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Vocalizations of *Hypsiboas beckeri* and *H. stenocephalus* (Anura: Hylidae), two species of the *H. polytaenius* group from southeastern Brazil

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Abstract

Vocalizations of *Hypsiboas beckeri* and *H. stenocephalus* (Anura: Hylidae), two species of the *H. polytaenius* group from southeastern Brazil. The analysis of anuran vocalizations is an important taxonomic tool, especially within complexes of morphologically similar species. *Hypsiboas beckeri* and *H. stenocephalus* are syntopic in their type locality (Poços de Caldas, state of Minas Gerais, Brazil), and both belong to the *H. polytaenius* species group. Analyzed calls of these species showed that a previous acoustic description for *H. beckeri*, from another locality, probably represents a distinct species. The calls of topotypical *H. beckeri* and *H. stenocephalus* differed substantially from each other, and also could be differentiated from other species of the *H. polytaenius* group. Additionally, we present the first record of *H. beckeri* for the State of São Paulo. The conservation status of both studied species varies among the available red lists and should thus be revisited in the future according to new taxonomic and distributional information.

Keywords: Atlantic Forest, bioacoustics, conservation, *Hypsiboas pulchellus* clade, Serra da Mantiqueira, taxonomy.

Resumo

Vocalizações de *Hypsiboas beckeri* e *H. stenocephalus* (Anura: Hylidae), duas espécies do grupo de *H. polytaenius* do sudeste do Brasil. A análise das vocalizações dos anuros é uma importante ferramenta taxonômica, especialmente em complexos de espécies morfologicamente similares. *Hypsiboas beckeri* e *H. stenocephalus* são sintópicas em sua localidade-tipo (Poços de Caldas, estado

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de Minas Gerais, Brasil), e ambas pertencem ao grupo de *H. polytaenius*. Os cantos analisados dessas espécies mostraram que uma descrição acústica prévia de *H. beckeri*, de outra localidade, provavelmente representa uma espécie distinta. Os cantos de *H. beckeri* e *H. stenocephalus* topotípicas diferiram substancialmente um do outro e também poderiam ser diferenciados dos cantos de outras espécies do grupo *H. polytaenius*. Adicionalmente, apresentamos o primeiro registro de *H. beckeri* para o estado de São Paulo. O status de conservação de ambas as espécies estudadas varia entre as listas vermelhas disponíveis, e deveria portanto ser revisto no futuro de acordo com novas informações taxonômicas e de distribuição.

Palavras-chave: bioacústica, clado de *Hypsiboas pulchellus*, conservação, Mata Atlântica, Serra da Mantiqueira, taxonomia.

Introduction

The speciose Neotropical genus *Hypsiboas* subsumes an assemblage of slender and longitudinally striped treefrogs, formally known as the *H. polytaenius* group (Cruz and Caramaschi 1998, Caramaschi and Cruz 1999, 2004a, 2013). It is regarded as a monophyletic group within the *H. pulchellus* clade (Faivovich *et al.* 2004, 2005) and currently comprises 12 species: *H. bandeirantes* Caramaschi and Cruz, 2013; *H. beckeri* (Caramaschi and Cruz, 2004); *H. botumirim* Caramaschi, Cruz and Nascimento, 2009; *H. buriti* (Caramaschi and Cruz, 1999); *H. cipoensis* (Lutz, 1968); *H. goianus* (Lutz, 1968); *H. latistriatus* (Caramaschi and Cruz, 2004); *H. leptolineatus* (Braun and Braun, 1977); *H. jaguariaivensis* Caramaschi, Cruz and Segalla, 2010; *H. phaeopleura* (Caramaschi and Cruz, 2000); *H. polytaenius* (Cope, 1870) and *H. stenocephalus* (Caramaschi and Cruz, 1999).

Among the species of the *Hypsiboas polytaenius* group, the calls of *H. bandeirantes*, *H. beckeri*, *H. botumirim*, *H. cipoensis*, *H. goianus*, *H. phaeopleura* and *H. polytaenius* have been described (Haddad *et al.* 1988, Heyer *et al.* 1990, Guimarães *et al.* 2001, Menin *et al.* 2004, Acioli and Toledo 2008, Caramaschi *et al.* 2009, Pinheiro *et al.* 2012). There are two call types reported so far: the "a" call or harsh call, presented by all these species, emitted as a single pulsed note (*H. bandeirantes*, *H. beckeri*, *H. botumirim* and *H. polytaenius*) or a sequence of about 2–3 short notes (*H. cipoensis*, *H. goianus* and *H. phaeopleura*), and the type "b" or trilled call, consisting of a sequence of rapidly repeated unpulsed notes, currently reported only for *H. bandeirantes*, *H. beckeri*, *H. goianus* and *H. polytaenius*. Additional call types (a third call) have been described, as the "encounter call" of *H. goianus* (Menin *et al.* 2004) and the "c" call of *H. polytaenius* (Pinheiro *et al.* 2012).

Hypsiboas beckeri and *H. stenocephalus* were described from the Poços de Caldas plateau, in the homonymous municipality, Minas Gerais state, southeastern Brazil (Caramaschi and Cruz 1999, 2004a). Both species have been reported from other localities, also in Minas Gerais (Acioli and Toledo 2008, Santos *et al.* 2009), and vocalizations of *H. beckeri* were described from São Thomé das Letras (Acioli and Toledo 2008). Nevertheless, a description of the vocalizations of topotypical specimens of *H. beckeri* is still not available, nor is there any acoustic description for *H. stenocephalus*.

Anuran vocalizations are critical for sexual selection and present species-specific attributes (Gerhardt and Bee 2007, Wells 2007), which have made their descriptions an important taxonomic tool (e.g., Angulo and Reichle 2008, Glaw *et al.* 2010, Martins and Giaretta 2011, Carvalho and Giaretta 2013). Therefore, describing the calls of the remaining species and comparing topotypical data among populations might be relevant for the taxonomy of species in the *Hypsiboas polytaenius* group.

In this paper, we describe vocalizations of *Hypsiboas beckeri* and *H. stenocephalus*, based mainly on topotypes, and examine whether (i) calls of topotypical *H. beckeri* agree with those previously described from another locality and (ii) if the calls of both species allow them to be distinguished from each other, given that they are syntopic, as well as from other species of the group. Furthermore, we report on the first record of *H. beckeri* for the state of São Paulo (Municipality of Águas da Prata) and discuss the conservational implications of our findings.

