

An amphibian strategy for the consolidation of the Páramo de Sonsón action plan in Colombia

CONSERVATION LEADERSHIP PROGRAMME

CLP project 02143820

An amphibian strategy for the consolidation of the Páramo de Sonsón action plan in Colombia

Sonsón, Antioquia, Colombia

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11 July 2022

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Summary

Our purpose was to assess the population dynamics, behavior, and ecology of three threatened frog species (*Pristimantis leopardus, Andinobates opisthomelas*, and *Hyloscirtus antioquia*). These inhabit the new protected area "Regional District of Integrated Management (DRMI; Spanish acronym) Páramo de Vida Mataimá - Sonsón" that includes the Páramo de Sonsón. Our results suggest *A. opisthomelas* has a small population, despite being abundant in the restricted patch of forest where it inhabits. On the other hand, *H. antioquia* and *P. leopardus* are very scarce and show activity peaks in the rain, which makes their study difficult. Moreover, their habitats are difficult to access despite being large areas of protected forest. However, we were able to record calls of all three species and obtain microenvironmental and phenotypic data for these types and other threatened amphibians. Nonetheless, the loss and fragmentation observed in the habitat of *A. opisthomelas* deserve urgent measures to stop this issue. Likewise, possible local extinction of the population of *H. antioquia* studied was observed due to illegal logging within DRMI. Our action plan and the published results will be crucial for the different stakeholders as well as for the planning of resources and efforts aimed at the conservation of the DRMI and its threatened species.

Introduction

The small Páramo de Sonsón is a complex of at least five independent areas not linked between them covering 27.31 km^2 (CORNARE, 2014). This complex was protected within the Integrated Management Regional District (DRMI, Spanish acronyms) Páramo de Vida Mataimá – Sonsón together with the surrounding cloud forest in a great area of 406.47 km^2 (CORNARE, 2019). In the DRMI at least eighteen amphibian species inhabit, fourteen of them are threatened and little is known about their populations. Thus, the DRMI is one of the most important amphibian conservation areas in Colombia and the world. However, from the time when DRMI was created in 2019 and since the publication of its management plan (CORNARE, 2021), there has been little interest about the presence of threatened amphibian species within it. So much so that within the conservation objectives, there were no amphibians included, even though there are unique and endemic species such as *Atopophrynus syntomopus* (CR) (Lynch & Ruiz-Carranza, 1982), *Pristimantis bernali* (CR) (Lynch, 1986), and *P. leopardus* (VU) (Rivera-Correa et al. 2017). Moreover, within the amphibian chapter published in the management plan, only reptiles were mentioned (CORNARE, 2021), revealing little knowledge by the professionals who built this document.

This project focused on three species of threatened frogs: *Pristimantis leopardus* (VU) (Rivera-Correa et al. 2017), *Hyloscirtus antioquia* (VU) (Rivera-Correa & Faivovich, 2013), and *Andinobates opisthomelas* (VU) (Boulenger, 1899). We collected population information, morphology, calls, and microenvironments data. In addition to a strong socio-ecological component of conservation activities. Although other amphibian species were recorded during the field trips, several of them were threatened. We analyzed population dynamics and the spatial distribution model for the species *A. opisthomelas*. It is necessary to state that an important group of stakeholders was also involved, coming from academia (researchers from local, national, and international universities), the regional environmental agency (CORNARE), the mayor's office, teachers, and students from two schools. Thus, this project impacted a population of more than 1000 people distributed in more than 870 YouTube views of the “Second biodiversity meeting of Sonsón” that was held virtually, and also more than 170 students through virtual workshops. Likewise, two manuscripts have already been submitted; the first already accepted is the defensive behavior of *H. antioquia*, and the second is the call of *P. leopardus*. Finally, work is being done on the proposal of a management plan for all threatened amphibian species (fourteen) within the DRMI Páramo de Vida Mataimá - Sonsón and on the writing of several manuscripts, the first including the activity temperatures, microenvironmental temperatures, and the spatial distribution model of *A. opisthomelas*, the second is the population dynamics of *A. opisthomelas*, and the third is a review of the calls of *Andinobates* from the *bombetes* group.

Our results increased information about the behavior, ecology, and natural history of little-known threatened frog species. Likewise, it indicated the difficulty in accessing and recording some species. Finally, this project highlighted the importance of monitoring and conserving the DRMI Páramo de Vida Mataimá - Sonsón, given the significant number of threatened amphibian species found during field trips. Including *Rhinella ruizi* (VU) (Grant, 2000), *Pristimantis scoloblepharus* (EN) (Lynch, 1991), and *Pristimantis parectatus* (EN) (Lynch & Rueda-Almonacid, 1998), as well as two very abundant species of salamanders.

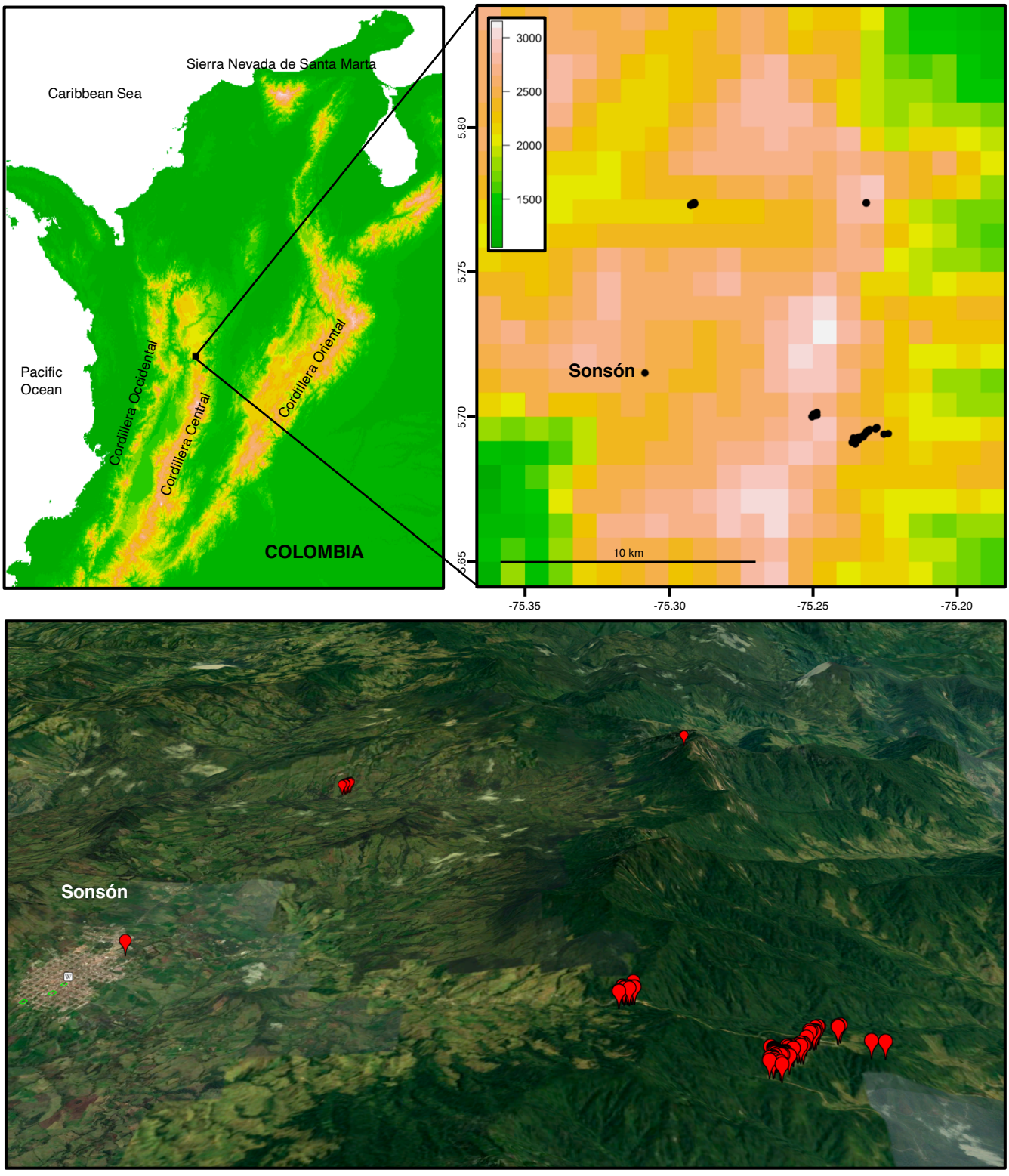


Figure 1. Project localities in Sonsón town (Antioquia, Colombia) and sampling points of the three focal species. Above left, part of Colombia highlighting the Sonsón town on the Central Cordillera. Above right, sampling points with elevation gradient (each pixel approximately 1 km^2). Below, LanSat image of the Páramo de Sonsón and the DRMI Páramo de Vida Mataimá – Sonsón (dark green). The red points above show *A. opisthomelas* and *P. leopardus* habitats (left and right, respectively). Below, Sonsón town (left) with the habitat of the *H. antioquia* (center and right) and other species recorded. The image

shows *A. opisthomelas* within an agricultural matrix outside of the Páramo de Sonsón and the DRMI, while *P. leopardus* and *H. antioquia* are within a big protected area.

Project members



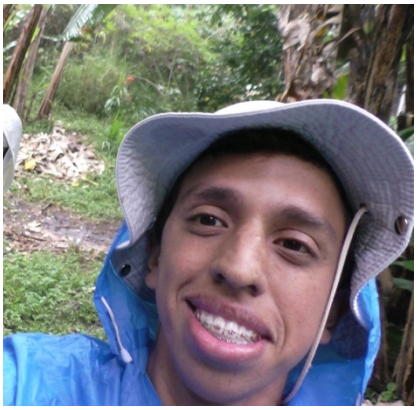
Gissella Pineda, MSc.
Giss currently works with the environment and rural development secretariat of Herveo (Tolima). Giss was the team leader and worked on the development of the maps and the spatial distribution model of *A. opisthomelas*. She also worked with the *P. leopardus* species and led the development of the Second Biodiversity Meeting of Sonsón together with the Universidad de Antioquia - Sonsón, CORNARE, and the Sonsón mayor's office.



Sigifredo Clavijo, MSc.
Sigi is finishing his master's degree in biological sciences at the Universidad del Tolima and currently works as an environmental consultant on different infrastructure projects. Sigi led the working group with the species *H. antioquia* and also recorded the species *R. ruizi*, *P. scoloblepharus*, *P. parectatus*, and found an apparently good populations of two species of salamanders of *Bolitoglossa* genus.



Ricardo Medina, MSc. PhD student
Ric began his doctoral studies on urban biological evolution and microbiome with amphibians. He was in charge of working with the species *A. opisthomelas* and also worked on the analysis of the species' calls and the consolidation of the Second biodiversity meeting of Sonsón. He leads the construction of the management plan for the amphibians of the DRMI Páramo de Vida - Mataimá in collaboration with Giss, Sigi, and two volunteers.







David Vargas, Bibiana Tovar, and Juan Pablo Carmona (biologists volunteers)

Aim and objectives

This project developed the following four objectives:

1. Determine the status populations of *Pristimantis leopardus, Hyloscirtus antioquia*, and *Andinobates opisthomelas* in the Páramo de Sonsón (Colombia).
2. Develop spatial distribution models based on published information (museums, journals, WorldClim data), and our environmental and distribution data that will be collected during the investigation.
3. Promote environmental education for the protection, monitoring, and management of the protection area and involve the students in scientific activities for they can monitor the threatened species.
4. Develop the second diversity meeting to involve stakeholders and together design the action plan for the focal species and the protected area.

Although no objective was changed there were partial adjustments during the implementation of the project the preventive measures against COVID-19 were taking place. First, the COVID-19 produced changes in the budget given that transportation costs doubled due to mobility restrictions between cities Ibagué - Medellín - Sonsón. Second, the restrictions restricted in-person workshops and only virtual activities were developed. About the surveys for the management plan, they could not be done with the student population within rural schools since access to people outside the institution was not allowed.

Due to the restrictions and variations during the implementation of the project, the changes in the objectives were as follows. For the first objective, it was only possible to determine the population dynamics of *A. opisthomelas*, because weather and logistical conditions prevented the recording of the species *P. leopardus*, and very few individuals of *H. antioquia* were recorded. In addition, for the *H. antioquia* species, one of the small streams where the monitoring was done was deforested for wood acquisition. Regarding the second objective, the spatial distribution model (SDM) was only done for the species *A. opisthomelas*. Although we were able to find the other two focal species, the four records of *P. leopardus* are within the same resolution grid as the historical reports (Fig. 1). This means that although there are more records, there are only four grids (pixels) to load the SDM, preventing the analysis. However, for *H. antioquia* the new records are within three resolution grids, although there are currently new reports in GBIF. Currently, we are developing an SDM for this species and we hope to be able to publish it together with *A. opisthomelas*. Likewise, with objective three, students were not involved in field activities, and all the workshops were done virtually due to the limitations caused by the pandemic. During the last two field trips, no workshops were held due to the students' vacation period, however the last objective was quite well given the great reception of the Second biodiversity meeting of Sonsón, this activity was only virtually.

Changes to original project plan

The main modifications to the objectives were previously described. These objectives were not changed, only partially modified due to the difficult access conditions and heavy rains in which two of the focal species (*P. leopardus* and *H. antioquia*) are most active. Thus, we worked in two groups of researchers for the species *A. opisthomelas* and *H. antioquia*.

The low abundance of *H. antioquia* and the complex conditions of its monitoring (nocturnal activity under rain and inhabiting streams that can be risky when sampling) prevented a good analysis of its population. Moreover, the point where population monitoring was being carried out was illegally deforested. Nonetheless, we have taken advantage of the abundance of salamanders and other species of *Pristimantis* frogs to take environmental and phenotypic data on these species and thus improve the knowledge about them. Although they were not part of the focal species of our project, there is also very little information on these species. Thus, we took advantage of this data to improve the knowledge of the amphibian fauna and make some reports.

Regarding the spatial model of distribution of these species, it is likely that one can be made for the species *H. antioquia* because we have new records and there are new reports in GBIF, so this SDM is currently being built for this species. The opposite is the case with *P. leopardus*, which despite having records, there are no new distribution areas reported and there are currently only four grids of resolution. The environmental activities and workshops were carried out virtually since in-person activities were not allowed.

