Diversity of Andean amphibians of the Tamá National Natural Park in Colombia: a survey for the presence of *Batrachochytrium dendrobatidis*

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Abstract
Diversity of Andean amphibians of the Tamá National Natural Park in Colombia: a survey for the presence of Batrachochytrium dendrobatidis.— Changes in diversity and possible decreases in populations of amphibians have not yet been determined in many areas in the Andes. This study aimed to develop an inventory of the biodiversity of amphibians in the Andean areas of the Tamá National Natural Park (Tamá NNP) and to evaluate the patterns of infection by *Batrachochytrium dendrobatidis* (*Bd*) in preserved and degraded areas. We performed samplings focused on three habitats (forest, open areas and streams) in four localities from 2,000 to 3,200 m in altitude. Fourteen species were recorded, 12 of which were positive for *Bd*. A total of 541 individuals were diagnosed and 100 were positive. Our analyses showed that preserved areas play an important role in keeping many individuals *Bd*–free as compared to those in degraded areas. This was the first study to evaluate diversity and infection by *Bd* in the northeast region of Colombia. Our findings may help improve our knowledge of the diversity of amphibian species in the area and facilitate the implementation of action plans to mitigate the causes associated with the decrease in amphibian populations.

Key words: Amphibians, Conservation, Chytridiomycosis, Diversity, Protected natural areas

Resumen
Diversidad de anfibios andinos del Parque Nacional Natural Tamá en Colombia: un estudio para determinar la presencia de Batrachochytrium dendrobatidis.— Son muchas las zonas de los Andes en las que aún no se han determinado los cambios en la diversidad ni la posible disminución de las poblaciones de anfibios. Este estudio pretende elaborar un inventario de la biodiversidad de anfibios en las áreas andinas del Parque Nacional Natural Tamá (PNN Tamá) y evaluar los patrones de infección por *Batrachochytrium dendrobatidis* (*Bd*) en áreas conservadas y degradadas. Se realizaron muestreos en tres hábitats (bosque, áreas abiertas y arroyos) en cuatro localidades entre los 2.000 y 3.200 m de altitud. Se registraron 14 especies de las cuales 12 fueron positivas para *Bd*; se diagnosticaron un total de 541 individuos de los que 100 resultaron positivos. Los análisis ponen de manifiesto que las áreas conservadas desempeñan un papel importante en el mantenimiento de un mayor número de individuos sin *Bd* en relación con las áreas degradadas. Este es el primer estudio en el que se evalúa la diversidad y la infección por *Bd* en la región nororiental de Colombia. Los resultados obtenidos pueden ayudar a mejorar nuestro conocimiento sobre la diversidad de especies de anfibios en la zona y facilitar la implementación de planes de acción encaminados a mitigar las causas asociadas con la disminución de las poblaciones de anfibios.

Palabras clave: Anfibios, Conservación, Quitridiomicosis, Diversidad, Áreas naturales protegidas

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Introduction

At least one–third of the 6,260 amphibian species assessed by the International Union for the Conservation of Nature are globally threatened (Zippel & Mendelson, 2008). Additionally, 159 species may be extinct, and 38 are confirmed as extinct (IUCN, 2012). The loss of amphibian diversity is associated with multiple factors such as destruction of habitat, introduction of competitors or predators, pollution, and other environmental risks (Alford & Richards, 1999; Blaustein & Kiesecker, 2002; Collins & Storfer, 2003; Corn, 2005; Allentoft & O'Brien, 2010; Hof et al., 2011). The major cause of amphibian species' decline towards extinction —occurring at an unprecedented rate in any taxonomic group in history— has been attributed to emerging diseases such as chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) (Longcore et al., 1999; Daszak et al., 1999; Lips, 1999; Gardner, 2001; Lips et al., 2005; Skerratt et al., 2007). *Batrachochytrium dendrobatidis* colonizes the keratinized surfaces of larval amphibians causing mutations in the oral disk structures of larval mouthparts that limit feeding. In later stages, chytridiomycosis causes thickening in the epidermis of juvenile and adult amphibians that often results in suffocation and heart failure leading to death (Berger et al., 1998; Bosch, 2003). Few studies, however, have linked other threats with possible increases in *Bd* infection, which could synergistically facilitate the spread of this pathogenic agent, and together negatively impact the ecology and long–term survival of various species (Kiesecker et al., 2001; Blaustein et al., 2003; Burrowes et al., 2004; Pounds et al., 2006; Lampo et al., 2006b; Alford et al., 2007). Moreover, it has been documented that amphibian declines are also occurring in protected areas where human impact is very low or absent (Gardner, 2001; Stuart et al., 2004; La Marca, 2007).

Colombia has 793 spp. of amphibians (Acosta–Galvis & Cuentas, 2016), ranking second place in amphibian diversity worldwide. As many as 275 of these species are under some level of threat, and 144 are data deficient (DD) (IUCN, 2012). As few studies have been conducted to date on the natural history, distribution, ecology and diversity of amphibians the process of assessing threats and documenting overall declines is limited, accounting for a lack of adequate management and conservation plans (Urbina–Cardona, 2008). At the regional level, the Department of Norte de Santander is a well–studied region, but there are great gaps in biological and ecological data that limit our knowledge of the conservation status of most species of amphibians in the region (Grant et al., 1994; Urbina–Cardona, 2008). Moreover, the number of species known to occur in Norte de Santander represents only 5.32% (38 spp.) of the amphibians described from Colombia (Acosta–Galvis, 2000; Armesto et al., 2009; Acevedo et al., 2011, 2013, 2014). This lack of information is due to multiple factors, such as political instability, societal problems, and lack of active herpetological research groups.

