



PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

Facultad de Ciencias Biológicas

Programa de Doctorado en Ciencias Biológicas

Mención Ecología

TESIS DOCTORAL

**“HISTORICAL BIOGEOGRAPHY, PHYLOGENETIC DIVERSITY AND EVOLUTION OF BODY SIZE IN
Pristimantis, THE WORLD’S MOST DIVERSE AMPHIBIAN GENUS”**

Por

ALDEMAR ALBERTO ACEVEDO RINCÓN

Diciembre, 2021



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Por

ALDEMAR ALBERTO ACEVEDO RINCÓN

Director de tesis: R. Eduardo Palma

Comisión de tesis:

Enrico Rezende

Patricio Plischoff

Marco Mendez

Diciembre, 2021



FACULTAD DE CIENCIAS BIOLÓGICAS
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Se firma la presente acta en la ciudad de Santiago, el día 14 de diciembre de 2021.

Dr. Juan Correa M.
Decano
Facultad de Ciencias Biológicas-UC

Dr. Enrico Rezende L.
Coordinador de Comité de Tesis
Facultad de Ciencias Biológicas-UC

Dr. Eduardo Palma V.
Director de Tesis
Facultad de Ciencias Biológicas-UC

Dr. Patricio Plischoff V.
Miembro Comité de Tesis
Facultad de Ciencias Biológicas-UC

Dr. Marco Méndez T.
Miembro Externo Comité de Tesis
Universidad de Chile

DEDICATORIA

Dedico mi tesis a mi mamá y papá, que partieron hace unos años; todo lo que me enseñaron me ha permitido ser una mejor persona cada día. A mi mamá Mary, que me ha dado todo su amor y a entregado su vida entera para que yo esté bien. A mis tíos Cesar y Tita por darme siempre su amor incondicional. A todos mis amigos que en la distancia siempre han tenido palabras de animo. A mi amor Moma, que siempre me ha brindado su cariño y compañía en los momentos más desafiantes. Y a nuestra hija gata Chuwe, que es nuestro apoyo y alegría diaria.



Pristimantis latidiscus amplexus. Illustration: Orlando Armesto.

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RESUMEN

La historia evolutiva de muchos organismos se relaciona con los progresivos eventos geológicos y climáticos sucedidos a diferentes escalas temporales y espaciales. En la mayoría de los casos, estos eventos han generado la formación de barreras, rutas migratorias y una amplia gama de diferentes climas. Ello, ha favorecido la diversificación de múltiples taxones de flora y fauna, generado patrones cambiantes de riqueza y distribución de especies, e influido en las variaciones de características ecológicas y morfológicas. Entre la vasta historia geológica de la tierra, el Neotrópico constituye la ecozona más rica en especies, donde la multiplicidad de taxones ha estado en continuos procesos de adaptación en conjunto con ambientes altamente dinámicos. Sin embargo, para muchos grupos taxonómicos, los procesos biogeográficos y evolutivos que facilitaron su diversificación espacio-temporal, siguen siendo desconocidos o poco entendidos. Por tanto, la integración entre múltiples factores, desde eventos paleogeográficos, la amplia gama de procesos ecológicos y la incorporación de datos moleculares, puede ayudarnos a responder preguntas relacionadas con los procesos que llevaron a la diversificación de especies, los patrones de distribución actuales y cambios en la dinámica evolutiva de diferentes taxones. En este contexto, utilicé como modelo de estudio las ranas del género *Pristimantis*, que entre otras cualidades se caracterizan por presentar un desarrollo directo, y por ser el género más diverso de todos los vertebrados, con alrededor de 600 especies conocidas. Este género es un grupo excelente para estudiar variaciones morfológicas interespecíficas, historia evolutiva y patrones de riqueza de especies, debido a su amplia distribución latitudinal y altitudinal, que abarca desde Centroamérica hasta las áreas andinas y amazónicas, y desde el nivel del mar hasta 4.500 m de altitud. Sin embargo, los estudios sobre este género se han centrado principalmente

en contextos taxonómicos, sistemáticos y biogeográficos. Por consiguiente, el objetivo general de esta tesis fue determinar los procesos ecológicos y evolutivos que permitieron la diversificación, composición y variación del tamaño corporal de las especies de *Pristimantis* (Anura: Strabomantidae) en el Neotrópico. Estos objetivos se abordarán en tres capítulos.

En el primero, puse a prueba si la diversificación de especies se ha producido a través de eventos sincrónicos con cambios geológicos en el Neotrópico, lo que habría permitido sucesivos eventos de colonización entre diferentes ecorregiones andinas y de tierras bajas. Combinamos datos publicados de seis genes mitocondriales más dos genes nucleares (RAG1 y Tyr) para 254 especies con datos recién generados para 50 especies de *Pristimantis* no secuenciadas previamente. El total analizado fue de 304 especies nombradas y candidatas. Nuestros resultados filogenéticos indicaron que este género se originó durante el Mioceno temprano en el noroeste de los Andes. Los patrones biogeográficos actuales habrían ocurrido como resultado de procesos de dispersión y vicarianza, impulsados predominantemente por el ascenso de los Andes. Nuestros hallazgos refuerzan la idea de que la evolución del paisaje al comienzo del Mioceno fue decisiva para aumentar los patrones de colonización del género *Pristimantis*. Hipotetizamos que Los Andes del norte y la Amazonía en el Mioceno medio, sirvieron como fuentes importantes de dispersión de *Pristimantis* hacia los Andes centrales, el Bosque Atlántico, los Andes venezolanos, el Escudo Guayanés y la Cordillera Oriental de Colombia. Además, describimos dos nuevas especies del género *Pristimantis* del margen occidental de la Cordillera Oriental de Colombia. La especie tiene una distribución simpátrica y se encuentra en los bosques tropicales húmedos de la parte baja del Parque Nacional Natural Tamá (PNN Tamá) y su zona de amortiguamiento. Las nuevas especies fueron descritas a partir de comparaciones morfológicas y reconstrucciones filogenéticas.

En el segundo capítulo, evaluamos si las variables ambientales impulsan la riqueza de especies y la diversidad filogenética. Múltiples factores históricos y ecológicos podrían explicar los patrones de diversidad en una región. Sin embargo, la riqueza de especies por sí sola no muestra el escenario completo en los procesos evolutivos de diferentes taxones. Compilamos un registro geográfico extenso para todas las especies de *Pristimantis* (600) e incluimos información filogenética para 304 especies del género. Con esto, realizamos dos análisis, uno evaluando si diferentes variables ambientales pueden ser buenos predictores de la riqueza de especies (SR, sigla en ingles). En segundo lugar, evaluamos si diferentes variables ambientales podrían predecir la diversidad filogenética (PD, sigla en ingles). Nuestros resultados mostraron que las ecorregiones con más SR no concuerdan con aquellas con valores de PD más altos (estandarizados). A su vez, encontramos que, para nueve ecorregiones neotropicales, la SR solo se predice por la aridez. Sin embargo, las variables de temperatura, precipitación, evaporación y altitud son buenos predictores de PD. Nuestros resultados muestran la importancia de incluir el componente evolutivo histórico para comprender los patrones de diversidad de grupos como *Pristimantis* que presentan una amplia distribución geográfica. A su vez, destacamos la importancia de los diferentes procesos históricos asociados a los eventos de dispersión y colonización como fuente relevante para comprender los valores de PD evidenciados en *Pristimantis*.

En el tercer capítulo, analizamos: (a) si *Pristimantis* se ajusta a la regla de Rensch, una tendencia de dimorfismo de tamaño sexual (SSD, sigla en ingles) a aumentar con el tamaño cuando los machos son del sexo más grande; (b) si las limitaciones ambientales asociadas a los gradientes de temperatura y humedad han influido en la variación del tamaño corporal; (c) qué modelo evolutivo explicaría mejor la variación de tamaño a lo largo de la filogenia y (d) cómo las tasas

de evolución del tamaño corporal han variado a lo largo del tiempo. El tamaño corporal es un rasgo organizativo clave, con profundas implicaciones ecológicas y evolutivas. Sin embargo, los factores ambientales y evolutivos que impulsan los patrones de tamaño corporal a nivel interespecífico siguen sin estar claros. Los análisis se basaron en dos fuentes de información, la primera que incluyó tamaños corporales de ~ 85% (495 especies) de especies conocidas del género, y una segunda que incorporó información filogenética molecular para 257 especies. Nuestros resultados mostraron que todas las especies de *Pristimantis* exhibieron un marcado SSD, pero no siguieron la regla de Rensch, ya que tendía a tener un sesgo hacia las hembras. Con base en dos enfoques analíticos (regresión filogenética escalonada y selección de modelos basada en la teoría de la información), encontramos que los modelos que mejor explicaban el tamaño corporal en machos, hembras y SSD contenían variaciones ambientales en temperatura, precipitación y elevación como predictores. A su vez, el tamaño corporal habría evolucionado hacia un óptimo, con una tasa de desaceleración de evolución diferenciada entre los grandes clados de *Pristimantis*.

Finalmente, esta tesis analizó el conjunto de datos filogenéticos más completo para *Pristimantis* hasta la fecha, incluidas 304 especies de 10 ecorregiones neotropicales. Esto nos ha permitido tener una mejor comprensión de los eventos biogeográficos, los cambios en los patrones de riqueza y diversidad filogenética y la evolución del tamaño corporal relacionados con los procesos evolutivos, geológicos y climáticos que han dado forma a la configuración de las especies de *Pristimantis*.

ABSTRACT

The evolutionary history of many organisms is related to progressive geological and climatic events at different temporal and spatial scales. In most cases, these events would have generated the formation of barriers, migratory routes, and different climates. This would have favored the diversification of multiple taxa, promoting changing patterns of species richness and distribution, and influenced variations in ecological and morphological characteristics. Among the plethora of ecoregions that have existed throughout Earth's history, the Neotropics constitute the most species-rich ecozone, where the diversity of taxa has undergone continuous adaptation processes in conjunction with highly dynamic environments. However, the biogeographic and evolutionary processes that have facilitated their spatio-temporal diversification remain unknown or poorly understood for many taxonomic groups. Therefore, the integration between multiple factors, from paleogeographic events, the wide range of ecological processes, and the incorporation of molecular data could help us to answer questions related to the processes that led to the diversification of species, their current distribution patterns, and changes in the evolutionary dynamics of different taxa.

In this context, I used frog species of the genus *Pristimantis* as a study model, that characterize by being forms of direct development, and by being the most diverse genus of all vertebrates with around 600 known species. Therefore, this genus is an excellent group to study interspecific morphological variations, evolutionary histories, and patterns of species richness, due to its wide latitudinal and altitudinal distribution, encompassing Central America to the Andean and Amazon areas, and from sea level up to 4,500 above sea level (a.s.l.). However, studies on this genus have focused mainly on taxonomic, systematic, and biogeographic contexts. Therefore, the general objective of this thesis was to determine the ecological and evolutionary processes

that induced the diversification, compositions, and variations of the body size of the species of *Pristimantis* (Anura: Strabomantidae) in the Neotropics. This major objective was addressed in three chapters.

First, we tested whether species diversification in the Neotropics has occurred through events that were synchronous with geological changes, allowing successive colonization events between different Andean and lowland ecoregions. We combined published data for six mitochondrial genes plus two nuclear genes (RAG1 and Tyr) for 254 species with newly generated data for 50 previously unsequenced *Pristimantis* species, for a total of 304 named and candidate species. Our phylogenetic results indicated that the genus originated during the early Miocene in the northwestern Andes. Current biogeographic patterns have resulted from dispersal and vicariant processes, driven predominantly by the rise of the Andes. Our findings reinforce the idea that the evolution of the landscape at the beginning of the Miocene was decisive in increasing the colonization patterns of the genus *Pristimantis*. The northern Andes and the Amazon would have served as essential sources of dispersal of *Pristimantis* to the central Andes, the Atlantic Forest, the Venezuelan Andes, the Guiana Shield, and the Eastern Cordillera of Colombia during the middle Miocene. In addition, we described two new species of the genus *Pristimantis* from the western margin of the Eastern Cordillera of Colombia. The species have a sympatric distribution and are found in the humid tropical forests of the lower part of the Tamá National Natural Park (PNN Tamá) and its buffer zone. The new species were described from morphological comparisons and a phylogenetic reconstruction.

In the second chapter, we assessed whether environmental variables drive species richness and phylogenetic diversity. Multiple historical and ecological factors can explain the diversity patterns in a region. However, species richness alone does not show the complete picture in the

evolutionary processes of different taxa. We compiled an extensive geographic record for all *Pristimantis* species (600) and included phylogenetic information for 304 species of the genus. With this, we performed two analyses; one, evaluating whether different environmental variables can be good predictors of species richness (SR); and the second, assessing whether different environmental variables could predict phylogenetic diversity (PD). Our results show that the ecoregions with more SR do not agree with those with higher PD values (standardized). In turn, we found that for nine Neotropical ecoregions, SR was only predicted by aridity. However, the variables of temperature, precipitation, evaporation, and altitude are good predictors of PD. Our results show the importance of including the historical evolutionary component to understand the diversity patterns of groups such as *Pristimantis*, which present a wide geographic distribution. In turn, we highlight the importance of the different historical processes associated with dispersal and colonization events as a relevant source to understand the PD values evidenced in *Pristimantis*.

In the third chapter, we analyzed: (a) whether *Pristimantis* conforms to Rensch's rule, a tendency for sexual size dimorphism (SSD) to increase with size when males are the larger sex; (b) whether the environmental limitations associated with temperature and humidity gradients have influenced variations in body size; (c) which evolutionary model best explains the variations in size along the phylogeny; and (d) how the rates of evolution of body size have varied over time. Body size is a key organizational trait with profound ecological and evolutionary implications. However, the environmental and evolutionary factors that drive body size patterns at the interspecific level remain unclear. The analyses were based on two sources of information. The first included body sizes of ~85% (495 species) of known species of the genus, and a second incorporated molecular phylogenetic information for 257 species. Our results show that all

Pristimantis species exhibited marked SSD but did not follow Rensch's rule, as it tended to have a bias towards females. Based on two analytical approaches (stepwise phylogenetic regression and model selection based on information theory), we found that the models that best explained body size in males, females, and SSD, contained environmental variations in temperature, precipitation, and elevation as predictors. In turn, body size has evolved towards an optimum, with a rate of deceleration of evolution differentiated between the large clades of *Pristimantis*. Finally, this thesis analyzed the most complete phylogenetic dataset for *Pristimantis* to date, including 304 species from 10 Neotropical ecoregions. This allowed us to better understand biogeographic events, changes in phylogenetic richness and diversity patterns, and body size evolution related to the evolutionary, geological, and climatic processes that have shaped the configuration of *Pristimantis*.

GENERAL INTRODUCTION

The wide range of taxa that shape life's complexity has been developed under multiple ecological and evolutionary scenarios that have molded the adaptation of species to changing ecosystems (Ricklefs and Schluter, 1993; Cracraft, 1994; Maurer, 1996). This has led to a central question: how are the patterns of distribution and assemblages of taxa changed over time in ecological and evolutionary contexts? (Cracraft, 1994). This is a key aspect for understanding the changes in the species pattern diversity on multiple spatio-temporal scales influenced by different scenarios. These may be expressed in an increase in species (radiation diversity) (Osborn, 1902; Gillespie et al., 2020), a decrease in species (reduction diversity), or maintenance over time (steady diversity; Cracraft, 1985; 1994). These processes are driven by multiple factors related to geological and climatic events and interactions between taxa within their communities (Mendoza-Henao and Garcia-R, 2021). Therefore, the integration of historical (e.g., orogenic events) and ecological (climatic regimes or interaction between species) episodes can help to understand why organisms are distributed where they are today, and how the taxa have assembled over time (Sanmartín, 2012).

Overall changes in biodiversity, framed by different ecologically and evolutionary views, have been considered from the perspective of diverse hypotheses: latitudinal diversity gradients (Mittelbach et al., 2007), the time and area (Wallace, 1878), the climatic variation (Haffer, 1969; Rohde, 1992), physiological tolerances (Janzen, 1967), biotic interactions (Dobzhansky, 1950), and geographical areas (Terborgh 1973). In addition, all hypotheses about biodiversity patterns are in some way related to geological scenarios over time and space, which have promoted the

different processes that explain the emergence, distribution, and establishment of diverse taxonomic groups (Hoorn et al., 2010). Therefore, a dynamic geological history, and the successive topographic complexity in different geographical contexts, have generated multiple scenarios (forming new habitats, climate gradients, and geographical barriers), thus promoting diversification processes in several taxa (Signor, 1990; Badgley, 2010; Velasco, 2018).

The Neotropics is one of the most interesting biogeographic ecozones in terms of diversity and biological evolution (Graham, 1997; Burnham and Graham, 1999; Myers et al., 2000; Rull, 2019; Antonelli and Sanmartín, 2011, Antonelli et al., 2015). Spanning a vast area stretching from northern Mexico to southern Brazil, the Neotropics comprise a wide heterogeneity of habitats (Morrone, 2014). The most species-rich biome in the region is the Andes, arising from several semi-independent orogenic systems and hosting many diverse ecosystems, ranging from lowland areas, predominantly with dry and subxerophytic forests, to puna and páramo habitats, 3000 m a.s.l.

Species diversification in the Neotropics has been analyzed in different ecological and evolutionary factors for different taxonomic groups (e.g., plants: Luebert and Weigend, 2014; reptiles: Esquerré et al., 2019; and birds: Lavinia et al., 2019). To this regard, a variety of hypotheses have been proposed regarding the processes and mechanisms that have given rise to the wide range of species that have occupied different geographical areas (Castroviejo-Fisher et al., 2014; Hazzi et al., 2018). Among these, two are recurrent hypotheses. First, geological and environmental shifts have promoted the emergence, distribution, and establishment of diverse taxonomic groups. These have mainly been influenced by the rise of the South American Andes

which began in the late Eocene (40 Ma ago) and intensified during the Miocene (20 Ma), triggering multiple environmental, spatial, and adaptive changes (Hoorn et al., 2010; Smith et al., 2014). Second, the time for speciation hypothesis sustains that early colonization events in different areas have increased species richness given the longer time that they have had for diversification (Gaston and Blackburn, 1996; Stephens and Wiens, 2003). Consequently, older taxa have lower extinction rates and larger geographic distribution sizes, in some cases acting as centers of origin for species diversity (Moreau and Bell, 2013).

In this thesis, I used amphibians as a model study for understanding diversification events and biogeographic patterns in the Neotropics (chapter 1), owing to their biological characteristics, such as limited dispersal capacity, preserved climatic requirements, and trait variations. Previous hypotheses have frequently provided explanations for diversification in some groups (e.g., Centrolenidae: Castroviejo-Fisher et al., 2014; Dendrobatidae: Guillory et al., 2019; Bufonidae: Maciel et al., 2010; Hylidae: Pirani et al., 2020; and salamanders: Jaramillo et al., 2020). In the Neotropical context, geological changes have shaped the adaptive scenarios for multiple taxa, allowing colonization between different ecoregions (e.g., Andes-Amazon), radiation patterns, and vicariant speciation from isolates between mountain ranges.

The Neotropics have a high amphibian richness; for example, anurans in the northern Andes (between Ecuador, Colombia, and Venezuela) account for 10.8% of the world's amphibian diversity (Armesto and Señaris, 2017). However, the richness patterns, their relationships with evolutionary history (phylogenetic diversity), and the factors that drive the number of species in time and space, continue to be a point of interest in megadiverse groups such as as

Pristimantis (Chapter 2). This genus is included in the clade Terrarana, consisting of Neotropical genera such as *Craugastor*, *Eleutherodactylus*, and *Strabomantis* among others (Hedges et al., 2008). *Pristimantis* comprises a large group of frogs with more than 540 recognized species (Frost, 2021), placing it as the genus of vertebrates with the highest richness of species. Their exceptional diversity is hypothesized by the non-dependence on water bodies for their reproduction, as they have direct development thus, facilitating the use of different niches (Hedges et al., 2008). The genus *Pristimantis* characterizes by the following features: a tympanic membrane and distinctive tympanic annulus; a smooth dorsum or shagreen; the presence or absence of a dorsal lateral fold; a commonly smooth belly but which may be weakly granular in some species; a slightly larger toe V than toe III (Hedges et al., 2008; De Oliveira et al., 2020). In turn, *Pristimantis* species exhibit a wide variation in body size, ranging from less than 15 mm to over 40 mm. However, the evolutionary and ecological aspects of body size have only been partially addressed (Zumel et al., 2021) (Chapter 3).

The geographical origin of *Pristimantis* has been proposed to the north of the South American Andes (Heinicke et al., 2007; Pinto-Sánchez et al., 2012). In turn, among the Neotropical amphibians, *Pristimantis* has a wide latitudinal distribution from Central America to Bolivia, in contrast to the majority of Neotropical amphibian genera which have more limited ranges of distribution (Pinto-Sánchez et al., 2012). Within *Pristimantis*, species richness is mostly concentrated in the Andean areas of Colombia and Ecuador (Frost, 2021; Meza-Joya and Torres, 2016). Therefore, the rise of the Andes may have favored their dispersal to different altitudinal and latitudinal bands (Mendoza et al., 2015; Meza-Joya and Torres, 2016).

Consequently, we evaluated three aspects related to the ecological and evolutionary processes involved in the historical biogeographic patterns (Chapter 1), variations in the richness and phylogenetic diversity of species (Chapter 2), and the evolution of body size (Chapter 3). To do this, we reconstructed a calibrated phylogenetic tree including molecular data from four partial mitochondrial genes and two nuclear genes, an extensive compilation of geographic records, environmental layers, and body size data for males and females for the majority of *Pristimantis* species. This allowed us to better understand the evolution of the genus *Pristimantis* and the importance of geological events in diversification within regions and dispersal patterns between Neotropical ecoregions. In turn, different climatic variables were found to be good predictors of richness and phylogenetic diversity. Finally, we demonstrated the importance of several environmental variables as relevant in body size patterns.

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**CHAPTER 1: GEOLOGICAL SHIFT SINCE THE EARLY MIOCENE EXPLAIN
BIOGEOGRAPHIC PATTERNS IN NEOTROPICAL RAIN FROGS OF THE GENUS
Pristimantis (ANURA: STRABOMANTIDAE)**

Aldemar A. Acevedo¹, R. Eduardo Palma¹, Angie Tovar^{2,3}, Andrew J. Crawford³

¹Laboratorio de Biología Evolutiva, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

²Facultad de Ciencias y Educación, Universidad Distrital Francisco José de Caldas, Bogotá, Colombia.

³Department of Biological Sciences, Universidad de los Andes, Bogotá, 111711, Colombia.

Abstract

Aim: The dynamic geology of the Neotropics has promoted the diversification of the regional flora and fauna by generating barriers, connections, and climatic variation that have driven speciation. However, for large taxonomic groups such as rain frogs of the genus *Pristimantis*, the most diverse genus of amphibians worldwide, some questions remain unanswered. We tested whether species diversification has occurred through events synchronous with geological changes in the Neotropics, which has allowed successive colonization events between different Andean and lowland ecoregions.

Location: Neotropics

Taxon: *Pristimantis* (Anura: Strabomantidae)

Methods: Combining new and published DNA sequence data, we reconstructed the most extensive time-calibrated phylogeny of *Pristimantis* to date. We integrate this phylogeny with species occurrence data (from Central America to Bolivia), and a model of regional connectivity through time. These were used to infer the historical origin and spatial diversification patterns of *Pristimantis*.

Result: Our time-calibrated phylogeny indicated that the time to most recent common ancestor (MRCA) of *Pristimantis* was during the early Miocene with an ancestral range in the northwestern Andes. Multiple diversification events between ecoregions during the early and middle Miocene were consistent with the intensifying mountain formation in the northern Andes and the emergence of the Pebas wetland in western Amazonia.

Main conclusions: Landscape evolution at the start of the Miocene was decisive in increasing the colonization patterns of the genus *Pristimantis*. The northern Andes and Amazonia in the middle Miocene served as important sources of *Pristimantis* dispersion towards the Central Andes, Atlantic Forest, Venezuelan Andes, Guiana Shield, and Eastern Cordillera of Colombia.

Keywords. Amphibians, diversification, historical biogeography, Terraranae.

Introduction

A key question in evolutionary biology involves how patterns of species diversity have emerged at different ecological and evolutionary scales (Dobzhansky, 1950; MacArthur, 1965; Whittaker, 1972). Among the variety of hypotheses concerning the mechanisms giving rise to biodiversity (see Mittelbach et al., 2007; Rull, 2020; Mendoza-Henao & Garcia-R, 2021), one is fundamental to understanding the macroevolutionary processes driving the variation in

species diversity within and between clades under biogeographical, ecological, and geological attributes (Schluter & Pennell, 2017; Rangel et al., 2018). The geological shifts hypothesis proposes that biodiversity patterns can be understood through the geological forces acting over time and space that have promoted the emergence, distribution, and establishment of diverse taxonomic groups (Hoorn et al., 2010). Dynamic geological history and the resulting topographic complexity in different geographical contexts promote diversification by forming new habitats, climate gradients, and geographical barriers (Signor, 1990; Badgley, 2010; Velasco, 2018). This allowed the periodic formation of a landscape that favored geographic islands between mountain systems and lowland areas that acted as barriers to species dispersal between areas and promoted vicarious speciation between mountain systems (Struwe et al., 2009; Hoorn et al., 2010).

The Neotropics is one of the most interesting biogeographic ecozones in terms of diversity, paleogeography, and biological evolution (Graham, 1997; Burnham & Graham, 1999; Myers et al., 2000; Rull, 2019; Antonelli & Sanmartín, 2011, Antonelli et al., 2015). The Neotropics span a vast area stretching from Mexico to Bolivia and comprise a wide heterogeneity of habitats due to a complex topography ranging from sea level to above 5000 m in altitude (Morrone, 2014a; Rull, 2020). Over the past 50 million years, the Neotropical landscape has changed continually with multiple geological events (Hoorn et al., 2010): the rise of the central and northern Andes of South America during the Paleogene, the intensification of mountain formation in the Northwestern Andes, the emergence of a large wetland developed in western Amazonia between the late Oligocene to early Miocene, and the closing of the Isthmus of Panama (Keigwin Jr, 1978; Bacon et al., 2015; Coates & Stallard, 2013; Montes et al., 2015). All of these events

promoted a wide variety of regional climates (Hoorn et al., 2010; Poulsen et al., 2010) and have been suggested as drivers of historical diversification and current patterns of species distributions (Gentry, 1982; Morrone, 2014b, Rull, 2007, 2020). Given this Neotropical context, this is a great place to study the hypotheses mentioned earlier.

Amphibians are an excellent model to understand the processes and patterns of biogeographic diversification in the Neotropics based on their long evolutionary history and biological characteristics, such as limited dispersal capacity, conserved climatic requirements, and occupation of different habitats and reproductive modes (Beebee, 1995; Forchhammer et al., 1998). Among the Neotropical amphibians, *Pristimantis* (Anura: Strabomantidae) Jiménez de la Espada 1870, dubbed ‘rain frogs’ in English, is the most diverse genus with more than 540 recognized species (Frost, 2021). They developed directly without passing through a tadpole stage and are included in the clade Terraranae formed by *Craugastor*, *Eleutherodactylus*, *Strabomantis*, and many other genera (Hedges et al., 2008). Phylogenetic studies have located the geographic origin of *Pristimantis* in South America (Heinicke et al., 2007; Pinto-Sánchez et al., 2012). Furthermore, along with considerable endemism, they have an extensive altitudinal range, from sea level to altitudes above 4,500 m a.s.l. in the ‘páramo’ (Heinicke et al., 2007; Meza-Joya & Torres, 2016).

Some studies have elucidated the evolutionary history of small portions of the diversity within *Pristimantis*. Mendoza et al. (2015) and Waddell et al. (2018) recognize the northwestern Andes of Colombia and Ecuador as the centers of origin for *Pristimantis*, and suggest that geological events in the Miocene, such as Andean orogeny and the concomitant formation and drainage of

the Pebas system could have influenced the diversification processes. However, the increase in the number of described species, as well as new molecular information from species that previously lacked DNA data, such as those from the Eastern Cordillera of Colombia, the Amazonian region, and the Atlantic Forest of Brazil, among other regions, provide new opportunities to address biogeographic hypotheses based on the evolutionary history of *Pristimantis*.

Here, we take a macroevolutionary approach using *Pristimantis* frogs as a model to investigate the diversification patterns that underlie the existing biodiversity in the context of Neotropical geological events (e.g., Andean orogeny, Pebas Formation, and closure of the Isthmus of Panama). We predict that the center of origin for species diversity are given by 1) dispersal patterns from the ecoregions of the Andes (northern and central Andes) to the lowlands (Amazonia and Chocó). To test this, we combined a new multi-locus DNA-sequence dataset with published sequences to reconstruct the most extensive calibrated phylogeny of *Pristimantis* to date (304 species in this study), in contrast to the 140 species analyzed by Waddell et al. (2018), and the 160 species studied by Mendoza et al. (2015). Finally, we integrated this phylogeny with species occurrence data, spatial information on ecoregions and their connectivity through time.

Materials and methods

Taxon sampling

The species included in this study covered the known geographic range of *Pristimantis*, from Central America to the South American Andes, Biogeographical Chocó, Amazonia, Guiana Shield, and Atlantic Forest of Brazil (Lynch & Duellman, 1997; Hedges et al., 2008) (Fig. 1). We generated a molecular data set (see molecular methods) for 304 species of *Pristimantis*. Of these, the sequences of 261 species were retrieved from GenBank (as of August 2020). The remaining sequences comprised 43 previously unsequenced species of *Pristimantis* collected in the last 10 years in multiple expeditions to the Colombian Eastern Andes, one of the ecoregions with the highest gaps in information for this genus. In total, our database covered approximately 64% of species of *Pristimantis*. Table S1 details the species included in this study and the GenBank accession numbers for all DNA sequences.

Species identification

Species identification for the 43 newly sequenced specimens was based on the following evidence: (1) comparison of the newly-collected specimens with morphological features of previously described species, as reported in the primary literature (i.e., original descriptions, redescrptions, or monographs). Character definitions follow Lynch & Duellman (1997) and Duellman & Lehr (2009), and (2) comparison with *Pristimantis* specimens deposited at the *Colección Herpetológica del Instituto de Ciencias Naturales* at the Universidad Nacional de Colombia (ICN-MHN-Her), the *Colección Herpetológica* at the Universidad de Pamplona

(MCNUP-H), *Museo de Historia Natural* at the Universidad Distrital Francisco José de Caldas (MHNUD); and the *Museo de Historia Natural C.J. Marinkelle* at the Universidad de los Andes (ANDES-A) (Table S2), (3) topologies resulting from phylogenetic inferences (maximum likelihood and Bayesian criteria, Fig. S1, as described below), (4) pairwise genetic divergence of at mitochondrial genes, using mean p-distance calculated in MEGA X (Kumar et al., 2018) (Table S3), (3) geographic information, evaluating the distribution patterns of the samples with the species known for the Eastern Andes of Colombia and the Venezuelan Andes. In addition, we review the corrections in the identification on the identity of some species of *Pristimantis* deposited in GenBank as suggested by González-Duran et al. (2017), Acevedo et al. (2020) and Rivera-Correa et al. (2021).

Molecular methods

Liver and muscle tissue samples collected from field-caught specimens were preserved in 95-99% ethanol and then stored at -20 °C. Genomic DNA was extracted using the QIAGEN DNeasy Blood and Tissue kit. The samples were amplified for fragments of four mitochondrial gene regions (12S, 16S, CYTB, and COI) and two nuclear genes (RAG1 and TYR) (Table 1). The general PCR cycles consisted of 2 min at 93 °C and 35 cycles of 30 s at 95 °C, 1 minute at 42 °C, 1:30 min at 72 °C, and a final extension step of 6 min at 72 °C. The purification and sequencing reactions were performed at MacroGen Inc.

The sequences were aligned using MUSCLE (Edgar, 2004) along with homologous sequences from other members of *Pristimantis* available in GenBank (Table S1). The alignments were

reviewed manually using the biological sequence alignment software BioEdit v7.2 (Hall et al., 2011). Subsequently, each coding region was translated into amino acids using MACSE v2.00 (Ranwez, et al., 2011) to identify possible stop codons and framework shifts. Lastly, aligned sequences for multiple loci were concatenated in SequenceMatrix v.1.8 (Vaidya et al., 2011).

Phylogenetic inference

Phylogenetic analysis was performed on the concatenated dataset for the eight partial gene sequences. For maximum likelihood and Bayesian inference methods, we selected the best partition scheme and the corresponding substitution models using PartitionFinder 2 (Lanfear et al., 2017) based on the Bayesian information criterion and using the greedy search algorithm (Lanfear et al., 2012). We assumed 19 possible partitions through the concatenated data matrix (by-gene and by-codon).

For maximum likelihood (ML) analysis, the software RAxML 8.1.11 (Stamatakis, 2014) was used in the online portal CIPRES (Miller et al., 2010). Each partition (12s16s, COI_pos1, COI_pos2, COI_pos3, CYTB_pos1, CYTB_pos2, CYTB_pos3, ND1_pos1, ND1_pos2, ND1_pos3, ND2_pos1, ND2_pos2, ND2_pos3, RAG1_pos1, RAG1_pos2, RAG1_pos3, TYR_pos1, TYR_pos2, TYR_pos3) was assigned a General Time Reversible model (GTR) combined with the proportion of invariable sites (+I) and with the rate heterogeneity across sites (+G), configured for RAxML as GTRGAMMAI and using the “-f a” option. The nodal support was evaluated with 1000 bootstrap replicas. We used four taxa as a paraphyletic outgroup (*Craugastor*, *Eleutherodactylus*, *Oreobates*, and *Tachiramantis*) all belonging to

Strabomantidae and closely related to *Pristimantis* (Heinicke et al., 2007). Previous studies suggest that the recently described genus, *Tachiramantis*, may be the most closely related to *Pristimantis* (Heinicke et al., 2015), but further testing is warranted, and thus we included multiple lineages in our outgroup.

Time-calibrated phylogeny

To estimate divergence times across our tree of *Pristimantis* species, we again used the Bayesian MCMC approach implemented in BEAST v1.10.4 (Suchard et al., 2018). The analysis assumed a relaxed clock model of absolute nucleotide substitution rates, a Yule speciation process (Drummond et al., 2006), and a partition scheme generated from PartitionFinder2 where the 16S-12S region was one partition and all protein coding genes were partitioned by codon position, within which each partition assumed a GTR+I+G model of relative substitution rates. The analysis was run for 100 million generations, sampling every 10 000 generations with a 10% burn-in. We assumed a lognormal prior distribution for the rate of substitution (ucl.d.mean) with mean = 0.01 substitutions per site per lineage per million years and SD = 0.333).

To estimate the posterior distribution of the age of each node, we implemented normally distributed *a priori* temporal constraints as secondary calibrations obtained from previous studies, as follows. (1) the time to most recent common ancestor (MRCA) of Eleutherodactylinae and *Pristimantis* was assumed to have a mean of 36.52 million years ago (Ma) (2.5% quantile = 26.56, 97.5% quantile = 50.81; Heinicke et al., 2007), (2) crown age (aka, MRCA) of *Pristimantis*: mean of 24.45 Ma (2.5% quantile = 17.30, 97.5% quantile = 34.82;

Heinicke et al., 2007), (3) the time to MRCA of the *P. pardalis* Species Group: mean of 8.6 Ma (2.5% quantile = 5.5, 97.5% quantile = 12.0; Pinto-Sánchez et al., 2012), and (4) the time to MRCA of the *P. taeniatus* Species Group: mean of 8.3 Ma (2.5% quantile = 5.6, 97.5% quantile = 11.2; Mendoza et al., 2015). We summarized posterior distributions and ensured convergence and adequate effective sample sizes using Tracer v1.10.4 (Suchard et al., 2018). After discarding the 10% burn-in iterations, the trees were scored and the posterior probabilities were summarized in TreeAnnotator v1.10.4 (Suchard et al., 2018).

Ancestral range reconstruction

To estimate ancestral ranges, we first retrieved the geographic records of all 304 ingroup species included in the phylogeny. A search was made as exhaustively as possible through several resources, including in the Global Biodiversity Information Facility (GBIF, <http://www.gbif.org>), original descriptions of each species, publications of locality records and range extensions, and reviews of biological collections (ICN-MHN-Her, MCNUP-H, MHNUD, and ANDES-A), in addition to our own field data. The localities for each species were mapped in QGIS v3.14.16 (Team, 2016) and individually curated to correct georeferencing errors and filter erroneous locations. The final database contained 7224 geographic records.

The total distributional range of *Pristimantis* was subdivided into 10 ecoregions, following Olson et al., (2001), as updated by Dinerstein et al. (2017). The following ecoregions were included: Amazonia (A), Central America (B), Central Andes (southern Ecuador, Peru, western Bolivia) (C), Chocó (western Ecuador, Pacific Colombia and the Darien in Panama) (D), Guiana Shield (E), Northwestern Andes (western and central Andean cordilleras of Colombia + the

Ecuadorian Andes) (F), Venezuelan Andes (G), Colombian Eastern Andes (H), Venezuelan Coastal Range (I), and the Atlantic Forest of Brazil (J) (Fig. 1) For each species included in the phylogeny, we generated a presence/absence matrix from the geographic data in each ecoregion (Table S4).

With the resulting matrix and the timetree estimated in BEAST (outgroup taxa were excluded), we estimated the ancestral ranges using a maximum likelihood approximation implemented in BioGeoBEARS (Matzke, 2016) executed in RASP 3.2 (Yu et al., 2015).

We used time stratification to implement likely changes over time in the probability of dispersal between the 10 geographic ecoregions defined for the Neotropics. The dispersal probabilities were based on the absence of ecoregions within certain periods of time, as follows: 1.0 for contiguous regions, 0.5 for regions separated by one region, and 0.1 for regions separated by more than two regions, based on the paleogeographic proposals of Díaz de Gamero, (1995) and Hoorn et al., (2010). We measured distances in three stages beginning at 22.5 Ma given the mean estimated depth of the *Pristimantis* phylogeny (Fig. 2). 1: From early to late Miocene (22.5 – 10 Ma) featuring northern Andes uplift (~ 12 Ma) and generation of the Pebas system. 2: Late Miocene (10 – 7 Ma) with the final Colombian Eastern Andes uplift in the north of the Andes and the reduction of the Pebas system. 3: From late Miocene to Pliocene-Pleistocene (7 – 0 Ma) when the Pebas system disappeared, the lowland rainforests expanded, closure of the Isthmus of Panama and the start of the Great American Biotic Interchange (GABI). See Table S5. Six biogeographic models (DEC, DEC+J, DIVALIKE, DIVALIKE+J, BAYAREALIKE, and BAYAREALIKE+J) were compared. The models were evaluated using the highest Akaike weight, which indicates the level of support for the most parsimonious model among the set of

candidate models, calculating the probability of each ancestral region for all nodes (Burnham et al, 2011). Lastly, we produced a lineages-through-time (LTT) plot for the whole *Pristimantis* tree with the ‘ltt’ function in the R package, Phytools (Revell, 2012).

Results

Phylogeny and taxonomy

The final alignment length consisting of portions of eight genes was 5391 base pairs (bp). Our resulting topology contained 304 species of *Pristimantis* (Table S1) including 26 that remain unidentified or are unconfirmed candidate species to be investigated in future studies. The topologies estimated by Bayesian (Fig. 2) and maximum likelihood (Fig. S1) criteria were broadly congruent with each other, as evidenced by the relative support values for each node estimated as posterior probabilities and bootstrap values, respectively (Fig. S1). However, The Bayesian tree (timetree) recovered an artificial group, containing just two species (*P. urichi* and *P. rozei*) distributed in the Antillean areas and coastal areas of Venezuela (Fig. 2). However, these two samples were not sisters in the ML tree, and in fact, were placed far apart on the phylogeny, and their position in all trees received very low bootstrap support or posterior probabilities. Given that these two samples are placed on very long external branches, their inferred status as sisters could be a consequence of long-branch attraction.

The following phylogenetic and biogeographic results follow the time-calibrated Bayesian consensus tree (Figs. 2, S1, S2). Our phylogeny recovered the species groups within *Pristimantis* as proposed previously by Padial, et al. (2014) and González-Durán et al. (2017) (Table 2).

However, new sequences of *P. carrangerorum* did not support its inclusion in the *P. conspicillatus* Species Group, *sensu* Padial et al. (2014). This species was more closely related to species that have not been assigned to specific groups, such as *P. anolirex*, *P. medemi*, and *P. lutitus*, and several undescribed species of the Colombian Eastern Andes included in the present analysis (Fig. 2, Fig. S1).

We subdivide our calibrated tree into three major clades, G1, G2, G3 (Fig. 2, Table 2) and we follow the taxonomy proposed by Padial et al. (2014).

Biogeographic history

The best-supported model for our data set was the dispersal–extinction–cladogenesis model plus a parameter incorporating the rate of ‘jump dispersal’ (DEC + J) (Table 3). A crown group G1 has a likely origin about 22.49 Ma with an estimated 95% Highest Posterior Density (HPD) = 17.12–28.18 Ma) during the early Miocene (Figs. 2, 3A). The most probable ancestral range of the most recent common ancestor (MRCA) of the genus corresponded to the Northwestern Andes (F) with a probability of 0.819, and from there, the ancestor dispersed to adjacent areas in the last 19 Ma to Amazonia (A), Chocó (D), Central Andes (C) and Colombian Eastern Andes (H) (Figs. 2, 3A). G1 consisted of a mixture of species distributed primarily in the northwestern Andes (e.g., *P. paisa*, *P. actites*, *P. calcaratus*) and in the Chocó (e.g., *P. cisnerosi*, *P. afrox*, *P. chocoensis*), plus one species from the Amazonia (*P. lathanites*) and two from the Colombian Eastern Andes, namely *P. sp. 15* and *P. fallax* (Fig. 2; Table 2).

The G2 clade was represented by two subclades with an ancestral range to the Amazonia around 19.4 Ma (Figs. 2, 3A). G2a, dates from 18.47 Ma (95% HDP = 13.8–23.43) (Table 2) from dispersal events to Central Andes (C), Venezuelan Andes (G), and the Chocó (D) (Figs. 2, 3A). Clade G2b dates from 17.95 Ma (95% HDP= 13.47–22.55 Ma) with a mix of ecoregions of the Venezuelan Coastal Range (I), Atlantic Forest (J), Amazonia (A), and Colombian Eastern Andes (H) (Figs. 2, 3A; Table 2).

Clade G3 has two subclades. The first, G3a, occurred at 16.61 Ma (95% HDP = 12.15–21.25 Ma) (Table 2), with an ancestral range represented by the Northwestern Andes (F) with a probability of 1.000. This clade was mostly represented by species distributed in the northwestern Andes of Colombia and Ecuador (e.g., *P. surdus*, *P. buckleyi*, *P. urani*).

Clade G3b dates from 17.3 Ma (95% HDP = 13.06–21.75) (Table 2), with an ancestral range represented by the Central Andes of Ecuador and Peru forming a group of mixed species from different ecoregion like the northwestern Andes (F), Central America (C), the Colombian Eastern Andes, Amazonia, and Guiana Shield (E). In addition, the Amazonian lineages are related to the colonization patterns of some species of the Central Andes (e.g., *P. platydactylus*, *P. salaputium*, *P. ashaninka*, *P. antisuyu*, and *P. cruciocularis*) and the source of the species of *Pristimantis* currently distributed in the Guiana Shield (E) (e.g., *P. jester*, *P. aureoventris*, and *P. yuruaniensis*), whose ancestor arrived just 6.62 Ma (95% HDP = 4.86–8.57 Ma) (Fig. 2, 3B).

Lineages distributed in the Eastern Andes of Colombia (H) and Chocó colonized from the Northwestern Andes (F) 10.89 Ma (95% HDP = 8.21–13.71 Ma). These events allowed new colonization of Amazonia and another of Central Andes from the Northwestern Andes 10.01

Ma (95% HDP = 7.52–12.62 Ma). In contrast, dispersal and vicariance events favored a recent dispersion pattern in the Colombian Eastern Andes 9.47 Ma (95% HDP = 7.16–11.94 Ma). Lastly, species in Central America such as *P. altae* and *P. pardalis* showed a pattern of dispersal from Chocó (*P. pirrensis*) 5.3 Ma (95% HDP = 3.48–7.44 Ma) (Figs. 2, 3B) as suggested by Ibáñez and Crawford (2004).

Lineage-Through Time analysis showed an increase in the number of lineages in the last 10 Ma, which corresponds to the diversification events that occurred above all in the Northwestern Andes and the Colombian Eastern Andes, corresponding mainly to clades G2 and G3 (Fig. 3B).

Discussion

Biogeographic patterns

Our results combining molecular phylogenetic and biogeographic data found that paleogeographic events such as the rise of the Andes and marine incursions during the early Miocene (23–10 Ma; *sensu* Hoorn et al., 2010) agreed with the diversification patterns of *Pristimantis* from the Northwestern Andes (F) to the lowland areas of Chocó (D), Central Andes (C), Amazonia (A), and Colombian Eastern Andes (H) under a mixture of dispersal and vicariance events (Figs. 2, 3A, B).

Our results support a crown most recent compared with previous timetree proposed by Mendoza et al. (2015), 28 Ma (95% HDP = 18.11 – 31.67, and Waddell et al. (2018), 28.97 Ma (95% HDP = 21.59 – 37.33 Ma) who place the origin of the genus during the late Oligocene. Although

our study was based on the same calibration points used by these two previous studies, our phylogenetic hypothesis derived from the results of the calibrated tree locates the origin of *Pristimantis* in the early Miocene (22.49 Ma (95% HDP = 17.12–28.18 Ma). Nevertheless, our biogeographic hypothesis coincides with the proposals of these authors, who suggested an ancestral range in the northwest of the Andes with subsequent dispersal events to the Chocó areas. Most likely, this new dating may be due to the inclusion of a higher number of *Pristimantis* species (304 species in this study) rather than the 140 species included in the study by Waddell et al. (2018) and the 160 species in the study by Mendoza et al. (2015).

The hypotheses related to the emergence of a heterogeneous range of habitats promoted by geological and climatic changes during the elevation of the Andes between the Oligocene and the Early Miocene (23–54 Ma) may explain the increase lineages through time in *Pristimantis* (Hutter et al., 2017). Subsequently, during the second stage of the rise of the Northwestern Andes, between the late Miocene and the Oligocene (12–4.5 Ma; Hoorn et al., 2010), *Pristimantis* diversification patterns favored vicariance processes, especially in the Colombian Eastern Andes (groups G2b and G4e), Venezuelan Andes, and Central Andes (group G2) (Figs. 2, 3A). Additionally, the geological events that occurred in the Amazonia (formation of the Pebas system, Fig. 2) have played a key role in species dispersal and vicariance processes to new geographic areas, as shown by the exchange of amphibian and other taxonomic lineages between the Amazonian and Andean areas (Antonelli et al., 2018).

Our results indicate that at least two events originated in Amazonia, one approximately 16 Ma, when Amazonian lineages arose from local speciation processes favored by the isolation generated by the Pebas system (Figs. 2, 3A), and another approximately 10–8 Ma during the

late Miocene, when Pebas system drained (Hoorn et al., 2010) favoring biotic interchanges between Andean areas and the Amazonia basin (Wesselingh & Salo, 2006), with dispersal events into the Central Andes and Guiana Shield areas (Figs. 2, 3B). Therefore, the specific Amazonia diversity composition has been partially influenced by exchanges between Amazonia and other adjacent areas (Fig. 3A) (Hughes et al., 2013; Antonelli et al., 2018; Réjaud et al., 2019). Therefore, the Miocene was a decisive period in the speciation processes of a large number of South American taxa Northwestern Andes (Santos et al., 2009), especially during the Middle Miocene when temperatures were warmer and humid (Shevenell et al., 2008). The latter conditions may have allowed the expansion of forested areas, resulting in a high diversification of species (Guo et al., 2020). Thus, the diversity of Amazonian *Pristimantis* (43 species included in this study) which have an older origin and were influenced by various paleogeographic events is underestimated, with a high percentage of species remaining undescribed in this ecoregion that is considered megadiverse (Da Silva et al., 2005), as shown for other amphibians such as salamanders of the genera *Bolitoglossa* (Jaramillo et al., 2020), *Allobates* (Réjaud et al., 2019) and dendrobatid species (Santos et al., 2009).

Conclusions

Our study analyzed the most complete phylogenetic dataset of *Pristimantis* to date, including 304 species from across 10 Neotropical ecoregions. The origin of *Pristimantis* dates back 22 Ma, during the early Miocene, with a possible ancestral area in the Northwestern Andes, whose lineages colonized into new geographic areas through mixed events of dispersal. Our results highlight the importance of the Andean zones that during the Miocene were important centers

of diversification of *Pristimantis* with dispersions towards low zones in the Amazonia and Chocó. In turn, with the successive orogenic events of the Middle Miocene, inter-Andean colonization events increased between regions such as the central Andes, the Venezuelan Andes, and the Eastern Andes of Colombia.

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Supporting information

Additional supporting information may be found in the Supporting Information section and all relevant data are available online on Dryad: <https://doi.org/10.5061/dryad.r7sqv9sd5>

Tables

Table 1. Primers used in this study for polymerase chain reaction analysis and DNA sequencing (F = forward, R = reverse)

Gene region	Primer name	Primer sequence (5'- 3')	Source
16S	16Sar (F)	CGC CTG TTT ATC AAA AAC AT	Palumbi et al. (1991)
	16Sbr ®	CCG GTC TGA ACT CAG ATC ACG T	Palumbi et al. (1991)
12S	12SL4E (F)	TAC ACA TGC AAG TYT CCG C	Heinicke et al. (2007)
	12SH10 ®	CAC YTT CCR GTR CRY TTA CCR TGT TAC GAC TT	Heinicke et al. (2007)
CYTB	MVZ15-L (F)	GAA CTA ATG GCC CAC ACW WTA CGN AA	Goebel et al. (1999)
	MVZ16-H ®	AAA TAG GAA RTA TCA YTC TGG TTT RAT	Goebel et al. (1999)
COI	dgLCO-1490	GGTCAACAAATCATAAAGAYATYGG	Meyer et al. (2005)
	dgHCO-2198	TAAACTTCAGGGTGACCAAARAAYCA	Meyer et al. (2005)
TYR	TYR1F (F)	GTT GTY GTA TCT ACC TCR CC	Heinicke et al. (2007)
	TYR1R ®	GMA GGG AAT GGT GAA RTT CTC	Heinicke et al. (2007)
RAG1	RAG1FF2 (F)	ATG CAT CRA AAA TTC ARC AAT	Heinicke et al. (2007)
	RAG1FR2 ®	CCY CCT TTR TTG ATA KGG WCA TA	Heinicke et al. (2007)

Table 2. Times of divergence (million years ago, Ma) for major groups in Fig. 2.

Clade	<i>Pristimantis</i> species group	Distribution
G1	<i>Pristimantis ridens</i> species group.	Chocó, Central America, Northwestern Andes, and Colombian Eastern Andes.
G2a	<i>Pristimantis danae</i> species group.	Central Andes, Venezuelan Andes, and Amazonia.
G2b	<i>Pristimantis conspicillatus</i> species group	Central Andes, Colombian Eastern Andes, Amazonia, Guiana Shield, Venezuelan Coastal Range, and Atlantic Forest.
G3a	<i>Pristimantis conspicillatus</i> species group <i>Pristimantis devillei</i> species group <i>Pristimantis leptolophus</i> species group	Northwestern Andes, and Colombian Eastern Andes.
G3b	<i>Pristimantis lacrimosus</i> species group, <i>Pristimantis galdi</i> species group <i>Pristimantis ridens</i> species group <i>Pristimantis (Huicundomantis) phoxocephalus</i> species	Northwestern Andes, Central Andes, and Chocó. Amazonia and Guiana Shield Colombian Eastern Andes Venezuelan Andes Guiana Shield

Table 3. Results of the BioGeoBEARS estimation of ancestral areas on the phylogeny of *Pristimantis*. Six models were tested and compared using the Akaike information criterion (AICc wt). d: dispersal; e: extinction; j: founder.

Model	Log Likelihood	Number of parameters	Parameters			AICc	AICc wt
			d	e	j		
DEC	-496.1	2	0.011	0.0080	0	996.3	7.9e-13
DEC+J	-467.2	3	0.0066	0.0027	0.019	940.5	1.00
DIVALIKE	-518.1	2	0.013	0.0074	0	1040	2.2e-22
DIVALIKE+J	-491.9	3	0.0075	0.0034	0.021	989.9	1.9e-11
BAYAREALIKE	-557.3	2	0.012	0.054	0	1119	2.1e-39
BAYAREALIKE+J	-491.8	3	0.0050	0.0066	0.026	989.6	2.2e-11

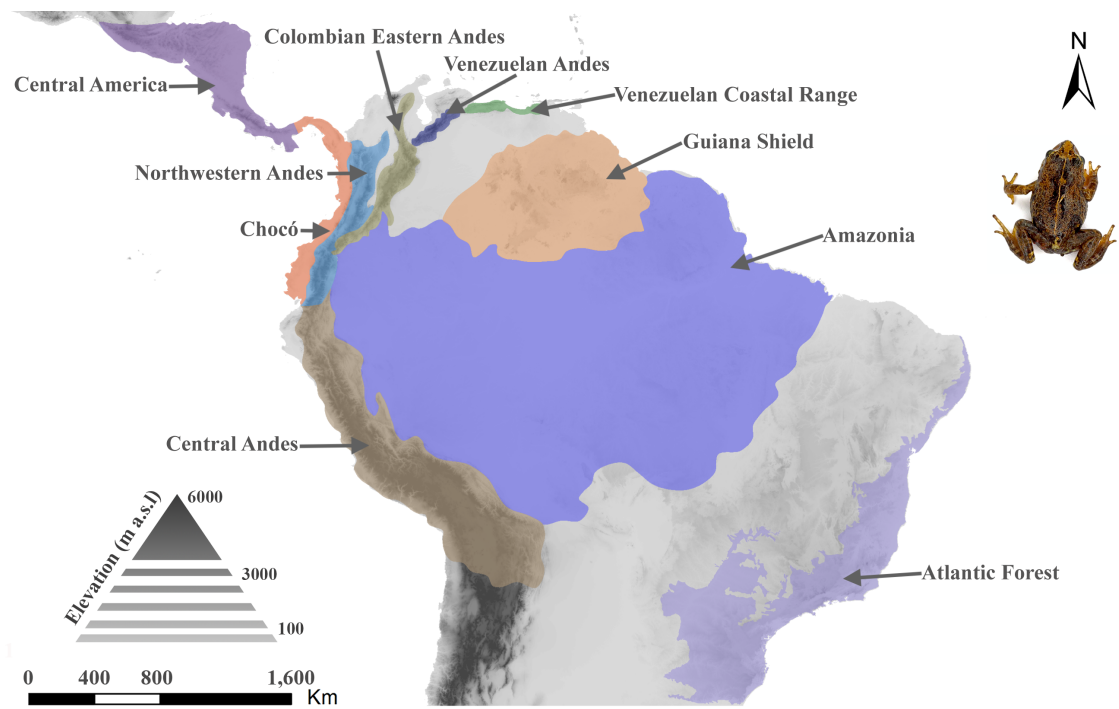


Fig 1. Map of the Neotropical realm divided into 10 ecoregions where *Pristimantis* occur.

