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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS  
(ZOOLOGIA)

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UMA NOVA ESPÉCIE DE *HYLODES* (ANURA, HYLODIDAE) DA SERRA DO  
JAPI: DESCRIÇÃO, MODO REPRODUTIVO E COMUNICAÇÃO

FÁBIO PERIN DE SÁ

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista Júlio de Mesquita Filho, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Zoologia).

Dezembro - 2013

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Orientador: Célio Fernando Baptista Haddad

Coorientadora: Juliana Zina Pereira Ramos

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Rio Claro, 12 de dezembro de 2013

Dedico à minha mãe e ao meu irmão. Meu núcleo.

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“Sapo é um pedaço de chão que pula.”

Manoel de Barros



## RESUMO

A espécie de *Hylodes* da Serra do Japi, que pertence ao grupo de *Hylodes lateristrigatus*, é uma rã-de-corredeira endêmica desta cadeia de montanhas no Estado de São Paulo, Brasil, e que apresenta machos territoriais e comportamento de corte elaborado. Apesar de ser morfológicamente similar à *H. amnicola*, *H. ornatus*, *H. perere* e *H. sazimai*, pode ser distinguida pelo seu padrão de coloração, suas características de canto de anúncio e por meio de análises moleculares. Dividido em dois capítulos, o presente estudo traz dados de taxonomia, sistemática filogenética, história natural e estado de conservação, além de informações referentes ao modo reprodutivo e à comunicação deste hilodídeo. No capítulo I são apresentadas a descrição da nova espécie assim como de seus girinos. Ainda, neste capítulo, são apresentados, pela primeira vez para uma espécie de *Hylodes*, detalhes da desova, características dos ovos fertilizados e o comportamento da fêmea auxiliando no fechamento da toca subaquática após a oviposição. O capítulo II aborda a comunicação desta rã-de-corredeira, sendo revelado o mais complexo repertório de sinais visuais entre os anfíbios anuros, incluindo cinco novos sinais visuais que são descritos. Nunca antes registrados para anuros, também são observados o uso individualizado dos sacos vocais pelos machos, de acordo com a posição do receptor, e a comunicação multimodal visual-tátil, realizada pelas fêmeas durante o ritual da corte. Por fim, fechando o capítulo II, uma revisão dos sinais visuais em rãs-de-corredeira neotropicais destaca a diversidade comportamental atualmente conhecida para a família Hylodidae. A presente dissertação de mestrado evidencia a importância da realização de mais estudos envolvendo: (1) taxonomia e filogenia integrativas com o gênero *Hylodes* unindo dados de morfologia, canto, distribuição geográfica e sequências de DNA, deste modo sendo reveladas potenciais espécies crípticas e, conseqüentemente, a real diversidade do gênero; e (2) a comunicação visual no gênero *Hylodes* e em anuros em geral, sobretudo em áreas tropicais onde os comportamentos tendem ser mais complexos, possivelmente em consequência do maior número de espécies e de grupos filogenéticos ou de limitações ecológicas como hábitos (e.g. diurnos) ou habitats reprodutivos (e.g. riachos de correnteza ruidosos).

**Palavras-chave:** Anfíbios. Comunicação acústica. Comunicação bimodal. Comunicação multimodal. Comunicação tátil. Comunicação visual. Controle do saco vocal. Desova. Girinos. *Hylodes* cf. *ornatus*. Mata Atlântica. Ovos.

## ABSTRACT

The *Hylodes* species from Serra do Japi mountain range, included in the *Hylodes lateristrigatus* group, is a torrent frog endemic to this mountain range in the São Paulo state, Brazil; it exhibits territorial males and elaborated courtship behavior. It is morphologically similar to *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai*; however, distinguishable by its color pattern, advertisement call, and DNA sequences. Divided into two chapters, we present data on taxonomy, phylogenetic systematic, natural history, and conservation status, besides information on the reproductive mode and communication of this hylodid. In the chapter I we present the descriptions of the new species, including the description of its tadpoles. Moreover, in this chapter we provide, for the first time for a *Hylodes* species, details of the spawning, traits of the fertilized eggs, and the behavior of the female helping to conceal the underwater constructed chamber after oviposition. In the chapter II we study the communication of this species of torrent frog, revealing the most complex visual displays repertoire among amphibian anurans, including five new displays that we describe. Never before registered for frogs, we also describe the individualized use of the vocal sacs by the males, according to the receiver's position, and the visual-tactile multimodal communication performed by females during the courtship. Finally, concluding the chapter II, a review on visual displays in Neotropical torrent frogs highlights the behavioral diversity currently known for the family Hylodidae. Our research evidences that it is important to conduct more studies comprising: (1) integrative taxonomy and phylogeny within the genus *Hylodes*, combining data on morphology, vocalizations, geographic distribution, and DNA sequences, thereby revealing potential cryptic species and, as a consequence, the true diversity of the genus; and (2) the visual communication in the genus *Hylodes* and frogs in general, mainly in tropical areas where the behaviors tend to be more complex, most likely due to the higher number of species and phylogenetic groups or ecological constraints as breeding habits (e.g. diurnal) or habitats (e.g. noisy fast streams).

**Key-words:** Acoustic communication. Amphibians. Atlantic forest. Bimodal communication. Eggs. *Hylodes* cf. *ornatus*. Multimodal communication. Spawning. Tactile communication. Tadpoles. Visual communication. Vocal sac control.

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## INTRODUÇÃO GERAL

### 1. HILODÍDEOS: AS RÃS-DE-CORRENTEZA NEOTROPICAIS

As florestas neotropicais conjuntamente abrigam uma das maiores biodiversidades do mundo (MYERS et al., 2000; PRIMACK e CORLETT, 2005). A Mata Atlântica, um dos biomas florestais neotropicais, é marcadamente biodiversa e ameaçada, sendo apontada como uma das áreas prioritárias para conservação no planeta (MITTERMEIER et al., 1998; MYERS et al., 2000). Os remanescentes de floresta Atlântica são calculados em cerca de 12% em relação a sua cobertura original, contudo ainda contemplam mais de 8.000 espécies endêmicas de plantas vasculares, anfíbios, répteis, aves e mamíferos (MORELLATO e HADDAD, 2000; RIBEIRO et al., 2009; RIBEIRO et al., 2011). Particularmente em relação aos anfíbios anuros, a alta diversidade e o elevado índice de endemismos na Mata Atlântica, bastante visíveis em áreas montanhosas do bioma, estão associados à elevada variedade de clima e ambiental, decorrentes principalmente de fatores como níveis de umidade, topografia acidentada e temperaturas variáveis, estas últimas decorrentes das variações tanto altitudinais como latitudinais (HADDAD e PRADO, 2005; HEYER e MAXSON, 1983; SILVA et al., 2012). Esta variedade de características ambientais proporciona tanto a existência de inúmeros microhabitats favoráveis ao desenvolvimento dos anuros como também condições limitantes para estes animais (por exemplo, riachos de correnteza), ambas relevantes na história evolutiva destes vertebrados, refletindo na diversidade em espécies, em biologies e em comportamentos (HADDAD e PRADO, 2005; HEYER et al., 1990; HÖDL, 1990). Haddad e Prado (2005) reconhecem, por exemplo, alta diversidade dos modos reprodutivos dos anuros na Mata Atlântica e ressaltam a relação com os variados microhabitats do bioma.

Estudos relacionados ao comportamento reprodutivo dos anuros evidenciam a função fundamental da comunicação acústica para a reprodução (WELLS, 1977a; 1977b; 2007). Contudo, a importância da comunicação visual foi recentemente reconhecida, especialmente em espécies diurnas que vivem e se reproduzem em ambientes ruidosos e apresentam comportamentos complexos (GRAFE e WANGER, 2007; GRAFE et al., 2012; HÖDL e AMÉZQUITA, 2001). A maior disponibilidade de luz facilita o uso do sentido da visão para a comunicação em anuros diurnos, conseqüentemente proporcionando o desenvolvimento de comunicação visual mais complexa (GRAFE e WANGER, 2007; HADDAD e GIARETTA, 1999; HÖDL e AMÉZQUITA, 2001). Já o alto nível de interferência sonora em riachos de correnteza atua como modulador dos cantos dos anuros nestes habitats, apresentando frequências sonoras que sobrepujam os ruídos ambientais (GRAFE et al., 2012; HADDAD e

GIARETTA, 1999; HARTMANN et al., 2006; HÖDL e AMÉZQUITA, 2001). Os diversos modos de comunicação permitem o reconhecimento coespecífico, inclusive de parceiros reprodutivamente maduros, além de atuarem como importantes mecanismos de isolamento pré-zigótico, conduzindo os processos evolutivos (HÖDL e AMÉZQUITA, 2001; PFENNIG e PFENNIG, 2012; WELLS, 2007). Os estudos da biologia e comportamento reprodutivos podem auxiliar na resolução de problemas taxonômicos, contribuindo para a identificação de espécies morfologicamente semelhantes. Trabalhos recentes sugerem que espécies podem ser classificadas com maior certeza taxonômica aliando dados moleculares a geográficos, morfológicos, ecológicos, reprodutivos e comportamentais (DESALLE et al., 2005).

Restrita à América do Sul, a família de anuros Hyloidae reúne os gêneros *Hylodes*, *Crossodactylus* e *Megaelosia*, sendo composta atualmente por 42 espécies (LAIA e ROCHA, 2012). Todos os hylodídeos conhecidos são reofílicos e ocorrem em riachos de montanhas, associados essencialmente aos domínios da Mata Atlântica (CANEDO e POMBAL, 2007; HADDAD e POMBAL, 1995; LYNCH, 1971; POMBAL et al., 2002). O gênero *Hylodes* é o mais diversificado na família, apresentando hoje 24 espécies (LAIA e ROCHA, 2012). É endêmico do Brasil e restrito à porção leste do país, ocorrendo desde o Estado do Espírito Santo, na região Sudeste do país, até o Rio Grande do Sul, região Sul, adentrando para o leste apenas no Estado de Minas Gerais (LAIA e ROCHA, 2012). Característica do gênero e exceção entre os anuros, os *Hylodes* apresentam hábitos diurnos (HADDAD e POMBAL, 1995; HADDAD et al., 1996; NASCIMENTO et al., 2001; POMBAL et al., 2002). As espécies de *Hylodes* também apresentam modo reprodutivo atípico que, além de *Crossodactylus*, só é conhecido em peixes: os ovos são depositados em câmaras subaquáticas construídas pelo macho e as larvas são exotróficas, vivendo nos riachos de correnteza (HADDAD e PRADO, 2005).

A Serra do Japi é uma área de altitude de Mata Atlântica do Estado de São Paulo que apresenta uma única espécie conhecida de anuro do gênero *Hylodes*, cuja posição taxonômica ainda se apresenta confusa. Com base em morfologia, Haddad e Sazima (1992) sugeriram se tratar de *Hylodes ornatus*, espécie conhecida apenas na sua localidade-tipo e proximidades (Parque Nacional do Itatiaia na Serra da Mantiqueira, entre os Estados de Minas Gerais e Rio de Janeiro). Entretanto, estes autores já observaram diferenças quanto ao padrão de coloração e sugeriram a possibilidade da existência de variações geográficas ou ainda de ambas se tratarem de espécies crípticas. Canedo (2008), em revisão taxonômica do gênero *Hylodes*, evidenciou que a espécie de *Hylodes* da Serra do Japi não apresenta algumas características diagnósticas do *H. ornatus* (faixa escura no dorso evidente e geralmente completa e ventre

predominantemente escuro em machos), não considerando as duas populações como pertencentes à mesma espécie. Segundo a mesma autora, a espécie de *Hylodes* da Serra do Japi pode ser considerada semelhante à *Hylodes amnicola*, *Hylodes perere* e *Hylodes sazimai*, diferindo destas apenas por seu menor tamanho. Apesar de bastante similares morfológicamente, as espécies *H. amnicola*, *H. sazimai* e *H. perere* podem ser diferenciadas pelos padrões de coloração dorsal e pelas vocalizações de anúncio (CANEDO, 2008). *Hylodes amnicola*, *H. ornatus*, *H. perere* e *H. sazimai* são espécies pertencentes ao grupo de *Hylodes lateristrigatus* e ocorrem na Serra da Mantiqueira e proximidades, compreendendo os Estados de Minas Gerais, Rio de Janeiro e São Paulo (LAIA e ROCHA, 2012).

## 2. OBJETIVOS E ESTRUTURAÇÃO DA DISSERTAÇÃO

Os objetivos deste mestrado foram aprofundar os conhecimentos sobre a taxonomia da espécie de *Hylodes* da Serra do Japi e o seu posicionamento dentre as espécies fenotipicamente similares em seu gênero, além do entendimento de seu modo reprodutivo e da sua comunicação. O trabalho está estruturado em dois capítulos, sendo que o primeiro está aceito para publicação na revista *Herpetologica*, porém, ainda em processo de revisão. Por meio de análises morfológicas, de canto de anúncio e moleculares, o capítulo I traz o reconhecimento da espécie de *Hylodes* da Serra do Japi como uma entidade nova, fornecendo também a descrição das larvas e do modo reprodutivo, com detalhes da desova, dos ovos e do comportamento inédito da fêmea auxiliando no fechamento da toca subaquática após a oviposição. O capítulo II traz a investigação da comunicação da espécie, revelando o mais complexo repertório de sinais visuais conhecido para anuros, assim como a descrição de cinco novos sinais para Anura. A partir dos comportamentos de territorialidade dos machos e do ritual de corte elaborada da espécie, é explorada pela primeira vez a comunicação multimodal para um hilotídeo. Temos no segundo capítulo os registros de comunicação bimodal visual-tátil, realizada pela fêmea, e visual-acústica, realizada pelo macho. Além disso, registramos a capacidade dos machos em controlar o uso dos sacos vocais (que é duplo nos machos de *Hylodes*) durante interações intraespecíficas em proximidade com o receptor, de tal forma que o macho emissor pode inflar cada saco vocal de modo independente, direcionando-os de acordo com a posição do receptor. Além da importância para os campos da taxonomia, sistemática, biologia e comportamento de anuros, a presente dissertação de mestrado também é relevante para a conservação da espécie de *Hylodes* da Serra do Japi. O nível de ameaça de cada espécie só pode ser mensurado com precisão por meio do conhecimento de sua distribuição geográfica, uso de hábitat e biologia (PIMENTA et al., 2005; YOUNG et al., 2001). Somado a isso, o número de relatos ao redor do mundo sobre a diminuição e desaparecimento de espécies de anuros é recorrente (e.g. CUELLO et al., 2009; MORRISON e HERO, 2003; VALERA-LEAL et al., 2011), sendo a perda de hábitats e mudanças climáticas os fatores apontados como principais responsáveis pelo desaparecimento e diminuição de populações de anuros na Mata Atlântica [e.g. *Flectonotus ohausi* em Boracéia, São Paulo (HEYER et al., 1988) e *Vitreorana eurygnatha* na Serra do Japi (HADDAD e SAZIMA, 1992)].



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**CAPÍTULO I**

A NEW SPECIES OF *HYLODES* (ANURA, HYLODIDAE)  
WITH THE DESCRIPTION OF ITS UNUSUAL REPRODUCTIVE MODE

FÁBIO P. DE SÁ; CLARISSA CANEDO; MARIANA L. LYRA; CÉLIO F. B. HADDAD

A NEW SPECIES OF *HYLODES* (ANURA, HYLODIDAE) WITH THE DESCRIPTION OF ITS UNUSUAL REPRODUCTIVE MODE

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## RESUMO

Uma nova espécie de *Hylodes* é descrita para a Mata Atlântica da Serra do Japi, município de Jundiá, Estado de São Paulo, Sudeste do Brasil. A nova espécie pertence ao grupo de *Hylodes lateristrigatus*, apresentando linha dorsolateral esbranquiçada. É diagnosticada pelo tamanho pequeno; polegares sem estruturas nupciais; superfícies dorsais relativamente lisas; padrão dorsal bastante variável; área interocular com manchas marrom escuras evidentes e de formas variáveis, podendo apresentar um padrão uniforme ou uma barra marrom escura interocular; dorso varia de um padrão uniforme até outro com grandes manchas marrom escuras evidentes e de formas variáveis; ventre predominantemente claro, de cor creme; e, em machos adultos, sacos vocais laterais pareados, amplamente expandidos. A nova espécie é morfológicamente similar a *H. amnicola*, *H. ornatus*, *H. perere* e *H. sazimai*, mas se distingue pelo padrão de coloração e características do canto de anúncio. Além disso, análises moleculares (sequências parciais de dois genes mitocondriais e um nuclear: COI, 16S e RAG1, respectivamente) indicam que a nova espécie é geneticamente distinta destas espécies mais similares morfológicamente. São também fornecidas a descrição dos girinos e informações sobre modo reprodutivo, história natural e conservação. São registrados, pela primeira vez, detalhes da reprodução em *Hylodes*, como a desova, o tamanho dos ovos e o comportamento da fêmea auxiliando no fechamento da toca subaquática após a oviposição.

**Palavras-chave:** Canibalismo. Desova. Girino. *Hylodes* cf. *ornatus*. *Hylodes* sp. (gr. *lateristrigatus*). Mata Atlântica. Ovos. Vocalização.

## ABSTRACT

We describe a new species of *Hylodes* from the Atlantic forest of Serra do Japi, Município de Jundiá, State of São Paulo, Southeastern Brazil. The new species belongs to the *Hylodes lateristrigatus* group, characterized by a whitish dorsolateral stripe. It is diagnosed by small size; thumbs without nuptial excrescences; relatively smooth dorsal surfaces; dorsal pattern quite variable; interocular area with evident dark brown blotches of variable shapes, may have a uniform pattern or an interocular dark brown bar; dorsum varying from uniform pattern to large evident dark brown blotches of variable shapes; venter primarily light cream colored; and, in adult males, paired lateral vocal sacs, widely expanded. The new species is morphologically similar to *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai*, distinguishing from them by slight differences in color pattern and advertisement call. We also determined that the new species is genetically distinct from the aforementioned species via molecular data (partial sequences of two mitochondrial genes and one nuclear gene: COI, 16S, and RAG1, respectively). We also describe the tadpoles and provide information on reproductive mode, natural history, and conservation. We provide the first details of the reproduction of a *Hylodes* species, like the spawning, the size of the eggs, and the behavior of a female helping to conceal the underwater chamber after oviposition.

**Key-words:** Atlantic forest. Cannibalism. Eggs. *Hylodes* cf. *ornatus*. *Hylodes* sp. (gr. *lateristrigatus*). Spawning. Tadpole. Vocalization.

## 1. INTRODUCTION

The Neotropical genera *Hylodes* Fitzinger, 1826, *Crossodactylus* Duméril and Bibron, 1841, and *Megaelosia* Miranda-Ribeiro, 1923 have been recognized as a monophyletic clade based on morphological and molecular studies (FROST et al., 2006; LYNCH, 1971; NUIN and DO VAL, 2005). This clade was recently proposed as the family Hylodidae (GRANT et al., 2006; PYRON and WINS, 2011). *Hylodes* is the most diverse genus of Hylodidae, currently containing 24 species (FROST, 2013). These species are arranged into four groups based on external morphology (HEYER, 1982): the monotypics *Hylodes glaber* and *Hylodes mertensi* groups; the *Hylodes nasus* group, containing *H. asper*, *H. cardosoi*, *H. dactylocinus*, and *H. nasus*; and the speciose *Hylodes lateristrigatus* group that contains 18 species: *H. amnicola*, *H. babax*, *H. charadranaetes*, *H. fredii*, *H. heyeri*, *H. lateristrigatus*, *H. magalhaesi*, *H. meridionalis*, *H. ornatus*, *H. otavioi*, *H. perere*, *H. perplicatus*, *H. phyllodes*, *H. pipilans*, *H. regius*, *H. sazimai*, *H. uai*, and *H. vanzolinii* (CANEDO and POMBAL, 2007; HADDAD et al., 1996; HEYER, 1982; POMBAL et al., 2002). The last group is characterized by small to moderately sized individuals with slender bodies, relatively smooth dorsal surfaces, and light dorsolateral stripes (HEYER, 1982).

