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Cuad. herpetol. 39 (2): 103-114 (*2025*)

Amphibians of caves in Serra do Ramalho karst area, a remarkable region of subterranean fauna in Brazil

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Recibida: 09 Enero 2025
Revisada: 29 Mayo 2025
Aceptada: 08 Junio 2025
Editor Asociado: J. Lescano

doi: 10.31017/CdH.2025.(2025-002)

ABSTRACT

Serra do Ramalho karst area is part of the Bambuí group, the largest geomorphological unit of Brazil. It is in southwestern state of Bahia, and extends over the municipalities of Carinhanha, Feira da Mata, Coribe, and Serra do Ramalho. We inspected 34 caves of Serra do Ramalho karst area, actively looking for amphibians. We were able to find amphibians in 12 caves. We sequenced some of the specimens found for the barcode mitochondrial *16S* to confirm identifications. We found 13 species of amphibians, all anurans: *Rhinella granulosa*, *Rhinella mirandaribeiroi*, *Oreobates remotus*, *Boana crepitans*, *Scinax* sp. 26, *Scinax x-signatus*, *Leptodactylus macrosternum*, *L. troglodytes*, *L. vastus*, *Physalaemus cicada*, *P. cuvieri*, *P. albifrons*, and *Odontophrynus carvalhoi*. As far as it is possible to say, these amphibians are using the cave to daily and seasonal retreat, where they can possibly protect themselves from the drought and heat of Caatinga during the day.

Key Words: Anurans; Cerrado-Caatinga; Limestone caves; Refugia; Bahia state

The Bambuí group is the largest geomorphological unit of Brazil (Assis *et al.*, 2023; Auler and Piló, 2015; Campos-Filho *et al.*, 2016), sheltering one of the most rich subterranean biodiversity of the country (Gallão and Bichuette, 2018), occupying part of the states of Bahia, Goiás, Minas Gerais, and Tocantins (Auler, 2002; Gallão and Bichuette, 2018). It is formed by carbonate-siliciclastic rocks dated from Late Proterozoic, around 950–600 My (Dardenne *et al.*, 1986, apud Campos-Filho *et al.*, 2016).

Serra do Ramalho karst area is part of the Bambuí group and is located in the Middle Rio São Francisco basin, in southwestern state of Bahia (Mattox *et al.*, 2008). It is situated in an ecotone of Caatinga and Cerrado phytophysionogmies. The climate is tropical dry, with a dry winter (March to October) and average annual precipitation of 640 mm ("Aw" type of Köppen-Geiger classification, Beck *et al.*, 2018). The lithology of the Serra do Ramalho karst area is limestone, and includes the municipalities of Carinhanha, Cocos, Feira da Mata, Coribe, and Serra do Ramalho, all in state of Bahia (Rubbioli *et al.*, 2019).

The fauna of the caves of Serra do Ramalho is well characterized, with examples of studies with Actinopterygii (Bichuette and Rizzato, 2012; Bichuette and Trajano, 2005; Mattox *et al.*, 2008; Trajano *et al.*, 2009), Arachnida (Assis *et al.*, 2021; Assis *et al.*, 2023;

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Baptista and Giupponi, 2002; Bertani *et al.*, 2018; Brescovit *et al.*, 2023), Crustacea (Fernandes *et al.*, 2019), Malacostraca (Campos-Filho *et al.*, 2022a; Campos-Filho *et al.*, 2022b; Cardoso *et al.*, 2022, 2023), Insecta (Cordeiro *et al.*, 2023; Lamas *et al.*, 2023; Oliveira *et al.*, 2023; Pellegrini *et al.*, 2022), and Mollusca (Salvador and Bichuette, 2024; Salvador *et al.*, 2023; Simone, 2012, 2013).

Amphibians in Brazilian caves are barely known, but they are very common, especially in the semi-arid ecosystems, where caves constitute a refugee from hot and dry climate. Considering complete publications about amphibians in Brazilian caves, there are reports in faunal lists (Dessen *et al.*, 1980; Pinto-da-Rocha, 1995; Trajano, 1987; Trajano and Bichuette, 2006; Trajano and Gnaspini-Netto, 1991), species descriptions (Canedo *et al.*, 2012; Pansonato *et al.*, 2020; Vaz-Silva *et al.*, 2018), fossil descriptions (Barcelos *et al.*, 2020; Barcelos and Verdade, 2022), distribution extension (Lima *et al.*, 2012; Motta *et al.*, 2020), auto-ecology (Andrade *et al.*, 2021), anuran richness and community ecology studies (Matavelli *et al.*, 2015; Santos *et al.*, 2022), a commentary (Sperandei *et al.*, 2023), and a distribution review (Sperandei *et al.*, 2024).

Although Serra do Ramalho caves are well known (Trajano and Bichuette, 2010a), there are no amphibian surveys for their caves. In this study, we aimed to invent the amphibians present in Serra do Ramalho karst area. We also briefly comment on amphibian occurrences in caves.

We conducted fieldwork in 34 caves in Serra do Ramalho region (some caves in Figs. 1 and 2, Appendix A1). We searched all the accessible areas of the caves, such as unconsolidated substrates, leaf litter, trunk debris, crevices in the walls, and water bodies, during daytime, mostly in the dry season. We manually collected most of the amphibians, but some were only photographed. We euthanized the collected amphibians with 5 % lidocaine. We removed fingertips, toe tips, or whole feet as tissue samples, and stored them in 100 % ethanol. Then we fixed the specimens in 10 % formalin or 70 % ethanol. The specimens are stored in 70 % ethanol in the collection of the Laboratório de Estudos Subterrâneos (LES, Universidade Federal de São Carlos, São Carlos, SP, Brazil; curator: M.E. Bichuette) and the Coleção de Anfíbios Célio F. B. Haddad (CFBH, Universidade Estadual Paulista, Rio Claro, SP, Brazil; curator: C.F.B. Haddad). The collections were approved by MMA-SISBio collection permit number 28992.

Since there are no amphibian species lists for the Serra do Ramalho karst region, we used specimens from the CFBH for taxonomic comparisons. To help with identification, we also performed a barcode analysis, described below.

From the tissue samples, we extracted total DNA with a protocol of ammonium acetate (adapted by Lyra *et al.*, 2017). With the total DNA, we directly amplified the part 3' of ribosomal *16S* rRNA (herein called *16S*-ARBR), with primers 16Sar-L and 16Sbr-H (Palumbi *et al.*, 2002). To perform the PCR, we used 10.0 μL per sample of Taq DNA Polymerase Master Mix RED (©Ampliqon), 10.0 μL per sample of autoclaved distilled water, 0.4 μL of each primer, and 1.0 μL of the concentrated total DNA. The temperature program of the PCR consisted of an initial denaturation step of 95 °C for three minutes; 40 cycles of 95 °C for 20 seconds, 50 °C for 20 seconds, and 68 °C for one minute; and a final extension of 68 °C for three minutes. We checked the amplicons with agarose gel 1 % in electrophoresis, and we purified the positive amplicons with Exonuclease and Alkaline Phosphatase. We sequenced the positive amplicons in only one direction (the forward one) at Macrogen Inc. (Seoul, South Korea). We manually trimmed each individual's chromatogram in Geneious (Dotmatics, 2023). To associate each individual with the most likely species, we used the selected fragment *16S*-ARBR in a BLAST® search (NCBI *et al.*, 2019) in Geneious (Dotmatics, 2023) in October, 2023. The GenBank accession numbers will be provided upon acceptance of the manuscript.

