DNA BARCODES AND DIVERSITY OF AMPHIBIANS AND REPTILES IN AGROECOSYSTEMS OF THE COLOMBIAN ANDES

CÓDIGOS DE BARRAS DE ADN Y DIVERSIDAD DE ANFIBIOS Y REPTILES EN UN AGROECOSISTEMA DE LOS ANDES COLOMBIANOS

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Resumen.– Buscamos caracterizar la diversidad de anfibios y reptiles desde una perspectiva ecológica, molecular y evolutiva asociada con plantaciones de aguacate cv. Hass (*Persea americana*) en el departamento de Risaralda, Colombia. Se implementaron dos períodos de muestreo entre 2019 y 2020 utilizando el muestreo por encuentros visuales; se utilizaron curvas de rarefacción y estimadores de riqueza de especies para evaluar la completitud del muestreo. La identificación del material se basó en caracteres morfológicos y en métodos de secuenciación de DNA para el marcador mitocondrial citocromo C oxidasa subunidad I (*COI*). Además, utilizamos secuencias de *COI* para construir filogenias y evaluar la diversidad filogenética (PD) de la comunidad. Se registraron siete especies de anfibios y 18 de reptiles, para las cuales se obtuvieron 49 secuencias de *COI*, de las cuales 15 representan el primer registro para Colombia. También encontramos 12 casos de incongruencia entre los métodos de identificación morfológica y de secuenciación de DNA, y proporcionamos una discusión sobre la identificación correcta. Finalmente, aunque comúnmente observamos una relación positiva entre la riqueza de especies y la PD, también encontramos que la PD puede proporcionar información valiosa cuando los valores de riqueza de especies no son informativos al comparar dos comunidades. Nuestros resultados respaldan la necesidad de un mayor esfuerzo de muestreo en el área para reconocer su verdadera diversidad, ya que esto permitirá una mejor comprensión de las dinámicas complejas presentes en paisajes modificados. Además, concluimos sobre el gran aporte de los estudios de secuenciación de DNA a una pequeña escala geográfica y soportamos el uso del marcador *COI* como una fuente adecuada de información para la identificación de especies y evaluación de la diversidad de comunidades desde una perspectiva histórica.

Palabras clave.– Diversidad filogenética, aguacate, *Persea americana*, códigos de barra, BOLD, Suramérica, herpetofauna, identificación de especies.

Abstract.– We aim to characterize the diversity of amphibians and reptiles associated with plantations of avocado cv. Hass (*Persea americana*) in the department of Risaralda, Colombia from an ecological, molecular, and evolutionary perspective. Two sampling periods were implemented between 2019 and 2020 using visual encounter surveys; rarefaction curves and species richness estimators were used to evaluate the completeness of the sampling. Identification of the material was based on both morphological characters as well as DNA barcoding methods sequencing the mitochondrial cytochrome C oxidase subunit I (*COI*) marker. We also used the

COI sequences to construct phylogenies and evaluate the phylogenetic diversity (PD) of the community. Seven amphibian and 18 reptile species were recorded, for which 49 *COI* sequences were obtained, 15 of which represent the first sequence barcoding records for Colombia. We also found 12 instances of incongruence between the morphological and DNA barcoding identification methods, and we provide a discussion on the correct identification. Finally, although we commonly observed a positive relationship between species richness and PD, we also found that PD can provide valuable information when species richness values are uninformative at comparing two communities. Our results support the need for a greater sampling effort in the area to recognize its true diversity, as this will allow a better understanding of the complex dynamics present in modified landscapes. Also, we conclude with the great contribution of DNA barcoding studies at a small geographical scale and support the use of the *COI* marker as a suitable source of information for species identification and evaluating the diversity of communities from a historical perspective.

Keywords.– Phylogenetic diversity, avocado, *Persea americana*, BOLD, South America, herpetofauna, species identification.

INTRODUCTION

Amphibians and reptiles are groups of vertebrates that, although widely distributed on the planet, experience their most outstanding diversity in the Neotropics, mainly in lowland rainforests and cloud forests (Uetz, 2020; Frost, 2023). Colombia is the second and third most diverse country worldwide for amphibians (863 species) and reptiles (617 species), respectively (Pérez-Santos & Moreno 1988; Acosta-Galvis, 2000; Uetz, 2020; Frost, 2023). Such diversity has been the result of multiple historical processes that determine the diversification of species (Wiens, 2011), and the identification of resultant patterns from an evolutionary dimension can be achieved through the concept of phylogenetic diversity (PD) (Faith, 1992). Thanks to the theoretical and practical advances of phylogenetic systematics, it is possible to understand the relative importance of evolutionary and ecological forces in shaping biological communities (Martin, 2002; Webb et al., 2002; Cavender-Bares & Wilczek, 2003). Studies on PD have focused mainly on groups such as plants, birds, and mammals, and assemblages at large geographic scales or in priority areas for conservation (Posadas et al., 2001; Webb et al., 2002), whereas understanding and describing the PD of communities at local scales, especially in amphibians and reptiles, or in agroecological systems, has received very little attention.

Although intuitive, to understand the historical processes that have shaped ecological communities, it is necessary to clearly define the species composition of such assemblages; nonetheless, our knowledge on the distribution of species is still sparse, and the species identification of groups with complex morphology and taxonomy remains a challenge. For instance, the composition and structure of amphibian and reptile communities associated with coffee plantations (in the northern Andes) are well documented (Paéz et al., 2002; Palacio-Baena et al., 2006; Rojas-Morales et al., 2011; Rojas-Morales et al., 2014; Vargas-Salinas & Aponte-Gutiérrez, 2016; Román-Palacios et al., 2017; Duarte-Marín et al., 2018); however, other areas above the coffee line (~1,800 m a.s.l.) have received less attention (Rueda-Almonacid, 1999; Bernal & Lynch, 2008), and no sampling that documents the diversity of these lineages in highland regions dedicated to agriculture has been implemented. Consequently, to recognize the diversity of this region, it is of utmost importance not only to develop biotic inventories, but also to implement rigorous methods for species identification.

In this case, DNA barcoding provides an operational framework for species identification, increasing the speed, objectivity, and efficiency of the process (Hebert et al., 2003; Collins et al., 2012). However, despite its relevance, the herpetological diversity present in Colombia is poorly represented in the Barcode of Life data system (BOLD Systems, https://www.boldsystems.org/; see Guarnizo et al., 2015; Gaytán et al., 2020), and thus the creation of DNA barcodes for this fauna is essential. Herein, we developed an inventory of amphibians and reptiles in forests associated with avocado (*Persea americana*) cv. Hass plantations and used DNA barcodes to corroborate the taxonomic identity of the species. Subsequently, we implemented the PD as a measure to compare the diversity between sampling sites.

MATERIALS AND METHODS

Study site

Fieldwork was done in four production units (PU) —Huertos, Pradera, Teresita, Playa Rica— of avocado (*Persea americana*) cv. Hass on the eastern slope of the Western Cordillera of Colombia (Department of Risaralda, municipalities of Guática and Quinchía; Fig. 1). The four PUs are close to each other (1.5–3.0 km apart) and have a total area of 3,560,000 m², of which 940,000 m² (26.5 %) are forests (Huertos: 2,320,000 m² total area, 30.0 % forest cover; Pradera: 250,000.0 m², 18.0 % forest; Teresita:

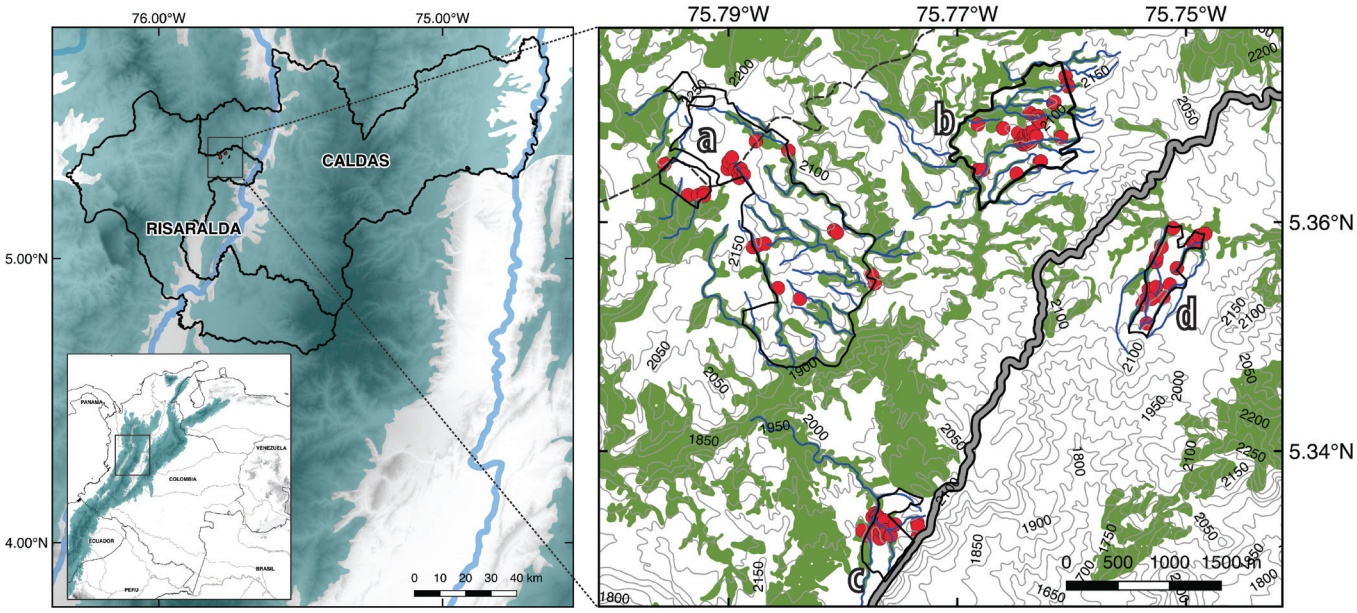

Figura 1. Izquierda: Área de estudio en Colombia y el departamento de Risaralda. Derecha: Detalle de las Unidades Productivas (Huertos, A; Playa Rica, B; Pradera, C; Teresita, D) mostrando cobertura forestal (verde), cuerpos de agua (azul), puntos de muestreo (rojo) y caminos primarios (gris).

