Reassessment of the taxonomic status of *Pseudopaludicola parnaiba* (Anura, Leptodactylidae, Leiuperinae), with the description of a new cryptic species from the Brazilian Cerrado

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Abstract. The Neotropical frog genus *Pseudopaludicola* includes 25 species distributed throughout South America. Herein we review the taxonomic status of *P. parnaiba* relative to *P. canga* and the specific identity of the population treated in previous studies as *Pseudopaludicola* sp. 3 from Barreirinhas in the Brazilian state of Maranhão. The lack of differentiation in advertisement call, morphology, and mitochondrial markers from topotypes and different populations rejects the status of *P. parnaiba* and *Pseudopaludicola* sp. 3 from Barreirinhas as distinct species. For these reasons, we suggest to formally...
consider *P. parnaiba* as a junior synonym of *P. canga*. We also found that a population previously reported as *P. facureae* from central Brazil (Palmeiras de Goiás, Goiás) corresponds to a cryptic species that we describe here as a new species. Lastly, we provide for the first time the phylogenetic positions of *P. giarettai*, *P. llanera* and *P. pusilla*.

**Keywords.** Integrative taxonomy, *Pseudopaludicola canga*, mitochondrial DNA, morphologically cryptic species.

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**Introduction**

Cerrado is the largest savanna formation in South America and is among the most threatened biodiversity hotspots on Earth (Myers *et al.* 2000), mainly due to habitat loss caused by agribusiness expansion, infrastructure development and limited conservation incentives (Strassburg *et al.* 2017). In the few last decades, the knowledge on anuran species richness of the Cerrado has expanded increasingly fast, indicating that species composition in the region needs to be urgently documented given the intensive anthropogenic activities developed in the region (Valdujo *et al.* 2013; Strassburg *et al.* 2017). The increase in species richness is partly due to the recognition of cryptic species in this formation (e.g., Vaz-Silva & Maciel 2011; Haga *et al.* 2017). Fišer *et al.* (2018) highlighted the importance of research efforts for fully integrating cryptic species into biodiversity science, thereby fostering a better understanding of the heterogeneous role of speciation in biodiversity pattern and process. Integrative taxonomy can notably improve the knowledge of frog diversity through species descriptions and delimitation, especially when dealing with morphologically cryptic species groups, recurrently reported for the frog genus *Pseudopaludicola* Miranda-Ribeiro, 1926 (e.g., Andrade *et al.* 2016, 2018a, 2018b, 2019; Pansonato *et al.* 2016; Cardozo *et al.* 2018).

The genus *Pseudopaludicola* includes 25 species (Frost 2020) that occur throughout most of South America (Lynch 1989). *Pseudopaludicola* is a monophyletic genus supported by the presence of a hypertrophied antebrachial tubercle (Lynch 1989) and some osteological features (Lobo 1995), as well as by molecular evidence based on mitochondrial DNA (Veiga-Menoncello *et al.* 2014). Veiga-Menoncello *et al.* (2014) indicated a clade including three nominal species, *P. canga* Giaretta & Kokubum, 2003, *P. facureae* Andrade & Carvalho, 2013, *P. atragula* Pansonato, Mudrek, Veiga-Menoncello, Rossa-Feres, Martins & Strüssmann, 2014, and a putative species, *Pseudopaludicola* sp. 3 (aff. *canga*) from Barreirinhas, MA, northern Brazil. Since then, the putative species has been treated as a species yet to be described (Veiga-Menoncello *et al.* 2014; Pansonato *et al.* 2014a; Andrade *et al.* 2016, 2018a, 2018b, 2019; Cardozo *et al.* 2018).

*Pseudopaludicola parnaiba* Roberto, Cardozo & Ávila, 2013 is known only from its type locality, the municipality of Ribeiro Gonçalves, PI, Brazil (Roberto *et al.* 2013). This species is supposedly closely related to *P. canga*, *P. facureae* and *P. atragula*, but, to date, there is no molecular evidence available for a phylogenetic assessment. Based on acoustic and morphological traits, Carvalho *et al.* (2015a) stated that this species could not be distinguished from *P. canga*, and suggested that an integrative reassessment of the taxonomic status of *P. parnaiba* in relation to *P. canga* was necessary. *Pseudopaludicola giarettai* Carvalho, 2012 is a well-characterized species, based on acoustic and morphological data (Carvalho 2012; Carvalho *et al.* 2015b). However, the phylogenetic position of the species remains to be tested (Andrade *et al.* 2018a, 2018b, 2019).
Carvalho et al. (2015a) also characterized the call of a population of *Pseudopaludicola* from the municipality of Palmeiras de Goiás, GO, Brazil, with the same trilled advertisement call pattern as *P. facureae*. Herein we combined acoustical, morphological and genetic evidence to (1) review the taxonomic status of *P. parnaiba* based on novel information from topotypes and additional populations of *P. canga*; (2) evaluate the specific identities of the population treated as *Pseudopaludicola* sp. 3 (sensu Veiga-Menoncello et al. 2014) and of the population from Palmeiras de Goiás; and (3) assess for the first time the phylogenetic positions of *P. giarettai*, *P. pusilla* (Ruthven, 1916) and *P. llanera* Lynch, 1989, providing the most complete mitochondrial phylogeny of the genus so far, with 21 species sampled. Our results revealed a cryptic species closely related to *P. atragula* and *P. facureae*, which we describe here as new.

**Material and methods**

**Reference specimens**

Specimens of the type series of the new species were collected and recorded in Palmeiras de Goiás (16°46′59″ S, 49°52′2″ W; 652 m above sea level (a.s.l.); datum = WGS84; Fig. 1), GO, Brazil. Type specimens are deposited in the amphibian collection of Museu de Zoologia “Adão José Cardoso” (ZUEC) of the Universidade Estadual de Campinas (UNICAMP), Campinas, SP, and in the Collection of frogs of the Museu de Biodiversidade do Cerrado (AAG-UFU), Universidade Federal de Uberlândia (UFU), Uberlândia, MG, both in Brazil.

We collected and recorded specimens of *P. parnaiba* at its type locality (7°35′42.19″ S, 45°20′36.66″ W, 203 m a.s.l.; datum = WGS84) on 10 February 2019. Also, we collected and recorded individuals at the locality of the three sequenced specimens (ZUEC 13858–60) of *Pseudopaludicola* sp. 3 (Veiga-Menoncello et al. 2014), in the Tabocas Village, municipality of Barreirinhas, MA, Brazil (3°0′28.96″ S, 43°8′29.56″ W, 43 m a.s.l.; datum = WGS84) on 13 February 2019. In addition, we found another population of *Pseudopaludicola* sp. 3 about 20 km north from Tabocas Village, in the municipality of Santo Amaro do Maranhão, MA, Brazil (2°49′22″ S, 43°7′27″ W, 18 m a.s.l.; datum = WGS84). This last population was assigned to *Pseudopaludicola* sp. 3 based on its geographical proximity (see Fig. 1), besides the morphological and acoustic similarities.

Specimens were collected under authorization number #30059-12 issued by SISBio / ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). According 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 euthanized by applying 5% lidocaine to the skin. After that, we collected muscle tissue for genetic analyses, fixed specimens in 10% formalin and transferred them to 70% ethanol for permanent storage. The new species hypothesis is in accordance to the General Lineage Concept, which treats species as separately evolving metapopulation lineages (de Queiroz 1998, 2007).

**Morphometry**

We measured 11 adult males and five adult females (type series) of the new species under a stereo microscope Zeiss Stemi 2000 coupled to an ocular micrometer; except snout–vent length which was taken with a Mitutoyo Absolute digital caliper (to the nearest 0.1 mm) under a stereo microscope. Twelve morphometric traits were measured following Watters et al. (2016): snout–vent length, head length, head width, eye diameter, interorbital distance, eye–nostril distance, snout length, internarial distance, hand length, thigh length, tibia length and foot length. Tarsus length was measured following Heyer et al. (1990). Terminal phalanges or expanded toe tips were verified by clearing and staining. These procedures were conducted following the protocols of Taylor & Van Dyke (1985). Shape of the
snout in dorsal and lateral views follows Heyer et al. (1990). Further details on examined specimens are in Appendix 1.

Bioacoustics

We recorded vocalizations with two digital recorders at 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 a Sennheiser ME66/K6 or ME 67/K6 directional microphones (Sennheiser electronic GmbH & Co. KG, Germany). Directional microphones were positioned about 1.5 m from the calling male. 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.

Fig. 1. Partial map of South America showing the Brazilian domains and samples of the species included in our molecular, morphological and acoustic comparisons. The type localities of the species are indicated with stars: *Pseudopaludicola coracoralinae* sp. nov. in Palmeiras de Goiás, GO (red star), *P. facureae* Andrade & Carvalho, 2013 in Uberlândia, MG (blue star), *P. canga* Giaretta & Kokubum, 2003 in Marabá, PA (yellow star) and *P. parnaiha* Roberto, Cardozo & Ávila, 2013 in Ribeiro Gonçalves, PI (green star). Municipalities: 1 = Barreirinhas (MA); 2 = Santo Amaro do Maranhão (MA); 3 = Aragominas (TO); 4 = Palmas (TO); 5 = Mateiros (TO); 6 = São Desidério (BA). Veiga-Menoncello et al. (2014) first noticed a taxonomic unit which they called as *Pseudopaludicola* sp. 3 from Barreirinhas, MA. Since then it has been treated as a species not yet formally described. It is represented here as *P. canga* from the localities 1 and 2.
brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked) and grid spacing (spectral resolution) = 43.1 Hz. Raven obtained the peaks of dominant frequency through its “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. We assessed frequency modulation through the “1st Quartile Frequency” and “3rd Quartile Frequency” functions; these Raven functions provide the frequencies that divide the selection into two frequency intervals containing 25 and 75% of the energy in the selection, respectively (Charif et al. 2010). We generated call figures using the Seewave ver. 1.6 package (Sueur et al. 2008) in R ver. 3.5.3 64-bit (R Core Team 2019). Seewave settings were: Hanning window, 90% overlap and 256 points resolution (FFT). We also assessed the between-male call variation through the coefficients of variation (CV = (SD/mean) × 100). We considered only the stereotyped non-pulsed notes to calculate the CV values of the species, not the introductory notes. 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%.

Temporal traits were measured on oscillograms and the spectral traits were measured on spectrograms. Details for acoustic terminology employed here for the species are available in Supplementary file 1. Pulse terminology follows Magalhães et al. (2014); note and call terminologies follow Köhler et al. (2017). We calculated means and standard deviations considering mean values of individual males, whereas the range (variation) encompasses the minimum and the maximum values for all call samples. For multivariate analyses, we considered only the stereotyped notes, not the introductory notes, because the introductory notes are very irregular and have no clear pattern. Sound files are deposited in Arquivo Sonoro da Coleção de Anuros da Universidade Federal de Uberlândia at UFU and in Fonoteca Neotropical Jacques Vielliard (FNJV) at UNICAMP, both in Brazil.

We recorded the vocalizations of 18 males of the new species in the municipality of Palmeiras de Goiás, GO. Further details of all the analyzed sound files of the new species are given in Appendix 2. We reanalyzed the recordings of 10 topotypical males of the original description of *P. facureae* (Andrade & Carvalho 2013) for comparisons with the new species. Additionally, we analyzed nine topotypical males of *P. facureae* that we recorded on 15 March 2019 at Jardim Karaíba neighborhood, municipality of Uberlândia, MG, Brazil. The recordings of four of these males of *P. facureae* are deposited at FNJV (FNJV 40312–5) and the other five are at Arquivo Sonoro da Coleção de Anuros (UFU).

We recorded and analyzed the vocalizations of 16 topotypical males of *P. parnaiba* (FNJV 40328–43). In order to maintain the reliability of acoustic comparisons with this species, as well as to guarantee the integrative approach, we used only acoustic data from males of populations from which we also have fragments of mitochondrial DNA. Therefore, we carried out our comparative analyzes based on calls from 12 males of *Pseudopaludicola* sp. 3 (FNJV 40316–27) of Veiga-Menoncello *et al.* (2014) from Barreirinhas and 17 males from Santo Amaro (FNJV 40344–60), both in MA, Brazil; three males of *P. cf. canga* from Aragominas, two males from Mateiros and two males from Palmas, all in TO, Brazil; and three topotypical males of *P. canga* from Marabá, PA, Brazil. Topotypical males of *P. canga* were recorded by Adão José Cardoso on July and August 1984 with an Uher analog tape recorder and an Uher M-538 microphone. Additionally, we analyzed recordings of trilled calls of the two males from the municipality of São Desidério, BA, Brazil. We did not include these last specimens in the multivariate analyses because we did not have their genetic information.

