

# Rediscovery of *Dendropsophus tintinnabulum* (Anura: Hylidae) in the upper Rio Negro Drainage (Amazonas, Brazil), with a description of its advertisement call and external morphology

Bernardo Franco da Veiga Teixeira<sup>1,2</sup> and Ariovaldo Antonio Giaretta<sup>1</sup>

<sup>1</sup> Laboratório de Taxonomia, Sistemática e Ecologia Comportamental de Anuros Neotropicais, Faculdade de Ciências Integradas do Pontal, Universidade Federal de Uberlândia, Rua 20, 1.600, Bairro Tupã, 38304-402, Ituiutaba, MG, Brazil.

<sup>2</sup> Programa de Pós-graduação em Biologia Comparada, Universidade de São Paulo, Departamento de Biologia/FFCLRP, Avenida dos Bandeirantes, 3900, 14040-901, Ribeirão Preto, SP, Brazil. E-mail: [teixeirabfv@gmail.com](mailto:teixeirabfv@gmail.com).

## Abstract

**Rediscovery of *Dendropsophus tintinnabulum* (Anura: Hylidae) in the upper Rio Negro Drainage (Amazonas, Brazil), with a description of its advertisement call and external morphology.** *Dendropsophus tintinnabulum* is one of six species of the genus unassigned to any group. Voucher specimens and recordings of this taxon obtained during field work in the municipality of São Gabriel da Cachoeira and elsewhere in the district of Cucuí in Amazonas state, Brazil, permit a description of the advertisement call and external morphological features. Based on overall similarities in size, dorsal color, body shape, advertisement call, and preliminary genomic results, *D. tintinnabulum* is tentatively assigned to the *D. microcephalus* Group.

**Keywords:** Amazon, bioacoustics, *Dendropsophus microcephalus* Group.

## Resumo

**Redescoberta de *Dendropsophus tintinnabulum* (Anura: Hylidae) na bacia do Alto Rio Negro (Amazonas, Brasil) com a descrição do canto de anúncio e da morfologia externa.** *Dendropsophus tintinnabulum* é uma das seis espécies de *Dendropsophus* que ainda não foi atribuída a nenhum dos grupos de espécies atualmente aceitos. Espécimes-testemunhos e gravações desse táxon obtidos durante trabalho de campo no município de São Gabriel da Cachoeira e no distrito de Cucuí, estado do Amazonas, Brasil, permitem a descrição do canto de anúncio e de caracteres da morfologia externa. A partir de todas as similaridades em relação ao tamanho corporal, padrão de coloração, formato do corpo, canto e dados genômicos preliminares, alocamos tentativamente *D. tintinnabulum* no grupo de *D. microcephalus*.

**Palavras-chave:** Amazônia, bioacústica, grupo de *Dendropsophus microcephalus*.

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## Introduction

As presently defined, the genus *Dendropsophus* Fitzinger, 1843 comprises almost 100 species placed in nine species groups (Faivovich *et al.* 2005) and six species unassigned to any group. One of these species, *D. tintinnabulum* (Melin, 1941), was described from northern Brazil based on collections from field expeditions to tropical regions of South America during the years 1923–1926. Since then, the species rarely has been reported in the literature (Lutz 1973, Azevedo-Ramos and Galatti 2002). During a field trip in the upper Rio Negro, we collected and recorded individuals of *Dendropsophus* that we assigned to *D. tintinnabulum* based on the original description and area of occurrence. Herein, 65 years after the original description, we describe the advertisement call and external morphological features based on recently collected examples of this poorly known Amazonian species.

## Materials and Methods

Fieldwork was conducted in the municipality of São Gabriel da Cachoeira (0°09'43" S, 66°59'32" W; 79 m a.s.l.; 27 June 2014) and elsewhere in the district of Cucuí (1°11'55" S, 66°50'13" W; 88 m a.s.l.; 29 June 2014) in Amazonas state, Brazil.

Calls were recorded with a digital recorder (Marantz PMD 671) at a 48.0 kHz sampling rate and a 16-bit resolution (mono WAVE format file) coupled to a directional microphone (Sennheiser K6/ME67). Acoustic variables were analyzed using Raven Pro 1.5 for Windows from The Cornell Lab of Ornithology (Bioacoustic Research Program 2012); settings were Hann window function at FFT width 1024 samples, 3 dB filter bandwidth 244 Hz, overlap (locked) 85%, and DFT size (locked) 1,024 samples. All other settings followed the “default” of Raven Pro. Terminology of the call descriptions follows that of Duellman and Trueb (1994). Dominant frequency was obtained using the function “Peak frequency.” Pulse repetition rate was quantified as the number of pulses divided by note duration.

