# Final Evaluation Report

| Your Details | |
|---|---|
| **Full Name** | Ricardo Medina |
| **Project Title** | Short-term population dynamics and microenvironment of *Andinobates aff tolimensis* |
| **Application ID** | 38948-1 |
| **Date of this Report** | 16 May, 2024 |

**1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.**

| Objective | Not achieved | Partially achieved | Fully achieved | Comments |
|---|---|---|---|---|
| Determine short-term population dynamics of *Andinobates aff tolimensis* of a probably new population with capture-mark-recapture methods | | | ■ | Eight field trips were made from April 2023 to November 2023, each lasting 5 days, with a total of 37 capture-mark-recapture events. Currently submitted manuscript. |
| Determine temporal and spectral features of the calls of *Andinobates aff tolimensis* | | | ■ | 41 calls from 17 *Andinobates aff. tolimensis* frogs were analysed. |
| Determine the morphology of *Andinobates aff tolimensis* | | | ■ | 376 individuals of *Andinobates aff. tolimensis* were measured, weighed and body temperature data were collected. |
| Contrast new and published sequences of mitochondrial genes to determine the status of a probably new population of *Andinobates aff tolimensis* | | | ■ | Two samples of *Andinobates aff. tolimensis* (Cytb gene), two of *Pristimantis tribulosus* (16S gene) (first sequences published of this species), two of *Nymphargus rosada* (16S gene), and two of *Centrolene antioquiensis* (16S gene) were sequenced. The sequences will be released upon acceptance of the manuscripts |
| Monitor microenvironment temperatures | | | ■ | Microenvironmental temperatures were recorded at 1.5 m above the ground, inside burrows, and underwater for 1 |

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| | | | | year with six HOBO MX2201 dataloggers. Currently, temperatures continue to be recorded for future studies and a second phase of Rufford grants |
| Evaluate the presence of *Batrachochytrium dendrobatidis* (Bd) | | | | Swabs were made with MW113 for these species: *A. aff. tolimensis*, *P. tribulosus*, *N. rosada*, *C. antioquiensis,* and *Pristimantis* sp. These samples were stored dry at -70 °C in the Universidad del Tolima. During 2024, Bd analysis will be carried out, with other samples that will be collected from other areas and species with a new grant that is already available. |
| Promote environmental education with students and different stakeholders, involving them in field trips and environmental workshops | | | | Although the proposed environmental education workshops were completed, it was not possible to do field trips with the locals because the field site represented a high risk for the students due to the steep slope. |

**2. Describe the three most important outcomes of your project.**

**a)** Practically all the objectives were met with very good results. The population dynamics evaluation was carried out and a manuscript was submitted, which we will share with Rufford as soon as possible. The new population of *Andinobates* was analysed genetically, morphologically, and behaviorally; this manuscript is also ready for submission. The manuscript reporting new amphibian species in Padua has been accepted (see section 10, Any other comments?). Only the evaluation of Bd could not be completed. Additionally, there is significant acceptance and interest from students

and the community regarding the threatened Andean frog species found in Padua. Our reports and research are the first conducted on amphibians in this area.

**b)** A strong and solid relationship was established with the Institución Educativa Juan XXIII. An agreement of understanding is currently being developed between the institution and the NGO we created, TropiCall Foundation, to conduct new research in 2024.

**c)** Although it was not part of the objectives, we found three additional frog species, *Centrolene antioquiensis*, *Pristimantis tribulosus*, and *Nymphargus rosada*. The latter two are categorised by the IUCN as Critically Endangered and Vulnerable, respectively. These findings, along with the new population of *Andinobates aff. tolimensis*, suggest that the forest remnants in Padua may be crucial for the conservation of little known and endangered species. This manuscript has already been accepted and is soon to be published (see section 10).

**3. Explain any unforeseen difficulties that arose during the project and how these were tackled.**

We do not consider that the project per se has encountered difficulties. Prior knowledge of the species and methods allowed us to achieve the expected results and obtain sufficient and relevant information for publication in peer-reviewed journals and other formats (web pages, stickers, posters, field guides, etc.).

We faced only one issue, the loss of a HOBO MX2201 data logger, which was subsequently replaced. Logistically, the area where the species are found is complex (see preliminary report). Despite the short distance to the urban area, the difficult topography poses significant physical challenges and risks, particularly for untrained individuals such as students. Consequently, students could not be involved in field trips, despite the excellent support and interest from students and teachers in the science area.

