Conservation Assessment of Amphibian in Tama Bi-National Park, Colombia –Venezuela

Final Project Report
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Conservation Leadership Programme

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Tamá Bi-National Park, Colombia-Venezuela

(June 2010 – June 2011)

By

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PROJECT MEMBERS

Aldemar A. Acevedo
Biologist, MSc. Ecology
Colombian
Project role: principal investigator
Aldemar's interests are focused on the conservation of amphibians and reptiles, mainly with epidemiology, ecology, natural history, biogeography, evolution and environmental education in rural communities..

Karen lizeth Silva
Biologist
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Project role: Co-investigator
Karen's interests include conservation, ecology and natural history of amphibians and reptiles.

Rosmery Franco
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Colombian
Project role: Co-investigator
Rosmery's interests include conservation and taxonomy of amphibians and reptiles.

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Aldemar A. Acevdo, Karen Sila & Rosmery Franco

SUMMARY

The Amphibian Conservation project of Tamá, aims to identify and prioritize species of amphibians that are at risk of threat and identify priority areas for conservation, identifying areas infected by the disease of amphibians "Chytridiomycosis" and also evaluate other factors that threaten amphibian populations, such microclimatic changes and habitat degradation. We recorded 31 species of amphibians, 10 new records for the Tamá Park and two new species of salamanders. In terms of biodiversity is a high value for this region, because the information about the status of amphibians and diversity is scarce. We recorded the highest number of species infected by the chytrid fungus in Colombia, registering a total of 23 species of amphibians distributed between 600 to 3200 m altitude, which together with factors such as deforestation of forests are probably the main threats impacting amphibian species in the region. Social programs have focused on integration with local residents, conducting workshops and creating a direct contact with current conservation issues.

INTRODUCTION

Amphibians are considered the most threatened group of vertebrates (Stuart et al. 2004; Beebee & Griffiths 2005; Hof et al. 2011). According to the IUCN Red List, 32% of the world's amphibian species are threatened or extinct. The Causes of these declines are a matter of continued research, but probably include climate change, land-use change and spread of the pathogenic fungal disease chytridiomycosis (Berger et al. 1999; Daszak et al. 1999; Stuart et al. 2004; Lips et al. 2006b; Skerratt et al. 2007; Wake & Vredenburg 2008; Rodder et al. 2009, Hof et al. 2011). The chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* (Bd) and is perhaps the most decisive factor in the decline of amphibian populations (Berger et al. 1998; Bosch et al. 2001; Bonaccorso & Guayasamin 2003; Lips et al. 2006a).

In Central and South America, the decrease of at

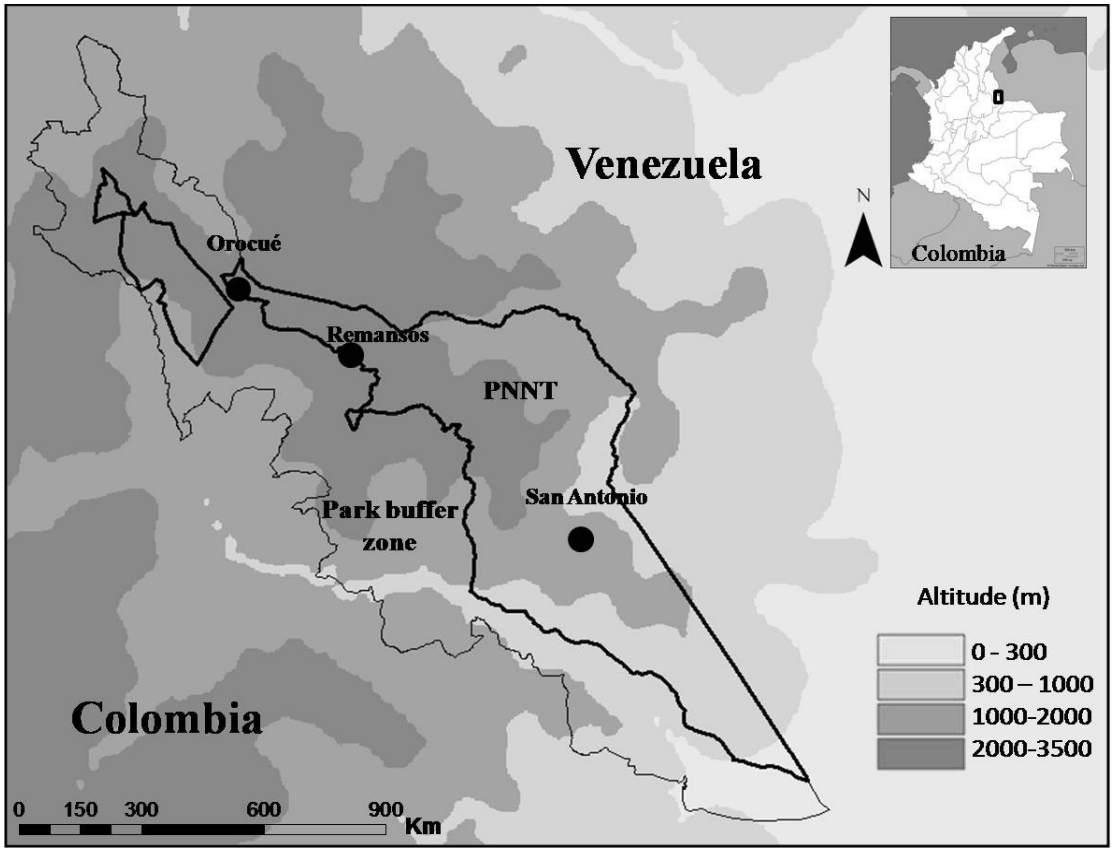

Figure 1. Tamá Bi-National Park. Study area.

least 56 species of amphibians have been associated with the presence of Bd (Berger et al. 1998; Lips 1999; Bonaccorso & Guayasamin 2003; Lips et al. 2003; Burrowes et al. 2004; Lips et al. 2004; La Marca et al. 2005; Carnaval et al. 2006; Lampo et al. 2006b; Lips et al. 2006a). In Colombia, studies on the presence and impact of Bd have been limited to western and central ranges, reporting 19 infected species (Ruiz & Rueda-Almonacid 2008; Velasquez et al. 2008), however the situation of the vast majority of species in Colombia is uncertain, having more than 129 species with data deficient (DD) (IUCN 2011).

Historical evidence has shown that the fungus has persisted in the Andes of Venezuela for over 19 years (Hanselmann et al 2004; Lampo et al. 2006b; Lampo and Señaris 2006; Sanchez et al. 2008). This reference is important because the Andes of Venezuela is geographically near of Tamá National Park (PNNT) (<100 km), located in the northeastern region of Colombia and Venezuela (Figure 1). PNNT is one of the least studied areas in which biodiversity studies and conservation. Studies in herpetology are limited to single inventories (Lynch 1996) so the diversity and conservation status is unknown. The PNNT has been the refuge of the years of illegal

armed groups closely related to the presence of illicit crops, especially in lowland areas (200-700m), which has few studies on the diversity, ecology and conservation of amphibians and other groups of fauna and flora.

The potential threat of Bd, especially in the Andean region and the nascent state of knowledge on the epidemiology of Bd, reinforcing the need for surveillance of infections in natural populations and monitoring the impact of this disease in potentially vulnerable areas (Daszak et al. 2007; Sanchez et al. 2008). A first step towards the identification of conservation priorities is the epidemiological risk assessment for amphibians for Bd Andes. We extend the number of amphibian species previously reported for PNNT, diagnose infected amphibian species in three different altitudes, between different habitats and substrates. At the same time assess the relationship between Bd regarding human impact factors such as temperature, humidity and altitude.

AIM AND OBJECTIVES

General. To determine the impact of chytridiomycosis infection in amphibians of the Tamá Bi-National Park (Colombia-Venezuela) and to evaluate the relationship with disturbed habitats and change climate to identify priority areas for special protection.

