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The advertisement calls of *Pristimantis galdi* Jiménez de la Espada, 1870 and *Pristimantis katoptroides* (Flores, 1988) (Anura, Strabomantidae)

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Abstract

In this study we describe for the first time the calls of *Pristimantis galdi* and *Pristimantis katoptroides*. Recordings were obtained in Sangay National Park, Ecuador. We highlight the importance of recording *P. galdi* since its call has been recorded after 153 years of having been described as a species. The call of *P. galdi* consists of 7 to 9 short notes, the sounds of which are similar to a hammer hitting a nail, with a mean dominant frequency of 2.39 kHz. In turn, the call of *P. katoptroides* consists of a single note, the sound of which is similar to a metallic "tic", with a mean dominant frequency of 1.74 kHz. We compared the advertisement calls of *P. galdi*, *P. katoptroides* and *P. roni* as these species share similar morphological characteristics and are grouped in the spiny green frog's ecotype. Despite these morphological similarities, their advertisement calls are different. Obtaining calls of *Pristimantis* species in Ecuador might prove difficult with short-term studies due to the great sampling efforts that may be needed to get these recordings. Therefore, implementing active and passive monitoring could help improve our knowledge of acoustic signals in Ecuador's rainfrogs.

Key Words

acoustic communication, calls, Sangay National Park, spiny green frogs

Acoustic communication is one of the Anurans' most important and conspicuous ethological traits, through which they transmit messages between conspecifics (Duellman and Trueb 1994; Angulo 2006; Wells 2007). Acoustic emissions respond to a specific social context and function (Toledo et al. 2015), where the advertisement call is the most commonly heard one in nature. This type of call announces sexual receptivity, position in a territory, and individual size (Gerhardt and Huber 2002; Wells 2007; Toledo et al. 2015; Köhler et al. 2017). In general, the advertisement call consists of a repetitive series of notes or pulses and, as a result of this stereotypical structure, tends to have a high degree of specificity among species (Gerhardt 2001; Wells and Schwartz 2007). These characteristics have allowed the use of the advertisement call as an identification feature among species (Angulo 2006). Moreover, analyzing and determining call variations has allowed for gaining a better understanding of the diversity of certain groups. Especially in those where morphological characteristics are insufficient to differentiate and diagnose species. (Angulo and Reichle 2008; Rivera-Correa et al. 2022).

PENSOFT.

Pristimantis Jiménez de la Espada, 1870 is a genus of anuran restricted to the New World, known as the most speciose vertebrate genus (595 species; Frost 2023). Nearly 20 species of *Pristimantis* have been described in the last two years in Ecuador, thereby making it the country with the highest description rate per year of this genus (Reyes-Puig and Mancero 2022). In fact, Ecuador counts 254 species, out of which 61.8% (157 species) are endemic (Ron et al. 2022). Despite this considerable rate of discoveries and publications, a large percentage of the vocalizations of the *Pristimantis* individuals of Ecuador remains unknown (Batallas and Brito 2016). In this study, we describe for the first time the advertisement calls of *Pristimantis galdi* Jiménez de la Espada, 1870 and *Pristimantis katoptroides* (Flores, 1988). Furthermore, we compare these results with the call of *Pristimantis roni* Yanez-Muñoz, Bejarano-Muñoz, Brito-M and Batallas-Revelo, 2014, since it is a species with some morphological resemblance.

Fieldwork was conducted in June and September of 2014 in the lower zone of Sangay National Park (administrative jurisdiction comprising the eastern foothills of the park within an altitudinal range of 600 to 1800 m), which corresponds to the Subtropical zoogeographic region (Albuja et al. 2012). The ecosystem comprises montane evergreen forest on the southern slope of the Eastern Andes (Ministerio Del Ambiente del Ecuador 2013). The habitat is characterized by trees whose gnarled trunks and branches support several species of epiphytes such as orchids and bromeliads. The emergent vegetation reaches up to 30 m in height, and there is a visual predominance of romerillo (*Prumnopitys montana*) and palma real (*Dictyocaryum lamarckianum*) trees. In the understory, there is visual predominance of herbaceous plants of the Araceae and Marantaceae families (Brito et al. 2017).

