Assessing the effect of environmental variables on microhabitat selection and distribution for seven amphibian species in Colombia using *in-situ* and MaxEnt approaches

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ABSTRACT

Colombia is recognized as one of the most biologically diverse places in the world with a high level of endemism. In addition, it is the country with the highest number of amphibian species, but also the highest number of threatened species. Knowledge of amphibian biological and ecological requirements is therefore of high priority. This study has shown that using in situ observations and bioinformatics can be useful for future conservation strategies. Here, I evaluated the possible effect of environmental variables on amphibian distribution and microhabitat selection for seven focal amphibian species (D. labialis; H. bogotensis; H. subpunctatus; P. miyatai; P. uisae; R. palmatus; and P. lynchi) in Colombia. In particular, this study showed that temperature and vegetation cover were the two most important environmental variables affecting occurrence distribution and microhabitat selection in situ for the species Pristimantis miyatai. Consistent with this, the predictions of the MaxEnt models showed that temperature seasonality was the most influential predictor that determined the distribution of the selected amphibian species. In addition, this information proved to be of vital value in reassessing conservation species status and defining priority conservation areas. According to the area of occurrence (AOO), the extent of occurrence (EOO), and the MaxEnt projected EOO, this study proposed a new conservation status for four of the focal species and, furthermore, it highlighted some priority areas in Colombia of high species richness for future conservation strategies. In conclusion, this work increases our knowledge on the effects of environmental variables on amphibian distribution and microhabitat selection in Colombia.
ABSTRACTO

Colombia es reconocido como uno de los lugares con mayor diversidad biológica en el mundo con un alto nivel de endemismo. Además, es el país con el mayor número de especies de anfibios, pero también el mayor número de especies amenazadas. El conocimiento de los requisitos biológicos y ecológicos de los anfibios es por lo tanto de alta prioridad. Este estudio ha mostrado que el uso observaciones in-situ y bioinformática pude ser útil para futuras estrategias de conservación de especies. Aquí evalué los posibles efectos de las variables ambientales sobre la distribución de anfibios y la selección de microhábitats para siete especies focales (D. labialis; H. bogotensis; H. subpunctatus; P. miyatai; P. uisae; R. palmatus; and P. lynchi) en Colombia. En particular, este estudio mostró que la temperatura y la cubierta vegetal fueron las dos variablesambientales más importantes que afectan la distribución de detecciones de individuos y la selección de microhábitats in-situ de la especie Pristimantis miyatai. En concordancia con esto, las predicciones de los modelos MaxEnt mostraron la estacionalidad de la temperatura como el predictor más influyente en determinar la distribución de todas las especies de anfibios seleccionadas. Además, esta información demostró tener un valor vital para la reevaluar el rango de conservación de las especies y definir áreas de conservación prioritarias. Según el área de ocurrencia (AOO), el grado de ocurrencia (EOO) y el EOO proyectado de MaxEnt, este estudio propuso un nuevo rango de conservación para cuatro de las especies seleccionadas y, además, destacó algunas áreas de prioridad en Colombia con gran riqueza de especies para futuras estrategias de conservación. En conclusión, este trabajo aumenta nuestro conocimiento sobre los efectos de las variables ambientales sobre la distribución de anfibios y la selección de microhábitats en Colombia.
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1. INTRODUCTION

For effective development of biodiversity conservation strategies, identifying and understanding species’ geographic distribution is essential, particularly for species of great concern, such as threatened or range-restricted species. It is also important to determine which species are most vulnerable to environmental changes, such as increasing temperatures or changes in habitat structure. Hence, in order to implement efficient conservation strategies, it is imperative to identify the environmental variables that strongly affect species distribution patterns. This will be increasingly important under current global climate change scenarios, as we will need to forecast potential effects of climate change on species or species assemblages in order to implement suitable protection strategies. These issues appear particularly applicable to the Andean region of Colombia.

Colombia's territory only accounts for 1% of the world's surface (WorldBank 2017), but the particular biological and topographical conditions of the country make it one of the most biodiverse places in the world. Colombia’s biodiversity is only rivalled by countries like Brazil, Australia, Indonesia, China and Mexico (Butler 2016; Worldatlas 2017). Colombia has the highest number of bird and orchid species in the world and the second highest number of butterflies, plants, and amphibians (CBD 2017). Based on the International Union of Conservation and Nature (IUCN) diversity data, Colombia has 806 species of amphibians (IUCN 2017b), of which 749 species are frogs and toads and 50% are endemic to Colombia (Acosta-Galvis 2000). Ecosystems in the Andes mountains hold the highest amphibian species richness in the country (Armenteras D et al. 2017; Navas 2006). The geological and environmental conditions of the Andes are extremely heterogeneous both in terms of physical structure and in terms of climate. Such heterogeneity suggests complex habitats that could support more niches and wider means to exploit resources, thereby promoting species diversity and in some cases endemism (Ricketts et al. 2005; Stuart et al. 2008). This is the case for amphibians, the most endemic taxonomic group in the Andes (Armenteras D et al. 2017; Cavelier J et al. 2001; Stuart et al. 2008). However, the Andes mountains of Colombia also hold the country’s main cities and half of its rural population (Eraso Rodríguez et al. 2013). Increasing pressure from human population growth, largely through agricultural expansion (Eraso Rodríguez et al. 2013), is
rapidly deteriorating the Andean ecosystems, with 70% of natural vegetation in the tropical Andes dramatically reduced in recent years (CBD 2017) and an increasing number of species being threatened with extinction.

As for amphibians worldwide, there is a trend of population decline in the past decade (Stuart et al. 2008; Whiles et al. 2006). Amphibians are vertebrates that live all over the world in a wide variety of aquatic and terrestrial habitats (Unrine et al. 2007; Whiles et al. 2006). Frogs and toads (Anura), salamanders and newts (Caudata), and caecilians (Gymnophiona) are all part of this group, but almost 90 percent of all amphibians are anurans (McDiarmid & Mitchell 2000; Stuart et al. 2008). All amphibian species are ectotherms that rely on environmental conditions to regulate their body temperature (Pough 1983), and they portray a variety of reproduction modes (Stuart et al. 2008; Touchon & Warkentin 2008). Amphibian reproduction mode is influenced by different factors, such as humidity (Silva et al. 2012) or habitat structure (Almeida-Gomes & Rocha 2015). They play an important role in the dynamics of an ecosystem as predators, but are also an important source of food for other animals, such birds and snakes (Blaustein AR et al. 2011; Pearman 1997; Whiles et al. 2006). Amphibians are thus crucial for the trophic flow of energy and ecosystem processes.

Amphibian populations are declining and rates of species extinction rapidly increasing. Based on IUCN Red list, nearly one third of the world’s amphibian species are threatened with extinction (IUCN 2017b). There is a general agreement that such declines are strongly influenced by habitat loss or fragmentation, invasive species, pollution and climate change (Blaustein AR et al. 2011; Leary et al. 2018; Stuart et al. 2004). Further assessment and understanding of how these threats independently or synergistically affect amphibian population declines is still necessary. This is particularly true for Andes mountain ecosystems where the reported number of species with declining populations has rapidly increased in past decade (Mittermeier et al. 1998; Stuart et al. 2008). However, what is more worrisome is the fact that many of the threats affecting such populations remain unidentified (Stuart et al. 2004). Yet, one of the most accepted hypothesis to explain the rapid decline posits that the decrease in populations is a result of a combination of factors. Changes in habitat and environmental niches are compromising the natural requirements and the essential biological processes for amphibian species to successfully survive (Blaustein AR et al. 2011; Collins & Storfer 2003). Evaluating amphibian populations’
ecological and environmental requirements provides a better-understanding and therefore an improved basis for the development of conservation strategies. Further, due to their crucial role in the environment, understanding amphibians offers a better assessment not only concerning this taxonomic group, but of the entire ecosystem. Increasing anthropogenic pressures and drastic climatic changes in the Tropical Andes question the future of amphibian populations in this region. Knowledge on amphibian biological and ecological requirements is therefore of high priority.

Initially, in order to understand amphibian biology, ecology and distribution patterns, it is important to consider the landscape features of the Andes and the origins of this mountain chain. The Andes is the longest mountain chain in the world with 7,232 kilometers in length and with some of the highest elevations reaching 6,962 m.a.s.l (Wee 2017). It has been classified into three sub-regions based on its climate defined characteristics: Tropical Andes (11°N-20°S), Dry Andes (20°-30°S) and Wet Andes (35°-55°S). Based on the wide elevational range of the current Andes mountains, amphibian adaptation to moderate elevations is probably ancient and biome specific, thus explaining the niche specialization for each species and hence high levels of endemism (Navas 2006) in places like tropical Andes (Cuesta et al. 2012). There are records indicating that some amphibians, especially anurans, had likely adapted to moderate elevations by the end of the Jurassic period (Carroll 1988). However, most amphibian species radiations in the high Andes occurred in more recent times, after the second Andes uplifting that formed a complex mosaic of high mountains and valleys (Duellman 1979; Navas 2006). Isolation played a crucial role on species radiation and it is therefore not surprising to find very diverse modern Andean amphibian communities at high elevations, as it is the case of some representatives in the genus *Pristimantis*. However, high geographical replacement of species and endemism seen in the tropical Andes may suggests a tradeoff between the species’ ability to survive in tropical Andes biomes with very narrow physiological and biological tolerance ranges, such temperature ranges.

There are some important aspects in the biology and physiology of amphibians that make them an important environmental indicator (Simon Edina et al. 2011; Welsh & Ollivier 1998) and therefore an important element for scientific research. In order to understand the high
susceptibility of amphibians to environmental variations and to understand their responses, aspects such as life cycles, reproductive modes, or the permeable skin physiology in different habitats need to be considered. Therefore, the first step towards understanding the mechanism behind the amphibian population decline is to examine the different biotic and abiotic variables that affect the life histories at a given site along with the habitat structural changes and its effects on the local and regional communities (Wiens 2011). As most amphibian species are sensitive to changes in their environment, the development and implementation of effective management strategies for rare and threatened species must utilize data on the geographic distribution and habitat characteristics of the species in question (Ramírez 2017). In the past decades, several methods to evaluate species distribution based on species occurrence and environmental variables have been developed. However, for many species, a lack of on-the-ground data can be challenging. Nevertheless, by using available data on species occurrence, it is possible to infer the potential presence of species elsewhere (Tole 2006). Among these methods, Ecological Niche Modelling (ENM) plays an important role in the evaluation of species distributions.