This study was designed to develop an inventory of the amphibian biodiversity in Andean areas of the Tamá National Natural Park (Tamá NNP) and to assess the conservation status of amphibians in this area, with particular emphasis on *Bd* infections. Our goal was to improve our knowledge about the richness of amphibian species in order to implement immediate action plans to mitigate the causes associated with their decline.

Material and methods

Study sites

Tamá National Natural Park consists of 48,000 hectares and is located in the northeastern corner of Colombia, in the municipalities of Toledo and Herrán, Department of Norte de Santander. The temperature varies between 5–25°C, with elevations ranging from 350 to 3,450 m (Londoño, 2005). The study area was divided into four sampling localities (fig. 1): (1) Paramo of Tamá (7° 23' 31.04'' N–72° 23' 32.99'' W) at 3,200 m of elevation with a mean temperature of 10.3°C and relative humidity of 94.62%, the vegetation type is dominated by *Espeletia brassicoidea,* the following areas correspond to Andean and high Andean forests; (2) Orocué (7° 25' 7.44'' N–72° 26' 2.05'' W), located in the municipality of Herrán from 2,400 to 2,600 m of elevation, the mean temperature is 12.34°C, and the relative humidity is 97.19%; (3) Asiria de Belén (7° 18' 20.07'' N–72° 22' 21.73'' W), located in the municipality of Toledo at 2,700 m of elevation, with a mean temperature of 16.19°C and a relative humidity of 79.79%; and (4) Los Remansos (7° 21' 23.69'' N–72° 28' 18.84'' W), located in the municipality of Toledo from 2,000 to 2,300 m of elevation, with a mean temperature of 15.6°C and relative humidity of 94.27%.

Fieldwork

Between August 2010 and April 2011, we made monthly visits to Orocué, La Asiria, Los Remansos and the Paramo of Tamá, additional visits were conducted between February 2014 and August 2015. In each area, we took samples from three habitat types (Andean forests, streams and open areas). Sampling was conducted along three replicated linear transects within each habitat type, with a distance of 100 m long and 2 m wide, between 9:00 h–12:00 h, 14:00 h–16:00 h and 18:00 h–24:00 h. We looked for amphibians among leaf litter and under rocks and fallen trees using Visual Encounter Surveys–VES (Crump & Scott, 1994; Heyer et al., 1994; Urbina–Cardona et al., 2006; Caceres–Andrade & Urbina–Cardona, 2009). For each amphibian recorded we drew a circular area of 100 m in diameter around it, and we quantified the degree of habitat loss, measured as the percentage of non–natural vegetation cover (Becker & Zamudio, 2011).

Amphibian swabbing

We collected skin swabs to detect *Bd* on individual amphibians. We used a sterile swab (Fine Tip Sterile MW100–Medical Wire) to rub the groin, palms, fingers

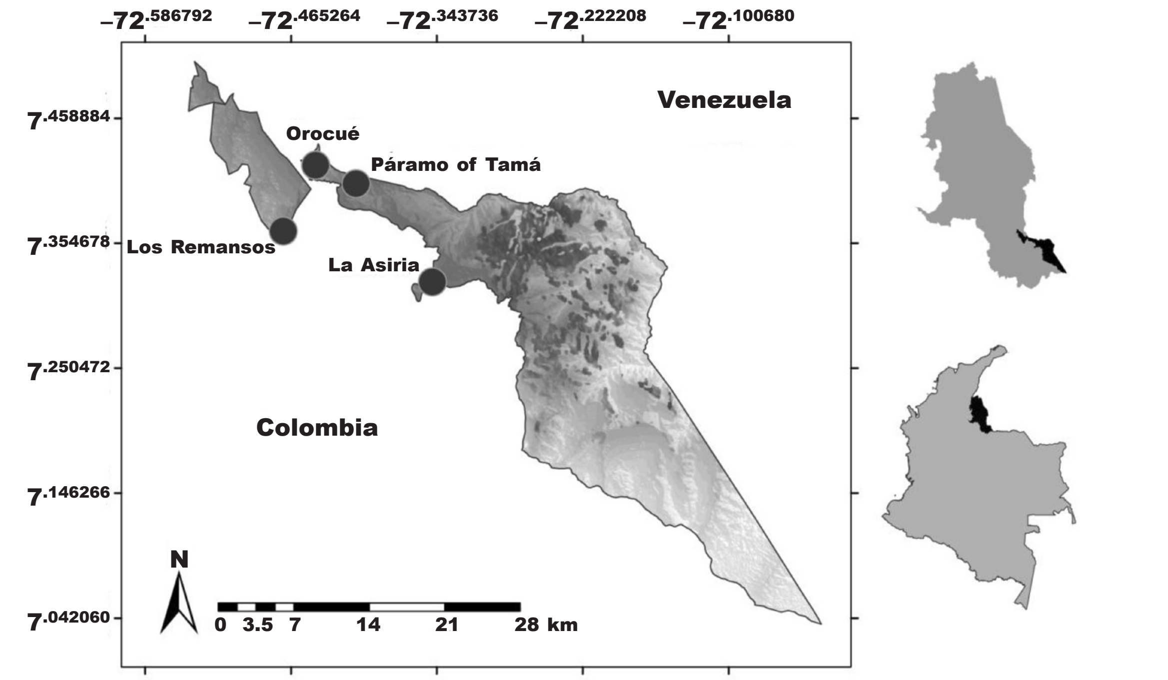

Fig. 1. Study area and sampling localities. Tamá National Natural Park, Colombia.

Fig. 1. Área de estudio y localidades de muestreo. Parque Nacional Natural Tamá, Colombia.

and legs 30 times. Samples were stored at 4°C (Hyatt et al., 2007).

