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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,

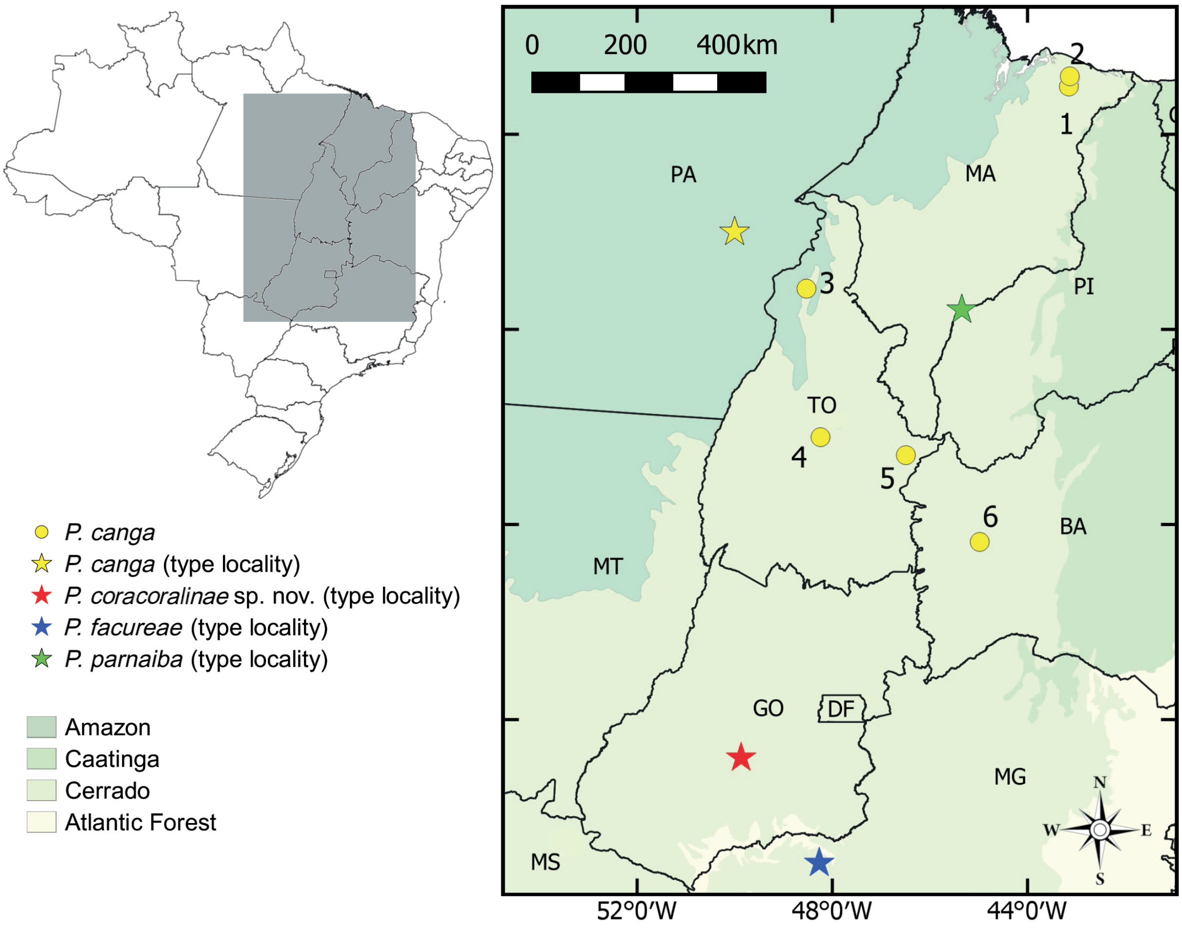

Fig. 1. Partial map of South America showing the Brazilian domains and samples of the species included in our molecular, morphological and acoustic comparisons. The type localities of the species are indicated with stars: *Pseudopaludicola coracoralinae* sp. nov. in Palmeiras de Goiás, GO (red star), *P. facureae* Andrade & Carvalho, 2013 in Uberlândia, MG (blue star), *P. canga* Giaretta & Kokubum, 2003 in Marabá, PA (yellow star) and *P. parnaiba* 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 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

INI = internote interval
ISI = interseries interval
MaxDF = max. reached dominant frequency

