

NEW DISTRIBUTION RECORDS WITH NOTES ON THE NATURAL HISTORY OF THREATENED FROGS (*PRISTIMANTIS TRIBULOSUS*, *NYMPHARGUS ROSADA*, AND *CENTROLENE ANTIOQUIENSIS*) IN NORTHERN TOLIMA, COLOMBIA

NUEVOS REGISTROS DE DISTRIBUCIÓN CON NOTAS DE HISTORIA NATURAL EN RANAS AMENAZADAS (*PRISTIMANTIS TRIBULOSUS*, *NYMPHARGUS ROSADA* Y *CENTROLENE ANTIOQUIENSIS*) AL NORTE DEL TOLIMA, COLOMBIA

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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. (Alcaldía Municipal de Herveo, 2005; González, 2001). 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 adenobranchia*, 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. adenobranchia* (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 h in the Corregimiento de Padua (Herveo, Colombia) (5.132855° N, 75.143863° W, 2,077 m a.s.l., 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) (Fig. 1). 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 stereo microscope. 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

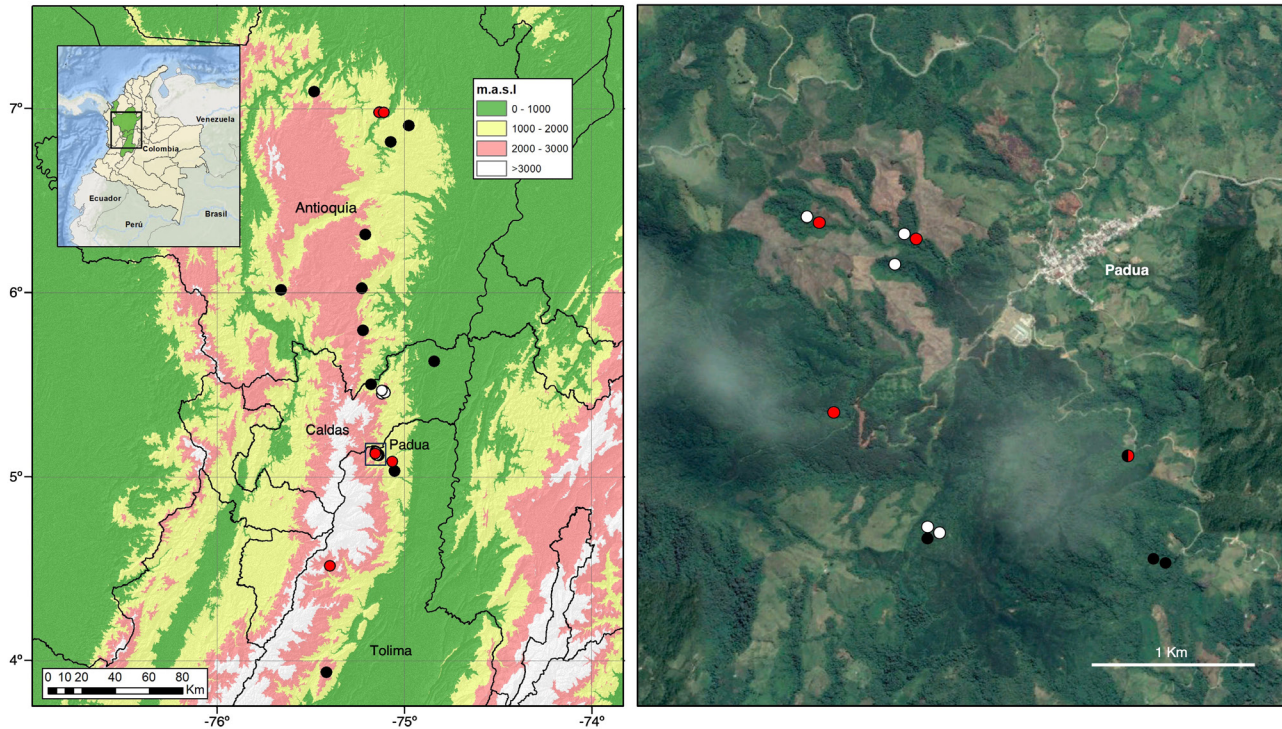


Figura 1. Mapa de distribución histórica y nuevos registros para las especies *P. tribulosus*, *N. rosada* y *C. antioquiensis*. A la izquierda la distribución de las tres especies en Colombia. A la derecha la distribución de las especies en el Corregimiento de Padua (Herveo, Tolima, Colombia). Puntos negros: *N. rosada*, rojos: *C. antioquiensis* y blancos: *P. tribulosus*. A la derecha, la imagen satelital es tomada de Google Earth Pro v7.3.4.8248. La imagen muestra la fuerte deforestación y pérdida de hábitat en el Corregimiento de Padua. Las áreas con cobertura verde oscura son cultivos introducidos de pino (*Pinus patula* y *Pinus maximinoii*). En la imagen de la derecha el punto rojo y negro indica un pequeño arroyo donde se observaron en simpatria *C. antioquiensis* y *N. rosada*.

