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Improved local inventory and regional contextualization for anuran diversity assessment at an endangered habitat in southeastern Brazil

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ABSTRACT

Amphibians are suffering population declines around the world and the main causes are related to human activities, especially those involving direct habitat destruction. On the other hand, we are far behind in our knowledge of species distribution, natural history and even taxonomy. Some areas are under high levels of threat due to their economic relevance, as is the case for the Quadrilátero Ferrífero, a biodiverse area that is rich in iron deposits in the southernmost highlands of the Espinhaço Mountain Range, in southeastern Brazil. Using four lakes in the Quadrilátero Ferrífero as an example, we aimed to employ two approaches that we believe can improve short-term species inventories such as those needed to meet legal requirements for potentially impacting enterprises (e.g. mining). These approaches were (1) combination of tadpole and adult frog inventories and (2) regional contextualization of local species assemblages. We found adult frog and tadpole sampling to be complementary and representative of all anuran life stages. We also found the studied habitats to be considerably different from all other sampled habitats in the Quadrilátero Ferrífero (which also differed substantially among them). This high spatial heterogeneity could not be explained by geographic distance, even accounting for the effects of different types of bodies of water and sampling methods among sites. These findings have important conservation implications. Thorough inventories including both adult and tadpole stages are recommended for any new potentially destructive enterprise to be implemented in the Quadrilátero Ferrífero, because endemic species and unique anuran assemblages are likely to be lost.

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Introduction

Frequent reports of amphibian population declines have put the urgency for their conservation in the spotlight, stressing the need to increase efforts to sample species diversity, abundance and distribution (Young et al. 2001). In Latin America, many areas

still need to be sampled for amphibians (Young et al. 2001; Silvano and Segalla 2005), and many species are considered as Data Deficient (DD; IUCN 2015). It is broadly recognized that species inventories and basic information on species' natural history provide essential information for amphibian conservation in Brazil (Eterovick et al. 2005). However, such studies are still insufficient considering the great species richness in the country (1026 species; Segalla et al. 2014) and the lack of information is potentially detrimental to species conservation (Silvano and Segalla 2005).

For anuran inventories to be effective they need to be conducted year-round, and they should include all available life stages (Silva 2010). However, many studies focus just on the adult stage (e.g. Canelas and Bertoluci 2007). For several areas, the only data available come from short-term inventories (a few days of sampling) aimed to meet the legal requirements for the licensing of enterprises such as hydroelectric dam construction (P.C.E. & F.S.F.L. pers. obs.). Anuran larvae are very different from the adults, but they are often poorly known. They usually remain in bodies of water for longer periods than adults, so making them easier to locate and sample. For instance, males of species that are explosive breeders may call at breeding sites for just a few days, whereas their tadpoles remain at the site for weeks or months until metamorphosis (McDiarmid and Altig 1999). Tadpoles of different species may look similar to each other upon first examination, but there are identifiable morphological features of tadpoles that can be used to distinguish species (e.g. Pezzuti 2011). Tadpole sampling and identification may accelerate species inventories, which is especially important when sampling time is restricted (Pezzuti 2011). The importance of tadpole identification (Boulenger 1892) and the inventory of tadpole diversity (Taylor 1942; Duellman 1970) has been long recognized, but still many inventory studies overlook this life stage (e.g. Canelas and Bertoluci 2007; Bertoluci et al. 2009; São-Pedro and Feio 2011).

The Quadrilátero Ferrífero (QFe) is a good example of an area where inventory efforts need to be maximized in the short-term. It represents the southern portion of the Espinhaço Mountain Range, in Minas Gerais State, southeastern Brazil. Its mountains are rich in huge iron ore deposits that make it one of the most important mineral provinces in the country (Carmo 2010). The vegetation that develops on superficial iron crusts, known as '*canga*', has several adaptations to tolerate harsh environmental conditions such as nutrient-poor, acidic soils with low water content and high levels of heavy metals (Giulietti et al. 1997; Jacobi et al. 2007). The *canga* vegetation is one of the most threatened and least known floras in Brazil (Jacobi and Carmo 2008). The situation is aggravated by its restricted distribution – the main locations in Brazil are Carajás in the Amazon, the QFe, Corumbá in Mato Grosso do Sul State (Jacobi et al. 2007; Carmo 2010); and northern Minas Gerais State (Marcelo F. Vasconcelos and Flávio Carmo, pers. comm.) – and its association with the iron deposits, which are the focus of mining activities that are destructive to the habitat (Jacobi and Carmo 2008). As pointed out by Silvano and Segalla (2005) and Vitt and Caldwell (2009), habitat loss is the main threat to amphibian conservation in Brazil.