Methodology

Field work sampling

Eight field trips were done between November 2020 and January 2022 in Sonsón (Antioquia) with three days (21 sampling events) for population analysis of the species *A. opisthomelas* and *H. antioquia* (Fig. 1). Although previously, during three field trips we tried to record the species *P. leopardus*, it was not possible due to weather conditions and the difficult access path to the two areas (Cerro la Vieja and Cerro las Palomas) where this species has been historically recorded. Thus, field trips were discarded due to the potential risk that only two researchers worked with this species. Finally, in December 2021, a final expedition was made to Cerro la Vieja where the call was recorded and four data loggers were left to monitor the environmental and microhabitat temperatures of this species.

Phenotypical analysis

We recorded spontaneous advertisement call of the species *P. leopardus, A. opisthomelas,* and *H. antioquia* with an Audio-Technica AT897 microphone coupled to a boompole K-Tek KE-89CC in a ZOOM H6 recorder positioned at approximately 70 cm directly in front every male calling. Due to temperature dependence for metabolic regulation in ectotherms, we also recorded air, substrate, and body temperature with a Hygro-Thermometer RH101 Extech IR and Klein tools IR5 dual laser infrared thermometer, and the record were filtered from apparent conspecific interactions considering that anurans can adjust their call features based on environmental factors (Ey & Fischer 2009) and social context (Wells 2007). For spectral and temporal analysis, we used Raven Pro v1.6.3 (Center for Conservation Bioacoustics, 2014) with 44.1 kHz and 16 bits. The spectral parameters were analyzed with a Fast Fourier transformation under the Blackman algorithm with 724 samples by a window, a frequency grid with 2,048 samples using the discrete Fourier transform algorithm (DFT), and default values for other parameters. Call parameters measured were specific to every species and some of them were call duration (CD), inter-note interval duration (IN), peak frequency (PF), lowest frequency

(LF), highest frequency (HF), and bandwidth (BW). All spectral parameters were measured depending on the species 25 and 15 decibels below peak frequency.

Furthermore, we recorded the snout-vent length (SVL) measures with a Mitutoyo Absolute CD-6" CSX digital caliper (nearest 0.01 mm) by Ricardo Medina (*A. opisth*omelas) and Sigifredo Clavijo (*H. antioquia* and other species). We also weighed frogs and salamanders with a pocket scale (nearest 0.01 g). Finally, some amphibians were euthanized with benzocaine, fixed in 10 % formalin, and stored in 70 % ethanol at the Colección Zoológica de la Universidad del Tolima, and a copy of the call recorded of *P. leopardus* was deposited at Fonoteca Zoológica (www.fonozoo.com) of the Museo Nacional de Ciencias Naturales, Madrid, Spain (Code 14202).

Finally, the presence of fluorescence in amphibians recorded was determined by visual inspection of individuals using a handheld LED UV-flashlight (provided in the visual implant elastomer tags kit) with a peak intensity of 405 nm. All fieldwork was carried out in strict adherence to the guidelines for work with amphibians and reptiles (Beaupre et al. 2004) under permit of collect and management was granted to the Grupo de Herpetología, Etología y Eco-fisiología of the Universidad del Tolima by the ANLA agency (Resolución Nº 02252 de 2019).

Capture-Mark-Recapture analysis

We used capture-mark-recapture (CMR) method in MARK software (White, 2014) with a closed population model (Williams et al. 2002) to determine the population parameters of *A. opisthomelas*. Recaptures were determined by non-invasive photographic identification method (Meekan et al. 2006) with ventral spotting pattern present on each individual, although we initially use visual implant elastomer tags (Anholt et al. 1998) these were discarded because the ventral spotting pattern was consistent over time. Both seasons (wet and dry) were explored, however, there were apparently no changes. This could be due to the fact that in 2021 there was the atmospheric phenomenon of La Niña, so a change marked by the seasons was not found. We make random time-standardized transects (five hours each, 7:00 - 12:00) using the visual encounter survey method (Crump & Scott, 1994) within a small patch of 1.2 hectares. *A. opisthomelas* males were determined through engaging in reproductive activities such as transporting tadpoles, calling, or by vocal sacs present in males that were actively calling (Duellman, 1966). However, as we were not able to differentiate between sexes using external characters in *A. opisthomelas*, we pooled all individuals for further analyses. Despite currently our models are being adjusted, we will estimate apparent survival probabilities (Φ), recapture probabilities (p), and the realized population growth rate (λ) for each interval between capture occasions, as a function of the adult class, time, season, and SVL acting additively (no interaction effects) in models that included more than one variable per parameter. We will use the Akaike Information Criterion (AIC) to evaluate the models (Akaike, 1987) which incorporate the uncertainty in the process of parameter estimation (Burnham & Anderson, 2004). Likewise, the Akaike information criterion corrected for a small sample size (AICc; Akaike, 1973; Burnham & Anderson, 2004). We will calculate model-specific Akaike weights and if more than one model is chosen, we will calculate weighted averages.

Spatial Distribution Models (SDM) for *A. opisthomelas*

To create the spatial distribution model of *A. opisth*omelas we compiled distribution data from our field data, published literature, and specimens housed at the herpetological collections report on GBIF

(https://www.gbif.org/occurrence/search?taxon_key=7773159&taxon_key=8855870&taxon_key=9309439&occurrence_status=present) (Accessed 15 May 2021). Furthermore, due to our knowledge about *A. opisthomelas* distribution, we filtered data occurrences from Western Cordillera and limited data only to Antioquia records because it excludes large regions where the species is likely absent (i.e., areas where the species has not been collected historically (Brotons et al. 2004), or inventory pseudo-absences (Elith & Leathwick, 2007). We used the elevation and 19 WorldClim v2 variables (Fick & Hijmans, 2017) for each sampling locality for the 1970–2000 period, at 30s resolution. We generated SDMs using the software MaxEnt (Phillips et al. 2006). We calculated the spatial correlations (Pearson coefficient) between variables using ENMTools (Warren et al. 2010). We used contribution scores and scores from spatial correlations to reduce predictors from the full model. We eliminated variables with low contribution and permutation importance scores (< 5%) in the full model and retained environmental variables with the highest scores. Thus, we generated a minimum convex polygon (MCP) using Quantum GIS software (QGIS Development Team. 2019) to create the smallest convex polygon enclosing all occurrence sites.

Microenvironmental analysis

Several temperature data were recorded in this project. We obtained hundreds of records of substrate, body, and environmental temperatures for many amphibian species. The methodology for these records is indicated in the phenotypic analysis. However, we also recorded 8563 microenvironmental temperatures from six MX2201 HOBO data loggers. Three of them were left within the microhabitat used (burrows) by *A. opisthomelas* and three around 1.5 m on the ground. Likewise, six data loggers were left to record *H. antioquia*, although all were left on the same microhabitat because no differences were observed in the use of habitat unlike *A. opisthomelas*. However, only two recorded 7720 hours of data, due to four were lost by illegal clearing of the forest. Finally, four data loggers were set down to record microenvironmental temperature data inside water holes in bromeliad leaf axils where *P. leopardus* calls were recorded and approximately 1.5 m above the ground. We will be monitoring all data loggers and we hope that they can be maintained in the middle- (5 years) and long- term (10 years), for future analysis of climatic change.

Environmental workshops and Second Biodiversity Meeting of Sonsón

For the environmental workshops at the schools, we only worked with schools in the Sonsón town due to the fact that all workshops were done virtually by COVID-19 emergency. Initially, we contacted the secretary of education of the Sonsón town, however, we did not get an answer so we individually contacted different science teachers from schools. Although Sonsón has a large student population, only two educational institutions allowed the workshops to take place, the first on ecosystem services and biodiversity and the second on climate change and biodiversity. We also made in-person surveys to assess the threats to the focal species in the field with students and people from the villages. Likewise, with the help of some teachers, we conducted tests and surveys within rural schools. A final workshop about the final results has not been done because the management plan has not yet been completed and delivered.

Likewise, the Second biodiversity meeting of Sonsón was held virtually, this event took place over three days (1-3 June 2021) in the morning and had significant support from the regional environmental agency (CORNARE). In addition, there was a great academic participation with 19 researchers in

biodiversity, 13 of which were from universities, research centers, and national environmental agencies and NGOs, the other six were from universities and international institutions that kindly joined this event. Topics ranged from local research in the Páramo de Sonsón with plants, amphibians, and birds, to macroecological research on biodiversity with birds and amphibians, fire ecology, management measures and monitoring of biodiversity in protected areas, invasive species, the biodiversity understanding of the Tropical Andes from the fossil record, up to conservation psychology. This objective was a great achievement with more than 400 people connecting during the three days of the event and currently with more than 1000 views of the recorded presentations (https://www.youtube.com/watch?v=_iKB4segPuc&t=333s&ab_channel=Cornare, https://www.youtube.com/watch?v=JFtlyM60nSg&t=3076s&ab_channel=Cornare, https://www.youtube.com/watch?v=D1z8CLABq1s&t=11175s&ab_channel=Cornare).

Currently, we are writing the amphibian management plan for fourteen amphibians threatened species within the DRMI Páramo de Vida Mataimá – Sonsón. This will take the results of the perceptions of amphibians' species by locals and recommendations of researchers from WCS-Colombia, Instituto Humboldt, Universidad de Antioquia, and Universidad del Tolima.

Outputs and results

1. **Determine the status populations of *Pristimantis leopardus, Hyloscirtus antioquia*, and *Andinobates opisthomelas* in the Páramo de Sonsón (Colombia).**

133 individuals of *A. opisthomelas* were captured, with 22% recaptures, during 21 sampling events with 18 juveniles recorded (13.5% of the population). Recapture determination was successful through the photographic method (Fig. 1, 2), indicating this non-invasive method can be perfectly replicated due to spotted ventral pattern do not change on time. Moreover, the recapture rate was high with individuals recorded up to 8 times within 21 events; also indicating that these individuals survive more than one year in the field (personal observations). Regarding how they use the habitat, our results indicate that the distribution is not homogeneous throughout the patch where they occur (Fig. 4). The data suggest that juveniles and small individuals occupy the habitat with the highest elevation (Fig. 4).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
1		Rec1	Rec2	Rec3	Rec4	Rec5	Rec6	Rec7	Rec8	Rec9	Rec10	Rec11	Rec12	Rec13	Rec14	Rec15	Rec17	Rec18	Rec19	Rec20	Rec21				Total			
2	Ind001	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	1	0				8	d1	5	Ind001 (r001, r032, r049, r21, r059a, r079, R101, R129), Ind077, Ind049, Ind032, Ind1
3	Ind002	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1	d2	1	Ind002 (R002)
4	Ind003	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind003 (R003)
5	Ind004	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				2		2	Ind004 (R004, r048), Ind048
6	Ind005	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind005 (R005)
7	Ind006	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind006 (R006)
8	Ind007	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				2		2	Ind007 (R007, R026), Ind026
9	Ind008	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0				6		4	Ind008 (R008, r027, r6, r050a, R099, R124), Ind062, Ind101, Ind027
10	Ind009	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind009 (R009)
11	Ind010	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind010 (R010)
12	Ind011	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind011 (R011)
13	Ind012	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				2	d3	2	Ind012 (R012, R022), Ind022
14	Ind013	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind013 (R013)
15	Ind014	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0				3		2	Ind014 (R030, r014, R095), Ind030
16	Ind015	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0				3		2	Ind015 (R015, r024, R085), Ind024
17	Ind016	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind016 (R016)
18	Ind017	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0				6		5	Ind017 (R017, R053, r040a, r5, r058a, R087), Ind091, Ind061, Ind052, Ind108
19	Ind018	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind018 (R018)
20	Ind019	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind019 (R019)
21	Ind020	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind020 (R020)
22	Ind021	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind021 (R021)
23	Ind022	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1	d4	1	Ind023 (R023)
24	Ind023	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0				2		2	Ind025 (R025, r044a), Ind095
25	Ind024	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind028 (R028)
26	Ind025	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind029 (R029)
27	Ind026	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0				4		1	Ind031 (R031, R086, R100, R127)
28	Ind027	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0				2		1	Ind033 (R033,R074)
29	Ind028	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1		1	Ind034 (R034)
30	Ind029	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				1	d5	1	Ind035 (R035)
31	Ind030	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0				2		2	Ind036 (R036, r12) Ind068

Figure 2. Image of table of individuals capture-recaptures of *Andinobates opisthomelas*. The first individual (Fig. 3) was recapture from November 2021 to January 2022.

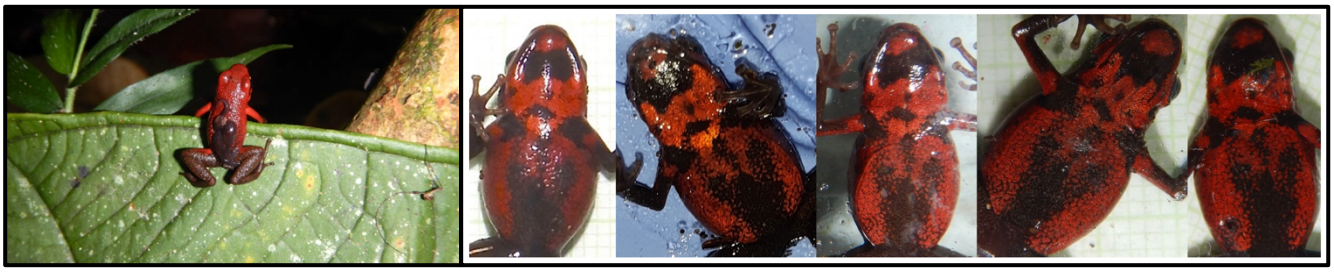


Figure 3. *Andinobates opisthomelas* dorsal and ventral color pattern. Left, male carrying three tadpoles and right, the first individual with the ventral spotted pattern at different event records.

