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SHORT NOTE

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First record of Colombian red-eyed tree frog *Agalychnis terranova* from the Sierra Nevada de Santa Marta, Colombia with a description of its advertisement call

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ABSTRACT - Based on collected specimens and phylogenetic information, we provide the first record of the Colombian red-eyed tree frog, *Agalychnis terranova*, from the Sierra Nevada de Santa Marta in northern Colombia. This species is known from several localities on the middle Magdalena river valley and the Pacific lowlands. With this new record, the known geographic distribution of this frog is extended about 370 km north-west from its previous northernmost record. Additionally, we describe for the first time the tonal advertisement call of this species, which consisted of one or two notes with a total duration of 0.52 s and dominant frequency of about 1.74 kHz.

INTRODUCTION

The genus *Agalychnis* Cope, 1864 (Anura: Phyllomedusidae) includes 14 recognised species, ranging from the lowlands of the south Pacific coast of Mexico, extending through the lowlands of Central America, along the Pacific coast to north-west Ecuador, and the lowlands along the Eastern Andes from Colombia and Venezuela to Peru and the Upper Amazon basin. Half of the known species of *Agalychnis* occur in Colombia: *A. callidryas* (Cope, 1862), *A. buckleyi* and *A. lemur* (Boulenger, 1882), *A. spurrelli* Boulenger, 1913, *A. psilopygion* (Cannatella, 1980), including two species restricted to the country: *A. danieli* (Ruiz-Carranza, Hernández-Camacho & Rueda-Almonacid, 1988) and *A. terranova* Rivera-Correa, Duarte-Cubides, Rueda-Almonacid & Daza, 2013.

The Colombian red-eyed tree frog *Agalychnis terranova*, was described using material collected from three localities on the middle Magdalena river valley (Magdalena province), Colombia (Rivera-Correa et al., 2013). Belonging to the *A. callidryas* group, *A. terranova* is characterised by a red iris with golden reticulations in the eyelid membrane, usually showing a slim dorsally green body that sometimes has white warts, and with a head that is wider than the body. The ventral body area is cream-coloured and the body extremities and flanks are orange with some reduced white warts. The head is slightly sloping in lateral view, with a rounded snout in dorsal view. This medium sized frog does not have calcars or tubercles on the legs, and the parotid glands are absents (Rivera et al., 2013).

Currently, this species has been reported in different localities in the middle of the Magdalena river valley region, in the departments of Antioquia, Cundinamarca and Santander. (Rivera-Correa et al., 2013; Guarnizo et al., 2015). Later, its distribution was extended to the Chocó province (Palacios-Rodríguez et al., 2016).

Figure 1. Adult male of *Agalychnis terranova* (CBUMAG:ANF:01173, SVL 43.7 mm) from south-western foothills of the Sierra Nevada de Santa Marta

During an amphibian field survey between 3rd to 5th September 2019, we discovered a new population of *Agalychnis terranova* (Fig. 1.) in the south-western foothills of the Sierra Nevada de Santa Marta, near the rural school of vereda Tierras Nuevas, Corregimiento Villa Germania, Municipio de Valledupar, Departamento del Cesar, Colombia (10° 14'25'' N, 73° 45'27'' W, 874 m altitude, Fig.