Two calls from a *Pristimantis galdi* individual collected at Sardinayacu lake complex (2°04'20.5"S, 78°12'52.4"W, 1,800 m) were recorded on June 24, 2014, between 14:00–16:00h. Meanwhile, 30 calls from three *Pristimantis katoptroides* individuals collected at Danu (2°04'45.3"S, 78°09'37.3"W, 1,360 m) were recorded on September 21, 2014, between 19:00–20:00h. The calls were obtained using a digital recorder (Olympus WS–802), coupled to a directional microphone (Sennheiser ME 66–K6), placed between 1–3m from the active individual. All recordings were made at a sampling rate of 44.1 kHz and 16 "bits" resolution, saving the audio files in the uncompressed WAV format. It is also worth noting that no fixed hours were set for recording during fieldwork, and it was rather the vocal activity of species that influenced the sampling effort. Collected specimens were sacrificed according to Chen and Combs (1999) protocols and preserved following Simmons (2002). They are deposited at the División de Herpetología del Instituto Nacional de Biodiversidad (DHMECN), Quito, Ecuador. The recordings are deposited at the Fonoteca Zoológica (www.fonozoo.com) del Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain (Appendix 1). Identification of specimens was based on morphological characteristics described in Lynch and Duellman, (1980), Flores (1988), and Duellman and Lehr (2009).

The spectral and temporal properties of the advertisement calls were analyzed with the software Raven 1.6 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology 2022). The spectrograms were obtained using the Hann window at 90% overlap and 512 samples of DFT size. The parameters analyzed were: (1) Dominant frequency of the entire call and each of the elements emitted in it; (2) Harmonics; (3) Duration of calls; (4) Intervals between calls; (5) Calls/minute; (6) Notes/call; (7) Duration of notes; (8) Intervals between notes; (9) Notes/second. Definitions, terminology and measurements of acoustic parameters follow the terms of Köhler et al. (2017), and Sueur (2018). The figures were processed in R software (R Core Team 2022). For this, the audio files were imported with the tuneR package version 1.4.1 (Ligges et al. 2018). Subsequently, the oscillogram and spectrogram were created using the Seewave package version 2.2.0 (Sueur et al. 2008). With the values of the analyzed parameters, the measures of central tendency (means) and dispersion (maximum, minimum, and standard deviation) were calculated.

Pristimantis galdi Jiménez de la Espada, 1870 (Fig. 1A)

The recorded male was calling from shrub vegetation approximately 150 cm above the ground. The advertisement call (Fig. 2A, Table 1) consists of 7 to 9 notes. Call duration ranges from 1730 to 2017 ms. The mean note duration is 44.62 ± 12.50 ms, with a mean rate of 4 ± 0.96 notes/second. The mean interval between notes duration is 217.57 ± 54.16 ms. The mean dominant frequency is 2.39 ± 0.16 kHz, showing a partial harmonic whose mean frequency is 4.76 ± 0.36 kHz. Its calls are a constant metallic sound, similar to a hammer hitting a nail.

Pristimantis katoptroides (Flores 1988) (Fig. 1B)

Recorded males were calling perched on tree branches, about 2–4 m above the ground. This species vocalizes in choruses of about seven to ten males, often observed in smaller groups of up to four individuals in the same tree. The advertisement call consists of a single note with a mean duration of 54.73 ± 5.69 ms. The mean interval between notes is 44.84 ± 25.14 ms, and mean rate is 1.80 ± 0.99 calls/minute. The mean dominant frequency is 1.74 ± 0.11 kHz, showing four partial harmonics in the spectrogram (Fig. 2B, Table 1). The calls have a metallic sound, onomatopoeically resembling the "tic" of a bell.

We describe for the first time the spectral and temporal parameters of the advertisement call of *Pristimantis galdi* and *Pristimantis katoptroides*. The former is assigned to the *Pristimantis* (*Pristimantis*) *galdi* species group (Hedges et al. 2008; Padial et al. 2014), where other species' calls remain unknown. Both, *P. galdi* and *P. katoptroides* (not assigned to any group), are placed in the spiny green frog's ecotype (Yanez-Muñoz et al. 2014). Within this ecotype, *Pristimantis roni* is morphologically similar to *P. katoptroides* (Yanez-Muñoz et al. 2014). However, these species with similar morphological characteristics differ in the properties of their advertisement calls. The

