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Unexpected biogeographical patterns? The case of the apparently widespread *Trachycephalus typhonius* (Anura: Hylidae)

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Taxonomic and spatial biases limit the understanding of biodiversity patterns, particularly in the Neotropics, with consequences in systematics, biogeography and conservation. Here, we address the impact of these biases by focusing on the widespread species *Trachycephalus typhonius*. Despite its common occurrence across its range, misidentifications and cryptic diversity have obscured its true phylogenetic and biogeographic histories. Through extensive field sampling and molecular analysis of cis- and trans-Andean localities, we revealed substantial genetic divergence within *T. typhonius*, which suggests the presence of multiple cryptic species. The samples from Orinoquia (cis-Andes) and Magdalena Valley (trans-Andes) in Colombia, differ from *T. typhonius* sensu stricto in Guyana. At least three lineages were observed for *T. typhonius* across the continent, suggesting a complex diversification pattern shaped by major Neotropical barriers. Our results show the influence of both Andes and Amazon-Orinoquia on the phylogeographic structure and provide insights into the biogeographic history of *T. typhonius* sensu lato. We discuss the importance of addressing taxonomic and spatial biases, and highlight how geographic barriers and historical processes have influenced diversification in the Neotropics. Our findings illustrate the need for comprehensive sampling and molecular studies for 'common' species, to unravel hidden diversity, which is crucial for accurate biodiversity assessments, understanding evolutionary processes, refine biogeographic models, reassess species distributions and inform conservation strategies. Este resumen traducido al español está disponible en supplementary materials.

Keywords: cryptic diversity, Amazon, phylogeography, sampling biases, Neotropics

INTRODUCTION

Ecological and behavioural barriers to species dispersal cause patterns of genetic differentiation of lineages through vicariance processes (Guevara-Andino et al., 2024) as a result of the geologic and climatic history over the past several million years (Gutiérrez-García & Vázquez-Domínguez, 2013). Geographic structure has a broad spectrum of effects on dispersal and isolation patterns in widespread lowland species. The strength of the barrier varies with the dispersal mode and elevational range of the species (Smith et al., 2014; Salgado-Roa et al., 2024). For instance, the Andes range is a well-known barrier to species dispersal in the Neotropics, splitting lowland lineages into what is known as cis- and trans-Andean biotas (relative to Amazonas) (Gregory-Wodzick, 2000; Hoorn et al., 2010). Although broad distributions might suggest gene flow across populations, several studies on Amazonian amphibians have revealed deep genetic divergences between lineages (Rojas et al., 2018; Jaramillo et al., 2020; Vacher et al., 2020), highlighting the cryptic diversity hidden and the role of historical barriers even within continuous distributions and habitats. A challenge arises with high diversity of cryptic species groups, where their morphologically indistinguishable traits hides the identity and distribution of the actual lineages (Fouquet et al., 2021).

Understanding biodiversity patterns in the Neotropics faces multiple challenges, including taxonomic and spatial biases (Gotelli et al., 2023), known as the Linnean and Wallacean shortfalls respectively (Whittaker et al., 2005; Riddle et al., 2008; Vasconcelos et al., 2019). Regarding the Linnean shortfall, species rarely encountered in the field tend to be sought after and are therefore over-represented in museum collections relative to their true abundance (Kruckeberg & Rabinowitz, 1985) while common species tend to be under-represented. The consequences of such bias can obscure critical trends within species complexes that are perceived as common (Troudet et al., 2017).

There is a clear bias in biodiversity research towards rare species, ignoring common ones because they are considered non-charismatic (Magurran & Henderson, 2003; Monsarrat & Kerley, 2018). Thus, it overlooks the cryptic diversity, as has been demonstrated in species such as *Boana platanera* with *B. xerophylla* (Escalona et al., 2021), *Hyalinobatrachium tatayoi* with *H. fleischmanni* (Mendoza-Henao et al., 2020), five

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lineages hidden within *Ischnocnema guentheri* (Gehara et al., 2013) and four lineages hidden within *Centrolene buckleyi* (Amador et al., 2018; Franco-Mena et al., 2024). Even among potentially extinct *Atelopus* species, some were described as new to science after spending many years in collections under uncertain identifications (e.g. *A. ignescens* and *A. ardila*) (Coloma et al., 2010). Identification of these cases would increase the known amphibian diversity in the Neotropics by at least 25% over the currently recognised species (Lyra et al., 2017).

Related to the Wallacean shortfall (spatial bias), the availability of information is unevenly distributed across the globe, particularly the lack of data in species-rich tropics (Boitani et al., 2011). Sampling efforts are usually biased by multiple barriers, including geographical location, accessibility (Oliveira et al., 2016) and socioeconomic disparities (Amano & Sutherland, 2013). Herpetological studies in South America often reflect a broader pattern of taxonomic and systematic research gaps in tropical regions, where key areas remain under-represented (Collen et al., 2008; Vásquez-Restrepo, 2021). A prominent example occurs in the north part of South America, in the limit between Central and South America, whose under-sampling regarding the vicinity is known as the "Colombian gap" (Vasquez-Restrepo, 2021). The exclusion or even the limited inclusion or representation of Colombian data in large-scale studies (e.g. Rivadeneira et al., 2018; Fouquet et al., 2021; Ttito & Catenazzi, 2021; Menéndez-Guerrero et al., 2024; Varela-Jaramillo et al., 2025) might bias the conclusions of new species descriptions and biogeographic studies. Complex geological processes such as the stepwise uplift of the Northern Andes and the subsequent isolation of the Amazon basin (Hoorn et al., 2010) have a central role in Neotropical diversification and continental biogeographic processes (Hazzi et al., 2018; Meseguer et al., 2022), creating ecological corridors and barriers, driving species evolution and dispersal across the continent (Pinto-Sánchez et al., 2012; Suarez et al., 2015; Mendoza et al., 2015; Hutter et al., 2017).

