First record of the torrent frog *Hylodes heyeri* (Anura, Hylodidae) in Santa Catarina State, South Brazil and acoustic comparison with the cryptic species *Hylodes perplicatus* (Anura, Hylodidae)

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Resumo

Primeiro registro da rã-das-corredeiras Hylodes heyeri (Anura, Hylodidae) para o estado de Santa Catarina, sul do Brasil e comparação acústica com a espécie críptica Hylodes perplicatus (Anura, Hylodidae). Realizamos o primeiro registro da rã-das-corredeiras Hylodes heyeri para o estado de Santa Catarina, sul do Brasil, conhecida anteriormente de localidades ao longo da Mata Atlântica somente nos estados de São Paulo e Paraná. Devido à sua semelhança morfológica e por agora poderem ser encontradas em simpatria, nós também efetuamos uma comparação acústica entre os cantos de anúncio de H. heyeri e H. perplicatus. Há uma maior variação na duração do canto de ambas as espécies, do que consta na literatura, provavelmente relacionada à variação na temperatura do ar. Para distinguir as duas espécies, além das diferenças nas frequências dominantes, um parâmetro temporal que pode ser utilizado é o intervalo entre as notas, porém não a duração do canto devido à influência da temperatura do ar. Essas descobertas reforçam a urgência de mais atividades de campo para o aumento do conhecimento sobre a distribuição geográfica de anuros, sua conservação e chamamos a atenção para a importância de análises acústicas detalhadas para distinguir espécies crípticas.

Palavras-chave: Bioacústica; Canto de anúncio; Distribuição geográfica; Espécies crípticas; Vocalizações

Abstract

Herein, we report the first record of the torrent frog *Hylodes heyeri* in Santa Catarina State, South Brazil, previously known from localities along the Atlantic Rainforest in the states of São Paulo and Paraná. We also performed an acoustic comparison between the calls of *H. heyeri* and *H. perplicatus* due to their morphological similarity and because now they could be found in sympatry. There was a greater variation in advertisement call duration for both species, than previously stated in the literature, probably related to variation in air temperature. To distinguish the two species, besides the differences in dominant frequency, a temporal parameter that could be used was the interval between notes, but call duration should not be used due to the influence of air temperature. Our

findings reinforce the urgency for more field activities to enhance our knowledge of the geographic distribution of anuran amphibians, to promote their conservation, and to call attention to the importance of detailed acoustic analysis to distinguish cryptic species.

Key words: Advertisement call; Bioacoustic; Cryptic species; Geographic distribution; Vocalizations

Introduction

Torrent frogs of the genus *Hylodes* Fitzinger, 1826 represent 24 endemic species of Brazil, mainly associated with the Brazilian Atlantic Forest (HADDAD; POMBAL JR., 1995; LAIA; ROCHA, 2012). These frogs are considered rheophilic, wary, and diurnal, calling mainly during the day, yet with some exceptions calling into the first hours of the night (HADDAD et al., 1996; LAIA; ROCHA, 2012; LINGNAU et al., 2013).

Hylodes heyeri Haddad, Pombal & Bastos (1996) is allocated in the H. lateristrigatus group, along with 17 other species (HADDAD et al., 1996; COSTA et al., 2009). The species was described from Caverna do Diabo, municipality of Eldorado, São Paulo State, Brazil. Later its geographic distribution was extended southward down to the municipality of Guaratuba in Paraná State (LINGNAU, 2004) and northward up to Parque Estadual Turístico do Alto Ribeira, São Paulo State (ARAÚJO et al., 2010).

Another species of the genus, *Hylodes perplicatus* (Miranda-Ribeiro, 1926), occurs not far from these localities, in Santa Catarina State. Pavan et al. (2001) were the first to mention a morphological similarity between *H. heyeri* and *H. perplicatus*. Due to this morphological similarity, Haddad et al. (2003) provided a redescription of *H. perplicatus*, and in comparing the advertisement calls of the two species, they found that these were clearly two distinct species. Besides differences in advertisement calls, to date, the two species are geographically separated: *H. heyeri* occurs from southern São Paulo State down to southern Paraná State, whereas *H. perplicatus* is restricted to Santa Catarina around the type-locality in Corupá and São Bento do Sul (HADDAD et al., 2003; FROST, 2014).