Figure 1. Left: Study area in Colombia and the department of Risaralda. Right: Detail of the Productive Units (Huertos, A; Playa Rica, B; Pradera, C; Teresita, D) showing forest cover (green), water bodies (blue), sampling points (red), and primary roads (gray).

210,000.0 m², 9.0 % forest; Playa Rica: 780,000.0 m², 23.0 % forest). The landscape is composed of a matrix of avocado trees and native forest fragments in different successional stages (Fig. 2). The life zone corresponds to humid montane forest (bh-M), ranging in elevation between 1,900–2,300 m a.s.l., with a mean annual temperature between 15–17 °C and annual precipitation between 2,200–2,500 mm (Fick & Hijmans, 2017).

Fieldwork

Two sampling periods were conducted, the first during the rainy season (between November 4th and 28th of 2019) and the second during the dry season (February 5th and 29th of 2020). The sampling effort was homogeneous across the four PUs, with the same number of hours/person/day at each PU (nine h/2 researchers/day). We implemented visual encounter surveys (VES) to detect and capture amphibians and reptiles (Crump & Scott, 1994; Urbina-Cardona et al., 2015).

Because the total area of the four PUs was small, the quadrat traversal method was implemented at predefined time intervals, covering diurnal (07:00–10:00 and 12:00–14:00) and nocturnal (20:00–02:00 am) periods (Corn & Bury, 1990; Aguirre-León, 2011). Different available microhabitats were sampled (e.g., leaf litter, tree trunks, stones, and streams) to eliminate the effect of ecological specialization on detectability.

Specimen collection and taxonomic identification

For each individual, we recorded sex, age class, date, time, geographic coordinates, elevation, and a description of the vegetation cover and microhabitat where it was found. Captured individuals were taken to a controlled environment where they were photographed and sacrificed following the Colombian legislation for wildlife management (Law 84 of 1989-Chapter V; Decree 1076 of 2015). Each specimen was measured, all the taxonomically informative characters were described, and a tissue sample (liver or muscle) was collected and preserved in 96 % ethanol. All specimens were fixed in airtight chambers with a 10 % buffered formalin solution for two or three days until transferred to 75 % ethanol. The collected specimens are deposited at the Colección Biológica Universidad EAFIT (Medellín, Colombia).

Species richness

To evaluate the effectiveness and completeness of sampling, we used rarefaction curves and species richness estimators (Colwell et al., 2004; Ficetola et al., 2013; Hernández-Ordóñez et al., 2015). Rarefaction curves based on species accumulation were generated from the number of individuals captured for each species using the Mao Tau estimator (*Sest*, Colwell et al., 2004, 2012). Also, we estimated the species richness at each PU—and

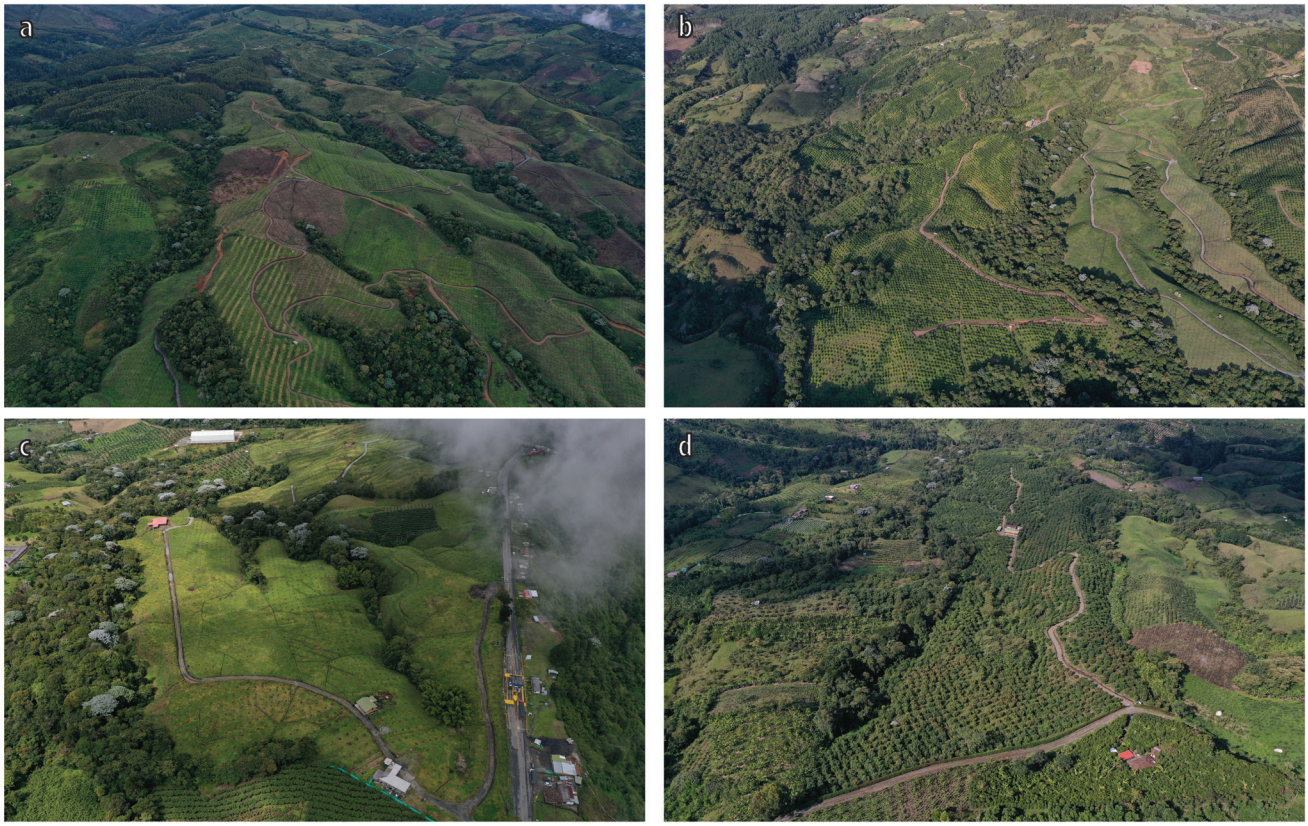

Figura 2. Cobertura vegetal asociada a la Unidades Productivas (PU) en el área de estudio. Huertos (A), Playa Rica (B), Pradera (C), Teresita (D).

Figure 2. Aerial photographs showing vegetation cover of the Productive Units (PU) in the study area. Huertos (A), Playa Rica (B), Pradera (C), Teresita (D).

in the overall community—by implementing non-parametric estimators based on abundance (Chao 1) and occurrence (Chao 2 and Jack 2; Colwell et al., 2004, 2012). The species richness estimators and their standard deviation were performed with a randomized sample of 100 replicates in EstimateS 9.1.0 (http://viceroy.eeb.uconn.edu/EstimateS; Colwell, 2013). Finally, to evaluate significant differences in the richness among sites, we used a non-parametric Kruskal-Wallis analysis of variance (ANOVA; Zar, 1996).

Laboratory work

To generate barcodes, DNA was extracted using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) following the manufacturer's instructions. We PCR-amplified a fragment of the mitochondrial marker Cytochrome C Oxidase Subunit I (*COI*) in 25 µl reactions using the primers and thermocycling conditions described in Folmer et al. (1994) and Nagy et al. (2012) for amphibians and reptiles, respectively. All primers were synthesized with M13 tails to improve sequencing (Ivanova et al., 2007). The resulting amplicons were sequenced using the M13 sequences on an ABI-3730xl automated sequencer (MCLAB, San Francisco, CA, USA). Chromatograms were edited and assembled in GENEIOUS® 9.1.8 (http://www.geneious.com), and low-quality sequences (e.g., chromatograms with double peaks or lack of open reading frame) were excluded from the analysis as they might represent pseudogenes (Song et al., 2008).