Current knowledge of the milky frog *Trachycephalus typhonius* (Anura: Hylidae) illustrates the taxonomic and spatial bias that widespread amphibians encounter. According to Frost (2025), this widespread species ranges in natural savannas and in lowlands from sea level to 800 m a.s.l. from southern Mexico to the north of Argentina, and was described by Linnaeus (1758) based on specimens from Paranamaribo, Suriname (Lavilla et al., 2010). Given their tolerance to human land transformation (Castellanos-Durán & Giraldo-Palacio, 2022; IUCN, 2023), the species is commonly reported in rapid inventories, but no detailed diagnostic characters are considered for individual determination, resulting in mistakes in species determination and profound consequences on the distribution of the species. Despite the possible or confirmed presence of multiple *Trachycephalus* species in Colombia (*T. coriaceus, T. jordani, T. resinifictrix, T. typhonius, T. venezolanus* and *T. cunauaru*) (Lynch, 2005; Frost, 2025), a rapid comparison of museum specimens assigned to *T. typhonius* from the Colombian Amazon shows several misidentifications, even considering the notorious differences in external morphology (Fig. 1).

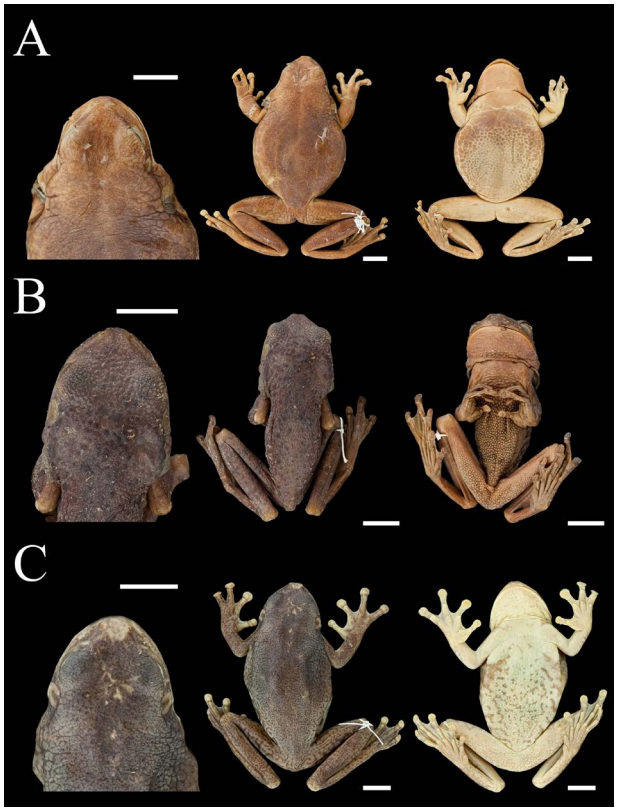

Figure 1. Morphological variability in museum specimens from Colombian Amazon formerly mis-assigned to *Trachycephalus typhonius*. **A.** IAvH-Am-02308 corresponds to *T.* cf. *coriaceus*, **B.** IAvH-Am-04632 corresponds to *T.* cf. *cunauaru* and **C.** IAvH-Am-04916 does not match the character combination of any of the species present in Colombia. **A.** and **B.** were collected in the Amacayacu National Natural Park and **C.** in Leticia municipality. White bars indicate 1 cm.

The paraphyletic condition of *T. typhonius* was also revealed in a molecular study carried out using samples from Ecuador (Ron et al., 2016). Ecuadorian Chocó sequences are related to cis-Andean *Trachycephalus*, whilst Amazonian *T. typhonius* (Peru and Ecuador) are related to *T. resinifictrix* and Venezuelan *T. typhonius*. In this study, Ron et al. (2016) assigned the name *T. macrotis* to Ecuadorian and Peruvian cis-Andean populations based on political boundaries rather than biological and geographical criteria pertinent to species delimitation. The study did not include samples from Central America and north-western South America; therefore, no definitive name applies to cis- and trans-Andean and Mesoamerican populations (see also Lavilla et al., 2010 for a nomenclatural history).

Molecular species identification using mtDNA helps resolve cases related to population structure, historical biogeographic barriers and cryptic diversity (Gehara et al., 2013; Guarnizo et al., 2015; Dudoit et al., 2018). Mitochondrial DNA markers have consistently been used to decipher genetic structuring in amphibian lineages (García-R et al., 2012; Crawford et al., 2013;

Franco-Mena et al., 2023). In this study, we used two mitochondrial regions to describe the genetic structure of *T. typhonius* sensu lato, proposing a hypothesis for its evolutionary history; and we use this as a case study to advocate for deeper research on widely distributed species. The inclusion of new molecular data across the species' range revealed previously unrecognised phylogeographic structure, clarified relationships among regional populations and highlighted inconsistencies in current taxonomy (Faivovich et al., 2005; Castellanos-Durán & Giraldo-Palacio, 2022). A careful study along its distributional range (including cis-Andes and trans-Andes) is not only taxonomically relevant but might also help elucidate the phylogenetic and biogeographic history of this clade (Blotto et al., 2021). We expect that the current genetic structure of *T. typhonius* reflects a vicariance event driven by the northern Andes as the main dispersal barrier, followed by secondary genetic structure for cis-Andes lineages.