Sound figures were obtained in the Seewave package (Version 1.5.9) (Sueur *et al.* 2008), R (Version 2.12.1) platform (R Development Core Team 2014), using Hanning window function, 85% overlap, and 256 points resolution (FFT).

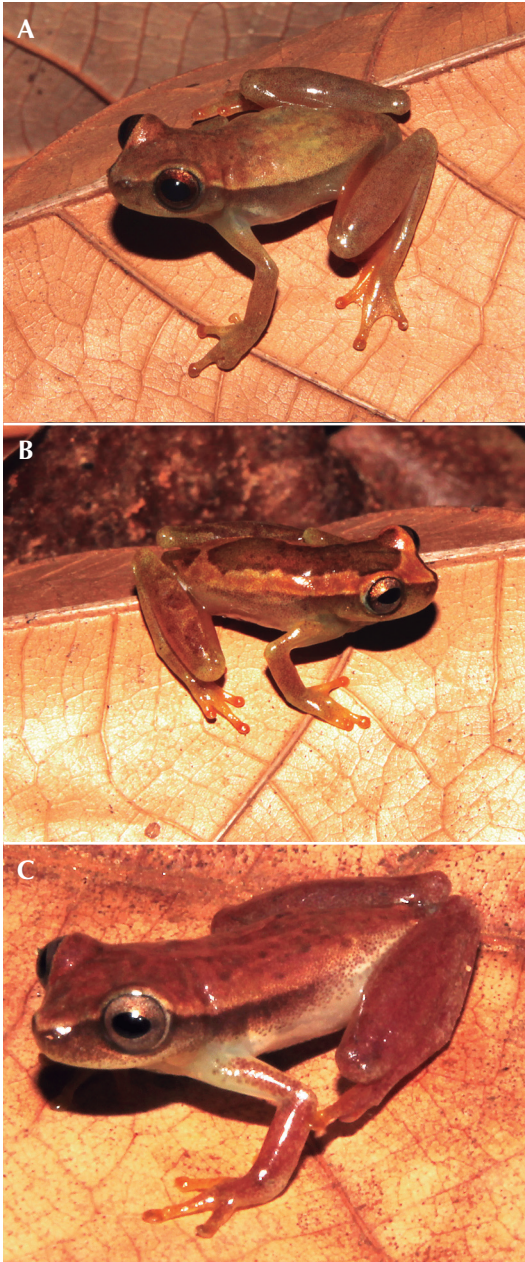
Specimens were euthanized in 5% lidocaine, fixed in 10% formalin and preserved in 70% ethanol, and identified based on Melin’s (1941) description of *Dendropsophus tintinnabulum*. Measurements were made with an ocular micrometer coupled to a stereomicroscope, and those greater than 10 mm were taken with calipers accurate to 0.1 mm. Nine measurements follow those defined by Duellman (1970): snout-vent length (SVL), head length (HL), head width (HW), tympanum diameter (TD), eye diameter (ED), interorbital distance (IOD), internarial distance (IND), shank length (SL) (= tibia length), and foot length (FL) and two measurements are those of Heyer *et al.* (1990)—thigh length (TL) and hand length (HAL). Eye-nostril distance (END) is as defined by Napoli and Caramaschi (1998).

Specimens examined and recordings along with the voucher specimens (Appendix I) are in the collection of frogs of the Universidade Federal de Uberlândia (AAG-UFU), Uberlândia municipality, Minas Gerais state, Brazil. Additional specimens examined are deposited in the following zoological collections: Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), and Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ), Brazil. We were unable to obtain data for the three syntypes of *D. tintinnabulum* at Museum of Natural History of Gothenburg, Sweden. Preliminary results based on genetic data are not presented here because a phylogenetic study has been undertaken by another research group.

## Results

### *Species Identification*

The series of frogs from the municipality of São Gabriel da Cachoeira and its environs in the



**Figure 1.** *Dendropsophus tintinnabulum* from the municipality of São Gabriel da Cachoeira, Amazonas state, Brazil. (A) AAG-UFU 3883; SVL = 17.4 mm; (B) AAG-UFU 3884; SVL = 19.2 mm; (C) AAG-UFU 3854; SVL = 18.4 mm (voucher).