Samples were processed in the Laboratory of Ecophysiology, Behavior and Herpetology at the Universidad de los Andes and the Laboratory of Ecology and Biogeography at the Universidad de Pamplona in Colombia, and conventional polymerase chain reaction (PCR) was used to identify *Bd* (Annis et al., 2004; Hyatt et al., 2007). We used the primers developed by Annis et al. (2004) to amplify the ITS1–ITS2 region specifically in *B. dendrobatidis*: Bd1a (5'– CAGTGTGCCATATGTCACG–3') and Bd2a (5'–CATGGTTCATATCTGTCCAG–3').

Data analysis

Diversity analyses were performed assigning the samples to the different habitats (Andean forests, streams and open areas). Species richness was analyzed using coverage of extrapolation and interpolation of the collected species (Chao & Jost, 2012). For each habitat, we estimated ^{0}D true diversity index (species richness), ^{1}D represented by relative abundances Shannon (exp H') and ^{2}D represented by abundant species (inverse of Simpson) (Jost, 2007). Prevalence of *Bd* was calculated by dividing the number of positive amphibians by the total number of amphibians sampled. To examine the structure of each habitat we constructed rank–abundance curves to compare patterns of abundance–dominance for the whole data set and for the *Bd* infected amphibians alone. To identify differences between amphibian abundances we performed a Kruskal–Wallis analysis. Finally, we performed a Spearman rank correlation between the abundance of amphibian species diagnosed (positive and negative) in conserved areas (with a continuous Andean forest area) and degraded areas (fragmented forests, pastures, crops and forests is done burnt).

Biosecurity protocols

All biosecurity measures were applied throughout this investigation to prevent accidental spread of amphibian pathogens both among amphibians and places (Phillott et al., 2010). Each collected specimen was handled using a new pair of disposable gloves, and transported in separate bags. A bleach solution was used to wash all materials and clothes exposed to field amphibians or environmental substrates before leaving each study site.

Results

We recorded 541 individuals belonging to 14 species of five families. The family with the most species records and number of individuals was Craugastoridae (9 spp.), representing 81% of the individuals sampled in the Tamá NNP, followed by the families Plethodontidae (1 sp., 10%) and Hylidae (2 spp., 6%) (table 1).

Table 1. Amphibian species recorded in the Tamá NNP, Colombia: HBd. Habitats with *Bd* prevalence; 1. Orocué; 2. Paramo of Tamá; 3. La Asiria; 4. Los Remansos; F. Andean forest; S. Streams; O. Open areas. (The letter in parentheses for each of the species represent its location in the rank–abundance, fig. 4.)

Tabla 1. Especies de anfibios registradas en el PNN Tamá, Colombia: HBd. Hábitats con prevalencia de Bd*; 1. Orocué; 2. Páramo del Tamá; 3. La Asiria; 4. Los Remansos; F. Bosque andino; S. Arroyos; O. Áreas abiertas. (La letra entre paréntesis en cada una de las especies representa su ubicación en el rango abundancia, fig. 4.)*

		Sites							
Family	Species	1	2	3	4	F	S	O	HBd
Craugastoridae	*Tachiramantis douglasi* (g)	X				X	X	X	25
	Pristimantis melanoproctus (j)	X		X	X	X	X	X	11
	Pristimantis mondolfii (k)			X	X	X	X	X	15
	Pristimantis anolirex (f)		X					X	3
	Pristimantis frater (h)			X	X	X	X		2
	Pristimantis nicefori (l)		X	X				X	0
	Pristimantis gryllus (i)			X	X	X	X		9
	Pristimantis sp. 1 (m)			X		X			0
	Pristimantis sp. 3 (n)	X	X			X	X	X	4
Hylidae	*Dendropsophus labialis* (c)				X			X	5
	Dendropsophus pelidna (d)	X						X	3
Centrolenidae	*Centrolene daidaleum* (d)				X		X		5
Hemiphractidae	*Gastrotheca helenae* (e)		X	X			X	X	2
Plethodontidae	*Bolitoglossa tamaense* (a)			X	X	X	X	X	16

Species richness

The entire sampling for each habitat showed values of 92% for the Andean forest habitat, 86% for the stream habitat and 75% for the open area habitat, meaning that a representative proportion of amphibians in the Andean areas from the Tamá were captured (fig. 2). By comparing the richness (Sobs: number of species observed) between habitats we observed that the Andean forest had the highest abundance with nine species, followed by the open area with eight species. The stream area had seven species, and the richness observed as the estimate was similar, indicating no differences in habitat (df = 3, p = 0.058).

Diversity profiles

The true diversity index ^{0}D, which represents richness of species, shows that the Andean forest had the most effective species (9 sp.), followed by the open area (8 sp.), and finally streams (7 sp.) (fig. 3). The diversity of order ^{1}D, represented by the most frequent species according to Shannon, indicated that the open area had the highest diversity with 4.9 effective species, leaving a maximum of 3.5 and 3.4 effective species for Andean forest and streams areas, respectively (fig. 3). Finally, the diversity index of order ^{2}D, based on the species dominance according to the inverse of Simpson, revealed very similar trends to ^{1}D, with the open area having the highest number of effective species (4.3). Streams and Andean forest areas showed differentiation based on common species, with Andean forest showing the highest equitability and open area showing the lowest equitability and highest dominance of two species (fig. 3).

According to the order ^{0}D, the highest contrast was between Andean forest, being 1.28 times more diverse than streams, and open areas covering 88% of the diversity of order ^{0}D present in the Andean forest. Finally, based on the most frequent species in the Shannon index (exp H') of order ^{1}D, the open area had 1.36 times more diversity than Andean forest and stream (fig. 3).