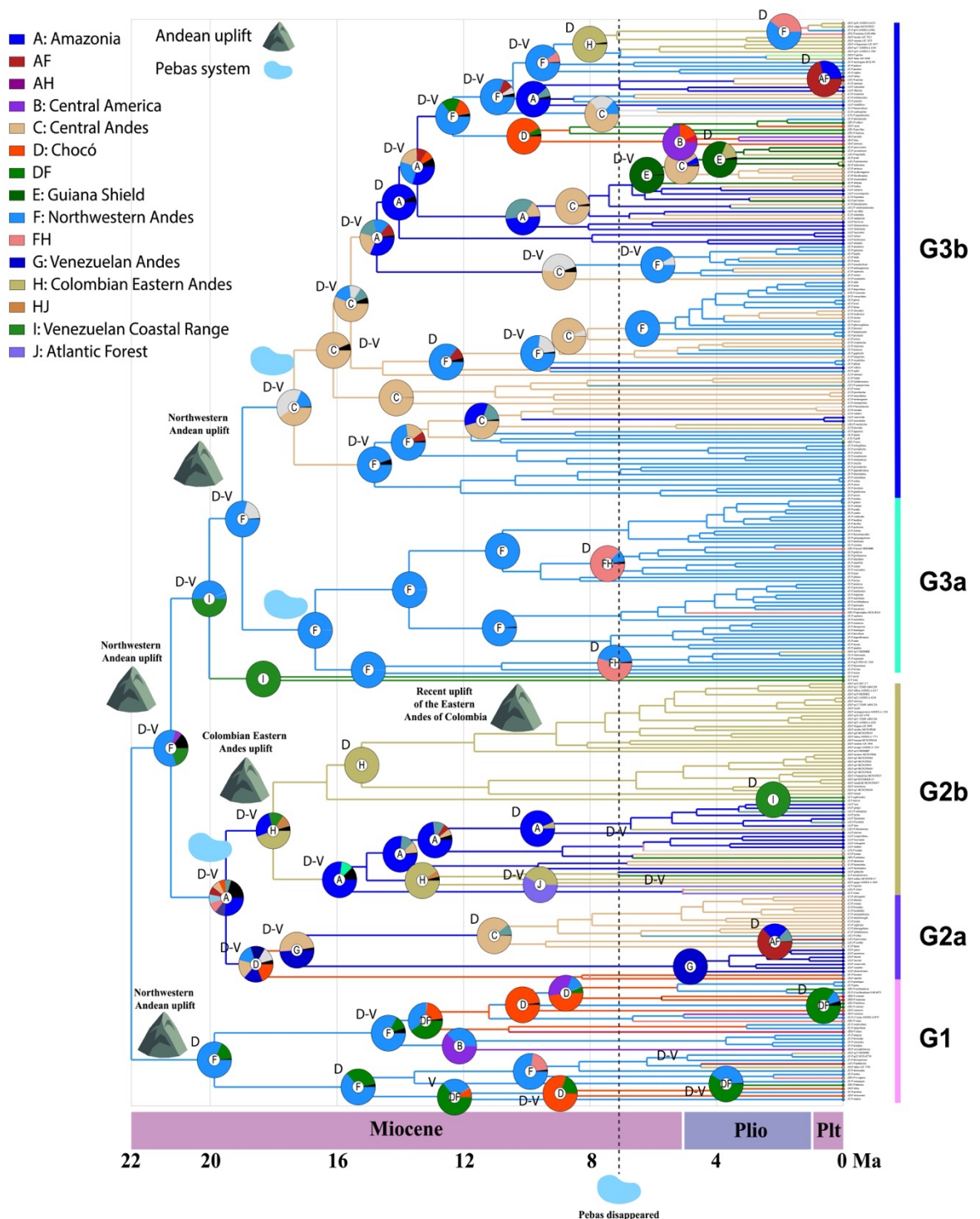


Fig 2. Reconstruction of the ancestral area from BioGeoBEARS on the Bayesian consensus tree. The model with the best fit was DEC + J. The most probable biogeographic areas are shown in the circles (the map of Fig. 1 shows the location of the study areas). The letters on each circle represent either (D) dispersal or (V) vicariance. Species with voucher numbers were collected and sequenced for this work (Table S1).

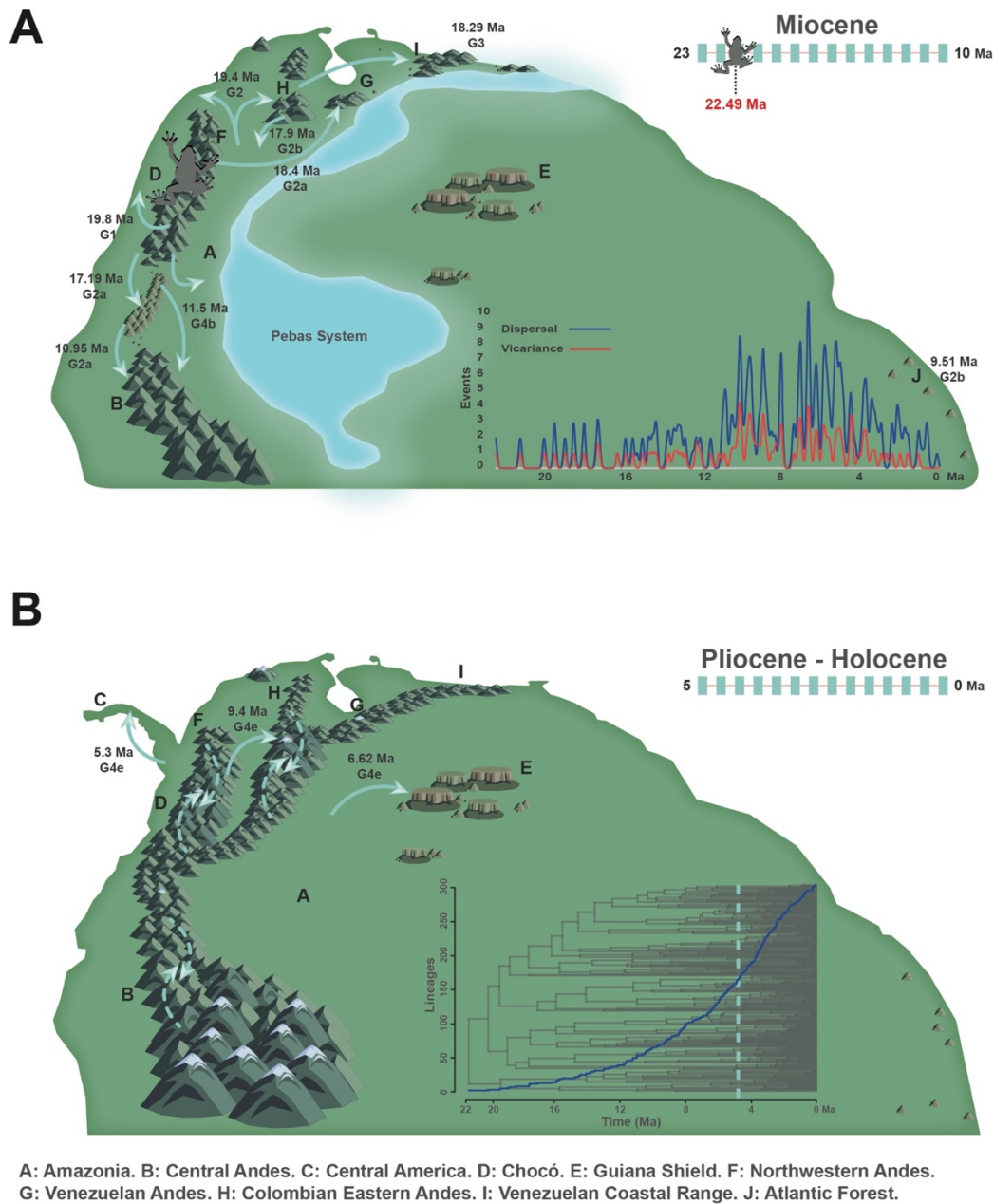


Fig 3. Schematic representation of the biogeographic patterns of *Pristimantis* in the Neotropical region resulting from the calibration and reconstruction of the ancestral area using BioGeoBEARS. The biogeographic events are based on proposals by Hoorn et al. (2010). A. Miocene, representing the elevation of the Andean areas in the northern and central Andes and the presence of wetland areas in the western Amazonia rainforest. B. The mega-wetland disappeared, and the mainland rainforests expanded; the formation of the Isthmus of Panama and the current conformation of the Andes and Amazonas.

Supplementary information

Table S1. *Pristimantis* species included in the phylogenetic analyzes with the respective Genbank accession numbers linked to the NCBI website: <https://www.ncbi.nlm.nih.gov/genbank/>

Taxon	12S	16S	CO1	CYTB	ND1	ND2	RAG1	TYR
		<u>MK211634.</u>						
<i>Craugastor podiciferus</i>	<u>EF493360.1</u>	<u>1</u>	-	-	-	-	<u>EF493450.1</u>	<u>EF493481.1</u>
<i>Eleutherodactylus caribe</i>	<u>EF493385.1</u>	<u>EF493385.1</u>	-	-	-	-	-	<u>EF493472.1</u>
<i>Eleutherodactylus marnockii</i>	<u>EF493820.1</u>	<u>EF493642.1</u>	-	-	-	-	-	<u>EF493476.1</u>
<i>Oreobates cruralis</i>	<u>EU186666.1</u>	<u>EU186666.1</u>	-	<u>EU368881.1</u>	-	-	<u>KY672963.1</u>	<u>EU186764.1</u>
<i>T. douglasi</i> ANDES-A 1932	-	-	-	-	-	-	-	-
<i>T. lentiginosus</i>	<u>KP297386.1</u>	<u>KP297386.1</u>	-	-	-	-	<u>KP297388.1</u>	<u>KP297390.1</u>
<i>T. prolixodiscus</i>	<u>KP297385.1</u>	<u>KP297385.1</u>	-	-	-	-	<u>KP297387.1</u>	<u>KP297389.1</u>
<i>P. abakapa</i>	-	<u>JQ742162.1</u>	-	-	<u>JQ742336.1</u>	-	-	-
<i>P. acatallelus</i>	-	<u>JN104675.1</u>	<u>JN371120.1</u>	-	-	-	-	<u>KT898368.1</u>
<i>P. acerus</i>	<u>EF493678.1</u>	<u>EF493678.1</u>	-	-	-	-	-	-
<i>P. achatinus</i>	<u>EF493827.1</u>	<u>JN104676.1</u>	<u>JN371121.1</u>	-	-	-	<u>JQ025168.1</u> <u>MH481368.</u>	<u>KT898335.1</u>
<i>P. achuar</i>	-	<u>EU130626.1</u>	-	<u>EU130668.1</u>	-	-	<u>1</u>	-
<i>P. actites</i>	<u>EF493696.1</u>	<u>EF493696.1</u>	-	-	-	-	<u>EF493432.1</u>	<u>EF493494.1</u>
<i>P. acuminatus</i>	-	<u>EU130579.1</u>	-	<u>EU130577.1</u>	-	-	-	-
<i>P. adiaastolus</i>	<u>AY964086.1</u>	-	-	-	-	-	-	-
<i>P. afrox</i>	-	-	-	-	-	-	<u>MT372578.1</u>	-
<i>P. albertus</i>	<u>EU186695.1</u>	-	-	-	-	-	-	-
<i>P. altae</i>	<u>JN991496.1</u>	-	<u>JN991361.1</u>	-	-	<u>EU443185.1</u> <u>GU168782.</u>	<u>JQ025174.1</u>	<u>JN991560.1</u>
<i>P. altamazonicus</i>	<u>EF493670.1</u>	<u>EF493670.1</u>	<u>MF118717.1</u>	<u>1</u> <u>MH516238.</u>	-	<u>1</u>	<u>MF118735.1</u> <u>MH481369.</u>	<u>MF118706.1</u>
<i>P. altamnis</i>	-	<u>EU130617.1</u>	-	<u>EU130673.1</u>	-	-	<u>1</u>	-

<i>P. andinognomus</i>	-	<u>KY967671.1</u>	-	-	<u>KY967652.1</u>	-	<u>KY967688.1</u>	-
<i>P. angustilineatus</i>	-	<u>JN104677.1</u>	<u>JN371123.1</u>	-	-	-	-	<u>KT898341.1</u>
<i>P. aniptopalmatus</i>	<u>EF493390.1</u>	<u>EF493390.1</u>	-	-	-	-	-	-
		<u>MG820148.</u>	<u>MG820176.</u>					
<i>P. antisuyu</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-
<i>P. appendiculatus</i>	<u>EF493524.1</u>	<u>EF493524.1</u>	-	-	-	-	-	-
<i>P. ardalonychus</i>	<u>EU186664.1</u>	<u>EU186664.1</u>	-	-	-	-	-	-
<i>P. ashaninka</i>	-	<u>KY006110.1</u>	-	-	-	-	-	-
		<u>MK881444.</u>					<u>MK881347.</u>	
<i>P. atillo</i>	-	<u>1</u>	-	-	-	-	<u>1</u>	-
		<u>MK881473.</u>			<u>MK881471.</u>		<u>MK881364.</u>	
<i>P. atratus</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. attenboroughi</i>	<u>KY594763.1</u>	<u>KY594755.1</u>	<u>KY962784.1</u>	-	-	-	<u>KY962760.1</u>	-
<i>P. aureoventris</i>	-	<u>JQ742151.1</u>	-	-	<u>JQ742333.1</u>	-	-	-
<i>P. bambu</i>	<u>JF906319.1</u>	-	-	-	<u>KY967654.1</u>	-	<u>KY967693.1</u>	-
		<u>MK881420.</u>					<u>MK881325.</u>	
<i>P. bicantus</i>	-	<u>1</u>	-	-	-	-	<u>1</u>	-
<i>P. bipunctatus</i>	<u>EF493702.1</u>	<u>EF493702.1</u>	<u>KY962785.1</u>	-	-	-	<u>KY962765.1</u>	<u>EF493492.1</u>
<i>P. boulengeri</i>	-	<u>KY494232.1</u>	<u>KU724444.1</u>	<u>DQ195480.1</u>	-	-	<u>KY494205.1</u>	<u>KY494217.1</u>
<i>P. bounides</i>	-	<u>KY962797.1</u>	<u>KY962789.1</u>	-	-	-	<u>KY962771.1</u>	-
<i>P. brevicrus</i>	-	<u>MF118702.1</u>	<u>MF118727.1</u>	-	-	-	<u>MF118751.1</u>	-
<i>P. brevifrons</i>	<u>JN991498.1</u>	<u>JN370957.1</u>	<u>JN371048.1</u>	-	-	-	-	<u>KT898343.1</u>
<i>P. briceni</i>	<u>JX155297.1</u>	<u>JX155297.1</u>	-	-	-	-	-	-
<i>P. bromeliaceus</i>	<u>EF493351.1</u>	<u>EF493351.1</u>	-	-	-	-	-	-
			<u>MG820165.</u>					
<i>P. buccinator</i>	-	<u>EU712630.1</u>	<u>1</u>	-	-	-	<u>KY672966.1</u>	<u>KY681071.1</u>
<i>P. buckleyi</i>	<u>EF493350.1</u>	<u>DQ679379.1</u>	-	-	-	-	-	-
<i>P. buenaventura</i>	<u>KU999242.1</u>	<u>KU999170.1</u>	-	-	-	-	-	-
<i>P. cajamarcensis</i>	<u>EF493823.1</u>	<u>EF493663.1</u>	-	-	-	-	-	-
	<u>MK993332.</u>	<u>MK604536.</u>					<u>MK602184.</u>	
<i>P. cajanuma</i>	<u>1</u>	<u>1</u>	-	-	-	-	<u>1</u>	-

<i>P. calcaratus</i>	-	<u>JN104657.1</u>	-	-	-	-	<u>KT898307.1</u>	<u>KT898346.1</u>
<i>P. calcarulatus</i>	<u>EF493523.1</u>	<u>EF493523.1</u>	-	-	-	-	-	-
<i>P. capitonis</i>	-	-	-	-	-	-	<u>KT898298.1</u>	<u>KT898333.1</u>
<i>P. caprifer</i>	<u>EF493391.1</u>	<u>EF493391.1</u>	-	-	-	-	-	-
<i>P. carvalhoi</i>	-	<u>DQ195454.1</u>	<u>KY672983.1</u>	<u>DQ195481.1</u>	-	-	<u>KY672967.1</u>	<u>KY681072.1</u>
<i>P. caryophyllaceus</i>	<u>EU186686.1</u>	<u>EU186686.1</u>	<u>FJ766776.1</u>	-	-	-	<u>KJ201964.1</u>	<u>JN991563.1</u>
<i>P. cedros</i>	-	<u>EF493523.1</u>	-	-	-	-	-	-
<i>P. celator</i>	<u>EF493685.1</u>	<u>EF493685.1</u>	-	-	-	-	-	-
<i>P. cerasinus</i>	<u>JN991502.1</u>	<u>FJ784387.1</u>	<u>FJ766786.1</u>	-	-	<u>EU443194.1</u>	<u>JQ025178.1</u>	<u>JN991565.1</u>
<i>P. ceuthospilus</i>	<u>EF493520.1</u>	<u>EF493520.1</u>	-	-	-	-	-	-
<i>P. chalceus</i>	<u>EF493675.1</u>	<u>EF493675.1</u>	-	-	-	-	-	-
<i>P. chiastonotus</i>	-	<u>JN691273.1</u>	-	-	-	-	-	<u>JN692005.1</u>
<i>P. chloronotus</i>	<u>AY326007.1</u>	<u>AY326007.1</u>	-	-	-	-	-	-
<i>P. chocoensis</i>	-	-	-	-	-	-	<u>MT372629.1</u>	-
		<u>MK881477.</u>			<u>MK881476.</u>		<u>MK881369.</u>	
<i>P. chomskyi</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. cisnerosi</i>	-	<u>MT372697.1</u>	-	-	<u>MT372570.1</u>	-	<u>MT372625.1</u>	-
<i>P. citriogaster</i>	<u>EF493700.1</u>	<u>EF493700.1</u>	-	-	-	-	-	-
<i>P. colomai</i>	<u>EF493354.1</u>	<u>EF493354.1</u>	-	-	-	-	<u>EF493440.1</u>	<u>EF493502.1</u>
<i>P. condor</i>	<u>EF493701.1</u>	<u>EF493701.1</u>	-	-	-	-	<u>EF493443.1</u>	<u>EF493504.1</u>
<i>P. conservatio</i>	<u>JX155287.1</u>	<u>JX155287.1</u>	-	-	-	-	-	-
<i>P. conspicillatus</i>	<u>EF493529.1</u>	<u>EF493529.1</u>	-	-	-	-	<u>EF493437.1</u>	<u>EF493499.1</u>
<i>P. cremnobates</i>	<u>EF493528.1</u>	<u>EF493528.1</u>	-	-	-	-	<u>EF493424.1</u>	<u>EF493486.1</u>
<i>P. crenunguis</i>	<u>EF493693.1</u>	-	-	-	-	-	-	-
				<u>MH516243.</u>				
<i>P. croceinguinis</i>	<u>EF493669.1</u>	<u>EF493665.1</u>	<u>KY962787.1</u>	<u>1</u>	-	-	<u>KY962767.1</u>	-
<i>P. crucifer</i>	<u>EU186736.1</u>	<u>EU186718.1</u>	-	-	-	-	-	-
<i>P. cruciocularis</i>	<u>EU186656.1</u>	<u>KY006095.1</u>	<u>KY962788.1</u>	-	-	-	<u>KY962768.1</u>	-
<i>P. cruentus</i>	<u>EF493697.1</u>	<u>EF493697.1</u>	<u>KC129259.1</u>	-	<u>FJ882747.1</u>	<u>EU443188.1</u>	<u>JQ025181.1</u>	<u>JN991569.1</u>

<i>P. cryophilus</i>	<u>EF493672.1</u>	<u>EF493672.1</u>	-	-	-	-	-	-
		<u>MK881475.</u>				<u>MK881475.</u>	<u>MK881365.</u>	
<i>P. cryptomelas</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. curtipes</i>	<u>EF493513.1</u>	<u>EF493513.1</u>	-	-	<u>AY819473.1</u>	-	<u>KX525470.1</u>	<u>EF493497.1</u>
<i>P. danae</i>	-	<u>KY652652.1</u>	<u>KY672984.1</u>	<u>EF636949.1</u>	-	-	<u>KY672968.1</u>	<u>KY681073.1</u>
<i>P. delius</i>	-	<u>KP064150.1</u>	<u>KP064162.1</u>	-	-	-	<u>MF118753.1</u>	-
<i>P. devillei</i>	<u>EF493688.1</u>	<u>EF493688.1</u>	-	-	-	-	-	-
<i>P. diadematus</i>	<u>EU186668.1</u>	<u>EU186668.1</u>	-	-	-	-	-	-
<i>P. dissimulatus</i>	<u>EF493522.1</u>	<u>EF493522.1</u>	-	-	-	-	-	-
<i>P. dorado</i>	-	<u>KU496877.1</u>	<u>KU496873.1</u>	-	-	-	-	-
<i>P. dorsopictus</i>	<u>KP082864.1</u>	<u>KU724440.1</u>	<u>KU724448.1</u>	-	-	-	-	<u>KP082879.1</u>
<i>P. duellmani</i>	<u>AY326003.1</u>	<u>AY326003.1</u>	-	-	-	-	<u>EF493438.1</u>	<u>EF493500.1</u>
<i>P. ecuadorensis</i>	<u>KX785339.1</u>	-	-	-	<u>KX785348.1</u>	-	-	-
<i>P. eremitus</i>	-	<u>MT508757.1</u>	-	-	-	-	-	-
<i>P. eriphus</i>	<u>EU186671.1</u>	<u>EU186671.1</u>	-	<u>DQ195484.1</u>	-	-	-	-
		<u>MG820143.</u>	<u>MG820170.</u>					
<i>P. erythroinguinis</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-
<i>P. erythropleura</i>	<u>JN991509.1</u>	<u>JN371036.1</u>	<u>JN371127.1</u>	-	-	-	<u>JQ025182.1</u>	<u>KT898349.1</u>
<i>P. euphronides</i>	<u>EF493527.1</u>	<u>EF493527.1</u>	-	-	-	-	<u>EF493427.1</u>	<u>EF493489.1</u>
<i>P. fenestratus</i>	<u>EF493703.1</u>	<u>EF493703.1</u>	<u>KU494666.1</u>	<u>EF636949.1</u>	-	-	-	-
		<u>MN080223.</u>						
<i>P. ferwerdai</i>	-	<u>1</u>	-	-	-	-	-	-
		<u>MG820163.</u>	<u>MG820188.</u>					
<i>P. flavobracatus</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-
		<u>MK881480.</u>					<u>MK881355.</u>	
<i>P. gagliardoi</i>	-	<u>1</u>	-	-	-	-	<u>1</u>	-
<i>P. galdi</i>	<u>EU186670.1</u>	<u>EU186670.1</u>	-	-	-	-	-	<u>EU186767.1</u>
<i>P. gentryi</i>	<u>EF493511.1</u>	<u>EF493511.1</u>	-	-	-	-	-	-
<i>P. ginesi</i>	<u>JX155295.1</u>	<u>JX155295.1</u>	-	-	-	-	-	-
		<u>MK992573.</u>	<u>MN010717.</u>					
<i>P. giorgii</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-

<i>P. glandulosus</i>	<u>EF493676.1</u>	<u>EF493676.1</u>	-	-	-	-	-	-
		<u>MK881402.</u>			<u>MK881402.</u>		<u>MK881316.</u>	
<i>P. gloria</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
	<u>MH306194.</u>	<u>MH306193.</u>						
<i>P. gralarias</i>	<u>1</u>	<u>1</u>	-	-	-	-	-	-
<i>P. gryllus</i>	<u>JX306022.1</u>	<u>JX306022.1</u>	-	-	-	-	-	-
<i>P. gutturalis</i>	<u>JN690705.1</u>	<u>JN691313.1</u>	-	-	-	-	-	<u>JN692012.1</u>
		<u>MK881504.</u>			<u>MK881504.</u>		<u>MK881387.</u>	
<i>P. hampatusami</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. hectus</i>	-	<u>JN104680.1</u>	<u>JN371130.1</u>	-	-	-	-	<u>KT898352.1</u>
<i>P. humboldti</i>	-	<u>KY962799.1</u>	<u>KY962792.1</u>	-	-	-	<u>KY962776.1</u>	-
<i>P. imitatrix</i>	<u>EF493824.1</u>	<u>EF493667.1</u>	-	-	-	-	-	-
<i>P. inguinalis</i>	<u>EU186676.1</u>	<u>EU186676.1</u>	-	-	-	-	-	<u>JN692014.1</u>
<i>P. inusitatus</i>	<u>EF493677.1</u>	<u>EF493677.1</u>	-	-	-	-	-	-
<i>P. jaguensis</i>	<u>KP082862.1</u>	<u>KP082873.1</u>	<u>KP082877.1</u>	-	-	-	-	<u>KP082878.1</u>
<i>P. jester</i>	-	<u>JQ742169.1</u>	-	-	<u>JQ742341.1</u>	-	-	-
		<u>MK881468.</u>			<u>MK881466.</u>		<u>MK881360.</u>	
<i>P. jimenezi</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. juanchoi</i>	-	<u>JN104681.1</u>	-	-	-	-	-	<u>KT898353.1</u>
<i>P. jubatus</i>	-	<u>JN370977.1</u>	<u>JN371075.1</u>	-	-	-	-	<u>KT898356.1</u>
<i>P. kelephas</i>	-	<u>JN104662.1</u>	-	-	-	-	-	<u>KT898359.1</u>
<i>P. kichwarum</i>	-	<u>EU130582.1</u>	-	<u>EU130636.1</u>	-	-	-	-
<i>P. koehleri</i>	<u>FJ438810.1</u>	-	-	-	-	-	-	-
<i>P. labiosus</i>	<u>EF493694.1</u>	<u>MT372686.1</u>	-	-	-	-	-	-
<i>P. lancinii</i>	<u>JX155284.1</u>	<u>JX155284.1</u>	-	-	-	-	-	-
<i>P. lanthanites</i>	<u>EF493695.1</u>	<u>EF493695.1</u>	-	-	-	-	-	-
<i>P. lasalleorum</i>	<u>KY494221.1</u>	<u>KY494221.1</u>	<u>KY627812.1</u>	-	-	-	-	-
<i>P. latidiscus</i>	<u>EF493698.1</u>	<u>EF493698.1</u>	-	-	-	-	-	-
		<u>MN865341.</u>	<u>MN010741.</u>					
<i>P. latro</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-
<i>P. leoni</i>	<u>EF493684.1</u>	<u>EF493684.1</u>	-	-	-	-	<u>EF493433.1</u>	<u>EF493495.1</u>

<i>P. leopardus</i>	-	<u>KY627792.1</u>	<u>KY627819.1</u>	-	-	-	-	-
<i>P. leptolophus</i>	<u>KY494226.1</u>	-	-	-	-	-	<u>KY494216.1</u>	-
				<u>MH516245.</u>			<u>MH481370.</u>	
<i>P. librarius</i>	<u>JN991515.1</u>	<u>JN991451.1</u>	<u>JN991379.1</u>	<u>1</u>	-	-	<u>1</u>	<u>JN991571.1</u>
<i>P. lindae</i>	-	<u>KY652653.1</u>	<u>KY672985.1</u>	-	-	-	<u>KY672969.1</u>	<u>KY681074.1</u>
<i>P. lirellus</i>	<u>EF493521.1</u>	<u>EF493521.1</u>	-	-	-	-	-	-
		<u>MK881497.</u>			<u>MK881497.</u>		<u>MK881382.</u>	
<i>P. lividus</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. llojsintuta</i>	-	<u>EU712641.1</u>	-	-	-	-	-	-
<i>P. luscombei</i>	<u>KP064143.1</u>	<u>KP064156.1</u>	-	-	-	-	-	-
<i>P. luteolateralis</i>	<u>EF493517.1</u>	<u>EF493517.1</u>	-	-	-	-	-	-
<i>P. lutitus</i> ANDES-A 1771	-	<u>KP149401.1</u>	-	-	-	-	-	-
		<u>MK881424.</u>			<u>MK881421.</u>		<u>MK881326.</u>	
<i>P. lutzae</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. lymani</i>	<u>EF493392.1</u>	<u>EF493392.1</u>	-	-	-	-	-	-
<i>P. lynchi</i>	-	<u>DQ195463.1</u>	-	-	-	-	-	-
<i>P. maculosus</i>	-	<u>KY494240.1</u>	-	-	-	-	<u>KY494210.1</u>	-
<i>P. malkini</i>	<u>EU186663.1</u>	<u>EU186663.1</u>	-	-	-	-	-	-
		<u>MK391386.</u>			<u>MK391386.</u>		<u>MK391384.</u>	
<i>P. mallii</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. marmoratus</i>	<u>EU186692.1</u>	<u>EU186692.1</u>	-	-	-	-	-	<u>JN692010.1</u>
				<u>MH516247.</u>				
<i>P. martiae</i>	<u>JN991517.1</u>	-	<u>JN991380.1</u>	<u>1</u>	-	-	<u>MF118755.1</u>	<u>JN991572.1</u>
<i>P. matidiktyo</i>	<u>KP064140.1</u>	<u>KP064147.1</u>	<u>KP064159.1</u>	-	-	-	-	-
<i>P. mazar</i>	-	<u>KY967664.1</u>	-	-	<u>KY967647.1</u>	-	<u>KY967685.1</u>	-
<i>P. melanogaster</i>	<u>EF493826.1</u>	<u>EF493664.1</u>	-	-	-	-	-	-
<i>P. mendax</i>	<u>EU186659.1</u>	<u>EU186659.1</u>	-	-	-	-	-	-
<i>P. merostictus</i>	-	<u>DQ195465.1</u>	-	<u>DQ195488.1</u>	-	-	-	-
		<u>MK391385.</u>			<u>MK391385.</u>		<u>MK391383.</u>	
<i>P. miktos</i>	-	<u>1</u>	<u>KP064163.1</u>	-	<u>1</u>	-	<u>1</u>	<u>MF118703.1</u>
<i>P. mindo</i>	-	<u>KF801581.1</u>	-	-	-	-	-	-

<i>P. minutulus</i>	<u>EU186657.1</u>	<u>EU186657.1</u>	-	-	-	-	-	-
		<u>MK992550.</u>	<u>MN010697.</u>					
<i>P. moa</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-
<i>P. moro</i>	<u>JN991520.1</u>	<u>JN991453.1</u>	<u>JN991384.1</u>	-	-	-	<u>JQ025192.1</u>	<u>JN991575.1</u>
		<u>MK881489.</u>						
<i>P. multicolor</i>	-	<u>1</u>	-	-	-	-	-	-
<i>P. muranunka</i>	-	<u>KY967661.1</u>	-	-	<u>KY967642.1</u>	-	<u>KY967680.1</u>	-
		<u>MK881501.</u>			<u>MK881501.</u>		<u>MK881386.</u>	
<i>P. muscosus</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
						<u>AY273103.</u>		
<i>P. museosus</i>	<u>JN991521.1</u>	<u>KC014941.1</u>	-	-	-	<u>1</u>	<u>JQ025193.1</u>	<u>JN991576.1</u>
	<u>KM675434.</u>	<u>KM675458.</u>						
<i>P. mutabilis</i>	<u>1</u>	<u>1</u>	-	-	-	-	-	-
<i>P. myops</i>	-	<u>JN104682.1</u>	<u>JN371132.1</u>	-	-	-	-	<u>KT898361.1</u>
		<u>MK881436.</u>			<u>MK881436.</u>		<u>MK881336.</u>	
<i>P. nangaritza</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. nervicus</i>	<u>JN991522.1</u>	<u>JN991456.1</u>	<u>JN991386.1</u>	-	-	-	<u>JQ025194.1</u>	<u>JN991577.1</u>
<i>P. nietoi</i>	<u>KU999286.1</u>	<u>KU999214.1</u>	-	-	-	-	-	-
<i>P. nyctophylax</i>	<u>EF493526.1</u>	<u>EF493526.1</u>	-	-	-	-	<u>EF493425.1</u>	<u>EF493487.1</u>
<i>P. ockendeni</i>	<u>EF493519.1</u>	<u>EF493519.1</u>	<u>KY672986.1</u>	-	-	-	<u>KY672970.1</u>	<u>KY681075.1</u>
<i>P. ocreatus</i>	<u>EF493682.1</u>	<u>EF493682.1</u>	-	-	-	-	-	-
		<u>MK881398.</u>					<u>MK881312.</u>	
<i>P. omeviridis</i>	-	<u>1</u>	-	-	-	-	<u>1</u>	-
<i>P. orcesi</i>	<u>EF493679.1</u>	<u>EF493679.1</u>	-	-	-	-	-	-
<i>P. orestes</i>	<u>EF493388.1</u>	<u>EF493388.1</u>	-	-	<u>KY967651.1</u>	-	<u>KY967689.1</u>	-
<i>P. ornatissimus</i>	<u>KX785338.1</u>	-	-	-	<u>KU720480.1</u>	-	-	-
<i>P. ornatus</i>	<u>EU186660.1</u>	<u>EU186660.1</u>	-	-	-	-	-	-
<i>P. orpacobates</i>	-	-	<u>JN371133.1</u>	-	-	-	-	<u>KT898363.1</u>
<i>P. paisa</i>	<u>JN991524.1</u>	<u>JN991477.1</u>	<u>JN991389.1</u>	-	-	-	-	<u>JN991578.1</u>
<i>P. palmeri</i>	-	<u>JN371003.1</u>	<u>JN371118.1</u>	-	-	-	-	<u>KT898366.1</u>
<i>P. paramerus</i>	<u>JX155279.1</u>	<u>JX155279.1</u>	-	-	-	-	-	-
						<u>AY273102.</u>		
<i>P. pardalis</i>	<u>JN991527.1</u>	<u>FJ784590.1</u>	<u>FJ766804.1</u>	-	-	<u>1</u>	<u>JQ025198.1</u>	<u>JN991579.1</u>

<i>P. parectatus</i>	<u>KY494222.1</u>	<u>KY494222.1</u>	<u>KY627826.1</u>	-	-	-	<u>KY494207.1</u>	<u>KY494220.1</u>
<i>P. parvillus</i>	<u>EF493352.1</u>	<u>EF493352.1</u>	-	-	-	-	-	-
<i>P. penelopus</i>	-	-	<u>KY652629.1</u>	-	-	-	-	-
<i>P. peraticus</i>	<u>KY494224.1</u>	<u>KY494224.1</u>	-	-	-	-	<u>KY494208.1</u>	-
<i>P. permixtus</i>	-	<u>DQ195467.1</u>	-	-	-	-	-	-
<i>P. peruvianus</i>	<u>EF493707.1</u>	<u>EF493707.1</u>	<u>JN991392.1</u>	<u>DQ195492.1</u>	-	-	<u>EF493436.1</u>	<u>EF493498.1</u>
<i>P. petrobardus</i>	<u>EF493825.1</u>	<u>EF493367.1</u>	-	-	-	-	-	-
<i>P. pharangobates</i>	-	<u>KY652655.1</u> <u>MK881427.</u>	<u>KY672987.1</u>	-	-	-	<u>KY681088.1</u> <u>MK881332.</u>	<u>KY681076.1</u>
<i>P. philipi</i>	-	<u>1</u> <u>MK881507.</u>	-	-	<u>1</u> <u>MK881507.</u>	-	<u>1</u> <u>MK881390.</u>	-
<i>P. phoxocephalus</i>	-	<u>1</u> <u>MK881399.</u>	-	-	<u>1</u> <u>MK881399.</u>	-	<u>1</u> <u>MK881313.</u>	-
<i>P. pichincha</i>	-	<u>1</u> <u>MK992524.</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. pictus</i>	-	<u>1</u>	<u>MN010670.</u>	-	-	-	-	-
<i>P. pirrensis</i>	<u>JN991528.1</u>	<u>JN991462.1</u>	<u>JN991393.1</u>	-	-	<u>EU443190.1</u>	<u>JQ025199.1</u>	<u>JN991580.1</u>
<i>P. platydactylus</i>	<u>FJ438811.1</u>	<u>EU192255.1</u>	<u>JN991394.1</u>	<u>EF636948.1</u>	-	-	<u>KY672971.1</u>	<u>KY681077.1</u>
<i>P. pleurostriatus</i>	<u>JX155278.1</u>	<u>JX155292.1</u>	-	-	-	-	-	-
<i>P. pluvialis</i>	-	<u>KX155578.1</u> <u>MK992577.</u>	<u>KX155585.1</u> <u>MN010732.</u>	-	-	-	<u>KY962770.1</u>	-
<i>P. pluvian</i>	-	<u>1</u>	<u>1</u>	-	-	-	-	-
<i>P. prolatus</i>	<u>EU186701.1</u>	<u>EU186701.1</u>	-	-	-	-	-	-
<i>P. prometeii</i>	-	<u>KX525478.1</u>	-	-	-	-	<u>KX525471.1</u>	-
<i>P. ptochus</i>	<u>JN991530.1</u>	-	<u>JN991395.1</u>	-	-	-	-	<u>JN991581.1</u>
<i>P. puipui</i>	-	<u>KY962800.1</u>	-	-	-	-	<u>KY962777.1</u>	-
<i>P. pulvinatus</i>	<u>EU186741.1</u>	-	-	-	-	-	-	-
<i>P. pycnodermis</i>	<u>EF493680.1</u>	<u>EF493680.1</u>	-	-	-	-	-	-
<i>P. pyrrhomerus</i>	<u>EF493683.1</u>	<u>EF493683.1</u>	-	-	-	-	<u>MN068025.</u> <u>1</u>	-
<i>P. quantus</i>	-	<u>JN104684.1</u>	<u>JN371136.1</u>	-	-	-	-	<u>KT898367.1</u>

							<u>MH481371.</u>	
<i>P. quaquaversus</i>	-	<u>EU130580.1</u>	<u>JN991396.1</u>	<u>EU130578.1</u>	-	-	<u>1</u>	<u>JN991582.1</u>
<i>P. quinquagesimus</i>	<u>EF493690.1</u>	<u>EF493690.1</u>	-	-	-	-	-	-
	<u>MK993337.</u>	<u>MK604544.</u>						
<i>P. quintanai</i>	<u>1</u>	<u>1</u>	-	-	-	-	-	-
<i>P. reichlei</i>	<u>EF493707.1</u>	<u>EF493707.1</u>	<u>KY672989.1</u>	-	-	-	<u>KY672972.1</u>	<u>KY681078.1</u>
<i>P. rhabdocnemus</i>	<u>EU186724.1</u>	<u>KY006107.1</u>	-	-	-	-	-	-
<i>P. rhabdolaemus</i>	-	<u>EF493706.1</u>	-	-	-	-	-	-
<i>P. rhodoplichus</i>	<u>EF493674.1</u>	<u>EF493674.1</u>	-	-	-	-	-	-
<i>P. ridens</i>	<u>EF493355.1</u>	<u>EF493355.1</u>	<u>FJ766808.1</u>	-	-	<u>EU443160.1</u>	<u>JQ025204.1</u>	<u>JN991586.1</u>
<i>P. riveti</i>	<u>EF493348.1</u>	<u>EF493348.1</u>	-	-	-	-	-	-
<i>P. rozei</i>	<u>EF493691.1</u>	<u>EF493691.1</u>	-	-	-	-	<u>EF493429.1</u>	<u>EF493491.1</u>
					<u>MK881407.</u>		<u>MK881320.</u>	
<i>P. rubicundus</i>	-	<u>MT372715.1</u>	-	-	<u>1</u>	-	<u>1</u>	-
		<u>MN954203.</u>						
<i>P. rupicola</i>	-	<u>1</u>	-	-	-	-	-	-
<i>P. sagittulus</i>	<u>EF493705.1</u>	<u>EF493705.1</u>	-	-	-	-	<u>EF493439.1</u>	<u>EF493501.1</u>
<i>P. salaputium</i>	-	-	<u>KY672990.1</u>	-	-	-	<u>KY672973.1</u>	<u>KY681079.1</u>
<i>P. saltissimus</i>	<u>EU186693.1</u>	<u>EU186693.1</u>	-	-	<u>JQ742340.1</u>	-	-	-
<i>P. samaipatae</i>	<u>FJ438814.1</u>	-	-	<u>EU368890.1</u>	-	-	-	-
		<u>MN078266.</u>						
<i>P. satagius</i>	-	<u>1</u>	-	-	-	-	-	-
	<u>MK993329.</u>	<u>MK604534.</u>						
<i>P. saturninoi</i>	<u>1</u>	<u>1</u>	-	-	-	-	-	-
<i>P. schultei</i>	<u>EF493681.1</u>	<u>EF493681.1</u>	-	-	-	-	-	-
<i>P. scoloblepharus</i>	-	<u>KY494236.1</u>	<u>KY627834.1</u>	-	-	-	<u>KY494213.1</u>	<u>KY494218.1</u>
<i>P. shrevei</i>	<u>EF493692.1</u>	<u>EF493692.1</u>	-	-	-	-	-	-
<i>P. simonbolivari</i>	<u>EF493671.1</u>	<u>EF493671.1</u>	-	-	<u>KY967657.1</u>	-	<u>KY967695.1</u>	-
<i>P. simonsii</i>	<u>EU186665.1</u>	<u>EU186665.1</u>	-	-	-	-	-	-
<i>P. simoterus</i>	-	<u>DQ195471.1</u>	-	<u>DQ195495.1</u>	-	-	-	-
<i>P. skydmainos</i>	<u>EF493393.1</u>	<u>EF493393.1</u>	-	-	-	-	-	-

	<u>KM675429.</u>	<u>KM675449.</u>							
<i>P. sobetes</i>	<u>1</u>	<u>1</u>	-	-	-	-	-	-	-
<i>P. spinosus</i>	<u>EF493673.1</u>	<u>EF493673.1</u>	-	-	-	-	-	-	-
		<u>MK881393.</u>			<u>MK881393.</u>		<u>MK881308.</u>		
<i>P. sternothylax</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-	-
<i>P. stictogaster</i>	<u>EF493704.1</u>	<u>EF493704.1</u>	-	-	-	-	<u>EF493445.1</u>	<u>EF493506.1</u>	
<i>P. subsigillatus</i>	<u>EF493525.1</u>	<u>EF493525.1</u>	-	-	-	-	-	-	-
<i>P. suetus</i>	<u>JN991537.1</u>	<u>JN991469.1</u>	-	-	-	-	-	-	-
<i>P. supernatis</i>	<u>AY326005.1</u>	<u>AY326005.1</u>	-	-	-	-	-	-	-
<i>P. surdus</i>	<u>EF493687.1</u>	<u>EF493687.1</u>	-	-	-	-	-	-	-
<i>P. terraebolivaris</i>	<u>EU186650.1</u>	<u>EU186650.1</u>	-	-	-	-	-	-	-
		<u>MK881478.</u>							
<i>P. teslai</i>	-	<u>1</u>	-	-	-	-	-	-	-
<i>P. thectopternus</i>	-	<u>JN104685.1</u>	-	-	-	-	-	<u>KT898369.1</u>	
<i>P. thymalopsoides</i>	<u>EF493514.1</u>	<u>EF493514.1</u>	-	-	-	-	-	-	-
<i>P. thymelensis</i>	<u>EF493516.1</u>	<u>EF493516.1</u>	<u>JX564889.1</u>	-	<u>JX564889.1</u>	<u>JX564889.1</u>	-	<u>EF493503.1</u>	
	<u>MH668161.</u>	<u>MH668276.</u>					<u>MH708576.</u>		
<i>P. tiktik</i>	<u>1</u>	<u>1</u>	-	-	-	-	<u>1</u>	-	-
		<u>MK881499.</u>			<u>MK881418.</u>		<u>MK881385.</u>		
<i>P. tinguchaca</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-	-
<i>P. toftae</i>	<u>EF493353.1</u>	<u>EF493353.1</u>	<u>KY672991.1</u>	-	-	-	<u>KY672974.1</u>	<u>KY681080.1</u>	
		<u>MK881492.</u>			<u>MK881492.</u>		<u>MK881380.</u>		
<i>P. torresi</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-	-
		<u>MK881505.</u>			<u>MK881406.</u>		<u>MK881388.</u>		
<i>P. totoroi</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-	-
<i>P. trepidotus</i>	<u>EF493515.1</u>	<u>EF493515.1</u>	-	-	-	-	-	-	-
<i>P. truebae</i>	<u>EF493512.1</u>	<u>EF493512.1</u>	-	-	-	-	-	-	-
<i>P. urani</i>	-	<u>KU724441.1</u>	<u>KU724450.1</u>	-	-	-	-	-	-
<i>P. uranobates</i>	<u>KY494225.1</u>	<u>KY494225.1</u>	<u>KY627836.1</u>	-	-	-	<u>KY494214.1</u>	-	-
<i>P. urichi</i>	<u>EF493699.1</u>	<u>EF493699.1</u>	-	-	-	-	<u>EF493426.1</u>	<u>EF493488.1</u>	
<i>P. vanadise</i>	<u>JX155296.1</u>	<u>JX155296.1</u>	-	-	-	-	-	-	-
<i>P. ventrimarmoratus</i>	<u>JF906310.1</u>	-	-	-	-	-	-	-	-

<i>P. verecundus</i>	<u>EF493686.1</u>	<u>EF493686.1</u>	-	-	-	-	-	-
		<u>MK881467.</u>				<u>MK881469.</u>	<u>MK881361.</u>	
<i>P. verrucolatus</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
		<u>MK881479.</u>			<u>MK881474.</u>		<u>MK881367.</u>	
<i>P. versicolor</i>	<u>EF493389.1</u>	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. vertebralis</i>	<u>EF493689.1</u>	<u>EF493689.1</u>	-	-	-	-	-	-
<i>P. viejas</i>	<u>JN991547.1</u>	<u>JN991475.1</u>	<u>KY652635.1</u>	-	-	-	<u>JQ025211.1</u>	<u>JN991596.1</u>
<i>P. vilarsi</i>	-	<u>KP149438.1</u>	<u>KP149232.1</u>	-	-	-	-	-
<i>P. vinhai</i>	<u>JX267343.1</u>	-	-	-	-	-	<u>JX267591.1</u>	<u>JX267726.1</u>
<i>P. w-nigrum</i>	<u>AY326004.1</u>	<u>AY326004.1</u>	<u>JN371140.1</u>	<u>KC857704.1</u>	-	-	-	<u>KT898370.1</u>
<i>P. walkeri</i>	<u>KU999291.1</u>	<u>KU999230.1</u>	-	-	-	-	<u>EF493428.1</u>	<u>EF493490.1</u>
<i>P. wiensi</i>	<u>EF493377.1</u>	<u>EF493668.1</u>	-	-	-	-	-	-
<i>P. yantzaza</i>	-	-	-	-	-	-	<u>MF615251.1</u>	-
		<u>MK881506.</u>				<u>MK881506.</u>	<u>MK881389.</u>	
<i>P. yumbo</i>	-	<u>1</u>	-	-	<u>1</u>	-	<u>1</u>	-
<i>P. yuruaniensis</i>	-	<u>JQ742160.1</u>	-	-	<u>JQ742335.1</u>	-	-	-
<i>P. zeuctotylus</i>	<u>EU186678.1</u>	<u>EU186678.1</u>	-	-	-	-	-	<u>JN691914.1</u>
<i>P. zophus</i>	<u>JN991548.1</u>	<u>JN991480.1</u>	<u>JN991413.1</u>	-	-	-	<u>JQ025214.1</u>	<u>JN991598.1</u>
<i>P. affinis</i> ANDES-A 4217	-	-	-	-	-	-	-	-
	<u>MN215421.</u>	<u>MN215441.</u>			<u>MN218386.</u>		<u>MN225954.</u>	<u>MN218371.</u>
<i>P. anolirex</i> MCNUPH66	<u>1</u>	<u>1</u>	-	<u>1</u>	-	-	<u>1</u>	<u>1</u>
	<u>MN215410.</u>	<u>MN215429.</u>			<u>MN218376.</u>		<u>MN225947.</u>	<u>MN218365.</u>
<i>P. ardilae</i> MCNUPH117	<u>1</u>	<u>1</u>	-	<u>1</u>	-	-	<u>1</u>	<u>1</u>
<i>P. bicolor</i> ANDES-A 4939	-	-	-	-	-	-	-	-
	<u>MN215407.</u>	<u>MN215432.</u>			<u>MN218375.</u>		<u>MN225942.</u>	<u>MN218360.</u>
<i>P. bowara</i> MCNUPH160	<u>1</u>	<u>1</u>	-	<u>1</u>	-	-	<u>1</u>	<u>1</u>
<i>P. carrangerorum</i> ANDES-A 1254	-	-	-	-	-	-	-	-
<i>P. cf batrachites</i> MCNUPH37	-	-	-	-	-	-	-	-
<i>P. cf bogotensis</i> ANDES-A 4840	-	-	-	-	-	-	-	-
<i>P. cf erythropleura</i> EAB 6873	-	-	-	-	-	-	-	-
<i>P. cf viejas</i> ANDES-A 6850	-	-	-	-	-	-	-	-

<i>P. elegans</i> ANDES-A 4932	-	-	-	-	-	-	-	-
<i>P. fallax</i> ANDES-A 3045	-	-	-	-	-	-	-	-
<i>P. frater</i> ANDES-A 4845	-	-	-	-	-	-	-	-
<i>P. gaigei</i> ANDES-A 3045	-	-	-	-	-	-	-	-
<i>P. medemi</i> ANDES-A 4812	-	-	-	-	-	-	-	-
<i>P. miyatai</i> AJC 3473	-	-	-	-	-	-	-	-
	<u>MN215420.</u>			<u>MN218385.</u>			<u>MN225953.</u>	<u>MN218370.</u>
<i>P. mondolfii</i> MCNUPH457	<u>1</u>	-	-	<u>1</u>	-	-	<u>1</u>	<u>1</u>
<i>P. sp25</i> PSO-ZC 3528	-	-	-	-	-	-	-	-
	<u>MN215418.</u>	<u>MN215436.</u>	<u>MN218387.</u>	<u>MN218383.</u>			<u>MN225949.</u>	<u>MN218368.</u>
<i>P. nicefori</i> MCNUPH48	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>	-	-	<u>1</u>	<u>1</u>
<i>P. savagei</i> ANDES-A 1255	-	-	-	-	-	-	-	-
<i>P. sp1</i> MCNUPH439	-	-	-	-	-	-	-	-
<i>P. sp10</i> ANDES-A 4770	-	-	-	-	-	-	-	-
<i>P. sp11</i> TEMP-ABM 299	-	-	-	-	-	-	-	-
<i>P. sp12</i> TEMP-ABM 236	-	-	-	-	-	-	-	-
<i>P. sp13</i> MUD-H628	-	-	-	-	-	-	-	-
<i>P. sp14</i> MUD-H627	-	-	-	-	-	-	-	-
<i>P. leptolophus</i> MUD-H618	-	-	-	-	-	-	-	-
<i>P. myersi</i> MUD-H640	-	-	-	-	-	-	-	-
<i>P. sp15</i> MUD-H921	-	-	-	-	-	-	-	-
<i>P. sp16</i> ANDES-A3962	-	-	-	-	-	-	-	-
<i>P. sp17</i> ANDES-A 4183	-	-	-	-	-	-	-	-
<i>P. sp2</i> MCNUPH584	-	-	-	-	-	-	-	-
<i>P. sp18</i> JDF 117	-	-	-	-	-	-	-	-
<i>P. sp19</i> MUD-H612	-	-	-	-	-	-	-	-
<i>P. sp20</i> ANDES-A 4230	-	-	-	-	-	-	-	-
<i>P. sp21</i> TEMP-ABM 206	-	-	-	-	-	-	-	-
<i>P. sp22</i> MUD-H758	-	-	-	-	-	-	-	-

<i>P. sp23</i> ANDES-A 4202	-	-	-	-	-	-	-	-
<i>P. sp24</i> ANDES-A 3961	-	-	-	-	-	-	-	-
<i>P. sp3</i> MCNUPH54	-	-	-	-	-	-	-	-
<i>P. sp4</i> MCNUPH443	-	-	-	-	-	-	-	-
<i>P. sp5</i> MCNUPH30	-	-	-	-	-	-	-	-
<i>P. sp6</i> HUPAR050-19	-	-	-	-	-	-	-	-
<i>P. sp7</i> ANDES-A4232	-	-	-	-	-	-	-	-
<i>P. sp8</i> MCNUPH525	-	-	-	-	-	-	-	-
<i>P. sp9</i> MCNUPH42	-	-	-	-	-	-	-	-
<i>P. taeniatus</i> EAB 6866	-	-	-	-	-	-	-	-
<i>P. unistrigatus</i> BCQ 991	-	-	-	-	-	-	-	-
	<u>MN215417.</u>	<u>MN215439.</u>					<u>MN225951.</u>	
<i>P. yukpa</i> MCNUPH29	<u>1</u>	<u>1</u>	-	-	-	-	<u>1</u>	-

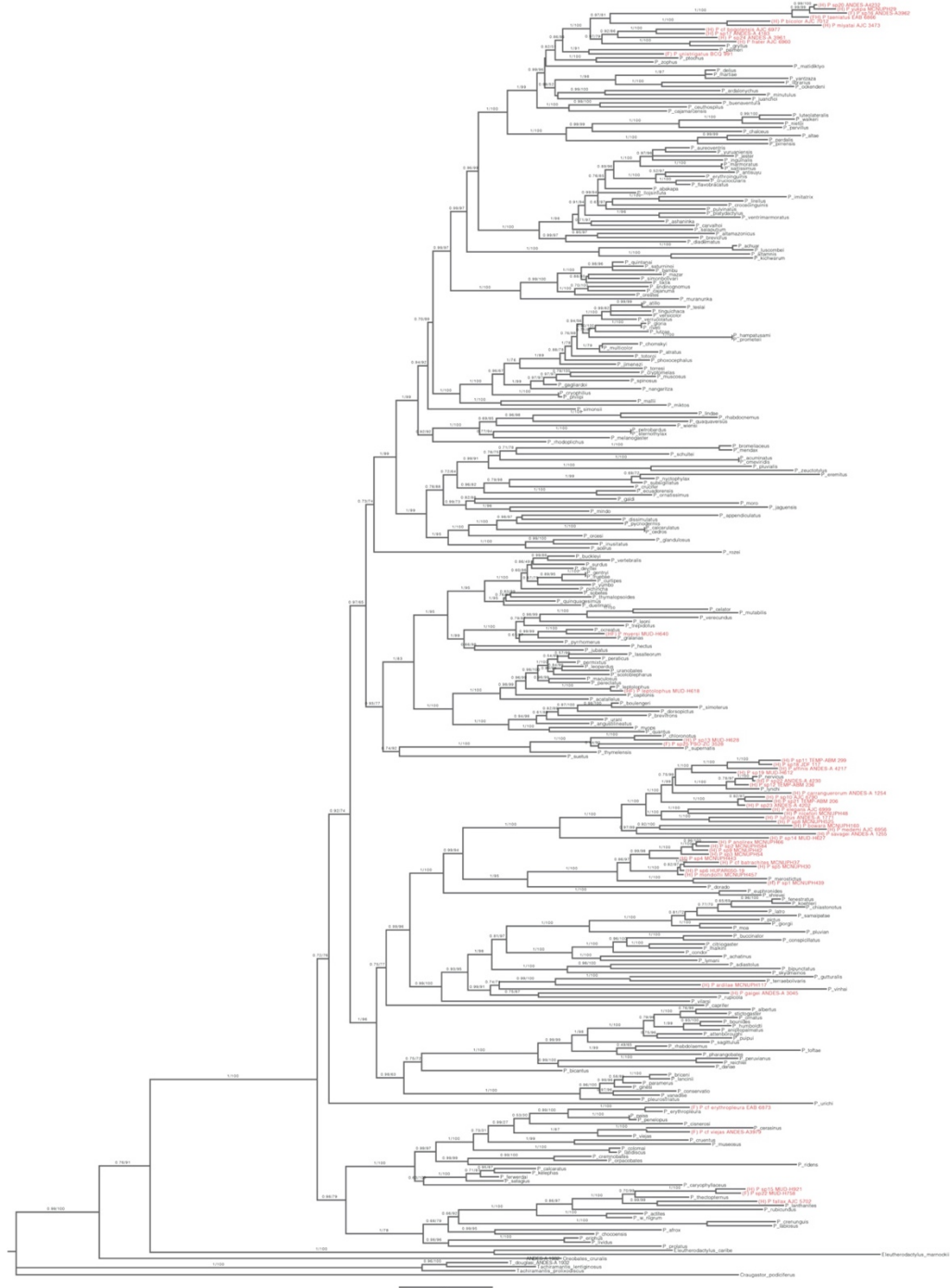


Fig S1. Phylogenetic relationships for 304 *Pristimantis* species obtained from a maximum likelihood analysis. Numbers to the left of slash are bootstrap values and to the right are posterior probability. In red, species sequenced in this work.

