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Inconspicuous, but not forgotten: another new Amazonian hylid frog in the *Dendropsophus microcephalus* species group from northern Bolivia

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Abstract. Recent molecular phylogenetic studies have identified numerous divergent lineages within the hylid genus *Dendropsopus*, especially within its most speciose *D. microcephalus* species group, many of which likely deserve species status. Herein, based on molecular genetics, morphology and bioacoustics, we provide evidence for the presence of another species-level lineage in this group of frogs collected in the Departamento Pando, northern Bolivia, and describe it as a new species, *Dendropsophus jamesi* sp. n. Phylogenetic analyses place the new species as the sister taxon to *D. riveroi*, with *D. shiwiarum*, *D. reichlei*, *D. coffea*, *D. walfordi* and *D. nanus* being other closely related species. The new species is distinguished from other species in the group by substantial differentiation in the 16S rRNA gene (3.3–10.5% uncorrected p-distance) and the combination of following characters: small adult male size (snout–vent lengths 17.6–18.4 mm), snout acuminate in dorsal view and rounded in lateral view, supratympanic fold distinct, rounded discs on Finger III and Toe IV, presence of a cream to white subocular spot, bronze-brown iris in life, whitish canthal line sharply outlining loreal and dorsal head coloration, and an advertisement call consisting of a short pulsed note (22–44 ms duration), each containing 7–12 pulses, and repeated in series at inter-call intervals of 185–270 ms. We discuss our findings in view of the challenges involved in completing the inventory of small Amazonian *Dendropsophus* and emphasize the importance of including data from topotypes to achieve taxonomic progress.

Key words. Amphibia, Anura, Hylidae, *Dendropsophus jamesi* sp. n., *Dendropsophus riveroi*, bioacoustics, molecular genetics, morphology, taxonomy.

Introduction

Dendropsophus Fitzinger, 1843 is a species-rich genus of Central and South American hylid frogs currently containing 105 valid species (Frost 2025). Recently, nine species groups have been defined or re-defined within the genus based on molecular phylogeny and a comprehensive set of phenomic data by Orrico et al. (2021), with the *D. microcephalus* species group being the most speciose one. Frogs of the *D. microcephalus* group are small in body size and most of them have brown to yellow dorsal coloration, with exception of the *D. rubicundulus* clade having green dorsi (Orrico et al. 2021). Their smallness, combined with both similar external morphology and intra-specific variation, makes them a rather challenging group for taxonomists, especially because advertisement calls among species in this group are known to be very similar (see e.g., Köhler et al. 2005, Moravec et al. 2008, Ortega-Andrade & Ron 2013, Seger et al. 2021). Molecular phylogenetic studies have identified numerous unnamed divergent lineages within the *D. microcephalus* group, many of them probably deserving species rank and thus indicating that the actual species numbers are likely distinctly higher than reflected by current alpha taxonomy in this group of frogs (e.g., Jansen et al. 2019, Ferrão et al. 2020, Orrico et al. 2021, Seger et al. 2021). However, allocating existing species names to identified molecular lineages is far from trivial and mostly possible only by comparison of newly collected vouchers to topotypic material of respective nominal taxa (e.g., Teixeira & Giaretta 2015, Seger et al. 2021).

Challenged with these difficulties and the increasing number of recognized species, the description and naming of new species in the *D. microcephalus* group requires an integrative approach, preferably including morphological data from name-bearing types, sequence data from topotypes and call recordings of genotyped voucher specimens.

Given the vast dimensions of the Amazonian region, the difficulties in accessing some type localities, and other various problems associated with searching for a certain species in the field and recording its advertisement calls, collecting all the necessary comparative data for a taxonomic treatment can be a long journey.

This was exactly the case with a small, inconspicuous species of *Dendropsophus* observed during fieldwork in 2007 near a small forest settlement named Palmira on the left bank of the Rio Beni in northeastern Bolivia. Morphological and bioacoustic data obtained at that time already suggested that this population might represent a new undescribed species (see Moravec et al. 2008), but it took another eighteen years to obtain all the data needed to provide evidence for the specific distinctness from already known species, justifying its formal description.

In this study, we provide independent lines of evidence for the species-level divergence of this small *Dendropsophus* from Palmira, northeastern Bolivia, by integrating results from molecular genetics, morphological comparisons and analyses of advertisement calls, and consequently describe and name it as a new species.

Material and methods

Fieldwork and voucher specimens

Fieldwork was conducted during rainy seasons. Specimens were observed and collected during opportunistic searches at night using torches and headlamps. The collected specimens were euthanized using chlorobutanol solution, fixed in 90% ethanol, and stored in 70% ethanol. Tissue samples for DNA analyses were taken prior to fixation and preserved in 99.8% ethanol. Collected specimens were deposited in the following collections: National Museum Prague, vertebrate collection (NMP-P6V); Hessisches Landesmuseum Darmstadt (HLMD), and Zoologisches Forschungsmuseum A. Koenig, Bonn (ZFMK). Other museum abbreviations used follow those in Frost (2025). Specimens examined for this study are listed in the Appendix.

Morphology

Morphological measurements are given in millimeters (mm) and were taken to the nearest 0.1 mm under a dissecting microscope using digital calipers. Notes on colour in life were taken from field notes and digital colour images. Measurement abbreviations used throughout the text are: EN, eye to nostril distance; ED, horizontal eye diameter; ELW, upper eyelid width; FL, foot length as the distance from the heel to the tip of the fourth toe; HL, head length as the straight line distance from the posterior edge of the jaw articulation to the tip of the snout; HW, greatest head width at midlevel of eyes; IOD, interorbital distance; SVL, snout–vent length; TD, horizontal tympanum diameter; and TL, tibia length. The format for the description is that of Moravec et al. (2008). Webbing formulae follow the standards of Myers & Duellman (1982), whereas all other terminology is that of Duellman (1970).

Molecular genetics

Our molecular genetic analysis primarily aimed at the identification of lineage divergence among focal samples of *Dendropsophus* using the mitochondrial 16S rRNA (16S) gene. For representative taxon sampling, we used BLAST searches (Altschul et al. 1990) of newly generated 16S sequences of the focal samples against the GenBank nucleotide archive and downloaded a representative selection of sequences of identified nominal species in the *Dendropsophus microcephalus* species group (sensu Orrico et al. 2021). A sequence of *Dendropsophus manonegra*, a member of the *D. leucophyllatus* species group, was added as outgroup taxon. Newly obtained sequences were submitted to GenBank (accession numbers: PV744317–PV744334). A table with all the samples used in the analyses was uploaded to the Zenodo repository and is accessible under https://doi.org/10.5281/zenodo.15096523.

Genomic DNA was extracted from tissue samples using Qiagen DNeasy Blood and Tissue Kit. We targeted a fragment of the mitochondrial 16S rRNA gene with primers and PCR conditions adapted from previous studies (Palumbi et al. 1991, Moravec et al. 2009). The Sanger sequencing was performed by Macrogen, Inc. (Amsterdam, the Netherlands). All chromatograms of newly generated sequences were visually checked using Geneious Prime v. 2023.0 (https://www.geneious.com).

Sequences were aligned using the Muscle algorithm as implemented in MEGA7 (Kumar et al. 2016). The alignment was then trimmed to a total length of 556 bp, to minimize missing data but keep the maximum of sequences in the data set. Based on the Bayesian Information Criterion as implemented in MEGA7 we chose a GTR+G substitution model as best fitting the data set. This substitution model was then used to infer a Maximum Likelihood (ML) tree in RAxML v8 (Stamatakis 2014) as implemented in raxmlGUI v2.0 (Edler et al. 2021), assessing node support with 500 non-parametric ML bootstrap replicates. Uncorrected pairwise distances among the sequences (p-distances) were calculated in MEGA7.

