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Geographic call variation in the Granular Glassfrog, *Cochranella granulosa* (Taylor, 1949), and a new distribution record in Colombia

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Abstract. We describe the advertisement call of a newly discovered Colombian population of *Cochranella granulosa* and compare it with available acoustic data closest to the type locality. We conducted a series of principal component analyses to explore the variation in the spectral and temporal features of the call. Despite the structural similarities, we found differences between the two populations of *C. granulosa* in most spectral variables and one temporal variable. In addition, we narrow the 950 km gap between the Panamanian and Ecuadorian records of *C. granulosa* and comment on previous records.

Keywords. Advertisement call, Biogeographic Chocó, Bioacoustic, Centrolenidae, Distribution gap

Introduction

Centrolenidae Taylor, 1951 is a monophyletic family of neotropical amphibians commonly known as glassfrogs due to the transparency of their ventral skin (Guayasamin et al., 2009). This characteristic, together with the forward-facing eyes, generally green dorsal colouration, and egg deposition out of the water near streams, makes most members of this family easy to identify (Guayasamin et al., 2009). This family presents a stereotyped call consisting of either a non-pulsed call with moderate amplitude modulation (Tic call type), a non-pulsed call without amplitude modulation (Tii call type), or a pulsed call with amplitude modulation (Trii call type), with some species exhibiting a combination of these (Duarte-Marín et al., 2022).

The genus *Cochranella* Taylor, 1951 is composed of eight species characterised by the absence of humeral spines, white digestive tract, lobed liver covered by a transparent hepatic peritoneum, ventral parietal peritoneum white anteriorly and transparent posteriorly, moderate to extensive webbing between Fingers III and IV (Taylor, 1951; Guayasamin et al., 2009; Duarte-Marín et al., 2022), and a Trii-type advertisement call (Duarte-Marín et al., 2022). *Cochranella* spp. are distributed along the humid lowland and premontane forests from Honduras to Panamá in Central America, and in Colombia, Ecuador, Perú, Bolivia, and Brazil in South America (Guayasamin et al., 2009). Five species are currently known from Colombia: *Cochranella euknemos* (Savage and Starrett, 1967) distributed along the humid lowland and premontane forests from Costa Rica, Panamá and in the Pacific region in Colombia (Jaramillo-Martinez et al., 2015); *Cochranella mache* Guayasamin and Bonaccorso, 2004 which inhabits the Pacific lowlands and premontane forests of Colombia and Ecuador (Jaramillo-Martinez et al., 2015; Molina-Zuluaga et al., 2017, Frost, 2023); *Cochranella resplendens* (Lynch and Duellman, 1973) occurs in the humid lowland and premontane forests of Colombia, Ecuador, Peru, Bolivia and northern Brazil (Molina-Zuluaga et al., 2017; Rojas-Padilla et al., 2019; Costa-Campos et al., 2020; Frost, 2023); *Cochranella litoralis* (Ruiz-Carranza and Lynch, 1996) distributed in the

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humid lowland forests of the Pacific region of Colombia and Ecuador (Guayasamin et al., 2006; Molina-Zuluaga et al., 2017; Rojas-Padilla et al., 2019; Costa-Campos et al., 2020; Frost, 2023); and, *Cochranella granulosa* (Taylor, 1949) is found in the humid lowlands and premontane forests from Honduras to Ecuador (Guayasamin et al., 2006; Culebras et al., 2020).

Cochranella granulosa was originally described from Los Diamantes, Guápiles, Cantón de Pococí, Provincia Limón, Costa Rica (Savage, 1974). In Colombia, *C. granulosa* is only known from a single specimen collected in Tanelita River, Balboa township, Unguía municipality, Chocó department (field number MAR 1049; Fig. 1; Díaz-Ricaurte and Guevara-Molina, 2020). There is a significant distributional gap southward, with the distance from Tanelita river to the closest known locality of the species in northern Ecuador (Fig. 1; Guayasamin et al., 2020) being ~813 km along the Chocó biogeographic region.

