



A new species of *Pseudopaludicola* Miranda-Ribeiro (Anura: Leptodactylidae: Leiuperinae) from eastern Brazil, with novel data on the advertisement call of *Pseudopaludicola falcipes* (Hensel)

FELIPE SILVA DE ANDRADE^{1,2,3,7}, ISABELLE AQUEMI HAGA², MARIANA LÚCIO LYRA⁴,
FELIPE SÁ FORTES LEITE⁵, AXEL KWET⁶, CÉLIO FERNANDO BAPTISTA HADDAD⁴,
LUÍS FELIPE TOLEDO¹ & ARIIVALDO ANTONIO GIARETTA²

¹Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brasil

²Laboratório de Taxonomia e Sistemática de Anuros Neotropicais (LTSAN), Faculdade de Ciências Integradas do Pontal, Universidade Federal de Uberlândia (UFU), Ituiutaba, Minas Gerais, Brasil

³Programa de Pós-Graduação em Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brasil

⁴Universidade Estadual Paulista (UNESP), Departamento de Zoologia e Centro de Aquicultura (CAUNESP), Instituto de Biociências, Rio Claro, São Paulo, Brasil

⁵Sagarana Lab, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Viçosa, Campus Florestal, Florestal, Minas Gerais, Brasil

⁶Haldenstr. 28, 70736 Fellbach, Germany

⁷Corresponding author. E-mail: felipe_andrade@ymail.com

Abstract

The Neotropical genus *Pseudopaludicola* includes 21 species, which occur throughout South America. Recent studies suggested that the population of Andaraí, in the state of Bahia, is an undescribed species, related to *P. pocoto*. Herein we formally describe this new species from lowlands of eastern Brazil. Recognition of this new species is supported by adult morphology, advertisement call, karyotype, and molecular data. It is diagnosed mainly by its small size, terminal phalanges knobbed (lack any expansion of the digital tips), proportionally short hindlimbs, 11 pairs of chromosomes, and advertisement call composed of series of three-pulsed notes, emitted at a high rate. In addition, we report for the first time the presence of *P. pocoto* in the *campo rupestre* (rupestrian grasslands) of Chapada Diamantina, a population with a much darker dorsal coloration than the population from the type locality. We also redescribed the advertisement call of *P. falcipes* based on recordings from topotypic males.

Key words: Advertisement call, bioacoustics, integrative taxonomy, *Pseudopaludicola pocoto*, morphologically cryptic species

Introduction

The dwarf swamp frogs of the genus *Pseudopaludicola* Miranda-Ribeiro are currently represented by 21 species (Frost 2018), which occur throughout South America, east of the Andes (Lynch 1989). *Pseudopaludicola* is a monophyletic group supported by a set of osteological (Lobo 1995), morphological (Lynch 1989; Lobo 1995), and molecular features (Veiga-Menoncello *et al.* 2014). Veiga-Menoncello *et al.* (2014) recovered four clades within *Pseudopaludicola*, in which the species of each clade share the same number of chromosomes, varying from $2n = 16$ to 22. Veiga-Menoncello *et al.* (2014) and Andrade *et al.* (2016) pointed out to several populations that could actually be considered as new species and stated that taxonomic studies within this genus are still needed. These two studies and a previous one (Duarte *et al.* 2010) specifically highlighted a putative new species that occurs in Andaraí, state of Bahia, Brazil, which is hereby described.

The most closely related taxa to this new species is *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad, and Garda, a widely distributed species known from seven Brazilian states along the Caatinga and Atlantic Forest domains (Andrade *et al.* 2017a), which had its advertisement call and tadpole already described (Magalhães *et al.* 2014). Another related species is *P. falcipes* (Hensel), which had its advertisement call briefly described based on a recording from Novo Hamburgo, state of Rio Grande do Sul, Brazil (Haddad & Cardoso 1987). *Pseudopaludicola falcipes* was the first described species in the genus and has historically undergone many taxonomic changes (Frost 2018). However, Langone *et al.* (2015) discussed on the types and addressed the misidentification in the literature. Lastly, Langone *et al.* (2016) stated that *P. falcipes* is distributed across the Pampean grasslands, thereby restricting its distribution.

Through a combination of phenotypic and genetic evidence we formally propose a name to the population recognized by Duarte *et al.* (2010), Veiga-Menoncello *et al.* (2014), and Andrade *et al.* (2016) as a putative new taxon. In order to strength the diagnosis of the new form we also further describe the advertisement call of *P. falcipes* from four localities in the state of Rio Grande do Sul (RS) and three localities from Argentina. The state of Rio Grande do Sul is attributed as the type locality for *P. falcipes* (Langone *et al.* 2015). In addition, we report, for the first time, the presence of *P. pocoto* in the *campo rupestre* (rupestrian grasslands) of Chapada Diamantina.

Material and methods

Reference specimens. Specimens of the type series of the new species were collected and recorded by F. S. Andrade and A. A. Giaretta in Andaraí (12°48'22.28" S, 41°19'59.47" W; 416 m above sea level [a.s.l.]; datum = WGS84), Chapada Diamantina in the state of Bahia, Brazil. We also collected and recorded individuals of *P. pocoto* from Pico das Almas (13°31'10.61" S, 41°56'59.60" W; 1545 m a.s.l.; datum = WGS84), Rio de Contas, Chapada Diamantina, state of Bahia (Figure 1). Examined individuals were collected under permit number #30059-9 issued by SISBio/Instituto Chico Mendes de Conservação da Biodiversidade. Individuals were killed by applying 5% lidocaine to their skin. We fixed specimens in 10% formalin and transferred them to 70% ethanol for permanent storage. Type specimens are deposited in the amphibian collection of Museu de Zoologia “Adão José Cardoso” (ZUEC) of the Universidade Estadual de Campinas (Unicamp), Campinas, state of São Paulo and in the amphibian collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, state of Minas Gerais, both in Brazil. We collected muscle tissue from the thigh of some individuals just after killing and stored samples in absolute ethanol.

Morphometry. We measured 14 adult males and five adult females (type series) under a stereomicroscope Zeiss Stemi 2000 coupled to an ocular micrometer; except SVL, which was measured with a Mitutoyo Absolute digital caliper (to the nearest 0.1 mm) under a stereomicroscope. Twelve morphometric traits were measured following Watters *et al.* (2016): snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), interorbital distance (IOD), eye-nostril distance (END), snout length (SL), internarial distance (IND), hand length (HAL), thigh length (TL), tibia length (TBL), and foot length (FL). Tarsus length (TAL) was measured following Heyer *et al.* (1990). Shape of the snout in dorsal and lateral views follows Heyer *et al.* (1990).

For comparisons with the most closely related species, we measured nine *Pseudopaludicola pocoto* type individuals (including the holotype) plus 15 specimens from the municipality of Rio de Contas, state of Bahia and 20 topotypes of *P. mineira* from the municipality of Santana do Riacho, Serra do Cipó, state of Minas Gerais. In addition, we measured 19 specimens of *P. falcipes* from Eldorado do Sul, Novo Hamburgo, Porto Alegre, and Viamão, all in the state of Rio Grande do Sul (RS). Further details on examined specimens are in Appendix I. Raw measurement data of the new species can be found online as Supplementary Material published with this paper.

Bioacoustics. We recorded 16 males and analyzed 308 pulsed notes. We recorded calls with two Marantz PMD 671 recorders (Marantz, Japan) at a sampling rate of 44.1 kHz and a sample size of 16 bits. One of the recorders was coupled to a ME67/K6 Sennheiser directional microphone (Sennheiser electronic GmbH & Co. KG, Germany) and the other to a ME66/K6 Sennheiser directional microphone, both positioned about 1.5 m from the calling male. Recordings were made on 26 November 2016 between 19:16–21:07 h. We analyzed calls with Raven Pro 1.5, 64-bit version (Bioacoustics Research Program 2014) with the following settings: window type = Hann; window size = 256 samples; 3 dB filter bandwidth = 248 Hz; brightness = 50%; contrast = 50%; overlap = 85% (locked); DFT size = 1024 samples (locked); grid spacing (spectral resolution) = 43.1 Hz. Temporal traits were measured on

oscillograms and spectral traits were measured on spectrograms. Raven obtained the peaks of dominant frequency through the “Peak Frequency (Hz)” function; the frequency values with 5 and 95% of call energy were obtained by “Frequency 5% (Hz)” and “Frequency 95% (Hz)” functions; and were considered as the minimum and maximum dominant frequencies (Hz), respectively. Call duration was considered the time extent that males spent emitting the series of three-pulsed notes. We generated call figures using the Seewave v.1.6 package (Sueur *et al.* 2008) in R version 3.4.1 64-bit (R Core Team 2017). Seewave settings were: Hanning window, 90% overlap, and 512 points resolution (FFT). Pulse terminology follows Magalhães *et al.* (2014), and overall acoustic terminology follows Köhler *et al.* (2017). We calculated means and standard deviations considering mean values of individual males, whereas the range (variation) encompasses the minimum and maximum values for all call samples. Pulse rate was calculated as number of pulses per note/note duration. Raw measurement data of the new species can be found online as Supplementary Material published with this paper.

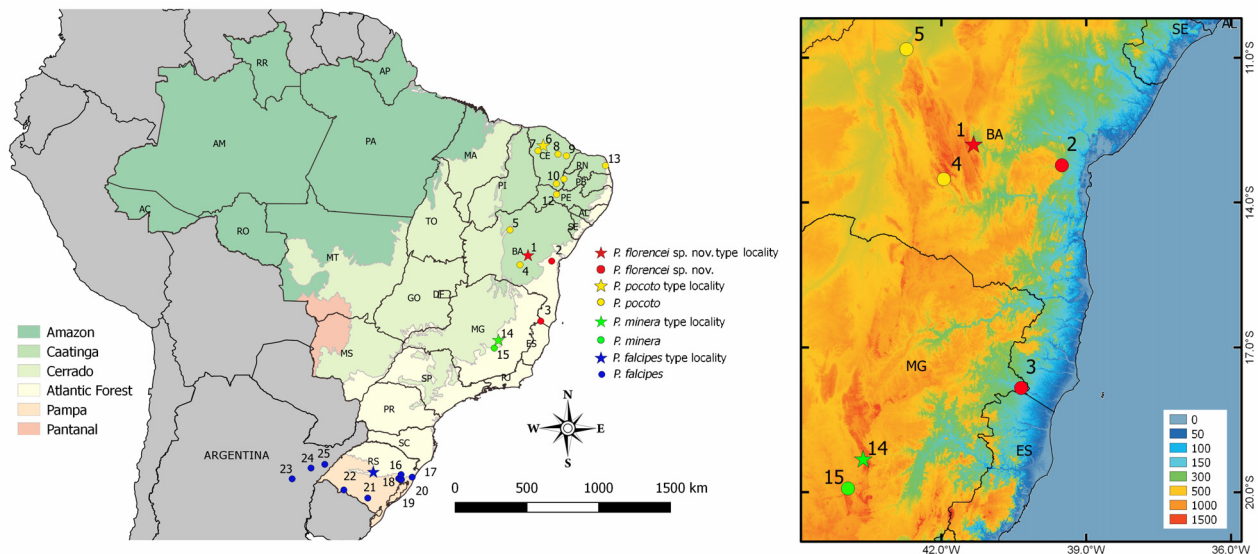


FIGURE 1. Partial map of South America showing the Brazilian domains and samples included in our molecular, morphological, and acoustic comparisons. The type locality of *Pseudopaludicola florencei* **sp. nov.** in Andaraí, state of Bahia, Brazil is indicated with a red star. The inset shows the hypsometric map of the Espinhaço range where tree species occur: the new species, *P. mineira* in the Serra do Cipó mountain range (MG) (green symbols), and *P. pocoto* in the Pico das Almas, Serra das Almas, Rio de Contas (BA) (yellow symbols). Municipalities: (1) Andaraí, BA; (2) Mutuípe, BA; (3) Nanuque, MG; (4) Rio de Contas, BA; (5) Xique-Xique, BA; (6) Santa Quitéria, CE; (7) Novas Russas, CE; (8) Quixadá, CE; (9) Morada Nova, CE; (10) Missão Velha, CE; (11) Cajazeiras, PB; (12) Salgueiro, PE; (13) Macaíba, RN; (14) Jaboticatubas (Serra do Cipó mountain range), MG; (15) Belo Horizonte, MG; (16) Novo Hamburgo, RS; (17) Osório, RS; (18) Eldorado do Sul, RS; (19) Porto Alegre, RS; (20) Viamão, RS; (21) Candiota, RS; (22) Santana do Livramento, RS; (23) Esquina, Corrientes; (24) Mercedes, Corrientes; (25) San Martin, Corrientes. Localities from 23 to 25 are in Argentina. Brazilian states: Bahia—BA; Ceará—CE; Minas Gerais—MG; Paraíba—PB; Pernambuco—PE; Piauí—PI; Rio Grande do Norte—RN; Rio Grande do Sul—RS. Legend contains the Brazilian domains and altitude in meters of the hypsometric map. See further details on the entire distribution of *P. pocoto* in Andrade *et al.* (2017a) and Silva *et al.* (2017), and for *P. falcipes* in Langone *et al.* (2015, 2016). The blue star is the centroid point of the state of Rio Grande do Sul (type locality of *P. falcipes*, according Langone *et al.* 2015).

For acoustic comparisons, we also recorded and analyzed 12 males of *P. pocoto* from Pico das Almas, Rio de Contas, state of Bahia. Additionally, we analyzed the recordings of five males of *P. pocoto* from Xique-Xique (those described in Andrade *et al.* 2017a); plus the original recordings of eight males published by Magalhães *et al.* (2014). Recordings of 12 males of *P. falcipes* were analyzed: three from Corrientes, Argentina; and nine from Candiota, Novo Hamburgo, Osório, and Viamão, all in the state of Rio Grande do Sul, Brazil. We also analyzed recordings from three males of *P. mineira* from Serra do Cipó and Belo Horizonte, both in the state of Minas Gerais.

Sound files are deposited in the Arquivo Sonoro da Coleção de Anuros da Universidade Federal de Uberlândia at UFU and at Fonoteca Neotropical Jacques Vielliard (FNJV) at Unicamp. Voucher specimens for call recordings are: *Pseudopaludicola* new species, ZUEC 23513, 23515, 23519–21; *P. pocoto* from Pico das Almas, Rio de Contas, state of Bahia, ZUEC 23506–10. Further details of the analyzed sound files are in Appendix 2.