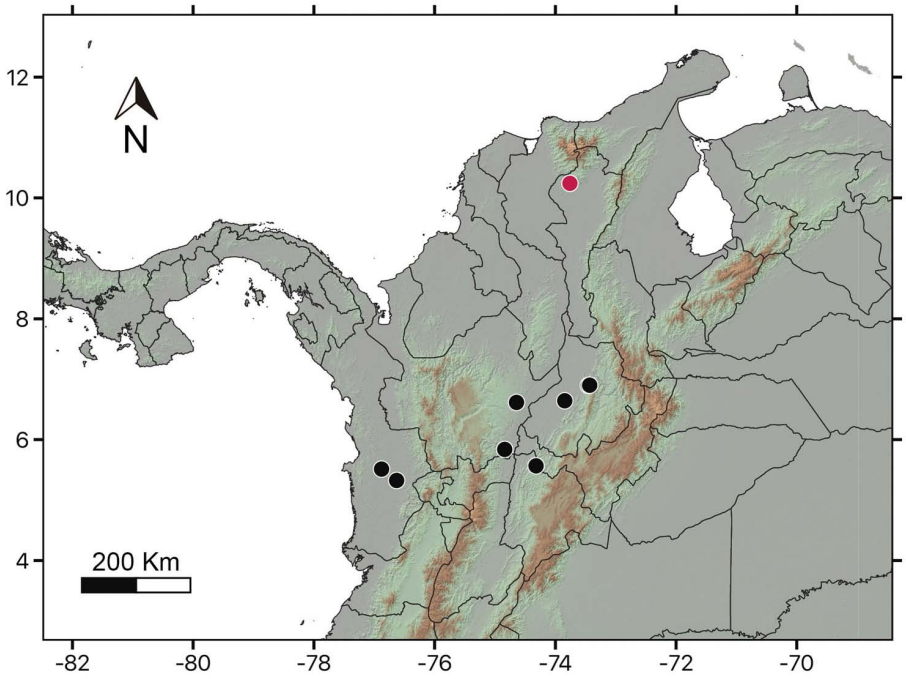

Figure 2. Known distribution of *Agalychnis terranova* in Colombia- black circles = previous records; red circle = new record in the Sierra Nevada de Santa Marta

Figure 3. Habitat of *Agalychnis terranova* in the Sierra Nevada de Santa Marta, Colombia 874 m altitude

2; Supplementary Material Table S1). This population was found in a small and degraded dry forest fragment where heavy rains had occurred in previous days, flooding much of the soil (Fig. 3). At this location, about 5 males were heard calling from the branches of trees at more than three meters high. Two individuals were captured, euthanised with a 2 % lidocaine solution, fixed in a 10 % formaldehyde solution, stored in 70 % ethanol and deposited in the herpetology section of the Centro de Colecciones Biológicas de la Universidad del Magdalena (vouchers CBUMAG:ANF:01173 and 01174). Prior to formalin fixation, muscle tissue was collected from these voucher specimens and stored in 98 % ethanol.

To confirm the species identity using genetic information, we conducted a phylogenetic analysis using a fragment of the ribosomal 16S gene. Nine species of *Agalychnis* with available genetic information were included (Supplementary Table S2). PCR and sequencing followed the protocols by Rivera & Daza (2020). The assembled matrix comprised 33 terminals and 839 sites, including 11 outgroups within Phyllomedusidae. The tree was rooted using *Cruziohyla calcarifer*. The best model of evolution was obtained using ModelFinder (Kalyaanamoorthy et al., 2017) and a maximum likelihood tree was obtained using IQTREE 1.6.12 (Nguyen et al., 2015). Nodal support was obtained using the ultrafast bootstrap method after 5000 pseudoreplicates (Hoang et al., 2018). Intraspecific variation within *A. terranova* was estimated from the ml distance matrix from IQTREE.

The inferred phylogenetic tree (lnL=-4532.8) agrees with previous studies within *Agalychnis* and confirms the identity of *A. terranova* on the Sierra Nevada de Santa Marta (Fig. 4). Intraspecific variation including the sample from the new locality is very low ranging from 0.0 to 0.58 percent.

Calls from three individuals were recorded with a Sennheiser ME66/K6 directional microphone connected to a Zoom H4N digital recorder. Recording settings were 96 kHz sampling rate and 16-bit resolution. The air temperature and relative humidity were also recorded using a thermo-hygrometer RH 101 Extech IR. All recordings were analyzed using Raven Pro 1.5 software for Windows (Bioacoustics Research Program 2014 in Hann's sampling window, FFT window size of 512 points. Call features definitions follow Köhler et al. (2017). According to Köhler et al. (2017), call dominant frequency was considered as the frequency with the greatest amount of acoustic energy, note as the sound unit produced by a single expiratory event of the frog, and pulse as a single unbroken wave train isolated in time by amplitude