MATERIALS & METHODS

Field sampling

We performed searches for data available in BOLD and GenBank databases for *T. typhonius* and other *Trachycephalus* species. The search returned samples from several countries and just one sequence available from Colombia. To uncover this bias, we processed tissue samples obtained during biodiversity inventory explorations in nine localities in Colombia (Table S1 in supplementary materials), including sites in the Caribbean (one sample) and Middle Magdalena Valley (seven samples, trans-Andean localities) and Orinoquian region (six samples, cis-Andean localities). Individuals were surveyed visually and auditorily (Angulo et al., 2006) in each site during free searches and transects between 18:00 h and 23:00 h, identifying microhabitats such as streams, ditches, lagoons and flooded areas. Each path had a variable length between 600 m and 2 km. A subsample of the total number of individuals found in the field was collected, and live photographs were taken of at least one specimen per species to obtain colouration data. Subsequently, the individuals were euthanised by applying topical 20% benzocaine following the protocol of Chen & Combs (1999). A sample of liver or muscle by specimen was stored in 96% ethanol solution and cryopreserved in the tissue collection of the Humboldt Institute (IAvH-CT) (Gonzalez & Arenas-Castro, 2017). Corresponding specimens were fixed with 10% analytical formalin, preserved in 70% ethanol (Cortez et al., 2006) and deposited in the amphibian collections of the Humboldt Institute (IAvH-Am).

Molecular analysis

DNA extraction from tissue was performed following the methodology described by Ivanova et al. (2006). Quantification of DNA concentration in the samples was estimated using the NanoDrop One instrument from Thermo Fisher Scientific. After extraction, amplification of mitochondrial genetic regions cytochrome c oxidase subunit 1 (COI) and 16S rRNA, was accomplished using a set of specific primers. For the COI region, a primer cocktail with LCO1490, HCO2198, Lep-F1 and Lep-R1 was used (Folmer et al., 1994; Hebert et al., 2004). On its part, for the 16S region, the primers 16SA-L and 16SB-H were employed (Palumbi et al., 1991).

The thermal cycling conditions for COI amplification involved an initial denaturation step at 94 °C for 3 minutes, followed by 5 cycles of denaturation at 94 °C for 30 seconds, annealing at 45 °C for 40 seconds, and extension at 72 °C for 1 minute. Subsequently, 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 51 °C for 40 seconds, and extension at 72 °C for 1 minute were carried out, followed by a final extension step at 72 °C for 5 minutes. On the other hand, for the 16S region, the PCR cycling conditions included an initial denaturation at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 40 seconds, and extension at 72 °C for 2 minutes, with a final extension step at 72 °C for 5 minutes.

Verification of amplification was carried out by visualising the PCR products by electrophoresis on 1.5% (w/v) agarose gels stained with GelRed® by Biotium. The successful amplicons were further purified enzymatically utilising the Exo-SAP protocol. Cleaned PCR products underwent Sanger sequencing, using an ABI 3500 XL genetic analyser from Applied Biosystem. The obtained chromatograms were then assembled and manually edited using the Geneious v10.2 software (Kearse et al., 2012). Nucleotide-sequences, traces files and images were uploaded to BOLD Systems (www.boldsystems.org/) and are available in the data set DS-TRCIH24 (Table S1 in supplementary material; dx.doi.org/10.5883/DS-TRCIH24).

Sequence divergence was estimated with Kimura's 2-parameter (K2P) nucleotide evolution model implemented by BOLD. To graphically represent the species divergence, we generated a Neighbour-Joining (NJ) tree based on K2P sequence divergence. Node support was computed with 1,000 bootstrap replicates. We compared the genetic distances from the lineages obtained in the delimitation analysis (based on strict consensus) from DNA barcoding in anurans (Lyra et al., 2017; Amador et al., 2018). To do so, we calculated the K2P-distances for 16S and COI genes in MEGA version 11 (Tamura et al., 2021).

Cluster analyses were conducted for the species dataset. Automatic Barcode Gap Discovery (ABGD, Puillandre et al., 2012) partitions clusters into candidate species based on a threshold obtained from the distribution of pairwise genetic distances within the same data set (Paz & Crawford, 2012; Guarnizo et al., 2015). This analysis was conducted with default parameters using the K2P model. Also, we used the Barcode Index Number (BIN) provided by BOLD Systems, which implements RESL (Refined Single Linkage; Ratnasingham & Hebert, 2013) to assign DNA barcodes to likely taxonomic entities available in the database. The BIN algorithm was designed to provide a conservative estimate of the actual species count (Floren et al., 2020).

