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## A New Species of *Pseudopaludicola* Miranda-Ribeiro, 1926 (Anura, Leptodactylidae) from Tocantins State, Brazil

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**ABSTRACT.**—The number of described species of *Pseudopaludicola* has increased at unprecedented rates over the past two decades. This increase was mainly driven by the sampling effort in regions historically neglected and the use of evidence from bioacoustic, genetic, and morphological datasets combined. Here, we describe a new species of *Pseudopaludicola* from a transitional zone between Amazonia and Cerrado in western Tocantins State as revealed through morphological, molecular, and bioacoustic analysis. *Pseudopaludicola javae* sp. nov. is distinguished from its congeners by its small size; knobbed terminal phalanges; smooth upper eyelids and heel; relatively short hind limbs; smooth, whitish, and subgular inflated vocal sac with few melanophores around the jaw; and advertisement call composed of an irregular series of multipulsed notes with 10–17 nonconcatenated pulses separated by intervals of 3–55 ms, emitted at a rate of 27–51 pulses/sec. The new species occurs in four Tocantins municipalities, all in the Araguaia–Tocantins interfluvium. We also provide the first records of *Pseudopaludicola jazmynmcdonaldae* outside the type locality (Caseara Municipality, Tocantins State), extending its distribution nearly 245 km southward from Caseara. *Pseudopaludicola javae* sp. nov. is the second species of *Pseudopaludicola* described recently from western Tocantins.

**RESUMO.**—O número de espécies de *Pseudopaludicola* descritas cresceu a taxas sem precedentes nas últimas duas décadas. Esse aumento foi impulsionado principalmente pelo esforço de amostragem em regiões historicamente negligenciadas e pelo uso de evidências bioacústicas, genéticas e morfológicas. Nós descrevemos uma nova espécie de *Pseudopaludicola* de uma zona de transição entre a Amazônia e o Cerrado no oeste do estado do Tocantins revelada através de análises morfológicas, moleculares e bioacústicas. *Pseudopaludicola javae* sp. nov. é diagnosticada de seus congêneres por seu pequeno tamanho, falanges terminais simples, pálpebras superiores e calcanhar lisos, membros posteriores relativamente curtos, saco vocal subgular, liso e esbranquiçado quando inflado com poucos melanóforos ao redor da mandíbula, e canto de anúncio composto por séries irregulares de notas com 10–17 pulsos não concatenados separados por intervalos de 3–55 ms, emitidos a uma taxa de 27–51 pulsos/s. A nova espécie ocorre em quatro municípios do Tocantins, todos no interflúvio Araguaia-Tocantins. Também fornecemos aqui os primeiros registros de *P. jazmynmcdonaldae* fora da localidade tipo (município de Caseara, estado do Tocantins), estendendo sua distribuição em cerca de 245 km em linha reta ao sul de Caseara. *Pseudopaludicola javae* sp. nov. é a segunda espécie de *Pseudopaludicola* descrita para o oeste do Tocantins nos últimos anos.

*Pseudopaludicola* is a monophyletic genus supported by the presence of a hypertrophied antebrachial tubercle (Lynch, 1989), osteological features (Lobo, 1995), and mitochondrial DNA (mtDNA) sequence (Veiga-Menoncello et al., 2014). Since the description of the first species of *Pseudopaludicola* 155 years ago, the diversity of this genus has reached 25 species (Frost, 2023), of which 60% were named just in the last two decades (Frost, 2023). This exceptional species description rate has resulted primarily from increases in sampling effort and the implementation of different sources of evidence to determine the taxonomic status of some *Pseudopaludicola* populations, especially acoustic and molecular data (e.g., Cardozo and Toledo, 2013; Andrade et al., 2019, 2020a). Additionally, this high rate of species discovery also indicates that new species likely remain to be discovered. Their small body size and conserved external morphology, as well as the existence of many poorly sampled

areas across their range, are among the main challenges in describing the hidden diversity of these dwarf swamp frogs (Diniz-Filho et al., 2005; Veiga-Menoncello et al., 2014; Andrade et al., 2020a; Porto et al., 2022).

Despite some disagreement about internal relationships, most researchers, via phylogenetic hypotheses, regard *Pseudopaludicola* as monophyletic (Lynch, 1989; Lobo, 1995; Pyron and Wiens, 2011; Fouquet et al., 2013; Veiga-Menoncello et al., 2014). In the comprehensive phylogenetic study of *Pseudopaludicola* provided by Veiga-Menoncello et al. (2014), many species and unassigned terminals were included in a phylogenetic context for the first time, and the resulting phylogeny supported four major clades within the genus. Ten new species have been described since the phylogeny proposed by Veiga-Menoncello et al. (2014), and three remain without mtDNA sequences available, namely, *Pseudopaludicola ibisoroca*, *Pseudopaludicola ceratophyes*, and *Pseudopaludicola hyleaustralis* (Andrade et al., 2020a). The most recent molecular phylogeny of the genus included 22 of the 25 described species (Andrade et al., 2020a)

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and recovered essentially the same topology as the previous work of Veiga-Menoncello et al. (2014). However, some nodes still had low bootstrap support, possibly because of the absence of terminals (i.e., unknown species) or the need for more molecular markers, especially nuclear ones (Veiga-Menoncello et al., 2014; Andrade et al., 2020a). Thus, the description of new species by using molecular markers to contextualize their phylogenetic positions may be essential for improving our understanding of the relationships within *Pseudopaludicola*.

Recent field sampling efforts have been performed in historically neglected regions, such as Tocantins State, resulting in many new species descriptions (Andrade et al., 2019; Oliveira et al., 2020; Silva et al., 2020a,b). At least four species of *Pseudopaludicola* are known for Tocantins state, as follows: *Pseudopaludicola canga*, *Pseudopaludicola jaredi*, *Pseudopaludicola jazmynmcdonaldae*, and *Pseudopaludicola mystacalis* (Silva et al., 2020b). *Pseudopaludicola jazmynmcdonaldae* was the most recently described among these species and remains restricted to its type locality, namely, Caseara Municipality, western Tocantins State (Andrade et al., 2019). This municipality is in the Araguaia-Tocantins interfluvium; it is one of the largest drainage systems of Brazil that is severely affected by agribusiness, mining, and hydropower activities (Bezerra and Gonzaga, 2019; Pelicice et al., 2021). Western Tocantins State is more influenced by the Araguaia River and its main tributaries (e.g., the Coco, Riozinho, Formoso, and Javaés rivers), a drainage system characterized by extensive floodplains, including both seasonally flooded forests (“igapós”) and grasslands.

During the last 2 years, new samplings performed in the Araguaia-Tocantins interfluvium yielded new records for different *Pseudopaludicola* populations. After carefully comparing them through morphological, acoustic, and molecular evidence, we found that one of them could not be assigned to any described species. Here, we describe it as a new species of *Pseudopaludicola* from western Tocantins State. Additionally, we also provide new records for *P. jazmynmcdonaldae* through Tocantins State, which are the first ones outside of its type locality.

#### MATERIAL AND METHODS

**Study Area and Sampling.**—Specimens of the type series were collected in the municipalities of Marianópolis (March and April 2021) and Lagoa da Confusão (March 2021 and January 2022), western Tocantins State, an ecotonal zone between Amazonia and Cerrado (Fig. 1; see voucher numbers for calls and tissue samples in Appendices 1–3). Additional samples were collected in Dueré (March 2021), Cristalândia (January 2022), and Couto Magalhães (March 2022) municipalities, Tocantins State. Specimens were collected during visual surveys in swampy environments between 1600 and 2100 h.

Frogs were euthanized by applying 5% lidocaine to their skin following the institutional recommendations and in agreement with the Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists (Herpetological Animal Care and Use Committee, 2004). We preserved specimens in 10% formalin and stored them in 70% ethanol. We collected muscle tissue from the thighs of some individuals just after euthanasia and stored these samples in 99% ethanol. Specimens were deposited in Coleção Herpetológica da Universidade Federal da Paraíba, João Pessoa, Paraíba (CHUFPB). Additional information on all specimens examined is provided in Appendix 1.

**Morphometry.**—We measured the following 13 morphometric traits: snout-vent length (SVL), head length, head width, eye diameter, interorbital distance, eye-nostril distance, snout length, internarial distance, hand length, thigh length, tibia length (= shank length), tarsus length, and foot length (Liu et al., 2022). We followed Heyer et al. (1990) for snout shape nomenclature. SVL was measured using a Mitutoyo digital caliper ( $\pm 0.01$  mm); all other traits we measured under a Leica-EZ4D stereomicroscope coupled to an ocular micrometer. Individuals were sexed by analyzing the presence of nuptial pads, vocal sac, and vocal slits (checked under the stereomicroscope).

**Bioacoustics.**—We recorded calls from 20 males with a digital recorder (DR 40 WL) at a sampling rate of 44.1 kHz and a sample size of 16 bits. Recordings of the new species were made between 28 February and 2 March 2021 from 1659–1915 h, and 25–30°C. We analyzed calls using Raven Pro 1.6 (Bioacoustics Research Program, 2014), 64-bit version, with the following settings: window type = Hann, window size = 256 samples, 3-dB filter bandwidth = 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), color map = “Cool,” DFT size = 1024 samples (locked), and grid spacing (spectral resolution) = 46.9 Hz. We analyzed temporal traits in oscillograms and spectral traits in spectrograms (Köhler et al., 2017). We used the “Peak Frequency” function to determine the peak of dominant frequency; frequency values with 5 and 95% of call energy were obtained by “Frequency 5% (Hz)” and “Frequency 95% (Hz)” functions (Charif et al., 2010) and were considered as the minimum and maximum dominant frequencies (Hz), respectively. Frequency modulation was accessed through the “1st Quartile Frequency” and “3rd Quartile Frequency” functions; these Raven functions provide the frequencies that break the selection into two frequency intervals containing 25 and 75% of the energy in the selection, respectively (Charif et al., 2010). We used the package Seewave v. 2.1.8 package (Sueur et al., 2012), R platform (version 4.1.1 “Kick Things”) to generate sound figures. Seewave settings for the spectrograms were as follows: Hanning window, 85% overlap, and 512 points resolution. Sound files analyzed are listed in Appendix 2 and were deposited in the Fonoteca Neotropical Jacques Viellard (FNJV; labels FNJV0051321–FNJV0051340).

We calculated means and standard deviations considering mean values of individual males, whereas the range encompasses the minimum and the maximum values for all call samples. Note and call terminologies follow those from Köhler et al. (2017), and pulse terminology follows that from Magalhães et al. (2014).

