A new tuberculated *Pristimantis* (Anura, Terrarana, Strabomantidae) from the Venezuelan Andes, redescription of *Pristimantis pleurostriatus*, and variation within *Pristimantis vanadisae*

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Abstract

A new tuberculated *Pristimantis* is described from the eastern versant of the Venezuelan Andes. The new species is found in cloud forest at around 1600 masl on the eastern side of the Cordillera de Mérida. It is distinguished from other similar tuberculated species by its round, ill-defined canthus rostralis, ill-defined canthal stripe, and absence of pale spots on the groin and posterior surface of thighs. *Pristimantis pleurostriatus* is a poorly known species found in cloud forest on the western slopes of the Venezuelan Andes. We redescribe the species based on topotypic specimens. *Pristimantis vanadisae* is a polychromatic species varying dramatically in pattern; four chromotypes are described. Molecular data are presented which distinguish among tuberculated and other species of *Pristimantis* in the Cordillera de Mérida. Molecular data also support placement of *Mucubatrachus* and *Paramophrynella* in *Pristimantis*.

Key words: new species, *Pristimantis*, *Eleutherodactylus*, Andes, cloud forest, *P. pleurostriatus*, *P. vanadisae*; Mérida, Venezuela

Resumen

Se describe una nueva especie tuberculada de *Pristimantis* de la vertiente este de los Andes venezolanos. La nueva especie se halla en selva nublada a 1600 m en la vertiente oriental de la Cordillera de Mérida. Se distingue de especies similares por su canto rostral poco definido, línea cantal pobrememente definida, y ausencia de manchas en zonas ocultas de muslos e ingles. *Pristimantis pleurostriatus* es una especie poco conocida de selva nublada en la vertiente oeste de los Andes venezolanos. Redescribimos la especie en base a especímenes topotipicos. *Pristimantis vanadisae* es una especie simpátrica policromática que varía dramáticamente en patrón de coloración; distinguimos aquí cuatro cromotipos. Presentamos además datos moleculares para distinguir entre las especies de piel tuberculada de la Cordillera de Mérida, que forman un clado no descrito que incluye varias especies andinas venezolanas, y se apoya la hipótesis sobre la posición de *Mucubatrachus* y *Paramophrynella* dentro de *Pristimantis*.


Introduction

The species-level diversity of Strabomantidae is far from being completely documented, especially in those countries where research on the genus has been limited (Venezuela, Perú, Bolivia; Barrio-Amorós 2009; Duellman
advantage herein to comment about the intraspecific variation within genetically. In Venezuela 56 species of Pristimantis collected (Barrio-Amorós & Molina 2010). Among these was another the eastern slopes of the Cordillera de Mérida, Calderas area, Estado Barinas, 17 species of amphibians were

Furthermore, during a RAP (Rapid Assessment Programme) made in July 2008 by Conservation International in operation base during its Amphibian Projects (www.andigena.org), and subsequently identified as freshly collected tubercled from La Carbonera, an area that was chosen by Fundacion AndigenA as one species in the Venezuelan Andes shares those characters. These specimens were compared with a new series of were found at the collection of vertebrates in the Universidad de los Andes (CVULA), confirming that more than distinctive species, conical heel tubercles and prominent ulnar and tarsal tubercles include only a single and apparently highly awaiting proper attention. Venezuelan Andes Pristimantis known to show clear diagnostic characters such as conical heel tubercles and prominent ulnar and tarsal tubercles include only a single and apparently highly distinctive species, P. vanadisae (La Marca, 1984). However, some specimens of a similar tuberculated species were found at the collection of vertebrates in the Universidad de los Andes (CVULA), confirming that more than one species in the Venezuelan Andes shares those characters. These specimens were compared with a new series of freshly collected tubercled Pristimantis from La Carbonera, an area that was chosen by Fundacion AndigenA as operation base during its Amphibian Projects (www.andigena.org), and subsequently identified as P. pleurostriatus, a rare and poorly known species vaguely described by Rivero (1984 “1982”), and known solely by two specimens. Furthermore, during a RAP (Rapid Assessment Programme) made in July 2008 by Conservation International in the eastern slopes of the Cordillera de Mérida, Calderas area, Estado Barinas, 17 species of amphibians were collected (Barrio-Amorós & Molina 2010). Among these was another Pristimantis, morphologically similar to P. vanadisae with clearly distinct characters, which is described herein.

While P. vanadisae is widespread through most of the central Cordillera de Mérida, P. pleurostriatus was only known from its original description (Rivero 1984 1982) and seems to be highly restricted and quite rare. From now on, we refer to P. vanadisae, P. pleurostriatus and the new species described herein as the tuberculated Venezuelan Andean Pristimantis.

In addition to describing this new species, and redescribing the little-known P. pleurostriatus, we also take advantage herein to comment about the intraspecific variation within P. vanadisae to help further research on the tuberculated species of Pristimantis from the cloud forest of the Venezuelan Andes.

Material and methods

External morphology and vocalization. All measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological terms follow Lynch and Duellman (1997). Comparisons are based on published descriptions by La Marca (1984) and Rivero (1984 “1982”), as well as preserved material from CVULA (Colección de Vertebrados, Facultad de Ciencias, Universidad de los Andes, Mérida, Venezuela). Measurements of adult frogs follow Barrio-Amorós et al. (2010a) and include the following. 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 classical measurements including IOD (inter orbital distance) and UEW (upper eyelid width) were not 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 their primary sexual attributes determined (testis, oviducts).

Calls were recorded with a Tascam handy recorder DR 100, with a cardioid microphone Senheiser M68, and analyzed with Praat 5.2.35 (http://www.fon.hum.uva.nl/praat/). Data on the temperature were also registered from the substrate where the frogs were calling. The dominant frequency was determined using the spectrogram slice view. Call parameters measured include (1) pulse duration, (2) interval between pulses or time between the beginning of the first pulse and the next, (3) number of pulses per second, and (4) dominant and fundamental frequencies.

