ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 37: 421–428 (2024)
DOI 10.3897/herpetozoa.37.e134923

Vocalizations of *Atelopus ignescens* (Cornalia, 1849): The repertoire of a resilient species that whispers not to disappear

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https://zoobank.org/1A600734-07C3-4287-B737-B43F78279706

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Academic editor: Bibiana Rojas ♦ **Received** 17 August 2024 ♦ **Accepted** 3 December 2024 ♦ **Published** 18 December 2024

Abstract

The *Atelopus* (or harlequin frogs) have become a symbol of the ongoing efforts to conserve biodiversity in Ecuador over the past few decades. *Atelopus ignescens*, also known as jambato, was previously considered to be an extinct species until it was rediscovered in the Andean locality of Angamarca, Ecuador. This study presents and describes the calls of *Atelopus ignescens* for the first time. These are low intensity sounds that comprise a repertoire of three distinct call types: tonal, short and pulsed call. They have different spectral and temporal characteristics. The most notable difference in their structure is the presence or absence of pulses and the frequency modulation observed in tonal calls. In terms of spectral characteristics, the mean dominant frequency is 1.7 kHz, with up to 11 harmonics visible. A peculiar feature of the Jambato is the absence of a tympanic middle ear, a characteristic observed in the majority of *Atelopus* species. The absence of these structures, however, does not mean that harlequin toads are deaf since they seem to have extratympanic mechanisms of audition. It is our hope that our contribution will stimulate further research into the bioacoustics and behavior of this endangered and relatively unknown group of anurans.

Key Words

acoustic communication, bioacoustics, harlequin frogs, jambato, vocal repertoire,

Atelopus ignescens (Cornalia, 1849) is a species of diurnal anuran endemic to Ecuador that belongs to the family Bufonidae. The species is characterized by a medium size, a black coloration, and a reddish belly (Coloma et al. 2000). It is more commonly known by its vernacular name, "jambato," which is derived from the Kichwa terminology utilized by indigenous communities to refer to any members of this group of anurans as "sapo" or "rana" (Coloma and Guayasamin 2021). *Atelopus ignescens* was regarded as the most common, abundant, and widely distributed of the high Andean species in Ecuador (Peters 1973; Coloma et al. 2000). However, in the early 1980s, the species experienced a precipitous decline in population, and it was believed to reach extinction (Ron et al. 2003). This decline is not unique to this species. Throughout the Neotropics, a critical decline in populations of *Atelopus* species has been reported as a result of multiple impacts (e.g. infectious diseases caused by

the *Batrachochytrium dendrobatidis* fungus, destruction of their habitats due to the expansion of the agricultural frontier and the overpopulation of introduced species such as *Oncorhynchus mykiss*) (La Marca et al. 2005; Pounds et al. 2006; Scheele et al. 2019; Forero Rodríguez et al. 2024). The history of the Jambato is not solely defined by this alarming and catastrophic disappearance. There are also fantastic accounts of its rediscovery and resilience amid a complex environmental crisis that continues to affect amphibians globally (Coloma 2016; Jaynes et al. 2022; Vega-Yánez et al. 2024; Yánez-Muñoz 2024).

The behavior of the *Atelopus* species is characterized by multimodal communication, which integrates auditory and visual signals (Cocroft et al. 1990; Lötters 1996). Acoustic communication has been described for 24 out of 100 species of its total diversity (Lötters et al. 2019; Rueda-Solano et al. 2020). In contrast to information on taxonomy, conservation, status and certain aspects of natural history (e.g. Coloma et al. 2010; Yánez-Muñoz et al. 2010; Tapia et al. 2017; Ortega-Andrade et al. 2021; Lötters et al. 2023; Vega-Yánez et al. 2024), the existing knowledge on the bioacoustics and behavioral characteristics of the *Atelopus* species of Ecuador is notably scarce.

The majority of *Atelopus* species lack a tympanic middle ear (Lötters 1996), which renders acoustic communication enigmatic, and perhaps explains the scarcity of studies in this subject (Womack et al. 2016, 2017, 2018). In the present study, we describe the vocal repertoire of *Atelopus ignescens*, the most emblematic anuran in Ecuador.