Survey of *Batrachochytrium dendrobatidis*

We found 100 individuals from 12 species were infected with *Bd* out of 541 (table 1). The locality of Los Remansos had the largest prevalence of *Bd* (30%, n = 132), followed by the locality of Paramo of Tamá (25%, n = 31), Asiria (22%, n = 23) and Orocué (12%, n = 29). The most prevalent species were *Bolitoglossa tamaense* (26%), *Pristimantis mondolfii* (22%) and *Tachiramantis douglasi* (9%) (table 1).

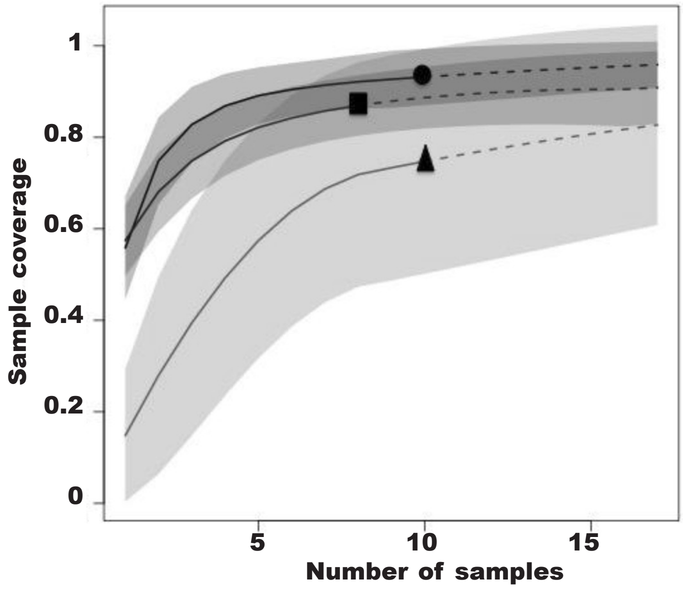

Fig. 2. Plot of sample coverage for amphibian species, with 95% confidence intervals: ● Andean forest; ■ Streams; ▲ Open areas.

Fig. 2. Cobertura de la muestra para las especies de anfibios, con intervalos de confianza del 95%: ● Bosque andino; ■ Arroyos; ▲ Áreas abiertas.

Variations between the abundance of the species recorded for the Andean Forest were similar for both the non–infected (fig. 4A) and the infected species (fig. 4B), with high prevalence of species of the genus *Pristimantis*; moreover, *T. douglasi* was the most abundant followed by *P. melanoproctus*, *P. mondolfii* and *P. gryllus*. On the other hand, for the Stream habitat only *B. tamaense* presented high values of abundance,

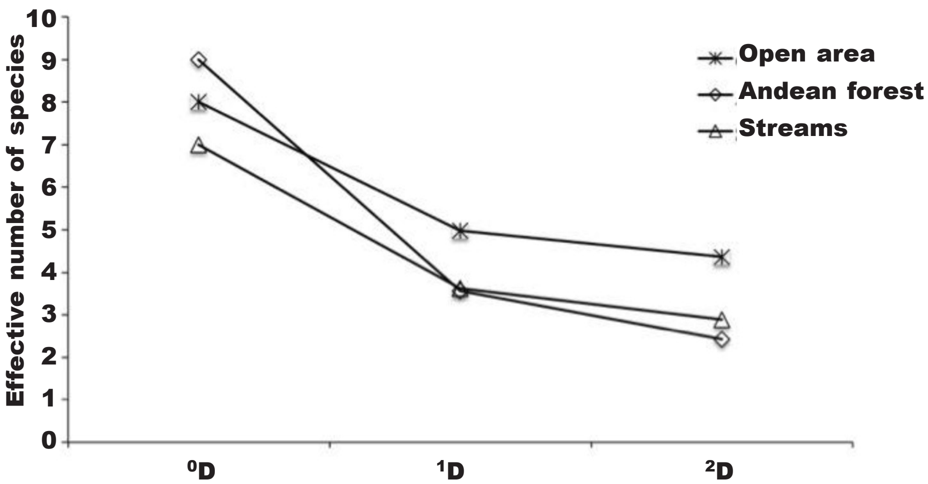

Fig. 3. Diversity profiles for three types of habitat (Andean forest, streams and open area) of Tamá NNP: ^{0}D. Species richness; ^{1}D. Relative abundance, exp H'; and ^{2}D. Abundant species.

Fig. 3. Perfiles de diversidad para tres tipos de hábitat (bosque andino, arroyos y área abierta) del PNN Tamá: ^{0}D. Riqueza de especies; ^{1}D. Abundancia relativa, exp H'; ^{2}D. Especies abundantes.

