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Extinction risk or lack of sampling in a threatened species: Genetic structure and environmental suitability of the neotropical frog *Pristimantis penelopus* (Anura: Craugastoridae)

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ABSTRACT

IUCN Red Lists have been a valuable tool to prioritize conservation plans in endemic neotropical frogs. However, many areas in this region are poorly known in terms of their diversity and endemism. Based on examined museum specimens of the threatened species Pristimantis penelopus *we revised its geographic distribution and determined the habitat suitability using niche modeling techniques. Using a mitochondrial fragment of COI gene, we determine the phylogenetic position and the extent of the genetic variation across its distribution in Colombia. We present the first records of* P. penelopus *for the Cordillera Oriental, the western versant of Cordillera Occidental and the northern portion of the Cauca river basin. Based on the molecular phylogenetic analysis,* Pristimantis penelopus *belongs to the* P. ridens *series* sensu *Padial* et al. *(2014). The mean of intraspecific genetic variation is 2.1% and the variation among population ranges between 2.3 and 3.5%. The genetic distance between the western populations and the Magdalena Valley populations suggests a potential phylogeographic break in northwestern Antioquia. We expand the realized distribution by 258 kilometers north, 200 km east and 223 km northwest. Based on our results and according to the IUCN criteria we propose a new category for the species and highlight the need to increase the surveys in poorly known regions to better understand the geographic distribution and conservation status of listed species.*

Key-Words: Colombia; IUCN Red List; Niche modeling; Phylogeography; Terrarana.

INTRODUCTION

The IUCN Red List of Threatened Species highlights which species are at the greatest risk of extinction and seeks to promote their conservation (Collar, 1996; Rodríguez *et al.,* 2006). To determine the conservation status of a species, IUCN uses objective criteria and the expert opinion regarding population

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declines, geographic range (*i.e.,* extent of occurrence and area of occupancy), number of mature individuals, population size and extinction probability based on quantitative analyses (IUCN, 2001).

In the Neotropical region, evidence about population trends and species distribution of amphibians is scant as many species are only known from few individuals (*i.e.,* the type series). Many regions have not been adequately sampled and information on the distribution range and area of occupancy is anecdotal and fragmented. This limited information hampers the appropriate assessment of the species conservation status. As a consequence, conservation efforts would be incorrectly implemented for species with an equivocal conservation status. Given the scarce information regarding population trends in neotropical amphibians, species description and occurrence records are the most important sources of information for species conservation assessments.

The frog genus *Pristimantis* Jiménez de la Espada, 1870, a very species-rich lineage with more than 470 described species, is distributed throughout southern Central America and northern South America (Padial *et al.,* 2014). Currently, 162 species are listed in one of the three IUCN threat categories and most of them under the B criterion (reduced extent of presence or area of occupancy; IUCN, 2016). All listed *Pristimantis* have restricted geographic distribution and face some habitat loss across their range. As a case, *Pristimantis penelopus* (Lynch & Rueda-Almonacid, 1999) is endemic to Colombia and is listed by IUCN as Vulnerable (VU B1ab (iii)) because its extent of occurrence is less than 20,000 km², it is known from fewer than ten locations, and there is continuing decline in the extent and quality of its habitat on the Cordillera Central of the Colombian Andes (Castro *et al.,* 2004).

Here, we evaluate the extent of occurrence of *Pristimantis penelopus* (Lynch & Rueda-Almonacid, 1999) based on recent collecting efforts in northwestern Colombia. Based on this new information in addition to genetic analysis and ecological niche modeling, we reassess its conservation category according to the IUCN Red List criteria. Although, new *Pristimantis* species are described every year, we show that an extensive geographic sampling and an integrative approach (*e.g.,* environmental and genetic) will help to reassess the conservation status of many other threatened species.

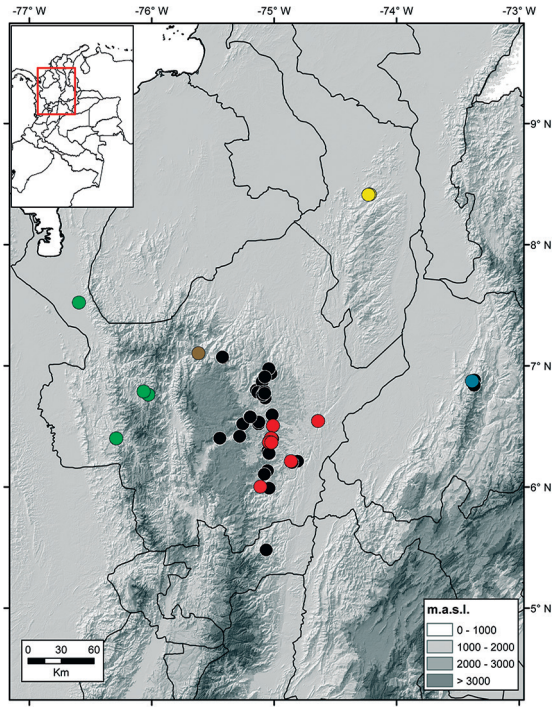

FIGURE 1: Geographic sampling of *Pristimantis penelopus.* Colored circles indicate sequenced specimens. Different colors represent the populations used in the genetic analysis (see Figure 2 for color codes).

MATERIALS AND METHODS

Specimen records

We collated records for *P. penelopus* from the GBIF database (www.gbif.org) from three biological collections: Instituto de Ciencias Naturales (ICN), Instituto Alexander von Humboldt (IAvH), and Museo de Herpetología Universidad de Antioquia (MHUA). Because records from the MHUA collection represent 73% out of all records for this species, we checked all specimens to corroborate taxonomic identity and we obtained 318 records for *P. penelopus.* Twenty-two remaining records are deposited at IAvH (17 records) and ICN (five records). In total, 316 confirmed records were obtained and mapped (Fig. 1).