Specifics. 1. To determine the distribution and composition of amphibians. 2. To determine the incidence and prevalence of Chytridiomycosis in amphibian populations. 3. To evaluate microclimatic fluctuations in potential habitats for amphibian populations. 4. To generate a map of the distribution of species and areas threatened by infection of chytridiomycosis. 5. To Increase public awareness of the importance of conserving amphibian populations and their habitat.

METHODOLOGY

Study area. Tamá National Park (PNNT) (Norte de Santander state, Colombia, 7°22'N, 72°28'W): 1. Orocué (2400-3200 m; Andean forest and moorland) 2. Los Remansos (2000-2700 m; Andean forest, y 3. San Antonio (600- 800 m; tropical forest) (Figure 1).

Distribution and composition of amphibians. Field trips August of 2010 and April of 2011, conducted six field trips, sampling day and night in three habitats (forest, stream and grassland) with a sampling effort of 360 man / hours. implemented by each habaitat six transects 100 m apart from each other by 200 m for a total of 600 m. For each amphibian we record the date, time, type of substrate (leaf (H), litter (Hj), soil (S), rock (R), stem (T) and water (A)), height above the ground, weight (g), canopy cover (%).

Incidence and prevalence of Chytridiomycosis. We sampled at least one individual of each species for the detection of Bd. Samples were taken using a sterile cotton swab. Each animal was rubbed ten times in different areas (Figure 2). The swabs were stored in sterile Eppendorf tubes and kept refrigerated at -15 ° C. All subjects were handled with gloves following the biosafety protocol to prevent transmission of the fungus between individuals and localities (Aguirre & Lampo 2006). We diagnosed 206 samples of 31 species of amphibians using the technique of chain reaction (PCR) following the protocol of Annis et al. (2004). The products were visualized on agarose gel 1%.

Figure 2. Skin smear to *Batrachochytrium dendrobatidis* samples using swab.

Microclimatic. In each locality, we taked temperature and relative humidity data using data loggers with continuous registration each month. Additionally were also collected data about structural aspects related to the average height of the tree stratum, shrub and sub-arboreal stratum.

Map of the distribution. Using the data collected we categorized the study area according to the distribution, number of infected species, and habitat type, through the delimitation of polygons in a geographic information system.

Activities with local communities. Through briefings and divulgation of educational materials four times a year we conformed working groups with regional environmental organizations to take special actions in highly infected areas. Workshops delivered to the park rangers and inhabitants of the region were key to engage stakeholders. Special workshops with park authorities were also carried out to propose special insitu measures to minimize fungus spread.

Data analysis. The proportion of individuals infected with Bd for localities, habitats, and substrates was determined by dividing the number of infected individuals by the total examined. The differences in the frequency of infection sites, habitats and substrates were determined by a Chi-square test. To evaluate the relationship of infection (positive or negative) for habitats and substrates, we performed a factorial analysis of variance (two-way ANOVA), with a subsequent test of Duncan's multiple range at a probability level of $P \leq 0.05$.

Estimate of abundance categories for the species (common, common, uncommon, and rare) from the encounter rate. We take as basis the total number of individuals recorded for each species by all observers, divided by the duration of the observation period (Rueda-Almonacid et al. 2006).

We used binary logistic regression model to assess the relationship between infection (positive=1 and negative=0) and independent explanatory variables.

OUTPUTS AND RESULTS

Outputs of ecological sciences

Distribution and composition of amphibians. We recorded 31 species of amphibians, of which ten are new records for the PNNT and two new species, -*Bolitoglossa tamense* sp. nov. and *Bolitoglossa leandrae* sp. nov- (Acevedo A. in preparation, Appendice 5; Figure 3). The localities do not share species, however, Orocue and Remansos have in common two frogs genus *Dendropsophus* and *Pristimantis*. San Antonio has the highest number of species (n = 15) followed by Remansos (n = 8) and Orocué (n = 8) (Figure 4; Table 1).

Figure 3. New species of *Bolitoglossa*. A. *Bolitoglossa tamaense* sp. nov B. *Bolitoglossa leandrae* sp. nov

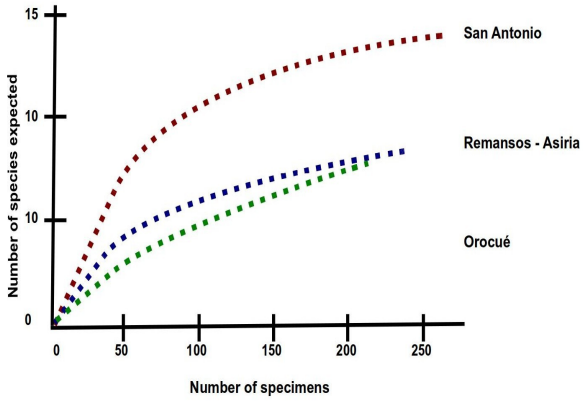

Figure 4. Species Accumulation Curves

Table 1. Distribution, abundance categories and *Batrachochytrium dendrobatidis* infection in the localities; Orocué (Oro), San Antonio (Sant) and Remansos (Rem) and habitats Forest (For), Stream (Stre) and Grassland (Grass).

Localities	Habtat	Species	% Positive Bd	Specimens examined	Abndance categories	IUCN categories
Orocué	For-Grass	*Dendropsophus pelidna* *	40	5	Frequent	Least Concern
	Grass	*Gastrotheca helenae* *	50	2	Uncommon	Data Deficient
	Grass	*Pristimantis anolirex* *	0	0	Uncommon	Near Threatened
	For-Stre	*Pristimantis douglasi* *	39	31	Common	Vulnerable
	Grass	*Pristimantis nicefori*	*0*	*0*	Rare	Least Concern
	Grass	*Pristimantis sp3* **	0	0	Rare	Data Deficient
	For	*Pristimantis sp6* **	100	1	Rare	Data Deficient
	For	*Pristimantis sp7* **	0	0	Rare	Data Deficient
Rem	Bo-Stre-Grass	*Bolitoglossa tamaense*	50	24	Frequent	
	Stre	*Centrolene sp1*	100	1	Rare	Data Deficient
	Grass	*Dendropsophus labialis*	*100*	*4*	Frequent	
	Grass	*Dendropsophus sp2*	*75*	*4*	Uncommon	Data Deficient
	For-Stre	*Pristimantis sp1*	*75*	*32*	Frequent	Data Deficient
	Grass-Stre	*Pristimantis sp2*	*50*	*2*	Uncommon	Data Deficient
	For	*Pristimantis sp4*	*50*	*2*	Uncommon	Data Deficient
	For	*Pristimantis sp5*	*0*	*0*	Uncommon	Data Deficient
Sant	Stre	*Allobates sp1*	*25*	*8*	Frequent	Data Deficient
	For	*Allobates sp2* **	50	4	Uncommon	Data Deficient
	Grass	*Allobates sp3* **	100	1	Rare	Data Deficient
	For	*Bolitoglossa leandrae Sp. nov* **	25	4	Uncommon	
	Stre	*Caecilia subnigricans* **	0	0	Rare	Least Concern
	Stre	*Centrolene sp2* **	50	4	Uncommon	Data Deficient
	Grass	*Dendropsophus sp1*	*50*	*2*	Uncommon	Data Deficient

	For-Grass	*Hypsiboas crepitans*	43	7	Uncommon	Least Concern
	Stre-Grass	*Hypsiboas lanciformis*	*60*	*5*	Uncommon	Least Concern
	Stre- Grass	*Leptodactylus colombiensis*	*38*	*6*	Uncommon	Least Concern
	Bo-Stre-Grass	*Leptodactylus bolivianus*	*53*	*34*	Common	Data Deficient
	For-Grass	*Leptodactylus sp2*	*0*	*0*	Uncommon	Data Deficient
	Stre	*Rhaebo glaberrimus* **	0	0	Uncommon	Least Concern
	Grass	*Scinax flavidus* **	3	3	Uncommon	Least Concern
	For	*Flectonotus pygmaeus* *	*1*	*2*	Uncommon	Least Concern

*** Amphibian species previously recorded for the Tamá Park**

**** New records of amphibians for Tamá Park**

Unmarked species are under review.