The advertisement call of anuran males is a species-specific trait that can be used as a tool for species identification and delimitation (Köhler et al., 2017; Emmrich et al., 2020), and like most life history traits, it can be coerced by environmental variables, exhibiting variation throughout the geographic distribution of each species (Köhler et al., 2017). It is therefore important to characterise intraspecific variation in widely distributed species to improve tools for species recognition and delimitation, as well as to elucidate and understand evolutionary processes.

For *Cochranella* species distributed in Colombia, only the advertisement calls of *C. euknemos, C. mache,* and *C. granulosa* have been reported (Rivera-Correa et al., 2021). For *C. granulosa*, however, the described advertisement call corresponds to data from populations in Costa Rica and central Panama (Ibañez, 1991, 1993; Kubicki, 2007). Here we describe the first advertisement call from a Colombian *C. granulosa* population that was newly discovered and compare it with the available acoustic data for the species.

Materials and methods

Study Area. Between June and November 2022, we conducted a herpetofauna survey following the auditory band transects and visual encounter surveys methodologies (Crump and Scott, 1994), in the Corregimiento of Huina, Bahía Solano municipality, Chocó department, Colombia. The collected *Cochranella* specimens were prepared following Heyer et al. (1994) and are stored in the Amphibian collection of Universidad EAFIT (EAFIT-Am), Medellín, Colombia (Appendix 1). We verified the identity of the individuals using the diagnostic characters described by Taylor (1951), Savage (2002), and Guayasamin et al. (2020).

Acoustics. Acoustic data were recorded using a Zoom H5 digital recorder coupled to an XYH-5 microphone capsule, in stereo mode at 44.1 kHz sampling frequency and 24-bit precision. The recorder was placed 50–100 cm from each of the recorded specimens. Calls of *C. granulosa* from Costa Rica were downloaded from Macaulay Library (ML), Cornell Laboratory of Ornithology and our newly recorded calls from the Colombian population were stored in the Banco de Sonidos OcainaCua, Museo de Ciencias Naturales de La Salle, respectively (Appendix 1). Two of the downloaded recordings had voucher specimens stored in the United States National Museum, as these specimens were collected by other researchers and seemingly have

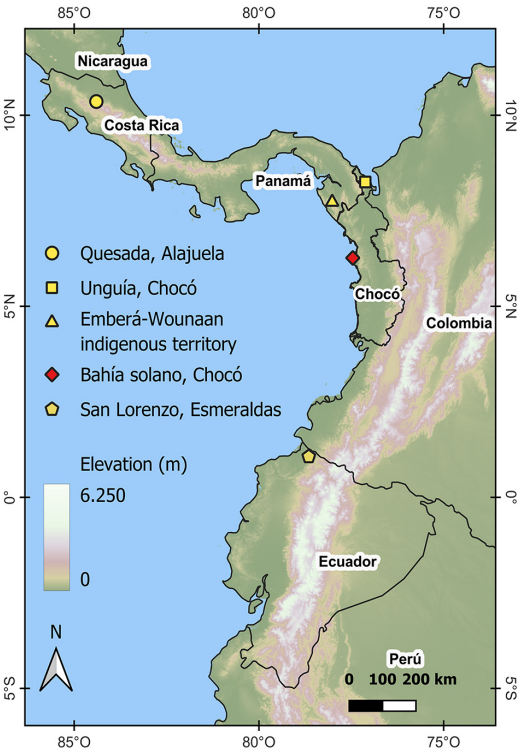

Figure 1. Known distribution of *Cochranella granulosa* in Colombia. New distribution record of *C. granulosa* for Colombia (red rhombus). The yellow circle is the Costa Rican population compared acoustically with the new Colombian population.

not been used in a publication, we also verified their identities.

