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New records and distribution extensions of the glassfrogs *Hyalinobatrachium taylori* (Goin, 1968) and *H. tricolor* Castroviejo-Fisher, Vilà, Ayarzagüena, Blanc & Ernst, 2011 (Anura, Centrolenidae) in Amapá, Brazil

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Abstract

Based on field surveys undertaken in two conservation areas, we report new distribution data of *Hyalinobatrachium taylori* (Goin, 1968) and *H. tricolor* Castroviejo-Fisher, Vilà, Ayarzagüena, Blanc & Ernst, 2011 from the state of Amapá, northern Brazil. We provide acoustic data from these new populations. These are the first records of *H. taylori* and *H. tricolor* from Amapá, extending the geographic distributions of these species by 317 km from Mitaraka and 320 km from Saut Grand Machicou, both in French Guiana, respectively.

Keywords

Amazonia, bioacoustics, range extension, tropical rainforest

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Introduction

The Neotropical amphibian family Centrolenidae, known as glassfrogs, encompasses 12 genera (Guayasamin et al. 2009), including *Hyalinobatrachium* Ruiz-Carranza & Lynch, 1991, which is represented by 33 species (Castroviejo-Fischer et al. 2011; Frost 2020). Six species of *Hyalinobatrachium* occur in Brazil (Segalla et al. 2019): *H. cappellei* (Van Lidth de Jeude, 1904), *H. carlesvilai* Castroviejo-Fisher, Padial, Chaparro, Aguayo & De la Riva, 2009, *H. iaspidiense* (Ayarzaguena, 1992), *H. mondolfii* Señaris & Ayarzaguena, 2001, *H. muiraquitan* Oliveira & Hernández-Ruz, 2017, and *H. munozorum* (Lynch & Duellman, 1973). However, there are three species of *Hyalinobatrachium* occurring in French Guiana (Vacher et al. 2020) but unreported from the Brazilian state of Amapá. As French Guiana borders Amapá, the presence of additional *Hyalinobatrachium* species in the state is likely (Silva e Silva and Costa-Campos 2016; Figueiredo et al. 2020).

Glassfrogs are nocturnal and associated with riparian vegetation; they generally deposit their clutches on plants or rocks overhanging water bodies (Noronha and Rodrigues 2018). On hatching, tadpoles drop into the water to complete their development (Kubicki 2007). The identification of closely related *Hyalinobatrachium* species can be challenging because they often have very similar morphology. Moreover, they are challenging to collect as they often call from the vegetation several meters above the water. Consequently, the geographic distributions of many species remain poorly documented (Silva e Silva and Costa-Campos 2016; Costa-Campos et al. 2020a, 2020b). Nevertheless, the examination of molecular and acoustic traits have fostered biodiscovery, the description of new species, and improved understanding of the geographic distribution of many species in this group (Kok and Castroviejo-Fisher 2008; Castroviejo-Fisher et al. 2011; Guayasamin et al. 2019).

Hyalinobatrachium taylori (Goin, 1968) is a small glassfrog having a snout–vent length (SVL) in adult males of 18.1–19.4 mm and in females of 20.6 mm (Castroviejo-Fisher et al. 2011). This species usually uses vegetation 2–30 m above streams for reproduction. Egg clutches are deposited on the undersides of leaves, and males call on the upper sides. This species occurs along New River, Guyana (coordinates not available for the type locality; Castroviejo-Fisher et al. 2011) and occupies a wide distribution from the western tepuis (table-top mountains found in the Guiana Highlands) to the eastern Guiana Shield at elevations of 200–2000 m and the Amazonia–Cerrado ecotone. It has been found in French Guiana, Guyana, Venezuela, Suriname, and adjacent Brazil (Noonan and Bonett 2003; Castroviejo-Fisher et al. 2011; Frost 2020; Silva et al. 2020; Vacher et al. 2020).

Hyalinobatrachium tricolor Castroviejo-Fisher, Vilà, Ayarzagüena, Blanc & Ernst, 2011 is a small glassfrog (SVL 20.3–21.0 mm in adult males; unknown in females; Castroviejo-Fisher et al. 2011). Males call on vegetation 4–5 m above streams 0.5–1.5 m in depth. The species was described from Crique Wapou, Kaw, French Guiana and occurs at low elevation (0–100 m) (Castroviejo-Fisher et al. 2011; Vacher et al. 2020).

