Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 13 (4): 135–139
https://doi.org/10.15560/13.4.135

PENSOFT

First record of *Boana alfaroi* (Caminer & Ron, 2014) (Anura: Hylidae) in Brazil

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Abstract
We report the occurrence of the Amazonian hylid *Boana alfaroi* outside of Ecuador for the first time. The locality (Assis Brasil, state of Acre) lies in northern Brazil, approximately 1,280 km southeast from the easternmost records in Ecuador. Additionally, we describe the vocalization of a male of *B. alfaroi* from the Brazilian population, including a second type of note besides the advertisement note. *Boana alfaroi* may occur across Amazonian lowland forests of Ecuador, northern Peru, and the Brazilian states of Acre and Amazonas.

Key words
Amazon Rainforest, biodiversity, *Boana calcarata–B. fasciata* complex, Brazilian Amazon, state of Acre, vocalization.

Academic editor: Raul F. D. Sales | Received 9 April 2017 | Accepted 7 June 2017 | Published 21 July 2017

Citation: Carvalho TR, Bang DL, Teixeira BFV, Giaretta AA (2017) First record of *Boana alfaroi* (Caminer & Ron, 2014) (Anura: Hylidae) in Brazil. Check List 13 (4): 135–139. https://doi.org/10.15560/13.4.135

Introduction

Boana alfaroi (Caminer & Ron, 2014) is 1 of the 4 species recently described in the revisionary study of the Amazonian *B. calcarata–B. fasciata* complex (Caminer and Ron 2014). Although this species was thought to be restricted to the Ecuadorian Amazon in the northeastern portion of the country, Caminer and Ron (2014) suggested that it could occur in Peru. Herein we report the first record of *B. alfaroi* from outside of Ecuador. This new record is from the municipality of Assis Brasil, on the tripoint of Brazil, Peru, and Bolivia, in the Brazilian Amazon.

Methods

The fieldwork was conducted on 11 and 12 February 2017 in the municipality of Assis Brasil (10.9393° S, 069.5712° W [datum WGS84]; 236 m above sea level), state of Acre, northern Brazil.

Calls were recorded with a ME67/K6 Sennheiser microphone coupled to a Marantz PMD 671 digital recorder. Recording settings were 44.1-kHz sampling rate and 16-bit resolution. The recordings were made under direct visualization and the microphone was positioned about 1 m from the voucher male. Calls of

a single male were analyzed in Soundruler (Gridi-Papp 2007) under the following settings: 256 points resolution (FFT), 90% overlap, window type Hann. The sound figure was generated in R (R Core Team 2014) through seewave package (Sueur et al. 2008) using a FFT = 256 points and 90% overlap. A high-band pass filter up to 500 Hz was applied to the cut from which the sound figure was generated. The call traits analyzed are as follows: temporal traits (note duration, internote interval, pulses per note, and pulse rate, defined as the number of pulses - 1 divided by the duration between the onset of first and last pulses); spectral traits (dominant frequency). Four recordings ("a–d") of a single male are housed in the acoustic database in the A. A. Giaretta laboratory under the identifier: Boana_alfaroiAssisBrasilAC1TRC_AAGm671. A male (AAG-UFU 5917) was recorded from 20:00–20:14 h on 12 February 2017. The air temperature was 26.2 °C. The distribution map of *B. alfaroi* was based on the coordinates provided by Caminer and Ron (2014) and our new record, which are listed in Table 1. Collection permit was issued by Instituto Chico Mendes de Conservação da Biodiversidade through Sistema de Autorização e Informação em Biodiversidade: SISBIO/ICMBio #30059–8.

Results

Boana alfaroi (Fig. 1) is distinguished from all other species of the *B. calcarata–B. fasciata* complex [*B. almendarizae* (Caminer & Ron, 2014), *B. calcarata* (Troschel, 1848), *B. fasciata* (Günther, 1858), *B. maculateralis* (Caminer & Ron, 2014); except *B. tetete* (Caminer & Ron, 2014)] by the following combination of traits: (1) absence of a well-developed calcar (only a small tubercle on heel); (2) iris upper band yellow in life and ill-defined; (3) presence of brown flecks on the neck and chest; (4) flanks with dark brown irregular spots; and (5) hidden surface of thighs with dark brown irregular spots.