Materials and Methods

We conducted field surveys between 2008 and 2015 in the Municipality of Poços de Caldas, state of Minas Gerais (ca. 21°47' S, 46°33' W, 1230 m a.s.l.) and in the neighboring Municipality of Águas da Prata, state of São Paulo (ca. 21°56' S, 46°43' W, 830 m a.s.l.). Both localities are characterized by Atlantic Forest remnants, including altitudinal fields, within the Serra da Mantiqueira mountain range, southeastern Brazil.

For call recordings, we used Marantz PMD671 and M-audio Microtrack II digital recorders, adjusted to a sample rate of 44.1 or 48 kHz and 16-bit resolution, coupled, respectively, to Sennheiser ME67/K6 or ME66/K6 directional microphones. For call analyses, we used the software Raven Pro v. 1.4 (Bioacoustics Research Program 2011) with window type = Hann, DFT and window size = 256 samples, grid spacing (spectral resolution) = 172 or 188 Hz (for 44.1 and 48 kHz recordings, respectively), overlap = 89.8%, hop size (temporal resolution) = 0.59 ms, window brightness = 55% and contrast = 70%. We measured and counted temporal traits on waveforms and spectral traits on spectrograms through the "peak frequency" function. In order to remove background noise and highlight only the frequency peaks, we generated power spectra with a 55 dB clipping. Measured call traits are presented as mean ± standard deviation (SD) and ranges (minimum–maximum). Overall means and SDs are based on means from each male, whereas ranges comprise the entire dataset.

Anurans may present a relatively diverse acoustic repertoire (Wells 2007), and analyzing the function of call types is beyond the aim of this paper. For taxonomic purposes, defining comparable units is a basic requirement for an accurate result. Thus, for the descriptions and comparisons of acoustic characters, we adopted the mechanistic definition, based on the concept of a note as the unit of sound produced during a single airflow cycle (McLister *et al.* 1995, Robillard *et al.* 2006), which followed the terminology used by Pinheiro *et al.* (2012) for the *H. polytaenius* group. We determined the number of notes and pulses per second by dividing their numbers by call and note durations, respectively. Call rate (calls per minute) was achieved by counting the number of calls in the recording, then applying a cross multiplication to fit one minute, as necessary.

We identified recorded adult males as *Hypsiboas beckeri* or *H. stenocephalus* based on diagnostic traits provided in their original descriptions (Caramaschi and Cruz 1999, 2004a). More specifically, these species can be differentiated by the whitened supra-cloacal crest, present in *H. beckeri* and absent in *H. stenocephalus*, and by the ratio of head width (HW) and snout-vent length (SVL), greater in the former than in the latter.

Voucher specimens and sound files are deposited in the Collection of Amphibians of the Museu de Biodiversidade do Cerrado (AAG-UFU), Universidade Federal de Uberlândia, Municipality of Uberlândia, state of Minas Gerais, Brazil (see Appendices I and II).

Results

Species Identifications

Based on morphological differences, we identified two species of the *Hypsiboas polytaenius* group in the study sites (Figures 1

and 2), which coincided with the number of vocalization patterns recorded.

Specimens of *Hypsiboas beckeri* had a whitened supra-cloacal crest and proportionally wider heads (SVL: 27.2 mm ± 1.3 [25.0–29.1]; HW: 8.7 mm ± 0.5 [7.8–9.4]; HW/SVL: 0.32 ± 0.01 [0.30–0.35]; *N* = 17 adult males).

Individuals of *Hypsiboas stenocephalus* lacked supra-cloacal crests and had proportionally narrower heads (SVL: 30.3 mm ± 1.3 [28.4–31.9]; HW: 8.1 mm ± 0.4 [7.6–8.7]; HW/SVL: 0.27 ± 0.01 [0.26–0.28]; *N* = 5 adult males).

Habitat

We found adult males of *Hypsiboas beckeri* and *H. stenocephalus* calling at night, after the beginning of the rainy season in the region. In Poços de Caldas (*N* = 3 sites) and Águas da Prata (*N* = 1 site), *H. beckeri* seemed to be a relatively common species, found calling perched on bushes or grasses at the margins of artificial ponds, or along streamlets (ca. 50 cm wide, 50 cm deep) within wet areas surrounded by cattle pastures. *Hypsiboas stenocephalus* was less abundant, found only in the latter habitat, syntopically with *H. beckeri*. Other syntopic calling frogs were *Aplastodiscus perviridis*, *Physalaemus cuvieri*, *P. jordanensis* and *Odontophrynus americanus*.

Vocalizations of Hypsiboas beckeri

Both the "a" and "b" call types were present (Figure 3). In the complete dataset recorded (*N* = 379 calls), there were three times more "a" calls (*N* = 285; 75.2%) than "b" calls (*N* = 94; 24.8%). Descriptive statistics of call traits are detailed in Tables 1 and 2.

The "a" call was a pulsed note emitted singly and irregularly repeated (*N* = 143 notes; 50.2% of recorded calls), or in fast sequences of two (*N* = 124; 43.5%) to three notes (*N* = 18; 6.3%) separated by short intervals within the sequence (118.0–354.0 ms). In one occasion, two males were recorded interacting with each other by emitting longer sequences of "a" calls (ca. 10 calls each).

The "b" call was composed of several short, unpulsed notes, with slightly lower amplitude

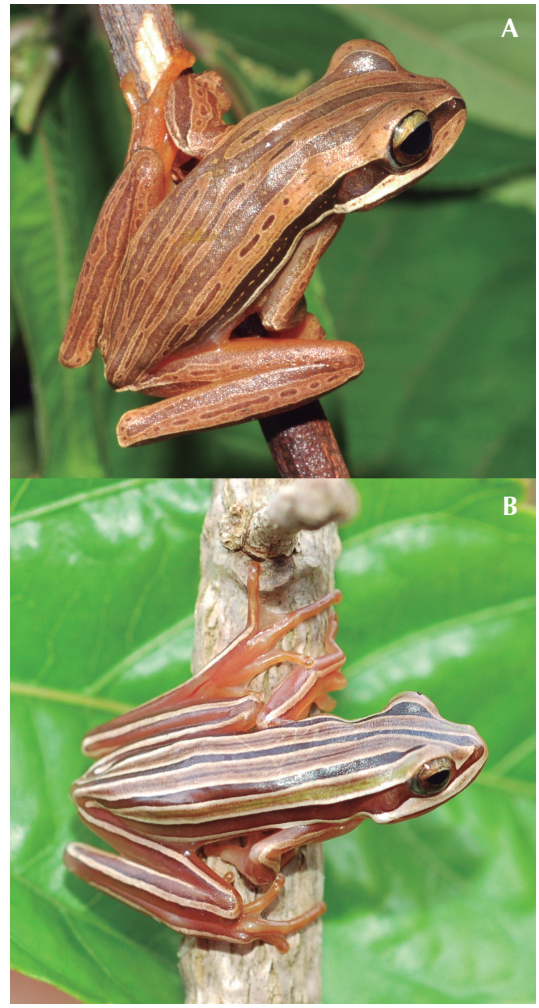

Figure 1. Specimens of *Hypsiboas beckeri* and *H. stenocephalus*, in life. (**A**) *Hypsiboas beckeri*, voucher AAG-UFU 4805, SVL = 28.5 mm. (**B**) *Hypsiboas stenocephalus*, voucher AAG-UFU 5071, SVL = 29.3 mm. Both specimens are topotypes (Poços de Caldas, Minas Gerais state, Brazil).

