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DIVERSITY, STRUCTURE AND NATURAL HISTORY OF AMPHIBIANS IN THE UPPER CLARO RIVER BASIN, A BUFFER ZONE OF THE NATIONAL NATURAL PARK LOS NEVADOS, CENTRAL CORDILLERA OF COLOMBIA

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Abstract: We present an assessment on composition, diversity and structure of amphibians in three zones along an elevation gradient (2,400–3,000 m) in the Central Cordillera of Colombia. For this purpose, we carried out two field trips in November 2014 and February 2015, covering rainy and dry seasons, respectively. Diurnal (08:00–12:00 h) and nocturnal (18:00–22:00 h) visual encounter surveys were made without spatial restrictions. The diversity for each zone (alpha) and for the entire landscape (gamma) was evaluated by the effective number of species, and the structure of the communities was analyzed by range-abundance curves. The inequality factor for each of the sampling zones was also calculated. A total of 15 species belonging to seven genera and three families were recorded, all of the order Anura. Craugastoridae with 11 species (73.3% of richness) and *Pristimantis* (eight species) were the most diverse family and genus, respectively. The average alpha diversity per zone was 6.6 effective species, with zone A being the most diverse with eight species. In terms of beta diversity we found 2.5 effective communities at the landscape level, and differences between zones are given by the rare species, while the most abundant ones (e.g., *Pristimantis uranobates*) are shared between them. It is presumed that the greater diversity of zone A is due to the lower elevation and better state of conservation if compared to the other two zones. Of the total species recorded, three are threatened with extinction: Endangered (*Hypodactylus latens*, *Osornophryne percrassa*), and Critically Endangered (*Niceforonia adenobranchia*). The finding of three yet undescribed species is highlighted.

Keywords: Andean landscapes, diversity, endemic species, threatened amphibians.

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INTRODUCTION

The study of biodiversity in the mega-diverse countries is a condition without which development models could not be implemented in a sustainable manner (Erhlich & Wilson 1991; Adams et al. 2004; Greene et al. 2005). Therefore, to carry out research that generates knowledge about diversity, distribution, uses and threats to biodiversity, it is an obligation to delineate these models at different scales. Colombia is characterized by its high biodiversity, highlighting a considerable richness of anuran amphibians (757 species with 354 endemics; Acosta & Cuentas 2018), being the second richest amphibian country in the world after Brazil (Frost 2017). Contrary to this, near to 28% of Colombian amphibian species are threatened with extinction (Acosta & Cuentas 2018) due to different factors, such as climate change, habitat transformation, chytridiomycosis, and introduction of exotic species (Stuart et al. 2008). The highest proportion of threatened species is concentrated in the Andean region, where the greatest diversity occurs with more than 400 species (Lynch et al. 1997; Acosta 2000; Kattan et al. 2004; Bernal & Lynch 2008; Acosta & Cuentas 2018).

Located in Central Cordillera and delimited from east to west by the basins of the Magdalena and Cauca rivers, respectively, the department of Caldas is one of the smallest departments (geographic political units) of Colombia. Despite having a small geographic area (7888 km²), its amphibian fauna represents approximately 14% of the known richness for Colombia (Acosta 2009; Rojas-Morales et al. 2014a). Such diversity can be attributed to its location within the north Andean region, which represents the global Hotspot (Myers et al. 2000) with the highest amphibian diversity in the world (Hutter et al. 2017). In some areas above 2,500 m of elevation and up to the Páramo level (3,200–3,700 m), there is still an information gap regarding composition and diversity of amphibians. At this area, landscape configuration has been modified since the mid-nineteenth century to expand livestock farms, and subsequently extensive potato crops (*Solanum tuberosum*). This has considerably transformed the original habitat of this mountain region (Valencia 1985; Márquez 2001).

Although in recent years there have been numerous studies to quantify the amphibian's diversity at different spatial scales in Colombia particularly to compare between natural and modified habitats (e.g., Arroyo et al. 2003; Urbina & Londoño 2003; Cadavid et al. 2005; García et al. 2007; Cáceres-Andrade & Urbina-Cardona 2009;

Osorno et al. 2011), the metrics used are not the most appropriate because they do not represent the diversity per se of the communities. Indexes such as Shannon or Simpson dominance are measures of entropy, but do not correspond with the number of effective elements that make up a biological community. For that reason it is difficult to interpret and compare results from different studies with this mathematical application (Ricotta 2003; Jost 2006; Moreno et al. 2011). Only until very recent years, a different approach has been used to measure the diversity (including amphibians), taking into account the conversion of indexes into values of number of effective species, to represent the species diversity (Jost 2006; Rös et al. 2012; Acevedo et al. 2016; Méndez-Narváez & Bolívar-G 2016; Vargas-Salinas & Aponte-Gutiérrez 2016; Casas-Pinilla et al. 2017). The approach proposed by Jost (2006) is more effective to account diversity, because results are given in number of species, which facilitates interpretation of results.

In this paper we present an analysis of amphibian diversity and structure, between three zones of an Andean river in the Central Cordillera of Colombia. We follow Jost's proposal (Jost 2006) for the analysis of species diversity, assuming the effective number of species as the diversity measurement. In addition, we provide information on natural history, distribution, and identification of the threatened species encountered in the study.

MATERIALS AND METHODS

Study area

The study was carried out in the area of the geothermal project of the "Nereidas Valley", located in the upper Claro River basin, Playa Larga Village, municipality of Villamaría, department of Caldas, Colombia, on the western slope of the Central Cordillera. This area occupies approximately 8 km² of extension, and is located within the buffer zone of Los Nevados National Natural Park (Fig. 1). Fieldwork was carried out specifically in the farms "La Laguna" (zone A), "Playa Larga" (zone B) and "Laguna Alta" (zone C). The sampling zones are between 2,400–3,000 m of elevation, with a temperature and a relative humidity ranging between 8–16 °C and 78–93%, respectively. The average annual rainfall reaches 2,076 mm, with two rainy periods between March–May and October–November (Acosta-Galvis 2009). Two life zones are recognized in this area (sensu Holdridge 1982), which correspond to the very humid low montane forest (bmh-MB) from

2,400–2,600 m, and the pluvial montane forest (bp-M) above this elevation, in the Zone B.

Landscape at this area is dominated by pastures for livestock (> 70% of the landscape) with introduced grass species such as *Pennisetum clandestinum*, *Holcus lanatus*, and *Paspalum* sp. (Poaceae). Secondary forests are the main natural coverage, restricted to the hilltops and stream borders that normally do not exceed 100m in width. All this area is drained by the Claro River, and Molinos and Nereidas streams, which make up the upper Claro River basin (Image 1).

Zone A - “La Cadena”: (4.919°N & -75.455°W, WGS84, 2,420m). This is a secondary forest fragment in advanced state of succession with low anthropic intervention located in a narrow canyon with a steep slope (> 50%). The forest shows four differentiable strata with trees that reach up to 50m height (*Ceroxylon quindiuense*) (Image 2a). Highest strata are represented by species such as *Weinmannia pubescens*, *W. elliptica* (Cunoniaceae), *Brunellia comocladifolia* (Brunelliaceae), *Faramea flavicans* (Rubiaceae), *Guarea kunthiana*

(Meliaceae), *Lippia schlimii* (Verbenaceae), *Drimys granadensis* (Winteraceae), *Oreopanax floribundus* (Araliaceae), *Turpinia occidentalis* (Staphyleaceae), *Ficus gigantocyce* (Moraceae) and *C. quindiuense* (Arecaceae). The understory is rich in herbaceous and growing trees, mainly of the genus *Piper* spp. (Piperaceae) and *Miconia* spp. (Melastomataceae), covered prolifically by mosses and lichens, as well as epiphytes such as bromeliads (*Mezobromelia capituligera*, *Guzmania multiflora*), and orchids (*Odontoglossum* spp., *Cyrtorchilus diceratum*). This zone has a thick layer of leaf litter that remained humid during the two field trips. Inside the forest runs “La Cadena”, a 2.5–3 m wide and 15–60 cm deep stream, which flows into the Claro River almost 300m downstream from this zone.

