORREST 1994). The environmental pressures on signals are often studied as background noise effects or in relation to signal attenuation and degradation patterns. Sound attenuation is usually greater than expected in spherical spreading conditions, due to sound absorption, scattering, reflection, and refraction caused by the environment, and this additional increase is called excess attenuation (FORREST 1994; GERHARDT & HUBER 2002).

Sound scattering and reflection also play a role in call degradation, defined as the decreasing of call integrity by losing definition in temporal traits and amplitude patterns (FORREST 1994). Local vegetation structure can increase the signal attenuation and degradation, decreasing the acoustic transmission distance and the signal accuracy. Temporal and spectral components of acoustic signals are differentially affected by environmental conditions; for example, higher frequencies attenuate more rapidly than lower frequencies in any environment (MORTON 1975; GERHARDT & HUBER 2002; ELLINGER & HÖDL 2003). In the Acoustic Adaptation Hypothesis (AAH), selection in the local environment would drive the selection for call design (MORTON 1975). Commonly, AAH is tested by field playback experiments in order to quantify the signal attenuation and degradation, comparing the habitat where the species lives with a contrasting habitat, usually, forest vs. open area. AAH predicts that, in comparison with densely vegetated areas (e.g., a forest), calls in open areas will 1) be shorter in length, 2) have a higher repetition rate, 3) have increased frequency modulation, 4) have a higher minimum frequency, 5) have a higher maximum frequency, 6) have a higher dominant frequency, and 7) a wider frequency bandwidth (MORTON 1975). However, the results concerning AAH are, in general, ambiguous, and that the response to environmental pressure may be varying on a fine scale that is usually not tested (BONCORAGLIO & SAINO 2007; EY & FISHER 2009).

Anuran advertisement calls have the primary functions of attracting females and announcing territory possession to other males (DUELLMAN & TRUEB 1994). The advertisement call may transmit information about the calling male, and females might base their mate-choice on that information. Male body size can be informed in the advertisement call, because in anurans, the dominant frequency is often inversely related to the body size, with larger males producing lower-frequency calls than smaller males (e.g., ZIMMERMAN 1983; RYAN 1988; GERHARDT 1991). Thus, anuran calls could be under different evolutionary pressures, e.g., sexual selection, species recognition, morphology, and phylogeny (RYAN 1986). Advertisement call production is costly (for a review see, WELLS 2007), as is a heterospecific mating (PANHUIS et al. 2001). Because of its importance to the mating system, it is expected that (i) the transmission distance of advertisement calls would be maximised, facilitating the call detection and recognition by conspecific females, thus avoiding energy wasting; and (ii) that the transmission distance would vary according to the surrounding environmental characteristics. EY & FISHER (2009) encountered only three articles on AAH in anurans, that indicated some environmental effect on advertisement call traits, but the general findings about AAH in anurans were inconclusive.

Here we will review a larger number of studies of AAH and environmental influences on anuran calls, to attempt to identify generalisations that are well supported, and to indicate how studies may be improved to allow better evaluations of AAH.

MATERIALS AND METHODS

We systematically searched in the Web of Knowledge database (http://apps.isiknowledge.com) with combinations in triads with the words “habitat”, “environment*”, “adaptation”, “acoustic*”, “call”, “acoustic communication” and “anura*”. Some articles that were not found by the systematic search were included, in order to complete the list of publications about acoustic adaptation in anurans and environmental effects on advertisement calls. These articles were found by checking references in related articles, theses, and occasional searches in Google (www.google.com) and ScienceDirect (www.sciencedirect.com). Articles consulted are summarised in Table 1. The main temporal and spectral call traits evaluated are described in Table 2. Call traits specific to one or a few species were not included. Because phylogenetic history and body size can affect the call structure, we recorded whether the studies included phylogeny and body size in their analyses.

RESULTS

We found 12 articles dealing with the environmental effects on anuran advertisement call traits and acoustic adaptation hypothesis in anurans (Table 1).

Environmental effects on specific temporal and spectral traits (for a description of call traits, see Table 2) were tested in six studies. Sometimes, one article analysed more than one temporal and spectral trait, and therefore the number of studies investigating temporal and spectral traits and the number of call traits considered were not the same. For temporal traits, environmental effects were found nine times (from four studies), four times for call duration, twice for pulse rate, once for call rate, once for inter-call interval, and once for inter-note inteval. In three tests (from two studies), temporal traits were not consistent with AAH predictions. These studies investigated: pulse rate (n = 1), call rate (n = 1), and call duration (n = 1).

For spectral traits, support for the AAH predictions was found three times (three studies) for dominant frequency (higher frequencies in open areas), twice for frequency modulation, and once for frequency bandwidth. In one study, frequency modulation was inversely related to microhabitat characteristics (more complex environments showing higher-frequency modulation). In three tests (from two studies) there was no response related to AAH predictions for dominant frequency (n = 1) or frequency bandwidth (n = 2). No relationship was found between environment and number of notes per call (n = 1), and the number of different notes added to the advertisement call (n = 1). Also, environment influenced call intensity (n = 1), and the number of different notes added to the advertisement call (n = 1).

The remaining studies (n = 6) analysed environmental effects on call degradation by field playback experiments of call propagation, and calculated cross-correlation coefficients to quantify the effects of attenuation and degradation (n = 5), or by model testing (n = 1); they did not report their results on specific call traits. Four of these studies did not find a relationship between environment and call degradation, and in one study, the environment apparently influenced the call propagation pattern (RYAN et al. 1990). The negative results (i.e., the environment was not affecting the call propagation pattern) in RYAN & SULLIVAN (1989) were attributed to the high similarity between the studied areas.

The power of results extrapolation depends on project design. We list four characteristics that deserve special attention: environment representation, body size, sample size, and phylogenetic inference.

(1) Environment representation

The environment was represented as a qualitative variable in eight studies. Only two studies quantified the vegetation structure around the calling site (CASTELLANO et al. 2003; ZIEGLER et al. 2011), and one the proportions of water and air in the holes used as calling sites (LARDNER & LAKIM 2009). In one case, the environment was also represented as the background noise (CASTELLANO et al. 2003). Although background noise can be a source of selection for call traits, it is not the focus of this review, and additional studies on this theme were not analysed (e.g., HÖDL & AMÉZQUITA 2001; PENNA et al. 2005; PREININGER et al. 2007).

(2) Body size

The body-size information was included in four studies, and in one study (BEVIER et al. 2008) morphology was represented by body mass. In these studies, the inverse relationship between the call-dominant frequency and body size or body mass was evident.

(3) Sample size

Usually, the experimental design consisted in testing call propagation or comparing call traits between one open area and one forested area. The number of species tested varied from 1 to 95, and from 1 to 51 species per area.

(4) Phylogenetic inference

Interspecific datasets were analysed in eight studies, and four studies analysed intraspecific datasets. Only three studies considered the phylogenetic influence on signal structure through species relationships (genus and family), but the authors did not use phylogenetic comparative methods to incorporate or exclude the species' phylogenetic relationships in the statistical analysis (ZIMMERMANN 1983; BOSH & DE LA RIVA 2004; BEVIER et al. 2008).

In summary, six articles found results compatible with AAH or at least showed the existence of an acoustical response flexibility related to environmental characteristics. No evidence supporting AAH or environmental influence was reported in six studies. The only interspecific study showing an environmental effect on call trait, analysed three environmental types (ecoregion, macrohabitat, and microhabitat), and found a weak relationship that was contrary to that expected for AAH for frequency modulation with microhabitat (BOSH & DE LA RIVA 2004). Like BOSH & DE LA RIVA (2004), who found an environmental effect on call trait contrary to AAH, ZIMMERMANN (1983) explained the relationship of spectral traits with the environment as a confounding effect with body size and phylogenetic effects.

DISCUSSION

The results of the articles that we reviewed showed that there is no general consensus about the importance of the environment as an evolutionary pressure affecting the evolution of advertisement calls in anurans. However, it is not possible to be sure whether this reflects different biology or different project designs. Advertisement calls were well represented in the studies, where spectral and temporal traits were tested for a similar number of times. Nevertheless, call intensity and structural traits were used less often (e.g., the addition of different notes to the call).

Biological concerns

Because the advertisement call is composed by temporal and spectral traits, its response to evolutionary pressures may vary within call traits. As a consequence, the acoustic signal design will be a result of the trade-off between the evolutionary pressures on call traits. Anuran advertisement call traits may exercise different roles in species recognition and sexual selection, and they may be classified as static or dynamic according to their coefficient of variation within and between males (GERHARDT 1991). Static traits are those with low variability within and between males, such as spectral traits, and dynamic traits are those with high variability within and between males, such as temporal traits (GERHARDT 1991). GERHARDT (1991) suggested that static traits should be used in species recognition, whereas dynamic traits would be more important for sexual selection. Therefore, call traits may vary according to the evolutionary pressures, and sexual selection can be stronger than environmental pressures when the call trait plays a role in mate-choice.

The relationship between dominant frequency and body size is well documented in anurans (e.g., ZIMMERMAN 1983; RYAN 1988; GERHARDT 1991). The reviewed studies that included body size in their statistical analyses, found the expected inverse relationship between the body size and dominant frequency. However, they failed to find a relationship between dominant frequency and environment. Possibly, in these cases, morphology represented by body size is a greater evolutionary pressure on call frequencies than is the environment itself. BEVIER et al. (2008) found environment effect on dominant frequency and included body mass in their statistical analyses, but the results were contrary to the expected by AAH, species occurring in open areas had higher body mass and produced lower-frequency calls. On the other hand, in studies where a dominant frequency was related to the environmental type or condition, the body size was not evaluated.

Background noise is a source of selection and can favour higher frequencies than the noise frequencies, even higher than those expected based on body size (PREININGER et al. 2007). It might also favour the appearance of other communication systems, such as the use of visual signals (for a review, see HÖDL & AMÉZQUITA 2001), or ultrasonic sounds (FENG et al. 2006). Background noise along with signal reverberations produced by the local vegetation may contribute to the degradation of temporal structure of *Hyla chrysoscelis* advertisement calls by "filling" the intervals between pulses, but despite the loss in pulse structure, degraded calls still elicited female phonotaxis (KUCZYNSKI et al. 2010).

Methodological concerns

This review showed contrasting results concerning the effects of the local environment and the AAH on anuran advertisement calls. Thus, we are not able to predict the call adjustment to the environment in anurans as a whole. Nevertheless, the evidence is very limited, and project designs may explain many of the discrepancies. The best environment-fitted acoustic signal (i.e., with high performance in the species' own habitat), as suggested by MORTON (1975) and ENDLER (1992), is rarely found to be inclusive for a well studied species group such as birds (for a review on AAH in birds, see BONCORAGLIO & SANIO 2007; EY & FISHER 2009). The AAH was based solely on call-propagation performance in very discrepant environments: a densely vegetated habitat, such as a forest, and an open area, such as grasslands. This was a very important and fundamental step for our understanding of bioacoustics and animal acoustic communication, but why has the experimental design not improved since then? Below, we list and discuss the four main points that we believe need to be taken into account for a successful test of AAH.

Study argument 1 - Environment representation. The way that the environment is represented is extremely important for the interpretation of results. Usually, the vegetation structure is summarised as a qualitative trait. The qualitative representation of habitat types could be masking the effective environment that a small frog uses to communicate. The need for more detailed vegetation information has been pointed out previously by BOSH & DE LA RIVA (2004), WELLS (2007), and EY & FISHER (2009). To investigate the environmental effects on call propagation, more precise measurements of vegetation structure are required, i.e., to characterise the vegetation microstructure. A more precise environment representation will allow a better hypothesis testing. Environment representations may be done in a variety of ways, ZIEGLER et al. (2011) measured the cover percentage inside sampling quadrats distributed in a 2 m radius around male calling site, CASTELLANO et al. (2003) represented the environment, constituted of open grasslands, by the percentage of vegetation cover, and the vegetation height, both measured in quadrats distributed along the propagation paths, transects 32 m long. The study site and, mainly, study question will determine the best representation for the local environment conditions. In a forested area, for example, to combine techniques to measure herbs, grass, and shrubs and techniques to measure trees would be desirable. But the study question will dictate how the measures should be done. Numerous field techniques to measure vegetation are found in botanical literature. For large interspecific datasets, where habitat representation in categories is used, the statistical analysis must include information on phylogeny.

Study argument 2 - The study should explicitly examine body-size effects. The perfect scenario would be to use individuals of the same species occurring in forested and open areas. However, as this scenario is difficult to find, pairs of species with similar body sizes should be used, as well as inclusion of body size as a covariate in analyses, which also helps to minimise unwanted effects of body size on the analysis.

Study argument 3 - The number of sample units. Avoiding pseudoreplication and, consequently, misinterpretation of collected data (for a review see HURLBERT 1984) is the principal challenge in all project designs. Further research must take into consideration increasing the number of sample units, i.e., the number of sampled areas. For example, call propagation playback experiments might be performed in several forested and open areas, rather than pseudoreplicates in one location. There is no magic number for how many sample units must be used. However, it is imperative to increase the number of sites above the numbers that were used in previous studies (usually the comparison of one forest with one open area).

Study argument 4 - The study should take into account phylogenetic effects. Depending upon the theoretical or field design, different approaches may be adopted to incorporate or exclude phylogenetic effects. Comparative phylogenetic methods (e.g., phylogenetic independent contrasts, Hansen's adaptation test) are preferable in theoretical studies. In field playback experiments, phylogenetic differences can be controlled by selecting species that occur in both forested and open areas. The ideal scenario would be to use individuals of the same species that live in both types of area. Such a scenario is not easy to find, and alternatively, pairs of sister-species or the closest phylogenetically related species could be used. The inclusion of different species-pairs is enough to achieve species replication.

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Table 1.

Environmental effects on advertisement call traits of anurans. The response to environment is show by: (-) no environment influence reported, (+) environment influence reported. Accordance to AAH follows the authors conclusion: (-) results not support AAH or any environment effect, (+) some support for AAH conditions or environment effect.

Taxa	Intra/interspecific dataset	Body size	Phylogenetic inference	Habitat description	Call duration	Call rate	Pulse rate	Intercall interval	Internote interval	Dominant frequency	Frequency bandwidth	Frequency modulation	Call intensity	Number of notes	Number of different notes	Accordance to AAH	Reference
56 Amazonian species	inter	yes	yes	Open Forest			-			+	+	+				-	ZIMMERMANN 1983
Ranidella riparia; R. signifera	inter	no	no	Rock Mud and reeds	+		+				-					+	ODENDAAL et al. 1986
Bufo woodhousii; B. valliceps	inter	yes	no	Two sites												-	RYAN & SULLIVAN 1989
Acris crepitans	intra	no	no	Open Forest												+	RYAN et al. 1990
5 Chilean species	inter	yes	no	Brush Water												-	PENNA & SOLÍS 1998
22 Panamian species	inter	no	no	Open Forest												-	KIME et al. 2000
Metaphrynella sundana	intra	no	no	Air depth inside a hole	+			+		+			+			+	LARDNER & LAKIM 2002
Pseudacris crucifer crucifer	intra	no	no	Models of tress, shrubs and ponds												+	PARRIS 2002

				cover												
3 taxa *Bufo viridis* complex	inter	no	no	% covering 1m^2 quadrats and background noise											-	CASTELLANO et al. 2003
95 Bolivian species	inter	no	yes	Ecoregion Macrohabitat Microhabitat	-	-				-	-	+	-	-	-	BOSCH & DE LA RIVA 2004
8 *Scinax* species	inter	no [*]	yes	Open Forest	+	+	+			+				+	+	BEVIER et al. 2008
Hypsiboas pulchellus	intra	yes	no	Vegetation cover, used in a path analysis											+	ZIEGLER et al. 2011
	intra	yes	no	% covering 25x25cm quadrats	+				+						+	

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[*] BEVIER et al. (2008) presented body mass measurements.

Table 2.

Description of temporal and spectral call traits considered in the revised articles. Most of them were presented in BOSH & DE LA RIVA (2004).