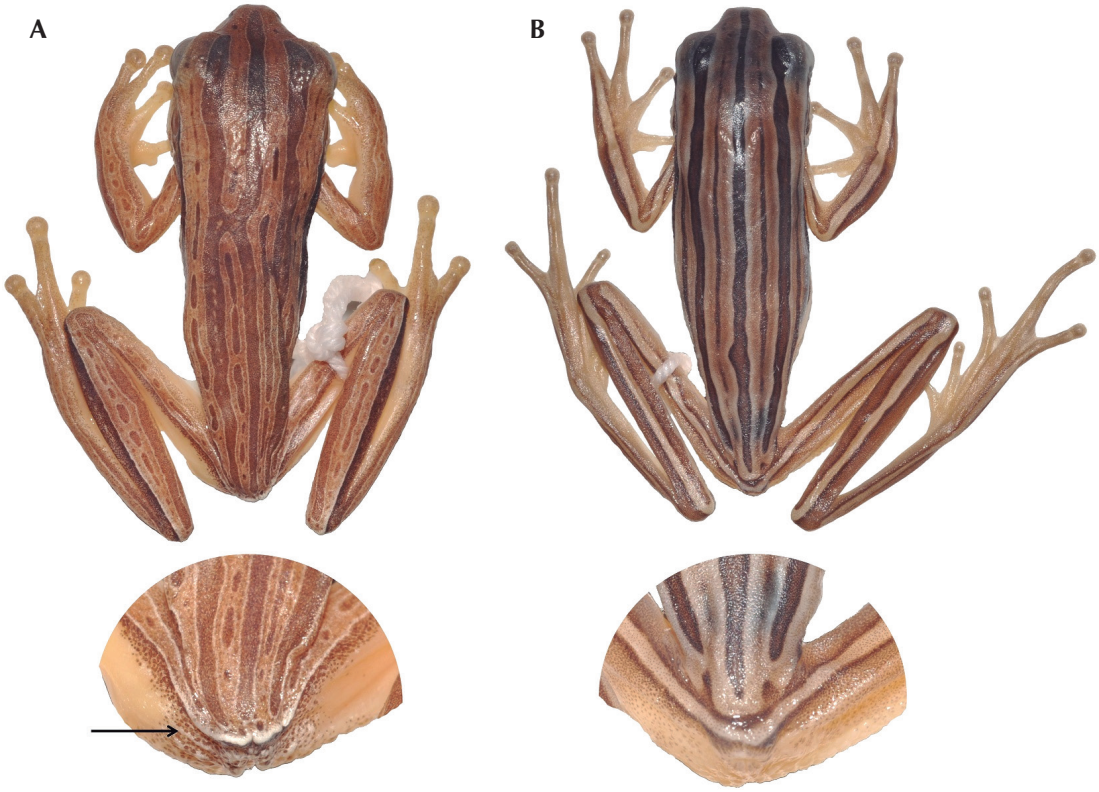

Figure 2. Preserved topotypes of *Hypsiboas beckeri* and *H. stenocephalus*, with the cloacal region in detail. (**A**) *Hypsiboas beckeri*, voucher AAG-UFU 4805, SVL = 28.5 mm. (**B**) *Hypsiboas stenocephalus*, voucher AAG-UFU 5071, SVL = 29.3 mm. Note the whitened flap pointed by the arrow in A (supra-cloacal crest).

and frequency than "a" calls. Usually, they were emitted after "a" calls, but also appeared isolated. It consisted of a trill of 7–24 irregularly spaced notes, with internote breaks tending to decrease towards the end of the call (internote breaks from 2.0 to 244.0 ms), the shorter ones about 2.0–5.0 ms, or even undetectable.

In both call types, three harmonics were noticed, and the fundamental one was the most emphasized (dominant frequency).

Vocalizations of Hypsiboas stenocephalus

Both the "a" and "b" call types occurred (Figure 4), but among 128 calls recorded, there was only one "b" call. Descriptive statistics of call traits are detailed in Tables 1 and 2.

The "a" call had 2–4 pulsed notes and was emitted singly and irregularly repeated (N = 44 calls; 34.6% of the recorded calls), or composing call groups of 2–8 calls in sequence (N = 83; 65.4%), separated by regular intervals (300.0–460.0 ms). Mostly, calls had two (N = 71 calls, 55.9% of the dataset) or three notes (N = 55, 43.3%), but a single call contained four notes. The first note was less intense (lower amplitude), shorter and with fewer pulses than the last notes, and middle notes, when present, were more similar to the first note in duration and number of pulses, but with higher amplitude. Spectro-