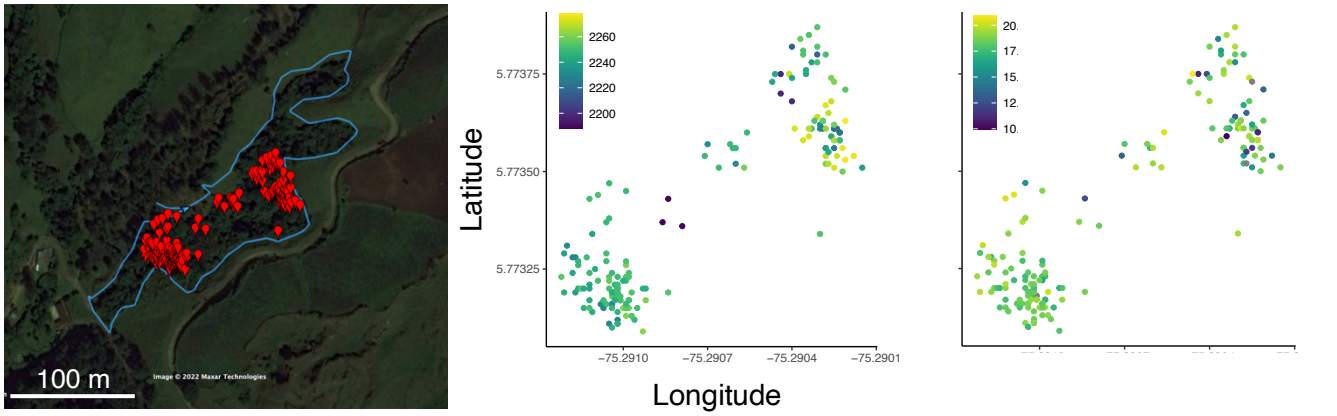


Figure 4. Spatial distribution and forest patch where *Andinobates opisthomelas* was recorded. Left, patch polygon where *A. opisthomelas* was captured. The white line indicates the scale. The patch had 1.2 hectares (0.012 km^2). Middle, elevational distribution (in meters) of every single individual captured in the patch. Right, spatial distribution of the snout-vent length (SVL, in mm) measures.

In addition to the population dynamics analysis, eleven calls of *A. opisthomelas* (Table 1), seven of *H. antioquia*, and one of *P. leopardus* were also recorded (Fig. 5). With regard to the calls of *A. opisthomelas*, a review of the calls of the *bombetes* group is being written together with researchers from the Universidad del Quindío (Colombia). Likewise, the call of *P. leopardus* was submitted to review by the journal Zootaxa (Appendices B). This call already is on the repository of the Fonoteca Zoológica (www.fonozoo.com) sound library, for free access (code 14202).

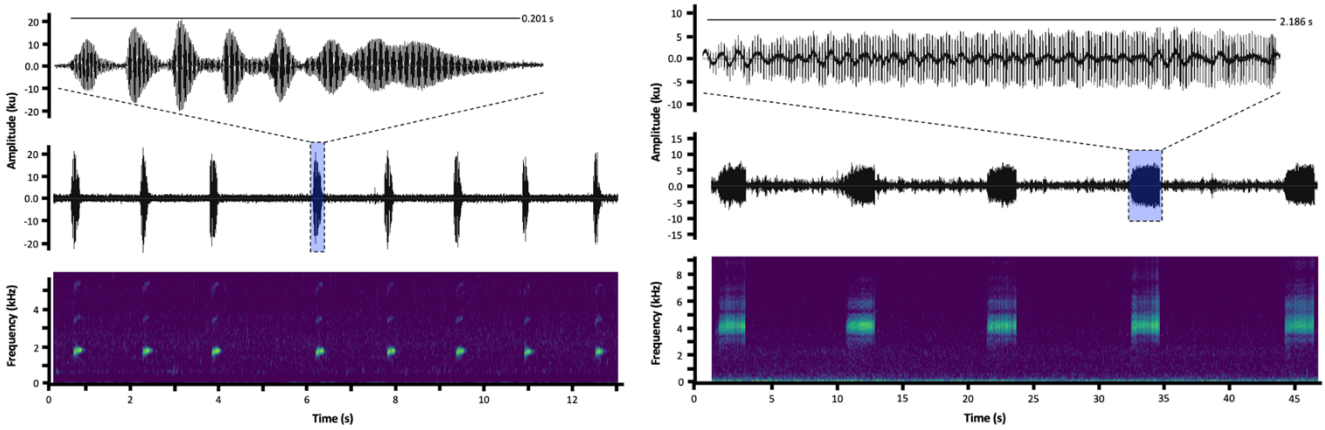


Figure 5. Call features of *Hyloscirtus antioquia* and *Andinobates opisthomelas***.** Left, call of *H. antioquia.* This call belongs to first individual recorded, who was recaptured on day three. The temporal and spectral features of the call are shown, as well as the duration. Right, call of *A. opisthomelas*. Although many calls of *A. opisthomelas* were heard, only the call of the first individual was recorded on day one. Analyses were made in Raven Pro v1.6. Analysis and results are presented in Table 1.

Table 1. Resume of the calls features recorded. Calls were recorded to 44,1 kHz and 16 bits and analyzed in Raven Pro v1.6. Spectral parameters as peak, low, and high frequency were analyzed in Blackman algorithm taken 10 dB below peak power. Pulse rate was calculated as #Pulses/Call duration. Call duration is in seconds and peak, low, and high frequency are in kHz.

Species		**Parameters**					
		# Pulses	**Pulse rate**	**Call duration**	**Peak frequency**	**Low frequency**	**High frequency**
A.	Range	160 - 250	91.0 - 103.0	1.66 - 2.56	4.23 - 4.92	3.69 - 4.01	4.64 – 5.28
opisthomelas	$\overline{X}$	201	95.9	2.09	4.55	4.01	4.93
N = 11	SD	32.96	3.88	0.33	0.20	0.24	0.21
H. antioquia	Range	6 – 9	36.4 – 50.0	0.15 – 0.22	1.72 – 1.81	0.97 – 1.37	1.95 – 2.30
N = 7	$\overline{X}$	8	42.8	0.18	1.73	1.21	2.05
	SD	1.07	5.2	0.02	0.03	0.18	0.13

Body and environmental temperatures were also analyzed (*for A. opisthomelas* and other amphibians' species) and although there was a strong dependence of body temperature on the environmental temperature (Fig. 6, 7, 8), we were able to determine differences between both temperatures for the species *A. opisthomelas* (Fig. 7) unlike other amphibian species (Fig. 8). On the other hand, unfortunately we did not find any fluorescent response in the animals tested, considering that we found

lizards of *Anolis* genus, snakes of *Atractus* genus, and all amphibian species (at least two salamanders' species and seven frogs (Fig. 9).

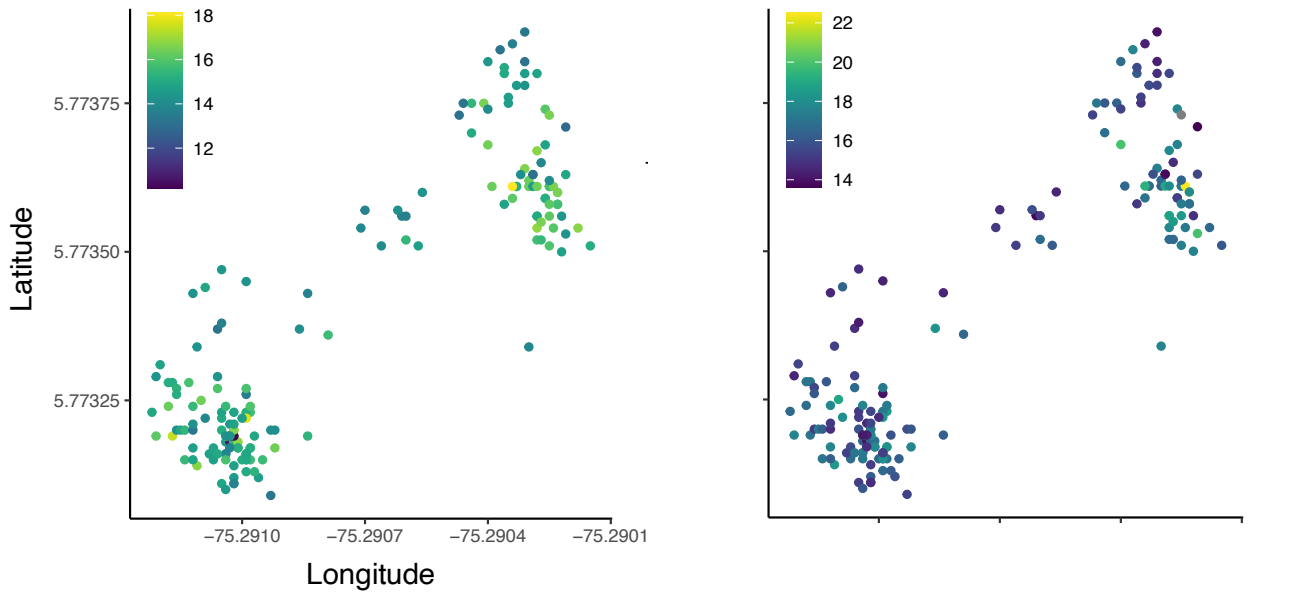


Figure 6. Spatial distribution of temperature records of *Andinobates opisthomelas*. Left, Substrate temperature (°C). Right, body temperature.

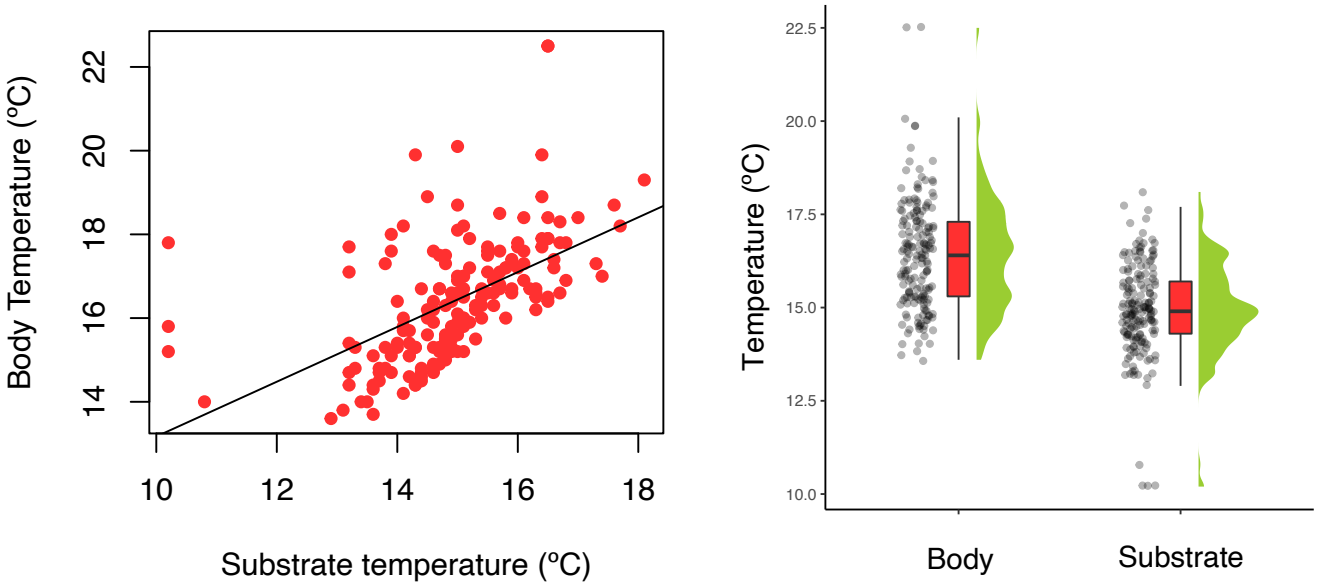


Figure 7. Relation between environmental and body temperature of *Andinobates opisthomelas*. Left, positive correlation and dependence of body temperature on substrate temperature. Right, differences between body and substrate temperatures.

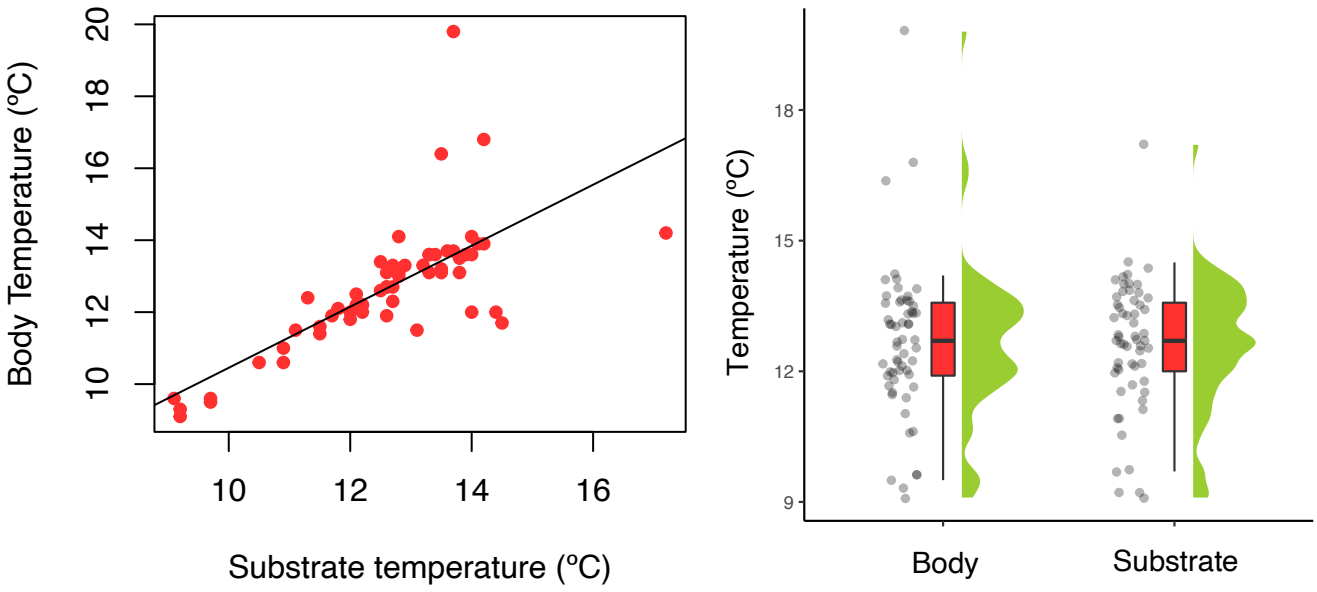


Figure 8. Relation between environmental and body temperature of other amphibian species. Left, positive correlation and dependence of body temperature on the substrate temperature. Right, no differences were observed between body and substrate temperatures. The records represent the species *H. antioquia, P. leopardus, P. scoloblepharus, P. parectatus, P. aff permixtus, P. aff bernali, R. ruizi, B. aff ramosi*, and *Bolitoglossa aff vallecula.*

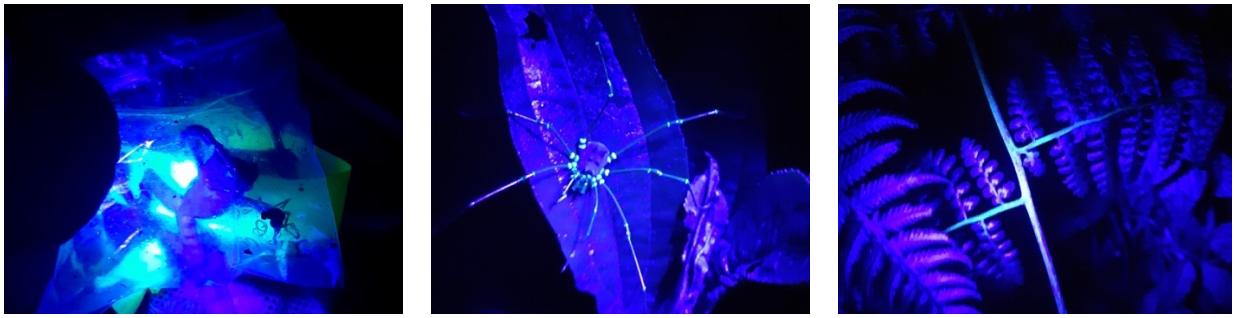


Figure 9. Fluorescent test with handheld LED UV-flashlight (peak intensity of 405 nm). Left, a negative test for *H. antioquia*; center, a positive test of harvestmen (Opilion); right, a positive test for fern branch. No amphibian or reptile tested (nine species) in the Páramo de Sonsón has shown a positive result for fluorescence.