In this paper, we report the first record of *Hylodes heyeri* in Santa Catarina State, in localities in sympatry with *H. perplicatus*, which also led us to comment about the variation in advertisement calls of *H. heyeri* and *H.*

perplicatus and reinforce the importance of detailed acoustic analysis for species in the genus *Hylodes*.

Materials and Methods

Two field expeditions, each lasting two days, were made to the regions of Rio do Júlio (26°17'45"S, 49°06'31"W; altitude of 712 m), and of Castelo dos Bugres (26°13'34"S, 49°03'08"W; altitude of 846 m), both in the mountains of Serra Dona Francisca, municipality of Joinville, Santa Catarina during October and December 2013. A great portion of Serra Dona Francisca is part of a municipal reserve, Área de Proteção Ambiental (APA) da Serra Dona Francisca.

Specimens of H. heyeri and H. perplicatus were found on the basis of acoustic survey, and their vocalizations were then recorded. Advertisement calls were recorded with a Tascam DR-40 recorder and Rode NTG-2 microphone. Sonograms and oscillograms were made with the software Avisoft-SASLab Light with fast Fourier transformation of 256 points, 50% overlap, and Hamming window. Dominant frequencies were obtained with Cool Edit 96 with fast Fourier transformation of 1024 points. Call terminology was according to Heyer et al. (1990), as also used by Lingnau et al. (2008; 2013). We also compared our recordings obtained at Serra Dona Francisca to previous recordings of *H. heyeri* from Morretes, Paraná State (LINGNAU; BASTOS, 2007) and of H. perplicatus from São Bento do Sul, Santa Catarina State (HADDAD et al., 2003).

Identification was based mainly on acoustic parameters, because as already mentioned by Haddad et al. (2003), an analysis of advertisement calls is crucial to defining the taxonomic position of some amphibians, particularly for *Hylodes* species. Vouchers were collected with license ICMBIO 26957-1, and they were housed in the herpetological collection at Universidade Tecnológica Federal do Paraná, Campus Francisco

Beltrão (RLUTF 921 – 923: *H. heyeri* and RLUTF 1001: *H. perplicatus*). Species identification was confirmed by comparison of vocalization recordings with the literature and morphological comparison with specimens from CFBH (Célio F. B. Haddad collection, Departamento de Zoologia, I.B., Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil) and ZUEC (Museu de História Natural, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil): *Hylodes heyeri*: CFBH 2466, 2467, 2468; ZUEC 8238, 8240, 8242, 8243, 8249, 8250, 8253; *Hylodes perplicatus*: CFBH 3570, 3572-3574.

Results and Discussion

During field trips, we found five males of *H. heyeri* (Figure 1A) in the region of Rio do Júlio, Serra Dona Francisca. Calling males were found on rocks and branches along the margin of an affluent of Rio do Júlio. We recorded and collected the advertisement calls of three males (RLUTF 921 – SVL 35.06 mm, RLUTF 922 – SVL 36.28 mm and RLUTF 923 – SVL 35.83 mm). This record extends the distribution of *H. heyeri* approximately 50 km (straight line) from the municipality of Guaratuba, State of Paraná (LINGNAU, 2004), and it is the first record of this species in Santa Catarina State (Figure 2). With this new data, we can consider that *H. heyeri* occurs along the Atlantic Forest,

from southern São Paulo State up to northern Santa Catarina.

Around 8 km (straight line) from Rio do Júlio, in the locality of Castelo dos Bugres, Serra Dona Francisca, we heard three individuals of *H. perplicatus* (Figure 1B). We recorded and collected the advertisement calls of one male (RLUTF 1001 – SVL 43.89 mm). The main acoustic parameters of *H. heyeri* and *H. perplicatus* are presented in Table 1.