Bioacoustics

Vocalizations were recorded in the field using a Marantz PMD660 digital recorder with a Stage Line ECM-950 directional microphone (recordings obtained at 44.1 KHz and 16-bit resolution, saved as uncompressed wave format on SD card), and Aiwa HS-F150 and Sony WM-D6C tape recorders with the directional microphone Sennheiser Me-80 attached. Recordings were sampled or re-sampled at 22.05 kHz and 32-bit resolution and analyzed using the software Cool Edit Pro 2.0. Temporal parameters were measured in the oscillograms. Frequency information was obtained

through Fast Fourier Transformation (FFT; width 1024 points) at Hanning window function. Spectrograms were produced at Blackman window function with 256 bands resolution. For a clearer graphical presentation, in most cases, careful band-pass filtering was applied to remove background sounds, applied only to frequencies outside the prevalent bandwidths of calls. Temporal measurements are summarized as range with mean ± standard deviation in parentheses. Description, terminology, and methods follow those recommended by Köhler et al. (2017), using the call-centered terminological scheme. Unless otherwise noted, all call parameters mentioned herein were measured using the same methodology, even if call descriptions derived from the same recordings were previously published. Statistical analyses were carried out in Statistica (Statsoft Inc), version 7.1. Representative sections of formerly unpublished call recordings were archived in the Zenodo repository under https://doi.org/10.5281/zenodo.15096523.

Nomenclatural act

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural act it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub: 23041777-C598-425E-96D1-A3479BD9D231. The electronic edition of this work was published in a journal with an ISSN, has been archived and is available from the following digital repositories: zenodo.org, salamandrajournal.com.

Results

Molecular genetics

Our Maximum Likelihood tree (Fig. 1) recovered a topology of the *Dendropsophus microcephalus* species group which is largely in agreement with those of Orrico et al. (2021) and Whitcher et al. (2025) in generally revealing similar species content of major clades. However, several basal nodes received no or only low support and the phylogenetic position of some taxa (e.g., *D. gaucheri*) is ambiguous. All samples of the focal population from Palmira form one cluster with high bootstrap support (91%) and show very low within-lineage differentiation (0.0–0.2% uncorrected p-distance). They are thus regarded conspecific. The Palmira samples are sister to an unidentified sample from Buenavista, Departamento Santa Cruz, Bolivia (bootstrap value 96%), and these two together are sister to a clade containing samples of *D. riveroi* and a sample from northern Bolivia we here refer to as *D.* cf. *riveroi* (bootstrap value 78%). All these mentioned taxa together are sister to a clade containing *D. shiwiarum*, *D. reichlei*, *D. coffea*, *D. walfordi* and *D. nanus*, but this relationship received no support. The *walfordi-nanus* subclade contains a sequence (GenBank accession number MT992110) labelled *D. elianeae*, which we consider to represent a misidentification, as other analyses (Jansen et al. 2019, Orrico et al. 2021, Nakamura et al. 2025, Whitcher et al. 2025) revealed *D. elianeae* as a member of the *D. rubicundulus* clade and only distantly related to *D. walfordi* and *D. nanus*.

Our focal lineage from Palmira, Bolivia, had uncorrected pairwise distances in the studied 16S gene fragment of 3.3–3.6% to its sister lineage *D. riveroi*, 6.0–7.5% to *D. shiwiarum*, 5.8–6.5% to *D. reichlei*, 5.6–5.7% to *D. coffea*, 5.9–6.1% to *D. walfordi*, 6.2–6.5% to *D. nanus* and 5.7–10.5% to all other samples of nominal species in the *D. microcephalus* group included in our analysis. Uncorrected p-distance of our Palmira samples to the most closely related sample from Buenavista, Departamento Santa Cruz, Bolivia, is 1.3–1.5%. This low value may argue for conspecifity of the Palmira and Buenavista populations, but given the geographical distance of ca. 850 km between both localities and the fact that specimens from Buenavista seem to slightly differ in color pattern (M. Jansen pers. comm.), we highlight the Buenavista population as in need of investigation to confirm or reject conspecifity. Uncorrected p-distance of *D. riveroi* to *D.* cf. *riveroi* from northern Bolivia (Bolpebra) ranges from 2.5–2.7%. Remarkably, our analysis revealed pronounced genetic distances within several nominal species, namely 4.5% in *D. shiwiarum*, 4.6% in *D. reichlei*, and 5.3% in *D. rhodopeplus*.

Bioacoustics

Advertisement calls known for species in the *Dendropsophus microcephalus* group are generally rather similar in character, namely consisting of short pulsed or pulsatile notes repeated singly, in groups, or short to long series (e.g., Moravec et al. 2006, 2008, Ortega-Andrade & Ron 2013, Ferrão et al. 2020, Seger et al. 2021). Differences between calls of different species are mainly quantitative rather than qualitative (sensu Köhler et al. 2017).

Analysis and comparisons of the advertisement calls of species related to the focal lineage from Palmira, Departamento Pando, Bolivia, revealed only subtle differences. For example, in pulsed calls from that clade, pulse rate within calls among all analysed species is in the same general range, as is call duration for several taxa studied (see Table 1, Fig. 2). Calls of the focal lineage are most similar to calls of its sister lineage, *D. riveroi* (Fig. 3). Calls of *D. riveroi* from 36 km southeast of Pucallpa, Departamento Ucayali, Peru (species identification of this population verified by genetic data; see Fig. 1) are very similar in call duration, inter-call intervals, and number of pulses per call (Table 1). Dominant frequency is slightly higher in *D. riveroi* when compared to calls from Palmira, Bolivia (5717–5845 versus 5072–5555 Hz). Mann-Whitney U tests revealed statistically significant differences between the calls of *D. riveroi* and the focal lineage in call duration (Z = -4.20;

Figure 1. Maximum Likelihood phylogenetic tree of selected samples of *the Dendropsophus microcephalus* species group, inferred from an alignment of the mitochondrial 16S rRNA gene. Numbers at nodes are rounded bootstrap values in percent (500 non-parametric bootstrap replicates; not shown if < 40%) as calculated with RaxML. The taxon name is followed by GenBank accession number, or voucher number for newly produced sequences (terminals in bold font), and sample locality. A sample of *Dendropsophus manonegra* was used to root the tree (not shown for better graphical presentation). Inset photos depict a paratopotype of *D. jamesi* sp. n. and *D. riveroi* from near its type locality Leticia, Colombia, in life.

Table 1. Numerical parameters of advertisement calls of selected species and populations in the *Dendropsophus microcephalus* group. Inter-call intervals were measured in highly motivated call series only; pulse rate was calculated within calls. * = poor quality recording (values with some caveat); n.a. = not applicable.

	number of calls/ males analyzed	call duration (= note duration) [ms]	inter-call interval [ms]	pulses/call	pulses/s	dominant frequency [Hz]
D. jamesi sp. n. Palmira, Bolivia	78/5	22–42 (30.3±6.2)	185–262 (205.4±24.3)	7–12 (9.6±1.3)	333–417 (358.6±33.3)	5072–5555 (5344±180)
D. jamesi sp. n. Cobija, Bolivia	39/2	28–44 (33.6±3.8)	205–270 (233.6±21.2)	9–12 (10.2±1.0)	333–375 (346.4±17.2)	5265–5437 (5368±51)
D. riveroi SE Pucallpa, Peru	33/2	32–43 (37.7±3.4)	165–202 (183.9±12.1)	8–11 (9.5±1.0)	250–333 (277.8±34.4)	5717–5845 (5758±34)
D. cf. *riveroi* Bolpebra, Bolivia*	12/1	39–47 (43.3±3.4)	223–561 (366.0±141.3)	10–12 (11.0±0.8)	ca. 250–360	6750–7115 (6853±128)
D. shiwiarum Yasuní, Ecuador	10/1	18–26 (22.6±2.4)	2069–3091 (2651.0±376.7)	6–8 (7.2±0.6)	292–333 (312.3±17.1)	4448–5242 (4983±358)
D. reichlei Limón, Bolivia	6/1	68–112 (92.6±13.1)	152–163 (157.5±7.8)	24–30 (27.0±2.6)	312–375 (337.4±23.7)	6212–6634 (6363±114)
D. coffea Sapecho, Bolivia	42/2	38–63 (51.0±8.1)	84–98 (90.9±5.2)	n.a. (pulsatile)	n.a. (pulsatile)	5737–5989 (5858±94)
D. cf. *nanus* EB Beni, Bolivia	9/1	9–15 (13.1±2.0)	241–324 (275.9±29.6)	n.a. (pulsatile)	n.a. (pulsatile)	4641–4920 (4791±103)

