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First record of *Pseudopaludicola boliviana* Parker, 1927 (Anura, Leptodactylidae, Leiuperinae) in the Brazilian state of Amapá, with comments on its advertisement call and distribution

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Abstract: We provide the first record of *Pseudopaludicola boliviana* from the state of Amapá (municipality of Macapá), northern Brazil. It is also the species easternmost record, extending its known geographic distribution in Brazil about 1,260 km from the state of Roraima, northern Brazil. Additionally, we also provide a call description for this population of *P. boliviana* and make comparisons with the available acoustic data for the species from Bolivia and Argentina. This new record fills a gap in the distribution of *P. boliviana* in northern Brazil.

Key words: Acoustics, eastern Amazon; geographic distribution; new state record

Pseudopaludicola boliviana was described from Santa Cruz, Bolivia (Parker 1927), and assigned to the *P. pusilla* group mainly by the presence of T-shaped terminal phalanges, expanded toe tips (disks or pads), and tubercles on the upper eyelids and heels (Lynch 1989; Cardozo and Suarez 2012). This species has a cis-Andean distribution associated with open environments from northern South America (Colombia and Venezuela; Lynch 1989), across Bolivia (De la Riva et al. 2000), to the south (Paraguay and Argentina; Lobo 1991; 1994). The advertisement call of this species has already been described twice before, once at or near the type locality (Santa Cruz, Bolivia; Márquez et al. 1995) and later from Corrientes, Argentina (Duré et al. 2004). These calls diverge in both temporal and spectral call traits.

During recent anuran field surveys (2013 and 2014) in the state of Amapá (municipality of Macapá), we recorded and collected specimens of a *Pseudopaludicola* species that we identified as *P. boliviana* based on its distinctive advertisement call pattern and external morphology. Here, we document the first record of *P. boliviana* from the state of Amapá, northern Brazil, and provide new advertisement call and distributional data for this population.

Fieldwork was carried out from March 2013 to November 2014 in three areas (Figure 1): Campo Experimental de Cerrado da Empresa Brasileira de Pesquisa Agropecuária/EMBRAPA (00°23′05″N, 051°02′02.0″W, datum: WGS84; locality 32 in Figure 1); floodplains of the Curiaú River (00°09′01″N, 051°02′18″W, datum: WGS84; locality 33), Curiaú Protection Area; floodplains of the Matapi River, District of Ariri (00°17′58″N, 051°07′48″W, datum: WGS84; locality 34).

Specimens were collected under the following ICMBio/SISBIO permits: #37907-1, #41586-1, #45990-1. Voucher specimens were deposited in the Herpetological Collection of the Universidade Federal do Amapá (Amazon Savanna: CECCAMPOS 1153: SVL=12.1 mm; CECCAMPOS 1154: SVL=11.3 mm; CECCAMPOS 1157: SVL=13.8 mm. Floodplains of the Curiaú River: CECCAMPOS 1155: SVL=12.3 mm; CECCAMPOS 1242: SVL=12.7 mm. Floodplains of the Matapi River: CECCAMPOS 1237: SVL=13.7 mm; CECCAMPOS 1238: SVL=12.8 mm; CECCAMPOS 1239: SVL=14.4 mm).

Additionally, we also obtained distributional records at SpeciesLink database (SpeciesLink 2015) from three Brazilian herpetological collections (UFMT, Coleção Zoológica, Setor de Herpetologia-Répteis da Universidade