Twelve out of the 34 studied caves presented amphibians (Fig. 3): Gruna Vila Nova, Gruna Google, Gruna do Cocho, Gruna do Domingão, Gruna da Altina and Gruna Bem Bom (Carinhanha); Gruna da Cacimbinha, Gruna Chico Pernambuco and Gruna Mamona (Coribe); Gruna Serra Solta II (Serra do Ramalho); Gruna do Engrunado and Gruna da Boca da Lapa (Feira da Mata). The amphibians found were all anurans, and correspond to 13 species distributed in five families: *Rhinella granulosa* (Spix, 1824), *R. mirandaribeiroi* (Gallardo, 1965) (Bufonidae); *Oreobates remotus* Teixeira, Amaro, Recoder, Sena, and Rodrigues, 2012 (Craugastoridae); *Boana crepitans* (Wied-Neuwied, 1824), *Scinax* sp. 26 (sensu Araujo-Vieira *et al.*, 2023), *Scinax x-signatus* (Spix, 1824) (Hylidae); *Leptodactylus macrosternum* Miranda-Ribeiro, 1926, *L. troglodytes* Lutz, 1926, *L. vastus* Lutz, 1930, *Physalaemus cicada* Bokermann, 1966, *P. cuvieri* Fitzinger, 1826, *P. albifrons* (Spix, 1824)

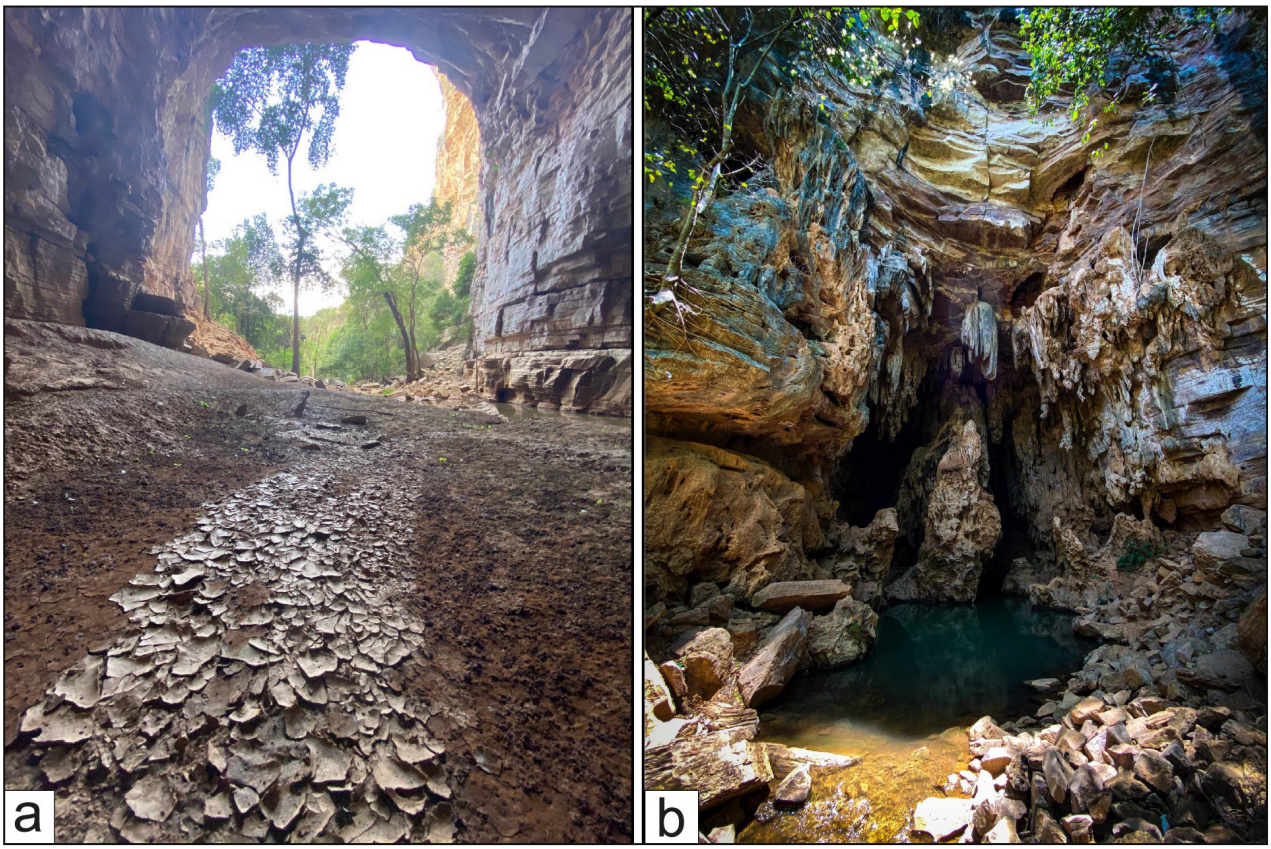

Figure 1. Some of the sampled caves in Serra do Ramalho karst area: (a) Gruna da Mamona, photo of Laboratório de Estudos Subterrâneos, UFSCar, São Carlos, Brazil; (b) Gruna Boca da Lapa, photo of A.O. Lobo.

(Leptodactylidae); and *Odontophrynus carvalhoi* Savage and Cei, 1925 (Odontophrynidae) (Table 1).

All the caves in which we found anurans possess permanent lentic water bodies, except for Gruna Google (Carinhanha), where we found the two *Odontophrynus* tadpoles (CFBH 32595), in which the lentic water body is temporary. All the species we found are nocturnal and reproduce during rainy season. This is a common pattern for anurans in arid and semi-arid environments (Hillman *et al.*, 2009). We found all the animals during the day, suggesting that the caves function as shelter in diurnal retreat. We also found all the animals in the dry season, probably hiding from the epigean drought while they are not in reproductive season. In Gruna da Cacimbinha, we found specimens that we could not collect, and we identified them as *Leptodactylus* cf. *macrosternum* (one individual) and *Leptodactylus* cf. *vastus* (a population of around 30 individuals, some of them were juveniles; Figs. 4C and 4D).

We found *Rhinella granulosa* in two caves (Gruna do Domingão and Gruna do Cocho, Carinhanha). This species is distributed in northeast Brazil and commonly found in epigean environments (Pereyra *et al.*, 2016). Matavelli *et al.* (2015) and Santos *et al.* (2022) also reported this species in caves in Caatinga. We confirmed with barcode the identity of the individual of *R. granulosa* from Gruna do Cocho (CFBH 32599, Table A2). We found *Rhinella mirandaribeiroi* in Gruna Serra Solta II (Serra do Ramalho municipality). *Rhinella mirandaribeiroi* is a species from Cerrado domain, and is known from Cerrado in Bahia (Narvaes and Rodrigues, 2009).

Oreobates remotus was described for Cavernas do Peruaçu National Park (Teixeira Jr. *et al.*, 2012), a federal conservation unit inserted in Bambuí geomorphological group, and is also registered in the vicinities of the National Park (Frost, 2025). *Oreobates antrum* Vaz-Silva, Maciel, Andrade, and Amaro, 2018, the sister species of *O. remotus*, is also registered in caves (Motta *et al.*, 2020; Vaz-Silva *et al.*, 2018). Another species of this genus, *Oreobates heterodactylus* (Miranda-Ribeiro, 1937), was described from a cave in the state of Mato Grosso (Gruta Fazendinha, Cáceres; Bokermann, 1966). These occurrences of *Oreobates* in caves suggest a

Figure 2. Some of the sampled caves in Serra do Ramalho karst area: (a) Salon of the Gruna da Serra Verde, showing a researcher collecting; (b) Entrance of the Gruna do Govi; (c) Gruna Bem Bom; (d) Gruna do Engrunado, with a researcher. Photos of Laboratório de Estudos Subterrâneos, UFSCar, São Carlos, Brazil.

pattern of the genus in the preference for shelter in subterranean environments. We found three individuals of *O. remotus* in caves of Serra do Ramalho karst area (Gruna do Engrunado, Fig. 4B, Feira da Mata; Gruna Bem Bom, Carinhanha), all confirmed by barcode (Table A2).

Boana crepitans is found in open areas in Caatinga, Cerrado, and in the Atlantic Forest (Orrico *et al.*, 2017). This is a species from the *Boana faber* group, firstly described for Bahia state, and well known in Brazilian Northeast (Frost, 2025). We found only one individual, in Gruna Bem Bom (Carinhanha), confirmed by barcode (LESV 754, Table A2). As far as we know, this is the first record of this species in caves.

