Phyllomedusa 24(2):255–269, 2025

ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v24i2p255-269

Description of the advertisement call of *Dendropsophus frosti* (Anura: Hylidae) as a phenotypic evidence of its phylogenetic relationships

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Abstract

Description of the advertisement call of *Dendropsophus frosti* (Anura: Hylidae) as a phenotypic evidence of its phylogenetic relationships. For amphibians, gaps in knowledge about natural history and geographic distribution are among the major causes that blur our understanding of their diversity. These key pieces of information contribute to our understanding off taxonomy, systematics, ecology, and conservation. Herein, we provide new information on the geographic distribution and describe for the first time the advertisement call of *Dendropsophus frosti* from western Amazonia. The call consists of two types of short notes, a trilled multipulsed note with a duration ranging from 158 to 297 ms (note type A), and a high-pitched pulsatile note with a duration of about 9 ms (note type B). The dominant frequency of note type A is about 4260 Hz, while that of note type B is around 4330 Hz. The notes are emitted in two call arrangements, a complex call formed by one note type A followed by one or two type B notes or a composite call formed by two to seven type B notes. Overall, the call structure of *D. frosti,* with those two types of notes emitted in complex or composite calls, is shared with closely related species from the *D. parviceps* group, showing a phylogenetic signal. Our results suggest that acoustic characters may represent phylogenetic informative traits for the *Dendropsophus parviceps* group,

Received 15 April 2025
Accepted 20 October 2025
Distributed December 2025

supporting the current taxonomy. Additionally, our findings extended the known geographic distribution by approximately 700 km eastward. Our results highlight once again the necessity of biological surveys in remote areas of Amazonia, where many knowledge gaps persist.

Keywords: Bioacoustics, *Dendropsophus parviceps* group, Raunkiæran shortfall, Wallacean shortfall.

Resumo

Descrição do canto nupcial de *Dendropsophus frosti* (Anura: Hylidae) como uma evidência fenotípica das suas relações filogenéticas. Para os anfíbios, as lacunas no conhecimento sobre a história natural e a distribuição geográfica estão entre as principais causas que obscurecem a nossa compreensão da sua diversidade. Essas são informações fundamentais para a taxonomia, sistemática, ecologia e conservação. Aqui, fornecemos novas informações sobre a distribuição geográfica e descrevemos, pela primeira vez, o canto de acasalamento do *Dendropsophus frosti*, da Amazônia ocidental. O canto consiste em dois tipos de notas curtas, uma nota trilada multipulsionada com duração variando de 158 a 297 ms (nota tipo A) e uma nota pulsante aguda com duração de cerca de 9 ms (nota tipo B). A frequência dominante da nota tipo A é de cerca de 4260 Hz, enquanto a da nota tipo B é de cerca de 4330 Hz. As notas são emitidas em dois arranjos de cantos, um canto complexo formado por uma nota A seguida por uma ou duas notas do tipo B, ou um canto composto formado por duas a sete notas do tipo B. No geral, a estrutura de vocalização de *D. frosti*, com esses dois tipos de notas emitidas em cantos complexos ou compostos, é compartilhada com espécies intimamente relacionadas do grupo *Dendropsophus parviceps*, mostrando sinal filogenético. Os nossos resultados sugerem que as características acústicas podem representar um caráter filogenético informativo para o grupo *D. parviceps*, apoiando a taxonomia atual. Além disso, nossas descobertas ampliaram a distribuição geográfica conhecida em aproximadamente 700 km a leste. Nossos resultados destacam mais uma vez a importância dos levantamentos biológicos em áreas remotas da Amazônia, onde persistem muitas lacunas de conhecimento.

Palavras-chave: Bioacústica, Déficit de Raunkiæran, Déficit de Wallacean, Grupo *Dendropsophus parviceps*.

Introduction

The lack of information on life-history traits and geographic distribution of species, known respectively as the Raunkiæran and Wallacean shortfalls, creates biases in our understanding of biodiversity and compromises knowledge in ecology, evolution, and conservation (Yang *et al.* 2013, Hortal *et al.* 2015). Sampling biases are well-known causes of shortfalls in anuran biodiversity, especially in regions like the Amazon rainforest, which harbors high species richness and encompasses large unexplored areas (Azevedo-Ramos and Gallati 2002, Peloso 2010, Mayer *et al.* 2019, Guerra *et al.* 2020, Moraes *et al.* 2022).

Vocalization and acoustic traits are crucial for studies of behavior, physiology, reproductive biology, ecology, evolutionary history, and taxonomy of amphibians (Wells 1977, Duellman and Trueb 1994, Kohler *et al.* 2017). Among the different acoustic signals, the advertisement call is the most extensively studied acoustic signal emitted by frogs (Wells 1977, Gerhardt 1994). These traits are influenced by various selective pressures and contain phylogenetic signals in frog phylogeny (Gingras *et al.* 2013, Forti *et al.* 2015), which varies in taxonomic scale (e.g. Forti *et al.* 2017). Despite advances in the last decades in the number of studies and described calls, a significant gap in the knowledge of this trait remains. The advertisement call is unknown in about 35% of anuran species (Guerra *et al.* 2018).