	Call Trait	Description
Temporal	Call duration	The call length from its onset until the end.
	Number of notes	Number of notes within a call.
	Call rate	Number of calls emitted per minute.
	Pulse rate	Number of pulses emitted per second.
	Intercall interval	Interval between two consecutive calls.
	Internote interval	Interval between two consecutive notes.
Spectral	Dominant frequency	The call frequency value with the highest energy in the call.
	Frequency bandwidth	The difference between the upper and lower call frequency.
	Frequency modulation	The changing of call frequency.
	Call intensity	The intensity of call, measured in dB.
	Number of different notes	Number of different notes composing an advertisement call.

CAPÍTULO 2

Erdtmann, L.K.; Costeira, J.M. & Lima, A.P. A macroevolutionary approach to environmental and morphological effects on the evolution of advertisement calls in tree-frogs (Anura: Hylidae).
Submetido a *Animal Behaviour*.

A macroevolutionary approach to environmental and morphological effects on the evolution of advertisement calls in tree-frogs (Anura: Hylidae)

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Local environment may be a strong selective force on acoustic signals used in long-range communication, because these signals are particularly affected by the environmental characteristics of their propagation path, such as temperature, vegetation structure, and background noise. Natural or anthropogenic background noise also exerts a strong pressure on acoustic communication. Morphology constitutes a constraint on acoustic signal production and evolution. Call frequencies in frogs are generally associated with body size, but frogs that live near waterfalls may communicate with frequencies higher than the waterfall noise frequencies, independent of their body size. This study evaluated the effects of environment and morphology on tree-frog (Hylidae) call evolution, through a macroevolutionary approach based on traditional statistical and phylogenetic comparative methods [i.e., phylogenetic independent contrasts (PIC), trait-correlated evolution, and Hansen's adaptation test]. We tested four evolutionary models based on Brownian motion and Ornstein-Uhlenbeck processes. The models were selected based on likelihood ratio tests, Akaike Information Criterion, and Bayesian Information Criterion. All the results pointed to an adaptive model of evolution. Our raw-data analysis indicated that habitat type influenced peak frequency and body size, and calling site influenced pulse rate. We found habitat effects on peak frequency and body size, and of calling site on pulse rate, also after taking into account phylogeny with phylogenetic ANOVAs. Variation in body size was related to variation in peak frequency (considering both raw data and PIC). Ornstein-Uhlenbeck models were selected for all call traits, suggesting a stabilising selection or strong directional evolution, with one or two adaptive peaks.

Keywords: acoustic adaptation; information-theory approach; model-based comparative analysis; selective regimes; advertisement call evolution; Hylidae; Anura

Advertisement calls are the principal acoustic signals in anuran communication. They may contain information about the signaller's identity, species identity, location, body size, and sexual status (Gerhardt & Huber 2002). Acoustic signal evolution may be constrained by sexual selection, morphology, physiology, phylogeny, predation, parasitism, and environment (Ryan 1986; Endler 1992; Forrest 1994). Advertisement calls are composed of spectral and temporal traits, which may respond differently to evolutionary pressures (e.g., Cocroft & Ryan 1995; McCraken & Sheldon 1997; Erdtmann & Amézquita 2009). Spectral traits usually show low variability in the same male and between males, and these relatively static traits are commonly involved in species recognition; whereas temporal traits are more dynamic, with wide variability in the same male and between males, and are often associated with the mate-choice process (Gerhardt 1991). Sexual selection may drive rapid evolutionary changes, leading to speciation (Panhuis et al. 2001), as proposed for populations of the Amazonian frog *Physalaemus petersii*, based on field playback experiments, genetic data, and computer simulations (Boul et al. 2007). Evolutionary forces acting on advertisement calls are not exclusive, and different forces may select in opposite directions (Ryan 1986). In the túngara frog *Physalaemus pustulosus*, the addition of high-frequency notes, called “chucks”, increases female attraction, and this preference would skew sexual selection towards “chuck calling” males (Ryan & Rand 1993); however, this type of call also increases the chances of attracting parasites (e.g., Bernal et al. 2006) and predators (e.g., Tuttle & Ryan 1981).

The local environment may act as a strong selective force on acoustic signals used in long-range communication, because these signals are strongly affected by the propagation path, which may be modified by temperature, vegetation structure, and background noise. Local vegetation structure can increase the signal attenuation and degradation, decreasing the acoustic transmission distance and signal accuracy. Temporal and spectral components of acoustic signals are affected differently by environmental conditions. Higher frequencies attenuate faster than lower frequencies in any environment (Morton 1975; Gerhardt & Huber 2002; Barker 2008). In the Acoustic Adaptation Hypothesis (AAH), selection in the local environment would drive the selection of call design (Morton 1975). AAH predicts that in an open area, the call will a) be shorter in length, b) have a higher repetition rate, c) show frequency modulation, and have d) a higher maximum frequency, e) a higher minimum frequency, f) a higher peak frequency, and g) a wider frequency bandwidth, than in areas with dense vegetation such as forests (Morton 1975). Acoustic adaptation has been reported in a variety of bird species, some mammals, crickets, and less commonly in anurans. Two reviews on this topic showed that the AAH can be overestimated even for bird species, where the hypothesis was most extensively studied (Boncoraglio & Saino 2007; Ey & Fisher 2009). AAH testing in anurans is usually weak because of the absence of standardised experimental design and macroevolutionary studies. Few field studies have corroborated AAH in anurans (Ryan et al. 1990; Bosch & De la Riva 2004), and anuran call plasticity in response to environmental characteristics has been reported only twice (Lardner & Lakim 2002; Ziegler et al. 2011). Environmental pressure on anuran call evolution has never been tested in a macroevolutionary approach.

Background noise constitutes a strong pressure on acoustic communication, specially for animals living in noisy habitats, such as waterfalls. In the waterfall habitat, the background noise is constant and may lead to the development of new communication systems, such as visual signalling (for a review, see Hödl & Amézquita 2001) and the use of ultrasonic calls (Feng et al. 2006). Some waterfall-dwelling frogs, independent of their body size, produce advertisement calls with frequencies higher than the waterfall noise frequency (Preininger et al. 2007). Conspecific and heterospecific calls also constitute background noise, interfering with anuran call production and call detection (e.g., Wollerman & Wiley 2002; Wong et al. 2009). Anthropogenic sounds, such as traffic noise from roads, may affect call traits (Hoskin

& Goosem 2010) or the signal active space (Bee & Swanson 2007).

Morphology is a constraint on sound production. In anurans, relationships between body size and peak frequency are widespread (e.g., Zimmerman 1983; Ryan 1988; Gerhardt 1991), with larger males producing calls with lower frequencies. Whereas spectral traits are constrained by morphology, temporal traits often are constrained by behaviour and physiology, such as stridulation and expiratory capacity (Ryan & Kime 2003). Cocroft & Ryan (1995) found that call traits related to morphological traits are more evolutionarily conservative than are call traits related to behavioural or physiological traits. Zimmermann (1983) compared 56 Amazonian species from open areas and forest, and postulated that anuran species occupying open areas would be smaller than forest species, and that body size would be inversely related to call peak frequency. Zimmermann's (1983) findings are in concordance with AAH predictions, because high frequencies degrade faster than low frequencies in densely vegetated areas (Morton 1975). However, her analysis also suggested that body-size constraints on call frequency were more common than habitat effects, and that phylogeny may play a major role in species call diversification.

Also, some variations in traits result from stochastic processes, such as random genetic drift or fluctuating directional evolution, with the direction of selection varying randomly across phylogeny (Ord & Martins 2006). Phylogenetic Comparative Methods (PCMs) have been developed to consider the statistical non-independence of sample units (i.e., the species). Incorporating phylogeny into the analysis allows better understanding of patterns of ecological, physiological and behavioural evolution, and allows stronger tests of hypotheses with an interspecific data set.

Hylidae is the largest anuran family, with 901 described species (Frost 2011). It is cosmopolitan, except for the Arctic and Antarctic regions, and species in the family occur in a wide range of habitats and microhabitats and have a wide range of body sizes (Duellman &

Trueb 1994). Faivovich et al. (2005) re-analysed hylid systematics, and Wiens et al. (2010) presented a new phylogenetic hypothesis, based on more species and using a different tree-construction method. Robillard et al. (2006), using the molecular phylogeny of Faivovich et al. (2005), found a mismatch between call structure and the mechanism of sound production of North American hylid tree-frogs, suggesting that mechanistic traits are more conservative.

In this study, we evaluated the effects of environment and morphology on tree-frog (Hylidae) call evolution, through a macroevolutionary approach based on traditional statistics and PCMs (i.e., phylogenetic independent contrasts, trait-correlated evolution, and Hansen's adaptation test). AAH predicts temporal and spectral adaptation to habitat type, spectral adaptation to calling-site type, and body-size adaptation to habitat but not to calling site. Morphology influence predicts body size effects on call spectral traits.

METHODS

The data set

To test the acoustic-adaptation hypothesis, we composed a data matrix with 76 hylid species, three acoustic traits, two ecological traits, and one morphological trait.

The acoustic data set was created by combining published articles on calls or species descriptions, with call analysis from commercially available audio CDs (Ibáñez et al. 1999; Marty & Gaucher 1999; Read 2000; Cocroft et al. 2001; Haddad et al. 2005). The advertisement call was described based on three continuous traits: call duration, pulse rate (number of pulses per second), and peak frequency (the call frequency value with the highest energy). The number of calls analysed per individual as well as the number of individuals analysed per species varied according to the number of calls recorded or the number of calls analysed in the published descriptions. The temporal and spectral call traits were analysed by LKE, using Raven Pro 1.4 sound analysis software (Charif et al. 2008). Spectra were analysed with the use of a fast Fourier transform size of 2048 points, frequency resolution of 80 Hz, overlap of 80%, and Blackman window.

The ecological matrix was represented by Habitat, with two categories “forest” and “open areas”; and Calling Site, with two categories, “lentic” (calling sites near lentic environments, i.e., quiet environments) and “lotic” (calling sites near lotic environments, i.e., noisy environments). Species that can exploit both habitat types or calling sites were included only in the cases where the recording site was described in the call recordings or descriptions. Whenever possible, the ecological information was obtained directly from the data associated with the call recording. When detailed recording information was not available, the data were collected from alternative literature, and checked against the International Union for Conservation of Nature (IUCN) Red List of threatened species data set (IUCN 2010).

To control for possible pleiotropic effects on variation in call traits, we included a morphological trait, body size, measured as the snout-vent length (SVL). SVL information was obtained from the recording information or call description, or from alternative literature when unavailable from the call source.

Phylogenetic Tree

We compared the phylogenetic hypotheses of Faivovich et al. (2005) and Wiens et al. (2010). Our first composite tree (Fig. 1a) contained 76 species, 74 from the molecular phylogenetic tree developed by Faivovich et al. (2005), and two species, *Hypsiboas curupi* (Garcia et al. 2007) and *Hypsiboas caipora* (Antunes et al. 2008), from the phylogenetic hypothesis for the *Hypsiboas pulchellus* group proposed by Antunes et al. (2008). For the second tree (Fig. 1b), based on the hypotheses of Wiens et al. (2010), we included 73 of the species used in the first tree. For the subset of species included in this study, the tree based on Wiens et al. (2010) differed from that of Faivovich et al. (2005) in the exclusion of three species (*Bokermannohyla pseudopseudis*, *Aplastodiscus erhardti*, *Hypsiboas caipora*), and the positions of *Hypsiboas lanciformis, Hypsiboas rufitelus, Hypsiboas semiguttatus, Hypsiboas curupi, Dendropsophus anceps, Smilisca baudinii*, *Itapotihyla langsdorffii*, *Trachycephalus hadroceps*, and *Osteocephalus leprieurii*; the *Scarthyla-Lysapsus-Pseudis* group as the sister group of the *Dendropsophus* clade; and the *Sphaenorhynchus* clade as the sister group of the *Scinax* clade.

Because the branch-length information was not provided in the published articles (Faivovich et al. 2005; Antunes et al. 2008; Wiens et al. 2010), we set all branch lengths equal to one, and used phylogenetic comparative methods that maximise the fit of data to the tree, simulating different evolutionary scenarios and providing a robust approximation to the branch lengths (see below).

Statistical Analyses

To test if Habitat and Calling Site affect call traits and body size, we performed a Kruskal-Wallis tests with the continuous call traits and body size. The influence of body size on variation in call traits was evaluated by linear regressions. Call duration, peak frequency, pulse rate, and body size were $\log_{10}$ (x+1) transformed to satisfy normality assumptions. The data set used in the standard statistical analyses contained 76 species.

In order to control for the possible effects of the evolutionary history shared between species, we calculated Phylogenetic Independent Contrasts (PIC) (Felsenstein 1985) for all the continuous traits and also for Habitat and Calling Site, using the ape 2.5-3 package (Paradis et al. 2010) in R 2.10.1 (R Development Core Team 2009). To correct for phylogenetic dependency, we analysed the relationship between the contrasts in SVL and the contrasts of all the call traits using linear regressions. The habitat and calling-site effects were tested again, but now controlling for phylogenetic effects, using the calculated PIC in linear regressions. To reinforce the results found and to control for certain methodological pitfalls (e.g., PIC calculated for discrete traits), we performed phylogenetic ANOVAs as proposed by Garland et al. (1993) and implemented in the geiger 1.3-1 package (Harmon et al. 2009) for R 2.10.1 (R Development Core Team 2009). The sample size for these analyses was 66 independent contrasts for the tree of Faivovich et al. (2005), and 65 independent contrasts for the tree based on Wiens et al. (2010).

A Brownian-motion model of trait evolution is assumed in the majority of phylogenetic comparative methods, including the PIC. Nevertheless, in some cases the use of a neutral model of evolution may not be the most appropriate approach, particularly if selection is persistent over time, causing correlated evolutionary changes between phylogenetic branches, and if different taxa are under the same selective regime (Felsenstein 1985). Both cases are expected to occur under natural selection (Butler & King 2004). Hansen (1997) incorporated selection into the model by considering the existence of adaptive optima using the Ornstein-Uhlenbeck process. In the Ornstein-Uhlenbeck model, phenotypes oscillate around an adaptive optimum by random drift, and selection prevents these phenotypes from straying from the optimum, in what is known as the “rubber band” effect. When the selection force is weak, tending to zero, the Ornstein-Uhlenbeck model resembles the Brownian motion model.

We used Hansen's Adaptation Test to test for acoustic adaptation in the family Hylidae to habitat types and calling sites, considering four evolutionary models (Fig. 2). The first was the Brownian Motion (BM) model. Secondly, models for the Ornstein-Uhlenbeck process had one optimum assigned for all species (OU) or with two optima (OU-2). For Habitat, the optima were forest and open areas for the tips and forest for the internal branches, representing the ancestral condition for the family; for Calling Site, the optima of tips were lentic and lotic, of which lentic environments were considered the ancestral condition and were assigned to the internal branches. The fourth model was Ornstein-Uhlenbeck with ancestral reconstruction

(OU-LP), where ancestral reconstruction was carried out for all the internal nodes by linear parsimony (Fig. 2) using the ape 2.5-3 package (Paradis et al. 2010) in R 2.10.1 (R Development Core Team 2009). In the cases where ape 2.5-3 (Paradis et al. 2010) failed to calculate the ancestral nodes, ancestral reconstruction was estimated using Mesquite 2.73 (Maddison & Maddison 2010). Model selection was based on a Likelihood Ratio Test (LR), where each OU-model was tested against the BM-model, Akaike Information Criteria corrected for small sample size (AIC_c) (Burnham & Anderson 2004), and Bayesian Information Criteria (BIC) (Burnham & Anderson 2004), as proposed by Butler & King (2004). The best model was considered that with the lowest value. To choose between models, we calculated the difference between the alternative models, to determine the model with the lowest value, by the formula:

$\Delta_i = AIC_i - AIC_{min}$,

where AIC_i is the AIC_c of the alternative model, and AIC_{min} is the AIC_c of the model with the lowest AIC value. The Δ_i values are shown in a continuum information, and the criteria for model selection are widely discussed (e.g., Burnham & Anderson 2004; Hegyi & Garamszegi 2011; Symonds & Moussalli 2011; Burham et al. 2011). In this study, we will consider models with $\Delta_i \leq 2$ as well supported and the best model, models with $\Delta_i \leq 7$, as having good support, and models with $\Delta_i > 10$ without substantial support. BIC model selection followed a similar ranking criteria (Burnham & Anderson 2004). Therefore, the best model was selected based on the model ranking and on the concordance between the information criteria.

