

Exploring Blind Spots in Amazonian Anuran Diversity: An Assessment of Research and Conservation Needs

Brena da Silva GONÇALVES*¹, Cristian de Sales DAMBROS², Fernanda P. WERNECK^{1,3}

¹ Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Zoologia, Manaus - AM, Brazil

² Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Biodiversidade Animal, Santa Maria - RS Brazil

³ Instituto Nacional de Pesquisas da Amazônia, Coordenação de Biodiversidade, Programa de Coleções Científicas Biológicas, Manaus - AM, Brazil

* Correspondence author: brenasgoncalves@outlook.com

ABSTRACT

The Amazonian megadiversity remains understudied and underestimated due to the region's vast size, environmental heterogeneity, and inaccessibility. With increasing threats to the biome, it is crucial to map current knowledge before it is lost. This study addresses Linnean (species description), Wallacean (species distribution), and Darwinian (genetic knowledge) shortfalls for Amazonian anurans, quantifying what remains unknown. We reviewed species descriptions, analyzed occurrence data, and evaluated available genetic sequences and phylogenetic uncertainty. Species descriptions have grown over time, peaking around 2000. We estimate that 1,525 species exist in Amazonia, but only 766 (based in the species shapefiles) or 810 (based in the occurrence data) were found. Richness was highest in northern and western Amazonia, while remote areas far from urban centers remain undersampled. Only two genes (mitochondrial 12S and 16S) have been sequenced for more than half of the known species and are available in open databases, while phylogenetic uncertainty predominates in most clades. Regions such as Gurupa Várzea and Cordillera Central Paramo are especially under-sampled. Our results show that taxonomic, distribution, and genetic shortfalls are complementary rather than overlapping, indicating that different regions and species groups require different research strategies. This study highlights major knowledge gaps and provides a framework to guide future efforts in taxonomy, biogeography, and evolutionary studies of Amazonian anurans, supporting conservation planning in one of the most diverse regions on Earth for one of the most diverse and threatened taxonomic groups.

KEYWORDS: knowledge gaps, amazon, frogs, shortfalls, biodiversity

Explorando pontos cegos na diversidade de Anuros Amazônicos: Uma avaliação das Necessidades de Pesquisa e Conservação

RESUMO

A megadiversidade amazônica ainda é pouco estudada e subestimada, devido ao vasto tamanho da região, à heterogeneidade ambiental e à sua difícil acessibilidade. Com as crescentes ameaças ao bioma, é fundamental mapear o conhecimento atual antes que seja perdido. Este estudo aborda as deficiências Linneanas (descrição de espécies), Wallaceanas (distribuição de espécies) e Darwinianas (conhecimento genético) dos anuros amazônicos, quantificando o que permanece desconhecido. Nós revisamos as descrições de espécies, analisamos dados de ocorrência e avaliamos as sequências genéticas disponíveis, bem como a incerteza filogenética. As descrições aumentaram ao longo do tempo, com pico por volta de 2000. Estimamos que existam 1.525 espécies na Amazônia, mas apenas 766 (com base nos shapefiles de espécies) ou 810 (com base em dados de ocorrência) foram encontradas. A maior riqueza foi registrada nas porções norte e oeste da Amazônia, enquanto regiões remotas e distantes de centros urbanos permanecem subamostradas. Apenas dois genes mitocondriais (12S e 16S) foram sequenciados para mais da metade das espécies conhecidas e estão disponíveis em repositórios públicos, enquanto a incerteza filogenética predomina na maioria dos clad. Regiões como a Várzea de Gurupa e o Páramo da Cordillera Central são particularmente pouco amostradas. Nossos resultados indicam que as deficiências taxonômicas, de distribuição e genéticas são complementares, e não sobrepostas, o que sugere que diferentes regiões e grupos de espécies demandam estratégias de pesquisa distintas. Este estudo destaca lacunas críticas de conhecimento e fornece uma base para orientar esforços futuros em taxonomia, biogeografia e estudos evolutivos de anuros amazônicos, contribuindo para o planejamento da conservação em uma das regiões mais biodiversas e para um dos grupos taxonômicos mais diversos e ameaçados do planeta.

PALAVRAS-CHAVE: lacunas de conhecimento, Amazônia, anuros, deficiências, biodiversidade

CITE AS: Gonçalves, B.S.; Dambros, C.S.; Werneck, F.P. 2025. Exploring Blind Spots in Amazonian Anuran Diversity: An Assessment of Research and Conservation Needs. *Acta Amazonica* 55: e55bc25007.

INTRODUCTION

Discovering and describing the planet's extant species remains a primary goal for many biologists and taxonomists. This is no easy task, and numerous challenges persist along the way. These include declining funding for basic research, taxonomic impediments, the systematic loss of natural habitats, and climate change (IUCN 2024). Such factors remove biological communities from the researchers' scope and can even lead to the extinction of many species before they are even known. As biodiversity conservation is a global priority for maintaining the environmental balance, ecosystem services, and human well-being (Joly *et al.* 2019), quantifying and identifying the current knowledge gaps in biodiversity may be key to defining future research priorities and effective conservation programs.

The Neotropics are widely recognized for harboring the greatest species richness on the planet. Located in northern South America, the Amazon is the largest tropical rainforest in the world and contains approximately 10% of all known biodiversity (Antonelli *et al.* 2018). The term Amazon can simultaneously refer to the Amazon River basin and the Amazonia biome. This biome covers about 40% of South America's territory and is shared by nine countries: Brazil, Peru, Colombia, Venezuela, Ecuador, Bolivia, Guyana, Suriname, and French Guiana (Vergara *et al.* 2022). Due to its vast extent and long paleoenvironmental history, the biome exhibits physical, chemical, and geological variations, which result in a range of distinct environments across the region, including different types of ecoregions, like forests, savannas, high-altitude areas, and wetlands (Igapó and Várzea) (Vergara *et al.* 2022, Darella-Filho *et al.* 2024). This environmental diversity enables the biome to support the world's largest number of species of plants, birds, freshwater fish, insects, and anuran amphibians (Antonelli *et al.* 2018, Vergara *et al.* 2022, Guayasamin *et al.* 2024). Estimates have shown that the species richness in the biome is even greater, and current richness numbers are likely largely underestimated (Camacho-Rozo and Urbina-Cardona 2024). However, not even such a rich environment remains unaffected by climate change and human activities (Albert *et al.* 2023). Climate change and anthropogenic pressures like deforestation are not spatially uniform across the Amazon — regions such as the “arc of deforestation” experience disproportionately high impacts (Silva Junior *et al.* 2021). These pressures may lead to local extinction, loss of phylogenetic diversity, phylogenetic endemism (Alves-Ferreira *et al.* 2025) or force species to shift their ranges in search of suitable habitats (Azevedo *et al.* 2024, Alves-Ferreira *et al.* 2025). Biological groups with limited mobility and habitat specificity may have increased extinction risks (Hof *et al.* 2011). Therefore, identifying knowledge shortfalls is essential for anticipating biodiversity responses to environmental change and for identifying potential climate refugia for conservation planning (Alves-Ferreira *et al.* 2025).

Biodiversity is a complex term and encompasses more than just species richness — it includes, for example, phylogenetic diversity (Faith 1992), functional traits (Cadotte *et al.* 2011), and ecological interactions (Tylianakis *et al.* 2008). Such facets demand integrative approaches to assess knowledge gaps (Hortal *et al.* 2015). Knowledge gaps (also named shortfalls or deficits) represent the difference between what has already been discovered (current knowledge) and the complete (ideal) knowledge of a biological group at a given moment, usually in the present day (Hortal *et al.* 2015). These gaps include the need to adequately catalog species richness (Linnaean shortfall), map their geographic distributions (Wallacean shortfall), understand their evolutionary processes and phylogenetic relationships (Darwinian shortfall), investigate their functional traits and ecological roles (Raunkiaeran shortfall), and their abiotic tolerances (Hutchinsonian shortfall) (Hortal *et al.* 2015, Lees *et al.* 2020, IUCN 2024). These interconnected gaps directly affect our ability to understand the drivers and preserve both species and Amazonian ecosystems.

Anurans are the most endangered group of vertebrates, with approximately 40% of known species facing some level of threat (Anuniação *et al.* 2024, IUCN 2024). Due to biological characteristics, such as permeable skin, low mobility, and a biphasic life cycle, they are strongly dependent on water bodies and humid environments for their reproduction and survival (Duellman and Trueb 1994). Yet, this dependency varies among taxa, with some lineages being able to reproduce in less humid habitats, such as larvae with endotrophic diet, direct development, and foam nest construction (Haddad and Prado 2005). Additionally, for species that are more dependent on water, the functional connectivity between aquatic and terrestrial habitats can be as critical as water availability itself. This makes them vulnerable to environmental shifts, such as water pollution, contamination, pathogen exposure, habitat loss, and climate change (Luedtke *et al.* 2023). Thus, the conservation of anuran species is essential for sustaining ecosystem functioning, given their unique ecological roles. As keystone species, they regulate arthropod population, contribute to nutrient recycling in both aquatic and terrestrial ecosystems, and can act as ecological indicators (Duellman and Trueb 1994, Ceron *et al.* 2023).

