

Combining citizen science, phylogenetics, and bioacoustics to inform taxonomy and conservation of the Near Threatened *Proceratophrys paviotii* (Anura, Odontophrynidae)

João Victor Andrade Lacerda¹, Diego J. Santana², Carla Guimarães², Alice Zanoni dos Santos³, Alan P. Araujo⁴, Natalia Pirani Ghilardi-Lopes⁵ and Sarah Mângia²

¹ National Institute of the Atlantic Forest, Santa Teresa, Espírito Santo, Brazil

² Matinguari Lab, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

³ Instituto Federal do Espírito Santo campus Santa Teresa, Santa Teresa, Espírito Santo, Brazil

⁴ Laboratório de Herpetologia e Comportamento Animal, Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Campus Samambaia, Goiânia, Goiás, Brazil

⁵ Center for Natural and Human Sciences, Federal University of ABC (UFABC), São Bernardo do Campo, São Paulo, Brazil

ABSTRACT

Herein, based on novel data gathered by citizen scientists and specialists, we contribute to the improvement of scientific knowledge and conservation of the Near Threatened *Proceratophrys paviotii* in order to: 1) test for the first time the phylogenetic position and a species delimitation of *P. paviotii* through a molecular approach; 2) describe a larger sample of its advertisement call to properly encompass the species intraspecific variation; 3) describe for the first time the *P. paviotii* release call; and 4) provide novel insights on the species conservation status. Our 16S tree confidently grouped *P. paviotii* with *P. cururu*, *P. renalis*, and *P. laticeps*. The average sequence divergence between *P. paviotii* and its congeners ranged from 2.2% (*P. laticeps*) to 9.1% (*P. redacta*). Advertisement calls consisted of a single note with duration of 0.26–0.58 s, 17–41 pulses emitted at rate of 54.19–77.49 pulses/s and peak frequency of 775.19–947.46 Hz. Release calls consisted of a single note with duration of 0.04–0.43 s, 2–13 pulses emitted at rate of 21.17–81.58 pulses/s and peak frequency of 689.1–1,722.6 Hz. Additionally, our study strongly supports the notion that Citizen Science approaches can yield invaluable information concerning species' geographic distribution and conservation.

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Corresponding author

João Victor Andrade Lacerda,
lacerdajva@gmail.com

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INTRODUCTION

Amphibians are among the most endangered animal groups on the planet, with 41% of their species classified as threatened with extinction, including many Neotropical lineages under some risk (Luedtke et al., 2023). The Neotropical genus *Proceratophrys*

Miranda-Ribeiro, 1920 occurs in Brazil, Argentina, Paraguay and possibly Bolivia, and currently includes 43 species (Frost, 2024). Eight species of *Proceratophrys* are threatened with extinction, classified as Critically Endangered (CR), Vulnerable (VU), or Endangered (EN). Two species of *Proceratophrys* are classified as Near Threatened (NT). A taxon is classified as Near Threatened (NT) when it is close to or likely to qualify for a threatened category in the near future (IUCN, 2024). Therefore, these species require special attention and focus from both the scientific community and conservation initiatives.

Evolutionary studies have played an important role in anuran taxonomy. Several populations recovered as independent evolutionary units have supported the description of new taxa (e.g., Ferreira et al., 2023; Folly et al., 2024). Conversely, taxa that have not been recovered as reciprocally monophyletic have led to synonymizations (e.g., Faivovich et al., 2021; Pereira et al., 2022). Among *Proceratophrys*, the same scenarios are observed with species that have been recently described (Santana et al., 2021a, 2021b; Mângia et al., 2022) and synonymized (Mângia et al., 2020) supported by phylogenetic approaches. These studies have great potential to impact conservation status, given that we protect nominal species.

Citizen Science (CS) approaches have great potential in providing information on several biological groups, such as fungi, plants and animals, even on data deficient, rare, or threatened species (e.g., Mori & Menchetti, 2014; Campanaro et al., 2017; Irga, Barker & Torpy, 2018; Heard, Chen & Wen, 2019; Pirota et al., 2020; Deacon, Govender & Samways, 2023; Farquhar et al., 2023; Krueger et al., 2023; Lacerda et al., 2023), once such projects have encouraged the participation of the public in different stages of scientific projects, such as gathering and/or analyzing biological data (Bonney et al., 2009).

Citizen Science projects focused on anurans have contributed to a vast range of issues in science and conservation (e.g., Pittman & Dorcas, 2006; Price & Dorcas, 2011; Cosentino et al., 2014; Westgate et al., 2015; Sterrett et al., 2019; Antúnez-Fonseca et al., 2021; Ceriaco et al., 2021; Lee et al., 2021; Forti et al., 2022a, 2022b; Glorioso et al., 2022; Forti & Szabo, 2023). The public usually contributes by sending pictures and thus providing data on species occurrence *via* digital platforms, such as the iNaturalist (e.g., Forti et al., 2022a, 2022b; Forti & Szabo, 2023). This active involvement of the public in sharing visual data enables researchers to gain insights into the distribution and presence of anuran species. However, while the pictures are invaluable for assessing anuran's identity, CS projects often overlook a critical aspect: a vast vocal repertoire of this group, emitted depending on its social context, such as reproductive, aggressive, defensive, and feeding calls (Köhler et al., 2017). In anurans, advertisement calls are heritable and usually species-specific (Duellman & Trueb, 1994; Wells, 2007). Despite the importance of bioacoustics for taxonomic and conservation purposes, only a few CS projects focus on receiving anurans audio files from the public. For instance, the FrogID project gathers audio files containing anuran vocalization from Australian citizens (<https://www.frogid.net.au/>) (Rowley et al., 2019). This project has contributed to several scientific and conservation issues, such as: fire effect (Rowley, Callaghan & Cornwell, 2020), invasive species (Rowley & Callaghan, 2022), species geographic distribution (Cutajar et al., 2022), behavior (Thompson et al., 2022), urban impact (Liu et al., 2022), among others.

Since 2020, we have been conducting a CS project entitled *Cantoria de Quintal* (translated as “Songs from the Back Yard”) in the municipality of Santa Teresa (Lacerda et al., 2023; Lacerda, Santos & Lima, 2023). We have encouraged the public to record anurans from their yards and/or surroundings using smartphones. The *Cantoria de Quintal* project aims to investigate scientific and conservation issues, such as species distribution, whether threatened species also occur in unprotected areas, urban and deforestation impacts, among others. However, species identification through audio files is not always easy and can be hampered when dealing with rare or poorly known species.

During 2020–2023 we received 42 audio files as part of the *Cantoria de Quintal* project from 10 citizen scientists. These files contained calls that we initially categorized as belonging to *Proceratophrys* sp.. After assessing literature information (Cruz, Prado & Izecksohn, 2005; Ferreira et al., 2019a), we assigned those records to *Proceratophrys* sp. (cf. *paviotii*). We were unable to confirm its identification to the species level due to two main reasons: 1) the vocalization of *P. paviotii* is not available for comparison in any existing public audio collections; and 2) the call description provided by Cruz, Prado & Izecksohn (2005) in the species’ original description is based on only seven calls from a single male, which certainly do not properly encompass the species’ intra-specific variation. Motivated by the challenge of properly identifying the citizen scientists’ records, we initiated an integrative investigation on the *Proceratophrys paviotii* taxonomy.

Proceratophrys paviotii (Fig. 1A) is a Near Threatened species (IUCN, 2024) that occurs in the central and northern State of Espírito Santo (Prado & Pombal, 2008; Almeida, Gasparini & Peloso, 2011; Peres & Simon, 2011; Figs. 1B–1D). This species has never been included in phylogenetic or molecular species delimitation approaches so far (see Mângia et al., 2020; Santana et al., 2021a, 2021b; Mângia et al., 2022). Therefore, its phylogenetic position has not been properly tested.

Efficient conservation predictions and strategies depend on filling knowledge gaps such as on its taxonomic identity (Linnean shortfall), geographic distribution (Wallacean shortfall), and evolution (Darwinian shortfall) (see Hortal et al., 2015). Therefore, combining data provided by citizen scientists and specialists, in this study we: 1) test for the first time the phylogenetic position and a species delimitation of *Proceratophrys paviotii* through a molecular approach; 2) describe a larger sample of its advertisement call to properly encompass the species intraspecific variation; 3) describe for the first time the *P. paviotii* release call; and 4) provide novel occurrence data and insights on the species conservation status. Thus, herein we contribute to the improvement of scientific knowledge and conservation of the Near Threatened *Proceratophrys paviotii*.

MATERIALS AND METHODS

Study area

The Municipality of Santa Teresa, State of Espírito Santo, located within the Atlantic Forest of Southeastern Brazil (Fig. 1), is known for its rich biodiversity across various biological groups (e.g., Thomaz & Monteiro, 1997; Brown & Freitas, 2000; Passamani, Mendes & Chiarello, 2000; Wendt et al., 2010; Gatti et al., 2014; Novaes et al., 2016). With 109 amphibian species, Santa Teresa stands out for harboring one of the highest diversities