Additionally, we aimed to conduct more environmental education workshops and training in monitoring methods. However, the former director was not receptive to providing additional opportunities for student engagement. This issue is likely to be resolved, as there has been a recent change in personnel at the educational institution (Institución Educativa Juan XXIII, https://www.facebook.com/IeJuanXXIIIPadua/?locale=es_LA). The current science

teachers and the academic coordinator are biologists who graduated from the Universidad del Tolima, and the school principal has also been replaced. With the new director, Leonardo Machado, we are currently negotiating an understanding agreement to develop new activities at the school.

**4. Describe the involvement of local communities and how they have benefitted from the project.**

No amphibian species had been reported in the Padua area prior to this study. Consequently, there were no publications on the amphibian diversity of this key area before our work.

We distributed 1,000 stickers to the local community and the school. Although no meetings were held with parents, the workshops and educational materials were well received, as evidenced by recognition from several parents. The activities were unique and highly engaging for the students, who informed their parents about the project and the biodiversity of Padua, thereby indirectly educating them.

In the coming months, as part of our commitments, we will disseminate the findings and results of our work through the Herveo mayor's website. This has been delayed allowing us to support the information with published scientific articles, enhancing the impact and providing valuable resources to decision-makers and stakeholders.

In some workshops, participants had the opportunity to observe live species (*Nymphargus rosada*, *Centrolene antioquiensis*, *Pristimantis tribulosus*, *Andinobates aff. tolimensis*, *Atractus* sp., and *Erythrolamprus mimus*) from the area, which created a greater impact.

We were also invited as jurors in October 2023 to participate in the Institución Educativa Juan XXIII science fair, suggesting an excellent relationship between our research team and the school.

Undoubtedly, our results will have significant impacts in the coming months as they are published and disseminated within the educational institution and through the mayor's office.

**5. Are there any plans to continue this work?**

Due to the significant results of the project, it is imperative for us to continue and seek resources to keep monitoring threatened species. The area showed substantial potential for ongoing conservation efforts with threatened species, reporting not only the focal species but also two additional species in the Vulnerable and Critically Endangered categories. Additionally, we have established an excellent relationship between the educational institution and local community, and ourselves as researchers, and the foundation we represent.

When we finish publishing the results, we will be writing a new proposal for Rufford, probably focusing on improving the methods and developing new research. This proposal aims to bring the species and local biodiversity closer to the people of Padua and improve dissemination so that our methods can be replicated in other areas and with other species.

Finally, the project is ongoing as we have decided to maintain remote microenvironmental monitoring. The six data loggers, installed for over a year, will continue to record microenvironmental temperatures. This data will support a potential new Rufford grant application, enabling us to investigate the effects of global warming on *Andinobates* through thermal performance tests on both adults and tadpoles.

**6. How do you plan to share the results of your work with others?**

Currently, three manuscripts have been written, two of which have been submitted, and one has already been accepted. The accepted manuscript is attached below in point 10. Additionally, we will be presenting our results at the Bioacoustics Congress in November this year. In 2023, we also developed stickers, posters, and workshops with locals to disseminate information about the focal species. Finally, multimedia material is being developed to ensure that anyone can access the information collected in Padua. Thus, being able to impact all possible actors, academia, decision makers, the local community, and other stakeholders interested in developing research, monitoring, and conservation in Padua.

**7. Looking ahead, what do you feel are the important next steps?**

First, publish the research results and disseminate them locally through multimedia channels, radio, and result-sharing sessions. Deliver the findings to stakeholders and

decision makers, including the mayor of Herveo and Institución Educativa Juan XXIII. This will create an atmosphere of improved knowledge regarding the area's species and biodiversity.

Second, secure additional resources to further research, monitoring, and conservation efforts. Develop a proposal that involves local actors (students, teachers, and stakeholders) as researchers, possibly through citizen science initiatives and community monitoring.

While the methodologies proposed for studying *A. aff. tolimensis* worked perfectly, the behavior, ecology, and biology of other reported threatened species may require different approaches. Thus, we must consider new methodologies for better monitoring and understanding population status, behaviour and distribution. Environmental DNA analysis, genomic data for estimating effective population sizes, and passive acoustic monitoring could be more effective given the limitations identified in our initial phase.