In comparing the data from Table 1 and Figure 3 with previous call descriptions of H. heyeri, we noted that the advertisement calls described here were longer than those reported from the type-locality by Haddad et al. (1996) (0.72 – 1.53 s at type-locality) and from Morretes by Lingnau and Bastos (2007) (0.69 - 1.86 s)at Morretes). This should be interpreted with caution, because as shown by Lingnau and Bastos (2007), the call duration in *H. heyeri* is strongly correlated with air temperature: at lower temperatures males show longer duration of advertisement calls. Probably due to this strong correlation with air temperature, males at Rio do Júlio (at 19°C) emitted longer advertisement calls as males from Morretes (20-25.2°C) and Eldorado (21-23°C). However, temporal parameters such as note duration, interval between notes and, most importantly, spectral parameters such as dominant frequency were similar at the three localities.

FIGURE 1: Males of *Hylodes heyeri* (A), and *H. perplicatus* (B). *Hylodes heyeri* – RLUTF923, from Rio do Júlio, Serra Dona Francisca, municipality of Joinville, Santa Catarina. *Hylodes perplicatus* (no voucher) from municipality of São Bento do Sul, Santa Catarina.

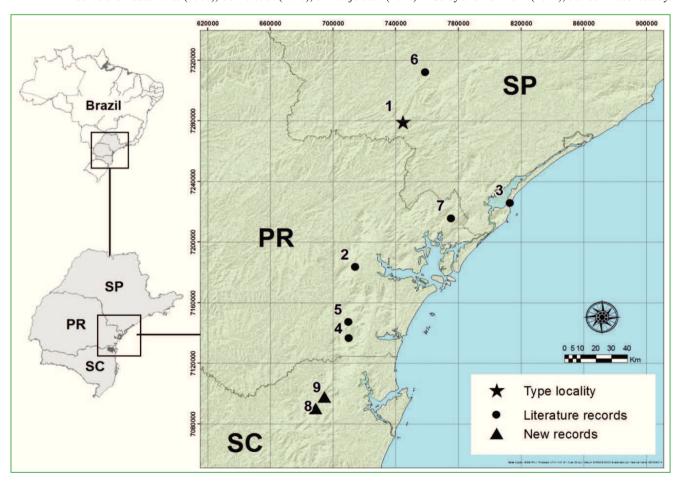




TABLE 1: Acoustic parameters of the advertisement call of *Hylodes heyeri* and *H. perplicatus* at Serra Dona Francisca, municipality of Joinville, Santa Catarina, Brazil.

	Hylodes heyeri			Hylodes perplicatus		
	Mean ± SD	Minimum – Maximum	Calls / ♂ recorded	Mean ± SD	Minimum – Maximum	Calls / ♂ recorded
Call duration (s)	2.03 ± 0.34	1.12-2.49	15/3	1.01 ± 0.18	0.75-1.25	5/1
Number of notes per call	44.93 ± 7.77	26-56	15/3	16.8 ± 2.58	13-20	5/1
Duration of notes (s)	0.026 ± 0.004	0.014-0.034	15/3	0.021 ± 0.004	0.015-0.027	5/1
Interval between notes (s)	0.024 ± 0.003	0.018-0.031	15/3	0.04 ± 0.009	0.033-0.057	5/1
Interval between calls (s)	27.72 ± 10.17	15.69-54.61	15/3	37.89 ± 11.05	27.6-56.58	5/1
Dominant frequency (kHz)	4.39 ± 0.36	3.89-4.89	15/3	3.028 ± 0.1	2.91-3.13	5/1

FIGURE 2: Map of the current geographic distribution of *Hylodes heyeri*. 1. Eldorado; 2. Morretes; 3. Cananeia; 4. Guaratuba; 5. São José dos Pinhais; 6. PETAR – Parque Estadual Turístico do Alto Ribeira; 7. Guaraqueçaba; 8. Rio do Júlio, Joinville; 9. Castelo dos Bugres, Joinville. Data from: 1. Haddad et al. (1996); 2. Lingnau (2000); 3. Toledo and Lingnau (2002); 4. Lingnau (2004); 5. Conte and Rossa-Feres (2006), Cunha et al. (2010); 6. Araújo et al. (2010) 7. Garey and Hartmann (2012); 8 and 9. Present study.



