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PRIMER REGISTRO DE LA RANA DE LLUVIA, *PRISTIMANTIS MUSEOSUS* (IBAÑEZ, JARAMILLO & AROSEMENA, 1994), EN COSTA RICA

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Resumen.—Reportamos el primer registro para Costa Rica de la hermosa rana de desarrollo directo *Pristimantis museosus* (Anura: Strabomantidae). Encontramos una hembra adulta en Alto Urén, en la Cordillera de Talamanca cerca de la frontera entre Costa Rica y Panamá. Este espécimen concuerda morfológicamente y filogenéticamente con otros *P. museosus*, incluyendo aquellos de la localidad tipo. Encontramos que *P. museosus* está formada por dos subclados, separados entre sí por distancias genéticas de 3.2 % en el gen 16S y 7.1 % en el citocromo oxidasa I (COI); el espécimen de Costa Rica se agrupa con aquellos del este de Panamá, incluyendo los más cercanos a la localidad tipo. También encontramos que otras especies dentro del grupo de especies *P. ridens* tienen alto nivel de variación genética con distancias genéticas que alcanzan hasta 14.5 % en el gen 16S y 19.1 % en el COI dentro de las especies actuales. Lo anterior podría estar enmascarando linajes crípticos dentro de las especies como reconocidas actualmente.

Palabras clave.—Código de barras de ADN, Panamá, Strabomantidae, Talamanca.

Abstract.— We report the first record of the direct-developing frog *Pristimantis museosus* (Anura: Strabomantidae) in Costa Rica. We found an adult female near the Costa Rica-Panamá border in Alto Urén, Caribbean slope of Talamanca Mountain Range. This specimen agrees morphologically and phylogenetically with other *P. museosus*, including the type series. *Pristimantis museosus* is formed by two subclades, separated by genetic distances of 3.2 % in 16S and 7.1 % in cytochrome oxidase I (COI); the specimen from Costa Rica is grouped within the subclade including those specimens from eastern Panamá, nearest to type locality. Also, we found that other species in the *P. ridens* Species Group have a high-level of genetic variation with genetic distances reaching up to 14.5 % in 16S and 19.1 % in COI under current taxonomy. This may mask cryptic lineages under the currently recognized taxa.

Keywords.— DNA barcoding, Panama, Strabomantidae, Talamanca.

One of the most remarkable examples of speciation in the Neotropics is the radiation of direct-developing frogs, including approximately 1,266 species in seven families and 29 genera (Frost, 2024). This group of frogs includes the most diverse genus of vertebrates in the world, *Pristimantis* Jiménez de la Espada, 1870; with ca. 612 species (Frost, 2024) distributed mainly in South America (Padial et al., 2014). The direct-developing frogs correspond with a high percentage of the amphibian diversity

in the Neotropics, for example, these frogs correspond with the 13.6 % of the anuran diversity in Brazil, 34.4 % in Colombia, 44.9 % in Ecuador, 37.5 % in Peru, and 31.1 % in Panamá (Frost, 2024). Costa Rica hosts 220 amphibian species (AmphibiaWeb, 2024; Frost, 2024) and this number grows each year as new species are described (Arias et al., 2024) and as new country records for previously described species are made (Pinto et al., 2024). Direct-developing frogs (i.e., *Craugastor* Cope, 1862; *Diasporus*

Hedges Duellman & Heinicke, 2008; *Eleutherodactylus* Duméril & Bibron, 1841; *Pristimantis*; and *Strabomantis* Peters, 1863) make up 31.6 % of the anuran's diversity in Costa Rica. According to Frost (2024), the genus *Pristimantis* contains 10 species in Costa Rica: *P. altae* (Dunn, 1942), *P. caryophyllaceus* (Barbour, 1928), *P. cerasinus* (Cope, 1875), *P. cruentus* (Peters, 1873), *P. educatoris* Ryan, Lips & Giermakowski, 2010, *P. gaigei* (Dunn, 1931), *P. moro* (Savage, 1965), *P. pardalis* (Barbour, 1928), *P. ridens* (Cope, 1866), and *P. taeniatus* (Boulenger, 1912). However, we follow Batista et al. (2014) who synonymized *P. educatoris* under *P. caryophyllaceus*. Previous studies suggest that the diversity of the genus may be underestimated due to notable morphological variation in species like *P. cruentus* (Savage, 2002) and the relatively low exploration effort in unexplored regions (Arias et al., 2024).