Species in the genus *Hylodes* are distributed throughout the Atlantic forest in eastern Brazil from the State of Espírito Santo to the State of Rio Grande do Sul (LINGNAU et al., 2008; NASCIMENTO et al., 2001; SAZIMA and BOKERMANN, 1982). *Hylodes* species are rheophilic with their reproductive biology strongly associated with torrent streams (LINGNAU et al., 2008; NASCIMENTO et al., 2001; POMBAL et al. 2002). Few *Hylodes* species have been observed in courtship and spawning, but past studies have already revealed that their reproductive mode involves aquatic unpigmented eggs and early larval stages in an underwater constructed chamber and exotrophic tadpoles in lotic water (e.g. HADDAD and GIARETTA, 1999; HADDAD and PRADO, 2005; NARVAES and RODRIGUES, 2005).

Herein, based on external morphology, advertisement call, and molecular evidence, we describe a new species of *Hylodes*, belonging to the *H. lateristrigatus* group. This new species occurs in the Atlantic forest of Serra do Japi, west of the Atlantic Plateau, in the State of São Paulo, Southeastern Brazil. We also provide, for the first time for any species in the *Hylodes* genus, information on the reproductive behavior of a female and traits of the spawning and eggs.



## 2. MATERIALS AND METHODS

Adult specimens, tadpoles, and eggs were collected and killed by anesthetic overdose. Tissues samples from livers and muscles were removed from recently killed adults and tadpoles and maintained in 100% ethyl alcohol. Adults were then fixed in 10% formalin and preserved in 70% ethyl alcohol. Tadpoles and eggs were fixed and preserved in 5% formalin. All adults, tadpoles, eggs, and tissue samples collected for the description of the new species and molecular comparisons following protocols approved by the Ethics Committee on Animal Use of Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil. These specimens and samples are housed in the Célio F. B. Haddad collection (CFBH), located in Departamento de Zoologia, Instituto de Biociências, of the same university.

We measured 11 standard metric traits for adults, following Duellman (2001): SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), TD (tympanum diameter), END (eye–nostril distance), IOD (interorbital distance), IND (internostril distance), THL (thigh length), TBL (tibia length), and FL (foot length). Definition of snout shape, for both dorsal and lateral views, follows Heyer et al. (1990). Measurements for tadpoles follow McDiarmid and Altig (1999): TL (total length), BL (body length), BH (body height; highest measure), IND (internostril distance; between centers of the nostrils), BW (body width; highest measure), IOD (interorbital distance; between centers of the pupil), END (eye–nostril distance; between center of the pupil and center of the nostril), and ED (eye diameter). Tadpole stages follow Gosner (1960) and tadpoles tooth row formula follows Altig (1970). We measured adult, tadpole, and egg traits using an ocular micrometer in a Zeiss stereomicroscope, except for SVL, HL, HW, THL, TBL, TL, and BL that we measured to the nearest 0.01 mm using a caliper. All measurements are expressed in millimeters. Drawings of the adult male and tadpole were made using a Zeiss stereomicroscope with a drawing tube.

Specimens used in the description are in the Célio F. B. Haddad collection (CFBH), Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil. Specimens examined for comparisons are listed in the *Appendix*. Institutional abbreviations are as listed at Sabaj Pérez (2010), except for MCT-PUCRS instead of MCP (Museu de Ciências e Tecnologia, Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil).

We recorded male vocalizations with a Marantz digital recorder (PMD-660), equipped with a Sennheiser external unidirectional microphone (ME-66). We measured air and water temperatures at the time of each recording. We analyzed the calls using a sampling frequency rate of 44.1 kHz and 16 bit resolution in the mono pattern with Raven Pro 1.4 (Cornell Lab of

Ornithology, Bioacoustics Research Program). We analyzed seven advertisement calls parameters: call duration (s), inter-calls duration (s), number of notes per call, note repetition rate (notes per s), note duration (s), inter-notes duration (s), and dominant frequency range (kHz). We described behaviors using focal animal and all occurrence sampling methods (ALTMANN, 1974; LEHNER, 1979).

Considering the phenotypic similarity that we found among the new species and *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai* we incorporated a molecular analysis exclusively for them. We included *H. nasus* and *H. pipilans* as outgroups. We obtained tissue samples of each analyzed species from their type localities from CFBH and MNRJ tissue collections (Table 1).

Table 1. *Hylodes* specimens included in the genetic analysis, collecting localities, and GenBank accession numbers (to be provided upon manuscript acceptance) for sequences generated in this study. Collecting localities correspond to the type localities for each species. We used tissue samples from a paratopotype of *H. pipilans* and topotypes for the remaining species.

Species	Voucher number	GenBank number			Locality
		COI	16S	RAG1	
<i>H. amnicola</i>	CFBH 30971				Ibitipoca, Lima Duarte, MG, Brazil
<i>Hylodes</i> sp. nov.	CFBH 33850				Serra do Japi, Jundiá, SP, Brazil
<i>H. nasus</i>	MNRJ 35435				Tijuca, RJ, Brazil
<i>H. ornatus</i>	CFBH 34905				Itatiaia, Itamonte, MG, Brazil
<i>H. perere</i>	CFBH 31106				Santa Bárbara do Monte Verde, MG, Brazil
<i>H. pipilans</i>	MNRJ 37307				Serra dos Órgãos, Teresópolis, RJ, Brazil
<i>H. sazimai</i>	CFBH 29586				Serra das Cabras, Campinas, SP, Brazil

DNA extraction, amplification, sequencing, and sequence alignment were performed as described in Canedo and Haddad (2012). GenBank accession numbers for sequences generated in this study are provided in Table 1. We analyzed partial sequences of two mitochondrial genes, Cytochrome oxidase c subunit I (COI) and 16S ribosomal RNA (16S), and one nuclear gene, the recombination activating gene 1 (RAG1). Table 2 lists the primers

used. We estimated genetic distances for mitochondrial genes using the Kimura two-parameter distance (KIMURA, 1980), with Standard Errors estimated using 1000 bootstraps in Mega 5.0 (TAMURA et al., 2011).

To elucidate phylogenetic relationships, we performed a Bayesian analysis in BEAST (DRUMMOND et al., 2012). For protein-coding genes (COI and RAG1), we analyzed the datasets by partitioning by gene and codon position; however, 16S was partitioned by gene only, for a total of seven partitions in the combined datasets. Using Mega 5.0 (TAMURA et al., 2011), we selected the best fit evolutionary model according to the Bayesian Information Criterion (BIC). Analyses were conducted under the GTR + G model for 16S and under the HKY + G for COI and RAG1, with a coalescent constant size prior starting with a randomly generated tree. Chains ran for 20 million generations and tree parameters were sampled every 2000 generations; 25% of initial values were discarded as burn-in. We confirmed convergence of the runs using Tracer software (RAMBAUT and DRUMMOND, 2007) and summarized the tree in TreeAnnotator 1.6.2 using the maximum clade credibility option as target tree type and mean heights for node heights.

Table 2. Primers used for amplification and sequencing reaction in the present study.

Primer	Direction	Gene	Sequence	Reference
LCO1490	Forward	COI	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HCO2198	Reverse	COI	TAAACTTCAGGGTGACCAAAAATCA	Folmer et al. (1994)
12SL13	Forward	16S	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller and Hedges (1998)
16sTitus1	Reverse	16S	GGTGGCTGCTTTTAGGCC	Titus and Larson (1996)
16sL2A	Forward	16S	CCAAACGAGCCTAGTGATAGCTGGTT	Hedges (1994)
16S-H10	Reverse	16S	TGCTTACGCTACCTTTGCACGGT	Hedges (1994)
16sAR	Forward	16S	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
16sWilk2	Reverse	16S	GACCTGGATTACTCCGGTCTGA	Wilkinson et al. (1996)
AmpF2	Forward	RAG1	ACNGGNMGICARATCTTYCARCC	Chiari et al. (2004)
AmpR1	Reverse	RAG1	AACTACGCTGCATTKCCAATRTCACA	Chiari et al. (2004)

### 3. SPECIES DESCRIPTION

#### 3.1. *Hylodes* sp. nov.

*Hylodes* cf. *ornatus*; Haddad and Sazima (1992)

*Hylodes ornatus*; Ribeiro et al. (2005)

*Hylodes ornatus*; Schloegel et al. (2012)

*Hylodes* cf. *ornatus*; Vieira et al. (2013)

*Hylodes ornatus*; Rosenblum et al. (2013)

#### 3.2. Specimens examined

CFBH 33850 (Figs. 1–3), an adult male, collected by Fábio P. de Sá and Adriana T. Salomão on 15 March 2012 at Serra do Japi (23°13'40"S, 46°57'59"W; 890 m above sea level; datum = WGS84), Município de Jundiá, State of São Paulo, Southeastern Brazil. In addition, eleven adult males (CFBH 693, 723, collected 11 March 1988 and 6 March 1989 respectively by Célio F. B. Haddad; CFBH 1245–49, collected 23 January 1991 by José P. Pombal Jr. and Célio F. B. Haddad; CFBH 33853–54, collected between 7 and 8 March 2012 by Fábio P. de Sá; CFBH 33855, collected 21 February 2011 by Fábio P. de Sá and Luiza R. Cholak; CFBH 33861, collected 7 March 2012 by Fábio P. de Sá) and four adult females (CFBH 724, collected 6 March 1989 by Célio F. B. Haddad; CFBH 3711, collected 2 November 1998 by Paulo C. A. Garcia and Célio F. B. Haddad; CFBH 33851, collected 14 March 2012 by Fábio P. de Sá and Adriana T. Salomão; CFBH 33857, collected 27 March 2011 by Fábio P. de Sá).

#### 3.3. Diagnosis

*Hylodes* sp. nov. is a slender species with relatively smooth dorsal surfaces (Fig. 2A). The new species is distinguished from all other species in the genus by the following combination of characters: (1) thumbs without nuptial excrescences; (2) dorsolateral fold continuously extending from supratympanic fold to groin; tubercles absent externally to the dorsolateral fold; (3) light dorsolateral stripes usually evident from shoulder to groin, become

gradually more apparent as they approach the posterior; (4) venter mainly light with dark spots, anastomosed or small dots, with weakly defined boundaries (Fig. 2B); (5) small size (SVL of males = 22.9–25.8 mm and SVL of females = 26.4–28.0 mm; see the statistics of variation in Table 3); (6) paired lateral vocal sacs, widely expanded externally, in adult males; (7) advertisement call (see details below); (8) scutes on upper surfaces of finger discs range from indistinct to somewhat evident; (9) dorsal pattern quite variable; interocular area with evident dark blotches of variable shapes, may have a uniform pattern or an interocular dark bar; dorsum varying from a uniform pattern to large evident dark blotches of variable shapes; (10) evident fringes on fingers and toes of males (wide and well-developed) and females (less developed); (11) small tubercles uniformly spread on the edge of upper lip in males; (12) evident light area on ventral half of lateral side of head, wider between nostril and eye; silver whitish in life (Fig. 1).



Figure 1. *Hylodes* sp. nov. (CFBH 33850; SVL = 24.5 mm); live adult male. Serra do Japi, Município de Jundiá, State of São Paulo, Southeastern Brazil.



Figure 2. *Hylodes* sp. nov., CFBH 33850, adult male in (A) dorsal and (B) ventral views. Scale bar = 5 mm.

Table 3. Measurements (in millimeters) of males ( $n = 12$ ) and females ( $n = 4$ ) of the type series of *Hylodes* sp. nov. ( $\bar{X}$  = mean; SD = standard deviation). Character abbreviations defined in *Materials and Methods* section.

	Males			Females		
	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range
SVL	24.7	0.76	22.9–25.8	27.1	0.65	26.4–28.0
HL	8.7	0.74	7.84–9.9	10.0	0.66	9.16–10.7
HW	8.16	0.32	7.73–8.62	8.72	0.36	8.31–9.16
ED	3.28	0.2	2.92–3.61	3.35	0.21	3.21–3.65
TD	1.63	0.2	1.28–1.95	1.83	0.17	1.66–2.07
END	1.6	0.16	1.3–1.87	1.96	0.07	1.9–2.06
IOD	2.57	0.34	1.99–3.02	3.01	0.27	2.69–3.3
IND	3.32	0.19	2.97–3.57	3.48	0.2	3.2–3.66
THL	12.5	0.37	12.0–13.0	13.7	0.24	13.5–14.0
TBL	13.0	0.38	12.1–13.8	14.8	0.52	14.2–15.4
FL	12.1	0.49	11.3–13.3	13.6	0.28	13.3–13.9

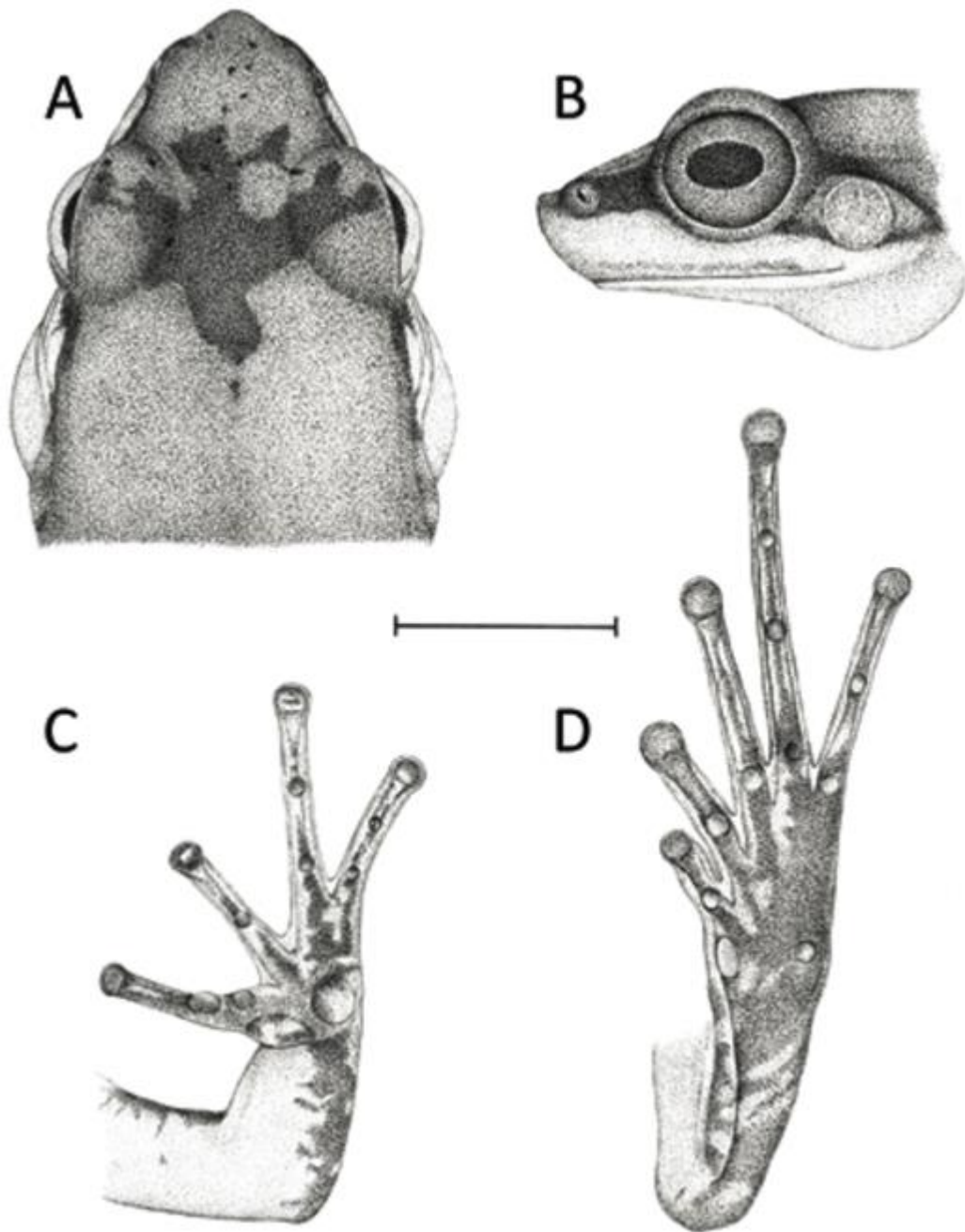


Figure 3. *Hylodes* sp. nov., CFBH 33850, adult male. (A) Dorsal and (B) lateral views of head and ventral views of (C) left hand and (D) left foot. Scale bar = 5 mm.

#### 3.4. Comparisons with other species

*Hylodes* sp. nov. can be easily distinguished from the species of the *H. nasus* group (*H. asper*, *H. cardosoi*, *H. dactylocinus*, and *H. nasus*) by relatively smoother dorsal surfaces (dorsal surfaces are rugose in the species of *H. nasus* group; LINGNAU et al., 2008; PAVAN et al., 2001; personal observations), dorsolateral fold continuously extending, light dorsolateral stripe usually evident, and tubercles absent externally to the dorsolateral fold (the

species of *H. nasus* group exhibit light tubercles scattered in the dorsolateral region, lacking dorsolateral fold and light dorsolateral stripe; PAVAN et al., 2001; LINGNAU et al., 2008; personal observations). Males of the new species can be easily distinguished from males of *H. fredei*, *H. phyllodes*, and *H. pipilans* by the absence of nuptial tubercles in the thumbs (thumbs with nuptial tubercles in *H. fredei*, *H. phyllodes*, and *H. pipilans*; CANEDO and POMBAL, 2007). *Hylodes* sp. nov. (males SVL 22.9–25.8 mm and females SVL 26.4–28.0 mm) also has a smaller size than *H. fredei* (*H. fredei* males SVL 32.2–36.7 mm and females SVL 34.5–37.5 mm). Compared to the following species, *Hylodes* sp. nov. has a lighter venter in adult males and a smaller size: *H. mertensi* (females SVL 51.5–54.5 mm; venter mainly dark with light blotches in adult males; BOKERMANN, 1956), *H. uai* (males SVL 30.9–33.6 mm and females SVL 36.4–36.6 mm; venter with proportional dark and light blotches in adult males; NASCIMENTO et al., 2001), *H. regius* (males SVL 30.7–33.5 mm and females SVL 36.2–38.0 mm; venter mainly dark with light dots in adult males; GOUVÊA, 1979), *H. otavioi* (males SVL 30.5–32.9 mm and female SVL 35.8 mm; venter with proportional dark and light blotches in adult males; SAZIMA and BOKERMANN, 1982), *H. babax* (male SVL 29.9 mm; venter mainly dark with light blotches in adult males; HEYER, 1982), and *H. magalhaesi* (males SVL 27.2–31.2 mm and females SVL 30.8–34.3 mm; venter mainly dark with light dots in adult males; BOKERMANN, 1964). The new species has a smaller size compared to *H. perplicatus* (males SVL 34.2–41.7 mm and females SVL 41.4–45.6 mm), *H. lateristrigatus* (males SVL 32.7–41.1 mm and females SVL 37.5–40.4 mm), *H. meridionalis* (males SVL 32.4–38.4 mm and females SVL 40.0–43.7 mm), *H. heyeri* (males SVL 32.1–42.0 mm and females SVL 39.0–45.7 mm), and *H. charadranaetes* (males SVL 28.6–34.6 mm and females SVL 32.7–35.7 mm). Finally, the new species has a lighter venter, smaller size, and paired lateral vocal sacs in adult males, as opposed to *H. glaber* (males SVL 32.5–38.6 mm and females SVL 36.0–40.0 mm; venter with proportional dark and light blotches; absence of vocal sacs in adult males; MIRANDA-RIBEIRO, 1926) and *H. vanzolinii* (male SVL 31.3 mm and female SVL 27.8 mm; venter mainly dark with light dots; absence of vocal sacs in adult male; HEYER, 1982).

*Hylodes* sp. nov. is morphologically similar to *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai*. However, the new species can be distinguished by its mainly light venter, variable interocular patterns, and dark, blotchy dorsal area. *Hylodes amnicola* exhibits an interocular dark bar (POMBAL et al., 2002). *Hylodes sazimai* exhibits a uniform dorsal pattern and an interocular bar exclusively along the anterior edges (HADDAD and POMBAL, 1995).



*Hylodes ornatus* adult males exhibit a mainly dark venter and a dark blotch along medial and longitudinal dorsal area (BOKERMANN, 1967). The new species also can be distinguished from *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai* via advertisement call (see Table 4) and by molecular evidence (see *Genetic analysis*).

Table 4. Advertisement calls of *Hylodes* sp. nov. and the most morphologically similar species: *H. amnicola* (in POMBAL et al., 2002), *H. ornatus* (in BILATE et al., 2012), *H. perere* (in SILVA and BENMAMAN, 2008), and *H. sazimai* (in HADDAD and POMBAL, 1995). ( $\bar{X}$  = mean; SD = standard deviation).

