UNIVERSIDAD TECNOLÓGICA INDOAMÉRICA

FACULTAD DE CIENCIAS DEL MEDIO AMBIENTE

CARRERA DE INGENIERÍA EN BIODIVERSIDAD Y RECURSOS GENÉTICOS

TEMA:

RELACIONES FILOGENÉTICAS Y DIVERSIDAD DE RANAS ANDINAS DEL GRUPO *PRISTIMANTIS MYERSI* (ANURA: CRAUGASTORIDAE)

Trabajo de titulación previo a la obtención del título de Ingeniero en Biodiversidad y Recursos Genéticos

Autor(a)

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PhD. Guayasamin Juan Manuel

QUITO – ECUADOR

2020

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PHYLOGENETIC RELATIONSHIPS AND DIVERSITY OF ANDEAN FROGS OF THE *PRISTIMANTIS MYERSI* GROUP (ANURA: CRAUGASTORIDAE).

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DEDICATORIA

To Marcelo, Rosa and Carolina Franco.

To my advisors in herpetology, ichthyology and conservation: Juan Manuel Guayasamin, Ibon Tobes Sesma, Ana Falconí and Juan Pablo Reyes-Puig.

To the pachamama, because "The earth does not belong to us, we belong to the earth".

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RESUMEN EJECUTIVO

Pristimantis es el género de ranas terrestres más diverso y ha sido dividido en grupos taxonómicos. A menudo la taxonomía de estos grupos es problemática debido a la convergencia de caracteres morfológicos, ausencia de información genética o a identificaciones erróneas. En este trabajo me enfoco en el grupo *Pristimantis myersi*, el cual contiene ranas distribuidas en los Andes de Ecuador y Colombia. La matriz genética incluye todas las especies para las cuales se contaban con secuencias en repositorios globales (GenBank), complementadas por 166 secuencias nuevas. Los análisis filogenéticos recuperan al grupo *myersi* como monofilético, aunque excluye algunas especies (*P. albujai*, *P. bicantus*, *P. sambalan* y *P. nelsongalloi*). Con estos resultados, el grupo *Pristimantis myersi* está conformado por 19 especies + 12 especies posiblemente nuevas para la ciencia. Este estudio también pone en evidencia la diversidad críptica existen en algunas especies del grupo: *P. hectus*, *P. festae*, *P. gladiator*, *P. onorei* y *P. verecundus*. Los resultados de esta investigación ponen en evidencia la necesidad de formalmente redefinir estos taxones, lo cual, sin duda, significará un cambio en el estatus de conservación de estas especies.

Descriptores:

Ecuador, Filogenia, Anura, Craugastoridae, *Pristimantis.*

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ABSTRACT

Pristimantis is the most diverse genus of terrestrial frogs. Historically, in order to facilitate species identification, it has been divided into several phenetic groups. However, in the light of phylogenetic analyses, many of these groups have turn out polyphyletic, denoting a high degree of morphological convergence and limited number of diagnostic traits. In this study, I focus on the group *Pristimantis myersi*, which contains frogs distributed in the Andes of Ecuador and Colombia. The genetic matrix includes all species for which sequences were available in global repositories (GenBank), supplemented by 166 new sequences. Phylogenetic analyses recover the *myersi* group as monophyletic, after excluding the following species: *P. albujai*, *P. bicantus*, *P. sambalan* and P. *nelsongalloi*. With these results, the *Pristimantis myersi* group is composed by 19 species + 12 species possibly new to science. Also, molecular evidence suggests cryptic diversity in the species currently recognized as *P. hectus*, *P. festae*, *P. gladiator*, *P. onorei* and *P. verecundus*. The results of this study shows the need to redefine these taxa, which will undoubtedly mean a change in the conservation status of these species.

Keywords:

Ecuador, Phylogeny, Anura, Craugastoridae, *Pristimantis*

Reviewed by:

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CHAPTER I: INTRODUCTION

Pristimantis Jimenez de la Espada (1870) is the most diverse genus of terrestrial frogs, with approximately 556 species described (Frost, 2020). It is distributed from Honduras to southern Brazil, but this genus concentrates a substantial part of its richness in the Andes of Colombia, Ecuador, and Peru (Duellman & Lehr, 2009; Frost, 2020; Hedges et al., 2008; Heinicke et al., 2007; Lynch & Duellman, 1997).

The taxonomy of *Pristimantis* is challenging because of its high diversity, intraspecific variation and few external diagnostic morphological characters (Duellman & Lehr, 2009; Guayasamin & Funk, 2009; Lynch & Duellman, 1997). Recent studies have shown that *Pristimantis* species richness is underestimated by the existence of morphologically cryptic species (Elmer & Cannatella, 2009; Hutter & Guayasamin, 2015; Ortega et al., 2015; Padial & de la Riva, 2009; Páez & Ron, 2019; Yánez-Muñoz et al., 2010). Cryptic species are two or more distinct species that are morphologically similar in such a way that they are incorrectly classified as a single species (Bickford et al., 2007). Cryptic species may seem identical, but; differences in their evolutionary history, behavior or biogeography confirm their recognition as separate species (De Queiroz, 2007; Lukhtanov, 2019; Simpson, 1961). The existence of cryptic species is problematic for taxonomists, who, by failing to distinguish between different taxa, underestimate species diversity, thus hindering conservation efforts (de Oliveira et al., 2019; Fouquet et al., 2007; Vacher et al., 2020). Also, inventories have increased in recent years and, as a result, numerous new species of *Pristimantis* have been described (de Oliveria et al., 2020; Hutter & Guayasamin, 2015; Lehr et al., 2017; Navarrete et al., 2016; Páez & Ron, 2019; Reyes-Puig et al., 2019; Székely et al., 2016; Trevisan et al., 2020).

Conceptual advances in the definition of species (De Queiroz, 2007), combining an integrative approach including the use of DNA sequences; morphological and behavioral data has allowed objective delimitation of species. However, the inference of phylogenetic relationships, based on molecular data, is still pending for most *Pristimantis* species, despite several recent efforts (Bickford et al., 2007; Hedges et al., 2008; Heinecke, 2007; Padial et al., 2010a; Padial et al., 2014b), which limits the ability to identify possible cryptic species.

In order to facilitate the taxonomy of this genus, numerous groups of species have been defined (Hedges et al., 2008; Lynch, 1976a; Lynch, 1976b; Lynch & Duellman, 1997; Páez & Ron, 2019; Pinto-Sánchez et al., 2012). Hedges et al. (2008) and Páez & Ron (2019), recognized four subgenera within *Pristimantis* (*Hypodyction*, *Pristimantis*, *Yunganastes* and *Huicundomantis*), with a total of 17 species groups (Lynch & Duellman, 1997) for the subgenus *Pristimantis* (*bellona*, *chalceus*, *conspicillatus, curtipes*, *devillei*, *frater*, *galdi*, *lacrimosus*, *leptolophus*, *loustes*, *myersi*, *orcesi*, *orestes*, *phoxocephalus*, *peruvianus*, *surdus* and *unistrigatus*).

The present study focused in the *Pristimantis myersi* group. The group inhabit the montane forests, rainforests and paramo of Colombia and Ecuador (Hedges et al., 2008). Currently, this group is composed of 24 species: *P. albujai, P. bicantus*, *P. celator*, *P. festae*, *P. floridus*, *P. gladiator*, *P. gralarias*, *P. hectus*, *P.jubatus*, *P. lucidosignatus*, *P. leoni*, *P. munozi*, *P. mutabilis, P. myersi*, *P. nelsongalloi*, *P. ocreatus*, *P. onorei*, *P. pyrrhomerus*, *P. repens*, *P. sambalan*, *P. scopaeus*, *P. sirnigeli*, *P. verecundus* and *P. xeniolum*. Twenty of those 24 species are found in Ecuador between 900 and 4150 m.a.s.l. (Frost, 2020) (Table 1).

The frogs of *P. myersi* group are characterized by the following features: small size (snout-vent length, SVL less than 28 mm), robust bodies, absence of a cranial crest, relatively narrow heads and short snouts, short to moderately long limbs, toe I shorter than toe II, toe V slightly longer than toe III and not extending to the proximal edge of the distal subarticular tuber of toe IV and narrow, rounded digital discs (Hedges et al., 2008; Lynch & Duellman, 1997; Ron et al., 2019). The *P. myersi* group species can be markedly cryptic (Yánez-Muñoz et al., 2010) and, at the same time, phenotypically variable. Therefore, these morphological characteristics represent an important source of taxonomic confusion. Currently, only seven species out of the 24 species in the group have been included in phylogenetic analyses (Guayasamin & Funk, 2009; Guayasamin et al., 2015; Guayasamin et al., 2018; Hedges et al., 2008; Padial et al., 2014b; Pyron & Wiens, 2011; Rojas-Runjaic et al., 2014).

This study aims to assess the phylogeny and species diversity of the *Pristimantis myersi* group through a much broader sampling with a greater representation of taxa and localities. This phylogenetic information will allow: (i) to define the number of species of the group, (ii) infer evolutionary relationships between species, (iii) establish species diversity from a molecular perspective. Clarifying these phylogenetic uncertainties is important, not only to delimit species within the group, but for conservation purposes, such as reassessing the conservation status of species and adequately guiding conservation strategies.

Table 1. Species in the *Pristimantis myersi* group, as defined herein, with information on the distribution, type locality, habitat and elevation.

Species	Distribution	Type locality	Habitat	Elevation	Sources
Pristimantis celator	Andes, northern in Ecuador and southern Colombia (Department of Nariño).	Ecuador, Imbabura, La Delicia, Cordillera de Intag.	Secondary forest: in terrestrial bromeliads.	1750 – 2800	Frost, 2020; Lynch, 1976c.
Pristimantis festae	On the eastern slope of the Ecuadorian Andes, Sucumbíos, Napo and Tungurahua provinces and near southern Colombia.	Ecuador, Papallacta.	Primary and secondary forest: under rocks or logs in grasslands.	2360 – 3650	Frost, 2020; Peracca, 1904.
Pristimantis floridus	Western flank of the Andes of Ecuador, Cotopaxi, Imbabura, and Pichincha provinces.	Ecuador, Cotopaxi, Sigchos.	Secondary and primary forest: in herbaceous vegetation (<60 cm) and on the forest floor.	700 – 2000	Frost, 2020; Lynch & Duellman, 1997.
Pristimantis gladiator	On the Amazonian slopes of the Andes Napo and Imbabura provinces; adjacent Putumayo, Colombia.	Ecuador, Napo, Cuyuja.	Secondary forest: grasslands, associated with rocks and fallen logs.	2270 – 2910	Frost, 2020; Lynch, 1976a.
Pristimantis gralarias	Known only from the type locality.	Ecuador, Pichincha, Las Gralarias.	Primary forest: on vegetation (<90 cm).	2192	Frost, 2020; Guayasamin et al., 2018.
Pristimantis hectus	Western slope of the Cordillera Occidental in the Department of Nariño, Colombia; northwestern Ecuador.	Colombia, Reserva La Planada.	Secondary forest: associated with forest leaf litter.	1200 – 2020	Frost, 2020; Lynch & Burrowes, 1990.
Pristimantis jubatus	Known only from the type locality.	Colombia, El Tambo, Munchique National Park.	Secondary forest: on vegetation within (< 2 cm) of the ground.	2550 – 2750	Frost, 2020; Garcia & Lynch, 2006.
Pristimantis leoni	Amazonian slopes of the Andes in northern Ecuador and southern Colombia.	Ecuador, Imbabura, Mojanda.	Secondary forests: under rocks and logs and in low vegetation (< 30 cm).	1960 – 3400	Frost, 2020; Lynch, 1976b.
Pristimantis munozi	Known only from the type locality.	Ecuador, Pichincha, Verdecocha.	Primary and secondary forest: scrubland, grassland, bamboo patches and mosses.	2851	Frost, 2020; Rojas-Runjaic, et al., 2014.
Pristimantis mutabilis	Ecuador, Pichincha and Imbabura provinces.	Ecuador, Pichincha, Las Gralarias.	Primary and secondary forests: on the surfaces of leaves (<100 cm).	1850 – 2063	Frost, 2020; Guayasamin et al., 2015.
Pristimantis myersi	On the southern, Cordillera Central in Colombia and in the Andes of Ecuador, Imbabura and Sucumbíos provinces.	Colombia, Nariño.	Primary and secondary forest: under rocks, logs and thick grass.	2800 – 3470	Frost, 2020; Goin & Cochran, 1963.
Pristimantis ocreatus	Northern Ecuador.	Ecuador, Carchi, Tufiño.	Primary and secondary forests: in humid moors and sub-moors.	3000 – 4150	Frost, 2020; Lynch, 1981.

Pristimantis onorei	Ecuador, Santo Domingo de los Tsáchilas, Pichincha and Cotopaxi provinces.	Ecuador, Pichincha, Tandapi.	Primary forest: associated with forest leaf litter and vegetation below 60 cm.	2115	Rödder & Schmitz, 2009; this study.
Pristimantis pyrrhomerus	Ecuador, Pichincha, Imbabura, Carchi, Cotopaxi and Bolívar provinces.	Ecuador, Cotopaxi, Pilaló.	Secondary forest: found under rocks, logs, wood chips along the road and grasslands (<100 cm).	2075 – 3000	Frost, 2020; Lynch, 1976a.
Pristimantis repens	Colombia, Pasto and La Cruz, Department of Nariño.	Colombia, Nariño.	Between high Andean forest and paramo is not only depending on the elevation and vegetation criteria.	3220 – 3300	Frost, 2020; Lynch, 1984.
Pristimantis scopaeus	Known only from the type locality.	Colombia, Municipal of Cajamarca.	Secondary forest: in terrestrial and arboreal microhabitats.	3580 – 3600	Frost, 2020; Lynch et al., 1996.
Pristimantis sirnigeli	Ecuador, Pichincha and Imbabura provinces.	Ecuador, Pichincha, Cordillera de Saloya.	Secondary forest: in herbaceous leaves (<100 cm).	2800 – 3050	Frost, 2020; Yánez-Muñoz et al., 2010.
Pristimantis verecundus	On the western flank of the Andes in southern Colombia (Nariño Department) and Ecuador (Carchi, Pichincha, and Cotopaxi, provinces).	Colombia, La Planada.	Secondary forest: on vegetation leaves (<150 cm).	900 – 2020	Frost, 2020; Lynch & Burrowes, 1990.
Pristimantis xeniolum	Known only from the type locality.	Colombia, Valle del Cauca	Paramo: grassland.	3300 – 3600	Frost, 2020; Lynch, 2001.

CHAPTER II: METHODS

Taxon sampling

We follow the taxonomic proposal of Hedges et al. (2008) regarding to family and genus allocation, and species groups arrangement. Where groups of species are seen as tentative units between subgenus and species until their monophyly can be proven. The genetic matrix includes all species for which sequences (700) were available in global repositories (GenBank, http://www.ncbi.nlm.nih.gov/genbank/) and supplemented by new mitochondrial DNA 16S sequences from 166 individuals were obtained in the Laboratorio de Biología Evolutiva, Universidad San Francisco de Quito USFQ (LBE-USFQ) and Laboratorio Molecular del BioCamb, Universidad Tecnológica Indoamérica, representing 19 species (Appendix 1). Voucher specimens of the new sequences are deposited at the Museo de la Universidad Tecnológica Indoamérica, Quito (MZUTI); Museo de Zoología, of the Universidad San Francisco de Quito (ZSFQ) and División de Herpetología of the Instituto Nacional de Biodiversidad (DHMECN-INABIO).

DNA sequence data includes 19 of 24 species currently assigned to the *Pristimantis myersi* group: *P. albujai*, *P. bicantus*, *P. celator*, *P. festae*, *P. gladiator*, *P. gralarias*, *P. hectus*, *P. jubatus*, *P. leoni*, *P. munozi*, *P. mutabilis*, *P. myersi*, *P. nelsongalloi*, *P. pyrrhomerus*, *P. sirnigeli*, *P. ocreatus*, *P. onorei*, *P. sambalan*, and *P. verecundus*, which represent coverage of 83% of the group (Appendix 1).

As outgroups, we included sequences of 10 species closely related to the genus *Pristimantis* (*Craugastor daryi*, *C. longirostris*, *Diasporus hylaeformis*, *D. vocator*, *Lynchius flavomaculatus*, *L. nebulanastes*, *Oreobates cruralis*, *O. saxatilis*, *Phrynopus auriculatus*, and *P. bracki*), as well as, sequences of *Agalychnis callidryas* (Hylidae, Phyllomedusinae), and *Litoria caerulea* (Hylidae, Pelodryadinae) (Pinto-Sánchez et al., 2012).