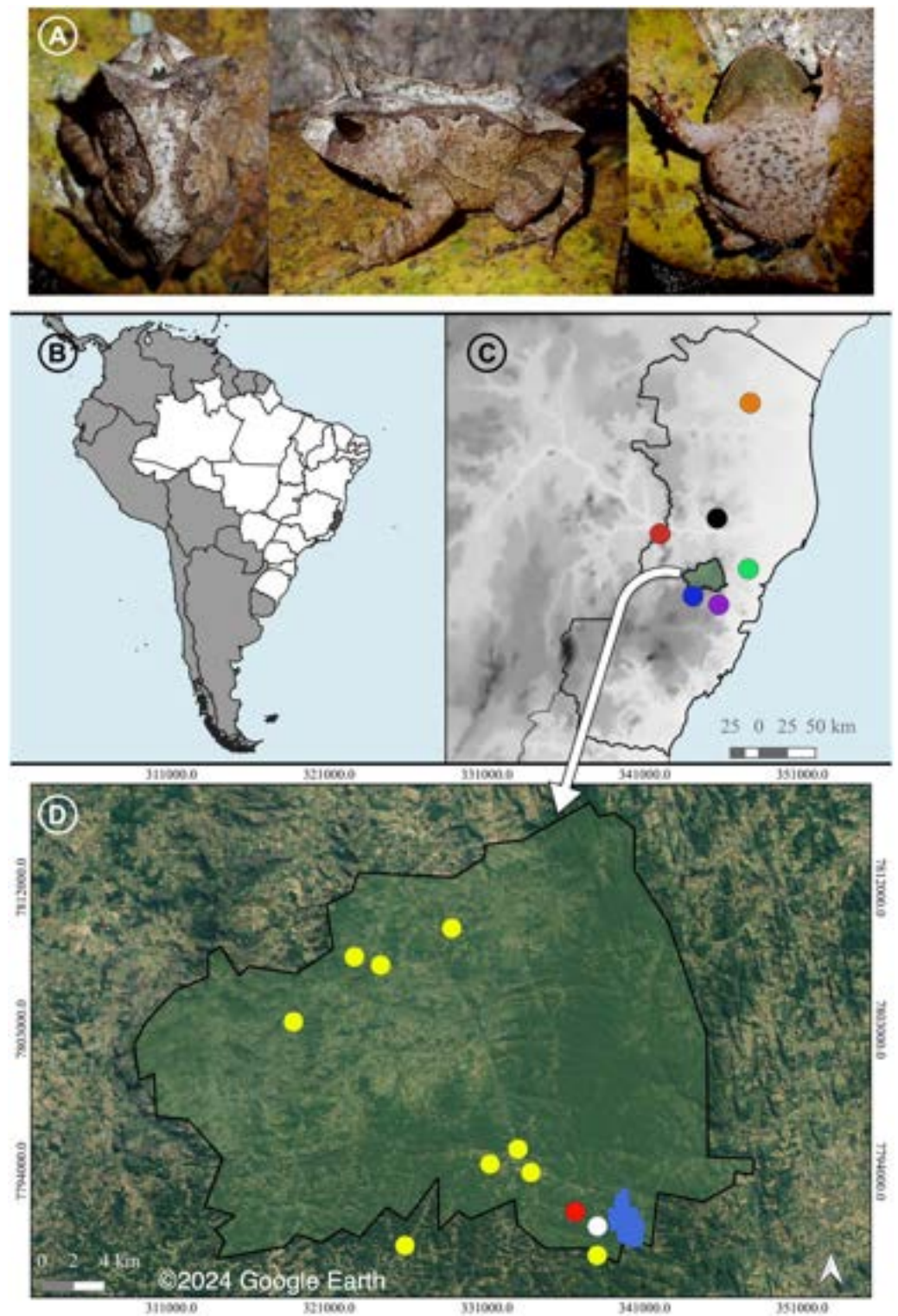



Figure 1 A recorded male and the distribution of *Proceratophrys pavotii*. (A) A recorded male of *Proceratophrys pavotii* (MBML 12369; snout-vent-length, SVL 44.1 mm): dorsal, lateral and ventral views. (B) State of Espírito Santo (shaded) in Southeastern Brazil. (C) Municipality of Santa Teresa (shaded) and the known distribution of *Proceratophrys pavotii* in the State of Espírito Santo: Municipality of Aracruz (green circle; Prado & Pombal (2008)), Municipality of Baixo Guandu (red circle; Prado & Pombal (2008)), Municipality of Santa Leopoldina (purple circle; Prado & Pombal (2008)),

Figure 1 (continued)

Municipality of Santa Maria do Jetibá (blue circle; *Almeida, Gasparini & Peloso (2011)*), Municipality of Marilândia (black circle; *Almeida, Gasparini & Peloso (2011)*), and Municipality of Pinheiros (red circle; *Peres & Simon (2011)*). (D) Municipality of Santa Teresa, type locality of *P. paviotii* Estação Biológica Santa Lucia (blue); records of *P. paviotii* made through audio files sent by citizen scientists (yellow); records of *P. paviotii* made by formal scientists while visiting a citizen scientists' property (red); and records of *P. paviotii* made by formal scientists (white). Map builded using the Google Satellite tool from QGIS 3.22.2: source IBGE (<https://www.ibge.gov.br/geociencias/organizacao-do-territorio/malhas-territoriais/15774-malhas.html>). © 2024 Google Earth. Full-size  DOI: 10.7717/peerj.17990/fig-1

of this group in the world (*Ferreira et al., 2019a; Lacerda et al., 2021*). Notably, Santa Teresa is the only place where six species of *Proceratophrys* can be found in sympatry (*Prado & Pombal, 2008; Ferreira et al., 2019a*): *Proceratophrys boiei* (Wied-Neuwied, 1824); *P. laticeps* Izecksohn & Peixoto, 1981; *P. moehringi* *Weygoldt & Peixoto, 1985*; *P. paviotii* *Cruz, Prado & Izecksohn, 2005*; *P. phyllostomus* *Izecksohn, Cruz & Peixoto, 1998*; and *P. schirchi* Miranda-Ribeiro, 1937.

Phylogenetic inference and genetic distances

We extracted DNA from tissue samples (liver) using the QIAGEN DNeasy Blood and Tissue Kit (Valencia, California, USA) following the manufacturer's protocol. Next, we amplified a fragment of the mitochondrial 16S gene using primers 16Sar and 16Sbr (*Palumbi et al., 1991*). The PCR protocol was configured with one initial phase of 94 °C for 3 min, followed by 35 cycles of 94 °C for 20 s, 50 °C for 20 s, 72 °C for 40 s, and a final extension phase of 72 °C for 5 min. Purification of PCR products and sequencing were performed by ACGTene Análises Moleculares Ltda. (Alvorada, Rio Grande do Sul, Brazil).

We combined our newly generated 16S sequences with comparable 16S sequences of *Proceratophrys* individuals available on GenBank, and also included the outgroups *Odontophrynus* spp., *Macrogenioglottus alipioi*, *Cycloramphus acangatan* and *Thoropa miliaris*, which are available in GenBank ([Document S1](#)). We aligned 16S mtDNA gene fragments using the MAFFT algorithm (*Katoh & Toh, 2008*) in Geneious v9.0.5 with default settings. The final dataset comprised 84 sequences of a 498 base-pair (bp) fragment of the 16S gene. All GenBank accession numbers and genetic voucher samples used here are listed in the [Supplemental Material](#). We used the Bayesian Information Criterion in jModelTest (*Darriba et al., 2012*) to determine that GTR+I+G was the best model of nucleotide substitution for our 16S data set. To estimate phylogenetic relationships, we used Bayesian Inference (BI) in BEAST v2.6.6 (*Bouckaert et al., 2019*) for 20 million generations, sampling every 2,000, using a Yule speciation prior, implementing a relaxed clock log normal. We checked for stationarity by visually inspecting trace plots and ensuring that all effective sample sizes were above 200 in Tracer v1.7.1 (*Rambaut et al., 2018*). 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 v2.6.6 (*Bouckaert et al., 2019*). We calculated sequence divergences (uncorrected *p*-distances) among species/individuals using MEGA v10.1.1 (*Kumar et al., 2018*). In order to explore the relationship among haplotypes, we estimated haplotype networks among species closely related to the *P. paviotii* for the 16S mtDNA gene in POPART

Table 1 Data on geographic occurrence of *Proceratophrys paviotii*. Municipality, file voucher, coordinates, and area description. An asterisk (*) indicates files that were included in the bioacoustical analysis.

Municipality	Voucher	Coordinates	Area description
Santa Teresa	FNJV 60391	19°56'20"S; 40°36'46"W	A wetland inside the urban area
	FNJV 60388*	19°56'19"S; 40°36'45"W	A street located at the border of the urban area (rainwater stored by the sidewalk channel)
	FNJV 60397	19°56'38"S; 40°35'17"W	A vacant lot located at the border of the urban area
	FNJV 60396	19°49'10"S; 40°41'37"W	A yard located at a rural area
	FNJV 60389*, 60390*, 60392–60395	19°49'27"S; 40°40'40"W	A yard located at a rural area
	–	19°48'03"S; 40°38'01"W	A yard located at a rural area
	–	19°51'21"S; 40°43'39"W	A yard located at a rural area
	–	19°55'54"S; 40°35'42"W	A street located at the border of the urban area
	FNJV 60377–60383*	19°58'00"S; 40°33'41"W	A yard located at a rural area
	FNJV 60384–60387*	19°58'30"S; 40°32'53"W	Rainwater stored at a roadside
Santa Leopoldina	–	19°59'37"S; 40°32'41"W	A yard located at a forest edge
Santa Maria de Jetibá	–	19°59'11"S; 40°39'58"W	A yard located at a rural area

(Leigh & Bryant, 2015) using the median-joining network method. We identified each species using different colors in the haplotype network. All molecular protocols follow previous studies on *Proceratophrys* taxonomy (e.g., Santana et al., 2021a, 2021b; Mângia et al., 2022).

Bioacoustics

Records (Table 1) made by specialists were performed with Tascam DR-40 and Tascam DR-05 recorders at a sampling rate of 44.1 kHz and 24 bits resolution (FNJV 60377–60383, 60384–60387). One male (MBML 12366) emitted release calls when handled (FNJV 60382). The citizen scientists' files were recorded using smartphones: *Motorola Moto G7 plus* (FNJV 60388, 60391), *Samsung Galaxy A21s* (FNJV 60397) and other two unspecified models (FNJV 60389–60390, 60392–60396). These files were sent via *Whatsapp* app (.ogg format) and then converted to .wav format. Bioacoustic analyses were performed using Raven Pro 1.5 software (*Bioacoustics Research Program*, 2014). Spectrograms were produced using Hann window type, DFT 256 sample size, and time grid overlap of 80.1%. Figures were built using *seewave* and *tuneR* packages in R (*R Core Team*, 2017), with window length = 512, overlap = 80.1%. Bioacoustic terminology follows the call-centered approach proposed by Köhler et al. (2017). We calculated call duration (s), number of pulses per call (pulses/call), pulse emission rate per call (pulses/s), pulse duration (s), interval between pulses (s), peak frequency (Hz), frequency 5% (Hz), and frequency 95% (Hz). Quantitative parameters are presented as minimum–maximum (mean ± standard deviation; n = sample size). Low frequencies up to 200 Hz (safely below the minimum frequency reached by *P. paviotii*) were high-pass filtered to decrease background noise in the recording files using Raven Pro 1.5. Recordings were then compared to the literature available for other *Proceratophrys* species. Vocalization recordings were deposited at Fonoteca Neotropical Jacques Viellard (FNJV; <https://www2.ib.unicamp.br/fnjv/>).

We examined the variability of acoustic parameters in male calls, focusing on both intra-individual and inter-individual levels. For assessing the intra-individual coefficient of variation (CV_{intra}), we utilized a dataset of 105 calls from 11 individuals and computed the mean (\bar{X}) and standard deviation (SD) of individual calls using the formula $CV = (SD/\bar{X}) \times 100$. To determine inter-individual variation (CV_{inter}), we evaluated the mean and standard deviation of parameter values across all individuals, including two cases where only one call was available for each. Parameters with CV values $\leq 5\%$ are considered static, while those with CV values $\geq 12\%$ are considered dynamic (Gerhardt, 1991). We calculated both CV_{intra} and CV_{inter} for six parameters (note duration, peak frequency, 5% frequency, 95% frequency, pulse number, and pulses/second). Additionally, to discern whether intra-individual variation was greater than inter-individual variation, we computed the CV_{intra}/CV_{inter} ratio. If CV_{intra}/CV_{inter} is >1 , it means a greater variation between males (inter-individual) than an intra-individual variation (Gerhardt, 1991; Moser et al., 2022).