Incidence and Prevalence of chytridiomycosis. *B. dendrobatidis* was detected in three locations. 23 species of the 31 diagnosed were positive for fungus, grouped into ten genera and two orders (Table 1).

101 specimens were positive for Bd, which corresponds to 49% of individuals examined (n = 206). 88 positive samples are anurans and 13 correspond to the order Caudata. Eight species were negative for Bd, of which seven are anurans (*Leptodactylus* sp2, *Pristimantis anolirex*, *Pristimantis nicefori*, *Pristimantis* sp3, *Pristimantis* sp5, *Pristimantis* sp7, *Rhaebo glaberrimus*) and the only species recorded in the order Gymnophiona, *Caecilia subnigricans* (Table 1). All positive species are new records of Bd infections in amphibians.

With respect to the number of infected species and specimens by habitat, grassland had the highest proportion with 52.1% (n = 12) and 47 % (n = 53). However, in each case (specimens or species) are not significant differences between habitats (Figure 5).

The substrate leaf had the highest number of individuals and species infected 65.3% (n = 66) and 82% (n = 19) respectively, showing significant differences compared to other substrates (F = 10, p = 0.001) (Figure 6).

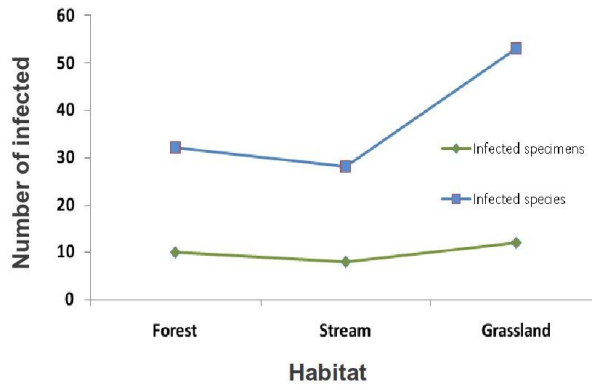

Figure 5. Number of specimens and species infected on each habitat.

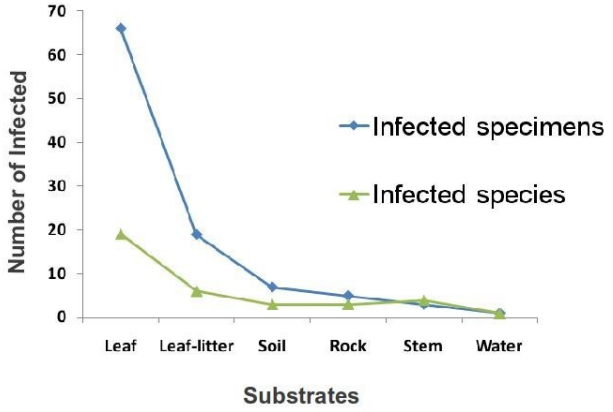

Figure 6. Number of specimens and species infected on each substrate.

Binary logistic regression. Considering the three locations: the explanatory variables in the model are the presence of livestock, people, and streams and grassland habitats (Table 2).

Considering the locations Orocue and Remansos (2000-3200 m): the explanatory variables of the model were the habitats of forest, grassland and streams and the presence of cattle (Table 2).

Orocué: the model developed on infected individuals identified grassland habitats and streams, soil substrate and relative humidity as explanatory variables in the model (Table 2).

Remansos: the model developed on infected individuals identified perch height, temperature and relative humidity as explanatory variables in the model (Table 2).

Genus: *Pristimantis*, the explanatory variables of the model were the habitats of grassland, forest and streams and the presence of cattle. *Leptodactylus*, the forest and grassland habitats. *Bolitoglossa*, the presence of people was the explanatory variable in the model (Table 2).

The model developed for the city of San Antonio was no explanatory variables.

In all test models Hosmer-Lemeshow goodness was not significant indicating a good fit to the model.

Considering the altitude: The model grouping species between 2000 and 3200 m was significant (R2 = 0.098, p = 0.0021).

Microclimatic. Affected individuals recorded a minimum temperature of 7.55 C° in the grassland and the maximum temperature of 29.45 C in the forest (Table 3).

Map of the distribution. Remansos locality registered the highest proportion of infected individuals (45.5%, n = 46) followed by San

Antonio locality (38.6%, n = 39) and Orocué locality (16%, n = 16). However, these differences between locations were not statistically significant. Similarly, the localities were no significant differences in the number of infected species, Orocué (17.3%, n = 4), Remansos (30.4%, n = 7) and San Antonio (52.1%, n = 12). (Figure 7).

Table 2. Logistic regression model ($p<0.05$) based on the diagnosis of *B. dendrobatidis* of 31 species of amphibians.

Variables		Localities[a]					Genus[d]		
		Oro	Rem	Sant	Oro-Rem[b]	Oro-Rem-Sant[c]	Pristimantis	Leptodactlus	Bolitoglossa
Habitat	Forest	-	-	-	0.05	-	0.06	-	-
	Stream	0.028	-	-	0.012	0.014	0.01	0.007	-
	Graasland	0.016	-	-	0.005	0.003	0.05	0.024	-
Substrates	Soil	0.015	-	-	-	-	-	-	-
Physical	Altitude		0.04	-	-	-	-	-	-
	Temperatur		0.04	-	-	-	-	-	-
	Humidity	0.023	0.05	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	
Anthropic	Persons		-	-	-	0.025	-	-	0.038
	Cattle		0.01	-	0.004	0.026	0.017	-	-
	Houses		0.03	-	-	-	-	-	-
			-	-	-	-	-	-	-
	X^2	5.125	6.02	0.57	0.210	0.78	0.513	0.024	0.861
	Odds ratio	0.500	1.84	0.84	1.088	0.962	0.842	0.952	

Different models were made for each case.
a. For each location separately.
b. Location of Orocué and backwaters.
c. The three locations.
d. The most representative genera

Table 3. Descriptive analysis of the temperature ranges (°C) for individuals infected with *B. dendrobatidis*.

Habitat	Maximun	Minimun	Mean	Standard deviation
Forest	11.86	27.62	17.27	5.020
Stream	11.82	29.45	18.99	5.66
Grassland	7.55	25.61	17.67	5.578