**Statistical analysis**

We sought for morphometric and acoustic discriminations among species by applying the function randomForest (RF) (randomForest ver. 4.6-14 package; Liaw & Wiener 2002) which constructs many (e.g., 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 function proximityPlot (rfPermute ver. 2.1.6 package; Archer 2018) creates a plot of RF proximity scores using multi-dimensional scaling. The direct or indirect packages for this discriminant analyses were run in R ver. 3.5.3 64-bit (R Core Team 2019).

For the morphometric multivariate analysis between new species and P. facureae, we used snout–vent length, head length, head width, eye diameter, eye–nostril distance, internarial distance, hand length, thigh length, tibia length and foot length. For the acoustic multivariate analysis and statistical tests, we used note duration, internote interval, number of notes per minute, number of notes per series, series duration, interseries interval, number of series per call, peak of dominant frequency, and minimum and maximum of dominant frequency. Acoustic traits were tested for statistical significance of differences between 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. We considered significance when \( P \leq 0.05 \).

Sequence analyses and phylogenetic inferences

For the taxonomic evaluations, we collected new tissue samples for specimens from Palmeiras de Goiás, GO; topotypes of P. parnaiba; specimens of P. canga from three different localities in TO; specimens of Pseudopaludicola sp. 3 of Veiga-Menoncello et al. (2014) from Barreirinhas and Santo Amaro do Maranhão, both in MA; and topotypes and other specimens of P. giarettai from Grande Sertão Veredas National Park, MG, Brazil (Appendix 3).

We extracted total DNA from newly collected samples using a standard ammonium acetate precipitation method (Maniatis et al. 1982; adapted by Lyra et al. 2017). We amplified a fragment of mitochondrial DNA including the partial sequences of 12S rRNA, tRNA-val and 16S rRNA genes (H1 fragment, \(~ 2450 \text{ bp}\); see Appendix 4 for primers used) for all species. PCR products were purified using enzymatic reaction and sent to Macrogen Inc. Republic of Korea, to be sequenced in an ABI 3730 automated DNA sequencer. New DNA sequences were edited for quality and assembled using Geneious ver. 11 (Biomatter) and submitted to GenBank (Appendix 3).

The new sequences were combined with the sequences available in GenBank for Pseudopaludicola spp. from previous works and 19 outgroups (Appendix 3), totalizing 94 samples. The H1 fragment was aligned using MAFFT ver. 7.25 using E-INS-I strategy (Katoh & Standley 2013). We performed Maximum Likelihood (ML) analysis with RAXML ver. 8.2.12 (Stamatakis 2014), searching for the most likely tree with 1000 replicates and using the GTRCAT substitution model. We then estimated node support with 1000 non-parametric bootstrap replicates under the same model. Analyses were run in the CIPRES Science Gateway (Miller et al. 2010). We edited the most likely tree in FigTree ver. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

The maximum genetic distances within species and/or populations and minimum genetic distances between species were calculated for the 16S fragment flanked by primers 16Sar-L and 16Sbr-H, since this fragment was available for all samples included in the analyses. Estimates were done using the package Spider in R ver. 3.6.1 (Brown et al. 2012; R Core Team 2019), uncorrected p-distances and the alignment obtained with MAFFT. Gaps and missing data were treated as pairwise deletions in uncorrected p-distances.

Abbreviations of acoustic traits

\[
\begin{align*}
\text{INI} & = \text{internote interval} \\
\text{ISI} & = \text{interseries interval} \\
\text{MaxDF} & = \text{max. reached dominant frequency}
\end{align*}
\]
MinDF = min. reached dominant frequency
ND = note duration
NM = notes/minute
NNS = number of notes per series
NSC = number of series per call
PDF = peak of dominant frequency
SND = series of notes duration

Abbreviations of morphometric traits
ED = eye diameter
END = eye–nostril distance
FL = foot length
HAL = hand length
HL = head length
HW = head width
IND = internarial distance
IOD = interorbital distance
SL = snout length
SVL = snout–vent length
TAL = tarsus length
TBL = tibia length
TL = thigh length

Repositories
AAG-UFU = Collection of frogs of the Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil
ANDES-A = Museo de Historia Natural ANDES at the Universidad de los Andes, Colombia
CFBH = Célio F.B. Haddad Collection, Departamento de Biodiversidade, Universidade Estadual Paulista, Campus de Rio Claro, São Paulo, Brazil
CFBH-T = Célio F.B. Haddad Tissues Collection, Departamento de Biodiversidade, Universidade Estadual Paulista, Campus de Rio Claro, São Paulo, Brazil
MACN = Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina
MLP-DB = Museo de La Plata, La Plata, Argentina
MNRJ = Museu Nacional/Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
SMRP = Shirlei Maria Recco-Pimentel Collection, Departamento de Biologia Estrutural e Funcional, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil
UFM = Amphibian Collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil
UFU = Universidade Federal de Uberlândia, Brazil
FNJV = Fonoteca Neotropical Jacques Vielliard, Universidade Estadual de Campinas, São Paulo, Brazil
DZSRJP = Amphibian Collection of the Departamento de Zoologia e Botânica, Universidade Estadual Paulista, São José do Rio Preto, São Paulo, Brazil
ZUEC = Museu de Zoologia “prof. Adão José Cardoso”, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil
ZUFMS = Zoological collection (ZUFMS) of the Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brazil
Brazilian states
BA = Bahia
CE = Ceará
ES = Espírito Santo
GO = Goiás
MA = Maranhão
MG = Minas Gerais
MT = Mato Grosso
MS = Mato Grosso do Sul
PA = Pará
PI = Piauí
RS = Rio Grande do Sul
SP = São Paulo
TO = Tocantins

Results

Taxonomic status of Pseudopaludicola parnaiba and Pseudopaludicola sp. 3 of Veiga-Menoncello et al. (2014) from Barreirinhas, state of Maranhão

Based on acoustics traits, we were unable to discriminate topotypical males of P. parnaiba from those of Pseudopaludicola sp. 3 from Barreirinhas and Santo Amaro do Maranhão (Table 1). The RF multivariate approach applied to acoustic data indicated a broad overlap between these two partitions (Fig. 2), with a considerable classification error (Table 2). Moreover, the results were very similar when comparing both P. parnaiba and Pseudopaludicola sp. 3 with P. canga (topotypical and non-topotypical males from TO), with a broad overlap of these four partitions (Fig. 2). All three topotypes of P. canga were correctly classified, while the other three groupings had classification errors (Table 2). In contrast, the raw data of their variables overlapped with those of P. parnaiba, Pseudopaludicola sp. 3 and P. canga from TO (Table 1).

Furthermore, the specimens of Pseudopaludicola sp. 3 and P. parnaiba were nested together with specimens of P. canga from TO in the topology of the phylogenetic tree (Fig. 3). Based on the 16S fragment, the minimum uncorrected p-distance was 0.21% between Pseudopaludicola sp. 3 and P. parnaiba, and 2.27% between Pseudopaludicola sp. 3 and P. canga from the type locality (Supplementary file 2). The genetic distance between P. parnaiba and P. canga from the type locality was 1.86% (Supplementary file 2).

In short, the acoustic and genetic evidence did not support a novel specific identity for Pseudopaludicola sp. 3 of Veiga-Menoncello et al. (2014). Also, the values of all traits of the analyzed calls of the two males from São Desidério, BA, overlapped with those described for P. canga in the present study (Table 1). Therefore, P. canga is the most suitable taxonomic identity for the populations of Pseudopaludicola sp. 3 of Veiga-Menoncello et al. (2014) and this one from São Desidério.

Phylogenetic inference for Pseudopaludicola

The final alignment used for phylogenetic inference contained 2499 bp, and the tree obtained (Fig. 3) recovered basically the same topologies and interspecific relationships inferred in previous analyses of Pseudopaludicola (Veiga-Menoncello et al. 2014; Andrade et al. 2016, 2018a, 2018b, 2019). The genus was recovered as monophyletic, composed of two major clades (Supplementary file 3). One major clade, identified as I (2n = 22) by Veiga-Menoncello et al. (2014), included two subclades. One subclade is well-supported (Bootstrap Support, BS = 99) and is composed of P. falcipes (Hensel, 1867),
Table 1. Advertisement call traits based on the recordings of topotypical males of *Pseudopaludicola parnaiba* Roberto, Cardozo & Ávila, 2013 and *P. canga* Giaretta & Kokubum, 2003; males of *Pseudopaludicola* sp. 3 from Barreirinhas and Santo Amaro do Maranhão; three males from Aragominas, two from Mateiros and other two from Palmas, all these seven are non-topotypical males of *P. canga* from TO; and additional non-topotypical males of *P. canga* from São Desidério, BA, Brazil. Values presented as mean ± standard deviation (minimum–maximum). n = analyzed males (analyzed notes).

|                  | *P. parnaiba* | *P. canga* | *Pseudopaludicola* sp. 3 |
|------------------|---------------|------------|--------------------------|
|                  | Topotypes     | State of   | São Desidério,           |
|                  |               | Tocantins  | state of Bahia           |
| Analyzed males (n) | 16            | 3          | 2                        |
| Analyzed notes (n)  | 312           | 196        | 140                      |
|                   | 7             | 2          | 40                       |
|                   | 29            |            | 570                      |
| Call duration (s)  | 16.7 ± 11.4   | 10.9 ± 5.6 | 15.2 ± 5.3               |
|                   | (2.5–43.3)    | (6.6–17.7) | (7.4–24.9)               |
| Series of notes duration (s) | 0.7 ± 0.3 | 0.5 ± 0.1 | 1.9 ± 0.9 |
|                   | (0.1–5.9)     | (0.2–0.6)  | (0.3–7.3)                |
| Interseries interval (s) | 0.7 ± 0.1 | 2.0 ± 0.5 | 0.9 ± 0.3 |
|                   | (0.2–2.2)     | (0.6–6.6)  | (0.4–2.1)                |
| Number of series per call | 12.9 ± 7.3 | 4.7 ± 1.5 | 8.0 ± 4.8 |
|                   | (3.0–30.0)    | (3.0–8.0)  | (4.0–17.0)               |
| Number of notes per series | 8.6 ± 2.8 | 6.8 ± 1.2 | 21.6 ± 10.1 |
|                   | (2.0–61.0)    | (3.0–9.0)  | (5.0–85.0)               |
| Note duration (ms) | 25 ± 4        | 29 ± 4     | 26 ± 2                   |
|                   | (14–42)       | (21–45)    | (18–33)                  |
| Internote interval (ms) | 56 ± 11 | 48 ± 7   | 61 ± 6                   |
|                   | (29–110)      | (36–73)    | (45–118)                 |
| Notes/minute      | 843.2 ± 154.4 | 862.8 ± 91.5 | 727.7 ± 47.8             |
|                   | (557.1–1285.7)| (676.7–944.9)| (582.0–891.7)            |
|                   | 727.7 ± 47.8 | (744.2–919.2)| (736.5–1097.9)           |
| Min. reached dominant frequency (kHz) | 4.40 ± 0.15 | 3.77 ± 0.13 | 4.14 ± 0.22 |
| Max. reached dominant frequency (kHz) | 4.80 ± 0.21 | 4.07 ± 0.08 | 4.56 ± 0.23 |
| 1st Quartile Frequency (kHz) | 4.52 ± 0.15 | 3.84 ± 0.13 | 4.26 ± 0.23 |
| 3rd Quartile Frequency (kHz) | 4.70 ± 0.18 | 3.96 ± 0.12 | 4.43 ± 0.24 |
| Peak of harmonic frequency (kHz) | 9.20 ± 0.40 | 7.77 ± 0.11 | 8.47 ± 0.22 |
| Air temperature (°C) | 26.8–29.6 | –         | 24.0–28.8               |
| Water temperature (°C) | 27.8 | –         | 25–26                   |

Air and water temperature ranges for the different localities are given.
Table 2. Confusion matrix for the comparisons with *Pseudopaludicola parnaiba* Roberto, Cardozo & Ávila, 2013 based on acoustic dataset by means of a randomForests model. Settings: number of tree permutations = 1000; number of variables tried at each split = 3.0; error rate = 30.91%.

|                        | *P. canga* | *P. parnaiba* | *Pseudopaludicola* | *P. canga* from the state of Tocantins | classification error |
|------------------------|------------|---------------|--------------------|----------------------------------------|---------------------|
| *P. canga*             | 3          | 0             | 0                  | 0                                      | 0%                  |
| *P. parnaiba*          | 0          | 9             | 7                  | 0                                      | 44%                 |
| *Pseudopaludicola* sp. 3 | 0          | 6             | 23                 | 0                                      | 21%                 |
| *P. canga* from the state of Tocantins | 0          | 1             | 3                  | 3                                      | 57%                 |

*P. florencei* Andrade, Haga, Lyra, Leite, Kvet, Haddad, Toledo & Giaretta, 2018, *P. restinga* Cardozo, Baldo, Pupin, Gasparini & Haddad, 2018, *P. pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014, *P. matuta* Andrade, Haga, Lyra, Carvalho, Haddad, Giaretta & Toledo, 2018, *P. mineira* Lobo, 1994, *P. murundu* Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-Pimentel & Haddad, 2010, *P. saltica* (Cope, 1887) and *P. jaredi* Andrade, Magalhães, Nunes-de-Almeida, Veiga-Menoncello, Santana, Garda, Loebmann, Recco-Pimentel, Giaretta & Toledo, 2016 (Fig. 3). *Pseudopaludicola falcipes* is a sister taxon of all other species in this subclade, but the relationships among *P. florencei* + *P. restinga* + *P. pocoto*, *P. matuta* + *P. mineira*, and *P. murundu* + *P. saltica* + *P. jaredi* have a moderate bootstrap support (BS = 88). The other subclade contains four members of the five known members of the *P. pusilla* species group (*sensu* Lynch 1989), *P. llanera*, *P. boliviana* Parker, 1927, *P. motorzinho* Pansonato, Veiga-Menoncello, Mudrek, Jansen, Recco-Pimentel, Martins & Strüssmann, 2016 and *P. pusilla*, but with a low bootstrap support (BS = 43; Fig. 3).