DNA barcoding and species identification

We obtained the Barcode Index Number (BIN) for each *COI* sequence; this identifier assigns sequences to operational taxonomic units (OTU), which are expected to correspond closely to species (Ratnasingham & Hebert, 2013). Additionally, we used the animal identification tool of BOLD Systems (Batch ID), using the database of barcode records (queried in August 2021) at the species level with a similarity threshold of 98 % (Guarnizo et al., 2015). DNA barcoding assigns a taxonomic identification to each sequence according to the BIN's first record (specimen and associated sequence); however, because this first record is not free of misidentifications (Collins & Cruickshank, 2012),

to corroborate the BIN identification, we implemented an independent identification through morphological characters. For amphibians, we followed Cochran & Goin (1970), Lynch & Rueda-Almoacid (1998, 1999), Savage (2002a), Savage (2002b), and Heyer (2005). For reptiles, we used the works of Peters & Orejas-M (1970), Ayala & Castro (1983), Perez-S & Moreno (1988), Harris (1994), Savage (2002a), Campbell & Lamar (2004), Romero-M et al. (2008), Velasco et al. (2010) and Moreno-A & Quintero-C (2015). The systematic arrangement for amphibians follows Frost (2023), for lizards we follow Uetz (2020), and for snakes Wallach et al. (2015).

Phylogenetic Diversity

Phylogenetic diversity (PD) is a measure of diversity calculated by summing the lengths of all branches of a topology across a set of species (Faith, 1992; Pellens & Grandcolas, 2016). We performed phylogenetic analyses using *COI* sequences to calculate the PD for each biotic group (amphibians and reptiles separately) and to compare PD values between PUs. For these analyses, a single representative sequence was used for each species; however, because it was not possible to obtain amplifications for some reptile species, we used sequences available in BOLD Systems or GenBank repositories. To root the topologies, we used the species *Dendropsophus columbianus* for amphibians, whereas our representative species of the clade Lepidosauria were used for the reptiles. All *COI* sequences were aligned using default parameters in MUSCLE (Edgar, 2004) implemented in GENEIOUS 9.1.8. The DNA evolution model that best-fit our data was evaluated in jModelTest2 using the Bayesian Information Criterion (BIC; Darriba et al., 2012). Five independent Maximum Likelihood (ML) searches were performed, and nodal support was assessed by 1,000 pseudo-replicated bootstrap analyses (BS) in GARLI 2.0 (Zwickl, 2006). Support values were annotated to the best-scoring ML tree using SUMTREES 3.3.1 (Sukumaran & Holder, 2010). With the resulting phylogenetic tree for each clade, we calculated the abundance-weighted PD of amphibian and reptile species for each PU in the R software (R Core Team, 2021) using the "Picante" package (Kembel et al., 2010).

RESULTS

Species composition and richness

A total of 228 individuals comprising seven amphibian species and 18 reptile species were recorded (Appendix 1). Across the four PUs, the number of species of amphibians per PU varied between four and six, while reptile richness per PU varied between seven and ten species. Even though Huertos (the largest PU with the most extensive forest cover) showed the greatest diversity of reptiles (10 species), there was no other apparent pattern in species richness associated with the area or forest cover across PUs for reptiles or amphibians (Table 1 and Appendix 1). All amphibians found (141 individuals) comprise the order Anura, specifically the families Bufonidae, Centrolenidae, Dendrobatidae, Hylidae, and Strabomantidae (Fig. 3, Table 1, and Appendix 1). The most diverse family was Strabomantidae, with three species (42.8 % of encountered amphibian diversity), while the other four amphibian families were represented by only one species each. Four of the seven anuran species were encountered in all four PUs, while two species were unique to a particular PU (Appendix 1 and 2).

For reptiles, all recorded species comprise the order Squamata, including eight species of lizards (suborder Lacertilia) and 10 species of snakes (suborder Serpentes; Fig. 4, Table 1, and

Tabla 1. Índices de diversidad para anfibios y reptiles por cada PU (Unidad Productiva) en el Departmento of Risaralda, municipalidades de Guática y Quinchía, Colombia.
Table 1. Diversity indices for amphibians and reptiles at each PU (Production Unit), in the Department of Risaralda, municipalities of Guática and Quinchía, Colombia.

Biotic group	Indices	Production Unit				
		Huertos	Pradera	Teresita	Playa Rica	Community
Amphibians	Phylogenetic Diversity	6.4	7.01	7.98	6.59	8.18
	Species Richness	4	5	6	5	7
	No. Individuals	39	37	26	39	141
Reptiles	Phylogenetic Diversity	7.46	4.86	4.94	5.24	9.59
	Species Richness	10	9	7	8	18
	No. Individuals	29	18	19	21	87

Appendix 1). The most species-rich reptile family was Dipsadidae (6 species, 33.3 % of the encountered reptile diversity), followed by Anolidae with 5 species (27.7 %), Gymnophtalmidae with two species (11.1 %), while the families Anomalepididae, Colubridae, Elapidae, Gekkonidae, and Viperidae each included one species (5.55 % of encountered reptile diversity; Appendix 1). One

Figura 3. Especies de anfibios registrados en este estudio. (A) *Rhinella horribilis*, (B) *Centrolene savagei*, (C) *Leucostethus fraterdanieli*, (D) *Dendropsophus columbianus*, (E) *Pristimantis achatinus*, (F-L) diferentes morfotipos de *Pristimantis palmeri*.

Figure 3. Amphibian species recorded in this study. (A) *Rhinella horribilis*, (B) *Centrolene savagei*, (C) *Leucostethus fraterdanieli*, (D) *Dendropsophus columbianus*, (E) *Pristimantis achatinus*, (F-L) different morphotypes of *Pristimantis palmeri*.

Figura 4. Especies de reptiles registrados en este estudio. (A) juvenil de *Atractus lehmanni*, (B) adulto de *Atractus lehmanni*, (C) juvenil de *Clelia equatoriana*, (D) *Dipsas sanctijoannis*, (E) *Erythrolamprus epinephelus*, (F) *Erythrolamprus bizona*, (G) *Micrurus mipartitus*, (H) *Bothriechis schlegelii*, (I) *Riama columbiana*, (J) *Pholidobolus vertebralis*, (K) *Lepidoblepharis duolepis*, (L) *Anolis ventrimaculatus*, (M) *Anolis danieli*, (N) *Anolis eulaemus*, (O) *Anolis mariarum.*

Figure 4. Reptile species recorded in this study. (A) juvenile of *Atractus lehmanni*, (B) adult of *Atractus lehmanni*, (C) juvenile of *Clelia equatoriana*, (D) *Dipsas sanctijoannis*, (E) *Erythrolamprus epinephelus*, (F) *Erythrolamprus bizona*, (G) *Micrurus mipartitus*, (H) *Bothriechis schlegelii*, (I) *Riama columbiana*, (J) *Pholidobolus vertebralis*, (K) *Lepidoblepharis duolepis*, (L) *Anolis ventrimaculatus, (M) Anolis danieli, (N) Anolis eulaemus, (O) Anolis mariarum.*

snake species (*Atractus lehmanni*) and two species of *Anolis* (*A. mariarum* and *A. ventrimaculatus*) were shared between the four PUs, whereas ten species were recorded from a single locality (Appendix 1 and 3). Despite some aforementioned differences in species composition, our analysis of variance indicates that these are not significant (X^2 = 1.255; p = 0.685), and as such, hereafter we focus on presenting and discussing our findings considering the four PUs as a single community.

The rarefaction curves for both groups did not evidence an asymptotic behavior (Fig. 5). This pattern of richness estimators reflects a greater expected number of species than encountered (Table 2). For amphibians, our sampling represents between 65–88 % of the expected diversity (up to four species estimated to have gone unsampled); similarly, for the reptiles, our work recorded between 63–76 % of the expected diversity, which means that at least 11 more species could be present in the area (Table 2). In terms of abundance, 80 % of the amphibian records (113 of 141) correspond to three species: *Pristimantis palmeri* was the most frequently recorded, followed by *P. achatinus*, and *Leucostethus fraterdanieli* (Appendix 1 and 2). For reptiles, the most abundant reptile species were *Anolis ventrimaculatus*, *A. mariarum*, and *Atractus lehmanni*, which represented 58.6 % of the total number of records (51 of 87; Appendix 1 and 3); conversely, seven species of lizards and snakes were recorded as singletons, representing locally rare or less abundant species (Appendix 3).

DNA barcoding

We obtained 49 *COI* sequences (between 571 and 711 bp) representing 20 of the 25 species (80 %) of amphibians and reptiles identified morphologically (Appendix 4). All the sequences and metadata were uploaded into the BOLD Systems and are accessible with the codes CARHE001-21–CARHE051-21 (Appendix 4). Noteworthy, 15 sequences (from nine species) represent the first record of this marker for Colombia, and 25 sequences (from 13 species) obtained a percentage of identity <98 %, which produced 13 new BINs (Appendix 4). We found 12 instances of inconsistency between our morphological and DNA-barcode identification methods; however, only two of these inconsistencies obtained an identity value >98 %, which corresponded to outdated taxonomies of the BOLD repository (Appendix 4). The other ten individuals with inconsistent identities showed identity values below 98 %. All 12 cases where a lack of consensus was found between identification methods are discussed below.

