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RESEARCH PAPER

New species of *Rupirana* (Heyer, 1999) (Anura, Leptodactylidae) from Serra do Assuruá, center-north of Bahia state, Brazil

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Abstract. The Caatinga, a semi-arid region with drought-adapted vegetation, supports unique biodiversity, including numerous endemic species. Serra do Assuruá in Bahia is one such high-altitude area, rich in ecological niches but largely understudied, especially regarding amphibians, and lacks conservation units. During fieldwork, we discovered a new *Rupirana* species approximately 200 km beyond its known range. Previously, *Rupirana* had remained monotypic for over two decades, restricted to the *campo rupestre* ecosystem in the northern mountain ranges of Chapada Diamantina, Bahia. This unexpected finding in Serra do Assuruá prompted detailed morphological, acoustic, and molecular analyses, confirming it as a new endemic species for the region. While wind farm expansion in remote areas like the Caatinga has enabled important scientific discoveries, including this *Rupirana*, it also poses risks to biodiversity through habitat changes. These findings highlight the need for enhanced conservation planning in the Caatinga biome, as such discoveries underscore both the ecological value and the vulnerability of these fragile ecosystems. Therefore, balancing renewable energy development with conservation efforts through rigorous environmental assessments is essential to protect these fragile ecosystems.

Key words: amphibia, Caatinga, conservation, taxonomy

Introduction

The Caatinga, located in Northeastern Brazil, is characterised by a semi-arid climate with high solar radiation, low relative humidity, limited cloud cover, and erratic and infrequent rainfall (Prado 2003). Its landscape is dominated by deciduous xerophytic vegetation, including cacti, shrubs, and small trees, which thrive in the face of a severe water deficit due to intense and unpredictable dry seasons (Cole 1960, Ab'Sáber 1998). Despite its extreme environmental conditions, the Caatinga is home to a significant and diverse flora and fauna (da Silva et al. 2017). Such biota occupies a variety of altitudinal zones, where numerous endemic amphibian species are found (Garda et al. 2017).

The altitudinal areas of the Caatinga in Bahia include the Chapada Diamantina, which constitutes the northern portion of the Espinhaço mountain

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range, characterised mainly by the *campos rupestres* (rupestrian fields), the predominant formation above 1,000 m in elevation (Schaefer et al. 2016, Miola et al. 2021). These *campos rupestres* are defined by a mosaic of grassy-shrubby vegetation interspersed with rocky outcrops, creating unique ecological niches. Recently, other high-altitude areas within the Caatinga have gained attention due to their biological significance and rich fauna (Garda et al. 2017, Cavalheri et al. 2024). One of these areas is Serra do Assuruá, located in the municipality of Gentio do Ouro, within the Médio São Francisco and Ibipeba regions. This area reaches elevations of up to 1,200 m, with typical Caatinga vegetation growing on rocky outcrops, and is considered of extreme importance for biological conservation (Tabarelli & da Silva 2003). However, despite the recognised ecological value of this and other similar regions, studies on anuran species remain scarce. This lack of research has created significant gaps in our understanding of the biological diversity and broader ecological dynamics in these critical habitats.

There are notable biotic similarities between the high-altitude regions of Chapada Diamantina and Serra do Assuruá, although the latter remains relatively understudied. For instance, the lizard *Tropidurus pinima* is found in both regions, yet these populations are considered distinct lineages due to geographical isolation (Werneck et al. 2015). Similarly, lizards of the genus *Eurolophosaurus* inhabit both areas, suggesting a shared biogeographical history (Passoni et al. 2008). However, amphibian surveys have not yet been conducted in Serra do Assuruá, leaving open the possibility that this region may harbour sister lineages to those in Chapada Diamantina. Given the geographic and ecological similarities between these areas, further research is crucial to elucidate the evolutionary relationships and uncover the biodiversity of the Serra do Assuruá habitats may support.

The Serra do Assuruá exhibits a remarkable level of endemism and is distinguished by the frequent discovery of new species, particularly among arthropods (e.g. Botero-Trujillo et al. 2017, Esposito et al. 2017) and lizards (e.g. Siedchlag et al. 2010, Arias et al. 2018, Recoder & Rodrigues 2020). However, despite the recognition of its biological significance and its relatively well-preserved nature, with virtually untouched areas, no conservation units have been established to protect the unique and endangered fauna of this region. It is imperative to underscore that the amphibian fauna within this area remains largely unexplored.

Among the typical fauna of the high-altitude areas in Chapada Diamantina is *Rupirana* (Heyer, 1999), a monotypic genus restricted to the *campo rupestre* ecosystem within the northern mountain ranges of this region. The species *Rupirana cardosoi* has been recorded in the municipalities of Morro do Chapéu, Palmeiras, Lençóis, and Mucugê (the type locality) (Heyer 1999, Juncá 2005). However, recent research has revealed that the population in Morro do Chapéu represents a distinct lineage within the *Rupirana* genus (Santos et al. 2020a). This discovery highlights the 'sky island' effect in Chapada Diamantina, where populations become geographically isolated, driving speciation. Similar patterns of diversification have been documented in other amphibian species inhabiting this region (e.g. *Bokermannohyla* spp. – de Oliveira et al. 2021, *Pristimantis* spp. – Trevisan et al. 2020).

During field expeditions to sites in Serra do Assuruá, within the municipality of Gentio do Ouro, situated in the central-north region of Bahia state, we encountered individuals belonging to the genus *Rupirana*. The occurrence of a *Rupirana* population in an area approximately 200 km distant from its known distribution range, on a distinct mountain peak adjacent to the Dunas of São Francisco, raised questions about its taxonomy. To evaluate this, our research encompassed a comprehensive investigation of the collected individuals, integrating morphological, acoustic, and molecular data analyses. Here, we described this population as a new and endemic species of *Rupirana* for Serra do Assuruá.