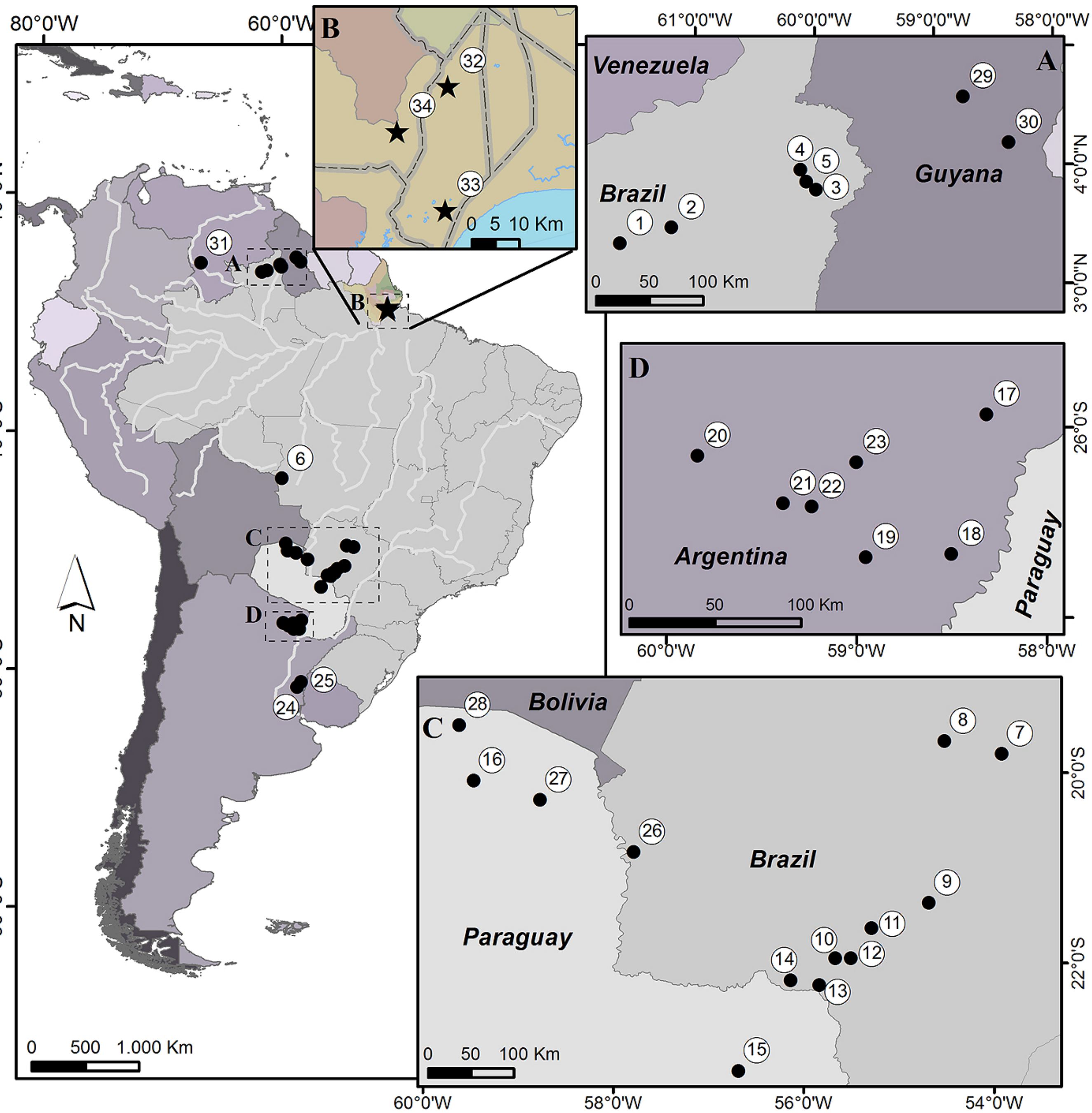

Figure 1. Geographic distribution map of *Pseudopaludicola boliviana* in South America. New distributional records are for the Brazilian state of Amapá (indicated in green on the map). Distribution records and their respective coordinates are provided in the Appendix (Table A1).

Federal de Mato Grosso; ZUEC-AMP, Coleção de Anfíbios do Museu de Zoologia da Universidade Estadual de Campinas; FNJV, Fonoteca Neotropical "Jacques Vielliard") and literature records (Parker 1935; Hoogmoed 1979; Lobo 1991; Lobo 1994; De la Riva et al. 2000; Cardozo and Lobo 2009).

Calls of one male were recorded using a Sennheiser® K6/ME67 directional microphone and a Marantz® PMD 670 digital recorder set at a sampling rate of 44.1 kHz and a sample size of 16 bits, and analyzed with Raven Pro 32-bit 1.5 beta version (Bioacoustics Research Program 2014). Temporal traits were manually measured from oscillograms, spectral traits from spectrograms (dominant frequency was obtained through the "Peak Frequency" measurement function). Raven Pro settings: window size = 256 samples; window type = Hann; 3dB filter bandwidth = 248 Hz; overlap = 85%; hop size = 0.86 ms; DFT size = 1024 samples; grid spacing = 43.1 Hz. Sound figures were generated with Seewave package version 1.7.3 (Sueur et al. 2008), R version 3.1.0 (R Core Team 2015), with the following settings: window type = Hann; window length = 128 samples (FFT); overlap = 85%. Acoustic definitions and terminology mostly follow those of Cocroft and Ryan (1995). Because there was no between-pulse interval within notes, we adopted the term "period" to refer to the time comprised from

Figure 2. *Pseudopaludicola boliviana* pair in amplexus. Notice the distinctive morphological features: conical tubercle on heels, tubercles on upper eyelids, and expanded toe tips.

beginning to end of one pulse, which coincides with the beginning of the next. Voucher male for the sound recording: CECCAMPOS 1156 (SVL 13.3 mm).