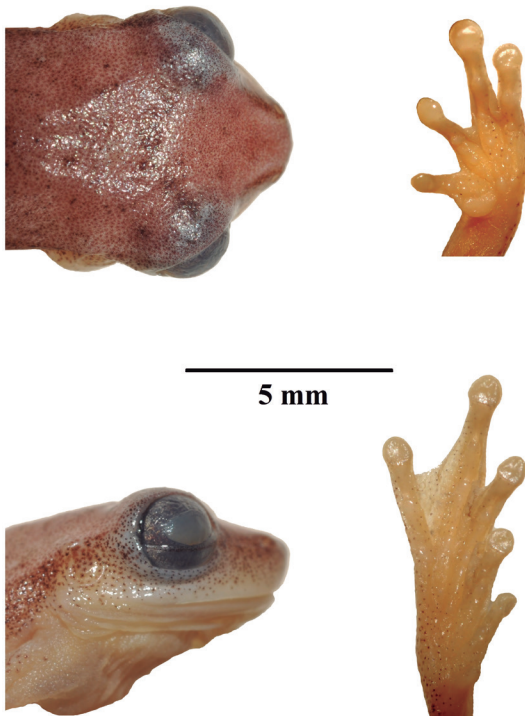
district of Cucuí was assigned to *D. tintinnabulum* based on the following combination of characters: snout-vent length ranging from 16.4–19.2 mm (Table 1); head broader than body, vomerine teeth barely visible; snout triangular to rounded; large eyes, protruding beyond lateral sides of head; tympanum discretely visible; a small knob is present at the mandibular symphysis; prepollical tubercle large; slight, elliptical medial metatarsal tubercle, and lateral tubercle and tarsal fold absent. Skin smooth; belly and medial parts of thighs granular; axillary fold distinct; single and subgular vocal sac, yellowish orange; dorsal color in life varying from yellowish orange to greenish brown; dorsal color pattern varying from immaculate to possessing irregularly distributed brown dots; some frogs with broad stripe between eyes and along dorsum; dots variably present on shanks; toes and fingers orange; diffuse brown broad band along dorsolateral limits of body extending from the snout tip toward sacral region, upper edged by a light-colored stripe (Figures 1, 2).

*Advertisement Call of Dendropsophus tintinnabulum* (N = 3 recorded males)

The advertisement call (Figure 3) consists of one type of note with a pulsed structure emitted in groups (series) or isolated (mean: 1.35 notes per call; range: 1–4 notes; SD = 0.13; N = 60). Average call parameters are as follow: note duration, 16 ms (range: 10–21 ms; SD = 1.7; N = 80); number of pulses/note, 3 (range: 2–4; SD = 3.2; N = 78); pulse duration, 5.5 ms (range: 3.0–10.0; SD = 0.5; N = 47); pulse repetition rate, 188.9 pulses/s (range: 133.3–266.7; SD = 11.4; N = 78); and note repetition rate (within a series), 7.0 notes/s (range: 5.3–8.4; SD = 0.5; N = 15); inter-note interval in series of notes, 229 ms (range: 206–263; SD = 4; N = 20); and dominant frequency, 5691 Hz (range: 5343–5906; SD = 56.8; N = 82). A weak frequency peak occurs at about 12 kHz (2 of 3 males) and a third peak with relevant energy is present (2 of 3 males) in 17033 Hz (range: 16688–17344; SD = 195.1; N = 38). Individual values are presented in Table 2.

**Table 1.** Morphometric variables of *Dendropsophus tintinnabulum* (N = 8 males) from municipality of São Gabriel da Cachoeira, Amazonas state, Brazil.