Considering this situation of high biological importance and high levels of threat, we intended to test the complementarity of adult and tadpole sampling for an effective anuran species inventory in time intervals up to a year within the QFe. We conducted anuran species inventories at four *canga* lakes using both adult and tadpole sampling to test the efficiency/complementarity of these two methods. This approach may aid

anuran species-richness assessment in the region because activities such as mining and urbanization are spreading and inventories legally required for implementation of such enterprises are often based on short sampling periods (a few days of sampling, sometimes not even in the rainy season; LMP, AML, FSFL, PCE, pers. obs.). We chose lakes in which to conduct this study because we expected anuran breeding periods to be shorter in such habitats, based on personal observations during previous studies in the QFe region (Kopp and Eterovick 2006; Afonso and Eterovick 2007), thereby increasing the likelihood that inventories based only on the adult stage would be biased.

To evaluate the uniqueness of a newly inventoried area in the landscape where it is located, we gathered all the information on amphibian species composition for localities within the QFe that was available in the literature and compared it with our four sampled lakes to observe whether they were similar to areas already inventoried or not. A unique area should differ from other known areas in the region and deserve more attention for conservation. Uniqueness could be a result of special habitats/species or a lack of inventories at similar neighbouring sites in a spatially structured landscape. We tested for a possible relationship between geographic distance and fauna similarity, which would indicate spatial structure of anuran assemblages in the region.

Material and methods

Study site

The Espinhaço Mountain Range represents the most extensive and continuous mountain chain in Brazil (Almeida-Abreu and Renger 2002). It extends for about 1000 km northwards from central Minas Gerais State to the Chapada Diamantina in Bahia State. The QFe constitutes the southernmost portion of the Espinhaço highlands. Several unique habitats occur in its *canga* formations including caves, lakes, crevices and puddles (Carmo 2010). They shelter natural communities with high conservation value because of the presence of rare and endemic species (e.g. see Carmo 2010 for plants; Leite et al. 2008 for anurans). Furthermore, the *cangas* are of special geological and archaeological importance, and hold large underground aquifers (used by the human population), further justifying the need to plan responsible exploitation of natural resources (Carmo 2010).

We conducted anuran species inventories at four lakes (Table 1) surrounded by *canga* vegetation, located between the municipalities of Mariana and Catas Altas. The region has a seasonal climate with a dry season from April to September and a rainy season from October to March. Mean annual precipitation is 1500 mm (INMET 2007). The climate is considered Cwb according to Köppen's classification (Sá-Júnior et al. 2012).

Sampling procedures

Three of us sampled the four lakes during two nights and two days every month from January 2011 to January 2012. We sampled each lake, once at night and once during the day, each month. We recorded calling males and gravid females, amplexant pairs, egg clutches, tadpoles and froglets. We searched for tadpoles with nets during the day and at night (about 90 minutes per sampling period). For the other life stages, we used visual

Table 1. Characterization of four lakes at the Quadrilátero Ferrífero, southeastern Brazil, sampled from January 2011 to March 2012; observed (Obs) and estimated (mean \pm SD; Jackknife I with 1000 simulations) species richness based on records of adult frogs, tadpoles and all life stages combined.

Lakes	Coordinates	Altitude (m)	Observations	Adults			Tadpoles			All life stages		
				Obs	Estimated	Obs	Obs	Estimated	Obs	Obs	Estimated	Obs
L1	20.1256°S, 43.3892°W	918	Semi-permanent lake (maximum depth less than 15 cm from July to September). Perimeter of 80.7 m and maximum depth of 1 m in December (peak of rainy season).	14	14.0 \pm 0.0	13	13.9 \pm 0.9	15	15.0 \pm 0.0			
L2	20.1283°S, 43.3852°W	916	Permanent lake. Perimeter of 614 m, maximum depth of 1.5 m in December.	9	9.0 \pm 0.0	8	8.9 \pm 0.9	9	9.0 \pm 0.0			
L3	20.1617°S, 43.4322°W	916	Temporary lake (dried completely from July to September). Perimeter of 535 m and maximum depth of 2 m in December.	12	12.0 \pm 0.0	11	13.8 \pm 1.5	13	13.0 \pm 0.0			
L4	20.1625°S, 43.4083°W	873	Permanent lake. Perimeter of 254 m, maximum depth of 3 m in December. Connected to another lake by a ditch of slow-flowing water surrounded by a riparian forest.	20	20.0 \pm 0.0	17	18.9 \pm 1.3	21	21.0 \pm 0.0			

search and auditory search (for adult males) methods concomitantly and searched the whole margin of each lake walking or with an inflatable boat when vegetation hampered access by land. Searches lasted about 3 hours per lake and focused on adult frogs, although we also recorded other life stages observed. Hence, total sampling effort at each lake was 108 person/hours for tadpoles and 108 person/hours for adults. We euthanized voucher specimens with 20% benzocaine, preserved them according to Heyer et al. (1994), and deposited them in the amphibian (post-metamorphic) and tadpole collections of the Herpetological Collection of the Centre of Taxonomic Collections of the Universidade Federal de Minas Gerais (UFMG; see Appendix 1). Whenever necessary, tadpoles were raised until metamorphosis to confirm species identity. We followed the classification adopted by Frost (2015).