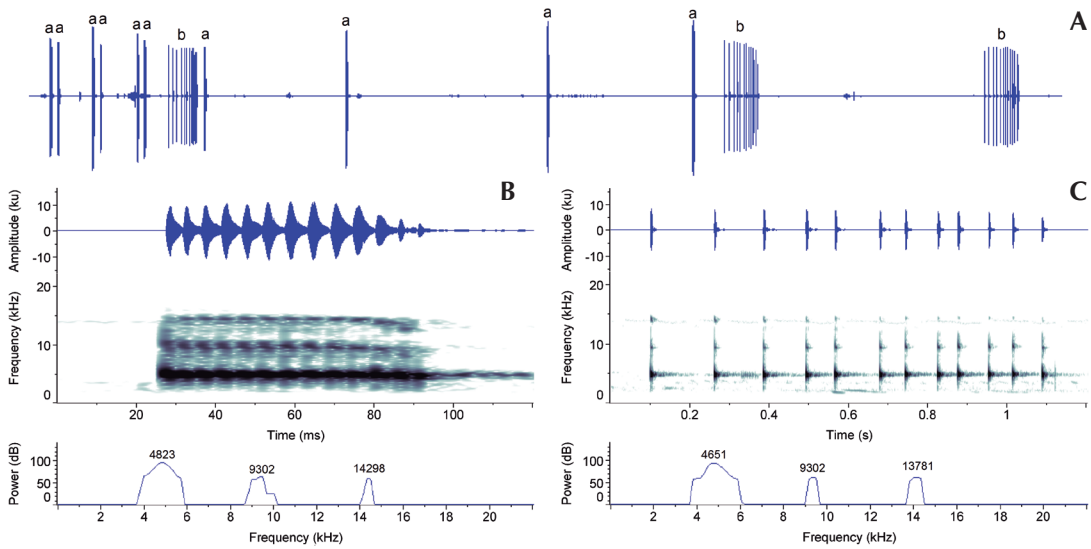

Figure 3. Vocalizations of *Hypsiboas beckeri*. (**A**) Waveform (30 s) with several "a" and "b" calls. (**B, C**) Call types "a" and "b" in detail, respectively (from top to bottom: waveforms, spectrograms and power spectra). Voucher specimen AAG-UFU 4676 (details of recording in Appendix I).

grams showed 2–3 distinct harmonics, with the fundamental one corresponding to the dominant frequency.

The only "b" call recorded presented 10 short, unpulsed notes, with amplitude and frequency similar to "a" calls. Notes were irregularly spaced, with internote breaks of 25.0–29.0 ms or very short/unmeasurable. Dominant frequency corresponded to the fundamental harmonic, and two others were detected.

Discussion

The calls of topotypical *Hypsiboas beckeri* differed substantially from specimens from São Thomé das Letras (Acioli and Toledo 2008). The dominant frequency in the latter was between 6890–7320 Hz in "a" calls and 6460–7320 Hz in "b" calls, whereas, in topotypes, it was between 4307–5063 Hz in "a" calls and 4134–4875 Hz in "b" calls. The "b" calls of specimens from São Thomé das Letras had a maximum of six notes (mean = 3.4), against 7–24 in topotypes (mean = 14.0). These differences are consistent and substantial enough to conclude it is very unlikely that they represent the same species.

Compared to the other species of the *Hypsiboas polytaenius* group, topotypical *H. beckeri* resembled *H. bandeirantes*, *H. botumirim* and *H. polytaenius* by the "a" calls usually consisting of a single pulsed note, eventually appearing as a group of these notes separated by short intervals. It differed from the calls of *H. bandeirantes* (described as *H.* aff. *polytaenius* in Pinheiro *et al.* 2012) and *H. polytaenius* (Pinheiro *et al.* 2012) by having dominant frequency in the "a" calls between 3938–5063 Hz (5340–5857 Hz in *H. bandeirantes* and 5813–7313 Hz in *H. polytaenius*) and between 3938–4875 Hz in the "b" calls (5340–5513 Hz in *H. bandeirantes* and 5438–6750 Hz in *H. polytaenius*); from *H.*

Table 1. Advertisement call characters of the "a" call (harsh call) of *Hypsiboas stenocephalus* and *H. beckeri* from three localities in southeastern Brazil (MG: state of Minas Gerais; SP: state of São Paulo). Data presented as mean ± standard deviation (minimum–maximum); sample size. *We measured only breaks within note sequences.

Call traits	***Hypsiboas stenocephalus***	***Hypsiboas beckeri***		
	Poços de Caldas (MG) *N* = 5 males	Poços de Caldas (MG) *N* = 13 males	Águas da Prata (SP) *N* = 1 male	São Thomé das Letras (MG) (Acioli and Toledo 2008)
Structure	Group of pulsed notes	Single pulsed note	Single pulsed note	Single pulsed note
Call duration	312.8 ± 74.0 (165.0–588.0); 93	= note duration	= note duration	= note duration
Number of notes	2.65 ± 0.42 (2–4); 93	1	1	1
Duration of notes (ms)	First: 22.5 ± 3.6 (15.0–36.0); 67 Middle: 24.7 ± 3.6 (15.0–36.0); 42 Last: 36.0 ± 5.5 (26.0–71.0); 70	63.9 ± 7.9 (35.0–104.0); 118	60.7 ± 9.6 (45.0–79.0); 30	110.0 ± 10.0 (90.0–130.0); 9
Number of pulses in notes	First: 2.3 ± 0.36 (2–3); 67 Middle: 2.6 ± 0.28 (2–4); 42 Last: 4.1 ± 0.47 (3–5); 70	12.1 ± 1.7 (6–18); 109	10.5 ± 2.9 (4–15); 30	16.9 ± 3.9 (11–23); 9
Pulses per second	108.8 ± 7.1 (86.9–134.6); 63	197.9 ± 16.7 (88.9–314.8); 109	171.9 ± 34.9 (88.8–229.2); 30	-
Pulse duration	8.8 ± 1.2 (5.0–13.0); 106	5.9 ± 0.9 (3.0–12.0); 163	6.3 ± 0.2 (4.0–9.0); 35	-
Internote breaks (ms)*	143.6 ± 15.3 (62.0–186.0); 105	220.6 ± 42.7 (118.0–354.0); 46	185.6 ± 43.6 (139.0–280.0); 24	510.0 ± 880.0 (160.0–1530.0); 9
Intercall breaks within call groups (ms)	345.6 ± 31.3 (300.0–460.0); 38	not present	not present	not present
Calls per minute	7.4 ± 2.9 (5.0–10.7); 5	10.5 ± 6.2 (3.0–23.3); 13	23.6; 1	-
Dominant frequency (Hz)	3874 ± 100 (3563–4134); 108	4723 ± 65 (4307–5063); 118	4237 ± 105 (3938–4313); 30	7100 ± 140 (6890–7320); 9
2nd harmonic (Hz)	7594 ± 156 (7125–8250); 53	9467 ± 209 (8613–10688); 49	8303 ± 87 (8250–8437); 14	-
3rd harmonic (Hz)	11084 ± 309 (9188–11886); 50	13952 ± 256 (12188–15188); 49	12535 ± 242 (12188–12937); 14	-
Air temperature (°C)	20.3 ± 1.0 (18.8–21.0)	16.4 ± 3.8 (12.0–22.0)	12.5	14.0

Table 2. Advertisement call traits of the "b" call (trilled call) of *Hypsiboas stenocephalus* and *H. beckeri* from three localities in southeastern Brazil (MG: Minas Gerais state; SP: São Paulo state). Data presented as mean ± standard deviation (minimum–maximum); sample size. *Authors considered it occasionally pulsed, which we interpreted, in our analyses, as common notes separated by very short intervals.