On 19 April 2024 at 21:00 h, we found a unique *Pristimantis* that we tentatively identified as *P. museosus* (Ibañez, Jaramillo & Arosemena, 1994). We found the individual during a dry night,

on the upper surface of a leaf at a height of about 2 m above the ground. The site is a mature forest near a small river in Provincia de Limón, Cantón de Talamanca, Distrito de Telire, Alto Urén (9.431° N; 82.978° W; WGS 84; 700 m a.s.l.; Fig. 1). The specimen, an adult female, was collected and humanely euthanized using a topical anesthetic. Following the euthanasia, a tissue sample was taken and stored in 96 % ethanol. The specimen was fixed in a 10% formalin solution and transferred into 70 % ethanol for long-term preservation. The specimen was deposited in the herpetological collection of Museo de Zoología at Universidad de Costa Rica (UCR). Museum collection acronyms follow Frost (2024).

To confirm the identity of the specimen UCR24442, tentatively identified as *Pristimantis museosus*, we performed a study of external morphology characters based on the most conspicuous phenotypic traits mentioned in the original description of *P. museosus* (Ibañez et al., 1994). We performed the measurements

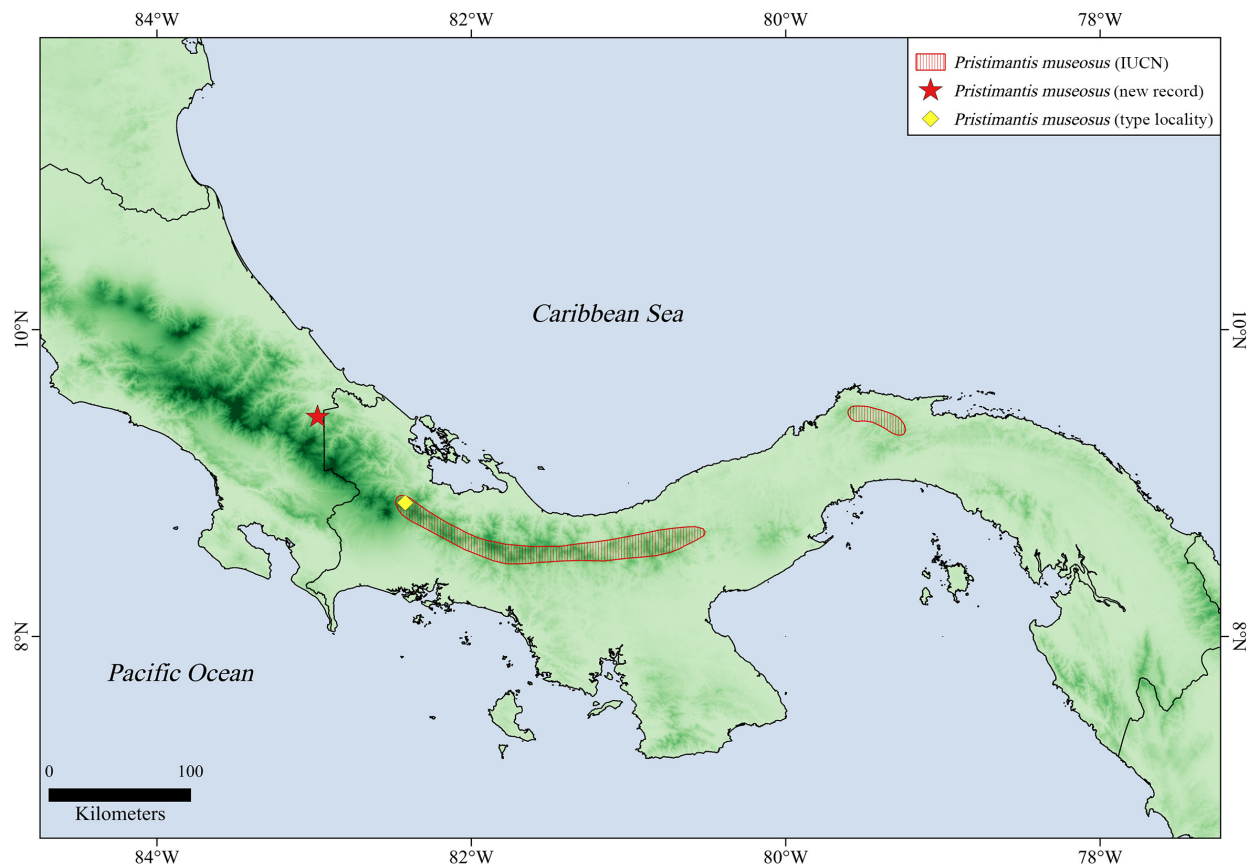


Figura 1. Distribución geográfica conocida para *Pristimantis museosus* (sombra). La estrella roja corresponde con el nuevo registro, en Alto Urén, Limón, Costa Rica. Fuente: La Lista Roja de la IUCN, descarga de datos el 20 de julio de 2024. <https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T56780A54350678.en>