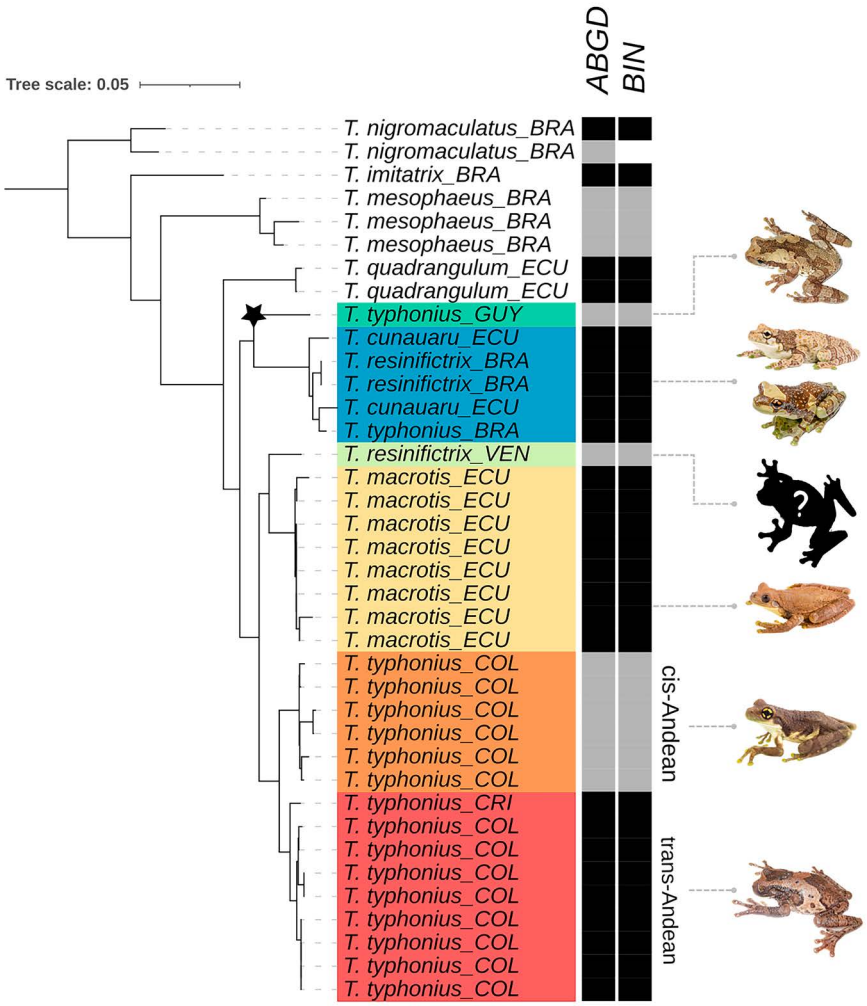

Figure 2. Automatic Barcode Gap Discovery (ABGD) and Barcode Index Number (BIN) clustering with COI for *Trachycephalus* species used in the study. The star indicates the *T. typhonius* sensu stricto from Guyana. See Table S1 in supplementary materials for localities.

The two genes were concatenated by using the cbind function in the ape package in R (Paradis et al., 2004) and phylogenetic tree was estimated by implementing Maximum likelihood analysis in RAxML 8.2.10 (Stamatakis, 2006) using the GTR evolution model, partitioning the dataset in the two genes independently. Maximum likelihood analysis was conducted using 10,000 rapid bootstrap analyses, the GTR+GAMMA evolution model and summarised support for the best tree.

We used the multi-rate tree process PTP (mPTP) model (Kapli et al., 2017) in the web server https://mptp.h-its.org on the multilocus tree inferred by Maximum Likelihood. This analysis incorporates different coalescence rates between clades, allowing for different levels of intraspecific genetic diversity (Kapli et al., 2017). Also, it uses the number of substitutions to identify significant changes in branching rates in a phylogeny, thereby they do not depend on the accuracy of ultrametric tree estimations. In addition, it may outperform other species delimitation methods when evolutionary distances are small (Pons et al., 2006).

RESULTS

We generated a final alignment of 1232 base pairs (bp) for 70 samples from nine species: *T. typhonius, T. coriaceus, T. nigromaculatus, T. imitatrix, T. mesophaeus, T. quadrangulum, T. cunauaru, T. resinifictrix* and *T. macrotis* including samples from Bolivia (3), Brazil (23), Colombia (14), Costa Rica (1), Ecuador (13), French Guiana (10), Guiana (2), Peru (1), Venezuela (2) and one sample from an unknown locality (Table S1 in supplementary materials). The obtained sequences represent unique haplotypes of the single genes. We did not detect any stop codons in the protein-coding gene (COI).

The ABGD analysis for COI sequences returns 11 candidate species with prior intraspecific divergences between 0.0017 and 0.0077. Under this scenario, cis- and trans- Andean samples became split into two isolated lineages (Fig. 2). The ABGD recovers only four species for intraspecific divergences between 0.0129 and 0.035, merging all samples of *T. macrotis, T. typhonius, T. cunauaru, T. quadrangulum* and *T. resinifictrix* in a single lineage.

