UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Tese de Doutorado

Aspectos eco-evolutivos da sinalização visual na comunicação intraespecífica de anfíbios anuros

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Porto Alegre, fevereiro de 2019.

Aspectos eco-evolutivos da sinalização visual na comunicação intraespecífica de anfíbios anuros

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Doutor em Ciências com ênfase em Ecologia.

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Porto Alegre, fevereiro de 2019.

Dedico à Raíssa de 4 anos atrás que
com entusiasmo
acreditou neste trabalho
e em mim.

AGRADECIMENTOS

Agradeço aos meus orientadores, Sandra e Rafael, por me ensinarem que o melhor trabalho é aquele que se faz entre amigos. Ao PPG Ecologia-UFRGS e à CAPES pela confiança profissional e pelo suporte financeiro ao longo do doutorado. Agradeço ao ICMBio e à equipe da Flona de São Franscisco de Paula-RS, onde eu pude contemplar a dança e os sons dos sapos. À equipe do LEPeC (UFRGS) e da Fonoteca Zoológica (MNCN-CSIC, Madrid) pelo ambiente acolhedor e estimulante. Agradeço também aos professores e colegas das inúmeras disciplinas que realizei, especialmente do estágio docência e da monitoria nos cursos de campo que me encheram de admiração e motivação pela docência.

Pelos prazerosos bate-papos científicos, meus agradecimentos ao Leandro Duarte, Camila Both, Pedro Ivo Simões, Peter Narins, Guilherme Seger, Vanderlei Debastiani, Maria João Pereira, Valentina Caorsi e Luísa Lermen. E aos membros da minha banca avaliadora por terem aceitado participar dessa fase tão importante da minha vida acadêmica.

Ninguém está só no mundo, mas eu tive o privilégio de estar muito bem acompanhada. Com amor, um agradecimento especial aos meus pais, à Amora, ao Leandro e ao Michael. À Valentina, Pedro, Elisa e Elizabeth, agradeço por terem sido meus alicerces enquanto eu, cambaleando, escrevia a tese neste último ano. Por fim, agradeço à Sandra e ao Rafa pela parceria; meus exemplos de profissionalismo com afeto.

RESUMO

Em anuros, a sinalização visual é principalmente relacionada ao hábito diurno, à reprodução em ambientes ruidosos (que podem reduzir a eficiência dos sinais acústicos) e à coloração corporal conspícua. Tais atributos diferem dos estados ancestrais previstos para o grupo – hábito noturno, reprodução em poças e coloração críptica. Nesta tese, primeiramente, eu reivindiquei mais atenção a este comportamento inexplorado dos anuros descrevendo o rico repertório visual de uma espécie Neotropical. Em segundo lugar, discuti a importância de distinguir pistas visuais (e.g., atividades deslocadas) de sinais visuais (com função de comunicação) empregando um método com apresentações de espelhos em três espécies de hilídeos Neotropicais. Em terceiro, eu utilizei duas abordagens para testar o *trade-off* entre pistas visuais e sinais acústicos a nível de indivíduo e espécie, descobrindo que pistas visuais não são uma alternativa, mas provavelmente, um complemento à sinalização acústica em anuros. Finalmente, em uma revisão, compilei pistas e sinais visuais previamente descritos para 159 espécies de anuros e realizei análises comparativas para testar os efeitos da filogenia, do ambiente e da coloração sobre a variação do repertório visual. Concluí que as pistas visuais parecem evoluir independentemente em diferentes linhagens, provavelmente como atividades deslocadas, não submetidas a forte seleção. No entanto, em algumas linhagens específicas, tais pistas tornaram-se sinais visuais verdadeiros, que evoluem por seleção fracamente mediada pelo ambiente, mas não pela coloração corporal.

Palavras-chave: sinais visuais, pistas visuais, ecologia comportamental, anuros, evolução de sinais, sinais acústicos.

ABSTRACT

In anurans, visual signaling is mainly related to diurnal habits, reproduction next to water background noise (which can reduce the efficiency of the acoustic signals), and conspicuous coloration. Such traits contradict anuran ancestral features – nocturnal activity, reproduction in ponds and cryptic coloration. First, I claimed for more attention to this unexplored anuran behavior by describing the fantastic and rich visual repertoire of a Neotropical diurnal frog, which reproduces in fast-streams. Second, I discussed the importance in distinguish visual cues (e.g., displacement activities) from visual signals (communication function). For that, I used testes with self-mirror presentation in three Neotropical anuran species. Third, I used two approaches to test the trade-off between visual cues and acoustic signals in the individual and species levels. I found that visual cues are not an alternative, but are possibly complementarity to acoustic signaling in anurans. Finally, in a review, I presented the diversity of visual cues and visual signals previously reported to 159 anuran species during reproductive and aggressive interactions. I performed comparative analyses to test for the effects of phylogenetic history, environment context, and conspicuous color pattern on the variation of visual repertoire. I concluded that visual cue repertoires seem to evolve independently in different anuran lineages, likely as displacement activities, not subjected to strong selection. Yet, in some specific lineages, such cues became true visual signals, which evolve by selection weakly mediated by the environmental context, but not by the dorsal pattern coloration.

Key words: visual signals, visual cues, behavioral ecology, anurans, evolution of signals, acoustic signals.

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INTRODUÇÃO GERAL

Ecologia pode ser definida como “o estudo científico da distribuição e abundância dos organismos e das interações que determinam a distribuição e a abundância” (BEGON et al., 2008; p. IX). Esta área da ciência pode ser dividida em três níveis – organismo, populações e comunidades, sendo que o foco de estudo na ecologia do organismo é os efeitos do ambiente sobre os indivíduos, e vice-versa. Entretanto, os indivíduos são como são, e interagem com o ambiente e com outros indivíduos da forma que interagem, devido às restrições determinadas por sua história evolutiva (BEGON et al., 2008). Neste contexto, na ecologia comportamental, os ecólogos estudam as bases evolutivas do comportamento animal e suas relações com pressões ecológicas (DAVIES et al., 2012).

Segundo Nikolaas “Niko” Tinbergen (1907-1988), podemos estudar o comportamento animal a partir de quatro perguntas principais: (1) O que é (como isto é causado fisiologicamente)? (2) Qual a função (qual é o valor adaptativo)? (3) Como evoluiu (qual sua história filogenética)? e (4) Como se desenvolve no organismo (qual é a sua ontogenia)? (BURKHARDT, 2014). Um notável comportamento animal é a comunicação, uma vez que está presente, de diversas formas (e.g., acústica, visual, química, etc.), em quase todas as interações entre os indivíduos (DAVIES et al., 2012).

O processo de comunicação envolve transferência de informação de um emissor a um receptor por meio de sinais especificamente projetados (SEYFARTH e CHENEY, 2017). Sendo que sinais que maximizam sua detectabilidade são selecionados; sendo que diferentes sinais são mais detectáveis em diferentes ambientes, uma vez que os ambientes diferem nas propriedades de transmissão. Esta divergência nos sinais, e na percepção dos mesmos, pode, por exemplo, resultar em uma menor atratividade entre indivíduos de diferentes populações durante a reprodução e, portanto, desencadear em um processo de isolamento reprodutivo (NOSIL, 2012).

Em anfíbios anuros, a eficiência na comunicação intraespecífica está diretamente relacionada ao sucesso reprodutivo dos indivíduos, uma vez que o macho deve ser capaz tanto de defender seu território de outros machos quanto de atrair fêmeas coespecíficas (WELLS, 2010). A comunicação acústica em anuros oferece oportunidades de seleção sexual de machos pelas fêmeas (MÁRQUEZ, 1995), e também pode atuar na defesa de território (CHUANG et al., 2017). Entretanto, apesar da comunicação em anfíbios anuros ser baseada, principalmente, na emissão de sinais sonoros (WELLS, 2010), há espécies que aparentemente não vocalizam (WALDMAN e BISHOP, 2004), ou que o fazem muito pouco (LINGNAU et al., 2008). Diferentes pressões ecológicas (e.g., risco de predação e fatores ambientais) podem ser responsáveis por esta redução na expressão acústica (WELLS, 1977a; TUTTLE e RYAN, 1982). Nestes casos, outras modalidades de sinais, como a sinalização visual, podem ser selecionadas.

De acordo com Hödl e Amézquita (2001), um sinal visual fornece uma pista visual durante uma interação, e para ser eficiente deve ser redundante, visível e estereotipado, além de provocar uma resposta imediata do receptor. Neste contexto, parece razoável que diferentes sinais visuais dinâmicos (sinais que podem ser “ligados” e “desligados” pelo emissor, GRAFE et al., 2012; e.g. movimentação dos membros e exibição do saco vocal inflado, HARTMANN et al., 2005) e diferentes padrões de coloração (que incluem cores altamente chamativas; HOFFMAN e BLOUIN, 2000; e.g. coloração conspícua da garganta/saco vocal, BURROWES, 2000) possam desempenhar funções importantes durante as interações sociais em anuros.

Acredita-se que os sinais evoluíram a partir de pistas pré-existentes ou de outros sinais (HÖDL e AMÉZQUITA, 2001). Uma vez que os modos de sinalização visual são diversos e amplamente difundidos nos anuros (HÖDL e AMÉZQUITA, 2001), diversos fatores podem ter favorecido a evolução de sinais visuais no grupo. Contrariamente às condições ancestrais dos anuros (hábito noturno, reprodução em poças e coloração críptica; DUELLMAN e TRUEB, 1994), a comunicação visual está, sobretudo, relacionada ao hábito diurno (e.g., *Allobates femoralis*; NARINS et al., 2003), reprodução próxima a quedas d'água (e.g., *Staurois parvus*; GRAFE e TONY, 2017) e coloração conspícua (e.g., *Phrynobatrachus krefftii*; HIRSCHMANN e HÖDL, 2006). A intensidade de luz no ambiente e a conspicuidade dos indivíduos podem facilitar a transferência de sinais visuais. E a sinalização visual pode ter evoluído como um modo alternativo ao sinal acústico em ambientes ruidosos, como cachoeiras (HÖDL e AMÉZQUITA, 2001; GRAFE e TONY, 2017).

Entretanto, a correta identificação de um sinal não é algo trivial. Por exemplo, durante as interações sociais os indivíduos podem exibir comportamentos involuntários, fora de contexto, que não são utilizados para a comunicação (e.g. *Hypsiboas albomarginatus*, FURTADO e NOMURA, 2014). No entanto, estas exibições visuais podem vir a se tornar sinais verdadeiros, uma vez que os padrões motores complexos são muitas vezes modificados, ou ritualizados, a partir de padrões motores antecedentes (ROSENTHAL, 2007). Logo, a compreensão da comunicação visual em anuros requer análises precisas, com testes de hipóteses, dos comportamentos e do contexto ecológico e evolutivo em que esses sinais são realizados.

Objetivo da Tese

O objetivo geral da tese de doutorado é compreender o contexto ecológico e evolutivo da sinalização visual na comunicação intraespecífica dos anfíbios anuros.

Tendo como base três das quatro Perguntas de Tinbergen sobre como estudar o comportamento animal, “O que é?”, “Qual a função?” e “Como evoluiu?”; os objetivos específicos são:

- Apresentar a diversidade do repertório visual em anuros;
- Verificar a função das pistas visuais na defesa territorial de anuros noturnos;
- Averiguar o papel da atividade acústica na diversidade e taxa de emissão de pistas visuais;
- Discutir a importância de se distinguir pistas visuais de sinais visuais;
- Descrever os modelos de evolução das pistas visuais e dos sinais visuais nos anuros atuais.
- Investigar o efeito da filogenia, do ambiente, e da coloração corporal no repertório visual dos anuros atuais.

Estrutura da Tese

A tese “Aspectos eco-evolutivos da sinalização visual na comunicação intraespecífica de anfíbios anuros” está estruturada em quatro capítulos:

Primeiro capítulo: “*Neotropical dancing frog: the rich repertoire of visual displays in an hylodine species*”. Artigo aceito para publicação na revista *Journal of Ethology*. Neste capítulo eu descrevo pela primeira vez um comportamento visual diverso e sofisticado em uma espécie de anuro endêmico do sul do Brasil, *Hylodes meridionalis*. Este estudo reforça que a comunicação visual em anuros pode ser mais comum do que se esperava, e também reivindica mais atenção dos etólogos para esse comportamento ainda pouco explorado no grupo.

Segundo capítulo: "*In front of a mirror: visual displays may not be aggressive signals in nocturnal tree frogs*", publicado em 2017 na revista *Journal of Natural History*. Neste capítulo eu utilizo espelhos para estudar o comportamento visual de três espécies de anuros do Pantanal Mato-Grossense. Também discuto a importância de se identificar sinais corretamente e de distinguir pistas visuais (e.g. atividades deslocadas) de sinais visuais (com função de comunicação) durante interações sociais em anuros.

Terceiro capítulo: "*Visual cues are not an alternative to acoustic signalling in anurans*". Este estudo foi desenvolvido durante o doutorado sanduíche no Museu Nacional de Ciências Naturais, em Madrid, Espanha, sob a supervisão do Dr. Rafael Márquez. Neste capítulo eu demonstro que não há relação entre uma baixa taxa de emissão de cantos e um maior repertório visual, utilizando para isto dados comportamentais sobre 69 espécies de anuros. Adicionalmente, descrevo como machos da espécie *Hylodes meridionalis* utilizam as duas modalidades (acústica e visual) para demonstrar suas disposições agressivas. Portanto, a sinalização visual provavelmente não é uma alternativa, mas uma complementariedade à sinalização acústica neste grupo. Após a incorporação das sugestões da banca de avaliação, este capítulo será finalizado e submetido à revista *Animal Behaviour*.

Quarto capítulo: "*Visual behavior during intraspecific interactions in anurans: phylogeny, environment and color factors*". Neste estudo, apresento a diversidade de pistas e sinais visuais previamente descritas em diferentes contextos sociais em anfíbios anuros. Descobri que pistas visuais têm um baixo sinal filogenético e sua evolução é mais bem explicada por um modelo de evolução neutra, enquanto sinais visuais são caracterizados por um alto sinal filogenético e evolução estabilizadora. O ambiente de transmissão e o padrão de coloração corporal explicaram uma porcentagem muito pequena da variação observada no repertório visual das 159 espécies estudadas. Este capítulo conta com a colaboração do Prof. Dr. Leandro Duarte (UFRGS), do Dr. Vanderlei J. Debastiani (UFRGS), e da Luísa Lermen (UFRGS). Esse capítulo será futuramente submetido à revista *Biological Reviews*.

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CAPÍTULO 1

Neotropical dancing frog: the rich repertoire of visual displays in an hylodine species

Neotropical dancing frog: the rich repertoire of visual displays in an hylodine species♣

Raíssa Furtado[1,*], Luísa N. Lermen[1], Rafael Márquez[2] & Sandra M. Hartz[1]

Abstract During reproductive season, males usually must defend their territory against competitor males and also attract females for reproduction. Acoustic signals evolved as an alternative to physical attacks, reducing injuries to both opponents during territorial dispute, and also are the primarily trait used by female frogs to select males. However, some recent evidences indicate that visual signalling may also be important during social interactions in frogs. In this study we describe for the first time a sophisticated visual behaviour of *Hylodes meridionalis*, a diurnal species endemic to the southern Atlantic Forest inhabiting fast streams. We submitted resident males to mirror self-image presentations to simulate the presence of an intruder male on their territories. Furthermore, we collected observations from close-range interactions between individuals of this very shy species. We observed seven types of visual displays: toe flagging (slow up-and-down movements of one or more toes), arm lifting (rapid up-and-down movements of one arm), leg lifting (rapid up-and-down movements of one leg), arm waving (lifting an arm and waving it in an arc), both legs kicking (stretching rapidly towards the back both hind limbs), foot flagging (raising slowly one hind limb in a semi-arch movement), and throat display (pulsation of one or both paired lateral

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♣ Este capítulo segue as normas de idioma e formatação da revista *Journal of Ethology*, onde foi aceito para publicação em 04 de janeiro de 2019.

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vocal sacs without sound production). Only ‘both legs kicking’ was displayed exclusively by females, and toe flagging and foot flagging were displayed by males only during agonistic interactions. The emission of visual displays (7 types, 117 events) was much greater than acoustic signals (3 types, 66 events). Our data demonstrate that the visual repertoire in the genus *Hylodes* is richer than recorded and that the visual behaviour in anurans can be more common than previously believed. Therefore, this characterization study helps to improve our understanding of the function of the rich repertoire of visual displays in frog species and also claims for more attention from ethologists by this poorly explored anuran behaviour.

Keywords: visual displays; territoriality; courtship; limb lifting; foot flagging; arm waving, *Hylodes*, Anuran.

Introduction

Communication involves information transfer between individuals by means of signals, which act by modifying the behaviour of the receiver, and can occur at both intra- and interspecific levels (Rendall et al., 2009). In anuran amphibians, intraspecific communication occurs mainly during the reproductive season, when males typically attract females for mating and defend their territory against any possible competing males (Wells, 2010). In most species, males produce advertisement calls to attract females and for territorial spacing among males, territorial calls during territorial dispute, and courtship calls for close-range communication between male and female, stimulating and orientating females (Wells, 2010; Toledo et al., 2015). Although anurans communicate mostly by acoustic signals (Ryan, 2001), some studies suggest that visual signalling also can be important during social interactions (e.g., *Micrixalus saxicola*, Preininger et al., 2013; *Staurois parvus*, Grafe and

Tony, 2017).

According to Hödl and Amézquita (2001), for a visual signal to be effective it must be redundant, visible and stereotyped, besides eliciting an immediate response in the receptor. Sexual selection and intraspecific competition, such as territorial disputes among males, can constitute pressures for the evolution and divergence of characters (Nosil, 2012). In the past years, several visual displays have been described for anurans in different social contexts (Hödl & Amézquita, 2001; Biju et al., 2014; Furtado et al., 2017). Specifically, in the anuran family Hylodidae, among the dynamic behaviours displayed during intraspecific communication, limb movements and posture raising are the most common visual displays reported during aggressive and/or reproductive interactions (Caldart et al., 2014; Forti & Castanho, 2012; de Sá et al., 2016).

The Hylodidae family is a monophyletic group with three genera (*Crossodactylus*, *Hylodes* and *Megaelosia*) and 47 known species (Frost, 2019). To date, visual displays have been reported for all hylodines (10 species) in which this behaviour was investigated: *Crossodactylus schmidti* (Caldart et al., 2014), *C. gaudichaudii* (Weygoldt & Silva, 1992), *Hylodes japi* (de Sá et al., 2016), *H. perere* (Silva & Benmaman, 2008), *H. dactylocinus* (Narvaes & Rodrigues, 2005), *H. asper* (Haddad & Giaretta, 1999; Hartmann et al., 2005), *H. nasus* (Wogel et al., 2004), *H. cardosoi* (Forti & Castanho, 2012), *H. heyeri* (Lingnau, 2003), and *H. phyllodes* (Hartmann et al., 2005). In 2001, Hödl and Amézquita suggested that the genus *Hylodes* is the most promising group to study the evolution of visual communication in anurans due to their diurnality and reproduction in noisy streams. The daylight facilitates the signal visualization and information transfer; and noisy environments, such as waterfalls, can reduce the efficiency of acoustic signals, favouring the use of visual signals (Hödl & Amézquita, 2001; Caldart et al., 2014). For example, males of *Staurois parvus* have diurnal habit and they increase foot flagging display and decrease advertisement call emission, during intraspecific interactions when submitted to high levels of stream noise (Grafe and Tony, 2017).

Hylodes meridionalis (Mertens, 1927) belongs to the *Hylodes lateristrigatus* group (Frost, 2019) and is endemic of the southern mountain slopes of the Atlantic Forest (Kwet et al., 2010). During the reproductive season (October–February), males call during daytime on fast streams of clean water, mostly on rocks or perched on fallen logs (Kwet et al., 2010). *Hylodes meridionalis* males are territorial and they defend their calling sites using acoustic signals (Lingnau et al., 2013). Advertisement and territorial calls were described by Lingnau et al. (2013). Kwet et al. (2010) observed leg movements displayed by individuals in natural conditions. However, the visual repertoire of *H. meridionalis* was not previously described in detail.

Several sample designs can be employed to study anuran visual behaviour, for example: (1) observations of natural encounters between individuals (Wogel et al., 2004); (2) mirror self-image presentations (Pombal et al., 1994); (3) picture or video presentations (Reichert & Höbel, 2015); (4) introducing adult males/females next to resident individuals (Lindquist & Hetherington, 1998); and (5) presentations of artificial models (Preininger et al., 2013). In the present study, we used mirror self-image presentations to simulate the presence of conspecific intruder males (i.e., the reflection in the mirror simulates the presence of a signal receiver; Furtado et al., 2017) with the purpose of describing the visual repertoire of *H. meridionalis* males during agonistic interactions. We also describe types and frequency of visual displays during natural close-range encounters between individuals. Comments on other aspects of natural history, as acoustic communication, are also presented.

Materials and methods

Study site

This study was conducted in January, September and November 2016 and February 2017, during the course of non-consecutive 20 days with an average of five to six work hours in the field per day, in three fast streams in the Atlantic Forest, in the São Francisco de Paula National Forest (Flona-SFP), southern of Brazil (29°29’13.3” S and 050°13’12” W). Flona-SFP has an area of 1606 ha and 56% of that being native forests (Narvaes et al., 2005); the vegetation is composed mostly by a mosaic of Araucaria moist forests, steppe formations, and introduced *Pinus* plantations (Backes et al., 2005). The Flona-SFP region has a temperate climate with an average annual rainfall of 2200 mm and a mean temperature of 14.5 °C (Ferreira & Eggers, 2008). However, during the data collection (summer season), the air temperature varied from 17 to 24.4 ºC and the relative humidity varied from 78 to 96% (measured with a TFA Digital Thermo-Hygrometer). The relief in Flona-SFP is wavy permeated by some rivers, where we can find groups of *Hylodes meridionalis* individuals feeding and calling (pers. obs.).

Data collection

We observed 18 males and two females of *Hylodes meridionalis* (87 min of video-recordings) in natural conditions. We recognized the males by call producing; and we identified the females by the absence of call producing and also by the absence of aggressive response from males when these individuals approached. Observations occurred during the afternoon, between 13h and 18h. Focal males were selected arbitrarily, the approximation was very gentle, and images were captured using a video camera (Panasonic HC-W850) positioned at least 1.5 m from the individual.

We submitted males to mirror self-image presentations to simulate the presence of an intruder male in the resident's territory (Figure 1). For that, we gently positioned in the visual field of the resident male a mirror (15 x 15 cm) supported by a retractable handle 1 m long. The mirror was 15–25 cm from the actively calling resident male, at an angle of approximately 45° in relation to the male's body position to avoid a possible blind spot at 0° (directly in front of the animal; Fite, 1973, Furtado et al., 2017). The behavioural responses of each focal male were recorded for at least 3 min and both visual and acoustic responses were measured through these recordings. Additionally, during the field work, we also observed close-range interactions between individuals (one male-female, and three male-male interactions) and recorded and scored these natural encounters. The visual responses were classified according to the motor patterns described by Hödl and Amézquita (2001), Hartmann et al. (2005), Caldart et al. (2014), and de Sá et al. (2016), and the acoustic responses following Lingnau et al. (2013).

Figure 1 Mirror self-image presentation to a male of *Hylodes meridionalis* in the São Francisco de Paula National Forest, southern Brazil. The reflection of the animal in the mirror (15 x 15 cm), positioned at an angle of approximately 45° in relation to the male body position, simulated the presence of an intruder male in the resident territory.

After the recording, the individuals were captured to measure their snout-vent length (SVL) using a paquimeter Mitutoyo, precision 0.02 mm. To prevent observing the same individual more than once, we placed the focal animals in terrariums until the end of each sampling period (not exceeding 3 days). All the animals were released exactly at their capture site, with apparent good health. Each sampling period was conducted in a different stream or in a different section of the same stream. The possibility of observing the same male in different sampling sites is low because this genus is characterized by strong territoriality (Haddad & Giaretta, 1999; Lingnau, 2003; Wogel et al., 2004; Forti & Castanho, 2012; de Sá et al., 2016).

Results

We localized and observed *Hylodes meridionalis* individuals only in the native forest, where males called in full daylight on fast streams of clean water, mostly on rocks or perched on fallen logs. The present study is the first recording *H. meridionalis* males in calling activity during September. The females were observed during the months of November 2016 and February 2017. Although we did not observe males vocalizing alone during the field work (the groups observed were formed by three to seven males in calling activity), the distance between males was more than 2 m. The males and one female were found very close to the water (only a few centimetres of distance) or with the posterior part of the body underwater (Figure 1 and 2). The other female was recorded while sitting inside a small cave formed by a big rock above the ground, 2 m away from the water. Although we could not capture the females to measure the SVL, our observations indicate that possibly the females (N = 2) were larger than males (SVL: 39.38 ± 1.96 mm; N = 8 males measured; Figure 2). We also observed lighter body coloration in females when compared to males (Figure 2).

This species can be characterized by being very shy. The males stopped calling and jumped into the water even before we arrived to the margin of the fast stream. However, if observers remained motionless and in silence, they often returned to the initial position after some minutes. Contrary to males, females did not return to the initial position after escaping from our approach. Additionally, females were observed only twice during the field work, and no amplexus, eggs or tadpoles were seen by us.

Figure 2 Male (smaller and darker colour; snout-vent length = 39.2 mm) and female (larger and lighter colour) of *Hylodes meridionalis* in the São Francisco de Paula National Forest, southern Brazil.

Visual repertoire

Despite the difficulty of observing *H. meridionalis* in natural conditions, we reported a large visual repertoire with seven visual displays performed by males and females:

– Toe flagging. Slow up-and-down movements of one or more toes. Toes may be moved independently, without a fixed sequence, or in sequence in a wave-like pattern. Toe flagging was performed with right, left or both feet, and it was mainly performed immediately before or after foot flagging display (see descriptions below; Video S1). In two occasions male displayed toe flagging and emitted advertisement calls simultaneously. During our observations, only one focal male performed toe flagging (Male 3), but in a highly repetitive way (10 events) during a close-range agonistic interaction with another male (Table 1).

– Arm lifting. Rapid up-and-down movement of one arm, without extending it (Video S1 – intruder male, Video S2). It was a high-speed display and it was performed with right or left arms. Additionally, the behaviour was observed immediately before or after leg lifting display (see descriptions below). This behaviour was performed by seven males during agonistic context and by one female during reproductive contexts (Table 1).

– Leg lifting. Rapid up-and-down movement of one leg, without extending it (Video S2). It was a high-speed display and it was performed with right or left limbs. This behaviour can be performed immediately before or after arm lifting display. Leg lifting was performed by ten males during both agonistic and reproductive contexts and by one female during reproductive context (Table 1). During our study we observed the same male performing both arm and leg lifting displays (Table 1, Video S2).

– Arm waving. Lifting an arm and waving it up and down in a gentle arc beside the head (Video S3). Both right and left arms were used to perform a high speed arm waving. Arm waving was performed by seven males during both agonistic and reproductive contexts and by one female during reproductive context (Table 1). Arm waving was the most frequent visual display during our study (49 events, 41.88% of visual displays).

– Both legs kicking. Stretching rapidly both hind limbs at the same time towards the back above the ground and returning them to the normal position, as if the individual was kicking the air (Video S4). ‘Both legs kicking’ was performed only once by a female during a very close-range male-female interaction (Table 1). It was performed five seconds after the male displayed arm waving (Video S4). The male did not visually or acoustically respond to ‘both legs kicking’ display and after some time (approximately 80 seconds) he moved away from the female, who did not follow him. Some minutes later the male returned close the female and both restarted to display visually to each other.

– Foot flagging. Raising slowly one hind limb, in a semi-arc movement, above the substrate level and returning it to the body side (Video S1). Foot flagging was performed with the right or left leg, and sometimes there was alternation of sides (Video S5). The lifted foot could simultaneously perform toe flagging. Foot flagging was performed by four males exclusively during agonistic encounters (Table 1).

– Throat display. Pulsation of the throat (inflation and deflation of the vocal sac) without audible sound production. It was performed once or repeated several times (Video S6). Contrary to the production of advertisement calls, when both paired lateral vocal sacs are inflated (Video S7), throat display can be performed by inflation of only one lateral vocal sac (Video S6). Throat display can be performed by males during both agonistic and reproductive contexts (Table 1), but in different ways. During agonistic interaction, one male performed ten throat displays consecutively inflating only the left vocal sac (Video S8), and other male performed two throat displays alternating with territorial calls (it was not possible to determine if the male inflated one or both vocal sacs, Video S8; Table 1). However, when in presence of a female, the male performed only one throat display, inflating only the left vocal sac, immediately after leg lifting and before the emission of unknown vocalizations (see details below; Video S9).

Territorial dispute between males

In total, we observed six types of visual displays performed by males during territorial dispute: toe flagging, arm lifting, leg lifting, arm waving, foot flagging, and throat display (99 events; Table 1). Arm waving behaviour was the most frequent visual display during both mirror self-image presentations and male-male interactions (Table 1). Although the emission of acoustic signals (63 events) was much less frequent than visual displays (117 events), we video-recorded two known acoustic signals for males of *H. meridionalis*: advertisement call (Video S7) and territorial call (Video S8; Lingnau et al., 2013). Advertisement call was the most frequent acoustic signals emitted by males during aggressive interactions (50 events, 79.4% of calls produced; Table 1). Additionally, the simultaneous emission of advertisement calls and toe flagging (2 events, 2 males) was only produced by resident males after approximation of intruding males.

Table 1 Emission of visual and acoustic behaviours and physical attacks by 18 males and one female of *Hylodes meridionalis* during agonistic and reproductive interactions. Agonistic interactions consisted in the presentation of mirror self-image to simulate the presence of an intruder male in their territories and in observations of close-range male-male interactions. Reproductive interactions consisted in observations of close-range male-female interaction. Time represents the duration of observation (minutes:seconds).