**Molecular phylogeny.** We performed molecular phylogenetic analyses to assess 1) the genetic distinctiveness of the new taxon from *Pristimantis vanadisae* and 2) the placement of it and other sampled Venezuelan taxa within *Pristimantis*. Tissue samples were obtained from an exemplar of the putatively new taxon, along with series of the similar species *P. vanadisae* (including one individual from the type locality) and several other Venezuelan *Pristimantis*. Sequences of the complete 12S and 16S rRNA genes were targeted for analysis. DNA extraction, PCR, and sequencing follow established protocols and used the Terrarana-specific PCR primers 12L29E, 12H46E, 12L4E, 12L34, 16H50, 16H48E, 16L19, 16H36E, 16L34, 16H47 (Heinicke et al. 2007, 2009; Hedges et al. 2008). All newly generated sequences are deposited in GenBank (accession numbers JX155277–JX155297). In addition to newly generated sequences, sequences of several *Pristimantis* representing major clades as found by Hedges et al. (2008) were obtained from GenBank, along with sequences for exemplars of the related genera *Hypodactylus* Hedges, Duellman, & Heinicke, *Oreobates* Jiménez de la Espada, *Phrynopus* Peters, and *Strabomantis* Peters. A sequence of the craugastorid *Craugastor podiciferus* (Cope) was used to root the tree.

Nucleotide sequences were aligned using MUSCLE (Edgar 2004) as implemented in MEGA 5 (Tamura et al. 2011), with poorly conserved regions deleted prior to phylogenetic analyses. The final aligned dataset includes 32 taxa and 2471 nucleotide characters (849 parsimony-informative), and is available through the Dryad data repository (doi:10.5061/dryad.6ff85). Phylogenetic analyses were performed employing unpartitioned likelihood and parsimony criteria. The best-fitting model for use in the ML analysis was identified based on the Akaike Information Criterion in jModelTest 0.1.1 (Posada 2008). This was identified as the TIM1 + G model (Posada 2003). The likelihood analysis was performed in PhyML 2.4.4 (Guindon & Gascuel 2003). The TN93 + G substitution model was used (Tamura & Nei 1993), with model parameters set to the values identified in jModelTest, assuming a BIONJ starting tree (Gascuel 1997). The TN93 model is the most similar model to TIM1 built into PhyML, differing in including one instead of two transversion parameters. The maximum parsimony analysis was performed in MEGA 5. A close-neighbor interchange search was implemented, with 10 random-addition replicates. For both the likelihood and parsimony analyses, branch support was assessed via 1000 bootstrap replicates (Felsenstein 1985).

**Results**

*Pristimantis conservatio* sp. nov.

**Holotype:** CVULA 7174, an adult female from a cloud forest immediately above the hamlet of Los Alcaravanes at Aguas Blancas, 1640 m, 856°43’N, 7025°54’W; Ramal de Calderas, Estado Barinas, Venezuela, taken on 8 of September 2008 by C. L. Barrio-Amorós.

**Paratypes:** An adult male of 21.7 mm SVL, CVULA 7175, with the same locality and collection data as the holotype.

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**Pristimantis FROM ANDEAN VENEZUELA**

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Referred specimens: A juvenile specimen of 14.1 mm SVL, CVULA 7176, with the same collection data as the holotype.

Diagnosis: *Pristimantis conservatio* is a moderately sized (only adult male 21.7 mm, only female 33.8 mm) member of the *unistrigatus* species group sensu Lynch and Duellman (1997) and Hedges et al. (2008). It is diagnosed by (1) dorsal skin shagreen with a well defined middorsal raphe; no occipital ridges; ventral skin areolate; flanks with white speculate tubercles; (2) tympanum distinct, with well-defined tympanic annulus, 36%–38.9% of the ED; (3) snout subacuminate in dorsal and lateral views; canthus rostralis rounded, not well defined; (4) upper eyelid smooth in preservative, with a few low tubercles, one usually larger and conical in life, on the posterior edge of the eyelid; (5) choanae small, round; dentigerous processes of the vomers inconspicuous, covered by palatal shelf; tongue large, longer than wide, cordiform, posterior half free; (6) males without vocal slits; no apparent nuptial pads; (7) finger I shorter than II; (8) fingers without lateral keels; (9) unlta tubercles present; (10) tarsal tubercles and conical heel tubercles present; (11) two metatarsal tubercles, inner oval, large; outer conical, small; (12) toes with well-marked lateral keels; webbing absent; toes III, IV and V with broad discs, slightly smaller than those on fingers III and IV; (13) in life, color dorsally dark to pale brown with no evident pattern, black supratympanic stripe, lip bars, flank diagonal stripes, and hind limbs bars; ventrally reddish with a profusion of small brown to black spots and marks. Iris dark copper reticulated by fine black venation; iris periphery blue. In preserve, colors fade to dull gray; ventrally dirty white with darker marks.