Figure 1. Known geographic distribution of *Pristimantis museosus* (shadow). The red star marks the new record in Alto Urén, Limón, Costa Rica. Source: The IUCN Red List, data download on 2024-07-20. <https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T56780A54350678.en>

shown by Ibañez et al. (1994) and added the width of finger III (WF3) and width of finger IV (WF4). Additionally, we obtained partial sequences of the large subunit ribosomal RNA (16S) and cytochrome oxidase I (COI) mitochondrial genes. We used the primers 16Sar and 16Sbr (Palumbi et al., 1991) for 16S and dgLCO and dgHCO (Meyer, 2003) for COI. The protocols for DNA extraction, amplification, sequencing, and editing are from Arias et al. (2018). We compared the DNA sequences for UCR24442 with selected 16S and COI sequences available on GenBank for members of the *P. ridens* Species Group in Costa Rica and Panamá. The list of vouchers and GenBank accession numbers used in this study are provided in Appendix 1, detailed molecular laboratory techniques are provided in the primary source (Crawford et al., 2010, 2013; Pinto-Sánchez et al., 2012; Batista

et al., 2014; Paz et al., 2015; Sepulveda-Seguro et al., 2022). We used a sequence of *P. taeniatus* as an outgroup following Padial et al. (2014). We analyzed mtDNA concatenated using Maximum Likelihood (ML) with RAxML v.8 (Stamatakis, 2014) and Bayesian phylogenetic analysis with MrBayes 3.2.6 (Ronquist et al., 2012), run on the CIPRES portal (Miller et al., 2010). Arias et al. (2019) provided detailed protocols for alignment, searching for the best partition scheme, determining the best model of sequence evolution for each partition, and conducting phylogenetic inference using RAxML and MrBayes.

The resulting data matrix had a total sequence length of 1,264 bp, including gaps: 606 bp for 16S and 658 bp for COI. Three partitions were identified with these models: GTR+G

