

A new minute Andean *Pristimantis* (Anura: Strabomantidae) from Venezuela

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Abstract

A new minute Andean *Pristimantis* (Anura: Strabomantidae) from Venezuela. A new species of *Pristimantis* is described from the Venezuelan Andes. The new species is the smallest in its genus known in Venezuela and belongs to the *Pristimantis unistrigatus* Group. It differs from the rest of Venezuelan Andean congeners in body size (mean male SVL < 21.3 mm, female SVL < 26.3 mm), expanded discs on fingers and toes, absence of dorsolateral folds, and a distinctive call consisting in 2–5 cricket-like short notes. The new species inhabits the southwestern part of the Cordillera de Mérida in Venezuela and the Venezuelan side of the Cordillera Oriental de Colombia, and could be present on the Colombian portion of the cordillera as well.

Keywords: Andes, Colombia, new species, *Pristimantis unistrigatus*.

Resumen

A new minute Andean *Pristimantis* (Anura: Strabomantidae) from Venezuela. Se describe una nueva especie de *Pristimantis* de los Andes de Venezuela. La nueva especie es la más pequeña del género conocida en Venezuela y pertenece al grupo de *Pristimantis unistrigatus*. Difiere del resto de congéneres andinos en Venezuela por su reducido tamaño corporal (LRC en machos < 21.3 mm, LRC en hembras < 26.3 mm de media), discos expandidos en los dedos de las manos y pies, ausencia de pliegues dorsolaterales y canto distintivo consistente en 2–5 notas cortas parecidas a un grillo. La nueva especie habita en la parte suroccidental de la Cordillera de Mérida en Venezuela y en el extremo nororiental de la Cordillera Oriental de Colombia en su parte venezolana, por lo que pudiera estar presente en Colombia.

Palabras Clave: Andes, Colombia, nueva especie, *Pristimantis unistrigatus*.

Resumo

Um nova espécie de *Pristimantis* (Anura: Strabomantidae) dos Andes da Venezuela. Descrevemos uma nova espécie de *Pristimantis* dos Andes da Venezuela. A nova espécie é a menor conhecida em seu gênero na Venezuela e pertence ao grupo de *Pristimantis unistrigatus*. Difere de suas congêneres andinas da Venezuela pelo tamanho corporal (SVL médio dos machos < 21.3 mm e das fêmeas < 26.3 mm), discos expandidos nos dedos das mãos e dos pés, ausência de dobras dorsolaterais e um canto distinto que consiste em 2–5 notas curtas semelhante ao de um grilo. A nova espécie habita a porção sudoeste da Cordilheira de Mérida, na Venezuela, e a vertente venezuelana da Cordilheira Oriental da Colômbia, podendo também estar presente na parte colombiana dessa cordilheira.

Palavras-chave: Andes, Colômbia, nova espécie, *Pristimantis unistrigatus*.

Received 22 May 2012.

Accepted 10 December 2012.

Distributed December 2012.

Introduction

Knowledge of the diversity of *Pristimantis* in Venezuela has changed considerably in recent years, from 16 species of *Eleutherodactylus* reported by Rivero (1961) to the current number of 61 species of *Terrarana* (Barrio-Amorós *in press*). The Venezuelan Andes show the greatest species richness of Venezuelan *Pristimantis*, with 28 species (Barrio-Amorós 2009, *in press*). We herein describe another species, endemic to the Cordillera de Mérida and the Venezuelan part of the Cordillera Oriental de Colombia, in the states of Mérida and Táchira. The peculiar call of the new species is heard from sunset to approximately 21:00 h along roads, in areas of disturbed and virgin cloud forests. Despite being an abundant species, its cricket-like call has probably confused previous herpetologists and this may explain why it has gone unnoticed until now. Collection of calling males, however, has proven a difficult matter, and therefore the type series is small. This new species has minute body size and is easily distinguished from congeners found in the Cordillera de Mérida and Sierra de Perijá in Venezuela, and in Cordillera Oriental de Colombia (J. D. Lynch, pers. comm.).