Laboratory procedures

Samples of 37 individuals from three species: *Pristimantis penelopus, Pristimantis erythropleura* (Boulenger, 1896), and *Pristimantis viejas* (Lynch & Rueda-Almonacid, 1999) were sequenced. Samples of *P. penelopus* come from 15 localities across its range (Fig. 1). Total genomic DNA was extracted from tissue samples (muscle) using the Qiagen DNeasy kit (QIAGEN). A fragment of the mitochondrial genome corresponding to the cytochrome oxidase I gene (COI) was amplified via PCR using the primers dgLCO-dgHCO (Meyer,

2003). All PCR products were sequenced at the Macrogen sequencing facility (www.macrogen.com). Raw sequence chromatographs were edited using Geneious 8.1.4 (Kearse *et al.,* 2012) and aligned with the program MUSCLE using default parameters (Edgar, 2004). All sequences generated in this study were deposited in GenBank (Supplementary Material).

Phylogenetic analysis

We combined previously published COI sequences with the new sequences generated for this study to create a matrix with a total of 109 terminals (Supplementary Material). Pinto-Sánchez *et al.* (2014) and later Padial *et al.* (2014) incorrectly included in their phylogenetic analyses the voucher AJC1344 (MHUAA48119) as *Pristimantis paisa.* However, after specimen examination and a molecular analysis, we unambiguously identified this specimen as *Pristimantis penelopus.* We used their phylogenetic hypotheses to determine the taxon sampling for our genetic analysis. We included available intraspecific sampling for the species most closely related to *P. penelopus: P. erythropleura, P. cruentus* (Peters, 1873), *P. latidiscus* (Boulenger, 1898), and *P. museosus* (Ibáñez *et al.,* 1994). This group represents the *P. ridens* series *sensu* Padial *et al.* (2014). Based on Pinto-Sánchez *et al.* (2014) and our preliminary analyses with the entire genus, we used *Pristimantis viejas* as the outgroup. We simultaneously inferred the best model of evolution and the Maximum Likelihood tree using the program IQ-TREE (Nguyen *et al.,* 2015). Nodal support was estimated using the ultrafast bootstrap implemented in IQ-TREE (Minh *et al.,* 2013). In addition, we inferred a Bayesian phylogenetic tree using the Markov chain Monte Carlo method implemented in the program BEAST 1.8.3 (Drummond *et al.,* 2012). We implemented the GTR+G+I model of evolution for the entire dataset as suggested by IQ-TREE. We initiated two independent runs from a starting random tree for 10 million generations sampling every 1,000 generations. On each run, the first two million generations were discarded as burn-in and the remaining samples were combined. Nodal support as Bayesian posterior probabilities were annotated on the maximum clade credibility tree.

Population genetic analysis

A haplotype network of *P. penelopus* samples was obtained using the median joining method (Bandelt *et al.,* 1999) implemented in the program PopART (Leigh, 2016). In PopART, we implemented an AMOVA test (Excoffier *et al.,* 1992) to stablish the molecular variation among geographic region, among localities and within localities. Uncorrected genetic distances among populations and within *P. penelopus* were estimated in Mega (Kumar *et al.,* 2016). The correlation between geographic and genetic distances was determined using a Mantel test with randomization, which tests for significance of a regression using a randomized permutation procedure to account for the potential non-independence among samples (Wang, 2013). This method was implemented in zt with 10,000 permutations (Bonnet & de Peer, 2002).

TABLE 1: Number of individuals, haplotypes and localities by geographic region of *Pristimantis penelopus.*

	# individuals	# localities	# haplotypes
Central	19	7	9
Western	5	3	4
Northern	3	1	2
Eastern	6	3	2
Cauca	1	1	1

Environmental niche modeling

We compiled occurrence records for *P. penelopus* as we mentioned before and only used for niche modeling those records corroborated by us using morphological and genetic data (53 unique locality records, Table 1). We buffered (~ 225 km of radius) each occurrence points to delimitate the background area to calibrate our niche model. We generated potential geographical distributions for this species using several algorithms (*e.g.,* GAM, GLM, Maxent, Mars, SVM) implemented in the sdm R package (Naimi & Aráujo, 2016) using 10 replicates of subsampling splitting occurrence data in 70% for training and 30% for testing. We used only 11 of 19 bioclimatic variables which were the least correlated between them (bio1, bio2, bio3, bio4, bio8, bio9, bio12, bio15, bio16, bio17, bio19). The best model was selected as the one that maximizes validation metrics of both: Area under the curve (AUC) and the True Skill Statistics (TSS) (Allouche *et al.,* 2006). The best model was one generated by Maxent and we reclassified this to a binary prediction (*i.e.,* presence-absence) using the minimum training presence threshold. We calculated the potential presence area (km^2) using ArcMap 10.2 (ESRI, 2011).

RESULTS

The aligned matrix included 688 sites. Phylogenetic relationships within the *P. ridens* series is in agreement with previous studies (Padial *et al.,* 2014; Pinto-Sánchez *et al.,* 2014). *Pristimantis penelopus* is recovered as monophyletic and its sister species is *P. erythropleura* (Fig. 2). Maximum likelihood and Bayesian trees show a phylogeographic structure in *P. penelopus* where the regions west of Cordillera Occidental, Bolivar, Santander and Antioquia represent different clades. However, two haplotypes from Antioquia are more related to Santander and Bolivar respectively (Fig. 2).