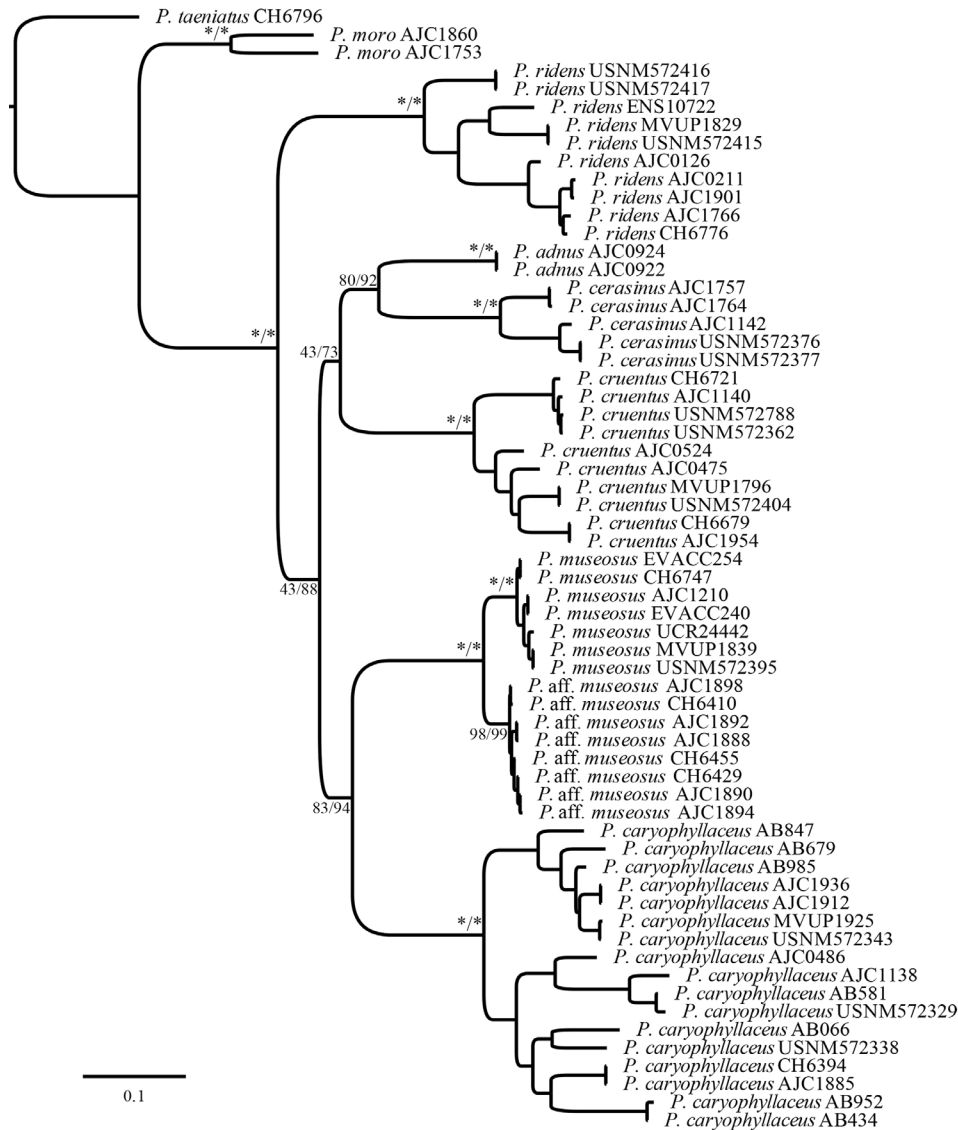


Figura 2. Inferencia filogenética bayesiana mostrando la relación del espécimen costarricense de *Pristimantis museosus* (UCR24442) entre miembros del grupo de especies *P. ridens* basándose en los fragmentos de los genes de ADN mitocondrial 16S y COI. Las proporciones de Bootstrap del análisis de máxima verosimilitud y las probabilidades posteriores (multiplicadas por 100) del análisis de MrBayes se muestran separadas por una barra inclinada (izquierda y derecha respectivamente). La barra de escala se refiere a las sustituciones estimadas por sitio. Los asteriscos representan un soporte de 100.

Figure 2. Bayesian phylogenetic inference shows the relationship of the Costa Rican specimen of *Pristimantis museosus* (UCR24442) among members of the *P. ridens* Species Group based on the 16S and COI mitochondrial DNA gene fragments. Bootstrap proportions from maximum likelihood and posterior probabilities (multiplied by 100) from MrBayes analysis are shown separated by a slash (left and right sides, respectively). The scale bar refers to the estimated substitutions per site. The support values of nodes within species are not shown. The asterisks represent support of 100.



for 16S + COI codon position 1, SYM+G for codon position 2 of COI, and HKY+I for COI codon position 3. The phylogenies from Garli and MrBayes were almost identical in topology (Fig. 2), both found the same phylogenetic relationships among the species, but minor differences were found within *Pristimantis caryophyllaceus* and *P. cruentus*. The phylogeny shows UCR24442 to be conspecific with *P. museosus*. This confirms the taxon's presence in Costa Rica.

The specimen UCR24442 matches the combination of diagnostic morphological characters, color patterns, and measurements (Table 1) as described by Ibáñez et al. (1994). The collected female has a strongly tuberculate dorsum; subacuminate snout in dorsal view; a pointed heel tubercle; well-developed accessory palmar tubercles; outer tarsal tubercles in series; distinct pointed tubercles on the upper eyelid; pads of fingers III and IV strongly expanded; green dorsum with brown markings in life (Fig. 3); purple color in preservative (Fig. 4); and a gold iris with a black reticulum. The female collected in Costa Rica is larger (SVL = 38.9 mm vs. 26.1 mm in adult male holotype) showing possible sexually dimorphic variation. In addition, the female lack of nuptial pads and vocal slits.