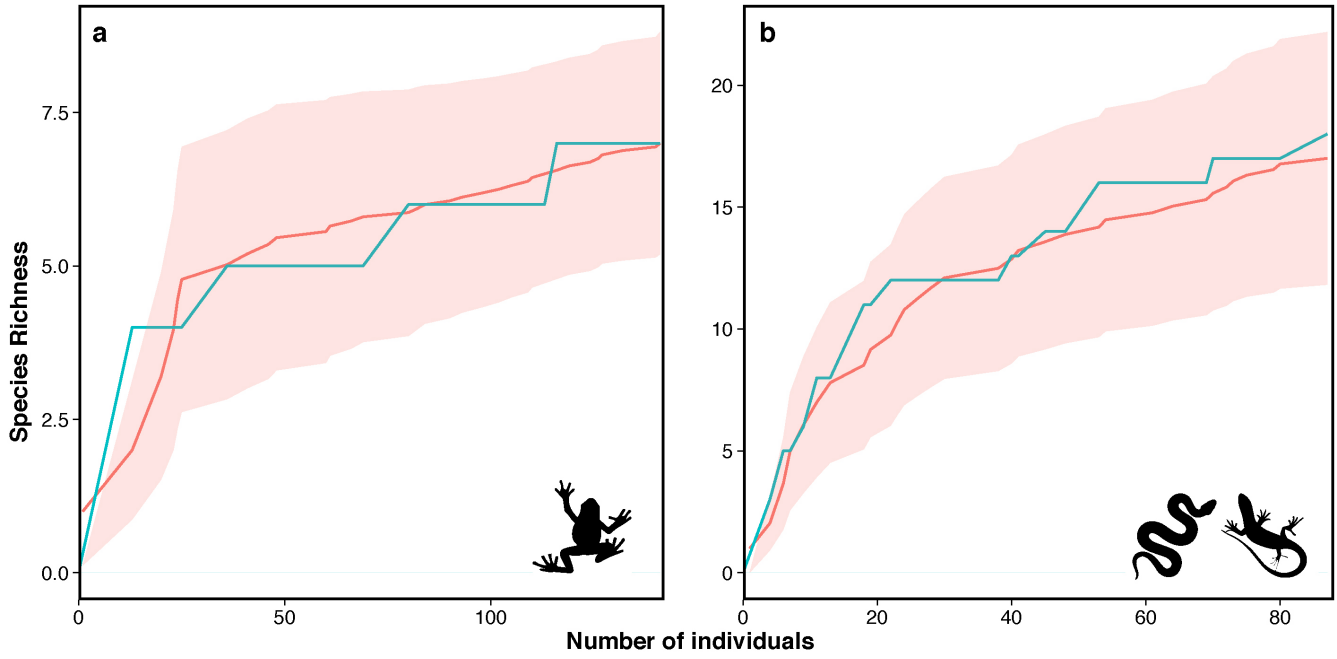

Figura 5. Curva de rarefacción para especies de anfibios (A) y reptiles (B). Línea roja: riqueza de especies esperada (Sest); línea azul: riqueza de especies observada (Sobs); área sombreada: 95 % intervalo de confianza de la riqueza esperada de especies.

Figure 5. Species rarefaction curves for amphibians (A) and reptiles (B). Red line: expected species richness (Sest); blue line: observed species richness (Sobs); shaded area: 95 % confidence interval of expected species richness.

Tabla 2. Índices de riqueza no paramétricos ponderados por la abundancia de anfibios y reptiles.

Table 2. Non-parametric richness indices weighted by abundance for amphibians and reptiles.

Biotic group	Diversity indices				
	$S_{(obs)}$	$S_{(est)}$	Chao-1	Chao-2	Jack-2
Amphibians	7	7	7.99	7.97	10.81
Reptiles	18	17	27.37	23.77	28.51

Phylogenetic diversity

Our final matrices for phylogenetic reconstructions included sequences from seven amphibian species (100 % completeness) and 15 reptile species (83.3 % completeness). We were unable to sequence the species *Anolis mariarum*, *Anolis* sp., and *Riama columbiana* (Appendix 5). For amphibians, the phylogenetic diversity of the four PUs (in decreasing order) was as follows: Teresita (PD=7.98), Pradera (PD=7.01), Playa Rica (PD=6.59), and Huertos (PD=6.40; Table 1); while for the reptiles, Huertos obtained the highest phylogenetic diversity (PD=7.46), followed by Playa Rica (PD=5.24), Teresita (PD=4.94), and Pradera (PD=4.86; Table 1).

DISCUSSION

Species composition and richness

The diversity of amphibians and reptiles in the Andean region, specifically the coffee axis in the departments of Antioquia, Caldas, and Quindío, is well-known (Paéz et al., 2002; Palacio-Baena et al., 2006; Rojas-Morales et al., 201, 2014; Vargas-Salinas & Aponte-Gutiérrez, 2016; Román-Palacios et al., 2017; Duarte-Marín et al., 2018); however, no relevant efforts have characterized the herpetological diversity in the department of Risaralda. For example, a data query at the SiB Colombia (https://www.gbif.org/) recovered only one record of amphibians and one record of reptiles for the municipality of Quinchía (*Pristimantis achatinus* and *Oxyrhopus petolarius*), and no records from Guática. In contrast, one study from the coffee axis documented the presence of 36 species of amphibians and 38 species of reptiles in an area within the department of Caldas between 700–5,300 m a.s.l., which is in close proximity to our study area (Rojas-Morales et al., 2014). Although the latter study recorded greater species richness than ours, their elevational range was also greater; nonetheless, it failed to document some species that our study found, such as the blind snake *Trilepida macrolepis*, the lizards *Anolis danieli* and *A. mariarum*, or the ground snake *Atractus lehmanni*. Conversely, Rojas-Morales et al. (2014) recorded "common" species of open areas, such as *Chironius monticola*, *Lampropeltis triangulum*, and *Mastigodryas boddaerti*, which were not recorded in our study. Extracting the reported species from Rojas-Morales et al. (2014) from a similar elevational gradient (1,900–2,300 m a.s.l.) as evaluated in our study, we observe that our study recorded only 38.8 % of the amphibian diversity (7 of 18 species) and 75 % of the reptile diversity (18 of 25 species); however, these differences in species richness may be explained by the differential scopes of study, as Rojas-Morales et al. (2014) was primarily based on a literature review of an area with extensive native forests (~150,000 ha), whereas our study involved fieldwork in an area mainly composed of avocado crops with only 355 ha of fragmented native forest.

Although our study is one of the few that contributes to the knowledge of the herpetological diversity in Risaralda, the true species richness of our study area is clearly underrepresented (Fig. 5, Table 2). It has been shown that the number of singletons and doubletons influences (positively) the magnitude of the richness estimates from Chao1 and Jack2 (Gotelli & Colwell, 2011). Not surprisingly, these two estimates showed the highest species richness considering that our sampling included 11 instances of singletons and doubletons (Appendix 1). These results support the need for additional sampling efforts that can overcome all factors affecting the detectability of species, such as climatic seasonality, rainfall regime, pathogens and parasites, demographic dynamics, habitat conservation status, and forest cover representativeness (only 26.5 % in our study site) (Kéry & Schmid, 2004).

Our analyses did not find the differences in species composition among the four PUs to be significant, even though only five species (20 %) were found to be common between the four areas, while six species (24 %) were exclusively found in a particular PU. These latter species are not characterized as rare or not abundant (Appendix 1); on the contrary, they are commonly found in Andean forests (Rojas-Morales et al., 2014), except for the blind snake *Trilepida macrolepis*, which is very inconspicuous

due to its fossorial habits (Savage, 2002a). The lack of significant differences between PUs could be explained by their proximity and the similarity of their abiotic conditions (see study area, Fig. 2), and based on the absence of an asymptotic behavior in the species rarefaction curves, future sampling might prove the presence of higher (shared) diversity across sampling sites.

DNA barcoding

Field identifications showed a strong correspondence (96.9 %) with the identifications provided by the DNA barcodes; however, 12 instances of inconsistency between the two methods were recovered (Appendix 4). Within these cases, two species exhibited >99 % similarity between our sequences and the BOLD-based identification, and we attribute this difference to outdated taxonomy in the BOLD portal rather than real incongruences between identification methods. In the first case, our morphological identification of *Rhinella horribilis* was assigned under BIN BOLD:AAB1186 to the name *R. marina*; however, using multiples lines of evidence, Acevedo et al. (2016) removed *R. horribilis* from the synonymy of *R. marina*, and allocated the cis-Andean populations to the nominal form *R. horribilis*, while the trans-Andean populations were assigned to the nominal form *R. marina*. Consequently, our population (and its morphological characters) corresponds to *R. horribilis*. Second, sequences from the morphologically identified *Leucostethus fraterdanieli* were assigned to *Colostethus fraterdanieli* using DNA barcoding (BIN BOLD:AAE9581); however, Marin et al. (2018) through morphology and genetic evidence transferred the *C. fraterdanieli*