P < 0.0001), interval (Z = 4.55; P < 0.0001), pulses per second (Z = 4.16; P < 0.0001), and dominant frequency (Z = -4.44; P < 0.0001). As elaborated in the Discussion, due to the limited data available we cannot exclude that this pattern in part reflects individual differences or influences of temperature and body size. However, of perhaps higher importance taxonomically are the distinct differences in amplitude modulation within calls. In calls from Palmira, all pulses are equal in duration (2–3 ms) and clearly separated, whereas in calls of *D. riveroi* the terminal pulse

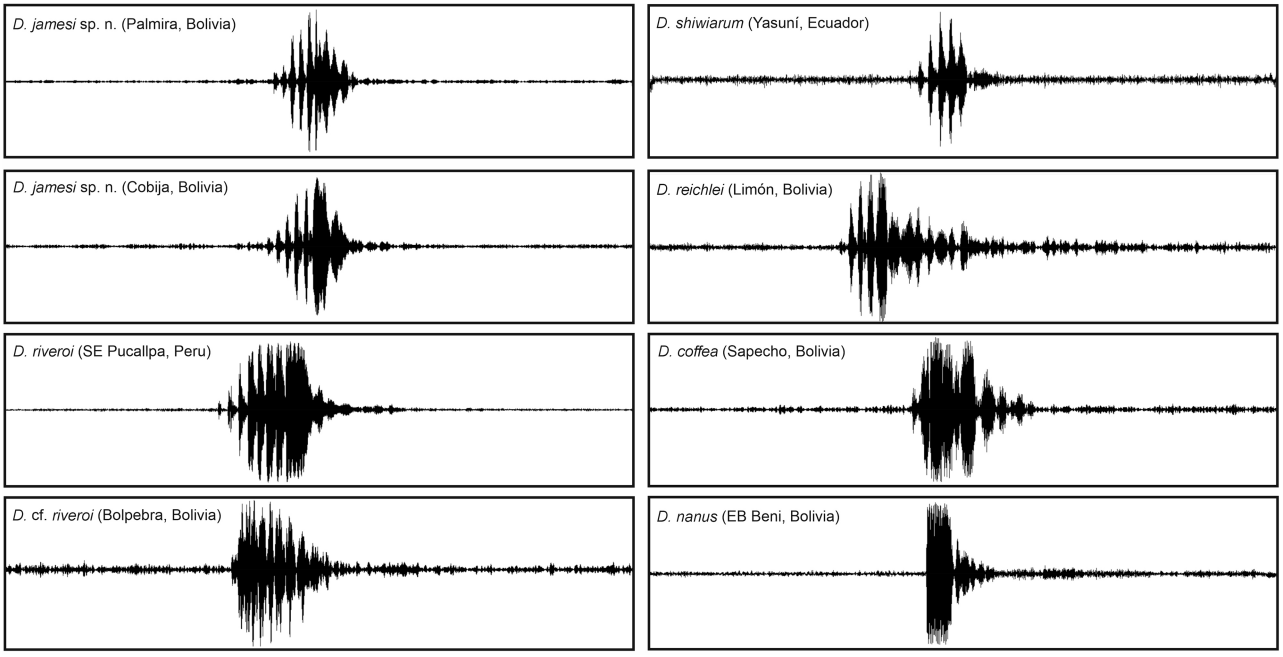

Figure 2. Comparative oscillograms of single advertisement calls of species in the *Dendropsophus microcephalus* group, all at 200 ms time scale (x-axis; y-axis refers to relative amplitude).

of each call is distinctly longer (7–12 ms) than preceding pulses (2–3 ms) (Figs 2, 3). This inconsistent pulse duration within calls of *D. riveroi* is unlikely to represent an artifact, as recordings are of good quality and the motivation of the calling males recorded was high. Despite the similarities in numerical parameters, to the human ear, both calls sound clearly different, with those of the focal lineage sounding 'smooth' and 'even', and the *D. riveroi* call sounding 'harsh' with more 'attack'. We assume that these differences in sound are mainly due to the differences in pulse structure of the calls, which would constitute a qualitative difference sensu Köhler et al. (2017).

From advertisement calls of other related lineages, calls of the focal lineage differ as follows: from *D. shiwiarum* from Yasuní National Park, Ecuador, by distinctly shorter inter-call intervals (185–262 versus 2069–3091 ms); from topotypic *D. reichlei* by shorter call duration (22–44 versus 68–112 ms) and lower number of pulses per call (7–12 versus 24–30); from topotypic *D. coffea* by longer inter-call intervals (185–270 versus 84–98 ms) and calls being distinctly pulsed (versus pulsatile); and from *D.* cf. *nanus* from Estación Biologica del Beni, Bolivia, by longer call duration (22–44 versus 9–15 ms) and calls being distinctly pulsed (versus pulsatile) (but see Seger et al. 2021). Calls of the focal lineage furthermore differ from calls of a population at Bolpebra, Departamento Pando, Bolivia, here referred to as *D.* cf. *riveroi*, by distinctly lower dominant frequency (5072–5555 versus 6750–7115 Hz), despite similar body sizes of calling males (see Table 1).

Morphology

Comparison of the external morphology of the focal specimens with that of closely related species, including respective type specimens, revealed partly subtle but constant differences in colour pattern, snout shape and certain other characters (see Diagnosis below) concordant with the phylogenetic lineages identified. In summary, results from molecular genetics, bioacoustics and morphology are congruent in supporting evolutionary lineage divergence of focal samples, and consequently we describe this lineage as a new species.

Taxonomy

***Dendropsophus jamesi* sp. n.**

ZooBank LSID: urn:lsid:zoobank.org:act:
8358B127-C3D5-4AF8-A300-7DF5282D7988

Remarks: This species has previously been referred to as *Hyla riveroi* by Köhler & Lötters (1999), De la Riva et al. (2000; partim), Márquez et al. (2002; partim), and *Dendropsophus* sp. by Moravec et al. (2008, 2011).

Holotype: NMP-P6V 73618/1, adult male (Figs 4a–c, 5), from the vicinity of the settlement of Palmira, 10°35' S, 65°44' W, ca. 140 m a.s.l., Provincia Federico Roman, Departamento Pando, Bolivia, collected on 18 November 2007 by J. Moravec, M. Guerrero-Reinhard and G. Calderon.