Zone B - “Playa Larga”: (4.911°N & -75.428°W, WGS84, 3,000m). This area corresponds to a farm dedicated exclusively to cattle raising for milk production, whose forest fragments are characterized by presenting early stages of succession, dominated by species such as *Chusquea* spp. (Poaceae), *Erato vulcanica*

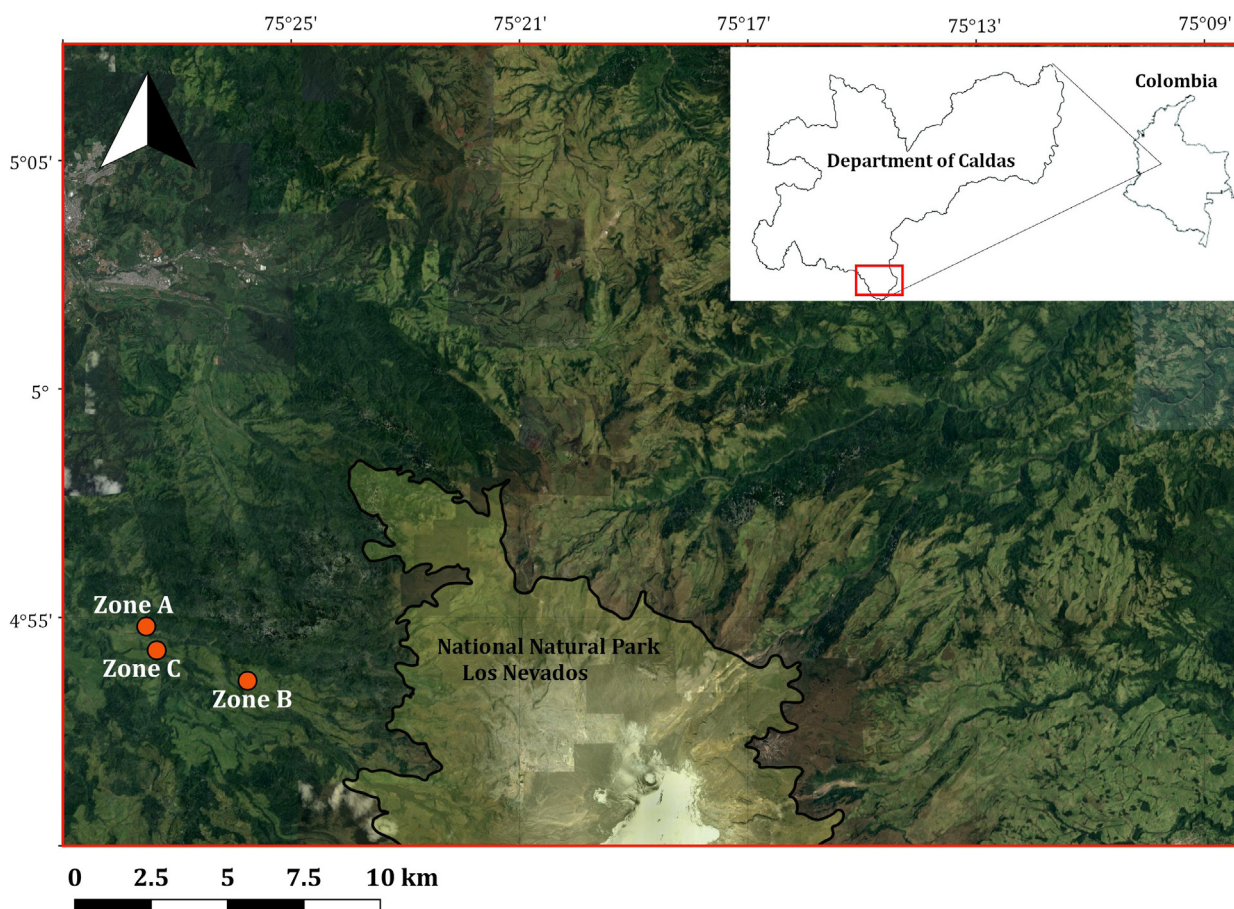


Figure 1. Study area indicating the sampling zones (red dots). Los Nevados National Natural Park is highlighted in light yellow.

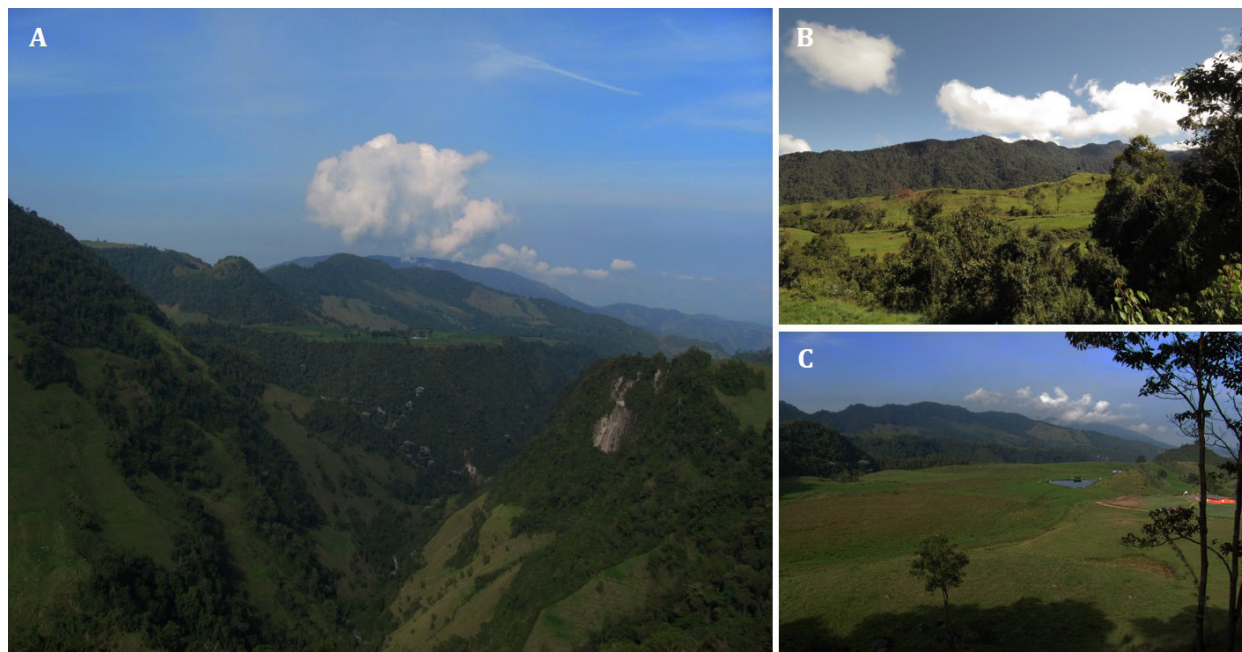


Image 1. Study area in the upper Claro River basin, municipality of Villamaría, department of Caldas, Colombia.

(A) Canyon of the Claro River at 2,500m elevation, showing the abrupt geomorphology in this area. (B) Zone B at 3,000m, evidencing the dominance of pastures for livestock; on the background there is a block of forest corresponding to the Forest Protective Reserve of the CHEC. (C) Zone C at 2,400m, showing the transformation of the natural forest to pastures and crops of potatoes (*Solanum tuberosum*) and onion (*Allium fistulosum*). © Julián Andrés Rojas.

(Asteraceae), *Solanum* spp. (Solanaceae), *Iresine* spp. (Amaranthaceae), *Gunnera* spp. (Gunneraceae), with a low presence of arboreal and shrub species; however, in the surroundings there are considerable forested areas restricted to the hilltops of the mountains, with slopes greater than 70% (Image 2b) crossed by streams that fall in cascades into the Claro River.

Zone C - "Laguna Alta": (4.925°N & -75.458°W, WGS84, 2,380m). This area also corresponds to a cattle farm for milk production, comprising a homogeneous matrix of introduced pastures. The forests are associated with the edge of the Claro River canyon.

Fieldwork

We carried out two field trips between November 11–17, 2014, and February 1–6 in 2015, covering rainy and dry periods, respectively. During each field trip the three zones were sampled, investing two consecutive days/night for each one. For the observation and capture of amphibians, diurnal (08:00–12:00 h) and nocturnal (18:30–22:00 h) samplings were carried out by two people, using the methodology of visual encounters surveys (VES) (Crump & Scott 1994), covering all possible habitats such as streams, inside forest, and open areas. The samplings were made from the ground to 2.5m above, being therefore an understory herpetofaunal

research.

Amphibians were manually captured, and kept in plastic bags with vegetation inside to maintain humidity. For all individuals we registered their snout-vent length (SVL) (mm) and weight (g). In addition to this, the specific microhabitat was recorded classified as bare soil, litter, rock, trunk, leaf and water. Height of the perch and the distance to the nearest water body (m), besides temperature (°C) and relative humidity (%) for each register point, were registered too. We follow Frost (2017) for the taxonomic arrangement and for species identification we used the works of Cochran & Goin (1970), Lynch (1975, 1989, 1991), Lynch et al. (1994), Ardila-Robayo et al. (1996), Kaplan (1997), and Rivera-Correa & Faivovich (2013). Individuals were identified in the field as far as possible, but some specimens with taxonomic uncertainty were collected with the permission granted by the environmental authority (Corporación Autónoma Regional de Caldas - Resolution 1166 of 09 October 2014). Collected individuals were euthanized and prepared following the animal care protocol (Cortés et al. 2006). Specimens were deposited in the Herpetological Collection of the Museo de Historia Natural of the Universidad de Caldas (MHN-UCa), Colombia.