We used the Raven Pro software (version 1.6.4) (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2023) for the analyses of the calls, applying a Hann window with 50% overlap and a DFT size of 512 samples. The temporal features of the call (call duration, note duration, and inter-note interval) were measured in oscillograms, and power spectra diagrams were used to obtain the spectral features of the call (dominant frequency, low frequency, and high frequency), which were measured at 20 dB below the peak intensity of the dominant call frequency. Oscillogram and spectrogram graphs were obtained using R software (version 4.2.2) (R Core Team, 2023) with the package Seewave (version 2.1.5) (Sueur et al., 2021), using a Hanning window type with 90% overlap, and a Fast Fourier transform (FFT) window length of 512 samples. Audio files in WAV format were imported with the tuneR package (version 1.3.3) (Ligges et al., 2023).

To explore the variation in the acoustic signals of the recordings from the Colombian and Costa Rican populations of *C. granulosa*, we conducted a series of principal component analyses (PCA), based on the covariance matrix of the six measured variables (call duration, note duration, inter-note interval, dominant frequency, low frequency, and high frequency). For the descriptive statistics, we calculated the mean of the spectral and temporal parameter of the call and note for each male and then we calculated a new mean and standard deviation for the population. As the number of notes in the advertisement call varies, we used the two-note call for the main analysis, as it is the most common call in the Costa Rica population, our smallest data set. In species with calls consisting of two or more notes, the values of note duration and call duration could exhibit variation (Köhler et al., 2017), thus we performed three separate PCA analyses, the first for the first note, the second for the second note and the third for the entire call, respectively. Acoustic definitions, terminology, and measurements follow Köhler et al., (2017) and Emmrich et al., (2020).

Results

New distribution record. A population of actively calling individuals of *C. granulosa* was found on 21 August 2022 in a streamlet named "Riachuelos de Juná" surrounded by secondary vegetation, in the corregimiento of Huina, Bahia Solano, Chocó, Colombia (6.26489, -77.45901; 78 m elevation; WGS 84; Fig. 1). Two individuals were recorded and collected: the first one was found at 23:00 h on 21 August 2022, calling from the upper surface of leaves about four meters high (EAFIT-Am 654; Fig. 2A), the second individual was found at 21:00 h on 2 November 2022, also calling from the upper surface of leaves on a fallen tree about three meters high (EAFIT-Am 674; Fig. 2B). The morphological diagnostic traits used to assign the individuals to *C. granulosa* were: granular dorsal skin texture, snout slightly sloping in lateral view, reduced white ventral peritoneum, white digestive tract, yellowish-green hands and feet, and dark green to bluish-green dorsum with scattered dark blue spots, the latter being a variable trait among the populations of Central America, Colombia, and Ecuador (Taylor, 1951; Savage, 2002). No other known glassfrog species from the Chocó biogeographic region has a similar combination of traits.

Call description. We recorded a total of four specimens, of which two were not collected (Appendix 1). The advertisement call of *C. granulosa* from the Huina population consists of one to five (Moda = 3) loud, frequency-modulated, rapidly repeated, pulsed, usually overlapped notes (Fig. 3). The first note is longer than the others (Table 1), and calls composed of one or five notes are rare. Therefore, the description focusses on the three notes calls parameters. Calling males were observed from 17:40–03:00 hrs.

Acoustic analysis. Online recordings for *C. granulosa* were limited to a Costa Rican population and were stored in the Macaulay Library, thus our comparison is limited to that population. In the PCA analysis for the two-note call, the first two components explain 94.7 % of the total variation (Fig. 4A). For the first principal component axis (PC1), the low frequency, dominant frequency, and high frequency have the highest loading values, explaining the majority of this axis' variation, whereas inter-note interval, delta of frequency, and high frequency contributed more to explaining the variation of the second PC axis (Appendix 2).

In the PCA analysis of the first note, the first two PC axes explain 91.3 % of the variation (Fig. 4B). Low frequency, high frequency, and dominant frequency have the highest loading values explaining most of the variation for the first PC axis, whereas delta frequency, note duration, and high frequency have the highest loading values explaining most of the variation for second PC2 axis (Appendix 2).