We recorded vocalizations of the jambato from the Andean locality of Angamarca, province of Cotopaxi, Ecuador (1°7'51.31"S, 78°54'16.85"W; 2962 m; Fig. 1). The area is situated in the western foothills of the An-

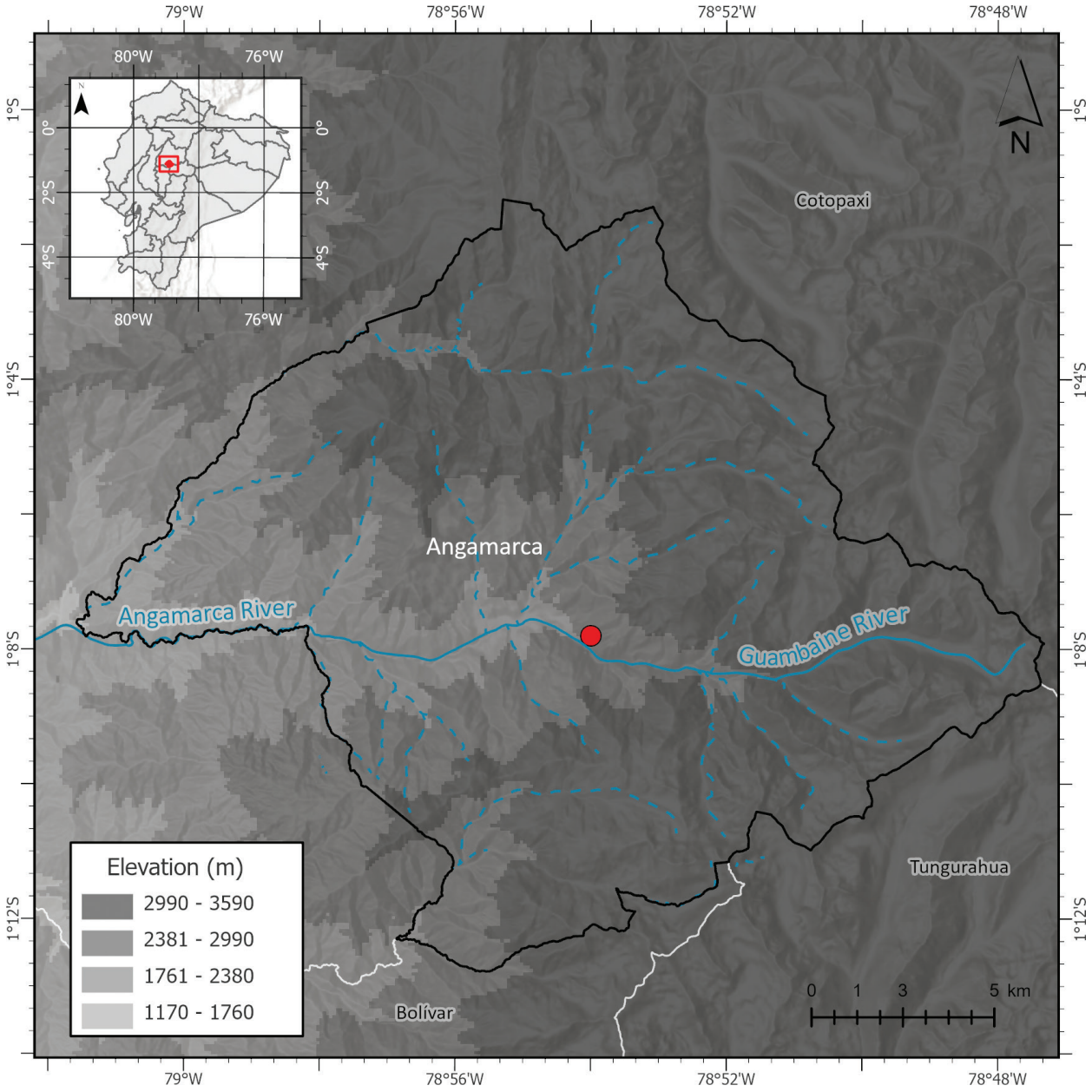

Figure 1. Map of the location of Angamarca (Ecuador), where the recordings of *Atelopus ignescens* were made.

des Mountains, belonging to the evergreen shrubland and paramo grassland ecosystem. The habitat is predominantly characterized by agricultural mosaic areas, grasslands and páramo vegetation (Vega-Yánez et al. 2024).