Dendropsophus Fitzinger, 1843 comprises 106 recognized species (Frost 2025) and nine species groups (*sensu* Orrico *et al.* 2021). One of these groups, the *Dendropsophus parviceps* group, has received significant attention in recent years, including descriptions of new species (e.g., Motta *et al.* 2012, Fouquet *et al.* 2015, Rivadeneira *et al.* 2018), taxonomic revisions (Fouquet *et al.* 2011, Orrico *et al.* 2013, 2021, Melo-Sampaio 2023), and studies of new natural history data (Forti *et al.* 2015, Orrico *et al.* 2021). Currently, this group includes 19 species: *Dendropsophus bokermanni* (Goin, 1960); *Dendropsophus brevifrons* (Duellman and Crump, 1974); *Dendropsophus counani* Fouquet, Orrico, Ernst, Blanc, Martinez, Vacher, Rodrigues, Ouboter, Jairam, and Ron, 2015; *Dendropsophus frosti* Motta, Castroviejo-Fisher, Venegas, Orrico, and Padial, 2012; *Dendropsophus garagoensis* (Kaplan, 1991); *Dendropsophus giesleri* (Mertens, 1950); *Dendropsophus grandisonae* (Goin, 1966); *Dendropsophus kamagarini* Rivadeneira, Venegas, and Ron, 2018; *Dendropsohus kubricki* Rivadeneira, Venegas, and Ron, 2018; *Dendropsophus luteoocellatus* (Roux, 1927); *Dendropsohus microps* (Peters, 1872); *Dendropsophus padreluna* (Kaplan and Ruiz-Carranza, 1997); *Dendropsophus parviceps* (Boulenger, 1882); *Dendropsophus pauiniensis* (Heyer, 1977); *Dendropsophus praestans* (Duellman and Trueb, 1983); *Dendropsophus subocularis* (Dunn, 1934); *Dendropsophus timbeba* (Martins and Cardoso, 1987); *Dendropsophus virolinensis* (Kaplan and Ruiz-Carranza, 1997); and *Dendropsophus yaracuyanus* (Mijares-Urrutia and Rivero, 2000). It also includes three nominal clades (*D. microps, D. garagoensis,* and *D. subocularis* clades), which highlight morphological similarities or phylogenetic relationships with taxonomic implications (see Orrico *et al.* 2021).

Dendropsophus frosti is a small treefrog in the *D. parviceps* group, closely related phylogenetically to the *D. subocularis* clade (Motta *et al.* 2012, Orrico *et al.* 2021). The species is known only from a few localities in the western Amazonian lowlands. Until recently, it was known to occur only in Colombia and Peru, but Koch *et al.* (2023) reported it from Tabatinga and Japurá municipalites, Amazonas state, Brazil. Despite its relative well-resolved phylogenetic relationship (Motta *et al.* 2012, Fouquet *et al.* 2015, Orrico *et al.* 2021), *D. frosti* does not share any of the known phenotypic synapomorphies of the *D. parviceps* group (Orrico *et al.* 2021). Furthermore, its reproductive biology, vocalization, tadpole morphology, and even its geographic range remain poorly known.

Given the importance of these features for *Dendropsophus* species, natural history, taxonomy, phylogeny and systematics (Orrico *et al.* 2009, Hepp *et al.* 2012, Teixeira *et al.* 2015), herein we describe the advertisement call of *D. frosti,* recorded from males recently collected near the type locality and from a new occurrence in Central Amazonia. Additionally, we reassess its habitat, calling sites, and geographic distribution, and discuss the role of these traits in the taxonomy of the *D. parviceps* group.

Materials and Methods

Study Site and Advertisement Call Recordings

We collected our data from three different expeditions conducted in the Brazilian Amazon, state of Amazonas, over the last ten years. Fieldwork was first carried out on the right bank of Japurá River (01°50'46.10'' S, 69°01'46.30'' W, 103 m a.s.l.), near Vila Bittencourt village, between 14 August and 20 September 2014, where we collected eight specimens of *D. frosti.* This locality is close to that reported by Koch *et al.* (2023), which is based on a single specimen. We describe the habitat where we found the additional specimens. A second expedition was conducted in the Parque Nacional do Jaú (hereafter PNJ), a protected area in Central Amazonia, about 200 km up the Negro River from Manaus. Fieldwork at PNJ took place from 16 to 28 February 2017, along the sampling sites on the banks of the Jaú River. On February 24, at one of the sampling sites (02°17'39" S, 62°27'21" W, 41 m a.s.l.), we collected 14 specimens and recorded calling males after a heavy afternoon rainfall. The recordings were made using a Marantz PMD620 recorder with an internal microphone. The final expedition occurred on 5 May 2018 during the rainy season, in a forest fragment in the area of Comando de Fronteira Solimões / 8º Batalhão de Infantaria de Selva of the Brazilian Army (04°14'52.77" S, 69°55'51.05'' W, 83 m a.s.l.), near the limits of Tabatinga municipality, approximately 15 km straight line from the type-locality of the species. Four specimens were collected and calling males were recorded after rainfall between 19:00 h and 23:00 h, using a Zoom H1/Handy recorder. Additionally, E. Koch provided us with a recording of an unvouchered male specimen from the same locality. Koch's recording was obtained on April 29, 2021, after heavy rainfall, using WavePad free recording app (NCH Software 2015) on a smartphone with an internal microphone. All recordings were made at 44,100 Hz sampling rate and 16-bits resolution. Digital copies of all recordings analyzed are deposited in the Coleção Bioacústica of Universidade Federal de Minas Gerais (CBUFMG), Belo Horizonte, Minas Gerais state, Brazil (Appendix I).