Species comparison: This species is compared (characters of *P. conservatio* in parentheses) with the most similar tuberculated species *P. vanadisa* and *P. pleurostriatus* as well as other *Pristimantis* from the Venezuelan Andes and adjacent areas in Colombia. Nonetheless, the Venezuelan Andes (comprising the cordillera de Mérida but not the Perijá range) is isolated from the Cordillera oriental de Colombia by a low and dry valley (Depresión del Táchira) and only a few widespread amphibian species are known to share both cordilleras. The species has no flash marks on the goin and hidden surfaces of hind limbs (present on *P. lentiginosus* [Rivero 1984 1982”], *P. melanoproctus* [Rivero 1984 1982"], *P. monolofii* [Rivero 1984 1982”], and *P. vanadisa*), furthermore, none of these mentioned species have unlta, tarsal or heel tubercles (present and conspicuous in *P. conservatio*). The former *tubernasus* group of Rivero (*P. prolixodiscus* [Lynch, 1978], *P. tubernasus* [Rivero 1984 1982”]) presents a pointed tubercle on the tip of snout (absent). *Pristimantis ameliae* Barrio-Amorós 2011 2012 has dorsolateral folds (absent), lacks unlta, tarsal and heel tubercles (present), and vestigial webbing (absent). *Pristimantis batrachites* (Lynch 2003) from Cordillera oriental de Colombia has an acuminate dorsal and protruding in lateral views (subacuminate dorsally and laterally), canthus rostralis sharp (rounded, ill-defined), upper eyelid lacking tubercles (present), vocal slits present (absent), and is smaller, with females up to 21.7 mm (33.8 mm). The most similar species morphologically is *Pristimantis vanadisa*, which is a somewhat smaller frog, with males up to 19.8 mm, mean 18.60.7 mm; n=9 (only male known 21.1), females up to 31.9 mm, mean 28.22.1 mm; n=50 (only female known 33.8); well defined and angular canthus rostralis (round, ill defined), cahtal stripe always present and well defined (present but ill-defined; never complete in three animals), yellow spots on the goin and posterior surface of thighs usually outlined with black (absent). *Pristimantis pleurostriatus* is a similar-sized frog, differing by having a round snout dorsally and laterally (subacuminate), a patterned flank with diagonal stripes (absent to ill-defined); and a white/black marbled belly (reddish with a profusion of small brown to black spots and marks), and occurs on the opposite side of the Cordillera de Mérida, the eastern versant (western versant).

Description of the holotype. Head longer than wide, head width 34.3% of SVL. Snout subacuminate in dorsal and lateral views: EN equal to ED; nostrils non-protuberant, directed dorsolaterally; canthus rostralis rounded and ill-defined, loreal region slightly concave. Upper eyelid with low tubercles, one conical, larger but also low on the posterior part of the eyelid; tubercles absent on head. Cranial crests absent. Tympanum distinct, 38.9% of ED, its posterior-dorsal part obscured by a supratympanic fold; one enlarged post-rectal tubercle. Choanae small, rounded, not concealed by palatal shelf of maxillary arch; vomerine dentigerous processes inconspicuous, not visible, only slightly apparent by touch under palatal shelf, posterior and medial to choanae; vocal slits absent, vocal sac absent. Tongue slightly cordiform, posterior half free.

Dorsal skin shagreen in preservative (tuberculate in life; Fig. 1a) with only a few small conical tubercles or spicules dorsally posterior to the head, tubercles also widespread on the flanks; occipital ridges not visible; middorsal raphe present, well-marked; dorsolateral folds absent; throat, chest and inferior part of thighs smooth, venter areolate; unlta and tarsal tubercles present, not prominent, conical heel tubercle low.
Relative length of appressed fingers III>IV>II>I; first finger reaching half of the disc on finger II when approached. Finger discs much broader than long, disc on finger III of right hand 3.1 times wider than adjacent phalanx; horizontally oval except on Finger II, which is rounded; disc on Finger I is unexpanded. Lateral fringes on fingers absent. Palmar and thenar tubercles distinct, the first deeply bifid, the last ovoid. Subarticular tubercles protuberant, single, round. Supernumerary tubercles protuberant, in rows under each finger.

Hind limbs relatively short; shank 47.3% of SVL. Heel just overlaps the eye when held to the sagittal plane. Relative lengths of appressed toes IV>V>III>II>I. IV toe disc slightly smaller than III finger disc. Toes with fine lateral fringes, no basal webbing. Discs horizontally oval, wider than long, except on toe I, which is round. Inner metatarsal tubercle large, protuberant, oval; outer distinct, conical; subarticular tubercles protuberant, single, round; supernumerary tubercles distinct, in rows under each toe.

Color in life: The female holotype in life (Fig. 1a) is dark brown dorsally, with a well defined blackish interorbital bar. The canthal stripes are not complete, broken in the middle, black; the supra tympanic stripe is dark brown dorsally surrounded by black, as are two ill-defined upper lip bars; the upper lip background is orange brown. The flanks are lighter than the dorsum, with ill-defined blackish diagonal stripes, and a profusion of small
white spots, apparently coinciding with small conical tubercles. Arms are orange-brown with white small spots; forearms are dark brown. Thighs and shanks are dark brown cross-barred with black. Finger and toe pads are variable, from light orange-brown to dark brown. Ventrally (Fig. 1b) the background is pale reddish brown with dark brown to black irregular and indefinite marks; the posterior part of the belly is reddish, as is the hind limbs background, reticulated with white. Iris dark copper reticulated by fine black venation; pupil black; the iris periphery is pale blue.

**Color preserved.** Dorsal color is dull grayish brown, with no evident marks; the only darker areas are the supratympanic stripe and limb bars. The posterior parts of the thighs are dirty white with a suffusion of dark spots. Ventrally, throat, chest, belly and under surfaces of hind limbs are dirty white with dark brown profusion of spots and irregular marks.

**Measurements of holotype (in mm):** SVL: 33.8; ShL 16; HeL: 12.5; HW: 11.6; EN: 4; ED: 3.8; TD: 1.5; FD: 1.7; T4D: 1.6; 1FiL: 4.5; 2FiL: 5.1.

**Variation:** Only two other specimens are known; one male (with enlarged greyish testicles) of 21.7 mm SVL (Fig. 1c); and a juvenile of 14.1 mm. The male is consistent with all characters of the female holotype, including the coloration, just being a little paler, and the dark diagonal stripes on the flanks are better defined. The iris periphery is pale blue. Vocal slits and sac are absent.