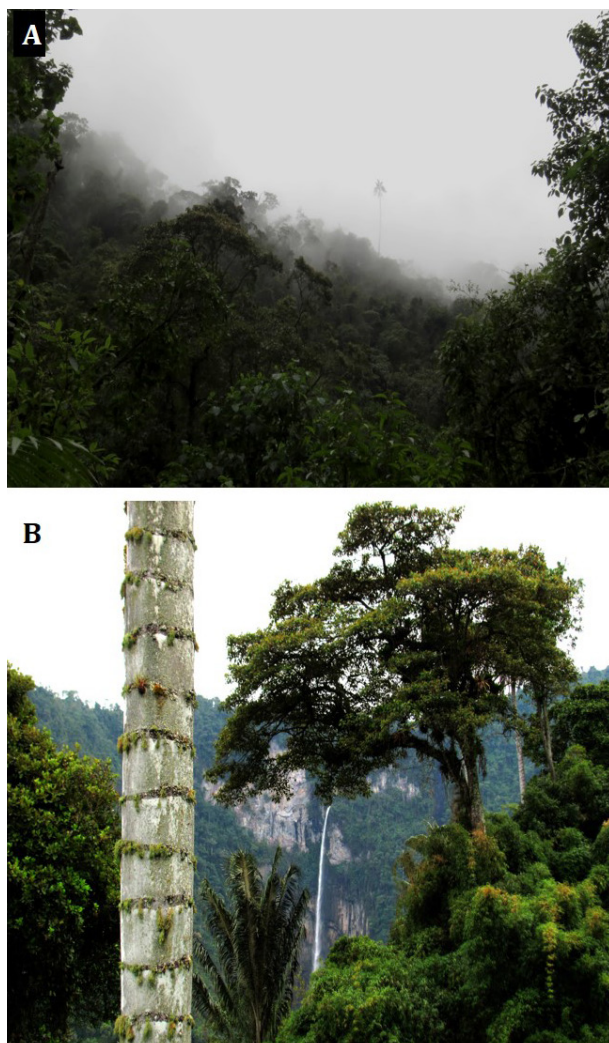


Image 2. Natural habitats sampled in the upper basin of the Claro River, municipality of Villamaría, department of Caldas, Colombia. (A) Interior of the forest in zone A. A semi-permanent fog covered the forest (cloud forest). (B) Zone B showing the beauty of the landscape characterized by the forest, steep slopes and waterfalls. © Julián Andrés Rojas (A) and César Duque Castrillón (B).

Data analysis

Diversity was evaluated in terms of the effective number of species “ qD ” (Jost 2006), an approach that is equivalent to Hill’s numbers (Hill 1973). qD is an ecological measure to quantify and compare the diversity expressed in biologically interpretable units (effective number of species) (Jost 2006). Three orders of diversity q were taken into account: order 0 (0D , species richness), 1 (1D , Shannon’s entropy exponential) and 2 (2D , Simpson’s inverse). Exponent q determines

the influence of the species abundance on the values of diversity. In this way the species richness (0D) is not sensitive to the abundance of species and gives disproportionate weights to the rare species (Jost 2006). In 1D (Diversity of Shannon), the contribution of each species is commensurate with its abundance in the community, and thus can be interpreted as the typical diversity or the number of common species in the community (Jost 2006). Finally, 2D can be interpreted as the number of “very abundant” or “dominant” species in the community (Jost 2006). The completeness was evaluated as percentage of the community represented in the total of captured individuals (Chao & Jost 2012). The sampling coverage varies between 0% (low completeness) to 100% (high completeness). The estimation of the sampling coverage and diversity were made with the iNEXT package for R (Hsieh et al. 2016).

The structure of communities was analyzed through range-abundance graphs using the relative abundances of each species. This is to observe dominant and rare species and the equity of the sampled communities. The inequality value for each one of the sampling zones was also calculated using the inequality factor $IF_{0,q}$, using in this case the values of 2D (Jost 2010):

$$IF_{0,2} = {}^0D / {}^2D$$

Where 0D corresponds to the values of species richness and 2D to the values of the second order diversity. A scale that oscillates between zero and one was standardized, where zero corresponds to low values of inequality and one to high values. For this the standardized formula ($IR_{0,q}$) was used for the value previously calculated ($IF_{0,q}$) and the number of species (S):

$$IR_{0,q} = (IF_{0,q} - 1) (S - 1)$$

To check whether the species showed any preference for a given geographical zone a chi-square based test of association was performed on the contingency matrix containing the species with their respective abundance in a given zone (Table 1). Correspondence analysis was performed to visualize the distribution of species in the three zones. Statistical analysis was performed in PAST 3.19 (Hammer et al. 2001). As the threatened species that were recorded are poorly represented in literature, we also present a description with useful characteristics for their identification in the field, and specific annotations about their distribution and natural history (Appendix II, Images 5–8).

RESULTS

Richness and species composition

After 96 hours/man in general for all sites (32h/site), 77 individuals were recorded, belonging to 15 species, seven genera and three families, all of the order Anura (Table 1). Craugastoridae was the best represented family, with 73.3% (11 species) of the records, and *Pristimantis* was the richest genus with eight species (Image 3); one of them represents a new species in the process of taxonomical description (González-Durán pers. comm.). The Hylidae family was represented by two species: *Dendropsophus bogerti* (Cochran & Goin 1970) and *Colomascirtus larinopygion* (Duellman 1973), as well as the family Bufonidae with *Osornophryne percrassa* Ruiz & Hernández, 1976 and *Rhinella* sp. The species *Rhinella* sp. and *Niceforonia* sp. also represent undescribed taxa (Image 4). The most abundant species in all sites was *Pristimantis uranobates* (n = 19), followed by *P. paisa* (n = 15) and *P. permixtus* (n = 14). The less abundant species were *Hypodactylus latens* (Lynch 1989), *P. thectopternus* (Lynch 1965), *Niceforonia* sp., *Pristimantis* sp. 1 (Image 3F) and *Pristimantis* sp. 3 (Image 3H), which had a single register during the entire study.

Sampling coverage and structure of the community

For zone A, sampling coverage reached 89%, with a total of 29 individuals, belonging to eight species, four genera and three families. Craugastoridae family was the most representative (75% of the species); *P. uranobates* (n = 11) was the most abundant species followed by *C. larinopygion* (n = 8) and *P. paisa* (n = 4). Species such as *H. latens* and *P. permixtus* had a single record (Fig. 2A, B). The value of the inequality factor for this zone was 0.08, being the site with the lowest value (Table 2). At this zone, *P. uranobates* and *C. larinopygion* accounted for 27% of the total abundances, followed by *P. paisa* representing 13%, *Rhinella* sp. and *P. boulengeri* 10%, and the rest of the species 3.4% (Fig. 2). For zone B, 93% sampling coverage was obtained, with 31 individuals represented by seven species, three genera and two families (Table 1). The most abundant species was *P. permixtus* (n = 13), followed by *P. uranobates* (n = 8) and *Pristimantis* sp. 2 (n = 4). The species that had a single record in this site were *Niceforonia* sp. and *P. paisa* (Fig. 2). The value of the inequality factor for this site was 0.15 (Table 2). In zone C, a total of 17 individuals were found distributed in five species, two genera and two families. The most abundant species was *P. paisa*

Table 1. Anuran species and number of individuals by zone in the upper Claro River basin, municipality of Villamaría, department of Caldas, Colombia. Threat categories are according to IUCN 2017 (International Union for Conservation of Nature). Categories represent Not evaluated (NE); Least Concern (LC); Near Threatened (NT); Endangered (EN); Critically Endangered (CR).