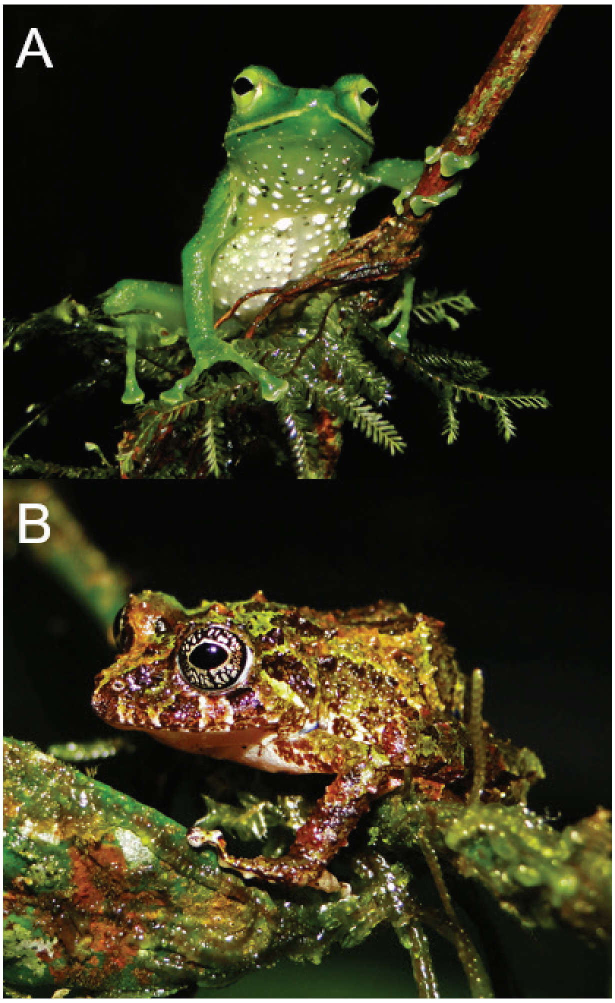

Figure 1. Individuals in their natural habitat. **A.** Adult male of *Pristimantis galdi* (DHMECN 12189, SVL 20 mm) from Sardinayacu, Parque Nacional Sangay, Ecuador; **B.** Adult male of *Pristimantis katoptroides* (DHMECN 12169, SVL 28.15 mm) from Danu, Parque Nacional Sangay, Ecuador.

call structure in both species is different, as *P. roni* emits several notes while *P. katoptroides* emits one. The calls of *P. katoptroides* are shorter, with longer intervals between calls and a lower number of emissions per minute. Finally, the dominant frequency in *P. katoptroides* is lower than that of *P. roni*. In the case of *P. galdi*, the call structure is similar (both species emit several notes), and the dominant frequency is lower than that of *P. roni* (see Fig. 3, Table 1 for further details). The advertisement calls from the three compared species share that they resemble a metallic sound (an onomatopoeic sound of a "Tic").

Among cryptic species, the use of acoustic diagnostic features for identification could be an alternative to morphometric and molecular diagnosis (Padial et al. 2009; Rivera-Correa et al. 2022). Even though morphologically similar, *P. galdi*, *P. katoptroides*, and *P. roni* have calls with different features, which supports the specificity of their acoustic signals at the species level. However, the magnitude of cryptic diversity and their advertisement calls remain largely unknown. (Funk et al. 2012; Reyes-Puig and Mancero 2022). Direct developing frogs, as is the case of *Pristimantis* species, do not conform to chorus aggregations and individuals are dispersed in their habitat (Duellman and Lehr 2009). Therefore, obtaining recordings of their calls could be more difficult due to their life history traits. In fact, the data collected in this study comes from two of the 17 expeditions conducted between 2010 to 2016 in Sangay National Park and buffer zones (Brito et al. 2017).

