

GEOGRAPHIC DISTRIBUTION, ADVERTISEMENT CALL DESCRIPTION, AND PHYLOGENETIC POSITION OF *PRISTIMANTIS TAENIATUS* (ANURA: CRAUGASTORIDAE)

DISTRIBUCIÓN GEOGRÁFICA, DESCRIPCIÓN DEL LLAMADO DE ANUNCIO Y POSICIÓN FILOGENÉTICA DE *PRISTIMANTIS TAENIATUS* (ANURA: CRAUGASTORIDAE)

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Resumen.— *Pristimantis taeniatus* (Boulenger 1912) es una rana terrestre relativamente común en Colombia y Panamá, recientemente registrada en Costa Rica. Sin embargo, el estatus taxonómico y la posición filogenética de las poblaciones en Costa Rica son actualmente desconocidos. Aquí nosotros reportamos *P. taeniatus* de dos nuevas localidades en Costa Rica, describimos su canto de anuncio y revisamos su posición filogenética dentro de su rango de distribución a partir de secuencias moleculares disponibles y algunas previstas por nosotros. Nosotros encontramos que *P. taeniatus* está formado por dos clados con profundas distancias genéticas que podrían representar distintas especies.

Palabras clave.— América Central Ístmica, bioacústica, código de barras, Panamá, Terrarana.

Abstract.— *Pristimantis taeniatus* (Boulenger 1912) is a relatively common terrarana in Colombia and Panama, recently recorded from Costa Rica. However, the taxonomic status and the phylogenetic position of the Costa Rican population are currently unknown. Here, we report *P. taeniatus* from two new localities in Costa Rica, describe the advertisement call and assess the phylogenetic position within its distribution range using available molecular sequences and some newly generated sequences for this study. We found that *P. taeniatus* is formed by two clades with deep genetic distances that might represent different species.

Keywords.— Barcoding, bicoustics, Isthmian Central America, Panama, Terrarana.

INTRODUCTION

Morphological similarities found among the direct-developing frogs (Hedges et al., 2008; Heinicke et al., 2018) has resulted in the presence of several cryptic species masked within current names in several genera (Padial & De la Riva 2009; Arias et al., 2019). *Pristimantis* is a genus of direct-developing frogs that includes 587

species distributed in the Neotropic (AmphibiaWeb 2022). The phylogenetic position of many *Pristimantis* species is unknown therefore, intraspecific variation and masking of species within current names is unknown. Boulenger (1912) described *Hylodes taeniatus* based on specimens from the San Juan River, Chocó,

Colombia. Currently the species is in the genus *Pristimantis* (Hedges et al., 2008). It is a relatively common species found in Colombia and Panamá (Lynch & Ardila-Robayo, 1999; IUCN, 2018; Batista et al., 2020; Acosta-Galvis, 2021). Four specimens (UCR20301–3, UCR21250) collected in 2008 from Potrero Grande on the Southern Pacific, Costa Rica, were tentatively referred to *P. taeniatus* (Bolaños et al., 2011; Leenders, 2016; IUCN, 2018). In addition, Gómez-Hoyos et al. (2018) reported the species from La Palmira, Coto Brus, Puntarenas, Costa Rica, detected through a citizen science initiative. Batista et al. (2020) reported this species from Western Panama, near the Costa Rica-Panama border, however, they suggested that those populations could represent an unnamed species. The presence of *P. taeniatus* in Costa Rica is not easy to assess taxonomically. Leenders (2016) stated that the distribution range for this species in Costa Rica is unknown, while Frost (2022) does not report the species for the country. Determining the evolutionary relationships and other species-specific attributes (e.g., morphology and bioacoustics) of the populations reported in the country could give insights on their taxonomic status. However, to date the phylogenetic position as well as other diagnostic characteristics of the Costa Rican populations are unknown. Here, we report *P. taeniatus* from two new localities in Costa Rica, describe its advertisement call and assess the phylogenetic position within the species along most of its distribution.