	<i>H. sp. nov.</i> (at 24°C)			<i>H. amnicola</i> (at 18°C)			<i>H. ornatus</i> (at 15°C)			<i>H. perere</i> (at 23–25°C)			<i>H. sazimai</i> (at 22°C)		
	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range
Call duration (s)	2.9	0.46	1.4–3.8	3.2	0.25	2.8–3.8	2.8	0.66	1.8–3.3	2.5	0.43	1.8–3.3	2	-	-
Inter-calls duration (s)	8.4	3.08	5.9–19.4	-	-	-	-	-	-	-	-	-	9.9	1.27	-
Notes per call	48.3	5.19	38–60	57.3	4.52	48–65	39.4	9.6	24–47 *	34.5	5.75	24–44	31.7	2.15	28–35
Rate notes / s	16.6	0.76	15.8–18	18.1	0.5	17.0–18.6	14.2	0.13	14–14.2	13.7	0.32	13.2–14.5	14.5	-	-
Note duration (s)	0.03	0.004	0.02–0.04	0.02	0.004	0.02–0.03	0.02	0.003	0.01–0.03	0.05	0.01	0.03–0.06	-	-	0.04–0.05
Inter-notes duration (s)	0.03	0.006	0.02–0.04	0.04	0.003	0.03–0.04	0.06	0.003	0.05–0.06	0.03	0.005	0.02–0.04	-	-	-
D. frequency (kHz)	6.2	0.17	5.8–6.6	-	-	5.1–6.2	5.3	0.18	5.1–6.4	-	-	4.8–6.2	-	-	4–5.5

\* Correction of range of notes per call for *H. ornatus* (M. BILATE, personal communication).

### 3.5. Genetic analysis

Sequence divergences between *H. amnicola*, *Hylodes* sp. nov., *H. ornatus*, *H. perere*, and *H. sazimai* (outgroup taxa excluded) ranged from 7.2% (*H. amnicola* vs. *H. perere*) to 16.2% (*Hylodes* sp. nov. vs. *H. amnicola*) in COI and from 3% (*H. amnicola* vs. *H. perere*) to 5.9% (*Hylodes* sp. nov. vs. *H. perere*) in 16S (Table 5). The tree topology obtained from the combined dataset (COI, 16S, and RAG1) is shown in Fig. 4. Analysis shows each species as a single evolutionary lineage, but we found small posterior probabilities (< 0.9) for phylogenetic relationships among *H. amnicola*, *Hylodes* sp. nov., *H. ornatus*, *H. perere*, and *H. sazimai*, except the clade that consists of *H. amnicola* and *H. perere* (0.99).

Table 5. Genetic distances (mean  $\pm$  standard error) among *Hylodes amnicola*, *Hylodes* sp. nov., *H. nasus*, *H. ornatus*, *H. perere*, *H. pipilans*, and *H. sazimai* for COI (upper-right diagonal) and 16S (lower-left diagonal).

	<i>H. amnicola</i>	<i>Hylodes</i> sp. nov.	<i>H. ornatus</i>	<i>H. perere</i>	<i>H. sazimai</i>	<i>H. nasus</i>	<i>H. pipilans</i>
<i>H. amnicola</i>		0.162 $\pm$ 0.018	0.127 $\pm$ 0.015	0.072 $\pm$ 0.011	0.122 $\pm$ 0.015	0.190 $\pm$ 0.019	0.207 $\pm$ 0.02
<i>Hylodes</i> sp. nov.	0.053 $\pm$ 0.006		0.161 $\pm$ 0.017	0.133 $\pm$ 0.016	0.135 $\pm$ 0.016	0.218 $\pm$ 0.021	0.218 $\pm$ 0.021
<i>H. ornatus</i>	0.047 $\pm$ 0.006	0.050 $\pm$ 0.006		0.133 $\pm$ 0.016	0.136 $\pm$ 0.016	0.211 $\pm$ 0.02	0.209 $\pm$ 0.021
<i>H. perere</i>	0.030 $\pm$ 0.005	0.059 $\pm$ 0.007	0.050 $\pm$ 0.006		0.101 $\pm$ 0.014	0.214 $\pm$ 0.022	0.2 $\pm$ 0.02
<i>H. sazimai</i>	0.041 $\pm$ 0.005	0.055 $\pm$ 0.006	0.045 $\pm$ 0.006	0.048 $\pm$ 0.005		0.212 $\pm$ 0.021	0.212 $\pm$ 0.021
<i>H. nasus</i>	0.125 $\pm$ 0.01	0.128 $\pm$ 0.01	0.122 $\pm$ 0.009	0.125 $\pm$ 0.01	0.120 $\pm$ 0.01		0.222 $\pm$ 0.022
<i>H. pipilans</i>	0.117 $\pm$ 0.01	0.123 $\pm$ 0.01	0.110 $\pm$ 0.009	0.122 $\pm$ 0.009	0.116 $\pm$ 0.009	0.155 $\pm$ 0.011	

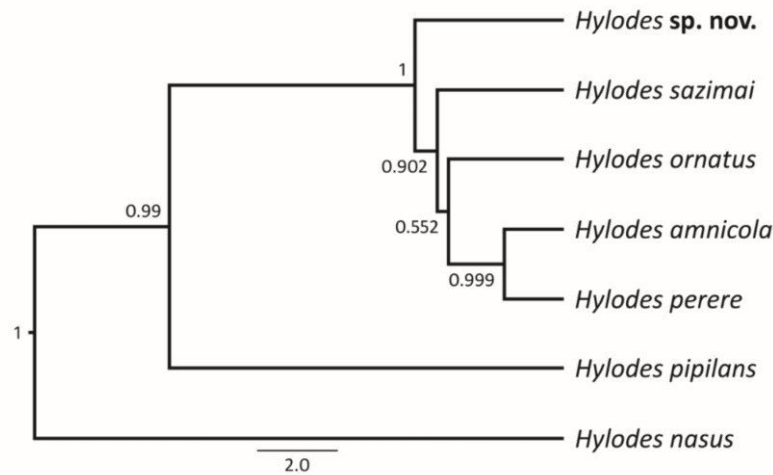


Figure 4. Phylogenetic tree obtained from a combined dataset (COI, 16S, and RAG1) using BI and ML analyses. Despite we obtained small posterior probabilities and bootstrap values for phylogenetic relationships, we present genetic evidence confirming each species as a single evolutionary lineage, comparing *Hylodes* sp. nov. with the following morphologically similar species: *Hylodes amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai*. *Hylodes nasus* and *H. pipilans* were added as outgroups.

### 3.6. Description of the specimen CFBH 33850

Small size; body slender (Fig. 2); head longer than wide; snout intermediate between pointed and mucronate in dorsal view and acute in lateral view (Figs. 3A, B); nostrils elliptical, non-protruding, laterally directed; canthus rostralis distinct, almost straight; loreal region concave; tympanum visible, nearly round, medium sized, nearly half the diameter of the eye; weak supratympanic fold; mental gland visible as acini spread in the gular region;

dorsolateral fold continuously (although somewhat indistinctly) extending from supratympanic fold to groin; tubercles absent externally to the dorsolateral fold; paired lateral vocal sacs, widely expanded externally; vocal slits present; tongue large, nearly ovoid, free in its final 1 / 3; vomerine teeth in two small series between choanae; choanae small and nearly round; maxillary teeth present; row of small tubercles uniformly spread on edge of upper lip. Arms and forearms slender; subarticular tubercles single, round (Fig. 3C); outer metacarpal tubercle large, round; inner metacarpal tubercle medium-sized, elliptical; thumbs without nuptial asperities, tubercles, or spines; discs on finger tips small, nearly oval from ventral view; scutes on upper surfaces of finger discs weakly developed; relative lengths of Fingers II < I  $\approx$  IV < III; Fingers I – IV bear lateral fringe. Legs robust; foot with elongated oval-shaped inner metatarsal tubercle (Fig. 3D) and a smaller protruding round outer metatarsal tubercle; subarticular tubercles single and protruding; relative lengths of Toes I < II < III  $\approx$  V < IV; toes exhibit extensive lateral fringe; tarsal flap extensive, distally continuous with fringe on the inner side of Toe I; toe discs nearly round; disc of Toe V smaller than other toe discs; developed scutes on upper surfaces of toe discs. Skin almost smooth on dorsum and flanks; slightly rugose on the posterior region of the body; undersurfaces smooth; weakly rugose texture near vent and on ventral surfaces of thighs.

### **3.7. Measurements of the specimen CFBH 33850 (mm)**

SVL 24.75; HL 9.90; HW 8.55; ED 3.29; TD 1.95; END 1.63; IOD 2.42; IND 3.45; THL 13.00; TBL 12.85; FL 11.90.

### **3.8. Coloration of the specimen CFBH 33850 in preservative**

Dorsum grayish-light brown with indistinct patterns of grayish-dark brown blotches in the interocular area and anterior region; whitish line extending from the tip of snout to the groin, line is faint from snout tip to arm and becoming strong from the arm region to the groin; dark brown lateral stripe extending from tip of snout, through nostril, eye, above of the tympanum and ends above vocal sac; a whitish lateral stripe occurs below the dark brown stripe and extending from tip of snout to protrusion of arm; lips gray; upper surfaces of arm, hand, thigh, tibia, tarsus, and foot pale gray; thigh with four dark gray transverse bars; tibia with three dark gray transverse bars; tarsus and foot with two dark gray transverse bars; small

warts on flanks and posterior region of the body dark gray; coccygeal region blackish; venter whitish with irregular small dark blotches (Fig. 2).

### **3.9. Coloration of the specimen CFBH 33850 in life**

The colors, although more vivid and contrasting, are essentially the same, comparing to the colors in preservative, excepting: copper iris; dorsum and upper surfaces of thigh, tibia, tarsus, and foot reddish brown; lateral stripe that extending from tip of snout to protrusion of arm (below the dark brown stripe) whitish silver; lips unpigmented and pale brown; dark brown transverse bars on thigh, tibia, tarsus, and foot; small warts on flanks and posterior region of the body dark reddish brown; yellowish coccygeal region; venter cream with irregular small gray blotches (Fig. 1).

### **3.10. Variation in preservative of the specimens examined**

Dorsum pale brown to gray; interocular area with evident dark blotches of variable shapes; interocular area may have a uniform pattern or a dark bar; dorsum varying from a uniform pattern to large evident dark blotches of variable shapes; one to five transverse bars of variable widths occur on thigh; coccygeal region blackish to gray; undersurfaces cream to whitish, with dark blotches ranging from brownish to grayish, also with variable intensity and shapes. Females are larger than males in SVL and do not have vocal slits and vocal sacs. Fringes evident on fingers and toes of males and females, more developed in males. See Table 3 for measurements of 12 males and 4 females (species series examined).

### **3.11. Distribution**

*Hylodes* sp. nov. is known only from the mountains of Serra do Japi, west of the Atlantic Plateau, Município de Jundiá, State of São Paulo, Southeastern Brazil (Fig. 5); an ecotonal region, mainly covered by seasonal semideciduous mesophytic forests (LEITÃO-FILHO, 1992).

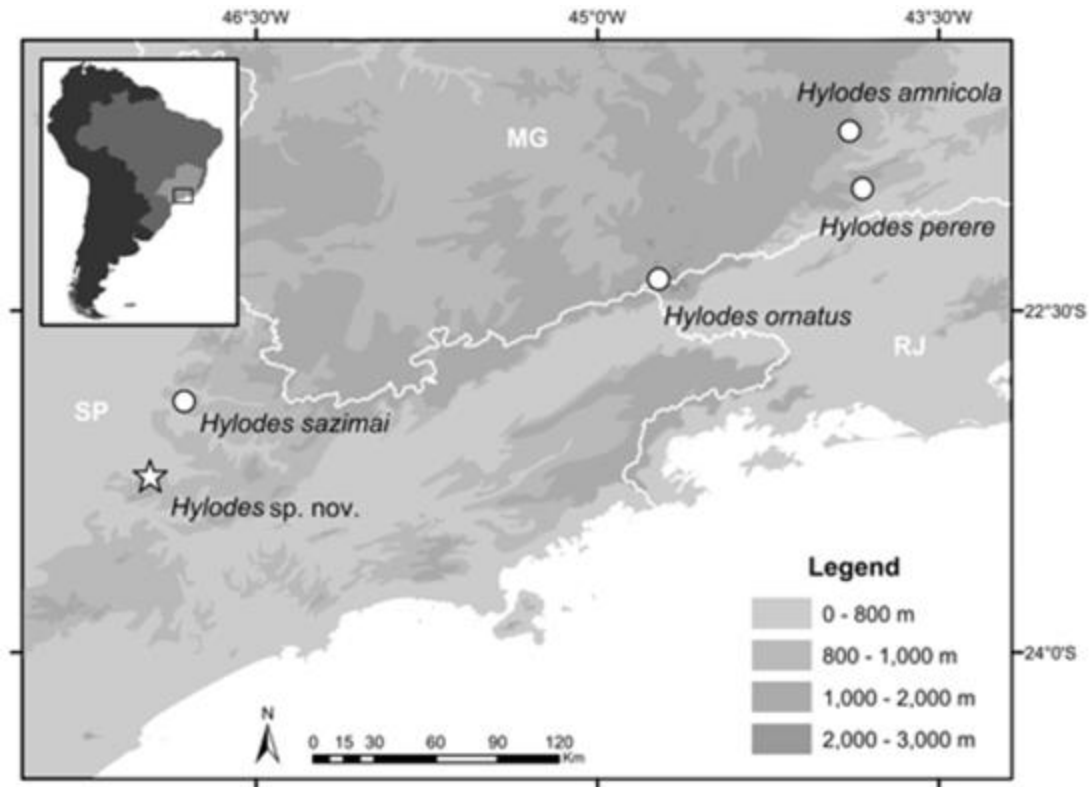


Figure 5. Type localities of *Hylodes* sp. nov. and the following morphologically similar species: *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai*. All are distributed in montane habitats of the Atlantic forest in eastern Brazil. Their maximum geographic ranges do not overlap. Tissue samples used in the genetic analysis are from the type localities.

### 3.12. Advertisement call

Males call sporadically. At an air temperature of 24°C and at a water temperature of 19.5°C, call duration is 1.36–3.83 s ( $2.92 \pm 0.46$ ,  $n = 34$  calls from four males); calls occur at intervals of 5.91–19.42 s ( $8.43 \pm 3.08$ ,  $n = 31$  intervals from four males); 38–60 notes per call ( $48.31 \pm 5.19$ ,  $n = 32$  calls from four males), given at a rate of 15.84–17.99 / s ( $16.6 \pm 0.76$ ,  $n = 32$  calls from four males); note duration is 0.017–0.039 s ( $0.025 \pm 0.004$ ,  $n = 90$  notes of nine calls from five males); notes given at intervals of 0.021–0.042 s ( $0.03 \pm 0.006$ ,  $n = 90$  intervals of nine calls from five males); each note consists of a rising frequency–modulated whistle; the dominant frequency occurs in the third harmonic and ranges from 5.8–6.6 kHz ( $6.2 \pm 0.173$ ,  $n = 90$  call notes from five males); the first note may be lower, with a dominant frequency range of 4.9–6.4 kHz ( $5.9 \pm 0.297$ ,  $n = 33$  call notes from five males). The advertisement call has harmonic structure; only the third harmonic is apparent (Fig. 6).

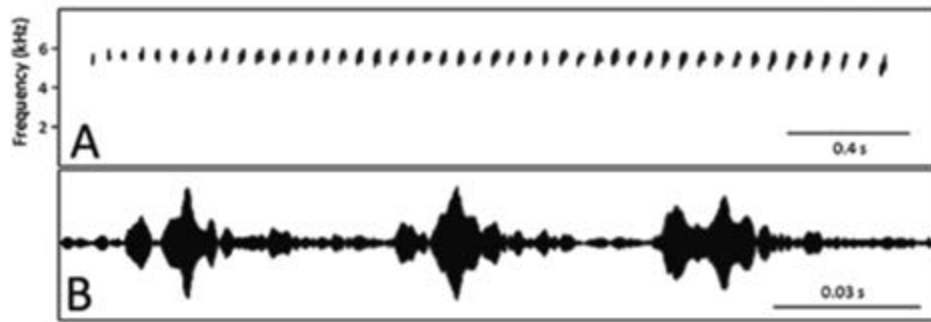


Figure 6. Advertisement call of *Hylodes* sp. nov. (A) Spectrogram of one call and (B) waveform of three notes of the advertisement call. Vocalization recorded on 15 April 2011; air temperature 24°C and water temperature 19.5°C.

*Hylodes* sp. nov. can be distinguished from morphologically similar species, particularly *H. amnicola*, *H. ornatus*, *H. perere*, and *H. sazimai*, by its advertisement call (see call comparisons in Table 4). The call notes of *H. amnicola* occur at a higher rate / s (18.1) and in shorter duration (0.015–0.034 s) (POMBAL et al., 2002). The call notes of *H. perere* occur less frequently per call (24–44), at lower rate / s (13.7), and for a longer duration (0.03–0.06 s) (SILVA and BENMAMAN, 2008). The call notes of *H. sazimai* occur less frequently per call (28–35), at a lower rate / s (14.5), for a longer duration (0.04–0.05 s), and at a lower dominant frequency (4.0–5.5 kHz) (HADDAD and POMBAL, 1995). Finally, the call notes of *H. ornatus* occur less frequently per call (24–47), at a lower rate / s (14.2), for a shorter duration (0.01–0.03 s), and with longer intervals duration (0.05–0.06 s) (BILATE et al., 2012).

### 3.13. Tadpoles

Aquatic tadpoles were collected at the type locality on 16 May 2011 by Fábio P. de Sá. The following description is based on tadpoles (lot CFBH 33865) in developmental Stage 27. The values are provided as mean  $\pm$  standard deviation ( $n$ ; range) in mm. TL  $54.4 \pm 4.31$  (11; 48.1–60.4); BL  $19.2 \pm 1.57$  (11; 16.5–21.3); BH  $7.3 \pm 1.29$  (11; 5.2–9.1); BW  $9.6 \pm 1.27$  (11; 7.6–11.5); IND  $3.9 \pm 0.22$  (11; 3.5–4.2); IOD  $5.1 \pm 0.42$  (11; 4.4–5.6); END  $2.9 \pm 0.19$  (11; 2.6–3.2); ED  $2.0 \pm 0.1$  (11; 1.8–2.2). Body oval in dorsal, ventral, and lateral views, widest posteriorly (Figs. 7A–C); snout rounded in dorsal and lateral views; eyes small, dorsolateral; nostrils dorsolateral, small, rounded, nearly midway between the eyes and the tip of the snout;

spiracle sinistral, situated at midbody, opening posterodorsal; cloacal tube short, opening dextral, attached to ventral fin; caudal musculature robust, gradually tapers to pointed tip; dorsal fin higher than ventral. Lateral line system composed of 12 lateral lines, six on each side of the body and tail. Body has a ventral depression anterior to the coiled intestine. Oral disc is directed ventrally and bordered by two rows of small papillae interrupted on the anterior labium (Fig. 7D); tooth row formula 2 (2) / 3 (1); jaw sheaths strongly developed and serrated, posterior jaw sheath V-shaped. The descriptions of the keratinized pieces of the mouth are based on one specimen, since almost all collected tadpoles exhibited damaged mouth structures, possibly due to infection by *Batrachochytrium dendrobatidis*, as already related for the new species (as *Hylodes ornatus* in SCHLOEGEL et al., 2012 and ROSENBLUM et al., 2013; and as *Hylodes cf. ornatus* in VIEIRA et al., 2013).

In preservative, dorsum and flanks are brown to dark brown; ventral surfaces brownish gray; edge of spiracle unpigmented and whitish; fins brownish gray and translucent, both with dark brown irregular blotches, more concentrated in the posterior region; iris black; edge of nostril black. In life, posterior dorsal part of the body and anterior dorsal part of the tail are brown with interspersed tones of yellow.

*Hylodes* sp. nov. tadpoles feed diurnally and nocturnally, but are more active and exposed at night. Tadpoles frequent benthic surface in the same torrent streams where adults breed, particularly among rocks and accumulated leaves. From February 2011 to May 2012 we observed tadpoles in high abundance; tadpoles were only less abundant in January.

