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

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Abstract. *Bokermannohyla martinsi* is an endemic anuran from the mountains of the southernmost portion of the Espinhaço range in southeastern Brazil. This region is known as the Iron Quadrangle (Quadrilátero Ferrífero) due to its iron-rich outcrops and is under intensive mining exploration, which threatens its caves. Here, we investigated whether males, females, and juveniles of *B. martinsi* use iron caves throughout the year, exhibit cave fidelity, distribute themselves differently within caves, and prefer to use caves that are microclimatically stable. We sampled 10 caves during eight 15-day periods regularly spaced throughout one year. Frogs used caves throughout the year and exhibited cave fidelity. They preferred the dysphotic zone, and young individuals remained closer to the entrance of the cave and to the ground, maybe due to their lower locomotory/climbing ability compared to adult frogs. *Bokermannohyla martinsi* preferred more stable caves (those with lower temperature and humidity variation), and the use of caves by females was more intensive during the dry and cold season, which may be related to their shorter permanence at breeding sites (streams). Our results show that caves are important habitats for *B. martinsi*, as it may be the case with other species, too, and their destruction should thus be evaluated and weighted with greater care when planning their mining.

Key words. Amphibia, cave habitat, frog, site fidelity.

Introduction

A broad definition of "cave" as provided by Culver & Pipan (2019) is that it is "a natural opening in solid rock with areas of complete darkness, and larger than a few millimeters in diameter". Subsurface habitats include both large (actual caves) and small cavities (interstitial habitats; Culver & Pipan 2019). Cave use in animals can be classified according to their dependence on a hypogean (subterranean) habitat. Based on an adaptation of the Schinner-Racovitza system (Holsinger & Culver 1988) provided by Sket (2008), (1) troglobionts are obligatory inhabitants of the hypogean habitat; (2) eutroglophiles are epigean (surface) species with subterranean population(s); (3) subtroglophiles may inhabit subterranean habitats for varied periods of time, but also need to exploit the epigean habitat for some resources; and (4) trogloxenes only occur sporadically underground. However, varied combinations of morphological, physiological, and behavioural adaptations influence the level of association of species with caves,

making it sometimes difficult to classify them in the absence of complete information (Howarth & Moldovan 2018).

Different from Europe, where the amphibian fauna includes troglobiont species and other species well known to maintain populations in caves (e.g., Blackburn 2019, Lunghi et al. 2020), many frogs in South America and other regions have frequently been classified as accidental cave occupants when found in caves (Trajano 1986, Trajano & Gnaspini-Netto 1990, Pinto-Da-Rocha 1995, Trajano & Bichuette 2006). However, several studies have demonstrated that some species may use caves regularly during parts of or their whole lifecycle (Resetarits Jr. 1986; Miller et al. 2008, Espino-Del-Castillo et al. 2009, Suwannapoom et al., 2018), being thus classified as trogloxenes or subtroglophiles (sensu Sket 2008).

Anurans were recorded at higher frequencies during autumn and winter in temperate caves, when environmental conditions are harsher outside, although they may also use cooler cave microhabitats during periods of high external

temperatures (Resetaritz Jr. 1986, Fenolio et al. 2005, Lunghi et al. 2017). However, standardized monitoring studies that relate anuran presence in caves to climatic variables are rare, especially from tropical regions (Espino-Del-Castillo et al. 2009). Anurans are sensitive to temperature and humidity conditions due to their highly permeable skin (Wells 2007), and caves may be useful shelters for these animals during harsh weather (Matavelli et al. 2015). Caves have more stable microclimatic conditions, meaning they have little temperature variation and high humidity compared to the outside (Culver & Pipan 2019). There is great variation in microclimatic features among caves, with long caves with small entrances being more stable than short caves with large entrances, as the latter are more influenced from outside conditions (Ferreira 2004, Pellegrini et al. 2016). Although iron caves are usually not very deep (Oliveira et al. 2011), they can have humidity and temperature conditions similar to those of caves from other lithologies, becoming relatively stable with increased distance from the entrance (Ferreira 2005).

Brazil hosts the highest amphibian richness in the world, with 1,091 known species (Segalla et al. 2019), 5% (54 species distributed in 19 genera and 11 families) of which have already been recorded from inside caves (Matavelli et al. 2015). Iron caves have been used by 23 of these species (14 genera and 10 families), more than any other cave lithology (Matavelli et al. 2015). Iron caves are usually shallow and relatively short, with a mean development of 25 m in the Iron Quadrangle in southeastern Brazil (Oliveira et al. 2011). However, these caves shelter a high invertebrate diversity, including troglobiont species (Ferreira 2005). The Iron Quadrangle is a unique tropical iron-rich rock outcrop with characteristic vegetation formations and high species diversity (Jacobi et al. 2007, Leite et al. 2008), but is greatly endangered by the expansion of mining activities (Jacobi et al. 2007).

Bokermannohyla martinsi (Bokermann, 1964) is endemic to the Iron Quadrangle in southeastern Brazil (Pinheiro et al. 2014), where it has been recorded only from above 800 m a.s.l. (Caramaschi et al. 2010). It breeds in permanent streams surrounded by riparian forests (Bokermann 1964, Caramaschi et al. 2010). Although it is listed as 'of least concern' in the IUCN Red List of Threatened Species, it is classified as 'near threatened' in the Brazilian List of Endangered Species (Haddad et al. 2016) due to its restricted distribution and intensive habitat destruction (mostly by mining activities) (Canelas & Bertoluci 2007, Haddad et al. 2016). Matavelli et al. (2015) recorded *B. martinsi* in iron caves, and it has been recorded also in limestone and quartzitic caves (M. C. M. Andrade, pers. obs.). The Iron Quadrangle is the region with the second highest number of iron caves in Brazil and the one with the highest concentration, encompassing 1083 known caves in an area of 490 km² , following the Carajás region, with 1787 known caves in an area of 4196 km² (Oliveira et al. 2011, Rubbioli et al. 2019). Information on the importance of these caves to *B. martinsi*, and other anuran species, could be useful to understand the general

Table 1. Features of sampled caves at the Serra do Gandarela National Park, southeastern Brazil including cave identification (Cave id.), area, altitude above sea level (alt.), linear extension (LE), number of entrances, and summed extension of all entrances (ent/extent), Environmental Stability Index (ESI), mean temperature amplitude (MTA), and mean humidity amplitude (MHA) measured within caves, and number of individuals of *Bokermannohyla martinsi* recorded from September 2017 to August 2018.

Cave id.	Area	alt. (m)	LE (m)	ent/ extent	ESI	MTA	MHA	Number of frogs
Apol-25	1	1607	12.3	3/3.26	1.41	0.87	1.21	3
SG-16	1	1618	51.1	3/5.77	1.89	1.04	1.23	11
SG-20	1	1601	33.3	5/6.2	1.04	1.23	2.83	3
SG-51	1	1627	55.4	1/4.2	2.58	0.16	0.40	20
SG-17	1	1602	7.4	3/4.75	0.29	1.67	4.22	0
SG-18	1	1603	9.0	3/2.97	0.89	1.08	1.88	0
SG-19	1	1602	8.2	4/5.27	0.90	0.75	1.48	0
Gand-056	2	1517	9.9	1/2.75	1.77	0.61	3.32	5
Gand-096	2	1554	45.0	5/5.18	1.91	0.85	2.06	20
Gand-008	2	1552	98.9	8/6.38	2.66	0.50	0.94	39

importance of caves as habitats for these animals, especially in regions where both caves and species that use them are endangered.