Materials and Methods

All measurements were taken with digital calliper to the nearest 0.1 mm on preserved specimens. Morphological terms follow Lynch and Duellman (1997). Comparisons were taken from Lynch (2003) and Rivero (1982) and preserved material from CVULA (Colección de Vertebrados, Facultad de Ciencias, Universidad de los Andes, Mérida, Venezuela) (Appendix I). Measurements of adult frogs follow Barrio-Amorós *et al.* (2010a) and are: SVL: straight length from tip of snout to vent; ShL: shank length from outer edge of flexed knee to heel; HeL: head length from tip of snout to the posterior border of skull (posterior edge of prootic, noted through the skin); HW: head width between angle of jaws; InD: inter-narial distance between centers

of nares; EN: distance of anterior edge of eye to nostril; ED: horizontal eye diameter; TD: horizontal tympanum diameter; ETS: distance between the anterior edge of the eye to the tip of snout; FD: disc width of Finger III; T4D: disc width of Toe IV; 1FiL: length of Finger I from inner edge of thenar tubercle to tip of disc; 2FiL: length of Finger II from the junction of Finger I and III to the tip of finger disc. Some traditional measurements like IOD (inter orbital distance) and UEW (upper eyelid width) are not being used, as we consider them highly variable due to preservation artifacts. Sex was determined, when possible, directly in the field by observation of calling males; non-calling specimens were dissected and its primary sexual traits determined (presence of mature testis or oviducts).

Calls were recorded with a Sony TCM-353V Mini disc recorder and a Sony F-V5 microphone; calls were analyzed and graphics elaborated using Raven Pro 1.4 (Cornell University, Ithaca, New York).

Methods used for the collection and analysis of the new DNA sequences are detailed elsewhere (Heinicke *et al.* 2007, Hedges *et al.* 2008, Hedges and Conn 2012). Contiguous sequences of three mitochondrial genes, 12S ribosomal RNA, tRNA Valine, and 16S ribosomal RNA, were aligned using MUSCLE (Edgar 2004) in MEGA 5 (Tamura *et al.* 2011), with poorly conserved regions deleted prior to phylogenetic analyses. The final aligned dataset included 31 taxa and 2578 nucleotide sites. Phylogenetic analyses were performed with maximum likelihood in MEGA 5 (Tamura *et al.* 2011), unpartitioned, using the best-fitting evolutionary model under the Bayesian Information Criterion, the GTR + I + Γ option (general time reversible + invariant sites + gamma distribution of changes). Gaps were treated as missing data. All parameters for the ML analyses were estimated by the program during the run. Branch support in the trees was provided by bootstrap analysis (500 replicates).

The following sequences were used (Genbank accession numbers in parentheses) from earlier

studies (Heinicke *et al.* 2007, Hedges *et al.* 2008, Barrio-Amorós *et al.* in review): *Pristimantis ardalonychus* (EU186664); *P. bipunctatus* (EF493702); *P. briceni* (JX155297); *P. bromeliaceus* (EF493351); *P. ceuthospilus* (EF493520); *P. chalceus* (EF493675); *P. conspicillatus* (EF493529); *P. curtipes* (EF493513); *P. galdi* (EU186670); *P. ginesi* (JX155295); *P. lancinii* (JX155294); *P. leoni* (EF493684); *P. luteolateralis* (EF493517); *P. malkini* (EU186663); *P. nyctophylax* (EF493526); *P. ockendeni* (EF493519); *P. paramerus* (JX155279); *P. parvillus* (EF493352); *P. phoxocephalus* (EF493349); *P. spinosus* (EF493673); *P. thymalopsoides* (EF493514); *P. thymelensis* (EF493516); *P. unistrigatus* (EF493387); *P. verecundus* (EF493686); *P. versicolor* (EF493389); *P. walkerii* (EF493518); *Strabomantis anomalus* (EF493534). The following two new sequences were collected for this study: *P. gryllus* (JX306022; CVULA 8343 from type locality); and *P. yukpa* (JX306020–21, to be deposited, from Kiriponsa, Río Tokuko, Municipio Machiques de Perijá, Sierra de Perijá, Estado Zulia, Venezuela).

Results

Pristimantis gryllus sp. nov.

Andean cricket frog; Ranita grillo andina.

Holotype.—CVULA 8343, an adult female from cloud forest along the road from Estanques to Paramo La Tosta, 8.4333 N, 71.5000 W, 1320 m, Estado Mérida, Venezuela, obtained on 30 of August 2007 by C. L. Barrio-Amorós.

Paratopotype.—CVULA 8344, adult male with same data as holotype.

Paratypes.—One adult female, CVULA 8346, and an adult male, CVULA 8347, from La Macana, aprox. 6 km SE from Santa Cruz de Mora, Estado Mérida, collected by J. M. Guayasamin and C. L. Barrio-Amorós on 12 June 2006; one adult male, CVULA 8345, from Los Ranchos, aprox. 6.5 km NW from Santa Cruz de Mora, Estado Mérida, collected by J. C.

Santos and C. L. Barrio-Amorós on 6 June 2007.

Referred specimens.—An adult male, CVULA 8348, from Siberia, Estado Táchira; and a juvenile specimen of 14.0 mm SVL, CVULA 8349, with the same data as the holotype.