From the closely related *B. tetete*, the most characteristic traits are the advertisement call and the diameter of the tympanum. All 6 specimens collected by us are in agreement with the snout–vent length (SVL 32.8–35.5 mm, mean = 34.2, SD = 1.0; n = 6 adult males) and tympanum diameter (TD 1.9–2.2 mm, mean = 2.0, SD = 0.1; n = 6 adult males) provided for *B. alfaroi* (Caminer and Ron 2014). *Boana alfaroi* may also be distinguished from *B. tetete* (mean male TD/SVL = 0.08, SD = 0.009; n = 5; Caminer and Ron 2014) by a smaller tympanum in relation to SVL (TD/SVL 0.05–0.06, mean = 0.06, SD = 0.003; n = 6 adult males).

Two other species of the *B. calcarata–B. fasciata* complex are also similar to *B. alfaroi*. *Boana steinbachi* (Boulenger, 1905) is a Bolivian species that was revalidated by Caminer and Ron (2014), based on the combination of ill-defined calcars and prominent and abundant supernumerary tubercles on hands (Caminer and Ron 2014). Supernumerary tubercles on the hands are present in *B. alfaroi*, but not as well-developed as they are in *B. steinbachi* (see Caminer and Ron 2014: fig. 11). *Boana dentei* (Bokermann, 1967), which was described from an Amazonian region in the Brazilian state of Amapá, is allied to the *B. calcarata–B. fasciata* complex. *Boana alfaroi* is distinguished from *B. dentei* by its different color patterns of the posterior thigh: in the former, there are dark brown irregular spots on a yellow background color; in the latter, thighs have black transverse stripes on a whitish background color (Bokermann 1967).

Table 1. Coordinates plotted on the distribution map of *Boana alfaroi* (Figure 3). All records from Ecuador were obtained in the original description (Caminer and Ron 2014).

Country	Province/state	Locality	Latitude (° S)	Longitude (° W)
Ecuador	Orellana	Parque Nacional Yasuní (type locality)	00.6893	076.4290
Ecuador	Orellana	Estación Científica Yasuní	00.6748	076.3844
Ecuador	Orellana	Río Napo (Nuevo Rocafuerte)	00.9192	075.4010
Ecuador	Orellana	Río Napo (Huiririma)	00.7116	075.6239
Ecuador	Orellana	Río Napo (San Vicente)	00.6790	075.6511
Ecuador	Orellana	Río Napo (Chiroisla)	00.5756	075.8998
Ecuador	Orellana	Río Napo (Edén)	00.4983	076.0711
Ecuador	Orellana	Río Napo (Añangu)	00.5249	076.3844
Ecuador	Orellana	Río Napo (La Primavera)	00.4442	076.7868
Ecuador	Orellana	Río Napo (La Primavera, El Descanso)	00.4310	076.7864
Ecuador	Orellana	El Coca	00.4778	076.9898
Ecuador	Orellana	Nuevo Rocafuerte (Tambococha)	00.9783	075.4256
Ecuador	Orellana	Nuevo Rocafuerte (Alto Florencia)	00.8966	075.4370
Ecuador	Sucumbíos	Playas del Cuyabeno	00.2654	075.8917
Ecuador	Sucumbíos	Puerto Bolívar	00.0886	076.1420
Ecuador	Sucumbíos	Río Napo (La Selva Lodge)	00.5086	076.3649
Ecuador	Sucumbíos	Río Napo (Pañacocha)	00.4712	076.6667
Ecuador	Sucumbíos	Nueva Loja	00.0917	076.8901
Ecuador	Sucumbíos	Pañacocha (Moretal Sur)	00.2758	075.9352
Ecuador	Sucumbíos	Limoncocha (La Selva Lodge)	00.4981	076.3738
Brazil	Acre	Assis Brasil [new record in this study]	10.9393	069.5712