The second major clade also includes two well supported subclades: one composed of *P. ameghini* (Cope, 1887) and *P. ternetzi* Miranda-Ribeiro, 1937 (clade II (2n = 20) from Veiga-Menoncello et al. 2014; Fig. 3). The other subclade includes *P. canga*, *P. facureae*, *P. atragula*, *Pseudopaludicola* sp. (Palmeiras de Goiás, GO), *P. mystacalis* (Cope, 1887), *P. jazymnmcdonaldae* Andrade, Silva, Koroiva, Fadel & Santana, 2019 and *P. giarettai* (clades III (2n = 18) and IV (2n = 16) of Veiga-Menoncello et al. 2014; Fig. 3). In this subclade, *P. canga* was recovered as a sister taxon of *P. facureae* + *P. atragula* + *Pseudopaludicola* sp. (Palmeiras de Goiás, GO), with a maximum bootstrap support for these relationships (Fig. 3); and *P. giarettai* was recovered as a sister taxon of *P. mystacalis* + *P. jazymnmcdonaldae*, with a moderate support (BS = 88; Fig. 3). The four sequences of the specimens of *P. giarettai* from the type locality and Grande Sertão Veredas National Park were nested together in the topology (Fig. 3). This park is 400 km north of the type locality.

**Taxonomic accounts**

**Synonymy**

The acoustic and genetic analyses did not allow the discrimination between *P. parnaiba* and *P. canga*. Given that the phylogenetic positions and call data could not distinguish these two species, no current evidence remains to consider *P. parnaiba* as a different species from *P. canga*, and we consider that *P. parnaiba* Roberto, Cardozo & Ávila, 2013 should be treated as a junior synonym of *P. canga* Giaretta & Kokubum, 2003, following the Principle of Priority of the International Code of Zoological Nomenclature (ICZN 1999, Article 23).
Fig. 2. A. First and second dimensions of the Multidimensional scaling on the proximity scores from the randomForest analysis considering acoustic traits of adult males of *Pseudopaludicola canga* Giaretta & Kokubum, 2003 from the type locality (red dots), *P. parnaiba* Roberto, Cardozo & Ávila, 2013 from the type locality (green dots), *Pseudopaludicola* sp. 3 *sensu* Veiga-Menoncello et al. 2014 (blue dots) and *P. canga* from TO (purple dots). Each dot represents an adult male. The circles around the dots represent how males were classified. B. Dotcharts of variable importance score considering acoustic traits as indicated by the randomForest analysis.
Fig. 3. Maximum likelihood tree recovered for the phylogenetic relationships of *Pseudopaludicola* Miranda-Ribeiro, 1926 based on the 12S rRNA, tRNA-val and 16S rRNA mitochondrial genes. Numbers near the nodes are bootstrap values and black dots represent bootstrap = 100; support below species level is not shown.
Species description

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 coracoralinae* sp. nov.
urn:lsid:zoobank.org:act:FAB2ABCB-37D5-429C-9628-9628-91BB62B185B6
Figs 4–5; Tables 3–4

*Pseudopaludicola facureae* from Palmeiras de Goiás, GO — Carvalho et al. 2015a: 267, 271, table 4, appendix 1–2.

Diagnosis

*Pseudopaludicola coracoralinae* sp. nov. is assigned to *Pseudopaludicola* by having a hypertrophied antebrachial tubercle (see Lynch 1989; Lobo 1995) and by its phylogenetic position within the genus. The new species is characterized by the following combination of characters: (1) upper eyelids smooth, without enlarged palpebral tubercles; (2) heel smooth, without conical tubercle; (3) single, subgular vocal sac, cream-colored with white or off-white warts; (4) terminal phalanges knobbed, without T-shaped terminal phalanges or expanded toe tips; (5) relative short hind limbs (tibio-tarsal articulation just reaching the corner of the mouth); (6) trilled advertisement call pattern, composed of 2–6 well-defined series of tonal notes, having each series of 7–116 notes, emitted at rates of 1485–2077 notes per minute.

Etymology

The specific name honors Anna Lins dos Guimarães Peixoto Bretas, better known by her pseudonym Cora Coralina. She was a simple woman, a Brazilian candy maker, writer and poetess. She was born and raised on the banks of the Vermelho River, in the municipality of Goiás, GO, and lived apart from urban centers. Cora Coralina studied until the third year of elementary school and did a typing course at the age of 70, due to a requirement of the publisher that would publish her first book. She is considered one of the most influential Brazilian writers. Although Cora Coralina wrote her first verses during her adolescence, she had her first book (Poemas dos Becos de Goiás e Estórias Mais) published in June 1965, when she was 75 years old. In 1984, the Brazilian Union of Writers awarded her the “literary personality of the year”. Following that honor, Carlos Drummond de Andrade, another distinguished Brazilian poet, said: “I admire Cora Coralina and her mastery of living in a state of grace with her poetry. Her verse is like running waters, her lyricism has the power and delicacy of the natural world.”

Type material

Holotype  
BRAZIL • adult ♂; state of Goiás, municipality of Palmeiras de Goiás; 16°46′59″ S, 49°52′2″ W; 652 m a.s.l. (Fig. 1); 14 Mar. 2019; F.S. Andrade and I.A. Haga leg.; GenBank: MT385245; ZUEC 24704 (Figs 4, 5A).

Paratypes  
BRAZIL • 6 adult ♂♂; same data as for holotype; GenBank: MT385243, MT385244; ZUEC 24701 to 24703, 24707 to 24709 • 5 adult ♀♀; same data as for holotype; ZUEC 24705, 24706, 24710 to 24712 • 4 adult ♂♂; state of Goiás, municipality of Palmeiras de Goiás; 16°50′48″ S, 49°51′51″ W; 611 m a.s.l.;
Type locality
Brazil, GO, municipality of Palmeiras de Goiás (16°46′59″ S, 49°52′2″ W; 652 m a.s.l.; Fig. 1).

Description of the holotype
Body elliptic and broad (Fig. 4A–B; Table 3). Head elliptical, slightly wider than long. Snout subovoid in dorsal view and rounded in profile (Fig. 4C–D). Eye not protuberant. Eye diameter almost equal to interorbital distance. Interorbital area flat. Pupil rounded. Upper eyelid without tubercles. Nostril not protuberant and closer to snout tip than to eye. Canthus rostralis rounded, smooth. Loreal region slightly concave. Single subgular vocal sac, externally expanded with warty texture. Choanae rounded, well separated from each other. Vocal slits present. Tympanum membrane and annulus absent. Discrete tympanic ridge from behind eye to proximal portion of arm insertion. Mouth opening ventral. Vomerine teeth absent. Tongue ovoid, longer than wide, free posteriorly, without pigmentation at its base. Lateral of head and flanks with discrete granules. One ovoid antebrachial tubercle presents in first quarter of forearm. Finger and toe tips not expanded. Outer and inner metacarpal tubercles well-defined; outer metacarpal tubercle rounded and inner metacarpal tubercle ovoid. Fingers with single and rounded subarticular tubercles. Supernumerary tubercles absent on palm of hand. Thumb with discrete, keratinized, light brown nuptial pad, extending from base of hand to proximal limit of terminal phalanx, covering almost entire external portion of finger. Webbing absent between fingers. Relative finger lengths, when adpressed one to another: I < II < IV < III (Fig. 4E). Outer metatarsal tubercle well defined, conical, smaller than ovoid inner metatarsal tubercle. Toes with well-defined, single, enlarged, rounded subarticular tubercles. Supernumerary tubercles absent on sole of foot. Toes webbed basally and fringed along their sides to almost their tips. Fringes developed on all toes (mainly on II, III, IV and V). External fringe on Toe V continues almost to outer metatarsal tubercle. Well-developed fold from internal metatarsal tubercle to mid-ventral tarsus, ending in protuberant tarsal tubercle. Relative toe lengths, when adpressed one to another: I < II < V < III < IV (Fig. 4F). Hind limb robust with tibiotarsal articulation just reaching posterior margins of eye. Thigh shorter than tibia. Foot longer than thigh. Heel without tubercles. Belly skin smooth. Abdominal fold present and complete. Well-defined vertebral stripe from snout tip to vent. Dorsal surfaces of head, body and limbs smooth. Paravertebral chevron-shaped dermal ridge from behind eye to scapular region. Cloacal region smooth (Fig. 4B). Measurements of the holotype showed in Table 3.

Color pattern of the holotype in preservative
Dorsum brown with dark brown and black gray blotches. Belly whitish (unpigmented). Vocal sac cream-colored with white or off-white warts. Dorsum darker than dorsal surfaces of limbs. Region between upper lip and eye with several rounded white blotches with alternating vertical grey and light beige stripes. Ventral faces of arm and leg unpigmented. Dorsum darker than dorsal surfaces of limbs. Region between upper lip and eye with several rounded white blotches with alternating vertical grey and light beige stripes. Color of sole of foot similar to that of dorsal region of hind limb. Dorsal face of arm light beige with several dark brown blotches. Dorsal face of limb light brown with dark brown transversal discontinuous stripes and with scattered brown blotches. Dark brown transverse stripes on arm (2–3), thigh (2–3), shank (2–3), foot (3–4). Light brown nuptial pads (Fig. 4).
Fig. 4. *Pseudopaludicola coracoralinae* sp. nov., holotype (ZUEC 24704, SVL = 13.1 mm), an adult ♂ from Palmeiras de Goiás, GO, Brazil. **A.** Dorsal view. **B.** Ventral view. **C.** Head, lateral view. **D.** Head, dorsal view. **E.** Right hand, ventral view. **F.** Right foot, ventral view. Scale bar (only for C, D, E, F) = 5 mm.
AAG-UFU 3393–96 have the region between upper lip and eye without alternating vertical stripes. The specimen ZUEC 24705 has no rounded white blotches on the region between upper lip and eye. The specimens ZUEC 24702, 24707–10, 24712 and AAG-UFU 3394 have dorsolateral stains on body, from posterior corner of eyes to almost the region of insertion of legs. The specimens ZUEC 24706, 24708–11 and AAG-UFU 3393 have no transverse stripes on arm.