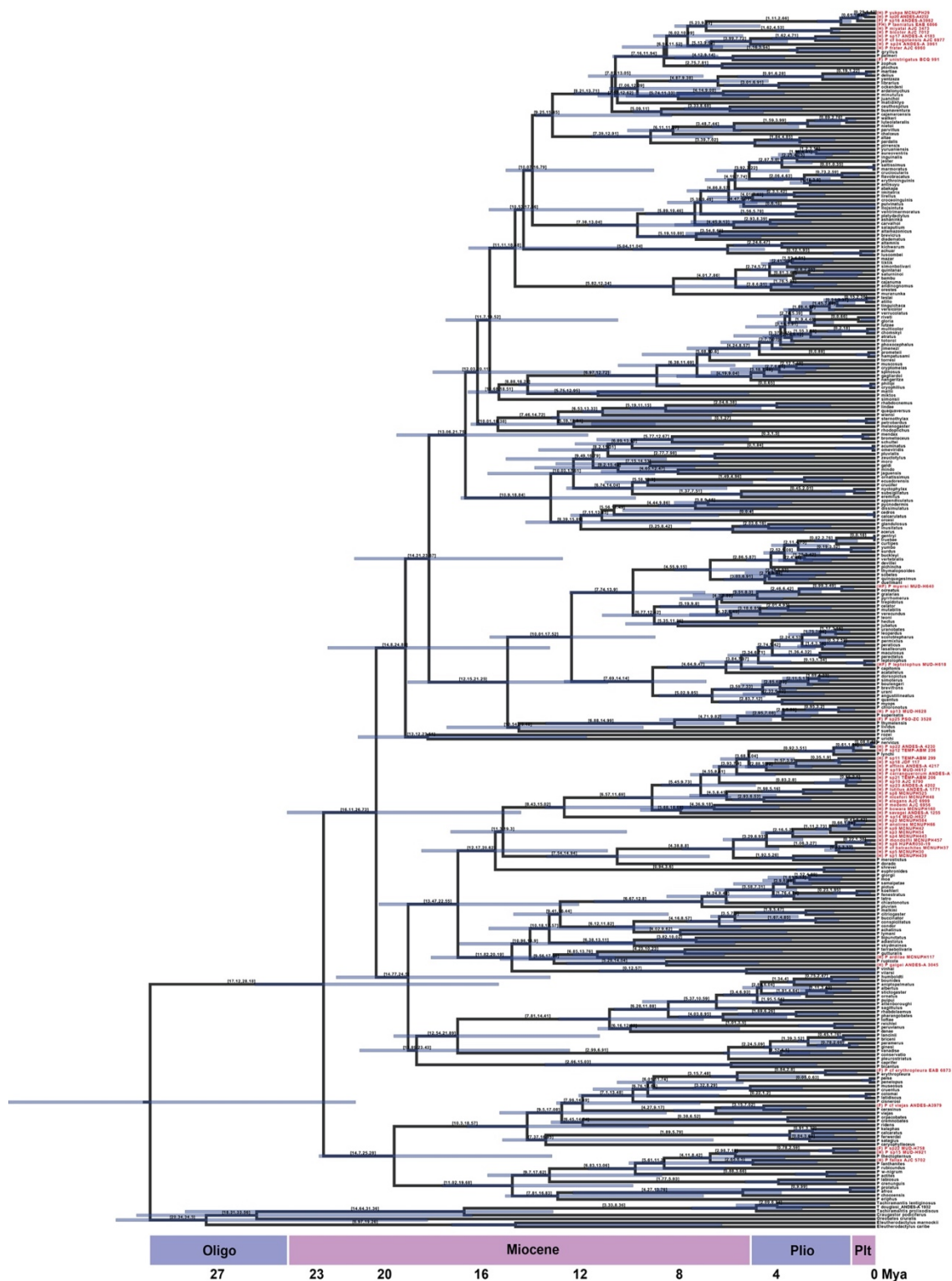


Fig S2. Phylogenetic timetree estimated in BEAST for 304 *Pristimantis* species, with the error bars representing the highest posterior density (HPD) interval at the 95% level.

Table S2. *Pristimantis* specimens examined for morphological comparisons.

Species	Voucher information
<i>Pristimantis anolirex</i>	ICN11375, ICN11380: COLOMBIA: Norte de Santander, Chitagá, Vereda Piedra, 2,770 m.a.s.l. ICN26212: COLOMBIA: Norte de Santander, Abrego, Cerro Oroque, 3,100 m.a.s.l. ICN10486, ICN10489, ICN10488: COLOMBIA: Norte de Santander, Herrán, Orocué, 2,300 m.a.s.l.
<i>Pristimantis batrachites</i>	ICN47890, ICN47891, ICN47888: COLOMBIA: Norte de Santander, Cucutilla, Vereda Carrizal. ICN26212: COLOMBIA: Norte de Santander, Abrego, Cerro Oroque, 3,100 m.a.s.l. ICN10486, ICN10489, ICN10488: COLOMBIA: Norte de Santander, Herrán, Orocué, 2,300 m.a.s.l.
<i>Pristimantis carrangerorum</i>	ICN5130, ICN5133, ICN5142, ICN5146: COLOMBIA: Boyaca, Pajarito, Corinto, 1,600 m.a.s.l.
<i>Pristimantis lutitus</i>	ICN5192, ICN51193: COLOMBIA: Santander, Charala, El Encino, 2,400 m.a.s.l.
<i>Pristimantis medemi</i>	ICN40589, ICN40591, 40593: COLOMBIA: Boyacá, Santa María, El Sendero.
<i>Pristimantis merostictus</i>	ICN01434, ICN11064: COLOMBIA: Boyacá, Duitama, Hacienda La Sierra, 2,400 m.a.s.l.
<i>Pristimantis nicefori</i>	ICN22295: COLOMBIA: Boyacá, Güicán, vereda El Tabor, 3,850 m.a.s.l. ICN22311, ICN20889: COLOMBIA: Boyacá, Güicán, vereda La Cueva, 3,900 m.a.s.l.
<i>Pristimantis tubernasus</i>	ICN10483: COLOMBIA: Norte de Santander, Herran, PNN Tamá, 2,300 m.a.s.l.
<i>Pristimantis merostictus</i>	ICN34254, ICN34234, ICN34236: COLOMBIA: Santander, Charalá, Duitama, 2,400 m.a.s.l.
<i>Tachiramantis lentiginosus</i>	ICN10503, ICN10499: COLOMBIA: Norte de Santander, Herrán, PNN Tamá, 2,300 m.a.s.l.
<i>Tachiramantis douglasi</i>	ICN15465, ICN15468, ICN15466: COLOMBIA: Santander, Bucaramanga/Pamplona, 2,580 m.a.s.l.
<i>Pristimantis frater</i>	ICN07173, ICN07172: COLOMBIA: Boyacá, Pajarito, 2,015 m.a.s.l.
<i>Pristimantis bacchus</i>	ICN33153, ICN33137, ICN33138: COLOMBIA: Santander, Charalá, 1,700-2400 m.a.s.l.
<i>Pristimantis elegans</i>	ICN41465: COLOMBIA: Cundinamarca, Páramo de Chingaza. ICN05745: COLOMBIA: Cundinamarca, Páramo de Sumapaz.
<i>Pristimantis taeniatus</i>	ICN43158, ICN43159, ICN43154: COLOMBIA: Santander, Suaita, San José de Suaita.
<i>Pristimantis w-nigrum</i>	ICN12398, ICN12388: COLOMBIA: Santander, Charalá, Virolín, 1750-1780 m.a.s.l.
<i>Pristimantis lynch</i>	ICN05907, ICN05908, ICN05909: COLOMBIA: Santander, Sauta, 3120-3250 m.a.s.l.
<i>Pristimantis miyatai</i>	ICN15267, ICN15270, ICN39110: COLOMBIA: Santander.
<i>Pristimantis bicolor</i>	ICN07419: COLOMBIA: Santander, Charalá.
<i>Pristimantis sp19</i>	MUD-H614, MUD-H637: Colombia, Huila, Garzón, PNR Cerro-Páramo de Miraflores, 2,743-3,400 m.a.s.l.
<i>Pristimantis bogotensis</i>	MUD-H603, MUD-H604: Cundinamarca, Matarrendondo, 3,332 m.a.s.l.
<i>Pristimantis sp13</i>	MUD-H631, MUD-H630, MUD-H636: Colombia, Huila, Garzón, PNR Cerro-Páramo de Miraflores, 3,050, 2935, 3400 m.a.s.l.
<i>Pristimantis leptolophus</i>	MUD-H873, MUD-H874, MUD-H858: Colombia, Huila, Teruel, La Armenia, 3026, 3027, 3035 m.a.s.l.

Table S3. Pairwise genetic distances (p-distance) among *Pristimantis* species based on 16s + 12s. (Table attached as excel file). Dryad Digital Repository: <https://doi.org/10.5061/dryad.r7sqv9sd5>

Table S4. Ecoregions assigned for each species of *Pristimantis* included in the analysis of ancestral ranges and phylogenetic diversity. A: Amazon. B: Central America. C: Central Andes. D: Chocó Biogeographical. E: Guiana Shield. F: Northern Andes. G: Venezuelan Andes. H: Colombian Eastern Andes. I: Venezuelan Coastal Range. J: Atlantic Forrest.

Species	Ecoregions
<i>P. abakapa</i>	E
<i>P. acatallelus</i>	F
<i>P. acerus</i>	F
<i>P. achatinus</i>	DF
<i>P. achuar</i>	A
<i>P. actites</i>	F
<i>P. acuminatus</i>	A
<i>P. adiaastolus</i>	C
<i>P. affinis</i> ANDES-A 4217	H
<i>P. afrox</i>	D
<i>P. albertus</i>	C
<i>P. altae</i>	B
<i>P. altamazonicus</i>	A
<i>P. altamnis</i>	A
<i>P. andinognomus</i>	C
<i>P. angustilineatus</i>	F
<i>P. aniptopalmatus</i>	C
<i>P. anolirex</i> MCNUPH66	H
<i>P. antisuyu</i>	C
<i>P. appendiculatus</i>	F
<i>P. ardalonychus</i>	C
<i>P. ardilae</i> MCNUPH117	H
<i>P. ashaninka</i>	C
<i>P. atillo</i>	F
<i>P. atratus</i>	C
<i>P. attenboroughi</i>	C
<i>P. aureoventris</i>	E
<i>P. bambu</i>	F
<i>P. bicantus</i>	F
<i>P. bicolor</i> AJC 7012	H
<i>P. bipunctatus</i>	C

<i>P. boulengeri</i>	F
<i>P. bounides</i>	C
<i>P. bowara</i> MCNUPH160	H
<i>P. brevicrus</i>	A
<i>P. brevifrons</i>	F
<i>P. briceni</i>	G
<i>P. bromeliaceus</i>	CF
<i>P. buccinator</i>	A
<i>P. buckleyi</i>	F
<i>P. buenaventura</i>	F
<i>P. cajamarcensis</i>	CF
<i>P. cajanuma</i>	C
<i>P. calcaratus</i>	F
<i>P. calcarulatus</i>	F
<i>P. capitonis</i>	F
<i>P. caprifer</i>	D
<i>P. carrangerorum</i> ANDES-A 1254	H
<i>P. carvalhoi</i>	A
<i>P. caryophyllaceus</i>	B
<i>P. cedros</i>	F
<i>P. celator</i>	F
<i>P. cerasinus</i>	B
<i>P. ceuthospilus</i>	C
<i>P. cf batrachites</i> MCNUPH37	H
<i>P. cf bogotensis</i> AJC 6977	H
<i>P. cf erythropleura</i> EAB 6873	F
<i>P. cf viejas</i> EAB 6850	F
<i>P. chalceus</i>	DF
<i>P. chiastonotus</i>	AE
<i>P. chloronotus</i>	F
<i>P. chocoensis</i>	D
<i>P. chomskyi</i>	C
<i>P. cisnerosi</i>	D
<i>P. citriogaster</i>	A
<i>P. colomai</i>	DF
<i>P. condor</i>	CF
<i>P. conservatio</i>	G
<i>P. conspicillatus</i>	A
<i>P. cremnobates</i>	F

<i>P. crenunguis</i>	F
<i>P. croceoinguinis</i>	A
<i>P. crucifer</i>	F
<i>P. cruciocularis</i>	C
<i>P. cruentus</i>	BD
<i>P. cryophilus</i>	F
<i>P. cryptomelas</i>	C
<i>P. curtipes</i>	F
<i>P. danae</i>	C
<i>P. delius</i>	A
<i>P. devillei</i>	F
<i>P. diadematus</i>	A
<i>P. dissimulatus</i>	F
<i>P. dorado</i>	H
<i>P. dorsopictus</i>	F
<i>P. duellmani</i>	F
<i>P. ecuadorensis</i>	F
<i>P. elegans</i> AJC 6999	H
<i>P. eremitus</i>	F
<i>P. eriphus</i>	F
<i>P. erythroinguinis</i>	C
<i>P. erythropleura</i>	DF
<i>P. euphronides</i>	I
<i>P. fallax</i> AJC 5702	H
<i>P. fenestratus</i>	A
<i>P. ferwerdai</i>	F
<i>P. flavobracatus</i>	C
<i>P. frater</i> AJC 6960	H
<i>P. gagliardoi</i>	F
<i>P. gaigei</i> ANDES-A 3045	H
<i>P. galdi</i>	CF
<i>P. gentryi</i>	F
<i>P. ginesi</i>	G
<i>P. giorgii</i>	A
<i>P. glandulosus</i>	F
<i>P. gloria</i>	F
<i>P. gralarias</i>	F
<i>P. gryllus</i>	HG
<i>P. gutturalis</i>	A

<i>P. hampatusami</i>	F
<i>P. hectus</i>	F
<i>P. humboldti</i>	C
<i>P. imitatrix</i>	A
<i>P. inguinalis</i>	AE
<i>P. inusitatus</i>	F
<i>P. jaguensis</i>	F
<i>P. jester</i>	E
<i>P. jimenezi</i>	F
<i>P. juanchoi</i>	F
<i>P. jubatus</i>	F
<i>P. kelephas</i>	F
<i>P. kichwarum</i>	A
<i>P. koehleri</i>	AC
<i>P. labiosus</i>	DF
<i>P. lancinii</i>	G
<i>P. lanthanites</i>	AF
<i>P. lasalleorum</i>	F
<i>P. latidiscus</i>	DF
<i>P. latro</i>	A
<i>P. leoni</i>	F
<i>P. leopardus</i>	F
<i>P. librarius</i>	A
<i>P. lindae</i>	C
<i>P. lirellus</i>	C
<i>P. lividus</i>	F
<i>P. llojsintuta</i>	C
<i>P. luscombei</i>	A
<i>P. luteolateralis</i>	F
<i>P. lutitus</i> ANDES-A 1771	H
<i>P. lutzae</i>	F
<i>P. lymani</i>	C
<i>P. lynchi</i>	H
<i>P. maculosus</i>	F
<i>P. malkini</i>	A
<i>P. mallii</i>	F
<i>P. marmoratus</i>	AE
<i>P. martiae</i>	AF
<i>P. matidiktyo</i>	A

<i>P. mazar</i>	F
<i>P. medemi</i> AJC 6956	H
<i>P. melanogaster</i>	C
<i>P. mendax</i>	C
<i>P. merostictus</i>	H
<i>P. miktos</i>	A
<i>P. mindo</i>	F
<i>P. minutulus</i>	C
<i>P. miyatai</i> AJC 3473	H
<i>P. moa</i>	A
<i>P. mondolfii</i> MCNUPH457	H
<i>P. moro</i>	BF
<i>P. multicolor</i>	C
<i>P. muranunka</i>	C
<i>P. muscosus</i>	C
<i>P. museosus</i>	BD
<i>P. mutabilis</i>	F
<i>P. sp25</i> PSO-ZC 3528	F
<i>P. myops</i>	F
<i>P. nangaritza</i>	C
<i>P. nervicus</i>	H
<i>P. nicefori</i> MCNUPH48	H
<i>P. nietoi</i>	D
<i>P. nyctophylax</i>	F
<i>P. ockendeni</i>	A
<i>P. ocreatus</i>	F
<i>P. omeviridis</i>	A
<i>P. orcesi</i>	F
<i>P. orestes</i>	F
<i>P. ornatissimus</i>	F
<i>P. ornatus</i>	C
<i>P. orpacobates</i>	F
<i>P. paisa</i>	F
<i>P. palmeri</i>	F
<i>P. paramerus</i>	G
<i>P. pardalis</i>	B
<i>P. parectatus</i>	F
<i>P. parvillus</i>	DF
<i>P. penelopus</i>	F

<i>P. peraticus</i>	F
<i>P. permixtus</i>	F
<i>P. peruvianus</i>	AF
<i>P. petrobardus</i>	C
<i>P. pharangobates</i>	C
<i>P. philipi</i>	F
<i>P. phoxocephalus</i>	F
<i>P. pichincha</i>	F
<i>P. pictus</i>	A
<i>P. pirrensis</i>	D
<i>P. platydactylus</i>	C
<i>P. pleurostriatus</i>	G
<i>P. pluvialis</i>	C
<i>P. pluvian</i>	A
<i>P. prolatus</i>	F
<i>P. ptochus</i>	F
<i>P. puipui</i>	C
<i>P. pulvinatus</i>	E
<i>P. pycnodermis</i>	F
<i>P. pyrrhomerus</i>	F
<i>P. quantus</i>	F
<i>P. quaquaversus</i>	AC
<i>P. quinquagesimus</i>	F
<i>P. quintanai</i>	F
<i>P. reichlei</i>	AC
<i>P. rhabdocnemus</i>	C
<i>P. rhabdolaemus</i>	C
<i>P. rhodoplichus</i>	C
<i>P. ridens</i>	BD
<i>P. riveti</i>	F
<i>P. rozei</i>	I
<i>P. rubicundus</i>	F
<i>P. rupicola</i>	J
<i>P. sagittulus</i>	C
<i>P. salaputium</i>	C
<i>P. saltissimus</i>	E
<i>P. samaipatae</i>	AC
<i>P. satagius</i>	F
<i>P. saturninoi</i>	F

<i>P. savagei</i> ANDES-A 1255	H
<i>P. schultei</i>	C
<i>P. scoloblepharus</i>	F
<i>P. shrevei</i>	I
<i>P. simonbolivari</i>	F
<i>P. simonsii</i>	C
<i>P. simoterus</i>	F
<i>P. skydmainos</i>	A
<i>P. sobetes</i>	F
<i>P. sp1</i> MCNUPH439	H
<i>P. sp10</i> AJC 6790	H
<i>P. sp11</i> TEMP-ABM 299	H
<i>P. sp12</i> TEMP-ABM 236	H
<i>P. sp13</i> MUD-H628	H
<i>P. sp14</i> MUD-H627	H
<i>P. leptolophus</i> MUD-H618	HF
<i>P. myersi</i> MUD-H640	HF
<i>P. sp15</i> MUD-H921	H
<i>P. sp16</i> ANDES-A3962	F
<i>P. sp17</i> ANDES-A 4183	H
<i>P. sp2</i> MCNUPH584	H
<i>P. sp18</i> JDF 117	H
<i>P. sp19</i> MUD-H612	H
<i>P. sp20</i> ANDES-A 4230	H
<i>P. sp21</i> TEMP-ABM 206	H
<i>P. sp22</i> MUD-H758	F
<i>P. sp23</i> ANDES-A 4202	H
<i>P. sp24</i> ANDES-A 3961	H
<i>P. sp3</i> MCNUPH54	H
<i>P. sp4</i> MCNUPH443	H
<i>P. sp5</i> MCNUPH30	H
<i>P. sp6</i> HUPAR050-19	H
<i>P. sp7</i> ANDES-A4232	H
<i>P. sp8</i> MCNUPH525	H
<i>P. sp9</i> MCNUPH42	H
<i>P. spinosus</i>	F
<i>P. sternothylax</i>	C
<i>P. stictogaster</i>	C
<i>P. subsigillatus</i>	F

<i>P. suetus</i>	F
<i>P. supernatis</i>	F
<i>P. surdus</i>	F
<i>P. taeniatus</i> EAB 6866	FH
<i>P. terraebolivaris</i>	I
<i>P. teslai</i>	F
<i>P. thectopternus</i>	F
<i>P. thymalopsoides</i>	F
<i>P. thymelensis</i>	F
<i>P. tiktik</i>	C
<i>P. tinguichaca</i>	F
<i>P. toftae</i>	AC
<i>P. torresi</i>	C
<i>P. totoroi</i>	F
<i>P. trepidotus</i>	F
<i>P. truebae</i>	F
<i>P. unistrigatus</i> BCQ 991	F
<i>P. urani</i>	F
<i>P. uranobates</i>	F
<i>P. urichi</i>	I
<i>P. vanadise</i>	G
<i>P. ventrimarmoratus</i>	AC
<i>P. verecundus</i>	F
<i>P. verrucolatus</i>	F
<i>P. versicolor</i>	CF
<i>P. vertebralis</i>	F
<i>P. viejas</i>	HF
<i>P. vilarsi</i>	AE
<i>P. vinhai</i>	J
<i>P. w-nigrum</i>	HF
<i>P. walkeri</i>	DF
<i>P. wiensi</i>	C
<i>P. yantzaza</i>	C
<i>P. yukpa</i> MCNUPH29	H
<i>P. yumbo</i>	F
<i>P. yuruaniensis</i>	E
<i>P. zeuctotylus</i>	AE
<i>P. zophus</i>	F
<i>P. prometeii</i>	F

Table S5. Time-stratified dispersion multiplier matrices for 10 ecoregions used for the estimation of ancestral ranges in BioGeobears: E: Amazonia. B: Central America. C: Central Andes. D: Chocó. E: Guiana Shield. F: Northwestern Andes. G: Venezuelan Andes. H: Colombian Eastern Andes. I: Venezuelan Coastal Range. J: Atlantic Forrest.

From Late Miocene to Pliocene-Pleistocene (7 - 0 Mya)	A	B	C	D	E	F	G	H	I	J
A	1	0,1	1	0,5	1	1	0,5	1	1	1
B	0,1	1	0,1	1	0,1	1	0,1	0,1	0,1	0,1
C	1	0,1	1	1	0,5	1	0,1	0,5	0,1	0,5
D	0,5	1	1	1	0,1	1	0,1	0,5	0,1	0,1
E	1	0,1	0,5	0,1	1	0,5	0,5	0,5	0,5	0,5
F	1	1	1	1	0,5	1	0,5	1	0,1	0,1
G	0,5	0,1	0,1	0,1	0,5	0,5	1	1	1	0,1
H	1	0,1	0,5	0,5	0,5	1	1	1	0,5	0,1
I	1	0,1	0,1	0,1	0,5	0,1	1	0,5	1	0,1
J	1	0,1	0,5	0,1	0,5	0,5	0,1	0,1	0,1	1
Late Miocene (10 - 7 Mya)	A	B	C	D	E	F	G	H	I	J
A	1	0,1	1	0,5	1	1	1	1	1	1
B	0,1	1	0,1	1	0,1	1	0,1	0,1	0,1	0,1
C	1	0,1	1	1	0,5	1	0,5	0,5	0,1	0,5
D	0,5	1	1	1	0,1	1	0,1	0,5	0,1	0,1
E	1	0,1	0,5	0,1	1	0,5	0,5	0,5	0,5	0,5
F	1	1	1	1	0,5	1	0,5	1	0,1	0,1
G	1	0,1	0,5	0,1	0,5	0,5	1	1	1	0,1
H	1	0,1	0,5	0,5	0,5	1	1	1	0,5	0,1
I	1	0,1	0,1	0,1	0,5	0,1	1	0,5	1	0,1
J	1	0,1	0,5	0,1	0,5	0,5	0,1	0,1	0,1	1
From early to late Miocene (22.5 - 10 Mya)	A	B	C	D	E	F	G	H	I	J
A	1	0,5	1	0,5	1	1	1	1	1	1
B	0,5	1	0,1	1	0,1	1	0,1	0,5	0,1	0,1
C	1	0,1	1	1	0,5	0,5	0,5	0,5	0,1	0,5
D	0,5	1	1	1	0,1	1	0,1	0,5	0,1	0,1
E	1	0,1	0,5	0,1	1	0,5	0,5	0,5	0,5	0,5
F	1	1	0,5	1	0,5	1	0,5	0,5	0,1	0,1
G	1	0,1	0,5	0,1	0,5	0,5	1	1	1	0,1
H	1	0,5	0,5	0,5	0,5	0,5	1	1	0,5	0,1
I	1	0,1	0,1	0,1	0,5	0,1	1	0,5	1	0,1
J	1	0,1	0,5	0,1	0,5	0,5	0,1	0,1	0,1	1

**PAPER 1: TWO NEW SPECIES OF *Pristimantis* (ANURA: CRAUGASTORIDAE)
WITH NOTES ON THE DISTRIBUTION OF THE GENUS IN NORTHEASTERN
COLOMBIA**

Aldemar A. Acevedo^{1,2,3}, Orlando Armesto^{1,2} & R. Eduardo Palma¹

¹Laboratorio de Biología Evolutiva, Departamento de Ecología, Facultad de Ciencias

Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Alameda 340, Santiago
6513677, Chile

²Grupo de Investigación en Ecología y Biogeografía, Universidad de Pamplona, Pamplona,
Colombia

³Corresponding author. E-mail: aeacevedo@uc.cl

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Abstract

We describe two new species of the genus *Pristimantis* from the western margin of the Eastern Cordillera of Colombia. The species have a sympatric distribution and are found in the humid tropical forests of the lower part of the Tamá National Natural Park (PNN Tamá) and its buffer zone. The new species were described from morphological comparisons and phylogenetic reconstruction from the sequencing of three mitochondrial and two nuclear genes. With these two new species, the number of *Pristimantis* species known in the department of Norte de Santander has increased to 10. In addition, we included new sequences for the species *P.*

anolirex, *P. nicefori*, *P. mondolfii*, and *P. yukpa*. Our explorations in the last decade indicate that there are probably between five and 10 species that have not been described in the northeastern part of Colombia, especially in areas where the armed conflict has predominated in the last five decades.

Keywords. Andes, Frogs, *Pristimantis conspicillatus* group, *Pristimantis ardilae* **sp. nov.**, *Pristimantis bowara* **sp. nov.**

Introduction

Colombia is classified as a megadiverse country for amphibians, with 838 species distributed in 14 families (Acosta-Galvis 2019), of which, Craugastoridae hosts the most significant richness with approximately 31% of the Colombian species. However, more than half of the craugastorid species are included within the genus *Pristimantis* Jiménez de la Espada, which covers approximately 500 species, of which 216 are present in Colombia (Heinicke et al. 2009; AmphibiaWeb 2019). The genus occupies a wide geographical area and is the most extensive of the genera of Neotropical amphibians, with species found in humid and forested environments of the Tropical Andes of Colombia, Ecuador, Peru, Venezuela, Bolivia and some areas of Central America (Lynch & Duellman 1997; Pinto-Sánchez et al. 2012; Meza-Joya & Torres 2016; Armesto & Señaris 2017). Additionally, members of this genus have an extensive altitudinal range, from sea level to altitudes above 4,500 m, with marked endemism in the high Andean areas and páramos (Heinicke et al. 2007; Meza-Joya & Torres 2016; Armesto & Señaris 2017).

Pristimantis is characterized by having a direct development and being associated with terrestrial areas, occupying a great variety of habitats that guarantee moisture zones for the survival of individuals from eggs to adults (Lynch & Duellman 1997; Padial et al. 2014). It has been proposed that the high species richness in the genus is related to its diversification rate, which is one of the highest among Neotropical vertebrates with an increase that has favored the colonization of different areas of South and Central America (Padial et al. 2014; Hutter et al. 2017).

The species described in this work were found in the department of Norte de Santander in the Eastern Cordillera of Colombia, one of the least known regions for *Pristimantis* and other groups of amphibians (Acevedo et al. 2014). To date, less than 10 species of *Pristimantis* frogs are known, which represents a very low richness compared to adjacent regions such as the department of Santander (northwest of Colombia), which records more than 20 species of *Pristimantis* (Frost 2019). However, it can be expected that this diversity has been underestimated. The different biomes in Norte de Santander such as dry forest, Andean forest, and high Andean and páramo, provide a wide range of ecological niches that would have favored the diversification of this group. However, public order problems as the armed conflicts in the last five decades have limited the development of adequate inventories, thus restricting the discovery of hidden amphibian diversity for the department. In this paper, we describe two new species of rainfrogs of the genus *Pristimantis* from lowland areas close to the Margua River in the Tamá National Natural Park and its buffer zone. In turn, we included new molecular sequences for some little-known species of *Pristimantis* in the northeastern region of Colombia and notes on the distribution of the genus *Pristimantis* for the department of Norte de Santander.

Materials and methods

Morphology

The species described in this work were collected in the northwestern part of the Eastern Cordillera of Colombia (Figure 1). The format for the description and diagnostic characters follows Lynch & Duellman (1997) and Duellman & Lehr (2009), and for the species associated with the *Pristimantis conspicillatus* group follows Padial and De la Riva (2009) and Padial et al. (2016). For each specimen, measurements and proportions were taken using digital calipers to the nearest 0.1 mm. They were verified using digital scaling with the software Fiji (Schindelin et al. 2012) on dorsal, frontal, and lateral views of the body and head, as well as the feet and hands. We measured the following characters according to Duellman & Lehr (2009): snout-vent length, SVL (straight length distance from tip of snout to vent); head length, HL (from angle of jaw to tip of snout); head width, HW (at level of angle of jaw); eye diameter, ED (distance between anterior and posterior borders of eyes); eye to nostril distance, END (straight line distance between anterior corner of orbit and posterior margin of external nares); inter-nostril distance, IND (distance between left and right nostrils); inter orbital distance, IOD (distance between left and right eyes); tympanum diameter, TD (distance between the anterior and posterior margins of the tympanic annulus); forearm length, FOL (the distance from the tip of the elbow to the proximal edge of the base palmar tubercle); length of the humerus, LHU (maximal length of the humerus measured from the proximal end of the humerus to the distal articular facet); foot length, FL (distance from proximal margin of inner metatarsal tubercle to tip of Toe IV); thigh length, THL (from vent opening to flexed knee); tibia length, TL (distance from the knee to the distal end of the tibia); disc diameter of finger three, DD3 (measured across

widest part of disk); and disc diameter of toe four, DF4. Length of Finger I, F1L (measured from outer edge of palmar tubercle to tip of the disc). The sex and maturity of specimens were verified with dissections of gonads, an examination of secondary sexual traits, such as the expansion of the vocal sac, and in the field by observing the calling activity. All specimens were deposited in the herpetological collection of the Museo de Ciencias Naturales of the Universidad de Pamplona, Colombia (MCNUP-H).

Molecular analyses

The collected *Pristimantis* frogs were euthanized with 2% lidocaine, fixed in 10% formalin, and stored in 70% ethanol. Samples of liver and muscle tissue obtained from the specimens collected were preserved in absolute ethanol and later stored at -20 °C. Genomic DNA was extracted using the QIAGEN DNeasyBlood and Tissue kits and stored at -20 °C. The samples of the new species were sequenced for three partial mitochondrial regions (12S, 16S, CYTB) and two nuclear genes (RAG1, TYR) (Table 1, Appendix 1). The general PCR cycles consisted of an initial 2 min at 93 °C and 35 cycles of 30 sec at 95 °C, 1 min at 42 °C, 1:30 min at 72 °C, and a final extension step of 6 min at 72 °C. The purification and sequencing reactions were performed by MacroGen Inc.

The new sequences were aligned using MUSCLE (Edgar 2004) with homologous sequences from other members of the genus *Pristimantis* available from GenBank (Appendix 2) and following the observations on the quality of the sequences suggested by González-Durán et al. (2017) and Hutter et al. (2017). The aligned sequences of multiple loci were concatenated by SequenceMatrix v.1.8 (Vaidya et al. 2011).

Best-fitting models and partitioning schemes were inferred using PartitionFinder 2 under the BIC criterion (Lanfear et al. 2016). To infer a Maximum Likelihood (ML) phylogeny, we used the program RaxML 8.1.11 (Stamatakis 2014) executed in the CIPRES portal (Miller et al. 2010), including 1,000 bootstrap replicates to evaluate the nodal support using the GTRGAMMA model for all partitions. We also inferred a Bayesian Inference phylogeny (BI) using MrBayes 3.2.7 (Ronquist et al. 2012) and four heated MCMC samples of every 1,000 generations for 50 million generations. For BI analysis, the best-fit models were GTR+I+G (12S, 16S), K81uf+G (CYTB), and K2P+I+G (RAG1, TYR). The phylogenies obtained (ML and BI) were visually compared to verify congruence between the topologies. Seven external groups were included according to previously published phylogenies (Heinicke et al. 2007; Hedges et al. 2008) (Appendix 2). Kimura two-parameter genetic distances (Kimura 1980) were based on the alignments for the 16S and 12S markers for a subset of the phylogeny that included 46 members of the genus *Pristimantis* that are phylogenetically related to the new species (Figure 2), in the program MEGA 5.0 (Tamura et al. 2011).

Results

Phylogenetic analyses

The entire dataset included 4,780 aligned sites. The phylogenetic tree inferred by ML (Figure 2) and BI (Figure 3) recovers, in general, well supported nodes within *Pristimantis* and agrees with previous studies (Padial et al. 2014). Regarding the clades of interest in this work, some species have changes in the position obtained with the inference by ML and BI (marked with a red asterisk in Figure 2 and 3). Despite this, the location of the new species remains constant with good bootstrap support values (Figure 2 and 3). The first species described here

(*Pristimantis ardilae*, **sp. nov.**) is most closely related to the *P. conspicillatus* species group given its morphological characters and phylogenetic position according to Padial et al. (2014). The sister species is *P. gutturalis* (Hoogmoed et al. 1977) (bootstrap, ML: 99; BI: 0.78, Figure 2) with a genetic distance of 3–8 %. In addition to the species of the *Pristimantis conspicillatus* group present in the Eastern Cordillera of Colombia, *P. carrangerorum* (Lynch 1994) has a genetic distance of 10%. However, this species was located on a separate clade along with *P. nervicus* (Lynch 1994), *P. lynchi* (Duellman & Simmons 1977) and *P. affinis* (Werner 1899) (Figure 2). On the other hand, the second species described here (*Pristimantis bowara* **sp. nov.**) was not assigned to a specific group, having as sister species *P. nicefori* (bootstrap, ML: 99; BI: 1, Figure 2) with a genetic distance of 20 %. Moreover to the sequences corresponding to the species described in this work, we included new sequences for geographically closed species located in the Eastern Cordillera of Colombia: *Pristimantis anolirex* (Lynch 1983), *P. nicefori* (Cochran & Goin 1970), *P. mondolfii* (Rivero 1984 “1982”), *P. yukpa* (Barrio-Amorós et al. 2008), and two species not described (*Pristimantis* sp1 and *Pristimantis* sp2) for the mitochondrial markers 12S, 16S, and CYTB and for the nuclear markers RAG1 and TYR. All sequences were deposited in GenBank (Table 1).

Systematics

Pristimantis ardilae, **sp. nov.**

(Figure 4A)

Holotype. An adult female (MCNUP-H 117, Figure 5A), collected on September 10, 2010, by Aldemar A. Acevedo, Karen Silva, and Rosmery Franco in the locality of San Antonio

(7.162172–72.25254; 612 m.a.s.l.), municipality of Toledo in the buffer zone of the Tamá National Natural Park (PNNT).

Paratypes. Two adult females (MCNUP-H 121 and 157), and two adult males (MCNUP-H 166 and 253) collected at the type locality on the same date of the holotype. One adult female (MCNUP-H 163), and two adult females (MCNUP-H 673 and 247), Collected on May 3, 2013, by Aldemar A. Acevedo in the low area of the PNNT, La Vega (7.13612–72.1843; 465 m.a.s.l.), municipality of Toledo in the buffer zone of the Tamá National Natural Park (PNNT).

Definition and diagnosis. *Pristimantis ardilae*, **sp. nov.** is assigned to the genus *Pristimantis* based on molecular phylogenetic relationships (Figure 2) and its morphological characteristics (Lynch & Duellman 1997; Duellman & Lehr 2009). We assign the new species to the *P. conspicillatus* species group based on position obtained in our phylogenetic analyses (Figure 2), and following Lynch & Duellman (1997) and Padial & De la Riva (2009) for having: (1) skin on dorsum shagreen; venter smooth to finely areolate whitish or yellowish coloration, and some black spots on the throat; short dorsolateral folds in scapular region. (2) tympanum distinct, round, $\sim 1/2$ – $1/3$ of the eye diameter; tympanic membrane and annulus present; supratympanic fold evident. (3) snout subacuminate in a dorsal view; rounded in profile (Figure 6A). (4) canthus rostralis defined, straight in dorsal view, slightly rounded in cross section; loreal region weakly concave. Nostrils slightly distinct, directed laterally. (5) upper eyelid without tubercles; cranial crests absent. (6) choanae small, round; dentigerous processes of vomers evident, with 5–6 teeth on the posterior margin; 12–16 premaxillary teeth. (7) vocal slits and nuptial pads present. (8) ED: 89% (females) and 96% (males) of the END. IOD: 81 %

(females) and 78 % (males) of the ED. (9) THL ~51% (females) and 46%(males) of the SVL. TL ~ 58% (females) and 56% (males) of the SVL. FL ~ 47 % (females) and 46 % (males) of the SVL. HW ~ 37% of the SVL in both females and males. (10) ulnar tubercles absent. (11) relative length of fingers: $II \leq I < IV < III$ (Figure 6A). Subarticular tubercles are round and upraised. The palmar tubercle is bifid, shaped similar to a heart; supernumerary tubercles is rounded and pronounced below the proximal subarticular tubercle of FIV; the thenar tubercle 80% of palmar tubercle. Finger fringes absent. FIII ~30% longer than FI. Disks on FIII–IV moderately expanded (state 2 according to Grant et al. 2006); disks on FI–II weakly expanded (state 1 according to Grant et al. 2006). (12) inner metatarsal tubercle ovoid; outer metatarsal tubercle small; tarsus without fold; relative length of toes: $I < II < III < V < IV$; Toe disks elliptical; Toe webbing absent (Figure 6A). (13) *Pristimantis ardilae* **sp. nov.** is apparently sexually dimorphic in size, with a mean SVL of 27.5 mm ($n = 4$) for males and 38.3 mm ($n = 4$) for females (Table 2). Of the 33 species that are part of the *P. conspicillatus* species group, only *P. carrangerorum*, and *P. medemi* (Lynch 1994) are found in the Eastern Cordillera of Colombia, the remaining species are found in distant biogeographic areas (Amazon and Cerrado, the Guianas and in the Atlantic Forest). Therefore, the comparison was based on *Pristimantis* species present in surrounding areas of the Northeast of Colombia and the Andes of Venezuela. The character state of the compared species is between parentheses. *Pristimantis ardilae* **sp. nov.** is distinguished from *P. carrangerorum* for having the first finger slightly longer than second (Finger $I < II$); fingers and toes without lateral fringes or keels (fingers and toes bearing fleshy lateral keels); Throat weakly spotted (heavily marble with brown). Tarsal fold absent (presente); *P. ardilae* **sp. nov.** is smaller (male SVL: 25.1–29.1, female SVL: 36.3–39.7) than *P. carrangerorum* (male SVL: 32.7 and female SVL: 41.7–50.7). *Pristimantis*

medemi is superficially similar to *P. ardilae* **sp. nov.** but differ in the states of the following characters: short dorsolateral folds in scapular region (absent); fingers and toes without lateral keels (fingers and toes bearing lateral keels). *Pristimantis ardilae* **sp. nov.** has no marks on the groin and hidden surfaces of the hind limbs (present in *P. melanoproctus* (Rivero 1984 “1982”), *P. mondolfii*, *P. anolirex*). It is a larger species (with a maximum SVL of 39.73 mm females; 29.1 mm males) than species that are geographically and phylogenetically close, (Table 3). *Pristimantis ardilae* **sp. nov.** does not have cranial crests (present in *P. anolirex*). Its first finger is longer than the second (the first finger is shorter than or about the same size as the second in *P. anolirex*, *P. mondolfii*, *P. melanoproctus*). Inner and outer edges of tarsus lacking tubercles (present in *P. tubernasus* (Rivero 1984 “1982”), *P. anolirex*, *P. nicefori*).

Description of the Holotype. SVL: 39.51 mm; upper eyelid tubercles absent in preservative (slightly visible in life); HL 38% of the SVL; HW 37% of the SVL; snout subacuminate in a dorsal view and rounded in profile; END 34% of the HW; tongue longer than wide. ED slightly shorter (88%) than the END; canthus rostralis distinguishable, straight in dorsal view and slightly rounded in cross-section; IND 70% of the END; IOD 96% of the IND. Cranial crests absent; supratympanic fold evident, tympanic annulus and tympanic membrane present, TD 34% of the ED; distance tympanum-eye: 2.96 mm. TD 9.8% of the HL. Choanae small, round; dentigerous processes of vomers with 6 teeth. Premaxilla with 16 teeth. Ulnar tubercles absent. Fingers and toes without lateral fringes. FI > FII; disks on FIII and FIV moderately expanded (state 2 according to Grant et al. 2006); relative size of the juxtaposed fingers: II < I < IV < III. Subarticular tubercles round and upraised, the largest one on FI. Palmar tubercle bifid; supernumerary tubercles distinct, protruding; thenar tubercle 80% the size of palmar tubercle.

Tarsal and calcar tubercles absent. Relative size of toes: $I < II < III < V < IV$; toe disks elliptical; toe webbing absent. LHU 66 % of the FOL.

Color in life. The dorsum usually intergrades yellowish brown tones against a predominantly dark brown back-ground. Head lighter cream and copper color than the dorsal part, with a large, dark, nearly triangular, interorbital spot. The upper lip has pale stripes. The flanks are predominantly lighter, often crossed by oblique brown bands, more evident toward the inguinal area. Ventral surface light cream; throat speckled with small dark brown spots. Upper arms and forearms cream color with brown to reddish transverse bands. The inguinal area is lighter with slightly mottled with cream.

Color in preservative. Specimens fixed with 10% formalin and stored in alcohol at 75% presented a dorsum with a dark brown coloration, with the head darker than the body, the lips maintained a pattern of light and brown stripes, although less defined than in life. The ventral part maintained a clear cream coloration, with a weak pattern towards the neck identifiable by small dark brown mottles. Slightly creamy extremities, but with a predominance of a dark copper color, the arms and hind limbs displayed weak traces of dark brown stripes.

Variation. *Pristimantis ardila* **sp. nov.** exhibits sexual dimorphism in body size with adult females reaching 39.7 mm SVL (MCNUP-H 163), adult males up to 29.1 mm SVL (MCNUP-H 253). In addition, the texture of skin dorsally is roughened and is bedecked with scattered tubercles, unlike females, whose back is usually smooth. Dorsal color pattern also differs between sexes; females tend to have a more uniform cream background on the dorsal surface and light copper stripes arranged towards the sides of the body. In contrast, the males typically

have a copper background on the dorsal with the dorsolateral folds with a strong reddish marking that extended from posterior edge of the upper eyelid to the sacrum.

Distribution and natural history. *Pristimantis ardilae* **sp. nov.** is only known from two localities: the settlements of San Antonio and La Vega, 9–11 km from the road Toledo-Saravena, near the Margua river. These two localities correspond to tropical humid forest and riparian zones of the lowlands (400 and 700 m.a.s.l.) from the western flank of the Eastern Cordillera, in the Tamá National Natural Park and its buffer zone, department Norte de Santander, Colombia (Figure 1). The individuals of *P. ardilae* **sp. nov.** were found among the riparian zone perched at a height between 1 and 2.5 meters from the ground. The highest activity was recorded between 8 and 11 at night. The species was found sympatrically with other amphibians such as *P. bowara* **sp. nov.**, *Rhaebo glaberrimus* (Günther), *Caecilia subnigricans* (Dunn), *Rulyrana flavopunctata* (Lynch & Duellman) and *Allobates algorei* (Barrio-Amorós & Santos).

Etymology. The specific epithet refers to the professor and herpetologist María Cristina Ardila-Robayo (15 February 1947–24 November 2017), who was a pioneer of herpetological studies in Colombia and contributed significantly to the knowledge of the diversity of Colombian amphibians, describing 15 species of *Pristimantis* frogs.

***Pristimantis bowara* sp. nov.**

(Figure 4B)

Holotype. An adult female (MCNUP-H 165, Figure 5B), collected on September 10, 2010, by Aldemar A. Acevedo, Karen Silva, and Rosmery Franco in the locality of San Antonio (7.16866–72.2655; 664 m.a.s.l.), municipality of Toledo in the buffer zone of the Tamá National Natural Park (PNNT), Colombia.

Paratypes. Two adult males (MCNUP-H 160 and 230), collected at the type locality on the same date of the holotype. Two adult males (MCNUP-H 303 and 304), collected on May 3, 2013, by Aldemar A. Acevedo in the low area of the PNNT, San Lorenzo (7.16615–72.2336; 648 m.a.s.l.), municipality of Toledo in the buffer zone of the Tamá National Natural Park (PNNT), Colombia.

Definition and diagnosis. *Pristimantis bowara*, **sp. nov.** is assigned to the genus *Pristimantis* based on molecular phylogenetic relationships (Figure 2) and its morphological characteristics (Lynch & Duellman 1997; Duellman & Lehr 2009). The new species was not assigned to any of the *Pristimantis* species group previously proposed by Padial et al. (2014), given the contrasting morphological traits among the species that are phylogenetically close (Figure 2). *Pristimantis bowara* **sp. nov.** is characterized by: (1) skin on dorsum smooth, and nearly uniform brown coloration; venter areolate with dark marks that extend in the throat. (2) dorsolateral folds absent. (3) tympanum distinct, round, $\sim 1/2$ – $1/3$ of the eye diameter; tympanic membrane and annulus present; supratympanic fold evident. (4) Snout subacuminate in a dorsal

view; protruding in profile (Figure 6B). (5) the canthus rostralis is recognizable and straight in a dorsal view and curved in cross section. (6) the loreal region was concave. (7) upper eyelid with small tubers only visible in life; cranial crests absent. (8) choanae small, round; dentigerous processes of vomers with 7–8 teeth; 14–18 premaxillary teeth. (9) Vocal slits and nuptial pads present. (10) ED: 96% (females) and 88% (males) of the END. IOD: 76% (females) and 87% (males) of the ED. (11) THL ~45% (females) and 43% (males) of the SVL. TL ~54% (females) and 50% (males) of the SVL. FL ~45% (females) and 42% (males) of the SVL. The HW ~41% in females and 36% males of the SVL. (12) ulnar tubercles absent. (13) relative length of fingers: $I \leq II < IV < III$ (Figure 6B). Subarticular tubercles are oval and upraised; on finger I, the subarticular tubercle is large and projected in the middle part of the finger, almost touching the thenar tubercle; the palmar tubercle is bifid and large, ~35% of the palm, and is more prominent in females than males; supernumerary tubercles are oval and pronounced; the thenar tubercle 90% of palmar tubercle. (14) finger fringes absent. FIII ~32% longer than FI. Disks on FIII–FIV greatly expanded (state 3 according to Grant et al. 2006); disk on FII moderately expanded (state 2); disk on FI weakly expanded (state 1). (15) inner and outer metatarsal tubercles conical; tarsus without fold; relative length of toes: $I < II < III < V < IV$; toe disks elliptical; basal webbing between toes III and IV, no more than 2% of the foot length (FL). (16) *Pristimantis bowara* **sp. nov.** is apparently sexually dimorphic in size, with a mean SVL of 26.6 mm ($n = 4$) for males and 39.7 mm ($n = 1$) for females (Table 4).

Comparisons with other species. *Pristimantis bowara* **sp. nov.** can be further distinguished from other *Pristimantis* species (characters in parentheses) from the northeastern region of Colombia and the Venezuelan Andes as follows, some summarized in Table 3. The species has

no marks on the groin and hidden surfaces of the hind limbs (present on *P. melanoproctus*, *P. mondolfii*, and *P. anolirex*). It is a larger species (with a maximum SVL of 39.7 mm for females and 25.5 mm for males) than species geographically and phylogenetically close, such as *P. anolirex*, *P. mondolfii*, *P. tubernasus*, *P. melanoproctus*, *P. batrachites* (Lynch 2013), *P. nicefori*, and slightly larger than the sympatric species *P. ardilae* **sp. nov.** described in this work (Table 3). The dorsal view of the snout is subacuminate (rounded in *P. nicefori* and acuminate in *P. merostictus* (Lynch 1984). Cranial crests absent (present in *P. anolirex*). The first and second fingers are equal or almost equal in length (the first finger is longer than the second in *P. medemi* and *P. ardilae* **sp. nov.**). There is an absence of outer tarsal tubercles (present in *P. anolirex*, *P. nicefori*, *P. nervicus*, *P. lynchi*, *P. merostictus*, and *P. tubernasus*); and an absence of heel tubercle (present in *P. gryllus* (Barrio-Amorós et al. 2012), *P. merostictus*, *P. anolirex*).

Description of the Holotype. SVL: 39.7 mm; upper eyelid tubercles absent; HL 33 % of the SVL; HW 41 % of the SVL; snout subacuminate in a dorsal view; protruding in profile; END 31% of the HW; tongue longer than wide; ED slightly shorter (96%) than the END; canthus rostralis distinguishable, straight in dorsal view and slightly rounded in cross section; IND 71% of the END; IOD 104% of the IND. Cranial crests absent; supratympanic fold evident, tympanic annulus and tympanic membrane present, TD 38% of the ED; distance tympanum-eye: 1.48 mm. TD 14% of the HL. Choanae small, rounded; dentigerous processes of vomers with seven teeth on the posterior margin; premaxillary with 18 teeth. Ulnar tubercles absent; fingers and toes without lateral fringes. FIII ~32% longer than FI. Disks on FIII–FIV greatly expanded (state 3); disk on FII moderately expanded (state 2); disk on FI weakly expanded (state 1); relative size of the juxtaposed fingers: $I \leq II < IV < III$. Subarticular tubercles ovoid

and upraised, the largest one on FI. Palmar tubercle bifid; supernumerary tubercles distinct, protruding, thenar tubercle 92% the size of palmar tubercle. Tarsal tubercles on the inner and outer edges absent. The heel lacked tubercles. Relative size of toes: $I < II < III < V < IV$; toe disks oval; basal webbing between TIII and TIV, ~1.5% of the foot. LHU 75% of the FOL; THL 18% slightly smaller than the TL.

Color in life. The dorsum usually intergrades copper red tones against a predominantly dark brown background (the holotype with a fine cream sagittal line crossed the entire body). Head copper and dark brown color, with an interorbital bar thin and dark. The upper lip has bars brown. The flanks are predominantly lighter, often crossed by oblique black bands, more evident toward the inguinal area. Ventral surface light cream colored with patches and dark brown spots scattered throughout the area; throat densely speckled with small dark brown spots. Upper arms and forearms brown. Thighs with a marked pattern of alternate stripes of copper and dark brown. The inguinal without evident patterns.

Color in preservative. Specimens fixed with 10% formalin and stored in alcohol at 75% presented a dorsum with a dark brown coloration, the head maintained a pattern of dark brown coloration similar to that of the body, the lips maintained a pattern of brown stripes, although less defined than in life. The ventral part maintained a light cream color, with a pattern towards the neck identifiable by a higher density of small speckles of dark brown color. The side stripes were more conspicuous. The arms and hind limbs displayed weak traces in the form of dark brown stripes.

Variation. *Pristimantis bowara* **sp. nov.** exhibits sexual dimorphism in body size SVL males 67% (largest specimen 25.5 mm, MCNUP-H 230) of the females (largest specimen 39.7 mm, MCNUP-H 165). Dorsal color pattern also differs between sexes; females tend to have a more uniform copper pattern. In contrast, the males typically have gray patterns and a coffee color that tended to be darker in preservative.

Distribution and natural history. *Pristimantis bowara* **sp. nov.** is only known from two localities: the tropical humid forest and riparian zones of the San Antonio and San Lorenzo settlements, 9.7 and 8.9 km respectively from the main road between the cities of Toledo and Saravena near the Margua River, in the lowlands of the Eastern Cordillera on its eastern flank in the Tamá National Natural Park and its buffer zone, department Norte de Santander, Colombia between 500 and 665 m.a.s.l. (Figure 1). The individuals of *P. bowara* **sp. nov.** were found mostly in the litter surrounding the riparian vegetation, some were perched at a height between 0.50 and 2 meters from the ground. The highest activity was recorded between 7 and 10 at night. The species was found in sympatry with other amphibians such as *P. ardilae* **sp. nov.**, *R. glaberrimus*, *R. flavopunctata* and *A. algorei*.

Etymology. The specific epithet *bowara* means jungle or forest in the U'wa dialect. The U'wa are an indigenous community that inhabit the humid forests of the Eastern Cordillera of the Andes, in the foothills of the Tamá National Natural Park, very close to where the species described in this work was found.

Discussion

We described two new species from the lowlands of Norte de Santander in Colombia. The species occurred sympatrically in the same biomes corresponding to vegetation structures of sub Andean and dry forests. Phylogenetic inferences (ML and BI) allocated as the sister species of *P. ardilae* **sp. nov.** to *P. gutturalis* (99 / 0.78, Figure 2), a species of wide geographical distribution found in Northern Brazil, southern French Guiana, and eastern Suriname (Frost 2019). Furthermore, *P. incertus* (Lutz 1927) varied in position, located near *P. ardilae* **sp. nov.** in the ML topology. Although, for BI it is more related to Amazonian lowlands species in central and southern Peru (Figure 3).

The position of *P. ardilae* **sp. nov.** agrees with the diagnostic characters assignable to the *P. conspicillatus* group (e.g., smooth to slightly granular belly, a distinct tympanic membrane, Finger I equal or longer than II). In turn, its phylogenetic position supports this assignment as defined by Padial et al. 2014. However, *P. ardilae* **sp. nov.** is one of the most geographically remote species concerning the other representatives of the *P. conspicillatus* group that are distributed mainly in the rainforest, in the Amazon basin in Brazil, Colombia, Ecuador and Peru (Frost 2019).

Only two species (*P. carrangerorum* and *P. medemi*) are geographically close, distributed in the Eastern Cordillera of Colombia, but they have particularly different taxonomic characters as discussed in the description section.

On the other hand, *Pristimantis bowara* **sp. nov.** is recovered as the sister species of *P. nicefori* (bootstrap, ML: 100; BI: 1, Figure 2), this species is distributed in high areas (over 3,000

m.a.s.l.) of the northeastern Cordillera of Colombia. Our phylogenetic analyzes located *Pristimantis bowara* **sp. nov.** in a clade along with species not assigned to particular groups according to previous analyzes (Padial et al. 2014). For example, *P. carranguerorum* assigned to the *P. conspicillatus* group by Hedges et al. (2008) and Padial et al. (2014), in our phylogenies it was located within the clade corresponding to *Pristimantis bowara* **sp. nov.** However, morphological characteristics of *P. carranguerorum* agree with the diagnostic assignable to the *P. conspicillatus* group (Padial & De la Riva 2009; Padial et al. 2016). Therefore, we suggest handling with caution the use of the sequences available in GenBank for *P. carranguerorum* as these may represent a mistaken identification. A similar case is the sequences of *P. anolirex* (DQ195478 and DQ195450) whose genetic distances are more related to *P. lutitus* (Lynch 1984) (1.2%). Therefore, it can be a mistaken identification, also suggested by Rivera-Correa et al. (2016). In this work, we provide new sequences (Table 1) corresponding to *P. anolirex* from the type locality (Chitagá, Departamento de Norte de Santander, Colombia), and other species poorly represented in the databases which will allow better phylogenetic estimates in the future. Some other cases that should be kept in mind when making phylogenetic interpretations in *Pristimantis*, are for example the available sequence of *P. permixtus* (DQ195467) assigned as an undescribed species by González-Durán et al. (2017). As well as sequences for *P. simoterus* (DQ195471, DQ195495) and *P. boulengeri* (DQ195452, DQ195480) whose similarity to each other is almost identical, therefore, the real identity cannot be known with certainty.

***Pristimantis* of Norte de Santander.** The department of Norte de Santander has, to date present 10 species of the genus *Pristimantis* (including those described in this work) distributed

between 100 to 4,600 m.a.s.l., covering all the biomes of the department from the dry forests of Cúcuta to the páramos ecosystems (Figure 5). The explorations in the last 10 years in different biomes of the northeast of the Eastern Cordillera of Colombia, have allowed to know a different prospect about the diversity of frogs of the genus *Pristimantis*. With at least twice the known species with varying patterns of colonization, diversification, and interesting systematic relationships between the biogeographical areas of the Eastern Cordillera.

Norte de Santander has three important areas in the biogeographical patterns of *Pristimantis* species: 1) the different altitudinal ranges of the PNN Tamá located in the Tamá Massif, between the municipalities Toledo and Herrán; 2) the different biomes between the cities of Cúcuta and Pamplona that cover a gradient of 300 to 2,600 m.a.s.l. in the Pamplonita River Basin; and 3) the páramos complexes of Almorzadero and Santurbán.