Crawford et al. (2013) evaluated the potential cryptic lineages within of *Pristimantis museosus*, they included samples from several localities in Panamá, including a site to 190 km east of type locality. Crawford et al. (2013) identified two well-supported clades using the same mitochondrial genes as those used herein. The specimen from Costa Rica grouped with the clade containing those specimens collected nearest of the

type locality. Thus, UCR24442 refers to the nominal name *P. museosus* with high confidence; the specimen from Costa Rica is separated of other *P. museosus* by genetic distances of 0.5–3.8 % in 16S and 1.2–7.9 % in COI. Our phylogenetic results agree with Crawford et al. (2013) in show that *P. museosus* has two clades: one includes samples from Central Panamá to Costa Rica, and the other includes samples from the Panamá-Colombia border region. The genetic distances between the two clades forming *P. museosus* are 3.2 % (16S) and 7.1 % (COI). It also highlights the genetic variation in other species from our analysis, especially within polymorphic species like *P. caryophyllaceus*, *P. cerasinus*, *P. cruentus*, and *P. ridens*. In those species the genetic distances within of current names reach up to 10.5 % (16S) and 19.1 % (COI) in *P. caryophyllaceus*, 5.2 % (16S) and 11.4 % (COI) in *P. cerasinus*, 14.5 % (16S) and 13.5 % (COI) in *P. cruentus*, and 8.6 % (16S) and 15.5 % (COI) in *P. ridens*. Ours results support previous analyses suggesting multiple cryptic lineages within these taxa (Savage, 2002; Wang et al., 2008; Crawford et al., 2013; Batista et al. 2014). Further clarification of the full taxonomic status of *P. museosus* and some clades within other species in the *P. ridens* Species Group is need. This new locality for *P. museosus* extends 88 km northwest from the nearest scientifically verified site for this species, the type locality. The specimen was found in a site covered by primary Tropical wet forest [Premontane transition] (Holdridge, 1967). This region has a dry season of 0-3 months; annual rainfall is 4,000 to 5,500 mm, and annual temperatures range from 21.5 °C to 24 °C (Bolaños et al., 2005). *Pristimantis museosus* may range northwest in Costa Rica, in the Talamanca foothills, following the Tropical wet forest life zone (Bolaños et al., 2005). Alto Urén and nearby areas contain microendemic

Tabla 1. Medidas en milímetros (mm) de la hembra de Costa Rica, el holotipo y los paratipos de *Pristimantis museosus*.

Tabla 1. Measurements in millimeters (mm) of the female from Costa Rica, the holotype, and paratypes of *Pristimantis museosus*.

	UCR24442 (female)	MVUP-1348 (holotype)	MVUP-1349 (paratype)	STRI-0081 (paratype)
Snout-vent length (SVL)	38.9	26.1	23.6	21.5
Shank	20.2	14.2	13.9	12.7
Head width (HW)	16.2	10.4	9.3	8.7
Head length (HL)	16.3	9.8	8.8	8.7
Upper eyelid width (EW)	3.3	-	-	1.9
Interorbital distance (IOD)	4.6	2.7	2.5	2.2
Eye diameter length (ED)	5.0	4.5	4.0	3.5
Tympanum length (TY)	1.2	0.9	0.8	0.8
Eye to nostril distance (EN)	5.0	3.4	3.2	3.0
Width of finger 3 (WF3)	2.4	-	-	-
Width of finger 4 (WF4)	2.0	-	-	-



Figura 3. Foto en vida del espécimen de *Pristimantis museosus* (UCR24442) de Alto Urén, Limón, Costa Rica. Foto: Stanley Salazar.

Figure 3. In-life photo of the specimen of *Pristimantis museosus* (UCR24442) from Alto Urén, Limón, Costa Rica. Photo: Stanley Salazar.