Figure 1. Adult males of *Boana alfaroi* from the municipality of Assis Brasil, State of Acre, northern Brazil. **A, B.** Specimen AAG-UFU 5919 (SVL 34.2 mm) in dorsolateral and ventral views from top to bottom. **C, D.** Specimen AAG-UFU 5917 (SVL 35.3 mm) in dorsolateral view and a detail of the hidden surface of thighs from top to bottom.

Six specimens were collected at night in a clearing of a secondary forest. Specimens were found perched up to 1.5 m high on broad leaves and twigs. The call voucher (AAG-UFU 5917; snout–vent length: 35.3 mm) was calling within 20 cm of the water surface in a flooded area. Males were heard calling infrequently.

The advertisement call of *B. alfaroi* is composed of a pulsed note (Fig. 2), whereas that of *B. tetete* is composed of 2 distinct note types, and the main note broadcast (type I note according to Caminer and Ron 2014) is a non-pulsed, beep-like note (Caminer and Ron 2014: fig. 13E–H). Different from the call description of *B. alfaroi* by Caminer and Ron (2014), the male recorded by us emitted 2 different types of notes. The main note broadcast by this male agrees with the previous description with respect to the temporal and spectral structures and

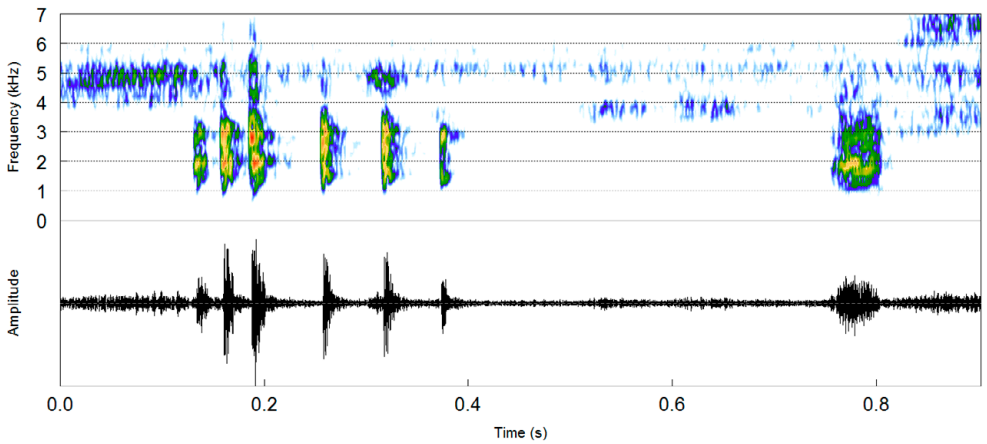

Figure 2. Vocalization of *Boana alfaroi* from Assis Brasil (Acre, Brazil): spectrogram (color palette in a relative dB scale: red = 0 dB, i.e. maximum energy) and oscillogram of a note type I followed by note type II. This cut was extracted from the recording "Boana_alfaroiAssisBrasilAC1dTRC_AAGm671".

is herein called the type I note as proposed for the most common note in the vocal repertoire of *B. tetete*. Additionally, a second type of note, herein referred as type II, was emitted 4 times shortly after the emission of type I notes; type II note was less often heard in the field. This note type is quite similar to the *B. tetete* type II note.

The type I note (n = 6) lasted 252–297 ms (mean = 278.8, SD = 22.3) and was emitted at irregular intervals (1 to several minutes in-between call emissions). The number of pulses was 6–7 (mean = 6.6, SD = 0.5); these were completely separated from each other along the note. There were 20.4–21.1 pulses per second (mean = 20.8, SD = 0.2). The dominant frequency varied from 1981–2670 Hz (mean = 2497.8, SD = 344.4). The type II note (n = 3) lasted 51–57 ms (mean = 54.0, SD = 3.0) and was always emitted shortly after the emission of the type I note. This note had weak and irregular amplitude modulations so pulse units could not be clearly quantified. The interval between type I and type II notes varied from 238–371 ms (mean = 299.0, SD = 67.2). The dominant frequency peaked at 1981 Hz (SD = 0).