A juvenile of 14.1 mm (Fig. 1d) is greenish laterally, with two orange dorsolateral stripes and a copper dorsum; the ulnar, tarsal and conical tubercles, as well as the enlarged tubercles on eyelids, are already well-developed; the flanks also bear the white small spiculated tubercles.

**Natural history:** All specimens were collected in cloud forest at ≈1600 masl, on upper leaves of bushes 0.30 m (juvenile) to 1.20 m (female) above ground. No calls were heard associated with the species. It appears to be a rare species, compared with relatively higher abundance of *P. vanadisae* in similar habitat. Congeners in the type locality include the sympatric *P. prolixodiscus* (Lynch, 1978) and the syntopic *P. yusti* (Barrio-Amorós & Chacón, 2004). Other anurans found in the type locality are *Allobates humilis* Rivero, *Aromobates* sp., *Hyloscirtus platidactylus* Boulenger, *Dendropsophus minutus* Peters, and *Gastrotheca nicefori* Gaige (Barrio-Amorós 2010).

**Distribution and natural history:** *Pristimantis conservatio* is known from a small area of the southeastern slopes of Paramo El Volcán, known as Aguas Blancas (Fig. 2). There is no reason why the species could be not more widespread along the eastern versant of the Cordillera de Mérida, with distribution similar to *P. yusti* (Barrio-Amorós & Chacón 2004). All three specimens were on leaves from 30 to 100 cm above the ground, in cloud forest not far from a small stream.

**Etymology:** From the Latin *conservatio* (or *conservationis*), meaning conservation or preservation. We dedicate this new species to the efforts made by Conservation International (CI) to protect the Earth’s natural heritage, both by promoting investigation and conservation. The species was collected during a RAP funded by CI’s Venezuelan office. We use the name as a nominative in apposition. The common English name Conservation landfrog; and the common Spanish name “Ranita nublada de los conservacionistas” are suggested. We use the English common name “landfrog” rather than “rainfrog” (as has been used by other authors) for terraranans because it is the English translation of "Terrarana." Also, terraranans are direct developers and breed on land, not using rain water, and often vocalize when there is no rain.

**Pristimantis pleurostriatus (Rivero, 1984 1982)***


_Pristimantis (Pristimantis) pleurostriatus_ — Hedges, Duellman, and Heinicke, 2008, _Zootaxa_, 1737: 118.

**Holotype:** UPR 4971, by original designation. Examined through photographs (Fig 3).

**Referred specimens:** seven (7) males (CVULA 7186–89, 7191–93) and one female (CVULA 7190) from cloud forest at Estancia la Bravera, 2352 m, 83°03’’N, 71°22’’59’’W, between San Eusebio and La Carbonera;
collected on 10 July 2007 by C. L. Barrio-Amorós. Three unsexed specimens (CVULA 4904, 4926, 6017) from La Carbonera, Mérida.

**FIGURE 2.** Distribution of tuberculated *Pristimantis* in the Cordillera de Mérida, northwestern Venezuela. Black square: 1: Black circle: *Pristimantis conservatio* sp. nov. 2: *Pristimantis pleurostriatus* (also locality for *P. vanadisae*). White circles: *Pristimantis vanadisae*. 3: La Mucuy. 4: San Javier. 5: Monte Zerpa. 6: La Macana.

**Diagnosis:** *Pristimantis pleurostriatus* is a small (males up to 25.3 mm SVL, only female known 31.1 mm) member of the *unistrigatus* species group *sensu* Lynch and Duellman (1997). It is diagnosed by (1) dorsal skin with a profusion of small tubercles, some larger and pungent especially on the anterior part of the body; on upper eyelid are some small tubercles, usually one pungent; no occipital ridges, shagreen posteriorly; ventral skin areolate; (2) tympanum distinct, with a tympanic annulus, 35.5–47.5% of the ED; (3) snout semi-rounded to rounded in dorsal view, rounded in profile; canthus rostralis rounded, not well defined; (4) upper eyelid smooth in preservative, with a few low tubercles, one usually larger and pungent in life, on the posterior edge of the eyelid; (5) choanae small, round to oval; dentigerous processes of the vomers inconspicuous, covered by palatal shelf on males, small on females; tongue large, longer than wide, cordiform, posterior half free; (6) males without vocal slits, subgular vocal sac present; no apparent nuptial pads; (7) finger I shorter than II; (8) fingers with lateral keels, less evident on well-preserved specimens; (9) ulnar tubercles present; (10) tarsal tubercles and conical heel tubercles present; (11) two metatarsal tubercles, inner oval, large; outer conical, small; (12) toes with well-marked lateral keels; webbing absent; toes III, IV and V with broad discs, slightly smaller than those on fingers III and IV; (13) in life, dorsal color light to orange-brown, with a post-occipital round mark, consistent brown diagonal stripes on whitish flanks, hidden surfaces of groin, thighs and shanks black with transverse white stripes or spots, and whitish venter with...
dark marbling on belly. Iris golden gray reticulated with fine venation; an orange dark brown area on each side of the black pupil; iris periphery blue. In preserve, same pattern as living frogs, but colors faded to greyish.

**FIGURE 3.** Holotype (UPR 4971) of *Eleutherodactylus pleurostriatus* (=now *Pristimantis pleurostriatus*). Photos courtesy of F. Bird.