Diagnosis.—*Pristimantis gryllus* is a small (males up to 21.3 mm, females up to 26.3 mm) member of the *unistrigatus* species Group *sensu* Lynch and Duellman (1997) and Hedges *et al.* (2008). It is diagnosed by the combination of the following traits: (1) Dorsal skin smooth in preservative, with no apparent middorsal raphe; no dorsolateral or occipital ridges; ventral skin smooth to areolate; dorsal skin slightly tubercular in life; (2) tympanum distinct (in some small specimens almost indistinct), with well defined tympanic annulus, 30% of the ED; (3) snout rounded in dorsal and lateral views; canthus rostralis rounded; (4) upper eyelid smooth in preservative, with one to few low tubercles, one usually larger and conical in life, on the posterior edge of the eyelid; (5) choanae round; dentigerous processes of the vomers absent; tongue rounded, posterior half free; (6) males with vocal slits and vocal sac; no apparent nuptial pads; (7) finger I shorter than II; (8) fingers without lateral keels; (9) ulnar tubercles absent; (10) tarsal tubercles and calcars absent in preservative, sometimes small or barely visible in life; (11) two metatarsal tubercles, inner oval, small; outer indistinct; (12) toes without lateral keels; webbing absent; toes III, IV and V with broad discs, equal in size than those on fingers III and IV; (13) in life, color dorsally variable, light brown, reddish brown, pale grey to dark grey, with a pattern consisting in diagonal stripes, dorsal chevrons, W-shape marking on scapula, or plain; vocal sac pale yellow. Iris pale to dark copper, reticulated by fine black venation; (14) small body size, adult males SVL = 17.1–21.3 ($N = 4$; mean = 18.5 ± 1.9); female SVL = 22.3–26.3 ($N = 2$; mean = 24.3 ± 2.8).

The molecular phylogenetic tree (Figure 3) supports the placement of *Pristimantis gryllus* in the *unistrigatus* Group. Although it clusters most closely with another Venezuelan species, *P.*

yupka, that relationship is only weakly supported. Other Venezuelan species of *Pristimantis* form a group elsewhere on the tree.

This species is compared with small species under 30 mm of *Pristimantis* from cloud forests in the Venezuelan Andes and nearby Colombia (characters of *Pristimantis gryllus* in parentheses). Páramo species, such as *P. anolirex* (Lynch), *P. boconoensis* (Rivero and Mayorga), *P. briceni* (Boulenger), *P. colostichos* (La Marca and Smith), *P. ginesi* (Rivero), *P. lancinii* (Donoso-Barros), *P. nicefori* (Cochran and Goin), and *P. paramerus* (Rivero), are found at higher altitudes [páramo dwellers, usually above 2500 m], are larger [*P. boconoensis* and *P. ginesi* only slightly larger], have finger and toe discs non expanded to weakly expanded (very expanded), and all species have very developed dorsolateral folds, middorsal raphe and/or large tubercles (dorsal skin smooth in preservative; in life can have small tubercles, middorsal raphe is almost indistinct and dorsolateral folds are generally absent).

The new species has no light marks on the groin and hidden surfaces of hind limbs, traits characteristic of *P. lentiginosus* (Rivero), *P. melanoproctus* (Rivero), and *P. mondolfii* (Rivero). *Pristimantis gryllus* lacks a pointed tubercle on the tip of snout, which differentiates it from species in the *tubernasus* Group of Rivero, *P. prolixodiscus* (Lynch) and *P. tubernasus* (Rivero). The ulnar, tarsal tubercles, and calcars of *Pristimantis gryllus* are only seen in life, lost in preservative, and are similar to those in *P. vanadisae* (La Marca), but this species is larger, with females up to 35 mm (up to 26.3 mm SVL), with a cherry colored belly (never this color), and white ocelli on groin (absent). *Pristimantis prolixodiscus* (Lynch), has quadrangular discs (horizontally oval), dorsal coloration green (variable, but never green), canthal and supratympanic stripes (absent). One species in Sierra de Perijá, another Andean range, *P. yupka* Barrio-Amorós, Rojas-Runjaic and Infante, is similar in size and shape, a little larger (males up to 27 mm, females up to 28.8

mm SVL), with a distinct middorsal raphe (absent), tympanum ill-defined (well distinct), basal web between toes VI and V (absent), and with small dentigerous processes of the vomers (absent). It may be the closest relative of *P. gryllus* (Figure 3). *Pristimantis ixalus* (Lynch), from North-eastern Colombia is a member of the *conspicillatus* group, thus having the Finger I longer than II (shorter), and is a much larger species, with a known female size of 45.4 mm SVL (females up to 26.3 mm SVL). *Pristimantis uisae* (Lynch) from North-eastern Colombia has vomerine odontophores (absent), males present nuptial pads (absent), fingers bearing lateral keels (absent), and is a slightly larger species, females up to 31.2 mm (females up to 26.3 mm SVL). *Pristimantis batrachites* (Lynch), from North-eastern Colombia, is a smaller species, with adult females up to 21.7 mm (up to 26.3 mm SVL), the upper eyelids are devoid of tubercles (small tubercles in life), males have nuptial pads (absent), fingers and toes bearing lateral fringes (absent), and have subconical ulnar and tarsal tubercles and a prominent heel tubercle (absent or barely visible).