			Behaviour										
Individual	Context	Time	Toe flagging	Arm lifting	Leg lifting	Arm waving	Both legs kicking	Foot flagging	Throat display	Advertisement call	Unknown call	Territorial call	Physical attack
Male 1	Mirror self-image	03:00	0	0	0	0	0	0	0	1	0	0	0
Male 2		03:00	0	0	0	5	0	1	0	7	0	0	1*
Male 3		03:00	1	2	1	0	0	0	0	7	0	0	1*
Male 4		03:00	0	0	0	0	0	0	0	0	0	0	0
Male 5		03:00	0	3	0	0	0	0	0	1	0	0	1*
Male 6		05:36	0	0	3	3	0	3	0	4	0	0	0
Male 7		03:00	0	1	0	0	0	0	0	2	0	0	0
Male 8		03:00	0	0	1	0	0	0	0	0	0	0	0
Male 9		03:01	0	0	0	0	0	0	0	2	0	0	0
Male 10		03:01	0	0	0	0	0	0	0	0	0	0	0
Male 11		07:45	0	0	0	7	0	0	0	4	0	0	0
Male 12		03:02	0	0	1	0	0	0	0	0	0	0	2*
Male 13		03:07	0	1	0	5	0	0	0	0	0	0	0
Male 3	Male-male interaction	10:25	10	0	1	10	0	8	0	19	0	0	1
Male 14**			0	3	1	4	0	2	0	3	0	0	0
Male 15	Male-male interaction	5:56	0	1	2	2	0	0	10	0	0	0	0
Male 16			0	1	2	0	0	0	0	0	0	0	0
Male 17	Male-male interaction	3:21	0	0	2	4	0	0	2	0	0	13	0
Male 18			0	0	0	0	0	0	0	0	0	0	0
Male 11	Male-female interaction	12:03	0	0	1	8	0	0	1	0	3	0	0
Female 1			0	1	1	5	1	0	0	0	0	0	0
Total:		78:17	11	13	16	49	1	14	13	50	3	13	6

*The male attacked the mirror. **Intruder male.

The males' aggressiveness during male-male competition could be identified not only by the production of visual displays and acoustic signals, but also by the observation of physical attacks against intruders. Four males of *H. meridionalis* jumped towards the mirror during recordings, probably physically attacking their reflection simulating a conspecific intruder male (Table 1; Video S7). Aggressive physical contact was also observed during close-range male-male interactions, but in a different way. We observed a male pushing away an intruder from his territory by putting its head under the intruder's head and pushing it out. After that, the resident male followed the intruder emitting advertisement calls, and performing toe flagging and foot flagging displays until the complete exit of the intruder from the rock (Video S10).

Male-female interaction

We observed only a single interaction between male and female (Figure 2). The female approached the male and both started to display visually to each other. During a period of approximately 15 min, the male emitted arm waving (8 events), leg lifting (1 event) and throat displays (1 event); and the female emitted arm waving (5 events), arm lifting (1 event), leg lifting (1 event), and both legs kicking (1 event; Table 1). The female emitted the 'both legs kicking' immediately after the male emitted an arm lifting display. During the exchange of visual displays, the distance between male and female animal varied from adjacent (Figure 2) to 25 cm.

After the performance of visual displays by both individuals that did not follow an apparent order, the male emitted four consecutive unknown vocalizations (Video

S9) immediately after the emission of leg lifting and throat display. In contrast to the production of advertisement calls, where both paired lateral vocal sacs are inflated, the male alternated the use of the vocal sacs to produce these undescribed vocalizations. After vocalizing, the male began to move towards the riverside. However, the female moved towards the opposite site and jumped into the water. In summary, no advertisement call was ever observed by *H. meridionalis* males during close-range interactions with a female.

Discussion

Almost all visual displays described for *Hylodes meridionalis* were previously described for other hylodine frogs (Table 2). ‘Both legs kicking’, however, was first reported for the genus *Hylodes* in the present study, and it was performed only by females of *H. meridionalis* (Table 2). These observations further emphasize the genus trend for visual communication that is mostly attributed to its characterized behavioural aspects of diurnality and reproduction in noisy streams (Haddad & Giaretta, 1999; de Sá et al., 2016). Environmental factors believed to be related to the evolution of visual signalling in anurans (Hödl & Amézquita, 2001), but not checked so far.

Table 2 Visual displays emitted by males (♂) and/or females (♀) of anurans species from the genus *Hylodes* (family Hylodidae) during agonistic (^{a}), reproductive (^{r}) or both (^{b}) social contexts.

Species	Toe/Finger trembling	Toe posture	Toe flagging	Limb lifting*	Arm waving	Leg stretching	Both legs kicking	Foot flagging	Mouth opening	Head bobbing	Head snaking	Throat display	Body lowering	Body raising	Upright posture	Back raising	Two legged pushing	Body jerking	References
Hylodes asper	–	–	♂^{a}	♂♀^{b}	–	♂♀^{b}	–	♂^{b}	–	–	–	–	–	♂^{b}	–	–	–	–	1, 2, 3, 4
H. japi	–	♂^{b}	♂^{b}	♂♀^{b}	♂♀^{b}	♂^{a}	–	♂^{b}	–	♂^{b}	♂^{r}	♂^{b}	♂^{a}	♂^{b}	♂^{b}	–	♂^{a}	♂♀^{b}	5
H. perere	–	–	–	–	–	♂	–	–	–	–	–	–	–	–	–	–	–	–	6
H. dactylocinus	♂	♂	♂	♂	–	♂	–	♂^{b}	–	–	–	–	–	–	–	♂^{a}	–	–	3,7
H. nasus	–	–	–	–	♂^{a}	♂^{a}	–	♂	–	–	–	–	–	–	♂^{a}	–	–	–	8
H. cardosoi	–	–	–	♂^{a}	–	♂^{b}	–	♂♀^{b}	–	–	–	–	–	♂^{b}	–	–	–	–	9,10
H. heyeri	–	♂	♂	♂^{a}	–	♂^{a}	–	–	♂	–	–	–	♂	–	♂	–	–	–	11
H. phyllodes	–	–	♂^{a}	♂	–	♂^{b}	–	♂	♂^{a}	–	♂	♂	♂^{a}	♂^{b}	♂^{a}	–	–	–	4,12
H. meridionalis	–	–	♂^{a}	♂♀^{b}	♂♀^{b}	–	♀^{r}	♂^{a}	–	–	–	♂^{b}	–	–	♂^{a}	–	–	–	Present study

*Including both arm and leg lifting behaviours.

References: [1]Heyer et al. (1990); [2]Haddad & Giaretta (1999); [3]Hödl & Amézquita (2001); [4]Hartmann et al. (2005); [5]de Sá et al. (2016); [6]Silva & Benmaman (2008); [7]Narvaes & Rodrigues (2005); [8]Wogel et al. (2004); [9]Lingnau et al. (2008); [10]Forti & Castanho (2012); [11]Lingnau (2003); [12]Forti (pers. obs.).

Arm waving was the most frequent visual display performed by *H. meridionalis*, especially during agonistic interactions between two males. For example, one focal male did not perform arm waving displays during mirror self-image presentations, but it performed several arm waving displays after the approach of another male into its territory. Therefore, it seems that the reflection in the mirror alone did not trigger an increase of the display of this particular behaviour. In addition, arm waving behaviour could be a visual display originating in the combination of rapid movement and colour contrast against background. In contrast to other anuran species (e.g., *Atelopus zeteki*, Lindquist & Hetherington, 1996, 1998; *Brachycephalus ephippium*, Pombal et al., 1994), the arm of *H. meridionalis* individuals moved fast during arm waving display. Conspicuous coloration in the supra-labial area in hylodines is commonly observed (Haddad et al., 2008). De Sá et al. (2016) suggested that the rapid arm movement associated with the colour contrast between the dark arm and the bright whitish-yellow coloration in the supra-labial area in *H. japi* can produce a flashing signal for the conspecific receiver. However, the present study is merely a starting point to elucidate the function of arm waving in *H. meridionalis* and future experimental studies are required.

Together, arm and leg lifting displays were the second most frequently performed visual display by males of *H. meridionalis*, and limb lifting displays was reported in other six hylodine frogs. In 2001, Hödl and Amézquita (2001) already suggested that limb lifting behaviour (including both arm and leg movements) was one of the most widespread visual displays in anurans. At the moment, this visual display was reported in species from eight anuran families (Micrixalidae, Biju et al., 2014; Ranidae, Stangel et al., 2015; Hylidae and Centrolenidae, Hartmann et al., 2005;

Aromobatidae, Narins et al., 2003; Dendrobatidae, Hödl & Amézquita, 2001; Bufonidae, Lindquist & Hetherington, 1996; and Hylodidae, present study). Although ethologists do not usually separate arm lifting from leg lifting during anuran visual repertoire description (e.g., Hödl & Amézquita, 2001; Hartmann et al., 2005) and individuals of *H. meridionalis* are able to perform both displays, de Sá et al. (2016) highlighted that individuals of *H. japi* were observed performing exclusively arm lifting displays. In addition, while leg stretching (stretching of only one leg) display was reported to all the other *Hylodes* species in which the visual communication was studied so far, especially performed by males. Here, we observed 'both legs kicking' performed by a female of *H. meridionalis*. However, the present study is not the first one to report 'both legs kicking' in the Hylodidae family. Caldart et al. (2014) described *Crossodactylus schmidti* individuals performing 'both legs kicking', but only during agonistic encounters between two males. Therefore, we suggest that future studies not only investigate the adaptive function of visual displays emitted by each sex of hylodines, but how the diversity of visual displays is distributed in the phylogeny of the group.

From the visual displays performed by males of *H. meridionalis* during agonistic contexts (toe flagging, arm lifting, leg lifting, arm waving, foot flagging, and throat display), throat display was the only one exclusively performed during close-range interaction between individuals. This result agrees with other studies (Pombal et al., 1994; Lindquist & Hetherington, 1998; Haddad & Giaretta, 1999; Furtado & Nomura, 2014) indicating that mirror self-image presentations can be a good method to investigate the visual repertoire in visually oriented animals. But it is not perfect. For future studies we strongly suggest the use of a proper control to confirm or reject any effect caused by the approximation of an object in the behaviour of focal animals. A control treatment would also make it possible to verify whether the individuals produce visual displays even in the absence of a signal receiver. In this case, visual displays would probably not represent visual signals, but displacement activities (Furtado & Nomura, 2014).

Displacement activities are unintentional behaviours with apparent irrelevance during ongoing activity (Tinbergen, 1952; Maestripieri et al., 1992). This kind of display apparently has no communication function, but can be very stereotyped and easily misinterpreted as a signal display (Furtado & Nomura, 2014). However, signals evolve from pre-existing cues, e.g., unintentional behaviours, or other signals (Tinbergen, 1952). Therefore, it is possible that some visual displays in *H. meridionalis* represent *bona fide* visual signals, but others not. In the last case, these displacement activities may have not been evolved to signals yet.

During our observations, a male of *H. meridionalis* inflated only one of his paired sacs during throat display after a female approached. This is the second anuran species known that can independently use each lateral vocal sac (this ability was first recorded to *H. japi* by de Sá et al. (2016) and discussed by Elias-Costa et al., 2017). We also described *H. meridionalis* males alternatively inflating the lateral vocal sacs during production of undescribed calls. Therefore, these results not only indicate that these animals can voluntarily control each vocal sac, but also that the use of a specific paired lateral vocal sac or both of them may be selected.

Species using visual signalling can also use acoustic signalling during social interactions (Narins et al., 2003). This makes it difficult to separate the function of each signal modality during communication (Amézquita & Hödl, 2004). Contrary to other anuran species also submitted to mirror presentations (e.g. *Boana albomarginatus*, Furtado & Nomura, 2014; *B. raniceps*, *Dendropsophus nanus* and *Lysapsus limellum*, Furtado et al., 2017), the visual displays were more frequent than acoustic signals in *H. meridionalis*. Since the visual displays actually represent displacement activities in *B. albomarginatus*, *B. raniceps*, *D. nanus* and *L. limellum* (Furtado & Nomura, 2014; Furtado et al., 2017), this result may indicate a high possibility that the visual displays performed by *H. meridionalis* individuals actually represent visual signals. Despite the low emission of calls by males of *H. meridionalis*, we recorded advertisement and territorial calls (described by Lingnau et al., 2013) and also one unknown type of call that was emitted only by a male during close-range male-female interaction. Therefore, we encourage future studies to confirm the production of courtship calls by males of *H. meridionalis*. In addition, bimodal stimulation (e.g. acoustic and visual signals) can result, for example, in the strongest behavioural response of focal animals (Narins et al., 2003). Therefore, the possibility that males of *H. meridionalis* could increase their aggressive response when confronted by a calling intruder male performing visual displays should be investigated in order to complement the findings reported in this study.

Visual displays during intraspecific communication have been reported for a small part of anuran diversity despite the evidence of its importance in the social interactions. In the present study we report a diverse visual repertoire in *H. meridionalis* during both aggressive and reproductive contexts, including a throat display inflating only one lateral vocal sac. Studies on natural history provide the primary information to elucidate the adaptive function and eco-evolutionary aspects of anuran behaviour, especially in this poorly explored field by ethologists.

Electronic Supplementary Material

Videos deposited in the Fonoteca Zoológica (Fonozoo), Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, Madrid, Spain.

Video S1 Resident male of *Hylodes meridionalis* performing toe flagging and foot flagging displays to a conspecific intruder male. The intruder male performed arm lifting at the beginning of the recording. Recorded on January 04, 2016 at 17:37h, air temperature of 20.9 ºC, in São Francisco de Paula National Forest, southern of Brazil. Fonoteca Zoológica Code: 10296MOV1.

Video S2 Male of *Hylodes meridionalis* performing leg and arm lifting movements as response to mirror self-image presentation. Recorded on January 06, 2016 at 15:25h, air temperature of 23.3 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10297MOV1.

Video S3 Male of *Hylodes meridionalis* performing arm waving displays as response to mirror self-image presentation. Recorded on November 13, 2016 at 17:04h, air temperature of 19.6 ºC, in São Francisco de Paula National Forest, southern of Brazil. Fonoteca Zoológica Code: 10298MOV1.

Video S4 Female of *Hylodes meridionalis* performing 'both legs kicking' in front of a conspecific male. Recorded on November 13, 2016 at 18:00h, air temperature of 19.6 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10299MOV1.

Video S5 Interaction between two calling males of *Hylodes meridionalis*. The resident male (bottom right corner of the video) performed foot flagging displays with alternation of feet. Recorded on January 04, 2016 at 17:37h, air temperature of 20.9 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10300MOV1.

Video S6 Male of *Hylodes meridionalis* performing throat displays during agonistic interaction with another male. Recorded on February 24, 2017 at 14:00h, air temperature of 24 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10302MOV1.

Video S7 Male of *Hylodes meridionalis* producing advertisement call and, immediately after that, jumping towards the mirror that was positioned in front of the focal animal to simulate an intruder male. Recorded on January 04, 2016 at 15:58h, air temperature of 23.6 ºC, in São Francisco de Paula National Forest, southern of Brazil. Fonoteca Zoológica Code: 10303MOV1.

Video S8 Male of *Hylodes meridionalis* alternating territorial calls and throat displays during agonistic interaction with another male. Recorded on February 24, 2017 at 15:00h, air temperature of 24 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10304MOV1.

Video S9 Male (bottom) of *Hylodes meridionalis* performing leg lifting and throat displays and, in sequence, producing courtship calls to a female (up) close to him. Recorded on November 13, 2016 at 18:00h, air temperature of 19.6 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10306MOV1.

Video S10 Agonistic interaction with physical attack between two males of *Hylodes meridionalis*. Recorded on January 04, 2016 at 17:37h, air temperature of 20.9 ºC, in São Francisco de Paula National Forest, southern Brazil. Fonoteca Zoológica Code: 10305MOV1.

Acknowledgments

This study was supported by the Graduate Program in Ecology of the Universidade Federal do Rio Grande do Sul (UFRGS). RF received a scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). LNL received a scholarship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). RM was funded by the Ministerio de Ciencia e Innovación, Spain (project TATANKA CGL2011-25062). SMH received a fellowship from the Brazilian Research Council (CNPq; grant 304820/2014-8). Juliana Heck provided assistance during field work. We thank Michael Bottesch and two anonymous reviewers for critically reading the manuscript.

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CAPÍTULO 2

In front of a mirror: visual displays may not be aggressive signals in nocturnal tree frogs

In front of a mirror: visual displays may not be aggressive signals in nocturnal tree frogs[♣]

Raíssa Furtado[3,*], Rafael Márquez[4], Sandra Maria Hartz[5]

Abstract Some evidence indicates that in anuran amphibians, visual signaling can be important during social interactions such as territorial disputes among males, especially in diurnal species. The correct identification of a signal is not a trivial matter. A visual signal provides a visual cue during a social interaction, and to be effective it must elicit an immediate response in the receiver. We tested the hypothesis that visual displays in an agonistic context constitute aggressive signals, in three nocturnal species of Hylidae. We predicted that the production of visual displays would increase in the presence of a conspecific intruder male. Males of *Hypsiboas raniceps*, *Dendropsophus nanus* and *Lysapsus limellum* were submitted to two treatments: (1) Self Image, a reflection in a mirror, simulating the presence of an intruder; and (2) Control, a black rectangle covering the mirror. We observed three visual displays: vocal-sac display (inflate the vocal sac

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[♣] Este capítulo segue as normas de idioma e formatação da revista *Journal of Natural History*, Print ISSN: 0022-2933, onde foi publicado em 2017: volume 51, números 7-8, pg. 443-454, DOI: 10.1080/00222933.2016.1262078.

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and maintain it inflated for some time), limb lifting (rapid up-and-down movements of one or more limbs), and toe/finger trembling (rapid up-and-down movements of one or more toes and/or fingers). This last display was observed only in *H. raniceps* males. Contrary to our hypothesis, the emission rates of all visual displays of the focal animals did not differ between treatments; and the behavioral response did not differ among species. Therefore, we suggest that these behaviors could not be used directly for communication in agonistic contexts, and may represent displacement activities (involuntary responses). Alternatively, an aggressive bimodal stimulus may be necessary to trigger a behavioral response by using visual signals during territory defense in these three species.

Keywords: vocal-sac display; limb lifting; toe/finger trembling; *Hypsiboas raniceps*; *Dendropsophus nanus*; *Lysapsus limellum*

Introduction

Communication involves information transfer between individuals by means of signals, which act by modifying the behavior of the receiver, and can occur at both intra- and interspecific levels (Sebeok 1968; Maynard-Smith and Harper 2003; Bradbury and Vehrencamp 2011). In anuran amphibians, intraspecific communication occurs mainly during the reproductive season, when the males typically attract conspecific females for mating and defend their territory from possible competing males (Wells 2007). Although anurans communicate mostly by emitting acoustic signals (Duellman and Trueb 1994; Wells 2007), some evidence indicates that visual signaling also can be important during social interactions (Hödl and Amézquita 2001; Hartmann et al. 2005).

Intraspecific competition such as territorial disputes among males can constitute a pressure for the evolution and divergence of characters (Nosil 2012), and in recent years, several visual displays have been described for amphibian anurans in agonistic contexts (Dyson et al. 2013; Bee et al. 2016). Among the dynamic behaviors displayed during intraspecific communication, rapid up-and-down movement of one or more limbs (‘limb lifting’ behavior) is one of the most common visual displays in anurans (Hartmann et al. 2005; Souza 2014), and is usually associated with aggressive interactions between males (Hödl and Amézquita 2001; Preininger et al. 2013). Also, rapid up-and-down movements of toes/fingers (without otherwise moving the limb; Hödl and Amézquita 2001) were described in several anuran species during agonistic context (Hartmann et al. 2005; Toledo et al. 2007; Souza 2014). Besides the visual cue, toe/finger movements has been associated with vibrational signaling (during intraspecific – Narins 1995, and interspecific communication – Sloggett and Zeilstra 2008) and with high levels of anxiety (Furtado and Nomura 2014).

Visual signaling in anurans is not restricted to limb movements. Some evidence indicates that conspicuous coloration of the throat in males can indicate the individual’s reproductive disposition to both receivers, females and potential rivals (Hirschmann and Hödl 2006; Sztatecsny et al. 2010; Gomez et al. 2011). Other studies indicate that the male vocal-sac display (inflate the vocal sac, with or without vocalizing, and maintain the vocal sac inflated for some time; adapted from Hartmann et al. 2005) can visually convey information about the individual's sex, intensifying the acoustic signal emitted by the male during territorial defense (Narins et al. 2003; de Luna et al. 2010; Preininger et al. 2013). In addition, although anurans can visually recognize an individual as the same species and sex even at night, as demonstrated by experiments with mirrors (Furtado and Nomura 2014), the role of visual perception of a potential reproductive competitor during social interactions remains little explored.

According to Hödl and Amézquita (2001), a visual signal provides a visual cue during a social interaction, and in order to be effective it must be redundant, visible and stereotyped, besides eliciting an immediate response in the receiver. The correct identification of a signal is not a trivial matter. For example, during social interactions individuals can exhibit involuntary behaviors, and although the motor pattern resembles visual signals emitted by other species, these behaviors are not used for communication (Tinbergen 1952; Furtado and Nomura 2014). Thus, studies of visual communication in anurans require accurate analysis testing the behaviors and ecological contexts in which these signals are performed.

Our purpose was to describe the visual repertoire, during agonistic interactions, of three nocturnal hylid species that reproduce in lentic environments: *Hypsiboas raniceps* Cope, 1862, *Dendropsophus nanus* (Boulenger, 1889) and *Lysapsus limellum* Cope, 1862. We also tested the hypothesis that the visual displays constitute aggressive visual signals. We predicted that the emission rate of visual displays by the resident male would increase in the presence of a conspecific intruder male in its territory; or alternatively, that the visual displays might actually represent displacement activities.

Materials and methods

Study site

This study was completed between 30 September and 6 October 2015 in a permanent pond in the southeastern Pantanal, in the Base de Estudos do Pantanal (BEP) of the Federal University of Mato Grosso do Sul, Corumbá, state of Mato Grosso do Sul, Brazil (19°34’37” S and 057°01’09” W). The Pantanal has an area of approximately 140,000 km^2, with elevations ranging from 75 to 200 m above sea level. The seasonal climate is characterized by a hot and humid summer from October to March, and a dry winter from May to September (Alvares et al. 2013; Prado and Haddad 2005). The predominant biome in the BEP is the Cerrado, with patches of semi-deciduous forest, gallery forests and grassland (Prado 2003).

Study animals

Hypsiboas raniceps Cope, 1862 (Figure 1a) belongs to the *H. albopunctatus* group and is widely distributed in South America (Frost 2015), where it inhabits open areas in northern Argentina, eastern and midwestern Brazil, eastern Bolivia, Paraguay, Venezuela and French Guyana (Uetanabaro et al. 2008). During the reproductive season (September-March), males call at night on the shores of ponds or wetlands (Uetanabaro et al. 2008), mostly perched on shrubby or emergent vegetation (Guimarães and Bastos 2003). Males are territorial and defend their calling sites using chases, acoustic signals and physical fighting (Guimarães and Bastos 2003). The advertisement and territorial calls, as well as the vocal-sac display associated with aggressive interactions, were described by Guimarães and Bastos (2003). The authors observed males of *H. raniceps* inflating the vocal sac during calling activity and maintaining it inflated for some time, even without emitting vocalizations, after agonistic interactions between competitor males.

Dendropsophus nanus (Boulenger, 1889) (Figure 1b) generally occurs in lentic environments in southern, southeastern and midwestern Brazil, central Paraguay, northern Argentina, Uruguay and eastern Bolivia. Males call year-round perched on grasses or emergent aquatic plants in ponds, usually a few centimeters above the water surface (Prado 2003; Uetanabaro et al. 2008). Although two types of notes have been described for the advertisement call (Martins and Jim 2003), no visual display has described for *D. nanus*.

Lysapsus limellum Cope, 1862 (Figure 1c) occurs throughout the Pantanal, from southern and central Brazil, Uruguay, Paraguay and Bolivia to northern Argentina. This semi-aquatic species can be found both day and night in lentic water bodies of open areas (Uetanabaro et al. 2008). Males call while perched on macrophyte leaves during the year (Prado 2003; Uetanabaro et al. 2008). Two types of calls, possibly advertisement and territorial, were described for the species (Santana et al. 2013). However, no visual display has been described for *L. limellum*.

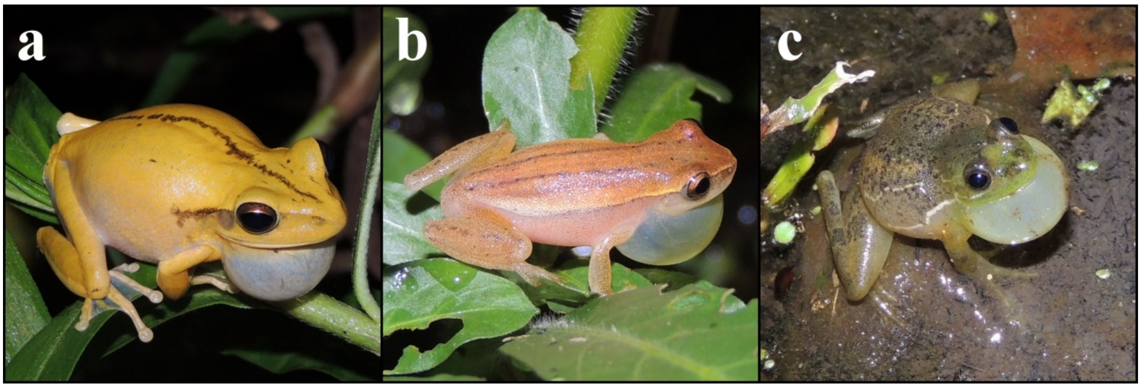

Figure 1 Males of (a) *Hypsiboas raniceps*, (b) *Dendropsophus nanus* and (c) *Lysapsus limellum* located in the southeastern Pantanal, Corumbá, state of Mato Grosso do Sul, Brazil. Note that all males perform the vocal-sac display behavior (inflate the vocal sac, with or without vocalization, and maintain it inflated for some time).

Data collection

We observed males of *H. raniceps* (36 min of recordings; 6 males), *D. nanus* (36 min of recordings; 6 males) and *L. limellum* (30 min of recordings; 5 males) in natural conditions, using focal animal sampling (Lehner 1996). Observations began at 20:00 h, generally 2 h after the first males started to call. Individuals were selected arbitrarily, mainly those that were in a suitable position either on the ground or on vegetation and at least 3 m distant from other males, and were located with a common flashlight. After the focal animal was selected, all flashlights were turned off to reduce the stress on the animal, and images were captured using a video camera (Panasonic HC-W850) with infrared light, positioned at least 1 m from the focal animal. Visual displays emitted by focal individuals of each species are represented by short videos available in the supplemental online material.

To simulate the presence of an intruder, the experiment consisted of two treatments for each resident male: (1) Self Image, a mirror (14 x 8 cm) supported by a retractable handle 1 m long, positioned in the visual field of the resident male; and (2) Control, with the mirror completely covered with a black rectangle (14 x 8 cm). The mirror was 25-30 cm from the actively calling resident male, at an angle of approximately 45° in relation to the male's body position to avoid a possibly blind spot at 0º (directly in front of the animal, Fite 1973). The reflection of the animal in the mirror simulated the presence of an intruder male (Lindquist and Hetherington 1998; Haddad and Giaretta 1999; Furtado and Nomura 2014; Figure 2). Each individual was subjected to each treatment. Each treatment lasted 3 min, with 2-min intervals between treatments. The order of treatments was previously randomized for each focal animal.

Figure 2 Self-Image treatment applied to a male of *Hypsiboas raniceps* in the southeastern Pantanal, Corumbá, state of Mato Grosso do Sul, Brazil. The reflection of the animal in the mirror (14 x 8 cm), positioned at an angle of approximately 45° in relation to the male's body position, simulated the presence of an intruder male in the resident's territory.

The visual responses were classified according to motor patterns described by Hödl and Amézquita (2001) and Hartmann et al. (2005). We described the behavioral repertoires of males of *H. raniceps*, *D. nanus* and *L. limellum* during agonistic interactions (Table 1) and we calculated the mean emission rate per minute per behavior in each treatment. For the vocal-sac display we calculated the proportion between display time and total treatment time.

Table 1 Visual repertoire of males of *Hypsiboas raniceps*, *Dendropsophus nanus* and *Lysapsus limellum* during agonistic interactions.

Behavior	Description	Species
Vocal-sac display	Inflate the vocal sac, with or without vocalizing, and maintain the vocal sac inflated for some time. The male may call while the vocal sac remains inflated.	*H. raniceps* *D. nanus* *L. limellum*
Limb lifting	Rapid up-and-down movements of one or more limbs (fore- or hind limb), without extending it.	*H. raniceps* *D. nanus* *L. limellum*
Toe/finger trembling	Rapid up-and-down movements of one or more toes and/or fingers, without otherwise moving the limb.	*H. raniceps*

Data analysis

To test our hypothesis that the repetition rate of each visual display (response variable) would differ between treatments (predictor variable with two levels: Self Image and Control), we used repeated-measures ANOVAs (Sokal and Rohlf 1995) with interaction between treatments and the species sampled. Since we were interested in how each behavioral response changed between the treatments, each response variable was tested separately (Huberty and Morris 1989). Statistical tests were performed and graphs generated in the software R, version 3.2.0 (R Development Core Team 2015).

Results

Males of *H. raniceps* and *D. nanus* called perched on emergent vegetation of lentic water bodies, a few centimeters (up to 0.5 m) above the water, while males of *L. limellum* called on macrophytes floating on the water surface. Although the total number of visual displays (139 events in 102 minutes of recordings) was much lower than the number of acoustic signals (1,206 events in 102 minutes of recordings), we recorded one known visual display type for males of *H. raniceps*, the vocal-sac display (Guimarães and Bastos 2003; Table 1); and also two types of visual display for each species, which are described here for the first time: toe/finger trembling and limb lifting (for *H. raniceps*; and vocal-sac display and limb lifting for *D. nanus* and *L. limellum* (Table 1).

The behavior of vocal-sac display consisted in the act of inflate the vocal sac, with or without vocalizing, and maintain the vocal sac inflated for some time. Eight of 17 focal males (five males of *H. raniceps* and three males of *D. nanus*) maintained the vocal sac inflated throughout the experiment, and males might call while the vocal sac remains inflated (see Videos 1, 2 and 3 on Supplementary Online Material). Limb lifting behavior, in turn, consisted in rapid up-and-down movements of one or more limbs (fore- or hind limb), without extending it (Videos 2 and 3 – Supplementary Online Material). We did not observe males of *H. raniceps*, *D. nanus* and *L. limellum* emitting another visual or acoustic signal at the same time that limb liftings. Finally, toe/finger trembling behavior consisted in rapid up-and-down movements of one or more toes and/or fingers, without otherwise moving the limb. Although different phalanges can be display during toe/finger trembling, the most conspicuous was the middle finger (see Video 2 on Supplementary Online Material).

The visual display most frequently observed during the experiments was the vocal-sac display (Figure 1), observed in 16 of 17 focal animals. However, the use of vocal-sac display, limb lifting and toe/finger trembling behavior by males of *H. raniceps*, *D. nanus* and *L. limellum* did not differ either between treatments (Self Image and Control) or among species (Table 2; Figure 3).