Expand the proposal's scope from focusing on a single species (*Andinobates aff. tolimensis*) to encompassing a key conservation area and multiple taxa. The northern region of Tolima, with elevations ranging from 1500 to 4500 m, presents significant potential for biodiversity monitoring and conservation of threatened species.

Third, seek new strategic alliances among local actors, academia (Universidad del Tolima, Universidad de Ibagué, Universidad de Manizales, other Universities, and research centers), and decision makers (mayor of Herveo and the regional environmental agency CORTOLIMA) to ensure that the results influence public policies and territorial planning.

**8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?**

Certainly, we have included the Rufford Foundation logo in each of the activities, presentations, and workshops we carry out in our project. This includes advertising for the training courses, academic talks, stickers about the threatened amphibian species (see preliminary report, https://www.rufford.org/projects/ricardo-medina/short-term-population-dynamics-and-microenvironment-andinobates-aff-tolimensis/), and soon on the posters that will be delivered with QR codes to access more information (calls, photos, maps, and taxonomic descriptions) about the area and the species. Likewise,

we mentioned the Rufford Foundation's name as the primary financiers in all manuscripts and accepted publications (see below, 10.).

**9. Provide a full list of all the members of your team and their role in the project.**

| Name | Role | Qualifications | Affiliation |
|---|---|---|---|
| Ricardo Medina | Team leader | MSc. BS. | TropiCall NGO and Grupo de Herpetología, Etología y Eco-fisiololía, Universidad del Tolima |
| Bibiana Tovar | Researcher | BS. | TropiCall NGO and Grupo de Herpetología, Etología y Eco-fisiololía, Universidad del Tolima |
| Manuel H. Bernal | Collaborator and adviser | PhD. | Grupo de Herpetología, Etología y Eco-fisiololía, Universidad del Tolima |
| Willy Reyes | Collaborator | MSc. student | TropiCall NGO and Universidad del Tolima |
| Carlos Galindo | Collaborator | MSc. | TropiCall NGO and Grupo de Herpetología, Etología y Eco-fisiololía, Universidad del Tolima |
| José Jaime Zuñiga | Adviser | PhD. | Faculty of Science, Universidad Nacional Autónoma de México (UNAM) |

**10. Any other comments?**

First manuscript accepted to publishing in Revista Latinoamericana de Herpetología

**New distribution records with notes on the natural history of threatened frogs (*Pristimantis tribulosus*, *Nymphargus rosada*, and *Centrolene antioquiensis*) in northern Tolima, Colombia.**

Ricardo Medina[1,2,*], Bibiana Tovar[1,2], Willy Fernando Reyes-Torres[2] & Manuel H. Bernal[1]
[1]*Grupo de Herpetología, Etología y Eco-fisiología, Universidad del Tolima, Ibagué, Tolima, Colombia*
[2]*TropiCall: Conservación, Desarrollo e Investigación de los Andes Tropicales*
*Correspondence: ramedinar1@gmail.com

The town of Herveo in the Department of Tolima, located in the Central Cordillera of the Tropical Andes in Colombia, is characterized by a wide topographic and environmental heterogeneity, ranging from 1,500 to 4,450 m.a.s.l. (INGEOMINAS, 2001; Alcaldía Municipal de Herveo, 2005). Historically, the biodiversity in this area has been poorly studied due to its complex topography and, until a few years ago, the influence of the armed conflict in Colombia (Pérez-Salazar, 2010). In Herveo, twelve species of

amphibians have been reported from four families (Bufonidae: *Osornophryne percrassa*, Centrolenidae: *Espadarana prosoblepon*, Dendrobatidae: *Hyloxalus lehmanni*, Hylidae: *Boana platanera*, Strabomantidae: *Pristimantis taeniatus*, *P. permixtus*, *P. w-nigrum*, *P. viejas*, *P. gaigei*, *P. simoterus*, *Niceforonia adenobrachia*, and *N. latens*) (Ardila-Robayo et al., 1996. Clavijo-Garzón et al., 2018; Lynch, 1980; Ruiz-Carranza & Hernández-Camacho, 1976), all belonging to the Anura order. Three of these species are listed as threatened: *O. percrassa* (Vulnerable), *N. latens* (Vulnerable), and *N. adenobrachia* (Endangered) (Gómez et al., 2017a, b, c). This suggests that this area is an important habitat for the conservation of threatened species.