Hansen's adaptation test was implemented in the ouch 2.7-1 package (King & Butler 2009) in R 2.10.1 (R Development Core Team 2009). Sample size equalled 76 species for all call traits and body size; Pulse Rate had a sample size of 63 species.

RESULTS

Habitat Effect

Without considering phylogeny, habitat had an effect on peak frequency (Kruskal-Wallis test, $X^2_1 = 10.99$, $P = 0.001$) and body size (Kruskal-Wallis test, $X^2_1 = 8.56$, $P = 0.003$) (Table 1), where larger males with lower peak frequency calls were found in forested areas.

When phylogeny was controlled by calculating PIC (Table 2), PIC habitat was related only to PIC peak frequency (Faivovich et al. 2005: $F_{1,64} = 7.91$, $R^2 = 0.11$, $P = 0.006$; Wiens et al., 2010: $F_{1,63} = 8.39$, $R^2 = 0.12$, $P = 0.005$). However, the phylogenetic ANOVAs (Table 3) indicated a relationship between habitat and peak frequency (Faivovich et al. 2005: $F_{1,64} = 13.55$, $P = 0.01$; Wiens et al. 2010: $F_{1,63} = 14.21$, $P = 0.009$) and body size (Faivovich et al. 2005: $F_{1,64} = 9.22$, $P = 0.04$; Wiens et al. 2010: $F_{1,63} = 9.58$, $P = 0.04$).

According to Hansen's Adaptation Test for call duration ($\log_{10}$ -transformed), the best evolutionary model was OU, but other models had good support, OU-2 and OU-LP (tree of Faivovich et al. 2005: LR, AIC_c, BIC), OU-2 and OU-LP (tree of Wiens et al. 2010: LR, AIC_c). For peak frequency ($\log_{10}$ -transformed), the best models were OU-LP with the tree of Faivovich et al. (2005), and OU-2 with the tree of Wiens et al. (2010). However, all criteria also indicated that OU-2 was a good model for the tree of Faivovich et al. (2005). For body size ($\log_{10}$ -transformed), the best model followed by the good models for the tree of Faivovich et al. (2005) were OU-LP (LR, AIC_c, BIC), BM and OU (BIC); and for the tree of Wiens et al. (2011), OU-2 (LR, AIC_c, BIC), OU (AIC_c, BIC), and OU-LP (AIC_c). For pulse rate ($\log_{10}$ (x+1)-transformed), the best model, followed by the good models, was OU (LR, AIC_c, BIC), OU-2 (LR, AIC_c), and OU-LP (LR, AIC_c) (Tables 4 and 5).

Calling Site Effect

Calling site influenced pulse rate (Kruskal-Wallis test, $X^2_1 = 8.06$, $P = 0.005$) (Table 1), but had no effect on call duration, peak frequency, or body size (Table 1).

PIC calling site showed no influence on PIC of any call trait or body size (Table 2). However, Phylogenetic ANOVA indicated a relationship between the calling site and pulse rate for the Faivovich et al. (2005) phylogenetic tree ($F_{1,64} = 12.10$, $P = 0.04$) (Table 3).

The test for adaptation to calling site for call duration ($\log_{10}$ -transformed) gave most support to the OU evolutionary model, followed by OU-2 and OU-LP (LR, $\mathrm{AIC_c}$). The best-supported model for peak frequency ($\log_{10}$ -transformed) was OU-2 (LR, $\mathrm{AIC_c}$, BIC), followed by OU ($\mathrm{AIC_c}$, BIC); and, only for the tree of Faivovich et al. (2005), the OU-LP model ($\mathrm{AIC_c}$). For body size ($\log_{10}$ -transformed) considering the tree of Faivovich et al. (2005), the best-supported model was OU (LR, $\mathrm{AIC_c}$, BIC), followed by BM (BIC), OU-2 (LR, $\mathrm{AIC_c}$), and OU-LP (LR, $\mathrm{AIC_c}$); and, considering the tree of Wiens et al. (2010), OU ($\mathrm{AIC_c}$, BIC), followed by OU-2 ($\mathrm{AIC_c}$) and OU-LP (LR, $\mathrm{AIC_c}$). The best-supported model for pulse rate ($\log_{10}$ (x+1)-transformed) was OU, followed by OU-2 and OU-LP (LR, $\mathrm{AIC_c}$) (Tables 6 and 7).

Body Size Effect

The expected inverse relationship between body size and peak frequency was found for the raw data (Faivovich et al. 2005: $F_{1,78} = 35.91$, $R^2 = 0.31$, $P = 6.01\text{e-}08$; Wiens et al. 2010: $F_{1,63} = 38.69$, $R^2 = 0.38$, $P = 4.47\text{e-}08$) (Fig. 3). When controlling for phylogenetic effects by using independent contrasts (Fig.3), an inverse relationship between PIC body size and PIC peak frequency was found (Faivovich et al. 2005: $F_{1,78} = 11.03$, $R^2 = 0.12$, $P = 0.001$; Wiens et al. 2010: $F_{1,63} = 8.84$, $R^2 = 0.12$, $P = 0.004$).

Body size had no effects on the remaining acoustic traits, call duration (raw data: Faivovich et al. 2005: $F_{1,78} = 0.19$, $R^2 = 0.002$, $P = 0.67$; Wiens et al. 2010: $F_{1,63} = 0.05$, $R^2 = 0.0008$, $P = 0.82$; PIC: Faivovich et al. 2005: $F_{1,78} = 0.67$, $R^2 = 0.008$, $P = 0.41$; Wiens et al. 2010: $F_{1,63} = 1.5$, $R^2 = 0.02$, $P = 0.22$) or pulse rate (raw data: Faivovich et al. 2005: $F_{1,78} =$

0.37, $R^2 = 0.005$, $P = 0.54$; Wiens et al. 2010: $F_{1,63} = 0.15$, $R^2 = 0.002$, $P = 0.7$; PIC: Faivovich et al. 2005: $F_{1,78} = 0.31$, $R^2 = 0.004$, $P = 0.58$; Wiens et al. 2010: $F_{1,63} = 0.41$, $R^2 = 0.006$, $P = 0.52$).

Phylogenetic Tree Effect

Similar results were found for the two phylogenetic trees used. Correlated trait evolution, based on PIC values calculated for both trees, was similar. However, the magnitude of effect was lower for the Wiens et al. (2010) phylogenetic hypotheses than for the tree of Faivovich et al. (2005). OU models had the best support for both phylogenetic trees.

DISCUSSION

All the results pointed to an adaptive model of evolution. McCracken & Sheldon (1997) identified ecological and phylogenetic components in avian call traits, based on predictions about call characteristics and environmental structure. Our raw-data analysis indicated that habitat type influenced peak frequency and body size, in agreement with the suggestions of Zimmerman (1983); and calling site influenced pulse rate. However, phylogeny seems to have an important role in anuran call evolution, e.g., a strong phylogenetic signal was found for call traits of dart-poison frogs (Dendrobatidae) (Erdtmann & Amézquita 2009). Goicoechea et al. (2010) suggested that selection is not strong enough to erase the phylogenetic signal in anuran calls. Robillard et al. (2006) found that the North-American tree-frog call structure contains less phylogenetic information than the mechanism that produces the call.

It is important to incorporate phylogenetic information in statistical analyses. Although the strength of the relationship between environment and call traits diminishes or disappears when we include the phylogenetic information in the analyses by using the phylogenetic independent contrasts in linear regression, some habitat and calling site effects were still found when using phylogenetically independent contrasts, and phylogenetic ANOVAs generated results similar to raw data analyses. However, the analyses based on PIC might not be capturing all trait variation, because PIC assumes a Brownian motion model of evolution; and the results from Hansen's adaptation test indicated that the best evolutionary model fitting our data would be a non-Brownian motion model of evolution (OU, OU-2, or OU-LP model).

The relationship between habitat and peak frequency indicates agreement with AAH predictions that lower frequencies will be emitted in forested areas, and higher frequencies in open areas. Nevertheless, there are pleiotropic effects of body size on spectral traits. We found that variation in body size affects variation in peak frequency (considering both raw data and PIC), but there is also a relationship between habitat and body size. Morphological traits are known to constrain call-trait evolution in birds (e.g., bill size: Podos 2001; bill size and body mass: Seddon 2005) and in anurans (Cocroft & Ryan 1995; Robillard et al. 2006; Erdtmann & Amézquita 2009). The Hansen adaptation test identified Brownian motion as a probable evolutionary model only for body size, indicating that this trait could be more conservative than others, as predicted by theory, where, generally, morphological traits are more conservative than behavioural traits (see Blomberg et al. 2003, for a comparison of phylogenetic signal between traits).

We found a relationship between calling site and pulse rate (raw data and phylogenetic ANOVAs), but not the expected relationship with peak frequency. Rapid frequency modulation is not expected in densely forested environments because of reverberations (Richards & Wiley 1980), which can modify the temporal content of the call, and the same principle may apply to lotic calling sites if these consist of many rocks. However, reverberation effects are not always negative; reverberation increases the note tail in the Green Hylia (*Hylia prasina*, Aves: Passeriformes), and males may perceive this information, since they are more responsive to longer note tails (Slabbekoorn et al. 2002).

It was not possible to determine the most appropriate evolutionary model underlying the evolution of call traits, since several of the models tested were good predictors of hylid call evolution. Ornstein-Uhlenbeck models were selected for all call traits, suggesting a stabilising selection or strong directional evolution, with one or two adaptive peaks. In response to habitat types, the best models pointed to the existence of two evolutionary peaks for peak frequency (OU-LP and OU-2) and body size (OU-LP and OU-2). In this case, selection on body size could be driving peak frequency evolution in a pleiotropic way, despite a true adaptation of peak frequency to habitat. Although it was the best-supported model for body size, the BM-model also had a good BIC ranking and cannot be discarded as a possible model underlying body-size evolution. We believe that this morphological trait may have a stronger phylogenetic signal than the call traits studied. Morphological traits usually tend to have stronger phylogenetic signals than behavioural traits (Blomberg et al. 2003; Diniz-Filho & Nabout 2009), a pattern also reported for anurans (Cocroft & Ryan 1995; Robillard et al. 2006). The best evolutionary model for peak frequency in response to calling site (OU-2) suggests the existence of two adaptive optima, but lotic sites had a lower optimum value than lentic sites (data not shown), in disagreement with our prediction about frequency displacement caused by background noise. However, the categorical representation of background noise may hide the real information on intensity of background noise, once slow-flow streams may produce low background noise and may not constitute a selective pressure to communication.

Differences in tree topology (Faivovich et al. 2005 and Wiens et al. 2010) did not modify the major conclusions of this study. However, traits more closely related to phylogeny may be more affected by the topological differences, as was apparent for peak frequency and body size. These traits showed greater differences between trees in the ranking of evolutionary models.

We evaluated environmental effects on hylid call traits, considering both raw data and PCMs. Analyses from raw data, PIC, and phylogenetic ANOVAs showed similar results. Results from Hansen Adaptation tests partially agreed with the results of our other analyses. However, few studies have used this type of analysis, and we have no other study of acoustic signal evolution for comparison. Other PCMs based on the OU-model (e.g., Phylogenetic General Least Squares - PGLS) are available, but implementation of the Hansen test, as proposed by Butler & King (2004), allows tests of complex evolutionary models and estimates strength of drift, strength of selection, and the values for adaptive optima for each model tested. It is a useful method to reveal details of evolutionary models and test hypotheses. Simpler models, i.e., a two-regimen model, can also be used by the Hansen test implemented in Compare 4.6b (Martins 2004).

In summary, habitat type did not affect temporal traits (raw data, PIC, and phylogenetic ANOVAs), but temporal-trait evolution fit an adaptive model of evolution. The evolution of peak frequency is linked to body-size evolution, and may not constitute a true adaptation. We did not find peak frequency displacement, with higher frequencies in lotic sites, but did find a response of body size to habitat type (raw data, PIC, and phylogenetic ANOVAs), so variation in body size may be driving differences in peak frequency. In this study, we provide a first insight into the influence of environment on anuran-call evolution. Further studies should evaluate the potential for adaptation within hylid clades, which would help us to identify in which clades the strength of selection is stronger, and refine our knowledge about anuran-call evolution.

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Table 1. Habitat and calling site effects on call traits and body size. In bold, statistically significant results for Kruskal-Wallis test performed with raw data.

Traits	Habitat		Calling Site	
	X^2	P	X^2	P
Call duration	0.035	0.852	0.164	0.685
Pulse rate	0.814	0.367	**8.063**	**0.005**
Peak frequency	**10.987**	**0.001**	0.823	0.364
Body size	**8.560**	**0.003**	0.123	0.725

Table 2. Linear regressions between independent contrasts (PIC) of Habitat and Calling Site and the PIC of continuous call traits and body size, comparing the results found using both phylogenetic trees, Faivovich *et al*. (2005) and Wiens *et al*. (2010). Statistically significant results, in bold.

Trait X	Trait Y	*F*	d.f.	R^2	*P*
Faivovich *et al*. (2005) tree					
IC.Habitat	IC.Call duration	3.16	64	0.05	0.08
IC.Habitat	IC.Pulse rate	0.006	64	0.0001	0.94
IC.Habitat	**IC.Peak frequency**	**7.91**	**64**	**0.11**	**0.006**
IC.Habitat	IC.SVL	1.83	64	0.03	0.18
IC.Calling site	IC.Call duration	0.87	64	0.01	0.35
IC.Calling site	IC.Pulse rate	0.06	64	0.001	0.80
IC.Calling site	IC.Peak frequency	2.73	64	0.04	0.10
IC.Calling site	IC.SVL	0.13	64	0.002	0.72
Wiens *et al*. (2010) tree					
IC.Habitat	IC.Call duration	2.52	63	0.04	0.12
IC.Habitat	IC.Pulse rate	0.04	63	0.0006	0.85
IC.Habitat	**IC.Peak frequency**	**8.39**	**63**	**0.12**	**0.005**
IC.Habitat	IC.SVL	1.09	63	0.02	0.30
IC.Calling site	IC.Call duration	1.36	63	0.02	0.25
IC.Calling site	IC.Pulse rate	0.31	63	0.005	0.58
IC.Calling site	IC.Peak frequency	1.87	63	0.03	0.18
IC.Calling site	IC.SVL	0.01	63	0.0002	0.91

Table 3. Results from standard ANOVAs and Phylogenetic ANOVAs, comparing both phylogenetic trees used in the analyses. In bold, the statistically significant results at 0.05.

Traits	Habitat			Calling Site			
	F	*P*	Phylogenetic *P*	*F*	*P*	Phylogenetic *P*	d.f.
Faivovich *et al*. (2005)							
Call duration	0.27	0.61	0.76	0.29	0.59	0.78	64
Pulse rate	1.92	0.17	0.37	**12.10**	**0.0009**	**0.04**	64
Peak frequency	**13.55**	**0.0005**	**0.01**	1.39	0.24	0.52	64
Body size	**9.22**	**0.003**	**0.04**	1.64	0.20	0.48	64
Wiens *et al*. (2010)							
Call duration	0.17	0.68	0.78	0.08	0.77	0.88	63
Pulse rate	1.55	0.22	0.41	10.08	0.002	0.08	63
Peak frequency	**14.21**	**0.0004**	**0.009**	1.82	0.18	0.49	63
Body size	**9.58**	**0.003**	**0.04**	2.01	0.16	0.46	63

Table 4. Hansen adaptation test results. Evolutionary models considering Habitat effects and Faivovich *et al*. (2005) phylogenetic tree. In bold, the best model for Likelihood Ratio test (LRT), Akaike Information Criterion (AICc), and Bayesian Information Criterion (BIC).