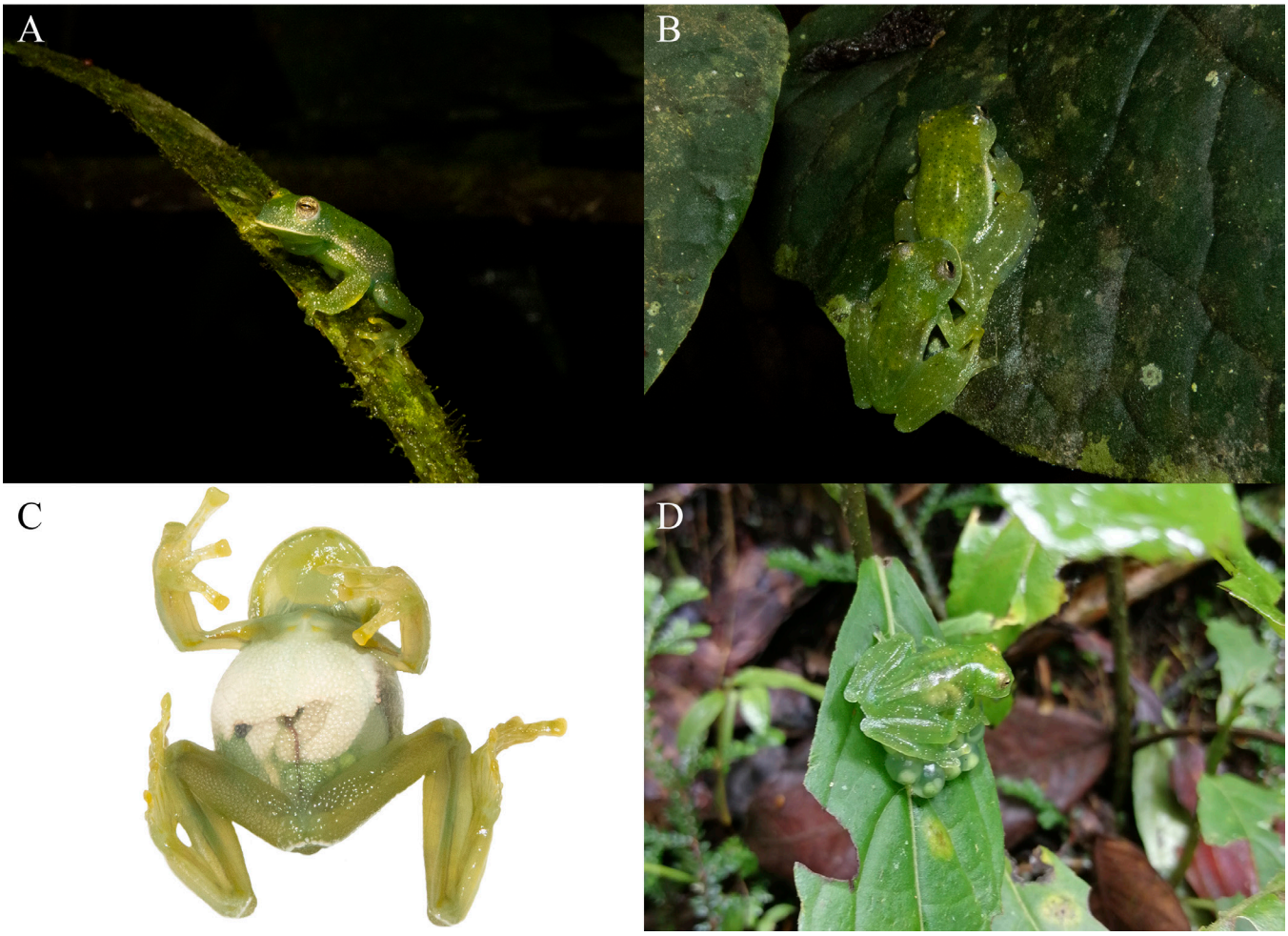

Figura 6. (A) Macho de *Centrolene savagei* posado sobre una hoja a 1,62 m sobre el nivel del suelo durante la actividad de canto; (B) pareja amplectante de *C. savagei*; (C) vista ventral de una hembra fértil de *C. savagei*; (D) cuidado parental de un macho de *C. savagei* sobre una nidada de huevos, que muestra el sustrato de oviposición y el color verde de la yema.

Figure 6. (A) *Centrolene savagei* male perching on a leaf at 1.62m above ground level during song activity; (B) amplectant *C. savagei* pair; (C) ventral view of fertile *C. savagei* female; (D) parental care of a male *C. savagei* over a clutch of eggs, showing oviposition substrate and green yolk color.

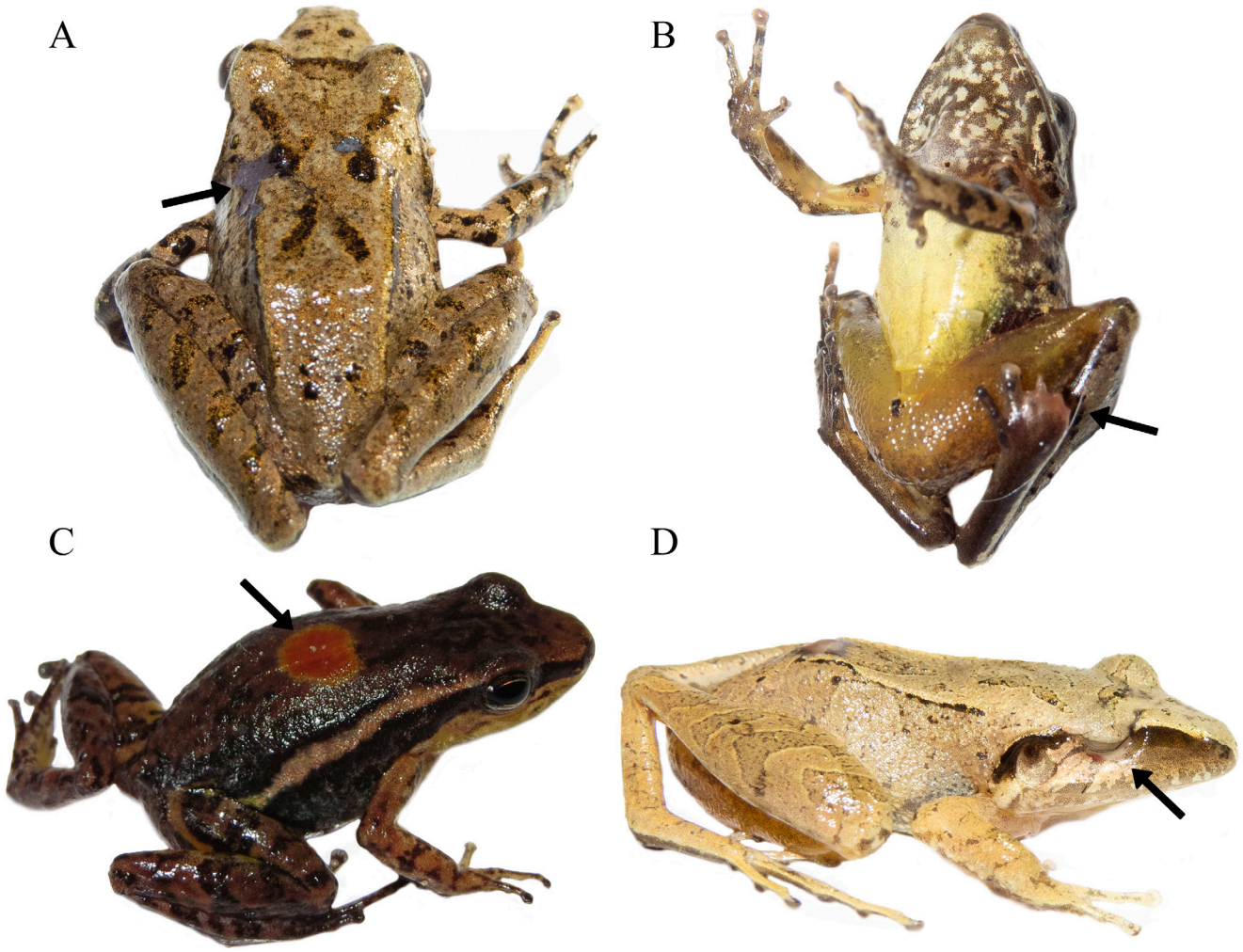

Figura 7. Individuos con malformaciones registradas en el área de estudio. (A) Desprendimiento de tejido cutáneo en *Pristimantis achatinus*; (B) pie izquierdo en vista plantar de *P. achatinus* con ausencia parcial de los dígitos I-II (braquidactilia) y ausencia total de los dígitos III-IV-V (ectrodactilia); (C) manchas de coloración inusual en el dorso de *Leucostethus fraterdanieli*; (D) anoftalmia en *P. achatinus*.

Figure 7. Individuals with malformations recorded in the study area. (A) Detachment of skin tissue in *Pristimantis achatinus*; (B) left foot in plantar view of *P. achatinus* with partial absence of digits I-II (brachydactyly), and total absence of digits III-IV-V (ectrodactyly); (C) unusual coloration spots on the dorsum of *Leucostethus fraterdanieli*; (D) anophthalmia in *P. achatinus*.

complex to the genus *Leucostethus*. Additionally, these authors demonstrate that the *Leucostethus* genus is not restricted to the Cis-Andean region of the western Amazon in Ecuador and Peru (Grant et al., 2017) but also occurs in the Andes of Colombia. Consequently, we follow Marin et al. (2018) as an up-to-date reference for the genus-level allocation of this species.