Advertisement call

The advertisement call of the new species (total duration: 1.3–25.8 s) consists of 2–6 series of stereotyped tonal notes (non-pulsed) that last 0.2–4.1 s, separated by intervals of 0.4–6.7 s. Before the emission of the series of stereotyped tonal notes, 12–40 (mean = 22.1, SD = 9.8) isolated notes with irregular structure, duration and interval are emitted, herein referred to as introductory notes (Fig. 6A). Introductory notes last 4–24 ms (mean 12, SD = 3), separated by intervals of 49–477 ms (mean = 146, SD = 27), and their dominant frequency peaks between 3.62–5.16 kHz (mean = 4.39, SD = 0.24). In contrast, within the series of stereotyped tonal notes, the notes have regular structure, duration and interval. These notes last 11–21 ms, separated by intervals of 12–61 ms, and are released at a rate of 1484.7–2076.6 notes per minute; notes have a slight increase in amplitude until the end of the first quartile of their durations, in the last quartile of their durations the notes suffer a decrease in amplitude (Fig. 6B). Dominant frequency peaks are between 4.18 and 5.06 kHz; the minimum frequency ranges between 3.84 and 4.59 kHz and the maximum frequency ranges between 4.41 and 5.44 kHz. The notes have a slight increase in frequency along their durations; on average, the notes have an increase of 275 Hz from the first to the third quartiles of frequencies (Table 4). The dominant frequency coincides with the fundamental harmonic, and the second harmonic ranges between 8.34 and 10.50 kHz (Fig. 6B). Air temperature of recorded calls varied from 22.2 to 26.0°C. Traits that were classified as static (between-male CV < 11%) to *P. coracoralinae* sp. nov. were note duration, notes per minute and all spectral traits. The other traits were classified as dynamic. Call quantitative traits and CV values are summarized in Table 4.
Differential diagnosis

*Pseudopaludicola coracoralinae* sp. nov. is promptly diagnosed from the *P. pusilla* species group (*sensu* Lynch 1989), which includes *P. boliviana*, *P. ceratophyes* Rivero & Serna, 1985, *P. llanera*, *P. pusilla* and *P. motorzinho*, by the absence of either T-shaped terminal phalanges or expanded toe tips (discs or pads). The new species has terminal phalanges knobbed, similar in shape to those of *P. falcipes* (Cardozo & Suárez 2012: fig. 2B). 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 2018).

![Fig. 5. Holotype and five paratypes of *Pseudopaludicola coracoralinae* sp. nov. in life. A. ZUEC 24704 (holotype, adult ♂ and call voucher), SVL = 13.1 mm. B. ZUEC 24703 (adult ♂ and call voucher), SVL = 13.2 mm. C. ZUEC 24707 (adult ♂), SVL = 12.5 mm. D. ZUEC 24712 (adult ♀), SVL = 16.0 mm. E. ZUEC 24705 (adult ♀), SVL = 16.8 mm. F. ZUEC 24706 (adult ♀), SVL = 17.0 mm.](image-url)
The new species also differs from P. boliviana, P. ceratophyes, P. llanera and P. motorzinho by having a smooth heel, without enlarged, conical tubercle (Lynch 1989; Pansonato et al. 2016).

*Pseudopaludicola coracoralinae* sp. nov. is promptly distinguished from the *P. saltica* species group that includes *P. saltica*, *P. murundu* and *P. jaredi*, by having short hind limbs (tibiotarsal articulation reaching near the corner of the mouth), whereas all three above-mentioned species have long hind limbs (tibiotarsal articulation extending beyond the tip of snout; Andrade et al. 2016).

The color and skin texture of the vocal sac of the *P. coracoralinae* sp. nov. is whitish cream with white or off-white warts (Fig. 4B), thereby distinguishing it from all congeners, except from *P. facureae*, *Pseudopaludicola ameghini*, *P. ternetzi*, *P. falcipes*, *P. giarettai*, *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strussmann & Martins, 2012, *P. canga*, *P. florencei*, *P. pocoto*, *P. mineira*, *P. restinga*, *P. matuta*, *P. mystacalis*, *P. ceratophyes*, *P. llanera*, *P. boliviana*, *P. motorzinho*, *P. ibisoroca* Pansonato, Veiga-Menoncello, Mudrek, Jansen, Recco-Pimentel, Martins & Strüssmann, 2016 and *P. saltica* have vocal sacs that are whitish, yellowish, or light cream with no warty texture (combined characters of the vocal sac of all above-mentioned species: Miranda-Ribeiro 1937; Ruthven 1916; Rivero & Serna 1985; Haddad & Cardoso 1987; Lynch 1989; Lobo 1994; Giaretta & Kokubum 2003; Carvalho 2012; Pansonato et al. 2012, 2013, 2016; Roberto et al. 2013; Magalhães et al. 2014; Carvalho et al. 2015b, Andrade et al. 2017a, 2018a, 2018b; Cardozo et al. 2018); *P. jazymynmcdonaldae* has a dark and smooth vocal sac with no warty texture (Andrade et al. 2019); and *P. atragula* has a white vocal sac with warty texture and dark-colored reticulations (Pansonato et al. 2014a).

![Fig. 6. A. Oscillogram of the entire advertisement call of *Pseudopaludicola coracoralinae* sp. nov. with the introductory notes followed by three series of tonal notes. B. Audiospectrogram (top) and corresponding oscillogram (bottom) detailing three notes. The male was recorded on 8 November 2016 at 18:08 h; air temperature 26°C, water temperature 29°C; recording_label: Pseudop_coracoralinaePalmeirasGoiasGO5cFSA_AAGmt.](image-url)
The trilled pattern of its advertisement call (presence of non-pulsed notes) promptly distinguishes the new species from all species of *Pseudopaludicola* that have notes with pulsatile structure (pulses separated by silence intervals or not): *P. ameghini*, *P. atragula*, *P. boliviana*, *P. falcipes*, *P. florencei*, *P. ibisoroca*, *P. jaredi*, *P. jazmynmcdonaldae*, *P. matuta*, *P. mineira*, *P. motorzinho*, *P. murundu*, *P. mystacalis*, *P. pocoto*, *P. restinga*, *P. saltica* and *P. ternetzi* (Haddad & Cardoso 1987; Duré et al. 2004; Pereira & Nascimento 2004; Pansonato et al. 2013, 2014a, 2014b, 2016; Magalhães et al. 2014; Andrade et al. 2016, 2017a, 2017b, 2018a, 2018b, 2019; Cardozo et al. 2018).

Table 4. Advertisement call traits based on the recordings of *Pseudopaludicola coracoralinae* sp. nov. and *P. facureae* Andrade & Carvalho, 2013, both from the type localities. Values presented as mean ± standard deviation (minimum–maximum). CV = between-male coefficient of variation; n = analyzed males (analyzed notes).

|                          | *P. coracoralinae* sp. nov. | *P. facureae* |
|--------------------------|-----------------------------|---------------|
| Call duration (s)        | 7.2 ± 6.4 (1.3–25.8)        | 12.3 ± 6.3 (4.7–22.6) |
| CV                       | 88.9%                       | 51.2%          |
| Series of notes duration (s) | 1.0 ± 0.6 (0.2–4.1)     | 0.5 ± 0.1 (0.1–3.5) |
| CV                       | 60.0%                       | 20.0%          |
| Interseries interval (s) | 1.0 ± 0.5 (0.4–6.7)        | 0.6 ± 0.1 (0.1–1.4) |
| CV                       | 50.0%                       | 16.7%          |
| Number of series per call | 3.2 ± 1.2 (2.0–6.0)       | 9.9 ± 5.9 (4.0–20.0) |
| CV                       | 37.5%                       | 59.6%          |
| Number of notes per series | 28.7 ± 15.7 (7.0–116.0)  | 12.7 ± 2.9 (2.0–74.0) |
| CV                       | 54.7%                       | 22.8%          |
| Note duration (ms)       | 15 ± 1 (11–21)             | 19 ± 3 (11–30)  |
| CV                       | 6.6%                        | 15.8%          |
| Interote interval (ms)   | 19 ± 4 (12–61)             | 24 ± 8 (11–156) |
| CV                       | 21.1%                       | 33.3%          |
| Notes/minute             | 1796.1 ± 123.1 (1484.7–2076.6) | 1381.4 ± 197.2 (512.1–1842.6) |
| CV                       | 6.8%                        | 14.3%          |
| Peak of dominant frequency (kHz) | 4.60 ± 0.22 (4.18–5.06) | 4.35 ± 0.25 (3.84–4.99) |
| CV                       | 4.8%                        | 5.7%           |
| Min. reached dominant frequency (kHz) | 4.27 ± 0.22 (3.84–4.59) | 4.11 ± 0.26 (3.61–4.74) |
| CV                       | 5.2%                        | 6.3%           |
| Max. reached dominant frequency (kHz) | 4.95 ± 0.27 (4.41–5.44) | 4.66 ± 0.29 (4.12–5.21) |
| CV                       | 5.5%                        | 6.2%           |
| 1st Quartile Frequency (kHz) | 4.46 ± 0.21 (4.12–4.87) | 4.26 ± 0.27 (3.75–4.87) |
| CV                       | 4.7%                        | 6.3%           |
| 3rd Quartile Frequency (kHz) | 4.73 ± 0.23 (4.26–5.16) | 4.47 ± 0.27 (3.94–5.08) |
| CV                       | 4.9%                        | 6.0%           |
| Peak of 2nd harmonic frequency (kHz) | 9.32 ± 0.57 (8.34–10.50) | 8.81 ± 0.57 (6.98–10.25) |
| CV                       | 6.1%                        | 5.5%           |
| Air temperature (°C)     | 22.2–26.0                   | 21.0–31.0      |
| Water temperature (°C)   | 24.2–29.0                   | 21.0–31.0      |

The trilled pattern of its advertisement call (presence of non-pulsed notes) promptly distinguishes the new species from all species of *Pseudopaludicola* that have notes with pulsatile structure (pulses separated by silence intervals or not): *P. ameghini*, *P. atragula*, *P. boliviana*, *P. falcipes*, *P. florencei*, *P. ibisoroca*, *P. jaredi*, *P. jazmynmcdonaldae*, *P. matuta*, *P. mineira*, *P. motorzinho*, *P. murundu*, *P. mystacalis*, *P. pocoto*, *P. restinga*, *P. saltica* and *P. ternetzi* (Haddad & Cardoso 1987; Duré et al. 2004; Pereira & Nascimento 2004; Pansonato et al. 2013, 2014a, 2014b, 2016; Magalhães et al. 2014; Andrade et al. 2016, 2017a, 2017b, 2018a, 2018b, 2019; Cardozo et al. 2018).
Acoustic comparison with its sister species

*Pseudopaludicola coracoralinae* sp. nov. and *P. facureae* are indistinguishable in external morphology, but the new species was recovered as a sister species of *P. facureae* + *P. atragula* (Fig. 3). Furthermore, the RF multivariate approach applied to morphometric data indicated a broad overlap between the two partitions (Fig. 7A–B), with a considerable classification error (Table 5). In relation to three species of *Pseudopaludicola* that share the trilled advertisement call pattern (*P. hyleaustralis, P. facureae* and *P. canga*), *P. facureae* is the one with the most similar call to that of *P. coracoralinae* sp. nov. The trait of notes per minute distinguishes the new species (1485–2077 notes per minute) from *P. canga* and *P. hyleaustralis* (368–1286 notes per minute; combined values, Table 1; see Carvalho et al. 2015a). The RF multivariate analysis on acoustic data indicated a complete segregation between *P. coracoralinae* sp. nov. and *P. facureae*, without any classification error (Table 5; Fig. 7C). Notes per minute (*P. coracoralinae* sp. nov. 1796 ± 123 (1485–2077) vs *P. facureae* 1383 ± 192 (512–1843)), number of series per call (*P. coracoralinae* sp. nov. 3 ± 1 (2–6) vs *P. facureae* 10 ± 6 (4–20)) and number of notes per series (*P. coracoralinae* sp. nov. 29 ± 16 (7–116) vs *P. facureae* 17 ± 18 (2–93)) were the main sources of variation in both variable importance measurements (Fig. 7D). In addition to these above-mentioned traits, we found differences (*P* ≤ 0.01) between these two species in note duration, internote interval, series of notes duration, interseries interval and dominant frequency.

Phylogenetic inference and genetic distances of the new species

*Pseudopaludicola coracoralinae* sp. nov. was recovered as a sister species of the *P. atragula* + *P. facureae* clade (Fig. 3). Uncorrected genetic distance between the *P. coracoralinae* sp. nov. and *P. atragula* was 4.5% (mean value), and from *P. facureae*, it was 4.9% (mean value). The maximum intraspecific distance was 0.4% (Supplementary file 2). No molecular data are available for *P. ceratophyes, P. hyleaustralis* and *P. ibisoroca*; however, the new species is easily diagnosed from these species by morphology and acoustics (see further details in Differential diagnosis section).

Natural history notes

Males of the new species were found calling in a partially flooded open area surrounded by a newly planted cornfield (corn stalk < 40 cm tall). We collected the holotype and ZUEC’s paratypes at this site. The AAG-UFU’s paratypes were collected in another partially flooded open area near to a permanent lagoon at the margins of the GO-156 highway. We observed three couples in axillary amplexus in the field. In our field recordings of vocalizations, the males were vocalizing well-spaced from each other, without any close-range encounters. The new species was observed syntopically with *Leptodactylus fuscus* (Schneider, 1799) and *Physalaemus marmoratus* (Reinhardt & Lütken, 1862) at its type locality. Curiously, the congener *Pseudopaludicola mystacalis* was observed about 50 meters in a similar partially flooded open area surrounded by the same cornfield. We heard and observed only *P. mystacalis* at this site, not *P. coracoralinae* sp. nov.

