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Molecular and morphological data support recognition of a new genus of New World direct-developing frog (Anura: Terrarana) from an under-sampled region of South America

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Abstract

We describe a new genus of New World direct-developing frog (Terrarana) from the northern Andes of Venezuela and adjacent Colombia. *Tachiramantis* gen. nov. includes three species formerly placed in the large genus *Pristimantis*. Molecular phylogenetic analysis of data from five nuclear and mitochondrial genes shows that *Tachiramantis* is not part of *Pristimantis* or any other named genus in its family (Craugastoridae or Strabomantidae). Morphological evidence further supports the distinctiveness of *Tachiramantis*, which has several aspects of skull morphology that are rare or absent in *Pristimantis* and synapomorphic for *Tachiramantis*, including frontoparietal-prootic fusion and degree of vomer development. The terminal phalanges, which narrow greatly before expanding at the tips, may represent an additional morphological synapomorphy. One species, *T. prolixodiscus*, also displays a fenestra between the posterior portions of the frontoparietals, a character state otherwise present in only 1 of 98 other sampled terraranan species. We use mapped ranges of most New World direct-developing frogs to show that *Tachiramantis* occurs in a geographic region that had been under-sampled in previous molecular studies of New World direct-developing frogs. Other under-sampled regions are identified in western Peru, Colombia, and northern Central America; these regions should provide fruitful target taxa for future phylogenetic studies.

Key words: *Tachiramantis*, *Pristimantis*, Andes, Venezuela, Colombia, phylogeny, osteology

Resumen

Describimos un Nuevo género de ranas de desarrollo directo del Nuevo Mundo (Terrarana) de los Andes del norte en Venezuela y adyacente Colombia. *Tachiramantis* gen. nov. incluye tres especies hasta ahora contenidas en el amplio género *Pristimantis*. Análisis de filogenia molecular de cinco genes nucleares y mitocondriales muestra que *Tachiramantis* no forma parte de *Pristimantis* ni de otro género conocido en la familia (Craugastoridae o Strabomantidae). Evidencia morfológica reafirma la distinción de *Tachiramantis*, el cual posee algunos aspectos de la morfología craneal que son raros y ausentes en *Pristimantis* y sinapomórficos para *Tachiramantis*, incluida la fusión frontoparietal con el proótico y el nivel de desarrollo del vómer. Las falanges terminales, que se estrechan notablemente antes de expandirse en la punta, pueden representar una sinapomorfía adicional. Una especie, *T. prolixodiscus*, muestra también una fenestra entre la porción posterior de los frontoparietales, un carácter sólo presente en una de 98 especies de terraranas estudiadas. Usamos distribuciones de muchas especies de ranas de desarrollo directo del Nuevo Mundo para mostrar que *Tachiramantis* ocurre en una región geográfica que ha sido poco muestreada en previos estudios moleculares de este tipo de ranas. Otras áreas poco muestreadas son identificadas en el oeste de Perú, Colombia y el norte de Centroamérica; estas regiones podrían proveer taxones de interés para futuros estudios filogenéticos.

Palabras clave: *Tachiramantis*, *Pristimantis*, Andes, Venezuela, Colombia, filogenia, osteología

Introduction

In the past decade, resolution of frog evolutionary relationships has been greatly aided by the availability and application of molecular data to address systematic problems (e.g. Blackburn & Duellman 2013, Frost *et al.* 2006, Hertwig *et al.* 2012). These data have been especially useful in defining relationships among species in groups that are diverse and lack easily characterized morphological traits to identify monophyletic lineages. One such group is Terrarana, a diverse (1,009 species) assemblage of New World direct-developing frogs recognized as belonging to up to five families: Brachycephalidae, Ceuthomantidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae (Heinicke *et al.* 2009; AmphibiaWeb 2014). In more recent phylogenetic analyses using additional data, Strabomantidae is recovered as paraphyletic (Pyron & Wiens 2011; Padial *et al.* 2014). Classifications based on these analyses consequently merge Strabomantidae or Strabomantidae + Ceuthomantidae into Craugastoridae (Frost 2014). Until recently, most terraranan frogs were lumped into a single genus, *Eleutherodactylus*, on the basis of shared phalangeal structure; recognized subgenera and species groups within *Eleutherodactylus* were based on a combination of data from external morphology, allozyme electrophoresis, osteology, and soft anatomy (e.g. Lynch 1971, 1976, 1986a; Savage 1987; Hedges 1989), but no explicit phylogenetic hypothesis was available for the group (Lynch & Duellman 1997). Molecular phylogenetic analyses have subsequently demonstrated that the former “*Eleutherodactylus*” is not monophyletic, and is instead comprised of numerous genera that exhibit significant geographic segregation (Heinicke *et al.* 2007). The four largest genera—*Craugastor*, *Eleutherodactylus*, *Ischnocnema*, and *Pristimantis*—are the dominant terraranan lineages in Central America, the West Indies, southeastern Brazil, and northern South America, respectively (Hedges *et al.* 2008).

Ongoing phylogenetic work has demonstrated that a full understanding of relationships within Terrarana is yet to be achieved. Since the initial revisionary work removing most terraranan species from *Eleutherodactylus* (Heinicke *et al.* 2007), subsequent molecular and morphological data have been used to identify numerous other previously unrecognized lineages. Even with these advances, much work is still needed to obtain a fully-realized understanding the relationships among Terrarana species, for several reasons. Most notably, molecular data are available for only about 500 named species (half of the named species), phylogenetic inference may be affected by the taxon and character sampling, alignment methods and optimality criteria used (Padial *et al.* 2014), the exceptional diversity of species in the largest genera (*Craugastor*, *Eleutherodactylus*, and *Pristimantis*) hinders identification of systematically useful morphological characters, and continued discovery of new species at a pace of ~15 species/year (Frost 2014) makes estimation of a comprehensive phylogeny a moving target. Regardless of these challenges, there is a common pattern among recent notable systematic discoveries in Terrarana. Specifically, several new terraranan genera have been recognized for newly discovered lineages from geographic regions that harbor significant species diversity but are peripheral to the centers of terraranan mega-diversity (Greater Antilles, Central America, upper Amazon and adjacent central Andes), and were under-sampled in previous molecular analyses. These include new discoveries of genera in the tepui region of southern Venezuela and Guyana (*Ceuthomantis*; Heinicke *et al.*, 2009), the southern Andes of Bolivia (*Yunganastes*; Padial *et al.* 2007), and the Atlantic Forests of Brazil (“*Eleutherodactylus*” *bilineatus* Bokermann; Canedo & Haddad 2012).

With this pattern of discovery in mind, we hypothesize that geographic regions that have been under-represented in molecular phylogenetic analyses are most likely to harbor unrecognized genera. We have therefore analyzed the ranges of known Terrarana species to identify such regions, with the aim of providing a framework for targeted sampling of molecular data of representative species. One such region we identify as under-sampled, which we target in this study, is the Cordillera de Mérida, the Venezuelan extension of the Andes. This cordillera harbors 27 species of *Pristimantis*, for which any genetic data have only recently been published (Barrio-Amorós *et al.* 2012, 2013). Several of these *Pristimantis* species are morphologically distinct and have sometimes been placed in the genera *Mucubatrachus* and *Paramophrynella* (La Marca 2007). We obtained genetic samples of nine terraranan species from this region to test their phylogenetic affinities. Our results show that, while seven species are correctly placed in *Pristimantis*, including those species in the putative genera *Mucubatrachus* and *Paramophrynella* (Barrio-Amorós *et al.* 2013), at least two Venezuelan “*Pristimantis*” species, along with a third from neighboring Colombia—“*P.*” *prolixodiscus* (Lynch), “*P.*” *lentiginosus* (Rivero), and “*P.*” *douglasi* (Lynch)—represent one of the most genetically divergent groups within the family Craugastoridae, with morphological examination further supporting their distinctiveness; we therefore describe a new genus to accommodate these species.

Methods

Identification of under-sampled regions. Regions in which terraranan species have been under-sampled in previous molecular phylogenetic studies were identified using Quantum GIS 1.8 (QGIS Development Team 2014). Shapefiles representing the mapped ranges of 865 terraranan species were obtained from the IUCN (IUCN 2014); distributions of species not mapped by IUCN were consulted using the Amphibian Species of the World database (Frost 2014) to ensure there is no extensive regional bias in the distributions of unmapped terraranan species. For those 865 species with available mapped ranges, inclusion in previous molecular phylogenetic studies was confirmed and scored based on the presence of sequences in the NCBI Taxonomy database (Benson *et al.* 2009). After scoring, the ranges of all species were overlaid on a grid with each cell measuring 2.5 degrees latitude and longitude. The number of species present and the number of species with available DNA sequences were determined for each grid cell. We then performed a linear regression of these data (number of species with available DNA sequences vs. total number of species) in Minitab 16. Exceptionally well-sampled and under-sampled regions were identified as the five percent of cells with the most extreme standardized residual values in the regression analysis.

Molecular data collection and analyses. For all species in the study, we sequenced the complete mitochondrial 12S and 16S ribosomal RNA genes, along with the intervening tRNA-Val gene. Preliminary maximum likelihood (ML) phylogenetic analyses revealed that most sampled species from the Venezuelan Andes are embedded in *Pristimantis*; these sequences have been published elsewhere (GenBank Accession numbers JX155277–JX155297; Barrio-Amorós *et al.*, 2013). For the two species belonging to a new genus, we also sequenced portions of two nuclear exons: RAG1 and TYR. Genomic DNA was extracted from frozen tissue samples using a Qiagen DNeasy Blood and Tissue kit. Following DNA extraction, PCR was performed under the following reaction conditions: initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of 94 °C (30 seconds), 50 °C (30 seconds), 72 °C (60 seconds), and a final extension at 72 °C (7 minutes). PCR primers were used in the following combinations: 12L29E/12H46E, 12.2L4E/16H50, 12L34/16H48E, 16L19/16H36E, 16L34/16H47, R182/R270, and TyrFE/TyrRE (primer sequences are listed in Hedges *et al.* 2008). Amplified PCR products were isolated and purified from agarose gels using Millipore Ultrafree-DA gel filters. Sequencing of purified products was performed by the Pennsylvania State University Nucleic Acids Facility. Sequence editing and assembly was then performed using BioEdit (Hall, 1999).