Material and Methods

Study area

The municipality of Gentio do Ouro is located in the central-north region of Bahia, Brazil, within the Serra do Assuruá (11°25′57.88′′ S, 42°30′28.56′′ W, 1,075 m a.s.l.) (Fig. 1). The area is characterised by elevations around 1,000 m a.s.l., featuring arboreal and shrubby Caatinga vegetation, typically associated with rocky outcrops. The hydrography consists of intermittent and seasonal watercourses that flow only during the rainy season, gradually drying up afterwards. The climate is predominantly semi-arid (Aw type – Equatorial savannah with dry winter – according to the Koppen classification), with an average temperature of 26 °C and an annual precipitation of approximately 700 mm (Álvares et al. 2013). In the higher-altitude areas, human impact is limited, with the presence of cattle and goats, artificial ponds, and sparse human settlements

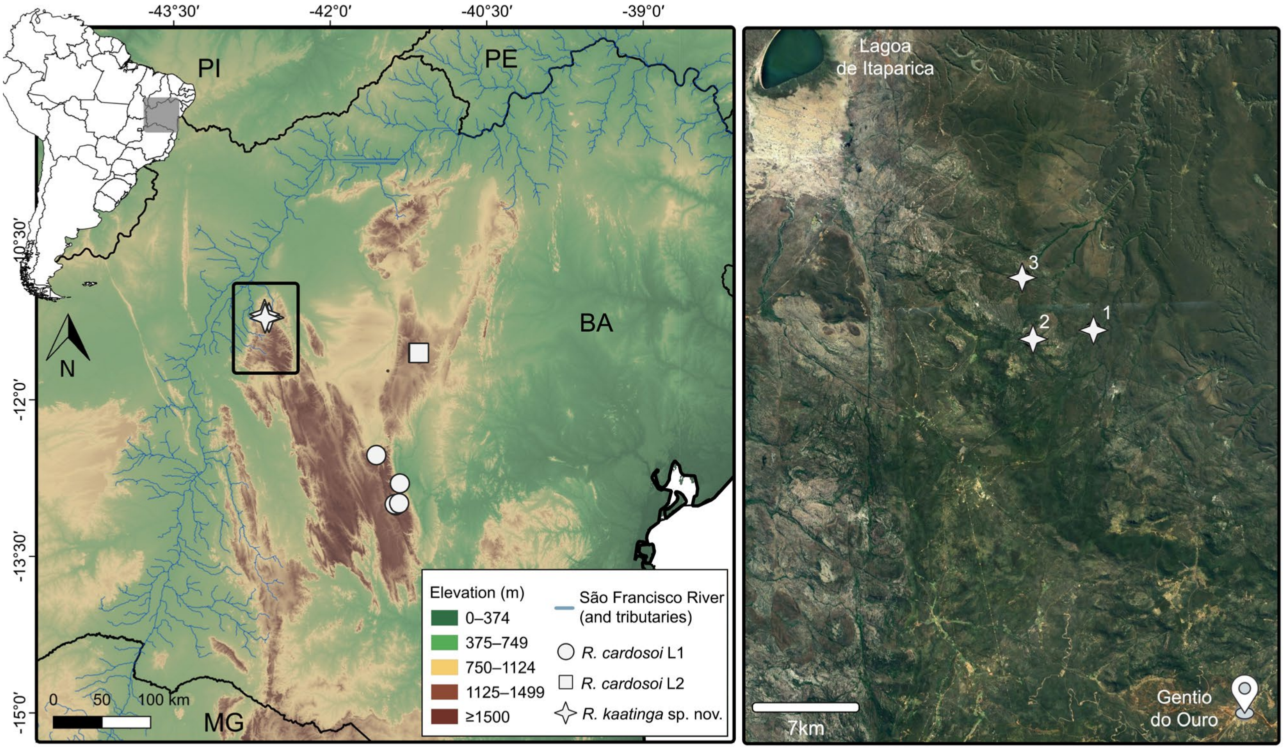

Fig. 1. Geographic distribution of *Rupirana kaatinga* sp. nov. from Serra do Assuruá, Gentio do Ouro municipality, Bahia state, Brazil.

observed in only a few locations. However, wind farms have recently surrounded the region, and new wind energy complexes are continuously expanding and developing.

Morphological and morphometric assessment

Specimens used in the description, and examined for comparisons, are housed in the following collections: Museu de Zoologia da Universidade Federal da Bahia (MZUFBA), Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS), Coleção Herpetológica da Universidade Federal da Paraíba (CHUFPB), and National Museum of Natural History, Division of Amphibians and Reptiles (UNMS). The analysed individuals originate from Brazil, specifically in the state of Bahia, with specimens collected in Andaraí (UFBA 16994-95, UFBA 17000-01, USNM 519755-58 (paratypes)), Lençóis (UFBA 17002-03), Mucugê (UFBA 16996-97), and Palmeiras (UFBA 16999, UFBA 17008-12, CHUFPB 24405, CHUFPB 24830, CHUFPB 24832, CHUFPB 24857, CHUFPB 26834, CHUFPB 26982, CHUFPB 27054, CHUFPB 27604).

We follow the terminology for morphological and morphometric characters of Heyer et al. (1990) and Heyer (1999). A single person (S. Mângia) took 14 measurements of 37 adult specimens of *R. cardosoi* (30 males, seven females) and five adult specimens of the new species (four males, one female), using a digital caliper (0.01 mm): snout-vent-length (SVL), head length (HL), head width (HW), interorbital distance (ID), tympanum diameter (TD), eye-nostril distance (END), internarial distance (IND), forearm length (FOL), hand length (HL), forearm + hand length (FOHL), thigh length (THL), tibia length (TL), foot length (FL), foot + tarsus length (FTL) (Table 1).

Acoustic analysis

We recorded the advertisement call of two males at Gentio do Ouro municipality, Bahia state, Brazil, by using a Sony Icd-px470 digital recorder. The male recorded on November 2021 (total of 40 calls) at 6:00 p.m. (11°10′43.81′′ S, 42°37′47.55′′ W, 868 m a.s.l.) was not collected. The male ZUFMS-AMP20347 (total of 40 calls) was recorded on 14 February 2022 at 10:00 a.m. (11°12′41.21′′ S, 42°35′16.96′′ W, 829 m a.s.l.).

We digitised recordings at 44.1 kHz with a resolution of 16 bits and analysed calls in Raven Pro 1.5 for Mac (Cornell Lab of Ornithology) with the following spectrogram settings: Hann window type, fast Fourier transform (FFT) window width – 256 samples, frame length – 100 samples, overlap – 50%, and discrete Fourier transform (DFT) size – 256 samples. All other settings followed the ‘default’ of Raven. We constructed audio spectrograms with the R package *Seewave* 1.7.3 (Sueur et al. 2008) in the R platform (ver. 3.6.1, R Development Core Team 2018) using

Table 1. Measurements (mm) of specimens of *Rupirana cardosoi* and *Rupirana kaatinga* sp. nov. Values are presented as mean ± SD (range).