Distribution

*Pseudopaludicola coracoralinae* sp. nov. is known only from the type locality (Fig. 1). However, we are aware of other populations that have trilled advertisement calls similar to those of *P. coracoralinae* sp. nov. and *P. facureae*. These populations occur in Limeira do Oeste, MG (Andrade & Carvalho 2013); Goianésia, Piracanjuba and in the Altamiro de Moura Pacheco State Park, all in GO, Brazil (Guimarães et al. 2001; Carvalho et al. 2015a; Ramalho et al. 2018). Goianésia is about 180 km northeast from the type locality of *P. coracoralinae* sp. nov., Piracanjuba is about 100 km southeast and the Altamiro de Moura Pacheco State Park is about 80 km northeast. Limeira do Oeste is closer to the type locality of *P. facureae*, about 250 km east. However, the specific identities of these populations will only be confirmed when their genetic information is available because they are morphologically and acoustically cryptic.
**Table 5.** Confusion matrix for *Pseudopaludicola coracoralinae* sp. nov. and *P. facureae* Andrade & Carvalho, 2013 based on morphometric and acoustic (values in bold) datasets by means of a randomForests model. Settings: number of tree permutations = 1000; number of variables tried at each split = 3.0; error rates = 29.2% | 0%.

|                | *P. coracoralinae* sp. nov. | *P. facureae* | classification error |
|----------------|-----------------------------|---------------|-----------------------|
| *P. coracoralinae* sp. nov. | 8 | 18 | 3 | 0 | 27% | 0% |
| *P. facureae* | 4 | 0 | 9 | 19 | 31% | 0% |

**Discussion**

The acoustic characterization of *P. parnaiba* showed here is in accordance with that of Carvalho *et al.* (2015a), and we were also unable to found reliable diagnostic characters between *P. parnaiba* and *P. canga*. In addition, the phylogenetic positions of the topotypes of *P. parnaiba* did not support the hypothesis that it evolved independently of *P. canga*. Therefore, *P. parnaiba* is regarded herein as a junior synonym of *P. canga*. Hence, the distribution of *P. canga* has increased considerably, occurring in four Brazilian states: PA (Marabá, type locality), MA (Barreirinhas and Santo Amaro do Maranhão), TO (Aragominas, Mateiros and Palmas) and PI (Ribeiro Goçalves).

We analyzed calls from São Desidério, BA, and concluded that these individuals represent *P. canga*, since the values of all their traits overlapped those described for this species in the present study. Therefore, this is the first record of *P. canga* for BA about 900 km southeast from the type locality. Oliveira *et al.* (2013) provided another locality for *P. canga* in PA, in the municipality of Brasil Novo. However, this record was based only on morphological information. Recently, Andrade *et al.* (2018c) reported on its larval morphology based on tadpoles from Mirador State Park, southern MA.

**Fig. 7.** A, C. First and second dimensions of the Multidimensional scaling on the proximity scores from the randomForest analysis of adult males of *Pseudopaludicola coracoralinae* sp. nov. from the type locality (red dots) and *P. facureae* Andrade & Carvalho, 2013 from the type locality (blue dots). A. Morphometric traits. C. Acoustic traits. Each dot represents an adult male. The circles around the dots represent how males were classified. B, D. Dotcharts of variable importance score as indicated by the randomForest analysis. B. Morphometric traits. D. Acoustic traits.
Pseudopaludicola coracoralinae sp. nov. and *P. facureae* are morphometrically indistinguishable from each other. However, the phylogenetic positions of these two cryptic sister species provides sufficient evidence to support our hypothesis that these lineages evolved independently. Moreover, based on the RF results of the acoustic comparison between *P. coracoralinae* sp. nov. and *P. facureae*, notes per minute was the main source of variation in both variable importance measurements. This trait was classified as static in *P. coracoralinae* sp. nov. (*sensu* Gerhardt 1991), due to its low between-male variability. Gerhardt (1991) pointed out that 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. Therefore, *P. coracoralinae* sp. nov. differs statistically from *P. facureae* in a temporal trait that is expected to be associated with their species recognition.

In addition, we found differences in the environment occupied by *P. coracoralinae* sp. nov. and *P. facureae*. The marshy areas of both sites where we recorded and collected *P. coracoralinae* sp. nov. in Palmeiras de Goiás were temporary (see details in the Natural history notes section). On the other hand, *P. facureae* was always found in permanent marshy areas, both in disturbed areas and along palm marshes (*Veredas*) in open grasslands (Giaretta & Facure 2009; Andrade & Carvalho 2013). Males of *P. facureae* called from slow flowing, shallow streamlets with clear water and a muddy bottom; they called sitting in or close to the water (Giaretta & Facure 2009). In addition, *P. facureae* can reproduce continuously all year round (Giaretta & Facure 2009). We did not visit the localities where *P. coracoralinae* sp. nov. occurs during the dry season, so we are unable to determine whether it breeds throughout the year as does *P. facureae*. In any case, the drying of temporary habitats should directly influence the reproduction of *P. coracoralinae* sp. nov.

Fišer *et al.* (2018) stated that evolutionary mechanisms leading to morphological similarity are heterogeneous, comprising recent divergence, niche conservatism and morphological convergence. Yet, those authors also argued that the biodiversity science is only just beginning to understand the ‘invisible’ world of cryptic species. They indicated that integrative approaches can reveal generalities in the speciation process, improving our understanding of the heterogeneity (species properties) in speciation, allowing a better integration in biodiversity science. Closely related species of *Pseudopaludicola* have similar external morphology and high intraspecific variation on dorsal color patterns (e.g., Andrade *et al.* 2017a), therefore, the association of multiple datasets is crucial for unequivocal identifications of *Pseudopaludicola* and to elucidate the hidden diversity within this Neotropical frog clade (Andrade *et al.* 2018a, 2018b, 2019). Thus, the identification of specimens of *Pseudopaludicola* based solely on their external morphology should be avoided in most cases. As an example, two recent studies failed in reporting the occurrence of *P. falcipes* for the Brazilian states GO and MG (Neves *et al.* 2019; Oliveira *et al.* 2019), because it is well-known that it does not occur in the Brazilian Cerrado (Langone *et al.* 2015, 2016).

The topology and interspecific relationships of the phylogeny proposed here for *Pseudopaludicola* species corroborated those topologies and relationships recovered previously (Veiga-Menoncello *et al.* 2014; Andrade *et al.* 2016, 2018a, 2018b, 2019). As in previously phylogenetic inferences, we were not able to find good support for nodes of some subclades of *Pseudopaludicola* (e.g., subclades of *P. mineira* + *P. matuta* and *P. florencei* + *P. restinga* + *P. pocoto*, and *P. saltica* species group). These low bootstrap values can be explained by the existence of unknown species that are not represented in the topologies available so far, i.e., the species richness of *Pseudopaludicola* still remains underestimated.

Here we include for the first time *P. pusilla* and *P. llanera* in a molecular phylogenetic inference of the genus. Although they are nested together with the other sampled species from *P. pusilla* group, it is worth highlighting the low support for this clade. However, we recall here that we obtained only the 16S mitochondrial sequences of *P. pusilla* and *P. llanera* from the GenBank database, which were made
available by Guarnizo et al. (2015). These authors preliminary identified and allocated to nominal species of their collected specimens using external morphology. They collected two specimens of *P. pusilla* in San Vicente, Santander, in the Magdalena River valley, western slope of the Eastern Cordillera (Colombia). Lynch (1989) restricted the distribution of *P. pusilla* to the lower and middle Magdalena River valley and to the Caribbean lowlands of northern Colombia and adjacent Venezuela. Therefore, we emphasize the need to sequence more markers for these Colombian specimens of *P. pusilla*, and further taxonomic studies with the species of this group, especially *P. llanera*, *P. pusilla* and *P. ceratophyes*. For example, there is no genetic information for *P. ceratophyes* yet. Also, acoustic data would be very important to better elucidate these taxonomic issues.

The increase of sampling efforts and the use of multiple datasets in *Pseudopaludicola* taxonomical studies have uncovered the striking species richness of these fascinating dwarf swamp frogs, especially in the last decade (e.g., Toledo et al. 2010; Pansonato et al. 2014a, 2016; Andrade et al. 2016, 2018a, 2018b, 2019; Cardozo et al. 2018). On the other hand, unsustainable agricultural activities, particularly soy production and cattle ranching, as well as burning of vegetation for charcoal, make the Cerrado one of the most threatened biodiversity hotspots (Strassburg et al. 2017). All these actions continue to pose a major threat to the Cerrado’s biodiversity and despite its environmental importance and uniqueness, it is one of the least protected formations in Brazil. The recognition of the *P. coracoralinae* sp. nov. is important to the knowledge of the frog richness and diversification patterns that operated in this region. Future phylogeographic studies would be valuable to shed light on the evolutionary process of *P. coracoralinae* sp. nov., *P. facureae* and *P. atragula*.

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**References**

Andrade F.S. & Carvalho T.R. 2013. A new species of *Pseudopaludicola* Miranda-Ribeiro (Leiuperinae: Leptodactylidae: Anura) from the Cerrado of southeastern Brazil. *Zootaxa* 3608 (5): 389–397. https://doi.org/10.11646/zootaxa.3608.5.7

Andrade F.S., Magalhães F.M., Nunes-de-Almeida C.H.L., Veiga-Menoncello A.C.P., Santana D.J., Garda A.A., Loebmann D., Recco-Pimentel S.M., Giaretta A.A. & Toledo L.F. 2016. A new species of long-legged *Pseudopaludicola* from northeastern Brazil (Anura, Leptodactylidae, Leiuperinae). *Salamandra* 52: 107–124.

Andrade F.S., Haga I.A., Bang D.L. & Giaretta A.A. 2017a. The differential acoustic diagnosis between two *Pseudopaludicola* sister species (Anura, Leptodactylidae, Leiuperinae). *Zootaxa* 4319 (2): 391–400. https://doi.org/10.11646/zootaxa.4319.2.12

Andrade F.S., Leite F.S.F., Carvalho T.R., Bernardes C.S. & Giaretta A.A. 2017b. First record of *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014 (Anura, Leptodactylidae, Leiuperinae) in Bahia state, northeastern Brazil, with further data on its advertisement call. *Check List* 13 (1): 2047. https://doi.org/10.15560/13.1.2047
Andrade F.S., Haga I.A., Lyra M.L., Leite F.S.F., Kwet A., Haddad C.F.B., Toledo L.F. & Giaretta A.A. 2018a. A new species of *Pseudopaludicola* Miranda-Ribeiro (Anura: Leptodactyliidae: Leiuperinae) from eastern Brazil, with novel data on the advertisement call of *Pseudopaludicola falciipes* (Hensel). *Zootaxa* 4433 (1): 71–100. https://doi.org/10.11646/zootaxa.4433.1.4

Andrade F.S., Haga I.A., Lyra M.L., Carvalho T.R., Haddad C.F.B., Giaretta A.A. & Toledo L.F. 2018b. A new species of *Pseudopaludicola* (Anura, Leptodactyliidae, Leiuperinae) from the state of Minas Gerais, Brazil. *European Journal of Taxonomy* 480: 1–25. https://doi.org/10.5852/ejt.2018.480

Andrade E.B., Ferreira J.S., Takazone A.M.G., Libório A.E.C. & Weber L.N. 2018c. Description of the tadpole of *Pseudopaludicola canga* Giaretta and Kokubum, 2003 (Anura: Leptodactyliidae). *South American Journal of Herpetology* 13: 64–72. https://doi.org/10.2994/SAJH-D-17-00032.1

Andrade F.S., Silva L.A., Koroiva R., Fadel R.M. & Santana D.J. 2019. A new species of *Pseudopaludicola* Miranda-Ribeiro, 1926 (Anura: Leptodactyliidae: Leiuperinae) from an Amazonia-Cerrado transitional zone, state of Tocantins, Brazil. *Journal of Herpetology* 53 (1): 68–80. https://doi.org/10.1670/18-125

Archer E. 2018. *rfPermute: Estimate Permutation p-values for Random Forest Importance Metrics (Computer software).* R package ver. 2.0.1. Available from https://CRAN.R-project.org/package=rfPermute [assessed 1 April 2019].

Bioacoustics Research Program. 2014. *Raven Pro: Interactive Sound Analysis Software, ver. 1.5.* The Cornell Lab of Ornithology, Ithaca, New York. Available from http://www.birds.cornell.edu/raven [accessed 5 Feb. 2015].