The vocalizations of an uncollected adult male of *A. ignescens* were recorded between 10:00–12:00 on December 8, 2021. The male was calling from grasslands and herbaceous vegetation at ground level. The calls were recorded using a cell phone, saving the audio files in FLAC format at a sampling rate of 44.1 kHz and 24 bits resolution. Prior to analysis, the audio files were converted to the WAV format and edited using the Adobe Audition CS6 software. The recordings are deposited at the Laboratorio de Biología Evolutiva de la Universidad San Francisco de Quito (LBE) and the Fonoteca Zoológica (www.fonozoo.com) del Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain (Appendix 1). The research was conducted under permits granted by the Ministerio de Ambiente, Agua y Transición Ecológica No. MAATE-DBI-CM-2022-0245.

The spectral and temporal parameters of calls were analyzed with the software Raven 1.6 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology 2024). We used a Hann window with 256 samples of the Fast Fourier Transformation (FFT), a 90% overlap, and a frequency grid with 512 samples of the Discrete Fourier Transformation (DFT). The analyzed parameters were: Call duration (**CD**), Rise time (**RT**), Decay time (**DT**) Intervals between calls (**IC**), Call rate (**CR**) Notes/call (**NC**), Note Duration (**ND**), Intervals between notes (**IN**), Note rate (**NR**) Pulse/note (**PN**), Pulse duration (**PD**) Intervals between pulses (**IP**), Pulse rate (**PR**), Dominant frequency (**DF**), Minimum frequency (**MinF**), Maximum frequency (**MaxF**), Frequency modulation (**FM**), Number of visible harmonics (**NH**), Harmonic frequency range, series of values that correspond to multiples of the fundamental frequency, with the value of the second and last harmonic taken as the reference (**HRF**). Definitions, terminology, and measurements of acoustic parameters follow the terms of Cocroft and Ryan (1995), Köhler et al. (2017), and Sueur (2018). The definition of call structure and the calculation of frequency modulation were based on Emmrich et al. (2020). In addition, to define the call types of the genus *Atelopus*, we follow the classification proposed by Cocroft et al. (1990). The oscillogram and spectrogram were obtained using R software (R Core Team, 2024), with the Seewave package version 2.2.3 (Sueur et al. 2008). To create the figures, we used a Hann window at 99% overlap with a size of 512 samples of the fast Fourier transform (FFT). The audio files in WAV format were imported with the tuneR package version 1.4.7 (Ligges et al. 2024). For each acoustic parameter, we calculated central tendency (means) and dispersion measures (maximum, minimum, and standard deviation).

The vocalizations of *Atelopus ignescens* comprises three distinct call types, ranging from non-pulsed to pulsed calls (Fig. 2, Table 1). They are low-intensity sounds, rendering them difficult to discern at close range. Intriguingly, their sounds remind us of rodent whispering (e.g. the vocalizations of *Rhipidomys albujai* described in Brito et al. (2017)). The mean call duration across these