Here, we report the first records of both *H. taylori* and *H. tricolor* from the state of Amapá, northern Brazil, and we provide acoustic data from these newly found populations.

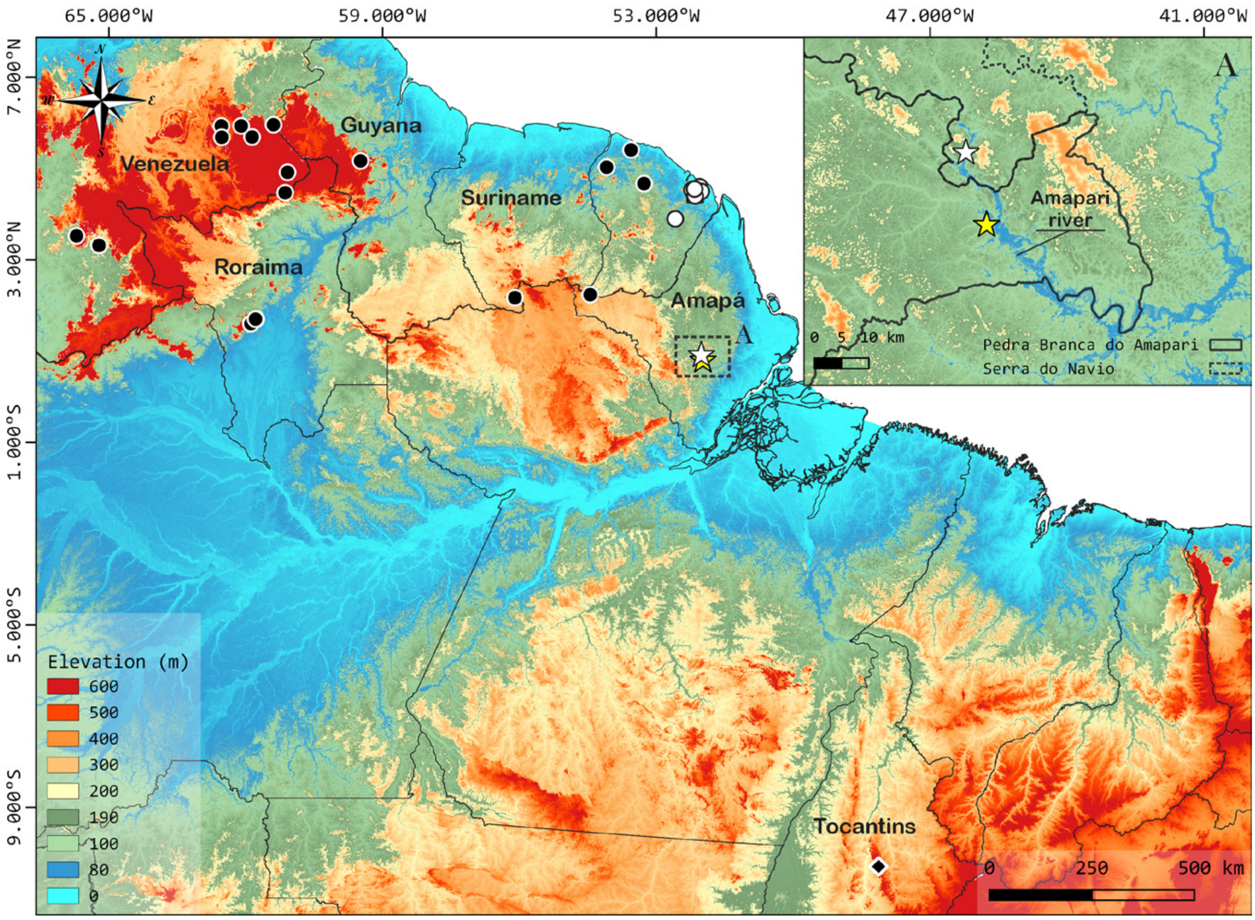

Figure 1. Known records of *Hyalinobatrachium taylori* (black circles) and *H. tricolor* (white circles). White square: type locality in Crique Wapou, Kaw, French Guiana (*H. tricolor*); *H. taylori coordinates not* available for type locality (Castroviejo-Fisher et al. 2011). Diamond: the occurrence in Tocatins may be a different species. New data for *H. taylori* (yellow star) and *H. tricolor* (white star) at Pedra Branca do Ampari and Serra do Navio, respectively, Amapá, Brazil (see Table 1 for details).