Discussion

The vocal repertoire of *B. alfaroi* and *B. tetete* share the type II note. This might indicate that this type of note in both species corresponds to an acoustic signal broadcast in an aggressive context, given that aggressive signals tend to be more conservative for species in a given taxonomic group (Wells 2007). On the other hand, type I notes of these species are certainly the major trait in their differential diagnosis in possessing distinct acoustic features: *B. alfaroi* has a pulsed note with wide bandwidth (Fig. 2), whereas *B. tetete* has a non-pulsed and tonal note (Caminer and Ron 2014).

Although the calls from the newly recorded population of *B. alfaroi* share temporal and spectral structures with the previous call description for this species (Caminer and Ron 2014), they differ especially in temporal traits. The calls from Ecuador have fewer pulses (4 or 5 pulses; referred as trill-like notes by Caminer and Ron 2014) and a shorter duration (160–190 ms) in comparison with the calls described here (6 or 7 pulses; note duration: 252–297 ms). One main difference is the interval between pulses, which tends to be longer between the last pulses in our sample (Fig. 2). In general, the calls analyzed here are much longer and also have a more variable dominant frequency in comparison with the previous call description from Ecuador. A more detailed study on acoustic variation in *B. alfaroi*, based on more samples would be useful, taking into consideration variations caused by extrinsic (e.g. effects of temperature) and intrinsic factors (e.g. body size and mass).

Another potentially undescribed species allied to the *B. calcarata–B. fasciata* complex was reported by Funk et al. (2012) from Cuzco Amazónico, Peru, which is closer to our record in Brazil than to *B. alfaroi*'s type locality (Ecuador). This lineage was recovered as the sister group to the clade *B. alfaroi* + *B. tetete* by Caminer and Ron (2014). Jansen et al. (2011) suggested that a lineage of *B. fasciata* from Buena Vista, Bolivia, probably corresponds to *B. steinbachi* in a narrow sense and might be resurrected. Thus, DNA barcoding of our Brazilian Amazon specimens may determine whether they are conspecific with *B. alfaroi* or with other lineages, for which gene sequences are available, within the *B. calcarata–B. fasciata* complex from Peru and Bolivia. Until more data are available, we assign specimens from Assis Brasil to *B. alfaroi* based on morphology, coloration, and vocalizations.

This new record for *B. alfaroi* extends this species' distribution approximately 1,280 km southeast from the easternmost records in the province of Orellana, eastern Ecuador, where it is known from along the Napo (Nuevo Rocafuerte) and Yasuní rivers (Nuevo Rocafuerte, Tambococha) on the border with Peru (Fig. 3; Table 1). This record is also the first time that this species has been found outside of Ecuador. *Boana alfaroi* is possibly more widespread in the lowland forests of the Brazilian Amazon, especially in Acre and western Amazonas; it may also be in the Peruvian Amazon in northern Peru.

Acknowledgements

Special thanks go to Moisés Barbosa de Souza for all the help and advice during our visit to Acre. We are grateful to an anonymous reviewer for providing helpful suggestions on the earlier version of this paper. Financial support was provided by CNPq and FAPEMIG. A research grant was provided to AAG by CNPq. Doctoral fellowships were provided by FAPESP (TRC; process #2012/15763–7) and CAPES (BFVT; "demanda social"); a Master's fellowship by CNPq (DLB; process #159817/2015–3).

Authors' Contributions

AAG coordinated the research project. TRC, DLB and BFVT collected data in the field. TRC identified the species and wrote the first version of the manuscript. All authors read, wrote and contributed to the subsequent versions.