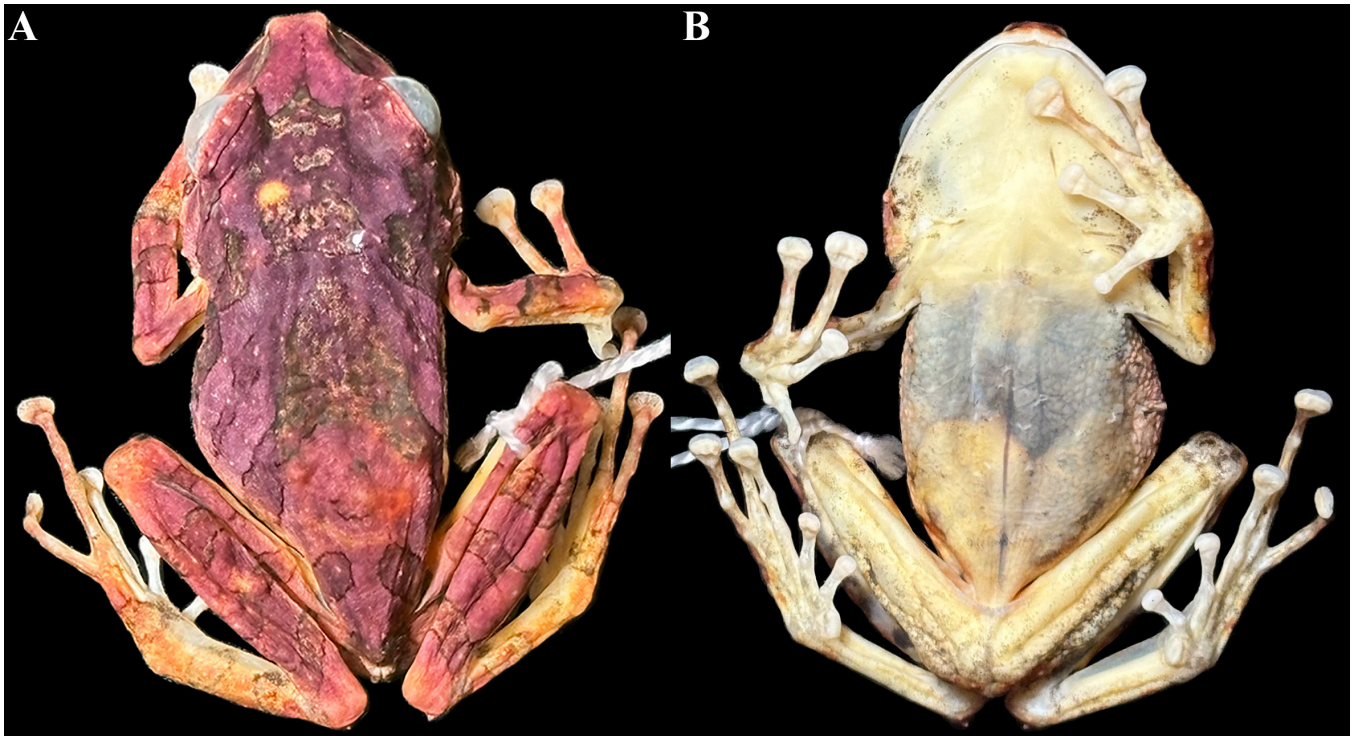


Figura 4. Fotografías del dorso (A) y vientre (B) del espécimen preservado de *Pristimantis museosus* (UCR24442). Fotos: Erick Arias.

Figure 4. Figure 4. Dorsal (A) and ventral (B) views of the preserved specimen of *Pristimantis museosus* (UCR24442). Photos: Erick Arias.

species, such as *D. lythodes* (Savage, 1968) and *Nototriton costaricense* Arias & Kubicki, 2018 (Savage, 1968; Arias & Kubicki, 2018); also, the only record of *P. pardalis* and *Bolitoglossa minutula* Wake, Brame & Duellman, 1973 on the Caribbean slope of Costa Rica (Arias, 2017). Given the potential for undiscovered species in this region, it is essential to prioritize an inventory project to document the diversity of its Herpetofauna.

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APPENDICES

Apéndice 1. Números de comprobantes institucionales, información de localidad y números de acceso de GenBank para los especímenes utilizados en los análisis filogenéticos moleculares. Las siglas de la colección del museo siguen a Frost (2024) con la adición de AJC = número de campo de Andrew J. Crawford; AB = números de campo de Abel Batista; CH = Círculo Herpetológico de Panamá, República de Panamá; ENS = números de campo de Eric Smith; EVACC = Número de muestra del Centro de Conservación de Anfibios El Valle, República de Panamá; MVUP = Museo de Vertebrados de la Universidad de Panamá, República de Panamá. CR = Costa Rica, HN = Honduras, PA = Panamá.

Appendix 1. Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum collection acronyms follow Frost (2024) with the addition of AJC = Andrew J. Crawford field number; AB = Abel Batista field numbers; CH = Círculo Herpetológico de Panamá, Republic of Panama; ENS = Eric Smith field numbers; EVACC = El Valle Amphibian Conservation Center sample number, Republic of Panama; MVUP = Museo de Vertebrados de la Universidad de Panamá, Republic of Panama. CR = Costa Rica, HN = Honduras, PA = Panamá.