Here, we investigated whether the use of caves by *B. martinsi* is intentional and not occasional. Thus, we tested the predictions that individuals of *B. martinsi* (1) use iron caves throughout the year, (2) exhibit cave fidelity, (3) go deeper into caves and are less active during periods with harsher external climatic conditions, and (4) prefer to use caves that are more stable regarding temperature and humidity and depending on external climatic conditions. We accounted for potential differences between sex/age groups (males, females and juveniles) in all analyses. We expected frogs to venture farther from the entrance in search of greater microclimatic stability when external humidity is low, which usually coincides with lower temperatures in the region. We also expected frogs to be less frequently active under such circumstances, as their breeding season (and expected higher activity levels) falls into the wet season.

Materials and methods
Study site

We sampled 10 natural iron caves in two different areas in the Serra do Gandarela National Park (20°6'3.24" S, 43°39'48.33" W), southeastern Brazil (Fig. 1, Table 1). This region is covered by typical ironstone outcrop plant communities (Jacobi et al. 2007) with Atlantic Forest on the northeastern slope and Cerrado formations in the southwestern portion (Lamounier 2009). This region has a wet season from October through March and a dry season from April through September.

The sampled caves have no bodies of water inside them; their humidity is generated by rain that permeates through fissures in the rock. The two sampled areas are separated by about 1 km (Fig. 1). One lies higher up on the terrain and encompasses seven caves (1601–1627 m a.s.l.), with distances between caves varying from 11 to 390 m. The other is on the slope and encompasses three caves (1517–1554 m a.s.l.), with distances between caves varying from nine to 14 m. The linear extension of the 10 caves varies from 7.4 to 98.9 m (mean = 33 m).

Species sampling

We sampled caves during eight periods between September 2017 and August 2018, with a minimum interval of 30 days between consecutive sampling periods. Two 15-day sampling periods were undertaken within each of four three-month periods that we considered as early wet season (October–December), late wet season (January–March), early dry season (April–June), and late dry season (July–September), respectively. During each 15-day sampling period, we sampled each cave for frogs on Days 1, 8 and 15, during the day. Sampling order was randomized among caves. We searched the whole extension of each cave from the floor to the ceiling visually with torchlights and listened for possible frog calls. We only moved forward after checking every surface that we could see, and these encompassed almost the entire expanses of the caves. Sampling time per cave lasted from 3 min. (SG-17, SG-18, and SG-19) to 3 hours and 18 min. (GAND 008), with a mean of 24 min. Frog call recording and the manipulation of specimens are included in these times. The total sampling effort summed up to 96 hours and 18 min/person.

We measured the snout–vent-length (SVL) of each frog and identified the sexes of sexually mature individuals as follows: males are smaller than females and have hypertrophied arms and an ossified prepolex (Bokermann 1964, Lima et al. 2013, Magalhães et al. 2018). Thus, we considered as adult males those individuals showing these fea-
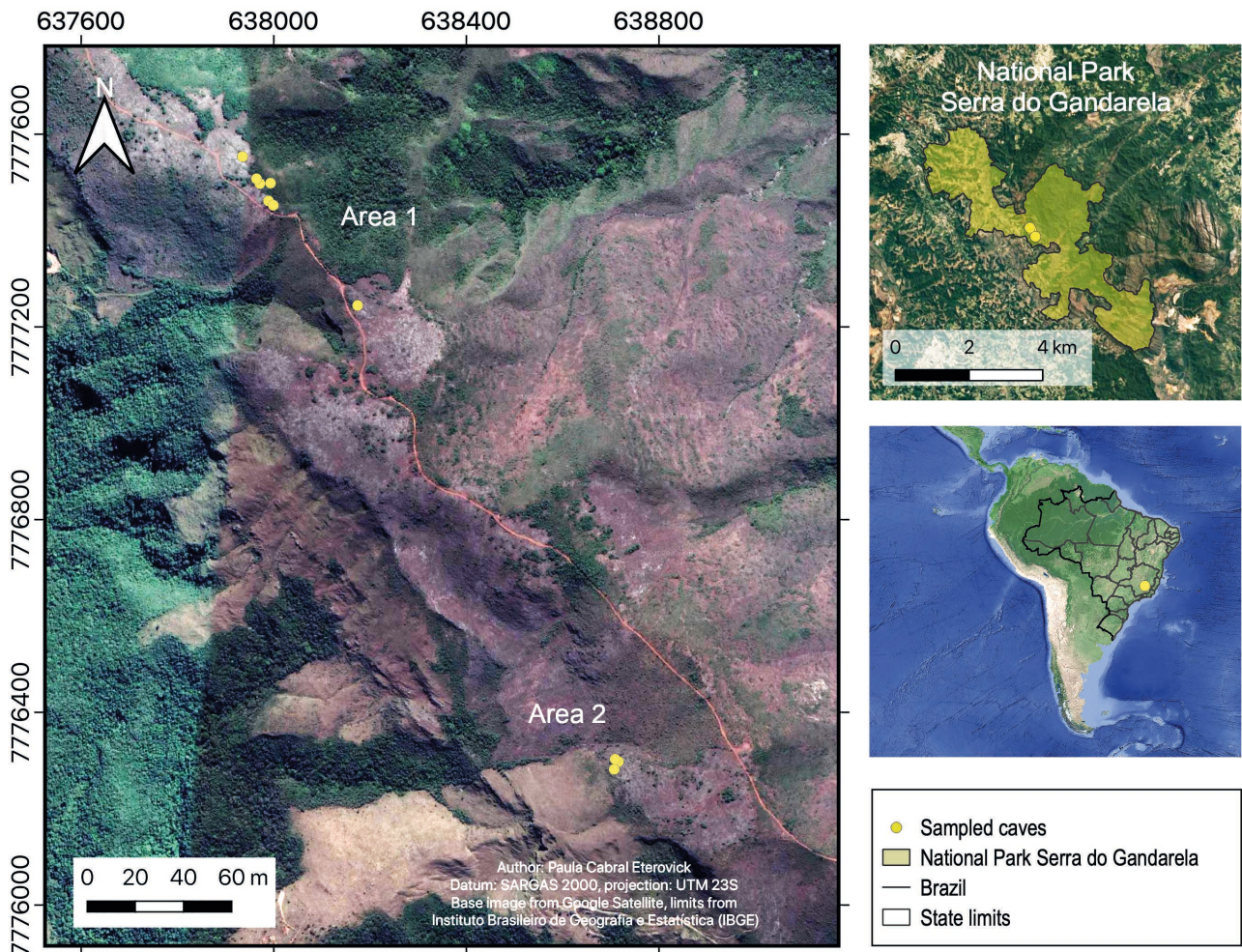

Figure 1. Location of the two areas and the ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil. The points in the maps with larger scales also represent the locations of the caves for reference (points overlap due to scale).

tures and as adult females those that were larger than the smallest adult male recorded (56 mm SVL) and did not present these features. Any individual smaller than 56 mm was considered juvenile.