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
UFMG = 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	Topotypes, state of Pará	State of Tocantins	São Desidério, state of Bahia	
Analyzed males (n)	16	3	7	2	29
Analyzed notes (n)	312	196	140	40	570
Call duration (s)	16.7 ± 11.4 (2.5–43.3)	10.9 ± 5.6 (6.6–17.7)	15.2 ± 5.3 (7.4–24.9)	27.6 ± 31.6 (5.2–49.9)	24.2 ± 13.1 (2.2–49.3)
Series of notes duration (s)	0.7 ± 0.3 (0.1–5.9)	0.5 ± 0.1 (0.2–0.6)	1.9 ± 0.9 (0.3–7.3)	1.2 ± 0.3 (0.7–1.8)	1.7 ± 2.0 (0.1–10.6)
Interseries interval (s)	0.7 ± 0.1 (0.2–2.2)	2.0 ± 0.5 (0.6–6.6)	0.9 ± 0.3 (0.4–2.1)	1.2 ± 0.0 (0.9–1.4)	0.5 ± 0.2 (0.2–1.7)
Number of series per call	12.9 ± 7.3 (3.0–30.0)	4.7 ± 1.5 (3.0–8.0)	8.0 ± 4.8 (4.0–17.0)	11.0 ± 11.3 (3.0–19.0)	18.9 ± 10.7 (4.0–37.0)
Number of notes per series	8.6 ± 2.8 (2.0–61.0)	6.8 ± 1.2 (3.0–9.0)	21.6 ± 10.1 (5.0–85.0)	16.3 ± 3.2 (11.0–23.0)	18.0 ± 17.1 (2.0–94.0)
Note duration (ms)	25 ± 4 (14–42)	29 ± 4 (21–45)	26 ± 2 (18–33)	20 ± 1 (17–22)	26 ± 2 (19–35)
Internote interval (ms)	56 ± 11 (29–110)	48 ± 7 (36–73)	61 ± 6 (45–118)	56 ± 8 (45–69)	66 ± 14 (27–152)
Notes / minute	843.2 ± 154.4 (557.1–1285.7)	862.8 ± 91.5 (676.7–944.9)	727.7 ± 47.8 (582.0–891.7)	815.4 ± 81.7 (744.2–919.2)	702.3 ± 114.4 (368.5–1097.9)
Peak of dominant frequency (kHz)	4.59 ± 0.17 (4.18–4.87)	3.87 ± 0.13 (3.75–4.13)	4.33 ± 0.23 (4.00–4.73)	4.16 ± 0.36 (3.84–4.50)	4.44 ± 0.25 (3.94–4.99)
Min. reached dominant frequency (kHz)	4.40 ± 0.15 (4.00–4.69)	3.77 ± 0.13 (3.66–4.00)	4.14 ± 0.22 (3.88–4.59)	4.00 ± 0.43 (3.66–4.31)	4.16 ± 0.27 (3.56–4.69)
Max. reached dominant frequency (kHz)	4.80 ± 0.21 (4.39–5.34)	4.07 ± 0.08 (3.96–4.26)	4.56 ± 0.23 (4.26–5.01)	4.40 ± 0.27 (4.12–4.59)	4.74 ± 0.27 (4.31–5.34)
1st Quartile Frequency (kHz)	4.52 ± 0.15 (4.13–4.78)	3.84 ± 0.13 (3.72–4.09)	4.26 ± 0.23 (3.96–4.73)	4.12 ± 0.41 (3.79–4.40)	4.33 ± 0.26 (3.75–4.82)
3rd Quartile Frequency (kHz)	4.70 ± 0.18 (4.26–5.06)	3.96 ± 0.12 (3.85–4.18)	4.43 ± 0.24 (4.13–4.87)	4.26 ± 0.34 (3.94–4.50)	4.59 ± 0.26 (4.03–5.16)
Peak of 2nd harmonic frequency (kHz)	9.20 ± 0.40 (8.35–10.22)	7.77 ± 0.11 (7.60–7.99)	8.47 ± 0.22 (7.97–8.74)	7.64–7.87 (N=1)	8.72 ± 0.46 (7.50–9.56)
Air temperature (°C)	26.8–29.6	26	24.0–28.8	26	27
Water temperature (°C)	27.8	–	25–26	30	27

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* sp. 3	*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, Kwet, 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. jazmynmcdonaldae* 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. jazmynmcdonaldae*, 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).