MATERIALS AND METHODS

On 29 May 2017 during fieldwork in Sabalito, Coto Brus, Puntarenas, Costa Rica, we discovered a population of an unknown *Pristimantis*. We collected one male which was euthanized with 5% lidocaine, fixed in 96% ethanol and later stored in 70% ethanol. From this specimen we extracted a muscle tissue sample from thigh that was stored in 96% ethanol. The voucher specimen is housed at the herpetological collection of Museo de Zoología at Universidad de Costa Rica (UCR 23184). Before collecting, the advertisement call of this male was recorded at 19:00 h, using a Tascam D-40 digital recorder (sampling rate: 44.1 kHz; accuracy: 24 bit; file format: WAV) from approximately 1 m of distance. The audio and a photo of the voucher is deposited at the Fonoteca Zoológica Animal Sound Library at the Museo Nacional de Ciencias Naturales of Madrid, Spain (www.fonozoo.com –Fz SOUND CODE 11981). We described this call following standard terminology and protocols (Köhler et al., 2017). We used the sound analysis software Raven Pro 1.6.1 to measure the following spectro-temporal parameters: duration (s), inter-note interval (s), maximum (kHz), minimum (kHz), and peak frequency (kHz). Spectrograms analysed were created using Raven's default settings (FFT Hanning;

Window size: 512 samples; Hop size: 256 samples; 50 % temporal overlap; 86.1 Hz of grid spacing). To visualize it we constructed a spectrogram and oscillogram using the R package seewave (Sueur et al., 2008).

We extracted total genomic DNA from the preserved tissue samples using the phenol-chloroform standard protocol (Sambrook & Russell, 2006). We included two additional specimens housed in UCR (UCR20301 and UCR20303) collection that were previously identified as *P. taeniatus* from Potrero Grande, Buenos Aires, Puntarenas, Costa Rica (9.1023° N, 83.1142° W, 1005 m a.s.l.). We obtained partial sequences (569 bp) of the large subunit ribosomal RNA (16S) mitochondrial gene for three specimens from southwestern Costa Rica (Fig. 1). The protocols for DNA extraction, amplification, sequencing, and editing follow those of Arias et al. (2018). The 16S sequences are available under GenBank accession numbers MT176435–MT176437. We compared the sequences here obtained with sequences available in GenBank of the 16S rRNA (16S) and cytochrome oxidase 1 (COI) mitochondrial genes for 43 specimens of closely related species to *P. taeniatus* following Acevedo et al. (2020) and including those samples referred by Pinto-Sánchez et al. (2012) as *P. taeniatus* and *Pristimantis* aff. *taeniatus*. We used a sequence of *P. pardalis* as outgroup following to Acevedo et al. (2020). Detailed molecular laboratory techniques and GenBank accession numbers for these sequences are provided in Pinto-Sánchez et al. (2012) and Acevedo et al. (2020). Phylogenetic analyses were performed using both the maximum likelihood and Bayesian analyses following Arias et al. (2019).

RESULTS

New records

COSTA RICA: Provincia de Puntarenas: Cantón de Coto Brus: Distrito de Sabalito: near Sabalito town (8.8215° N, 82.9150° W, 905 m a.s.l.). An adult male (UCR23184) collected on 29 May 2017 about 19:00 h. Several other individuals were heard calling hidden on leaves of ornamental plants in the coffee plantation borders. A disjunct population was located at Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: Ujarrás (9.28° N, 83.3° W, 900 m a.s.l.) on February 2019 through calls but no individuals were seen.