	R. kaatinga sp. nov.		*R. cardosoi*	
	Males (n = 4)	Female (n = 1)	Males (n = 30)	Females (n = 7)
SVL	28.25 ± 1.67 (26.41-30.45)	29.13	28.55 ± 1.14 (26.02-30.35)	33.96 ± 1.19 (31.82-35.25)
HL	9.24 ± 0.24 (9.01-9.53)	10.15	9.61 ± 0.47 (8.78-10.56)	10.79 ± 0.61 (9.93-11.85)
HW	10.46 ± 0.51 (10.05-11.16)	10.19	10.17 ± 0.56 (9.06-11.31)	11.31 ± 0.75 (10.05-12.08)
ID	5.18 ± 0.3 (4.9-5.6)	5.56	5.08 ± 0.69 (3.25-5.84)	5.67 ± 1.06 (3.37-6.58)
TD	2.08 ± 0.22 (1.8-2.27)	2.26	2.11 ± 0.26 (1.66-2.70)	2.40 ± 0.17 (2.17-2.61)
END	3.02 ± 0.39 (2.67-3.43)	3.62	3.31 ± 0.39 (2.68-4.14)	3.56 ± 0.22 (3.29-3.99)
IND	2.41 ± 0.2 (2.19-2.68)	2.99	2.34 ± 0.24 (1.96-2.93)	2.55 ± 0.26 (2.32-3.08)
FOL	5.95 ± 0.16 (5.72-6.09)	6.09	5.93 ± 0.52 (4.77-6.79)	6.55 ± 0.40 (5.94-7.21)
HL	7.53 ± 0.67 (6.89-8.43)	7.35	7.39 ± 0.46 (6.43-8.15)	8.39 ± 0.50 (7.46-8.94)
FOHL	13.62 ± 0.31 (13.34-14.05)	14.28	13.78 ± 0.65 (12.66-15.12)	15.39 ± 0.64 (14.61-16.42)
THL	13.49 ± 0.46 (12.89-14.02)	13.90	14.25 ± 0.85 (11.93-15.51)	15.38 ± 0.76 (13.96-16.28)
TL	13.93 ± 0.47 (13.33-14.47)	13.75	14.61 ± 0.71 (13.20-16.28)	15.96 ± 0.78 (15.04-17.12)
FL	13.8 ± 0.44 (13.31-14.29)	14.54	15.10 ± 1.01 (13.29-16.77)	16.64 ± 0.99 (15.25-17.73)
FTL	19.74 ± 0.89 (18.89-20.79)	20.00	21.86 ± 1.03 (19.87-24.04)	23.73 ± 1.22 (21.94-25.51)

the following settings: Hanning window, 256 points resolution (FFT) and 70% of overlap. We analysed the following acoustic parameters of Juncá & Lugli (2009): call duration (sec), interval between notes (sec), number of pulses per note, pulse rate (pulses/sec), and dominant frequency (Hz). Call terminology follows Köhler et al. (2017).

Molecular data

We sequenced two adult individuals of the new species collected at the type locality and two additional individuals of *R. cardosoi* from Palmeiras municipality (Table 2). We targeted a fragment of the 16S ribosomal RNA to infer the new species' phylogenetic position. Whole genomic DNA was extracted from muscle or liver tissues using a Qiagen DNeasy 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. 2002). Reactions contained 10 µl of GoTaq (R) G2 Green Master Mix, 6 µl of H_2O and 1 µl of each primer and 2 µl of template DNA. 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, and 72 °C for 40 s, with a final extension phase of 72 °C for 5 min. Purification of PCR products and DNA sequencing were performed by Eurofins Genomics Inc. (Louisville, Kentucky, USA).

We compared the newly generated 16S sequences with all *Rupirana* sequences with compatible fragment region deposited in GenBank and *Crossodactylodes itambe* (MN610848) as outgroup. We aligned the 16S mtDNA gene fragments using the MAFFT

Table 2. Genbank accession numbers of all terminals used in the molecular section of this study.

Species	Municipality, State	Voucher	Genbank accession no.	Reference
Rupirana kaatinga sp. nov.	Gentio do Ouro, BA	ZUFMS-AMP20347	PV155509	This study
Rupirana kaatinga sp. nov.	Gentio do Ouro, BA	ZUFMS-AMP20349	PV155510	This study
Rupirana Lineage2	Morro do Chapéu, BA	MTR_22434	MN917601	Santos et al. 2020a
Rupirana Lineage2	Morro do Chapéu, BA	MTR_22497	MN917602	Santos et al. 2020a
Rupirana cardosoi	Mucugê, BA	MTR_19805	MN917596	Santos et al. 2020a
Rupirana cardosoi	Mucugê, BA	MTR_19924	MN917597	Santos et al. 2020a
Rupirana cardosoi	Mucugê, BA	UFMG_4379	MN917599	Santos et al. 2020a
Rupirana cardosoi	Mucugê, BA	UFMG_4606	MN917600	Santos et al. 2020a
Rupirana cardosoi	Mucugê, BA	FJ117/MZFS 2415	KC593361	Fouquet et al. 2013
Rupirana cardosoi	Mucugê, BA	UFMG_4274	MN917598	Santos et al. 2020a
Rupirana cardosoi	Mucugê, BA	JC_1112	MN917595	Santos et al. 2020a
Rupirana cardosoi	Palmeiras, BA	AAGARDA_6897	PV155508	This study
Rupirana cardosoi	Palmeiras, BA	AAGARDA_6918	PV155507	This study
Crossodactylodes itambe	Santo Antônio do Itambé, MG	UFMG_13381	MN610848	Santos et al. 2020b

algorithm (Katoh et al. 2002) in Geneious v 9.0.5 with default settings. The final dataset comprised 13 sequences of a 531 base pairs (bp) fragment of the 16S gene. All GenBank accession numbers and genetic vouchers used here are listed in Table 2. We ran a Bayesian Inference (BI) performed in BEAST v. 2.6.7 (Bouckaert et al. 2019) for 20 million generations, sampling every 2,000 steps using a Yule Process tree prior. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v. 1.7.1 (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 v. 2.6.3 (Bouckaert et al. 2019). Using this tree, we implemented a bPTP (Bayesian implementation of the Poisson tree processes) species delimitation analysis (Zhang et al. 2013), which the calculations were performed on the bPTP web server (http://species.h-its.org/ptp/), with 500,000 MCMC generations, thinning set at 100 and burn-in at 10%. In addition, we ran a Generalized Mixed Yule Coalescent (GMYC) for species delimitation (Pons et al. 2006, Fujisawa & Barraclough 2013) in the R v. 4.1.1 (R Development Core Team 2018) by using the package *splits* (Ezard et al. 2017). We also performed an Assemble Species by Automatic Partitioning (ASAP, Puillandre et al. 2021), which is a distance-based method. The ASAP delimitation was performed on the online server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb), considering a simple distance model to compute the distances between samples and default parameters. We kept the delimitation scheme supported by the lowest ASAP score (Puillandre et al. 2021). Lastly, we calculated sequence divergences (uncorrected p-distances) among individuals using MEGA v. 10.1.1 (Kumar et al. 2018). Finally, we estimated haplotype networks among species of *Rupirana* for the 16S mtDNA gene in POPART (Leigh & Bryant 2015) using the median-joining network method. We identified each species using different colours in the haplotype network.