During a three-day field trip in May 2023, between 19:00 – 24:00 hours in the Corregimiento de Padua (Herveo, Colombia) (5.132855, -75.143863, 2077 m, WGS84), we recorded three species of anurans: three individuals of *Centrolene antioquiensis* (Noble, 1920), five of *Nymphargus rosada* (Ruiz-Carranza & Lynch, 1997), and five of *Pristimantis tribulosus* (Lynch & Rueda-Almonacid, 1997). We measured snout-vent length (SVL) of all specimens with a Mitutoyo Absolute CD-6" CSX digital caliper (Table 1) and recorded advertisement calls of one male of *C. antioquiensis* and one male of *N. rosada* (Fig. 2, Table 1) located at two small streams (Fig. 1). Calls were recorded with an Audio-Technica AT897 microphone in a ZOOM H6 recorder and for every single call, we recorded the air temperature with a Hygro-Thermometer RH101 Extech IR. Body and substrate temperatures were also recorded with a Klein tools IR5 dual laser infrared thermometer. Briefly, Raven Pro v1.6.5 (Cornell Lab of Ornithology, 2022) was used for acoustic analyses with 44.1 kHz sampling rate and 16 bits of resolution. 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. We extracted a suite of acoustic measures: call duration (CD), number of pulses (P), number of notes (N), peak frequency (PF), bandwidth (BW), and bandwidth 90% (BW90), Lowest frequency (LF), and highest frequency (HF) following Medina et al., (2021). All spectral traits were measured 30 and 20 decibels below peak frequency for *N. rosada* and *C. antioquiensis*, respectively. Copies of the calls recorded were deposited at Fonoteca Zoológica (www.fonozoo.com) of the Museo Nacional de Ciencias Naturales, Madrid, Spain (FZ-Sound-Code 14740 and 14741). The identification of *C. antioquiensis*, *N. rosada*, and *P. tribulosus* were carried out using morphological characteristics both life and ethanol-preserved specimens under a stereoscope. Additionally, the species were confirmed through spectral and temporal characteristics of their calls. The specimens were euthanized with 5% lidocaine, immersed in 10% ethanol (ETOH), and subsequently deposited in 70% ethanol within the Colección Zoológica de la Universidad del Tolima (CZUT) (Table 1). Fieldwork was carried out in strict adherence to the guidelines for work with amphibians and reptiles (Beaupre et al., 2004). Scientific collection permit was provided to Grupo de Herpetología, Etología y Eco-fisiología of the Universidad del Tolima by the ANLA agency (Resolución No 02252 de 2019).

*C. antioquiensis* was determined based on: 1) the absence of vomerine teeth, 2) small and curved humeral spine, 3) green bones in life, 4) ulnar fold, 5) absent membrane between fingers I and II, reduced membrane between the outer toes, and 6) type I nuptial outgrowths (Ruiz-Carranza & Lynch, 1997; Rivera-Correa, 2010). Furthermore, the call recorded in Padua (Fig. 2) showed a pair of notes, each note consisting of repeated pulses with the last one longest in duration, although with a peak frequency slightly higher than the reported by Duarte-Marín et al., (2022) (Table 2). On the other hand, *N. rosada* individuals showed: 1) Absence of vomerine teeth, 2) truncated rostrum in dorsal and lateral view, 3) visible tympanum, 4) pericardium and parietal peritoneum covered by iridophores, 5) absence of humeral spine, and 6) absent interdigital membranes between fingers I – III (Ruiz-Carranza & Lynch, 1997; Patiño-Ocampo et al., 2022). The call of *N. rosada* consisted of a single note, with a duration and peak frequency

similar to that reported by Duarte-Marín et al., (2022) (Table 2; Fig. 2). *P. tribulosus* was identified based on: 1) numerous conical tubercles, 2) snout long, acuminate in dorsal view, protruding in lateral profile; canthus rostralis sharp, 3) long row ulnar tubercles, including one on the elbow, 4) heel with large calcar tubercle, conical tubercles on the underside of tarsus, tubercles along inner and outer margins of tarsus, 5) metatarsal tubercles, 6) upper eyelid bearing large conical tubercle on posterior third, and 7) one or two conical tubercles on the knee. Regarding eye color, the original description does not report it, however, Duarte-Marín et al., (2018) show a light green iris, with reticulation in the lower part of the eye and a brown longitudinal triangular stripe. Observations of individuals from Padua show creamy white irises, reticulated in the lower part of the eye and with a burgundy longitudinal triangular stripe (Fig. 2). Lastly, the dorsal color pattern of one individual showed a thick cream-colored line from the interorbital area to the cloaca (Fig. 2). No calls of this species were recorded.