Statistical analysis. Considering the morphometric and acoustic (multivariate) datasets separately, we sought for discrimination among species by applying two functions: (1) randomForest (RF) (randomForest v. 4.6-12 package; Liaw & Wiener 2002) and (2) DAPC (Discriminant Analysis on Principal Components, adegenet v. 2.0.1 package; Jombart 2008; Jombart & Ahmed 2011). RandomForest algorithm constructs many (generally 1,000) classification trees using bootstrap samples of the data (each split using the best predictors among those randomly chosen at each node) then generating classifiers and aggregating results by voting to classes (Liaw & Wiener 2002). The classic Discriminant Analysis (DA) depends on multivariate normality (Pohar *et al.* 2004) and on a larger number of objects than variables. The multivariate normality of the original data was evaluated through the function mardiaTest (MVN package; Korkmaz *et al.* 2014). The DAPC performs analyzes on the Principal Component scores (Jombart 2008). Application of a DA on a few axes (preserving about 95% of the variance) of a Principal Component Analysis, as performed by DAPC, improves the imbalance between objects and traits (Jombart *et al.* 2010). Despite the lack of normality in both of our datasets (details not shown), the results of DAPC are presented within an exploratory context to assess the congruence between it and RandomForest discriminations. Additionally, we also perform two Principal Components Analyzes (PCA) based on morphometric and acoustic datasets using the function dudi.pca from ade4 package v. 1.7-10 (Dray & Dufour 2007), and they are presented as a supplementary figure. The direct or indirect packages related to the application of both discriminant functions were run in R.

For the multivariate analysis and statistical tests we used all the morphometric features detailed above, only for the holotype plus the paratypes. For the acoustic analyzes we used note duration, internote interval, number of pulses per note, pulse duration, interpulse interval, pulse rate, note rate, peak of dominant frequency, and minimum and maximum of dominant frequency. As environmental variables influence acoustic traits of anurans (Köhler *et al.* 2017), we incorporated air temperature as a continuous variable in our multivariate analyzes. Considering that both multivariate analyzes, to both datasets, were concordant in species discrimination, we presented the RF classification results in tables and DAPC in scatter plots.

Acoustic and morphometric traits were tested for statistical significance of differences among species through the Exact Wilcoxon Mann Whitney Rank Sum Test, function wilcox_test of the package Coin (Resampling Statistics model; Hothorn *et al.* 2008) in R. As these tests were done between species/populations pairs, the significance levels (“*P*”) were adjusted considering the number of pairings through the method of Holm (p.adjust function in R).

Phylogenetic inference and genetic distances. We generated new sequence data for three specimens of the new species and six specimens of *Pseudopaludicola pocoto* from different localities (Appendix 3). For that, we extracted total DNA using a standard ammonium acetate precipitation method (Maniatis *et al.* 1982; adapted by Lyra *et al.* 2017) and amplified two mitochondrial fragments (12S and 16S). We used primers 12S-L48 and 12S-H978 for 12S (Walker *et al.* 2018), 16Sa-L and 16Sb-H for 16S (Kessing *et al.* 1989), and used the PCR cycling conditions described in Lyra *et al.* (2017) for 16S for both fragments. PCR products were purified using enzymatic reaction and sent to Macrogen INC. South Korea, to be sequenced in an ABI 3730 automated DNA sequencer. New DNA sequences were edited for quality using Geneious V.6 (Biomatter).

For the phylogenetic inference we aligned the new sequences with the sequences available for *Pseudopaludicola* from previous works including 10 outgroups (Appendix 3) using MAFFT v.7.25 (Katoh & Standley 2013; default parameters). We determined optimal partitioning scheme and model choice for dataset in PartitionFinder (Lanfear *et al.* 2012) under the Bayesian Information Criterion (BIC). The Bayesian phylogenetic inference was performed with MrBayes v.3.2.6 (Ronquist *et al.* 2012) using two independent runs of eight chains. Chains were started from random trees and run for 10 million generations each, being sampled every 1,000 generations. We checked the accuracy of posterior distributions for all model parameters using Tracer v.1.6 (Rambaut *et al.* 2014), and considered it adequate if all ESS values were higher than 200. Twenty-five percent of the trees were discarded as ‘burn-in’ before generating a 50% majority rule consensus tree. We rooted the consensus tree with *Odontophrynus americanus*. Analyzes were run at the CIPRES Science Gateway (Miller *et al.* 2010) and the consensus tree was edited in FigTree v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>).

The average genetic distances within and between species were calculated for the 16S fragment flanked by primers 16Sa-L and 16Sb-H in software MEGA v.6 (Tamura *et al.* 2013). For that, we classified each specie as one group, aligned sequences with MAFFT and estimated uncorrected p-distance and variance by bootstrap method (1,000 replicates) using pairwise deletion.

Species description

Pseudopaludicola florencei sp. nov.

(Figures 2–3, Tables 1–2)

Pseudopaludicola sp. (Andaraí/BA): Duarte *et al.* 2010; Andrade *et al.* 2016

Pseudopaludicola sp. 1 (Andaraí/BA): Veiga-Menoncello *et al.* 2014

Holotype. Adult male (ZUEC 23521; Figures 2–3, call voucher) collected by F.S. Andrade and A.A. Giaretta in Andaraí (12°48'22.28" S, 41°19'59.47" W, 416 m a.s.l.), Chapada Diamantina, state of Bahia, Brazil on 26 November 2016.

Paratypes. Thirteen adult males: ZUEC 23512–3, 23515, 23518–20, 23522, 23524–5, 23527–30; and five adult females: ZUEC 23514, 23516–7, 23523, and 23526, all collected with the holotype. Seven specimens: UFMG 4310–6, collected at Área de Proteção Ambiental Marimbus-Iraquara, Andaraí, state of Bahia, Brazil (12°45'46" S, 41°17'48" W, 326 m a.s.l.; ca. 6 km from the type locality) on 26 January 2010 by F. S. F. Leite.

Additional specimens: Brazil: state of Bahia: municipality of Mutuípe: CFBH 29652 (adult male); state of Minas Gerais: municipality of Nanuque: CFBH 33235 (adult female).

Diagnosis. *Pseudopaludicola florencei* sp. nov. is assigned to the genus *Pseudopaludicola* by having a hypertrophied antibrachial tubercle (see Lynch 1989, Lobo 1995). The new species is characterized by the following combination of characters: (1) small size (SVL 12.8–14.8 mm in adult males); (2) upper eyelids smooth, with no palpebral tubercles; (3) heel smooth, with no enlarged conical tubercle; (4) relatively short hindlimbs (tibiotarsal articulation just reaching the posterior margin of eye); (5) 11 pairs of chromosomes (2n=22; see Duarte *et al.* 2010); and (6) advertisement call composed of regular series of three-pulsed notes, emitted at high rate.

Comparison with other species. *Pseudopaludicola florencei* sp. nov. is promptly diagnosed from the *P. pusilla* species group (see Lynch 1989), which includes *P. boliviana* Parker, *P. ceratophyes* Rivero and Serna, *P. llanera* Lynch, *P. pusilla* (Ruthven), and *P. motorzinho* Pansonato, Veiga-Menoncello, Mudrek, Jansen, Recco-Pimentel, Martins, and Strüssmann by its terminal phalanges knobbed; whereas the abovementioned species have T-shaped terminal phalanges or expanded toe tips (disks or pads). The tips of the phalanges of the new species are similar in shape to those of *P. falcipes* (Figure 2B in Cardozo & Suárez 2012). The new species is also distinguished from *P. ceratophyes* by having upper eyelids smooth; *P. ceratophyes* has upper eyelids with an enlarged palpebral tubercle (Lynch 1989). *Pseudopaludicola florencei* sp. nov. differs from *P. boliviana* and *P. motorzinho* also by the heel smooth, with no enlarged, conical tubercle on the heel (Pansonato *et al.* 2016).

Pseudopaludicola florencei sp. nov. is distinguished from *P. saltica* (Cope), *P. murundu* Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-Pimentel, and Haddad, and *P. jaredi* Andrade, Magalhães, Nunes-de-Almeida, Veiga-Menoncello, Santana, Garda, Loebmann, Recco-Pimentel, Giaretta, and Toledo (*P. saltica* species group) by having relatively short hindlimbs; i.e., the tibiotarsal articulation reaches the posterior margins of eye in the new species, whereas it reaches the tip of the snout in the *P. saltica* species group.

The chromosome number 2n = 22 distinguishes *Pseudopaludicola florencei* sp. nov. (as *Pseudopaludicola* sp. in Duarte *et al.* 2010) from *P. mystacalis* (Cope) (2n = 16); *P. canga* Giaretta and Kokubum, *P. facureae* Andrade and Carvalho, and *P. atragula* Pansonato, Mudrek, Veiga-Menoncello, Rossa-Feres, Martins, and Strüssmann (2n = 18); *P. ternetzi* Miranda-Ribeiro and *P. ameghini* (Cope) (2n = 20) (Duarte *et al.* 2010; Fávero *et al.* 2011; Cardozo *et al.* 2016). In addition, Duarte *et al.* (2010) highlighted that *P. florencei* sp. nov. has morphological polymorphisms in the chromosomes 7, 8, and 11, which distinguishes it from *P. mineira*. Moreover, these same authors stated that the two species are distinguished by polymorphisms in the heterochromatin distribution and by the NOR position in the pair 8, which was terminal in *P. mineira* and subterminal in *P. florencei* sp. nov..

Pseudopaludicola florencei sp. nov. is promptly distinguished from *P. canga* (Giaretta & Kokubum 2003; Pansonato *et al.* 2012; Carvalho *et al.* 2015a), *P. giarettai* Carvalho (Carvalho 2012; Carvalho *et al.* 2015b), *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strüssmann, and Martins (Pansonato *et al.* 2012), *P. facureae* (Andrade & Carvalho 2013; Carvalho *et al.* 2015a), and *P. parnaíba* Roberto, Cardozo, and Ávila (Roberto *et al.* 2013; Carvalho *et al.* 2015a) by possessing pulsed notes, whereas those species have calls composed of non-pulsed notes. Note structure (three non-concatenated pulses) distinguishes *P. florencei* sp. nov. from species with notes with concatenated pulses (= lack of interpulse interval; *sensu* Magalhães *et al.* 2014): *P. mystacalis* [12–14 concatenated pulses; Pansonato *et al.* 2013], *P. boliviana* [3–6; Duré *et al.* 2004], *P. ibisoroca* Pansonato, Veiga-

Menoncello, Mudrek, Jansen, Recco-Pimentel, Martins, and Strüssmann [3–12; Pansonato *et al.* 2016], and *P. motorzinho* [2–6; Pansonato *et al.* 2016].

The new species is distinguished from other congeners [values within square brackets] with notes with non-concatenated pulses by the following acoustic traits: *P. ternetzi* has shorter note duration (108–166 [32–80] ms) and interpulse interval (22–92 [1–14] ms), higher note (223–297 [606–921] notes per minute) and pulse rates (18–32 [61–139] pulses per second), and lower peak of dominant frequency (4608–5599 [3516–4500] Hz) (Andrade *et al.* 2017b); *P. ameghini* has shorter interpulse interval [1–23 ms], and higher note [348–452 notes per minute] and pulse rates [40–56 pulses per second], and lower peak of dominant frequency [3141–4312 Hz] (Andrade *et al.* 2017b); *P. atragula* has longer note duration [300–700 ms], higher number of pulses per note (3–4 [9–36]), lower note rate [42–98 notes per minute], and lower peak of dominant frequency [3618–4264 Hz] (Pansonato *et al.* 2014). The three species of the *P. saltica* species group (*P. saltica*, *P. murundu*, and *P. jaredi*) vary highly the number of pulses in their notes (2–7, combined values; Andrade *et al.* 2016); on the other hand, the new species has very stereotyped three-pulsed notes (Figure 4A). *Pseudopaludicola falcipes* may have notes with two or three pulses, but those with two pulses are more common (83 %, n = 240 analyzed notes; Figure 4D). In addition, the new species can be significantly differentiated from *P. falcipes* by having longer note duration and interpulse interval, and lower pulse and note rates (Wilcoxon-Mann-Whitney Test: $P < 0.01$; present study).

In comparison with the two phylogenetically close related species, the new species is distinguished from *P. mineira* by having stereotyped three-pulsed notes (only two notes of an individual have four pulses; n = 320 analyzed notes), whereas the *P. mineira* has stereotyped two-pulsed notes (Figure 4C). This character and its longer note duration (108–166 [42–87] ms) distinguish the new species from *P. mineira* (Pereira & Nascimento 2004; present study). *Pseudopaludicola pocoto* has also stereotyped three-pulsed notes (Figure 4B); however, *P. florencei* **sp. nov.** is readily distinguished from *P. pocoto* by having a higher note rate (223–297 [100–184] notes per minute). Additionally, *P. florencei* **sp. nov.** differs significantly from *P. mineira* by its note rate; and from *P. pocoto* by having shorter note duration, internote interval and interpulse interval, and higher pulse rate (Exact Wilcoxon-Mann-Whitney Test: $P < 0.01$).

Both multivariate approaches (randomForest and DAPC) to morphometry yielded no noticeable discrimination among species (Table 3; Figure 5A). However, the Exact Wilcoxon-Mann-Whitney Test on SVL revealed that *P. florencei* **sp. nov.** is larger than *P. pocoto*, and smaller than *P. mineira* and *P. falcipes* ($P < 0.01$). Regarding calls, the randomForest model resulted in total discrimination among *P. florencei* **sp. nov.** and its closer related species (*P. mineira* + *P. pocoto*) and *P. falcipes*, with all its individuals classified correctly (Table 3). The DAPC also revealed substantial differentiation among the new species and its closer related species (Figure 5B), with a greater separation along axis 1 (LD1 = 78.6 %; LD2 = 19.4 %). Number of pulses per note (66 %), pulse (12 %), and note (12 %) rates mainly accounted for species separation along LD1 (Figure 5B). Number of pulses per note (44 %), internote (27 %), and interpulse (18 %) intervals mainly accounted for species separation along LD2 (Figure 5B). See also the similar results presented in the performed PCAs (Appendix 4).

