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A taxonomic integrative approach of the anuran species from a Cerrado-Amazonian transitional area in western Tocantins state, Brazil

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Abstract

Due to their complexity environments, ecotonal areas harbor high levels of biodiversity. The Cerrado is geographically situated among other Brazilian biomes and exhibits extensive transitional areas with neighboring biomes. The state of Tocantins, in northern Brazil, has a vast transitional area between the Cerrado and Amazonia in the North and West. Despite the remarkable richness revealed in recent studies, the anuran fauna of Tocantins is likely underestimated, as several municipalities have knowledge gaps, particularly those located in these Cerrado-Amazonia transitional areas. Here, we improve the knowledge of amphibians' diversity in a Cerrado-Amazonia transitional area in western Tocantins state using integrative taxonomy. We conducted four field expeditions in the Caseara municipality, totaling 50 days of fieldwork. We performed diurnal and nocturnal active surveys and installed pitfall traps along an environmental gradient, ranging from typical Cerrado habitats to typical Amazon habitats. We combined morphology, bioacoustic, and mtDNA to improve the identification of the collected specimens. In total, we collected 738 vouchers assigned to 42 species belonging to seven families. We also present morphometric data for 38 of the recorded species (n = 609), acoustic data for 19 species, and 21 new 16S mtDNA sequences for seven species. These new data were essential to identify 38 of the 42 recorded species at the species level, including interesting new records for species like *Allobates crombiei* and *Scinax similis*. Four recorded species remained with an undefined status: *Boana* sp. (gr. *semilineata*), *Adenomera* sp. (aff. *hylaedactyla*), *Physalaemus* sp. (gr. *cuvieri*), and *Proceratophrys* sp. (cf. *ararype*). We encourage the use of similar approaches in species inventories to ensure taxonomic accuracy and to provide reliable data for conservation policies.

Key words: Amphibians, Bioacoustic, Biodiversity, Ecotone, Morphology, mtDNA

Introduction

Transitional areas are unique heterogeneous landscapes that enable faunal exchanges between biomes (Smith *et al*. 1997). These areas have garnered increased attention as significant centers for ecological and evolutionary processes (Matavelli *et al*. 2019). Despite harbor high levels of biodiversity, transitional areas also face critical threats due to human land use and climate changes, leading to organisms declining and/or local extinctions (Pinheiro & Dornas 2009; Collins 2010; Matavelli *et al.* 2019; Taylor *et al*. 2020). The decline in biodiversity is particularly alarming

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as numerous unknown and undescribed species may disappear even before their discovery, which demands urgent strategies to uncover and characterize the biological diversity and the unique ecological processes and dynamics exclusive to these complex areas (Lees & Pimm 2015). Thus, filling taxonomic gaps in these regions (e.g. Linnean shortfall, Hortal *et al*. 2015) is crucial, especially for amphibians, which are among the most threatened groups of organisms globally (Stuart *et al*. 2004; Wake & Vredenburg 2008; Whittaker *et al*. 2013).

The Cerrado biome is considered the most diverse and threatened savanna on Earth (Silva & Bates 2002), harboring a rich biodiversity distributed throughout its vast area (Françoso *et al*. 2020). This biome is also a global conservation priority area due to its high number of endemic species and the substantial rates of natural habitat loss (Myers *et al*. 2000; Norman 2003; Françoso *et al*. 2015; Damasco *et al*. 2018; Françoso *et al*. 2020, Ribeiro *et al*. 2020). Additionally, the geographical location of the Cerrado allows its contact with other large South American biomes, Amazonia, Caatinga, Chaco, Atlantic Forest, and Pantanal (Silva & Bates 2002), creating extensive transitional areas and allowing the coexistence of species with different ecological requirements (Nogueira *et al*. 2011; Valdujo *et al*. 2012; Guedes *et al*. 2014; Andrade *et al*. 2017). The Amazonia is the world's largest tropical forest, hosting an immense diversity of terrestrial and aquatic plants and animals but also faces alarming rates of deforestation in the last years (Sioli 1984; Margalef 1997; Hoorn *et al*. 2010; Silva Junior *et al*. 2020). Together, the Amazonia and Cerrado accounts more than 318 and 271 species of amphibians (a total of 589 species), respectively, nearly 50% of the currently Brazilian recognized (Toledo & Batista 2012; Segalla *et al*. 2021). The ecotonal area of the Cerrado-Amazonia Transition (CAT henceforward) exceeds 6,000 km of extension (Torello-Raventos *et al*. 2013). However, the boundary between forests and savannas cannot be defined by a simple line, as it is formed by an irregular mosaic of environments with unique characteristics along its limits (Marimon *et al*. 2006; Marques *et al*. 2019).

The Tocantins state is predominantly covered by Cerrado phytophysiognomies (nearly 92%), while the Amazon covers nearly 7% of its territory in northern and western regions, which creates an extensive transitional zone between these two large South American biomes (Ab'Saber 1977; Ab'Saber 2002; Sano *et al*. 2010). Currently, there are 98 amphibian species known to occur in the Tocantins, 23 of them being Cerrado endemic and 25 Amazonian endemic (Silva *et al*. 2020; Motta *et al.* 2020; Silva *et al.* 2023). However, this number is likely underestimated due to the lack of information on amphibian diversity in almost 60% of the municipalities in the state of Tocantins, particularly within the CAT (Silva *et al*. 2020). In the same study, the authors also highlighted the importance of further investigations of the identity of some amphibian lineages found in Tocantins using different lines of evidence, so that more accurate taxonomic determination could be provided. To achieve this objective, we further investigated an amphibian assemblage from Caseara municipality in western Tocantins state, combining morphological, acoustical, and molecular data, and providing a more detailed discussion on species that represented the most relevant records.

Materials and Methods

Study area and Sampling effort

We conducted the study in the municipality of Caseara, including the Reserva Particular do Patrimônio Natural (RPPN) Canto do Obrieni (~2,5 km²), as well as in three farms surrounding the RPPN, located in the Midwest region of Tocantins state, Brazil (Table 1). The region represents a transitional zone between the Cerrado and Amazon biomes (Fig. 1) and is characterized by an extensive remnant of Alluvial Semideciduous Seasonal Forest (Santiago and Pereira Júnior 2010), with forest formations (some seasonally flooded) and open areas (Cerrado and grassland formations) (Pinheiro & Dornas 2009; Tocantins 2016). According to the Köppen classification, the local climate is tropical, with a dry winter and wet summer (Aw), annual temperature varying between 24ºC and 26ºC, and the annual precipitation varies between 1,900 and 2,500 mm per year (Álvares *et al*. 2013). Although there are two protected areas nearby the sampling sites, the Parque Estadual do Cantão (890 km²) and the Parque Nacional do Araguaia (~5.500 km²), the study area includes only the recently created Reserva Particular do Patrimônio Natural (RPPN) Canto do Obrieni (~2,5 km²), which is situated within a matrix of pasture and monocultures (Ferreira *et al*. 2011).

Our sampling points were distributed in five areas: the Canadá, Santa Juliana, and Guaíra Farms, a natural pond next to a highway (here referred as lajedo), and in the RPPN Canto do Obrieni (Fig. 2; Table 1). We conducted

four field expeditions (FE) lasting between 11 and 14 each. The first one was performed in 2017 (FE I, from 19 to 30 November, rainy season), and the other three in 2018 (FE II in 22 January to 3 February, rainy season; FE III in 20 to 30 May, dry season; and FE IV in 11 to 24 November, rainy season), totaling 50 days of fieldwork. Four researchers conducted active surveys (in all five areas) in diurnal (2 hours in the morning and 2 hours in the afternoon) and nocturnal (~4 hours daily) periods, totaling 8 hours/day of sampling/researcher (i.e. 8 hours/day x 50 days x 4 researchers, totaling 1,600 hours of sampling). We also installed 11 pitfall trap stations in the RPPN Canto do Obrieni. Four stations were in open areas (Cerrado *stricto sensu*), each one displayed in a "Y" shape (Fig. 2F) and composed of four 40-liter buckets, placed five meters apart from each other. The seven remaining stations were installed in primary forest environments. Three stations were installed in lines, each one composed of five 60-liter buckets, five meters apart, and the other four were composed of three 60-liter buckets buried in line, with each extremity ending in two buckets laterally positioned (totaling seven buckets in each station). We opened the pitfalls on the first sampling day and closed them on the last day of each field expedition. We inspected the traps every day during all four expeditions, totalizing 44 nights of opened traps. This sampling effort totalized 2,596 buckets-day.

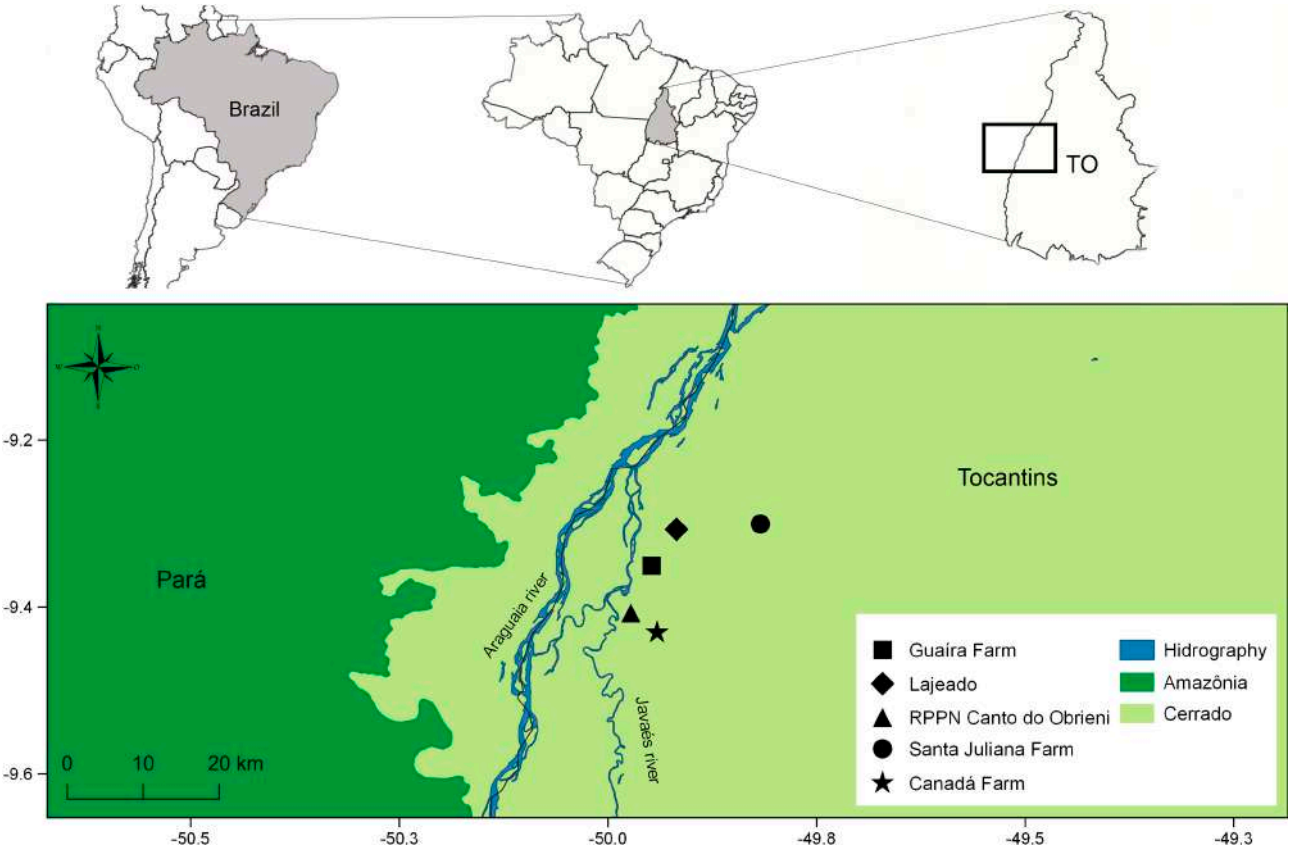

FIGURE 1. Sampling areas surveyed during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil.