Identification

The specimen of *P. taeniatus* from Sabalito (UCR23184; Fig. 2) agree with the description provided by Lynch (1980). The specimen from Sabalito agree with the photos shown by Leenders (2016), who stated that this species was recently reported from Costa Rica but did not indicate the distribution; those shown photos

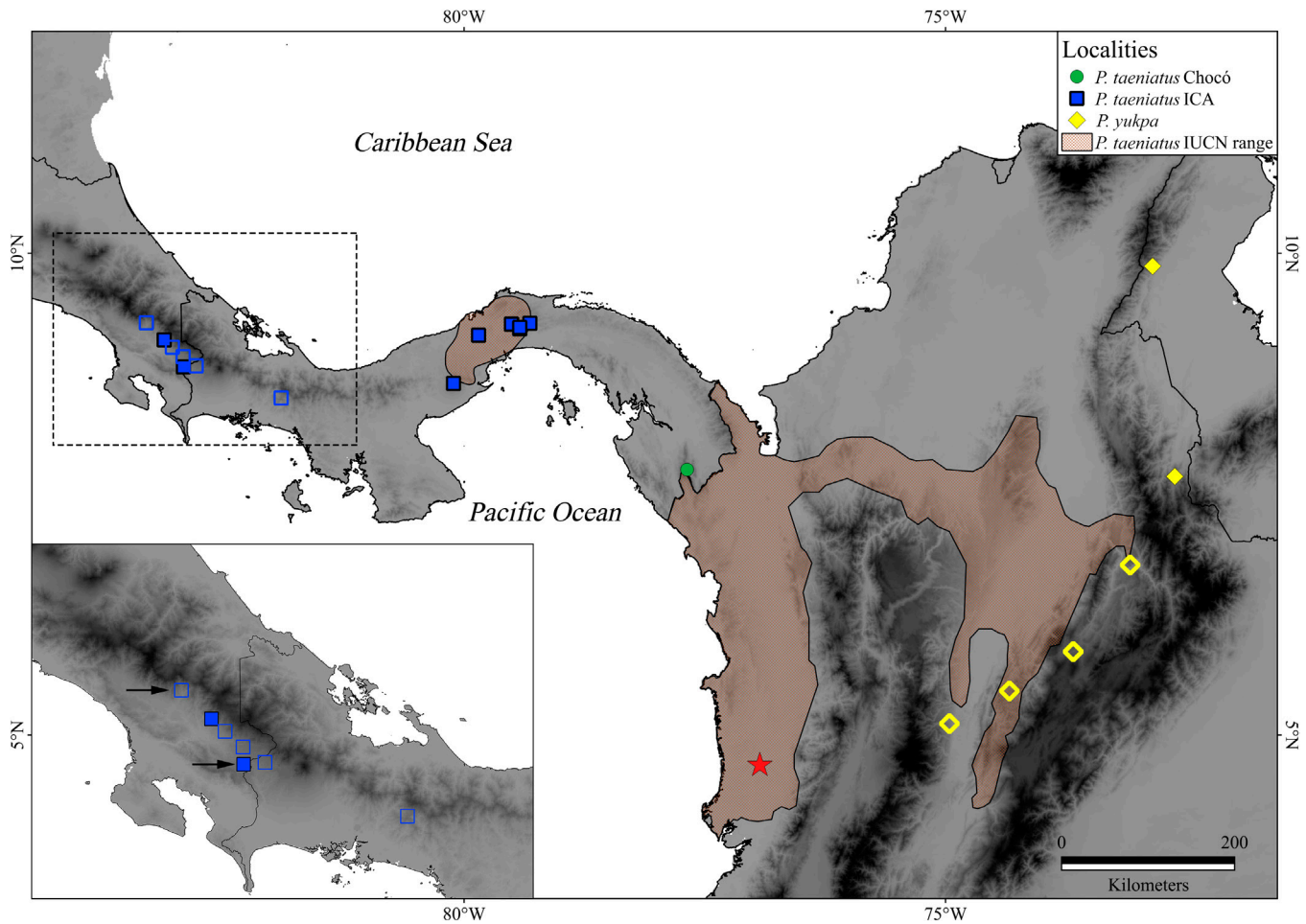


Figura 1. Mapa mostrando el rango de distribución conocido para *Pristimantis taeniatus*, incluyendo dos nuevas localidades reportadas en este trabajo (flechas) y de su relativo *P. yukpa*. Círculos y cuadrados rellenos indican las localidades con secuencias disponibles, cuadrados vacíos indican localidades sin datos genéticos; la estrella roja corresponde con la localidad tipo de *P. taeniatus*. Los diamantes muestran la distribución de *P. yukpa* (los diamantes rellenos corresponden a localidades previamente conocidas). El cuadro incrustado resalta la distribución en el suroeste de Costa Rica y el oeste de Panamá. La capa de distribución es cortesía de la Lista Roja de la IUCN (2018).

