First record of *Dendropsophus anataliasiasi* (Bokermann, 1972) (Anura, Hylidae) for the state of Pará, Brazil

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Resumo

Este estudo reporta o primeiro registro de *Dendropsophus anataliasiasi* para o estado do Pará, Brasil (município de Santana do Araguaia). Esta descoberta expande a distribuição geográfica conhecida da espécie, que habita o Cerrado, em aproximadamente 229 km para noroeste da localidade tipo. A identificação dos espécimes coletados foi confirmada por análises morfológicas e genéticas. Morfologicamente, os indivíduos apresentaram os caracteres diagnósticos da espécie (como listras dorsais), embora com leves variações nas medidas da cabeça. A análise de DNA confirmou sua identidade taxonômica, agrupando-a com as demais sequências conhecidas da espécie. Este achado representa a 196ª espécie de anfíbio documentada para o Pará.

Palavras-chave: Dendropsophini, Extensão Geográfica, 16S

Abstract

The study reports the first record of *Dendropsophus anataliasiasi* for the state of Pará, Brazil (municipality of Santana do Araguaia). This finding expands the known geographic distribution of the species, which inhabits the Cerrado, by approximately 229 km northwest of its type locality. The identification of the collected specimens was confirmed through morphological and genetic analyses. Morphologically, the individuals exhibited the species' diagnostic characters (such as dorsal stripes), while with slight variations in head measurements. The DNA analysis confirmed the taxonomic identity, with sequences clustering with known sequences for the species. This finding represents the 196th amphibian species documented for the state of Pará.

Key-words: Dendropsophini, Geographic Extension, 16S

The *Dendropsophus rubicundulus* subgroup comprises 11 species of small treefrogs distributed across the Brazilian Cerrado and adjacent regions of Argentina, Bolivia, and Paraguay (Orrico et al., 2021). One of those species, *Dendropsophus anataliasiasi* (Bokermann, 1972), is known for the Brazilian states of Goiás, Maranhão, Mato Grosso, and Tocantins (Napoli and Caramaschi, 1999; 2000; Arantes et al., 2023; Fig. 1). Morphologically, *D. anataliasiasi* is characterized by a snout-vent length (SVL) of 16.0–21.8 mm in males and 16.6–21.6 mm in females, a dorsum with nearly parallel dark brown stripes, the two anterior ones in close contact and continuous with the two sacral stripes, and a head longer than wide (Bokermann, 1972; Napoli and Caramaschi, 1999; 2000). The most recent international assessment of the species conservation status, by International Union for Conservation of Nature (IUCN), classified it as Least Concern (LC) of extinction (IUCN, 2023). Herein, we report the first record of *Dendropsophus anataliasiasi* from the state of Pará, Brazil, and provide an updated distribution map for the species. We also present morphometric data for the newly recorded specimens.

On January 25th, 2024, in the early evening, we observed many breeding individuals of *D. anataliasiasi* on the grassy vegetation at the margin of a large pond on a private farm (Fazenda Fartura; -9.738ºS, -50.188ºW), in the municipality of Santana do Araguaia, Pará state (Fig. 1), and collected five adult males. On the same day, a third-party brought us an additional adult male from a different site at the same farm (-9.838ºS, -50.334ºW), within the municipality of Santa Terezinha, Mato Grosso state (Fig 1). The specimens were euthanized with a topic solution of 5 % lidocaine, fixed in 10 % formaldehyde solution, stored in 70 % ethanol and deposited in the Museu de Zoologia of the Universidade de São Paulo (MZUSP, Brazil), under the accession numbers MZUSP 161273–8. For genetic analyses, we collected a piece of liver from each specimen before fixation and stored them in ethanol P.A.. Specimens were collected under permit ICMBio #10126–7 issued by the Chico Mendes Institute for Biodiversity Conservation.