Five species (*P. anolirex*, *P. nicefori*, *P. tubernasus*, *Pristimantis* sp1, *Pristimantis* sp2) are distributed from the transition zones of the High Andean forest (2,800 m.a.s.l.) to páramo (> 3,000 m.a.s.l.) (Figure 7). However, only *P. anolirex* has a distribution in different high areas of the department (e.g., PNN Tamá and Complejo de Almorzadero and Santurbán). *Pristimantis nicefori* has only been registered in our sampling for the type locality in the páramo biome from the Complejo del Almorzadero between Norte de Santander and Santander departments. *Pristimantis tubernasus* has its type locality in the state of Mérida in Venezuela (Rivero 1984), with records for Norte de Santander by Ruiz-Carranza et al. (1996) in the highlands of the Tama National Natural Park. However, multiple expeditions since 2010 in the locality reported for Colombia and around the Natural Park have not found this species. For the Sub Andean and

Andean zones (1,000–2,800 m.a.s.l.), *P. mondolfii*, and *P. melanoproctus* are distributed towards the PNN Tamá, whereas *P. gryllus* and *P. batrachites* are distributed towards the margins of the Santurban complex. In contrast, the species that are distributed in the lowland zones (0–1,000 m.a.s.l.) are *P. yukpa* in the dry forests of Cúcuta, and the new species *P. ardilae* **sp. nov.** and *P. bowara* **sp. nov.** in the PNN Tamá.

Conservation. Of the 10 species of *Pristimantis*, nine tested positive for presence of the pathogen *Batrachochytrium dendrobatidis* (*P. anolirex*, *P. nicefori*, *Pristimantis* sp1, *P. mondolfii*, *P. melanoproctus*, *P. gryllus*, *P. yukpa*, *P. ardilae* sp nov., and *P. bowara* **sp. nov.**) All infections were reported by Acevedo et al. (2016a) and Acevedo et al. (2016b). The areas of the páramos from Almorzadero and Santurbán, have suffered a progressive loss of plant cover of transitional forests towards the páramo with increasing agricultural areas, where species such as *P. nicefori*, *P. anolirex*, and one species not yet described (*Pristimantis* sp1) have been affected with decreases in the number of individuals registered in field sampling conducted between 2013 and 2016.

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Table 1. Species sequenced in this study for three mitochondrial and two nuclear partial genes. Localities and geographic coordinates are included.

Species	Locality	Geographical coordinates	Voucher	12S	16S	CYTB	RAG1	TYR
<i>Pristimantis ardilae</i> sp. nov.	San Antonio	7.162172, - 72.25254	MCNUP- H117	MN215410	MN215424	MN218376	MN225943	MN218361
	San Antonio	7.162172, - 72.25254	MCNUP- H121	MN215412	MN215427	MN218378	MN225945	MN218365
	San Antonio	7.162172, - 72.25254	MCNUP- H157	MN215411	MN215425	MN218377	MN225944	MN218362
	San Antonio	7.162172, - 72.25254	MCNUP- H166	MN215414	MN215429	MN218380	MN225947	MN218363
	San Antonio	7.162172, - 72.25254	MCNUP- H253	-	MN215426	-	-	-
	La Vega	7.13612, - 72.1843	MCNUP- H163	MN215413	MN215428	MN218379	MN225946	MN218364
<i>Pristimantis bowara</i> sp. nov.	San Antonio	7.16866, - 72.2655	MCNUP- H165	MN215406	MN215431	MN218372	MN225939	MN218357
	San Antonio	7.16866, - 72.2655	MCNUP- H160	MN215407	MN215432	MN218373	MN225940	MN218358
	San Antonio	7.16866, - 72.2655	MCNUP- H230	MN215408	MN215433	MN218374	MN225941	MN218360
	San Lorenzo	7.16615, - 72.2336	MCNUP- H303	-	MN215430	-	-	-
	San Lorenzo	7.16615, - 72.2336	MCNUP- H304	MN215409	MN215434	MN218375	MN225942	MN218359
<i>P. nicefori</i>	Presidente, Páramo de Almorzadero	7.39008, - 72.3945	MCNUP- H48	MN215418	MN215436	MN218383	MN225949	MN218368
<i>P. anolirex</i>	Chitagá, Páramo de Almorzadero	7.150928, - 72.6335	MCNUP- H67	MN215421	MN215441	MN218386	MN225954	MN218371
<i>P. mondolfii</i>	Asiria de Belen, PNN Tamá	7.3223888, -	MCNUP-	MN215420	MN215440	MN218385	MN225953	MN218370

		72.36497	H457					
<i>P. yukpa</i>	Bochalena	7.6845, -72.6194	MCNUP-H28	MN215416	MN215438	-	MN225950	-
<i>P. yukpa</i>	Bochalena	7.6845, -72.6194	MCNUP-H29	MN215417	MN215439	-	MN225951	-
<i>P. sp1</i>	Orocué, PNN Tamá	7.429583, -72.44533	MCNUP-H100	MN215415	MN215435	MN218381	MN225948	MN218366
<i>P. sp1</i>	Orocué, PNN Tamá	7.429583, -72.44533	MCNUP-H75	-	-	MN218382	-	MN218367
<i>P. sp2</i>	Páramo del Tamá, PNN Tamá	7.390086, -72.3945	MCNUP-H600	MN215419	MN215437	MN218384	MN225952	MN218369

Table 2. Morphological variation (mm) of adults specimens of *Pristimantis ardilae* **sp. nov.** (range, average $\pm$ SD).

Measurements	Female (5)	Males (5)
Snout-vent length (SVL)	36.3–39.7 (38.3 $\pm$ 1.6)	25.1–29.1 (27.5 $\pm$ 1.8)
Head length (HL)	12.1–15.7 (13.9 $\pm$ 1.7)	9.0–9.7 (9.1 $\pm$ 0.6)
Head width (HW)	13.0–15.3 (14.2 $\pm$ 1.0)	9.4–10.8 (10.2 $\pm$ 0.6)
Eye diameter (ED)	3.4–5.1 (4.2 $\pm$ 0.7)	3.2–3.6 (3.2 $\pm$ 0.4)
Eye to nostril distance (END)	4.3–5.1 (4.7 $\pm$ 0.4)	2.9–3.3 (3.1 $\pm$ 0.2)
Inter Nostril distance (IND)	2.9–3.6 (3.3 $\pm$ 0.3)	2.3–2.7 (2.4 $\pm$ 0.2)
Inter orbital diameter (IOD)	3.3–3.8 (3.4 $\pm$ 0.3)	2.3–2.7 (2.5 $\pm$ 0.2)
Tympanum diameter (TD)	1.3–1.7 (1.5 $\pm$ 0.1)	0.9–1.0 (0.9 $\pm$ 0.1)
Forearm length (FOL)	7.2–8.2 (7.8 $\pm$ 0.4)	5.0–6.2 (5.7 $\pm$ 0.6)
Length of the humerus (LHU)	5.1–5.7 (5.4 $\pm$ 0.2)	4.1–5.0 (4.6 $\pm$ 0.5)
Foot length (FL)	17.5–19.0 (18.0 $\pm$ 0.7)	11.8–13.3 (12.6 $\pm$ 0.9)
Thigh length (THL)	18.7–19.9 (19.5 $\pm$ 0.6)	11.4–13.4 (12.6 $\pm$ 1.0)
Tibia length (TL)	21.6–23.1 (22.5 $\pm$ 0.7)	13.4–16.8 (15.3 $\pm$ 1.7)
Disc diameter of the finger three (DD3)	1.0–1.2 (1.1 $\pm$ 0.1)	0.7–1.1 (0.8 $\pm$ 0.2)
Disc diameter toe four (DF4)	1.1–1.3 (1.2 $\pm$ 0.1)	0.7–0.9 (0.8 $\pm$ 0.1)

Table 3. Comparison of diagnostic characters of some species of the *Pristimantis conspicillatus* group and species from northeastern Colombia, including the new species: (1) Length of first and second fingers. (2) Belly skin texture. (3) Dorsal skin texture. (4) Dorsal tubercles. (5) Dorsolateral folds. (6) Finger fringes. (7) Toe fringes. (8) Basal toewebbing. (9) Throat color pattern. (10) Tarsal fold. (11) Colour pattern of posterior surfaces of thighs. (12) Nuptial pads. (13) SVL (males). (14) SVL (females). Character states: 1= present, 0= absent.

Species	1	2	3	4	5	6	7
<i>P. carranguerorum</i> * (N=3)	F I < II	smooth	shagreen	short folds	1	weak	weak
<i>P. medemi</i> * (N=3)	F I > II	slightly granular	shagreen	presence dorsal tubercles	0	weak	weak
<i>P. pedimontanus</i> * (N=7)	F I < II	granular	granular	presence dorsal tubercles	0	prominet	prominet
<i>P. anolirex</i> (N=8)	F I < II	slightly granular	granular	presence dorsal tubercles	1	prominet	prominet
<i>P. batrachites</i> * (N=3)	F I < II	slightly granular	smooth	presence dorsal tubercles	0	prominet	prominet
<i>P. melanoproctus</i> * (N=1)	F I < II	granular	shagreen	presence dorsal tubercles	1	weak	weak
<i>P. mondolfii</i> * (N=2)	F I < II	smooth, granular posterolaterally	granular	presence dorsal tubercles	0	weak	weak
<i>P. nicefori</i> (N=4)	F I < II	granular	granular	short folds	0	0	0
<i>P. tubernasus</i> * (N=1)	F I < II	coarsely shagreen	shagreen	presence dorsal tubercles	0	0	0
<i>P. lutitus</i> * (N=2)	F I < II	granular	shagreen	presence dorsal tubercles	0	-	-
<i>P. merostictus</i> * (N=3)	F I < II	granular	granular	presence dorsal tubercles	0	weak	weak
<i>P. dorado</i> ** (N=7)	F I < II	granular	shagreen	-	1	0	0
<i>P. ardilae</i> , sp. nov. (N=10)	F I > II	Smooth, slightly granular	shagreen	presence dorsal tubercles	1	0	0
<i>P. bowara</i> , sp. nov. (N=5)	F I < II	granular	smooth	short folds	0	0	0

TABLE 3. (Continued)

Species	8	9	10	11	12	13	14
<i>P. carrangerorum</i> * (N=3)	0	stained or variable	1	barely visible	1	32.7	41.7–50.7
<i>P. medemi</i> *	0	stained or variable	0	barely visible and Weakly spotted	1	29.4–32.9	39.8–43.1
<i>P. pedimontanus</i> * (N=7)	1	stained or variable	0	plain	1	26.2–33.6	38.8–44.8
<i>P. anolirex</i> (N=8)	0	stained	1	barely visible	1	24–31.8	35.5–40
<i>P. batrachites</i> * (N=3)	0	stained	1	barely visible and Weakly spotted	1	14–17	17.9–21.7
<i>P. melanoproctus</i> * (N=1)	1	stained	0	barely visible and Weakly spotted	-	12–14.3	20–28.2
<i>P. mondolfii</i> * (N=2)	1	stained	0	well-defined and spotted	-	24.5–37.2	31.2–38.5
<i>P. nicefori</i> (N=4)	1	weakly spotted	1	barely visible	1	22.5–26.5	29.2–31.2
<i>P. tubernasus</i> * (N=1)	0	stained	1	plain	0	26	22.5–24
<i>P. lutitus</i> * (N=2)	0	stained	1	barely visible	-	-	23.7–28
<i>P. merostictus</i> * (N=3)	0	stained or variable	1	Well-defined and spotted	0	16.5–22.5	31
<i>P. dorado</i> ** (N=7)	0	weakly spotted	0	plain or Weakly spotted	1	14.2–17.9	25.6
<i>P. ardilae</i> , sp. nov. (N=10)	0	weakly spotted	0	Well-defined	1	25.1–29.1	36.3–39.7
<i>P. bowara</i> , sp. nov. (N=5)	0	heavily spotted	0	Well-defined	1	28.4–25.5	39.7

*complementary information was taken from the original description.

**All information was taken from the original description.

Table 4. Morphological variation (mm) of adults specimens of *Pristimantis bowara* sp. nov. (range, average $\pm$ SD).

Measurements	Female (1)	Males (4)
Snout-vent length (SVL)	39.7	28.4–25.5 (26.6 $\pm$ 1.3)
Head length (HL)	13.2	9.4–8.1 (8.8 $\pm$ 0.5)
Head width (HW)	16.4	10.7–8.7 (9.7 $\pm$ 0.8)
Eye diameter (ED)	5.5	3.3–2.9 (3.1 $\pm$ 0.2)
Eye to nostril distance (END)	4.8	3.9–3.1 (3.5 $\pm$ 0.3)
Inter Nostril distance (IND)	3.7	2.2–1.9 (2.3 $\pm$ 0.3)
Inter orbital diameter (IOD)	3.8	3.0–2.5 (2.7 $\pm$ 0.3)
Tympanum diameter (TD)	1.9	1.7–1.1 (1.3 $\pm$ 0.3)
Forearm length (FOL)	9.5	6.4–5.8 (6.0 $\pm$ 0.3)
Length of the humerus (LHU)	7.2	4.3–4.0 (4.2 $\pm$ 0.1)
Foot length (FL)	18.1	11.6–10.8 (11.2 $\pm$ 0.4)
Thigh length (THL)	18.0	11.6–11.4 (11.6 $\pm$ 0.2)
Tibia length (TL)	21.7	13.9–12.8 (13.3 $\pm$ 0.6)
Disc diameter of the finger three (DD3)	1.2	0.9–0.8 (0.9 $\pm$ 0.1)
Disc diameter toe four (DF4)	1.1	0.9–0.7 (0.7 $\pm$ 0.1)

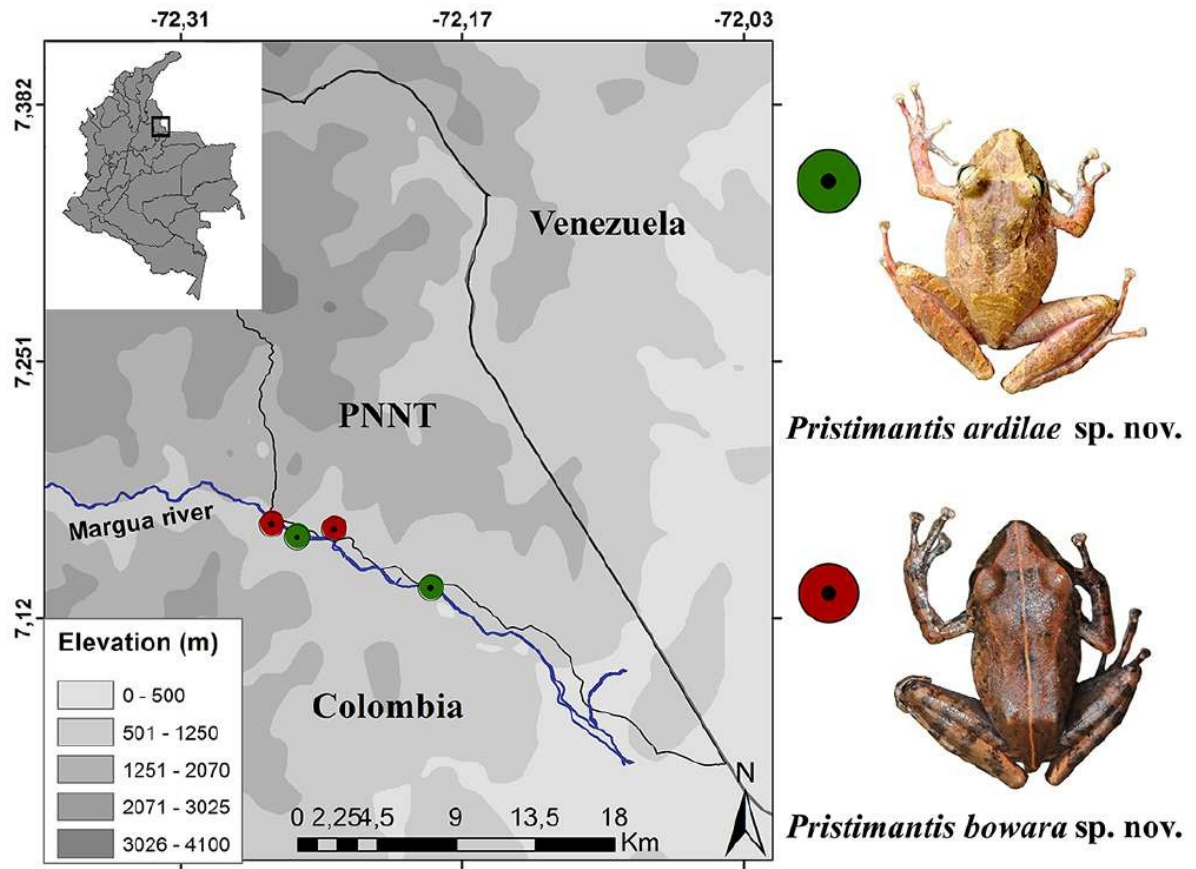


Fig 1. Map of the geographical records of the new species. Norte de Santander, Colombia.

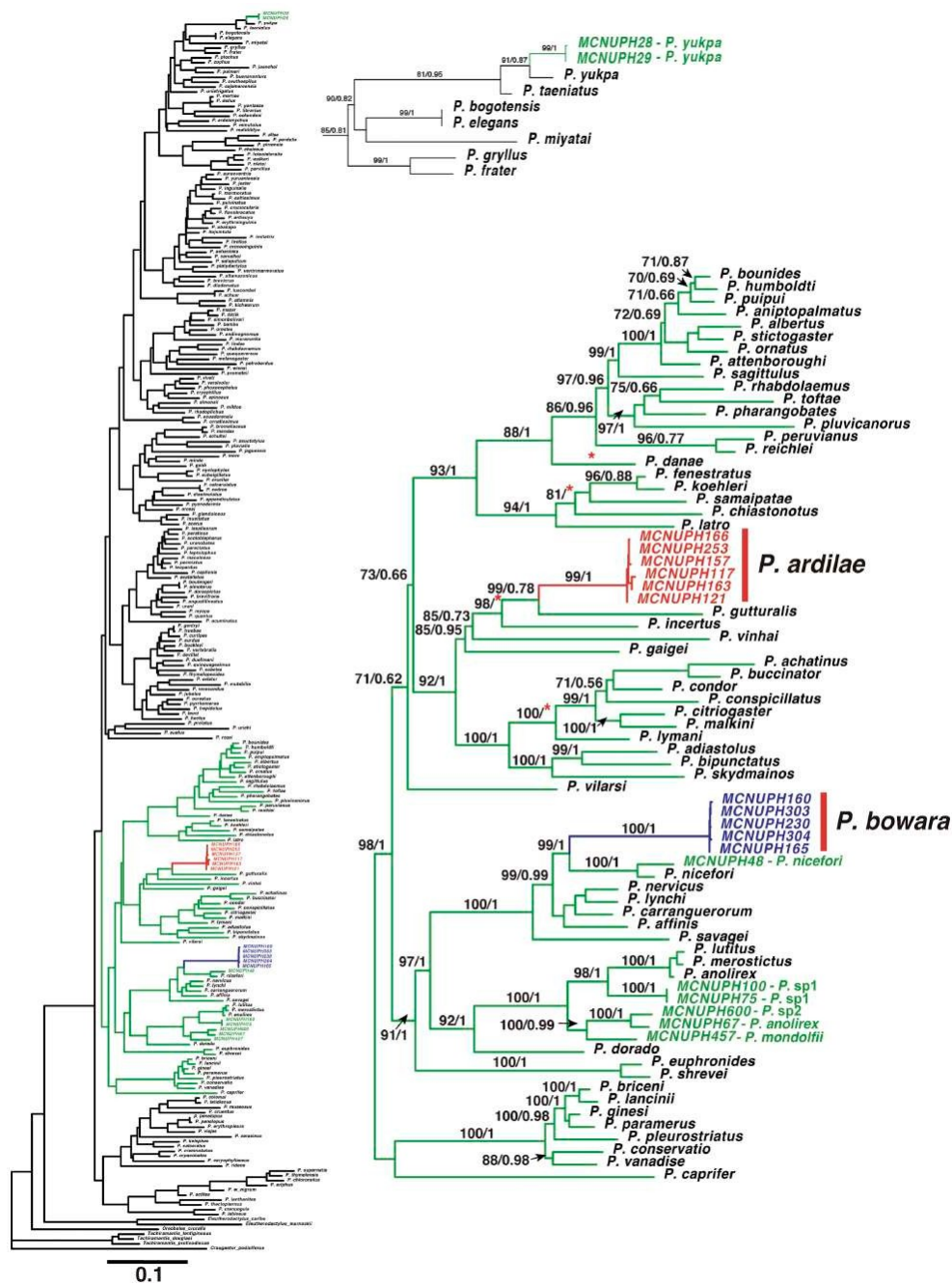


Fig 2. Phylogenetic relationships for some *Pristimantis* species in South America obtained from a maximum likelihood analysis. Numbers to the left of slash are bootstrap values and to the right are posterior probability. *Pristimantis ardilae* sp. nov. (red clade), *P. bowara* sp. nov. (blue clade). Additional species of *Pristimantis* from Norte de Santander appear as green labels.

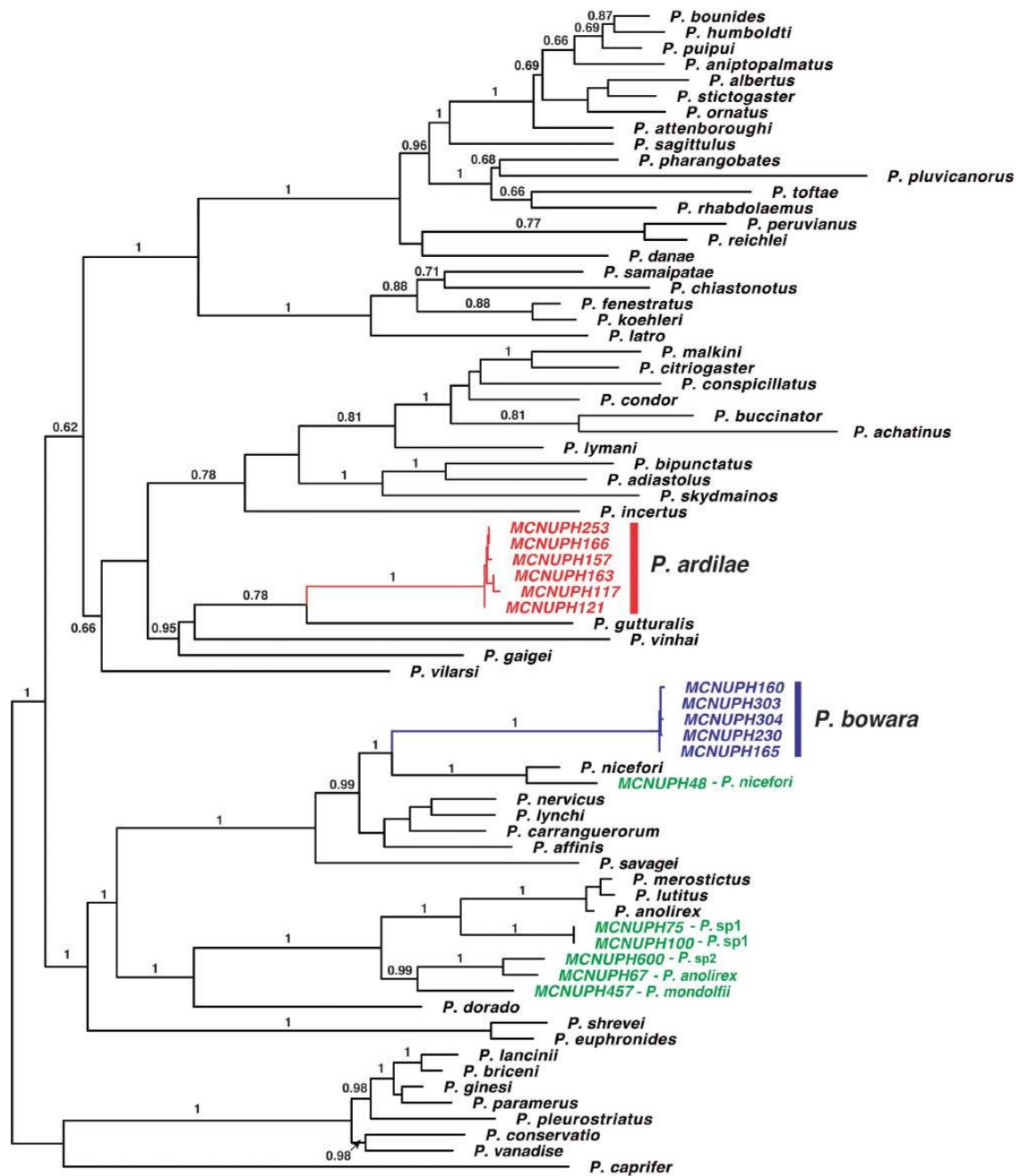


Fig 3. Phylogenetic relationships subset for some *Pristimantis* species obtained from a Bayesian Inference analysis. Numbers above branches are the highest posterior probability. *Pristimantis ardilae* sp. nov. (red clade), *P. bowara* sp. nov. (blue clade). Additional species of *Pristimantis* from Norte de Santander appear as green labels.



Fig 4. Photos of the specimens in life. A. *Pristimantis ardilae* **sp. nov.**, uncollected animals. B. *Pristimantis bowara* **sp. nov.**, holotype, MCNUP-H 165. Lower zone of the buffer zone of PNN Tamá, Colombia.

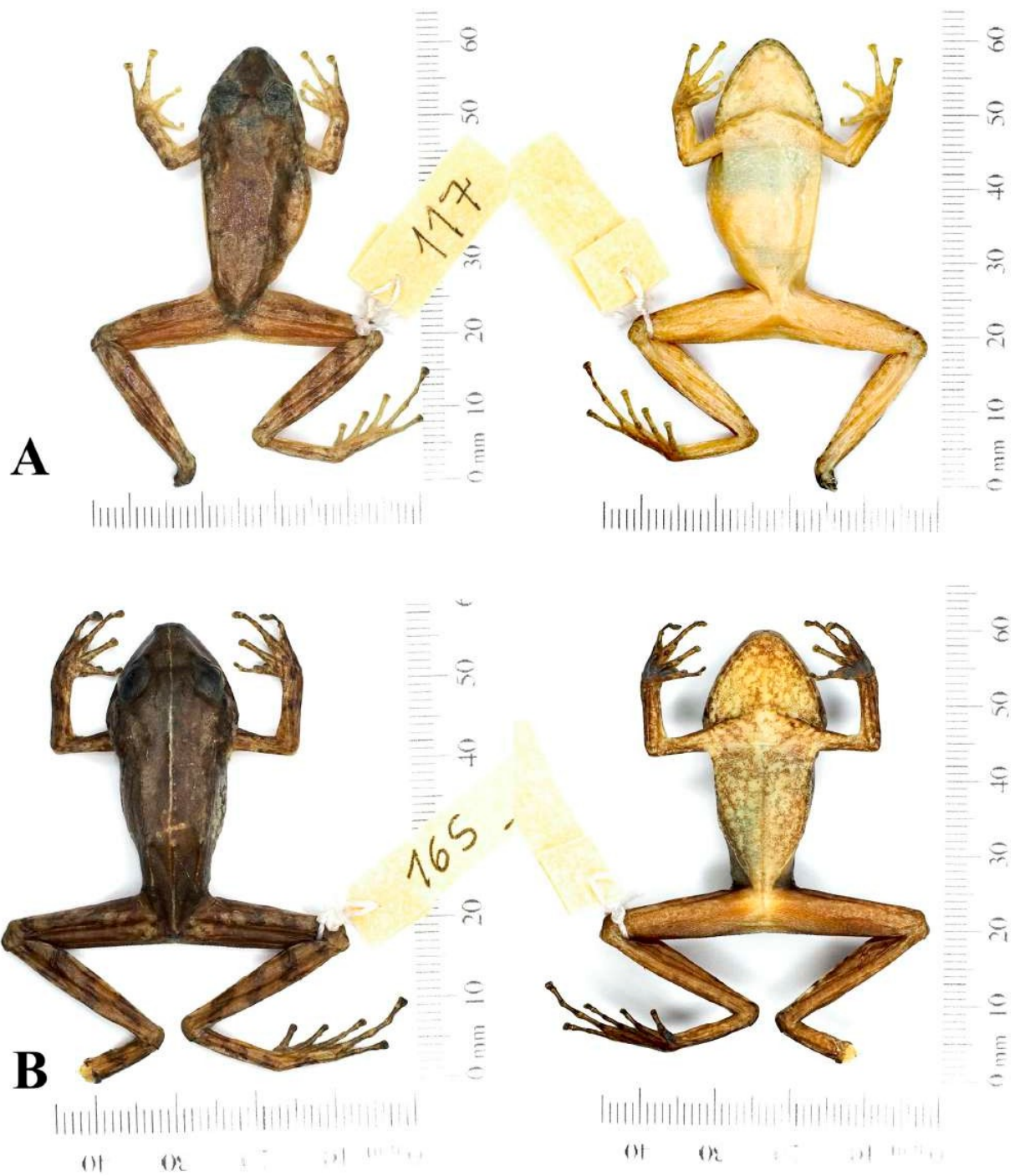


Fig 5. Photos of the holotype specimens. A. *Pristimantis ardilae* **sp. nov.** (MCNUP-H 117) B. *Pristimantis bowara* **sp. nov.** (MCNUP-H 165).

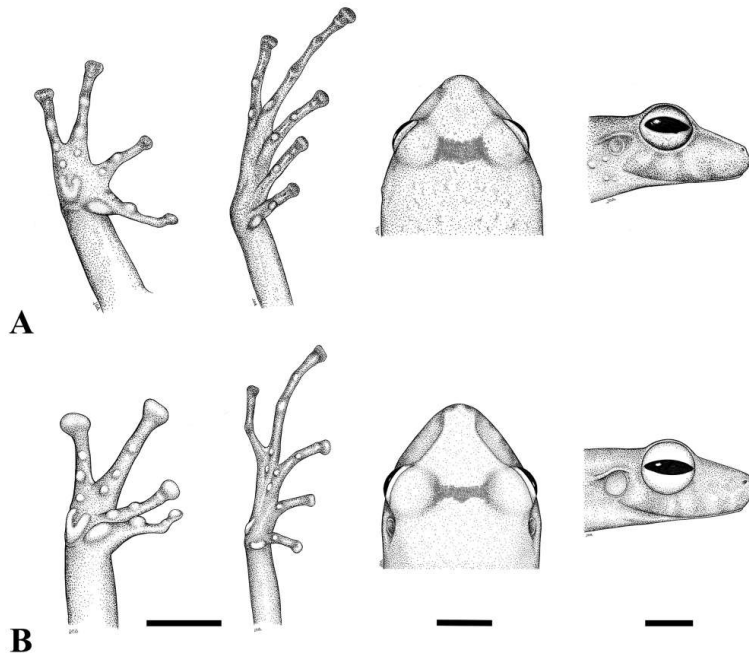


Fig 6. Illustration of the morphological characteristics of palmar surface, the foot and the head (lateral and dorsal view): A. *Pristimantis ardilae* **sp. nov.** B. *Pristimantis bowara* **sp. nov.** Scale bar = 5 mm.

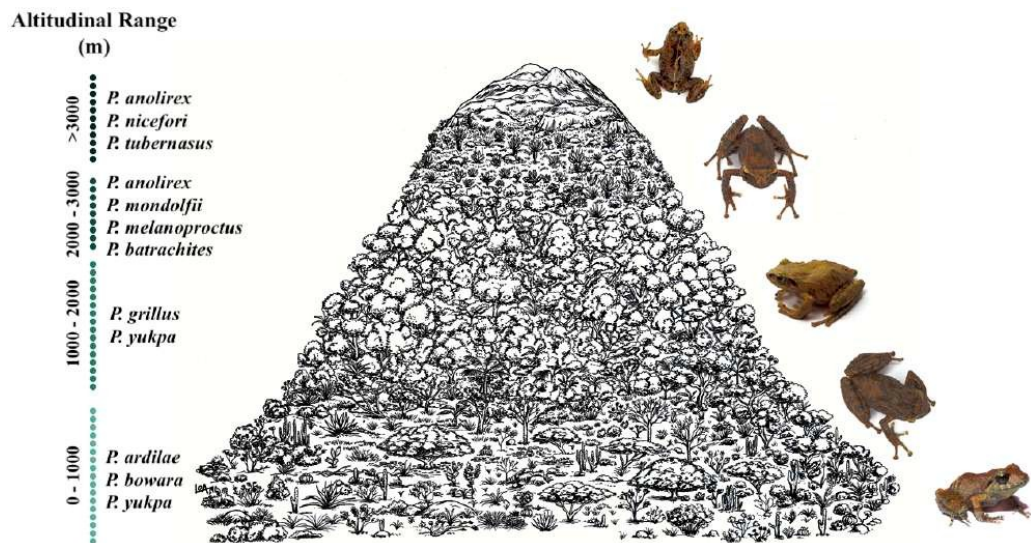


Fig 7. Altitudinal distribution of *Pristimantis* species in the department of Norte de Santander, Colombia.

Supplementary information

Table S1. Primers used in this study for PCR and DNA sequencing (F = forward, R = reverse).

Gene region	Primer name	Primer sequence (5'– 3')	Source
16S	16Sar (F)	CGC CTG TTT ATC AAA AAC AT	Palumbi et al. (1991)
	16Sbr (R)	CCG GTC TGA ACT CAG ATC ACG T	Palumbi et al. (1991)
12S	12SL4E (F)	TAC ACA TGC AAG TYT CCG C	Heinicke et al. (2007)
	12SH10 (R)	CAC YTT CCR GTR CRY TTA CCR TGT TAC GAC TT	Heinicke et al. (2007)
CYTB	MVZ15-L (F)	GAA CTA ATG GCC CAC ACW WTA CGN AA	Goebel et al. (1999)
	MVZ16-H (R)	AAA TAG GAA RTA TCA YTC TGG TTT RAT	Goebel et al. (1999)
TYR	TYR1F (F)	GTT GTY GTA TCT ACC TCR CC	Heinicke et al. (2007)
	TYR1R (R)	GMA GGG AAT GGT GAA RTT CTC	Heinicke et al. (2007)
RAG1	RAG1FF2 (F)	ATG CAT CRA AAA TTC ARC AAT	Heinicke et al. (2007)
	RAG1FR2 (R)	CCY CCT TTR TTG ATA KGG WCA TA	Heinicke et al. (2007)

Table S2. GenBank accession numbers for the species included in the phylogenetic analysis of this study.

Species	12S	16S	CYTB	TYR	RAG1
<i>Craugastor podiciferus</i>	EF493360	EF493360	GQ345197	EF493481	EF493450
<i>Eleutherodactylus caribe</i>	EF493385	EF493385	HQ831645	EF493472	EF493411
<i>Eleutherodactylus marnockii</i>	EF493820	EF493642	-	EF493476	EF493399
<i>Oreobates cruralis</i>	EU186666	EU186666	EU368881	KY681068	KY672963
<i>Tachiramantis lentiginosus</i>	KP297386	KP297386	-	KP297390	KP297388
<i>Tachiramantis prolixodiscus</i>	KP297385	KP297385	-	KP297389	KP297387
<i>Tachiramantis douglasi</i>	-	KP149286	-	-	-
<i>Pristimantis acatallelus</i>	-	JN104675	-	-	-
<i>Pristimantis acerus</i>	EF493678	EF493678	-	-	-
<i>Pristimantis achatinus</i>	EF493827	EF493660	KC857740	-	KC858083
<i>Pristimantis achuar</i>	-	EU130626	EU130679	-	-
<i>Pristimantis actites</i>	EF493696	EF493696	-	EF493494	EF493432
<i>Pristimantis acuminatus</i>	-	EU130579	EU130577	-	-
<i>Pristimantis adiaxolus</i>	AY964086	-	-	-	-
<i>Pristimantis affinis</i>	JN991487	JN991424	-	JN991554	-

<i>Pristimantis albertus</i>	EU186695	-	-	-	-
<i>Pristimantis altae</i>	JN991496	-	-	JN991560	JQ025174
<i>Pristimantis altamazonicus</i>	EF493670	EF493670	-	EU186778	EF493441
<i>Pristimantis altamnis</i>	-	EU130617	EU130665	-	-
<i>Pristimantis angustilineatus</i>	-	JN104677	-	-	-
<i>Pristimantis aniptopalmatus</i>	EU186694	EU186694	-	-	-
<i>Pristimantis anolirex*</i>	-	DQ195450	DQ195478	-	-
<i>Pristimantis appendiculatus</i>	EF493524	EF493524	-	-	-
<i>Pristimantis ardalonychus</i>	EU186664	EU186664	-	-	-
<i>Pristimantis attenboroughi</i>	KY594754	KY594762	-	-	KY962759
<i>Pristimantis aureoventris</i>	-	JQ742151	-	-	-
<i>Pristimantis bambu</i>	JF906319	-	-	-	-
<i>Pristimantis bipunctatus</i>	EF493702	EF493702	-	EF493492	EF493430
<i>Pristimantis bogotensis</i>	JN991497	DQ195451	DQ195479	-	-
<i>Pristimantis bounides</i>	KY962794	-	-	-	KY962771
<i>Pristimantis boulengeri</i>	-	DQ195452	DQ195480	-	-
<i>Pristimantis brevifrons</i>	JN991498	JN370957	-	-	-
<i>Pristimantis briceni</i>	JX155297	JX155297	-	-	-
<i>Pristimantis bromeliaceus</i>	EF493351	EF493351	-	-	-
<i>Pristimantis buccinator</i>	-	EU712630	-	-	-
<i>Pristimantis buckleyi</i>	EF493350	EF493350	-	-	-
<i>Pristimantis cajamarcensis</i>	EF493823	EF493663	-	-	-
<i>Pristimantis calcaratus</i>	-	JN104657	-	-	-
<i>Pristimantis calcarulatus</i>	-	EF493523	-	-	-
<i>Pristimantis caprifer</i>	EF493391	EF493391	-	-	-
<i>Pristimantis carrangerorum</i>	-	DQ195453	-	-	-
<i>Pristimantis carvalhoi</i>	-	DQ195454	DQ195481	-	-
<i>Pristimantis caryophyllaceus</i>	EU186686	EU186686	-	JN991563	JQ025175
<i>Pristimantis calcaratus</i>	-	EF493523	-	-	-
<i>Pristimantis celator</i>	EF493685	EF493685	-	-	-
<i>Pristimantis cerasinus</i>	JN991502	FJ784387	-	JN991565	JQ025178
<i>Pristimantis ceuthospilus</i>	EF493520	EF493520	-	-	-
<i>Pristimantis chalceus</i>	EF493675	EF493675	-	-	-
<i>Pristimantis chiastonotus</i>	-	JN691273	-	JN692009	-
<i>Pristimantis chloronotus</i>	AY326007	AY326007	-	-	-
<i>Pristimantis citriogaster</i>	EF493700	EF493700	-	-	-
<i>Pristimantis colomai</i>	EF493354	EF493354	-	EF493502	EF493440
<i>Pristimantis condor</i>	EF493701	EF493701	-	EF493504	EF493443
<i>Pristimantis conservatio</i>	JX155287	JX155281	-	-	-
<i>Pristimantis conspicillatus</i>	EF493529	EF493529	-	EF493499	EF493437
<i>Pristimantis cremnobates</i>	EF493528	EF493528	-	EF493486	EF493424
<i>Pristimantis crenunguis</i>	EF493693	EF493666	-	-	-

<i>Pristimantis croceoinguinis</i>	EF493669	EF493665	-	-	-
<i>Pristimantis crucifer</i>	EU186736	EU186718	-	-	-
<i>Pristimantis cruciocularis</i>	EU186656	EU186656	-	-	-
<i>Pristimantis cruentus</i>	EF493697	EF493697	-	JN991569	JQ025181
<i>Pristimantis cryophilus</i>	EF493672	EF493672	-	-	-
<i>Pristimantis curtipes</i>	EF493513	EF493513	-	EF493497	DQ679272
<i>Pristimantis danae</i>	-	EU192266	EF636950	-	-
<i>Pristimantis devillei</i>	EF493688	EF493688	-	-	-
<i>Pristimantis diadematus</i>	EU186668	EU186668	-	-	-
<i>Pristimantis dissimulatus</i>	EF493522	EF493522	-	-	-
<i>Pristimantis dorado</i>	KU496877	-	-	-	-
<i>Pristimantis duellmani</i>	AY326003	AY326003	-	EF493500	EF493438
<i>Pristimantis elegans</i>	-	DQ195457	DQ195483	-	-
<i>Pristimantis eriphus</i>	EU186671	EU186671	DQ195484	-	-
<i>Pristimantis erythropleura</i>	JN991509	JN371036	-	-	JQ025182
<i>Pristimantis euphronides</i>	EF493527	EF493527	-	EF493489	EF493427
<i>Pristimantis fenestratus</i>	FJ438809	EF493703	EF636949	-	-
<i>Pristimantis festae trepidotus</i>	EF493515	EF493515	-	-	-
<i>Pristimantis frater</i>	-	DQ195459	DQ195485	-	JQ025183
<i>Pristimantis gagei</i>	JN991511	JN991449	-	JN991570	JQ025185
<i>Pristimantis galdi</i>	EU186670	EU186670	-	EU186767	EU186746
<i>Pristimantis gentryi</i>	EF493511	EF493511	-	-	-
<i>Pristimantis ginesi</i>	JX155295	JX155295	-	-	-
<i>Pristimantis glandulosus</i>	EF493676	EF493676	-	-	-
<i>Pristimantis gryllus</i>	JX306022	JX306022	-	-	-
<i>Pristimantis gutturalis</i>	JN691313	JN690705	JN692012	JN692012	-
<i>Pristimantis hectus</i>	-	JN104680	-	-	-
<i>Pristimantis humboldti</i>	KY962798	-	-	KY962775	
<i>Pristimantis incertus</i>	EU186650	EU186650			
<i>Pristimantis imitatrix</i>	EF493824	EF493667	-	-	-
<i>Pristimantis inguinalis</i>	EU186676	EU186676	-	JN692014	-
<i>Pristimantis inusitatus</i>	EF493677	EF493677	-	-	-
<i>Pristimantis jester</i>	-	JQ742169	-	-	-
<i>Pristimantis jorgevelosai</i>	-	DQ195461	DQ195486	-	-
<i>Pristimantis juanchoi</i>	-	JN104681	-	-	-
<i>Pristimantis jubatus</i>	-	JN104663	-	-	-
<i>Pristimantis kelephas</i>	-	JN104660	-	-	-
<i>Pristimantis kichwarum</i>	-	EU130582	EF581021	-	-
<i>Pristimantis koehleri</i>	FJ438810	FJ438799	-	-	-
<i>Pristimantis labiosus</i>	EF493694	EF493694	-	-	-
<i>Pristimantis lancinii</i>	JX155294	JX155283	-	-	-
<i>Pristimantis lanthanites</i>	EF493695	EF493695	-	-	-

<i>Pristimantis latidiscus</i>	EF493698	EF493698	-	-	-
<i>Pristimantis latro</i>	KX242519	-	-	-	-
<i>Pristimantis leoni</i>	EF493684	EF493684	-	EF493495	EF493433
<i>Pristimantis librarius</i>	JN991515	JN991451	-	JN991571	JQ025188
<i>Pristimantis lirellus</i>	EF493521	EF493521	-	-	-
<i>Pristimantis llojsintuta</i>	-	EU712641	-	-	-
<i>Pristimantis luteolateralis</i>	EF493517	EF493517	-	-	-
<i>Pristimantis lutitus</i>	-	DQ195464	DQ195488	-	-
<i>Pristimantis lymani</i>	EF493392	EF493392	-	-	-
<i>Pristimantis lynchi</i>	-	DQ195463	-	-	-
<i>Pristimantis malkini</i>	EU186663	EU186663	-	-	-
<i>Pristimantis marmoratus</i>	EU186692	EU201063	-	JN692010	-
<i>Pristimantis martiae</i>	JN991516	-	-	JN991572	JQ025189
<i>Pristimantis mashpi</i>	KM675441	KM675466	-	-	-
<i>Pristimantis melanogaster</i>	EF493826	EF493664	-	-	-
<i>Pristimantis mendax</i>	EU186659	EU186659	-	-	-
<i>Pristimantis merostictus</i>	-	DQ195465	DQ195489	-	-
<i>Pristimantis mindo</i>	-	KF801581	-	-	-
<i>Pristimantis minutulus</i>	EU186657	EU186657	-	-	-
<i>Pristimantis miyatai</i>	JN991518	DQ195466	DQ195490	JN991573	-
<i>Pristimantis moro</i>	JN991520	JN991453	-	JN991575	JQ025192
<i>Pristimantis museosus</i>	JN991521	KC014940	-	JN991576	JQ025193
<i>Pristimantis mutabilis</i>	KM675434	KM675458	-	-	-
<i>Pristimantis myops</i>	-	JN104682	-	-	-
<i>Pristimantis nervicus</i>	JN991522	JN991456	-	JN991577	JQ025194
<i>Pristimantis nicefori</i>	-	-	DQ195491	-	-
<i>Pristimantis nyctophylax</i>	EF493526	EF493526	-	EF493487	EF493425
<i>Pristimantis ockendeni</i>	EF493519	EF493519	-	EF493496	EF493434
<i>Pristimantis ocreatus</i>	EF493682	EF493682	-	-	-
<i>Pristimantis orcesi</i>	EF493679	EF493679	-	-	-
<i>Pristimantis orestes</i>	EF493388	EF493388	-	-	-
<i>Pristimantis ornatus</i>	EU186660	EU186660	-	-	-
<i>Pristimantis orpacobates</i>	-	-	-	-	-
<i>Pristimantis pahuma</i>	-	EF493523	-	-	-
<i>Pristimantis paisa**</i>	JN991524	JN991459	-	JN991578	-
<i>Pristimantis palmeri</i>	-	JN371001	-	-	-
<i>Pristimantis paramerus</i>	JX155279	JX155279	-	-	-
<i>Pristimantis pardalis</i>	JN991527	FJ784386	-	JN991579	JQ025198
<i>Pristimantis parvillus</i>	EF493352	EF493352	-	-	-
<i>Pristimantis paulodutrai</i>	JX267297	JX267297	-	JX267707	JX267573

<i>Pristimantis permixtus</i> ***	-	DQ195467	-	-	-
<i>Pristimantis peruvianus</i>	-	JN991461	DQ195492	EF493498	EF493436
<i>Pristimantis petrobardus</i>	EF493825	EF493367	-	-	-
<i>Pristimantis pharangobates</i>	AY843586	AY843586	-	AY844035	-
<i>Pristimantis phoxocephalus</i>	EF493349	EF493349	-	-	-
<i>Pristimantis pirrensis</i>	JN991528	JN991462	-	JN991580	JQ025199
<i>Pristimantis platydactylus</i>	FJ438811	EU192255	EF636948	-	-
<i>Pristimantis pleurostriatus</i>	JX155292	JX155292	-	-	-
<i>Pristimantis pluvicanorus</i>	AY843586	AY843586	-	JF809896	JF809917
<i>Pristimantis prolatus</i>	EU186701	EU186701	-	-	-
<i>Pristimantis ptochus</i>	JN991530	-	-	JN991581	-
<i>Pristimantis puipui</i>	KY962800	-	-	-	KY962777
<i>Pristimantis pulvinatus</i>	EU186741	EU186723	-	-	-
<i>Pristimantis pycnodermis</i>	EF493680	EF493680	-	-	-
<i>Pristimantis pyrrhomerus</i>	EF493683	EF493683	-	-	-
<i>Pristimantis quantus</i>	-	JN104684	-	-	-
<i>Pristimantis quaquaversus</i>	-	EU130580	EU130578	JN991582	JQ025201
<i>Pristimantis quinquagesimus</i>	EF493690	EF493690	-	-	-
<i>Pristimantis ramagii</i>	JX267300	JX267299	-	JX267711	-
<i>Pristimantis reichlei</i>	EF493707	EF493707	-	EF493498	EF493436
<i>Pristimantis rhabdocnemus</i>	EU186724	EU186706	-	-	-
<i>Pristimantis rhabdolaemus</i>	EF493706	EF493706	-	-	-
<i>Pristimantis rhodoplichus</i>	EF493674	EF493674	-	-	-
<i>Pristimantis ridens</i>	EF493355	EF493355	-	JN991586	JQ025204
<i>Pristimantis riveti</i>	EF493348	EF493348	-	-	-
<i>Pristimantis rozei</i>	EF493691	EF493691	-	EF493491	EF493429
<i>Pristimantis sagittulus</i>	EF493705	EF493705	-	EF493501	EF493439
<i>Pristimantis saltissimus</i>	EU186693	EU186693	-	-	-
<i>Pristimantis samaipatae</i>	FJ438814	FJ438803	EU368890	-	-
<i>Pristimantis savagei</i>	JN991536	DQ195470	-	JN991587	JQ025205
<i>Pristimantis schultei</i>	EF493681	EF493681	-	-	-
<i>Pristimantis shrevei</i>	EF493692	EF493692	-	-	-
<i>Pristimantis simonbolivari</i>	EF493671	EF493671	-	-	-
<i>Pristimantis simonsii</i>	EU186665	EU186665	-	-	-
<i>Pristimantis simoterus</i>	-	DQ195471	DQ195495	-	-
<i>Pristimantis skydmainos</i>	EF493393	EF493393	-	-	-
<i>Pristimantis sobetes</i>	KM675428	KM675449	-	-	-
<i>Pristimantis spinosus</i>	EF493673	EF493673	-	-	-
<i>Pristimantis stictogaster</i>	EF493704	EF493704	-	EF493506	EF493445
<i>Pristimantis subsigillatus</i>	EF493525	EF493525	-	-	-

<i>Pristimantis suetus</i>	JN991537	JN991469	-	-	-
<i>Pristimantis supernatis</i>	AY326005	AY326005	-	-	-
<i>Pristimantis surdus</i>	EF493687	EF493687	-	-	-
<i>Pristimantis taeniatus</i>	JN991539	DQ195474	DQ195498	JN991592	JQ025210
<i>Pristimantis thectopternus</i>	-	JN104685	-	-	-
<i>Pristimantis thymalopsoides</i>	EF493514	EF493514	-	-	-
<i>Pristimantis thymelensis</i>	JX564889	JX564889	JX564889	EF493503	EF493442
<i>Pristimantis toftae</i>	EF493353	EF493353	-	-	-
<i>Pristimantis truebae</i>	EF493512	EF493512	-	-	-
<i>Pristimantis unistrigatus</i>	EF493387	EF493387	DQ195499	EF493505	EF493444
<i>Pristimantis uranobates</i>	-	DQ195476	-	-	-
<i>Pristimantis urichi</i>	EF493699	EF493699	-	EF493488	EF493426
<i>Pristimantis vanadise</i>	JX155291	JX155290	-	-	-
<i>Pristimantis ventrimarmoratus</i>	JF906310	-	-	-	-
<i>Pristimantis verecundus</i>	KM675424	KM675445	-	-	-
<i>Pristimantis versicolor</i>	EF493389	EF493389	-	EF493493	EF493431
<i>Pristimantis vertebralis</i>	EF493689	EF493689	-	-	-
<i>Pristimantis viejas</i>	JN991547	JN991475	-	JN991596	JQ025211
<i>Pristimantis vinhai</i>	JX267343	JX267343	-	JX267726	JX267591
<i>Pristimantis vilarsi</i>	KP149333	-	-	-	-
<i>Pristimantis w-nigrum</i>	AY326004	AY326004	DQ195501	-	KC858081
<i>Pristimantis walkeri</i>	EF493518	EF493518	-	EF493490	EF493428
<i>Pristimantis wiensi</i>	EF493377	EF493668	-	-	-
<i>Pristimantis yukpa</i>	JX306020	JX306021	-	-	-
<i>Pristimantis yuruniensis</i>	-	JQ742160	-	-	-
<i>Pristimantis zeuctotylus</i>	EU186678	EU186678	-	JN691903	-
<i>Pristimantis zophus</i>	JN991548	JN991480	-	JN991598	JQ025214

* Related to *P. lutitus* according to Rivera-Correa et al. (2016) and this work.

** *Pristimantis* sp.6. according to González-Durán et al. (2017).

*** *Pristimantis penelopus* according to Rivera-Correa et al. (2016).

Table S3. Specimens examined. Instituto de Ciencias Naturales—ICN, Bogotá.

Pristimantis anolirex: ICN11375, ICN11380: COLOMBIA: Norte de Santander, Chitagá, Vereda Piedra, 2,770 m.a.s.l., ICN26212: COLOMBIA: Norte de Santander, Abrego, Cerro Oroque, 3,100 m.a.s.l., ICN10486, ICN10489, ICN10488: COLOMBIA: Norte de Santander, Herrán, Orocué, 2,300 m.a.s.l. *Pristimantis batrachites*: ICN47890, ICN47891, ICN47888: COLOMBIA: Norte de Santander, Cucutilla, Vereda Carrizal. *Pristimantis carrangerorum*: ICN5130, ICN5133, ICN5142, ICN5146: COLOMBIA: Boyaca, Pajarito, Corinto, 1,600 m.a.s.l. *Pristimantis lutitus*: ICN5192, ICN51193: COLOMBIA: Santander, Charala, El Encino, 2,400 m.a.s.l. *Pristimantis medemi*: ICN40589, ICN40591, 40593: COLOMBIA: Boyacá, Santa María, El Sendero. *Pristimantis merostictus*: ICN01434, ICN11064: COLOMBIA: Boyacá, Duitama, Hacienda La Sierra, 2,400 m.a.s.l. *Pristimantis nicefori*: ICN22295: COLOMBIA: Boyacá, Güicán, vereda El Tabor, 3,850 m.a.s.l. ICN22311, ICN20889: COLOMBIA: Boyacá, Güicán, vereda La Cueva, 3,900 m.a.s.l. *Pristimantis tubernasus*: ICN10483: COLOMBIA: Nortede Santander, Herran, PNN Tamá, 2,300 m.a.s.l. *Pristimantis merostictus*: ICN34254, ICN34234, ICN34236: COLOMBIA: Santander, Charalá, Duitama, 2,400 m.a.s.l.

**CHAPTER 2: SPECIES RICHNESS AND PHYLOGENETIC DIVERSITY PATTERNS
EXPLAINED BY ENVIRONMENTAL PREDICTORS IN *Pristimantis*, THE MOST
DIVERSE AMPHIBIAN GENUS**

Aldemar A. Acevedo¹, R. Eduardo Palma¹

¹Laboratory of Evolutionary Biology, Department of Ecology, Faculty of Biological Sciences,
Pontificia Universidad Católica de Chile, Santiago, Chile.

Abstract

Multiple historical and ecological factors can explain the patterns of diversity in a region. However, species richness alone does not show the complete scenario in the evolutionary processes of different taxa. Therefore, to evaluate whether environmental variables drive species richness and phylogenetic diversity, we used the neotropical *Pristimantis* frogs as a study model. We compiled an extensive geographic record for all species of *Pristimantis* (600) and included phylogenetic information for 304 species of the genus. With this, we carry out two analyzes, one evaluating whether different environmental variables can be good predictors of species richness (SR). Second, we assessed whether different environmental variables could predict the phylogenetic diversity (PD). Our results showed that the ecoregions with more SRs do not agree with those with higher PD values (standardized). In turn, we find that for nine Neotropical ecoregions, SR is only predicted by aridity. However, the variables of temperature, precipitation, evaporation, and altitude are good predictors of PD for different PD metrics. Our results show the importance of including the historical component to understand the diversity patterns of

groups such as *Pristimantis* that present a wide geographic distribution. In turn, we highlight the importance of different historical processes associated with dispersal and colonization events as a relevant source to understand the PD values evidenced in *Pristimantis*.

Keywords. Climatic variables, diversity, evolution, frogs, Neotropics.