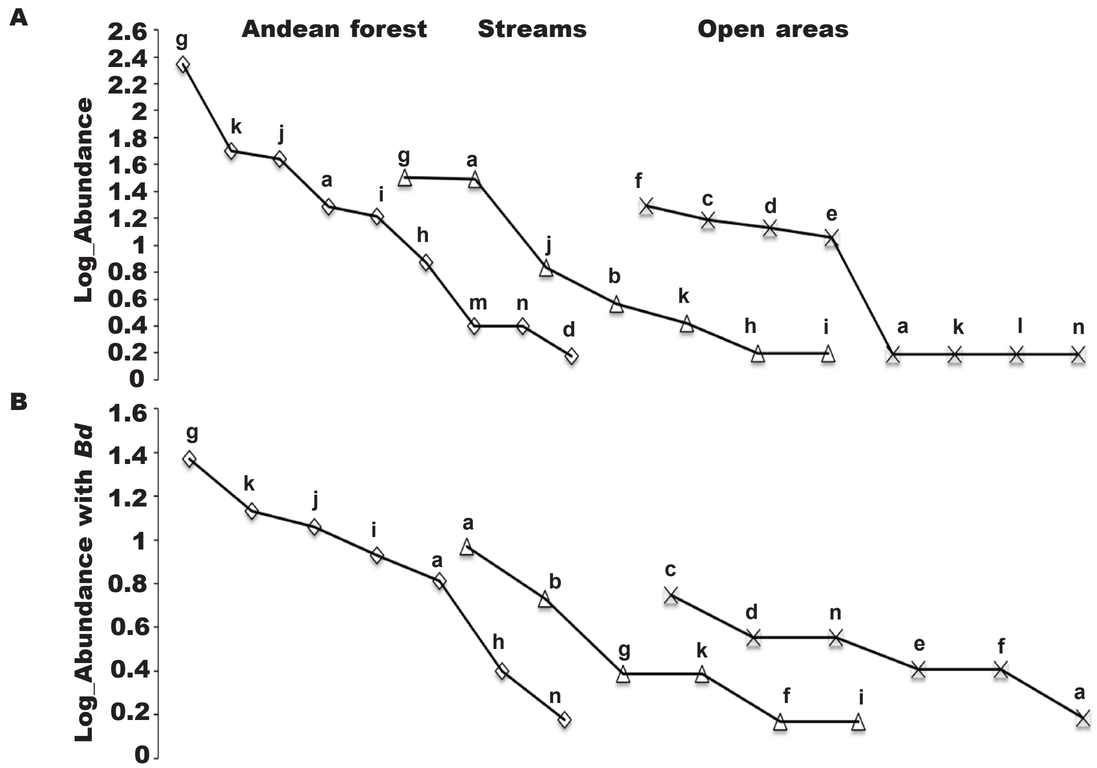

Fig. 4. Rank–abundance distributions for amphibian species in habitats of Andean forest, streams and open areas at the Tamá NNP: A. *Bd* negative species; B. *Bd* positive species. (For the abbreviations of species, see table 1.)

Fig. 4. Distribución de rango abundancia de las especies de anfibios presentes en los hábitats de bosque andino, arroyos y zonas abiertas en el PNN Tamá: A. Especies negativas para Bd*; B. Especies positivas para* Bd. *(Para las abreviaturas de las especies, véase la tabla 1.)*

both as infected and non–infected species; however, *P. mondolfii* and *P. gryllus* showed low values of abundance in both ranges, which is opposite to the results for the Andean forest. For the open areas, habitat species of the genus *Dendropsophus* were the most common, also having the highest abundance ranges of infection in such habitat, although some species of the genus *Pristimantis* and *B. tamaense* are less frequent in this habitat. Even though we observed differences in the variation of dominance of infected and non–infected species for *Bd* across habitats, such differences were not significantly different (χ^2 = 0.85, df = 1, p = 0.35).

Bd infection: preserved *vs.* degraded areas

Assessing the presence of positive and negative individuals for *Bd* in relation to the conserved and degraded areas, we observed a significant difference (ANOVA, F = 2.27, p = 0.05) for negative individuals for *Bd* (3.37 ± 0.5) and positive individuals for *Bd* (1.73 ± 0.5) recorded in the conserved areas (fig. 5). While in the degraded areas there were not significant differences in the variation of positive and negative individuals for *Bd* (fig. 5). In addition, when comparing negative individuals from conserved areas with negative individuals from degraded areas, we observed a significant difference (ANOVA, F = 2.53, p = 0.05), which suggests conserved areas play an important role in limiting infection events (fig. 5).

Discussion

The high areas of the Tamá NNP (above 2,000 m) are home to a wide diversity of amphibians representative of the Andean areas of the Colombian northeast, with predominance of species of the families Craugastoridae, and to a lesser degree, Hylidae, Centrolenidae, Hemiphractidae and Plethodontidae. The Colombian Andean mountain range is considered one of the richest in amphibian diversity (Lynch, 1998). However, the Eastern mountain range has been considered one of mountain chains with least diversity of amphibians with approximately 131 species. The high number of endemic species is emphasized

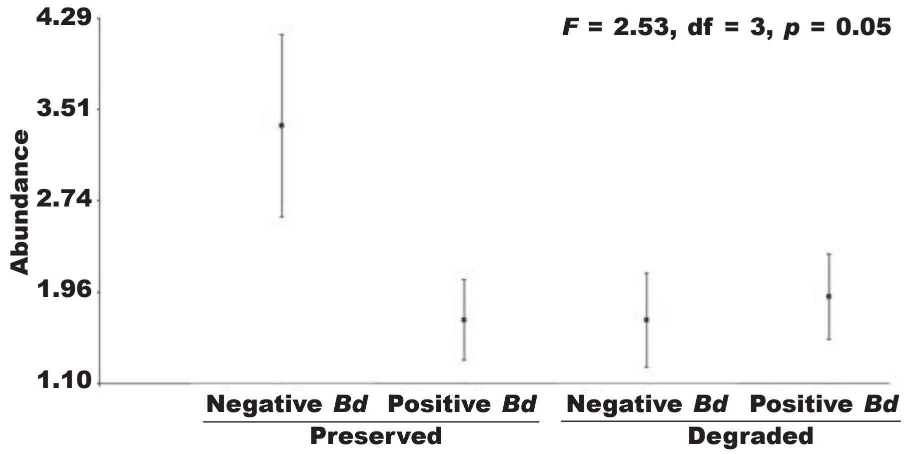

Fig. 5. Spearman rank correlation between amphibians diagnosed (negative, positive) in preserved and degraded areas.

