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Intraspecific variation in acoustic traits and body size, and new distributional records for *Pseudopaludicola giarettai* Carvalho, 2012 (Anura, Leptodactylidae, Leiuperinae): implications for its congeneric diagnosis

Thiago Ribeiro de Carvalho[1,2,4]
Lucas Borges Martins[1,2]
Bernardo Franco da Veiga Teixeira[1,2]
Leandro Braga Godinho[3]
Ariovaldo Antonio Giaretta[1]

ABSTRACT

In this paper, we provide an updated diagnosis for Pseudopaludicola giarettai *based on the morphometric and acoustic variation observed with the assessment of new populations, plus an expansion of its distribution range. Our results support that all acoustic variation observed might be attributed to intraspecific variation. The variation in body size and dorsal stripe patterns observed for* Pseudopaludicola giarettai *reinforces that the distinctive whistling advertisement call pattern is the most reliable evidence line to diagnose it from its congeners, whereas morphological (robust body, glandular dorsum) and morphometric (body size) features vary considerably within and among populations so that they should no longer be employed as diagnostic features of* Pseudopaludicola giarettai.

Key-Words: Bioacoustics; Minas Gerais; Taxonomy.

INTRODUCTION

The genus *Pseudopaludicola* currently comprises 18 species distributed throughout South America (Frost, 2014). This genus was traditionally defined by external morphology and osteological features (Lynch 1971, 1989). The monophyly of the genus was later assessed through phylogenetic analyses based on external morphology and osteology (Lobo, 1995), and, more recently, molecular data (Veiga-Menoncello *et al.,* 2014). In both Lobo's (1995) and Veiga-Menoncello *et al.*'s (2014) hypotheses, a few species were

1. Universidade Federal de Uberlândia – UFU, Laboratório de Taxonomia, Sistemática e Ecologia Comportamental de Anuros Neotropicais, Faculdade de Ciências Integradas do Pontal. Rua Vinte, 1.600, Bairro Tupã, CEP 38304-402, Ituiutaba, MG, Brasil.
2. Universidade de São Paulo – USP, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Departamento de Biologia, Programa de Pós-Graduação em Biologia Comparada. Avenida dos Bandeirantes, 3.900, Vila Monte Alegre, CEP 14040-901, Ribeirão Preto, SP, Brasil.
3. Universidade Federal de Viçosa – UFV, Departamento de Biologia Animal, Museu de Zoologia João Moojen. Avenida Peter Henry Rolfs, s/nº, Campus Universitário, CEP 36570-000, Viçosa, MG, Brasil.
4. E-mail to corresponding author: thiago_decarvalho@yahoo.com.br
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not tested for their phylogenetic position, including *P. giarettai* Carvalho, 2012, which possesses a unique, whistling advertisement call pattern in the genus, associated with a large size, robust body, warty dorsum, yellow vocal sac (Carvalho, 2012), a suite of features that is apparently more related to the "2n = 20" clade from Veiga-Menoncello et al. (2014), formed by *P. ameghini* Pansonato, Strüssman, Mudrek & Martins, 2013 and *P. ternetzi* Miranda-Ribeiro, 1937.

During recent field surveys (2013/2014) in the Cerrado of southeastern Brazil, we recorded and collected specimens of *Pseudopaludicola* assigned in the field to *P. giarettai* through its distinctive advertisement call pattern. We herein provide an updated diagnosis for *P. giarettai* based on the morphometric and acoustic variation observed with the assessment of new populations, plus an expansion of its distribution range.

MATERIAL AND METHODS

Field work was conducted in six municipalities of Minas Gerais State, southeastern Brazil: Buritis (15°21'29"S, 46°29'45"W; approx. 905 m a.s.l.); Buritizeiro (16°52'32"S, 45°05'02"W; approx. 700 m a.s.l.); Chapada Gaúcha, at the Parque Nacional Grande Sertão Veredas (15°10'51"S, 45°46'10"W; approx. 710 m a.s.l.); Coromandel (18°07'35"S, 47°11'60"W; approx. 730 m a.s.l.); Curvelo (18°46'09"S, 44°26'54"W; approx. 620 m a.s.l.); Unaí (16°43'33"S, 46°29'30"W; approx. 525 m a.s.l.). Examined specimens are listed in Appendix 1.

Calls were recorded using digital equipment (M-Audio Microtrack II and Marantz PMD 671) set at a sampling rate of 48.0 kHz and a resolution of 16 bits (mono WAVE file format), coupled to directional microphones (Sennheiser K6/ME66 and K6/ME67, respectively). At two study sites (Buritizeiro and Unaí), calls were recorded using an Olympus DM-420 Digital Voice Recorder coupled to an Audio-Technica Pro stereo condenser microphone set at a sampling rate of 32 kHz and a resolution of 24 bits (stereo WMA file format). Sound files were converted to mono WAVE file format prior to perform any sound analysis. Calls from two municipalities were provided by Diego J. Santana: i) Muriaé (Minas Gerais State; see Santana *et al.,* 2009), and ii) Flores de Goiás (Goiás State); recordings perfectly fitted *P. giarettai* distinctive calling pattern, so both populations were assigned by us to *P. giarettai,* but we excluded these data from acoustic comparisons since just a few calls of one male from each site were recorded.