**Species comparison:** This species is compared (characters of *P. pleurostriatus* in parentheses) with those of the *lentiginosus* group of Rivero (1984, 1982), *P. lentiginosus, P. mondolfii* and *P. melanoproctus*, and tuberculated similar species (*P. vanadisae* and *P. conservatio* sp. nov.) in the Cordillera de Mérida of Venezuela and Cordillera Oriental of Colombia. *Pristimantis ameliae* has dorsolateral folds (absent), lacks ulnar, tarsal and heel tubercles (present), webbing vestigial (absent). *Pristimantis batrachites* from Cordillera oriental of Colombia (Lynch 2003) has an acuminate dorsal and protruding in lateral views (subacuminate dorsally and rounded laterally), canthus rostralis sharp (rounded, ill-defined), upper eyelid lacking tubercles (present), vocal slits present (absent), and is smaller, with females up to 21.7 mm (31.1 mm). *Pristimantis pleurostriatus* differs markedly from *P. lentiginosus, P. mondolfii* and *P. melanoproctus* because these species lack conical heel tubercles and protuberant ulnar and tarsal tubercles (present), and all have light spots on the groin (absent). The most similar species, with which it is sympatric, is *P. vanadisae*. It is easily distinguishable because adult males are much smaller, up to 19.8 mm, mean 18.6 ± 0.7 mm; n=9 (up to 25.3 mm, mean 24.30 ± 0.7 mm; n=7); has a subovoid and longer snout (shorter and rounded), well-defined and angular canthus rostralis (round, ill defined), canthal stripe always present and well-defined (if
present, ill-defined; usually absent), an iris peripheric blue ring is lacking (present), belly without consistent pattern, usually cherry colored (marbled in black and white), and yellow spots on the groin and posterior surface of thighs usually outlined with black (absent).

**Description of the referred series.** Head slightly longer than wide, head width 35.7–39.9% of SVL. Snout subacuminate dorsally (Fig. 4a) and rounded in lateral view (Fig 2d, 3a), without pointed tubercles except one not collected juvenile specimen, which showed a distinct pungent papilla. EN shorter than ED; nostrils non-protuberant, directed dorsolaterally; canthus rostralis rounded but ill-defined, loreal region slightly concave. Upper eyelid with low tubercles, largest one rounded to conical on the posterior part of the eyelid; tubercles sparse on head, especially on the interorbital region. Cranial crests absent. Tympanum distinct, 37.5–45.4% of ED, tympanic annulus not well-defined; supratympanic ridge covered by small tubercles hiding a small portion of its posterodorsal section. Choanae small, rounded, not concealed by palatal shelf of maxillary arch; vomerine dentigerous processes usually inconspicuous, not visible, only apparent by touch under palatal shelf, posterior and medial to choanae. Males usually have dentigerous processes concealed by the palatal shelf. CVULA 7188 exposes one tooth on the left process; CVULA 7189 one on the left, two on the right; the female CVULA 7190 has exposed dentigerous processes, bearing on the left process two teeth and on the right four. Tongue barely cordiform, posterior half free; vocal slits absent, vocal sac present.

![Figure 4](image_url)

**FIGURE 4.** *Pristimantis pleurostriatus* in life. (a) male CVULA 7187; (b) female CVULA 7190, (c) male CVULA 7188, (d) uncollected calling male at the type locality.
Dorsal skin tuberculate, with dispersed small conical tubercles or spicules also covering the flanks; occipital ridges not defined; middorsal raphe present but vague; dorsolateral folds absent; throat and chest smooth, venter and inferior part of thighs areolate; ulnar, tarsal tubercles and conical heel tubercles present, prominent in males; in the female CVULA 7190 these tubercles are less developed.

Relative length of appressed fingers III>IV>II>I; first finger surpassing the disc on finger II when appressed to each other. Finger discs much broader than long, disc on finger III of right hand 2.5–2.8 times wider than adjacent phalanx; discs quadrangular except on Finger I, which is distinctly expanded but round and smaller than those on the other fingers. Lateral fringes on fingers ill-defined. Palmar and thenar tubercles distinct, the first deeply bifid, the last ovoid. Subarticular tubercles protuberant, single, round. Supernumerary tubercles protuberant, in rows under each finger.

Hind limbs relatively short; shank 49.2–55.4% of SVL. Heel reaches midway from eye to naris when held to the sagittal plane. Relative lengths of appressed toes IV>V>III>II>I. The IV toe disc slightly smaller than the III finger disc. Toes with lateral fringes, giving the appearance of basal webbing. Discs wider than long, except those on toes I and II, which are rounded; toe discs wider than phalanges, those on toes III, IV and V quadrangular. Inner metatarsal tubercle large, protuberant, oval; outer distinct, conical; subarticular tubercles protuberant, single, round; supernumerary tubercles distinct, in rows under each toe.

**Color in life and variation:** CVULA 7187 in life (Fig. 4a) is dark brown dorsally, on the head is a brown light interorbital bar and frontal; the upper eyelids and the anterior edge of the interorbital bar are greenish. The canthal and supra-tympanic stripes are dark brown, as are three upper lip bars. There is a much darker brown dorsal chevron; the flanks are white-barred with dark brown stripes. Hind limbs are orange-brown crossbarred with dark brown. Finger pads are greenish. Ventrally the throat and chest are translucent white, while the belly is white with a striking black marbling. Iris golden gray reticulated by fine venation with an orange dark brown area on each side of the black pupil; the iris periphery is blue.