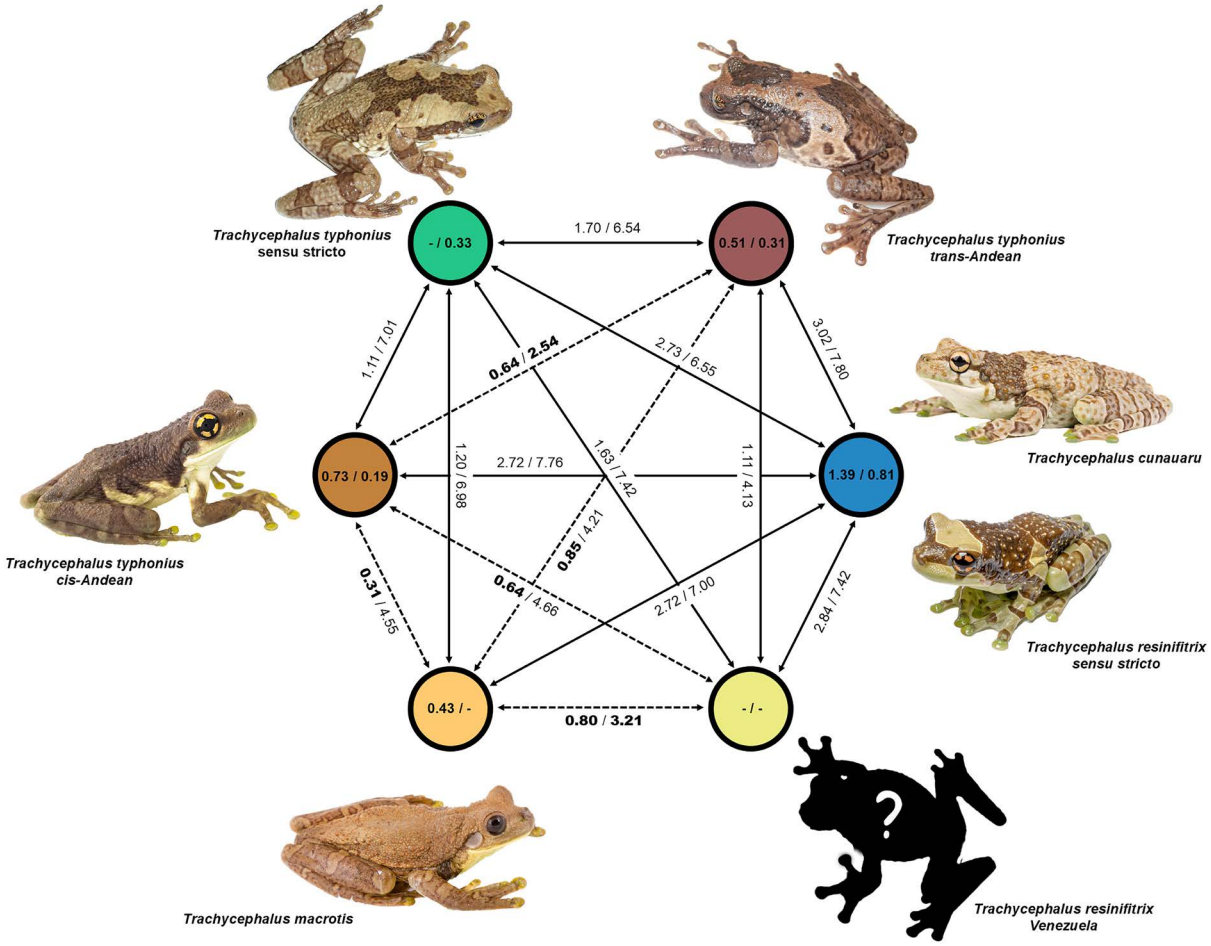

Figure 3. Mean K2P distances among OTUs for 16S (upper) and COI (lower) mitochondrial genes.

The BIN analysis for COI returns ten candidate species (Fig. 2). In some cases, a single BIN grouped more than one species. For example, BIN BOLD:ACA4687 includes *T. cunauaru* (Ecuador), *T. resinifictrix* (Brazil) and *T. typhonius* (Brazil). Also, sequences from one species were assigned to different BINs, for example *T. resinifictrix* from Brazil (BOLD:ACA4687) were grouped in a different BIN from Venezuelan sequences (BOLD:ACA5927) of the same species. Colombian samples were divided into different BINs based on their cis- or trans-Andean distribution. The Costa Rican sample was grouped in the same BIN as the trans-Andean samples from Colombia. Six BINs were consistently grouped into a single taxonomic entity in each case, with no mixing of other species: *T. macrotis* (BOLD:ADK1552), *T. mesophaeus* (BOLD:ACA3812), *T. nigromaculatus* (BOLD:ACF9093), *T. quadrangulum* (BOLD:ADJ5861), *T. imitatrix* (BOLD:ACF7612) and the type sample of *T. typhonius* from Guyana (BOLD:ACH6375).

The K2P distances amongst all OTUs suggested by BOLD and ABGD were relatively low (Fig. 3) ranging between 2.5% and 7.8% for COI and between 0.3% and 3.0% for 16S. The cis- and trans- Andean samples showed the shorter distances between them (2.5% for COI and 0.6% for 16S), followed by *T. macrotis* and Venezuelan *T. resinifictrix* (3.2% for COI and 0.7% for 16S).

Based on the results of the Maximum Likelihood tree for COI and 16S, sequences assigned to *T. typhonius* fall on three different branches in the tree (Fig. 4). The analysis merged Colombian sequences assigned to *T. typhonius* in a single clade, with a clear split of cis-Andean and trans-Andean sequences in two independent lineages. These two clades were nested into a clade composed of the Ecuadorian *T. macrotis* and *T. resinifictrix* from Venezuela. The complete clade of the former sequences was nested to a clade covering the *T. typhonius* from the type locality, Guiana, French Guiana and Rorarima state in Brazil. All these samples are sisters of a clade composed of *T. typhonius* and *T. resinifictrix* from Bolivia, Pará, Mato Grosso and Amazonas states in Brazil, plus *T. cunauaru* from Ecuador.

The ML implementation of PTP applied to the ML tree delimited *T. typhonius* samples in three lineages with high support values (0.99 – 1): the first lineage includes *T. macrotis*, all Colombian samples and *T. resinifictrix* from Venezuela (AMNH-131201). The second lineage includes samples assigned to *T. cunauaru, T. resinifictrix* (excluding the sample from Venezuela), *T. typhonius* from Bolivia and Brazil (excluding samples from Roraima state). The third lineage includes *T. typhonius* from Guiana, French Guiana, and Roraima state in Brazil.