The Amazon environmental heterogeneity and abiotic conditions has fostered anuran extraordinary diversification, making them one of the richest vertebrate groups in the biome (Frost 2024). Recently studies revealed the presence of cryptic species, which are morphologically similar and genetically distinct, and whose identification is hindered by the lack of phylogenetic studies (Ferrão *et al.* 2018, Rojas *et al.* 2018, Carvalho *et al.* 2019, Lima *et al.* 2020, Mônico *et al.* 2024), and the diversity of specialized reproductive modes (Haddad and Prado 2005, Nunes-de-Almeida *et al.* 2021). However, current knowledge about frogs is unevenly distributed, many species are known from very limited records, or are poorly

ecologically and evolutionarily characterized, with historically underexplored regions throughout the Amazonia (Bickford *et al.* 2007, Camacho-Rozo and Urbina-Cardona 2024, Penhacek *et al.* 2024). Considering the accelerating habitat loss and climate change driven by anthropogenic pressures, studying this biological community is of utmost importance to identify these blind spots in knowledge gaps.

Therefore, this study aims to identify and map the Linnaean, Wallacean, and Darwinian gaps regarding Amazonian frogs, based on literature and available online data, to highlight priority regions for future research and contribute to the understanding of one of the planet's most threatened biomes. Based on previous studies and accessibility patterns, we expected to find greater shortfalls in remote areas, with higher taxonomic and distribution knowledge concentrated around urban centers and well-studied regions (Hortal *et al.* 2015, Carvalho *et al.* 2023).

MATERIAL AND METHODS

Study area

We investigated shortfalls in biodiversity knowledge in the Amazon biome (WWF 2021), which encompasses nine countries (Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guyana, Peru, Suriname, and Venezuela), and covers an area of seven million ha (Vergara *et al.* 2022). Although regional weather is broadly warm (average $\sim 25^{\circ}\text{C}$) with mean precipitation of $\sim 2,300\text{mm year}^{-1}$ (Fisch *et al.* 1998), the Amazon exhibits pronounced climatic heterogeneity, especially in temperature and precipitation seasonality across regions, influenced by heterogeneous topography, from 0 to 100m a.s.l. to mountains higher than 3,000m a.s.l. (Hoorn *et al.* 2010, Flores *et al.* 2024). The Amazon biome also includes different vegetation types, from Dense and Open Ombrophilous Forests, to Deciduous Seasonal Forest, and to open vegetation types, such as Campinaranas, Savannas, Steppe-savanna, and Pioneer Formations (IBGE 2023).

Linnean shortfall

To evaluate the Linnean shortfall, we recorded the number of species currently occurring in the region, the number of available papers describing species over time, and estimated the complete number of species existing in the Amazonia. To determine the number of occurring species, we considered the anurans' shapefiles distributed in the Amazonia (IUCN 2024). First, we downloaded the species range shapefiles from the South American anurans, overlapped these with the Amazonia area shapefile (WWF 2019), and removed species that don't have distribution in the study area. Also, we only considered an 'Amazonian species' shapefiles with at least 50% of its distribution inside the Amazonian area. We removed the synonyms, checked, and updated the names following the Amphibian Species of the World (Frost 2024).

To compile studies about anuran species in the Amazonia region, we conducted a systematic literature search Google Scholar using the query '(anura OR anurans OR frogs OR sapos OR rãs OR pererecas) AND (Amazonia OR Amazon OR Amazônia OR Floresta Amazônica)', covering the period from 1700 to 2018. The starting point was chosen to include the first known species description from the region (Linnaeus 1758). We used the Zotero browser extension to automatically retrieve documents in PDF format. We obtained 1,114 documents, including, articles, thesis, dissertations, notes and books. To select only description papers, we applied a filtering script in R using regular expressions that searched for key terms such as "n.sp.", "sp.nov.", and "new.species" in the texts. This methodology didn't count how many species were described in each paper, only if at least one species was. We excluded 824 documents that did not include new species descriptions, and our selection criteria focused on descriptions based on specimens collected in the Amazon biome. We narrowed our temporal analyses from 1950 to 2021 to compare them to actual and estimated species numbers. To estimate the complete number of species that have yet to be described and predict when this number might be reached, we extrapolated the current species count using the Jackknife richness estimator (Oksanen *et al.* 2024). Based on the time range covered by the previously surveyed papers, we selected 1700 as the start year and 2018 as the end year. This estimator uses the number of described species in this period as a sample and assumes that the annual descriptions result from homogeneous levels of sampling effort. The species curve can be extrapolated by considering different sampling effort values and accounting for heterogeneous sampling efforts. We used the *specpool* function of the *vegan* package, version 2.6-4 (Oksanen *et al.* 2024). Additionally, to determine the year in which the predicted number of species can be reached, we applied the adaptation of the density-dependent model proposed by (Lu and He 2017) based on the work of Gotelli (2008):

$$N_t = K / [1 + (K/N_0 - 1) e^{(-rt)}]$$

where K is the number of possibly existing species (calculated as the sum of Jackknife estimate plus the number of species described before 1974), N is the number of species described over time (t), N_0 is the number of valid species described until $t = 0$, and r is the theoretical constant representing the rate of species description. We assessed the difference in sampling efforts by using multiple values for r (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5), as proposed by Freitas *et al.* (2020).

Wallacean shortfall

To assess the Wallacean shortfall, we used a different type of data to obtain the number of species in the Amazonia. We downloaded the occurrence of Anuran data available to the Amazon region from the Global Biodiversity Information

Facility (GBIF 2024), and the 49 Amazonian ecoregions shapefiles' from the World Wildlife Fund database (WWF 2019). The ecoregion shapefiles were obtained from Olson *et al.* (2001), which classify ecoregions based on ecological, vegetation, and biogeographical characteristics. These ecoregions encompass diverse environments such as floodplain forests, *terra-firme* forests, and non-forest areas like white-sand vegetation, savannas and pioneer formations. The occurrence data (GBIF data) encompasses information from preserved specimens, published observations, and biological collections. Although public databases may include taxonomic or spatial inaccuracies, we minimized these issues through a rigorous data cleaning process and taxonomic standardization, following widely adopted practices in large-scale biodiversity research. First, we removed duplicated latitude-longitude coordinates, species names, and records lacking species identification. Second, we reviewed and updated the nomenclature according to Frost (2024), the most up-to-date nomenclature, and excluded instances where nomenclature could not be corrected or was incorrectly identified. At the end, we added a column corresponding to their respective ecoregions to the occurrence data. We adopted a two-scale approach to better characterize the Wallacean shortfall in Amazonia. First, we used a $1 \times 1^\circ$ grid over the entire biome to provide a broad overview of species richness and sampling completeness across space. Second, we analyzed the same information by ecoregions to account for environmental and biogeographic heterogeneity across the Amazon. This dual-scale framework allows for identifying both general patterns and more localized inventory gaps.

We checked if the occurrence points were localized within the grid area and excluded those that were not. We utilized all unique occurrence points to calculate species richness per cell and ecoregion and to generate species accumulation curves (SAC). We employed accumulation curves as a proxy for assessing the deficit of inventories for cells and ecoregions. Cells or ecoregions with curves equal to or below 0.1 were considered well sampled (i.e. less than 10% of species still need to be described). SACs ranging from 0.1 to 0.5 were considered reasonably sampled, requiring additional sampling for completeness. Also, SACs above 0.5 were considered poor sampling and required great sampling effort.

Darwinian shortfall

To assess the lack of knowledge regarding Amazonian anurans' phylogenetic relationships, we employed two approaches: (i) identify the proportion of species with genetic sequences sequenced and accessible at open databases and (ii) identify and quantify phylogenetic uncertainty by measuring how variable each species' position is across multiple phylogenetic trees. To determine the proportion of sequences, we used the matrix provided in the supplementary material of Jetz and Pyron (2018), counting only the sequences for anuran

species recorded in the Amazonia, and then comparing this count to the number of species estimated on Wallacean shortfall previously. To identify the phylogenetic uncertainty, we first selected a sample of 100 phylogenies from the 10,000 posterior distribution trees of Jetz and Pyron (2018). Subsequently, we computed the distance between all tip pairs from each phylogeny using *cophenetic.phylo* function of the *ape* package (Paradis and Schliep 2019). We excluded from the matrix the non-amazonian species distances, which did not match with our species list (using occurrence data). Then we calculated the standard deviation (sd) from the trees using the distance matrix, as a proxy for the uncertain phylogenetic position of the species. Therefore, higher values of sd mean a higher uncertainty in the species relationships between the compared trees, while lower values correspond to higher confidence in the phylogenetic relationships between species.

All the analyses were done in the R software (R Core Team 2024).

RESULTS

Linnean shortfall

From the 1,114 published articles about Amazonian anurans that we analyzed, only 131 were recovered as species description papers. The first of them was published in 1758 (Linnaeus 1758). We did not recover papers for most of the years before 1850, but we recovered at least one article for almost all the subsequent years (Figure 1a). The average number of articles describing Amazonian anuran species per year increased over time, starting with an average of 2 articles in the early 1800s and reaching 7.5 around 2000, and the highest number was 49 in 2007 (Figure 1a).

When we compared the data over the years, we noticed some discrepancies between the data downloaded and the description papers filtered. The number of species described followed a gradual increase pattern until approximately 1960 when the cumulative number of species described turned into an exponential curve (Figure 1b), with a decrease of the increment around 2000, with stagnation and decrease in 2021. A similar pattern of increase was found for the papers but in a short period. The number of studies about anurans increased slowly around 1980. After 2000 the increase was exponential with no signal of stagnation (Figure 1b). However, the number of descriptive studies gradually increased after 2000, reaching 131 papers (Figure 1b).