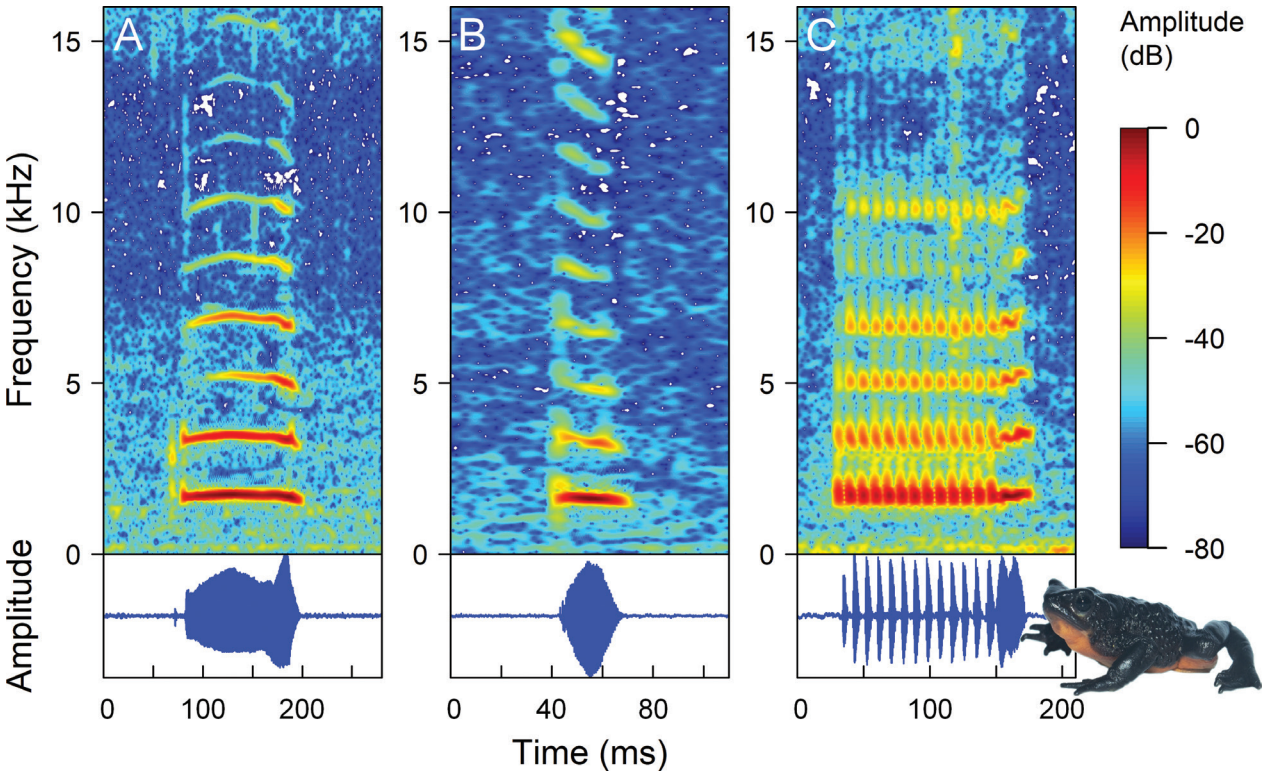

Figure 2. Call types present in *Atelopus ignescens* vocalization. **A.** Tonal call (Tc); **B.** Short call (Stc); **C.** Pulsed call (Pc). Spectrograms obtained using the Hann window at 99% overlap and 512 samples of FFT siz

Table 1. Spectral and temporal values of *Atelopus ignescens*. The abbreviations used in the parameters correspond to: DF = Dominant frequency; MinF = Minimum frequency; MaxF = Maximum frequency; FM = Frequency modulation; NH = Number of visible harmonics; HRF = Harmonic Frequency Range; CD = Call duration; RT = Rise time; DT = Decay time; IC = Intervals between calls; CR = Call rate; NC = Notes/call; ND = Note duration; IN = Intervals between notes; NR = Note rate; PN = Pulses/Note; PN = Pulse duration; IP = Interval between pulses; PR = Pulse rate. The abbreviations used in the call types correspond to: Tc = Tonal call; Stc = Short call; Pc = Pulsed call. The abbreviations used in units of measurement correspond to: kHz = kilohertz; ms = milliseconds; s = seconds; /min = per minute; /s = per second. We indicate: number of specimens/calls/notes/pulses.