Taxa	Zone A	Zone B	Zone C	Total	Code	IUCN Red List
Bufonidae						
<i>Osornophryne percrassa</i>	-	2	-	2	A	EN
<i>Rhinella</i> sp.	3	-	-	3	B	
Craugastoridae						
<i>Hypodactylus latens</i>	1	-	-	1	C	EN
<i>Niceforonia</i> sp.	-	1	-	1	D	
<i>Niceforonia adenobranchia</i>	-	2	-	2	E	CR
<i>Pristimantis boulengeri</i>	3	-	-	3	F	LC
<i>Pristimantis paisa</i>	4	1	10	15	G	LC
<i>Pristimantis permixtus</i>	1	13	-	14	H	LC
<i>Pristimantis</i> sp. 1	1	-	-	1	I	
<i>Pristimantis</i> sp. 2	-	4	-	4	J	
<i>Pristimantis</i> sp. 3	-	-	1	1	K	
<i>Pristimantis thectopternus</i>	-	-	1	1	L	LC
<i>Pristimantis uranobates</i>	8	8	3	19	M	LC
Hylidae						
<i>Dendropsophus bogerti</i>	-	-	2	2	N	LC
<i>Colomascirtus larinopygion</i>	8	-	-	8	O	NT
Total	29	31	17	77		



Image 3. Species of the genus *Pristimantis* (Craugastoridae) recorded during this study. (a) *P. boulengeri* SVL 23,4mm; (b) *P. paisa* SVL 25,6mm; (c) *P. permixtus* SVL 25,5 mm; (d) *P. thectopternus* SVL 24,3mm; (e) *P. uranobates* (green morph) SVL 20,3mm; (f) *Pristimantis* sp. 1 SVL 22,7mm; (g) *Pristimantis* sp. 2 SVL 26mm; (h) *Pristimantis* sp. 3 SVL 58mm. © Julián Andrés Rojas.

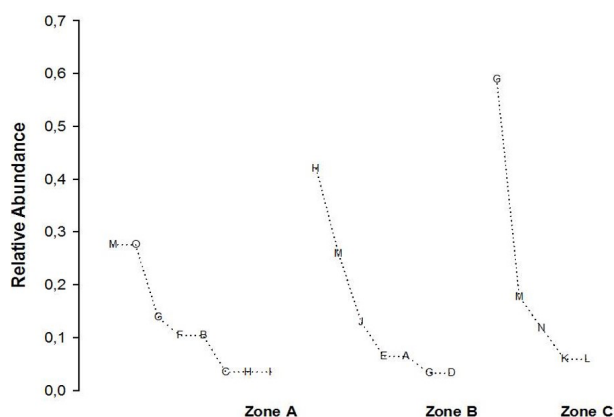


Figure 2. Range-abundance amphibian curves for each zone in the upper Claro River basin, municipality of Villamaría, department of Caldas, Colombia. Letters represent species code, see Table 1.

($n = 10$) followed by *P. uranobates* ($n = 3$) and *D. bogerti* ($n = 2$). *Pristimantis thectopternus* and *Pristimantis* sp. 3 had a single record (Fig. 2). The sampling coverage for this site was 88% and the inequality value was 0.25, this being the highest value among all sites (Table 2).

Diversity profiles

The species richness (0D) shows that zone A has a greater number of effective species (8), followed by zone B (7) and finally zone C (5) (Fig. 3). In the same way, the diversity of order 1D indicates that zone A presents a greater number of effective species with respect to the other two zones with 6.1, followed by zone B with 4.7 and zone C with 3.3 effective species (Fig. 3). When expressing this results in equivalences, zone B may contain 77% of the diversity found for zone A. On the other hand, it is observed that zone A presented almost twice the diversity of species with respect to zone C, and when comparing zones B and C, it was found that the latter may contain 70% of the diversity recorded for zone B. With the order 2D measure of diversity, all the sites result with a lower number of effective species (Fig. 3), since this measure focuses only on the most abundant species (Jost 2006). With this measure of diversity zone A reached 5.1 effective species, being the most diverse zone followed by zone B with 3.7 effective species, and finally zone C with 2.5 effective species. This is due to the fact that in zone A (which presented the lowest values of inequity, see Table 2), the most abundant species represent 27% of the total of individuals, while for zone B the most abundant species represent 41% of the total, and in zone C 58%; this leads to greater equity in the distribution of abundances among the species common to zone A compared to the other two sites.

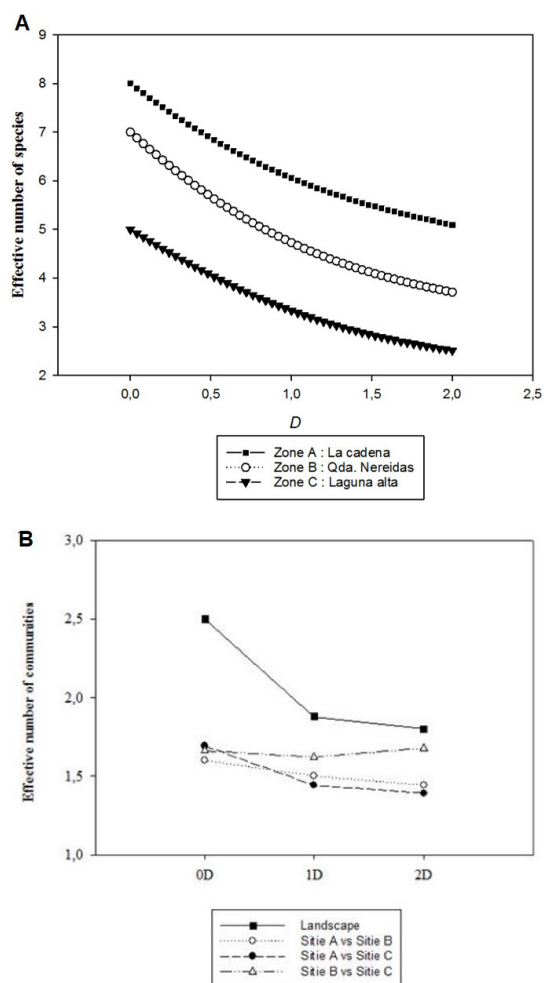


Figure 3. Amphibian diversity profiles for three zones in the upper Claro River basin, municipality of Villamaría, department of Caldas, Colombia. (A) Diversity in effective number of species; (B) Diversity in terms of communities.

Table 2. Values of the inequality factor for the three sampled zones in the upper Claro River basin, municipality of Villamaría, department of Caldas, Colombia.

Factor of inequality	$IF_{0.2}$	$RI_{0.2}$
Zone A-La Cadena	1.57	0.08
Zone B-Playa Larga	1.89	0.15
Zone C-Laguna Alta	1.99	0.25

With respect to beta diversity, it was found that there are 2.5 effective communities at the landscape level (0D), 1.9 according to the diversity 1D , and 1.8 according to 2D , respectively. These results indicate that the differences between the sampled areas are given for the rare species, while some abundant species are shared, as *P. uranobates*, which was the most

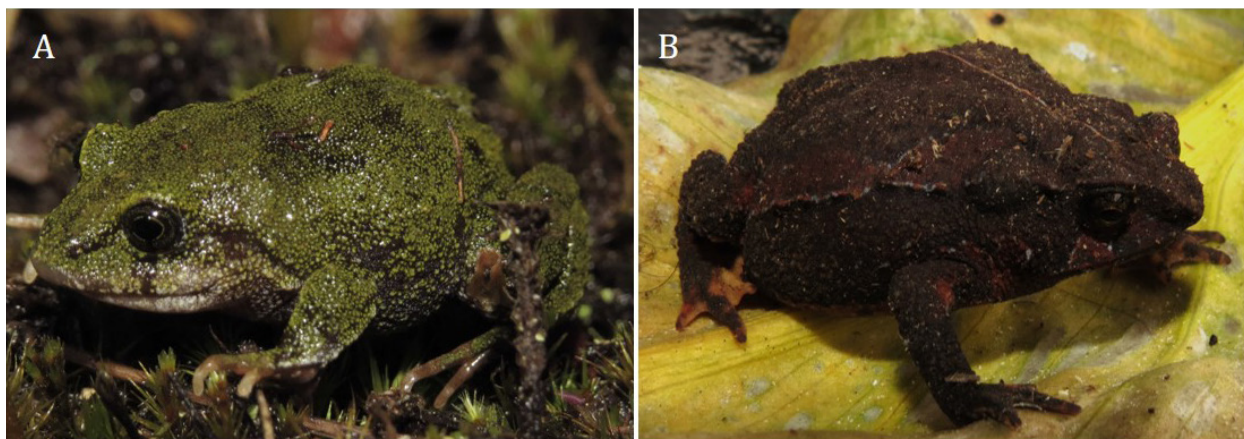


Image 4. Undescribed amphibian species registered for the upper Claro River basin, municipality of Villamaria, department of Caldas, Colombia. (A) *Niceforonia* sp. (Craugastoridae) SVL 22.9mm; (B) *Rhinella* sp. (Bufonidae) SVL 34.1mm. © Julián Andrés Rojas.

abundant species being found in all the sampling zones (Tabla 1, Fig. 2). When making comparisons between pairs of zones, it is observed that for zone A the results of 1D and 2D for beta diversity are lower than the 0D expression (Fig. 3). This shows that zone A differs from the other two mainly because of its rare species ($^1D < ^0D$), and some of the most abundant species are still shared ($^2D < ^1D$). When comparing zone B with respect to C, it is observed that the values of 1D (1.62 effective communities) are lower than for species richness 0D (1.66 effective communities), while the values of 2D are greater (1.68 effective communities). This indicates that the differences between these two sites are given to a greater extent by the abundant species ($^2D > ^0D$), although some of these are shared ($^1D < ^0D$). The most abundant species for zone B and C were *P. permixtus* and *P. paisa*, respectively (Fig. 2).