Table 2 Repeated-measures ANOVAs comparing the emission of vocal-sac display (display time/total time), limb lifting (events/min) and toe/finger trembling (events/min) displays by males of *Hypsiboas raniceps* (N=6), *Dendropsophus nanus* (N=6) and *Lysapsus limellum* (N=5) in response to two experimental treatments, Self-Image and Control. The results show the variances between treatments and also among species, but considering the individuals as blocks. DF = degrees of freedom.

Behaviour	Predictor variable	Estimate	Error	DF	*t*	*P*
Vocal-sac display	Intercept	0.85	0.44	25	1.90	0.06
	Treatment	0.14	0.28	25	0.53	0.59
	Species	-0.11	0.15	25	-0.74	0.46
	Treatment:Species	-0.006	0.09	25	-0.06	0.94
Limb lifting	Intercept	0.34	0.30	25	1.13	0.26
	Treatment	-0.15	0.19	25	-0.81	0.42
	Species	-0.11	0.10	25	-1.04	0.30
	Treatment:Species	0.06	0.06	25	1.02	0.31
Toe/finger trembling	Intercept	5.01	2.24	25	2.23	0.03
	Treatment	-0.86	1.41	25	-0.61	0.54
	Species	-1.42	0.77	25	-1.82	0.07
	Treatment:Species	0.24	0.49	25	0.49	0.62

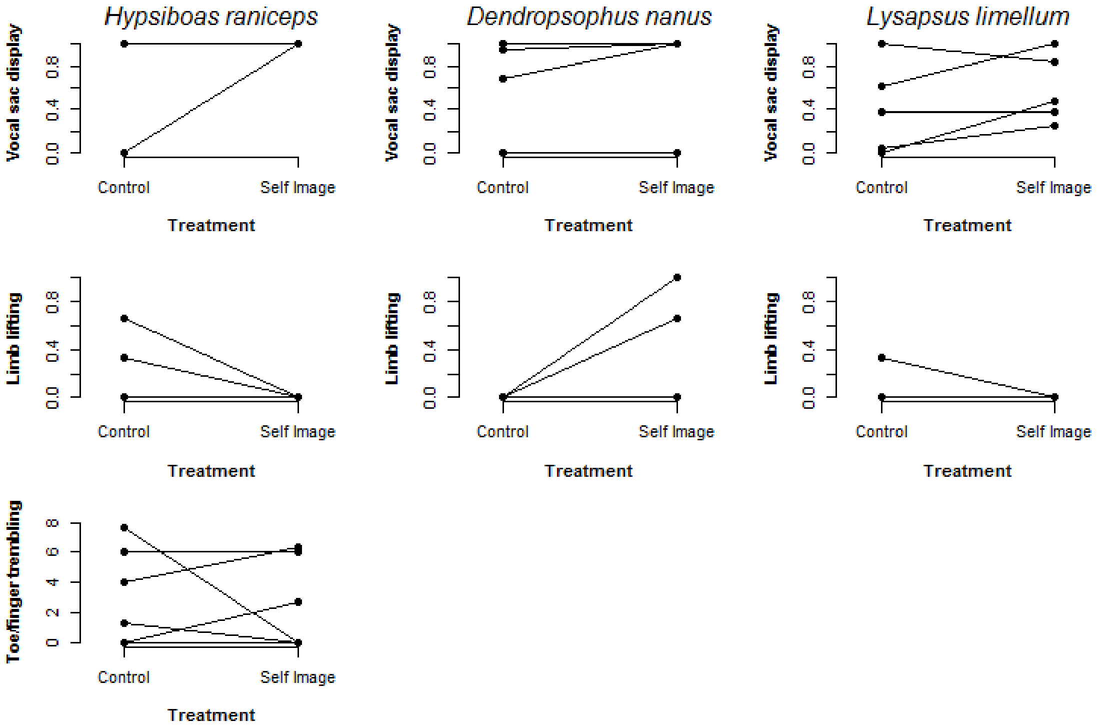

Figure 3 Rate of vocal-sac (emission time/total time), limb lifting (events/min) and toe/finger trembling (events/min) displays by males of *Hypsiboas raniceps* (N=6), *Dendropsophus nanus* (N=6) and *Lysapsus limellum* (N=5) subjected to two treatments (Self Image and Control) in the southeastern Pantanal, Corumbá, state of Mato Grosso do Sul, Brazil. Toe/finger trembling behavior was performed only by males of *H. raniceps* during the experiments. Each pair of points represents one individual; and points may overlap each other. The frequency of behaviors did not differ, either between treatments or among species.

Discussion

The observation of visual behaviors, for the first time, in all three anuran species studied here demonstrates that it is possible to make discoveries about natural history even with low sample sizes. For example, for fewer than ten focal animals, Montanarin et al. (2011) described several visual displays, and Furtado and Nomura (2014) observed significant behavioral changes in response to mirror-experiments in anuran species. However, it is prudent not to exclude the possibility that males of *H. raniceps*, *D. nanus* and *L. limellum* may be using other visual displays during agonistic interactions, such as those described for other anurans (see review with description of 18 visual display patterns, Hödl and Amézquita 2001).

The emission of vocal-sac display, limb lifting and toe/finger trembling behaviors, during intraspecific interactions, were previously reported to many hylid species (Table 3). In *Aplastodiscus eugenioi* (Hartmann et al. 2004, 2005) and *Hypsiboas curupi* (Lipinski et al. 2012), at least one of these behaviors was emitted by males during reproductive contexts (females as receivers). However, in all the other hylid species studied at the moment (16 species, 6 genera; Table 3) the males were observed emitting vocal-sac display, limb lifting or toe/finger trembling behaviors during male-male interactions (Hödl and Amézquita 2001; Amézquita and Hödl 2004; Hartmann et al. 2005; Toledo and Haddad 2005; Giasson and Haddad 2006; Toledo et al. 2007; Miranda et al. 2008; Barros and Feio 2011; Lipinski et al. 2012; Furtado and Nomura 2014; Horne et al. 2014; Souza 2014).

Table 3 Emission of toe/finger trembling, limb lifting and/or vocal-sac display behaviors by males of tree frogs (Anura: Hylidae) during aggressive (Aggr.) and/or reproductive (Repr.) contexts.

Species	Vocal-sac display	Limb lifting	Toe/Finger trembling	References
Bokermannohyla sapiranga	-	Aggr.	-	1
Aplastodiscus eugenioi	Repr.	Repr.	-	2,3
Aplastodiscus perviridis	-	Aggr.	-	4
Hypsiboas raniceps	Aggr.	Aggr.	Aggr.	Present study
Hypsiboas albopunctatus	Aggr.	Aggr.	Aggr.	1,4
Hypsiboas albomarginatus	Aggr.	Aggr.	Aggr.	3,5,6
Hypsiboas faber	Aggr.	Aggr.	Aggr.	7
Hypsiboas lundii	Aggr.	Aggr.	Aggr.	7
Hypsiboas curupi	Aggr./Repr.	-	-	8
Hypsiboas leptolineatus	Aggr.	Aggr.	-	7
Hypsiboas goianus	Aggr.	Aggr.	Aggr.	1
Hypsiboas bischoffi	Aggr.	Aggr.	Aggr.	4,7
Scinax eurydice	-	Aggr.	-	3
Scinax maracaya	-	Aggr.	-	9
Scinax fuscomarginatus	-	Aggr.	-	10
Scinax nasicus	-	Aggr.	-	7
Lysapsus limellum	Aggr.	Aggr.	-	Present study
Dendropsophus parviceps	-	-	Aggr.	11,12
Dendropsophus werneri	-	Aggr.	-	13
Dendropsophus nanus	Aggr.	Aggr.	-	Present study
Acris blanchardi	Aggr.	Aggr.	-	14

References: [1]Souza (2014); [2]Hartmann et al. (2004); [3]Hartmann et al. (2005); [4]Toledo et al. (2007); [5]Giasson and Haddad (2006); [6]Furtado and Nomura (2014); [7]Furtado (pers. obs.); [8]Lipinski et al. (2012); [9]Barros and Feio (2011); [10]Toledo and Haddad (2005); [11]Hödl and Amézquita (2001); [12]Amézquita and Hödl (2004); [13]Miranda et al. (2008); [14]Horne et al. (2014).

Although previous studies have associated visual signals with agonistic interactions in nocturnal tree frogs (e.g., *Dendropsophus parviceps*, Amézquita and Hödl 2004; *Agalychnis callidryas*, Caldwell et al. 2010; *Bokermannohyla sapiranga*, Souza 2014), we experimentally demonstrated that only visual cues of a reproductive competitor were not sufficient to increase the frequency of visual displays in males of *Hypsiboas raniceps*, *Dendropsophus nanus* and *Lysapsus limellum*. Thus, our results indicate that: (i) the visual displays emitted by males of these species are not used for communication during aggressive intraspecific interactions (Furtado and Nomura 2014); or (ii) a bimodal aggressive stimulus may be necessary to trigger a behavioral response by using visual signals during territory defense (Narins et al. 2003, 2005).

As demonstrated in other nocturnal species of the family Hylidae (e.g., *Hypsiboas albomarginatus*, Furtado and Nomura 2014; *H. goianus* and *H. albopunctatus*, Souza 2014), the motor patterns, generally associated with visual signaling in diurnal species, observed in our study can actually represent unintentional behavioral responses. Although displacement activities might be a good indicator of anxiety levels, they are not communication signals (Maestripieri et al. 1992). Therefore, the vocal-sac display, toe/finger-trembling and limb-lifting behaviors performed by males of *H. raniceps*, *D. nanus* and *L. limellum* might represent a reflexive response derived from increased anxiety due to the unpredictability of antagonistic social interactions. However, these motor patterns could evolve to become “bona fide” visual signals, since studies with ritualization of a displacement activity during evolution reveal an increase in the differences between the ritualized behavior and the original (reviewed by Tinbergen 1952).

Alternatively, the visual displays emitted by males of *H. raniceps*, *D. nanus* and *L. limellum* during aggressive intraspecific interactions may have signal function in other contexts. For example, the focal males could actually be sending the visual message to females (e.g. *Engystomops pustulosus*, Rosenthal et al. 2004; *Dryophytes* *versicolor*, Reichert and Höbel 2015) and not to the self-image reflected on the mirror. Or even these behaviors can be associated with interspecific interactions. Sloggett and Zeilstra (2008), for example, suggested the use of toe movements as an alternative predatory function in anurans. Although the present study is a starting point to elucidate the function of visual displays emitted by males of these species, future experimental studies are required.

However, since species using visual signaling also use acoustic signaling during social interactions (e.g. *Hylodes asper*, Haddad and Giaretta 1999; *Micrixalus saxicola*, Krishna and Krishna 2006), it is difficult to separate the function of each signal in anuran communication (Amézquita and Hödl 2004). For example, in *Epipedobates femoralis,* a bimodal stimulus consisting of both visual (vocal-sac pulsation) and acoustic signals is necessary to elicit an aggressive response by the receiver resident male (Narins et al. 2003). Additionally, males of *H. albomarginatus* did not respond visually, but rather acoustically (by increasing the emission of aggressive calls) to visual stimuli simulating conspecific males (Furtado and Nomura 2014). We found that a unimodal stimulus of a conspecific intruder did not trigger the visual aggressive response by resident males of *H. raniceps*, *D. nanus* and *L. limellum*. However, our results do not exclude the possibility that visual stimuli lead to acoustic modulations or to bimodal stimuli (e.g. visual and acoustic signals combined), resulting in an increase in the frequency of visual displays by males of these three species.

We concluded that the visual displays emitted during agonistic interactions by three species of nocturnal tree frogs that reproduce in lentic environments, *Hypsiboas raniceps*, *Dendropsophus nanus* and *Lysapsus limellum*, probably represent displacement activities. However, in view of the diversity of visual displays and their use during social interactions, these behaviors should be investigated in other social and environmental contexts, including bimodal experiments, and in other species of amphibians.

Supplementary Online Material

Videos deposited in the Fonoteca Zoológica (Fonozoo), Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, Madrid, Spain.

Video 1 Experiment with a male of *Hypsiboas raniceps* performing a vocal-sac display and emitting acoustic signals. Fonoteca Zoológica Code: 10313MOV1.

Video 2 Male of *Hypsiboas raniceps* calling and performing toe/finger trembling (00:06 min and 00:12 min) and limb lifting (00:16 min) behaviors. Fonoteca Zoológica Code: 10314MOV1.

Video 3 Male of *Dendropsophus nanus* calling and using vocal-sac display and limb lifting (00:06 min) behaviors. Fonoteca Zoológica Code: 10315MOV1.

Acknowledgments

This study was supported by the Graduate Program in Ecology and Conservation of the Universidade Federal do Mato Grosso do Sul (UFMS). RF received a scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). RM was funded by the Ministerio de Ciencia e Innovación, Spain (project TATANKA CGL2011-25062). SMH received a fellowship from the Brazilian Research Council – CNPq (grant

304820/2014-8). We thank all colleagues and professors, especially Augusto Ribas, of the Ecology of the Pantanal Field Course (EcoPan 2015), for encouragement and critical suggestions. We thank the two unidentified reviewers for critically reading the manuscript. Juan Fernando Cuestas provided assistance during field work.

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CAPÍTULO 3

Visual cues are not an alternative to acoustic signalling in anurans

Visual cues are not an alternative to acoustic signalling in anurans♣

Raíssa Furtado[6,*], Sandra M. Hartz[6], Rafael Márquez[7]

Abstract Acoustic signalling is the ancestral and predominant form of communication in anurans. However, in some situations, like in noisy habitats, exclusive acoustic signalling may be disadvantageous for individual reproductive success. Additionally, there are species that rarely vocalize or have low intensity calls. Recently, studies described a rich repertoire of visual cues in many anuran species. Could visual signalling be used as an alternative to acoustic signalling in anurans? We used two approaches in order to answer this question. We (1) analysed pre-existing data on 69 species by compiling the visual repertoire (28 types of visual cues) from the literature and measuring call rates by analysing recordings deposited in the Fonoteca Zoológica (MNCN-CSIC). We predicted a larger repertoire of visual cues in species that produce less calls per minute during intraspecific communication. Controlling for phylogenetic effect, we found no trade-off between call rate and visual repertoire diversity. In a behavioural approach, we (2) submitted males of *Hylodes meridionalis* (which reproduce in fast-streams) to mirror presentations to test for alternative signalling. Their reflection in the mirror simulated a conspecific intruder (signal receiver). We

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♣ Este capítulo segue as normas de idioma e formatação da revista *Animal Behaviour*.

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predicted a higher chance to observe visual cues in males that emitted less calls. We found no trade-off between call rate and the majority of visual cues observed (arm lifting, leg lifting, arm waving, and foot flagging). However, toe flagging displays and jumps toward the mirror, i.e. highly aggressive behaviour against an intruder, increased with increasing call rate. According to our results, males of *H. meridionalis* use both visual cues and acoustic signals to manifest their aggressive disposition. So low call emission may not be related with the use of visual cues by anurans. Therefore, visual cues are probably not an alternative, but complementary to acoustic signalling during intraspecific communication in anurans.

Keywords: visual displays, call rate, advertisement call, toe flagging, mirror presentation, frogs.

Introduction

Acoustic signalling is the predominant communication modality in anuran amphibians and probably is an ancestral character (Wells, 1977a). Depending on the social context, different types of calls can be emitted. The advertisement call, emitted by males, is the most frequently produced and it is considered the most relevant one (Toledo et al., 2014). Advertisement calls are usually emitted for long distance communication and its main functions are attraction of conspecific females for reproduction and territorial spacing among neighbouring males (Márquez et al., 2008; Toledo et al., 2014). Therefore, acoustic signals, especially the advertisement call, are crucial for the recognition of mating partners and for evolutionary processes as sexual selection and speciation (Ryan, 1988; Wilkins & Safran, 2013).

However, there are cases in which communication exclusively by acoustic signals may not be so advantageous for the individual reproductive success. For instance, there are anuran species that apparently do not produce calls during social interactions (e.g. *Leiopelma hamiltoni*, Waldman & Bishop, 2004), other species vocalize only sporadically (e.g. *Hylodes cardosoi*, Lingnau et al., 2008; *Sechellophryne gardineri*, Boistel et al., 2013), or have calls with very low intensities (e.g. *Chalcorana chalconota*, Márquez & Eekhout, 2006). Different ecological pressures, e.g. predation risk, parasitism and environmental factors, may be selecting this reduction in acoustic expression (Wells, 1977a; Tuttle & Ryan, 1982). Background noise might interfere with the perception of acoustic signalling, but not on the perception of other signal modalities. For example, males of *Staurois parvus* reduce its calling activity as a response to increase in stream noise (Grafe & Tony, 2017). Nevertheless, this diurnal species found a curious alternative to communicate under noisy environmental conditions by increasing the emission of foot flagging displays (visual modality; Grafe & Tony, 2017). Therefore, other communication modalities (e.g., visual, de Sá et al., 2016; chemical, Starnberger et al., 2013; and vibrational, Caldwell et al. 2010) may have evolved as an alternative to acoustic signalling in anurans.

In a review about visual signalling in anurans, Hödl and Amézquita (2001) requested more attention for this poorly explored anuran behaviour. Since then, the visual repertoire, varying from absence of visual cues (e.g. *Pithecopus megacephalus*, Oliveira et al., 2012; *Micrixalus sali*, Biju et al., 2014) to a rich visual repertoire (e.g. *Hylodes japi*, de Sá et al., 2016; *Hylodes meridionalis*, Souza, 2019), has been described for many species during social contexts. Different types of visual cues can be observed in anurans, including limb movements (e.g. limb lifting, foot flagging), body movements (e.g. upright posture, vocal sac display) and colouration (e.g., colour changing, throat colouration display) (Hödl & Amézquita, 2001; Hartmann et al., 2005; Biju et al., 2014; de Sá et al., 2016). However, the factors that could explain the variation on visual repertoire diversity in anurans remain unknown.

Species using visual signalling may also use acoustic signals to communicate (e.g. *Allobates femoralis*, Narins et al., 2003). Therefore, it is important to understand not only the role of each modality, but also the relationship between visual and acoustic signalling in anuran communication. Visual signalling could increase the strength of messages sent by acoustic signals (Narins et al., 2003). For example, males of *Bokermannohyla sapiranga* are characterized by emitting low advertisement and territorial call rates, but their aggressiveness peaks when a conspecific intruder approaches emitting both visual and acoustic signals (Souza, 2014). Additionally, there are evidences that visual signalling can act as a localization cue of the sender (Narins et al., 2003) and help during sex recognition among individuals (Sztatecsny et al., 2012). Thus, multimodal communication may have been selected in species which males have low calling activity, since low advertisement call rate is less attractive to females than high call rate (e.g. Márquez et al., 2008), and may also impairs the signal orientation quality of females in a chorus (Beckers & Schul, 2004).

We used two approaches to test the hypothesis that visual cues can be an alternative to acoustic signalling during intraspecific communication in anuran amphibians. First, we verified if there is a trade-off on species level between the emission rate of advertisement calls given by males and the previous reported visual repertoire diversity in 69 species. Controlling for phylogenetic effect, we predict to observe a larger visual repertoire in species which males emit less advertisement calls per minute (call rate) during social interactions. In this case, the acoustic activity could explain the variation on visual repertoire diversity observed in anurans. Secondly, we selected one anuran species (*Hylodes meridionalis*) which males produce calls in noisy environments (Kwet et al., 2010) and perform different visual cues during social interactions (Souza, 2019) to investigate if there is a trade-off at the individual level between the call rate and the emission rate of visual cues. The prediction if there is a trade-off is that visual signals will be used more often by males of *H. meridionalis* that emit less calls per minute when submitted to aggressive stimuli using mirror presentations in order to simulate the presence of an intruder. Alternatively, the visual cues may be used as a complement to acoustic signalling in anurans. Under this hypothesis, the prediction would be that the visual repertoire diversity and emission rate of visual cues would increase with increasing in call rate.

Methods

Call Rate X Visual Repertoire Diversity

To test if there is a trade-off between visual repertoire diversity and call rate in anuran species, we first compiled the visual cues observed during intraspecific communication reported in the literature. We searched the Web of Sciences database (Thompson Reuters) and Goggle scholar on 2016, without restrictions on publication year and using the following combination of keywords: (visual) AND (signal OR display OR cue OR communication) AND (anuran OR frog OR toad OR tree frog). From the search results, we selected those papers whose authors mentioned at least the presence or absence of visual cues in males and/or females during social interactions. We compiled the following information from each selected paper to create our database: studied species; presence or absence of visual cue(s); and type(s) of visual cue(s). Additionally, we consulted experienced researchers (Dr. Fausto Nomura, Dr. Albert S. Feng, Dr. Lucas R. Forti, and MSc. Camila I. Medeiros) to contribute with species without any visual cues during intraspecific communication; since the absence of behaviour is not commonly mentioned in papers.

The visual cues were classified according to the motor patterns described by Hödl and Amézquita (2001), Hartmann et al. (2005), Starnberger et al. (2011), Souza (2014, 2019), and de Sá et al. (2016). Because of the lack of experimental studies testing the communication function of each visual cue, we could not distinguish between visual cues and visual signals for carrying out the statistical analyses (in our database, visual signals were confirmed only in nine species; Table S1). We considered visual cue as any stereotypical behaviour (visual display that can be "on" and "off" by the sender) or morphological (e.g., colour changing) trait visually transmitted, with exception of body size. On the other hand, visual signal is a specific type of visual cue selected to transmit information between individuals (communication function, Hödl & Amézquita, 2001). In summary, our database was composed of species without any visual cue ("zero class") and species with at least one visual cue (including visual signal) previously reported.

To determine the call rate of advertisement calls for each species of our visual database, we analysed 282 recordings deposited by researchers at Fonoteca Zoológica (Fonozoo, Museo Nacional de Ciencias Naturales, CSIC, Madrid). For the recording selections we gave priority to high quality recordings (those where it was easier to recognize the focal male call) and the audio file format (.wav). We followed call descriptions published in scientific papers for correct identification of advertisement calls in the recordings. To determine the advertisement call rate for each species, we analysed recordings of up to 10 males, mostly of them from different study sites, per species. We visually counted, for each male, the number of calls per minute in a Dell P41G computer with Windows 10 operating system using Raven Pro 64 1.4 software.

To control for phylogenetic effect (similar behaviour in phylogenetically closely-related species) in our analysis, we built a phylogenetic tree for the 69 species included in our database based on a published molecular phylogeny and dated super-tree for amphibians (Pyron, 2014). For those species of our database that did not appear in the super-tree proposed by Pyron (2014), we inferred their position in the phylogenetic super-tree based on previous knowledge about the taxonomic relationships established by molecular, morphological and/or natural history data (Faivovich, 2002; Faivovich et al., 2005; Fabri, 2013; Lourenço et al., 2015; Frost, 2019). We used a semi-parametric method based on Penalized Likelihood Approach to estimate the age of included nodes (function ‘chronopl’ of package ape in software R; Sanderson, 2002; Paradis, Claude & Strimmer, 2004; R Core Team, 2018). After including all species in the super-tree and adjusting node ages, we excluded all other species present in the super-tree but absent in our database. By doing so, we obtained a dated phylogenetic sub-tree containing only the species included in our database.

Finally, we used a Phylogenetic Generalized Linear Model (function ‘phyloglm’ of package *phylolm* in software R; Ho & Ané, 2014) to verify if anuran species in which males emit less advertisement calls per minute (predictor variable: call rate) have a larger visual repertoire (response variable: number of different visual cues) controlling the phylogenetic effect. Since the response variable is a quantitative class number (counting data), we used the "poisson_GEE" method, that solves the generalized estimating equations (GEE) for Poisson regression (Ho & Ané, 2014).

Call Rate X Emission Rate of Visual Displays

To investigate if there is a trade-off between the emission of acoustic signals and the emission of visual displays at the individual level, we observed males of *Hylodes meridionalis* (Family Hylodidae) in their natural habit. *Hylodes meridionalis* is an endemic frog from the south mountains of the Brazilian Atlantic Forest. During reproductive season, males of this species call during daytime on rocky rivulets of clean water (Kwet et al., 2010). Advertisement and territorial calls were described by Lingnau et al. (2013). More recently, Souza (2019) reported a rich visual repertoire, including limbs and throat movements performed by males and females during close distance interactions. Moreover, they found a greater emission of visual displays than acoustic signals by individuals during social interactions in natural conditions (Souza, 2019). The representative use of both communication modalities (visual and acoustic) makes this species the perfect study animal to test our hypothesis.

We recorded males of *H. meridionalis* in January, September and November 2016 and February 2017 in three fast streams in the Atlantic Forest, in the São Francisco de Paula National Forest, southern Brazil (29°29’13.3” S and 50°13’12” W). Observations occurred in the field between 13h and 18h. We selected focal males in the field arbitrarily and recordings were captured using a video camera (Panasonic HC-W850) positioned at least 1.5 m from the individual.

Since communication occurs when one individual affects another and the receiver responds to the stimulus (Maynard-Smith & Harper, 2003), we submitted the focal males to mirror self-image presentations to simulate the presence of a conspecific receiver. Therefore, the reflection simulates an intruder male in the resident's territory. For that, we gently positioned a mirror in the visual field of actively calling resident males. The mirror (15 x 15 cm) was positioned at a distance of 15–25 cm, and at an angle of approximately 45° in relation to the male's body position to avoid a possible blind spot at 0° (directly in front of the animal; Fite, 1973; Furtado et al., 2017). The visual and acoustic responses of each focal animal were recorded for at least 3 minutes; and females were not observed close to focal males during the recordings.

To prevent observing the same individual more than once, after each recording we placed the focal animals in terrariums until the end of each sampling period (not exceeding 3 consecutive days). All the animals were released exactly at their capture site, with apparent good health; and each sampling period was conducted in a different stream or in a different section of the same stream.

The visual responses were classified according to the motor patterns described by Souza (2019), and the acoustic responses following Lingnau et al. (2013). We analysed the video images in a Dell P41G computer with Windows 10 operating system using VLC Media Player software to determine the emission rate of each visual display and acoustic signal. We used Pearson Correlations (function ‘cor.test’ of package *stats* in software R) to check if there was a correlation between the rate of visual displays emissions (in total and each type individually) and the advertisement call rate performed by males of *H. meridionalis* presented with aggressive stimulus. The statistical analyses and graphs were performed in the R environment (R Core Team, 2018).

Results

Call Rate X Visual Repertoire Diversity

Our database was composed of 69 anuran species from 15 families (Figure 1). We compiled 28 different visual cues used during social interactions by males and females from the literature (a total of 53 articles; Table S1). We classified the visual cues in four categories: (1) limb movements, with nine types of visual displays; (2) body movements – stationary, with 14 types of visual displays; (3) body movements – displacement, with three types of visual displays; and (4) colouration, with only two types of visual cues (display of throat colouration and colour change) (Table S1).

The visual repertoire varied from absence of visual cues (13 species) to 12 visual cues in *Hylodes phyllodes* (Table S1). The rarest visual cues were toe posture, hind foot lifting, head bobbing, head snaking, and body wiping displays, performed by only one species each; and the most common visual cue was limb lifting display, performed by 40.6% of studied species (28 species; Table S1). About the acoustic data, the advertisement call rate varied from 1.08 calls/min in *Hylodes meridionalis* to 219.74 calls/min in *Scinax nasicus* (Figure 1). However, despite the great variation of visual repertoire and call activity observed in the studied species (Figure 1), we found no relationship between visual repertoire diversity and the advertisement call rate (phyloglm: $Z = 1.17$; $P = 0.23$).

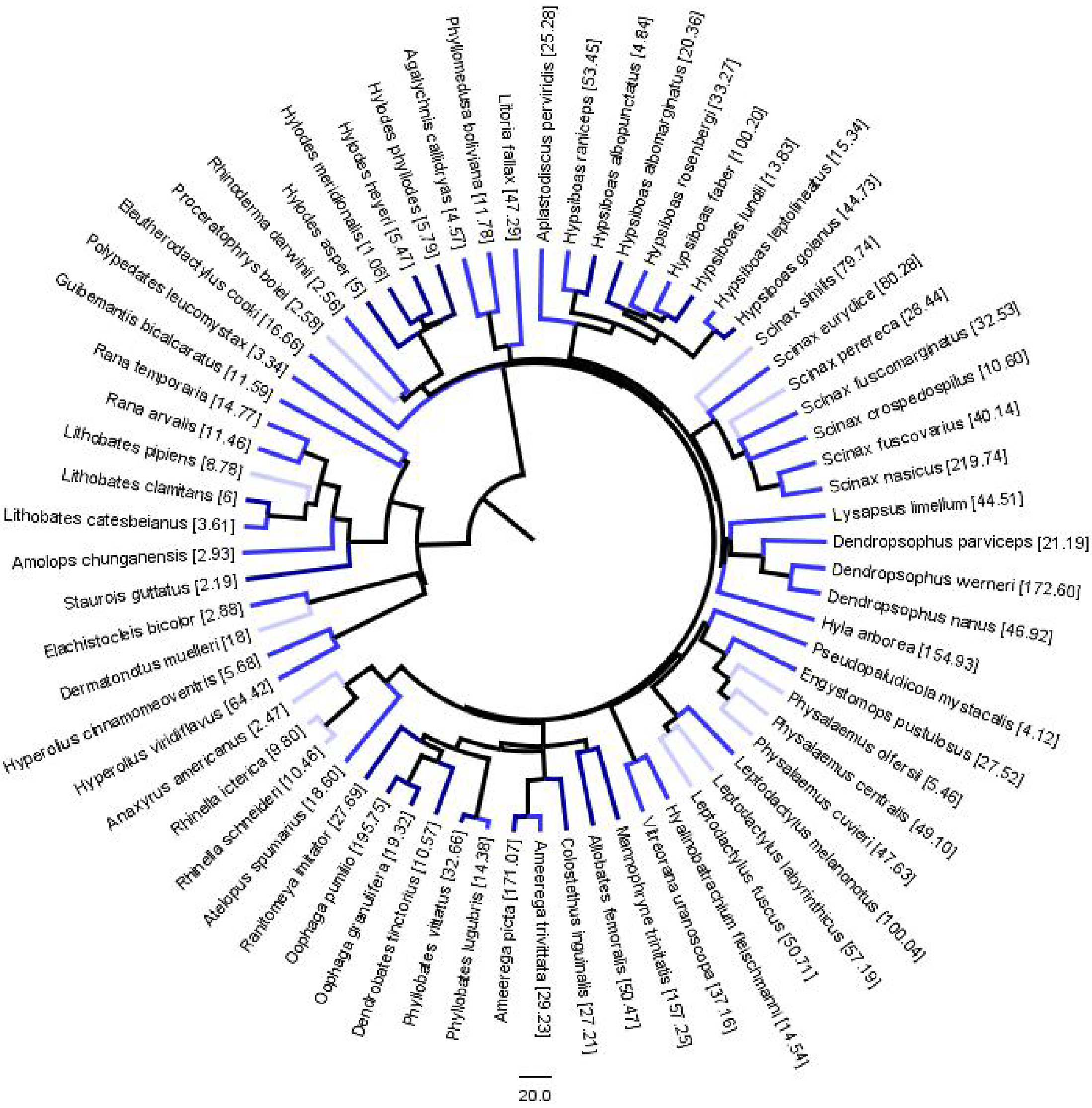

Figure 1 Phylogenetic relationships among anuran species (69 species, 15 families) included in the study based on data from Pyron (2014), Faivovich (2002), Faivovich et al. (2005), Fabri (2013), Lourenço et al. (2015), and Frost (2019). The intensity of blue colour of the terminal branches indicates the visual repertoire diversity (total of 28 visual cues). The database is composed by species without any visual cues (very light blue) to species with 12 visual cues (very dark blue). Numbers between [ ] indicate the advertisement call rate (number of calls per minute) measured from recordings deposited in Fonoteca Zoológica (MNCN, CSIC, Madrid).