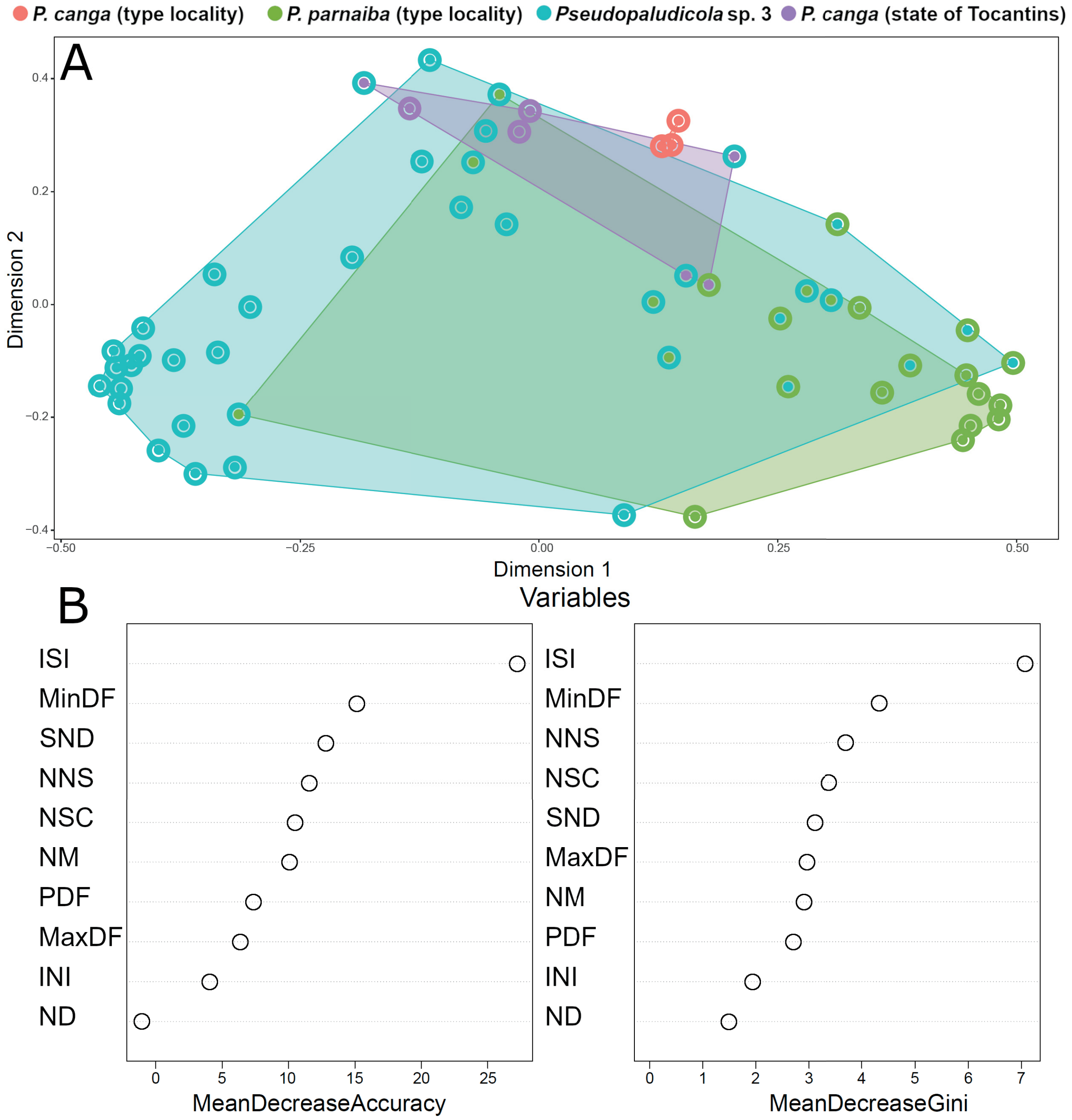

Fig. 2. A. First and second dimensions of the Multidimensional scaling on the proximity scores from the randomForest analysis considering acoustic traits of adult males of *Pseudopaludicola canga* Giaretta & Kokubum, 2003 from the type locality (red dots), *P. parnaiba* Roberto, Cardozo & Ávila, 2013 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.