### 3.14. Reproductive mode

*Hylodes* sp. nov. lays small spawning of large aquatic and unpigmented eggs inside a constructed underwater chamber; tadpoles are exotrophic in lotic water. Male chooses an area on the bottom of torrent streams and construct an underwater chamber by excavating the sand around small, submerged rocks. The chamber is totally permeable, filled by the stream water; apparently is only large enough for one male and female to fit inside. A short, narrow tunnel entrance provides access to the chamber. Based on observations, the male and female must enter one at a time. After oviposition, mate couple conceals the entrance of the chamber.

The male conducts the female until what we interpreted as potential oviposition sites during the underwater part of courtship behavior ( $n = 3$  courtships). Male dives and visits

several underwater sites, followed by the female; tactile stimulation occurs between the couple by touching themselves mutually with arms, legs, and dorsal and ventral surfaces of their bodies, but without amplexus ( $n = 3$  courtships). Apparently the couple explores some possible oviposition sites before deciding where they will lay the eggs ( $n = 3$  courtships). After they choose a site for the eggs, the male excavates a chamber in about one or two minutes. The male finishes construction and enters first, immediately followed by the female ( $n = 2$  courtships). When the male is conducting the female until the reproductive site, female can refuse the male, going away ( $n = 1$  courtship).

After oviposition, male and female leave the chamber. The male remains close to the entrance ( $n = 1$  courtship) and begins to conceal the entrance aperture by pushing sand from the bottom of the stream into the opening, first with his legs and feet, later with his arms and hands ( $n = 1$  courtship). The female returns and, apparently guided by the male, verifies the entrance of the chamber, also helping to conceal it, using her hands, moving her arms ( $n = 1$  courtship). When the chamber is sealed, the male returns to the surface; the female assess the blocked entrance and also leaves the chamber area ( $n = 1$  courtship). We observed one complete courtship event, which lasted an underwater total of 37 min. Conduction of female by the male until the chamber lasted approximately 5 min. Once inside the chamber, oviposition lasted 27 min. Concealment of the chamber entrance by male and female lasted 5 min. Males and females intermittently swam to the surface to breathe at all points of courtship, with the exception of oviposition while in the chamber ( $n = 3$  courtships).

After the only fully observed courtship event, we excavated the underwater chamber and collected the spawning, which consisted of 11 eggs. It was not possible to measure the chamber because the walls collapsed during the excavation. Mean egg diameter was  $3.47 \pm 0.4$  mm ( $n = 4$ ; 2.48–3.47 mm) and mean gelatinous capsule diameter was  $8.03 \pm 0.19$  mm ( $n = 3$ ; 7.81–8.16 mm). Eggs were unpigmented, exhibiting a cream whitish color (Fig. 8). In four days, fertilized eggs reached developmental Stage 14 (GOSNER, 1960). Stage 25 was the earliest larval stage observed swimming in the torrent stream; aquatic larva most likely remain inside the excavated chamber during the early development stages. In laboratory conditions, we observed tadpoles eating a fertilized conspecific egg. Based on our observations, *Hylodes* sp. nov. tadpoles are benthic, exotrophic, omnivorous, and occasionally cannibalistic.



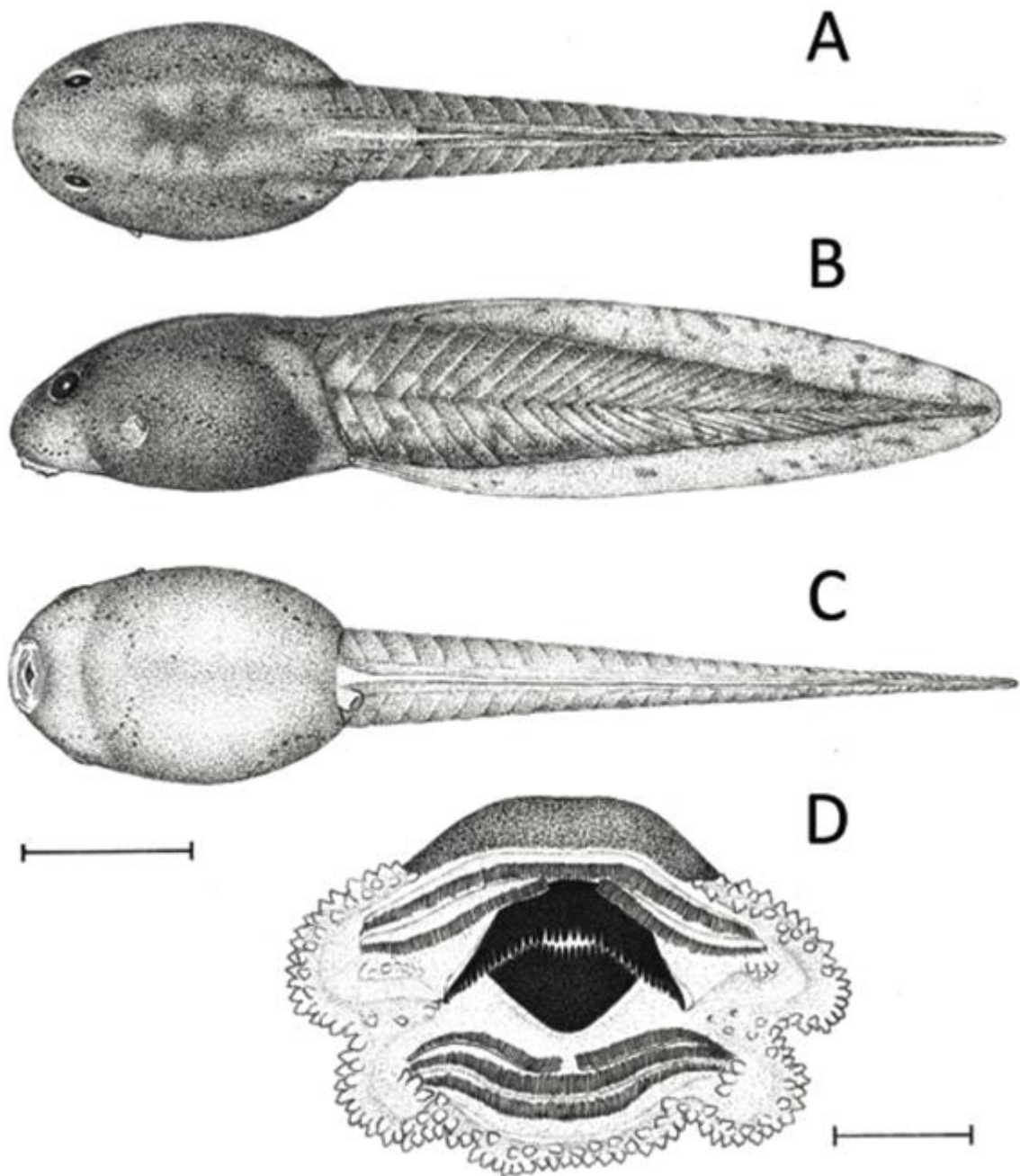


Figure 7. Tadpole of *Hylodes* sp. nov. CFBH 33865, in developmental Stage 27. (A) Dorsal, (B) lateral, and (C) ventral views; scale bar represents 10 mm. (D) Detail of the oral disc; scale bar represents 1 mm.

### 3.15. Natural history and conservation

*Hylodes* sp. nov. specimens were found along torrent streams between 850–1050 m. The new species appears to be mainly diurnal; however, during the breeding season, we recorded at least a few individuals calling at night as well. We observed (although less intense) crepuscular and nocturnal reproductive activity. Additionally, of the three observed

courtship events, two occurred during the day (15:00 h and 17:00 h) and one occurred at a dark night (4:00 h); the first courtship ended with rejection by the female, while the other two resulted in effective reproduction. Males call from land on the margins of torrent streams and emergent rocks, trunks, branches, and leaves located on the margins or in the middle of torrent streams. When disturbed, males usually hide in small crevices or dive into the water; after a few minutes, they return to the same calling site and begin to call again.



Figure 8. Four eggs of *Hylodes* sp. nov. from a spawning of 11. We uncovered eggs from an underwater chamber. Serra do Japi, Município de Jundiá, State of São Paulo, Southeastern Brazil.

Although Serra do Japi is considered a protected area in Brazil, it is currently under strong human development pressure, due to its close proximity to large urban centers. Serra do Japi supports an extraordinary diversity of flora and fauna (MORELLATO, 1992; VASCONCELLOS-NETO et al., 2012), and the discovery of a new anuran species endemic to this locality reinforces the urgent need for effective conservation in the area.

#### 4. DISCUSSION

*Hylodes* sp. nov. was previously identified as *Hylodes* cf. *ornatus* by Haddad and Sazima (1992). Although these authors mentioned differences in coloration between *Hylodes* specimens from Serra do Japi and *H. ornatus*, they mainly emphasized strong resemblance between them. The same authors considered the possibility of *Hylodes* populations from Serra do Japi and Itatiaia being “cryptic species” or a “geographical variation of the same species”. Indeed, due to their high level of similarity, *Hylodes* sp. nov. can only be distinguished from *H. ornatus* (as well as from *H. amnicola*, *H. perere*, and *H. sazimai*) by comparing dorsal and ventral color patterns, advertisement call, and molecular evidence; the latter suggests independent lineages with relatively high values of divergence (see VENCES et al., 2009).

The present study improves our knowledge of the diversity and the taxonomy of *Hylodes*, besides to amplify our understanding on the biology and behavior of the genus. As far as we know, the knowledge on reproductive mode in the entire Hylodidae family is currently based on records of *Crossodactylus gaudichaudii* in captivity (WEYGOLDT and CARVALHO-E-SILVA, 1991), and now six species of *Hylodes* in nature, *H. phyllodes* (FARIA et al., 1993), *H. asper* (HADDAD and GIARETTA, 1999), *H. heyeri* (LINGNAU, 2003), *H. dactylocinus* (NARVAES and RODRIGUES, 2005), *H. cardosoi* (FORTI and CASTANHO, 2012), and *Hylodes* sp. nov. (present study). These species represent about 16% of hylodids and no information exists regarding the reproductive biology of any species in the genus *Megaelosia*. Despite this lack of data, it is still reasonable to suppose that species within *Crossodactylus* and *Hylodes* exhibit a single reproductive mode, as previously suggested by Haddad and Prado (2005). We believe that concealing eggs inside an underwater chamber may increase the protection of offspring against: (1) strong torrent flow, (2) heterospecific predators, and (3) cannibalism.

Although the life histories of *Hylodes* species have still not been adequately investigated, their reproductive biology has likely influenced diversification the most throughout the Atlantic forest, as this rheophilic genus is strongly associated with torrent streams (e.g. LINGNAU et al., 2008; NASCIMENTO et al., 2001; POMBAL et al., 2002; present study). Furthermore, given the high level of Atlantic forest endemism (CARNAVAL and MORITZ, 2008) and the increasing trend of new hylodid species being described (LAIA and ROCHA, 2012), the taxonomic diversity of *Hylodes* is clearly underestimated. As we demonstrate in the present study, molecular evidence continues to aid in revealing cryptic

diversity. Future taxonomic and phylogenetic studies must integrate natural history, morphology, advertisement call, geographical distribution, and DNA sequence data (see DAYRAT, 2005; PADIAL et al., 2010; WILL et al., 2005) in order to reveal the true diversity of *Hylodes* and relationships among its species.

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## APPENDIX: ADDITIONAL SPECIMENS EXAMINED

*Hylodes amnicola*: State of Minas Gerais: Lima Duarte, Parque Estadual do Ibitipoca MNRJ 24859 (holotype), MNRJ 24846–47, 24860–61, 25650–52, 25655, 26309, 26858 (paratypes), CFBH 30971. *Hylodes asper*: State of Rio de Janeiro: Duque de Caxias, Barro Branco MNRJ 666, 1276, 1517, 1554, 1980, 8116, 8187, 8189, 10473; Duque de Caxias MNRJ 2316; Mangaratiba, Praia Grande MNRJ 33090; Mangaratiba, RESECO Rio das Pedras MNRJ 1682–83; Parati, Tarituba MNRJ 32859; Parati MNRJ 1375, 1631, 7568–72; Teresópolis EI 8787, MNRJ 2008, 31106–08, 33476, MZUSP 2038, 53426; Matas de Salú MNRJ 2071. State of São Paulo: Serra da Bocaina MZUSP 23410, 23413–14, 23409, 23411, 23435, 23437–38, 23446, 23450, 23471–72, 23476–77, 23480, 23482–84, 23486; São José do Barreiro, Serra da Bocaina, Campo de Fruticultura MZUSP 76965–66, 76970, 76975; Bananal, Serra da Bocaina, Posto de Biologia e Criação de Truta EI 1310, 2569; Caraguatatuba MZUSP 9988; Ilha Bela MZUSP 1484, 9979; Salesópolis, Boracéia MZUSP 1753, 1761, 1766, 1768, 1784–85, 1787–88, 1793–94, 1797, 1802–03, 1806–07, 1809, 1812, 1814, 4033, 4035, 4037, 4039, 23563–64, 23755, 23759, 23761, 23764, 27983, 37586–87, 37661–63, 37665–66, 37711, 37764; Santo André, Paranapiacaba MZUSP 8852–54, 23497, 23499, 23501, 23506; Ubatuba, Picinguaba MZUSP 130196–97; Caminho do Mar MZUSP 10012, 10236, 10238, 21897–99, 21903, 21905, 23551, 64748, 21907–08, 23554–55, 97601. *Hylodes babax*: State of Minas Gerais: Alto Caparaó, Parque Nacional do Caparaó MZUSP 57949 (holotype). *Hylodes cardosoi*: State of Paraná: Morretes, Porto de Cima MNRJ 29751 (holotype), 28533–34, 29752, 29754–55; Morretes, Marumbi, Rio Taquaral MNRJ 38061–65, 39250, 40264–65; Morretes, Marumbi DZSJRP 5211, 5214, 5217, DZSJRP 5221–22, 5224–25, 5233–34; Morretes, São João da Graciosa MZUSP 60685–88, 60690–92, 60695–96; Morretes DZSJRP 5289–90, 5292, 5294–95, 5308–11, 5314–15, MZUSP 112585–88 (paratypes), DZSJRP 5288, 5291, 5296–5298, 5304–07, 5312–13, 5316, MZUSP 112586, 112588. State of São Paulo: Apiaí, Gruta dos Caboclos MZUSP 21882, 21886, 21888; Apiaí MZUFV 4740–41, 4753; Capão Bonito MZUFV 5120–21; Iporanga CFBH 8275–76. *Hylodes charadranaetes*: State of Rio de Janeiro: Teresópolis, PARNA Serra dos Órgãos, Abaixo do km 4 da subida MNRJ 31842–43; Teresópolis, PARNA Serra dos Órgãos, Córrego Frio MNRJ 31844–47; Teresópolis, PARNA Serra dos Órgãos, Entre Fagundes e Barreira MNRJ 41949; Teresópolis, PARNA Serra dos Órgãos, Garrafão MNRJ 45750; Teresópolis, PARNA Serra dos Órgãos EI 2572–74, 8753, 10121–22, MNRJ 31175, 31836–37, 45667–70, 45672, 45756–68, 45770–72, 45773–78; PARNA Serra dos Órgãos, MNRJ 31831, 33740, 35114,

41962, 41964; PARNA Serra dos Órgãos, Ponto de abastecimento de água MNRJ 45753–55; Teresópolis, Soberbo MNRJ 31830; Teresópolis MNRJ 548, 3308, 31758, 31760, 31763, 31833, 45769; Serra dos Órgãos MNRJ 31176, MZUSP 475; close to Teresópolis, Alto do Soberbo MZUSP 60663–66 (paratypes); Nova Friburgo MNRJ 27074; Casemiro de Abreu, Morro de São João MNRJ 40626–28. *Hylodes dactylocinus*: State of São Paulo: Peruíbe, Estação Ecológica Juréia-Itatins MZUSP 89904 (holotype), MNRJ 31199, MZUSP 89901, 89903, 89906, 89909–14 (paratypes). *Hylodes fredii*: State of Rio de Janeiro: Angra dos Reis, Ilha Grande MNRJ 36077 (holotype), MNRJ 10895, 18809, 35191–204, 35206–09, 35210–11, 35214, 35217, 35220–22, 35246–48, 35250–54, 35256–62, 38936 (paratypes). *Hylodes glaber*: State of Minas Gerais: Itamonte, PARNA Itatiaia, Brejo da Lapa MNRJ 3284 (holotype of *E. pulchra*); State not indicated: Planalto de Itatiaia, Brejo da Lapa, km 10 MNRJ 41891, 41893–95. State of Rio de Janeiro: Resende, PARNA Itatiaia, Estrada das Agulhas Negras EI 7049–50, 7052; Resende, PARNA Itatiaia, Rodovia das Agulhas Negras, km 10 EI 8581, 9851; PARNA Itatiaia MNRJ 3564, 3900, 15239–40, 31120, 31123–25, 31181–83, MZUSP 12732, 12735–36, 58994. *Hylodes heyeri*: PARANÁ: Morretes, Instituto Agrônômico do Paraná (IAPAR) MNRJ 31805–06, 39240–48; Morretes, Rio Taquaral MNRJ 39249; Morretes, Marumbi MNRJ 40267–70, 41968, MZUSP 112578, 112580–82, 112584; Banhado, MZUSP 112589. State of São Paulo: Eldorado, close to Caverna do Diabo MNRJ 17090 (holotype), MNRJ 17091 (paratype); Parque Estadual do Alto Ribeira MZUSP 93228; Apiaí, Bairro dos Caboclos MZUSP 13472. *Hylodes lateristrigatus*: State of Rio de Janeiro: Nova Friburgo MNRJ 23625–27, 33739; Tinguá, Nova Iguaçu MNRJ 31789; Teresópolis EI 8757, MNRJ 2007, 5055, 10497, 23628, 31835, 31839, 33741, 41867–68, MZUSP 53259–61. State of Espírito Santo: Cariacica, Duas Bocas MNRJ 30333, 31269; Santa Tereza MNRJ 28342–43, 28417, 30643–44, 30916, MZUSP 76369. *Hylodes magalhaesi*: State of Minas Gerais: Camanducaia, Monte Verde CFBH 3714, 7491, 8329–31, MNRJ 30374–75. State of São Paulo: Campos do Jordão MZUSP 73676 (holotype), EI 2577, MNRJ 3973, 14219, MZUSP 73622–29, 73677, 73705, 74439 (paratypes), EI 362; Campos do Jordão, Fazenda Lagoinha MZUSP 112658, 112661–62, 112664, 112665; Campos do Jordão, Horto Florestal CFBH 9921–26. *Hylodes meridionalis*: State of Rio Grande do Sul: São Francisco de Paula EI 9608, MCT-PUCRS 8317–21, MNRJ 41950, 41981, 41983–92, 41995–99, 42001–02, MZUSP 112851, 112853–55, 130380–81; Viamão, Parque Estadual Itapoã MZUSP 89938–39; Serra do Pinto, Terra de Areia MCT-PUCRS 1658–60. *Hylodes mertensi*: State of São Paulo: Caminho do Mar, km 47 MZUSP 74171 (holotype), MZUSP 10017. *Hylodes nasus*: State of Rio de Janeiro: Rio de Janeiro, Tijuca MNRJ 88, 1445, 1856, 1864, 1866, 1869,