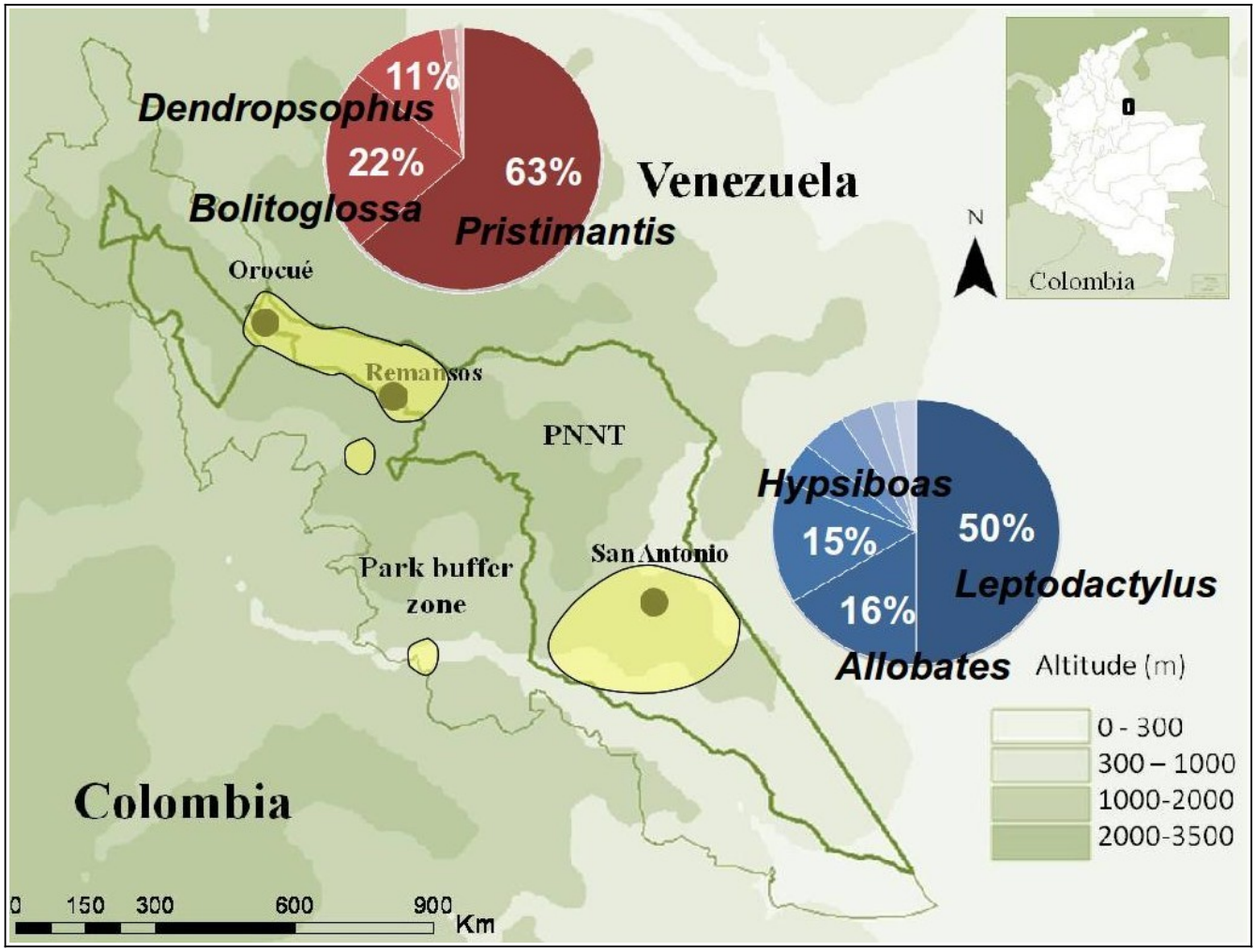

Figure 7. Map of the distribution of Bd in the Tamá Bi-National Park. Polygons were drawn from localities with records for Bd. Circle represents the percentage of the genus of amphibians with more records of Bd infection.

Outputs of social science activities

***Activities with local communities*.** Four Workshops were held with local communities, 1. Remansos, 2. San Antonio, 3. Toledo, 4. Orocué. A workshop on biosafety teccnicas was carried out with the rangers and rural community (Figure 8)

Education activities have achieved positive results, the active involvement of local communities generating and sharing knowledge iterations. It was agreed in conjunction with local communities, environmental agencies and the education sector to develop a management plan for the threatened habitats of PNNT, with the goal of increasing long-term restoration programs of

Figure 8. Workshops with local communities and park rangers.

forests, as necessary to ensure the survival amphibian species that coexist in these habitats.

Three posters were presented at the Latin American Congress of Herpetology in Curitiba, Brazil (Appendices 4). A scientific paper was published in -Boletín Científico, Centro de Museos, Universidad de Caldas- (Appendices). We are currently preparing the following publications: 1. article with the description of two new species of *Bolitoglossa*, 2. article on Bd infection 3. Guide Tamá amphibians.

ACHIEVEMENTS AND IMPACTS

We obtained the first records of *B. dendrobatidis* to the northeast of Colombia, at an estimated area of 700 km2 of PNNT. In this study 23 of 31 species we examined were positive for Bd, comprising eight families of amphibians (Hylidae, Strabomantidae, Caeciliaidae, Centrolenidae, Leptodactylidae, Dendrobatidae, Hemiphractidae and Plethodontidae). The fungus was recorded in all localities, distributed from 600 m to 3200 m altitude. In Orocué Bd reported for a marsupial frog *Gastrotheca helenae* almost unknown whose presence had not been reported for over two decades (Acevedo et al. 2011). The most frequent cases of Bd were presented at the altitude range between 2000 and 2700 m.

According to the logistic regression model (with three locations) of grassland habitats and streams and the presence of livestock and people influence infection by Bd, which is understandable given the increase in agricultural and livestock activities in these areas, resulting in the continuous movement of people and animals that act as dispersal mechanisms of chytrid fungus. The models developed for each location and gender, have different variables that explain the infection, indicating the independence of the action of the fungus by its geographical location, different groups of species and altitudinal ranges. However, amphibian species distributed in the localities above 2000 m there is a wide range of variables that are related to infection by Bd, possibly due to climatic conditions which creates a favorable environment for infection by the fungus.

Apparently there is a preference for low temperatures usually associated with mountains (Berger et al. 2004; Kriger & Hero 2008) suggested that Bd has become the main explanation for the decline of amphibian populations in high mountain areas (Daszak et al. 1999; La Marca et al. 2005, Pounds et al. 2006).

The grassland was the explanatory variable in five of the eight models, especially in the highlands (2400-3200m) of Orocue and Remansos. Bd could grow better in lotic waters as these remain lower temperatures, just as the type of reproduction may play an important role in the rate and spread of Bd, as the habitat and life history of amphibians are considered related to the larval stage, which is crucial at the time of infection (Kriger & Hero 2007).

18 of the 23 infected species have indirect development which suggests that the stage of development could facilitate the spread of Bd

Although adults are more sensitive to Bd infections, tadpoles become infected in the mouth, the only area that has keratin in the early stages of their life cycle (Bosch 2003) so that the larvae of amphibians could act as hosts to be reservoirs for Bd to infect uninfected adults (Woodhams & Alford 2005).

In terrestrial habitats, breeding sites of amphibians are distributed in the forests around water reservoirs, which could increase the rate of infection because of overcrowding. This host-parasite relationship, probably favor infection of amphibians that breed in permanent streams. It is unlikely that the fungus can infect a higher rate of terrestrial amphibians with direct development (Kriger & Hero 2007).

The lower zone (San Antonio), presents a very different group of species sharing only the genus Bolitoglossa with other localities. For this locality no variable explains the model, suggesting that these species are subject to different environmental and anthropogenic pressures and probably other factors not considered in this study may be affecting these populations of low areas. It is therefore important to consider the synergies between different factors, in turn generalized models that explain the spread and prevalence of Bd, could fall into the bias to misinterpretation, because the conditions from one to another location may vary considerably.

The different levels of infection attributable to natural history, habitat, breeding sites, food, microclimatic changes and human impact, favoring different routes of infection according to the scenario where the fungus interacts in a complex climate-human-species-habitat.

Therefore studies focused on establishing changes in weather patterns, land use, biology and behavioral aspects of the species and the historical estimate of infection by Bd for these areas, could provide information on how infection and scenarios generated by increases in the disease and its spread in different habitats.

CONCLUSION

Tamá Park has a great diversity of species of amphibians, our project reported several species that were not known for this area, two new species and several others to describe. However, threats to the conservation of amphibian species are remarkable, persisting fragmentation of forests, livestock and the presence of diseases such as chytridiomycosis, from the lowlands (600m) to the highlands (3200m). Our study, reported for the first time chytridiomycosis infection in 23 species of amphibians, the highest number for a region in Colombia, the threat is combined with the accelerating fragmentation of forests, the combination of these threats possibly this led to a deterioration amphibian populations, so it is necessary a reevaluation of the conservation status of several of these species where the majority are with "data deficient (DD)" according to the IUCN Red List.

We currently have an overview of biodiversity and threats, which information was void before the completion of this project, in turn advance in the processes of socialization and involvement of

local communities, academic and state. From these results will begin to concrete conservation programs for threatened species and habitats in the region.