Amphibians are the most threatened vertebrate group worldwide (Stuart et al., 2008; Luedtke et al., 2023). The most of threatened amphibians in Colombia are endemic, known from only one locality, and some occur in protected areas (Rueda-Almonacid et al., 2004). However, protected areas alone are not sufficient to ensure the survival and long-term conservation of species (Watson & Castillo, 2022; Williams et al., 2022). *P. tribulosus* is a species of rain frog categorized by the IUCN as Critically Endangered (Castro et al., 2017), endemic to the protected area Parque Nacional Natural (PNN) Selva de Florencia (Caldas, Colombia). Since its description in 1997, this frog has only been sporadically reported within the Selva de Florencia (Lynch & Rueda-Almonacid, 1997; Duarte-Marín et al., 2018), an area covering 100.2 km2 on the eastern flank of the Central Cordillera of the Tropical Andes in Colombia. Unfortunately, this protected area is not connected with other areas and there are no records of this species outside of its locality. Therefore, our findings in Padua represent the first report of *P. tribulosus* beyond its type locality. Thus, we extend the range of *P. tribulosus* to the south by approximately 38 km (linear distance) from Selva de Florencia. In addition, our report suggests that *P. tribulosus* is not necessarily endemic to the Selva de Florencia, and consequently, so nearby conserved areas could represent remnant populations for this species.

*N. rosada* and *C. antioquiensis* are classified as Vulnerable (Quevedo et al., 2017) and Near Threatened (Molina et al., 2017), respectively, and both are found in the Central Cordillera of Colombia. In Tolima, the Padua locality represents the third known occurrence of these species, marking the first report of sympatry (Fig. 1). This finding also suggest that the environmental conditions and habitat between Padua and Selva de Florencia, a protected area where these three species are also present, seem to be similar. Thus, conserving the small patches in this new locality becomes crucial for enhancing connectivity among protected areas and facilitating gene flow between populations of these species. On the other hand, the Guarinó River Canyon is a significant geographic barrier, which separates the Tolima populations from those of Selva de Florencia. This barrier may also contribute to a genetic structure between these populations, potentially explaining the observed variation in *P. tribulosus*. Future systematic studies employing genetic markers could validate the phenotypic differences detected.

We also observed a thanatosis behavior in *P. tribulosus* and a broad range of coloration patterns, suggesting a polymorphic variation in this species. Moreover, we noted a discernible

shift in coloration patterns between nocturnal activity and diurnal rest, with a darker color observed during the daytime.

We could not record the call of *P. tribulosus*, which may be due to a potential late-night or early-morning activity pattern. During our field trips, we sporadically heard a few calls both at night and during the day, likely from *P. tribulosus* as it was the only species found in this area. Given the limited observations of *P. tribulosus*, we plan to use passive acoustic monitoring in the future to capture the call and better understand its activity pattern.

While our records are favorable for the conservation of the threatened species *P. tribulosus* and *N. rosada*, extensive deforestation (Fig. 1) and the habitat shift into an agricultural matrix indicate potential displacement of these species to the edge of their habitat. Consequently, these species face ongoing significant threats from habitat loss within their distribution range. The amphibian species recorded in Herveo now stands at fifteen, including five threatened frogs. Herveo exhibits substantial environmental heterogeneity due to its extensive altitudinal range (around 3,000 m), suggesting that amphibian biodiversity in the area may be underestimated. Our current understanding is based on only three systematic expeditions conducted in Herveo over the past 60 years. Thus, it becomes imperative to undertake future expeditions to monitor the reported species. Moreover, these expeditions can identify new areas that may contribute to expand the records of amphibians, potentially supporting new protected areas or enhancing connectivity between these, contributing to the overall conservation efforts. Finally, we encourage future studies on these threatened frogs in ecology, behavior, physiology, evolution, natural history, and population trends to reduce their threat level and improve effective monitoring and conservation strategies.