Based on the IUCN species shapefiles, we identified 766 species of anurans with their ranges currently distributed in the Amazonian biome, while our estimation suggests there could be around 1,525 anurans species in the region (jack1 = 1525.872). When we project this result into the future, we estimate that we will achieve this species number between 2030 (best-case scenario of sampling efforts; $r = 0.5$) and 2100 (worst-case scenario; $r = 0.05$). However, under the assumption of maintaining the current trajectory (scenario with $r = 0.1$), this number may be reached by 2070 (Figure 2).

Wallacean shortfall

After filtering the occurrence data from GBIF, we obtained 35,498 occurrence points for 1,224 species of anurans distributed in the Amazon region, of which 27,018 were unique occurrences. These occurrences show a clear clustering pattern in the western, southwestern, central, and northeastern areas of Amazonia, particularly in Ecuador, Colombia, the Guianas, and near large urban centers in Brazil (Figure 3a).

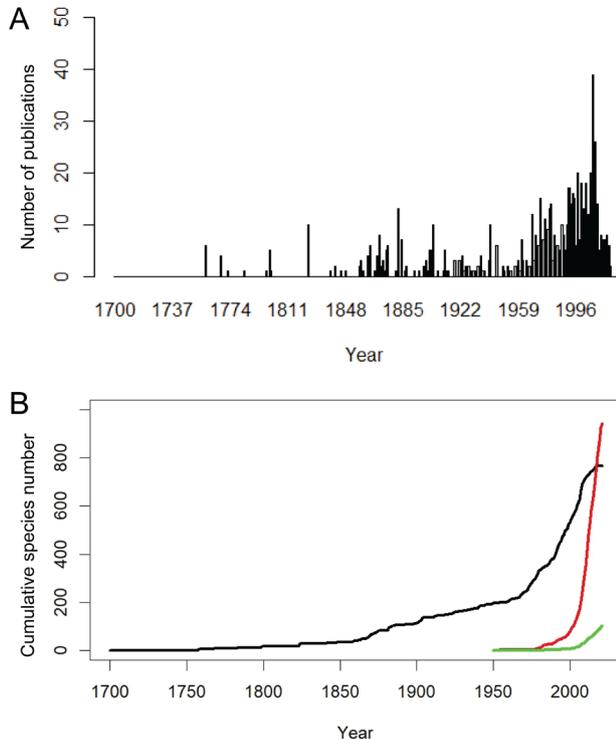


Figure 1. A - Historical trend in the number of publications about Amazonian anurans from 1700 to 2021. This graph illustrates a steady increase in research output, with notable peaks in recent decades. **B** - Temporal trends of the Linnean shortfalls for Amazonian anurans, with the cumulative number of species described following IUCN shapefiles (black line), number of articles studying anurans from Amazonia (red line), and cumulative number of articles describing Amazonian anurans (green line).

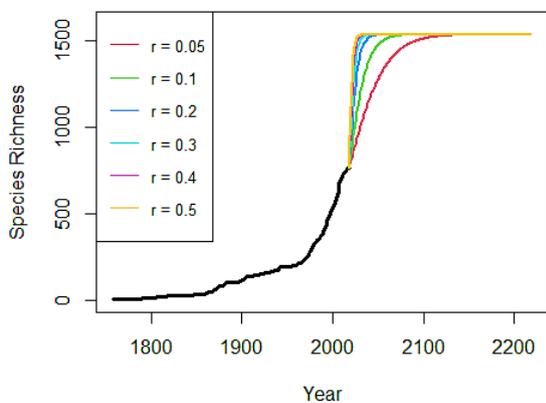
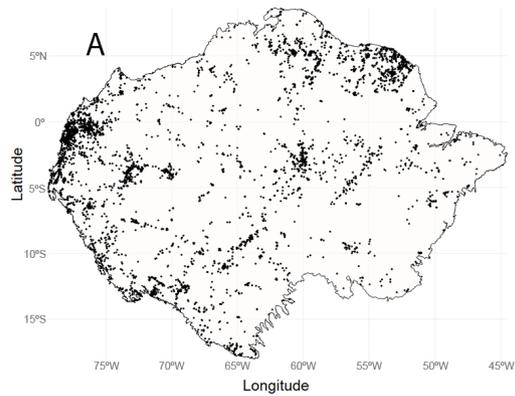
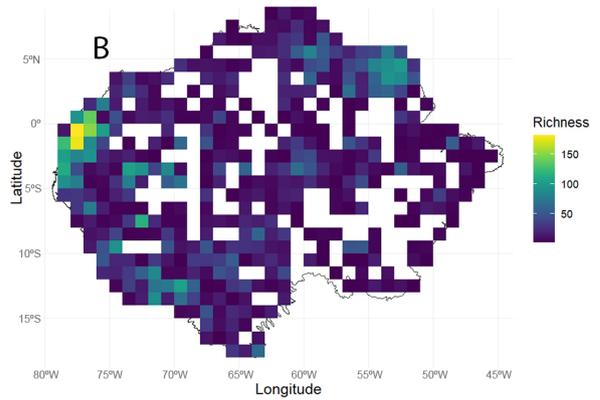


Figure 2. Estimation of Amazonian anuran's species richness, using six different values of sampling effort. Lower effort corresponds to $r = 0.05$ (red line) and higher effort to $r = 0.5$ (yellow line).

Unique occurrences distribution



Species richness distribution



Inventory deficit

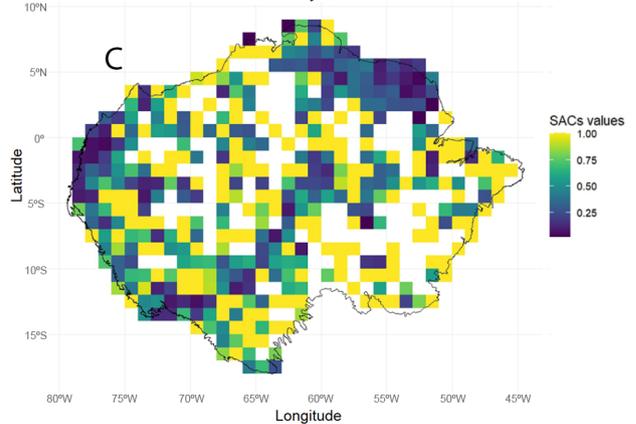


Figure 3. A - Geographic distribution of unique anuran species occurrences in the Amazon. Black dots represent occurrence locations, with a higher density of records in the northern and western regions. **B** - Anuran species richness distribution in the Amazon, by grid cells. Richness values correspond to raw species counts based on occurrence records. White cells denote lack of data. Higher species concentrations are found in the western Amazon, while central and eastern areas show lower richness or lack of data. **C** - Inventory completeness distribution in the Amazon. The map highlights the species accumulation curves (SACs) by grid cells. White cells denote lack of data. Poorly sampled cells are scattered throughout the Amazon, while well-sampled cells are concentrated in the northern and western regions.

These clusters coincide with regions of higher inferred species richness, with the highest richness recorded in extreme western Amazonia, where a single cell reached 181 species. The average richness per cell was 10 species (Figure 3b), and the richness gradient aligns with the west-to-east decline observed in other studies. From the 918 cells analyzed, SACs could not be calculated for 486 cells (52.94%) due to insufficient sampling. Among the remaining cells, 249 (27.12%) were classified as poorly sampled, 153 (16.66%) as reasonably sampled, and only 30 (3.26%) as well-sampled (Figure 3c). The well-sampled cells are concentrated in the western and northern regions, consistent with the richness pattern observed. These findings highlight the uneven sampling effort across Amazonia and a correlation between sampling effort and inferred richness, emphasizing the need for further sampling efforts in less-explored regions.

The two most recorded anuran species in Amazonia are responsible for almost 10% of the total occurrences: *Rhinella marina* Linnaeus, 1758 (4.58%) and *Rhinella margaritifera* Laurenti, 1768 (4.52%). Summed, the ten more common species correspond to 15% of the occurrences (Table 1, Figure 4). The most recorded families are Bufonidae (*Rhinella* genus), Hylidae (*Scinax*, *Osteocephalus*, *Boana*, and *Trachycephalus* genera), Aromobatidae (*Allobates* genus), and Leptodactylidae (*Adenomera* and *Leptodactylus* genera; Table 1).

We found a mean of 96.8 species for the 40 ecoregions calculated. The richest ecoregion was the Eastern Cordillera Real Montane Forests, in western Amazonia, with 343 species (Figure 5a), followed by the Southwestern Amazonia Moist Forests with 284 species. The Gurupa Várzea is the least rich ecoregion with only 2 species registered (Figure 5a), followed by the Cordillera Central Páramo with 3 species. Despite the localization of these low-richness areas it is possible to visualize a richness pattern across the Amazonia, with higher richness regions concentrated in the west and poorer regions in the east (Figure 5a).

Table 1. The top ten most common species registered in the compiled dataset, and their respective record amounts.