We euthanized the specimens with a topical application of 5% lidocaine (following resolution CFBio nº148/2012; CFB 2012), fixed in 10% formalin, and preserved in alcohol 70% (Papavero 1994). Before formalin application, we removed muscle and/or liver tissue from specimens and stored it in absolute ethanol. Voucher specimens and tissues are housed in the Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (ZUFMS) and Coleção de Tecidos do Laboratório Mapinguari (MAP-T), respectively (Table S1). Specimens were collected according to permits granted by ICMBio (63228-1). Taxonomy follows Frost *et al.* (2024) where taxonomic authorities and nomenclatural accounts of all species can be found.

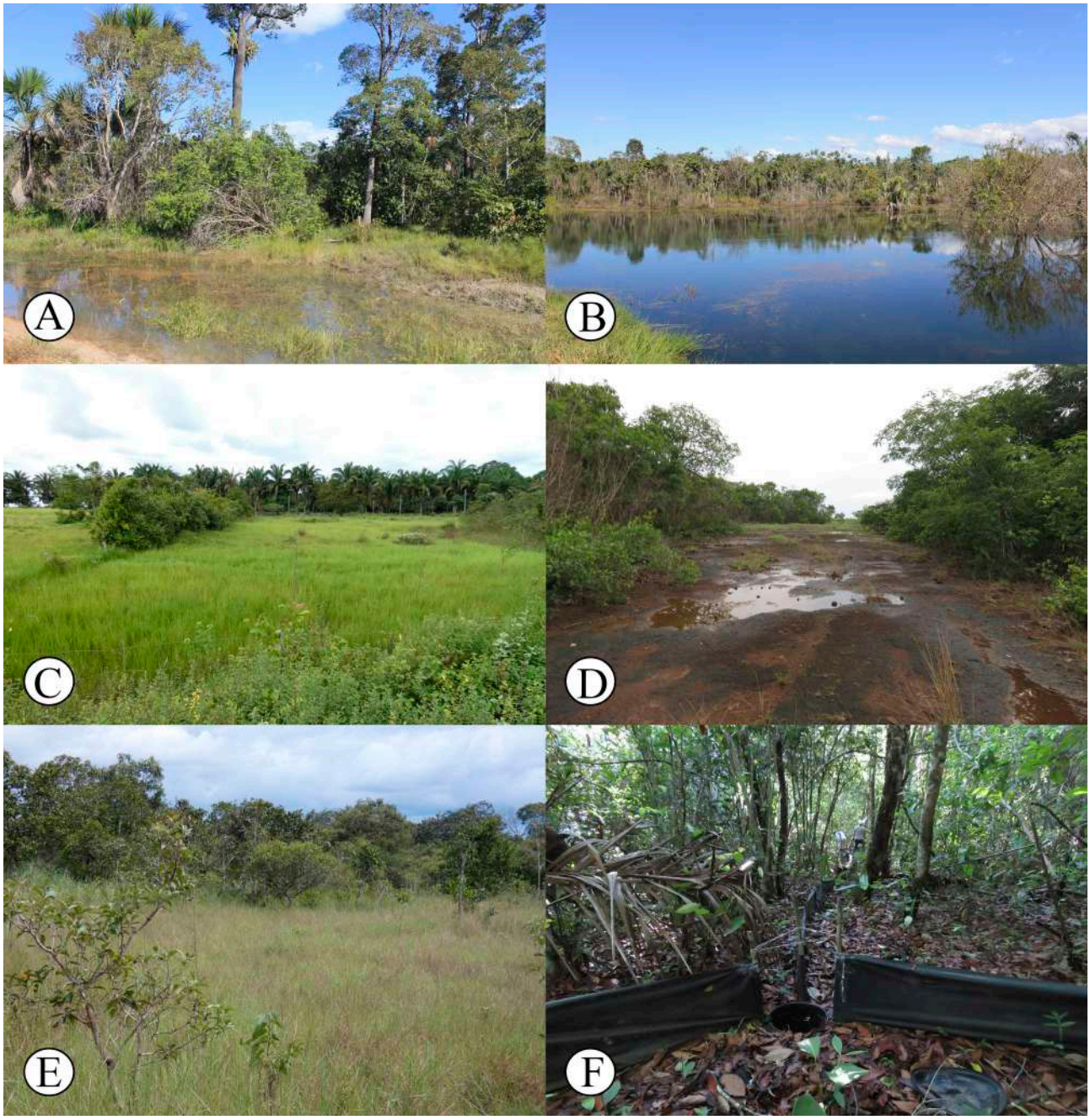

FIGURE 2. Overview of sampling areas surveyed during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil: A, B, C: Canadá Farm; D: Lajedo; E, F: RPPN Canto do Obrieni.

Morphology

We measured 609 adult specimens belonged to 38 species, following Watters *et al.* (2016): head length (HL); head width (HW); snout-vent length (SVL); snout length (SL); eye diameter (ED); upper eyelid width (UEW); internarial distance (IND); eye-nostril distance (END); interorbital distance (IOD); tympanum diameter (TD); tibia length (TL); foot length (FL); thigh length (THL); hand length (HAL); forearm length (FLL); finger IV disk width (Fin4DW). We evaluated other specific morphometric parameters of some species when necessary for proper taxonomic resolution (Table S2). We identified as adults those specimens engaged in vocalization activity or presenting developed secondary sexual characters, such as vocal sacs and vocal slits, nuptial pads, and the presence of eggs (females). We followed original species descriptions and taxonomic revisions for proper specimens' identification (e.g., Lourenço *et al*. 2015; Mângia *et al*. 2018).

TABLE 1. Description and coordinates of sample areas in the municipality of Caseara, Tocantins state, Brazil.

Area	Latitude	Longitude	Description
Guaíra Farm	-9.350560	-49.947597	Pond with sparse herbaceous vegetation and grasses, surrounded by pasture.
Santa Juliana Farm	-9.300474	-49.817380	Large and anthropized floodable area and legal reserve.
Canadá Farm	-9.430589	-49.941205	Seasonally flooded area (locally known as *veredas*) dominated by native grass and palm trees (*Mauritia flexuosa*) and surrounded by pasture.
RPPN Canto do Obrieni	-9.407044	-49.972592	Legal reserve: presence of open areas and gallery forest.
Lajedo	-9.306842	-49.917862	Large and seasonally flooded laterite inserted in a matrix of Cerrado *stricto sensu*.

Bioacoustics

We obtained 44 recordings for 21 species with a TASCAM DR-40 digital recorder, at sampling rate of 44.1 Hz and 16 bits' resolution (Table S3). We performed the bioacoustic analysis using the software Raven Pro 1.5 (Bioacoustics Research Program 2014) with the spectrogram settings on "default": window type = Hanning, window size = 512 samples, bandwidth = 124 Hz, overlap = 69.9%, DFT size = 1024 samples, hope size = 154 samples, grid spacing = 43.1 Hz. We obtained sound graphics using Seewave (Sueur *et al*. 2008) and tuneR (Ligges *et al*. 2014) packages of R platform (R Core Team 2017) with the following spectrogram parameters: Hanning window, FFT = 512, and 70% overlap. We adopted Köhler *et al.* (2017) definition of a call as an acoustic unit in frog vocalization, distinct from other calls by periods of silence (longer than the call itself), in accordance with the call-centered approach. We measured the call parameters following quantitative parameters considered in amphibian taxonomy: call duration (s), inter-call interval (s), pulse duration (s), pulse rate (pulses/s), dominant frequency (Hz), and other parameters as needed to compare with original descriptions, summarized in mean values and standard deviation. We compared the calls with the available literature and phonographic libraries for each species. The recordings used in the present study are in the online repository 10.5281/zenodo.14884692.

Molecular analyses

We extracted the genomic DNA of 21 specimens belonging to seven species using the phenol-chloroform protocol of Sambrook *et al*. (1989). We used the 16S ribosomal RNA mitochondrial (standard marker for amphibians; Vences *et al*. 2012), 16Sa/16Sb primer pair of Palumbi *et al*. (2002), following polymerase chain reaction (PCR) conditions described by Costa *et al*. (2016). The sequences were obtained by Sanger method using the ABI PRISM Big Dye Terminator V.3 Cycle Sequencing kit (Applied Biosystems, Waltham, Massachusetts, USA). Sequencing reactions were made in 96-well plates with a final volume of 10μL, containing 5 μL of sterile H_2O, 1.5 μL of sequencing buffer 5X, 0.5 μL of primer (10 μM), 1 μL of Big Dye mixture, and 2 μL of purified PCR products. The dye incorporation reactions followed 96°C/1 min; 35 cycles of 96°C/15 sec, 50°C/15 sec, and 60°C/4 min. The plates were precipitated in ethanol/EDTA, eluted with 10 μL Formamida Hi-Di and detected with an ABI 3500 genetic analyzer (Applied Biosystems), following the manufacturer's instructions. We edited and aligned the resulting sequences in the Geneious v.9.1.2 with the MUSCLE algorithm using default parameters (Edgar 2004). We deposited the sequences in the GenBank database (PV082231-PV082251, Table S4).