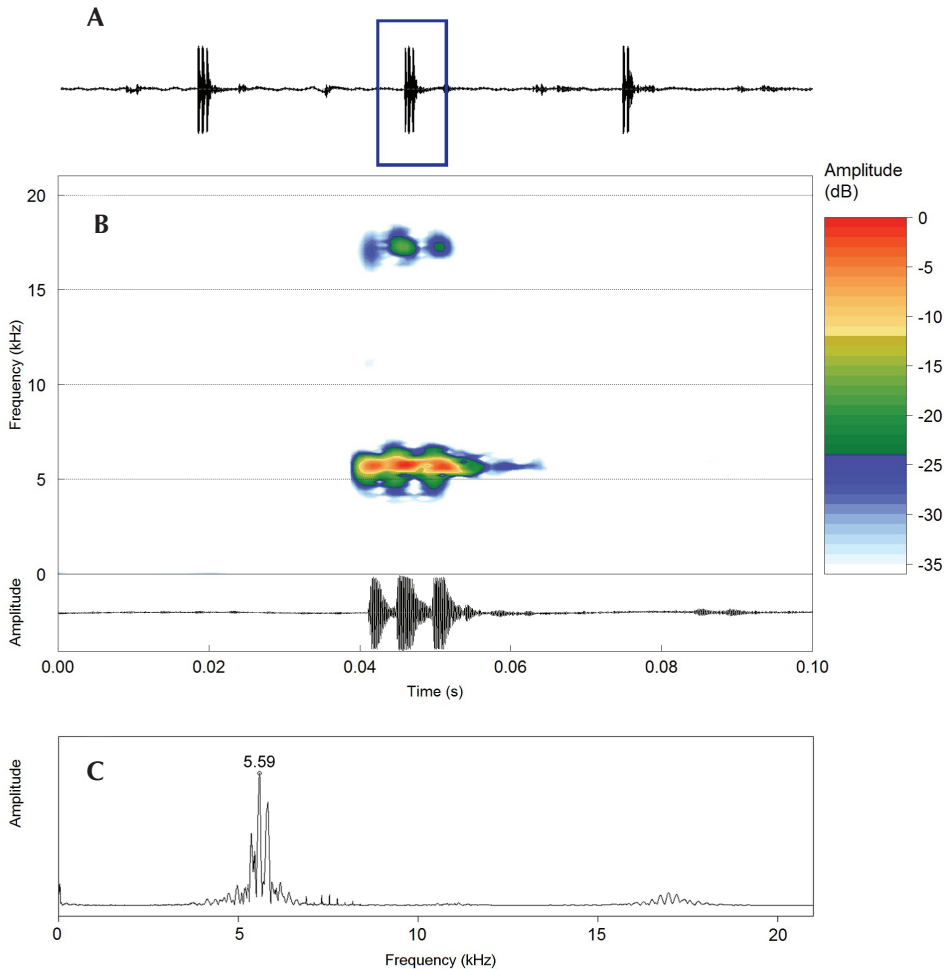
	Mean $\pm$ SD	Range
Snout-vent length	17.9 $\pm$ 0.8	16.4–19.2
Head length	5.7 $\pm$ 0.2	5.5–5.9
Head width	6.7 $\pm$ 0.3	6.0–7.0
Interorbital distance	2.1 $\pm$ 0.2	1.7–2.5
Internarial distance	1.3 $\pm$ 0.2	1.1–1.6
Eye-nostril distance	1.4 $\pm$ 0.1	1.2–1.5
Eye diameter	2.2 $\pm$ 0.1	2.1–2.5
Tympanum diameter	1.0 $\pm$ 0.1	0.8–1.2
Thigh length	9.7 $\pm$ 0.6	8.5–10.5
Shank length	9.9 $\pm$ 0.5	9.0–10.8
Hand length	5.2 $\pm$ 0.4	4.7–6.0
Foot length	8.0 $\pm$ 0.8	7.1–9.7

**Figure 2.** *Dendropsophus tintinnabulum* (AAG-UFU 3883), adult male from the municipality of São Gabriel da Cachoeira, Amazonas state, Brazil. Lateral and dorsal views of the head and palmar and plantar views of hand and foot, respectively.

Male *Dendropsophus tintinnabulum* call from perches on herbaceous vegetation 0.2–1.0 m above the ground along the river margins (Rio Negro), typically in “Igapó” flooded-forest environments. Syntopic species include *Scinax cruentommus* (Duellman, 1972), *Hypsiboas boans* (Linnaeus, 1758), *H. cinerascens* (Spix, 1824), *Leptodactylus validus* Garman, 1888, *Rhinella marina* (Linnaeus, 1758), *Rhinella* sp. (*R. margaritifera* group), and *Scinax ruber* (Laurenti, 1798).