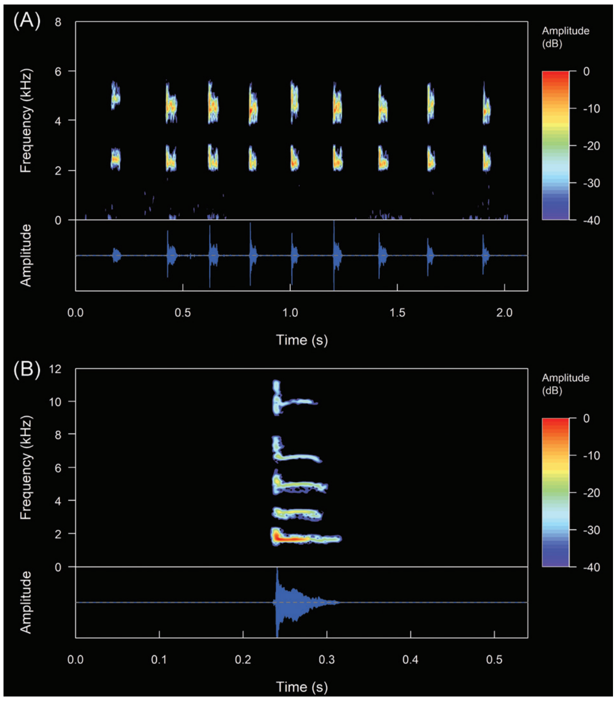

Figure 2. Spectograms and Oscillograms of the advertisement calls described in this study. **A.** *Pristimantis galdi* (DHMECN 12189, SVL 20 mm, 18 °C air temperature); **B.** *Pristimantis katoptroides* (DHMECN 12169, SVL 28.15 mm, 17.1 °C air temperature).

Table 1. Spectral and temporal values of *Pristimantis* calls belonging to the spiny green frog's ecotype (*sensu* Yánez-Muñoz et al. 2014). The abbreviations used in the parameters correspond to: kHz = kilohertz, ms = milliseconds, s = seconds. In the analyzed sample, n=number of specimens/calls/notes.

	P. galdi n=1/2/16	*P. katoptroides* n=3/30	*P. roni* n=1/9/50
Dominant frequency (kHz)	2.19–2.58 (2.39 ± 0.16)	1.60–2.06 (1.74 ± 0.11)	3.14–3.35 (3.32 ± 0.06)
Harmonic 1 (kHz)	4.35–5.34 (4.76 ± 0.36)	3.19–3.99 (3.43 ± 0.24)	–
Harmonic 2 (kHz)	–	4.94–5.09 (5.03 ± 0.05)	–
Harmonic 3 (kHz)	–	6.53–6.84 (6.65 ± 0.10)	–
Harmonic 4 (kHz)	–	8.80–9.91 (9.65 ± 0.42)	–
Call duration (ms)	1730–2017	42–64 (54.73 ± 5.69)	40–1247 (493.09 ± 341.67)
Interval between calls (s)	–	16.05–94.92 (44.84 ± 25.14)	2.09–7.93 (6.13 ± 1.71)
Call rate (calls/min)	–	0.64–3.72 (1.80 ± 0.99)	6.73–12.44 (8.79 ± 1.76)
Notes per call	7–9	1	1–5
Note duration (ms)	30–77 (44.62 ± 12.50)	–	29–45 (36.84 ± 4.68)
Interval between notes (ms)	151–325 (217.57 ± 54.16)	–	175–298 (249,56 ± 35.91)
Note rate (notes/s)	2.54–5.29 (4 ± 0.96)	–	–
Source	This work	This work	Yánez-Muñoz et al. 2014