The five individuals of *Scinax* found in Gruna Bem Bom (Carinhanha; two of them in Figs. 5B and 5C) are morphologically similar, of which two were barcoded (LESV 749 and 753). One of them (LESV 753) matched with *Scinax* sp. 26 (an unconfirmed candidate species—UCS—sensu Araujo-Vieira *et al.*, 2023) and the other matched with *Scinax x-signatus* (LESV 749). Since the taxonomic status of *Scinax* sp. 26 is a pending issue, we consider the other three individuals (not barcoded) as *Scinax x-signatus* (LESV 748, 750-751). These specimens must be further studied once the taxonomic limits between *Scinax* sp. 26 and *Scinax x-signatus* are better addressed. The species *Scinax x-signatus* and *S.* sp. 26 (sensu Araujo-Vieira *et al.*, 2023) are sister species and morphologically similar (Araujo-Vieira *et al.*, 2023; Araujo-Vieira *et al.*, 2020). Along with *Scinax fuscovarius* (Lutz, 1925), these two species form the *S. fuscovarius* group (Araujo-Vieira *et al.*, 2023). As these species lack morphological differences, it was only possible to recognize the occurrence of the two species in Gruna do Bem Bom through DNA barcode (Table A2). *Scinax x-signatus* is a

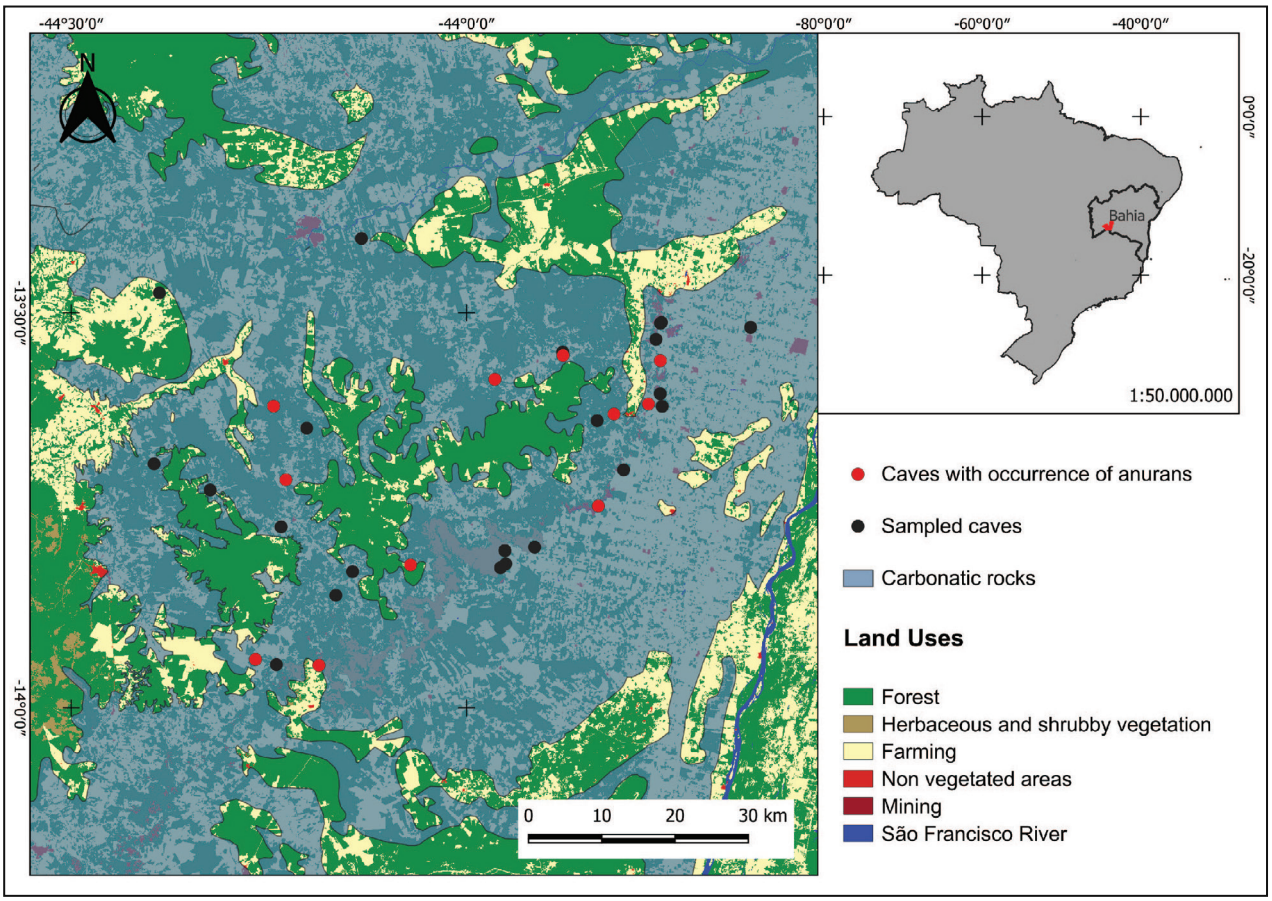

Figure 3. Distribution map of the studied caves, showing the caves in which we found amphibians (in red), regions with carbonatic rocks, and main land uses in the region.

species found in Caatinga, Atlantic Forest, Cerrado, and Amazon. It was reported as occurring in a cave (Sperandei *et al.*, 2023), but with no mention in which cave or biome. On the other hand, *Scinax* sp. 26 (sensu Araujo-Vieira *et al.*, 2023) was known only in epigean environments of Cavernas do Peruaçu National Park (Araujo-Vieira *et al.*, 2020) and from Caetité and Jacaraci municipalities, in the state of Bahia (Araujo-Vieira *et al.*, 2023).

Leptodactylus macrosternum is found in other parts of Carinhanha and Bom Jesus da Lapa municipalities (Magalhães *et al.*, 2020), localities close to Serra do Ramalho karst area. This species was also registered by Matavelli *et al.* (2015) and Santos *et al.* (2022) in caves in Caatinga and Cerrado. We registered this species in four caves: Gruna do Domingão (Carinhanha); Gruna do Engrunado and Gruna Boca da Lapa (Feira da Mata); Gruna da Mamona and Gruna da Cacimbinha (Coribe; the last one not collected, Fig. 4C), most of them confirmed by barcode (Tables 1 and A2). *Leptodactylus troglodytes* is distributed in Caatinga and Cerrado (Thomé *et al.*, 2021). We found this species in Gruna do Domingão (Carinhanha, Table 1). It has also been recorded in caves by Matavelli *et al.* (2015) and Santos *et al.* (2022). From the genus *Leptodactylus*, we also found *Leptodactylus vastus*, in Gruna Bem Bom (Carinhanha), and another population of around 30 individuals in Gruna da Cacimbinha (Coribe, not collected), including juveniles (Fig. 4D). This non-collected encounter is evidence that this species is using the cave as a shelter. *Leptodactylus vastus* was already recorded in the cave Toca da Raposa (Simão Dias municipality, state of Sergipe), where the authors state that the species was "a regular fauna" of the cave (A.S. Ferreira, M.A.T. Dantas & C.R. Donato, personal communication). This species was also recorded in the entrance of the cave Casa de Pedra (Itabaiana municipality, state of Sergipe) preying on bats (Gouveia *et al.*, 2009). Also, the species was found in a cave in Cerrado (Santos *et al.*, 2022).

Physalaemus cuvieri is a very common frog, known for Cerrado, Caatinga, and open areas in Atlantic Forest and Amazon (Frost, 2025). We