DNA extraction and PCR amplification for 16S rRNA gene

For DNA extraction, we followed the Guanidine thiocynate protocol designed by Peñafiel et al. (2019). Each extraction was quantified to estimate DNA concentration and quality. For the Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene, we used the protocol designed by Guayasamin et al. (2008).

For DNA amplification we used the primers 16SC (GTRGGCCTAAAGCAGCCAC) and 16Sbr-H (5' - CCG GTC TGA ACT CAG ACG T - 3') designed by Darst & Cannatella (2004) and Palumbi et al. (1991). Each PCR reaction contained a final concentration of 1.5 mM MgCl2, 0.5 mM dNTP, 0.25 U / μL of Taq (Invitrogen) DNA polymerase and 0.2 μM of each primer, in a total volume of 25 μL. The PCR protocol included an initial denaturation step of 4 minutes at 94°C; followed by 1 minute at 94°C, 30 seconds at 57°C, 2 minutes at 72°C for 30 cycles, and a final extension of 8 minutes at 72°C. The amplicon obtained was visualized by electrophoresis with a 2% agarose gel. The amplified samples were cleaned with Exosap and cycle sequencing reactions were performed by Macrogen Sequencing Labs (Macrogen Inc., Korea). All fragments were sequenced in both forward and reverse directions.

Sequence alignment and phylogenetic analyses

All chromatogram sequences were fully inspected, compared against their reverse complements to detect errors, and manually edited using the program GENEIOUS Pro v5.4.6 (Genematters Ltd.). Alignments were performed with the online program MAFFT v7 (Katoh & Standley, 2013), under G-INS-I strategy (Avaliable at: https://mafft.cbrc.jp/alignment/server/index.html), and visualized in MESQUITE project v3.6

(Maddison & Maddison, 2018). Uncorrected pairwise distances were calculated in PAUP*4.0a167 (Swofford, 2003). To obtain the best nucleotide substitution model, we used Model-Finder under the Bayesian information criterion (BIC) (Kalyaanamoorthy et al., 2017) for Maximum likelihood (ML) and Partition Finder to Bayesian Inference (BI) under the Bayesian information criterion (BIC) (Lanfear et al., 2012). Phylogenetic inference was inferred under a Maximum likelihood (ML) criterium.

In IQ-Tree v1.6.11 (Nguyen et al., 2015), we implemented the best model for 16S phylogenetic analysis which finds simultaneously the topology, branch lengths, and substitution model parameters that maximize the log-likelihood, using a stochastic genetic algorithm-like approach (Guindon & Gascuel, 2003). Node support was assessed via 10000 UFBoost replicates (Minh et al., 2013). Finally, all trees generated were visualized in FIGTREE v1.4.4 (Rambaut, 2017) and edited in ADOBE ILLUSTRATOR v24.1.2 (Adobe Systems Inc.) to obtain high quality figures.

Species concept and candidate species

Conceptually, a species was defined as a metapopulation lineage that evolves separately from others (De Queiroz, 2007). From an operational point of view, we identified candidate species based on molecular data. Using two approaches: genetic distances (a non-tree based method), and phylogenetic trees to verify the monophyly of species.

Candidate species classified as follows: (1) Confirmed Candidate Species (CCS): where there are clear differences in genetic characteristics and are consistent with differences in morphological characteristics; (2) Unconfirmed Candidate Species (UCC): genetic distances were >0.02, but no further information or consistency with morphological characteristics are available; and (3) Deep Conspecific Lineages (DCL): deep genealogical lineages above a typical threshold value for populations within one species; divergence might be related to geographic distance (Vieites et al., 2009).

Genetic distances are a powerful tool for identifying and discovering species. When gene flow is limited, and genetic distances should increase, as gene flow between populations is restricted (Janzen, 2004; Nei, 1972). Thus, genetic distances between populations of the same species should be smaller than between different species. We chose a lower threshold than that proposed by Fouquet et al. (2007) for amphibians (0.03). To identify candidate species, we set a 0.02 threshold of uncorrected distances for the gene 16S. Because, there is empirical evidence indicating that sister species in *Pristimantis* (e.g., Ortega et al., 2015; Padial & de la Riva, 2009) and other Neotropical frogs (e.g., Caminer & Ron 2014; Coloma et al., 2012; Funk et al., 2012; Jungfer et al., 2013) often differ by genetic distances <0.03.

CHAPTER III: RESULTS

Taxon and geographic sampling

Our sampling includes a nearly complete coverage of the infrageneric group *Pristimantis myersi* (80%, or 19 of 24 species). In our sampling, we were able to include sequences from populations from the type localities (or nearby) of all Ecuadorian species. Appendix 1 and Figure 1 summarize the geographic scope of our sampling in Ecuador.

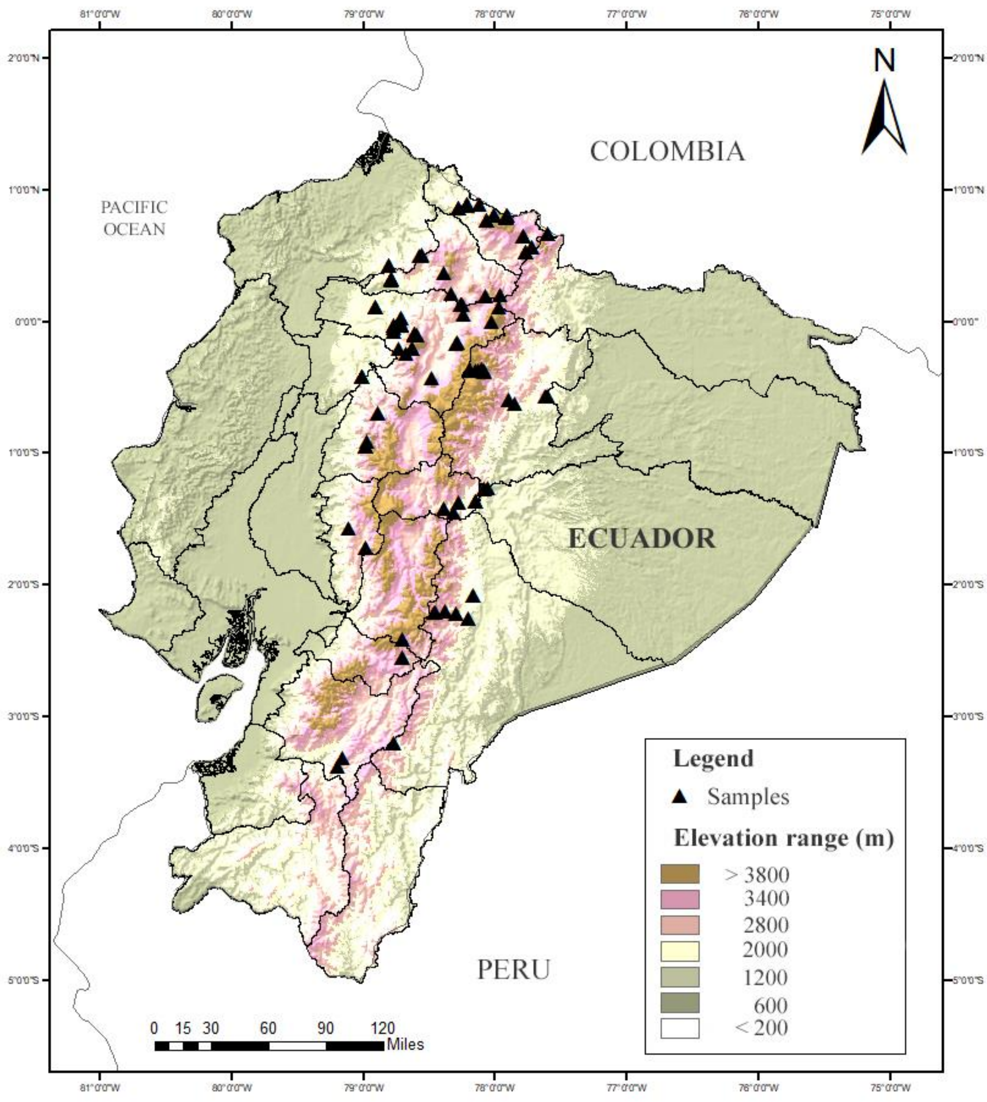

Figure 1. Distribution map of the sampled populations of species in the *Pristimantis myersi* group in Ecuador.

Monophyly and phylogenetic relationships among species of the *Pristimantis myersi* group

All sampled species (Figure 2) currently assigned to the *Pristimantis myersi* group (except *P. bicantus*, *P. nelsongalloi*, *P. albujai* and *P. sambalan*) (Figures 3 – 4) form a monophyletic group, with significant bootstrap support (100%). Based on our results, the species of the *Pristimantis myersi* group, contains 19 described species (*P. celator*, *P. festae, P. gladiator*, *P. gralarias*, *P. hectus*, *P. jubatus*, *P. leoni*, *P. lucidosignatus*, *P. munozi*, *P. mutabilis*, *P. myersi*, *P. pyrrhomerus*, *P. repens, P. sirnigeli*, *P. scopaeus, P. ocreatus, P. onorei, P. sirnigeli, P. verecundus* and *P. xeniolum),* as well as 12 new candidate species (Figures 3 and 4). This study did not consider samples of three species belonging to Colombia (*P. repens, P. scopaeus* and *P. xeniolum*). Therefore, we only mention above that the phylogeny is composed by 16 described species and 12 new candidate species.

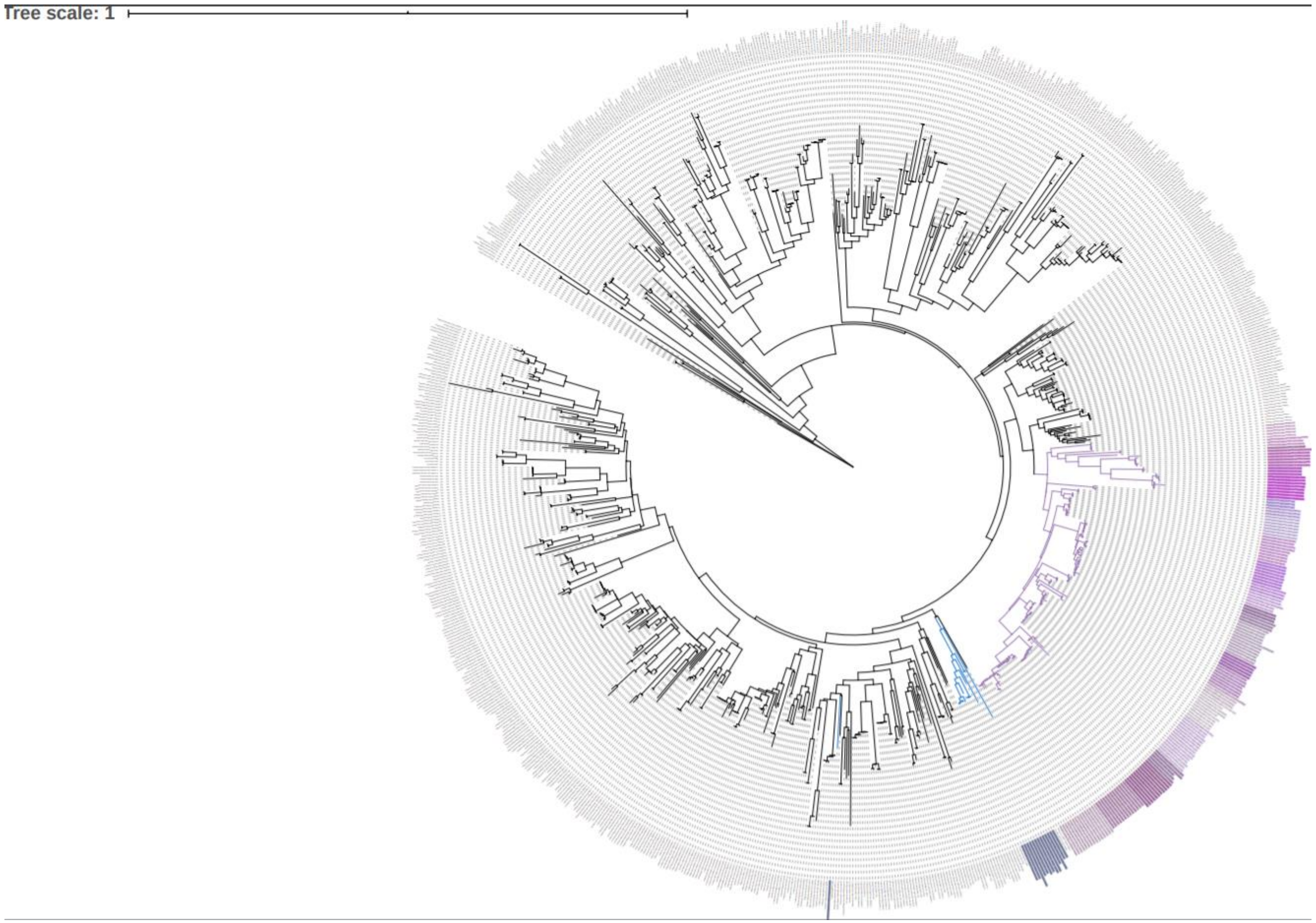

Figure 2. Maximum likelihood tree of the genus *Pristimantis* and outgroups based on the 16S mitochondrial gene. The *Pristimantis myersi* Group, as defined herein, is in purple. Species that we exclude from the group are shown in blue.

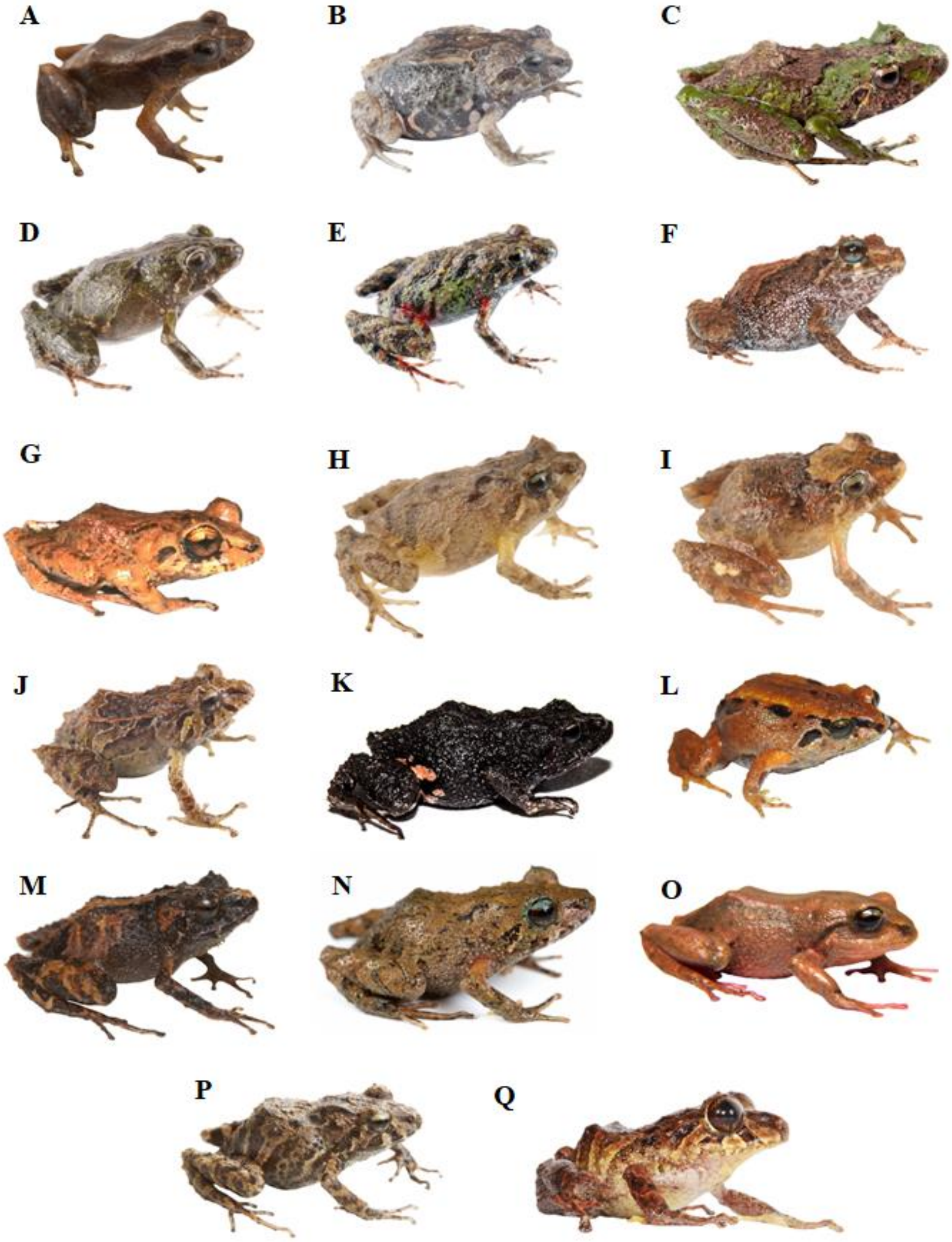

Figure 3. Morphological variation within the *Pristimantis myersi* species group. **A.** *Pristimantis celator*, **B.** *P. festae*, **C.** *P. floridus*, **D.** *P. gladiator*, **E.** *P. gralarias*, **F.** *P. hectus*, **G.** *P. jubatus*, **H.** *P. leoni*, **I.** *P. munozi*, **J.** *P. mutabilis*, **K.** *P. myersi*, **L.** *P. ocreatus*, **M.** *P. onorei*, **N.** *P. pyrrhomerus*, **O.** *P. scopaeus*, **P.** *P. sirnigeli* and **Q.** *P. verecundus*. Photos by Tropical Herping (A) (B) (C) (D) (E) (H) (I) (J) and (P), Sebastián Duarte (G) and (O), Francisco Muñoz and Daniela Franco (F) and (Q), Gustavo Pisso (K), Diego Batallas (L) and Juan Manuel Guayasamin (M) and (N).