Species description

Rupirana kaatinga sp. nov. (Figs. 2-4, Tables 1, 3)

Holotype: ZUFMS-AMP20347 (MAP8594), adult male, collected at Cafundó, Gentio do Ouro municipality, Bahia state, Brazil (11°12′41.21″ S, 42°35′16.96″ W, 829 m a.s.l.), on 14 February 2022, by S. Mângia and G. Amaral.

Paratypes: ZUFMS-AMP20349-50 (adult males), ZUFMS-AMP20348, adult female, collected at

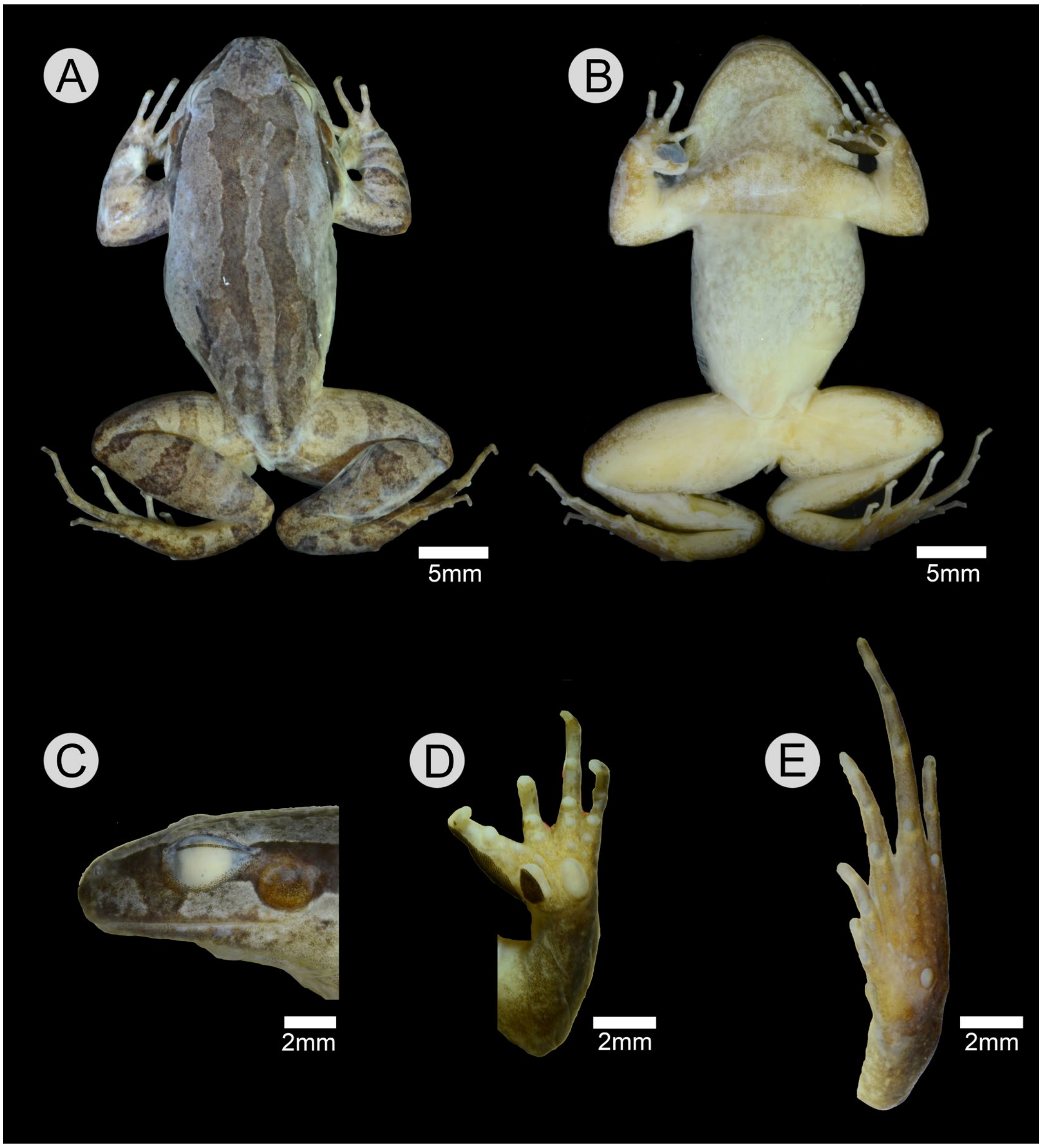

Fig. 2. *Rupirana kaatinga* sp. nov. (holotype, adult male, ZUFMS-AMP20347, field number MAP 8594, SVL 30.45 mm): A) dorsal and B) ventral views, C) lateral view of the head, ventral views of D) hand and E) foot.

Cafundó, Gentio do Ouro municipality, Bahia state, Brazil (11°10′43.81′′ S, 42°37′47.55′′ W, 868 m a.s.l.), on 15 November 2021, and ZUFMS-AMP20351-58 (juveniles) (11°13′0.74′′ S, 42°37′31.01′′ W, 969 m a.s.l.) on 8 February 2022, by S. Mângia and G. Amaral.

Diagnosis: Diagnosed by the following combination of characters: 1) snout vent-length of 26.41-30.45 mm in adult males, 2) snout round in profile and from above, 3) smooth skin on the dorsum, including the arms, legs, and upper eyelids, with only a few small, scattered tubercles, 4) belly and gular region is cream-coloured with light grey vermicular spots, 5) advertisement call lasting 0.075-0.135 s, with 20 pulses per call, 191.7 pulses/s, and a dominant frequency of 1,378.1-1,722.7 Hz.

Comparison with other species: Rupirana kaatinga sp. nov. differs from *R. cardosoi* by having smooth skin on the dorsum, including the arms, legs, and upper eyelids, with only a few small, scattered tubercles (the dorsum of *R. cardosoi*, including upper eyelid, is smooth but covered with numerous large, scattered white tubercles) (Figs. 4A, B). The advertisement call of *R. kaatinga* sp. nov. has a shorter average duration ($\bar{x}$ = 0.103 s) than *R. cardosoi*, which has a longer average duration ($\bar{x}$ = 0.176 s). *Rupirana kaatinga* sp. nov. also has a lower average number of pulses per call (20) than *R. cardosoi* (22.06). Additionally, the calls of *R. kaatinga* sp. nov. exhibit a higher pulse rate (191.7 pulses per sec) and dominant frequency range (1,378.1-1,722.7 Hz) compared to *R. cardosoi* (134.6 pulses/s, 960-1,450 Hz) (Fig. 5, Table 3).