Occurrence data through Citizen Science and by specialists

We received 42 audio files (.ogg format) through the *Cantoria de Quintal* project containing calls emitted by *Proceratophrys paviotii*. Files were sent by eight citizen scientists from eight distinct localities in the Municipality of Santa Teresa, one in Santa Maria de Jetibá, and one in Santa Leopoldina, State of Espírito Santo (Fig. 1; Table 1). The *Cantoria de Quintal* activities include visiting citizen scientists to provide environmental education and enhance their engagement in the project. During one of these visits (October 23rd, 2020), we found a population of *Proceratophrys paviotii* inhabiting the citizen scientist's yard and surroundings with several males in calling activity (FNJV 60377–60383). This property (19°58'00"S; 40°33'41"W) is located about 2.2 km straight line from Estação Biológica de Santa Lúcia (type locality of *P. paviotii*; Fig. 1). Males were calling from the banks of an anthropized sandy stream. Recordings took place from 06:00 to 08:30 pm, during a light rain, air temperature ca. 20 °C. Additionally, we found a second population of *Proceratophrys paviotii* (February 19th, 2021) with several males calling from the rainwater stored at a roadside about 800 m from the species type locality (Fig. 1). We recorded five of these males (FNJV 60384–60387). Recordings took place from 9:00 to 10:00 pm, during a light rain, air temperature ca. 20 °C.

Voucher specimens

Specimens collected as vouchers were anesthetized and killed with lidocaine 2%, fixed in formaldehyde 10%, and preserved in 70% ethanol at Museu de Biologia Prof. Mello Leitão (MBML) from Instituto Nacional da Mata Atlântica (INMA) (MBML 12366–12370). Before fixation, specimens had tissue (liver) samples extracted and stored in 96% ethanol for whole genomic DNA extraction. We have followed CONCEA (Jared et al., 2023) for guidelines for the ethical treatment. The Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio #63575-5) and animal research ethics committee of the Universidade de Vila Velha (CEUAUVV #491-2018) provided sampling permits.

Table 2 Mean *p*-distance for mtDNA 16S of *Proceratophrys paviotii* to other species of the genus.

Species	<i>p</i> -distance	Species	<i>p</i> -distance
<i>P. aff. ararype</i>	0.076	<i>P. kaingang</i>	0.051
<i>P. appendiculata</i>	0.028	<i>P. korekore</i>	0.077
<i>P. ararype</i>	0.081	<i>P. laticeps</i>	0.022
<i>P. avelinoi</i>	0.059	<i>P. mantiqueira</i>	0.038
<i>P. belzebul</i>	0.035	<i>P. melanopogon</i>	0.041
<i>P. bigibbosa</i>	0.046	<i>P. minuta</i>	0.080
<i>P. boiei</i> N1	0.056	<i>P. moratoi</i>	0.068
<i>P. boiei</i> N2	0.064	<i>P. pombali</i>	0.049
<i>P. boiei</i> S	0.037	<i>P. redacta</i>	0.091
<i>P. brauni</i>	0.060	<i>P. renalis</i>	0.030
<i>P. concavitympanum</i>	0.086	<i>P. salvatori</i>	0.067
<i>P. cristiceps</i>	0.087	<i>P. schirchi</i>	0.073
<i>P. cururu</i>	0.027	<i>P. strussmannae</i>	0.069
<i>P. goyana</i>	0.036	<i>P. tupinamba</i>	0.024
<i>P. itamari</i>	0.034	<i>P. velhochico</i>	0.082
<i>P. izecksohni</i>	0.033		

group (PP = 1.00). However, the relationship between these clades was not strongly supported (PP = 0.69). Due to the utilization of a single mtDNA locus for species barcoding, our tree had low posterior probabilities for several nodes within *Proceratophrys*, which is expected. The average sequence divergence between *P. paviotii* and its congeners ranged from 2.2% (*P. laticeps*) to 9.1% (*P. redacta*) (Table 2). Furthermore, the mitochondrial haplotype network, based on a fragment of the 16S gene of *P. paviotii* and its closely related species (see Fig. 3), shows seven distinct mitochondrial lineages, with no haplotype sharing between them.

Bioacoustics

We analyzed a total of 107 advertisement calls emitted by 13 males of *Proceratophrys paviotii* (Table 3). Calls consisted of a single note with duration of 0.26–0.58 s ($0.46 \text{ s} \pm 0.07$; $n = 107$ calls), 17–41 pulses ($31.17 \text{ pulses} \pm 5.23$; $n = 104$ calls), pulse duration of 0.007–0.026 s ($0.012 \text{ s} \pm 0.002$; $n = 755$ pulses), pulses emitted at rate of 54.19–77.49 pulses/s ($68.32 \text{ pulses/s} \pm 6.64$; $n = 104$ calls). Calls had peak frequency of 775.19–947.46 Hz ($861.85 \text{ Hz} \pm 33.09$; $n = 101$ calls), frequency 5% of 656.25–861.33 Hz ($718.85 \text{ Hz} \pm 45.51$; $n = 101$ calls), and frequency 95% of 937.5–1,119.73 Hz ($1,001.91 \text{ Hz} \pm 54.84$; $n = 101$ calls). Calls have an ascendant amplitude modulation on its beginning and descendant amplitude modulation on its ending (Fig. 4A).

We analyzed a total of 55 release calls emitted by a single male of *Proceratophrys paviotii*. Release calls consisted of a single note with duration of 0.04–0.43 s ($0.15 \text{ s} \pm 0.07$; $n = 55$ calls), 2–13 pulses ($7.78 \text{ pulses} \pm 3.22$; $n = 55$ calls) with 0.003–0.023 s ($0.008 \text{ s} \pm 0.002$; $n = 427$ pulses) emitted at rate of 21.17–81.58 pulses/s ($51.56 \text{ s} \pm 11.9$; $n = 55$ calls).

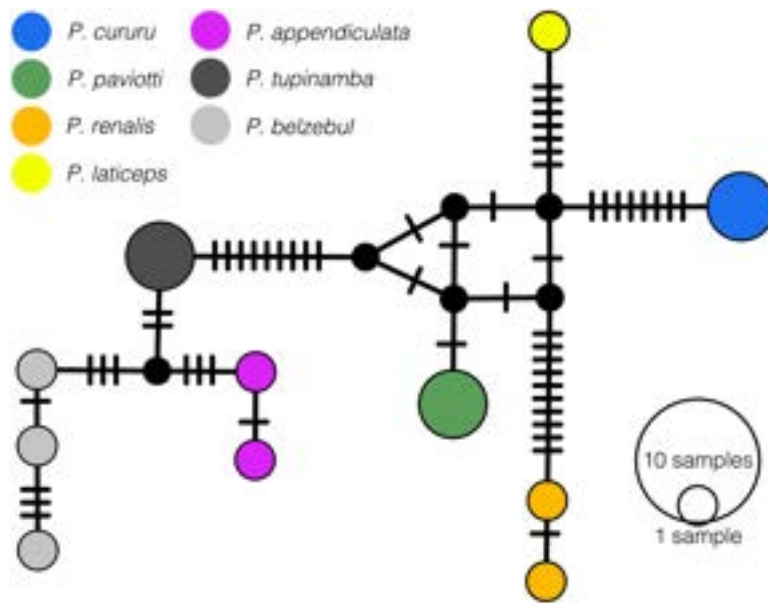


Figure 3 Median-joining haplotype network based on mtDNA 16S of *Proceratophrys paviotii*. Each haplotype circle is proportional to its frequency (indicated in legend). Each color represents distinct species, and black dots represent inferred unsampled or extinct haplotypes. Mutational steps between alleles are represented by lines. *Proceratophrys cururu* (blue), *P. paviotii* (green), *P. renalis* (orange), *P. laticeps* (yellow), *P. appendiculata* (pink), *P. tupinamba* (dark grey), *P. belzebul* (light grey). DJ Santana prepared the Haplotype network using POPART. [Full-size !\[\]\(52516a3edab5b871bdd69195863186f9_img.jpg\) DOI: 10.7717/peerj.17990/fig-3](https://doi.org/10.7717/peerj.17990/fig-3)

Release calls had peak frequency of 689.1–1,722.6 Hz (1,227.8 Hz \pm 424.3; $n = 55$ calls), frequency 5% of 516.8–861.3 Hz (667.1 Hz \pm 81.6; $n = 55$ calls), and frequency 95% of 1,205.9–3,445.3 Hz (2,211.7 Hz \pm 322.7; $n = 55$ calls). Release calls had irregular patterns of amplitude and frequency modulation. Isolated pulses were sporadically emitted between calls (Fig. 4B).

The CV_{intra} demonstrated a static variation in peak frequency, 5% frequency, 95% frequency and pulse rate, and an intermediate variation in note duration and number of pulses per call. On the other hand, in a CV_{inter} approach, note duration and number of pulses per call were both considered dynamic; pulse rate was considered intermediate; and peak frequency, 5% frequency, 95% frequency were considered static (Table 4). Additionally, the ratio between CV_{inter} and CV_{intra} was greater than one (>1) in all parameters, so it was considered a dynamic variation.

The advertisement call of *Proceratophrys paviotii* differs from those of *P. carranca*, *P. goyana*, *P. rotundipalpebra*, and *P. vielliardi* by its single note call pattern (multi noted call pattern in these species). The advertisement call of *P. paviotii* differs from those of *P. appendiculata*, *P. bigibbosa*, *P. boiei*, *P. brauni*, *P. cururu*, and *P. moehringi* by its shorter notes. The advertisement call of *P. paviotii* differs from those of *P. carranca* and *P. goyana* by its longer notes. The advertisement call of *P. paviotii* differs from those of *P. appendiculata* and *P. cristiceps* by its lower number of pulses per note. It differs from those of *P. bigibbosa*, *P. boiei*, *P. brauni*, *P. cururu*, *P. moehringi*, and *P. palustris* by its higher pulse emission rate. It differs from those of *P. ararype*, *P. carranca*,

Table 3 Quantitative parameters (presented as range) of the advertisement call of *Proceratophrys* species. When more than one source was available, we combined the values provided in a single range. Values in bold do not overlap with *P. paviotii*.