For the second note PCA analysis, the first two PC

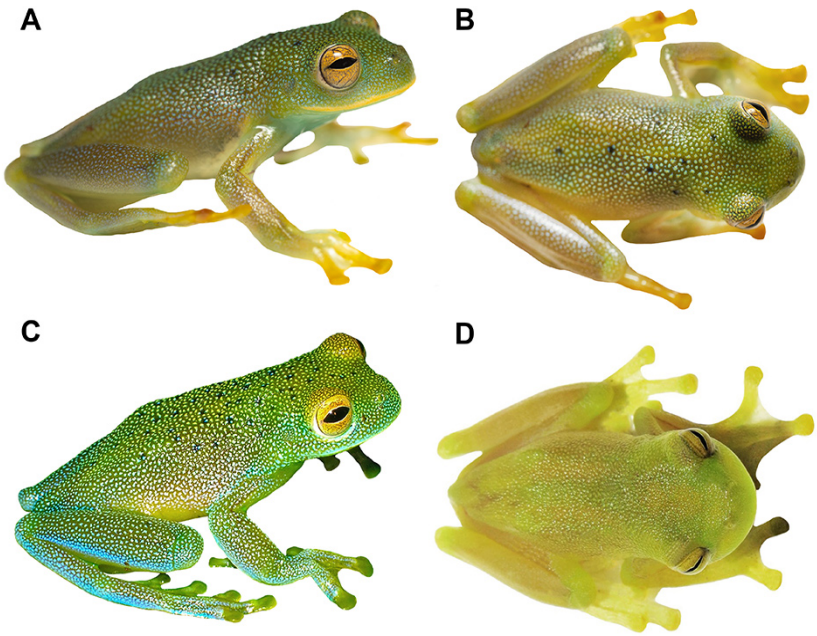

Figure 2. Specimens of *Cochranella granulosa* from different localities in Central and South America. (A) Lateral and (B) dorsal views of EAFIT-Am 674 from Bahía Solano, Chocó, Colombia. (C) Dorsolateral view of a specimen from Cerro Musún, Nicaragua (not collected specimen). And (D) dorsal view of an individual from Ecuador (ZSFQ 5380). Photos Juan-P. Durango (A, B), Javier Sunyer (C), and Daniela Franco-Mena (D).

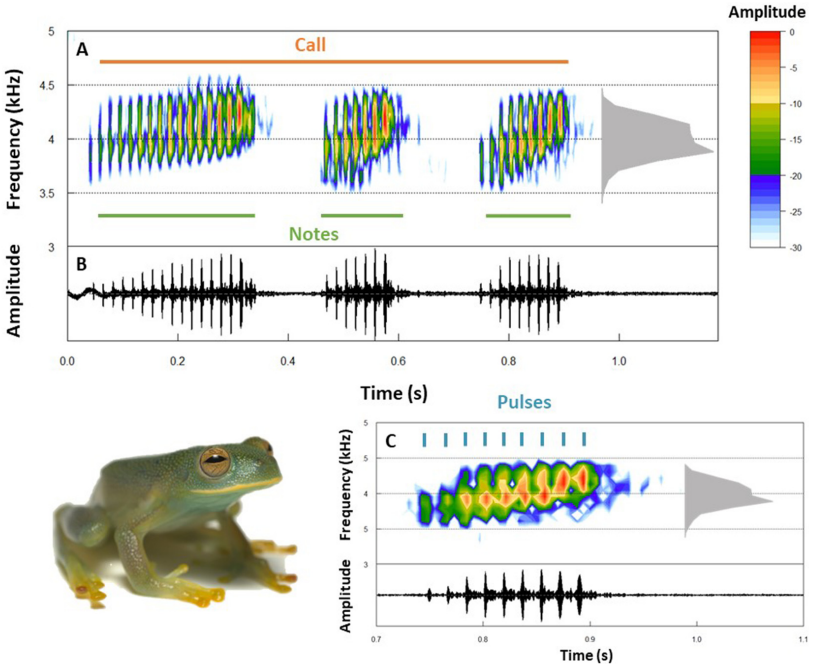

Figure 3. Advertisement call of *Cochranella granulosa* (EAFIT-Am 674) from Bahía Solano, Chocó, Colombia. (A) Spectrogram and mean frequency spectrum —grey shadow— (B) oscillogram and (C) zoom of third note. Photo by Juan-P. Durango.

