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A new species of *Pseudopaludicola* (Anura, Leptodactylidae, Leiuperinae) from the state of Minas Gerais, Brazil

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Abstract. The genus of Neotropical frogs *Pseudopaludicola* Miranda-Ribeiro, 1926 includes 23 species, which occur throughout South America. Herein we describe a new species of *Pseudopaludicola* from the central region of the state of Minas Gerais (southeastern Brazil). This new species is distinguished by the adult morphology, the advertisement call, and molecular data. It is diagnosed mainly by its small size, terminal phalanges knobbed (lack of any expansion of the digital tips), proportionally short hind limbs, and its advertisement call composed of series of two-pulsed notes, emitted at a high repetition rate. In addition, we report the occurrence of the new species in the southern foothills of the Serra do Cipó National Park, at elevations lower than 800 m above sea level (a.s.l). The sister species of this new taxon is *P. mineira* Lobo, 1994, which occurs in rupestrian grasslands above 1200 m a.s.l. at the Serra do Cipó National Park and in Serra do Cabral, both in the state of Minas Gerais, Brazil. We also redescribe the advertisement call of *P. mineira* based on recordings from topotypical males and compare it to calls of related species.

Keywords. Advertisement call, integrative taxonomy, *Pseudopaludicola matuta* sp. nov., *Pseudopaludicola mineira*, Serra do Cipó National Park.

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Introduction

The monophyletic genus *Pseudopaludicola* Miranda-Ribeiro, 1926 comprises 23 species (Frost 2018) that occur throughout South America, east of the Andes (Lynch 1989). Lynch (1989) proposed the *P. pusilla* group that includes nowadays five species: *P. boliviana* Parker, 1927, *P. ceratophyes* Rivero & Serna, 1985, *P. llanera* Lynch, 1989, *P. pusilla* (Ruthven, 1916) and *P. motorzinho* Pansonato, Veiga-Menoncello, Mudrek, Jansen, Recco-Pimentel, Martins & Strüssmann, 2016, all sharing T-shaped terminal phalanges. Lobo (1995), Veiga-Menoncello *et al.* (2014) and Andrade *et al.* (2016, 2018), based on phylogenetic analyses, recovered *Pseudopaludicola* and the *P. pusilla* group as clades. Toledo (2010) proposed the *P. saltica* group, which contains currently three species, sharing the synapomorphy of relatively long hind limbs: *P. saltica* (Cope, 1887), *P. murundu* Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-Pimentel & Haddad, 2010, and *P. jaredi* Andrade, Magalhães, Nunes-de-Almeida, Veiga-Menoncello, Santana, Garda, Loebmann, Recco-Pimentel, Giaretta & Toledo, 2016. This group was later corroborated by the phylogenetic analysis of Veiga-Menoncello *et al.* (2014). In the last eight years 12 Brazilian species of the genus *Pseudopaludicola* were described, suggesting that species diversity within this genus is still incompletely understood (Toledo *et al.* 2010; Pansonato *et al.* 2012, 2014, 2016; Carvalho 2012; Andrade & Carvalho 2013; Roberto *et al.* 2013; Magalhães *et al.* 2014; Andrade *et al.* 2016, 2018; Cardozo *et al.* 2018).

Pseudopaludicola mineira Lobo, 1994 is a species known only from the type locality, in the *Campo Rupestre* (rupestrian grasslands) of the Serra do Cipó National Park, the southern portion of the Espinhaço mountain range, and in Serra do Cabral, municipality of Buenópolis, both in the state of Minas Gerais, southeastern Brazil (Lobo 1994). The advertisement call of this species was described based on two males from its type locality (Pereira & Nascimento 2004). Pereira & Nascimento (2004) compared *P. mineira* acoustically to *P. falcipes* (Hensel, 1867), *P. mystacalis* (Cope, 1887), *P. saltica*, and *P. facureae* Andrade & Carvalho, 2013 (referred as *P.* aff. *saltica*). Veiga-Menoncello *et al.* (2014) and Andrade *et al.* (2016, 2018) were unable to completely resolve the phylogenetic relationships of *P. mineira* within the clade with species that presented the karyotype number of 2n = 22.

Regional sympatry or even syntopic occurrence of two or more species of *Pseudopaludicola* has been reported in the literature (e.g., Giaretta & Facure 2009; Pansonato *et al.* 2013). Carvalho (2012) briefly reported the existence of a population of *Pseudopaludicola* sp. (*P.* aff. *mineira*) that occurs in syntopy with *P. giarettai* Carvalho, 2012 at its type locality (municipality of Curvelo, Minas Gerais, Brazil). In addition, we found a second population of the same species in the southern foothills of the Serra do Cipó National Park, Minas Gerais, Brazil. We, therefore, evaluated it based on morphological, bioacoustic, and genetic data in order to assess its taxonomic status, which revealed this to be an undescribed species. Thus, we describe it as a new taxon, sister to *P. mineira*. In order to strengthen the diagnosis of the new species, we also provide novel acoustic data for topotypical males of *P. mineira*.

Material and Methods

Reference specimens

Examined individuals of the new species were collected under permit #62146-1 issued by SISBio/Instituto Chico Mendes de Conservação da Biodiversidade. Accordingly to current legislation, the access to the National System for the Management of Genetic Heritage and Associated Traditional Knowledge was registered (SISGen #A2FCFCC). Individuals were killed by applying 5% lidocaine to their skin. We collected muscle tissue for genetic analyses. After that, we fixed specimens in 10% formalin and transferred them to 70% ethanol for permanent storage. Type specimens are deposited in the amphibian collection of the Museu de Zoologia "Adão José Cardoso" (ZUEC) at the Universidade Estadual de Campinas (Unicamp), Campinas, state of São Paulo, and in the collection of frogs of the Museu de Biodiversidade do Cerrado (AAG-UFU) at the Universidade Federal de Uberlândia (UFU), Uberlândia, state of Minas Gerais, both in Brazil. The new species hypothesis follows to the General Lineage Concept, which treats species as separately evolving metapopulation lineages (de Queiroz 1998, 2007).

Morphometric analyses

We measured 16 adult males and five adult females of the new species from the municipality of Curvelo, Minas Gerais, Brazil. We measured three adult males and three adult females of the new species from the southern foothills of the Serra do Cipó National Park (lowland area, 797 m a.s.l.), municipality of Santana do Riacho, Minas Gerais, Brazil (Fig. 1). For comparison with its most closely related species, we measured all four types (MZUSP 56858–61) and 18 topotypes of *P. mineira* designated by Lobo (1994) (MZUSP 56768–72, 56867, 57719–23, 57725–7, 57729–30). In addition, we measured additional 20 topotypes of *P. mineira* from the highlands of the Serra do Cipó National Park. Further details on examined specimens are given in Appendix I.

We measured specimens under a stereomicroscope Zeiss Stemi DV4 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).

Bioacoustics

We recorded calls with two digital recorders at a sampling rate of 44.1 kHz and a sample size of 16 bits: Marantz PMD 661MKII (Marantz, Japan) and M-audio Microtrack II (M-audio, USA), both with Sennheiser ME66/K6 directional microphone (Sennheiser electronic GmbH & Co. KG, Germany). Directional microphones were positioned about 1.5 m from the calling male. We analysed calls with Raven Pro v. 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%" and "Frequency 95%" functions; and were considered as the minimum and maximum frequencies (Hz), respectively.

Call duration was considered the total time that males spent emitting the series of two-pulsed notes. Pulse rate was quantified as the number of pulses of a note divided by the duration of this same note. 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 of the new species considering mean values of all individual males, whereas the range presented (variation) encompasses the minimum and maximum values for all call samples. For each advertisement call, we analysed 20 notes and all pulse/interpulse intervals that comprise these notes. We also assessed the within-male call variation through the coefficients of variation [CV = (SD/mean) × 100]. We considered only the stereotyped two-pulsed