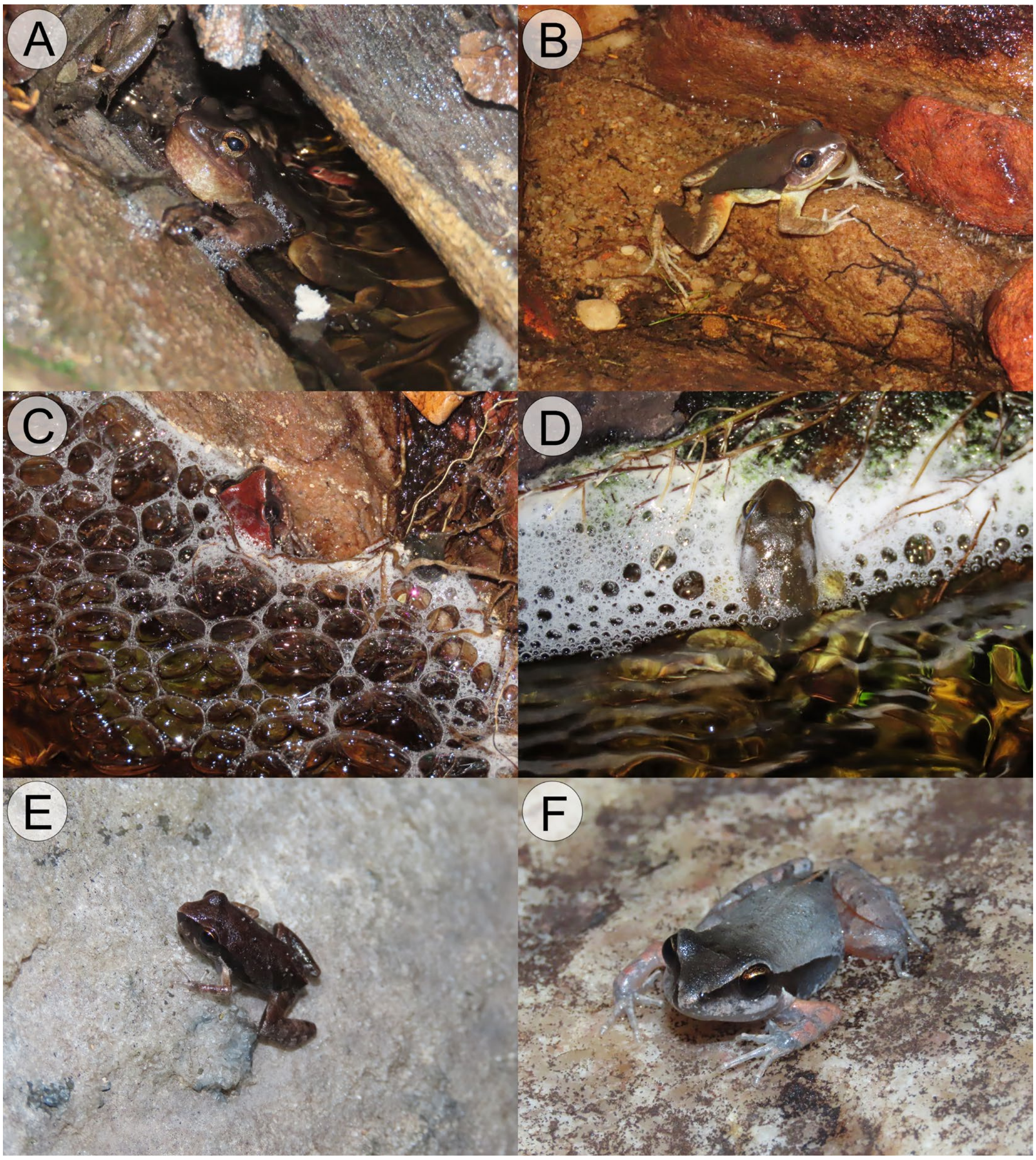

Fig. 3. Live specimens of *Rupirana kaatinga* sp. nov.: A) adult male (ZUFMS-AMP20347, field number MAP 8594, SVL 30.45 mm), B), C) and D) adult males not collected, E) paratype (juvenile ZUFMS-AMP20354, field number MAP 8599), and F) juvenile not collected.

Description of the holotype: Adult male; snout round in profile and from above; head wider than longer, head length 32% of SVL; nostrils anterolateral, near tip of snout, 2.74 mm distant from the eye; canthus rostralis indistinct; loreal obtuse; tympanum distinct, rounded; supra-tympanic fold distinct from behind eye to shoulder, bordering tympanum dorsally; tongue elongate, triangular, with slight emargination on anterior edge; vomerine teeth in two small transverse patches in line with posterior borders of small, round choanae, vomerine tooth patches separated from each other by about width of single tooth patch; vocal slits elongate, lateral to tongue, on the floor of mouth; vocal sac single, subgular, indicated externally by lateral skin folds/wrinkles; finger lengths I ~ II < III > IV; fingers free of web; tips of fingers rounded, not expanded; inner and outer palmar tubercle ovoid, with similar sizes; subarticular tubercles moderately developed, slightly pungent; no supernumerary tubercles; accessory palmar tubercles present, two in line with each digit; thumb with extensive keratinised sandpaper-appearing asperity from penultimate phalanx to base of thumb, a second ovoid patch on inner palmar tubercle; smooth skin on the dorsum, including the arms, legs, and upper eyelids, with only a few small, scattered tubercles; body lacking any obvious glands; venter smooth; belly and gular region is cream-coloured with very light gray vermicular spots; toe lengths I < II < III < IV > V; tips of toes rounded, not expanded; toes with