Call traits	***Hypsiboas stenocephalus***	***Hypsiboas beckeri***		
	Poços de Caldas (MG) *N* = 1 male	Poços de Caldas (MG) *N* = 13 males	Águas da Prata (SP) *N* = 1 male	São Thomé das Letras (MG) Acioli and Toledo (2008)
Structure	Trill of unpulsed notes	Trill of unpulsed notes	Trill of unpulsed notes	Trill of unpulsed notes*
Call duration (ms)	287.0; 1	1015.0 ± 147.0 (530.0–1945.0); 66	779.8 ± 223.8 (508.0–1223.0); 22	-
Notes per call	10; 1	14.0 ± 2.3 (7–24); 66	12.1 ± 4.7 (6–22); 22	3.4 ± 2.0 (0–6); 11
Notes per second	34.8; 1	14.1 ± 1.5 (7.3–20.6); 66	15.3 ± 3.2 (11.8–25.1); 22	-
Note duration (ms)	13.8 ± 1.4 (12.0–16.0); 10	20.9 ± 4.6 (10.0–49.0); 210	20.3 ± 3.9 (11.0–29.0); 49	20.0 ± 0.0 (10.0–30.0); 11
Internote breaks (ms)	27.0 ± 2.0 (25.0–29.0); 5	66.8 ± 33.8 (2.0–244.0); 206	65.1 ± 44.2 (3.0–194.0);45	110.0 ± 80.0 (20.0–230.0); 11
Calls per minute	< 1	4.1 ± 2.4 (1.4–10.6); 13	5.1 ± 1.3 (4.2–6.0); 2	-
Dominant Frequency (Hz)	3862 ± 202 (3562–4125); 10	4555 ± 117 (4134–4875); 205	4144 ± 139 (3938–4500); 49	7020 ± 240 (6460–7320); 11
2nd harmonic (Hz)	7664 ± 234 (7312–7875); 8	9093 ± 168 (8613–9938); 53	8475 ± 262 (8250–9000); 10	-
3rd harmonic (Hz)	10714 ± 489 (9938–11438); 7	13486 ± 170 (12747–14470); 54	12393 ± 241 (12188–12750); 10	-
Air temperature (°C)	20.3 ± 1.0 (18.8–21.0)	16.4 ± 3.8 (12.0–22.0)	12.5	14.0

botumirim (Caramaschi et al. 2009), it differed by the "a" calls tending to have more pulses (mean = 11.9; 4–18 pulses *vs.* mean = 3.8; 3–5 pulses in *H. botumirim*) and for the "b" calls unknown in *H. botumirim*.

Hypsiboas stenocephalus presented the "a" call as a sequence of 2–4 short notes, similar to *H. cipoensis*, *H. goianus* and *H. phaeopleura*. While in *H. goianus* and *H. phaeopleura* notes are unpulsed (Guimarães *et al.* 2001, Menin *et al.* 2004, Pinheiro *et al.* 2012), they are pulsed in *H. cipoensis* from Serra da Canastra (Haddad *et al.* 1988), as we found for *H. stenocephalus*. Nevertheless, a more detailed comparison

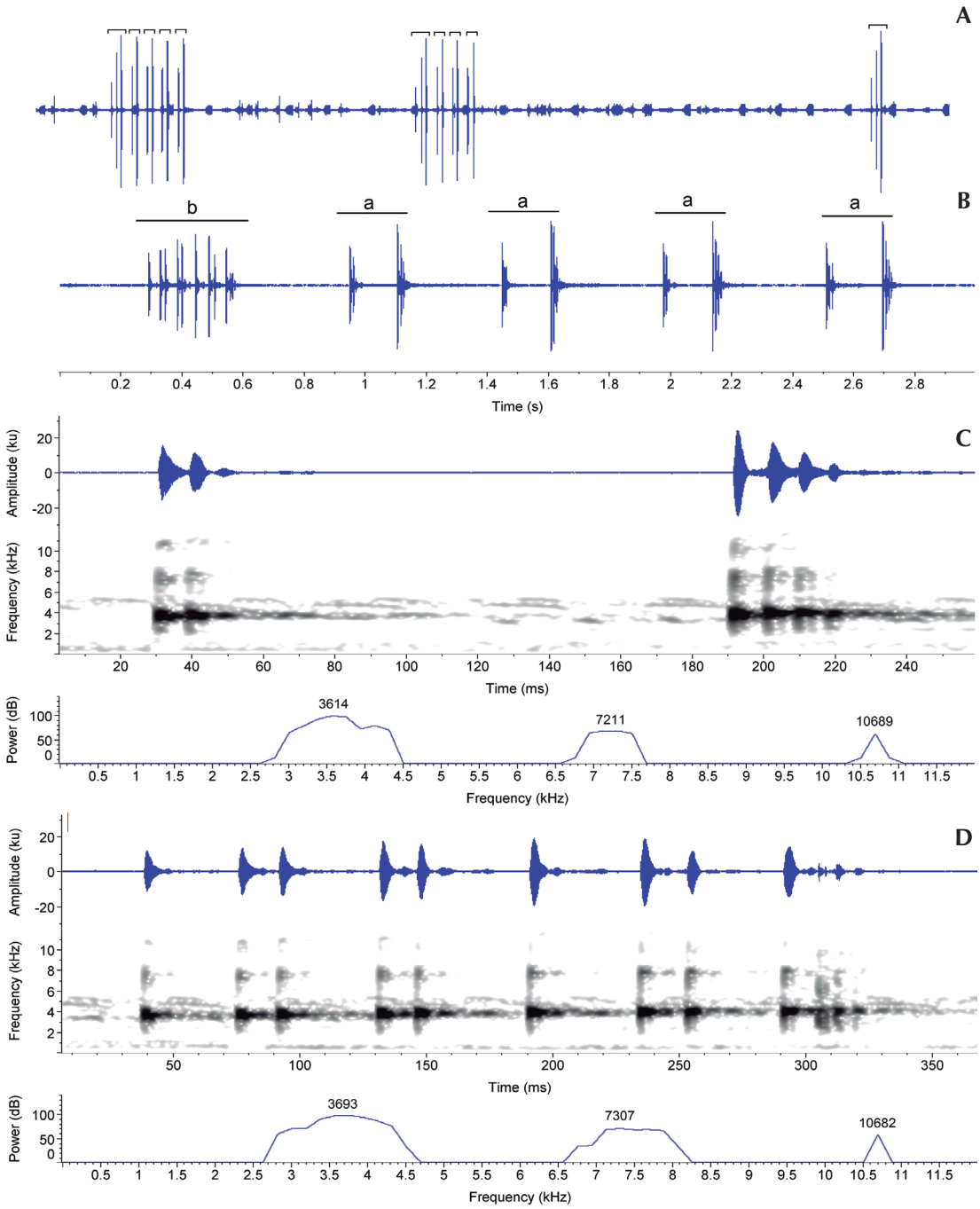

Figure 4. Vocalizations of *Hypsiboas stenocephalus*. (**A**) Waveform (30 s) evidencing the structure of "a" calls: a call group with five calls, followed by another with four calls and an isolated call. (**B**) Waveform showing a "b" call followed by four two-note "a" calls. (**C, D**) Call types "a" and "b" in detail, respectively (from top to bottom: waveforms, spectrograms and power spectra). Unvouchered recording #3 (details in Appendix I).