We applied the function randomForest (randomForest v. 4.6-14 package; further details available in Liaw and Wiener, 2002) to look for acoustic discrimination among the new species and the closely related or acoustically similar *P. mystacalis*, *P. jazmynmcdonaldae*, and *Pseudopaludicola atragula*. For these comparisons, we used the dataset of Andrade et al. (2019), totaling 20 males from the new species, 24 from *P. jazmynmcdonaldae*, and 4 from *P. atragula*. In addition, we included in this dataset new calls from the sister taxon of the new species, namely, 17 males of *P. mystacalis* from the municipalities of Içém (São Paulo State;  $n = 12$ ), Barra do Garças ( $n = 2$ ), and Pontal do Araguaia ( $n = 3$ ) (Mato Grosso State). The ProximityPlot function (rfPermute v. 2.2 package; Archer, 2018) creates a plot of randomForest proximity scores using multidimensional scaling. For statistical tests of differences between calls of the new species and *P. atragula* (which had the call most similar to the new species: see Results), we used the following acoustic traits: pulse duration, interpulse interval, pulse rate, note



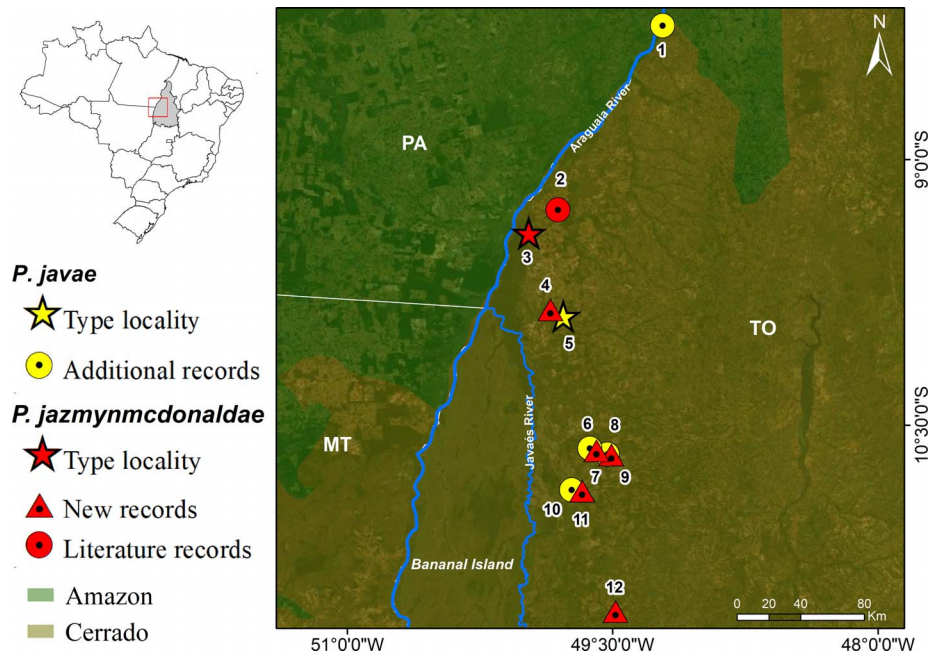


FIG. 1. Geographic distribution of *Pseudopaludicola javae* sp. nov. and *P. jazmynmcdonaldae*. Abbreviations: MT = Mato Grosso State; PA = Pará State; TO = Tocantins State. Municipalities: Couto Magalhães = 1; Caseara = 2–3; Marianópolis = 4–5; Lagoa da Confusão = 6–7 and 10–11; Cristalândia = 8–9; Dueré = 12.

duration, internote interval, notes per minute, pulses per note, and dominant frequency. We tested these traits for statistically significant differences between *P. atragula* and the new species with the exact Wilcoxon Mann-Whitney *U*-test using the Coin 1.4-2 package (Resampling Statistics model; Hothorn et al., 2008; function “wilcox\_test”) in R. The direct or indirect packages for this discriminant analysis were run in R v. 4.1.1 64-bit (R Core Team, 2021).

**Molecular Analysis.**—We extracted DNA from muscle samples by using the sodium–chloride salt precipitation method (Bruford et al., 1992). We then amplified mitochondrial 16S fragments by using primers 16Sar and 16Sbr (Palumbi, 1996). For the polymerase chain reaction (PCR) amplification, we used 7.5  $\mu$ L of Taq DNA Polymerase Master Mix (Ampliqon), 0.4  $\mu$ L of each primer (forward/backward), and 1–2  $\mu$ L of DNA and supplemented with Milli Q water for a final reaction volume of 15  $\mu$ L. PCR reactions included one initial cycle of 94°C for 3 min; followed by 35 cycles of 94°C for 20 sec, 48°C for 20 sec, and 68°C for 40 sec; and a final extension cycle of 68°C for 5 min. The purification of PCR products and sequencing was performed by Macrogen Inc.

To estimate phylogenetic relationships of lineages addressed here, we compared a fragment of the 16S mitochondrial gene with all *Pseudopaludicola* species with compatible fragments deposited in GenBank. We aligned 16S mtDNA gene fragments using the MAFFT algorithm (Katoh and Standley, 2013) in Geneious v. 9.0.5 with default settings. We aligned our 16S sequences with sequences available in GenBank for other species of *Pseudopaludicola* (ingroup) and *Physalaemus cuvieri*, *Pleurodema brachyops*, and *Leptodactylus pentadactylus* as outgroups, totaling 83 individuals and 537 bp. Because our interest was interspecies relationships and not intraspecific genetic structure, we constructed a simplified alignment with two to three sequences per species, when possible, resulting in a final alignment of 53 individuals and 537 bp. Both alignments led to the same relationships among species (Supplementary Figs. S1–

S2). All GenBank accession numbers and genetic vouchers used here are listed in Appendix 3.

To infer phylogenetic relationships, we performed 3 independent runs in BEAST v. 2.6.6 (Bouckaert et al., 2019) for 50 million generations, sampled trees every 2,000 steps, and logged results every 3,000 steps. We used Beast Model Test with Transition x Transversion split to simultaneously evaluate phylogenetic relationships and the sequence evolution model with the Yule Process tree prior. We used a strict molecular clock, with a mutation rate of 0.00555 mutations per million years (following Fouquet et al., 2012) after finding that the hypothesis of a strict clock could not be rejected for our dataset based on a likelihood ratio test (see Results). We checked for stationarity by visually inspecting trace plots for each run and ensuring that all values of effective sample sizes were above 200 in Tracer v. 1.7.1 (Rambaut et al., 2018). We checked for convergence among runs by comparing parameter estimates in Tracer v. 1.7.1. We used LogCombiner v. 2.6.6 to combine logged trees from the three runs and discarded the first 20% as burn-in. Finally, we built a maximum clade credibility tree with median node ages constructed in TreeAnnotator v. 2.6.6 (Bouckaert et al., 2019) and visualized trees in FigTree v. 1.4.4.

## RESULTS

### *Pseudopaludicola javae* sp. nov.

(Figs. 2–6; Tables 1–3)

**Holotype.**—CHUFPB31044 adult male, Fazenda Escondida,  $-9.884426^{\circ}$ ,  $-49.779139^{\circ}$  (WGS84), 179 m a.s.l., Tocantins State, Marianópolis Municipality, Brazil (Fig. 1) collected on 2 February 2021 by L. A. Silva, S. P. Dantas, and E. P. Neto (Figs. 2, 3B, 4, 5).

**Paratopotypes.**—Eighteen adult males; same data as for holotype: CHUFPB31033, 31045 to 31050, 31053, 31056 to 31060, 31062, 31063, 31065, 31067, and 31070. One adult female; same data as for the holotype: CHUFPB31042. Collected between 28 February and 2 March 2021.

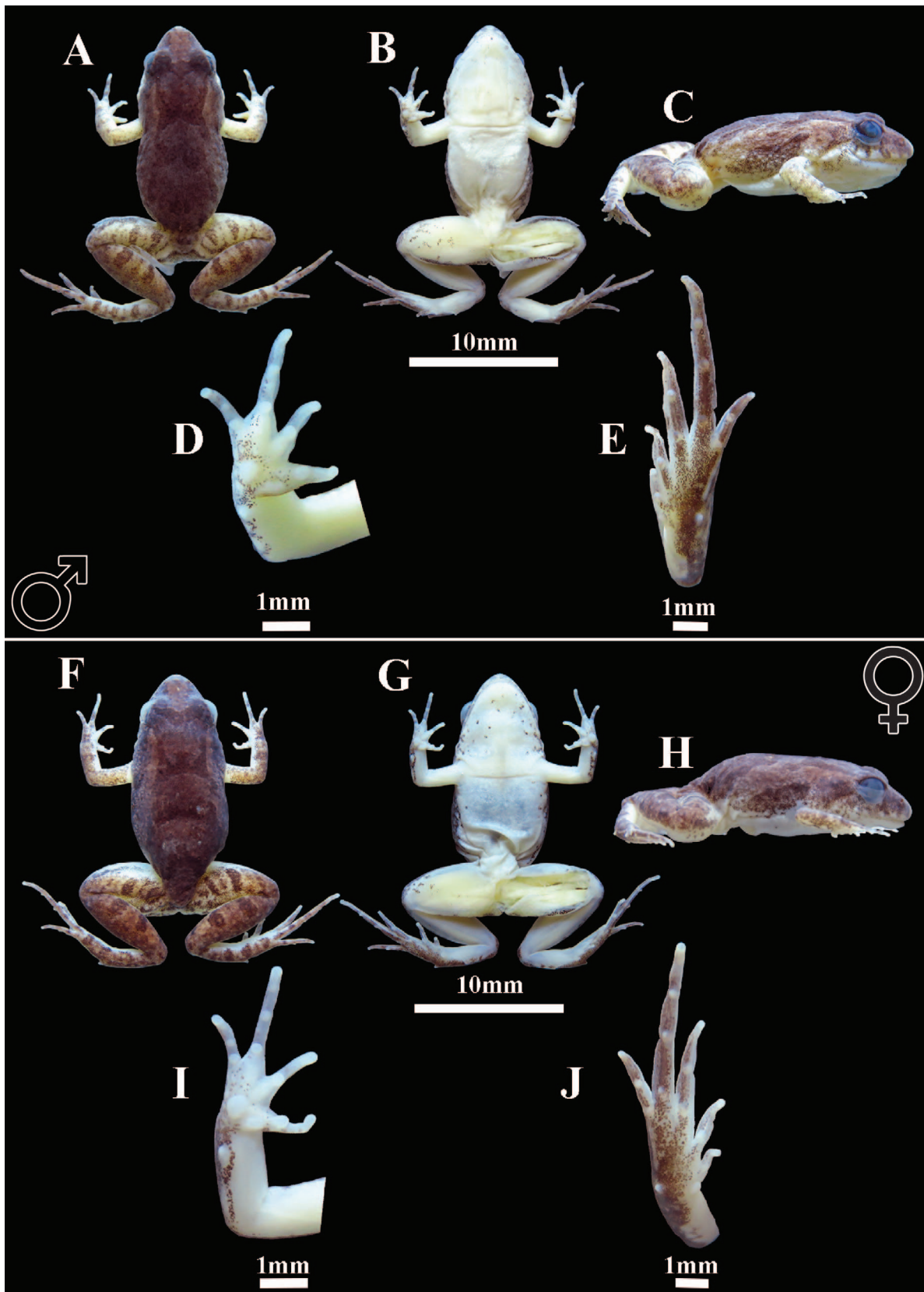


FIG. 2. Holotype of *Pseudopaludicola javae* sp. nov. (above, CHUFPB31044; SVL = 15.1 mm, A-E) and paratype female (below, F-J, CHUFPB31042; SVL = 14.8 mm) in preservative. (A) Dorsal body, (B) ventral body, (C) profile body; (D) palm of right hand, and (E) sole of left foot of the holotype. (F) Dorsal body, (G) ventral body, (H) profile body; (I) palm of right hand, and (J) sole of right foot of the female CHUFPB31042.





FIG. 3. Variation in life of *Pseudopaludicola javae* sp. nov. (A) Male CHUFPB31055 (SVL = 13.8), (B) holotype male CHUFPB31044 (SVL = 15.1), (C) male CHUFPB31056 (SVL = 13.2), (D) unvouchered male, (E) male CHUFPB31043 (SVL = 14.3), (F) male CHUFPB31059 (SVL = 15.4), (G) male CHUFPB31062 (SVL = 14.0), and (H) female CHUFPB31042 (SVL = 14.8). Specimens A, D, and E are from Lagoa da Confusão; the remaining specimens are from Marianópolis Municipality, all Tocantins State.