To further investigate the taxonomic status of the collected specimens (Table 3), we compared the mtDNA sequences generated in the present study for each focal lineage with those available for closely related taxa in GenBank (Benson *et al*. 2014). First, we used BLAST (available at https://blast.ncbi.nlm.nih.gov/Blast.cgi) to preliminarily check the identity of sequenced specimens and selected sequences with similarity above 96% to assemble the alignments of each one; for other species, we based on recent molecular phylogenetic taxonomic studies. The outgroups were set based on the literature available, and/or available sequences on Genbank. Then, we performed a Bayesian phylogenetic analysis in BEAST 1.8 (Drummond *et al*. 2012), using the best-fit model Bayesian Information Criterion (BIC), estimated in the jModelTest (Darriba *et al*. 2012). For each analysis we

ran 50 million generations, sampling every 1000 steps using a tree prior Yule Process. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size (ESS) were above 200 in Tracer v.1.5 (Rambaut & Drummond 2007). The first 10% of sampled genealogies were discarded as burn-in, and the maximum clade credibility tree with median node ages was calculated with TreeAnnotator v1.8 (Drummond *et al*. 2012). We also calculated sequence divergence (uncorrected p-distance) among species/individuals for the 16S mtDNA using MEGA v 6.06 (Tamura *et al*. 2013). The alignments used in this study are available in the Zenodo online repository at https://doi.org10.5281/zenodo.14884692.

Richness distribution and conservation status

We performed an accumulation curve with Estimates Statistical Program v.8.2.0 (Colwell 2009), using a presence/absence matrix (Gotelli & Colwell, 2001), through 1000 randomizations versus sampling effort. We used the species richness estimator Jackknife 1 to determine the expected richness (Colwell and Coddington, 1994; Colwell and Elsensohn, 2014). Based on Valdujo *et al*. (2012), we attributed the following distribution categories for each recorded species: CE–endemic to Cerrado: any species restricted to Cerrado (including narrow endemics or at least highly associated to the biome limits); WD–widespread (generalist species that consistently occur in more than two biomes, including both open and forested biomes); OD–diagonal belt of open biomes (species that occur in Caatinga, Cerrado, and Chaco biomes); and AM–Amazonia-Cerrado (species that occur in both Amazonia and Cerrado biomes). We also categorized the environment where each species was recorded as FF–forest formations (e.g., gallery forest); G–generalists (species recorded in more than one type of environment); AE–species associated with anthropic environments (e.g., pasture); and OA–species recorded only in open areas (e.g., Cerrado *stricto sensu* and field formations). The conservation status of species followed the classification proposed by the International Union for Conservation of Nature (IUCN 2024) and the Brazilian Red List of Endangered Species (MMA 2022). We also compared our results with those found in other CAT areas (see Table 2).

TABLE 2. Number of amphibian species recorded on transitional areas in Brazil (abbreviations for Brazilian states: BA: Bahia; CE: Ceará; MS: Mato Grosso do Sul; MT: Mato Grosso; PE: Pernambuco; PI: Piauí; RN: Rio Grande do Norte; SE: Sergipe; TO: Tocantins).

Ecotone	Locality	Number of species	Sampling effort	Reference
Cerrado/Amazonia	Caseara, TO	43	5 points; 50 days; rainy and dry season; 400 hours/person; active surveys and 2.596 buckets/day	Present work
Cerrado/Amazonia	Fazenda Tanguro, MT	35	No information	Oliveira *et al.* 2010
Cerrado/Amazonia	Parque Estadual Do Mirador, MA	31	7 points; 192 days; rainy and dry season; active surveys; 144 hours/person	Andrade *et al.* 2017
Cerrado/Amazonia	Various localities, including Tocantins state	14	11 points; 101 days; active surveys and search in database; rainy season	Valdujo *et al.* 2012
Cerrado/Chaco		10		
Cerrado/Caatinga		9		
Atlantic Forest/ Cerrado		33		
Cerrado/Caatinga/ Restinga	São Gonçalo do Amarante, CE	23	3 points; 72 days; active surveys and 15 traps/day; rainy season;108 hours/person	Borges-Leite *et al.* 2014
Cerrado/Amazon/ Caatinga	Santo Antônio dos Lopes, MA	37	No information	Pinto *et al.* 2019

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TABLE 2. (Continued)

Ecotone	Locality	Number of species	Sampling effort	Reference
Cerrado/Caatinga/ Restinga	Northeastern Maranhão	50	24 points; 24 days; rainy season; active surveys	Matavelli *et al.* 2019
Tropical Dry Forest/ Caatinga	Parque Nacional da Serra das Confusões, PI	19	16 points; rainy and dry season; active surveys and pitfall only in 7 points	Dal Vechio *et al.* 2016
Cerrado/Pantanal	UHE Ponte de Pedra, Itiquira, MS-MT	33	3 months of faunal inventory; 8 months of faunal monitoring; 20 stations of pitfalls; 180 buckets; 720 open buckets	Silva-Jr. *et al.* 2009
Atlantic forest/ Cerrado	Central-eastern of Paraná state	42	36 days; 66 water bodies; 66 hours	Batista and Bastos 2014
Atlantic forest/ Caatinga	Serra do Brejo Novo – Jequié, BA	32	42 pitfalls days; 3 points; rainy and dry season; active surveys duration not specified	Silva *et al.* 2013
Atlantic forest /Caatinga	Parque Nacional Serra de Itabaiana, SE	23	No information	Carvalho *et al.* 2005
Atlantic forest/ Caatinga	Serra da Jibóia, BA	30	12 days; active surveys and database search	Juncá 2006
Atlantic forest/ Caatinga	Macaíba, RN	34	Active surveys in transects that crossed several temporary and semi-permanent ponds; sampling effort not specified	Magalhães *et al.* 2013
Atlantic forest/ Caatinga	APA Delta Do Parnaíba, PI	21	Sampling effort non-regular between three years; 7 points; active surveys	Loebmann and Mai 2008
Coastal forest/ Caatinga	Fazenda Maturi, CE	20	1 month	Borges-Nojosa *et al.* 2010
Coastal forest/ Caatinga	Fazenda Formosa, CE	20		
Cerrado/Pantanal	Parque Nacional da Serra da Bodoquena	38	450 man/hour, 12 sampling days during dry season and 13 sampling days during rainy season. Active search	

Results

We collected a total of 738 amphibian specimens, distributed in 42 species and seven families (Fig. 3–7, Table 3). The most diverse families were Hylidae (17 species, 41% of the total number of species) and Leptodactylidae (16 species, 38%), followed by Bufonidae (4 species, 10%), Microhylidae (2 species, 5%) three other families with one species each (e.g., Aromobatidae, Odontophrynidae, and Strabomantidae, 2% each). We found similar numbers of species in each field expedition (33, 35, 30, and 33 species, respectively), with some species being collected in only one expedition, such as *Rhaebo guttatus* (FE IV, rainy season), *Lithodytes lineatus* (FE III, dry season), and *Proceratophrys* sp. (cf. *ararype*) (FE II, rainy season). On the other hand, most species were recorded in all field expeditions (e.g., *Allobates crombiei*, *Dendropsophus cruzi*, *Lysapsus caraya*, *Leptodactylus macrosternum*, *Pseudopaludicola canga*, and *Elachistocleis magna*). Based on the rarefaction curves, we found a local richness close to the theoretical real diversity (Figure 8).

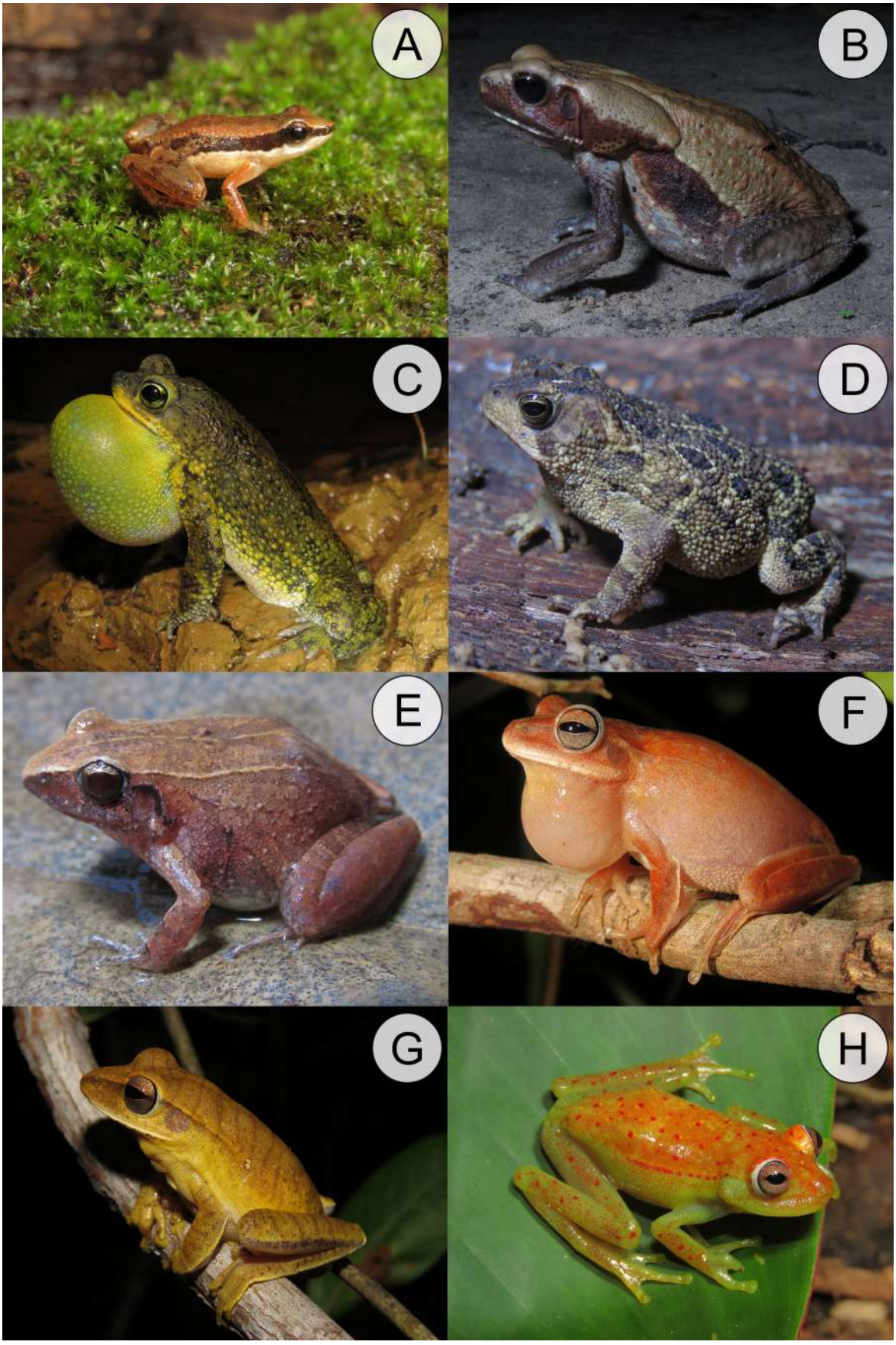

FIGURE 3. Amphibian species recorded during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil. A: *Allobates crombiei*; B: *Rhaebo guttatus*; C: *Rhinella mirandaribeiroi*; D: *Rhinella ocellata*; E: *Barycholos ternetzi*; F: *Boana caiapo*; G: *Boana multifasciata*; H: *Boana punctata*.