ENM is a great tool and has a diverse applications in ecology, evolution and conservation biology (Arntzen 2006; Guisan & Huiller 2005). Currently, increasing technological advances and the availability of open access databases, such online museums records and occurrence data points, have allowed for an easier way to improve species conservation approaches based on predictions of species’ distributions. ENM uses occurrence data with a complement of ecological variables to evaluate the probability of a species to occur somewhere else based on specific habitat characteristics. This approach has been a conservation instrument to evaluate species’ distribution, to detect undocumented populations, to recognize the effects of climate change or land cover changes on species distribution, as well as to evaluate the effects of alien species (Hijmans & Graham 2006). In the context of this study, the importance of ENM lies in its capacity to identify areas of possible habitat suitability for selected amphibian species and areas of high conservation priority based on species richness. Therefore, this study uses Maximum Entropy Species Distribution Modeling (MaxEnt) to evaluate habitat suitability for selected amphibian species across the Colombian Andes. Such an approach represents an opportunity to develop distribution information at any required scale, for local, regional, or national conservation objectives.
Even though there is a great interest to increase the knowledge that exist about amphibians in Colombia, there is evidently a lack of sufficient biological records. For example, while the genus *Pristimantis* encompasses more than 27% of Colombian total amphibian fauna with more than 200 species, there is little or no information on their ecology and natural history (Ramírez 2017). More scientific research in the region is needed to build comprehensive and effective local, regional, and national short and long-term conservation strategies (Acosta-Galvis 2017; Ramírez 2017). The tropical Andes is the region with the highest number of amphibian species in the world of which 68.3% are endemic. This region also supports the highest number of endemic plants on the planet (20,000 species). Despite such diversity and high levels of endemism, the tropical Andes also ranks as one of the most severely threatened areas in the world (CBD 2017). Although mountain amphibians are threatened by habitat loss and other anthropogenic factors as most other species, they are additionally threatened by factors and interactions still not fully clear (Collins & Storfer 2003; Leary et al. 2018; Stuart et al. 2004). In addition, while we know that many amphibian species are listed under threatened status, amphibians are largely listed as Data Deficient (DD) under the IUCN criteria (IUCN 2012). Lack of sufficient data is of a common and significant concern in the tropical Andes ecosystems as most of the amphibian species are niche specialist and have small geographical ranges. This makes them highly susceptible to extinction (Wake & Vredenburg 2008), as it is the case of the Miyatai’s robber frog (*Pristimantis miyatai*) (Lynch J.D. & Ardila 1999), the Lynchis robber frog (*Pristimantis lynchi* or *Pristimantis uisae*) (Ramírez 2017). In many cases, species go extinct before there is any knowledge about them (Lees & Pimm 2015).

Therefore, any information that improves the current knowledge on species ecology is of fundamental value for the conservation of Colombian Andes amphibians as well as the conservation of Andes ecosystems (CBD 2017). Determining potential biological consequences of forecasted environmental change and its effects on environmental dynamics is increasingly important. Thus, to speculate with confidence on the possible status of a species based on the current understanding of its biology, ecology, behaviour and physiology could be very beneficial for future conservation approaches. This study aims to investigate biological and ecological information on seven selected amphibian species in the area of Boyacá, Colombia, by assessing the effects of environmental variables and habitat structure and by using Ecological Niche Modeling (ENM) predictions on possible suitable habitat distribution patterns. More specifically,
this study attempts to do this by identifying a set of environmental predictors that are most likely to affect selected species of amphibians and calculating the Extent of Occurrence (EOO) and Area of Occupancy (AOO) and the Extent of Occurrence generated by MaxEnt (ENM-EOO) for each selected species. In addition, I assess current conservation status of the species, evaluate the effectiveness of protected areas for conservation of the species, and identify areas of high conservation priority for selected amphibian species. The results will be valuable for better local, regional, and national amphibian species conservation strategies in Colombia.
2. MATERIALS AND METHODS

2.1 STUDY AREA

Colombia is located in northwest corner of South America (Figure 1a). It is considered one of the most megadiverse countries in the world, harboring at least 10 percent of the planet’s biodiversity (Ministerio de Ambiente y Desarrollo Sostenible 2014). This study focused on the Andean region of Colombia, which extents about 278,600 km² (Figure 2a) approximately 24.5% of the country’s geographical area (Etter & Wyngaarden 2000). The Andes mountains cut across Colombia from north to south and are divided into three main mountain ranges: The Western, Central and Eastern Cordillera. This region includes some of the most biodiverse areas (Figure 2b) due to high levels of endemism and species richness (Myers et al. 2000; Potes 2013). The high tropical Andes ecosystem provide variety of ecosystem services, such as water and nutrient cycling. Due to the region’s geology, topography and unique matrix of microclimates the tropical Andes assembles a remarkable diversity of ecosystems, such as cloud forest, puna grasslands, wetlands and deserts (Figure 2a). Throughout the tropical Andes, rainfall is relatively high depending on the slope of exposure, while temperature is highly regulated by altitude (Garreaud 2009). In the tropical Andes, very low temperatures can be found above 3,500 m.a.s.l. where snow clad mountains are present in some places. Throughout the tropical Andes, the extreme diurnal fluctuation of atmospheric temperature may have stronger impact and higher ecological implications than annual and seasonal temperature. In the same fashion, rainfall patterns and temperature regimes have an impact on vegetation structure and ecosystem functioning.
Figure 1: The geographic location of Colombia (a) and the department of Boyacá identified by the red polygon (b).

Figure 2: Map of Tropical Andes region of Colombia with main ecosystems (a), and Tropical Andes biodiversity hotspot (b). Source: Grid-Arendal.no and natureserve.org
The current study was conducted in situ in the province of Ricaurte, in the department of Boyacá (5°32’N 73°22’W) (Figure 1b). The study area was centered on the eastern Cordillera, and field efforts were concentrated on three specific areas based on the level of humidity (Figure 3 and 4), elevation, water presence, and accessibility. This region is characterized by high level of biodiversity and endemism, but also it has shown possible high influence of extreme environmental changes on species responses and species extinction rates (Malcolm et al. 2006). This study concentrated in situ efforts within the Province of Ricaurte in Boyacá, but the Ecological Niche Modelling process was not restricted to Boyacá. ENM was performed for the entire Colombian national territory, thus allowing habitat suitability maps to be projected across all of Colombia.

**Sampling sites**

The research was conducted during November and December in 2016. Amphibian surveys were performed in distinct habitats and with varied biotic factors from north to south. All sites were located between 2100 and 2500 m.a.s.l. Most of these areas are part of an ecological belt called Guantiva-La Rusia-Iguaque with high conservation status (Córdoba-Córdoba et al. 2017). Throughout the region, rainfall and dry (less rainy) season patterns are strongly influenced and determined by oceanic and continental air masses (Bendix et al. 2005). Most of the region of central and western Colombia experiences bimodal annual precipitation cycles with two rainy seasons (February to May and October to November) and two dry seasons (June to September and December to January) (Poveda et al. 2005). However, the eastern slopes of the Andes experience higher precipitation due to the winds from the Amazon basin, which usually carries high quantities of water vapor (Emck 2007). In general, Boyacá experiences two peaks of higher relative humidity during the year, first in May to August and another second one, not as pronounced, in November. Annual relative humidity in Boyacá is estimated between 70-95% based on IDEAM (2018).
Figure 3: (a) Three polygons showing the study area in the Ricaurte Province based on a humidity gradient: High humidity - Moniquira/Arcabuco in green; Moderate humidity - Gachantiva in blue; and low humidity - Raquira in red. (b) The tree location sites (dots) and the three humidity belts present in the Province of Ricaurte; lemon-colour area represents lower humidity; green area represents moderate humidity and dark green represents high humidity. Yellow numbers show Annual mean precipitation (mm). Source: created on GoogleEarth, 2016.

Figure 4: Three habitats based on a humidity gradient where amphibian surveys were carried out in the province of Ricaurte, Boyacá. Photos: Guillermo Alba
High Humidity Sampling Area

The study area on the northern region in the municipalities of Arcabuco and Moniquira had high humidity conditions and amphibian surveys were conducted in the regional park Serrania El Peligro (UTM 5.819011, -73.499615) with a total area of 2647 ha (Figure 4). The sampling site is a patch of high Andean forest dominated by *Quercus humboldtii* at an elevation between 2300-2500 m.a.s.l, with average annual precipitation of 2000 mm (Figure 3b) and with a median monthly temperature from 6 to 12°C. There is a steep slope throughout the site and the site has a series of temporary creeks and waterfalls pouring downhill into the Pomeque River only really noticeable after heavy rain episodes. In times of less rainfall, the only body of water is the Pomeque river that runs perennially and perpendicular to the site on the southern edge Figure 5a).

Moderate Humidity Sample Area

The study area of intermediate humidity was located in Alto Gachantiva (UTM 5.683796, -73.559643), between Villa de Leyva and Santa Sofia. This is a high-altitude valley of 2150 to 2200 m.a.s.l. with an annual precipitation of 1400 mm and median monthly temperature from 12 to 18 °C (Figure 3b and 4). The surveyed area was along a canyon and ecological corridor dominated by oak forest, secondary mixed forest and some regenerating vegetation (Córdoba-Córdoba et al. 2017). The survey area contained a small tributary crossing the entire site eventually pouring into the Moniquira River on the south west (Figure 5b).