For the intraspecific variation analyses we used a matrix with 460 base pairs of COI obtained from 34 individuals. A total of 42 variable nucleotide sites were found with no insertions or deletions and 18 different haplotypes were identified. All haplotypes were restricted to a single geographic region and we did not find dominant haplotypes. The median-joining network showed a pattern with phylogeographic structure in accordance with the phylogenetic tree (Fig. 2). The AMOVA results for the five geographic

TABLE 2: Uncorrected genetic distances among *Pristimantis penelopus* populations based on a 688 bp fragment of the mitochondrial gene COI. Numbers on the diagonal represent the intraspecific variation.

	Central	Eastern	Northern	Western	Cauca
Central	(0.008)				
Eastern	0.023	(0.001)			
Northern	0.024	0.023	(0.001)		
Western	0.034	0.035	0.033	(0.009)	
Cauca	0.028	0.031	0.031	0.025	(—)

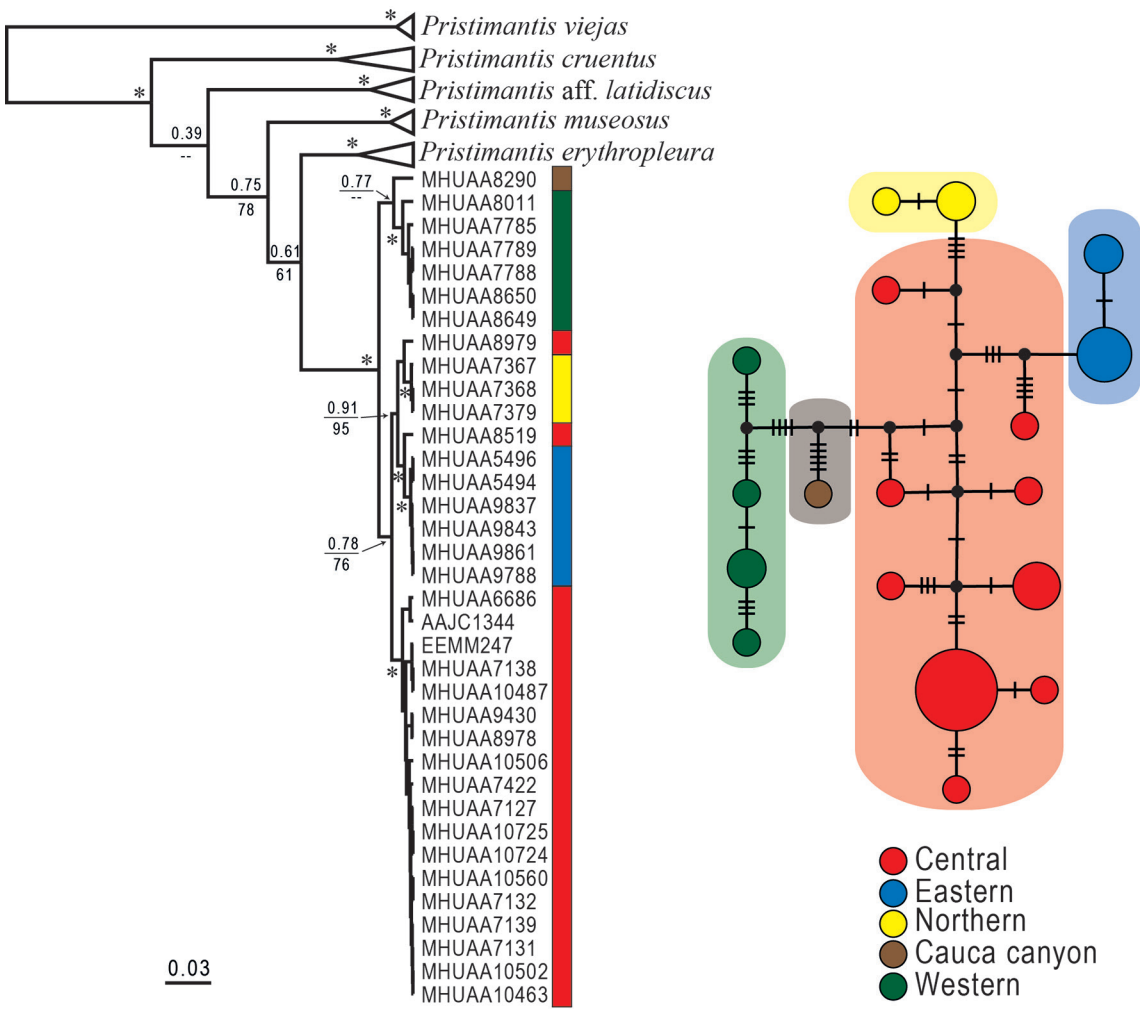

FIGURE 2: (Left) Maximum clade credibility tree depicting the phylogenetic position of *Pristimantis penelopus* within the *P. ridens* series. Numbers on nodes indicate posterior probabilities. Numbers below nodes represent nodal support using the ultrafast bootstrap (see methods). Asterisks indicate nodal support above 95% in both Bayesian and ML methods. (Right) Haplotype network based on 460 bp of the COI region. Numbers of mutational steps are shown on the lines connecting haplotypes. Colors refer to geographic locations shown in Figure 1.

FIGURE 3: Phenotypic variation of *Pristimantis penelopus* across its distribution. Localitites are shown in Appendix 1.

regions estimated that 64.7% of the genetic variation occurred among geographic regions, 33.8% among localities and 1.6% within populations. The genetic variation we found follows an isolation by distance pattern (IBD; $r = 0.77$, $P < 0.0001$). The mean genetic distance between geographic regions ranged from 2.3% and 3.5% (Table 2).

The potential geographic distribution of *Pristimantis penelopus* encompass the central and northern Andes in Colombian and southern Venezuela (Fig. 4). Its distribution area estimated from the environmental niche model is ~ 191535 km². The model has a relative good performance (AUC = 0.93; TSS = 0.77).