2671, 3089, 10192–93, 10195, 10207, 10213, 12492, 13698–99, 13703–09, 18478, 29210, 31853–54, 31875, 31877, 31880, 31884, 31932–36, 31938–39, 32075–84, 33743–44, 34198, 34200–01, 35113, 35298, 35434, 35485, 35488–90. *Hylodes ornatus*: State of Minas Gerais: Vargem Grande, Estrada Brejo da Lapa MNRJ 33405; Itamonte, PARNA Itatiaia, close to Brejo da Lapa, km 7 CFBH 3569, 34905. State of Rio de Janeiro: Resende, PARNA Itatiaia, Estrada das Agulhas Negras EI 7043; Resende, PARNA Itatiaia, Estrada das Agulhas Negras, km 10, EI 7044–48, 9840; Resende, PARNA Itatiaia, Estrada do Planalto de Itatiaia, km 9 MNRJ 32070; Resende, PARNA Itatiaia, Planalto de Itatiaia, Estrada do Registro, km 11 MNRJ 14521–22; Subida para o Alto / Planalto Itatiaia MNRJ 3550, 41930–31, 41933, 41935; Alto Itatiaia MNRJ 31115, 32069, 41927–28; Planalto de Itatiaia, PARNA Itatiaia MZUSP 73679 (holotype), MZUSP 73682–83, 73871–75, 73878–79, 73875, 73877, 73880–81, (paratypes); Itatiaia, Brejo da Lapa MNRJ 41877, MZUSP 60843–45; Itatiaia, Macieira MNRJ 41919; PARNA Itatiaia MZUSP 41916, 96195, 130384–86; Itatiaia MNRJ 41921–22, 41926. *Hylodes otavioi*: State of Minas Gerais: Jaboticatubas, Serra do Cipó MNRJ 4163 (holotype), MZUSP 73575–77. *Hylodes perere*: State of Minas Gerais: Arantina CFBH 22830–33; Santa Bárbara do Monte Verde, Serra Negra CFBH 24473–74, 31106–08. *Hylodes perplicatus*: State of Santa Catarina: Corupá MNRJ 89 (lectotype), MNRJ 545, 5589, 5594–95, 5600–01, 5606, 5608, 5610, 5613, 5615, 5617, 5621 (paralectotypes); São Bento do Sul, Estrada do Saraiva MNRJ 41878–80, 41882; São Bento do Sul CFBH 3570, 3572–74, MNRJ 30587; Jardim, MZUSP 60697; Botuverá Parque Municipal de Botuverá MZUSP 133226; Timbé do Sul MCT-PUCRS 3314, MZUSP 60698, 60700; Serra da Subida, Caminho Blumenau - Lages MNRJ 41948. *Hylodes phyllodes*: State of Rio de Janeiro: Parati MNRJ 1256, 31849–52; Parati, Tarituba MNRJ 33393; Angra dos Reis, Estrada entre Angra dos Reis e Lídice MNRJ 30933; Mangaratiba, RESECO Rio das Pedras MNRJ 37900–01, 41680–81; Matas de Salú MNRJ 2074, 10623. State of São Paulo: Salesópolis, Boracéia MZUSP 59934 (holotype); MZUSP 1700–02, 1704–06, 1708–11, 1714, 1716–21, 3308, 3527, 4040–43, 23050–53, 23679–81, 23686–88, 23690–91, 23693–97, 36874, 37573, 37575–79, 37581–83, 37585, 37678, 37680, 37683–87, 37702–03, 38854 (paratypes); Santo André, Paranapiacaba, Pedra Lisa CFBH 856, 868; Cubatão, COPEBRÁS CFBH 9226, 11345, 11356–57, 11359, 11549, 11551, MZUSP 11365, 11381; Cubatão MZUSP 64747; Cubatão, Caminho do Mar, km 47 MZUSP 10216; São Sebastião MZUSP 58717; Ilha Bela, Ilha dos Búzios MZUSP 23952, 23954; Ilha Bela MNRJ 24303–04, MZUSP 51669; Ubatuba, Praia Vermelha do Sul MZUSP 84582; Ubatuba, Picinguaba CFBH 1418–19, 1421, 1505–06, 3948, 3962, 4258, 12817–18; Ubatuba, Picinguaba, Cascata da estrada para Cambury CFBH 12810–11;

Ubatuba, Picinguaba, Poço do Amor CFBH 12809, 12814, 12816; Ubatuba, Picinguaba, riacho próximo à cachoeira da escada MNRJ 34482–83; Ubatuba, Cassandoquinha, Trilha a direita da praia MNRJ 40173–74; Ubatuba, Maranduba, Trilha após a praia Caçandoquinha MNRJ 34481; Cunha CFBH 10874; Parque Estadual da Serra do Mar, Núcleo Cunha-Indaiá, Trilha Rio Bonito CFBH 12192; São José do Barreiro, Serra da Bocaina, Campo de Fruticultura MZUSP 113986. *Hylodes pipilans*: State of Rio de Janeiro: Guapimirim, PARNA Serra dos Órgãos, próximo ao Rio Soberbo MNRJ 33765 (holotype), MNRJ 31928–31, 33742, 33764, 33766, 35989–90, 37306–07, 39371, 39374 (paratypes). *Hylodes regius*: State of Minas Gerais: Itamonte, Vargem Grande MNRJ 4110 (holotype); MNRJ 4109, 4111 (paratypes), Brejo da Lapa MNRJ 41913–14. Stated not indicated: Alto Itatiaia MNRJ 41886. *Hylodes sazimai*: State of São Paulo: Campinas, Joaquim Egídio, Observatório de Capricórnio MNRJ 15869 (paratype), CFBH 3708–3709; Campinas, Sousas MNRJ 34697; Monte Alegre do Sul, MZUSP 2332, 2340. *Hylodes uai*: State of Minas Gerais: Belo Horizonte, Mangabeiras MNRJ 23771 (holotype), MNRJ 23772–75, 23777 (paratype); Morro do Pilar, Rio Mata-Cavalo MNRJ 41454–56; Catas Altas, Serra do Caraça MZUSP 23861–64, MZUSP 23861. *Hylodes vanzolinii*: State of Minas Gerais: Alto Caparaó, Parque Nacional do Caparaó, MZUSP 57950 (holotype); MZUSP 52923 (paratype).

## CAPÍTULO II

COMPLEX COMMUNICATION IN A BRAZILIAN TORRENT FROG,  
GENUS *HYLODES*, AND A REVIEW OF VISUAL COMMUNICATION IN HYLODIDS  
(ANURA)

FÁBIO P. DE SÁ; JULIANA ZINA; CÉLIO F. B. HADDAD



COMPLEX COMMUNICATION IN A BRAZILIAN TORRENT FROG, GENUS *HYLODES*, AND A REVIEW OF VISUAL COMMUNICATION IN HYLODIDS (ANURA)

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## RESUMO

A comunicação intraespecífica em anuros exerce um papel importante na sinalização e defesa territorial, no reconhecimento sexual e coespecífico e como um mecanismo de isolamento reprodutivo pré-zigótico. Neste estudo é investigada a comunicação intraespecífica em *Hylodes* sp. (gr. *lateristrigatus*), uma espécie de rã-de-corredeira brasileira com machos territoriais e com comportamento de corte elaborado. É apresentado o repertório de sinais acústicos assim como o mais complexo repertório de sinais visuais entre anuros, incluindo cinco novos sinais visuais aqui descritos. A comunicação bimodal visual-tátil é descrita pela primeira vez entre os anuros, realizada pela fêmea durante a corte. Nas sinalizações visuais, o macho escolhe o membro locomotor ou saco vocal (que é duplo em *Hylodes*) que será usado (direito, esquerdo ou ambos) de acordo com a posição do receptor coespecífico. Possivelmente este direcionamento melhora a performance individual. O movimento e a cor dos sacos vocais devem ser importantes para a comunicação visual em *Hylodes* sp. (gr. *lateristrigatus*), mesmo quando há emissão sonora. Por fim, é apresentada uma revisão de sinais visuais em rãs-de-corredeira neotropicais, destacando a extraordinária diversidade comportamental na família Hylodidae. Este estudo indica que a complexidade da comunicação visual em hilodídeos, assim como em anuros em geral, ainda está subestimada, principalmente nos trópicos. Nas regiões tropicais a comunicação tende ser mais complexa, possivelmente correlacionada com o maior número de espécies e de grupos filogenéticos ou as limitações ecológicas, tais como hábitos diurnos e riachos de correnteza como habitats reprodutivos.

**Palavras-chave:** Amphibia. Comunicação bimodal. Comunicação multimodal. Comunicação tátil. Comunicação visual. Controle do saco vocal. *Hylodes* sp. (gr. *lateristrigatus*). Mata Atlântica.

## ABSTRACT

Intraspecific communication in frogs plays an important role in territorial signaling and defense, conspecific and sexual recognition, and as a pre-zygotic isolation mechanism. We investigate the intraspecific communication in *Hylodes* sp. (gr. *lateristrigatus*), a Brazilian torrent frog with territorial males and an elaborated courtship behavior. We describe the repertoire of acoustic signals as well as the most complex repertoire of visual displays in anurans, including five new visual displays. We describe the visual-tactile bimodal communication for the first time in frogs, in the present case performed by the female during the courtship. We also verify that, for visual signaling, the male chooses the limb or vocal sac (which is double in *Hylodes*) that will be used (right, left, or both) according to the position of the conspecific receptor. Most likely this orientation improves and optimizes individual performance. It is also considered evidence that the vocal sac movement and color must be important for visual communication in *Hylodes* sp. (gr. *lateristrigatus*), even with sound production. Finally, we provide a review of visual displays in Neotropical torrent frogs, highlighting the extraordinary behavioral diversity in the family Hylodidae. Our study indicates that the complexity of communication in hylodids, as well as in anurans in general, is still underestimated, mainly in the tropics. In tropical regions the communication tends to be more complex, most likely correlated to the higher number of species and phylogenetic groups or ecological constraints, such as diurnal habits and fast streams as breeding habitats.

**Key-words:** Amphibia. Atlantic forest. Bimodal communication. *Hylodes* sp. (gr. *lateristrigatus*). Multimodal communication. Tactile communication. Visual communication. Vocal sac control.

## 1. INTRODUCTION

Intraspecific communication assumes a sender transferring a message to a receiver via codified signals that both are able to understand (BRADBURY and VEHCAMP, 1998; HÖDL and AMÉZQUITA, 2001). In turn, multimodal communication presupposes that individuals use two or more sensory modalities to compound combined and more complex signals for message transfer. Multimodal signaling is known for several animal groups, such as wolf spiders (HEBETS et al., 2013; TAYLOR et al., 2005), fishes (MARUSKA et al., 2012; MCLENNAN, 2003), squirrels (PARTAN et al., 2009; PARTAN et al., 2010), and birds (DALZIELL et al., 2013; SEIBT and WICKLER, 1977). Acoustic communication is currently considered the main intraspecific mode of communication among most frog species and has been historically more studied than other modes of communication (DORCAS et al., 2009; NARINS et al., 2007). However, researches focusing on visual communication have increased more recently, revealing that this communication mode is also relevant for several diurnal and nocturnal frog families (e.g. HADDAD and GIARETTA, 1999; STARNBERGER et al., 2011; ZINA and HADDAD, 2007). Even more recently, studies on multimodal frog communication are providing considerable progresses in the comprehension of intraspecific anuran communication (e.g. GOMEZ et al., 2011; GRAFE and WANGER, 2007; PREININGER et al., 2013).

Hödl and Amézquita (2001) conducted the last review on visual communication in anurans, organizing the visual displays of several distinct species and evidencing the great visual communication complexity in frogs. Since that compilation, many new records on visual displays have been published for several anuran species like *Hypsiboas albomarginatus* (GIASSON and HADDAD, 2006), *Nectophrynoides tornieri* (STARNBERGER et al., 2011), and hylodid frogs like *Hylodes cardosoi* (FORTI and CASTANHO, 2012) and *Hylodes phyllodes* (HARTMANN et al., 2005). The diversity on visual display repertoires is variable among frog species and families, suggesting several cases of independent evolution (see HARTMANN et al., 2005; HÖDL and AMÉZQUITA, 2001). The evolution of visual communication in frogs apparently was favored by particular ecological traits and the repertoires of visual displays are more complex in diurnal species that breed in noisy environments, like *Hylodes asper* (HADDAD and GIARETTA, 1999), *Micrixalus saxicola* (KRISHNA and KRISHNA, 2006), and *Staurois parvus* (GRAFE et al., 2012).

Some specific groups of anurans were indicated as potentially important to the understanding of the evolution of communication in this vertebrates (see HÖDL and AMÉZQUITA, 2001). Among those anurans are the species of Brazilian montane fast streams dwellers, genus *Hylodes*. In fact species of *Hylodes* (Hylodidae) are still understudied and are excellent models to investigate communication, since they usually vocalize and have an extraordinary repertoire of visual displays associated to distinct social and breeding contexts (FORTI and CASTANHO, 2012; HADDAD and GIARETTA, 1999; HARTMANN et al., 2005; NARVAES and RODRIGUES, 2005).

Herein we investigated the communication of the Brazilian torrent frog *Hylodes* sp. (gr. *lateristrigatus*), an endemic species to the montane Atlantic forest of Southeastern Brazil with territorial males and an elaborated courtship behavior. Specifically our goals were to: (1) characterize the visual displays and acoustic signals performed by males and females of *Hylodes* sp. (gr. *lateristrigatus*), identifying the respective roles and associated social contexts; (2) describe the *Hylodes* sp. (gr. *lateristrigatus*) elaborated courtship behavior, investigating the visual-tactile bimodal communication performed by females; and (3) characterize the way used by senders to control signal emission according to the position of the receiver. Moreover we provide a review of the current knowledge on visual signaling in Neotropical torrent frogs based on literature and new data, highlighting the behavioral diversity in this family (Hylodidae). Our study indicates that, in hylodids and in anurans in general, visual communication is certainly more widespread and complex than we currently know.

## **2. MATERIALS AND METHODS**

### **2.1. Study site**

The study was conducted in the biological reserve of Serra do Japi, Município de Jundiaí, state of São Paulo, Southeastern Brazil, an ecotonal region mainly covered by seasonal semideciduous mesophytic forests (LEITÃO-FILHO, 1992). It is an important remnant of the threatened Atlantic forest, still supporting a high diversity of flora and fauna (MORELLATO, 1992, VASCONCELLOS-NETO et al., 2012). We concentrated our field studies in the Ribeirão Ermida, a fast stream in the north part of the reserve (23°13'S,

46°58'W; 880 m elev.). Additional information on the study site can be found in Morellato (1992) and Vasconcellos-Neto et al. (2012).

## 2.2. Study species

*Hylodes* sp. (gr. *lateristrigatus*) is a small Brazilian torrent frog endemic to the Serra do Japi. This species is mainly diurnal and, as all the other hylodids, exhibits reophilic habits. Its reproductive biology is associated to montane fast streams, where they construct underwater chambers for egg deposition and have exotrophic tadpoles (see *Chapter I*). Although this species is mainly diurnal, it may also exhibit crepuscular and nocturnal reproductive activity (*Chapter I*, HADDAD and SAZIMA, 1992).

## 2.3. Behavioral observations, visual displays repertoire, and signal directionality analysis

We spent 93 days and 206 h collecting data in the field, in a total of 17 visits (in February 2010, and from February 2011 to April 2012). To record the behaviors we used focal animal and all occurrence sampling methods (ALTMANN, 1974; LEHNER, 1979). For the behavioral descriptions of males and females we used video recordings obtained in the field with a Sony HDR-XR550V camcorder (2011, Sony Co., Japan) and complemented with observations. We followed the approach of Hödl and Amézquita (2001) to include behavioral events as intraspecific visual displays into our analyses. Basically, the behavioral events were considered as a visual display when they fit on the following predictions: (1) behavior may provide a visual cue during intraspecific interaction; (2) must be redundant, conspicuous, and stereotyped; and (3) may likely provoke an immediate response by the receiver benefiting the sender (see HÖDL and AMÉZQUITA, 2001). We identified the visual displays of *Hylodes* sp. (gr. *lateristrigatus*) based on the available reviews of anuran visual displays (HARTMANN et al., 2005; HÖDL and AMÉZQUITA, 2001).

We also conducted an analysis of limb/vocal sac used in the close-range signaling to intruder males. We counted the side of the body (which limb and which vocal sac) was used in the signaling by resident males within three different situations: with the intruder male at the left side of the resident male, in front of the resident male, and at the right side of the

resident male. We excluded those signals that were not detected only from one side: advertisement calls (always emitted by using both vocal sacs), body stationary visual displays (excepting throat display, which was included in the analysis), and non-stationary visual displays.

## 2.4. Acoustic signals analyses

We recorded male vocalizations with a Marantz PMD-660 digital recorder (2011, Marantz, Japan) and a Sennheiser ME-66 external unidirectional microphone (2011, Sennheiser electronic GmbH & Co. KG, Germany) positioned 1.0 m from calling males. We measured air and water temperatures at the time of each recording. We analyzed the calls using a sampling frequency rate of 44.1 kHz and 16 bit resolution in the mono pattern with Raven Pro 1.4 (2010, Cornell Lab of Ornithology, Bioacoustics Research Program, US), excepting advertisement call, which is already described in *Chapter I*. We describe the calls analyzing six parameters: call duration (s), inter-calls duration (s), number of notes per call, note duration (s), inter-notes duration (s), and dominant frequency range (kHz). We could define the functions of each call according to the context in which they were emitted.

## 3. RESULTS

### 3.1. Ecology and communication

The communication of *Hylodes* sp. (gr. *lateristrigatus*) is based on visual, acoustic, and tactile signals, as described below. These signals are easily observed when the densities of males are high, making intraspecific interactions more frequent. Males call in all months, except October. However, the breeding season occurs at the end of the rainy season (February–April), when we observed males calling in a chorus and performing visual displays. Within this period, we observed intense male-male interaction and competition. In addition, we registered pair formation, courtship, mating, and oviposition only in these three months. We also recorded three courtship events: two during the day (15:00 h and 17:00 h) and one in a dark night (4:00 h), the first with rejection by the female and the other two with effective reproduction confirmed by oviposition (see *Chapter I*). *Hylodes* sp. (gr. *lateristrigatus*) exhibits three diurnal peaks of calling activity: in the beginning of the day,

starting one hour before sunrise (between 5:00 h and 8:00 h), in the middle of the day (between 10:00 h and 13:00 h), and in the afternoon, until one hour before sunset (between 15:00 h and 17:00 h). Males strongly decrease calling activities after sunset, during the breeding period, at least a few individuals call sporadically all night long. Males adopt and defend as territories the margins of fast streams as well as emergent rocks, trunks, branches, and leaves located on the margins or in the middle of fast streams, using those as calling and courtship sites, and for feeding activity.

### 3.2. Visual displays repertoire

*Hylodes* sp. (gr. *lateristrigatus*) presents cryptic brown and reddish brown dorsal coloration, resembling the environment where they live. However, contrasting to the general body color and environmental background, they exhibit an evident bright whitish silver stripe on the upper-lip region, and venter surface cream with irregular small darkish spots, sometimes forming a longitudinal thin darkish stripe in the throat region. Males also have bright whitish double vocal sac, mainly evident when it is inflated, and toe tips whitish silver, all contrasting to their body color and habitat background. Males and females of *Hylodes* sp. (gr. *lateristrigatus*) performed a diverse visual intraspecific communication. We observed 68 *Hylodes* sp. (gr. *lateristrigatus*) individuals and registered a total of 19 different visual displays associated to four behavioral contexts: announcement, far-range agonistic, close-range agonistic, and courtship. Announcement context consists on nonaggressive behaviors, where only one male was calling or two neighbor males, without territory invasion, were interacting acoustically, calling in antiphony. When a resident male identifies an intruder conspecific male inside its territory, immediately the resident changes its behaviors, apparently defending the territory, what we called as far-range agonistic context. The close-range agonistic context is observed when two males are closely interacting, even without physical contact, being a resident male defending a territory against an intruder male. Finally, we considered a courtship context when the male perceives a female in the territory until the couple enters into an underwater constructed chamber to lay the eggs.

The observed males of *Hylodes* sp. (gr. *lateristrigatus*) ( $N = 65$ ) performed 19 visual displays involving movements with limbs (toes, feet, hands, legs, and arms), body, vocal sacs, and head, besides stereotyped walking and jumping displays. Males perform visual displays during announcement, agonistic, and/or courtship contexts. The observed females ( $N = 3$ )



performed a total of three distinct visual displays, recorded only during courtship events using hands, arms, and the body. We identified the visual displays of *Hylodes* sp. (gr. *lateristrigatus*) based on the available reviews of anuran visual displays (see *Behavioral observations and visual displays repertoire*), except for toes posture, two-armed impulse, head bobbing, head snaking, and truncated walking. These five behaviors did not fit into any previous described display category and are described herein as new visual displays for frogs. All visual displays performed by *Hylodes* sp. (gr. *lateristrigatus*) are described in Table 1. The functions of visual displays could be also provided via comparisons of the contexts in which each display was executed.

Table 1. Visual displays of *Hylodes* sp. (gr. *lateristrigatus*) during intraspecific communication in announcement, agonistic, and/or courtship contexts. In Serra do Japi, Município de Jundiá, São Paulo, Brazil.