Parameters	Call (general)	Call Types		
	n = 1/15/15/27	Tc	Sc	Pc
DF (kHz)	1.5–1.8 (1.7 ± 0.07)	1.64–1.81 (1.71 ± 0.06)	1.46–1.81 (1.60 ± 0.09)	1.55–1.72 (1.68 ± 0.05)
MinF (kHz)	1.03–1.64 (1.50 ± 0.12)	1.55–1.72 (1.64 ± 0.08)	1.29–1.55 (1.44 ± 0.06)	1.03–1.64 (1.51 ± 0.14)
MaxF (kHz)	1.55–2.07 (1.78 ± 0.12)	1.64–1.89 (1.71 ± 0.10	1.55–1.98 (1.70 ± 0.10)	1.72–2.07 (1.83 ± 0.12)
FM (Hz/ms)	0–14.38 (5.64 ± 4.26)	0.27–1.56 (0.91 ± 0.48)	1.71–14.38 (7.93 ± 3.34)	0–0.92
NH	5–11 (8.86 ± 1.63)	6–10 (9.67 ± 0.52)	8–11 (9.76 ± 0.83)	5–10 (8.11 ± 1.80)
HRF (kHz)	2.93–17.14	3.27–17.05	2.93–17.05	3.93–17.14
CD (ms)	27–1716 (370 ± 460.98)	111–322 (209.33 ± 81.94)	21–51 (33.82 ± 10.20)	189–137
RT %	18–91 (42.68 ± 16.44)	18–52 (35.75 ± 14.2)	30–70 (47.63 ± 11.13)	20–67 (35.83 ± 12.25)
DT%	9–82 (57.32 ± 16.44)	48–82 (64.25 ± 14.2)	30–70 (52.38 ± 11.13)	33–80 (64.17 ± 12.25)
IC (s)	2283–19178 (7465.54 ± 5193.34)	–	–	–
CR (/min)	3.13–25.97 (11.38 ± 7.19)	–	–	–
NC	1–4 (1.56 ± 0.89)	–	–	–
ND(ms)	21–266 (66.33 ± 72.83)	–	–	–
IN (ms)	41–612 (418.11 ± 170.87)	–	–	–
NR (/s)	1.57–10.87 (2.87 ± 3.01)	–	–	–
PN	13–15	–	–	–
PD (ms)	3–21 (7.67 ± 4.26)	–	–	–
IP (ms)	2–7 (4.76 ± 1.33)	–	–	–
PR (/s)	62.5–142.86 (90.20 ± 24.74)	–	–	–