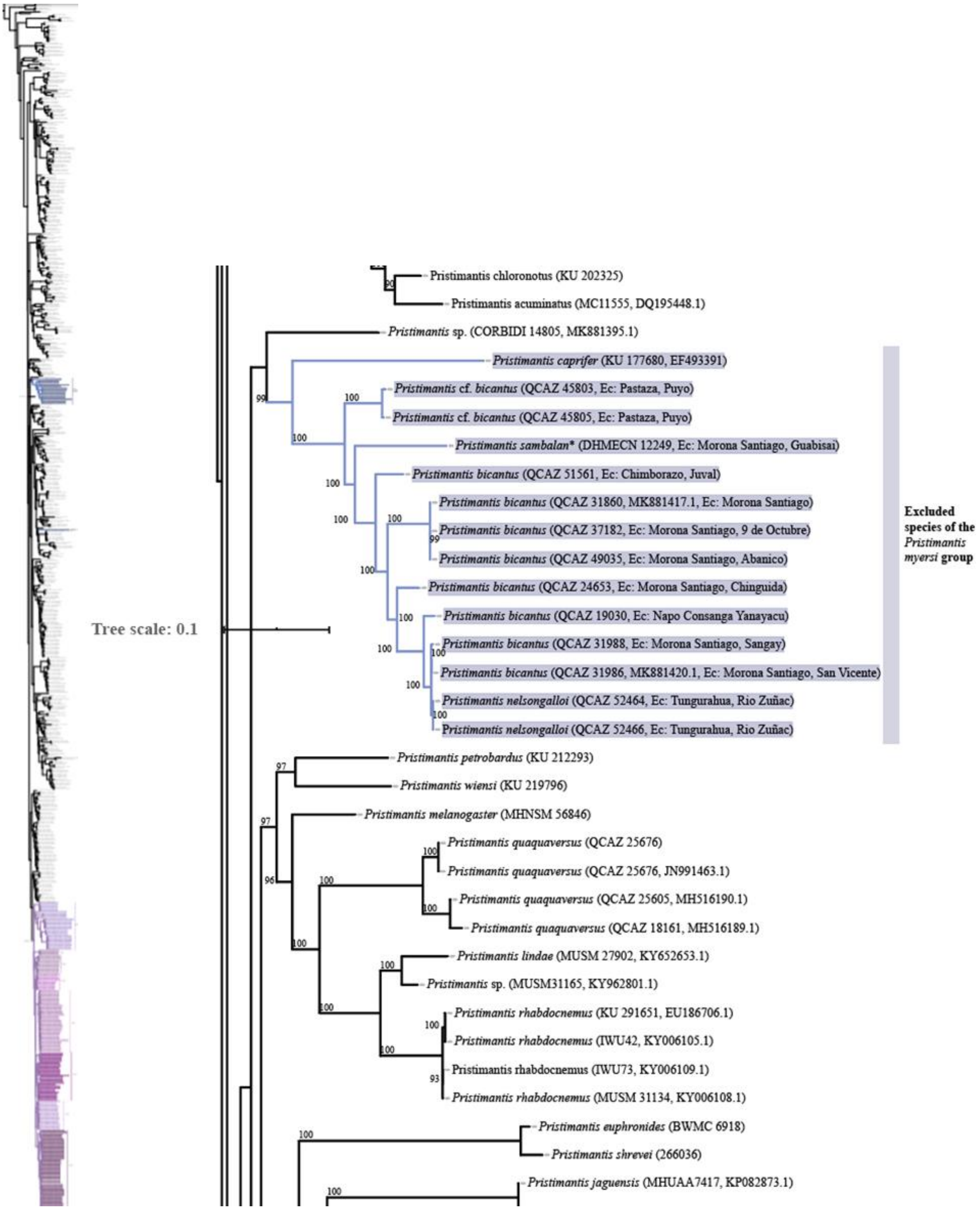

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Figure 4. Inferred ML consensus tree, showing species of *P. myersi* clade (in purple). Species in blue represented taxa that were previously considered part of the *myersi* group, but that are shown to be part of a distant clade; the species are *P. bicantus*, *P. nelsongalloi*, *P. albujai* and *P. sambalan*. Support values are shown for clades with bootstrap > 50%.

