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SHORT NOTE

Release call of *Trachycephalus typhonius* (Anura, Hylidae) in the Cerrado, Central-western Brazil

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Abstract. Anurans exhibit a diverse acoustic repertoire, and the release call is emitted when an individual is improperly clasped, often by males mistakenly grasping other males or unreceptive females during amplexus. The release call also serves as a crucial source for taxonomic resolution. However, for certain widespread hylid genera like *Trachycephalus*, knowledge about their release calls is limited or non-existent. *Trachycephalus* comprises 18 species, yet only one has its release call described. This study presents the first description of the release call of *T. typhonius* from a population in the Cerrado of Central-Western Brazil. Its release call is composed of a multipulsed note, distinguishing it from the only other known call in the genus (*T.* '*vermiculatus*'). Given the differences in the release call parameters between these species, such variations could serve as diagnostic criteria for distinguishing the two lineages. Additional descriptions of release calls, including from more *T. typhonius* and *T.* '*vermiculatus*' populations, are essential for species diagnosis and taxonomic classification.

Key words: amphibia, bioacoustics, milk frog, vocalisation

Anurans are widely known for their reproductive vocalisation repertoire (Duellman & Trueb 1994). The advertisement call, emitted by males to attract females during the breeding season, is among the most extensively studied and well-known reproductive calls (Guerra et al. 2018). Additionally, there are other types of calls used in different social contexts. These calls can be categorised into distinct groups such as reproductive, aggressive, defensive, and feeding, each with its corresponding subcategories (e.g. reproductive calls are divided into advertisement, courtship, amplectant, release, post-oviposition male resealing, and rain calls) (Toledo et al. 2015, Köhler et al. 2017). Among this rich repertoire, the release call is emitted by an individual when it is improperly clasped by another, usually occurring when males mistakenly clasp other males or unreceptive females for amplexus purposes (Gerhardt 1994). The release call is considered an important source for taxonomic resolution, as it can differ among anuran species and may contain phylogenetic information to help distinguish closely related taxa (e.g. cryptic species) (Vieira et al. 2014, Mângia et al. 2019). Furthermore, release calls have the potential to facilitate interspecific communication in heterospecific amplexus (Leary 2001).

Despite the importance of the release call, few studies have focused on this aspect of neotropical

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hylid ecology. Moreover, for certain widespread and even common hylid genera, such as *Trachycephalus* (Tschudi, 1838), the existing knowledge is either insufficient or non-existent. The genus *Trachycephalus* comprises 18 species (Frost 2024), with only one species having its release call described: *T.* '*vermiculatus*' (Barrio-Amorós & Guell 2023). The most widespread species in the genus is *T. typhonius*, which exhibits a wide distribution across Brazil, French Guyana, Guyana, Suriname, Argentina, Bolivia, and Venezuela (IUCN SSC 2023, Frost 2024) and shows explosive breeding behaviour (Prado et al. 2005). This species is known to have unresolved taxonomic issues and is currently recognised as a species complex. One of the few studies attempting to organise these populations was conducted by Ron et al. (2016), focusing on populations in Ecuador and Peru. The authors revalidated *T. quadrangulum* for populations in western Ecuador and *T. macrotis* in the Amazon of Ecuador and Peru. However, no comprehensive review covers the entire geographical distribution of *T. typhonius*. Therefore, the name *T. typhonius* continues to be widely used for populations in Brazil (Vaz-Silva et al. 2020, de Souza et al. 2022), underscoring the need for a more comprehensive understanding of its systematics. The significance of bioacoustics data, particularly the release call and the requirement for additional information for taxonomic resolution within *T. typhonius* (*sensu lato*), are highlighted. Herein, we provide the first description of its release call from a population in the Cerrado region of Central-Western Brazil.

We collected two males of *T. typhonius* on January 27th and 29th, 2024, in the Parque Natural Municipal do Pombo (20°22'25'' S; 52°36'3'' W, datum WGS84), municipality of Três Lagoas, state of Mato Grosso do Sul, Brazil. On 30th January 2024, at approximately 10:00 a.m., while being handled for photography, the frogs began to emit release calls. At that time, the air temperature was 33.3 °C, with a relative humidity of 49% (parameters collected in the Água Clara weather station, located about 25 km from the Park, INMET 2024). Each male was gently held to simulate amplexus, with the thumb and index finger positioned in the frog's axillae, while the calls were recorded using a Tascam DR-40 digital recorder. We analysed the recordings with the software *Raven Pro* version 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics 2024), with the following settings: window type – Hann, window size – 216 samples, 3dB filter bandwidth – 294 Hz, brightness – 50%, contrast – 50%, overlap – 50%, DFT size – 256 samples, grid spacing – 172 Hz. We analysed the