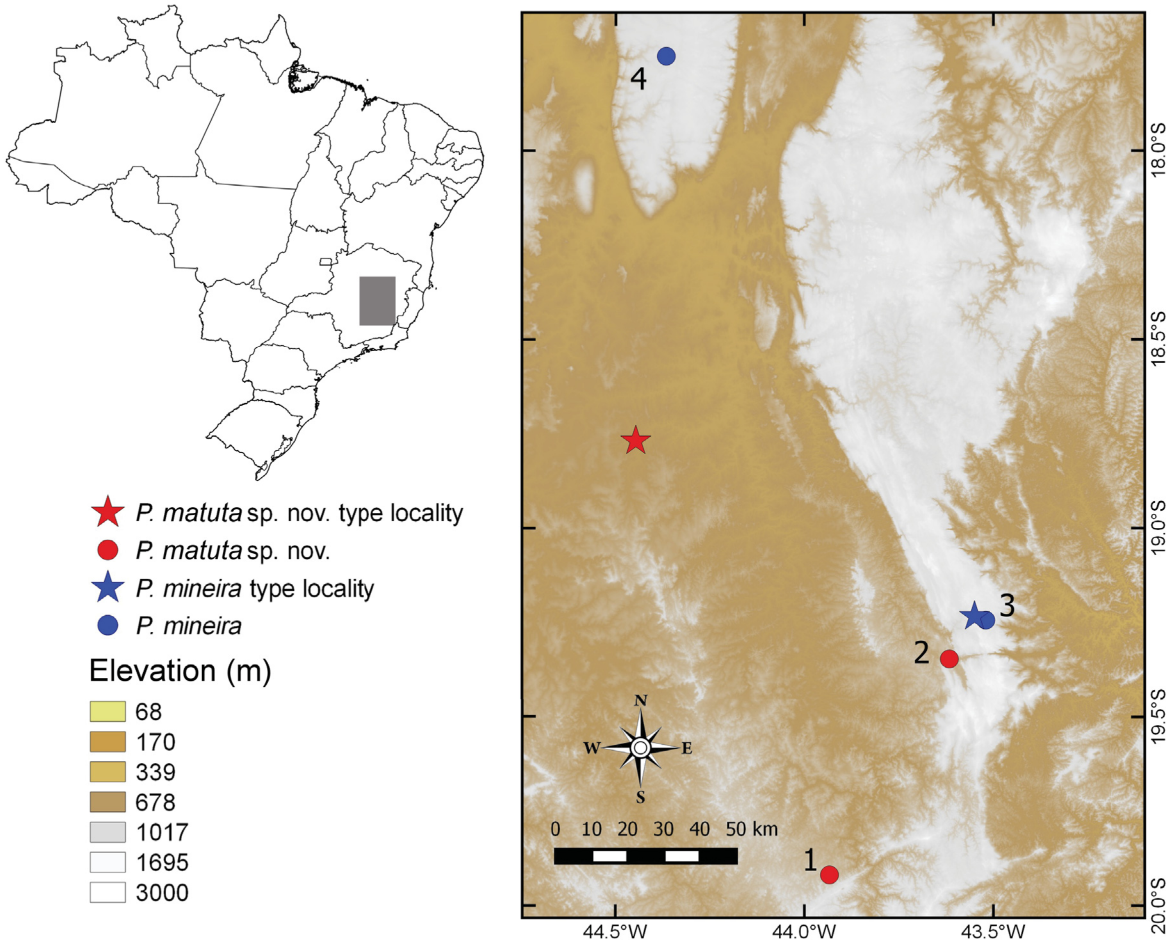

Fig. 1. Hypsometric map of the region highlighted on the map of Brazil (left), showing the samples of *Pseudopaludicola matuta* sp. nov. included in our molecular, morphological, and acoustic comparisons. The type locality of *P. matuta* sp. nov. in the municipality of Curvelo, is indicated with a red star. The map also highlights the known distribution (blue symbols) of *P. mineira* Lobo, 1994 and our record for this species. The type locality of *P. mineira* in the Serra do Cipó National Park is indicated with a blue star (Lobo 1994). Numbers: (1) Belo Horizonte; (2) lowlands of the Serra do Cipó National Park (near the park's headquarters, in a lowland area), municipality of Santana do Riacho; (3) highlands of the Serra do Cipó National Park, municipality of Santana do Riacho (our data); (4) Serra do Cabral, municipality of Buenópolis (Lobo 1994). All represented points are in the state of Minas Gerais, Brazil. Legend also contains the altitude in meters of the hypsometric map.

notes to calculate the CV values of the species, not the introductory notes. The average was calculated for each trait from individual male CV values. Acoustic traits with low within-male CV values (usually less than 5%) were classified as static and traits with higher values (usually greater than 12%) as dynamic (Gerhardt 1991). We established one note per minute measurement for each analysed male; therefore, we checked the variability of this temporal trait through between-male coefficient of variation. Gerhardt (1991) reported that between-male coefficients of variation of static acoustic properties were less than 11%, whereas coefficients of variation of dynamic properties exceeded 15%.

We recorded 23 males of the new species in the municipality of Curvelo, state of Minas Gerais. We recorded seven males of the new species from the southern foothills of the Serra do Cipó National Park (lowland area, 797 m a.s.l.), municipality of Santana do Riacho, Minas Gerais. In addition, we analysed one recording (FNJV 31921) of a male from Pampulha, municipality of Belo Horizonte, Minas Gerais, identified previously as *P. mineira* and recorded by Werner C. A. Bokermann on 10 Feb. 1965. For acoustic comparison, we recorded and analysed seven topotypical males of *P. mineira* from rupestrian grasslands (1294 m a.s.l.) of the Serra do Cipó National Park. Additionally, we also analysed two recordings (FNJV 32050, 31899) of two topotypical males of *P. mineira* recorded by Werner C. A. Bokermann and Ivan Sazima.

The sound files are deposited in the Arquivo Sonoro da Coleção de Anuros da Universidade Federal de Uberlândia at UFU, Minas Gerais, Brazil and in the Fonoteca Neotropical Jacques Vielliard (FNJV) at Universidade Estadual de Campinas, São Paulo, Brazil. Voucher specimens for call recordings and further details of the analysed sound files are given in Supplementary File 1. Voucher specimens for call recordings and GenBank are ZUEC 24303–4, 24308, 24324, and 24327 (new species); and ZUEC 24330–1 (*P. mineira*).

Statistical analysis

We sought for discrimination between the new species and *P. mineira* by applying the randomForest (RF) model (randomForest v. 4.6-12 package; Liaw & Wiener 2002) considering the morphometric and acoustic data separately. The randomForest algorithm (Breiman 2001) constructs many (generally 1000) 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 (further details in Liaw & Wiener 2002). The RF results include an estimate of distances among the objects and a Multidimensional Scaling Analysis (MDS). This analysis can be plotted with the "proximityPlot" function of the package 'rfPermute' v. 2.1.5 (Archer 2016), which also allows the results to be displayed graphically. Analyses were conducted in R.

For the multivariate analysis and statistical tests we used all the morphometric features of adult males detailed above. For the acoustic analyses, we used note duration, internote interval, number of pulses per note, pulse duration, interpulse interval, pulse rate, note rate, and peak 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 analyses. For the multivariate analysis and statistical tests we used the averaged values of each adult male. Acoustic and morphometric traits were tested for statistical significance of differences between species using the Exact Wilcoxon Mann Whitney Rank Sum Test, function "wilcox_test" of the package Coin (Resampling Statistics model; Hothorn *et al.* 2008) in R.

Phylogenetic inference and genetic distances

We generated new sequence data for 11 specimens: nine of the new species (four from the type locality and five from southern foothills of the Serra do Cipó National Park), and two specimens of *P. mineira* from the type locality (Supplementary File 2). We extracted total DNA using a standard ammonium acetate precipitation method (Maniatis *et al.* 1982; adapted as in Lyra *et al.* 2017) and amplified two mitochondrial fragments (12S rRNA and 16S rRNA genes). For the 12S fragment, we used primers 12S-L48 and 12S-H978 (Walker *et al.* 2018), and for the 16S fragment, primers 12L13 (Feller & Hedges 1998), H10 (Hedges 1994), 16Sa-L and 16Sb-H (Kessing *et al.* 1989). PCR products were purified by enzymatic reaction and sent to Macrogen Inc. South Korea for sequencing. The new DNA sequences were quality trimmed and assembled using Geneious V.6 (Biomatter).

For phylogenetic inference we used MAFFT v. 7.25 (Katoh & Standley 2013; default parameters) to align the new sequences with the sequences available for *Pseudopaludicola* spp., including 10 outgroups (Supplementary File 2). Phylogenetic analyses were performed under Bayesian Inference (BI) and Maximum Likelihood (ML) with the software MrBayes v. 3.2.6 (Ronquist *et al.* 2012) and PhyML v. 3.0 (Guindon *et al.* 2010), respectively.

The BI analysis was performed using two independent runs of 10 million generations, starting with random trees and four Markov chains (one cold), sampled every 1000 generations. We used GTR + Gamma as evolution model, selected in PartitionFinder (Lanfear *et al.* 2012) under the Bayesian Information Criterion (BIC). Twenty-five percent of generations and trees were discarded as 'burnin' and runs were considered convergent if standard deviation of split frequencies was lower than 0.01. Analyses were run at the CIPRES Science Gateway (Miller *et al.* 2010). The ML analysis was conducted online using Smart Model Selection option (Lefort *et al.* 2017; available at http://www.atgc-montpellier.fr/phyml-sms/), which includes automatic selection of the best-fit substitution model for a given alignment. Tree searching was improved using Nearest Neighbor Interchanges (NNIs) and branch support estimated using aLRT SH-like tests. 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, since this fragment was available for all samples included in the analyses. Estimates were done using the alignment generated in MAFFT v.7.25 imported in MEGA v.6 (Tamura *et al.* 2013), and using uncorrected p-distance with pairwise deletion and variance estimated by bootstrap method (1000 replicates).

Results

Class Amphibia Linnaeus, 1758
Order Anura Fischer von Waldheim, 1813
Family Leptodactylidae Werner, 1896 (1838)
Subfamily Leiuperinae Bonaparte, 1850
Genus *Pseudopaludicola* Miranda-Ribeiro, 1926

Pseudopaludicola matuta sp. nov.

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Figs 2–5, Tables 1–4

Pseudopaludicola sp. – Carvalho (2012): 52, Natural History Section.
Pseudopaludicola mineira from Pampulha, Belo Horizonte, Minas Gerais – Andrade *et al.* 2018: 87, Discussion Section.

Diagnosis

Pseudopaludicola matuta sp. nov. is assigned to the genus *Pseudopaludicola* by having a hypertrophied antebrachial tubercle (see Lynch 1989, Lobo 1995). The new species is characterized by the following combination of characters: (1) small size (SVL 12.8–14.2 mm in adult males); (2) absence of both, enlarged palpebral tubercles and an enlarged conical tubercle on heel; (3) relatively short hind limbs (tibiotarsal articulation only reaching the posterior margin of the eye); and (4) advertisement call composed of regular series of stereotyped two-pulsed notes, with notes having a short duration and emitted at a higher rate (notes per minute).