We obtained a total of four recordings, from which we analyzed the calls emitted by seven males (Appendix I). Four individuals were recorded simultaneously by APA in Tabatinga in 2018 (CBUFMG 1119; Appendix I). Only two of the recorded males were collected, while the remaining data came from unvouchered individuals. Specimens were collected following standard procedures and subsequent fixation in formalin. Specimens were deposited in Zoological Collection Paulo Burnheim of Universidade Federal do Amazonas (CZPB-AA), Manaus, Amazonas, Brazil; Collection of Amphibians and Reptiles of Instituto Nacional de Pesquisas da Amazonia (INPA-H), Manaus; and Coleção Herpetológica Osvaldo Rodrigues da Cunha, Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil (Appendix II). Collecting permits were obtained from Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO-ICMBio).

Acoustic Analysis

We analyzed 17 advertisement calls (*N* = 7 males) with Raven Pro 1.5 from the Cornell Lab of Ornithology (Bioacoustics Research Program 2017). The call description followed a note-centered approach, with terminology based on Köhler *et al.* (2017). We report temporal features such as call duration, number of notes, note repetition rate (NRR), internote interval, note duration, and number of pulses per note; for spectral features we report lowe and high frequencies, and bandwidth calculated from these variables. Additionally, we measured dominant frequency using the function Peak Frequency option and the bandwidth of the most concentrated energy using the Frequency 5%, Frequency 95% and Bandwidth 90% functions. Temporal variables were taken from oscillograms, and spectral variables were taken from spectrograms with the following settings: window type = Blackman, window size = 256 samples, 3 dB bandwidth filter in 283 Hz, overlap = 80%, hop size 1.16 ms, frequency resolution DFT size = 2048 samples, and grid spacing 21.5 Hz. To avoid overlapping background noises, we measured upper and lower frequencies 20 dB below the peak frequency using the power spectrum tool in Raven. Sound figures were obtained with Seewave package v.2.0.2 (Sueur *et al.* 2008), on R environment (R Development Core Team, 2011). The Seewave settings were Blackman window, 512 points of resolution (FFT) and 80% overlap.

Results

All collected specimens and recorded individuals share morphological characteristics consistent with the diagnosis of *D. frosti* provided by Motta *et al.* (2012). Similarities include coloration patterns at night (dorsum yellow without contrasting coloration of the flanks; Figure 1A) and during the day (dorsum brown, ventral surface pale yellow, a dark brown coloration, near black, in the thighs, shanks, lateral surface of the body and head and inner parts of feet and hands; Figures 1B, C). These features unequivocally confirm the identification of the specimens, and the calling males recorded in this study.

Advertisement Call Description

The call of *D. frosti* consists of two distinct types of short notes, referred to hereafter as type A and type B notes. These notes are emitted alternately at varying rates in two different arrangements, referred to here as Call type I and Call type II (Figure 2; Table 1). Note type A is a longer trill, i.e., a multipulsed note (χ = 23.3 pulses, SD = 4.4 *N* = 7), with duration that varies from 158 to 297 ms (χ = 215, SD = 54, *N* = 7), distinct pulses with amplitude modulation that increase in intensity in the first third of the note to the highest peak in the middle of the note, decreasing during the last pulses (Figure 2). Note type B is a high-pitched pulsatile short note, with duration varying from 8 to 17 ms (χ = 12, SD = 3, *N* = 8) in the Call type I, and 4 ms to 14 ms (χ = 9 ms, SD = 3, *N* = 33) in Call type II. As mentioned above, the notes are emitted in two different arrangements: Call type I consists of a complex call formed by one type A note, followed by one or two type B notes, with internote interval varying from 93 to 112 ms (χ = 105, SD = 7, *N* = 7) and duration ca. 272 to 505 ms (χ = 351 ms, SD = 82 ms, *N* = 7) resulting in NRR about 3 notes per second (χ = 3.38, SD = 0.54, *N* = 7) Call type II, which consists of a composite call formed by a series of 2 to 7 uniform pulsatile pitched notes (Figure 2), during ca. 124 to 648 ms depending on the number of notes emitted (see Table 1), and with note repetition rate about eight notes/sec (χ = 8.51, SD = 0.57, *N* = 10).

Both note types have no frequency modulation and present similar spectral structure (Table 1). Note type A has a dominant frequency between 4048.2 to 4435.8 Hz (χ = 4260.5 Hz, SD = 134,1, *N* = 7) and the following pulsatile notes observed in Call type I have a dominant frequency varying