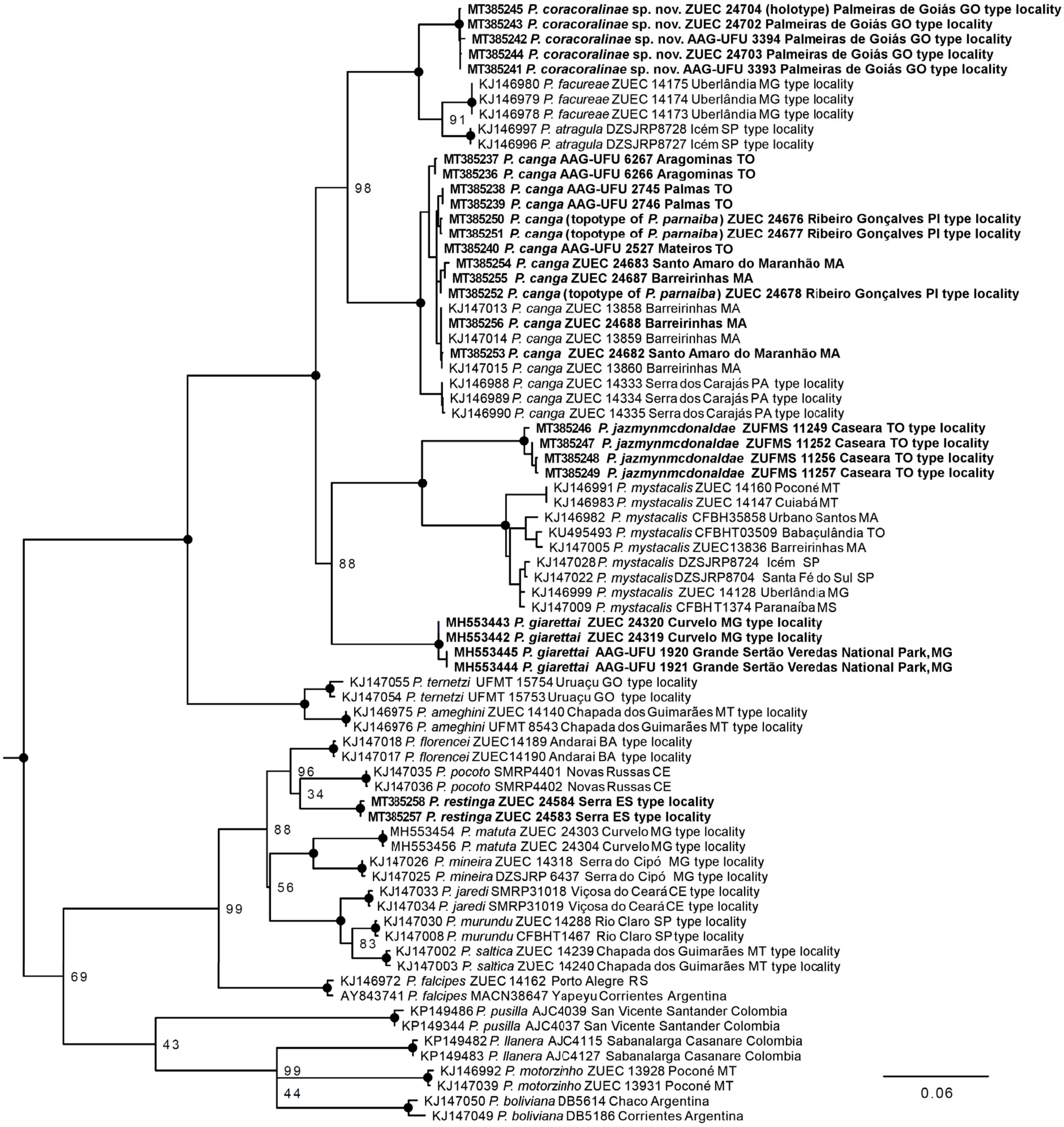

Fig. 3. Maximum likelihood tree recovered for the phylogenetic relationships of *Pseudopaludicola* Miranda-Ribeiro, 1926 based on the 12S rRNA, tRNA-val and 16S rRNA mitochondrial genes. Numbers near the nodes are bootstrap values and black dots represent bootstrap = 100; support below species level 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-9628-91BB62B185B6
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.;

18 Dec. 2013; T.R. de Carvalho and L.B. Martins leg; GenBank: MT385241, MT385242; AAG-UFU 3393 to 3396.

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. Foot longer than tibia. 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. Palm of hand brown, pigmented. Sole of foot brown, pigmented and darker than hand, arm and hind limb. 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).

Variation in the type series

Dorsal surface of body varies from brown to dark brown, with black or dark brown irregular blotches (Fig. 5). The specimen ZUEC 24705 does not have well-defined light vertebral line. The specimens ZUEC 24705–06, 24708–10 and AAG-UFU 3396 have no paravertebral chevron-shaped dermal ridges from behind the eyes to the scapular region. The specimens ZUEC 24702–03, 24706, 24710–12 and