Low Humidity Sample Area

The low humidity site was dominated by desert habitat and was located in the southern region between Sachica, Sutamarchan and Raquira (UTM 5.559913, -73.605376) at altitudes ranging from 2200 to 2300 m.a.s.l. and annual precipitation of 800 mm and a median monthly temperature from 18 to 24 °C. The southern region is predominately sub-xerophilic habitat, such as La Candelaria Desert, with some open shrubs vegetation structure. There is a dominating shrub steppe grassland associated with this area (Figure 3b and 4). The study area lies on a hilly
area where a small perennial pond is present on the highest point, while there is a small river flowing at lower elevation (Figure 5c).

2.2 FIELD SAMPLING

2.2.1. Study Design

In order to determine amphibian species presence in the study area, active searches were carried out. At each study site (high humidity; moderate humidity; and low humidity), six 50m x 20m sampling transects were established (Figure 5). Locations within the sites were chosen based on proximity to water bodies (rivers, creeks, lakes) and/or habitat characteristics presumed good for tropical Andes amphibians, thus maximizing species detection. Each site was surveyed during four interspersed periods and each survey period lasted three days. Each study site was thus surveyed a total of 12 days. Every day before morning survey, three transects were randomly picked using a dice. The chosen morning transects were also surveyed at night. Morning surveys were performed from 8.00 hr to not later than 13.00 hr, and night surveys from 18.00 hr to 22.00 hr using headlights or flashlights. Active search was done under surfaces and vegetation. Objects such as logs, rocks, stumps, and tree bark were searched as suggested by Crump and Scott Jr (1994); Eekhout (2010). This method focused on the most important habitat components while leaving enough time to move along the transect. Using GPS units (Garmin eTrex Vista), start and end points and elevation were recorded. Survey times were recorded and total survey time (survey time minus stops) calculated. Searching periods were aimed to be approximately an hour per transect. For each survey, all individuals encountered were noted and the detection location recorded. In addition, location in the vertical forest strata where the animal was heard, seen, or captured was recorded using the forest community stratification methodology by Mueller-Dombois and Ellenberg (1974). For the purpose of this study the layer legends have been modified to numeric references, 1 to 4 (Figure 6). In addition, for each survey, the following information was recorded for every detection: observer, site/transect, date, weather, altitude, time, species, detection type (e.g., visual, auditory, capture), and UTM location. For each capture, additional information was added, such as age class (juvenile, sub-adult, adult), snout-vent length and weight, and a photo-voucher was taken. The time taken for species identification and data collecting was not included in the total searched time.
Figure 5: Maps showing the transect layout in the High Humidity (a), Moderate humidity (b), and low humidity (c) sites. Source: Created with GoogleEarth 2017

Figure 6: Diagram of forest community stratification. 1. Tree layer: >5m; 2. Shrub Layer: 30cm-5m; 3. Herb Layer <30cm; 4. Moss, Lichen, or Debris: Decaying wood, stones, or exposed rocks. Source: Image modified from Elke Freese at http://commons.wikimedia.org/
Habitat Assessment

Habitat assessment was performed at each transect using procedures following Freitas et al. (2002) and Ralph et al. (1993). Along each transect of 50m long, I evaluated microhabitat based on plant cover at every 10m with a total of six measuring stations per transect. At each station, I used a measuring tape to measure 3m in each cardinal direction and marked each end with a piece of flagging tape. Each station thereby contained five points (a centre point and a point in each cardinal direction) and covered an area of $36m^2$. Then by using a 0.50x0.50 frame divided by hundred open squares (Figure 7a), I measured plant cover, litter cover, rock cover, and vegetation obstruction at three heights, 0.50m, 1.00m, and 1.50m (Figure 7b). Each measurement consists of counting the number of squares visually obstructed, at least 50% obstructed space or more; all squares obstructed defined one hundred percent obstruction. In order to measure Plant, Litter, and Rock cover, I held the frame horizontally to the ground with extended arms as closer to my knees (Figure 7b1). All measurements were done at all five points and bare ground measurement was added to the measured area for a total of a hundred percent (Plant + litter + Rock + Bare Cover = 100%). In addition, canopy cover and canopy closure were measured, where canopy cover is always measured in a vertical direction, while canopy closure involves an angle of view (Jennings et al. 1999). Canopy cover was measured by placing the frame above the head (Figure 7b2) and counting percentage of canopy covering the frame’s squares. Vegetation obstruction was measured only at the center points of each stations in the four cardinal directions. I measured it by placing the frame vertically at 0.50m, 1.00m, and 1.50m from the ground (Figure 7b3-5) and estimating the percentage of vegetation obstruction focusing on three meters range between the center and the cardinal point marking flag. In addition, I measured canopy closure with a spherical forest densitometer using the method developed by Lemon (1956). I used the same five points, and measured canopy closure by holding the densitometer elbow height and counting the dots not covered. Then, I multiplied the result by 1.04 and subtracted it to 100 to get percentage of canopy cover.

In addition, I estimated basal area along each transect by measuring and recording all plant species greater than 5.0cm in DBH (diameter at breast height); everything below 1.0m in height was considered understory. In addition, tree height was estimated and recorded.
Figure 7: (a). Square used to measure habitat structure. (b). Observers posture 1. Vegetation cover; 2. Canopy cover; 3-5 Vertical Cover (0.50m; 1.0m; 1.50m) Source: (Freitas et al. 2002).

Weather Monitoring

GPS unit (Garmin eTrex Vista) was used to obtain the longitude, latitude, and elevation of each transect. Soil moisture records were taken with Soil Moisture Meter (model 7028C) every 10m along each transect and read as 1-3 dry, 4-7 moist, and 8-10 wet. Outdoor thermometer and humidity sensors were placed in November and left *in-situ* throughout the study period. Relative humidity and temperature were recorded at the beginning and at the end of each amphibian survey. Rain gauges were placed within the study area and were checked and recorded before or after surveying that area.

2.2.2 Study Taxon

Colombia has a wide variety of climates and characteristics that have made this country one of the most biodiverse. This is why Colombia has the second highest number of amphibian species in the world, with the Andes region being the most Anuran species richness in the country (Acosta-Galvis 2000; Acosta-Galvis 2017; Bernal & Lynch 2008). Increasing knowledge about their distribution and ecology can provide insights for better conservation strategies. In the Andes, despite the high species diversity, there are few studies documenting patterns of amphibian diversity in some regions like Boyacá (Arbeláez–Cortés 2013). The scarce knowledge of amphibian diversity and distribution patterns in the region represents an obstacle to evaluate their conservation status and to ensure community representativeness in protected areas and to formulate conservation strategies (Gómez–Hoyos et al. 2017).
In Colombia there are around 714 amphibian species described so far, and the country has the second highest number of endemic species in the world (IUNC 2017). There is therefore a critical demand for conservation as 214 amphibian species are currently threatened. Yet, while Colombian amphibian diversity contributes greatly to the world’s diversity, it is Andean amphibian diversity that is the most significant (Duellman 1979; Lynch et al. 1997). Most amphibians in the tropical Andes occur in forest habitats and only twenty percent can survive in secondary habitats (Stuart et al. 2008). Dramatic changes in topography and microclimates along the Andean mountain gradient has provoked narrow ranges among numerous amphibians making them extra susceptible to external threats (IUNC 2017). Most amphibians utilize freshwater habitats like ponds, streams, marshes and swamps. However, many Andes amphibians have adapted to areas with no permanent body of water, but highly humid microhabitats, as it is the case of some species in the genus *Pristimantis* (Arroyo et al. 2008). Amphibians in the Andes portray different reproduction modes across habitats and include larval development, direct development and live-bearing (Navas 2006). However, Andes amphibians of high elevation generally have direct development.

2.3 STATISTICAL ANALYSIS OF FIELD DATA

Since there are many possible predictor variables and relatively few samples, Partial Least Squares (PLS) regression was used to reduce variables to a smaller set of uncorrelated components and test correlation (Mevik & Wehrens 2007). The analysis was divided in two parts and were performed using the “pls” and “plsvarsel” packages on R. The first part of the analysis predicted which environmental variables strongly affect amphibian occurrences, in this case *P. miyatai*. Due to the low number of occurrences across sample transects (poor replication) within the low and moderate humidity sites, only data from the high humidity site was used for this analysis. The model contained number of individuals as a response, and temperature, relative humidity, elevation, soil moisture, % plant cover, % litter cover, % rock cover, % bare ground, canopy cover, canopy closure, DBH, tree height, vertical cover at 0.50m, vertical cover at 1.00m and vertical cover at 1.50m as factors. Significance threshold for Loading Weights, Regression Coefficients, Variable Importance on Projection and Significant multivariable correlation were automatically run by the “plsvarsel” package to select the most significant environmental

16
variables. Variables that had a significant effect were subsequently plotted against the response variable (number of individuals).

The second part of the analysis evaluated the environmental variables that affect amphibian microhabitat selection along the vertical height gradient. The model contained vertical height occurrences as a response, and temperature, relative humidity, elevation, soil moisture, % plant cover, % litter cover, % rock cover, % bare ground canopy cover, canopy closure, DBH, tree height, vertical cover at 0.50m, vertical cover at 1.00m, vertical cover at 1.50m as factors and the analysis performed as described above. Subsequently, a GLM was performed to evaluate the relationship between the significant variables and height occurrences of *P. miyatai*. All statistical analyses were performed with R version 3.4.2.

2.4 ECOLOGICAL NICHE MODEL (ENM)

For the Ecological Niche Model, I chose seven amphibians species (*D. labialis; H. bogotensis; H. subpunctatus; P. miyatai; P. uisae; R. palmaus;* and *P. lynchi*) based on level of local concern and the likelihood of occurrence in the focal area of Boyacá, Colombia. (Table 3 and Figure 8). In addition, some of these species lack current or sufficient data for future conservation actions, as is the case for *P. uisae* and *P. lynchi* listed under IUCN Red list as data deficient (IUCN 2017b).