Consistent color features are the presence of a round light post-occipital spot crossed by the anterior part of the middorsal raphe, in all males, from most visible in the holotype and CVULA 7193 to less visible in CVULA 7188 and absent in CVULA 7192. The pattern is much darker on the female (Fig. 4b) and not easily distinguished. All seven males and the only female have the striking diagonal dark brown stripes bordered by white on the flanks, being lighter on CVULA 7189 and darker on CVULA 7190, with more contrast on CVULA 7188 (Fig. 4c) and CVULA 7193. One male photographed at night (Fig 4d) is green dorsally, including hind limbs and arms. The contrasting diagonal stripes on the flanks are bright white. In all specimens there is a black background on the anterior and posterior surfaces of the thighs, crossed by transverse white stripes, or dotted with white spots. However, this cannot be confused with the flash marks found in other species. A very distinct specimen is CVULA 7188 (Fig. 4c), exhibiting light brown to orange-brown with a complex design of dorsal undulating wavy stripes surrounded by white lines. Other specimens can be less contrasting, with a pale brown-orange dorsum, shanks and tarsi, with diagonal white stripes on dark brown flanks. Ventrally, all specimens have a distinguishing marbled pattern, but this pattern can be less or more contrasting. CVULA 7188, 7192 and 7193, darker on CVULA 7192 and 7193, and lighter on 7189. The female CVULA 7190 is greyish brown ventrally, also with a striking dark marbled pattern. Some juveniles are grey with black broken canthal and complete supratympanic stripes, some orange irregular spots on the dorsum and shanks, white arms, but always with the distinct diagonal stripes on the flanks.

**Remarks:** The original description by Rivero (1984 1982) was based on only two specimens (holotype and paratype), and these apparently are atypical compared with the larger series examined for this work. Rivero mentioned that those specimens have vomerine odontoids, while in our sample they are usually absent, and if present, small. He mentions as well a smooth dorsal skin, with just a few tubercles, while our sample is quite tubercular. The most striking difference is that he does not mention especially the presence of tarsal and conical heel tubercles, when in our sample they are well-developed. And finally, the color reported for the holotype in life is not close to what we observed in the studied sample plus many other specimens not collected. Rivero describes a longitudinally striped pattern; with a vertebral line, paravertebral and dorsolateral stripes, and purple color beneath. In no specimen from our sample have we observed this pattern and color. However, we are hesitant to describe a new species at this time based in these discrepancies without direct examination of the holotype. Rivero (1984 “1982”) also gives contradictory data about the presence of vocal slits for *P. pleurostriatus*. On page 89 he states that the “dimorfismo sexual consistente, hasta donde se sabe, de un saco vocal subgular muy bien diferenciado,
 pero sin hendeduras bucales ("sexual dimorphism consists, as far as is known, of a well-differentiated vocal sac, but without vocal slits") while on page 122 he states that the species differs from others "en tener los machos saco y hendeduras bucales ("in the males having vocal sac and slits"). Without examining the holotype at UPR (Universidad de Puerto Rico), is impossible to state which one is the correct character.

FIGURE 5. (a) oscillogram and (a') spectrogram of one pulsed note of a sequence of the call of *Pristimantis pleurostriatus*. Background noise has been strongly reduced and frequencies below 100-1500 Hz were filtered from the spectrogram.
Natural history: The males call from 1830h to at least 2100h, after light rains in the evening, from heights of 1 to 3 m, easily visible on bushes without much foliage (Fig. 4d). Three specimens were heard calling from bromeliads, one 3 m above the forest soil. The only female was on a leaf 50 cm above the ground, in the same microhabitat where all *P. vanadisae* from that site were observed. In our experience males of this species are never found less than 1 m from the ground, while the sympatric *P. vanadisae* is usually perched from 0.25 to 1.5 m above the forest floor. *Pristimantis pleurostriatus* appears to be rarer than sympatric *P. vanadisae* (8 specimens collected...
of *P. pleurostriatus* versus 24 *P. vanadisae* in the same period of time). *Pristimantis pleurostriatus* is highly seasonal, appearing only in the wet season (never seen or heard by us in March, December or January), whereas *P. vanadisae* can be seen throughout the year. Other amphibians observed at La Bravera and surroundings include *Centrolene altitudinale* (Rivero), *C. venezuelense* (Rivero), *Hyalinobatrachium duranti* (Rivero), *Gastrotheca nicefori* Gaige, *Dendropsophus meridensis* (Rivero), *Hyloscirtus platydictylus* (Boulenger), *Bolitoglossa orestes* Brame & Wake, and the introduced *Lithobates catesbeianus* (Shaw). The area was once habitat for *Atelopus carbonerensis* Rivero, which is currently believed to be extinct at this locality (C.L. Barrio-Amorós, pers. obs.).

**Vocalization:** Two series of vocalizations were recorded at 13.6 °C the night of 3 July 2012. The call consists of one single pulsed note, that can be repeated up to 11 times, but most common is a call of one to four notes. If single, it is repeated as a short and low whistle (see Fig. 5 for single pulse oscillogram (a) and spectrogram (a')). Fig. 6 depicts a series of 12 pulsed calls, (a) oscillogram and (a') spectrogram. The fundamental frequency is at 2009 Hz, the dominant frequency is at 2316 Hz; the duration of the twelve-note call is 1.65 sec. Note and inter-note duration with their mean, SD and range in seconds are indicated as follows: (1) note duration, n=12, 0.03 0.01 (0.01 – 0.04) and (2) inter-note, n= 11, 0.20 0.03 (0.15–0.25). The recorded call can be heard at AmphibiaWeb.

**Distribution:** *Pristimantis pleurostriatus* is known from the cloud forests surrounding Páramo El Tambor, at the southwestern edge of the Sierra de la Culata of the Cordillera de Merida (Fig. 2). The cloud forests around Páramo El Tambor are isolated and some other amphibians are known to be endemic, like *Atelopus carbonerensis*, *Aromobates meridensis* and *A. mayorgai* (Rivero, 1978).

**Etymology:** *pleurostriatus*, from Greek *pleuron*, meaning side, and from Latin *striatus*, meaning stripes, due to the evident diagonal stripes on the flanks of all specimens studied.

### Variation within *Pristimantis vanadisae*

There are two available descriptions of *P. vanadisae*: that of *Eleutherodactylus vanadise* by La Marca (1984) and that of *Eleutherodactylus cerasoventris* by Rivero (1984 “1982”). Even though the descriptions were based on 29 and 36 specimens, the variation in nature is not fully represented. We describe herein chromatic variation of this species more in accordance with the spectrum exhibited in wild populations.