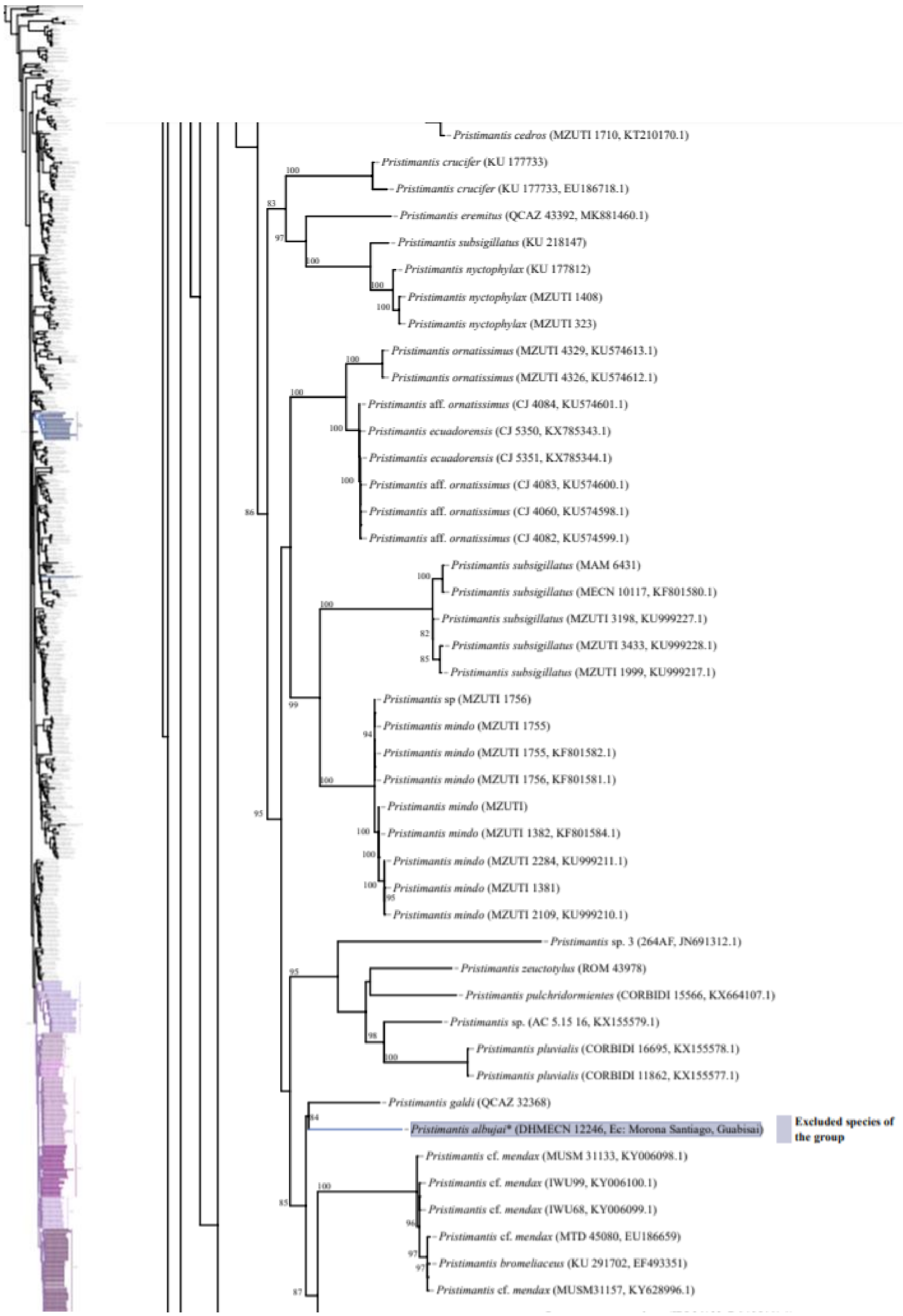

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Figure 4. Continued

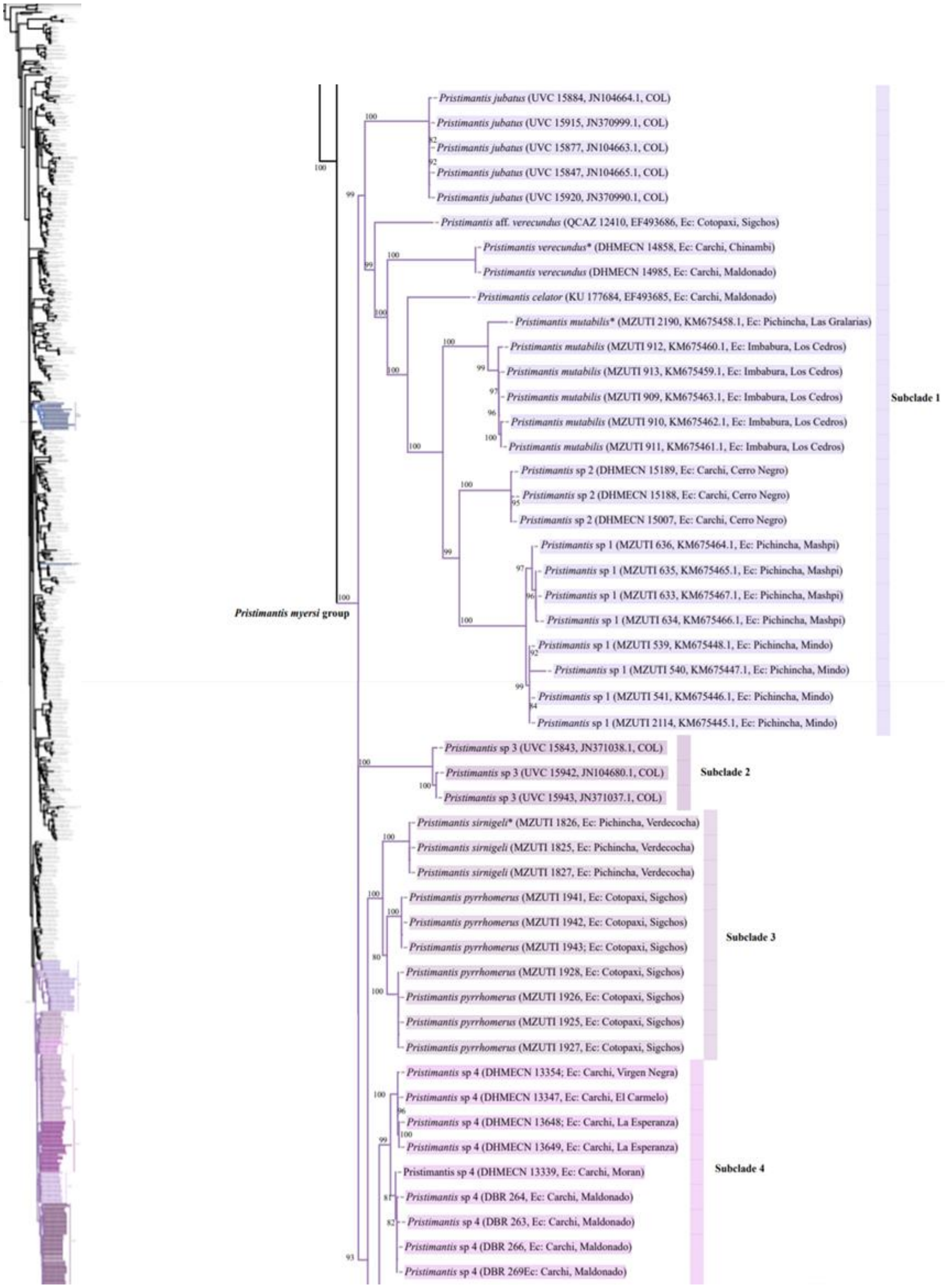

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Figure 4. Continued

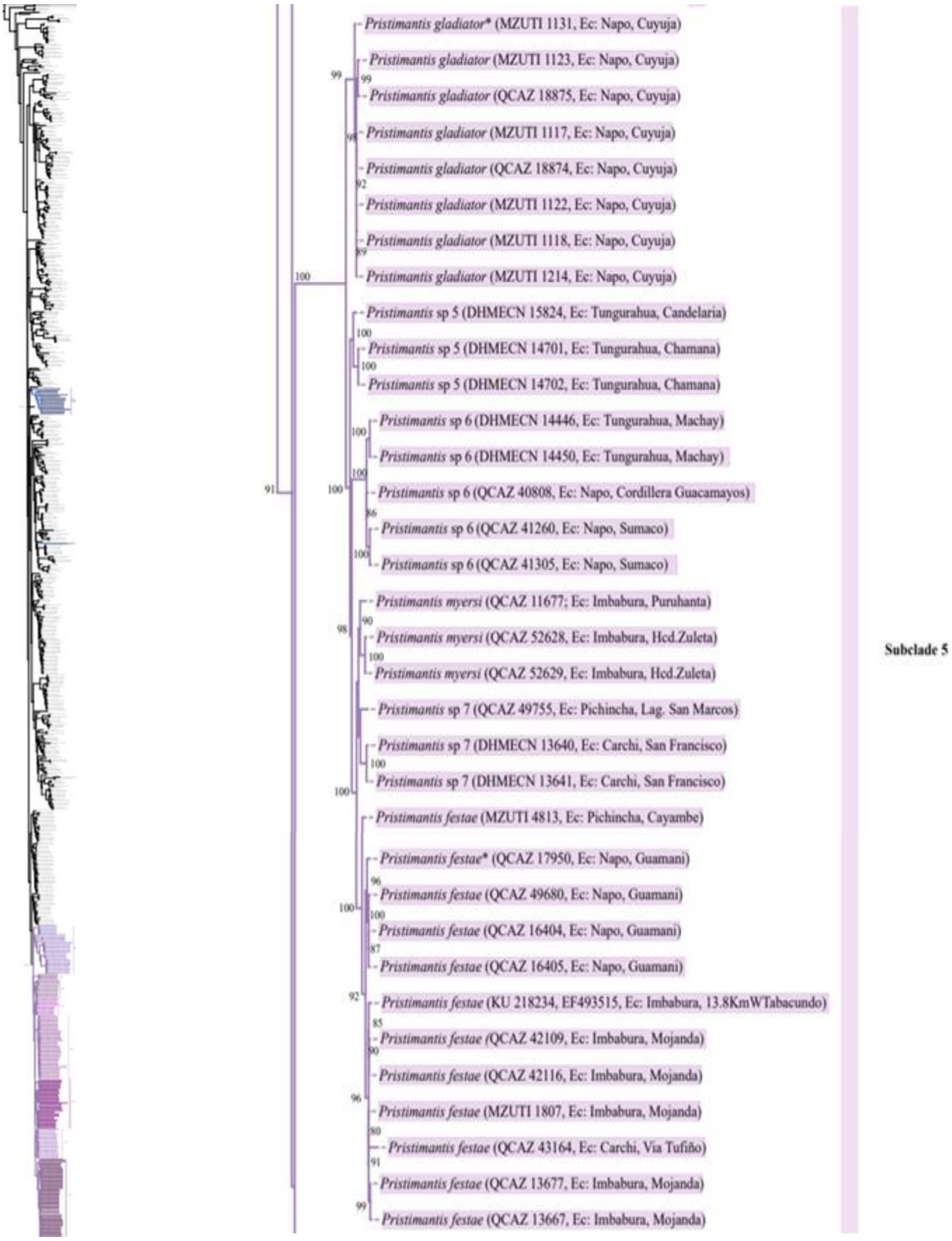

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Figure 4. Continued

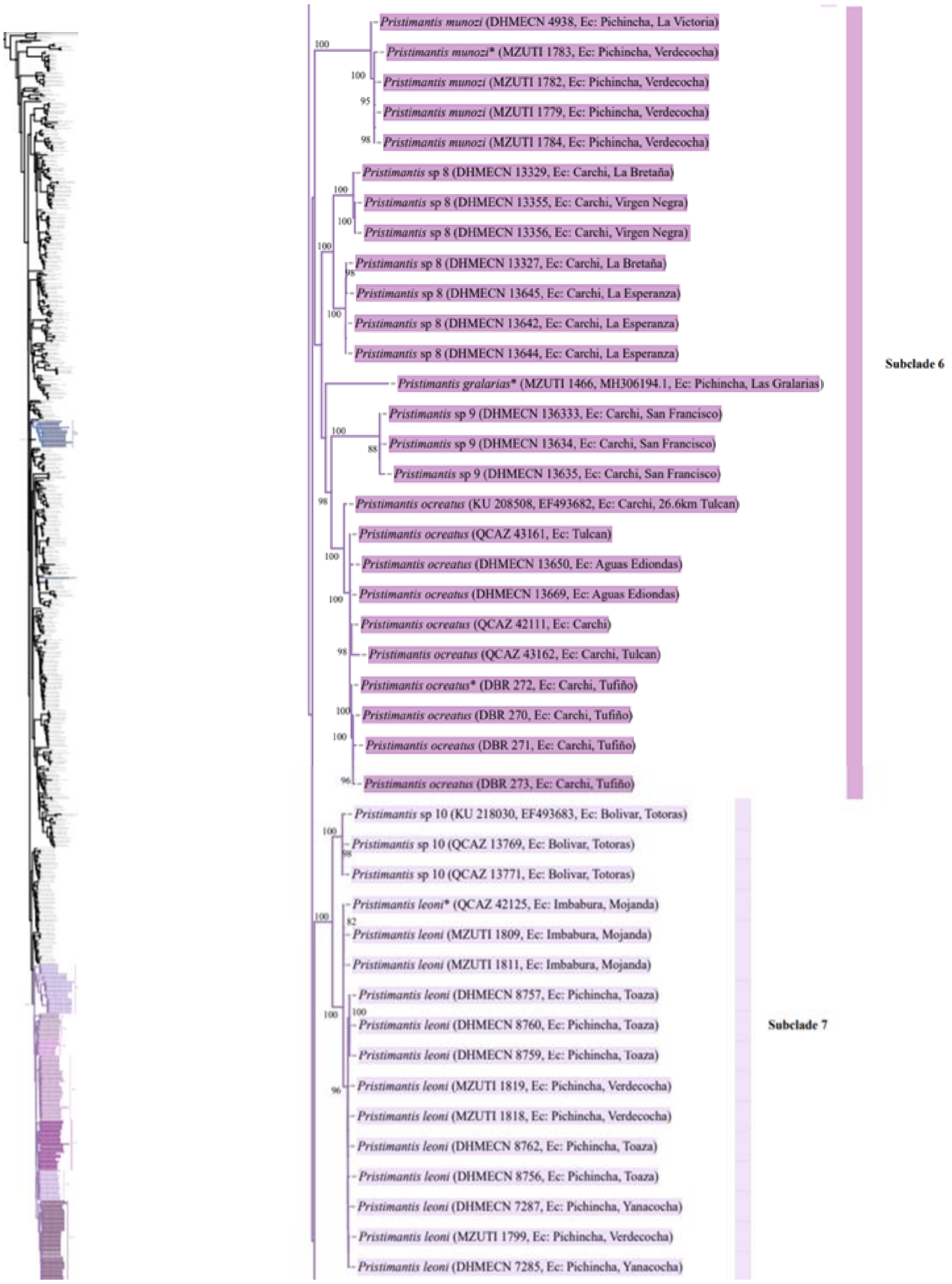

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Figure 4. Continued

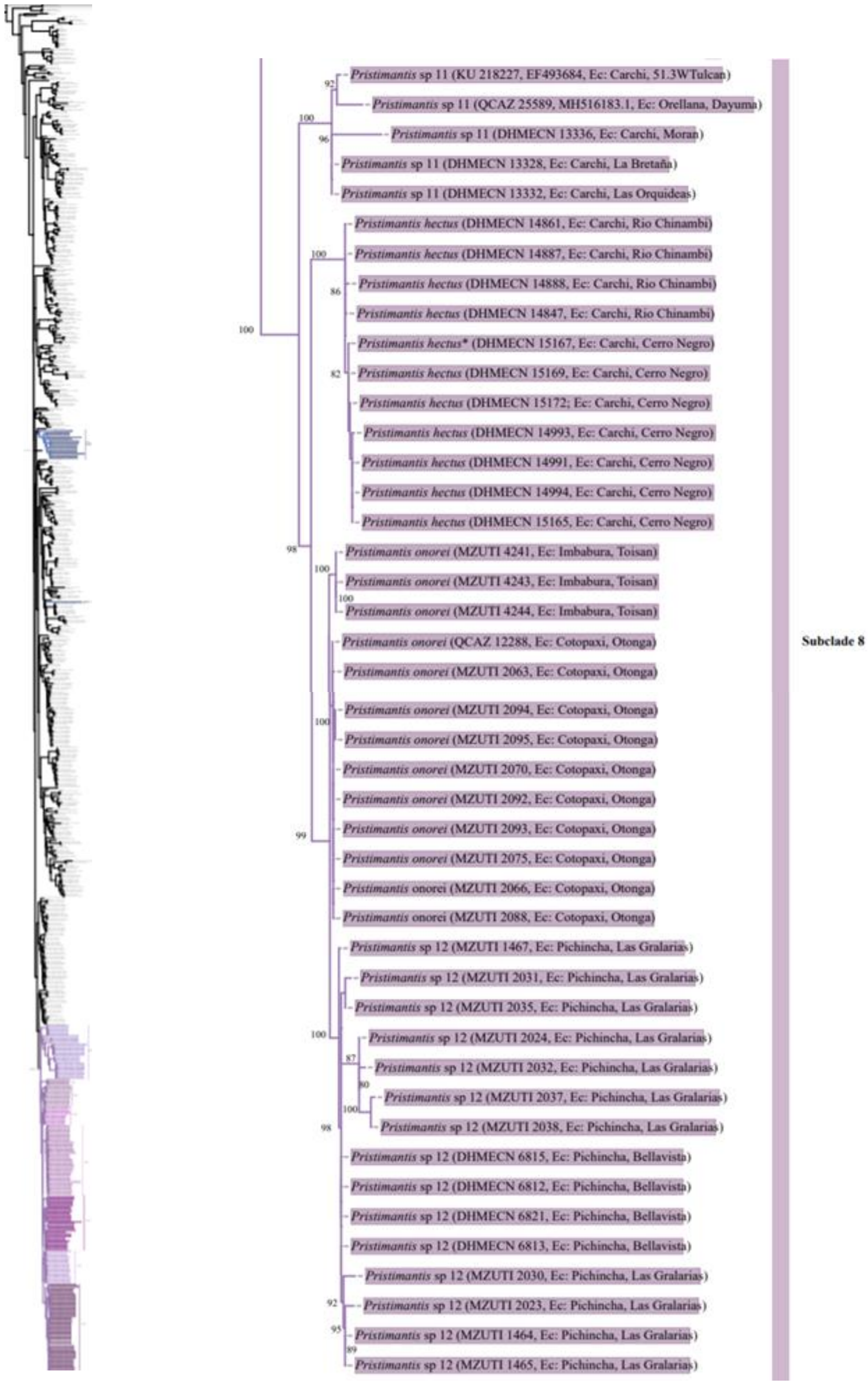

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Figure 4. Continued

Table 2. Uncorrected genetic distance (16S) among taxa that form part of the *Pristimantis myersi* species group, as defined herein. **1.** *Pristimantis celator*; **2.** *P. festae*; **3.** *P. gladiator*; **4.** *P. gralarias*; **5.** *P. hectus*; **6.** *P. jubatus*; **7.** *P. leoni*; **8.** *P. munozi*; 9. *P. mutabilis*; 10. *P. myersi*; **11.** *P. ocreatus*; **12.** *P. onorei*; **13.** *P. pyrrhomerus*; **14.** *P. sirnigeli*; **15.** *P.* aff. *verecundus*; **16.** *P. verecundus*; **17.** *Pristimantis* sp. 1; **18.** *Pristimantis* sp. 2; **19.** *Pristimantis* sp. 3; **20.** *Pristimantis* sp. 4; **21.** *Pristimantis* sp. 5; **22.** *Pristimantis* sp. 6; **23.** *Pristimantis* sp. 7; **24.** *Pristimantis* sp. 8; **25.** *Pristimantis* sp. 9; **26.** *Pristimantis* sp. 10; **27.** *Pristimantis* sp. 11; **28.** *Pristimantis* sp. 12.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
1	-																										
2	0.09	-																									
3	0.09	0.02	-																								
4	0.07	0.08	0.07	-																							
5	0.07	0.08	0.08	0.07	-																						
6	0.07	0.09	0.08	0.08	0.08	-																					
7	0.07	0.07	0.06	0.06	0.06	0.07	-																				
8	0.08	0.08	0.07	0.07	0.08	0.09	0.06	-																			
9	0.07	0.13	0.13	0.12	0.12	0.10	0.12	0.11	-																		
10	0.09	0.01	0.02	0.07	0.09	0.08	0.07	0.08	0.13	-																	
11	0.08	0.07	0.07	0.06	0.07	0.07	0.04	0.06	0.11	0.08	-																
12	0.08	0.08	0.07	0.07	0.03	0.07	0.06	0.08	0.12	0.08	0.06	-															
13	0.07	0.07	0.07	0.07	0.07	0.07	0.05	0.06	0.11	0.07	0.06	0.06	-														
14	0.08	0.08	0.07	0.07	0.08	0.07	0.06	0.06	0.11	0.08	0.07	0.07	0.03	-													
15	0.07	0.07	0.06	0.06	0.07	0.05	0.06	0.07	0.08	0.07	0.06	0.06	0.05	0.06	-												
16	0.09	0.13	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.13	0.12	0.12	0.11	0.13	0.08	-											
17	0.07	0.11	0.12	0.11	0.11	0.10	0.11	0.11	0.10	0.12	0.10	0.12	0.11	0.11	0.08	0.13	-										
18	0.07	0.12	0.12	0.11	0.13	0.12	0.11	0.11	0.10	0.12	0.11	0.13	0.12	0.12	0.09	0.13	0.08	-									
19	0.06	0.10	0.09	0.08	0.08	0.08	0.07	0.08	0.12	0.09	0.08	0.08	0.07	0.08	0.05	0.11	0.12	0.12	-								
20	0.07	0.06	0.05	0.06	0.06	0.07	0.04	0.05	0.11	0.06	0.05	0.06	0.04	0.05	0.05	0.10	0.10	0.11	0.08	-							
21	0.09	0.02	0.02	0.07	0.08	0.08	0.06	0.07	0.13	0.02	0.07	0.08	0.07	0.08	0.07	0.13	0.12	0.11	0.09	0.05	-						
22	0.09	0.02	0.02	0.08	0.09	0.09	0.07	0.07	0.13	0.02	0.07	0.08	0.07	0.09	0.07	0.13	0.11	0.12	0.09	0.06	0.02	-					
23	0.09	0.01	0.02	0.08	0.09	0.09	0.07	0.07	0.13	0.01	0.07	0.08	0.08	0.08	0.07	0.13	0.12	0.12	0.09	0.05	0.02	0.02	-				

24	0.07	0.07	0.06	0.07	0.08	0.08	0.05	0.06	0.12	0.07	0.05	0.07	0.06	0.07	0.07	0.11	0.11	0.11	0.07	0.05	0.07	0.07	0.07	-			
25	0.09	0.08	0.08	0.07	0.09	0.08	0.06	0.07	0.12	0.08	0.05	0.09	0.07	0.08	0.07	0.13	0.11	0.11	0.08	0.06	0.07	0.07	0.08	0.06	-		
26	0.07	0.07	0.06	0.06	0.06	0.07	0.02	0.06	0.11	0.07	0.05	0.06	0.04	0.06	0.05	0.11	0.11	0.11	0.07	0.04	0.06	0.07	0.07	0.05	0.06	-	
27	0.08	0.09	0.08	0.08	0.06	0.09	0.07	0.08	0.12	0.09	0.06	0.04	0.07	0.08	0.07	0.13	0.11	0.12	0.10	0.07	0.08	0.09	0.09	0.07	0.08	0.06	-
28	0.09	0.10	0.08	0.09	0.05	0.09	0.07	0.08	0.13	0.10	0.07	0.03	0.08	0.09	0.08	0.13	0.12	0.13	0.09	0.07	0.09	0.09	0.09	0.08	0.09	0.08	0.05

With a relatively good population sampling of several species of the *P. myersi* group, we found in the resulting topology, at least 28 (16 species and 12 new candidate species) well-supported lineages (Figure 4). In order to facilitate visualization and the discussion of biogeographic patterns, we organized these lineages in eight evolutionary subclades. Genetic distance within subclades is an average 0.2% (0.0–1.2). Many of the subclades correspond to lineages that are also diagnosable by morphology, but others are composed by cryptic species. Uncorrected pairwise distances for the 16S dataset are presented in Table 2. Below we present our results exclusively for the subclades that represent species complexes.

Subclade 1 (Figure 5): Well-supported clade (99%), composed by *P. jubatus* (Cauca, Colombia), *Pristimantis* aff. *verecundus* (Cotopaxi province, Ecuador), *Pristimantis verecundus sensu stricto* (Carchi province, Ecuador), *P. celator* (Carchi province, Ecuador), *P. mutabilis sensu stricto* (Pichincha province, Ecuador), and two potentially undescribed species, *Pristimantis* sp. 1 (Pichincha province, Ecuador) and *Pristimantis* sp. 2 (Carchi province, Ecuador). Genetic distances within this subclade are 0.05–0.13% (Table 2). In addition, the populations of *P. verecundus* are distributed only to the north of Ecuador (Carchi province) and south of Colombia.

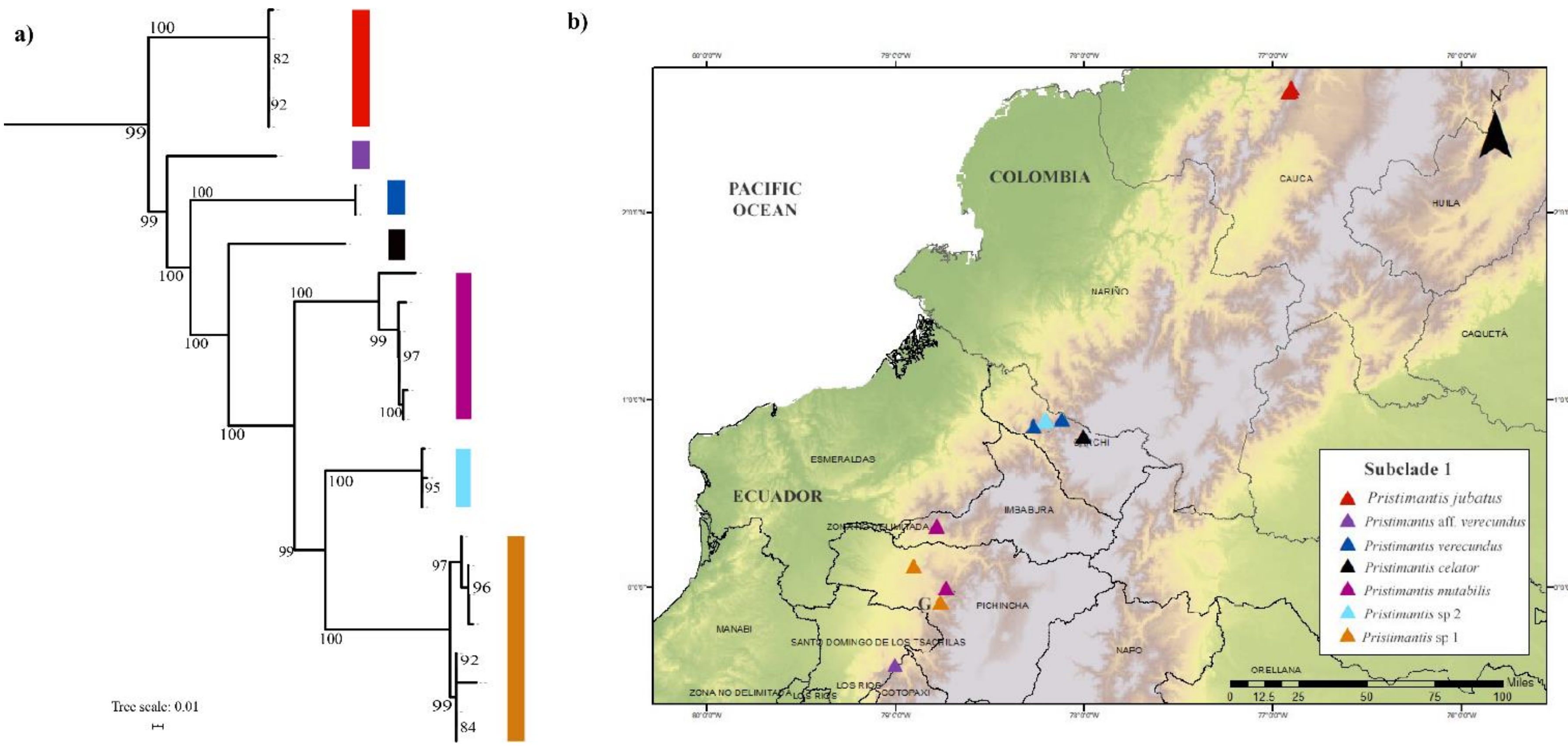

Figure 5. Phylogeny and distribution of *Pristimantis myersi* group, Subclade 1. Colors of lineages in the phylogenetic tree correspond to colors of triangles on the map.