Taxonomic account

Based on a full consensus of the molecular evidence available, we update the information on distribution range of two formerly described species (*T. typhonius* and *T. macrotis*).

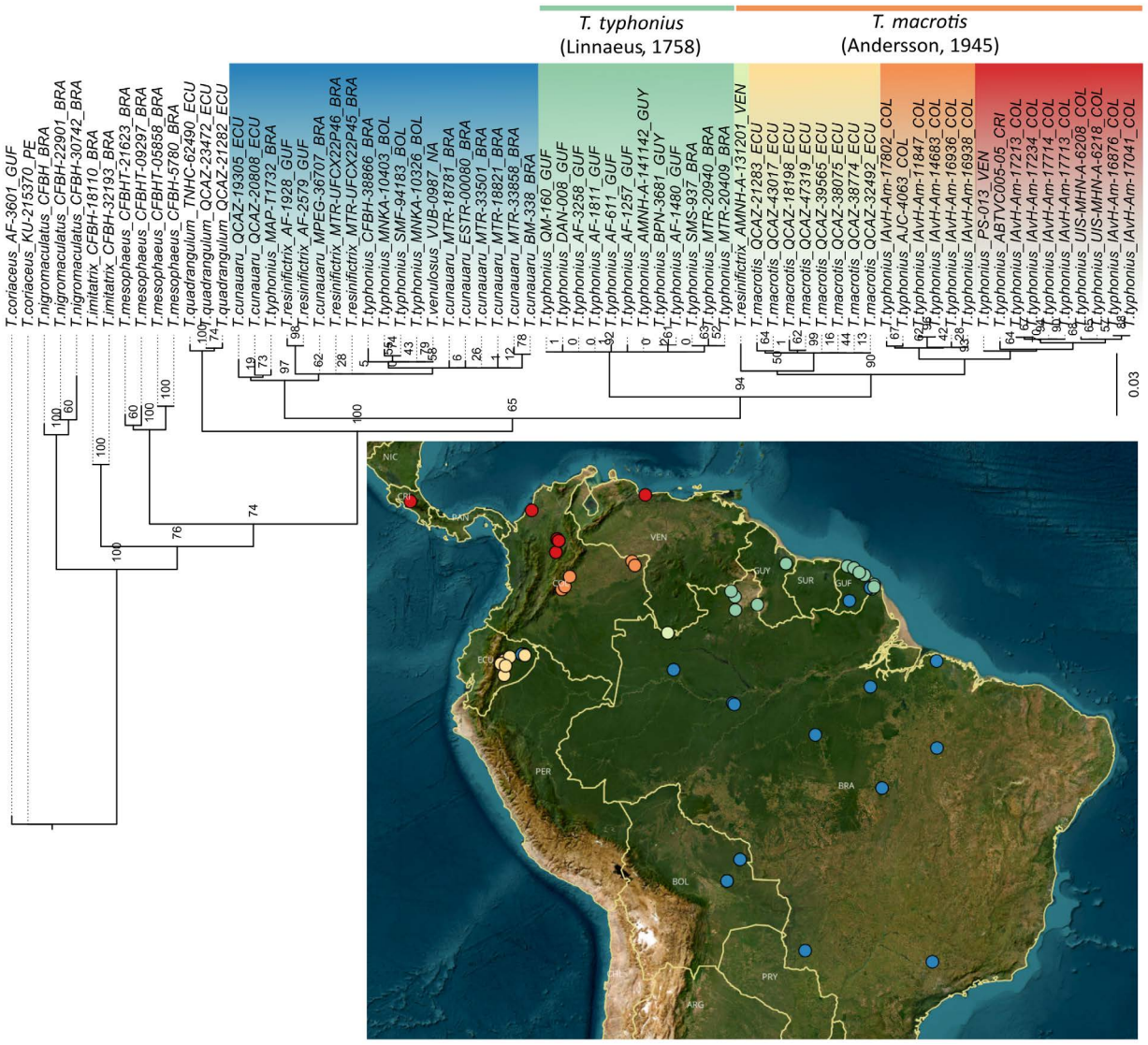

Figure 4. Maximum Likelihood tree for 16S and COI and location of all *Trahycephlalus typhonius* samples and relatives by OTU assignment (ABGD and BOLD). The upper bars indicate the clustering generated by mPTP. See Table S1 in supplementary materials.

Trachycephalus typhonius (Linnaeus, 1758)
Holotype. AMNH-A 141142
Type locality. Number 1, Kerkplein Street, Paranamaribo, Suriname

New data
Distribution. *Trachycephalus typhonius* ranges in the Guiana Shield, covering lowlands of French Guiana, Suriname, Guyana and Mato Grosso State in Brazil.

Trachycephalus macrotis (Andersson, 1945)
Holotype. NHRM 1958
Type locality. 'Rio Pastaza, Watershed', eastern Ecuador.

New data
Distribution. *Trachycephalus macrotis* ranges from Ecuador and adjacent north-eastern Peru, Orinoquia savannah in Colombia and Venezuela, the trans-Andes dry and humid forest of Upper and Middle Magdalena and Caribbean dry forest in Colombia, extending its range up to Costa Rica.

DISCUSSION

Our work fills the gap in the distribution range for the northern part of South American populations for *T. typhonius* by including samples from cis-Andean and trans-Andean populations in Colombia. The patterns detected solely by mtDNA reveals useful information about the species identity of samples assigned to *T. typhonius* and provides insights into the biogeographical patterns of lineage isolation in its distribution range.