The new sequences (GenBank Accession numbers KP297385–KP297390), along with sequences of *Ceuthomantis smaragdinus*, *Yunganastes fraudator*, and 20 species of *Pristimantis* from the Andes of Venezuela and adjacent Colombia were added to the 80-species, combined gene data matrix of Hedges *et al.* (2008). The assembled data set (Table 1) includes representatives of all genera of terraranan frogs for which at least 12S and 16S sequence data are available, including 43 representative *Pristimantis* species encompassing the breadth of phylogenetic diversity in the genus. Sequence data were aligned using MAFFT (Katoh *et al.* 2002) under default parameters.

Phylogenetic analyses were performed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). In ML and BI analyses, alignment gaps were treated as missing data rather than a fifth state, and polymorphic sites in the nuclear loci were considered as ambiguous bases. Alignment gaps were treated as a fifth state in the MP analysis. For the model-based ML and BI criteria, PartitionFinder (Lanfear *et al.* 2012) was used to identify best-fitting partition scheme and models of evolution for each locus under the Bayesian Information Criterion (BIC). Eight potential data blocks were considered (12S/tRNA-Val/16S stems, 12S/tRNA-Val/16S loops, and codon positions 1, 2, and 3 of the RAG-1 and TYR genes, respectively), and a greedy search scheme was employed. The MP analysis was performed using PAUP 4b10 (Swofford 2003), and used a series of 100 heuristic searches using tree bisection-reconnection branch swapping on random starting trees, with topological support based on a series of 1000 bootstrap replicates using the same search strategy (e.g., 100 heuristic searches per bootstrap). The ML analysis was performed using Garli 2.01 (Zwickl 2006). A series of 100 searches were performed to identify the best tree, with search termination thresholds and starting tree conditions maintained at default values. 1000 bootstrap replicates were used to assess topological support. In addition to the primary analysis, ML analyses were also performed on the three individual loci to ensure that there was no significant conflicting signal among the loci from coalescent processes. The Bayesian analysis was performed in MrBayes 3.2 (Ronquist *et al.* 2012). Two parallel runs were performed, with three heated and one cold chain, for 11 million

generations, with a sampling frequency of 1000 generations per sample. The first 1000 samples, representing one million generations, were discarded as burn-in. Standard deviations of split frequencies, potential scale reduction factors, and effective sample sizes as computed in Tracer 1.5 (Rambaut & Drummond 2007) were consulted to confirm that six million generations adequately sampled the posterior probability distribution.

TABLE 1. GenBank accession numbers of sequences used in phylogenetic analyses.

Species	GenBank Accession Number		
	12S/tRNA-Val/16S	RAG1	TYR
<i>Adelophryne gutturosa</i>	EU186679	EU186751	EU186772
<i>Agalychnis callidryas</i>	DQ283423	EF493362	DQ283018
<i>Barycholos pulcher</i>	EU186727/EU186709	EU186744	EU186765
<i>Brachycephalus ephippium</i>	AY326008/DQ283091	EU186761	DQ282917
<i>Bryophryne cophites</i>	EF493537	EF493423	EF493508
<i>Ceuthomantis smaragdinus</i>	EU186677	EU186750	EU186771
<i>Craugastor daryi</i>	EF493531	EF493452	EF493480
<i>Craugastor longirostris</i>	EF493395	EF493454	EF493482
<i>Craugastor montanus</i>	EF493530	EF493453	EF493478
<i>Craugastor podiciferus</i>	EF493360	EF493450	EF493481
<i>Craugastor pygmaeus</i>	EF493711	EF493451	EF493479
<i>Craugastor spatulatus</i>	EU186674	EU186749	EU186770
<i>Craugastor uno</i>	EU186673	EU186748	EU186769
<i>Diasporus diastema</i>	EU186682	EU186752	EU186773
<i>Eleutherodactylus abboti</i>	EF493540	EF493412	EF493457
<i>Eleutherodactylus albipes</i>	EF493386	EF493409	EF493475
<i>Eleutherodactylus alcoae</i>	EF493382	EF493406	EF493469
<i>Eleutherodactylus auriculatus</i>	EF493344	EF493417	EF493458
<i>Eleutherodactylus caribe</i>	EF493385	EF493411	EF493472
<i>Eleutherodactylus cooki</i>	EF493539	EF493413	EF493455
<i>Eleutherodactylus counouspeus</i>	EF493719	EU186760	
<i>Eleutherodactylus feichtingeri</i>	EF493345	EF493408	EF493467
<i>Eleutherodactylus gossei</i>	EF493716	EF493410	EF493466
<i>Eleutherodactylus griphus</i>	EF493381	EF493415	EF493465
<i>Eleutherodactylus inoptatus</i>	EF493380	EF493405	EF493463
<i>Eleutherodactylus lamprotes</i>	EF493379	EU186759	EF493460
<i>Eleutherodactylus leberi</i>	EF493342	EF493403	EF493459
<i>Eleutherodactylus lentus</i>	EF493717	EF493418	EF493471
<i>Eleutherodactylus leoncei</i>	EF493375/EF493715	EF493404	EF493468
<i>Eleutherodactylus luteolus</i>	EF493545	EU186757	EF493464
<i>Eleutherodactylus marnockii</i>	EF493820/EF493642	EF493399	EF493476
<i>Eleutherodactylus martinicensis</i>	EF493343	EF493419	EF493456
<i>Eleutherodactylus planirostris</i>	EF493346	EF493396	EF493470
<i>Eleutherodactylus richmondi</i>	EF493541	EU186758	EF493461
<i>Eleutherodactylus thorectes</i>	EF493384	EF493416	EF493473
<i>Eleutherodactylus unicolor</i>	EF493542	EF493398	EF493462

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TABLE 1. (Continued)

Species	GenBank Accession Number		
	12S/tRNA-Val/16S	RAG1	TYR
<i>Eleutherodactylus zeus</i>	EF493718	EF493402	EF493477
<i>Eleutherodactylus zugi</i>	EF493347	EF493401	EF493474
<i>Haddadus binotatus</i>	EF493361	EF493397	DQ282918
<i>Holoaden bradei</i>	EF493378/EF493366	EF493449	EU186779
<i>Holoaden leuderwaldi</i>	EU186728/EU186710	EU186747	EU186768
<i>Hypodactylus brunneus</i>	EF493357	EF493422	EF493484
<i>Hypodactylus dolops</i>	EF493394	EF493414	EF493483
<i>Hypodactylus peraccai</i>	EF493710	EF493420	EF493485
<i>Ischnocnema guentheri</i>	EF493533	EF493407	EF493510
<i>Ischnocnema parva</i>	EF493532	EF493400	EF493509
<i>Lithobates catesbeianus</i>	DQ283257	EF493448	DQ282959
<i>Litoria caerulea</i>	AY843692	EF493446	AY844131
<i>Lynchius flavomaculatus</i>	EU186667	EU186745	EU186766
<i>Noblella lochites</i>	EU186699	EU186756	EU186777
<i>Oreobates cruralis</i>	EU186666	EU186743	EU186764
<i>Oreobates saxatilis</i>	EU186726/EU186708	EU186742	EU186763
<i>Phrynopus bracki</i>	EF493709	EF493421	EF493507
<i>Phyzelaphryne miriame</i>	EU186689	EU186753	EU186774
<i>Pristimantis actites</i>	EF493696	EF493432	EF493494
<i>Pristimantis altamazonicus</i>	EF493670	EF493441	EU186778
<i>Pristimantis anolirex</i>	DQ195450		
<i>Pristimantis bipunctatus</i>	EF493702	EF493430	EF493492
<i>Pristimantis bricenei</i>	JX155297		
<i>Pristimantis carrangeuerorum</i>	DQ195453		
<i>Pristimantis charlottevillensis</i>	EU186650		
<i>Pristimantis colomai</i>	EF493354	EF493440	EF493502
<i>Pristimantis condor</i>	EF493701	EF493443	EF493504
<i>Pristimantis conservatio</i>	JX155287		
<i>Pristimantis conspicillatus</i>	EF493529	EF493437	EF493499
<i>Pristimantis cremnobates</i>	EF493528	EF493424	EF493486
<i>Pristimantis curtipes</i>	EF493513	EF493435	EF493497
<i>Pristimantis duellmani</i>	AY326003	EF493438	EF493500
<i>Pristimantis elegans</i>	DQ195457		
<i>Pristimantis euphronides</i>	EF493527	EF493427	EF493489
<i>Pristimantis gaigeae</i>	JN991514/FJ784461	JQ025184	JN991570
<i>Pristimantis galdi</i>	EU186670	EU186746	EU186767
<i>Pristimantis ginesi</i>	JX155295		
<i>Pristimantis jorgevelosai</i>	DQ195461		
<i>Pristimantis lancinii</i>	JX155294		
<i>Pristimantis leoni</i>	EF493684	EF493433	EF493495
<i>Pristimantis lutitus</i>	DQ195464		

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TABLE 1. (Continued)

Species	GenBank Accession Number		
	12S/tRNA-Val/16S	RAG1	TYR
<i>Pristimantis lynchi</i>	DQ195463		
<i>Pristimantis merostictus</i>	DQ195465		
<i>Pristimantis miyatai</i>	JN991518/JN991452		JN991573
<i>Pristimantis nervicus</i>	JN991522/JN991456	JQ025194	JN991577
<i>Pristimantis nyctophylax</i>	EF493526	EF493425	EF493487
<i>Pristimantis ockendeni</i>	EF493519	EF493434	EF493496
<i>Pristimantis paramerus</i>	JX155279		
<i>Pristimantis pleurostriatus</i>	JX155278		
<i>Pristimantis reichlei</i>	EF493707	EF493436	EF493498
<i>Pristimantis sagittulus</i>	EF493705	EF493439	EF493501
<i>Pristimantis stictogaster</i>	EF493704	EF493445	EF493506
<i>Pristimantis taeniatus</i>	JN991538/JN991470	JQ025208	JN991588
<i>Pristimantis thymelensis</i>	EF493516	EF493442	EF493503
<i>Pristimantis turpinorum</i>	EF493691	EF493429	EF493491
<i>Pristimantis unistrigatus</i>	EF493387	EF493444	EF493505
<i>Pristimantis urichi</i>	EF493699	EF493426	EF493488
<i>Pristimantis vanadise</i>	JX155296		
<i>Pristimantis versicolor</i>	EF493389	EF493431	EF493493
<i>Pristimantis walkeri</i>	EF493518	EF493428	EF493490
<i>Pristimantis w-nigrum</i>	AY326004		
<i>Psychrophrynella usurpator</i>	EF493714	EU186762	EU186780
<i>Psychrophrynella wettsteini</i>	EU186696	EU186755	EU186776
<i>Strabomantis anomalus</i>	EF493534	EF493447	
<i>Strabomantis biporcatus</i>	EU186691	EU186754	EU186775
<i>Tachiramantis douglasi</i>	DQ195456		
<i>Tachiramantis lentiginosus</i>	KP297386	KP297388	KP297390
<i>Tachiramantis prolixodiscus</i>	KP297385	KP297387	KP297389
<i>Yunganastes fraudator</i>	JF809938/FJ5390656	JF809916	JF809895