The individuals of *Pseudopaludicola boliviana* from the state of Amapá (Figure 2) exhibited variation in morphology and color, such as the presence or absence of vertebral stripe, interorbital blotch, glandular folds, and the extent of glands on dorsum, whereas the morphological features of conical tubercles on heels, tubercles evident on upper eyelids (but not elongated), and expanded toe tips (disks or pads) were present in all examined specimens, which represent diagnostic characters for this species (Lynch 1989). Calling males had white vocal sacs.

Males of *Pseudopaludicola boliviana* called during daytime and decreased call activity after nightfall in all three areas. Males mostly called at the margins of shallow temporary pools and floodplains. At these sites, males were in calling activity from 17:30 to 22:40 h during the entire dry season, and maintained their activity as long as the reproductive sites were flooded (usually from July to November). We also observed the following syntopic species in calling activity: *Rhinella major* (Muller & Helmich, 1936), *Dendropsophus* cf. *nanus* (Boulenger, 1889), *Scinax nebulosus* (Spix, 1824), *S. ruber* (Laurenti, 1768), and *Leptodactylus fuscus* (Schneider, 1799).

The advertisement call of *Pseudopaludicola boliviana* (Figure 3) consists of a stereotyped four-note structure (n = 50 calls). Notes are separated by quite short intervals (Figure 3B) or apparently no interval among each other. Relative amplitude of the notes was variable within calls. Notes are formed by incomplete pulses (< 100% between-pulse amplitude modulation). Call duration ranged from 87 to 98 ms (mean = 92.1, SD = 2.6; n = 50), separated by intervals from 129 to 655 ms (mean = 207.1, SD = 97.5; n = 49). Calls were emitted continuously at a rate of 3.3 calls per second. The first note had 4 to 6 pulses (mean = 4.7, SD = 0.7; n = 20), and its period ranged from 19 to 23 ms (mean = 21.3, SD = 0.9; n = 20); the second note had 5 to 7 pulses (mean = 5.9, SD = 0.6; n = 20), its period ranged from 18 to 26 ms (mean = 21.6, SD = 2.0; n = 20); the third note had 5 to 8 pulses (mean = 6.8, SD = 0.7; n = 20), its period ranged from 20 to 29 ms (mean =