Figure 1. Map showing the known distribution range for *Pristimantis taeniatus*, including two new localities reported in this work (arrows) and its closed relative *P. yukpa*. Solid circles and squares indicate the localities with sequences available, empty squares indicate localities without genetic data; the red star corresponds to type locality of *P. taeniatus*. The diamonds shown the distribution of *P. yukpa* (the solid diamonds correspond to previously known localities). The inset map highlights the distribution in southwestern Costa Rica and western Panamá. The distribution shape is courtesy of the IUCN Red List (2018).

are from Panamanian individuals (see photos of *P. taeniatus* in AmphibiaWeb, 2022).

The specimens from Costa Rica are small (males SVL = 25.70 ± 0.56 mm, n = 3, female SVL = 30.4 mm, n = 1); head relatively narrow, HW = 38.4 ± 0.2 % of SVL; snout rounded to subovoid in the dorsal view, rounded in profile; snout relatively long (HL = 34.7 ± 0.6 % of SL), with nostrils directed laterally; in ventral view, tip of snout protruding markedly beyond edge of lower lip. Internarial area convex; canthus rostralis rounded; loreal region slightly concave; vomerine teeth transverse, in two fascicles

behind choanae; choanae moderately large; no vocal slits. Eye large (EN/ED = 85.9%), protruding beyond dorsal outline of head in ventral view, directed laterally. Tympanic membrane distinct, covered in skin; tympanic annulus prominent, round (TY/ED = 37.1–45.1%). Skin on dorsal and lateral surfaces of head smooth. Upper eyelid smooth, with one-three small tubercles. Two-paired postrectal tubercle, postero-ventral to tympanum. Skin on dorsum and limbs smooth to shagreen with a W-shaped scapular fold; two tubercles sacral. Skin of chest and throat smooth, venter coarsely areolate with low granules; ventral surfaces of thighs areolate; skin of groin smooth. Flanks smooth.



Figura 2. Macho adulto de *Pristimantis taeniatus* (UCR23184) encontrado en Coto brus, Costa Rica. Foto: Cesar Barrio-Amorós.

Figure 2. An adult male *Pristimantis taeniatus* (UCR23184) found on Coto Brus, Costa Rica. Photo: Cesar Barrio-Amorós.

Discoïdal fold complete. Forelimb relatively short and slim; fingers moderately long and slim with evident lateral fringes. Discs present, expanded truncate; all fingers with grooves; pads truncate. Supernumerary tubercles absent; one-two small and rounded accessory palmar tubercles; subarticular tubercles rounded in basal outline, slightly projecting in form, and obtuse in profile; thenar tubercle elongate and palmar tubercle hear-shape, fat, palmar slightly larger than thenar. Ulnar fold absent.

Fingers not webbed. Nuptial pads in adult males. Legs relatively long and slim, toes with evident lateral fringes; heel with two globular tubercles. Discs and grooves on all toes, expanded truncate on Toe IV and IV; pads truncate. Supernumerary tubercles absent; plantar tubercles small and rounded; subarticular tubercles ovoid in basal outline, projecting in form, and obtuse in profile; inner metatarsal tubercle elongate, flat; outer metatarsal tubercle rounded, globular; outer

metatarsal tubercle much smaller than inner; inner edge tarsal with an incomplete fold. Dorsum brown suffused with red with dark brown blotch covering the W-shaped fold and the sacral tubercles; dark brown stripe covering the supratympanic fold and the top half of the tympanum; dark brown band in the loreal region, lips with dark brown marks; dark brown interorbital line; arm and posterior surfaces of thighs with dark brown stripes.