Morphological data collection and comparisons. In order to identify diagnostic morphological characters (i.e., synapomorphies) for the new genus, external observations were made in specimens of both “*P.*” *lentiginosus* (CVULA 9100, adult male) and “*P.*” *prolixodiscus* (CBA 7510, adult female; KU 132724, immature male; KU 132727, juvenile unsexed; KU 132728, adult male; KU 132729, adult male) under a Leica stereo-zoom microscope. External examination focused on characters that have proven diagnostic among other terraranan genera including the following: head shape, tympanum condition, presence of cranial crests, degree of development of dentigerous processes of vomers, presence and development of terminal digital discs, relative lengths of digits, presence of tubercles, presence of circumferential grooves, presence of webbing between digits, presence of dermal folds, and skin texture. Character states for these were compared against published definitions of other South American terraranan genera (Padial *et al.* 2008, Hedges *et al.* 2008, Heinicke *et al.* 2009) as well as *Pristimantis* from the Cordillera de Mérida (Barrio-Amorós 2012, Barrio-Amorós & Chacón-Ortiz, 2004, Barrio-Amorós *et al.* 2012, Barrio-Amorós *et al.* 2013, Cochran & Goin 1970, La Marca 2004, 2005, 2007, La Marca & Smith 1982, Lynch 1978, 1996, Rivero 1984), and museum specimens (see Appendix).

In addition, a high-resolution x-ray computed tomograph (CT) scan of “*P.*” *prolixodiscus* specimen KU132729 was made in order to obtain diagnostic osteological data. The scan was obtained using a GE phoenix v | tome | x

s240 CT scanner housed at the American Museum of Natural History Microscopy and Imaging Facility at voxel dimensions of 0.03 mm. Image slices were assembled for visualization using OsiriX software (Rosset *et al.* 2004). Comparisons were made with 98 other species of Terrarana based on images obtained from the literature, including one *Atopophrynus* (Myers & Ford 1986), one *Barycholos* (Lynch 1971), two *Brachycephalus* (Alves *et al.* 2006), one *Bryophryne* (Lynch 1975a), one *Ceuthomantis* (Heinicke *et al.* 2009), five *Craugastor* (Lynch 1971, Lynch 2000), one *Diasporus* (Lynch 1971), one *Dischidodactylus* (Lynch 1979a), 14 *Eleutherodactylus* (Díaz *et al.* 2001, 2003, 2005, Heinicke *et al.* 2009, Lynch 1971), one *Euparkerella* (Lynch 1971), one *Haddadus* (Heinicke *et al.* 2009), one *Holoaden* (Lynch 1971), one *Hypodactylus* (Cannatella 1984), one *Ischnocnema* (Heinicke *et al.* 2009), three *Lynchius* (Lynch 1975a, Cannatella 1984), two *Niceforonia* (Lynch 1968, 1971), two *Noblella* (Lynch 1975a, 1986b), one *Oreobates* (Lynch 1971), two *Phrynopus* (Lynch 1986b, Trueb & Lehr 2008), 45 *Pristimantis* (La Marca 1984; Lynch 1968, 1971, 1979b, 1992, 1995, Lynch & Ruiz-Carranza 1985, Lynch & Trueb 1980, Lynch *et al.* 1994, Guayasamin 2004a, b, Avilán & Hoyos 2006, Heinicke *et al.* 2009, Flores & Vigle 1994, Teran-Valdez & Guayasamin 2010, Wiens & Coloma 1992, Flores & Rodríguez 1997), one “*Pristimantis*” (Lynch 1996), nine *Strabomantis* (Lynch 1975b, 1997), and one *Yunganastes* (De la Riva & Lynch 1997). A total of 17 skull characters showing variation within the sample were scored, including (states given in parentheses) skull width/length ratio (wider than long/equal/longer than wide), rostrum length (short/moderate/long), presence and position of frontoparietal fontanelle (posterior/anterior/narrow opening along entire length/absent), frontoparietal/prootic fusion (fused/not fused), nasal size (large/medium/small), nasal contact (narrow separation/wide separation/contact), overlap of sphenethmoid with nasals and frontoparietal (sphenethmoid overlaps both/overlaps nasals only/overlaps frontoparietals only/overlaps neither), premaxillary tooth number (actual count), vomerine tooth development (few/many/none), vomer size (nearly surround choanae/half surround choanae/poorly developed), palatine size (narrow/wide), shape of zygomatic ramus of squamosal (pointed + long/blunt + long/pointed + short/blunt + short), angle of alary process of parasphenoid (oblique angle/right angle), width of parasphenoid (wide/narrow), presence of anterior narrowing of parasphenoid (present/absent), length of parasphenoid (reaches palatines/shorter than palatines/longer than palatines), and degree of maxilla/nasal contact (narrow separation/wide separation/absent). Skull length was measured from the occipital condyle to the anterior edge of premaxilla, while width was measured at the quadratojugal/maxilla articulation. The final character matrix is available at MorphoBank (<http://morphobank.org>, project 2089).

Results

Regional sampling of Terraranan species. When a 2.5 degree grid is overlaid on the composite distribution of terraranan species, a total of 287 grid cells overlap the range of at least one terraranan species (Fig. 1). Individual grid cells overlap the ranges of as many as 109 species (80°–77.5° W, 2.5°–0° S; central Ecuador), but far fewer species occur throughout most of the range of Terrarana (mean total species per grid cell = 9.4; median = 4). The number of species per grid cell sampled in previous phylogenetic analyses is similarly variable, ranging from zero to the 70 species sampled in central Ecuador (mean species sampled per cell = 6.6, median = 3). There is a strong linear relationship between the two values ($R^2 = .88$, $p = 0.000$). Based on our regression analysis, the geographic areas that have been most thoroughly sampled in previous molecular phylogenetic analyses of Terrarana (those with the most positive standardized residual values) are concentrated in two regions: the Greater Antilles and southern Mesoamerica (Fig. 1). The grid cell encompassing the Cordillera de Mérida of Venezuela (72.5°–70° W, 7.5°–10° N), which we targeted for additional sampling, is identified among the under-sampled regions, with only 3 of 26 mapped species appearing in phylogenetic analyses prior to this study and our 2013 study (Barrio-Amorós *et al.* 2013). Most other under-sampled regions are also distributed along the Andes of South America, including the Sierra Nevada de Santa Marta in northern Colombia, the western and northwestern Andes of Colombia, and northwestern and southwestern areas of Peru. The only significantly under-sampled region outside the Andes is a northern Mesoamerican region including parts of Belize, Guatemala, and Honduras.

Phylogeny. The final aligned data set measures 4,043 bp, including 2,912 bp of 12S/tRNA-Val/16S data, 638 bp of RAG1, and 493 bp of TYR. A total of 2,772 sites are variable and 2,334 sites are parsimony informative. The best partitioning scheme under the BIC divided the data set into four partitions: 12S/tRNA-Val/16S stems, 12S/tRNA-Val/16S loops, RAG1/TYR codon positions 1 and 2, and RAG1/TYR codon position 3. Best-fitting models

were identified as SYM + I + G, GTR + I + G, TVM + I + G, and K81 + G, respectively, and were implemented in the ML and BI analyses. The Bayesian analysis reached convergence, as evidenced by effective sample size values (>3600 for all parameters), potential scale reduction factors (=1.000 for all parameters), and average standard deviation of split frequencies (≤ 0.003). Preliminary single-locus analyses revealed no significant conflicting signal among the loci: all genera represented by multiple taxa were recovered as monophyletic in the single-locus analyses, although relationships among genera vary (see below).

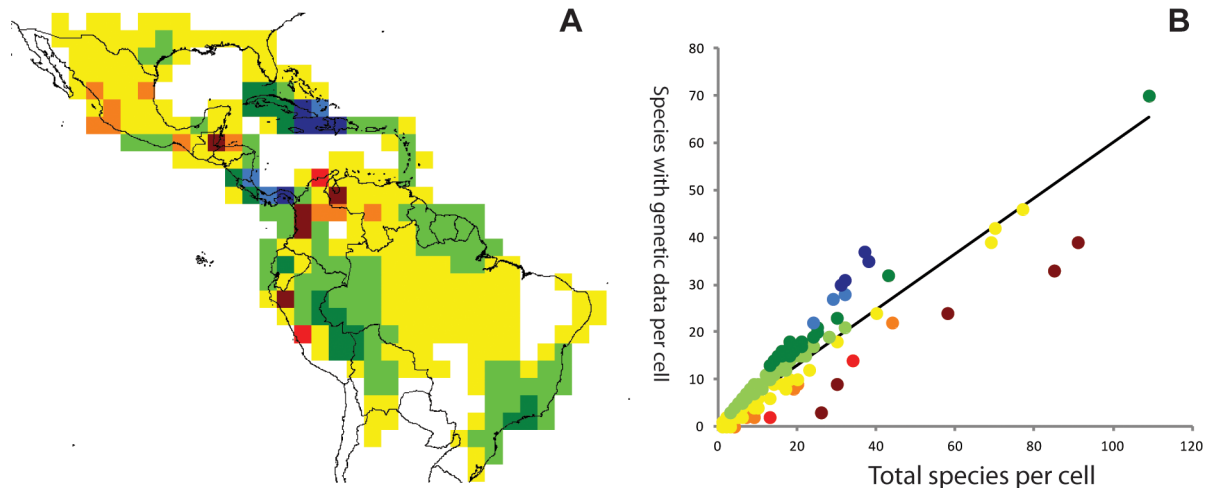


FIGURE 1. (A) 2.5 degree grid cell map of terraranan distribution, depicting degree of sampling in each grid cell as standardized residuals of a linear regression of the number of species with available DNA sequences vs. total number of species. (B) Scatterplot of number of species with available DNA sequences vs. total number of species for all 287 grid cells.