Table 1. Descriptive statistics of call parameters of individuals of *C. granulosa* from Bahía Solano - Chocó, and Alajuela - Costa Rica. Range (mean ± SD; *n*). *n* = the number of calls or notes measured.

Parameters	Colombia	Costa Rica
Two notes call duration (s)	0.36 – 0.74 (0.53 ± 0.10; 9)	0.65 – 1.00 (0.75 ± 0.12; 8)
Three notes call duration (s)	0.62 – 0.93 (0.80 ± 0.08; 23)	—
Two notes call Inter–note interval (s)	0.09 – 0.16 (0.11 ± 0.02; 9)	0.03 – 0.31 (0.18 ±0.12; 8)
First note duration (s)	0.12 – 0.44 (0.27 ± 0.05; 41)	0.11 – 0.61 (0.31 ± 0.12; 18)
Second note duration (s)	0.07 – 0.23 (0.15 ± 0.03; 41)	0.16 – 0.36 (0.23 ± 0.07; 9)
Third note duration (s)	0.11–0.23 (0.16 ± 0.03; 32)	—
Two notes call dominant frequency (Hz)	3531.4 – 4134.3 (3818.5 ± 215.3; 9)	4048.2 – 4478.9 (4198.9 ± 170.7; 8)
Three notes call dominant frequency (Hz)	3617.5 – 4134.3 (3834.7 ± 179.6; 23)	—
First note dominant frequency (Hz)	3617.5 – 4478.9 (3983.1 ± 248.7; 41)	4048.2 – 4478.9 (4206.1 ± 154.2; 18)
Second note dominant frequency (Hz)	3531.4 – 4220.5 (3887.3 ± 180.4; 41)	4048.2 – 4565.0 (4210.9 ± 189.9; 9)
Third note dominant frequency (Hz)	3617.5 – 4392.7 (3873.5 ± 219.5; 32)	—
First note low frequency (Hz)	3125.76 – 3894.39 (3489.86 ± 209.93; 41)	3508.83 – 4120.09 (3773.32 ± 218.68)
Second note low frequency (Hz)	3083.50 – 3877.08 (3437.96 ± 221.64; 41)	3503.86 – 4110.8 (3749.73 ± 232.95)
Third note low frequency (Hz)	3116.38 – 3871.88 (3411.71 ± 199.39; 32)	—
First note high frequency (Hz)	4199.00 – 4735.81 (4457.86 ± 147.77; 41)	4411.89 – 5184.6 (4613.679 ± 208.92)
Second note high frequency (Hz)	4052.94 – 4650.97 (4375.37 ± 160.35; 41)	4342.42 – 4966.07 (4551.09 ± 236.6)
Third note high frequency (Hz)	3951.31 – 4752.11 (4319.87 ± 209.87; 32)	—
Two notes call low frequency (Hz)	3098.42 – 3530.61 (3305.48 ± 115.60; 9)	3538.6 – 4058.3 (3753.58 ± 208.81)
Three notes call low frequency (Hz)	3155.04 – 3533.64 (3344.80 ± 111.33; 23)	—
Two notes call high frequency (Hz)	4201.54 – 4531.76 (4383.92 ± 117.81; 9)	4392.04 – 4965.28 (4642.48 ± 224.86)
Three notes call high frequency (Hz)	4198.87 – 4526.10 (4402.44 ± 92.53; 23)	—

axes explain 99 % of the total variation (Fig. 4C). The dominant frequency, low frequency, and high frequency have the highest loading values explaining most of the variation for the first PC axis, whereas delta frequency, note duration, and high frequency have the highest loadings for the second PC axis (Appendix 2).