Species	Notes/ call	Note duration (s)	Pulses/ note	Pulses/s	Dominant frequency (kHz)	Source
<i>P. paviotii</i>	1	0.26–0.58	17–41	54.2–77.5	0.775–0.947	Present study
<i>P. paviotii</i>	1	0.34–0.43	26–32	x	0.660–1.280	<i>Cruz, Prado & Izecksohn (2005)</i>
<i>P. appendiculata</i>	1	1.32–2.41	51–129	30.0–65.4	0.562–0.656	<i>Dias et al. (2013)</i>
<i>P. ararype</i>	1	0.37–0.65	38–65	95.7–102.7	1.033–1.378	<i>Mângia et al. (2018)</i>
<i>P. avelinoi</i>	1	0.22–0.75	23–70	64.0–72.0	1.050–2.300	<i>Kwet & Baldo (2003)</i>
<i>P. bigibbosa</i>	1	1.60–1.90	40–45	23.0–27.0	1.050	<i>Kwet & Faivovich (2001)</i>
<i>P. boiei</i>	1	0.70–0.80	30–35	45.0	0.350–1.350	<i>Heyer et al. (1990)</i>
<i>P. brauni</i>	1	0.70–0.90	24–28	35.0–40.0	1.350	<i>Kwet & Faivovich (2001)</i>
<i>P. carranca</i>	1–10	0.04–0.19	5–21	95.2–131.5	1.033–1.378	<i>Godinho et al. (2013)</i>
<i>P. concavitympanum</i>	1	0.18–0.50	19–51	100.0–119.7	754.6–1,186.3	<i>Santana et al. (2010)</i>
<i>P. cristiceps</i>	1	0.52–0.79	33–69	78.5–91.8	0.860–1.030	<i>Nunes & Juncá (2006), Nunes et al. (2015)</i>
<i>P. cururu</i>	1	1.20	x	45.0	0.600–1.000	<i>Eterovick & Sazima (1998)</i>
<i>P. goyana</i>	1–34	0.06–0.24	7–23	83.3–120.5	0.937–1.125	<i>Martins & Giaretta (2013)</i>
<i>P. huntingtoni</i>	1	0.2–0.3	19–25	x	1.095–1.3445	<i>Ávila, Pansonato & Strüßmann (2012)</i>
<i>P. itamari</i>	1	0.4–0.8	20–42	49.0–55.0	1.033–1.205	<i>Mângia et al. (2014)</i>
<i>P. korekore</i>	1	0.16–0.33	18–32	96.4–111.1	0.861	<i>Santana et al. (2021b)</i>
<i>P. laticeps</i>	1	0.49–1.57	28–94	44.0–74.4	0.340–0.680	<i>Araújo et al. (2021), Martins & Giaretta (2021), Sichieri et al. (2021)</i>
<i>P. mantiqueira</i>	1	0.17–0.48	12–41	68.0–96.0	0.999–1.274	<i>Mângia, Santana & Feio (2010)</i>
<i>P. melanopogon</i>	1	0.40–0.80	20–38	40.0–55.0	0.831–1.033	<i>Mângia et al. (2014)</i>
<i>P. minuta</i>	1	0.40–0.72	30–52	66.8–75.2	1.980–2.070	<i>Nascimento et al. (2019)</i>
<i>P. moehringi</i>	1	3.50–4.00	x	33.0–40.0	0.200–0.700	<i>Weygoldt & Peixoto (1985)</i>
<i>P. moratoi</i>	1	0.14–0.33	12–26	65.0–103.0	1.153–1.594	<i>Brasileiro, Martins & Jim (2008), Martins & Giaretta (2012), Magalhães et al. (2020)</i>
<i>P. palustris</i>	1	0.33–0.75	12–26	32.0–37.0	1.464	<i>Martins & Giaretta (2012)</i>
<i>P. pombali</i>	1	0.30–0.60	30–49	76.2–88.8	1.312–1.550	<i>Malagoli, Mângia & Haddad (2016)</i>
<i>P. redacta</i>	1	0.15–0.85	13–74	84.8–106.7	1.697–2.142	<i>Simões et al. (2020)</i>
<i>P. renalis</i>	1	0.15–0.46	13–30	61.5–86.1	0.689–1.033	<i>Santana et al. (2011)</i>
<i>P. rotundipalpebra</i>	1–24	0.04–0.33	4–32	78.1–130.4	1.125–1.453	<i>Martins & Giaretta (2013)</i>
<i>P. salvatori</i>	1	0.19–0.42	15–25	54.0–61.0	1.572–1.875	<i>Bastos et al. (2011), Magalhães et al. (2020)</i>
<i>P. sanctaritae</i>	1	0.20–0.90	31–94	102.3–142.4	0.950–1.290	<i>Cruz & Napoli (2010)</i>
<i>P. schirchi</i>	1–6	0.18–0.45	15–31	66.0–93.4	0.861–1.810	<i>Nascimento et al. (2019), Sichieri et al. (2021)</i>
<i>P. velhochico</i>	1	0.32–0.51	29–46	85.4–96.5	1,312.5	<i>Mângia et al. (2022)</i>
<i>P. vielliardi</i>	3–20	0.04–0.30	4–30	95.6–118.8	1.022–1.291	<i>Martins & Giaretta (2011)</i>

P. concavitympanum, *P. cristiceps*, *P. goyana*, *P. korekore*, *P. redacta*, *P. rotundipalpebra*, *P. sanctaritae*, *P. velhochico*, and *P. vielliardi* by its lower pulse emission rate. The advertisement call of *P. paviotii* differs from those of *P. brauni*, *P. minuta*, *P. moratoi*, *P. palustris*, *P. pombali*, *P. redacta*, *P. salvatori*, and *P. velhochico* by its lower peak of frequency. The advertisement call of *P. paviotii* differs from those of *P. appendiculata* and

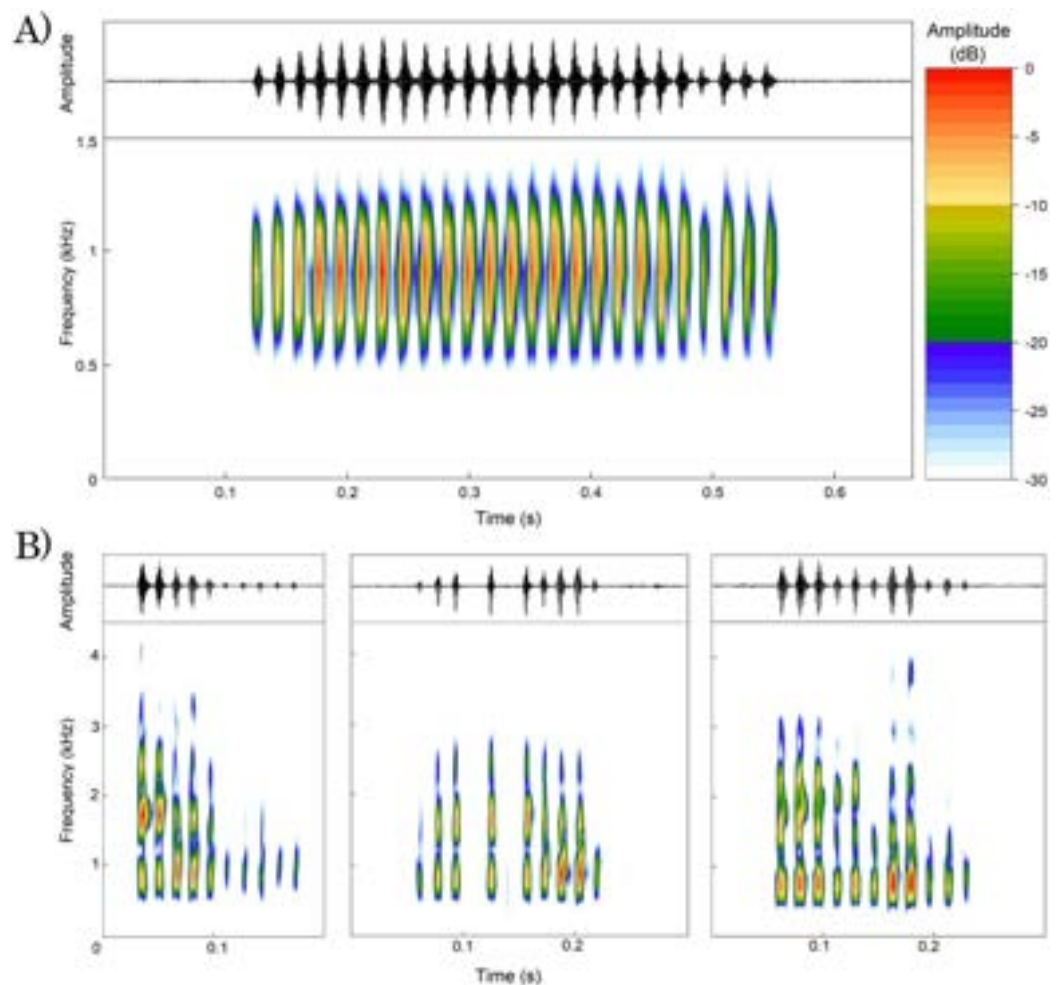


Figure 4 Oscillogram and spectrogram of *Proceratophrys paviotii* recorded at Santa Teresa, State of Espírito Santo, Southeastern Brazil. (A) Advertisement call; and (B) three release calls showing different patterns of amplitude and frequency modulation. The scale of amplitudes is decibels (dB), where colors represent different amplitude levels. The scale ranges from 0 to 30 dB, with warm colors indicating lower amplitudes and cool colors indicating higher amplitudes. The colors are distributed as follows: Red for 0 dB, Orange for 5 dB, Yellow for 10 dB, Light Green for 15 dB, Dark Blue for 20 dB, Light Blue for 25 dB, and White for 30 dB.

Full-size [DOI: 10.7717/peerj.17990/fig-4](https://doi.org/10.7717/peerj.17990/fig-4)

Table 4 Intra- and interindividual coefficients of variation of the advertisement calls of *Proceratophrys paviotii* from the Municipality of Santa Teresa, State of Espírito Santo, Southeast Brazil. *Static variables.

Acoustic signal	CVintra	CVinter	CVinter/CVintra
Note duration	9.3	14.6	1.5
Peak frequency	1.6*	3.8*	2.3
5% frequency	2.8*	5.9*	2.1
95% frequency	3.9*	5.4*	1.4
Pulses/call	8.6	16.8	2.0
Pulse rate	2.1*	9.7	4.4

P. moehringi by its higher peak of frequency. For detailed comparison and literature sources, see [Table 3](#).

DISCUSSION

Data gathered by citizen scientists were crucial in discovering new populations of *Proceratophrys paviotii*, enabling valuable systematic evaluations. Our phylogenetic analysis grouped *Proceratophrys paviotii* with *P. cururu*, *P. renalis*, and *P. laticeps* (PP = 0.91) in a clade, and *P. appendiculata*, *P. belzebul*, and *P. tupinamba* in a sister clade (PP = 1.00). Sequence divergence ranged from 2.2% to 9.1%. Advertisement calls consisted of single-note calls lasting 0.26–0.58 s with 17–41 pulses emitted at a rate of 54.19–77.49 pulses/s, and peak of frequency at 775.19–947.46 Hz. Additionally, the analysis of 55 release calls from a single male showed durations of 0.04–0.43 s with 2–13 pulses emitted at a rate of 21.17–81.58 pulses/s, and peak of frequency at 689.1–1,722.6 Hz. Variation analysis indicated dynamic traits.