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**Table 1.** New records of distribution of three frogs in Herveo town (Tolima, Colombia). CZUT: code of the Colección Zoológica de la Universidad del Tolima. SVL = Snout-vent length. Wg = Weight in grams. T = Temperature, b = body, s = substrate, e = environment. RH = Relative humidity. Asterisks (*) indicate species with call recorded (see Table 2, Fig. 2). The last record of *C. antioquiensis* was not collected.

| Voucher | Species | SVL (mm) | Wg (g) | T.b (ºC) | T.s (ºC) | T.e (ºC) | RH (%) | Coordinates | | Elevation |
|---|---|---|---|---|---|---|---|---|---|---|
| CZUT-3161 | *Pristimantis tribulosus* | 30.54 | 1.80 | 17.8 | 17.5 | 19.7 | 30 | 5.13324 | -75.15196 | 2213 |
| CZUT-3165 | *Pristimantis tribulosus* | 15.86 | - | 16.7 | 16.6 | 18 | 55 | 5.13534 | -75.15479 | 2252 |
| CZUT-3166 | *Pristimantis tribulosus* | 27.77 | - | 16.7 | 16.8 | 15.9 | 88 | 5.1192 | -75.15069 | 2164 |
| CZUT-3164 | *Pristimantis tribulosus* | 17.53 | 0.57 | 15.8 | 15.8 | 18.8 | 46 | 5.13539 | -75.15479 | 2253 |
| CZUT-3162 | *Pristimantis tribulosus* | 29.41 | 2.35 | - | 19.8 | 19.3 | 47 | 5.11938 | -75.15031 | 2039 |
| CZUT-3163 | *Pristimantis tribulosus* | 29.44 | 2.68 | - | 18.0 | 19.8 | 46 | 5.11837 | -75.15044 | 2039 |
| CZUT-3157 | *Nymphargus rosada* | 26.26 | 1.30 | - | - | - | - | 5.11716 | -75.13875 | 1895 |
| CZUT-3155 | *Nymphargus rosada* | 26.70 | 1.59 | - | - | - | - | 5.11799 | -75.13946 | 1881 |
| CZUT-3158 | *Nymphargus rosada** | 26.78 | 1.25 | - | - | 18.8 | 62 | 5.11785 | -75.13921 | 1898 |
| CZUT-3156 | *Nymphargus rosada* | 25.53 | 1.10 | - | - | - | - | 5.11777 | -75.13944 | 1884 |
| CZUT-3159 | *Nymphargus rosada* | 26.57 | 1.20 | - | - | - | - | 5.11785 | -75.13921 | 1898 |
| CZUT-3160 | *Centrolene antioquiensis** | 21.81 | 1.05 | 15.2 | 15.0 | 20.3 | 41 | 5.14059 | -75.15445 | 2218 |
| CZUT-3152 | *Centrolene antioquiensis* | 22.30 | 0.86 | 13.9 | 13.9 | 19.6 | 38 | 5.13486 | -75.15444 | 2226 |
| - | *Centrolene antioquiensis* | - | - | - | - | - | - | 5.12674 | -75.15557 | 2278 |

**Table 2.** A comparative suite of acoustic measures. Units of call duration (CD) is in seconds. All spectral parameters are in kHz. (*) indicates not reported data. Environmental temperature and relative humidity of *N. rosada* were 18.8 ºC and 62%, respectively. Environmental temperature, substrate temperature, body temperature, and relative humidity of *C. antioquensis* were 20.3 ºC, 15 ºC, 15.2 ºC, and 41%, respectively. See text for parameter description.

| **Parameter** | | ***Nymphargus rosada*** | | ***Centrolene antioquiensis*** | |
|---|---|---|---|---|---|
| | | This study (N=23) | Duarte-Marín et al., 2022 | This study (N=14) | Duarte-Marín et al., 2022 |
| **CD** | Mean | 0.057 ± 0.002 | 0.060 ± 0.004 | 0.035 ± 0.003 | * |
| | range | 0.052 – 0.063 | 0.049 - 0.068 | 0.031 – 0.040 | * |
| **N** | Mean | 1 | 1 | 2 | 2.5 ± 0.3 |
| | range | 1 | 1 | 2 | 1 - 4 |
| **P** | Mean | * | * | 5 ± 1 | 7.4 ± 2.5 |
| | range | * | * | 4 - 7 | 5 -12 |
| **PF** | Mean | 3.583 ± 0.014 | 3.94 ± 0.11 | 7.117 ± 0.137 | 6.99 ± 0.02 |
| | range | 3.553 – 3.618 | 3.79 – 4.04 | 6.891 – 7.321 | 6.965 –7.02 |
| **LF** | Mean | 3.386 ± 0.023 | * | 6.607 ± 0.177 | * |
| | range | 3.309 – 2.411 | * | 6.247 – 6.857 | * |
| **HF** | Mean | 3.763 ± 0.012 | * | 7.860 ± 0.093 | * |
| | range | 3.743 – 3.783 | * | 7.689 – 8.011 | * |
| **BW** | Mean | 0.377 ± 0.027 | * | 1.253 ± 0.150 | * |
| | range | 0.345 – 0.469 | * | 0.982 – 1.548 | * |
| **BW90%** | Mean | 0.167 ± 0.013 | * | 0.658 ± 0.055 | * |
| | range | 0.151 – 0.194 | * | 0.517 – 0.754 | * |