between *H. cipoensis* and *H. stenocephalus* is still not possible, since the only acoustic description for *H. cipoensis* is relatively brief and calls of specimens from the type locality remain unknown. Another issue that deserves attention is that "b" calls were not reported yet for *H. cipoensis* and *H. phaeopleura*, so that additional studies are still necessary to confirm if these species do not produce this call type or if it is simply rare, as in *H. stenocephalus*.

In addition to quantitative differences, such as note duration and number of pulses per note in "a" calls, the vocalizations of *Hypsiboas beckeri* and *H. stenocephalus* differed substantially from each other in structure, as the "a" call of the former was a single note and that of the latter a series of 2–4 short notes (which varied in amplitude, duration and number of pulses from the first to the last note). Furthermore, in addition to the call of *H. stenocephalus* consisting of a group of notes, the calls themselves could be emitted in groups of up to eight calls, a structure not observed in *H beckeri*. The acoustic differentiation between these species was already expected, since when closely related species occur in syntopy, evolutionary forces such as reproductive character displacement and reinforcement may cause or maintain divergence in traits related to reproduction, diminishing the probability of heterospecific pairings and its deleterious consequences (Blair 1974, Grenat *et al.* 2013, Höbel and Gerhardt 2003, Malone *et al.* 2014).

Among the recorded specimens of *Hypsiboas beckeri*, we recorded the first specimen of this species for the state of São Paulo (Águas da Prata). Although the region of Poços de Caldas represents a traditional sampling site for anurans in Minas Gerais state (Andrade and Cardoso 1987, Cardoso and Martins 1987, Cardoso *et al.* 1989, Cardoso and Haddad 1992, Giaretta and Sazima 1993, Vasconcelos and Giaretta 2003, Giaretta and Oliveira 2007, Santos *et al.* 2009, Martins and Giaretta 2012a), adjacent areas across the border in São Paulo state are relatively poorly sampled. This paucity of samplings in northeastern São Paulo state, near the borders with Minas Gerais state, was already reported by Rossa-Feres *et al.* (2011), and new records of anurans have been constantly reported for the area (Araujo *et al.* 2007a, b, Martins and Silva 2009, Martins and Giaretta 2012b).

New state records like this may not represent outstanding distribution extensions, but are very relevant for conservation purposes, since they reinforce the need of additional species inventories and establishment of conservation policies for the region. As described in the São Paulo state Constitution and more recent legislation (Estado de São Paulo 1989, 2005), the government is responsible for the protection of local species and their habitats, and might also promote species inventories and regularly update the list of threatened species in the state.

Both *Hypsiboas beckeri* and *H. stenocephalus* appear as threatened (Vulnerable) in the red list of species from Minas Gerais state (Biodiversitas 2007, Estado de Minas Gerais 2010), although they are not in the national red list (Ministério do Meio Ambiente 2014) and are cited as Data Deficient in the IUCN red list (Caramaschi and Cruz 2004b, Stuart 2006). Of the 10 anuran species on the Minas Gerais state red list, seven were described from Poços de Caldas [besides *H. beckeri* and *H. stenocephalus*, *Bokermannohyla vulcaniae* (Vasconcelos and Giaretta, 2005); *Phyllomedusa ayeaye* (Lutz, 1966); *Proceratophrys palustris* Giaretta and Sazima, 1993; *Scinax caldarum* (Lutz, 1968); *Scinax ranki* (Andrade and Cardoso, 1987)], and are mostly considered as restricted to the area or known only from a few other localities, demonstrating why this region, including adjacent areas in São Paulo state (Águas da Prata, São José do Rio Pardo), is among the priority areas for conservation of Brazilian biodiversity (Ministério do Meio Ambiente 2007).

Analysis of vocalizations appears to be a helpful tool for the taxonomy of the *Hypsiboas polytaenius* group. Future studies might focus on describing calls of species that remain unknown

(*H. jaguariaivensis*, *H. latistriatus*, *H. leptolineatus*), as well as comparing topotypical data to additional populations, which could uncover cryptic diversity and undescribed species. If necessary, conservation assessments might be revisited in the future in order to accommodate new taxonomic and distributional knowledge.

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References

Acioli, E. C. S. and L. F. Toledo. 2008. Amphibia, Anura, Hylidae, *Hypsiboas beckeri:* filling gap and description of its advertisement call. *Check List 4:* 182–184.

Andrade, G. V. and A. J. Cardoso. 1987. Reconhecimento do grupo *rizibilis:* descrição de uma nova espécie de *Hyla* (Amphibia, Anura). *Revista Brasileira de Zoologia 3:* 433–440.

Angulo, A. and S. Reichle. 2008. Acoustic signals, species diagnosis, and species concepts: the case of a new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia. *Zoological Journal of the Linnean Society 152:* 59–77.

Araujo, C. O., T. H. Condez, and C. F. B. Haddad. 2007a. Amphibia, Anura, *Barycholos ternetzi*, *Chaunus rubescens*, and *Scinax canastrensis:* distribution extension, new state record. *Check List 3:* 153–155.

Araujo, C. O., T. H. Condez, and C. F. B. Haddad. 2007b. Amphibia, Anura, *Phyllomedusa ayeaye* (B. Lutz, 1966): distribution extension, new state record, and geographic distribution map. *Check List 3:* 156–158.

Bioacoustics Research Program. 2011. Raven Pro: interactive sound analysis software, Version 1.4. URL: http://www.birds.cornell.edu/raven.

Biodiversitas. 2007. *Revisão das Listas das Espécies da Flora e da Fauna Ameaçadas de Extinção do Estado de Minas Gerais: Relatório Final, Volume 3*. Belo Horizonte. Fundação Biodiversitas. 142 pp.

Blair, W. F. 1974. Character displacement in frogs. *American Zoologist 14:* 1119–1125.