DISCUSSION

Piristimantis penelopus distribution has been restricted to the sub-Andean forests of the eastern slopes of the Cordillera Central in Antioquia and Caldas Departments (Lynch & Rueda-Almonacid, 1999; Bernal & Lynch, 2008) at elevations between 1,180 to 1,500 m. Several authors (Castro *et al.,* 2004; Stu-

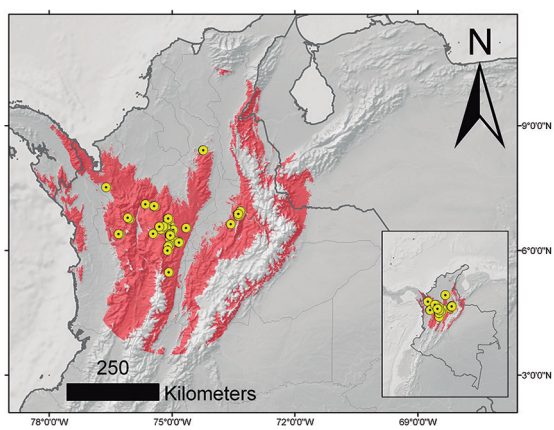

FIGURE 4: Potential distribution of *Pristimantis penelopus* based on ecological niche modeling (red). Yellow dots represents occurrence localities used to calibrate the model. See main text for details.

art *et al.,* 2008; Llano-Mejía *et al.,* 2010) mentioned the presence of *P. penelopus* in Tolima department but no voucher specimens support its presence. Combining specimen examination, genetic analysis, and niche modeling, we show that *Pristimantis penelopus* is distributed along the eastern flank of the Cordillera Central in Caldas and Antioquia, the western flank of the Cordillera Oriental in Santander, north of Serranía San Lucas in Bolívar, and the western flank of the Cordillera Central in Antioquia. Our findings expand the known distribution 258 kilometers north, 200 km east and 223 km northwest relative to the closest edge in the previously known distribution range for the species (Fig. 4). Altitudinal distribution is also extended as the original range changed from 1,180-1,500 m to 94-1,720 m.

We present molecular evidence that allows us to clarify the phylogenetic position of *Pristimantis penelopus,* the interpopulation genetic variation, and the distribution range. Phylogenetic relationships within *Pristimantis* are still incompletely understood because of the lack of adequate taxon sampling and the high levels of cryptic diversity and taxonomic complexity in this highly diverse clade (Fig. 3; Rivera-Correa & Daza, 2016). Although we used only a fragment of the mitochondrial genome to determine the phylogenetic position of *P. penelopus,* our results are in agree with the more extensive sampling of taxa and genes in a large *Pristimantis* phylogeny (*i.e.,* Pinto-Sánchez *et al.* 2014). The sister species to *P. penelopus* is *P. erythropleura* (Boulenger, 1896). This later species is distributed mainly on the western flank of the Cordillera Occidental of Colombia at elevations from 1,200-2,600 m (Frost, 2016). The genetic variation within *Pristimantis* species in the *P. ridens* group are fairly similar (Fig. 2).

The presence of a distinct clade in the lowlands of the western flank on the Cordillera Occidental suggest that a potential phylogeographic break might occur between the Chocó and the Magdalena lowlands, likely due to a conspicuous geographical/ecological barrier hard to delimitate. In some cases, this putative break would result in sister species on both sides of the barrier (*e.g., Allobates talamancae* and *Allobates niputidea, Agalychnis callidryas* and *Agalychnis terranova*). In contrast, western and eastern populations of *Pristimantis penelopus* correspond to the same species as the observed intraspecific variation is similar to the one found in other *Pristimantis* species (Crawford *et al.,* 2010; García-R. *et al.,* 2012; Fig. 2). Although we do not have quantitative measures of morphological variation across this break, specimen examination also indicates that populations from both sides represent one single species. More sampling effort in the northern cordilleras Occidental and Central and the Cauca canyon will illuminate the presence and location of this hypothetical phylogeographic break and its role in species divergence.

Pristimantis penelopus is currently categorized as a Vulnerable species (VU B1ab (iii)). Before our study, fewer than ten localities are known for the species and the extent of occurrence (EOO) was less than 20,000 km². In addition, the species has been considered to be rare and restricted to mature forest (Castro *et al.,* 2004). The continued decline in the extent and quality of its habitat across its range was considered an indirect evidence of population decline. Our results show that *P. penelopus* occurs in at least 26 localities and the EOO is more than seven times larger than originally considered. The species has been found in secondary forests, and all specimens deposited in the MHUA collection provide indirect evidence that the species is not rare. Therefore, based on our results, we suggest that this species is assigned to the Least Concern category according to IUCN guidelines.

CONCLUSION

Our findings highlight the need for thorough sampling in poorly studied regions in the Neotropics. Taxonomic studies using multiple lines of evidence (morphology, genetics and environmental suitability) are necessary to uncover geographic structure and distributional ranges of poorly known amphibians. Lastly, conservation biology should not only deal with enhancing the persistence of local and rare populations but also by exploring undersampled areas that might lead to discover new populations and in turn will decrease the extinction risks of the species across its distribution.