Revealing cryptic diversity on 'widespread' species is a common trend in Neotropical amphibians (Lyra et al., 2017). Based on the analysis of the mtDNA, our results support the inference that Colombian populations represent a different lineage from *T. typhonius* sensu stricto, and that cis-Andean and trans-Andean Colombian populations might be considered as a single OTU, different from *T. macrotis*. Interestingly, owing to the low variation in 16S sequences (Fig. 2), there is no consensus on molecular delimitation tests for lineages of *T. macrotis* and Colombian *T. typhonius*. Whilst mPTP

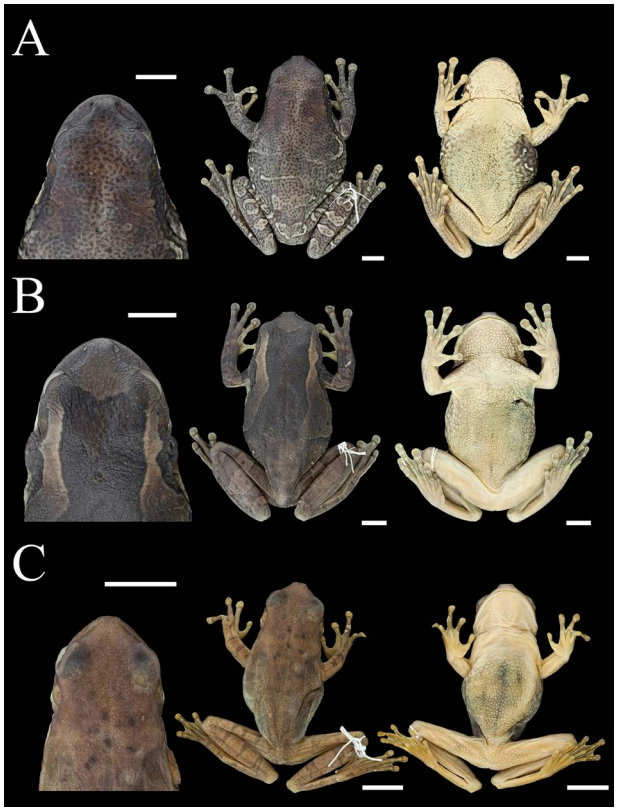

Figure 5. Morphological variability of Colombian *Trachycephalus* "*macrotis"* populations. **A.** IAvH-Am-14683 from Puerto Carreño, Vichada, **B.** IAvH-Am-16876 from Puerto Wilches, Santander, **C.** IAvH-Am-11417 from Puerto Nariño, Amazonas. **A.** and **C.** correspond to cis-Andean, Orinoco and Amazonian populations, respectively, **B.** corresponds to a trans-Andean population. White bars indicate 1 cm.

returned a single lineage, ABGD and BIN suggested three lineages for the same samples. This discordance presents a challenge for species delimitation based solely on molecular evidence.

Appealing to cryptic diversity might drive taxonomic inflation, especially in the face of limited geographic sampling and a lack of clear diagnostic characters, leading in some cases to the refutation of newly described species (Arias-Cárdenas et al., 2024). Previous studies have delimited Neotropical anuran species with a COI K2P distance greater than 4%; however, this threshold is usually subject to discussion (Lyra et al., 2017). Whilst this threshold was sufficient to split the *Boana platanera* and *B. xerophylla* (Escalona et al., 2021), the independence of lineages was recently discussed in depth in the case of *Dendropsophus molitor - D. luddeckei* (Arias-Cardenas et al., 2024).

In this scenario, we suggest that the taxonomy of the *T. typhonius* complex be reviewed and examined more thoroughly. Our results reveal some differences that might serve as a starting point for this purpose. Considering the morphological variation of this species (Fig. 5), an integrative approach with multiple lines of evidence (e.g. including tadpole morphology and bioacoustics) may better clarify their taxonomy and lead to formal species descriptions (Franco-Mena et al., 2024). Further emphasis should be placed on poorly sampled localities to ensure that they represent real gaps in the distribution of species and not biases due to sampling efforts (Kieswetter & Schneider, 2013).

By including *T. typhonius* of several other localities available in public repositories published by Jansen et al. (2011) and Vacher et al. (2020), our analysis recovered enough data to support a single lineage including individuals from Brazil, Bolivia assigned to *T. typhonius, T. cunauaru* from Ecuador, and *T. resinifictrix* from Brazil (including the type locality). These results suggest that specimens identified as *T. typhonius* from the western, central and southern Amazonian must be assigned to *T. cunauaru* or *T. resinifictrix*. In this case, given that *T. cunauaru* and *T. macrotis* are sympatric in some western Amazon areas (Ron et al., 2016) and given that these two species can be easily differentiable by external morphology, a careful examination of museum specimens might help on species assignment of both lineages.

What does the incorporation of our samples tell us about Andes and Amazon biogeographic patterns?

The addition of our data reveals an interesting biogeographical pattern that aligns with the current biogeographical regionalisation in which Orinoquia and the trans-Andean lowlands constitute a biogeographical domain different from the Amazon (Morrone, 2014). As expected, cis-Andes and trans-Andes can be differentiated by COI sequences and wide literature is available about the vicariant effect of the Andes on both highland and lowland species (Bagley & Johnson, 2014; Mendoza et al., 2019). However, the Colombian cis-Andes samples formerly assigned to *T. typhonius* did not group with cis-Andes of the Amazon region (Fig. 3). Thus, 1) the higher genetic similarity between cis-Andes and trans-Andes *T. typhonius* and 2) the deep divergence amongst north and south cis-Andean samples found here suggest an ancient isolation within cis-Andean lineages and a recent dispersal-vicariance process across the Andes, challenging the classical and simple scenario of mere vicariance by mountain systems (Salgado-Roa et al., 2024).