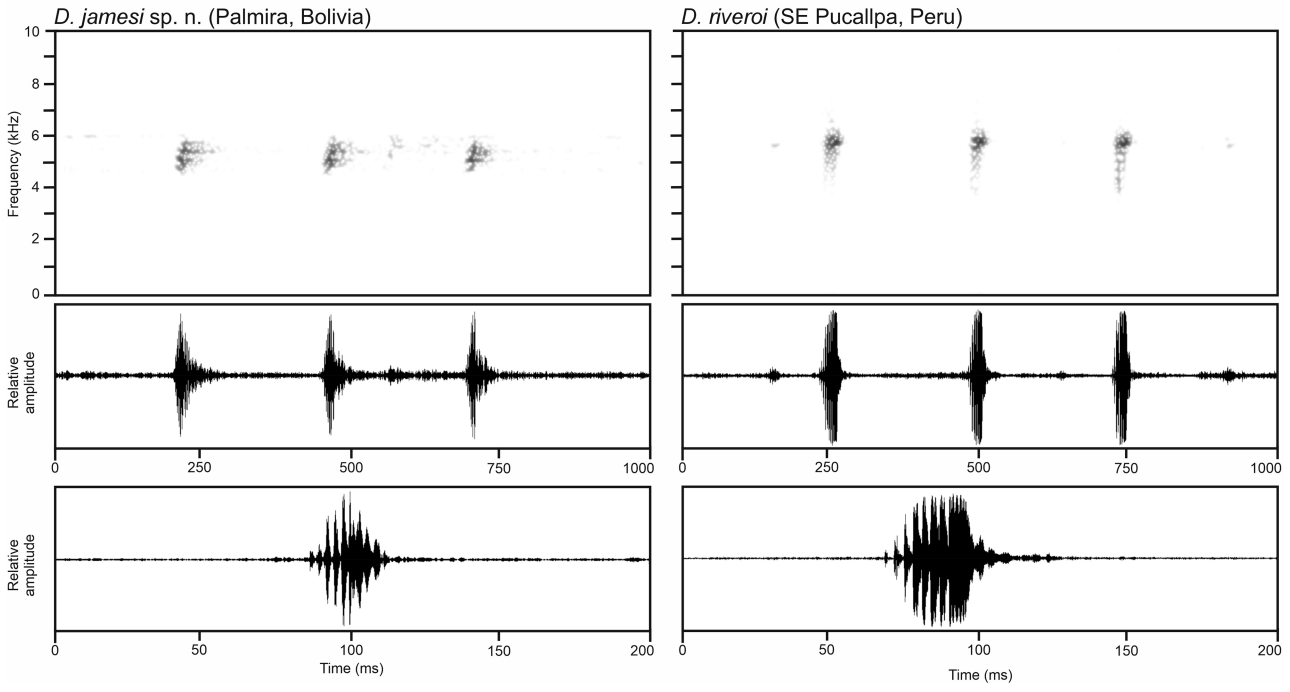

Figure 3. Audiospectrograms and corresponding oscillograms of three calls emitted in motivated call series (top) of *Dendropsophus jamesi* sp. n. from the type locality Palmira, Departamento Pando, Bolivia, and *D. riveroi* from 36 km southeast of Pucallpa, Departamento Ucayali, Peru. Oscillograms at the bottom each show a single call at 200 ms time scale.

Paratopotypes: NMP-P6V 73618/2–4, NMP-P6V 77078–77079, HLMD-RA 3240–3241, seven adult males, same locality and collecting data as the holotype.

Referred specimens: ZFMK 67145–67148, four adult males, from the vicinity of Cobija, ca. 11°02' S, 68°46' W, 220 m a.s.l., Provincia Nicolás Suárez, Departamento Pando, Bolivia, collected on 13 January 1998 by J. Köhler and S. Lötters. Specimens not genotyped; species identity derived from morphology and bioacoustics.

Definition: A species in the *Dendropsophus microcephalus* species group, as derived from molecular phylogenetic relationships and morphological similarities, distinguished from the other members of the group by the following combination of characters: (1) small size, SVL 17.6–18.4 mm (N = 8) in males (females unknown), head slightly narrower than body; (2) snout short, acuminate in dorsal view, rounded in lateral view; (3) canthus rostralis moderately expressed, slightly rounded in cross-section; loreal region slightly concave; (4) tympanum evident, round, about one third of eye

Figure 4. Adult males of *Dendropsophus jamesi* sp. n. in live: (a–c) holotype NMP-P6V 73618/1 in dorsolateral and ventral views; (d) paratopotype NMP-P6V 73618/2 in dorsolateral view; and (e) male specimen (one from the series ZFMK 67145–67148) from the vicinity of Cobija, Departamento Pando, Bolivia, in dorsolateral view (mirrored). Not to scale.

length, tympanic annulus indistinct; supratympanic fold distinct; (5) vomerine odontophores small, prominent, separated medially, in the middle between choanae; (6) skin on dorsal surfaces finely shagreen, with scattered low minute tubercles; upper eyelid lacking enlarged tubercles; (7) tarsal fold and tubercles on outer edge of tarsus absent; ulnar folds and tubercles absent; (8) axillary membrane extensively developed; (9) fingers about one third webbed; toes about three fourth webbed; (10) distal subarticular tubercle under fourth finger slightly bifid; (11) pectoral glands lacking; (12) generally darker coloration of the loreal-tympanic region contrasting to lighter dorsal head coloration, loreal region sharply outlined from the dorsal head coloration by a narrow whitish canthal line, one white to cream spot below the eye, another smaller white to cream spot bellow the nostril in some individuals; (13) in life, ground coloration of dorsum light brown to cinnamon brown by day, dorsal pattern consists of more or less distinct dark brown flecks and markings forming a narrow dark brown interorbital bar, X-shaped mark in scapular region, irregular, usually inverted V-shaped mark in sacral region, and dark brown bars on extremities; head dark brown laterally; flanks translucent pink ventrally and posteriorly, without chromatophores; hidden surfaces of thighs yellowish brown; (14) in life, throat yellow in males; belly white in pectoral and central part, translucent fleshy pink in posterior and lateral parts; ventral surfaces of thighs translucent fleshy pink; (15) in life, iris bronze-brown; bones white; (16) advertisement call consisting of a short pulsed note (22–44 ms duration), each containing 7–12 pulses, repeated in series at inter-call intervals of 185–270 ms.

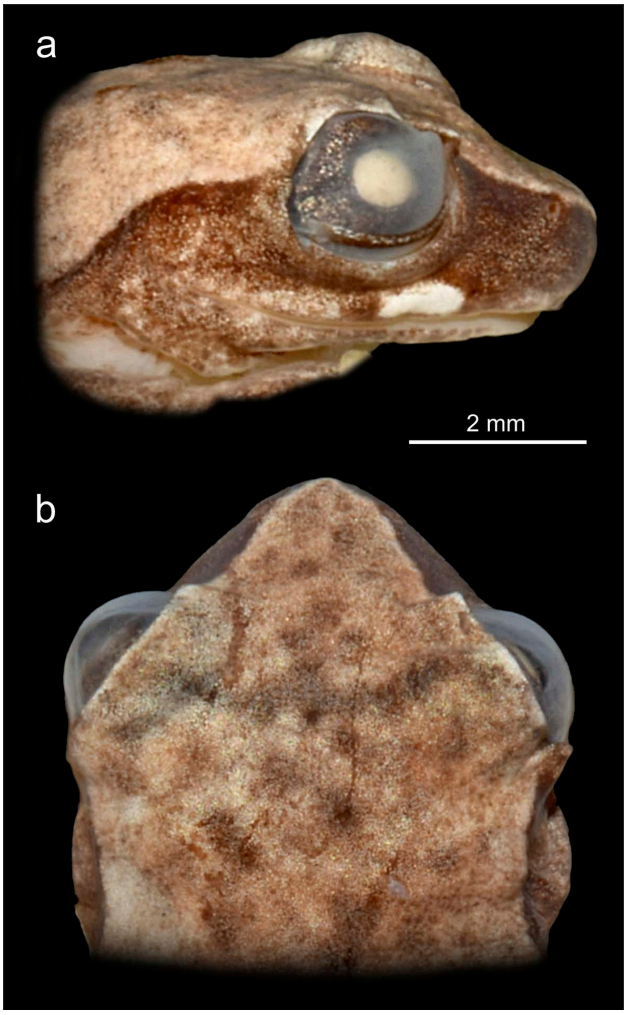

Figure 5. Head of the preserved holotype of *Dendropsophus jamesi* sp. n. (NMP-P6V 73618/1) in (a) lateral profile and (b) dorsal view.