Methods

We conducted nocturnal visual encounter surveys for amphibians at two locations in Amapá, northern Brazil: Parque Natural Municipal do Cancão, municipality of Serra do Navio, on 7–12 March 2018, and Reserva Extrativista Beija-Flor Brilho de Fogo, municipality of Pedra Branca do Amapari, on 20–25 May 2019 (Fig. 1, Table 1).

Collected specimens were sacrificed using 5% lidocaine chlorhydrate, fixed in 10% formalin solution, and preserved in 70% ethanol. The specimens were collected under the Instituto Chico Mendes de Conservação da Biodiversidade/Sistema de Autorização e Informação em Biodiversidade (ICMBio/SISBIO permit #48102-2), and for each individual we collected muscle tissue samples before fixing the specimens and storing in 100% ethanol. We identified specimens of *H. taylori* and *H. tricolor* using the original species' descriptions by Castroviejo-Fisher et al. (2011). To confirm our identifications we checked acoustic parameters, because morphological characters are not sufficient to distinguish centrolenid species from one another (Castroviejo-Fisher et al. 2011). Specimens are deposited in the Herpetological Collection of the Universidade Federal do Amapá (CECC).

Bioacoustics. Vocalizations were recorded using a Marantz PMD 670 digital recorder (sampling rate: 44.1 kHz; resolution: 16 bits) and a Sennheiser ME67/K6 unidirectional microphone. Microphone was positioned at approximately 1 m from calling individuals. Bioacoustic analysis were conducted in Raven Pro v. 1.6 (Bioacoustics Research Program - Raven Pro 2019) with the following settings: window type Hann and Fast Fourier Transform (FFT) of 1024 samples; 3 dB filter bandwidth of 61.9 Hz; time grid resolution with overlap of 90%, and hop size of 2.31 ms; frequency grid resolution with discrete Fourier transform of 1024 and grid spacing of 44.1 Hz. Temporal measurements were manually obtained from the oscillogram and spectral measurements from the spectrogram. The following acoustic traits were measured following Castroviejo-Fisher et al. (2011): call duration; number of notes; note duration; interval between notes (i.e., silent period from the end of a note to the beginning of the next one); minimum, maximum, and dominant frequency (obtained with frequency 5%, 95%, and peak frequency functions, respectively). Harmonics were observed in calls of both species, but we refrained from measuring frequency values of harmonics in calls of *H. taylori*, as we observed artifacts in the spectrogram (i.e., aliasing) due to saturation of the microphone during recording; this could lead to spurious interpretation of these frequency components. Sound figures were generated in R v. 4.0.2 (R Development Core Team 2019) using Seewave (Sueur et al. 2008)

Table 1. Geographic coordinates from literature records and field surveys. * = type locality; coordinates of the type locality of *H. taylori* are not available. All coordinates use the WGS84 datum.