Calls were analyzed with the software Raven Pro version 1.5, 32-bit version (Bioacoustics Research Program, 2012). Temporal and spectral traits were measured from spectrograms; temporal traits were manually measured, and spectral traits (dominant and other frequencies) were obtained with the Peak Frequency measurement. Raven Pro settings: window type = Hann; window size = 1024 samples; 3 dB filter bandwidth = 67.4 Hz; brightness = 50%; contrast = 50%; overlap (locked) = 85%; hop size (temporal resolution = 3.21 ms); DFT size (locked) = 1024 samples (spectral resolution = 46.9 Hz). Calls of *P. giarettai* from its original description (Carvalho, 2012) were reanalyzed under the analytical standards and software settings presented earlier. Acoustic terminology is defined in Table 1. Mean and standard deviation (SD) given along the text and tables were calculated from individual mean values.

The overall thermal dependency of temporal variables of the advertisement call of *P. giarettai* was performed in two steps using Vegan package (Oksanen *et al.,* 2013) version 2.0-10, R platform (R Core Team, 2014): a Principal Component Analysis (PCA) was performed on a correlation matrix from untransformed individual mean values. Then, a correlation analysis between recording air temperature and the first component scores (PC1) was performed to identify potential temperature-dependent variables. This statistical approach was not likewise applied to calling male size (SVL) dependency of spectral traits of its call due to the lack of either a minimum sample size or a non-correspondence of voucher males and recordings. See Appendices 2-3 for the first three principal component scores, eigenvalues, eigenvectors, proportional explained variation, and mean individual values. Further, the potential association between linear geographic distance and variation in acoustic traits was assessed through a Mantel test applied on Euclidean distance matrices with 10,000 Monte Carlo permutations, using ade4 package (Dray & Dufour, 2007) version 1.6-2, R platform.

Specimens are housed in the Collection of Amphibians of the Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia (AAG-UFU), Municipality of Uberlândia, and at the Museu de Zoologia João Moojen, Universidade Federal de Viçosa (MZUFV), Municipality of Viçosa, both in Minas Gerais, Brazil. Call voucher males were registered under the following accession numbers: AAG-UFU 1772 (Buritis), AAG-UFU 1920-1924 (Chapada Gaúcha), AAG-UFU 3565 (Coromandel), AAG-UFU 0309-0313 (Curvelo), MZUFV 14207, 14210 (Buritizeiro), MZUFV 14878, 14882 (Unaí).

TABLE 1: Acoustic terminology for *Pseudopaludicola giarettai* advertisement call.

CALL TRAITS	DEFINITION
Call duration	Time from beginning to end of one call; Call = note
Call interval	Time from end of one call to beginning of the next call
Call rise time	Time from beginning of call to point of maximum amplitude
Dominant frequency	Frequency with greatest energy, determined from entire call
Fundamental frequency	Frequency with the greatest amount of sound energy in the first harmonic
Frequency modulation	Frequency at end of call minus that of beginning of call
Call rate	(Total number of calls – 1) / time from beginning of first call to beginning of last call

RESULTS AND DISCUSSION

Distribution

Pseudopaludicola giarettai was previously reported only for its type locality (Curvelo) and from Buritizeiro, *ca.* 160 km northward to the type locality (Carvalho, 2012). We extend its distribution range to localities in northwestern Minas Gerais (Buritis, Chapada Gaúcha, and Unaí), *ca.* 300 km westward (Coromandel), and *ca.* 340 km southeastward (Muriaé) to its type locality. Besides, Flores de Goiás, located in northern Goiás (central Brazil), represents the first record outside Minas Gerais, *ca.* 550 km northwestward to *P. giarettai* type locality (Fig. 1).

Color patterns and body size

Although the holotype of *P. giarettai* neither possesses vertebral pin-stripe nor dorsolateral stripes, some paratypes, on the other hand, possess at least one of the two characters (Carvalho, 2012). In other populations, the presence of these characters was more regularly observed (Table 2; Fig. 2). Regarding body size, *P. giarettai* was considered one