Description of the holotype: Body elliptical and broad (Figure 2A–B; Table 1). Head elliptical, slightly wider than long. Snout subovoid in dorsal view and rounded in profile (Figure 2C–D). Eye not protuberant. Eye diameter equal to the interorbital distance. Interorbital area flat. Pupil rounded. Upper eyelid without tubercles. Nostril not protuberant and closer to the snout tip than to the eye. *Canthus rostralis* rounded, smooth. Loreal region slightly concave. Single subgular vocal sac, externally expanded and with discrete longitudinal folds. Choanae rounded well separated from each other. Vocal slits present. Tympanum indistinct. A discrete dermal fold extending from the posterior margin of the eye to the insertion of the arm. Mouth opening ventral. Vomerine teeth absent (unnoticeable also to the touch). Tongue elliptical, longer than wide, free posteriorly, without pigmentation at its base. Flanks with discrete granules. One ovoid antibrachial tubercle present in the first quarter of the forearm and a second ovoid tubercle closer to elbow. Finger and toe tips not expanded. Outer and inner metacarpal tubercles well-defined, outer metacarpal tubercle ovoid and inner metacarpal tubercle elliptical. Fingers with single and rounded subarticular tubercles. Palm of hand smooth, with no supernumerary tubercles. Thumb with a keratinized, light brown nuptial pad, extending from the base of the hand to the proximal limit of the terminal phalanx, covering almost the entire external portion of the finger. Webbing absent between fingers. Relative finger lengths, when adpressed one to another: $I < II < IV < III$ (Figure 2E). Outer metatarsal tubercle well defined, conical. Inner metatarsal tubercle elliptical. The internal metatarsal tubercle larger than the external. Toes with well-defined, single, enlarged, and rounded subarticular tubercles. Sole of the foot smooth, with no supernumerary tubercles.

Toes webbed basally and fringed along their sides to almost their tips. Fringes developed on all toes (mainly II, III, and IV). External fringe on Toe V continues almost to the outer metatarsal tubercle. Well-developed fold from internal metatarsal tubercle to the mid-ventral tarsus, ending in a tarsal tubercle poor protuberant. Relative toe lengths, when addressed one to another: $I < II < V < III < IV$ (Figure 2F). Hind limb robust and moderately long with the tibio-tarsal articulation just reaching the posterior margins of eye. Thigh shorter than tibia. Foot longer than thigh and tibia. Tubercle absent on calcaneus. Belly skin smooth. Abdominal fold present. Dorsal surfaces of head, body, and limbs smooth. Dorsal surface of body interspersed with some discrete tubercles. Cloacal region smooth (Figure 2B). Measurements of the holotype presented in Table 1.

Color pattern of the holotype in preservative. Dorsum grayish with dark grey, white, and brown blotches. Brown dorsolateral stains on body, from posterior corner of eyes to the region of insertion of legs. Belly whitish (unpigmented). Throat whitish, pigmented (with small black dots scattered). Dorsum darker than the dorsal surfaces of limbs. Region between upper lip and eyes with alternating vertical grey and light beige stripes. Ventral faces of arms and legs unpigmented, except of the thigh (pigmented as in throat). Palm of hand pigmented. Sole of foot pigmented and darker than hands, arms, and legs. Color of the sole of the foot similar to that of dorsal leg. Dorsal faces of arms light grayish with dark brown blotches. Dorsal faces of legs light grayish with dark brown transversal discontinuous stripes and with scattered brown blotches. Transverse stripes on thighs (2–3), shanks (3–4), feet (3–4). Dark brown nuptial pads (Figure 2A).

Variation in type series. Dorsal surface of body varies from dark grey to dark brown, with black or dark brown irregular blotches (Figure 3). Ventral surface pigmented (with small black dots scattered) on throat, chest, and belly (ZUEC 23512–3, 23515–8, 23520, 23522–3, 23525–8, 23530). The specimen ZUEC 23520 has a brown vertebral line, and the specimens ZUEC 23512, 23514, and 23520 have white scattered blotches on dorsum. The specimens ZUEC 23512–4, 23516–7, 23519, 23521–5, 23528, and 23530 do not have dorsolateral stains on body, from posterior corner of eyes to the region of insertion of legs. The specimens ZUEC 23514, 23523–4, and 23529–30 have a discrete tympanic ridge from behind the eyes to the proximal portion of the arms. The specimens ZUEC 23525–8 have white blotches on the region between the mouth corner and the insertion of the arms. The specimen ZUEC 23512 has a large black blotch on dorsum. The specimen ZUEC 23527 has two large black blotches in the posterior portion of the dorsum, slightly above the cloacal region. Females have a more robust body and no nuptial pads, vocal sac and vocal slits.

Vocalizations. *Pseudopaludicola florencei* **sp. nov.** emits an advertisement call of variable duration that can be long (1.6–22.4 s), consisting of series of three-pulsed notes (1–5 series of three-pulsed notes per call) that lasts 0.4–22.4 s, separated by intervals of 103–561 ms (Figure 4A). Notes last 108–166 ms separated by intervals of 64–141 ms, and are released at a rate of 223–297 notes per minute; notes have a slightly increase in amplitude from the first to the second pulses (= midpoint of note), followed by a decrease in amplitude regarding the second and third pulses (see oscillogram in Figure 4A). Notes are composed of three non-concatenated pulses (Figure 4A); notes with four pulses were rarely found (only two notes of an individual). Pulses vary from 4–16 ms, separated by intervals of 22–92 ms, and are released at a rate of 18–32 pulses per second (Figure 4A). Dominant (= fundamental) frequency peaks are between 4608–5599 Hz; minimum frequency ranges 4005–5125 Hz, and the maximum frequency ranges 4996–6804 Hz. Notes present up to two harmonics; the second ranging from 8915–10982 Hz (mean = 9834; SD = 525) (Figure 4A). Air temperature of recorded calls varied from 22 to 23.5 °C. Call quantitative traits are summarized in Table 2.

An additional pulsed note was noticed shortly after four advertisement calls from two recorded males (Figure 6) and other males heard in the field. Regular notes can gradually gain pulses until they become this note type (Figure 6). This note ($n = 10$) has a duration ranging from 68–116 ms (mean = 83 ± 16), is composed of 7–14 pulses (mean = 10.9 ± 2.1), lasting 2–9 ms (mean = 5 ± 1), is separated by intervals of 0–22 ms (mean = 4 ± 1), and is emitted at rates of 93–157 pulses per second (mean = 134 ± 22). The dominant frequency varies from 4436–4823 Hz (mean = 4630 ± 158); minimum frequency ranges 3747–3833 Hz (mean = 3781 ± 34) and maximum frequency ranges 4996–5125 Hz (mean = 5060 ± 42) (Figure 6). Another emphasized frequency band may be present, peaking from 8398–8570 Hz (mean = 8488 ± 59).

Distribution. *Pseudopaludicola florencei* **sp. nov.** is known from its type locality, and in the municipality of Mutuípe, state of Bahia, Northeastern Brazil, and in the municipality of Nanuque, state of Minas Gerais, Southeastern Brazil (Darío Cardozo pers. Communication; see Figure 1). Mutuípe and Nanuque are situated about 200 km southeast and 560 km south of the type locality, respectively.

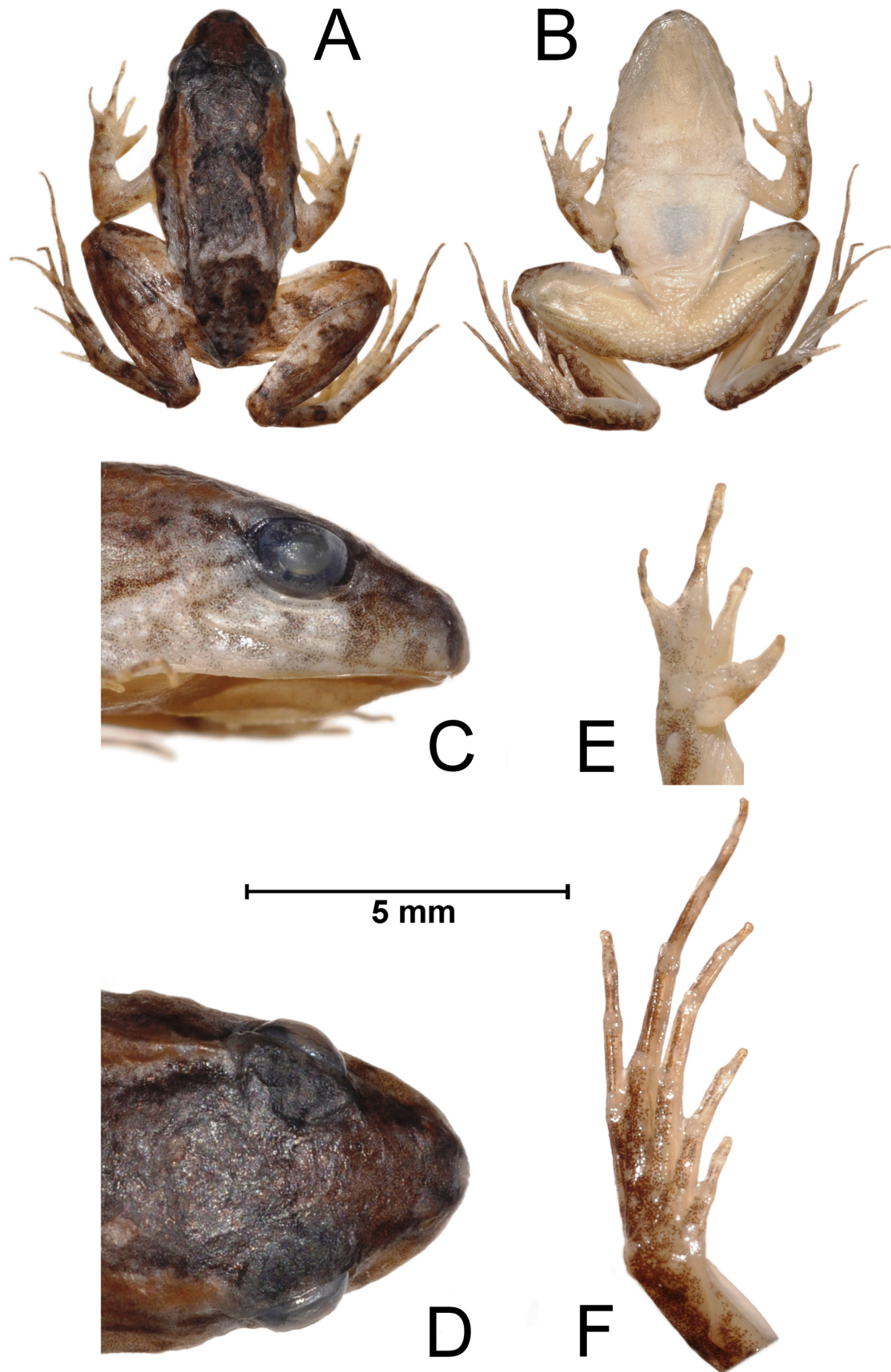


FIGURE 2. *Pseudopaludicola florencei* sp. nov. holotype (ZUEC 23521, SVL = 12.9 mm), an adult male. Dorsal (A) and ventral (B) views; (C) lateral and (D) dorsal views of head; ventral views of (E) hand and (F) foot. Scale bar = 5 mm.

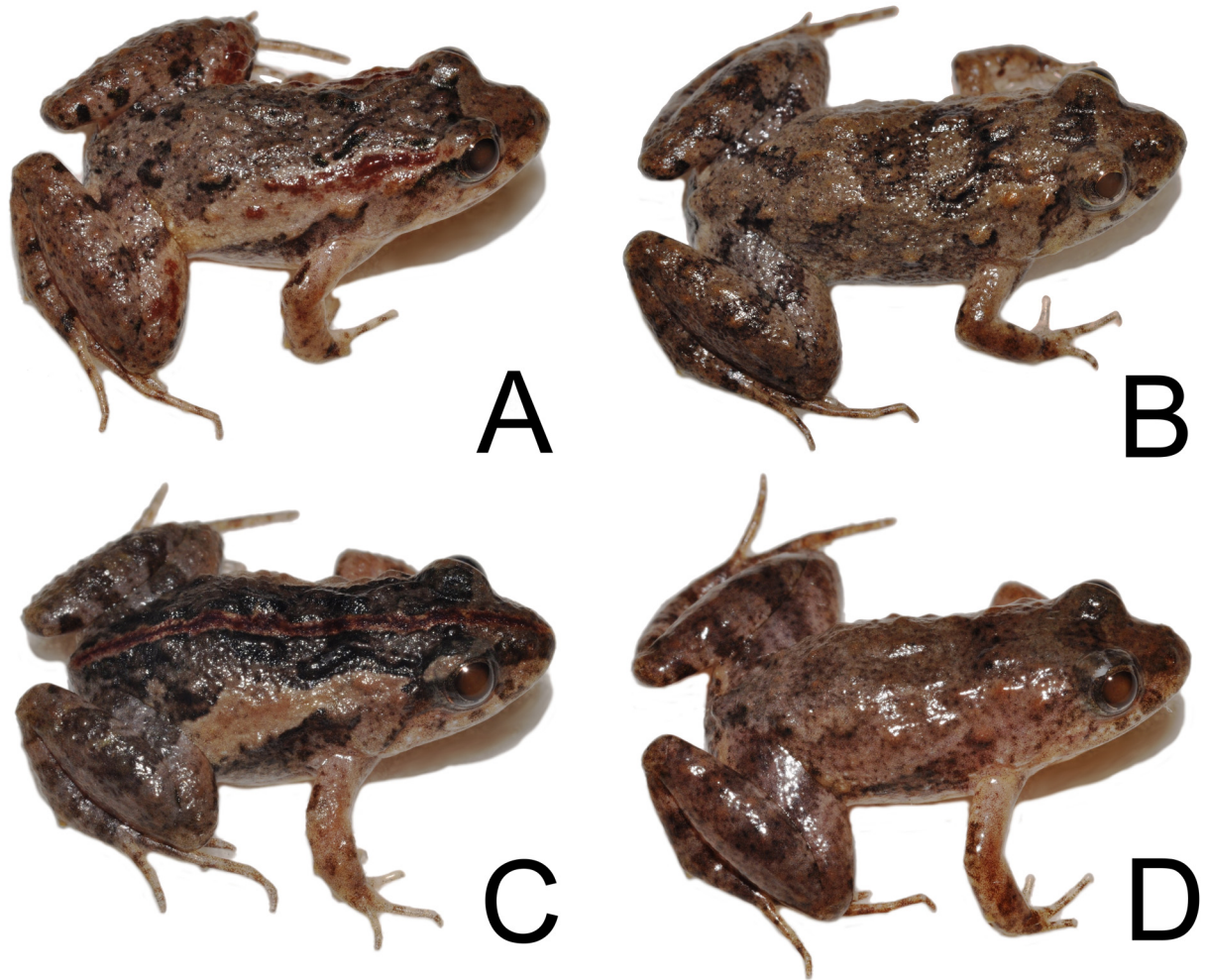


FIGURE 3. Holotype and three paratypes of *Pseudopaludicola florencei* **sp. nov.** in life. (A) ZUEC 23521 (holotype, adult male, call voucher, SVL = 12.9 mm), (B) ZUEC 23523 (adult female, SVL = 15.5 mm), (C) ZUEC 23520 (adult male, call voucher, SVL = 13.2 mm), and (D) ZUEC 23522 (adult male, SVL = 12.8 mm).