Call trait	Model	-2logL	d.f.	LRT	*P*	AICc	Δ AICc	BIC	Δ BIC
Log call duration	BM	236.91	2			241.08	21.88	245.57	19.71
	OU	212.86	3	24.05	< 0.0005	**219.20**	0	**225.86**	0
	OU-2	212.63	4	24.28	< 0.0005	221.19	1.99	**225.86**	0
	OU-LP	**212.55**	4	24.36	< 0.0005	221.11	1.91	**225.86**	0
Log peak frequency	BM	106.93	2			111.09	12.01	115.59	7.75
	OU	99.46	3	7.47	< 0.01	105.79	6.71	112.45	4.61
	OU-2	91.67	4	15.26	< 0.0005	100.23	1.15	108.99	1.15
	OU-LP	**90.51**	4	16.42	< 0.0005	**99.08**	0	**107.84**	0
Log body size	BM	61.03	2			65.19	6.74	69.69	2.48
	OU	54.69	3	6.34	< 0.02	61.03	2.58	67.69	0.48
	OU-2	52.76	4	8.27	< 0.02	61.32	2.87	70.08	2.87
	OU-LP	**49.89**	4	11.14	< 0.005	**58.45**	0	**67.21**	0
Log pulse rate	BM	263.19	2			267.39	5.88	271.48	3.95
	OU	255.10	3	8.09	< 0.025	**261.51**	0	**267.53**	0
	OU-2	255.08	4	8.11	< 0.05	263.77	2.26	271.65	4.12
	OU-LP	**254.54**	4	8.65	< 0.05	263.23	1.72	271.11	3.58

Table 5. Hansen adaptation test results. Evolutionary models considering Habitat effects and Wiens *et al.* (2010) phylogenetic tree. In bold, the best model for Likelihood Ratio test (LRT), Akaike Information Criterion (AICc), and Bayesian Information Criterion (BIC).

Call trait	Model	-2logL	d.f.	LRT	*P*	AICc	Δ AICc	BIC	Δ BIC
Log call Duration	BM	228.06	2			232.23	27.79	236.64	25.68
	OU	198.09	3	29.97	< 0.0005	**204.44**	0	**210.96**	0
	OU-2	**198.04**	4	30.02	< 0.0005	206.63	2.19	215.20	4.24
	OU-LP	198.09	4	29.97	< 0.0005	206.68	2.24	215.25	4.29
Log peak frequency	BM	101.28	2			105.45	11.67	109.86	7.5
	OU	93.02	3	8.26	< 0.005	99.37	5.59	105.89	3.53
	OU-2	**85.19**	4	16.09	< 0.0005	**93.78**	0	**102.36**	0
	OU-LP	92.10	4	9.18	< 0.01	100.69	6.91	109.26	6.9
Log body size	BM	60.80	2			64.97	4.79	69.38	2.68
	OU	53.83	3	6.97	< 0.01	**60.18**	0	**66.70**	0
	OU-2	**51.78**	4	9.02	< 0.02	60.37	0.19	68.94	2.24
	OU-LP	53.82	4	6.98	< 0.05	62.41	2.23	70.98	4.28
Log pulse rate	BM	270.83	2			275.03	10.68	279.08	8.76
	OU	257.94	3	12.89	< 0.005	**264.35**	0	**270.32**	0
	OU-2	257.84	4	12.99	< 0.005	266.54	2.19	274.34	4.02
	OU-LP	**257.07**	4	13.76	< 0.005	265.77	1.42	273.58	3.26

Table 6. Hansen adaptation test results. Evolutionary models considering Calling site effects and Faivovich *et al*. (2005) phylogenetic tree. In bold, the best model for Likelihood Ratio test (LRT), Akaike Information Criterion (AICc), and Bayesian Information Criterion (BIC).

Call trait	Model	-2logL	d.f.	LRT	*P*	AICc	Δ AICc	BIC	Δ BIC
Log call duration	BM	236.91	2			241.08	21.88	245.57	19.71
	OU	212.86	3	24.05	< 0.005	**219.20**	0	**225.86**	0
	OU-2	**212.85**	4	24.06	< 0.005	221.41	2.21	230.17	4.31
	OU-LP	212.86	4	24.05	< 0.005	221.43	2.23	230.19	4.33
Log Peak frequency	BM	106.93	2			111.09	6.04	115.59	3.14
	OU	99.46	3	7.47	< 0.01	105.80	0.75	**112.45**	0
	OU-2	**96.49**	4	10.44	< 0.01	**105.05**	0	113.81	1.36
	OU-LP	98.40	4	8.53	< 0.02	106.96	1.91	115.72	3.27
Log body size	BM	61.03	2			65.19	4.16	69.69	2
	OU	54.69	3	6.34	< 0.02	**61.03**	0	**67.69**	0
	OU-2	**54.67**	4	6.36	< 0.05	63.23	2.2	71.99	4.3
	OU-LP	54.69	4	6.34	< 0.05	63.26	2.23	72.02	4.33
Log pulse rate	BM	263.19	2			267.39	5.88	271.48	3.95
	OU	255.10	3	8.09	< 0.02	**261.51**	0	**267.53**	0
	OU-2	**255.05**	4	8.14	< 0.05	263.74	2.23	271.63	4.1
	OU-LP	255.08	4	8.11	< 0.05	263.77	2.26	271.65	4.12

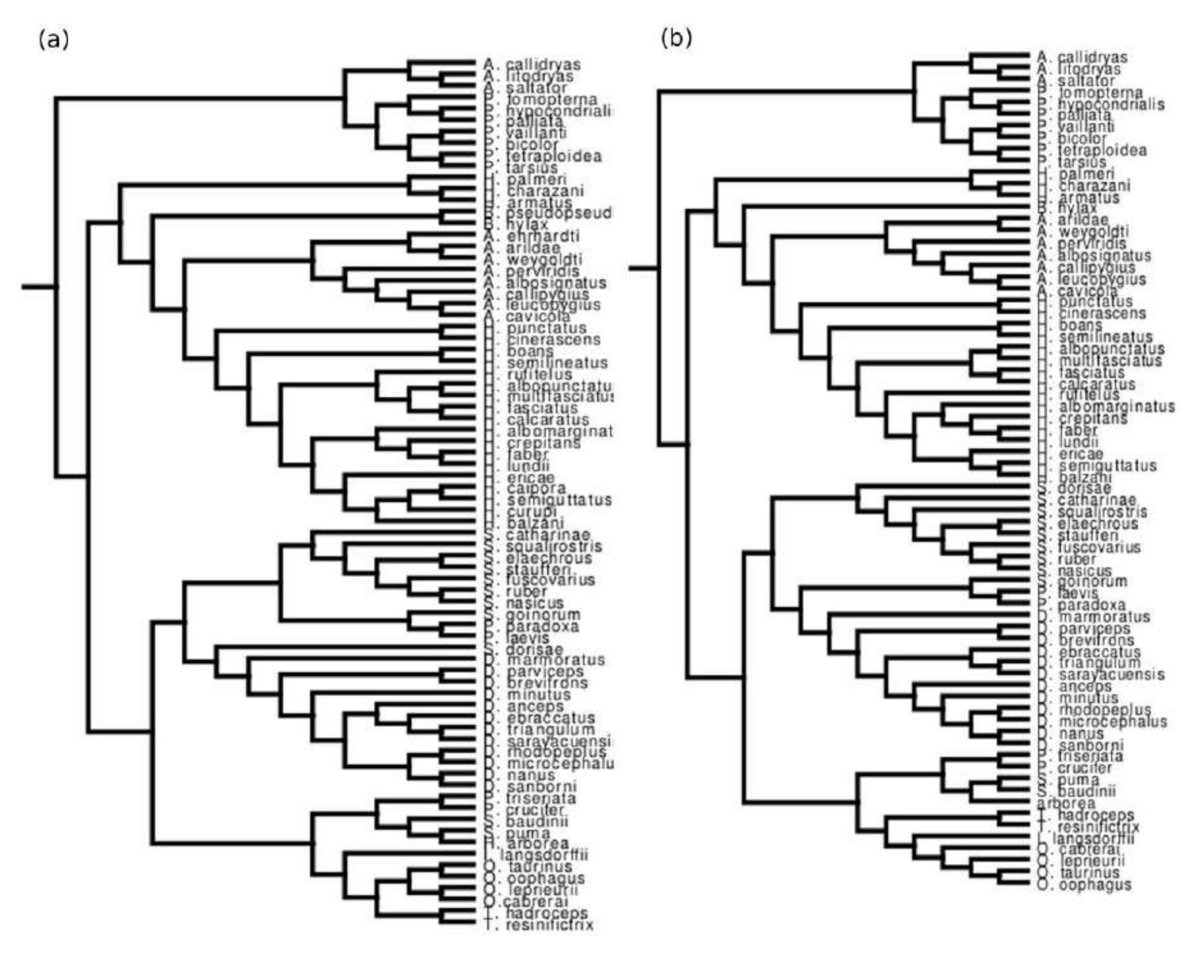
Table 7. Hansen adaptation test results. Evolutionary models considering Calling site effects and Wiens *et al*. (2010) phylogenetic tree. In bold, the best model for Likelihood Ratio test (LRT), Akaike Information Criterion (AICc), and Bayesian Information Criterion (BIC).

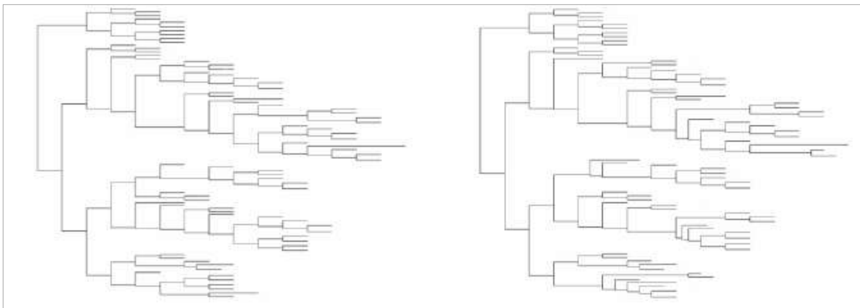
Call trait	Model	-2logL	d.f.	LRT	*P*	AICc	Δ AICc	BIC	Δ BIC
Log call duration	BM	228.06	2			232.23	27.79	236.64	25.68
	OU	198.09	3	29.97	< 0.0005	**204.44**	0	**210.96**	0
	OU-2	197.99	4	30.07	< 0.0005	206.58	2.14	215.15	4.19
	OU-LP	**197.92**	4	30.14	< 0.0005	206.51	2.07	215.08	4.12
Log peak frequency	BM	101.28	2			105.45	7.15	109.86	3.97
	OU	93.02	3	8.26	< 0.005	99.37	1.07	**105.89**	0
	OU-2	**89.71**	4	11.57	< 0.005	**98.30**	0	106.88	0.99
	OU-LP	93.02	4	8.26	< 0.02	101.61	3.31	110.18	4.29
Log body size	BM	60.80	2			64.97	4.79	69.38	2.68
	OU	53.83	3	6.97	< 0.01	**60.18**	0	**66.70**	0
	OU-2	53.82	4	6.98	< 0.05	62.41	2.23	70.98	4.28
	OU-LP	**52.65**	4	8.15	< 0.02	61.24	1.06	69.81	3.11
Log pulse rate	BM	270.83	2			275.03	10.68	279.08	8.76
	OU	257.94	3	12.89	< 0.005	**264.35**	0	**270.32**	0
	OU-2	**257.54**	4	13.29	< 0.005	266.24	1.89	274.05	3.73
	OU-LP	257.91	4	12.92	< 0.005	266.61	2.26	274.42	4.1

Figure 1. Hylidae phylogenetic trees used in this study, (a) based on Faivovich et al. (2005), with 76 species, and (b) based on Wiens et al. (2010), with 73 species.

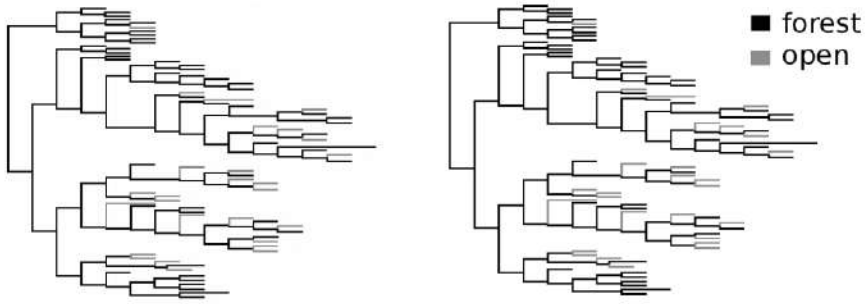
Figure 2. Evolutionary models tested considering, at left, Faivovich et al. (2005) tree, and at right, Wiens et al. (2010) tree. (a) Brownian motion and Ornstein-Uhlenbek (OU) with a single optimum, (b) OU-2, with two optima for habitat, (c) OU-LP, with linear parsimony reconstruction and two optima for habitat, (d) OU-2, with two optima for calling site, and (e) OU-LP, with linear parsimony reconstruction and two optima for calling site. The branch lengths are scaled, the distance from tips to root is one.

Figure 3. Linear regressions of body size (SVL) and peak frequency, considering (a) the data set related to the Faivovich et al. (2005) tree and (c) the Wiens et al. (2010) tree. And, relationship between phylogenetic independent contrasts (PIC) of body size (SVL) and PIC of peak frequency for (b) tree of Faivovich et al. (2005), and (d) tree of Wiens et al. (2010).

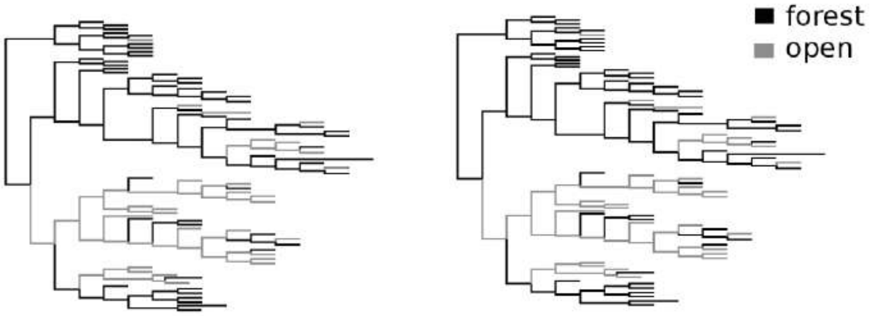
(a)
A. callidryas
A. litodryas
A. saltator
P. tomopterna
P. hypocondriali
P. palliata
P. vaillanti
P. bicolor
P. tetraploidea
P. tarsius
H. palmeri
H. charazani
H. armatus
B. pseudopseud
B. hylax
A. ehrhardti
A. arildae
A. weygoldti
A. perviridis
A. albosignatus
A. callipygius
A. leucopygius
A. cavicola
H. punctatus
H. cinerascens
H. boans
H. semilineatus
H. rufitelus
H. albopunctatu
H. multifasciatu
H. fasciatus
H. calcaratus
H. albomarginat
H. crepitans
H. faber
H. lundii
H. ericae
H. caipora
H. semiguttatus
H. curupi
H. balzani
S. catharinae
S. squalirostris
S. elaechrous
S. staufferi
S. fuscovarius
S. ruber
S. nasicus
S. goinorum
P. paradoxa
P. laevis
S. dorisae
D. marmoratus
D. parviceps
D. brevifrons
D. minutus
D. anceps
D. ebraccatus
D. triangulum
D. sarayacuensi
D. rhodopeplus
D. microcephalu
D. nanus
D. sanborni
P. triseriata
P. crucifer
S. baudinii
S. puma
H. arborea
L. langsdorffii
O. taurinus
O. oophagus
O. leprieurii
O.cabrerai
T. hadroceps
T. resinifictrix
(b)
A. callidryas
A. litodryas
A. saltator
P. tomopterna
P. hypocondrialis
P. palliata
P. vaillanti
P. bicolor
P. tetraploidea
P. tarsius
H. palmeri
H. charazani
H. armatus
B. hylax
A. arildae
A. weygoldti
A. perviridis
A. albosignatus
A. callipygius
A. leucopygius
A. cavicola
H. punctatus
H. cinerascens
H. boans
H. semilineatus
H. albopunctatus
H. multifasciatus
H. fasciatus
H. calcaratus
H. rufitelus
H. albomarginatus
H. crepitans
H. faber
H. lundii
H. ericae
H. semiguttatus
H. balzani
S. dorisae
S. catharinae
S. squalirostris
S. elaechrous
S. staufferi
S. fuscovarius
S. ruber
S. nasicus
S. goinorum
P. laevis
P. paradoxa
D. marmoratus
D. parviceps
D. brevifrons
D. ebraccatus
D. triangulum
D. sarayacuensis
D. anceps
D. minutus
D. rhodopeplus
D. microcephalus
D. nanus
D. sanborni
P. triseriata
P. crucifer
S. puma
S. baudinii
arborea
T. hadroceps
T. resinifictrix
L. langsdorffii
O. cabrerai
O. leprieurii
O. taurinus
O. oophagus