PROBLEMS ENCOUNTERED AND LESSONS LEARNT

Which project activities and outcomes went well and why?

Although we have achieved all the goals, we will like cover more areas and work with more communities. We hope that a future project to expand the scope of work in the more remote areas of difficult access.

Please detail any problems that the project encountered or deviations from original project plans. Describe how these problems were addressed and what solutions were found to deal with these issues.

The main problems we have encountered in implementing the project, are references to travel to the areas of work, last minute changes of schedule and social problems in the areas of work.

The main problems and solutions are:

1. Remote workstations: The sampling sites are difficult to access and movement and the assistance of the authorities of the park is limited, so we've enlisted the help of local residents with transportation to sites, holiday animals load, and assistance as guides.

2. Presence of protests from local people and indigenous communities: In some cases there have been protests by local communities, affecting our travel to work areas, we have stayed in the homes of local people waiting to pass the protest the area, this has allowed us to live directly with the local people, creating relationships directly with the social problems of the region.

3. Presence of illegal armed groups: In some areas especially in the lower part of the park (San Antonio) remain illegal armed groups, which restrict us deeper expeditions in the park.

Most problems have been resolved through direct support of local people working directly in the integration; we call this process "social integration of conservation".

Briefly assess the specific project methodologies and conservation tools used.

The methodologies used are standard for sampling amphibians. Transects were conducted in each habitat in order to maintain the same sampling unit, so it can be repeated over time and

compare population changes that can be evaluated statistically.

With regard to the diagnosis of chytridiomycosis, the sampling was done with all biosecurity protocols to prevent transmission between individuals and prevent the dispersion of the fungus to other locations. The sampling methods were not invasive.

For the workshops we use conservation lectures, pamphlets and recreational games with local communities. The workshop on recognition of images and sounds was the most comfortable among the communities, by integrating visual and practical way our experiences in the field and transmit them to the communities.

Please state important lessons which have been learnt through the course of the project and provide recommendations for future enhancement or modification to the project activities and outcomes.

The main lesson learned is that if we want to do conservation work that has a lasting success, the work with communities is essential, but not through one-way communication, must have a dialogue where the exchange of knowledge prevails, integrating with the communities, their problems, know their interests and learn from them,so local communities are more receptive, interested in listening to outsiders who come to talk about conservation, forests and need to keep them healthy. We have learned that conservation is a process where they become friends, sharing and making local communities feel they are part of the process and the land they walk on is his house that he shares with many living things.

IN THE FUTURE

The identification of a large number of amphibian species which were unknown in the region has generated interest from several environmental organizations to promote conservation programs in the area. We are in the process of making the first management plan for threatened habitats and amphibians in the region in order to legally engage environmental stakeholders, and to disseminate conservation strategies in conjunction with local communities.

Several species are not currently evaluated (IUCN categories), and other species are data deficient, so we consider it necessary reassessment of the conservation category for some species of amphibians. The high percentage of infection by chytridiomycosis is a clear indicator of the conservation problems that we face together with the processes of fragmentation of forests. This project addresses the first phase to identify priority species for conservation and generate possible solutions that deserve future studies to continue with a second phase that allows more practical conservation actions for amphibians of Tama.

ACKNOWLEDGEMENTS

We express our thanks to Conservation Leadership Programme, Save Our Species, IdeaWild and all donors for funding this project. Thanks to Tamá Park and the National Parks Authorities for logistic support. Special thanks to Dr. Diego J. Lizcano for their support, suggestions and for your confidence. Thanks to all members of the group of Ecology and Biogeography, University of Pamplona. Special thanks to Dr. Adolfo Amezquita and Vicky Flechas at the University of the Andes of Colombia for its support in the diagnosis of chytridiomycosis. Finally, special thanks to all rural communities and children who joined us and opened their minds to the world of conservation, for giving us their support, knowledge and friendship during the execution of this project.

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ADDRESS LIST AND WEB LINKS

inaturalist
Page Tamá Amphibian Project
http://www.inaturalist.org/places/50249#taxon=20978

http://www.inaturalist.org/observations/aldemar

Herpetological Society of Colombia

http://acherpetologia.org/

Grupo de Investigación en Ecología y Biogeografia – Universidad de Pamplona

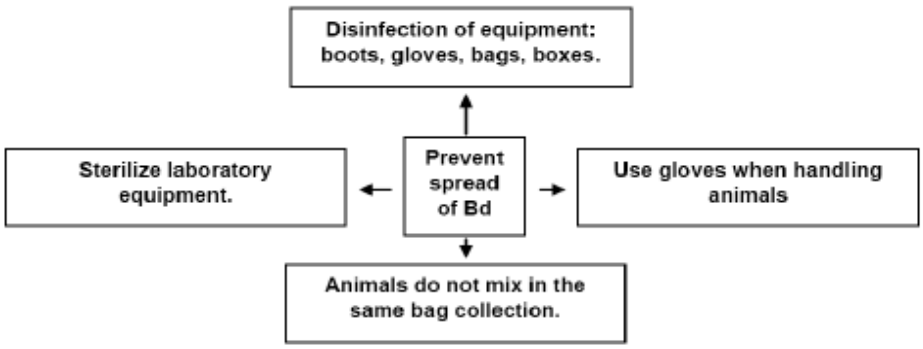
https://sites.google.com/site/giebupa/

Video Tamá

www.youtube.com/watch?v=U0fdIhoyqpI

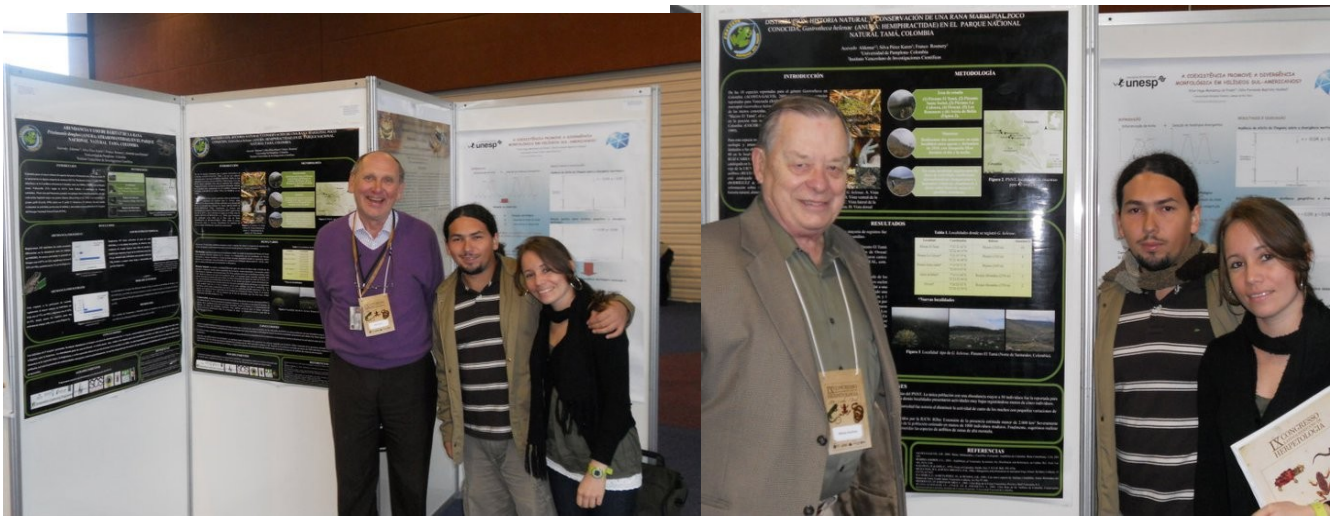
APPENDICES

Appendice 1. Brochure invitation to the first workshop of conservation and biosecurity.