Call Rate X Emission Rate of Visual Displays

We observed advertisement calls (24 events, 8 males) and five types of visual displays performed by 13 males of *Hylodes meridionalis*: (1) Toe flagging – slow up-and-down movements of all toes of one foot (1 event). (2) Arm lifting – rapid up-and-down movement of one arm, without extending it (4 events, 3 males). (3) Leg lifting – rapid up-and-down movement of one leg, without extending it (3 events, 3 males). (4) Arm waving – lifting an arm and waving it up and down in an arch beside the head (20 events, 13 males). Arm lifting, leg lifting and arm waving were high-speed displays performed with right or left limbs. (5) Foot flagging – raising slowly one hind limb, in a semi-arc movement, above the substrate level and returning it to the body side (4 events, 2 males). Foot flagging was performed with the right or left leg, and sometimes there was alternation of sides.

We found no correlation between the emission rate of the majority of visual displays (arm lifting, leg lifting, arm waving and foot flagging) and the advertisement call rate in *H. meridionalis* (Table 1). However, the emission of toe flagging display was correlated with advertisement call rate (Table 1). As opposed to our trade-off prediction, the only toe flagging observed during our study was performed by the same male which produced the highest advertisement call rate (2.33 events/min).

Furthermore, we observed eight males jumping towards or attacking the mirror, which was positioned in their territory to simulate a conspecific intruder male. While these behaviours do neither represent visual nor acoustic signals, they suggest the aggressive disposition of these resident males. This very aggressive response had a positive and strong correlation with advertisement call rate in *H. meridionalis* (Table 1, Figure 2), but not with toe flagging display (Pearson's r = 0.03, *t* = 0.1, df = 11, *p* = 0.91).

Table 1 Pearson Correlations comparing the emission per minute of visual cues and jumps toward the mirror with the advertisement call rate of 13 males of *Hylodes meridionalis* submitted to mirror self-image presentations in the Brazilian Atlantic Forest. (df) and (*) means degrees of freedom and significant values, respectively.

	Behaviour	Pearson's r	*t*	df	*p*
Visual Response	All visual cues	-0.02	-0.06	11	0.94
	Toe flagging	0.63	2.74	11	0.02*
	Arm lifting	0.19	0.66	11	0.52
	Leg lifting	0.05	0.19	11	0.85
	Arm waving	-0.13	-0.44	11	0.67
	Foot flagging	<0.01	0.02	11	0.98
Jump towards the mirror		0.73	3.51	11	0.004*

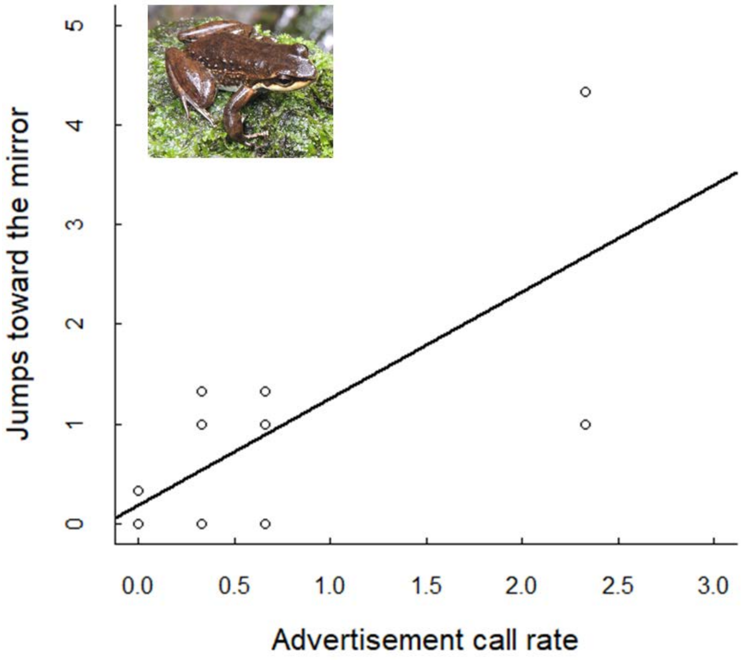

Figure 2 Number of jumps toward the mirror per minute performed by 13 males of *Hylodes meridionalis* in relation to their advertisement call rate (calls/minute). The focal males were submitted to mirror self-image presentations, in the Brazilian Atlantic Forest, to simulate the presence of a conspecific intruder male into their territory. The jump behaviour includes jumps closer to the mirror and jumps attacking the mirror. Jumps and advertisement call rate were positively correlated (y = 1.07x + 0.19).

Discussion

Call Rate X Visual Repertoire Diversity

Our results indicate that low call emission is not a sufficient trigger for the use of different visual cues by anurans. Therefore, visual cues may not be an alternative to acoustic signalling during intraspecific communication in anurans. Commonly more than one signal modality, differing in signal efficacy and receiver perception, is used during social interactions (Higham & Hebets, 2013). Multimodal signals, for example, can be classified as redundant or non-redundant, depending on signal information content. Redundant signals have equivalent effects in the receiver, reinforcing the message. But non-redundant signals have different effects in the receiver ("multiple messages"), or even a third complete new effect when combined (Partan & Marler, 2005). Thus, the successful use of acoustic signals by anurans may have not been a barrier against the evolution of visual signals during intraspecific communication.

Despite the fact that our database included species representing many anuran families (15 out of 56 families), it represented less than 1% of anuran diversity known to date (69 out of a total 7027 species; Frost, 2019). However, though small, our sample of species showed a great variety of visual cues in most studied species (56 from 69 species had at least one visual cue previous reported). This shows that despite the efforts of Hödl and Amézquita´s (2001) claim for more attention to this fascinating but poorly-explored anuran behaviour, we still have much more to discover. For example, the present study is pioneer in considering the study of visual behaviour of anurans in association with acoustic signalling on a macro scale. The effect of other acoustic parameters besides call rate in the visual behaviour of anurans remains unclear, as the evolutionary aspects of both signalling modalities in multimodal communication.

In this context, amplitude of the calls (in decibels) might be an acoustic parameter able to better explain the presence of visual cues in anurans than call rate. Visual signalling in anurans has been related to short distance interactions, mostly up to 50 cm between sender and receiver (Hödl & Amézquita, 2001). The distance between individuals can, in its turn, be strongly related to acoustic signals emitted by males. The lower the call amplitude, the shorter is its range (Wells, 1977b). For example, during the chorus formation species which males produce calls with low amplitude have smaller territorial spacing than species which males produce calls with high amplitude (Murphy & Floyd, 2005). Therefore, production of calls with low amplitude results in social interactions at short distances (Wells, 1977b; Murphy & Floyd, 2005), which might favour the use of visual cues as a complementary signal modality.

Finally, the environment is one of the most important factors explaining character divergence (Nosil, 2012; Goutte et al., 2016). During the communication process, the environmental conditions (which depend of animal behaviour) are crucial to determine the quality and effectiveness of signal transmission and detection (Endler, 1999). Therefore, signals that maximize their detectability will be selected. However, the most detectable signals are not the same in different environments; and different signal modalities have different detectability in the same environment (Endler, 1999; Nosil, 2012). For example, background noise affects the transmission and detection of acoustic signals (Vélez et al., 2013) and, therefore, its evolution (Goutte et al., 2016), but it does not affect visual signals. However, the hypothesis that environmental factor can explain the distribution of visual signals in the anuran taxa still needs to be tested.

Call Rate X Emission Rate of Visual Displays

Although territorial call was previously reported to *Hylodes meridionalis* (Lingnau et al., 2013), we did not record it during our study, suggesting that advertisement calls play an important role during aggressive encounters. Together with the increase in advertisement call rates, males showed their aggressive disposition by jumping towards the mirror (which the reflection was simulating a competitor male), and one male performed toe flagging display. Therefore, visual displays are not an alternative to acoustic signalling in *H. meridionalis* during agonistic interactions. Our results support the alternative hypothesis which is the visual signalling as a complementary communication modality to acoustic signalling in anurans.

Complex aggressive repertoire, formed by visual and acoustic signals, might have been selected as a strategy to avoid the risks of physical encounter between individuals. The use of different signal components during an agonistic encounter can offer different opportunities for the opponents to evaluate and make a decision between staying and increasing their aggressiveness or move away, in an escalation of aggressive behaviour (Martins et al., 1998). Thus, fights could happen as a last resort during agonistic encounters. In this context, the non-production of territorial calls by males of *H. meridionalis* during our study was unexpected. It is possible that an acoustic stimulus is required to induce this response, which was not used in our mirror experiments. Therefore, future studies are required to test the specific adaptive function of visual displays in *H. meridionalis*; and also to test the relation of visual and acoustic signals to escalations of aggressive behaviour.

Hylodes meridionalis is a diurnal frog that lives in noisy environments (Kwet et al., 2010); two traits commonly related with visual signalling (Hödl & Amézquita, 2001). These traits are also found in *Staurois parvus*, which males reduce its calling activity and increase visual signals as a response to increase in stream noise (Grafe & Tony, 2017). In an undisturbed environment context (absence of environment manipulation, beyond our presence), we found that males of *H. meridionalis* produce both acoustic and visual response against an intruder in its territory. However, if a disturbance in the environment (e.g., increase in background noise) reduces the quality and effectiveness of signal transmission and detection of one of the communication modality, it needs to be verified if the animals could react by emitting more the other signal modality as in *Staurois parvus*. Reinforcing the hypothesis of complementary function between visual and acoustic signalling in anuran communication.

Conclusion

We conclude that anuran call activity, specifically the advertisement call rate during social interactions, is not correlated with the visual repertoire observed during intraspecific communication when analysing many species. When focusing in one species, *Hylodes meridionalis*, we found that males emit both visual displays and acoustic calls as response to intruders, with also no correlation between the emission rates. Therefore, visual signalling might not serve as an alternative to acoustic signalling in anurans, but might be part of multimodal signalling during social interactions.

Acknowledgments

We are grateful to Fausto Nomura, Albert Feng, Lucas Forti, and Camila Medeiros for their contributing to our visual database. We thank Almudena Garcia for her support in Fonoteca Zoológica (MNCN, CSIC-Spain) and Luísa Lermen for her assistance during field work. We thank Michael Bottesch and Valentina Zaffaroni for critically reading the manuscript. RF received scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil). SMH received a fellowship from the Brazilian Research Council (CNPq; grant 304820/2014-8). RM was funded by the Ministerio de Ciencia e Innovación (project TATANKA CGL2011-25062, Spain).

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Supplementary Material

Table S1 Visual repertoire of 69 anuran species during intraspecific interactions.

	Limb movements									Body movements - Stationary														Body movements - Displacement			Colour		
Family Species	Toe/finger trembling	Toe posture	Toe flagging	Limb lifting	Arm waving	Face wiping	Hind foot lifting	Leg stretching	Foot flagging	Wiping	Mouth opening	Head bobbing	Head snaking	Throat display	Vocal sac display	Body lowering	Body rising	Upright posture	Back raising	Body inflation	Two legged pushing	Body jerking	Body wiping	Truncated walking	Running jumping display	Circling	Throat colouration	Colour changing	References
Hyperoliidae																													
Hyperolius viridiflavus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Hyperolius cinnamomeoventris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1,2
Microhylidae																													
Dermatonotus muelleri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^{PO}
Elachistocleis bicolor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Nomura^{PO}
Ranidae																													
Staurois guttatus	0	0	0	1^{m}	$1a^{m}$	0	0	$1r^{m}$	$2b^{b}$	0	1^{f}	0	0	1^{m}	0	0	0	1^{f}	0	0	0	0	0	0	0	0	1^{b}	0	3,4
Amolops chunganensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	Feng^{PO}
Lithobates catesbeianus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	5, Medeiros^{PO}
Lithobates clamitans	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	1^{m}	0	0	$1a^{m}$	0	0	0	0	0	0	$1a^{m}$	0	1^{m}	0	6,7
Lithobates pipiens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Feng^{PO}
Rana arvalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$2r^{m}$	8,9,10,11
Rana temporaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	1^{m}	12
Mantellidae																													
Guibemantis bicalcaratus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	13
Rhacophoridae																													

Polypedates leucomystax	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	Feng^{PO}
Eleutherodactylidae																													
Eleutherodactylus cooki	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$2r^{m}$	0	14
Odontophrynidae																													
Proceratophrys boiei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^{PO}
Rhinodermatidae																													
Rhinoderma darwinii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$1r^{m}$	15
Hylodidae																													
Hylodes asper	0	0	$1a^{m}$	$1b^{b}$	0	0	0	$1b^{b}$	$2b^{m}$	0	0	0	0	0	0	0	$2b^{m}$	0	0	0	0	0	0	0	0	0	0	0	6,16,17,18,19
Hylodes meridionalis	0	0	$1a^{m}$	$1a^{m}$	0	0	$1a^{m}$	$1a^{m}$	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	1^{m}	0	0	0	Furtado^{PO}
Hylodes heyeri	0	1^{m}	1^{m}	$1a^{m}$	0	0	0	$1a^{m}$	0	0	1^{m}	0	0	1^{m}	0	1^{m}	0	1^{m}	0	0	0	0	0	0	1^{m}	0	1^{m}	0	16,20
Hylodes phyllodes	0	0	$1a^{m}$	1^{m}	0	0	0	$1b^{m}$	1^{m}	0	$1a^{m}$	0	1^{m}	1^{m}	0	$1a^{m}$	$1b^{m}$	$1a^{m}$	0	0	0	0	0	0	1^{m}	0	1^{m}	0	16,19,Forti^{PO}
Hylidae																													
Agalychnis callidryas	0	0	0	0	0	0	0	$1a^{m}$	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	$2a^{m}$	0	0	0	0	0	0	21,22
Phyllomedusa boliviana	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23
Litoria fallax	0	0	0	0	0	0	0	$1a^{m}$	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6,24
Aplastodiscus perviridis	0	0	0	$1a^{m}$	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	25
Hypsiboas raniceps	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	26
Hypsiboas albopunctatus	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	1^{m}	0	25,27
Hypsiboas albomarginatus	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	$1a^{m}$	0	$1b^{m}$	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	19,28,29
Hypsiboas rosenbergi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	6
Hypsiboas faber	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	Furtado^{PO}
Hypsiboas lundii	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	Furtado^{PO}
Hypsiboas leptolineatus	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	Furtado^{PO}
Hypsiboas goianus	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	27
Scinax similis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^{PO}
Scinax eurydice	0	0	0	1^{m}	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
Scinax perereca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^{PO},Furtado^{PO}
Scinax fuscomarginatus	0	0	0	$1a^{m}$	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	30,31
Scinax crospedospilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Nomura^{PO}
Scinax fuscovarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	1^{m}	Nomura^{PO},Furtado^{PO}
Scinax nasicus	0	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Furtado^{PO}
Lysapsus limellum	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26
Dendropsophus parviceps	1^{m}	0	0	0	$1a^{b}$	0	0	0	$2a^{m}$	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6,32
Dendropsophus werneri	0	0	0	$1a^{m}$	0	0	0	$1b^{b}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33

Dendropsophus nanus	0	0	0	1a^m	0	0	0	0	0	0	0	0	0	0	1a^m	0	0	0	0	0	0	0	0	0	0	0	1^m	0	26
Hyla arborea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^m	1r	34,35,36,37,38
Leptodactylidae																													
Pseudopaludicola mystacalis	0	0	0	0	0	0	0	0	0	0	0	0	0	1a^m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39
Engystomops pustulosus	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^m	0	0	0	0	0	0	0	0	0	0	0	0	1^m	0	40,41,42,43
Physalaemus olfersii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO
Physalaemus centralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO
Physalaemus cuvieri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO
Leptodactylus melanonotus	1^m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^m	0	0	1^m	0	0	0	0	0	0	0	0	0	6
Leptodactylus labyrinthicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO
Leptodactylus fuscus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO
Centrolenidae																													
Hyalinobatrachium fleischmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^m	0	0	0	0	0	0	6
Vitreorana uranoscopa	0	0	0	1a^m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
Aromobatidae																													
Mannophryne trinitatis	1	0	0	0	0	0	0	0	0	0	0	0	0	1^b	0	0	1^b	1^b	0	0	0	0	0	0	1^m	0	1^m	1^m	6,44
Allobates femoralis	1	0	0	1r^m	0	0	0	1r^m	0	0	0	0	0	2a^m	0	1	0	1	0	0	0	0	1r^f	0	0	1r^m	0	0	6,45,46,47,48
Dendrobatidae																													
Colostethus inguinalis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^f	0	1	1^b	0	0	0	0	0	0	1^m	0	0	6
Ameerega trivittata	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	6
Ameerega picta	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	6
Phyllobates lugubris	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	6
Phyllobates vittatus	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	6
Dendrobates tinctorius	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	6
Oophaga pumilio	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1^m	1	0	1	0	0	0	0	1	0	0	0	6,49,50,51,52
Ranitomeya imitator	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	6
Bufonidae																													
Atelopus spumarius	0	0	0	0	1^m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	53
Rhinella schneideri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO
Rhinella icterica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nomura^PO,Furtado^PO
Anaxyrus americanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Feng^PO

Visual behaviour: 0 = absence; 1 = visual cue; 2 = visual signal. Social context: r = reproductive; a = agonistic; b = both contexts. Sender's gender: ^m = male; ^f = female; ^b = both male and female. References: ^PO = personal observation; Nomura = Dr. Fausto Nomura; Feng = Dr. Albert S. Feng; Medeiros = MSc. Camila Ineu Medeiros; Furtado = MSc. Raíssa Furtado; Forti = Dr. Lucas Rodriguez Forti.

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CAPÍTULO 4

Visual behavior during intraspecific interactions in anurans: phylogeny, environment and color factors

Visual behavior during intraspecific interactions in anurans: phylogeny, environment and color factors[♣]

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Abstract Visual signals are redundant, visible and stereotyped visual cues sent by an individual that provoke an immediate response from another individual receiving those signals through a specific environmental context. In anurans, there are evidences that visual communication probably is related to diurnal habits, reproduction next waterfalls and conspicuous coloration, despite their ancestral features being related to nocturnal habits, reproduction in lentic environments, and cryptic coloration. We reviewed the diversity of visual cues during intraspecific interactions in living anuran amphibians in relation to contrasting environment contexts and color patterns, and along the evolutionary history of the group. First, we discussed the importance of distinguishing visual cues (any morphological or behavioral traits that provides a visual cue) from visual signals (visual cues with communication function). Then, we compiled 29 different visual cues (limb movements, body movements and coloration), reported during reproductive and territorial contexts, and distributed among 129 anuran species

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[♣] Este capítulo segue as normas de idioma e formatação da revista *Biological Reviews*.

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from 21 families. We also performed comparative analyses to test for the effects of phylogenetic history, environment context, and conspicuous color pattern on the variation of visual repertoire in anuran taxa. The evolution of visual cues across anuran lineages was best explained by Brownian motion, suggesting a neutral evolutionary process. While environment explained the variation of visual cues repertoires better than phylogeny, most variation remained unexplained. Visual signal variation was best explained by the Orstein-Uhlenbeck evolutionary model, suggesting that stabilizing selection drove visual signaling in anurans. Indeed, phylogeny explained most variation of visual signals in anurans, while environmental and phylogeny-structured environmental effects explained only a small fraction of variation. Visual repertoire was only weakly explained by individual color pattern. Therefore, environmental factors and individual conspicuousness seem not to be the only selective forces required for the divergence and evolution of visual signals during intraspecific communication in anurans. In conclusion, visual cue repertoires seem to evolve independently in different anuran lineages, likely as displacement activities not subjected to strong selection. Yet, in some specific lineages, such cues become true visual signals that evolve by selection mediated by environmental context.

Keywords: visual signals, visual communication, phylogenetic signal, mode of evolution, diurnal habits, open habitat, natural noisy environment, conspicuousness.

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I. Introduction

'*The first aggressive act by the resident [male of Mannophryne herminae] is usually a hopping approach to the intruder and a challenge, in which the bright yellow throat is exhibited and slowly pulsated.*' – F. H. Test (1954, p. 140).

In anurans, the efficiency of intraspecific communication is directly related to individual reproductive success, since males must defend their territory from other males and also attract conspecific females (Wells, 2010). During the communication process, those signals that maximize their detectability will be selected. Since the environments differ in their transmission properties, distinct signals will be more detectable in different environments (Nosil, 2012). For example, males of *Mannophryne herminae* have a bright yellow throat that is visually very conspicuous against the background of their calling site – gray rocks (Test, 1954). Therefore, the divergence of intraspecific signals is driven mostly by environment, sexual selection and competition factors (Rundle & Nosil, 2005; Nosil, 2012).

Acoustic communication is probably an ancestral character in anurans and it offers opportunities for male sexual selection by females and also can act as territorial defense (Wells, 1977, 2010; Halliday, 1983; Ryan, 1988; Márquez, 1995; Márquez, Bosch & Eekhout, 2008). Therefore, acoustic signals are crucial in the recognition of conspecifics and in evolutionary processes such as sexual selection and speciation (Ryan, 1988). The relationship between acoustic parameters and the environmental factors in which the social interaction occurs is now well established (Haddad, 1995; Oseen & Wassersug, 2002; Bosch & De la Riva, 2004). However, the presence of phylogenetic signal in the acoustic parameters in anurans has only been investigated recently. There are evidences of strong association between the acoustic signal and the phylogenetic relationship between species (Erdtmann & Amézquita, 2009; Goicoechea, De La Riva & Padial, 2010; Gingras *et al*., 2013; Goutte *et al*., 2016; Sulbarán *et al*., 2018). Similarly to acoustic signals, studies indicate that visual cues also appear to be widely distributed among anurans (Hödl & Amézquita, 2001; Hartmann *et al*., 2005; Biju *et al*., 2014; de Sá *et al*., 2016; Furtado *et al*., 2017), and the visual repertoire during inter- and intraspecific interactions seems to be more similar within rather than between families (Hödl & Amézquita, 2001).

In unique detailed review about visual signaling in anurans, Hödl and Amézquita (2001) point out that visual signals provide redundant, visible and stereotyped visual cues during an interaction, which must provoke an immediate response from the receiver in order to be efficient. In this context, it seems reasonable to hypothesize that different dynamic visual cues (e.g. pulsation of the vocal sac by males of *Mannophryne herminae*, Test, 1954), different color patterns (e.g. bright yellow throat in males of *Mannophryne herminae*, Test, 1954) or both simultaneously (multimodal communication; see review by Higham & Hebets, 2013) can play important roles during social interactions in anurans. Hödl and Amézquita (2001) provided a detailed compilation of 18 types of visual cues distributed in 56 anurans species, performed during both inter- or intraspecific communication, with the aim to claim more attention for this poorly explored anuran behaviour.

Since then, visual communication in anurans has been mainly related to diurnal habits (e.g., *Allobates femoralis*; Narins, Hödl & Grabul, 2003), reproduction near noisy waterfalls (e.g., *Micrixalus saxicola*; Preininger *et al*., 2013a) and conspicuous coloration (e.g. *Phrynobatrachus krefftii*; Hirschmann & Hödl, 2006). Such ecological contexts and traits contradict some anuran ancestral features, such as nocturnal habit, reproduction in lentic environments, and cryptic coloration (Duellman & Trueb, 1994). Visual signaling may have evolved as an alternative to acoustic communication in noisy environments, such as waterfalls (Grafe *et al*., 2012). Furthermore, the intensity of light in the environment and the conspicuousness of individual color can facilitate the transfer of visual signals (Cole & Endler, 2016). Hödl and Amézquita (2001) observed a more complex visual repertoire in anuran species that breed in noisy environments or at terrestrial sites; suggesting that different selective forces may be acting in the evolution and divergence of visual signals in anurans. Therefore, a combination of environmental pressures may be the main factor to explain the evolution of visual signaling in the anuran taxa.

However, the identification and analysis of visual signals is not very simple. For example, during social interactions individuals can exhibit involuntary behaviors, irrelevant movements during ongoing activity (Tinbergen, 1952; Furtado & Nomura, 2014; Souza, 2014; Furtado *et al*., 2017). These unintentional behavioral responses ("displacement activities") can be very stereotyped and easily misinterpreted as a signal display (Maestripieri *et al*., 1992). However, there is evidence that signals can evolve from other signals or pre-existing cues, as displacement activities (Tinbergen, 1952; Rosenthal, 2007). Therefore, visual cues with apparent no function could evolve independently across anuran taxa, but in some lineages, such diffuse visual cues could be selected by ecological pressures and become true visual signals.

In this study we reviewed the diversity of visual cues during intraspecific communication between adults of living anuran amphibians in relation to environment context and color pattern along the evolutionary history of the group. First, we discussed about the concepts of: visual cue, visual display, visual signal, and displacement activity. Then, we compiled the diversity of visual cues and visual signals during reproductive and territorial contexts and its distribution among anuran taxa. This allowed us to check the model of evolution that best explained the variation found in the repertoire diversity of visual cues and visual signals. We expected to observe Neutral Evolution on visual cues and selection acting on the visual signals (Fig. 1).

We also performed comparative analyses to test for the effects of phylogenetic history, environment context, and conspicuous color pattern on the variation of visual repertoire in the anuran taxa. By doing so, we were able to test the following hypotheses: (1) the emission of visual signals, but not visual cues, is strongly influenced by phylogenetic relatedness among species; (2) combined environmental factors (e.g. social interactions during daytime and in open and noisy environments) and conspicuous color patterns are important factors to explain visual signal diversity, but not visual cue diversity; (3) environmental and color factors are mostly structured phylogenetically (Fig. 1).

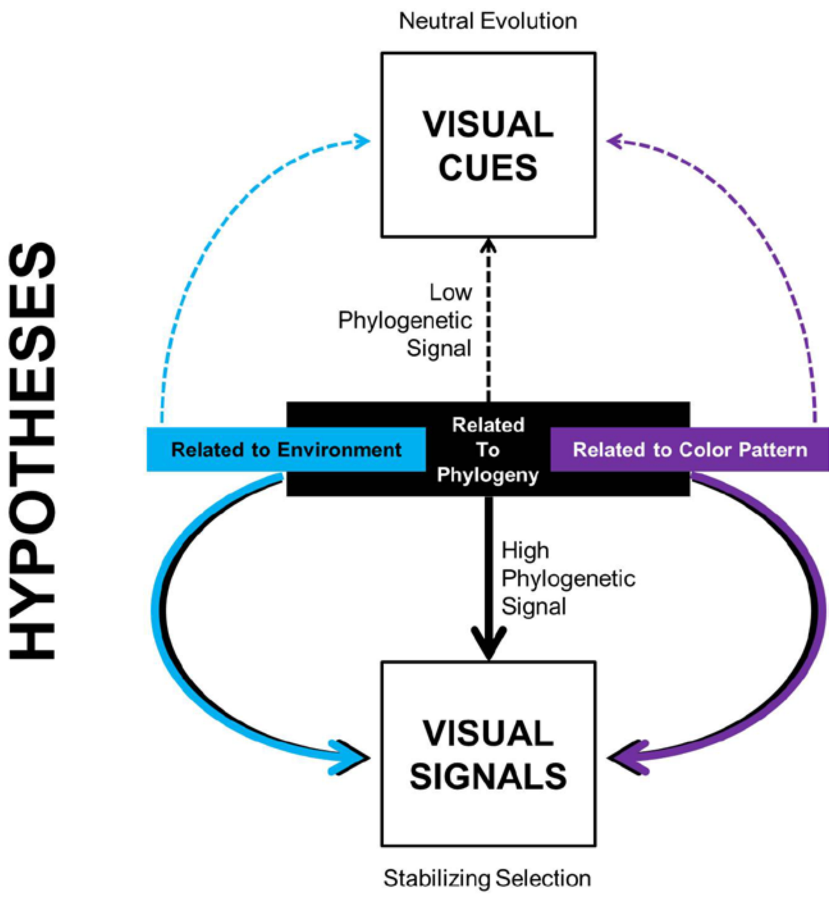


Fig. 1. Diagram of hypotheses. Diversity of visual cues and visual signals, during intraspecific communication in anurans, in relation to environment context and color pattern along the evolutionary history of the group. We expect that the models of evolution that best explain the variation in visual cues and visual signals are the Brownian Motion (Neutral Evolution) and the Ornstein-Uhlenbeck Model (Stabilizing Selection), respectively. Dotted arrows and complete arrows indicate weak and strong effects, respectively. Black, blue and purple colors indicate environmental, phylogenetic and color effects, respectively. Therefore, we have three main hypotheses: (1) the emission of visual signals, but not visual cues, is strongly influenced by phylogenetic relatedness among species; (2) environmental factors and conspicuous color patterns are important factors to explain visual signal diversity, but not visual cue diversity; (3) environmental and color factors are mostly structured phylogenetically.

II. Anuran Vision

In order to better identify and study the role of the visual modality in anuran communication, we must first try to understand how they possibly see the world. Amphibia is a very diverse group, and studies reveal a wide variation in visual capacity. However, in anurans, these studies have being limited to prey-catching contexts in only some species (e.g. *Lithobates pipiens*, *L. catesbeianus*, *Bufo bufo* and *Dryophytes cinereus*; see reviews by Stebbins and Cohen [1997], and Fite [2013]). Despite the evidence that anurans can have different visual capacities during prey-catching and mate choice (Yovanovich *et al*., 2017), we can state generally that the eyes of adult individuals are large and well-developed (Williams & Whitaker, 1994; Stebbins & Cohen, 1997; Fite, 2013).