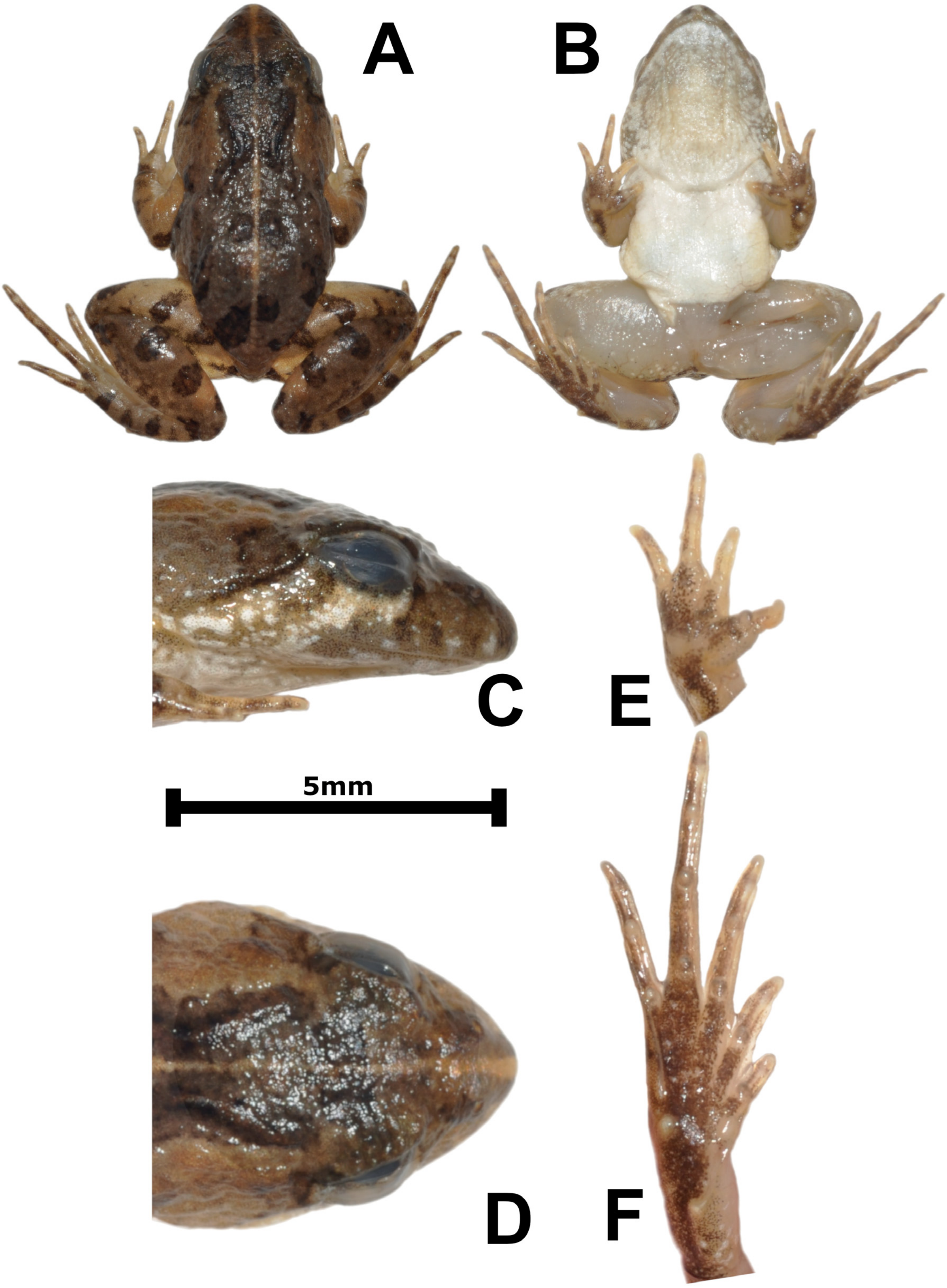

Fig. 4. *Pseudopaludicola coracoralinae* sp. nov., holotype (ZUEC 24704, SVL = 13.1 mm), an adult ♂ from Palmeiras de Goiás, GO, Brazil. **A**. Dorsal view. **B**. Ventral view. **C**. Head, lateral view. **D**. Head, dorsal view. **E**. Right hand, ventral view. **F**. Right foot, ventral view. Scale bar (only for C, D, E, F) = 5 mm.

Table 3. Morphometry of *Pseudopaludicola coracoralinae* sp. nov. type series (including the holotype) from Palmeiras de Goiás, GO, Brazil. Values presented in millimeters as mean ± standard deviation (minimum–maximum). n = number of measured specimens.

Characters	**Holotype**	**Males (n = 10)**	**Females (n = 5)**
Snout-vent length (SVL)	13.1	13.7 ± 0.8 (12.5–15.3)	16.8 ± 0.4 (16.0–17.2)
Head length (HL)	3.9	4.3 ± 0.2 (4.0–4.6)	4.8 ± 0.2 (4.6–5.0)
Head width (HW)	4.7	4.8 ± 0.2 (4.5–5.3)	5.5 ± 0.3 (5.0–5.7)
Eye diameter (ED)	1.5	1.6 ± 0.0 (1.5–1.6)	1.8 ± 0.2 (1.5–2.1)
Interorbital distance (IOD)	1.6	1.7 ± 0.2 (1.5–2.1)	2.0 ± 0.2 (1.7–2.1)
Eye-nostril distance (END)	1.0	1.1 ± 0.1 (1.0–1.2)	1.4 ± 0.2 (1.1–1.5)
Snout length (SL)	2.1	2.1 ± 0.1 (1.9–2.3)	2.4 ± 0.1 (2.2–2.5)
Internarial distance (IND)	1.2	1.2 ± 0.1 (1.0–1.4)	1.5 ± 0.0 (1.4–1.5)
Hand length (HAL)	3.6	3.9 ± 0.2 (3.5–4.2)	4.5 ± 0.2 (4.2–4.8)
Thigh length (TL)	6.2	6.5 ± 0.3 (6.0–7.0)	7.7 ± 0.3 (7.2–8.0)
Tibia length (TBL)	6.7	7.2 ± 0.3 (6.6–7.7)	8.4 ± 0.4 (8.0–9.0)
Tarsus length (TAL)	3.5	3.8 ± 0.3 (3.3–4.3)	4.4 ± 0.3 (4.2–4.9)
Foot length (FL)	7.2	7.9 ± 0.4 (7.3–8.5)	9.2 ± 0.5 (8.6–9.8)