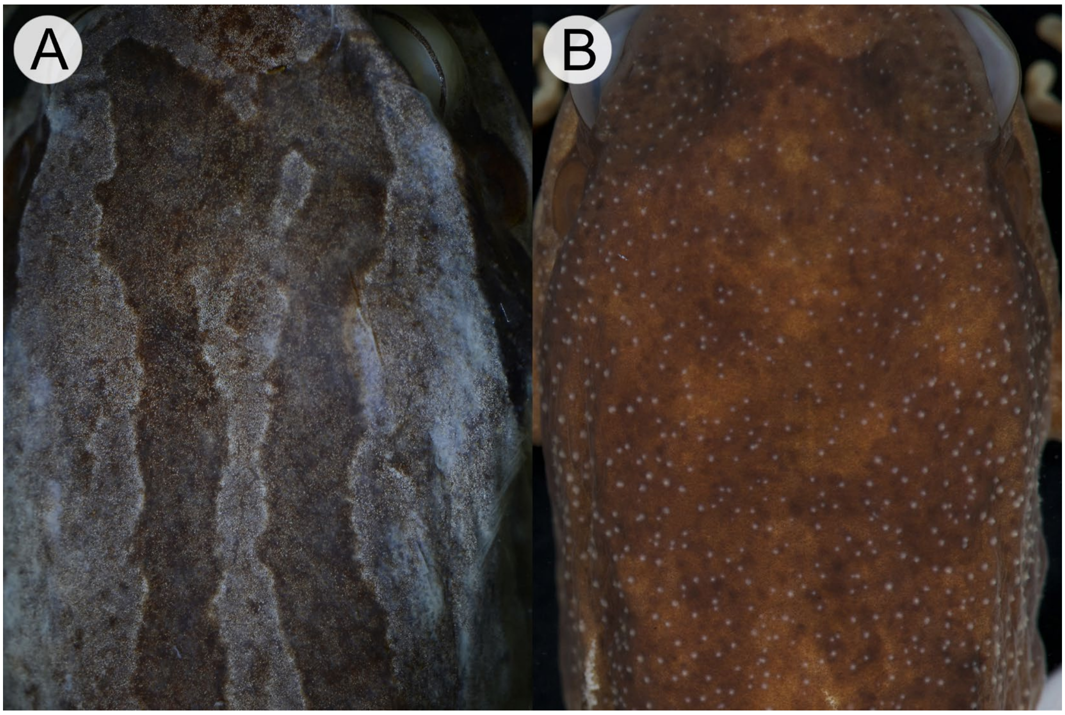

Fig. 4. Dorsal skin comparison between *Rupirana kaatinga* sp. nov. and *Rupirana cardosoi*: A) *Rupirana kaatinga* sp. nov. (holotype, adult male, ZUFMS-AMP20347, SVL 30.45 mm): smooth skin on the dorsum, including the arms, legs, and upper eyelids, with only a few small, scattered tubercles, B) *Rupirana cardosoi* (paratype, adult male, USNM519755, SVL 29.28 mm) dorsum, including upper eyelid, is smooth but covered with numerous large, scattered white tubercles.

Table 3. Acoustic parameters of the advertisement call of *Rupirana kaatinga* sp. nov. and *Rupirana cardosoi*. Values are presented as mean ± 1 SD (range). Numbers in bold stand for values that most distinguish the two species.

Taxon/parameters	*Rupirana kaatinga* sp. nov. (male not collected)	*Rupirana kaatinga* sp. nov. ZUFMS-AMP 20347	*Rupirana kaatinga* sp. nov. combined	*Rupirana cardosoi*
	n = 40 calls	n = 40 calls	n = 80 calls	n = 41 calls
Duration (s)	0.098 ± 0.01 (0.086-0.117)	0.107 ± 0.01 (0.075-0.140)	0.103 ± 0.01 (0.075-0.135)	0.176 ± 52.0 (0.071-0.277)
Pulses/call	19 ± 1 (16-22)	20 ± 2 (15-25)	20 ± 2 (15-25)	22.08 ± 7.25
Pulses/s	194.04 ± 8.6 (174.90-213.22)	189.27 ± 6.84 (177.65-200.85)	**191.7 ± 8.1** **(174.9-213.2)**	134.60 ± 7.61
Dominant Frequency (Hz)	1,559.0 ± 54.48 (1,378.1-1,722.7)	1,541.8 ± 77.53 (1,378.1-1,722.7)	**1,550.4 ± 66.1** **(1,378.1-1,722.7)**	1,290 ± 0.12 (960-1,450)
Location	Gentio do Ouro, Bahia	Gentio do Ouro, Bahia	Gentio do Ouro, Bahia	Mucugê and Palmeiras, Bahia
Reference	This study	This study	This study	Juncá & Lugli 2009

weak lateral ridges expanded into weak fringes at base of inner sides of toes I and III resulting in trace of strap-shaped basal web between toes I, II, and III; outer metatarsal tubercle small, round, about 1/3 size of small, ovoid inner metatarsal tubercle; distinct but relatively weak tarsal fold extending about 1/2 length of tarsus; sole of foot smooth; subarticular tubercles moderate, pungent.

Measurements of the holotype (in mm): SVL 30.45, HL 9.53, HW 11.16, ID 5.16, TD 2.02, END 2.70, IND 2.68, FOL 5.72, FOHL 13.45, HL 6.89, THL 12.89, TL 13.94, FL 13.31, FTL 18.89.

Colour in life of the holotype: The dorsal colouration of the specimen is predominantly grey, with two parallel lines of brown spots extending from the head

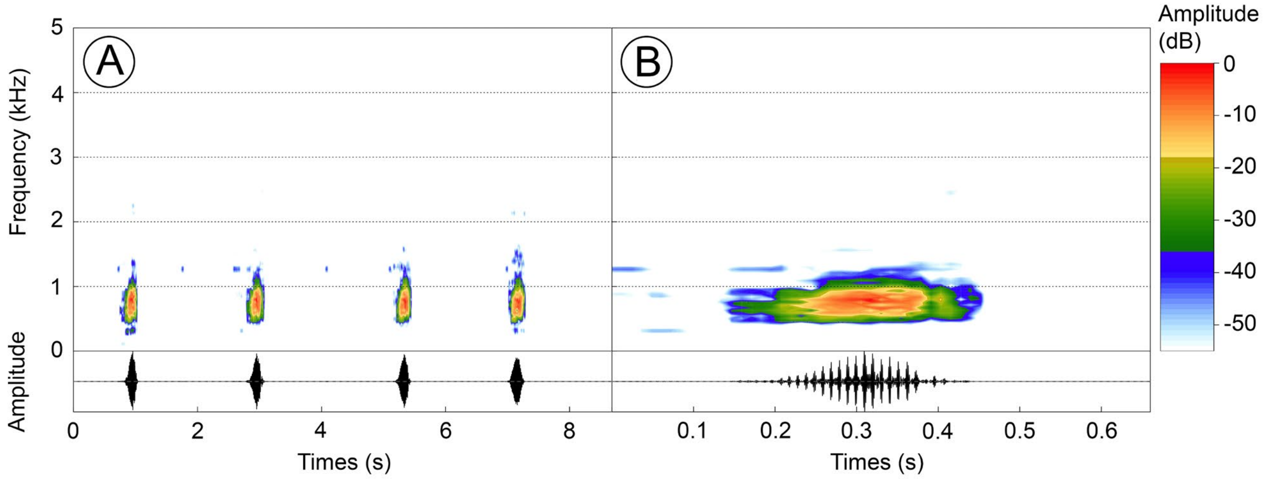

Fig. 5. Advertisement call of *Rupirana kaatinga* sp. nov. (holotype, adult male, ZUFMS-AMP20347, field number MAP 8594, SVL 30.45 mm). A) Oscillogram of four calls, B) spectrogram, oscillogram, and power spectrum of one call.