There was a significant association between the zones and the species (chi-square = 88.779, df = 28, $P < 0.0001$), indicating that the amphibians had a preference for a particular zone. Correspondence analysis (Fig. 4) showed that the amphibian fauna of the zones was distinct from each other. *Colomascirtus larinopygion*, *H. latens*, *P. boulengeri*, *Pristimantis* sp. 1 and *Rhinella* sp. were restricted to Zone A; *N. adenobrachia*, *Niceforonia* sp., *O. percrassa* and *Pristimantis* sp. 2 were restricted to Zone B, while *D. bogerti*, *P. thectopternus* and *Pristimantis* sp. 3 were restricted to Zone C. Although *P. paisa*, *P. permixtus* and *P. uranobates* were present in all three zones, *P. uranobates* was more ubiquitous in all three zones as compared to *P. permixtus* that was more abundant in Zone B and *P. paisa* that was more abundant in Zone C (Table 1).

Natural history observations and conservation status of the species

During fieldwork most of the individuals were observed at twilight and night hours, between 18:00–21:00 h, mainly stream-dwelling frogs such as *C. larinopygion*, which were observed by calling males. *Dendropsophus bogerti* was only observed in the flooded pastures during the first fieldtrip, formed by the constant rains during that period. Leaf-litter and understory frogs, like those of the genus *Pristimantis* (Image 3, Appendix I), were common both inside and on the edge of forests, and also in the pastures that surrounded them. Species such as *Rhinella* sp. and *H. latens* (Appendix I) were observed only during the day (10:30–12:00 h) on the litter or into the flooded soil. Typical nocturnal species, such as *P. uranobates* and *P. permixtus* were observed during the day, immobile on the ground, or hidden under leaf litter.

Of the 15 registered species, four are threatened with extinction according to the International Union for the Conservation of Nature (IUCN 2017). *Niceforonia adenobrachia* is Critically Endangered (CR) due to their very restricted distribution range ($< 100\text{km}^2$) (see Appendix II). *Hypodactylus latens* and *Osornophryne percrassa* (Appendix II) are Endangered (EN), both equally with restricted and fragmented distributions ($< 5000\text{km}^2$), and because the extension and quality of their habitat is decreasing in response to human intervention in cloud forests (Castro et al. 2004; IUCN SSC Amphibian Specialist Group 2015). Finally, the hyliid frog *C. larinopygion* is Near Threatened (NT) due to their fragmented distribution, although it is a relatively common species where it has been registered (Bolívar et al. 2010) (Appendix II). On the other hand, *Pristimantis*

frogs present a state of Least Concern (LC) and their populations apparently remain stable; however, how species are responding to habitat intervention at local scale is unknown. The undescribed species *Rhinella* sp., *Niceforonia* sp. and *Pristimantis* sp. 2 are not yet categorized and therefore their conservation status is unknown.

DISCUSSION

Richness and species composition

Among current ectothermic vertebrates, amphibians are the most diversified group in the high mountain ecosystems of the tropical Andes (Lynch 1999a; Lynch & Suárez-Mayorga 2002; Navas 2006; Bernal & Lynch 2008; Hutter et al. 2013), however, amphibian distribution patterns show that there is a decrease in diversity as elevation increases, similar to latitudinal reduction patterns (Lynch 1986). In this study we recorded 15 amphibian species with a representativeness between 89–93 % for the three evaluated zones, indicating that the fauna observed is a representative sample, but possibly some additional species can be found with an increasing sampling effort. Among the latter, members of the family Centrolenidae (glass frogs), which are known to be important components of the Andean amphibian communities (Guayasamin et al. 2009; Hutter et al. 2013; Rojas-Morales et al. 2014b; and references therein included), were not found in

this study. It is possible that species such as *Centrolene buckleyi* (Boulenger, 1882), *C. robledo* Ruiz-Carranza & Lynch, 1995, *C. quindianum* Ruiz-Carranza & Lynch, 1995 and *Nymphargus grandisonae* (Cochran & Goin, 1970) are also present in the study area, but because of their arboreal habits and possible seasonal reproduction, they were not observed during this study.

On the other hand, the richness and representativeness of the *Pristimantis* genus registered in this study, is not surprising because these frogs represent about a third of the known amphibians in Colombia, being highly diversified in Andean ecosystems (Acosta & Cuentas 2018). In addition, its greatest peak of diversity is between 1,750–2,400 m (Lynch & Duellman 1980, 1997; Lynch & Rueda-Almonacid 1997). The high diversity of these frogs seems to be associated with their reproductive modes, which include terrestrial eggs and direct development, which does not limit their reproduction to aquatic environments. In this way, these frogs can inhabit all terrestrial environments that have sufficient moisture for the development of eggs and survival of juveniles and adults (Lynch & Duellman 1980, 1997; Lynch & Rueda-Almonacid 1997; Lynch et al. 1997; Lynch 1999a, b; Duellman & Lehr 2009).

Diversity and structure of the community

In amphibians, species diversity is affected by ecological traits, such as forest structure, microclimate and topography (Vonesh 2001; Dixo & Martins 2008; Santori & McManus 2014; Meza-Joya & Torres 2016).

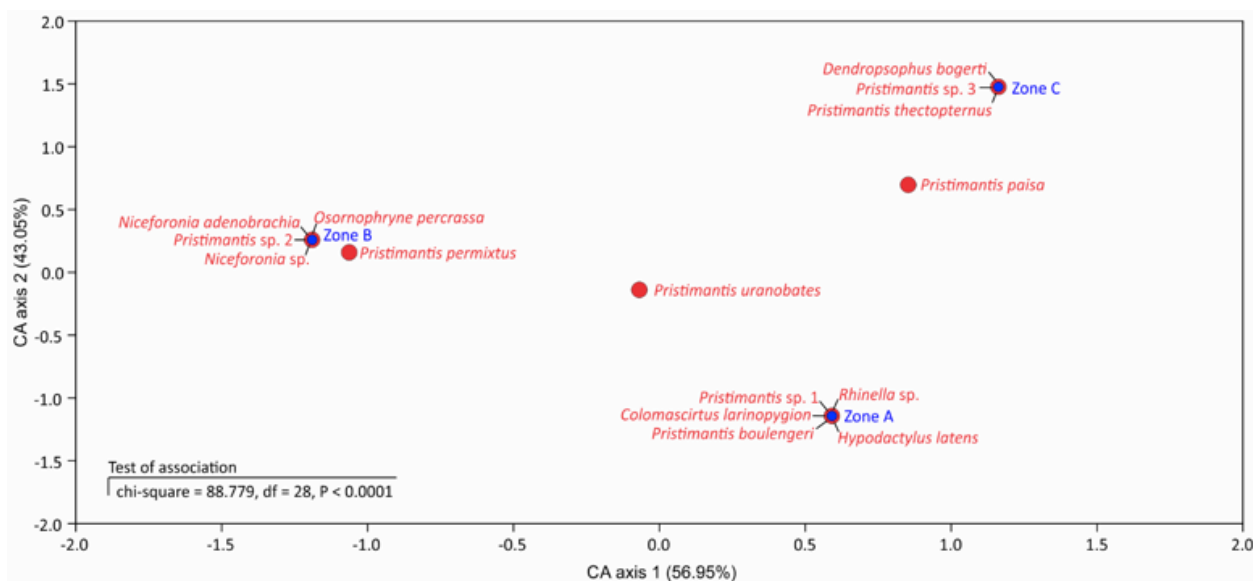


Figure 4. Correspondence analysis (CA) depicting the distribution of amphibians in the three zones evaluated. Test of association is shown in the inset. Percentage contribution by each CA axis is provided in parenthesis.