Pristimantis gryllus is unique among other *Pristimantis* from Venezuela and neighbouring Colombia in the following combination of characters: small size (males up to 21.3 mm, females up to 26.3 mm), dorsal skin smooth in preservative, slightly tubercular in life, snout rounded in dorsal and lateral views; vocal slits and single subgular vocal sac on males; absence of dentigerous processes of the vomers; ulnar, tarsal tubercles and calcars absent, all tubercles on hands and feet distinct and protuberant; fringes absent on fingers and toes, and a distinctive call consisting in 2–5 short cricket-like notes.

Description of the holotype (Figure 1A, B). Head slightly longer than wide, head width 36.1% of SVL. Snout rounded in dorsal and lateral views; EN equal than ED; nostrils non protuberant, directed dorsolaterally; canthus rostralis rounded, loreal region slightly concave. Upper eyelid with four low tubercles, none

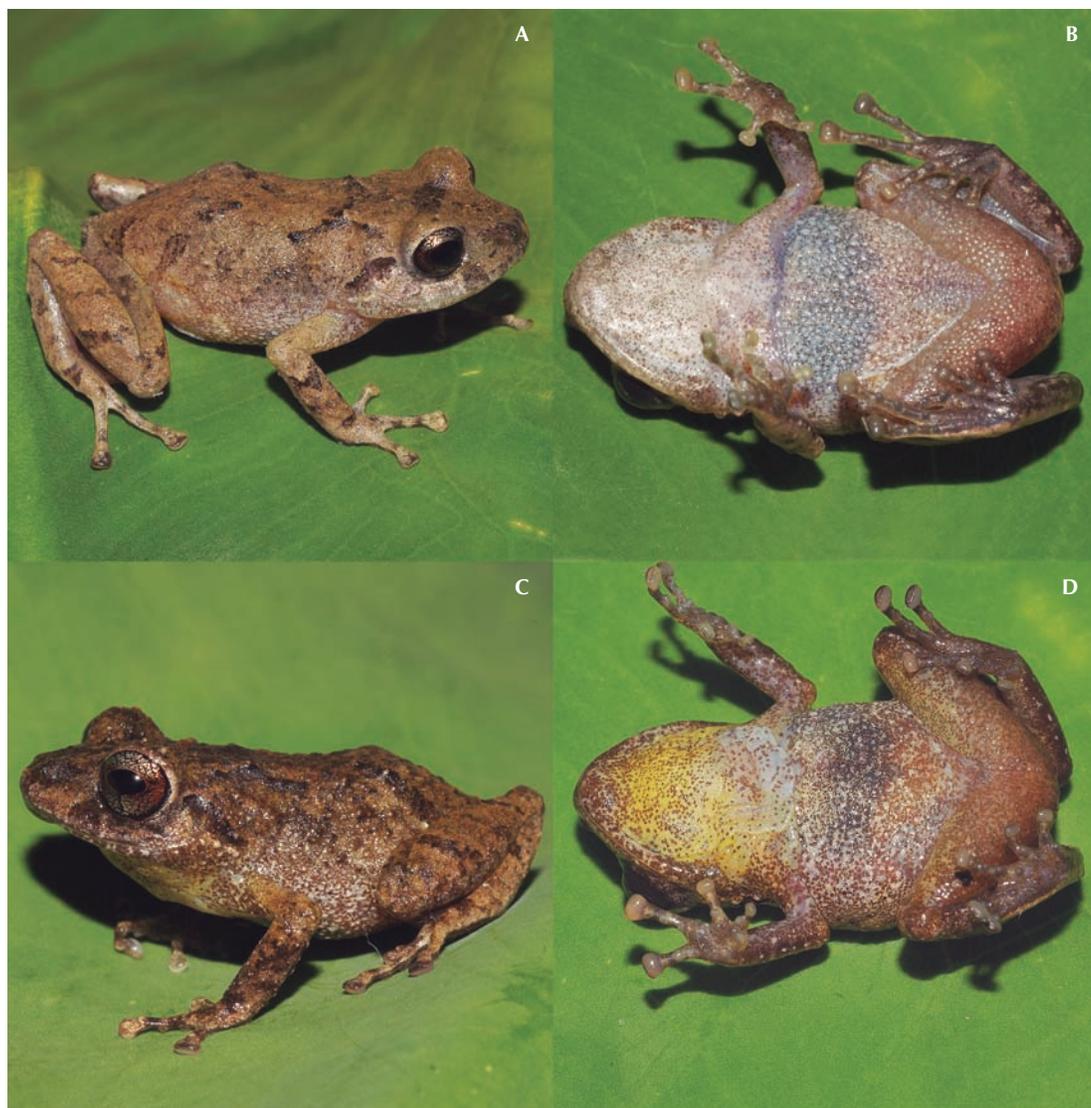


Figure 1. *Pristimantis gryllus* sp. nov., female holotype (A) dorsolateral view, (B) ventral view. Male paratype CVULA 8344, (C) dorsolateral view, (D) ventral view. Photos: Cesar L. Barrio-Amorós.

pungent, the posterior one larger; tubercles absent on head. Cranial crests not apparent. Tympanum small, ill-defined but always distinct under magnification, 30% of ED, its postero-dorsal part obscured by a supratympanic fold; two enlarged postrictal tubercles. Choanae

rounded, not concealed by palatal shelf of maxillary arch; vomerine dentigerous processes absent. Tongue rounded, posterior half free.

Dorsal skin smooth in preservative (slightly tubercular in life; Figure 1A); scapular ridges ill-defined (slightly defined in life; Figure 1A);

middorsal raphe absent in preservative (barely noticeable in life); dorsolateral folds absent; throat, chest and inferior part of thighs smooth, venter areolate (granular in life; Figure 1B); ulnar and tarsal tubercles absent, calcar absent.

Relative length of adressed fingers III > IV > II > I; first finger 90% of second finger length. Finger discs much broader than long, disc on finger III of right hand 3 times wider than adjacent phalanx; horizontally oval except on Finger I, which is rounded. Lateral fringes on fingers absent. Palmar tubercle indistinct, bifid; thenar tubercle distinct, ovoid. Subarticular tubercles large, protuberant, single, round on fingers III and IV, subconical on fingers I and II. Supernumerary tubercles large, low, in rows under each finger.