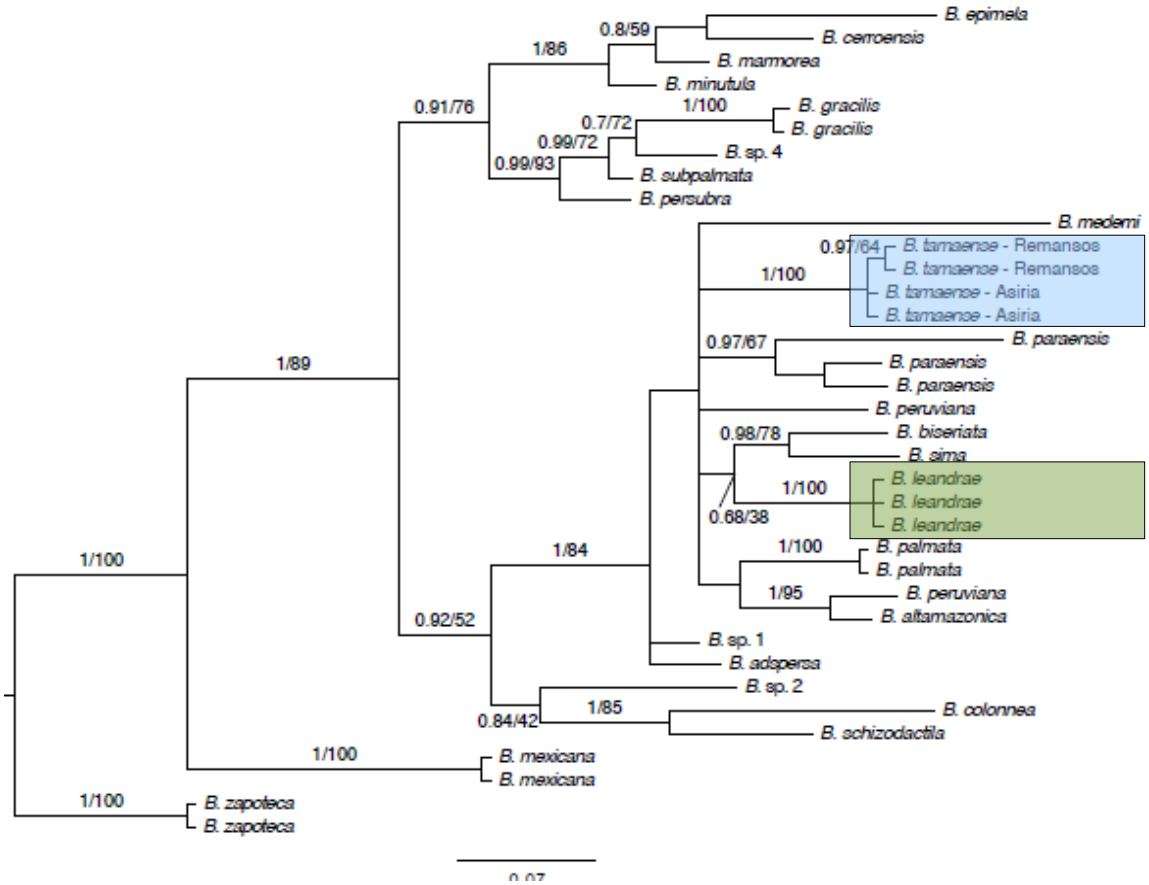


Appendice 2. Biosecurity protocol.

Appendice 3. Workshop and training.



Appendice 4. Posters in the Latin American Congress of Herpetology, Curitiba, Brazil.



Appendice 5. phylogenetic tree showing the new species of *Bolitoglossa*. *Bolitoglossa leandrae* and *Bolitoglossa tamaense*.

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BOLETÍN CIENTÍFICO
CENTRO DE MUSEOS
MUSEO DE HISTORIA NATURAL

DISTRIBUCIÓN, HISTORIA NATURAL Y CONSERVACIÓN DE UNA RANA MARSUPIAL POCO CONOCIDA, *GASTROTHECA HELENAE* (ANURA: HEMIPHRACTIDAE), EN EL PARQUE NACIONAL NATURAL TAMÁ, COLOMBIA*

Aldemar A. Acevedo[1,2], Karen Lizeth Silva[1], Rosmery Franco[1] y Diego J. Lizcano[1]

Resumen

Gastrotheca helenae es una rana marsupial poco conocida, dada su limitada distribución al estar restringida al complejo Macizo El Tamá compartido por Colombia y Venezuela. Evaluamos el estado actual de sus poblaciones mediante la búsqueda en nuevas localidades y describimos aspectos de la historia natural de la especie, con el fin de generar futuros planes de conservación para los anfibios de zonas altas de la Cordillera Nororiental.

Palabras clave: rana marsupial, *Gastrotheca helenae*, Parque Tamá, Colombia, Venezuela, conservación.

DISTRIBUTION, NATURAL HISTORY AND CONSERVATION OF THE NOT VERY WELL KNOWN MARSUPIAL FROG *GASTROTHECA HELENAE* (ANURA: HEMIPHRACTIDAE) IN THE TAMÁ NATIONAL PARK, COLOMBIA

Abstract

The marsupial frog *Gastrotheca helenae* is a not very well known species, as it has a limited distribution that is restricted to the Tamá massif complex shared by Colombia and Venezuela. We assessed the current status of their populations, by means of a search in new locations and we describe aspects of the species natural history, in order to generate future conservation plans for the amphibians in the highlands of the North Eastern Cordillera.

Key words: marsupial frog, *Gastrotheca helenae*, Tamá massif, Colombia, Venezuela, conservation.

INTRODUCCIÓN

Las ranas marsupiales del género *Gastrotheca* (Fitzinger, 1843) pertenecen a la familia Hemiphractidae, componen un grupo de especies que se distribuyen en Suramérica y Centroamérica, caracterizándose por tener una cavidad cutánea dorsal donde los huevos se desarrollan. En algunas especies los huevos eclosionan

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y los renacuajos completan su desarrollo en cuerpos de agua, mientras que en otras especies presentan desarrollo directo, los huevos son retenidos en la bolsa y eclosionan como ranitas (DUELLMAN & MANESS, 1980).

De las 18 especies reportadas para el género *Gastrotheca* en Colombia (ACOSTA-GALVIS, 2000) y de las seis especies reportadas para Venezuela (BARRIO-AMORÓS, 2004), la rana marsupial de El Tamá *Gastrotheca helenae* (DUNN, 1944) (Figura 1) es una de las menos conocidas. Esta rana es endémica del complejo "Macizo El Tamá", el cual es un ecosistema altoandino ubicado en la porción más septentrional de la Cordillera Oriental de Colombia (COCHRAN & GOIN, 1970; LA MARCA *et al.*, 1989).

Para esta especie no hay estudios puntuales sobre su distribución, ecología y amenazas; los registros para *G. helenae* están limitados a las observaciones aisladas realizados en la década de los 80 en la localidad tipo (Páramo El Tamá) (DUELLMAN & RUIZ-CARRANZA, 1986). Actualmente, *G. helenae* está catalogada en la categoría "Datos Deficientes (DD)" según la lista roja de la UICN y para Colombia no figura en el libro rojo de anfibios (RUEDA-ALMONACID *et al.*, 2004); para Venezuela está catalogada como (DD) en el libro rojo de fauna (RODRÍGUEZ & ROJAS-SUÁREZ, 2008). Dada la escasa información sobre esta especie, en este artículo describimos los aspectos de historia natural, distribución y estatus de conservación de *G. helenae*.

Figura 1. Individuos de *G. helenae*. **A:** Hembra. *Fotografía:* César Leal. **B:** Macho. *Fotografía:* Aldemar A. Acevedo.

MATERIALES Y MÉTODOS

Área de estudio

En el Parque Nacional Natural Tamá (PNNT) seleccionamos seis localidades distribuidas en el complejo de páramos El Tamá, el cual está ubicado al suroriente del departamento de Norte de Santander en la Cordillera Oriental de Colombia

(RANGEL-CH., 2000): 1) Páramo El Tamá, 2) Páramo Santa Isabel, 3) Páramo La Cabrera, 4) Orocué, 5) Los Remansos y 6) Asiria de Belén (Tabla 1).