RESUMEN

Las listas rojas de la IUCN han sido una herramienta fundamental para priorizar planes de conservación de ranas endémicas neotropicales. Sin embargo, muchas áreas en esta región han sido poco estudiadas y el endemismo puede ser un artificio del muestreo. A partir de especímenes de museo de la especie amenazada Pristimantis penelopus *revisamos su distribución geográfica y determinamos su hábitat potencial utilizando técnicas de modelo de nicho. A partir de un fragmento del gen mitocondrial COI, determinamos la posición filogenética de la especie y la magnitud de su variación genética*

a lo largo de su distribución en Colombia Presentamos los primeros registros de P. penelopus *en la Cordillera Oriental de los Andes, en la vertiente occidental de la Cordillera Occidental y en el norte de la cuenca del río Cauca. Teniendo en cuenta los análisis filogenéticos moleculares,* Pristimantis penelopus *pertenece al grupo* P. ridens sensu *Padial* et al. *(2014). La variación genética intraespecífica promedio fue de 2.1% y la variación entre poblaciones estuvo entre 2.3 y 3.5%. La distancia genética entre las poblaciones de la Cordillera Occidental y las poblaciones del Valle del Magdalena sugieren la presencia de un quiebre filogeográfico en el noroccidente de Antioquia. Expandimos la distribución realizada de* P. penelopus *258 kilómetros al norte, 200 km al este y 223 km al noroccidente. Teniendo en cuenta estos resultados y los criterios propuestos por la IUCN proponemos una nueva categoría de amenaza para la especie y resaltamos la necesidad de incrementar los muestreo en regions geográficas poco conocidas para comprender mejor la distribución geográfica y el estado de conservación de las especies incluidas en las Listas Rojas.*

Palabras-Clave: Colombia; Listas Rojas de la IUCN; Modelos de Nicho; Filogeografía; Terrarana.

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SUPPLEMENTARY MATERIAL

Voucher information of the *Pristimantis penelopus* specimens used in this study.

VOUCHER	DEPARTMENT	MUNICIPALITY	LATITUDE	LONGITUDE	ALTITUDE
MHUAA 07037	Antioquia	Alejandria	6.36699	-75.02892	1053
MHUAA 07126	Antioquia	Alejandria	6.36804	-75.02556	1198
MHUAA 07128	Antioquia	Alejandria	6.36699	-75.02892	1282
MHUAA 07132	Antioquia	Alejandria	6.36699	-75.02892	1282
MHUAA 07422	Antioquia	Alejandria	6.36930	-75.02520	1286
MHUAA 07424	Antioquia	Alejandria	6.36930	-75.02520	1286
MHUAA 07425	Antioquia	Alejandria	6.36930	-75.02520	1286
MHUAA 08495	Antioquia	Alejandria	6.39128	-75.03640	1291
MHUAA 08498	Antioquia	Alejandria	6.39128	-75.03640	1291
MHUAA 07127	Antioquia	Alejandria	6.36888	-75.02544	1300
MHUAA 07131	Antioquia	Alejandria	6.36888	-75.02544	1300
MHUAA 07139	Antioquia	Alejandria	6.37236	-75.04374	1307
MHUAA 08227	Antioquia	Alejandria	6.36725	-75.02723	1309
MHUAA 08487	Antioquia	Alejandria	6.36725	-75.02723	1309
MHUAA 08489	Antioquia	Alejandria	6.36725	-75.02723	1309
MHUAA 08588	Antioquia	Alejandria	6.36728	-75.02697	1313
MHUAA 01168	Antioquia	Amalfi	6.80900	-75.15150	900
MHUAA 01487	Antioquia	Amalfi	6.78605	-75.13393	940
MHUAA 01427	Antioquia	Amalfi	6.91194	-75.07833	980
MHUAA 01467	Antioquia	Amalfi	6.91194	-75.07833	980
MHUAA 01486	Antioquia	Amalfi	6.78950	-75.08003	1000
MHUAA 01160	Antioquia	Amalfi	6.91194	-75.07833	1050
MHUAA 01967	Antioquia	Amalfi	6.93860	-75.02917	1550
MHUAA 01968	Antioquia	Amalfi	6.93860	-75.02917	1550
MHUAA 03082	Antioquia	Amalfi	6.87361	-75.09881	1844
MHUAA 01973	Antioquia	Amalfi	6.80289	-75.14575	1850
MHUAA 02440	Antioquia	Amalfi	6.80289	-75.14575	1875
MHUAA 02441	Antioquia	Amalfi	6.80289	-75.14575	1875
MHUAA 09430	Antioquia	Amalfi	6.82106	-75.07454	1924
MHUAA 09436	Antioquia	Amalfi	6.82090	-75.07452	1935
MHUAA 04485	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04486	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04490	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04491	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04492	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04493	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04494	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 04597	Antioquia	Amalfi	6.78950	-75.08003	
MHUAA 04734	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 05368	Antioquia	Amalfi	6.97861	-75.04444	
MHUAA 05371	Antioquia	Amalfi	6.97861	-75.04444	
MHUAA 06101	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 06102	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 06103	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 06104	Antioquia	Amalfi	6.78605	-75.13393	
MHUAA 00012	Antioquia	Anori	7.07842	-75.15067	790