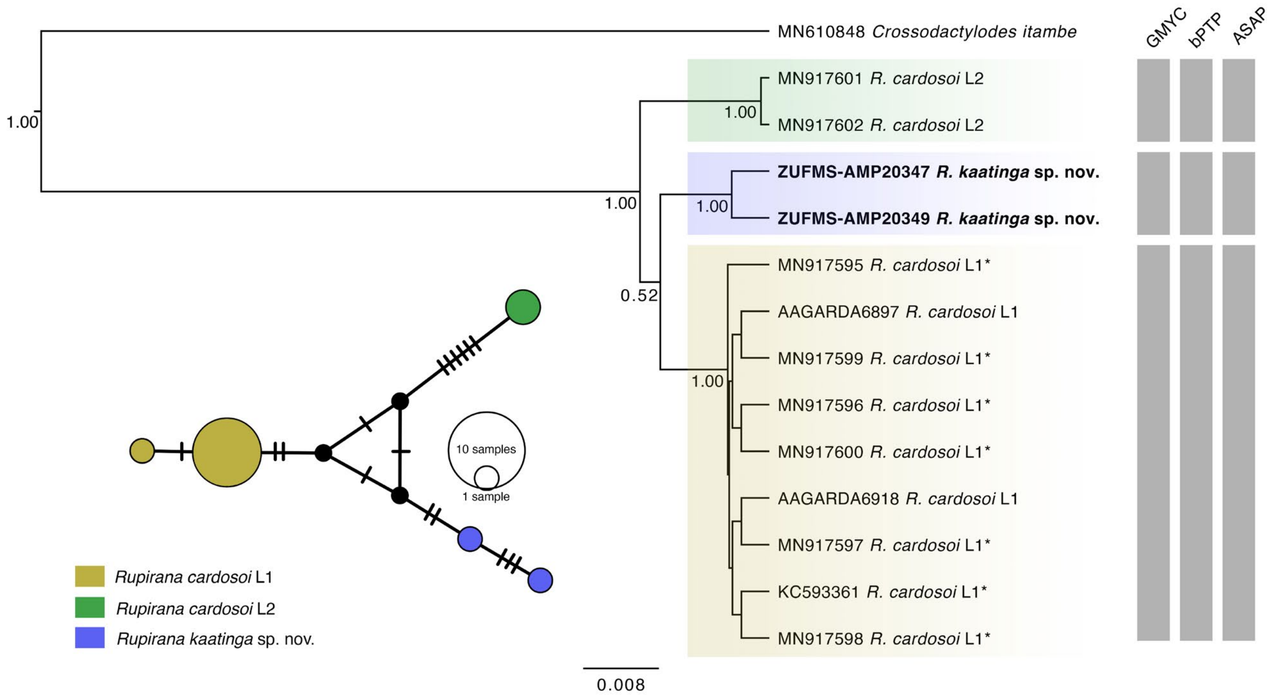

Fig. 6. Phylogeny of *Rupirana* lineages estimated in BEAST, with results from GMYC, bPTP, and ASAP analysis. Haplotype networks from neighbour-joining analysis for 16S rRNA. Crossed traits indicate additional mutational steps for branches with more than one mutation. Different colours indicate species-level units. The black dots are median vectors (hypothesised sequences). Asterisks indicate individuals from Mucugê (type locality of *Rupirana cardosoi*).

region to the sacral area (Fig. 3A). There is a dark lateral stripe that starts at the nostrils, crosses the eyes, passes through the tympanum (which is dark brown), and ends at the flank. The arms and legs display transverse dark bands, adding a camouflage pattern to the animal. The inner thighs and inguinal region are vibrant orange, contrasting with the rest of the body. The belly and gular region are cream-coloured with very light grey vermicular spots.

Colour in preservative of the holotype: The background and the spot colours remained almost the same, except for the tympanum, which became light brown, and the orange parts, which completely faded (Fig. 2).

Variation: The specimens show consistent morphological characteristics. The dorsal colour can vary among adults, with individuals exhibiting brownish (Figs. 3A, B) reddish (Fig. 3C), or greenish (Fig. 3D). Some juvenile individuals displayed orange transverse bands on their arms and legs while alive (Fig. 3F). Males can be distinguished from females by the presence of vocal slits, vocal sacs, and thumb asperities (Figs. 2B, D).

Fig. 7. Environments of *Rupirana kaatinga* sp. nov. in Serra do Assuruá, Gentio do Ouro municipality, Bahia state, Brazil: A) São João stream, where the holotype was collected, B) section where the São João stream, which serves as the species' breeding site, is crossed by a road, C) São João stream during the dry season (August 2021), and D) during the rainy season (February 2022), E) stream at Cafundó locality where most of the juveniles were collected, and F) landscape view of Cafundó.

Advertisement call: Based on the 80 calls from the two males recorded, the advertisement call of *R. kaatinga* sp. nov. is characterised by a single pulsed note (Fig. 5) lasting between 0.075 to 0.135 sec (0.103 s ± 0.01). Each call comprises 15-25 pulses (20 pulses/call ± 2), with the pulse rate ranging from 174.9 to 213.2 pulses per sec (191.7 pulses/s ± 8.1). The dominant frequency of these calls ranges from 1,378.1 to 1,722.7 Hz (1,550.4 Hz ± 67.1) (Fig. 5).

Molecular analysis: Our 16S tree identified three primary clades, which were further confirmed by the species delimitation analyses (Fig. 6). The mPTP (Score Null Model: –3.719), the GMYC (confidence interval: 10-13; likelihood ratio test: 16.604; *P* > 0.001), and the ASAP (asap score: 4.00; threshold distance: 0.075) species delimitation methods recovered the same three lineages. The average sequence divergence between *R. kaatinga* sp. nov. from its congeners ranged from 1.3-2.1% (*R. cardosoi*, population from Mucugê municipality) to 1.2-2.3% (*Rupirana* L2, population from Morro do Chapéu municipality). Furthermore, based on a fragment of the 16S gene, the mitochondrial haplotype network shows three distinct mitochondrial lineages for the genus *Rupirana*.

Geographic distribution: Rupirana kaatinga sp. nov. is only known to occur at three sites in Serra do Assuruá (above 800 m a.s.l.) within the high elevation areas of the municipality of Gentio do Ouro, Bahia state, Brazil (Fig. 1).

Etymology: The name 'Caatinga' is derived from the Tupi-Guarani language and means 'white forest' ('ka'a' (forest) + 'ting' (white) + '–a' (noun-forming suffix)). This name alludes to the whitish landscape presented by the vegetation during the dry season, when most plants shed their leaves, and the trunks become whitish and dry. The pronounced seasonality in this region significantly influences the life cycles of organisms inhabiting this type of environment to a lesser or more noticeable extent for some species, even determining whether a particular species is recorded in the area or not. We named this new species after the Caatinga region due to its endemism and strong connection to the cycles of rain and drought within this domain (see below). The specific epithet *kaatinga* is treated as a noun in apposition.