The phylogenies estimated in the combined ML and BI analyses (Fig. 2) are very similar to that presented by Hedges *et al.* (2008), as would be expected based on the largely overlapping data sets. All families as recognized by Hedges *et al.* (2008) and Heinicke *et al.* (2009) were recovered as monophyletic, in contrast to recent analyses (Pyron & Wiens 2011; Padial *et al.* 2014) finding Craugastoridae and Strabomantidae *sensu* Heinicke *et al.* (2009) to not be reciprocally monophyletic. We note, however, that the data set and analyses in this study were not designed to specifically address alternate family-level taxonomies for Terrarana, so we treat Strabomantidae as a synonym of Craugastoridae here to agree with the more recently-proposed taxonomies based on larger data sets (Frost 2014), although we do not preclude recognition of Strabomantidae in the future should new data support such an action. “*Pristimantis*” *prolixodiscus*, “*P.*” *lentiginosus*, and “*P.*” *douglasi* are embedded within Craugastoridae, with moderate support (BI/ML support values 1.0/63). In both phylogenies, these species are most closely related to genera in the subfamily Holoadeninae, but with poor support. While the exact phylogenetic position of these species within Craugastoridae is therefore ambiguous, there is strong evidence in the phylogeny indicating they are not part of *Pristimantis*. Specifically, *Pristimantis* and *Yunganastes* receive significant support as sister taxa (Bayesian/ML support values 1.0/99), and these genera in turn receive moderate support for being most closely related to *Oreobates*, *Lynchius*, and *Phrynopus* (support values 1.0/70). Other groupings of genera receiving strong support in both the BI and ML analyses include the grouping of *Oreobates*, *Lynchius*, and *Phrynopus* (1.0/100), a grouping of *Psychrophrynella* + *Holoaden* + *Bryophryne* + *Noblella* + *Barycholos* (1.0/96), and *Adelophryne* + *Phyzelaphryne* + *Diasporus* + *Eleutherodactylus* (1.0/99).

The MP phylogeny and single-locus ML phylogenies differ somewhat from the phylogeny recovered in the combined BI and ML analyses. The MP analysis identified nine equally-parsimonious trees (score = 31841), differing from one another mainly in the branching pattern within *Pristimantis*. Branches receiving strong support in the combined BI and ML analysis receive at least moderate support in the MP analysis (Fig. 2), while those receiving poor support in the ML analysis often do not appear in the MP trees. Most significantly, *Haddadus* is recovered as sister to *Brachycephalus* instead of *Craugastor* in the MP analysis (bootstrap value = 64), *Ceuthomantis* falls outside the remainder of Terrarana + the hylid outgroups (bootstrap value = 79), and “*Pristimantis*” *prolixodiscus*, “*P.*” *lentiginosus*, and “*P.*” *douglasi* are recovered as most closely related to *Craugastor*, with poor support (bootstrap value = 10).

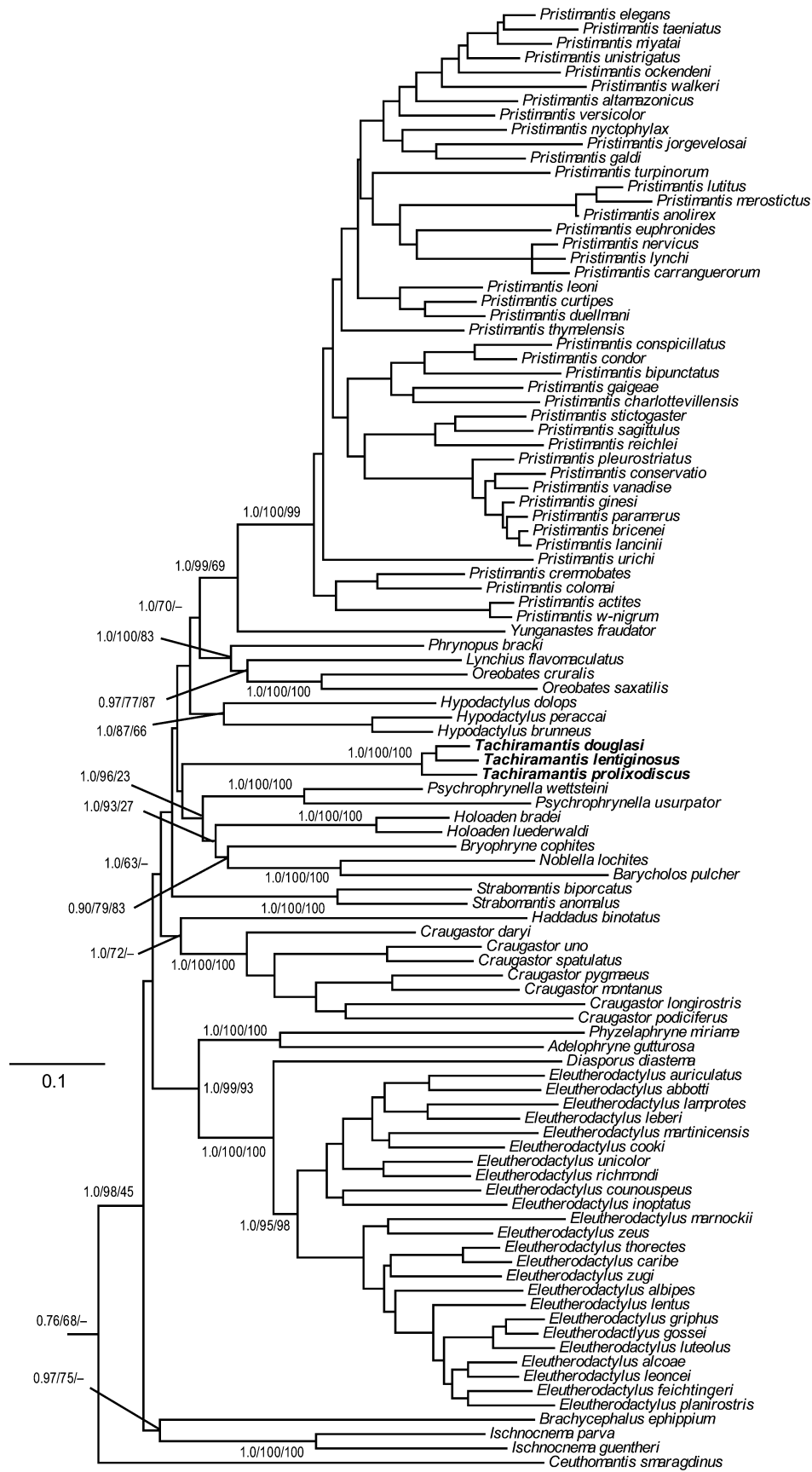


FIGURE 2. Maximum likelihood phylogeny of Terrarana, based on analysis of the genes 12S, tRNA-Val, 16S, RAG1, and TYR. Support values (Bayesian posterior probabilities/ML bootstrap support/MP bootstrap support) are indicated for nodes bearing on intergeneric relationships (i.e., at or above the genus level) with ML bootstrap support $\geq 50\%$.

The same pattern observed in the MP analysis occurs for *Ceuthomantis* in the single-locus ML 12S/tRNA-Val/16S and TYR analyses, but not the single-locus RAG1 analysis, in which it is part of a monophyletic Terrarana. *Haddadus* is sister to *Craugastor* only in the single-locus TYR analysis, otherwise grouping with *Brachycephalus* (12S/tRNA-Val/16S) or a large set of craugastorid genera (RAG1). The position of the three “*Pristimantis*” species also varies by locus: 12S/tRNA-Val/16S data alone group these species with *Ischnocnema*, RAG1 data place them as most closely related to a large set of craugastorid + brachycephalid genera, and TYR data group them with *Craugastor* + *Haddadus*. In contrast, intergeneric relationships receiving strong support in the combined BI and ML analyses typically occur in all three single-locus analyses. Of most importance, all single-locus analyses recover most *Pristimantis* + *Yunganastes* to be most closely related to one another, to the exclusion of the three “*Pristimantis*” species.

In sum, all phylogenetic analyses we performed show that “*Pristimantis*” *prolixodiscus*, “*P.*” *lentiginosus*, and “*P.*” *douglasi* are not closely related to other species included in *Pristimantis*. As we ascribe to a philosophy that named taxa should be monophyletic (Vences *et al.* 2013), we are compelled to restore the monophyly of *Pristimantis* and describe a new genus to accommodate these three species. Osteological evidence provides further support for this decision (see below).

Systematics

Tachiramantis gen. nov.

(Figs. 3–7)

Type species. *Eleutherodactylus prolixodiscus* Lynch, 1978 (Holotype: KU 132726)

Diagnosis. Species of *Tachiramantis* are moderately small craugastorid frogs (SVL 20–30 mm). The head is wider than long, with a moderately pointed snout; cranial crests absent; canthus rostralis well-defined; tympanum well-defined and large, approximately 2/5 to 3/4 diameter of eye; dentigerous processes of vomers well-developed, arranged obliquely, with few teeth. Overall body form is moderately robust, with limbs of moderate length; skin on the dorsum varies from smooth to finely granular, that on the venter is smooth to areolate or granular; dorsolateral folds are poorly-developed or absent; discoidal folds are present or absent; anal sheath or cloacal folds are absent. Males have vocal slits but only some species have an external vocal sac; nuptial pads are present in at least some species. Digits bear pads which are moderately dilated; digital fringes narrow or absent; circumferential grooves present; webbing is absent. Finger I is shorter than Finger II, Finger IV is much shorter than Finger III, and Toe III is shorter than Toe V. Tarsal folds present or absent; the inner metatarsal tubercle is larger than the outer, metacarpal tubercles are small. The color pattern is variable but usually includes white spots on the posterior surfaces of the thighs. In skull, the frontoparietals are fused to the prootics; a large fontanelle exists between posterior (not anterior) portions of frontoparietals in at least one species; vomers are strongly-developed and nearly surround choanae; the parasphenoid is broad, does not narrow anteriorly, reaches palatines, and has alary processes angled slightly backward obliquely. There are eight presacral vertebrae; no vertebrae are fused and neural spines are absent. Terminal phalanges are conical with minute, delicate T-shaped tips; intercalary elements on digits are absent.

Content. *Tachiramantis prolixodiscus* (Figs. 3–7), *T. douglasi* (Lynch), *T. lentiginosus* (Fig. 3).

Distribution. Known from both sides of the Táchira depression in the Cordillera de Mérida of Venezuela and adjacent portions of the Cordillera Oriental in Colombia (Fig. 8). *Tachiramantis prolixodiscus* has also been reported from the Sierra de Perijá along the Venezuela-Colombia border (Barrio-Amorós *et al.*, 2010).