Country	State, locality	Latitude	Longitude	Species	Source
Brazil	Amapá, Pedra Branca do Amapari, Reserva Extrativista Beija-Flor Brilho de Fogo	00.791°N	051.978°W	*H. taylori*	This study
Brazil	Roraima, Serra da Mocidade	01.600°N	061.885°W	*H. taylori*	Moraes et al. 2017
Brazil	Roraima, Serra da Mocidade	01.700°N	061.768°W	*H. taylori*	Moraes et al. 2017
Brazil	Roraima, Serra da Mocidade	01.700°N	061.785°W	*H. taylori*	Moraes et al. 2017
Brazil	Pacaraima	04.469°N	061.136°W	*H. taylori*	Vacher et al. 2020
Brazil	Tocantins, Palmas, Taquaruçu	10.296°S	048.128°W	*H. taylori*	Silva et al. 2020
French Guiana	Chutes Voltaire	05.029°N	054.089°W	*H. taylori*	Vacher et al. 2020
French Guiana	Trinité, Crique Grand Leblond	04.669°N	053.269°W	*H. taylori*	Castroviejo-Fisher et al. 2011
French Guiana	Mitaraka	02.235°N	054.449°W	*H. taylori*	Fouquet et al. 2019
French Guiana	Montagne de Fer	05.407°N	053.554°W	*H. taylori*	Vacher et al. 2020
Guyana	Kaieteur National Park	05.166°N	059.483°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Suriname	Sipaliwini, Apalagadi	02.170°N	056.099°W	*H. taylori*	Vacher et al. 2020
Venezuela	Amazonas, Cerro Marahuaca	03.524°N	065.708°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Venezuela	Amazonas, Cerro Duida	03.316°N	065.217°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Venezuela	Auyan-tepui, Campamento Guayaraca	05.685°N	062.525°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Venezuela	Auyan-tepui, central-west sector of Auyan-tepui	05.933°N	062.535°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Venezuela	Bolívar, La Escalera	05.959°N	061.391°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Venezuela	Bolívar, Quebrada Jaspe	05.933°N	062.100°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Venezuela	Bolívar, Salto Karuay	05.690°N	061.861°W	*H. taylori*	Guayasamin et al. 2008
Venezuela	San Ignacio de Yuraní	04.916°N	061.083°W	*H. taylori*	Castroviejo-Fisher et al. 2011
Brazil	Amapá, Serra do Navio, Parque Natural Municipal do Cancão	00.911°N	052.012°W	*H. tricolor*	This study
French Guiana	Kaw, Montagne	04.550°N	052.160°W	*H. tricolor*	Castroviejo-Fisher et al. 2011
French Guiana	Régina, Canal de Kaw	04.500°N	052.016°W	*H. tricolor*	Castroviejo-Fisher et al. 2011
French Guiana	Régina, Crique Patawa	04.505°N	052.091°W	*H. tricolor*	Castroviejo-Fisher et al. 2011
French Guiana	Régina, Crique Wapou	04.433°N	052.150°W	*H. tricolor**	Guayasamin et al. 2008
French Guiana	Régina, Crique Wapou	04.400°N	052.150°W	*H. tricolor*	Castroviejo-Fisher et al. 2011
French Guiana	Régina, Rivière de Kaw	04.600°N	052.050°W	*H. tricolor*	Castroviejo-Fisher et al. 2011
French Guiana	Roura, Kaw Mountain	04.533°N	052.216°W	*H. tricolor*	Castroviejo-Fisher et al. 2011
French Guiana	Saut Grand Machicou	03.897°N	052.583°W	*H. tricolor*	Vacher et al. 2020

and tuneR (Ligges et al. 2018) packages with the following settings: window Hann, overlap = 90%, and FFT size = 1024. Acoustic recordings were deposited in the Fonoteca Neotropical Jacques Vielliard (FNJV).

Results

Hyalinobatrachium taylori **(Goin, 1968)**
Figures 1, 2A

New record. BRAZIL • Amapá, municipality of Pedra Branca do Amapari, Reserva Extrativista Beija-Flor Brilho de Fogo, Água Fria stream; 00.7918°N, 051.9783°W; 110 m elev.; 25.V.2019; Carlos E. Costa-Campos leg.; found at night (21:00 h) perched on a leaf >1.5 m above the stream; CECC 3274, 1 adult ♂, SVL 19.0 mm.

Identification. The following combination of phenotypic characters differentiates *H. taylori* from all other congeneric species: green bones in life, iris grey with black reticulations, dorsum dark green (Castroviejo-Fisher et al. 2011). Our recordings of the advertisement call is also distinctive, showing values within the range of variation as described in the original description for call duration (0.652–1.052 s), number of notes (5–9), and dominant frequency (4010.0–4350.0 Hz) described by Castroviejo-Fisher et al. (2011).