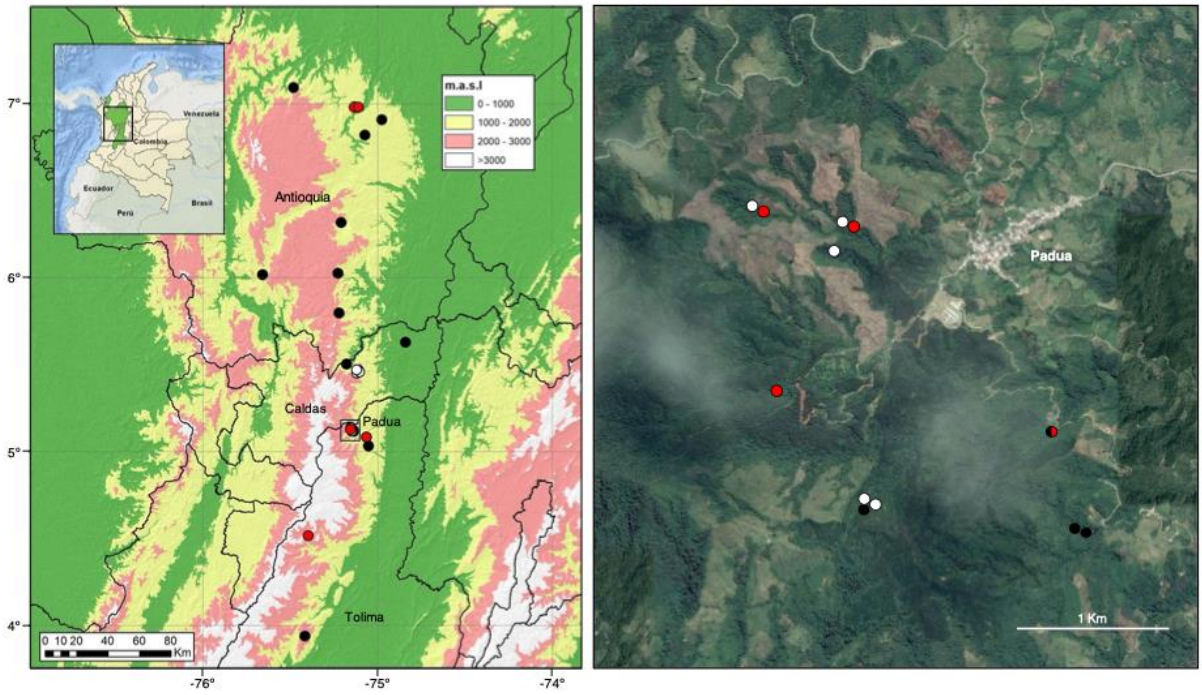


**Figure 1.** Historical distribution map and new records for the species *P. tribulosus*, *N. rosada* and *C. antioquiensis*. Left, distribution of the three species in Colombia. Right, distribution of species in Corregimiento de Padua (Herveo, Tolima, Colombia). Black dots: *N. rosada*, red dots: *C. antioquiensis*, and white dots: *P. tribulosus*. Right, satellite image taken from Google Earth Pro v7.3.4.8248. The image shows the strong deforestation and habitat loss in the Corregimiento of Padua. The areas with dark green forest cover represent introduced pine (*Pinus patula* and *Pinus maximinoii*) crops. Right image shows a small stream marked with black/red dot where *C. antioquiensis* and *N. rosada* were observed in sympatry.