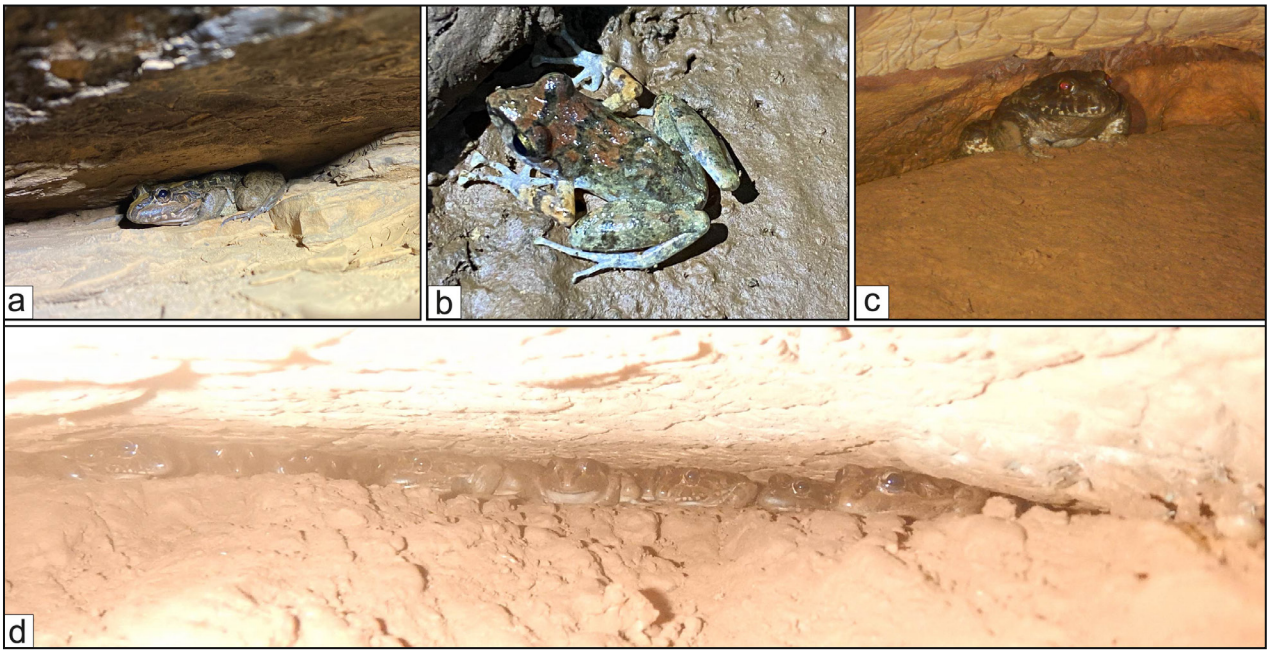

Figure 4. Anurans recorded in some caves in Coribe municipality, Bahia state, in Serra do Ramalho karst area: (a) *Leptodactylus macrosternum* in Gruna da Mamona; (b) *Orebates remotus* in Gruna do Engrunado; (c) *L. vastus* in Gruna da Cacimbinha, not collected; (d) Population of *L. macrosternum* in Gruna da Cacimbinha, not collected. Photos of Laboratório de Estudos Subterrâneos, UFSCar, São Carlos, Brazil.

found this species in Gruna Vila Nova and Gruna da Altina (Carinhanha). But it was already found in caves before, like the Pains karstic region (Ferreira *et al.*, 2022), and other unspecified caves (Matavelli *et al.*, 2015; Santos *et al.*, 2022; Sperandei *et al.*, 2023). Nevertheless, the other two species of the genus found in Serra do Ramalho karst area, *Physalaemus albifrons* (Gruna Serra Solta II, Serra do Ramalho) and *P. cicada* (Gruna Domingão, Carinhanha), have never been recorded in caves before. *Physalaemus albifrons* is a species from Caatinga, Atlantic Forest and ecotones (Palmeira *et al.*, 2011). And *Physalaemus cicada* is a species found in Caatinga, Cerrado and ecotones (Linares and Mello, 2011; Lisboa and Haddad, 2009).

Similarly, we found three individuals of *Odontophrynus* (CFBH 32944-32946; one of them in Fig. 6) in Gruna Chico Pernambuco (Coribe), that resembled *Odontophrynus carvalhoi*. One of them was barcoded and matched *Odontophrynus carvalhoi* (CFBH 32944, Table A2). The other two (CFBH 32945-32946) are morphologically similar to the barcoded one, so we considered them as *Odontophrynus carvalhoi*. In Gruna Google (Carinhanha), we found two tadpoles (CFBH 32595), identified as *Odontophrynus carvalhoi* following the description of Santos *et al.* (2017). *Odontophrynus carvalhoi* (Fig. 5) is a species from Caatinga and ecotones (Frost, 2025). It has never been registered for caves. We found adults in one cave (Gruta Chico Pernambuco, Coribe) and probably tadpoles in another cave (Gruna Google, Carinhanha). According to Haddad *et al.* (2013), *Odontophrynus carvalhoi* reproductive mode is defined by subaquatic eggs laid on bed of lotic water; offspring with indirect development, lecithotrophic nutrition, exotrophic, without parental feeding, and tadpoles developing in lotic water. This reproductive mode is currently the number 16 (sensu Nunes-de-Almeida *et al.*, 2021). Nevertheless, we found the tadpoles of *Odontophrynus carvalhoi* in Gruna Google (Carinhanha) in a lentic water. If we can confirm these tadpoles as *Odontophrynus carvalhoi*, this finding indicates some plasticity in the use of water bodies for reproduction in this species, allowing it to explore either lentic or lotic water for tadpole development. Another hypothesis is that these tadpoles entered in Gruna Google through the water flow or flood.

Anurans might enter in caves due to the more humid and cooler environment, and due to the presence of water bodies, especially in semi-arid biomes (Trajano and Bichuette, 2006). This is probably why recent reviews found more amphibians in caves of Caatinga and Cerrado than in caves of Amazonia

and Atlantic Forest, that are more humid (Matavelli *et al.*, 2015; Santos *et al.*, 2022). Anyhow, there is a consensus that amphibians seek shelter in caves (e.g. Andrade *et al.*, 2021; Bichuette *et al.*, 2022; Matavelli *et al.*, 2015; Santos *et al.*, 2022; Sperandei *et al.*, 2023; Trajano and Bichuette, 2006).

Some studies considered that the search for the caves by amphibians might be somewhat motivated and not accidental, occurring all-year around (Santos *et al.*, 2022; Sperandei *et al.*, 2023). An accidental animal in a subterranean environment is an organism that has entered into the hypogean areas through falls or dragged by water, or maybe an organism that has entered the cave looking for a colder and more humid place (Trajano, 2005, apud Trajano, 2012).

Although incipient, the studies of amphibians in Brazilian caves have raised some questions and hypotheses suggesting that the caves are used "systematically" by these amphibians (Sperandei *et al.*, 2024). But as most of the studies are species –or genus– lists, the questions and hypotheses are currently being raised about the possible uses that amphibians make of the caves. It is not possible yet to classify most Brazilian amphibians found in caves in Schiner-Racovitza classification (Racovitza, 1907; Schiner, 1854). Some populations of *Bokermannohyla martinsi* Bokermann, 1964 from Serra do Gandarela National Park, state of Minas Gerais (Andrade *et al.*, 2021), and a population of *Pipa* cf. *pipa* from Refugio Maroaga in the municipality of Presidente Figueiredo, state of Manaus (M. E. Bichuette, unpublished data) might deserve Schiner-Racovitza classification after careful studies.

To adequately classify amphibians as cave organisms in Schiner-Racovitza classification (Racovitza, 1907; Schiner, 1854), it is necessary to study them monthly, for some annual cycles (Trajano