Subclade 2 (Figure 6): This clade is formed by individuals previously identified as *Pristimantis hectus* (Cauca, Colombia). However, we considered this lineage as an undescribed taxon (*Pristimantis* sp. 3) that it is not closely related the sequences from populations collected nearby the type locality of *P. hectus*.

Subclade 3 (Figure 6): A clade composed by *Pristimantis pyrrhomerus* (Cotopaxi province, Ecuador), collected nearby to the type locality (east edge of Pilaló), and *P. sirnigeli*, collected from Pichincha (Reserva Ecológica Verdecocha, Pichincha, Ecuador). The validity of these two taxa are also well stablished morphologically; *Pristimantis pyrrhomerus* is easily distinguished from *P. sirnigeli* (the latter exhibiting longer and thinner fingers and more expanded digital discs than *P. pyrrhomerus*) and genetic distances between lineages are 0.03% (Table 2).

Subclade 4 (Figure 6): Composed only by one new candidate species *Pristimantis* sp. 4 from Carchi province, Ecuador.

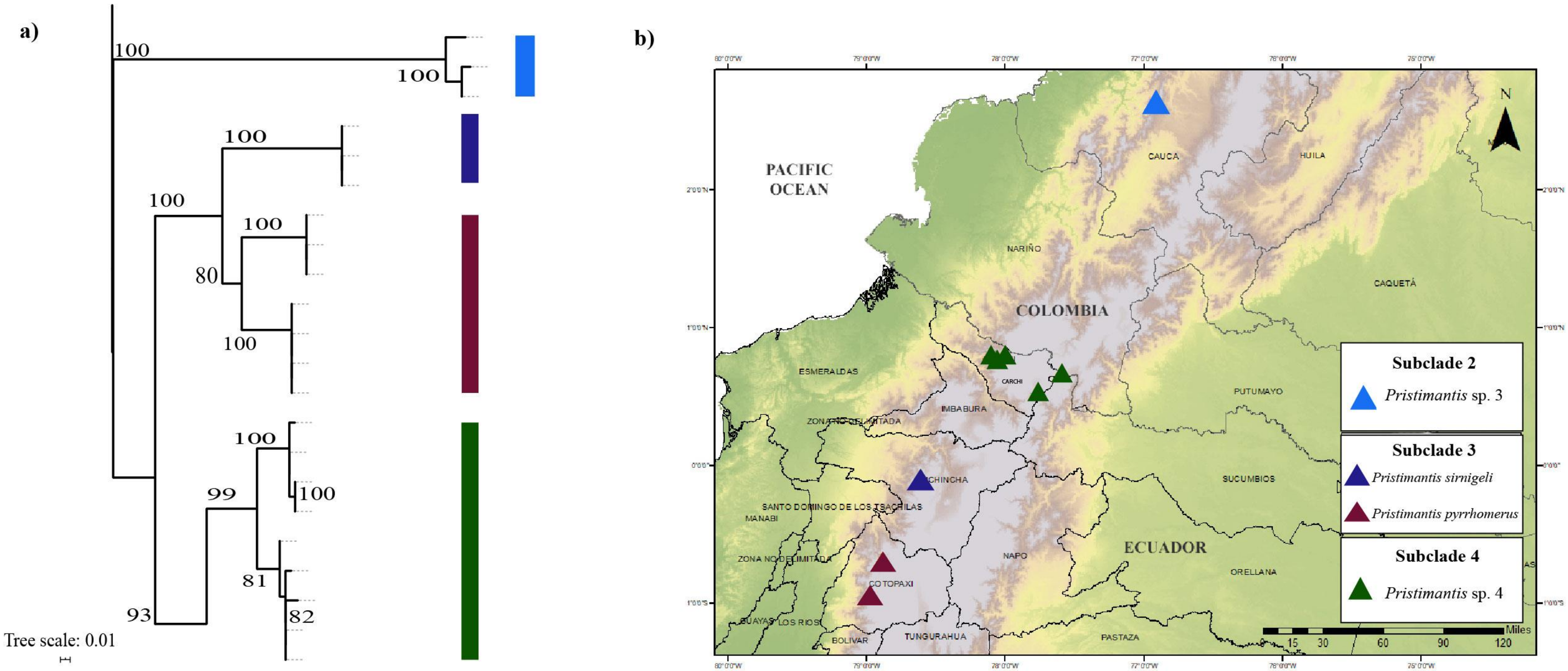

Figure 6. Phylogeny and distribution of *Pristimantis myersi* group, Subclades 2–4. Colors of lineages in the phylogenetic tree correspond to colors of triangles on the map.

Subclade 5 (Figure 7): A well-supported clade (100%), formed by five lineages. The first lineage corresponds to *P. gladiator sensu stricto* and includes individuals from the type locality and nearby (Papallacta-Cuyuja, eastern versant of the Ecuadorian Andes). This lineage is sister to *P. myersi* (Imbabura province, Ecuador), *P. festae sensu stricto* (Napo province, Ecuador), *Pristimantis* sp. 7 (Carchi province, Ecuador), *Pristimantis* sp. 5 (Bosque Protector Cerro Candelaria, Tungurahua province, Ecuador) and *Pristimantis* sp. 6 (Reserva Machay, Tungurahua province, Ecuador). *Pristimantis* sp. 5 and sp. 6 are virtually indistinguishable in terms of morphology; these lineages are geographically close, being separated by the Pastaza river valley, exhibiting a 2% genetic divergence, we suspect they may be cryptic species.

Subclade 6 (Figure 8): A clade composed by *Pristimantis munozi* (Verdecocha, Pichincha province, Ecuador), *P. gralarias* (Reserva Las Gralarias, Pichincha province, Ecuador), *P. ocreatus* (Tufiño, Carchi province, Ecuador), *Pristimantis* sp. 8 (El Carmelo, Carchi province, Ecuador) and *Pristimantis* sp. 9 (Pioter, Carchi province, Ecuador). *Pristimantis* sp. 8 and sp. 9 are virtually distinguishable in terms of coloration pattern; these lineages are geographically close, being separated by the foothills of the Ecuadorian Andean valley, and genetic distances between lineages are 0.07% (Table 2).

Subclade 7 (Figure 9): A well-supported clade (bootstrap support = 100%) that is composed by *Pristimantis leoni* and *Pristimantis* sp. 10. The sampling of *P. leoni* contains individuals from six localities, including the type locality (Nudo de Mojanda), from the western slopes of the Ecuadorian Andes. *Pristimantis* sp. 10 is composed by individuals coming from Bosque Protector Cashca Totoras (Bolívar Province, southern Ecuador), also on the western versant. Based on its reciprocal monophyly with *P. leoni*, and their genetic distances (between-subclades 1.3–3%) we proposed that, *Pristimantis* sp. 10 is a candidate species, cryptic species and the southern taxonomic replacement of *P. leoni*.

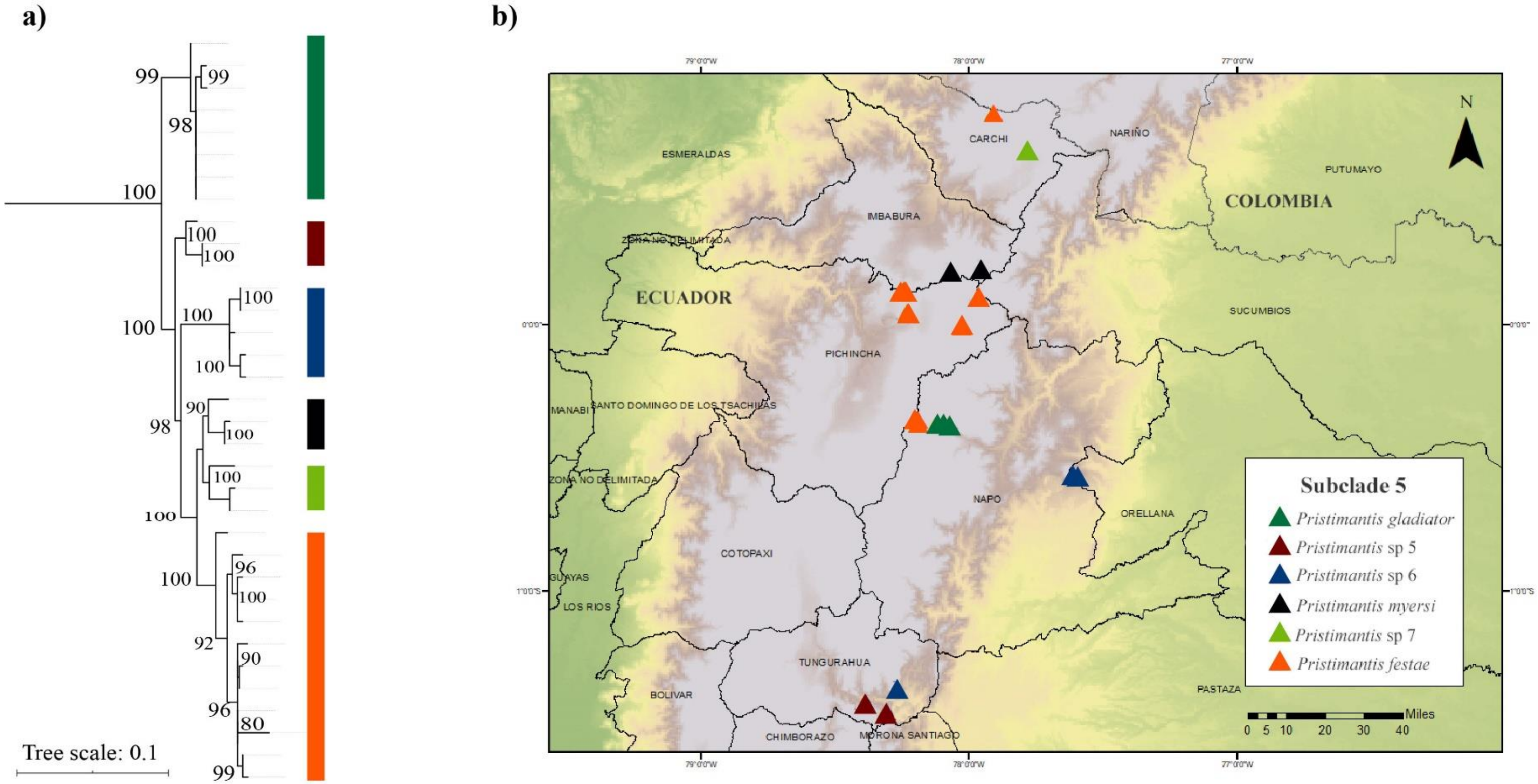

Figure 7. Phylogeny and distribution of *Pristimantis myersi* group, Subclade 5. Colors of lineages in the phylogenetic tree correspond to colors of triangles on the map.

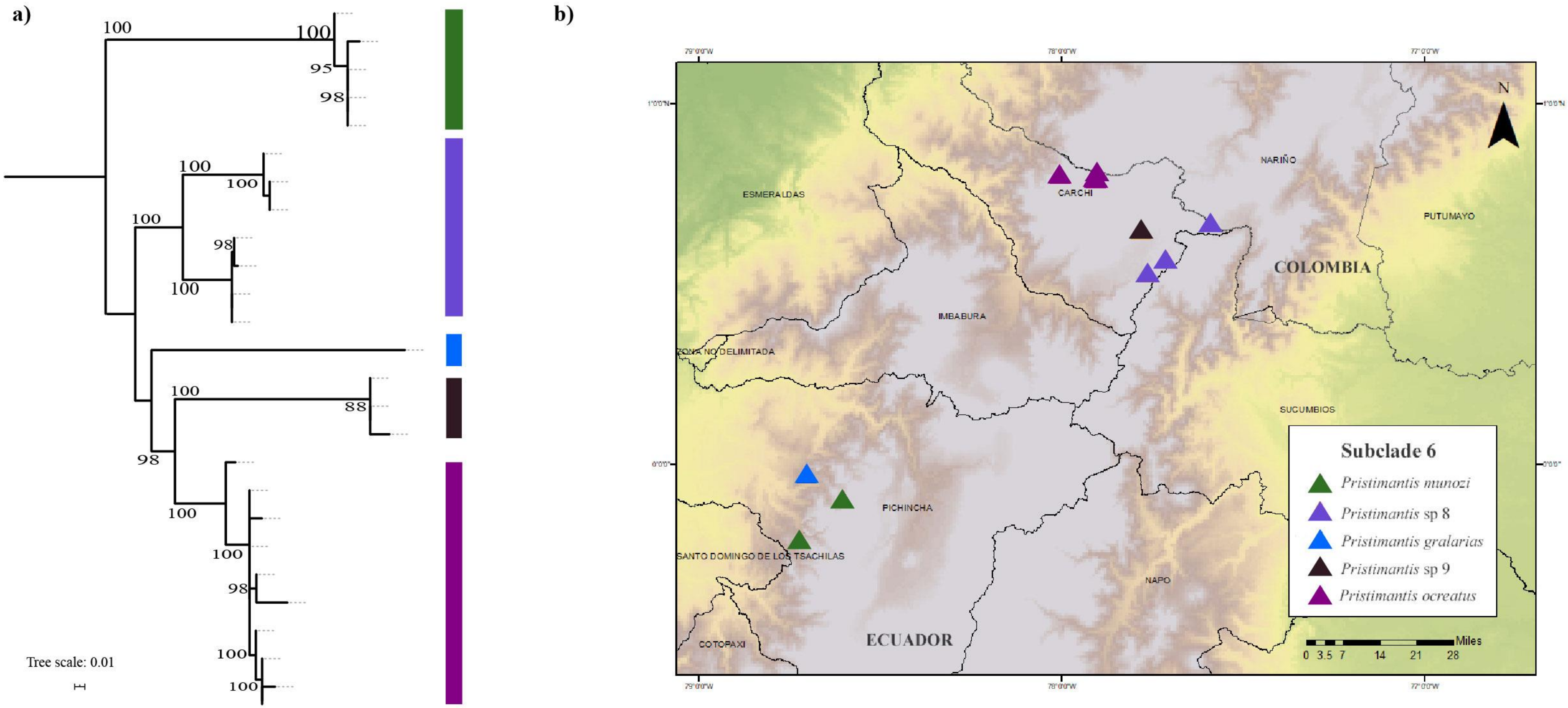

Figure 8. Phylogeny and distribution of *Pristimantis myersi* group, Subclade 6. Colors of lineages in the phylogenetic tree correspond to colors of triangles on the map.