We photographed the dorsal region and limbs of every individual for subsequent identification, as their colour patterns are highly variable and facilitate individual recognition (Fig. 2). This photographic identification method has already been proved successful in other anuran species and renders unnecessary the use of invasive marking techniques (Miranda et al. 2005, Kenyon et al. 2009, Lama et al. 2011, Russo et al. 2018) and minimizes the handling of individuals (Lunghi et al. 2020). We created a photo archive for the identification of recaptured individuals with no need for additional manipulation. We released each frog at the exact same spot of capture after taking measurements and pictures.

We recorded, for each frog, its distance from the nearest cave entrance (irrespective of its being usable to humans or not), height above the floor, and light incidence. We measured the distances with a laser distance meter (Leica Disto™ D8) with a range from 0.05 to 200 m, and a precision of 1.5 mm. We classified light incidence into three categories: (1) euphotic, close to the entrance with direct light incidence (that is, with insolation at some time during the day) and presence of photosynthetic organisms, (2) dysphotic, with indirect light incidence (with no direct insolation), and (3) aphotic, without any incidence of light (adapted from Soares et al. 2013). As the caves are not long (Table 1), only the three longest actually had aphotic zones. We also recorded the activity level of each individual at the moment of its discovery as inactive (resting with the eyes closed, body flattened to the surface, and limbs held close to the body, not responding to the torchlight) or active (awake with limbs extended to any extent and responsive to the torchlight).

Environmental features inside and outside caves

We recorded internal temperatures and humidity levels of the caves with data loggers (digital thermo hygrometers AKSO®, model AK174) placed on the floor in the middle of each cave's linear extension, which corresponded to the area where most frogs were located, based on our previous observations. We also placed identical data loggers outside each of the two groups of caves to record temperature and humidity. We set them up on the first day of each 15-day sampling period and retrieved them on the last day. The data loggers recorded the respective values every 30 minutes and we used mean values and standard deviations (SD) for each 15-day sampling period in our analyses (see Statistical analyses). We obtained data on mean temperature, mean humidity, and mean daily temperature and humidity amplitudes.
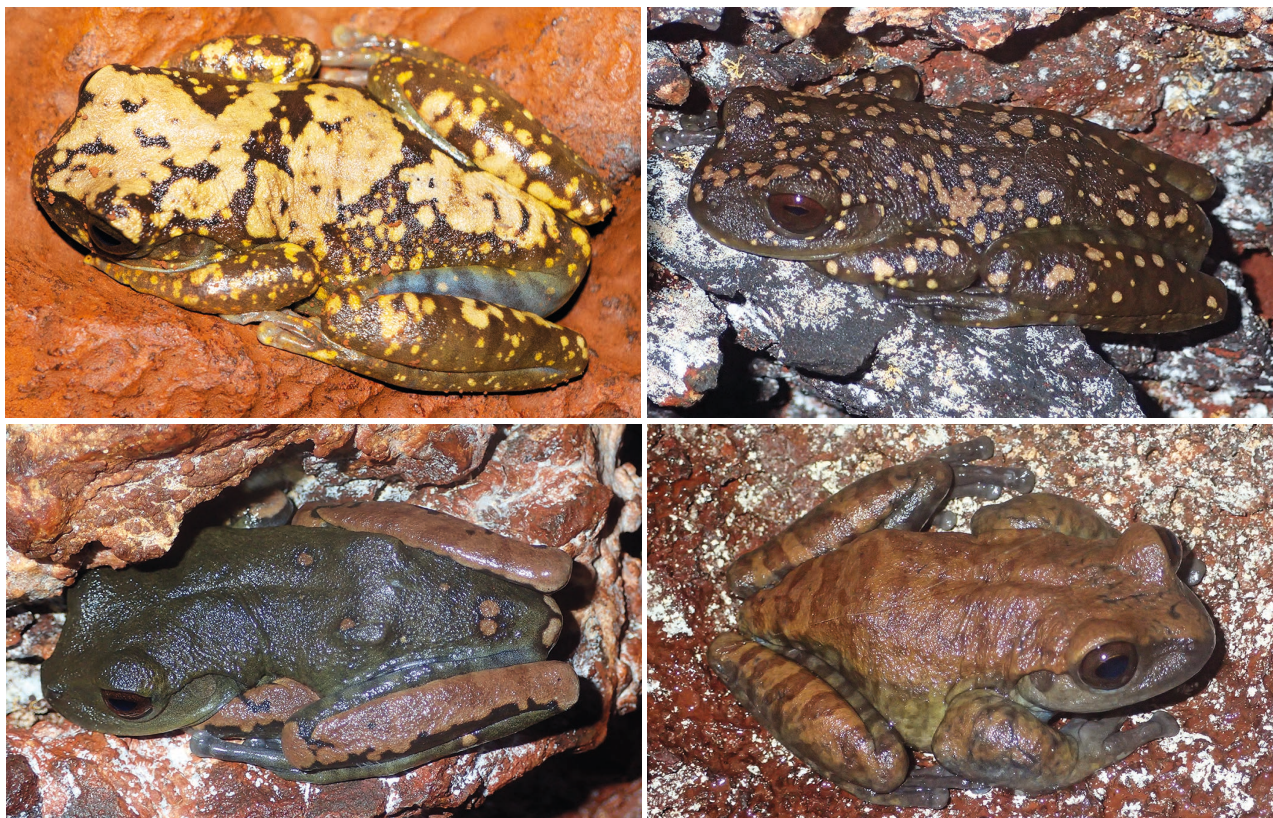

Figure 2. Individuals of *Bokermannohyla martinsi* illustrating the variation in dorsal colour patterns used for individual identification.

We calculated cave environmental stability using the Environmental Stability Index (ESI) proposed by Ferreira (2004). This index measures the influence of the external environment on the cave microclimate, taking into account the dimensions of the cave, as well as the number, positions, and sizes of entrances (Ferreira 2004, Bento et al. 2016, Pellegrini et al. 2016). For caves with a single entrance, ESI = ln (LE/EE), where LE = linear extension, and EE = entrance extension. The measurement of linear extension is obtained by summing up the longitudinal linear measurements of all conducts, irrespective of their inclination (Chabert & Watson 1981). We measured both LE and EE with the laser distance meter (Leica Disto™ D8).

For caves with more than one entrance, we applied

$$ESI = \ln \left(\frac{(LE/\Sigma EE)}{\frac{(NE)(DEE)}{LE}} \right)$$

where NE = number of entrances and DEE = mean of the distances from one entrance to all the others. Larger values of ESI indicate more stable caves. Caves with an ESI of between 1.00 and 3.50 are considered moderately stable, with large dysphotic zones (Ferreira 2004). We compared cave ESIs with the mean values of temperature and humidity amplitudes (MTA and MHA, respectively, that correspond to mean standard deviations) measured by us inside the caves per 15-day sampling period in order to confirm the applicability of the index (following Ferreira 2004). As we found ESIs to represent well cave stability concerning MTA (estimate = -0.77, t = -3.26, p = 0.011) and MHA (estimate = -0.44, t = -2.55, p = 0.034), we then used this index in subsequent analyses.

We obtained rainfall data from the meteorological station at the Santuário do Caraça Private Reserve (20°5'53.6" S, 43°29'17.0" W). We used mean values of the ten days prior to each 15-day sampling period in our analyses (see Statistical analyses). We considered this period to adequately represent the rainfall before our sampling periods, considering that it filled and maintained the water levels of the temporary streams where *B. martinsi* breeds at the study site (M. C. M. Andrade, pers. obs.) and thus likely influenced its activity levels.