Discussion

Kubicki (2007) described the advertisement call of Costa Rican *C. granulosa* population close to the type locality, but this description was poor as there is no methodology description, making comparisons virtually impossible. Moreover, it only accounts for call and notes duration, and "fundamental" frequency, which falls short for the now more detailed advertisement calls descriptions. The most comprehensive approaches to describe the *C. granulosa* advertisement call were made by Ibañez in several manuscripts on Panamanian populations (Ibañez, 1991, 1993; Ibañez et al., 1999), but these were not formal descriptions and rather studies that refer to some call parameters. Besides these, there were no descriptions on advertisement call variation for populations in South America, especially from Colombia, hindering comparisons.

Compared to Panamanian, Colombian *C. granulosa* populations from Huina have a wide variation in their acoustic signals, ranging from one up to five notes, with extensive overlapping of calls. The dominant frequency for the three-note calls in the Panamanian population occurs between 3.9 and 4.9 kHz (Ibañez, 1991, 1993; Ibañez et al., 1999), while in the Colombian population reported on here it is lower, ranging from 3.6 to 4.1 kHz. Kubicki (2007) described the advertisement call for Costa Rican populations of *C. granulosa* as a fast and sharp three-notes call (sometimes with five notes), with a duration of 0.8 s and a fundamental frequency for the three-notes call of 3.7 kHz. Kubicki's description is consistent with the spectral and temporal characterisation of the call from the Colombian population. All known populations (i.e., Costa Rica, Panama, and Colombia) share a particular acoustic pattern regarding the call structure, with males tending to emit monotonous calls at the beginning of the activity period, and progressing to more dense calls with up to five-note calls. As hypothesised by Ibañez (1991, 1993), the one-note call could be a specific behaviour used by males to detect other nearby males, since male choruses could be disadvantageous in attracting females. In contrast, the five-notes call is usually emitted at the peak of the

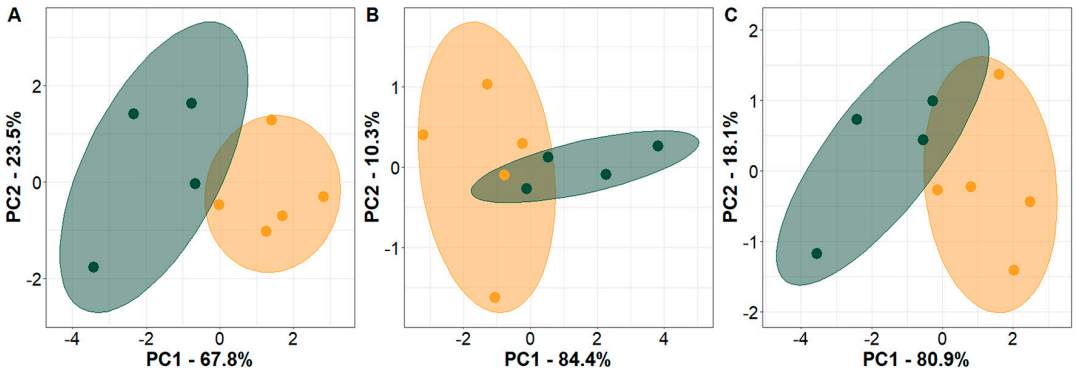

Figure 4. Clusters of the first two Principal Components Analysis (PCA) components showing the overall variation at the acoustic level (spectral and temporal characteristics) between Colombian (yellow) and Costa Rican (green) populations of *Cochranella granulosa*. (A) Two-note call, (B) first note and (C) second note.

species' acoustic activity, when several males compete through energetic calling (Ibañez, 1991, 1993).