VOUCHER	DEPARTMENT	MUNICIPALITY	LATITUDE	LONGITUDE	ALTITUDE
MHUAA 05233	Antioquia	Anori	6.98500	-75.08972	875
MHUAA 05234	Antioquia	Anori	6.98500	-75.08972	875
MHUAA 08571	Antioquia	Anori	7.07833	-75.12333	1443
MHUAA 03942	Antioquia	Anori	6.98690	-75.13750	1538
MHUAA 03943	Antioquia	Anori	6.98690	-75.13750	1538
MHUAA 03944	Antioquia	Anori	6.98690	-75.13750	1538
MHUAA 03955	Antioquia	Anori	6.81296	-75.05954	1538
MHUAA 03956	Antioquia	Anori	6.81296	-75.05954	1538
MHUAA 03957	Antioquia	Anori	6.81296	-75.05954	1538
MHUAA 05563	Antioquia	Anori	6.98690	-75.13750	1600
MHUAA 05564	Antioquia	Anori	6.98690	-75.13750	1600
MHUAA 05565	Antioquia	Anori	6.98690	-75.13750	1600
MHUAA 05566	Antioquia	Anori	6.98690	-75.13750	1600
MHUAA 05350	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05352	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05353	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05354	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05355	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05547	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05551	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05557	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05558	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05559	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05569	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05570	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05571	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05572	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05573	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05574	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05575	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 05582	Antioquia	Anori	6.97830	-75.11110	1650
MHUAA 08979	Antioquia	Anori	6.98047	-75.13625	1723
MHUAA 05050	Antioquia	Anori	6.98333	-75.13472	1728
MHUAA 04550	Antioquia	Anori	6.98333	-75.13472	1732
MHUAA 04551	Antioquia	Anori	6.98333	-75.13472	1732
MHUAA 04610	Antioquia	Anori	6.98333	-75.13472	1732
MHUAA 08978	Antioquia	Anori	6.97987	-75.13596	1764
MHUAA 03908	Antioquia	Anori	6.98500	-75.14111	1787
MHUAA 03909	Antioquia	Anori	6.98500	-75.14111	1787
MHUAA 03474	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03480	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03481	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03482	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03483	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03484	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03485	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03486	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03487	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03488	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03489	Antioquia	Anori	6.98778	-75.14330	

VOUCHER	DEPARTMENT	MUNICIPALITY	LATITUDE	LONGITUDE	ALTITUDE
MHUAA 03490	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03491	Antioquia	Anori	6.98333	-75.13472	
MHUAA 03492	Antioquia	Anori	6.98778	-75.14330	
MHUAA 03938	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03939	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03940	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03941	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03958	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03959	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03960	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03961	Antioquia	Anori	6.81296	-75.05954	
MHUAA 03962	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04272	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04273	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04274	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04275	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04276	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04277	Antioquia	Anori	6.81296	-75.05954	
MHUAA 04552	Antioquia	Anori	6.98778	-75.14330	
MHUAA 04553	Antioquia	Anori	6.98778	-75.14330	
MHUAA 05147	Antioquia	Anori	6.81296	-75.05954	
MHUAA 05580	Antioquia	Anori	6.97830	-75.11110	
MHUAA 08290	Antioquia	Briceno	7.12059	-75.63442	1139
MHUAA 08292	Antioquia	Briceno	7.12060	-75.64030	1230
MHUAA 08649	Antioquia	Canasgordas	6.79170	-76.06717	1055
MHUAA 08650	Antioquia	Canasgordas	6.76353	-76.02791	1278
MHUAA 06770	Antioquia	Chigorodo	7.52110	-76.59020	56
MHUAA 07785	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07788	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07789	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07790	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07791	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07794	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07796	Antioquia	Chigorodo	7.52509	-76.59398	94
MHUAA 07101	Antioquia	Cisneros	6.53770	-75.12575	1368
MHUAA 07100	Antioquia	Cisneros	6.53785	-75.12635	1381
MHUAA 08519	Antioquia	Cocorna	6.00215	-75.09375	988
MHUAA 07276	Antioquia	Don Matias	6.51181	-75.25195	1130
MHUAA 07277	Antioquia	Don Matias	6.51181	-75.25195	1130
MHUAA 07280	Antioquia	Don Matias	6.51181	-75.25195	1130
MHUAA 07281	Antioquia	Don Matias	6.51181	-75.25195	1130
MHUAA 07282	Antioquia	Don Matias	6.51181	-75.25195	1130
MHUAA 10260	Antioquia	Carmen de V.	6.02675	-75.22936	1835
MHUAA 05212	Antioquia	Girardota	6.40633	-75.44472	1521
MHUAA 06639	Antioquia	Gomez Plata	6.58472	-75.19889	1080
MHUAA 06642	Antioquia	Gomez Plata	6.58472	-75.19889	1080
MHUAA 07146	Antioquia	Gomez Plata	6.58472	-75.19889	1080
MHUAA 07147	Antioquia	Gomez Plata	6.58472	-75.19889	1080
MHUAA 07649	Antioquia	Gomez Plata	6.58472	-75.19889	1085
MHUAA 05834	Antioquia	Gomez Plata	6.58472	-75.19889	1093