Haddad et al. (2003) compared the advertisement calls of *H. heyeri* and *H. perplicatus* and argued that the advertisement call of *H. heyeri* was shorter than that of *H. perplicatus* based on their recordings. As observed now with our data, this is not always true, because there is a larger variation in call duration in both species, probably related to variation in air temperature. Based only on our recordings from Serra Dona Francisca, we could say that the advertisement call of *H. heyeri* is longer than that of *H. perplicatus* (the opposite of HADDAD et al., 2003), which is demonstrated by the data in Table 1 and Figure 3.

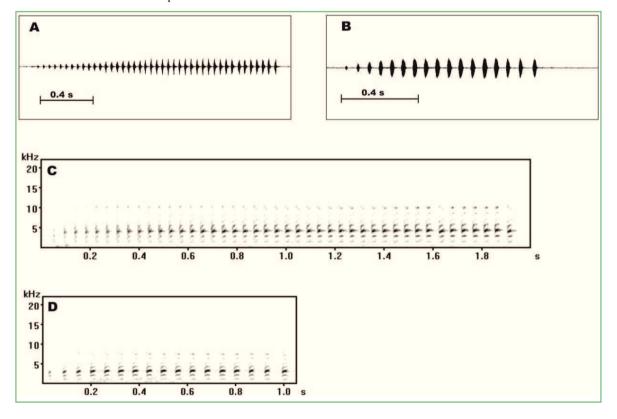
Hylodes heyeri and H. perplicatus are morphologically very similar, yet clearly distinct according to their vocalizations (HADDAD et al., 2003). Besides differences in their dominant frequency, a temporal parameter that could be used to distinguish the two species is the interval between notes, but call duration should not be used due to temperature

influence. In *H. heyeri*, the interval between notes of the advertisement call was smaller than in *H. perplicatus*, as already mentioned by Haddad et al. (2003), and we also verified this with our data from recordings of *H. heyeri* and *H. perplicatus* from Serra Dona Francisca, of *H. heyeri* from Morretes (LINGNAU; BASTOS, 2007) and of *H. perplicatus* from São Bento do Sul (HADDAD et al., 2003).

At both localities, Rio do Júlio and Castelo dos Bugres, we found *H. heyeri* and *H. perplicatus* in sympatry. It is very possible that both species could also occur in sympatry along other nearby rivers. Therefore caution is recommended in identifying species of *Hylodes*, particularly in the region of Serra Dona Francisca, where until recently, all specimens were identified as *H. perplicatus*.

Until now, only three species of *Hylodes* are recognized in Santa Catarina (*H. heyeri*, *H. meridionalis*

FIGURE 3: Advertisement calls of *Hylodes heyeri* and *H. perplicatus* from Serra Dona Francisca, municipality of Joinville, Santa Catarina, Brazil. (A) Oscillogram of *H. heyeri*; (B) oscillogram of *H. perplicatus*; (C) spectrogram of *H. heyeri*; and (D) spectrogram of *H. perplicatus*. *Hylodes heyeri* RLUTF 923, recorded at 19.0°C air temperature; *H. perplicatus* RLUTF 1001, recorded at 27.2°C air temperature.



and *H. perplicatus*). *Hylodes heyeri* and *H. perplicatus* are restricted to the northern part of the state (HADDAD et al., 2003; this work), and occurrences of *Hylodes* in the southern part have been reported to be *H. meridionalis* (LINGNAU et al., 2013 and references therein). Surely, in the next years, new species of *Hylodes* in Santa Catarina will be discovered, since it is unlikely that along 1250 km of coastal Atlantic Forest in Santa Catarina, there are only three species of *Hylodes*, while in São Paulo State, a 1241 km extent of coastal Atlantic Forest has 10 species of this genus (ROSSA-FERES et al., 2011).

Despite an increase in taxonomic and even ecological knowledge about species of *Hylodes* in recent years (e.g. CANEDO; POMBAL JR., 2007; LINGNAU et al., 2008; 2013; DA SILVA; BENMAMAN, 2008), there is still not enough knowledge about the species belonging to this genus and their distribution (LAIA; ROCHA, 2012). Our findings reinforce the urgency for more field activities to enhance our knowledge of the geographic distribution of anurans, to promote their conservation, and to call attention to the importance of detailed acoustic analysis to distinguish cryptic species.

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