Advertisement call. Call duration is 0.393–0.489 s (mean = 0.447, SD = 0.049, n = 3; Fig. 2A). Each call is composed of 6 or 7 notes (mean = 7, SD = 0.6) with visible harmonics. Each note duration is 0.024–0.046 s (mean = 0.039, SD = 0.005, n = 20), with intervals between notes of 0.013–0.050 s (mean = 0.033, SD = 0.011, n = 17). Frequency bandwidth is narrow, with minimum frequency ranging from 3876.0 to 4005.2 Hz (mean = 3947.8, SD = 65.8, n = 3) maximum frequency from 4177.4 to 4220.5 Hz (mean = 4206.1, SD = 24.9, n = 3), and dominant frequency from 4091.3 to 4134.4 Hz (mean = 4120.0, SD = 24.9, n = 3), corresponding to the first harmonic. Recording = FNJV 46269.

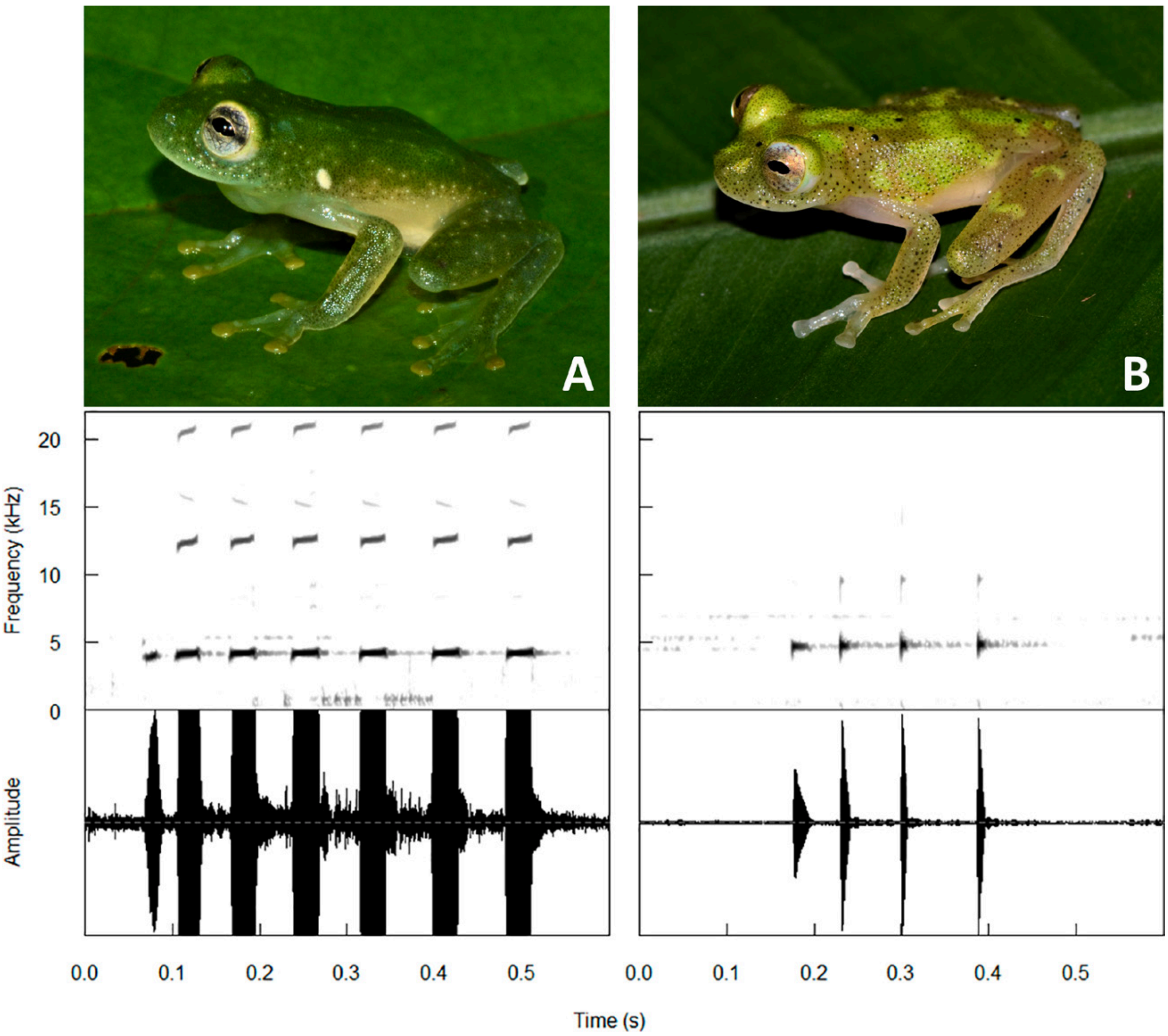

Figure 2. Spectrograms and respective oscillograms of the advertisement calls. **A.** *Hyalinobatrachium taylori* (21:00 h on 25 May 2019; ambient temperature 24.3 °C), at Pedra Branca do Amapari. **B.** *H. tricolor* (19:45 h on 11 February 2018; ambient temperature 25.6 °C) at Serra do Navio. Spectrograms were generated with a gray scale in which black = 0 dB.