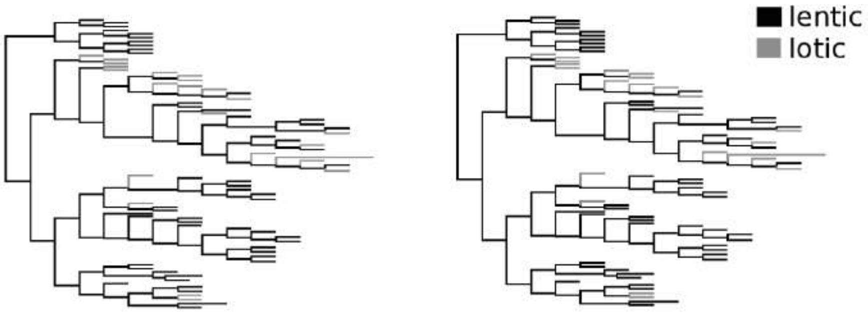
(b) OU - 2: habitat



(c) OU - LP: habitat



(d) OU - 2: calling site



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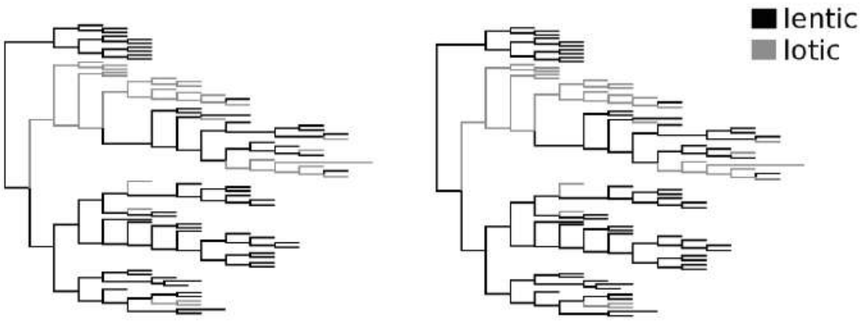

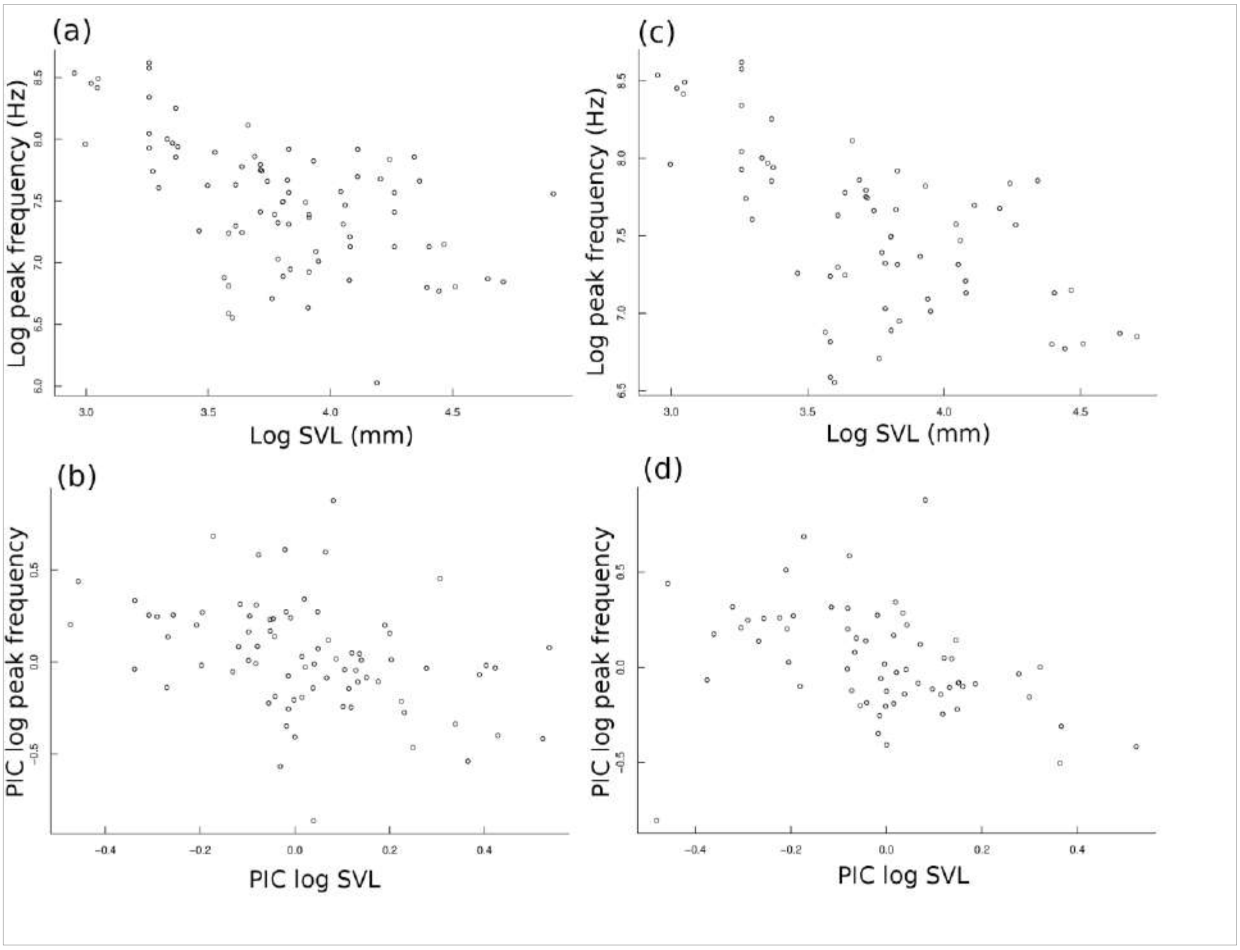
(a)
Log peak frequency (Hz)
Log SVL (mm)
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6.5
7.0
7.5
8.0
8.5
3.0
3.5
4.0
4.5
(b)
PIC log peak frequency
PIC log SVL
-0.5
0.0
0.5
-0.4
-0.2
0.0
0.2
0.4
(c)
Log peak frequency (Hz)
Log SVL (mm)
6.5
7.0
7.5
8.0
8.5
3.0
3.5
4.0
4.5
(d)
PIC log peak frequency
PIC log SVL
-0.5
0.0
0.5
-0.4
-0.2
0.0
0.2
0.4

CAPÍTULO 3

Erdtmann, L.K.; Simões, P.I.; Mello, A.C.; Lima, A.P. 2011. Do natural differences in acoustic signals really interfere in conspecific recognition in the pan-Amazonian frog *Allobates femoralis*? *Behaviour*, 148(4): 485-500.

Do natural differences in acoustic signals really interfere in conspecific recognition in the pan-Amazonian frog *Allobates femoralis*?

Short title: Conspecific recognition in a pan-Amazonian frog

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Summary

The call of the pan-Amazonian frog *Allobates femoralis* shows wide geographical variation, and males show a stereotyped and conspicuous phonotactic response to playback of conspecific calls. We evaluated the capacity of males of *A. femoralis* and a closely related species *A. hodli* to respond aggressively to natural conspecific and heterospecific calls varying in numbers of notes, by means of field playback experiments performed at two sites in the Brazilian Amazon. The first site, Cachoeira do Jirau (Porto Velho, Rondônia), is a parapatric contact zone between *A. femoralis* that use 4-note calls, and *A. hodli* with 2-note calls, where we performed cross-playbacks in both focal populations. The second site, the Reserva Florestal Adolpho Ducke (Manaus, Amazonas), contained only *A. femoralis* with 4-note calls. There, we broadcast natural stimuli of 2-note *A. hodli*, 3-note and 4-note *A. femoralis*, and 6-note *A. myersi*. We found that the phonotactic behavior of *A. femoralis* and *A. hodli* males did not differ toward conspecific and heterospecific stimuli, even in parapatry. Our results indicated that the evolutionary rates of call design and call perception are different, because the geographical variation in calls was not accompanied by variation in the males' aggressive behavior.

Keywords: species recognition, contact zone, playback, Dendrobatoidea, Anura.

Introduction

Advertisement calls are conspicuous acoustic signals emitted by males of most species of anurans. These calls generally have multiple purposes, such as female attraction and territory defense against conspecific males (Duellman & Trueb, 1994), and informing about a male's physical condition, identity, and location (Wells & Schwartz, 2007). Species recognition is especially important for species in which heterospecific mating is very costly (Panhuis et al., 2001), and for territorial males in order to avoid misidentification of competitors, thus saving energy and time (Gerhardt, 1999; Lemmon, 2009; Bernal et al., 2009). The geographical variation in advertisement calls between allopatric populations or sister-species may arise as a result of genetic drift, or from selective pressures exclusive to the geographical range occupied by each population (Littlejohn, 1988; Coyne & Orr, 2004). The occurrence of closely related species in sympatry may facilitate the divergence in signals and signal preferences between and within species (Gerhardt, 1999; Lemmon, 2009), favoring species-recognition traits over mate-quality traits, i.e., by character displacement.

The pan-Amazonian frog *Allobates femoralis* (Boulenger, 1884) is widely distributed throughout the Amazon non-flooded forests, is diurnal, and males defend a multi-purpose territory (Roithmair, 1992). Previous studies have shown males of *A. femoralis* defending territories for up to 90 days in a Peruvian Amazon population (Roithmair, 1992), and for more than 79 days in a population in French Guyana (Ringler et al., 2009). Geographical variation in number of notes in the advertisement call is known for *A. femoralis* (Hödl et al., 2004; Amézquita et al., 2005; Amézquita et al., 2006). Calls with 1 note are reported from the Parque Nacional Yasuní in Ecuador (Read, 2000), and along the Rio Juruá in Brazil (Simões & Lima, unpublished data); 3-note calls are known from the Panguana station in Peru (Hödl et al., 2004; Amézquita et al., 2006). Four-note calls are geographically widespread, being described from localities in the central Brazilian Amazon, the Rio Madeira basin, Colombia, and French Guyana (Hödl et al., 2004; Amézquita et al., 2006; Simões et al., 2008; Amézquita et al., 2009). A related species, *Allobates myersi* (Pyburn, 1981), has its known distribution restricted to the Colombian Amazon (Lötters et al., 2007; Frost, 2010), and was recently found in the municipaliy of São Gabriel da Cachoeira in the upper Rio Negro, state of Amazonas, Brazil (P. Simões & A. Lima, personal observations). Its advertisement call is very similar to that of *A. femoralis*, but is composed by a mean number of 6 notes (unpublished data) (Figure 1). A 2-note-call population formerly referred to as *A. femoralis*, which recently received species status (*Allobates hodli* Simões, Lima & Farias, 2010), occurs on the left bank of the upper Rio Madeira, and the southeastern part of the state of Acre, Brazil (Simões et al., 2010). On the left bank of the upper course of the Rio Madeira, a 2-note *A. hodli* population encounters a 4-note *A. femoralis* population at a narrow contact zone (Simões et al., 2008; Simões et al., 2010).

Exposure to conspecific calls above 68 dB sound pressure level (re 20 µPa) elicits phonotactic behavior in *A. femoralis* males, which consist of stopping calling, head and body orientation, and approaching the broadcasting loudspeaker (Narins et al., 2003). The recognition mechanism in *A. femoralis* has been studied in field experiments using synthetic calls directed to males, each time isolating the acoustic trait to be tested. For example, the number of notes and call peak frequency (Amézquita et al., 2005), variations in the frequency modulation of notes (Hödl et al., 2004), and the duration of the silent interval between notes (Göd et al., 2007) have been analyzed previously. The probability of response of *A. femoralis* males matched the main frequency value for a Colombian 4-note-call population, but also matched the range of frequency variation of heterospecific advertisement calls, indicating that the peak frequency alone was not sufficient for males to discriminate between conspecific and heterospecific calls (Amézquita et al., 2005). The changing of the typical ascending-frequency modulation in the advertisement calls of *A. femoralis* to a descending-frequency modulation or to an unmodulated call causes no differences in the response patterns of *A. femoralis* males (Hödl et al., 2004). Males of *A. femoralis* can recognize advertisement calls with a silent interval between notes that varyies up to 60% of the population mean value (Göd et al., 2007). Previous studies were not able to determine a particular acoustic trait that elicits accurate species recognition, and demonstrated that not all stereotyped traits are relevant for this purpose. Probably there is no single acoustic trait responsible for species recognition, which is, rather, achieved by the interaction or summing of the distinctive characteristics of multiple traits (Gerhardt & Huber, 2002; Göd et al., 2007).

When considering the variation in the number of notes per call (2-4 notes), differences in the recognition curves toward 2-note calls were found, and it was suggested that this differential response could indicate local adaptation to another species' calls or a reaction to a number of notes that is not typical of the focal population (Amézquita et al., 2005). In this study, we evaluated the male recognition capacity of two different populations of *A. femoralis* and one of *A. hodli*, toward natural conspecific and heterospecific advertisement calls, which have a notable geographical variation in the number of notes, from 2 to 6 notes per call. We expected that the remarkable differences in the advertisement calls would elicit more accurate call recognition, also meaning species or population recognition.

Material and methods

To test the effect of natural variation in advertisement calls, here represented as the variation in the number of notes constituting each call (acoustical analysis revealed that the observed variation in call traits between populations and species is related to the number of notes per call, Table 1), on the phonotactic behavior of *Allobates femoralis* males, we recorded advertisement calls in three distinct populations referred to as *A. femoralis*, in one population of *Allobates hodli* from the southwestern Brazilian Amazon (Simões et al., 2010), and in one population of *Allobates myersi*, a species taxonomically related to *A. femoralis* (Pyburn, 1981; Grant et al., 2006), found in the northwestern Brazilian Amazon (Figure 1). The localities sampled for advertisement calls cover most of the known acoustical variation in number of notes for the species (Amézquita et al., 2006, 2009). Across its distribution, *A. femoralis* shows high levels of genetic differentiation, and it is possible that phenotypic or genetically divergent groups will be assigned species status in the future (Grant et al., 2006; Santos et al., 2009). For purposes of taxonomic consistency, we treat all source and test populations (except the *A. myersi* and *A. hodli* populations) as *A. femoralis.*

Our data set included recordings from 14 individuals of *A. hodli*, which produces a 2-note advertisement call, and 14 individuals of a 4-note advertisement call population found at the extremes of an interpopulation contact zone at Cachoeira do Jirau (Jirau), Rondônia, Brazil (9.3206º S, 64.7225º W). This parapatric contact zone is located on the left bank of the Madeira River and coincides with the boundary between geomorphological units (Simões et al., 2008). To avoid sampling calls of hybrid individuals, males were recorded at least 1.2 km upstream and downstream from the area where the occurrence of acoustic morphotypes overlaps. Recordings were obtained from November 2004 to January 2005 by P. I. Simões and A. P. Lima.

Ten individuals from another 4-note call population were recorded at the Reserva Florestal Adolpho Ducke (Ducke), in Manaus, Brazil (2.9167º S, 59.9833º W) by A. P. Lima and L. K. Erdtmann in 2002 and 2008. Ten recordings of a 3-note advertisement call population used in this study were obtained by Adolfo Amézquita in 2002, at Panguana Station in Peru (9.6137° S, 74.9355°W). To obtain *A. myersi* calls, ten individuals were recorded at São Gabriel da Cachoeira, Amazonas, Brazil (0.1558° S, 67.0861° W), by A. P.

Lima and P. I. Simões in May 2008. Advertisement calls of *A. myersi* used in this study were formed by a first trill of four notes followed by one or more consecutive note pairs, or couplets. The addition of one couplet was the most common call structure, totaling six notes similar to those of *A. femoralis* populations in terms of frequency range and modulation (Table 1). Thus, *A. myersi* recordings were termed a 6-note advertisement call stimulus in the experiments described below.