VOUCHER	DEPARTMENT	MUNICIPALITY	LATITUDE	LONGITUDE	ALTITUDE
MHUAA 05967	Antioquia	Gomez Plata	6.58472	-75.19889	1093
MHUAA 05971	Antioquia	Gomez Plata	6.58472	-75.19889	1093
MHUAA 05973	Antioquia	Gomez Plata	6.58472	-75.19889	1093
MHUAA 08673	Antioquia	Gomez Plata	6.58139	-75.19623	1093
MHUAA 08165	Antioquia	Gomez Plata	6.58151	-75.19761	1103
MHUAA 08166	Antioquia	Gomez Plata	6.58151	-75.19761	1103
MHUAA 08168	Antioquia	Gomez Plata	6.58151	-75.19761	1103
MHUAA 05726	Antioquia	Gomez Plata	6.58472	-75.19889	
MHUAA 06276	Antioquia	Gomez Plata	6.58472	-75.19889	
MHUAA 06284	Antioquia	Gomez Plata	6.58472	-75.19889	
MHUAA 06287	Antioquia	Gomez Plata	6.58472	-75.19889	
MHUAA 08296	Antioquia	Granada	6.10671	-75.08196	1197
MHUAA 04811	Antioquia	Maceo	6.54690	-74.64360	500
MHUAA 04753	Antioquia	Mutata	7.20231	-76.44225	217
MHUAA 06918	Antioquia	Remedios			896
MHUAA 06916	Antioquia	Remedios			
MHUAA 08209	Antioquia	San Carlos	6.19570	-74.81670	613
MHUAA 07074	Antioquia	San Carlos	6.21561	-74.81189	795
MHUAA 07134	Antioquia	San Carlos	6.20671	-74.85500	824
MHUAA 07213	Antioquia	San Carlos	6.21678	-74.86497	851
MHUAA 07142	Antioquia	San Carlos	6.20441	-74.85477	858
MHUAA 08295	Antioquia	San Carlos	6.13667	-75.05630	1342
MHUAA 09748	Antioquia	San Rafael	6.28365	-74.92393	934
MHUAA 09735	Antioquia	San Rafael	6.32809	-75.01195	1008
MHUAA 09727	Antioquia	San Rafael	6.32860	-75.01146	1010
MHUAA 09737	Antioquia	San Rafael	6.32865	-75.01147	1013
MHUAA 09733	Antioquia	San Rafael	6.32831	-75.01188	1023
MHUAA 07034	Antioquia	San Rafael	6.34818	-75.00237	1030
MHUAA 07035	Antioquia	San Rafael	6.34818	-75.00237	1030
MHUAA 07036	Antioquia	San Rafael	6.34818	-75.00237	1030
MHUAA 07059	Antioquia	San Rafael	6.34818	-75.00237	1030
MHUAA 07060	Antioquia	San Rafael	6.34818	-75.00237	1030
MHUAA 09725	Antioquia	San Rafael	6.32834	-75.01184	1032
MHUAA 09734	Antioquia	San Rafael	6.32866	-75.01155	1034
MHUAA 09721	Antioquia	San Rafael	6.32820	-75.01186	1039
MHUAA 09722	Antioquia	San Rafael	6.32820	-75.01186	1039
MHUAA 09736	Antioquia	San Rafael	6.32820	-75.01186	1039
MHUAA 09760	Antioquia	San Rafael	6.29817	-74.91981	1151
MHUAA 09756	Antioquia	San Rafael	6.29771	-74.91969	1161
MHUAA 09753	Antioquia	San Rafael	6.29698	-74.91936	1173
MHUAA 09754	Antioquia	San Rafael	6.29647	-74.91934	1193
MHUAA 08172	Antioquia	San Rafael	6.28033	-75.04346	1233
MHUAA 08173	Antioquia	San Rafael	6.28033	-75.04346	1233
MHUAA 08174	Antioquia	San Rafael	6.28033	-75.04346	1233
MHUAA 06686	Antioquia	San Roque	6.49774	-74.95490	1074
MHUAA 06687	Antioquia	San Roque	6.49774	-74.95490	1074
MHUAA 07138	Antioquia	San Roque	6.40684	-75.02533	1107
MHUAA 08624	Antioquia	San Roque	6.47147	-74.85585	1108
MHUAA 08618	Antioquia	San Roque	6.47192	-74.88449	1172
MHUAA 08595	Antioquia	Santa R. de Osos	6.57869	-75.29236	1346

VOUCHER	DEPARTMENT	MUNICIPALITY	LATITUDE	LONGITUDE	ALTITUDE
MHUAA 08593	Antioquia	Santa R. de Osos	6.57975	-75.29783	1453
MHUAA 08594	Antioquia	Santa R. de Osos	6.57975	-75.29783	1453
MHUAA 08012	Antioquia	Urrao	6.40340	-76.29003	959
MHUAA 08011	Antioquia	Urrao	6.39946	-76.25956	1080
MHUAA 06866	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06867	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06869	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06877	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06878	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06879	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06880	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 06883	Antioquia	Yarumal	7.07592	-75.42171	1520
MHUAA 04087	Antioquia	Yarumal	7.06861	-75.41889	1720
MHUAA 01465	Antioquia	Yolombo	6.76241	-75.09489	950
MHUAA 08162	Antioquia	Yolombo	6.77641	-75.07880	963
MHUAA 08163	Antioquia	Yolombo	6.77641	-75.07880	963
MHUAA 01161	Antioquia	Yolombo	6.59793	-75.01852	1000
MHUAA 04484	Antioquia	Yolombo	6.73600	-75.07583	1102
MHUAA 00775	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 06108	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 06109	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 08018	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 08019	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 08020	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 08021	Antioquia	Yolombo	6.76241	-75.09489	1115
MHUAA 04731	Antioquia	Yolombo	6.73600	-75.07583	
MHUAA 04859	Antioquia	Yolombo	6.73600	-75.07583	
MHUAA 07367	Bolivar	Norosi	8.41510	-74.22115	881
MHUAA 07368	Bolivar	Norosi	8.41510	-74.22115	881
MHUAA 07369	Bolivar	Norosi	8.41510	-74.22115	881
MHUAA 07370	Bolivar	Norosi	8.41510	-74.22115	881
MHUAA 07364	Bolivar	Norosi	8.41318	-74.23162	885
MHUAA 07379	Bolivar	Norosi	8.41318	-74.23162	885
MHUAA 01795	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01796	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01797	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01799	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01800	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01801	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01803	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01824	Santander	Betulia	6.95339	-73.31842	1300
MHUAA 01802	Santander	Betulia	6.95339	-73.31842	1350
MHUAA 01794	Santander	Betulia	6.95339	-73.31842	1400
MHUAA 02309	Santander	Betulia	6.95339	-73.31842	1400
MHUAA 01804	Santander	C. de Chucuri	6.63958	-73.55200	850
MHUAA 01808	Santander	C. de Chucuri	6.63958	-73.55200	850
MHUAA 01805	Santander	C. de Chucuri	6.63958	-73.55200	1075
MHUAA 01806	Santander	C. de Chucuri	6.63958	-73.55200	1080
MHUAA 01807	Santander	C. de Chucuri	6.63958	-73.55200	1080
MHUAA 01825	Santander	C. de Chucuri	6.63958	-73.55200	1080