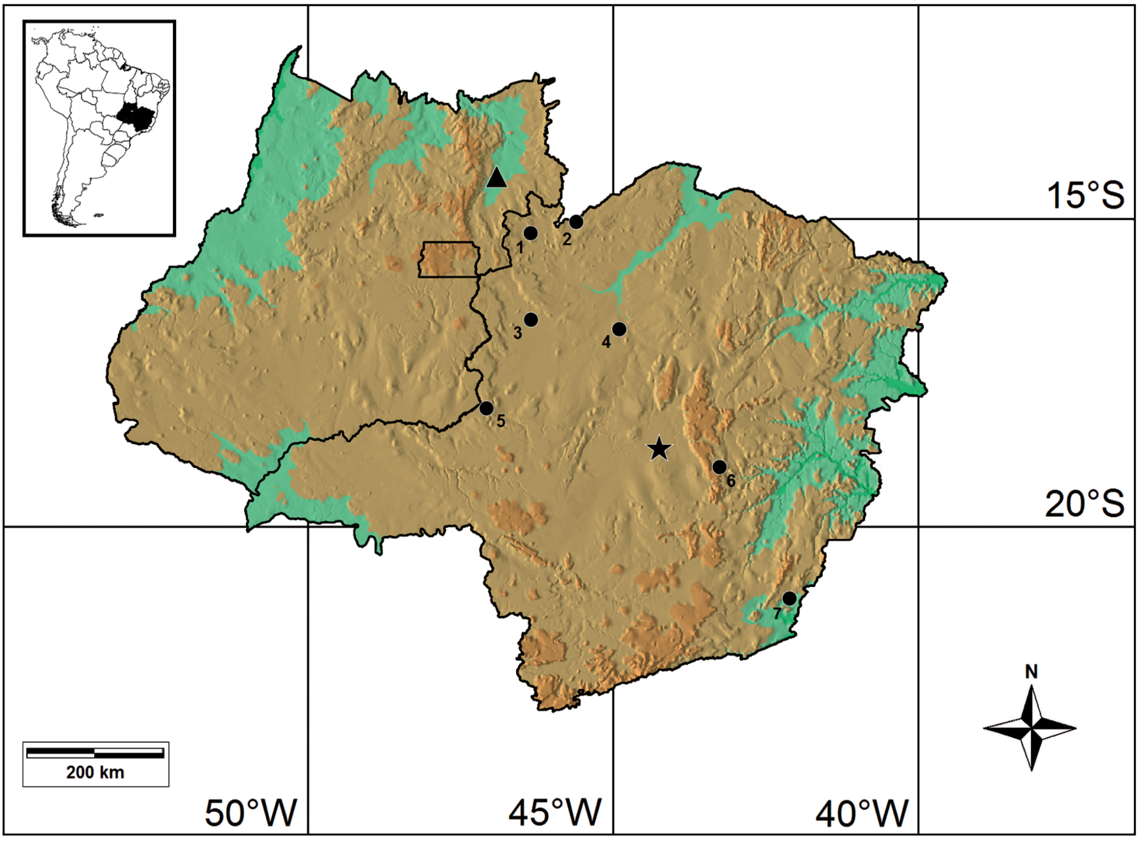

FIGURE 1: Distribution map of *Pseudopaludicola giarettai.* Star (Type locality: Curvelo, MG); Circles (localities in Minas Gerais): 1 (Buritis), 2 (Chapada Gaúcha), 3 (Unaí), 4 (Buritizeiro), 5 (Coromandel), 6 (Conceição do Mato Dentro; Pimenta *et al.,* 2014), 7 (Muriaé; Santana *et al.,* 2009); Triangle (Flores de Goiás, GO). MG = Minas Gerais; GO = Goiás.

TABLE 2: Presence of vertebral (pin-stripe) and/or dorsolateral stripes in adult specimens of *Pseudopaludicola giarettai* from six localities of Minas Gerais, southeastern Brazil. N = number of examined specimens; * Type specimens.

LOCALITY	PIN-STRIPE	DORSOLATERAL STRIPES	BOTH STRIPES	NO STRIPES
Curvelo (N = 8)*	0	1	0	7
Buritis (N = 7)	0	4	0	3
Buritizeiro (N = 9)	1	3	1	4
Chapada Gaúcha (N = 9)	1	4	1	3
Coromandel (N = 4)	0	0	0	4
Unaí (N = 6)	2	1	2	1

TABLE 3: Summary of snout-vent length (SVL) ranges (mm) for adult specimens of *Pseudopaludicola giarettai* from six localities of Minas Gerais, southeastern Brazil. Sample sizes in parentheses; * Type locality.

LOCALITY	*PSEUDOPALUDICOLA GIARETTAI*	
	MALES	FEMALES
Curvelo*	16.2-18.0 (10)	20.5 (1)
Buritis	13.8-16.3 (6)	15.6 (1)
Buritizeiro	13.4-15.9 (7)	16.3-16.4 (2)
Chapada Gaúcha	13.5-14.7 (7)	14.6-14.8 (2)
Coromandel	16.7 (1)	18.4-18.8 (3)
Unaí	14.7-16.2 (6)	—

of the largest *Pseudopaludicola* species according to its original description (Carvalho, 2012): adult male SVL 16.2-18.0 mm. However, adult males from Buritis (SVL 13.8-16.3 mm; N = 6), Buritizeiro (SVL 13.4-15.9 mm; N = 7), Chapada Gaúcha (SVL 13.5-14.7 mm; N = 7), and Unaí (SVL 14.7-16.2 mm; N = 6) are remarkably smaller than *P. giarettai* type series (Table 3), whereas the only adult male collected in Coromandel has a relatively larger size (SVL 16.7 mm), which fits male size range of its type series (Table 3).