Pristimantis taeniatus from Costa Rica have a relatively high level of intraspecific polymorphism; some specimens have tips of Finger III rounded (UCR23184), whereas others have tips expanded (UCR20301, UCR21250). The Sabalito male has elliptical and inclined tympanum while the Potrero Grande males have round tympanum. The specimens from Costa Rica are similar to other specimens from Panama (Amphibians of Panama, 2021).

The combination of the following characteristics distinguishes *P. taeniatus* from its congeners on Costa Rica: (1) presence of a W-shaped scapular fold covered with dark brown blotch; (2) presence of two tubercles sacral covered with dark brown blotches; (3) presence of small supraocular tubercles on upper eyelids; (4) presence of lateral fringes in fingers and toes; (5) presence of nuptial pads in males; and (6) lack of inguinal

white or orange flash coloration.

Molecular phylogenetic analysis

The resulting mitochondrial data matrix included 47 sequences with a total sequence length of 1238 bp including gaps; 580 bp for 16S and 658 bp for COI. The best strategy partition contains four partitions, one for 16S and one for each codon in COI. The

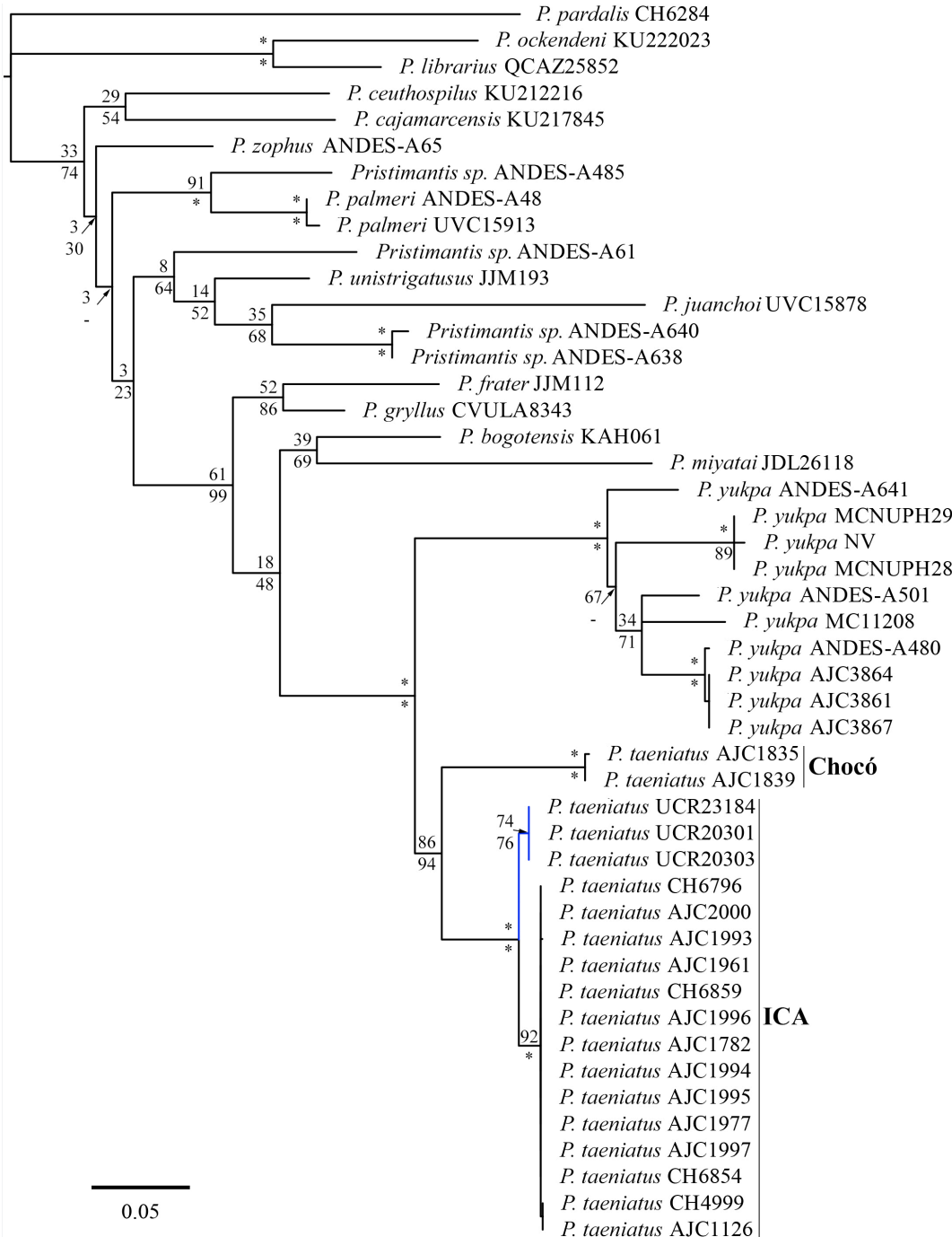


Figura 3. Filogenia de máxima verosimilitud (log likelihood = -5798.567851) mostrando la población de *Pristimantis* encontrada en Sabalito, Costa Rica dentro de *Pristimantis taeniatus* basado en los genes de ADN mitocondrial 16S y COI. Las proporciones de bootstraps son mostradas sobre las ramas y las probabilidades posteriores (multiplicadas por 100) del análisis en MrBayes son mostradas debajo de las ramas. La barra de escala representa las sustituciones estimadas por sitio. Los asteriscos representan soporte >95. NV = sin voucher.