The genus *Proceratophrys* currently encompasses 43 species with occurrences in Brazil, Argentina, Paraguay, and possibly in Bolivia ([Frost, 2024](#)). Historically, most *Proceratophrys* species have been arranged into four morphological groups, currently considered non-monophyletic: the *P. appendiculata* complex, the *P. bigibbosa* group, the *P. boiei* complex, and the *P. cristiceps* group ([Izecksohn, Cruz & Peixoto, 1998](#); [Giaretta, Bernarde & Kokubum, 2000](#); [Kwet & Faivovich, 2001](#); [Prado & Pombal, 2008](#)). We did not recover the monophyly of these phenotypic arrangements in accordance with previous studies ([Mângia et al., 2020](#); [Santana et al., 2021a, 2021b](#); [Mângia et al., 2022](#)).

Among anurans, genetic distance of 3% (16S rDNA) between taxa is commonly used as threshold to consider a lineage as candidate species ([Fouquet et al., 2007](#)) (*i.e.*, genetic distance below this threshold is commonly observed intraspecifically). Among *Proceratophrys* spp., four species showed genetic distance below this threshold compared to *P. paviotii*: *P. appendiculata*, *P. cururu*, *P. laticeps*, and *P. tupinamba*. In spite of this low genetic distance, we reinforce that *P. paviotii* can be easily diagnosed from these species based on the following combination of characters: presence of palpebral appendages and lack of rostral appendage ([Prado & Pombal, 2008](#)). In addition, other *Proceratophrys* were proven to be morphological, genetic, and geographically distinguished, and have been previously described or validated with values lower than 3% of genetic distance ([Dias et al., 2013](#); [Magalhães et al., 2020](#); [Santana et al., 2021a](#)).

Bioacoustic characters have commonly shown to be relevant in diagnosing species of *Proceratophrys* (*e.g.*, [Cruz & Napoli, 2010](#); [Martins & Giaretta, 2011](#); [Godinho et al., 2013](#); [Mângia et al., 2014, 2018, 2022](#)). However, 12 species of *Proceratophrys* still lack call description. Among the 31 species of *Proceratophrys* with described vocalization, *P. paviotii* stands out due to its notably subsampling. The description provided by [Cruz, Prado & Izecksohn \(2005\)](#) is based on seven calls emitted by a single male. Such a sample limitation certainly makes taxonomic comparisons questionable because it does not properly encompass intra-specific variation. Herein we increased samples of both the number of analyzed calls (seven calls in [Cruz, Prado & Izecksohn \(2005\)](#) and 107 in the present study) and recorded males (one in [Cruz, Prado & Izecksohn \(2005\)](#) and 13 in the

present study). Nevertheless, the advertisement call of *P. paviotii* distinguishes it from 22 out of 30 *Proceratophrys* spp. (Table 3). Given that the advertisement call of *P. paviotii* differentiates it from the majority of species within the genus based on various parameters, such as note duration, pulse emission rate per call, and dominant frequency, we reinforce the importance of using bioacoustics characters in diagnosing *Proceratophrys* species.

Spectral parameters and pulse rate are commonly considered static in amphibians while temporal parameters are classified as dynamic (Gerhardt, 1991; Bee et al., 2001). Similar pattern was observed for *Proceratophrys paviotii*. The ratio between CV_{inter} and CV_{intra} observed indicates that there is greater variation between males than the individual variation itself, as also observed in other species of anurans (e.g., Morais et al., 2012; Forti, Lingnau & Bertoluci, 2017). Furthermore, all advertisement call properties examined in this study exhibited greater variability inter-individuals than intra-individuals, suggesting the possibility of individual recognition (Bee et al., 2001; Pettitt, Bourne & Bee, 2013; Moser et al., 2022).

A taxon is classified as Near Threatened (NT) when is close to or likely to qualifying for a threatened category in the near future (IUCN, 2024). However, this does not appear to apply to *P. paviotii*. In several studies conducted across its distribution range in the central and northern parts of the State of Espírito Santo (Fig. 1) (Cruz, Prado & Izecksohn, 2005; Prado & Pombal, 2008; Almeida, Gasparini & Peloso, 2011; Peres & Simon, 2011; Zocca, Tonini & Ferreira, 2014; Silva et al., 2018; Ferreira et al., 2019a, 2019b), the species has been consistently observed within protected areas (Cruz, Prado & Izecksohn, 2005), as well as in disturbed areas such as coffee plantations (see Prado & Pombal (2008) comment on Teixeira & Coutinho (2002)), and even urban areas (present study). Thus, our results suggest that *Proceratophrys paviotii* should be classified as Least Concern regarding its conservation status in future evaluations. Additionally, we emphasize that, despite suggesting the classification as Least Concern, assessing the species under the Green Status framework can aid in monitoring the effectiveness of the conservation actions presented herein. This methodology aids in tracking how close the species is to being “Fully Recovered” and in devising more efficient conservation strategies (IUCN, 2024).

The *Cantoria de Quintal* CS project stands as a pioneering initiative in Brazil, with specific focus on frog vocalizations. Although the project is limited to the State of Espírito Santo, it has managed to gather calls from more than 40 anuran species, including the Near Threatened *Proceratophrys paviotii*. Our study strongly supports the notion that Citizen Science approaches can yield invaluable information concerning species’ geographic distribution and taxonomy. The data obtained through this participatory approach have provided important insights for conservation efforts, underscoring the potential significance of involving the public in scientific research and conservation initiatives.

Despite the many new studies using Citizen Science, this approach for Neotropical amphibians is still incipient. The approach used in “Cantoria de Quintal” was paramount in discovering new individuals of *Proceratophrys paviotii*. The genus *Proceratophrys* includes opportunistic breeders that call only after heavy rains (Prado & Pombal, 2005; Godinho et al., 2013; Mângia et al., 2014; Malagoli, Mângia & Haddad, 2016), and

many species in the genus have been described based on limited type series due its rarity (e.g., Prado & Pomal, 2008; Mângia et al., 2014). Therefore, initiatives such as “Cantoria de Quintal,” which utilize a contributive Citizen Science approach, should be encouraged in future studies, as they have demonstrated their effectiveness in targeting rare or explosively breeding species.

CONCLUSIONS

We conducted the first molecular analysis to determine the phylogenetic position and species delimitation of the Near Threatened *Proceratophrys paviotii*. Our findings did not support the monophyly of phenotypic arrangements within *Proceratophrys*, consistent with previous studies. The 16S tree confirmed *P. paviotii* as a valid species, grouping it with *P. cururu*, *P. renalis*, and *P. laticeps* in a clade. The advertisement call of *P. paviotii* distinguishes it from 22 other species within the genus based on various parameters, highlighting the importance of bioacoustic characters in diagnosing *Proceratophrys* species.

Although Near Threatened species are close to qualifying for higher risk categories, *P. paviotii* has been observed in both protected and disturbed areas, including coffee plantations and urban regions. Our results suggest that *P. paviotii* should be classified as Least Concern in future conservation assessments. We also emphasize the value of Citizen Science in gathering information on species distribution, taxonomy, and conservation, contributing to the scientific knowledge and conservation of *Proceratophrys paviotii*.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- João Victor Andrade Lacerda conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Diego J. Santana conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Carla Guimarães conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Alice Zanoni dos Santos performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Alan P. Araujo conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Natalia Pirani Ghilardi-Lopes conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
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DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The sequences generated from this study are available at GenBank: PP442191–PP442193 and in the [Supplemental Files](#).

Existing sequences used are available at GenBank: DQ283097, FJ685684, FJ685685, JX564880, KP295642, FJ685686, FJ685687, FJ685688, AY843704, FJ685694, KF214151, KF214152, KX858852, KX858853, KX858854, DQ283039, FJ685691, KP295643, KF214154, KF214155, KF214156, FJ685692, MG798659, MG798660, JN814630, JN814653, JN814662, JN814592, JN814620, JN814648, JN814586, JN814612, JN814660, KU495472, KX858855, FJ685695, MF953400, MF953401, FJ685696, KU495477, KU495478, FJ685697, KU495479, FJ685699, KF214142, KF214147, KF214157, KU495483, MW916089, MW916090, MW916088, MW889930, MW889928, MW889929, FJ685698, KF214143, KF214140, KF214149, JX982965, JX982966, FJ685689, MT196403, KF214144, KF214148, JX982967, JX982968, FJ685700, JN814584, MT196397, MT196399, FJ685701, KU495473, LTJV180, MHO117, MHO290, MHO309, KF214158, KF214159, KF214160, MT537176, MT537177, FJ685682.

Data Availability

The following information was supplied regarding data availability:

The audio files are available at [Fonoteca Neotropical Jacques Vieliard \(FNJV\)](#), search only using the number, not including the letters: FNJV_0060377, FNJV_0060378, FNJV_0060379, FNJV_0060380, FNJV_0060381, FNJV_0060382, FNJV_0060383, FNJV_0060384, FNJV_0060385, FNJV_0060386, FNJV_0060387, FNJV_0060388, FNJV_0060389, FNJV_0060390.

Supplemental Information

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REFERENCES

- Almeida AP, Gasparini JL, Peloso PLV. 2011. Frogs of the state of Espírito Santo, Southeastern Brazil—the need for looking at the “coldspots”. *Check List* 7(4):542–560 DOI 10.15560/7.4.542.
- Antúnez-Fonseca C, Juárez-Peña C, Sosa-Bartuano Á, Alvarado-Larios R, Sánchez-Trejo L, Vega-Rodríguez H. 2021. First records in El Salvador and new distribution records in Honduras for *Eleutherodactylus planirostris* Cope, 1862 (Anura, Eleutherodactylidae), with comments on its dispersal and natural history. *Caribbean Journal of Science* 51(1):37–43 DOI 10.18475/cjos.v51i1.a5.
- Araújo AP, Lacerda JVA, Zocca C, Ferreira RB. 2021. Vocal repertoire of the horned frog *Proceratophrys laticeps* (Amphibia: Anura: Odontophrynidae). *Zootaxa* 5032(1):147–150 DOI 10.11646/zootaxa.5032.1.10.
- Ávila RW, Pansonato A, Strüssmann C. 2012. A new species of *Proceratophrys* (Anura: Cycloramphidae) from Midwestern Brazil. *Journal of Herpetology* 46(4):466–472 DOI 10.1670/11-038.