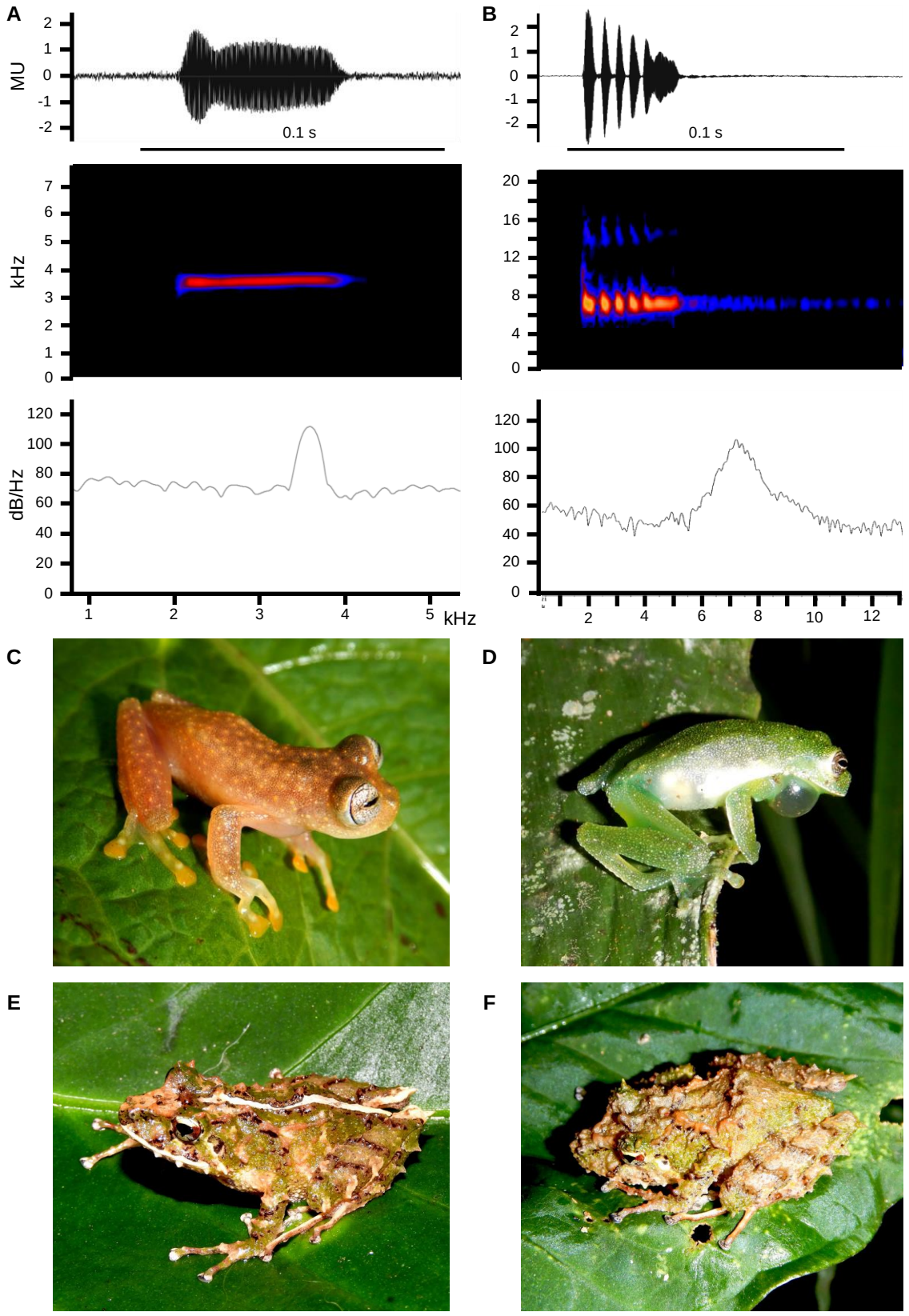


**Figure 2.** Advertisement calls and morphology of recorded species. A) *N. rosada*, B) *C. antioquiensis*, C) male calling of *N. rosada*, D) male calling of *C. antioquiensis*, E) and F) two females of *P. tribulosus*. Copies of calls recorded can be heard at www.fonozoo.com (FZ-Sound-Code

14740 and 14741). Calls are shown in waveform, spectrogram, and power spectrum. The dark blue highlights the bandwidth (BW) measured 30 (A) and 20 (B) decibels below peak frequency. B) waveform of a single note of *C. antioquiensis* call. The measurements of the calls can be seen in Table 2. Photos by Ricardo Medina