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#### Limbs

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1. Toe trembling ( $N = 87$ ; based on HARTMANN et al., 2005; HÖDL and AMÉZQUITA, 2001): wiggling, twitching, or vibrating the toes, rapidly and with the foot and leg motionless. Toes may be moved independently, without an order, or in a wave-pattern. Sometimes, only the fourth or fifth toes can be moved. The whitish silver upper surface of the toe tips (a male color dimorphism) is exposed during the display. Toe trembling is only performed by males with right or left toes. It is usually performed during the courtship behavior or when the resident male perceives a conspecific male entering its territory. However, is also performed during male-male close interactions and during announcement. Toe trembling is a long and short-range visual display, with courtship and agonistic functions.
2. Toe flagging ( $N = 47$ ; based on HARTMANN et al., 2005): lightly raising the toes from the substrate, keeping them raised, slowly flagging them in up and down slightly movements, and with foot and leg motionless. Toes may be moved independently, without an order. Sometimes, only the fourth or fifth toes can be moved. The whitish silver upper surface of the toe tips (a male color dimorphism) is exposed during the display. Toe flagging is only performed by males with right, left, or both feet toes simultaneously. It is usually performed during courtship, announcement (only with toes of right or left foot) or when the resident male perceives a conspecific male entering inside its territory (more frequently with the toes of both feet together). However, rarely it is observed during male-male close interactions. Toe flagging is a long and short-range visual display, with courtship, agonistic, and announcement functions.
3. Toes posture ( $N = 23$ ; new display for frogs; Figure 1.A): holding some seconds the foot up with frontal exposing of dorsal surfaces of foot and toes; or holding some seconds the foot up with the toes curved down, exposing dorsal surfaces of toes. The whitish silver

upper surface of the toe tips (a male color dimorphism) is exposed during the display. Toes posture is performed by males with right or left foot independently, or with both feet simultaneously. It is usually performed when the resident male perceives a conspecific male entering inside its territory. However, it is also executed during announcement and rarely during courtship. Toes posture is a long-range visual display, with agonistic and announcement functions.

4. Foot shaking or hand shaking ( $N = 36$ ; modified from HÖDL and AMÉZQUITA, 2001): rapidly moving one foot ( $N = 26$ ) or one hand ( $N = 10$ ) in an up and down movement, with the leg or arm motionless. It is a high-speed display. Foot shaking and hand shaking are only performed by males with right or left foot or hand. Foot shaking is usually performed when the resident male perceives a conspecific male entering its territory or during announcement, but is also observed during male-male or male-female close interactions. Foot shaking is a long and short-range visual display, with announcement and agonistic functions. Hand shaking is performed during the courtship or when the resident male perceives a conspecific male inside its territory. Hand shaking is a long and short-range visual display, with courtship and agonistic functions.
5. Foot wiping ( $N = 16$ ; based on HÖDL and AMÉZQUITA, 2001): rapidly and jerkily rubbing the foot on the ground and returning it back in the resting position. The foot is not raised and the leg and foot are motionless. It is a high-speed display. Foot wiping is only performed by males, with right or left foot. It is commonly performed during announcement contexts. Rarely observed when the resident male perceives a conspecific male entering inside its territory. Foot wiping is a long-range announcement visual display.
6. Leg stretching ( $N = 15$ ; based on HÖDL and AMÉZQUITA, 2001): stretching and keeping stretched back the leg on the ground for some seconds. Leg stretching is only performed by males with right, left, or both legs. It is performed when the resident male perceives a conspecific male entering inside its territory. Rarely executed during male-male close interactions. Leg stretching is a long-range agonistic visual display.
7. Foot flagging ( $N = 43$ ; based on HÖDL and AMÉZQUITA, 2001): slowly raising one leg, extending it out and back, lightly performing an arc in the air, and returning it back to the resting position. Whitish silver upper surface of the toe tips (a male color dimorphism) accentuates the display. Foot flagging is only performed by males with right or left leg, sometimes with regular alternation. It is observed in all behavioral contexts; however, it is rarely performed during courtship. Foot flagging is a long and short-range visual display, with announcement and agonistic functions.
8. Limb lifting ( $N = 91$ ; based on HARTMANN et al., 2005): rapidly moving up and down one leg, without extending it ( $N = 3$ ), or one arm, in the chest level ( $N = 88$ ). Leg and arm lifting are high-speedy displays. Foot lifting is rare and only performed by males during announcement. Arm lifting is more common and performed by males in all behavioral contexts and by females during the courtship. Male and female perform arm lifting with right or left arms. Arm lifting is the most common female visual display. Leg lifting is a long-range announcement visual display. Arm lifting is a long and short-range visual display, with courtship, agonistic, and announcement functions.

9. Arm waving ( $N = 37$ ; based on HÖDL and AMÉZQUITA, 2001): rapidly lifting one arm and waving it up and down in an arc forward, passing it in front of the head. When crossing in front of the face, the brown arm and hand contrast to the bright whitish silver stripe on the upper-lip region, producing a flashing-signal for the conspecific receiver, also contrasting to the background. It is performed by males during agonistic contexts and by females during the courtship. Male and female use both right and left arms, to perform it. Arm waving is the second most common female visual display. Arm waving is a short-range visual display, with agonistic and courtship functions.

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#### Body stationary

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10. Body lowering ( $N = 2$ ; based on HÖDL and AMÉZQUITA, 2001): lowering the whole body on the ground, totally hiding the cream ventral body. Only performed by males during male-male close agonistic interactions, body lowering is a short-range visual display, most likely signaling submission or lack of aggressiveness.
11. Upright posture ( $N = 16$ ; based on HÖDL and AMÉZQUITA, 2001): raising only the anterior part of the body by extension of the arms, exposing the cream throat and chest (anterior ventral body). Upright posture sometimes is preceded by two-armed impulse (see display description below), but not necessarily. Upright posture is only performed by males, usually during agonistic contexts; however, it is also executed during announcement. Upright posture is a long and short-range visual display, with agonistic and announcement functions.
12. Two-armed impulse ( $N = 6$ ; new display for frogs; Figure 1.B): boosting the whole body forward by impulsion via an up and down movement with both arms simultaneously, moving the body forward and raising the anterior part of the body. Necessarily two-armed impulse ends in an upright posture, exposing the cream throat and chest (anterior ventral body). However, the upright posture is not necessarily preceded by two-armed impulse. Two-armed impulse is only performed by males during agonistic contexts.
13. Head bobbing ( $N = 9$ ; new display for frogs; Figure 1.C): performing up or down jerky movements with the head without lifting either hands or feet off the ground neither moving the body. It is performed preceding callings by males. Head bobbing is rarely performed, but was recorded in all social-behavioral contexts studied.
14. Throat display ( $N = 146$ ; based on HÖDL and AMÉZQUITA, 2001): rapidly inflating and pulsating the vocal sac, without sound production. It is performed once or several quick and sequential times. The bright whitish double vocal sacs are clearly exposed during throat display, contrasting to the background and working as a flashlight with flashing signals. Throat display is only performed by males, inflating both vocal sacs or only one, right or left. Throat display is by far the most performed visual display in *Hylodes* sp. (gr. *lateristrigatus*). It is usually performed during male-male agonistic close interactions ( $N = 89$ ) or during the courtship ( $N = 40$ ). However, it is also observed during announcement ( $N = 9$ ) and when the resident male perceives a conspecific male entering inside its territory ( $N = 8$ ). Throat display is a short-range agonistic and courtship visual display.
15. Head snaking ( $N = 1$ ; new display for frogs; Figure 1.D): rapidly approaching a

conspecific female, raising the head up and moving it alternately sideways eight times (four times on each side), in a snakelike motion. It is performed with the throat at the level and in front of the female snout, very close to each other, but without touching. During head snaking sequential and conspicuous movements, the cream throat and chest (anterior ventral body) is closely exhibited for the female. Head snaking was recorded only once, performed by a male during courtship behavior. It is displayed preceding underwater courtship behaviors, when male is guiding the female. Head snaking is a short-range courtship visual display.

16. Body raising ( $N = 3$ ; based on HÖDL and AMÉZQUITA, 2001): raising the body by extension of the legs and arms. Body raising is a posture only performed by males. Rarely performed, it was recorded during announcement and male-male close interactions.
17. Body jerking ( $N = 51$ ; based on HÖDL and AMÉZQUITA, 2001): performing a jerky movement with the body, without lifting either hands or feet. It is performed in a forward and backward motion or up and down motion. Body jerking is usually performed by males during agonistic contexts; however it is observed during the courtship and was registered once during announcement. It is also performed by females in courtship. Body jerking is a long and short-range agonistic and courtship visual display.

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#### Body non-stationary

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18. Truncated walking ( $N = 13$ ; new display for frogs; Figure 1.E): 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 moved concomitantly with the left leg and vice-versa. Truncated walking is only performed by males, when the resident perceives a conspecific male entering inside its territory. Most likely, it is performed when the resident male is approaching the intruder. Truncated walking is a long-range agonistic visual display.
  19. Jump display ( $N = 3$ ; based on HARTMANN et al., 2005): jumping quickly sideways in front of a conspecific male, as a discontinuous movement. It is performed by one or more conspicuous quick jumps, none of them on the same side. Jump display is very rarely performed, only recorded thrice by males during close-range agonistic interactions. Jump display is a short-range agonistic visual display.
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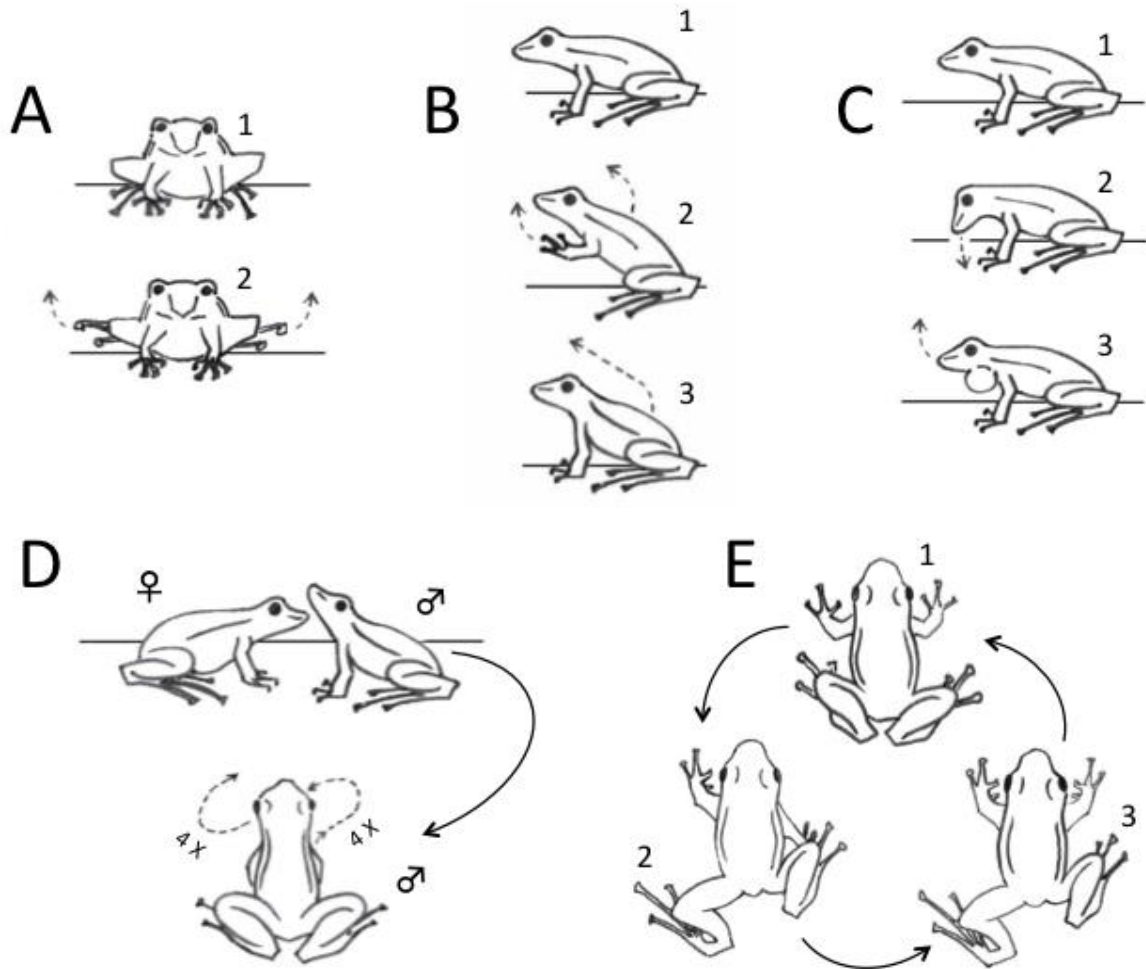


Figure 1. New visual displays for anurans, performed by males of *Hylodes* sp. (gr. *lateristrigatus*). (A) Toes posture; from the resting position (above; frontal view) raising the feet and holding some seconds the feet up with the toes curved down, exposing dorsal surfaces of toes (below; frontal view). (B) Two-armed impulse; from the resting position (above; lateral view) boosting the whole body forward by impulsions via an up and down movement with both arms simultaneously, moving the body forward and raising the anterior part of the body. (C) Head bobbing; from the resting position (above; lateral view) performing up or down jerky movements with the head without lifting either hands or feet off the ground neither moving the body; it is performed preceding callings by males. (D) Head snaking; rapidly approaching a conspecific female, raising the head up (above; lateral view of the couple) and moving it alternately sideways eight times (four times on each side), in a snakelike motion (below; dorsal view of the male); it is performed with the throat at the level and in front of the female snout, very close to each other, but without touching. (E) Truncated walking; lowering the body and walking ahead slowly, with alternation of legs and arms; it is performed with a moving and stopping pattern; the left arm is moved concomitantly with the right leg and vice-versa. In Serra do Japi, Município de Jundiaí, São Paulo, Brazil.

### 3.3. Acoustic signals repertoire

Males of *Hylodes* sp. (gr. *lateristrigatus*) expose their bright whitish vocal sacs when calling and also can combine acoustic signals to visual displays (bimodal communication). Besides the rich repertoire of visual displays, *Hylodes* sp. (gr. *lateristrigatus*) exhibits an also diverse acoustic repertoire composed by four call types: advertisement, peep, squeal, and courtship calls. Except for the advertisement call (see *Acoustic signals analyses in Chapter II*), the other calls of *Hylodes* sp. (gr. *lateristrigatus*) can be described as follows.

We recognize two types of territorial calls in *Hylodes* sp. (gr. *lateristrigatus*), peep (Figure 2.A) and squeal (Figure 2.B); however, both also have courtship function. The fourth call of *Hylodes* sp. (gr. *lateristrigatus*) that is triggered by the female visual-tactile stimulation ( $N = 15$ ) during the courtship (see below), is a courtship call (Figure 2.C). At an air temperature of 24°C and at a water temperature of 19.5°C the parameters of each call type are described below.

Peep calls are composed by frequency modulated peep notes. Peep call duration is 0.03–0.38 s ( $0.21 \pm 0.072$ ,  $N = 34$  calls from five males); calls are emitted at intervals of 1.04–1.86 s ( $1.37 \pm 0.23$ ,  $N = 31$  intervals from four males); each call is composed of 1–4 notes ( $2.63 \pm 0.71$ ,  $N = 32$  calls from five males); note duration is 0.019–0.042 s ( $0.028 \pm 0.005$ ,  $N = 90$  notes from 36 calls from five males); notes give at intervals of 0.05–0.11 s ( $0.075 \pm 0.014$ ,  $N = 90$  intervals of 45 calls from five males); the dominant frequency occurs in the third harmonic and ranges from 3.1–5.5 kHz ( $4.9 \pm 0.54$ ,  $N = 90$  call notes from five males). The peep call has harmonic structure; only the third harmonic is usually apparent.

The duration of squeal calls is 0.037–0.33 s ( $0.17 \pm 0.086$ ,  $N = 30$  calls from five males); calls are emitted at intervals of 0.6–7.46 s ( $1.23 \pm 1.23$ ,  $N = 30$  intervals, between the end of a squeal call and the beginning of a peep call, from five males); they are composed by only one note ( $N = 30$  calls from five males); the squeal note has the energy concentrated between 3.1–5.5 kHz ( $4.8 \pm 0.58$ ,  $N = 30$  call notes from five males). Peep and squeal calls can be emitted combined, with a peep preceding a squeal call.

The duration of courtship calls is 0.24–0.88 s ( $0.46 \pm 0.17$ ,  $N = 11$  calls from one male); each call has 4–6 notes ( $5.28 \pm 0.65$ ,  $N = 11$  calls from one male); note duration is 0.016–0.098 s ( $0.055 \pm 0.02$ ,  $N = 55$  notes from 11 calls from one male); notes given at intervals of 0.012–0.19 s ( $0.037 \pm 0.041$ ,  $N = 43$  intervals of 11 calls from one male); each

call is composed by frequency modulated notes, usually rising until the middle and lowering to the end; the dominant frequency is in the third harmonic and ranges from 3.6–5.7 kHz ( $4.9 \pm 0.51$ ,  $N = 57$  call notes from one male). The courtship call has harmonic structure; only the third harmonic is usually apparent.

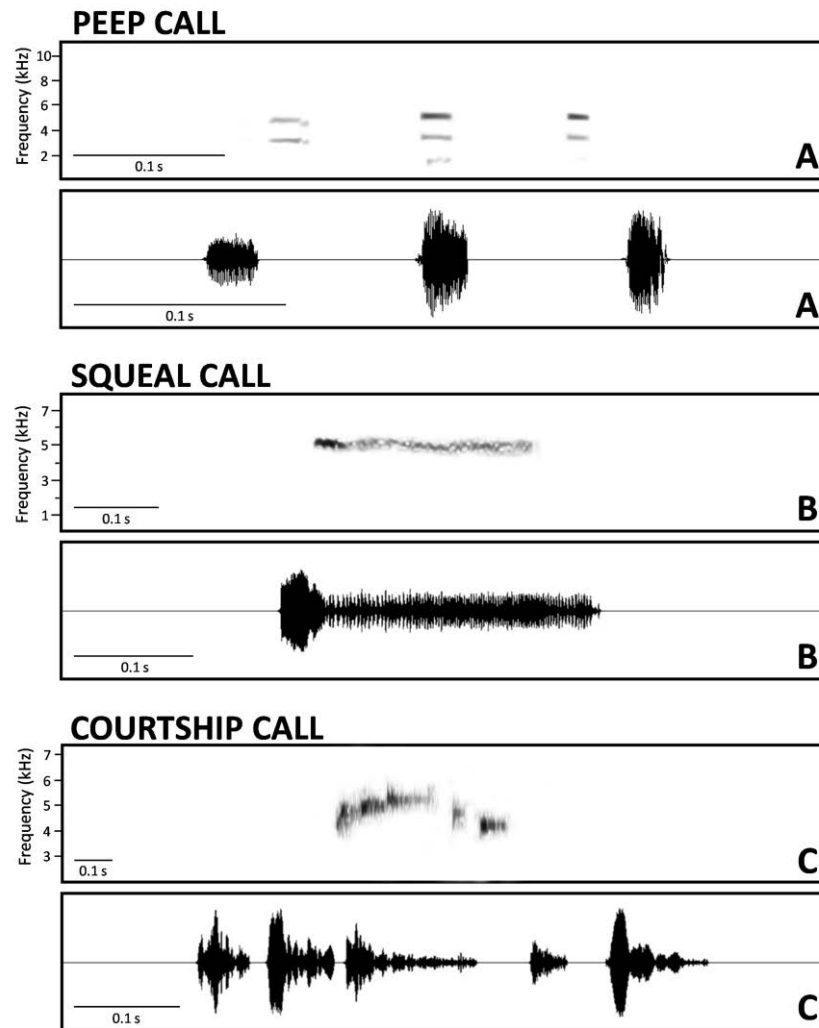


Figure 2. Vocalizations of *Hylodes* sp. (gr. *lateristrigatus*). (A) Above spectrogram and below waveform of a peep call composed by three notes. (B) Above spectrogram and below waveform of a squeal call. (C) Above spectrogram and below waveform of a courtship call composed by five notes. Peep and squeal calls recorded on 15 April 2011 and courtship call on 6 March 2012. For all vocalizations air temperature 24°C and water temperature 19.5°C. In Serra do Japi, Município de Jundiaí, São Paulo, Brazil.