Anurans can see in all directions at once, giving them one of the most expansive visual field among vertebrates (Fite, 1973, 2013; Stebbins & Cohen, 1997). The overlapping visual field of the two eyes (Fite, 1973) contributes to a stronger depth perception, being important to the judgment of distance (House, 2013). Anuran eyes are adapted to detect and rapidly respond to changes in the scenery, but often they have difficulty trying to observe a scene in stasis (Ewert, 2004). Thus, the best way for an anuran to visually perceive a prey, predator, potential mate or competitor is if it is moving across its visual field.

We can observe active anurans in a broad range of light-intensity environments, which depends on habitat choice and the extent of diurnal, crepuscular, nocturnal, and/or fossorial behavior of the individual. Therefore, we have species adapted for night or dim-light vision, probably an ancestor character in the group (Duellman & Trueb, 1994), but also species with the ability to see quite well in lighted surroundings (Stebbins & Cohen, 1997).

In addition to the variation in anuran color vision, many species are tetrachromatic, using four types of photoreceptors for a very sensitive sense of color: “green-sensitive” and “blue-sensitive” rods, active in dim light, and single and double cones, not active in dim light. The "green-sensitive" rods are photoreceptors common to all vertebrates, but the "blue-sensitive" rods are unique amphibian photoreceptors (Stebbins & Cohen, 1997; Yovanovich *et al*., 2017). Additionally, some aquatic species possess a third type of rod photoreceptor that contains the purple-receptive porphyropsin, which enables them to see in the ultraviolet range (Duellman & Trueb, 1994; Ries *et al*., 2008). Single and double cones contain a pigment that has peak absorbance at yellow wavelength, but the double cone also contains rhodopsin (Stebbins & Cohen, 1997). Recently, Yovanovich *et al*. (2017) verified that anurans can discriminate colors at very low light levels, even at the absolute visual threshold. Therefore, they can see colors event at night.

About the visual sense, it has been experimentally determined that anurans are neurologically more sensitive to movements, changes in light-intensity, and can identify the color and/or light contrast between an object and its background (Ansorge & Grusser-Cornehls, 1977; Ewert, 2004; Fite, 2013). Therefore, in a context of intraspecific interactions, we conclude that anurans have a good visual capacity adapted to different light-intensity environments, able to recognize a wide variety of colors, and a superior ability to recognize and respond to motion signals than to static signals.

III. What is a Visual Signal?

The communication process involves transfer of information through an environment from a sender to a receiver via signals specifically selected for each context. In turn, signals are features that act manipulating the receiver behavior (Endler, 1992, 1993a; Bradbury & Vehrencamp, 1998; Candolin, 2003; Maynard-Smith and Harper, 2003; Rendall, Owren & Ryan, 2009; Seyfarth & Cheney, 2016). Therefore, communication has been shaped by natural selection and signals, receptors, and behaviors are not suites of evolutionarily independent traits (Endler, 1992; Seyfarth & Cheney, 2016). For a signal to evolve it must be advantageous for both sender and receiver, which involves a co-evolutionary process (Endler, 1992; Maynard-Smith and Harper 2003; Seyfarth & Cheney, 2016).

Hödl and Amézquita (2001, p.122) '*define a visual signal if it is reported or personally observed that the behavioral event (1) provides a visual cue during an intra- or interspecific interaction, (2) is redundant, conspicuous, and stereotypical, and (3) most likely provokes an immediate response by the receiver that benefits the sender*'. In this context, it seems reasonable that different dynamic visual displays (displays that can be turned "on" and "off" by the sender, Hödl & Amézquita, 2001; Hartmann *et al*., 2005) and different color patterns (which include highly bright colors; Hoffman & Blouin, 2000; Rojas, 2016) can play important roles during social interactions in anurans. Also there is evidence that the visual cue of individual body size can influence the behavioral response of intraspecific receivers. For example, males of *Allobates femoralis* (Aromobatidae) attack small opponents more often than large opponents (de Luna, Hödl & Amézquita, 2010). However, body size was not included as visual cue in the present study.

Although the concept of visual signal is already well established, the identification of signals in non-human animals requires attention. For example, during social interactions anuran individuals can exhibit involuntary behaviors such as fast movements of limbs (e.g., *Hypsiboas albomarginatus*, Furtado & Nomura, 2014; *H. goianus* and *H. albopunctatus*, Souza, 2014; *H. raniceps*, *Dendropsophus nanus* and *Lysapsus limellum*, Furtado *et al.*, 2017). This kind of displacement activity is a behavioral pattern characterized by apparent irrelevance during ongoing activity and is elicited by motivational conflict (fight or flight) or in situations of stress, like when an animal is prevented from attaining its goal (Tinbergen, 1952; Maestripieri *et al.*, 1992; Troisi, 2002). These unintentional behavioral responses can be very stereotyped, with motor patterns focused mostly on one's own body such as self-touching (Troisi, 2002), and can be easily misinterpreted as a signal display (Maestripieri *et al.*, 1992). Therefore, it is important to emphasize that, although displacement activities might be a good indicator of stress levels (Troisi, 2002) they are not communication signals (information content, Maestripieri *et al.*, 1992).

However, visual signals assumedly evolved from pre-existing runways or other signals (Hödl & Amézquita, 2001). Thus, unintentional behavioral responses could evolve to become “bona fide” signals, since studies with ritualization of a displacement activity during evolution reveal an increase in the differences between the ritualized behavior and the original (Tinbergen, 1952; Rosenthal, 2007). The fact that signals may derive from displacement activities raises the question of how to make a distinction between them. Troisi (2002), for example, supports the idea that a displacement activity is always fragmentary and incomplete, when compared with the same activity carried out in its normal mood and context.

In summary, in the present study “visual cue” was considered as any morphological or behavioral trait that provides a visual cue during a social interaction, but it did not necessarily evolve for communication purpose (adapted from Davies *et al*., 2012). The kind of visual cue which can be turned “on” and turned “off” by the sender was termed “visual display” (adapted from Hödl & Amézquita, 2001). Visual cues (which may or may not be visual displays) that have at least partly been modified by natural selection for the purpose of communication were termed “visual signals” (adapted from Hödl & Amézquita, 2001 and Davies *et al*., 2012). Finally, when a visual display, demonstrably, had no communication function during an ongoing activity, it was classified as a “displacement activity”.

(1) How to Study Visual Signals?

Given the difficulty of accurately identifying a behavior as a visual signal (Hödl & Amézquita, 2001), studies of animal communication require accurate analysis testing the behaviors and ecological contexts in which these displays are performed. Therefore, it is important for studies on visual communication of anuran amphibians not being restricted to observations but also including hypothesis testing using in situ or ex situ experiments, in order to avoid misinterpretation of behavioral responses.

Several experimental designs can be employed depending on the question to be answered, for example: (1) mirror self-image presentations (territorial context) - the reflection in the mirror simulates the presence of a conspecific intruder; (2) picture or video presentations (both reproductive and territorial contexts) - simulation of a conspecific intruder or mate choice experiments; (3) introducing adult males/females next to resident males/females (both reproductive and territorial contexts); (4) presentations of artificial models (both reproductive and territorial contexts), simulation of a conspecific intruder or mate choice experiments; (5) playback experiments with conspecific calls (both reproductive and territorial contexts) - simulation of a conspecific intruder/neighbor or mate choice experiments; and (6) multimodal experiments, which includes visual (mirror self-image, picture, video or artificial model presentations) and other modality(ies) (usually acoustic) of stimuli (both reproductive and territorial contexts). A list of experimental studies which investigated the visual communication in anuran species is available in Table 1.

Table 1. Experimental designs in studies of visual signaling during intraspecific communication in anuran amphibians.

Experimental design	Species studied	Reference
Mirror self-image presentations	*Brachycephalus ephippium*	Pombal *et al.*, 1994
	Atelopus zeteki	Lindquist & Hetherington, 1998
	Hylodes asper	Haddad & Giaretta, 1999
	Hypsiboas albomarginatus	Furtado & Nomura, 2014
Picture or video presentations	*Hyla arborea*	Gomez *et al.*, 2009
	Hyla versicolor	Reichert & Höbel, 2015
Introducing adult males/females next to resident males/females	*Atelopus varius*	Crump, 1988
	Brachycephalus ephippium	Pombal *et al.*, 1994
	Atelopus zeteki	Lindquist & Hetherington, 1998
	Oophaga pumilio	Summers *et al.*, 1999; Maan & Cummings, 2008, 2009
	Dendropsophus parviceps	Amézquita & Hödl, 2004
	Hypsiboas albomarginatus	Giasson & Haddad, 2006
	Phrynobatrachus krefftii	Hirschmann & Hödl, 2006

	Rana arvalis	Ries *et al*., 2008
	Nectophrynoides asperginis	Arch, Richards-Zawaki & Feng, 2011
Presentation of artificial models	*Atelopus zeteki*	Criswell, 2008
	Agalychnis callidryas	Caldwell *et al*., 2010
Playback experiments with conspecific calls	*Atelopus zeteki*	Lindquist & Hetherington, 1996
	Aplastodiscus eugenioi	Hartmann *et al*., 2005
	Hypsiboas albomarginatus	Hartmann *et al*., 2005; Giasson & Haddad, 2006
	Vitreorana uranoscopa	Hartmann *et al*., 2005
	Hylodes asper	Hartmann *et al*., 2005
	Hylodes phyllodes	Hartmann *et al*., 2005
	Staurois guttatus	Grafe & Wanger, 2007
	Aplastodiscus perviridis	Toledo *et al*., 2007
	Hypsiboas albopunctatus	Toledo *et al*., 2007
	Hypsiboas bischoffi	Toledo *et al*., 2007
	Scinax maracaya	Barros & Feio, 2011
	Staurois parvus	Grafe *et al*., 2012
	Nectophrynoides tornieri	Starnberger *et al*., 2011
Multimodal experiments, which includes visual and other modality(ies) (usually acoustic) of stimuli	*Allobates femoralis*	Narins *et al*., 2003, 2005; de Luna *et al*., 2010
	Engystomops pustulosus	Rosenthal *et al*., 2004; Taylor *et al*., 2008; Taylor & Ryan, 2013
	Hylodes nasus	Wogel, Abrunhosa & Weber, 2004
	Scaphiopus couchii	Vásquez & Pfennig, 2007
	Hyla squirella	Taylor *et al*., 2007
	Agalychnis callidryas	Caldwell et al., 2010
	Micrixalus saxicola	Preininger *et al*., 2013a
	Bokermannohyla sapiranga	Souza, 2014
	Hypsiboas goianus	Souza, 2014
	Hypsiboas albopunctatus	Souza, 2014
	Hyla arborea	Troïanowski *et al*., 2014

During the experimental design, studies with visual stimuli share one main methodological question: where to place the visual stimulus (e.g. mirror or artificial model) in relation to the focal animal? Most visual interactions between conspecific anuran individuals were reported at short distances (less than 50 cm, see review by

Hödl & Amézquita, 2001). However, several factors can influence this distance. For example, diurnal species in which the interactions occur in a very visually unobstructed environment (e.g. without emerging vegetation), and at least the sender individual has a conspicuous color pattern, the visual interaction may occur in distances larger than 50 cm (e.g. *Staurois guttatus*, Grafe & Wanger, 2007; *S. latopalmatus*, Preininger, Boeckle & Hödl, 2009). However, in nocturnal species where social interactions occur in environments with dense vegetation, and whose individuals have cryptic color pattern, it is possible that the visual interactions may occur at distances shorter than 25 cm (e.g. *Dendropsophus parviceps*, Amézquita & Hödl, 2004). Therefore, is important to conduct previous observational studies, for each focal species, to determine the distance between focal animal and stimulus with more precision.

Additionally, the angle of the visual stimulus in relation to the focal animal body position may influence the quality of the signal. Different anuran species have different visual fields, mainly caused by the different positions of the eyes in the skull (Grüsser & Grüsser-Cornehls, 1976). Studies that test their ability to catch preys showed a lower visual capacity in the field directly in front of the focal animal (at 0°), in different anuran species (Fite, 1973; Grüsser & Grüsser-Cornehls, 1976). Those species with more lateral eyes have a higher blind spot at 0° (Grüsser & Grüsser-Cornehls, 1976). Therefore, we strongly suggest that visual experimental studies with anuran species avoid placing visual stimuli directly in front of the focal animal.

IV. Diversity of Visual Cues and Visual Signals in Anuran Taxa

(1) The Database

Visual cues observed during intraspecific interactions between adults of anuran species were compiled from the literature. We searched the Web of Sciences database (Thompson Reuters) and Goggle Scholar in 2016, without restrictions on publication year and using the following combination of keywords and wildcards: (visual) AND (signal* OR display* OR cue* OR communication*) AND (anuran* OR frog* OR toad* OR tree frog*). From the research results, we selected those papers whose authors mentioned at least the presence or absence of visual cues in males and/or females during social interactions. We compiled the following information from each selected paper to create our database: studied species; presence or absence of visual cue(s); type(s) of visual cue(s); if the visual cues have communication functions during social interactions (categories: (1) unknown function or no function; and (2) communication function); the general social context (reproductive: male-female interactions; and/or agonistic interactions) in which the visual cues were reported; and sender sex (female, male, or both).

In total, 99 papers published since 1954 were evaluated. Between 1954 and 2001 the description of visual behaviors in natural history studies in anurans was very sporadic (20 papers in 47 years, Fig. 2), and in only five of them the use of visual behaviors during social contexts was the aim of the study (Harding, 1982; Lindquist & Hetherington, 1996, 1998; Haddad & Giaretta, 1999; Summers *et al*., 1999). However, the number of papers in which the authors mentioned at least the visual cues during ongoing social interactions increased considerably after 2001 (79 papers in 15 years, Fig. 2), not coincidentally the year when the seminal book chapter entitled "Visual signaling in anuran amphibians" was published by W. Hödl and A. Amézquita (Hödl & Amézquita 2001).

Additionally, we consulted experienced researchers (Dr. Fausto Nomura, Dr. Albert S. Feng, Dr. Lucas R. Forti, Dr. Valentina Zaffaroni Caorsi, MSc. Fernando José María Rojas-Runjaic and MSc. Camila I. Medeiros) to contribute with species without any visual cues during intraspecific interactions ("zero class"); since the absence of behavior is not commonly mentioned in papers. We added to the database only those species in which the researchers have large experience in observing them in natural conditions. Therefore, our database was composed by species without any visual cue, species with at least one visual cue, and species with at least one visual signal during intraspecific interactions. However, it is important to mention two limitations of our database: (1) a visual cue could has been identified as absent in a species, but in fact it may occur, and may have not been observed until now; and (2) a visual cue could have been identified as a visual cue, but actually its signal function may have not been tested and proven until now.

Based on the bibliographical research and personal observations of researchers, our database was composed by 29 different visual cues distributed in 129 anuran species from 21 families (more details below). We cannot ignore a possible taxonomic bias in our database, especially because visual signaling is a relatively new study field in anurans. However, our aim in this review is motivate researchers to investigate the visual behavior in many species as possible, and do not restrict their questions because of limited data.

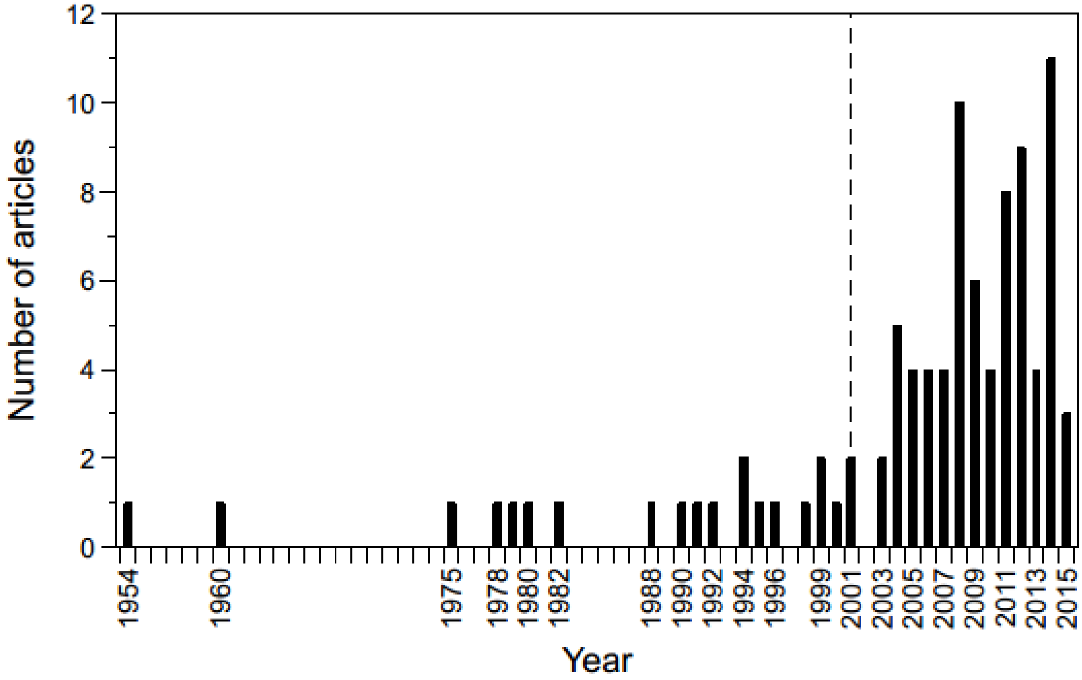


Fig. 2. Number of articles in which there at least mention of the presence or absence of visual cues during social interactions in anuran amphibians per year of publication. The dotted line indicates the year of publication of the review of visual communication in anurans, written by W. Hödl and A. Amézquita.

(2) The Visual Repertoire

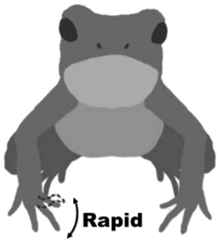
We compiled 29 different visual cues reported by previous studies during intraspecific interactions in anurans (Table 2). This diversity of visual cues was classified into four categories: (1) limb movements, dynamic behaviors (displays that can be turned "on" and "off" by the sender) moving any part or whole member of one or more limbs without moving the head and body trunk (10 types of visual cues); (2) body movements – stationary, dynamic behaviors moving any part of the body, with exception of limbs, or the whole body without displacement (13 types of visual cues); (3) body movements – displacement, stereotypical displacement behaviors (four types of visual cues); and (4) coloration, color patterns as visual cues (three types of visual cues) (Table 2).

Since the communication function was not confirmed for most visual cues described so far, we only provide the general social context in which each visual cue was reported. Therefore, from the 29 visual cues reported for anurans, 18 behaviors were reported during both reproductive (courtship interactions between males and females) and agonistic (territorial defense, most commonly observed between males) interactions (Table 2; Table S1). Five visual cues were reported exclusively during agonistic contexts (mouth opening, back rising, two legged pushing, truncated walking, and jumping running display, see descriptions in Table 2; Table S1); and other five visual cues were reported exclusively during reproductive contexts (head snaking, body inflation, body wiping, circling, and color change, see descriptions in Table 2; Table S1). However, the social context in which hind foot lifting and wiping displays (Table 2) were reported in the focal species was not specified by the authors of the selected papers to the present study (Table S1).

Table 2. Visual repertoire of anuran amphibians during intraspecific interactions.

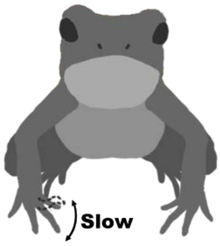
Limb movements

Toe/finger trembling[1,2,3]. Rapid up-and-down movements of one or more toes and/or fingers. It is a high-speed display. Toes/fingers may be moved independently, without an order, or in sequence in a wave-like pattern. Toe/finger trembling is performed with toes/finger of right or left limbs, or both toes/fingers at the same side simultaneously. Toe/finger trembling can be produced by both males and females, and it was observed during both agonistic and



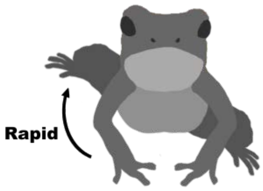
reproductive contexts (Table S1). As “toe trembling” in Hödl & Amézquita (2001).

Toe/finger flagging[1,2,3]**.** Slow up-and-down movements of one or more toes/or fingers. Toes/fingers may be moved independently, without an order, or in sequence in a wave-like pattern. Toe/finger flagging is performed with right, left, or both feet/hands simultaneously. This movement may be able to show the contrasting coloration between toe undersides and toe dorsal region. Toe flagging can be performed as part of the arm waving and hind foot rising displays (see descriptions below). Toe flagging can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As “toe flagging” in Hartmann *et al*. (2005), and as “toe trembling” in Hödl & Amézquita (2001).



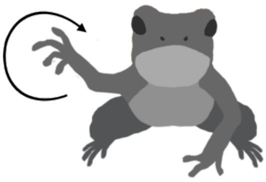
Toe posture[3]**.** Holding the foot up for some seconds with a frontal exposure of the dorsal surfaces of feet and toes; or holding the foot up for some seconds with toes curved down, exposing dorsal surfaces of toes. Whitish-silver dorsal surface of toe tips can be exposed during the display. Toe posture is performed with right or left foot independently, or with both feet simultaneously. This behavior was reported only for genus *Hylodes*. Toe posture was produced by males during both agonistic and reproductive contexts (Table S1).

Limb lifting[1,2,3]**.** Rapid up-and-down movements of one or more limbs (fore- and/or hind limbs), without extending them. It is a high-speed display. Limb lifting is performed with one of the right or left limbs, or both limbs at the same side simultaneously. Limb lifting can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As “limb shaking” in



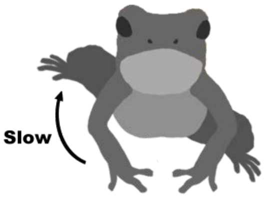
Hödl & Amézquita (2001), "tapping" in Preininger *et al*. (2013b), and as "foot shaking or hand shaking" and "arm lifting" in de Sá *et al*. (2016).

Arm waving[1,3]. Lifting slowly an arm and waving it up and down in an arc above or in front of the head. Both right and left arms are used to perform arm waving. The movements may or may not be performed in a temporal pattern. Arm waving may also be performed while the animal is walking and the lifted hand may perform simultaneous toe/finger flagging. In *Hylodes japi* (de Sá *et al*., 2016), arm waving was reported as a high speed movement. Arm waving can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As "semaphores" in Lindquist & Hetherington (1998) and Criswell (2008), and as "hand waving" in Meyer *et al*. (2012).

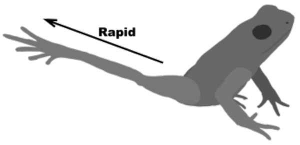


Face wiping[2]. Lifting an arm and touching the head with the hands, passing the hands on the eyes and snout, returning to the normal position. It seems as if the individual is cleaning its face. Both right and left arms are used to perform face wiping. The motion speed was not reported either as too slow or too fast. Face wiping can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1).

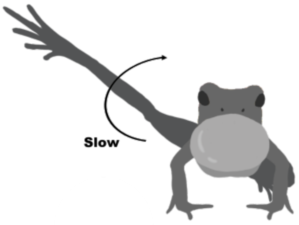
Hind foot lifting[1]. Slow up-and-down movements of one or two hind feet, without extending them. The motion may or may not be repeated before the limb is set back on the ground. The display of one hind foot can be followed by the other. The lifted foot may simultaneously perform toe/finger flagging. Hind foot lifting can be produced by males during agonistic context (Table S1).



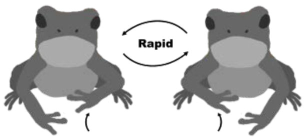
Leg stretching[1,2,3]. Stretching rapidly to back one or both hind limbs above or at the substrate level. The leg may or may not remain extended for some time. Leg stretching is performed by males with right, left, or both legs. Leg stretching can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As "leg-kicking" in Hartmann *et al.*, 2005.



Foot flagging[1,2,3]. Raising slowly one or both hind limbs, extending it/them out and back in an arc above the substrate level, and returning it/them to the body side. Foot flagging is performed with right or left leg, sometimes with regular alternation. The lifted foot may simultaneously perform toe/finger flagging. Foot flagging may be able to show the contrasting coloration between foot webbings and background (Stangel *et al.*, 2015; de Sá *et al.*, 2016). Foot flagging can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1).



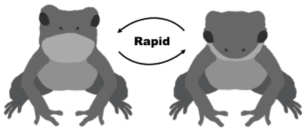
Wiping[1]. Moving rapidly one or more limbs (fore- and/or hind limb) upon the ground, without lifting it. The limbs are not fully extended; and the hands or feet remain in contact with the substrate during the motion. Wiping can be produced by both males and females (Table S1).



<u>Body movements – Stationary</u>

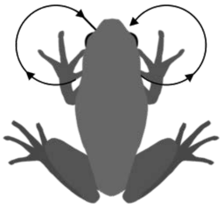
Mouth opening[2]. Opening and closing the mouth slowly or rapidly. The individual can repeat the motion many times or it can remain with the mouth open for some time. Mouth opening can be produced by both males and females, and it was produced during agonistic contexts (Table S1).

Head bobbing[3,4]. Rapid forward-backward or up-and-down movements of the head without lifting hands or feet off the

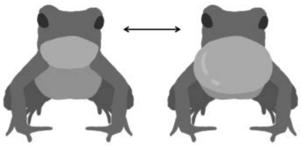


ground or moving the body. Head bobbing can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As “nod” in Souza (2014).

Head snaking[3]. Raising the head up and moving it to alternate sides eight times (four times on each side), in a snakelike motion. During head snaking the conspicuous throat coloration can be exhibited (“throat coloration”, see description below). Head snaking is a short-range courtship visual display emitted by males (Table S1).



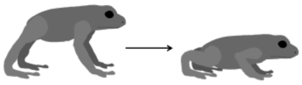
Throat display[1,2,3]. Pulsation (inflation and disinflation) of the throat with or without apparent sound production. It is performed once or repeated several times in quick succession. Throat display is usually emitted after the individual adopted an upright posture (see description below). We just considered throat display with sound production as visual cue when the visual modality is important to the signal transmission (Narins *et al.*, 2003) and not when it is just a production of the calling activity. The vocal sac can contrast with the background during the throat display because of the conspicuous coloration (“throat coloration”, see description below). Throat display can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1).



Vocal sac display[2]. Inflate the vocal sac, with or without apparent sound production, and remain the vocal sac inflates for some time. The male can call while keeps the vocal sac inflated. The vocal sac can contrast with the background during the vocal sac display because of the conspicuous coloration (“throat coloration”, see description below). Vocal sac display can be produced by males during both agonistic

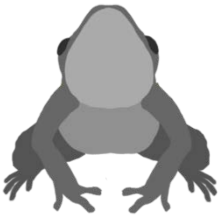
and reproductive contexts (Table S1).

Body lowering[1,2,5]. Lowering either the anterior part of the body or the whole body, pressing it against the substrate. The limbs may remain pressed to the sides of the body. Body lowering can be produced by males during both agonistic and reproductive contexts (Table S1). As "flat position" in Starnberger *et al*. (2011).



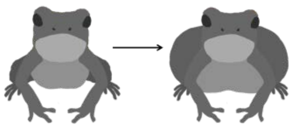
Body rising[1,2,5]. Elevating the body by extending all four legs. Sometimes the individual stands on the toes of the rear feet. Body rising may occur as a posture itself or it can precede body lowering (see description above) or body jerking (see description below) behaviors. Body rising can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As "body raising" in Hödl & Amézquita (2001) and Hartmann *et al*. (2005), and as "push-up" in Starnberger *et al*. (2011).

Upright posture[1,2,5]. Elevating the anterior part of the body by extending the forelimbs. As Hödl & Amézquita (2001), we did not consider the upright posture as a visual display when the extension of the arms was associated with the facilitation of calling activity. Upright posture may occurs merely as a posture or precede walking toward an intruder or displaying the throat. Upright posture can be produced by both males and females during agonistic contexts (Table S1). As "sit-up" in Starnberger *et al*. (2011).

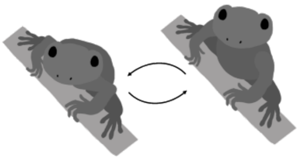


Back rising[1]. Elevating the posterior part of the body by extending the hind limbs. It may expose a particular coloration of the dorsal posterior end of the body. Back rising can be produced by both males and females, and it was observed during agonistic context (Table S1). As "back raising" in Hödl & Amézquita (2001).

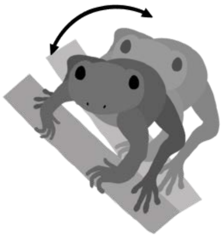
Body inflation[1]. Increasing the apparent body size by pumping air into the body. It is commonly performed after body rising (see description above). Body inflation can be produced by both males and females, and it was observed during reproductive context (Table S1).



Two armed impulse[1,3]. Rapid up-and-down movements of the anterior part of the body through jerky and repeated extensions of the forelimbs. Two legged pushing can be produced by both males and females, and it was observed during reproductive context (Table S1). As “two legged pushing” in Hödl & Amézquita (2001).



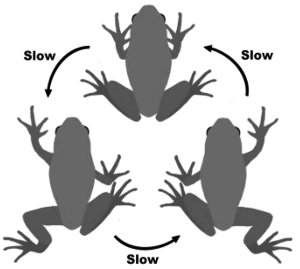
Body jerking[1,3]. Performing jerky movements with the body without lifting either hands or feet off the ground or perch. It can be associated with vibrational signaling (Caldwell *et al.*, 2010). Body jerking can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1).



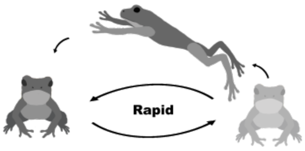
Body wiping[2]. Slow raising the limb laterally and touching its own dorsum and lateral parts with the hand/foot. Body wiping can be produced by both males and females during reproductive contexts (Table S1).

Body movements – Displacement

Truncated walking[3]. Lowering the body and walking ahead slowly, with alternation of legs and arms. Truncated walking is performed with a moving and stopping pattern. The right arm is with the left leg and vice-versa. Truncated walking can be produced by males during agonistic context (Table S1).

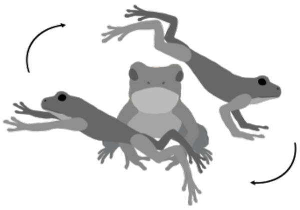


Running jumping display[1,2]. Running and/or jumping quickly back and forth or sideways along the substrate, calling perch or water, as a continuous or discontinuous movement. It may be accompanied by calls, hind foot lifting (see description



above), or by "four-feet" jumps. Running jumping display can be produced by both males and females, and it was observed during both agonistic and reproductive contexts (Table S1). As “jump display” in Hartmann *et al*. 2005; and as "splashing display" in Wells (1978) (“a male would make loud splashing noises by jumping up and down in the water”).