Advertisement call redescription (type locality: Curvelo, Minas Gerais)

Eight males were recorded (N = 400 calls). Advertisement call (Table 4; Figs. 3-4) consists of a whistle, i.e. non-pulsed signal, with a remarkable ascendant frequency modulation throughout its duration. Calls may have a slight upward amplitude modulation in their half or last third duration (see oscillogram in Fig. 4) or upward and downward amplitude modulations in their first third or half duration (see oscillogram in Fig. 3). Fundamental frequency (1st harmonic) always corresponds to the carrier frequency, and up to four higher harmonically related frequencies can be observed. Call duration varies from 101-212 ms (mean 174.4; SD = 11.5), whose rise time varies from 61-143 ms (mean 105.3; SD = 25.4), which corresponds approximately to 60% of average call duration. Call interval varies from 158-781 ms (mean 264.8; SD = 76.8). Call rate varies from 1.77-2.61 per second (mean 2.24; SD = 0.30). Dominant frequency (= fundamental frequency) varies from 3984-4594 Hz (mean 4294.3; SD = 142.6), with frequency modulation varying from 1214-1582 Hz (mean 1388.8; SD = 81.3); second, third, and fourth harmonic frequencies peak from 7172-9094 Hz, 10969-13922 Hz, and 15094-18281 Hz, respectively. Fifth harmonic was not measured once it can be in-

FIGURE 2: Adult specimens of *P. giarettai* in life from: Above – Parque Nacional Grande Sertão Veredas, Chapada Gaúcha, Minas Gerais (voucher male AAG-UFU 1920: CRC = 14.6 mm); Below – Coromandel (female AAG-UFU 3566: CRC 18.8 mm).

TABLE 4: Acoustic traits for *Pseudopaludicola giarettai* from Curvelo (type locality), and five additional municipalities of Minas Gerais, southeastern Brazil. Mean ± SD (minimum-maximum). N = number of recorded males (number of analyzed calls). H = harmonic peak frequency. * Dominant frequency (= Fundamental frequency); ** Obtained from calls of one male.

ACOUSTIC TRAITS	CURVELO N = 8 (400)	BURITIS N = 3 (148)	BURITIZEIRO N = 2 (35)	CHAPADA GAÚCHA N = 9 (391)	COROMANDEL N = 2 (30)	UNAÍ N = 2 (52)
Call duration (ms)	174.4 ± 11.5 (101-212)	213.9 ± 18.9 (188-260)	171.4 ± 1.0 (130-203)	199.9 ± 21.4 (118-255)	197.0 ± 6.9 (168-227)	161.0 ± 17.4 (136-186)
Call interval (ms)	264.8 ± 76.8 (158-781)	392.2 ± 75.4 (258-576)	246.1 ± 30.4 (182-319)	308.7 ± 37.5 (163-612)	377.3 ± 1.9 (227-567)	313.6 ± 69.9 (225-416)
Call rise time (ms)	105.3 ± 25.4 (61-143)	152.8 ± 9.8 (133-178)	75.8 ± 33.6 (44-107)	113.1 ± 20.3 (74-160)	116.9 ± 20.6 (95-138)	109.5 ± 27.1 (82-134)
Call rate/s	2.24 ± 0.30 (1.77-2.61)	1.71 ± 0.29 (1.38-1.92)	2.41 ± 0.05 (2.37-2.44)	1.97 ± 0.14 (1.66-2.23)	1.73 ± 0.00	2.09 ± 0.35 (1.84-2.33)
1st H (Hz)*	4294.3 ± 142.6 (3984-4594)	4344.6 ± 130.3 (4078-4500)	4134.9 ± 25.0 (3984-4594)	4673.8 ± 241.5 (4172-5016)	4322.8 ± 42.2 (4226-4406)	4522.3 ± 344.1 (4125-4781)
Frequency modulation (Hz)	1388.8 ± 81.3 (1241-1582)	1189.4 ± 107.8 (1025-1430)	1145.7 ± 328.1 (882-1430)	1477.5 ± 155.6 (1144-1854)	1453.9 ± 224.7 (1239-1669)	906.5 ± 112.4 (742-1011)
2nd H (Hz)	8590.4 ± 230.5 (7172-9094)	8671.6 ± 312.0 (8063-8953)	8552.3 ± 36.5 (7688-8813)	9512.0 ± 409.1 (8906-10266)	8681.4 ± 272.1 (7875-9234)	8657.8 ± 22.7 (8625-8672)**
3rd H (Hz)	12638.1 ± 878.8 (10969-13922)	12089.1 ± 867.3 (11672-13734)**	12384.4 ± 295.3 (12047-12703)**	13901.4 ± 1125.1 (11484-15375)	12585.9 ± 563.5 (12188-13078)	12557.8 ± 14.8 (12516-12563)**
4th H (Hz)	17272.8 ± 777.3 (15094-18281)	17601.6 ± 178.5 (17156-17813)**	—	18702.4 ± 1264.5 (15328-20484)	16609.4 ± 957.3 (15797-17906)**	16387.5 ± 131.1 (16078-16453)**
Harmonics	Up to 5	Up to 4	Up to 3	Up to 5	Up to 4	Up to 5
Air (°C)	24.0-35.0	23.5-24.8	26.0	34.0-36.0	24.7	28.0

complete in spectrogram screen [maximum frequency measurement allowed by a 48-kHz sampling rate (Nyquist of sampling rate) = 24 kHz].