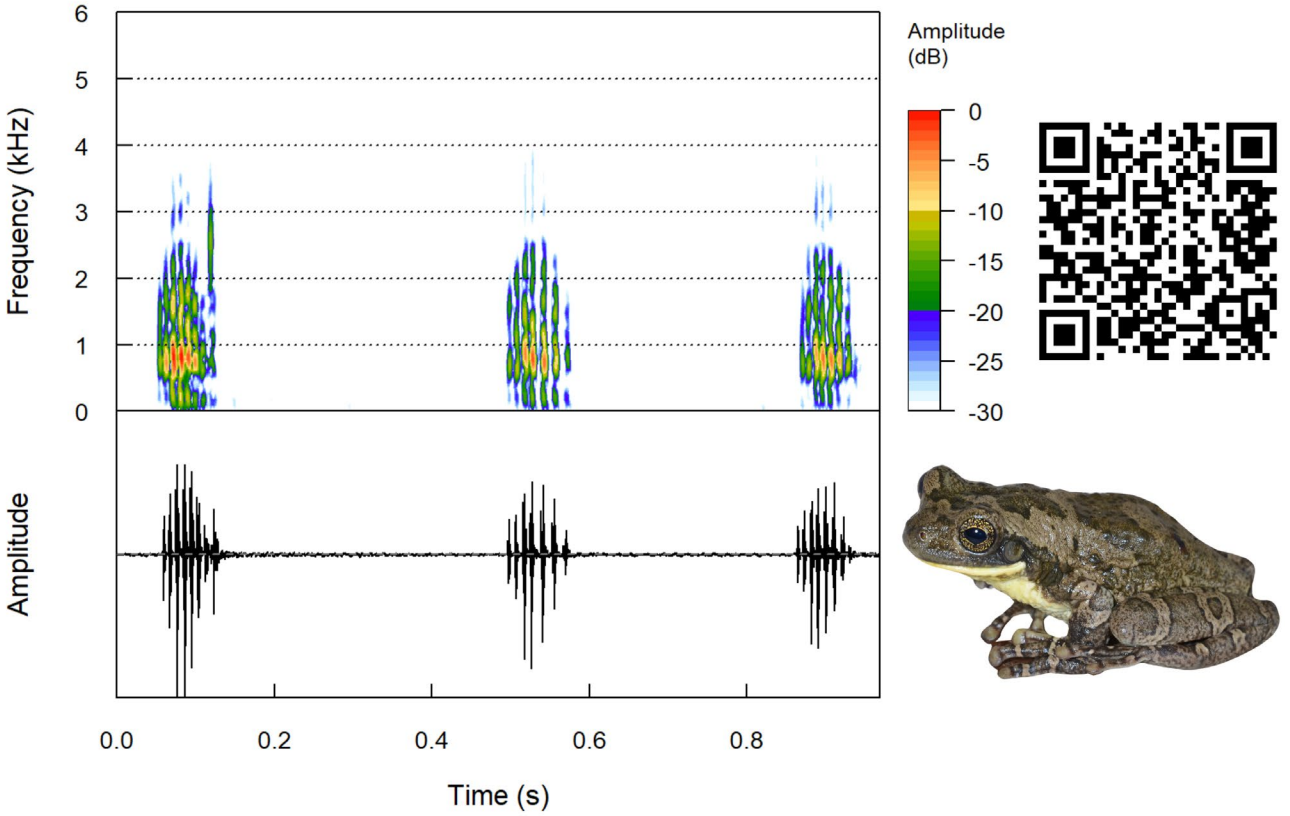

Fig. 1. Release call of *Trachycephalus typhonius* (ZUFMS-AMP19450, MAP-V 331, SVL 76.04 mm) from Parque Natural Municipal do Pombo, municipality of Três Lagoas, state of Mato Grosso do Sul, Brazil. Video available at https://youtu.be/XUDJUdbOy9c.

Table 1. Release and advertisement call of *Trachycephalus typhonius* and the release call of *Trachycephalus 'vermiculatus'*. Values are presented as mean, ± SD (range), SD – standard deviation; SVL – snout-vent length.