Introduction

Time and space are the main factors that lead to variations in the ability of speciation to increase species richness (SR) within a region (Rosenzweig, 1995). Therefore, understanding the variation in biodiversity at different scales is fundamental to the interpretation of biogeographic and adaptive patterns under different ecological and evolutionary contexts (Stephens & Wiens, 2003). Two hypotheses can explain SR patterns: first, the time for speciation hypothesis maintains that early colonization events can facilitate an increase in species richness simply by providing more time in an area, even if diversification rates are constant relative to those in younger, less species-rich regions (Gaston & Blackburn, 1996; Stephens & Wiens, 2003). Second, the montane species pump model predicts that high species richness occurs in mountain regions facilitated by topographic heterogeneity that increases allopatric and parapatric speciation (Moritz et al., 2000; García-Rodríguez et al., 2021). Nevertheless, species diversity is usually estimated using metrics such as SR because it is an easy measure to quantify in different communities (Fleishman et al., 2006). Additionally, it is correlated with a wide variety of ecological and climatic variables (Gotelli & Colwell, 2001; Boucher-Lalonde et al., 2014; Lean & Maclaurin, 2016). Nevertheless, with the introduction of new phylogenetic inferences

and increased accessibility to molecular data from different taxa in areas with limited information, the use of metrics such as phylogenetic diversity (PD) has increased in recent years (Lean & Maclaurin, 2016). Phylogenetic diversity is reflected as the sum of the lengths of the branches of the species that connect the evolutionary tree (Faith, 1992, 2006). This means that PD indicates branch length information based on the number of new features that emerge in the evolutionary context of terminal taxa in a region (Faith, 1992). With this, we can address issues related to the degree of evolutionary history present in one or more geographic areas (Miller et al., 2018).

Moreover, we highlight that PD is advantageous over SR, in using phylogenetic information when a clade presents taxonomic uncertainties or the species are difficult to identify (Isaac et al., 2004). Furthermore, different environmental variables have been identified as good predictors of PD (Earl et al., 2021; Paz et al., 2021), which can support climate-related hypotheses as potential drivers of diversity. For example, the role of climatic stability between regions has been relevant to understanding species diversity patterns (Graham et al., 2006). The lowest extinction rates are found in regions with higher climatic stability (Rohde 1992; Willig et al., 2003) as compared to regions that present variable climatic regimes generating spatial variation in phylogenetic diversity (Saladin et al., 2020). For example, bioregions closer to the equator have smaller temperature fluctuations during the year, thus experiencing higher climatic stability (Janzen 1967; Fine, 2015), which has probably contributed to the high values of diversity in these regions. However, on broader scales (e.g., the Neotropics) and between regions with different geological histories, the behavior of PD metrics with climatic predictors

has only been partially addressed (Paz et al., 2021). To date, these issues remain overlooked in the genus *Pristimantis*.

Among the Neotropics, the genus *Pristimantis* (Anura: Strabomantidae) is characterized as the most diverse, with approximately 600 recognized species (Heinicke et al. 2009, AmphibiaWeb 2021). It occupies a wide geographical area, being the most extensive among the neotropical amphibian genera, with species found in humid and forested environments of the Tropical Andes of Colombia, Ecuador, Peru, and some areas of Central America (Lynch and Duellman 1997; Pinto-Sánchez et al. 2012; Meza-Joya and Torres 2016). Additionally, it presents an extensive altitudinal range, from sea level to altitudes above 4500 m, with considerable endemism for the páramo areas (Heinicke et al. 2007; Meza-Joya and Torres 2016). Two factors have been proposed that would explain the high SR of this genus (Guayasamin et al. 2017; Hutter et al. 2017): the first refers to the diversification rate, which has been considered one of the highest among the Neotropical vertebrates, with an increase that has favored the colonization of different areas of South and Central America (Crawford 2003; Padial et al. 2014; Hutter et al. 2017); the second is related to orogenic changes in the northwestern Andes, which would have been the principal promoter of speciation in *Pristimantis* (Acevedo et al., in preparation).

We estimated the species richness for 10 neotropical ecoregions and evaluated whether the SR and PD were linearly related to each other. Subsequently, we conducted two analyses, including most of the *Pristimantis* species (c. 600) and integrating phylogenetic information for 304 species. For each analysis, different environmental predictors associated with 10 neotropical ecoregions were associated. With this, we evaluated whether several climatic variables could be

predictors of PD and SR. We hypothesized that variations in PD metrics (clustering or phylogenetic overdispersion) can be associated with a gradient pattern in the climatic regimes between neotropical ecoregions.

Materials and methods

Data collection

We collected 9183 geographic records comprising all *Pristimantis* species (600 species) (Table S1). The records were obtained through a search of different resources, including those in the database of the Global Biodiversity Information Facility (GBIF, <http://www.gbif.org>), original descriptions of each species, publications of records and extensions of distribution ranges, and reviews of biological collections (ICN-MHN-Her, MCNUP-H, MHNUD, and ANDES-A). The records were curated by eliminating incorrect coordinates, misassigned locations, and records outside the known range by comparing them with the distribution maps available on the IUCN portal (<https://www.iucnredlist.org>).

The total distributional range of *Pristimantis* was subdivided into 10 ecoregions, following Olson et al., (2001), as updated by Dinerstein et al. (2017). The following ecoregions were included: Amazonia (A), Central America (B), Central Andes (southern Ecuador, Peru, western Bolivia) (C), Chocó (western Ecuador, Pacific Colombia, and the Darien in Panama) (D), Guiana Shield (E), Northwestern Andes (western and central Andean cordilleras of Colombia + the Ecuadorian Andes) (F), Venezuelan Andes (G), Colombian Eastern Andes (H), Venezuelan

Coastal Range (I), and the Atlantic Forest of Brazil (J). The ecoregions were vectorized using DIVA-GIS 7.5 (Hijmans et al., 2005) (Fig. 1).

Species richness (SR)

We generated 100×100 -km cells throughout the entire species distribution (Central America to Central Andes in Peru and Bolivia). We then overlapped the geographic records and calculated SR as the total count of species within each grid-cell unit. We also estimated the richness of each ecoregion by overlapping each layer on the geographic records of *Pristimantis*. We used DIVA-GIS 7.5 (Hijmans et al., 2005) to visualize geographic records, estimate species richness, and export the map generated in R (R Core Team, 2019).

Phylogenetic diversity (PD)

We estimated Faith's measure of phylogenetic diversity (PD) (Faith, 1992) as the length of all phylogenetic branches connecting any set of two or more species. To estimate PD by ecoregion, we used the R package "picante" (Kembel et al., 2010), the TimeTree estimated for 304 species of *Pristimantis* generated by Acevedo et al. (in preparation), and a matrix of species presence/absence for the 10 ecoregions previously defined (Atlantic Forest was excluded as it only had four species) (Table S2). We estimated two indices of PD among species at different depths on the phylogenetic tree (Webb et al., 2002; Massante et al., 2019): 1) mean pairwise distance (MPD), which calculates the mean phylogenetic distance among all pairs of species within a community, and 2) mean nearest taxon distance (MNTD), which calculates

phylogenetic distances at a shallower level, between any focal species and its closest relative(s) (Tucker et al., 2017, Massante et al., 2019). To produce PD indices independent of species richness, we calculated the standardized effect sizes of PD (SES-PD) and both indices MPD (SES-MPD) and MNTD (SES-MNTD), comparing the PD values of the observed community with the null distribution of randomly assembled communities with equal richness (Kembel et al., 2010, Massante et al., 2019). SES can be defined as:

$$\text{ses.Metric} = \text{Metric}_{\text{obs}} - \text{mean}(\text{Metric}_{\text{null}}) / \text{sd}(\text{Metric}_{\text{null}})$$
 where $\text{Metric}_{\text{obs}}$ is the observed metric in a given assemblage, and $\text{Metric}_{\text{null}}$ is the same metric but calculated n times with n randomized assemblages (Mazel et al., 2016). We use the null model "independentswap," which randomizes the community data matrix with the independent swap algorithm (Gotelli 2000), maintaining the species occurrence frequency and sample species richness. Negative values of PD (<1) correspond to a phylogenetic clustering pattern, and positive values (>1) are consistent with a pattern of phylogenetic overdispersal that drives greater PD independently of SR (Webb, 2000).

Environmental variables

To explore the relationship between climatic variation and variation in our two standardized measures of PD and SR among the 10 ecoregions, we obtained the layers corresponding to annual mean values of temperature, precipitation, aridity index, and evapotranspiration potential, as well as elevation from the WorldClim database (<http://www.worldclim.org>) at a resolution of $\sim 1 \text{ km}^2$. Although some of these variables are correlated with each other (Table S3), these climatic components are key in the processes and ecological dynamics of amphibian populations (Duellman & Trueb 1986; Hillyard, 1999). For example, temperature and humidity

have been related to oxygen absorption, development, and locomotion, among other physiological processes (Hillman et al., 2009), and thus to migration, mating, and reproduction events (Beebee, 1995; Forchhammer et al., 1998). The average values of the climatic data were obtained by overlapping the matrix of geographic records for each *Pristimantis* species (9183 geographic records) on the polygons of the 10 previously established ecoregions (Table S4).

Analysis

We employed two analytical approaches; the first of these focused on evaluating whether climatic variables can be good predictors of SR. To this end, we included the species richness values for each ecoregion and contrasted them with the average values of the climatic variables through simple linear regressions (SLR) using the “lm” function in R (R Core Team, 2019). The second analysis included PD estimates (SES-MPD and SES-MNTD) for the 304 species of *Pristimantis* included in the phylogeny. We explored the relationships between PD as response variables and the climatic averages of each ecoregion as predictors through SLR. In turn, we used the same analysis to contrast the relationship between SR and PD to assess whether species richness can be a predictor of PD.

Results

Species richness and phylogenetic diversity

The SR of *Pristimantis* sampled in this work was concentrated in the Andean areas of the Northwestern Andes of Colombia and Ecuador, with values ranging from 25 to 69 species for each 100×100 -km cell (Fig. 1A). The Colombian Eastern Andes and Venezuelan Andes also hosted a high level of SR, with cell values ranging from 7 to 20 species (Fig. 1A). The total species richness by ecoregion can be seen in Fig. 1A.

The Colombian Eastern Andes was the area with the highest SES-PD (9.11), followed by the Venezuelan Andes (7.72). In contrast, the Northwestern Andes, Central Andes, Guiana Shield, and the Amazonia showed similar SES-PD values (5.82–6.78) (Fig. 1B; Table 1), and the Atlantic Forest, Venezuelan Coastal Range, Central America, and Chocó had the lowest SES-PD values (<3.14) (Fig. 1B; Table 1).

According to the two PD metrics, for SES-MPD the highest value was found in the Venezuelan Andes (12,548) followed by Colombian Eastern Andes (8912) and Central Andes (8445), while the lowest values were in lowland ecoregions (Table 1). For SES-MNTD the pattern was similar to that of SES-MPD except that the Colombian Eastern Andes (6819) had the highest value (Table 1). The regression analysis between SR and PD (not standardized) yielded a positive relationship ($F_{1,7} = 61.08$, $p = 0.000106$, $R^2 = 0.88$; Fig. 2); however, there was no relationship between SR and SES-PD ($F_{1,7} = 1.008$, $p = 0.34$, $R^2 = 0.009$; Fig. 5).

Species richness, phylogenetic diversity, and climate

SR by region did not show a relationship with any environmental variable (Table S5) except for the aridity index, which showed a negative relationship ($F_{1,7} = 8.625$, $p = 0.02181$, $R^2 = 0.488$; Fig. 3). In relation to environmental variables as predictors of PD, the annual mean temperature was negatively related to the PD indices SES-MNTD ($F_{1,7} = 13.16$, $p = 0.0084$, $R^2 = 0.60$; Fig. 4A) and SES-MPD ($F_{1,7} = 7.481$, $p = 0.029$, $R^2 = 0.44$; Fig. 4B). The ecoregions Colombian Eastern Andes, Venezuelan Andes, Northwestern Andes, and Central Andes had the highest values of SES-MNTD and SES-MPD, which corresponded to an annual mean temperature between 12°C and 16°C (Fig. 4A, B). By contrast, in ecoregions associated with medium- and low-elevation (0–2000 m a.s.l.) areas, the SES-MNTD and SES-MPD values were associated with temperatures above 22°C (Fig. 4A, B). The SES-MNTD index was negatively related to potential evapotranspiration ($F_{1,7} = 7.395$, $p = 0.029$, $R^2 = 0.44$; Fig. 5C), while SES-MPD was not ($F_{1,7} = 2.642$, $p = 0.1481$, $R^2 = 0.17$). The Colombian Eastern Andes ecoregion was associated with low potential evapotranspiration values, whereas medium- and low-altitude ecoregions were associated with potential evapotranspiration values ranging from 1100 to 1500 mm (Fig. 4C). Similarly, SES-MPD was negatively related to precipitation ($F_{1,7} = 9.078$, $p = 0.019$, $R^2 = 0.50$; Fig. 5D), while SES-MNTD was not ($F_{1,7} = 1.868$, $p = 0.214$, $R^2 = 0.09$). The Venezuelan Andes had the highest values of SES-MPD, and they had a low mean annual precipitation value (Fig. 4D), whereas values of SES-MPD associated with high precipitation corresponded to the regions of Amazonia, Chocó, and Central America (Fig. 4D).

Lastly, elevation was positively related to the indices SES-MNTD ($F_{1,7} = 11.5, p = 0.011, R^2 = 0.56$; Fig. 4E) and SES-MPD ($F_{1,7} = 8.976, p = 0.020, R^2 = 0.49$; Fig. 4F). In contrast to lowland ecoregions, areas such as the Northwestern Andes, Colombian Eastern Range, Central Andes, and Venezuelan Andes had high SES-MNTD and SES-MPD values (Fig. 4E, F). Regarding the aridity index, there was no linear relationship with SES-MPD ($F_{1,7} = 0.663, p = 0.442, R^2 = -0.043$) or with SES-MNTD ($F_{1,7} = 0.013, p = 0.91, R^2 = -0.140$).

Discussion

The Northwestern Andes is the ecoregion with the highest SR from the *Pristimantis*, consistent with the ancestral area where *Pristimantis* diversified (Acevedo et al., in preparation). The relation between SR and the ancestral area of the genus supports the time-for-speciation effect hypothesis, which suggests that in areas with a higher number of species, SR is correlated with evolutionary time, whereby species have had more time for speciation (Stephens & Wiens, 2003). This has allowed dispersion to multiple neotropical zones (Acevedo et al., in preparation) but has had an important influence of the ecoregion of The Amazonia and Colombian Eastern Andes in the current richness patterns present in *Pristimantis*. Therefore, regional history plays a relevant role in the configuration of distributions and SR (Jetz et al., 2004; Buckley & Jetz, 2007). However, the SR and SES-PD values do not follow a linear relationship: while the SR is concentrated in the Northwestern Andes, the SES-DP metrics found that the ecoregions of the Colombian Eastern Andes and Venezuelan Andes presented the highest values.

The high SES-PD values agree with biogeographic patterns on the multiple routes of dispersion and colonization that have occurred in the Colombian Eastern Andes, from the Northwestern Andes, Amazonia, and Venezuelan Andes at least in the last 15 Ma (Acevedo et al., in preparation). We found similar results when evaluating the phylogenetic structure at different evolutionary scales, which are sensitive to processes that operate for basal (SES-MPD) and terminal (SES-MNTD) structures of the phylogenetic tree (Webb et al. 2002). For all communities the values were positive, indicating patterns of over-dispersal, meaning that species would be more distantly related than expected to occur by chance (Webb, 2000). The evaluated metrics do not evidence phylogenetic clustering processes or processes that lead to cluster of overdispersion, in which some processes (e.g., environmental filtering) generate basal clustering, and others can create terminal over-dispersion on the shape and size of the phylogenetic tree (Webb, 2000), which suggests that the historical patterns of dispersal and colonization of *Pristimantis* between ecoregions have been relevant to the structure of SES-PD at different evolutionary scales.

Knowing the PD of a region or ecological community has various implications: PD can be used as a proxy for evolutionary potential, which is related to the capacities of communities to evolve in response to environmental changes (Winter et al., 2013). High PD values (distantly related species) should increase the chances that communities have higher evolutionary potential than is assumed for closely related species with a similar evolutionary potential (Winter et al., 2013), such as in the case of the Andean species of the Northwestern Andes, which have closer evolutionary relationships. Additionally, PD plays a key role in conservation decision-making because species that descend from long branches are more likely to maintain older traits than

those that descend from shorter ones (Faith, 1992; Veron et al., 2019). Lean & Maclaurin (2016) present a series of uses and scopes of PD for decision-making, threatened species, and ecological communities, which can be applied in the context of *Pristimantis* to prioritize areas for conservation.

Phylogenetic diversity and climatic variables

Of all the climatic variables examined, only the aridity index was a significant predictor of SR: as the aridity index increased, SR decreased. Therefore, water availability plays an important role in limiting SR in different geographical regions, which is related to the physiology of amphibians and their water requirements (Whittaker et al., 2007; Buckley & Jetz, 2007). However, when conducting the analyses with PD, our study revealed that climatic variables associated with temperature, precipitation, and evapotranspiration were negatively related to the two PD metrics, SES-MNTD and SES-MPD. When the values of these climatic variables increase, PD decreases, which is reflected in lowland ecoregions (e.g., Chocó or Amazonia). This indicates that changes in the conditions of these environmental variables have played an essential role in the phylogenetic structuring of *Pristimantis*. PD patterns in relation to environmental variables can be explained by the mix of divergent lineages that have dispersed between regions, which have experienced multiple geological and climatic events, especially in the Andean areas during the last 20 Ma (Graham, 2009). Elevation and PD were positively related, with high PD values and patterns that could be explained by the historical relationships of dispersal and colonization in the genus at the altitudinal level in Andean areas (Mendoza et al., 2015; Waddell et al., 2018). However, species from regions with low PD (e.g., Central

America, Chocó or Guiana Shield) have probably experienced restrictions on leaving their ancestral ranges, limitations related to different geological events, in situ speciation, or restrictions on species dispersal (Cooper et al., 2008).

Conclusions

The incorporation of PD has allowed us to address questions related to changes in evolutionary history among the most related or distant species of a community. All the ecoregions evaluated for *Pristimantis* show positive values for PD metrics, which translates into a greater phylogenetic overdispersion. Moreover, the Colombian Eastern Andes have the highest PD despite having fewer species reported than the Northwestern Andes, probably due to a complex geological, climatic, and evolutionary history that has favored colonization from the Northwestern and Amazonia in the last 20 to 12 Ma. Therefore, the settlement of lineages in high mountain areas (over 3000 m asl), endemism patterns, and relationships with Amazonia should be studied further to evaluate different local biogeographic hypotheses in different Andean contexts.

We recommend the use of PD metrics on SR, whose characteristics did not allow us to detect relationships with different environmental predictors. By contrast, the increase or decrease in PD metrics (SES-MNTD and SES-MPD) in relation to the climatic variables yielded a correlative pattern between the different ecoregions included in this study. The climatic variables evaluated in this work are good predictors of the PD of *Pristimantis*. We highlight the importance of climatic factors inherent in each ecoregion in conjunction with the evolutionary

history of *Pristimantis* to understanding the processes that shape PD. Our findings reinforce the idea that both ecological (changes in species richness, responses of communities to climatic variables, local adaptation, trait evolution) and geological events are relevant for understanding PD patterns and the evolutionary history of species between ecoregions, especially for genera with a wide geographic distribution such as *Pristimantis* and other groups of Neotropical amphibians.

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Data availability statement

The data that support the findings of this study are openly available in electronic supplementary information and in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.bnzs7h4cb>, <https://doi.org/10.5061/dryad.9cnp5hqk9>, <https://doi.org/10.5061/dryad.tx95x69zv>

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Tables

Table 1. Neotropical *Pristimantis* ecoregions with phylogenetic diversity (PD), species richness (SR) and standardized metric values (SES-MNTD, SES-MPD, SES-PD).

Ecoregion	SES-MNTD	SES-MPD	SES-PD	PD	SR
Amazonia	4.205	4.617	6.267	419.0658	73
Venezuelan Andes	5.06	12.548	8.138	60.31086	20
Central America	2.78	1.896	2.904	113.36577	12
Venezuelan Coastal Range	1.877	1.024	1.608	81.97498	19
Central Andes	4.297	8.445	6.822	532.68209	72
Chocó	2.91	1.923	3.889	205.71777	48
Colombian Eastern Andes	6.819	8.912	10.189	406.50461	98
Guiana Shield	3.103	7.723	5.392	142.93698	24
Northwestern Andes	4.046	4.866	6.054	967.05053	330

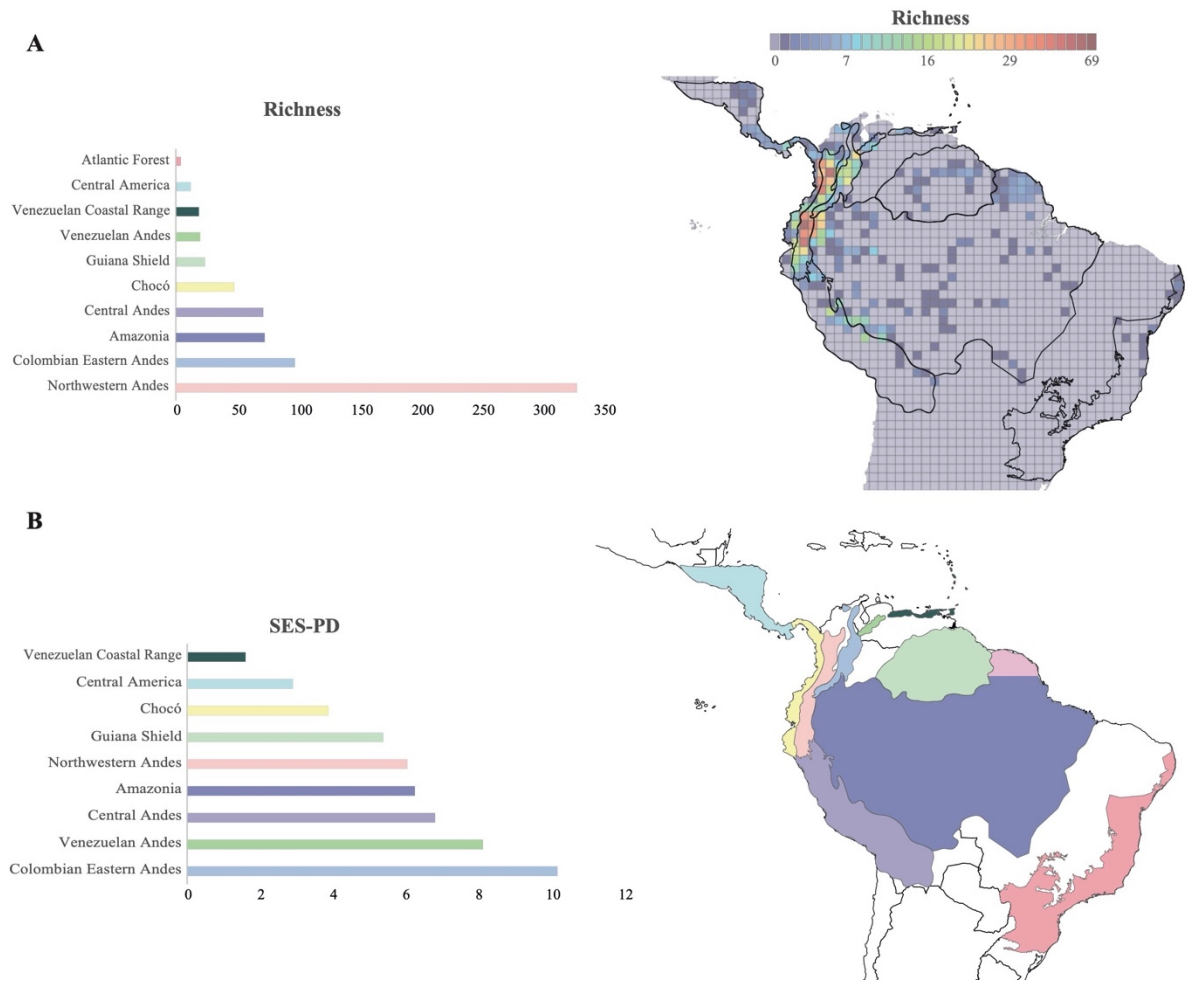


Fig 1. Neotropical ecoregions of *Pristimantis* distribution. A. Species richness (SR) by ecoregion and map with 100×100 -km cells indicating the SR (color bar). B. Standardized effect size of phylogenetic diversity (PD) for each ecoregion included in this study.

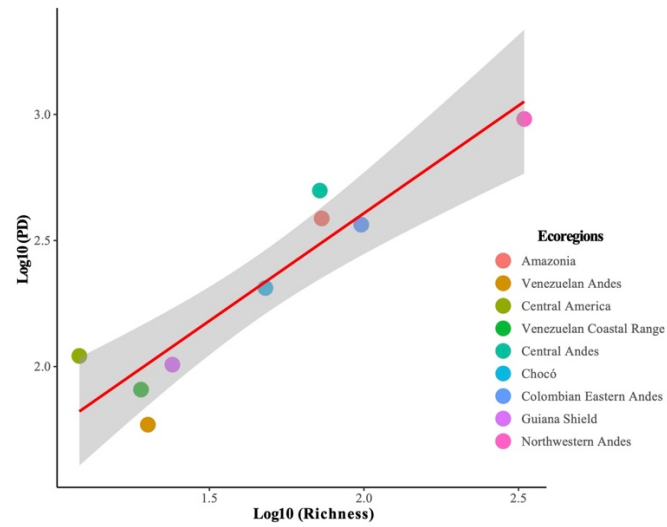


Fig 2. Linear regression between the mean value for a given climatic variable and standardized phylogenetic diversity indices, the standardized effect size-mean nearest taxon distance (SES-MNTD) and the standardized effect size-mean pairwise distance SES-MPD). The circles represent each ecoregion identified on the map. Mean climatic values are based on all locality records of *Pristimantis* in a given ecoregion, totalling 7224 records across the ten ecoregions.

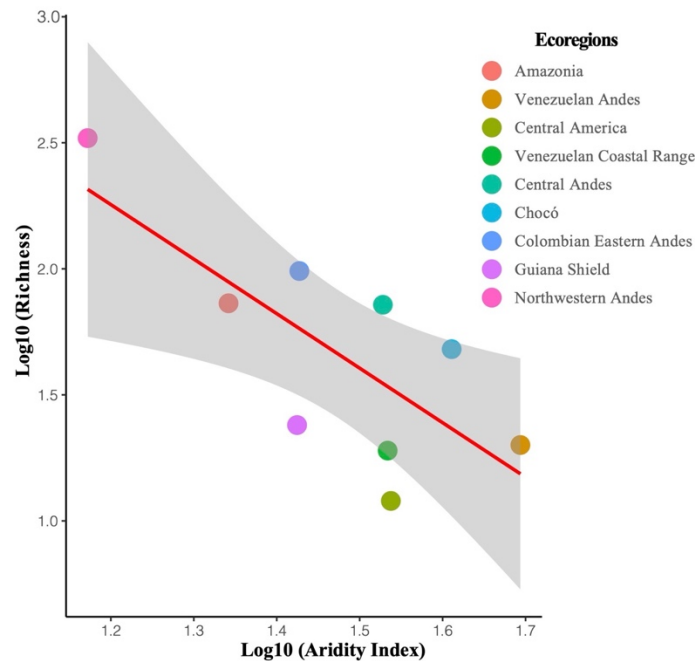


Fig 3. Linear regression between aridity index and species richness (SR) by ecoregions.

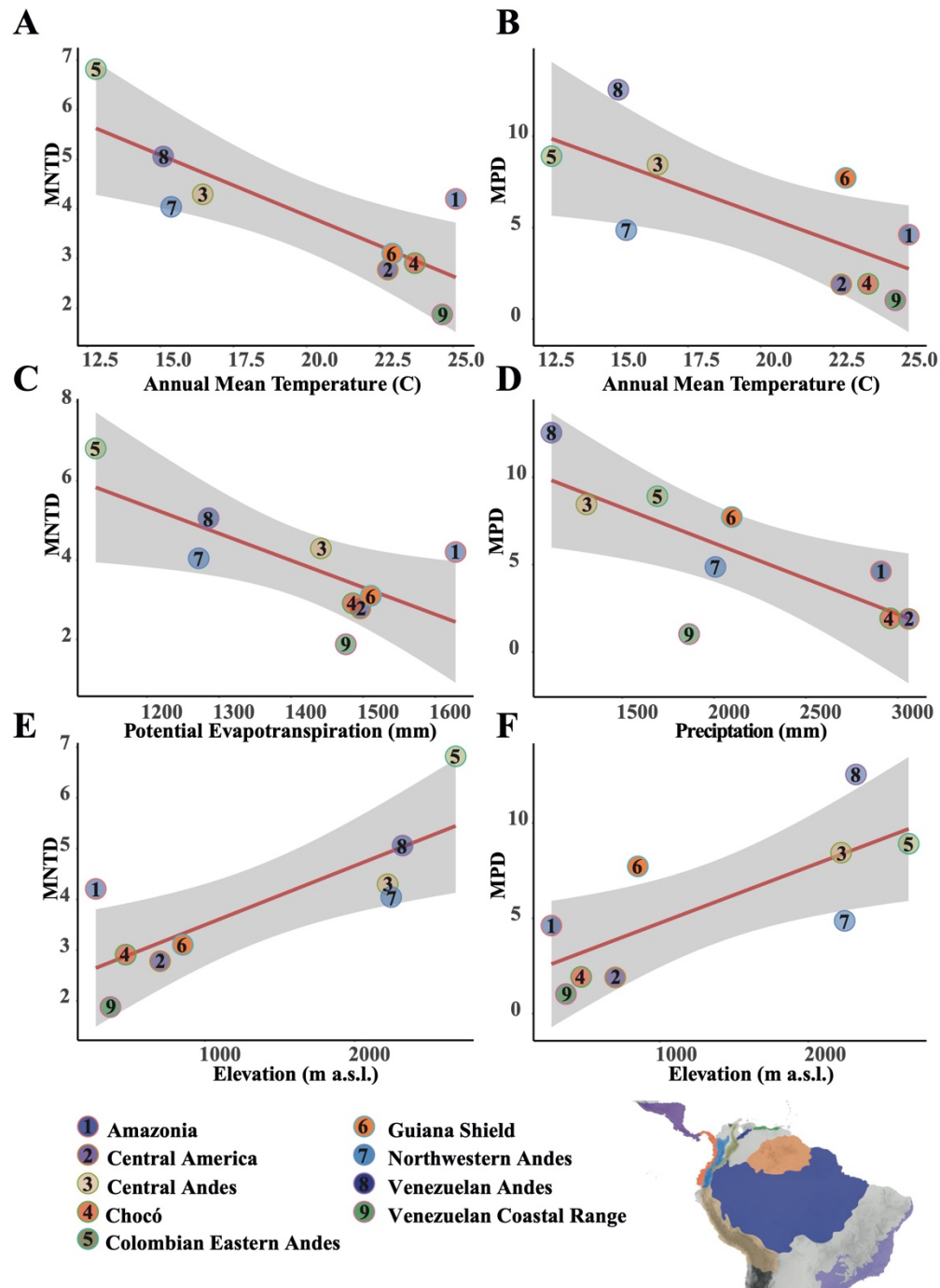


Fig 4. Linear regressions between the environmental predictors and the SES-MPD and SES-MNTD metrics by ecoregions indicated in numbers in the map at the bottom.

Supplementary information

Table S1. Database with 9183 geographic records that include all *Pristimantis* species (600 species). Dryad Digital Repository: <https://doi.org/10.5061/dryad.bnzs7h4cb>.

Table S2. Presence / absence matrix for 304 species of *Pristimantis* according to geographic records for nine neotropical ecoregions. Dryad Digital Repository: <https://doi.org/10.5061/dryad.9cnp5hqq9>.

Table S3. Pearson's correlation coefficients of the relationship between environmental variables for phylogenetic diversity analyzes.

	Annual Mean Temperature (AMT)	Annual Potential Evapotranspiration (AnnualPET)	Thornthwait e aridity index	Digital Elevation Model (DEM)	Climatic Moisture Index	Annual Precipitat ion	Precipitation of Wettest Month
Annual Mean Temperature (AMT)	1	0,9	-0,11	-0,99	0,3	0,66	0,67
Annual Potential Evapotranspiration (AnnualPET)	0,9	1	0,01	-0,86	0,03	0,44	0,44
Thornthwaite aridity index	-0,11	0,01	1	0,14	-0,65	-0,52	-0,27
Digital Elevation Model (DEM)	-0,99	-0,86	0,14	1	-0,35	-0,69	-0,7
Climatic Moisture Index	0,3	0,03	-0,65	-0,35	1	0,78	0,73
Annual Precipitation	0,66	0,44	-0,52	-0,69	0,78	1	0,91
Precipitation of Wettest Month	0,67	0,44	-0,27	-0,7	0,73	0,91	1

Table S4. Database for 600 species of *Pristimantis* with the averaged values of each climatic variable used in the SR and PD analyzes. Dryad Digital Repository: <https://doi.org/10.5061/dryad.tx95x69zv>.

Table S5. Linear regressions between species richness (SR) and environmental variables.

	$F_{1,7}$	p	R^2
DEM	0.8475	0.388	-0.019
BIO1	1.857	0.215	0.096
PET	0.6755	0.438	-0.0422
BIO12	0.003148	0.957	-0.142
ARidity	8.625	0.02181	0.488

**CHAPTER 3: BODY SIZE EVOLUTION AND ENVIRONMENTAL FACTORS
DRIVING SEXUAL BODY SIZE DIMORPHISM IN *Pristimantis*, THE WORLD'S
MOST DIVERSE AMPHIBIAN GENUS***

Aldemar A. Acevedo¹, R. Eduardo Palma¹, Miguel Ángel Olalla-Tárraga²

¹Laboratory of Evolutionary Biology, Department of Ecology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.

²Department of Biology and Geology, Physics & Inorganic Chemistry, Universidad Rey Juan Carlos, Móstoles, Spain.

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Abstract

Body size is a key organismal trait, with profound ecological and evolutionary implications. However, the environmental and evolutionary factors that drive body size patterns at the interspecific level remain unclear. Here, we explored these relationships phenotype-environment using neotropical frogs of the genus *Pristimantis*, the amphibian genus with most currently described species, as a study model. We analyzed: (a) whether this group conforms to Rensch's rule, a trend of sexual size dimorphism (SSD) to increase with size when males are the larger sex; (b) whether environmental constraints associated to temperature and humidity gradients have influenced body size variation; (c) which evolutionary model best accounts for size variation across the phylogeny and (d) how the rates of body size evolution have varied

over time. Analyses were based on two information sources, the first one including body sizes of ~85% (495 species) of known species in the genus, and a second one incorporating molecular phylogenetic information for 257 species. Our results showed that all *Pristimantis* species exhibited marked SSD, but did not follow Rensch's rule, as it tended to have a bias toward females. Based on two analytical approaches (stepwise phylogenetic regression and model selection based on information theory), we found that the models that best explained body size in males, females, and SSD contained environmental variations in temperature, precipitation, and elevation as predictors. In turn, body size has evolved toward an optimum, with a decelerating rate of evolution differentiated between the large *Pristimantis* clades.

Keywords. Frogs, Neotropics, sexual size dimorphism, trait evolution.

Introduction

Body size is perhaps the most evident trait for most organisms. In some cases, body size correlates with different ecological and evolutionary factors (LaBarbera, 1986; Peters & Peters, 1986; Klingenberg & Spence, 1997; Blanckenhorn, 2000; Sibly & Brown, 2007), which has generated a commonly repeated question: what are the ecological and evolutionary processes that drive body size limits? (Schmidt-Nielsen, 1975; Calder, 1996). This question has been approached from a few different perspectives, including studies on allometry and trends correlated with body size (Gould, 1966), relationships between physiological processes and body size (Kleiber, 1947; Gillooly et al., 2001; Gearty & Payne, 2020), and the processes responsible for evolutionary trends in body size (Maurer et al., 1992; Hone & Benton, 2005).

Similarly, questions related to the interspecific differences in body size, particularly relating to sexual size dimorphism (SSD; i.e., the differences in size between the sexes), continue to be an essential study subject for understanding how the sexes reach different optimal sizes and which ecological and evolutionary mechanisms drive these differences (Reeve & Fairbairn, 2001). Sexual size dimorphism is widespread in different animal groups and is variable even between closely related groups (Blanckenhorn, 2005; Wu et al., 2018). SSD varies in terms of its direction due to differences in the body size of different taxa of vertebrate and invertebrates, with SSD patterns tending to lean towards increased body size in males, but also producing inverse cases where the SSD is female-biased (Cox et al., 2007; Stillwell et al., 2010). This pattern, represented by the covariance between body size and SSD, is known as Rensch's rule (Rensch, 1950; 1960).

The drivers behind the variation in body size and its disparity between males and females have been associated with ecological, life history, and evolutionary factors (Shine, 1989). Therefore, the integration of ecological information (e.g., elevation, temperature, and precipitation) and evolutionary information (e.g., ancestral states and rates of trait evolution) can play a decisive role in understanding the forces driving the variations and evolution of body size between sexes (Ceballos et al., 2013; Amado et al., 2021). For example, Rensch's rule assumes that a larger body size in males is associated with a faster evolutionary rate, whereas a larger body size in females is associated with a slower evolutionary rate (Blanckenhorn et al., 2005; Wu et al., 2018), with a macroevolutionary trend where male-biased SSD drives the direction of SSD between related species (Starostová et al., 2010). However, other studies have rejected Rensch's rule, for example, in studies that have indicated inverse patterns where SSD is female-biased

(e.g., Herczeg et al., 2010; Liao & Chen, 2012; Cooper, 2018). Evolutionary contexts with an isometry between sexes indicate that changes in body size occurred at the same rate and thus equally influenced the evolution of SSD (e.g., Liao & Chen, 2012; Cheng & Kuntner, 2014), whereas evolutionary contexts with mixed SSD patterns resulted in phylogenetic lineages with species associated with a female-biased SSD and others with a male-biased SSD (Webb & Freckleton, 2007; Starostová et al., 2010).

Hypotheses associated with classical ecogeographic rules that relate geographical patterns in the variation of biological traits and environmental gradients are important for understanding interspecific patterns of body size-associated traits at different scales and ecological and evolutionary contexts (Gaston et al., 2008). Climatic variables have been found to be a major driver of broad-scale interspecific body size patterns for different taxonomic groups, including cold-blooded animals (e.g., Olalla-Tárraga & Rodríguez, 2007; Olalla-Tárraga et al., 2009; Gouveia & Correia, 2016; Pincheira-Donoso et al., 2019). In fact, two hypotheses have been recurrent related to body size in amphibians and both have to do with the importance of surface-to-volume ratios on the homeostatic capacity of organisms through its effects on thermoregulation and hydoregulation. According to the water availability hypothesis, larger organisms (i.e., those with a lower surface-to-volume ratio) would be favored in drier environments since they have lower rates of evaporative water loss and, hence, are more resistant to desiccation (Nevo, 1973; Ashton, 2002). In thermoregulating anurans, the heat balance hypothesis suggests that the higher heat retention capacity associated with a lower surface-to-volume ratio would benefit organisms with large body sizes in cold environments (Olalla-Tárraga & Rodríguez, 2007). This hypothesis, analogous to the classic heat conservation

explanation of Bergmann (1847) for size gradients in endotherms, can be extended to ectotherms below a certain body size threshold especially in the presence of compensatory physiological, morphological and/or behavioral mechanisms to reduce heating times (Olalla-Tárraga et al. 2006; Olalla-Tárraga et al., 2009). For example, heating rates are faster in dark ectotherms than in light-colored ones, which is particularly advantageous for the survival of larger species under low ambient temperature conditions (Trullas et al., 2007).

Using an ecological and evolutionary approach, we evaluated the patterns of interspecific variation in body size using the amphibian genus *Pristimantis* as model study. *Pristimantis* is the most diverse amphibian genus on the planet, with 584 known species (Frost, 2021). Therefore, this genus is an excellent group for studying large-scale interspecific variations associated with body size due to its wide latitudinal and altitudinal distribution, from Central America to the Andean and Amazonian areas (Acevedo et al., 2020), and from sea level up to 4,500 m (Heinicke et al., 2007). *Pristimantis* originated in South America during the early Miocene mainly associated with Andean orogenic events (Pinto-Sánchez et al., 2012; Acevedo et al., unpublished). Studies on this genus have mainly been focused on taxonomic, systematic, and biogeographical contexts. However, the patterns related to interspecific variations in body size have only been partially evaluated (Zumel et al., 2021), with the existing information on body size being found across species descriptions, natural history notes, and selected morphological studies.

We used two approaches to investigate body size patterns. First, we collected body size data for all known *Pristimantis* species (584 species) without phylogenetic information. Second we

included phylogenetic information on 257 species for which body size data and molecular information were available for each sex. We analyzed: (a) whether the SSD would conform to Rensch's rule, where SSD should increase with body size when SDD is male-biased; (b) whether male and female body size and SSD are driven by environmental factors, about which we predicted that bioclimatic patterns would be positively associated with variation in body size; (c) whether the body sizes of males and females evolve towards optimal body sizes (Ornstein-Uhlenbeck, OU), fit a neutral evolutionary model (Brownian Movement, BM), or followed adaptive radiation (early burst, EB); and (d) how the evolutionary rates of body size changed throughout evolutionary time with different patterns between males and females, for which we predicted a pattern of deceleration in the body size evolution rate alongside a decrease in both male and female body size.

Materials and methods

Datasets

We collected data on maximum body size, represented by snout-vent length (SVL), for adult males and females of all known *Pristimantis* species (584) described until July 2021. The maximum size was taken as a proxy for the potential size of organisms with indeterminate growth (Hariharan et al., 2016; Levy and Heald, 2016; Amado et al., 2019). We built two databases for developing the two analytical approaches. The first approach focused on ecological analysis without including a molecular phylogeny, the final database included 495 species (85% of the known species of the genus) the remaining 89 species only present data for

one of the sexes, therefore they were excluded (Table S1). For the second database, we used a subset of species on which we performed additional evolutionary analyses. Although there are molecular data available in different public repositories (e.g., Genbank) for approximately 300 *Pristimantis* species, we only included 257 species for which there were molecular data and body size measurements were available for both sexes (Table S2). Body sizes were log-transformed ($\log_{10}$) to reduce the effects on size distribution. The species included in this study represented the entire known distributional range for the genus, spanning from Central America to the Central Andes of Peru and Bolivia. Measurement data were compiled from the original descriptions of each species, field data, and museum records on the review and verification of specimens (Colección Herpetológica del Instituto de Ciencias Naturales, Universidad Nacional de Colombia; Colección Herpetológica de la Universidad de Pamplona; Museo de Historia Natural de la Universidad Distrital Francisco José de Caldas; and the Museo de Historia Natural de la Universidad de los Andes). Measurements were taken using a digital caliper to the nearest 0.01 mm following Watters et al. (2016).

To estimate the SSD for each species and to evaluate Rensch's rule, we used the index proposed by Lovich and Gibbons (1992):

$$\text{SDD} = (\text{size of largest sex} / \text{size of smallest sex}) + 1 \text{ if females are larger}$$

$$\text{SDD} = (\text{size of largest sex} / \text{size of smallest sex}) - 1 \text{ if males are larger}$$

Divergence Time Estimation

The divergence times for the 257 species that were included in the body size matrix were obtained from the concatenated dataset for six partial mitochondrial genes (12S, 16S, CYTB,

COI, ND1, and ND2; Table S3) and two partial nuclear genes (RAG1 and TYR; Table S3). We selected the best partition scheme and the corresponding substitution models using PartitionFinder 2 (Lanfear et al., 2017) with the Bayesian information criterion and the greedy algorithm (Lanfear et al., 2012). We assumed 19 possible partitions through the concatenated data matrix (by genes and codons).

Divergence times were estimated using a relaxed Bayesian clock implemented in BEAST 1.10.4 (Drummond & Rambaut, 2007), using a Yule speciation process (Drummond et al., 2006) with substitution rate variation. The analysis was performed using 100 million generations that were sampled every 1,000 generations. We based our analysis on four previously published divergence times: (a) the divergence time between eleutherodactylines and the South American clades of *Pristimantis*, 36.52 Mya (I.C. = 26.56–50.81) (Heinicke et al., 2007); (b) the most recent common ancestor of *Pristimantis*, 24.45 Mya (I.C. = 17.30–34.82) (Heinicke et al., 2007); (c) the divergence age of the species of *P. pardalis*, 8.6 Mya (I.C. = 5.5–12.0) (Pinto-Sánchez et al., 2012); and (d) the clade age of *P. taeniatus*, 8.3 Mya (I.C. = 5.6–11.2) (Mendoza et al., 2015). The phylogeny was rooted including four outgroups: *Tachiramantis*, *Oreobates*, *Eleutherodactylus*, and *Craugastor* (Heinicke et al., 2007). The trees were visualized using Figtree v.1.3.1 (Rambaut, 2018).

Cross-species analyses

We used a cross-species approach in which each species represented independent data (Olalla-Tárraga et al., 2010). We created an extensive compilation of the latitudinal and longitudinal

coordinates of the 495 species. The geographical occurrence data were obtained through different methods, such as from the Global Biodiversity Information Facility (<https://www.gbif.org>), the scientific literature, a review of biological collections (as done for body size), and our own field data. Records for each species were mapped in QGIS v3.14.16 (Team, 2016) and individually curated to correct georeferencing errors and eliminate erroneous locations. The final database contained 9,237 geographic records (Table S4 includes geographic information for all *Pristimantis* species).

We selected 24 climate variables at a resolution of 1 km², taken from Worldclim (<https://www.worldclim.org>) and ENVIREM (<https://envirem.github.io>). We excluded highly correlated variables from the analyses to minimize multicollinearity (Figure S1, Table S5). The resulting variables were used to test the two main hypotheses: (a) heat balance (James, 1970; Olalla-Tárraga & Rodríguez, 2007): annual mean temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), and temperature seasonality (BIO4); and (b) water availability (Ashton, 2002): annual precipitation (BIO12), precipitation seasonality (BIO15), and moisture index. Additionally, we included altitudinal information to estimate the available habitat formed by the interaction between the topography and macroclimate (Olalla-Tárraga et al., 2009; Hawkins & Diniz-Filho, 2006). We also subdivide the elevation into bands every 1,000 m a.s.l. according to Lynch (1999) and Navas (2002) who propose the elevation limit between Andean anurans and high mountain anurans at 3,000 m a.s.l. For each species, the variables were averaged based on the geographic occurrences (Table S1). The predictors were logarithmically transformed and then scaled to have a mean of zero and unit variance using the 'standardize' package in R (Eager, 2017).

Phylogenetic comparative analyses

We estimated the phylogenetic signal of the residual errors for the body sizes of males and females using the statistic lambda λ (Pagel's λ). When $\lambda = 1$, it signifies a phylogenetic relationship with similar trait patterns between species (Brownian motion model). When $\lambda = 0$, it signifies patterns of trait similarity between species that are independent of phylogeny (non-phylogenetic regression) (Pagel, 1999a; 1999b). The phylogenetic signal tests were implemented in R using the 'phylosig' function of the 'phytools' package (Revell, 2012).

We evaluated three evolutionary models for the body size of males and females: (a) a Brownian motion (BM) model, in which the variation of traits increases proportionally with time at different evolutionary rates (σ^2), following a random walk (Edwards & Cavalli-Sforza, 1964; Felsenstein, 1973); (b) a Ornstein-Uhlenbeck (OU) model, in which traits evolve toward an optimal type (Lande, 1976; Felsenstein, 1988); and (c) an early burst (EB) model, in which the diversification of traits is faster at the beginning of a lineage and slows down towards the present, with the differences between clades being maintained over time (Blomberg et al., 2003; Harmon et al., 2010). Each model was estimated using the 'fitContinuous' function of the 'phytools' package in R, and the best model was subsequently evaluated using the Akaike's weight value (AICw).

We mapped the ancestral states of male and female body size that were transformed to the log10 scale for the internal nodes of the calibrated phylogeny obtained in BEAST. We used the 'contMap' function in the R 'phytools' package (Revell, 2012). This function uses the maximum

probability to estimate the ancestral states, with the trait data for each species at the tips of the tree (Revell, 2013). The function was adjusted to the best evolutionary model for body size.

Bayesian analysis of macroevolutionary mixtures

We performed an analysis in BAMM v. 2.5.0 (Rabosky, 2014) to estimate and quantify the heterogeneity in the evolutionary rates of body size in males and females. BAMM uses reversible-jump Markov chain Monte Carlo (rjMCMC) to choose between models that vary in the number of evolutionary regimes (Rabosky, 2014; Rabosky et al., 2017). The priors for males (poissonRatePrior = 1.0, betaInitPrior = 0.054371, betaShiftPrior = 0.051182) and females (poissonRatePrior = 1.0, betaInitPrior = 0.027243, betaShiftPrior = 0.051182) were estimated using the BAMMtools v. 2.1.7 package (Rabosky et al., 2014) in R before analysis in BAMM. We used a consensus tree obtained previously in BEAST, and the chains were run for 100 million generations with a sampling frequency of 10,000. The first 10% of samples were discarded as burn-in. An effective sample size (ESS) > 200 of the log-likelihood and the number of shift events present in each sample were evaluated using the R package ‘coda’ (Plummer et al., 2006). The 95% credible set of distinct rate-shift configurations (CSSs) was obtained using the BAMMtools v. 2.1.7 package (Rabosky et al., 2014) and visualized on the calibrated tree.

Ecological and evolutionary analysis

To evaluate whether SSD patterns related to the increase, decrease, or isometry of the maximum log₁₀-transformed *Pristimantis* body size, we performed simple linear regressions using the

‘lm’ function in R (R Core Team, 2020) for the database that included all species. We also performed a phylogenetic generalized least squares (PGLS) regression including the calibrated phylogenetic tree. For both analyses, we placed females on the x-axis (Wu et al., 2018). We examined the slope of the regressions of male and female body size according to three scenarios following Fairbairn (1997, 2005): (a) Rensch’s rule, slope greater than one ($b > 1$); (b) inverse of Rensch’s rule, slope less than one ($b < 1$); and (c) isometry, slope equal to one ($b = 1$). Additionally, we performed a simple linear regression comparing the SSD values against the SVL log10 of males and females to evaluate the increase or decrease in SSD according to sex.

To determine the predictors that best explained the interspecific variation in body size, we performed two analyses, one with the database for the 495 *Pristimantis* species (Table S1) and another one including phylogenetic information and male and female body size of 257 species (Table S2). Analyses were performed separately for males, females, and SSD. For the first analysis (495 species), we selected models based on information theory criteria (Visser et al., 2018). We used the R ‘glmulti’ package (Calcagno, 2013), which finds the set of confidence models among all possible models. The best models were found by a genetic algorithm (GA), from which the multi-model average was derived using the ‘coef’ function. The GA incorporates an immigration operator, which allows the eliminated variables to be reconsidered. Immigration increases the level of randomization and, therefore, the probability of convergence of the model to a global optimum rather than a local one (Calcagno & de Mazancourt, 2010; Visser et al., 2018). We obtained the importance of the evaluated predictors, which was equal to the sum of the weights / probabilities of the models in which the variable appeared. Then, a variable with large weights that appeared in a set of candidate models received a high importance value

(Callaghan et al., 2013). We set the parameter level to 1 to include only the main effects, meaning that 2^8 represented the 256 possible models that were generated in the set of considered candidates. We used the function ‘crit = aicc’ (AICc or AIC corrected) to select the model and the multi-model inference, which were based on different predictors, accounted for all possible models that corresponded to different biological hypotheses, and varied in the degree to which they fit the available data (Garamszegi & Mundry, 2014).

The second analysis included a stepwise phylogenetic regression using the calibrated phylogeny and the databases for the 257 species that included the body size of males, females, and SSD in conjunction with the previously defined climatic variables (Table S2). The stepwise search mode was backward elimination starting from the full model, including all independent variables (BIO1 + BIO2 + BIO3 + BIO4 + BIO12 + BIO15 + DEM). The models were compared based on their AIC values.

Results

General pattern of SSD

Maximum body size varied considerably among *Pristimantis*, from 12.6 mm (*P. xeniolum*) to 50.8 mm (*P. labiosus*) in males and 15.3 mm (*P. coronatus*) to 69.3 mm (*P. lymani*) in females (Table S1). Our results showed that all 495 *Pristimantis* species evaluated in this study exhibited marked sexual dimorphism related to body size (Figure 1A). The species with the lowest SSD was *P. jabonensis* (0.003) and the highest was *P. latidiscus* (1.06) (Table S1). On average, SDD

was female-biased with a near-normal distribution (Figure 1B). The linear regression between the body sizes of males and females showed that the allometric slopes were less than 1 ($\beta = 0.83922 \pm 0.04967$, 95% CI = 0.8027025–0.8757443; $R^2 = 0.80$, $F_{1,493} = 2038$, $P < 0.001$; Figure 2A); this variation in SSD did not follow Rensch's rule. Additionally, there is no clear pattern with the species grouped by altitudinal bands (Figure 2A). Phylogenetically corrected analyses also showed a positive correlation between male and female sizes ($R^2 = 0.72$, $F_{1,255} = 686.4$, $P < 0.0001$). The relationship between female body size and SDD showed a positive regression ($\beta = 0.50318 \pm 0.1581$, 95% CI = 0.3869339–0.6194305; $R^2 = 0.12$, $F_{1,493} = 72.33$, $P < 0.001$) with an increase in SDD as the female body size increased, indicating a reversed Rensch's rule (Figure 2B).

Environmental predictors of body size

Our analyses (with and without phylogeny) found significant patterns that showed that SSD and body size of males and females were associated with climatic variation related to the heat balance and water availability hypotheses. SSD analysis conducted by selecting models based on information theory identified temperature-associated variables (BIO1: annual mean temperature; BIO4: temperature seasonality) as more relevant to explain SSD patterns in *Pristimantis*, with a greater relative importance of 0.99 for BIO1 (Figure 3A; Tables 1 and S6). For male body size, the best model included variables associated with precipitation (BIO15: precipitation seasonality), elevation (available habitat), and temperature (BIO1), with relative importance values of 0.99, 0.78, and 0.73, respectively (Figure 3B; Tables 1 and S6). For females, model selection yielded a similar result, but included BIO4 (temperature seasonality)

instead of BIO1. However, the relative importance values gave more weight to precipitation (BIO15) and elevation, with 0.98 and 0.81, respectively (Figure 3C; Tables 1 and S6). Based on the stepwise phylogenetic regression, the candidate model that best fitted the body sizes of males and females incorporated the variables BIO1, BIO2, BIO4, and altitude; for SSD, the best model included the variables BIO3 and BIO15 (Table 2).

The evolution of body size in *Pristimantis*

Ancestral state reconstruction

There were significant phylogenetic signals for the body size of males ($\lambda = 0.85$, $P < 0.001$) and females ($\lambda = 0.86$, $P < 0.001$). Evolutionary model selection showed that the OU model was the one that best fit the body size data in males (AIC: -520.9069) and females (AIC: -490.6076). Maximum likelihood continuous-character ancestral reconstruction for the maximum body size and fitting for the OU evolutionary model yielded an internal node value of 36.23 mm for females (95% CI: 28.36–44.10) (Figure 4A), while for males it was 26.97 (95% CI: 21.42–32.51) (Figure 4B). The general pattern showed that most of the body sizes among the different clades were close to the average size (female = 33.78, male = 25.19, SSD = 0.34) (Table 3).

Evolutionary rate of body size

Bayesian analysis of macroevolutionary mixtures (BAMM) supported changes in the evolution of body size, with the rates for males and females having a marginal probability of 0.988 (>

95% of all models sampled from the post-burn-in chain). A regimen change located at node 98 (20 Mya) between clades G1, G2, and G3 occurred in males and females. Regime change was associated with a reversal in the rate of body size evolution; thus, within these clades, the rates tended to decelerate over time (Figure 5A, B; Table S7). In turn, males and females showed different patterns in the rates of body size evolution, where females had higher evolutionary rates than males, and for both sexes there was evidence of decelerating rate of body size evolution (Figure 6A, B; Table S7).

Discussion

Body size of *Pristimantis* species ranged from 12.6 mm to 50.8 mm for males and between 15.3 mm and 73 mm for females. A recent study by Zumel et al. (2021) reported *P. nanus* (not included in this study) as the smallest species, with males being only 12.46 mm. This new species is part of the *P. trachyblepharis* species group, which is a group of micro-endemic taxa distributed in south-central Ecuador and northern Peru (Zumel et al., 2021). Our analyses did not identify any clades with a tendency to miniaturize, as size patterns varied within each clade (Figure 7, Figure S2). This implies that the species within each clade present multiple selective pressures that have determined the wide ranges of body size in *Pristimantis*.