## Discussion

The combination of snout-vent length, body shape, dorsal color patterns, and the large, prominent eyes of *Dendropsophus tintinnabulum* is most similar to this suite of characters in the Amazonian species of the *D. microcephalus* Group (i.e., *D. leali* [Bokermann, 1964]; *D. juliani* Moravec, Aparicio and Köhler, 2006; *D. reichlei* Moravec, Aparicio and Köhler, 2008; *D. riveroi* [Cochran and Goin, 1970]; and *D. coffea* Köhler, Jungfer, and Reichle, 2005). Based on these resemblances, the advertisement call, and the preliminary genetic analysis of *D.*



**Figure 3.** Advertisement call of *Dendropsophus tintinnabulum* from the municipality of São Gabriel da Cachoeira, Amazonas state, Brazil. Note the remarkably strong harmonic (3<sup>rd</sup> harmonic) that is about 17 kHz. (A) Waveform of a call sequence (1.8 s section); the note outlined is detailed in (B), along with its waveform, and (C) power spectrum. Sound file: Dendrop\_tintinnabSaoGabrielAM1aTRC\_AAGm671. Air temperature 30°C.

*tintinnabulum*, we tentatively assign *D. tintinnabulum* to the *D. microcephalus* Group.

*Dendropsophus tintinnabulum* differs morphologically from the other Amazonian members of the *D. microcephalus* Group as follows (*D. tintinnabulum* in brackets): *D. shiwiarum* Ortega-Andrade and Ron, 2013 and *D. ozzyi* Orrico, Peloso, Sturaro, Silva-Filho, Neckel-

Oliveira, Gordo, Faivovich, and Haddad, 2014 have pointed fingers discs (rounded in *D. tintinnabulum*); *D. juliani* has a green vocal sac (Moravec *et al.* 2006) (yellowish orange); *D. reichlei* has one or two small, white to cream spots below the eye (Moravec *et al.* 2008); and *D. microcephalus* (Cope, 1886), *D. nanus* (Boulenger, 1889), and *D. walfordi* (Bokermann,

**Table 2.** Advertisement call of *Dendropsophus tintinnabulum* specimens from São Gabriel da Cachoeira, Amazonas state, Brazil. Mean  $\pm$  SD (range) [number of analyzed calls].

Traits	AAG-UFU 3856 (SVL = 17.5 mm)	AAG-UFU 3857 (SVL = 17.7 mm)	AAG-UFU 3854 (SVL = 18.4 mm)
Note duration (ms)	17.5 $\pm$ 2.1 (13.0–21.0) [20]	16.1 $\pm$ 1.6 (12.0–19.0) [40]	14.2 $\pm$ 2.4 (10.0–19.0) [20]
Pulse duration (ms)	17.5 $\pm$ 2.1 (13.0–21.0) [16]	17.5 $\pm$ 2.1 (13.0–21.0) [16]	17.5 $\pm$ 2.1 (13.0–21.0) [15]
Number of pulses per note	5.9 $\pm$ 1.7 (5.0–10.0) [16]	4.9 $\pm$ 0.9 (4.0–7.0) [16]	5.6 $\pm$ 1.4 (3.0–9.0) [15]
Peak frequency (Hz)	5770 $\pm$ 79.7 (5625–5906) [20]	5639 $\pm$ 91.6 (5343–5765) [41]	5665 $\pm$ 91.6 (5390–5906)
Third harmonic (Hz)	17228 $\pm$ 71.8 (17109–17344) [20]	16839 $\pm$ 85.0 (16688–17016) [18]	Not detected
Pulse repetition rate	184.2 $\pm$ 21.0 (157.9–230.7) [20]	201.8 $\pm$ 28.3 (157.9–266.7) [38]	180.6 $\pm$ 22.2 (133.3–230.8) [20]
Call group rate (notes per s)	6.9 $\pm$ 0.8 (6.1–8.0) [3]	7.6 $\pm$ 0.6 (6.3–8.4) [8]	6.6 $\pm$ 1.2 (5.3–7.8) [4]
Number of notes per call	1.3 $\pm$ 0.6 (1.0–3.0) [16]	1.3 $\pm$ 0.5 (1.0–3.0) [30]	1.5 $\pm$ 0.9 (1.0–4.0) [14]
Number of notes per sequence	2.3 $\pm$ 0.5 (2.0–3.0) [3]	2.1 $\pm$ 0.3 (2.0–3.0) [8]	2.8 $\pm$ 0.8 (2.0–4.0) [4]
Internote interval in series (s)	0.23 $\pm$ 0.19 (0.21–0.26) [4]	0.22 $\pm$ 0.01 (0.20–0.25) [9]	0.23 $\pm$ 0.01 (0.22–0.24) [7]

1962) have numerous dotted lines on dorsum (dots irregularly distributed). We did not find any reliable qualitative morphological differences between *D. leali*, *D. coffea*, *D. riveroi*, and *D. tintinnabulum*.