### 3.4. Male communication: Signal choice and territoriality

Males defend territories as feeding and breeding (calling and courtship) sites. When a male perceives the presence of a conspecific male or female inside the territory, it readily increases the emission of peep, squeal, and/or courtship calls, also increasing the emission of visual displays. From the 65 males observed, 28 were in context of announcement, 10 in far-range agonistic, 24 in close-range agonistic, and three in courtship. Males exhibit an evident higher percent of use of acoustic signals than visual displays in all behavioral contexts, except for close-range agonistic, when visual and acoustic signals have almost the same representativeness (Figure 3). We could also verify differences in the call type emitted, according to each moment (Figure 4). During announcement context, males basically emit advertisement calls inflating both vocal sacs, most likely with functions of female attraction and territorial maintenance. Advertisement call is the acoustic signal less emitted during long-range agonistic contexts. It had almost the same representativeness as squeal call during close-range agonistic contexts. In long-range and close-range agonistic contexts peep call was the vocalization more frequently emitted. Although courtship call is only emitted during courtship, it is the less frequent call type within this context. During male-female close interactions, peep and squeal calls are the most frequent ones, evidencing that these calls also have courtship functions. The relative use of call types in the four distinct contexts are presented in Figure 4.

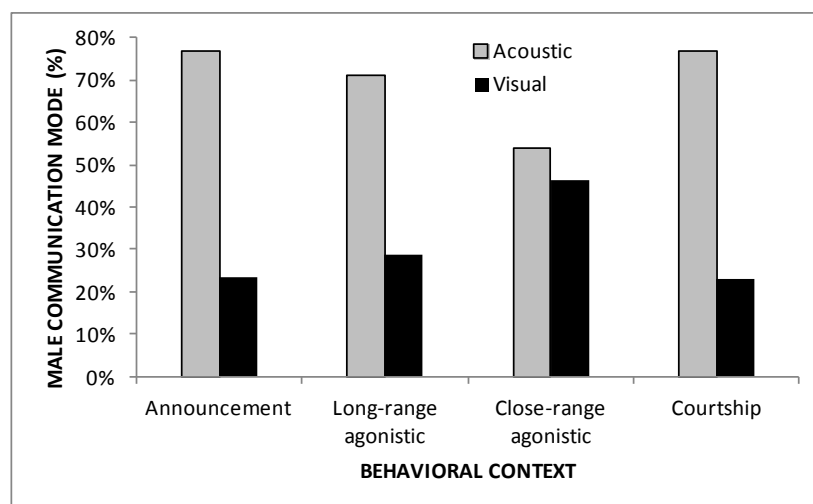


FIGURE 3. Relative importance (%) of visual and acoustic signals performed by males of *Hylodes* sp. (gr. *lateristrigatus*) in the context of announcement, long-range agonistic, close-range agonistic, and courtship. In Serra do Japi, Município de Jundiaí, São Paulo, Brazil.



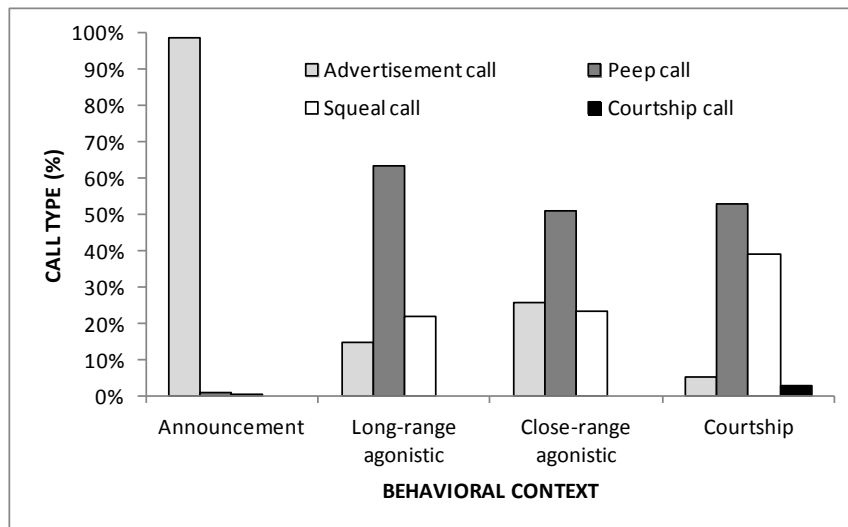


Figure 4. Relative importance (%) of advertisement, peep, squeal, and courtship calls of *Hylodes* sp. (gr. *lateristrigatus*), in announcement, long-range agonistic, close-range agonistic, and courtship contexts. In Serra do Japi, Município de Jundiaí, São Paulo, Brazil.

There are also differences in the visual displays used by males during the four distinct contexts (Table 2). For example, toes posture is observed during announcement and long-range agonistic contexts and less frequently during courtship; foot flagging is an announcement visual display, but it is also registered in the other three contexts; arm waving is performed by both males and females with exclusively agonistic and courtship functions, respectively; throat display is a significant visual display for both close interactions, close-range agonistic and courtship; and head snaking is only used by males in a specific moment during courtship, when the male is conducting the female for the dive.

Males of *Hylodes* sp. (gr. *lateristrigatus*) use acoustic and visual signals to maintain territories, avoiding fights. Fights are used if the signals do not work in deterring the territorial invasions. We observed only one physical confrontation between males that were disputing the same territory. In March 24 2011, two males of *Hylodes* sp. (gr. *lateristrigatus*) were in distinct small emerged rocks in the middle of the fast stream. Both were close to each other (50 cm apart) and using aggressive visual and acoustic signals, when one of them jumped over the other. The individual that jumped was larger (males were not measured). The larger male pushed the smaller with his snout and chest with forward and backward movements; both males were emitting squeal calls. The smaller male tried to stay on the rock; however, it was pushed down inside the water. This pushing fight lasted about 50 minutes. The smaller male

tried to climb up again, simultaneously pushing its opponent using the snout and the body. They kept in this dispute for more 10 minutes and the larger male again pushed the smaller down in the water and won the contest.

Table 2. Percentage of visual displays produced by males of *Hylodes* sp. (gr. *lateristrigatus*) in the communication contexts of announcement, long-range agonistic, close-range agonistic, and courtship. In Serra do Japi, Município de Jundiaí, São Paulo, Brazil.

Visual display	Behavioral contexts			
	Announcement (%)	Long-range agonistic (%)	Close-range agonistic (%)	Courtship (%)
Toe trembling	7.6	14.3	4.6	29.4
Toe flagging	9.8	6.4	1.1	15.3
Toes posture	8.7	8.6	0	1.7
Foot and hand shaking	7.6	6.4	4	7.3
Foot wiping	15.2	1.4	0	0
Leg stretching	0	8.6	1.7	0
Foot flagging	22.8	6.4	5.7	1.7
Limb lifting	9.8	6.4	7.5	14.7
Arm waving	1.1	5	5.7	0
Body lowering	0	0	1.1	0
Upright posture	3.3	5	3.4	0
Two-armed impulse	0	2.1	1.7	0
Head bobbing	2.2	2.9	1.1	5.6
Throat display	9.8	5.7	51.1	22.6
Head snaking	0	0	0	5.6
Body raising	1.1	0	1.1	0
Body jerking	1.1	11.4	8	6.2
Truncated walking	0	9.3	0	0
Jump display	0	0	1.7	0

### 3.5. Female-male communication: An elaborated courtship with bimodal signaling

Despite females of *Hylodes* sp. (gr. *lateristrigatus*) are very secretive, throughout our study we observed three couples in courtship. The three courtships events observed were partials, but together they complete the whole sequence of behaviors. The courtship is complex and comprises by visual, acoustic, and tactile signals. The male calls throughout the courtship, alternating among advertisement, peep, squeal, and courtship calls. During courtship, we observed males performing visual displays using toes, feet, hands, legs, arms, vocal sacs, head, and body. The female also performs visual displays; however, only with movements of hands, arms, and body. Moreover female also stimulates the male during the courtship by using tactile signals.

The courtship of *Hylodes* sp. (gr. *lateristrigatus*) takes place on land, always in the margin of a fast stream or close to it, inside the male's territory, excepting the last part that precedes oviposition, which occurs in an underwater constructed chamber, in the bottom of the fast stream. The announcement context is the pre-courtship step. The courtship behavioral sequence starts when the male perceives a female inside its territory. The complete courtship of *Hylodes* sp. (gr. *lateristrigatus*) may be summarized into three steps as follows: (1) evaluation, (2) acceptance, and (3) conduction. These steps are described in detail below, compiled from observations of three courtship events and totalizing 53 minutes.

(1) Evaluation: female is attracted by the male advertisement calls, enters the male's territory, and observes the male from afar. Immediately after perceiving the female presence, the male stops to emit advertisement calls, starts to perform visual displays, and drastically increases emission of peep and squeal calls. During the courtship, the male will emit advertisement calls only if a conspecific male approaches the mating couple. The female slowly starts to approach the male by sparse jumps, while the male courts the female. Sometimes, the male also will jump towards the female. During the approaching process, which lasts about two minutes, the male faces the female and alternates between acoustic and visual communication modes. The male starts the courtship performing visual displays (foot shaking and throat display). Then it alternates between peep call, squeal call, and visual displays, including toe trembling, toe flagging, toes posture, foot shaking, hand shaking, and foot flagging. As a result of the evaluation perform by the female, there are two mutually exclusive possibilities: the female does not become interested in that male and goes away, leaving the male territory; or female becomes interested and starts to approach the male.

When the male is refused, the female dives and stays motionless on the bottom of the fast stream. The male starts to pursue the female, diving and approaching it; however, female goes away by emerging and diving, still followed by the male, and definitely flees, going away by swimming on the bottom of the fast stream. The male emerges and returns to announcement context (pre-courtship), emitting advertisement calls and apparently waiting for another potential mate.

(2) Acceptance: when female is interested it approaches to the male side. At this moment, the female starts signaling by arm lifting. This visual display ends as a tactile signal, with the female putting one of its arms down over one of the male's foot, touching it. Immediately after that, the female touches the male dorsum (next to the head) with the gular region. The female will put its hands intermittently on the male's dorsum, but will keep the gular region continuously over the male until the next step of the courtship (conduction) (Figure 5). Male keeps performing intensively both peep and squeal calls (usually with regular alternation), intercalating them with visual displays (toe trembling, toe flagging, foot shaking, hand shaking, arm lifting, head bobbing, throat display, and body jerking), while female keeps motionless. Without an apparent order, the female performs the visual-tactile signals of arm lifting or arm waving (usually ending with the female touching the male's face, or its anterior dorsal body region, or its posterior dorsal body region, only depending on which arm was used to perform the signal), or body jerking (always with her gular region touching the male's dorsum). Even while performing arm visual-tactile signals, the female keeps its gular region on the male's dorsum. When stimulated by the visual-tactile signals performed by the female, sometimes the male readily respond with courtship calls ( $N = 15$ ). Male and female keep the visual-acoustic-tactile stimulation process for around 40 minutes. Only once during the courtship behavior step, the male performed body jerking and the female responded lightly moving her body few times forward and backward, consequently scrubbing her gular region on the male's back. Close to the end of this step, the female keeps simultaneously the gular region and both hands on the male's back.

(3) Conduction: at this moment, the male dives being followed by the female. They examine the underwater area close to the male's territory and take a decision concerning chamber construction, with the male excavating it. From this moment on there is no acoustic and visual signaling anymore. There are two possible situations in this courtship step: male and female already are located on the fast stream margin or they are next to the margin, but not on it. In the first case, without departing and maintaining physical contact with the female

(the female keeping the gular region on the male's back), the male lightly moves its body forward, consequently moving the female body together. Immediately the male dives followed by the female. In the second case, the male conducts the female until the place where they will dive. In this case, the male jumps in the direction of the stream, attracting the female by using emissions in similar proportions of advertisement, peep, and squeal calls. When male and female are about 2 cm apart, they keep facing each other for some seconds. The male jumps right in front of the female, rises its head, performs head snaking display, and jumps on the stream margin, now moving away from the female in about 30 cm. The male turns to the stream and emitting only advertisement calls attracts the female, which slowly approaches the male by sparse jumps until about 1 cm from it. When the female arrives on the margin, the male slowly enters in the water, diving next to the margin, and is followed by the female. The underwater part of the conduction step is composed only by tactile stimulation by mutual touches between the couple (described in details in the *Chapter I*). After submersion, the male guides the female, visiting together some potential oviposition sites to decide the place where the male will excavates the chamber. The male uses both hands to excavate the chamber. The male enters the chamber first, immediately followed by the female. They lay the eggs inside the underwater constructed chamber, but we could not observe the oviposition behaviors. Male and female, then, leave the chamber and conceal its entrance. Only the male remains close to the entrance and conceals the entrance aperture by pushing sand from the bottom of the stream into the opening. The male first uses its legs and feet, later its arms and hands. The female returns, verifies the entrance of the chamber and also helps to conceal it, using its hands, by movements of its arms. The conduction step lasts around 11 minutes in the total, six minutes on land and five minutes until the couple enters in the chamber. Inside the chamber, the oviposition behaviors last 27 minutes. The concealing of the chamber by the couple after oviposition lasts five minutes. From when the male perceives the female inside its territory until the chamber is concealed lasts 85 minutes, being 48 minutes on land and 37 minutes underwater.

The courtship calls are emitted only during the acceptance courtship step. During this step, the female keeps the gular region on the male's back. From video recording of one of the three courtship events observed, we quantified the signals displayed by females and which arms were used to perform arm lifting or arm waving (analyzed together). In this couple, the female's left arm is closer to the male's head and the female's right arm is closer to the posterior body side of the male (see positions of male and female during the acceptance step

in Figure 5). The male was able to see only the movements performed by the female's left arm. So, movements performed by the female's right arm could be only perceived by the male through tactile stimuli. Figure 6 exhibits the relative frequency of courtship calls emitted by the male ( $N = 15$ ), triggered by movements of the left and right arms of the female. We observe that both female's arms stimulated the male to emit courtship calls. However, the left arm was responsible to trigger 11 courtship calls while the right arm only four. Only the female tactile signals are able to trigger the courtship calls emitted by the male, though, combination of visual and tactile signals by the same arm presents a higher and well-succeeded male stimulation, with thrice better positive male reply. This result suggests the occurrence of bimodal communication in frogs by the combination of visual and tactile stimuli.



Figure 5. Female of *Hylodes* sp. (gr. *lateristrigatus*) touching the dorsum of the male with the gular region during the courtship. The male is calling only with the left vocal sac inflated (arrow), an evidence of the visual component of the bright whitish vocal sacs. Note that the female's left arm is closer to the male's head and that the female's right arm is close to the posterior body region of the male. In Serra do Japi, Município de Jundiá, São Paulo, Brazil.

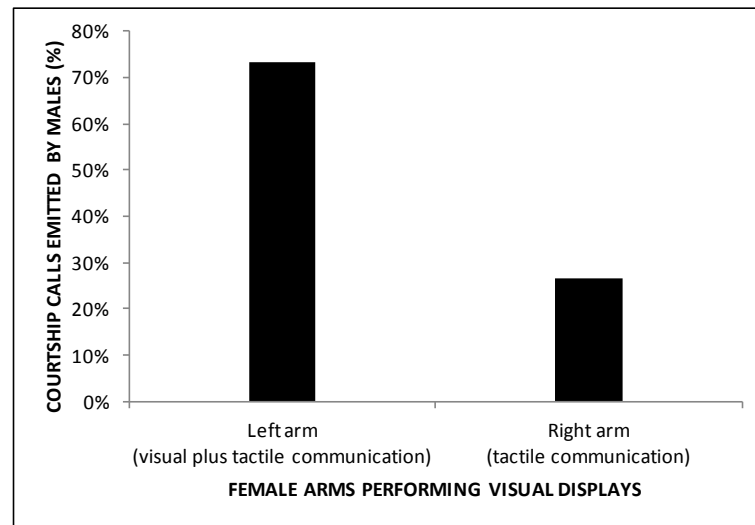


Figure 6. Frequencies (%) of courtship calls when the attracted female performs bimodal signals (visual plus tactile communication) and pure tactile signals, in *Hylodes* sp. (gr. *lateristrigatus*). In Serra do Japi, Município de Jundiá, São Paulo, Brazil. See text for details.

### 3.6. Male communication: Signal emission control and directionality

While the male is calling during announcement contexts it moves several times, usually in an angle of  $45^\circ$ . These movements apparently make the male more detectable to conspecifics, males and females. Furthermore there were two situations where males were calling partially submerged in the stream so that their inflated vocal sacs were in contact with water surface during emission of advertisement calls. The inflated vocal sacs touching the water surface produce distorted calls ( $N = 2$ ). In one of those situations, the male apparently perceived the distorted sound that was being produced and, during the intervals of its advertisement calls, it experiments new positions to call, trying to stay out of the water, apparently looking for a better position to call until producing the regular call, without the contact of the vocal sacs with the water surface. We also audibly observed that the male is able to control the intensity of its calls, sometimes clearly lowering the vocalization volume during close-range agonistic interactions or courtship interactions.

Finally, visual displays performed by limbs infrequently are executed with both arms or legs at the same time; the individual chooses one of the sides (right or left) to perform the display. In addition, males of *Hylodes* sp. (gr. *lateristrigatus*) apparently have control over which vocal sac they will use during acoustic and visual signaling. When calling or

performing the throat display, the male chooses to use both vocal sacs simultaneously or only one of them individually. However, the advertisement calls of *Hylodes* sp. (gr. *lateristrigatus*) are always executed by using both the vocal sacs ( $N = 329$  advertisement calls video recorded, from 65 males). From video recording analysis of 65 males we also observed that throat display and the other calls in the repertoire (peep, squeal, and courtship) can be executed with both vocal sacs simultaneously ( $N = 18$  throat displays, 314 peep calls, 124 squeal calls, and 6 courtship calls) or only with the right ( $N = 14$  throat displays, 30 peep calls, 7 squeal calls, and none courtship calls) or left ( $N = 72$  throat displays, 190 peep calls, 174 squeal calls, and 10 courtship calls).

We conduct an analysis of limb/vocal sac used in the close-range signaling to intruder males (see *Behavioral observations, visual displays repertoire, and signal directionality analysis*). We provide the result in the Figure 7. When resident males decide how to emit calls or perform displays that can be signaled by both, left or right vocal sacs or limbs, they do it based on conspecific receptor position, during the close-agonistic interactions.

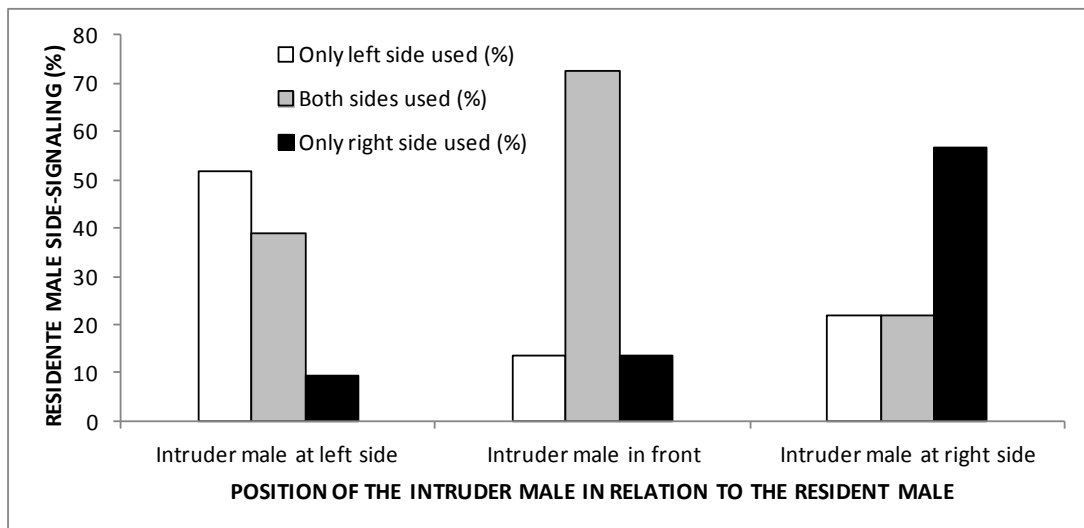


Figure 7. Resident male of *Hylodes* sp. (gr. *lateristrigatus*) choosing left or right sides to signalize during close-range agonistic context and in three distinct situations: intruder male at the left side of the resident male ( $N = 9$  males and 164 communication signals recorded), in front of the resident ( $N = 10$  males and 95 communication signals recorded), and at its right side ( $N = 5$  males and 23 communication signals recorded). In Serra do Japi, Município de Jundiá, São Paulo, Brazil.