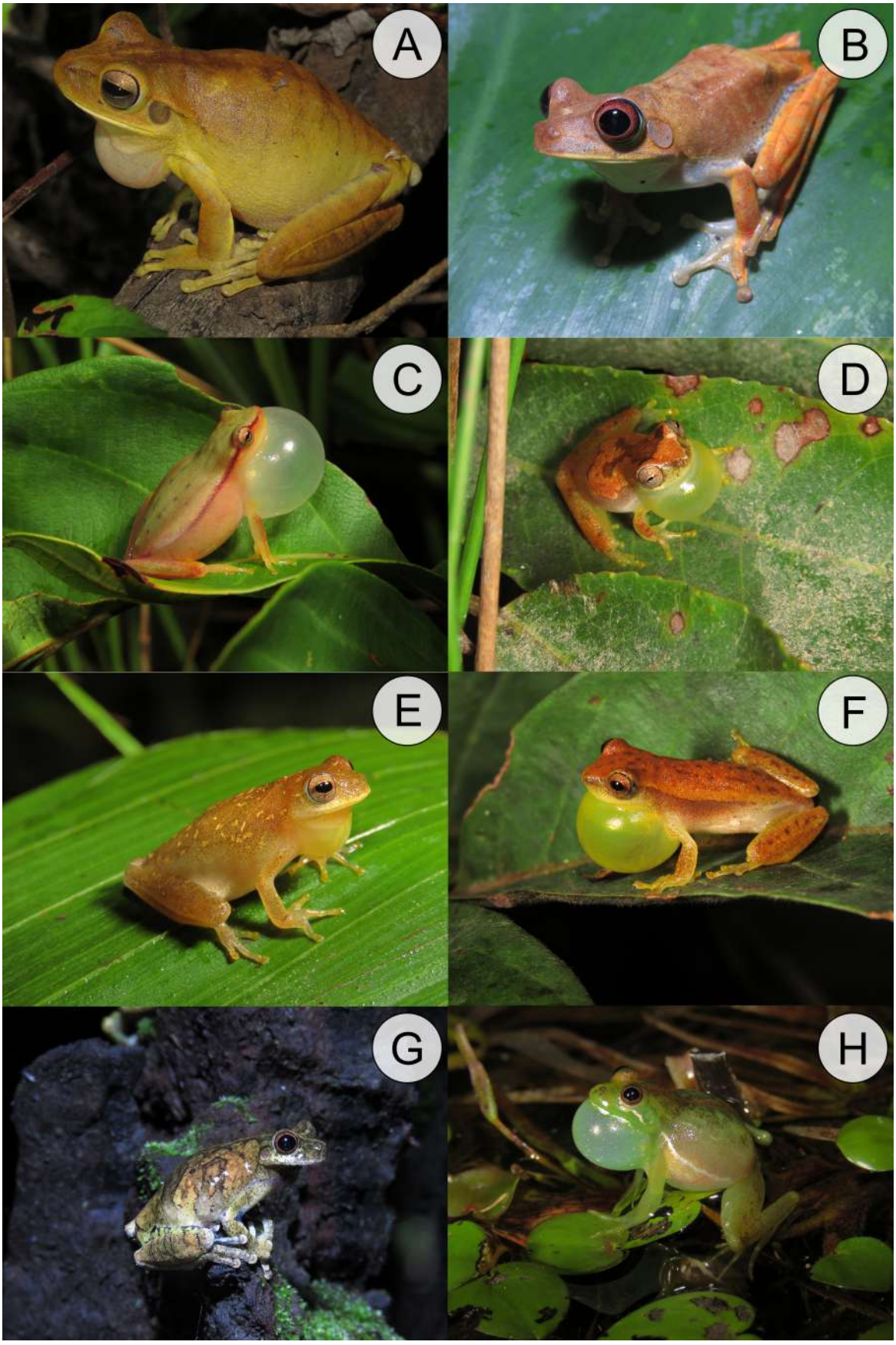

FIGURE 4. Amphibian species recorded during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil. A: *Boana raniceps*; B: *Boana* sp. (aff. *semilineata*); C: *Dendropsophus anataliasiasi*; D: *Dendropsophus cruzi*; E: *Dendropsophus minutus*; F: *Dendropsophus nanus*; G: *Dendropsophus melanargyreus*; H: *Lysapsus caraya*.

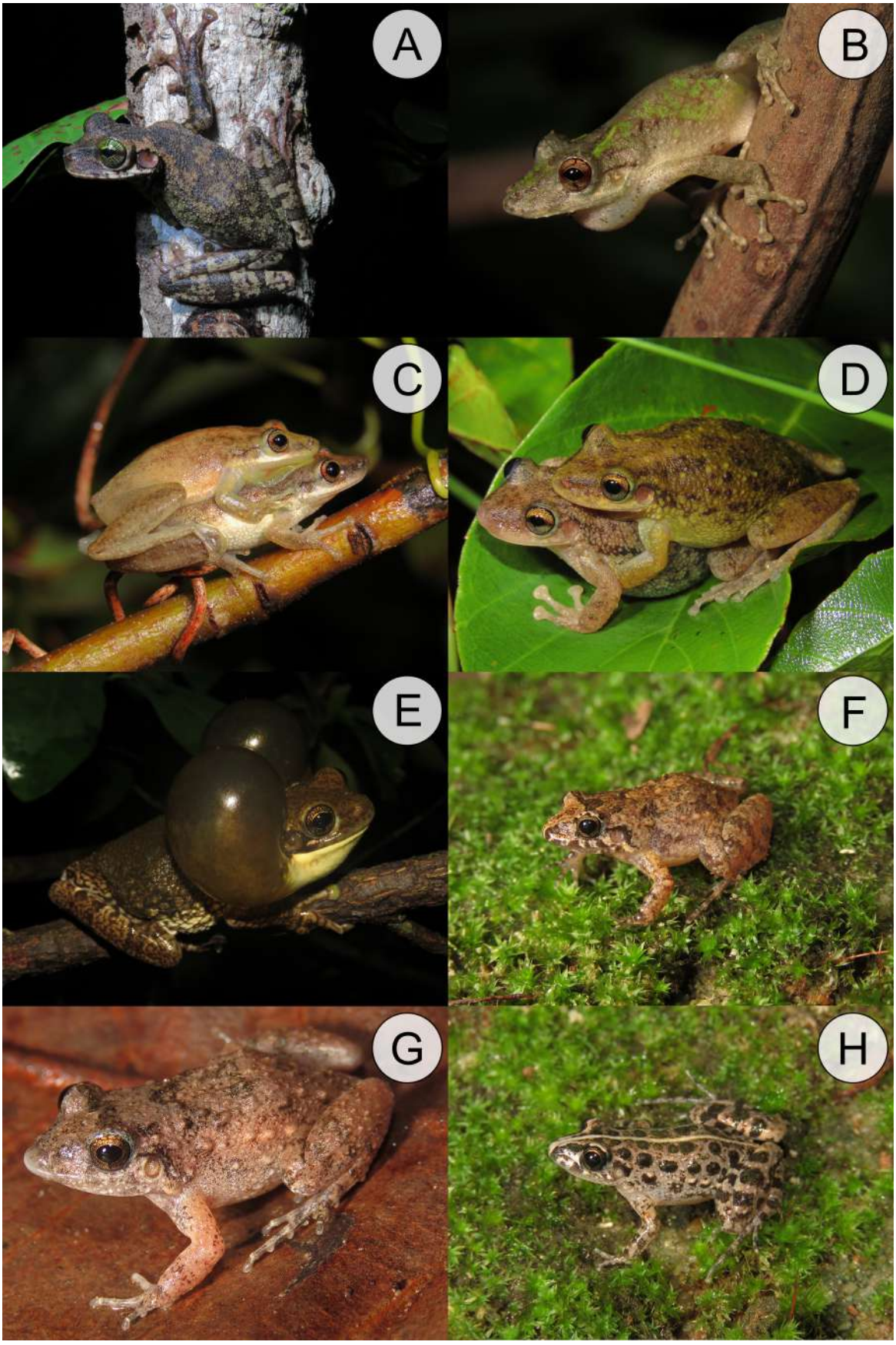

FIGURE 5. Amphibian species recorded during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil. A: *Osteocephalus taurinus*; B: *Scinax constrictus*; C: *Scinax fuscomarginatus*; D: *Scinax similis*; E: *Trachycephalus typhonius*; F: *Adenomera juikitan*; G: *Adenomera cotuba*; H: *Adenomera saci*.