Statistical analyses

We estimated species richness for each lake based on presence/absence data of (1) tadpoles, (2) adult anurans, and (3) tadpoles and adult anurans using Jackknife I in the software EstimateS 8.0.0 (Colwell 2006). We used the Jackknife I species-richness estimator because this estimator has been shown to perform well with small sample sizes and to provide good species-richness estimates (Walther and Morand 1998; Williams et al. 2007). For each of the three estimates, we performed 1000 randomizations using months as samples and we presented results as mean \pm SD. We worked with species presence/absence data instead of abundance because we combined different sampling techniques and counting individuals was not sufficiently precise for some of them (e.g. auditory sampling).

We found 12 studies in the literature with data on anuran species composition at nine localities from eight municipalities in the QFe: Rio Acima (Condomínio Canto das Águas; Grandinetti and Jacobi 2005); Ouro Branco (Serra de Ouro Branco; São-Pedro and Feio 2010, 2011); Catas Altas, including the site sampled in the present study and the RPPN Santuário do Caraça (Kopp and Eterovick 2006; Afonso and Eterovick 2007; Canelas and Bertoluci 2007); São Gonçalo and Santa Bárbara (Estação Ambiental de Peti; Bertoluci et al. 2009); Nova Lima, including Estação Ecológica de Fechos (Leite 2007) and Área de Proteção Mutuca (Nascimento et al. 1994); Brumadinho (Instituto Inhotim, Linares and Eterovick 2013); and Ouro Preto (Floresta Estadual Uaimií, Pirani 2011; see Figure 1, Table 2, and Appendix 2). We estimated dissimilarities among all the sampled amphibian assemblages considering each study separately (even the ones conducted at the same sites but in different bodies of water) using Jaccard's coefficient. We then performed a grouping analysis using the weighted pair group method with averaging (WPGMA; Quinn and Keough 2002) with the software SYSTAT (SYSTAT 2007). We conservatively included only native taxa with confirmed identities in this analysis. We also performed the same analysis with all taxa (except for three species reported as introduced by Linares and Eterovick 2013) and obtained the same groups (results not shown).

To test for the existence of spatial structure in the distribution of amphibian species within the QFe we compared the dissimilarity matrix obtained from the previous analysis with a matrix of geographic distances among localities using a partial Mantel test in the software PASSaGE (Rosenberg and Anderson 2011). To account for the effects of sampling methods and types of bodies of water sampled in each study, we built two