**Additional Specimens.**—Five adult males; Tocantins State, Lagoa da Confusão Municipality, Lago Verde Region, access road to Fazenda Campo Guápo;  $-10.866078^{\circ}$ ,  $-49.731674^{\circ}$  (WGS84); 182 m a.s.l.; 19 March 2021; S. P. Dantas and E. P. Neto: CHUFPB31034, 31043, 31054, 31055, and 31069. One adult female; same data as for the previous five specimens, CHUFPB31064.

**Diagnosis.**—The new species is characterized by the following set of characters: (1) small size (SVL 12.7–15.4 mm in adult

males); (2) terminal phalanges knobbed, with no T-shaped terminal phalanges or expanded toe tips; (3) upper eyelids smooth, with no palpebral tubercles; (4) heel smooth, with no enlarged conical tubercle; (5) relatively short hind limbs (tibiotarsal articulation reaching the corner of the mouth); (6) subgular, smooth, whitish vocal sac when inflated with few melanophores around the jaw, cream to yellowish when at rest; (7) advertisement call composed of an irregular series of 12–105 multipulsed notes (note duration range: 235–412 ms), emitted at

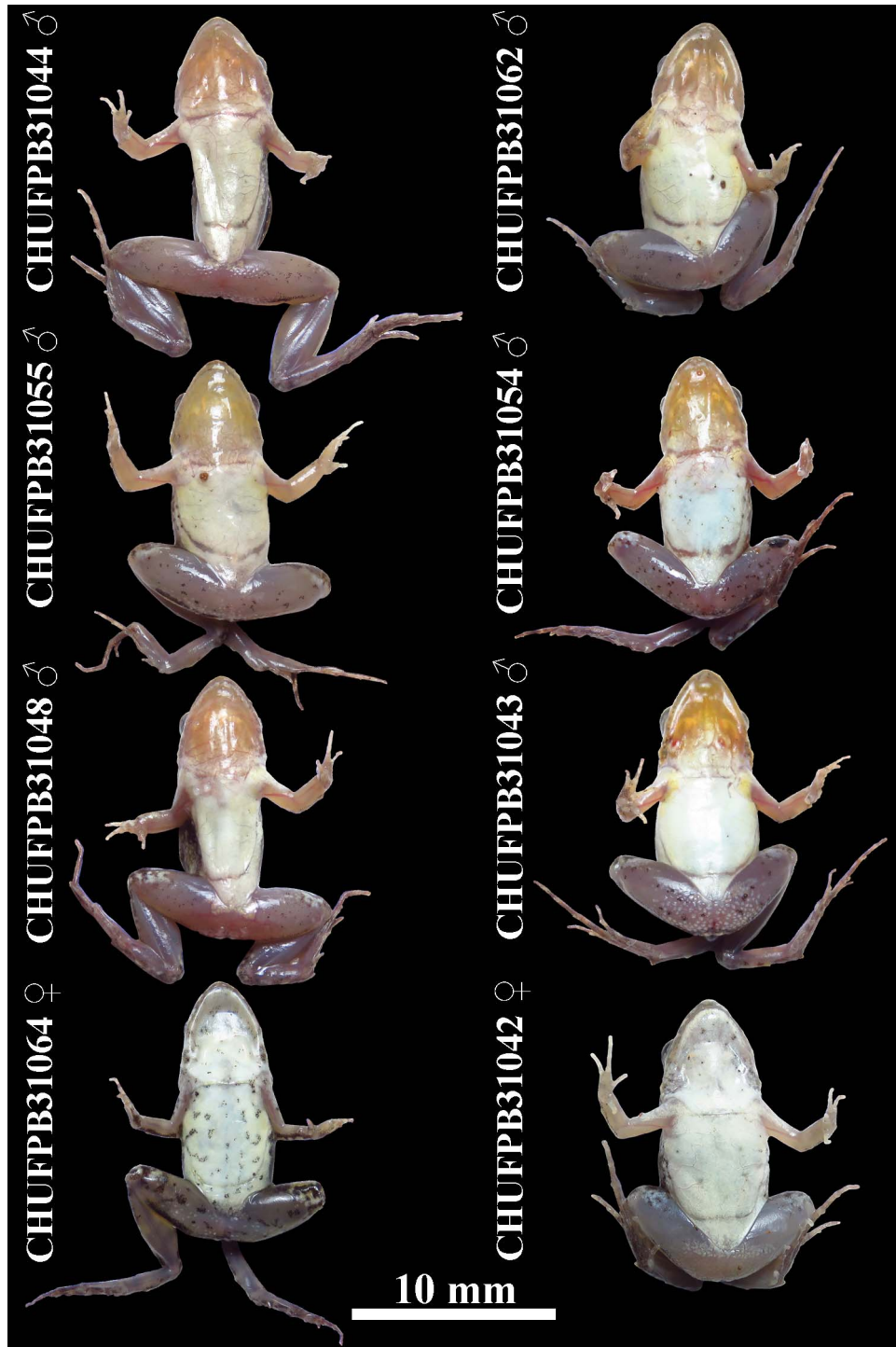


FIG. 4. Ventral variation in life of *Pseudopaludicola javae* sp. nov. The specimens are followed by the respective label and sex. Specimens from the type locality, Marianópolis Municipality, Tocantins State, as follows: CHUFPPB31042, CHUFPPB31044 (holotype), CHUFPPB31048, and CHUFPPB31062; Specimens from Lagoa da Confusão municipality, Tocantins State are as follows: CHUFPPB31043, CHUFPPB31054, CHUFPPB31055, and CHUFPPB31064.

low rate (43–71 notes/min), and with 10–17 nonconcatenated pulses separated by intervals of 3–55 ms, emitted at a rate of 27–51 pulses/sec.

*Description of Holotype*.—SVL 15.1 mm. Body elliptic and broad (Fig. 2A). Head slightly wider than long (Table 1). Snout subelliptical in dorsal view and rounded in profile (Fig. 2A–C). Eyes protuberant. Eye diameter slightly larger than interorbital distance. Interorbital area flat. Pupil rounded. Upper eyelid smooth. Nostril not protuberant and closer to snout tip than to

eye. Canthus rostralis rounded, smooth. Loreal region flat. Single subgular vocal sac, externally expanded. Choanae rounded, well separated from each other. Both vocal slits present. Tympanum membrane and annulus absent. Tympanic ridge absent. Mouth opening ventral. Vomerine teeth absent. Tongue ovoid, longer than wide, free posteriorly, without pigmentation at its base. Flanks with small, but discrete granules. Paravertebral chevron-shaped dermal ridges from behind the eyes to the scapular region barely visible. One ovoid antebrachial tubercle present at the first



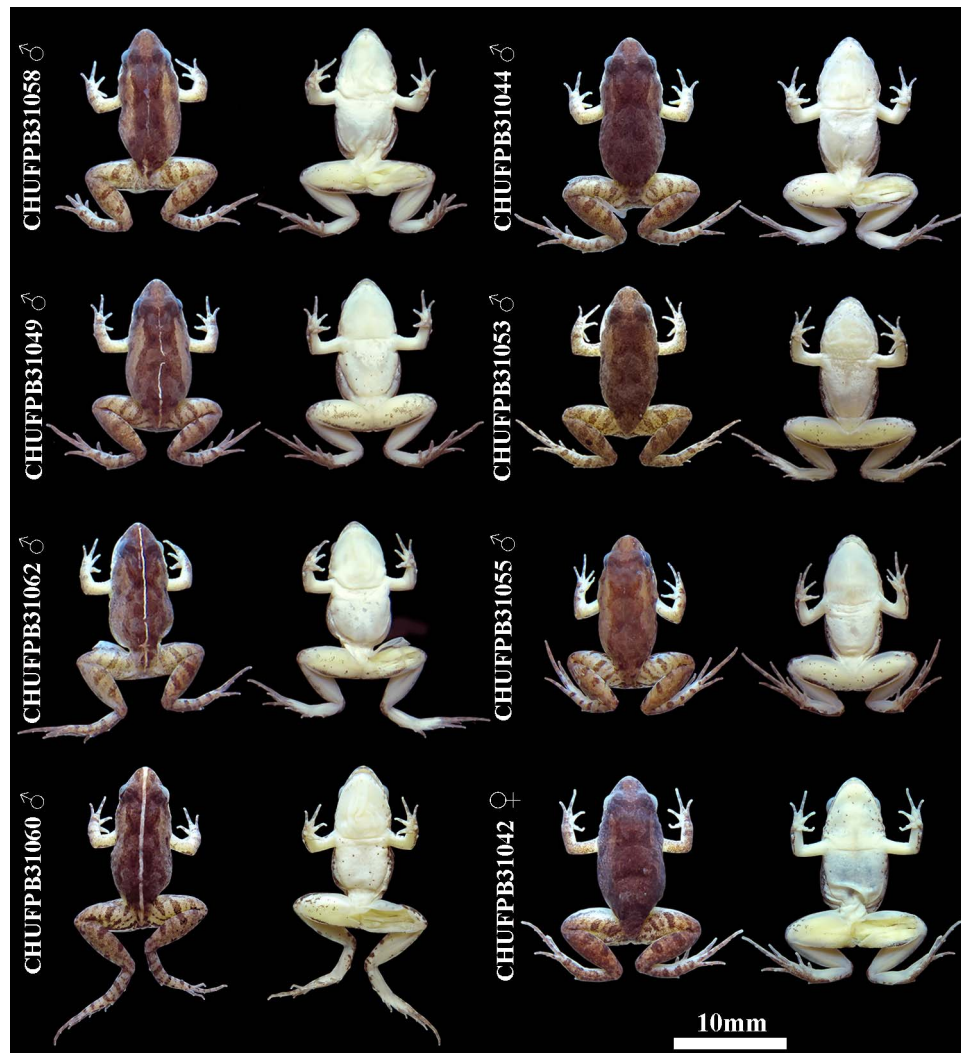


FIG. 5. Dorsal (left) and ventral (right) variation of *Pseudopaludicola javae* sp. nov. in preservative. The specimens are followed by the respective label and sex. The specimen CHUFPB31055 was collected in Lagoa da Confusão Municipality, Tocantins State; all other specimens are from the type locality, Marianópolis Municipality, Tocantins State.

quarter of the forearm (Fig. 2B). 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 (Fig. 2D). 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 the entire external portion of finger. Webbing absent between fingers. Relative finger lengths, when addressed to one another:  $I < II < IV < III$ . Outer metatarsal tubercle well-defined, conical, smaller than ovoid inner metatarsal tubercle. Toes with well-defined, single, enlarged, rounded subarticular tubercles (Fig. 2E). Sole of foot without supernumerary tubercles. 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 midventral tarsus, ending in protuberant tarsal tubercle. Relative toe lengths, when addressed one to another:  $I < II < V < III < IV$ . Hind limb robust with tibiotarsal articulation just reaching posterior margins of eye. Thigh slightly shorter than tibia. Foot longer than thigh. Foot slightly longer

than tibia. Heel without tubercles. Belly skin smooth. Abdominal fold present and complete. Dorsal surfaces of head, body, and limbs smooth. Cloacal region smooth. Measurements of the holotype shown in Table 1.