Caramaschi, U. and C. A. G. Cruz. 1999. Duas espécies novas do grupo de *Hyla polytaenia* Cope, 1870 do estado de Minas Gerais, Brasil (Amphibia, Anura, Hylidae). *Boletim do Museu Nacional 403:* 1–10.

Caramaschi, U. and C. A. G. Cruz. 2004a. Duas novas espécies de *Hyla* do grupo de *H. polytaenia* Cope, 1870 do sudeste do Brasil (Amphibia, Anura, Hylidae). *Arquivos do Museu Nacional 62:* 247–254.

Caramaschi, U. and C. A. G. Cruz. 2004b. *Hypsiboas stenocephalus*. IUCN red list of threatened species. Version 2014.3. Electronic Database accessible at http://www.iucnredlist.org/. Captured on 13 May 2015.

Caramaschi, U. and C. A. G. Cruz. 2013. A new species of the *Hypsiboas polytaenius* clade from southeastern Brazil (Anura: Hylidae). *South American Journal of Herpetology 8:* 121–126.

Caramaschi, U., C. A. G. Cruz, and L. B. Nascimento. 2009. A new species of *Hypsiboas* of the *H. polytaenius* clade from southeastern Brazil (Anura: Hylidae). *South American Journal of Herpetology 4:* 210–216.

Cardoso, A. J. and C. F. B. Haddad. 1992. Diversidade e turno de vocalizações de anuros em comunidade neotropical. *Acta Zoologica Lilloana 41:* 93–105.

Cardoso, A. J. and J. E. Martins. 1987. Diversidade de anuros durante o turno de vocalizações em comunidade neotropical. *Papéis Avulsos de Zoologia 36:* 279–285.

Cardoso, A. J., G. V. Andrade, and C. F. B. Haddad. 1989. Distribuição espacial em comunidades de anfíbios (Anura) no sudeste do Brasil. *Revista Brasileira de Biologia 49:* 241–249.

Carvalho, T. R. and A. A. Giaretta. 2013. Bioacoustics reveals two new syntopic species of *Adenomera* Steindachner (Anura: Leptodactylidae: Leptodactylinae) in the Cerrado of central Brazil. *Zootaxa 3731:* 533–551.

Cruz, C. A. G. and U. Caramaschi. 1998. Definição, composição e distribuição geográfica do grupo de *Hyla polytaenia* Cope, 1870 (Amphibia, Anura, Hylidae). *Boletim do Museu Nacional, Nova Série, Zoologia 392:* 1–19.

Estado de Minas Gerais. 2010. Deliberação Normativa COPAM nº 147, de 30 de abril de 2010: aprova a lista de espécies ameaçadas de extinção da fauna do estado de Minas Gerais. Diário do Executivo, Minas Gerais. Brasil.

Estado de São Paulo. 1989. Constituição do Estado de São Paulo: texto constitucional promulgado em 5 de outubro de 1989, com as alterações adotadas pelas Emendas Constitucionais nº 1/1990 a 28/2009. Assembleia Legislativa do Estado de São Paulo, São Paulo, Brasil.

Estado de São Paulo. 2005. Lei nº 11.977, de 25 de agosto de 2005: institui o Código de Proteção aos Animais do estado e dá outras providências. Assembleia Legislativa do Estado de São Paulo, São Paulo, Brasil.

Faivovich, J., P. C. A. Garcia, F. Ananias, L. Lanari, N. G. Basso, and W. C. Wheeler. 2004. A molecular perspective on the phylogeny of the *Hyla pulchella* species group (Anura, Hylidae). *Molecular Phylogenetics and Evolution 32:* 938–950.

Faivovich, J., C. F. B. Haddad, P. C. A. Garcia, D. R. Frost, J. A. Campbell, and W. C. Wheeler. 2005. Systematic review of the frog family Hylidae, with special reference to the Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History 294:* 1–240.

Gerhardt, H. C. and M. A. Bee. 2007. Recognition and localization of acoustic signals. Pp. 113–146 *in* P. M. Narins, A. S. Feng, R. R. Fay, and A. N. Popper (eds.), *Hearing and Sound Communication in Amphibians*. New York. Springer.

Giaretta, A. A. and I. Sazima. 1993. Nova espécie de *Proceratophrys* Mir. Rib. do sul de Minas Gerais, Brasil (Amphibia, Anura, Leptodactylidae). *Revista Brasileira de Biologia 53:* 13–19.

Giaretta, A. A. and L. E. Oliveira. 2007. *Phyllomedusa ayeaye* (Reticulate Leaf Frog), habitat and conservation. *Herpetological Review 38:* 441.

Glaw, F., J. Köhler, I. De la Riva, D. R. Vieites, and M. Vences. 2010. Integrative taxonomy of Malagasy treefrogs: combination of molecular genetics, bioacoustics and comparative morphology reveals twelve additional species of *Boophis*. *Zootaxa 2383:* 1–82.

Grenat, P. R., J. A. Valetti, and A. L. Martino. 2013. Intraspecific variation in advertisement call of *Odontophrynus cordobae* (Anura, Cycloramphidae): a multilevel and multifactor analysis. *Amphibia-Reptilia 34:* 471–482.

Guimarães, L. D., L. P. Lima, R. F. Juliano, and R. P. Bastos. 2001. Vocalizações de espécies de anuros (Amphibia) no Brasil central. *Boletim do Museu NacionalRio de Janeiro 474:* 1–16.

Haddad, C. F. B., G. V. Andrade, and A. J. Cardoso. 1988. Anfíbios anuros no Parque Nacional da Serra da Canastra, estado de Minas Gerais. *Brasil Florestal 64:* 9–20.

Heyer, W. R., A. S. Rand, C. A. G. Cruz, O. L. Peixoto, and C. E. Nelson. 1990. Frogs of Boracéia. *Arquivos de Zoologia 31:* 231–410.

Höbel, G. and H. C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution 57:* 894–904.

Malone, J. H., J. Ribado, and E. M. Lemmon. 2014. Sensory drive does not explain reproductive character displacement of male acoustic signals in the upland chorus frog (*Pseudacris feriarum*). *Evolution 68:* 1306–1319.

Martins, L. B. and A. A. Giaretta. 2011. A new species of *Proceratophrys* Miranda-Ribeiro (Amphibia: Anura: Cycloramphidae) from central Brazil. *Zootaxa 2880:* 41–50.

Martins, L. B. and A. A. Giaretta. 2012a. Advertisement calls of two species of *Proceratophrys* (Anura: Odontophrynidae) from Minas Gerais, Brazil, with comments on their distribution, taxonomy and conservation status. *South American Journal of Herpetology 7:* 203–212.