Natural history. The holotype and paratypes collected on November 2016 were recorded on the banks of a river that crosses the urban area of Andaraí. In this place, we observed hundreds of specimens of *P. florencei* **sp. nov.** occurring syntopically with *Dendropsophus* sp. and *Rhinella granulosa*. We also have some call recordings of the seven paratypes from Área de Proteção Ambiental Marimbus-Iraquara, which is a protected site.

In the field, we heard and recorded the additional pulsed note abovementioned. In all our field recordings, males were isolated at their calling sites, without any close-range encounters. However, there were dozens of males competing for calling sites at that night; it was possible to observe several males interacting physically. In these interactions, some males jumped quickly over the calling males, but no fighting was observed.

Etymology. The specific name honors Antoine Hercule Romuald Florence. Better known as Hercule Florence, a French artist, painter, polygrapher, and inventor, is acknowledged as the inventor of photography in Brazil in the 19th century. After his return from the Langsdorff's expedition (from 1826 to 1829), Florence developed a system able to properly describe animal sounds, transcribing them into a five line music staff (Florence 1831, 1876; Toledo & Araújo 2017). Such method, termed as “Zoophonie” by Florence, was the first universal method of describing animal sounds and he is therefore designated as the “father of bioacoustics” (Vielliard 1993; Toledo & Araújo 2017). At least these two techniques (photography and zoophony = bioacoustics) are fundamental for species description nowadays (Köhler *et al.* 2017). Specifically, bioacoustics has proved to be efficient in clarifying the taxonomy of the genus *Pseudopaludicola* (as in the present study).

TABLE 1. Morphometric characters of *Pseudopaludicola florencei* **sp. nov.** type series (including the holotype) from the municipality of Andaraí, state of Bahia, Brazil. Values presented in millimeters as mean \pm standard deviation (minimum–maximum); n = number of measured specimens.

Characters	Holotype	Males (n = 13)	Females (n = 5)
Snout-vent-length (SVL)	12.9	13.5 \pm 0.6 (12.8–14.8)	14.7 \pm 0.7 (13.8–15.5)
Head length (HL)	3.8	3.6 \pm 0.2 (3.4–3.9)	4.2 \pm 0.2 (4.0–4.4)
Head width (HW)	4.6	4.6 \pm 0.1 (4.4–4.8)	4.9 \pm 0.2 (4.7–5.1)
Eye diameter (ED)	1.5	1.5 \pm 0.1 (1.4–1.7)	1.6 \pm 0.1 (1.4–1.8)
Interorbital distance (IOD)	1.5	1.5 \pm 0.1 (1.3–1.6)	1.6 \pm 0.1 (1.6–1.7)
Eye-nostril distance (END)	1.1	1.1 \pm 0.0 (1.0–1.1)	1.2 \pm 0.1 (1.1–1.3)
Snout length (SL)	2.1	2.0 \pm 0.1 (1.9–2.2)	2.3 \pm 0.1 (2.2–2.4)
Internarial distance (IND)	1.2	1.1 \pm 0.1 (1.0–1.2)	1.2 \pm 0.1 (1.1–1.3)
Hand length (HAL)	3.8	3.8 \pm 0.2 (3.4–4.1)	4.1 \pm 0.1 (4.0–4.2)
Thigh Length (TL)	6.8	6.8 \pm 0.4 (5.8–7.1)	7.6 \pm 0.4 (6.9–8.0)
Tibia length (TBL)	7.3	7.1 \pm 0.4 (6.1–7.8)	8.0 \pm 0.3 (7.5–8.2)
Tarsus length (TAL)	3.5	3.6 \pm 0.3 (3.2–4.1)	4.1 \pm 0.2 (3.9–4.3)
Foot length (FL)	7.6	7.7 \pm 0.3 (7.0–8.4)	8.6 \pm 0.6 (7.7–9.1)

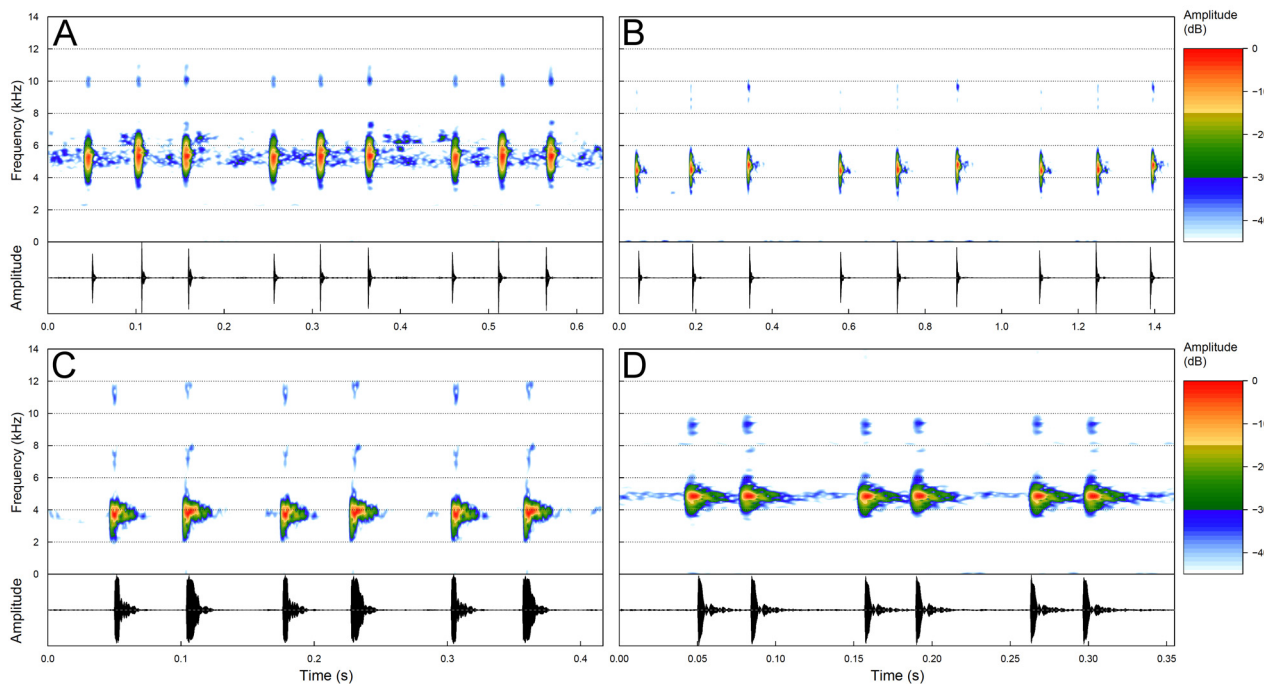


FIGURE 4. Audiospectrograms (above) and corresponding oscillograms (below) detailing the pulsed notes of the advertisement calls of four *Pseudopaludicola* species with non-concatenated pulses: (A) *Pseudopaludicola florencei* **sp. nov.** from the type locality with its three-pulsed notes (Pseudop_florenceiAndaraíBA2aAAGm671; 20:29 h, 26 Nov 2016; air 22°C); (B) *Pseudopaludicola pocoto* from Pico das Almas, Rio de Contas, state of Bahia (Pseudop_pocotoRioContasBA9bFSA_AAGm; 21:34 h, 25 Nov 2016; air 19.1°C); (C) *Pseudopaludicola mineira* from Serra do Cipó mountain range, state of Minas Gerais (FNJV 31899; 10 Feb 1964; air 24°C); and (D) *Pseudopaludicola falcipes* from Viamão, state of Rio Grande do Sul (*Pseudopaludicola_4A08*; 15:00 h; 28 Sep 1996; air 23°C).

Phylogenetic inferences and mitochondrial DNA divergences. We recovered the same tree topology inferred by Veiga-Menoncello *et al.* (2014) and Andrade *et al.* (2016) for the genus *Pseudopaludicola* (Appendix 5), where species that shows equal diploid chromosome numbers are recovered in well-supported clades. Among species with $2n = 22$ chromosomes (Figure 7), we recovered *P. florencei* **sp. nov.** as sister group of *P. pocoto* and

these two as sister group of *P. mineira*. But this last phylogenetic relationship was recovered with low posterior probabilities, as evidenced in previous studies. The average uncorrected p-distance between *P. florencei* **sp. nov.** and *P. pocoto* was 4.2 % (range 3.4–4.9%; Table 4). The overall genetic distances between all *Pseudopaludicola* species ranged from 1.8% (*P. canga* vs *P. sp.* from Barreirinhas, state of Maranhão) to 18 % (*P. atragula* vs *P. boliviana*). The intraspecific distance for *P. florencei* **sp. nov.** was 0.1% and it ranged from 0 to 1.5% in other species analyzed (Table 4).

TABLE 2. Advertisement call traits of *Pseudopaludicola florencei* **sp. nov.** from the type-locality; *P. pocoto* from Pico das Almas, Rio de Contas (Chapada Diamantina) and Xique-Xique, state of Bahia; and from those from the original description (Magalhães *et al.* 2014); and *P. falcipes* from Candiota, Novo Hamburgo, Osório, and Viamão, state of Rio Grande do Sul, Brazil; and from Corrientes, Argentina. Mean \pm SD (minimum–maximum). n = number of males recorded (number of analyzed notes).

Traits	<i>P. florencei</i> sp. nov. n = 16 (308)	<i>P. pocoto</i> n = 25 (500)	<i>P. falcipes</i> n = 12 (240)
Call duration (s)	10.4 \pm 4.9 (1.6–22.4)	58.6 \pm 40.1 (10.1–141.8)	47.3 \pm 44.3 (7.5–154.4)
Series of notes duration (s)	8.2 \pm 5.5 (0.4–22.4)	31.4 \pm 24.3 (1.0–96.0)	29.6 \pm 28.4 (2.8–110.7)
Interseries interval (s)	0.303 \pm 0.157 (0.103–0.561)	15.4 \pm 30.1 (0.5–101.1)	1.4 \pm 0.9 (0.25–4.4)
Series per call	1.6 \pm 1.2 (1–5)	1.9 \pm 0.8 (1–4)	1.8 \pm 1.4 (1–5)
Note duration (ms)	135 \pm 12 (108–166)	273 \pm 46 (122–397)	66 \pm 23 (32–167)
Internote interval (ms)	100 \pm 12 (64–141)	205 \pm 40 (116–297)	92 \pm 23 (45–143)
Pulse duration (ms)	9 \pm 2 (4–16)	10 \pm 3 (3–21)	9 \pm 3 (4–20)
Interpulse interval (ms)	54 \pm 6 (22–92)	122 \pm 23 (9–200)	37 \pm 14 (15–89)
Notes per minute	254.5 \pm 20.4 (223.1–297.3)	131.5 \pm 20.0 (100.0–184.2)	394.0 \pm 94.2 (238.1–535.1)
Pulse rate	22.4 \pm 2.1 (18.1–32.5)	11.3 \pm 2.0 (7.6–23.6)	36.2 \pm 7.8 (18.0–62.5)
Pulses per note	3.0 \pm 0.025 (3–4)	3.0 \pm 0.1 (2–4)	2.2 \pm 0.2 (2–3)
Peak of dominant frequency (Hz)	5044.3 \pm 261.3 (4608.1–5598.6)	5020.0 \pm 570.6 (4177.4–6460.0)	5133.0 \pm 318.6 (4687.5–5986.2)
Minimum dominant frequency (Hz)	4603.9 \pm 224.9 (4005.2–5124.9)	4430.9 \pm 934.6 (3359.2–5770.9)	4758.8 \pm 318.5 (4218.8–5598.6)
Maximum dominant frequency (Hz)	5488.2 \pm 353.4 (4995.7–6804.5)	5435.7 \pm 540.6 (4565.0–6804.5)	5484.3 \pm 428.2 (4995.7–6804.5)
Air temperature (°C)	22–23.5	19.1–24.9	16.0–25.5

TABLE 3. Confusion matrix for four *Pseudopaludicola* species based on morphometric and **acoustic (values in bold)** datasets by means of a Random Forests model. Settings: number of tree permutations = 1000; number of variables tried at each split = 3.0; error rates = 23.4 % | **7.1 %**.

	<i>P. florencei</i> sp. nov.	<i>P. falcipes</i>	<i>P. mineira</i>	<i>P. pocoto</i>	class.error
<i>P. florencei</i> sp. nov.	5 16	2 0	1 0	6 0	0.64 0.00
<i>P. falcipes</i>	1 1	17 11	1 0	0 0	0.10 0.08
<i>P. mineira</i>	2 0	0 3	17 0	1 0	0.15 1.00
<i>P. pocoto</i>	1 0	1 0	2 0	20 25	0.17 0.00

The advertisement call and acoustic diagnosis of *P. falcipes*. Quantitative traits are summarized in Table 2. Air temperature of recorded calls varied from 16.0–25.5 °C. *Pseudopaludicola falcipes* emits a long advertisement calls of variable duration that can be long (7.5–154.4 s), consisting of series of pulsed notes (1–5 series of notes per call) that lasts 2.8–110.7 s, separated by intervals of 0.25–4.4 s (Figure 4D). Notes last 32–167 ms separated by intervals of 45–143 ms and are released at a rate of 238–535 notes per minute. Eighty-three percent of all the analyzed notes (n = 320 notes) have two non-concatenated pulses, the other notes with three non-concatenated

pulses each. Pulses vary from 4–20 ms, separated by intervals of 15–89 ms, and are released at a rate of 18–62 pulses per second (Figure 4D). Dominant (= fundamental) frequency peaks between 4687–5986 Hz; minimum frequency ranges 4219–5599 Hz, and the maximum frequency ranges 4996–6804 Hz. Second harmonic peaks between 8441–11326 Hz (mean = 10188; SD = 666) (Figure 4D).