The PCA analysis for the acoustic variables grouped the sampled individuals into two clusters (with some minor overlap); these clusters match with the geographic origin of the populations (Colombia and Costa Rica; Fig. 4). Despite the structural similarity (Fig. 5), the exploratory analyses (i.e., PCA) show that there are slight differences between the two groups for most spectral variables and the inter-note interval between the two analysed populations of *C. granulosa* (Table 1, Fig. 4). The Panamanian populations studied by Ibañez (1991, 1993) and the Costa Rican calls we analysed have a higher dominant frequency than the Colombian population (Table 1). This pattern is confirmed by the contribution of the dominant frequency in the PCA analysis of the two-note calls (Fig. 4A).

Although it was not possible to determine and control for the effects of body size (SVL) on differences in spectral properties, as well as environmental or instrumental bias, we hypothesise that the differences we found may represent segregation in acoustic characters related to

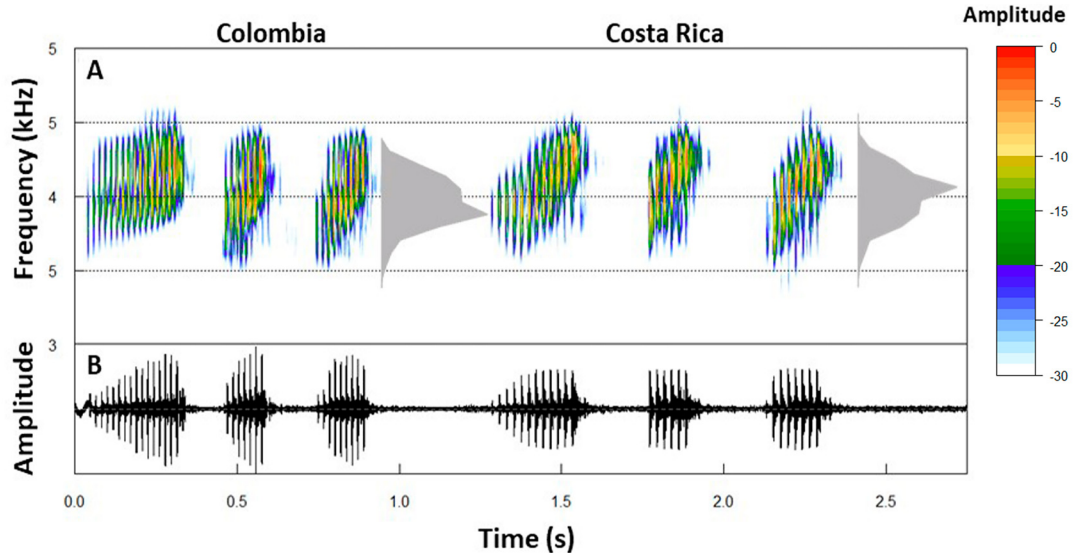

Figure 5. Comparison of the advertisement call of Colombian and Costa Rican populations of *Cochranella granulosa.* (A) Spectrogram and mean frequency spectrum —grey shadow— and (B) oscillogram.

the geographical distance between the populations. Our acoustic analysis is a relevant contribution to the understanding of intraspecific variation in *C. granulosa* and could help to address evolutionary, systematic, and ecological questions of this widely distributed species.

Our newly discovered population reduces the 950 km gap between Ecuadorian record for *C. granulosa* and its nearest neighbouring population in Panama (Culebras et al., 2020). However, further studies and sampling efforts are needed to determine and accurately document the presence of *C. granulosa* in the northern (approx. 222 km) and southern (approx. 590 km) regions of the Chocó biogeographical area (Fig. 1). The data presented here, for both individuals and calls, are based on voucher specimens currently deposited in official biological collections and natural history museums, and the accuracy of their taxonomic identification could be confirmed at any time in the future.