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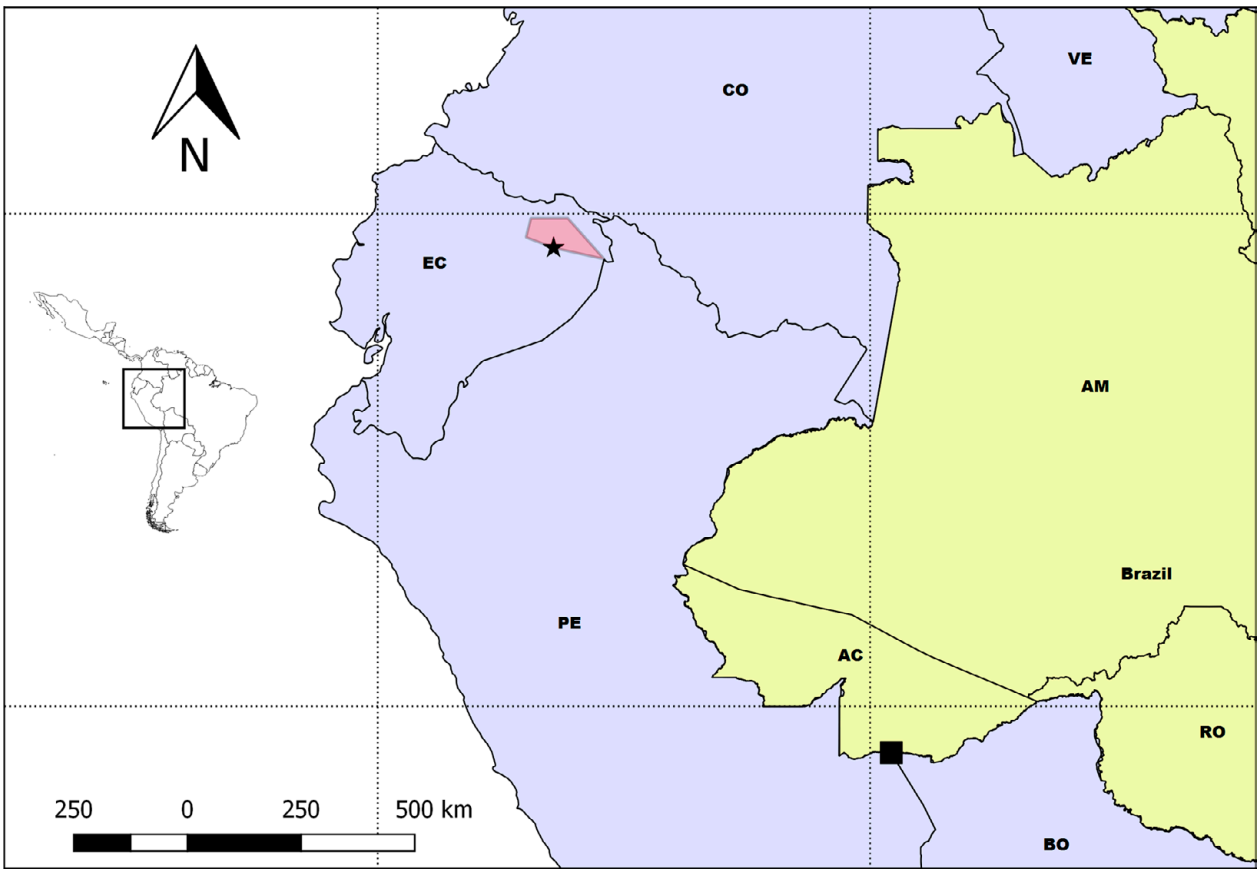

Figure 3. Distribution of *Boana alfaroi*. The star corresponds to the type locality (Parque Nacional Yasuní, Ecuador), and the polygon to the other Ecuadorian localities reported for this species in the original description (Caminer and Ron 2014; Table 1). The square is the new record in Assis Brasil (Acre, Brazil).

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