Hind limbs relatively short; shank 44.8% of SVL. Heel reaches the posterior edge of the eye when held to the sagittal plane. Relative lengths of adressed toes IV > V > III > II > I. Disc of toe IV slightly smaller than disc of finger III. Toes without lateral fringes, basal webbing between III–V. Discs horizontally oval, wider than long. Inner metatarsal tubercle small, protuberant, oval; outer indistinct; subarticular tubercles large, protuberant, single, round; supernumerary tubercles small, distinct, in rows under each toe.

Color in life. Dorsal background pale brown, with a wide, incomplete interorbital bar, a W-shaped scapular mark, two symmetrical paravertebral spots at mid dorsum, and two sacral elongated spots, all marks being dark grey (Figure 1A). Two narrow crossbars on each limb, and a few irregular dark spots on tarsi. On the head, the only consistent marking is a dark supratympanic fold, in many cases there is a complete or incomplete dark interorbital bar. Ventrally (Figure 1B); throat and chest white with a profusion of melanophores; skin on the anterior part of the belly somewhat transparent, white, and the posterior part of the belly and under surfaces of limbs pale brown. Palms and soles dark grey. Iris golden copper, reticulated by fine black venation; pupil black.

Color preserved. Dorsal color is dull greyish

brown, with the dark marks faded; ventral side dirty white without magnification; under microscope, a profusion of melanophores are spread throughout the throat, chest, belly and under surfaces of legs. Palms pale grey, soles dark grey; discs ventrally white.

Measurements of holotype (in mm). SVL: 26.3; ShL 11.8; FL: 9.3; HeL: 9.6; HW: 9.5; EN: 2.9; ED: 3.0; TD: 0.9; FD: 1.2; T4D: 1.1; 1FiL: 2.7; 2FiL: 3.0.

Variation. The other female is nearly identical, differing in small details, possibly due to preservation artifacts. Some notable differences are the subacuminate dorsal profile (vs. rounded), ill-defined cranial crests (not apparent in the holotype), low ulnar tubercles (absent in the holotype). Males are much smaller (SVL = 17.1–21.3 mm), having vocal sac and vocal slits, and a darker belly. Only two specimens (CVULA 8346 and 8347) have very low and almost indistinct scapular ridges. In life, dorsal skin can be shagreen to slightly tubercular (Figure 1C), usually without middorsal raphe.