Acoustic comparisons

Despite the advertisement call pattern shared among all populations, i.e. non-pulsed signal with notable ascendant frequency modulation throughout its duration, dominant frequency coincident with fundamental frequency, and higher harmonically related frequencies typically observed, quantitative (temporal and spectral) traits of *P. giarettai* call varied within and among populations (Table 4; Fig. 5). The first three PC axes explained approximately 87% of the overall variation. Type specimens and from Buritizeiro were partially separated from the other populations along PC1 (*ca.* 55% explained variation) mainly by temporal traits of call (higher call rate, and shorter call duration and interval), as well as faster call rise time, whereas type specimens and from Chapada Gaúcha were completely separated along PC2 (*ca.* 21% explained variation) from specimens from Buritizeiro and Unaí, mainly by a wider frequency modulation.

Considering acoustic variation represented in Fig. 5, it is possible to assume that the between-population variation was not great enough to discriminate any as a potential independent group with respect to acoustic information, thus we attributed it to intraspecific variation only. Populations from Coromandel, Buritis, and Unaí showed within-population acoustic variation that should possibly be explained by an insufficient sample size of recorded males (N = 2 males). The Mantel test showed no significant association between geographic distance and acoustic variation (Mantel r = -0.04; p = 0.77).

Effect of temperature on temporal traits of call

Air temperature could not explain the overall variation observed in all three temporal traits analyzed of *P. giarettai* call ($F_{1,24} = 3.61$; df = 24; p = 0.07).

Revised diagnosis

Considering the newly assessed intraspecific variation in dorsal stripe patterns, as well as morphometric and acoustic data of *P. giarettai,* an updated diagnosis is provided as follows: *Pseudopaludicola giarettai* is assigned to the genus *Pseudopaludicola* by the presence of distal hypertrophied antebrachial tubercles. The distinctive whistling advertisement call pattern of *P. giarettai* diagnoses it from all congeneric

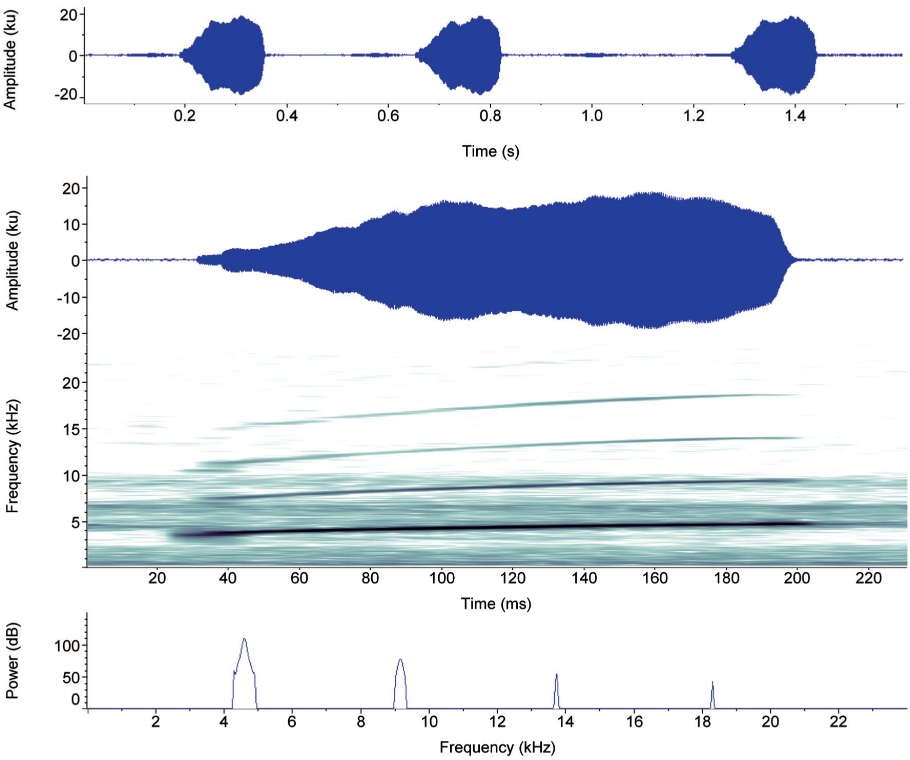

FIGURE 3: Advertisement call of *P. giarettai* from the type locality (Curvelo, Minas Gerais). From top to bottom: oscillogram section (*ca.* 1.6 s) depicting calling pattern, waveform, spectrogram, and power spectrum of the second advertisement from oscillogram section. Sound energy with relative amplitude below 40 dB was clipped to zero dB to remove background noise from power spectrum.