Statistical analyses

We first confirmed individual frog identities through comparison of the photographs taken and calculated the numbers of recaptures, frog relocations between caves, and the numbers of individuals of each age/sex group in specific caves during each sampling period.

We tested whether perch height above the ground and distance from the cave entrance (log-transformed) were influenced by external climatic factors. We used data from every record of cave use by individual frogs. We tested the roles of (1) sex/age group (adult male, female or juvenile), (2) activity level, (3) mean external temperature, (4) mean external humidity amplitude, and (5) mean rainfall in the

Table 2. Seasonal variation in cave use throughout the year represented by numbers of males, females, juveniles, and all individuals recorded in each seasonal period in all sampled caves at the National Park Serra do Gandarela, from September 2017 to August 2018. These numbers account for 429 records from 94 individuals (several of them recorded in more than one seasonal period).

Sex and age class (number of individuals)	Late dry season	Early wet season	Late wet season	Early dry season
Total adults	44	28	24	26
Adult males	26	19	15	15
Adult females	18	9	9	11
Juveniles	25	8	8	20
Total individuals	69	36	32	46

previous 10 days as explanatory variables for frog (1) distance (log-transformed) from the nearest entrance to the cave, and (2) perch height above the ground as dependent variables. We did not include the mean external temperature amplitude, because it was correlated to the mean external humidity amplitude (Spearman's rho = 0.880, P < 0.001), nor mean external humidity, because it also correlated with the mean external humidity range (Spearman's rho = -0.905, p < 0.001). We used individual identity as a random variable to account for repeated encounters. We built Linear Mixed-Effects Models (LMM) using the package lme4 (Bates et al. 2015) in R (R Core Team 2020). Model significance was assessed by comparison with a null model including only the random variable.

We looked for cave features and external climatic features that influenced the use of caves by males, females and juveniles of *B. martinsi* throughout the year. We used data from each of the 10 caves in each one of the eight sampling periods, so that the dependent variable was the number of individuals (separate analyses for males, females, and juveniles) detected in each cave during each sampling period. Repeated records of the same individual during the same sampling period were not considered. The explanatory variables were ESI, mean external temperature, mean external humidity amplitude, and mean rainfall during the preceding 10 days. We used cave identity and sampling period as random variables. We built Generalized Linear Mixed Models (GLMM) with the function glmer (family Poisson, link = "log") in the package lme4 of R (Bates et al. 2015). We used the package MuMIn (Bartoń 2019) to select the best models from the global model using AICc. We tested for residual overdispersion with the package DHARMa (Hartig 2021). We used the package emmeans (Lenth 2020) for post hoc tests.

Results

We obtained 429 records of 94 individuals of *B. martinsi* (Table 2) in seven out of the ten sampled caves. These included four caves from Area 1 and all the three caves of

Area 2 (Fig. 1), with a maximum of 39 individuals recorded in one cave (Table 1). The caves with more records and individuals were the ones with greater linear extension (Table 1). Only seven out of the 94 recorded frogs used two different caves during the study. Two of these moved from cave SG-20 to cave SG-16 (Fig. 1), whose entrances are separated by 115 m (consecutive individual records were 35 and 50 days apart). Three individuals moved between Gand-056 and Gand-008, separated by 12 m (consecutive individual records were 5 to 58 days apart), and two others moved between Gand-096 and Gand-008, separated by nine metres (both individual records were 31 days apart).

We recorded 12 individuals (13%) during the four sampling periods, nine (10%) during three periods, 38 (40%) during two periods, and 35 (37%) during only one of the sampling periods. A mean of 59% of individuals were captured during one period and again during the following
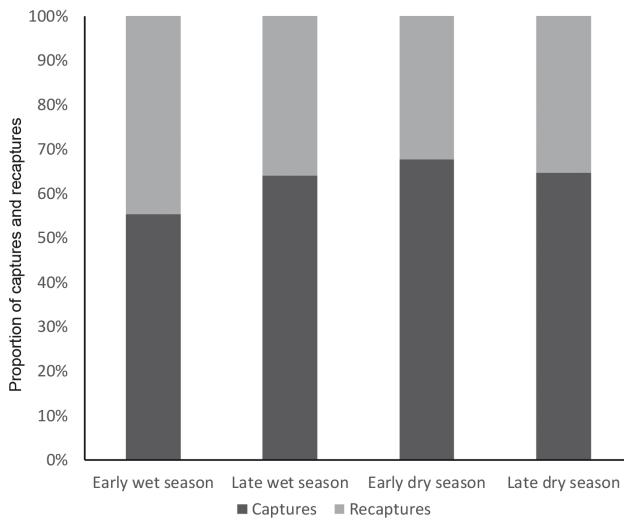

Figure 3. Number of individuals of *Bokermannohyla martinsi* captured during each season with the proportion representing recaptures from the previous season.

period, with the greatest recapture rates coinciding with the beginning of the wet season (66%) and the beginning of the dry season (63%; Fig. 3). The numbers of new juveniles increased at the end of the wet season and the beginning of the dry season, contributing to larger numbers of juveniles in caves recorded during these and subsequent months (February–August, Fig. 4), whereas the number of new adults increased from the beginning of the wet season until the beginning of the dry season, but we found no new adults at the end of the dry season. Individuals returned repeatedly to the very same spot where we had found them previously.

The mean distance of individuals of *B. martinsi* from the cave entrance was 7.28 m, corresponding to the dysphotic zone. Compared to the null model, only sex/age group explained variations in the distance of frogs to the cave entrance (Chi-Square = 10.71, df = 2, p = 0.005) with juveniles remaining closer to the entrance than females (post-hoc estimate = 0.21, se = 0.06, t = 3.31, p = 0.004) and males, although this result only approaches significance (post-hoc estimate = 0.43, se = 0.06, t = 2.26, p = 0.067). Males and females did not differ in their distances to the cave entrance (post-hoc estimate = 0.07, se = 0.05, t = 1.41, p = 0.341; Fig. 5A). Again, only sex/age group explained variations in the perch height of frogs above the ground (Chi-Square = 17.18, df = 2, p < 0.001) with juveniles remaining closer to the ground than both males (post-hoc estimate = -0.55, se = 0.15, t = 3.58, p = 0.002) and females (post-hoc estimate = -0.65, se = 0.16, t = 4.20, p < 0.001). Males and females did not differ in their perch height above the ground (post-hoc estimate = 0.10, se = 0.11, t = 0.93, p = 0.625; Fig. 5B). No other models showed better explanatory power than the null model.