*Variation.*—The female CHUFPB31042 (SVL = 14.8 mm) from Marianópolis was larger than 83% of the males, whereas the female CHUFPB31064 from Lagoa da Confusão (SVL = 13.7 mm) was smaller than 71% of the males (Table 1). Detailed morphometric measurements of the type series are shown in Table 1. The most pronounced variations were observed in dorsum color patterns (Figs. 3, 5). The state of the vertebral line varied almost in a continuum of complete (from the snout tip to the cloaca; three specimens: CHUFPB31048, CHUFPB31060, and CHUFPB31062) to absent (20 specimens of holotype condition: CHUFPB31033, 31034, 31042 to 31047, 31050, 31053 to 31057, 31059, 31063 to 31065, 31067, and 31069), including three specimens with incomplete vertebral lines (CHUFPB31058, CHUFPB31070, and CHUFPB31049; Fig. 5). In life, dorsum varies from light brown (e.g., CHUFPB31055) to dark brown (e.g., CHUFPB31044); dorsum with dark-brown blotches of different sizes, forms and positions (Figs. 3, 5). Paravertebral



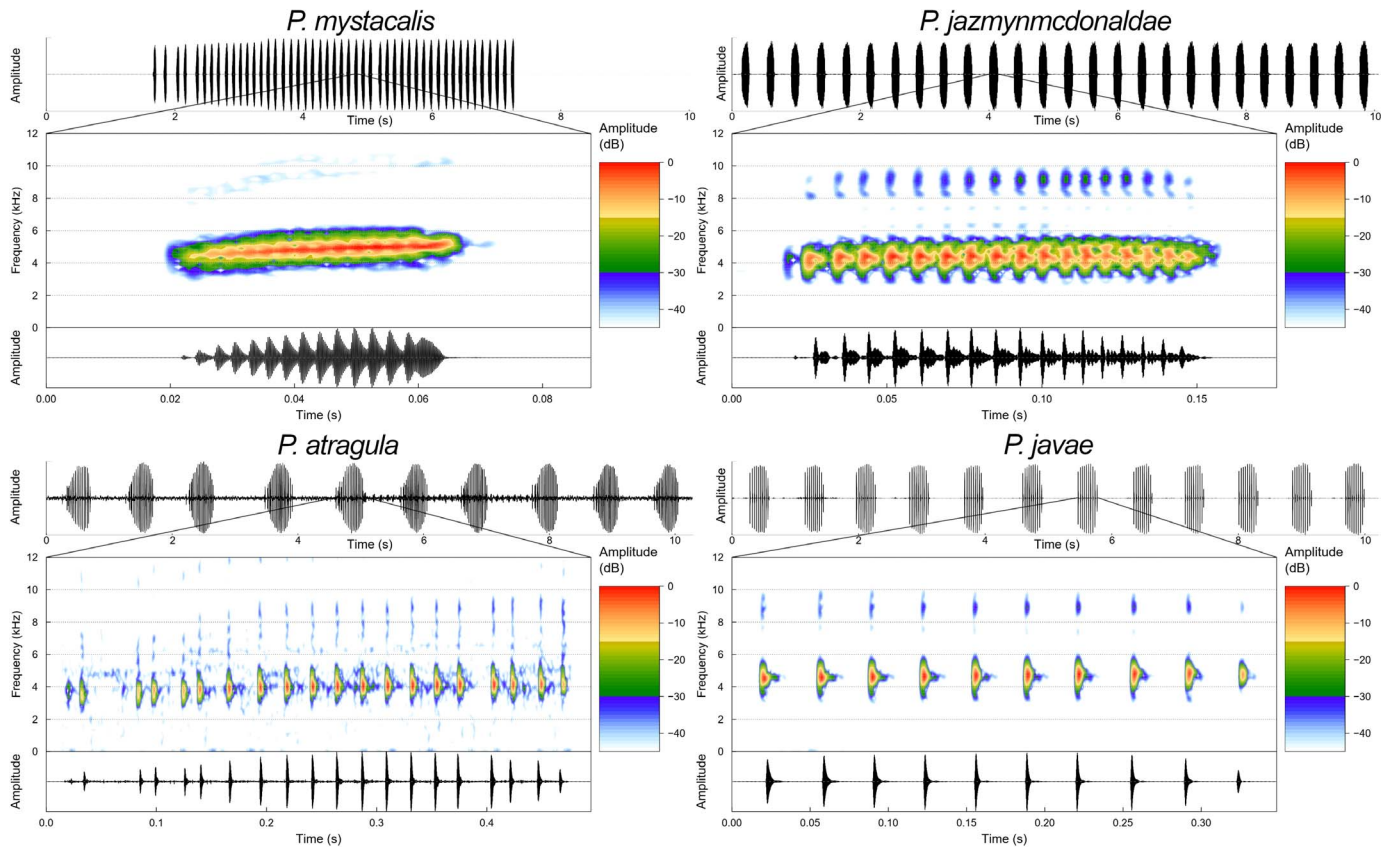


FIG. 6. A comparison of the advertisement calls of *Pseudopaludicola mystacalis*, *P. jazmynmcdonaldi*, *P. atragula*, and *P. javae* sp. nov. The oscillograms represent 10-sec segments of a series of advertisement calls from each species. Below is shown the corresponding audiospectrograms and oscillograms for the highlighted notes, detailing their pulsatile microstructures. The advertisement call of the paratype for the new species was recorded on 01 March 2021 at 1705 h; the air temperature was 25.0–28.0°C (recording label: FNVJ 0051324).

chevron-shaped dermal ridges from behind the eyes to the scapular region are more appreciable in life (e.g., Fig. 3F, CHUFPB31059). Dorsolateral stripe from the posterior margin of eye to the inguinal region ranging from light brown to orange brown (Fig. 3). Dorsal colors in preservative become faded. Belly can be immaculate whitish (e.g., holotype CHUFPB31044 and female CHUFPB31042) or whitish scattered with melanophores (e.g., male CHUFPB31060 and female CHUFPB31064; Fig. 4). Melanophores on ventral surface of thigh vary in a continuum of

absent (CHUFPB31042) to large and conspicuous (e.g., CHUFPB31064; Fig. 4). Gular region in live males is beige to yellowish with few melanophores near jaw (Fig. 4), more conspicuous during call activity (Figs. 3B–D). The specimen CHUFPB31055 does not have visible melanophores around the jaw even during call activity (Fig. 3A). In preservative, gular region whitish (Fig. 5). The two females exhibited a white gular region with few (CHUFPB31042) or many (CHUFPB31064) melanophores.

TABLE 1. Morphometric measurements of *Pseudopaludicola javae* sp. nov. from the type locality, Marianópolis Municipality ( $n = 20$ ), and from Lagoa da Confusão Municipality ( $n = 6$ ), Tocantins State, Brazil. Values are shown in millimeters; ranges are shown followed by mean and standard deviation in parentheses.

Measures	Marianópolis (type locality)			Lagoa da Confusão	
	Holotype (CHUFPB31044)	Males ( $n = 18$ )	Female (CHUFPB31042)	Males ( $n = 5$ )	Female (CHUFPB31064)
Snout–vent length	15.1	13.2–15.4 (14.1 ± 0.6)	14.8	12.7–14.3 (13.6 ± 0.6)	13.7
Head length	4.3	3.6–4.6 (4.0 ± 0.2)	4.3	3.5–4.1 (3.8 ± 0.3)	3.5
Head width	5.7	4.7–5.5 (5.2 ± 0.2)	5.6	4.7–4.9 (4.8 ± 0.1)	4.5
Eye diameter	1.7	1.5–1.8 (1.7 ± 0.1)	1.9	1.6–1.8 (1.7 ± 0.1)	1.6
Interorbital distance	1.2	0.8–1.2 (1.0 ± 0.1)	0.9	0.7–1.0 (0.9 ± 0.1)	0.8
Eye–nostril distance	1.3	1.0–1.2 (1.1 ± 0.1)	1.2	1.0–1.1 (1.1 ± 0.1)	1.0
Snout length	2.5	1.9–2.5 (2.2 ± 0.1)	2.2	1.7–2.2 (2.0 ± 0.2)	1.8
Internarial distance	1.1	1.0–1.2 (1.1 ± 0.1)	1.3	1.0–1.2 (1.1 ± 0.1)	1.0
Hand length	3.3	2.7–3.4 (3.1 ± 0.2)	3.4	3.0–3.4 (3.2 ± 0.2)	3.2
Thigh length	6.0	5.7–6.5 (6.1 ± 0.2)	6.4	5.6–5.9 (5.7 ± 0.1)	6.2
Tibia length (= shank length)	6.8	6.3–7.1 (6.6 ± 0.2)	7.1	6.2–6.8 (6.5 ± 0.3)	6.7
Tarsus length	3.8	2.9–4.0 (3.5 ± 0.3)	3.9	3.2–3.5 (3.4 ± 0.2)	3.6
Foot length	7.1	6.3–7.5 (6.9 ± 0.3)	7.5	6.8–7.7 (7.2 ± 0.4)	7.4

TABLE 2. Advertisement call traits of *Pseudopaludicola javae* sp. nov. based on the recordings of 20 males (200 notes) from Marianópolis (type locality;  $n = 18$ ) and Lagoa da Confusão ( $n = 2$ ) municipalities. Values are presented as mean  $\pm$  standard deviation (minimum–maximum).

Traits	Values
Call duration (sec)	50.0 $\pm$ 21.0 (29.2–94.8)
Series of notes duration (sec)	51.9 $\pm$ 21.3 (6.4–60.2)
Inter-series interval (sec)	12.1 $\pm$ 7.7 (2.5–13.7)
Note duration (ms)	320 $\pm$ 30 (235–412)
Inter-note interval (ms)	687 $\pm$ 129 (512–1,734)
Notes per minute	61.0 $\pm$ 7.1 (42.9–70.7)
Pulse duration (ms)	8 $\pm$ 2 (2–22)
Interpulse interval (ms)	19 $\pm$ 4 (3–55)
Pulses per second	39.0 $\pm$ 5.8 (27.2–51.1)
Pulses per note	12.4 $\pm$ 1.4 (10.0–17.0)
Dominant frequency (Hz)	4,618.2 $\pm$ 164.3 (4,177.4–5,124.9)
Min. dominant frequency reached (Hz)	4,174.9 $\pm$ 145.2 (3,832.9–4,478.9)
Max. dominant frequency reached (Hz)	4,996.1 $\pm$ 160.5 (4,651.2–5,383.3)
1st Quartile frequency	4,452.2 $\pm$ 149.7 (4,091.3–4,823.4)
3rd Quartile frequency	4,752.4 $\pm$ 154.2 (4,435.8–5,168.0)
Peak of 2nd harmonic frequency (kHz)	9,014.0 $\pm$ 373.1 (7,665.8–10,034.0)
Air temperature (°C)	26.5–29.6

*Color in Life of the Holotype.*—In life (Fig. 3B), dorsum brown with a dark-brown blotch like an inverted triangle between eyes, a “W”-like blotch on the first third of the dorsum, and many irregular blotches on the posterior dorsum. Brown dorsolateral stripes ranging from the posterior margin of the eye to the inguinal region. Ventrolateral region dark brown scattered with yellowish and whitish specks. Belly immaculate light cream (Fig. 4, top left). Whitish vocal sac when inflated with few melanophores concentrated around the jaw (Fig. 3B); yellowish when not inflated (Fig. 4, top left). Dorsum darker than dorsal surfaces of limbs. Upper lip with four white blotches separated by vertical dark gray stripes. Ventral surfaces of arms and legs unpigmented; thigh scattered with melanophores. Palm of hand light cream with melanophores mainly concentrated around the outer metacarpal tubercle. Sole of foot dark brown. Dorsal surface of arm light brown with few dark brown blotches, more conspicuous near the hand. Dorsal face of hind limb light brown with dark-brown transverse stripes and with scattered brown blotches. Light cream longitudinal stripe on posterior surface of thigh. Dark-brown transverse stripes on thigh (2), shank (3), and foot (3). Light-gray nuptial pads. In preservative, general color becomes faded, except the gular region, which turns whitish (Figs. 2A,B).