Brown S.D.J., Collins R.A., Boyer S., Lefort M.C., Malumbres-Olarte J., Vink C.J. & Cruickshank R.H. 2012. SPIDER: an R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources* 12: 562–565. http://doi.org/10.1111/j.1755-0998.2011.03108.x

Cardozo D. & Suárez P. 2012. Osteological description of *Pseudopaludicola canga* with implications for the taxonomic position of this taxon. *Zootaxa* 3515: 75–82. https://doi.org/10.11646/zootaxa.3515.1.6

Cardozo D.E., Baldo D., Pupin N., Gasparini J.L. & Haddad C.F.B. 2018. A new species of *Pseudopaludicola* (Anura, Leiuperinae) from Espirito Santo, Brazil. *PeerJ* 6: e4766. https://doi.org/10.7717/peerj.4766

Carvalho T.R. 2012. A new species of *Pseudopaludicola* Miranda-Ribeiro (Leiuperinae: Leptodactylidae: Anura) from the Cerrado of southeastern Brazil with a distinctive advertisement call pattern. *Zootaxa* 3328: 47–54. https://doi.org/10.11646/zootaxa.3328.1.4

Carvalho T.R., Teixeira B.F.V., Martins L.B. & Giaretta A.A. 2015a. Intraspecific variation and new distributional records for *Pseudopaludicola* species (Anura, Leptodactyliidae, Leiuperinae) with trilled advertisement call pattern: diagnostic characters revisited and taxonomic implications. *North-Western Journal of Zoology* 11: 262–273.

Carvalho T.R., Borges-Martins M., Teixeira B.F.V., Godinho L.B. & Giaretta A.A. 2015b. Intraspecific variation in acoustic traits and body size, and new distributional records for *Pseudopaludicola giarettai* Carvalho, 2012 (Anura, Leptodactyliidae, Leiuperinae): implications for its congeneric diagnosis. *Papéis Avulsos de Zoologia* 55: 245–254. https://doi.org/10.1590/0031-1049.2015.55.17

Charif R.A., Waack A.M. & Strickman L.M. 2010. *Raven Pro 1.4 User’s Manual.* Cornell Lab of Ornithology, Cornell University, USA. Available from http://ravensoundsoftware.com/wp-content/uploads/2017/11/Raven14UsersManual.pdf [assessed 3 Jun. 2020].
de Queiroz K. 1998. The general lineage concept of species, species criteria, and the process of speciation and terminological recommendations. In: Howard D.J. & Berlocher S.H. (eds) Endless Forms: Species and Speciation: 57–75. Oxford University Press, Oxford.

de Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology 56 (6): 879–886. https://doi.org/10.1080/10635150701701083

Duré M.I., Schaefer E.F., Hamann M.I. & Kehr A.I. 2004. Consideraciones ecológicas sobre la dieta, la reproducción y el parasitismo de *Pseudopaludicola boliviana* (Anura, Leptodactylidae) de Corrientes, Argentina. Phyllomedusa 3: 121–131. https://doi.org/10.11606/issn.2316-9079.v3i2p121-131

Fišer C., Robinson C.T. & Malard F. 2018. Cryptic species as a window into the paradigm shift of the species concept. Molecular Ecology 27 (3): 613–635. https://doi.org/10.1111/mec.14486

Frost D.R. 2020. Amphibian Species of the World: an Online Reference. ver. 6.0. American Museum of Natural History, New York. Available from http://research.amnh.org/herpetology/amphibia/index.html [accessed 3 Apr. 2020].

Gerhardt H.C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. Animal Behaviour 42: 615–635. https://doi.org/10.1016/S0003-3472(05)80245-3

Giaretta A.A. & Kokubum M.N.C. 2003. A new species of *Pseudopaludicola* (Anura, Leptodactylidae) from northern Brazil. Zootaxa 383: 1–8. https://doi.org/10.11646/zootaxa.383.1.1

Giaretta A.A. & Facure K.G. 2009. Habitat, egg-laying behaviour, eggs and tadpoles of four sympatric species of *Pseudopaludicola* (Anura, Leiuperidae). Journal of Natural History 43: 995–1009. https://doi.org/10.1080/00222930802702456

Guimarães L.D., Lima L.P., Juliano R.F. & Bastos R.P. 2001. Vocalizações de espécies de anuros (Amphibia) no Brasil Central. Boletim do Museu Nacional, Zoologia (Nova Série) 474: 1–15. https://doi.org/10.1590/S0073-47212003000200005

Guarnizo C.E., Paz A., Muñoz-Ortiz A., Flechas S.V., Méndez-Narváez J. & Crawford A.J. 2015. DNA Barcoding survey of anurans across the eastern cordillera of Colombia and the impact of the Andes on cryptic diversity. PLoS ONE 10 (5): e0127312. https://doi.org/10.1371/journal.pone.0127312

Haddad C.F.B. & Cardoso A.J. 1987. Taxonomia de três espécies de *Pseudopaludicola* (Anura, Leptodactylidae). Papéis Avulsos de Zoologia 36: 287–300.

Haga I.A., Andrade F.S., Bruschi D.P., Recco-Pimentel S.M. & Giaretta A.A. 2017. Unrevealing the leaf frogs Cerrado diversity: A new species of *Pithecopus* (Anura, Arboranae, Phyllomedusidae) from the Mato Grosso state, Brazil. PLoS ONE 12 (9): e0184631. https://doi.org/10.1371/journal.pone.0184631

Heyer W.R., Rand A.S., Cruz C.A.G., Peixoto O.L. & Nelson C.E. 1990. Frogs of Boracéia. Arquivos de Zoologia 31: 235–410.

Hothorn T., Hornik K., Van De Wiel M.A. & Zeileis A. 2008. Implementing a class of permutation tests: the coin package. Journal of Statistical Software 28: 1–23. https://doi.org/10.18637/jss.v028.i08

ICZN 1999. International Code of Zoological Nomenclature, 4th Edition: adopted by the International Union of Biological Sciences. The International Trust for Zoological Nomenclature. Available from https://www.iczn.org/the-code/the-international-code-of-zoological-nomenclature/the-code-online/ [accessed 26 Mar. 2020].

Katoh K. & Standley D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30 (4): 772–780. https://doi.org/10.1093/molbev/ms3010
Köhler J., Jansen M., Rodríguez A., Kok P.J.R., Toledo L.F., Emmrich M., Glaw F., Haddad C.F.B., Rödel M.O. & Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251: 1–124. https://doi.org/10.11646/zootaxa.4251.1.1

Langone J.A., Lavilla E.O., Sá R.O. & Cardozo D. 2015. Comments on the type locality, type series, and geographic distribution of *Pseudopaludicola falcipes* (Hensel, 1867) (Amphibia, Anura). *Zootaxa* 4058 (1): 145–150. https://doi.org/10.11646/zootaxa.4058.1.12

Langone J.A., Camargo A. & Sá R.O. 2016. High genetic diversity but low population structure in the frog *Pseudopaludicola falcipes* (Hensel, 1867) (Amphibia, Anura) from the Pampas of South America. *Molecular Phylogenetics and Evolution* 95: 137–151. https://doi.org/10.1016/j.ympev.2015.11.012

Liaw A. & Wiener M. 2002. Classification and regression by randomForest. *R News* 2: 18–22.

Lobo F. 1994. Descripción de una nueva especie de *Pseudopaludicola* (Anura: Leptodactylidae), redescrizión de *P. falcipes* (Hensel, 1867) y *P. saltica* (Cope, 1887). *Cuadernos de Herpetología* 8: 177–199.

Lobo F. 1995. Análisis filogenético del género *Pseudopaludicola* (Anura: Leptodactylidae). *Cuadernos de Herpetología* 9: 21–43.

Lynch J.D. 1989. A review of leptodactylid frogs of the genus *Pseudopaludicola* in northern South America. *Copeia* 3: 577–588. https://doi.org/10.2307/1445483

Lyra M.L., Haddad C.F.B. & Azeredo-Espin A.M.L. 2017. Meeting the challenge of DNA barcoding Neotropical amphibians: polymerase chain reaction optimization and new COI primers. *Molecular Ecology Resources* 17 (5): 966–980. https://doi.org/10.1111/1755-0998.12648

Magalhães F.M., Loebmann D., Kokubum M.N.C., Haddad C.F.B. & Garda A.A. 2014. A new species of *Pseudopaludicola* (Anura: Leptodactylidae: Leiuperinae) from northeastern Brazil. *Herpetologica* 70: 77–88. https://doi.org/10.1655/HERPETOLOGICA-D-13-00054

Maniatis T., Fritsch E.F. & Sambrook J. 1982. *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory, New York.

Miller M.A., Pfieffer W. & Schwartz T. 2010. *Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees*. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans. https://doi.org/10.1109/GCE.2010.5676129

Miranda-Ribeiro A. 1937. Alguns batrachios novos das colleções do Museo Nacional. *O Campo* 8: 66–69.

Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B. & Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. https://doi.org/10.1038/35002501

Neves M.O., Yves A., Pereira E.A., Alves L., Vasques J.B., Coelho J.F.T. & Silva P.S. 2019. Herpetofauna in a highly endangered area: the Triângulo Mineiro region, in Minas Gerais State, Brazil. *Herpetozoa* 32: 113–123. https://doi.org/10.3897/herpetozoa.32.e35641

Oliveira E.A., Hernández-Ruz E.J., de Carvalho J.C. & Sanches D. 2013. Geographic distribution: *Pseudopaludicola canga*. *Herpetological Review* 44: 104.

Oliveira S.R., Santos C.E., Souza A.O., Lima-Ribeiro M.S., Vergilio K., Zórtea M., Silva D.A., Melo F.R., Guilherme F.A.G., Vaz-Silva W. & Morais A.R. 2019. Species composition and reproductive modes of anurans from the southwestern state of Goiás, Brazil. *Herpetology Notes* 12: 491–499.

Pansonato A., Morais D.H., Ávila R.W., Kawashita-Ribeiro R.A., Strüssmann C. & Martins I.A. 2012. A new species of *Pseudopaludicola* Miranda-Ribeiro, 1926 (Anura: Leiuperidae) from the state of Mato
Grosso, Brazil, with comments on the geographic distribution of *Pseudopaludicola canga* Giaretta & Kokubum, 2003. *Zootaxa* 3523: 49–58. https://doi.org/10.11646/zootaxa.3523.1.5

Pansonato A., Strüssmann C., Mudrek J.R. & Martins I.A. 2013. Morphometric and bioacoustic data on three species of *Pseudopaludicola* Miranda-Ribeiro, 1926 (Anura: Leptodactylidae: Leiuperinae) described from Chapada dos Guimarães, Mato Grosso, Brazil, with the revalidation of *Pseudopaludicola ameghini* (Cope, 1887). *Zootaxa* 3620: 147–162. https://doi.org/10.11646/zootaxa.3620.1.7

Pansonato A., Mudrek J.R., Veiga-Menoncello A.C.P., Rossa-Feres D.C., Martins I.A. & Strüssmann C. 2014a. A new species of *Pseudopaludicola* Miranda-Ribeiro, 1926 (Anura: Leptodactylidae: Leiuperinae) from northwestern state of São Paulo, Brazil. *Zootaxa* 3861: 249–264. https://doi.org/10.11646/zootaxa.3861.3.3

Pansonato A., Mudrek J.R., Simioni F., Martins I.A. & Strüssmann C. 2014b. Geographical variation in morphological and bioacoustic traits of *Pseudopaludicola mystacalis* (Cope, 1887) and a reassessment of the taxonomic status of *Pseudopaludicola serrana* Toledo, 2010 (Anura: Leptodactylidae: Leiuperinae). *Advances in Zoology* 2014: 1–13. https://doi.org/10.1155/2014/563165

Pansonato A., Veiga-Menoncello A.C.P., Mudrek J.R., Jasen M., Recco-Pimentel S.M., Martins I.A. & Strüssmann C. 2016. Two new species of *Pseudopaludicola* (Anura: Leptodactylidae: Leiuperinae) from eastern Bolivia and western Brazil. *Herpetologica* 72: 235–255. https://doi.org/10.1655/Herpetologica-D-14-00047.1

Pereira E.G. & Nascimento L.B. 2004. Descrição da vocalização e do girino de *Pseudopaludicola mineira* Lobo, 1994, com notas sobre a morfologia de adultos (Amphibia, Anura, Leptodactylidae). *Arquivos do Museu nacional* 62: 233–240.

R Core Team 2019. *R: A Language and Environment for Statistical Computing*. ver. 3.5.3. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/ [accessed 1 Jan. 2019].