Acoustically, *Dendropsophus tintinnabulum* can be distinguished from the other Amazonian species of the *D. microcephalus* Group that have advertisement calls composed of one type of note. *Dendropsophus juliani* has a lower dominant frequency (dominant frequency 3300–4400 Hz vs. 5484–5812 Hz in *D. tintinnabulum*; Moravec *et al.* 2006). *Dendropsophus ozyzi* has a higher dominant frequency (9130–10136 Hz; Orrico *et al.* 2014). The note duration in other species of the group are as follow: *D. reichlei*, 68–112 ms (Moravec *et al.* 2008); 38 ms in *D. walfordi* (De la Riva *et al.* 1997); 53 ms in *D. shiwiarum* (Ortega-Andrade and Ron 2013); 34–92 ms in *D. leali* (Marquez *et al.* 1993); 20–29

ms in *D. riveroi* (Marquez *et al.* 1993); and 60–116 ms in *D. coffea* (Köhler *et al.* 2005). The note duration is 10–21 ms in *D. tintinnabulum*.

In comparison with the species that occur in the adjacent biomes, the advertisement call of *Dendropsophus tintinnabulum* is most like those of *D. cachimbo* (Napoli and Caramaschi, 1999), *D. cruzi* (Pombal and Bastos, 1998), and *D. pseudomeridianus* (Cruz, Caramaschi, and Dias, 2000). In *D. tintinnabulum*, the dominant frequency (5484–5812 Hz) is higher than in *D. cachimbo* (3890–4406 Hz; Teixeira and Giaretta 2015). The note duration of *D. tintinnabulum* (10–21 ms) longer than in *D. cruzi* (7–9 ms; Pombal and Bastos 1998). Although there is an overlap in ranges of call values between *D. tintinnabulum* and *D. pseudomeridianus*, the former has a lower mean of pulse repetition rate (189 pulses/s) than *D. pseudomeridianus* (477 pulses/s; Caram *et al.* 2014).

*Dendropsophus tintinnabulum* was described from Uaupés River (“some days’ journey north of Ipanoré”, Melin 1941 page 29). Our record from Cucuí extends the distribution of the species to the upper Rio Negro to the Brazilian/Venezuelan/Colombian borders, around 200 km northeast of the type locality (Appendix I). *Dendropsophus tintinnabulum* probably has a wider distribution along the Rio Negro Drainage. Perhaps our data will be helpful in the assessment of additional distributional records for this Amazonian frog species.

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**Appendix I. Specimens examined.**

*Dendropsophus tintinnabulum*: BRAZIL: AMAZONAS: São Gabriel da Cachoeira: AAG-UFU 3854–3857, Distrito de Cucuí 3882–3884; Taracá: MZUSP 118212–118213.

*Dendropsophus leali*: type specimens: MZUSP 74210 (Ex. WCAB 10397); MZUSP 74483–74486 (EX. WCAB 10379, 10388, 10390, 10394).

*Dendropsophus riveroi*: COLOMBIA: AMAZONAS: Leticia MZUSP 95883–95886 (Ex. WCAB 11751–11754) (Topotypes).

*Dendropsophus cruzi*: type specimens: MNRJ 21782; GOIÁS: Silvânia: AAG-UFU 1987–1990 (Topotypes).

*Dendropsophus walfordi*: type series: MZUSP 73652 (Ex. WCAB 8436); MZUSP 74019–74041; topotype specimens: AAG-UFU 5306–5327.

**Appendix II. AAG-UFU collection voucher number and labels of the related analyzed sound files.**

Voucher number	Sound file labels
3856	Dendrop_tintinnabSaoGabrielAM1aTRC_AAGm671
3857	Dendrop_tintinnabSaoGabrielAM2TRC_AAGm671
3854	Dendropsophus_tintinnabulum_SaoGrabrielDaCachoeira_AM_1b_BFVT_AAG_m671