*Comparisons With Other Species.*—*Pseudopaludicola javae* sp. nov. is easily distinguished from the *Pseudopaludicola pusilla* species group (*Pseudopaludicola boliviana*, *Pseudopaludicola ceratophyes*, *Pseudopaludicola llanera*, *P. pusilla*, and *Pseudopaludicola motorzinho*) by the absence of either T-shaped terminal phalanges or

expanded toe tips (discs or pads). Terminal phalanges of the new species are knobbed, like in *Pseudopaludicola falcipes* (Cardozo and Lobo, 2009; Cardozo and Suárez, 2012; Fig. 2B). *Pseudopaludicola ceratophyes* and *P. llanera* also have upper eyelids with enlarged palpebral tubercles (Lynch, 1989), whereas the new species has smooth upper eyelids. Additionally, *P. boliviana*, *P. ceratophyes*, *P. pusilla*, and *P. motorzinho* present a conical tubercle on the heel, whereas the new species has smooth heels (Lynch, 1989; Cardozo and Lobo, 2009; Pansonato et al., 2016).

From the *Pseudopaludicola saltica* clade, which includes *P. jaredi*, *Pseudopaludicola murundu*, and *P. saltica*, *P. javae* sp. nov. is distinguished by having short hind limbs (tibiotarsal articulation reaching near the corner of the mouth), whereas the three long-legged species have the tibiotarsal articulation extending beyond the tip of snout (Lobo, 1995; Andrade et al., 2016a).

The vocal sac of *Pseudopaludicola javae* sp. nov. is subgular, smooth, and whitish when inflated (cream to yellowish when at rest) with few melanophores around the jaw that become poorly visible at rest. *Pseudopaludicola atragula*, *Pseudopaludicola coracoralinae*, and *Pseudopaludicola facureae* have vocal sacs with a warty texture (Andrade and Carvalho, 2013; Pansonato et al., 2014; Andrade et al., 2020a). In addition, *P. atragula* has a vocal sac with dark reticulations (Pansonato et al., 2014), and *P. jazmynmcdonaldae* has a dark vocal sac (Andrade et al., 2019).

*Pseudopaludicola javae* sp. nov. is smaller than *Pseudopaludicola ameghini* (males with SVL of 14.1–19.3, females with SVL of 18.5–22.5, Pansonato et al., 2013) and *Pseudopaludicola ternetzi* (females with SVL of 19.7–20.1, Caramaschi and Pombal, 2011; males with SVL of 14.0–18.1, Andrade et al., 2017) with SVL of 12.7–15.4 mm in males and 13.7–14.8 mm in females.

The following 11 species can be regarded as morphologically cryptic with respect to *P. javae* sp. nov., and a more reliable diagnosis depends on acoustic evidence: *P. canga*, *P. falcipes*, *Pseudopaludicola florencei*, *Pseudopaludicola giarettai*, *Pseudopaludicola hyleaustralis*, *Pseudopaludicola ibisoroca*, *Pseudopaludicola matuta*, *Pseudopaludicola mineira*, *P. mystacalis*, *Pseudopaludicola pocoto*, and *Pseudopaludicola restinga*. Below, we provide the acoustic diagnosis of *P. javae* sp. nov. in relation to its congeners.

The new species has a call composed of an irregular series of 12–105 multipulsed notes (note duration range: 235–412 ms), emitted at rates of 43–71 notes/min; notes have 10–17 non-concatenated pulses separated by intervals of 1–31 ms, and emitted at rate of 27–51 pulses/sec. *Pseudopaludicola javae* sp. nov. is easily distinguished from species that have a trilled advertisement call pattern, that is, calls with nonpulsed notes. Notably, *P. canga* (Giaretta and Kokubum, 2003; Pansonato et al., 2012; Roberto et al., 2013; Carvalho et al., 2015b; Andrade et al., 2020a), *P. coracoralinae* (Andrade et al., 2020a), *P. facureae* (Andrade and Carvalho, 2013; Carvalho et al., 2015a,b), *P. giarettai* (Carvalho, 2012; Carvalho et al., 2015a,b), and *P. hyleaustralis* (Pansonato et al., 2012) have trilled advertisement calls (i.e., calls with nonpulsed notes). In contrast with the new species, *P. boliviana*, *P. ibisoroca*, *P. motorzinho*, and *P. mystacalis*

TABLE 3. Confusion matrix for the comparisons of *Pseudopaludicola atragula*, *P. mystacalis*, and *P. jazmynmcdonaldae* with *P. javae* sp. nov. based on the acoustic dataset by means of a randomForest model. Settings: number of tree permutations = 1,000; number of variables tried at each split = 2.0; error rate = 3.08%.

	<i>P. atragula</i>	<i>P. jazmynmcdonaldae</i>	<i>P. javae</i> sp. nov.	<i>P. mystacalis</i>	Classification error
<i>P. atragula</i>	2	0	2	0	50%
<i>P. jazmynmcdonaldae</i>	0	24	0	0	0%
<i>P. javae</i> sp. nov.	0	0	20	0	0%
<i>P. mystacalis</i>	0	0	0	17	0%

have calls composed of pulsed notes, with concatenated pulses (lack of silent intervals between them; Duré et al., 2004; Pansonato et al., 2013, 2016).

The new species is easily distinguished from other species lacking concatenated pulses by temporal traits, especially in the number of pulses per note. *Pseudopaludicola falcipes*, *P. mineira*, *P. restinga*, and *P. matuta* have calls composed of a series of two-pulsed stereotyped notes, emitted at higher rates of 238–535 notes/min (combined values; Haddad and Cardoso, 1987; Pereira and Nascimento, 2004; Andrade et al., 2018a,b; Cardozo et al., 2018). *Pseudopaludicola pocoto* and *P. florencei* have calls composed of stereotyped notes with three pulses (Magalhães et al., 2014; Andrade et al., 2017; Andrade et al., 2018b). The number of pulses in the notes emitted by the long-legged *P. saltica*, *P. murundu*, and *P. jaredi* varies from two to seven (combined values; Andrade et al., 2016a). *Pseudopaludicola ternetzi* and *P. ameghini* have calls composed of pulsed notes with three to six pulses each, emitted at higher rates of 348–921 notes/min (combined values; Andrade et al., 2017).

Among the species that share advertisement calls with pulsatile structure, *P. jazmynmcdonaldae* and *P. atragula* have the most similar calls compared to that of *P. javae* sp. nov. *Pseudopaludicola jazmynmcdonaldae* has a shorter note duration (58–174 ms), more notes per minute (106.8–189.5), and a higher rate of pulses per second (Andrade et al., 2019). Therefore, the new species is easily distinguished from *P. jazmynmcdonaldae* by temporal traits because of the lack of overlap in these parameters. Environmental conditions can affect frog calls, but air temperature during field recordings of these species overlapped significantly (Andrade et al., 2019). We were not able, however, to identify acoustic characters that discriminate the new species from *P. atragula*. Still, even with overlaps, we found significant differences in note duration ( $P = 0.04$ ;  $Z = 2.01$ ), the number of pulses per note ( $P < 0.01$ ;  $Z = 3.09$ ), pulse duration ( $P = 0.02$ ;  $Z = -2.4013$ ), interpulse interval ( $P < 0.01$ ;  $Z = -3.0764$ ), and pulse rate ( $P < 0.01$ ;  $Z = 3.02$ ). The randomForest model resulted in total discrimination (100%) between *P. javae* sp. nov. and *P. jazmynmcdonaldae* and partial discrimination between the new species and *P. atragula* (50%), with two males of *P. atragula* misclassified (see Fig. 8; see Table 3). Interpulse interval, pulse rate, notes per minute, and note duration were the main sources of variation in both variable importance measurements (see Fig. 8). It is worth highlighting that morphological (vocal sac texture and color) and phylogenetic position (Fig. 7) provided sufficient evidence to support our hypothesis that *P. javae* sp. nov. is evolving independently from *P. atragula*. The following three *Pseudopaludicola* species have no published information on their advertisement calls, which all belong to the *P. pusilla* group: *P. ceratophyes*, *P. llanera*, and *P. pusilla*. These species are easily diagnosed from *P. javae* sp. nov. based on morphological and genetic/phylogenetic evidence.

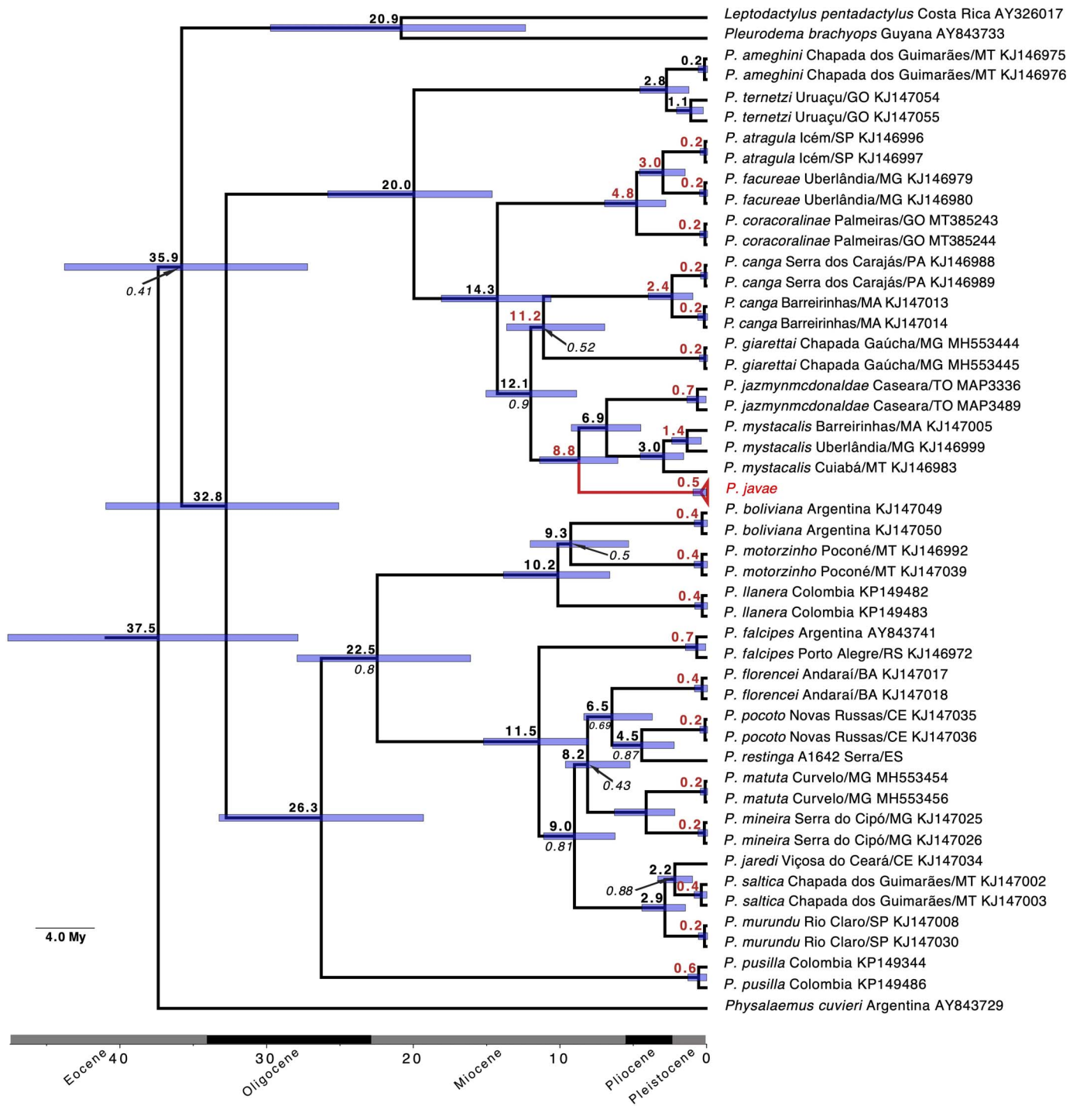
**Advertisement Call.**—Twenty males were recorded ( $n = 200$  notes and 2,475 pulses analyzed). We summarize quantitative call traits in Table 2. The advertisement call (total duration: 29–95 sec) consists of irregular series of notes that last 6–60 sec, separated by interseries intervals of 2.5–14 sec. Series have 12–105 (mean = 50; SD = 23) stereotyped multipulsed notes per series. These notes last 235–412 ms, separated by intervals of 512–1,734 ms, and are emitted at a rate of 43–71 notes/min; notes have a slight increase in amplitude from the beginning to the midpoint of the note, followed by a decrease in amplitude until the end (Fig. 6). Notes are composed of 10–17 nonconcatenated pulses (Fig. 6). Pulses