We report four different patterns, each of which occurs at multiple localities (Fig 7). Our description is based on a sample of 98 adults and subadults (specimens over 15 mm) of *P. vanadisae* from four localities: San Javier del Valle, Sierra La Culata, approx. 2000 m (n=11), La Bravera, between San Eusebio and La Carbonera, 2200 m (n=11), La Mucuy Alta, type locality of *P. vanadisae*, 2300 m (n=25), and Monte Zerpa, northeast of Merida city, 2000 m (n=61). Fig. 2 shows all localities.

**Pattern A** (plain) (Fig 7a,b): dorsum unicolor, dark green, beige, pale yellow, or gray, patternless except for ill-defined leg cross bars, and normally pigmented groin and posterior surfaces of thighs (usually black, cherry red with pale spots in adults, lacking this pattern in juveniles). Canthal and supratympanic stripes are always present and well defined (usually black but also dark brown). Forty-seven specimens in our sample have this pattern, representing 47.9%. The following specimens have this pattern: CVULA 0186, 0283, 0632, 1118, 1163, 1234, 1513–14, 1642, 1648–49, 1651–52, 1680, 1682, 1684–85, 1687, 1690, 1692–94, 2014, 2020, 2022, 2208, 2317, 2356, 2428–29, 2431, 2605, 3115, 3118, 3190–21, 3123–24, 3127–29, 7185, 7200–1, 7203, 7205.

**Pattern B** (dorsoconcolor) (Fig. 7c): Dorsum (including snout, eyelids, head and back) beige, cream or pale yellow. Flanks pale brown, delimited from the dorsum by the dorsolateral folds, sometimes plain, sometimes with diagonal bars. Hind limbs with or without cross bars. Canthal and supratympanic stripes are always present and well defined. This is a less abundant pattern, found only in 3 of 98 specimens (3%). The following specimens have this pattern: CVULA 0185, 7196 and 7204.

**Pattern C** (spotted) (Fig. 7e, f): This is the most complex and variable pattern. Dorsal color variable, yellow, green, brown, or grey in different tonalities, but always spotted on dorsum, head (always with an interorbital bar, canthal and supratympanic stripes), and cross-barred on limbs. Usually a black or dark brown “W on the scapular area. Twenty-eight of 98 (28.5%) specimens bear this pattern. The following specimens exhibit pattern C: CVULA 0745–46, 0878, 1122, 1166, 1235, 1643, 1645–46, 1653, 2017, 2019, 2355, 2358, 2430, 3107–08, 3110–11, 3113, 3116–17, 3125–26, 7184, 7194, 7198, 7206, 7981.
FIGURE 7. Pattern variation in *Pristimantis vanadisae*. (a) Uncatalogued specimen photographed alive, and (b) CVULA 7203: Pattern A. (c) CVULA 7204: Pattern B. (d) CVULA 7199: Pattern D. (e) CVULA 7206 and (f) CVULA 7198: Pattern C.
**Pattern D** (dorsolateral stripes) (Fig. 7d): Main dorsal and flank color uniform, brown to grey. Interorbital bar and limb cross bars ill-defined. Two dorsolateral stripes, usually narrow, extend from the canthal above eyelid to the supratympanic stripe to the mid body, sometimes reaching sacrum area, yellow to orange. While this pattern is rarely seen in La Bravera (1 of 11=9%), it is more common at Monte Zerpa (16 of 61=26.2%). In total, this pattern represents 20.4% of the sample. Samples with pattern D: CVULA 0285, 0879, 1116–17, 1650, 1681, 1683, 1686, 1688, 1691, 1695, 2018, 2021, 2156, 2353–54, 2357, 3114, 7119, 7199.

Independent of the pattern variation, all specimens show canthal and supratympanic black stripes, clearly diagnostic when compared to sympatric *P. pleurostriatus*, and the groin and posterior surface of the thighs are dark with pale spots.

**Relationships of *P. conservatio* and other Venezuelan *Pristimantis* within Terraranas**

There are no known synapomorphies for *Pristimantis*. However, Hedges *et al.* (2008) list the following combination of features as typical for *Pristimantis*: head as wide as body, tympanic membrane differentiated, dentigerous process of vomers present, terminal discs of digits expanded, bearing well defined circumferential grooves, toe V usually longer than toe III. *Pristimantis conservatio* conforms to these characteristics, and molecular phylogenetic analyses additionally place this species within *Pristimantis* (Fig. 8). We assign tentatively *P. conservatio* to the *P. unistrigatus* species group as defined by Lynch & Duellman (1997) and Hedges *et al.* (2008) by having slender bodies, being small to medium sized frogs, with short snouts and moderately long limbs, having finger I shorter than finger II, toe V longer than toe III, the digital discs expanded, the tympanic annulus and membrane present, without cranial crests, vomerine teeth and vocal sacs present.

We caution, however, that most of the classical species groups of *Pristimantis* based on morphology (Lynch & Duellman 1997) have yet to be supported in molecular phylogenies (Hedges *et al.* 2008; Pinto-Sánchez *et al.* 2012). Pinto-Sánchez *et al.* (2012) attributed, incorrectly, those morphologically-based species groups to Hedges *et al.* (2008). Those latter authors only listed the groups defined by earlier authors (Lynch & Duellman 1997), for convenience, pointing out that "none of these phenetic groups has been clearly distinguished in various phylogenetic analyses" (Hedges *et al.* 2008). Pinto-Sánchez *et al.* (2012) concluded that "the subgeneric taxonomy of *Pristimantis* is clearly flawed," but this was based largely on their misunderstandings of the earlier work. Neither those authors, or Hedges *et al.* (2008), sampled taxa sufficiently for a reclassification of *Pristimantis*, and therefore claims of rejection or support of supraspecific taxa (Pinto-Sánchez *et al.* 2012) are premature.