Differential diagnosis

Pseudopaludicola matuta sp. nov. is promptly distinguished from the species of the *P. saltica* group (*P. saltica*, *P. murundu*, and *P. jaredi*) by having relatively short hind limbs; i.e., the tibiotarsal articulation reaches the posterior margin of the eye in the new species, whereas it reaches the tip of the snout in species of the *P. saltica* group (Andrade *et al.* 2016).

Pseudopaludicola matuta sp. nov. is distinguished from species of the *P. pusilla* group (*P. boliviana*, *P. ceratophyes*, *P. llanera*, *P. pusilla*, and *P. motorzinho*) by the absence of either T-shaped terminal phalanges or expanded toe tips (disks or pads). The tips of the phalanges of the new species are not T-shaped; they are similar in shape to those of *P. falcipes* (fig. 2B in Cardozo & Suárez 2012). The new species is also distinguished from *P. ceratophyes* by the absence of an enlarged palpebral tubercle (Lynch 1989). *Pseudopaludicola matuta* sp. nov. differs from *P. boliviana* and *P. motorzinho* by the absence of an enlarged, conical tubercle on the heel (Pansonato *et al.* 2016).

Pseudopaludicola matuta sp. nov. has an advertisement call composed of series of notes with two non-concatenated pulses each (Fig. 4A–B); therefore, it is promptly distinguished from all species with advertisement call composed of non-pulsed notes: *P. canga* Giaretta & Kokubum, 2003 (Giaretta & Kokubum 2003; Pansonato *et al.* 2012; Carvalho *et al.* 2015a), *P. giarettai* (Carvalho 2012; Carvalho *et al.* 2015b), *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strüssmann & Martins, 2012 (Pansonato *et al.* 2012), *P. facureae* (Andrade & Carvalho 2013; Carvalho *et al.* 2015a), and *P. parnaiba* Roberto, Cardozo & Ávila, 2013 (Roberto *et al.* 2013; Carvalho *et al.* 2015a); and from all 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 & Strüssmann, 2016 [3–12; Pansonato *et al.* 2016], and *P. motorzinho* [2–6; Pansonato *et al.* 2016].

Pseudopaludicola matuta sp. nov. is distinguished from other congeners with notes with non-concatenated pulses (values within square brackets "[ ]") by the following acoustic traits: *P. ameghini* (Cope, 1887) has longer note duration (27–52 [60–121] ms), higher number of pulses per note (2 [3–5]), and lower note rate (542–1101 [348–452] notes per minute) (Andrade *et al.* 2017a); *P. ternetzi* Miranda-Ribeiro, 1937 has higher number of pulses per note [3–6] (Andrade *et al.* 2017a); *P. atragula* Pansonato, Mudrek, Veiga-Menoncello, Rossa-Feres, Martins & Strüssmann, 2014 has longer note duration [300–700 ms], higher number of pulses per note [9–36], and lower note rate [42–98 notes per minute] (Pansonato *et al.* 2014). The three species of the *P. saltica* species group have lower note rate [180–480 notes per minute, combined values], and vary highly the number of pulses in their notes (2–7 pulses per note, combined values; Andrade *et al.* 2016). *Pseudopaludicola falcipes* and *P. restinga* Cardozo, Baldo, Pupin, Gasparini & Haddad, 2018 have lower note rates [238–535 notes per minute] (Andrade *et al.* 2018; Cardozo *et al.* 2018). *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014 and *P. florencei* Andrade, Haga, Lyra, Leite, Kwet, Haddad, Toledo & Giaretta, 2018 have stereotyped three-pulsed notes along their series of notes, longer note duration [108–397 ms, combined values], and lower note rate [100–297 notes per minute, combined values] (Magalhães *et al.* 2014; Andrade *et al.* 2017b, 2018). In comparison with the phylogenetically close related species, the new species is distinguished from *P. mineira* by having shorter note duration [50–114 ms] and lower note rate [282–485 notes per minute] (Table 2). The advertisement call of *P. ceratophyes*, *P. llanera*, and *P. pusilla* are unknown; however, there are strong morphological differences among them and *P. matuta* sp. nov.

Table 1. Morphometry of the type series and additional specimens of *Pseudopaludicola matuta* sp. nov., and of specimens of *P. mineira* Lobo, 1994 (including types and additional specimens designated by Lobo 1994). Values presented in millimeters as mean ± standard deviation (minimum–maximum); n = number of measured specimens.

Characters	Holotype	Type series		Additional specimens		*P. mineira*
		Males (n = 15)	Females (n = 5)	Males (n = 3)	Females (n = 3)	Males (n = 38)
Snout-vent length	13.0	13.5 ± 0.4 (12.8–14.3)	15.4 ± 0.6 (14.7–16.0)	13.4 ± 0.3 (13.1–13.6)	15.0 ± 0.3 (14.7–15.3)	13.6 ± 0.7 (12.2–14.5)
Head length	4.1	3.9 ± 0.2 (3.6–4.3)	4.4 ± 0.2 (4.2–4.6)	3.8 ± 0.2 (3.7–4.0)	4.4 ± 0.2 (4.2–4.5)	3.5 ± 0.2 (3.2–4.0)
Head width	4.7	4.8 ± 0.1 (4.5–4.9)	5.4 ± 0.2 (5.2–5.5)	4.6 ± 0.2 (4.5–4.8)	5.1 ± 0.2 (4.9–5.2)	4.6 ± 0.2 (4.4–4.9)
Eye diameter	1.6	1.5 ± 0.1 (1.4–1.6)	1.6 ± 0.1 (1.5–1.7)	1.5 ± 0.0 (1.5–1.5)	1.7 ± 0.1 (1.6–1.7)	1.5 ± 0.1 (1.4–1.7)
Interorbital distance	1.5	1.4 ± 0.1 (1.2–1.7)	1.7 ± 0.1 (1.6–1.8)	1.5 ± 0.1 (1.4–1.5)	1.6 ± 0.1 (1.5–1.6)	1.4 ± 0.1 (1.2–1.5)
Eye-nostril distance	1.1	1.1 ± 0.1 (0.9–1.4)	1.2 ± 0.2 (1.0–1.4)	1.2 ± 0.1 (1.1–1.2)	1.3 ± 0.1 (1.2–1.4)	1.1 ± 0.1 (0.9–1.2)
Snout length	2.1	2.0 ± 0.1 (1.7–2.2)	2.3 ± 0.2 (2.1–2.5)	2.0 ± 0.1 (2.0–2.1)	2.2 ± 0.1 (2.1–2.3)	2.0 ± 0.1 (1.8–2.2)
Internarial distance	1.3	1.2 ± 0.1 (0.9–1.4)	1.3 ± 0.1 (1.2–1.5)	1.2 ± 0.1 (1.1–1.2)	1.3 ± 0.1 (1.3–1.4)	1.0 ± 0.1 (0.9–1.1)
Hand length	3.7	3.7 ± 0.2 (3.3–4.0)	4.2 ± 0.3 (3.7–4.4)	3.7 ± 0.3 (3.5–4.0)	4.2 ± 0.2 (4.0–4.3)	3.7 ± 0.2 (3.2–4.0)
Thigh Length	6.7	6.3 ± 0.2 (5.9–6.7)	7.0 ± 0.5 (6.2–7.6)	6.2 ± 0.1 (6.1–6.2)	7.1 ± 0.3 (6.8–7.4)	6.5 ± 0.3 (6.0–7.1)
Tibia length	7.2	6.9 ± 0.4 (6.2–7.5)	7.8 ± 0.2 (7.5–8.0)	6.6 ± 0.2 (6.4–6.8)	7.7 ± 0.3 (7.4–8.0)	7.2 ± 0.3 (6.6–7.7)
Tarsus length	3.9	3.7 ± 0.3 (3.4–4.1)	4.4 ± 0.2 (4.1–4.6)	3.8 ± 0.1 (3.7–3.9)	4.4 ± 0.4 (4.0–4.8)	3.7 ± 0.2 (3.3–4.2)
Foot length	7.8	7.2 ± 0.3 (6.4–7.7)	8.1 ± 0.4 (7.6–8.5)	7.3 ± 0.1 (7.2–7.4)	8.3 ± 0.4 (7.9–8.6)	7.3 ± 0.3 (6.5–7.8)

In relation to the most closely related species, *P. mineira*, the randomForest multivariate approach applied to morphometric data indicated a broad overlap between the two partitions (Fig. 5A–B), with a considerable classification error (Table 3). We found no statistical significance in any trait. Therefore, we were unable to distinguish *P. matuta* sp. nov. from *P. mineira* based on morphometric or any other feature of external morphology or coloration. In contrast, the RandomForest multivariate approach on acoustic data highlighted a full segregation between *P. matuta* sp. nov. and *P. mineira* (Table 3, Fig. 5C), without any classification error. Pulse rate, note duration, notes per minute, and interpulse interval are the main sources of variation in both variable importance measurements (Fig. 5D). The trait of notes per minute for *P. mineira* did not overlap the values for the new species either. It is noteworthy that the air temperature overlapped at the time of the field recordings for both species (Table 2), and is among the variables that least explained the variation of the dataset, with low importance measures as produced by randomForest (Fig. 5D). Therefore, we do not attribute this found acoustic difference to the influence of the air temperature. In addition, there was only a slight overlap between both species in note duration (see Table 2). The new species can be significantly differentiated from *P. mineira* in the following traits: note duration, internote interval, interpulse interval, pulse rate, and dominant frequency (for all these traits, Wilcoxon-Mann-Whitney Tests had $P < 0.01$).

Etymology

The feminine noun *matuta* in Brazilian Portuguese means rustic, provincial, related to those who live in the countryside.