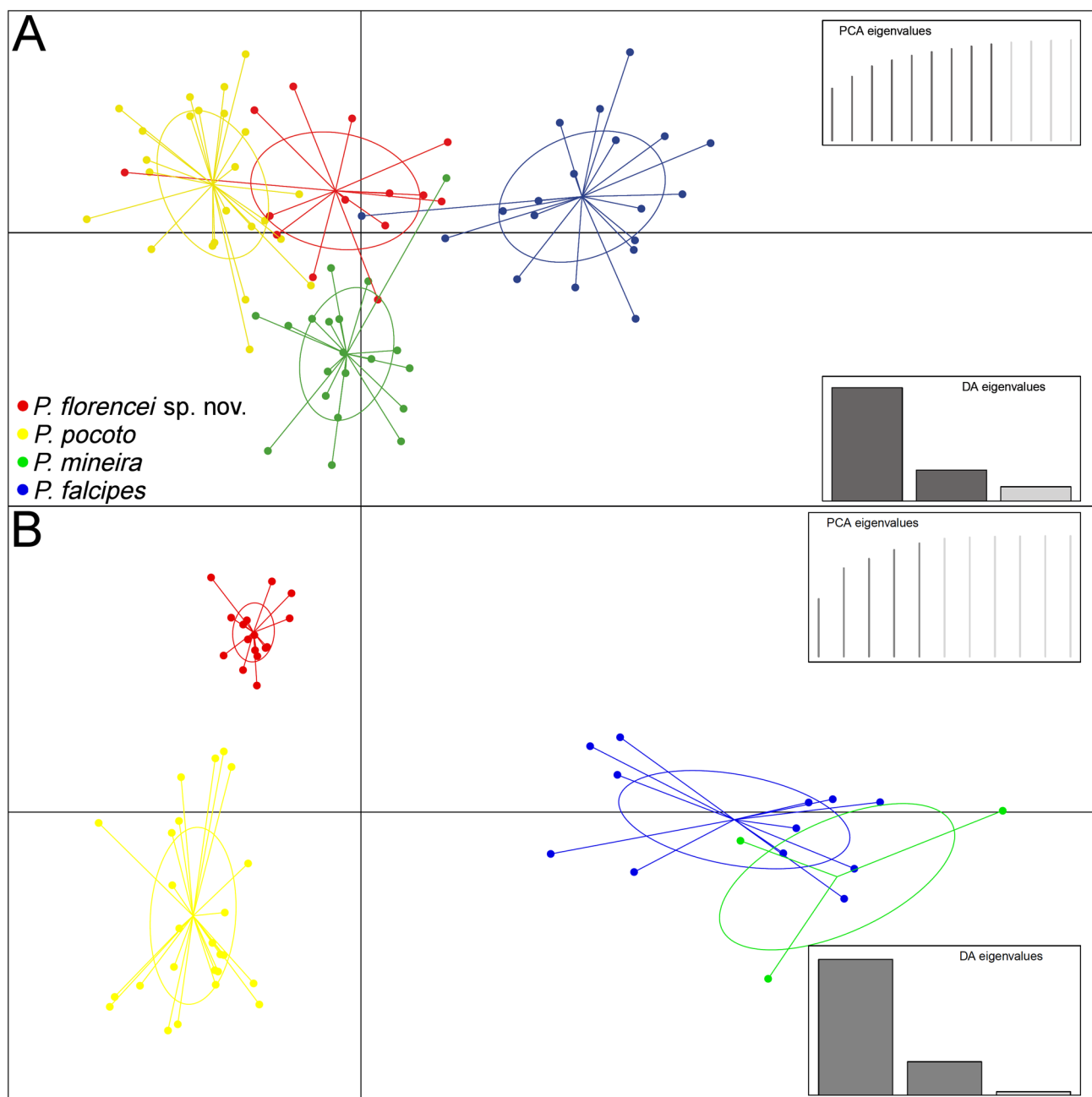


FIGURE 5. Scatterplot of the discriminant analyzes (DAPC) on the morphometric and acoustic datasets of *Pseudopaludicola florencei* sp. nov., *P. pocoto*, *P. mineira*, and *P. falcipes*. (A) The two first axes on the morphometric data (9 first PCs, 96% retained variance). Variance explained by the axes: LD1 = 71.6 % (F-statistic = 101.9) and LD2 = 19.5 % (F-statistic = 27.7). (B) The two first axes on the acoustic data (5 first PCs, 94% retained variance). LD1 = 78.6 % (F-statistic = 220.4) and LD2 = 19.4 % (F-statistic = 54.3).

Like the new species, *P. falcipes* can be distinguished from species that have non-pulsed structure or with concatenated pulses (with lack of interpulse interval): *P. canga*, *P. giarettai*, *P. hyleaustralis*, *P. facureae*, *P. parnaiba*, *P. mystacalis*, *P. boliviana*, *P. ibisoroca*, and *P. motorzinho*. *Pseudopaludicola falcipes* distinguishes from other congeners [values within square brackets] by the following acoustic traits: *P. ternetzi* has shorter interpulse interval (15–89 [1–14] ms), higher note (per minute) and pulse rates (238–535 [606–921] notes per minute; 18–62 [61–139] pulses per second), and a lower peak of dominant frequency (4688–5986 [3516–4500] Hz)

(Andrade *et al.* 2017b); *P. ameghini* has shorter interpulse interval [1–23 ms] and lower peak of dominant frequency [3141–4312 Hz] (Andrade *et al.* 2017b); *P. atragula* has longer note duration (32–167 [300–700 ms]), higher number of pulses per note (2–3 [9–36]), lower note rate [42–98 notes per minute], and lower peak of dominant frequency [3618–4264 Hz] (Pansonato *et al.* 2014). From the three long-legged species (*P. saltica*, *P. murundu*, and *P. jaredi*), *P. falcipes* is distinguished by having low variation in the number of pulses per note (2–3) (Andrade *et al.* 2016). *Pseudopaludicola pocoto* has a lower note rate [100–184 notes per minute] (present study). In addition, *P. falcipes* differs significantly from *P. pocoto* by its shorter note duration, interpulse and internote intervals, and higher pulse and note rates (Exact Wilcoxon-Mann-Whitney Test: $P < 0.01$). There is no acoustic difference that distinguishes *P. falcipes* from *P. mineira* (Table 3; Figures 4C–D and 5).

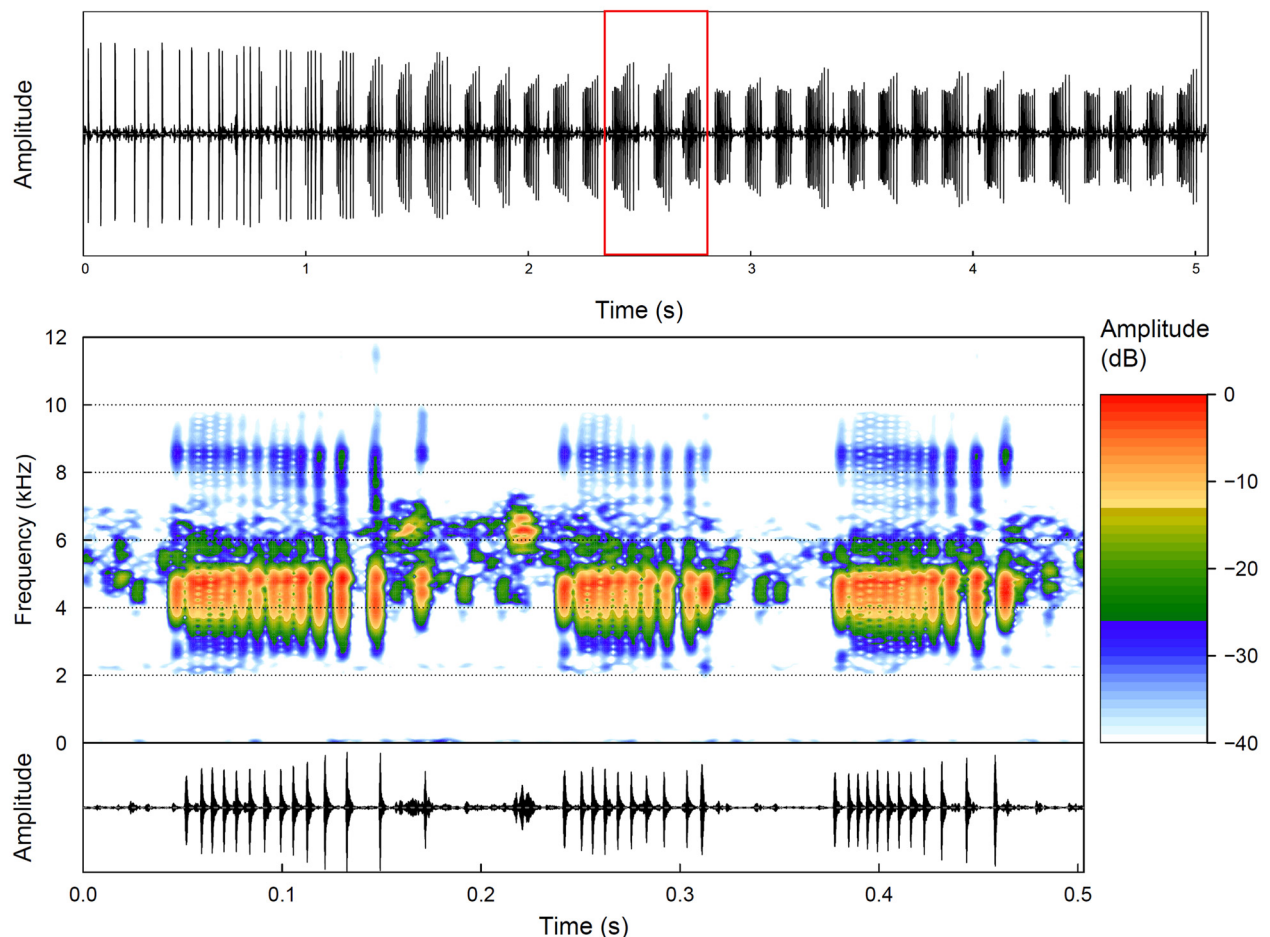


FIGURE 6. Oscillogram detailing the distinct pulsed notes of *Pseudopaludicola florencei* **sp. nov.** that can be emitted shortly after the advertisement call (above). Audiospectrogram and corresponding oscillogram detailing three of these notes (below). Note the differences in the temporal domain compared to notes of the advertisement call. Sound file: Pseudop_florenceiBA14aFSA_AAGm.wav; 19:58 h; 26 Nov 2016; air 23.5°C.

New distributional data and color variation of *P. pocoto*. We reported, for the first time, the occurrence of *P. pocoto* at an altitude of 1545 m in the *campo rupestre* (or rupestrian grasslands; Silveira *et al.* 2016) from Pico das Almas, Rio de Contas, Chapada Diamantina (Espinhaço range), state of Bahia. The previous maximum recorded altitude for this species was around 550 m, in Custódia, state of Pernambuco (Lantyer-Silva *et al.* 2016). Therefore, this new record extends about 1,000 m the altitudinal distribution of *P. pocoto*. In the phylogenetic inferences provided by Veiga-Menoncello *et al.* (2014) and Andrade *et al.* (2016), there is a specimen (ZUEC 14192; GenBank number KJ147016) from Andaraí recovered together with those of *P. pocoto*. In our analyzes this specimen was also recovered within *P. pocoto*. Although we did not hear or collect *P. pocoto* during fieldwork in Andaraí, it is possible that the two species occur sympatrically in this locality. The three specimens from Pico das Almas were also recovered also within *P. pocoto* (Figure 7), but with a small genetic distance that might reflect

population structure. Until now, we were unable to distinguish this population from the others of *P. pocoto* based on acoustic comparisons. Therefore, acoustic and genetic evidence support the occurrence of this species in Chapada Diamantina.

Furthermore, we observed that the specimens of *P. pocoto* from Pico das Almas have a darker color pattern (Figure 8) than that described for the species (Magalhães *et al.* 2014). In this population, the dorsal surface varies from dark grey to dark brown, with black or dark brown irregular blotches. Ventral surface varies from whitish to light beige, highly pigmented. The pigmentation on ventral surface may be present on throat, chest, and belly or still may be absent. Vertebral line may be absent, being present on only six males. Some specimens have enlarged white blotches on dorsum and/or dorsal surface of hind limbs, and white blotches on the region between the mouth corner and the insertion of the arms.