Etymology. *Tachira* refers to the Táchira depression, which lies between the Andean ranges in which all referred species occur. The word Táchira is ultimately derived from the Timoto-Cuica word *tachure*, which refers to a medicinal plant that grows in this region. The word *mantis*, from Greek, means treefrog. The name is masculine.

Remarks. In external morphology *Tachiramantis* can be readily distinguished from most craugastorid genera (Hedges *et al.* 2008) by the combination of having a broad head, well-developed tympanum, and expanded digital tips with pads separated by circumferential grooves. Among craugastorid genera sharing these characteristics,

Noblella differs from *Tachiramantis* in lacking dentigerous processes of the vomers and having terminal phalanges minimally expanded, and the genera *Yunganastes* and *Strabomantis* differ from *Tachiramantis* in having Finger I longer than Finger II and Toe III longer than Toe V. In addition, *Strabomantis* species are typically large (SVL 30–106 mm), heavy-bodied, and have cranial crests.

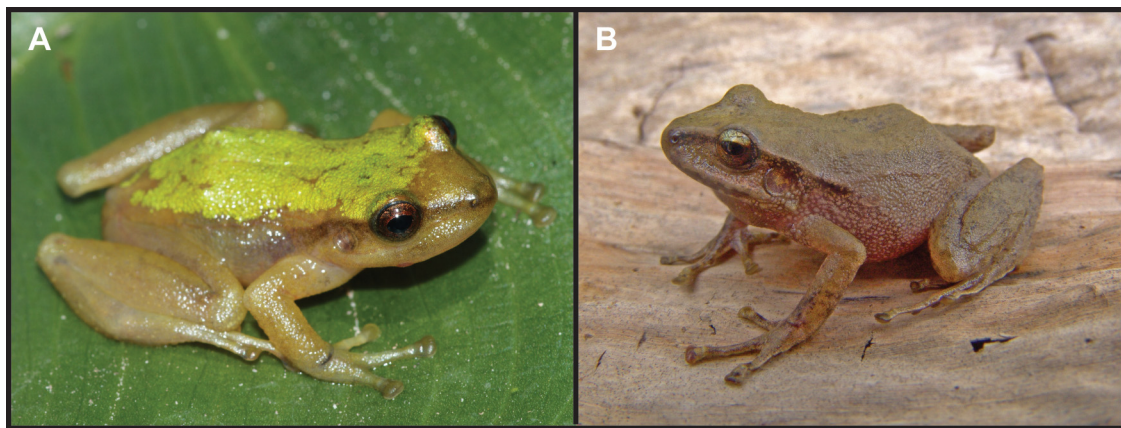


FIGURE 3. *Tachiramantis* species in life. (A) *Tachiramantis prolixodiscus* specimen CBA 7510, adult female from Calderas, Barinas, Venezuela. (B) *Tachiramantis lentiginosus* specimen CVULA 9100, adult male from Guaraque, Mérida, Venezuela.



FIGURE 4. *Tachiramantis prolixodiscus* specimen KU 132729, adult male paratype of the type species of *Tachiramantis*.

As might be expected given the constituent species were previously placed in *Pristimantis*, there are no obvious external characteristics that universally distinguish *Tachiramantis* from *Pristimantis*. However, compared to the ~25 recognized *Pristimantis* occurring in adjacent regions of Venezuela and Colombia, *Tachiramantis* does differ from most in having finger IV much shorter than (about 2/3 the length of) finger III. The lack of well-developed dorsolateral or pre-cloacal folds, lack of cranial crests, presence of vocal slits, and lack of extremely expanded digital discs distinguishes *Tachiramantis* from most other *Pristimantis* in adjacent Venezuela and Colombia except for *P. melanoproctus* (Rivero), *P. monodolphi* (Rivero), *P. nicefori*, and *P. tubernasus*.

Osteological characters more readily distinguish *Tachiramantis* from *Pristimantis* and other terraranan genera (Figs. 5–7; see morphobank project for data matrix). Most notably, investigation of the osteology of *T. prolixodiscus* revealed a suite of characters that are rare or absent in *Pristimantis*: presence of a posterior frontoparietal fontanelle (Fig. 5A), frontoparietal-prootic fusion (Fig. 5A), parasphenoid shape (broad, does not narrow anteriorly, and reaches forward to level of palatines; Fig. 5B), and vomers that nearly surround the choanae (Fig. 5B). The large fontanelle between the posterior portions of the frontoparietals was not otherwise observed in a sampling of 45 *Pristimantis* species and only occurred in one of 53 other sampled terraranans (*Elutherodactylus intermedius*). In other terraranans characterized by the presence of a frontoparietal fontanelle, such as species in the *Pristimantis orcesi* group (Guayasamin 2004b), the opening is between the anterior portions of the frontoparietals. A brief description of the cranial osteology of *Eleutherodactylus chlorosoma* (= *T. prolixodiscus*) by Rivero (1984) states that there is no frontoparietal fontanelle. However, this statement apparently only refers to a typical anterior

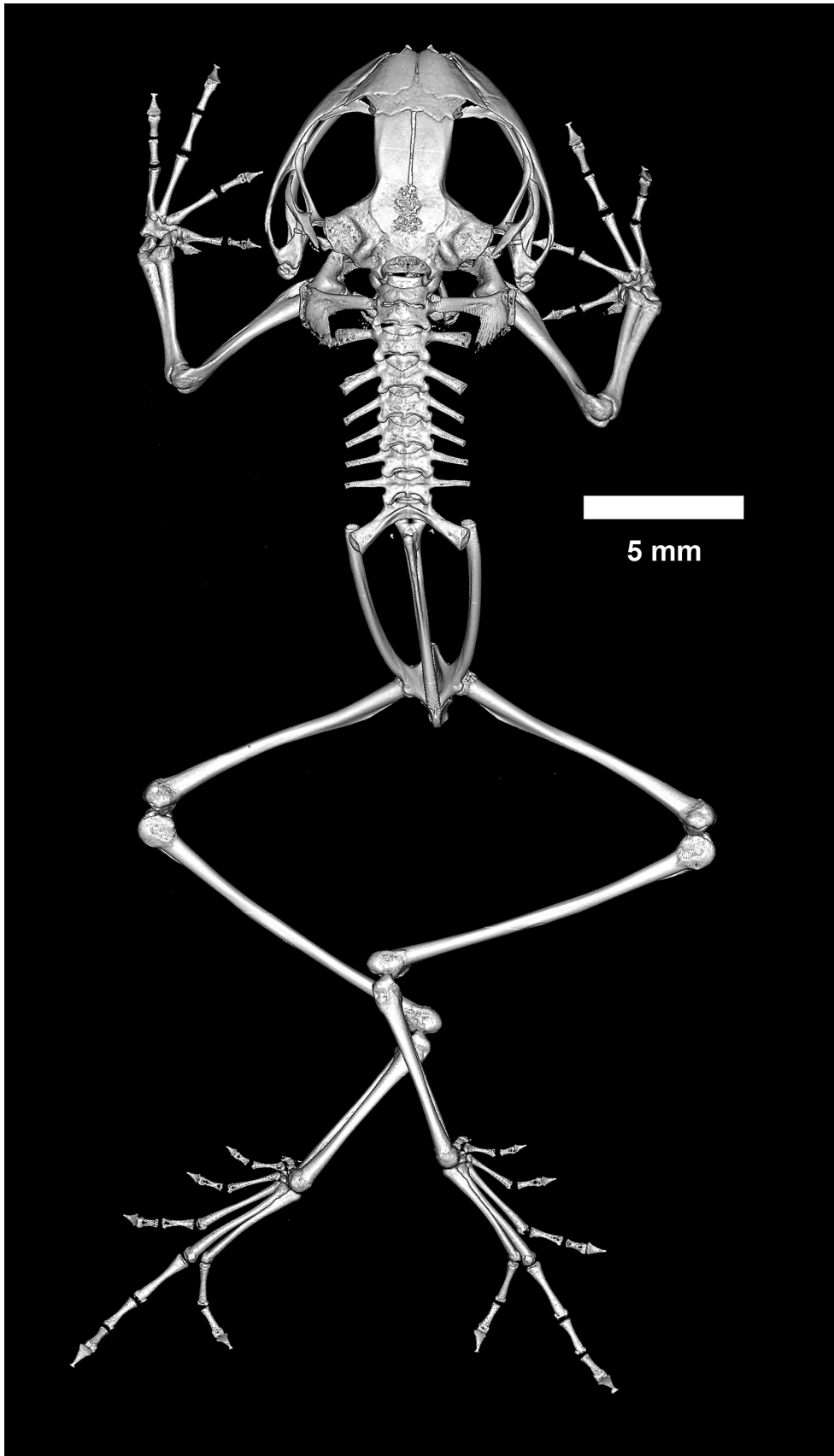


FIGURE 5. High resolution X-ray computed tomography of *Tachiramantis prolixodiscus* (KU 132729, adult male); dorsal view.