Circling[1]. Moving around another individual or simply pivoting around its own axis. It is mostly performed as a discontinuous movement and combined with body lowering (see description above). Circling can be produced by both males and females, and it was observed during reproductive contexts (Table S1).

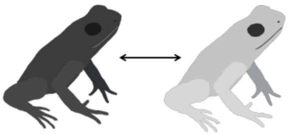


<u>**Coloration**</u>

Color pattern. Any visual cue associated with the color pattern at the dorsal surface of individual, as hue, brightness and marks, that there is evidence of its importance to the intraspecific communication. Color pattern was reported in both males and females (Table S1).

Throat coloration. Very colorful, brightness and conspicuous throat coloration in relation to dorsal body surface and/or background. This visual cue becomes more stereotyped when the animal adopts the upright posture (see description above). Throat coloration was reported in both males and females (Table S1).

Color change[1]. Changing the color and/or brightness of the whole body, or at least the dorsal and/or throat surface, in a short period of time; and the reverse change occurs sometime after the social interaction. Color change was reported in both males and females during reproductive contexts (Table S1).



Description based on: [1]Hödl & Amézquita (2001); [2]Hartmann *et al*. (2005); [3]de Sá, Zina & Haddad (2016); [4]Furtado (2014); [5]Starnberger *et al*. (2011).

(a) Limb Movements

Limb lifting behavior (Table 2) was the most common visual cue in anurans, reported for 67 species (Table S1). From 67 species that produced limb lifting behavior, only in 38 species the authors made clear in which general social context the visual cue was observed (Table S1). And from these 38 species, 31 species produce limb lifting exclusively during agonistic contexts (Table S1). In addition, there are evidences of signal function of limb lifting displays during territorial disputes between males of *Micrixalus saxicola*. In this species, limb liftings are especially produced by intruders in an occupied territory (Preininger *et al*., 2013b). Therefore, the rapid up-and-down movements of one or more limbs can be strongly associated with aggressive interactions, especially between males.

Toe/finger trembling (Table 2) was also one of the most common visual cues in anurans (40 species; Table S1). Both limb lifting and toe/finger trembling displays differ from the others mainly because of their high speed. Even when the motion was recorded by a video camera and viewed after by a trained observer, it can easily remain unnoticed. Despite limb lifting display seeming to be important during territory dispute in *Micrixalus saxicola* (Preininger *et al*., 2013b), no communication function between conspecifics has been associated with toe/finger trembling display to date.

Besides the visual cue, limb movements have been associated with vibrational signaling [during both intraspecific (Narins, 1995; Hartmann *et al*., 2005) and interspecific (Sloggett & Zeilstra, 2008) contexts] and unintentional responses (Furtado & Nomura, 2014). For example, females of *Polypedates leucomystax* are stationary and they emit toe/finger trembling displays from their perches (dense mats of floating vegetation; Narins, 1995). Therefore, Narins (1995) suggests that the toe/finger trembling may functions as a vibrational signal indicating the female's presence to neighboring males. On the other hand, Furtado and Nomura (2014) had not found difference in the emission rate of limb lifting and toe/finger trembling displays by males of *Hypsiboas albomarginatus* when submitted to presence or absence of an intruder in their territories. This result indicates that the rapid limb movements are not used to communicate during agonistic contexts in *H. albomarginatus* and, probably, they should be considered displacement activities.

Two of the most stereotyped visual cues in anurans are foot flagging and arm waving displays (see table 2 for detailed descriptions). Both behaviors are characterized by slow movements of the limbs and their signal function has already been identified in seven anuran species (foot flagging: *Micrixalus saxicola*, Preininger *et al*., 2013a; *Staurois latopalmatus*, Preininger *et al*., 2009; *S. guttatus*, Grafe & Wanger, 2007; *S. parvus*, Grafe *et al*., 2012; *Hylodes asper*, Haddad & Giaretta, 1999; *Dendropsophus parviceps*, Amézquita & Hödl, 2004; and arm waving: *Atelopus zeteki*, Criswell, 2008). Visual cues characterized by rapid movements of the limbs (limb lifting and leg stretching, Table 2) were associated with signal function only in one anuran species until now (*Micrixalus saxicola*, Preininger *et al*., 2013b), and it may actually be associated with vibrational signaling (Narins, 1995; Caldwell *et al*., 2010). Thus, perhaps not only the movement, but its speed may be an important factor for a dynamic visual signal to evolve in anurans.

(b) Body Movements – Stationary

From the visual cues associated with the animal posture, the upright posture (Table 2) was the most representative visual cue in anuran taxa (second most common visual cue reported in 51 species; Table S1). However, the signal function of upright posture was confirmed only for one anuran species to date. Upright posture probably has a similar function as aggressive calls in *Bokermannohyla sapiranga*, since males produce more upright postures when submitted to both visual and acoustic aggressive stimuli simultaneously (Souza, 2014). A particularity of upright posture display is the exhibition of the throat region (see the illustration for "upright posture" in Table 2). For example, males of *Bokermannohyla sapiranga* have the vocal sac zone much more conspicuous than the dorsal surface (Brandão *et al*. 2012). Thus, upright posture may act as an intermediary signal to increase the visibility of male throat to a reproductive competitor. In this case, visual signaling can occur from joining two visual cues: a motor cue, upright posture, and a color cue, throat coloration (Table 2). Additionally, body rising (Table 2) was also associated with aggressive disposition in *Hylodes asper* (Haddad & Giaretta, 1999). Since males adopted that posture only during close interactions for territory dispute. Therefore, elevating the anterior part or the whole body is possibly associated to aggressive posture display in some anurans.

The exhibition of male vocal sac (vocal sac display or throat display, Table 2) can send different messages for each conspecific receivers (males or females). For example, vocal sac display is the most emitted aggressive signal during territorial dispute between males of *Phrynobatrachus krefftii* (Hirschmann & Hödl, 2006). In addition, resident males of *Allobates femoralis* are more aggressive (physical attacks) against intruder males that pulse the vocal sac simultaneously with the call emission (Narins *et al*., 2003). On the other hand, females of *Dryophytes versicolor* (Reichert & Höbel, 2015) and *Engystomops pustulosus* (Rosenthal *et al*., 2004) prefer males that emit advertisement calls accompanied by vocal sac inflation. Therefore, the vocal sac is not only important for sound production in anurans, but also for sending visual messages during distinct social interactions among individuals.

Besides the toe/finger trembling, another visual behavior also has been associated with vibrational signaling: successive body jerking displays (Table 2). Caldwell *et al*. (2010) demonstrated that both visual cue and vibrations in vegetation, resulted from body jerking displays, are aggressive signals in *Agalychnis callidryas*. The authors submitted resident males to playback experiments with a robotic model frog (visual stimulus) and/or an electrodynamic shaker (vibrational stimulus). And each stimulus, separately, was sufficient to increase the aggressive response of males, especially body jerking displays. However, the most aggressive response was observed when the bimodal stimulus was presented to focal animals. Therefore, one behavior ("body jerking") can represent multimodal signals (visual and vibrational) with redundant information (aggressive disposition), which increase the accuracy of the information transfer (hypothesis of redundant multimodal signals, Hebets and Papaj 2005).

(c) Body Movements – Displacement

None of the stereotyped displacements (Table 2) has been associated with communication function during intraspecific interactions in anurans to date. From the four categories of visual cues, “body movements – displacement” was the least produced by anuran species: truncated walking (lowering the body and walking ahead slowly, see description in Table 2) was reported for only one species (*Hylodes japi*, de Sá *et al*., 2016), running jumping displays in 10 species (Table S1), and circling in 16 species (Table S1).

(d) Coloration

In comparison to body movements as visual cues performed during intraspecific interactions (e.g., Hödl & Amézquita, 2001) and the color pattern as defense strategic during prey-predator interactions (e.g., Toledo & Haddad, 2009), the use of color patterns as visual cues during intraspecific interactions in anurans has received little attention by ethologists. For example, Hödl and Amézquita (2001) only considered the change in color (see description in Table 2) as color visual cue in their review. Here we presented three types of visual cues from the remarkable variety of color patterns in anuran amphibians: color pattern, throat coloration, and color change (Table 2).

All the three color visual cues were already associated with signal function in some anuran species. In at least five species (*Oophaga pumilio*, Summers *et al*., 1999, Maan & Cummings, 2008, 2009; *Dryophytes squirellus*, Taylor, Buchanan & Doherty, 2007; *Scaphiopus couchii*, Vásquez & Pfenning, 2007; *Hyla arborea*, Gomez *et al*., 2009; *Agalychnis callidryas*, Jacobs *et al*. 2016) some visual cue, associated with color dorsal surface of males, was proved to be an important criterion during male selection by females for reproduction. Throat coloration (Table 2), in turn, was associated with signal function during both territorial dispute between males (*Micrixalus saxicola*, Preininger *et al*., 2013a) and male selection by females (*Eleutherodactylus cooki*, Burrowes, 2000; *Hyla arborea*, Gomez *et al*., 2009). And color change (Table 2) in *Rana arvalis* is a visual signal that permits sex recognition for both receivers (males and females) in large breeding aggregations, since only males turn blue just before the reproductive period (Sztatecsny *et al*., 2012). However, there is still much to discover about the role of anuran behavior in color perception and consequently the role of the variety of color patterns in the communication and reproductive success of individuals.

(3) Distribution of Visual Cues and Visual Signals in Anuran Taxa

Visual cues during intraspecific interactions have been reported for 159 anuran species distributed in 21 families. Although our database represent only ~2.4% of anuran extant diversity (7 027 species, 56 families; Frost, 2019), it is well distributed in the anuran phylogeny at family level (Fig 3). The most representative anuran family in our database was Hylidae (45 species, 14 genera), followed by Dendrobatidae (27 species, 8 genera), Bufonidae (15 species, 7 genera), Ranidae (13 species, 7 genera), Hylodidae (11 species, 2 genera), Leptodactylidae (10 species, 4 genera), Micrixalidae (10 species, 1 genus), Aromobatidae (7 species, 4 genera), Centrolenidae (5 species, 4 genera), Hyperoliidae (3 species, 1 genus), Microhylidae (2 species, 2 genera), and Rhacophoridae (2 species, 2 genera) (Table S1). The families Scaphiopodidae, Phrynobatrachidae, Dicroglossidae, Mantellidae, Myobatrachidae, Brachycephalidae, Eleutherodactylidae, Odontophrynidae and Rhinodermatidae were represented by only one species each in our database (Table S1).

We built a phylogenetic tree for the 159 species included in our database based on a molecular phylogeny and dated super-tree for amphibians (3309 species, ~45% of amphibian extant diversity; Pyron, 2014). For those species (38 species) that did not appear in the super-tree proposed by Pyron (2014), we inferred their position in the phylogenetic super-tree based on previous knowledge about the taxonomic relationships established by molecular, morphological and/or natural history data (Faivovich, 2002; Faivovich *et al*., 2005; Maciel *et al*., 2010; Fabri, 2013; Biju *et al*., 2014; de Sá *et al*., 2014; Lourenço *et al*., 2015; Poyarkov *et al*., 2015; Frost, 2019). We used a semi-parametric method based on Penalized Likelihood Approach to estimate the age of included nodes (function 'chronopl' of package *ape* in software R; Sanderson, 2002; Paradis, Claude & Strimmer, 2004; R Core Team, 2016). After including all 159 in the super-tree and adjusting node ages, we excluded all other species present in the super-tree but absent in our database. By doing so we obtained a dated phylogenetic sub-tree containing 159 anuran species (Fig. 4).

From these 159 anuran species, 23 species corresponded to the "zero class", i.e. those species for which no visual cue was observed, at the moment, during social interactions. The most representative genera in the "zero class" groups were: *Rhinella*, *Physalaemus* and *Leptodactylus* (Table S1, Fig. 4). However, we believe that the real number of species with absence of visual cues during social interactions is much larger, but the absence of behavior does not attract researchers' attention and also it is not commonly reported in papers. Other 127 species (~80% of the database) had at least one visual cue previously reported (Table S1, Fig. 4). Finally, 19 species had one or more visual signals with proven communication function during intraspecific communication (Table S1, Fig. 4). Again, we believe that the real number of species which use visual signals to communicate with each other is much higher; we just have to discover it.

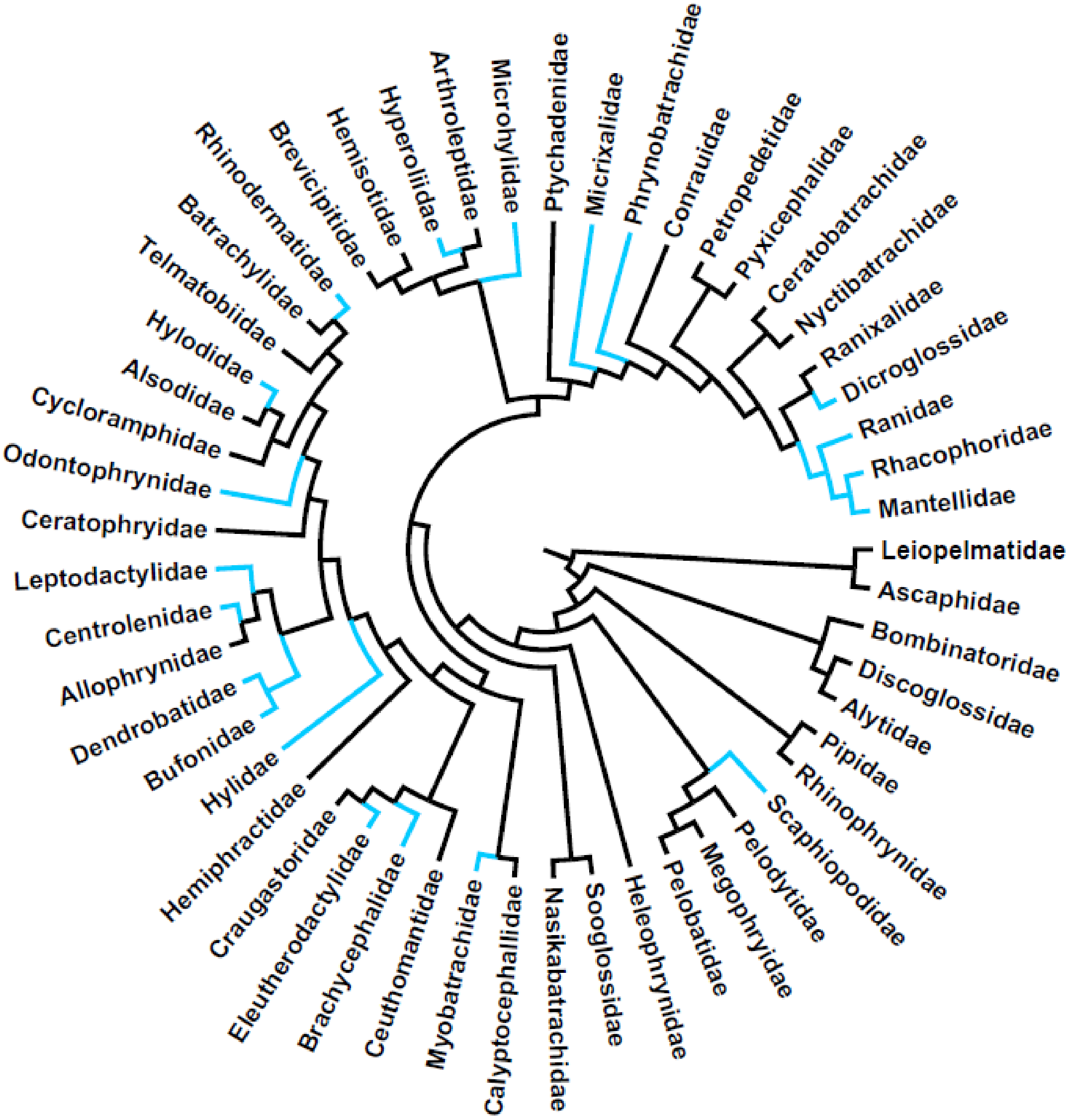


Fig. 3. Tree topology based on data from Pyron and Wiens (2011) representing the phylogenetic relationships among anuran families. In light blue the families with at least one species includied in our visual database (159 anuran species in the total). The visual database is composed by species without any visual cue, species with at least one visual cue, and species with at least one visual signal (visual cue with communication function) during intraspecific interactions.

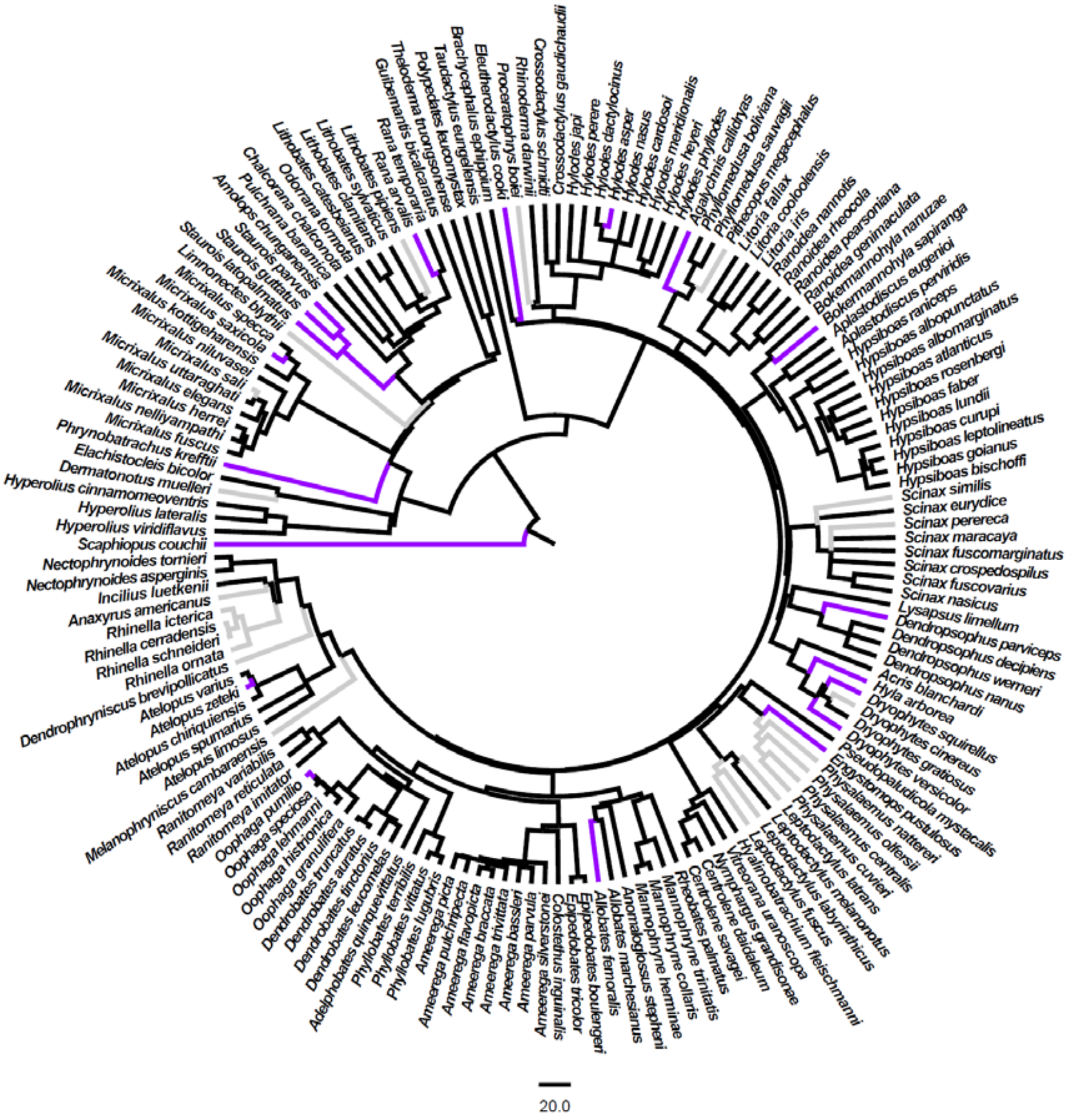


Fig. 4. Phylogenetic relationships among the anuran species (159 species, 21 families) included in the study based on data from Pyron (2014), Faivovich (2002), Faivovich *et al*. (2005), Maciel *et al*. (2010), Fabri (2013), Biju *et al*. (2014), de Sá *et al*. (2014), Lourenço *et al*. 2015, Poyarkov *et al*. (2015), and Frost (2019). Gray lines indicate species which no presence of visual cues; black lines indicate species which at least one visual cue; and violet lines indicate species which at least one visual signal (visual cue with communication function) during intraspecific interactions.

In most of the species included in our database the visual behavior was only superficially studied. Those studies include observations and analyses not directly addressing visual communication (Table S1). However, in all the more intensely studied species (those species with four or more papers about their visual communication) at least one “bona-fide” visual signal was discovered during social interactions: *Micrixalus saxicola* (Micrixalidae; visual signals: limb lifting, foot flagging, and throat coloration), *Staurois parvus* (Ranidae; visual signal: foot flagging), *Rana arvalis* (Ranidae; visual signal: color change), *Hylodes asper* (Hylodidae; visual signals: limb lifting, foot flagging, and body raising), *Hyla arborea* (Hylidae; visual signals: color pattern, and throat coloration), *Engystomops pustulosus* (Leptodactylidae: visual signal: throat display), *Allobates femoralis* (Aromobatidae; visual signal: throat display), *Oophaga pumilio* (Dendrobatidae; visual signal: color pattern), *Atelopus zeteki* (Bufonidae; visual signal: arm waving) (see Table S1 for more details and references). This relationship between number of studies and discovery of visual signals can be interpreted in two ways. First, ethologists may invest more studies in those species for which previous studies suggest visual signaling. Second, the use of visual signals by anurans may be more common than we imagined, we just need looking for them carefully. In either case, more studies are necessary to complete our knowledge about visual signaling in anuran communication.

We used a Principal Coordinate Analysis (PCoA) to visually represent the variation in the repertoire of visual signals in the anuran taxa reported to date (Legendre & Legendre, 2012). The First PCoA axis separated anuran species for which no visual signals were reported from anuran species for which visual signaling was reported during social interactions (Fig. 5). Along the second PCoA axis, three main groups were formed according to the visual repertoire (Table 2): (1) foot flagging behavior, with *Dendropsophus parviceps* (Hylidae) very close to *Staurois guttatus*, *S. latopalmatus* and *S. parvus* (Ranidae), and *Hylodes asper* (Hylodidae) and *Micrixalus saxicola* (Micrixalidae) a little far away from the others; (2) color pattern, with *Dryophytes squirellus* (Hylidae), *Oophaga pumilio* (Dendrobatidae) and *Scaphiopus couchii* (Scaphiopodidae) very close to each other, and *Agalychnis callidryas* (Hylidae) and *Hyla arborea* (Hylidae) a little far away from the others; and (3) throat display [*Allobates femoralis* (Aromobatidae), *Dryophytes versicolor* (Hylidae) and *Engystomops pustulosus* (Leptodactylidae)] and throat coloration [*Eleutherodactylus cooki* (Eleutherodactylidae) and *Hyla arborea* (Hylidae)] next to others visual signals as arm waving (*Atelopus zeteki*, Bufonidae), upright posture (*Bokermannohyla sapiranga*, Hylidae), vocal sac display (*Phrynobatrachus krefftii*, Phrynobatrachidae), and color change (*Rana arvalis*, Ranidae) (Fig. 5). “Foot flagging group” and “color pattern group” were positioned in opposite sides, and the others signals (e.g., throat display and throat coloration) between then. The family Hylidae, the most representative anuran family in our database, was the only one with species represented in all PCoA groups (Fig. 5).

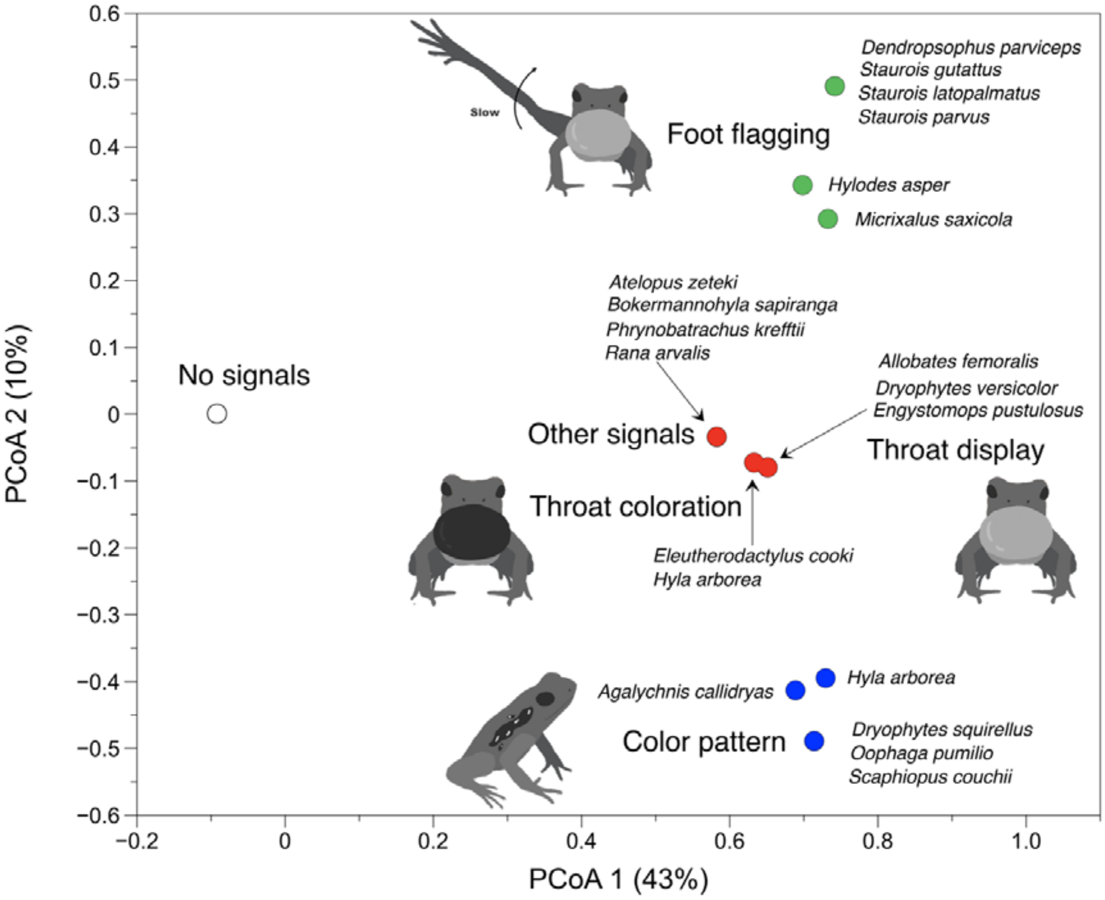


Fig. 5. Principal Coordinate Analysis of the visual signals, visual cues with communication function, emitted during intraspecific communication by anuran amphibians (159 species, 21 families). Green circles indicate species that emit “foot flagging” displays. Blue circles indicate species that the “color pattern” has signal function during social interactions. And red circles indicate species that emit “throat coloration”, “throat display” or other type of signals (arm waving: *Atelopus zeteki*; upright posture: *Bokermannohyla sapiranga*; vocal sac display: *Phrynobatrachus krefftii*; and color change: *Rana arvalis*).

V. Eco-Evolutionary Aspects of Visual Behavior

Signals evolve from pre-existing cues or other signals (Tinbergen, 1952; Hödl & Amézquita, 2001; Rosenthal, 2007). Indeed, both visual cues and visual signals are widely distributed in the anuran taxa (Fig. 3, Fig. 4, Table S1). Several factors may be acting for visual cues to evolve into visual signals during intraspecific communication in anurans (Hödl & Amézquita, 2001). Besides sexual selection and competition, the environment is one of the most important factors explaining character divergence (Rundle & Nosil, 2005; Nosil, 2012).

Although our database represents only 2% of the known diversity of anurans, we checked the models of evolution that best explain the variation in the repertoire diversity of visual cues and visual signals reported so far. We also analyzed the effects of the phylogeny, environment context and the contrast between the animal and background environment colors on the emission of visual behaviors during intraspecific communication in anurans. Furthermore, since species are not evolutionary independent units, comparative analyses where species are observations should explicitly consider phylogenetic relationships among species in order to control for inflated type I error (Harvey & Pagel 1991).

(1) Models of Evolution and Phylogenetic Signal

There is evidence that anurans can perform visual behaviors without an apparent function during an ongoing activity (Furtado *et al.*, 2017) and that “bona fide” signals can evolve from these pre-existing cues (Rosenthal, 2007). Therefore, we investigated the models of evolution that best explain the variation in the repertoire diversity of visual cues and visual signals during intraspecific interactions. We expected that the visual cue repertoire evolve independently across anuran taxa, likely as displacement activities not subjected to strong selection. In this case, visual cues in anurans would be less conserved along phylogeny, showing a low phylogenetic signal. However, we expected that in some specific lineages, these visual cues would become "bona fide" visual signals evolving by selection forces. Because of the presence of an adaptive function, the visual signals would provide an evolutionary advantage for the individuals, resulting in their conservation along the phylogeny (high phylogenetic signal) (Fig. 1).

We first created two binary matrices from our visual database (Table S1): (1) presence or absence of visual cues (29 types of visual cues, Table 2) and (2) presence or absence of visual signals in each of the 159 anuran species. We used the 1-Jaccard index to compute pairwise dissimilarity matrices between species based on visual cues or visual signals, using the function 'vegdist' of package *vegan* in software R; Oksanen *et al.*, 2016; R Core Team, 2016).

Based on the sub-tree built for the 159 species of our database (Fig. 4), we computed phylogenetic variance-covariance matrices among species under Brownian Motion (BM) or Ornstein-Uhlenbeck (OU) models of evolution. Variance-covariance structures (corPhyl) were obtained using the functions 'corBrownian' and 'corMartins' of package *ape*, respectively. Function 'vcv'of *ape* was then used to extract variance-covariance matrices based on corPhyl objects (Paradis et al., 2004; R Core Team, 2016). While BM model assumes that trait divergence between a pair of species is linearly related to the time since its evolutionary divergence (Felsenstein 1985), OU model corresponds to a BM process model with stabilizing selection toward a trait optimum (θ) (Butler & King, 2004). The values of the traits are thus constrained around a given value and the strength of this constraint is controlled by the selection parameter α. An α-value of 0.001 reflects a very weak constraint of the trait towards the optimum θ, whereas an α of 100 corresponds to instantaneous transition to θ; if α equals 0, the OU model is reduced to a BM model (Butler & King, 2004; Goutte *et al.*, 2016). In this study we used three different α-values in order to estimate the strength of stabilizing selection: 0.001, 0.01, and 0.1.