2.4.1 Species Occurrence Data

Species occurrence data were obtained from the field conducted surveys and online databases, such as the GBIF database (GBIF.org), iNaturalist (inaturalist.org) and published literature (Ramírez 2017). A list of occurrence points (latitude and longitude) of each of the seven selected amphibian species were extracted and converted to .CSV format.

2.4.2 Environmental Variables Selection

Twenty-two environmental variables were considered for the ecological niche modeling as potential predictors of amphibian species habitat. The 19 bioclimatic variables (Table 1) were
used to describe temperature and precipitation patterns and were obtained from the Worldclim database version 1.4 (worldbioclim.org). This is a set of global climate grids with multi-spatial resolutions and multi-temporal periods developed by Hijmans et al. (2005). Other important variables included in the model were the Aridity index (AI), which defines the potential level of dryness in a defined space (Maliva & Thomas M. Missimer 2012), and Potential evapotranspiration (PET), which is highly related to AI when developing climate analysis. The data used were obtained from the CGIAR-CSI dataset (http://www.cgiar-csi.org) developed by Zomer et al. (2008). Lastly, altitude data was obtained through the Global Digital Elevation Model (DEM) by USGS (https://earthexplorer.usgs.gov/). All environmental variable datasets used were in ASCII raster format. For the purpose of statistical comparison, all the layers have the same projection system, same geographic limit, and cell size. The raster layers used for ENM were 30 arc second (~1 km²) resolution.

Table 1: Environmental data used for ENM.

<table>
<thead>
<tr>
<th>CODES</th>
<th>Environmental Predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIO 1</td>
<td>Annual Mean Temperature</td>
</tr>
<tr>
<td>BIO 2</td>
<td>Mean Diurnal Range (Mean of monthly (max temp - min temp))</td>
</tr>
<tr>
<td>BIO 3</td>
<td>Isothermality (BIO2/BIO7) (* 100)</td>
</tr>
<tr>
<td>BIO 4</td>
<td>Temperature Seasonality (standard deviation *100)</td>
</tr>
<tr>
<td>BIO 5</td>
<td>Max Temperature of Warmest Month</td>
</tr>
<tr>
<td>BIO 6</td>
<td>Min Temperature of Coldest Month</td>
</tr>
<tr>
<td>BIO 7</td>
<td>Temperature Annual Range (BIO5-BIO6)</td>
</tr>
<tr>
<td>BIO 8</td>
<td>Mean Temperature of Wettest Quarter</td>
</tr>
<tr>
<td>BIO 9</td>
<td>Mean Temperature of Driest Quarter</td>
</tr>
<tr>
<td>BIO 10</td>
<td>Mean Temperature of Warmest Quarter</td>
</tr>
<tr>
<td>BIO 11</td>
<td>Mean Temperature of Coldest Quarter</td>
</tr>
<tr>
<td>BIO 12</td>
<td>Annual Precipitation</td>
</tr>
<tr>
<td>BIO 13</td>
<td>Precipitation of Wettest Month</td>
</tr>
<tr>
<td>BIO 14</td>
<td>Precipitation of Driest Month</td>
</tr>
<tr>
<td>BIO 15</td>
<td>Precipitation Seasonality (Coefficient of Variation)</td>
</tr>
<tr>
<td>BIO 16</td>
<td>Precipitation of Wettest Quarter</td>
</tr>
<tr>
<td>BIO 17</td>
<td>Precipitation of Driest Quarter</td>
</tr>
<tr>
<td>BIO 18</td>
<td>Precipitation of Warmest Quarter</td>
</tr>
<tr>
<td>BIO 19</td>
<td>Precipitation of Coldest Quarter</td>
</tr>
<tr>
<td>AI_yr</td>
<td>Aridity</td>
</tr>
<tr>
<td>PET</td>
<td>Potential Evapo-transpiration</td>
</tr>
<tr>
<td>Alt</td>
<td>Altitude</td>
</tr>
</tbody>
</table>
2.4.3 Assessing Correlation between Environmental Variables

To evaluate the collinearity between different environmental predictors, I performed a correlation analysis in Excel. Any environmental predictor with a correlation value higher than 0.75 or less than -0.75 were considered highly correlated (Rissler et al. 2006). For any pair of variables highly correlated, the most biologically significant and easy to predict of the two was chosen for MaxEnt analysis. This step of the process was based on literature and expert information with the idea of preserving as many parameters that characterize as much environmental information as possible.

2.4.4 Maximum Entropy (MaxEnt) Model

MaxEnt (version 3.4.0) was used to estimate the probability of habitat suitability for seven selected amphibian species based on presence records. After inputting the file of species presence localities and the raster grids of the selected environmental variable into the model, the program runs a uniform distribution generated over pixels in the grid and performs a number of repetitions, which aims to increase the probability of sample species locations improving the fit to the data (Merow et al. 2013). Then MaxEnt generates a coloured image with estimated probability of a species’ presence between 0 (not present) and 1 (present). Based on this map, I was able to make a distinction between suitability among different areas where high suitability showed red colour and poor suitability blue colour. I ran the model for every selected amphibian species separately using the uncorrelated environmental variables. The MaxEnt model creates a probability niche distribution across the entire study area, which allows comparisons between regions, such as the Colombian Andes. In addition, MaxEnt provides an analysis of the contribution of each environmental variable based on a jackknife analysis of the gain. The gain is an indication of how much better than random the model fit is.

**Contribution of Environmental Variables**

The MaxEnt model estimates the percentage of the relative contribution of each environmental variable by adding the increment value of the specific variable has on the fitness of the model. In case the effect is negative, the value is subtracted from the total percentage
contribution of the variable. The percentage value is a good indication of the influence each particular variable has on the model’s prediction. However, it does not infer that variables with the highest values are more important to the species than other variables.

**Jackknife Test**

The MaxEnt model evaluates the importance of each environmental variable through a Jackknife approach. This process of evaluation leaves out one of the variables and reruns the model using the remaining variables. In the same way, it keeps one selected variable in isolation and reruns the model leaving out all of the other variables. Therefore, the level of importance of each environmental variable can be calculated by subtracting the gain value of each variable to the total gain value. Environmental variables with the highest gain value carry the most meaningful information to the model.

**2.4.5 Binary Maps (Presence/Absence)**

The MaxEnt model uses the environmental predictors selected per species and produces a continuous occurrence probability map. The minimum training presence cloglog threshold (Table 2) was adopted to convert maps from continuous suitability maps to presence/absence maps. Although there is not a defined optimal threshold for binary depictions of presence/absence maps, the approach by Phillips (2006) was used.

The idea to generate presence-absence maps is to depict the potential distribution of each species based on identified levels of suitability areas. Presence-absence maps were determined by allocating a value of zero (0) to all the pixels with a value under the chosen threshold (cut-off value), signifying the probable absence of the species. Any value above the chosen threshold value was assigned a value of one (1), signifying the probable presence of the species. Consequently, species richness maps were created by overlapping distribution maps of all of the species.
2.4.6 Model Evaluation: ROC/AUC

The Receiver Operating Characteristic (ROC) is used to predict the diagnostic ability of the predicted model. The Area Under Curve (AUC) is used to measure the model performance and accuracy. AUC values are generated from ROC plots using MaxEnt and use a ranking value, from 0 (poor discrimination) to 1 (perfect discrimination; Engler et al. 2004). Due to the characteristics of using presence-only data, such as sampling bias, this evaluation measures the ability of the model to discriminate between suitable environment and just random background pixel (Phillips 2006) rather than presence from absence.

2.5. GEOGRAPHICAL DISTRIBUTION AND RISK STATUS ASSESSMENT

2.5.1 Geographic Range

The distribution range for the seven selected amphibian species was evaluated using Extent of Occurrence (EOO) and Area of Occupancy (AOO) methods. These methods are commonly used by the IUCN to evaluate the conservation status of a species using an open source Geospatial Conservation Assessment Tool, GeoCAT (http://geocat.kew.org/) and 2km x 2km grid size as recommended by IUCN (IUCN 2012). In addition, distribution range was evaluated using Extent of Occurrence calculated by the MaxEnt Ecological Niche Model (ENM).

EOO is the area which lies within the outermost geographical limits to the occurrence of the species or convex polygon fencing a set of distribution points (Willis et al. 2003). It is the potential area where the species has been known, inferred or projected to be present (IUCN 2012).

### TABLE 2: Minimum Training and 10 percent training presence threshold used for binary maps

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Minimum Training Presence</th>
<th>10 percent training presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dendropsophus labialis</td>
<td>0.0197</td>
<td>0.1282</td>
</tr>
<tr>
<td>2</td>
<td>Hyloscirtus bogotensis</td>
<td>0.0157</td>
<td>0.3032</td>
</tr>
<tr>
<td>3</td>
<td>Hyloalus subpunctatus</td>
<td>0.0211</td>
<td>0.2041</td>
</tr>
<tr>
<td>4</td>
<td>Pristimantis miyatai</td>
<td>0.04</td>
<td>0.2715</td>
</tr>
<tr>
<td>5</td>
<td>Pristimantis uisae</td>
<td>0.0815</td>
<td>0.3336</td>
</tr>
<tr>
<td>6</td>
<td>Rheobate palmatus</td>
<td>0.0142</td>
<td>0.3784</td>
</tr>
</tbody>
</table>
AOO is the area within those outermost limits where the species actually occur (Bachman S et al. 2011). It is the measure that reflects the area where the species is actually present through its EOO excluding unsuitable or unoccupied habitat (IUCN 2012). It is calculated by the sum of occupied grid cells. An appropriate grid cell dimension should be ideally the tenth of the length of the EOO polygon (Willis et al. 2003).

EOO-Environmental Niche Model was calculated after creating suitability maps produced by MaxEnt into binary, presence/absence (section 2.4.5.3.) maps using minimum presence training area threshold.