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 de 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.

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Centrolene 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 (Rivera-Correa, 2010; Ruiz-Carranza & Lynch, 1997). 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 (Patiño-Ocampo et al., 2022; Ruiz-Carranza & Lynch, 1997). 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

Tabla 1. Nuevos registros de distribución de tres ranas en el municipio de Herveo (Tolima, Colombia). CZUT = código de la Colección Zoológica de la Universidad del Tolima. SVL = longitud rostro cloaca. Wg = peso en gramos. T = Temperatura, b = cuerpo, s = sustrato, e = ambiente. RH = humedad relativa. Los asteriscos (*) indican los cantos registrados (Tabla 2, Fig. 2). El último registro de *C. antioquiensis* no fue colectado. Elevación en m s.n.m.

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. Elevation in m a.s.l.

Voucher	Species	SVL (mm)	Wg (g)	T.b (°C)	T.s (°C)	T.e (°C)	RH (%)	Coordinates	Elevation
CZUT-3161	<i>Pristimantis tribulosus</i>	30.54	1.8	17.8	17.5	19.7	30	5.13324 -75.15196	2,213
CZUT-3165	<i>Pristimantis tribulosus</i>	15.86	-	16.7	16.6	18	55	5.13534 -75.15479	2,252
CZUT-3166	<i>Pristimantis tribulosus</i>	27.77	-	16.7	16.8	15.9	88	5.1192 -75.15069	2,164
CZUT-3164	<i>Pristimantis tribulosus</i>	17.53	0.57	15.8	15.8	18.8	46	5.13539 -75.15479	2,253
CZUT-3162	<i>Pristimantis tribulosus</i>	29.41	2.35	-	19.8	19.3	47	5.11938 -75.15031	2,039
CZUT-3163	<i>Pristimantis tribulosus</i>	29.44	2.68	-	18	19.8	46	5.11837 -75.15044	2,039
CZUT-3157	<i>Nymphargus rosada</i>	26.26	1.3	-	-	-	-	5.11716 -75.13875	1,895
CZUT-3155	<i>Nymphargus rosada</i>	26.7	1.59	-	-	-	-	5.11799 -75.13946	1,881
CZUT-3158	<i>Nymphargus rosada</i> *	26.78	1.25	-	-	18.8	62	5.11785 -75.13921	1,898
CZUT-3156	<i>Nymphargus rosada</i>	25.53	1.1	-	-	-	-	5.11777 -75.13944	1,884
CZUT-3159	<i>Nymphargus rosada</i>	26.57	1.2	-	-	-	-	5.11785 -75.13921	1,898
CZUT-3160	<i>Centrolene antioquiensis</i> *	21.81	1.05	15.2	15	20.3	41	5.14059 -75.15445	2,218
CZUT-3152	<i>Centrolene antioquiensis</i>	22.3	0.86	13.9	13.9	19.6	38	5.13486 -75.15444	2,226
-	<i>Centrolene antioquiensis</i>	-	-	-	-	-	-	5.12674 -75.15557	2,278

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 (Luedtke et al., 2023; Stuart et al., 2008). 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). *Pristimantis 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 (Duarte-Marín et al., 2018; Lynch & Rueda-Almonacid, 1997), 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.