Fig. 5. Correlación de rangos de Spearman entre anfibios diagnosticados (positivo y negativo) en áreas conservadas y degradadas.

(76 species) (Bernal & Lynch, 2008). However, the diversity of amphibians tends to decrease the higher the altitude (above 2,500 m), possibly associated with restrictive environmental conditions and physiological conditions for the amphibians (Navas, 1999, 2003). The Earth's elevated transformation processes can generate drastic changes in the diversity and stability of Andean amphibian populations.

The results of our evaluation of infection by *Bd* show a differential pattern between infection events that occur in preserved areas and those in areas with some degree of intervention. Here, preserved areas may play an important role in safeguarding several species and decreasing possible events of contagion, as is the case of species of the genus *Pristimantis*, *Bolitoglossa*, and *Gastrotheca*. This observation contrasts with the intervened areas where no significant differences were seen between the number of positive and negative individuals for *Bd*. These results support previous studies showing that loss of habitat is negatively associated with the occurrence of *Bd*, and the prevalence and intensity of infection in populations of tropical amphibians (Becker & Zamudio, 2011; Becker et al., in press). Therefore, the decreases that have occurred in pristine areas present a non–random pattern with regards to ecological preferences, geographic intervals and taxonomical groups of the species affected (Stuart et al., 2004; Skerratt et al., 2007).

The conservation status of the landscape within Tamá NNP is considered to be good, with 70% of its forests in pristine condition (Londoño, 2005). However, most amphibian species reported in this study face ongoing threats, which vary in their long–term impacts on conservation. El Tamá NNP has moderate human disturbance due to agriculture, cattle, and deforestation (Garavito et al., 2012); the isolated forest remnants have undergone modifications in their biotic components due to edge effects, potentially altering the distributional patterns of species and negatively impacting population dynamics (Marsh & Pearman, 1997). While the impacts of human activities are considered to be the major threats to amphibians living within Tamá NNP, this study indicates the fungus *Batrachochytrium dendrobatidis* (*Bd*), known to occur in 37 countries across five continents (Kriger & Hero, 2009) and cause rapid irreversible amphibian extinctions, is also a serious threat in the park. This study detected *Bd* in 12 out of 14 species. Moreover, the locality of Los Remansos has a high percentage of *Bd* infection in the three habitats. In the locality of Paramo of Tamá, the incidence of *Bd* was relatively low.

The presence of *Bd* has not been well documented in Colombia even though the country has one of the richest diversities of amphibians in the world. None of amphibians showed external signs of chytridiomycosis disease from *Bd*, such as lethargy, abnormal body position, loss of reflexes, or anorexia (Berger et al., 1998). However, it is important to note that *Bd* is known to kill rapidly. Therefore this is the most alarming report of *Bd* infection in Colombia to date. The first report of *Bd* from Colombia was made from the histological inspection of 672 specimens preserved in scientific collections. However, only three of the 53 frog species examined were infected with *Bd* (Ruiz & Rueda–Almonacid, 2008). A second histological study was conducted in the Western Cordillera in the Department of Valle de Cauca, where 22 out of 466 individuals were infected with *Bd* (Velasquez et al., 2008). In the Eastern Andean region of Colombia, 222 amphibians were tested for the presence of *Bd*. Sixteen of the 222 individuals tested positive for *Bd* (Quintero, 2008). The most recent report from the

Central Andean, Eastern Orinoco, and Colombian Amazon analyzed 336 samples of 57 species, and three species were diagnosed as infected with *Bd* (Vasquez–Ochoa et al., 2012).

Recent studies in the Venezuelan Andes (Hanselmann et al., 2004; Lampo et al., 2006a, 2006b) suggest that *Bd* has been present in the state of Mérida for the last 24 years and is currently at endemic levels in several species of amphibians (Sánchez et al., 2008). The possible expansion from the Venezuelan Andes to the northeastern area of the Colombian Andes could thus explain the cases of infection reported in this study. However, as historic comparative data about the levels of amphibians populations in the region are lacking, we cannot estimate the possible time span when *Bd* first appeared in the area. It is therefore essential to implement an epidemiological supervision plan for the species reported here. Such a plan could detect increases in the prevalence of infection in populations of amphibians that might indicate the appearance of an epidemic at an early stage, facilitating strategies or measures to lower the impact that this may have on amphibian population sizes and possible extinction events.

Changes in vegetation cover due to accelerated anthropic processes may generate imbalances in the intrinsic response of each population of amphibians in Andean areas. Fragmentation of Andean ecosystems could consequently increase the rates of infection by *Bd* in amphibians of the high Andes. Thus, the generation of ecological restoration programs would function as an adequate strategy to buffer the accelerated effects of infection by *Bd*, allowing the recovery of species that are vulnerable to population decline.

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References

Acevedo, A., Franco, R. & Silva, K., 2014. Nuevos registros de especies del género *Pristimantis* (Anura: Craugastoridae) para el nororiente de Colombia. *Revista de Biodiversidad Neotropical*, 4: 162–169.

Acevedo, A., Silva, K., Franco, R. & Lizcano, D., 2011. Distribución, historia natural y conservación de una rana marsupial poco conocida, *Gastrotheca helenae* (Anura: Hemipractidae), en el Parque Nacional Natural Tamá – Colombia. *Bol. Cient. Mus. Hist. Nat.*, 15: 68–74.

Acevedo, A., Wake, D., Márquez, R., Silva, K., Franco R. & Amézquita, A., 2013. Two new species of salamanders, genus *Bolitoglossa* (Amphibia: Plethodontidae), from the Eastern Colombian Andes. *Zootaxa,* 3609: 069–084.