vary from 2–22 ms, separated by intervals of 3–55 ms, and are released at a rate of 27–51 pulses/sec (Fig. 6). Each note has a slight increase in frequency along its duration; on average, notes have an increase of 300 Hz from the first to the third quartile frequencies. Dominant (= fundamental) frequency peaks are between 4,177 and 5,125 Hz (mean = 4,618); minimum frequency ranges are between 3,833 and 4,479 Hz (mean = 4,175), and maximum frequency ranges are between 4,651 and 5,383 Hz (mean = 4,996). Notes present up to two harmonics, with the second ranging 7,666–10,034 Hz (Fig. 6). The air temperature of recorded calls varied from 25 to 30°C. Air temperature had no observable effect on call traits (see previous section). A video of the holotype (CHUFPB31044) emitting the advertisement call is available in Supplementary Material and at 10.6084/m9.figshare.22189945. We also recorded a specimen (unvouchered) emitting aggressive notes when another male vocalized nearby (Supplementary Material, 10.6084/m9.figshare.22189957). The male CHUFPB31058 was recorded inflating the vocal sac without producing any audible sound (Supplementary Material, 10.6084/m9.figshare.22189960). An unvouchered specimen from Lagoa da Confusão was also filmed (Supplementary Material, 10.6084/m9.figshare.22190014). Lastly, a video of the northernmost record of *P. javae* sp. nov. from Couto de Magalhães Municipality, Tocantins State, is also available (Supplementary Material; 10.6084/m9.figshare.22190050).

**Phylogenetic Relationships.**—Our resulting maximum clade credibility tree had most nodes highly supported ( $>0.95$ ; Fig. 7 and Supplementary Fig. S1). We ran a maximum likelihood analysis in MEGA (using a GTR + G + I model selected in the same software) and compared likelihoods of the resulting tree with and without enforcing a strict molecular clock. The null hypothesis of the equal evolutionary rate throughout the tree could not be rejected at a 5% significance level ( $P = 0.0703$ ). We, therefore, conducted subsequent analyses by using a strict clock model. *Pseudopaludicola javae* sp. nov. forms a clade with *P. mystacalis* and *P. jazmynmcdonaldae*. It diverged from these species around 8.8 mya (range = 4.2–16.8 mya) in the Miocene (most likely) or early Pliocene. Deeper nodes in the phylogeny had wider confidence intervals, as expected, and some lacked significant support, probably because of the accumulation of synonymous substitutions. Our results match previous phylogenetic relationships published for the genus (Andrade et al., 2020a). However, a few internal nodes are less supported, as expected, because we used a smaller fragment. For example, *P. pusilla* was not part of the species group that harbors its name in our topology (although, the node where it is placed had a low posterior probability of 0.8). Still, in the previous phylogeny where *P. pusilla* was monophyletic, the clade also had low support (bootstrap 43; Andrade et al., 2020a). Hence, the monophyly of the *P. pusilla* species group remains contentious. A better understanding of the phylogenetic relationships among species in the genus depends, therefore, on a larger molecular dataset. *Pseudopaludicola javae* sp. nov., nevertheless, is molecularly distinct and diverged millions of years ago from its common ancestor within the *P. mystacalis* + *P. jazmynmcdonaldae* clade.

**Etymology.**—The Javaé are an ethnic group that has been historically present in the regions surrounding the middle Araguaia River, primarily on the world's largest fluvial island, Bananal Island. This island is flanked on its west side by the Araguaia River and to the east by the Javaés River, which is an anabranch of the Araguaia, also known as “Braço Menor do Araguaia.” Besides the Javaé, Bananal Island is also home to two





Time (Millions of Years before present)

FIG. 7. Phylogenetic relationships inferred for the species of the genus *Pseudopaludicola* based on a fragment of 16S mtDNA. Numbers in bold above branches indicate the mean age of the node to the right, whereas italicized numbers below branches indicate posterior probabilities for branches with posterior probabilities of  $<0.99$ ; all other branches have posterior probabilities of  $>0.99$ . Codes to the right of species names are GenBank accession numbers. For Brazilian species, names are followed by their corresponding municipality/state abbreviation. Species from other countries are followed by country name. The collapsed branch of *P. javae* sp. nov. contains five terminals, including four from the type locality, Marianópolis Municipality, Tocantins State (the female CHUFPB31042 and the males CHUFPB31044 [holotype], CHUFPB31046, and CHUFPB31047), and one from Lagoa da Confusão Municipality, Tocantins State (female CHUFPB31064).

other indigenous groups, as follows: Karajá and Xambioá. The Javaé people refer to themselves as *Inĩ*, meaning “human being.” More than 10 Javaé villages are present on Bananal Island, and the closest village to the type locality is “Boto Velho” village

(Inãwébohona in the *Inĩ* language), about 100 km away. Thus, the specific name is in recognition of this singular Brazilian ethnic group that historically occupied the region where the new species is found.

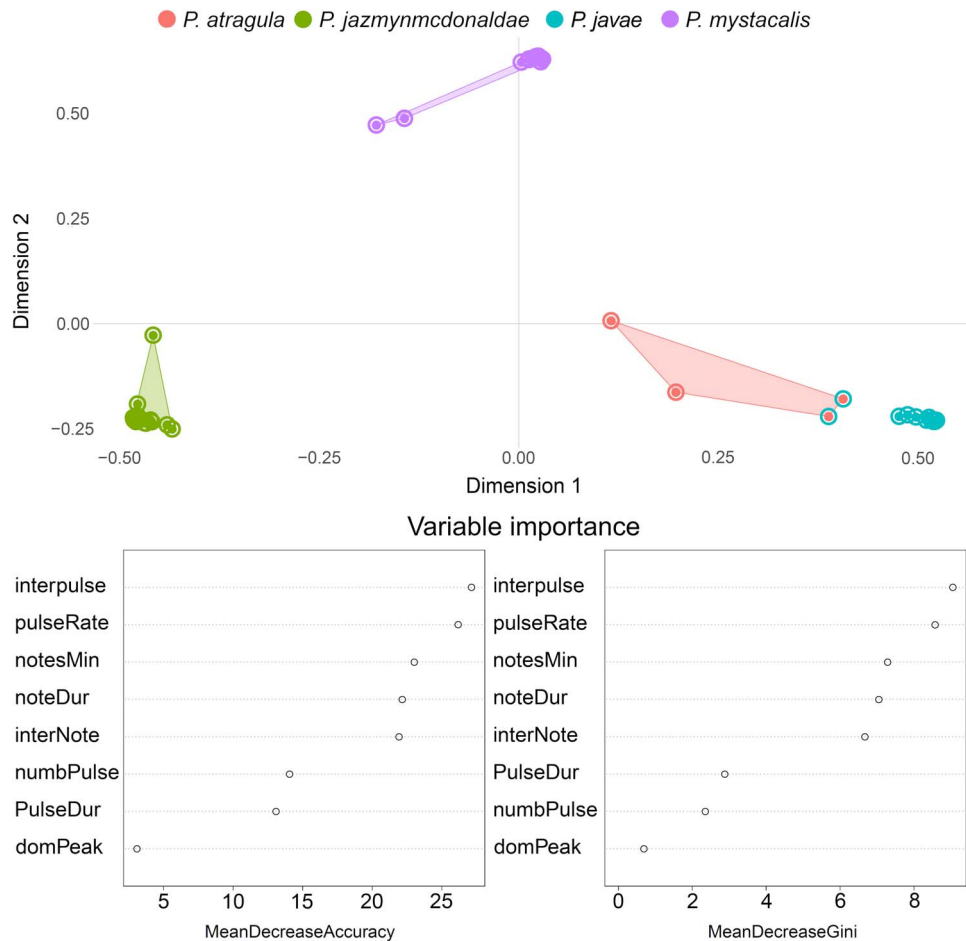


FIG. 8. First and second dimensions of the multidimensional scaling on the proximity scores from the randomForest analysis considering acoustic traits of adult males of *Pseudopaludicola javae* sp. nov. (blue dots), *P. atragula* (red dots), *P. jazmynmcdonaldae* (green dots), and *P. mystacalis* (purple dots). The color of the circles around dots represents the species the randomForest algorithm attributed to each individual. Dot charts of the variable importance score are shown below, considering acoustic traits as indicated by the randomForest analysis. Mean decreased accuracy and mean decrease Gini are coefficients that measure how variables contribute to nodes and leaves homogeneities in the resulting randomForest.

*Geographic Distribution and Natural History.*—*Pseudopaludicola javae* sp. nov. is currently found in four municipalities in western Tocantins State, namely, Marianópolis (type locality), Cristalândia (nearly 90 km due south from the type locality), Lagoa da Confusão (nearly 110 km due south from the type locality), and Couto Magalhães (nearly 192 km due north from the type locality). The new species was found in both anthropized (pastures, margins of roads, and soybean and rice crops) and natural areas. Natural environments consisted of open vegetation dominated by grasses that become partially flooded during the rainy season. Male activity initiated around 1500 h, with a peak at 1800–2000 h. Few specimens were heard after 2200 h. Calling males were found on exposed ground and between grasses, usually calling on top of small clay mounds likely constructed by ants or earthworms. At the type locality, congeners found syntopically were *P. canga*, *P. jazmynmcdonaldae*, and *P. aff. saltica*. In Lagoa da Confusão, we found *P. javae* sp. nov. syntopically with *P. jazmynmcdonaldae* and *P. mystacalis*. In Cristalândia, the new species was syntopic with *P. jazmynmcdonaldae*. As can be observed in the species comparison section, *P. javae* sp. nov. can be easily distinguished from syntopic congeners through morphological and/or acoustic traits. Furthermore, all syntopic congeners are either distantly related to or form a monophyletic clade with the new species in our topology. Other syntopic

anuran species were *Adenomera saci*, *Boana caiapo*, *Dendropsophus anataliasiasi*, *Rhinella mirandaribeiroi*, and *Scinax fuscomarginatus*.