Species name	Family	Occurrence records
<i>Rhinella marina</i>	Bufonidae	1322
<i>Rhinella margaritifera</i>	Bufonidae	1305
<i>Scinax ruber</i>	Hylidae	956
<i>Osteocephalus taurinus</i>	Hylidae	648
<i>Boana boans</i>	Hylidae	461
<i>Boana lanciformis</i>	Hylidae	409
<i>Allobates femoralis</i>	Aromobatidae	390
<i>Trachycephalus typhonius</i>	Hylidae	383
<i>Adenomera andreae</i>	Leptodactylidae	376
<i>Leptodactylus pentadactylus</i>	Leptodactylidae	363

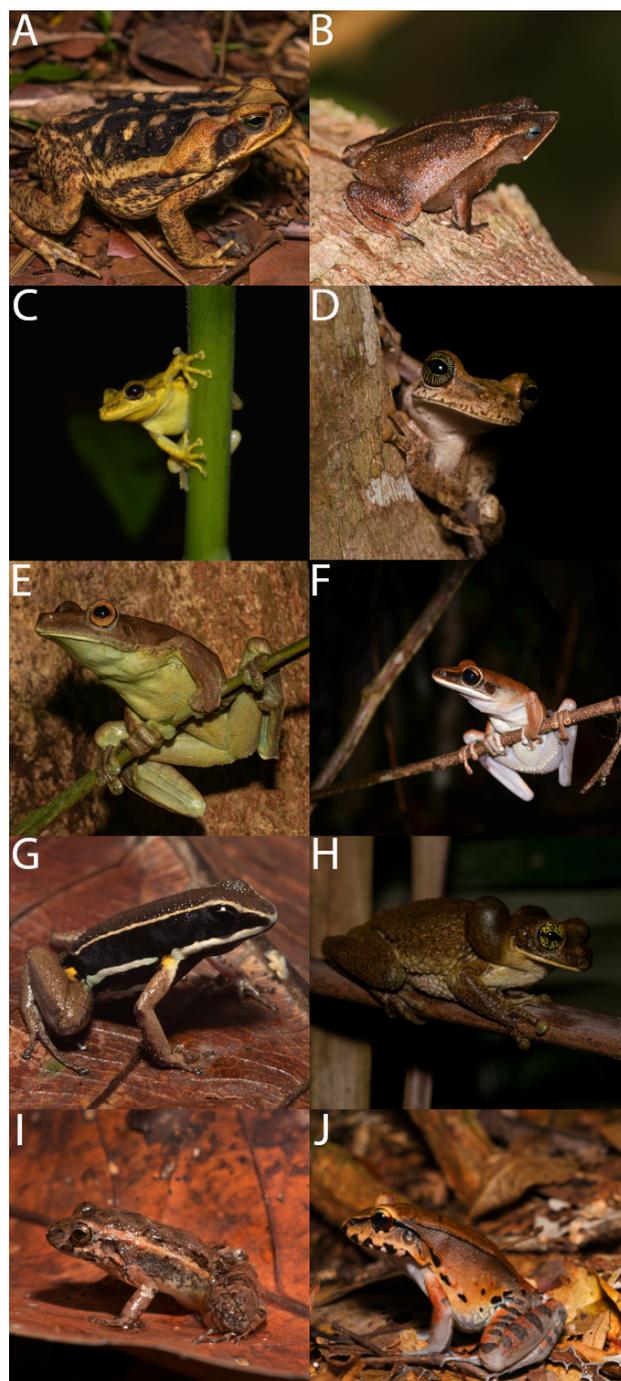


Figure 4. The ten most commonly registered species. **A** – *Rhinella marina*, **B** – *Rhinella margaritifera*, **C** – *Scinax ruber*, **D** – *Osteocephalus taurinus*, **E** – *Boana boans*, **F** – *Boana lanciformis*, **G** – *Allobates femoralis*, **H** – *Trachycephalus typhonius*, **I** – *Adenomera andreae*, **J** – *Leptodactylus pentadactylus*. Picture credits: A/B/C/D/H/J – Lywouty Nascimento; E/I – Lucas Rosado; F – Jordana Guimaraes; G – Igor Yuri Fernandes.

From the 49 ecoregions, SACs could be calculated for 40 (81.63%). Among these, 11 ecoregions (22.45%) were considered well-sampled (depicted in dark purple; Figure 5b), while 24 (48.98%) were reasonably sampled (depicted

in purple). Only five ecoregions (10.20%) were poorly sampled (depicted in yellow and green; Figure 5b). The most well-sampled ecoregion was the Guianan Lowland Moist Forests (SAC = 0.007; Figure 5b), followed by Napo Moist Forest. Conversely, the Gurupa Várzea (SAC = 1.00) and Cordillera Central Páramo (SAC = 1.00) were the poorest sampled (Figure 5b). Following the richness pattern, the

most well-sampled ecoregions are concentrated in the western part of the Amazon, except for the Guianan Lowland Moist Forests in the northeast. In contrast, the Gurupa Várzea, the most poorly sampled ecoregion (depicted in yellow, SAC = 1.00), is situated close to the well-sampled areas, highlighting a poorly explored or underrepresented area in the data.

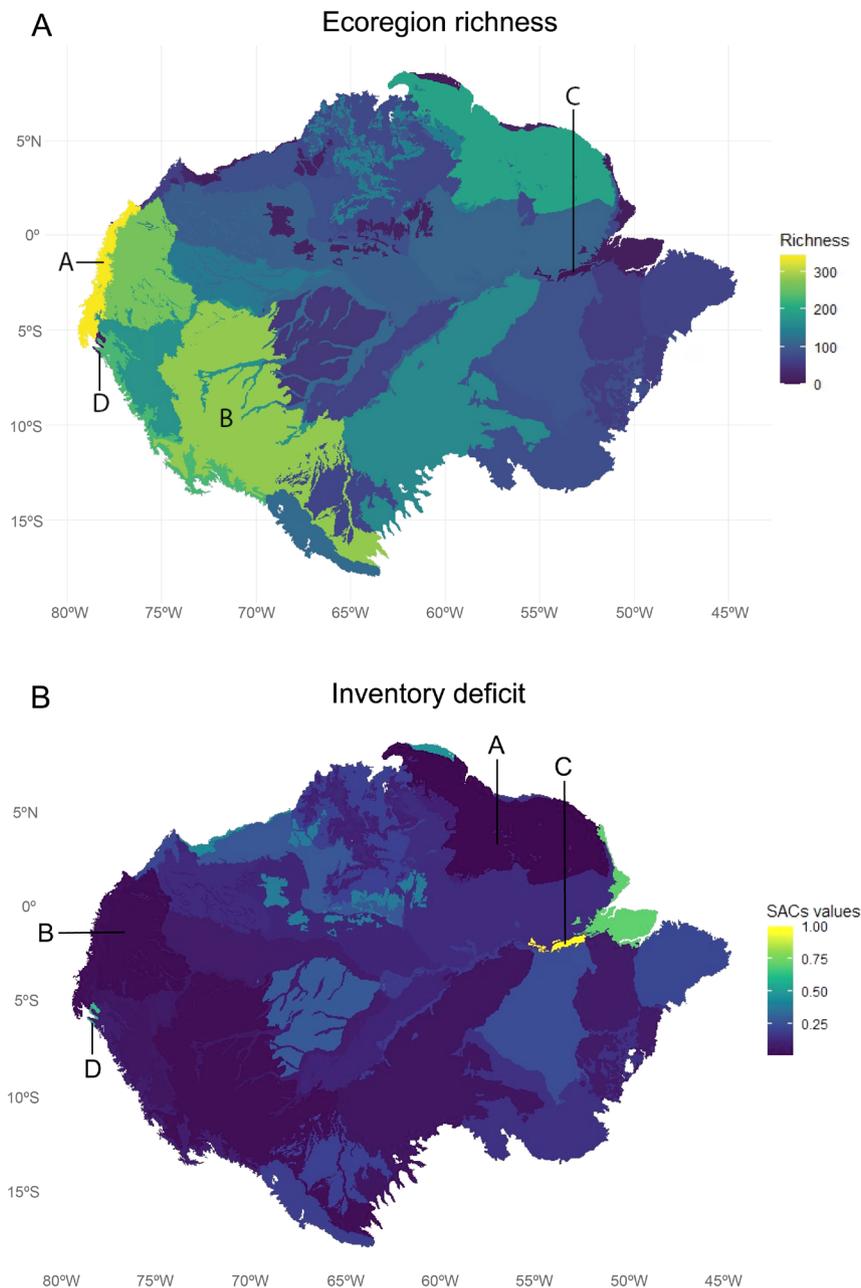


Figure 5. A) Species richness distribution per ecoregion, based on the occurrence data. The letters correspond to the ecoregions: **A** - Eastern Cordillera Real Montane Forests, **B** - Southwestern Amazonia Moist Forests, **C** - Gurupa Várzea, and **D** - Cordillera Central Páramo. B) Inventory Deficit in Amazonian Ecoregions. The map displays the standardized inventory completeness (SAC) values across Amazonian ecoregions, representing sampling coverage. Lighter tones (yellow) indicate lower completeness (higher inventory deficit) and thus a greater need for additional inventories, while darker tones (purple) represent better-sampled areas. The letters indicate the poorer and richer ecoregions: **A** - Guianan Lowland Moist Forests, **B** - Napo Moist Forest, **C** - Gurupa Várzea and **D** - Cordillera Central Páramo.

Darwinian shortfall

As expected, the most abundant sequences in data repositories are for the mitochondrial genes 16S and 12S (Figure 6), available for more than 500 of the currently recognized species, even though this estimate does not correspond to one-third of the estimated richness. The genes RAG1 (nuclear) and cytb (mitochondrial) were the next most frequent sequences in data repositories, sequenced for around 200 species. The less sequenced are the SLC8A3 and BDNF genes, known for less than 50 species (Figure 6).