Ramalho W.P., França D.P.F., Guerra V., Marciano R., Vale N.C. & Silva H.L.R. 2018. Herpetofauna of Parque Estadual Altamiro de Moura Pacheco: one of the last remnants of seasonal forest in the core region of the Brazilian Cerrado. *Papéis Avulsos de Zoologia* 58: e20185851. http://doi.org/10.11606/1807-0205/2018.58.51

Rivero J.A. & Serna M.A. 1985. Una nueva *Pseudopaludicola* (Amphibia: Leptodactylidae) cornuda del sureste de Colombia. *Caribbean Journal of Science* 20:169–171.

Roberto I.J., Cardozo D. & Ávila R.W. 2013. A new species of *Pseudopaludicola* (Anura, Leiuperidae) from western Piauí State, Northeast Brazil. *Zootaxa* 3636: 348–360. https://doi.org/10.11646/zootaxa.3636.2.6

Ruthven A.G. 1916. A new species of *Paludicola* from Colombia. *Occasional Papers of the Museum of Zoology, University of Michigan* 30: 1–3.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Strassburg B.B.N., Brooks T., Feltran-Barbieri R., Iribarrem A., Crouzeilles R., Loyola R., Latawiec A.E., Filho F.J.B.O., Scaramuzza C.A.M., Scarano F.R., Soares-Filho B. & Balmford A. 2017. Moment of truth for the Cerrado hotspot. *Nature Ecology & Evolution* 1: 0099. https://doi.org/10.1038/s41559-017-0099

Sueur J., Aubin T. & Simonis C. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18: 213–226. https://doi.org/10.1080/09524622.2008.9753600
Taylor W.R. & Van Dyke G.C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* IX (2): 107–119.

Toledo L.F., Siqueira S., Duarte T.C., Veiga-Menoncello A.C.P., Recco-Pimentel S.M. & Haddad C.F.B. 2010. Description of a new species of *Pseudopaludicola* Miranda-Ribeiro, 1926 from the State of São Paulo, Southeastern Brazil (Anura, Leiuperidae). *Zootaxa* 2496: 38–480. https://doi.org/10.11646/zootaxa.2496.1.2

Vaidya G., Lohman D.J. & Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Valdujo P.H., Carnaval A.C.O.Q. & Graham C. 2013. Environmental correlates of anuran beta diversity in the Brazilian Cerrado. *Ecography* 35: 1–10. https://doi.org/10.1111/j.1600-0587.2012.07374.x

Vaz-Silva W. & Maciel N.M. 2011. A new cryptic species of *Ameerega* (Anura: Dendrobatidae) from Brazilian Cerrado. *Zootaxa* 2826: 57–68. https://doi.org/10.11646/zootaxa.2826.1.3

Veiga-Menoncello A.C.P., Lourenço L.B., Strüssmann C., Rossa-Feres D.C., Andrade G.V., Giaretta A.A. & Recco-Pimentel S.M. 2014. A phylogenetic analysis of *Pseudopaludicola* (Anura) providing evidence of progressive chromosome reduction. *Zoologica Scripta* 43: 261–272. https://doi.org/10.1111/zsc.12048

Walker M., Lyra M.L. & Haddad C.F.B. 2018. Phylogenetic relationships and cryptic species diversity in the Brazilian Egg-Brooding tree frog, genus *Fritziana* Mello-Leitão 1937 (Anura: Hemiphractidae). *Molecular Phylogenetic and Evolution* 123: 59–72. https://doi.org/10.1016/j.ympev.2018.02.012

Watters J.L., Cummings S.T., Flanagan R.L. & Siler C.D. 2016. Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa* 4072 (4): 477–495. http://doi.org/10.11646/zootaxa.4072.4.6

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Appendix 1. Specimens examined.

Pseudopaludicola ameghini
BRAZIL – Mato Grosso • Chapada dos Guimarães (type locality); ZUEC 14138, 14139, 14141 to 14145.

Pseudopaludicola atragula
BRAZIL – São Paulo • Icém (type locality); AAG-UFU 5103 to 5105, 5107, 5109.

Pseudopaludicola boliviana
ARGENTINA – Chaco Province • Antequera; MNRJ 75298. – Corrientes Province • Itá-Ibaté; MNRJ 75299. – Santa Fé Province • General Obligado; MNRJ 75300. – Formosa Province • Tres Marias; MNRJ 75301 • Riacho Formosa; MNRJ 75303 • Tatané; MNRJ 75304 to 75308 • Virasol; MNRJ 75309 to 75312 • Colonia Salvación; MNRJ 75322.

Pseudopaludicola canga
BRAZIL – Pará • Marabá, Serra dos Carajás (type locality); ZUEC 9990, 10034, 14370, 14372 to 14374, 14378. – Maranhão • Barreirinhas; ZUEC 24687 to 24695 • Santo Amaro do Maranhão; ZUEC 24682 to 24686.

Pseudopaludicola coracoralinae sp. nov.
BRAZIL – Goiás • Palmeiras de Goiás (type locality); ZUEC 24701 to 24712 • Same collection data as for preceding; AAG-UFU 3393 to 3396.

Pseudopaludicola facureae
BRAZIL – Minas Gerais • Uberlândia (type locality); AAG-UFU 0853 to 0855 • Same collection data as for preceding; ZUEC 13651, 13652, 14215, 14218, 14219, 14221, 14224.

Pseudopaludicola falcipes
BRAZIL – Rio Grande do Sul • Eldorado do Sul; ZUEC 11468 • Novo Hamburgo; ZUEC 4876 • Porto Alegre; ZUEC 10387, 10388, 13999, 13000, 14002, 14003, 14005, 14006, 14008, 14009, 14016, 14017, 14022, 14162 to 14166, 14168 • Santana do Livramento; ZUEC 10355, 10356 • Viamão; ZUEC 5297 to 5299.

Pseudopaludicola florencei
BRAZIL – Bahia • Andaraí (type locality); ZUEC 23512 to 23530 • Same collection data as for preceding; UFMG 4310 to 4316.

Pseudopaludicola giarettai
BRAZIL – Minas Gerais • Curvelo (type locality); AAG-UFU 0309 to 0317 • Same collection data as for preceding; ZUEC 24319 to 24322.

Pseudopaludicola jaredi
BRAZIL – Ceará • Viçosa do Ceará (type locality); CFBH 32609, 32614, 32617 to 32625 • Same collection data as for preceding; ZUEC 20477 to 20484 • Nísia Floresta; ZUEC 21858 to 21872.
Pseudopaludicola jazmynmcdonaldae
BRAZIL – Tocantins • Caseara (type locality); ZUFMS-AMP 11247 to 11258 • Same collection data as for preceding; ZUEC 24457 to 24464.

Pseudopaludicola matuta
BRAZIL – Minas Gerais • Curvelo (type locality); ZUEC 24302 to 24306, 24308 to 24310, 24313, 24315 to 24318.

Pseudopaludicola mineira
BRAZIL – Minas Gerais • Jaboticatubas (Serra do Cipó, type locality); ZUEC 1570, 1572 to 1589, 1591.

Pseudopaludicola murundu
BRAZIL – São Paulo • Águas de Santa Bárbara; ZUEC 20507, 20508 • Rio Claro (type locality); AAG-UFU 5125, 5126, CFBH 8235 to 8242 • Same collection data as for preceding; ZUEC 14284 to 14290. – Minas Gerais • Brumadinho; ZUEC 16396 to 16398, 16442, 16443, 19549, 19551, 19555, 19557 to 19578, 19560 • Santana do Riacho; ZUEC 2323 • São João del-Rei; ZUEC 16447 to 16452, 16455, 16456.

Pseudopaludicola mystacalis
BRAZIL – Goiás • Itapirapuã; ZUEC 10222. – Mato Grosso • Cáceres; ZUEC 10286 • Chapada dos Guimarães (type locality); ZUEC 5115, 5117, 5119, 5121, 10685. – Mato Grosso do Sul • Três Lagoas; ZUEC 16720, 16949. – Tocantins • Formoso do Araguaia; ZUEC 10154.

Pseudopaludicola parnaiba (junior synonym of P. canga)
BRAZIL – Piauí • Ribeiro Gonçalves (type locality); ZUEC 24673 to 24680.

Pseudopaludicola pocoto
BRAZIL – Ceará • Novas Russas; CFBH 20285 to 20287 • Santa Quitéria (type locality); CFBH 26842 to 26847. – Bahia • Rio de Contas; ZUEC 25506 to 25510 • Same collection data as for preceding; UFMG 5902 to 5911.

Pseudopaludicola saltica
BRAZIL – Mato Grosso • Chapada dos Guimarães (type locality); ZUEC 14228, 14230 to 14233, 14235, 14239, 14240, 14244, 14247, 14272, 5134 to 51346, 5854, 5855. – Minas Gerais • Uberlândia; AAG-UFU 2308, 2630, 4598, 4631, 4735, 4707 to 4711.

Pseudopaludicola ternetzi
BRAZIL – Goiás • Uruaçu (type locality); MNRJ 445 to 447, 5460 to 5462, 5442. – Minas Gerais • Uberlândia; ZUEC 14036 to 14039, 14170, 14171. – Tocantins • Formoso do Araguaia; ZUEC 10140 to 10143, 10145, 10147, 10150, 10153.
### Appendix 2.

Sound files (wav format) of *Pseudopaludicola coracoralinae* sp. nov. in this study.

| Label                                      | Date    | Time   | Air (°C) | Water (°C) | Recorder – microphone                  | Voucher     |
|--------------------------------------------|---------|--------|----------|------------|----------------------------------------|-------------|
| Pseudop_coraPalmeirasGoiasGO1aTRC_AAGm671 | 18 Dec. 2013 | 21:37  | 23.8     | -          | Marantz PMD 671 – ME67/K6 Sennheiser    | AAG-UFU 3393 |
| Pseudop_coraPalmeirasGoiasGO2TRC_AAGm671 | 18 Dec. 2013 | 20:46  | 23.8     | -          | Marantz PMD 671 – ME67/K6 Sennheiser    | AAG-UFU 3394 |
| Pseudop_coraPalmeirasGoiasGO3aTRC_AAGm671 | 18 Dec. 2013 | 21:49  | 23.8     | -          | Marantz PMD 671 – ME67/K6 Sennheiser    | AAG-UFU 3395 |
| Pseudop_coraPalmeirasGoiasGO4aFSA_AAGmt   | 07 Nov. 2016 | 20:55  | 22.2     | 24.2       | M-audio Microtrack II – ME66/K6 Sennheiser | –           |
| Pseudop_coraPalmeirasGoiasGO5cFSA_AAGmt   | 08 Nov. 2016 | 18:08  | 26.0     | 29.0       | M-audio Microtrack II – ME66/K6 Sennheiser | –           |
| Pseudop_coraPalmeirasGoiasGO6aFSA_AAGmt   | 08 Nov. 2016 | 18:10  | 26.0     | 29.0       | M-audio Microtrack II – ME66/K6 Sennheiser | –           |
| FNJV 40300                                 | 14 Mar. 2019 | 20:55  | 25.4     | 25.6       | M-audio Microtrack II – ME66/K6 Sennheiser | ZUEC 24704  |
| FNJV 40301                                 | 14 Mar. 2019 | 20:47  | 25.4     | 25.6       | M-audio Microtrack II – ME66/K6 Sennheiser | –           |
| FNJV 40302                                 | 14 Mar. 2019 | 19:44  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
| FNJV 40303                                 | 14 Mar. 2019 | 19:59  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
| FNJV 40304                                 | 14 Mar. 2019 | 19:38  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
| FNJV 40305                                 | 14 Mar. 2019 | 20:07  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | ZUEC 24701  |
| FNJV 40306                                 | 14 Mar. 2019 | 20:13  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
| FNJV 40307                                 | 14 Mar. 2019 | 20:15  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
| FNJV 40308                                 | 14 Mar. 2019 | 20:24  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | ZUEC 24702  |
| FNJV 40309                                 | 14 Mar. 2019 | 20:35  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
| FNJV 40310                                 | 14 Mar. 2019 | 20:47  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | ZUEC 24703  |
| FNJV 40311                                 | 14 Mar. 2019 | 21:02  | 25.4     | 25.6       | Marantz PMD 661 MKII – ME66/K6 Sennheiser | –           |
Appendix 3 (continued on next two pages). Specimens of *Pseudopaludicola* Miranda-Ribeiro, 1926 used for phylogenetic inferences with voucher number, sample locality and GenBank accession number. The sequences provided for the present study are in bold. Outgroups are also listed.