All recordings were made with a Sony WM D6C (Sony Corp., Japan) cassette tape recorder or a Marantz PMD 660 digital recorder (DM Professional, U.S.A.), and AKG D5 (AKG Acoustics GMBH, Austria) or Sennheiser K6/ME66 (Sennheiser Electronic Corporation, U.S.A.) directional microphones. Cassette tape recordings were digitized at 22 kHz using the software Raven 1.2 (Charif et al., 2004). Digital recordings were made at 44 kHz and 16-bit resolution, and analyzed using Raven 1.2 (Charif et al., 2004).

The recordings were used as natural stimuli for field playback experiments performed with three focal populations: the 2-note and 4-note advertisement call populations at Jirau, and the 4-note advertisement call population at Ducke. The populations at Jirau were tested in two short field trips during 11 days in January 2008 and seven days in February 2009. The experiments at Ducke were done weekly from December 2008 to March 2009. The focal populations at Jirau and Ducke are separated by at least 1000 km across the Madeira-Purus interfluve and by the Amazon River, at the southern end of Manaus. Whereas Jirau represents the contact zone between two very distinct populations, the 4-note call population at Ducke is highly allopatric in relation to other *A. femoralis* acoustic phenotypes.

Each stimulus represents a different individual, and all stimuli were edited to control for the number of calls emitted in each calling bout and the duration of silent intervals between calling bouts, using Raven 1.2 (Charif et al., 2004). A calling bout was constituted by 20 calls followed by a 20-s silent interval. In order to construct each stimulus, we used calls from the core of the original calling bout recorded, thus avoiding warm-up and final calls, which generally show wider frequency variation (Gerhardt & Huber, 2002). The number of calls and duration of silent intervals were approximated based on available recordings of complete call bouts and silent intervals for the source populations. Two Ducke recordings that contained excessive background noise were edited further. We used Audacity 1.3.4-beta (Audacity Team, 2008) to filter for frequencies below 300 Hz, and the resulting filtered recordings were used in the playback experiments. We did not control stimuli for any additional acoustic trait, because these represent natural recordings, including the within- and between-individual call variation observed in the source populations.

The design of playback experiments varied between study sites to explore the simpatry and allopatry condition. At Jirau, 2-note and 4-note stimuli recorded upstream and downstream from the contact zone were broadcast to 2-note- and 4-note-call focal males in the core area of the parapatric contact zone. At Ducke, 4-note-call focal males were tested with stimuli constructed from recordings of 2-note calls from the Jirau population, 3-note calls from the Panguana population, 6-note calls of *A. myersi*, as well as 4-note calls from males of the same population.

Focal males were located through their advertisement calls. Once spotted by one of the researchers, their initial position was marked with a small wooden stick. The playback experiment started when the focal male resumed calling activity. Stimuli were broadcast with a G-flash wma-mp3 player (Maxfield, Germany) connected to Sony SRS-M30 battery-powered loudspeakers (Sony Corp., Thailand), positioned 1.5 m from the focal male. To avoid pseudoreplication, each focal male was tested once. In addition to flagging the initial position of males, differences in lateral lines and patterning of flash marks on the thighs allowed us to discriminate between tested and untested individuals. During the recording of stimuli, the average air temperature at calling sites of recorded males was of 27.3°C ± 1.5°C (23.5 –

29.8°C) at Jirau, and of 26°C ± 1.26°C (23 – 30°C) at Ducke. As air temperatures did not significantly oscillate among recording sessions at each locality or between localities, we did not control for air temperature at the time of playback experiments.

At Jirau, differences in male phonotactic behavior elicited by playbacks of two classes of stimuli (calls from the same population *versus* calls from the alternate population) were measured as the relative number of males that approached the loudspeakers. We considered that a male approached the loudspeakers when the individual advanced until it reached a 30-cm radius around the loudspeakers. To test for differences between intra- and interspecific recognition patterns, we performed a Fisher's exact test considering the number of approaches to the loudspeakers in intraspecific and interspecific categories. Additionally, we measured latency to the first movement (here considered the time elapsed from the start of the experiment to the first orientation, jumping, or antiphonal calling movement), and latency to approach (here considered as the time elapsed between the start of the experiment and the male approaching the loudspeakers). The existence of differences in behavioral responses between different classes of stimuli was tested with the non-parametric Kruskal-Wallis test. The experiment ended when the focal male approached the loudspeakers or, alternatively, five minutes after the start of the experiment if males did not respond to the playback. Because only a few individuals used antiphonal calls as a response to acoustic stimuli, we did not include the number of antiphonal calls in any of the tests regarding the Jirau and Ducke populations.

At Ducke, the phonotactic response to the different classes of stimuli tested (two, three, four, and six-note calls) was measured as latency to orientation, latency to jump, latency to approach, and number of antiphonal calls produced by focal males during the experiment. The experiments ended when the focal male approached a 30-cm radius around the loudspeakers. In order to control for male motivational state, always when a male did not approach the loudspeakers, we performed a control period, with the broadcast of a natural recording from the Ducke population, subsequent to the stimulus period. After the experiment, the distance between the loudspeakers and the initial focal-male position was confirmed, and the Sound Pressure Level (SPL) at the initial focal-male position was measured in dB with a Voltcraft SL-100 sound-level meter (re 20 µPa, peak intensity, fast time, resolution of 0.1 dB). At Jirau, the distance between the focal male and the loudspeakers (1.5 m) was measured prior to the start of the experiment, and stimuli intensity was controlled by previously fixing the loudspeakers and wma-mp3 player volume controls at around 74 dB. The existence of differences in phonotactic response in relation to stimuli classes at Ducke was tested using the non-parametric Kruskal-Wallis test, considering stimuli intensity measured after the experiment and the distance from the focal male to the loudspeakers as covariables. Note that the variable 'latency to movement' measured at Jirau and 'latency to orientation' measured at Ducke, can be considered as homologous traits, because at Ducke the first movement in all experiments except one was the orientation. All the statistical analyses were implemented and performed in R 2.9.1 (R Development Core Team, 2009).

The number of playbacks was defined based on previous studies which found significant results with similar number of experiments (e.g., 9 – 15 experiments in Narins et al., 2003; 14 positive and/or negative results experiments in Hödl et al., 2004; 10 males tested in Luna et al., 2010). In this way, the total number of experiments executed in this study was 10 tested males in 2-note population at Jirau, five of them were presented with 4-note stimuli and five with 2-note stimuli, and 18 males were tested in the 4-note population, nine of them presented with 4-note stimuli and nine with 2-note stimuli. At Ducke, we tested 37 males, 10 males per class of stimulus (2-note, 3-note and 6-note), and 7 males to the control (4-note calls from Ducke).

Results

We found no difference between the phonotactic behavior of males from the two tested *A. femoralis* populations and one *A. hodli* population and the natural calls from different populations of *A. femoralis* and *A. myersi*. At Cachoeira do Jirau, 2-note and 4-note males showed similar phonotactic responses to calls from their own population and calls from the parapatric population (Fisher's exact test $p = 0.45$, $N = 14$), considering either the latency to movement ($\chi^2 = 1.11$, $p = 0.77$, $N = 28$, 10 males tested in 2-note population and 18 males tested in 4-note population) or the latency to approach ($\chi^2 = 2.66$, $p = 0.45$, $N = 15$, 10 males tested in 2-note population and 18 males tested in 4-note population) (Figure 2).

At Ducke, the male response patterns did not differ between the 2-note, 3-note, and 6-note stimuli and the control (4-note calls from Ducke) for latency to orientation ($\chi^2 = 19.25$, $p = 0.74$, $N = 37$), latency to jump ($\chi^2 = 17.73$, $p = 0.82$, $N = 37$), and latency to approach ($\chi^2 = 25.01$, $p = 0.40$, $N = 33$), these analyses included 10 males per stimulus class and seven for the control (Figure 3).

At Jirau, 28 experiments were performed. In all of them, the males presented any behavioral change, orientation or jump, from the initial behavior, just calling.13 resulted in males that did not approach the loudspeakers, and of these, five showed antiphonal behavior (emission of advertisement calls from its own population) in response to the broadcast stimulus, four of them belonged to a 2-note population, and called in response to 2-note ($N = 2$) and 4-note stimuli ($N = 2$). The number of antiphonal calls varied from 35 to 100 during the playback experiments. At Ducke, we performed 37 playback experiments, 10 for each type of stimulus and 7 for the control. Four males did not approach the loudspeakers during the five minutes of stimulus playback (two for 6-note, one for 2-note, and one for 3-note call stimulus), however all these males approached the loudspeakers during the subsequent control period. Only one male was recorded as having antiphonal behavior; it did not approach the loudspeakers but emitted 56 calls during the 6-note stimulus playback. Another calling male was observed, but it approached the loudspeakers and emitted only five calls during the stimulus (3-note advertisement call) period.

Discussion

We found that males of the Amazonian frog *A. femoralis* showed similar phonotactic behavior toward natural stimuli from conspecific and heterospecifc populations, varying mainly in the number of notes emitted per call. This result suggests that evolutionary rates are different for signal production and male signal perception, because the geographical variation in the advertisement call was not related to a concomitant variation in signal recognition.

The variation in the advertisement calls of *A. femoralis* has been attributed, at least in part, to stochastic processes, although not excluding the hypothesis that selective effects could act on some call traits, generating the remaining call variation (Amézquita et al., 2009). Regarding the evolutionary mechanism underlying call selection in *A. femoralis,* Grether et al. (2009) proposed that the best model is the divergent Agonistic Character Displacement (ACD), based on the sympatry with *Amereega trivittata* (see Amézquita et al., 2006). However, our results for species that are more closely related to *A. femoralis* do not support the ACD hypothesis. The recognition of heterospecific and conspecific calls suggests that the advertisement calls of *A. femoralis* and *A. hodli* have little effect on the male species recognition mechanism. A similar pattern is observed among males of the field cricket *Gryllus texensis*, which courted equally conspecific and heterospecific females in sympatry and allopatry (Gray, 2004). Although females of *G. texensis* and males and females of *G. rubens* preferred conspecifics, again there were no differences between the response pattern in sympatry and allopatry (Gray, 2004). It has been postulated that heterospecific competition in *A. femoralis* is regulated by the competition for acoustic space and oviposition sites (Göd et al, 2007), once the reproductive success in this species is related to the size and ownership of one territory (Roithmair, 1992). At Jirau, the simpatric species, *A. hodli*, presents a similar ecology and use of space of *A. femoralis*. However, the only contact zone known for this species-group is located at Jirau. At Reserva Ducke, *A. femoralis* males are not sympatric to any closely-related species with similar ecology.

Signal detection and perception can vary according to the receiving gender, because the associated cost of an error in recognition can differ between the sexes (Searcy & Brenowitz, 1988). According to this theory, females would show a more accurate response to signals than would males (Searcy & Brenowitz, 1988; Bernal et al., 2007; but see Espinedo et al., 2010). Our experiments were performed only with males of *A. femoralis*, because this species has a very complex courtship behavior, where the female can remain in a male's territory for two, and up to four days before mating (Roithmair, 1994). After that, the male guides the female through his territory for at least 1 day before oviposition (Montanarin et al., 2010). In contrast to males, *A. femoralis* females do not exhibit a stereotyped phonotaxis behavior, restricting the utility of simple playback experiments in tests of sex differences in call recognition. Field observations provide support to the development of experimental designs that are more appropriate for tests of female *A. femoralis* recognition, as well as possible selection between conspecific and heterospecific signals. These questions remain unclear, and deserve more attention in order to establish the relative role of sexual selection in speciation within this species group.

The acoustic environment to which an individual is exposed includes much more than its own species' calls, and the individual must be able to identify conspecific calls, predators, heterospecifics, and any potential danger (Dall et al., 2005). The ability to detect and discriminate between distinct environmental sounds can be evolutionarily advantageous (Hödl et al., 2004). A good example is the European smooth newt *Lissotriton vulgaris*, which may access habitat-quality information during migration by the heterospecific calling of the common toad *Bufo bufo* (Pupin et al., 2007). In this sense, the recognition space will be as wide as the amount and variety of sounds found in the local environment, constrained by the species' physiology and neurology.

Although often proposed as a key trait promoting or reinforcing species isolation and diversification in contact zones between related taxa (e.g., Höbel & Gerhardt, 2003; Hoskin et al., 2005; Lemmon, 2009), the role of acoustic signals in impairing the recognition of interspecific individuals as conspecifics may be overestimated. Our results suggest that advertisement call variation can contribute to the behavioral reproductive isolation between closely related species, without necessarily being reflected in the tuning of aggressive response toward more similar acoustic signals, even when the defense of food and reproductive resources within a male's territory is at stake. Although the stochastic process may be related to call evolution, the pattern of recognition of sound cues by *A. femoralis* and *A. hodli* males did not concord with the call evolution observed within the *A. femoralis* species group. Identifying the precise role of each acoustic trait in the long-range and short-range communication between *A. femoralis* males and, importantly, between males and females, is a key step that will help us to disentangle patterns of advertisement-call evolution from the evolution of call perception, providing valuable insights into the evolution of this communication.

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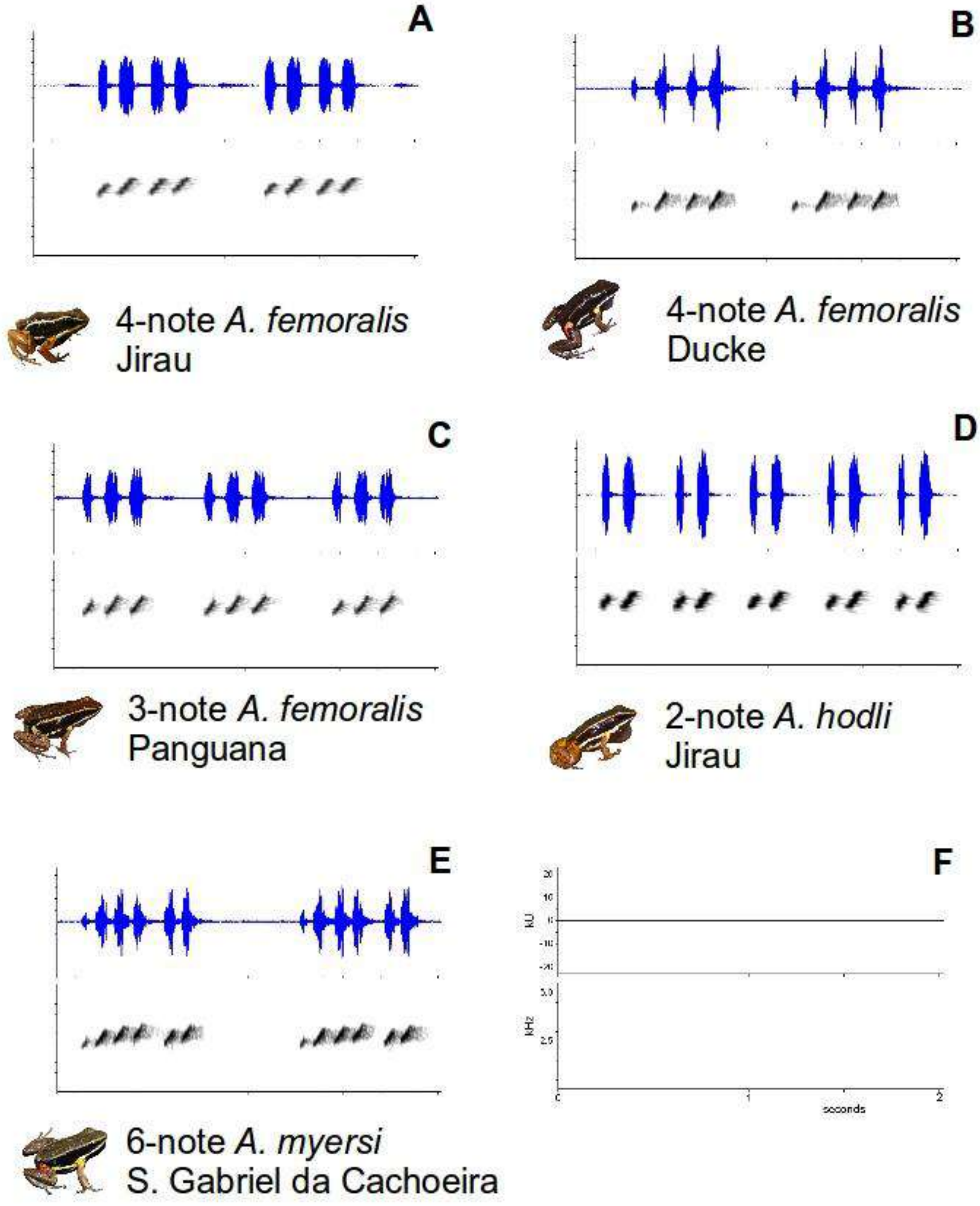
Table 1. Acoustic traits of the natural stimuli used in playback experiments at Jirau and Ducke sites. Each trait is represented by the mean ± standard deviation, and the minimum and maximum values. The advertisement calls were analysed in Raven 1.2 using Blackmann window, 80% overlapping, and a fast Fourier transform of frequency resolution of 80 Hz and 2048 points. Note that call duration and intercall interval, but not peak frequency, increases with the number of notes per call.