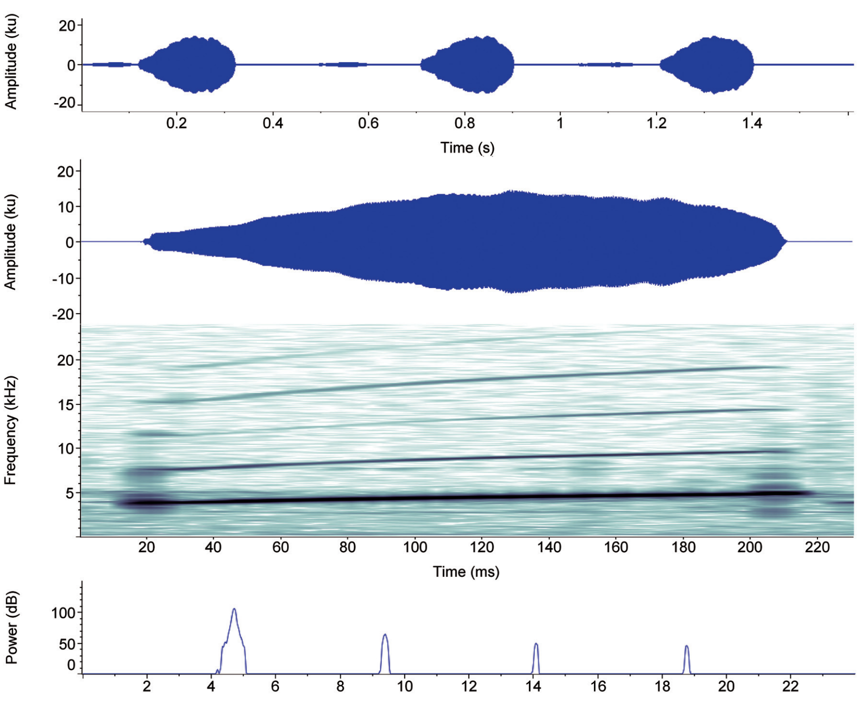

FIGURE 4: Advertisement call of *P. giarettai* from the Parque Nacional Grande Sertão Veredas (Chapada Gaúcha, Minas Gerais). From top to bottom: oscillogram section (*ca.* 1.6 s) depicting calling pattern, waveform, spectrogram, and power spectrum of the second advertisement from oscillogram section. Sound energy with relative amplitude below 40 dB was clipped to zero dB to remove background noise from power spectrum.

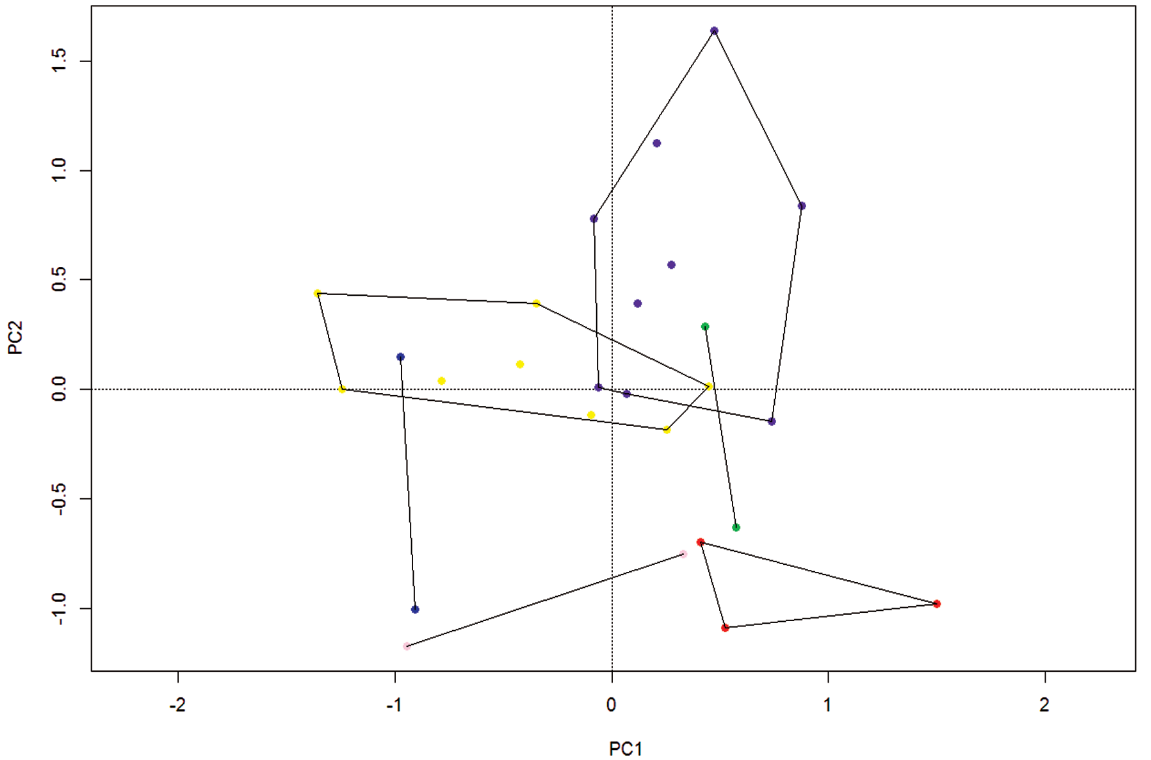

FIGURE 5: Scatterplot of the first two principal component scores (PCs) from acoustic traits of six populations of *P. giarettai.* Yellow (Curvelo; type locality); purple (Chapada Gaúcha); green (Coromandel); red (Buritis); blue (Buritizeiro); pink (Unaí).