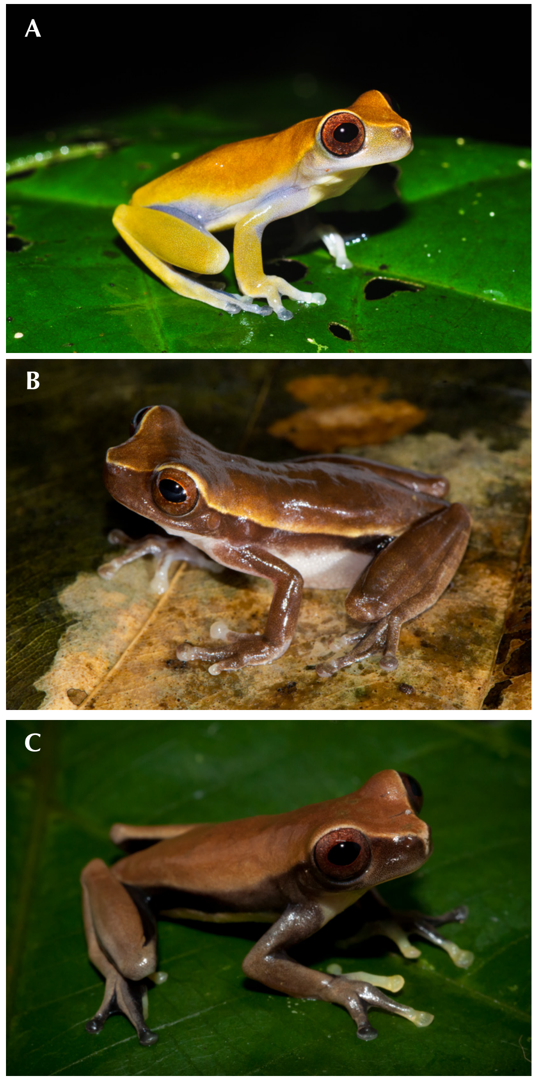

Figure 1. Specimens of *D. frosti* observed and collected from (**A**) Japurá River, near Villa Bittencourt village (INPA-H 38287), (**B**) Parque Nacional do Jaú-PNJ (MPEG 41866), and (**C**) Tabatinga (INPA-H 47002), AM, all localities in the Brazilian Amazon.

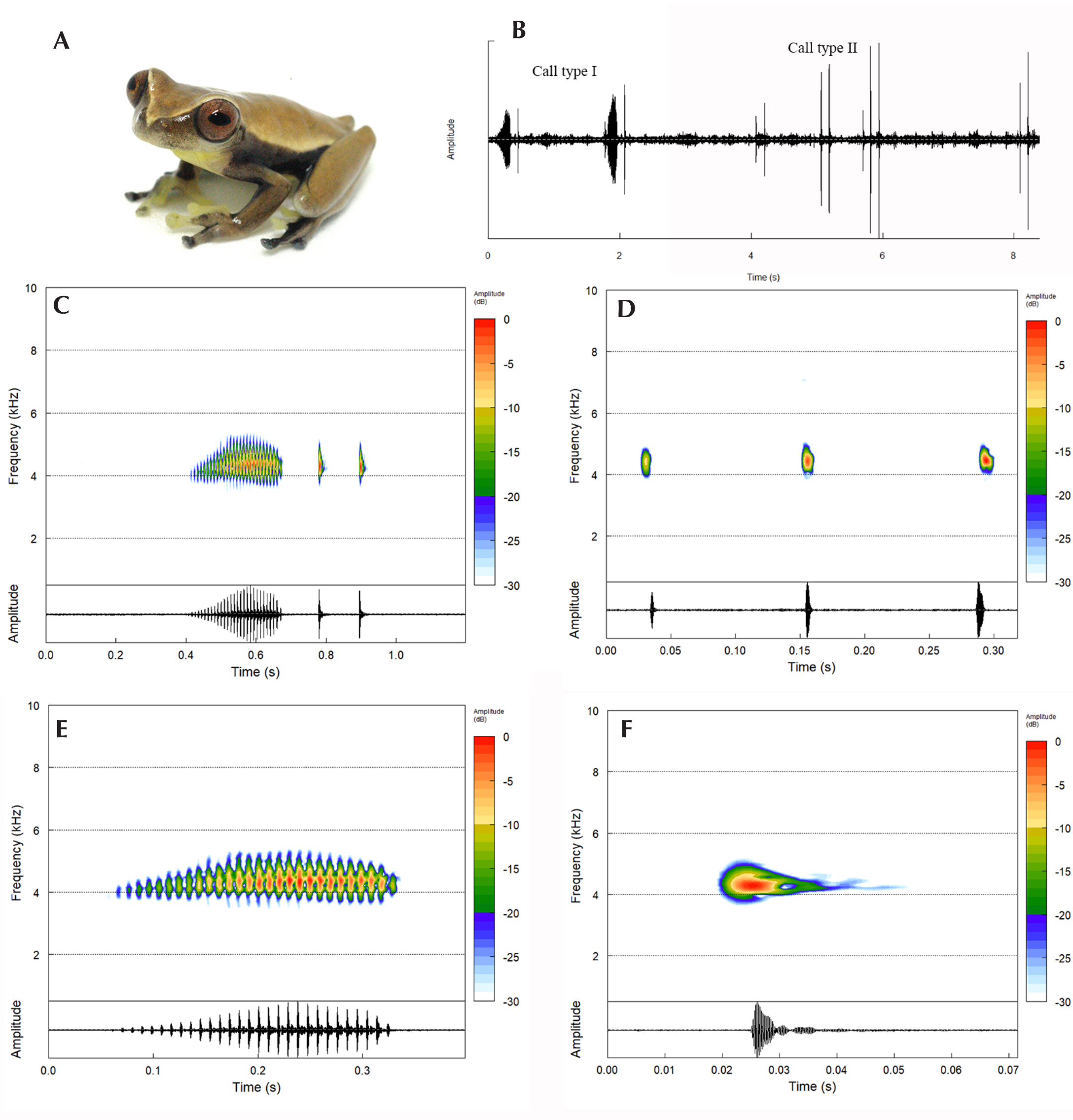

Figure 2. The advertisement call of *D. frosti:* (**A**) a recorded specimen INPA-H 47001; (**B**) Audiospectrogram highlighting the two call arrangements; (**C**) Waveform and spectrogram of Call type I; (**D**) Waveform and spectrogram Call type II; Waveform and spectrogram of notes, type A (**E**) and type B (**F**).

from 4306.6 to 4392.8 (χ = 4332.5 Hz, SD = 38.5, *N* = 8). In Call type II, the note type B shows a dominant frequency in a similar bandwidth varying from 4199 to 4392.8 Hz along the notes (χ = 4334.2 Hz, SD = 41, *N* = 19). In two calls recorded from PNJ (CBUFMG #1122; Appendix I) we observed a harmonic structure in both notes of Call type I, with the first harmonic

Table 1. Temporal and spectral traits of the advertisement call of *Dendropsophus frosti*. Call types and notes were recorded from seven males. Temporal traits of Call type II are presented for the three different arrangements (number of notes) observed. (*) denotes values obtained from 19 notes with better resolution on spectrogram (**) denotes values obtained from the notes of Call type I with visible harmonic structure.