Figure 3. Maximum likelihood phylogeny (log likelihood = -5798.567851) showing the population of *Pristimantis* from Sabalito, Costa Rica within *Pristimantis taeniatus* based on 16S and COI mitochondrial DNA genes. Bootstraps proportions are shown above branches and posterior probabilities (multiplied by 100) from MrBayes analysis are shown below branches. The scale bar refers to the estimated substitutions per site. The asterisks represent support of >95. NV = no voucher.

following substitution models were selected: SYM+I+G for 16S, TrN+G for COI codon position 1, K80+I for COI codon position 2, and F81+I for COI codon position 3. The ML and Bayesian trees were concordant in supporting the tree in figure 3. The phylogeny shows that the samples referred to *P. taeniatus* are grouped in two well-supported clades (Fig. 3). The first clade (hereafter called Chocó clade) is formed by two samples from Darien, Panama and the second clade contains the samples from

Isthmian Central America -central Panama and those from Costa Rica- (hereafter ICA clade). Genetic distances between ICA clade and Chocó clade are 5.9 – 6.8 % (16S) and 13.8–14.5 % (COI). These two clades that we referred as *P. taeniatus* form the sister clade to *P. yukpa* Barrio-Amorós, Rojas-Runjaic & Infante-Rivero, 2008 from Cordillera Central, Colombia including the samples from Magdalena Valley, previously considered as *P. taeniatus*. These two species (*P. taeniatus* and *P. yukpa*) form a well-supported