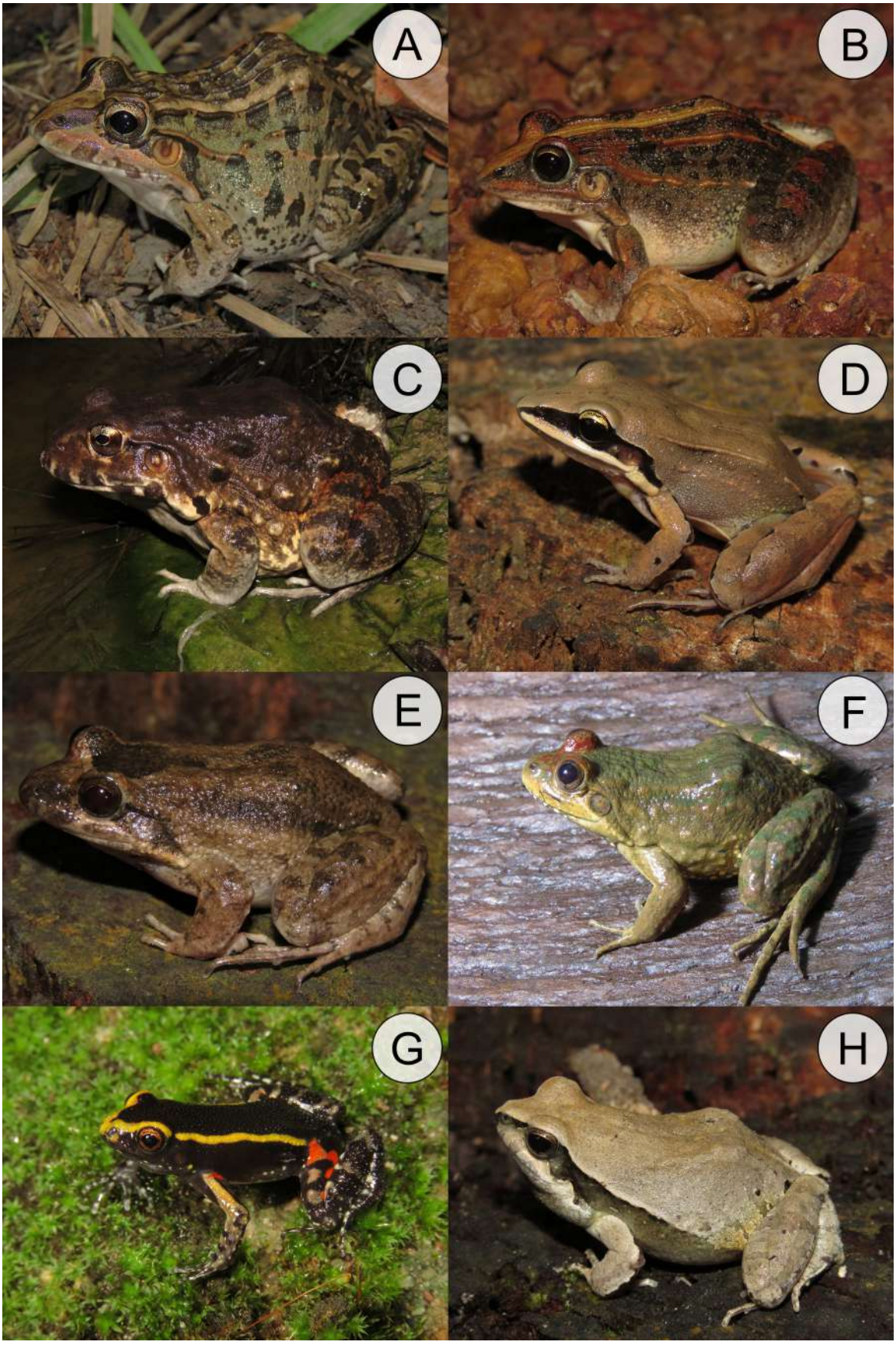

FIGURE 6. Amphibian species recorded during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil. A: *Leptodactylus macrosternum*; B: *Leptodactylus fuscus*; C: *Leptodactylus labyrinthicus*; D: *Leptodactylus mystaceus*; E: *Leptodactylus natalensis*; F: *Leptodactylus pustulatus*; G: *Lithodytes lineatus*; H: *Physalaemus centralis*.

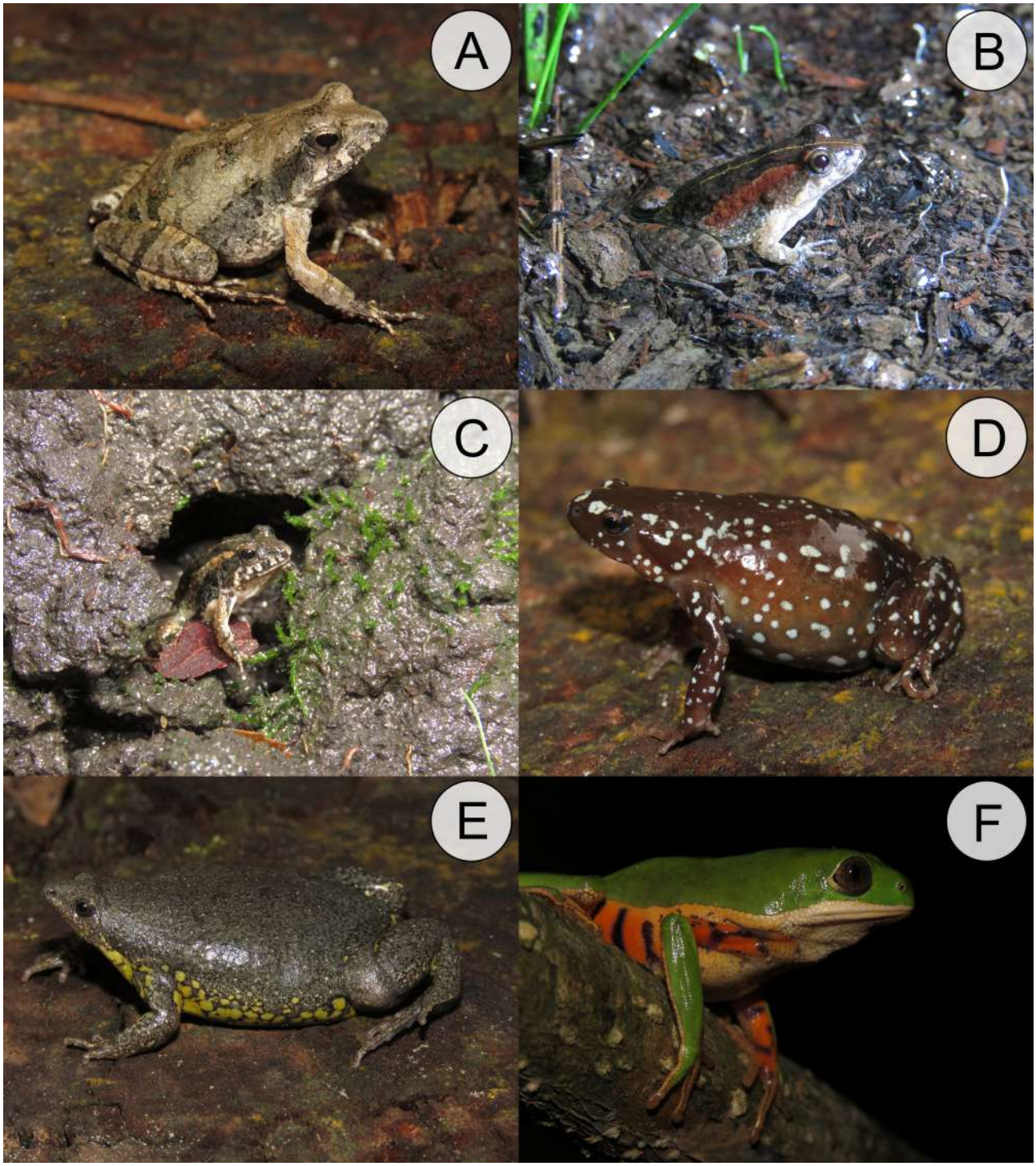

FIGURE 7. Amphibian species recorded during the amphibian's inventory at the Caseara municipality, western Tocantins state, Brazil. A: *Physalaemus* sp. (aff. *cuvieri*); B: *Pseudopaludicola canga*; C: *Pseudopaludicola jazmynmcdonaldae*; D: *Chiasmocleis centralis*; E: *Elachistocleis magna*; F: *Pithecopus hypochondrialis*.

Comparing the species richness at each sampling point, Canadá Farm exhibited the highest amphibian diversity with 35 species, followed by RPPN Canto do Obrieni with 34 species, Santa Juliana with 21 species, Guaíra Farms with 17 species, and lajedo with just three species (Table 3). Regarding the species distribution within the environmental mosaic of the study area, five were recorded exclusively in forested formations (*A. crombiei*, *R. guttatus*, *Barycholos ternetzi*, *Osteocephalus taurinus*, and *L. lineatus*), whereas eight only in anthropic environments (*Rhinella diptycha*, *Boana* sp. [aff. *semilineata*], *Dendropsophus nanus*, *Lysapsus caraya*, *Scinax fuscomarginatus*, *Adenomera* sp. [aff.

hylaedactyla], *Leptodactylus fuscus*, and *Leptodactylus pustulatus*). The remaining 27 species were observed in two or more different vegetation types. Following the distribution categories, 13 species are considered widespread, nine occur in both Amazonia and Cerrado biomes, and 14 are endemic to the Cerrado (Table 3).

None of the species recorded in the present study is listed in the Brazilian Red List of Endangered Species (MMA 2022). According to the IUCN Red List of Threatened Species (2024), 34 of the 42 species were classified as least concern (LC), as the widely distributed *Boana raniceps* and *Leptodactylus macrosternum*. On the other hand, two other small bodied Leptodactylidae species raise more concerns, *Adenomera cotuba* and *Pseudopaludicola jazmynmcdonaldae*. Both species were described relatively recently from Cerrado regions under severe anthropic impact, especially the conversion of natural habitat into pastures and agricultural lands. In fact, *P. jazmynmcdonaldae* is still known to occurs only in the vicinities of its type locality, the Caseara municipality, in areas also extensively modified by the agribusiness. The Microhylidae *Chiasmocleis centralis* was the only data deficient (DD) species (Table 2). Lastly, the remaining four species recorded in the study area were not identified at the species level, and no conservation status was applicable: *Adenomera* sp. (aff. *hylaedactyla*), *Boana* sp. (gr. *semilineata*), *Physalaemus* sp. (gr. *cuvieri*), and *Proceratophrys* sp. (cf. *ararype*).

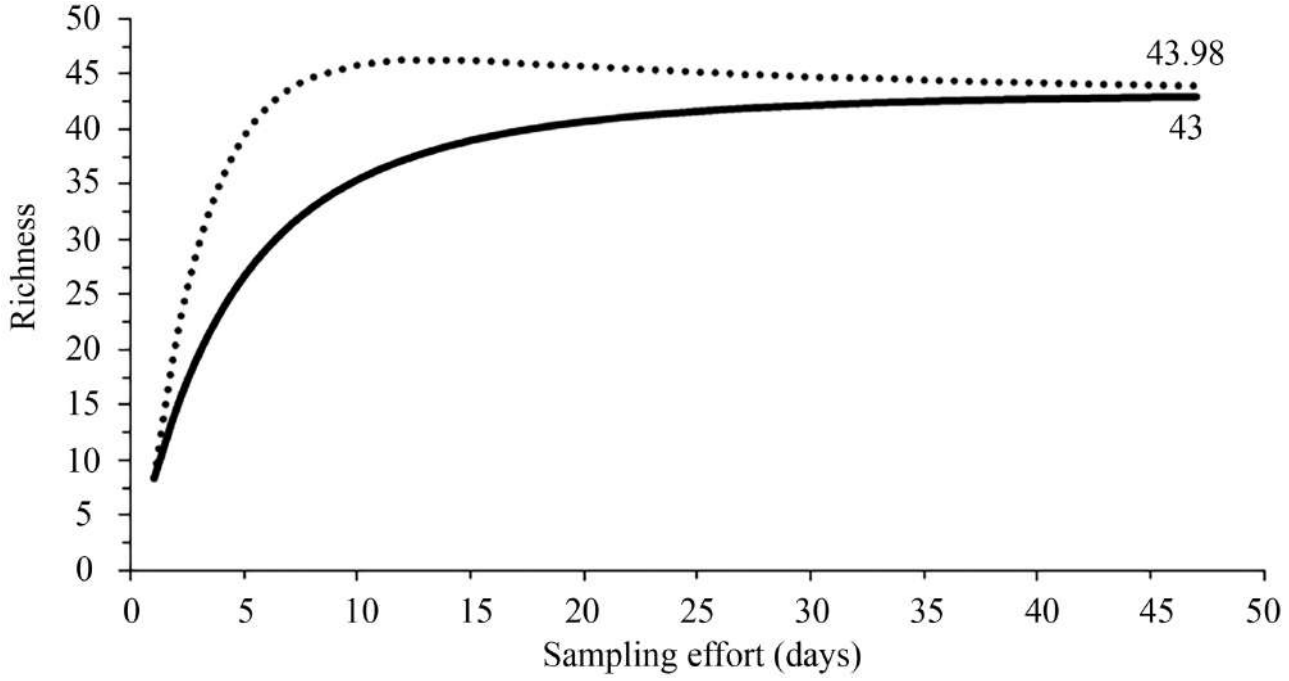

FIGURE 8. Species accumulation curve of amphibian species from Caseara municipality, Tocantins state, Brazil.