Pristimantis body size patterns exhibited marked SSDs. However, the genus conforms to a female-biased inverse Rensch rule ($b < 1$). This is consistent with previously documented patterns for the three amphibian orders (Pincheira-Donoso et al., 2021). This deviation of SSD towards females would be related to fecundity selection, which describes the fitness advantages

that result from the selection of traits that increase the number of offspring per reproductive period (Woolbright, 1983; Roff, 2002; Nali et al., 2014; Pincheira-Donoso et al., 2021). However, despite knowing that all *Pristimantis* species have direct egg development, the clutch size of most species is unknown, although estimates for a few species have been between 30–38 eggs per clutch (Hill et al., 2010; Rojas-Rivera et al., 2011; Granados-Pérez & Ramirez-Pinilla, 2020). Other explanations have attributed the variations in body size between males and females to the energy requirements associated with reproductive behavior. In males, investment is usually related to territorial defense, agonistic, and advertising behavior, whereas females invest more in body growth (Woolbright, 1983). Accordingly, variations in body size have several ecological and evolutionary implications. For example, larger amphibians tend to require lower metabolic rates for maintaining their temperature compared to smaller amphibians (Levy & Heald, 2016). However, our results suggest that environmental conditions associated with temperature are decisive for body size differences between males and females. SSD in *Pristimantis* is driven by temperature seasonality (BIO4), which is a measure of temperature change over the year (O'Donnell & Ignizio, 2012), and annual mean temperature (BIO1), which is the average maximum and minimum temperature for the warmest and coldest months, respectively (O'Donnell & Ignizio, 2012). Previous studies have shown that temperature seasonality is related to body size in amphibians, and this relationship would be associated with the phenomenon that shapes latitudinal gradients (Valenzuela-Sánchez et al., 2015; Pincheira-Donoso et al., 2021). However, over 60% of *Pristimantis* species are distributed in Andean areas, where local temperature variations tend to be relatively stable (Parsons, 1982). Therefore, the annual mean temperature may be more related to SSD patterns in *Pristimantis*, especially because this variable presented an importance of 0.99. However, around 18% (90) of the species

are restricted to the páramo zone (> 3000 m a.s.l.), which includes more fluctuating thermal landscapes that can influence the physiological patterns relevant to body size differentiation (Navas et al., 2013) that varies between 12 and 50 mm.

The climatic predictors that best explain body size for each sex are precipitation seasonality and elevation. Although mean annual precipitation is used most often to describe different ecological relationships, the dynamic of temporal variation in precipitation under different geographical settings can influence ecosystem responses and the morphological trait patterns of species (Swemmer et al., 2007). Additionally, elevation also drives body size in *Pristimantis* males and females, possibly due to the habitat availability hypothesis, which predicts larger body sizes in lowlands relative to mountains (Olalla-Tárraga et al., 2009). However, *Pristimantis* does not exhibit a clear body size pattern across elevational gradients, neither with a trend toward decreasing nor one toward increasing body size. This may be due to the broad Andean bioclimatic landscapes that are driven by topographic heterogeneity between flanks and mountain ranges (Kattan et al., 2004).

Evolution of Pristimantis body size

The phylogenetic signal of body size for males and females was high (Pagel's $\lambda > 0.85$), which supports the findings previously reported by Zumel et al. (2021) and those reported for amphibians in general (Babich et al., 2021). However, for other neotropical amphibians, such as the genus *Boana* which has a similar distribution to *Pristimantis*, a low phylogenetic signal for species has been reported, having large variations in body size with random patterns of trait

variation (Gouveia and Correia, 2016). Nevertheless, the high phylogenetic signal found for *Pristimantis* males and females indicates that the evolutionary patterns associated with body size are narrow, with decreasing biological similarity as the evolutionary distance between species increases (Revell et al., 2008; Kamilar & Cooper, 2013). Body size conforms to an OU evolutionary model, which evolves toward an optimal ecophysiological phenotype (Lande, 1976; Felsenstein, 1988), instead of an incremental change model (Brownian motion) or adaptive radiation (early burst). Under a macroevolutionary framework, Landis and Schraiber (2017) found that clades of modern vertebrates fit better through pulsed processes (i.e., Lévy jump processes) than with other evolutionary models. However, for the amphibian superfamily Hyloidea, which includes *Pristimantis* (Landis & Schraiber, 2017), no particular model was selected because there was ambiguity among the evaluated models. However, other studies have also supported OU as the evolutionary model associated with body size in amphibians (Babich et al., 2021) and turtles (Ceballos et al., 2013). This trend towards an optimum can be explained by peaks in an adaptive landscape, where there have been a large number of selective demands (genetic and environmental) for body size on a macroevolutionary time scale (Hansen, 1997; Butler & King, 2004).

Evolution rate of body size

Body size evolution rates for male and female *Pristimantis* suggested a regimen change associated with progressive rate deceleration, with a break between clades G1–G2 and G3 (Figure 5A, B; Table 3). Changes in body size evolution rates of *Pristimantis* may be associated with historical biogeographic patterns that have shaped its diversification patterns. Clades G1–

G2 showed the highest ranges of body size evolution rates, between 5.2750–5.6506 for males and 9.0240–11.1643 for females (Table 3). These early clades consist mainly of species from the ecoregions of the Northwestern Andes ecoregions, which have been suggested as a possible center of origin of *Pristimantis* (Mendoza et al., 2015; Acevedo et al., unpublished). Subsequent dispersal patterns with the colonization of ecoregions such as Chocó, Amazonia, the Colombian Eastern Andes, Central Andes, the Venezuelan Andes, the Atlantic Forest, Northern South American montane coastal areas, and the Guiana Shield have shaped clades G1–G2 (Acevedo et al., unpublished). Clade G3 (Figure 5A, B; Table 3) exhibited evolution rates with a tendency to slow down between 3.2704–3.0947 for males and 6.9802–6.4955 for females (Table 3). Clade G3 comprises species from the Northwestern Andes, the Colombian Eastern Andes, Amazonia, Central Andes, Central America, and the Guiana Shield. The deceleration pattern is explained by the trend towards an evolutionary optimum in body size that *Pristimantis* species have experienced, where forces acted to reduce phenotypic variation. In contrast, the initial acceleration may be the response of the ancestral lineages to some selective pressure experienced in the early stages of genus diversification. This is supported by the OU evolutionary model found for *Pristimantis*.

Females had higher evolutionary rates than males. For both sexes, there was a pattern of deceleration in the rates of body size evolution (Figure 6A, B), and this was associated with reduced body size (Figure 6C). This finding is in contrast with patterns found in mammals, for example, where the deceleration body size evolution rates is associated with an increase in size (Baker et al., 2015). However, at the macroevolutionary level for different amphibian groups, the patterns of the evolutionary rates of traits associated with body size are not yet clear.

Therefore, future studies must test ecophysiological hypotheses, niches, and other ecological attributes to obtain a general understanding of the changes in the patterns of body size evolution rates. Our results provide an approach for understanding the relationship between body size and its evolutionary rates in the world's most diverse amphibian genus.

In conclusion, our results support the evolution of body size and SSD variation in *Pristimantis*, suggesting that environmental variables can drive and/or maintain the degree of body size divergence between sexes. The rates of body size evolution show a deceleration over time with a slight increase in body size. In turn, body size trends toward optimal evolution. These results contribute to knowing the factors involved in body size and its evolution, helping to generate future hypotheses related to sexual and natural selection or macroevolution tendency in SSD.

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Data availability statement

The data that support the findings of this study are openly available in electronic supplementary information and in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.xgxd254hk>, <https://doi.org/10.5061/dryad.f7m0cfxxq>, <https://doi.org/10.5061/dryad.80gb5mkrx>.

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Tables

Table 1. Selecting models based on information theory indicating the combination of environmental variables for males, females, and SSD. Only the best models of 256 possible models are shown.

Multivariate models	β	Std. Error	95% CI (lower, upper)	Importance	AICc
SDI					
BIO1	0.0434	0.0113	(0.0212, 0.0656)	0.9980	-379.7360
BIO4	-0.0079	0.0101	(-0.0277, -0.0119)	0.5372	
SVL Males					
BIO15	0.0199	0.0053	(0.0096, 0.0302)	0.9970	-770.9435
DEM	-0.0156	0.0118	(-0.0386, 0.0075)	0.7814	
BIO1	-0.0138	0.0116	(-0.0365, 0.0089)	0.7360	
SVL Females					
BIO15	0.0180	0.0057	(0.0068, 0.0292)	0.9868	-702.0437
DEM	-0.0132	0.0089	(-0.0306, 0.0043)	0.8154	
BIO4	0.0030	0.0053	(-0.0074, 0.0135)	0.4093	

Table 2. Best model by stepwise phylogenetic regression for males, females and SSD indicated by the AICc value.

GLM models + phylogenetic tree	β	AICc
SDI		
BIO3	0.02712334	-174.21
BIO15	0.01941823	
Males		
Bio1	-0.02222096	-503.39
Bio2	0.01660305	
Bio4	-0.01595270	
DEM	-0.02089806	
Females		
Bio1	-0.01591907	-479.2
Bio2	0.01979775	
Bio4	-0.02465312	
DEM	-0.01612496	

Table 3. The major phylogenetic clades associated with body size ancestral states, body size evolution rates, age of nodes and ecoregions for males, females, and SSD.

Clade	Body size ancestral states			Body size evolution rates		Age of the Major clade (Ma)	Ecoregion
	Male	Female	SSD	Male	Female		
G1	28.53, 95% CI: 22.74-34.33	38.46, 95% CI: 30.24-46.69	0.35, 95% CI: 0.16-0.54	5.6506	9.8752	19.872	Northwestern Andes, Chocó, Central America, Amazonia, Colombian Eastern Andes.
G2a	26.14, 95% CI: 21.06-31.23	35.38, 95% CI: 28.17 - 42.59	0.35, 95% CI: 0.19-0.52	5.2750	9.0240	18.532	Central Andes, Northwestern Andes, Venezuelan Andes, Chocó, Amazonia.
G2b	26.75, 95% CI: 22.08-31.43	36.73, 95% CI: 30.10-43.37	0.37, 95% CI: 0.22-0.53	5.4816	11.1643	18.014	Chocó, Amazonia, Colombian Eastern Andes, Guiana Shield, Central Andes, Atlantic Forest, Northern Sudamerican montane coastal.
G3a	23.70, 95% CI: 18.67-28.72	31.57, 95% CI: 24.44-38.70	0.33, 95% CI: 0.17-0.49	3.2704	6.9802	16.684	Northwestern Andes, Colombian Eastern Andes.
G3b	24.74, 95% CI: 20.55-28.92	32.71, 95% CI: 26.76-38.65	0.32, 95% CI: 0.19-0.46	3.0947	6.4955	17.355	Northwestern Andes, Amazonia, Central Andes, Central America, Guiana Shield, Colombian Eastern Andes, Chocó, Venezuelan Andes.

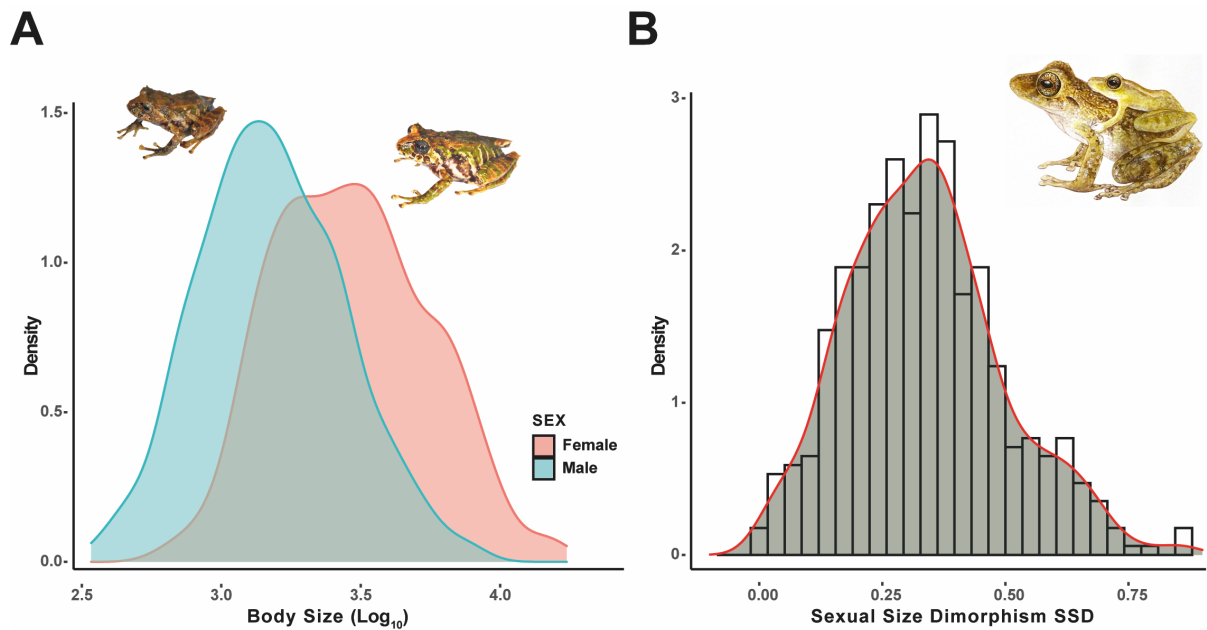


Fig 1. A. Frequency distribution of the log10 maximum body size for males and females. B. Sexual size dimorphism with a fit of normal distribution curve. Images of *Pristimantis scoloblepharus* (Photos: Rivera, M.)

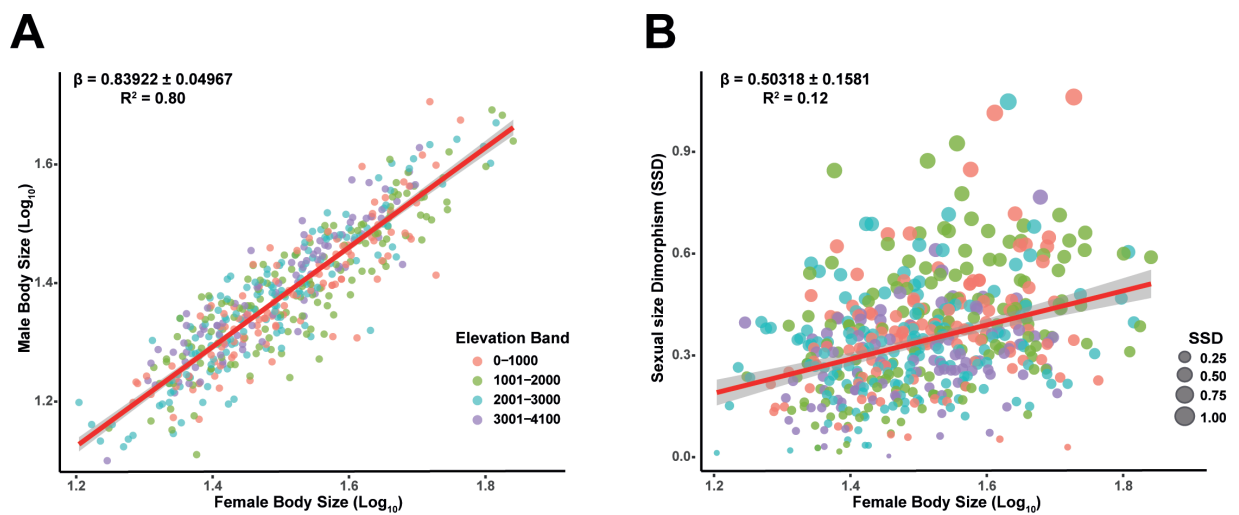


Fig 2. A. Linear regression with 95% confidence interval. B: SSD-size relationship with female-biased SSD vs. log10 body size of females, the gray circles represent the SSD increased by 0.25 mm. Each dot represents one species based on the maximum body size of males and females. The colors of each point represent the altitudinal band divided every 1000 m a.s.l.

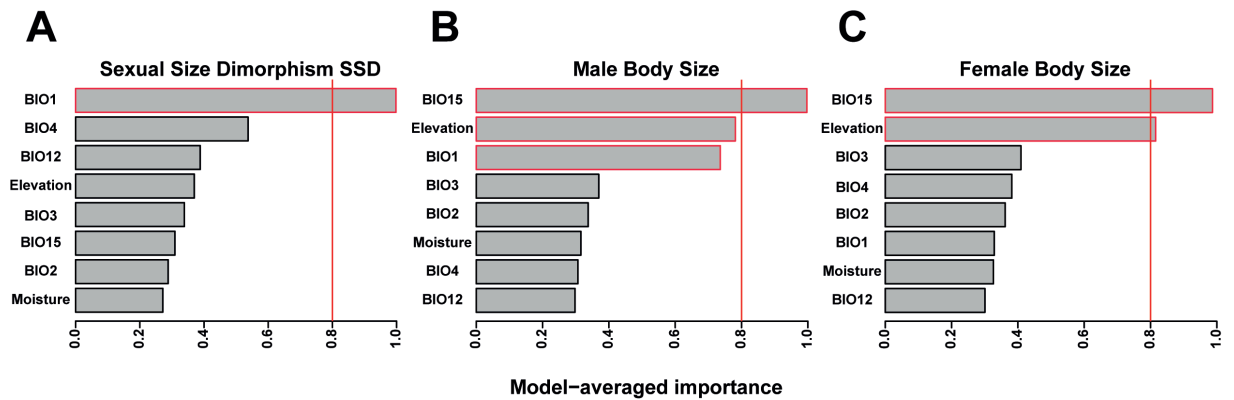


Fig 3. Relative importance of environmental variables according to the selection of models based on information theory. A. Variables selected for SSD. B. Variables selected for males. C. Variables selected for females.

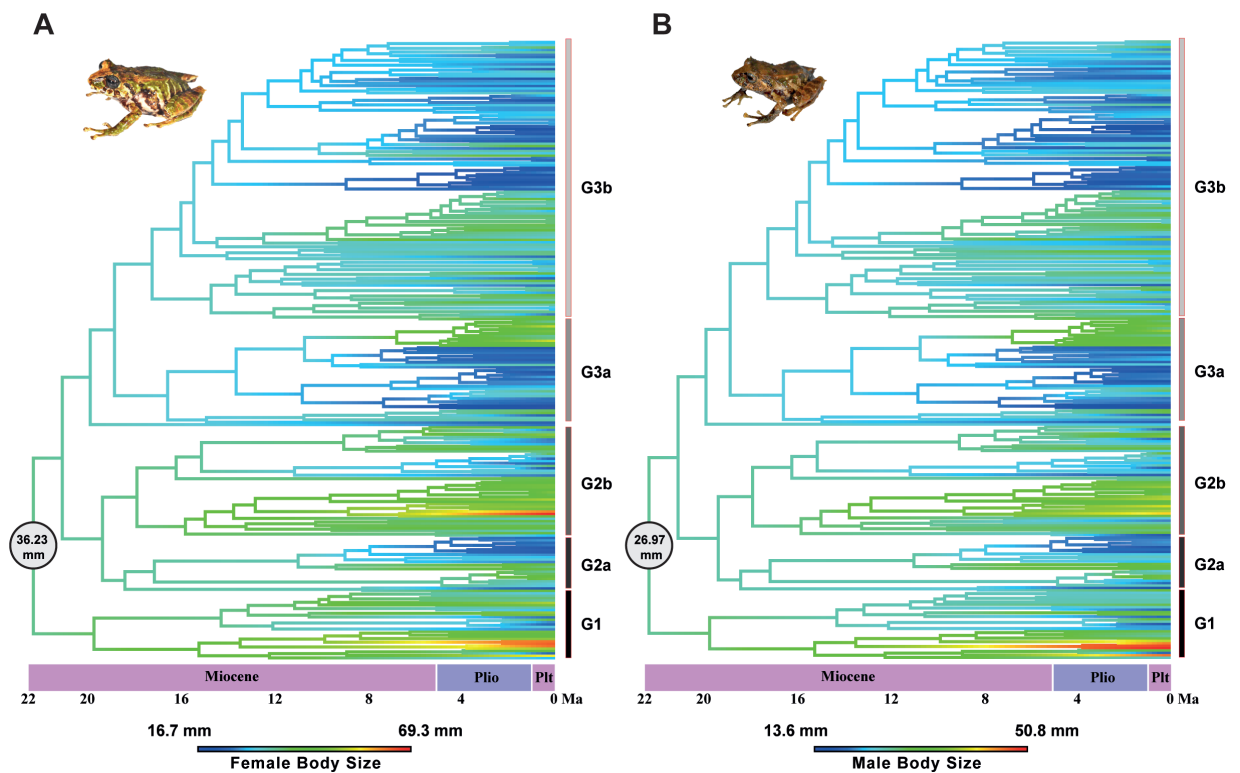


Fig 4. Maximum likelihood continuous-character ancestral reconstruction for the maximum body size for males (A) and females (B) and fitting for the OU evolutionary model. The horizontal bar indicates the corresponding clade. The color gradient indicates the body size range. Images of *Pristimantis scoloblepharus* (Photos: Rivera, M.)

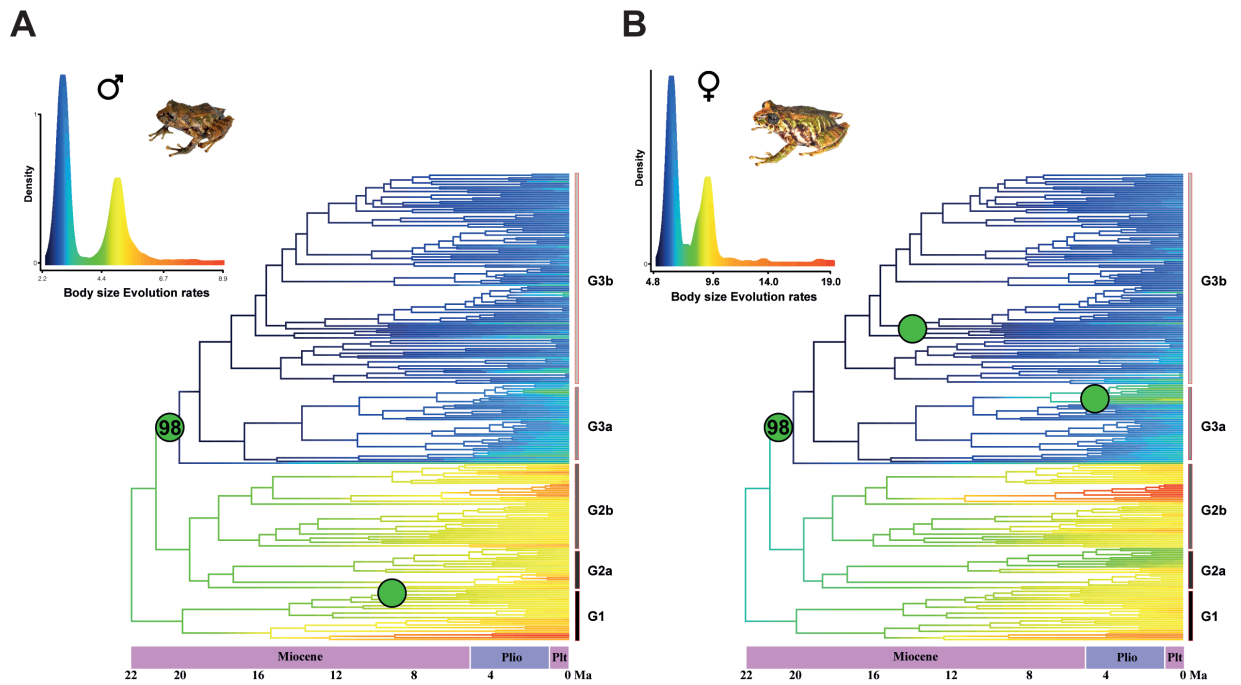


Fig 5. Bayesian analysis of macroevolutionary mixtures (BAMM) for males and females showing changes in body size evolution rates (green dots) for males (A) and females (B). Top of each phylogenetic tree with the histogram represented by the density of body size evolution rates. The horizontal bar indicates the corresponding clade. Images of *Pristimantis scoloblepharus* (Photos: Rivera, M.)

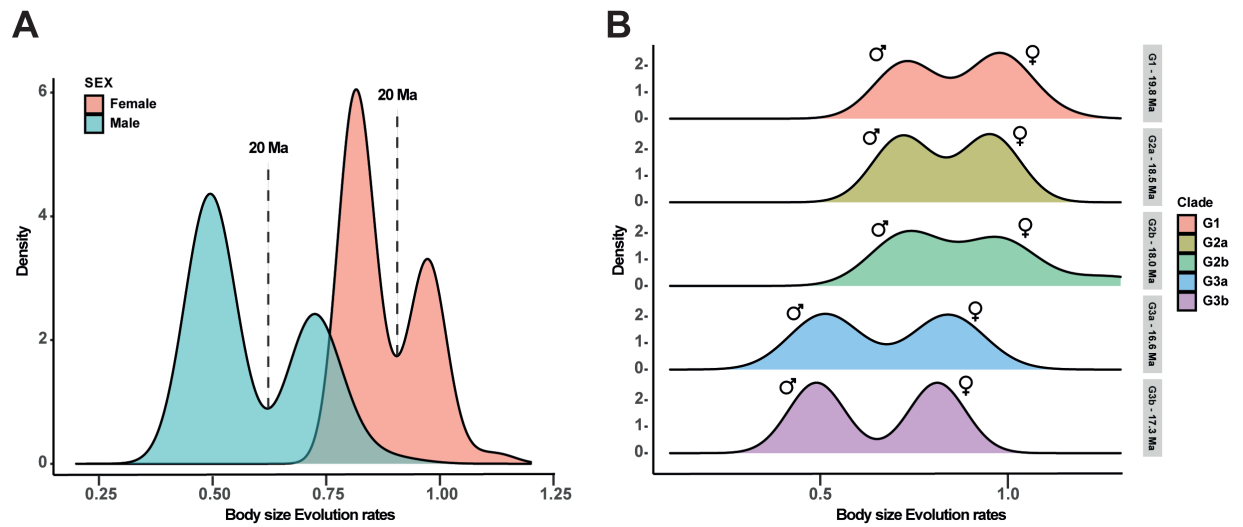


Fig 6. A. Histogram showing the density of body size evolution rates for males and females. Dotted line indicates the age (Ma) that represents the change in the rate regime (Figure 5). B. Histograms by phylogenetic clades showing the density of body size evolution rates for males and females, the horizontal bars indicate the clade with its age (Ma)

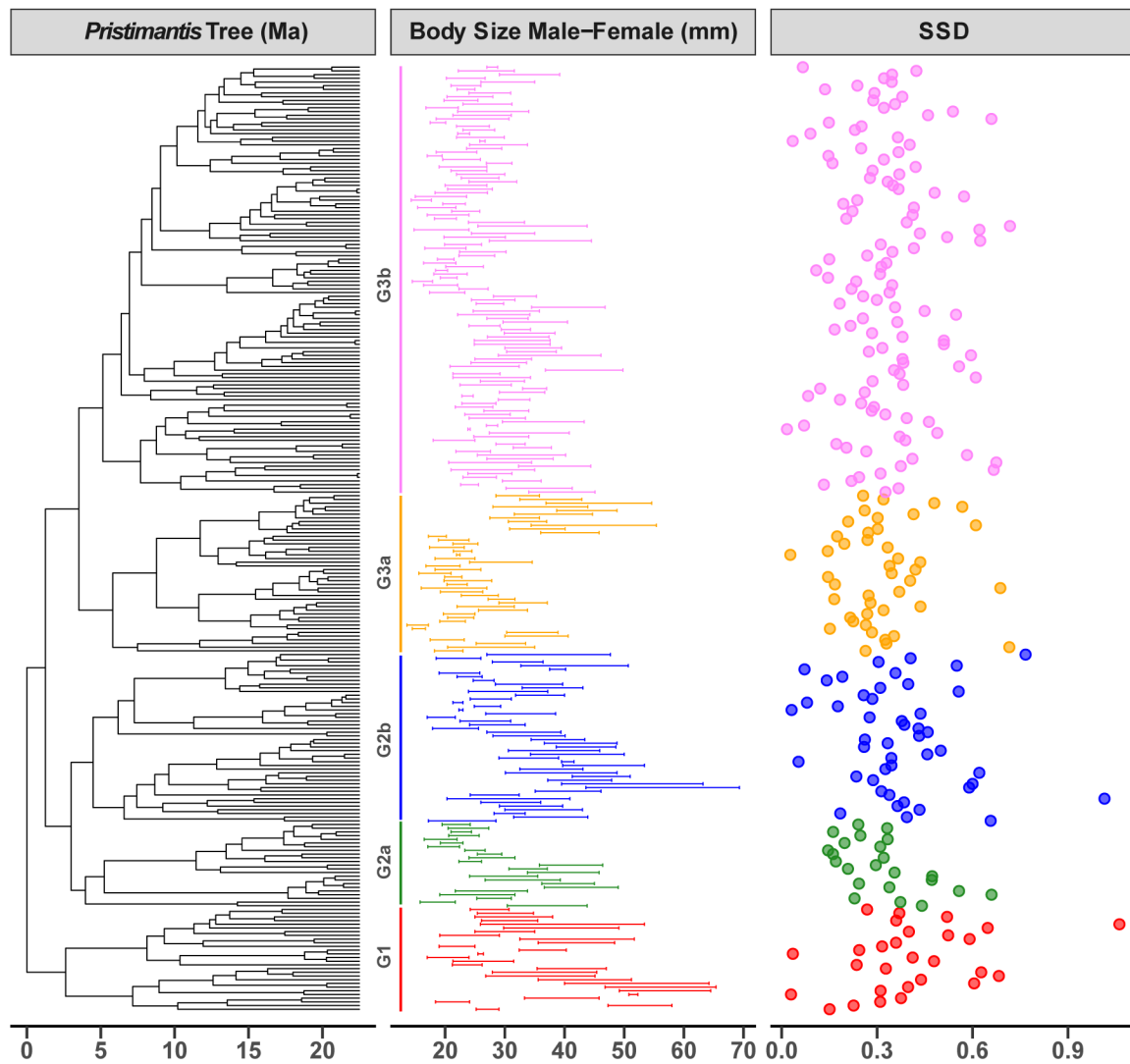


Fig 7. Calibrated phylogeny of *Pristimantis* with the range of body size for males and females and SSD. Each color represents a clade.

Supplementary information

Table S1. Database for 495 species of *Pristimantis* with body size of males and females (mm), SSD and climatic variables scaled. Dryad Digital Repository:

<https://doi.org/10.5061/dryad.xgxd254hk>.

Table S2. Database for 257 species of *Pristimantis* for evolutionary analyzes. Body size of males and females (mm), SSD and climatic variables scaled.

Species	SVL Max Males	SVL Max Females	SSD	Bands	LOG Moistur e	Log DEM	Log BIO1	Log BIO2	Log BIO3	Log BIO4	Log BIO12	Log BIO15
<i>P. abakapa</i>	21.1	25.8	0.22274 8815	2001- 3000	0.72540 9412	0.47968 1477	0.45361 5793	0.53568 3246	0.52207 7245	0.77757 9905	0.63498 2663	1.06985 219
<i>P. acatallelus</i>	27.2	31.7	0.16544 1176	2001- 3000	0.77679 7172	0.42158 5219	0.07335 7403	0.82888 0601	0.97718 9998	1.14208 7092	0.57582 5884	0.46139 6744
<i>P. acerus</i>	34	45.1	0.32647 0588	2001- 3000	1.13018 8891	0.75867 6518	1.09017 4011	1.42804 1968	0.52733 568	0.06534 6339	0.67247 6151	2.02576 4458
<i>P. achatinus</i>	35.1	46.1	0.31339 0313	1001- 2000	0.33541 7512	0.27971 2148	0.72410 0678	0.60726 3492	0.36341 8395	0.02164 4381	0.66667 8421	0.64865 8285
<i>P. achuar</i>	16.6	23.5	0.41566 2651	0- 1000	0.62929 2121	2.03335 7464	1.30092 9341	0.36712 9087	1.04175 2223	0.58947 0073	1.49854 2279	1.92320 8307
<i>P. actites</i>	40	64.2	0.605 0.605	2001- 3000	0.38331 3763	0.51983 3709	0.33662 6197	0.10028 7715	0.52578 8107	0.58400 6779	0.75093 1152	1.27633 6607
<i>P. acuminatus</i>	24.01	33.45	0.39316 9513	0- 1000	0.77220 4343	1.91985 7225	1.26697 7464	0.65715 3794	1.17027 7821	0.49760 8484	1.63743 6462	1.86565 918
<i>P. affinis ANDES-A 4217</i>	27	47.7	0.76666 6667	3001- 4100	0.28204 1282	0.85269 49	0.00054 82	1.38417 0348	0.56963 5487	0.20960 4018	0.06325 9697	0.51352 7522
<i>P. afrox</i>	33.3	45.8	0.37537 5375	0- 1000	1.09094 6198	2.22315 6694	1.27832 5581	0.95201 4215	0.77518 1603	0.78446 5395	2.06019 6684	0.92135 0835
<i>P. albertus</i>	19.5	24.2	0.24102 5641	1001- 2000	1.42509 7625	0.29771 351	0.28889 1382	1.87145 8213	0.26044 941	1.43421 4567	0.28454 459	0.47229 6374
<i>P. altae</i>	21	27	0.28571 4286	0- 1000	0.93183 8526	0.52997 1478	0.84987 5661	0.66778 7581	1.51057 527	1.45255 6742	1.63956 5493	0.02874 1626

					-	-	-	-	-	-	-	-
<i>P. altamazonicus</i>	19.8	30.1	0.52020 0- 202 1000	0.50682 2.00132 7572 3585	1.30399 0.26533 6416 1029	0.95398 0.78552 099 131	1.39296 1.04814 2114 503					
<i>P. altamnis</i>	22.3	28.3	0.26905 0- 8296 1000	0.84826 1.26789 3946 7306	1.13862 0.85324 0077 6009	0.37505 0.01437 404 8968	1.56729 1.67785 1359 551					
<i>P. andinognomus</i>	14.5	17.9	0.23448 2001- 2759 3000	1.64790 0.61288 4302 6189	0.17788 0.40750 4399 2908	0.36424 0.57187 5494 5846	0.62442 0.07792 5072 5278					
<i>P. angustilineatus</i>	20.4	24.8	0.21568 2001- 6275 3000	0.90180 0.35296 5628 3601	0.02149 1.09410 7712 7719	0.97370 1.06976 3894 9539	0.65939 0.62756 3391 0888					
<i>P. aniptopalmatus</i>	16.5	22	0.33333 2001- 3333 3000	0.01353 0.62622 2323 7832	0.12578 2.04175 6031 0476	0.11582 1.12859 4415 0062	1.12915 0.65256 7205 5729					
<i>P. anolirex</i> <i>MCNUPH66</i>	31.8	40	0.25786 3001- 1635 4100	0.76509 0.85382 3848 8283	1.41427 1.58341 8684 7702	0.63852 0.77767 9053 42	0.81333 0.94622 3803 3001					
<i>P. antisuyu</i>	15	23.6	0.57333 1001- 3333 2000	0.68758 0.14781 4716 3922	0.45574 1.55224 632 217	1.22341 1.65977 022 7184	0.07278 0.40252 9168 1905					
<i>P. appendiculatus</i>	21	35	0.66666 1001- 6667 2000	0.16563 0.26244 3339 6208	0.07697 0.68797 3892 0263	1.07369 1.22829 636 2341	0.32643 0.91492 6953 8004					
<i>P. ardalonychus</i>	21.9	27.4	0.25114 0- 1553 1000	1.03929 0.71046 2874 6951	1.12920 1.17142 4138 6422	0.46216 0.03339 2676 4659	0.40511 1.26700 593 6091					
<i>P. ardilae</i> <i>MCNUPH117</i>	29.1	39.7	0.36426 0- 1168 1000	0.98747 0.77276 6149 3506	0.92910 0.22894 7832 6486	0.16987 0.56468 6976 221	0.48762 0.54110 6461 9332					
<i>P. atillo</i>	28.1	35.3	0.25622 3001- 7758 4100	3.63156 0.97859 9915 1733	2.12160 0.35025 8285 2766	0.16855 0.29036 9262 2111	0.59269 0.95074 3279 488					
<i>P. atratus</i>	24	29.2	0.21666 2001- 6667 3000	0.60842 0.74695 8937 4135	0.61112 0.67413 3131 3099	0.26046 0.85124 7541 9795	0.99288 0.43966 0437 8603					
<i>P. attenboroughi</i>	19.2	23	0.19791 3001- 6667 4100	0.49786 1.08453 3592 7678	1.35008 2.90892 2163 8873	0.54380 0.83150 2336 8792	1.14386 0.85423 0503 4165					

								-	-			
<i>P. aureoventris</i>	22.7	29	0.27753 304	1001- 2000	0.36857 2135	0.26278 27	0.40414 8329	0.20100 0632	0.46069 7009	0.42289 8344	0.10019 0888	0.79222 7806
					-	-	-	-	-	-	-	-
<i>P. bambu</i>	20.1	26.4	0.31343 2836	2001- 3000	0.06339 0847	0.79682 6129	0.86296 486	0.67127 3507	0.71262 6747	0.03459 5053	1.40896 3403	1.63792 5985
					-	-	-	-	-	-	-	-
<i>P. bicantus</i>	15.8	21.7	0.37341 7722	2001- 3000	0.50930 6702	0.38552 2781	0.00512 1069	0.54584 7396	0.89998 1367	0.44192 8444	0.65259 8846	0.81332 1039
<i>P. bicolor ANDES-A 4939</i>	29.1	39.2	0.34707 9038	1001- 2000	0.00876 3768	0.01067 67	0.63903 8624	0.50503 5846	0.70550 2595	0.46521 2042	0.30233 7845	0.18491 3108
					-	-	-	-	-	-	-	-
<i>P. boulengeri</i>	25.6	33.8	0.32031 25	2001- 3000	0.16483 9733	0.72143 4056	0.93260 409	0.94582 9968	1.43680 9303	1.90164 8858	0.15948 3447	0.00244 7129
					-	-	-	-	-	-	-	-
<i>P. bounides</i>	21	24.4	0.16190 4762	3001- 4100	0.32866 4231	1.01611 5424	1.24917 8909	2.65873 2217	0.18670 5612	0.89845 9297	1.19605 6626	0.88886 4013
<i>P. bowara MCNUPH160</i>	28.4	39.7	0.39788 7324	0- 1000	0.98485 2308	0.72921 12	0.90143 4874	0.26652 0371	0.18114 0135	0.53556 7473	0.60567 7627	0.51644 673
					-	-	-	-	-	-	-	-
<i>P. brevicrus</i>	24.4	35	0.43442 623	0- 1000	0.86985 4288	1.45929 4431	1.18350 4866	0.72597 5382	1.29956 9547	0.54950 724	1.74474 1735	1.64548 1959
					-	-	-	-	-	-	-	-
<i>P. brevifrons</i>	19.7	25	0.26903 5533	2001- 3000	0.29588 1075	0.38292 6239	0.02005 868	0.63515 5738	1.14650 2939	1.30445 4442	0.25087 4464	0.32586 4545
					-	-	-	-	-	-	-	-
<i>P. briceni</i>	36.2	45	0.24309 3923	3001- 4100	0.22486 6478	0.93714 5972	1.38301 2856	1.21832 4562	0.31698 7495	0.63765 5342	1.30958 1905	0.73602 9898
					-	-	-	-	-	-	-	-
<i>P. bromeliaceus</i>	22.8	28.5	2001- 0.25	3000	0.34415 5449	0.39906 4142	0.02103 0334	0.84353 5632	0.20705 7962	0.21388 745	0.73445 5943	0.87503 1111
					-	-	-	-	-	-	-	-
<i>P. buckleyi</i>	38.7	48.8	0.26098 1912	3001- 4100	0.34615 8149	0.88945 5195	1.56466 1136	0.12416 7873	0.42656 9342	0.12834 9389	0.25820 9266	1.02241 9457
					-	-	-	-	-	-	-	-
<i>P. buenaventura</i>	21.9	29.9	0.36529 6804	0- 1000	0.05327 3027	1.09948 2578	1.12208 1653	0.99570 7289	0.34230 7232	0.76436 4329	1.59431 9862	2.13131 2473

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<i>P. cajamarcensis</i>	24.1	33.8	0.40248 9627	2001- 3000	0.12600 7509	0.53807 5129	0.13078 6072	1.25591 5078	0.33257 8285	0.34709 2039	1.39005 8532	0.83489 9751
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<i>P. cajanuma</i>	16.4	22.1	0.34756 0976	3001- 4100	0.96380 8213	0.83006 5728	1.13331 8782	0.59129 5454	0.09291 2842	0.23614 6447	1.05769 6756	0.21883 1933
					-		-			-		-
<i>P. calcaratus</i>	17	24	0.41176 4706	2001- 3000	0.00565 7755	0.39402 1285	0.00625 2555	0.59749 1226	1.19714 8667	1.34903 4975	0.18896 7616	0.21101 7309
					-		-			-		-
<i>P. calcarulatus</i>	23.8	31.2	0.31092 437	1001- 2000	0.28146 4156	0.24423 2213	0.12192 4221	0.74459 2422	1.06395 2521	1.15250 0808	0.45928 3786	0.98369 2892
					-		-			-		-
<i>P. capitonis</i>	22.7	28.9	0.27312 7753	2001- 3000	0.40513 3191	0.59984 8085	0.56223 6635	0.65613 2513	1.31186 6142	1.48391 2614	0.05209 5998	0.20969 3369
					-		-			-		-
<i>P. caprifer</i>	30.4	43.8	0.44078 9474	0- 1000	0.55856 7988	1.20872 8625	1.14052 6	0.67044 8442	0.73602 8382	0.75809 5661	1.61203 4686	0.01720 2038
<i>P. carrangerorum ANDES-A 1254</i>	32.7	50.7	0.55045 8716	1001- 2000	1.00363 2851	0.08744 0916	0.71621 6705	0.43255 431	0.62742 7498	0.79121 0613	0.91019 9548	0.76135 7889
					-		-			-		-
<i>P. carvalhoi</i>	14.8	24	0.62162 1622	0- 1000	0.02620 8384	1.74188 8623	1.31807 382	0.14527 6369	0.87607 7334	0.98617 9604	0.95477 981	0.24737 3186
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<i>P. caryophyllaceus</i>	21.2	26.2	0.23584 9057	0- 1000	0.87508 0613	0.46536 9635	0.76798 4272	0.37271 5919	0.95314 1462	1.18227 6058	1.53376 2804	0.13720 6273
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<i>P. cedros</i>	23	28.6	0.24347 8261	1001- 2000	0.47545 4157	0.19022 3674	0.24431 1346	0.81123 2603	0.46548 9214	0.64769 6244	0.30146 1249	0.92917 9188
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<i>P. celator</i>	21.4	24.5	0.14485 9813	2001- 3000	1.73101 8132	0.62667 7389	0.60857 8112	0.03409 2992	1.01996 8548	0.86481 4903	0.75018 2205	0.55500 0406
					-		-			-		-
<i>P. cerasinus</i>	25	35	0- 0.4	1000	0.73154 7387	1.61721 3495	1.21134 8327	0.97231 885	2.11347 101	1.72415 382	1.34017 9985	0.30423 884
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<i>P. ceuthospilus</i>	25.8	26.7	0.03488 3721	2001- 3000	0.23914 0392	0.71600 592	0.52445 5982	0.66300 0635	0.66043 1875	1.74476 4915	1.43218 459	1.21116 5364

<i>P. cf batrachites</i>					-		-	-	-	-	-	-
<i>MCNUPH37</i>	17	21.7	0.27647 0588	2001- 3000	1.17514 2371	0.54079 5969	0.45070 3452	1.42103 1243	0.26290 4805	0.11614 961	0.49792 6628	0.84359 3732
<i>P. cf bogotensis</i>					-		-	-	-	-	-	-
<i>ANDES-A 4840</i>	26	35	0.34615 3846	3001- 4100	0.04403 582	0.84476 5625	1.30080 2042	1.09309 3443	0.50317 1321	0.19823 3606	0.43948 936	0.41832 4239
<i>P. cf erythropleura</i>								-		-		-
<i>EAB 6873</i>	25.4	34.8	0.37007 874	1001- 2000	0.51522 4424	0.26752 5869	0.25073 1693	0.52788 466	0.92526 9701	0.78973 2983	0.60022 6784	0.29626 3145
<i>P. cf viejas ANDES-A</i>								-				-
<i>6850</i>	19.1	29.1	0.52356 0209	1001- 2000	0.80486 4565	0.18971 5026	0.85905 332	0.23097 4207	0.53121 223	0.07745 9348	1.10803 878	0.26654 4679
<i>P. chalceus</i>	26.9	31.2	0.15985 1301	0- 1000	0.55272 1674	0.48144 6701	0.81589 1284	0.79961 9477	0.20517 6922	0.07768 2316	1.03608 4097	0.85630 7709
<i>P. chiastonotus</i>	39.7	53.4	0.34508 8161	0- 1000	0.58782 33	2.50890 8406	1.31969 4726	0.30622 6236	1.06641 6209	0.60343 8934	1.08422 8624	0.50738 0324
<i>P. chloronotus</i>	30.3	38.9	0.28382 8383	3001- 4100	0.27408 0304	0.88198 4417	1.56386 3964	0.30085 9509	0.07311 9856	0.42674 2853	0.18296 2842	1.25312 0402
<i>P. chocoensis</i>	47.3	58	0.22621 5645	0- 1000	0.61646 7346	2.02280 2374	1.19508 6953	1.02414 045	0.23545 4127	0.00493 406	0.49536 4382	0.45711 1249
<i>P. cisnerosi</i>	29.8	49.1	0.64765 1007	0- 1000	0.75367 7596	2.70349 4312	1.30830 3892	1.00414 211	0.85061 829	0.58838 5486	1.39178 88	0.63347 4823
<i>P. citriogaster</i>	41.3	51	0.23486 6828	0- 1000	0.23202 5547	0.88922 9536	1.16361 5461	0.84224 0139	0.41040 569	0.06009 8779	0.62442 7757	1.28245 9456
<i>P. condor</i>	39.5	63.2		1001- 2000	0.69654 6067	0.13320 7377	0.44357 2657	0.72571 0676	0.08347 5964	0.44541 9789	0.14726 6751	0.76604 5452
<i>P. conservatio</i>	21.7	33.8	0.55760 3687	0- 1000	0.25264 748	0.97713 548	1.19966 9806	0.28713 1243	1.04893 7613	0.43260 472	0.47486 4854	0.78417 9008
<i>P. conspicillatus</i>	30.1	48.8	0.62126 2458	0- 1000	0.66036 5547	1.46646 6084	1.18999 1522	0.28205 2583	0.71765 7786	0.46030 3631	1.60340 5814	1.31014 141

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<i>P. cremnobates</i>	32.5	51.7	0.59076 9231	1001- 2000	0.87092 4931	0.07716 3669	0.47577 8683	0.36274 0262	0.89696 6982	1.21538 4679	1.00216 542	1.20760 7153
<i>P. crenunguis</i>	49.2	64.5	0.31097 561	1001- 2000	0.49361 2811	0.13780 0842	0.22883 9033	0.78282 5996	1.00821 1714	1.09593 7696	0.67247 4223	1.04279 1168
<i>P. croceinguinis</i>	18.2	21.9	0.20329 6703	0- 1000	0.72540 9412	1.75465 5792	1.25199 5352	0.51462 3333	0.76616 3591	0.44582 9913	1.51818 0945	1.67604 2348
<i>P. crucifer</i>	20.6	34.5	0.67475 7282	1001- 2000	0.37473 3757	0.21834 6721	0.15034 3838	0.55882 6214	1.05120 0466	1.13079 4766	0.35974 7716	0.99906 2897
<i>P. cruciocularis</i>	15.4	21.8	0.41558 4416	1001- 2000	1.29443 1342	0.19971 0863	0.33598 459	2.33615 5037	0.02895 5724	1.22872 4631	0.22812 0263	0.40895 0037
<i>P. cruentus</i>	25	38	0- 0.52	1000	0.85224 1584	0.63460 1923	0.88880 4413	0.44910 639	1.04969 2333	1.21047 6522	1.37288 3663	0.58732 0523
<i>P. cryophilus</i>	36.8	49.8	0.35326 087	3001- 4100	1.15623 23	1.06316 2321	2.48049 1266	1.07933 1731	0.43763 021	0.98690 7346	0.94287 0975	1.28291 8937
<i>P. cryptomelas</i>	30.3	38.6	0.27392 7393	2001- 3000	0.43620 0636	0.72124 1867	0.57553 8271	0.88278 0685	0.22380 9457	0.70512 8131	1.07528 3026	0.43353 3084
<i>P. curtipes</i>	32.5	42.9	3001- 0.32	4100	0.33414 1101	0.98146 355	1.90400 0343	0.91439 2442	0.30789 247	0.02302 1208	1.37264 063	0.54791 5083
<i>P. danae</i>	33.8	45.8	0.35502 9586	1001- 2000	0.12173 8305	0.30987 7001	0.79130 5061	1.18575 3622	1.59416 8949	1.86944 1261	0.56048 8653	0.41525 2435
<i>P. devillei</i>	31.6	44.7	0.41455 6962	2001- 3000	1.33809 6124	0.75541 3635	1.02960 3703	1.49950 8966	0.70847 0302	0.13564 028	0.58624 1299	2.25646 906
<i>P. diadematus</i>	27.4	44.5	0.62408 7591	0- 1000	0.65432 0617	1.38620 6106	1.17320 5643	0.03442 967	0.45494 0564	0.55904 2015	1.62485 0983	1.09316 2664
<i>P. dorado</i>	17.9	25.6	0.43016 7598	2001- 3000	0.72540 9412	0.70612 1291	0.70882 0675	1.25680 0852	1.22408 6395	0.06449 8617	0.33595 6445	0.54721 9788

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<i>P. dorsopictus</i>	22	31.6	0.43636 3636	2001- 3000	0.70623 4859	0.71704 5128	0.80924 2738	0.89190 0476	0.98507 6579	1.21164 0023	0.43740 1405	0.12029 6364
<i>P. duellmani</i>	36	45.8	0.27222 2222	2001- 3000	0.34415 5449	0.43728 1455	0.16913 9492	0.43039 0539	1.25471 1372	1.40287 4928	0.11678 3072	0.76915 6908
<i>P. ecuadorensis</i>	25.4	40.2	0.58267 7165	1001- 2000	0.57460 2772	0.05328 3185	0.35892 2686	0.80181 8565	0.88620 3965	0.94651 0788	0.77823 7816	1.34381 0552
<i>P. elegans ANDES-A 4932</i>	37.5	40.2	0.072	3001- 4100	0.05277 8465	0.94908 1816	1.86835 347	1.58502 485	1.08836 2304	0.01825 0586	0.22119 3874	0.61327 4062
<i>P. eremitus</i>	21.8	27.6	0.26605 5046	2001- 3000	0.28321 3936	0.62172 2565	0.54847 136	0.04508 2153	1.47336 62	1.49459 2372	0.85546 2581	0.63348 4806
<i>P. eriphus</i>	25.2	29	0.15079 3651	2001- 3000	0.02995 118	0.45001 7954	0.25625 4538	0.80888 0975	0.99123 2236	0.38651 0331	0.18898 113	1.46616 5758
<i>P. erythroinguinis</i>	14.3	17.7	0.23776 2238	1001- 2000	0.42305 447	0.28800 9994	0.81275 9816	0.94596 1121	1.86083 1525	1.57614 3363	0.89844 7341	0.36406 5358
<i>P. euphronides</i>	27	39.4	0.45925 9259	0- 1000	0.17564 0379	1.65787 1742	1.29355 7254	1.68340 9517	1.95516 1796	1.64901 2441	0.03516 7967	0.17119 2582
<i>P. fallax ANDES-A 3045</i>	26.8	45.1	0.68283 5821	1001- 2000	0.67696 0616	0.13311 4734	0.80574 9753	0.29484 72	0.60154 7329	0.02249 1955	1.00661 1622	0.22870 1086
<i>P. fenestratus</i>	34.3	50	0.45772 5948	0- 1000	0.53752 3896	2.12873 6811	1.31942 7241	0.78770 5644	0.90086 8505	1.48765 0674	0.50974 9483	0.91586 6892
<i>P. ferwerdai</i>	25.5	26.4	0.03529 4118	2001- 3000	0.93674 3569	0.41831 2342	0.03503 8705	0.88705 5091	0.83693 701	1.20873 9118	0.69657 8449	0.74785 8038
<i>P. flavobracatus</i>	19.6	23.4	0.19387 7551	1001- 2000	0.34415 5449	0.24161 7377	0.33212 7995	2.26576 3306	0.17752 9183	1.23700 7816	0.67283 8449	0.77840 259
<i>P. frater ANDES-A 4845</i>	22	25	0.13636 3636	1001- 2000	1.01077 5438	0.19377 8427	0.78036 6961	0.59123 9608	0.27172 9036	0.42966 1427	0.96648 4226	0.69153 7937

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<i>P. gagliardoi</i>	24.33	33.64	0.38265 5158	2001- 3000	0.11513 1263	0.80368 6246	0.95440 5588	0.73309 5274	0.59949 1099	0.01155 7533	1.31433 3785	1.33370 7095
<i>P. gaigei ANDES-A 3045</i>	30	43	0.43333 3333	0- 1000	0.43116 9674	1.02215 0454	1.23517 9485	0.29502 6038	0.42013 97	0.10814 255	1.00895 8375	0.14098 5477
<i>P. galdi</i>	24.8	34	0.37096 7742	1001- 2000	0.59987 4609	0.12389 5424	0.29462 4804	0.28248 4934	0.36097 8234	0.42709 6977	0.72738 3185	0.78435 7244
<i>P. gentryi</i>	28.5	35.8	0.25614 0351	3001- 4100	0.39493 7489	1.01295 5842	2.04559 8238	0.79624 7938	0.87989 6402	0.60200 1742	1.71352 3671	0.29489 735
<i>P. ginesi</i>	24.1	35.5	0.47302 9046	3001- 4100	2.68129 4025	1.16148 4933	2.90738 4503	1.92058 2762	1.01940 0878	0.09426 892	1.31195 6646	1.26640 6224
<i>P. giorgii</i>	36.6	48.8	0.33333 3333	0- 1000	0.03457 6415	3.74686 2902	1.41192 7787	0.44364 7857	0.44143 6428	0.25179 6254	0.81706 7873	1.58639 8223
<i>P. glandulosus</i>	30.2	41.3	0.36754 9669	2001- 3000	2.02190 0554	0.65519 2637	0.63985 1887	1.35775 3084	1.06573 4634	0.55925 1468	0.34635 4575	1.85892 237
<i>P. gloria</i>	24.7	35.8	0.44939 2713	2001- 3000	0.26839 2621	0.79701 9933	0.90034 6049	1.21831 0989	1.18661 0059	1.12116 7113	1.45647 8746	1.44678 3094
<i>P. gryllus</i>	21	26	0.23809 5238	1001- 2000	0.80852 3242	0.06715 5528	0.81188 9913	0.20219 6085	0.26409 359	0.71584 2108	0.88302 9347	1.02042 4084
<i>P. gutturalis</i>	20.3	40.9	1.01477 8325	0- 1000	0.44351 6913	2.12079 0942	1.29193 4571	0.11828 1062	0.88489 2587	0.67590 3539	1.01118 9456	0.51689 1017
<i>P. hampatusami</i>	24.9	37.6	0.51004 0161	0- 1000	0.26348 7578	0.71695 6578	1.00638 6882	1.36172 7277	0.53620 4249	0.02276 5775	1.27736 4636	2.30338 8309
<i>P. hectus</i>	16.8	22.5	0.33928 5714	2001- 3000	0.42661 0784	0.52465 7329	0.27635 7787	0.46656 1963	0.98371 8995	1.34630 1931	0.28076 0941	0.02638 9132
<i>P. humboldti</i>	20.6	25.7	0.24757 2816	3001- 4100	0.22486 6478	0.96477 4346	1.03361 4338	2.87979 3576	0.19652 4124	1.15051 5716	1.18139 9745	0.84316 2484