Likelihood and parsimony analyses recovered highly concordant trees (Fig. 8). *Pristimantis conservatio* is confirmed as a member of *Pristimantis*, with monophyly of the genus receiving significant support. The species is also distinct from *P. vanadisae*, with uncorrected pairwise genetic distances of 5%–6%, vs. 0%–2% within *P. vanadisae*. These distances are somewhat smaller than distances between *Pristimantis* species for more rapidly-evolving mitochondrial genes like ND2 or cytochrome b (Elmer *et al.* 2007, Wang *et al.* 2008), but are consistent with species-level differences in homologous 12S and 16S gene regions observed among sets of related species of *Pristimantis* (e.g. Padial & De la Riva, 2009). For example, inspection of a dataset of 12S and 16S sequences included in Hedges *et al.* (2008) reveals 47 *Pristimantis* species pairs having uncorrected pairwise 12S/16S genetic distances at or below 5% (out of 5460 possible pairwise comparisons; the dataset included 105 *Pristimantis* species).

The series of individuals representing *P. vanadisae* is monophyletic, and includes individuals exhibiting pattern forms A, C, and D, confirming the presence of pattern polymorphism in *P. vanadisae*. CVULA 7184 from Altos de San Luis, was identified as *P. aff. vanadisae*, due to its similar morphology, but in our phylogeny is sister to *P. conservatio*, with a pairwise genetic distance of 3.4% from *P. conservatio*, vs. 5.0% from *P. vanadisae*. This point to another similar species still to be described. Additional Venezuelan taxa recovered as nested within *Pristimantis* include *P. ginesi* (Rivero, 1964), *P. briceni* (Boulenger, 1903), *P. lancinii* (Donoso-Barros, 1968), *P. paramerus* (Rivero, 1982), and *P. pleurostriatus*. *Eupsophus ginesi (=Pristimantis ginesi)* is the type species of *Paramophrynella* La Marca, 2007, supporting placement of this genus in the synonymy of *Pristimantis*, following the nomenclature of Hedges *et al.* (2008). *Pristimantis briceni*, *P. lancinii* and *P. paramerus* were placed in *Macubatrachus* by La Marca (2007), with *Hyloides briceni* (=*Pristimantis briceni*) designated as type species. While these three species plus an undescribed species form a monophyletic grouping in our phylogeny, it is clear
that this genus also is embedded within *Pristimantis sensu* Hedges *et al.* (2008). As recognition of either genus at this time would render *Pristimantis* paraphyletic, we advocate maintaining both *Mucubatrachus* and *Paramophrynella* in the synonymy of *Pristimantis* at present. Recognition of one or both at the genus or subgenus level may be warranted in the future considering the current unwieldy nature of *Pristimantis*, with well over 400 recognized species (Frost 2013). However, such action should only be taken in the context of a broadly-sampled revisionary work.

**FIGURE 8.** Maximum likelihood phylogeny of strabomantid frogs, rooted with *Craugastor podiciferus*. Bootstrap support values (likelihood/parsimony) are indicated at nodes. Pattern variants of *Pristimantis vanadisae* are noted. Museum, or when not available, field voucher numbers (for newly sequenced specimens) and GenBank accession numbers (bold; for all taxa in tree) are given. For newly-sequenced specimens, locality data are provided in species accounts, Appendix 1, or Appendix 2.

**Discussion**

In this work we attempt to clarify the taxonomy of the tuberculated *Pristimantis* from the Cordillera de Mérida (Andean Venezuela). Until now, only one species was known to have ulnar, tarsal and conical heel tubercles: *P. vanadisae*. This species is distributed throughout the central Cordillera de Mérida, both at Sierra Nevada and Sierra de la Culata, and the name *P. vanadisae* has been associated with most specimens encountered with such tubercles in Venezuelan collections (e.g. CVULA). The reality is more complex, as *P. vanadisae* is polychromatic, varying
within and among populations, while *P. pleurostriatus* and *P. conservatio* sp. nov. also are characterized by such tubercles. Rivero (1984 1982, Rivero & Segu 1989) also commented on the apparent disproportionate sex ratio in favor of the females of *Eleutherodactylus cerasoventris* (=*P. vanadisae*), of which he never saw a male. This was taken by Rivero (Rivero & Segu 1989) as a suggestion that *P. vanadisae* could be a parthenogenetic species, as he dissected 104 specimens and all proved to be females. La Marca (1984) in the original description of *P. vanadisae*, mention three males vs. 26 females, additional evidence of biased sex ratio. Even if males are extremely rare, the fact that no call can be associated with *P. vanadisae* is apparently true; the senior author never has associated a call with any known population of *P. vanadisae* (La Macana, La Bravera, Monte Zerpa, La Mucuy). Our experience is different with *P. pleurostriatus*, of which the majority of the animals known are males (nine males, one female); and almost all were calling when captured.

*Pristimantis pleurostriatus* is not a common species, and its limited area of distribution suggests that it be categorized as VU B2ab(i,ii) under the IUCN criteria (Stuart et al. 2008). On the other hand, *P. conservatio* may be found in similar areas of cloud forest in the eastern versant of the Cordillera de Mérida, as there are no significant geographic barriers to suggest that its distribution should be so limited. We suggest classifying it as DD (data deficient).

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APPENDIX 1. Specimens examined.


APPENDIX 2. Additional specimens newly sequenced for phylogenetic analyses.

Pristimantis aff. briceni. CVULA 8352: Piñango village, 2400 m. CVULA 8353: Páramo de Piñango, 3400 m. Pristimantis briceni. CVULA 8364: trail from Monterrey to Cabaña del Cura, 2600 m, Sierra de la Culata, Cordillera de Mérida, Estado Mérida.