Another significant result was the description of the call and the record of *P. leopardus* species (Fig. 10), which was elusive during most sampling and field trips. After four field trips, this objective could be achieved, and although logistically it is unlikely to carry out a population analysis through traditional capture and recapture methods (Fig. 11), the recording of the call and the activity conditions determined by our observations allow us to suggest that automated acoustic methods could be tested with this species. Moreover, we also left four data loggers to monitor the microenvironment of this species.

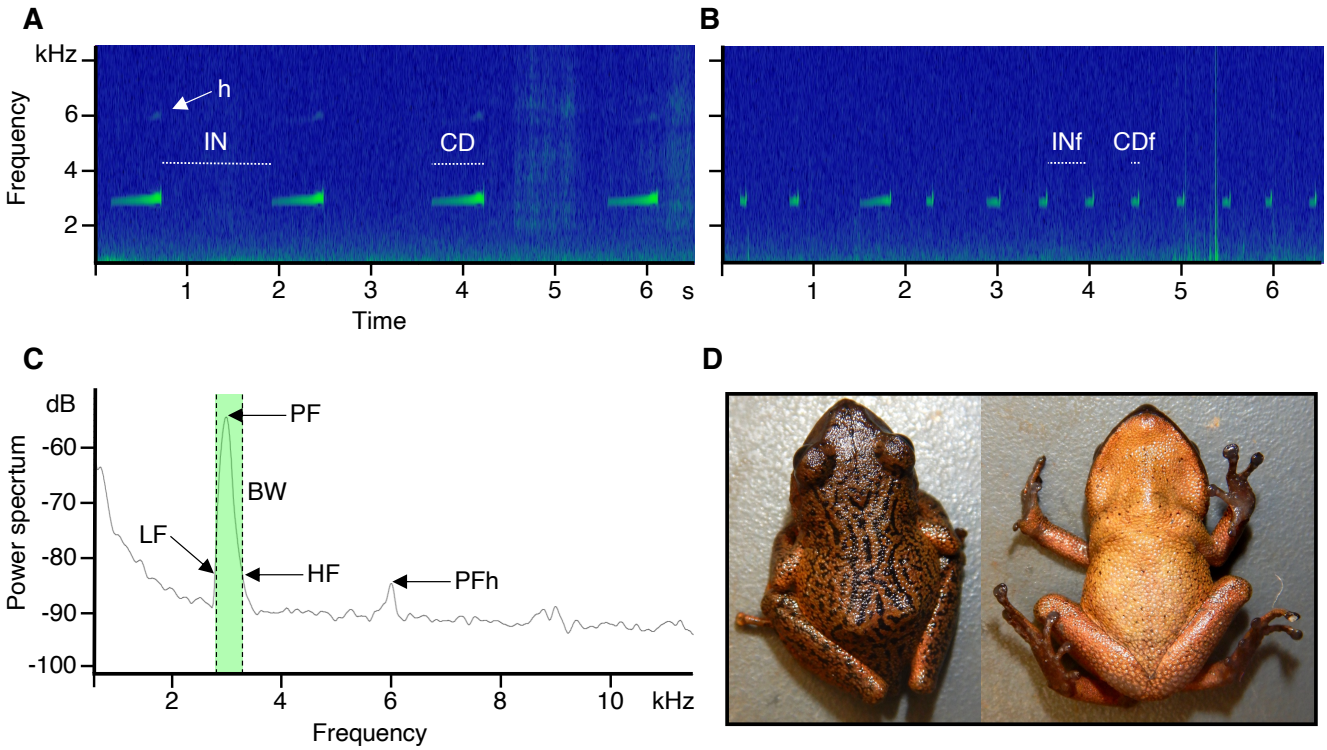


Figure 10. Call features of *Pristimantis leopardus.* The image description is shown in the manuscript attached below (Appendices B).

We also record more amphibian species in our samplings (Fig. 12). Within these several threatened species and even a significant number of salamanders (>20). Which suggests a strong conservation strategy for this habitat given the importance it represents for many more threatened species.

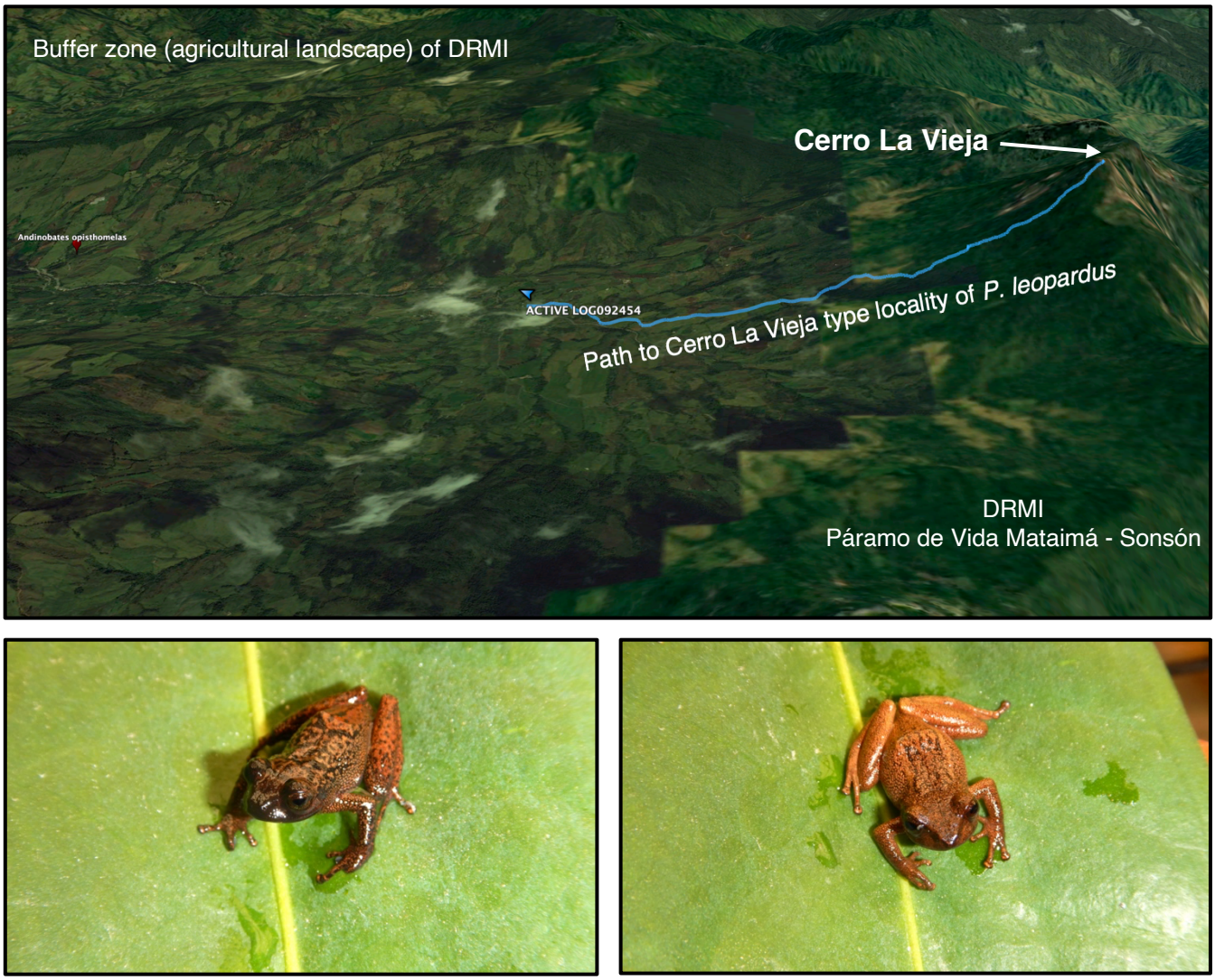


Figure 11. Landscape features of the *Pristimantis leopardus* habitat. Above, the LanSat image shows the track to access Cerro La Vieja habitat of *P. leopardus*. The approximate distance covered is 4.18 km which begins in the Manzanares Centro village at 2382 m and ends at the top of the mountain at 3243 m. The site where the population of *A. opisthomelas* was analyzed can be seen on the right of the image. Below, two *P. leopardus* males captured. This species has only been recorded at the top of this mountain and at Cerro de las Palomas above 3000 m. To access both locations it is impossible to use horses to carry equipment and food due to the features of the landscape and the path.



Figure 12. Other amphibian species recorded in the Páramo de Sonsón. Above, *Pristimantis parectatus* (EN), *Pristimantis scoloblepharus* (EN), and *Anolis marmoratus* (LC), respectively. Middle, *Pristimantis permixtus* (LC), *Bolitoglossa aff ramosi* (LC), and *Bolitoglossa aff vallecula* (LC), respectively. Below, *Pristimantis aff bernali* (CR), *Rhinella ruizi* (EN), and *Rhinella aff ruizi* juvenile (EN), respectively. In total we recorded 33 salamanders, 40 other *Pristimantis* species, and 3 *Rhinella ruizi*.

Although the population analysis and the recording of different ecological and phenotypic data were contemplated to be carried out with the three focal species, these were only consistent for *A. opisthomelas*. Regarding *H. antioquia* during the first four field trips (12 sampling events), data from 10 individuals (Fig. 13) were recorded. However, on the fifth field trip, illegal logging (Fig. 14) was observed in the habitat where this species was being recorded, due to which four data loggers were lost and data on this species were not recorded again, suggesting a possible local extinction of this population of *H. antioquia*. However, calls were heard in other small streams that were recorded and reported to expand the distribution of this species and to create a future SDM. Our unfortunate results to evaluate the population of this species suggest that perhaps an automatic acoustic recording method in several streams along the Páramo de Sonsón could be a better strategy for monitoring and determining the population dynamics for this species, avoiding risk for researchers.



Figure 13. Capture-mark-recapture records of *Hyloscirtus antioquia*. From left to right, the first three photos show the same male capture and the last photo shows a different male.



Figure 14. Habitat loss for *Hyloscirtus antioquia*. The figure shows two small streams that were illegal clearing between July and August (2021), in these streams four temperature data loggers to record environmental temperature for *H. antioquia* were lost. Red arrows indicate the small streams. The photos below represent the same stream. Calls were not heard again on these small streams.

2. Develop spatial distribution models based on published information (museums, journals, WorldClim data), and our environmental and distribution data that will be collected during the investigation.

The spatial model of distribution for the *A. opisthomelas* species shows a large potential area for this species. However, this area has historically been transformed into an agricultural and livestock matrix (Amézquita et al. 2013), which implies that its populations are probably highly fragmented. Our SDM

shows *A. opisthomelas* in Sonsón town is at the limits of the potential distribution due to poor habitat suitability (Fig. 15). These results may have strong evolutionary implications since strong local adaptation is expected in these populations that move away from the center of their distribution range (Sexton et al. 2009). Although we hoped to be able to use our local temperature data to improve the SDM of the species, this option was not possible due to the poor representativeness of our data in the distribution of the species. However, we have recorded important data on the temperatures of the microenvironment used by *A. opisthomelas* and *H. antioquia* (Fig. 16, 17). Furthermore, we are currently monitoring temperatures for *P. leopardus*. With this objective we hope to be able to determine in the medium (5 years) and long term (10 years) how much these temperatures have varied for the species that inhabit there.