Acosta–Galvis, A. R., 2000. Ranas, salamandras y caecilias (Tretrápoda: Amphibia) de Colombia. *Biota Colombiana,* 1: 289–319.

Acosta-Galvis, A. R. & Cuentas, D., 2016. *Lista de los Anfibios de Colombia:* Referencia en línea V.05.2015.0. Retrieved from http://www.batrachia.com. [Accessed on 18 January 2016] Batrachia, Villa de Leyva, Boyacá, Colombia.

Alford, R. A., Bradfield, K. S. & Richards, S. J., 2007. Global warming and amphibian losses. *Nature*, 447: E3–E4.

Alford, R. A. & Richard, S., 1999. Global amphibian declines: A problem in applied ecology. *Annu. Rev. Ecol. Syst.*, 30: 133–165.

Allentoft, M. E. & O'Brien, J., 2010. Global amphibian declines, loss of genetic diversity and fitness: a review. *Diversity,* 2: 47–71.

Annis, S., Dastoor, F., Ziel, H., Daszak, P. & Longcore, J. E., 2004. A DNA–based assay identifies *Batrachochytrium dendrobatidis* in amphibians. *Journal of Wildlife Diseases*, 40: 420–428.

Armesto, O., Esteban, J. B. & Torrado, R., 2009. Fauna de anfibios del municipio de Cúcuta, Norte de Santander. *Herpetotrópicos,* 5: 57–63.

Becker, C. G., Rodríguez, D., Longo, A. V., Toledo, L. F., Lambertini, C., Leite, D. S., Haddad, C. F. B. & Zamudio, K. R. (in press). Deforestation, host community structure, and disease risk in temperate and tropical amphibians. *Basic and Applied Ecology*.

Becker, C. G. & Zamudio, K. R., 2011. Tropical amphibian populations experience higher disease risk in natural habitats. *PNAS, Proceedings of the National Academy of Sciences*, 108: 9893–9898.

Bernal, M. H. & Lynch, J. D., 2008. Review and Analysis of Altitudinal Distribution of the Andean Anurans in Colombia. *Zootaxa*. 1826: 1–25.

Blaustein, A. R. & Kiesecker, J. M., 2002. Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters*, 5: 597–608.

Blaustein, A. R., Romansic, J. M., Kiesecker, J. M. & Hatch, A. C., 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity & Distributions*, 9: 123–140.

Berger, L., Speare, R., Daszak, P., Green, D. E., Cuningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G. & Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forest of Australia and Central America. *PNAS, Proceedings of the National Academy of Sciences,* 95: 9031–9036.

Bosch, J., 2003. Nuevas amenazas para los anfibios: Enfermedades emergentes. *Munibe* 16: 55–71.

Burrowes, P. A., Joglar, R. L. & Green, D. E., 2004. Potential causes for amphibian declines in Puerto

Rico. *Herpetologica*, 60: 141–154.

Cáceres–Andrade, S. P. & Urbina–Cardona, J. N., 2009. Ensamblajes de anuros de sistemas productivos y bosques en el piedemonte llanero, departamento del Meta, Colombia. *Caldasia*, 31: 175–194.

Chao, A. & Jost, L., 2012. Coverage–based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology*, 93: 2533–2547.

Collins, J. P. & Storfer, A., 2003. A Global amphibian declines: sorting the hypotheses. *Diversity and Distributions*, 9: 89–98.

Corn, P. S., 2005. Climate change and amphibians. *Animal Biodiversity and Conservation*, 28.1: 59–67.

Crump, M. L. & Scott, N. Y., 1994. Visual encounter surveys. In: *Measuring and monitoring biological diversity: standard methods for amphibians*: 84–92 (W. Heyer, M. A. Donnelley, R. A. Mcdiarmid, L. C. Hayek, & M. C. Foster, Eds.). Smithsonian Institution, USA.

Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E. & Speare, R., 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases,* 5: 735–748.

Garavito, T. N., Álvarez, E., Arango, S., Araújo, M., Blundo, C., Boza, T. E., La Torre, M. A., Gaviria, J., Gutiérrez, N., Jørgensen, P. M., León, B., López, R., Malizia, L., Millán, B., Moraes, M., Pacheco, S., Rey, J. M., Reynel, C., Timaná de la flor, M., Ulloa, C., Vacas, O. & Newton, A. C., 2012. Evaluación del estado de conservación de los bosques montanos en los Andes Tropicales. *Ecosistemas*, 21: 148–166.

Gardner, T., 2001. Declining amphibian populations: a global phenomenon in conservation biology. *Animal Biodiversity and Conservation*, 24.2: 25–44.

Grant, B. W., Brown, K. L. & Ferguson, G. W., 1994. Changes in amphibian biodiversity associated with 25 years of pine forest regeneration: implications for biodiversity management. In: *Biological diversity: problems and challenges*: 355–367 (S. K. Majumdar, F. J. Brenner, J. E. Lovich, J. F. Schalles & E. W. Miller, Eds.). The Pennsylvania Academy of Science, York, PA.

Hanselmann, R., Rodríguez, A., Lampo, M., Fajardo–Ramos, L., Aguirre, A. A., Kilpatrick, A. M., Rodríguez, J. P. & Daszak, P., 2004. Presence of an emerging pathogen of amphibians in introduced bullfrogs *Rana catesbeiana* in Venezuela. *Biological Conservation*, 120: 115–119.

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. C. & Foster, M. S., 1994. *Measuring and monitoring biological diversity: Standard methods for amphibians*. Smithsonian Institution Press, Washington, DC.