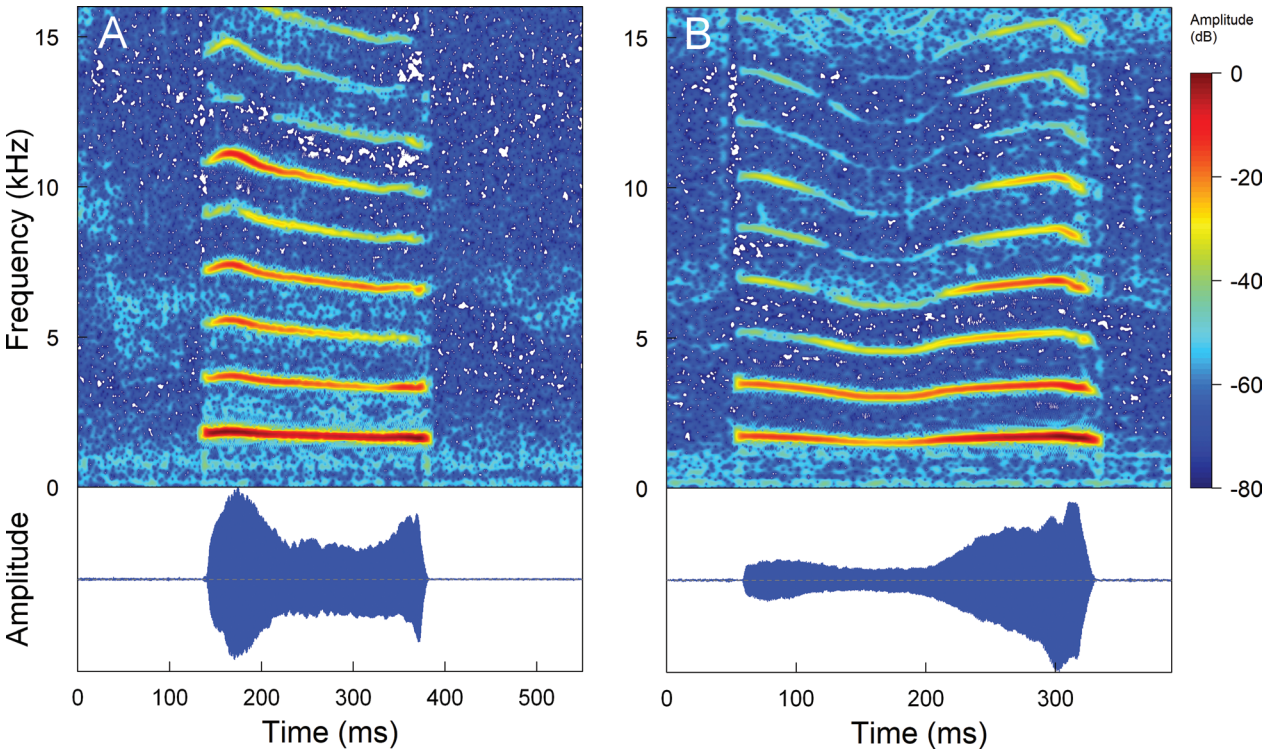

Figure 3. Variability of the tonal call of Atelopus ignescens, based on frequency modulation patterns **A.** Downward modulation tonal call; **B.** Complex modulation tonal call (upward-downward). Spectrograms obtained using the Hann window at 99% overlap and 512 samples of FFT size.

vocalizations in general is 370 ± 460.98 ms, emitted at mean intervals of 7465.54 ± 5193.34 ms, with a mean rate of 11.38 ± 7.19 calls/minute. The calls are composed of a mean of 1.56 ± 0.89 notes. The mean note duration is 66.33 ± 72.83 ms, emitted at mean intervals of 418.11 ± 170.87 ms, with a mean rate of 2.87 ± 3.01 notes/second. Some calls are composed of 13–15 pulses. The mean pulse duration is 7.67 ± 4.26 ms, emitted

at mean intervals of 4.76 ± 1.33 ms, with a mean rate of 90.20 ± 24.74 pulses/second. The calls are downward frequency modulated (non-frequency modulated in pulsed calls), with a mean frequency modulation of 1.66 ± 0.07 Hz/ms. The mean dominant frequency is 1.66 ± 0.07 kHz. The mean minimum frequency is 1.50 ± 0.12 kHz, while the mean maximum frequency is 1.78 ± 0.12 kHz. Up to 11harmonics are visible, with the second having a mean frequency of 3.32 ± 0.15 kHz (ranging from 2.93–3.62 kHz) and the eleventh a mean frequency of 16.71 ± 0.34 kHz (ranging from 16.37–17.05 kHz) (Fig. 2, Table 1).

The vocalizations of *Atelopus ignescens* lack a stereotyped structural pattern. They are classified into three distinct types of calls based on their structural and temporal characteristics. Two of the types of vocalizations are non-pulsed calls. 1) *Tonal calls* (Tc; Fig. 2A) are defined as having a duration exceeding 100 ms. The majority of calls exhibit a downward modulated frequency, which in some instances can be a complex modulation between upward-downward (Fig. 3). The amplitude envelope demonstrates a mean rise of 35.75% and a mean decay of 64.25% of the total call time. This indicates that this particular type of call reaches its maximum amplitude in a relatively short period of time, with a subsequent progressive decay. 2) *Short calls* (Sc; Fig. 2B) are calls characterized by a duration not exceeding 100 ms and a downward frequency modulation. The amplitude envelope demonstrates a mean rise of 47.63% and a mean decay of 52.38% of the total call time. This indicates that in this type of call, the time to reach its maximum amplitude and subsequent decay is progressive. 3) *Pulsed calls* (Pc; Fig. 2C). In contrast, this type of call is characterized by repetitive and continuous bursts of emissions or pulses, with a duration of no more than 21 ms. The amplitude envelope exhibits a mean rise of 35.83% and a mean decay of 64.17% over the total time. This suggests that the pulses reach their maximum amplitude in a relatively short time, with a progressive decay.

In this study we present for the first time the description of the spectral and temporal parameters of *Atelopus ignescens* calls. A repertoire composed of three structural calls types (i.e., tonal call, short call, pulsed call). The vocalizations described in previous studies indicate that species of the genus *Atelopus* have more than one call type in their repertoire (e.g. Lötters et al. 1999; Granda-Rodríguez et al. 2020; Jorge et al. 2020; Rueda-Solano et al. 2020). Conversely, few species emit only one type of call (see Lötters et al. 2019). The *Atelopus ignescens* repertoire is comprised of three temporally distinct call types. While the classification system initially proposed by Cocroft et al. (1990) was considered, the *Atelopus ignescens* call is frequency modulated and thus does not have pure tones. Consequently, the classification criteria were developed based on calls that exhibited both tonal and pulsed characteristics. Although an emission pattern has been defined and characterized in the calls of the genus *Atelopus*, which is useful for unifying criteria for comparison, the behavioral contexts of each of its species have been poorly studied, defined, and understood (Lötters et al. 2019).