3. RESULTS

3.1 SPECIES SELECTION

Three amphibian species were recorded: *Pristimantis miyatai* at the high humidity site, *Rheobate palmatus* at the moderate humidity site and *Dendropsophus labialis* at the low humidity site. All species found are classified in the Order Anura and are endemic to Colombia. At high humidity sites, 25 detections were recorded, at least once, in all six transects. Most individuals detected were adults, but occasionally young ones were also recorded. At moderate humidity sites, all 70 detections were recorded, and they were all found in a small perennial pond. Eighteen tadpoles were captured and measured, while 52 were only visually detected. At low humidity sites, 7 adult frogs were captured and measured, 2 were visually detected and 13 were detected via calls. All records, except one adult frog captured, came from the same transect in close proximity to a perennial pond.

Low and moderate humidity areas had most of the occurrence records from one of the six transects and thus suffer from a low number of replications and poor statistical power. Therefore, only data from the high humidity area were for analysing species occurrence and microhabitat selection. For the ENM, four more amphibian species, based on local conservation value and limited occurrence data, were added to the three species detected during fieldwork to increase sample size (Table 3 and Figure 8). In addition, occurrence records for all seven species were
investigated, resulting in *Pristimantis lynchi* being excluded from MaxEnt model analysis due to a lack of sufficient occurrence data after the thinning process.

**Table 3:** List of selected amphibian species in Ricaurte, Boyacá, with elevational ranges and total of records from GBIF, iNaturalist, published literature and field surveys collected for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Family</th>
<th>Elevational Range</th>
<th>Total of records</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Dendropsophus labialis</em> *</td>
<td>Green Dotted Treefrog</td>
<td>Hylidae</td>
<td>1600-3650</td>
</tr>
<tr>
<td>2</td>
<td><em>Hyloclerista bogotensis</em></td>
<td>Bogota Treefrog</td>
<td>Hylidae</td>
<td>2410-3520</td>
</tr>
<tr>
<td>3</td>
<td><em>Hyloclerista subpunctatus</em></td>
<td>Bogota Rocket Frog</td>
<td>Dendrobatidae</td>
<td>1750-4020</td>
</tr>
<tr>
<td>4</td>
<td><em>Pristimantis miyatai</em> *</td>
<td>Miyata's Robber Frog</td>
<td>Craugastoridae</td>
<td>1720-2400</td>
</tr>
<tr>
<td>5</td>
<td><em>Pristimantis uisae</em></td>
<td>unknown</td>
<td>Craugastoridae</td>
<td>2400-2700</td>
</tr>
<tr>
<td>6</td>
<td><em>Rheobates palmatus</em> *</td>
<td>Palm Rocket Frog</td>
<td>Aromobatidae</td>
<td>350-2500</td>
</tr>
<tr>
<td>7</td>
<td><em>Pristimantis lynchi</em> *</td>
<td>Lynch's Robber Frog</td>
<td>Craugastoridae</td>
<td>1600-3580</td>
</tr>
</tbody>
</table>

* Found in active surveys
△ After point thinning process there is insufficient occurrence data (< 5 points) to run MaxEnt model analysis.

*Dendropsophus labialis* (photo by Andres Acosta)  
*Hyloclerista bogotensis* (photo by Hugo Bernal)
3.2 ENVIRONMENTAL VARIABLES AFFECTING AMPHIBIAN OCCURRENCE IN SITU

Partial Least Square regression analysis showed that temperature has a significant effect on amphibian occurrence. In this case, Load Weight (lw), Regression Coefficient (rc) and Variable Importance of Projection (vip) were located above the 0.75 significance threshold.
(Figure 9). Vertical cover at 0.50m (V_05) was found significant by Significant Multivariate Correlation (sms) (Figure 9). At Serrania del Peligro, temperature and vertical cover at 0.50 m (V_05) are thus the most significant environmental variables affecting the occurrence of *P. miyatai*.

![Figure 9](image.png)

**Figure 9:** Partial Least Square regression analysis of environmental variables and their effect on the occurrence of *P. miyatai* based on automatic model choice defined threshold for Load Weights (lw), Regression Coefficients (rc), Variable Importance on Projection (vip) and Significant Multivariate Correlation (sms). Dashed line represent threshold for significant effects - i.e. scores closer to +/- 1 can be considered significant.

In fact, number of *P. miyatai* individuals increases as temperature increases, but only until the temperature reaches 15°C (Figure 10a). As temperature continues to increase, passing 15°C, the number of individuals drop (Figure 10a). The same analysis performed on vertical cover at 0.50cm shows that increasing vegetation cover percentage at 0.50 increases the number of individuals. However, 30 and 40% cover seem to be the most desirable condition for *P. miyatai* at this study site (Figure 10b).
3.3 ENVIRONMENTAL VARIABLES AFFECTING AMPHIBIAN MICROHABITAT SELECTION

Partial Least Square regression analysis showed that temperature has a significant effect on the vertical preference of *P. miyatai*. Temperature came out significant for three tests - Variable Importance on Projection (vip), Load Weight (lw), and Regression Coefficients (rc) where the significance value was above the 0.75 threshold for vip and below -0.75 for lw and rc (Figure 11). At Serrania El Peligro, temperature is thus the most significant variable affecting *P. mitayai* microhabitat preference on a vertical gradient in the forest.

**Figure 10**: The relationship between *P. miyatai* occurrence and a) temperature and b) vertical cover at 0.50m.
**Figure 11:** Partial Least Square regression analysis of environmental variables and their effect on amphibian vertical strata microhabitat selection, based on threshold for Load Weights (lw), Regression Coefficients (rc), Variable Importance on Projection (vip) and Significant Multivariate Correlation (sms). Dashed line represent threshold for significant effects - i.e. scores closer to +/- 1 can be considered significant.

**Figure 12:** The relationship between temperature and *Pristimantis miyatai* microhabitat selection along a vertical gradient in the forest.
The model indicates that individuals of \textit{P. miyatai} will be perched at lower heights as temperature increases (Figure 12). There is high possibility that individuals of this species will prefer to stay higher than 200 cm when the temperature drops to 12°C or lower. In contrast, individuals will stay lower in the forest as the temperature increases to above 15°C.

### 3.4 THE CONTRIBUTION OF ENVIRONMENTAL VARIABLES

Based on the correlation matrix (section 2.4.3) of the 22 environmental variables, a list of selected environmental predictors and the percentage contribution of each variable per species after running MaxEnt model are listed in Table 7.

<table>
<thead>
<tr>
<th>CODES</th>
<th>Environmental Predictors</th>
<th>\textit{D. labialis}</th>
<th>\textit{H. bogotensis}</th>
<th>\textit{H. subpunctatus}</th>
<th>\textit{P. miyatai}</th>
<th>\textit{P. usae}</th>
<th>\textit{R. palmatus}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIO 1</td>
<td>Annual Mean Temperature</td>
<td>23.2</td>
<td>27.5</td>
<td>18.6</td>
<td>13.2</td>
<td>24.7</td>
<td>7.1</td>
</tr>
<tr>
<td>BIO 2</td>
<td>Mean Diurnal Range (Mean of monthly</td>
<td>2.9</td>
<td>1.8</td>
<td>-</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(max temp - min temp))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 3</td>
<td>Isothermality (BIO2/BIO7) (* 100)</td>
<td>2.6</td>
<td>1.3</td>
<td>8.4</td>
<td>-</td>
<td>-</td>
<td>7.2</td>
</tr>
<tr>
<td>BIO 4</td>
<td>Temperature Seasonality (standard deviation</td>
<td>63.6</td>
<td>53.7</td>
<td>57.4</td>
<td>72.1</td>
<td>69.3</td>
<td>66.8</td>
</tr>
<tr>
<td></td>
<td>*100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 5</td>
<td>Max Temperature of Warmest Month</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 6</td>
<td>Min Temperature of Coldest Month</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 7</td>
<td>Temperature Annual Range (BIOS-BIO6)</td>
<td>-</td>
<td>0.9</td>
<td>6.9</td>
<td>-</td>
<td>3.3</td>
<td>1.4</td>
</tr>
<tr>
<td>BIO 8</td>
<td>Mean Temperature of Wettest Quarter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 9</td>
<td>Mean Temperature of Driest Quarter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 10</td>
<td>Mean Temperature of Warmest Quarter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 11</td>
<td>Mean Temperature of Coldest Quarter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 12</td>
<td>Annual Precipitation</td>
<td>0</td>
<td>0.2</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>BIO 13</td>
<td>Precipitation of Wettest Month</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 14</td>
<td>Precipitation of Driest Month</td>
<td>5</td>
<td>-</td>
<td>5.5</td>
<td>9.2</td>
<td>-</td>
<td>9.6</td>
</tr>
<tr>
<td>BIO 15</td>
<td>Precipitation Seasonality (Coefficient of</td>
<td>2.6</td>
<td>3.3</td>
<td>2.6</td>
<td>3.2</td>
<td>-</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>Variation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO 16</td>
<td>Precipitation of Wettest Quarter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>BIO 17</td>
<td>Precipitation of Driest Quarter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>BIO 18</td>
<td>Precipitation of Warmest Quarter</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
<td>1.1</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>BIO 19</td>
<td>Precipitation of Coldest Quarter</td>
<td>-</td>
<td>11.3</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>AI yr</td>
<td>Aridity</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>PET</td>
<td>Potential Evapo-transpiration</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>Altitude</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to the MaxEnt estimation of relative contribution of environmental variables to the models, it is apparent that temperature seasonality (Bio_04) and Annual Mean Temperature (Bio_01) have the highest contribution to the models for all species. However, the third most significant variable appears to be exclusive to each species, except for \textit{D. labialis} and \textit{P. miyatai} where Precipitation of Driest Month (Bio_14) is the third most influential variable.
3.5 JACKKNIFE TEST

According to the Jackknife test for all species analyzed, Temperature Seasonality (Bio_04) is the most influential predictor having the highest gain value in isolation, thus having the most meaningful information for all the models (Figure 13). Annual Mean Temperature (BIO_01) shows the largest decrease in gain value when omitted for *D. labialis*, *H. bogotensis*, *H. subpunctatus*, *P. uisae*, and *R. palmatus*, which underlines significant information not present in the other predictors. Contrastingly, temperature seasonality portrays a decrease in gain value when omitted for *P. miyatai* (Figure 13d).