In this study, the zone A showed the highest diversity among all sites, with higher values in the three orders of diversity q , as well as the lowest values for the inequality factor (Table 2). This result may be due to the interaction of several factors, such as environmental conditions and elevation (2,400m); in addition, this is the area where the least intervened ecosystem persists. In spite of diversity values in zone A, it is important to emphasize that between this zone and zone B there was not a significant difference respect to the species richness, but they are not similar in species composition, mainly due to rare and threatened species. This is important, since the loss of these habitats due to deforestation could lead to the local extinction of some of these populations (Dodson & Gentry 1991). Unlike these two zones, zone C presented the lowest values of diversity, probably because this area has a greater degree of anthropogenic intervention, evidenced in large areas of open grasslands, with a minimum proportion of native forest.

On the other hand, although the elevation gradient between the zones is low (600m), 2.5 effective communities were found (beta diversity at the landscape level), and the differences between zones are given for its rare species in greater measure (Fig. 4). It is very likely that the 2.5 effective communities in this gradient correspond to the change in life zones across the landscape. In high mountain ecosystems it has been recognized that species composition can be relatively heterogeneous between localities at small spatial scales (Moen et al. 2009). We suggest that the 2.5 effective communities are composed, on the one hand, of species belonging to higher elevation ecosystems (Páramos, and high Andean forest), such as *O. percrassa* and *N. adenobrachia* (Ardila-Robayo et al. 1996; Bernal & Lynch 2008), and *Rhinella* sp., and *C. larinopygion* to medium elevation Andean forest. These species in turn were rare species within the sampling zones. On the other hand, it was found that *Pristimantis* frogs are important elements of the communities in all the zones evaluated. These frogs, as already mentioned, are the most specious genus in the Neotropics (Lynch & Rueda-Almonacid 1997; Santori & McManus 2014); in addition, most of their species are highly generalistic in the use of their resources, found in a wide range of habitats and microhabitats, and feeding on a great variety of prey (Arroyo et al. 2008; García et al. 2015; Santori & McManus 2014; Gutiérrez-Cárdenas et al. 2016). In the case of *P. uranobates*, which was the most abundant species and was also found in all zones, it has been reported that it may tolerate intervened environments and also have a wide altitudinal distribution (Lynch

1991; Lynch et al. 1996).

Conservation status of species

Of all species recorded in this study, three of them are threatened with extinction: two Endangered (EN) and one Critically Endangered (CR) (Table 1, Appendix II). The record of these species within the buffer zone of Los Nevados National Natural Park is highly significant, because the buffer zones have a management policy that permits the development of productive activities, such as cattle breeding and agriculture. Records of *Niceforonia* sp. (Fig. 5A) and *N. adenobrachia* (Appendix II) are of great value, because the former is an undescribed species and possibly has a restricted distribution like the other species of the genus (see Acosta-Galvis 2000); in the case of *N. adenobrachia*, this register represents one of the few known populations for the species (see Appendix II). It is very probable that the distribution of these threatened species (and also of *Hypodactylus latens*) encompasses the strict conservation area of the Los Nevados National Natural Park. For this reason, we suggest that it is important to delimit areas of protection within the farms, mainly the forest associated to creeks and streams, in order to establish a limit for the advance of human activities that threaten biodiversity outside of protected areas. In addition, it is important to restore the structural connectivity between forest fragments to mitigate the fragmentation of amphibian populations due to deforestation. Other recognized threats to amphibians such as climate change evidenced in extreme seasons (e.g., extended droughts), the introduction of exotic species, and emerging diseases such as chytridiomycosis, however, represent a latent risk for amphibian communities even within protected areas (see Lips et al. 2003, 2004; La Marca et al. 2005; Lampo et al. 2006; Acevedo et al. 2016).

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Appendix 1. Ecological traits for the anurans recorded in the upper Claro River basin, municipality of Villamaría, department of Caldas, Colombia. The values correspond to the data range. Activity: calling (C), motionless (I), movement (M), perched (P).

Family	Species	Substrate	Perch height (m)	Distance from water (m)	Activity
Bufonidae	<i>Osornophryne percrassa</i>	Moss, leaf-litter	0	4–70	C, I
	<i>Rhinella</i> sp.	Leaf-litter, fallen trunk	0	12–15	M
Craugastoridae	<i>Hypodactylus latens</i>	Muddy ground	0	3	M
	<i>Niceforonia adenobranchia</i>	Fallen trunk, moss	0.25–1	70–80	M, I
	<i>Niceforonia</i> sp.	Moss	0	30	M
	<i>Pristimantis boulengeri</i>	Leaf-litter, ferns	0.9–1.65	2–15	C, M
	<i>Pristimantis paísa</i>	Pastures, ground	0–1	0–>100	C, I, P, M
	<i>Pristimantis permixtus</i>	Pastures, moss, leafs, ferns	0–1.02	13–80	C, I, P, M
	<i>Pristimantis uranobates</i>	Leaf-litter, pasture, leafs, ferns	0–2.5	0–>100	C, I, P, M
	<i>Pristimantis thectopternus</i>	Leaf-litter	0	>100	M
	<i>Pristimantis</i> sp. 1	Ground	0	>100	M
	<i>Pristimantis</i> sp. 2	Bamboo leafs	0.3–1.3	50–100	C, P, M
	<i>Pristimantis</i> sp. 3	Pasture	0.3	>100	
Hylidae	<i>Dendropsophus bogerti</i>	Flooded pasture	0	0	C
	<i>Colomascirtus larinopygion</i>	Leafs, branches, ferns	0–>5	0–3	C

Appendix 2. Particular comments on threatened amphibians recorded in this study.

Amphibia: Anura

Family Bufonidae

Osornophryne percrassa

Ruiz-Carranza & Hernández-Camacho, 1976

Herveo Plump Toad (Image 5)

Conservation status: Endangered (EN) B1ab(iii)**Abundance in the study area:** Rare. During fieldwork only two individuals were recorded, both on moss and litter at the edge of the secondary forest in zone B (3,000m).**Morphological characters:** A small toad with short limbs that limit it to an exclusively slow locomotion (Ruiz-Carranza & Hernández-Camacho 1976). It does not present parotoid glands as well as tympanic rings; manual and pedal fingers very reduced and little differentiated, wrapped by a thick integument like a palm that reaches almost to the distal end of the fingers. The skin of the whole body is covered with tubercles of variable size and shape, and ventrally more uniform in number and arrangement. It presents a uniform dark dorsal coloration, variable between black, dark brown and olive, and ventrally present yellow spots of variable size, especially concentrated in the posterior region of the belly, forearms and thighs (Image 5) (Ruiz-Carranza & Hernández-Camacho 1976; Vanegas-Guerrero & Fernández-Roldán 2014).**Distribution:** This species is endemic to Colombia, being known for the Páramo and cloud forest biomes on both sides of the Central Cordillera, from 2,700–3,840 m (Bernal & Lynch 2008, Vanegas-Guerrero et al. 2016). Politically it is registered in the departments of Antioquia, Caldas, Risaralda, Quindío, Tolima, and Valle del Cauca (Vanegas-Guerrero et al. 2016; Mantilla-Castaño et al. in press).

Family Craugastoridae

Hypodactylus latens (Lynch, 1989)

Boqueron Robber Frog (Image 6)

Conservation status: Endangered (EN) B1ab(iii)**Abundance in the study area:** Very rare, although this may be a result of its cryptic behavior. A single individual (Image 6) was registered during the field work, specifically in zone A (2,400m) buried in marshy ground inside secondary forest (10:55h; 16.6°C, 91% HR). It is presumed that its presence is restricted to forests with adequate

moisture conditions and vegetation cover.