Acoustic variables	**Advertisement Call type I Males (*N* = 4) / Call (*N* = 7)**	**Advertisement Call type II Males (*N* = 5) / Call (*N* = 10)**	
Call type	Complex	Composite multinote	
Number of notes	2–3	2–7	
Call duration (ms)	351 ± 82 (272–505)	Two notes (*N* = 5) Three notes (*N* = 3) Seven notes (*N* = 2)	129 ± 4 (124–134) 260 ± 8 (252–267) 646 ± 4 (643–648)
Internote interval (ms)	108 ± 5 (102–112)	112 ± 3 (107–125)	
NRR (notes/sec)	3.4 ± 0.39 (2.8–3.82)	8.26 ± 0.26 (7.81–8.55)	
Note	***N* = 7**	***N* = 8**	***N* = 33**
Note type	(A) Multipulsed	(B) pulsatile	(B) pulsatile
Note duration (ms)	188 ± 33 (158 – 244)	11 ± 3(8–15)	9 ± 3 (4–14)
Number of pulses/note	21 ± 2 (19–25)	Unpulsed	Unpulsed
Low frequency (Hz)	3692.4 ± 63 (3598.1–3752.4)	3882.7 ± 117.9 (3711.7–3987.9)	* 3922.2 ± 94.8 (3644.5–4091.3)
High Frequency (Hz)	4937.0 ± 169.6 (4648.3–5084.6)	4826.5 ± 145.6 (4592.7–4974.4)	* 4795.6 ± 85.8 (4586.6–5023.7)
Bandwidth (Hz)	1244.6 ± 204.7 (921.0–1486.7)	943.8 ± 250 (604.9–1262.7)	* 873.4 ± 163.5 (495.3–1248.1)
Dominant Frequency (Hz)	4310.9 ± 110.2 (4134.4–4435.8)	4332.5 ± 38.5 (4306.6–4392.8)	* 4344.0 ± 41.7 (4199.0–4392.8)
Frequency 5% (Hz)	4013.8 ± 58.2 (3940.6–4069.8)	4091.3 ± 80.6 (3962.1–4177.4)	* 4115.1 ± 41.8 (4005.2–4199.0)
Frequency 95% (Hz)	4646.8 ± 108.1 (4478.9–4758.8)	4578.0 ± 99.4 (4457.4–4715.8)	* 4586.6 ± 50.3 (4543.5–4737.3)
Bandwidth 90% (Hz)	633.1 ± 119.5 (516.8–818.3)	486.7 ± 160.1 (323.0–753.7)	* 471.5 ± 65.3 (409.1–646.0)
2nd Harmonic Frequency (Hz)**	8613 ± 685.2	8089.3 ± 442.5	NA
3rd Harmonic Frequency (Hz)**	11627.8 ± 1279	12511 ± 30.2	NA

and fundamental frequency equal to the dominant frequency of both notes, and secondary harmonics having a frequency ca. 8128 Hz and 11627 Hz, respectively, for the trilled note, and ca. 8089 Hz and 12510 Hz for the pulsatile notes.

Comparisons with Closely Related Species

The overall acoustic structure of the *D. frosti* call, including its complex and composite calls with two types of notes, is similar to those described for the most closely related species in the *D. parviceps* group (Table 2). The call of *D. frosti* can be distinguished from that of *D. subocularis* by the presence of the pulsatile notes, which are absent in *D. subocularis.* Additionally, the *D. frosti* call is differentiated by its shorter multispulsed note (trill) duration compared to *D. brevifrons* (ca. 460 ms), *D. counani* (ca. 300 ms) and *D. subocularis* (ca. 530 ms). Conversely, the trill note in *D. frosti* is longer (approximately 215 ms) than in *D. bokermani* (approximately 200 ms), *D. parviceps* (approximately 140 ms), *D. kamagarini* (approximately 140), and *D. kubricki* (approximately 190 ms).

Regarding spectral structure, the call of *D. frosti* has a dominant frequency lower than that of *D. parviceps* (around 6300 Hz), but higher than those of *D. subocularis, D. counani,* and *D. kamagarini,* which all have dominant frequencies below 4000 Hz. Among the remaining species, the dominant frequency varies between 4000 Hz and 4600 Hz, which fits with the variation that we described. Besides *D. frosti,* a harmonic structure has been observed in the *D. counani* call (two secondary harmonics with approximately 8000 Hz and 11000 Hz; Fouquet *et al.* 2015), *D. giesleri,* with approximately 6000–10000 Hz harmonics (Heyer 1980), and *D. microps* with harmonics in around 8000 Hz and 11500 Hz (Forti *et al.* 2015).

Habitat and Geographic Distribution

In all our observations of *D. frosti,* individuals were engaged in breeding activity, perched on vegetation above large temporary ponds within "terra-firme" forests (non-seasonally flooded habitat). Calling males were found perched on fallen logs beside the ponds or on vegetation at heights ranging from a few centimeters to 2m above the water, and females were found around the males, in the vegetation. We also found other species of frogs sharing this habitat in all reported sites, including *Dendropsophus sarayacuensis* (Shreve, 1935), *Dendropsophus* cf. *parviceps, Dendropsophus minutus* (Peters, 1872), and *Callimedusa tomopterna* (Cope, 1868).