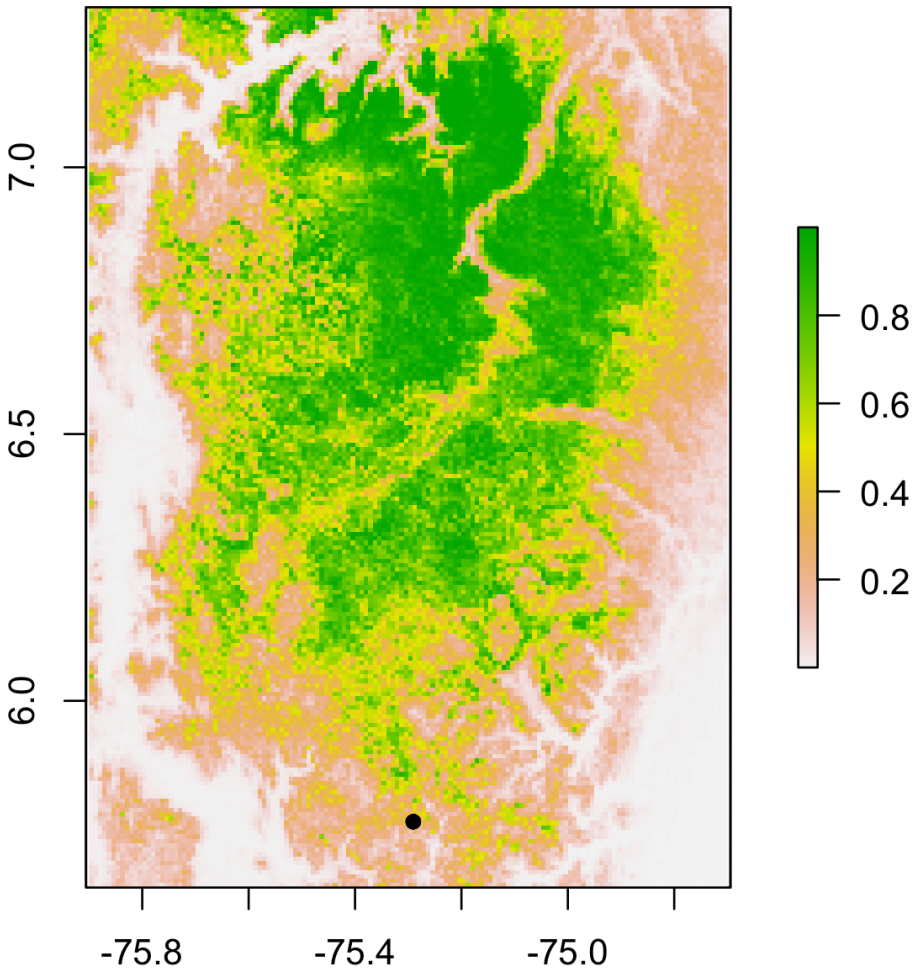


Figure 15. Spatial Distribution Model for *Andinobates opisthomelas*. Black point indicates the locality in Sonsón (Tasajo village). The image shows that the Tasajo population in Sonsón is at the limit of the suitable habitat for *A. opisthomelas*. Every pixel indicates 1 km^2. The scale shows the suitable habitat.

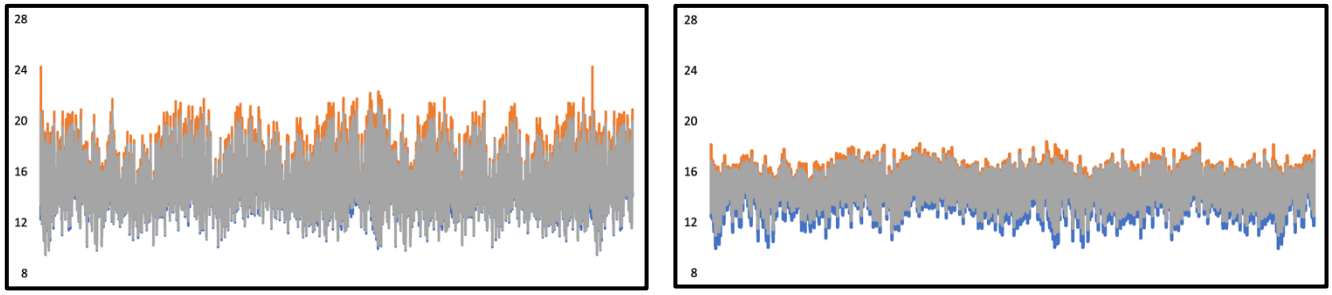


Figure 16. Microenvironmental temperatures of *Andinobates opisthomelas*. Left, temperatures recorded around 1.5 meters above ground. Right, temperatures recorded within three burrows inhabit by *A. opisthomelas* frogs. The HOBO MX2201 data loggers recorded 8563 hours. Both scales are standardized. Records were from 14 January 2021 to 7 January 2022. Orange, gray, and blue were the maximum, average, and minimum temperatures recorded of each hour respectively. Maximum temperature recorded on left was 24.6 (11/29/21, 14:00), with 15.2 in average, and 9.5ºC (1/17/21, 7:00) of minimal; and maximum temperature recorded on right was 18.4 (7/31/21, 13:00), with 14.2 in average, and 9.8ºC (12/2/21, 4:00) of minimal.

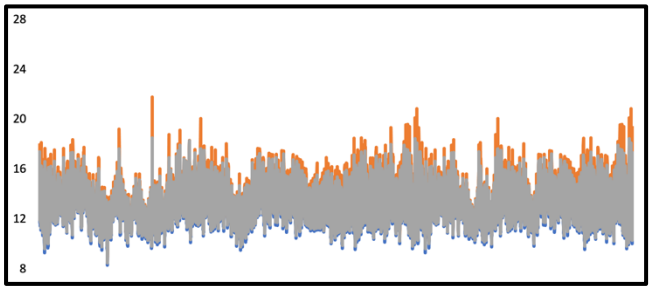
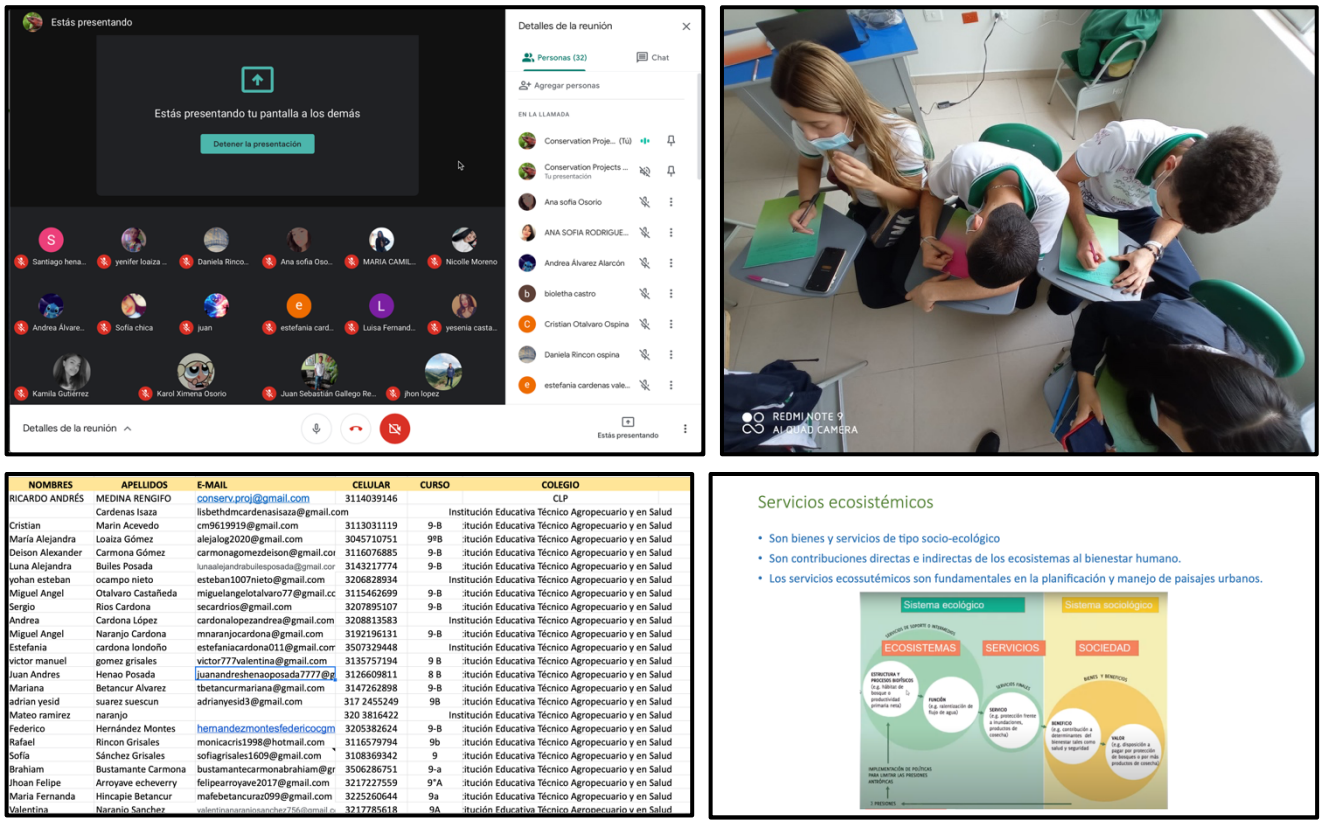


Figure 17. Microenvironmental temperatures of *Hyloscirtus antioquia*. Temperatures recorded around 1.5 meters above ground. The image shows 7720 hours recorded of two HOBO MX2201 data loggers. Four dataloggers were lost due to illegal clearing of the forest. Records were from 14 January to 8 August 2021. Orange, gray, and blue were the maximum, average, and minimum temperatures recorded of each hour respectively. Maximum temperature recorded was 21.8 (3/16/2021, 11:00), with 13.1 in average, and 8.3ºC (2/20/21, 7:00) of minimal.

3. Promote environmental education for the protection, monitoring, and management of the protection area and involve the students in scientific activities for they can monitor the threatened species.

Initially, this project contemplated the support in the field activities of the student population as a strategy of conservation and appropriation of the project. However, due to the COVID-19 emergency, this activity had to be modified. Even today, access to educational facilities by external agents not necessary to educational establishments is not allowed. For this reason, only virtual activities were done with more than 170 students from two schools (Fig. 18). Although during the field trip in November 2021, it was possible to made surveys on the perception of threats and knowledge of the species in two rural villages (Vereda Tasajo, habitat of *A. opisthomelas*; and Vereda la Paloma, habitat of *H. antioquia*)

with students who were passing through and local people (Fig. 19). In total 63 surveys were made and these were tabulated for the creation of the management plan.



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Figure 18. Environmental education virtual workshop and school activities. The initial proposal included teaching environmental workshops in small rural schools and institutions in Sonsón town, and working with small groups of students for field trips. However, due to COVID-19 emergency, in-person activities were not able in these institutions. This prevented involving students in field trips and giving workshops in rural schools due to low internet coverage. Nonetheless, virtual workshops as shown above were made with more than 170 students (right; above and below). Also, thanks to the support of teacher Luis Eduardo Díaz, professor of Science at the IETAS school, some tests (right image) were done to determine the students' perception of the amphibian species of the Páramo. The image below shows a slide of the ecosystem services workshop.







Figure 19. Threat perception survey and knowledge of the Páramo de Sonsón amphibian species. 63 surveys were developed in the villages of Tasajo (top; N 5.77214, W -75.29294), La Paloma (right; N 5.69596, W -75.23041), and Manzanares Centro (bottom; N 5.76823, W -75.26205).

Based on our environmental education workshops an important behavior change was done. We achieve the concession by the Mr. Alejandro Osa, owner of the patch where *A. opisthomelas* was studied, to put a fence in this area and prevent the entry of cattle and the clearing of the forest for the wood (Fig. 20). However, in the future, it is expected to increase this area and be able to connect it with other adjacent fragments.



Figure 20. Habitat loss for *Andinobates opisthomelas***.** Above, the intervention made in June (2021) to create a road and space to build a small room for avocado storage. The red arrow (above) indicates the area where a barrier was placed (below photo) to protect the small forest fragment where *A. opisthomelas* inhabits, preventing the entry of people, animals, and cattle.

4. Develop the second diversity meeting to involve stakeholders and together design the action plan for the focal species and the protected area.

We developed the Second biodiversity meeting of Sonsón on June 1, 2, and 3, 2021. This was successful due to the great reception by different stakeholders such as CORNARE (the regional environmental agency), the Universidad de Antioquia, and different universities and institutes from Colombia and international (Fig. 21). The topics were pertinent with information that was even relevant to Sonsón, such as the presence of invasive species and their management in the municipality. Although one purpose was to be able to institutionalize this event in order to continue it in the coming years, there was little interest from the Sonsón mayor's office. It is likely that there will be strong pressure against any project or initiative that seeks to conserve the Paramo de Sonsón and the DRMI, given the strong growth of the economy as a result of agricultural activities such as avocado crops. Although the

agricultural tradition of Sonsón has not been the avocado, since its entry into the municipality its growth has been exponential and its consequences for the conservation of the DRMI have not yet been evaluated. This topic will be deepened in the management plan. However, we believe that the lack of interest in maintaining biodiversity meetings may be due in part to the reluctance of farmers, avocado entrepreneurs, and even the mayor's office itself, which sees this activity as an engine of development.

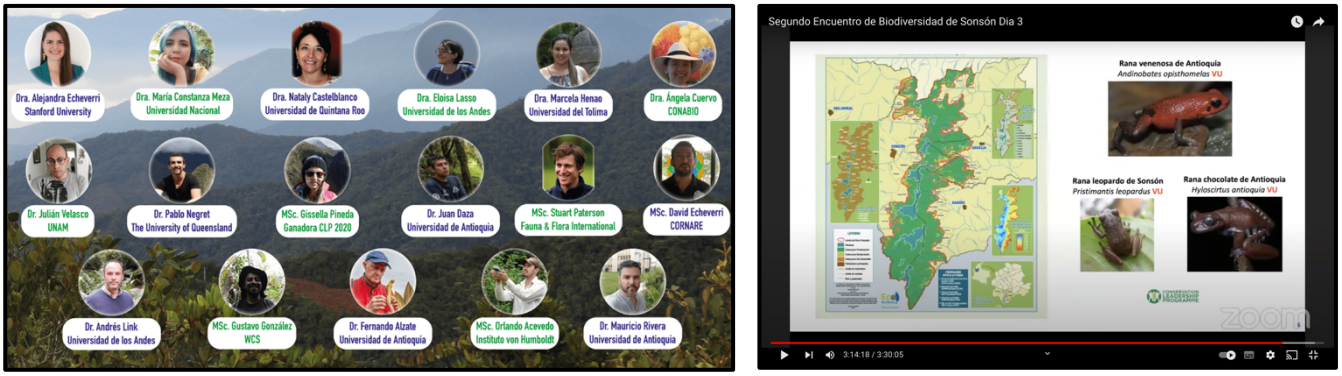


Figure 21. Second biodiversity meeting of Sonsón. Announcement images used to promote the second biodiversity meeting of Sonsón. Left, the poster of invited panelists. Right, our speak about the CLP project(https://www.youtube.com/watch?v=D1z8CLABq1s&t=11175s). The programming of the second biodiversity meeting of Sonsón, this is on the website of the regional environmental agency CORNARE (https://www.cornare.gov.co/evento/segundo-encuentro-de-biodiversidad-de-sonson/).