#### 4. DISCUSSION

*Hylodes* sp. (gr. *lateristrigatus*) exhibits an extraordinary diverse communication involving a rich repertoire of visual and acoustic intraspecific signals that are and even more complex during the courtship, when individuals also include tactile signaling. Males and females are cryptic, with dorsal coloration resembling the background and substrates where they live (see *chapter I*). However, when observed from the frontal view, as they are observed by conspecific individuals, their cream ventral bodies contrast to the dark background. While the dorsal coloration works as camouflage for predators, the disruptive ventral pattern plays a role of intraspecific visual cue, in particular for the visual displays of body lowering, upright posture, and head snaking. These three displays associated, respectively, to subordination, territoriality, and female stimulation, evidence that ventral body color may transfer visual messages during intraspecific interactions. During body lowering, the male hides its cream venter, what can be interpreted as a submissive display, consequently avoiding agonistic behaviors and fights, expressing non-aggressive intentions during the close-range territorial interaction of conspecific males. As two opposite examples, upright posture and head snaking are visual displays that express, respectively, aggressive and courtship messages via the cream venter exposure.

Like the contrasting color patterns, movements, postures, or combinations of those also works as conspicuous traits and can shape visual displays. Head snaking is a stereotypic movement intensified by the cream ventral coloration. Foot and hand shaking, foot wiping, limb lifting, head bobbing, body jerking, and jump display are examples of visual displays that are conspicuous only due to movements. Leg stretching, two-armed impulse, and truncated walking are conspicuous in consequence of the association of movements and postures. Body raising is a conspicuous display only due to posture. Toe trembling, toe flagging, and foot flagging are examples of displays composed by movements that are even more evidenced in consequence of the contrasting bright whitish-silver color of the toe tips. In toes posture, the posture adopted by the male exposes the contrasting color of the toe tips. We observed a sexual dimorphism associated to communication in *Hylodes* sp. (gr. *lateristrigatus*). Apparently there is an association between behavior and color. An example is that only the males perform visual displays using toes and only they have the whitish-silver contrasting toe tips, while the females have cryptic toe tips coloration. Arm waving performed by *Hylodes* sp. (gr. *lateristrigatus*) females and males is also a visual display involving association of movement and color. Females and males perform arm waving with a quick

movement, producing a flashing signal by passing the brown arm in front of the whitish-silver upper-lip region. Likewise, movement and color associations in visual signaling are also observed for other anuran species. *Brachycephalus ephippium* is a tiny diurnal frog that also performs arm waving; however, in this case the display is somewhat different from that observed in *Hylodes* sp. (gr. *lateristrigatus*), since *B. ephippium* displays arm waving slowly, passing its yellow arm in front of its black eye (POMBAL et al., 1994).

Throat display also involves association of movement and color for visual signaling in *Hylodes* sp. (gr. *lateristrigatus*). Throat display is described as movements of the bright whitish vocal sacs, also producing flashing signals. We observed that males are able to control their double vocal sacs individually, inflating only the right sac, only the left sac, or both simultaneously, according to the location of conspecific receiver. Males controlling and directing signal emissions is also evidenced and corroborated by: turns during announcement contexts, call volume control during close-range intraspecific interactions, better positioning when the advertisement call is distorted, and selection of the communication mode or signal type that better fits in each particular behavioral context. When the male is controlling and directing its signals, most likely there is a trade-off between energy demands and information transfer success. Within this context, the control of signals improves and optimizes individual performance of the males. During visual displays, it is also reasonable that males take the decisions on which limb will be used according to the location of the conspecific receiver.

Vocal sacs control is executed either when performing a visual display (throat display) or emitting acoustic signals (peep, squeal, and courtship calls), with exception of advertisement calls that are always emitted by using both vocal sacs. The advertisement call is emitted usually in announcement contexts, when the male is alone in its territory; if there are not receivers, the male does not have to emit visual signals using the vocal sacs. The acoustic component during advertisement call emission, a usually a long-range signal, may be more important than the visual component. If the receptor cannot see the emitter, the signal is purely acoustic; from the moment that the receptor sees the emitter, the signal becomes bimodal, since beyond the acoustical information it will have a visual information by the pulsating vocal sacs. In particular during close intraspecific interactions, the male uses its vision and/or audition to localize the conspecific receptor, directing its vocal sacs during the signal emission. The bright vocal sacs act as visual cues either when males are performing the pure visual display (throat display, a unimodal visual communication) or when males are calling. During callings, the vocal sacs color and movement (visual components) and the call

sound (acoustic component) act concomitantly in a synergic bimodal communication. We believe that, for *Hylodes* sp. (gr. *lateristrigatus*), the vocal sacs color and movements are not only an epiphenomenon of the acoustic signaling, but they help with detection and discrimination of the emitter within the complex background noise of the habitat. The male control on which vocal sac is inflated, even when it is calling, further confirms our suggestion.

During the courtship behavior, we report two visual displays performed by the female and usually integrated to tactile signaling: the arm lifting and arm waving. The tactile and visual components of the movements performed by the female act together in synergy, working as a bimodal signal. Our study is the first to demonstrate visual-tactile signaling as a type of multimodal communication in frogs. This is the second evidence of bimodal communication in *Hylodes* sp. (gr. *lateristrigatus*) (the first was discussed in the preceding paragraph). Similarly to the visual-acoustic communication, if the male (receptor) cannot see the visual component, the signal is purely tactile; if the male sees the movement of the tactile-visual signaling, the signal becomes bimodal. Only the tactile component of the signal from the female is sufficient to trigger the courtship call from the male. However, when the tactile and visual components are associated, as a unique signal, they trigger almost thrice more courtship calls from the males. We conclude that the movement is not only an epiphenomenon of the tactile signaling, but rather a visual component that amplifies the tactile component during the female-male communication.

By comparing our data and the literature, we observe the similarity of communication among hylodid species. For example, repertoire and functions of acoustic signals are alike among *Hylodes* sp. (gr. *lateristrigatus*), *H. asper* (HADDAD and GIARETTA, 1999), *H. heyeri* (LINGNAU and BASTOS, 2007), *H. meridionalis* (LINGNAU et al., 2013), and *H. phyllodes* (HARTMANN et al., 2006). Tactile stimuli executed by male and female is also usually observed in the courtship of torrent frog species (e.g. *Hylodes asper* and *Hylodes phyllodes*; HADDAD and GIARETTA, 1999 and FARIA et al., 1993, respectively).

From 42 species currently recognized for the family Hylodidae (FROST, 2013), only nine species (including the species studied in the present work) had their visual repertoires studied (Table 3). In addition, we have data on visual communication for only one *Crossodactylus* species (WEYGOLDT and CARVALHO-E-SILVA, 1991) and no information on communication in *Megaelosia*. It is reasonable to believe that the lack of

information on the communication of hylodids is a consequence of their wary and secretive behaviors, making studies difficult, either in the field or in captivity. Among the hylodids, the repertoire of visual displays is more complex in *Hylodes* sp. (gr. *lateristrigatus*) (Table 3). Currently, *Hylodes* sp. (gr. *lateristrigatus*) is the species with the most diverse visual displays repertoire within the order Anura. In addition, the five new visual displays that we described and categorized here correspond to 21.7% of the visual displays recognized for torrent frogs and around 13.8% for anurans. However, since this species was the most studied, this could be a consequence of the higher time spent on observations of its behavior (see Table 3). The less the species was observed poorer its repertoire of visual displays, as *H. perere*; those that had more observations present the most diverse repertoires, such as *H. dactylocinus*, *H. phyllodes*, and *Hylodes* sp. (gr. *lateristrigatus*). Therefore, we believe that our result on visual communication is not an exception among the hylodids and anurans in general, but a consequence of the time invested to understand the behaviors of *Hylodes* sp. (gr. *lateristrigatus*). Some behaviors, as arm lifting and arm waving, are only distinguishable by use of video. Moreover, another visual displays of the torrent frogs are only performed during specific and rare situations. Head snaking, for example, was recorded only twice among all studies with hylodids, once during a *H. phyllodes* courtship and in the present study. The accepted male is the only individual that performs head snaking, and also only executes it during the courtship, when the couple is located far from the fast stream margin. Furthermore, as far as we know, at least three from the five new visual displays herein described (toes posture, two-armed impulse, and head snaking) were previously mentioned in literature (FARIA et al., 1993; NARVAES and RODRIGUES, 2005); however, the authors did not describe them as new displays. It is plausible to expect that the same complexity in the visual communication observed for *Hylodes* sp. (gr. *lateristrigatus*) is similarly widespread within the family Hylodidae, at least for *Hylodes* species. The high complexity of the visual communication system may be a pattern for Brazilian torrent frogs, most likely a phylogenetic trait of this genus. Neotropical torrent frogs deserve more attention and new studies on their communication can even uncover another new behaviors and patterns.

Table 3. Diversity of intraspecific visual communication in Neotropical torrent frogs (Anura, Hylodidae), based on literature survey and new data. All displays here considered were recorded during intraspecific communication in announcement, agonistic, and/or courtship contexts.

Visual displays	<i>Crossodactylus gaudichaudii</i> <sup>1</sup>	<i>Hylodes asper</i> <sup>2</sup>	<i>Hylodes cardosoi</i> <sup>3</sup>	<i>Hylodes dactylocinus</i> <sup>4</sup>	<i>Hylodes nasus</i> <sup>5</sup>	<i>Hylodes heyeri</i> <sup>6</sup>	<i>Hylodes</i> sp. (gr. <i>lateristrigatus</i> ) <sup>7</sup>	<i>Hylodes perere</i> <sup>8</sup>	<i>Hylodes phyllodes</i> <sup>9</sup>
Toe trembling	-	Males	Males	Males	Males	Males	Males	Males	-
Toe flagging	-	Males	Males	-	-	-	Males	-	Males
Toes posture	-	-	-	Males	-	-	Males	-	-
Hind foot lifting	Males	-	-	-	-	-	-	-	-
Foot and hand shaking	-	Males	-	Males	-	-	Males	-	-
Foot wiping	-	-	-	-	-	-	Males	-	-
Leg kicking	-	-	Males	-	-	-	-	-	-
Leg stretching	Males	Both	Males	Males	Males	Males	Males	Males	Males
Foot flagging	-	Males	Both	Males	Males	-	Males	-	Males
Limb lifting	-	Both	Males	Males	-	Males	Both	-	Males
Arm waving	Males	-	-	-	Males	-	Both	-	-
Body lowering	-	-	-	-	-	-	Males	-	Males
Upright posture	-	-	-	-	Males	Males	Males	-	Males
Two-armed impulse	-	-	-	Males	-	-	Males	-	-
Head bobbing	-	-	-	-	-	-	Males	-	-
Throat display	-	-	-	-	-	-	Males	-	Males
Head snaking	-	-	-	-	-	-	Males	-	Males
Mouth gaping	-	-	-	-	-	-	-	-	Males
Body raising	-	Males	Males	Males	-	-	Males	-	Males
Back raising	-	-	-	Males	-	-	-	-	-
Body jerking	Males	-	-	-	-	Males	Both	-	-
Truncated walking	-	-	-	-	-	-	Males	-	-
Jump display	-	-	-	-	-	-	Males	-	Males
Female displays ( <i>N</i> )	0	2	1	0	0	0	3	0	0
Male displays ( <i>N</i> )	4	7	7	9	5	5	19	2	11
Total ( <i>N</i> )	4	7	7	9	5	5	19	2	11

Reference: 1. Weygoldt and Carvalho-e-Silva (1991); 2. Haddad and Giaretta (1999), Hartmann et al. (2005), Heyer et al. (1990), Hödl et al. (1997), Lingnau (2003); 3. Forti and Castanho (2012), Lingnau et al. (2008); 4. Lingnau (2003), Narvaes (1997), Narvaes and Rodrigues (2005), Pavan et al. (2001); 5. Pavan, personal observation in Pavan et al. (2001), Wogel et al. (2004); 6. Lingnau (2003); 7. present study; 8. Silva and Benmaman (2008); 9. Faria et al. (1993), Hartmann et al. (2005).

Hödl and Amézquita (2001) showed that the shift to diurnality facilitated the evolution of visual communication in frogs. They suggested that visual repertoires tend to be more complex in species that breed at noisy streams and even more complex in species that breed and feed at the same terrestrial sites. Indeed, even with new several records of visual displays performed by distinct species, the group of the aromobatids/dendrobatids still presents one of the most complex visual communications among frogs. However, comparatively, the group of the hylodids starts to exhibit an even more elevated complexity, as observed in the repertoires of *Hylodes phyllodes* and *Hylodes* sp. (gr. *lateristrigatus*) (see Table 3).

It is plausible to expect complex visual communications well-developed and modulated by the environment in fast stream-dweller diurnal frogs, as hylodids (e.g. HADDAD and GIARETTA, 1999; HARTMANN et al., 2006; NARVAES and RODRIGUES, 2005). In comparison with Brazilian torrent frogs, another diurnal torrent frogs in the world, such as the Indian torrent frogs (*Micrixalus* spp., Micrixalidae family; GURURAJA, 2010; KRISHNA and KRISHNA, 2006; PREININGER et al., 2013; VASUDEVAN, 2001) and the Bornean torrent frogs (*Staurois* spp., Ranidae family; BOECKLE et al., 2009; GRAFE and WANGER, 2007; GRAFE et al., 2012; PREININGER et al., 2009), present similar complexities in communication, as adaptive convergences. These three tropical genera, despite from distinct parts of the world, share similarities in the daytime breeding habits, breeding habitats, reproductive modes, conspicuous visual displays (e.g. foot flagging), vast repertoires of visual displays, patterns of behavioral communication, and multimodal communications. Added to visual and acoustic, tactile signaling also composes the courtship ritual in hylodids (FARIA et al., 1993; HADDAD and GIARETTA, 1999; LINGNAU, 2003; WEYGOLDT and CARVALHO-E-SILVA, 1991; present study). These three communication modes (visual, acoustic, and tactile) are also recorded during the courtship behaviors of other frog families in which the male conducts the female until the oviposition site. Some examples are: *Allobates femoralis* (Aromobatidae; MONTANARIN et al., 2011), *Ameerega braccata* (Dendrobatidae; FORTI et al., 2013), *Aplastodiscus arildae* (Hylidae; ZINA and HADDAD, 2007), *Aplastodiscus leucopygius* (Hylidae; HADDAD and SAWAYA, 2000), *Aplastodiscus perviridis* (Hylidae; HADDAD et al., 2005), *Leptodactylus fuscus* (Leptodactylidae; MARTINS, 1988), *Leptodactylus mystacinus* (Leptodactylidae; DE OLIVERIA FILHO and GIARETTA, 2008). During the conduction steps of the courtship, *Ameerega braccata* and *Aplastodiscus* spp. show exactly the same mate position that we

observed for *Hylodes* sp. (gr. *lateristrigatus*) (see Figure 5), what suggests behavioral convergence, since they are not closely related groups (FROST et al., 2006).

Moreover, in the last years, new windows are being opened concerning the evolution of communication in frogs by the study of other fascinating communication modes, which have been uncovered, for example: water wave communication in the basal frogs of the genus *Bombina* spp. (SEIDEL, 1999; SEIDEL et al., 2001), chemical communications in the basal species, *Leiopelma hamiltoni* (LEE and WALDMAN, 2002; WALDMAN and BISHOP, 2004), pure ultrasonic communication in the frog *Huia cavitympanum*, the first record for a non-mammalian vertebrate (ARCH et al., 2009), and vibrational communication in the arboreal frog (*Agalychnis callidryas*; CALDWELL et al., 2010). Among frogs, all those new communication modes above mentioned, as well as the control and directionality of the vocal sacs (as observed in the present study), and even new integrations between distinct communication modes (as visual-tactile bimodal signaling in females of *Hylodes* sp. (gr. *lateristrigatus*), as observed in the present study), are considerably improving our comprehension on the diversity and evolution of communication in anurans.

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## CONCLUSÕES FINAIS

- A rãzinha-de-correnteza do gênero *Hylodes*, que só é conhecida da Mata Atlântica da Serra do Japi, município de Jundiá, Estado de São Paulo, Sudeste do Brasil é, neste trabalho, reconhecida como uma espécie nova. Apesar de apresentar similaridade morfológica com *H. amnicola*, *H. ornatus*, *H. perere* e *H. sazimai*, a nova espécie se distingue pelo padrão de coloração e por características do canto de anúncio. Também, por meio de análises moleculares (sequências parciais de dois genes mitocondriais e um nuclear: COI, 16S e RAG1, respectivamente) se conclui que a nova espécie é geneticamente distinta destas espécies morfológicamente similares.
- A nova espécie de anuro da Serra do Japi pertence ao grupo de *Hylodes lateristrigatus*, possuindo listras dorsolaterais claras. É diagnosticada pelo tamanho pequeno; polegares sem estruturas nupciais; superfícies dorsais relativamente lisas; padrão dorsal bastante variável com área interocular apresentando manchas escuras evidentes e de formas variáveis, podendo apresentar um padrão uniforme ou uma barra escura interocular, e com o dorso variando de um padrão uniforme até outro com grandes manchas escuras evidentes e de formas variáveis; ventre predominantemente claro; machos adultos com sacos vocais laterais pareados, amplamente expandidos.
- A espécie deposita pequenas desovas compostas por ovos grandes dentro de uma câmara subaquática construída pelo macho. Os girinos vivem em águas lóxicas e são exotróficos. Macho e fêmea fecham a câmara conjuntamente após depositarem os ovos. Os girinos apresentam atividades de forrageamento durante o dia e durante a noite, mas são mais ativos e expostos de noite. São frequentemente encontrados na região bentônica dos riachos de correnteza da Serra do Japi, particularmente entre pequenas rochas ou folhas acumuladas.
- Os sinais de comunicação intraespecífica são mais facilmente observáveis durante os picos de atividade reprodutiva da espécie. Os machos de *Hylodes* sp. (gr. *lateristrigatus*) cantam todos os meses, exceto outubro. No entanto a temporada reprodutiva de fato ocorre no final da estação chuvosa, entre fevereiro e abril. Apresentam turno reprodutivo majoritariamente diurno. Machos e fêmeas são encontrados próximos aos riachos de correnteza da Serra do Japi.

- A partir de observações em campo foram registrados 19 diferentes sinais visuais associados a contextos de anúncio, agonístico de longa distância, agonístico de curta distância e corte. Os machos realizam os 19 sinais visuais nos quatro contextos acima mencionados. As fêmeas realizam três sinais visuais, apenas durante a corte.
- Dentre os 19 sinais apresentados pela espécie, cinco são considerados novos sinais para a ordem Anura: postura dos artelhos (*toes posture*), impulso de dois braços (*two-armed impulse*), aceno de cabeça (*head bobbing*), serpenteando a cabeça (*head snaking*) e caminhada truncada (*truncated walking*). O reconhecimento dos 19 sinais é baseado em diversos registros de comportamentos estereotipados, associados a determinados contextos sociais.
- Os machos de *Hylodes* sp. (gr. *lateristrigatus*) vocalizam quatro tipos distintos de cantos: o canto de anúncio (*advertisement call*); os cantos piado (*peep call*) e chiado (*squeal call*), que são emitidos em contextos agonísticos e de corte; e o canto de corte (*courtship call*), somente provocado pelas sinalizações tátil ou visual-tátil realizadas pela fêmea no ritual da corte.
- A corte é complexa e envolve sinais visuais, acústicos e táteis. Os machos vocalizam e sinalizam visualmente durante a corte. As fêmeas realizam sinais visuais e táteis. A corte se inicia e ocorre quase completamente em terra, sempre próximo ou na margem do riacho de correnteza, dentro do território do macho. Apenas a última etapa da corte é subaquática e precede a oviposição. A corte completa do *Hylodes* sp. (gr. *lateristrigatus*) pode ser sintetizada em três etapas: (1) avaliação, (2) aceitação e (3) condução.
- A sinalização tátil e a sinalização visual-tátil realizadas pela fêmea durante a corte disparam o canto de corte, emitido pelo macho. Entretanto, o estímulo visual-tátil provoca quase três vezes mais cantos de corte. Possivelmente os componentes visual e tátil atuam em sinergia, sendo esta a primeira evidência de comunicação bimodal visual-tátil para uma espécie de anuro.
- Durante as sinalizações visuais, o macho escolhe o membro locomotor ou saco vocal (que é duplo em *Hylodes*) que será usado (direito, esquerdo ou ambos) de acordo com a posição do receptor coespecífico. Possivelmente este direcionamento melhora e otimiza a performance individual. É também possível que o movimento e a cor dos sacos vocais sejam importantes para a comunicação visual em *Hylodes* sp. (gr. *lateristrigatus*), mesmo quando há emissão sonora.

- A complexidade da comunicação visual em hilodídeos, assim como em anuros em geral, ainda está subestimada, principalmente nos trópicos.