After randomization and filtering, we retained 100 trees and adjusted for the 1,024 species distributed across 15 families. The standard deviation (sd) values ranged from 0 to 33.48 among clades. It is observed that uncertainty tends to vary within clades, with some terminal species showing lower variation compared to basal species. Considering variation by family, the mean SD values ranged from 3.16 to 28.91 (Figure 7, Table 2). Pipidae showed the highest phylogenetic uncertainty (5 species, mean sd = 28.91), while Allophrynidae had the lowest value (2 species, mean sd = 3.16). There was a predominance of variation in species' positions within clades for most families (Figure 7). Despite encompassing widely recognized species complexes, more speciose families exhibited varied uncertainty values. For instance, the family Craugastoridae (268 species), which

Table 2. Summary of species richness and phylogenetic uncertainty (SD) across anuran families. The table lists the number of species and the mean standard deviation (SD) of phylogenetic uncertainty for each family included in the study. Families with fewer species, such as Pipidae (5 species), exhibited the highest SD values (28.92). Conversely, families with many species, such as Craugastoridae (268 species), showed moderate uncertainty (mean SD = 11.10). The family with the lowest phylogenetic uncertainty was Allophrynidae (2 species, mean SD = 3.17).

Family	Species number	SD
Pipidae	5	28.91805
Telmatobiidae	16	17.18807
Ceratophryidae	2	11.6809
Microhylidae	36	11.19286
Craugastoridae	268	11.103
Centrolenidae	59	10.76937
Leptodactylidae	86	10.4335
Hemiphractidae	54	10.13657
Hylidae	213	9.13767
Aromobatidae	59	8.020893
Bufo	100	7.95004
Dendrobatidae	91	6.434303
Eleutherodactylidae	8	5.947633
Phyllomedusidae	23	4.768688
Allophrynidae	2	3.167011

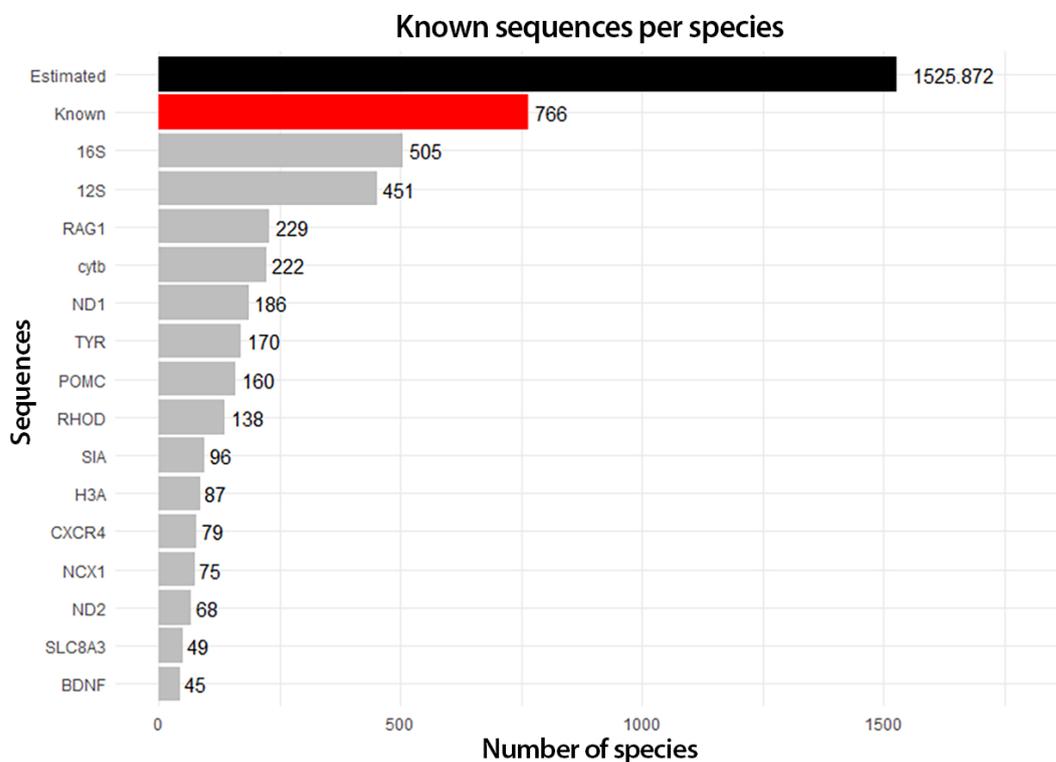


Figure 6. Bar chart showing the number of Amazonian anuran species for which genetic sequences are available. The first two bars represent the estimated total number of species in the region ("Estimated") and the number of currently described species ("Known"). The subsequent bars show the number of species with available sequences for specific mitochondrial (e.g., X16S, X12S, cytb) and nuclear (e.g., RAG1) genes. Bars are displayed in descending order of species count.

Phylogenetic uncertainty of anuran species

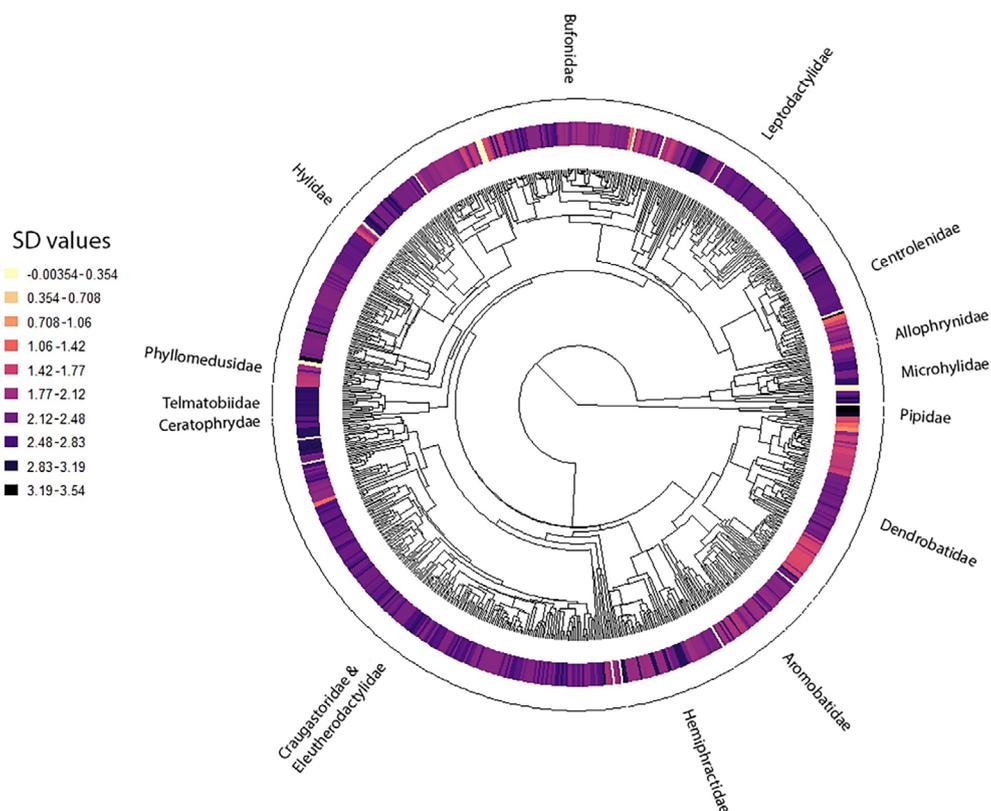


Figure 7. Phylogenetic trees show the variation in phylogenetic uncertainty among anuran species in the Amazon. The outer ring represents the mean standard deviation (SD) values of phylogenetic uncertainty for each species, with colors ranging from yellow (lower uncertainty) to dark purple (higher uncertainty). Families are labeled around the perimeter of the tree.

includes the genus *Pristimantis* – known for being one of the most challenging genera to identify – showed a mean sd value of 11.10, approximately one-third of the highest value observed for Pipidae (Table 2). Additionally, some families, such as Phyllomedusidae and Dendrobatidae, exhibit a more heterogeneous distribution, suggesting additional complexity in the phylogenetic placement of their species.

DISCUSSION

Although amphibians are relatively well-studied on a global scale, significant knowledge gaps persist in regions with high species richness and limited sampling, such as the Amazonia. In our study, we assessed the extent of these gaps by evaluating taxonomy, distribution, and phylogenetic knowledge of Amazonian anurans. We estimated that approximately 50.22% of the species remain undescribed, and show that species records are spatially clustered around urban centers, large rivers, and easily accessible locations. In addition, our analysis revealed considerable uncertainty regarding the phylogenetic positioning and the availability of genetic data,

challenging our understanding of Amazonian biodiversity distribution and its origins and evolutionary history.

The exponential growth in the number of species description publications along time, reported here, corresponds with trends observed for amphibians in Brazil (Guerra *et al.* 2020), South America (Vasconcelos *et al.* 2019), and globally (Frachea *et al.* 2024). Two major increases occurred: one in the early 20th century, and the second and largest occurred from the 2000s onwards, with the strengthening of scientific research and the expansion of Graduate Programs in Brazil and other Amazonian countries (CAPES 2024). This expansion enabled greater access to advanced molecular techniques and facilitated the development of integrative taxonomy approaches, which combines molecular, morphological, ecological and other data sources (Schlick-Steiner *et al.* 2010). Applying these new methods accelerated the identification of previously undescribed species and revealed cryptic speciation through DNA sequence divergence, a process that traditional taxonomic approaches alone cannot achieve as efficiently (Moraes *et al.* 2017, Kok *et al.* 2018, Mónico *et al.* 2024). In addition to academic training and access to

molecular tools, strengthening biodiversity research in the Amazon also depends on expanding long-term monitoring programs based on permanent sampling sites, consolidating the management of protected areas through science-based actions, and improving biodiversity assessments (e.g. Costa *et al.* 2015) sometimes associated with infrastructure licensing processes (Bergallo *et al.* 2023). Such efforts are fundamental not only to accelerate species descriptions but also to refine our understanding of species distributions, which remains one of the major challenges for Amazonian biodiversity.