Type material

Holotype

BRAZIL: adult ♂, Minas Gerais, municipality of Curvelo, Sítio Mato do Engenho, 18°46′07.6″ S, 44°26′50.7″ W, 620 m a.s.l., 29 Nov. 2017, F.S. Andrade & I.A. Haga leg. (ZUEC 24302; Figs 2–3, call voucher).

Paratopotypes

BRAZIL: 12 adult ♂♂, same collection data as the holotype (ZUEC 24303–6, 24308–10, 24313, 24315–8); 3 adult ♂♂, same locality as holotype, 21 Feb. 2011, T.R. Carvalho leg. (AAG-UFU 0308, 0386–7); 5 adult ♀♀, same collection data as the holotype (ZUEC 24307, 24311–2, 24314, 24323).

Type locality

BRAZIL: Minas Gerais, municipality of Curvelo, Sítio Mato do Engenho, 18°46′07.6″ S, 44°26′50.7″ W, 620 m a.s.l.

Other material examined

BRAZIL: 3 adult ♂♂, Minas Gerais, municipality of Santana do Riacho, district of Serra do Cipó, southern foothills of Serra do Cipó National Park, near Lagoa da Capivara (19°20’46.30” S, 43°36’59.62” W, 797 m a.s.l.), 2–3 Dec. 2017, F.S. Andrade & I.A. Haga leg. (ZUEC 24324, 24327, 24329); 3 adult ♀♀, same collection data as previous (ZUEC 24325–6, 24328).

Description of the holotype

Body elliptic and broad (Table 1, Fig. 2A–B). Head elliptical, slightly wider than long. Snout subovoid in dorsal view and rounded in profile (Fig. 2C–D). Eye not protuberant. Eye diameter almost 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 tympanic ridge from behind the eye to the proximal portion of the arm. Mouth opening ventral. Vomerine teeth absent. Tongue elliptical, longer than wide, posteriorly free, without pigmentation at its base. Flank with discrete granules. One ovoid antebrachial 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 rounded. Fingers with single and rounded subarticular tubercles. Supernumerary tubercles absent on palm of hand. 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 (Fig. 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. Supernumerary tubercles absent on sole of the foot. Toes webbed basally and fringed along their sides to almost their tips. Fringes developed on all toes (mainly II, III, IV, and V). 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 adpressed one to another: I < II <V < III < IV (Fig. 2F). Hind limb robust and moderately long with the tibiotarsal articulation just reaching the posterior margins of eye. Thigh shorter

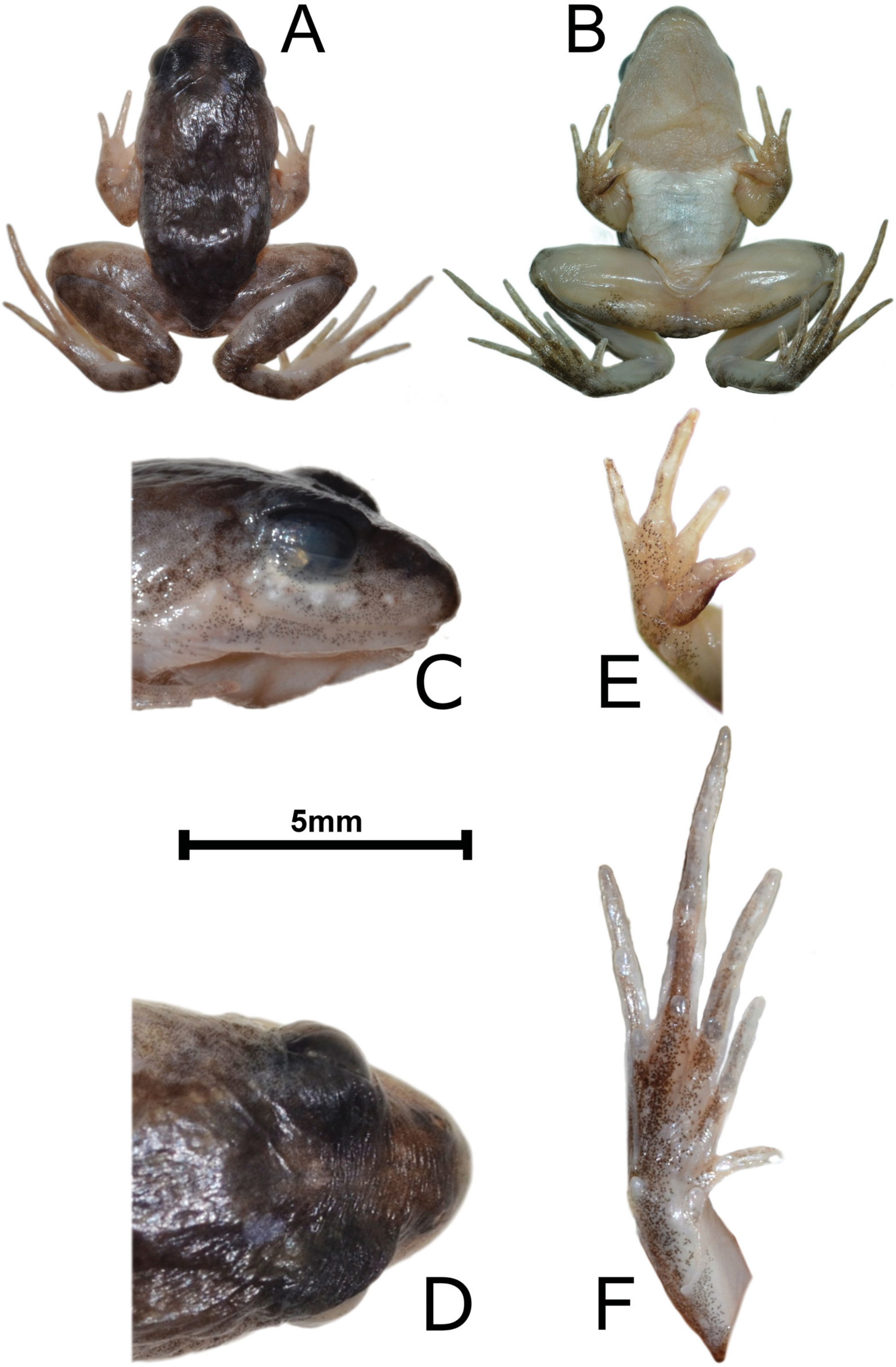

Fig. 2. *Pseudopaludicola matuta* sp. nov. holotype, adult ♂ (ZUEC 24302, SVL = 13.0 mm). **A**. Dorsal view. **B**. Ventral view. **C**. Head, lateral view. **D**. Head, dorsal view. **E**. Hand, ventral view. **F**. Foot, ventral view. Scale bar = 5 mm.

than tibia. Foot longer than thigh. Foot longer than tibia. Tubercle absent on heel. Belly skin smooth. Abdominal fold present and complete. Dorsal surfaces of head, body, and limbs smooth. Cloacal region smooth (Fig. 2B). Measurements of the holotype presented in Table 1.

Color pattern of the holotype in preservative

Dorsum grayish with dark gray, white, and brown blotches. Belly whitish (unpigmented). Throat light beige, unpigmented. Throat darker than belly. Dorsum darker than the dorsal surfaces of limbs. Region between upper lip and eye with several rounded white blotches. Ventral faces of arms and