Species	Institutional voucher	Collection locality	COI GB number	16S GB number	Original reference
<i>Pristimantis taeniatus</i>	CH6796	Cerro Azul, Chilibre, PA	KR863076	KR863333	Paz et al. (2015)
<i>P. adnus</i>	AJC0922	Serranía del Sapo, Darién, PA	ON008317	ON009871	Sepulveda-Seguro et al. (2022)
<i>P. adnus</i>	AJC0924	Serranía del Sapo, Darién, PA	ON008318	ON009872	Sepulveda-Seguro et al. (2022)
<i>P. moro</i>	AJC1753	Cerro Azul, Chilibre, PA	JN991383	JN991453	Pinto-Sánchez et al. (2012)
<i>P. moro</i>	AJC1860	Serranía de Pirre, Darién, PA	JN991384	JN991454	Pinto-Sánchez et al. (2012)
<i>P. caryophyllaceus</i>	AB066	Serranía Maje, PA	KJ201943	KJ201953	Batista et al. (2014)
<i>P. caryophyllaceus</i>	AB434	Cerro Sapo, Darién, PA	KJ201942	KJ201952	Batista et al. (2014)

Species	Institutional voucher	Collection locality	COI GB number	16S GB number	Original reference
<i>P. caryophyllaceus</i>	AB581	Donoso, Colón, PA	KJ201947	KJ201958	Batista et al. (2014)
<i>P. caryophyllaceus</i>	AB679	Wargandi, Darién, PA	KJ201951	KJ201962	Batista et al. (2014)
<i>P. caryophyllaceus</i>	AB847	Darién, PA	KJ201946	KJ201957	Batista et al. (2014)
<i>P. caryophyllaceus</i>	AB952	Darién, PA	KJ201948	KJ201959	Batista et al. (2014)
<i>P. caryophyllaceus</i>	AB985	Chepo, PA	KJ201945	KJ201955	Batista et al. (2014)
<i>P. caryophyllaceus</i>	AJC486	Cascajal, San José, CR	JN991363	JN991434	Pinto-Sánchez et al. (2012)
<i>P. caryophyllaceus</i>	AJC1138	Altos del María, PA	JN991364	JN991435	Pinto-Sánchez et al. (2012)
<i>P. caryophyllaceus</i>	AJC1885	Serranía de Pirre, Darién, PA	KR863036	KR863293	Paz et al. (2015)
<i>P. caryophyllaceus</i>	AJC1912	Brewster, Panamá, PA	KR863042	KR863299	Paz et al. (2015)
<i>P. caryophyllaceus</i>	AJC1936	Brewster, Panamá, PA	KR863044	KR863301	Paz et al. (2015)
<i>P. caryophyllaceus</i>	CH6394	Pinogana, Darién, PA	KR863043	KR863300	Paz et al. (2015)
<i>P. caryophyllaceus</i>	MVUP1925	El Copé, Coclé, PA	FJ766776	FJ784473	Crawford et al. (2010)
<i>P. caryophyllaceus</i>	USNM572329	El Copé, Coclé, PA	FJ766771	FJ784397	Crawford et al. (2010)
<i>P. caryophyllaceus</i>	USNM572338	El Copé, Coclé, PA	FJ766775	FJ784491	Crawford et al. (2010)
<i>P. caryophyllaceus</i>	USNM572343	El Copé, Coclé, PA	FJ766772	FJ784375	Crawford et al. (2010)
<i>P. cerasinus</i>	AJC1142	Altos del María, PA	JN991367	JN991438	Pinto-Sánchez et al. (2012)
<i>P. cerasinus</i>	AJC1757	Cerro Azul, Chilibre, PA	KR863049	KR863306	Paz et al. (2015)
<i>P. cerasinus</i>	AJC1764	Cerro Azul, Chilibre, PA	KR863045	KR863302	Paz et al. (2015)
<i>P. cerasinus</i>	USNM572376	El Copé, Coclé, PA	FJ766786	FJ784387	Crawford et al. (2010)
<i>P. cerasinus</i>	USNM572377	El Copé, Coclé, PA	FJ766785	FJ784391	Crawford et al. (2010)
<i>P. cruentus</i>	AJC0475	Tapantí, Cartago, CR	JN991370	JN991441	Pinto-Sánchez et al. (2012)
<i>P. cruentus</i>	AJC0524	Turrialba, Cartago, CR	JN991368	JN991440	Pinto-Sánchez et al. (2012)
<i>P. cruentus</i>	AJC1140	Altos del María, PA	KC129260	KC129372	Crawford et al. (2013)
<i>P. cruentus</i>	AJC1954	Brewster, Panamá, PA	KR863054	KR863311	Paz et al. (2015)
<i>P. cruentus</i>	CH6679	Brewster, Panamá, PA	KR863056	KR863313	Paz et al. (2015)
<i>P. cruentus</i>	CH6721	Brewster, Panamá, PA	JN991371	JN991442	Pinto-Sánchez et al. (2012)
<i>P. cruentus</i>	MVUP1796	El Copé, Coclé, PA	FJ766797	FJ784354	Crawford et al. (2010)
<i>P. cruentus</i>	USNM572362	El Copé, Coclé, PA	FJ766782	FJ784525	Crawford et al. (2010)
<i>P. cruentus</i>	USNM572404	El Copé, Coclé, PA	FJ766796	FJ784409	Crawford et al. (2010)
<i>P. cruentus</i>	USNM572788	El Copé, Coclé, PA	FJ766787	FJ784380	Crawford et al. (2010)
<i>P. ridens</i>	AJC0126	Río Claro, Puntarenas, CR	JN991400	JN991466	Pinto-Sánchez et al. (2012)
<i>P. ridens</i>	AJC0211	Nusagandi, Kuna Yala, PA	JN991399	JN991465	Pinto-Sánchez et al. (2012)
<i>P. ridens</i>	AJC1766	Cerro Azul, Chilibre, PA	KR863061	KR863318	Paz et al. (2015)
<i>P. ridens</i>	AJC1901	Brewster, Panamá, PA	KR863060	KR863317	Paz et al. (2015)