In the first case, the likely recent divergence between cis-Andes and trans-Andes suggests that the Andes range is a partial barrier to dispersal for lowland lineages followed by recent vicariance (Smith et al., 2014; Bemmels et al., 2018) and that there was probably short time for genetic differentiation, similar to the data found for *Hyalinobatrachium tatayoi* (Mendoza et al., 2019). Non-homogeneous elevation along the Andes (Gregory-Wodzicki, 2000) might act as a semi-permeable barrier that permits recent side-to-side dispersal (Scotti-Saintagne et al., 2013; Smith et al., 2014). In fact, there is evidence of historic ecological continuity across low-lying passes for other vertebrates, which promotes population connectivity across the Andes (Haffer, 1967; Cadena et al., 2016). Candidates for these low-lying passes (Hazzi et al., 2018) need to be tested with deeper phylogeographical approaches by including time-calibrated phylogenies to

nest the geological changes with the evolutionary history of the species.

In the second case, the deep divergence between the north and south cis-Andean lineages is a pattern that coincides with the profound differences between the Orinoquia Savanna and Amazonian Rainforest. Environmental barriers are important for philopatric and low-mobility species such as amphibians (Beebee, 2005), which are also highly susceptible to small changes in their environment (Navas, 2006). Here, the Amazon/Orinoquia transition explains genetic differentiation in broadly distributed lowland taxa in the Neotropics (Arbeláez-Cortés, 2020; Vacher et al., 2020) and the isolation of Orinoco populations in anurans coincides with phylogeographic patterns found for *Elachistochleis* sp. (Jowers et al., 2021) another 'widespread species' to be revealed paraphyletic on multiple isolated lineages. The Llanos of Venezuela and Colombia are one of the coldspots of new amphibian species in South America (Vasconcelos et al., 2019). Our findings can help not only elucidate the evolutionary history of certain lineages, but also serve as a case study for understanding the processes behind the poorly studied Orinoco Savanna, in terms of evolutionary and biogeographical processes (Jaramillo, 2023).

Lastly, the heterogeneity of lowland Amazonian habitats is sometimes underappreciated because the region has been wrongly perceived as a large and homogeneous ecosystem, and many taxa have been mistakenly considered widespread and generalist (Guayasamin et al., 2024). Here, the presence of multiple lineages assigned to *T. typhonius* in overlapping ranges for some parts of the Amazon region (Fig. 3) shows that these populations might host cryptic diversity, even beyond bioregionalisations suggested for birds and amphibians (Silva et al., 2019; Réjaud et al., 2020; Vacher et al., 2020). Data along all the distribution range for the three lineages assigned to *T. typhonius* and close-related species (*T. resinifictrix, T. cunauaru* and *T. macrotis*) provides an interesting study system to evaluate the role of Amazonian biogeographical areas on recent lineages speciation and diversification (Guayasamin et al., 2024).

It is important to keep expanding knowledge and avoid the Wallacean shortfall, particularly in species that can be complexes or have cryptic diversity. The limited availability of data from Colombia, particularly in herpetofaunal studies (Vásquez-Restrepo, 2021), constrains our understanding of key biogeographic patterns in northern South America, (Avendaño et al., 2017; Arbeláez-Cortés, 2020; González-Orozco, 2023). Through studies involving widely distributed species, the inclusion of data from this region could significantly enhance our understanding of the evolutionary history of Neotropical diversity. However, the barriers faced by the Global South including limited data accessibility, logistical challenges in site access, bureaucratic obstacles and linguistic barriers (Vásquez-Restrepo, 2021; Arenas-Castro et al., 2024) remain a gap in the capacity to generate information compared to the Global North (Amano & Sutherland, 2013; Ocampo-Ariza et al., 2023) (See supplementary materials for a Spanish version of this article). This gap continues to hinder access to the technology and supplies needed to obtain data and address the pressing need of megadiverse countries to understand their biodiversity to protect it. In this sense, it is important to promote and facilitate scientific collaboration between countries (Avendaño et al., 2017) for the inclusion of data from under-represented regions in future broad studies.

Better knowledge, better decisions for the conservation of herpetofauna

Ignoring the study of apparently 'common' and widely distributed species can hamper conservation efforts (Gaston & Fuller, 2008). Without clarity, not only on their taxonomy but also in key attributes such as geographical range, these species might not receive the required attention (Stuart et al., 2004). For example, species with Deficient Data (DD) or categorised as Least Concern (LC) may be overlooked where uncertainties exist about their true threat status (Howard & Bickford, 2014; Nori & Loyola, 2015). Even species that were once common can become suddenly depleted, such as the Panamanian golden frog *Atelopus zeteki* and the giant glassfrog *Centrolene geckoideum*. Describing new species from populations assigned to a species considered 'common' and 'widespread' species could be a future result of this study. Even if new species are not formally described yet, molecular data, together with biogeographic history, can guide the delimitation of Evolutionarily Significant Units (ESUs, Hutama et al., 2017). In this way, we provide resources to understand patterns of diversity and/or genetic structure of populations in the future and to complement decision-making in conservation strategies (e.g. Evolutionary Distinctiveness - ED, Evolutionarily Distinct and Globally Endangered - EDGE index) from biogeography or genetic conservation perspectives (Whittaker et al., 2005; Caviedes-Solis et al., 2020).

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