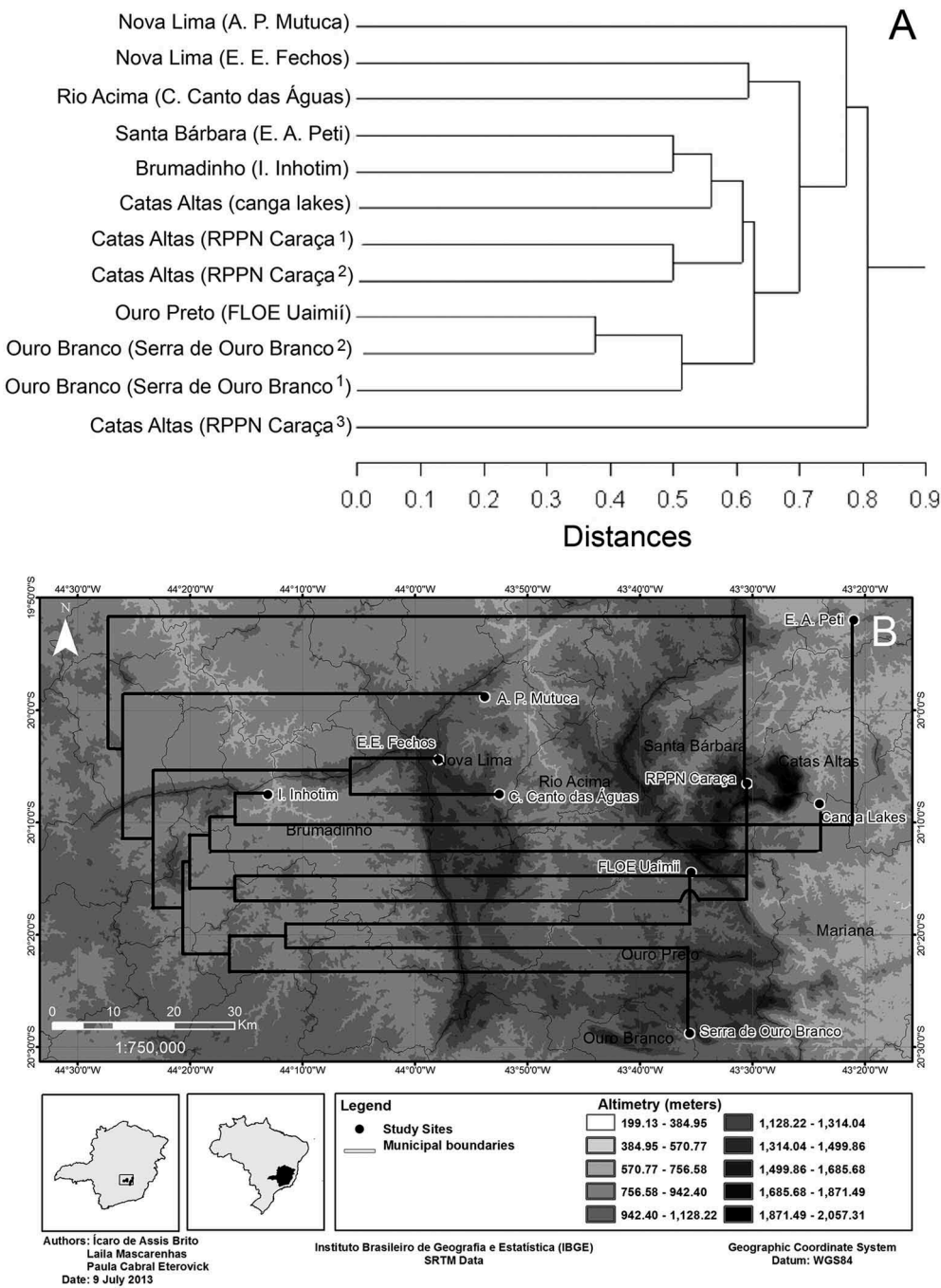


Figure 1. Result of the WPGMA (Jaccard's index) showing (A) dissimilarities among localities with published anuran inventories at the Quadrilátero Ferrífero, southeastern Brazil, and (B) their spatial distribution. Locality names are as in Table 2.

Table 2. Localities in the Quadrilátero Ferrífero, Minas Gerais state, with available species lists including details on sampling duration, types of habitats sampled, sampling methods, species richness and references.

Municipalities (localities)	Sampling period (months)	Sampled habitats	Species richness	Sampling methods	References
Catas Altas (<i>canga</i> lakes)	13	PL, TL, FO	29	AS, AUS, TS	Present study
Catas Altas (RPPN Caraça ¹)	12	PL, PS, SW	43	AS, AUS	Canelas and Bertoluci (2007)
Catas Altas (RPPN Caraça ²)	16	PL, TL, FO	22	AS, AUS, TS	Kopp and Eterovick (2006)
Catas Altas (RPPN Caraça ³)	15	PS	19	AS, AUS	Afonso and Eterovick (2007)
Ouro Branco (Serra de Ouro Branco ¹)	12	PL, PS, PT	28	AS, AUS, TS	São-Pedro and Feio (2010)
Ouro Branco (Serra de Ouro Branco ²)	12	PL, TL, PS, TS, SW, FO	47	AS, AUS	São-Pedro and Feio (2011)
São Gonçalo/ Sta Bárbara (E. A. Peti)	31	PL, TL, PS, SW	30	AS, TS	Bertoluci et al. (2009)
Rio Acima (C. Canto das Águas)	13	PL, PS, SW, FT	14	AS, AUS	Grandinetti and Jacobi (2005)
Nova Lima (A. P. Mutuca)	17	PL, PS, TS, DAM	9	AS, AUS, TS	Nascimento et al. (1994)
Nova Lima (E. E. Fechos)	28	PS, DAM	15	AS, AUS, TS	Leite (2007)
Brumadinho (I. Inhotim)	12	PL, PS, SW	32	AS, AUS, TS, PIT	Linares and Eterovick (2013)
Ouro Preto (FLOE Uaimi)	12	PS, SW	35	AS, AUS, TS	Pirani (2011)

Types of bodies of water: FT, fish farm tanks; PS, permanent stream; TS, temporary streams; PL, permanent lake; TL, temporary lake; SW, swamp or flooded area; DAM, lake formed by dam construction; FO, forest. Sampling methods: PIT, pitfall traps; AS, active search; AUS, auditory search; TS, tadpole sampling. Superscript numbers represent different studies at the same locality.

matrices of Jaccard dissimilarity from presence/absence data for (1) each type of body of water and (2) each type of sampling method per study/locality considered (see Table 2). We held both these matrices constant for the computation of a partial Mantel test between the main matrices (species dissimilarities and geographic distances). Significance was obtained based on 999 permutations.