The best model to explain cave use by males included only ESI (estimate = 2.95, se = 0.68, z = 4.32, p < 0.001; Supplementary document 1). Models including rainfall and mean humidity amplitude had similar AICc values (Table 3), however neither rainfall nor mean humidity amplitude alone explained a significant amount of variation in the number of males per cave. The best model to explain
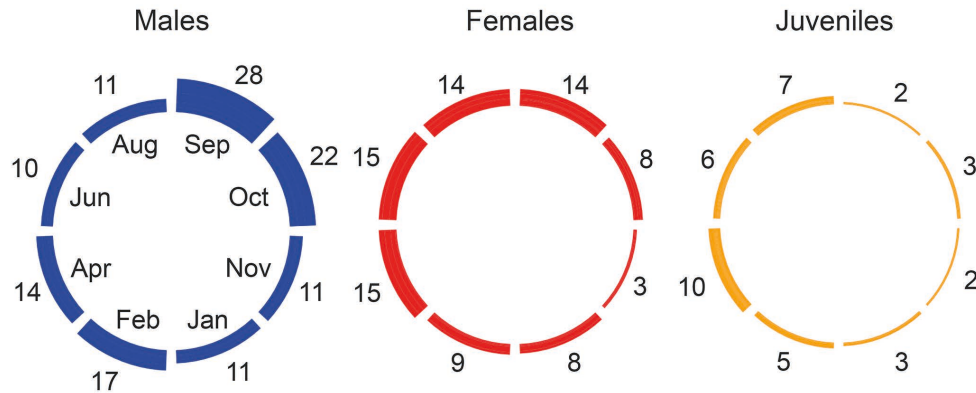

Figure 4. Numbers of individual males, females, and juveniles of *Bokermannohyla martinsi* recorded per sampling period in ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil.

the use of caves by females included ESI (estimate = 3.37, se = 0.96, z = 3.51, p < 0.001) and mean external temperature (estimate = -1.193, se = 0.07, z = -2.81, p = 0.005) with females choosing more stable caves during colder periods (recorded during the dry season, Fig. 4). This model presented deviant residual dispersion (Supplementary document 2). A model including just mean external temperature (estimate = -0.193, se = 0.07, z = -2.82, p = 0.004) had residuals fitted to expected values (Supplementary document 3). Models including rainfall and mean humidity range also had similar explanatory powers. Females tended to use caves more often during drier periods, but mean humidity amplitude alone did not explain the cave use by

females. The best model to explain the use of caves by juveniles included just ESI (estimate = 1.36, se = 0.51, z = 2.70, p = 0.007; Supplementary document 4). Other models including rainfall, mean temperature, and mean humidity amplitude had similar explanatory powers, however neither of these variables alone explained a significant amount of the variation in the cave use by juveniles.

Discussion

We recorded an intensive and continuous use of caves by the endemic frog *Bokermannohyla martinsi*, with seven out of the ten sampled caves hosting frogs (four of those during all sampling periods throughout the year) and 63% of all individuals recorded being found in caves during more than one sampling period. Site fidelity was also observed, with 52 (88%) recaptured frogs being found in the same caves. Recruitment of juveniles (70%) occurred at the end of the dry season, and they were likely from the previous rainy season, as some of them still had tail remnants.

Even though we do not know which proportion of the whole population uses caves, at least 94 individuals occurred in this habitat, which is more than we usually find in breeding habitats (streams) during year-round samplings in other localities (Afonso & Eterovick 2007, P. C. Eterovick, pers. obs.). Thus, the high abundance of *B. martinsi* in the studied caves corroborates the hypothesis that they use these habitats as shelters. During the whole study we never saw any potential predator inside the caves.

Bokermannohyla martinsi used more intensively the dysphotic zone, which has high humidity and moderate temperatures (Biswas 2014) as is usually observed in troglophilic and trogloxenic species (Lunghi et al. 2015, 2017), including other anurans (Oseen & Wassersug 2002, Lunghi et al. 2018). In these sectors, the frogs may also benefit from decreased visibility to visually orientated predators (Duellman & Trueb 1994, Oseen & Wassersug 2002) and also from moister and more stable microclimatic conditions compared to the euphotic zone (Hetem et al. 2012, Everall et al. 2015, Lunghi et al. 2018). Adult individuals of *B. martinsi* were found to have moved farther into the caves than juveniles, which could be due to the former's better locomotory performance resulting from their larger sizes and greater familiarity with the cave habitat due to previous visits, enabling them to reach more stable sectors.

Within the caves, juveniles tended to be closer to the ground than adults of *B. martinsi*. Spots higher up may be chosen during periods of lower activity, as has been observed in *Eleutherodactylus longipes* (Baird, 1859) (Espino-del-Castillo et al. 2009), however, active and inactive individuals of *B. martinsi* did not differ regarding their perch heights above the ground. For their part, juveniles may position themselves closer to the ground due to their less developed climbing skills, as opposed to adult frogs. Morphological features of *B. martinsi* such as digital disks, a camouflage colour pattern, and nocturnal habits have also
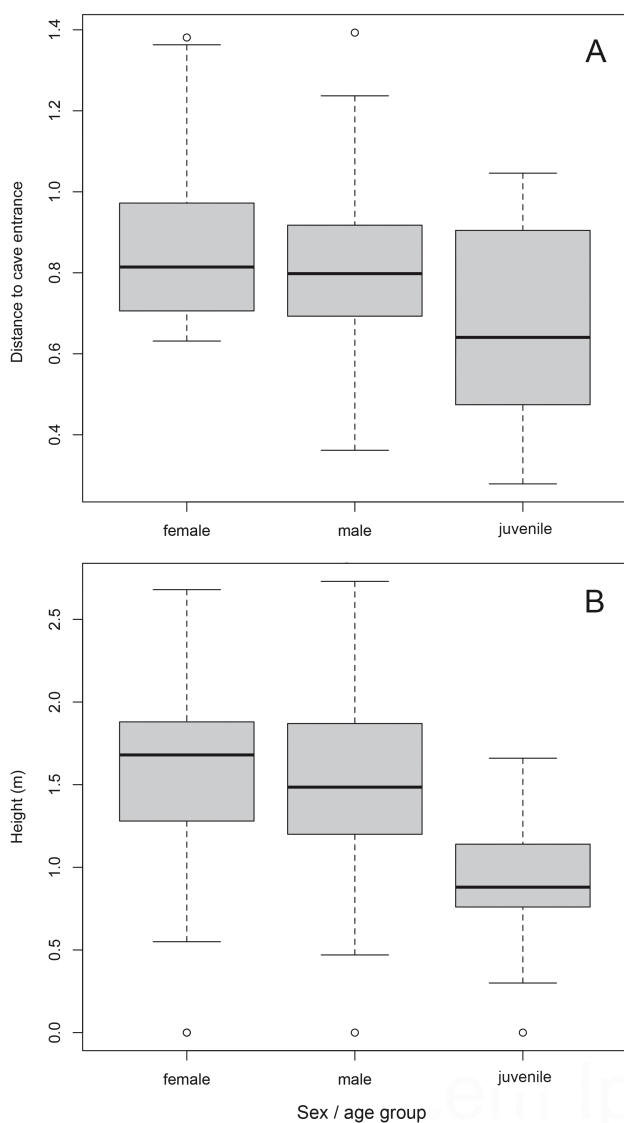

Figure 5. Distance (m; log-transformed) from cave entrance (A) and perch height (m) above the ground (B) of males, females and juveniles of *Bokermannohyla martinsi* recorded in ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil.

Table 3. Models built to evaluate the influence of cave ESI (Environmental Stability Index) and external climatic features: rainfall, external mean temperature (extmeantemp), and external mean humidity amplitude (extMHA) on use of caves (number of individuals using caves per sampling period) by males, females and juveniles of *Bokermannohyla martinsi* at the National Park Serra do Gandarela, southeastern Brazil, from August 2017 to August 2018. The selected model is presented in boldface. All models that differed from the best model by less than two AICc units are presented. Significance values for the increased explanatory power of the selected model against the corresponding null model are presented (see text for analysis details).