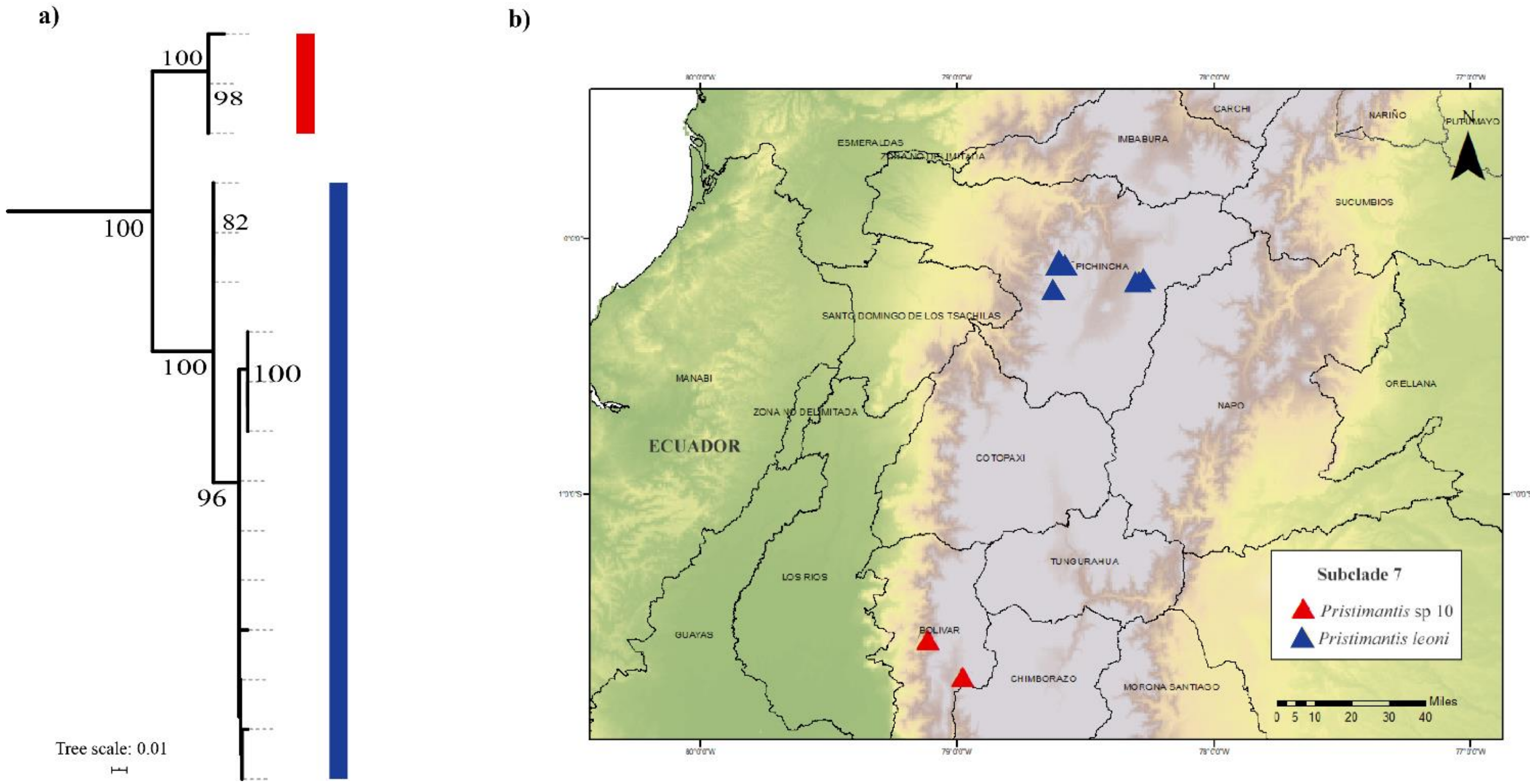

Figure 9. Phylogeny and distribution of *Pristimantis myersi* group, Subclade 7. Colors of lineages in the phylogenetic tree correspond to colors of triangles on the map.

Subclade 8 (Figure 10): A well-supported clade (bootstrap = 100%) composed by four lineages. *Pristimantis hectus sensu stricto* is represented by individuals found 100 km from the type locality (Reserva La Planada, Colombia). This lineage is sister to *P. onorei* (Reserva Otonga, Pichincha province, Ecuador), *Pristimantis* sp. 12 (Las Gralarias, Pichincha province, Ecuador) and *Pristimantis* sp. 11 (Moran, Carchi province, Ecuador). Our results show that *P. lucidosignatus* is genetically identical to *P. onorei* from Reserva Otonga (Table 3); morphologically, the most diagnostic trait in *P. lucidosignatus* is the presence of a light marking on the shank (Rodder & Schmitz, 2009), but this characteristic is actually polymorphic in *P. onorei*. Therefore, we place *P. lucidosignatus* Rodder & Schmitz 2009 under the synonymy of *P. onorei* Rodder & Schmitz (2009). In addition, we restrict *P. hectus* to northern Ecuador (Carchi province) and southern Colombia (Fig. 10).

Table 3. Samples of *Pristimantis onorei* and *P. lucidosignatus* included in the phylogeny; note that, genetically and morphologically, these two species turned out to be a single taxon.

Museum Code	Scientific Name (Before)	Scientific Name (This study)	Country	Locality	Longitude	Latitude	Elevation (m.s.a.l)
MZUTI 2063	*Pristimantis onorei*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2094	*Pristimantis lucidosignatus*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2095	*Pristimantis lucidosignatus*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2070	*Pristimantis onorei*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2092	*Pristimantis lucidosignatus*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115

MZUTI 2093	*Pristimantis lucidosignatus*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2075	*Pristimantis onorei*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2066	*Pristimantis onorei*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115
MZUTI 2088	*Pristimantis onorei*	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115

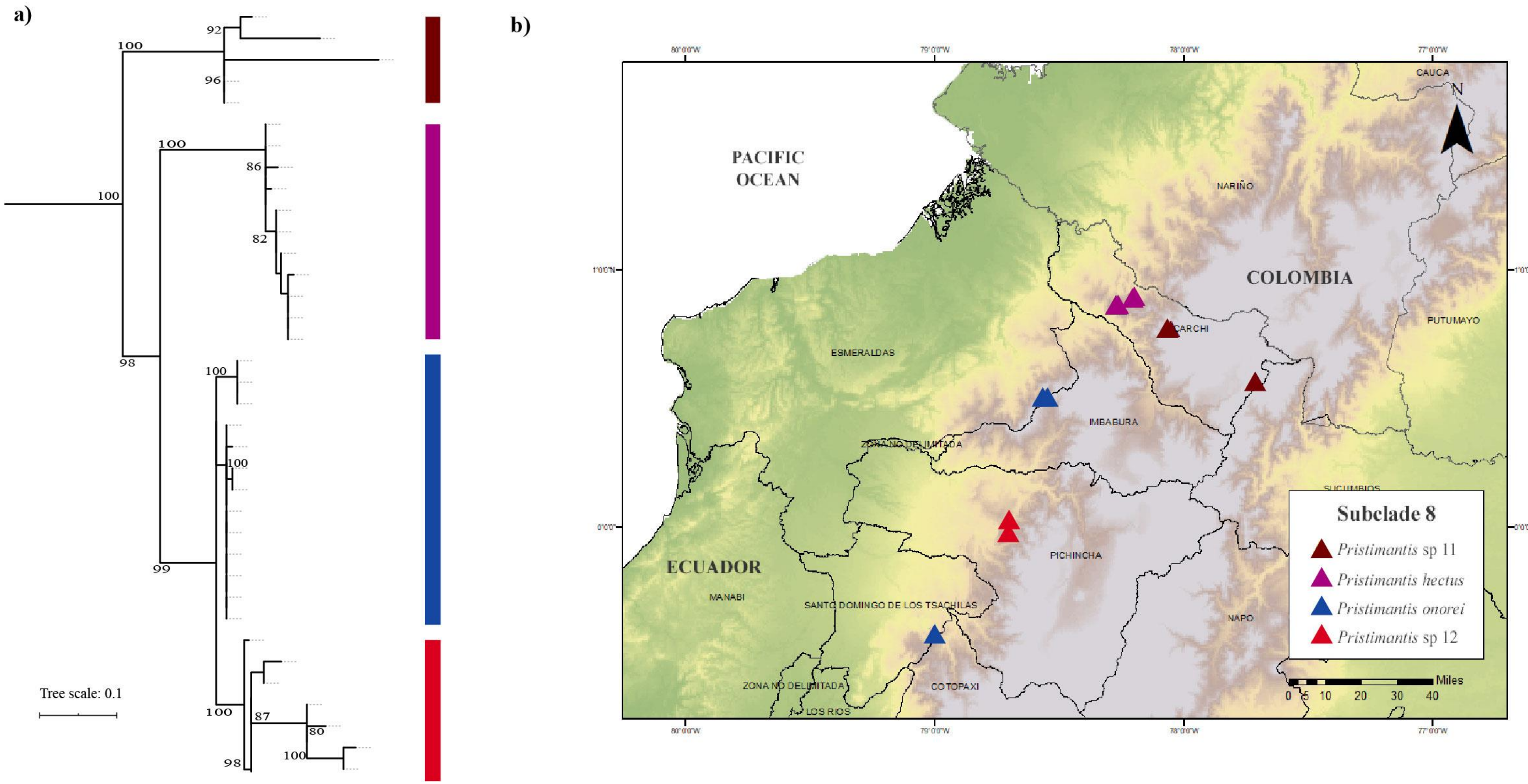

Figure 10. Phylogeny and distribution of *Pristimantis myersi* group, Subclade 8. Colors of lineages in the phylogenetic tree correspond to colors of triangles on the map. Note that individuals that were recognized as *P. lucidosignatus* (*Pristimantis* sp. 12) are, actually, a synonym of *P. onorei*.

CHAPTER IV: DISCUSSION

Monophyly, phylogenetic relationships, and diagnosis of the *Pristimantis myersi* group

Previous studies on the phylogenetic relationships of the genus *Pristimantis* inferred the *P. myersi* group —Hedges et al. (2008); *sensu* Lynch & Duellman (1997) — as monophyletic (Gonzales-Duran et al., 2017; Guayasamin et al., 2015; Guayasamin et al., 2018; Hedges et al., 2008; Heinicke et al., 2007; Pinto et al., 2012; Pyron & Wiens, 2011). However, all these studies had a very low taxon sampling of the group, including sequences of only up to six species: *P. festae*, *P. leoni*, *P. ocreatus*, *P. pyrrhomerus*, *P. myersi* and *P. gralarias*, representing 25% of the species currently assigned to this group. In contrast, the present analysis includes 19 of the 24 species allocated to the group (Appendix 1). Our topology supports the monophyly of the *Pristimantis myersi* group, excluding *P. albujai*, *P. bicantus. P. sambalan* and *P. nelsongalloi*. These four taxa were assigned to the *P. myersi* group based on morphologic traits (Brito et al., 2017; Guayasamin & Funk, 2009; Valencia et al., 2019). A clade formed by *Pristimantis bicantus*, *P. sambalan* and *P. nelsongalloi* is sister to a large assemblage integrated by several species of the *curtipes*, *devillei*, *surdus* and *unistrigatus* groups. *Pristimantis albujai* is sister to species in the *bromeliaceus, schultei* and *galdi* groups. Cases like this, where morphology converges in phylogenetically distant taxa is relatively common in the rainfrog family (Craugastoridae), where several phenetic groups have turn out to be non-monophyletic (Hedges et al., 2008; Padial et al., 2014b). Based on our results, and in order to maintain the *P. myersi* group monophyletic, we exclude *Pristimantis albujai*, *P. bicantus. P. sambalan and P. nelsongalloi* from the *myersi* group. We refrain to assign *Pristimantis albujai*, *P. bicantus. P. sambalan* and *P. nelsongalloi* to any other species group.

Regarding to *Pristimantis verecundus*, *P. celator* and *P. jubatus* are currently not assignable to any species group (Padial et al., 2014b). Nevertheless, morphologically these species match the diagnosis of the *P. myersi* species group in having robust bodies, short snouts, relative narrow heads, limbs moderately short, and Finger I shorter than II (García-R & Lynch, 2006; Lynch, 1976c; Lynch & Burrowes, 1990; Rojas-Runjaic et al., 2014). Based on the traits mentioned above, we include these three species in the *P. myersi* clade.

Thus, based on the species content of the *Pristimantis myersi* group proposed herein, the diagnosis of this clade is as follows (modified from Lynch, 1981; Hedges et al., 2008): small body size (SVL in females < 19.5 mm (range 15.8-34.6 mm) and in males < 16.4 mm (range 12.6-20.5 mm), short snout, robust body, Toe V longer than Toe III, Finger I shorter than II and cranial crests absent. In addition, all the species in the group (except the arboreal *P. mutabilis* and *P. verecundus*) are found on low vegetation (< 150 cm above the ground) or on the forest floor (Table 1). In addition, the *Pristimantis myersi* group contains (modified from Guayasamin et al., 2018; Lynch & Duellman 1997; Padial et al., 2014b): *P. celator*, *P. festae*, *P. floridus*, *P. gladiator*, *P. gralarias*, *P. hectus*, *P. jubatus*, *P. leoni*, *P. munozi*, *P. mutabilis*, *P. myersi*, *P. ocreatus*, *P. onorei*, *P. pyrrhomerus*, *P. repens*, *P. scopaeus*, *P. sirnigeli*, *P. verecundus* and *P. xeniolum* (Table 1 – 4).

Taxonomic remarks

Taxonomic remarks are provided for *Pristimantis lucidosignatus* and *P. onorei* were described by Rödder & Schimtz (2009); these authors noted that both species are syntopic and closely related. *Pristimantis lucidosignauts* is mainly distinguished from the former, and from all other species of the *Pristimantis myersi* group, by the absence of lateral fringes on fingers, and by the presence of prominent light markings on the shanks (Fig. 11). However, the presence of lateral keels (or fringes) on fingers can be verified in the topotypes and in the photographs of the right hand of the holotype presented in the original description (Rödder & Schimtz, 2009). The light markings on shanks are polymorphic in *P. onorei* (Fig. 11) and, thus, not valid as a diagnostic trait. Therefore, as mentioned above, we place *P. lucidosignatus* Rödder & Schimtz (2009) under the synonym of *P. onorei* Rödder & Schimtz (2009).

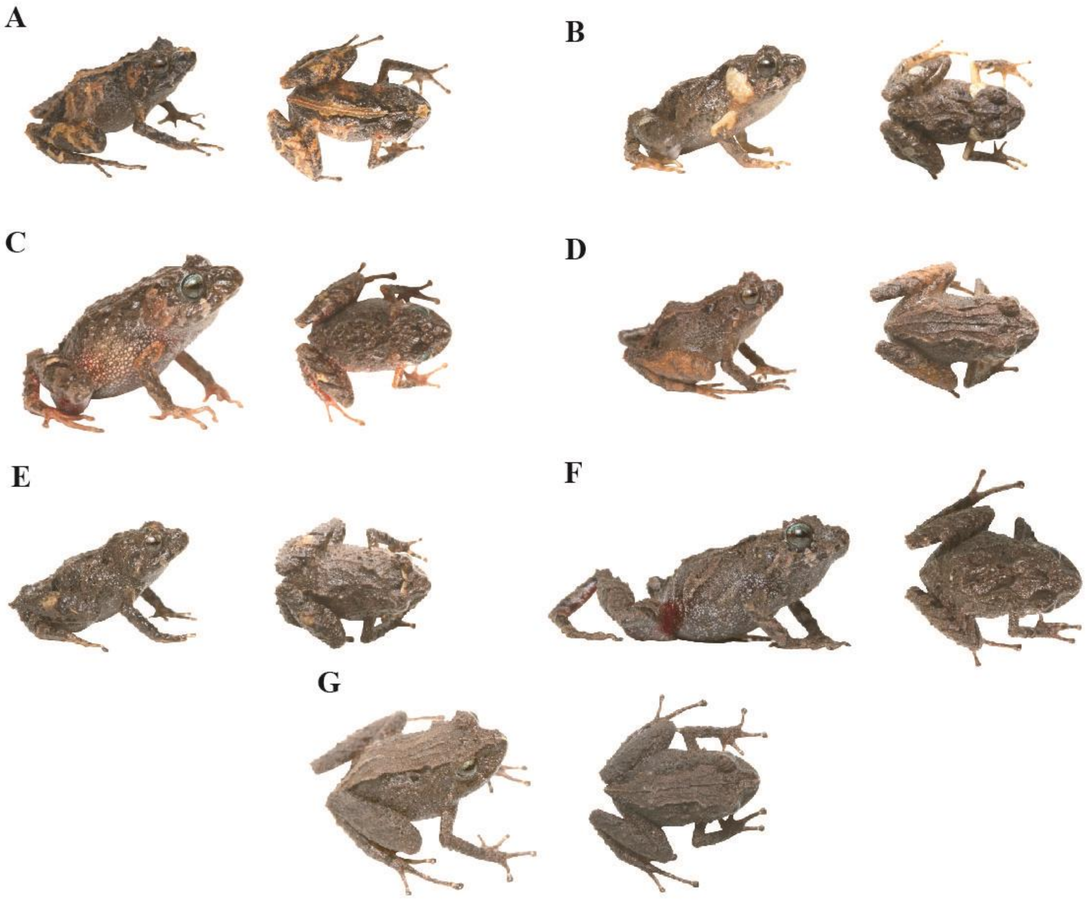

Figure 11. Dorsal and lateral variation in color pattern of *Pristimantis onorei*. First to fourth rows (from left to right): **A.** MZUTI 2063; **B.** MZUTI 2095 (male); **C.** MZUTI 2094 (female); **D.** MZUTI 2064; **E.** MZUTI 2065 (male); **F.** MZUTI 2062 (female); **G.** MZUTI 2066. Photos by Lucas Bustamante (not scale). Note that the color polymorphism of *P. onorei* includes that pattern reported for *P. lucidosignatus* (B, E); thus *P. lucidosignatus* is placed under the synonymy of *P. onorei*.