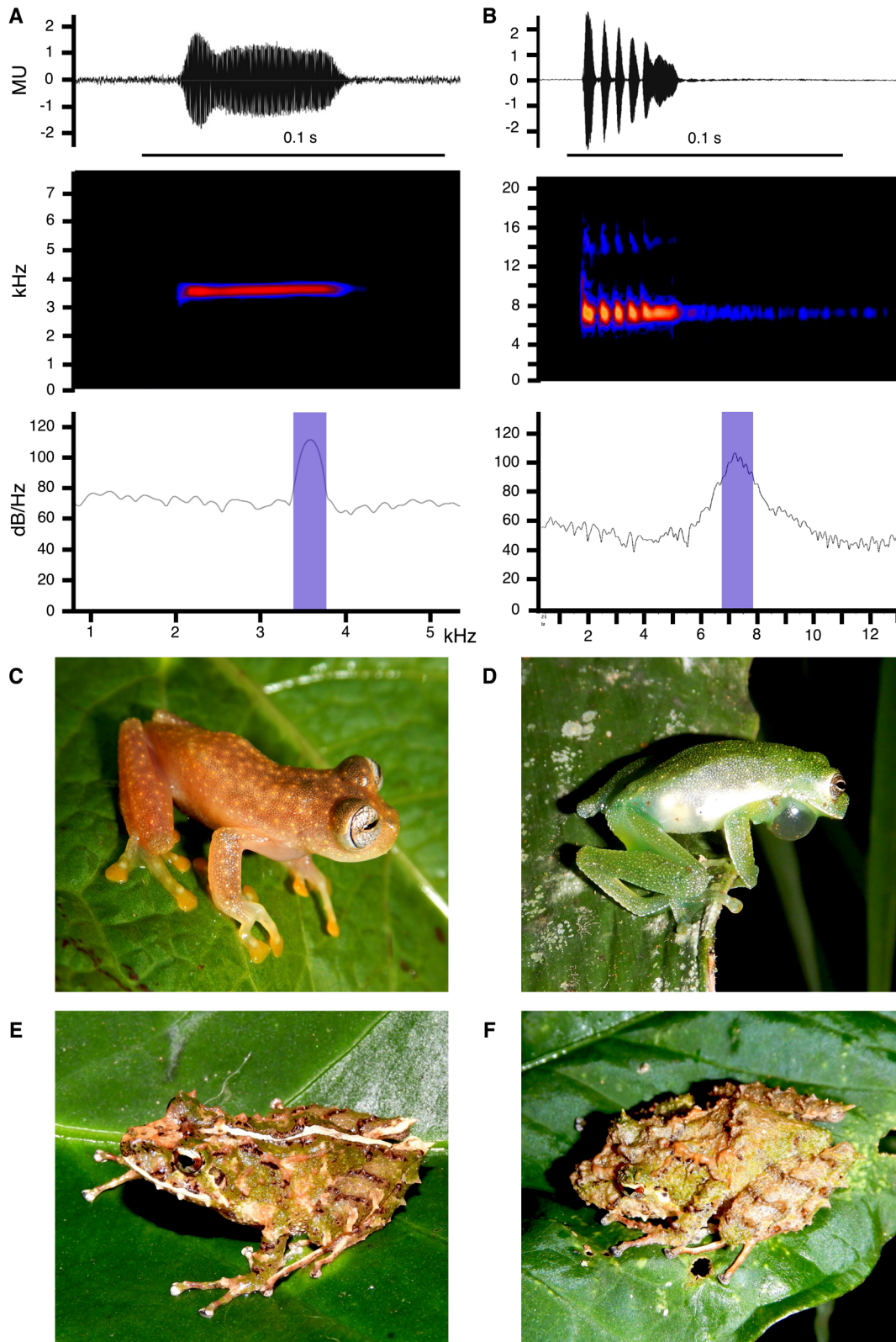


Figura 2. Cantos de anuncio y morfología de las especies registradas. A) *N. rosada*, B) *C. antioquiensis*, C) macho cantando de *N. rosada*, D) macho cantando de *C. antioquiensis*, E) y F) dos hembras de *P. tribulosus*. Los cantos registrados se pueden escuchar en www.fonozoo.com con los códigos FZ-Sound-Code 14740 and 14741. Los cantos se muestran en forma de onda, espectrograma y espectro de potencia. El azul oscuro destaca el ancho de banda (BW) medido 30 (A) y 20 (B) decibels por debajo de la frecuencia máxima. B) forma de la onda de una sola nota del canto de *C. antioquiensis*. Las mediciones de los cantos se pueden ver en la Tabla 2. Fotos: Ricardo Medina.

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: Ricardo Medina.

Tabla 2. Comparación de las variables acústicas. Duración del canto (CD) en segundos. Todos los parámetros espectrales se midieron en kHz. El (*) indica datos no reportados. La temperatura ambiental y humedad relativa de *N. rosada* fue de 18.8 °C and 62 %, respectivamente. La temperatura ambiental, del sustrato y del cuerpo, y la humedad relativa de *C. antioquiensis* fueron 20.3 °C, 15 °C, 15.2 °C, and 41 %, respectivamente. Los parámetros se describen en el texto.

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 °C and 62 %, respectively. Environmental temperature, substrate temperature, body temperature, and relative humidity of *C. antioquiensis* were 20.3 °C, 15 °C, 15.2 °C, and 41 %, respectively. See text for parameter description.

Parameter		<i>Nymphargus rosada</i>		<i>Centrolene antioquiensis</i>	
		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	0.044 - 0.046
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	*

Nymphargus 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 suggests 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 Guarínó River Canyon is a significant geographic barrier, which separates the Tolima populations from those of Selva de Florencia. This barrier may also contribute to 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 a.s.l.), 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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