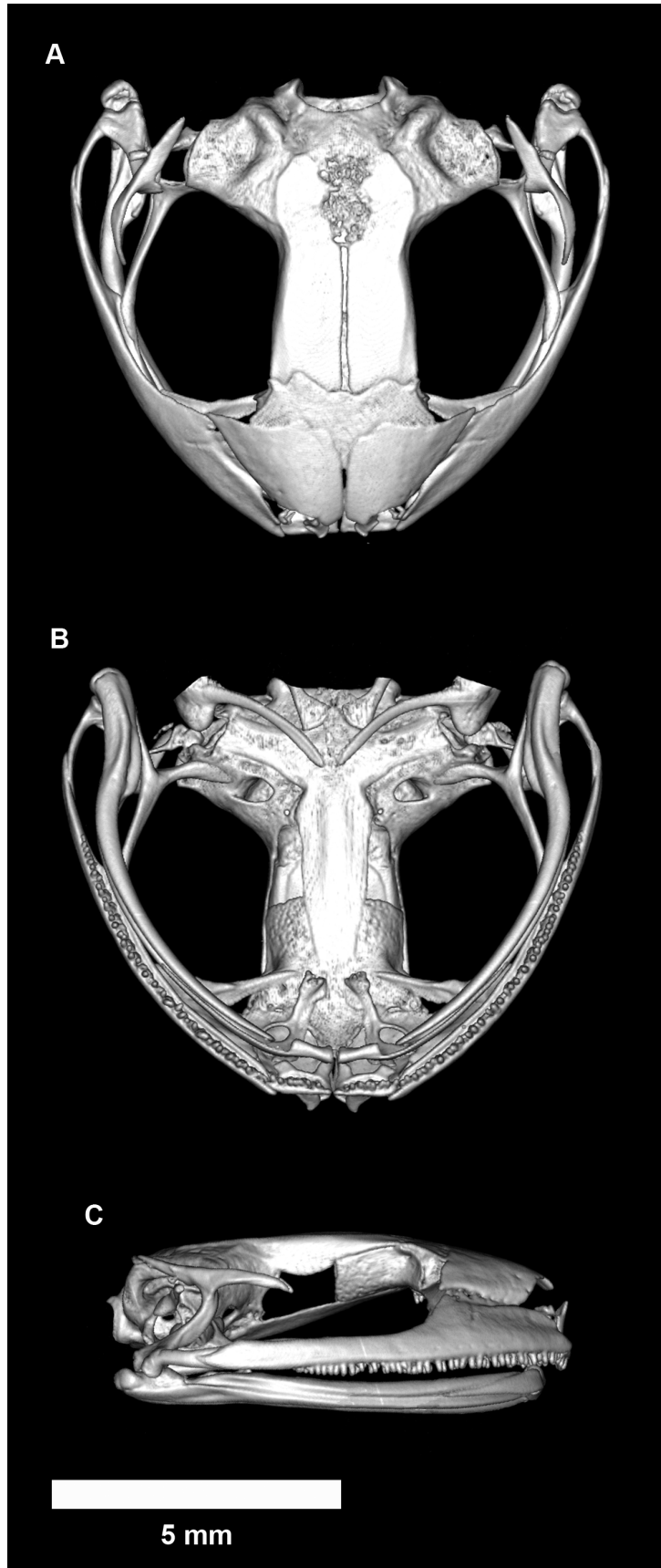


FIGURE 6. High resolution X-ray computed tomography of skull of *Tachiramantis prolixodiscus* (KU 132729, adult male); (A) dorsal view, (B) ventral view, (C) side view.

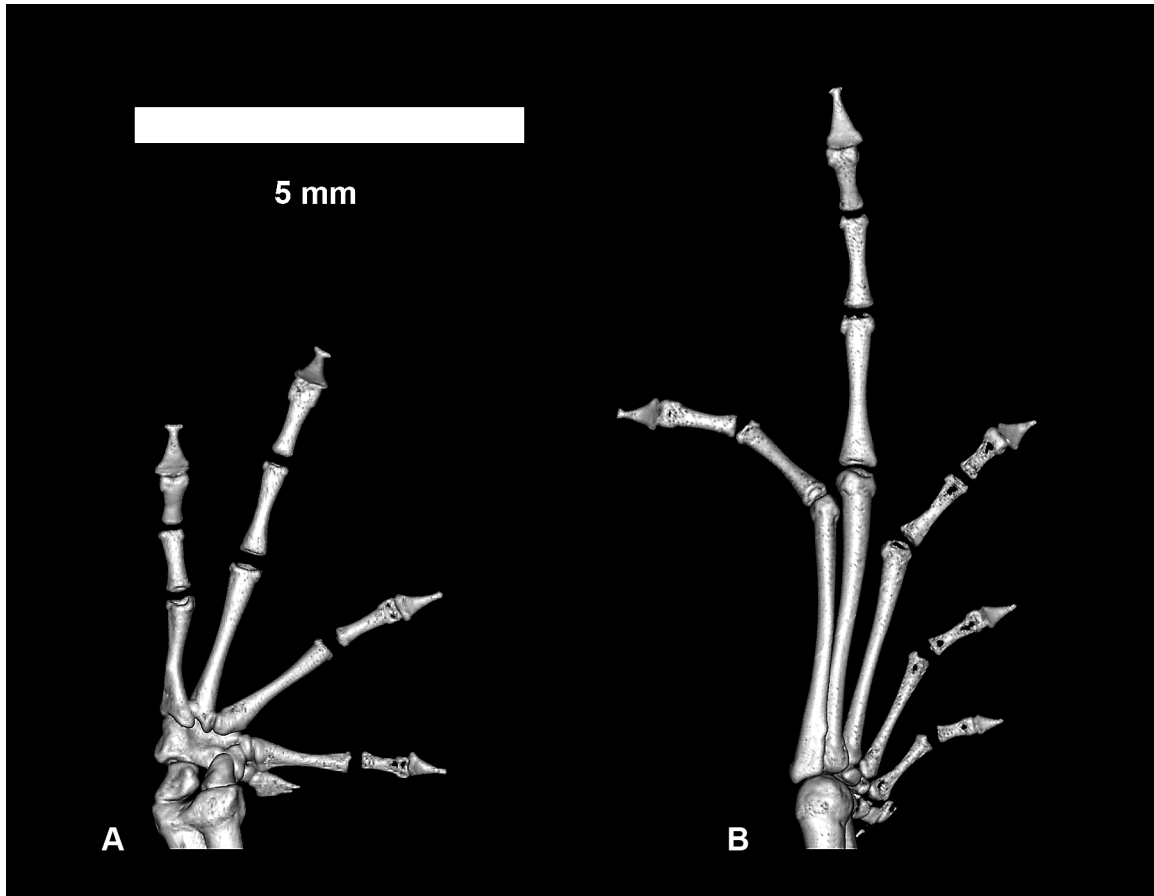


FIGURE 7. High resolution X-ray computed tomography of digits of *Tachiramantis prolixodiscus* (KU 132729, adult male); (A) manus, (B) pes.

opening: the same description states that the rear of the skull is shrunken (“apergaminado”), exposing parts of the telencephalon and optic lobes of the brain, the same region of the brain left uncovered by the fontanelle we observe in specimen KU 132729.

The other three skull characters listed in the preceding paragraph are all shared with *Tachiramantis douglasi*, which occurs sympatrically with *T. prolixodiscus* in Colombia. Vomeres that nearly surround the choanae were not otherwise documented in *Pristimantis*, and among all Terrarana were observed in only three species of *Eleutherodactylus* (subgenus *Euhayas*). The parasphenoid shape shared by *T. prolixodiscus* and *T. douglasi* is found in only one sampled *Pristimantis*: *P. tayrona*. This species from the Sierra Nevada de Santa Marta is externally similar to and was confused with *T. prolixodiscus* until being named by Lynch and Ruiz-Carranza (1985), but given differences in vomer shape and lack of frontoparietal/prootic fusion, we refrain from referring *P. tayrona* to *Tachiramantis* at this time. Fusion of the frontoparietal and prootics occurs in *Pristimantis*, but more rarely than in other terraranan genera, as only seven of 45 *Pristimantis* show this condition. In combination, these three skull characters distinguish *Tachiramantis* from all other sampled terraranans. The terminal phalanges of *T. prolixodiscus* also differ from the typical T-shaped condition described for most *Pristimantis* and other terraranans: rather than narrowing gradually from the proximal to distal end before broadening at the terminal expansions, the phalanges narrow dramatically before broadening at the delicate terminal expansions, such that the overall width of the terminal expansions of each phalanx is less than the width of the base of the phalanx (Fig. 7). Assuming the genus-level phylogeny of Terrarana presented in Fig. 2, fusion of the frontoparietals to the prootics and vomeres that nearly surround the choanae are unambiguous osteological synapomorphies for *Tachiramantis*. The parasphenoid shape characters are not synapomorphic of *Tachiramantis*, although they do serve to distinguish the genus from *Pristimantis*. The shape of the terminal phalanges may represent an additional osteological synapomorphy, but requires additional investigation for confirmation. None of the examined external morphological characters represent unambiguous synapomorphies.

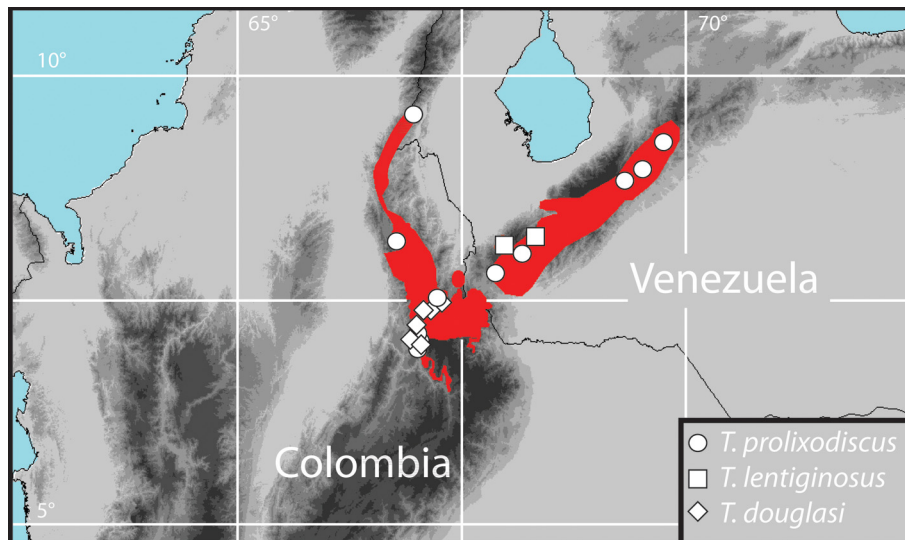


FIGURE 8. Distribution of *Tachiramantis* in the Cordillera de Mérida of Venezuela and the Cordillera Oriental of Colombia. The range gap corresponds to the relatively low-elevation Táchira Depression. Known point localities of each species are indicated, based on specimens listed in the Appendix, as well as those listed in Rivero (1984), and those with data on GBIF (www.gbif.org).

Several other *Pristimantis* species, in addition to *P. tayrona*, have at one time or another also been considered closely related to species we refer to *Tachiramantis*. Rivero (1984) suggested a close relationship between *T. prolixodiscus* (includes synonym *Eleutherodactylus chlorosoma* Rivero) and the externally similar, sympatric species *P. tubernasus* (Rivero) (includes synonym *Eleutherodactylus pulidoi* Rivero). A close relationship between these two species was argued against by Lynch (2003), who considered their similar morphologies to be adaptations for living in bromeliads, and justified treating them as not closely related based on the two species having differently-shaped tympani. Rivero (1984) also recognized a *lentiginosus* complex, including the species *P. melanoproctus* (Rivero), *P. mondolfii* (Rivero), and *P. vanadise* (La Marca), based on all four species having a color pattern that includes white spots on the rear of the thighs (*T. douglasi* also shares this pattern); subsequent genetic and morphological data show that *P. vanadise*, at least, is not closely related to *Tachiramantis* (Barrio-Amorós *et al.* 2013). While we have no genetic or skeletal data for *melanoproctus* or *mondolfii*, they bear a striking external resemblance to *T. lentiginosus*. We suspect both species are referable to *Tachiramantis*, but refrain from making this change until molecular or osteological data become available.