On the other hand, 10 of the mentioned inconsistencies were below the 98 % identity threshold suggested by BOLD (Appendix 4). In these instances, we retain our morphological identification according to the following considerations: 1) The morphologically identified *Centrolene savagei* was identified as *Cochranella savagei* under the BIN BOLD:ACK9980; however, we follow the taxonomical proposal for the family Centrolenidae of Guayasamín et al. (2009). 2) We captured a juvenile individual morphologically identified as *Pristimantis* sp., but the same individual was assigned to the name *Pristimantis* aff. *taeniatus* under BIN BOLD:AAV6492; however, due to the low percentage of identity (91.67 %) and the difficulty of observing diagnostic characters in the juvenile specimen, we opted to keep our taxonomic identification at the broader genus level. 3) The material identified morphologically as *Trilepida macrolepis* had

a very low identity (85 %) with the name assigned by the DNA barcode, *Rena humilis* (BIN BOLD: ADC8672); however, we retain our morphological identification due to the shared presence of the following characteristics in *T. macrolepis* description and our own material: supraocular scale present, rostral scale similar in size to supranasals, three supralabial scales, four infralabial scales, ventral scale row along the central axis of the tail, seven dorsal scale rows with dark brown and seven ventral scales with light brown (Pinto & Fernandes, 2017). Additionally, *R. humilis* is endemic to North America (Uetz, 2020). 4) The lizard *Anolis danieli* was assigned under the BIN BOLD:ACH6055 with an identity percentage of 93.81 %; however, this BIN does not have an assigned taxonomic identity. 5) Our material morphologically identified as *Anolis eulaemus* had an identity close to 89 % with *Anolis anoriensis* (BIN BOLD:ADM5331); in addition to the endemic distribution of *A. anoriensis* to the department of Antioquia (Velasco et al., 2010), both species can be easily distinguished by the color of their dewlap, with *A. eulaemus* having a brown dewlap (the same color as in our material), while *A. anoriensis* shows a green-colored dewlap (Velasco et al., 2010). 6) Our record of *Anolis* sp. is based on a juvenile specimen (EAFIT-R 0337), for which we were unable to observe reliable diagnostic characters. Although this individual was assigned to the taxon *A. calimae* according to the BIN BOLD:ACH5307, such identification is based on a very low identity (89.12 %). Therefore, given the lack of compelling morphological evidence, we decided to provide an identification only to the genus level. 7) Our morphology-based identification of *Atractus lehmanni* contrasts with the DNA barcoding identification of this taxon as *A. lasallei* (BOLD:ACL0112). Although the molecular identity is not low (97.6 %), we retain the morphological identification since our material exhibit the diagnostic characters of the original description: dorsal scale rows 17/17/17, tree temporals, two postocular, six infralabials (vs. 4 to 5 in *A. lasallei*), incomplete white nuchal collar (vs. absence of nuchal collar in *A. lasallei*), and black belly with square reddish-white spots (vs. yellow or cream belly with numerous black spots in the middle in *A. lasallei*) (Amaral, 1931; Boettger, 1898). However, we are aware that the taxonomy of the *Atractus genus* is problematic (Arteaga et al., 2017), and our identification could change with time. 8) The species *Dipsas sanctijoannis* was assigned under the BIN BOLD:ACJ9742 to the name *Dipsas* sp. In the absence of any contradictory results, we rely on our morphological identification based on the presence of a loreal scale, 1 to 3 preoculars, two postoculars, nine supralabials, 15 rows of dorsal scales in the middle, 70 to 98 divided subcaudals, and brown back with clear transverse bands, which have black or darker colored edges (Boulenger, 1911; Harvey, 2008). 9) *Erythrolamprus bizona* was assigned to the name *Lampropeltis triangulum* according to its BIN (BOLD:ACI6138); however, *Erythrolamprus* differs from *Lampropeltis* by some characters such as one preocular (vs. seven in *L. triangulum*), temporals 1+2 or 1+1+2 (vs. I + 2 or 2 + 3 in *L. triangulum*), presence of two black nuchal rings (vs. one nuchal ring in *Lampropeltis* species), and additionally *L. triangulum* is distributed exclusively in North America (Savage, 2002a; William, 1994). The high identity recovered by BOLD (96.77 %) suggests a likely misidentification of the original sequence in the portal. 10) Finally, the lizard *Lepidoblepharis duolepis* was identified as *Lepidoblepharis xanthostigma* according to the BIN BOLD:ACI5205; nonetheless, in Colombia, the latter species is distributed mainly in the lowlands of the Magdalena River Valley (as opposed to the higher-elevation populations of *L. duolepis*); furthermore, our specimens did not exhibit dark spots in the dewlap region and a pale "W" shaped band on the posterodorsal region of the head that are characteristic in *L. xanthostigma*, but are absent in *L. duolepis* (Ayala & Castro, 1983).

Phylogenetic diversity

In amphibians, a positive relationship was found between species richness (SR) and PD across PUs (Table 1). This finding is expected given the increased number of branches within the phylogeny as new species are added. In the event of two localities with equal SR, the higher PD is related to the branch lengths of the species included: the more distantly related the taxa are, the higher their branch lengths, and consequently the PD, will be. We observed one such instance of localities with the same SR: while Pradera showed a higher PD with species from four different families, Playa Rica exhibited a lower PD with species from only two families (Appendix 1). Conversely, in reptiles, no positive relationship was found between SR and PD (Table 1). This is most likely due to our taxon sampling lacking sequences from three species (*Anolis mariarum*, *Anolis* sp., and *Riama columbiana*), which impacts the branch lengths of the phylogeny (Appendix 5).

Given its straightforward implementation and understanding, SR is the most common measure to study biodiversity; however, the inclusion of PD metrics in biodiversity studies (e.g., inventories or monitoring) allows for a better understanding of species assemblages, as it considers not only the richness but also the evolutionary history of species (Cadotte et al., 2011; Belmaker & Jetz, 2013). Although the inclusion of PD in these types of studies is not new (Fenker et al., 2011; Gumbs et al., 2020; Paz et al., 2021), in Colombia is uncommon and has been restricted to a few examples in mammals (González-Caro et al., 2014; González-Maya et al., 2016); it is therefore necessary to expand this metric's implementation to inventories and monitoring projects of the country's herpetological diversity. We hope that our work can be taken as a proposal to include

this metric in future biotic inventories so that it can be used for comparative purposes in upcoming studies.

Conservation concerns: endemic and threatened species

Of the 25 species of amphibians and reptiles recorded, none are micro-endemic to the study area. However, *Centrolene savagei*, *Dendropsophus columbianus*, *Leucostethus fraterdanieli*, *Pristimantis palmeri*, and the reptiles *Anolis danieli*, *A. mariarum*, *Dipsas sanctijoannis*, *Lepidoblepharis duolepis*, and *Riama columbiana* are endemic to Colombia, specifically to highland Andean forests (Appendix 1; Frost, 2023). Among these, *Centrolene savagei* is the most sensitive in terms of its habitat requirements; this glass frog is distributed in the coffee axis in disturbed and undisturbed forests between 1,440–2,410 m a.s.l., it shows a preference for humid microhabitats, especially streams or lentic environments, and its presence has been considered a bioindicator of habitats with good quality (Vargas-Salinas et al., 2014). In the present study, we found 11 individuals of this species in all PUs and recorded four reproductive behaviors: vocalizations in males, one instance of amplexus, fertile females with eggs, and parental care behavior (Fig. 6). All these behaviors have been recently reported by Ospina-L et al. (2020), Navarro-Salcedo et al. (2021) and Vargas-Salinas et al. (2014, 2017).

None of recorded species are included in any of the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) or under any category of threat at the national level (Cruz-Rodríguez et al., 2017), but several species are found in the Red List of Threatened Species from the International Union for Conservation of Nature (IUCN) based on international evaluations (Appendix 1). Specifically, 19 species are categorized as "Least Concern" (IUCN, 2021), *Atractus lehmanni* as "Data Deficient" (IUCN, 2021), and *Riama columbiana* as an "Endangered" endemic species (IUCN, 2021). This latter lizard has a small area of distribution (1214 km2) and is restricted to the Andean Forest habitat in a narrow altitudinal strip that shows extensive fragmentation generated by both human and natural activities (Guhl, 2004; Frost, 2023).

Worldwide, much of the amphibian diversity is experiencing population declines associated with habitat degradation, emerging diseases and pathogens, invasive species, climate change, and pollutants (Luedtke et al., 2023). In particular, pesticides are a type of environmental contaminant that can cause significant mortalities, as various categories of amphibian body malformations have been documented and associated with the proximity of populations to agricultural systems (Davidson et al., 2002; Ankley et al., 2004; Lannoo, 2008; Lajmanovich et al., 2012). Although little information is known regarding these malformations in South American populations and their specific causes, there are some findings on the tolerance of embryos and tadpoles to different pH levels (Henao-García et al., 2011), the impact of agrochemicals on amphibian populations (Lajmanovich et al., 2012), and the possible consequences of using herbicides such as glyphosate in anurans (Ramírez-Jaramillo, 2019). In the present study, we recorded multiple instances of individuals exhibiting detachment of skin tissue (Fig. 7A), brachydactyly (metatarsal bones present but the number of phalanges reduced), ectrodactyly (total absence of some digits including the metatarsal bone; Fig. 7B), unusual coloration spots (Fig. 7C), and anophthalmia (absence of eyes; Fig. 7D; Cortés-Suárez, 2018).