**Figure 13:** Jackknife analysis for the most important environmental variables to the model: the contribution of each individual variable (blue bar), contribution when a given variable is excluded (green bar), entire set of variables (red bar). *Dendropsophus labialis* (a); *Hyloscirtus bogotensis* (b); *Hyloxalus subpunctatus* (c); *Pristimantis miyatai* (d); *Pristimantis uisae* (e); *Rheobate palmatus* (f).
3.6. BINARY MAPS (PRESENCE-ABSENCE) AND AMPHIBIAN SPECIES RICHNESS

Minimal training presence cloglog threshold (Table 5) for the six selected amphibian species were used to create binary maps from habitat suitability maps for each species (Figure 14a-f).
Figure 14: Predicted distribution maps (presence/absence) of selected species using MaxEnt model analysis. Grey colour = presence; Black colour = absence (left column). Actual species occurrence records using GEOCAT (right column). *Dendropsophus labialis* (a); *Hyloscirtus bogotensis* (b); *Hyloxalus subpunctatus* (c); *Pristimantis miyatai* (d); *Pristimantis uisae* (e); *Rheobate palmatus* (f).

Figure 14 shows that occurrence points, in most cases, fall within the high probability area (light grey areas) predicted by the MaxEnt model. Some exceptions occur for *P. uisae* along
the central region and for *D. labialis* and *R. palmatus* for Amazonia in the south.

In order to generate amphibian species richness distribution map, all presence-absence maps were overlapped (Figure 15). The areas with highest richness (red) fall within the Andes regions along the Eastern, Western and central Cordilleras. There is a small area to the north known as Sierra Nevada de Santa Marta also showing high species richness.

**Figure 15:** Species richness areas based on the binary maps of species distributions in relation to national protected areas. Source: protected areas were obtained at protectedplanet.net Oct 2017

### 3.7 MODEL EVALUATION

Area Under the Curve (AUC) values for all the selected species are listed in Table 9. All six amphibian species selected for the MaxEnt analysis have AUC values for training data higher than 0.99 and testing data higher than 0.98, indicating good model performance. The ROC/AUC values are shown in the Appendix 1.
### 3.8 GEOGRAPHIC RANGE AND RISK STATUS

After calculating the Extent of Occurrence (EOO) for all seven amphibian species using GEOCAT, five species (D. labialis, H. bogotensis, H. subpunctatus, P. miyatai and R. palmatus) portray high EOO values (Table 10), while P. uisae has an EOO value less than 50,000 km² and P. lynchi less than 1000 km². Area of Occurrence (AOO) for D. labialis, H. bogotensis, P. miyatai, P. uisae and P. lynchi have values less than 500 km² while R. palmatus H. subpunctatus have AOO values less than 2000 km². The Extent of Occurrence (EOO-ENM) calculated using MaxEnt minimum presence area threshold shows that most of the species have large ranges (more than 700,000 km²) expect for R. palmatus, which has less than 300,00 km². P. lynchi was not assessed it was excluded from the MaxEnt model analysis due to a lack of sufficient occurrence data after the thinning process (Table 10).

Table 10: Area of Occurrence (AOO), Extent of Occurrence (EOO) and Minimum Presence Area estimated with ENM for the seven frog species.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Current IUCN status</th>
<th>AOO (Km²)</th>
<th>IUCN Status⁴</th>
<th>EOO (km²)</th>
<th>Proposed Status</th>
<th>ENM-EOO (Km²)</th>
<th>% of Total Area*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dendropsophus labialis</td>
<td>LC</td>
<td>444,000</td>
<td>EN</td>
<td>539,505.12</td>
<td>LC</td>
<td>1,057,547.000</td>
<td>79.4</td>
</tr>
<tr>
<td>2</td>
<td>Hyloscirtus bogotensis</td>
<td>NT</td>
<td>164.000</td>
<td>EN</td>
<td>85,723.899</td>
<td>EN</td>
<td>704,920.000</td>
<td>52.9</td>
</tr>
<tr>
<td>3</td>
<td>Hylocaulus subpunctatus</td>
<td>LC</td>
<td>512.000</td>
<td>VU</td>
<td>766,384.788</td>
<td>LC</td>
<td>1,125,677.000</td>
<td>84.5</td>
</tr>
<tr>
<td>4</td>
<td>Pristimantis miyatai</td>
<td>LC</td>
<td>176.000</td>
<td>EN</td>
<td>148,393.71</td>
<td>EN</td>
<td>734,640.000</td>
<td>55.2</td>
</tr>
<tr>
<td>5</td>
<td>Pristimantis uisae</td>
<td>DD</td>
<td>96.000</td>
<td>EN</td>
<td>38,079.17</td>
<td>EN</td>
<td>970,106.000</td>
<td>72.8</td>
</tr>
<tr>
<td>6</td>
<td>Rheobate palmatus</td>
<td>LC</td>
<td>716.000</td>
<td>VU</td>
<td>425,343.31</td>
<td>LC</td>
<td>294,587.000</td>
<td>22.1</td>
</tr>
<tr>
<td>7</td>
<td>Pristimantis lynchi</td>
<td>DD</td>
<td>20.000</td>
<td>EN</td>
<td>858.387</td>
<td>EN</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Status using GEOCAT analysis based on AOO <5000Km² = NT. <2000 Km² = VU. <500 Km² = EN. <10 Km² = CR
NA: Not Assessed.
* based on Colombia total area of 1,331,822.00 Km²
4. DISCUSSION

Environmental Variables Affecting *Pristimantis miyatai* Occurrence Distribution and Microhabitat Selection in La Serrania El Peligro.

*Pristimantis miyatai* occurrence was largely influenced by temperature and vegetation cover. These results correspond well with the literature identifying temperature as one of the most influential variables affecting amphibian distribution (Berven 1982; Luddecke 1997; Navas 2002). Amphibians in particular, compared with other vertebrates, rely on external sources of heat and behavioural thermoregulation to control their body temperature (Duellman & Trueb 1997). Therefore, all aspects of amphibian life history are strongly impacted by environmental factors, such as temperature. Many crucial physiological processes, such as rate of oxygen uptake, locomotion, developmental rate, immune functions and water balance are regulated by temperature (Feder & Burggren 1992). Water balance highly influences amphibian physiology and behaviour by controlling the ability of the body to gain water through osmosis and lose it through evaporation (Hillyard 1999). Thus, temperature plays an important role for physiological processes in amphibians that seem to work synergistically or antagonistically influencing the organism’s behaviour and survival.

In ectothermic organisms, like amphibians, daily and seasonal temperature changes affect variation in the thermal tolerance (Rome et al. 1992). Amphibians must have different mechanism to counterbalance extreme changes in temperature and photoperiods. The tropical Andes experiences pronounced diurnal atmospheric variations, which influences temperature fluctuations between day and night - experiencing very warm temperatures during the day and very cold temperatures during the night (Garreaud 2009). Surprisingly, in La Serrania El Peligro, differences in average temperatures during the day and night were not statistically significant (p=0.060), which could suggest that *P. miyatai* is not likely to undergo thermal acclimation common in species inhabiting temperate regions and extreme habitats, such as deserts. The lack of extreme daily and seasonal temperature fluctuations could lead *P. miyatai* to have narrow thermal tolerance range and inhibit the species to quickly acclimatise to extreme temperatures, which is energy costly and unfavourable (Angilletta Jr. 2009). However, one caveat of the current study is that all of the temperature measurements were recorded during morning surveys.
and during night surveys. This could have created a bias towards midnight/early morning temperature records. Significant differences could have been detected if continuous temperature measurements were taken throughout the day. More detailed temperature records in situ are therefore necessary to fully understand the thermal tolerance and acclimation abilities of *P. miyatai*. Understanding the effects of temperature and thermal tolerance in amphibian species could be critical under current climate change scenarios.

Even though temperature has been recognized as one of the most influential variables affecting amphibian distribution, further evaluation by Navas (2006) proposed that maybe other factors affecting embryo development, such as UV radiation and water pH, may be a more limiting factor on amphibian species distribution. This idea was examined earlier by Blaustein and Belden (2003) when discussing the effects of UV radiation as an important factor for amphibian population decline. In addition, synergistic effects of temperature with other environmental variables is likely to be significant. For example, changes in relative humidity could have affected how frogs experience even small fluctuations in temperature (Hall & Root 1930) and thereby influence microhabitat selection. Nevertheless, as mentioned by Wells (2010), there are very few studies on the role of moisture and humidity for microhabitat selection in anurans. A relationship between temperature and humidity could explain why, at this site, all *P. miyatai* detections were recorded during dusk when temperatures were slightly higher compared to night temperatures, but still lower compared to day temperatures. Amphibians are humidity sensitive and most species show higher activity rates when moisture conditions are more favourable. This is shown by some species exhibiting more foraging activity on nights with higher cloud cover when humidity is high, compared with clearer and drier nights (Wells 2010). The fact that *Pristimantis* are predominantly limited to humid habitats (Duellman 1979; Lynch & Duellman 1997) suggests that a combination of climatic optimal conditions and local environmental characteristics are crucial in shaping its distribution. Adaptations, such as reproduction mode, to habitats lacking aquatic breeding sites or to acclimate to strong temperature fluctuations between day and night have allowed this species to survive in the Andes.
Vegetation cover plays an important role in thermoregulation and possibly for foraging mode and reproductive site selection (Deichmann et al. 2012; Toft 1981). For example, differences in the altitudinal gradient translates to drastic changes in habitat structure and community composition as shown through the transition from the Andean cloud forest at 3400-3600 m.a.s.l to a less diverse open alpine grassland (paramo) at 3800 m.a.s.l and above (Hofstede et al. 2014; Ramsay 2001). Such changes are correspondingly affecting plant productivity, which Deichmann et al. (2012) suggest has an indirect effect on amphibian biomass by reducing arthropods abundance. Therefore, lower productivity could result in higher interspecific and intraspecific competition, with knock-on effects on growth rate and possibly leading to smaller adult size as observed in salamanders (Bernardo and Agosta 2003). Consequently, body size changes could have an additional impact on thermal tolerance, as it has been extensively evaluated through the Bergmann (1847) rule on body size and Allen (1877) rule on body shapes - as both rules cause changes in the surface area to volume ratios. Smaller body size in amphibians could represent higher vulnerability to desiccation under extreme environments as experienced by salamanders (Ray 1958). However, as stated by Ashton (2002), anurans show weaker tendencies towards affinity with Bergmann’s rule in relation to latitude and elevation compared with other ectoderms. Therefore, caution and further evaluation is needed. Certain regions have a set of characteristics that affect each species differently (Leibold 1995; Pulliam 2000), thus creating species-specific diverse variations of tolerance or adaptations to a particular variable. In addition, vegetation composition and structure and the effects on amphibian communities have been shown to be vital. They not only determine the presence or absence of amphibian species, but predict amphibian species richness, abundance and composition as shown by Basham et al. (2016) who compared pasture, secondary forest and primary forest in the tropical Andes. Furthermore, they recognized that in species highly specialized, or like many forest-dwelling amphibians, structural changes in the vegetation become not only important but extremely species-specific, thereby causing higher vulnerability and lower adaptability to disturbances.