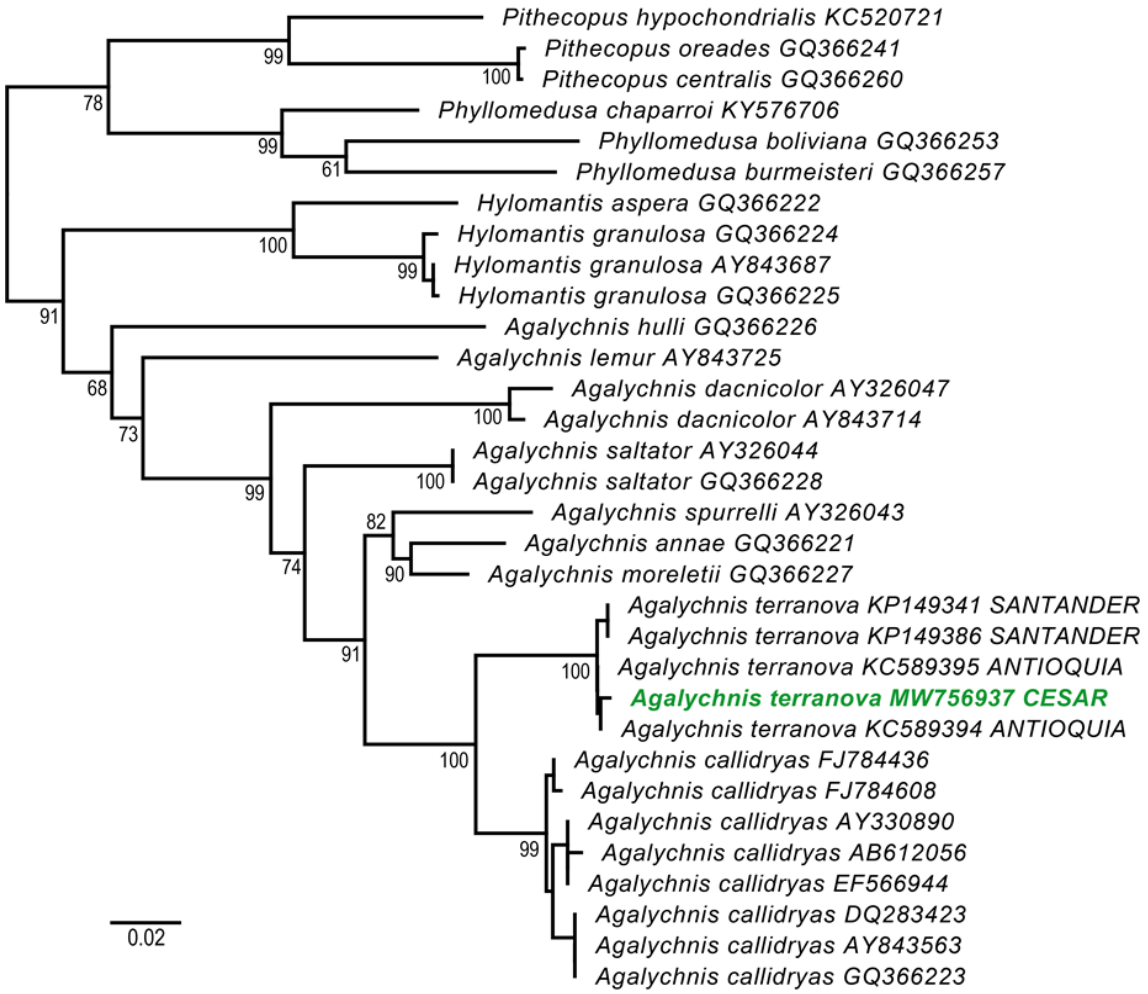

Figure 4. Maxium likelihood tree of Phyllomedusidae showing the phylogenetic position of *Agalychnis terranova* from the south-western foothills of the Sierra Nevada de Santa Marta. Numbers next to nodes represent ultrafast bootstrap support. See the Supplementary Material, Table S2.

reduction. We report numerical call features as mean ± SD and the respective range in parenthesis. The temporal call features (i.e., call duration, number and duration of notes and pulses) were measured in oscillograms; we used power spectra diagrams to calculate the call dominant frequency, and frequency bandwidth hereafter referred to as low frequency and high frequency, which were measured at 10 dB (re 10 mPA) below the peak intensity of the dominant call frequency. Graphs of oscillograms, spectrograms, and power spectra were elaborated with R 2.15.1 software using Seewave package (Sueur et al., 2008) settings (i.e., window name (Fourier transform window) = Hann; window length = 512 samples; and overlap = 90 %). The values of each call feature are presented with mean ±standard deviation (minimum - maximum). Five recording are housed at Fonoteca Zoológica (MNCN-CSIC): FZ SOUND CODE 12964 to 12968.

The tonal advertisement call of *Agalychnis terranova* (Fig. 5) consisted of one or two notes with similar dominant frequency of approx. 1.74 kHz and total call duration of 0.52 s, N = 7 calls. These calls were recorded from three solitary males perched between five and seven meters from the riparian vegetation. A summary of acoustic features is presented in the Table 1. According to the guild classification system proposed for anuran advertisement calls (Emmrich et al., 2020), *A. terranova* has an advertisement call of the guild E type, which corresponding to multi, uniform and no modulated notes.