Models to explain number of males in caves per sampling period	df	loglik	AICc	Delta	Weight	p
males ~ ESI+(1|cave)+(1|sampling)	4	-81.72	171.97	0.00	0.38	<0.001
males ~ ESI+rainfall+(1|cave)+(1|sampling)	5	-81.22	173.25	1.29	0.20	
males ~ ESI+extMHA+(1|cave)+(1|sampling)	5	-81.41	173.64	1.67	0.16	
Models to explain number of females in caves per sampling period						
females ~ ESI+extmeantemp+(1|cave)+(1|sampling)	5	-67.59	146.00	0.00	0.30	<0.001
females ~ ESI+rainfall+extMHA+(1|cave)+(1|sampling)	6	-66.76	146.68	0.68	0.21	
females ~ ESI+rainfall+(1|cave)+(1|sampling)	5	-68.07	146.94	0.95	0.19	
females ~ ESI+rainfall+extmeantemp+(1|cave)+(1|sampling)	6	-67.03	147.22	1.22	0.16	
females ~ ESI+rainfall+extmeantemp+extMHA+(1|cave)+(1|sampling)	7	-66.00	147.55	1.55	0.14	
Models to explain number of juveniles in caves per sampling period						
juveniles ~ ESI+(1|cave)+(1|sampling)	4	-64.75	138.04	0.00	0.24	0.007
juveniles ~ ESI+extmeantemp+extMHA(1|cave)+(1|sampling)	6	-62.77	138.70	0.66	0.17	
juveniles ~ ESI+extMHA(1|cave)+(1|sampling)	5	-64.21	139.22	1.19	0.13	
juveniles ~ ESI+extmeantemp (1|cave)+(1|sampling)	5	-64.25	139.32	1.28	0.13	
juveniles ~ ESI+rainfall+extMHA(1|cave)+(1|sampling)	6	-63.11	139.38	1.34	0.12	

been found in other anuran species that use caves (Biswas 2014, Luría-Manzano & Ramírez-Bautista 2017) and may represent important adaptations for cave users.

Although the positioning of frogs within the caves did not depend on external climatic conditions, the number of frogs using caves was higher during the dry season, when a greater recruitment of juveniles is expected and external climatic conditions are harsher. During this period, both adult frogs and juveniles may seek shelter in caves. This effect was significant for females of *B. martinsi*, which used caves more often during colder and dry months. Although the model including ESI to explain the use of caves by females had overdispersed residuals, this is not unusual in GLMMs and does not mean the model is unusable (Hartig 2021). This seasonal pattern was also observed for *Eleutherodactylus longipes* in a cave in Mexico (Espino-del-Castillo et al. 2009). In the Crimean mountains (Ukraine), amphibian species also used caves non-accidentally during the winter (non-breeding season), although breeding-related activities are likely to occur within caves in some species (Turbanov et al. 2019).

The lower numbers of records of *B. martinsi* in caves during the rainy summer is likely due to the frogs being outside in search of breeding habitats, with males remaining at calling sites for longer periods than females (PCE pers. obs.). Frogs may then seek temporary shelter in spots closer to streams during the daylight hours between consecutive nights of breeding activity. Although *B. mar-tinsi* uses permanent streams for reproduction (Bokermann 1964, Caramaschi et al. 2010), we found tadpoles in ephemeral streams in the vicinities of the studied caves. In Area 1, the closest streams are 120–180 m from the entrances of the caves, whereas in Area 2, the closest stream is about 160 m distant. These distances between streams and caves are within the possible reach of these amphibians, as is suggested by their moving 159–290 m between breeding habitats and terrestrial habitats (Semlitsch & Bodie 2003). However, we recorded *B. martinsi* also in caves with perennial bodies of water (one quartzitic and three dolomitic caves in the Serra do Gandarela and one quartzitic cave in the neighbouring Serra do Caraça). Thus, the possible use of caves for reproduction by *B. martinsi* is worth investigating.

Bokermannohyla martinsi used caves more intensively during dry periods. Although *B. martinsi* can breed all year round (with peaks in the rainy season) in permanent streams (Eterovick & Barata 2006), its breeding activities at our study site are restricted to the rainy season, when temporary streams carry water for tadpole development.

Bokermannohyla martinsi did not use caves with the smallest (< 1.00) ESI values and used more frequently caves with greater environmental stability. The preference of amphibians for caves with greater humidity and environmental stability was also observed in other field studies (Lunghi et al. 2014) and experimentally (Lüddecke 2003). Amphibian susceptibility to water loss via the skin

(Wells 2007) makes the use of caves advantageous due to their high humidity and stability (Biswas 2014), facilitating water condensation and absorption through the skin, even at sites with low water availability (Tracy et al. 2011). Additionally, prey availability and lower predation risk are likely advantages of caves (Biswas 2014).

This study underscores the importance of caves as habitats for anurans, corroborating many others that have demonstrated these animals not to be just occasional cave visitors (Resetarits Jr. 1986, Fenolio et al. 2005, Espino-del-Castillo et al. 2009, Biswas 2014, Lunghi et al. 2014, Matavelli et al. 2015, Turbanov et al. 2019). The practical application of such knowledge in the process of environmental licensing should afford caves greater appreciation and acknowledge their need of preservation, which is especially necessary in the Iron Quadrangle, considered one of the major areas of iron mining in the world (Pinheiro et al. 2014), where many caves exist and are threatened with destruction through mining. Brazilian legislation (Executive order #6640, 7 November 2008) already attributes a higher conservation value to caves hosting troglobionts and/or that represent essential habitats for populations of threatened species, amongst other features. The current assessment of the importance of cave habitats to the species that use them is, however, not satisfactory, making it difficult to apply the law properly (Cruz & Piló 2019). We hope our results, by adding to the knowledge of the importance of caves as habitats for species not usually considered cave inhabitants, will contribute to a greater appreciation of caves in the conservation of biodiversity.

References

Afonso, L. G. & P. C. Eterovick (2007): Microhabitat choice and differential use by anurans in forest streams in southeastern Brazil. – Journal of Natural History, **41**: 937–948.

Bartoń, K. (2019): MuMIn: Multi-Model Inference. R package version 1.43.6. – https://CRAN.R-project.org/package=MuMIn

Bates, D., M. Maechler, B. Bolker & S. Walker (2015): Fitting Linear Mixed-Effects Models using lme4. – Journal of Statistical Software, **67**: 1–48.

Bento, D. M., R. L. Ferreira, X. Prous, M. Souza-Silva, B. C. Bellini & M. Vasconcellos (2016): Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. – Journal of Cave and Karst Studies, **78**: 61–71.

Biswas, J. (2014): Occurrence and distribution of cave dwelling frogs of peninsular India. – Ambient Science, **1**: 17–25.

Blackburn, D. G. (2019): The oviparous olm: Analysis & refutation of claims for viviparity in the cave salamander *Proteus anguinus* (Amphibia: Proteidae). – Zoologischer Anzeiger, **281**: 16–23.

Bokermann, W. C. A. (1964): Dos nuevas especies de *Hyla* de Minas Gerais y notas sobre *Hyla alvarengai*. (Amphibia, Salientia, Hylidae). – Neotropica, **10**: 67–76.

Canelas, M. A. & J. Bertoluci (2007): Anurans of the Serra do Caraça, southeastern Brazil: species composition and phenological patterns of calling activity. – Iheringia, SérieZoologia, **97**: 21–26.