- Bastos RP, Signorelli L, Morais AR, Costa TB, Lima LP, Pombal JP. 2011.** Advertisement calls of three anuran species (Amphibia) from the Cerrado, Central Brazil. *South American Journal of Herpetology* **6**(2):67–72 DOI [10.2994/057.006.0204](https://doi.org/10.2994/057.006.0204).
- Bee MA, Kozich CE, Blackwell KJ, Gerhardt HC. 2001.** Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. *Ethology* **107**:65–84 DOI [10.1046/j.1439-0310.2001.00640.x](https://doi.org/10.1046/j.1439-0310.2001.00640.x).
- Bioacoustics Research Program. 2014.** Raven pro: interactive sound analysis software, version 1.5. Available at www.birds.cornell.edu/raven.
- Bonney R, Cooper CB, Dickinson J, Kelling S, Phillips T, Rosenberg KV, Shirk J. 2009.** Citizen science: a developing tool for expanding science knowledge and scientific literacy. *BioScience* **59**(11):977–984 DOI [10.1525/bio.2009.59.11.9](https://doi.org/10.1525/bio.2009.59.11.9).
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, Du Plessis L, Popinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu CH, Xie D, Zhang C, Stadler T, Drummond AJ. 2019.** BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLOS Computational Biology* **15**(4):e1006650 DOI [10.1371/journal.pcbi.1006650](https://doi.org/10.1371/journal.pcbi.1006650).
- Brasileiro CA, Martins IA, Jim J. 2008.** Amphibia, Anura, Cycloramphidae, *Odontophrynus moratoi*: distribution extension and advertisement call. *Check List* **4**(4):382 DOI [10.15560/4.4.382](https://doi.org/10.15560/4.4.382).
- Brown KS Jr, Freitas AVL. 2000.** Diversidade de *Lepidoptera* em Santa Teresa, Espírito Santo. *Boletim do Museu de Biologia Mello Leitão* **12**:71–118.
- Campanaro A, Hardersen S, De Zan LR, Antonini G, Bardiani M, Maura M, Maurizi E, Mosconi F, Zauli A, Bologna MA, Roversi PF, Peverieri GS, Mason F. 2017.** Analyses of occurrence data of protected insect species collected by citizens in Italy. *Nature Conservation* **20**:265–297 DOI [10.3897/natureconservation.20.12704](https://doi.org/10.3897/natureconservation.20.12704).
- Ceríaco LMP, Santos BS, Marques MP, Bauer AM, Tiutenko A. 2021.** Citizen Science meets specimens in old formalin filled jars: a new species of Banded Rubber Frog, genus *Phrynomantis* (Anura, Phrynomeridae) from Angola. *Alytes* **38**:18–48.
- Cosentino BJ, Marsh DM, Jones KS, Apodaca JJ, Bates C, Beach J, Beard KH, Becklin K, Bell JM, Crockett C, Fawson G, Fjelsted J, Forys EA, Genet KS, Grover M, Holmes J, Indeck K, Karraker NE, Kilpatrick ES, Langen TA, Mugel SG, Molina A, Vonesh JR, Weaver RJ, Willey A. 2014.** Citizen science reveals widespread negative effects of roads on amphibian distributions. *Biological Conservation* **180**(5):31–38 DOI [10.1016/j.biocon.2014.09.027](https://doi.org/10.1016/j.biocon.2014.09.027).
- Cruz CAG, Napoli MF. 2010.** A new species of smooth frog, genus *Proceratophrys* Miranda-Ribeiro (Amphibia: Anura: Cycloramphidae), from the Atlantic Rainforest of Eastern Bahia, Brazil. *Zootaxa* **2660**:57–67 DOI [10.11646/zootaxa.2660.1.5](https://doi.org/10.11646/zootaxa.2660.1.5).
- Cruz CAG, Prado GM, Izecksohn E. 2005.** Nova espécie de *Proceratophrys* Miranda-Ribeiro, 1920 do sudeste do Brasil (Amphibia, Anura, Leptodactylidae). *Arquivos do Museu Nacional do Rio de Janeiro* **63**:289–295.
- Cutajar TP, Portway CD, Gillard GL, Rowley JJJ. 2022.** Australian frog atlas: species' distribution maps informed by the FrogID dataset. *Technical Reports of the Australian Museum Online* **36**:1–48 DOI [10.3853/j.1835-4211.36.2022.1789](https://doi.org/10.3853/j.1835-4211.36.2022.1789).
- Darriba D, Taboada GL, Doallo R, Posada D. 2012.** JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**(8):772 DOI [10.1038/nmeth.2109](https://doi.org/10.1038/nmeth.2109).

- Deacon C, Govender S, Samways MJ. 2023.** Overcoming biases and identifying opportunities for citizen science to contribute more to global macroinvertebrate conservation. *Biodiversity and Conservation* **32(6)**:1789–1806 DOI [10.1007/s10531-023-02595-x](https://doi.org/10.1007/s10531-023-02595-x).
- Dias PHS, Hepp F, Carvalho-e-Silva AMPT, Carvalho-e-Silva SP. 2013.** Breeding biology and advertisement call of the horned leaf-frog, *Proceratophrys appendiculata* (Amphibia: Anura: Odontophrynidae). *Zoologia* **30(4)**:388–396 DOI [10.1590/S1984-46702013000400004](https://doi.org/10.1590/S1984-46702013000400004).
- Duellman WE, Trueb L. 1994.** *Biology of Amphibians*. Baltimore and London: The Johns Hopkins University press.
- Eterovick PC, Sazima I. 1998.** New species of *Proceratophrys* (Anura: Leptodactylidae) from southeastern Brazil. *Copeia* **1998**:159–164.
- Faivovich J, Pinheiro PDP, Lyra ML, Pereyra MO, Baldo D, Muñoz-Saravia A, Reichle S, Brandão RA, Giaretta AA, Thomé MTC, Chaparro JC, Baêta D, Widholzer RL, Baldo JL, Lehr E, Wheeler WC, Garcia PCA, Haddad CFB. 2021.** Phylogenetic relationships of the *Boana pulchella* Group (Anura: Hylidae). *Molecular Phylogenetics and Evolution* **155**:106981 DOI [10.1016/j.ympev.2020.106981](https://doi.org/10.1016/j.ympev.2020.106981).
- Farquhar JE, Carlesso A, Pili A, Gale N, Chapple DG. 2023.** Capturing uncatalogued distribution records to improve conservation assessments of data-deficient species: a case study using the glossy grass skink. *Animal Conservation* **27(1)**:124–137 DOI [10.1111/acv.12892](https://doi.org/10.1111/acv.12892).
- Ferreira RB, Mônico AT, Cruz CAG, Guidorizzi CE, Zocca C, Canedo C, Ornellas IS, Oliveira JCF, Tonini JFR, Lacerda JVA, Toledo LF, Peloso PLV, Taucce PPG, Lourenço-de-Moraes R, Silva-Soares T, Verdade VK, Pertel W. 2019b.** Anfíbios ameaçados de extinção no estado do Espírito Santo. In: Fraga CN, Formigoni MH, Chaves FG, eds. *Fauna e flora ameaçadas de extinção no estado do Espírito Santo*. Santa Teresa, ES, Brazil: Instituto Nacional da Mata Atlântica, 256–269.
- Ferreira RB, Mônico AT, Silva ET, Lirio FCF, Zocca C, Mageski MM, Tonini JFR, Beard KH, Duca C, Silva-Soares T. 2019a.** Amphibians of Santa Teresa, Brazil: the hotspot further evaluated. *ZooKeys* **857**:139–162 DOI [10.3897/zookeys.857.30302](https://doi.org/10.3897/zookeys.857.30302).
- Ferreira RB, Zocca C, Carvalho SEC, Haddad CF, Santos MTT. 2023.** A new species of the bromeligenous genus *Crossodactylodes* (Anura: Leptodactylidae: Paratelmatobiinae) from Southeastern Brazil. *Journal of Herpetology* **57**:373–380 DOI [10.1670/23-030](https://doi.org/10.1670/23-030).
- Folly M, Condez TH, Vrcibradic D, Rocha CFD, Machado AS, Lopes RT, Pombal JP Jr. 2024.** A new species of *Brachycephalus* (Anura, Brachycephalidae) from the northern portion of the state of Rio de Janeiro, Southeastern Brazil. *Vertebrate Zoology* **74**:1–21 DOI [10.3897/vz.74.e103573](https://doi.org/10.3897/vz.74.e103573).
- Forti LR, Hepp F, de Souza JM, Protazio A, Szabo JK. 2022a.** Climate drives anuran breeding phenology in a continental perspective as revealed by citizen-collected data. *Diversity and Distributions* **28(10)**:2094–2109 DOI [10.1111/ddi.13610](https://doi.org/10.1111/ddi.13610).
- Forti LR, Lingnau R, Bertoluci J. 2017.** Acoustic variation in the advertisement call of the Lime treefrog *Sphaenorhynchus caramaschii* (Anura: Hylidae). *Vertebrate Zoology* **67(2)**:197–205 DOI [10.3897/vz.67.e31586](https://doi.org/10.3897/vz.67.e31586).
- Forti LR, Pontes MR, Augusto-Alves G, Martins A, Hepp F, Szabo JK. 2022b.** Data collected by citizen scientists reveal the role of climate and phylogeny on the frequency of shelter types used by frogs across the Americas. *Zoology* **155(11)**:126052 DOI [10.1016/j.zool.2022.126052](https://doi.org/10.1016/j.zool.2022.126052).
- Forti LR, Szabo JK. 2023.** The iNaturalist platform as a source of data to study amphibians in Brazil. *Anais da Academia Brasileira de Ciências* **95(1)**:e20220828 DOI [10.1590/0001-3765202320220828](https://doi.org/10.1590/0001-3765202320220828).

- Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ. 2007.** Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLOS ONE* **2(10)**:e1109 DOI [10.1371/journal.pone.0001109](https://doi.org/10.1371/journal.pone.0001109).
- Frost DR. 2024.** *Amphibian species of the world: an online reference. Version 6.2 (March 05 2024)*. New York, USA: American Museum of Natural History. Electronic Database accessible at. Available at <https://amphibiansoftheworld.amnh.org/index.php>.
- Gatti A, Segatto B, Carnelli CC, Moreira DO. 2014.** Mamíferos de médio e grande porte da Reserva Biológica Augusto Ruschi, Espírito Santo. *Natureza on Line* **12**:61–68.
- Gerhardt HC. 1991.** Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behavior* **42(4)**:615–635 DOI [10.1016/S0003-3472\(05\)80245-3](https://doi.org/10.1016/S0003-3472(05)80245-3).
- Giaretta AA, Bernarde PS, Kokubum MNC. 2000.** A new species of *Proceratophrys* (Anura: Leptodactylidae) from the Amazon rain forest. *Journal of Herpetology* **34**:173–178 DOI [10.2307/1565412](https://doi.org/10.2307/1565412).
- Glorioso BM, Vanbergen P, Pilgrim S, Villermin B, Vanbergen E, Comeaux-Villermin K, Wood KD. 2022.** A citizen science herpetofaunal inventory of palmetto island state park in Southwest Louisiana, USA. *Herpetological Conservation and Biology* **17**:122–130.
- Godinho LB, Moura MR, Lacerda JVA, Feio RN. 2013.** A new species of *Proceratophrys* (Anura: Odontophrynidae) from the middle São Francisco River, southeastern Brazil. *Salamandra* **49**:63–73.
- Heard J, Chen JP, Wen CKC. 2019.** Citizen science yields first records of *Hippocampus japapigu* and *Hippocampus denise* (Syngnathidae) from Taiwan: a hotspot for pygmy seahorse diversity. *ZooKeys* **2019**:83–90 DOI [10.3897/zookeys.883.39662](https://doi.org/10.3897/zookeys.883.39662).
- Heyer WR, Rand AS, Cruz CAG, Peixoto OL, Nelson CE. 1990.** Frogs of Boracéia. *Arquivos de Zoologia* **31**:231–410.
- Hortal J, De Bello F, Diniz-Filho JAF, Lewinsohn TM, Lobo JM, Ladle RJ. 2015.** Seven shortfalls that beset large-scale knowledge of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **46(1)**:523–549 DOI [10.1146/annurev-ecolsys-112414-054400](https://doi.org/10.1146/annurev-ecolsys-112414-054400).
- Irga PJ, Barker K, Torpy FR. 2018.** Conservation mycology in Australia and the potential role of citizen science. *Conservation Biology* **32(5)**:1031–1037 DOI [10.1111/cobi.13121](https://doi.org/10.1111/cobi.13121).
- IUCN. 2024.** The IUCN Red List of Threatened Species. Version 2023-1. Available at <https://www.iucnredlist.org> (accessed 5 March 2024).
- Izecksohn E, Cruz CAG, Peixoto OL. 1998.** Sobre *Proceratophrys appendiculata* e algumas espécies afins (Amphibia; Anura; Leptodactylidae). *Revista Universidade Rural, Série Ciências da Vida* **20**:37–54.
- Jared CAGS, Grego KF, Antoniazzi MM, Sant'Anna SS, Santos SMA, Mattaraia VGM. 2023.** Anfíbios e Serpentes. In: Mattaraia VGM, cord, Viana AAB, De Angelis K, Braga LMGM, eds. *Guia brasileiro de produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica/Concea*. Brasília: Ministério da Ciência, Tecnologia e Inovação, 460–521.
- Katoh K, Toh H. 2008.** Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9(4)**:286–298 DOI [10.1093/bib/bbn013](https://doi.org/10.1093/bib/bbn013).
- Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M. 2017.** The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* **4251(1)**:1–124 DOI [10.11646/zootaxa.4251.1.1](https://doi.org/10.11646/zootaxa.4251.1.1).
- Krueger T, Robinson A, Bourke G, Fleischmann A. 2023.** Small leaves, big diversity: citizen science and taxonomic revision triples species number in the carnivorous *Drosera microphylla* complex (D. Section Ergaleium, Droseraceae). *Biology* **12(1)**:141 DOI [10.3390/biology12010141](https://doi.org/10.3390/biology12010141).

- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6):1547–1549 DOI 10.1093/molbev/msy096.
- Kwet A, Faivovich J. 2001. *Proceratophrys bigibbosa* species group (Anura: Leptodactylidae), with description of a new species. *Copeia* 2001:203–215 DOI 10.1643/0045-8511(2001)001[0203:PBSGAL]2.0.CO;2.
- Kwet A, Baldo D. 2003. Advertisement call of the leptodactylid frog *Proceratophrys avelinoi*. *Amphibia Reptilia* 24:104–107.
- Lacerda JVA, Ferreira RB, Araujo-Vieira K, Zocca C, Lourenço ACC. 2021. A new species of *Scinax* Wagler (Amphibia, Anura, Hylidae) from the Atlantic Forest, Southeastern Brazil. *Ichthyology and Herpetology* 109(2):522–536 DOI 10.1643/h2020091.
- Lacerda JVA, Koffler S, Gonzalez JD, Monteiro B, Kawabe LDA, Mendes M, Bravo P, Ghilardi-Lopes NP. 2023. Many possibilities for students as citizen scientists: a supplement to Forti (2023). *Biodiversity* 24(4):181–185 DOI 10.1080/14888386.2023.2256712.
- Lacerda JVA, Santos AZ, Lima IMS. 2023. *Coaxos de ciência e educação*. Brasil: Instituto Nacional da Mata Atlântica.
- Lee TS, Kahal NL, Kinas HL, Randall LA, Baker TM, Carney VA, Kendell K, Sanderson K, Duke D. 2021. Advancing amphibian conservation through Citizen science in urban municipalities. *Diversity* 13:211 DOI 10.3390/d13050211.
- Leigh JW, Bryant D. 2015. POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6(9):1110–1116 DOI 10.1111/2041-210X.12410.
- Liu G, Kingsford RT, Callaghan CT, Rowley JLL. 2022. Anthropogenic habitat modification alters calling phenology of frogs. *Global Change Biology* 28(21):6194–6208 DOI 10.1111/gcb.16367.
- Luedtke JA, Chanson J, Neam K, Hobin L, Maciel AO, Catenazzi A, Borzée A, Hamidy A, Aowphol A, Jean A, Sosa-Bartuano Á, Fong GA, de Silva A, Fouquet A, Angulo A, Kidov AA, Muñoz SA, Diesmos AC, Tominaga A, Shrestha B, Gratwicke B, Tjaturadi B, Martínez RCC, Vásquez ACR, Señaris C, Chandramouli SR, Strüssmann C, Cortez FCF, Azat C, Hoskin CJ, Hilton-Taylor C, Whyte DL, Gower DJ, Olson DH, Cisneros-Heredia DF, Santana DJ, Nagombi E, Najafi-Majd E, Quah ESH, Bolaños F, Xie F, Brusquetti F, Álvarez FS, Andreone F, Glaw F, Castañeda FE, Kraus F, Parra-Olea G, Chaves G, Medina-Rangel GF, González-Durán G, Ortega-Andrade HM, Machado IF, Das I, Dias IR, Urbina-Cardona JN, Crnobrnja-Isailović J, Yang JH, Jianping J, Wangyal JT, Rowley JLL, Measey J, Vasudevan K, Chan KO, Gururaja KV, Ovaska K, Warr LC, Canseco-Márquez L, Toledo LF, Díaz LM, Khan MMH, Meegaskumbura M, Acevedo ME, Napoli MF, Ponce MA, Vaira M, Lampo M, Yáñez-Muñoz MH, Scherz MD, Rödel MO, Matsui M, Fildor M, Kusrini MD, Ahmed MF, Rais M, Kouamé NGG, García N, Gonwouo NL, Burrowes PA, Imbun PY, Wagner P, Kok PJR, Joglar RL, Auguste RJ, Brandão RA, Ibáñez R, von May R, Hedges SB, Biju SD, Ganesh SR, Wren S, Das S, Flechas SV, Ashpole SL, Robleto-Hernández SJ, Loader SP, Incháustegui SJ, Garg S, Phimmachak S, Richards SJ, Slimani T, Osborne-Naikatini T, Abreu-Jardim TPF, Condez TH, De Carvalho TR, Cutajar TP, Pierson TW, Nguyen TQ, Kaya U, Yuan Z, Long B, Langhammer P, Stuart SN. 2023. Ongoing declines for the world's amphibians in the face of emerging threats. *Nature* 622(7982):308–314 DOI 10.1038/s41586-023-06578-4.
- Magalhães FM, Brandão RA, Garda AA, Mângia S. 2020. Revisiting the generic position and acoustic diagnosis of *Odontophrynus salvatori* (Anura: Odontophrynidae). *Herpetological Journal* 30(4):189–196 DOI 10.33256/hj30.4.189196.

- Mângia S, Koroiva R, Nunes PMS, Roberto IJ, Ávila RW, Sant'Anna AC, Santana DJ, Garda AA. 2018.** A new species of *Proceratophrys* (Amphibia: Anura: Odontophrynidae) from the Araripe Plateau, Ceará State, Northeastern Brazil. *Herpetologica* 74(3):255–268 DOI 10.1655/Herpetologica-D-16-00084.1.
- Mângia S, Magalhães FM, Leite FSF, Cavalheri DG, Garda AA. 2022.** A new species of *Proceratophrys* (Anura: Odontophrynidae) from Boqueirão da Onça, Northern Bahia State, Brazil. *Journal of Herpetology* 56(1):120–136 DOI 10.1670/20-070.
- Mângia S, Oliveira EF, Santana DJ, Koroiva R, Paiva F, Garda AA. 2020.** Revising the taxonomy of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura: Odontophrynidae) from the Brazilian semiarid Caatinga: morphology, calls and molecules support a single widespread species. *Journal of Zoological Systematics and Evolutionary Research* 58(4):1151–1172 DOI 10.1111/jzs.12365.
- Mângia S, Santana DJ, Cruz CAG, Feio RN. 2014.** Taxonomic review of *Proceratophrys melanopogon* (Miranda-Ribeiro, 1926) with description of four new species (Amphibia, Anura, Odontophrynidae). *Boletim do Museu Nacional* 531:1–33.
- Mângia S, Santana DJ, Feio RN. 2010.** Advertisement call of the cycloramphid toad *Proceratophrys melanopogon* (Miranda-Ribeiro, 1926). *South American Journal of Herpetology* 5(2):127–131 DOI 10.2994/057.005.0206.
- Malagoli LR, Mângia S, Haddad CFB. 2016.** The advertisement call of *Proceratophrys pombali* (Amphibia: Anura: Odontophrynidae) with comments on its distribution and natural history. *South American Journal of Herpetology* 11(1):18–24 DOI 10.2994/SAJH-D-15-00022.1.
- Martins LB, Giarretta AA. 2011.** A new species of *Proceratophrys* Miranda-Ribeiro (Amphibia: Anura: Cycloramphidae) from central Brazil. *Zootaxa* 50(1):41–50 DOI 10.11646/zootaxa.2880.1.4.
- Martins LB, Giarretta AA. 2012.** Advertisement calls of two species of *Proceratophrys* (Anura: Odontophrynidae) from Minas Gerais, Brazil, with comments on their distribution, taxonomy and conservation status. *South American Journal of Herpetology* 7(3):203–212 DOI 10.2994/057.007.0302.
- Martins LB, Giarretta AA. 2013.** Morphological and acoustic characterization of *Proceratophrys goyana* (Lissamphibia: Anura: Odontophrynidae), with the description of a sympatric and related new species. *Zootaxa* 3750(4):301–320 DOI 10.11646/zootaxa.3750.4.1.
- Martins LB, Giarretta AA. 2021.** The advertisement call of *Proceratophrys laticeps* Izecksohn & Peixoto, 1981 (Anura: Odontophrynidae). *Zootaxa* 5032(3):442–446 DOI 10.11646/zootaxa.5032.3.9.
- Morais AR, Batista VG, Gambale PG, Signorelli L, Bastos RP. 2012.** Acoustic communication in a Neotropical frog (*Dendropsophus minutus*): vocal repertoire, variability and individual discrimination. *Herpetological Journal* 22:249–257.
- Mori E, Menchetti M. 2014.** “Sometimes they come back”: citizen science reveals the presence of the Italian red squirrel in Campania. *Quaderni del museo civico di storia naturale di Ferrara* 2:91–94.
- Moser CF, Schuck LK, Olmedo GM, Lingnau R. 2022.** Individual variation in the advertisement call of *Aplastodiscus albosignatus* (Anura: Hylidae) is correlated with body size and environmental temperature. *Zoologia* 39:e21008 DOI 10.1590/S1984-4689.v39.e21008.
- Nascimento JS, Abreu RO, Menezes L, Trevisan CC, Solé M, Juncá FA, Napoli MF. 2019.** The advertisement call of *Proceratophrys minuta* Napoli, Cruz, Abreu, and Del Grande, 2011 (Anura: Odontophrynidae), with comments on acoustic parameters in the genus. *South American Journal of Herpetology* 14(1):24–36 DOI 10.2994/SAJH-D-17-00021.1.