*Remarks on the Geographic Distribution of Pseudopaludicola jazmynmcdonaldae.*—*Pseudopaludicola jazmynmcdonaldae* was previously known only from its type locality, Caseara municipality (Andrade et al., 2019; Silva et al., 2020b). We provide new records for this species from Marianópolis (nearly 56 km due south from the type locality), Cristalândia (nearly 147 km due south from the type locality), Lagoa da Confusão (nearly 163 km due south from the type locality), and Dueré (nearly 245 km due south from the type locality) municipalities. All these new localities are in the Araguaia River basin.

#### DISCUSSION

Most species in the genus *Pseudopaludicola*, with few exceptions, are morphologically cryptic. Exceptions include species of the *P. pusilla* group (*P. boliviana*, *P. ceratophyes*, *P. llanera*, *P. motorzinho*, and *P. pusilla*; all have T-shaped terminal phalanges on toes), the *P. saltica* clade (*P. jaredi*, *P. murundu*, and *P. saltica*; tibiotarsal articulation extending beyond the tip of snout), and the robust-bodied species with flat warts on the dorsum (*P. ameghini* and *P. ternetzi*; Lynch, 1989; Lobo, 1995; Cardozo and Toledo, 2013). Outside these 3 groups, the 15 remaining species are morphologically conserved with notable

chromatic variation, which ultimately hampers the use of traditional morphological characters for diagnosis. The only characters that seem to be intraspecifically consistent is vocal sac texture (warty or smooth) and color (concentration of melanophores), indicating their value to diagnose some *Pseudopaludicola* species.

*Pseudopaludicola javae* was recovered as a sister taxon of *P. jazmynmcdonaldae* + *P. mystacalis* with a high posterior probability. Despite being morphologically similar and, most importantly, closely related, these three species were found syntopically in our study area, which is unusual for *Pseudopaludicola* species (Veiga-Menoncello et al., 2014). In fact, *P. canga*, another morphologically similar but not closely related species, was found in syntopy with the aforementioned species in different sites in Tocantins State (see section on the geographic distribution of *P. javae*). This finding may be related to the strong acoustic barrier between species, which ensures reproductive isolation while allowing coexistence (Duellman and Trueb, 1986; Gerhardt and Huber, 2002; Köhler et al., 2017). Future researchers of biogeographic studies may determine whether these species diversified allopatrically with secondary contact or if they diversified syntopically. In any case, intraspecific acoustic recognition likely played an important role in reproductive isolation of these syntopic species.

Conversely, *P. atragula* and the new species are indistinguishable based on advertisement calls but are allopatrically distributed. *Pseudopaludicola atragula* is only known from the states of São Paulo and Goiás (Andrade et al., 2016b; Vaz-Silva et al., 2020). Although Goiás State is the southern neighbor to Tocantins State, *P. atragula* is found 1,000 km south of the type locality of the new species and in a different hydrographic basin that runs southward (Paraná-Paraguay River basin), separated from the Araguaia-Tocantins (part of the Amazon River basin) by the highlands of the Central Brazilian Plateau (Almeida et al., 2000). Indeed, the genetic structure in frogs is influenced by hydrographic basins, with within-basin migrations favored and with divergent lineages or different species in different rivers (Fonseca et al., 2021). The central Brazilian Plateau, furthermore, has been shown to limit or halt gene flow among species and populations (Oliveira et al., 2018; Camurugi et al., 2021). It is likely, therefore, that reproductive isolation between these species has been ensured by their contrasting geographic distributions, releasing pressure on advertisement calls. However, it is not possible to rule out convergence as the source of similarities, and such hypotheses need a well-resolved phylogeny for the genus to be tested rigorously.

The diversity of amphibians in Tocantins was poorly known until recently (Diniz-Filho et al., 2004), but this state is now known to be highly diverse, housing several undescribed taxa and recently described species (Andrade et al., 2019; Oliveira et al., 2020; Silva et al., 2020a). Despite an increase in sampling effort throughout the state in the past few years, this study is the first to document the lineage here described as *P. javae*. Notably, researchers of a previous study implemented five field expeditions in Caseara Municipality, a neighbor municipality of Marianópolis (*P. javae* type locality), but the only recorded congeners were *P. canga* and *P. jazmynmcdonaldae* (Andrade et al., 2019; Silva, pers. obs.). In contrast, new data presented here for Marianópolis confirmed four *Pseudopaludicola* species, namely, *P. canga*, *P. jazmynmcdonaldae*, *P. javae*, and *P. aff. saltica*. In addition to these species, we also recorded *P. mystacalis* in the Lagoa da Confusão Municipality, bringing the list of *Pseudopaludicola* species found in the Araguaia-Tocantins interfluvium to

five. Finally, *P. jaredi* was also recorded on the east bank of the Tocantins River (Andrade et al., 2020b), resulting in a noteworthy list of six *Pseudopaludicola* species for the state. The ongoing discovery of new species and new records for *Pseudopaludicola* species in Tocantins State further indicates that its actual amphibian diversity remains underestimated.

Tocantins State is part of a large region known as Matopiba, one of the last agricultural frontiers of Brazil, which has received substantial federal support to expand its production (Bezerra and Gonzaga, 2019). The most direct consequence of this expansion is the loss of natural areas and soil degradation. Indeed, between 2002 and 2013, Tocantins experienced an astonishing increase of 328% in cropland area and 29% in pastureland (Sano et al., 2019; Pelicice et al., 2021). The Araguaia-Tocantins interfluvium corresponds to 1 of 10 mesoregions of Matopiba (Occidental Tocantins), where the production of soy has consistently increased, with Lagoa da Confusão, Pium, Marianópolis, and Caseara municipalities as highlights in soy expansion (Araújo et al., 2019). In fact, the current level of anthropization of the Araguaia-Tocantins River basin requires urgent attention from conservation managers to safeguard the singular biodiversity of this region (Pelicice et al., 2021). Such high rates of habitat loss become more dramatic when we consider the high levels of biodiversity underestimation of the Neotropical Region and that many of the recently described and undescribed species already face extinction risk with few public policies to protect them (Fiser et al., 2018; Liu et al., 2022). In this sense, the effort to describe this hidden biodiversity should be seen as a high priority.

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## SUPPLEMENTARY DATA

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Supplementary Sound File 1 can be found online at <http://dx.doi.org/10.1670/22-062.S1>.

Supplementary Sound File 2 can be found online at <http://dx.doi.org/10.1670/22-062.S2>.

Supplementary Sound File 3 can be found online at <http://dx.doi.org/10.1670/22-062.S3>.

Supplementary Sound File 4 can be found online at <http://dx.doi.org/10.1670/22-062.S4>.

Supplementary Sound File 5 can be found online at <http://dx.doi.org/10.1670/22-062.S5>.

## APPENDIX 1

Museum and field series acronyms of specimens and tissues used in this study are as follows: ZUEC: Museu de Diversidade Biológica (MDBio), Área Zoologia, Universidade Estadual de Campinas (UNICAMP), São Paulo, Brazil; AAG-UFG: Collection of frogs of the Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil; MNRJ: Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro, Brazil; UFMG: Amphibian Collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil; CFBH: Célio F.B. Haddad Amphibian Collection, Universidade Estadual Paulista (Unesp), Rio Claro, State of São Paulo, Brazil; ZUFMS-AMP: Zoological collection (ZUFMS) of the Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, state of Mato Grosso do Sul, both in Brazil; CHUFPB – Herpetological collection of the Universidade Federal da Paraíba (UFPB), João Pessoa, Pernambuco, Brazil; DZSJRP: Amphibia adults collection of the Universidade Estadual Paulista: Campus de São José do Rio Preto (UNESP – IBILCE), São José do Rio Preto, São Paulo, Brazil; SMRP: Shirlei Maria Recco-Pimentel Amphibian Collection, Instituto de Biologia, UNICAMP, Campinas, São Paulo, Brazil; MACN: Museo Argentino de Ciencias



Naturales "Bernardino Rivadavia", Buenos Aires, Argentina; AJC: Andrew Jackson Crawford Amphibian Collection, Universidad de los Andes, Bogotá, Colombia; and CFBH-T: Célio F.B. Haddad Amphibian Collection (tissues database), Universidade Estadual Paulista (Unesp), Rio Claro, State of São Paulo, Brazil

*Specimens Examined.*—*Pseudopaludicola ameghini*. BRAZIL—Mato Grosso: Chapada dos Guimarães (type locality), ZUEC 14138, 14139, 14141–14145.

*Pseudopaludicola atragula*. BRAZIL—São Paulo: Icém (type locality), AAG-UFU 5103–5105, 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 75304–75308; Virasol, MNRJ 75309–75312; Colonia Salvación, MNRJ 75322.

*Pseudopaludicola canga*. BRAZIL—Pará: Marabá, Serra dos Carajás (type locality), ZUEC 9990, 10034, 14370, 14372–14374, 14378. Maranhão: Barreirinhas, ZUEC 24687–24695; Santo Amaro do Maranhão, ZUEC 24682–24686. Piauí: Ribeiro Gonçalves, ZUEC 24673–24680.

*Pseudopaludicola coracoralinae*. BRAZIL—Goiás: Palmeiras de Goiás (type locality), ZUEC 24701–24712; Same collection data as for preceding, AAG-UFU 3393–3396.

*Pseudopaludicola facureae*. BRAZIL—Minas Gerais: Uberlândia (type locality), AAG-UFU 0853–0855; 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, 14002, 14003, 14005, 14006, 14008, 14009, 14016, 14017, 14022, 14162–14166, 14168; Santana do Livramento, ZUEC 10355, 10356; Viamão, ZUEC 5297–5299.

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

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

*Pseudopaludicola Jaredi*. BRAZIL—Ceará: Viçosa do Ceará (type locality), CFBH 32609, 32614, 32617–32625; Same collection data as for preceding, ZUEC 20477–20484. Rio Grande do Norte: Nísia Floresta, ZUEC 21858–21872.

*Pseudopaludicola jazmynmcdonaldae*. BRAZIL—Tocantins: Caseara (type locality); ZUFMS-AMP 11247–11258; Same collection data as for preceding; ZUEC 24457–24464.

*Pseudopaludicola javae* sp. nov. BRAZIL—Tocantins: Marianópolis (type locality), CHUFPPB31033, 31042, 31044 (holotype)—31050, 31053, 31056–31060, 31062, 31063, 31065, 31067, and 31070; Lagoa da Confusão, CHUFPPB31034, 31043, 31054, 31055, 31064, and 31069.

*Pseudopaludicola matuta*. BRAZIL—Minas Gerais: Curvelo (type locality), ZUEC 24302–24306, 24308–24310, 24313, 24315–24318.

*Pseudopaludicola mineira*. BRAZIL—Minas Gerais: Jaboticatubas (Serra do Cipó, type locality), ZUEC 1570, 1572–1589, 1591.

*Pseudopaludicola murundu*. BRAZIL—São Paulo: Águas de Santa Bárbara ZUEC 20507, 20508; Rio Claro (type locality), AAG-UFU 5125, 5126, CFBH 8235–8242; Same collection data as for preceding, ZUEC 14284–14290. Minas Gerais: Brumadinho, ZUEC 16396–16398, 16442, 16443, 19549, 19551, 19555, 19557–19578, 19560; Santana do Riacho, ZUEC 2323; São João del-Rei, ZUEC 16447–16452, 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 pocoto*. BRAZIL—Ceará: Novas Russas, CFBH 20285–20287; Santa Quitéria (type locality), CFBH 26842–26847. Bahia: Rio de Contas, ZUEC 25506–25510; Same collection data as previous, UFMG 5902–5911.