Morphological characters: Small frog with a robust body and short extremities with thin, short, well-differentiated fingers without discs; the first manual finger longer than the second; absent manual and pedal membranes; tympanic ring absent. *Hypodactylus latens* has a finely granular skin, with relatively smooth flanks. Its coloration is variable, from light brown with a thin white line crossing the back, to dorsal uniform purple, with the exception of the groins, armpits and flanks that have yellow spots, which is characteristic of the species (Image 6). It also has a dark rostral band that is not very conspicuous and the edge of the eyelid is outlined in pale yellow. Iris reddish-brown to dark brown.**Distribution:** Endemic to high mountain ecosystems (cloud forests, subpáramo and páramo, between 2,600–3,200 m) in the Central Cordillera of Colombia. Politically it is registered in the departments of Antioquia, Caldas, Quindío and Tolima. The record in the study area was to 2,400m, 200m below the lowest known limit for the species.*Niceforonia adenobrachia*

(Ardila-Robayo, Ruiz-Carranza & Barrera-Rodríguez, 1996)

(Image 7)

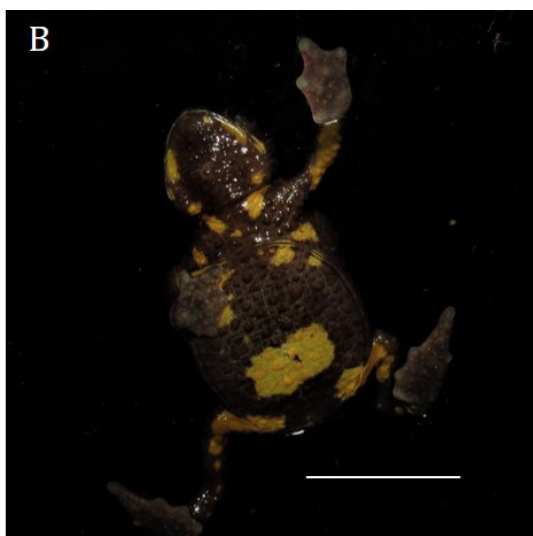
Conservation status: Critically Endangered (CR) B1ab(iii)+2ab(iii)**Abundance in the study area:** Very rare, although possibly due to its cryptic behavior. Two individuals (males) were registered during the field work, both in zone B (3,000m). Both individuals were active at night between 21:30–22:07 h (12–13.8 °C, 90–92 % RH). One of them was moving on a fallen trunk 80cm above the ground and more than 100m from the Nereidas Stream. The second individual was registered on moss that covered a rock on the edge of a secondary forest. This finding represents the fourth known population for the species, expanding its distribution in 23.7km to the southwest of its type locality on the western flank of the Central Cordillera.**Morphological characters:** A small frog (range SVL 14.5–23.1 mm, Ardila-Robayo et al. 1996), with a robust body, oval in shape, with the head narrower than the body. Dorsal skin smooth with few small and scattered tubercles; presents one or two tubercles in the eyelids, and a conical tubercle prominent in the heels; the skin of the belly is slightly granular; forearms widened in the males; the fingers of the hands are short, the first and the second of equal size and do

Image 5. Dorsolateral (a) and ventral view (b) of *Osornophryne percrassa* (SVL 21.3mm; weight 2.4g), from Nereidas Stream, Playa Larga Village, municipality of Villamaría, department of Caldas, Colombia. Scale bar 10mm. © Julián Andrés Rojas.

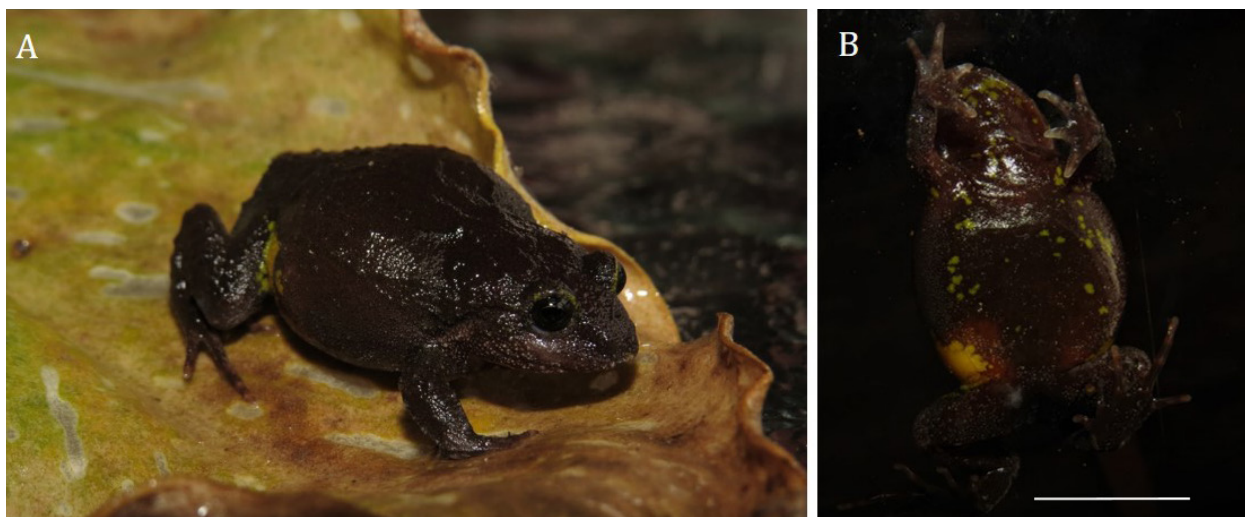


Image 6. Dorsolateral (a) and ventral views (b) of a *Hypodactylus latens* (female SVL 30.4mm; weight 2.7g) from La Cadena Stream, Playa Larga Village, municipality of Villamaría, department of Caldas, Colombia (MHN-UCa 0765). Scale bar 10mm. © Julián Andrés Rojas.



Image 7. Dorsolateral (a) and ventral view (b) of *Niceforonia adenobranchia* (male SVL 18.5mm; weight 0.8g) (MHN-UCa 0775), from the Nereidas Stream, Playa Larga Village, municipality of Villamaría, department of Caldas, Colombia. Scale bar 10mm. © Julián Andrés Rojas.

not have terminal discs; the toes are longer and thinner; absence of interdigital membranes in hands and feet. The face is short, sub-acuminate in dorsal view and inclined in lateral view. Tympanic ring absent. The coloration of the back varies from light to dark brown, with an axillary and inguinal cream spot; some individuals have a thin longitudinal white dorsal line. It presents a dark brown post-ocular band resembling a mask. Fingers and hands light orange. Copper iris with red crosslinks. The belly has a black and white crosslinking, accompanied by some dark brown spots on the reticulations, and some yellow spots of variable shape and arrangement in the interspaces (Image 7).

Distribution. Endemic to Colombia, *N. adenobranchia* is known from a small number of localities around Los Nevados National Natural Park, in the department of Caldas (this register), Tolima (Ardila-Robayo et al. 1996; Romero-García et al. 2015) and Quindío (Buitrago-González et al. 2016). Its type locality is the Cerro Bravo Volcano, Páramo of

Letras, Albania village, municipality of Herveo, department of Tolima, between 3,000–3,400 m), in the Central Cordillera of Colombia.

Family Hylidae

Colomascirtus larinopygion (Duellman, 1973) (Image 8)

Conservation status: Near Threatened (NT)

Abundance in the study area: A common species, inhabitant of primary and secondary forests associated with rapid flow streams. In the study area and Los Nevados National Natural Park, this is a characteristic element of the tree frogs associated with creeks and streams.

Morphological field characters: A medium-sized tree frog with a robust body (see Duellman 1973, Duellman & Berger 1982, Duellman & Hillis 1990, Rivera-Correa & Faivovich 2013 for a detailed description).

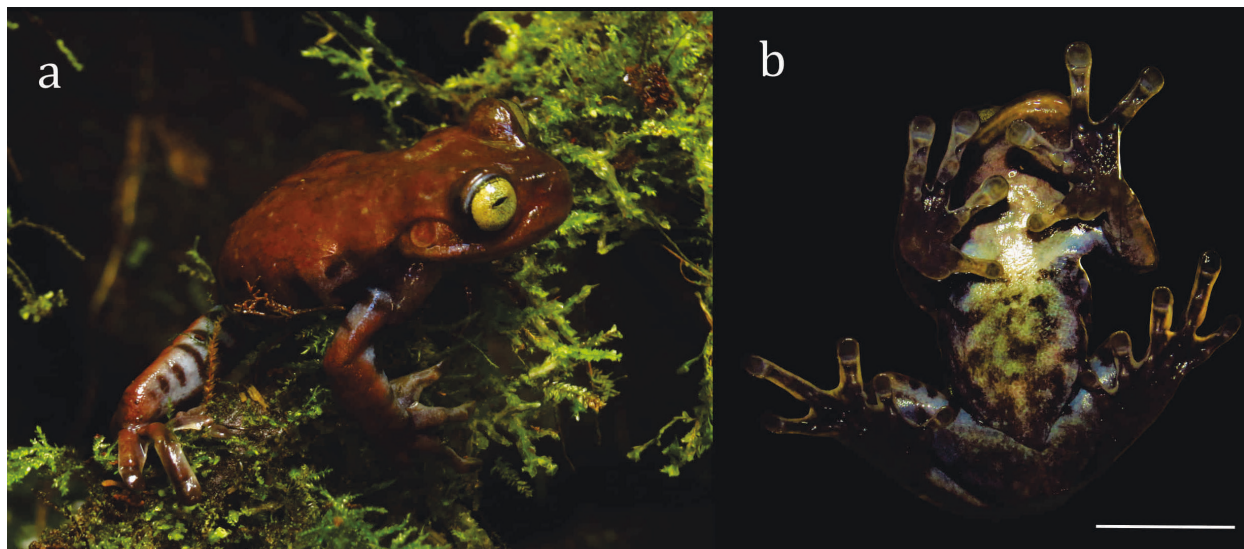


Image 8. Dorsolateral (a) and ventral view (b) of an *Colomascirtus larinopygion* (SVL 47.6mm, weight 3.7g), from La Cadena stream, Playa Larga village, municipality of Villamaría, department of Caldas, Colombia. © Julián Andrés Rojas.