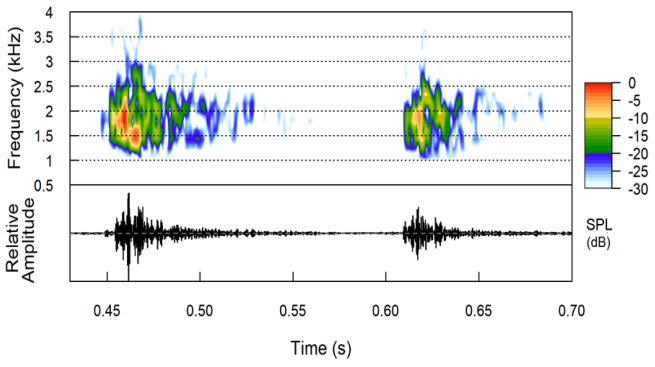

Figure 5. The tonal advertisement call of *Agalychnis terranova* (voucher CBUMAG:ANF:01173, recording time 18:10 h, air temperature 26.8 ˚C, air humidity 88 %) from the Sierra Nevada de Santa Marta, Colombia. Spectrogram (above), oscillogram (below).

Table 1. Summary of tonal call features for *Agalychnis terranova* from the Sierra Nevada de Santa Marta, Colombia. Data of seven calls from three individuals (including voucher CBUMAG:ANF:01173); air temperature 26.8 ˚C, relative humidity 88 %.

	Call duration (s)	Inter-Call intervals (s)	1˚ Note duration (s)	2˚ Note duration (s)	Inter-Note interval (s)	1˚ Note Dominant frequency (kHz)	1˚ Note Low frequency (kHz)	1˚ Note High frequency (kHz)	2˚ Note Dominant frequency (kHz)	2˚ Note Low frequency (kHz)	2˚ Note High frequency (kHz)
Mean	0.52	29.925	0.227	0.173	0.087	1.739	1.160	2.300	1.741	1.128	2.193
SD	0.15	2.07	0.05	0.06	0.03	0.20	0.06	0.16	0.19	0.08	±0.07
Min	0.03	17.97	0.03	0.047	0.03	1.37	1.060	2.660	1.360	1.040	2.080
Max	0.46	22.11	0.15	0.20	0.10	1.89	1.280	2.120	1.890	1.250	2.280

Table 2. Tonal call features in the *Agalychnis callydrias* species group. Species are sorted according to the phylogeny in Figure 4.

Species	Notes/Call	Call duration (s)	Dominant frequency (kHz)	Source
Agalychnis saltator Taylor, 1955	1 –2	0.08–0.12	1.844–1.89	Duellman (1970)
Agalychnis spurelli Boulenger, 1913	1	0.13–0.36	0.75–1.051	Cossio & Medina-Barcenas (2020)
Agalychnis annae (Duellman, 1963)	1	0.16–0.44	1.044–1.295	Duellman (1970)
Agalychnis moreletii (Duméril, 1853)	1	0.022–0.088	1.046–1.396	Duellman (1970); Briggs (2010)
Agalychnis terranova				
Rivera-Correa, Duarte-Cubides, Rueda-Almonacid & Daza, 2013	1–2	0.03–0.46	1.36–1.89	This Study
Agalychnis callidryas (Cope, 1862)	1–2	0.08-0.24	1.488–2.4	Duellman (1970); Lee (1996)

The advertisement call of *A. terranova* is similar to that of its sister species *A. callidryas* (Duellman, 1970; Lee, 1996); both show one or two notes and their call durations and dominant frequencies overlap. Likewise, when the call characteristics of *A. terranova* are compared with the other species of the *A. callidryas* group, several similarities are observed (Table 2).

This is the first record of *A. terranova* in the Sierra Nevada de Santa Marta and extends its geographical distribution over 370 km in a straight-line from San Vicente de Chucurí, Santander, Colombia, the north-easternmost locality previously reported in literature (Guarnizo et al., 2015). Furthermore, this record extends the presence of this species to a new biogeographic region, the Maracaibo province (Morrone, 2001), adding to the other two provinces (Chocó and Magdalena) where the species has been found. With the description of advertisement call of *A. terranova*, we contribute to acoustic studies of Colombian anuran species, mainly those that inhabit the dry forest (Vargas-Salinas et al., 2019).

The Colombian red-eyed tree frog, *A. terranova*, is included in the Near Threatened (NT) IUCN red list category in the most recent Colombian amphibian assessment. This is due to the continuous decline in the extent and quality of habitat in the few localities that are known for this species due to several human activities including mining and the construction of dams (IUCN SSC Amphibian Specialist Group 2017). Similar to the other known localities for the species, the south-western foothills of the Sierra Nevada de Santa Marta are being severely deforested to make way for livestock, crops, and mining, which may be affecting this particular population of *A. terranova*.

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