It is noteworthy to highlight that the individuals with these malformations were collected in the avocado (*Persea americana*) matrix and the ecotone between crop and forest edge, both areas with greater exposure to pesticides and other chemical substances compared to the forest interior. Although there is prevailing evidence on the effects of pesticides on amphibian populations (see above), our findings were accidental, and we did not test to confirm the causal relationship between these malformations and the pesticides used in the agricultural system where the fieldwork took place. Amphibians play an essential role in the trophic guild of aquatic and terrestrial habitats (being both consumers and prey), and their abundance in the tropics is relatively high, so the effects of amphibian losses are likely to extend to other taxa, such as reptiles and mammals (Zipkin et al., 2020). It is therefore essential to increase biological inventories conducted in agroecological systems, as this will provide a better understanding of the impact of modified landscapes on their herpetological communities.

CONCLUSIONS

The diversity of amphibians and reptiles in the department of Risaralda, Colombia, shows a deficit of knowledge compared to other nearby departments in the coffee-growing region. Using a combination of morphology and DNA barcoding, the present study reports seven species of amphibians (4 endemic to Colombia) and 18 species of reptiles (five endemic) from four agroecosystems in Risaralda, although the observed richness was almost certainly underestimated considering the non-asymptotic nature of the rarefaction curve. Our results support the need for greater sampling efforts to recognize the region's true biodiversity, as this will allow a better understanding of the complex dynamics that can be observed in modified landscapes. The presence of several individuals of the glass frog *Centrolene savagei* might be considered a sign of excellent habitat quality,

but on the contrary, we also found multiple individuals of amphibians with signs of body malformations that could be associated with the use of pesticides. These contradictory results demonstrate that we still need to understand the extent to which forest remnants associated with agroecosystems are important for the long-term viability of threatened species and the impacts that agricultural practices impose on those species.

Our study generated 49 total sequences, 15 of which represent the first DNA barcode for nine species in Colombia (three endemics), reinforcing the importance of not only implementing DNA barcoding studies at large geographical scales, but also in inventories at local scales such as this one (Bergsten et al., 2012). In addition, we show that the *COI* sequences generated for barcoding studies can also be used for downstream analyses like evaluating the PD of communities. The PD is a crucial component when maximizing biodiversity conservation efforts, as it can provide a better understanding of the long-term viability and response capacity of an ecosystem and the unique and irreplaceable evolutionary processes of each community.

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APPENDICES

Appendix 1. Species richness and abundance of amphibians and reptiles at each PU. *Endemic species to Colombia, IUCN threat categories: Data Deficient (DD) and Endangered (EN) (see Discussion).

Apéndice 1. Riqueza de especies y abundancia de anfibios y reptiles en cada UP. *Especies endémicas de Colombia, categorías de amenaza de la UICN: Datos insuficientes (DD) y En peligro de extinción (EN) (ver Discusión).

Order	Family	Species	Number of individuals per Production Unit				Total Individuals
			Huertos	La Pradera	La Teresita	Playa Rica	
Anura	Bufonidae	*Rhinella horribilis*			1		1
	Centrolenidae	*Centrolene savagei**	1	3	3	4	11
	Strabomantidae	*Pristimantis achatinus*	13	6	8	12	39
		*Pristimantis palmeri**	12	17	4	14	47
		Pristimantis sp.				1	1
	Dendrobatidae	*Leucostethus fraterdanieli**	13	4	2	8	27
	Hylidae	*Dendropsophus columbianus**		7	8		15

Appendix 1 (cont.). Species richness and abundance of amphibians and reptiles at each PU. *Endemic species to Colombia, IUCN threat categories: Data Deficient (DD) and Endangered (EN) (see Discussion).

Apéndice 1 (cont.). Riqueza de especies y abundancia de anfibios y reptiles en cada PU. *Especies endémicas de Colombia, categorías de amenaza de la UICN: Datos insuficientes (DD) y En peligro de extinción (EN) (ver Discusión).

Order	Family	Species	Number of individuals per Production Unit				Total Individuals
			Huertos	La Pradera	La Teresita	Playa Rica	
Squamata	Anomalepididae	*Trilepida macrolepis*	1				1
	Colubridae	*Dendrophidion* sp.		1			1
	Anolidae	*Anolis danieli**	1				1
		Anolis eulaemus	3		1	1	5
		*Anolis mariarum**	5	3	3	1	12
		Anolis sp.		1			1
		Anolis ventrimaculatus	4	5	6	9	24
	Dipsadidae	*Atractus lehmanni*[DD]	4	2	5	4	15
		Atractus sp.		1			1
		Clelia equatoriana			1		1
		*Dipsas sanctijoannis**				2	2
		Erythrolamprus bizona				1	1
		Erythrolamprus epinephelus	1	1	1		3
	Elapidae	*Micrurus mipartitus*		1			1
	Gekkonidae	*Lepidoblepharis duolepis**	5				5
	Gymnophtalmidae	*Pholidobolus vertebralis*	3		2	2	7
		*Riama columbiana**[EN]		3			3
	Viperidae	*Bothriechis schlegelii*	2			1	3

Appendix 2. Abundance and relative abundance (RA) of amphibian species recorded in each of the four PUs individually and across all four PUs. *Endemic species to Colombia.

Apéndice 2. Abundancia y abundancia relativa (RA) de especies de anfibios registradas en cada una de las cuatro UP individualmente y en las cuatro UP. *Especie endémica a Colombia.

Species	Huertos	RA	Pradera	RA	Teresita	RA	Playa Rica	RA	Total Ind.	RA Total
Rhinella horribilis	0	0	0	0	1	0.04	0	0	1	0.01
*Centrolene savagei**	1	0.03	3	0.08	3	0.12	4	0.1	11	0.08
Pristimantis achatinus	13	0.33	6	0.16	8	0.31	12	0.31	39	0.28
*Pristimantis palmeri**	12	0.31	17	0.46	4	0.15	14	0.36	47	0.33
Pristimantis sp.	0	0	0	0	0	0	1	0.03	1	0.01
*Leucostethus fraterdanieli**	13	0.33	4	0.11	2	0.08	8	0.21	27	0.19
*Dendropsophus columbianus**	0	0	7	0.19	8	0.31	0	0	15	0.11
Total	**39**	**1**	**37**	**1**	**26**	**1**	**39**	**1**	**141**	**1**

Appendix 3. Abundance and relative abundance (RA) of reptile species recorded in each of the four PUs individually and across all four PUs. *Endemic species to Colombia.

Apéndice 3. Abundancia y abundancia relativa (RA) de especies de reptiles registradas en cada una de las cuatro UP individualmente y en las cuatro UP. *Especie endémica a Colombia.

Species	Huertos	RA	Pradera	RA	Teresita	RA	Playa Rica	RA	Total Ind.	RA Total
Trilepida macrolepis	1	0.03	0	0	0	0	0	0	1	0.01
Dendrophidion sp.	0	0	1	0.06	0	0	0	0	1	0.01
*Anolis danieli**	1	0.03	0	0	0	0	0	0	1	0.01
Anolis eulaemus	3	0.1	0	0	1	0.05	1	0.05	5	0.06
*Anolis mariarum**	5	0.17	3	0.17	3	0.16	1	0.05	12	0.14
Anolis sp.	0	0	1	0.06	0	0	0	0	1	0.01
Anolis ventrimaculatus	4	0.14	5	0.28	6	0.32	9	0.43	24	0.28
*Atractus lehmanni^*	4	0.14	2	0.11	5	0.26	4	0.19	15	0.17
Atractus sp.	0	0	1	0.06	0	0	0	0	1	0.01
Clelia equatoriana	0	0	0	0	1	0.05	0	0	1	0.01
*Dipsas sanctijoannis**	0	0	0	0	0	0	2	0.1	2	0.02
Erythrolamprus bizona	0	0	0	0	0	0	1	0.05	1	0.01
Erythrolamprus epinephelus	1	0.03	1	0.06	1	0.05	0	0	3	0.03
Micrurus mipartitus	0	0	1	0.06	0	0	0	0	1	0.01
*Lepidoblepharis dualepis**	5	0.17	0	0	0	0	0	0	5	0.06
Pholidobolus vertebralis	3	0.1	0	0	2	0.11	2	0.1	7	0.08
*Riama columbiana**	0	0	3	0.17	0	0	0	0	3	0.03
Bothriechis schlegelii	2	0.07	0	0	0	0	1	0.05	3	0.03
Total	29	1	18	1	19	1	21	1	87	1

Appendix 4. Individuals sequenced in this study. New public BINs in BOLD Systems are shown in bold. *New sequences for Colombia. ^Inconsistencies between morphological identification and the DNA barcode identification provided by BOLD Systems (see Discussion).

Apéndice 4. Individuos secuenciados en este estudio. Los nuevos BIN públicos en BOLD Systems se muestran en negrita. *Nuevas secuencias para Colombia. ^Inconsistencias entre la identificación morfológica y la identificación del código de barras de DNA proporcionada por BOLD Systems (ver Discusión).