Individuals in the study area have an average of SVL 56.6 ± 3 mm ($n = 8$). This is an easily distinguishable species in the study area by the following set of characters: head as wide as the body, with the face truncated in dorsal and lateral view; protruding eyes, bordered by a black line on top and back; elliptical pupil and yellow iris; tympanic ring present; long and slender manual fingers, terminated in expanded discs; the discs of the pedial fingers are smaller than those of the hands. Thick forearms and thin, slender hind legs. Basal manual membranes and extended on the feet. Anal region markedly swollen and bordered by a white line. Skin smooth and slightly corrugated on the flanks. Dorsal coloration dark brown uniform, in some individuals purple; flanks, belly, and inner surface of the fingers, thighs and forearms pale blue color with black stripes that radiate from the belly. Ventrally the coloration is dark gray (Image 8).

Distribution. *Colomascirtus larinopygion* is the widest distributed species of the *larinopygion* group (Faivovich et al. 2005), inhabiting cloud forests of the northern Andes in Colombia and Ecuador. In Colombia it is distributed on both flanks of the Central and Western Cordilleras, between 1,950–3,100 m (Acosta 2000; Bernal & Lynch 2008; Rivera-Correa & Faivovich 2013). Politically it is registered in the departments of Antioquia, Caldas, Cauca, Huila, Quindío, Tolima and Valle del Cauca (Rivera-Correa & Faivovich 2013).



Spanish abstract: Resumen: Presentamos una evaluación sobre la composición, diversidad y estructura de los anfibios en tres zonas a lo largo de un gradiente de elevación (2,400–3,000 m) en la Cordillera Central de Colombia. Para esto, se llevaron a cabo dos jornadas de muestreo, una en noviembre 2014 y otra en febrero 2015, en períodos de lluvia y seco, respectivamente. Los muestreos fueron tanto diurnos (08:00–12:00 h) como nocturnos (18:00–22:00 h) usando la metodología de relevamiento por encuentros visuales, sin restricciones espaciales. La diversidad fue evaluada en términos del número efectivo de especies para cada zona (alfa) y para todo el paisaje en general (gamma), y la estructura de las comunidades fue analizada mediante curvas de rango-abundancia. Se calculó un factor de inequidad para cada zona de muestreo. Se registró un total de 15 especies pertenecientes a siete géneros y tres familias, todas del orden Anura. La familia Craugastoridae fue la más rica con 11 especies (73,3%), y el género más rico fue *Pristimantis* con ocho especies. La diversidad alfa promedio por zona fue de 6,6 especies efectivas, siendo la zona A la más diversa con ocho especies. En términos de la diversidad beta, existen 2,5 comunidades efectivas a nivel de todo el gradiente evaluado. Las diferencias de composición entre zonas están dadas por las especies raras, mientras que las especies más abundantes (p. ej. *Pristimantis uranobates*) son compartidas entre las mismas. Se presume que la mayor diversidad en la zona A es debido a su ubicación a menor elevación y por presentar un hábitat en mejor estado de conservación respecto a las otras dos zonas. Del total de especies registradas, dos están amenazadas de extinción: *Hypodactylus latens* (En Peligro), y *Niceforonia adenobranchia* (Peligro Crítico). Se resalta el hallazgo de tres especies indescritas.

Author Details: Julián A. Rojas-Morales has research interests on the natural history and biogeography of the tropical herpetofauna, particularly frogs and Neotropical snakes, aspects that he intends to integrate to develop programs and initiatives for biodiversity conservation. Currently, he teaches the courses for Managing of Biodiversity and Ecosystems conservation in the University of Santa Rosa de Cabal (UNISARC). Mateo Marín-Martínez he is a Colombian herpetologist focused on amphibian and reptile ecology. He is very interested in the study of trophic interactions and species diversity patterns. Currently, he is working in the project “Vertebrate fauna monitoring in the influence areas of ISAGEN at the east department of Caldas-Colombia”, supported by University of Caldas.



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Articles

‘Non-protected’ primates as bushmeat, pets and pests in southeastern Democratic Republic of Congo

– Paul Kaseya Kazaba, Pp. 13251–13260

Diversity, structure and natural history of amphibians in the upper Claro River basin, a buffer zone of the National Natural Park Los Nevados, Central Cordillera of Colombia

– Julián Andrés Rojas-Morales & Mateo Marín-Martínez, Pp. 13261–13277

Communications

Medium and large-sized mammals in an Atlantic Forest fragment of Brazil: recording of threatened species

– Vinícius Nunes Alves, Caroline Marques Maia, Telma Regina Alves & Renata Cristina Batista Fonseca, Pp. 13278–13286

Nuisance behaviors of macaques in Puerto Princesa Subterranean River National Park, Palawan, Philippines

– Lief Erikson Gamalo, Joselito Baril, Judeline Dimalibot, Augusto Asis, Brian Anas, Nevong Puna & Vachel Gay Paller, Pp. 13287–13294

Current data on the reproduction of Four-horned Antelope *Tetracerus quadricornis* in zoological parks

– Gérard Dubost, Stéphane Labes & Armelle Lutz, Pp. 13295–13303

Characterization of dorsal guard hair of the wild goats and sheep (Bovidae: Caprinae) occurring in the Himalaya and Western Ghats of India

– Manokaran Kamalakannan, Pp. 13304–13309

Rediscovery of the ‘extinct’ bee *Hesperocolletes douglasi* Michener, 1965 (Colletidae: Colletinae: Paracolletini) in Western Australia and first description of the female

– Juliana Pille Arnold, Mark V. Murphy, Raphael K. Didham & Terry F. Houston, Pp. 13310–13319

Butterflies of the myristica swamp forests of Shendurney Wildlife Sanctuary in the southern Western Ghats, Kerala, India

– Prabhakaran Chandrika Sujitha, Gopal Prasad & Kalesh Sadasivan, Pp. 13320–13333

Pollination ecology of three ecologically valuable carpetweed herbs, *Mollugo cerviana*, *M. nudicaulis* and *M. pentaphylla* (Molluginaceae)

– Maddala Sulakshana & Aluri Jacob Solomon Raju, Pp. 13334–13349

Sacred groves: a traditional way of conserving plant diversity in West Midnapore District, West Bengal, India

– Uday Kumar Sen, Pp. 13350–13359

Review

Media reporting on the protected areas in Maharashtra, India: a thematic analysis

– Trupthi Narayan & Pankaj Sekhsaria, Pp. 13360–13376

Short Communications

Avian survey in tourist sites near Putao in northern Myanmar

– Alexey E. Scopin, Vladimir N. Sotnikov, Dmitry V. Skumatov & Alexey A. Sergeev, Pp. 13377–13384

New record of Blue-eyed Eastern Spadefoot Toad *Leptobrachium bompui* (Amphibia: Megophryidae) from Sarpang District in Bhutan

– Jigme Tenzin & Jigme Tshelthrim Wangyal, Pp. 13385–13389

New record of Low’s Flatfaced Longhorn Beetle *Sarothrocerus lowii* White, 1846 (Coleoptera: Cerambycidae: Lamiinae: Lamiini) in Nagaland, India, along with first-time descriptions of male and female genitalia

– Kolla Sreedevi, Manisha Sharma & Hemant Vasant Ghatge, Pp. 13390–13394

On the rediscovery of *Onychomesa susainathani*, an emesine bug endemic to India (Heteroptera: Reduviidae: Emesinae)

– Hemant Vasant Ghatge & Balasaheb Sarode, Pp. 13395–13401

First record of the callinassid ghost shrimp *Neocallichirus jousseumei* (Nobili, 1904) (Decapoda: Axiidea) from India

– Imtiyaz Beleem, Paresh Poriya & Bharatsinh Gohil, Pp. 13402–13405

New distribution records of four species of crop wild relatives to India

– K. Pradheep, K. Joseph John, G.D. Harish, S.M. Sultan, I. Jaisankar, K. Naveen, S.P. Ahlawat & Manish Kanwat, Pp. 13406–13414

Note

Animal-fungal interactions 3: first report of mycophagy by the African Brush-tailed Porcupine *Atherurus africanus* Gray, 1842 (Mammalia: Rodentia: Hystricidae)

– Todd F. Elliott, Camille Truong, Olivier Séné & Terry W. Henkel, Pp. 13415–13418

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