Species accounts: remarks on taxonomy and distribution

In total, we measured 609 specimens belonging to 38 species. We recorded 44 calls from 21 species and sequenced the 16S fragment for 21 specimens representing seven species. Based on this dataset, we discuss the taxonomic remarks for some of the recorded species, incorporating acoustic data, details on geographic variation and genetic divergence (Tables S2, S3, S4).

Hylidae

Boana caiapo (Fig. 3F). This species was described based on morphological, acoustic, and osteological characteristics (Pinheiro *et al*. 2018). The authors identified two types of notes, A and B, which can be emitted separately or together. Although both types are very similar in structure and frequency, note A has a shorter duration and fewer pulses. Additionally, note A is emitted more frequently and sometimes in combination with note B, whereas note B is rarely emitted alone (Pinheiro *et al*. 2018). We observed only the note A in the advertisement call of *B. caiapo* from Caseara, which reached a higher number of pulses per call (78–195 pulses; 69–122 pulses in the original

description) (n = 169; Table S3). There are no female individuals in the type series of *B. caiapo*, and we provide here the measurements of four females, which are generally bigger than males (SVL 47.63–53.78 mm in females, n = 4; 39.88–49.51 mm in males, n = 39, present work; 42.2–51.2 mm in males, Pinheiro *et al*. 2018) (Table S2).

Boana sp. (aff. *semilineata*) (Fig. 4B). The *Boana semilineata* species group is mainly characterized by the presence of a reticulated lower eyelid membrane and is known to present several cryptic lineages (Fouquet *et al*. 2016; Peloso *et al*. 2018). The lineage of the *B. semilineata* group collected in Caseara municipality presents dark brown irregular bands on the dorsal surface of tibia and thighs, flanks black with white and yellowish spots, iris reddish surrounded by a black circle, and pupils black. Fouquet *et al*. (2016) identified an unnamed lineage with occurrence in Altamira municipality, Pará state, and Guaraí municipality, Tocantins state, called as *Boana* aff. *semilineata* 2. Silva *et al*. (2020) also recorded individuals that belong to this lineage in the municipalities of Ananás, Aragominas, Araguaína, Babaçulândia, Colinas do Tocantins, Muricilândia, Nazaré, and Santa Fé do Araguaia, all in the state of Tocantins. Our record may also represent this cryptic lineage, but we do not have acoustic and molecular data to confirm the identity of this population.

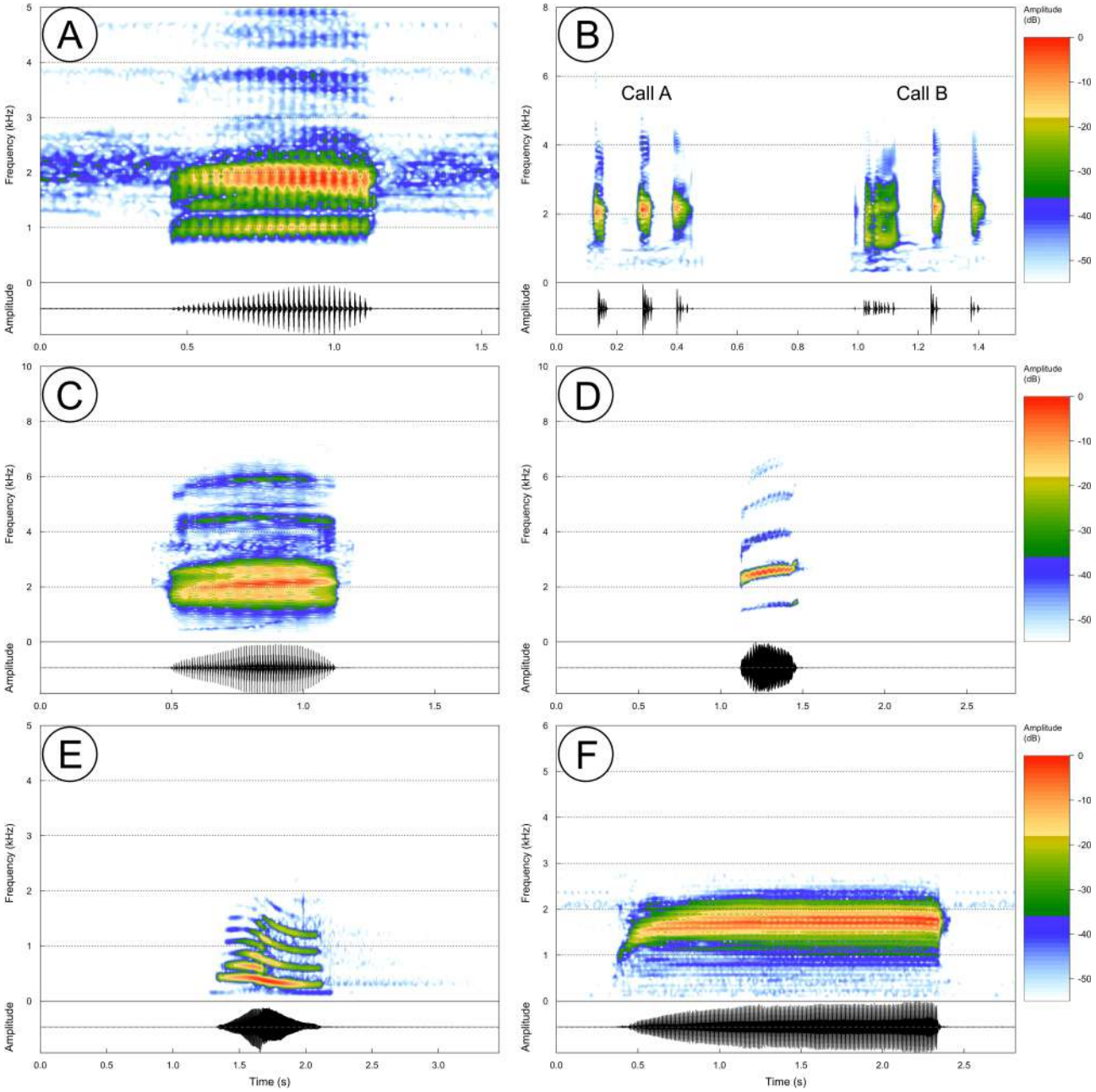

FIGURE 9. Spectrogram and oscillogram of the advertisement call of some species recorded at Caseara municipality, Tocantins state, Brazil. A: *Dendropsophus melanargyreus*; B: *Lysapsus caraya*; C: *Scinax fuscomarginatus*; D: *Adenomera juikitan*; E: *Physalaemus* sp. (gr. *cuvieri*); F: *Elachistocleis magna*.

TABLE 3. Amphibians recorded in Caseara municipality, Tocantins state, Brazil, from November 2017 to May 2018. Sampling areas: SJF = Santa Juliana Farm; CF = Canada Farm; GF = Guaíra Farm; RPPN = RPPN Obrieni Corner; LJ = Lajedo. Geographic distribution (GD): AM = species that occur in both Amazonia and Cerrado biomes; OD = diagonal belt of open biomes; EN = endemic species from Cerrado; WD = widespread. Environment: AT = anthropic (e.g. pasture), FF = Forest formation (e.g. gallery forest), OE = Open environments (e.g. Cerrado *stricto sensu*). Conservation Status (IUCN): DD = Data Deficient, EN = Endangered, NT = Near Threatened, NE = Not Evaluated, LC = Least Concern. Type of data: MM = morphometric, ML = morphologic, BA = bioacoustics, GN = genetic.

Taxa	SJF	CF	GF	RPPN	LJ	GD	Env.	IUCN	Data
Amphibia									
Aromobatidae									
Allobates crombiei (Morales, 2002 "2000")	-	X	-	X		AM	FF	LC	MM, ML, BA
Bufonidae									
Rhaebo guttatus (Schneider, 1799)	-	-	-	X		AM	FF	LC	MM, ML
Rhinella diptycha (Cope, 1862)	-	X	-	X		WD	AT	LC	MM, ML
Rhinella mirandaribeiroi (Gallardo, 1965)	-	X	X	X		EN	AT	LC	MM, ML
Rhinella ocellata (Günther, 1859 "1858")	-	-	-	X		EN	AT, OE	LC	MM, ML, BA
Hylidae									
Boana caiapo Pinheiro, Cintra, Valdujo, Silva, Martins, Silva & Garcia, 2018	X	X	X	-		AM	AT, FF, OE	LC	MM, ML, BA, GN
Boana multifasciata (Günther, 1859 "1858")	X	X	X	X		WD	AT, FF, OE	LC	MM, ML
Boana punctata (Schneider, 1799)	X	X	X	-		WD	AT, FF	LC	MM, ML
Boana raniceps (Cope, 1862)	-	X	X	-		WD	AT, OE	LC	MM, ML, BA
Boana sp. (gr. *semilineata*)	X	-	-	-		AM	AT	-	MM, ML
Dendropsophus anataliasiasi (Bokermann, 1972)	-	X	-	X		EN	AT, OE	LC	MM, ML
Dendropsophus cruzi (Pombal & Bastos, 1998)	X	X	-	X		EN	AT	LC	MM, ML, BA
Dendropsophus melanargyreus (Cope, 1887)	-	X	-	X		AM	AT, FF	LC	MM, ML, BA
Dendropsophus minutus	-	X	-	X		WD	AT, FF	LC	MM, ML