| Species               | Voucher/tissue | Locality                           | 12S-tval-16S/ [12S, 16S] |
|-----------------------|----------------|------------------------------------|-------------------------|
| *P. ameghini*         | ZUEC 14140     | Chapada dos Guimarães, MT, type locality | KJ146975                |
| *P. ameghini*         | UFMT 8543      | Chapada dos Guimarães, MT, type locality | KJ146976                |
| *P. atragula*         | DZSJRP 8727    | Icém, SP, type locality             | KJ146996                |
| *P. atragula*         | DZSJRP 8728    | Icém, SP, type locality             | KJ146997                |
| *P. canga*            | ZUEC 14333     | Serra dos Carajás, PA, type locality | KJ146988                |
| *P. canga*            | ZUEC 14334     | Serra dos Carajás, PA, type locality | KJ146989                |
| *P. canga*            | ZUEC 14335     | Serra dos Carajás, PA, type locality | KJ146990                |
| *P. canga*            | AAG-UFU 6266   | Aragominas, TO                      | MT385236                |
| *P. canga*            | AAG-UFU 6267   | Aragominas, TO                      | MT385237                |
| *P. canga*            | AAG-UFU 2745   | Palmas, TO                          | MT385238                |
| *P. canga*            | AAG-UFU 2746   | Palmas, TO                          | MT385239                |
| *P. canga*            | AAG-UFU 2527   | Mateiros, GO                        | MT385240                |
| *P. coracoralinae*    | AAG-UFU 3393   | Palmeiras de Goiás, GO, type locality | MT385241                |
| *P. coracoralinae*    | AAG-UFU 3394   | Palmeiras de Goiás, GO, type locality | MT385242                |
| *P. coracoralinae*    | ZUEC 24702     | Palmeiras de Goiás, GO, type locality | MT385243                |
| *P. coracoralinae*    | ZUEC 24703     | Palmeiras de Goiás, GO, type locality | MT385244                |
| *P. coracoralinae*    | ZUEC 24704     | Palmeiras de Goiás, GO, type locality | MT385245                |
| *P. facureae*         | ZUEC 14173     | Uberlândia, MG, type locality       | KJ146978                |
| *P. facureae*         | ZUEC 14174     | Uberlândia, MG, type locality       | KJ146979                |
| *P. facureae*         | ZUEC 14175     | Uberlândia, MG, type locality       | KJ146980                |
| *P. giarettai*        | AAG-UFU 1920   | Grande Sertão Veredas National Park, MG | MH533445               |
| *P. giarettai*        | AAG-UFU 1921   | Grande Sertão Veredas National Park, MG | MH533444               |
| *P. giarettai*        | ZUEC 24319     | Curvelo, MG, type locality          | MH533442                |
| *P. giarettai*        | ZUEC 24320     | Curvelo, MG, type locality          | MH533443                |
| *P. jazymymcdonaldae* | ZUFMS 11249    | Caseara, TO, type locality          | MT385246                |
| *P. jazymymcdonaldae* | ZUFMS 11252    | Caseara, TO, type locality          | MT385247                |
| *P. jazymymcdonaldae* | ZUFMS 11256    | Caseara, TO, type locality          | MT385248                |
| *P. jazymymcdonaldae* | ZUFMS 11257    | Caseara, TO, type locality          | MT385249                |
| *P. mystacalis*       | CFBH 35858     | Urbano Santos, MA                   | KJ146982                |
| *P. mystacalis*       | ZUEC 14147     | Cuiabá, MT                           | KJ146983                |
| *P. mystacalis*       | ZUEC 14160     | Poconé, MT                           | KJ146991                |
| *P. mystacalis*       | ZUEC 14128     | Uberlândia, MG                       | KJ146999                |
| *P. mystacalis*       | ZUEC 13836     | Barreirinhos, MA                    | KJ147005                |
| *P. mystacalis*       | CFBH-T 1374    | Paranaíba, MS                        | KJ147009                |
| *P. mystacalis*       | DZSJRP 8704    | Santa Fé do Sul, SP                  | KJ147022                |
| *P. mystacalis*       | DZSJRP 8724    | Icém, SP                             | KJ147028                |
| *P. mystacalis*       | CFBH-T 03509   | Babaquândia, TO                      | KU495493                |
| *P. canga*            | ZUEC 13858     | Barreirinhos, MA                    | KJ147013                |
Appendix 3 (continued). Specimens of *Pseudopaludicola* Miranda-Ribeiro, 1926 used for phylogenetic inferences with voucher number, sample locality and GenBank accession number. The sequences provided for the present study are in bold. Outgroups are also listed.

| Species       | Voucher/tissue | Locality                      | 12S-tval-16S/ [12S, 16S] |
|---------------|----------------|--------------------------------|--------------------------|
| *P. canga*    | ZUEC 13859     | Barreirinhas, MA               | KJ147014                 |
| *P. canga*    | ZUEC 13860     | Barreirinhas, MA               | KJ147015                 |
| *P. canga* (topotype of *P. parnaiba*) | ZUEC 24676     | Ribeiro Gonçalves, PI, type locality | MT385250 |
| *P. canga* (topotype of *P. parnaiba*) | ZUEC 24677     | Ribeiro Gonçalves, PI, type locality | MT385251 |
| *P. canga* (topotype of *P. parnaiba*) | ZUEC 24678     | Ribeiro Gonçalves, PI, type locality | MT385252 |
| *P. canga*    | ZUEC 24682     | Santo Amaro do Maranhão, MA    | MT385253                 |
| *P. canga*    | ZUEC 24683     | Santo Amaro do Maranhão, MA    | MT385254                 |
| *P. canga*    | ZUEC 24687     | Barreirinhas, MA               | MT385255                 |
| *P. canga*    | ZUEC 24688     | Barreirinhas, MA               | MT385256                 |
| *P. ternetzi* | UFMT 15753     | Uruaçu, GO, type locality      | KJ147054                 |
| *P. ternetzi* | UFMT 15754     | Uruaçu, GO, type locality      | KJ147055                 |
| *P. falcipes* | MACN 38647     | Yapeyu, Corrientes, Argentina  | AY843741                 |
| *P. falcipes* | ZUEC 14162     | Porto Alegre, RS               | KJ146972                 |
| *P. flore cei*| ZUEC 14190     | Andarai, BA, type locality     | KJ147017                 |
| *P. flore cei*| ZUEC 14189     | Andarai, BA, type locality     | KJ147018                 |
| *P. jaredi*   | SMRP 310.18    | Viçosa do Ceará, CE, type locality | KJ147033 |
| *P. jaredi*   | SMRP 310.19    | Viçosa do Ceará, CE, type locality | KJ147034 |
| *P. murundu*  | CFBH-T 1467    | Rio Claro, SP, type locality   | KJ147008                 |
| *P. murundu*  | ZUEC 14288     | Rio Claro, SP, type locality   | KJ147030                 |
| *P. mineira*  | DZSJRP 6437    | Serra do Cipó, MG, type locality | KJ147025 |
| *P. mineira*  | ZUEC 14318     | Serra do Cipó, MG, type locality | KJ147026 |
| *P. matuta*   | ZUEC 24303     | Curvelo, MG, type locality     | MH553454                 |
| *P. matuta*   | ZUEC 24304     | Curvelo, MG, type locality     | MH553456                 |
| *P. pocoto*   | SMRP 440.1     | Novas Russas, CE               | KJ147035                 |
| *P. pocoto*   | SMRP 440.2     | Novas Russas, CE               | KJ147036                 |
| *P. restinga* | ZUEC 24583     | Serra, ES, type locality       | MT385257                 |
| *P. restinga* | ZUEC 24584     | Serra, ES, type locality       | MT385258                 |
| *P. saltica*  | ZUEC 14239     | Chapada dos Guimarães, MT, type locality | KJ147002 |
| *P. saltica*  | ZUEC 14240     | Chapada dos Guimarães, MT, type locality | KJ147003 |
| *P. boliviana*| MLP-DB 5186    | Corrientes, Argentina          | KJ147049                 |
| *P. boliviana*| MLP-DB 5614    | Chaco, Argentina               | KJ147050                 |
| *P. llanera*  | ANDES-A 1253   | Sabanalarga, Casanare, Colombia | KP149482 |
| *P. llanera*  | ANDES-A 1114   | Sabanalarga, Casanare, Colombia | KP149483 |
| *P. motorzinho* | ZUEC 13928 | Poconé, MT                      | KJ146992 |
| *P. motor zinho* | ZUEC 13931   | Poconé, MT                      | KJ147039 |
| *P. pusilla*  | ANDES-A 1791   | San Vicente, Santander, Colombia | KP149486 |
Appendix 3 (continued). Specimens of *Pseudopaludicola* Miranda-Ribeiro, 1926 used for phylogenetic inferences with voucher number, sample locality and GenBank accession number. The sequences provided for the present study are in bold. Outgroups are also listed.

| Species                          | Voucher/tissue | Locality                                      | 12S-tval-16S/ [12S, 16S] |
|----------------------------------|----------------|-----------------------------------------------|--------------------------|
| *P. pusilla*                     | ANDES-A 1790   | San Vicente, Santander, Colombia              | KP149344                 |
| *Alsodes gargola*               | outgroup       |                                               | AY843565                 |
| *Allophryne ruthveni*            | outgroup       |                                               | AY843564                 |
| *Adenomera hylaedactyla*         | outgroup       |                                               | DQ283063                 |
| *Crossodactylodes bokermanni*    | outgroup       |                                               | KC593358                 |
| *Edalorhina perezi*              | outgroup       |                                               | AY843585                 |
| *Engystomops petersi*            | outgroup       |                                               | FJ668193                 |
| *Hydrolaetare caparum*           | outgroup       |                                               | KC603953, KC603954       |
| *Leptodactylus latrans*         | outgroup       |                                               | AY843688                 |
| *Leptodactylus pentadactylus*    | outgroup       |                                               | AY326017                 |
| *Lithodytes lineatus*            | outgroup       |                                               | AY843690                 |
| *Odontophrynus americanus*       | outgroup       |                                               | AY843704                 |
| *Paratelmatobius gaigeae*        | outgroup       |                                               | EU224397                 |
| *Physalaemus cuvieri*            | outgroup       |                                               | AY843729                 |
| *Physalaemus nattereri*          | outgroup       |                                               | DQ37208                  |
| *Pleurodema brachyops*           | outgroup       |                                               | AY843733                 |
| *Proceratophrys avelinoi*        | outgroup       |                                               | KP295643                 |
| *Rupirana cardosoi*              | outgroup       |                                               | KC593361                 |
| *Scythrophrys sawayae*           | outgroup       |                                               | DQ283099                 |
| *Vitreorana eurygnatha*          | outgroup       |                                               | AY843595                 |
Appendix 4. Primers used to amplify the mitochondrial fragments 12S, tRNA-val, 16S.

| Name   | Sequence (5'-3') | Author               |
|--------|------------------|----------------------|
| MVZ59  | ATAGCACGTAAAAYGCTDAGATG | Graybeal 1997       |
| tRNA-Val | GGTGTAAGCGARAGGGCTTGGTTAAG | Goebel et al. 1999 |
| 12S-L13 | TTAGAAGAGGCAAGTCGTAACATGGTA | Feller & Hedges 1998 |
| 16S-H10 | TGCTTACGCTACCTTTGCCAGGT | Hedges 1994         |
| 16Sa-L  | CGCCTGTATCTAAAAACAT | Palumbi et al. 1991 |
| 16Sb-H  | CCCGTCTGAACTCACGATACGT | Palumbi et al. 1991 |

Graybeal A. 1997. Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypothesis characterizing their radiation. Zoological Journal of the Linnean Society 119: 297–338. https://doi.org/10.1111/j.1096-3642.1997.tb00139.x

Goebel A.M., Donnelly J.M. & Atz M.E. 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. Molecular Phylogenetics and Evolution 11: 163–199. https://doi.org/10.1006/mpev.1998.0538

Feller A.E. & Hedges S.B. 1998. Molecular evidence for the early history of living amphibians. Molecular Phylogenetics and Evolution 9: 509–516. https://doi.org/10.1006/mpev.1998.0500

Hedges S.B. 1994. Molecular evidence for the origin of birds. Proceedings of the Natural Academy of Science 91: 2621–2624. https://doi.org/10.1073/pnas.91.7.2621

Palumbi S.R., Martins A., Romano S., McMillan W.O., Stice L. & Grabowski G. 1991. The Simple Fool’S Guide to PCR, ver. 2.0. University of Hawaii, Honolulu. Privately published, compiled by S. Palumbi.
Supplementary material

Supplementary file 1. Acoustic terminology employed.

Supplementary file 2. Estimates of evolutionary divergence between sequences of the species from *Pseudopaludicola canga* clade.

Supplementary file 3. Phylogenetic relationships of the *Pseudopaludicola* species based on the 12S rRNA, tRNA-val and 16S rRNA mitochondrial genes fragment, including the 19 outgroups. Numbers near the nodes are the support values from a maximum likelihood bootstrap analysis. Black dots represent bootstrap = 100; support below species level is not shown.