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<i>P. inguinalis</i>	20	27	0.35	0- 1000	0.52592 4582	2.00575 6504	1.27308 3382	0.02565 2252	0.90089 1312	0.67773 0935	1.01303 0724	0.49453 0579
<i>P. inusitatus</i>	22.6	25.6	0.13274 3363	2001- 3000	0.06339 0847	0.56433 9191	0.50800 8048	0.85489 7235	0.93525 7485	0.21101 9726	0.03683 1745	1.47646 4553
<i>P. jaguensis</i>	23.8	24.2	0.01680 6723	1001- 2000	0.75367 7596	0.26875 2526	0.35587 4475	0.18391 4341	0.71496 7387	0.14729 2966	1.23947 473	0.25300 5371
<i>P. jimenezi</i>	27.1	37.4	0.38007 3801	2001- 3000	0.27266 1826	0.60053 8	0.46496 5783	0.93643 4566	0.64605 5396	0.70686 7214	2.07712 7999	1.92123 6095
<i>P. juanchoi</i>	23	28.3	0.23043 4783	1001- 2000	0.60483 5336	0.22419 3503	0.32052 1787	0.20100 2627	1.22500 6433	1.31715 8107	0.07871 5279	0.17456 6085
<i>P. jubatus</i>	24.1	34.6	0.43568 4647	2001- 3000	0.96501 1754	0.76296 2889	0.94768 4831	0.73372 6568	0.96349 4166	1.55532 4891	0.47030 8607	0.35386 7706
<i>P. kelephas</i>	21.3	31.5	0.47887 3239	2001- 3000	1.00329 9732	0.42781 6935	0.14549 6568	1.23134 2075	0.96891 8629	1.15909 9419	0.48300 8467	0.73670 4522
<i>P. kichwarum</i>	22.4	30.2	0.34821 4286	0- 1000	0.87107 7812	1.58571 9809	1.18147 8307	0.67132 6653	0.79412 5521	0.39049 1403	1.85433 7016	1.64738 9737
<i>P. koehlerii</i>	29	39	0.34482 7586	0- 1000	0.25264 748	0.80405 444	0.94073 9395	1.22286 1322	4.77475 4772	4.03093 6651	1.49211 3381	0.96747 9997
<i>P. labiosus</i>	50.8	52.3	0.02952 7559	0- 1000	0.77105 3727	1.40332 9651	0.56799 4507	0.90934 4238	0.15036 1719	0.44680 7256	1.58369 1718	0.95008 1206
<i>P. lancinii</i>	36.6	49	0.33879 7814	3001- 4100	0.06339 0847	0.97261 9031	2.12782 4779	1.71755 9828	0.82077 1997	0.13442 4098	1.43385 2031	1.09054 348
<i>P. lanthanites</i>	27.9	45.4	0.62724 0143	0- 1000	0.98322 1184	0.84685 4521	0.96628 2808	0.35074 7689	0.41964 5596	0.19953 0424	1.85775 4317	1.80583 3463
<i>P. lasalleorum</i>	15.6	21	0.34615 3846	2001- 3000	0.86125 8911	0.60214 6193	0.30085 8509	1.08068 5278	0.88383 0821	0.81190 186	0.55780 3307	0.14419 873

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<i>P. latidiscus</i>	25.9	53.4	1.06177 6062	0- 1000	1.03521 3657	1.73411 0967	1.24683 3186	0.81658 2752	0.18453 7818	0.05679 7938	2.21785 8728	0.16685 2942
						-	-	-	-	-	-	-
<i>P. latro</i>	39.5	41.6	0.05316 4557	0- 1000	0.87413 1853	1.79789 6588	0.85908 1126	0.92531 3218	0.99957 0447	0.25749 5956	1.67776 3355	1.78690 8189
						-	-	-	-	-	-	-
<i>P. leoni</i>	18.3	25	0.36612 0219	2001- 3000	0.70465 8983	0.78345 0865	1.11722 5688	0.04627 6591	0.10311 1507	0.15065 1074	0.69802 0261	1.04086 5543
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<i>P. leopardus</i>	19.9	22.8	0.14572 8643	3001- 4100	0.83514 463	0.85907 1126	1.24454 0295	0.76742 1941	1.59124 5243	1.43400 4652	0.54270 8541	0.84630 8503
						-	-	-	-	-	-	-
<i>P. librarius</i>	18.5	30.7	0.65945 9459	0- 1000	0.87199 4737	1.64201 9385	1.20988 3205	0.73786 3143	0.82346 4991	0.32416 362	1.84010 7508	1.74169 4654
						-	-	-	-	-	-	-
<i>P. lirellus</i>	17	24	0.41176 4706	0- 1000	0.57431 3129	0.67827 7443	1.16092 141	1.17073 7708	0.49241 8946	0.17201 4234	0.16268 2038	1.01423 0829
						-	-	-	-	-	-	-
<i>P. lividus</i>	20.4	35	0.71568 6275	2001- 3000	1.91408 4106	0.79527 4518	0.10644 1477	1.59423 2711	0.72829 626	0.14903 0246	0.55965 5708	2.76146 1182
						-	-	-	-	-	-	-
<i>P. luscombei</i>	19.9	26.1	0.31155 7789	0- 1000	0.40513 3191	2.11333 09	1.34187 4277	0.01759 9587	0.64345 6221	0.62520 4407	1.27818 9067	1.95000 3161
						-	-	-	-	-	-	-
<i>P. luteolateralis</i>	23.6	29.5	1001- 0.25 2000		0.53533 0849	0.19961 7773	0.18442 4236	0.80182 1113	1.02923 735	1.12891 0608	0.72442 3769	1.03827 7388
						-	-	-	-	-	-	-
<i>P. lutitus ANDES-A 1771</i>	24.7	28.2	0.14170 0405	2001- 3000	1.27538 9947	0.53891 0923	0.38532 8322	1.11658 5199	0.36912 629	0.42219 3816	0.71785 97	0.74031 4345
						-	-	-	-	-	-	-
<i>P. lutzae</i>	27	33.9	0.25555 5556	3001- 4100	1.64790 4302	1.00087 9348	2.08497 0633	0.97799 1194	0.89102 3224	1.06679 6273	1.10292 0219	1.11670 0917
						-	-	-	-	-	-	-
<i>P. lymani</i>	43.6	69.3	0.58944 9541	1001- 2000	0.16041 5604	0.30374 4692	0.21501 8745	1.16338 4334	0.38619 01	0.09934 8253	1.49234 6543	0.95964 2662
						-	-	-	-	-	-	-
<i>P. lynchi</i>	27.9	36.4	0.30465 9498	3001- 4100	0.25139 8261	0.85601 185	1.49803 9031	1.61732 5305	1.60536 9473	0.44882 4673	0.77994 8023	0.94383 1775

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<i>P. maculosus</i>	19.2	26.3	0.36979 1667	2001- 3000	0.78137 4665	0.68836 7558	0.63203 3172	0.23953 9506	0.49350 1737	0.21201 14	0.57493 6914	0.33286 007
						-		-	-			-
<i>P. malkini</i>	37.2	47.9	0.28763 4409	0- 1000	0.71793 8232	1.99928 9963	1.29558 1164	0.62264 7362	0.86821 7517	0.37141 4204	1.65416 9607	1.72891 8904
						-		-	-			-
<i>P. mallii</i>	21.3	34.3	0.61032 8638	2001- 3000	0.11513 1263	0.41615 5225	0.11524 7944	0.15385 3207	0.56396 8229	0.10966 0112	0.19233 0234	0.10010 0129
						-		-	-			-
<i>P. marmoratus</i>	20.4	27.9	0.36764 7059	0- 1000	0.48419 3997	1.57109 3925	1.21341 6066	0.15843 8976	0.90472 8885	0.58740 1514	0.93086 4211	0.68407 5665
						-		-	-			-
<i>P. martiae</i>	16.8	22.2	0.32142 8571	0- 1000	0.87839 6111	1.35054 3749	1.14446 586	0.53473 2529	0.90618 9782	0.43470 3655	1.77319 6755	1.89941 2555
						-		-	-			-
<i>P. matidiktyo</i>	22.1	24.1	0.09049 7738	0- 1000	0.89952 0694	1.47970 1855	1.16278 1879	0.64694 6551	0.72236 8033	0.38511 6415	2.01425 1333	1.80703 5233
						-		-	-			-
<i>P. mazar</i>	18.1	23.7	0.30939 2265	3001- 4100	0.65788 7706	0.84354 3817	1.26251 4217	0.58875 8702	0.73615 4329	0.29092 3032	1.28479 0508	1.44152 0226
<i>P. medemi ANDES-A 4812</i>	32.9	43.1	0.31003 0395	0- 1000	0.97344 6853	0.54520 1571	0.99585 6913	0.41041 0214	1.07750 8229	1.28630 9318	1.22126 9903	0.91215 6512
						-		-	-			-
<i>P. melanogaster</i>	22.8	24.7	0.08333 3333	3001- 4100	1.42509 7625	0.91776 9772	1.04332 7139	2.28888 8926	1.26814 0411	0.17027 4659	0.68191 4053	0.26438 314
						-		-	-			-
<i>P. mendax</i>	21.7	28	0.29032 2581	2001- 3000	0.18172 0027	0.57217 5765	0.14080 8519	2.61816 8661	0.86342 7061	1.57548 1731	1.12382 371	0.86931 7468
						-		-	-			-
<i>P. merostictus</i>	22.5	31	0.37777 7778	2001- 3000	0.64724 632	0.57733 6105	0.35457 2465	0.64722 4928	0.14450 5071	0.08077 188	0.56972 3374	0.72813 1974
						-		-	-			-
<i>P. miktos</i>	21.3	29.2	0.37089 2019	0- 1000	0.52592 4582	2.45223 9711	1.36569 3132	0.02802 0099	0.85229 5341	0.77329 7889	1.30378 0322	2.31789 4229
						-		-	-			-
<i>P. mindo</i>	27.4	40.8	0.48905 1095	2001- 3000	0.21172 0313	0.70023 3787	0.35870 4512	1.11262 3146	1.31318 492	1.45441 578	1.58896 9278	0.67029 0437

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<i>P. minutulus</i>	17.5	20.1	0.14857 1429	0- 1000	1.73101 8132	0.54593 7805	1.14467 8887	1.34017 4917	0.46386 8326	0.25813 5954	0.13645 2085	0.52533 3977
<i>P. miyatai AJC 3473</i>	20.2	26.7	0.32178 2178	2001- 3000	0.80475 2634	0.40663 9727	0.00868 6792	0.71410 8316	0.24924 7911	0.19902 1759	0.36154 4869	0.32650 4463
<i>P. moa</i>	34.4	43.4	0.26162 7907	0- 1000	4.58184 5808	2.39326 5624	1.38525 4727	1.66682 6617	0.73489 3037	1.26140 1606	0.02441 592	1.44844 4009
<i>P. moro</i>	18	25	0.38888 8889	1001- 2000	0.92197 5474	0.17747 7593	0.65383 7119	0.98184 1953	0.43182 1257	0.47726 9556	1.12156 439	0.50443 7374
<i>P. multicolor</i>	29.7	40.5	0.36363 6364	3001- 4100	0.01353 2323	0.94773 331	1.31239 021	0.53062 5571	1.17972 2448	1.55317 8932	1.53612 1064	1.25676 4188
<i>P. muranunka</i>	17.4	23.3	0.33908 046	2001- 3000	1.81949 7756	0.59153 6655	0.20693 3108	0.12277 4642	0.92467 2369	0.30994 5801	0.46489 1928	1.61107 8586
<i>P. muscosus</i>	28.9	46.1	0.59515 5709	2001- 3000	1.56954 2502	0.51835 3035	0.00283 2885	0.55089 3507	0.47764 0404	0.41854 4389	0.66921 797	0.08501 6123
<i>P. museosus</i>	26.1	35.5	0.36015 3257	0- 1000	0.89005 4891	0.57097 942	0.90440 9275	1.16148 9798	0.70837 7112	0.54311 6397	1.19939 7665	0.68493 2278
<i>P. mutabilis</i>	17.4	23.2	0.33333 3333	1001- 2000	0.23233 6615	0.30353 2738	0.12220 8326	0.66533 2384	1.13717 4778	1.21905 8946	0.46630 8367	0.85139 7543
<i>P. myersi MUD-H640</i>	17.5	23.2	0.32571 4286	3001- 4100	0.57869 3328	0.89434 5113	1.55599 261	0.40484 9625	0.11607 7248	0.42595 9125	0.08378 7929	1.33859 3831
<i>P. myops</i>	13.6	17.2	0.26470 5882	2001- 3000	0.98485 2308	0.43579 6616	0.12603 3924	1.19643 9231	0.98521 9805	1.14231 6505	0.51134 9112	0.72756 9774
<i>P. nangaritza</i>	20.8	32.4	0.55769 2308	1001- 2000	0.06339 0847	0.21941 4759	0.57977 9727	0.80891 9553	0.42576 1344	0.56573 5225	0.73897 6484	1.87609 1259
<i>P. nervicus</i>	18.5	26	0.40540 5405	3001- 4100	0.25767 8526	0.91062 7166	0.61001 6705	1.56470 3714	1.41691 5855	0.12368 1625	0.16290 0905	0.91403 1912

<i>P. nicefori</i>							-	-	-	-	-	-
<i>MCNUPH48</i>	19	25.8	0.35789 4737	3001- 4100	0.34307 3103	0.97046 9724	2.18723 4472	2.09650 1168	1.93856 1871	0.47255 8088	0.51033 6781	1.00650 3106
<i>P. nietoi</i>	17	19.5	0.14705 8824	0- 1000	0.69654 6067	1.50668 9025	1.10499 3539	1.15319 7687	0.04512 4311	0.29568 7358	0.38734 8461	0.99522 2375
<i>P. nyctophylax</i>	31.4	37.8	0.20382 1656	1001- 2000	0.47856 6436	0.13936 2611	0.21817 5878	0.75892 1094	1.06583 1139	1.16455 1795	0.65878 5026	1.09110 4454
<i>P. ockendeni</i>	21.3	31.1	0.46009 3897	0- 1000	0.38294 0933	1.96582 7075	1.31039 5689	0.09024 0115	0.80869 6879	0.86160 5468	1.24649 6256	0.55897 1334
<i>P. ocreatus</i>	17.2	20.2	0.17441 8605	3001- 4100	0.82853 7587	1.05377 1365	2.61005 4875	0.15789 63	0.11419 263	0.03701 4682	0.39946 2842	1.50456 8576
<i>P. omeviridis</i>	23.31	30.91	0.32604 0326	0- 1000	0.75367 7596	2.11821 3043	1.32483 5969	0.43334 5665	2.12099 8803	1.33635 8745	1.48135 4831	0.28919 2011
<i>P. orcesi</i>	29.6	36.1	0.21959 4595	3001- 4100	0.52129 9347	0.94182 5529	1.68313 5918	0.94357 7225	0.31685 4481	0.07552 0665	1.50755 1848	0.42524 5185
<i>P. orestes</i>	22.3	27.2	0.21973 0942	2001- 3000	0.05413 3925	0.76635 1327	0.83730 9156	1.25238 5327	0.93402 006	0.80041 5913	1.52244 2552	0.69792 6087
<i>P. ornatissimus</i>	27	38.1	0.41111 1111	1001- 2000	0.57814 0729	0.21103 0493	0.55156 3447	0.59783 8706	0.79359 7555	0.62956 7357	0.62444 2269	1.10771 4398
<i>P. ornatus</i>	20.5	27.3	0.33170 7317	3001- 4100	0.01353 2323	0.92749 8688	1.06828 45	2.77021 8045	1.08436 1123	1.45942 1793	1.77228 5986	1.32288 4337
<i>P. orpacobates</i>	35.6	48.4	0.35955 0562	1001- 2000	0.09346 0975	0.11125 6864	0.40660 1904	0.32376 6712	1.07125 1731	1.03024 0703	0.52585 8177	0.23713 2233
<i>P. paisa</i>	24.2	30.7	0.26859 5041	2001- 3000	0.78880 1691	0.45462 8711	0.08134 404	0.17395 2941	0.75970 3275	0.20971 1329	0.87845 1235	0.35841 9121
<i>P. palmeri</i>	20.3	28	0.37931 0345	1001- 2000	0.01835 9986	0.22861 0027	0.28924 8719	0.48261 3765	1.07321 8132	1.08782 5107	0.27212 0715	0.27869 3592

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<i>P. paramerus</i>	26.7	39.3	0.47191 0112	3001- 4100	0.34415 5449	1.00412 08	2.14811 148	1.65407 9097	0.90310 8469	0.04631 103	1.62668 0986	1.07297 213
<i>P. pardalis</i>	19	27	0.42105 2632	0- 1000	0.88688 5046	0.47947 278	0.86694 6551	0.30914 2027	0.85258 1793	1.08693 5068	1.34038 1664	0.96075 5541
<i>P. parectatus</i>	16	27	0.6875	2001- 3000	0.80894 9867	0.77109 7619	0.92421 3707	0.78864 2986	1.39014 7266	1.26717 5963	0.65169 7388	0.49392 8198
<i>P. parvillus</i>	19.6	25.9	0.32142 8571	0- 1000	0.58650 6971	0.96115 2506	0.91689 2255	1.11025 0731	0.47760 4704	0.76583 5554	0.90170 0523	1.47605 6448
<i>P. peraticus</i>	18.3	26	0.42076 5027	3001- 4100	0.67531 5792	0.98503 1909	2.16315 8802	0.64201 8694	1.55865 021	1.93581 3233	0.46065 6979	0.27291 486
<i>P. peruvianus</i>	35.8	46.4	0.29608 9385	1001- 2000	0.44659 0812	0.00709 8529	0.43184 8496	0.05056 7552	0.11339 0892	0.31863 523	0.79684 2806	0.87246 2083
<i>P. pharangobates</i>	25.4	29.5	0.16141 7323	1001- 2000	3.63156 9915	0.24350 0657	0.27920 3044	1.18630 9984	2.01515 9344	2.00518 0083	0.57698 6163	0.45747 7324
<i>P. phoxocephalus</i>	29.9	38.4	0.28428 0936	2001- 3000	0.05949 0536	0.64056 1347	0.60678 8392	0.56569 6404	0.03574 4749	0.21299 0559	1.45706 1783	0.67651 6609
<i>P. pichincha</i>	27.5	35.8	0.30181 8182	3001- 4100	0.47482 1732	0.94671 298	1.63308 5251	0.79431 8606	1.95010 8763	2.36694 5854	1.05130 652	0.01535 6943
<i>P. pictus</i>	30.6	45.9	0- 0.5	1000	0.47482 1732	1.74188 8623	1.45524 9861	1.17255 5689	1.67946 1469	1.18646 4446	0.57173 3834	1.39202 9457
<i>P. pirrensis</i>	21.9	30	0.36986 3014	0- 1000	0.69654 6067	1.75608 3156	1.28476 8552	1.18976 7855	0.14939 105	0.11356 1592	0.72127 4422	0.97114 9314
<i>P. platydactylus</i>	23.9	33.3	0.39330 5439	1001- 2000	0.79509 7956	0.05217 25	0.55659 091	1.82541 0642	1.42300 3047	2.34912 4277	0.59240 1456	0.69828 9947
<i>P. pleurostriatus</i>	25.3	31.1	0.22924 9012	0- 1000	0.69654 6067	0.50372 3835	0.81827 2697	0.01208 3169	0.17107 5245	0.60702 1715	0.67827 9621	1.11084 8035

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<i>P. pluvialis</i>	26.9	28.8	0.07063 197	1001- 2000	0.52592 4582	0.41892 643	1.63217 7483	0.84283 9404	1.56788 6287	1.57754 8934	1.11342 768	0.32728 167
					-	-			-			
<i>P. pluvian</i>	32.5	43.1	0.32615 3846	0- 1000	1.73101 8132	2.24753 3778	1.36219 7059	1.20003 4978	0.82369 0043	0.17075 3357	0.16709 5183	1.54541 324
					-	-			-			
<i>P. prolatus</i>	18.4	24.1	0.30978 2609	1001- 2000	0.71393 6892	0.01237 6701	0.43814 3788	0.42033 94	1.15415 0853	1.34353 7105	0.85507 91	1.17921 2765
					-	-			-			
<i>P. prometeii</i>	24.9	37.6	0.51004 0161	0- 1000	0.16890 1225	0.80405 444	0.98703 8575	1.44655 0279	0.70592 4104	0.62965 294	1.09640 6355	2.23230 2614
					-	-			-			
<i>P. ptochus</i>	19.8	25.5	0.28787 8788	2001- 3000	0.74967 4795	0.44019 2533	0.16716 6244	1.18833 8511	1.08027 9908	1.19062 7297	0.41116 0202	0.50984 0113
					-	-			-			
<i>P. puipui</i>	17.1	22.4	0.30994 152	3001- 4100	0.50953 1394	1.09853 7152	1.50353 407	2.65690 311	0.50902 5552	0.72576 5259	1.17354 517	0.95301 5504
					-	-			-			
<i>P. pycnodermis</i>	32.3	44.4	0.37461 3003	3001- 4100	1.03560 2538	0.84704 7839	1.00055 1948	0.84698 2605	0.75680 3475	0.73978 8718	1.06838 5807	1.49209 5265
					-	-			-			
<i>P. pyrrhomerus</i>	18.9	24	0.26984 127	2001- 3000	0.05075 5195	0.78748 4903	1.08536 8284	0.07450 2177	0.54885 4693	0.67483 5442	1.97461 0342	1.21547 9228
					-	-			-			
<i>P. quantus</i>	14.5	16.7	0.15172 4138	2001- 3000	0.93060 9518	0.38884 414	0.07381 4399	1.15153 9932	0.98857 566	1.13021 1237	0.57640 3537	0.72694 3041
					-	-			-			
<i>P. quaquaversus</i>	22.5	31.1	0.38222 2222	0- 1000	0.93148 7492	0.45360 1171	0.76573 6821	0.36378 512	0.06312 1435	0.12666 5135	1.68529 3036	1.46105 2141
					-	-			-			
<i>P. quinquagesimus</i>	30.8	40.1	0.30194 8052	1001- 2000	0.17439 6056	0.23158 4157	0.14137 2097	0.62678 2845	1.02967 0688	1.23076 7582	0.22399 4147	0.82147 8664
					-	-			-			
<i>P. quintanai</i>	16.4	21.8	0.32926 8293	2001- 3000	1.91408 4106	0.61901 0906	0.47521 809	1.23650 731	0.71719 8364	0.49143 7543	1.05983 0693	0.69346 6937
					-	-			-			
<i>P. reichlei</i>	30.7	37.1	0.20846 9055	0- 1000	0.19152 0667	1.54710 9673	1.25137 4612	0.80425 5583	0.90618 1953	1.70434 8814	0.84036 5864	0.59901 8871

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<i>P. rhabdolaemus</i>	24	31.7	0.32083 3333	2001- 3000	0.20129 4723	0.38810 6889	0.33768 6537	1.86872 0429	0.35809 9539	1.39626 7667	0.78602 259	0.69459 3472
<i>P. rhodoplichus</i>	28.9	34.2	0.18339 1003	3001- 4100	0.33100 9284	0.84966 8865	0.76538 8802	0.95547 3068	0.92860 8196	1.48112 5494	1.46914 3645	1.16749 3016
<i>P. ridens</i>	19	25	0.31578 9474	0- 1000	0.69638 4902	1.06692 7718	1.08638 601	0.70545 5079	1.76879 6615	1.64743 4707	1.29629 377	0.60370 4458
<i>P. riveti</i>	22.09	34.2	0.54821 1861	3001- 4100	0.74074 386	0.95093 1634	1.75365 0109	0.77632 055	0.57913 4702	0.79927 4014	1.07199 7271	1.36244 5813
<i>P. rubicundus</i>	35.6	51.2	0.43820 2247	1001- 2000	1.07628 3399	0.16846 7025	0.57035 9725	0.35688 9965	0.40519 7551	0.07013 7068	1.75403 334	1.65703 6895
<i>P. rupicola</i>	28.2	33.4	0.18439 7163	1001- 2000	0.26806 8997	0.00755 2795	0.29754 713	0.91164 7784	2.12367 4995	2.68792 0149	1.22177 3631	1.30909 1482
<i>P. sagittulus</i>	23.3	26.7	0.14592 2747	1001- 2000	0.40793 2451	0.33538 4413	0.26563 9783	2.55446 0379	0.15348 0401	1.15522 2781	0.80319 1732	0.83245 4856
<i>P. saltissimus</i>	18.3	27.1	0.48087 4317	1001- 2000	0.58518 9375	0.14272 8754	0.69849 412	0.19435 1931	0.52489 2067	0.71119 9878	0.13345 1537	1.50381 9108
<i>P. samaipatae</i>	38.6	48.6	0.25906 7358	1001- 2000	0.78137 4665	0.02443 038	0.42799 5442	2.70934 4844	3.72991 2187	3.64381 2848	2.33387 1175	1.39051 2171
<i>P. satagius</i>	32.4	40.3	0.24382 716	3001- 4100	0.96100 8953	0.87584 0022	1.26484 2517	0.56184 2856	1.11714 2278	1.14985 4234	0.62925 4997	0.00447 3778
<i>P. saturninoi</i>	18.7	21.5	0.14973 262	1001- 2000	0.75367 7596	0.10389 6982	0.48484 9534	0.05056 9576	0.54712 087	0.21535 3887	0.74472 1706	1.16501 275
<i>P. savagei ANDES-A 1255</i>	23.9	37.2	0.55648 5356	1001- 2000	0.98571 1571	0.03792 927	0.64112 2262	0.67979 9946	0.63627 8792	0.65332 5206	1.05191 801	0.67003 358
<i>P. schultei</i>	26.5	34	0.28301 8868	2001- 3000	0.11513 1263	0.67292 878	0.30802 5694	1.82401 1167	0.83492 7505	0.29468 1133	1.05024 3163	0.16257 9045

						-	-	-	-	-	-	-
<i>P. scoloblepharus</i>	20.3	23.7	0.16748 7685	2001- 3000	0.80852 3242	0.74037 3574	0.86547 3311	1.01017 7404	1.13780 8828	1.22559 5002	0.75970 1533	0.71840 5126
<i>P. shrevei</i>	28	40.1	0.43214 2857	0- 1000	0.94892 9946	0.59886 0174	0.92402 1328	2.08741 0493	2.46872 6689	1.92480 7441	0.41548 6119	0.16906 6467
<i>P. simonbolivari</i>	19.2	22	0.14583 3333	3001- 4100	1.29443 1342	0.94807 322	1.75682 778	0.38153 7955	0.41995 2519	0.50302 008	2.27951 9846	0.84148 742
<i>P. simonsii</i>	25.9	33.3	0.28571 4286	3001- 4100	1.39124 5084	1.00759 5201	1.45644 5824	2.31048 6491	1.19215 167	0.07128 2182	1.05050 8957	0.62258 5055
<i>P. simoterus</i>	29	37.1	0.27931 0345	3001- 4100	0.91510 2264	1.03214 6953	2.46947 9647	1.13512 699	1.51799 3584	1.95699 414	0.45986 6976	0.07364 6379
<i>P. skydmainos</i>	24.2	32.4	0.33884 2975	0- 1000	0.00134 5946	1.65244 029	1.27025 3778	0.49240 2425	0.76211 871	1.28485 8531	0.93478 9304	0.13352 7624
<i>P. sobetes</i>	30.6	37	0.20915 0327	1001- 2000	0.19780 1834	0.22133 4729	0.11060 7258	0.61540 5761	1.16533 2569	1.20675 8341	0.39042 7408	1.09219 0591
<i>P. sp1 MCNUPH439</i>	24.1	33.4	0.38589 2116	2001- 3000	1.29443 1342	0.77286 753	1.26465 7625	2.05419 7254	0.21221 2228	0.31291 8479	0.17616 8833	1.00990 7143
<i>P. sp2 MCNUPH584</i>	24.2	31.1	0.28512 3967	3001- 4100	0.69762 8413	0.87493 4925	1.70779 4297	2.02622 89	0.03193 82	0.69457 5087	0.17722 1645	1.11754 6167
<i>P. sp3 MCNUPH54</i>	24.9	29.3	0.17670 6827	3001- 4100	0.39170 7905	0.87012 5988	1.64034 139	1.39304 4991	1.03882 9326	1.13829 4372	1.27074 0207	0.32888 3577
<i>P. sp4 MCNUPH443</i>	22.3	23	0.03139 0135	3001- 4100	0.40793 2451	0.87764 8077	1.14000 2778	2.08741 5299	0.08748 68	0.56573 5225	0.48153 6992	1.05576 7189
<i>P. sp5 MCNUPH30</i>	26.8	38.5	0.43656 7164	3001- 4100	1.20076 8505	0.84023 0554	1.51835 6789	1.23251 2309	0.94897 6592	0.91477 0424	0.38107 2237	1.23044 1765
<i>P. sp8 MCNUPH525</i>	22	26.2	0.19090 9091	2001- 3000	0.78074 224	0.61538 5385	0.62638 8991	1.74342 4722	0.63255 168	0.33432 5104	0.20151 889	1.00093 9619

					-	-	-	-	-	-	-	-
<i>P. sp9 MCNUPH42</i>	21.3	23	0.07981 2207	3001- 4100	0.06339 0847	0.98255 6368	2.02088 6287	2.09510 1809	0.35789 849	0.92563 1892	0.05906 7111	0.56459 0977
					-	-	-	-	-	-	-	-
<i>P. spinosus</i>	25	34.5	0.38 3000	2001- 3000	0.34415 5449	0.40439 9988	0.14412 2225	1.15418 7886	0.05487 3002	0.07871 1254	0.73215 4685	0.42075 9309
					-	-	-	-	-	-	-	-
<i>P. sternothylax</i>	29.1	36.7	0.26116 8385	3001- 4100	0.33100 9284	0.84093 5422	0.95073 7715	0.50515 3134	0.62114 8236	1.57208 4692	1.64302 9707	1.41896 78
					-	-	-	-	-	-	-	-
<i>P. subsigillatus</i>	28.5	33.4	0.17192 9825	0- 1000	0.63223 4722	1.47291 8692	1.05429 9254	1.24049 3901	0.92009 0029	1.12676 5545	1.01139 0479	1.67502 3586
					-	-	-	-	-	-	-	-
<i>P. suetus</i>	18.2	23	0.26373 6264	2001- 3000	1.07628 3399	0.35280 3122	0.23169 3842	0.80675 0543	0.43252 3095	0.27093 5316	0.94319 7214	0.23432 1185
					-	-	-	-	-	-	-	-
<i>P. supernatis</i>	30	40.6	0.35333 3333	2001- 3000	0.10253 4313	0.76631 285	1.00069 8639	0.16710 1563	0.29794 7663	0.02753 0976	0.07824 6129	0.93339 9856
					-	-	-	-	-	-	-	-
<i>P. surdus</i>	36.9	54.6	0.47967 4797	2001- 3000	0.45591 1665	0.64010 7695	0.59129 8196	0.07609 4164	1.25458 972	1.34012 0114	0.58037 0821	0.65540 6352
					-	-	-	-	-	-	-	-
<i>P. taeniatus EAB 6866</i>	22.2	31.6	0.42342 3423	1001- 2000	0.64586 1147	0.24120 9949	0.86543 4265	0.31397 1596	0.51148 9542	0.02116 3672	1.00437 9759	0.00137 747
					-	-	-	-	-	-	-	-
<i>P. terraebolivaris</i>	26	36	0.38461 5385	0- 1000	0.39001 7731	0.74501 5351	0.89516 2892	0.31078 2073	1.20280 8986	1.64676 7023	0.93526 4963	0.55386 6829
					-	-	-	-	-	-	-	-
<i>P. thectoptyernus</i>	35.4	47	0.32768 3616	1001- 2000	0.22436 9415	0.31569 533	0.13273 6551	0.58907 4613	0.96926 4993	1.02864 2847	0.24509 5746	0.17375 3836
					-	-	-	-	-	-	-	-
<i>P. thymalopsoides</i>	34.4	55.4	0.61046 5116	1001- 2000	0.44074 4499	0.16632 516	0.28128 3094	0.73010 7457	1.09574 2961	1.10801 3577	0.67008 4347	1.13575 672
					-	-	-	-	-	-	-	-
<i>P. thymelensis</i>	25.2	33.5	0.32936 5079	3001- 4100	0.02336 0022	1.04993 6377	2.47134 1584	0.94358 8599	0.05950 7509	0.21372 9013	1.11643 9523	1.48037 1251
					-	-	-	-	-	-	-	-
<i>P. tiktik</i>	18.4	20.4	0.10869 5652	3001- 4100	0.86922 1863	0.89236 7859	1.32186 3269	0.81537 2494	1.00106 8747	0.92832 6395	1.30326 0929	0.71751 4748

					-	-	-	-	-	-	-	-
<i>P. tinguichaca</i>	24.4	31.7	0.29918 0328	2001- 3000	0.10637 1116	0.72930 5859	0.81753 4842	0.76662 9999	0.01966 8029	0.52094 085	0.88831 6913	0.71452 3382
<i>P. toftae</i>	22.3	26.1	0.17040 3587	0- 1000	0.25190 7953	1.33040 5367	1.21502 2822	0.80114 0508	0.78765 0563	1.59213 21	0.74369 7646	0.55952 0145
<i>P. torresi</i>	30	39.5	0.31666 6667	2001- 3000	0.69762 8413	0.70276 0695	0.19248 2988	0.23421 9751	1.10247 8836	0.18199 3119	1.72368 7743	1.96980 3042
<i>P. totoroi</i>	29.4	34.3	0.16666 6667	2001- 3000	0.86922 1863	0.76039 8319	0.95126 6022	0.09636 7769	0.69898 5257	0.63730 2162	1.99000 3569	1.24451 1265
<i>P. trepidotus</i>	21.3	25.5	0.19718 3099	3001- 4100	0.18730 3627	0.94603 2256	1.92539 4547	1.26673 1752	0.24110 1472	0.47933 5191	0.69224 2162	2.37058 8054
<i>P. unistrigatus BCQ 991</i>	24	31	0.29166 6667	2001- 3000	0.13731 4863	0.72950 3832	0.72984 1773	0.75392 2266	0.54754 1787	0.44347 4267	1.11469 4172	0.25722 3318
<i>P. urani</i>	19.1	23.4	0.22513 089	2001- 3000	0.72540 9412	0.48985 1757	0.08318 8843	0.48085 1081	1.04579 4853	0.48369 9559	0.62142 2054	0.03274 7234
<i>P. uranobates</i>	19.8	27.8	0.40404 0404	3001- 4100	0.73465 81	0.87130 1499	0.27063 4466	1.01892 622	1.37357 4403	1.65567 467	0.01271 2821	0.08845 1091
<i>P. urichi</i>	23	25	0.08695 6522	0- 1000	0.30560 2345	2.01010 1821	1.30603 5601	1.49048 5724	1.06446 202	1.16751 3751	0.37669 1191	0.21742 4423
<i>P. vanadise</i>	19.1	31.7	0.65968 5864	1001- 2000	1.29443 1342	0.17601 0776	0.32428 4975	0.94131 8971	0.54296 7059	0.63854 4222	0.93003 9509	0.83386 3538
<i>P. ventrimarmoratus</i>	25.5	43.8	0.71764 7059	0- 1000	0.32807 675	1.34522 9953	1.21296 3187	0.35589 4865	0.73165 5005	1.11707 5467	1.02329 5402	0.11505 4326
<i>P. verecundus</i>	21.9	22.5	0.02739 726	1001- 2000	0.29238 8184	0.31787 9747	0.39240 9004	0.52872 1468	0.90553 8759	1.04820 5782	0.03804 0985	0.50512 7969
<i>P. verrucolatus</i>	34.5	46.8	0.35652 1739	3001- 4100	1.21376 3467	0.99426 0076	1.89598 9908	0.73720 7179	0.52009 9015	0.80550 4371	1.19982 9528	0.15083 7074

					-	-	-	-	-	-	-	-
<i>P. versicolor</i>	25.2	29.8	0.18253 9683	2001- 3000	0.60448 5243	0.59886 017	0.27222 546	0.71091 5905	0.44433 4645	0.05886 3796	0.89751 7778	0.62164 6783
<i>P. vertebralis</i>	28	43.9	0.56785 7143	2001- 3000	0.31945 2831	0.51479 162	0.34670 8317	0.45467 8208	1.02662 2858	1.12783 7687	0.65227 5819	1.19842 7316
<i>P. vilarsi</i>	31.5	43.9	0.39365 0794	0- 1000	0.48584 5879	2.05147 9703	1.39866 5052	0.43926 2939	1.60107 384	1.05539 1248	1.11844 2743	0.00994 4811
<i>P. vinhai</i>	17.2	28.5	0.65697 6744	0- 1000	0.80960 5588	3.54398 3408	1.29949 6574	0.54676 8092	3.02721 4267	2.33130 0596	0.12227 4581	0.98715 1086
<i>P. w-nigrum</i>	46.8	65.4	0.39743 5897	2001- 3000	0.25869 241	0.38007 416	0.01262 2781	0.43510 6674	0.90817 4314	0.94934 1857	0.26169 8051	0.12216 5074
<i>P. walkeri</i>	18.5	25.3	0.36756 7568	0- 1000	0.55813 2003	1.21409 6679	0.98238 8933	1.08706 7513	0.61502 419	0.83652 7758	0.83448 9834	1.62834 6049
<i>P. wiensi</i>	33	37	0.12121 2121	2001- 3000	0.21172 0313	0.67508 5492	0.42472 7898	0.55089 3507	0.59615 4693	2.02498 756	1.39169 6427	1.15723 2712
<i>P. yantzaza</i>	22.1	34	0.53846 1538	1001- 2000	2.68129 4025	0.30932 2659	0.29580 1924	0.58120 3538	0.83084 6835	0.55447 1256	0.63688 1513	1.11644 4696
<i>P. yukpa MCNUPH29</i>	27	28.8	0.06666 6667	0- 1000	0.52722 1422	0.58105 4426	0.94156 9052	0.11603 0308	0.43082 2845	0.71400 5387	0.55491 2088	0.85578 9477
<i>P. yuruanensis</i>	24	32	0.33333 3333	1001- 2000	0.21172 0313	0.18363 4871	0.37877 3478	0.03410 6186	0.43383 7996	0.44670 0908	0.08710 4059	0.36161 0242
<i>P. zeuctotylus</i>	29.6	43.3	0.46283 7838	0- 1000	0.44598 1168	2.57399 8463	1.34447 4845	0.20423 7588	1.07948 6374	0.69293 8978	0.96844 7755	0.56181 279
<i>P. zophus</i>	23	31.2	0.35652 1739	2001- 3000	0.87697 6165	0.57997 0433	0.37580 1135	0.76430 3894	1.11813 4691	1.05038 8048	0.74030 5076	0.10030 1321

Table S3. *Pristimantis* species included in the phylogenetic analyzes with the respective Genbank accession numbers linked to the NCBI website: <https://www.ncbi.nlm.nih.gov/genbank/>

Taxon	12S	16S	CO1	CYTB	ND1	ND2	RAG1	TYR
<i>Craugastor podiciferus</i>	EF493360.1	MK211634.1	-	-	-	-	EF493450.1	EF493481.1
<i>Eleutherodactylus caribe</i>	EF493385.1	EF493385.1	-	-	-	-	-	EF493472.1
<i>Eleutherodactylus marnockii</i>	EF493820.1	EF493642.1	-	-	-	-	-	EF493476.1
<i>Oreobates cruralis</i>	EU186666.1	EU186666.1	-	EU368881.1	-	-	KY672963.1	EU186764.1
<i>T. douglasi</i> ANDES-A 1932	-	-	-	-	-	-	-	-
<i>T. lentiginosus</i>	KP297386.1	KP297386.1	-	-	-	-	KP297388.1	KP297390.1
<i>T. prolixodiscus</i>	KP297385.1	KP297385.1	-	-	-	-	KP297387.1	KP297389.1
<i>P. abakapa</i>	-	JQ742162.1	-	-	JQ742336.1	-	-	-
<i>P. acatallelus</i>	-	JN104675.1	JN371120.1	-	-	-	-	KT898368.1
<i>P. acerus</i>	EF493678.1	EF493678.1	-	-	-	-	-	-
<i>P. achatinus</i>	EF493827.1	JN104676.1	JN371121.1	-	-	-	JQ025168.1	KT898335.1
<i>P. achuar</i>	-	EU130626.1	-	EU130668.1	-	-	MH481368.1	-
<i>P. actites</i>	EF493696.1	EF493696.1	-	-	-	-	EF493432.1	EF493494.1
<i>P. acuminatus</i>	-	EU130579.1	-	EU130577.1	-	-	-	-
<i>P. afrox</i>	-	-	-	-	-	-	MT372578.1	-
<i>P. albertus</i>	EU186695.1	-	-	-	-	-	-	-
<i>P. altae</i>	JN991496.1	-	JN991361.1	-	-	EU443185.1	JQ025174.1	JN991560.1
<i>P. altamazonicus</i>	EF493670.1	EF493670.1	MF118717.1	MH516238.1	-	GU168782.1	MF118735.1	MF118706.1
<i>P. altamnis</i>	-	EU130617.1	-	EU130673.1	-	-	MH481369.1	-
<i>P. andinognomus</i>	-	KY967671.1	-	-	KY967652.1	-	KY967688.1	-
<i>P. angustilineatus</i>	-	JN104677.1	JN371123.1	-	-	-	-	KT898341.1
<i>P. aniptopalatus</i>	EF493390.1	EF493390.1	-	-	-	-	-	-

<i>P. antisuyu</i>	-	<u>MG820148.</u> <u>1</u>	<u>MG820176.</u> <u>1</u>	-	-	-	-	-
<i>P. appendiculatus</i>	<u>EF493524.1</u>	<u>EF493524.1</u>	-	-	-	-	-	-
<i>P. ardalonychus</i>	<u>EU186664.1</u>	<u>EU186664.1</u>	-	-	-	-	-	-
<i>P. atillo</i>	-	<u>MK881444.</u> <u>1</u>	-	-	-	-	<u>MK881347.</u> <u>1</u>	-
<i>P. atratus</i>	-	<u>MK881473.</u> <u>1</u>	-	-	<u>MK881471.</u> <u>1</u>	-	<u>MK881364.</u> <u>1</u>	-
<i>P. attenboroughi</i>	<u>KY594763.1</u>	<u>KY594755.1</u>	<u>KY962784.1</u>	-	-	-	<u>KY962760.1</u>	-
<i>P. aureoventris</i>	-	<u>JQ742151.1</u>	-	-	<u>JQ742333.1</u>	-	-	-
<i>P. bambu</i>	<u>JF906319.1</u>	-	-	-	<u>KY967654.1</u>	-	<u>KY967693.1</u>	-
<i>P. bicantus</i>	-	<u>MK881420.</u> <u>1</u>	-	-	-	-	<u>MK881325.</u> <u>1</u>	-
<i>P. boulengeri</i>	-	<u>KY494232.1</u>	<u>KU724444.1</u>	<u>DQ195480.1</u>	-	-	<u>KY494205.1</u>	<u>KY494217.1</u>
<i>P. bounides</i>	-	<u>KY962797.1</u>	<u>KY962789.1</u>	-	-	-	<u>KY962771.1</u>	-
<i>P. brevicrus</i>	-	<u>MF118702.1</u>	<u>MF118727.1</u>	-	-	-	<u>MF118751.1</u>	-
<i>P. brevifrons</i>	<u>JN991498.1</u>	<u>JN370957.1</u>	<u>JN371048.1</u>	-	-	-	-	<u>KT898343.1</u>
<i>P. briceni</i>	<u>JX155297.1</u>	<u>JX155297.1</u>	-	-	-	-	-	-
<i>P. bromeliaceus</i>	<u>EF493351.1</u>	<u>EF493351.1</u>	-	-	-	-	-	-
<i>P. buckleyi</i>	<u>EF493350.1</u>	<u>DQ679379.1</u>	-	-	-	-	-	-
<i>P. buenaventura</i>	<u>KU999242.1</u>	<u>KU999170.1</u>	-	-	-	-	-	-
<i>P. cajamarcensis</i>	<u>EF493823.1</u>	<u>EF493663.1</u>	-	-	-	-	-	-
<i>P. cajanuma</i>	<u>MK993332.</u> <u>1</u>	<u>MK604536.</u> <u>1</u>	-	-	-	-	<u>MK602184.</u> <u>1</u>	-
<i>P. calcaratus</i>	-	<u>JN104657.1</u>	-	-	-	-	<u>KT898307.1</u>	<u>KT898346.1</u>
<i>P. calcarulatus</i>	<u>EF493523.1</u>	<u>EF493523.1</u>	-	-	-	-	-	-
<i>P. capitonis</i>	-	-	-	-	-	-	<u>KT898298.1</u>	<u>KT898333.1</u>
<i>P. caprifer</i>	<u>EF493391.1</u>	<u>EF493391.1</u>	-	-	-	-	-	-
<i>P. carvalhoi</i>	-	<u>DQ195454.1</u>	<u>KY672983.1</u>	<u>DQ195481.1</u>	-	-	<u>KY672967.1</u>	<u>KY681072.1</u>
<i>P. caryophyllaceus</i>	<u>EU186686.1</u>	<u>EU186686.1</u>	<u>FJ766776.1</u>	-	-	-	<u>KJ201964.1</u>	<u>JN991563.1</u>
<i>P. cedros</i>	-	<u>EF493523.1</u>	-	-	-	-	-	-

<i>P. celator</i>	<u>EF493685.1</u>	<u>EF493685.1</u>	-	-	-	-	-	-
<i>P. cerasinus</i>	<u>JN991502.1</u>	<u>FJ784387.1</u>	<u>FJ766786.1</u>	-	-	<u>EU443194.1</u>	<u>JQ025178.1</u>	<u>JN991565.1</u>
<i>P. ceuthospilus</i>	<u>EF493520.1</u>	<u>EF493520.1</u>	-	-	-	-	-	-
<i>P. chalceus</i>	<u>EF493675.1</u>	<u>EF493675.1</u>	-	-	-	-	-	-
<i>P. chiastonotus</i>	-	<u>JN691273.1</u>	-	-	-	-	-	<u>JN692005.1</u>
<i>P. chloronotus</i>	<u>AY326007.1</u>	<u>AY326007.1</u>	-	-	-	-	-	-
<i>P. chocoensis</i>	-	-	-	-	-	-	<u>MT372629.1</u>	-
<i>P. cisnerosi</i>	-	<u>MT372697.1</u>	-	-	<u>MT372570.1</u>	-	<u>MT372625.1</u>	-
<i>P. citriogaster</i>	<u>EF493700.1</u>	<u>EF493700.1</u>	-	-	-	-	-	-
<i>P. condor</i>	<u>EF493701.1</u>	<u>EF493701.1</u>	-	-	-	-	<u>EF493443.1</u>	<u>EF493504.1</u>
<i>P. conservatio</i>	<u>JX155287.1</u>	<u>JX155287.1</u>	-	-	-	-	-	-
<i>P. conspicillatus</i>	<u>EF493529.1</u>	<u>EF493529.1</u>	-	-	-	-	<u>EF493437.1</u>	<u>EF493499.1</u>
<i>P. cremnobates</i>	<u>EF493528.1</u>	<u>EF493528.1</u>	-	-	-	-	<u>EF493424.1</u>	<u>EF493486.1</u>
<i>P. crenunguis</i>	<u>EF493693.1</u>	-	-	-	-	-	-	-
<i>P. croceoinguinis</i>	<u>EF493669.1</u>	<u>EF493665.1</u>	<u>KY962787.1</u>	<u>MH516243.1</u>	-	-	<u>KY962767.1</u>	-
<i>P. crucifer</i>	<u>EU186736.1</u>	<u>EU186718.1</u>	-	-	-	-	-	-
<i>P. cruciocularis</i>	<u>EU186656.1</u>	<u>KY006095.1</u>	<u>KY962788.1</u>	-	-	-	<u>KY962768.1</u>	-
<i>P. cruentus</i>	<u>EF493697.1</u>	<u>EF493697.1</u>	<u>KC129259.1</u>	-	<u>FJ882747.1</u>	<u>EU443188.1</u>	<u>JQ025181.1</u>	<u>JN991569.1</u>
<i>P. cryophilus</i>	<u>EF493672.1</u>	<u>EF493672.1</u>	-	-	-	-	-	-
<i>P. cryptomelas</i>	-	<u>MK881475.1</u>	-	-	<u>MK881475.1</u>	-	<u>MK881365.1</u>	-
<i>P. curtipes</i>	<u>EF493513.1</u>	<u>EF493513.1</u>	-	-	<u>AY819473.1</u>	-	<u>KX525470.1</u>	<u>EF493497.1</u>
<i>P. danae</i>	-	<u>KY652652.1</u>	<u>KY672984.1</u>	<u>EF636949.1</u>	-	-	<u>KY672968.1</u>	<u>KY681073.1</u>
<i>P. devillei</i>	<u>EF493688.1</u>	<u>EF493688.1</u>	-	-	-	-	-	-
<i>P. diadematus</i>	<u>EU186668.1</u>	<u>EU186668.1</u>	-	-	-	-	-	-
<i>P. dorado</i>	-	<u>KU496877.1</u>	<u>KU496873.1</u>	-	-	-	-	-
<i>P. dorsopictus</i>	<u>KP082864.1</u>	<u>KU724440.1</u>	<u>KU724448.1</u>	-	-	-	-	<u>KP082879.1</u>
<i>P. duellmani</i>	<u>AY326003.1</u>	<u>AY326003.1</u>	-	-	-	-	<u>EF493438.1</u>	<u>EF493500.1</u>

<i>P. ecuadorensis</i>	<u>KX785339.1</u>	-	-	-	<u>KX785348.1</u>	-	-	-
<i>P. eremitus</i>	-	<u>MT508757.1</u>	-	-	-	-	-	-
<i>P. eriphus</i>	<u>EU186671.1</u>	<u>EU186671.1</u>	-	<u>DQ195484.1</u>	-	-	-	-
<i>P. erythroinguinis</i>	-	<u>MG820143.</u> <u>1</u>	<u>MG820170.</u> <u>1</u>	-	-	-	-	-
<i>P. euphronides</i>	<u>EF493527.1</u>	<u>EF493527.1</u>	-	-	-	<u>EF493427.1</u>	<u>EF493489.1</u>	-
<i>P. fenestratus</i>	<u>EF493703.1</u>	<u>EF493703.1</u>	<u>KU494666.1</u>	<u>EF636949.1</u>	-	-	-	-
<i>P. ferwerdai</i>	-	<u>MN080223.</u> <u>1</u>	-	-	-	-	-	-
<i>P. flavobracatus</i>	-	<u>MG820163.</u> <u>1</u>	<u>MG820188.</u> <u>1</u>	-	-	-	-	-
<i>P. gagliardoi</i>	-	<u>MK881480.</u> <u>1</u>	-	-	-	<u>MK881355.</u> <u>1</u>	-	-
<i>P. galdi</i>	<u>EU186670.1</u>	<u>EU186670.1</u>	-	-	-	-	<u>EU186767.1</u>	-
<i>P. gentryi</i>	<u>EF493511.1</u>	<u>EF493511.1</u>	-	-	-	-	-	-
<i>P. ginesi</i>	<u>JX155295.1</u>	<u>JX155295.1</u>	-	-	-	-	-	-
<i>P. giorgii</i>	-	<u>MK992573.</u> <u>1</u>	<u>MN010717.</u> <u>1</u>	-	-	-	-	-
<i>P. glandulosus</i>	<u>EF493676.1</u>	<u>EF493676.1</u>	-	-	-	-	-	-
<i>P. gloria</i>	-	<u>MK881402.</u> <u>1</u>	-	-	<u>MK881402.</u> <u>1</u>	<u>MK881316.</u> <u>1</u>	-	-
<i>P. gryllus</i>	<u>JX306022.1</u>	<u>JX306022.1</u>	-	-	-	-	-	-
<i>P. gutturalis</i>	<u>JN690705.1</u>	<u>JN691313.1</u>	-	-	-	-	<u>JN692012.1</u>	-
<i>P. hampatusami</i>	-	<u>MK881504.</u> <u>1</u>	-	-	<u>MK881504.</u> <u>1</u>	<u>MK881387.</u> <u>1</u>	-	-
<i>P. hectus</i>	-	<u>JN104680.1</u>	<u>JN371130.1</u>	-	-	-	<u>KT898352.1</u>	-
<i>P. humboldti</i>	-	<u>KY962799.1</u>	<u>KY962792.1</u>	-	-	<u>KY962776.1</u>	-	-
<i>P. inguinalis</i>	<u>EU186676.1</u>	<u>EU186676.1</u>	-	-	-	-	<u>JN692014.1</u>	-
<i>P. inusitatus</i>	<u>EF493677.1</u>	<u>EF493677.1</u>	-	-	-	-	-	-
<i>P. jaguensis</i>	<u>KP082862.1</u>	<u>KP082873.1</u>	<u>KP082877.1</u>	-	-	-	<u>KP082878.1</u>	-
<i>P. jimenezi</i>	-	<u>MK881468.</u> <u>1</u>	-	-	<u>MK881466.</u> <u>1</u>	<u>MK881360.</u> <u>1</u>	-	-
<i>P. juanchoi</i>	-	<u>JN104681.1</u>	-	-	-	-	<u>KT898353.1</u>	-

<i>P. jubatus</i>	-	<u>JN370977.1</u>	<u>JN371075.1</u>	-	-	-	-	<u>KT898356.1</u>
<i>P. kelephas</i>	-	<u>JN104662.1</u>	-	-	-	-	-	<u>KT898359.1</u>
<i>P. kichwarum</i>	-	<u>EU130582.1</u>	-	<u>EU130636.1</u>	-	-	-	-
<i>P. koehlerii</i>	<u>FJ438810.1</u>	-	-	-	-	-	-	-
<i>P. labiosus</i>	<u>EF493694.1</u>	<u>MT372686.1</u>	-	-	-	-	-	-
<i>P. lancinii</i>	<u>JX155284.1</u>	<u>JX155284.1</u>	-	-	-	-	-	-
<i>P. lanthanites</i>	<u>EF493695.1</u>	<u>EF493695.1</u>	-	-	-	-	-	-
<i>P. lasalleorum</i>	<u>KY494221.1</u>	<u>KY494221.1</u>	<u>KY627812.1</u>	-	-	-	-	-
<i>P. latidiscus</i>	<u>EF493698.1</u>	<u>EF493698.1</u>	-	-	-	-	-	-
<i>P. latro</i>	-	<u>MN865341.1</u>	<u>MN010741.1</u>	-	-	-	-	-
<i>P. leoni</i>	<u>EF493684.1</u>	<u>EF493684.1</u>	-	-	-	-	<u>EF493433.1</u>	<u>EF493495.1</u>
<i>P. leopardus</i>	-	<u>KY627792.1</u>	<u>KY627819.1</u>	-	-	-	-	-
<i>P. librarius</i>	<u>JN991515.1</u>	<u>JN991451.1</u>	<u>JN991379.1</u>	<u>MH516245.1</u>	-	-	<u>MH481370.1</u>	<u>JN991571.1</u>
<i>P. lirellus</i>	<u>EF493521.1</u>	<u>EF493521.1</u>	-	-	-	-	-	-
<i>P. lividus</i>	-	<u>MK881497.1</u>	-	-	<u>MK881497.1</u>	-	<u>MK881382.1</u>	-
<i>P. luscombei</i>	<u>KP064143.1</u>	<u>KP064156.1</u>	-	-	-	-	-	-
<i>P. luteolateralis</i>	<u>EF493517.1</u>	<u>EF493517.1</u>	-	-	-	-	-	-
<i>P. lutitus</i> ANDES-A 1771	-	<u>KP149401.1</u>	-	-	-	-	-	-
<i>P. lutzae</i>	-	<u>MK881424.1</u>	-	-	<u>MK881421.1</u>	-	<u>MK881326.1</u>	-
<i>P. lymani</i>	<u>EF493392.1</u>	<u>EF493392.1</u>	-	-	-	-	-	-
<i>P. lynchi</i>	-	<u>DQ195463.1</u>	-	-	-	-	-	-
<i>P. maculosus</i>	-	<u>KY494240.1</u>	-	-	-	-	<u>KY494210.1</u>	-
<i>P. malkini</i>	<u>EU186663.1</u>	<u>EU186663.1</u>	-	-	-	-	-	-
<i>P. mallii</i>	-	<u>MK391386.1</u>	-	-	<u>MK391386.1</u>	-	<u>MK391384.1</u>	-
<i>P. marmoratus</i>	<u>EU186692.1</u>	<u>EU186692.1</u>	-	-	-	-	-	<u>JN692010.1</u>