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TABLE 3. (Continued)

Taxa	SJF	CF	GF	RPPN	LJ	GD	Env.	IUCN	Data
Dendropsophus nanus (Boulenger, 1889)	X	X	-	-		WD	AT	LC	MM, ML
Lysapsus caraya Gallardo, 1964	X	X	X	-		EN	AT	LC	MM, ML, BA
Pithecopus hypochondrialis (Daudin, 1800)	-	X	-	X		AM	AT, FF, OA	LC	MM, ML
Osteocephalus taurinus Steindachner, 1862	-	X	X	X		AM	FF	LC	MM, ML
Scinax constrictus Lima, Bastos & Giaretta, 2004	X	X	X	X		EN	AT, OE	LC	MM, ML, BA, GN
Scinax fuscomarginatus (A. Lutz, 1925)	-	X	X	X		WD	AT	LC	MM, ML, BA, GN
Scinax similis (Cochran, 1952)	-	-	-	X		-	FF	LC	MM, ML, BA, GN
Trachycephalus typhonius (Linnaeus, 1758)	X	X	-	-		WD	AT, FF	LC	MM, ML
Leptodactylidae									
Adenomera cotuba Carvalho & Giaretta, 2013	X	X	-	X		EN	AT, OE	NT	MM, ML, BA
Adenomera juikitam Carvalho & Giaretta, 2013	-	X	-	X		EN	AT, OE	LC	MM, ML, BA
Adenomera saci Carvalho & Giaretta, 2013	-	X	-	X		EN	AT, OE	LC	MM, ML, BA
Adenomera sp. (aff. *hylaedactyla*)	-	X	-	-		-	AT	-	MM, ML
Leptodactylus fuscus (Schneider, 1799)	X	X	-	X		WD	AT	LC	MM, ML, BA
Leptodactylus labyrinthicus (Spix, 1824)	X	X	X	X		WD	AT, FF	LC	MM, ML
Leptodactylus macrosternum Miranda-Ribeiro, 1926	X	X	X	X	X	DA	AT, FF	LC	MM, ML
Leptodactylus mystaceus (Spix, 1824)	-	X	-	X		WD	AT, FF	LC	MM, ML, BA
Leptodactylus podicipinus (Cope, 1862)	X	X	X	X		WD	AT, FF, OE	LC	MM, ML
Leptodactylus pustulatus (Peters, 1870)	X	X	X	-		EN	AT	LC	MM, ML

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TABLE 3. (Continued)

Taxa	SJF	CF	GF	RPPN	LJ	GD	Env.	IUCN	Data
Leptodactylus natalensis Lutz, 1930	-	-	-	X		-	AT, FF	LC	ML, NA
Lithodytes lineatus (Schneider, 1799)	-	-	-	X		AM	FF	LC	MM, ML
Physalaemus centralis Bokermann, 1962	X	X	X	X		EN	AT, FF	LC	MM, ML, BA, GN
Physalaemus sp. (gr. *cuvieri*)	-	X	-	X	X	-	AT, FF	-	MM, ML, BA, GN
Pseudopaludicola canga Giaretta & Kokubum, 2003	X	X	-	X		AM	AT, OE	LC	MM, ML, BA
Pseudopaludicola jazmynmcdonaldae Andrade, Silva, Koroiva, Fadel, and Santana, 2019	X	X	-	X		EN	AT, OE	EN	MM, ML
Microhylidae									
Chiasmocleis centralis Bokermann, 1952	-	-	-	X		EN	FF, OE	DD	MM, ML
Elachistocleis magna Toledo, 2010	X	X	-	X		-	AT, FF, OE	LC	MM, ML, BA, GN
Odontophrynidae									
Proceratophrys sp. (cf. *ararype*)	-	-	-	X		-	FF, OE	-	MM, ML, BA, GN
Strabomantidae									
Barycholos ternetzi (Miranda Ribeiro, 1937)	X	X	X	X		EN	FF	LC	MM, ML
Total	21	35	16	34	3	-	-	-	

Dendropsophus melanargyreus (Fig. 4E). We identified individuals of *Dendropsophus melanargyreus* in the study area according to morphological characteristics described by Caramaschi and Jim (1983). This species occurs in the states of Goiás and Mato Grosso states to São Paulo, Minas Gerais, and northeastern Brazil; Surinam and French Guiana, Bolivia, and the Pantanal of northeastern Paraguay (Neves *et al*. 2020; Frost 2024). Despite this reported distribution, there is few information available about geographic variation of this species. The advertisement call of *D. melanargyreus* is currently described for two localities, Puerto Almacén, Bolivia and Belém municipality, Pará state, Brazil, both in the Amazon biome (Duelmann & Pyles 1983; Marquéz *et al*. 1993). Here we update the description of the advertisement call of this species, providing data for a new location at the CAT. The call of the species from the study area is composed of one pulsed note emitted sporadically, with duration ranging from 0.32 to 0.48 s (0.38 ± 0.04). The calls present 36 to 48 pulses (40 ± 2.5), with pulse rate ranging from 98 to 119 pulses per second (106.2 ± 7.7). The dominant frequency varies from 3359.2 to 3876.0 Hz (3566.7 ± 146) (Fig. 9A, Table S3). The advertisement call from Caseara is more similar to the calls provided for Bolivia (0.37–0.46 s, 34–39 pulses/call, 83–97 pulses/s, and 3036–3661 Hz), and differs significantly in all parameters from the Belém population (0.25–0.33 s, 127–140 pulses/s, 2093–2399 Hz). These call differences and the gap of knowledge about *D. melanargyreus* suggest that more than one species may be hidden under this name (Faivovich *et al*. 2005; Pyron & Wiens 2011; Fouquet *et al*. 2011; Medeiros *et al*. 2013).

Dendropsophus minutus (Fig. 4F). This is a small hylid frog, thought to be widely distributed in South America. However, Gehara *et al*. (2014) recovered a high genetic diversity within *Dendropsophus minutus* suggesting the existence of numerous distinct species. The authors recovered 43 lineages, including the population from Caseara municipality, designated as lineage 36, which is widely distributed across several Brazilian states.

Lysapsus caraya (Fig. 4H). The advertisement call of this species was first described to Araguapaz municipality, Goiás state, characterized by a pulsed call, composed of three notes with different note durations and pulse numbers (Bastos *et al*. 2011). Santana *et al*. (2013) described the advertisement call of four species of *Lysapsus* and found two types of call (A and B) for each species, except for *L. caraya* (only type A). According to the authors, the call A is emitted more frequently and may be classified as an advertisement call composed of several pulses, which may be concatenated or not; and call B, subdivided in notes x and y, it may be the result of choruses with intense social interactions among individuals. In the present study, we recorded the two types of calls for *L. caraya* (Fig. 9B, Table S3). The call A presents duration of 0.082 to 0.205 s (0.141 ± 0.035), with the number of pulses ranging from 8 to 17 (12.5 ± 24) and dominant frequency ranging from 4312.5 to 4781.2 Hz (4593.8 ± 119.9) (Fig. 9B). The duration of call B ranges from 0.111 to 0.211 s (0.171 ± 0.024) and dominant frequency from 4406.2 to 4875.0 Hz (4651.8 ± 127.4) (Fig. 9B). The first note (x) of call B presents a duration of 0.018 to 0.060 s (0.043 ± 0.013), with 5 to 16 pulses per note (10.5 ± 3.2), and dominant frequency ranging from 2718.8 to 4593.8 Hz (3850.4 ± 801.8). The duration of the second note (y) ranges from 0.026 to 0.088 s (0.064 ± 0.021), 4 to 8 number of pulses per note (6.0 ± 1.0), and dominant frequency ranging from 4406.2 to 4875.0 Hz (4654 ± 149.8). The values of call A overlap with those described by Santana *et al*. (2013), and both calls can be emitted alone or together, with several arrangements (e.g. A, B, A+B, A+B+B, B+A, A+A, B+B, B+B+B). We compared the call B of *L. caraya* with the same call of *L. bolivianus* and *L. laevis*, that present structure similarities. The entire call B duration of *L. caraya* overlaps with both species, and there are similarities between the parameters analyzed in the two types of notes (x and y). The number of pulses of the note x of *L. caraya* overlaps with *L. bolivianus* (5–16 and 6–22 pulses, respectively), and the duration of the note y of *L. caraya* overlaps with *L. laevis* (0.03–0.09 s and 0.03–0.13 s, respectively).

Scinax fuscomarginatus (Fig. 5C). This species presents a rich genetic structure and extensive geographical distribution, showing variation in morphological and call parameters throughout its distribution (Toledo & Haddad 2005; Brusquetti *et al*. 2014). The advertisement call from Caseara is composed of multipulsed note, with a duration of 0.27 to 0.33 s (0.30 ± 0.02), 53 to 65 pulses per call (58.6 ± 3.5), a pulse rate ranging from 187.3 to 205.9 (196.2 ± 3.6), and a dominant frequency ranging from 4134.4 to 4306.6 Hz (4300 ± 33.8) (Fig. 9C, Table S3). We focused our comparisons with two other localities of Cerrado and Amazon biomes, close to the studied area: Formoso do Araguaia municipality, Tocantins state, and Altamira municipality, Pará state (~300 km and ~700 km from Caseara, respectively). The call is consistent with both populations, but its duration is more similar to that of Tocantins population (0.27–0.36 **s,** 183.1–195.3 pulses/s, 3444.4–3888.9 Hz) than to the Altamira population (0.74–0.94 s, 165.2–201.2 pulses/s, 2888.9–4222.2 Hz).

Scinax similis. This species was mainly found in open and anthropized areas. Its SVL ranged from 32.8–39.1 mm in males and 27.1–37.7 mm in females (Table S2). In life, lineage presents gray to yellowish dorsum, with a

light cream belly. The *Scinax similis* reported here is conspecific regard to the population misidentified by Silva *et al*. (2020) as *S. x-signatus* (figure 4J in the mentioned study). The advertisement call of this population presents a duration range from 0.15 to 0.29 s, with 6 to 11 pulses per call, pulse rate from 35.2 to 40.9 pulses/s, and dominant frequency ranging from 1033.6 to 4048 Hz (Table S3).

Leptodactylidae

Adenomera juikitam (Fig. 5F). We found specimens in the study area with morphometric values and morphologic characters overlapping with the description of *Adenomera juikitam* (Carvalho & Giaretta 2013). Despite that, we observed differences in the fundamental and dominant frequency peaks in the advertisement call from Caseara (2906.2–3187.5 Hz and 5343.8–5812.5 Hz) and the original description from Teresina de Goiás municipality, state of Goiás (1880–2110 Hz and 3700–4170 Hz) (Fig. 9D, Table S3). In the study area, this species was found in sympatry with the congeneric species *A. cotuba*.