Osteology of *Tachiramantis prolixodiscus*. The skull of *T. prolixodiscus* (Fig. 6) is broad, with the length of the skull only 86% the greatest width of the skull. The skull narrows quite gradually anteriorly, with the head width at the level of the palatines being 91% the width at the level of jaw articulation. The rostrum is short (27% of skull length), which, in combination with the skull's width, gives an overall blunt appearance.

The braincase combines well- and poorly ossified elements. The anterior portion of the braincase is fully enclosed by the sphenethmoid, which broadly overlaps the frontoparietals and nasals dorsally and the parasphenoid ventrally, but only extends posteriorly to approximately the level of the midpoint of the orbit, resulting in enlarged optic fenestrae. The posterior portion of the braincase is also fully enclosed, by the fused prootics and exoccipitals. These, in turn, are fused to the frontoparietals. The frontoparietals are relatively large, and narrowly separated anteriorly; there is a broad, non-ossified fontanelle between the posterior third of the frontoparietals, leaving the optic tectum of the brain exposed. At the floor of the braincase, the parasphenoid is large and quite broad. Anteriorly, it does not narrow appreciably and reaches to the level of the palatines. Posteriorly, the alary processes are well-developed, and directed somewhat posteriorly at an oblique angle.

The nasals are large, nearly triangular in shape, and narrowly separated from one another and from the robust pars facialis of the maxillae. The maxillae broadly articulate with the quadratojugal, and bear large, peglike teeth. The premaxillae bear a small number (7) of similarly-shaped teeth; vomerine teeth are also few in number. The vomers themselves are large with broad pre- and post-choanal processes that combine to almost fully enclose the choanae. In contrast, the dentigerous processes of the vomers are relatively narrow, though elongate. The palatines

are long, narrow, and pointed, and angled posteriorly, terminating adjacent to the dentigerous processes of the vomers. The pterygoids are triradiate, each with a long, thin anterior ramus articulating with the maxilla just anterior to the level of the midpoint of the orbit, a short, stout medial ramus broadly articulating with the prootic, and a more gracile, short posterior ramus articulating with the squamosal. Each squamosal is itself robust, with a long, narrow, pointed zygomatic ramus, and a similarly-shaped otic ramus that deflects medially but remains quite distant of the prootic. In the mandible, the angulosplenials are very well-developed and sigmoidal in shape, articulating broadly with the relatively small dentaries anterior of the level of the orbits and are only narrowly separated from the mentomeckelian bones. The only ossified portions of the hyoid apparatus are the two posteromedial processes, which are slightly expanded anteriorly and posteriorly and are moderately separated from one another at their anterior ends.

There are eight presacral vertebrae, none of which are fused. All presacral vertebrae save the atlas have well-developed transverse processes; those on vertebrae 4–6 are deflected posteriorly. Transverse processes on vertebrae 4–8 are similar in size, with those on vertebra 3 being larger and those on vertebra 2 being smaller. Neural spines are absent. The sacral diapophyses are relatively narrow and are uniform in width. The sacrum has a bicondylar articulation with the urostyle, which is 89% of the length of the presacral vertebral column and extends posteriorly to the ischium. In the pectoral girdle, the clavicles are long and curved, moderately separated from one another medially. Coracoids are stout and hourglass-shaped, with the sternal ends more broadly expanded than the scapular end. The scapulae are narrow, while each cleithrum is long and very slender, broadly articulating with a partially mineralized suprascapula. Ossification of the suprascapula is concentrated in the areas of articulation with the scapula and cleithrum, resulting in an “L” shaped appearance of the suprascapula + cleithrum. There is no mineralized sternum or omosternum. The remainder of the appendicular skeleton is well-ossified, with the exception of some phalanges, which apparently have remained partially cartilaginous. The phalangeal formulae are 2/2/3/3 (manus) and 2/2/3/4/3 (pes); relative digital lengths are IV>V>III>II (manus; frogs do not have digit I) and IV>V>III>II>I (pes). Terminal phalanges are conical in shape, each narrowing to a minute terminus with delicate T-shaped expansions that do not reach the width of the base of the terminal phalanges.

Discussion

The discovery of *Tachiramantis* highlights the still-evolving nature of our understanding of evolutionary relationships among species of Terrarana. *Tachiramantis* is the fourth new terraranan genus to be documented subsequent to the revision of Hedges *et al.* (2008). In addition, a number of other species have been re-classified based on new genetic evidence, including species being moved to/from *Oreobates* (Padiál *et al.* 2012), *Haddadus aramunha* Cassimiro, Verdade & Rodrigues being moved from *Strabomantis* (Amaro *et al.* 2013), *Mucubatrachus* and *Paramophrynella* being synonymized with *Pristimantis* (Barrio-Amorós *et al.* 2013), and several *Pristimantis* removed from *Ischnocnema* (Canedo & Haddad 2012). All of these taxa occur in regions that were poorly-sampled in the phylogenetic analysis of Hedges *et al.* (2008), and as demonstrated by this study, we expect that new higher-level taxonomic discoveries will occur in regions that remain poorly-sampled. Colombia in particular seems to hold promise in this regard. That is not to say that systematic work should be ignored in better-sampled regions. Even in the Greater Antilles, which has historically been the most intensively collected and studied area inhabited by terraranans, new species continue to be discovered (e.g., *Eleutherodactylus juanariveroi* Rios-López and Thomas, *E. feichtingeri* Díaz, Hedges, and Schmid). Population-level studies, many in areas that could be considered well-sampled, have also documented numerous cryptic species that await formal naming (Wang *et al.* 2008, Rodríguez *et al.* 2010, Heinicke *et al.* 2011, Fouquet *et al.* 2012, Streicher *et al.* 2014). Thus, while continuous research from multiple groups has provided a general framework of evolutionary relationships within Terrarana, there is still much refinement to be made.

However, with DNA sequence data now available for ~500 species, and general patterns of relationships established, the opportunity exists to conduct broad analyses of evolutionary patterns in Terrarana. Having a molecular phylogenetic framework should prove especially useful for detailed studies of morphology, which, with some exceptions (e.g. Guayasamin 2004b, Hedges 1989, Lynch 1986a) have encountered difficulty in placing morphological variation among terraranans into an evolutionary context. As shown in this study, Terrarana encompasses species with extensive variation in osteology, at least some of which is phylogenetically informative

and may be useful in determining the evolutionary relationships of species for which genetic data are not available (Trueb & Lehr 2008). Similarly, recent work has also demonstrated the presence of numerous synapomorphies of the soft anatomy within Terrarana (Taboada *et al.*, 2013). Going forward, both osteology and soft anatomy may be key evidence in defining evolutionary units within *Pristimantis*, which, with 467 species currently divided into demonstrably non-monophyletic phenetic species groups, is still unwieldy. The description of *Tachiramanits* represents only a small step in this process.

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APPENDIX

Specimens examined. Institutional codes are as follows: CVULA (Colección de Vertebrados, Facultad de Ciencias, Universidad de los Andes, Mérida, Venezuela), EBRG (Museo de la Estación Biología de Rancho Grande, Maracay, Aragua, Venezuela), KU (Kansas University Natural History Museum, Lawrence, USA), MBLUZ (Museo de Biología de la Universidad del Zulia, Maracaibo, Venezuela), MBUCV (Museo de Biología de la Universidad Central de Venezuela, Caracas, Venezuela), MHNLS (Museo de Historia Natural La Salle, Caracas, Venezuela).