<i>P. martiae</i>	<u>JN991517.1</u>	-	<u>JN991380.1</u>	<u>MH516247.1</u>	-	-	<u>MF118755.1</u>	<u>JN991572.1</u>
<i>P. matidiktyo</i>	<u>KP064140.1</u>	<u>KP064147.1</u>	<u>KP064159.1</u>	-	-	-	-	-
<i>P. mazar</i>	-	<u>KY967664.1</u>	-	-	<u>KY967647.1</u>	-	<u>KY967685.1</u>	-
<i>P. melanogaster</i>	<u>EF493826.1</u>	<u>EF493664.1</u>	-	-	-	-	-	-
<i>P. mendax</i>	<u>EU186659.1</u>	<u>EU186659.1</u>	-	-	-	-	-	-
<i>P. merostictus</i>	-	<u>DQ195465.1</u>	-	<u>DQ195488.1</u>	-	-	-	-
<i>P. miktos</i>	-	<u>MK391385.1</u>	<u>KP064163.1</u>	-	<u>MK391385.1</u>	-	<u>MK391383.1</u>	<u>MF118703.1</u>
<i>P. mindo</i>	-	<u>KF801581.1</u>	-	-	-	-	-	-
<i>P. minutulus</i>	<u>EU186657.1</u>	<u>EU186657.1</u>	-	-	-	-	-	-
<i>P. moa</i>	-	<u>MK992550.1</u>	<u>MN010697.1</u>	-	-	-	-	-
<i>P. moro</i>	<u>JN991520.1</u>	<u>JN991453.1</u>	<u>JN991384.1</u>	-	-	-	<u>JQ025192.1</u>	<u>JN991575.1</u>
<i>P. multicolor</i>	-	<u>MK881489.1</u>	-	-	-	-	-	-
<i>P. muranunka</i>	-	<u>KY967661.1</u>	-	-	<u>KY967642.1</u>	-	<u>KY967680.1</u>	-
<i>P. muscosus</i>	-	<u>MK881501.1</u>	-	-	<u>MK881501.1</u>	-	<u>MK881386.1</u>	-
<i>P. museosus</i>	<u>JN991521.1</u>	<u>KC014941.1</u>	-	-	-	<u>AY273103.1</u>	<u>JQ025193.1</u>	<u>JN991576.1</u>
<i>P. mutabilis</i>	<u>KM675434.1</u>	<u>KM675458.1</u>	-	-	-	-	-	-
<i>P. myops</i>	-	<u>JN104682.1</u>	<u>JN371132.1</u>	-	-	-	-	<u>KT898361.1</u>
<i>P. nangaritza</i>	-	<u>MK881436.1</u>	-	-	<u>MK881436.1</u>	-	<u>MK881336.1</u>	-
<i>P. nervicus</i>	<u>JN991522.1</u>	<u>JN991456.1</u>	<u>JN991386.1</u>	-	-	-	<u>JQ025194.1</u>	<u>JN991577.1</u>
<i>P. nietoi</i>	<u>KU999286.1</u>	<u>KU999214.1</u>	-	-	-	-	-	-
<i>P. nyctophylax</i>	<u>EF493526.1</u>	<u>EF493526.1</u>	-	-	-	-	<u>EF493425.1</u>	<u>EF493487.1</u>
<i>P. ockendeni</i>	<u>EF493519.1</u>	<u>EF493519.1</u>	<u>KY672986.1</u>	-	-	-	<u>KY672970.1</u>	<u>KY681075.1</u>
<i>P. ocreatus</i>	<u>EF493682.1</u>	<u>EF493682.1</u>	-	-	-	-	-	-
<i>P. omeviridis</i>	-	<u>MK881398.1</u>	-	-	-	-	<u>MK881312.1</u>	-

<i>P. orcesi</i>	<u>EF493679.1</u>	<u>EF493679.1</u>	-	-	-	-	-	-
<i>P. orestes</i>	<u>EF493388.1</u>	<u>EF493388.1</u>	-	-	<u>KY967651.1</u>	-	<u>KY967689.1</u>	-
<i>P. ornatissimus</i>	<u>KX785338.1</u>	-	-	-	<u>KU720480.1</u>	-	-	-
<i>P. ornatus</i>	<u>EU186660.1</u>	<u>EU186660.1</u>	-	-	-	-	-	-
<i>P. orpacobates</i>	-	-	<u>JN371133.1</u>	-	-	-	-	<u>KT898363.1</u>
<i>P. paisa</i>	<u>JN991524.1</u>	<u>JN991477.1</u>	<u>JN991389.1</u>	-	-	-	-	<u>JN991578.1</u>
<i>P. palmeri</i>	-	<u>JN371003.1</u>	<u>JN371118.1</u>	-	-	-	-	<u>KT898366.1</u>
<i>P. paramerus</i>	<u>JX155279.1</u>	<u>JX155279.1</u>	-	-	-	-	-	-
<i>P. pardalis</i>	<u>JN991527.1</u>	<u>FJ784590.1</u>	<u>FJ766804.1</u>	-	-	<u>AY273102.1</u>	<u>JQ025198.1</u>	<u>JN991579.1</u>
<i>P. parectatus</i>	<u>KY494222.1</u>	<u>KY494222.1</u>	<u>KY627826.1</u>	-	-	-	<u>KY494207.1</u>	<u>KY494220.1</u>
<i>P. parvillus</i>	<u>EF493352.1</u>	<u>EF493352.1</u>	-	-	-	-	-	-
<i>P. peraticus</i>	<u>KY494224.1</u>	<u>KY494224.1</u>	-	-	-	-	<u>KY494208.1</u>	-
<i>P. peruvianus</i>	<u>EF493707.1</u>	<u>EF493707.1</u>	<u>JN991392.1</u>	<u>DQ195492.1</u>	-	-	<u>EF493436.1</u>	<u>EF493498.1</u>
<i>P. pharangobates</i>	-	<u>KY652655.1</u>	<u>KY672987.1</u>	-	-	-	<u>KY681088.1</u>	<u>KY681076.1</u>
<i>P. phoxocephalus</i>	-	<u>MK881507.1</u>	-	-	<u>MK881507.1</u>	-	<u>MK881390.1</u>	-
<i>P. pichincha</i>	-	<u>MK881399.1</u>	-	-	<u>MK881399.1</u>	-	<u>MK881313.1</u>	-
<i>P. pictus</i>	-	<u>MK992524.1</u>	<u>MN010670.1</u>	-	-	-	-	-
<i>P. pirrensis</i>	<u>JN991528.1</u>	<u>JN991462.1</u>	<u>JN991393.1</u>	-	-	<u>EU443190.1</u>	<u>JQ025199.1</u>	<u>JN991580.1</u>
<i>P. platydactylus</i>	<u>FJ438811.1</u>	<u>EU192255.1</u>	<u>JN991394.1</u>	<u>EF636948.1</u>	-	-	<u>KY672971.1</u>	<u>KY681077.1</u>
<i>P. pleurostriatus</i>	<u>JX155278.1</u>	<u>JX155292.1</u>	-	-	-	-	-	-
<i>P. pluvialis</i>	-	<u>KX155578.1</u>	<u>KX155585.1</u>	-	-	-	<u>KY962770.1</u>	-
<i>P. pluvian</i>	-	<u>MK992577.1</u>	<u>MN010732.1</u>	-	-	-	-	-
<i>P. prolatus</i>	<u>EU186701.1</u>	<u>EU186701.1</u>	-	-	-	-	-	-
<i>P. prometeii</i>	-	<u>KX525478.1</u>	-	-	-	-	<u>KX525471.1</u>	-
<i>P. ptochus</i>	<u>JN991530.1</u>	-	<u>JN991395.1</u>	-	-	-	-	<u>JN991581.1</u>
<i>P. puipui</i>	-	<u>KY962800.1</u>	-	-	-	-	<u>KY962777.1</u>	-

<i>P. pycnodermis</i>	<u>EF493680.1</u>	<u>EF493680.1</u>	-	-	-	-	-	-
<i>P. pyrrhomerus</i>	<u>EF493683.1</u>	<u>EF493683.1</u>	-	-	-	-	<u>MN068025.1</u>	-
<i>P. quantus</i>	-	<u>JN104684.1</u>	<u>JN371136.1</u>	-	-	-	-	<u>KT898367.1</u>
<i>P. quaquaversus</i>	-	<u>EU130580.1</u>	<u>JN991396.1</u>	<u>EU130578.1</u>	-	-	<u>MH481371.1</u>	<u>JN991582.1</u>
<i>P. quinquagesimus</i>	<u>EF493690.1</u>	<u>EF493690.1</u>	-	-	-	-	-	-
<i>P. quintanai</i>	<u>MK993337.1</u>	<u>MK604544.1</u>	-	-	-	-	-	-
<i>P. reichlei</i>	<u>EF493707.1</u>	<u>EF493707.1</u>	<u>KY672989.1</u>	-	-	-	<u>KY672972.1</u>	<u>KY681078.1</u>
<i>P. rhabdolaemus</i>	-	<u>EF493706.1</u>	-	-	-	-	-	-
<i>P. rhodoplichus</i>	<u>EF493674.1</u>	<u>EF493674.1</u>	-	-	-	-	-	-
<i>P. ridens</i>	<u>EF493355.1</u>	<u>EF493355.1</u>	<u>FJ766808.1</u>	-	-	<u>EU443160.1</u>	<u>JQ025204.1</u>	<u>JN991586.1</u>
<i>P. riveti</i>	<u>EF493348.1</u>	<u>EF493348.1</u>	-	-	-	-	-	-
<i>P. rubicundus</i>	-	<u>MT372715.1</u>	-	-	<u>MK881407.1</u>	-	<u>MK881320.1</u>	-
<i>P. rupicola</i>	-	<u>MN954203.1</u>	-	-	-	-	-	-
<i>P. sagittulus</i>	<u>EF493705.1</u>	<u>EF493705.1</u>	-	-	-	-	<u>EF493439.1</u>	<u>EF493501.1</u>
<i>P. saltissimus</i>	<u>EU186693.1</u>	<u>EU186693.1</u>	-	-	<u>JQ742340.1</u>	-	-	-
<i>P. samaipatae</i>	<u>FJ438814.1</u>	-	-	<u>EU368890.1</u>	-	-	-	-
<i>P. satagius</i>	-	<u>MN078266.1</u>	-	-	-	-	-	-
<i>P. saturninoi</i>	<u>MK993329.1</u>	<u>MK604534.1</u>	-	-	-	-	-	-
<i>P. schultei</i>	<u>EF493681.1</u>	<u>EF493681.1</u>	-	-	-	-	-	-
<i>P. scoloblepharus</i>	-	<u>KY494236.1</u>	<u>KY627834.1</u>	-	-	-	<u>KY494213.1</u>	<u>KY494218.1</u>
<i>P. shrevei</i>	<u>EF493692.1</u>	<u>EF493692.1</u>	-	-	-	-	-	-
<i>P. simonbolivari</i>	<u>EF493671.1</u>	<u>EF493671.1</u>	-	-	<u>KY967657.1</u>	-	<u>KY967695.1</u>	-
<i>P. simonsii</i>	<u>EU186665.1</u>	<u>EU186665.1</u>	-	-	-	-	-	-
<i>P. simoterus</i>	-	<u>DQ195471.1</u>	-	<u>DQ195495.1</u>	-	-	-	-
<i>P. skydmainos</i>	<u>EF493393.1</u>	<u>EF493393.1</u>	-	-	-	-	-	-

<i>P. sobetes</i>	<u>KM675429.</u> <u>1</u>	<u>KM675449.</u> <u>1</u>	-	-	-	-	-	-
<i>P. spinosus</i>	<u>EF493673.1</u>	<u>EF493673.1</u>	-	-	-	-	-	-
<i>P. sternothylax</i>	-	<u>MK881393.</u> <u>1</u>	-	-	<u>MK881393.</u> <u>1</u>	-	<u>MK881308.</u> <u>1</u>	-
<i>P. subsigillatus</i>	<u>EF493525.1</u>	<u>EF493525.1</u>	-	-	-	-	-	-
<i>P. suetus</i>	<u>JN991537.1</u>	<u>JN991469.1</u>	-	-	-	-	-	-
<i>P. supernatis</i>	<u>AY326005.1</u>	<u>AY326005.1</u>	-	-	-	-	-	-
<i>P. surdus</i>	<u>EF493687.1</u>	<u>EF493687.1</u>	-	-	-	-	-	-
<i>P. terraebolivaris</i>	<u>EU186650.1</u>	<u>EU186650.1</u>	-	-	-	-	-	-
<i>P. thectopternus</i>	-	<u>JN104685.1</u>	-	-	-	-	-	<u>KT898369.1</u>
<i>P. thymalopsoides</i>	<u>EF493514.1</u>	<u>EF493514.1</u>	-	-	-	-	-	-
<i>P. thymelensis</i>	<u>EF493516.1</u>	<u>EF493516.1</u>	<u>JX564889.1</u>	-	<u>JX564889.1</u>	<u>JX564889.1</u>	-	<u>EF493503.1</u>
<i>P. tiktik</i>	<u>MH668161.</u> <u>1</u>	<u>MH668276.</u> <u>1</u>	-	-	-	-	<u>MH708576.</u> <u>1</u>	-
<i>P. tinguichaca</i>	-	<u>MK881499.</u> <u>1</u>	-	-	<u>MK881418.</u> <u>1</u>	-	<u>MK881385.</u> <u>1</u>	-
<i>P. toftae</i>	<u>EF493353.1</u>	<u>EF493353.1</u>	<u>KY672991.1</u>	-	-	-	<u>KY672974.1</u>	<u>KY681080.1</u>
<i>P. torresi</i>	-	<u>MK881492.</u> <u>1</u>	-	-	<u>MK881492.</u> <u>1</u>	-	<u>MK881380.</u> <u>1</u>	-
<i>P. totoroi</i>	-	<u>MK881505.</u> <u>1</u>	-	-	<u>MK881406.</u> <u>1</u>	-	<u>MK881388.</u> <u>1</u>	-
<i>P. trepidotus</i>	<u>EF493515.1</u>	<u>EF493515.1</u>	-	-	-	-	-	-
<i>P. urani</i>	-	<u>KU724441.1</u>	<u>KU724450.1</u>	-	-	-	-	-
<i>P. uranobates</i>	<u>KY494225.1</u>	<u>KY494225.1</u>	<u>KY627836.1</u>	-	-	-	<u>KY494214.1</u>	-
<i>P. urichi</i>	<u>EF493699.1</u>	<u>EF493699.1</u>	-	-	-	-	<u>EF493426.1</u>	<u>EF493488.1</u>
<i>P. vanadise</i>	<u>JX155296.1</u>	<u>JX155296.1</u>	-	-	-	-	-	-
<i>P. ventrimarmoratus</i>	<u>JF906310.1</u>	-	-	-	-	-	-	-
<i>P. verecundus</i>	<u>EF493686.1</u>	<u>EF493686.1</u>	-	-	-	-	-	-
<i>P. verrucolatus</i>	-	<u>MK881467.</u> <u>1</u>	-	-	<u>MK881469.</u> <u>1</u>	-	<u>MK881361.</u> <u>1</u>	-
<i>P. versicolor</i>	<u>EF493389.1</u>	<u>MK881479.</u> <u>1</u>	-	-	<u>MK881474.</u> <u>1</u>	-	<u>MK881367.</u> <u>1</u>	-

<i>P. vertebralis</i>	<u>EF493689.1</u>	<u>EF493689.1</u>	-	-	-	-	-	-
<i>P. vilarsi</i>	-	<u>KP149438.1</u>	<u>KP149232.1</u>	-	-	-	-	-
<i>P. vinhai</i>	<u>JX267343.1</u>	-	-	-	-	-	<u>JX267591.1</u>	<u>JX267726.1</u>
<i>P. w-nigrum</i>	<u>AY326004.1</u>	<u>AY326004.1</u>	<u>JN371140.1</u>	<u>KC857704.1</u>	-	-	-	<u>KT898370.1</u>
<i>P. walkeri</i>	<u>KU999291.1</u>	<u>KU999230.1</u>	-	-	-	-	<u>EF493428.1</u>	<u>EF493490.1</u>
<i>P. wiensi</i>	<u>EF493377.1</u>	<u>EF493668.1</u>	-	-	-	-	-	-
<i>P. yantzaza</i>	-	-	-	-	-	-	<u>MF615251.1</u>	-
<i>P. yuruaniensis</i>	-	<u>JQ742160.1</u>	-	-	<u>JQ742335.1</u>	-	-	-
<i>P. zeuctotylus</i>	<u>EU186678.1</u>	<u>EU186678.1</u>	-	-	-	-	-	<u>JN691914.1</u>
<i>P. zophus</i>	<u>JN991548.1</u>	<u>JN991480.1</u>	<u>JN991413.1</u>	-	-	-	<u>JQ025214.1</u>	<u>JN991598.1</u>
<i>P. affinis</i> ANDES-A 4217	-	-	-	-	-	-	-	-
<i>P. anolirex</i> MCNUPH66	<u>MN215421.</u> <u>1</u>	<u>MN215441.</u> <u>1</u>	-	<u>MN218386.</u> <u>1</u>	-	-	<u>MN225954.</u> <u>1</u>	<u>MN218371.</u> <u>1</u>
<i>P. ardilae</i> MCNUPH117	<u>MN215410.</u> <u>1</u>	<u>MN215429.</u> <u>1</u>	-	<u>MN218376.</u> <u>1</u>	-	-	<u>MN225947.</u> <u>1</u>	<u>MN218365.</u> <u>1</u>
<i>P. bicolor</i> ANDES-A 4939	-	-	-	-	-	-	-	-
<i>P. bowara</i> MCNUPH160	<u>MN215407.</u> <u>1</u>	<u>MN215432.</u> <u>1</u>	-	<u>MN218375.</u> <u>1</u>	-	-	<u>MN225942.</u> <u>1</u>	<u>MN218360.</u> <u>1</u>
<i>P. carrangerorum</i> ANDES-A 1254	-	-	-	-	-	-	-	-
<i>P. cf batrachites</i> MCNUPH37	-	-	-	-	-	-	-	-
<i>P. cf bogotensis</i> ANDES-A 4840	-	-	-	-	-	-	-	-
<i>P. cf erythropleura</i> EAB 6873	-	-	-	-	-	-	-	-
<i>P. cf viejas</i> ANDES-A 6850	-	-	-	-	-	-	-	-
<i>P. elegans</i> ANDES-A 4932	-	-	-	-	-	-	-	-
<i>P. fallax</i> ANDES-A 3045	-	-	-	-	-	-	-	-
<i>P. frater</i> ANDES-A 4845	-	-	-	-	-	-	-	-
<i>P. gaigei</i> ANDES-A 3045	-	-	-	-	-	-	-	-
<i>P. medemi</i> ANDES-A 4812	-	-	-	-	-	-	-	-
<i>P. miyatai</i> AJC 3473	-	-	-	-	-	-	-	-

	<u>MN215418.</u>	<u>MN215436.</u>	<u>MN218387.</u>	<u>MN218383.</u>			<u>MN225949.</u>	<u>MN218368.</u>
<i>P. nicefori</i> MCNUPH48	<u>1</u>	<u>1</u>	<u>1</u>	<u>1</u>	-	-	<u>1</u>	<u>1</u>
<i>P. savagei</i> ANDES-A 1255	-	-	-	-	-	-	-	-
<i>P. sp1</i> MCNUPH439	-	-	-	-	-	-	-	-
<i>P. myersi</i> MUD-H640	-	-	-	-	-	-	-	-
<i>P. sp2</i> MCNUPH584	-	-	-	-	-	-	-	-
<i>P. sp3</i> MCNUPH54	-	-	-	-	-	-	-	-
<i>P. sp4</i> MCNUPH443	-	-	-	-	-	-	-	-
<i>P. sp5</i> MCNUPH30	-	-	-	-	-	-	-	-
<i>P. sp8</i> MCNUPH525	-	-	-	-	-	-	-	-
<i>P. sp9</i> MCNUPH42	-	-	-	-	-	-	-	-
<i>P. taeniatus</i> EAB 6866	-	-	-	-	-	-	-	-
<i>P. unistrigatus</i> BCQ 991	-	-	-	-	-	-	-	-
<i>P. yukpa</i> MCNUPH29	<u>MN215417.</u> <u>1</u>	<u>MN215439.</u> <u>1</u>	-	-	-	-	<u>MN225951.</u> <u>1</u>	-

Table S4. Database with geographic records (longitude, latitude) for 495 species of *Pristimantis*. Dryad Digital Repository: <https://doi.org/10.5061/dryad.f7m0cfxxq>

Table S5. Pearson's correlation coefficients of the relationship between environmental variables. Dryad Digital Repository: <https://doi.org/10.5061/dryad.80gb5mkrx>

Table S6. Results of the multimodel inference for males, females, and SSD with the importance values of all the environmental variables analyzed.

SSD	Estimate	Std. Error	z value	Pr(> z)	ci.lb	ci.ub	Importance
(Intercept)	0.3422	0.0074	46.3321	0	0.3278	0.3567	1
LOGBIO1_Scale	0.0434	0.0113	3.8356	0.0001	0.0212	0.0656	0.998
LOGBIO4_Scale	-0.0079	0.0101	-0.7815	0.4345	-0.0277	0.0119	0.5372
LOGBIO12_Scale	0.0043	0.0075	0.5695	0.569	-0.0104	0.0189	0.3882
LOGDEM_1_Scale	0.0046	0.0086	0.5404	0.5889	-0.0122	0.0214	0.3698
LOGBIO3_Scale	0.0011	0.0051	0.2062	0.8366	-0.009	0.0111	0.3386
LOGBIO15_Scale	-0.0015	0.0037	-0.4086	0.6828	-0.0087	0.0057	0.3093
LOGBIO2_Scale	-0.0008	0.0032	-0.2369	0.8127	-0.007	0.0055	0.2881
LOGMoisture_1_Scale	0.0004	0.0026	0.1626	0.8709	-0.0046	0.0055	0.2716

Males	Estimate	Std. Error	z value	Pr(> z)	ci.lb	ci.ub	Importance
(Intercept)	1.3811	0.005	277.748	0	1.3713	1.3908	1
LOGBIO15_Scale	0.0199	0.0053	3.7711	0.0002	0.0096	0.0302	0.997
LOGDEM_1_Scale	-0.0156	0.0118	-1.3236	0.1856	-0.0386	0.0075	0.7814
LOGBIO1_Scale	-0.0138	0.0116	-1.1887	0.2345	-0.0365	0.0089	0.736

LOGBIO3_Scale	0.0023	0.0043	0.535	0.5927	-0.0062	0.0108	0.3695
LOGBIO2_Scale	-0.0016	0.0032	-0.4867	0.6265	-0.0079	0.0048	0.3374
LOGMoisture_1_Scale	0.0012	0.0027	0.4368	0.6622	-0.0042	0.0065	0.3155
LOGBIO4_Scale	-0.0006	0.0028	-0.2055	0.8372	-0.0062	0.005	0.3065
LOGBIO12_Scale	-0.001	0.0032	-0.3129	0.7544	-0.0072	0.0052	0.2978

Females	Estimate	Std. Error	z value	Pr(> z)	ci.lb	ci.ub	Importance
(Intercept)	1.5056	0.0053	282.488	0	1.4951	1.516	1
LOGBIO15_Scale	0.018	0.0057	3.1451	0.0017	0.0068	0.0292	0.9868
LOGDEM_1_Scale	-0.0132	0.0089	-1.4786	0.1392	-0.0306	0.0043	0.8154
LOGBIO3_Scale	0.003	0.0053	0.5698	0.5688	-0.0074	0.0135	0.4093
LOGBIO4_Scale	-0.0024	0.0049	-0.4831	0.629	-0.0119	0.0072	0.3817
LOGBIO2_Scale	-0.002	0.0039	-0.5256	0.5992	-0.0096	0.0056	0.3616
LOGBIO1_Scale	-0.0005	0.0038	-0.1348	0.8927	-0.0079	0.0069	0.3288
LOGMoisture_1_Scale	0.0014	0.0031	0.4618	0.6442	-0.0047	0.0075	0.3262
LOGBIO12_Scale	0.0002	0.0028	0.0787	0.9373	-0.0053	0.0057	0.3005

Table S7. Evolution rates of body size for males and females for 257 species of *Pristimantis*. Each species has the associated phylogenetic clade, distribution ecoregions, and age (Ma) of the major phylogenetic clades.

Species	Evolution Rates SVL-Females	Evolution Rates SVL-Males	Clade	Ecoregion	Clade Age (Ma)
<i>P. actites</i>	9.512171	5.748921	G1	Northwestern Andes	19.872
<i>P. afrox</i>	12.446033	7.914128	G1	Chocó	19.872
<i>P. calcaratus</i>	9.506787	5.163201	G1	Northwestern Andes	19.872
<i>P. caryophyllaceus</i>	9.760402	5.24558	G1	Central America	19.872
<i>P. cerasinus</i>	9.329345	5.191085	G1	Central America	19.872
<i>P. cf_erythropleura_EAB_6873</i>	9.310652	5.061522	G1	Northwestern Andes	19.872
<i>P. cf_viejas_EAB_6850</i>	9.357956	5.184619	G1	Northwestern Andes	19.872
<i>P. chocoensis</i>	11.583553	7.571389	G1	Chocó	19.872
<i>P. cisnerosi</i>	9.633041	5.369014	G1	Chocó	19.872
<i>P. cremnobates</i>	9.49941	5.160773	G1	Northwestern Andes	19.872
<i>P. crenunguis</i>	10.326049	5.701081	G1	Northwestern Andes	19.872
<i>P. cruentus</i>	9.412674	5.117103	G1	Central America-Chocó	19.872
<i>P. eriphus</i>	10.065217	6.003064	G1	Northwestern Andes	19.872
<i>P. fallax_AJC_5702</i>	9.37286	5.561999	G1	Colombian Eastern Andes	19.872
<i>P. ferwerdai</i>	9.751789	5.254135	G1	Northwestern Andes	19.872
<i>P. kelephas</i>	9.501039	5.160889	G1	Northwestern Andes	19.872
<i>P. labiosus</i>	9.720551	5.644869	G1	Chocó-Northwestern Andes	19.872
<i>P. lanthanites</i>	9.388528	5.536025	G1	Amazonia-Northwestern Andes	19.872
<i>P. latidiscus</i>	9.646143	5.059515	G1	Chocó-Northwestern Andes	19.872
<i>P. museosus</i>	9.333641	5.040371	G1	Central America-Chocó	19.872
<i>P. orpacobates</i>	9.578442	5.146317	G1	Northwestern Andes	19.872
<i>P. paisa</i>	9.407084	5.06922	G1	Northwestern Andes	19.872
<i>P. prolatus</i>	13.253077	8.869386	G1	Northwestern Andes	19.872
<i>P. ridens</i>	9.575514	5.181924	G1	Central America-Chocó	19.872

<i>P. rubicundus</i>	9.39537	5.481185	G1	Northwestern Andes	19.872
<i>P. satagius</i>	10.067723	5.289508	G1	Northwestern Andes	19.872
<i>P. thectopternus</i>	9.33728	5.721844	G1	Northwestern Andes	19.872
<i>P. w-nigrum</i>	9.435461	5.768667	G1	Northwestern Andes-Colombian Eastern Andes	19.872
<i>P. albertus</i>	8.502011	4.997849	G2a	Central Andes	18.532
<i>P. aniptopalmatus</i>	8.496144	5.013833	G2a	Central Andes	18.532
<i>P. attenboroughi</i>	8.49341	4.992889	G2a	Central Andes	18.532
<i>P. bicantus</i>	9.58605	5.393458	G2a	Northwestern Andes	18.532
<i>P. bounides</i>	8.512014	4.998446	G2a	Central Andes	18.532
<i>P. briceni</i>	9.324526	6.170482	G2a	Venezuelan Andes	18.532
<i>P. caprifer</i>	9.416585	5.550783	G2a	Chocó	18.532
<i>P. conservatio</i>	9.277891	5.383805	G2a	Venezuelan Andes	18.532
<i>P. danae</i>	9.62334	5.107098	G2a	Central Andes	18.532
<i>P. ginesi</i>	9.3105	5.466995	G2a	Venezuelan Andes	18.532
<i>P. humboldti</i>	8.501185	4.998561	G2a	Central Andes	18.532
<i>P. lancinii</i>	9.422764	6.171916	G2a	Venezuelan Andes	18.532
<i>P. ornatus</i>	8.4765	4.99593	G2a	Central Andes	18.532
<i>P. paramerus</i>	9.294382	5.499594	G2a	Venezuelan Andes	18.532
<i>P. peruvianus</i>	9.679426	5.11985	G2a	Amazonia-Northwestern Andes	18.532
<i>P. pharangobates</i>	8.611991	5.231267	G2a	Central Andes	18.532
<i>P. pleurostriatus</i>	9.143825	5.22045	G2a	Venezuelan Andes	18.532
<i>P. puipui</i>	8.541972	5.086567	G2a	Central Andes	18.532
<i>P. reichlei</i>	9.763013	5.244588	G2a	Amazonia-Central Andes	18.532
<i>P. rhabdolaemus</i>	8.87899	5.08136	G2a	Central Andes	18.532
<i>P. sagittulus</i>	8.658198	5.13209	G2a	Central Andes	18.532
<i>P. toftae</i>	8.800726	5.01443	G2a	Amazonia-Central Andes	18.532
<i>P. vanadise</i>	9.238825	5.45502	G2a	Venezuelan Andes	18.532
<i>P. achatinus</i>	9.855723	5.087823	G2b	Chocó-Northwestern Andes	15.917

<i>P. ardilae</i> _MCNUPH117	9.212583	5.372215	G2b	Colombian Eastern Andes	15.917
<i>P. chiastonotus</i>	9.246482	5.117891	G2b	Amazonia-Guiana Shield	15.917
<i>P. citriogaster</i>	9.472241	5.105381	G2b	Amazonia	15.917
<i>P. condor</i>	9.807313	5.141323	G2b	Central Andes-Northwestern Andes	15.917
<i>P. conspicillatus</i>	9.459781	5.227005	G2b	Amazonia	15.917
<i>P. fenestratus</i>	9.876382	5.24274	G2b	Amazonia	15.917
<i>P. gaigei</i> _AJC_4250	9.320323	5.187079	G2b	Colombian Eastern Andes	15.917
<i>P. giorgii</i>	9.154371	5.13124	G2b	Amazonia	15.917
<i>P. gutturalis</i>	9.138264	5.218473	G2b	Amazonia	15.917
<i>P. koehleri</i>	9.869305	5.300795	G2b	Amazonia-Central Andes	15.917
<i>P. latro</i>	9.355884	5.218229	G2b	Amazonia	15.917
<i>P. lymani</i>	10.817913	5.158563	G2b	Central Andes	15.917
<i>P. malkini</i>	9.379263	5.152156	G2b	Amazonia	15.917
<i>P. moa</i>	9.174037	5.19666	G2b	Amazonia	15.917
<i>P. pictus</i>	9.216253	5.121585	G2b	Amazonia	15.917
<i>P. pluvian</i>	9.417496	5.128634	G2b	Amazonia	15.917
<i>P. rupicola</i>	9.235866	5.301103	G2b	Atlantic Forest	15.917
<i>P. samaipatae</i>	9.159753	5.107222	G2b	Amazonia-Central Andes	15.917
<i>P. skydmainos</i>	9.478371	5.102881	G2b	Amazonia	15.917
<i>P. terraebolivaris</i>	9.306715	5.182314	G2b	Northern Sudamerican montane coastal	15.917
<i>P. vilarsi</i>	9.31331	5.914939	G2b	Amazonia-Guiana Shield	15.917
<i>P. vinhai</i>	9.33033	5.64959	G2b	Atlantic Forest	15.917
<i>P. affinis</i> _EAB_004	9.667327	5.35713	G2b	Colombian Eastern Andes	16.318
<i>P. anolirex</i> _MCNUPH66	19.185214	7.386947	G2b	Colombian Eastern Andes	16.318
<i>P. bowara</i> _MCNUPH160	9.536679	5.175247	G2b	Colombian Eastern Andes	16.318
<i>P. carrangerorum</i> _LSB_385	10.071224	5.400614	G2b	Colombian Eastern Andes	16.318
<i>P. cf_batrachites</i> _MCNUPH37	19.022541	6.676212	G2b	Colombian Eastern Andes	16.318
<i>P. dorado</i>	11.189342	5.223418	G2b	Colombian Eastern Andes	16.318

<i>P. elegans_AJC_6999</i>	9.52673	6.431317	G2b	Colombian Eastern Andes	16.318
<i>P. euphronides</i>	9.626438	5.115924	G2b	Northern Sudamerican montane coastal	16.318
<i>P. lutitus_AJC_3490</i>	9.437978	5.264836	G2b	Colombian Eastern Andes	16.318
<i>P. lynchi</i>	9.831527	5.421929	G2b	Colombian Eastern Andes	16.318
<i>P. medemi_AJC_6956</i>	9.522805	5.574284	G2b	Colombian Eastern Andes	16.318
<i>P. merostictus</i>	13.768978	5.479254	G2b	Colombian Eastern Andes	16.318
<i>P. nervicus</i>	9.930254	5.572889	G2b	Colombian Eastern Andes	16.318
<i>P. nicefori_MCNUPH48</i>	9.53623	5.70323	G2b	Colombian Eastern Andes	16.318
<i>P. savagei_LSB_383</i>	9.455047	5.316842	G2b	Colombian Eastern Andes	16.318
<i>P. shrevei</i>	9.397546	5.133366	G2b	Northern Sudamerican montane coastal	16.318
<i>P. sp1_MCNUPH439</i>	13.774103	5.481896	G2b	Colombian Eastern Andes	16.318
<i>P. sp2_MCNUPH584</i>	19.114385	6.256868	G2b	Colombian Eastern Andes	16.318
<i>P. sp3_MCNUPH54</i>	18.711861	6.043992	G2b	Colombian Eastern Andes	16.318
<i>P. sp4_MCNUPH443</i>	18.158757	6.132519	G2b	Colombian Eastern Andes	16.318
<i>P. sp5_MCNUPH30</i>	18.879689	6.254101	G2b	Colombian Eastern Andes	16.318
<i>P. sp8_MCNUPH525</i>	9.515235	5.253448	G2b	Colombian Eastern Andes	16.318
<i>P. sp9_MCNUPH42</i>	19.102947	6.132364	G2b	Colombian Eastern Andes	16.318
<i>P. urichi</i>	7.837245	3.859343	G	Northern Sudamerican montane coastal	
<i>P. acatallelus</i>	6.933206	3.209008	G3a	Northwestern Andes	16.684
<i>P. angustilineatus</i>	6.646394	3.166248	G3a	Northwestern Andes	16.684
<i>P. boulengeri</i>	6.865908	3.175625	G3a	Northwestern Andes	16.684
<i>P. brevifrons</i>	6.84116	3.256496	G3a	Northwestern Andes	16.684
<i>P. buckleyi</i>	7.617699	3.972772	G3a	Northwestern Andes	16.684
<i>P. capitonis</i>	6.670855	3.175915	G3a	Northwestern Andes	16.684
<i>P. celator</i>	6.607712	3.139692	G3a	Northwestern Andes	16.684
<i>P. chloronotus</i>	6.848224	3.351916	G3a	Northwestern Andes	16.684
<i>P. curtipes</i>	7.440064	3.250735	G3a	Northwestern Andes	16.684
<i>P. devillei</i>	7.43949	3.365212	G3a	Northwestern Andes	16.684

<i>P. dorsopictus</i>	6.836436	3.233411	G3a	Northwestern Andes	16.684
<i>P. duellmani</i>	7.547302	3.20804	G3a	Northwestern Andes	16.684
<i>P. gentryi</i>	7.468946	3.28395	G3a	Northwestern Andes	16.684
<i>P. hectus</i>	6.630661	3.375599	G3a	Northwestern Andes	16.684
<i>P. jubatus</i>	6.870318	3.162509	G3a	Northwestern Andes	16.684
<i>P. lasalleorum</i>	6.691611	3.228111	G3a	Northwestern Andes	16.684
<i>P. leoni</i>	6.768398	3.141685	G3a	Northwestern Andes	16.684
<i>P. leopardus</i>	6.594546	3.146355	G3a	Northwestern Andes	16.684
<i>P. lividus</i>	6.749502	3.579326	G3a	Northwestern Andes	16.684
<i>P. maculosus</i>	6.687785	3.222957	G3a	Northwestern Andes	16.684
<i>P. mutabilis</i>	6.60049	3.164991	G3a	Northwestern Andes	16.684
<i>P. myersi_BCQ_829</i>	6.911503	3.789056	G3a	Northwestern Andes-Colombian Eastern Andes	16.684
<i>P. myops</i>	6.697035	3.199652	G3a	Northwestern Andes	16.684
<i>P. ocreatus</i>	6.649721	3.254573	G3a	Northwestern Andes	16.684
<i>P. parectatus</i>	6.629135	3.13198	G3a	Northwestern Andes	16.684
<i>P. peraticus</i>	6.642508	3.196763	G3a	Northwestern Andes	16.684
<i>P. pichincha</i>	7.434293	3.293894	G3a	Northwestern Andes	16.684
<i>P. pyrrhomerus</i>	6.670317	3.142204	G3a	Northwestern Andes	16.684
<i>P. quantus</i>	6.791674	3.230943	G3a	Northwestern Andes	16.684
<i>P. quinquagesimus</i>	7.500304	3.220214	G3a	Northwestern Andes	16.684
<i>P. scoloblepharus</i>	6.595097	3.133671	G3a	Northwestern Andes	16.684
<i>P. simoterus</i>	6.867348	3.223839	G3a	Northwestern Andes	16.684
<i>P. sobetes</i>	7.806482	3.169911	G3a	Northwestern Andes	16.684
<i>P. suetus</i>	6.895837	3.255289	G3a	Northwestern Andes	16.684
<i>P. supernatis</i>	6.802764	3.379722	G3a	Northwestern Andes	16.684
<i>P. surdus</i>	8.071242	3.306976	G3a	Northwestern Andes	16.684
<i>P. thymalopsoides</i>	8.745174	3.228351	G3a	Northwestern Andes	16.684
<i>P. thymelensis</i>	6.740189	3.183026	G3a	Northwestern Andes	16.684

<i>P. trepidotus</i>	6.835565	3.194726	G3a	Northwestern Andes	16.684
<i>P. urani</i>	6.740239	3.275742	G3a	Northwestern Andes	16.684
<i>P. uranobates</i>	6.663215	3.13266	G3a	Northwestern Andes	16.684
<i>P. verecundus</i>	6.6895	3.153891	G3a	Northwestern Andes	16.684
<i>P. vertebralis</i>	7.414623	3.721988	G3a	Northwestern Andes	16.684
<i>P. acerus</i>	6.785098	3.220414	G3b	Northwestern Andes	14.807
<i>P. acuminatus</i>	6.452449	2.997418	G3b	Amazonia	14.807
<i>P. appendiculatus</i>	6.618155	3.399897	G3b	Northwestern Andes	14.807
<i>P. bromeliaceus</i>	6.429642	3.028166	G3b	Central Andes-Northwestern Andes	14.807
<i>P. calcarulatus</i>	7.24012	3.045168	G3b	Northwestern Andes	14.807
<i>P. cedros</i>	7.24012	3.045168	G3b	Northwestern Andes	14.807
<i>P. crucifer</i>	6.493837	3.324809	G3b	Northwestern Andes	14.807
<i>P. ecuadorensis</i>	6.456782	3.083787	G3b	Northwestern Andes	14.807
<i>P. eremitus</i>	6.870413	3.127998	G3b	Northwestern Andes	14.807
<i>P. galdi</i>	6.662921	3.065929	G3b	Central Andes-Northwestern Andes	14.807
<i>P. glandulosus</i>	7.106913	3.065408	G3b	Northwestern Andes	14.807
<i>P. inusitatus</i>	7.540872	3.144332	G3b	Northwestern Andes	14.807
<i>P. jaguensis</i>	6.638077	3.032914	G3b	Northwestern Andes	14.807
<i>P. mendax</i>	6.429642	3.028166	G3b	Central Andes	14.807
<i>P. mindo</i>	6.741254	3.097181	G3b	Northwestern Andes	14.807
<i>P. moro</i>	6.834073	3.217288	G3b	Central America-Northwestern Andes	14.807
<i>P. nyctophylax</i>	6.695649	3.13097	G3b	Northwestern Andes	14.807
<i>P. omeviridis</i>	6.455092	2.997418	G3b	Amazonia	14.807
<i>P. orcesi</i>	6.640537	3.020216	G3b	Northwestern Andes	14.807
<i>P. ornatissimus</i>	6.462567	3.008955	G3b	Northwestern Andes	14.807
<i>P. pluvialis</i>	6.755569	3.107747	G3b	Central Andes	14.807
<i>P. pycnodermis</i>	6.621394	3.367958	G3b	Northwestern Andes	14.807
<i>P. schultei</i>	6.699935	3.104532	G3b	Central Andes	14.807

<i>P. subsigillatus</i>	6.69328	3.191967	G3b	Northwestern Andes	14.807
<i>P. zeuctotylus</i>	6.627484	3.047773	G3b	Amazonia-Guiana Shield	14.807
<i>P. melanogaster</i>	6.476916	3.31749	G3b	Central Andes	14.807
<i>P. quaquaversus</i>	6.495565	3.137878	G3b	Amazonia-Central Andes	14.807
<i>P. rhodoplichus</i>	6.573501	3.361153	G3b	Central Andes	14.807
<i>P. sternothylax</i>	6.484509	3.065939	G3b	Central Andes	14.807
<i>P. wiensi</i>	6.419348	3.164022	G3b	Central Andes	14.807
<i>P. atillo</i>	6.298457	3.003387	G3b	Northwestern Andes	14.807
<i>P. atratus</i>	6.416505	3.085288	G3b	Central Andes	14.807
<i>P. cryophilus</i>	7.357211	3.386255	G3b	Northwestern Andes	14.807
<i>P. cryptomelas</i>	6.38144	3.056317	G3b	Central Andes	14.807
<i>P. gagliardi</i>	6.35374	3.105376	G3b	Northwestern Andes	14.807
<i>P. gloria</i>	6.303714	3.340591	G3b	Northwestern Andes	14.807
<i>P. hampatusami</i>	6.291103	3.000249	G3b	Northwestern Andes	14.807
<i>P. jimenezi</i>	6.449159	2.999208	G3b	Northwestern Andes	14.807
<i>P. lutzae</i>	6.313356	3.010434	G3b	Northwestern Andes	14.807
<i>P. mallii</i>	6.532001	3.035317	G3b	Northwestern Andes	14.807
<i>P. miktos</i>	6.543744	3.013137	G3b	Amazonia	14.807
<i>P. multicolor</i>	6.365955	3.000249	G3b	Central Andes	14.807
<i>P. muscosus</i>	6.503241	3.016381	G3b	Central Andes	14.807
<i>P. nangaritza</i>	6.414299	3.046253	G3b	Central Andes	14.807
<i>P. phoxocephalus</i>	6.413342	3.087991	G3b	Northwestern Andes	14.807
<i>P. prometeii</i>	6.291103	3.000249	G3b	Northwestern Andes	14.807
<i>P. riveti</i>	6.303714	3.312504	G3b	Northwestern Andes	14.807
<i>P. simonsii</i>	6.394231	3.023988	G3b	Central Andes	14.807
<i>P. spinosus</i>	6.66845	3.084051	G3b	Northwestern Andes	14.807
<i>P. tinguichaca</i>	6.3609	3.020963	G3b	Northwestern Andes	14.807
<i>P. torresi</i>	6.41429	3.111909	G3b	Central Andes	14.807

<i>P. totoroi</i>	6.335741	3.10388	G3b	Northwestern Andes	14.807
<i>P. verrucolatus</i>	7.264729	3.55773	G3b	Northwestern Andes	14.807
<i>P. versicolor</i>	6.311378	3.002925	G3b	Central Andes-Northwestern Andes	14.807
<i>P. abakapa</i>	6.389008	3.107818	G3b	Guiana Shield	14.807
<i>P. achuar</i>	6.309689	3.093239	G3b	Amazonia	14.807
<i>P. altae</i>	6.273215	3.057805	G3b	Central America	14.807
<i>P. altamazonicus</i>	6.43202	3.067219	G3b	Amazonia	14.807
<i>P. altamnis</i>	6.278028	3.047484	G3b	Amazonia	14.807
<i>P. andinognomus</i>	6.43212	3.039054	G3b	Central Andes	14.807
<i>P. antisuyu</i>	6.304637	3.004094	G3b	Central Andes	14.807
<i>P. ardalonychus</i>	6.417169	3.043803	G3b	Central Andes	14.807
<i>P. aureoventris</i>	6.36855	3.004177	G3b	Guiana Shield	14.807
<i>P. bambu</i>	6.524348	3.00555	G3b	Northwestern Andes	14.807
<i>P. bicolor_AJC_7012</i>	6.492537	3.102921	G3b	Colombian Eastern Andes	14.807
<i>P. brevicrus</i>	6.348888	3.185752	G3b	Amazonia	14.807
<i>P. buenaventura</i>	6.352659	3.056975	G3b	Northwestern Andes	14.807
<i>P. cajamarcensis</i>	6.397642	3.079625	G3b	Central Andes-Northwestern Andes	14.807
<i>P. cajanuma</i>	6.287311	3.008971	G3b	Central Andes	14.807
<i>P. carvalhoi</i>	6.331262	3.066445	G3b	Amazonia	14.807
<i>P. ceuthospilus</i>	6.480572	3.157205	G3b	Central Andes	14.807
<i>P. cf_bogotensis_AJC_6977</i>	6.378701	3.283239	G3b	Colombian Eastern Andes	14.807
<i>P. chalceus</i>	6.494179	3.05796	G3b	Chocó-Northwestern Andes	14.807
<i>P. croceinguinis</i>	6.778298	3.083837	G3b	Amazonia	14.807
<i>P. cruciocularis</i>	6.339143	3.024084	G3b	Central Andes	14.807
<i>P. diadematus</i>	6.811743	3.127451	G3b	Amazonia	14.807
<i>P. erythroinguinis</i>	6.557886	3.029762	G3b	Central Andes	14.807
<i>P. flavobracatus</i>	6.45125	3.024084	G3b	Central Andes	14.807
<i>P. frater_AJC_6960</i>	6.343667	3.088307	G3b	Colombian Eastern Andes	14.807

<i>P. gryllus</i>	6.330653	3.003105	G3b	Colombian Eastern Andes-Venezuelan Andes	14.807
<i>P. inguinalis</i>	6.418253	3.004177	G3b	Amazonia-Guiana Shield	14.807
<i>P. juanchoi</i>	6.549139	3.068454	G3b	Northwestern Andes	14.807
<i>P. kichwarum</i>	6.393076	3.17307	G3b	Amazonia	14.807
<i>P. librarius</i>	6.40626	3.042668	G3b	Amazonia	14.807
<i>P. lirellus</i>	6.474373	3.23337	G3b	Central Andes	14.807
<i>P. luscombei</i>	6.358713	3.093239	G3b	Amazonia	14.807
<i>P. luteolateralis</i>	6.417409	2.997631	G3b	Northwestern Andes	14.807
<i>P. marmoratus</i>	6.296957	3.10581	G3b	Amazonia-Guiana Shield	14.807
<i>P. martiae</i>	6.524754	3.016165	G3b	Amazonia-Northwestern Andes	14.807
<i>P. matidiktyo</i>	6.577592	3.091664	G3b	Amazonia	14.807
<i>P. mazar</i>	6.338066	2.997154	G3b	Northwestern Andes	14.807
<i>P. minutulus</i>	6.618286	3.043715	G3b	Central Andes	14.807
<i>P. miyatai_AJC_3473</i>	6.537013	3.04777	G3b	Colombian Eastern Andes	14.807
<i>P. muranunka</i>	6.331565	3.328803	G3b	Central Andes	14.807
<i>P. nietoi</i>	6.389974	3.075257	G3b	Chocó	14.807
<i>P. ockendeni</i>	6.466488	3.022447	G3b	Amazonia	14.807
<i>P. orestes</i>	6.295206	3.152787	G3b	Northwestern Andes	14.807
<i>P. palmeri</i>	6.347858	3.205649	G3b	Northwestern Andes	14.807
<i>P. pardalis</i>	6.307761	3.023666	G3b	Central America	14.807
<i>P. parvillus</i>	6.341428	3.01242	G3b	Chocó-Northwestern Andes	14.807
<i>P. pirrensis</i>	6.391038	3.017868	G3b	Chocó	14.807
<i>P. platydactylus</i>	6.35971	3.060001	G3b	Central Andes	14.807
<i>P. ptochus</i>	6.468007	3.130007	G3b	Northwestern Andes	14.807
<i>P. quintanai</i>	6.297877	3.010262	G3b	Northwestern Andes	14.807
<i>P. saltissimus</i>	6.296957	3.164519	G3b	Guiana Shield	14.807
<i>P. saturninoi</i>	6.282884	3.0682	G3b	Northwestern Andes	14.807
<i>P. simonbolivari</i>	6.345212	3.088235	G3b	Northwestern Andes	14.807

<i>P. taeniatus_EAB_6866</i>	6.397727	3.074311	G3b	Northwestern Andes-Colombian Eastern Andes	14.807
<i>P. tiktik</i>	6.31372	3.237866	G3b	Central Andes	14.807
<i>P. unistrigatus_BCQ_991</i>	6.438941	2.997219	G3b	Northwestern Andes	14.807
<i>P. ventrimarmoratus</i>	6.657715	3.043598	G3b	Amazonia-Central Andes	14.807
<i>P. walkeri</i>	6.344958	3.027693	G3b	Chocó-Northwestern Andes	14.807
<i>P. yantzaza</i>	6.3474	3.042058	G3b	Central Andes	14.807
<i>P. yukpa_MCNUPH29</i>	6.542729	3.094229	G3b	Colombian Eastern Andes	14.807
<i>P. yuruniensis</i>	6.454345	3.004177	G3b	Guiana Shield	14.807
<i>P. zophus</i>	6.324818	2.996816	G3b	Northwestern Andes	14.807

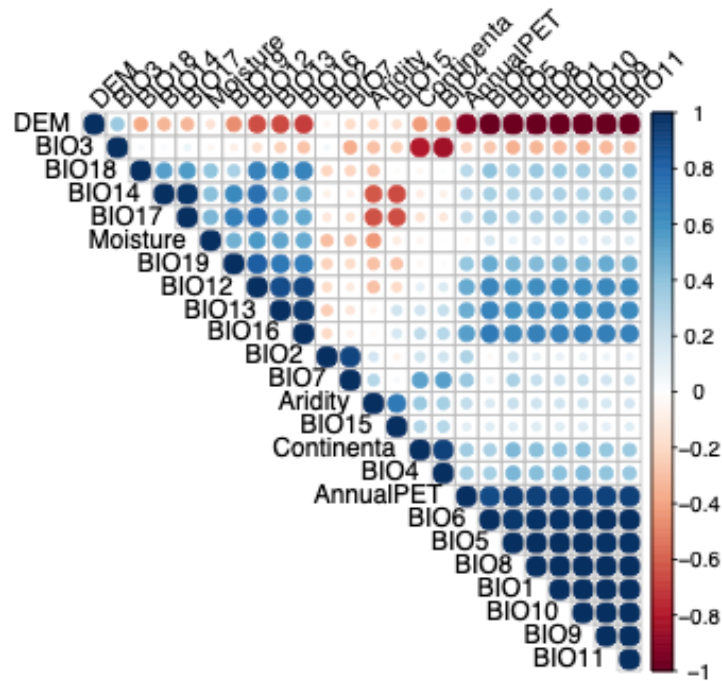


Fig S1. Pearson's correlation coefficients of the relationship between environmental variables.

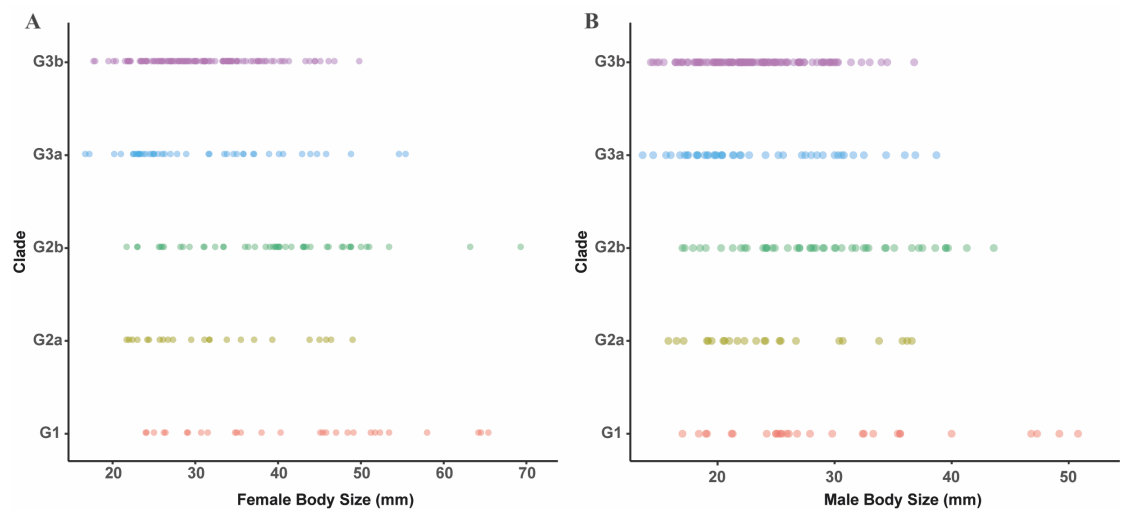


Fig S2. Body size ranges according to the major phylogenetic clades for females (A) and males (B)

GENERAL CONCLUSIONS

Understanding the spatial and historical configuration of the Neotropical biota involves the integration of multiple pathways of evidence that are supported by hypotheses based mainly on geological events that have shaped the processes of diversification, dispersal routes, local adaptations, and variations in different morphological and ecological traits. In this sense, this thesis analyzed the most complete phylogenetic dataset of *Pristimantis* to date, including 304 species from across 10 Neotropical ecoregions. The origin of *Pristimantis* was shown to date back to 22 Ma, during the early Miocene, with a possible ancestral area in the Northwestern Andes, whose lineages colonized new geographic areas through mixed dispersal events. Our results highlighted the importance of the Andean zones which were important centers of diversification of *Pristimantis* during the Miocene, with dispersal events towards low zones in the Amazonia and Chocó. With the successive orogenic events of the Middle Miocene, inter-Andean colonization events increased between regions such as the central Andes, the Venezuelan Andes, and the Eastern Andes of Colombia.

In turn, changes in the patterns of species richness have been linked to both evolutionary and geological historical processes which have shaped the configuration of species in different ecoregions. However, the incorporation of SR alone does not show a complete picture of the processes that have taken place in the structuring of the differential patterns of the number of species between different ecoregions. Therefore, the incorporation of PD allowed us to address questions related to changes in evolutionary history among the most related or distant species of a community. All the ecoregions evaluated for *Pristimantis* showed positive values of PD

metrics, translating into a greater phylogenetic overdispersion. Moreover, the Colombian Eastern Andes had the highest PD despite having fewer reported species than the northwestern Andes, probably due to a complex geological, climatic, and evolutionary history that favored colonization from the northwest and Amazonia over the last 20 to 12 Ma. Therefore, the settlement of lineages in high mountain areas (over 3000 m a.s.l.), endemism patterns, and relationships with Amazonia should be further studied to evaluate different local biogeographic hypotheses in different Andean contexts.

We recommend the use of PD metrics on SR, whose characteristics did not allow us to detect relationships with different environmental predictors. By contrast, the increase or decrease in PD metrics (SES-MNTD and SES-MPD) in relation to the climatic variables, yielded a correlative pattern between the different ecoregions included in this study. The climatic variables evaluated in this work are good predictors of the PD of *Pristimantis*. We highlight the importance of climatic factors inherent in each ecoregion in conjunction with the evolutionary history of *Pristimantis* to understand the processes that shape PD. Our findings reinforce the idea that both, ecological (changes in species richness, response of communities to climatic variables, local adaptation, trait evolution) and geological events, are relevant for understanding PD patterns and the evolutionary history of species between ecoregions. This is especially relevant for genera with a wide geographic distribution such as *Pristimantis* and other groups of Neotropical amphibians.

Regarding the evolution of body size and SSD variations in *Pristimantis*, our results support that environmental variables may drive and/or maintain the degree of body size divergence

between sexes. The rates of body size evolution show a deceleration over time with a slight increase in body size. In turn, body size trends toward optimal evolution. These results contribute to identifying the factors involved in body size and its evolution, helping to generate future hypotheses related to sexual and natural selection or macroevolutionary tendencies in SSD.