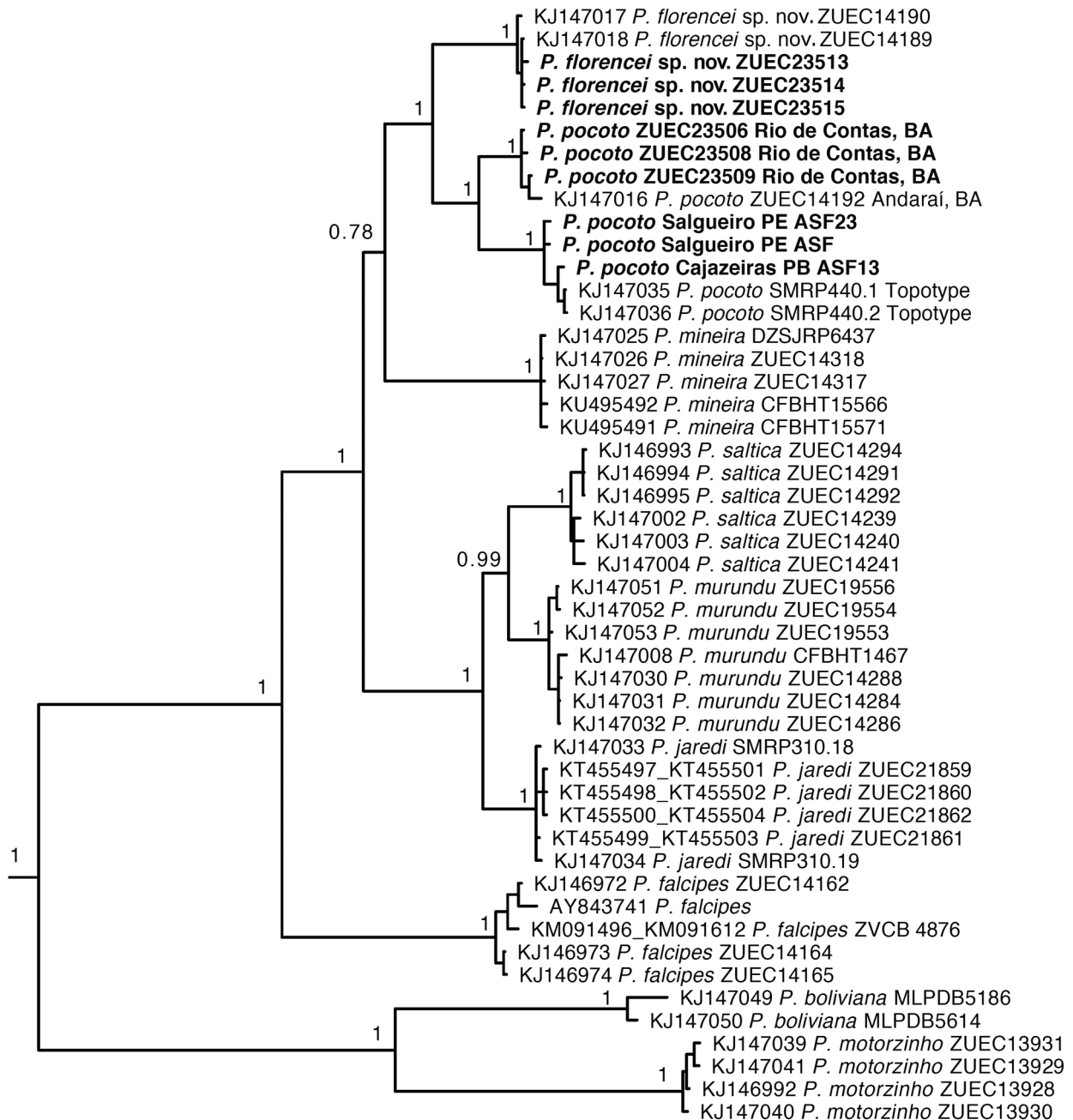


FIGURE 7. Phylogenetic relationships of the *Pseudopaludicola* species from the $2n = 22$ clade based in the 12S and 16S rDNA mitochondrial genes. Bayesian posterior probabilities are given near the nodes.

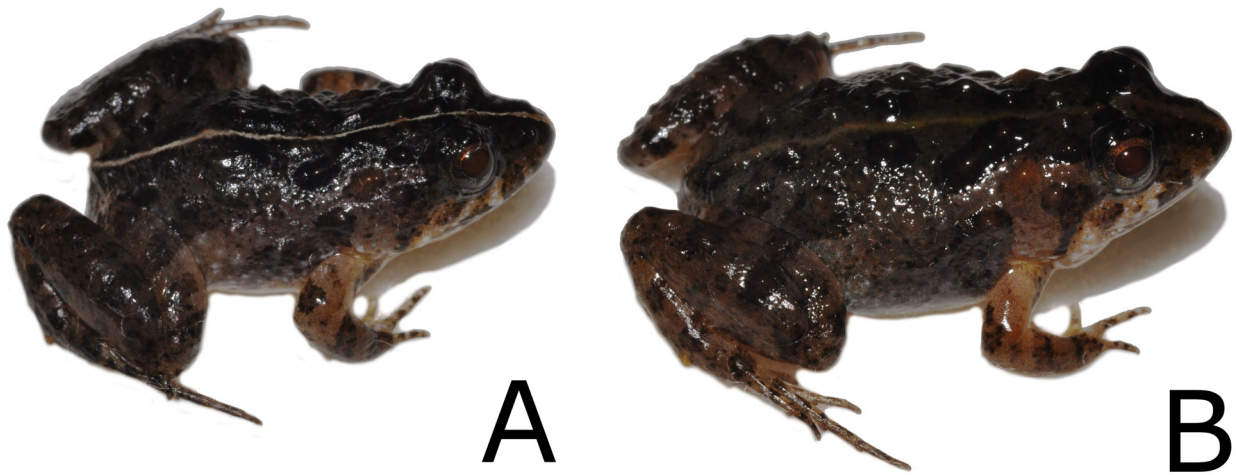


FIGURE 8. Live specimens of *Pseudopaludicola pocoto* from Pico das Almas, Rio de Contas, state of Bahia, Brazil. (A) ZUEC 23510 (adult male, call voucher, SVL = 12.3 mm); (B) ZUEC 23511 (adult female, SVL = 14.8 mm).

Discussion

Advertisement call temporal traits, mainly a non-overlapping note rate, properly distinguish the new species from its more phylogenetically close related species, *P. pocoto*. On the other hand, spectral traits were unable to distinguish them. Such difference between the applicability of temporal and spectral traits to distinguish sister species of *Pseudopaludicola* was also reported for *P. motorzinho* and *P. boliviana* (Pansonato *et al.* 2016), *P. atragula* and *P. facureae* (Pansonato *et al.* 2014), and *P. ternetzi* and *P. ameghini* (Andrade *et al.* 2017b). Therefore, we suggest that temporal traits, instead of spectral ones, are more informative for species recognition in this genus. It is noteworthy that the air temperature overlapped at the time of the field recordings of the four species (new species, *P. pocoto*, *P. falcipes* and *P. mineira*; see Table 2) and is among the variables that least explained the variation of the dataset, with low importance measures as produced by randomForest and DAPC results. Therefore, we do not attribute the found acoustic differences to air temperature differences.

Santana & Tostes (2007) reported territorial behavior in *P. mystacalis*, in which dominant calling male jumped quickly over the other males who were trying to stay in its calling site. Santana & Tostes (2007) also reported quick male fights. The territorial behavior described here for the new species is very similar to that described by Santana & Tostes (2007); however, we do not observe male fights during the field works. In most anuran species, aggressive and advertisement calls have similar dominant frequencies, but differ in temporal traits (Wells 2007). The notes of the advertisement call and the additional notes of *P. florencei* **sp. nov.** are temporally distinct, mostly by the higher number of pulses per note, shorter interpulse interval, and higher pulse rate in the additional notes. Therefore, we suggest that males of *P. florencei* **sp. nov.** emit additional notes at the end of the advertisement call when they interact aggressively with conspecific males. Indeed, future studies can better explore this issue by providing more conclusive results and discussion.

The acoustic characterization of *P. falcipes* presented here is in accordance with that of Haddad & Cardoso (1987), and provides a diagnostic support for this species with almost all congeners, except for *P. mineira*. Although these two species are allopatric (Lobo 1994; Langone *et al.* 2016) and have a high genetic divergence (Andrade *et al.* 2016), we are unable to distinguish them acoustically. Lobo (1994) considered that *P. mineira* can be distinguished from *P. falcipes* by having a wider head and abdominal fold (absent or incomplete in *P. falcipes*). There is a high overlap in the head width values from topotypes of these two species obtained by us (*P. mineira*: 4.5–4.9 mm; *P. falcipes*: 4.4–4.8 mm). However, based on our analysis, the absent or incomplete abdominal fold in *P. falcipes* distinguishes this species from *P. mineira*, therefore, it seems to be the only reliable diagnostic external morphology character for them. Given that our acoustic sample is low for *P. mineira*, future studies are need to better state that their calls are identical.

TABLE 4. Estimates of intra and interspecific uncorrected P distances for *Pseudopaludicola* 16S fragment (576 bp). Dist: distances; SD: Standard error estimates. Pflo—*P. florencei* sp. nov.; Ppoc—*P. pocoto*; Pmin—*P. mineira*; Pfal—*P. falcipes*; Pmur—*P. murundu*; Ppsal—*P. saltica*; Pjar—*P. jaredi*; Pbol—*P. boliviana*; Pmot—*P. motorzinho*; Pame—*P. ameghini*; Pter—*P. ternetzi*; Pfac—*P. facureae*; Patr—*P. atragula*; Pcan—*P. canga*; Pbar—*Pseudopaludicola* sp. (Barreirinhas, MA); Pmys—*P. mystacalis*. Values for the new species are in bold.

	Intra [dist, SD]	Inter [dist\SD]															
		Pflo	Ppoc	Pmin	Pfal	Pmur	Psal	Pjar	Pbol	Pmot	Pame	Pter	Pfac	Patr	Pcan	Pbar	Pmys
Pflo	[0.001, 0.001]		0.007	0.006	0.011	0.009	0.009	0.010	0.013	0.011	0.013	0.013	0.015	0.016	0.014	0.015	0.015
Ppoc	[0.014, 0.003]	0.041		0.007	0.011	0.009	0.010	0.010	0.013	0.012	0.013	0.014	0.015	0.016	0.016	0.016	0.016
Pmin	[0, 0]	0.043	0.042		0.010	0.008	0.009	0.009	0.012	0.011	0.013	0.013	0.015	0.016	0.014	0.015	0.015
Pfal	[0.004, 0.002]	0.078	0.078	0.070		0.010	0.013	0.012	0.012	0.013	0.013	0.013	0.015	0.016	0.014	0.014	0.014
Pmur	[0.002, 0.001]	0.054	0.055	0.045	0.070		0.007	0.006	0.011	0.012	0.011	0.012	0.014	0.015	0.014	0.014	0.014
Psal	[0.003, 0.001]	0.062	0.062	0.048	0.088	0.025		0.007	0.012	0.013	0.012	0.013	0.014	0.015	0.015	0.015	0.015
Pjar	[0, 0]	0.064	0.067	0.050	0.089	0.022	0.026		0.012	0.012	0.013	0.014	0.016	0.016	0.016	0.016	0.016
Pbol	[0.005, 0.003]	0.119	0.120	0.114	0.112	0.115	0.126	0.129		0.011	0.012	0.012	0.015	0.015	0.014	0.014	0.014
Pmot	[0.002, 0.001]	0.129	0.135	0.127	0.129	0.123	0.138	0.131	0.074		0.012	0.012	0.015	0.016	0.014	0.014	0.013
Pame	[0.003, 0.001]	0.129	0.133	0.120	0.126	0.110	0.123	0.119	0.124	0.120		0.005	0.013	0.013	0.010	0.010	0.011
Pter	[0.004, 0.001]	0.134	0.139	0.126	0.130	0.116	0.129	0.125	0.123	0.117	0.015		0.013	0.013	0.010	0.010	0.011
Pfac	[0.008, 0.003]	0.173	0.173	0.160	0.158	0.144	0.146	0.160	0.171	0.162	0.106	0.110		0.008	0.011	0.010	0.011
Patr	[0, 0]	0.173	0.171	0.158	0.164	0.142	0.141	0.159	0.180	0.172	0.102	0.106	0.037		0.011	0.010	0.011
Pcan	[0, 0]	0.154	0.157	0.151	0.137	0.128	0.141	0.151	0.155	0.154	0.086	0.082	0.084	0.081		0.005	0.011
Pbar	[0.001, 0.001]	0.155	0.150	0.147	0.135	0.123	0.138	0.146	0.152	0.154	0.081	0.080	0.074	0.072	0.018		0.010
Pmys	[0.015, 0.003]	0.145	0.152	0.135	0.141	0.127	0.136	0.152	0.146	0.154	0.091	0.093	0.091	0.091	0.072	0.069	

We analyzed one specimen of *P. mineira* from Pampulha, Belo Horizonte, state of Minas Gerais recorded by W. C. A. Bokermann on 10 February 1965. However, it is not possible to find this species nowadays in this locality (F. S. F. Leite pers. observations). In addition, this individual presents a considerable variation in some temporal traits of the advertisement call (e.g., pulse and note rates) when compared to the two males recorded in Serra do Cipó (type locality). As in previous studies (Veiga-Menoncello *et al.* 2014; Andrade *et al.* 2016), we were unable to completely resolve the phylogenetic relationship of *P. mineira* within the clade $2n = 22$, using mitochondrial data.

The intraspecific color variation we found between lowland and highland populations of *P. pocoto* was also reported for others animal groups (e.g., the fly *Drosophila melanogaster*; see Pool & Aquadro 2007; the lizard *Psammodromus algirus*; Reguera *et al.* 2014). For example, Reguera *et al.* (2014) demonstrated that the dorsal coloration of *P. algirus*, a Mediterranean mountain lizard, changes in an altitudinal gradient, which means that individuals from highland populations are more darker than individuals from mid and low-altitude populations. These authors suggested that the variation fits the “protection against UV damage hypothesis” (Clusella-Trullas *et al.* 2007). This hypothesis could also explain the variation found in specimens of *P. pocoto* from Pico das Almas, since they are darker than lower altitudinal specimens. But it is important to note that more studies are needed to better understand the morphological variation among populations of *P. pocoto*, even because this variation was never reported for any Brazilian frog species.

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APPENDIX I. Examined specimens.