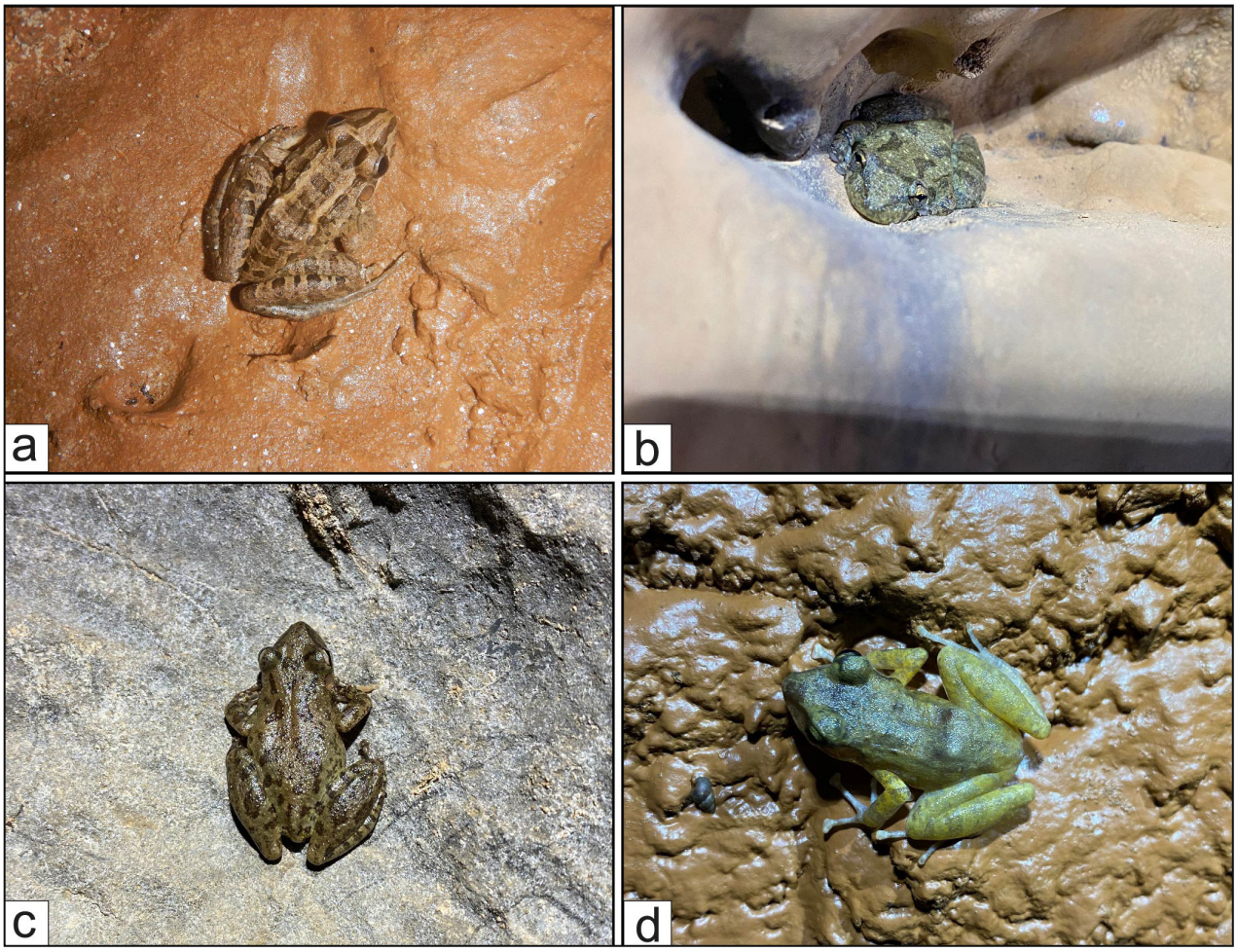

Figure 5. Anurans recorded in some caves in Serra do Ramalho karst area: (a) *Leptodactylus macrosternum* in twilight zone of Gruna Boca da Lapa (Feira da Mata, Bahia state); (b) *Scinax* cf. *x-signatus* in Gruna do Bem Bom (Carinhanha, Bahia state); (c) *Scinax* cf. *x-signatus* in Gruna do Bem Bom (Carinhanha, Bahia state); (d) *Oreobates remotus* in Gruna do Bem Bom (Carinhanha, Bahia state). Photos of Laboratório de Estudos Subterrâneos, UFSCar, São Carlos, Brazil.

and Bichuette, 2010b). We also consider that these studies must be done with samplings during the day (the period in which most amphibians are resting in shelters) and during the night (the period in which most amphibians are in activity). The difficulty here is that cave and subterranean specialists usually sample the caves during the day, and during dry season. During the day and during dry season, most of the amphibians are in fact sheltering, and not in activity.

Anyhow, regardless of the temporal aspects of the sampling, researchers should always remember that each cave has its own faunistic and diversity peculiarities (Trajano and Bichuette, 2006). This also means that each amphibian population found in a cave will be unique, and it could be difficult to define patterns of occupation beyond the amphibian search for shelter.

The physiological, morphological, and behavioral adaptations of anurans in the xeric environment of Caatinga are still mostly unknown (Jared *et al.*, 2019). The rain in Caatinga is markedly seasonal and finding a suitable site to retreat is necessary for these animals to survive (Navas *et al.*, 2004). The caves of Serra do Ramalho karst area have probably been used by anurans as a site for diurnal and winter retreat away from the adverse conditions of the Caatinga environment. To study the habitats and microhabitats used by Caatinga anurans during dry season and hot days is necessary to propose hypotheses regarding the physiology of these animals (Navas *et al.*, 2004). As several species of anurans use caves during part of their life cycles, the protection of this type of environment is necessary for the conservation of anurans.

Figure 6. *Odontophrynus carvalhoi* from Gruna Chico Pernambuco (Coribe, Bahia state), in Serra do Ramalho karst area. Photo of Pedro Pereira Rizatto.

Acknowledgments

The research was partially granted by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade)/Vale S.A. (TCCE 02/2020), under the projects "Estudos para definição de áreas prioritárias para a Conservação de Proteção do Patrimônio Espeleológico na Serra do Ramalho – BA", executed by the Grupo Bambuí de Pesquisas Espeleológicas (GBPE), "Teste de metodologias propostas em Legislação Ambiental relacionadas à fauna subterrânea e proposição de novas áreas prioritárias para conservação de cavernas"), executed by M.E.B. and post-doctoral scholarship to J.E.G., with operational management carried out by the IABS (Instituto Brasileiro de Desenvolvimento e Sustentabilidade). Financial support was also granted by FAPESP to M.E.B. (2010/08459-4), C.F.B.H. (2013/50741-7, and 2021/10639-5), and A.F.S. (2021/12455-9), and CNPq to C.F.B.H. (304713/2023-6) and A.F.S. (141454/2015-6). We thank M. Vaini for help processing the specimens; K. Araujo-Vieira and T. Carvalho for help with amphibian identifications; and M.V.S.A. Duarte for confection of map of Fig. 3. We thank ICMBio/SISBIO for collect permissions (20165 and 28992).

Literature cited

Andrade, M.C.M.d.; Costa, J.C.R. & Eterovick, P.C. 2021. Fidelity in the use of iron caves by *Bokermannohyla martinsi* (Anura: Hylidae): a step further in unveiling the importance of Brazilian caves for the herpetofauna. *Salamandra* 57: 502-512.

Araujo-Vieira, K.; Lourenço, A.C.C.; Lacerda, J.V.A.; Lyra, M.L.; Blotto, B.L.; Ron, S.R.; Baldo, D.; Pereyra, M.O.; Suárez-Mayorga, Á.M.; Baêta, D.; Ferreira, R.B.; Barrio-Amorós, C.L.; Borteiro, C.; Brandão, R.A.; Brasileiro, C.A.; Donnelly, M.A.; Dubeux, M.J.M.; Köhler, J.; Kolenc, F.; Fortes Leite, F.S.; Maciel, N.M.; Nunes, I.; Orrico, V.G.D.; Peloso, P.; Pezzuti, T.L.; Reichle, S.; Rojas-Runjaic, F.J.M.; Da Silva, H.R.; Sturaro, M.J.; Langone, J.A.; Garcia, P.C.A.; Rodrigues, M.T.; Frost, D.R.; Wheeler, W.C.; Grant, T.; Pombal, J.P.; Haddad, C.F.B. & Faivovich, J. 2023. Treefrog diversity in the Neotropics: phylogenetic relationships of Scinaxini (Anura: Hylidae: Hylinae). *South American Journal of Herpetology* 27: 1-145.

Araujo-Vieira, K.; Pombal Jr, J.P.; Caramaschi, U.; Novaes-e-Fagundes, G.; Orrico, V.G.D. & Faivovich, J. 2020. A neotype for *Hyla x-signata* Spix, 1824 (Amphibia, Anura, Hylidae). *Papéis Avulsos de Zoologia* 60: e20206056.

Assis, L.d.; von Schimonsky, D.M. & Bichuette, M.E. 2021. The first troglobitic *Pseudochthonius* Balzan, 1892 (Pseudoscorpiones, Chthoniidae) from the karst area of Serra do Ramalho, Brazil: a threatened species. *Subterranean Biology* 40: 109-128.

Assis, L.d.; von Schimonsky, D.M.; Gallão, J.E. & Bichuette, M.E. 2023. Contribution to the knowledge of Brazilian

troglobitic Pseudoscorpiones (Arachnida): description of *Pseudochthonius lubueno* sp. nov. (Chthoniidae) from Serra do Ramalho karst area, state of Bahia, Brazil. *Zoologia* 40: e22048.

Auler, A.S. 2002. Karst areas in Brazil and the potential for major caves - an overview. *Boletín de la Sociedad Venezolana de Espeleología* 36: 29-35.

Auler, A.S. & Piló, L.B. 2015. Lagoa Santa Karst: cradle of Brazilian cave studies. *In*: Vieira, B.C.; Salgado, A.A.R. & Santos, L.J.C. (eds.), Landscapes and Landforms of Brazil. Springer Science and Business Media Dordrecht.

Baptista, R.L.C. & Giupponi, A.P.d.L. 2002. A new troglomorphic *Charinus* from Brazil (Arachnida: Amblypygi: Charinidae). *Revista Ibérica de Aracnología* 6: 105-110.

Barcelos, L.A.; Almeida-Silva, D.; Santos, C.M.D. & Verdade, V.K. 2020. Description of a new species of fossil *Ceratophrys* (Anura: Ceratophryidae) from Versalles Cave, São Paulo, Brazil. *Journal of Vertebrate Paleontology* 40: e1811293.

Barcelos, L.A. & Verdade, V.K. 2022. A new fossil of *Ceratophrys* Wied-Neuwied (Ceratophryidae: Anura) from Southeastern Brazil. *Zootaxa* 5094: 495-500.