Speciation

Lynch & Duellman (1997) described three general patterns of speciation for Ecuadorian *Pristimantis*: latitudinal, altitudinal, and trans-Andean replacement. Our observations match with Lynch & Duellman (1997) in that species of the *P. myersi* group mainly follow a pattern of latitudinal replacement rather than altitudinal or trans-Andean. This pattern agrees with the hypothesis that long montane ranges are fragmented by narrow transverse valleys, promoting allopatric divergence by limiting contact among contiguous populations and that distinctive environmental conditions, moisture and not temperature, as the limiting climatic factor determining the occurrence of these species in high Andean and explain the limited distribution of numerous montane frogs species (Burrowes et al., 2020; De la Riva, 2020; Graves, 1988; Remsem, 1984; Wollenberg et al., 2008). A scenario of mostly allopatric speciation also would explain the presence of cryptic species, since selective pressures (biotic and abiotic) are similar for allopatric species, which means that ancestral morphologies and behaviors are retained (Graham et al., 2004; Peterson et al., 1999).

The pattern of diversification of the *Pristimantis myersi* group is congruent with other studies from mountainous areas, which report allopatric speciation (e.g., Arteaga et al., 2016; Coloma et al., 2012; Guayasamin et al., 2017; Guayasamin et al., 2020; Jungfer et al., 2013; Lynch & Duellman, 1997; Yánez et al., 2018). Some river canyons that disrupt gene flow in some species are Pastaza, Mira, Guayllabamba and Jubones (Krabbe, 2008; Prieto et al., 2018). Most sister species within the Andean *Pristimantis myersi* group fit into this speciation pattern, although there are also examples of species that have reached higher ecological zones (e.g., *P. festae*, habits only paramo). The canyon of the Pastaza River separates *Pristimantis sp. 4 and sp. 5*, and it seems that amphibian microendemisms might mirror those found in orchids and angiosperms (Jost, 2004; Jost & Shepard, 2017; Matsuda, 2018).

Diversity of the *Pristimantis myersi* group

Numerous *Pristimantis* species remains undescribed because of the limited number of useful morphological traits for such a diverse genus. Then, it should not surprise us that cryptic diversity is rampant when using alternative approaches, such as molecular phylogenies (Díaz et al., 2012; Fouquet et al., 2012; Guayasamin et al., 2017; Hutter et al., 2017; Kieswetter & Schneider, 2013; Padial & De la Riva, 2009; Padial et al., 2012; Páez & Ron, 2019; Urgiles et al., 2019).

Based on the results of this study, the number of species of the *Pristimantis myersi* group, as defined herein, would increase from 19 species to 31 (19 already recognized + 12 new candidate species). Thus, our study shows that 39% of the diversity of the *P. myersi* group remains undescribed. Previous estimates of cryptic diversity on Neotropical amphibians range between 22–400%, but these studies are restricted to the Amazon region and are based on non-terraranans species (Fouquet et al., 2007; Funk et al., 2011; Gehara et al., 2014; Jaramillo et al., 2020; Jungfer et al., 2013; Rojas et al., 2018). To date, no estimation of cryptic diversity for the Andean amphibians has been published. Then, our estimates, although restricted to a relatively small group, may apply to other Andean anurans with similar biological characteristics (e.g. Terrarana).

A limitation of this study is that it is based on a single mitochondrial gene (16S) because of funding limitations. However, 16S is a gene with numerous virtues (see Rubinoff & Holland, 2005), including: (i) it is the gene with the highest number of sequences available in public repositories (i.e., Genbank), facilitating comparisons across taxa and geography; (ii) it is ideal to study closely related taxa because of the very low recombination rate (Piganeau et al., 2004), maternal inheritance, simple genetic structure, reduced effective population size, and rapid evolution (Avise et al., 1983; Moritz et al., 1987). The main limitation of using a single gene is the risk that the inferred gene tree may be different from true species tree. Therefore, it is advisable to use more than one marker, coming from different organelles (e.g. nuclear, mitochondrial DNA) (Hills, 2019; Nakhleh, 2013; Vences et al., 2005). Nevertheless, is important to note that mitochondrial genes are informative and are the backbone for most amphibian phylogenies (see Frost et al., 2006; Pyron & Wiens, 2011; Guayasamin et al., 2020), and that inferred mitochondrial trees are hypothesis that should be tested with larger datasets.

Conservation implications

Clarifying taxonomic uncertainties is imperative, not only to know the real species richness within the group, but also for conservation purposes. Our study provides information that affects the conservation status of several species. Currently, the conservation status of five species of the *Pristimantis myersi* group have not been even assessed by the IUCN (Table 4). Moreover, the extinction risk of the several species requires a re-evaluation for the following reasons: (1) most species in the *Pristimantis myersi* group have distribution ranges that are actually smaller than currently recognized; (2) most species inhabit areas of paramo and montane forest that are confronted by severe anthropogenic factors (Menéndez-Guerrero & Graham, 2013); (3) several species (e.g. *Pristimantis festae*, *P. hectus* and *P. verecundus*) actually represent species complexes. Specifically, we consider that seven species within the group need to be re-evaluated in their IUCN conservation status because they exhibit smaller distributions than previously thought. These species are: *Pristimantis festae*, *P. gladiator*, *P. hectus*, *P. leoni*, *P. ocreatus*, *P. pyrrhomerus*, *P. sirnigeli* and *P. verecundus* (see Table 4). Finally, if the lineages identified as putative species are described, this would require assessing their conservation status and, hopefully, delimiting priority areas for their conservation.

Table 4. Species in the *Pristimantis myersi* group, with information on the distribution, distribution (this study), IUCN conservation status, and observations regarding the IUCN status. (Conservation status follows IUCN categories: Least concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN) and No Evaluated (NE)) and No information (−).

Species	Distribution	IUCN	Observations IUCN	Sources
P. celator	Southern Colombia, Department of Nariño and Andes, northern in Ecuador, Carchi, Imbabura, Pichincha and Cotopaxi provinces.	VU	—	Frost, 2020; IUCN, 2019a; Lynch, 1976c.
P. festae	On the eastern slope of the Ecuadorian Andes, Sucumbíos, Napo and Tungurahua provinces and near southern Colombia. This study: Carchi, Pichincha, Imbabura, Napo and excluded Tungurahua province.	EN	Requires re-evaluation	Coloma et al., 2004a; Frost, 2020; Peracca, 1904.
P. floridus	Western flank of the Andes of Ecuador, Cotopaxi, Imbabura, and Pichincha provinces.	VU	—	Frost, 2020; Lynch & Duellman, 1997; Lynch et al., 2004.
P. gladiator	On the Amazonian slopes of the Andes Carchi, Napo and Imbabura provinces; adjacent Putumayo, Colombia. This study: Only in Napo province Ecuador, and the other localities excluded.	VU	Requires re-evaluation	Frost, 2020; IUCN, 2019b; Lynch, 1976a.
P. gralarias	Known only from the type locality, Ecuador (Pichincha province).	NE	—	Frost, 2020; Guayasamin et al., 2018.
P. hectus	Western slope of the Cordillera Occidental in the Department of Nariño, Colombia; northwestern Ecuador (Esmeraldas and Imbabura provinces). This study: Carchi province, Ecuador.	VU	Requires re-evaluation	Frost, 2020; IUCN, 2019c; Lynch & Burrowes, 1990.
P. jubatus	Known only from the type locality, Colombia (El Tambo, Munchique National Park).	NT	—	Frost, 2020; Garcia & Lynch, 2006; IUCN, 2017.
P. leoni	Southern Colombia and Amazonian slopes of the Andes in northern Ecuador (Carchi, Imbabura, Pichincha, Sucumbíos and Santo Domingo de los Tsáchilas provinces). This study: Only in the Imbabura and Pichincha provinces, Ecuador.	LC	Requires re-evaluation	Castro et al., 2010; Frost, 2020; Lynch, 1976.
P. munozi	Known only from the type locality (Pichincha province, Ecuador)	NE	—	Frost, 2020; Rojas-Runjaic et al., 2014.

P. mutabilis	Ecuador, Pichincha and Imbabura provinces.	EN	—	Frost, 2020; Guayasamin et al., 2015; IUCN, 2016.
P. myersi	On the southern, Cordillera Central in Colombia and in the Andes of Ecuador, Imbabura and Sucumbíos provinces.	LC	—	Frost, 2020; Goin & Cochran, 1963.
P. ocreatus	Ecuador, Carchi, Imbabura, Napo and Cotopaxi provinces. This study: Carchi province and we excluded the others provinces.	EN	Requires re-evaluation	Coloma et al., 2004b; Frost, 2020; Lynch, 1981.
P. onorei	Ecuador, Santo Domingo de los Tsáchilas, Pichincha and Cotopaxi provinces.	NE	—	Frost, 2020; Rödder & Schmitz, 2009.
P. pyrrhomerus	Ecuador, Pichincha, Imbabura, Carchi, Cotopaxi and Bolívar provinces. This study: Only Cotopaxi province, Ecuador.	EN	Requires re-evaluation	Coloma et al., 2004c; Frost, 2020; Lynch, 1976b.
P. repens	Colombia, Pasto and La Cruz, Department of Nariño.	EN	—	Frost, 2020; IUCN, 2019d; Lynch, 1984.
P. scopaeus	Known only from the type locality, Colombia (Municipal of Cajamarca).	LC	—	Frost, 2020; IUCN, 2019e; Lynch et al., 1996.
P. sirnigeli	Ecuador, Pichincha and Imbabura provinces. This study: Pichincha province, Ecuador.	NE	Requires re-evaluation	Frost, 2020; Yánez-Muñoz et al., 2011.
P. verecundus	On the western flank of the Andes in southern Colombia (Nariño Department) and Ecuador (Carchi, Cotopaxi, Esmeraldas, Pichincha, Imbabura, Santo Domingo de los Tsáchilas provinces). This study: Only in Carchi province, Ecuador. We excluded the other provinces.	NT	Requires re-evaluation	Frost, 2020; IUCN; 2018; Lynch & Burrowes, 1990.
P. xeniolum	Known only from the type locality, Colombia (Department of Valle del Cauca).	VU	—	Frost, 2020; IUCN, 2019f; Lynch, 2001.

CHAPTER V: CONCLUSIONS AND RECOMMENDATIONS

Pristimantis myersi group, after the exclusion of *P. albujai*, *P. bicantus*, *P. nelsongalloi* and *P. sambalan*, is monophyletic. Based on genetic evidence, we place *P. lucidosignatus* under the synonymy of *P. onorei*. Our study substantially contributes to better understand the relationships among the species of the *Pristimantis myersi* group. Our estimate of genetic divergences and topologies, inferred from the ribosomal 16S rRNA, provides a relatively well-supported dataset, but in order to add robustness to our analyses, and given the highly structured topology, geographic and genetic sampling must be improved. In the species of the *P. myersi* group mainly follow a pattern of latitudinal replacement rather than altitudinal or trans-Andean, promoting allopatric divergence. A scenario of mostly allopatric speciation also would explain the presence of cryptic species. In addition, obtaining calls would help solving species boundaries, particularly among sympatric lineages. We recommended an exhaustive taxonomic review of the *Pristimantis myersi* species group, as well as a re-evaluation of the conservation status of each species given the data provided herein.

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Appendix 1. Localities and voucher information of the new sequences generated in this study. **DHMECN:** División de Herpetología Museo Ecuatoriano de Ciencias Naturales; Quito, Ecuador. **MZUTI:** Museo de Zoología de la Universidad Tecnológica Indoamérica; Quito, Ecuador; **QCAZ**: Museo de Zoología de la Pontificia Universidad Católica de Ecuador, Quito, Ecuador.

Collection Code	Catalog Number	Scientific Name	Country	Locality	Longitude	Latitude	Elevation	GenBank
UVC	15847	*Pristimantis jubatus*	Colombia	Cali	2.6642	-76.9025	–	JN104665.1
UVC	15884	*Pristimantis jubatus*	Colombia	Cali	2.6464	-76.9086	–	JN104664.1
UVC	15877	*Pristimantis jubatus*	Colombia	Cali	2.6381	-76.9150	–	JN104663.1
UVC	15915	*Pristimantis jubatus*	Colombia	Cali	2.6642	-76.9025	–	JN370999.1
UVC	15920	*Pristimantis jubatus*	Colombia	Cali	2.6464	-76.9086	–	JN370990.1
QCAZ	12410	*Pristimantis* aff. *verecundus*	Ecuador	Cotopaxi, Otonga	-0.4189	-79.0039	1500	EF493686
DHMECN	14858	*Pristimantis verecundus*	Ecuador	Carchi, Maldonado	0.8609	-78.2724	–	–
DHMECN	14985	*Pristimantis verecundus*	Ecuador	Carchi, Maldonado	0.8940	-78.1176	–	–
KU	177684	*Pristimantis celator*	Ecuador	Carchi, Maldonado	0.8030	-78.0070	55	EF493685
MZUTI	2190	*Pristimantis mutabilis*	Ecuador	Pichincha, Reserva Las Gralarias	-0.0084	-78.7305	2063	KM675458.1
MZUTI	912	*Pristimantis mutabilis*	Ecuador	Imbabura, Bosque Protectos Los Cedros	0.3194	-78.7851	1845	KM675460.1
MZUTI	913	*Pristimantis mutabilis*	Ecuador	Imbabura, Bosque Protectos Los Cedros	0.3249	-78.7809	1651	KM675459.1
MZUTI	909	*Pristimantis mutabilis*	Ecuador	Imbabura, Bosque Protectos Los Cedros	0.3197	-78.7858	1880	KM675463.1
MZUTI	910	*Pristimantis mutabilis*	Ecuador	Imbabura, Bosque Protectos Los Cedros	0.3197	-78.7858	1880	KM675462.1
MZUTI	911	*Pristimantis mutabilis*	Ecuador	Imbabura, Bosque Protectos Los Cedros	0.3194	-78.7851	1845	KM675461.1
DHMECN	15189	*Pristimantis* sp 2	Ecuador	Carchi, Cerro Negro	0.8859	-78.2076	2062	–
DHMECN	15188	*Pristimantis* sp. 2	Ecuador	Carchi, Cerro Negro	0.8849	-78.2046	–	–
DHMECN	15007	*Pristimantis* sp. 2	Ecuador	Carchi, Cerro Negro	0.8919	-78.2031	2150	–