Caramaschi, U., L. B. Nascimento, P. C. Eterovick & D. Silvano (2010): *Bokermannohyla martinsi*. – The IUCN Red List of Threatened Species 2010: e.T55549A11330848. – https://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T55549A11330848.en, accessed on 17 June 2020.

Chabert, C. & R. A. Watson (1981): Mapping and measuring caves, a conceptual analysis. – NSS Bulletin, **43**: 3–11.

Costa, T. R. N., A. C. O. Q. Carnaval & L. F. Toledo (2012): Mudanças climáticas e seus impactos sobre os anfíbios brasileiros. – Revista da Biologia, **8**: 33–37.

Cruz, J. B. & L. B. Piló (2019): Espeleologia e Licenciamento Ambiental. – ICMBio, Brasília, Brazil.

Culver, D. C. & T. Pipan (2019): The subterranean domain. – pp. 1–23 in: Culver, D. C. & T. Pipan (eds): The biology of caves and other subterranean habitats, Second edition. – Oxford University Press, New York.

Duellman, W. E. & L. Trueb (1994): Biology of Amphibians. – The John Hopkins University Press, Baltimore and London.

Espino-Del-Castillo, A., G. Castaño-Meneses, M. J. Dávila-Montes, M. Miranda-Anaya, J. B. Morales-Malacara & R. Paredes-León (2009): Seasonal distribution and circadian activity in the troglophile long-footed robber frog, *Eleutherodactylus longipes* (Anura: Brachycephalidae) at Los Riscos Cave, Querétaro, Mexico: Field and laboratory studies. – Journal of Cave and Karst Studies, **71**: 24–31.

Eterovick, P. C. & I. M. Barata (2006): Distribution of tadpoles within and among Brazilian streams: the influence of predators, habitat size and heterogeneity. – Herpetologica, **62**: 365–377.

Everall, N. C., M. F. Johnson, R. L. Wilby & C. J. Bennett (2015): Detecting phenology change in the mayfly *Ephemera danica*: responses to spatial and temporal water temperature variations. – Ecological Entomology, **40**: 95–105.

Fenolio, D. B., G. O. Graening & J. F. Stout (2005): Seasonal movement patterns of pickerel frogs (*Rana palustris*) in an Ozark cave and trophic implications supported by stable isotope evidence. – Southwestern Naturalist, **50**: 385–390.

Ferreira, R. L. (2004): A medida da complexidade ecológica e suas aplicações na conservação e manejo de ecossistemas subterrâneos. – PhD Dissertation, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Ferreira, R. L. (2005): A vida subterrânea nos campos ferruginosos. – O Carste, **17**: 106–115.

HADDAD, C. F. B., I. F. MACHADO, J. G. R. GIOVANELLI, Y. S. L. BATAUS, V. M. UHLIG, F. A. Q. BATISTA, A. O. MACIEL, C. A. G. CRUZ, D. LOEBMANN, D. L. SILVANO, E. M. L. GONSALES, F. NOMURA, F. S. F. LEITE, H. B. A. PINTO, I. B. AMARAL, L. B. NASCIMENTO, M. J. STURARO, M. S. HOOGMOED, M. S. KIENLE, M. B. SOUZA, N. M. MACIEL, P. COLOMBO, R. N. FEIO, R. LINGNAU & R. P. BASTOS (2016): *Bokermannohyla martinsi* (Bokermann, 1964). Processo de avaliação do risco de extinção da fauna brasileira. – ICMBio. – http://www.icmbio.gov.br/portal/faunabrasileira/carga-estado-de-conservacao/8134-anfibios-bokermannohyla-martinsi, accessed on 17 June 2020.

HARTIG, F. (2021): DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.3. – http://florianhartig.github.io/DHARMa/

HETEM, R. S., W. M. STRAUSS, L. G. FICK, S. K. MALONEY, L. C. R. MEYER, M. SHOBRAK, A. FULLER & D. MITCHELL (2012): Activity re-assignment and microclimate selection of free-living Arabian oryx: responses that could minimise the effects of climate change on homeostasis? – Zoology (Jena), **115**: 411–416. doi: 10.1016/j.zool.2012.04.005

HOLSINGER, J. R. & D. C. CULVER (1988): The invertebrate cave fauna of Virginia and a part of Eastern Tennessee: Zoogeography and ecology. – Brimleyana, **14**: 1–162.

HOWARTH, F. G. & O. T. MOLDOVAN (2018): The ecological classification of cave animals and their adaptations. – pp. 41–67 in: MOLDOVAN, O. T., L. KOVÁČ & S. HALSE (eds): Cave Ecology, Springer, Berlin.

JACOBI, C. M., F. F. CARMO, R. C. VINCENT & J. R. STEHMANN (2007): Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem. – Biodiversity and Conservation, **16**: 2185–2200.

KENYON, N., A. D. PHILLOTT & R. A. ALFORD (2009): Evaluation of the photographic identification method (PIM) as a tool to identify adult *Litoria genimaculata* (Anura: Hylidae). – Herpetological Conservation & Biology, **4**: 403–410.

KOLLER, K. (2017): Underground occurrences of three species of amphibians and reptiles with special emphasis on *Rana latastei* (Amphibia: Anura). – North-Western Journal of Zoology, **13**: 176–179.

LAMA, F. L., M. D. ROCHA, M. A. ANDRADE & L. B. NASCIMENTO (2011): The use of photography to identify individual tree frogs by their natural marks. – SAJH, **6**: 198–204.

LAMOUNIER, W. (2009): Patrimônio natural da Serra do Gandarela e seu entorno: análise ambiental como subsídio para a criação de unidades de conservação no Quadrilátero Ferrífero–Minas Gerais. – Unpubl. Masters Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

LEITE, F. S. F., F. A. JUNCÁ & P. C. ETEROVICK (2008): Status do conhecimento, endemismo e conservação dos anfíbios anuros da Serra do Espinhaço, Brasil. – Megadiversidade, **4**: 182–200.

LENTH, R. (2020): emmeans: Estimated Marginal Means, aka Least-SquaresMeans. R package version 1.5.1. – https://CRAN.R-project.org/package=emmeans

LIMA, N. G. S., R. C. L. LIMA, J. E. M. DIAS, P. F. TORRES & P. C. ETEROVICK (2013): Spatial niche variation in two sympatric species of *Bokermannohyla* (Anura: Hylidae) in southeastern Brazil. – Journal of Natural History, **48**: 229–240.

LÜDDECKE, H. (2003): Space use, cave choice, and spatial learning in the dendrobatid frog *Colostethus palmatus*. – Amphibia-Reptilia, **24**: 37–46.

LUNGHI, E., R. MANENTI & F. G. FICETOLA (2014): Do cave features affect underground habitat exploitation by non-troglodyte species? – Acta Oecologica, **55**: 29–35.

LUNGHI, E., R. MANENTI & F. G. FICETOLA (2015): Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection? – PeerJ., **3**: e1122.doi: 10.7717/peerj.1122

LUNGHI, E., R. MANENTI & F. G. FICETOLA (2017): Cave features, seasonality and subterranean distribution of non-obligate cave dwellers. – PeerJ., **5**: e3169.doi: 10.7717/peerj.3169

LUNGHI, E., G. BRUNI, F. G. FICETOLA & R. MANENTI (2018): Is the Italian stream frog (*Rana italica* Dubois, 1987) an opportunistic exploiter of cave twilight zone?– Subterranean Biology, **25**: 49–60.