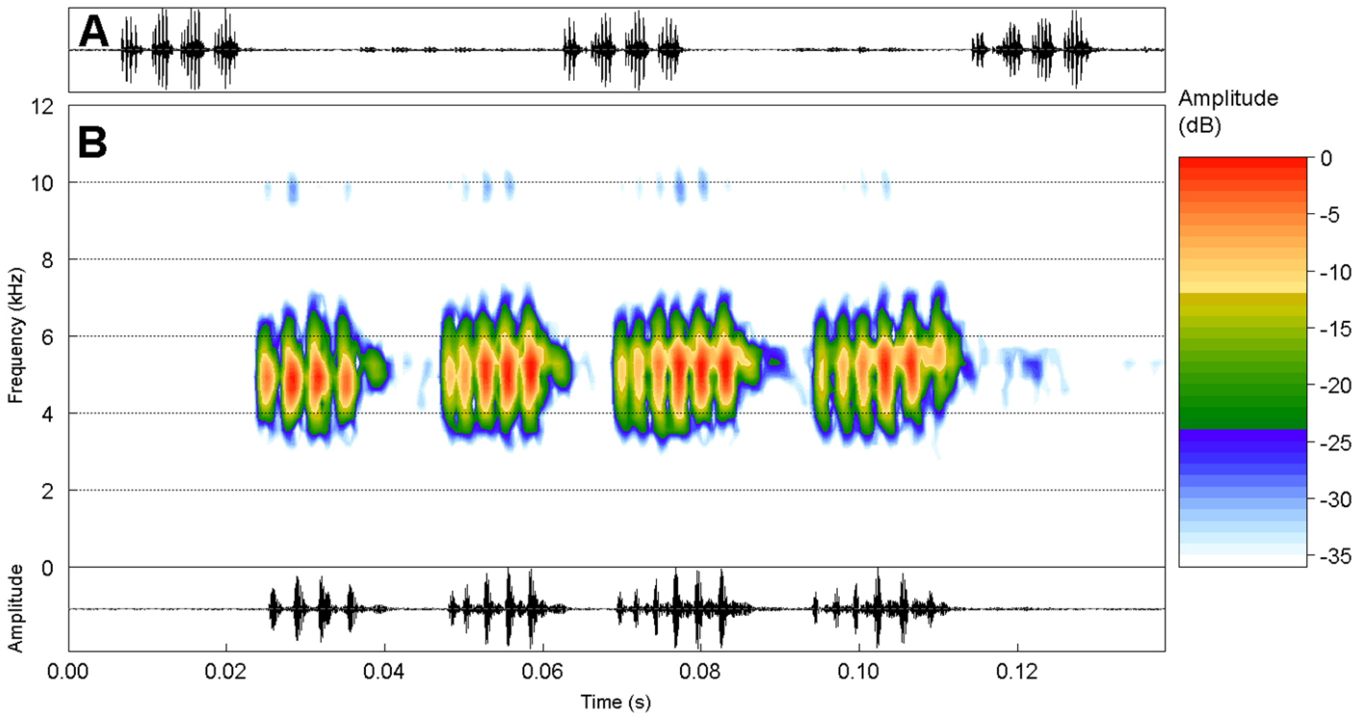

Figure 3. (**A**) Waveform section (ca. 815 ms) of three advertisement calls of *Pseudopaludicola boliviana*, and (**B**) spectrogram and respective oscillogram of the first call in A. Notice the stereotyped four-note structure, and an emphasized frequency band at approximately 10 kHz. Air temperature: 27.5°C.

24.6, SD = 2.2; n = 20); the fourth note had 5 to 8 pulses (mean = 6.8, SD = 0.8; n = 20), its period ranged from 22 to 29 ms (mean = 25.1, SD = 1.8; n = 20). Minimum frequency of calls ranged from 3514 to 3840 Hz (mean = 3674.1, SD = 98.1; n = 20), maximum frequency from 6966 to 7390 Hz (mean = 7153.3, SD = 123.1; n = 20), and dominant frequency from 4737 to 5297 (mean = 5090.5, SD = 136.5; n = 50). Notes had a slightly ascending frequency modulation from the first to the third or fourth note; emphasized frequency (peak frequency) increasing up to 200 Hz approximately. Two emphasized frequency bands at approximately 10 kHz (Figure 3) and 15 kHz (indistinctly visible in the spectrogram frame, not figured) were also observed (higher harmonics).

In comparison with the acoustic data described for a population of *Pseudopaludicola boliviana* from Bolivia (Márquez et al. 1995), our calls (state of Amapá, northern Brazil) had the same four-note call pattern, temporal traits of note duration and pulses per note had similar values as well. Dominant frequency, on the other hand, was remarkably lower (4119 to 4543 Hz; Márquez et al. 1995) compared to our values (4737 to 5297 Hz). Larger sample sizes for both populations are required prior to any assumption so that individual variation and effects of body size on spectral call traits can be assessed and tested.

In comparison with the call described from Argentina (Duré et al. 2004), temporal traits of call duration, call interval, and notes per call mostly overlapped, but duration of 1st to 4th notes were considerably shorter than our values. This might be explained due to different analytic procedures. We measured note period (from beginning of one note to beginning of the next; see Jang et al. 2011 for definition) instead of note duration (beginning to end of one note) given that intervals between notes were often barely identified or inexistent in our sample, whereas inter-note intervals in calls analyzed by Duré et al. (2004) seemed to be more discernible based on their Figure 3B. With respect to call frequency, minimum and dominant frequencies had quite similar values, but maximum frequency of their calls presented an expressive lower value range (< 6000 Hz) compared to ours (6966 to 7390 Hz). These authors did not provide their software settings for call analysis. Differences in spectral resolution might explain the diverging values observed for maximum frequency, but the other spectral traits were quite similar. It might be worth for future studies to stress that their Figures 1 and 2 have a time scale problem, being that Figures 1A and 2A portrayed the temporal and spectral structures, respectively, of a series of five calls, and Figures 1B and 2B should probably have provided the temporal and spectral structure, respectively, of one call at higher resolutions, but time scales in all figures (1A–B, 2A–B) 1A and 1B are the same. Plus, spectral structure of the portrayed call in Figure 2B is completely uninformative due to the lack of resolution.

The new distributional record of *Pseudopaludicola boliviana* is the first for the state of Amapá and represents the easternmost occurrence for this species, whose distribution range in northern South America encompasses (east of the Andes) Colombia, Venezuela, Guyana, Suriname (referred to as *P. pusilla* in Hoogmoed 1979), as well as northern Brazil in the state of Roraima (Parker 1935; Lobo 1991). The new record extends the known distribution of this species eastward by about 1,260 km in Brazil.