Beck, H.E.; Zimmermann, N.E.; McVicar, T.R.; Vergopolan, N.; Berg, A. & Wood, E.F. 2018. Present and future Koppen-Geiger climate classification maps at 1-km resolution. *Scientific Data* 5: 180214.

Bertani, R.; von Schimonsky, D.M.; Gallao, J.E. & Bichuette, M.E. 2018. Four new troglophilic species of *Loxosceles* Heinecken & Lowe, 1832: contributions to the knowledge of recluse spiders from Brazilian caves (Araneae, Sicariidae). *Zookeys* 806: 47-72.

Bichuette, M.E. & Rizzato, P.P. 2012. A new species of cave catfish from Brazil, *Trichomycterus rubbioli* sp.n., from Serra do Ramalho karstic area, São Francisco River basin, Bahia state (Siluriformes: Trichomycteridae). *Zootaxa* 3480: 48-66.

Bichuette, M.E.; Sperandei, V.d.F.; Bertolini, D.L.V.B.V.; Simão, P.X.A. & Sabbag, A.F. 2022. Frogs of seven granitic caves on Santa Catarina Island, Florianópolis municipality, Santa Catarina state, southern Brazil. *Herpetology Notes* 15: 353-359.

Bichuette, M.E. & Trajano, E. 2005. A new cave species of *Rhamdia* (Siluriformes: Heptapteridae) from Serra do Ramalho, northeastern Brazil, with notes on ecology and behavior. *Neotropical Ichthyology* 3: 587-595.

Bokermann, W.C.A. 1966. Lista anotada das localidades tipo de anfíbios brasileiros. Serviço de Documentação - RUSP. São Paulo.

Brescovit, A.D.; Gallão, J.E. & Cizauskas, I. 2023. A new species of *Pinelema* Wang & Li, 2012, a relictual telemid spider inhabiting caves in Brazil (Araneae, Telemidae). *Zootaxa* 5383: 352-364.

Campos-Filho, I.S.; Bichuette, M.E. & Taiti, S. 2016. Three new species of terrestrial isopods (Crustacea, Isopoda, Oniscidea) from Brazilian caves. *Nauplius* 24: e2016001.

Campos-Filho, I.S.; Gallo, J.S.; Gallão, J.E.; Torres, D.F.; Carpio-Diaz, Y.M.; Lopez-Orozco, C.M.; Borja-Arrieta, R.; Taiti, S. & Bichuette, M.E. 2022a. Expanding the knowledge on the diversity of the cavernicolous Styloniscidae Vandel, 1952 (Oniscidea, Synocheta) from Brazil, with descriptions of two new species from the semiarid karst regions. *Zookeys* 1101: 35-55.

Campos-Filho, I.S.; Gallo, J.S.; Gallão, J.E.; Torres, D.F.; Horta, L.; Carpio-Díaz, Y.M.; López-Orozco, C.M.; Borja-Arrieta, R.; Aguiar, J.O. & Bichuette, M.E. 2022b. Unique and fragile diversity emerges from Brazilian caves - two new amphibious species of *Xangoniscus* Campos-Filho, Araujo & Taiti, 2014 (Oniscidea, Styloniscidae) from Serra do Ramalho karst area, state of Bahia, Brazil. *Subterranean Biology* 42: 1-22.

Canedo, C.; Targino, M.; Leite, F.S.F. & Haddad, C.F.B. 2012. A new species of *Ischnocnema* (Anura) from the São Francisco basin karst region, Brazil. *Herpetologica* 68: 393-400.

Cardoso, G.M.; Bastos-Pereira, R. & Ferreira, R.L. 2022. A new species of *Chaimowiczia* from the karstic Serra do Ramalho plateau, Brazil (Oniscidea, Synocheta, Styloniscidae). *Subterranean Biology* 42: 139-149.

Cardoso, G.M.; Bastos-Pereira, R. & Ferreira, R.L. 2023. Cave-dwellers *Diploexochus* (Isopoda, Armadillidae): new species and new records of the genus from Brazil. *Nauplius* 31: e2023008.

Cordeiro, I.R.S.; Bichuette, M.E. & Moreira, F.F.F. 2023. A new species of *Spelaeometra* Polhemus & Ferreira, 2018 (Hemiptera: Heteroptera: Hydrometridae) from a hotspot of troglobites in Brazil, Serra do Ramalho karst area. *Animals* 13: 3199.

Dessen, E.M.B.; Eston, V.R.; Silva, M.S.; Temperini-Beck, M.T. & Trajano, E. 1980. Levantamento preliminar da fauna de cavernas de algumas regiões do Brasil. *Ciência e Cultura* 32: 714-725.

Dotmatics. 2023. Geneious Prime. Versão 2023.2.1.

Fernandes, C.S.; Campos-Filho, I.S.; Araujo, P.B. & Bichuette, M.E. 2019. Synopsis of terrestrial isopods (Crustacea: Isopoda: Oniscidea) from Brazilian caves, with emphasis on new records from north, midwest, northeast and southeast regions. *Journal of Natural History* 53: 1095-1129.

Ferreira, R.L.; Souza-Silva, M. & Zampaulo, R.d.A. 2022. A vida subterrânea no carste de Pains: biodiversidade, ameaças e conservação de fauna em uma notável paisagem cárstica tropical. *In*: Piló, L.B.Cruz, J.B. (eds.), A região cárstica de Pains. ICMBio. Brasília

Frost, D.R. 2025. Amphibian Species of the World: an Online Reference. Version 6.2. Avaiable at: https://amphibiansoftheworld.amnh.org/index.php. Access in: April 4th, 2025.

Gallão, J.E. & Bichuette, M.E. 2018. Brazilian obligatory subterranean fauna and threats to the hypogean environment. *Zookeys* 1-23.

Gouveia, S.F.; Rocha, P.A.d.; Mikalauskas, J.S. & Silveira, V.V.-B. 2009. *Rhinella jimi* (cururu toad) and *Leptodactylus vastus* (Northeastern pepper frog). Predation on bats. *Herpetological Review* 40: 210.

Haddad, C.F.B.; Toledo, L.F.; Prado, C.P.A.; Loebmann, D.; Gasparini, J.L. & Sazima, I. 2013. Guia dos Anfíbios da Mata Atlântica - Diversidade e Biologia. Anolis Books. São Paulo.

Hillman, S.S.; Withers, P.C.; Drewes, R.C. & Hillyard, S.D. 2009. Ecological and environmental physiology of amphibians. Oxford University Press Inc. New York.

Jared, C.; Mailho-Fontana, P.L.; Mendelson, J. & Antoniazzi, M.M. 2019. Life history of frogs of the Brazilian semi-arid (Caatinga), with emphasis in aestivation. *Acta Zoologica* 101: 302-310.

Lamas, C.J.E.; Mendes, L.L.; Falaschi, R.L. & Evenhuis, N.L. 2023. A second new species of *Tamanduamyia* Rafael

and Limeira-de-Oliveira (Diptera, Mythicomyiidae, Mythicomyiinae) from Brazil, with discussion of the possible first cave dwelling record of a micro-bee fly. *Neotropical Entomology* 52: 560-570.

Lima, A.M.X.; Araujo, C.d.O. & Verdade, V.K. 2012. *Cycloramphus eleutherodactylus* (alto button frog): calling among rocks and caves. *Natural History Notes* 39-42.

Linares, A.M. & Mello, H.E.S.d. 2011. *Physalaemus cicada* Bokermann, 1966 (Anura: Leiuperidae): Distribution extension with new south limit and geographic distribution map. *Check List* 7: 859-861.

Lisboa, B.S. & Haddad, C.F.B. 2009. Amphiba, Anura, Leiuperidae, *Physalaemus cicada* Bokermann, 1966: Distribution extension and geographic distribution map. *Check List* 5: 699-701.

Lyra, M.L.; Haddad, C.F.B. & de Azeredo-Espin, A.M.L. 2017. Meeting the challenge of DNA barcoding Neotropical amphibians: polymerase chain reaction optimization and new COI primers. *Molecular Ecology Resources* 17: 966-980.

Magalhães, F.d.M.; Lyra, M.L.; Carvalho, T.R.d.; Baldo, D.; Brusquetti, F.; Burella, P.; Colli, G.R.; Gehara, M.C.; Giaretta, A.A.; Haddad, C.F.B.; Langone, J.A.; López, J.A.; Napoli, M.F.; Santana, D.J.; de Sá, R.O. & Garda, A.A. 2020. Taxonomic review of South American butter frogs: phylogeny, geography patterns, and species delimitation in the *Leptodactylus latrans* species group (Anura: Leptodactylidae). *Herpetological Monographs* 34: 131-177.