Acoustic parameters/Rate	Release call			Advertisement call
	T. typhonius (ZUFMS-AMP19450)	*T. typhonius* (ZUFMS-AMP19451)	*T. 'vermiculatus'*	*T. typhonius*
n (calls)	50	29	-	-
SLV (mm)	76.04	82.12	-	-
Duration (s)	0.066 ± 0.012 (0.036-0.090)	0.086 ± 0.016 (0.057-0.117)	(0.06-0.09)	0.454 (0.343-0.598)
Pulse/call	6.26 ± 0.803 (5-8)	6 ± 0.802 (5-8)	-	68 (52-87)
Pulse/second	0.010 ± 0.001 (0.006-0.014)	0.014 ± 0.003 (0.009-0.019)	-	149 (123-204)
Dominant Frequency (Hz)	794.144 ± 46.984 (689.06-947.46)	843.507 ± 53.391 (689.06- 861.33)	(430.00-516.00)	2143 (1.705-2.750)
Reference	Present study	Present Study	Barrio-Amorós & Güell (2023)	Zaracho et al. (2018)

following acoustic parameters: call duration (s), pulses per call, pulse rate (pulses per second), and dominant frequency (Hz). The oscillogram and spectrogram illustrations were generated using the package *seewave* version 2.2.3 (Sueur et al. 2023) on the R platform version 4.3.3 (R Development Core Team 2024). *Seewave* settings employed were window name (Fourier transformation window) – Hanning; window length – 512 samples; overlap – 90%. Voucher specimens were measured (snout-vent length) using a digital calliper and were housed at the herpetological collection of the Federal University of Mato Grosso do Sul (ZUFMS-AMP19450 and ZUFMS-19451), Brazil. Recordings were stored as uncompressed wave files at the Mapinguari Sound Library of the Federal University of Mato Grosso do Sul (MAP-V 331 and MAP-V 332).

The release call of both males of *T. typhonius* (Fig. 1) is characterised by a multipulsed note with a duration of 0.036-0.117 s (0.073 ± 0.017), and a dominant frequency ranging from 689.06 to 947.46 Hz (812.26 ± 54.62) (Table 1). These calls consist of 5-8 pulses (6.16 ± 0.81) emitted at a rate of 0.006-0.019 pulses/s (0.012 ± 0.003). Comparatively, the advertisement call of *T. typhonius* exhibits a similar multipulsed note but with a longer duration (0.343-0.598 s), more pulses per call (52-87), a higher pulse rate (123-204 pulses/s), and a dominant frequency between 1,705 and 2,750 Hz (Zaracho et al. 2018) (Table 1).

In comparison to the release call of *T. 'vermiculatus'*, the only known in the genus, the release call of *T. typhonius* exhibits a higher dominant frequency (689.06-947.46 Hz; 430.00-516.00 Hz in *T. 'vermiculatus'*). Both males of *T. typhonius* recorded in the present study emitted the release calls inflating only one of their two vocal sacs (video available at https://youtu.be/XUDJUdbOy9c), while the release calls registered by Barrio-Amorós & Guell (2023), both vocal sacs of *T. 'vermiculatus'* were inflated, however with about half their maximum volume.

Currently, *T. 'vermiculatus'* serves as a placeholder taxon encompassing all available names formerly attributed to *Trachycephalus typhonius*, ranging from Chocoan South America to southern and eastern Mexico (Frost 2024). According to Ron et al. (2016), Central American populations of this species may not be conspecific with *T. typhonius*, the previously assigned name, suggesting the possibility of these populations receiving a distinct designation, potentially *T. vermiculatus* (Barrio-Amorós & Guell 2023). Assuming the distinctiveness of these

populations and their recognition as a species complex, with only two species currently reclassified for South America, there is a pressing need for a comprehensive review addressing the entire distribution range of this species. The differences in release calls observed here could serve as diagnostic synapomorphies to distinguish between the lineages. Among the 18 known species of *Trachycephalus*, release calls are currently described for two species, including the one presented in this study. Given the potential for taxonomic classification in the release call, it is crucial to provide additional descriptions to differentiate between species, including more *T. typhonius* and *T. 'vermiculatus'* populations. When combined with advertisement calls, it is evident that such calls can significantly contribute to *Trachycephalus* systematics.

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Author Contributions

P.N. Lopes: collected and analysed the data, generated the oscillogram and spectrogram illustrations, prepared figures and tables, wrote the manuscript and approved the final draft. A. Varago: collected the data, recorded the video, analysed the calls on the software, analysed the data, prepared figures and tables, wrote the manuscript and approved the final draft. J. Portilho: collected the data, reviewed drafts of the manuscript and approved the final draft. D.J. Santana: collected and analysed the data, took photos, recorded the calls, reviewed drafts of the manuscript, and approved the final draft. S. Mângia: collected and analysed the data, supervised the analysis and the writing of the manuscript, reviewed drafts of the paper, and approved the final draft.

Data Availability Statement

The data and RScript supporting this study's findings are available at: https://github.com/Rhinella85/Release_Call_Trachycephalus_typhonius.

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