LUNGHI, E., S. GIACHELLO, Y. ZHAO, C. CORTI, G. F. FICETOLA & R. MANENTI (2020): Photographic database of the European cave salamanders, genus *Hydromantes*. – Scientific Data, **7**: 171.

LURÍA-MANZANO, R. & A. RAMÍREZ-BAUTISTA (2017): Diet comparison between rainforest and cave populations of *Craugastor alfredi* (Anura: Craugastoridae): does diet vary in contrasting habitats? – Journal of Natural History, **51**: 2345–2354.

MAGALHÃES, R. F., J. V. A. LACERDA, L. P. REIS, P. C. A. GARCIA & P. D. PINHEIRO (2018): Sexual dimorphism in *Bokermannohyla martinsi* (Bokermann, 1964) (Anura, Hylidae) with a report of male-male combat. – SAJH, **13**: 202–210.

MATAVELLI, R., A. M. CAMPOS, R. N. FEIO & R. L. FERREIRA (2015): Occurrence of anurans in Brazilian caves. – Acta Carsologica, **44**: 107–120.

MILLER, B. T., M. L. NIEMILLER & R. G. REYNOLDS (2008): Observations on egg-laying behavior and interactions among attending female red salamanders (*Pseudotriton ruber*) with comments on the use of caves by this species. – Herpetological Conservation and Biology, **3**: 203–210.

MIRANDA, T., M. EBNER, M. SOLÉ & A. KWET (2005): Estimativa populacional de *Pseudis cardosoi* (Anura, Hylidae), com emprego de método fotográfico para reconhecimento individual. – Biociências, **13**: 49–54.

OLIVEIRA, O. A. B., J. P. R. OLIVITO & D. RODRIGUES-SILVA (2011): Caracterização da Unidade Espelológica e das Unidades Geomorfológicas da Região do Quadrilátero Ferrífero-MG. – Espeleo-Tema, **22**: 61–80.

OSEEN, K. L. & R. J. WASSERSUG (2002): Environmental factors influencing calling in sympatric anurans. – Oecologia, **133**: 616–625.

PECL, G. T., M. B. ARAÚJO, J. D. BELL, J. BLANCHARD, T. C. BONEBRAKE, C. CHEN, T. D. CLARK, R. K. COLWELL, F. DANIELSEN, B. EVENGÅRD, L. FALCONI, S. FERRIER, S. FRUSHER, R. A. GARCIA, R. B.GRIFFIS, A. J. HOBDAY, C. JANION-SCHEEPERS, M. A. JARZYNA, S. JENNINGS, J. LENOIR, H. I. LINNETVED, V. Y. MARTIN, P. C. McCORMACK, J. McDONALD, N. J. MITCHELL, T. MUSTONEN, J. M. PANDOLFI, N. PETTORELLI, E. POPOVA, S. A. ROBINSON, B. R. SCHEFFERS, J. D. SHAW, C. J. B. SORTE, J. M. STRUGNELL, J. M. SUNDAY, M. N. TUANMU, A. VERGÉS, C. VILLANUEVA, T. WERNBERG, E. WAPSTRA & S. E. WILLIAMS (2017): Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. – Science, **355**: 6332, eaai9214. doi: 10.1126/science.aai9214

PELLEGRINI, T. G., L. P. SALES, P. AGUIAR & R. L. FERREIRA (2016): Linking spatial scale dependence of land-use descriptors and

invertebrate cave community composition. – Subterranean Biology, **18**: 17–38

Pinheiro, P. D. P., P. P. G. Taucce, F. S. F. Leite & P. C. A. Garcia (2014): The advertisement call of the endemic *Bokermannohyla martinsi* (Bokermann, 1964) (Anura: Hylidae) from southern Espinhaço range, southeastern Brazil. – Zootaxa, **3815**: 147–150.

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

R Core Team (2020): R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Version 3.6.3. – https://cran.r-project.org/

Resetarits Jr, W. J. (1986): Ecology of cave use by the frog *Rana palustris*. – The American Midland Naturalist, **116**: 256–266.

Romero, A. (2009): Cave Biology: Life in Darkness. – Cambridge University Press, Cambridge.

Rubbioli, E., A. Auler, D. Menin & R. Brandi (2019): Cavernas-Atlas do Brasil Subterrâneo. – ICMBio, Brasília, DF, Brazil.

Russo, V. G., L. F. Russo, L. Coppari & T. Notomista (2018): First record of *Bombina variegata* (Linnaeus, 1758) in a cave environment. – Herpetology Notes, **11**: 967–969.

Segalla, M. V., U. Caramaschi, C. A. G. Cruz, P. C. A. Garcia, T. Grant, C. F. B. Haddad, D. J. Santana, L. F. Toledo & J. A. Langone (2019): Brazilian amphibians: List of species. – Herpetologia Brasileira, **8**: 65–96.

Semlitsch, R. D. & J. R. Bodie (2003): Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. – Conservation Biology, **17**: 1219–1228.

Sket, B. (2008): Can we agree on an ecological classification of subterranean animals? – Journal of Natural History, **42**: 1459–1563.

Soares, L. G. S., A. F. Van de Koken, M. B. Timo & R. P. Silva (2013): Fauna de invertebrados em cavernas de calcário na região de Córrego Fundo, Minas Gerais, Brasil. – Revista Eletrônica de Biologia, **6**: 48–68.

Suwannapoom, C., M. Sumontha, J. Tunprasert, T. Ruangsuwan, P. Pawangkhanant, D. V. Korost, S. Nikolay & A. Poyarkov (2018): A striking new genus and species of cave-dwelling frog (Amphibia: Anura: Microhylidae: Asterophryinae) from Thailand. – PeerJ., **6**: e4422; DOI 10.7717/peerj.4422

Tracy, C. R., N. Laurence & K. A. Christian (2011): Condensation onto the skin as a means for water gain by tree frogs in tropical Australia. – American Naturalist, **178**: 553–558.

Trajano, E. (1986): Fauna cavernícola brasileira: composição e caracterização preliminar. – Revista Brasileira de Zoologia, **3**: 533–561.

Trajano, E. & P. Gnaspini-Netto (1990): Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos táxons. – Revista Brasileira de Zoologia, **3**: 383–407.

Trajano, E. & M. E. Bichuette (2006): Biologia Subterrânea: Introdução. – Ed. Redespeleo, São Paulo, Brazil.

Turbanov, I. S., O. V. Kukushkin & R. S. Vargovitsh (2019): Amphibians and Reptiles in the subterranean cavities of the Crimean Mountains. – Russian Journal of Herpetology, **26**: 29–53.

Wells, K. D. (2007): The ecology and behavior of amphibians. – The University of Chicago Press, Chicago, U.S.A.

Supplementary data

The following data are available online:

Supplementary document 1. Residual diagnostics for the model selected to explain the use of caves by males of *Bokermannohyla martinsi* in ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil.

Supplementary document 2. Residual diagnostics for the model selected to explain the use of caves by females of *Bokermannohyla martinsi* in ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil.

Supplementary document 3. Residual diagnostics for a model including just external mean temperature to explain the use of caves by females of *Bokermannohyla martinsi* in ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil.

Supplementary document 4. Residual diagnostics for the model selected to explain the use of caves by juveniles of *Bokermannohyla martinsi* in ten sampled caves in the National Park Serra do Gandarela, southeastern Brazil.