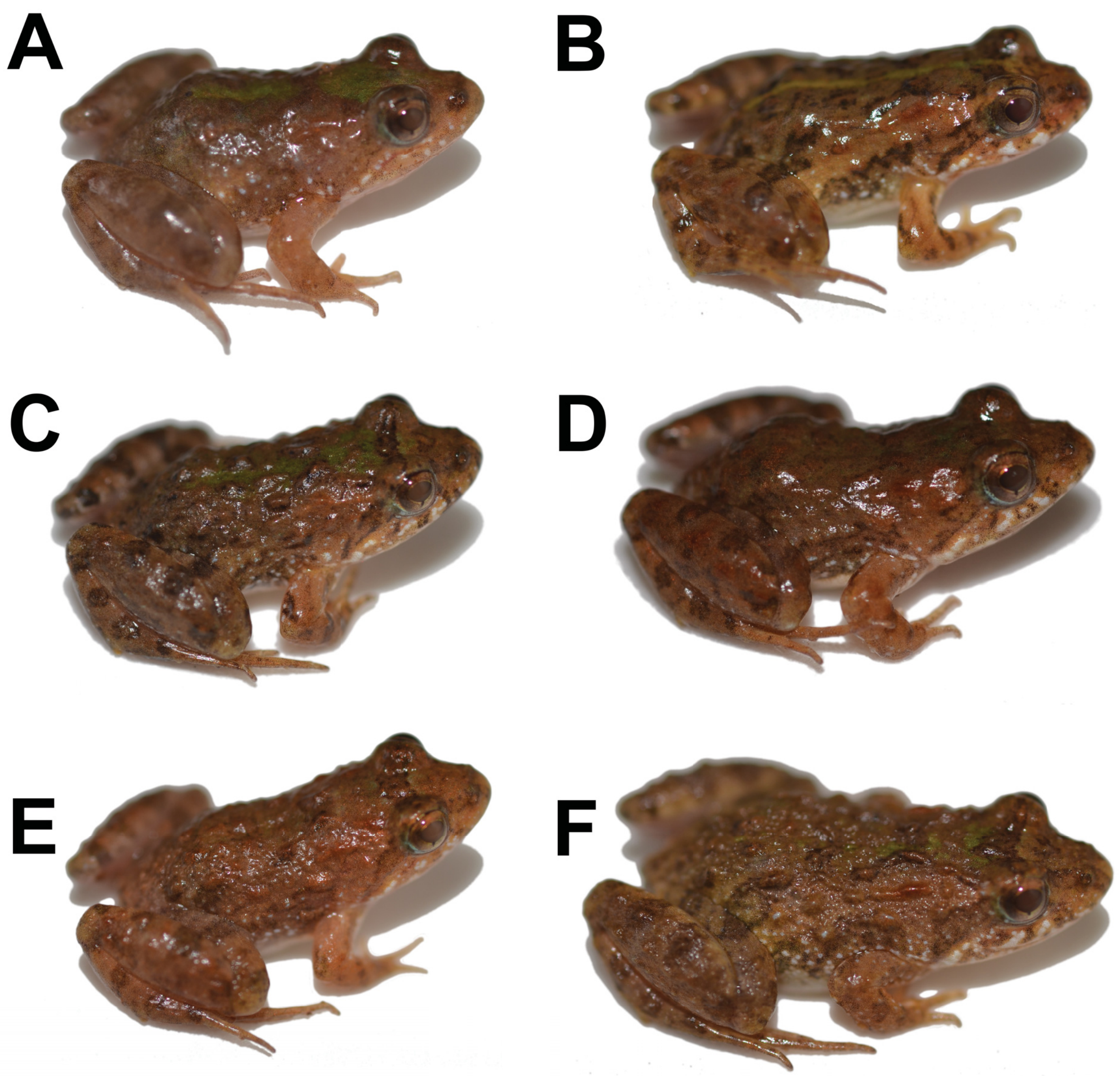

Fig. 3. Holotype and five paratypes of *Pseudopaludicola matuta* sp. nov. in life. **A**. Specimen ZUEC 24302 (holotype, adult ♂, call voucher; SVL = 13.0 mm). **B**. Specimen ZUEC 24304 (adult ♂, call and GenBank voucher; SVL = 13.3 mm). **C**. Specimen ZUEC 24303 (adult ♂, call and GenBank voucher; SVL = 12.8 mm). **D**. Specimen ZUEC 24305 (adult ♂ and call voucher; SVL = 13.4 mm). **E**. Specimen ZUEC 24308 (adult ♂, call and GenBank voucher; SVL = 13.5 mm). **F**. Specimen ZUEC 24307 (adult ♀ and GenBank voucher; SVL = 15.2 mm).

Table 2. Advertisement call traits of *Pseudopaludicola matuta* sp. nov. from the type locality, lowlands of the Serra do Cipó National Park (southern foothills), municipality of Santana do Riacho, and municipality of Belo Horizonte; and of *P. mineira* Lobo, 1994 from its type locality, in the highlands of Serra do Cipó; all in the Brazilian state of Minas Gerais. Mean ± SD (minimum–maximum); n = number of males recorded (number of analysed notes).

Traits	*P. matuta* sp. nov.		*P. mineira*
	Type locality n = 23 (460)	Other localities n = 8 (160)	Type locality n = 9 (180)
Call duration (s)	26.1 ± 18.7 (3.6–66.2)	44.4 ± 34.9 (19.7–69.1)	–
Series of notes duration (s)	9.4 ± 6.5 (0.2–34.0)	23.7 ± 14.5 (7.4–46.0)	14.5 ± 15.7 (0.4–44.3)
Interseries interval (s)	0.6 ± 0.5 (0.2–2.0)	0.6 ± 0.3 (0.3–0.8)	1.9 ± 0.0 (0.3–10.9)
Series per call	3.4 ± 2.0 (1–9)	2.5 ± 0.7 (1–3)	-
Note duration (ms)	37 ± 3 (27–46)	41 ± 4 (34–52)	69 ± 8 (50–114)
Internote interval (ms)	29 ± 6 (17–72)	49 ± 9 (35–76)	74 ± 20 (42–159)
Pulse duration (ms)	9 ± 2 (4–15)	6 ± 1 (3–10)	10 ± 3 (4–22)
Interpulse interval (ms)	18 ± 3 (11–31)	30 ± 4 (22–40)	47 ± 10 (5–94)
Notes per minute	928.6 ± 85.1 (738.0–1101.4)	655.2 ± 75.4 (541.9–736.6)	427.1 ± 64.2 (282.1–485.1)
Pulse rate	54.7 ± 5.9 (43.5–74.1)	48.8 ± 4.4 (38.5–58.8)	29.8 ± 3.0 (17.5–117.6)
Pulses per note	2.0 ± 0.0 (2–2)	2.0 ± 0.0 (2–2)	2.0 ± 0.1 (2–3)
Dominant frequency (kHz)	4.5 ± 0.2 (3.9–5.1)	4.7 ± 0.1 (4.6–5.0)	3.8 ± 0.2 (3.6–4.3)
Minimum frequency (kHz)	4.0 ± 0.3 (2.9–4.6)	4.1 ± 0.3 (1.8–4.4)	3.2 ± 0.2 (2.3–3.9)
Maximum frequency (kHz)	4.9 ± 0.2 (4.4–5.5)	5.1 ± 0.1 (4.9–5.2)	4.2 ± 0.1 (4.0–4.5)
Air temperature (°C)	24.0–25.2	20.5–23.0	17.0–24.0

Table 3. Confusion matrix for two sister species of *Pseudopaludicola* based on morphometric and **acoustic (values in bold)** data by means of a Random Forests model. Settings: number of tree permutations = 1000; number of variables tried at each split = 3.0; error rates = 8.77% | **0.00%**.

	P. matuta sp. nov.	*P. mineira*	class.error
P. matuta sp. nov.	15 | **31**	4 | **0**	0.21 | **0.00**
P. mineira	1 | **0**	37 | **9**	0.03 | **0.00**

Table 4. Estimates of uncorrected p-distances for the 16S fragment between species of *Pseudopaludicola*. Standard error estimate(s) are shown above the diagonal. There was a total of 576 positions in the final dataset. Dist: distances; SD: Standard error estimates. Pmat: *P. matuta* sp. nov.; Pmin: *P. mineira*; Psal: *P. saltica*; Pmur: *P. murundu*; Pjar: *P. jaredi*; Ppoc: *P. pocoto*; Pres: *P. restinga*; Pflo: *P. florencei*; Pfal: *P. falcipes*; Pmot: *P. motorzinho*; Pbol: *P. boliviana*; 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.

		Inter (SD)																
	Intra (SD)	Pmat	Pmin	Psal	Pmur	Pjar	Ppoc	Pres	Pflo	Pfal	Pmot	Pbol	Pame	Pter	Pfac	Patr	Pcan	Pbar	Pmys
Pmat	0.001 (0.001)		0.007	0.009	0.009	0.01	0.009	0.009	0.01	0.011	0.015	0.014	0.014	0.014	0.014	0.014	0.015	0.015	0.014
Pmin	0.003 (0.002)	**0.035**		0.01	0.009	0.01	0.007	0.008	0.008	0.01	0.014	0.013	0.014	0.013	0.014	0.014	0.013	0.014	0.014
Psal	0.003 (0.001)	**0.063**	0.056		0.006	0.007	0.009	0.01	0.011	0.012	0.014	0.014	0.014	0.012	0.013	0.012	0.012	0.013	0.013
Pmur	0.002 (0.001)	**0.055**	0.048	0.025		0.006	0.009	0.01	0.01	0.011	0.014	0.013	0.013	0.012	0.012	0.012	0.012	0.012	0.013
Pjar	0 (0)	**0.062**	0.056	0.026	0.022		0.01	0.01	0.011	0.012	0.014	0.014	0.014	0.013	0.015	0.014	0.014	0.014	0.015
Ppoc	0.01 (0.003)	**0.061**	0.045	0.06	0.054	0.064		0.006	0.008	0.01	0.014	0.014	0.014	0.013	0.014	0.014	0.013	0.013	0.014
Pres	0.002 (0.002)	**0.059**	0.046	0.062	0.056	0.068	0.035		0.007	0.011	0.013	0.013	0.013	0.013	0.014	0.013	0.013	0.013	0.014
Pflo	0.001 (0.001)	**0.073**	0.046	0.066	0.06	0.072	0.039	0.031		0.011	0.013	0.013	0.014	0.014	0.014	0.013	0.013	0.014	0.014
Pfal	0.004 (0.002)	**0.084**	0.072	0.088	0.07	0.087	0.075	0.076	0.08		0.015	0.014	0.014	0.014	0.015	0.014	0.014	0.015	0.016
Pmot	0.002 (0.001)	**0.134**	0.134	0.142	0.127	0.134	0.139	0.131	0.131	0.134		0.01	0.014	0.014	0.013	0.015	0.015	0.014	0.013
Pbol	0.005 (0.003)	**0.129**	0.118	0.131	0.12	0.137	0.125	0.125	0.12	0.12	0.07		0.013	0.013	0.014	0.014	0.014	0.013	0.014
Pame	0.003 (0.002)	**0.139**	0.119	0.123	0.112	0.121	0.13	0.127	0.125	0.118	0.11	0.127		0.005	0.013	0.012	0.011	0.011	0.012
Pter	0.004 (0.001)	**0.138**	0.123	0.124	0.114	0.125	0.133	0.125	0.127	0.12	0.112	0.128	0.015		0.013	0.013	0.011	0.012	0.012
Pfac	0 (0)	**0.178**	0.17	0.152	0.147	0.163	0.167	0.162	0.168	0.154	0.153	0.163	0.104	0.108		0.007	0.011	0.011	0.011
Patr	0 (0)	**0.173**	0.167	0.147	0.144	0.161	0.166	0.162	0.168	0.158	0.163	0.171	0.102	0.106	0.032		0.01	0.009	0.01
Pcan	0 (0)	**0.164**	0.151	0.14	0.128	0.151	0.152	0.145	0.151	0.129	0.147	0.152	0.089	0.085	0.083	0.079		0.006	0.011
Pbar	0.001 (0.001)	**0.160**	0.15	0.136	0.125	0.148	0.146	0.148	0.152	0.13	0.145	0.15	0.082	0.081	0.076	0.072	0.018		0.011
Pmys	0.015 (0.003)	**0.161**	0.146	0.143	0.133	0.153	0.162	0.157	0.15	0.147	0.148	0.157	0.096	0.098	0.095	0.09	0.08	0.078	

legs unpigmented, except that of the thigh (slight pigmented). Palm of hand pigmented. Sole of foot pigmented and darker than hands, arms, and hind limbs. Color of the sole of the foot similar to that of the dorsum of hind limb. Dorsal faces of arms light brown with several dark brown blotches. Dorsal faces of legs light grayish with scattered brown blotches. Dark brown nuptial pads (Fig. 2).