species with described calls (unknown calls: *P. ceratophyes, P. llanera,* and *P. pusilla*). This non-pulsed call structure distinguishes *P. giarettai* from the pulsed calls of *P. ameghini* (Pansonato *et al.*, 2013), *P. atragula* (Pansonato *et al.*, 2014a), *P. boliviana* (Duré *et al.*, 2004), *P. falcipes* (Haddad & Cardoso, 1987), *P. mineira* (Pereira & Nascimento, 2004), *P. murundu* (Toledo *et al.*, 2010), *P. mystacalis* (Pansonato *et al.*, 2013), *P. saltica* (Pansonato *et al.*, 2013), and *P. ternetzi* (Cardozo & Toledo, 2013). Regarding *Pseudopaludicola* species with non-pulsed structure (*P. canga, P. facureae, P. hyleaustralis,* and *P. parnaiba*), *P. giarettai* can easily be distinguished from all four species by its distinctive long-lasting (combined value range: 101-260 ms; Table 3) whistling call with a remarkable ascendant frequency modulation, and emitted in an intermittent calling pattern, whereas calls of all four species are emitted in a trilled calling pattern (well-defined series of non-pulsed notes) with short-lasting notes (maximum note duration: 50 ms; see Table 2 in Pansonato *et al.*, 2012), having no or slight frequency modulation (Giaretta & Kokubum, 2003; Pansonato *et al.*, 2012; Andrade & Carvalho, 2013; Roberto *et al.*, 2013).

FIGURE 6: Typical breeding habitat of *P. giarettai:* a permanent pond associated with a Buriti palm grove marsh at the Parque Nacional Grande Sertão Veredas (Municipality of Chapada Gaúcha), northwestern Minas Gerais, southeastern Brazil.

Notes on natural history

Males tend to call during daytime and decrease or cease call activity after nightfall. Males call on the margins of artificial ponds or slow-flowing streamlets with clean water and muddy/sandy bottom, either on water surface among grassy vegetation or at their border on the ground, almost always in association with Buriti (*Mauritia flexuosa*) palm grove marshes (Fig. 6).

DISCUSSION

A better sample of calls from each population should be assessed prior to any assumption on the association of temperature and temporal traits of *P. giarettai* call, as well as to test the effect of water temperature on them, given that this species may call on water surface in association with vegetation. Although the exploratory test (PCA) recovered acoustic variation both within and among populations of *P. giarettai,* it was insufficient to raise doubts on the assignment of all populations enclosed in a single taxonomic unit. Thus, our results support that all acoustic variation observed might be attributed to intraspecific variation.

The remarkable morphological and morphometric intraspecific variation observed for *P. giarettai* reinforces that the distinctive advertisement call pattern is the most straightforward evidence line, perhaps the unique unambiguous character, to diagnose it from its congeners, whereas morphological (robust body, glandular dorsum) and morphometric (body size) features show such variation, especially for non-topotypical populations, so that they should no longer be employed as reliable diagnostic features for *P. giarettai.* Still, the color feature of yellow vocal sac was observed in all specimens of *P. giarettai,* which might be helpful to distinguish this species besides its distinctive whistling advertisement call.

Morphological/morphometric features might have little contribution to assess specific identity of *Pseudopaludicola,* whereas acoustic information has revealed cryptic diversity and has contributed to the reassessment of taxonomic status within this Neotropical frog group. In the last four years, six species were described (in chronological order: *P. giarettai, P. hyleaustralis, P. facureae, P. parnaiba, P. pocoto,* and *P. atragula;* Carvalho, 2012; Pansonato *et al.,* 2012, 2014a; Andrade & Carvalho, 2013; Roberto *et al.,* 2013; Magalhães *et al.,* 2014), one revalidated (*P. ameghini;* Pansonato *et al.,* 2013), and two invalidated (*P. riopiedadensis* and *P. serrana;* Cardozo & Toledo, 2013; Pansonato *et al.,* 2014b) taking into account this line of evidence. Therefore, it is essential to obtain, whenever it is possible, the association of advertisement call pattern to *Pseudopaludicola* specimens to unambiguous assessment of their species identity.

RESUMO

Nesse trabalho, nós apresentamos uma diagnose atualizada para Pseudopaludicola giarettai *a partir das variações morfométrica e acústica encontradas com a amostragem de novas populações, além da expansão de sua distribuição geográfica. Nossos resultados suportam que toda a variação acústica observada pode ser atribuída a variação intraespecífica. A variação em tamanho e padrão de faixas dorsais observada em* Pseudopaludicola giarettai *reforça que o padrão assobiado distintivo de canto de anúncio representa a linha de evidência mais confiável para diagnosticar a espécie de seus congêneres, enquanto as características morfológicas (corpo robusto, dorso glandular) e morfométricas (tamanho do corpo) variam consideravelmente dentro e entre populações de modo que não devem mais ser utilizadas como características diagnósticas para* Pseudopaludicola giarettai.