- Pristimantis anolirex*: KU 168626. 18.5 km S Chitaga, 2850 m, Departamento Norte de Santander, Colombia.
- Pristimantis ameliae*: CVULA 7117–8. San Javier del Valle, 2500 m, Estado Merida, Venezuela.
- Pristimantis auricarens*: EBRG 2724–7. Auyantepui, 1750 m, Estado Bolívar, Venezuela.
- Pristimantis boconoensis*: CVULA 8371–72. Páramo de Boconó, Estado Trujillo, Venezuela.
- Pristimantis briceni*: CVULA 2752–56. Páramo La Culata. CVULA 8364–65. trail from Monterrey to Cabaña del Cura, 2600 m, Sierra de la Culata, Cordillera de Mérida, Estado Mérida, Venezuela.
- Pristimantis cantitans*: EBRG 3003. Cima Cerro Yaví, 2150 m, Estado Amazonas, Venezuela.
- Pristimantis conservatio*: CVULA 7174–6. Cloud forest immediately above the hamlet of Los Alcaravanes, 1640 m, 8°56'43"N, 70°25'54"W; Ramal de Calderas, Estado Barinas, Venezuela.
- Pristimantis fasciatus*: MHNLS 18466. Kunana, cuenca del Río Negro, Sierra de Perijá, Municipio Machiques de Perijá, Estado Zulia, Venezuela. MHNLS 18470. Fundo “El Progreso”, Río Socuy upper basin, Sierra de Perijá, Municipio Jesús E. Lossada, Estado Zulia, Venezuela. MHNLS 18540. Cascada Kusare, near Ipika, Río Tokuko basin, Sierra de Perijá, Municipio Machiques de Perijá, Estado Zulia, Venezuela.
- Pristimantis ginesi*: CVULA 1567. Páramo de Mucubají; 5950–59, Laguna Santo Cristo, Sierra Nevada, Estado Mérida, Venezuela.
- Pristimantis gryllus*: CVULA 8343–4. Road from Estanques to Paramo La Tosta, 1320 m, Estado Mérida, Venezuela. CVULA 8346–47. La Macana, approx. 6 km SE from Santa Cruz de Mora, Estado Mérida, Venezuela. CVULA 8345. Los Ranchos, approx. 6.5 km NW Santa Cruz de Mora, Estado Mérida, Venezuela. CVULA 8348. Siberia, Estado Táchira, Venezuela.
- Pristimantis lancinii*. CVULA 5578–79, La Corcovada, Venezuela. CVULA 2622, 5822, Páramo de Mucubají, Estado Mérida, Venezuela. CVULA 0621, 0995–98, 1000, 1007–08, 1010–11, 1993, 1995–96, 5826–27. Páramo de Mucubají, Estado Mérida, Venezuela.
- Pristimantis lassoalcalai*: MHNLS 18893–8, 18460, 18877. Cerro Las Antenas, 1827 m, Sierra de Perijá, Municipio Rosario de Perijá, Estado Zulia, Venezuela.
- Pristimantis marahuaka*: MHNLS 12854, 12856–58. Cumbre Sur Cerro Marahuaka, 2650 m, Estado Amazonas, Venezuela. MA-21 (personal collection of José Ayarzagüena; to be deposited in MHNLS), from Marahuaka Summit, Estado Amazonas, Venezuela.
- Pristimantis melanoproctus*: KU 181023–26. 15 km SSW Zumbador, 1919 m, Estado Táchira, Venezuela.
- Pristimantis mondolfii*: KU 181017–22. 3 km SE Zumbador, 2650 m, Estado Táchira, Venezuela.
- Pristimantis nicefori*: CVULA 5558–60. El Alto, km 5, carretera Capilla-Cocuy, Departamento Boyacá, Colombia.
- Pristimantis paramerus*: CVULA 766–67, 990–93, 1003–05, 1859–60, 1994, 5828–34, 5836, 5838–48, 5850–54. La Corcovada, Paramo de Mucubají, Estado Mérida, Venezuela.
- Pristimantis pleurostriatus*. CVULA 7186–89, 7190–93. Cloud forest at Estancia la Bravera, 2352 m, between San Eusebio and La Carbonera, Estado Mérida, Venezuela.
- Pristimantis pulvinatus*: MHNLS 4734. Carretera San Isidro-Santa Elena de Uairén, 800 m, Estado Bolívar, Venezuela.
- Pristimantis aff. pulvinatus*: EBRG 2730. Auyantepui, camp 4, 1600 m, Estado Bolívar, Venezuela.
- Pristimantis rhigophilus*: CVULA 6854. Páramo de Guaramacal, Estado Trujillo, Venezuela.
- Pristimantis rivasi*: MHNLS 18445, MHNLS 18797, 18872 18459, 18835–36, 18865–66, 18860. Cerro Las Antenas, Sierra de Perijá, Municipio Rosario de Perijá, Estado Zulia. MHNLS 19010–12. Near Yurumuto (Yukpa indigenos village), 1640 m, Río Tokuko basin, Sierra de Perijá, Municipio Machiques de Perijá, Estado Zulia, Venezuela.
- Pristimantis sarisarinama*: EBRG 4668–75. Sima Mayor, Sarisariñama-tepui, 1100 m, Estado Bolívar, Venezuela.
- Pristimantis turik*: MBLUZ 155. Cueva del Agua (Northern wall Cave), Mesa Turik, Municipio Rosario de Perijá, Sierra de Perijá, Estado Zulia, Venezuela.
- Pristimantis vanadise*: CVULA 6169–70. Pozo Azul, Los Curos, Estado Mérida, Venezuela. CVULA 0185, 0283, 2191, 4926–27, 6084. La Carbonera, Estado Mérida, Venezuela. CVULA 0632, 2353. San Javier del Valle, La Culata, Estado Mérida, Venezuela. CVULA 3107. Via El Morro, Estado Mérida, Venezuela. CVULA 0186, 0285, 1116–24, 1163–67, 1234–35, 1642–55, 1681–90, 2014, 2317, 2016–23, 2156–57, 2208–09, 2354–62, 2605. Monte Zerpa, NW of the city of Mérida, Estado Mérida, Venezuela. CVULA 0745–47, 3108–21, 3123–27. La Mucuy Alta, Tabay, Venezuela. CVULA 7184–85. Altos de San Luis, La Azulita, Estado Mérida, Venezuela. CVULA 7194–96, 7198–206, 7206. La Bravera, way from Mérida to La Azulita, 2200 m, Estado Mérida, Venezuela.
- Pristimantis vilarsi*: EBRG 637–8, 644, 647. Base cerro Yapacana, Atabapo, Amazonas, Venezuela. EBRG 651. Caño Yagua, Chipital, Atabapo, 120 m, Amazonas, Venezuela. EBRG 653. Serranía Parú, Atabapo, Amazonas, Venezuela. EBRG 1137–8. Río Puruname, Atabapo, Amazonas, Venezuela. EBRG 1249. Río Guayapo, Salto Moriche, Amazonas, Venezuela. EBRG 1335–1339, 1349–50. Río Autana, raudal Ceguera, 100 m, Amazonas, Venezuela. EBRG 1378 Río

Autana, raudal Perez, 100 m, Amazonas, Venezuela. EBRG 1384–5. Río Autana, 2 km W boca caño Cabeza de Manteco, Atures, Amazonas, Venezuela. EBRG 1404, 1406 Río Sipapo, 5 km arriba boca Río Guayabo, Atures, Amazonas, Venezuela. EBRG 1929–30, 1935. Mavaquita, upper Orinoco, Amazonas, Venezuela. EBRG 2004–5. Raudales Atures, 12 km S Pto Ayacucho, Orinoco River, Amazonas, Venezuela. EBRG 2130. Río Asisa, Atabapo, Amazonas, Venezuela. EBRG 2530. Alto Orinoco, 400 m, Amazonas, Venezuela. EBRG 2900–2. Campo turístico Yutajé, Río Yutajé, 150 m, Amazonas, Venezuela. EBRG 3662. Lago Leopoldo (Paraka- Wachoi), Serranía Sipapo, 300 m, Amazonas, Venezuela. MHNLS 17049. Valle en Serranía de Unturán, 200 m, Amazonas, Venezuela. MHNLS 11167. Cerro Parú, 710 m, Amazonas, Venezuela. MHNLS 13874. Cerro Duida, 1000 m, Amazonas, Venezuela. MHNLS 11627. Middle Mavaca River, one hour upriver from Makarapiwei, Amazonas, Venezuela. EBRG 1797–8, Serranía Los Pijiguaos, 600 m, Bolívar, Venezuela. EBRG 1855, 1859, 1861. Los Pijiguaos, 550 m, Bolívar, Venezuela. EBRG 1874. Alto Río Cuao, Bolívar, Venezuela. EBRG 1882. Serranía Pijiguaos, Distrito Cedeño, Bolívar, Venezuela. EBRG 2162, 2166. Río Tabaro, ca. Boca Nichare, Reserva Forestal Caura, 100 m, Bolívar, Venezuela. EBRG 1517–8. Hacienda Sagitario, approx. 90 km NNE Puerto Ayacucho, Bolívar, Venezuela. EBRG 2083. Caño Icutú, tributary of Río Nichare, afl. Río Caura, 200 m, Bolívar, Venezuela. EBRG 2089. Entreríos, Río Caura, 350 m, Bolívar, Venezuela. EBRG 2104. Cerro Mocho, Bolívar, Venezuela. EBRG 2428–29, 2432–5, 2439–41. Cacurí, Sierra Maigualida, 890 m, Bolívar, Venezuela. MBUCV 6588. Summit Cerro Santa Rosa, El Triunfo, Serranía del Supamo, 700 m, Bolívar, Venezuela. MBUCV 5540. Río Paragua, entre Guaiquinima y Río Karún, Bolívar, Venezuela. CVULA 6527–8. Triunfo, Serranía del Supamo, 350 m, Bolívar, Venezuela. CVULA 6529–31. Santa Maria de Erebató, Río Erebató, alto Caura, Bolívar, Venezuela. MHNLS 9981–83. Fundo El Carmen, Río Parguaza, Bolívar, Venezuela. MHNLS 11893. Los Pijiguaos, 160 km SW of Caicara del Orinoco, Bolívar, Venezuela. MHNLS 11889. Trapichote II, Los Pijiguaos, Bolívar, Venezuela.

Pristimantis w-nigrum: CVULA 3097-98. Páez, km 34, Belcázar-Tacuayo. Departamento Cauca, Colombia.

Pristimantis yaviensis: EBRG 3007, 3015. Cima Cerro Yaví, 2150 m, Estado Amazonas, Venezuela.

Pristimantis yukpa: MHNLS 18525. Kiriponsa, Yukpa indigenous village, Río Tokuko basin, Municipio Machiques de Perijá, Sierra de Perijá, Estado Zulia, Venezuela. MBLUZ A-023-25. Caño María Lionza, San José de los Altos, Sierra de Perijá, Municipio Jesús Enrique Lossada, Estado Zulia, Venezuela. MHNLS 18115-18118, 18485-18489: Ipika, Yukpa indigenous village, Río Tukuko basin, Municipio Machiques de Perijá, Sierra de Perijá, Estado Zulia, Venezuela.

Pristimantis yuruaniensis: MHNLS 12800. Yuruani-tepui, Estado Bolívar, Venezuela.

Strabomantis biporcatus: CVULA 2726–67. Rancho Grande, 1000 m, Estado Aragua, Venezuela. EBRG 68–69, 1198. Rancho Grande, 1200 m, Venezuela. EBRG 2303. Campamento Palmichal, Pico San Marcos, 780 m, Estado Carabobo, Venezuela. MHNLS 3680. Bárbula, 1000 m, Venezuela. MHNLS 4799. Cerro Pelón, La Florida, Serranía de Barbula, 1100 m, Venezuela. MHNLS 3116–67. La Toma, Capaya, 250 m, Estado Miranda, Venezuela. EBRG 2386, 2498. Cerro el Humo, NE Las Melenas, Península de Paria, 1100 m, Estado Sucre, Venezuela. MHNLS 11439. Canales de Naguayatá, 720 m, Estado Vargas, Venezuela. EBRG 2796. Hacienda El Jaguar, 15 km NNW Aroa, 700 m, Estado Yaracuy, Venezuela.

Tachiramantis lentiginosus: CVULA 9100. Guaraque, Estado Mérida, Venezuela.

Tachiramantis prolixodiscus: EBRG 202. Sierra de Perijá, 2000 m, Estado Zulia, Venezuela. EBRG 5227-28. Buenos Aires, Parque Nacional Dinira, Estado Lara, Venezuela, 1900 m. KU 132724, 132727–9. 30 km ENE Bucaramanga, 2485 m, Departamento Santander, Colombia. CBA 7510 (C. Barrio-Amorós field number; specimen deposited at MBUCV and awaiting accessioning). Calderas, Barinas, Venezuela.