Additional surveys are still required to better understand the distribution pattern of *Pseudopaludicola boliviana*, whose known range has a considerable gap between Cerrado and Chaco formations (Argentina, Paraguay, and Bolivia) and the savanna and open grassfield formations in northern South America, split by a wide region covered with forested environments (Amazon Rainforest) in northern Brazil (Figure 1).

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APPENDIX

Table A1. Geographic coordinates (latitude and longitude in degrees and minutes format) from literature and Specieslink records for the occurrence of *Pseudopaludicola boliviana*. Numbers refer to those plotted on the map (Figure 1).

No.	Country	State/province	Locality	Latitude	Longitutde	Source
1	Brazil	Roraima	Ilha de Maracá	03°20′ N	061°38′ W	SpeciesLink database, Coleção de Anfíbios do Museu da UNICAMP, ZUE-AMP 6781
2		Roraima	Surumu	03°28′ N	061°12′ W	Lobo 1994
3		Roraima	Caracaraña	03°47′ N	059°59′ W	
4		Roraima	Boa Vista	03°57′ N	060°07′ W	SpeciesLink database, Fonoteca Neotropical Jacques Vielliard, FNJV 11048
5		Mato Grosso	Bonfim	03°51′ N	060°04′ W	Lobo 1994
6		Mato Grosso do Sul	Vila Bela da Santíssima Trindade	14°00′ S	059°57′ W	Lobo 1994
7		Mato Grosso do Sul	Estancia Caimán	19°48′ S	053°55′ W	Lobo 1994
8		Mato Grosso do Sul	Fazenda Nhumirim, Nhecolância	19°40′ S	054°31′ W	SpeciesLink database, Coleção Zoológica da UFMT, UFMT-A 7097
9	Paraguay		Departamento Caaguazú	21°22′ S	054°41′ W	Lobo 1994
10			Departamento Concepción	21°57′ S	055°40′ W	Lobo 1994
11			Departamento Central, Villeta	21°38′ S	055°17′ W	Lobo 1994
12			Departamento San Pedro, Villa del Rosario	21°57′ S	055°30′ W	Lobo 1994
13			Estancia La Golondrina	22°14′ S	055°50′ W	Lobo 1994
14			Departamento Amambay, Bella Vista Norte	22°11′ S	056°08′ W	Lobo 1994
15			Departamento Cordillera, Caraguatay, Estancia Saladillo	23°08′ S	056°41′ W	Lobo 1994
16			Departamento Alto Paraguay	20°05′ S	059°28′ W	Lobo 1994
17	Argentina	Chaco	Departamento Bermejo	25°56′ S	058°19′ W	Cardoso and Lobo 2009
18		Chaco	Departamento San Fernando	26°40′ S	058°30′ W	Cardoso and Lobo 2009
19		Chaco	Departamento Comandante Fernandez, Roque Saenz Peña	26°41′ S	058°57′ W	Cardoso and Lobo 2009
20		Chaco	Departamento General Güemes	26°09′ S	059°50′ W	Cardoso and Lobo 2009
21		Corrientes	Departamento Ituzaingó	26°24′ S	059°23′ W	Cardoso and Lobo 2009
22		Formosa	Departamento Laishi	26°25′ S	059°14′ W	Cardoso and Lobo 2009
23		Formosa	Departamento Pilcomayo	26°11′ S	059°00′ W	SpeciesLink database, Coleção de Anfíbios do Museu da UNICAMP, ZUE-AMP 18993
24		Formosa	Departamento De Garay, Cayastá	31°34′ S	058°40′ W	Cardoso and Lobo 2009
25		Formosa	Departamento General Obligado, Santa Fé	31°08′ S	058°21′ W	Cardoso and Lobo 2009
26	Bolivia		Departamento La Paz, Ixiamas	20°50′ S	057°47′ W	Cardoso and Lobo 2009
27			Departamento Santa Cruz de la Sierra	20°17′ S	058°46′ W	Cardoso and Lobo 2009
28			Departamento El Beni, Estancia San Miguel	19°30′ S	059°37′ W	Cardoso and Lobo 2009
29	Guyana		Rupununi Savanna	04°34′ N	058°45′ W	Cardoso and Lobo 2009
30	Venezuela		Amazonas, Santa Bárbara	04°11′ N	058°22′ W	Cardoso and Lobo 2009
31	Colombia		La Uribe, Meta	04°06′ N	066°46′ W	SpeciesLink database, Fundación Puerto Rastrojo, FPR-Colombia 6351