Despite this recent history of capacity building (e.g., investment increase and training of new taxonomists) and the number of described species, we estimate that, at the current rate and research landscape, it would still take approximately 50 years to describe all Amazonian anuran species. The slow pace at which new species are described may be due to the difficulty in obtaining all necessary information about new species (holotypes, paratypes, and occurrence points) and accurately delineating the distribution area of known species. These challenges are primarily due to limited financial resources aimed at fulfilling specific gaps, logistical problems, and even a shortage of taxonomists (Drew 2011, Löbl *et al.* 2023). Therefore, there is a need to expand taxonomist training and increase financial investment at steady rates specifically to sample new areas. Knowing and describing species becomes urgent in the current climate change and habitat destruction scenario that directly threatens biodiversity even before it is fully understood (Löbl *et al.* 2023, Liu *et al.* 2024). Our results suggest that, despite the current number of universities and research institutes in the Amazonian region, specific policies, establishment of cross-countries research networks and investments are crucial for accelerating taxonomic studies within these institutions.

In addition to the large number of amazonian anuran species yet to be described, the known distribution of species has not been evenly recorded across the biome, with a significant concentration of records and studies around major urban centers, areas near large rivers, and roads, similar to what has been found for other taxa. These findings are consistent with distribution patterns observed in other taxonomic groups such as bats, angiosperms, arthropods, and birds (Hopkins 2007, Oliveira *et al.* 2016, Santos *et al.* 2020). Many species have narrow ranges, often restricted to specific ecoregions or interfluves, while few are considered broadly distributed. Conversely, there are also cases where species initially thought to have narrow distributions, such as *Cruziophyla craspedopus*, were later found to be widely distributed as sampling expanded (Bitar *et al.* 2015). This highlights the critical role of detectability and sampling effort in shaping our current understanding of species distributions. This suggests that the species distribution knowledge is more challenging than the taxonomic shortfall (Lees *et al.* 2020). It depends on refining information for already known species and delineating

occurrence areas for undescribed species. Even for well-known and widely distributed species, conducting studies in remote areas can lead to the identification of cryptic species (species complexes) that were originally recognized under a single name (Guerra *et al.* 2020, Rainha *et al.* 2021, Frachea *et al.* 2024), as seen in some species of the genera *Allobates* (Lima *et al.* 2020), *Pristimantis* (Ortega-Andrade *et al.* 2015, Mônico *et al.* 2024) and *Anomaloglossus* (Vacher *et al.* 2017).

According to our criteria, only 3.26% of the cells and 22.43% of the ecoregions were considered well-sampled, while 43.78% of the cells and 58.78% of the ecoregions were poorly to reasonably sampled. This pattern aligns with recent analyses highlighting persistent sampling gaps across the Amazon, which are driven by a combination of logistical barriers, financial limitations and spatial biases (Carvalho *et al.* 2023). In Amazonia, the primary limiting factor for biological studies is low financial investment, which in 2010 was estimated to only US\$ 0.01 per hectare (Magnusson *et al.* 2013). More recently, investment per project has been estimated as approximately US\$ 0.13 per square kilometer (Stegmann *et al.* 2024). Expeditions to remote areas are extremely expensive, logistically challenging, and require extensive planning and execution periods (Moraes *et al.* 2017, Carvalho *et al.* 2023, FAPESP 2024).

Even when expeditions are feasible, the detection of anurans can be difficult depending on the habitat, due to their small size, restricted geographic distribution, and morphological and behavioral traits (Barnett *et al.* 2023, Guedes *et al.* 2024). Additionally, it is important to note that, as with any analysis based on presence-only data, the absence of a species in a given cell does not necessarily represent a true absence but may reflect limited detectability or sampling effort. Therefore, the patterns of species richness and distribution described here are likely to capture a combination of biological reality and the spatial heterogeneity of sampling effort across the Amazon. Many species are active only during specific reproductive periods or times of the day and exhibit camouflage strategies that hinder their detection (Rojas 2017). Hence, expeditions to sample remote areas can provide previously unknown information about local and regional diversity (Metcalf *et al.* 2020, Moraes *et al.* 2022). Therefore, coordinated efforts among researchers, funding agencies, and governments are essential to expand knowledge about biodiversity and species distribution in the Amazon.

The Darwinian shortfall adds a further layer of complexity to biodiversity research. We found that only two genes (12S and 16S) are available for more than half of the known species, a similar result previously found by Mayer *et al.* (2019). The absence of molecular data limits our understanding of biodiversity in several ways. First, it underestimates the genetic diversity present in populations. Second, it hinders the discovery of cryptic species, which are more appropriately

detectable by sampling multiple genes and explicit use of species delimitation approaches (Rodríguez *et al.* 2017). Lastly, many genetic differences may be missed by simpler methodologies, making it necessary to employ more robust approaches (e.g., multilocus, genomic-level datasets or integrative taxonomy) to avoid species overlapping and establish a more solid foundation for taxonomic classification (Wiens 2007). Given our results on phylogenetic uncertainty, it is likely that the absence or incomplete sampling of genes is responsible for the inconsistent positioning of some taxa in the phylogeny. The lack of molecular data, combined with issues like misidentification, convergent traits, sequencing errors, and limited analytical methods, makes it harder to determine the correct position of species in the phylogeny (Wiens 2007, Rangel *et al.* 2015, Som 2015, Coates *et al.* 2018, Rancilhac *et al.* 2024).

Our results show that the absence of molecular data occurs for more than half of the estimated species, even considering the most common genes, and such absences may reflect low confidence in the phylogenetic position of many Amazonian species. Maintaining the Darwinian shortfall directly impacts the conservation and management of species and their environments. Undetected species may lack the necessary protection, and genetically distinct populations could be mismanaged as a single group, raising their extinction risks (Coates *et al.* 2018). This gap will only be minimized with significant efforts and investments to collect as much molecular data as possible and to use approaches that account for the complexity of the biology of this group.

CONCLUSIONS

Our study integrates multiple data sources to expose persistent knowledge shortfalls and inform future research priorities for Amazonian anurans. The gaps identified across taxonomy, geographic distribution, and molecular data reveal critical biodiversity blind spots — including under-sampled ecoregions such as Gurupa Várzea and Cordillera Central Páramo, overlooked families like Pipidae and Telmatobiidae, and poorly represented molecular datasets, particularly nuclear and genomic data. These deficiencies not only hinder a comprehensive understanding of Amazonian amphibian diversity but may also compromise effective conservation strategies. To address these challenges, we stress the need to expand field sampling in poorly explored regions, adopt integrative taxonomic approaches to uncover hidden diversity, and prioritize molecular research to strengthen phylogenetic and evolutionary insights. Finally, identifying conservation priority areas and implementing long-term monitoring programs are essential steps to ensure the protection of both Amazonian amphibians and the broader ecosystem they inhabit.

ACKNOWLEDGMENTS

BSG acknowledges *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for the doctoral and sandwich Ph.D. scholarships (grants 88887.633128/2021-00 and 88881.690402/2022-01) and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for the professional scholarship (406239/2022-3 and 407160/2023-0) that supported the development of this work. FPW thanks CNPq (productivity fellowship 307695/2023-9) and *Fundação de Amparo à Pesquisa do Estado do Amazonas* (FAPEAM, 1.02.016301.04697/2022-45). We are also grateful to Josué Azevedo, Erik Choueri, and Gabriel Costa for their valuable suggestions and assistance with the analyses, as well as to Jordana Guimarães, Lywouty Nascimento, Lucas Rosado and Igor Yuri Fernandes for providing the anuran pictures that added a visually appealing touch to this article.

REFERENCES

- Albert, J.S.; Carnaval, A.C.; Flantua, S.G.A.; Lohmann, L.G.; Ribas, C.C.; Riff, D.; *et al.* 2023. Human impacts outpace natural processes in the Amazon. *Science* 379: eabo5003.
- Alves-Ferreira, G.; Heming, N.M.; Talora, D.; Keitt, T.H.; Solé, M.; Zamudio, K.R. 2025. Climate change is projected to shrink phylogenetic endemism of Neotropical frogs. *Nature Communications* 16: 3713.
- Antonelli, A.; Zizka, A.; Carvalho, F.A.; Scharn, R.; Bacon, C.D.; Silvestro, D.; *et al.* 2018. Amazonia is the primary source of Neotropical biodiversity. *Proceedings of the National Academy of Sciences* 115: 6034–6039.
- Anuniação, P.R.; Valencia-Zuleta, A.; Signorelli, L.; Martins, M.; Bastos, R.P.; Bataus, Y.; *et al.* 2024. Amphibian conservation status in Brazil: Spatial patterns, threats, and challenges. *Journal for Nature Conservation* 79: 126611.
- Azevedo, J.A.R.; Faurby, S.; Colli, G.R.; Antonelli, A.; Werneck, F.P. 2024. Deforestation limits evolutionary rescue under climate change in Amazonian lizards. *Diversity and Distributions* 30: e13860.
- Barnett, J.B.; Yeager, J.; McEwen, B.L.; Kinley, I.; Anderson, H.M.; Guevara, J. 2023. Size-dependent colouration balances conspicuous aposematism and camouflage. *Journal of Evolutionary Biology* 36: 1010–1019.
- Bergallo, H.G.; Rosa, C.; Ochoa, A.C.; Manzatto, A.G.; Guimaraes, A.F.; Banhos, A.; *et al.* 2023. Long-term Ecological Research: Chasing fashions or being prepared for fashion changes? *Anais da Academia Brasileira de Ciências* 95: e20230051.
- Bickford, D.; Lohman, D.J.; Sodhi, N.S.; Ng, P.K.L.; Meier, R.; Winker, K.; *et al.* 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22: 148–155.
- Bitar, Y.O.D.C.; Silva, K.R.A.; Filho, H.F.D.S.; Pinheiro, L.C. 2015. Amphibia, Anura, Hylidae, *Cruziohyla craspedopus* (Funkhouser, 1957): distribution extension and first record from the state of Pará, Brazil. *Check List* 11: 1574.
- Cadotte, M.W.; Carscadden, K.; Mirotchnick, N. 2011. Beyond species: functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology* 48: 1079–1087.