Matavelli, R.; Campos, A.M.; Feio, R.N. & Ferreira, R.L. 2015. Occurrence of anurans in Brazilian caves. *Acta Carsologica* 44: 107-120.

Mattox, G.M.T.; Bichuette, M.E.; Secutti, S. & Trajano, E. 2008. Surface and subterranean ichthyofauna in the Serra do Ramalho karst area, northeastern Brazil, with updated lists of Brazilian troglobitic and troglophilic fishes. *Biota Neotropica* 8: 145-152.

Motta, A.P.; Lyra, M.; Gazoni, T.; Parise-Maltempi, P.P. & Haddad, C.F.B. 2020. New distribution records of *Oreobates antrum* Vaz-Silva, Maciel, Andrade, and Amaro, 2018, a cave-associated species from the Seasonally Dry Tropical Forest in the Brazilian Cerrado (Anura: Brachycephalidae: Craugastoridae). *Herpetology Notes* 13: 561-563.

Narvaes, P. & Rodrigues, M.T. 2009. Taxonomic revision of *Rhinella granulosa* species group (Amphibia, Anura, Bufonidae), with a description of a new species. *Arquivos de Zoologia* 40: 1-73.

Navas, C.A.; Antoniazzi, M.M. & Jared, C. 2004. A preliminary assessment of anuran physiological and morphological adaptation to the Caatinga, a Brazilian semi-arid environment. *International Congress Series* 1275: 298-305.

NCBI; Camacho, C.; Madden, T.; Tao, T.; Agarwala, R. & Morgulis, A. 2019. BLAST Command Line Applications User Manual. National Center for Biotechnology Information. Bethesda, MD.

Nunes-de-Almeida, C.H.L.; Haddad, C.F.B. & Toledo, L.F. 2021. A revised classification of the amphibian reproductive modes. *Salamandra* 57: 413-427.

Oliveira, L.A.d.; Hoyos-Benjumea, D.; Zepon, T.; Bichuette, M.E. & Vieira, L. 2023. New records of ground beetles genera (Coleoptera: Carabidae: Scaritinae: Clivinini) from Brazilian caves. *EntomoBrasilis* 16: 1-11.

Orrico, V.G.D.; Nunes, I.; Mattedi, C.; Fouquet, A.; Lemos, A.W.; Rivera-Correa, M.; Lyra, M.L.; Loebmann, D.; Pimenta, B.V.S.; Caramaschi, U.; Rodrigues, M.T. & Haddad, C.F.B. 2017. Integrative taxonomy supports the existence of two distinct species within *Hypsiboas crepitans* (Anura: Hylidae). *Salamandra* 53: 99-113.

Palmeira, C.N.S.; Gonçalves, U. & Paz, V.N. 2011. *Physalaemus albifrons* Spix, 1824 (Anura: Leiuperidae): Distribution and geographic extension. *Check List* 7: 839-840.

Palumbi, S.; Martin, A.; Romano, S.; McMillan, W.O.; Stice, L. & Grabowski, G. 2002. The simple fool's guide to PCR. University of Hawaii. Honolulu, Hawaii.

Pansonato, A.; Motta, A.; Cacciali, P.; Haddad, C.F.B.; Strüssmann, C. & Jansen, M. 2020. On the identity of species of *Oreobates* (Anura: Craugastoridae) from Central South America, with the description of a new species from Bolivia. *Journal of Herpetology* 54: 393-412.

Pellegrini, T.G.; Bichuette, M.E. & Vieira, L. 2022. *Coarazuphium bambui* (Carabidae: Zuphiini), a new cave-dwelling beetle from the threatened region of Serra do Ramalho, Brazil. *Zootaxa* 5129: 557-568.

Pereyra, M.O.; Baldo, D.; Blotto, B.L.; Iglesias, P.P.; Thomé, M.T.C.; Haddad, C.F.B.; Barrio-Amorós, C.; Ibáñez, R. & Faivovich, J. 2016. Phylogenetic relationships of toads of the *Rhinella granulosa* group (Anura: Bufonidae): a molecular perspective with comments on hybridization and introgression. *Cladistics* 32: 36-53.

Pinto-da-Rocha, R. 1995. Sinopse da fauna cavernícola do Brasil (1907-1994). *Papéis Avulsos de Zoologia* 39: 61-173.

Racovitza, E.G. 1907. Essai sur les problèmes biospéologiques. *Archives de Zoologie Experimentale et Génerale* 6: 371-488.

Rubbioli, E.; Auler, A.; Menin, D. & Brandi, R. 2019. Cavernas. Atlas do Brasil subterrâneo. Instituto Chico Mendes de Conservação da Biodiversidade. Brasília, Brasil.

Salvador, R.B. & Bichuette, M.E. 2024. *Idiopyrgus* Pilsbry, 1911 (Gastropoda, Tomichiidae): a relict genus radiating into subterranean environments. *Zoosystematics and Evolution* 100: 1543-1556.

Salvador, R.B.; Santos Silva, F. & Bichuette, M.E. 2023. Taxonomic study on a collection of terrestrial and freshwater gastropods from caves in Bahia state, Brazil, with the description of a new species. *Folia Malacologica* 31: 48-60.

Santos, D.L.; Andrade, S.P.; Rocha, C.F.; Maciel, N.M.; Caramaschi, U. & Vaz-Silva, W. 2017. Redescription of the tadpole of *Odontophrynus carvalhoi* Savage and Cei, 1965 (Anura, Odontophrynidae) with comments on the geographical distribution of the species. *Zootaxa* 4323: 419-422.

Santos, T.d.; Marques de Souza, A.; Bondezan, F.L. & Eterovick, P.C. 2022. Going underground: what the natural history traits of cave users can tell us about cave use propensity. *Journal of Herpetology* 56: 153-163.

Schiner, J.R. 1854. Fauna der Adelsberger-, Lueger- und Magdalen-Grotte. *Verhandlungen der Zoologisch-Botanischen Gesellschaft* 3: 1-40.

Simone, L.R.L. 2012. A new genus and species of cavernicolous Pomatiopsidae (Mollusca, Caenogastropoda) in Bahia, Brazil. *Papéis Avulsos de Zoologia* 52: 515-524.

Simone, L.R.L. 2013. *Habeas*, a new genus of Diplommatinidae from central Bahia, Brazil (Caenogastropodae), with description of

three new species. *Journal of Conchology* 41: 519-525.

Sperandei, V.d.F.; Alvarenga, D.d.A.; Araújo, M.S.d. & Pereira, C.C. 2023. Are caves true habitats for anurans or more a favorable rocky environment? A discussion of habitat occupation by frogs in Neotropical caves. *Neotropical Biology and Conservation* 18: 131-137.

Sperandei, V.d.F.; Vaz-Silva, W. & Leonardo Tejerina-Garro, F. 2024. Characterization of anuran fauna inside caves in Brazil. *Biota Neotropica* 24: e20241657.

Teixeira Jr., M.; Amaro, R.C.; Recoder, R.S.; Sena, M.A.d. & Rodrigues, M.T. 2012. A relict new species of *Oreobates* (Anura, Strabomantidae) from the seasonally dry tropical forests of Minas Gerais, Brazil, and its implication to the biogeography of the genus and that of South American dry forests. *Zootaxa* 3158: 37-52.

Thomé, M.T.C.; Carstens, B.C.; Rodrigues, M.T.; Alexandrino, J. & Haddad, C.F.B. 2021. Genomic data from the Brazilian sibilator frog reveal contrasting pleistocene dynamics and regionalism in two South American dry biomes. *Journal of Biogeography* 48: 1112-1123.

Trajano, E. 1987. Fauna cavernícola brasileira: composição e caracterização preliminar. *Revista Brasileira de Zoologia* 3: 533-561.

Trajano, E. 2012. Ecological classification of subterranean organisms. *In*: White, W.B.Culver, D.C. (eds.), Encyclopedia of Caves. Elsevier Academic Press. Amsterdan

Trajano, E. & Bichuette, M.E. 2006. Biologia subterrânea: introdução. Redespeleo Brasil. São Paulo.

Trajano, E. & Bichuette, M.E. 2010a. Diversity of Brazilian subterranean invertebrates, with a list of troglomorphic taxa. *Subterranean Biology* 7: 1-16.