Hyalinobatrachium tricolor **Castroviejo-Fisher, Vilà, Ayarzagüena, Blanc & Ernst, 2011**
Figures 1, 2B

New record. BRAZIL • Amapá, municipality of Serra do Navio, village of Pedra Preta; Amapari River; 00.9008°N, 052.0134°W; 140 m elev.; 11.II.2018; Jackson C. Sousa leg.; found at night (19:45 h) on leaves on vegetation 3.5 m above the ground; CECC 2949, 1 adult ♂, SVL 20.6 mm.

Identification. The following combination of phenotypic characters differentiates *H. tricolor* from all other congeneric species: bones white in life, dorsum in life light green with lime green and brown patches and brown. Its advertisement call also has a multi-note structure and also showed values within the range of variation for call duration (0.19–0.20 s), number of notes (4), and dominant frequency (4628.11–4903.07 Hz) described by Castroviejo-Fisher et al. (2011).

Advertisement call. Call duration is 0.219–0.220 s (mean = 0.219, SD = 0.001, *n* = 2; Fig. 2B). Each call is composed of four notes with visible harmonics. Each note duration is 0.007–0.020 s (mean = 0.012, SD = 0.005, *n* = 6), with intervals between notes of 0.033–0.079 s (mean = 0.057, SD = 0.019, *n* = 17). Frequency bandwidth is narrow ranging from 4565.0 to 4866.5 Hz (*n* = 2), and dominant frequency from 4694.2 to 4737.3 Hz (mean = 4715.7, SD = 30.5, *n* = 2), corresponding to the first harmonic. Recording = FNJV 46270.

Discussion

Calls of both *Hyalinobatrachium* species reported herein are in agreement with the unique multi-note pattern and other acoustic traits described for *H. taylori* and *H. tricolor* (Castroviejo-Fisher et al. 2011). Thus, the calls provide unambiguous evidence for the identification of the populations reported here. These records represent the first reports for *H. taylori* and *H. tricolor* in Amapá. We found *H. taylori* in a primary forest without anthropogenic disturbance, while *H. tricolor* was recorded in a riparian zone on the right bank of the Amapari River, which has some anthropic disturbance. *Hyalinobatrachium taylori* and *H. tricolor* are Least Concern according to the International Union for the Conservation of Nature Red List (IUCN SSC Amphibian Specialist Group 2018, 2020a). *Hyalinobatrachium taylori* has been reported from the state of Tocantins (Silva et al. 2020). This is the southernmost occurrence of this species and would seem to represent a disjunct population, as it is 1,500 km from populations of *H. taylori* in French Guiana, Suriname, Guyana, and Venezuela. Silva et al. (2020) did not include call data and discussed the possibility that this Tocantins population could represent a distinct, yet undescribed species. Our records of *H. taylori* in Amapá, however, is much closer to populations occurring in French Guiana, Suriname, Guyana, and Venezuela. Our new record of *H. taylori* extends the geographic distribution of this species southeast by approximately 317 km from Mitaraka, French Guiana, the closest previously known occurrence. The new record of *H. tricolor* is 320 km from Saut Grand Machicou, French Guiana. Our new records suggest that these species may be more widespread on the Guiana Shield than previously assumed (Castroviejo-Fisher et al. 2011). Additional sampling effort would probably find other populations in the areas between the previously known occurrences and our new record of *H. tricolor*.

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Authors' Contributions

CECC, VAMBF, and RTP conducted the fieldwork, collected the specimens, and wrote the text. AF revised the manuscript. DLB conducted bioacoustic analysis. VAMBF and CECC made the map and photographed the specimens. All authors have read and approved the submitted manuscript.

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