- Camacho-Rozo, C.P.; Urbina-Cardona, N. 2024. Major knowledge shortfalls for Colombian Amazonian anurans: Implications for conservation. *Austral Ecology* 49: e13564.
- CAPES. 2024. *GEOCAPES - Sistema de Informações Georreferenciadas*. (<https://geocapes.capes.gov.br/geocapes/>). Accessed on 28 Nov. 2024.
- Carvalho, R.L.; Resende, A.F.; Barlow, J.; França, F.M.; Moura, M.R.; Maciel, R.; et al. 2023. Pervasive gaps in Amazonian ecological research. *Current Biology* 33: 3495–3504.e4.
- Carvalho, T.R.D.; Giaretta, A.A.; Angulo, A.; Haddad, C.F.B.; Peloso, P.L.V. 2019. A New Amazonian Species of *Adenomera* (Anura: Leptodactylidae) from the Brazilian State of Pará: A Tody-Tyrant Voice in a Frog. *American Museum Novitates* 3919: 1.
- Ceron, K.; Paula, T.; Scheibler, P.; Fadel, R.; Guimarães, C.D.S.; Silva, L.A.; et al. 2023. Trophic ecology of small to large hylids from an Amazonia-Cerrado transitional zone in Brazil. *Food Webs* 36: e00295.
- Coates, D.J.; Byrne, M.; Moritz, C. 2018. Genetic Diversity and Conservation Units: Dealing With the Species-Population Continuum in the Age of Genomics. *Frontiers in Ecology and Evolution* 6: 165.
- Costa, F.V.; Costa, F.R.C.; Magnusson, W.E.; Franklin, E.; Zuanon, J.; Cintra, R.; et al. 2015. Synthesis of the first 10 years of long-term ecological research in Amazonian Forest ecosystem – implications for conservation and management. *Perspectives in Ecology and Conservation* 13: 3–14.
- Darela-Filho, J.P.; Rammig, A.; Fleischer, K.; Reichert, T.; Lugli, L.F.; Quesada, C.A.; et al. 2024. Reference maps of soil phosphorus for the pan-Amazon region. *Earth System Science Data* 16: 715–729.
- Drew, L.W. 2011. Are We Losing the Science of Taxonomy? *BioScience* 61: 942–946.
- Duellman, W.E.; Trueb, L. 1994. *Biology of amphibians*. Johns Hopkins university press, Baltimore London, 670p.
- Faith, D.P. 1992. Conservation evaluation and phylogenetic diversity. *Biological Conservation* 61: 1–10.
- FAPESP. 2024. *O desafio de planejar uma expedição científica à Amazônia*. AGÊNCIA FAPESP. (<https://agencia.fapesp.br/o-desafio-de-planejar-uma-expedicao-cientifica-a-amazonia/53354>). Accessed on 20 Dec. 2024.
- Ferrão, M.; Moravec, J.; Kaefer, I.L.; de Fraga, R.; Lima, A.P. 2018. New species of *Scinax* (Anura: Hylidae) with red-striped eyes from Brazilian Amazonia. *Journal of Herpetology* 52: 472–488.
- Fisch, G.; Marengo, J.A.; Nobre, C.A. 1998. Uma revisão geral sobre o clima da Amazônia. *Acta Amazonica* 28: 101–101.
- Flores, B.M.; Montoya, E.; Sakschewski, B.; Nascimento, N.; Staal, A.; Betts, R.A.; et al. 2024. Critical transitions in the Amazon forest system. *Nature* 626: 555–564.
- Frachea, A.; Amori, G.; Battisti, C.; Luiselli, L. 2024. Temporal and biogeographical patterns of new species descriptions in worldwide amphibians. *Rendiconti Lincei. Scienze Fisiche e Naturali* 35: 553–562.
- Freitas, T.M.S.; Stropp, J.; Calegari, B.B.; Calatayud, J.; De Marco, P.; Montag, L.F.A.; et al. 2020. Quantifying shortfalls in the knowledge on Neotropical Auchenipteridae fishes. *Fish and Fisheries* 22: 87–104.
- Frost, D.R. 2024. *Amphibian Species of the World*. (<https://amphibiansoftheworld.amnh.org/>). Accessed on 27 Nov. 2024.
- GBIF. 2024. *Global Biodiversity Information Facility*. (<https://www.gbif.org/>). Accessed on 27 Nov. 2024.
- Gotelli, N.J. 2008. *A Primer of Ecology*. 4th ed. Sinauer Associates, Sunderland, MA, 291p.
- Guayasamin, J.M.; Ribas, C.C.; Carnaval, A.C.; Carrillo, J.D.; Hoorn, C.; Lohmann, L.G.; et al. 2024. Evolution of Amazonian biodiversity: A review. *Acta Amazonica* 54: e54bc21360.
- Guedes, J.J.M.; Diniz-Filho, J.A.F.; Moura, M.R. 2024. Macroecological correlates of Darwinian shortfalls across terrestrial vertebrates. *Biology Letters* 20: 20240216.
- Guerra, V.; Jardim, L.; Llusia, D.; Márquez, R.; Bastos, R.P. 2020. Knowledge status and trends in description of amphibian species in Brazil. *Ecological Indicators* 118: 106754.
- Haddad, C.F.B.; Prado, C.P.A. 2005. Reproductive Modes in Frogs and Their Unexpected Diversity in the Atlantic Forest of Brazil. *BioScience* 55: 207.
- Hof, C.; Araújo, M.B.; Jetz, W.; Rahbek, C. 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature* 480: 516–519.
- Hoorn, C.; Wesselingh, F.P.; Ter Steege, H.; Bermudez, M.A.; Mora, A.; Sevink, J.; et al. 2010. Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science* 330: 927–931.
- Hopkins, M.J.G. 2007. Modelling the known and unknown plant biodiversity of the Amazon Basin. *Journal of Biogeography* 34: 1400–1411.
- Hortal, J.; De Bello, F.; Diniz-Filho, J.A.F.; Lewinsohn, T.M.; Lobo, J.M.; Ladle, R.J. 2015. Seven Shortfalls that Beset Large-Scale Knowledge of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 46: 523–549.
- IBGE. 2023. *BDIA - Banco de Dados e Informações Ambientais*. (<https://bdiaweb.ibge.gov.br/#/consulta/vegetacao>). Accessed on 04 Aug. 2025.
- IUCN. 2024. *The IUCN Red List of Threatened Species*. IUCN Red List of Threatened Species. (<https://www.iucnredlist.org/en>). Accessed on 27 Nov. 2024.
- Jetz, W.; Pyron, R.A. 2018. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution* 2: 850–858.
- Joly, C.A.; Scarano, F.R.; Bustamante, M.; Gadda, T.M.C.; Metzger, J.P.W.; Seixas, C.S.; et al. 2019. Brazilian assessment on biodiversity and ecosystem services: summary for policy makers. *Biota Neotropica* 19: e20190865.
- Kok, P.J.R.; Nicolai, M.P.J.; Lathrop, A.; MacCulloch, R.D. 2018. *Anomaloglossus meansi* sp. n., a new Pantepui species of the *Anomaloglossus* beebei group (Anura, Aromobatidae). *ZooKeys* 759: 99–116.
- Lees, A.C.; Rosenberg, K.V.; Ruiz-Gutierrez, V.; Marsden, S.; Schulenberg, T.S.; Rodewald, A.D. 2020. A roadmap to