Physalaemus sp. (gr. *cuvieri*) (Fig. 7A). The genus *Physalaemus* Fitzinger, 1826 has a complex taxonomic history, and some authors highlight the presence of cryptic lineages under the name of *P. cuvieri* Fitzinger, 1826 (Lourenço *et al*. 2015; Oliveira Miranda *et al*. 2019; Nascimento *et al*. 2019). Lourenço *et al*. (2015) recovered three unnamed lineages of *Physalaemus cuvieri*, including one population from Porto Nacional municipality, Tocantins state (named therein as "lineage 3"), ~230 km southeastern from Caseara municipality. According to our molecular data, *Physalaemus* sp. (gr. *cuvieri*) was recovered as the sister taxon of lineage 3, with a genetic distance ranging from 11.2% to 12.3% (Supplementary material, Table S5). The advertisement call of *Physalaemus* sp. (gr. *cuvieri*) is composed by one single non-pulsed note, with six harmonics characterized by descending frequency modulation. The call presents duration ranges from 0.36–0.78 s (0.67 ± 0.1 s), dominant frequency ranges from 689.1–818.3 Hz (719.3 Hz ± 44.8). The minimum frequency ranges from 516.8–602.9 Hz (557.1 Hz ± 13.9) and the maximum frequency ranges from 1421.2–2153.3 Hz (1595.3 Hz ± 185.3) (Fig. 9E, Table S3). The taxonomic status of *P. cuvieri* remains unclear, especially because there are several uncertainties on its type series and type locality. This scenario hampers the definition of which these lineages should bear the name *P. cuvieri* and which ones should be described as new species. In any case, in this study we first diagnose the Caseara population as an additional lineage of the *P. cuvieri* complex.

Microhylidae

Elachistocleis magna (Fig. 7E). The genus *Elachistocleis* Parker, 1927 currently comprises 18 species widely distributed throughout the Neotropical region from Panama to central Argentina (Frost *et al*. 2024). The taxonomy of this genus has been discussed in the last decade and still comprise many cryptic species, unnamed lineages, and synonyms (Toledo *et al*. 2010; De Sá *et al*. 2012; Peloso *et al*. 2016; Pansonato *et al*. 2018; Novaes-e-Fagundes *et al*. 2022). Recently, a comprehensive study also shed light on the fact that many characters traditionally used to diagnose *Elachistocleis* species failed to accurately delimit the current valid species (Novaes-e-Fagundes *et al*. 2022). Our gene tree recovered the *Elachistocleis* population from Caseara as *Elachistocleis magna* Toledo, 2010 (Supplementary material). The patterns of ventral color of Caseara population are highly variable, ranging from immaculate to spotted. These intraspecific variable patterns are known for the genus, and the use of additional data is necessary to identify species of this genus (Pereyra *et al*. 2013; Novaes-e-Fagundes *et al*. 2022). The calls of *Elachistocleis magna* from Caseara is similar with those presented by Marinho *et al*. (2018), except for the higher dominant frequency (3656.2 to 3962.1 Hz; 3140.0 to 3750.0 Hz in *E. magna* from Espigão d'Oeste, Rondônia) (Fig. 7F, Table S3).

Odontophrynidae

Proceratophrys sp. (cf. *ararype*). Several species of the genus *Proceratophrys* Miranda-Ribeiro, 1920 have been recently described by integrative approaches, using morphological, molecular, and acoustic data (Teixeira *et al*.

2012; Brandão *et al*. 2013; Dias *et al*. 2013; Mângia *et al*. 2014, 2018, 2022). Our molecular data recovered the *Proceratophrys* population from Caseara closely related to *P. ararype* Mângia, Koroiva, Nunes, Roberto, Ávila, Sant'Anna, Santana, and Garda, 2018, with 0.8% of genetic distance (Supplementary material, Table S5). Regarding the advertisement call, the parameters of *Proceratophrys* sp. (cf. *ararype*) overlap with those presented by Mângia *et al*. (2018) for *P. ararype* (Table S3). In contrast, we found some differences between Caseara and Chapada do Araripe populations, such as the female size (SVL 37.9–42.6 mm and 51.3–57.0 mm, respectively), the proportion of the head length in relation to SVL (40% and 46%, respectively), head largest than wider, and the snout shape in dorsal and ventral views (triangular in Caseara individuals, and rounded in individuals from Araripe) (Table S2). There is a sampling gap between the type locality of *P. ararype* and Caseara (~1200 km), suggesting that we might be evaluating extremes of the species distribution. However, further investigations are needed to clarify this issue. For these reasons, we consider the individuals recorded in the present study as *Proceratophrys* sp. (cf. *ararype*).

Discussion

The richness found in the present study (42 species) is in agreement with the habitat heterogeneity hypothesis, that suggests that complex habitats offer a great variety of niches, allowing the presence of a diverse number of species (Bazzaz 1975; Tews *et al*. 2004). This finding aligns with previous studies conducted in transitional areas, where landscape heterogeneity enables the presence of species typical of both Cerrado and Amazon regions (e.g. Valdujo *et al*. 2012; Andrade *et al*. 2017; Matavelli *et al*. 2019). The amphibian richness uncovered in our work represents the largest assemblage of CAT areas (e.g. Valdujo *et al*. 2012; Silva *et al*. 2013; Dal Vechio *et al*. 2016; Andrade *et al*. 2017). In comparison to transitional areas involving other biomes, a higher number of species was recorded in Maranhão (50 species), which can be explained to the confluence of three distinct environments (areas of Cerrado, Caatinga, and Restinga) and the extensive survey area comprised of 24 sampling points (Kark 2017; Matavelli *et al*. 2019). In relation to other regions within Tocantins state, Caseara municipality showcases a comparable or even superior species count (e.g. Valdujo *et al*. 2012; Silva *et al*. 2020). In fact, the study area harbors approximately 50% of the known amphibian diversity within the state and 20% of that found in the Cerrado (Valdujo *et al*. 2012; Silva *et al*. 2020). Research focusing on amphibian diversity in transitional areas remains limited, with most investigations concentrated in the northeast region, between the Atlantic Forest and adjacent biomes (e.g. Juncá, 2006; Loebmann & Mai 2008; Borges-Nojosa *et al*. 2010; Magalhães *et al*. 2013; Batista & Bastos 2014) (Table 2).

None of the species we documented were found in all environments within the studied area. We observed that forest-associated species, such as *Allobates crombiei*, *Lithodytes lineatus*, and *Osteocephalus taurinus*, also inhabited open environments. Additionally, species typically found in swamp areas during the rainy season, such as *Pseudopaludicola canga* and *P. jazmynmcdonaldae*, migrated to the gallery forest during the dry season. These spatial occupation patterns suggest a complex biogeographic dynamic in the region. For example, *A. crombiei* and *O. taurinus* are ideal models for investigating the chronology of occupation in the northern Cerrado by typically Amazonian species (Silva *et al.* 2020).

Many recent studies have questioned conventional approaches to amphibian species identification solely relying on morphological data (e.g. Funk *et al*. 2011; Bruschi *et al*. 2014; Brusquetti *et al*. 2014; Caminer & Ron 2014; Carvalho *et al*. 2015; Lourenço *et al*. 2015; de Sá *et al*. 2019). Integrative use of molecular and acoustic approaches frequently supports the review of morphological characters, updating species identification and delimitation based on independent lines of evolutionary evidence (Fouquet *et al*. 2007; Elmer & Cannatella 2008; Padial & De la Riva 2009; Funk *et al*. 2011; Moraes *et al*. 2017). This is the case of the *Proceratophrys* population reported here, wich is related to *P. ararype*, exhibiting divergent morphological characters but low genetic distances and similar advertisement call parameters. However, it is well established that within the genus *Proceratophrys*, distinct species can exhibit even lower genetic distances (e.g. Dias *et al.* 2013). Therefore, we recommend further investigation to clarify the taxonomic status of these species, especially considering that *P. ararype* is classified as Critically Endangered (IUCN, 2024) and is restricted to a few locations within the Araripe Plateau (Mângia *et al.* 2018). Furthermore, *Trachycephalus typhonius* also deserves special attention, as it may constitute a cryptic species complex, as suggested by Zaracho et al. (2018). However, our study was limited to collecting morphometric and morphologic data for *T. typhonius*. Therefore, future studies should explore the possibility to clarify the genetic diversity and geographical distribution of this species.

Acoustic communication is the most common among anurans and act as an isolating mechanism, being a relevant data for taxonomic delimitation and the recognition of cryptic species (Gerhardt 1988; Duellman & Trueb 1994; Padial *et al*. 2009; Glaw *et al*. 2010; Köhler *et al*. 2017). In addition, widespread species are exposed to different evolutionary pressures and may present geographic variation on advertisement call, which can be the case of *S. fuscomarginatus* (Pombal Jr. *et al*. 1995; Toledo & Haddad 2005; Jang *et al*. 2011; Wilkins *et al*. 2013, Brusquetti *et al*. 2014; Mângia *et al*. 2017). Additionally, the similarity found between the calls of this species from Caseara and Pará populations reinforce the Amazonian influence in the studied area. We compared the advertisement call of *D. melanargyreus* with the only two calls descriptions available, both from the Amazonia (Bolivia and Belém), which indicated the Caseara population possibly as conspecific with the Bolivia population (~1700 km from our population). The call differences suggest that the status of this species deserves further investigation, especially considering the gap of knowledge among the populations.

Amphibians are the most vulnerable group of vertebrates, due to low dispersal abilities and their dependence on specific environments and conditions to complete the life cycle (Duellman & Trueb 1994; Luedtke *et al*. 2023). Therefore, these organisms are threatened by several factors and are declining at a fast rate, reinforcing the importance and urgency to deeply investigate the amphibian richness and its taxonomic and phylogenetic diversity, especially in unprotected areas (Hortal *et al*. 2015). This increases the importance of the study area as a refuge, mainly because CAT areas are under constant pressure and some lineages can disappear even before it is formally described (Marques *et al*. 2019).

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Data Availability

Supplementary Tables S1–S4, which include summary data on the collected specimens, morphometric and bioacoustic measurements, GenBank accession numbers, and Figure S1, are available on Zenodo at https://doi.org/10.5281/zenodo.14884692.

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SUPPLEMENTARY MATERIAL. FIGURE S1. Gene tree based on the 16S mtDNA of some species from Caseara municipality, Tocantins state. A: *Scinax*; B: *Physalaemus*; C: *Elachistocleis*; D: *Proceratophrys*. The GenBank accession numbers appear after the names of downloaded sequences and the voucher numbers of the individuals of the present work. (Can be downloaded at the DOI landing page of this paper)