Variation in the type series

Dorsal surface of body varies from dark gray to dark brown, with black or dark brown irregular blotches (Fig. 3). The specimens ZUEC 24303–18 and 24323 have transverse stripes on thighs, shanks, and feet. The specimens ZUEC 24304, 24310–3, 24317–8 have a well-defined light vertebral line. The specimens ZUEC 24304, 24310–3, 24315, 24317–8, and 24323 have dorsolateral stains on body, from posterior corner of eyes to almost the region of insertion of legs. The specimens ZUEC 24304–5, 24307–8, 24311, 24315–8, 24323 also have white blotches on the region between the posterior corner of eyes and the region of insertion of arms. The specimen ZUEC 24312 has the region between upper lip and eye with alternating vertical gray and light beige stripes. Females have a more robust body and lack nuptial pads, vocal sac, and vocal slits.

Advertisement call

Pseudopaludicola matuta sp. nov. emits the advertisement call with highly variable duration (3.6–66.2 s), consisting of series of stereotyped two-pulsed notes (1–9 series of two-pulsed notes per call) that lasts 0.2–34.0 s, separated by intervals of 0.2–1.9 s (Fig. 4A). Before the emission of the series of stereotyped two-pulsed notes, 1–10 (mean = 4.4, SD = 3.3) isolated pulsed notes with irregular structure, duration, interval, and number of pulses are emitted, herein referred to as introductory notes (Fig. 4A). Introductory notes last 29–117 ms (mean 64, SD = 24), separated by intervals of 37–495 ms (mean = 213, SD = 111), and are composed of 2–5 non-concatenated pulses (mean = 3.3, SD = 0.7). These pulses vary from 4–16 ms (mean = 9, SD = 2), separated by intervals of 1–36 ms (mean = 16, SD = 6). Dominant frequency peaks are between 4.1–4.5 kHz (mean = 4.4, SD = 0.2). On the other hand, within the series of stereotyped two-pulsed notes, the notes have regular structure, duration, interval, and number of pulses. These notes last 27–52 ms, separated by intervals of 17–76 ms, and are released at a rate of 542–1,101 notes per minute; notes have a slightly increase in amplitude from the first to the second pulse (see oscillogram in Fig. 4B). Pulses vary from 4–15 ms, separated by intervals of 11–40 ms, and are released at a rate of 43–74 pulses per second (Fig. 2B). Dominant (= fundamental) frequency peaks are between 3.9–5.1 kHz; the minimum frequency ranges between 1.8–4.6 kHz and the maximum frequency ranges from 4.4–5.5 kHz. Notes have up to three harmonics; the second ranging from 6.9–9.8 kHz (mean = 8.7; SD = 0.4). Air temperature of recorded calls varied from 24.0 to 25.2°C. Call quantitative traits are summarized in Table 2.

Traits that were classified as static (within-male CV < 5%) to *P. matuta* sp. nov. were note duration (mean = 4.2%, SD = 1.8 [1.6–7.4]), number of pulses per note (mean = 0%), pulse rate (mean = 4.2%, SD = 1.8 [1.6–7.4]), and dominant peak (mean = 0.9%, SD = 0.7 [0.0–3.0]). The other traits were classified as dynamic (CV < 7.4%). Additionally, the between-male coefficient of notes per minute was equal to 9.2%, this low variability among males allowed us to classify this trait as static.

We noticed a remarkable intraspecific variation in the advertisement call of *P. matuta* sp. nov., in which the three males of the population from the southern foothills of the Serra do Cipó National Park have a lower note rate (529–737 notes per minute) than those of male types (738–1101 notes per minute). It is noteworthy that the air temperatures measured during the recordings in these two localities did not overlap, 24.0–25.2°C at type locality, and 20.5–23.0°C in lowlands of the Serra do Cipó National Park.

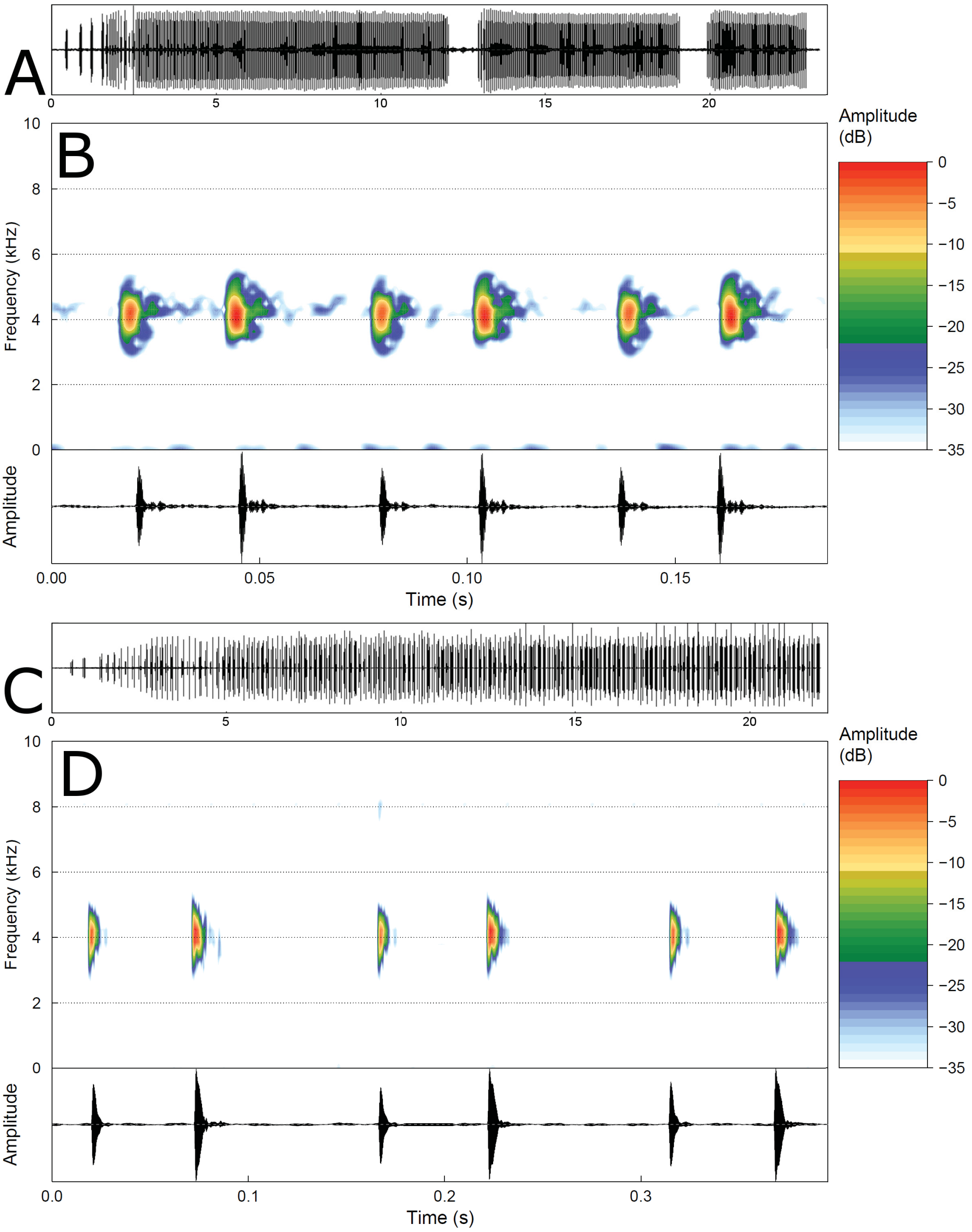

Fig. 4. **A**. Oscillogram of the entire advertisement call with the introductory notes and three series of two-pulsed notes of *Pseudopaludicola matuta* sp. nov. **B**. Audiospectrogram (above) and corresponding oscillogram (below) detailing three two-pulsed notes of the holotype (ZUEC 24302). The holotype was recorded on 29 Nov. 2017 at 19:19 h; air temperature 25.2°C (recording_label: Pseudop_matutaCurveloMG10iIAH_AAGmt). **C**. Oscillogram of the entire advertisement call with a single series of two-pulsed notes of *P. mineira* Lobo, 1994. **D**. Audiospectrogram (above) and corresponding oscillogram (below) detailing three two-pulsed notes of a topotypical male (ZUEC 24330; GenBank voucher). The topotype was recorded on 4 Dec. 2017 at 19:28 h; air temperature 21.0°C (recording_label: Psedop_mineiraSantanadoRiachoMG2aIAH_AAGm661MK2). Note the remarkable difference in the time spent emitting the same three two-pulsed notes between these two sister species, *P. mineira* spends about twice as much time than *P. matuta* sp. nov.