Recently, Barrio-Amorós et al. (2023) reported the presence of *C. granulosa* in Colombia in the municipalities of Capurganá (near the Panamanian border) and Nuquí (central Chocó department). These records are based solely on *in situ* photographs (Barrio-Amorós et al., 2023, Figures 1–2), without any additional support or physical evidence. Photographs do not provide the same quality of information as voucher specimens (texture and shape), and do not allow to assess internal morphological characters. Occurrence records, especially for understudied species, require the collection of voucher specimens, which are key to confirming (or rejecting) taxonomic identity (Amorim et al., 2016; Grandcolas, 2017; Troudet et al., 2018). Vouchers from previously unknown localities do allow for further development of morphologic (internal and external characters), evolutionary and conservation studies of species (Bortolus, 2008, Pleijel et al., 2008; National Academies of Sciences Enginering and Medicine, 2020; Hilton et al., 2021; Rohwer et al., 2022). Therefore, taxonomic uncertainty cannot be excluded when referring to the records of *C. granulosa* reported by Barrio-Amorós et al. (2023), since review and corroboration of the presence/absence of characters in specimens will not be available. We would like to clarify that photographic records are a powerful complementary tool, or useful only for species who are well sampled throughout their distribution, that are diagnosable with external morphology, and in studies were the presence of a species is widely documented for the studied area.

Conservation status assessments mainly focus on species occurrence data (i.e., Area of Occupancy and Extent of Occurrence), and the only way to assess, with certainty, the presence of *C. granulosa* along the Colombian Pacific lowlands are using voucher specimens, as provided here. These vouchers corroborate Barrio-Amorós et al.'s (2023) reports of *C. granulosa* in Colombia and add another population, meaning that the species' distribution within Colombia is larger. Thus, this new vouchered report of *C. granulosa* is an important step towards a better understanding of the species' distribution and allows for a more informed assessment of its conservation status in Colombia.

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Appendix 1

Specimen (*) and recording (+) vouchers employed in this study. COLOMBIA: Chocó department, Bahía Solano, Corregimiento of Huina, 6.26489, -77.45901 (EAFIT-Am 654*, 674*; BSOC 200–203+). COSTA RICA: Alajuela Province, Quesada district, ca. 5.6 mi NE of Tesalia, 10.358333, -84.4, (USNM 219308–219309*; ML 212751+, 212758+); Puntarenas Province, Rincón de Osa (ML 213409–213410+).

Appendix 2

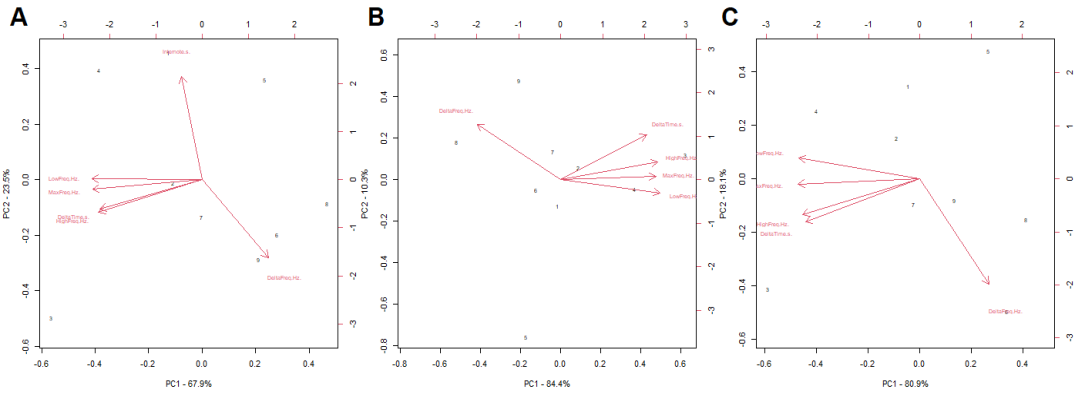

Figure 1. Biplot showing variables and their contribution for the variance on PC 1 and 2 for (A) a Two notes call, (B) First note, and (C) Second note of the advertisement call of *C. granulosa*..

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