- Novaes TD, Silva JN, Flores FM, Passamani JA, Magnago GR, Simon JE. 2016.** Comunidade de aves da Reserva Biológica Augusto Ruschi, município de Santa Teresa, Espírito Santo, Brasil. *Atualidades Ornitológicas On-Line* **194**:39–51.
- Nunes I, Juncá FA. 2006.** Canto de anúncio de três espécies da família Leptodactylidae no Estado da Bahia, Nordeste do Brasil (Amphibia, Anura, Leptodactylidae), com considerações sobre suas posições taxonômicas. *Arquivos do Museu Nacional* **64**:151–157.
- Nunes I, Loebmann D, Cruz CAG, Haddad CFB. 2015.** Advertisement call, colour variation, natural history, and geographic distribution of *Proceratophrys caramaschii* (Anura: Odontophrynidae). *Salamandra* **51**:103–110.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991.** *The simple fool's guide to PCR version 2*. Honolulu: University of Hawaii.
- Passamani M, Mendes SL, Chiarello AG. 2000.** Non-volant mammals of the Estação Biológica de Santa Lúcia and adjacent areas of Santa Teresa, Espírito Santo, Brazil. *Boletim do Museu de Biologia Mello Leitão* **12**:201–214.
- Pereira EA, Ceron K, Silva HR, Santana DJ. 2022.** The dispersal between Amazonia and Atlantic Forest during the Early Neogene revealed by the biogeography of the treefrog tribe Sphaenorhynchini (Anura, Hylidae). *Ecology and Evolution* **12**(4):e8754
DOI [10.1002/ece3.8754](https://doi.org/10.1002/ece3.8754).
- Peres J, Simon JE. 2011.** Geographic distribution: *Proceratophrys paviotii*. *Herpetological Review* **42**:108.
- Pettitt BA, Bourne GR, Bee MA. 2013.** Advertisement call variation in the golden rocket frog (*Anomaloglossus beebei*): evidence for individual distinctiveness. *Ethology* **119**(3):244–256
DOI [10.1111/eth.12058](https://doi.org/10.1111/eth.12058).
- Pirotta V, Reynolds W, Ross G, Jonsen I, Grech A, Slip D, Harcourt R. 2020.** A citizen science approach to long-term monitoring of humpback whales (*Megaptera novaeangliae*) off Sydney. *Australia Marine Mammal Science* **36**(2):472–485 DOI [10.1111/mms.12651](https://doi.org/10.1111/mms.12651).
- Pittman SE, Dorcas ME. 2006.** Catawaba river corridor coverboard program: a citizen science approach to amphibian and reptile inventory. *Journal of the North Carolina Academy of Science* **122**:142–151.
- Prado GM, Pombal JP Jr. 2005.** Distribuição espacial e temporal dos anuros em um brejo da Reserva Biológica de Duas Bocas, Sudeste do Brasil. *Arquivos do Museu Nacional* **63**:685–705.
- Prado GM, Pombal JP Jr. 2008.** Espécies de *Proceratophrys* Miranda-Ribeiro, 1920 com apêndices palpebrais (Anura; Cycloramphidae). *Arquivos de Zoologia* **39**(1):1–85
DOI [10.11606/issn.2176-7793.v39i1p1-85](https://doi.org/10.11606/issn.2176-7793.v39i1p1-85).
- Price SJ, Dorcas ME. 2011.** The Carolina Herp Atlas: an online, citizen-science approach to document amphibian and reptile occurrences. *Herpetological Conservation and Biology* **6**:287–296.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018.** Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**(5):901–904
DOI [10.1093/sysbio/syy032](https://doi.org/10.1093/sysbio/syy032).
- R Core Team. 2017.** *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at <http://www.R-project.org/>.
- Rowley JLL, Callaghan CT. 2022.** Tracking the spread of the eastern dwarf tree frog (*Litoria fallax*) in Australia using citizen science. *Australian Journal of Zoology* **70**(6):204–210
DOI [10.1071/zo23012](https://doi.org/10.1071/zo23012).

- Rowley JJJ, Callaghan CT, Cornwell WK. 2020. Widespread short-term persistence of frog species after the 2019–2020 bushfires in eastern Australia revealed by citizen science. *Conservation Science and Practice* 2(11):1–8 DOI 10.1111/csp2.287.
- Rowley JJJ, Callaghan CT, Cutajar T, Portway C, Potter K, Mahony S, Trembath DF, Flemons P, Woods A. 2019. FrogID: citizen scientists provide validated biodiversity data on frogs of Australia. *Herpetological Conservation and Biology* 14:155–170.
- Santana DJ, de São-Pedro VA, Bernarde PS, Feio RN. 2010. Descrição do canto de anúncio e dimorfismo sexual em *Proceratophrys concavitympanum* Giaretta, Bernarde & Kokubum, 2000. *Papéis Avulsos de Zoologia* 50(11):167–174 DOI 10.1590/s0031-10492010001100001.
- Santana DJ, Mângia S, da Silva Alves Saccol S, dos Santos TG. 2021a. A new species of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura, Odontophrynidae) of the *P. bigibbosa* species group from Southern Brazil. *Vertebrate Zoology* 71:387–401 DOI 10.3897/VZ.71.E67894.
- Santana DJ, Rodrigues R, De Albuquerque RL, Laranjeiras DO, Protázio ADS, França FGR, Mesquita DO. 2011. The advertisement call of *Proceratophrys renalis* (Miranda-Ribeiro, 1920) (Amphibia: Anura: Cycloramphidae). *Zootaxa* 68(1):67–68 DOI 10.11646/zootaxa.2809.1.6.
- Santana DJ, Silva LAd, Sant’Anna AC, Shepard DB, Mângia S. 2021b. A new species of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura, Odontophrynidae) from Southern Amazonia, Brazil. *PeerJ* 9:e12012 DOI 10.7717/peerj.12012.
- Sichieri G, Cruz CAG, Pimenta BVS, Nunes I. 2021. Advertisement call description of two *Proceratophrys* species (Anura: Odontophrynidae). *Zootaxa* 4975(2):397–400 DOI 10.11646/zootaxa.4975.2.10.
- Silva ET, Peixoto MAA, Leite FSF, Feio RN, Garcia PCA. 2018. Anuran distribution in a highly diverse region of the Atlantic Forest: the mantiqueira mountain range in Southeastern Brazil. *Herpetologica* 74(4):294–305 DOI 10.1655/0018-0831.294.
- Simões CRMA, Pontes BES, Trevisan CC, Abreu RO, Juncá FA, Solé M, Araújo CB, Napoli MF. 2020. The advertisement call of *Proceratophrys redacta* (Anura, Odontophrynidae). *Zootaxa* 4750(3):447–450 DOI 10.11646/zootaxa.4750.3.14.
- Sterrett SC, Katz RA, Fields WR, Campbell Grant EH. 2019. The contribution of road-based citizen science to the conservation of pond-breeding amphibians. *Journal of Applied Ecology* 56(4):988–995 DOI 10.1111/1365-2664.13330.
- Teixeira RL, Coutinho ES. 2002. Hábito alimentar de *Proceratophrys boiei* (Wied) (Amphibia, Anura, Leptodactylidae) em Santa Teresa, Espírito Santo, sudeste do Brasil. *Boletim do Museu de Biologia Mello Leitão* 14:13–20.
- Thomaz L, Monteiro R. 1997. Composição florística da Mata Atlântica de encosta da Estação Biológica de Santa Lúcia, município de Santa Teresa-ES. *Boletim do Museu de Biologia Mello Leitão* 7:3–48.
- Thompson MM, Rowley JJJ, Poore AGB, Callaghan CT. 2022. Citizen science reveals meteorological determinants of frog calling at a continental scale. *Diversity and Distributions* 28(11):2375–2387 DOI 10.1111/ddi.13634.
- Wells KD. 2007. *The ecology and behavior of amphibians*. Chicago: University of Chicago press.
- Wendt T, Coser TS, Fernandes HB, Martinelli G. 2010. Bromeliaceae do município de Santa Teresa, Espírito Santo: lista de espécies, distribuição, conservação e comentários taxonômicos. *Boletim do Museu de Biologia Mello Leitão* 27:21–53.
- Westgate MJ, Scheele BC, Ikin K, Hofer AM, Beaty RM, Evans M, Osborne W, Hunter D, Rayner L, Driscoll DA. 2015. Citizen science program shows urban areas have lower occurrence of frog species, but not accelerated declines. *PLOS ONE* 10(11):e0140973 DOI 10.1371/journal.pone.0140973.

Weygoldt P, Peixoto OL. 1985. A new species of horned toad (*Proceratophrys*) from Espírito Santo, Brazil (Amphibia: Salientia: Leptodactylidae). *Senckenbergiana-Biologica* **66**:1–8.

Zocca C, Tonini JFR, Ferreira RB. 2014. Uso do espaço por anuros em ambiente urbano de Santa Teresa, Espírito Santo. *Boletim do Museu de Biologia Mello Leitão* **35**:105–117.