In addition, this study suggests that P. miyatai use of the vertical forest strata is strongly dictated by temperature and time of day. P. miyatai assemblages show a microhabitat preferences for sites between 0.5 and 1.5 meters in height, which is consistent with other studies of Pristimantis assemblages that indicate a preference for perching within 1.5 meters of the
When temperatures were below 12°C, individuals were most likely encountered in the higher portions of the vegetation above 2.0 meters. As the temperature increased during the day, frogs appeared to descend to perch in the lower vegetation strata, which agrees with the notion that humidity is greatest closer to the ground. This apparent thermoregulatory behaviour concurs with previous observations of day and night temperature fluctuations and their effects on amphibians in high tropical mountain regions (Ghalambor et al. 2006). A similar trend has also been observed in other taxa, such as Neotropical birds (Walther (2002). It is likely that microhabitat height selection is determined by a thermoregulation mechanism to maximize activity at dusk when frogs show the highest activity rate and assumed optimal body temperature. This was shown by the highest frog occurrences within 1.5 meters at dusk when temperatures were 14°C and 15°C. Therefore, in order to regulate body temperature *P. miyatai* seem to actively select particularly warm microhabitats with optimal moist substrates. Navas (1996) evaluated microhabitat selection of some high Andes *Pristimantis* species, such *P. bogotensis*, an ecologically similar species to *P. miyatai*, and found that they were thermoconformes with body temperatures very similar to substrate temperatures. This is relevant for *P. miyatai* as it seems that they descent from higher vegetation strata as temperatures increase during the day, most likely to find optimal temperature lower down where they spend most of the nocturnal activity. In accordance with Navas’ observations (Navas 1996), as night proceeds and temperature decrease most frogs should find refuge in microhabitats that can maintain their body temperatures and reduce desiccation from the wind. In this study, it is evident that *P. miyatai* finds prime microhabitat between 0.5m-1.5 meters from the ground with 30-40% vegetation cover the most suitable. This idea can also be examined by the fact that the peak of activity for most frogs (18.00-21.00 hours), which requires optimal body temperature, were between 0.5m-1.5 meters height from the ground. Similar results were observed by Arroyo et al. (2008) on diet, microhabitat and time of activity of *Pristimantis*. Moreover, after peak activity hours, it was difficult to find frogs, suggesting that as temperatures started to decrease below 12°C, most frogs looked for refuge within vegetation micro-refugia or above 2m height, thus out of the observers reach. Frogs could still be very active even in higher strata but based on calling activity this appears unlikely. In addition to microhabitat being selected for thermoregulation of optimal active body temperature, it has also been proposed as an important amphibian micro-refugia under extreme climate conditions.
conditions. In a study on thermally buffered microhabitats, González del Pliego et al. (2016) exposed the importance of microhabitats providing shelter for species with narrow ranges and tolerance limitations that under extreme climate conditions will be destined to extinction. This might be the case of *P. miyatai*, which has a quite narrow altitudinal range of about 1720-2400 m.a.s.l and inhabits high mountain forest with relative cold conditions (Bernal & Lynch 2008; Mendoza et al. 2015).

Another approach to understanding microhabitat use is related to foraging and food sources. Perhaps *P. miyatai* utilize the most vegetation strata with 30-40% cover, as temperature and humidity are more stable than in open microhabitats, such as ground level. Therefore, favouring such a microhabitat supports easier regulation and maintenance of optimal body temperature. This translates into effective locomotion (Feder & Burggren 1992) and thus higher foraging activity and efficiency. In addition, Arroyo et al. (2008) shows that *P. miyatai* diet is commonly based on collembolans, coleopterans, and isopods, which are more abundant in the lower vegetation (Johnson & Catley 2005). High selectivity for microhabitats within 1.5 meters that hold 30-40 percent vegetation cover suggests that such selective process could be driven by a combination of foraging, morphological and structural characteristics. This is the case for *Eleutherodactylus* coqui, which not only shows such links, but also higher hatching success with low trade-off between parental care and mate attraction (Townsend 1989). Breeding site preference may also play a role, but further study on breeding and nesting site preferences for *P. miyatai* are necessary to evaluate this. In addition, it should be mentioned that based on the fact that all of the encounters recorded in this study were during the night and found in microhabitat above ground, *P. miyatai* assemblages at La Serrania El Peligro appear to be primarily arboreal and nocturnal.

**Amphibian Species Distribution Model and Environmental Predictors**

The MaxEnt models successfully predicted potential suitable habitat for *D. labialis, H. bogotensis, H. subpunctatus, P. miyatai, P. uisae* and *R. palmatus*, as represented by the high AUC values. Following the methodology of Engler et al. (2004), models that maintain AUC values higher than 0.9 are consider having a good fit while models with AUC values lower than 0.7 are considered to have a poor performance. Of all the species tested in the current study, the
model for *H. subpunctatus* had the lowest AUC training (0.991) and AUC test (0.988), underlining the high performance of the models. In addition, the results show that in the case of *P. uisae* with 13 records, the MaxEnt model also performed well, despite other model approaches showing problems with small sample sizes (Hernandez et al. 2006).

The jackknife test showed that temperature seasonality was the most important predictor of habitat distribution for all six species, which matches the heuristic test of percentage contribution. While both tests provided good information, the jackknife test shows which variables have the most useful information independent of the others, while the heuristic test does not make that distinction. This is important as the model could give a higher percentage to a variable based on its correlated effect with another variable. These results are valuable, but they should be considered based on the biological role of the variable for the species in question. For this study, the results agree with most of the literature that define temperature as one of the most influential variables on amphibian distribution and seasonality, and playing a crucial role for anurans living at high elevations (Navas 2006). Although not unusual, this result is significant in a climate context. Colombia is a tropical country located in the equatorial region, therefore lacking seasons but experiencing high rainfall periods and low rainfall periods. In addition, the heterogeneous Colombian topography creates various microclimatic systems depending on altitudinal levels. Therefore, Colombia has particular characteristics under a climatic phenomenon, such as El Niño or La Niña, where changing sea temperatures and wind currents reduce precipitation periods and increase temperature or increase precipitation periods and decrease average temperatures, respectively. In Colombia, the regions most affected by these phenomena are the Caribbean, Pacific and Andes regions. Throughout these events, average temperatures may increase with more than 0.3°C during El Niño and decrease with 0.2°C during La Niña in areas like the Andes (Appendix 2) (IDEAM 2018).

The strong influence of temperature seasonality found in this study therefore suggests that such climate phenomena could play an important role in amphibian species distributions. The dynamic of this possible interaction could be based on the hypothesis that limited seasonal temperature variations in places like the Andes has reduced species distribution to narrow ranges (Janzen 1967). This suggests that the impact of sudden changes in temperature seasonality by El Niño or La Niña could break temperature stratification niches across elevations. This could have
an effect as observed in the second most recent amphibian radiation in the Andes, which was
driven by a contraction and expansion of habitat changes, thus allowing speciation through
vicariance (Navas 2006). Although a phenomenon like El Niño or La Niña appears to be random
and limited in time, not enough to produce evolutionary changes through speciation, it is
certainly an important force that could influence faster amphibian adaptations through
climate change projections in the Andes for the end of the 21st century and predicted that
projected changes in temperatures and precipitation patterns over the Andes would have similar
c characteristics as produced by El Niño. Therefore, under sudden and unpredicted climatic events
or under more predicted long-term trends, climate should be regarded as an important element of
amphibian species conservation.

The MaxEnt models were highly successful in predicting the known distribution of the
six selected amphibian species. In addition, the models identified numerous potential suitable
areas, indicating that even poorly occurrence-represented species like *H. bogotensis, P. uisae,*
and *R. palmatus* could have a much larger expected range. In general, all the projections of all
the models showed low suitability in areas like La Guajira, which is mostly desert and xeric.
Interestingly, regions like southern Amazonia and southern Chocó, also showed low suitability
even though they are considered areas of high species diversity (Arbeláez–Cortés 2013). This
shows that even though there are areas known for high biodiversity, some species-specific
aspects may play an important role in the presence and conservation of these species. For
example, most of these species in this study are known to inhabit mountain regions with
relatively low temperatures, while areas like Chocó and Amazon have average annual
temperatures of 30°C (IDEAM 2018), possibly restricting their distribution. While all of the
species distribution projections seem not to follow general Colombian vertebrate species richness
patterns, they all seem to follow the patterns of vertebrate species endemism distribution (Forero-
Medina & Joppa 2010a). All selected species are known to be endemic to Colombian and they
play a crucial role contributing to the rich genetic diversity (Myers et al. 2000). Nonetheless,
endemic species face high vulnerability to extinction based on reduced range, specific habitat
requirements, and small population sizes. This observation also agrees with the low occurrence
records shown by *H. bogotensis, P. uisae, R. palmatus* and *P. lynchii,* possibly indicating a
restricted distribution and abundance (Brown 1984; Holt et al. 1997), but not certainly. These species also have very narrow elevation ranges and show a preference for humid Andes mountain habitats (Bernal & Lynch 2008; Lynch & Duellman 1997; Navas 2006; Ramírez 2017; Ramírez Pinilla et al. 2004) - with the exception of *R. palmatus*, which exhibits more generalist characteristics with a wider elevation range (350-2500 m.a.s.l). However, as observed by Lynch (2006), this species is quite susceptible to anthropogenic disturbances in the habitat.