- identifying and filling shortfalls in Neotropical ornithology. *The Auk* 137: ukaa048.
- Lima, A.P.; Ferrão, M.; Lacerda da Silva, D. 2020. Not as widespread as thought: Integrative taxonomy reveals cryptic diversity in the Amazonian nurse frog *Allobates tiniae* Melo-Sampaio, Oliveira and Prates, 2018 and description of a new species. *Journal of Zoological Systematics and Evolutionary Research* 58: 1173–1194.
- Linnaeus, C. 1758. *Systema naturae per regna tria naturae :secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. 10th ed. Impensis Direct. Laurentii Salvii, Stockholm, Sweden, .
- Liu, D.; Thompson, J.R.; Gao, J.; Shen, H. 2024. Train young scientists in taxonomy to help solve the biodiversity crisis. *Nature* 626: 954–954.
- Löbl, I.; Klausnitzer, B.; Hartmann, M.; Krell, F. 2023. The Silent Extinction of Species and Taxonomists—An Appeal to Science Policymakers and Legislators. *Diversity* 15: 1053.
- Lu, M.; He, F. 2017. Estimating regional species richness: The case of China’s vascular plant species. *Global Ecology and Biogeography* 26: 835–845.
- Luedtke, J.A.; Chanson, J.; Neam, K.; Hobin, L.; Maciel, A.O.; Catenazzi, A.; *et al.* 2023. Ongoing declines for the world’s amphibians in the face of emerging threats. *Nature* 622: 308–314.
- Magnusson, W.; Braga-Neto, R.; Pezzini, F.; Baccaro, F.; Bergallo, H.G.; Penha, J.; *et al.* 2013. Biodiversidade e Monitoramento Ambiental Integrado. .
- Mayer, M.; Fonte, L.F.M.; Lötters, S. 2019. Mind the gap! A review of Amazonian anurans in GenBank. 55: 89–96.
- Metcalf, M.; Marsh, A.; Torres, E.; Graham, D.; Gunnels, C. 2020. Herpetofauna of the Santa Cruz Forest Preserve in the Peruvian Amazon Basin. *Herpetology Notes* 13: 753–767.
- Mônico, A.T.; Koch, E.D.; Ferrão, M.; Fernandes, I.Y.; Marques, G.M.G.; Chaparro, J.C.; *et al.* 2024. The small and inconspicuous majority: Revealing the megadiversity and historical biogeography of the *Pristimantis unistrigatus* species group (Anura, Strabomantidae). *Molecular Phylogenetics and Evolution* 201: 108203.
- Moraes, L.J.C.L.; de Almeida, A.P.; de Fraga, R.; Rojas, R.R.; Pirani, R.M.; Silva, A.A.A.; *et al.* 2017. Integrative overview of the herpetofauna from Serra da Mocidade, a granitic mountain range in northern Brazil. *ZooKeys*: 103.
- Moraes, L.J.C.L.; Rainha, R.N.; Werneck, F.P.; Oliveira, A.F.S.; Gascon, C.; Carvalho, V.T. 2022. Amphibians and reptiles from a protected area in western Brazilian Amazonia (Reserva Extrativista do Baixo Juruá). *Papéis Avulsos de Zoologia* 62: e202262054.
- Nunes-de-Almeida, C.H.L.; Haddad, C.F.B.; Toledo, L.F. 2021. A revised classification of the amphibian reproductive modes. *SALAMANDRA German Journal of Herpetology* 57: 413–427.
- Oksanen, J.; Simpson, G.L.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; *et al.* 2024. vegan: Community Ecology Package. : 2.6-8.
- Oliveira, U.; Paglia, A.P.; Brescovit, A.D.; de Carvalho, C.J.B.; Silva, D.P.; Rezende, D.T.; *et al.* 2016. The strong influence of collection bias on biodiversity knowledge shortfalls of Brazilian terrestrial biodiversity. *Diversity and Distributions* 22: 1232–1244.
- Olson, D.M.; Dinerstein, E.; Wikramanayake, E.D.; Burgess, N.D.; Powell, G.V.N.; Underwood, E.C.; *et al.* 2001. Terrestrial ecoregions of the world: A new map of life on Earth. *BioScience* 51: 933–938.
- Ortega-Andrade, H.M.; Rojas-Soto, O.R.; Valencia, J.H.; Espinosa De Los Monteros, A.; Morrone, J.J.; Ron, S.R.; *et al.* 2015. Insights from Integrative Systematics Reveal Cryptic Diversity in *Pristimantis* Frogs (Anura: Craugastoridae) from the Upper Amazon Basin. *PLOS ONE* 10: e0143392.
- Paradis, E.; Schliep, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35: 526–528.
- Penhacek, M.; Souza, T.; Santos, J.; Guerra, V.; Castro-Souza, R.; Rodrigues, D. 2024. Amazonian amphibians: diversity, spatial distribution patterns, conservation and sampling deficits. *Biodiversity Data Journal* 12: e109785.
- R Core Team. 2024. *R: The R Project for Statistical Computing*. (<https://www.r-project.org/>). Accessed on 27 Nov. 2024.
- Rainha, R.N.; Martinez, P.A.; Moraes, L.J.C.L.; Castro, K.M.S.A.; Réjaud, A.; Fouquet, A.; *et al.* 2021. Subtle environmental variation affects phenotypic differentiation of shallow divergent treefrog lineages in Amazonia. *Biological Journal of the Linnean Society* 134: 177–197.
- Rancilhac, L.; Enbody, E.D.; Harris, R.; Saitoh, T.; Irestedt, M.; Liu, Y.; *et al.* 2024. Introgression Underlies Phylogenetic Uncertainty But Not Parallel Plumage Evolution in a Recent Songbird Radiation. *Systematic Biology* 73: 12–25.
- Rangel, T.F.; Colwell, R.K.; Graves, G.R.; Fučíková, K.; Rahbek, C.; Diniz-Filho, J.A.F. 2015. Phylogenetic uncertainty revisited: Implications for ecological analyses. *Evolution* 69: 1301–1312.
- Rodríguez, A.; Dugo-Cota, A.; Montero-Mendieta, S.; Gonzalez-Voyer, A.; Bosch, A.; Vences, M.; *et al.* 2017. Cryptic within cryptic: genetics, morphometrics, and bioacoustics delimitate a new species of *Eleutherodactylus* (Anura: Eleutherodactylidae) from Eastern Cuba. *Zootaxa* 4221.
- Rojas, B. 2017. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. *Biological Reviews* 92: 1059–1080.
- Rojas, R.R.; Fouquet, A.; Ron, S.R.; Hernández-Ruz, E.J.; Melo-Sampaio, P.R.; Chaparro, J.C.; *et al.* 2018. A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura: Bufonidae). *PeerJ* 6: e4941.
- Santos, T.C.M.; Lopes, G.P.; Rabelo, R.M.; Giannini, T.C. 2020. Bats in Three Protected Areas of The Central Amazon Ecological Corridor in Brazil. *Acta Chiropterologica* 21: 425.
- Schlick-Steiner, B.C.; Steiner, F.M.; Seifert, B.; Stauffer, C.; Christian, E.; Crozier, R.H. 2010. Integrative Taxonomy: A Multisource Approach to Exploring Biodiversity. *Annual Review of Entomology* 55: 421–438.
- Silva Junior, C.H.L.; Pessôa, A.C.M.; Carvalho, N.S.; Reis, J.B.C.; Anderson, L.O.; Aragão, L.E.O.C. 2021. The Brazilian Amazon deforestation rate in 2020 is the greatest of the decade. *Nature Ecology & Evolution* 5: 144–145.
- Som, A. 2015. Causes, consequences and solutions of phylogenetic incongruence. *Briefings in Bioinformatics* 16: 536–548.

- Stegmann, L.F.; França, F.M.; Carvalho, R.L.; Barlow, J.; Berenguer, E.; Castello, L.; *et al.* 2024. Brazilian public funding for biodiversity research in the Amazon. *Perspectives in Ecology and Conservation* 22: 1–7.
- Tylianakis, J.M.; Didham, R.K.; Bascompte, J.; Wardle, D.A. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11: 1351–1363.
- Vacher, J.P.; Kok, P.J.R.; Rodrigues, M.T.; Lima, J.D.; Lorenzini, A.; Martinez, Q.; *et al.* 2017. Cryptic diversity in Amazonian frogs: Integrative taxonomy of the genus *Anomaloglossus* (Amphibia: Anura: Aromobatidae) reveals a unique case of diversification within the Guiana Shield. *Molecular Phylogenetics and Evolution* 112: 158–173.
- Vasconcelos, T.S.; da Silva, F.R.; dos Santos, T.G.; Prado, V.H.M.; Provete, D.B. 2019. *Biogeographic Patterns of South American Anurans*. Springer International Publishing, Cham, .
- Vergara, A.; Arias, M.; Gachet, B.; Naranjo, L.G.; Román, L.; Surkin, J.; *et al.* 2022. *Living Amazon Report 2022*. WWF, Quito, 98p.
- Wiens, J.J. 2007. Species Delimitation: New Approaches for Discovering Diversity. *Systematic Biology* 56: 875–878.
- WWF. 2019. *Amazon_Rainforest (FeatureServer)*. (https://services2.arcgis.com/j80Jz20at6Bi0thr/arcgis/rest/services/Amazon_Rainforest/FeatureServer). Accessed on 27 Nov. 2024.
- WWF. 2021. Amazon Ecoregion shapefile [geospatial data]. Available from: <https://www.arcgis.com/home/item.html?id=841600854d934d7d8a1656eee32d5847>. Accessed on 08 Sep 2021.

RECEIVED: 12/01/2025

ACCEPTED: 29/07/2025

ASSOCIATE EDITOR: Paulo Estefano Bobrowiec 

DATA AVAILABILITY: The data that support the findings of this study are available upon request from the corresponding author, Brena da Silva Gonçalves.

AUTHOR CONTRIBUTIONS:

GONÇALVES, B.S.: Conceptualization, Methodology, Formal analysis, Data Curation, Writing - Original Draft, Writing - review & editing.

DAMBROS, C.S.: Conceptualization, Methodology, Formal analysis, Writing - original draft.

WERNECK, F.P.: Conceptualization, Formal analysis, Visualization, Writing - review & editing.