VOUCHER	DEPARTMENT	MUNICIPALITY	LATITUDE	LONGITUDE	ALTITUDE
MHUAA 01826	Santander	C. de Chucuri	6.63958	-73.55200	1080
MHUAA 01827	Santander	C. de Chucuri	6.63958	-73.55200	1080
MHUAA 01828	Santander	C. de Chucuri	6.63958	-73.55200	1080
MHUAA 09861	Santander	San V. de Chucuri	6.79158	-73.47604	1234
MHUAA 05494	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05495	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05496	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05497	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05498	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05499	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05500	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05501	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05502	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05509	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05510	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05511	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05512	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05513	Santander	San V. de Chucuri	6.87667	-73.38528	1258
MHUAA 05507	Santander	San V. de Chucuri	6.88639	-73.36639	1314
MHUAA 05508	Santander	San V. de Chucuri	6.88639	-73.36639	1314
MHUAA 09851	Santander	San V. de Chucuri	6.79363	-73.47725	1336
MHUAA 09858	Santander	San V. de Chucuri	6.79363	-73.47725	1336
MHUAA 09776	Santander	San V. de Chucuri	6.79277	-73.47946	1425
MHUAA 09772	Santander	San V. de Chucuri	6.79299	-73.47943	1436
MHUAA 09788	Santander	San V. de Chucuri	6.79499	-73.47968	1450
MHUAA 09836	Santander	San V. de Chucuri	6.79677	-73.47838	1466
MHUAA 09837	Santander	San V. de Chucuri	6.79677	-73.47838	1466
MHUAA 09843	Santander	San V. de Chucuri	6.79677	-73.47838	1466
MHUAA 05503	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05504	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05505	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05506	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05639	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05640	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05641	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05642	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05643	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05644	Santander	San V. de Chucuri	6.84333	-73.36917	1692
MHUAA 05645	Santander	San V. de Chucuri	6.84333	-73.36917	1692

GenBank accession numbers for the COI fragment used in this study. Species from the *Pristimantis ridens* series were obtained from Crawford *et al.* (2013; http://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12054/suppinfo).

Species	Voucher	Locality	COI
Pristimantis erythropleura	MHUAA 8112	Urrao, Antioquia	KY652598
Pristimantis erythropleura	MHUAA 8114	Urrao, Antioquia	KY652599
Pristimantis erythropleura	nrps0055	Valle del Cauca	JN991372
Pristimantis erythropleura	nrps0057	Valle del Cauca	JN991373
Pristimantis erythropleura	UVC15886	Valle del Cauca	JN371127
Pristimantis erythropleura	UVC15933	Valle del Cauca	JN371126
Pristimantis penelopus	AJC1344	Antioquia	JN991389
Pristimantis penelopus	EMM247	Antioquia	JN991412
Pristimantis penelopus	MHUAA 5494	Santander	KY652600
Pristimantis penelopus	MHUAA 5496	Santander	KY652601
Pristimantis penelopus	MHUAA 6686	Antioquia	KY652602
Pristimantis penelopus	MHUAA 7127	Antioquia	KY652603
Pristimantis penelopus	MHUAA 7131	Antioquia	KY652604
Pristimantis penelopus	MHUAA 7132	Antioquia	KY652605
Pristimantis penelopus	MHUAA 7138	Antioquia	KY652606
Pristimantis penelopus	MHUAA 7139	Antioquia	KY652607
Pristimantis penelopus	MHUAA 7367	Bolívar	KY652608
Pristimantis penelopus	MHUAA 7368	Bolívar	KY652609
Pristimantis penelopus	MHUAA 7379	Bolívar	KY652610
Pristimantis penelopus	MHUAA 7422	Antioquia	KY652611
Pristimantis penelopus	MHUAA 7785	Antioquia	KY652612
Pristimantis penelopus	MHUAA 7788	Antioquia	KY652613
Pristimantis penelopus	MHUAA 7789	Antioquia	KY652614
Pristimantis penelopus	MHUAA 8011	Antioquia	KY652615
Pristimantis penelopus	MHUAA 8290	Antioquia	KY652616
Pristimantis penelopus	MHUAA 8519	Antioquia	KY652617
Pristimantis penelopus	MHUAA 8649	Antioquia	KY652618
Pristimantis penelopus	MHUAA 8650	Antioquia	KY652619
Pristimantis penelopus	MHUAA 8978	Antioquia	KY652620
Pristimantis penelopus	MHUAA 8979	Antioquia	KY652621
Pristimantis penelopus	MHUAA 9430	Antioquia	KY652622
Pristimantis penelopus	MHUAA 9788	Santander	KY652623
Pristimantis penelopus	MHUAA 9837	Santander	KY652624
Pristimantis penelopus	MHUAA 9843	Santander	KY652625
Pristimantis penelopus	MHUAA 9861	Santander	KY652626
Pristimantis penelopus	MHUAA 10463	Antioquia	KY652627
Pristimantis penelopus	MHUAA 10487	Antioquia	KY652628
Pristimantis penelopus	MHUAA 10502	Antioquia	KY652629
Pristimantis penelopus	MHUAA 10506	Antioquia	KY652630
Pristimantis penelopus	MHUAA 10560	Antioquia	KY652631
Pristimantis penelopus	MHUAA 10724	Antioquia	KY652632
Pristimantis penelopus	MHUAA 10725	Antioquia	KY652633
Pristimantis viejas	MHUAA 6597	Antioquia	KY652634
Pristimantis viejas	MHUAA 7119	Antioquia	KY652635