Two of the localities accessed in this study were recently reported as new occurrences of the species (Koch *et al.* 2023). Here, we present a new record for the species from Central Amazonia, at the PNJ. Our record extends the distribution of *D. frosti* about 733 km east of the nearest known occurrence on the right bank of Japurá River and about 856 km east from the type-locality, in Letícia, Colombia, thereby establishing the easternmost limit of the known geographic range of the species (Figure 3).

Discussion

The lack of information on anuran advertisement calls is tipically associated with species with limited knowledge of their geographic distribution and general biology or with species that occur in remote, hard-to-access areas (Guerra *et al.* 2018). Our findings specifically augment the biological understanding of the poorly documented species *D. frosti,* previously known only from its type series.

The advertisement call structure of *D. frosti* described herein closely resembles that of other species within the *D. parviceps* group. These calls have been characterized as "short insect-like chirps" (*D. brevifrons,* Duellman 1978), "the chirping of a cricket" (*D. microps,* Lutz 1973), "ratling buzz of pulsed notes" (*D. timbeba,* Duellman 2005), "a high-pitched 'creeeek' followed or not by a series of shorter notes, "eek-eek-eek" (*D. pauiniensis* = *D. koechlini*, Duellman and Trueb 1989), "train" (*D. microps,* Forti *et al.* 2015), "trills" and "clicks" (*D. counani,* Fouquet *et al.* 2015), and characterized by primary and secondary notes (Duellman and Crump 1974, Duellman 1978). These different terminologies describe the acoustic signal of multipulsed and pulsatile notes emitted either randomly, in a series of pulsatile notes, or in a complex combination, similar to that we identified for *D. frosti,* although with variations in temporal and spectral note features. Notably, *D. microps* stands out for having a call structure with two different types of notes: a pulsed note with fused pulses and another shorter note with evident pulses (Forti *et al.* 2015). The lack of standardized terminology, and analyses of those calls make homology assessments dificult (Hepp *et al.* 2012).

Advertisement call structure and properties within *Dendropsophus* have been reported as a phylogenetically informative trait in *D. marmoratus* (Laurenti, 1768) (Orrico *et al.* 2009,

Table 2. Comparisons of temporal and spectral acoustic traits of closely related species oft he *Dendropsophus parviceps* group. Data obtained from literature ([1]Duellman 1970, [2]Duellman and Crump 1974, [3]Duellman 1978, [4]Martins and Cardoso 1987, [5]Heyer 1980, [6]Duellman and Pyles 1983, [7]Duellman and Trueb 1989, [8]Duellman 2005, [9]Forti *et al.* 2015, [10]Fouquet *et al.* 2015, [11]Rivadaneira *et al.* 2018). Abbreviations: NA = not available; A = the multipulsed note (trill); B = the high-pitched notes (secondary notes); numbers denote the source. * The complex call of *D. microps* described by Forti *et al.* (2015) is formed by one to five notes, and two kinds of notes: a longer note with fused pulses and the other note with evident pulses (B').

Species (Source)	Call type	Call duration (ms)	Note duration (ms)	Dominant frequency (Hz)
D. frosti (present study)	complex	272–502 (2–3 notes)	A = 158–297	A = 4048–4435
	composite multinote	129–646 (2–7 notes)	B = 9–12	B = 4258–4344
D. bokermanni (2, 6, 8,10)	complex	NA	A = 230–280	A = 4000–4652
	composite multinote	620 (5–7 notes)	B = 40	B= 4300
D. brevifrons (2, 3, 6, 8)	complex	750 (4–5 notes)	A = 430–490	4254–5115
	composite multinote		B = ~15	
D. counani (10)	complex	NA	A = 210–350	3780–3970
	composite multinote	330-540 (3–6 notes)	B = NA	
D. giesleri (5)	Single multipulsed note	300	300	3600
D. microps (9)	Single multipulsed note (trill)	670	A = 670	A = 4913
	complex *	8–1930 (1–5 notes)	B' = 340	B' = 4972
D. parviceps (11)	complex	260–2120	A = 60–240	A = 5081–6869
	composite multinote	NA	B = 28–82	B = 4866–6922
D. pauiniensis (7, 8)	complex	NA	A = 220–400	A = 4500
	composite multinote	NA	B = 20	B = NA
D. kamagarini (11)	complex	260–810	A = 90–200	A = 3164–4306
	composite multinote	NA	B = 30–100	B = 3164–4532
D. kubrickyi (11)	complex	230–630	A = 100–300	A = 3542–4394
	composite multinote	NA	B = 30–90	B = 3703–4500
D. subocularis (1)	Single multipulsed note (trill)	530	530	2200
D. timbeba (4, 8)	Single multipulsed note (trill)	400–600	400–600	3000–4200