MZUTI	636	*Pristimantis* sp. 1	Ecuador	Pichincha, Pachijal	0.1119	-78.9028	1241	KM675464.1
MZUTI	635	*Pristimantis* sp. 1	Ecuador	Pichincha, Pachijal	0.1119	-78.9028	1241	KM675465.1
MZUTI	633	*Pristimantis* sp. 1	Ecuador	Pichincha, Pachijal	0.1119	-78.9028	1241	KM675467.1
MZUTI	634	*Pristimantis* sp. 1	Ecuador	Pichincha, Pachijal	0.1119	-78.9028	1241	KM675466.1
MZUTI	539	*Pristimantis* sp. 1	Ecuador	Pichincha, Pachijal	-0.0285	-78.7659	1521	KM675448.1
MZUTI	540	*Pristimantis* sp. 1	Ecuador	Pichincha, Mindo	-0.0792	-78.7634	1404	KM675447.1
MZUTI	541	*Pristimantis* sp. 1	Ecuador	Pichincha, Mindo	-0.0792	-78.7634	1404	KM675446.1
MZUTI	2114	*Pristimantis* sp. 1	Ecuador	Pichincha, Mindo	-0.0283	-78.7682	–	KM675445.1
UVC	15942	*Pristimantis* sp. 3	Colombia	Cauca	2.6381	-76.9150	–	JN104680.1
UVC	15943	*Pristimantis* sp. 3	Colombia	Cauca	2.6381	-76.9150	–	JN371037.1
UVC	15843	*Pristimantis* sp. 3	Colombia	Cauca	2.6381	-76.9150	–	JN371038.1
MZUTI	1825	*Pristimantis sirnigeli*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.1044	-78.6101	3084	–
MZUTI	1826	*Pristimantis sirnigeli*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.1044	-78.6101	3084	–
MZUTI	1827	*Pristimantis sirnigeli*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.1033	-78.6103	3078	–
MZUTI	1925	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Sigchos	-0.7015	-78.8830	2803	–
MZUTI	1926	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Sigchos	-0.7015	-78.8830	2803	–
MZUTI	1927	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Sigchos	-0.7015	-78.8830	2803	–
MZUTI	1928	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Sigchos	-0.1033	-78.6103	2803	–
MZUTI	1941	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Pilaló	-0.9464	-78.9733	2720	–
MZUTI	1942	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Pilaló	-0.9464	-78.9733	2720	–
MZUTI	1943	*Pristimantis pyrrhomerus*	Ecuador	Cotopaxi, Pilaló	-0.9464	-78.9733	2720	–
DHMECN	13354	*Pristimantis* sp. 4	Ecuador	Carchi, El Carmelo	0.6700	-77.5904	–	–
DHMECN	13347	*Pristimantis* sp. 4	Ecuador	Carchi, El Carmelo	0.6700	-77.5904	–	–
DHMECN	13648	*Pristimantis* sp. 4	Ecuador	Carchi, San José	0.5315	-77.7629	–	–

DHMECN	13649	*Pristimantis* sp. 4	Ecuador	Carchi, San José	0.5315	-77.7629	–	–
DHMECN	13339	*Pristimantis* sp. 4	Ecuador	Carchi, Moran	0.7693	-78.0537	–	–
DBR	264	*Pristimantis* sp. 4	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3362	–
DBR	263	*Pristimantis* sp. 4	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3362	–
DBR	266	*Pristimantis* sp. 4	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3362	–
DBR	269	*Pristimantis* sp. 4	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3362	–
MZUTI	1131	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3764	-78.0747	2708	–
MZUTI	1123	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3677	-78.0931	2820	–
QCAZ	18875	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3781	-78.0742	2717	–
MZUTI	1117	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3758	-78.1217	2973	–
QCAZ	18874	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3781	-78.0742	2717	–
MZUTI	1122	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3677	-78.0931	2820	–
MZUTI	1118	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3758	-78.1217	2973	–
MZUTI	1214	*Pristimantis gladiator*	Ecuador	Napo, Cuyuja	-0.3677	-78.0931	2820	–
DHMECN	15824	*Pristimantis* sp. 5	Ecuador	Tungurahua, Reserva Candelaria	-1.4516	-78.3082	–	–
DHMECN	14701	*Pristimantis* sp. 5	Ecuador	Tungurahua, Reserva Candelaria	-1.4206	-78.3875	–	–
DHMECN	14702	*Pristimantis* sp. 5	Ecuador	Tungurahua, Reserva Chamana	-1.4206	-78.3875	–	–
QCAZ	41260	*Pristimantis* sp. 6	Ecuador	Napo, Pacto-Sumaco	-0.5640	-77.6155	2775	–
QCAZ	41305	*Pristimantis* sp. 6	Ecuador	Napo, Pacto-Sumaco	-0.5696	-77.5941	2300	–
QCAZ	40808	*Pristimantis* sp. 6	Ecuador	Napo, Cordillera de los Guacamayos	-0.6240	-77.8409	2294	–
DHMECN	14446	*Pristimantis* sp. 6	Ecuador	Tungurahua, Reserva Machay	-1.370100000	-78.26830000	2220	–
DHMECN	14450	*Pristimantis* sp. 6	Ecuador	Tungurahua, Reserva Machay	-1.370100000	-78.26830000	2781	–
QCAZ	11677	*Pristimantis myersi*	Ecuador	Imbabura, Laguna de Puruhanta	0.2050	-77.9545	2800	–

QCAZ	52628	*Pristimantis myersi*	Ecuador	Imbabura, Hacienda Zuleta	0.1944	-78.0655	2955	–
QCAZ	52629	*Pristimantis myersi*	Ecuador	Imbabura, Hacienda Zuleta	0.1944	-78.0655	2955	–
DHMECN	13640	*Pristimantis* sp. 7	Ecuador	Carchi, Pioter	0.6500	-77.7833	-	–
DHMECN	13641	*Pristimantis* sp. 7	Ecuador	Carchi, Pioter	0.6500	-77.7833	-	–
QCAZ	49755	*Pristimantis* sp. 7	Ecuador	Pichincha, Laguna San Marcos	0.1057	-77.9631	3140	–
MZUTI	4813	*Pristimantis festae*	Ecuador	Pichincha, Cayambe	-0.0042	-78.0253	4240	–
QCAZ	17950	*Pristimantis festae*	Ecuador	Napo, Guamaní	-0.3703	-78.1923	3730	–
QCAZ	49680	*Pristimantis festae*	Ecuador	Napo, Guamaní	-0.3703	-78.1923	3950	–
QCAZ	16404	*Pristimantis festae*	Ecuador	Napo, Guamaní	-0.3590	-78.1903	4000	–
QCAZ	16405	*Pristimantis festae*	Ecuador	Napo, Guamaní	-0.3590	-78.1903	4000	–
KU	218234	*Pristimantis festae*	Ecuador	Imbabura, Tabacundo	0.0500	-78.2300	3002	EF493515
QCAZ	42109	*Pristimantis festae*	Ecuador	Imbabura, Mojanda	0.1267	-78.2561	3834	–
QCAZ	42116	*Pristimantis festae*	Ecuador	Imbabura, Mojanda	0.1267	-78.2561	3834	–
MZUTI	1807	*Pristimantis festae*	Ecuador	Imbabura, Mojanda	0.1267	-78.2561	3557	–
QCAZ	43164	*Pristimantis festae*	Ecuador	Carchi, Maldonado	0.7939	-77.9111	3841	–
QCAZ	13667	*Pristimantis festae*	Ecuador	Imbabura, Mojanda	0.1297	-78.2400	3500	–
QCAZ	13677	*Pristimantis festae*	Ecuador	Imbabura, Mojanda	0.1297	-78.2400	3500	–
DHMECN	4938	*Pristimantis munozi*	Ecuador	Pichincha, La Victoria	-0.2090	-78.7229	2048	–
MZUTI	1783	*Pristimantis munozi*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.0964	-78.6043	2855	–
MZUTI	1782	*Pristimantis munozi*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.0964	-78.6043	2855	–
MZUTI	1779	*Pristimantis munozi*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.0964	-78.6043	2855	–
MZUTI	1784	*Pristimantis munozi*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.0964	-78.6043	2855	–
DHMECN	13329	*Pristimantis* sp. 8	Ecuador	Carchi, Cerro La Bretaña	0.5689	-77.7142	–	–
DHMECN	13355	*Pristimantis* sp. 8	Ecuador	Carchi, El Carmelo	0.6700	-77.5904	–	–
DHMECN	13356	*Pristimantis* sp. 8	Ecuador	Carchi, El Carmelo	0.6700	-77.5904	–	–

DHMECN	13327	*Pristimantis* sp. 8	Ecuador	Carchi, Cerro La Bretaña	0.5689	-77.7142	–	–
DHMECN	13645	*Pristimantis* sp. 8	Ecuador	Carchi, Loma la Esperanza	0.5315	-77.7629	–	–
DHMECN	13642	*Pristimantis* sp. 8	Ecuador	Carchi, Loma la Esperanza	0.5315	-77.7629	–	–
DHMECN	13644	*Pristimantis* sp. 8	Ecuador	Carchi, Loma la Esperanza	0.5315	-77.7629	–	–
MZUTI	1466	*Pristimantis gralarias*	Ecuador	Pichincha, Las Gralarias	-0.0275	-78.7048	2192	–
DHMECN	13633	*Pristimantis* sp. 9	Ecuador	Carchi, Pioter	0.6500	-77.7833	–	–
DHMECN	13634	*Pristimantis* sp. 9	Ecuador	Carchi, Pioter	0.6500	-77.7833	–	–
DHMECN	13635	*Pristimantis* sp. 9	Ecuador	Carchi, Pioter	0.6500	-77.7833	–	–
KU	208508	*Pristimantis ocreatus*	Ecuador	Carchi, Vía Tulcan	0.7920	-77.9069	–	EF493682
QCAZ	43161	*Pristimantis ocreatus*	Ecuador	Carchi, Maldonado	0.7920	-77.9069	3817	–
DHMECN	13650	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.8106	-77.9043	3595	–
DHMECN	13669	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.8106	-77.9043	3595	–
QCAZ	42111	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.7939	-77.9111	3834	–
QCAZ	43162	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.7939	-77.9111	3841	–
DBR	272	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3785	–
DBR	270	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3785	–
DBR	271	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3785	–
DBR	273	*Pristimantis ocreatus*	Ecuador	Carchi, Tufiño	0.8028	-78.0047	3785	–
KU	218030	*Pristimantis* sp. 10	Ecuador	Bolívar, Bosque Protector Cashca Totoras	-1.5700	-79.1100	1996	EF493683
QCAZ	13769	*Pristimantis* sp. 10	Ecuador	Bolívar, Bosque Protector Cashca Totoras	-1.7180	-78.9766	2900	–
QCAZ	13771	*Pristimantis* sp. 10	Ecuador	Bolívar, Bosque Protector Cashca Totoras	-1.7180	-78.9766	2900	–
QCAZ	42125	*Pristimantis leoni*	Ecuador	Imbabura, Nudo Norte Mojanda	-0.1663	-78.2913	3385	–
MZUTI	1809	*Pristimantis leoni*	Ecuador	Imbabura, Nudo Norte Mojanda	-0.1605	-78.2827	3557	–
MZUTI	1811	*Pristimantis leoni*	Ecuador	Imbabura, Nudo Norte Mojanda	-0.1605	-78.2827	3557	–

DHMECN	8757	*Pristimantis leoni*	Ecuador	Pichincha, Valle del Toaza	-0.2011	-78.6231	3420	–
DHMECN	8760	*Pristimantis leoni*	Ecuador	Pichincha, Valle del Toaza	-0.2011	-78.6231	3420	–
DHMECN	8759	*Pristimantis leoni*	Ecuador	Pichincha, Valle del Toaza	-0.2011	-78.6231	3420	–
MZUTI	1818	*Pristimantis leoni*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.1033	-78.6103	3078	–
MZUTI	1819	*Pristimantis leoni*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.1033	-78.6103	3078	–
DHMECN	8762	*Pristimantis leoni*	Ecuador	Pichincha, Valle del Toaza	-0.2011	-78.6231	3420	–
DHMECN	8756	*Pristimantis leoni*	Ecuador	Pichincha, Valle del Toaza	-0.2011	-78.6231	3420	–
MZUTI	1799	*Pristimantis leoni*	Ecuador	Pichincha, Reserva Ecológica Verdecocha	-0.0964	-78.6043	2855	–
DHMECN	7285	*Pristimantis leoni*	Ecuador	Pichincha, Reserva Biológica Yanacocha	-0.1039	-78.5850	3000	–
DHMECN	7287	*Pristimantis leoni*	Ecuador	Pichincha, Reserva Biológica Yanacocha	-0.1039	-78.5850	3000	–
KU	218227	*Pristimantis* sp. 11	Ecuador	Carchi, Tulcan	0.7693	-78.0537	55	EF493684
QCAZ	25589	*Pristimantis* sp. 11	Ecuador	Orellana, Dayuma	–	–	–	MH516183.1
DHMECN	13336	*Pristimantis* sp. 11	Ecuador	Carchi, Moran	0.7693	-78.0537	–	–
DHMECN	13328	*Pristimantis* sp. 11	Ecuador	Carchi, Moran	0.5689	-77.7142	–	–
DHMECN	13332	*Pristimantis* sp. 11	Ecuador	Carchi, Moran	0.7693	-78.0537	–	–
DHMECN	14861	*Pristimantis hectus*	Ecuador	Carchi, Rio Chinambi	0.8610	-78.2695	2177	–
DHMECN	14887	*Pristimantis hectus*	Ecuador	Carchi, Rio Chinambi	0.8610	-78.2741	2176	–
DHMECN	14888	*Pristimantis hectus*	Ecuador	Carchi, Rio Chinambi	0.8606	-78.2733	2172	–
DHMECN	14847	*Pristimantis hectus*	Ecuador	Carchi, Rio Chinambi	0.8627	-78.2676	2184	–
DHMECN	15167	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8859	-78.2076	1976	–
DHMECN	15169	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8850	-78.2075	2000	–
DHMECN	15172	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8828	-78.2096	2107	–
DHMECN	14993	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8919	-78.2031	2000	–

DHMECN	14991	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8919	-78.2031	2000	–
DHMECN	14994	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8919	-78.2031	2140	–
DHMECN	15165	*Pristimantis hectus*	Ecuador	Carchi, Cerro Negro	0.8867	-78.2073	1898	–
MZUTI	4241	*Pristimantis onorei*	Ecuador	Imbabura, Toisán	0.5032	-78.5700	2575	–
MZUTI	4243	*Pristimantis onorei*	Ecuador	Imbabura, Toisán	0.5044	-78.5461	2575	–
MZUTI	4244	*Pristimantis onorei*	Ecuador	Imbabura, Toisán	0.5047	-78.5500	2558	–
QCAZ	12288	*Pristimantis onorei*	Ecuador	Cotopaxi, Sigchos	-0.4194	-79.0033	2000	–
MZUTI	2063	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2094	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2095	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2070	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2092	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2093	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2075	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2066	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	2088	*Pristimantis onorei*	Ecuador	Cotopaxi, Reserva Otonga	-0.4155	-79.0048	2115	–
MZUTI	1467	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	0.0275	-78.7048	–	–
MZUTI	2031	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	2035	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	2024	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	2032	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	2037	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	2038	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
DHMECN	6815	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Bellavista	-0.0241	-78.7082	2375	–

DHMECN	6812	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Bellavista	-0.0241	-78.7082	2375	–
DHMECN	6821	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Bellavista	-0.0241	-78.7082	2375	–
DHMECN	6813	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Bellavista	-0.024092	-78.7082	2375	–
MZUTI	2030	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	2023	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	-0.0256	-78.7039	2136	–
MZUTI	1464	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	0.0275	-78.7048	–	–
MZUTI	1465	*Pristimantis* sp. 12	Ecuador	Pichincha, Reserva Las Gralarias	0.0275	-78.7048	–	–
DHMECN	12246	*Pristimantis albujai*	Ecuador	Morona Santiago, Parque Nacional Sangay	-2.0793	-78.1604	3650	–
DHMECN	12249	*Pristimantis sambalan*	Ecuador	Morona Santiago, Parque Nacional Sangay	-2.2061	-78.4527	3337	–
QCAZ	31988	*Pristimantis bicantus*	Ecuador	Napo, Cosanga	-0.5992	-77.8897	2000	–
QCAZ	24653	*Pristimantis bicantus*	Ecuador	Morona Santiago, Chinguinda	-3.2004	-78.7631	2521	–
QCAZ	31988	*Pristimantis bicantus*	Ecuador	Morona Santiago, Parque Nacional Sangay	-2.2002	-78.3706	2371	–
QCAZ	37182	*Pristimantis bicantus*	Ecuador	Morona Santiago, 9 de Octubre	-2.2216	-78.2883	1729	–
QCAZ	45803	*Pristimantis bicantus*	Ecuador	Pastaza, Parque Nacional Llanganates	-1.2762	-78.0725	2266	–
QCAZ	45805	*Pristimantis bicantus*	Ecuador	Pastaza, Parque Nacional Llanganates	-1.2762	-78.0725	2266	–
QCAZ	49035	*Pristimantis bicantus*	Ecuador	Morona Santiago, Bosque Protector Abanico	-2.2538	-78.1989	1647	–
QCAZ	51559	*Pristimantis bicantus*	Ecuador	Cañar, San Antonio de Juval	-2.4122	-78.6960	–	–
QCAZ	51561	*Pristimantis bicantus*	Ecuador	Cañar, San Antonio de Juval	-2.4122	-78.6960	2451	–
QCAZ	52464	*Pristimantis nelsongalloi*	Ecuador	Tungurahua, Reserva Río Zuñac	-1.3674	-78.1457	2140	–

QCAZ	52466	*Pristimantis nelsongalloi*	Ecuador	Tungurahua, Reserva Río Zuñac	-1.3674	-78.1457	2140	–