Diagnosis: Our molecular phylogenetic analysis recovers the new species *Dendropsophus jamesi* as most closely related to *D. riveroi*, *D. shiwiarum*, *D. reichlei*, *D. coffea*, *D. nanus*, and *D. walfordi*. It differs from all these species by substantial differentiation in the 16S gene (see above) and, based on own comparison of specimens and literature data, as follows: it differs from *D. riveroi* by exhibiting a cream to white subocular spot (versus lacking), bronze-brown iris in life (versus golden-yellow), whitish canthal line sharply outlining loreal and dorsal head coloration (versus canthal line lacking), canthus rostralis moderately expressed, slightly rounded in cross-section (versus less sharply defined and distinctly rounded in cross-section), snout acuminate in dorsal view (versus truncate), supratympanic fold distinct (versus indistinct) (Cochran & Goin 1970, Ortega-Andrade & Ron 2013; Figs 6, 7), and details in the advertisement call, namely all pulses within calls equal in duration (2–3 ms) and clearly separated (versus modulated pulse duration, 2–12 ms; Fig. 3); it differs from *D. shiwiarum* by shorter inter-call intervals in regular series of advertisement calls (185–270 versus 814–3091 ms), snout acuminate in dorsal view and rounded in lateral profile (versus truncate in both dorsal and lateral views), and rounded discs on Finger III and Toe IV (versus discs with pointed tip) (Ortega-Andrade & Ron 2013); it differs from *D. reichlei* by shorter duration of advertisement calls (22–44 versus 68–112 ms), lower number of pulses per call (7–12 versus 16–30), snout rounded in profile (versus truncate), dark brown X-shaped and inverted V-shaped marks in scapular and sacral region (versus absent), and white belly in life (versus bright yellow) (Moravec et al. 2008); it differs from *D. coffea* by distinctly pulsed advertisement calls (versus pulsatile), smaller adult male size (SVL 17.6–18.4 versus 21.1–21.2 mm), and snout rounded in profile (versus truncate and slightly protruding) (Köhler et al. 2005); it differs from *D. nanus* and *D. walfordi* by exhibiting a cream to white subocular spot (versus lacking), by a short, acuminate snout in dorsal view (versus longer, more pointed snout), dorsum with dark interorbital bar and X-shaped dark marking in scapular region (versus dark interorbital bar lacking, multiple thin dark longitudinal lines) and differences in the advertisement calls (for comparison see Seger et al. 2021). Moreover, the new species differs from *D. gaucheri*, a species recovered as being closely related to the focal clade mentioned above by other studies

(Fouquet et al. 2011, Orrico et al. 2021, Whitcher et al. 2025), by a rounded snout in lateral profile (versus truncate, slightly protruding), upper eyelid lacking tubercles (versus bearing few enlarged tubercles), and details of dorsal color pattern (Lescure & Marty 2000).

Description of the holotype: Body moderately robust; head slightly narrower than body, slightly shorter than wide, widest below eyes; snout acuminate in dorsal view, rounded in lateral profile (Fig. 5); distance from nostril to eye shorter than diameter of eye; canthus rostralis moderately expressed, slightly rounded in cross-section; loreal region slightly concave; lips slightly flared; internarial area slightly depressed; nostrils barely protuberant, directed dorsolaterally; interorbital area flat, IOD 150.0% of ELW; eye large, strongly protuberant, its diameter about four times depth of lip below eye; tympanic membrane small, round, barely evident, its diameter about one third of eye diameter, separated from eye by ca. 138% of its diameter; tympanic annulus indistinct; supratympanic fold evident, slightly obscuring upper and posterior edge of tympanum. Arm slender, not hypertrophied; axillary membrane extending nearly to elbow joint; ulnar folds and tubercles absent; fingers of medium length, bearing small, round discs; relative length of fingers 1<2<4<3; diameter of disc on third finger about the size of tympanum; subarticular tubercles small to medium sized, round to ovoid, distal ones of first and fourth fingers prominent, slightly bifid in fourth finger; supernumerary tubercles barely evident; palmar tubercle medium sized, flat, elliptical; prepollical tubercle large, flat, elliptical, lacking nuptial excrescences and glands; fingers about one third webbed; webbing basal between fingers one and two; webbing formula of fingers $II2^{-}–3III2^{1/5}–2^{+}IV$. Legs moderately long, slender; heels overlapping when limbs flexed perpendicular to axis of body; tarsal fold and tarsal tubercles absent; toes moderately long, bearing round discs slightly smaller than those of fingers; relative length of toes 1<2<5<3<4; outer metatarsal tubercle small, barely evident; inner metatarsal tubercle large, flat, elliptical; distal subarticular tubercle of the first toe large, ovoid, ca. 65% of the length of inner metatarsal tubercle, extending distally under the second phalange; remaining subarticular tubercles medium sized to small, prominent, ovoid; supernumerary tubercles obscure; toes three fourth webbed;

Figure 6. Living adult males of *Dendropsophus riveroi*: (a, b) individual from 8 km north of Leticia (Tanimboca), Departamento Amazonas, Colombia, in dorsolateral and ventral views (courtesy of J. M. Padial); (c) *D. riveroi* (NMP-P6V 74912/1) from 36 km southeast of Pucallpa, Departamento Ucayali, Peru; and (d) specimen (NMP-P6V 72570) from Bolpebra, Departamento Pando, Bolivia, here tentatively referred to as *D.* cf. *riveroi* (uncorrected p-distance to nominal *D. riveroi* 2.5–2.7%). Not to scale.

webbing formula of toes I2⁻–2II1–1⁺III2–2IV2⁺–1$^{1/3}$V. Skin on dorsum, head, and dorsal surfaces of limbs finely shagreen, with scattered low minute tubercles, but upper eyelid lacking enlarged tubercles; skin on flanks smooth; skin on venter coarsely granular; skin on throat smooth; skin on lower surfaces of thighs smooth. Cloacal opening directed posteriorly at upper level of thighs; moderately long simple cloacal sheath covering cloacal opening; cloacal folds and tubercles absent. Tongue nearly round, slightly notched posteriorly, posterior and lateral margins not attached to floor of mouth; vomerine odontophores prominent, separated medially, about half size of choanae, bearing teeth, in the middle between choanae; choanae medium sized, oval to rectangular; vocal slits long, extending from the middle of lateral base of tongue to angle of jaws; vocal sac large, single, median, subgular.

Measurements (in mm): SVL 18.0; HL 6.4; HW 6.5; EN 1.6; ED 2.5; TD 0.8; ELW 1.6; IOD 2.4; TL 9.0; FL 12.5.

In ethanol, dorsal surfaces of head, body, and limbs pinkish brown with conspicuous dark brown flecks and markings, a narrow interorbital bar, X-shaped mark in scapular region, inverted V-shaped mark in sacral region, and dark brown bars on extremities are present. Loreal region dark brown sharply outlined from lighter dorsal head coloration by a narrow whitish canthal line extending from tip of snout to margin of upper eyelid. Region around nostrils dark brown. Upper lip whitish, with scattered melanophores and distinct white spot below the eye (the spot outlined by dark brown); a smaller white spot present below the nostril on the left lip. Tympanic region dark brown. Dark brown stripe extending from posterior edge of eye across tympanic fold to mid-side of flanks. Flanks whitish, translucent ventrally and posteriorly. Dorsal surfaces of hands, feet and webbing light brown, covered with melanophores (melanophores reduced on inner two fingers and three toes). Posterior surfaces of thighs light brown with dense light brown chromatophores. Cloacal sheath dark brown. Throat yellowish, with scattered melanophores distally. Belly and ventral surfaces of limbs whitish. Palmar and plantar surfaces with scattered melanophores.