Species	Institutional voucher	Collection locality	COI GB number	16S GB number	Original reference
<i>P. ridens</i>	CH6776	Cerro Azul, Chilibre, PA	KR863062	KR863319	Paz et al. (2015)
<i>P. ridens</i>	ENS10722	Sierra de Agalta, Olancho, HN	JN991398	JN991464	Pinto-Sánchez et al. (2012)
<i>P. ridens</i>	MVUP1829	El Copé, Coclé, PA	FJ766806	FJ784398	Crawford et al. (2010)
<i>P. ridens</i>	USNM572415	El Copé, Coclé, PA	FJ766805	FJ784399	Crawford et al. (2010)
<i>P. ridens</i>	USNM572416	El Copé, Coclé, PA	FJ766808	FJ784388	Crawford et al. (2010)
<i>P. ridens</i>	USNM572417	El Copé, Coclé, PA	FJ766807	FJ784389	Crawford et al. (2010)
<i>P. aff. museosus</i>	AJC1888	Cana, Darién, PA	KC129246	KC129358	Crawford et al. (2013)
<i>P. aff. museosus</i>	AJC1890	Cana, Darién, PA	KC129242	KC129354	Crawford et al. (2013)
<i>P. aff. museosus</i>	AJC1892	Cana, Darién, PA	KC129243	KC129355	Crawford et al. (2013)
<i>P. aff. museosus</i>	AJC1894	Cana, Darién, PA	KC129248	KC129360	Crawford et al. (2013)
<i>P. aff. museosus</i>	AJC1898	Cana, Darién, PA	KC129250	KC129362	Crawford et al. (2013)
<i>P. aff. museosus</i>	CH6410	Cana, Darién, PA	KC129244	KC129356	Crawford et al. (2013)
<i>P. aff. museosus</i>	CH6429	Cana, Darién, PA	KC129249	KC129361	Crawford et al. (2013)
<i>P. aff. museosus</i>	CH6455	Cana, Darién, PA	KC129241	KC129353	Crawford et al. (2013)
<i>P. museosus</i>	AJC1210	Altos del María, PA	JN991385	JN991455	Pinto-Sánchez et al. (2012)
<i>P. museosus</i>	EVACC240	Altos del María, Panamá, PA	KC014758	KC014951	Crawford et al. (2013)
<i>P. museosus</i>	EVACC254	Brewster, Panamá, PA	KC014744	KC014943	Crawford et al. (2013)
<i>P. museosus</i>	CH6747	Brewster, Panamá, PA	KC129262	KC129374	Crawford et al. (2013)
<i>P. museosus</i>	MVUP1839	El Copé, Coclé, PA	FJ766795	FJ784430	Crawford et al. (2010)
<i>P. museosus</i>	UCR24442	Talamanca, Limón, CR	PQ610213	PQ611590	This study
<i>P. museosus</i>	USNM572395	El Copé, Coclé, PA	FJ766793	FJ784559	Crawford et al. (2010)