Environment and habitat: We found *R. kaatinga* sp. nov. in temporary streams, characterised as seasonal lotic streams with flowing water primarily in the months of December, January, and February (Fig. 7). Males of *R. kaatinga* sp. nov. were observed emitting calls while positioned with the posterior half of their bodies underwater. They preferred concealed locations, often nestled beneath leaves and small rocks. This calling behaviour was observed during the daytime (approximately 10:00 a.m.) and evening (between 6:00 and 10:00 p.m.). We documented the breeding activities of the new species, including calling and reproduction, spanned from November 2021 to February 2022. Our data reveal distinctive temporal patterns in the calling behaviour of *R. kaatinga* sp. nov. In November, we documented the presence of males actively calling. However, by February, calling males were notably absent, and instead, we observed numerous juveniles occupying the same locations. In addition, during field expeditions conducted in May/June 2021 and August/September 2021, we did not observe any presence of *R. kaatinga* sp. nov. individuals at the study site, which had no water flowing. This temporal disparity in their occurrence highlights the seasonality of their presence in the area (Figs. 7C, D).

We observed *R. kaatinga* sp. nov. co-occurring with other anuran species in the same habitat. These syntopic species included *Corythomantis greeningi*, *Leptodactylus troglodytes*, *L. vastus*, and *Scinax x-signatus*, all of which were in calling activities during the rainy season.

Discussion

The genus *Rupirana* was originally described based on specimens collected in the municipality of Mucugê (Heyer 1999), an upland area in the Chapada Diamantina, Bahia state, and it remained monotypic for over two decades. Recently, however, a study revealed that a population from isolated mountains in Morro do Chapéu, also in Chapada Diamantina, represents a new lineage within the genus (Santos et al. 2020a). The discovery of a third lineage in another isolated mountain, located west of the Chapada Diamantina in Serra do Assuruá, supports a biogeographical scenario known as 'sky islands'.

This 'sky islands' scenario, which has already been documented for other frog species in the genus *Bokermannohyla* (de Oliveira et al. 2021), *Scinax* (Santana et al. 2024), *Leptodactylus* (Carvalho et al. 2021), and *Pristimantis* (Trevisan et al. 2020), suggests that isolated populations on mountain tops act as refuges, promoting diversification. Nonetheless, the newly discovered lineage of *Rupirana* is the first record of the genus outside the Chapada Diamantina. This finding raises the possibility that other frog genera, besides *Rupirana*, might also inhabit Serra do Assuruá and be phylogenetically related to populations from the sky islands of Chapada Diamantina.

The discovery also highlights a critical issue of knowledge gaps in the Caatinga (Linnean and Wallacean shortfalls, Hortal et al. 2015), where vast regions remain underexplored, including areas that are conservation priorities (see Fig. 5.2 in Garda et al. 2017). In Bahia state alone, Tabarelli & Silva (2003) identified 27 priority areas for biodiversity conservation in the Caatinga, which include the municipality of Gentio do Ouro, the type locality of *R. kaatinga* sp. nov. This region was classified as of extreme biological importance, with recommendations for the establishment of areas of integral protection. The discovery of a new species in this area emphasises the need for increased sampling efforts, as it suggests that other undescribed species may also inhabit these poorly studied regions. Furthermore, Garda et al. (2017) mapped potential species richness across the Caatinga, identifying Serra do Assuruá as having a high potential to harbour a diverse anuran fauna, while the surrounding lowlands are expected to support fewer species.

The recent escalation in wind farm construction across Brazil, particularly in isolated regions such as the high-elevation areas in the Caatinga, presents unique opportunities for scientific discovery, given the mandatory monitoring that comes with these enterprises. Wind farm development has facilitated access to previously difficult areas to explore, leading to significant biological discoveries, including range extensions and even the identification of new species (Mott et al. 2008, Ribeiro et al. 2020, Oliveira et al. 2023). Notably, the discovery of *R. kaatinga* sp. nov. in the Serra do Assuruá was made possible due to wind farm development in this isolated and hard-to-access region. These findings are invaluable, as they contribute to our understanding of biodiversity in regions that remain largely under-sampled.

However, while these projects bring new opportunities for biodiversity sampling, they also pose ecological challenges. The rapid expansion of wind farms in the Caatinga, one of the least explored ecosystems in Brazil (da Silva et al. 2017), raises concerns about the potential loss of biodiversity due to habitat modification. Although renewable energy initiatives are often hailed for their low carbon footprint and environmental benefits, they can also cause unintended consequences for local ecosystems (Dean 2008, Borowski 2019, Velilla et al. 2021). The construction of wind farms has already resulted in significant landscape alterations, and there is increasing evidence that this may lead to local extinctions or significant changes in population dynamics, especially in species that rely on the fragile habitats found in these isolated regions (e.g. Bernard et al. 2014, Falavigna et al. 2020, Turkovska et al. 2021).

The discovery of *R. kaatinga* sp. nov. in the Serra do Assuruá – a region undergoing rapid transformation due to wind farm development – highlights these concerns. While the discovery is scientifically exciting, it underscores the urgent need to carefully monitor the impacts of such development on local biodiversity. Without comprehensive biological inventories and ongoing monitoring programs before and after wind farm construction, we risk losing species before we even fully understand their ecological roles.

To mitigate these risks, it is essential to establish a balance between renewable energy development and biodiversity conservation. Conducting thorough environmental assessments, including surveys of species presence and population health, is critical to minimising the ecological impact of wind farm projects. The data collected from these studies should be made readily available to researchers and policymakers to ensure that conservation strategies are informed and effective. In addition, specific mitigation strategies and guidelines should be proposed to address the ecological challenges associated with wind farm development. These could include measures such as habitat restoration projects to offset habitat loss, the careful siting of wind turbines to avoid critical habitats, and the implementation of biodiversity monitoring protocols tailored to local ecosystems. Integrating these strategies into environmental assessments makes it possible to reduce the negative impacts of wind farm projects while maximising their potential as opportunities for scientific discovery.

Ultimately, the goal should be to achieve harmony between infrastructure development and environmental stewardship. By investing in research and monitoring, we can ensure that wind farm construction contributes to both energy sustainability and the preservation of biodiversity, proving that economic development and conservation can go hand in hand. Future research should address knowledge gaps in the Caatinga, particularly through comprehensive biodiversity surveys and genetic studies. These efforts are crucial to better understand the species richness and ecological dynamics of this unique biome and inform more effective conservation strategies. Expanding these studies to similar underexplored biomes could further enhance our understanding of biodiversity in fragile and remote ecosystems while guiding responsible infrastructure development across Brazil.

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Author Contributions

S. Mângia collected and analysed the data, supervised the analysis, prepared figures and tables, wrote the manuscript and approved the final draft. G. do Amaral collected the data and wrote and approved the final draft. M. Müller extracted and amplified the DNA samples, prepared tables and approved the final draft. D.J. Santana analysed the data, conducted the molecular analysis, prepared figures and tables, wrote the manuscript and approved the final draft.

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