Martins, L. B. and A. A. Giaretta. 2012b. *Ameerega flavopicta* (Lutz, 1925): first dart-poison frog (Anura: Dendrobatidae) recorded for the state of São Paulo, Brazil, with comments on its advertisement calls and taxonomy. *Check List 8:* 502–504.

Martins, L. B. and W. R. Silva. 2009. Amphibia, Anura, Leptodactylidae, *Leptodactylus syphax*: new state record. *Check List 5:* 433–435.

McLister, J. D., E. D. Stevens, and J. P. Bogart. 1995. Comparative contractile dynamics of calling and locomotor muscle in three hylid frogs. *The Journal of Experimental Biology 198:* 1527–1538.

Menin, M., R. A. Silva, and A. A. Giaretta. 2004. Reproductive biology of *Hyla goiana* (Anura, Hylidae). *Iheringia Série Zoologia 94:* 49–52.

Ministério do Meio Ambiente. 2007. Áreas prioritárias para conservação, uso sustentável e repartição de benefícios da biodiversidade brasileira: atualização Portaria MMA n° 9, de 23 de janeiro de 2007. Secretaria de Biodiversidade e Florestas, Ministério do Meio Ambiente, Brasília, Brasil.

Ministério do Meio Ambiente. 2014. Portaria do Ministério do Meio Ambiente (MMA) n°444. *Diário Oficial da União 245:* 121–126.

Pinheiro, P. D. P., T. L. Pezzuti, and P. C. A. Garcia. 2012. The tadpole and vocalizations of *Hypsiboas polytaenius* (Cope, 1870) (Anura, Hylidae, Hylinae). *South American Journal of Herpetology 7:* 123–133.

Robillard, T., G. Höbel, and H. C. Gerhardt. 2006. Evolution of advertisement signal in North American hylid frogs: vocalizations as end-products of calling behavior. *Cladistics 22:* 533–545.

Rossa-Feres, D. C., R. J. Sawaya, J. Faivovich, J. G. R. Giovanelli, C. A. Brasileiro, L. Schiesari, J. Alexandrino, and C. F. B. Haddad. 2011. Anfíbios do estado de São Paulo, Brasil: conhecimento atual e perspectivas. *Biota Neotropica 11:* 1–19.

Santos, J. T., E. F. Oliveira, V. A. São-Pedro, A. C. Monteiro-Leonel, and R. N. Feio. 2009. Amphibia, Anura, *Hypsiboas stenocephalus:* distribution extension and geographic distribution map. *Check List 5:* 27–31.

Stuart, S. 2006. *Hypsiboas beckeri*. The IUCN red list of threatened species Version 2014.3. Electronic Database accessible at http://www.iucnredlist.org/. Captured on 13 May 2015.

Vasconcelos, E. G. and A. A. Giaretta. 2003. A new species of *Hyla* (Anura: Hylidae) from southeastern Brazil. *Revista Española de Herpetologia 17:* 21–27.

Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. Chicago. University of Chicago Press. 1148 pp.

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Appendix I. *Recordings analyzed in this work. Sound files are labelled as: species (e.g., Hypsib_beckeri); locality (e.g., PocosCaldasMG); number of the recorded specimen for the species and locality (1, 2 etc.), with letters (a, b, c etc.), when present, indicating different sound files for the same specimen; initials of the authors; equipment abbreviation ("mt" for M-audio Microtrack II and "M671" for Marantz PMD671).*

Sound files	Voucher specimen	Date recorded	Time (hh:mm)	Air temperature (ºC)
***Hypsiboas beckeri*; Poços de Caldas, Minas Gerais state, Brazil**				
Hypsib_beckeriPocosCaldasMG1AAGmt	AAG-UFU 4676	08 October 2008	–	14.0
Hypsib_beckeriPocosCaldasMG2TRC_AAGmt	AAG-UFU 4805	10 October 2009	22:30	12.0
Hypsib_beckeriPocosCaldasMG3TRC_AAGmt	–	10 October 2009	22:32	12.0
Hypsib_beckeriPocosCaldasMG4TRC_AAGmt	–	08 November 2009	22:05	18.6
Hypsib_beckeriPocosCaldasMG5TRC_AAGmt	–	08 November 2009	22:08	18.6
Hypsib_beckeriPocosCaldasMG6TRC_AAGmt	–	08 November 2009	22:12	18.6
Hypsib_beckeriPocosCaldasMG7TRC_AAGmt	–	08 November 2009	22:16	18.6
Hypsib_beckeriPocosCaldasMG8TRC_AAGmt	–	08 November 2009	22:22	18.6
Hypsib_beckeriPocosCaldasMG9AAGm671	–	21 September 2014	20:35	12.5
Hypsib_beckeriPocosCaldasMG10AAGm671	–	21 September 2014	20:47	12.7
Hypsib_beckeriPocosCaldasMG11AAGm671	AAG-UFU 3910	21 September 2014	20:53	12.7
Hypsib_beckeriPocosCaldasMG12AAGm671	–	26 October 2014	23:13	22.0
Hypsib_beckeriPocosCaldasMG13AAGm671	–	26 October 2014	23:15	22.0
***Hypsiboas beckeri*; Águas da Prata, São Paulo state, Brazil**				
Hypsib_beckeriAguasPrataSP1a-bAAGm671	AAG-UFU 3911	21/09/2014	21:56	12.5
***Hypsiboas stenocephalus*; Poços de Caldas, Minas Gerais state, Brazil**				
Hypsib_stenocepPocosCaldasMG1AAGmt	AAG-UFU 4375	29 January 2009	21:05	19.5
Hypsib_stenocepPocosCaldasMG2a-fLBM_AAGmt	AAG-UFU 4827	09 November 2009	21:45	18.8
Hypsib_stenocepPocosCaldasMG3a-dAAGm671	–	01 January 2015	19:31	21.0
Hypsib_stenocepPocosCaldasMG4a-bAAGm671	AAG-UFU 5071	01 January 2015	19:36	21.0
Hypsib_stenocepPocosCaldasMG5a-bAAGm671	AAG-UFU 5072	01 January 2015	20:18	21.0

Appendix II. *Examined specimens for species identifications.*

Hypsiboas beckeri. BRAZIL: **Minas Gerais**, Poços de Caldas: AAG-UFU 1843–1853, 3910, 3922, 4676, 4805, 4822–4826; São Paulo state, Águas da Prata: AAG-UFU 3911.

Hypsiboas stenocephalus. BRAZIL, **Minas Gerais**, Poços de Caldas: AAG-UFU 4374, 4375, 4827, 5071, 5072.