Population	Number of Notes	Call Duration (s)	Intercall Interval (s)	Peak Frequency (Hz)
A. hodli Jirau	2	0.16 ± 0.011 (0.14 – 0.18)	0.22 ± 0.032 (0.17 – 0.28)	3428.6 ± 125.2 (3149.2 – 3576.3)
A. femoralis Panguana	3	0.33 ± 0.029 (0.29 – 0.42)	0.37 ± 0.033 (0.32 – 0.43)	3238.7 ± 273.9 (2853.1 – 3707.3)
A. femoralis Jirau	4	0.49 ± 0.024 (0.46 – 0.54)	0.44 ± 0.049 (0.36 – 0.54)	3435.6 ± 162.8 (3075.6 – 3730.6)
A. femoralis Ducke	4	0.53 ± 0.03 (0.49 – 0.57)	0.51 ± 0.08 (0.43 – 0.65)	2865.4 ± 218.2 (2548.1 – 3222.8)
A. myersi São Gabriel da Cachoeira	6	0.61 ± 0.042 (0.53 – 0.67)	0.79 ± 0.109 (0.64 – 0.93)	2859.4 ± 138.2 (2662.9 – 3078.1)

Figure 1. Waveforms and spectrograms of stimuli used in the playback experiments, including the three focal populations. (A) 4-note advertisement calls of *Allobates femoralis* from Jirau, (B) 4-note calls of *A. femoralis* from Ducke, (C) 3-note calls of *A. femoralis* from Panguana, (D) 2-note calls of *A. hodli* from Jirau, and (E) 6-note calls of *A. myersi* from São Gabriel da Cachoeira. In (F) the scale of amplitude (kU), frequency (kHz), and time (s) for these acoustic graphs.

Figure 2. Latency of response of *A. femoralis* and *A. hodli* to natural conspecific and heterospecific stimuli in field cross-playback experiments at Jirau. (A) Latency to movement, and (B) latency to approach the loudspeakers, where 2-2 represents the response of *A. hodli* males when presented with its own calls, 2-4 the response of *A. hodli* males when presented with the parapatric *A. femoralis* calls, 4-4 that of *A. femoralis* males presented with *A. femoralis* calls, and 4-2 that of *A. femoralis* males presented with *A. hodli* calls.

Figure 3. Latency of reaction of *A. femoralis* males to natural conspecific and heterospecific stimuli in field playback experiments at the Ducke field station. (A) Latency to orientation, (B) latency to jump, and (C) latency to approach the loudspeakers, toward advertisement calls of 2-note *A. hodli*, 3-note *A. femoralis*, 6-note *A. myersi*, and the control call, 4-note Ducke's *A. femoralis.*



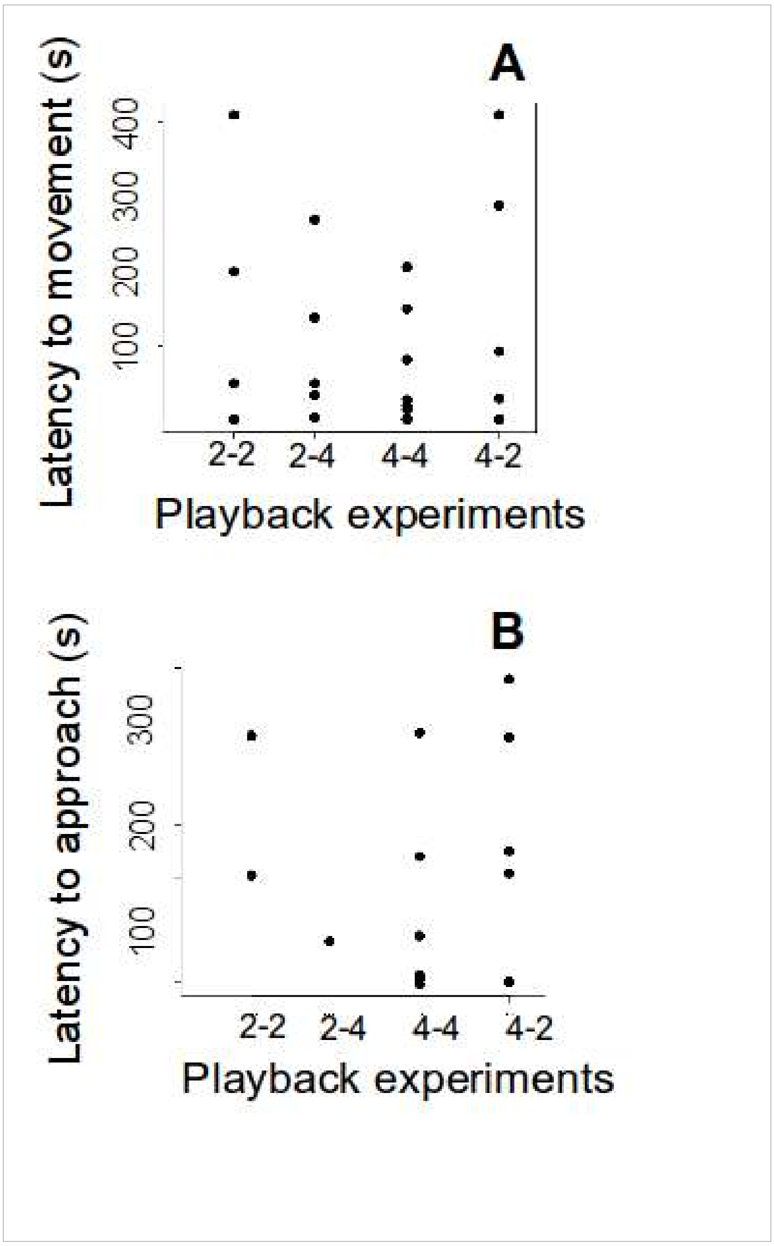
4-note *A. femoralis*
Jirau

4-note *A. femoralis*
Ducke

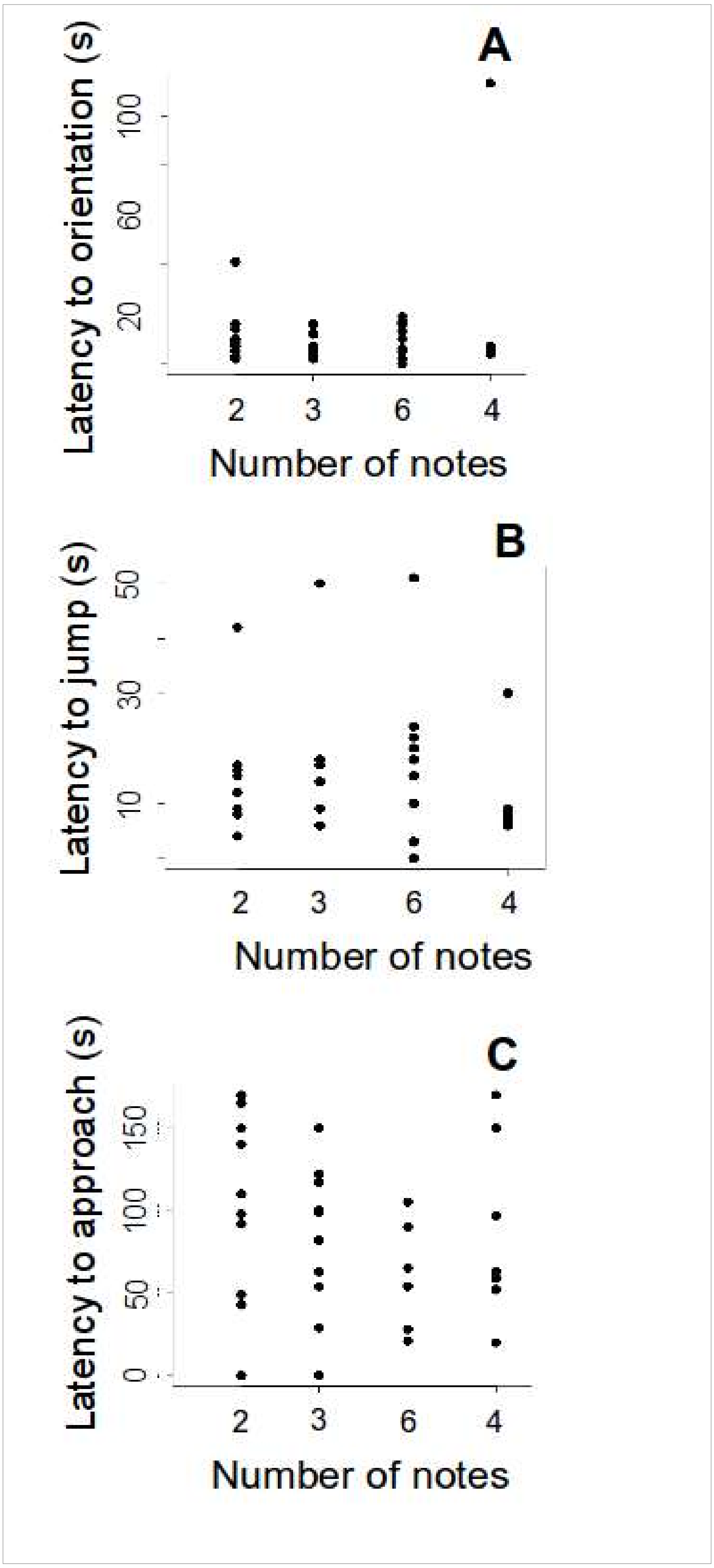
3-note *A. femoralis*
Panguana

2-note *A. hodli*
Jirau

6-note *A. myersi*
S. Gabriel da Cachoeira



A
Latency to movement (s)
100
200
300
400
2-2
2-4
4-4
4-2
Playback experiments
B
Latency to approach (s)
100
200
300
2-2
2-4
4-4
4-2
Playback experiments


A
Latency to orientation (s)
20
60
100
2
3
6
4
Number of notes
B
Latency to jump (s)
10
30
50
2
3
6
4
Number of notes
C
Latency to approach (s)
0
50
100
150
2
3
6
4
Number of notes

SÍNTESE

A Hipótese de Adaptação Acústica apesar de ser amplamente conhecida e testada em aves, foi fracamente investigada em anuros. Poucos são os registros dos efeitos da vegetação sobre as características e propagação do canto de anúncio. A falta de consistência entre os resultados encontrados, mesmo para organismos melhor estudados como as aves, mostra que o assunto merece maior atenção. Novos projetos deverão considerar uma padronização na metodologia e um desenho experimental mais criterioso. É importante ressaltar a importância da escala em que a estrutura da vegetação é representada, a replicação dos experimentos e das unidades amostrais, o controle para os efeitos filogenéticos e para efeitos do tamanho corporal.

Esta primeira análise macroevolutiva das pressões seletivas sobre o canto de anúncio na família Hylidae mostrou que um modelo adaptativo é o que melhor explica a evolução das características temporais, duração do canto e taxa de emissão de pulsos. Enquanto a evolução da frequência dominante parece estar relacionada com a evolução do tamanho corporal, uma característica mais conservativa cujo padrão evolutivo poderia seguir o esperado de acordo com o modelo Browniano.

A variação geográfica no canto de *Allobates femoralis* é notória e bem reportada, porém este foi o primeiro estudo utilizando gravações naturais testando os padrões de reconhecimento coespecífico e heteroespecífico nesta espécie e em *A. hodli*. Os resultados encontrados foram surpreendentes uma vez que o nível de agressividade apresentado pelos machos de *A. femoralis* e *A. hodli* foi similar entre os diferentes estímulos testados. A similaridade na resposta agressiva manteve-se nas duas situações estudadas, simpatria e alopatria.

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ANEXOS*

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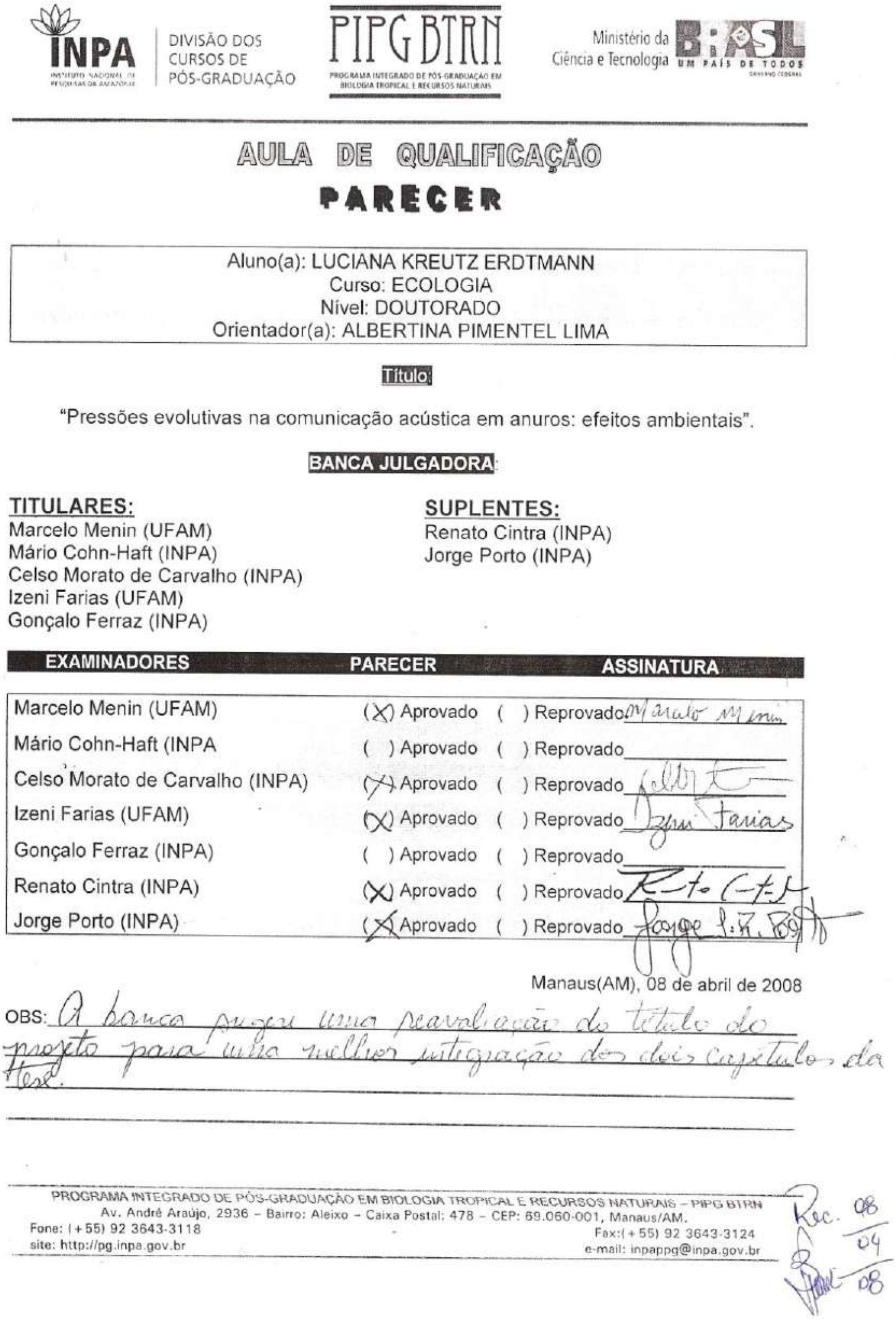
* Pareceres emitidos pelas bancas examinadoras da aula de qualificação, da versão escrita da tese e da defesa pública da tese, respectivamente



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Ministério da Ciência e Tecnologia
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AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): LUCIANA KREUTZ ERDTMANN
Curso: ECOLOGIA
Nível: DOUTORADO
Orientador(a): ALBERTINA PIMENTEL LIMA

Título:

"Pressões evolutivas na comunicação acústica em anuros: efeitos ambientais".