Pseudopaludicola florencei **sp. nov.**: Brazil: Bahia: Andaraí: urban area: ZUEC 23512–30; Área de Proteção Ambiental Marimbus-Iraquara: UFMG 4310–16; Mutuípe: CFBH 29652; Minas Gerais: Nanuque: CFBH 33235. *Pseudopaludicola pocoto*: Brazil: Ceará: Novas Russas: CFBH 20285–7; Santa Quitéria (type locality): CFBH 26842–7; Bahia: Rio de Contas: ZUEC 25506–10, UFMG 5902–11. *Pseudopaludicola mineira*: Brazil: Minas Gerais: Jaboticatubas (Serra do Cipó): ZUEC 1570, 1572–89, 1591. *Pseudopaludicola falcipes*: Brazil: Rio Grande do Sul: Eldorado do Sul: ZUEC 11468; Novo Hamburgo: ZUEC 4876; Porto Alegre: ZUEC 10387–8, 13999–4000, 14002–3, 14005–6, 14008–9, 14016–7, 14022, 14162–66, 14168; Santana do Livramento: ZUEC 10355–6; Viamão: ZUEC 5297–9. *Pseudopaludicola ameghini*: Brazil: Mato Grosso: Chapada dos Guimarães: ZUEC 14138–9, 14141–5. *Pseudopaludicola boliviana*: Argentina: Chaco: Departamento San Fernando: Antequera: MNRJ 75298; Corrientes: Departamento General Paz: Itaibaté: MNRJ 75299; Santa Fé: Departamento General Obligado: MNRJ 75300; Formosa: Departamento Formosa: Três Marias: MNRJ 75301; Riacho Formosa: MNRJ 75303; Tatoné: MNRJ 75304–8; Formosa: Departamento Pilagas: Virasol: MNRJ 75309–12; Departamento Patiño: Colônia Salvación: MNRJ 75322. *Pseudopaludicola canga*: Brazil: Pará: Marabá: Serra dos Carajás: ZUEC 9990, 10034, 14370, 14372–4, 14378. *Pseudopaludicola facureae*: Brazil: Minas Gerais: Uberlândia: AAG-UFU 0853–5, ZUEC 13651–2, 14215, 14218–9, 14221, 14224. *Pseudopaludicola jaredi*: Brazil: Ceará: Viçosa do Ceará: CFBH 32609, 32614, 32617–25, ZUEC 20477–84; Nísia Floresta: ZUEC 21858–72. *Pseudopaludicola murundu*: Brazil: São Paulo: Águas de Santa Bárbara: ZUEC 20507–8; Rio Claro: AAG-UFU 5125–6, CFBH 8235–42, ZUEC 14284–90; Minas Gerais: Brumadinho: ZUEC 16396–8, 16442–3, 19549, 19551, 19555, 19557–78, 19560; Santana do Riacho: ZUEC 2323; São João del Rei: ZUEC 16447–52; 16455–6. *Pseudopaludicola mystacalis*: Brazil: Goiás: Itapirapuã: ZUEC 10222; Mato Grosso: Cáceres: ZUEC 10286; Chapada dos Guimarães: ZUEC 5115, 5117, 5119, 5121, 10685; Mato Grosso do Sul: Três Lagoas: ZUEC 16720, 16949; Tocantins: Formoso do Araguaia: ZUEC 10154. *Pseudopaludicola saltica*: Brazil: Mato Grosso: Chapada dos Guimarães: ZUEC 14228, 14230–3, 14235, 14239–40, 14244, 14247, 14272, 5134–46, 5854–5; Minas Gerais: Uberlândia: AAG-UFU 2308, 2630, 4598, 4631, 4735, 4707–11. *Pseudopaludicola ternetzi*: Brazil: Goiás: Uruaçu: MNRJ 445–47, 5460–62, 5442; Minas Gerais: Uberlândia: ZUEC 14036–9, 14170–1; Tocantins: Formoso do Araguaia: ZUEC 10140–3, 10145, 10147, 10150, 10153.

APPENDIX 2. Analyzed sound files (.wav format) of the four *Pseudopaludicola* species: *P. florencei* sp. nov., *P. pocoto*, *P. mineira*, and *P. faicipes*. All files deposited at Arquivo Sonoro da Coleção de Anuros da Universidade Federal de Uberlândia (AAG-UFU; Universidade Federal de Uberlândia, Brazil) or at the Fonoteca Neotropical Jacques Vieillard (FNJV; Universidade Estadual de Campinas, Brazil).

Label	Date	Municipality (state)	Time	Air (°C)	Recorder - microphone	Voucher
Pseudop_florenceiBA1aAAGm671.wav	26 November 2016	Andaraí (BA)	20:28	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA2bAAGm671.wav	26 November 2016	Andaraí (BA)	20:32	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA4aAAGm671.wav	26 November 2016	Andaraí (BA)	20:37	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA5aAAGm671.wav	26 November 2016	Andaraí (BA)	20:37	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA6aAAGm671.wav	26 November 2016	Andaraí (BA)	20:40	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA7aAAGm671.wav	26 November 2016	Andaraí (BA)	20:44	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA8aAAGm671.wav	26 November 2016	Andaraí (BA)	20:50	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA9aAAGm671.wav	26 November 2016	Andaraí (BA)	20:57	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA10aAAGm671.wav	26 November 2016	Andaraí (BA)	21:07	22.0	Marantz PMD 671 -ME67/K6 Sennheiser	-
Pseudop_florenceiBA11aFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	19:16	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23513
Pseudop_florenceiBA13bFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	19:44	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23515
Pseudop_florenceiBA14cFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	19:59	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	-
Pseudop_florenceiBA15aFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	20:10	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23520
Pseudop_florenceiBA16aFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	20:18	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	-
Pseudop_florenceiBA17aFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	20:34	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23521
Pseudop_florenceiBA18aFSA_AAAGm671.wav	26 November 2016	Andaraí (BA)	20:53	23.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23519
VHZ 109	01 March 2012	Esquina, Corrientes (AR)	-	16	M-audio Microtrack II -ME66/K6 Sennheiser	-
VHZ 135	23 September 2012	Mercedes, Corrientes (AR)	-	19.5	M-audio Microtrack II -ME66/K6 Sennheiser	-
VHZ 174	21 September 2013	San Martin, Corrientes (AR)	-	17.3	M-audio Microtrack II -ME66/K6 Sennheiser	-
FNJV 31298	07 February 1982	Nova Hamburgo (RS)	21:00	25	Uher 4000 - DB 88 Sennheiser	-

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APPENDIX 2. (Continued)

Label	Date	Municipality (state)	Time	Air (°C)	Recorder - microphone	Voucher
FNVJ 31299	17 December 1982	Viamão (RS)	22:00	24	Uher 4000 - DB 88	-
Pseudopaludicola_4A08_kwet	28 September 1996	Viamão (RS)	15:00	23.0	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudopaludicola_4A10_chorus_kwet	28 September 1996	Viamão (RS)	17:00	22.5	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudopaludicola_4B02_kwet	29 September 1996	Viamão (RS)	00:10	16.5	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudopaludicola_5B21_chorus_kwet	05 October 1996	Viamão (RS)	23:00	20	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudopaludicola_8B18_kwet	21 December 1997	Osório (RS)	22:15	25.5	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudopaludicola_9A18_kwet	02 January 1998	Viamão (RS)	21:45	23.5	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudopaludicola_10B02_kwet	21 December 1999	Candiota (RS)	01:00	21	Sony WM-D6C tape recorder - ME66/K6 Sennheiser	-
Pseudop_pocotoRioContasBA1aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	18:49	20.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23509
Pseudop_pocotoRioContasBA2aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	19:14	20.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23506
Pseudop_pocotoRioContasBA3bFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	19:42	20.5	Marantz PMD 671 -ME66/K6 Sennheiser	-
Pseudop_pocotoRioContasBA4aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	19:50	20.5	Marantz PMD 671 -ME66/K6 Sennheiser	-
Pseudop_pocotoRioContasBA5aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	19:54	20.5	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23510
Pseudop_pocotoRioContasBA6aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	19:59	19.1	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23507
Pseudop_pocotoRioContasBA7aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	20:42	19.1	Marantz PMD 671 -ME66/K6 Sennheiser	-
Pseudop_pocotoRioContasBA8aFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	21:00	19.1	Marantz PMD 671 -ME66/K6 Sennheiser	ZUEC 23508
Pseudop_pocotoRioContasBA9bFSA_AAGm671.wav	25 November 2016	Rio de Contas (BA)	21:34	19.1	Marantz PMD 671 -ME66/K6 Sennheiser	-
Pseudopaludicola sp., Pico das Almas, Rio de Contas, BA, indiv. 3.WAV	09 January 2010	Rio de Contas (BA)	-	20	Marantz PMD 660 -ME66/K6 Sennheiser	-
Pseudopaludicola sp., Pico das Almas, Rio de Contas, BA, indiv. 1.WAV	09 January 2010	Rio de Contas (BA)	-	20	Marantz PMD 660 -ME66/K6 Sennheiser	-
Pseudopaludicola sp., Pico das Almas, Rio de Contas, BA, indiv. 2.WAV	09 January 2010	Rio de Contas (BA)	-	20	Marantz PMD 660 -ME66/K6 Sennheiser	-
ASUFRN145	20 April 2009	Macatiba (RN)	17:54	24	Marantz PMD 661 - ME66/K6 Sennheiser	-

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APPENDIX 2. (Continued)

Label	Date	Municipality (state)	Time	Air (°C)	Recorder - microphone	Voucher
ASUFRN146	20 April 2009	Macaíba (RN)	18:01	24	Marantz PMD 661 - ME66/K6	-
ASUFRN153	28 April 2009	Macaíba (RN)	20:00	24.3	Sennheiser Marantz PMD 661 - ME66/K6	-
ASUFRN224	15 March 2012	Missão Velha (CE)	-	-	Sennheiser Olympus S11 with a built-in microphone	-
ASUFRN225	27 March 2008	Morada Nova (CE)	-	-	Sony TCM 5000 EV - ME66/K6	-
ASUFRN226	15 March 2008	Novas Russas (CE)	-	-	Sennheiser Sony TCM 5000 EV - ME66/K6	-
ASUFRN227	07 February 2013	Macaíba (RN)	-	23.4	Sennheiser Marantz PMD 661 - ME66/K6	-
ASUFRN612	13 April 2015	Quixadá (CE)	-	24.9	-	-
Pseudop_pocotoXiqueXiqueBA1cCBS_AA.Gm.wav	05 April 2015	Xique Xique (BA)	22:52	27	Marantz PMD 670 -ME67/K6	-
Pseudop_pocotoXiqueXiqueBA2cCBS_AA.Gm.wav	05 April 2015	Xique Xique (BA)	23:00	27	Sennheiser Marantz PMD 670 -ME67/K6	-
Pseudop_pocotoXiqueXiqueBA3bCBS_AA.Gm.wav	05 April 2015	Xique Xique (BA)	23:05	27	Sennheiser Marantz PMD 670 -ME67/K6	-
Pseudop_pocotoXiqueXiqueBA4bCBS_AA.Gm.wav	05 April 2015	Xique Xique (BA)	23:17	27	Sennheiser Marantz PMD 670 -ME67/K6	-
Pseudop_pocotoXiqueXiqueBA5cCBS_AA.Gm.wav	05 April 2015	Xique Xique (BA)	23:47	27	Sennheiser Marantz PMD 670 -ME67/K6	-
FNJV 32050	03 November 1972	Serra do Cipó (MG)	21:00	17	Uher 4000	-
FNJV 31899	10 February 1964	Serra do Cipó (MG)	-	24	Uher 4000	-
FNJV 31921	10 February 1965	Belo Horizonte (MG)	-	20.5	Uher 4000	-

All files deposited at (Abbreviation to collections): AAG sound collection (Universidade Federal de Uberlândia, Brazil); Fonoteca Neotropical Jacques Viellard (FNVJ) (Universidade Estadual de Campinas, Brazil); VHZ sound collection (Victor Hugo Zaracho, Laboratorio de Herpetología, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste); Axel Kwet sound collection (State Museum of Natural History in Stuttgart); ASUFRN (Arquivos Sonoros da Universidade Federal do Rio Grande do Norte); Felipe Leite sound collection (Universidade Federal de Viçosa). Recordings made with tape recorders were digitized at 44.1 kHz and 16-bit resolution.

APPENDIX 3. GenBank details: species, voucher number, sample locality, accession number, and authors of the sequences used for phylogenetic inferences. Bold lines are the new mitochondrial sequences provided in this study.

Species	Voucher/tissue	Group	Local	12S-tvaI-16S/[12S, 16S]
<i>Pseudopaludicola florencei</i> sp. nov.	ZUEC 23513	2N=22	Andaraí, BA	MG025519, MG025525
<i>Pseudopaludicola florencei</i> sp. nov.	ZUEC 23514	2N=22	Andaraí, BA	MG025520, MG025526
<i>Pseudopaludicola florencei</i> sp. nov.	ZUEC 23515	2N=22	Andaraí, BA	MG025521, MG025527
<i>Pseudopaludicola florencei</i> sp. nov.	ZUEC 14192	2N=22	Andaraí, BA	KJ147016
<i>Pseudopaludicola florencei</i> sp. nov.	ZUEC 14190	2N=22	Andaraí, BA	KJ147017
<i>Pseudopaludicola florencei</i> sp. nov.	ZUEC 14189	2N=22	Andaraí, BA	KJ147018
<i>Pseudopaludicola pocoto</i>	ZUEC 23506	2N=22	Pico das Almas, Rio de Contas, BA	MG025522, MG025528
<i>Pseudopaludicola pocoto</i>	ZUEC 23508	2N=22	Pico das Almas, Rio de Contas, BA	MG025523, MG025529
<i>Pseudopaludicola pocoto</i>	ZUEC 23509	2N=22	Pico das Almas, Rio de Contas, BA	MG025524, MG025530
<i>Pseudopaludicola pocoto</i>	3660 MSFH	2N=22	Salgueiro, PE	MG025531 [16S]
<i>Pseudopaludicola pocoto</i>	ASF	2N=22	Salgueiro, PE	MG025532 [16S]
<i>Pseudopaludicola pocoto</i>	3669 MSFH	2N=22	Cajazeiras, PB	MG025533 [16S]
<i>Pseudopaludicola pocoto</i>	SMRP 440.1	2N=22	Novas Russas, CE	KJ147035
<i>Pseudopaludicola pocoto</i>	SMRP 440.2	2N=22	Novas Russas, CE	KJ147036
<i>Pseudopaludicola mineira</i>	DZSJP 6437	2N=22	Serra do Cipó, MG	KJ147025
<i>Pseudopaludicola mineira</i>	ZUEC 14318	2N=22	Serra do Cipó, MG	KJ147026
<i>Pseudopaludicola mineira</i>	ZUEC 14317	2N=22	Serra do Cipó, MG	KJ147027
<i>Pseudopaludicola motorzinho</i>	ZUEC 13931	2N=22	Poconé, MT	KJ147039
<i>Pseudopaludicola motorzinho</i>	ZUEC 13930	2N=22	Poconé, MT	KJ147040
<i>Pseudopaludicola motorzinho</i>	ZUEC 13929	2N=22	Poconé, MT	KJ147041
<i>Pseudopaludicola motorzinho</i>	ZUEC 13928	2N=22	Poconé, MT	KJ146992
<i>Pseudopaludicola murundu</i>	CFBH-T1467	2N=22	Rio Claro, SP	KJ147008
<i>Pseudopaludicola murundu</i>	ZUEC 14288	2N=22	Rio Claro, SP	KJ147030
<i>Pseudopaludicola murundu</i>	ZUEC 14284	2N=22	Rio Claro, SP	KJ147031
<i>Pseudopaludicola murundu</i>	ZUEC 14286	2N=22	Rio Claro, SP	KJ147032
<i>Pseudopaludicola murundu</i>	ZUEC 19556	2N=22	Serra da Moeda, MG	KJ147051
<i>Pseudopaludicola murundu</i>	ZUEC 19554	2N=22	Serra da Moeda, MG	KJ147052
<i>Pseudopaludicola murundu</i>	ZUEC 19553	2N=22	Serra da Moeda, MG	KJ147053
<i>Pseudopaludicola saltica</i>	ZUEC 14294	2N=22	Uberlândia, MG	KJ146993