Each variance-covariance matrix thereby obtained was converted into a distance matrix (Legendre & Legendre, 2012). For each variance-covariance matrix, phylogenetic eigenvectors representing phylogenetic relationships among species based on different evolutionary models (BM, $OU_{0.001}$, $OU_{0.01}$ an $OU_{0.1}$) were extracted from the respective phylogenetic distance matrix (Diniz-Filho, Sant'Ana & Bini, 1998). Then, we performed linear regressions on dissimilarity matrices (MacArdle & Anderson, 2001) taking the pairwise dissimilarities among species based on visual cues or visual signals as response variables and phylogenetic eigenvectors as predictors. This procedure enabled us to find the combination of phylogenetic eigenvectors that maximized the F-value of regressions by means of a non-sequential selection of phylogenetic eigenvectors (Diniz-Filho *et al.*, 2011). An R function implementing the analysis is available in the Supplementary Material. We used the function 'adonis' of package vegan in software R to performed dissimilarity-based regressions (Oksanen *et al.*, 2016; R Core Team, 2016).

This procedure allowed us to evaluate the best evolutionary model explaining the variation of visual cues or signals between anuran species. Such evaluation was made based on the maximum F-value found between the four different evolutionary models (BM, $OU_{0.001}$, $OU_{0.01}$ an $OU_{0.1}$).

The evolutionary model that best explained the variation in the visual cues in anuran amphibians was Brownian motion (Table 3; Fig. 6). This suggests that the evolution of visual cues in anurans probably occurred by neutral evolution, since the variation in the occurrence of visual cues between species was merely a function of their evolutionary distance, without any evidence of strong selection. As expected, visual cues in anurans have low phylogenetic signal ($F = 10.63$, $R^2 = 0.06$, $p = 0.001$; Fig. 6).

Table 3. Statistical models for the evolution of visual cues and visual signals during intraspecific communication in anuran amphibians (159 species, 21families). Estimated model = without processing of phylogenetic data. BM = evolution model by Brownian motion; OU = Ornstein-Uhlenbeck model, stabilizing selection. $OU_{0.001}$ = OU model with 0.001 alfa-value. $OU_{0.01}$ = OU model with 0.01 alfa-value. $OU_{0.1}$ = OU model with 0.1 alfa-value. Nvectors = number of selected phylogenetic eigenvectors by non-sequential selection. Df = degree of freedom. SS = sum of squares. MS = mean of squares.

	Model	Nvectors	Df	SS	MS	F
Visual Cues	BM	1	1	4.13	4.13	10.63
	$OU_{0.001}$	1	1	4.10	4.10	10.54
	$OU_{0.01}$	1	1	3.49	3.49	8.88
	$OU_{0.1}$	140	140	64.24	0.46	8.70
Visual Signals	BM	34	34	13.87	0.41	13.38
	$OU_{0.001}$	32	32	14.25	0.44	16.46
	$OU_{0.01}$	1	1	1.80	1.80	17.8
	$OU_{0.1}$	67	67	16.95	0.25	32.50

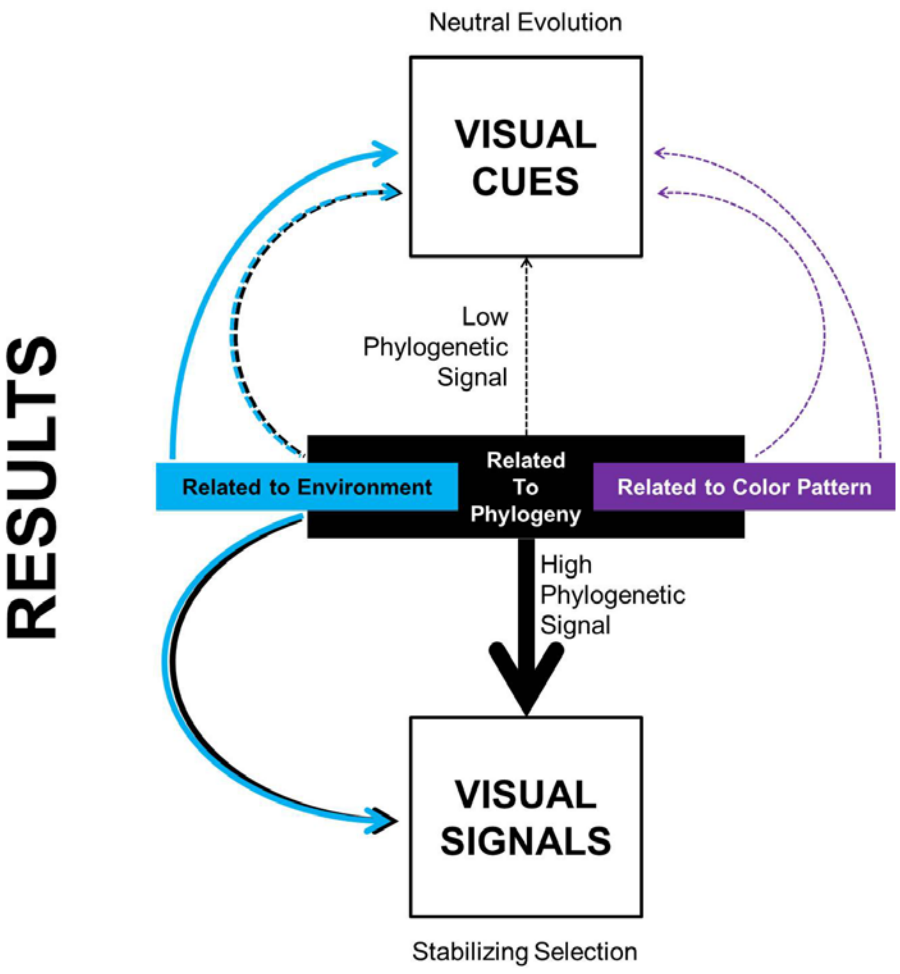


Fig. 6. Diagram of results: diversity of visual cues and visual signals, during intraspecific communication in anurans, in relation to environment context and color pattern along the evolutionary history of the group. The model of evolution that best explains the variation in the visual cues and visual signals are the Brownian Motion (Neutral Evolution) and the Ornstein-Uhlenbeck Model (Stabilizing Selection), respectively. Dotted arrows and complete arrows indicate weak and strong effects, respectively. The width of the arrows indicates the strength of the effect. Black, blue and purple colors indicate environmental, phylogenetic and color effects, respectively. Therefore: (1) the emission of visual signals, but not visual cues, is strongly influenced by phylogenetic relatedness among species; (2) the environmental effect was higher than the phylogenetic signal on the variation on visual cues; (3) The effect of the

environmental context on visual signals diversity is low and mostly phylogenetically structured; (4) the effect of color conspicuousness of the individual dorsal surface on visual cues diversity is very low, and it on visual signals is almost inexistent.

On the other hand, the evolutionary model that best explained the variation in visual signals was the Ornstein-Uhlenbeck model with the highest alpha parameter ($OU_{0.1}$; Table 3; Fig. 6). This result suggests that the evolution of visual signals probably occurred by stabilizing selection. Therefore, when a given visual cue conferred adaptive advantage for a species by gaining a communication function and was thereby selected, such function tended to be kept along the subsequent diversification of that anuran lineage. Furthermore, visual signals showed high phylogenetic signal ($F = 32.50$, $R^2 = 0.96$, $p = 0.001$; Fig. 6). Therefore, closely-related species had a more similar visual signal repertoire than distant-related ones.

Our results suggest that visual cues reported for anuran species that are phylogenetically closely-related to others for which the signal function from the same visual cue was already proved possibly also represent true visual signals. For example, males of *Micrixalus saxicola* emit foot flagging and throat coloration displays (see descriptions in table 2) as aggressive signals against intruders (Preininger *et al*., 2013b). And the same behaviors were reported for other eight species of genus *Micrixalus* (Biju *et al*., 2014; Table S1). Therefore, there is a great possibility that foot flagging and throat coloration displays in several *Micrixalus* species (“Dancing Frogs of India”, Biju *et al*., 2014) also represent aggressive signals during territorial defense.

We also recommend experimental studies for the purpose of confirming the communication function of foot flagging behaviors in the genera *Hylodes* (Hylodidae) and *Staurois* (Ranidae). Foot flagging behavior was reported for seven species of the genus *Hylodes* (Table S1); including *Hylodes asper*, for which foot flagging represent a territorial signal (Haddad & Giaretta, 1999). Moreover, the communication function of foot flagging behavior was reported for all species of genus *Staurois* studied so far (Grafe & Wanger, 2007; Preininger *et al*., 2009; Grafe *et al*., 2012; Stangel *et al*. 2015; Table S1). Additionally, our results suggest that color pattern can be an important visual signal during intraspecific communication in the genera *Dryophytes* (Hylidae) and *Oophaga* (Dendrobatidae) (Table S1, Fig. 5). Therefore, we strongly suggest these five anuran genera – *Micrixalus*, *Hylodes*, *Staurois*, *Dryophytes* and *Oophaga* – to study the evolutionary aspects of a particular visual signal. However, the family Hylidae maybe is the best choice to study how and why different visual signals evolved in the same clade, since we found a great diversity of visual cues and visual signals in tree frogs (Fig. 5, Table S1).

(2) Environment Context

Environment conditions during signal transmission and detection can affect the quality and effectiveness of received signals because both can alter the perceived form of the signal. Besides the signal form, signal effectiveness will depend of animal behaviors that determine the environmental conditions during signal transmission (Endler, 1992, 1999). Therefore, signals that maximize their detectability will be selected. However, the most detectable signals are not the same in different environments, since the environments contrast in transmission properties (Endler, 1999; Nosil, 2012). This difference in signals, and the perception thereof, is typically limited by the phenotypic attributes of the individual or species (Hödl & Amézquita, 2001) and might, for example, trigger a process of speciation (Nosil, 2012).

Several environmental factors may have influenced the effectiveness of visual signal transmission and, consequently, the divergence and evolution of visual signaling in anuran amphibians (Hödl & Amézquita, 2001). The level of visibility in the environment between individuals probably is the main factor for visual signaling to occur. The visibility level in an environment is determined by both light intensity and density of visual obstacles between individuals during an ongoing interaction (Endler, 1993b, 1999; Cole & Endler, 2016). Additionally, visual signaling may have evolved as an alternative or complementarity to acoustic signaling in anuran species that reproduce in a noisy environment, such as waterfalls (Hödl & Amézquita, 2001).

Visual signaling in anuran amphibians is mainly associated with diurnal habitat (Hödl and Amézquita 2001). The more light available during the day facilitates the signal transmission and perception of the signals by receivers (Endler, 1993b, 1999; Cole & Endler, 2016). For example, while in nocturnal species the visual displays were reported at interindividual distances shorter than 30 cm (e.g. *Dendropsophus parviceps*, Amézquita & Hödl, 2004), in diurnal species visual communication can occur at distances larger than 50 cm (e.g. *Staurois guttatus*, Grafe & Wanger, 2007; *S. latopalmatus*, Preininger *et al*. 2009). It is believed that nocturnal habitat is an ancestral character and it is widely distributed in anuran taxa (Duellman & Trueb, 1994). In the present study, from 19 anuran species that use visual signals to communicate with each other (Fig. 4 and Fig. 5), 10 species emit visual signals during daytime (Table S1).

Clear vision between individuals during a social interaction can also be an important factor to an efficient visual signal transmission. Open environments, such as grasslands, offer few visual barriers (Endler, 1992), and maybe facilitate the use of visual signals by anurans (Hödl & Amézquita, 2001; Amézquita & Hödl, 2004; Hartmann *et al.*, 2005). The use of rocks and high perches as microhabitats can also reduce visual barriers, caused by emergent vegetation, between individuals during an interaction. For example, from 19 anuran species that demonstrably use visual signals during intraspecific communication, males of 11 species used rocks and/or perches as calling site during the reproductive period (Table S1).

Natural selection favors signals, receptors, and signaling behavior that maximize the received signal relative to background noise and minimize signal degradation (Endler, 1992, 1999). It is well know that background noise can affect acoustic activity in anurans (Bosch & De la Riva, 2004; Halfwerk *et al.*, 2015; Grafe *et al.*, 2012; Goutte, Dubois, & Legendre, 2013). For example, the level of background noise (e.g. reproductive choruses and waterfalls) has been noted as an important criterion for microhabitat selection during male calling activity (Goutte *et al.*, 2013). Therefore, visual signaling may have evolved as alternative or complementarily to acoustic signaling in anuran species that reproduce in noisy environments, like waterfalls or fast-flowing streams (Hödl & Amézquita, 2001; Grafe *et al.*, 2012; Caldart, Iop & Cechin, 2014). For example, in an environment characterized by continuous noise, males of *Staurois parvus* probably produce advertisement calls as alerting signals to aggressive visual signals, which are foot flagging displays (Grafe *et al.*, 2012).

(a) Visual Behavior: Environment Context *versus* Phylogeny

Here we investigated if the environment context in which the social interaction occurs affects the visual behavior during intraspecific interactions in anuran amphibians. We predicted a more diverse visual repertoire in species where social interactions occur: (1) during daytime; (2) in open habitats, as grasslands; (3) in visually unobstructed calling sites, as rocks and high perches; (4) and in noisy environments, as next to waterfalls or fast-flowing streams. We expected that the environment context could explain more the presence of visual signals than visual cues in anurans. Since visual signals have high phylogenetic signal, the effect of environmental context on visual signal variation among species may likely show some overlap with phylogenetically-structured visual signal variation (Fig. 1).

For each species included in our visual database, we compiled from literature (e.g. Hödl & Amézquita, 2001) and online databases (e.g. The IUCN Red List, AmphibiaWeb, iNaturalist) the following information about the environment context during reproductive period to construct a binary matrix where activity period (diurnal and/or nocturnal), habitat (forest and/or open habitat), calling site (water, ground, rock and/or perch) and proximity to lotic environments (e.g. waterfalls and fast-flowing streams) were taken as environmental variables for each species.

We performed linear regressions on dissimilarity matrices (MacArdle & Anderson 2001) taking the pairwise dissimilarities among species based on visual cues or visual signals as response variables and either (a) the environmental matrix, (b) the selected phylogenetic eigenvectors corresponding to the best evolutionary model (see Table 3), or (c) both, as predictors. We used the method of variation partitioning

(Desdevises *et al*. 2003) to estimate the influences of either environment or phylogeny, as well as their shared influence, on visual behavior of anuran amphibians.

The environmental effect (F = 3.26, R^2 = 0.18, p = 0.001) was higher than the phylogenetic signal (F = 10.63, R^2 = 0.06, p = 0.001) on the variation on visual cues during intraspecific interactions in anurans (Fig. 6). This suggests that the environment context (activity period, habitat, calling site and water body) in which the social interactions occurs may better explain the presence of visual cues than phylogenetic relationship between anuran species. However, 80% of variation in visual cues repertoire was not explained by the statistical model (Table 4). Thus, besides environment context, other factors such as individual conspicuous color, male-male competition and sexual selection may be also acting in the repertoire of visual cues during intraspecific interaction.

Additionally, the environmental effect in the visual signal repertoire (F = 1.93, R^2 = 0.11, p = 0.001) was low, and mostly phylogenetically-structured (Table 4; Fig. 6). Although previous studies have associated the evolution of visual signals with environments with high visibility and background noise (Hödl & Amézquita, 2001; Preininger *et al*., 2009; Grafe *et al*., 2012; Caldart *et al*., 2014). Our results suggest that phylogenetic relationships among anuran species are more important to the presence of visual signals than reproduction during daytime, in open habitats and microhabitats (rocks and perches as calling site), and next to waterfalls.

Table 4. Partitioning the variation of visual cues and visual signals, emitted during intraspecific communication in anuran amphibians (159 species, 21 families), among environment context (activity period, habitat, calling site and water background noise) and phylogenetic components.

	Related to Environment Context			Unexplained
		Related to Phylogeny		
Visual Cues	0.14	0.04	0.02	0.80
Visual Signals	< 0.01	0.11	0.85	0.03

(3) Color as Visual Cue

The sense organs do not pass a complete perception of the world, but a representation that selects and emphasizes aspects that are important for the individual survival, reproduction and foraging (Crescitelli, 2013). By adaptive forces, the coloration of individuals can be selected from the colors present in the background environment (Endler, 1978, 1980; Cuthill, et al. 2017). This combination of color between individual and background may, for example, make the animal cryptic to predators (Degani & Biton, 2013; Polo-Cavia *et al.*, 2016) or even highlight the animal in relation to the environment, making it conspicuous to both intraspecific (e.g., blue coloration pattern against brown substrate in *Rana arvalis*; Ries *et al.*, 2008; Sztatecsny *et al.*, 2012) and interspecific receivers (e.g. aposematic color pattern as defense strategy against predation; Maan & Cummings, 2012).

The most representative color pattern in anurans is the cryptic coloration, which is mainly related to the evolutionary pressure that the visual ability of the predators exerts under their prey (Toledo & Haddad, 2009; Polo-Cavia *et al.*, 2016;

Rojas, 2016). However, there are indications that conspicuous patterns of coloration may play an important role in both male sexual selection by females (e.g. *Dryophytes squirellus*, Taylor *et al*., 2007; *Scaphiopus couchii*, Vásquez & Pfennig, 2007) and territorial dispute between males (e.g. *Micrixalus saxicola*, Preininger *et al*., 2013a) even in anuran species with cryptic coloration. Therefore, where is the balance between "not being seen" by predators and "being seen" by potential reproductive partners and/or co-specific competitors?

It is expected that species whose cryptic coloring evolved as a defense strategy against predator attacks ("not being seen") present alternatives (behavioral, morphological and/or physiological) to become conspicuous ("being seen") by conspecifics during social interactions. Three visual strategies related to body color pattern may have been selected to optimize both chances of survival and reproduction in anuran amphibians: color pattern with marks as visual cues; voluntary display of conspicuous parts of the body; and body color change.

Cryptic color pattern that includes marks may have been selected by both predation and sexual selection forces. For example, females of *Hyla arborea* and *Dryophytes squirellus* (arboreal species with green and cryptic dorsal color patterns) prefer males with conspicuous lateral body stripe (Taylor *et al*., 2007; Gomez *et al*., 2009). Therefore, a particular characteristic in the cryptic color pattern may represent a visual signal – providing a conspicuous visual cue which provokes an immediate response by the receiver (Hödl & Amézquita, 2001) – during a social interaction (“being seen”) without hampering the defense strategy (“not being seen” by predators).

The behavior of intentionally exposing some conspicuous and usually hidden part of the body can make an individual conspicuous, even possessing a predominant cryptic coloration in a given environment. For example, the conspicuous throat coloration in anuran males was already proved as visual signal in male sexual selection (mate choice) by females (e.g. *Eleutherodactylus cooki*, Burrowes, 2000; *Hyla arborea*, Gomez *et al*., 2009) and in territorial disputes between males (e.g. *Micrixalus saxicola*, Preininger *et al*., 2013a). Throat coloration display (Table 2) was also reported in other 38 anuran species (Table S1), but its function during social interactions still needs to be investigated. Additionally, several anuran species had conspicuous coloration and/or conspicuous marks in their flanks (e.g. genera *Phyllomedusa* and *Agalychnis*; Rojas, 2016), ventral surface (e.g. genus *Bombina*, Groza *et al*., 2007; and genus *Melanophryniscus*, Caorsi *et al*., 2014), or foot webbings (e.g. *Staurois guttatus* and *S. parvus*, Stangel *et al*., 2015) which are exposed only during body and/or limb movements (Hödl & Amézquita, 2001; Rojas, 2016). In all cases it is possible for the individual to quickly return to the resting position (vocal sac deflated, head down, and limbs close to the body) at the slightest sign of predators, and decrease their conspicuousness ("not being seen"). This strategy allows the animal to have a greater control over the time it will be exposed/visible to predators than in color conspicuous dorsal surface.

Finally, there are anuran species whose individuals not only can change all their body coloration and/or brightness to a more conspicuous one, and they may do it in a short period of time (e.g. *Incilius luetkenii*, Doucet & Mennill, 2009; *Scinax fuscomarginatus*, Toledo & Haddad, 2009; *Rana temporaria*, Sztatecsny *et al*., 2010; *Rhinoderma darwinii*, Bourke *et al*., 2011; *Rana arvalis*, Sztatecsny *et al*., 2012; *Dryophytes versicolor*, Reichert & Höbel, 2015; Table S1). For example, males of *Rana arvalis* change their body color pattern from brown to blue just before the explosive reproductive season, while females remain with cryptic coloration (Sztatecsny *et al.*, 2012). This color change behavior (Table 2) only in males can prevent males from spending time and energy trying to copulate with other males by mistake (Sztatecsny *et al.*, 2012); in addition it can facilitate the location of partners by both sexes (Sztatecsny *et al.*, 2010). This can be a good strategy especially in anurans species with high density of agglomerated individuals (e.g. explosive reproduction). Therefore, the body color change to a more conspicuous one will increase the opportunity of mating ("being seen"). However, when out of reproductive context, individuals can increase their survivor rates using a cryptic coloration as defense strategy ("not being seen").

On the other hand, aposematism is an anti-predator strategy through which preys warn predators about their unprofitability by means of conspicuous color patterns that act as warning signals (Toledo & Haddad, 2009; Rojas, 2016). In this case, the individual wants to "being seen" and to be avoided by predators. This contrasting color, selected by interspecific pressures, may unintentionally make the animal more conspicuous also to conspecific receivers. And the individual color conspicuity can facilitate the transfer of motor visual intraspecific signals (Cole & Endler, 2016). Besides the color contrast, the position of aposematic animals on the substrate may favor the transmission of visual signals, since being in more exposed sites is likely not to be a problem for them. In Hödl and Amézquita (2001), from 56 anuran species included in their database about visual signaling, 30 species (more than half) had aposematic coloration as defense strategy. In spite of this, cryptic coloration is an ancestral condition (Duellman & Trueb, 1994) and the most representative color pattern in anurans (Toledo & Haddad, 2009; Rojas, 2016). Therefore, the individual conspicuity from aposematic coloration perhaps facilitated the evolution of visual signals in anuran amphibians.

Additionally, it is possible that the visual signals in anurans evolved from pre-existing runways or other signals (Hödl & Amézquita, 2001). Thus, the color pattern used as visual signal during intraspecific communication in anurans may have evolved from different forces than the social interactions. For example, *Oophaga pumilio* (Dendrobatidae) is a poison frog with different aposematic color and patterns among isolated populations (e.g., orange and green morphs; Summers *et al*., 1999). During mate choice experiments, females of *Oophaga pumilio* prefer males with the color pattern from their own population (Summers *et al*., 1999). The authors asked if divergence in aposematic coloration drove divergence in female preference, or divergence in female preference drove divergence in aposematic color pattern. Therefore, interspecific (e.g., prey-predator interactions) and intraspecific (e.g., sexual selection and competition for mates) pressures in the evolution of visual signaling in anuran amphibians may be more interconnected than we imagined.

(a) Visual Behavior: Color Pattern *versus* Phylogeny

We investigated if body color patterns affected the visual behavior during intraspecific interactions in anuran amphibians. We expected that color conspicuousness of the dorsal surface could explain more the variation in visual signal repertoire than in visual cues among anuran taxa. Since visual signals have high phylogenetic signal, the effect of the conspicuousness color pattern on visual signal variation among species may likely show some overlap with phylogenetically-structured visual signal variation (Fig. 1).

For each species included in our visual database, we compiled from literature (e.g. Hödl & Amézquita, 2001) and online databases (e.g. The IUCN Red List, AmphibiaWeb, iNaturalist) the following information about the color pattern to construct a binary vector: color conspicuousness – aposematic color pattern of the dorsal surface – or not conspicuousness – cryptic pattern of the dorsal surface. We performed linear regressions on dissimilarity matrices (MacArdle & Anderson 2001) taking the pairwise dissimilarities among species based on visual cues or visual signals as response variables and either (a) the color pattern, (b) the selected phylogenetic eigenvectors corresponding to the best evolutionary model (see Table 3), or (c) both, as predictors. We used the method of variation partitioning (Desdevises *et al*. 2003) to estimate the influences of either color pattern or phylogeny, as well as their shared influence, on visual behavior of anuran amphibians.

The variation of visual cues during intraspecific interactions in anurans species was weakly explained by color conspicuousness of dorsal surfaces ($F = 8.89$, $R^2 = 0.05$, $p = 0.001$; Fig. 6). Together, color pattern and phylogeny explained just 8% of the visual cues variation (Table 5; Fig. 6). It is important to remember that visual cue is any morphological or behavioral trait occurring during a visually-mediated social interaction and that it is not, necessarily, associated with communication function. Therefore, a fraction of the visual cues in anurans might represent displacement activities (Furtado & Nomura, 2014; Souza, 2014), which are behaviors without implications to the individual reproductive success (Tinbergen, 1952; Maestripieri *et al*., 1992) and probably maintained in the group by genetic drift (present study).

The variation of intraspecific visual signals in anurans was very weakly explained by color pattern (F = 0.88, R^2 = 0.005, p = 0.39). And, contrary to expectations, the percentage of explication shared by dorsal color pattern and phylogenetic relationships was also very low (less than 1%; Table 5). This result only reinforces that phylogeny seems to be the most important factor in visual signaling during intraspecific communication in anuran amphibians. Therefore, color conspicuousness of the dorsal surface seems not to be an important factor for the emission of both visual cues and visual signals during social contexts in anuran amphibians (Fig. 6). However, is important to consider that the color pattern classification of anuran dorsal surface into “conspicuous” or “not conspicuous” was based on human perception. For example, Ries *et al*. (2008) detected reflection of bright ultraviolet-blue spectrum from the skin of males *Rana arvalis* (Ranidae) that was not perceived by human eyes.

Table 5. Partitioning the variation of visual cues and visual signals, emitted during intraspecific communication in anuran amphibians (159 species, 21 families), among color pattern of the individual dorsal surfaces (binary variable: conspicuous or not conspicuous) and phylogenetic components.

<table>
<tr><td></td><td colspan="2">Related to Color Pattern</td><td></td><td rowspan="2">Unexplained</td></tr>
<tr><td></td><td></td><td colspan="2">Related to Phylogeny</td></tr>
<tr><td>Visual Cues</td><td>0.02</td><td>0.03</td><td>0.03</td><td>0.92</td></tr>
<tr><td>Visual Signals</td><td>< 0.01</td><td>< 0.01</td><td>0.95</td><td>0.04</td></tr>
</table>

VI. Future Directions

Divergence of intraspecific signals is driven mostly by environment, sexual selection and competition factors (Rundle & Nosil, 2005; Nosil, 2012). In the present study, we found that environmental context and individual color conspicuousness do not seem to be as important to explain the variation in visual repertoire of anurans as we expected. However, our study was limited by the absence of information about visual behavior of the most of the known anuran species. Therefore, we recommend that future studies, hopefully with a greater volume of data, investigate not only the role of environmental context, but the male selection by females and the territorial dispute among individuals, in the evolution and divergence of intraspecific visual signals in anurans. Additionally, our results indicated that detailed studies with the genera *Micrixalus* (Micrixalidae), *Hylodes* (Hylodidae), *Staurois* (Ranidae), *Dryophytes* (Hylidae) and *Oophaga* (Dendrobatidae) may provide a starting point to elucidate the evolution of visual signaling in anuran amphibians.

Anuran species using visual signaling also use acoustic signaling during social interactions (e.g. *Allobates femoralis*, Narins *et al*., 2003). And there is also evidence of other modalities of communication, as vibrational (e.g. *Agalychnis callidryas*, Caldwell *et al*., 2010) and chemical signals (e.g. *Hyperolius cinnamomeoventris*, Starnberger *et al*., 2013), acting with the visual signaling in anuran species. This makes it difficult to separate the function of each modality of signal during anuran communication (Amézquita & Hödl, 2004). According to Amézquita and Hödl (2004), visual and acoustic signaling in anurans may either represent multimodal signals in which the signals are used to send redundant information, increasing the accuracy of the information transfer, or potentially used to send multiple sets of information simultaneously (Johnstone, 1996; Partan & Marler, 2005). Therefore, we strongly suggest studies with more than one modality of communication to complete our knowledge about visual signaling in anuran amphibians.

VII. Conclusions

Visual cues repertoire is diverse (including limb movements, body movements, and color patterns) and is widely distributed in anuran taxa. However, visual cue repertoires seem to evolve independently in different anuran lineages, likely as displacement activities not subjected to strong selection. Yet, in some specific lineages, such cues become true visual signals that evolve by selection weakly mediated by environmental context. Therefore, contrary to what was suggested in previous studies, we concluded that environmental context (activity period, habitat, calling site and water body) and individual color conspicuousness (aposematic or cryptic) seem not to be the only selective forces required for the divergence and evolution of visual signals during intraspecific communication in Anura.

VIII. Acknowledgements

We thank Peter Narins for encouragement and critical suggestions. We are grateful to Fausto Nomura, Albert S. Feng, Lucas R. Forti, Fernando J. M. Rojas-Runjaic, Valentina Z. Caorsi and Camila Ineu Medeiros for their contributing to our database. We thank Guilherme D. S. Seger for his support during phylogenetic tree manipulation. RF and

LNL received a scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). RM was funded by the Ministerio de Ciencia e Innovación, Spain (project TATANKA CGL2011-25062). SMH received a fellowship from the Brazilian Research Council (CNPq; grant 304820/2014-8).

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X. Supplementary Material

Table S1. Distribution of visual cues, observed during intraspecific interactions, among anuran taxa.