The vocalizations of *Atelopus* species from Ecuador remain largely unknown, with only those of *Atelopus exiguus* having been described (Coloma et al. 2000) out of the 25 species in Ecuador (Centro Jambatu 2020). Notably, *A. ignescens* was the first amphibian species described in Ecuador, and had its call formally documented only after 175 years (Cornalia 1849). This lack of information, perhaps related to declining populations (La Marca et al. 2005; Scheele et al. 2019; Lotters et al. 2023) can lead to misconceptions about species' vocalization capabilities (Batallas and Brito 2022). The absence of a tympanic middle ear in *Atelopus* (McDiarmid 1971) may further reinforce the notion that they might be deaf and that acoustic communication is insignificant (Cocroft et al. 1990). The *Atelopus* calls are characterized as inconspicuous and unremarkable (Cocroft et al. 1990; Rivera-Correa et al. 2021). In fact, the short non-pulsed call was not discernible at the time of recording the vocalizations of *A. ignescens*. This former type of call is consistent with the anecdotal description by Peters (1973), who describes them as a slight, low sound that is difficult to hear even at close range. However, more studies are needed to understand extra tympanic mechanisms for hearing in the absence of a middle ear and the relative importance of acoustic communication in this group (e.g. Boistel et al. 2011; Womack et al. 2018).

It is noteworthy that the Jambato, like the majority of the genus *Atelopus*, is distinguished by the absence of a tympanic middle ear (McDiarmid 1971). This trait renders their acoustic communication particularly intriguing, as they would be considered deaf subjectively. The limited knowledge of these endangered anurans presents opportunities for diverse studies on their behavior and communication.

Atelopus ignescens has become the most representative species for conservation in Ecuador and the Neotropics. Beyond behavioral characteristics, we endow this iconic species with an acoustic identity. We hope that this contribution will establish a baseline for acoustic knowledge of this species, thus facilitating future monitoring and discovery of new populations using passive acoustic methods. Furthermore, acoustic data may serve as a crucial component in evaluating the potential taxonomic issues that remain unresolved in this species (see Guayasamin et al. 2010). This knowledge is incorporated into the efforts to comprehend and preserve jambato, ensuring that its subtle calls are not lost on truly deaf ears.

Acknowledgments

First of all, we thank the community of Angamarca for their collaboration and interest in the conservation of the Jambato. We thank the members of the Alianza Jambato, with whom we had the opportunity to embark on fieldwork and engage in shared experiences. To Mario Yánez, for his valuable stories and advice on the conservation of this species, which gave us enough encouragement to

shape this work. Special thanks to Laura González of the Fonoteca Zoológica de Madrid for her assistance in the assignment of collection numbers and the generation of QR codes. We thank Bibiana Rojas and two anonymous reviewers for their valuable and constructive comments that helped to improve the quality of this manuscript. This study was partially funded by Universidad San Francisco de Quito USFQ (Grants HUBI 5466, 16871, 17857). We also extend our appreciation to all the researchers around the globe for their efforts over the past decades to prevent the extinction of this species.

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Appendix 1

Recordings of an uncollected specimen of *Atelopus ignescens*. The associated codes correspond to three recordings, which were deposited at the Laboratorio de Biología Evolutiva (LAB) and are available at the Fonoteca Zoológica de Madrid (FZ):

A **B** **C**

Figure A1. Codes correspond to three recordings, available at the Fonoteca Zoológica de Madrid (FZ). **A.** LBE-C 061, FZ 14845, https://lc.cx/La_JkA; **B.** LBE-C 062; FZ 14846; https://lc.cx/G2D01L; **C.** LBE-C 063; FZ 14847; https://lc.cx/AGXuN4.