Communication & application of results

We have only communicated the results through the Second biodiversity meeting of Sonsón and partial (https://www.youtube.com/watch?v=D1z8CLABq1s&t=11175s) results during our workshops with educational institutions (Fig. 18). By the end of October, we will deliver our management plan to CORNARE and CLP, and stickers will be delivered to the schools. We are designing stickers with QR code (Fig. 22) so that they allow easy access to the management plan and thus have a better spreading of it. For this activity, we will invest the last money saved. Moreover, we hope to be able to participate in some congresses in the future to socialize the results. Finally, the scientific publications are in progress, with one accepted, another under review, and another in preparation (see Appendices).



Figure 22. Stickers designed with threatened frog species. Left, *Pristimantis leop*ardus (VU), Center, *Hyloscirtus antioquia* (VU), and Right, *Andinobates opisthomelas* (VU). The QR code is an example, but it will allow quick access to the management plan for threatened amphibian species in the DRMI. For this, we hope to be able to use the CLP platform to store the management plan and through the QR of the stickers, people can download this document.

Monitoring and evaluation

We were only able to evaluate the environmental education workshops at the IETAS institution, thanks to the support of science teacher Luis Eduardo Díaz, who made tests and gave us their results (Fig. 18). With this evaluation it was verified that the students understood the topics. Our results quickly indicated that it was not going to be possible to analyze the population of the species *P. leopardus* (Fig. 11) and *H. antioquia* due to the difficult access and strong weather conditions in which both species inhabit. Thus, our plan moved to record all amphibian species that could be found in the habitat of *H. antioquia* (Fig. 12).

Achievements and impacts

1. A spatial distribution model was developed for the species *Andinobates opisthomelas* (Fig. 15). This has made it possible to observe that the populations of this species in Sonsón are really at the limit of the suitable habitat.
2. A good number of phenotypic and environmental data have been recorded for the species *A. opisthomelas, H. antioquia*, *P. leopardus*, and other non-focal species observed (Fig. 6, 7, 8, 16, 17;

Table 1; Appendices) This work will contribute like few others with microenvironmental information not evaluated in amphibians in Colombia, as well as a robust phenotypic database.

3. An excellent Second biodiversity meeting of Sonsón was held (Fig. 21) that allowed us to look again at the biodiversity of Sonsón.
4. It has been possible to register other threatened species with phenotypic and environmental data (Fig. 9, 12). This will allow us to contribute new reports and unknown information on the species.
5. It has been possible to record calls of the three focal species *A. opisthomelas, P. leopardus,* and *H. antioquia* (Table 1; Fig. 5; Appendices B). We were recently invited to collaborate with a review of the calls of *Andinobates* thanks to our experience in bioacoustics and the records we have made of several species of *Andinobates*.
6. The call description of the species *P. leopardus* is currently in review by Zootaxa journal (Appendices B).
7. The defensive behavior of *H. antioquia* was accepted to publication in the Revista Latinoamericana de Herpetología journal (Appendices A).
8. We have data about the spatial distribution and how *A. opisthomelas* uses the habitat (Fig. 4). In addition, we have been able to observe and help prevent habitat loss by committing farm owners to fence the small remaining forest and (Fig. 20).
9. We have good data to make a population analysis of *A. opisthomelas* (Fig 2, 3, 4, 6, 7, 15).
10. We have been found that there are differences in how *A. opistho*melas uses the microenvironment (Fig. 16).
11. We have left four data loggers to record the temperature of the microhabitat of *P. leopardus*.
12. We have verified that there is strong anthropic pressure due to changes in land use on the population of *A. opisthomelas* (Fig. 1), which contrasts with *H. anti*oquia, which is very rare but its habitat is very little intervened.
13. Despite the logistical problem of not being able to do in-person environmental education workshops, we had a good reception from teachers and students (Fig. 18, 19).
14. We estimate to be able to publish at least three scientific publications, the population analysis of *A. opisthomelas,* the revision of Andinobates calls, and the spatial distribution model with data on microenvironmental and body temperatures. Moreover, the management plan that is our main result will be able by the end of October (2022) and will be shared through CLP site.
15. We were able to fence the habitat where we evaluated the population of *A. opisthomelas* (Fig. 20). This at least ensures that there will be no access by livestock or people. It also avoids clearing for the use of wood.

Capacity development and leadership capabilities

This project showed us that we have good leadership skills and a call to work as a team. We are good at writing and reporting findings in scientific journals. We know how to take advantage of the opportunities that present themselves through the difficulties of the initial plans. We are very good at explaining complex topics to people with little academic knowledge such as rural people and first-year students. We have good physical condition and easily accommodate intense field work and adverse conditions. We have now the ability to identify two more threatened species (*P. parectatus* and *P. scoloblepharus*) (Fig. 12). We were able to create a good relationship of trust and work with the staff of CORNARE and the University of Antioquia - Sonsón.

For me, it was important to lead this work, despite the fact that all our plans were accommodated due to the unexpected COVID-19, I think that together with my colleagues we made the best decisions to do good fieldwork. I learned a lot about leadership and organization, I was able to do very good contacts with other researchers and broaden my research perspectives. In addition, Sonsón is always a wonderful place to work and enjoy. I would have loved to be able to do much more important work with the student population and locals to create better awareness about amphibian conservation. Sonsón has great potential. I hope to return soon to work more with Sonsón people. **Giss**

For me as a herpetologist, being able to register threatened species and work on their conservation was very important. Thanks to the CLP workshops I am sure that I can do a better job. Also, DRMI is an area that deserves a lot of work given the number of threatened species, which I hope to be able to do in the future. I think one strength I brought to the team was the ability to analyze data and write, **Sigi**

For me, returning to Sonsón has always been important given the potential this area has for working on research and conservation. This time it was more challenging since we were not able to have much contact with people and students, which always improves conservation projects. Unlike my teammates, the species I worked with was much easier to sample, although the threats to it are much more observable given the extreme loss of habitat where it occurs. On the other hand, I think that my greatest growth in this project was the ability to convene and create collaboration networks with other researchers, stakeholders, and local people. **Ricardo**

The experience lived as a field assistant in the project allowed me to learn a little more about the importance of the conservation of anuran species in the country, and also allowed me to learn about the richness and biodiversity of this area, both due to the geographical conditions and the fauna present there and also due to the cultural diversity of Sonsón, knowledge allows us to conserve, and said knowledge must be freely accessible, especially for the locals. Finally, I was able to do my first scientific publication on the defense behavior of Hyloscirtus antioquia. **Bibiana**

I am a botanist from Sonsón and I work there as a field guide. However, I had the opportunity to accompany some field trips and finally, we were able to record the call of the Sonsón leopard frog species. Learning about bioacoustics and how difficult it was to record the species seemed very interesting to me. The habitat of this species is at the top of the highest mountains where it is necessary to walk for about 4 and 7 hours. On the last field trip, it rained very hard throughout the night, but when it did not rain, we were able to go out to record the call. I'm still working at Sonsón and I've been taking data from the data loggers that are still here. I also had the fortune to participate in my first publication, thanks to the team. **Juan Pablo**

For me, it was a great experience to work in the field and learn about threatened species that I did not know and the field methodologies. As an anecdote, I got sick with COVID-19 during a field trip and we had to leave late at night from the field to a hotel in Sonsón. I will always remember Sonsón for the pretty cool species, landscapes, cold, and for the good feeling of the people. The work in the field was challenging, due to the rains, but I would return to Sonsón. **David**

Conclusion

All our objectives were partially met. First, we were able to record the three focal species, this is important given that they are threatened species (Fig. 3, 11, 13). In addition, we were able to understand a little better the ecology and behavior of the species. For example, the habitat of *P. leopardus* and *H. antioquia* is quite well preserved and is very large (Fig. 1), but the species are difficult to record and they don't seem to be very abundant. The species *P. leopardus* is very difficult to sample due to the restricted habitat (top of mountains above 3000 m), the difficult path (Fig. 11), and weather conditions. It is very difficult to estimate with our observations whether it is abundant or not. Unlike the agricultural matrix and the highly fragmented patches inhabited by *A. opisthomelas* (Fig. 1), but with a much easier population to sample and with more individuals (Fig. 3) than those recorded for the other focal species. However, with a recovery rate of 22% and just over 130 individuals recorded, it can be estimated that the population of *A. opisthomelas* may be quite small. Thus, connectivity measures for the populations of *A. opisthomelas* are urgent, due to the strong loss of habitat and because the population recorded in this study is outside the protected area of the DRMI (Fig. 1, 20). Likewise, we were also able to record the calls of the three species and even described the call of *P. leopardus* (Appendices B).

The few historical records and our observations for the species *P. leopardus* and *H. antioquia* did not allow us to create Spatial Distribution Models (SDM) for these species. Unlike *A. opisthomelas*, which has a very wide habitat potential on the Cordillera Central (Fig. 15). However, our results indicate that the populations of *A. opisthomelas* that inhabit Sonsón are at the limit of the suitable habitat (Fig. 15). Fine microenvironmental data are scarce for threatened or non-threatened species, here we highlight that our data indicate that the microhabitat of *A. opisthomelas* (burrows) is a temperature buffer that reduces maximum temperatures by up to 6 degrees (max temp burrows = 18.4ºC; 1.5 m above ground 24.6ºC) (Fig. 16), while the minimum temperatures were practically the same (min temp burrows = 10ºC; 1.5 m above ground 9.5ºC), with an average body temperature of 16.4ºC (± 1.5ºC) (Fig. 7). The microenvironmental results indicate that the use of the habitat in *A. opisthomelas* is important when it comes to compensating for the temperature changes that can be recorded in the habitat, more so as this is a diurnal species. Despite the wide suitable habitat estimated in *A. opisthomelas*, if the populations are connected to each other, is a question to be resolved in the future. Furthermore, the combination of the SDM and microenvironmental results, together with genomic analysis will allow us in the future to understand the adaptive capacity and response to climate change in *A. opisthomelas*. On the other hand, the maximum and minimum temperatures of the microenvironment for *H. antioquia* were 21.8 and 8.3ºC (Fig. 17), respectively, while the average body temperature was 15.1ºC (±1.9). In *H. antioquia*, the limitation may be given more by the minimum temperatures, since this species is nocturnal and as an ectotherm, it depends on the environmental temperature to regulate metabolism. Our observations are contradictory since the conditions in which *H. antioquia* presented the most vocal activity were under heavy rain and fog, which implies lower temperatures and therefore a challenge for the thermoregulation of this species. Unfortunately, only a few body temperature records were obtained from *P. leopardus* with an average of 9.7ºC (±0.7), while microenvironmental temperature records were only taken at the end of the field trips, so these results will be known later.

We consider that COVID-19 did not have a significant impact on field trips, but it had on communication and conservation activities, and on increased transportation costs. We do not believe that the record of the species could have improved if the pandemic had not occurred, instead, we believe that perhaps

other methodologies such as automatic recording systems for species such as *P. leopardus* and *H. antioquia* can be much more cost-effective and avoid exposing researchers to environmental conditions (heavy rains, landslides, rising streams) that can be difficult and risky. On the other hand, COVID-19 had a strong impact on the environmental strategy, communication, and in general the socialization of the project. The Second Biodiversity Meeting of Sonsón (Fig. 21), the environmental workshops with students (Fig. 18), and the meetings with stakeholders such as CORNARE and the Universidad de Antioquia were virtual. However, there was a genuine interest on their part to work on the conservation of DRMI and its species and the students were very receptive to conservation issues.

It is clear that the DRMI is a biodiversity hotspot and represents a fundamental area for the conservation of many threatened amphibian species, as well as other vertebrates, plants, insects, microorganisms, and ecosystem services. Nonetheless, despite being in a protected area, the threats to the amphibian species are still persistent (Fig. 1, 4, 14, 20). Moreover, other threats that are not so easy to determine, such as the effects of agrochemicals, introduced invasive species such as rainbow trout, climate change, and the presence of pathogens such as *Batrachochytrium dendrobatidis*, should be a priority in future research.

Problems encountered and lessons learnt

- **Which project activities and outcomes went well and why?**

The population evaluation of the species *A. opisthomelas* has gone very well, and all the associated data (phenotypic, ecological, spatial) are quite robust. This species is diurnal, very active (constantly singing, even on warm days), and despite the strong reduction of the habitat, it is abundant. Thus, the biology and ecology of the species have made its study favorable. Furthermore, as it is diurnal and is not associated with streams, it is possible to sample it even on days of heavy rain.

The development of the virtual workshops has been possible only with the students of the town because the rural schools do not have internet connection. However, there has been a positive response from both teachers and students, providing the spaces and participating actively. The reason that this objective has worked is perhaps due to the good relationships we have with some teachers, which allows us to have the spaces to work.

The spatial distribution model for *A. opisthomelas* has worked quite well. This is explained because there is a good record in databases and museums. Currently, we are also making a model for *H. antioquia* since there are already more reports in GBIF and we have also added some resolution grids that may allow us to make a good inference.

Microenvironmental monitoring for *A. opisthomelas* and *H. antioquia* is going quite well, despite the loss of some data loggers. We have even been able to find very interesting results for the species *A. opisthomelas*. These good results are due to the fact that we have bought very good quality equipment, we also take advantage of the ecology of the species to test hypotheses and not only record descriptive data. Furthermore, since December 2021 we are recording temperature data with the same methodology of *A. opisthomelas* for *P. leopardus*.

The Second biodiversity meeting of Sonsón was a success because we were very aware of the entire process. In addition, we take advantage of the experience of CORNARE and the Universidad de Antioquia staff in organizing virtual events and managing platforms. We also take advantage of our good relationships with some researchers who supported us even inviting researchers from other countries. Thus, we transform a possible disadvantage into an advantage.

Regarding the publication of results, one article was accepted and another is under review. We still have information to publish perhaps three more articles. This is because despite the fact that our objectives were not fully met, that is, the three species could not be analyzed, we have taken full advantage of any other data that we have recorded of the focal species and other species found.

- **Which project activities and outcomes have been problematic and in what way, and how has this been overcome?**

The activities associated with the species *H. antioquia* and *P. leopardus* have been quite problematic. This is because the species are not so common, they are nocturnal and in the case of *H. antioquia* it occurs in streams that are very difficult and risky to sample at night. Likewise, *P. leopardus* is only found on the top of the mountains and access is difficult with more than four hours walking, without the possibility of carrying equipment on horseback, and the area to camping at night for several days is very irregular due to the thick presence of Páramo plants as *Espeletia restricta* (big monks endemics from Sonsón). Furthermore, these two species inhabit in an area where it rains quite often or there is thick fog.