	Limb movements										Body movements - Stationary													Body movements - Displacement			Coloration								
Family Species	Toe/finger trembling	Toe posture	Toe flagging	Limb lifting	Arm waving	Face wiping	Hind foot lifting	Leg stretching	Foot flagging	Wiping	Mouth opening	Head bobbing	Head snaking	Throat display	Vocal sac display	Body lowering	Body rising	Upright posture	Back raising	Body inflation	Two legged pushing	Body jerking	Body wiping	Truncated walking	Running jumping display	Circling	Color pattern	Throat coloration	Color changing	Activity period	Habitat	Calling site	Water body	Dorsal coloration	References
Scaphiopodidae																																			
Scaphiopus couchii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	0	0	I	O	W	Le	Cr	1
Hyperoliidae																																			
Hyperolius viridiflavus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	N	F/O	P	Le	Co	2
Hyperolius lateralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	N	F	P	Le	Cr	2
Hyperolius cinnamomeoventris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	N	F/O	P	Le	Cr	2,3
Microhylidae																																			
Dermatonotus muelleri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	G	Le	Cr	Nomura^{PO}
Elachistocleis bicolor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	N	O	G	Le	Cr	Nomura^{PO}
Phrynobatrachidae																																			
Phrynobatrachus krefftii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2a^{m}	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	W	Lo	Cr	4
Micrixalidae																																			
Micrixalus fuscus	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Micrixalus nelliyampathi	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Micrixalus herrei	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Micrixalus elegans	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Micrixalus uttaraghati	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Micrixalus Sali	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Cr	5
Micrixalus niluvasei	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Micrixalus kottigeharensis	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5

Micrixalus saxicola	0	0	0	2a^{m}	0	0	0	0	2a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2a^{m}	0	D	F	R	Lo	Cr	5,6,7,8,9
Micrixalus specca	0	0	0	1a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	5
Dicroglossidae																																			
Limnonectes blythii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	W/G	Lo	Cr	10
Ranidae																																			
Staurois latopalmatus	0	0	0	0	1a^{m}	0	0	0	2a^{m}	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	10,11
Staurois guttatus	0	0	0	1^{m}	1a^{m}	0	0	1r^{m}	2b^{b}	0	1^{f}	0	0	1^{m}	0	0	0	1^{f}	0	0	0	0	0	0	0	0	0	1^{b}	0	I	F	R	Lo	Cr	12,13
Staurois parvus	0	0	0	1^{m}	1a^{m}	0	0	0	2^{b}	0	1^{b}	0	0	0	0	1a^{m}	0	1^{b}	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Cr	10,13, 14,15
Amolops chunganensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	G	Lo	Cr	Feng^{PO}
Pulchrana baramica	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	16
Chalcorana chalconota	0	0	0	1a^{m}	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	17
Odorrana tormota	1r^{f}	0	0	0	0	0	0	0	0	0	0	1r^{f}	0	0	0	0	0	0	0	1r^{f}	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	Feng^{PO}
Lithobates catesbeianus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	I	O	W	Le	Cr	8, Medeiros^{PO}
Lithobates clamitans	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	1^{m}	0	0	1a^{m}	0	0	0	0	0	0	1a^{m}	0	0	1^{m}	0	N	O	W	Le	Cr	10,18
Lithobates sylvaticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{b}	D	F	G	Le	Cr	19
Lithobates pipiens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	I	O	W/G	Le	Cr	Feng^{PO}
Rana arvalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	N	O	W	Le	Co	20,21,22,23
Rana temporaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	1^{m}	N	O	W	Le	Cr	24
Mantellidae																																			
Guibemantis bicalcaratus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	I	F	P	Le	Cr	25
Rhacophoridae																																			
Theloderma truongsonense	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	26
Polypedates leucomystax	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F	P	Le	Cr	Feng^{PO}
Myobatrachidae																																			
Taudactylus eungellensis	0	0	0	0	0	0	0	1^{m}	1^{m}	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	10
Brachycephalidae																																			
Brachycephalus ephippium	0	0	0	0	1b^{b}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Lo	Co	10,27
Eleutherodactylidae																																			
Eleutherodactylus cooki	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	0	N	C	G	Lo	Cr	28
Odontophrynidae																																			
Proceratophrys boiei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	G	Lo	Cr	Nomura^{PO}
Rhinodermatidae																																			
Rhinoderma darwinii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1r^{m}	D	F	G	Lo	Cr	29
Hylodidae																																			
Crossodactylus schmidti	1a^{m}	0	1b^{b}	1b^{b}	0	0	0	1a^{m}	0	0	0	0	0	0	0	1b^{m}	0	1b^{b}	0	0	0	1b^{b}	0	0	1b^{b}	0	0	0	0	D	F	R	Lo	Cr	30,31
Crossodactylus gaudichaudii	0	0	0	0	1^{m}	0	1^{m}	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	D	F	R	Lo	Cr	10,31,32,33
Hylodes japi	0	1b^{m}	1b^{m}	1b^{b}	1b^{b}	0	0	1a^{m}	1b^{m}	0	0	1b^{m}	1r^{m}	1b^{m}	0	1a^{m}	1b^{m}	1b^{m}	0	0	1a^{m}	1b^{b}	0	1a^{m}	1a^{m}	0	0	0	0	D	F	R	Lo	Cr	31
Hylodes perere	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	I	F	R	Lo	Cr	31,34

Hylodes dactylocinus	1^{m}	1^{m}	1^{m}	1^{m}	0	0	0	1^{m}	$1b^{m}$	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Cr	10,31,35
Hylodes asper	0	0	$1a^{m}$	$1b^{b}$	0	0	0	$1b^{b}$	$2b^{m}$	0	0	0	0	0	0	0	$2b^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Cr	10,31,36,37,38
Hylodes nasus	0	0	0	0	$1a^{m}$	0	0	$1a^{m}$	1^{m}	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	1^{m}	0	D	F	R	Lo	Cr	31,33
Hylodes cardosoi	0	0	0	$1a^{m}$	0	0	0	$1b^{m}$	$1b^{b}$	0	0	0	0	0	0	0	$1b^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Cr	31,39,40
Hylodes meridionalis	0	0	$1a^{m}$	$1a^{m}$	0	0	$1a^{m}$	$1a^{m}$	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	1^{m}	0	0	0	0	D	F	R	Lo	Cr	Furtado^{PO}
Hylodes heyeri	0	1^{m}	1^{m}	$1a^{m}$	0	0	0	$1a^{m}$	0	0	1^{m}	0	0	1^{m}	0	1^{m}	0	1^{m}	0	0	0	0	0	0	1^{m}	0	0	1^{m}	0	D	F	R	Lo	Cr	31,41
Hylodes phyllodes	0	0	$1a^{m}$	1^{m}	0	0	0	$1b^{m}$	1^{m}	0	$1a^{m}$	0	1^{m}	1^{m}	0	$1a^{m}$	$1b^{m}$	$1a^{m}$	0	0	0	0	0	0	1^{m}	0	0	1^{m}	0	D	F	R	Lo	Cr	31,38,Forti^{PO}
Hylidae																																			
Agalychnis callidryas	0	0	0	0	0	0	0	$1a^{m}$	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	$2a^{m}$	0	0	0	0	$2r^{m}$	0	0	N	F	P	Le	Cr	42,43
Phyllomedusa boliviana	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Cr	44
Phyllomedusa sauvagii	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	P	Le	Cr	10
Pithecopus megacephalus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	45
Litoria fallax	0	0	0	0	0	0	0	$1a^{m}$	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Cr	10,46
Litoria cooloolensis	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F	P	Le	Cr	46
Litoria iris	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	N	F	P	Lo	Cr	46
Ranoidea nannotis	0	0	0	0	1^{m}	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	R	Lo	Cr	10
Ranoidea rheocola	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	R	Lo	Cr	10
Ranoidea pearsoniana	0	0	0	0	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	R/P	Lo	Cr	46
Ranoidea genimaculata	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	R	Lo	Cr	10
Bokermannohyla nanuzae	0	0	0	0	0	$1r^{f}$	0	0	0	0	0	0	0	0	0	$1r^{f}$	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	R/P	Lo	Cr	47
Bokermannohyla sapiranga	0	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	$2a^{m}$	0	0	0	0	0	0	0	0	0	0	0	N	F	G	Lo	Cr	48
Aplastodiscus eugenioi	0	0	0	$1r^{b}$	0	$1r^{m}$	0	0	$1r^{m}$	0	0	0	0	0	$1r^{m}$	$1r^{m}$	$1b^{f}$	$1a^{b}$	0	0	0	0	$1r^{m}$	0	0	0	0	1^{m}	0	N	F	P	Le	Cr	38,49
Aplastodiscus perviridis	0	0	0	$1a^{m}$	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F/O	P	Le	Cr	50
Hypsiboas raniceps	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F	P	Le	Cr	Furtado^{PO}
Hypsiboas albopunctatus	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F	G/P	Le	Cr	48,50
Hypsiboas albomarginatus	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	$1a^{m}$	0	$1b^{m}$	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Cr	38,51,52
Hypsiboas atlanticus	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	N	F	W/P	Le	Cr	53
Hypsiboas rosenbergi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	10
Hypsiboas faber	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F/O	G/P	Le	Cr	Furtado^{PO}
Hypsiboas lundii	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	Furtado^{PO}
Hypsiboas curupi	0	0	0	0	0	$1r^{b}$	0	$1r^{b}$	0	0	$1a^{m}$	0	0	$1b^{m}$	$1b^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	54
Hypsiboas leptolineatus	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	O	P	Le	Cr	Furtado^{PO}
Hypsiboas goianus	$1a^{m}$	0	0	$1a^{m}$	0	$1a^{m}$	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Co	48
Hypsiboas bischoffi	$1a^{m}$	0	0	$1a^{m}$	0	0	0	$1a^{m}$	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	O	P	Le	Co	50,Furtado^{PO}
Scinax similis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	P	Le	Cr	Nomura^{PO}
Scinax eurydice	0	0	0	1^{m}	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Cr	38
Scinax perereca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Cr	Nomura^{PO},Furtado^{PO}
Scinax maracaya	0	0	0	$1a^{m}$	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	G/P	Le	Cr	55

Scinax fuscomarginatus	0	0	0	1a^{m}	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	N	O	P	Le	Cr	56,57
Scinax crospedospilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	N	O	P	Le	Cr	Nomura^{PO}
Scinax fuscovarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	1^{m}	N	O	G	Le	Cr	Nomura^{PO},Furtado^{PO}
Scinax nasicus	0	0	0	1a^{m}	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	P	Le	Cr	Furtado^{PO}
Lysapsus limellum	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	W	Le	Cr	Furtado^{PO}
Dendropsophus parviceps	1^{m}	0	0	0	1a^{b}	0	0	0	2a^{m}	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	G/P	Le	Cr	10,58
Dendropsophus decipiens	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F/O	P	Le	Co	59
Dendropsophus werneri	0	0	0	1a^{m}	0	0	0	1b^{b}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Le	Cr	60
Dendropsophus nanus	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F/O	P	Le	Cr	Furtado^{PO}
Acris blanchardi	0	0	0	1a^{m}	0	0	0	1a^{m}	0	0	1a^{m}	0	0	0	1a^{m}	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	I	F	G/P	Le	Cr	61
Hyla arborea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	2r^{m}	1r	N	F/O	W/G/P	Le	Cr	62,63,64,65,66
Dryophytes squirellus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	0	0	N	F	P	Le	Cr	67
Dryophytes cinereus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	W/P	Le	Cr	Feng^{PO}
Dryophytes gratiosus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	I	O	P	Le	Cr	Feng^{PO}
Dryophytes versicolor	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	N	F	G/R/P	Le	Cr	68
Leptodactylidae																																			
Pseudopaludicola mystacalis	0	0	0	0	0	0	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	O	W	Le	Cr	69
Engystomops pustulosus	0	0	0	0	0	0	0	0	0	0	0	0	0	2r^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	N	F/O	W	Le	Cr	70,71,72,73
Physalaemus nattereri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	G	Le	Cr	Nomura^{PO}
Physalaemus olfersii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	G	Le	Cr	Nomura^{PO}
Physalaemus centralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	I	O	G	Le	Cr	Nomura^{PO}
Physalaemus cuvieri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	G	Le	Cr	Nomura^{PO}
Leptodactylus latrans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	I	F/O	W	Le	Cr	Nomura^{PO}
Leptodactylus melanonotus	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	N	F/O	W/G	Le	Cr	10
Leptodactylus labyrinthicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	I	O	W/G	Le	Cr	Nomura^{PO}
Leptodactylus fuscus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	G	Le	Cr	Nomura^{PO}
Centrolenidae																																			
Hyalinobatrachium fleischmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	0	0	0	0	0	0	0	N	F	P	Lo	Cr	10
Vitreorana uranoscopa	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	38
Nymphargus grandisonae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	N	F	P	Lo	Cr	74
Centrolene daidaleum	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	P	Lo	Cr	Rojas-Runjaic^{PO}
Centrolene savagei	0	0	0	1r^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1r^{m}	0	0	0	0	0	0	0	N	F	P	Lo	Cr	75
Aromobatidae																																			
Rheobates palmatus	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	1^{f}	1^{b}	1^{b}	0	0	0	0	0	0	0	0	0	0	1^{m}	D	F	G	Lo	Cr	10
Mannophryne trinitatis	1	0	0	0	0	0	0	0	0	0	0	0	0	1^{b}	0	0	1^{b}	1^{b}	0	0	0	0	0	0	1^{m}	0	0	1^{m}	1^{m}	D	F	P	Lo	Cr	10,76
Mannophryne collaris	1	0	0	0	0	0	0	0	0	0	0	0	0	1a^{f}	0	0	1^{b}	1^{f}	0	0	0	1^{m}	0	0	1^{m}	0	0	1^{f}	1^{m}	D	F	R	Lo	Cr	10,77
Mannophryne herminae	0	0	0	0	0	0	0	0	0	0	0	0	0	1a	0	0	0	1a	0	0	0	0	0	0	0	0	0	1a	0	D	F	R	Lo	Cr	78,79
Anomaloglossus stepheni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{f}	1^{m}	0	0	0	0	0	0	0	1^{m}	1^{b}	0	0	0	D	F	G	-	Cr	10

Allobates marchesianus	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{f}	0	1^{f}	0	1^{f}	0	0	0	0	0	0	0	1^{b}	0	0	0	D	F	G	Le	Cr	10
Allobates femoralis	1	0	0	$1r^{m}$	0	0	0	$1r^{m}$	0	0	0	0	0	$2a^{m}$	0	1	0	1	0	0	0	0	$1r^{f}$	0	0	$1r^{m}$	$1a^{m}$	0	0	D	F	G	Le	Cr	10,80,81,82,83
Dendrobatidae																																			
Epipedobates boulengeri	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	D	F/O	G	Lo	Co	10
Epipedobates tricolor	1	0	0	1^{b}	0	0	0	0	0	1^{f}	0	0	0	0	0	1^{f}	1^{m}	1^{m}	0	1^{m}	0	0	0	0	0	1^{f}	0	0	0	D	F	G	Lo	Co	10
Colostethus inguinalis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{f}	0	1	1^{b}	0	0	0	0	0	0	1^{m}	0	0	0	D	F	R	Lo	Cr	10
Ameerega silverstonei	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Lo	Co	10
Ameerega parvula	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Lo	Co	84,85
Ameerega bassleri	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	D	F	G	Lo	Co	10
Ameerega trivittata	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Le	Co	10
Ameerega braccata	0	0	0	$1r^{b}$	0	0	0	$1r^{b}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	P	Lo	Co	Forti^{PO}
Ameerega flavopicta	0	0	0	0	0	0	0	$1r^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Co	86
Ameerega pulchripecta	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Le	Co	10
Ameerega picta	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	D	F	G	Le	Cr	10
Phyllobates lugubris	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	D	F	R	Lo	Co	10
Phyllobates vittatus	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	D	F	G	Lo	Co	10
Phyllobates terribilis	1	0	0	1^{b}	1^{b}	0	0	0	0	0	0	0	0	0	0	1^{m}	1^{m}	1^{m}	0	1^{m}	1	0	0	0	0	1^{f}	0	0	0	D	F	G	-	Co	10
Adelphobates quinquevittatus	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	0	D	F	G	-	Co	10
Dendrobates leucomelas	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Lo	Co	10
Dendrobates tinctorius	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	D	F	G	Lo	Co	10
Dendrobates auratus	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	D	F	G/P	-	Co	10
Dendrobates truncatus	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	D	F	G	Le	Co	10
Oophaga granulifera	1	0	0	1^{m}	0	0	0	0	0	1	0	0	0	0	0	0	0	1^{m}	1^{f}	0	0	0	0	0	0	0	0	0	0	D	F	G	-	Co	10
Oophaga histrionica	1	0	0	1^{b}	0	0	0	0	0	1^{b}	0	0	0	0	0	1^{b}	1^{m}	1^{m}	0	1	1^{b}	0	0	0	0	1	1^{f}	0	0	D	F	G/P	-	Co	10
Oophaga lehmanni	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	1	0	0	0	D	F	G	-	Co	10
Oophaga speciosa	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	D	F	G	-	Co	10
Oophaga pumilio	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	1	0	1	0	0	0	0	1	0	$2r^{m}$	0	0	D	F	G	-	Co	10,87,88,89,90
Ranitomeya imitator	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	0	D	F	P	Le	Co	10
Ranitomeya reticulata	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	1	0	0	0	D	F	G	-	Co	10
Ranitomeya variabilis	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	D	F	G	Le	Co	10
Bufonidae																																			
Melanophryniscus cambaraensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	G	Le	Cr	Caorsi^{PO}
Atelopus limosus	0	0	0	0	1^{m}	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	D	F	R	Lo	Cr	10,91
Atelopus spumarius	0	0	0	0	1^{m}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	D	F	G	Lo	Co	92
Atelopus chiriquiensis	0	0	0	0	$1b^{m}$	0	0	0	0	0	0	0	0	0	0	$1a^{m}$	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Co	10,92,93
Atelopus zeteki	0	0	0	1	$2b^{b}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Co	10,92,94,95
Atelopus varius	0	0	0	0	$1a^{b}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	R	Lo	Co	10,96
Dendrophryniscus brevipollicatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	F	G/P	-	Cr	Nomura^{PO}

Rhinella ornata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F	G	Le	Cr	Nomura^{PO}
Rhinella schneideri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	F/O	W/G	Le	Cr	Nomura^{PO}
Rhinella cerradensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	W/G	Le	Cr	Nomura^{PO}
Rhinella icterica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	I	F/O	W/G	Lo	Cr	Nomura^{PO},Furtado^{PO}
Anaxyrus americanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	O	W/G	Le	Cr	Feng^{PO}
Incilius luetkenii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1r^{m}	D	F	G	Le	Co	97
Nectophrynoides asperginis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1a^{m}	0	0	0	0	0	0	0	0	1b^{m}	0	0	D	O	W	Lo	Co	98,Feng^{PO}
Nectophrynoides tornieri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{m}	1^{m}	1^{m}	0	0	0	0	0	0	0	0	0	0	0	N	F	W	-	Cr	99

Visual behavior: 0 = absence; 1 = visual display; 2 = visual signal. Social context: r = reproductive; a = agonistic; b = both contexts. Sender's gender: ^{m} = male; ^{f} = female; ^{b} = both male and female. Activity period: D = diurnal; N = nocturnal. Habitat: F = forest; O = open habitat. Calling site: W = water (partially submerged or floating); G = ground; R = rock; P = perched on vegetation. Type of water body for oviposition: Le = lentic; Lo = lotic; NA = oviposition do not occurs in bodies water (e.g., trunks, leaves and litter). Dorsal coloration: Cr = cryptic; Co = conspicuous. References: ^{PO} = personal observation; Nomura = Dr. Fausto Nomura; Feng = Dr. Albert S. Feng; Medeiros = MSc. Camila Ineu Medeiros; Furtado = MSc. Raíssa Furtado; Forti = Dr. Lucas Rodriguez Forti; Rojas-Runjaic = MSc. Fernando José María Rojas-Runjaic; Caorsi = MSc. Valentina Zaffaroni Caorsi.

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R function. Phylogenetic Eigenvector Regression (PVR) and eigenvector selection using Analysis of Variance with Distance Matrices (adonis).

```
## Required Package
require(vegan)

## Description
# Phylogenetic Eigenvector Regression (PVR) and eigenvector selection using Analysis of Variance with Distance Matrices (adonis).

## Arguments
# traits.dist = Traits distance matrix (class dist).
# dist = Phylogenetic distance matrix (class dist).
# cumulative = Percentage of variation in the phylogenetic distances considered in the analysis. Cumulative percentage must be higher than the cumulative percentage of the first two eigenvalues, and less than 1.

## Note
# The sequence of species in the traits distance matrix must be the same as that in the phylogenetic distance matrix.

PVR_adonis<-function(traits.dist,dist,cumulative=0.99){
        if((0<=cumulative & cumulative<=1)==FALSE){
```

```
		stop("\n Cumulative percentage must be higher than the cumulative
percentage of the first two eigenvalues, and less than 1\n")
	}
	dist<-as.matrix(dist)
	ordination<-wcmdscale(sqrt(dist),eig=TRUE)
	values<-ordination$eig[which((ordination$eig>=0)==TRUE)]
	vectors<-ordination$points
	colnames(vectors)=colnames(vectors,do.NULL=FALSE,prefix="Axis.")
	relative<-values/sum(values)
	if(cumulative<sum(relative[1:2])){
		print(paste("Relative eigenvalue for axis 1 =",relative[1]))
		print(paste("Relative eigenvalue for axis 2 =",relative[2]))
		stop("\n Cumulative percentage must be higher than the cumulative
percentage of the first two eigenvalues\n")
	}
	cumulative2<-as.vector(rep(NA,length(values)))
	for (i in 1:length(values)){
		cumulative2[i]<-sum((values/sum(values))[1:i])
	}
	use<-which((cumulative2<=cumulative)==TRUE)
	if(cumulative==1){
		use<-1:(length(values)-1)
	}
	values2<-cbind(values,relative,cumulative2)
```

```
   colnames(values2)=c("Eigenvalues","Relative_eig","Cumul_eig")

   rownames(values2)=1:length(values)

   x<-vectors[,use]

   fac<-length(use)

   result.seq<-matrix(NA,nrow=fac,ncol=1)

   for(i in 1:fac){

           mod_temp <- vegan::adonis(traits.dist ~ x[,1:i], permutations = 1)

result.seq[i,] <- mod_temp$aov.tab$F.Model[1]

   }

   rownames(result.seq)=rownames(result.seq,FALSE,prefix="PRV.")

   colnames(result.seq)="F.Model"

   result.unique<-matrix(NA,nrow=fac,ncol=1)

   for(i in 1:fac){

           mod_temp <- vegan::adonis(traits.dist ~ x[,i,drop=FALSE], permutations = 1)

result.unique[i,] <- mod_temp$aov.tab$F.Model[1]

   }

   rownames(result.unique)=rownames(result.unique,FALSE,prefix="PRV.")

   colnames(result.unique)="F.Model"

   remainder<-1:fac

   result.f<-matrix(NA,nrow=fac,ncol=1)

   result.pvr<-matrix(NA,nrow=fac,ncol=1)

   included=NULL

   k = 0
```

```
    repeat{
        k = k+1
        result.temp<-matrix(NA,nrow=1,ncol=length(remainder))
        colnames(result.temp)<-remainder
        for(i in 1:length(remainder)){
            included.temp<-c(included,remainder[i])
            mod_temp <- vegan::adonis(traits.dist ~
x[,included.temp,drop=FALSE], permutations = 1)
      result.temp[,i] <- mod_temp$aov.tab$F.Model[1]
            included<-
c(included,as.numeric(colnames(result.temp)[which(result.temp==max(result.temp))])
)
            remainder<-setdiff(1:fac,included)
        }
        result.f[k,]<-max(result.temp)
        result.pvr[k,]<-paste(included,sep="",collapse=" ")
        if (length(included)==fac) break
    }
    rownames(result.f)=rownames(result.f,FALSE,prefix="Step.")
    colnames(result.f)="F.Model"
    colnames(result.pvr)<-"PVRs"
    rownames(result.pvr)=rownames(result.f,FALSE,prefix="Step.")
    RES.seq<-cbind(result.seq)
    RES.unique<-cbind(result.unique)
```

```
        RES.step<-cbind(result.pvr,result.f)
        res.x<-cbind(values2[1:fac,3])
        rownames(res.x)=rownames(res.x,do.NULL=FALSE,prefix="Axis.")
        colnames(res.x)="Cumulative"
        inf<-values2[fac,3]
        RES<-
list(values=values2,vectors=vectors,inf.cumulative=inf,n.axis.considered=fac,
RES.seq=RES.seq,RES.unique=RES.unique, RES.step=RES.step)
        class(RES)<-"pvr"
        return(RES)
}


## Values
# values = Eigenvalues, relative eigenvalues and cumulative eigenvalues for the PCoA
of phylogenetic distance matrix.
# vectors = The principal coordinates with positive eigenvalues.
# inf.cumulative = Percentage of the variation in the phylogenetic distances considered
in the analysis (approximately the specified in cumulative argument),
# n.axis.considered = Number of axes considered.
# RES.seq = F value for sequential approach using all PVR axes (PVR 1, PVR 1 + PVR 2,
...).
# RES.unique = F value for each PVR axis.
```

```
# RES.step = F value for non-sequential approach, that uses the combination of PVRs axes that maximize the F value. The selection finishes using all PVRs considered. The max F value can be selected manually in the results.

## Examples
require(ape)
dtraits<-vegdist(cbind(rnorm(12)),method="euclidean")
dtraits
dphylo<-cophenetic(rcoal(12))
dphylo
Res<-PVR_adonis(dtraits,dphylo)
Res
Res$RES.step[order(as.numeric(Res$RES.step[,2]),decreasing=TRUE)[1],] # Combination of PVRs with the max F

plot(Res$RES.seq[,1],type="b",main="Sequential",xlab="PVR",ylab="F value")
plot(Res$RES.unique[,1],type="b",main="Unique",xlab="PVR",ylab="F value")
plot(as.numeric(Res$RES.step[,2]),type="b",main="Non-sequential",xlab="PVR",ylab="F value")
```

CONSIDERAÇÕES FINAIS

Estudos sobre história natural fornecem a matéria-prima necessária para a elaboração de perguntas e hipóteses, principalmente em áreas com escassez de conhecimento como a sinalização visual em anuros. Dentre as 7.023 espécies de anuros descritas até o momento (*Amphibian Species of the World 6.0, an Online Reference*), a presença ou ausência de pistas visuais durante interações sociais foram relatadas em apenas 159. Se 2% da diversidade conhecida de anuros já foram capazes de nos fascinar com um rico repertório visual, o que as outras 98% das espécies têm a nos ensinar?

Inspirada pela primeira pergunta de Tinbergen, “O que é?”, observei e descrevi o comportamento visual de uma espécie de rã-de-corredeira diurna. Além de um repertório visual diverso, descobri que machos emitem mais *displays* visuais do que cantos durante contextos agressivos. Entretanto, há um aparente consenso entre os herpetólogos de que a sinalização acústica é a principal forma de comunicação em anuros. Será que a sinalização visual não seria mais relevante do que se imaginava?

Todavia, a identificação de um sinal visual não é algo trivial. Para identificar “Qual a função?” (segunda pergunta de Tinbergen) de um comportamento é necessário realizar testes de hipóteses com metodologias apropriadas, a fim de evitar conclusões precipitadas baseadas apenas na percepção humana. Portanto, submeti machos de três espécies de hilídeos noturnos, e que se reproduzem em poças, a um experimento com espelhos simulando interações agonísticas. Em todas as espécies estudadas, os *displays* visuais emitidos parecem não possuir função de comunicação. Neste caso, os *displays* visuais não representam sinais visuais, mas possivelmente atividades deslocadas (aparentemente sem função em uma atividade em andamento).

Visto que alguns destes comportamentos representam, comprovadamente, sinais visuais verdadeiros em outros anuros, este resultado me levou a questionar quais pressões (“Como evoluiu?” – terceira pergunta de Tinbergen) estão atuando na evolução de sinais visuais a partir de pistas pré-existentes (e.g. atividades deslocadas).

Durante a realização desses dois primeiros estudos, constatei que as três espécies de hilídeos observadas possuíam um repertório visual consideravelmente menor (2-3 comportamentos) do que a espécie de rã estudada anteriormente (7 comportamentos). Além disso, os machos dos hilídeos vocalizavam em taxas muito maiores (e.g., *Lysapsus limellum*: 44,5 cantos/minuto) do que a rã-de-corredeira (*Hylodes meridionalis*: 1,1 cantos/minuto). Poderia a sinalização visual ser um modo de comunicação alternativo à sinalização acústica em anuros? Para responder esta questão, compilei o repertório visual de 69 espécies e analisei seus respectivos cantos. Entretanto, não encontrei maiores repertórios visuais em espécies que produziam menos cantos por minuto durante interações sociais. Adicionalmente, o macho de rã-de-corredeira com maior atividade acústica em resposta à presença de um competidor, também foi aquele que emitiu mais *toe flaggings*. Isto sugere que a sinalização visual pode não ser uma alternativa, mas um complemento à sinalização acústica em anuros.

Uma vez que o ambiente normalmente exerce fortes pressões sobre a evolução e divergência de caracteres, seria a associação entre hábito diurno e reprodução em ambiente ruidoso o fator-chave que explicaria o rico repertório visual da rã-de-corredeira? Estes atributos são diferentes daqueles dos hilídeos pesquisados neste estudo, que também são tidos como atributos das linhagens de anuros ancestrais (hábito noturno e reprodução em poças). Posto isto, compilei dados da literatura para averiguar o efeito do ambiente e da filogenia na variação de pistas e sinais visuais em anuros. Conforme o esperado, eu encontrei que as pistas visuais parecem evoluir independentemente em diferentes linhagens, mas que em algumas linhagens específicas estas pistas vieram a se tornar sinais visuais verdadeiros. Entretanto, o ambiente explicou muito pouco da variação de sinais visuais observada em 159 espécies incluídas no estudo. Portanto, o ambiente parece não ser a única força seletiva necessária para a divergência e evolução de sinais visuais durante a comunicação intraespecífica nos anuros.

Com o desenvolvimento desta tese de doutorado eu pude concluir que ainda sabemos muito pouco sobre o fascinante comportamento visual dos anuros e que estudos descritivos devem ser incentivados. Entretanto, ressalvo cuidado durante a interpretação deste tipo de dado comportamental, uma vez que nem todos os *displays* visuais emitidos possuem um significado inserido. Também pude constatar que provavelmente não há uma relação antagônica entre o repertório visual e a atividade acústica nos anuros. Isto sugere que os indivíduos utilizam ambas as modalidades para se comunicar, e que futuros trabalhos deveriam incorporar abordagens multimodais a fim de estudar a comunicação do grupo de maneira mais precisa. Apesar das limitações impostas pelo volume modesto de dados, encontrei que, em algumas linhagens específicas, pistas visuais tornam-se sinais visuais que evoluem por seleção fracamente mediada pelo ambiente. Portanto, outras pressões seletivas, como a seleção sexual e a territorialidade, devem ser investigadas para elucidar a evolução e divergência de sinais visuais durante a comunicação intraespecífica em anuros.