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APPENDIX 3. (Continued)

Species	Voucher/tissue	Group	Local	12S-tval-16S/[12S, 16S]
<i>Pseudopaludicola saltica</i>	ZUEC 14291	2N=22	Uberlândia, MG	KJ146994
<i>Pseudopaludicola saltica</i>	ZUEC 14292	2N=22	Uberlândia, MG	KJ146995
<i>Pseudopaludicola saltica</i>	ZUEC 14239	2N=22	Chapada dos Guimarães, MT	KJ147002
<i>Pseudopaludicola saltica</i>	ZUEC 14240	2N=22	Chapada dos Guimarães, MT	KJ147003
<i>Pseudopaludicola saltica</i>	ZUEC 14241	2N=22	Chapada dos Guimarães, MT	KJ147004
<i>Pseudopaludicola boliviana</i>	MLP-DB 5186	2N=22	Corrientes, Argentina	KJ147049
<i>Pseudopaludicola boliviana</i>	MLP-DB 5614	2N=22	Chaco, Argentina	KJ147050
<i>Pseudopaludicola falcipectes</i>	ZVCB 4876	2N=22		KM091496, KM091612
<i>Pseudopaludicola falcipectes</i>	ZUEC 14162	2N=22	Porto Alegre, RS	KJ146972
<i>Pseudopaludicola falcipectes</i>	ZUEC 14164	2N=22	Porto Alegre, RS	KJ146973
<i>Pseudopaludicola falcipectes</i>	ZUEC 14165	2N=22	Porto Alegre, RS	KJ146974
<i>Pseudopaludicola jaredi</i>	SMRP 310.18	2N=22	Viçosa do Ceará, CE	KJ147033
<i>Pseudopaludicola jaredi</i>	SMRP 310.19	2N=22	Viçosa do Ceará, CE	KJ147034
<i>Pseudopaludicola jaredi</i>	ZUEC 21859	2N=22	Nisia Floresta, RN	KT455497, KT455501
<i>Pseudopaludicola jaredi</i>	ZUEC 21860	2N=22	Nisia Floresta, RN	KT455498, KT455502
<i>Pseudopaludicola jaredi</i>	ZUEC 21861	2N=22	Nisia Floresta, RN	KT455499, KT455503
<i>Pseudopaludicola jaredi</i>	ZUEC 21862	2N=22	Nisia Floresta, RN	KT455500, KT455504
<i>Pseudopaludicola ameghini</i>	ZUEC 14140	2N=20	Chapada dos Guimarães, MT	KJ146975
<i>Pseudopaludicola ameghini</i>	UFMT 8543	2N=20	Chapada dos Guimarães, MT	KJ146976
<i>Pseudopaludicola ameghini</i>	ZUEC 14138	2N=20	Chapada dos Guimarães, MT	KJ146977
<i>Pseudopaludicola ameghini</i>	ZUEC 13924	2N=20	Vila Bela da Santíssima Trindade, MT	KJ147045
<i>Pseudopaludicola ameghini</i>	ZUEC 13925	2N=20	Vila Bela da Santíssima Trindade, MT	KJ147046
<i>Pseudopaludicola ameghini</i>	ZUEC 13923	2N=20	Vila Bela da Santíssima Trindade, MT	KJ147047
<i>Pseudopaludicola ameghini</i>	ZUEC 14146	2N=20	Vila Bela da Santíssima Trindade, MT	KJ147048
<i>Pseudopaludicola ternetzi</i>	ZUEC 14169	2N=20	Uberlândia, MG	KJ146986
<i>Pseudopaludicola ternetzi</i>	ZUEC 14171	2N=20	Uberlândia, MG	KJ146987
<i>Pseudopaludicola ternetzi</i>	ZUEC 14172	2N=20	Uberlândia, MG	KJ147042
<i>Pseudopaludicola ternetzi</i>	SMRP 266.6	2N=20	Uberlândia, MG	KJ147043

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APPENDIX 3. (Continued)

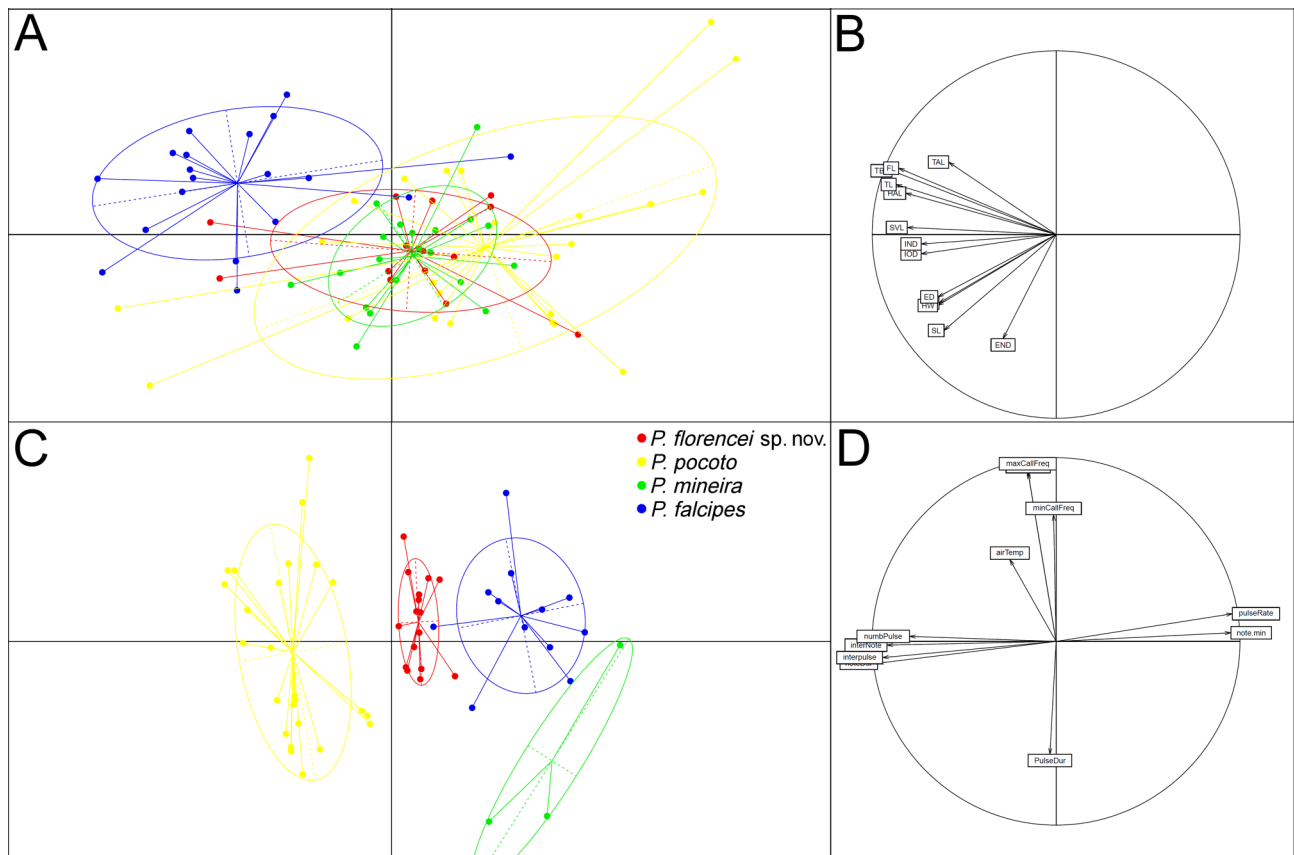
Species	Voucher/tissue	Group	Local	12S-tval-16S/[12S, 16S]
<i>Pseudopaludicola ternetzi</i>	DZSJRP 6456	2N=20	Icém, SP	KJ147010
<i>Pseudopaludicola ternetzi</i>	DZSJRP 6446	2N=20	Icém, SP	KJ147011
<i>Pseudopaludicola ternetzi</i>	DZSJRP 6445	2N=20	Icém, SP	KJ147012
<i>Pseudopaludicola ternetzi</i>	DZSJRP 8723	2N=20	Icém, SP	KJ147029
<i>Pseudopaludicola ternetzi</i>	UFMT 15753	2N=20	Uruaçu, GO	KJ147054
<i>Pseudopaludicola ternetzi</i>	UFMT 15754	2N=20	Uruaçu, GO	KJ147055
<i>Pseudopaludicola ternetzi</i>	UFMT 18061	2N=20	Uruaçu, GO	KJ147056
<i>Pseudopaludicola canga</i>	ZUEC 14333	2N=18	Serra dos Carajás, PA	KJ146988
<i>Pseudopaludicola canga</i>	ZUEC 14334	2N=18	Serra dos Carajás, PA	KJ146989
<i>Pseudopaludicola canga</i>	ZUEC 14335	2N=18	Serra dos Carajás, PA	KJ146990
<i>Pseudopaludicola facureae</i>	ZUEC 14173	2N=18	Uberlândia, MG	KJ146978
<i>Pseudopaludicola facureae</i>	ZUEC 14174	2N=18	Uberlândia, MG	KJ146979
<i>Pseudopaludicola facureae</i>	ZUEC 14175	2N=18	Uberlândia, MG	KJ146980
<i>Pseudopaludicola atragula</i>	DZSJRP 8727	2N=18	Icém, SP	KJ146996
<i>Pseudopaludicola atragula</i>	DZSJRP 8728	2N=18	Icém, SP	KJ146997
<i>Pseudopaludicola atragula</i>	DZSJRP 8747	2N=18	Icém, SP	KJ146998
<i>Pseudopaludicola</i> sp.	ZUEC 13858	2N=18	Barreirinhas, MA	KJ147013
<i>Pseudopaludicola</i> sp.	ZUEC 13859	2N=18	Barreirinhas, MA	KJ147014
<i>Pseudopaludicola</i> sp.	ZUEC 13860	2N=18	Barreirinhas, MA	KJ147015
<i>Pseudopaludicola mystacalis</i>	ZUEC 14147	2N=16	Cuiabá, MT	KJ146983
<i>Pseudopaludicola mystacalis</i>	ZUEC 14148	2N=16	Cuiabá, MT	KJ146984
<i>Pseudopaludicola mystacalis</i>	ZUEC 14149	2N=16	Cuiabá, MT	KJ146985
<i>Pseudopaludicola mystacalis</i>	ZUEC 14160	2N=16	Poconé, MT	KJ146991
<i>Pseudopaludicola mystacalis</i>	ZUEC 14128	2N=16	Uberlândia, MG	KJ146999
<i>Pseudopaludicola mystacalis</i>	ZUEC 14129	2N=16	Uberlândia, MG	KJ147000
<i>Pseudopaludicola mystacalis</i>	ZUEC 14130	2N=16	Uberlândia, MG	KJ147001
<i>Pseudopaludicola mystacalis</i>	ZUEC 13836	2N=16	Barreirinhas, MA	KJ147005
<i>Pseudopaludicola mystacalis</i>	ZUEC 13837	2N=16	Barreirinhas, MA	KJ147006
<i>Pseudopaludicola mystacalis</i>	ZUEC 13838	2N=16	Barreirinhas, MA	KJ147007

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APPENDIX 3. (Continued)

Species	Voucher/tissue	Group	Local	12S-tval-16S/[12S, 16S]
<i>Pseudopaludicola mystacalis</i>	CFBH-T1374	2N=16	Paranaíba, MS	KJ147009
<i>Pseudopaludicola mystacalis</i>	ZUEC 13872	2N=16	Barreirinhas, MA	KJ147019
<i>Pseudopaludicola mystacalis</i>	ZUEC 20542	2N=16	Barreirinhas, MA	KJ147020
<i>Pseudopaludicola mystacalis</i>	ZUEC 13873	2N=16	Barreirinhas, MA	KJ147021
<i>Pseudopaludicola mystacalis</i>	DZSIRP 8704	2N=16	Santa Fé do Sul, SP	KJ147022
<i>Pseudopaludicola mystacalis</i>	DZSIRP 8694	2N=16	Santa Fé do Sul, SP	KJ147023
<i>Pseudopaludicola mystacalis</i>	DZSIRP 8696	2N=16	Santa Fé do Sul, SP	KJ147024
<i>Pseudopaludicola mystacalis</i>	DZSIRP 8724	2N=16	Icém, SP	KJ147028
<i>Pseudopaludicola mystacalis</i>	DZSIRP 8726	2N=16	Icém, SP	KJ147037
<i>Pseudopaludicola mystacalis</i>	DZSIRP 8725	2N=16	Icém, SP	KJ147038
<i>Pseudopaludicola mystacalis</i>	ZUEC 13869	2N=16	Urbano Santos - São Felipe, MA	KJ146981
<i>Pseudopaludicola mystacalis</i>	CFBH 35858	2N=16	Urbano Santos - São Felipe, MA	KJ146982
<i>Pseudopaludicola mystacalis</i>	ZUEC 13868	2N=16	Urbano Santos - São Felipe, MA	KJ147044
OUT groups				
<i>Odontophrynus americanus</i>				AY843704
<i>Alsodes gargola</i>				AY843565
<i>Crossodactylus schmidti</i>				AY843579
<i>Adenomera hylaetactyla</i>				DQ283063
<i>Leptodactylus pentadactylus</i>				AY326017
<i>Paratelmatobius gaigeae</i>				EU224397
<i>Edalorhina perezii</i>				AY843585
<i>Engystomops pustulosus</i>				DQ337249
<i>Physalaemus nattereri</i>				AY326020
<i>Pleurodema brachyops</i>				AY843733

APPENDIX 4. Scatterplots and respective scatter diagrams of a correlation circle of the two first principal component scores (PCs) based on morphometric (A and B) and acoustic datasets (C and D). Variance explained by the axes in A: PC1 = 51.7 % and PC2 = 11.9 %; variance explained by the axes in C: PC1 = 47.7 % and PC2 = 25.6 %.



APPENDIX 5. Phylogenetic relationships of the species of the genus *Pseudopaludicola* based on the 12S and 16S rDNA mitochondrial genes. Bayesian posterior probabilities are given near the nodes.