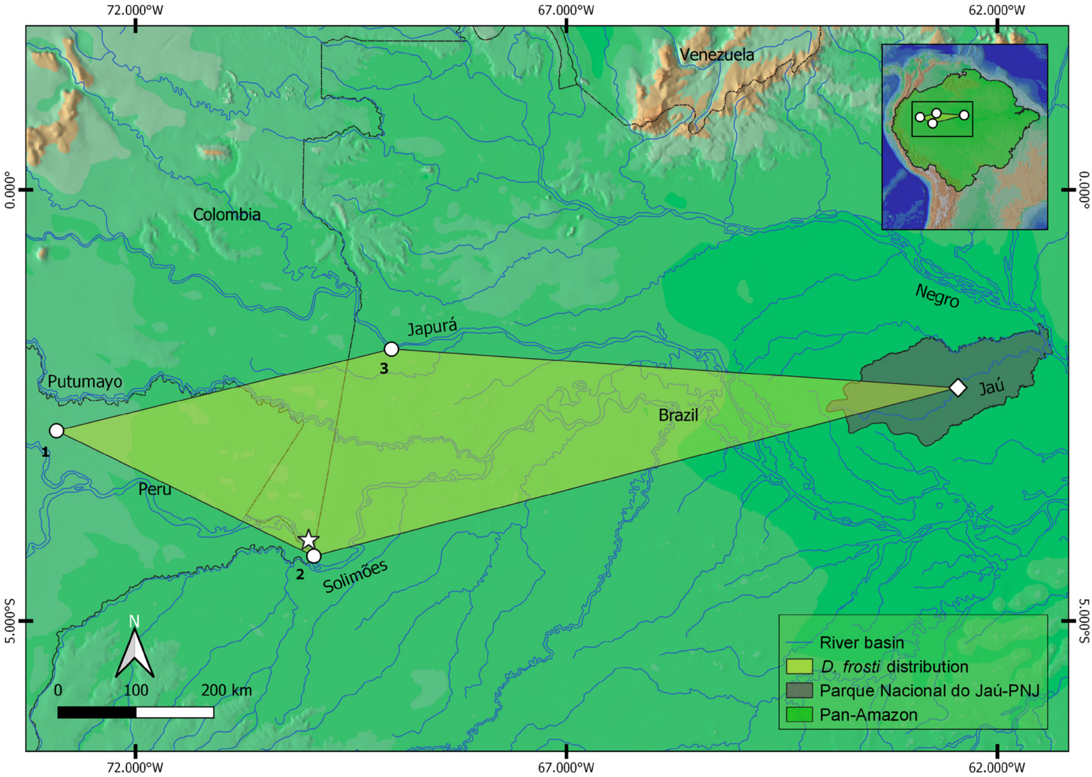

Figure 3. Geographic distribution of *Dendropsophus frosti* in northwestern Amazonia, highlighting the new record in Parque Nacional do Jaú (PNJ), which extends its distribution in ca.733 km to the nearest known occurrence. White star denotes the type locality in Leticia, Colombia (Motta *et al.* 2012); dots denote the known occurrences in (1) Piedras, Peru, near the Putumayo River (Motta *et al.* 2012); (2) Tabatinga municipality, Brazil (Koch *et al.* 2023, this study) and (3) near Vila Bittencourt village, Brazil, on the right bank of Japura River (Koch *et al.* 2023; this study). Diamond denotes the new occurrence in the PNJ.

Hepp *et al.* 2012), *D. microcephalus* (Cope, 1886) (Duellman 1970, Teixeira *et al.* 2013, Teixeira and Giaretta 2015), *D. leucophyllatus* (Beireis, 1783) (Forti *et al.* 2017), and *D. parviceps* groups (Duellman and Crump 1974), indicating it as synapomorphy for these groups, or for the clades with closely related species within the groups (e.g. Hepp *et al.* 2012). Our findings provide the first phenotypic evidence that supports the phylogenetic relationship of *D. frosti* within the *D. parviceps* group, with *D. bokermanni* as the closely related species from *D. subocularis* clade *sensu* Orrico *et al.* (2021). The advertisement call along with a terrestrial egg clutch reported by Koch *et al.* (2023), could be considered as two synapomorphies that *D. frosti* shares with the *D. subocularis* clade (Orrico *et al.* 2021).

Dendropsophus frosti inhabits large temporary ponds within forests, often near major river channels, as reported previously (Motta *et al.* 2012, Koch *et al.* 2023) and corroborated by our study. In all records, we observed the syntopic occurrence of other species of *Dendropsophus* from the *D. leucophyllatus* and *D. microcephalus* groups, as well as closely related species from the *D. parviceps* group (*D. parviceps* complex). This syntopic occurrence has also been noted in other instances, with sympatric and occasionally syntopic occurrences between *D. parviceps, D. brevifrons, D. bokermanni,* and *D. pauiniensis* (previously recognized as *D. koechlini*) in Ecuador and Peru (Duellman 1978, Duellman and Trueb 1989, Duellman 2005).

Regarding the distribution of species from the *D. parviceps* group, Orrico *et al.* (2021) described it as patchy, with species found in different domains of South America, most notably in Amazonia. *Dendropsophus frosti* was previously known only from the western Amazonian lowlands, with scattered records near its type-locality (Motta *et al.* 2012, Koch *et al.* 2023). The population from PNJ extends the known distribution to the interfluve between the Japurá and Negro rivers, suggesting it may be more widely distributed in northwestern Amazonia, as observed in many other hylid species (e.g. Carvalho *et al.* 2015, Ferrão *et al.* 2019, Almeida *et al.* 2021). Our study once again highlights the necessity of expeditions and fieldwork in remote areas in Amazonia to address knowledge shortfalls, such as the Raunkiæran and Wallacean shortfalls, in anuran biodiversity.

Finally, the overall similarities observed in habitat used by the species (including syntopic and sympatric occurrences), combined with the patterns of acoustic traits observed in the *D. parviceps* group, especially within the *D. subocularis* clade, may offer clues about sympatric speciation driven by sexual selection (Gerhardt 1994, Higashi *et al.* 1999). This ideia has also been suggested for the *D. microps* clade (Forti *et al.* 2015) and could be properly tested in a more comprehensive study.