In life, dorsum light brown with a similar pattern of dark markings and spots as in the preserved specimen. Dorsal surfaces of hands and feet including tips of toes and fingers light brown to whitish, covered with dark brown melanophores. Flanks ventrally and posteriorly translucent fleshy pink without chromatophores. Hidden surfaces of thighs yellowish brown. Throat yellow with dark brown melanophores distally. Belly white in pectoral and central part, translucent fleshy pink in posterior and lateral parts. Ventral surfaces of thighs translucent fleshy pink with dark brown melanophores posteriorly. Palmar and plantar surfaces fleshy pink with scattered dark brown melanophores. Iris bronze-brown. Bones white.

Variation: Variation of measurements and body proportions of eight adult male type specimens is given in Table 2. Dorsal pattern varies mostly regarding shape and distinctness of the dark brown interorbital bar, the marks in the scapular and sacral regions and presence of other smaller markings. Color pattern of the paratype NMP-P6V 73618/2 is shown in Figure 4d. Some variation is evident in size and distinctiveness of the white spots below the eyes. The spots are present in all paratypes, but they are barely evident in the paratype NMP-P6V 73618/3 (on the right side) and in the paratype NMP-P6V 77078 (on both sides). The type specimens also vary in the presence and shape of the white spots below the nostrils. Two paratypes (NMP-P6V 73618/2, 73618/4) have the white spots below both nostrils, the holotype and one paratype (HLMD-RA 3240) have only one white spot below the left nostril and in all remaining paratypes these spots are barely evident or absent. The finger and toe webbing formulae vary as follows:

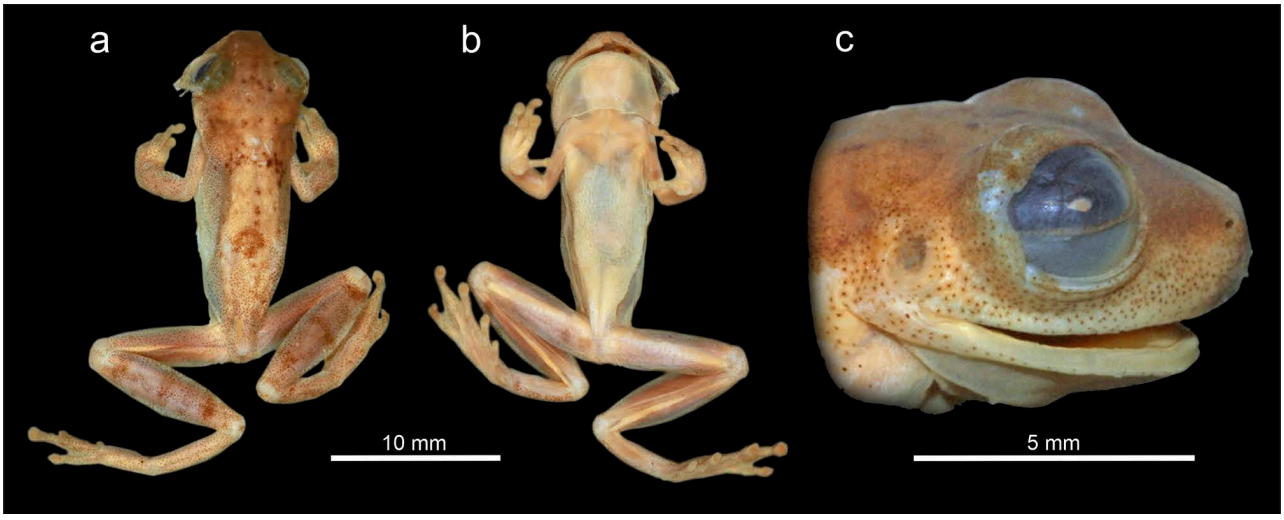

Figure 7. Preserved holotype of *Hyla riveroi* Cochran & Goin, 1970 (CM 37433) from Leticia, Amazonas, Colombia, in (a) dorsal and (b) ventral views, and (c) lateral profile of head.

Table 2. Variation of morphological measurements (in mm) and proportions of the type series of *Dendropsophus jamesi* sp. n. (adult males, N = 8). See text for abbreviations.

	Range	Mean (±SD)
SVL	17.6–18.4	18.1±0.31
HL	6.1–6.5	6.4±0.14
HW	6.3–6.6	6.5±0.10
EN	1.5–1.6	1.6±0.05
ED	2.4–2.7	2.5±0.09
TD	0.8–1.1	0.9±0.11
ELW	1.4–1.6	1.5±0.08
IOD	2.4–2.5	2.4±0.06
TL	8.8–9.3	9.1±0.18
FL	12.0–13.1	12.6±0.38
HL/SVL	0.35–0.37	0.35
HW/SVL	0.35–0.37	0.36
HW/HL	1.00–1.03	1.01
EN/ED	0.59–0.67	0.62
ED/HL	0.38–0.42	0.39
ED/HW	0.38–0.42	0.39
TD/ED	0.32–0.44	0.37
ELW/IOD	0.61–0.67	0.63
IOD/ED	0.89–1.00	0.96
TL/SVL	0.50–0.51	0.51
FL/SVL	0.68–0.71	0.70

II ($1^{1/4}$–2^{+})–($2^{3/4}$–3) III ($2^{1/4}$–3)–(2^{-}–2^{+}) IV and I (1^{+}–$1^{1/2}$)–(2^{-}–2) II (1^{-}–$1^{1/4}$)–(2^{-}–2^{+}) III (1^{-}–$1^{1/3}$)–(2^{-}–$2^{1/2}$) IV (2^{-}–$2^{1/4}$)–(1^{-}–1^{+}) V. Specimens collected near Cobija (ZFMK 67145–67148), Departamento Pando, Bolivia, here assigned to *D. jamesi* based on bioacoustics and morphology, generally agree in color pattern with those from the type locality and all exhibit a dark side of the head and distinct white subocular flecks. However, the dark markings on dorsal surfaces, namely interorbital bar, other dorsal marks, and dark crossbands on limbs are far less contrasting when compared to the types, albeit always recognizable. In contrast to the types, the finger and toe discs exhibit a yellow tint in life when viewed from above and lack white spots below the nostril (Fig. 4e). Females are unknown.

Vocalization: Advertisement calls of *Dendropsophus jamesi* were recorded on 19 November 2007 at the type locality (ambient temperature 25.0°C). Males were calling at a forest swamp in a small chorus. Because of the presence of numerous calling males, calls in the recording are not referable to individuals, but the type series was collected from the chorus, leaving no doubt that the calls described herein actually correspond to *D. jamesi*. Calls consist of a high-pitched short pulsed note, emitted at irregular intervals. In fully motivated calling, calls are emitted in rather regular series containing up to 20 calls, but more frequently we observed short series of 2–4 calls. Calls exhibit some clear amplitude modulation, with maximum energy present in the middle of the call. Pulses are clearly separated and countable, but tend to partly fuse in the second half of the call (Fig. 3). Numerical parameters for 78 analyzed calls emitted by several different males are as follows: call duration (= note duration), 22–42 ms (30.3 ± 6.2 ms); inter-call interval in highly motivated call series, 180–262 ms (205.4 ± 24.3 ms); pulses/call, 6–12 (9.6 ± 1.3); pulses/second within calls, 333–417 (358.6 ± 33.3); dominant frequency 5072–5555 Hz (5344 ± 180 Hz); prevalent bandwidth 3200–6500 Hz.

Calls recorded in the vicinity of Cobija, Departamento Pando, Bolivia, on 13 January 1998 (ambient temperature 23.2°C), from two males contained in the series ZFMK 67145–67148 agree with those at the type locality in all general characters. Numerical parameters for 39 analyzed calls are as follows: call duration (= note duration), 28–44 ms (33.6 ± 3.8 ms); inter-call interval in highly motivated call series, 205–270 ms (233.6 ± 21.2 ms); pulses/call, 9–12 (10.2 ± 1.0); pulses/second within calls, 333–375 (346.4 ± 17.2); dominant frequency 5265–5437 Hz (5368 ± 51 Hz); prevalent bandwidth 4000–6600 Hz.