Furthermore, ENM may be used to identify areas where there is a high possibility of population persistence, suitable habitats for species reintroductions, and areas of high species richness and conservation value (Guisan & Huiller 2005; Joshi et al. 2017). The predicted richness patterns presented herein show moderate to high species richness along the south of the Andes regions and towards the east of Colombia (across the departments of Caquetá, Guaviare, Vaupez and Guania). These areas have also been shown in other analysis to be highly diverse (Arbeláez–Cortés 2013; Forero-Medina & Joppa 2010b) (Appendix 3 and 4) and the Andean region is known as a biodiversity hotspot (Myers et al. 2000). This study showed that projected areas of high species richness along the Andes cordilleras fall under areas where conservation measurements have been implemented, such as protected areas, which account for about 11.27 percent of Colombian territory (SINAP 2002-2009). Yet, the Colombian Amazon region, such as Caquetá, remains poorly studied and protected due to the political instability and violent civil unrest of the past sixty years (Arbeláez–Cortés (2013). In addition, areas like Antioquia show very few protected areas, which contrast with the fact that the department shows a high number of studies on conservation and diversity that compares with its high levels of biodiversity (Arbeláez–Cortés 2013). However, after political and social reforms, Colombia has opened its doors to peace agreements and also to science. Approaches like the ones used in the current thesis can be used to design local and national conservation management efforts in areas poorly explored, such as the Caquetá region, and to prioritize efforts by targeting highly suitable areas.

This study of species distribution based on MaxEnt presented herein is a useful guide to areas where these endemic species could possibly be located. This approach offers an easier way to assess locations for conservation strategies where they are difficult to evaluate due to inaccessibility or lack of information. Nevertheless, MaxEnt showed some restrictions in this study. One of the main concerns with ENM approaches is the requirement of species presence
and absence data and its effects on model performance. Different modeling methods offset the lack of absence data by using various statistical approaches, which are influenced by the number of occurrence points and spatial scale (Pearson et al. 2007). Thus, making occurrence data available is critical to current modelling approaches.

However, other assessments could be considered for species with low occurrence records, such as *P. lynchi* that was unfit for the MaxEnt analysis in the current work. One idea would be the one proposed by Pearson et al. (2007) in a study of cryptic geckos in Madagascar with less than 25 records, which successfully used a jackknife validation approach on k-fold partitioning. Gómez–Hoyos et al. (2017) also found positive results for species with less than ten occurrence records by defining species distribution with relation to the species percentage records within the Regional System of Protected Areas and defined its representativeness. Such approaches, even though novel or less robust, offer a good estimation for species distribution otherwise undervalued or ignored. However, such evaluations were beyond the scope of the current thesis.

**Distribution Range and Conservation Status**

The results from calculating Area of Occupancy (AOO) and Extent of Occupancy (EOO) showed that *P. lynchi, P. uisae, H. bogotensis*, and *P. miyatai* have the smallest range of occupancy of all seven selected amphibian species. This result could possibly be a result of low occurrence records from all four species. However, this is unlikely since *P. miyatai* has the most occurrence records among all seven species. This implies that the narrow range of occupancy for *P. miyatai* is realistic and not an artefact of occurrence records. However, results such as *P. lynchi*, which has the narrowest range of occupancy, but also, the lowest number occurrence records, should be taken with caution as occupancy range might change with increase of occurrence records, but not certainty.

All seven focused species have been assessed by the IUCN (2017a) under the global amphibian assessment and a conservation status has been assigned based on the IUCN conservation status criteria. Four of the seven focal species are listed as least concern (LC): *D. labialis, H. subpunctatus, R. palmatus* and *P. miyatai*, with the last one, being downgraded from near threatened (NT) in 2017. In addition, IUCN highlights that all species, although stable and common, maintain a very narrow extent of occurrence (EOO). This is relevant to a species like
*H. bogotensis* which is listed as near threatened (NT) already, and thus with low EOO and habitat declining it could be at higher risk to become rapidly listed as vulnerable (VU). *P. uisae* and *P. lynchi* are listed as data deficient (DD) and any information would therefore be highly valuable.

This was evident on two of the focal species, *P. uisae* and *P. lynchi*, which although relatively newly described, there is not much information and still under IUCN Red List status of data deficient since 2004 (Lynch 2004; Ramírez Pinilla et al. 2004). Nevertheless, there has been important efforts to increase basic species data and knowledge, such as the work on *P. uisae* resulting in new occurrence records and an elevation range extension Ramírez (2017).

Using occurrence data, I evaluated and reassessed the IUCN Red List Category and proposed a different status for the focal species. However, EOO has limitations with tendencies to overestimate the species range or include significant areas of unsuitable habitat (Gaston 1991; Gaston 1994). Given that the potential Extend of Occupancy generated by MaxEnt (ENM-EOO) increased species occupancy by 30-70%, compared with EOO generated by actual species occurrence points, these results should be interpreted with caution. EOO generated by MaxEnt (ENM-EOO) could potentially overestimate by including habitat discontinuities at much finer scales or topographical barriers impossible for some species to overcome. For example, *P. miyatai* results show an increment in ENM-EOO of 44.1% when its area of occurrence (AOO) is as low as 0.12% of its EOO. Based on the natural history of the species, it has a very narrow elevation range (1700-2400 m.a.sl.) and temperature tolerance range, and it would be very unlikely to find it in areas such the west/south of Colombia where elevations do not exceed 500 m.a.s.l and temperatures are relatively high. Similarly, this is the case for *H. bogotensis* with a ENM-EOO increment of 46.5%, but AOO as low as 0.19%. This outcome becomes even more critical for species that are possibly considered very specialist and cryptic, such as *P. uisae*. Although *P. uisae* shows low AOO and EOO, there is an increase of 70% under ENM-EOO. This can be misleading as *P. uisae* seems to have a very narrow temperature tolerance range, reaffirmed by its very narrow EOO of 2.8%. We therefore need to be conservative when using such measurements as it could be detrimental for the conservation of the species. Therefore, I have focused primarily on Area Of Occurrence (AAO) as a conservation approach, because it is the area over which species actually occur and generally correlates with population size (Joseph
It can also provide a better judgement on the species population susceptibility to decline or extinction. Nonetheless, using the three different range measurements in combination could create a more integrated and functional approach.

Results show that all species have low AOO, but their EOO is relatively variable with *H. subpunctatus* the highest (57.5%) and *P. lynchii* (0.06%) the lowest. Generally, species with a low EOO should have higher conservation value (Joshi et al. (2017)). If species with low EOO are very specific in abiotic requirements, such as rain regimes or land cover, then its subsistence is even more at risk. Thus, *H. bogotensis, P. miyatai, P. uisae, and P. lynchii* having a low EOO, and very low AOO should be reassessed to endangered (EN) based on IUCN Red List criteria A3, B1a, B2a (IUCN 2012). This analysis, plus the limited dispersal ability, narrow elevational range, high habitat specialization, rapidly increasing habitat loss, and climate change (Almeida-Gomes & Rocha 2015; Donnelly & Crump 1998; Stuart et al. 2008; Whiles et al. 2006) reiterates the high vulnerability of these amphibian species populations.
5. CONCLUSION

In conclusion, this study shows that *in situ* evaluation of environmental variables that define amphibian species distribution, are important to understand the basic requirements needed for species conservation. However, complementary and cost-efficient approaches, such as Ecological Niche Modelling, offer a practical and effective tool to predict the possible distribution of species over a greater area. I found that such approaches may influence management and IUCN Red List status of Andean amphibian species and protection strategies. MaxEnt projections were highly accurate with known species distributions, reaffirming high rich species diversity along the Andean Cordilleras. Also, it offered new possible research windows in regions like Caquetá and Antioquia where high species richness is present, but low conservation efforts apparent. The models showed that environmental variables, such as temperature and precipitation, play an important role in amphibian species distribution in the Andes. Nonetheless, complex biotic and abiotic interaction may be crucial, and results should be interpreted with caution. Yet, results suggest that climate change is likely to affect future species distributions and MaxEnt could provide a predictable tool to project such changes. In general, Colombia offers interesting opportunities for novel conservation biodiversity approaches and Andean amphibian species provide a good study system on which to tests such approaches.
6. REFERENCE


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APPENDIX 1:

Cross-validated ROC/AUC curve for the performance of all six MaxEnt amphibian species models. It runs the model multiple times using all data for validation. Each figure shows the mean AUC and the summary response curves with one standard deviation error bars.

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APPENDIX 2:

APPENDIX 2: Most drastic Temperatures change predictions under El Nino (A) and La Nina (B). Colombian and Department of Boyaca. Source: IDEAM - Interactive Climate Atlas of Colombia (Instituto de Hidrologia, Metereologia y Estudios Ambientales) http://atlas.ideam.gov.co/visorAtlasClimatologico.html
APPENDIX 3: Distribution of richness, endemics, and threat for vertebrate species in Colombia. A) species richness (amphibians, mammals, birds), B) number of threatened species, and C) number of endemic species in Colombia. Source: (Forero-Medina & Joppa 2010a)
APPENDIX 4: Geographic patterns of the total number of studies on Colombian biodiversity from 1990 to 2011, and the Simpson diversity index for each Colombian department and ocean. Source: (Arbeláez–Cortés 2013)