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

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

#### APPENDIX 2. Sound files of *Pseudopaludicola javae* sp. nov. from Tocantins State.

FNJV label	Voucher	Locality	Municipality	Date	Hour	Temperature (°C)
FNJV0051321	CHUFPPB31069	Região de Lago Verde	Lagoa da Confusão	19 Mar 2021	1659	25.0–28.0
FNJV0051322	CHUFPPB31043	Região de Lago Verde	Lagoa da Confusão	19 Mar 2021	1705	25.0–28.0
FNJV0051323	CHUFPPB31056	Fazenda Escondida	Marianópolis	28 Feb 2021	1915	25.0–28.0
FNJV0051324	CHUFPPB31060	Fazenda Escondida	Marianópolis	01 Mar 2021	1705	25.0–28.0
FNJV0051325	unvouchered	Fazenda Escondida	Marianópolis	01 Mar 2021	1715	25.0–28.0
FNJV0051326	unvouchered	Fazenda Escondida	Marianópolis	01 Mar 2021	1754	25.0–28.0
FNJV0051327	CHUFPPB31065	Fazenda Escondida	Marianópolis	01 Mar 2021	1800	25.0–28.0
FNJV0051328	unvouchered	Fazenda Escondida	Marianópolis	01 Mar 2021	1807	25.0–28.0
FNJV0051329	Unvouchered	Fazenda Escondida	Marianópolis	01 Mar 2021	1819	25.0–28.0
FNJV0051330	CHUFPPB31047	Fazenda Escondida	Marianópolis	01 Mar 2021	1825	25.0–28.0
FNJV0051331	CHUFPPB31048	Fazenda Escondida	Marianópolis	01 Mar 2021	1832	25.0–28.0
FNJV0051332	Unvouchered	Fazenda Escondida	Marianópolis	01 Mar 2021	1837	25.0–28.0
FNJV0051333	Unvouchered	Fazenda Escondida	Marianópolis	02 Mar 2021	1800	29.6
FNJV0051334	Unvouchered	Fazenda Escondida	Marianópolis	02 Mar 2021	1815	29.3
FNJV0051335	CHUFPPB31063	Fazenda Escondida	Marianópolis	02 Mar 2021	1821	28.8
FNJV0051336	Unvouchered	Fazenda Escondida	Marianópolis	02 Mar 2021	1835	27.6
FNJV0051337	Unvouchered	Fazenda Escondida	Marianópolis	02 Mar 2021	1842	27.3
FNJV0051338	CHUFPPB31044 (holotype)	Fazenda Escondida	Marianópolis	02 Mar 2021	1846	27.3
FNJV0051339	CHUFPPB31062	Fazenda Escondida	Marianópolis	02 Mar 2021	1903	26.5
FNJV0051340	CHUFPPB31058	Fazenda Escondida	Marianópolis	02 Mar 2021	1914	26.5



APPENDIX 3. GenBank accession numbers, voucher information, and sample locality of sequences used for phylogenetic inferences.

Species	Voucher/tissue	Locality	GenBank accession number
<i>P. ameghini</i>	ZUEC 14140	Chapada dos Guimarães, MT, type locality	KJ146975
<i>P. ameghini</i>	UFMT 8543	Chapada dos Guimarães, MT, type locality	KJ146976
<i>P. atragula</i>	DZSJRP 8727	Icém, SP, type locality	KJ146996
<i>P. atragula</i>	DZSJRP 8728	Icém, SP, type locality	KJ146997
<i>P. boliviana</i>	MLP-DB 5614	Chaco, Argentina	KJ147050
<i>P. boliviana</i>	MLP-DB 5186	Corrientes, Argentina	KJ147049
<i>P. canga</i>	ZUEC 24676	Ribeiro Gonçalves, PI, type locality	MT385250
<i>P. canga</i>	ZUEC 24677	Ribeiro Gonçalves, PI, type locality	MT385251
<i>P. canga</i>	ZUEC 24678	Ribeiro Gonçalves, PI, type locality	MT385252
<i>P. canga</i>	AAG-UFU 6266	Aragominas, TO	MT385236
<i>P. canga</i>	AAG-UFU 6267	Aragominas, TO	MT385237
<i>P. canga</i>	ZUEC 13858	Barreirinhas, MA	KJ147013
<i>P. canga</i>	ZUEC 13859	Barreirinhas, MA	KJ147014
<i>P. canga</i>	ZUEC 13860	Barreirinhas, MA	KJ147015
<i>P. canga</i>	ZUEC 24687	Barreirinhas, MA	MT385255
<i>P. canga</i>	ZUEC 24688	Barreirinhas, MA	MT385256
<i>P. canga</i>	AAG-UFU 2527	Mateiros, GO	MT385240
<i>P. canga</i>	AAG-UFU 2745	Palmas, TO	MT385238
<i>P. canga</i>	AAG-UFU 2746	Palmas, TO	MT385239
<i>P. canga</i>	ZUEC 24682	Santo Amaro do Maranhão, MA	MT385253
<i>P. canga</i>	ZUEC 24683	Santo Amaro do Maranhão, MA	MT385254
<i>P. canga</i>	ZUEC 14333	Serra dos Carajás, PA, type locality	KJ146988
<i>P. canga</i>	ZUEC 14334	Serra dos Carajás, PA, type locality	KJ146989
<i>P. canga</i>	ZUEC 14335	Serra dos Carajás, PA, type locality	KJ146990
<i>P. coracoralinae</i>	AAG-UFU 3393	Palmeiras de Goiás, GO, type locality	MT385241
<i>P. coracoralinae</i>	AAG-UFU 3394	Palmeiras de Goiás, GO, type locality	MT385242
<i>P. coracoralinae</i>	ZUEC 24702	Palmeiras de Goiás, GO, type locality	MT385243
<i>P. coracoralinae</i>	ZUEC 24703	Palmeiras de Goiás, GO, type locality	MT385244
<i>P. coracoralinae</i> (holotype)	ZUEC 24704	Palmeiras de Goiás, GO, type locality	MT385245
<i>P. facureae</i>	ZUEC 14173	Uberlândia, MG, type locality	KJ146978
<i>P. facureae</i>	ZUEC 14174	Uberlândia, MG, type locality	KJ146979
<i>P. facureae</i>	ZUEC 14175	Uberlândia, MG, type locality	KJ146980
<i>P. falcipes</i>	MACN 38647	Yapeyu, Corrientes, Argentina	AY843741
<i>P. falcipes</i>	ZUEC 14162	Porto Alegre, RS	KJ146972
<i>P. florencei</i>	ZUEC 14190	Andaraí, BA, type locality	KJ147017
<i>P. florencei</i>	ZUEC 14189	Andaraí, BA, type locality	KJ147018
<i>P. giarettai</i>	ZUEC 24319	Curvelo, MG, type locality	MH553442
<i>P. giarettai</i>	ZUEC 24320	Curvelo, MG, type locality	MH553443
<i>P. giarettai</i>	AAG-UFU 1920	Grande Sertão Veredas National Park, MG	MH553445
<i>P. giarettai</i>	AAG-UFU 1921	Grande Sertão Veredas National Park, MG	MH553444
<i>P. jaredi</i>	SMRP 310.18	Viçosa do Ceará, CE, type locality	KJ147033
<i>P. jaredi</i>	SMRP 310.19	Viçosa do Ceará, CE, type locality	KJ147034
<i>P. jazmynmcdonaldae</i>	ZUFMS 11249	Caseara, TO, type locality	MT385246
<i>P. jazmynmcdonaldae</i>	ZUFMS 11252	Caseara, TO, type locality	MT385247
<i>P. jazmynmcdonaldae</i>	ZUFMS 11256	Caseara, TO, type locality	MT385248
<i>P. jazmynmcdonaldae</i>	ZUFMS 11257	Caseara, TO, type locality	MT385249
<i>P. javae</i>	CHUFPB31047	Marianópolis, TO, type locality	To be submitted
<i>P. javae</i> (holotype)	CHUFPB31044	Marianópolis, TO, type locality	To be submitted
<i>P. javae</i>	CHUFPB31042	Marianópolis, TO, type locality	To be submitted
<i>P. javae</i>	CHUFPB31046	Marianópolis, TO, type locality	To be submitted
<i>P. javae</i>	CHUFPB31064	Lagoa da Confusão, TO	To be submitted
<i>P. llanera</i>	AJC 4115	Sabanalarga, Casanare, Colombia	KP149482
<i>P. llanera</i>	AJC 4127	Sabanalarga, Casanare, Colombia	KP149483
<i>P. matuta</i>	ZUEC 24303	Curvelo, MG, type locality	MH553454
<i>P. matuta</i>	ZUEC 24304	Curvelo, MG, type locality	MH553456
<i>P. mineira</i>	DZSJRP 6437	Serra do Cipó, MG, type locality	KJ147025
<i>P. mineira</i>	ZUEC 14318	Serra do Cipó, MG, type locality	KJ147026
<i>P. motorzinho</i>	ZUEC 13928	Poconé, MT	KJ146992
<i>P. motorzinho</i>	ZUEC 13931	Poconé, MT	KJ147039
<i>P. murundu</i>	CFBH-T 1467	Rio Claro, SP, type locality	KJ147008
<i>P. murundu</i>	ZUEC 14288	Rio Claro, SP, type locality	KJ147030
<i>P. mystacalis</i>	CFBH-T 03509	Babaçulândia, TO	KU495493
<i>P. mystacalis</i>	ZUEC 13836	Barreirinhas, MA	KJ147005
<i>P. mystacalis</i>	ZUEC 14147	Cuiabá, MT	KJ146983
<i>P. mystacalis</i>	DZSJRP 8724	Icém, SP	KJ147028
<i>P. mystacalis</i>	CFBH-T 1374	Paranaíba, MS	KJ147009
<i>P. mystacalis</i>	ZUEC 14160	Poconé, MT	KJ146991
<i>P. mystacalis</i>	DZSJRP 8704	Santa Fé do Sul, SP	KJ147022
<i>P. mystacalis</i>	ZUEC 14128	Uberlândia, MG	KJ146999
<i>P. mystacalis</i>	CFBH 35858	Urbano Santos, MA	KJ146982
<i>P. pocoto</i>	SMRP 440.1	Novas Russas, CE	KJ147035
<i>P. pocoto</i>	SMRP 440.2	Novas Russas, CE	KJ147036

APPENDIX 3. GenBank accession numbers, voucher information, and sample locality of sequences used for phylogenetic inferences.

Species	Voucher/tissue	Locality	GenBank accession number
<i>P. pusilla</i>	AJC 4039	San Vicente, Santander, Colombia	KP149486
<i>P. pusilla</i>	AJC 4037	San Vicente, Santander, Colombia	KP149344
<i>P. restinga</i>	ZUEC 24583	Serra, ES, type locality	MT385257
<i>P. restinga</i>	ZUEC 24584	Serra, ES, type locality	MT385258
<i>P. saltica</i>	ZUEC 14239	Chapada dos Guimarães, MT, type locality	KJ147002
<i>P. saltica</i>	ZUEC 14240	Chapada dos Guimarães, MT, type locality	KJ147003
<i>P. ternetzi</i>	UFMT 15753	Uruaçu, GO, type locality	KJ147054
<i>P. ternetzi</i>	UFMT 15754	Uruaçu, GO, type locality	KJ147055
<i>Leptodactylus pentadactylus</i>	outgroup	Costa Rica, Limon	AY326017
<i>Physalaemus cuvieri</i>	outgroup	Argentina, Misiones	AY843729
<i>Pleurodema brachyops</i>	outgroup	Guyana, Southern Rupununi Savannah	AY843733