Distribution, ecology and threat status: The new species is known from its type locality, lying on the left bank of the Rio Beni in northeastern Bolivia, and from the vicinity of the town Cobija (specimens not barcoded, identification based on call analyses) in northwestern Bolivia (Fig. 8). Due to the geographical proximity of both localities to the Bolivian–Brazilian border, the occurrence of *D. jamesi* in Brazil is highly expectable. The habitat at the type locality had character of a permanently flooded swampy forest. The open flooded areas contained many fallen dead trees covered with herbaceous plants, while the more solid banks were covered with dense, impenetrable forest containing palms and herbaceous lianas (mostly family Araceae) climbing the higher strata (Fig. 9). Tens of calling males of *D. jamesi* were observed at the locality during two consecutive nights on 18–19 November 2007. The males called from low herbaceous plants growing close to the water (up to 100 cm above the water surface). Other syntopic hylid species included *Boana punctata*, *Dendropsophus juliani*, *D.* cf. *salli*, *Scinax garbei*, and *Sphaenorhynchus lacteus.* At Cobija, males were calling from bushes within a swampy area at the edge of the town. There, the species occurred in sympatry with the hylid species *Boana lanciformis*, *B. punctata, Dendropsophus joannae, D. kamagarini*, and *Scinax* sp. 27 (sensu Araujo-Vieira et al. 2023). Reproductive mode and tadpoles are unknown. Considering the sparse data available, we here classify *Dendropsophus jamesi* as Data Deficient according to the IUCN Red List criteria.

Etymology: The species name is a patronym for our late friend and colleague James Aparicio Effen, researcher at the Colección Boliviana de Fauna, La Paz, Bolivia, in recognition of many years of fruitful collaboration and his contributions to the knowledge of the Bolivian herpetofauna.

Discussion

With the description of *Dendropsophus jamesi*, we added another Amazonian species to the *D. microcephalus* group. Although we realized at the time of its discovery that the respective population from Palmira, Bolivia, is not assignable to any known nominal species, clarifying its taxonomic status turned out to be a complex task. Taxonomic treatment was hampered by the lack of comparative data of morphologically similar species and the fact that in the literature such small *Dendrosophus* exhibiting white subocular flecks occurring in Bolivia, Ecuador and Peru were partly referred to as *D. riveroi* (e.g., Duellman 1978, Rodríguez & Duellman 1994, Duellman & Mendelson 1995, Köhler & Lötters 1999), adding to uncertainty about the identity of this nominal taxon. Köhler et al. (2005) already assumed that likely more than one species is contained in the name *D. riveroi* as referred to at that time. This assumption was corroborated by Ortega-Andrade & Ron (2013), who described *D. shiwiarum* from Ecuador and diagnosed it against *D. riveroi*, including comparison with *D. riveroi* type material. Similarly, our examination of the *D. riveroi* holotype and additional genotyped specimens revealed morphological characters that are not in agreement with *D. jamesi*, most obviously among these is the lack of a white or cream-coloured subocular fleck and a barely expressed supratympanic fold, both present in the new species. Apparently, none of the specimens referable to nominal *D. riveroi* exhibit such a white fleck or spot below the eye (see also photos in Gagliardi-Urrutia et al. 2022:107).

Our study revealed morphological differences, slight advertisement call differences and the 16S uncorrected p-distances of 3.3–3.6% between *D. riveroi* and *D. jamesi*. In general, in many if not all amphibians, reproductive isolation is stochastically correlated with genetic divergence which in turn is proportional to time since divergence (e.g., Malone & Fontenot 2008, Dufresnes et al. 2021),

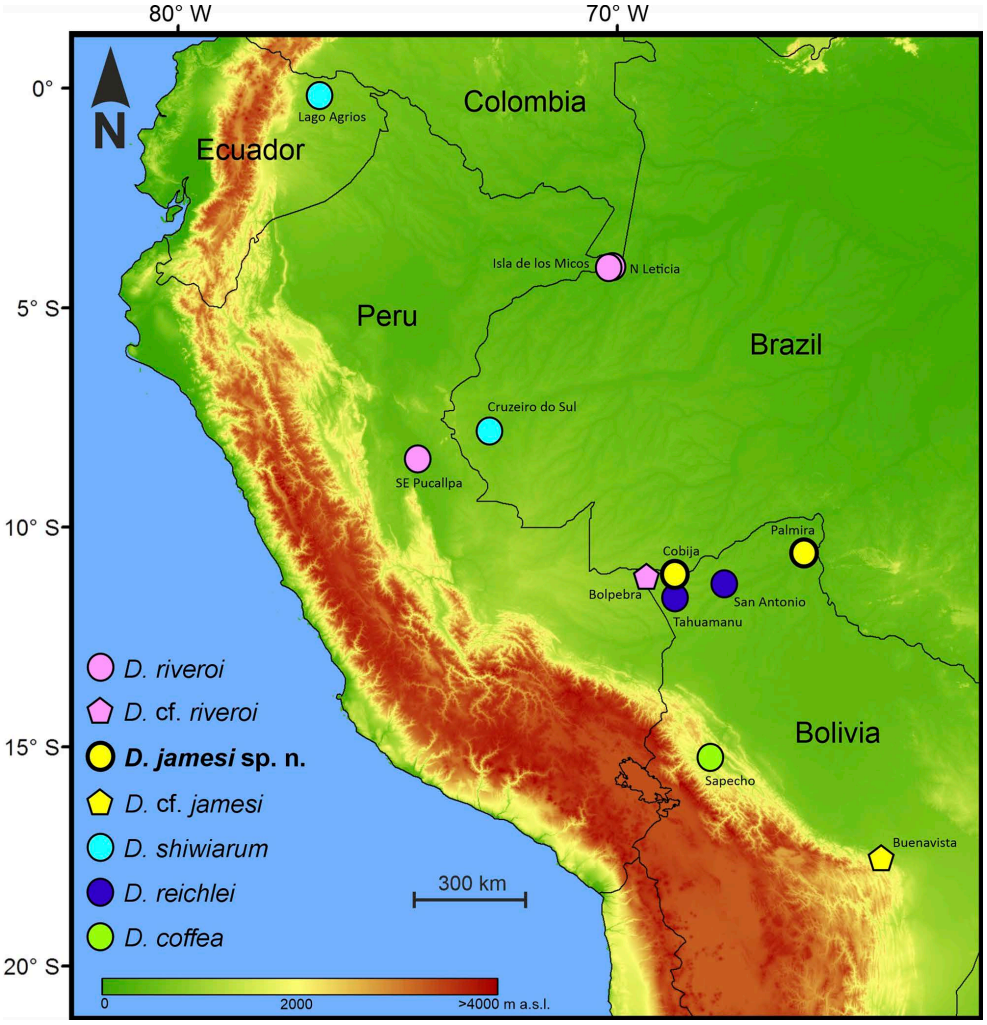

Figure 8. Map of central-western South America showing localities of selected samples of the *Dendropsophus microcephalus* species group used in the phylogenetic analysis or referred to in the text.

and higher genetic divergences thus correspond to a higher likelihood of completion of species formation. The genetic distances observed herein are only slightly above the 3% point of reference empirically determined to characterize lineages in Neotropical frogs that usually are diverged at the species level (Fouquet et al. 2007), but they correspond to those between other closely related sister species in the *D. microcephalus* species group (e.g., Ferrão et al. 2020). We emphasize that we consider the concordance between slight but consistent morphological, bioacoustic and genetic differences as main argument to support a status of *D. riveroi* and *D. jamesi* as separate, distinct species.