PALAVRAS-CHAVE: Bioacústica; Minas Gerais; Taxonomia.

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APPENDIX 1

List of examined specimens

Pseudopaludicola giarettai – BRAZIL: Minas Gerais: Curvelo (holotype: AAG-UFU 0312; paratypes: AAG-UFU 0309-0311, 0313-0316); Buritis (AAG-UFU 1772-1778); Buritizeiro (AAG-UFU 4580, MZUFV 14203-14210); Chapada Gaúcha (AAG-UFU 1920-1928); Coromandel (AAG-UFU 3565-3568); Unaí (MZUFV 14878-14883).

APPENDIX 2

Mean individual values, scores, eigenvalues, and proportional explained variation (%) of the first three principal components. CUR (Curvelo), BUR (Buritis), BUZ (Buritizeiro), CHG (Chapada Gaúcha), COR (Coromandel), UNA (Unaí). T = recording air temperature (°C), CD = call duration (s), CI = call interval (s), CRT = call rise time (s), CRS = call rate/s, DF = dominant frequency (Hz), FM = frequency modulation (Hz).

Individuals	T	CD	CI	CRT	CRS	DF	FM	PC1	PC2	PC3
CUR 1	24.0	0.179	0.406	119.0	1.8	4436.2	1516.4	0.446	0.015	-0.088
CUR 2	33.0	0.180	0.229	114.7	2.2	4365.0	1468.2	-0.347	0.392	-0.175
CUR 3	33.0	0.177	0.206	120.8	2.2	4376.2	1331.6	-0.426	0.113	0.144
CUR 4	35.0	0.152	0.194	64.8	2.6	4109.1	1453.4	-1.358	0.440	-0.539
CUR 5	35.0	0.167	0.216	65.0	2.6	4080.9	1284.3	-1.243	-0.001	-0.625
CUR 6	35.0	0.170	0.219	109.5	2.5	4212.2	1344.9	-0.786	0.040	-0.199
CUR 7	24.0	0.180	0.322	122.4	2.2	4330.3	1380.1	-0.097	-0.118	-0.104
CUR 8	24.0	0.190	0.327	126.2	1.9	4444.5	1331.6	0.255	-0.185	0.072
BUR 1	24.8	0.206	0.364	155.9	1.9	4234.7	1136.9	0.525	-1.089	-0.255
BUR 2	23.5	0.200	0.335	141.9	1.9	4488.5	1117.9	0.411	-0.696	0.403
BUR 3	23.5	0.235	0.478	160.7	1.4	4310.6	1313.3	1.499	-0.978	-0.855
BUZ 1	26.0	0.222	0.243	118.5	2.0	4896.3	1741.6	-0.909	-1.003	0.207
BUZ 2	26.0	0.225	0.334	144.5	1.9	4751.7	1633.0	-0.975	0.149	-0.857
CHG 1	36.0	0.185	0.304	116.7	2.1	4845.0	1627.9	0.473	1.639	-0.037
CHG 2	36.0	0.168	0.308	86.4	2.0	4895.6	1415.7	0.877	0.839	-0.146
CHG 3	36.0	0.219	0.309	80.6	1.9	4250.6	1358.6	0.209	1.124	0.634
CHG 4	36.0	0.175	0.340	119.1	2.0	4918.1	1411.0	-0.081	0.781	1.178
CHG 5	34.0	0.209	0.372	133.7	1.7	4467.5	1408.7	0.070	-0.017	-1.196
CHG 6	34.0	0.207	0.282	105.8	2.0	4477.2	1444.4	0.275	0.568	1.300
CHG 7	34.0	0.188	0.285	113.0	2.1	4562.5	1256.1	0.736	-0.145	-0.291
CHG 8	34.0	0.171	0.225	99.5	2.4	4152.6	913.7	0.118	0.392	-0.417
CHG 9	34.0	0.172	0.268	52.0	2.4	4117.2	1377.7	-0.064	0.008	0.437
COR 1	24.7	0.192	0.379	102.3	1.7	4352.7	1612.8	0.432	0.285	-0.749
COR 2	24.7	0.202	0.376	131.5	1.7	4293.0	1295.0	0.573	-0.629	-0.430
UNA 1	28.0	0.149	0.264	90.3	2.3	4279.0	827.0	-0.944	-1.174	0.952
UNA 2	28.0	0.173	0.363	128.7	1.8	4765.6	986.0	0.331	-0.751	1.637
Eigenvalues	—	—	—	—	—	—	—	3.268	1.229	0.749
% variation	—	—	—	—	—	—	—	54.5	20.5	12.5

APPENDIX 3

Eigenvectors from *P. giarettai* acoustic traits in the first three principal components.

Acoustic traits	Eigenvectors		
	PC1	PC2	PC3
Call duration	1.161	0.124	-0.596
Call interval	1.212	-0.432	-0.023
Call rise time	1.163	-0.347	0.242
Call rate/s	-1.352	0.213	0.017
Dominant frequency	0.706	0.786	0.939
Frequency modulation	0.418	1.234	-0.482