Acknowledgments

We are thankful for fieldwork assistance from the local communities during all expeditions. APA thanks Fabiano Waldez and Weslei Valteran for logistic assistance during the fieldwork in Tabatinga. Also, APA thanks the Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq) for a postdoctoral fellowship (CNPq 25/2021 Pós-doutorado Junior PDJ 2021, process #15972/2022-6). RWA thanks CNPq for a research grant (PQ 305988/2018-2, 307722/2021-0). PP was supported by an award from the Maxwell/Hanrahan Foundation. MJS was financially supported by CNPq (process # 434362/2018-2). Fieldwork at Japurá river was financialy supported by CNPq (SISBIOTA Program #533348/2010) and Fundação de Amparo à Pesquisa do Estado do Amazonas—FAPEAM (Edital FAPEAM/SISBIOTA). The expedition to Parque Nacional do Jaú was supported by grants from the National Geographic Society and the Smithsonian Institution. Fieldwork at Tabatinga/AM received financial support from CNPq (Edital Universal #405640/2016-1). This work was suported by the Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq) under the grants (process #15972/2022-6; #PQ 305988/2018-2, 307722/2021-0; #434362/2018–2; #533348/2010; #405640/2016-1); Fundação de Amparo à Pesquisa do Estado do Amazonas—FAPEAM (#FAPEAM/SISBIOTA), the National Geographic Society and the Smithsonian Institution.

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Editor: Ariovaldo Giaretta

Appendix I. *Collection records of the recordings analyzed in the present study with catalog number, recording file identification, and localities where recordings were made. All recordings were deposited in Coleção Bioacústica da Universidade Federal de Minas Gerais (CBUFMG).*

Catalog number	Voucher Number	File-record name	Locality
CBUFMG 1119	INPA-H 470001 INPA-H 470002	DR_APA01_Dendrop frosti_TBT_050518	Tabatinga, Amazonas state, Brazil
CBUFMG 1120	Unvouchered specimen	DR_APA02_EK_D_frosti_Tabatinga_2021	Tabatinga, Amazonas state, Brazil
CBUFMG 1121	Unvouchered specimen	PLPDR 066 - Dendropsophus frosti - Parque Nacional do Jau - PNJAU029_nv	PNJ, Amazonas state, Brazil
CBUFMG 1122	Unvouchered specimen	PLPDR 067 - Dendropsophus frosti - Parque Nacional do Jau - PNJAU031	PNJ, Amazonas state, Brazil

Appendix II. *Museum numbers and occurrence data of the specimens of* Dendropsophus frosti *collected during three fieldwork expeditions in western Amazonia, indicating voucher information, localities, and coordinates. Recorded specimens in bold. Legend to localities:* PNJ, *Parque Nacional do Jaú, AM, Brazil;* T, *Tabatinga, AM, Brazil;* JR, *Japurá River, Vila Bittencourt, AM, Brazil.*

Voucher Number	Field Number	Locality	Geographic Coordinates
MPEG 41861	PLVP 681	PNJ	02°17'39" S, 62°27'21" W
MPEG 41862	PLVP 682	PNJ	02°17'39" S, 62°27'21" W
MPEG 41863	PLVP 683	PNJ	02°17'39" S, 62°27'21" W
MPEG 41864	PLVP 684	PNJ	02°17'39" S, 62°27'21" W
MPEG 41866	PLVP 720	PNJ	02°17'39" S, 62°27'21" W
MPEG 41867	PLVP 721	PNJ	02°17'39" S, 62°27'21" W
MPEG 41868	PLVP 722	PNJ	02°17'39" S, 62°27'21" W
MPEG 41869	PLVP 723	PNJ	02°17'39" S, 62°27'21" W
MPEG 41870	PLVP 724	PNJ	02°17'39" S, 62°27'21" W
MPEG 41865	PLVP 725	PNJ	02°17'39" S, 62°27'21" W
MPEG 41872	PLVP 726	PNJ	02°17'39" S, 62°27'21" W
MPEG 41871	PLVP 727	PNJ	02°17'39" S, 62°27'21" W
MPEG 41873	PLVP 728	PNJ	02°17'39" S, 62°27'21" W
MPEG 41874	PLVP 729	PNJ	02°17'39" S, 62°27'21" W
***INPA-H 47001**	**RSOL 22**	**T**	**04°14'52.77" S, 69°55'51.05" W**
***INPA-H 47002**	**RSOL 24**	**T**	**04°14'52.77" S, 69°55'51.05" W**
INPA-H 47003	RSOL 25	T	04°14'52.77" S, 69°55'51.05" W
INPA-H 47004	RSOL 32	T	04°14'52.77" S, 69°55'51.05" W
CZPB-AA 1555	CTGA-N 1893	JR	01°50'46.10" S, 69°01'46.30" W
INPA-H 38286	CTGA-N 1912	JR	01°50'46.10" S, 69°01'46.30" W
INPA-H 38288	CTGA-N 1935	JR	01°50'46.10" S, 69°01'46.30" W
INPA-H 38287	CTGA-N 2189	JR	01°50'46.10" S, 69°01'46.30" W
CZPB-AA 1554	CTGA-N 2190	JR	01°50'46.10" S, 69°01'46.30" W
CZPB-AA 1552	CTGA-N 2191	JR	01°50'46.10" S, 69°01'46.30" W
CZPB-AA 1553	CTGA-N 2192	JR	01°50'46.10" S, 69°01'46.30" W
INPA-H 38285	CTGA-N 2220	JR	01°50'46.10" S, 69°01'46.30" W