BANCA JULGADORA:

TITULARES:	SUPLENTES:
Marcelo Menin (UFAM)	Renato Cintra (INPA)
Mário Cohn-Haft (INPA)	Jorge Porto (INPA)
Celso Morato de Carvalho (INPA)	
Izeni Farias (UFAM)	
Gonçalo Ferraz (INPA)	

EXAMINADORES	PARECER		ASSINATURA
Marcelo Menin (UFAM)	(X) Aprovado	( ) Reprovado	Marcelo Menin
Mário Cohn-Haft (INPA	( ) Aprovado	( ) Reprovado	
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Gonçalo Ferraz (INPA)	( ) Aprovado	( ) Reprovado	
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Jorge Porto (INPA)	(X) Aprovado	( ) Reprovado	Jorge I. R. Porto

Manaus(AM), 08 de abril de 2008

OBS: A banca sugere uma reavaliação do título do projeto para uma melhor integração dos dois capítulos da tese.

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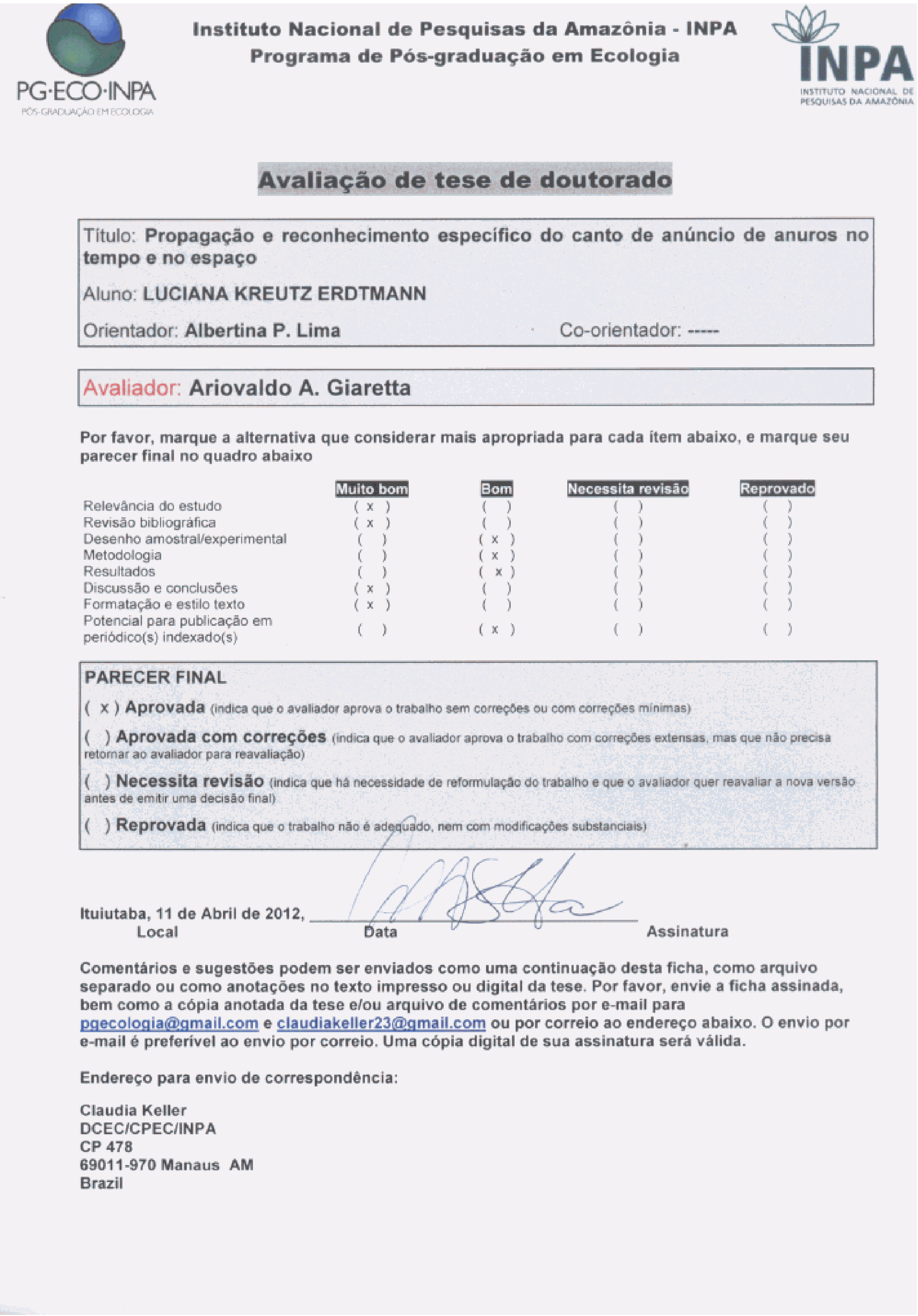

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Avaliação de tese de doutorado

Título: **Propagação e reconhecimento específico do canto de anúncio de anuros no tempo e no espaço**

Aluno: **LUCIANA KREUTZ ERDTMANN**

Orientador: **Albertina P. Lima** Co-orientador: -----

Avaliador: **Ariovaldo A. Giaretta**

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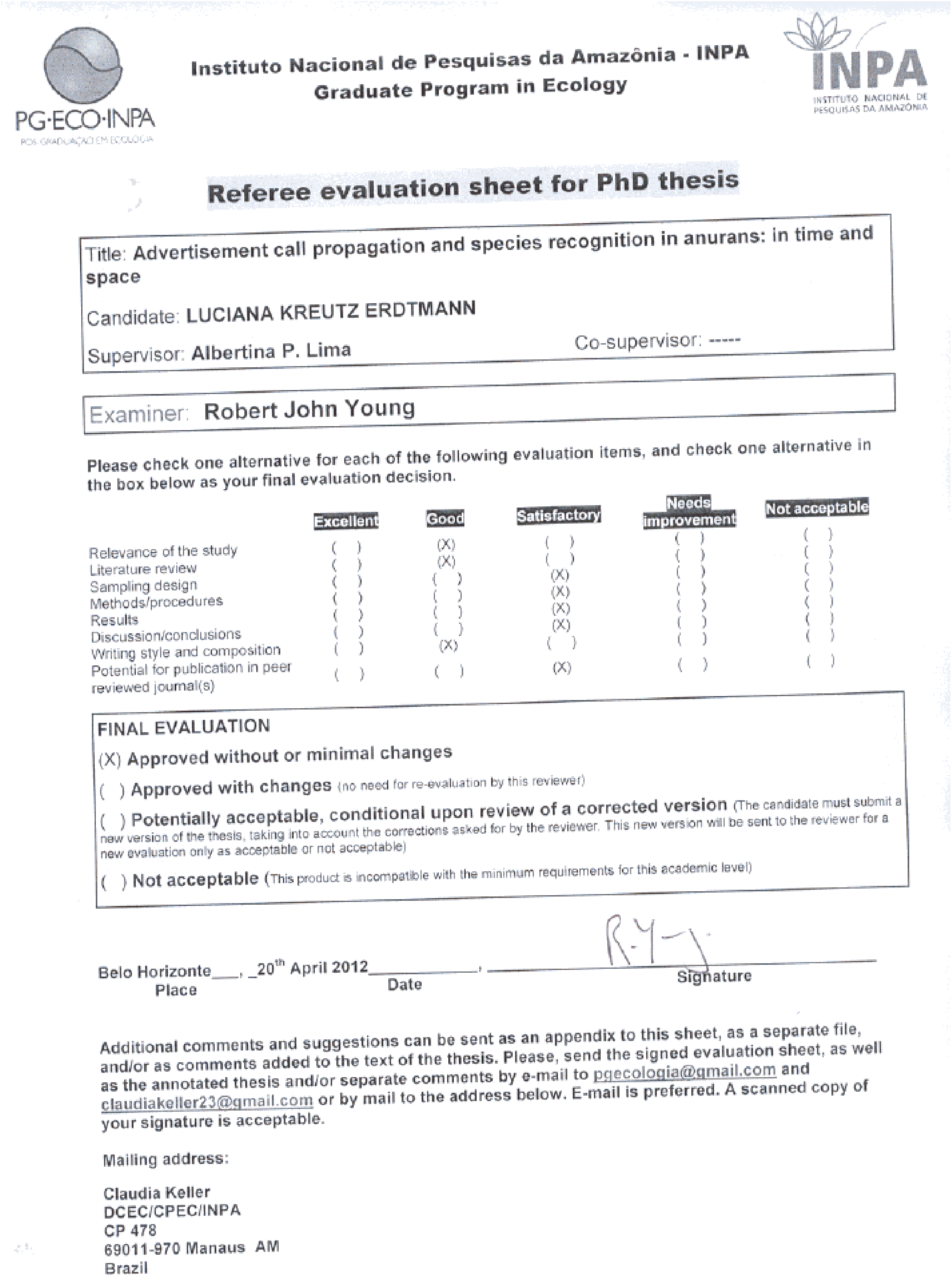

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Referee evaluation sheet for PhD thesis

Title: **Advertisement call propagation and species recognition in anurans: in time and space**

Candidate: **LUCIANA KREUTZ ERDTMANN**

Supervisor: **Albertina P. Lima** Co-supervisor: -----

Examiner: **Robert John Young**

Please check one alternative for each of the following evaluation items, and check one alternative in the box below as your final evaluation decision.

	Excellent	**Good**	**Satisfactory**	**Needs improvement**	**Not acceptable**
Relevance of the study	( )	(X)	( )	( )	( )
Literature review	( )	(X)	( )	( )	( )
Sampling design	( )	( )	(X)	( )	( )
Methods/procedures	( )	( )	(X)	( )	( )
Results	( )	( )	(X)	( )	( )
Discussion/conclusions	( )	( )	(X)	( )	( )
Writing style and composition	( )	(X)	( )	( )	( )
Potential for publication in peer reviewed journal(s)	( )	( )	(X)	( )	( )

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Graduate Program in Ecology

Referee evaluation sheet for PhD thesis

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Candidate: **LUCIANA KREUTZ ERDTMANN**

Supervisor: **Albertina P. Lima** Co-supervisor: **-----**

Examiner: David Luther

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Relevance of the study	( )	( )	( )	( )	( )
Literature review	( )	(X)	( )	( )	( )
Sampling design	( )	(X)	( )	( )	( )
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Avaliação de tese de doutorado

Título: **Propagação e reconhecimento específico do canto de anúncio de anuros no tempo e no espaço**

Aluno: **LUCIANA KREUTZ ERDTMANN**

Orientador: **Albertina P. Lima** Co-orientador: **-----**

Avaliador: **Jeff Podos**

Por favor, marque a alternativa que considerar mais apropriada para cada ítem abaixo, e marque seu parecer final no quadro abaixo

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Orientador: **Albertina P. Lima** Co-orientador: **-----**

Avaliador: **Maria Luisa da Silva (UFPA)**

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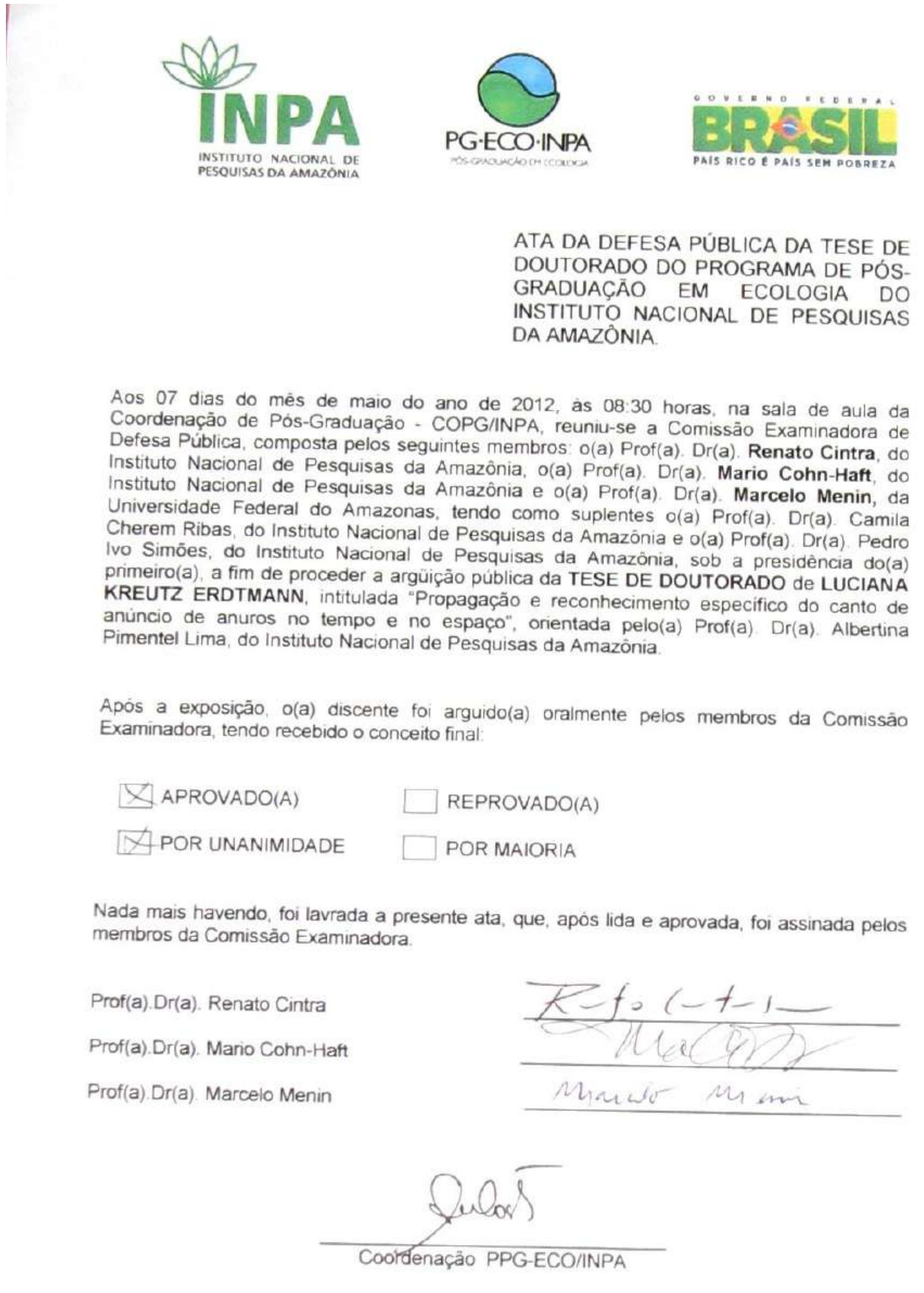

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 07 dias do mês de maio do ano de 2012, às 08:30 horas, na sala de aula da Coordenação de Pós-Graduação - COPG/INPA, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Renato Cintra**, do Instituto Nacional de Pesquisas da Amazônia, o(a) Prof(a). Dr(a). **Mario Cohn-Haft**, do Instituto Nacional de Pesquisas da Amazônia e o(a) Prof(a). Dr(a). **Marcelo Menin**, da Universidade Federal do Amazonas, tendo como suplentes o(a) Prof(a). Dr(a). Camila Cherem Ribas, do Instituto Nacional de Pesquisas da Amazônia e o(a) Prof(a). Dr(a). Pedro Ivo Simões, do Instituto Nacional de Pesquisas da Amazônia, sob a presidência do(a) primeiro(a), a fim de proceder a argüição pública da **TESE DE DOUTORADO** de **LUCIANA KREUTZ ERDTMANN**, intitulada "Propagação e reconhecimento específico do canto de anúncio de anuros no tempo e no espaço", orientada pelo(a) Prof(a). Dr(a). Albertina Pimentel Lima, do Instituto Nacional de Pesquisas da Amazônia.

Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☒ APROVADO(A) ☐ REPROVADO(A)

☒ POR UNANIMIDADE ☐ POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). Renato Cintra

Prof(a).Dr(a). Mario Cohn-Haft

Prof(a).Dr(a). Marcelo Menin

Coordenação PPG-ECO/INPA