Hyatt, A. D., Boyle, D. G., Olsen, V., Boyle, D. B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, J. M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F. & Colling, A., 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, 73: 175–192.

Hof, C., Levinsky, I., Araújo, M. B. & Rahbek, C., 2011. Rethinking species' ability to cope with rapid climate change. *Global Change Biology*, 17: 2987–2990.

IUCN, Conservation International, and NatureServe 2012. Global Amphibian Assessment. Available from http://www.iucnredlist.org/initiatives/amphibians/analysis/geographic–patterns#diversity [Accessed on 4 January 2013].

Jost, L., 2007. Partitioning diversity into independent alpha and beta components. *Ecology*, 88: 2427–2439.

Kiesecker, J. M., Blaustein, A. R. & Belden, L. K., 2001. Complex causes of amphibian population declines. *Nature*, 410: 681–684.

Kriger, K. M. & Hero, J. M., 2009. Chytridiomycosis, amphibian extinction, and lesson for the prevention of future panzootics. *EcoHealth,* 6: 148–151.

La Marca, E., 2007. Estatus de poblaciones de ranas de la familia Dendrobatidae (Amphibia: Anura) en sus localidades tipo en los Andes de Venezuela. *Herpetotrópicos*, 2: 73–81.

Lampo, M., Rodríguez–Contreras, A., La Marca, E. & Daszak, P., 2006a. A chytridiomycosis outbreak and a severe dry season precede the disappearance of *Atelopus* species from the Venezuelan Andes. *Herpetological Journal*, 16: 395–402.

– 2006b. A chytridiomycosis epidemic and a severe dry season precede the disappearance of *Atelopus* species from the Venezuelan Andes. *Herpetological Journal*, 16: 395–402.

Lips, K. R., 1999. Mass mortality and populations declines of anurans at an upland site in western Panama. *Conservation Biology*, 13: 117–125.

Lips, K., Burrowes, P. A., Mendelson, J. & Parra–Olea, G., 2005. Amphibian population declines is Latin America: A synthesis. *Biotrópica,* 37: 222–226.

Londoño, J. M., 2005. *Plan de Manejo Tamá*. Documento ejecutivo. Bogotá, Colombia.

Longcore, J., Pessier, A. & Nichols, D., 1999. *Batrachochytrium dendrobatidis* gen et sp nov., a Chytrid pathogenic to amphibians. *Mycologia*, 91: 219–227.

Lynch, J. D., 1998. La riqueza de la fauna anura de los Andes Colombianos. *Innovación y Ciencia,* 7: 46–51.

Marsh, D. M. & Pearman, P. B., 1997. Effect of habitat fragmentation on the abundance of two species of Leptodactylidae frogs in an Andean montane forest. *Conservation Biology*, 11: 1323–1358.

Navas, C. A., 1999. Biodiversidad de anfibios y reptiles en el páramo: una visión ecofisiológica. *Rev. Acad. Colomb. Cienc.,* 23*:* 465–474.

– 2003. Herpetological diversity along Andean elevational gradients: links with physiological ecology and evolutionary physiology. *Comp. Biochem. Physiol*., 133: 469–485.

Phillott, A. D., Spear, R., Hines, H. B., Meyer, E., Skerratt, L. F., McDonald, K. R., Cashins, S. D., Mendez, D. & Berger, L., 2010. Minimising exposure of amphibians to pathogens during field studies. *Diseases of Aquatic Organisms*, 92: 175–185.

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino–Viteri, A., Puschendorf, R., Santiago, S. R., Sanchez–Azofeifa, G. A., Still,

C. J. & Young, B. E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, 439: 161–167.

Quintero, M. P., 2008. Estimating infection level and vulnerability of Andean frogs to the pathogenic fungus *Batrachochytrium dendrobatidis*. Master Thesis, Universidad de los Andes.

Ruiz, A. & Rueda–Almonacid, J. V., 2008. *Batrachochytrium dendrobatidis* and Chytridiomycosis in Anuran Amphibians of Colombia. *EcoHealth*, 5: 27–33.

Sánchez, D., Chacón–Ortiz, A., León, F., Han, B. A. & Lampo, M., 2008. Widespread ocurrence of an emerging pathogen in amphibian communities of the Venezuela Andes. *Biological Conservation*, 141: 2898–2905.

Skerratt, L. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Phillott, A. D., Hines, H. B. & Kenyon, N., 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, 4: 125–134.

Stuart, S., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fishman, D. L. & Waller, R. W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science*, 306: 1783–1786.

Urbina–Cardona, J. N., 2008. Conservación de la herpetofauna neotropical: líneas de investigación y desafíos. *Tropical Conservation Science*, 1: 359–375.

Urbina–Cardona, J. N., Olivares–Pérez, M. I. & Reynoso, V. H., 2006. Herpetofauna diversity and microenvironment correlates across the pasture–edge–interior gradient in tropical rainforest fragments in the region of Los Tuxtlas, Veracruz. *Biological Conservation*, 132: 61–75.

Vásquez–Ochoa, A., Bahamón, P., Prada, L. D. & Franco–Correa, M., 2012. Detección y cuantificación de *Batrachochytrium dendrobatidis* en anfibios de las regiones Andina Central, Oriental, Orinoquía y Amazonía de Colombia. *Herpetotrópicos*, 8: 13–21.

Velásquez, B., Castro, F., Bolívar, W. & Herrera, M. I., 2008. Infección por el hongo quitrido *Batrachochytrium dendrobatidis* en anuros de la Cordillera Occidental de Colombia. *Herpetrópicos* 4: 65–70.

Zippel, K. C. & Mendelson III, J. R., 2008. The amphibian extinction crisis: a call to action. *Herpetological Review*, 39: 23–29.