To confirm species identification, specimens were morphologically compared with the original *D. anataliasiasi* description, following the nomenclature used in the diagnosis (Bokermann, 1972; Napoli and Caramaschi, 2000). Eighteen morphometric measurements were taken (in millimeters) by GNCC: SVL (snout–vent length); HW (head width); HL (head length); ED (eye diameter); UEW (upper eyelid width); IOD (interorbital distance); END (eye–nostril distance); IND (internarial distance); THL (thigh length); TD (tympanum diameter); NSD (nostril to tip of snout distance); UAR (upper arm length); FAR (forearm length); HAL (hand length); 3FD (third finger disk diameter); TL (tibia length); FL (foot length); and 4TD (fourth toe disk diameter). We measured all characters on the right side of each specimen using a Mitutoyo digital caliper accurate to 0.1 mm at Laboratório de Herpetologia of the Universidade de São Paulo (USP) under a Nikon binocular stereomicroscope (Model C-PS). The dorsal color pattern was examined and compared with descriptions available in the literature (Napoli and Caramaschi, 1999). We conducted DNA-based identification for two specimens (MZUSP 161273–4). Genomic DNA was extracted following the protocol described by Fetzner (1999) and we amplified fragments of the final portion of 16S rRNA gene using forward (5'-CGCCTGTTTATCAAAAACAT-3') and reverse (5'-CCGGTCTGAACTCAGATCACGT-3') primers (16Sar-L and 16Sbr-H, respectively; Palumbi et al. 1991). The amplification, through polymerase chain reaction (PCR), consisted of initial denaturation at 95 ºC for 3 min, 45 cycles of a denaturing step of 30 s at 95 °C, annealing at 50 °C for 40 s and extension at 72 °C for 40 s with final extension at 72 ºC for 5 min. PCR products were purified with Exonuclease I and Alkaline Phosphatase (ExoSaP protocol). Sequencing was performed using the BigDye Terminator 3.1 Cycle Sequencing kit (Applied Biosystems), followed by analysis in ABI Prism Genetic Analyzer (3100, Applied Biosystems) at the Instituto de Biociências of the Universidade de São Paulo (USP, Brazil). We used Geneious v.6 (Kearse et al., 2012) to edit the raw sequences. The sequences were deposited in the GenBank online repository (GB) (accession numbers: PV920703–4). After editing the sequences, we submitted them to a BLAST® search under the Standard Nucleotide BLAST function (*blastn*, Johnson et al., 2008). For each analyzed sample, we downloaded the 100 most similar sequences from the GB online repository (Clark et al., 2016) and aligned the dataset using the MAFFT online server with default parameters, except using the E-INS-I strategy for the RNA. Uncorrected pairwise genetic distances were estimated for the alignment using MEGA v.11 (Tamura et al., 2021) with pairwise deletion. Following Fouquet et

al. (2007), we considered the maximum of 3 % of genetic distance as a threshold to interspecific differentiation.

All specimens exhibited the diagnostic characters of the species, including acuminate snouts, mid-dorsal pin stripes, and a SVL range of 20.5–21.9 mm (Fig 2, Tab. 1). Most morphometric measurements also fell within the known range of intraspecific variation (Bokermann, 1972; Napoli and Caramaschi, 1999), except for the UAR, which was slightly reduced in all specimens. Despite this discrepancy, we consider these measurements to fall within the species' expected variation, as one of the specimens with reduced UAR was among those molecularly identified (MZUSP 161273). In fact, the two newly generated DNA sequences confirmed the taxonomic identity of *D. anataliasiasi*, as they fully clustered with previously published sequences of this species by Orrico et al. (2021) and Arantes et al. (2023), with uncorrected pairwise genetic distances ranging from 0.2 % to 0.5 %.