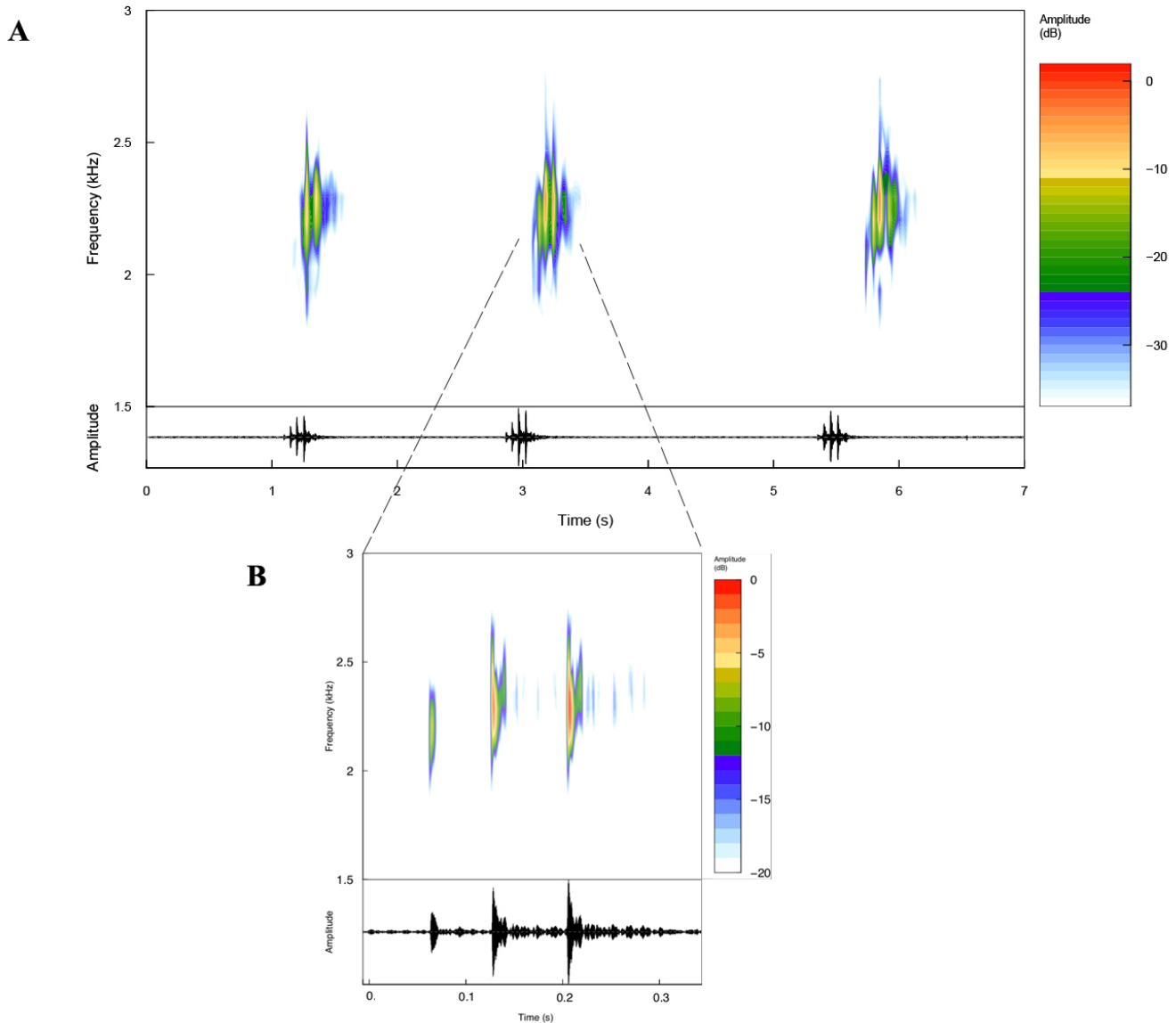


Figura 4. A) Espectrograma y oscilograma del canto de anuncio completo de *Pristimantis taeniatus* (UCR23184, SVL = 26.18 mm, 21.5 °C) consistiendo de tres notas. B) Detalle de una de estas notas.

Figure 4. A) Spectrogram and oscillogram of the full advertisement call of *Pristimantis taeniatus* (UCR23184, SVL = 26.18 mm, 21.5 °C) consisting of three notes. B) Detail of one of the notes.

clade that includes to *P. bogotensis* (Peters, 1863), *P. frater* (Werner, 1899), *P. gryllus* Barrio-Amorós, Guayasamin & Hedges, 2012, and *P. miyatai* (Lynch, 1984). Also, the phylogeny shows that the specimens previously referred as *P. aff. taeniatus* correspond to unnamed species and/or some species of *Pristimantis* from Colombia that could not be taxonomically determined.