The workshop to establish the management plan for the focal species could not be held either because the meeting format was made virtual. However, on the advice of WCS officials, we try to build this management plan under Open Standards for the Practice of Conservation with the communities surrounding the study areas and the Páramo de Sonsón. This methodology did not work out very well either because it was not possible to hold group meetings in different villages. We were only able to access students and locals who were passing through, for this reason only 63 surveys could be carried out.

The Páramo de Sonsón is an area with a high number of threatened and little-studied species. We have taken advantage of the unsuccessful field trips for *H. antioquia* to record other species, thus we have been able to find a significant number of Salamanders (33), *Pristimantis* genus frogs (40), and *Rhinella* (3) individuals. Some of them are listed as threatened.

- **Briefly assess the specific project methodologies and conservation tools used.**

Although our non-invasive methodology was initially reticent, our observations and results indicate that it is possible to capture-mark-recapture (CMR) these species using only coloration patterns. However, in future studies, we would not recommend the use of traditional CMR with *P. leopardus* or *H. antioquia* based on our results. At least for the DRMI area, the access and weather conditions are very difficult and risky for researchers. Nonetheless, other research and conservation methodologies, questions, or problems should be tested.

We did not have any problems with the equipment in its operation, they showed good performance in the field and the use of waterproof cameras was fundamental because during almost all field trips there was rain or fog. Regarding the calling recording equipment, this worked perfectly. The calls were analyzed and we hope to publish the call of *P. leopardus* soon (currently under review in Zootaxa, Appendices B).

Perhaps we should have used more media such as radio or even local television channels, made videos, or podcasts to better explain the project, and held different environmental education workshops in response to the COVID-19 restrictions. However, we believe that in the future everything learned can be improved in a more effective strategy of mass disclosure.

- **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.**

We have learned positive lessons such as the organization and development of virtual meetings with researchers. We have also been very organized with data collection and equipment management so we have very safe and relevant information.

We know that making a previous field trip to recognize if the species are abundant can be fundamental. Although we had previously observed all three species, our observations had been sporadic. However, for a systematic work such as a population analysis, a more effective methodology, even changing the focal species for other more abundant. For example, while trying to analyze the population of *H. antioquia*, we were able to record a large number of salamanders and *P. scoloblepharus* which are endangered.

Some species present really great challenges due to their ecology and biology. Furthermore, the weather conditions of the Páramo de Sonsón at night are generally dominated by rain and fog, even in the dry season. This creates a quite risky scenario to work with, perhaps a passive acoustic monitoring methodology should be implemented in future studies with species such as *P. leopardus* and *H. antioquia* and thus reduce the risk both for investigators and to avoid the damage or loss of equipment.

We have seen that there has been significant development and progress in Sonsón town. This is mainly due to the change in the agricultural tradition. Currently, big areas of avocado culture have been implemented, generating better income for farmers. Furthermore, big international companies have bought land and made strong investments in the municipality. Hence, we believe that there is a clear lack of interest on the part of the town administration to support research and conservation activities. It has been very difficult to get support from the local administration. This scenario is worrying because it exerts great pressure on the protected area with the apparent complicity of local authorities.

In the near future, questions should be resolved about How agrochemicals may be acting on the populations of amphibians threatened in the DRMI? *A. opisthomelas* is a widely reported species in Antioquia, however, could it be a cryptic species? If so, the limits of this species and the population's genetic structure should be recognized to know if it is necessary to consider independent management

units. Does the fragmentation of the habitat of *A. opisthomelas* have an effect on the functional connectivity and gene flow of this species? And What is the adaptive evolutionary potential of the threatened amphibian species in the DRMI Páramo de Vida Mataimá - Sonsón? These are some of the urgent questions to answer in order to better direct resources and conservation efforts.

In the future

We continue to monitor temperatures and as we have proposed, we hope to do so in the medium (5 years) and long term (10 years). For this, we will be reviewing the data loggers every six months and changing the batteries. However, to continue monitoring the species and develop a connectivity program for the remaining forest patches to reduce the pressure on the *A. opisthomelas* species, much more work, and resources are needed. For now, we do not have more grants, we only have the capacity to visit the sites sporadically with our own resources. We have continued trying to obtain more resources but still without positive results. We hope to apply a follow-up to the CLP during the next round in order to continue monitoring and address new questions and resolve some conservation problems detected.

We continue writing and analyzing data to increase scientific publications and knowledge of the species and the conservation area. Finally, by the end of October, we will be delivering the management plan to CORNARE and a short visit will be made to Sonsón to deliver posters and socialize this document. A copy will also be sent to CLP. This plan is the document that summarizes the findings and directs the resources and conservation efforts for the threatened amphibian species in the DRMI Páramo de Vida Mataimá - Sonsón.

Financial report

Itemized expenses	Total CLP Requested (USD)*	Total CLP Spent (USD)	% Difference	Details & Justification (Justification must be provided if figure in column D is +/- 25%)	Proposed Spending (Preliminary Report Only)
PHASE I - PROJECT PREPARATION					
Communications (telephone/internet/postage)	200.00	200.00	0%		
Field guide books, maps, journal articles and other printed materials					
Insurance					
Visas and permits					
Team training					
Reconnaissance					
Other (Phase 1)					
EQUIPMENT					
Scientific/field equipment and supplies	3,745.00	3206.66	-14%	The soundmeter was not bought because this needs to be calibrated at each field trip and we need to buy the calibrator, this will increase the cost above $500 taking into account customs ($10%), shipment ($), and tax ($19%). In addition, we save money to pay customs, shipping, and taxes that were not accounting in the initial proposal.	
Photographic equipment	495.00	730.00	47%	We changed the brand of cameras because we need a better macro photo with low light performance. These were more expensive, but we have saved m	
Camping equipment	435.00	550.32	27%	The equipment was a little more expensive; however, we have saved money from equipment to compensate for this excess	
Boat/engine/truck (including car hire)					
Other (Equipment)					
PHASE II - IMPLEMENTATION					
Accommodation for team members and local guides	2,400.00	2824.00	18%		
Food for team members and local guides	3,360.00	4020.00	20%		
Travel and local transportation (including fuel)	2,160.00	2548.80	18%		
Customs and/or port duties		696.58		Unfortunately, this expense was not taken into account and could not be avoided. However, this value has not affected any field trips or the objectives	
Workshops	1005		-100%		
Outreach/Education activities and materials (brochures, posters, video, t-shirts, etc.)	200.00		-100%	This money plus $23.64 has not yet been used, as the stickers will be delivered together with the DRMI amphibian management plan in mid-September.	
Other (Phase 2)					
PHASE III - POST-PROJECT EXPENSES					
Administration					
Report production and results dissemination	1,000.00		-100%	This money ($984) was used for the extra cost of travel and food. The budget exceeded these expenses due to the Covid-19 emergency. Even after ma	
Other (Phase 3)					
Total	**15,000.00**	**14,776.36**			

* The money that has not yet been spent will be invested in stickers with the focal species (Fig. 22) that will be delivered at the end of October in the municipality of Sonsón, together with the management plan for the threatened amphibian species.

Appendices

Output	Number	Additional Information
Number of CLP Partner Staff involved in mentoring the Project	2	Stuart Paterson and the CLP alumni Jenny Gallo were speakers at the Second Biodiversity Meeting of Sonsón (Fig. 21)
Number of species assessments contributed to (E.g. IUCN assessments)	1	Initially, three threatened frog species (*H. antioquia, P. leopardus, and A. opisthomelas*) were going to be evaluated for their population dynamics. However, only data for *A. opisthomelas*. Although we were able to record data for the other two species that may be important for their knowledge and natural history (Fig. 2, 3, 4)
Number of site assessments contributed to (E.g. IBA assessments)	0	The Páramo de Sonsón is an IBA and AZE
Number of NGOs established	0	We are creating the statutes and legal documents to create a corporation (NGO) and constitute ourselves as a stakeholder that contributes to public policies for the conservation measures, as a consulting entity within the medium- and long-term management of the DRMI Páramo de Vida Mataimá - Sonsón
Amount of extra funding leveraged ($)	$850	We got some extra funds from IdeaWild to be able to leave some camera traps and to be able to understand which species co-occur with the

		threatened focal species and even which could be their possible predators.
Number of species discovered/rediscovered	3	We have been able to record and confirm the presence of the following three species of threatened frogs *Rhinella ruizi* (VU), *Pristimantis scoloblepharus* (EN), and *Pristimantis parectatus* (EN) (Fig. 12)
Number of sites designated as important for biodiversity (e.g. IBA/Ramsar designation)	0	The Páramo de Sonsón had already been designated as an IBA and AZE in previous years
Number of species/sites legally protected for biodiversity	0	Although in 2019 the Páramo de Sonsón was covered within the DRMI Páramo de Vida Mataimá - Sonsón, this kind of reserve allows farming, cattle, fish farming, and logging. Furthermore, the management plan created by CORNARE for this reserve did not include any amphibian species within its conservation objectives.
Number of stakeholders actively engaged in species/site conservation management	3	CORNARE, Sonsón's town hall, and the Universidad de Antioquia - Sonsón campus
Number of species/site management plans/strategies developed	1	The management plan for the fourteen threatened amphibian species within the DRMI will be able by the end of October
Number of stakeholders reached	4	CORNARE, Sonsón's town hall, the Universidad de Antioquia - Sonsón campus, and community in general and students.

Examples of stakeholder behavior change brought about by the project.	1	Due to the fact that in-person activities could not be carried out with the students, participatory monitoring could not be done, nor could the student ecological group be created. However, we were able to convince the owner of the property (Mr. Alejandro Osa) where *A. opisthomelas* inhabit to fence off this small patch. An urgent measure due the isolation of the patch, the presence of cattle, and logging.
Examples of policy change brought about by the project	0	Beyond the Second Sonsón Biodiversity Meeting, Sonsón's town hall had no participation.
Number of jobs created	0	
Number of academic papers published	1	One accepted (Appendices A), One in review (Appendices B), and two writing
Number of conferences where project results have been presented	1	Partial results were showed in the Second Biodiversity Meeting of Sonsón

DEFENSIVE BEHAVIOR OF THE ANTIOQUIA CHOCOLATE FROG (*Hyloscirtus antioquia*)

Abstract.— We present the description of defensive behavior in the Colombian chocolate frog (*Hyloscirtus antioquia*), observed in seven males who performed a change in body posture. The defensive behavior corresponded to body-contraction, chin-tucking, and eye protection (closed eyes), accompanied by skin secretions and an intense odor after they were captured and handled. Suggesting that the combination of defensive postures and skin secretions could limit predation in this species, as occurs in other anurans.

Keywords.— *Hyloscirtus*, Amphibian, Defensive behavior, Predation, Secretions, Odor, Colombia.
Resumen.— Presentamos la descripción del comportamiento defensivo de la rana chocolate Colombiana *(Hyloscirtus antioquia),* observado en siete machos quienes realizaron un cambio de su postura corporal. El comportamiento defensivo correspondió a la contracción del cuerpo, el ocultamiento del rostro y la protección de los ojos (cerrados), acompañados de secreciones cutáneas y un olor intenso después de que fueron capturados y manipulados. Esto sugiere que la combinación de posturas defensivas y secreciones cutáneas podría limitar la depredación en esta especie, como ocurre en otros anuros.
Palabras clave.— *Hyloscirtus*, Anfibios, Comportamiento defensivo, Depredación, Secreciones, Olor, Colombia.

Predation is an important force structuring communities, especially in groups of concern such as amphibians (Albecker & Vance-Chalcraft, 2015), however, there must be a balance between food searching and predation risk in spatially and temporally shifting predator environments. In order to optimize this tradeoff, prey needs to be able to display an appropriate response based on the degree of predation risk (Albecker & Vance-Chalcraft, 2015). Anurans have evolved a broad number of defensive behaviors (Wells, 2007; Toledo et al., 2011; Ferreira et al., 2019) and are used in both tadpoles and adult frogs (Toledo et al., 2011). Toledo et al. (2011) suggest that behavior defenses include at least 30 different types of strategies, while Ferreira et al. (2019) have classified antipredator behavior in 12 categories. In both reviews the contraction category is a frequent behavior, has been documented in at least 149 species of anurans, mainly from the Hylidae (N= 48) and Bufonidae (N= 25) families. Contraction behavior is described as the contraction of all limbs and arching of the body and is usually associated with ventral flexion of the head, eyes either opened or closed and skin secretions (Toledo et al., 2011; Ferreira et al., 2019).

Based on above, we describe the post-metamorphic defensive behavior of the Antioquia chocolate frog (*Hyloscirtus antioquia),* a Vulnerable species (IUCN, 2018) distributed in the northern sections of the Cordillera Central of Colombia (Rivera-Correa & Faivovich, 2013). Its habitat is the cloud forest of the high mountains where they inhabit in or alongside streams and puddles or wetlands perched up to 3 meters high. Their elevation range consists of the upper limit at 3,200 meters and the lower limit at 2,500 meters (Rivera-Correa & Faivovich, 2013). This species has been only recorded in the Departamento de Antioquia on the Cordillera Central and it is probably the southern geographic limits are the Arma and Samaná rivers canyons. The genus *Hyloscirtus* contains 38 species of stream-dwelling treefrogs divided into the *H. armatus, H. bogotensis*, and *H. larinopygion* groups, and only the defensive behavior of *H. larinopygion*, the sister species of *H. antioquia*, has been reported (Duarte-Marín et al., 2019).

We documented the defensive behavior displayed by *Hyloscirtus antioquia* in seven individuals between 6:00 p.m. and 11:00 p.m. in the months of November (2020), January, March, August and November (2021) in two localities (N 5.7006, W -75.25082, 2742 m and N 5.6923, -75.23752, 2559 m, WGS84) of the Páramo de Sonsón (Sonsón, Antioquia). The individuals were found calling on the vegetation, captured and handled to be measured, weighed, photographed, the temperature was taken, and released in the same place.