Although we herein detected and described slight differences in the advertisement calls of the two species, it is obvious that more comprehensive biacoustic studies of genotyped voucher specimens are desired to confirm differentiation in calls. Specifically, analysing call recordings of various genotyped males per species, each with precise measurements of body temperature and body size and ideally from different populations, would allow comparisons in multivariate models that take confounding factors such as individual, temperature, body size and population into account and quantify the amount of variation truly corresponding to interspecific differences. Until such analyses become available, we here followed Köhler et al. (2017) in relying on qualitative call differences, instead of quantitative differences of uncertain relevance despite their statistical significance.

Figure 9. Habitat of *Dendropsophus jamesi* sp. n. at the type locality Palmira, Departamento Pando, Bolivia, 18 November 2007: (a) general aspect of the swamp and surrounding forest; (b) details of the vegetation at the edge of the swamp from where males were calling.

Even if we provided data in support of the existence of an undescribed taxon, our knowledge about species in the *D. microcephalus* group is fragmentary and studies (including ours herein) frequently suffer from limited geographical coverage as well as the lack of genotyped voucher specimens. Little is known about intra-specific variation of nominal species and their geographical distribution. In many cases, like in the case of *D. jamesi*, new species in this group were described from single or few nearby localities (e.g., Bokermann 1962, Cochran & Goin 1970, Köhler & Lötters 2001, Köhler et al. 2005, Moravec et al. 2006, 2008, Jansen et al. 2019). After establishment of such new names, subsequent research has to clarify the allocation of other known populations to respective species, which is not an easy task given the difficulties involved in this group of tiny frogs (see Introduction). However, with the implementation of molecular genetics in taxonomy and sequencing of newly collected topotypic specimens (e.g., Seger et al. 2021, Jaramillo et al. 2021, Ferreira et al. 2024), or the application of 'museomics' to obtain DNA from historical name-bearing types (e.g., Scherz et al. 2020, Vences et al. 2021, Köhler et al. 2024, Nakamura et al. 2025), chances to clarify the taxonomic status of certain populations dramatically increased. For our study, despite indications from external morphology, the availability of sequences from topotypic *D. riveroi* was crucial to evaluate the status of the focal lineage from northern Bolivia, to confirm the presence of *D. riveroi* in central Peru (near Pucallpa, Departamento Ucayali), and to allocate a call recording to this nominal taxon. As a general result of the accumulation of sequence data, many populations of small *Dendropsophus*, formerly unidentified or misidentified, could be unequivocally allocated to a certain nominal species. This is exemplified by the recent record of *D. joannae* (originally described from Cobija, northern Bolivia; Köhler & Lötters 2001) in the Departamento Loreto, northern Peru (Gagliardi-Urrutia et al. 2022; photograps on page 101 in this book erroneously figure a different species [likely *D. brevifrons*], G. Gagliardi-Urrutia pers. comm.) and in the Departamento Huánuco, central Peru (this study). Therefore, we assume that future studies will also identify *D. jamesi* at additional localities in the Amazon basin, at least in adjacent Brazil and Peru, some of them possibly having been formerly referred to as '*D. riveroi* with white subocular spots'.

Nevertheless, there are more complicated cases involved, as demonstrated by the presence of a population from Bolpebra, Departamento Pando, Bolivia, which differs from topotypic *D. riveroi* by 2.7% uncorrected p-dis-

tance in the 16S gene, exhibits a somewhat different color pattern compared to nominal *D. riveroi* (Fig. 6d), and has an advertisement call with distinctly higher dominant frequency (at similar body size). It is therefore herein referred to as *D.* cf. *riveroi*. Unfortunately, our call recording of the single collected male of this population is of too poor quality to evaluate it for differences in pulse structure with respect to calls of nominal *D. riveroi*. More fieldwork and sampling is needed to evaluate the status of this Bolpebra population, as well as that of the population from Buenavista, Departamento Santa Cruz, Bolivia, that we here tentatively refer to as *D.* cf. *jamesi*.

On the other hand, it is quite clear that the inventory of species in the *D. microcephalus* group is far from complete. Even our rather limited sampling revealed pronounced genetic differentiation in the 16S gene within nominal species, such as *D. reichlei*, *D. rhodopeplus* and *D. shiwiarum*, with uncorrected p-distances within taxa amounting to 4.6, 5.3, and 4.5%, respectively. These results, together with those of other studies (e.g., Gehara et al. 2014, Orrico et al. 2021, Whitcher et al. 2025), provide rather strong indication for the presence of undescribed species diversity in *Dendropsophus* frogs and it is far from daring to foresee that many more such cases will become evident with more comprehensive sampling. There is still a long journey ahead for taxonomists to complete the inventory of small Amazonian *Dendropsophus*.

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Appendix
Additional specimens examined

Dendropsophus coffea: BOLIVIA: La Paz: 55 km on road from Caranavi to Palos Blancos, 800 m, NKA 6538 (holotype), ZFMK 80590 (paratype); 5 km N Río Beni bridge, near Sapecho, 550 m, CBF 5538, ZFMK 82182 (paratypes).

Dendropsophus joannae: BOLIVIA: Pando: Bolpebra, 250 m, NMP-P6V 72562; Cobija, 250 m, CBF 3323 (holotype), CBF 3324–3326, KU 224701–224703, ZFMK 67119–67120, 67121–67124 (paratypes), NMP-P6V 74252/1; Nacebe, 170 m, NMP-P6V 72169/1–2; PERU: Huánuco: ACP Panguana, 260 m, MUSM 42083.

Dendropsophus juliani: BOLIVIA: Pando: vicinity of the settlement of Barracón on the road from Cobija to Riberalta, 160 m, CBF 5923 (holotype), CBF 5924–5927, NMP-P6V 72799/1–3, HLMD-RA-3051 (paratypes).

Dendropsophus leali: BOLIVIA: Beni: El Porvenir, 300 m, CBF 2449–2450, ZFMK 62826; Puerto Almacén, Río Ibaré, 300 m, ZFMK 60721–60722; Cochabamba: 6.5 km N Chipiriri, 260 m, KU 136281–136294; BRAZIL: Rondônia: Forte Príncipe de Beira, KU 92058–92059 (paratypes).

Dendropsophus meridianus: BRAZIL: Rio de Janeiro: 20 km N of Rio de Janeiro, ZFMK 39499–39500.

Dendropsophus microcephalus: COSTA RICA: Guanacaste: Colorado, ZFMK 62142–62148; VENEZUELA: Sucre: Parare, ZFMK 36085–36094.

Dendropsophus minimus: BRAZIL: Amazonas: Taperinha (near Santarem), NMW 19436 (holotype).

Dendropsophus nanus: BOLIVIA: Beni: Puerto Almacén, ZFMK 60458–60462; 6.5 km NE of Riberalta, 175 m, NMP-P6V 70693/1–3, 2 km SW of Riberalta, 175 m, NMP-P6V 70694; Santa Cruz: Buenavista, 250 m, ZFMK 80011–80014; San Ramón, ZFMK 60391–60392; La Florida, ZFMK 60374–60381; Santa Cruz de la Sierra, 400 m, ZFMK 67001; PARAGUAY: Chaco: 23 km S of Filadelfia, ZFMK 53262–53266.

Dendropsophus reichlei: BOLIVIA: Pando: vicinity of the settlement of Limón, CBF 6073 (holotype); surroundings of the settlement San Antonio de Filadelfia, CBF 6074–6075, NMP6V 73617/1–2, HLMD-RA-3065 (paratypes); Estación Biológica Tahuamanu, NMP-P6V 73617/2.

Dendropsophus riveroi: COLOMBIA: Amazonas: Leticia, CM 37433 (holotype); PERU: Ucayali: 36 km SE of Pucallpa, 150 m, NMP-P6V 74912/1–3.

Dendropsophus cf. *riveroi*: BOLIVIA: Pando: Bolpebra, 250 m, NMP-P6V 72570.

Dendropsophus walfordi: BRAZIL: Rondônia: Forte Príncipe da Beira, MZUSP 73652 [WCAB 8436] (holotype; photos only).