Trajano, E. & Bichuette, M.E. 2010b. Relevance of caves: why environmental studies have been inadequate. *Espeleo-Tema* 21: 105-112.

Trajano, E. & Gnaspini-Netto, P. 1991. Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos taxons. *Revista Brasileira de Zoologia* 7: 383-407.

Trajano, E.; Secutti, S. & Bichuette, M.E. 2009. Natural history and population data of fishes in caves of the Serra do Ramalho karst area, Middle São Francisco basin, northeastern Brazil. *Biota Neotropica* 9: 129-133.

Vaz-Silva, W.; Maciel, N.M.; de Andrade, S.P. & Amaro, R.C. 2018. A new cryptic species of *Oreobates* (Anura: Craugastoridae) from the seasonally dry tropical forest of central Brazil. *Zootaxa* 4441: 89-108.

Appendix

Table A1. Caves sampled in the Serra do Ramalho, a limestone karst area, part of the Bambuí group. Each cave is with its respective "ID" number, elevation (in meters, a.s.l.), and coordinates (latitude and longitude, WGS84). Caves in bold are the caves in which we found amphibians.

ID	Cave	Elevation (m a.s.l.)	Latitude	Longitude
1	Gruna Baixão da Canoa	–	13°51'27.6000"S	44°09'55.3000"W
2	Gruna do Chico Pernambuco	697	13°49'09.0000"S	44°04'14.9000"W
3	Gruna do Domingão	799	13°44'40.7000"S	43°49'59.7000"W
4	Caverna Viração	702	13°32'58.8000"S	43°52'42.1000"W
5	Gruna Água Fina	803	13°41'55.6000"S	43°48'04.5000"W
6	Gruna Bem Bom	691	13°35'03.3000"S	43°57'51.2000"W
7	Gruna Boca da Lapa	544	13°56'46.3843"S	44°11'11.9794"W
8	Gruna da Água Clara	491	13°48'03.1478"S	43°57'05.5534"W
9	Gruna da Água Escura I	482	13°49'03.6997"S	43°57'01.3195"W
10	Gruna da Água Espalhada	–	13°41'28.4000"S	44°23'43.1000"W
11	Gruna da Altina	496	13°33'38.1000"S	43°45'17.2000"W
12	Gruna da Cacimbinha	676	13°42'41.1523"S	44°13'42.6306"W
13	Gruna da Mamona	573	13°37'05.1861"S	44°14'38.9007"W
14	Gruna da Pingueira II	715	13°49'39.4445"S	44°08'39.1169"W
15	Gruna da Serra Solta II	–	13°30'43.7000"S	43°45'12.3000"W
16	Gruna da Serra Solta III	506	13°30'47.6821"S	43°45'17.0714"W
17	Gruna da Serra Verde	–	13°43'27.9000"S	44°19'27.6000"W
18	Gruna das Três Cobras	804	13°37'06.3000"S	43°45'09.1000"W
19	Gruna do Cesário	457	13°31'06.1105"S	43°38'26.2070"W
20	Gruna do Cocho	514	13°36'55.6000"S	43°46'11.7000"W
21	Gruna do Enfurnado	638	13°38'45.8000"S	44°12'07.9000"W
22	Gruna do Engrunado	693	13°56'19.7664"S	44°16'01.1646"W
23	Gruna do Govi	684	13°56'43.3004"S	44°14'25.9451"W
24	Gruna do Ibelmon	–	13°24'21.5000"S	44°07'58.4000"W

25	Gruna do Mandiaçu	–	13°32'00.7000"S	43°45'37.2000"W
26	Gruna do Zeferini	708	13°46'15.6879"S	44°14'04.5732"W
27	Gruna dos Índios	494	13°48'04.7076"S	43°57'04.9468"W
28	Gruna Google	803	13°37'41.6000"S	43°48'48.3000"W
29	Gruna Grande	493	13°36'08.8977"S	43°45'18.1887"W
30	Gruna Pedro Cassiano	–	13°47'48.0000"S	43°54'50.0000"W
31	Gruna Vandercir I e II	725	13°38'11.4000"S	43°50'05.1000"W
32	Gruna Vila Nova	720	13°33'14.2673"S	43°52'40.7352"W
33	Lapa dos Peixes	487	13°49'22.0928"S	43°57'25.1839"W
34	Sistema Euzébio	–	13°28'29.2638"S	44°23'19.0320"W

Table A2. Specimens collected from Serra do Ramalho, part of the Bambuí group, with their respective specimen number in zoological collections, locality, municipality in Bahia, and collection date. The column "Barcode" marks the specimens that we confirmed through 16S sequencing. An asterisk (*) denote the species of *Scinax* that grouped with S. "sp. 26" from the study of Araujo-Vieira *et al.* (2023). The acronym LESV stands for Laboratório de Estudos Subterrâneos Vertebrate Collection (Universidade Federal de São Carlos, São Carlos, SP, Brazil; curator: M.E. Bichuette), and the acronym CFBH stands for the Coleção de Anfíbios Célio F. B. Haddad (CFBH, Universidade Estadual Paulista, Rio Claro, SP, Brazil; curator: C.F.B. Haddad).

Specimen number	Species	Locality	Municipality	Collection date	Barcoded
CFBH 32594	*Physalaemus cuvieri*	Gruna Vila Nova	Carinhanha	11.ix.2008	
CFBH 32595	*Odontophrynus carvalhoi*	Gruna Google	Carinhanha	13.ix.2008	
CFBH 32599	*Rhinella granulosa*	Gruna do Cocho	Carinhanha	02.vi.2012	X
CFBH 32942	*Leptodactylus macrosternum*	Gruna do Domingão	Carinhanha	27.vii.2012	X
CFBH 32943	*Leptodactylus macrosternum*	Gruna do Domingão	Carinhanha	27.vii.2012	X
CFBH 32944	*Odontophrynus* cf. *carvalhoi*	Gruna Chico Pernambuco	Coribe	28.vii.2012	X
CFBH 32945	*Odontophrynus* cf. *carvalhoi*	Gruna Chico Pernambuco	Coribe	28.vii.2012	
CFBH 32946	*Odontophrynus* cf. *carvalhoi*	Gruna Chico Pernambuco	Coribe	28.vii.2012	
CFBH 40905	*Physalaemus cuvieri*	Gruna da Altina	Carinhanha	27.xi.2015	
CFBH 48263	*Rhinella mirandaribeiroi*	Gruna Serra Solta II	Serra do Ramalho	24.x.2023	
CFBH 48264	*Physalaemus albifrons*	Gruna Serra Solta II	Serra do Ramalho	24.x.2023	
LESV 663	*Rhinella granulosa*	Gruna do Domingão	Carinhanha	01.viii.2019	
LESV 671	*Leptodactylus troglodytes*	Gruna do Domingão	Carinhanha	01.viii.2019	
LESV 671	*Physalaemus cicada*	Gruna do Domingão	Carinhanha	01.viii.2019	
LESV 710	*Leptodactylus macrosternum*	Gruna do Engrunado	Feira da Mata	13.x.2020	X
LESV 712	*Leptodactylus macrosternum*	Gruna da Boca da Lapa	Feira da Mata	15.x.2020	
LESV 713	*Oreobates remotus*	Gruna do Engrunado	Feira da Mata	13.x.2020	X
LESV 714	*Oreobates remotus*	Gruna do Engrunado	Feira da Mata	13.x.2020	X
LESV 748	*Scinax x-signatus*	Gruna Bem Bom	Carinhanha	07.vii.2021	
LESV 749	*Scinax x-signatus*	Gruna Bem Bom	Carinhanha	07.vii.2021	X
LESV 750	*Scinax x-signatus*	Gruna Bem Bom	Carinhanha	07.vii.2021	
LESV 751	*Scinax x-signatus*	Gruna Bem Bom	Carinhanha	07.vii.2021	
LESV 752	*Oreobates remotus*	Gruna Bem Bom	Carinhanha	07.vii.2021	X
LESV 753	*Scinax* sp. *26**	Gruna Bem Bom	Carinhanha	07.vii.2021	X
LESV 754	*Boana crepitans*	Gruna Bem Bom	Carinhanha	07.vii.2021	X
LESV 755	*Leptodactylus vastus*	Gruna da Mamona	Coribe	09.vii.2021	
LESV 756	*Leptodactylus macrosternum*	Gruna da Mamona	Coribe	09.vii.2021	X