The chromatic variation is conspicuous. Dorsal background color can vary: pale brown, reddish brown, pale grey to dark grey, with a pattern consisting of diagonal stripes, dorsal chevrons, W-shape marking on scapula, or plain (Figure 2A, B, C). Vocal sac is pale yellow (Figure 1D). There is no marked variation on the ventral side color. Both males and females have a great profusion of melanophores, both on the throat, chest and belly. The expanded vocal sac of the males shows enlarged melanophores.

Natural history. Males call actively in wet months (May to October) from dusk (ca. 18:00 h) to ca. 21:00 h. They are usually exposed on debris, small leaves on low plants (0.20–0.60 cm high) or directly on the ground on taluses of roads. We found a solitary male calling on a *Pinus* plantation on the ground in Siberia, Táchira (CVULA 8349, Figure 2D). Females are sometimes close to active calling males.

Etymology. The specific epithet *gryllus* (Latin, a masculine noun in the nominative case, in apposition, for “cricket”) refers to the small



Figure 2. *Pristimantis gryllus* sp. nov. (A, B, C) different patterns from La Macana, estado Mérida. (D) specimen (CVULA 8348) from Siberia, Estado Táchira. A, D, males; B, C, females. Specimens not preserved. Photos: Cesar L. Barrio-Amorós.

size of this species, as well as for the cryptic call resembling that of a cricket.

Vocalization. The calls of Venezuelan terraranans are poorly known, precluding comparison with other species. Several calls of *Pristimantis gryllus* were recorded at 21.4°C on the night of

26 July 2008. The call is a cricket-like “tick” with no frequency modulation, given as 2–5 short notes, each with two pulses separated by 6 milliseconds (ms). Figure 4 shows a representative call with three double-pulsed notes. Based on analysis of our recording of six calls, the

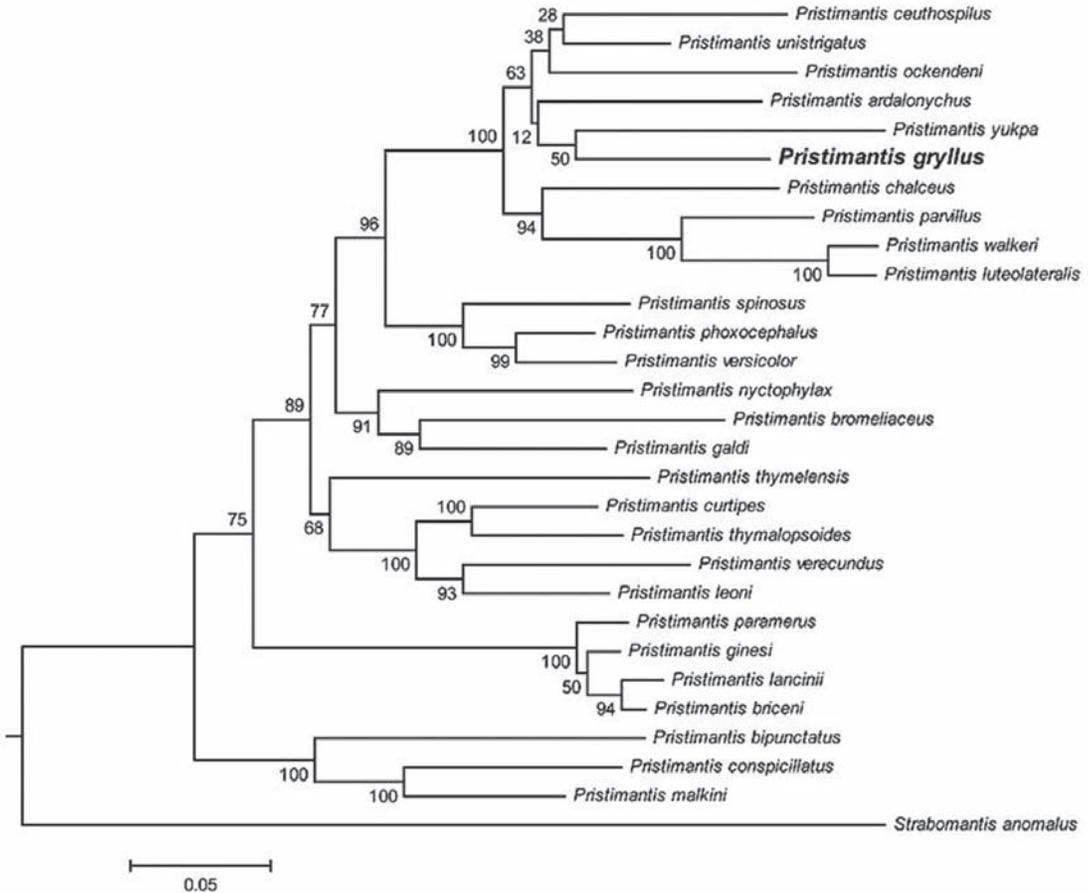


Figure 3. Maximum-likelihood tree inferred from DNA sequences of three mitochondrial genes (12S and 16S ribosomal RNA, and tRNA Valine) showing the phylogenetic position of *Pristimantis gryllus* sp. nov. with respect to 27 congeneric species. A scale bar indicates sequence divergence. The numbers at nodes are bootstrap support values for the clade defined by the node. The tree is rooted with the species *Strabomantis anomalus*.