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

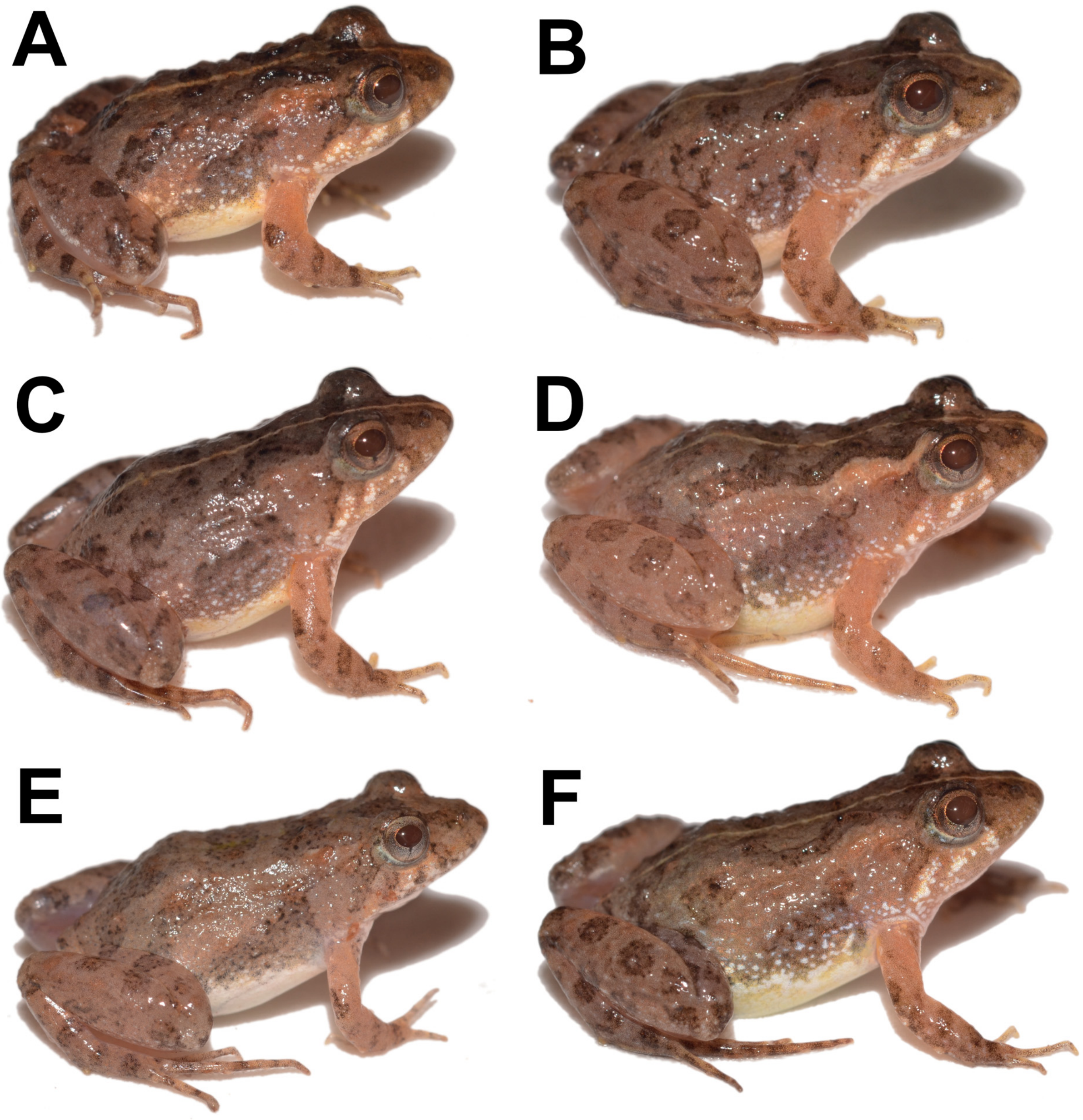

Fig. 5. Holotype and five paratypes of *Pseudopaludicola coracoralinae* sp. nov. in life. **A**. ZUEC 24704 (holotype, adult ♂ and call voucher), SVL = 13.1 mm. **B**. ZUEC 24703 (adult ♂ and call voucher), SVL = 13.2 mm. **C**. ZUEC 24707 (adult ♂), SVL = 12.5 mm. **D**. ZUEC 24712 (adult ♀), SVL = 16.0 mm. **E**. ZUEC 24705 (adult ♀), SVL = 16.8 mm. **F**. ZUEC 24706 (adult ♀), SVL = 17.0 mm.

1989). 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. jazmynmcdonaldae* 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).