Muestreo

Realizamos dos muestreos en cada localidad entre agosto y diciembre de 2010, con búsqueda libre durante el día y la noche. En cada localidad registramos las horas de actividad, condiciones microclimáticas (temperatura y humedad relativa), abundancia, datos sobre historia natural y condiciones del hábitat. Adicionalmente a partir de muestras de frotis cutáneo realizamos diagnósticos para *Batrachochytrium dendrobatidis* (Bd) mediante la técnica de Reacción en Cadena de la Polimerasa convencional (PCR).

RESULTADOS

Se registraron 70 individuos mediante encuentro visual y registro de cantos. La mayoría de registros fueron en zonas de páramo, siendo menos frecuentes en zonas de pre-páramo y bosque altoandino (Tabla 1).

Tabla 1. Localidades con registros de *G. helenae*.

Localidad	Coordenadas	Hábitat
Páramo El Tamá	7°23'33.14"N 72°22'46.15"O	Páramo (3200 m)
Páramo La Cabrera	7°21'57.75"N 72°21'46.48"O	Páramo (3300 m)
Páramo Santa Isabel	7°19'54.71"N 72°20'0.03"O	Páramo (3600 m)
Asiria de Belén	7°19'51.04"N 72°23'25.96"O	Bosque Altoandino (2700 m)
Orocué	7°24'22.92"N 72°26'12.50"O	Bosque Altoandino (2700 m)
Los Remansos*	7°20'35.82"N 72°25'53.75"O	Bosque Altoandino (2400 m)

*Sin registros de *G. helenae*.

Descripción

G. helenae puede ser diferenciada de las especies de *Gastrotheca* de Colombia y Venezuela por los siguientes caracteres morfológicos (modificado de COCHRAN & GOIN, 1970): los machos de *G. helenae* presentan una longitud rostro-cloaca de 45 mm y las hembras 49 mm. Dermis craneal libre, piel superior suave al igual que la piel de la garganta y pecho, presentan una bolsa (resguardar huevos) sobre la parte posterior del dorso, hocico cortó, dientes vomerianos en dos series pequeñas, canthus rostralis bien definido, tímpano alrededor de un tercio del diámetro del ojo, tímpano separado del ojo por una distancia casi igual a dos veces su propio

diámetro, mandíbula superior no se extiende más allá de la mandíbula inferior, dedos completamente libres, cuarto dedo considerablemente más largo que el segundo, arco frontoparietal completo en la parte posterior del cráneo (Figura 2).

Coloración

Presenta una coloración amarilla brillante en forma de manchas dispersas en el dorso, acompañadas con franjas café oscuro; la zona ventral presenta una coloración crema con tonos azulados en las extremidades acentuándose en las manos. El patrón de coloración varía entre sexos, los machos presentan una coloración más definida con manchas amarillas y franjas dorso laterales oscuras, por su parte las hembras presentan un patrón de coloración dorsal menos definido con manchas amarillas dispersas por su cuerpo (Figura 2).

Distribución

G. helenae presenta una distribución restringida, las únicas poblaciones conocidas estaban registradas para el Páramo El Tamá, el cual es compartido por Colombia y Venezuela. Reportamos cinco nuevas localidades (Tabla 1), siendo la localidad del Páramo El Tamá la que presentó mayores registros de *G. helenae* en comparación con las localidades de Orocué (sub-páramo y bosque altoandino) y Asiria de Belén (bosque altoandino) donde se registraron cantos aislados con individuos cantando en el sotobosque; una sola hembra fue registrada en el Páramo Santa Isabel (C. LEAL, com. pers.) y ningún individuo para la localidad de Los Remansos.

Historia natural

Registramos la mayor frecuencia de cantos entre las 10 p.m. y 1 a.m., incrementando su actividad luego de las lluvias, en las noches con fuertes vientos disminuyen por completo la actividad de canto, durante el día se les encuentra reposando dentro del musgo; se registraron cantos aislados entre las seis y ocho de la mañana y en las tardes nubladas con lluvias moderadas. La actividad de canto de *G. helenae* está determinada por marcadas condiciones microclimáticas, los individuos suelen cantar a temperaturas entre los 6 y 8°C con condiciones de humedad relativa superiores a 78%. En agosto, en la época de lluvia registramos el mayor número de individuos (n = 45) en contraste con el mes de diciembre que fue más seco, donde registramos individuos aislados (n = 11).

A diferencia de la mayoría de especies de *Gastrotheca*, las cuales suelen cantar en el dosel arbóreo (VALERA-LEAL *et al.*, 2011), *G. helenae* genera un canto fuerte de dos notas continuas, vocalizando a nivel del suelo en zonas de laderas altas al borde de los pajonales y frailejones, donde suelen resguardarse de los fuertes vientos paramunos (Figura 3). Los machos suelen estar dispersos, separados a una distancia entre 20 a 30 m, generan coros que se distribuyen similar a una "ola" donde un grupo de un sector de la población canta y el siguiente grupo responde siguiendo una cadena hasta que la respuesta de canto retorna, este comportamiento se puede apreciar es coros de *G. ovifera* en Venezuela hasta en un rango de 2 km (A.A. ACEVEDO, com. pers.) y *G. nicefori* (C.L. BARRIO-AMORÓS, com. pers.). Las poblaciones de *G. helenae* del Páramo comparten territorio con dos especies del género *Pristimantis* (*P. anolirex* y *P. nicefori*) y con una especie de *Pristimantis* sp. de las localidades con bosque altoandino.

Amenazas para la conservación

Antes de la formación del PNNT en 1977, las poblaciones de *G. helenae* del Páramo El Tamá probablemente presentaron un alto impacto generado por la ganadería y movilizaciones humanas, registrándose más de 200 bestias de carga y ganado por semana, los cuales cruzaban la frontera por el río Oirá de Colombia hacia Venezuela (L.A. GONZÁLEZ, com. pers.). Actualmente, *G. helenae* presenta una distribución limitada a menos de 1.000 km^2 según las localidades reportadas en este estudio.

A pesar de que la mayoría de localidades están dentro de un área protegida (PNNT), las poblaciones de *G. helenae* presentan las siguientes amenazas: 1) incendios en las zonas de páramo, 2) quema, tala y ganadería en las zonas de bosque altoandino (Asiria de Belén) y 3) fragmentación de hábitats, y 4) Bd; en todas las localidades registramos individuos positivos para otras especies de anfibios, siendo positivo para Bd un individuo de *G. helenae* en la localidad Páramo El Tamá.

Figura 2. *G. helenae*. **A:** vista ventral. **B:** vista ventral de la mano. **C:** vista lateral de la cabeza. **D:** vista dorsal. *Fotografías A, B, C:* César Leal. *Fotografía D:* Aldemar A. Acevedo.

Figura 3. Hábitat de *G. helenae* en la localidad tipo "Páramo El Tamá". *Fotografía:* Rosmery Franco.

DISCUSIÓN

Nuestros resultados indican la presencia de poblaciones de *G. helenae* en varias localidades de las zonas altas del PNNT, la única población con una abundancia mayor a 60 individuos fue la reportada para el Páramo El Tamá colombiano, probablemente al ser la localidad más conservada dada su dificultad de acceso; las demás localidades presentaron actividades muy bajas registrándose menos de cinco individuos.

G. helenae es muy susceptible a cambios en las condiciones microclimáticas, lo que pudiera estar afectando a corto plazo su supervivencia dadas las fluctuaciones climáticas cambiantes que se están registrando (CORN & MUTHS, 2002). La dependencia de la temperatura y humedad fue notoria al disminuir la actividad de canto de los machos con pequeñas variaciones de temperatura.