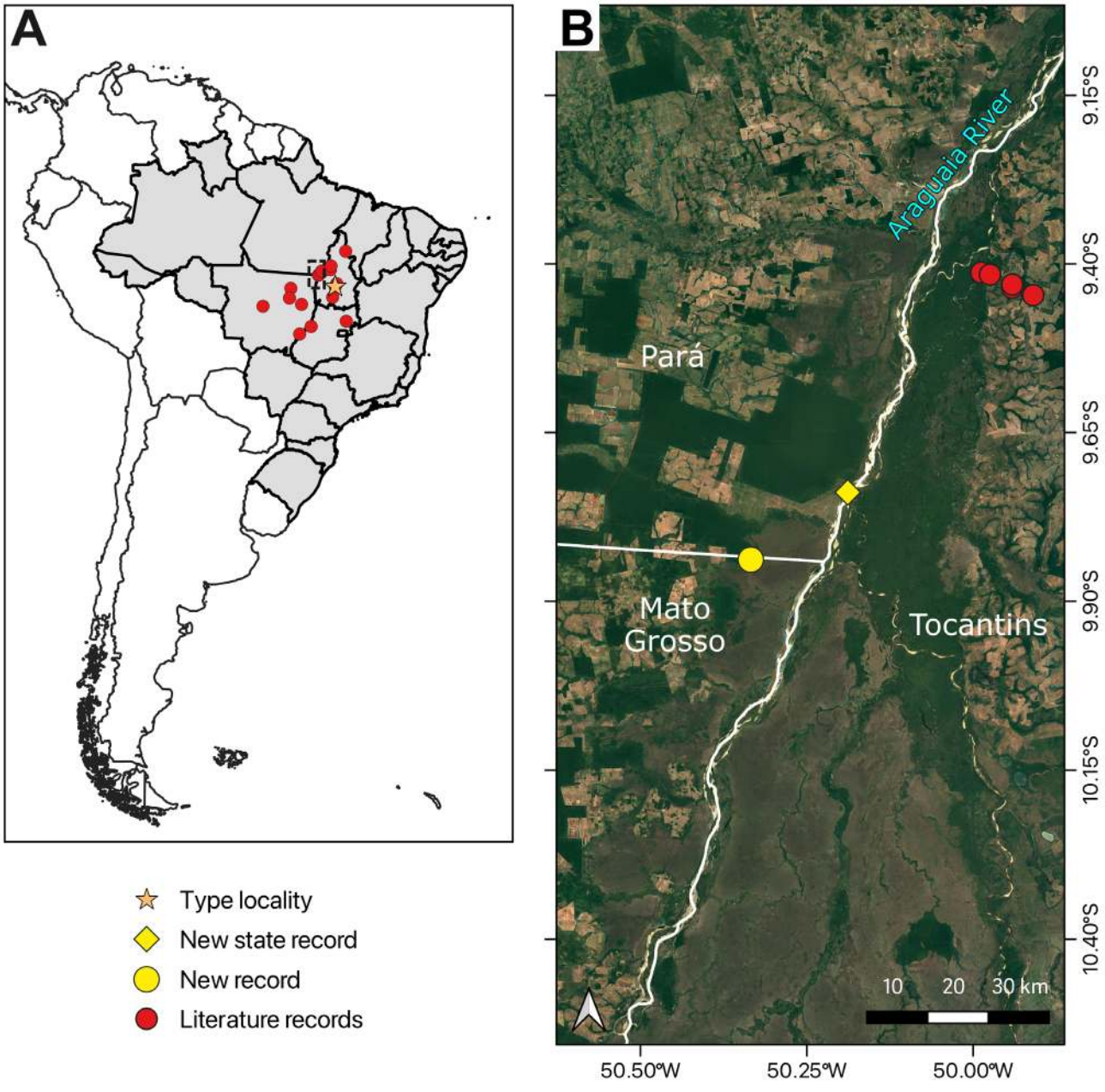

Figure 1. Updated geographic distribution of *Dendropsophus anataliasiasi* in central Brazil, with our new records highlighted in yellow from Santa Terezinha municipality, Mato Grosso state and from Santana do Araguaia municipality, Pará state. Background of the inset map from Google Earth (Map data ©2020 Google/Landsat/Copernicus).

Our records of *D. anataliasiasi* from the municipality of Santana do Araguaia, Pará state, extend the known geographic distribution of the species by approximately 40 km from its nearest previously known locality, in Caseara, Tocantins state, and 229 km northwest of its type locality, in Brejinho de Nazaré, Tocantins state (Fig. 1). In a recent review of amphibians from the Pará state, Cassundé et al. (2022) did not include *D. anataliasiasi*. Therefore, our record represents the 196th amphibian species documented for the state and may contribute to future state-level conservation assessments.

Figure 2. Newly recorded specimens of *Dendropsophus anataliasiasi* in Brazil.
A – MZUSP 161273; field number MTR 41824, male, from Santa Terezinha municipality, Mato Grosso state (diurnal coloration). **B** – Unvouchered male from Santana do Araguaia municipality, Pará state (nocturnal coloration). Not on scale.

Table 1. Morphometric measurements of newly collected specimens of *Dendropsophus anataliasiasi*, from the municipalities of Santa Terezinha (Mato Grosso state) and Santana do Araguaia (Pará state), Brazil. Values are shown in millimeters.

Measurements	**MZUSP 161273**	**MZUSP 161274**	**MZUSP 161275**	**MZUSP 161276**	**MZUSP 161277**	**MZUSP 161278**
Snout–vent length (SVL)	21.9	21.7	21.2	21.0	20.5	21.8
Head width (HW)	5.4	5.6	5.3	5.3	5.6	5.5
Head length (HL)	5.6	5.9	5.7	5.9	5.9	5.6
Eye diameter (ED)	2.0	1.7	1.9	1.8	2.3	2.0
Upper eyelid width (UEW)	1.3	1.4	1.2	1.4	1.5	1.5
Interorbital distance (IOD)	1.4	1.6	1.6	1.5	1.6	1.5
Eye–nostril distance (END)	1.4	1.6	1.3	1.5	1.5	1.4
Internarial distance (IND)	1.5	1.4	1.2	1.5	1.4	1.4
Thigh length (THL)	9.3	9.5	9.3	9.6	9.1	8.8
Tympanum diameter (TD)	0.8	0.7	0.8	0.9	0.8	0.6
Nostril to tip of snout distance (NSD)	0.8	0.7	0.8	0.7	0.7	0.7
Upper arm (UAR)	4.0	4.2	4.3	4.0	3.5	4.2
Forearm (FAR)	3.5	3.8	3.8	3.5	3.5	3.2
Hand length (HAL)	5.2	5.3	5.2	4.8	5.1	5.4
Third finger disk diameter (3FD)	0.7	0.8	0.6	0.7	0.7	0.7
Tibia length (TL)	9.5	9.5	9.4	9.1	9.1	9.1
Foot length (FL)	13.4	13.1	12.9	12.9	12.7	13.0
Fourth toe disk diameter (4TD)	0.7	0.5	0.4	0.6	0.7	0.7

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