BOLD ID	Morphological identification	Identity (%)	BIN	Barcode identification at BOLD Systems
CARHE010-21	*Rhinella horribilis* ^	99.39	BOLD:AAB1186	*Rhinella marina*
CARHE007-21	*Centrolene savagei*^	94.79	BOLD:ACK9980	*Cochranella savagei*
CARHE021-21	*Centrolene savagei*^	94.79	BOLD:ACK9980	*Cochranella savagei*
CARHE017-21	*Pristimantis achatinus*	100	BOLD:ACH5902	*Pristimantis achatinus*
CARHE020-21	*Pristimantis achatinus*	100	BOLD:ACH5902	*Pristimantis achatinus*
CARHE009-21	*Pristimantis achatinus*	100	BOLD:ACH5902	*Pristimantis achatinus*
CARHE023-21	*Pristimantis palmeri*	100	BOLD:AAT9612	*Pristimantis palmeri*
CARHE001-21	*Pristimantis palmeri*	99.85	BOLD:AAT9612	*Pristimantis palmeri*
CARHE002-21	*Pristimantis palmeri*	99.85	BOLD:AAT9612	*Pristimantis palmeri*
CARHE006-21	*Pristimantis palmeri*	100	BOLD:AAT9612	*Pristimantis palmeri*
CARHE008-21	*Pristimantis palmeri*	100	BOLD:AAT9612	*Pristimantis palmeri*
CARHE012-21	*Pristimantis palmeri*	100	BOLD:AAT9612	*Pristimantis palmeri*
CARHE014-21	*Pristimantis palmeri*	99.85	BOLD:AAT9612	*Pristimantis palmeri*
CARHE015-21	*Pristimantis palmeri*	99.85	BOLD:AAT9612	*Pristimantis palmeri*
CARHE016-21	*Pristimantis palmeri*	99.85	BOLD:AAT9612	*Pristimantis palmeri*
CARHE024-21	*Pristimantis palmeri*	100	BOLD:AAT9612	*Pristimantis palmeri*
CARHE018-21	*Pristimantis* sp.*^	91.67	BOLD:AAV6492	*Pristimantis* aff. *taeniatus*
CARHE003-21	*Leucostethus fraterdanieli*^	99.84	BOLD:AAE9581	*Colostethus fraterdanieli*
CARHE004-21	*Leucostethus fraterdanieli*^	99.69	BOLD:AAE9581	*Colostethus fraterdanieli*
CARHE013-21	*Leucostethus fraterdanieli*^	99.69	BOLD:AAE9581	*Colostethus fraterdanieli*
CARHE005-21	*Dendropsophus columbianus*	98.93	BOLD:ACC1738	*Dendropsophus columbianus*
CARHE011-21	*Dendropsophus columbianus*	99.06	BOLD:ACC1738	*Dendropsophus columbianus*
CARHE019-21	*Dendropsophus columbianus*	99.22	BOLD:ACC1738	*Dendropsophus columbianus*
CARHE022-21	*Dendropsophus columbianus*	99.22	BOLD:ACC1738	*Dendropsophus columbianus*
CARHE040-21	*Trilepida macrolepis* ^	85.45	BOLD:ADC8672	*Rena humilis*
CARHE039-21	*Anolis danieli*^	93.81	BOLD:ACH6055	-

Appendix 4 (cont.). Individuals sequenced in this study. New public BINs in BOLD Systems are shown in bold. *New sequences for Colombia. ^Inconsistencies between morphological identification and the DNA barcode identification provided by BOLD Systems (see Discussion).

Apéndice 4 (cont.). Individuos secuenciados en este estudio. Los nuevos BIN públicos en BOLD Systems se muestran en negrita. *Nuevas secuencias para Colombia. ^Inconsistencias entre la identificación morfológica y la identificación del código de barras de DNA proporcionada por BOLD Systems (ver Discusión).

BOLD ID	Morphological identification	Identity (%)	BIN	Barcode identification at BOLD Systems
CARHE036-21	*Anolis eulaemus*^	89.67	BOLD:ADM5331	*Anolis anoriensis*
CARHE043-21	*Anolis eulaemus*^	89.52	BOLD:ADM5331	*Anolis anoriensis*
CARHE029-21	*Anolis* sp.^	89.12	BOLD:ACH5307	*Anolis calimae*
CARHE025-21	*Anolis ventrimaculatus*	94.2	BOLD:ACI0307	*Anolis ventrimaculatus*
CARHE044-21	*Anolis ventrimaculatus*	94.36	BOLD:ACI0307	*Anolis ventrimaculatus*
CARHE049-21	*Anolis ventrimaculatus*	94.22	BOLD:ACI0307	*Anolis ventrimaculatus*
CARHE050-21	*Anolis ventrimaculatus*	94.39	BOLD:ACI0307	*Anolis ventrimaculatus*
CARHE051-21	*Anolis ventrimaculatus*	94.36	BOLD:ACI0307	*Anolis ventrimaculatus*
CARHE038-21	*Atractus lehmanni*^	97.64	BOLD:ACL0112	*Atractus lasallei*
CARHE045-21	*Atractus lehmanni*^	97.66	BOLD:ACL0112	*Atractus lasallei*
CARHE027-21	*Atractus lehmanni*^	97.66	BOLD:ACL0112	*Atractus lasallei*
CARHE032-21	*Atractus lehmanni*^	97.66	BOLD:ACL0112	*Atractus lasallei*
CARHE033-21	*Clelia equatoriana*	97.86	BOLD:ACK9778	*Clelia equatoriana*
CARHE047-21	*Dipsas sanctijoannis**^	91.25	BOLD:ACJ9742	*Dipsas* sp.
CARHE048-21	*Erythrolamprus bizona*^	96.77	BOLD:ACI6138	*Lampropeltis triangulum*
CARHE031-21	*Erythrolamprus epinephelus*	99.16	BOLD:ACK9765	*Erythrolamprus epinephelus*
CARHE034-21	*Erythrolamprus epinephelus*	99.49	BOLD:ACK9765	*Erythrolamprus epinephelus*
CARHE026-21	*Micrurus mipartitus*	99.01	BOLD:ACI5866	*Micrurus mipartitus*
CARHE030-21	*Lepidoblepharis duolepis**^	83.83	BOLD:ACI5205	*Lepidoblepharis xanthostigma*
CARHE037-21	*Lepidoblepharis duolepis**^	83.83	BOLD:ACI5205	*Lepidoblepharis xanthostigma*
CARHE035-21	*Bothriechis schlegelii*	95.19	BOLD:ACJ9812	*Bothriechis schlegelii*
CARHE042-21	*Bothriechis schlegelii*	95.5	BOLD:ACJ9812	*Bothriechis schlegelii*
CARHE046-21	*Bothriechis schlegelii*	95.5	BOLD:ACJ9812	*Bothriechis schlegelii*

Appendix 5. *COI* sequences used for the phylogenetic reconstruction.

Apéndice 5. Secuencias *COI* utilizadas para la reconstrucción filogenética.

Order	Family	Identification	Voucher	BOLD/ GenBank	*COI* (pb)	Source
Anura	Bufonidae	*Rhinella horribilis*	JCA1353	CARHE010-21	663	This study
	Centrolenidae	*Centrolene savagei*	JCA1303	CARHE007-21	663	This study
	Strabomantidae	*Pristimantis achatinus*	JCA1331	CARHE009-21	663	This study
		Pristimantis palmeri	JCA1413	CARHE014-21	663	This study
		Pristimantis sp.	JCA1430	CARHE018-21	663	This study
	Dendrobatidae	*Leucostethus fraterdanieli*	JCA1273	CARHE003-21	663	This study
	Hylidae	*Dendropsophus columbianus*	JCA1448	CARHE022-21	663	This study
Squamata	Leptotyphlopidae	*Trilepida macrolepis*		CARHE040-21	663	
			JCA1385			This study
	Colubridae	*Dendrophidion percarinatum*	CH:5403	MH140114	654	Mulcahy et al. 2018
	Dactyloidae	*Anolis danieli*	JCA1384	CARHE039-21	588	This study
		Anolis eulaemus	JCA1372	CARHE036-21	631	This study
		Anolis sp.	JCA1304	CARHE029-21	605	This study
		Anolis ventrimaculatus	JCA1254	CARHE025-21	663	This study
	Dipsadidae	*Atractus lehmanni*	JCA1406	CARHE045-21	663	This study
		Clelia equatoriana	JCA1361	CARHE033-21	661	This study
		Dipsas sanctijoannis	JCA1416	CARHE047-21	660	This study
		Erythrolamprus bizona	JCA1421	CARHE048-21	658	This study
		Erythrolamprus epinephelus	JCA1367	CARHE034-21	663	This study
	Elapidae	*Micrurus mipartitus*	JCA1278	CARHE026-21	651	This study
	Gekkonidae	*Lepidoblepharis duolepis*	JCA1320	CARHE030-21	663	This study
	Gymnophtalmidae	*Pholidobolus vertebralis*	CH:5627	MH140319	654	Mulcahy et al. 2018
	Viperidae	*Bothriechis schlegelii*	JCA1368	CARHE035-21	649	This study