number of notes per call is 2.2 and the dominant frequency is 3.28 (3.03–3.46) kilohertz (kHz), usually with harmonics at 6.39 (6.26–6.69) kHz and 9.85 (9.68–10.2) kHz. The call duration is 177 (15–307) ms, the note duration is 20 (15–26) ms, and the note interval is 117 (109–132) ms. The call interval appeared to be 1–2 seconds, but it was not possible to associate calls with specific individuals in the recording.

Distribution. The species has been found in seven localities throughout the south-western portion of the Cordillera de Mérida, in Mérida and Táchira states (Figure 5), at elevations between 900 to 2020 m. Aside from the specimens in the type series (from three different populations), we recently identified two more populations in Estado Táchira, one recognized by its distinctive call in the city of San Cristobal,

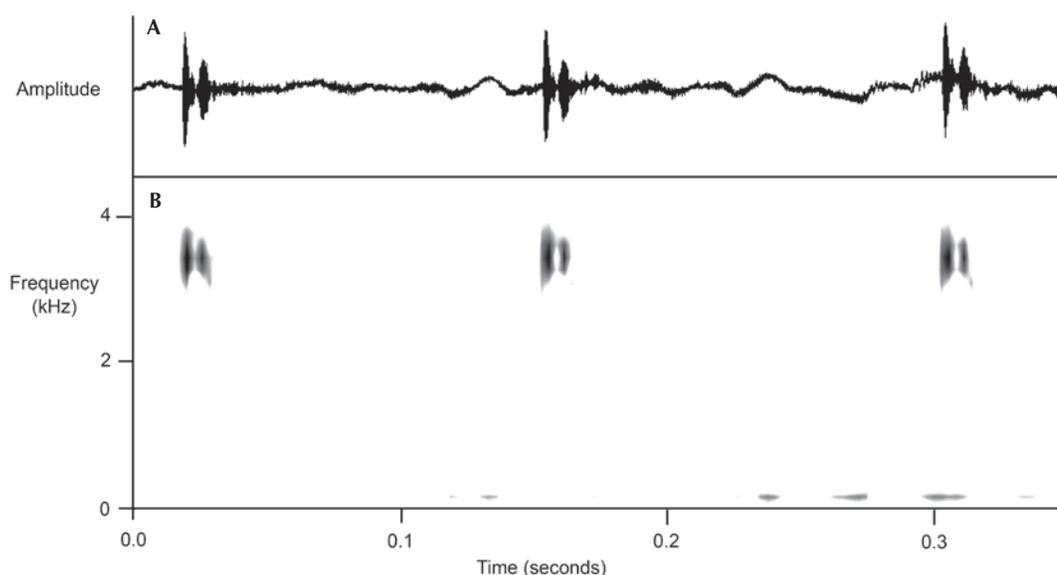


Figure 4. Sonogram of a call consisting in three notes of *Pristimantis gryllus* sp. nov. Unvouchered call.

urbanization Pirineos 1 (7.7666 N, -72.2000 W, 1010 m), and another at Matamula, (7.2833 N, -72.4333 W, 2020 m), where we observed numerous individuals. Because of the closeness of the southwesternmost locality to Colombia, we suspect that *Pristimantis gryllus* could also be present at least in Departamento Norte de Santander.

Discussion

Pristimantis gryllus is the smallest species of the genus currently known from the Venezuelan Andes, with males being mature at 17.4 mm SVL (observed calling males). Adult males of *P. ginesi* (the next smallest species) have a minimum SVL of 20.5 mm. *Pristimantis gryllus* is the only known representative of the large *unistrigatus* Group of *Pristimantis* (sensu Hedges *et al.* 2008) in the Cordillera de Mérida. All other species already sequenced (Figure 3) belong to two well-differentiated clades. This raises the question, beyond the scope of this

work, of the origin of the terraranan fauna in the Venezuelan Andes. Furthermore, many species from the same general area were placed by previous authors in the *unistrigatus* Group, but the group is so large now that the definition (Lynch and Duellman 1997) is in need of revision. Hedges *et al.* (2008) demonstrated that the *unistrigatus* Group is not a natural (monophyletic) clade, but they did not have sufficient representation in their molecular phylogeny to attempt a redefinition.