Acoustics

The advertisement call of *P. taeniatus* has an approximate duration of 0.25 seconds and consists of multiple (three in this case) tonal and modulate notes that increase in amplitude to the previous note (Fig. 4). Duration of each note was 0.410 secs, 0.352 secs and 0.320 secs. The first and the second note were separated by 1.295 secs whereas the second and the third by 2.126 secs. The notes have a mean duration of 0.361 ± 0.045 secs, with short inter-note intervals of 1.711 ± 0.578 . Notes are produced in a range between 1651.9 ± 18.1 and 2635.2 ± 53.6 with a peak frequency of 2196.4 Hz. Although the third note reaches slightly higher frequencies, peak frequency was the same for all notes.

DISCUSSION

The preliminary phylogenetic evidence suggests that *P. taeniatus* is formed by two clades that could have resulted in divergence as a consequence of geographic distance (Slatkin 1993). However, the deep genetic distances found suggest that we may be dealing with a case of cryptic diversity that requires further studies integrating phenotypic-level information throughout the distribution of the species. The genetic distances among the two clades found are above the thresholds of 3% in 16S and 10% in COI mitochondrial genes suggested by Fouquet et al. (2007) and Vences et al. (2005), respectively, to define candidate species.

We consider, the two clades within *P. taeniatus* should be analysed using an integrative taxonomy approach, combining information on morphology, DNA sequences, acoustics, or other independent evidence sources (Padial et al. 2010). Especially important is the inclusion of sequences of specimens from the type locality and other sites on the Chocó to delimit the phylogenetic position of *P. taeniatus* [sensu stricto].

The call of *P. taeniatus* (referred in the publication as *Eleutherodactylus ockendeni*) was first described onomatopoeically as a “wrack-ak-ak-ak” by Duellman (1967). Later, Ibañez et al. (1999) provided a spectrogram for this species that visually coincides in dominant frequency with the call we recorded. However, these authors described it as a high-pitched long thrill with a larger number of notes (Ibañez et al., 1999). Although the available sample is limited, the calls from Costa Rica and

Magdalena Valley are different in structure, with 3 elements in Costa Rica (Fig. 3) but 4–6 elements in those specimens from Ibagué, Colombia (Bernal et al., 2004). For these specimens, reported dominant frequency is 1800 hz, way lower than the measured here for the Costa Rican call. Note duration is similar for both the call from Costa Rica and the call from Ibagué, 0.361 and 0.372 secs, respectively. Nevertheless, it is necessary to determine the taxonomic status of the species recorded by Bernal et al. (2004), due that it is possible that this call correspond to *P. yukpa* (see below) or another species of *Pristimantis*. Also, it is necessary to record the call of the specimens from the Chocó clade and compare it with those shown by us.

The evidence shown suggests that the populations from Costa Rica and western Panama could be referred to *P. taeniatus* with high confidence, although in the future these populations could be considered a different species. In Costa Rica *P. taeniatus* is known only of the Premontane Rainforest (900 – 1,000 m a.s.l.), therefore we suggested that for this country the species is restricted to Valle del General on Southwestern; it is possible that this species extends its distribution range 50 km to the North to Perez Zeledón. Although the specimens used by Batista et al. (2020) were not available for our phylogenetic analysis, it is very plausible that these specimens correspond to the ICA clade. In addition, the specimens from Magdalena Valley, Colombia used by Pinto-Sánchez et al. (2012) -referred as *P. taeniatus*- correspond with *P. yukpa*, extending 386 km to the Southeast the known distribution for this latter species (Acevedo et al. 2020). Therefore, it is necessary to evaluate the taxonomy and distribution of *P. taeniatus* in the Northwestern Colombia as the species could be restricted to Colombian Chocó.

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