Phylogenetic inferences and mitochondrial DNA divergences

The inferred tree topology agreed with previous phylogenetic analyses of *Pseudopaludicola* (Veiga-Menoncello *et al.* 2014; Andrade *et al.* 2016, 2018), and was very similar between BI and ML inferences (Fig. 6). The new species was found as sister clade of *P. mineira* (Fig. 6). Uncorrected genetic distance between the new species and *P. mineira* was 3.5% (mean value) and maximum intraspecific distance was 0.1% (Table 4). No molecular data are available for *P. ceratophyes*, *P. llanera*, *P. pusilla*, *P. hyleaustralis*, *P. parnaiba*, *P. giarettai*, *P. ibisoroca*; however, the new species is strongly diagnosed from these species by morphology and acoustics (see further details in Differential diagnosis section).

Natural history notes

We recorded specimens of *P. matuta* sp. nov. in an area with relatively dense vegetation. In this site, there were grasses, shrub vegetation, and some spaced trees of 5–7 m in height, with the soil well soaked. Males of other *Pseudopaludicola* species usually call exposed in open areas. Curiously, two other specimens of *P. matuta* sp. nov. were recorded a few meters from this above-mentioned site in an open and waterlogged area, where *P. giarettai* also occurs syntopically. In both places, we observed dozens of specimens of *P. matuta* sp. nov.

However, during the fieldwork in the lowlands of the Serra do Cipó National Park, we were able to find a single small population near Lagoa da Capivara and Cipó River. During the two sampling nights, it was possible to hear only eight males, which were recorded. They were not excited and were vocalizing well-spaced from each other. This social condition may have influenced the intraspecific acoustic variation described by us. Besides that, air temperature was lower (without overlap) during the fieldwork in the lowlands of the Serra do Cipó National Park.

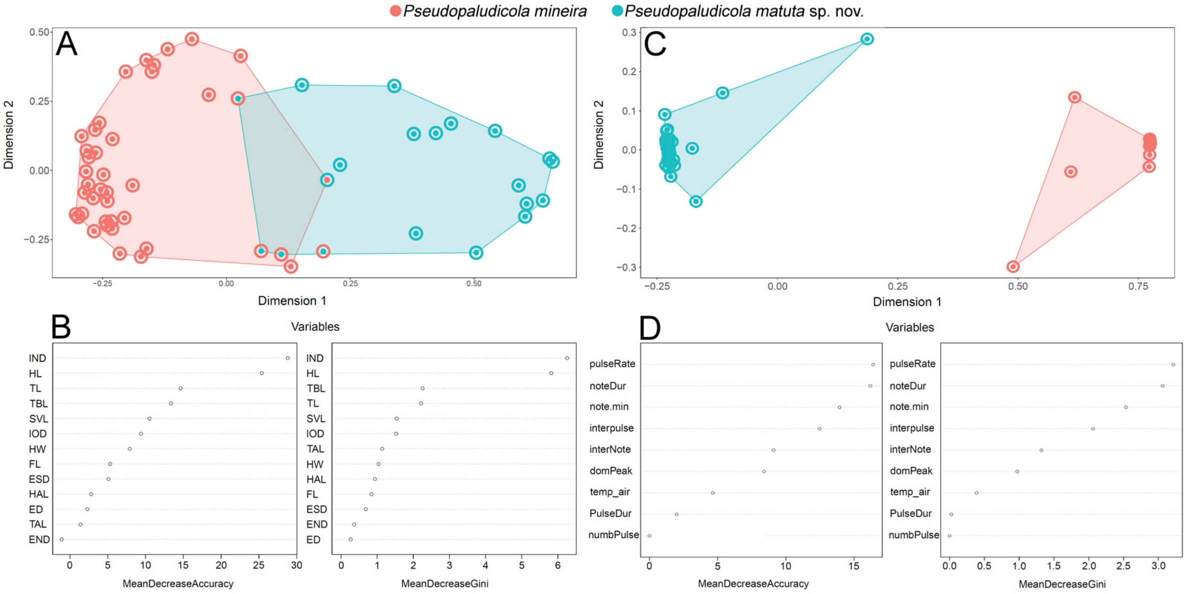

Fig. 5. First and second dimensions of the Multidimensional scaling on the proximity scores from the Random Forest analysis considering morphometric (**A**) and acoustic (**C**) traits of adult males of *Pseudopaludicola matuta* sp. nov. (blue dots) and *P. mineira* Lobo, 1994 (red dots). Dotcharts of variable importance score considering morphometric (**B**) and acoustic (**D**) traits as indicated by the Random Forest analysis. Each dot represents an adult male.

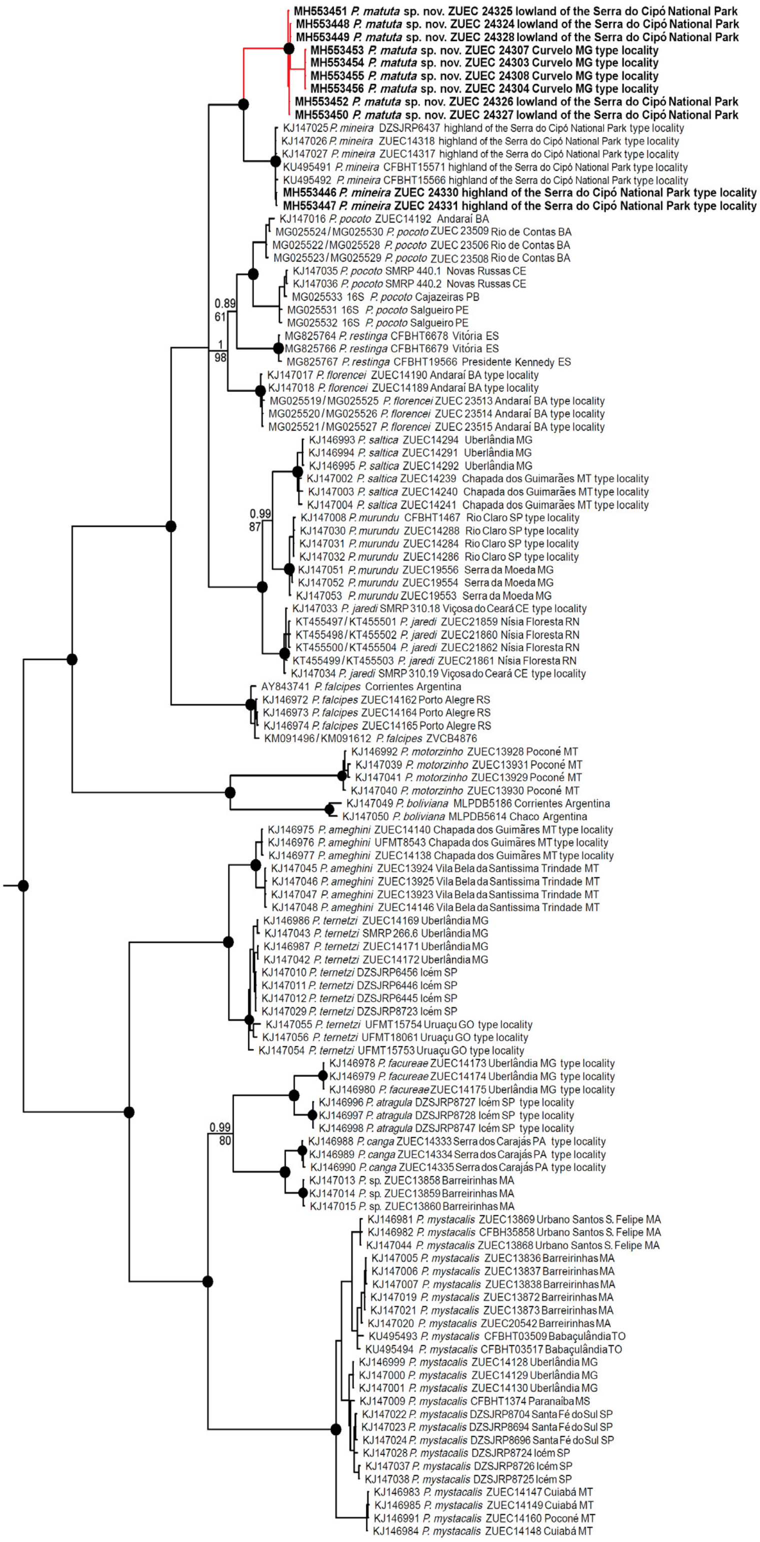

Fig. 6. 50% majority rule consensus tree based on the 12S and 16S rDNA mitochondrial genes of the species of *Pseudopaludicola* from Bayesian inference analysis. Numbers above branches are posterior probabilities (PP) and numbers below branches are the support values from a maximum likelihood bootstrap analysis. Black dots represent PP = 1 and bootstrap = 100; no support below species level is shown.

Distribution

Pseudopaludicola matuta sp. nov. is known from its type locality, lowlands of the Serra do Cipó National Park (southern foothills), district of Serra do Cipó, municipality of Santana do Riacho, and in the municipality of Belo Horizonte from a report in the 1960s (Fig. 1). However, it is not possible to find this species nowadays in the municipality of Belo Horizonte (F.S.F. Leite, pers. obs.). Lobo (1994) reported the occurrence of *P. mineira* in the Serra do Cipó National Park (at 1264 m a.s.l., type locality) and in Serra do Cabral (at 1104 m a.s.l.), a nearby mountainous area but isolated from the main Espinhaço mountain range (Fig. 1). The altitudes we found *P. matuta* sp. nov. vary between 620 and 883 m a.s.l. In addition, in the type locality and where we collected *P. mineira* during our fieldwork are only about 14 km northeast from the site where we recorded *P. matuta* sp. nov. (797 m a.s.l., Fig. 1); however, the elevational difference between these two sites is about 460 m. We did not find *P. matuta* sp. nov. during our fieldwork in the rupestrian grasslands of the Serra do Cipó National Park. Therefore, it seems that *P. mineira* is restricted to the higher rupestrian grasslands of the Serra do Cipó National Park and Serra do Cabral (Lobo 1994), whereas *P. matuta* sp. nov. occurs in adjacent lowland regions to the west and south of this unique Brazilian mountain range.