Se han reportado especies de anfibios que han presentado cambios en el tiempo de apareamiento dadas las variaciones climáticas (DONNELLY & CRUMP, 1998; BLAUSTEIN *et al.*, 2001; CORN & MUTHS, 2002), por lo tanto, recomendamos realizar estudios más detallados sobre las implicaciones de los cambios en las condiciones climáticas sobre las poblaciones de *G. helenae*.

Estado de conservación

A partir de nuestras observaciones, sugerimos que dicha especie sea catalogada como Vulnerable (VU) siguiendo los criterios sugeridos por la IUCN: B2ba: Extensión de la presencia estimada menor de 2.000 km^2 Severamente fragmentada; C2ai: Se estima que ninguna subpoblación contiene más de 1000 individuos maduros y D1: Tamaño de la población estimado en menos de 1000 individuos maduros. Finalmente, sugerimos realizar más exploraciones a las zonas del Macizo El Tamá, y evaluar otros aspectos de amenazas a las que podrían estar sometidas las especies de anfibios de zonas de alta montaña.

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Expresamos nuestro agradecimiento a los funcionarios del PNNT, especialmente especialmente a Harold Moreno Valderrama por su colaboración en este trabajo, a Don Luis Alfonso González por ser nuestro guía y alojarnos en su casa, a los campesinos de las zonas, al Lic. Orlando Armesto por su colaboración en campo, y a Don César Leal por las fotografías del ejemplar de Santa Isabel y su ayuda en campo. A la Universidad de Pamplona y a *Conservation Leadership Programme* y *Save Our Species*, por el financiamiento dado al proyecto "0621310 - 2010 - Evaluación del estado de conservación de los Anfibios de El Tamá".

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Abundancia y uso de hábitat de la rana Pristimantis douglasi (Anura: Strabomantidae) en el Parque Nacional Natural Tamá, Colombia.

Grupo Taxonômico: Anfíbios - Anuros **Categoria:** Ecologia / Historia Natural

Pristimantis douglasi se distribuye en la Cordillera Oriental de Colombia, entre los 1800m y 2550m, está catalogada como Vulnerable (VU) según la IUCN. Suele habitar el sotobosque de bosques nublados. Estudiamos la actividad, preferencia de hábitat, microhábitat y microclima en una población del Parque Nacional Natural Tamá (PNNT) en la localidad de Orocué (2440m) mediante muestreos en 3 tipos de hábitat (bosque-río-pastizal) llevados a cabo en agosto y diciembre de 2010. Registramos 252 individuos presentando diferencias entre hábitats (p=0,005), la mayor abundancia se presentó en bosque (n=221), seguido por río (30) y pastizal (n=1). Con respecto a la preferencia de sustrato registramos el mayor número de individuos en hoja (n=193) y hojarasca (n=37). P. douglasi presenta diferencias de actividad (p=0,02), con mayor actividad durante la noche (n=212) que durante el día (n=30). Con respecto a las condiciones microclimáticas (T y Hr) no se presentó una relación de estas variables con la abundancia y actividad. La población de P. douglasi en esta localidad está relativamente estable, la localidad presenta zonas de bosques conservadas con escaso impacto antrópico permitiendo la conservación de esta especie. Los autores están evaluando otras amenazas como la presencia de quitridiomicosis.
Expresamos nuestro agradecimiento a Conservation Leadership Programe, Save Our Species y al PNNT por el financiamiento dado.

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Distribución, historia natural y conservación de una rana marsupial poco conocida, Gastrotheca helenae (Anura: Hemiphractidae) en el Parque Natural Nacional Tamá

Grupo Taxonômico: Anfíbios - Anuros **Categoria:** Ecologia / Historia Natural

De las 18 especies reportadas para el género Gastrotheca en Colombia y de las 6 especies reportadas para Venezuela, la rana marsupial del Tamá Gastrotheca helenae es una de las menos conocidas, dada su limitada distribución al estar restringida al complejo de Páramos del Tamá compartidos por Colombia y Venezuela. Evaluamos el estado actual de sus poblaciones mediante la búsqueda en nuevas localidades y describimos aspectos de la historia natural de la especie, con el fin de generar futuros planes de conservación para los anfibios de zonas altas de la Cordillera Nororiental de Colombia. Registramos 70 individuos en 5 localidades mediante encuentro visual y el registro de cantos. La mayoría de registros fueron en zonas de Páramo (3200m), siendo menos frecuentes en zonas de pre-páramo (2900m) y bosque altoandino (2700m). Los machos suelen estar dispersos, separados a una distancia entre 20 a 30m la mayor frecuencia de cantos fue entre las 10p.m y el 1a.m, incrementando su actividad luego de las lluvias. Durante el día se les encuentra reposando dentro del musgo, suelen presentarse cantos aislados entre las 6 y 8 de la mañana y en las tardes nubladas con lluvias moderadas. La actividad de canto de G. helenae está determinada por marcadas condiciones microclimáticas, los individuos suelen cantar a temperaturas entre los 6 y 8°C con condiciones de Hr superiores a 78%. G. helenae genera un canto fuerte de dos notas continuas, vocalizando a nivel del suelo en zonas de laderas altas al borde de los pajonales y frailejones donde suelen resguardarse de los fuertes vientos paramunos. Actualmente G. helenae está catalogada en la categoría Datos Deficientes (DD) según la lista roja de la UICN. A pesar que la mayoría de las localidades están dentro de un área protegida (Parque Nacional Natural Tamá -PNNT), las poblaciones de G. helenae presentan las siguientes amenazas: 1. Incendios en las zonas de páramo, 2. Quema, tala y ganadería en la zonas de bosque alto andino y 3. Fragmentación de hábitats, a su vez estimamos su distribución a menos de 1.000km2, por lo que sugerimos se reevalúe su estado de conservación. Expresamos nuestro agradecimiento a Conservation Leadership Programe, Save Our Species y al PNNT por el financiamiento dado.

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Preferencia de hábitat y sustrato en anfibios de tres localidades del Parque Nacional Natural Tamà, Colombia.

Grupo Taxonômico: Anfíbios - Anuros **Categoria:** Ecologia / Historia Natural

La amplia distribución de los anfibios se debe a las diferentes adaptaciones que han desarrollado durante su historia de vida, como son adaptaciones morfológicas, fisiológicas y ecológicas, las cuales les han permitido colonizar y utilizar un gran número de hábitats y microhábitats que hace posible mantener una alta densidad poblacional y una explotación óptima de recursos disponibles. Mediante este estudio analizamos la composición de anfibios frente al uso de hábitat y de sustratos en tres localidades del Parque Nacional Natural Tamà (PNNT). Se llevaron a cabo dos muestreos por localidad entre agosto de 2010 y abril de 2011 ubicando tres transectos lineales por hábitat. Realizamos observaciones continuas diurnas y nocturnas registrando los tipos de sustrato sobre el que se encontraban los anfibios (hoja, hojarasca, suelo desnudo, roca, suelo cubierto, tallo, musgo, agua y tronco). Registramos 794 individuos pertenecientes a 27 especies, 9 familias y a 3 ordenes (Anura, Caudata y Gymnophiona). Los hábitats bosque, río y pastizal no presentaron diferencias en el número de especies ($p>0,05$), sin embargo la abundancia presentó diferencias para las localidades, hábitats y sustratos ($p<0,05$). Los sustratos con mayores registros fueron Hoja (individuos=394, especies=19) y hojarasca (individuos=95, especies=8) y el sustrato con menor registro fué tronco (individuos=9, especies=4). Se presentaron marcadas preferencias de las especies por los hábitats, con 7 especies exclusivas de bosque de los géneros Allobates, Bolitoglossa, Flectonotus y Pristimantis; 5 especies exclusivas de río de los géneros Allobates, Centrolene, Caecilia, Rhaebo, y 6 especies exclusivas de pastizal de los géneros Dendropsophus, Rhinella, Allobates, y Scinax. Expresamos nuestro agradecimiento a Conservation Leadership Programme, Save Our Species y al PNNT.

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