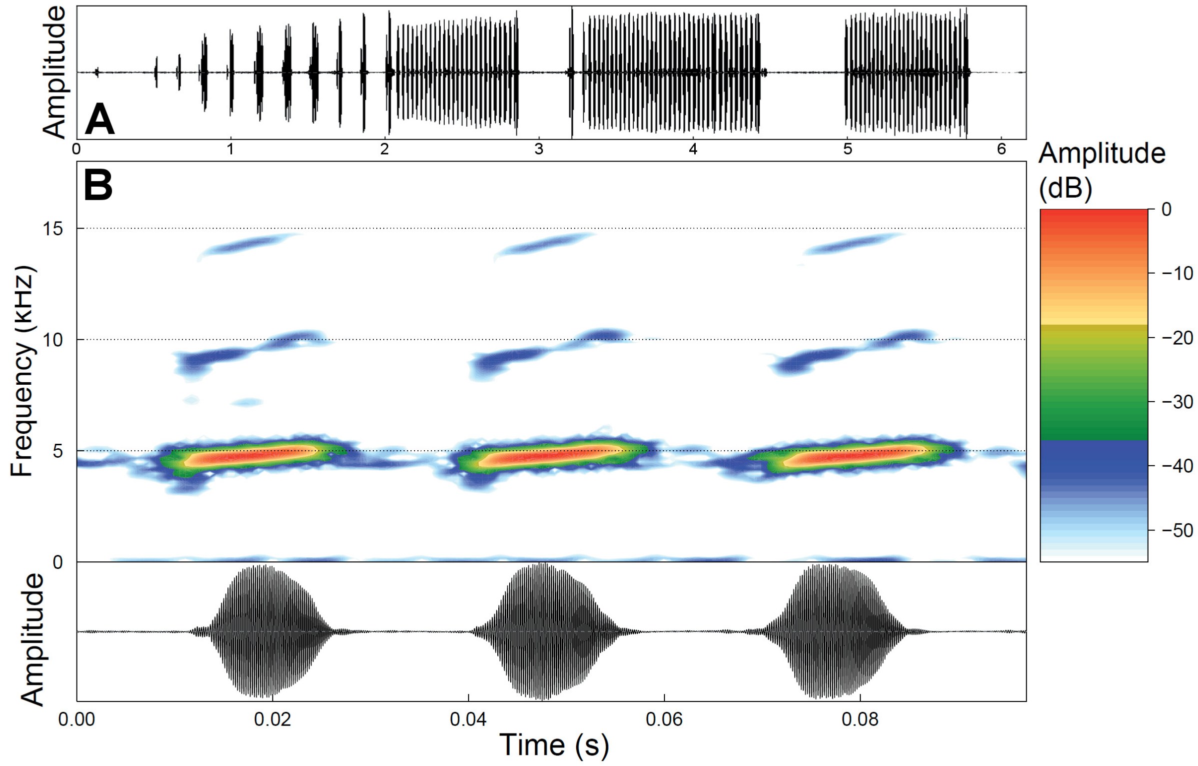

Fig. 6. A. Oscillogram of the entire advertisement call of *Pseudopaludicola coracoralinae* sp. nov. with the introductory notes followed by three series of tonal notes. **B**. Audiospectrogram (top) and corresponding oscillogram (bottom) detailing three notes. The male was recorded on 8 November 2016 at 18:08 h; air temperature 26°C, water temperature 29°C; recording_label: Pseudop_coracoralinaePalmeirasGoiasGO5cFSA_AAGmt.

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.** n = 18 (334)	***P. facureae*** n = 19 (372)
Call duration (s)	7.2 ± 6.4 (1.3–25.8) CV = 88.9%	12.3 ± 6.3 (4.7–22.6) CV = 51.2%
Series of notes duration (s)	1.0 ± 0.6 (0.2–4.1) CV = 60.0%	0.5 ± 0.1 (0.1–3.5) CV = 20.0%
Interseries interval (s)	1.0 ± 0.5 (0.4–6.7) CV = 50.0%	0.6 ± 0.1 (0.1–1.4) CV = 16.7%
Number of series per call	3.2 ± 1.2 (2.0–6.0) CV = 37.5%	9.9 ± 5.9 (4.0–20.0) CV = 59.6%
Number of notes per series	28.7 ± 15.7 (7.0–116.0) CV = 54.7%	12.7 ± 2.9 (2.0–74.0) CV = 22.8%
Note duration (ms)	15 ± 1 (11–21) CV = 6.6%	19 ± 3 (11–30) CV = 15.8%
Internote interval (ms)	19 ± 4 (12–61) CV = 21.1%	24 ± 8 (11–156) CV = 33.3%
Notes / minute	1796.1 ± 123.1 (1484.7–2076.6) CV = 6.8%	1381.4 ± 197.2 (512.1–1842.6) CV = 14.3%
Peak of dominant frequency (kHz)	4.60 ± 0.22 (4.18–5.06) CV = 4.8%	4.35 ± 0.25 (3.84–4.99) CV = 5.7%
Min. reached dominant frequency (kHz)	4.27 ± 0.22 (3.84–4.59) CV = 5.2%	4.11 ± 0.26 (3.61–4.74) CV = 6.3%
Max. reached dominant frequency (kHz)	4.95 ± 0.27 (4.41–5.44) CV = 5.5%	4.66 ± 0.29 (4.12–5.21) CV = 6.2%
1st Quartile Frequency (kHz)	4.46 ± 0.21 (4.12–4.87) CV = 4.7%	4.26 ± 0.27 (3.75–4.87) CV = 6.3%
3rd Quartile Frequency (kHz)	4.73 ± 0.23 (4.26–5.16) CV = 4.9%	4.47 ± 0.27 (3.94–5.08) CV = 6.0%
Peak of 2nd harmonic frequency (kHz)	9.32 ± 0.57 (8.34–10.50) CV = 6.1%	8.81 ± 0.57 (6.98–10.25) CV = 6.5%
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 ($P \leq 0.01$) 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 Gonçalves).