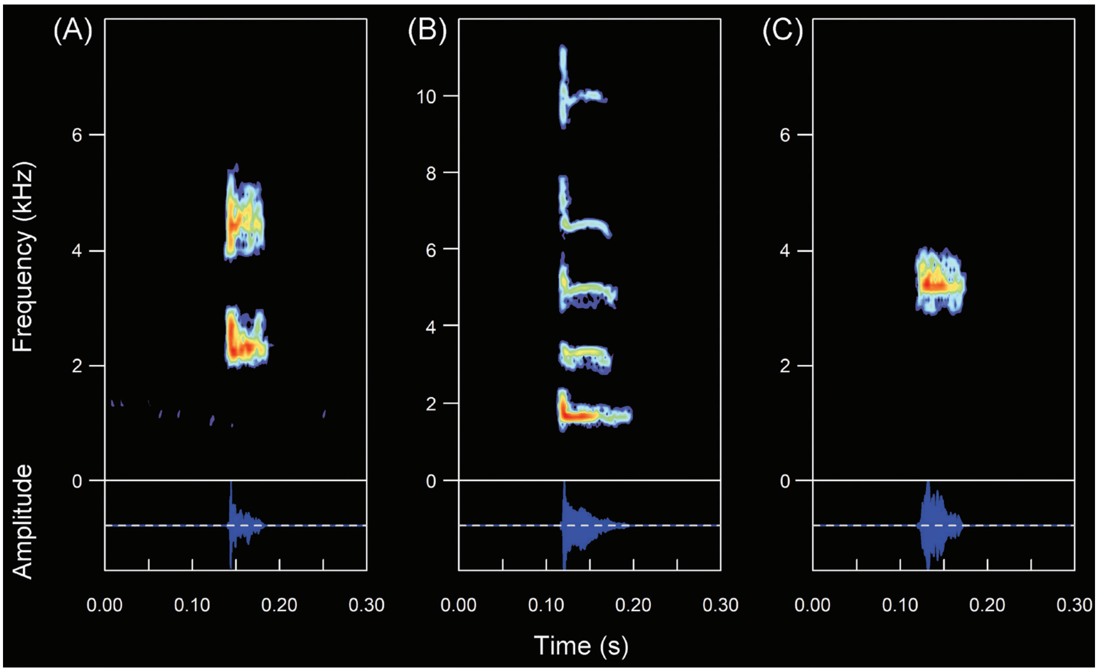

Figure 3. Comparative oscillograms and spectograms of advertisement calls of three *Pristimantis* species belonging to the spiny green frog's ecotype. **A.** *P. galdi* (DHMECN 12189, SVL 20 mm, 18 °C air temperature); **B.** *P. katoptroides* (DHMECN 12169, SVL 28.15 mm, 17.1 °C air temperature); **C.** *P. roni* (DHMECN 11322, SVL 16.55 mm, 15.6 °C air temperature).

Bioacoustics, behavioral and natural history information for species of the genus *Pristimantis* is very scarce, which contrasts with their abundant diversity (Hutter et al. 2016; Rivera-Correa et al. 2021). In Ecuador, such a contrast becomes more pronounced when taking into consideration the description rate of new species presented in recent years (Reyes-Puig and Mancero 2022). The fact that the call from a species such as *P. galdi*, which was described 153 years ago, has just been recorded for the first time suggests that recording the calls from *Pristimantis* species in Ecuador is difficult to obtain with short-term studies including direct visual encounters. Even though these methods are complementary and necessary for detecting and recording species in prospective phases (Boullhesen et al. 2021), complementary methos, such as passive acoustic monitoring, could help with recording the acoustic signals in one of Ecuador's most diverse and yet unknown groups of frogs.

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Appendix 1

Specimens recorded and collected:

Pristimantis galdi (Ecuador): Provincia de Morona Santiago, Complejo lacustre de Sardinayacu, Parque Nacional Sangay: DHMECN 12189, SVL 20 mm.46; FZ 11490–91

Pristimantis katoptroides (Ecuador): Provincia de Morona Santiago, Danu, Parque Nacional Sangay: DHMECN 12169, SVL 28.15 mm, FZ 11492; DHMECN 12170, SVL 24.05 mm, FZ 11493; DHMECN 12172, SVL 26.24 mm, FZ 11493.

Pristimantis roni (Ecuador): Provincia de Morona Santiago, Complejo lacustre de Sardinayacu, Parque Nacional Sangay: DHMECN 11322 SVL 16.55 mm, FZ 14208

Supplementary material 1

Pristimantis galdi recording 1. Fonozoo library code: FZ 11491

Authors: Diego Batallas, Jorge Brito
Data type: WAV file

Link: https://doi.org/10.3897/herpetozoa.36.e97705.suppl1

Supplementary material 2

Pristimantis galdi recording 2. Fonozoo library code: FZ 11490

Authors: Diego Batallas, Jorge Brito
Data type: WAV file

Link: https://doi.org/10.3897/herpetozoa.36.e97705.suppl2

Supplementary material 3

Pristimantis katoptroides recording 1. Fonozoo library code: FZ 11492

Authors: Diego Batallas, Jorge Brito
Data type: mp3 file

Link: https://doi.org/10.3897/herpetozoa.36.e97705.suppl3

Supplementary material 4

Pristimantis katoptroides recording 2. Fonozoo library code: FZ 11493

Authors: Diego Batallas, Jorge Brito
Data type: mp3 file

Link: https://doi.org/10.3897/herpetozoa.36.e97705.suppl4

Supplementary material 5

Pristimantis katoptroides recording 3. Fonozoo library code: FZ 11494

Authors: Diego Batallas, Jorge Brito
Data type: mp3 file

Link: https://doi.org/10.3897/herpetozoa.36.e97705.suppl5

Supplementary material 6

Pristimantis roni recording. Fonozoo library code: FZ 14208

Authors: Diego Batallas, Jorge Brito
Data type: WAV file

Link: https://doi.org/10.3897/herpetozoa.36.e97705.suppl6