The known species diversity of *Pristimantis* of the Venezuelan Andes has increased greatly in recent years as indicated by the recent description of 14 new species since 2000, totalizing now 29 known species (Barrio-Amorós 1998, Barrio-Amorós and Chacon 2004, Barrio-Amorós *et al.* 2007, 2010, La Marca 2007, unpubl. data). We expect many more new species to be discovered in cloud forests and páramos in the states of Apure, Táchira, Mérida, Trujillo and Lara, which have been poorly explored.

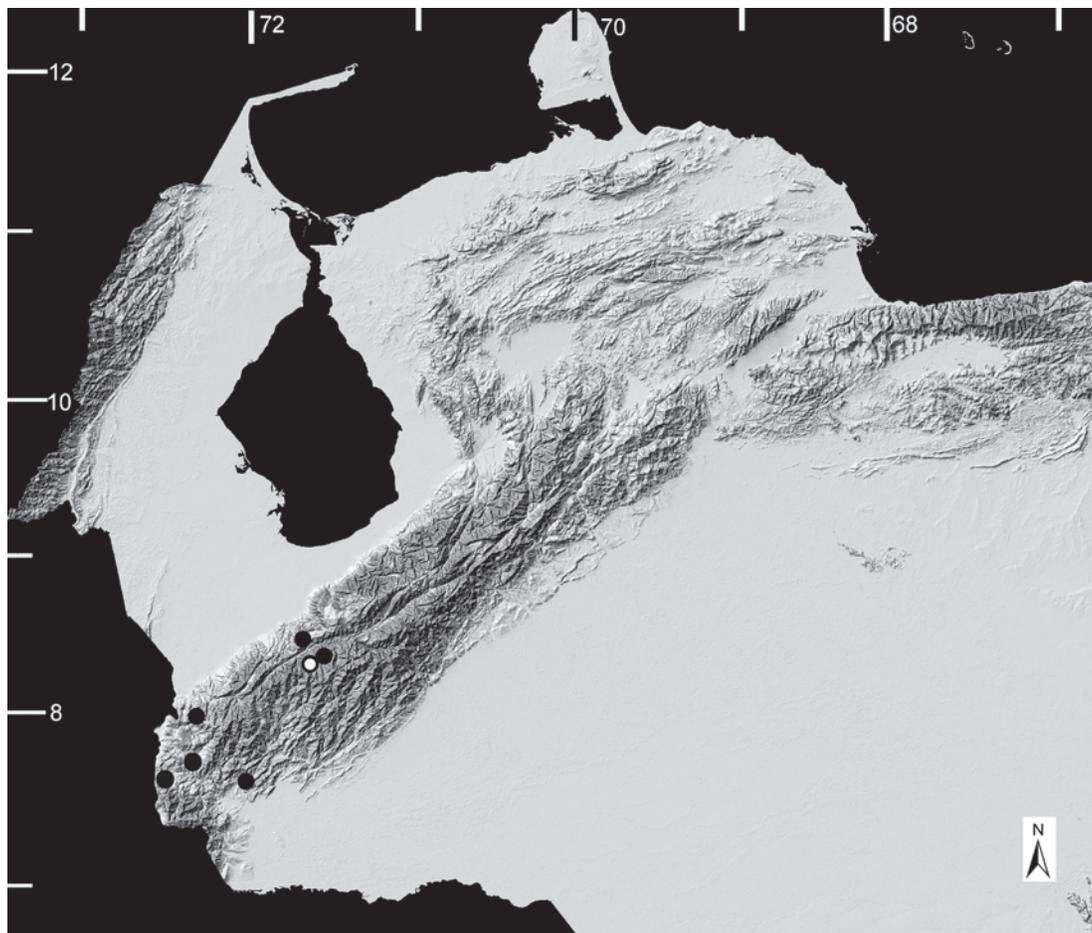


Figure 5. Map of northwestern Venezuela showing the distribution of *Pristimantis gryllus* sp. nov. in the Venezuelan Andes. The white circle represents the type locality.

Acknowledgments

CLBA thanks his field companions, Amelia Díaz de Pascual, Andres Chacón, Roger and Robert Manrique, the late Erik Arrieta, Juan Carlos Santos, Elisa Bonaccorso, Andres Orellana, William Tovar, and Valeria Bellazzini with whom he enjoyed the discovery of Andean pristine places in Venezuela. The specimens

were collected under permit 2231 by the Ministerio del Ambiente to CLBA, tissues were collected under access permit to Fundacion AndigenA, and exported legally to U. S. John Lynch commented on the distinctiveness of the new species compared with Colombian relatives. Angela Marion assisted in phylogenetic analyses. SBH was supported by grants from the U. S. National Science Foundation. 🐸

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