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.

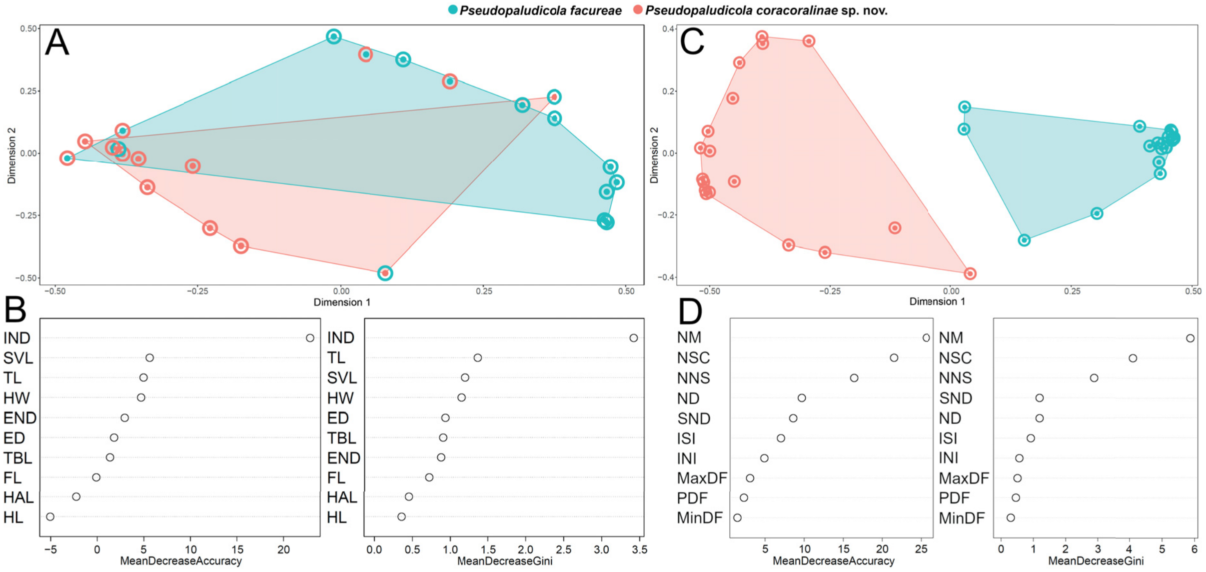

Fig. 7. A, **C**. First and second dimensions of the Multidimensional scaling on the proximity scores from the randomForest analysis of adult males of *Pseudopaludicola coracoralinae* sp. nov. from the type locality (red dots) and *P. facureae* Andrade & Carvalho, 2013 from the type locality (blue dots). **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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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 (holotype)**	**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**	**MH553445**
P. giarettai	**AAG-UFU 1921**	**Grande Sertão Veredas National Park, MG**	**MH553444**
P. giarettai	**ZUEC 24319**	**Curvelo, MG, type locality**	**MH553442**
P. giarettai	**ZUEC 24320**	**Curvelo, MG, type locality**	**MH553443**
P. jazmynmcdonaldae	**ZUFMS 11249**	**Caseara, TO, type locality**	**MT385246**
P. jazmynmcdonaldae	**ZUFMS 11252**	**Caseara, TO, type locality**	**MT385247**
P. jazmynmcdonaldae	**ZUFMS 11256**	**Caseara, TO, type locality**	**MT385248**
P. jazmynmcdonaldae	**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	Barreirinhas, 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	Babaçulândia, TO	KU495493
P. canga	ZUEC 13858	Barreirinhas, 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. florencei	ZUEC 14190	Andaraí, BA, type locality	KJ147017
P. florencei	ZUEC 14189	Andaraí, 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. motorzinho	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 caparu	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		DQ337208
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	GGTGTAAGCGARAGGCTTTKGTTAAG	Goebel *et al.* 1999
12S-L13	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller & Hedges 1998
16S-H10	TGCTTACGCTACCTTTGCACGGT	Hedges 1994
16Sa-L	CGCCTGTTTATCAAAAACAT	Palumbi *et al.* 1991
16Sb-H	CCCGTCTGAACTCAGATCACGT	Palumbi *et al.* 1991

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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

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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.