The advertisement call and acoustic diagnosis of *P. mineira*

Pseudopaludicola mineira also emits long advertisement calls, consisting of series of stereotyped two-pulsed notes that lasts 0.4–44.3 s, separated by intervals of 0.3–10.9 s (Fig. 4C). Notes last 50–114 ms separated by intervals of 42–159 ms and released at a rate of 282–485 notes per minute. Only six notes of one male have three non-concatenated pulses of all the analysed notes (n = 180 notes); the other notes have two non-concatenated pulses each. Pulses vary from 4–22 ms, are separated by intervals of 5–94 ms, and are released at a rate of 17–118 pulses per second (Fig. 4D). Dominant (= fundamental) frequency peaks between 3.6–4.3 kHz, the minimum frequency ranges 2.3–3.9 kHz, and the maximum frequency ranges 4.0–4.5 kHz. Second harmonic peaks between 6.8–8.1 kHz (mean = 7.1; SD = 0.07). *Pseudopaludicola mineira* also has introductory notes with irregular structure, duration, interval, and number of pulses. Quantitative traits are summarized in Table 2. Air temperature of recorded calls varied from 16.0 to 25.5°C.

Similarly to the new species, *P. mineira* 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 mineira* distinguishes from other congeners [values within square brackets] by the following acoustic traits: *P. ternetzi* has higher note rate (282–485 [606–921] notes per minute), and higher number of pulses per note (2–3 [3–6]) (Andrade *et al.* 2017a); *P. ameghini* has higher number of pulses per note [3–5] (Andrade *et al.* 2017a); *P. atragula* has longer note duration (50–144 [300–700 ms], and higher number of pulses per note [9–36], and lower note rate [42–98 notes per minute] (Pansonato *et al.* 2014). From the three long-legged species (*P. saltica*, *P. murundu*, and *P. jaredi*), *P. mineira* is distinguished by having low variation in the number of pulses per note [2–7] (Andrade *et al.* 2016). *Pseudopaludicola pocoto* and *P. florencei* have lower note rate [100–297 notes per minute, combined values], higher dominant frequency (3.6–4.3 [4.2–6.5] kHz), and stereotyped three-pulsed notes (Magalhães *et al.* 2014; Andrade *et al.* 2017b, 2018). *Pseudopaludicola mineira* differs from *P. falcipes* by its lower dominant frequency [4.7–6.0 kHz; Andrade *et al.* 2018; present study]; however, the advertisement calls of these two species are quite similar in the temporal domain. We were unable to distinguish acoustically *P. mineira* from *P. restinga* based on data from literature (Cardozo *et al.* 2018). Note emission rate is one of the most informative acoustic traits to diagnose closely related species of *Pseudopaludicola* (e.g., Andrade *et al.* 2017a, 2018). In Cardoso *et al.* (2018) there is no measure of this feature but an estimated value from their figured sounds is around 490 notes per minute, what is within the range we observed to *P. mineira*.

Discussion

The non-overlapping note rate distinguishes *Pseudopaludicola matuta* sp. nov. and *P. mineira*. This trait was classified as a static trait (*sensu* Gerhardt 1991) for both species, due to its low variability between-males in these species. The spectral and fine-scale temporal traits of the frog calls are usually important for species recognition, whereas variable temporal traits may be important for mate choice (Gerhardt 1991). Therefore, the species differ one from another in a temporal trait that is expected to be associated with their species recognition. In addition, note duration almost alone distinguishes *P. matuta* sp. nov. from *P. mineira*, and it was also classified as static for the new species. It is well known that environmental variables can influence the acoustic traits in anurans; however, we do not attribute the acoustic differences to air temperature differences, once the values overlapped at the time of the field recordings of the two species. However, air temperature may be a plausible explanation for the intraspecific variation in the note emission rate of *P. matuta* sp. nov. Temporal traits are more informative for species recognition than spectral traits in the genus *Pseudopaludicola*; especially the note rate that seems to be a reliable diagnostic character among closely related species.

The acoustic characterization of *Pseudopaludicola mineira* presented here is in accordance with that of Pereira & Nascimento (2004) and provides a diagnostic support for this species in comparison with all congeners, except *P. falcipes*. Andrade *et al.* (2018) were unable to distinguish acoustically *P. mineira* from *P. falcipes*. Based on a larger acoustic sample of topotypes of *P. mineira*, we observed that these two species have quite similar calls in the temporal domain, without differences in any trait. However, in the spectral domain *P. mineira* has a lower dominant frequency (3.8 ± 0.2 [3.6–4.3] kHz) compared to *P. falcipes* (5.1 ± 0.3 [4.7–6.0] kHz; Andrade *et al.* 2018). However, the values of dominant frequency of *P. mineira* presented in Pereira & Nascimento (2004) slightly overlap with those of *P. falcipes* in Andrade *et al.* (2018). As spectrograms are directly influenced by the settings used in acoustic analyses, as well as by the above-mentioned overlap with the data presented in Pereira & Nascimento (2004), we prefer not to assume here that the spectral differences found between *P. falcipes* and *P. mineira* are in fact diagnostic traits.

Andrade *et al.* (2018) pointed out that there is only one reliable diagnostic external morphology character between *P. mineira* and *P. falcipes*, the absent or incomplete abdominal fold in *P. falcipes*; whereas in *P. mineira* the abdominal fold is always complete, as all other species of *Pseudopaludicola*. *Pseudopaludicola mineira* and *P. falcipes* are allopatric species with high genetic divergence based on mitochondrial markers (Lobo 1994; Veiga-Menoncello *et al.* 2014; Langone *et al.* 2016; Andrade *et al.* 2018); nuclear genes will be informative to investigate if mitochondrial introgression is involved in this case. Cardozo *et al.* (2018) were unable to find acoustic differences among *P. mineira*, *P. restinga*, and *P. falcipes*. According to those authors, the most closely related taxon to *P. restinga* is *P. pocoto*, which has different advertisement calls. In our phylogenetic hypothesis, we recovered *P. restinga* closely related to *P. pocoto*, but with a relatively low support. In any case, this putative relationship needs to be better established in the future.

Previous studies (Veiga-Menoncello *et al.* 2014; Andrade *et al.* 2016, 2018) were unable to completely resolve the phylogenetic relationships of *P. mineira* within the clade with 2n = 22 chromosomes (*sensu* Veiga-Menoncello *et al.* 2014). Here we recovered *P. mineira* as sister clade of *P. matuta* sp. nov. with high support, but the relationship of *P. mineira* and *P. matuta* sp. nov. within the clade 2n = 22 remains uncertain. Andrade *et al.* (2018) analysed acoustically one specimen of *Pseudopaludicola* (called by them *P. mineira*) from Pampulha, Belo Horizonte, Minas Gerais, recorded by W. C. A. Bokermann on 10 Feb. 1965. These authors also reported that this individual presented 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 the Serra do Cipó National Park (type locality of *P. mineira*). *Pseudopaludicola matuta* sp.

nov. is actually the best taxonomic identity for this specimen, from Pampulha, Belo Horizonte, based on acoustic evidence.

Pseudopaludicola mineira is classified as least-concern and suggested to be endemic to the highlands of the Serra do Cipó mountain range, occurring in shallow and temporary swamps (Leite *et al.* 2008). Leite *et al.* (2008) also reported the occurrence of *P. mineira* in the municipalities of Diamantina, Felício dos Santos, and São Gonçalo do Rio Preto, all in the state of Minas Gerais. However, not all specimens examined by them are from the highlands of the Serra do Cipó National Park range (F.S.F. Leite, pers. com.). Therefore, we were unable to identify whether the specimens analysed by Leite *et al.* (2008) are *P. mineira* or *P. matuta* sp. nov. Future phylogeographic studies will be valuable to resolve the evolutionary history of these two sister species and the role of the Espinhaço mountain range in this context.

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Appendix I: Examined specimens

Pseudopaludicola matuta sp. nov.

BRAZIL: Minas Gerais, Curvelo (type locality): AAG-UFU 0308, 0386–7, and ZUEC 24302–12; Santana do Riacho (lowlands of the Serra do Cipó National Park): ZUEC 24324–9.

Pseudopaludicola mineira

BRAZIL: Minas Gerais, Santana do Riacho (highlands of the Serra do Cipó National Park - type locality): ZUEC 1570, 1572–89, 1591, 24330–31; MZUSP 56858–61 (type series), MZUSP 57719–24

Pseudopaludicola florencei

BRAZIL: Bahia, Andaraí, urban area: ZUEC 23512–30; Área de Proteção Ambiental Marimbus-Iraquara: UFMG 4310–6; 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 giarettai

BRAZIL: Minas Gerais, Curvelo: AAG-UFU 0309–17.

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, 14162–6, 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 0853–55, 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, 19557–8, 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, 5134–6, 5854–5; Minas Gerais, Uberlândia: AAG-UFU 2308, 2630, 4598, 4631, 4735, 4707–11.

Pseudopaludicola ternetzi

BRAZIL: Goiás, Uruaçu: MNRJ 445–7, 5460–2, 5442; Minas Gerais, Uberlândia: ZUEC 14036–9, 14170–1; Tocantins, Formoso do Araguaia: ZUEC 10140–3, 10145, 10147, 10150, and 10153.