The frogs had a snout-vent length (SVL) mean of 58.09 ± SD, 3.14 mm, a weight mean of 12.37 ± 0.61 g, a perch temperature mean 13.1ºC ± 0.75ºC, and body temperature mean 15.3ºC ± 2.68ºC. As a result of the capture and manipulation, all frogs remained motionless in the following posture for approximately five minutes even after being released on the ground. Individuals folded their limbs as close to their body, curled their body up and tucked their head between their forelimbs, keeping their snout pointed towards the ground and their eyes closed (Fig. 1A-B). The stance is similar to that described by Toledo et al. (2011) and Ferreira et al. (2019) on body contraction, chin-tucking, and eye protection. Duarte-Marín et al., (2019) reported body contraction in *H. larinopygion*, the sister species of *H. antioquia*. However, this posture differed between species, with a notable body arching observed in the Antioquia chocolate frog. These differences could be associated with the type of stimulus, the frequency, and the duration of the stimulus (Williams et al., 2000). The frogs emitted an intense odor when captured, even in the absence of defensive behavior, shared strategy with *H. larinopygion* (Duarte-Marín et al., 2019). Likewise, we observed the presence of a whitish viscous secretion, which caused the gloves to stick (Fig. 1C). Both the smell and the secretion had been reported by Rivera-Correa & Faivovich (2013) in the description of the species. They even indicate that the secretion is bitter, although we have no way to support this, they also describe a body-color change that was not observed by us. We also did not hear any type of distress call as has been recorded in the sister species *Hyloscirtus larinopygion* (Duarte-Marín et al., 2019).

On the basis of our observations, we encourage future studies to reveal if the behavior of *H. antioquia* in conjunction with the presence of the secretion and the odor really discourages potential predators. Furthermore, if these compounds can have some effect on microbiota, pathogens, and specifically on *Batrachochytrium dendrobatidis*, and what is the effect of this odor on other conspecifics and heterospecifics? We expect that a large number of future questions in chemical ecology and behavioral ecology will be resolved in the near future. However, unfortunately for future studies of this species, the second locality reported in this study was deforested (Fig. 1D) and frogs have not been recorded again on this small stream, which shows why this species is under threat.

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Figure 1. (A-B) Defensive behavior of *Hyloscirtus antioquia*. (C) On the gloves, it can see the whitish and sticky secretion produced by this species when captured. (D) habitat loss by wood clearance in the second locality.

Appendices B – Second manuscript in review by Zootaxa

The advertisement call of the threatened frog *Pristimantis leopardus* (Anura: Strabomantidae)

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The advertisement call in frogs is a behavioral mechanism that transmits information about species identity, position, body size, and sexual receptivity for hetero- and con-specifics (Wells & Scwartz 2007). Therefore, it is a fundamental trait to understand the identity of the species and works as a particular trait in its taxonomy, given its strong implication in speciation (Gerhardt 1994; Vences & Wake 2007; Wells 2007).

Pristimantis leopardus (Rivera-Correa *et al.* 2017) is a polymorphic Andean frog recently described that inhabits the Central Cordillera in Colombia. Despite this species is found within the Páramo de Vida Maitamá - Sonsón, an area of 406.47 km^2 with special conservation management (CORNARE, 2021), *P. leopardus* only occurs in two localities, the tips of the Cerro la Vieja and Cerro las Palomas in the Sonsón town (Antioquia, Colombia) (Rivera-Correa *et al.* 2017). Thus, this species was categorized as Vulnerable due to restricted area of occupancy (IUCN 2019) and since its description in 2017 very little about ecology, behavior, evolution, or distribution is known.

In a recent expedition on December 6 2021 in Sonsón town (Colombia) specifically at the Cerro la Vieja locality, we heard and recorded calls of *P. leopardus*. The individuals were detected by acoustic and visual encounters between 18:00 and 7:30 hours in a small Páramo patch (N 5.77333, W -75.23242; 3140 m) surrounded by plants, grass and trees below 1.6 m high of genus *Symbolanthus*, *Guzmania*, *Bejaria*, *Calamagrostis*, *Puya*, *Paepalanthus*, *Gaultheria*, *Badilloa*, and *Espeletia*. Although the locality was visited several times, only during heavy rains have we been able to hear and record the call of *P. leopardus*. Furthermore, under heavy fog even at noon, this frog is probably to be heard (JPC personal observations). However, we cannot rule out that this species has seasonal activity or that it only has a vocal behavior under particular weather conditions (*i.e.*, heavy rains and fog).

Fieldwork was carried out in strict adherence to the guidelines for work with amphibians and reptiles (Beaupre *et al.* 2004) and a spontaneous advertisement call of one male adult of *P. leopardus* was recorded with an Audio-Technica AT897 microphone coupled to a boompole K-Tek KE-89CC in a ZOOM H6 recorder positioned at approximately 70 cm directly in front of male calling. Due to temperature dependence for metabolic regulation in ectotherms, we also recorded air, substrate, and body temperature with a Hygro-Thermometer RH101 Extech IR, and the record was filtered from apparent conspecific interactions considering that anurans can adjust their call features based on environmental factors (Ey & Fischer 2009) and social context (Wells 2007). Raven Pro v1.6.3 (Center for Conservation Bioacoustics, 2014) was used for acoustic analyses with 44.1 kHz and 16 bits. The spectral parameters were analyzed with a Fast Fourier transformation under the Blackman algorithm with 724 samples by window, a frequency grid with 2,048 samples using the discrete Fourier transform algorithm (DFT), and default values for other parameters. Call parameters measured were: call duration (CD), inter-note interval duration (IN), peak frequency (PF), lowest frequency (LF), highest frequency (HF), bandwidth (BW), and bandwidth 90% (BW90). All spectral parameters were measured 25

decibels below peak frequency. Due to the call of *P. leopardus* change over time, we analyze the parameters with 10 calls at the beginning of the record and ten calls at the final of the record (parameters analyzed were CDf, INf, and PFf; see description above). Due to the apparent microphone failure, we were unable to describe the waveform and therefore a larger number of temporal parameters. Furthermore, some male adults not recorded were captured and photographed to measure the snout-vent length (SVL) with a Mitutoyo Absolute CD-6" CSX digital caliper (nearest 0.01 mm) by Ricardo Medina and were weighed with a pocket scale (nearest 0.01 g). Finally, they were euthanized with benzocaine, fixed in 10 % formalin, and stored in 70 % ethanol at the Colección Zoológica de la Universidad del Tolima (CZUT–A XXXX, XXXX, XXXX, XXXX) and a copy of the records was deposited at Fonoteca Zoológica (www.fonozoo.com) of the Museo Nacional de Ciencias Naturales, Madrid, Spain (Code 14202).

The advertisement call features of *P. leopardus* show it is a complex call since it changes as time passes (Fig. 1A-B). We recorded 1.26 minutes of calls at 2:14 am from a focal male (Fig. 1D) calling on Bromeliad leaf with air temperature 9.2 ºC, substrate temperature (leaf) 10.9 ºC, body temperature 10.6 ºC, relativity humidity 96%, weight 0.71 g, and SVL 21.04 mm. The call was recorded just after heavy rainy conditions and not in the summer season. During the recording we had some problems with the shotgun microphone; therefore, the waveform of the call is not distinguishable in the record, however, we suggest the call is perhaps composed of a single rising note, with a peak frequency (PF) of 2.97 kHz (± 0.03), inter-note interval duration (IN) 1.16 s (± 0.08), and a duration (CD) of 0.55 s (± 0.03) at the beginning of the call. However, all parameters change and decrease over time until the male stops calling as follows PFf 2.86 kHz (± 0.08), INf 0.40 s (± 0.03), and CDf of 0.11 s (± 0.01). A harmonic with a peak frequency of 5.95 kHz (± 0.10) was also observed (Fig. 1A, C). Other parameters are shown in Table 1.

We observed the call of *P. leopardus* is pretty different with respect to *P. uranobates* (Lynch 1991), the sister species (Rivera-Correa *et al.* 2017). Thus, the peak frequency of *P. uranobates* is 4.13 kHz (± 0.074) and the call duration is 0.022 s (±0.006) (Romero-García *et al.* 2015), suggesting a very small note with higher frequency respect to *P. leopardus* (Table 1). Likewise, the spectrogram indicates that the greatest energy is at the beginning of the note and this is lost as time passes (Romero-García *et al.* 2015, Fig. 1C), contrary to recorded in *P. leopardus* (Fig. 1A).

In addition to the call description, we were also able to take phenotypic and microenvironmental measures of four male individuals with SVL mean 21.56 mm (± 0.76; ranged 20.79 – 22.37), weight 0.69 g (± 0.09; ranged 0.56 – 0.75), body temperature 9.7 ºC (± 0.67; ranged 9.1 – 10.6), air temperature 9.3 ºC (± 0.10; ranged 9.2 – 9.4), substrate temperature 9.8 ºC (± 0.80; ranged 9.2 – 10.9), and relativity humidity 96.8 % (± 0.5; ranged 96 – 97). We also are currently automatically monitoring the microenvironmental temperatures of this species for future studies on climate change.

Overall, since the description of *P. leopardus* by Rivera-Correa *et al.* (2017) no study about its ecology, behavior, or evolution has been published. Furthermore, Rivera-Correa *et al.* (2017) indicate that they did not hear vocal behavior, this coincides with our observations given that after four field trips we were able to record only one call. Despite not being able to report the waveform, we expand the knowledge of *P. leopardus* a threatened species with the advertisement call description and encourage future behavioral studies and the use of automatic acoustic monitoring as a conservation measure given the difficult weather conditions and access to the localities.

Acknowledgements

The permit of collect and management was granted to the Grupo de Herpetología, Etología y Eco-fisiología of the Universidad del Tolima by the ANLA agency (Resolución Nº 02252 de 2019). This publication was financially supported by a grant from the Conservation Leadership Programme.

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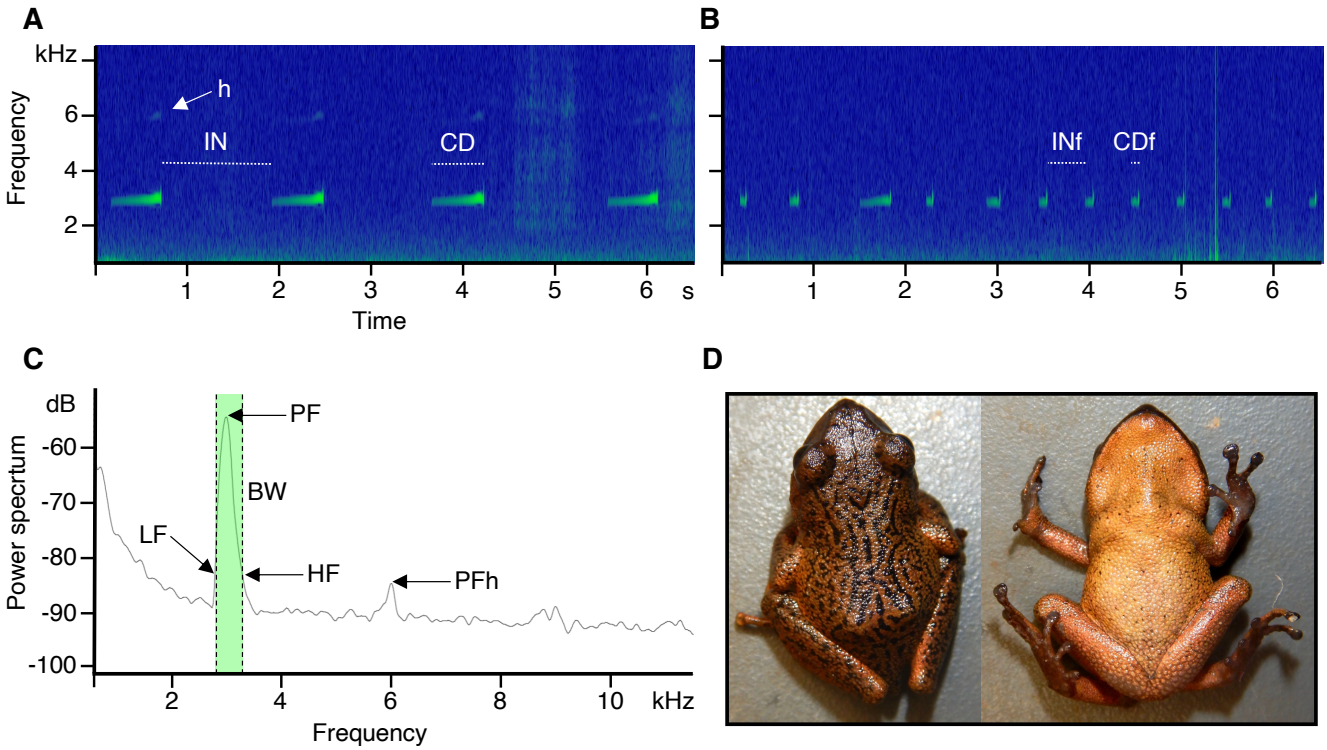


FIGURE 1. Advertisement call of *Pristimantis leopardus*. (A) Spectrogram of the advertisement call of *P. leopardus* just when starts calling, (B) Spectrogram of the advertisement call of *P. leopardus* when it is ending. The difference between CD and IN is observed. Both Spectrograms are at the same time scale with some temporal parameters shown, (C) it is shown how spectral parameters in the advertisement call of *P. leopardus* were measured with some parameters shown. Negative decibels (dB) were measured as FS/Hz. Green box the bandwidth (BW) measured 25 dB below peak frequency, and (D) Dorsal and ventral view of the male *P. leopardus* recorded. See above the description of call parameters.

TABLE 1. Temporal and spectral parameters of the advertisement call of *P. leopardus* recorded from Cerro La Vieja on the Páramo de Sonsón (Antioquia, Colombia). CD, IC, CDs, and ICs were measured in seconds, the other parameters in kHz. N = 10 calls recorded. SD = standard deviation. The (h) indicates the peak frequency of the harmonic. The (f) indicates the parameters measured on the notes at the final of the call. Parameters description above.

	Acoustic parameters										
	LF	**HF**	**PF**	**BW**	**BW90**	**PFh**	**CD**	**IN**	**PFf**	**CDf**	**INf**
Mean	2.70	3.27	2.97	0.57	0.18	5.95	0.55	1.16	2.86	0.11	0.40
Range	2.51 – 2.78	3.15 – 3.41	2.91 – 2.99	0.38 – 0.80	0.17 – 0.22	5.79 – 6.12	0.47 – 0.60	1.06 – 1.32	2.80 – 2.99	0.10 – 0.13	0.35 – 0.43
SD	0.09	0.09	0.03	0.15	0.02	0.10	0.03	0.08	0.08	0.01	0.03

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CONSERVATION LEADERSHIP PROGRAMME

An amphibian strategy for the consolidation of the Páramo de Sonsón action plan in Colombia