Results

We recorded 29 anuran species in the four sampled lakes, representing three families: Hylidae (19 species), Leptodactylidae (nine species) and Microhylidae (one species) (Table 3). Species accumulation curves based on records of adult frogs showed stabilization for all lakes (Figure 2). Curves based on records of tadpoles tended to stabilize, but indicated that there may be more species to be found if a great additional sampling effort was applied. Curves based on records of both tadpoles and adult frogs represented all life stages and showed stabilization and higher species richness than curves based on one life stage (Figure 2; Table 1).

From the species recorded as adults, we did not observe eight (*Phyllomedusa burmeisteri*, *Scinax* aff. *perereca*, *Scinax eurydice*, *Scinax fuscovarius*, *Leptodactylus furnarius*, *Leptodactylus fuscus*, *Leptodactylus labyrinthicus* and *Leptodactylus mystacinus*) in the tadpole stage in any of the lakes. We recorded 22 species in the tadpole stage in the four lakes combined, and we did not observe one of those as adult (*Scinax* gr. *ruber*). Estimates of species richness using records of anurans in all life stages (adults, tadpoles, egg clutches and froglets) produced the same results as estimates obtained using adult and tadpole records combined, because species recorded as eggs or froglets were also observed as adults and/or tadpoles in the same lake, in the same month (Table 3).

The comparison among anuran species inventories available for the QFe showed high levels of dissimilarity (Figure 1A). Similarity was greater than 50% only between FLOE Uaimií at Ouro Preto (Pirani 2011) and a locality at the Serra de Ouro Branco (São-Pedro and Feio 2011). A group was formed by two data sets from the RPPN Caraça (Kopp and Eterovick 2006; Canelas and Bertoluci 2007); however, a third study conducted at this site had a very different result (Afonso and Eterovick 2007). Although the latter was conducted only at streams, the data set provided by Canelas and Bertoluci (2007) included data from streams, lakes and swamps whereas the work by Kopp and Eterovick (2006) included just lakes. Our study site was grouped with E.A. Peti at Santa Bárbara (Bertoluci et al. 2009) and I. Inhotim at Brumadinho (Linares and Eterovick 2013), which is surprising because both sites have mostly forest formations whereas the study site is comprised of open habitats. However, the level of dissimilarity was close to 60% (Figure 1A). At Nova Lima, one locality (E.E. Fechos; Leite 2007) grouped with Rio Acima (C. Canto das Águas; Grandinetti and Jacobi 2005), whereas the other (A.P. Mutuca; Nascimento et al. 1994) was one of the most different from the remaining areas, with at least 80% dissimilarity (Figure 1A). Geographic distance did not explain dissimilarity levels among localities sampled in the QFe even accounting for the different types of bodies of water and sampling methods included in each study/locality ($Z = 39.04$; $r = 0.144$; $p = 0.187$; Figure 1B).

Table 3. Temporal distribution of anurans at four *canga* lakes at the Quadrilátero Ferrífero (southeastern Brazil) from January 2011 to January 2012.

Taxa	Jan	Feb	Mar	Apr	May	Jun	Jul**	Aug**	Sep**	Oct	Nov	Dec	Jan	Lakes
Family Hylidae														
<i>Bokermannohyla circumdata</i>	V, Voc, T	T	T	V, T	T	V, T	V, T, Fr	T	T	V, T	V, T	V, T, Fr	V, Voc, T	4*
<i>Dendropsophus decipiens</i>	V, Voc	V, Voc, E	T	V, Voc	T	V, Voc	V	Voc	V, Voc	V, Fr, E	V, Voc, T, Fr	V, Voc, T, Fr	T	4*
<i>Dendropsophus elegans</i>	V, Voc, T	V, Voc, T	V, Voc, T	V, Voc, T	T	Voc, T	Voc	Voc	V, Voc	V, Voc, T	V, Voc, T	V, Voc, T	V, Voc, T	1, 2, 3, 4
<i>Dendropsophus minutus</i>	V, Voc, T	V, Voc, T	V, Voc, T	V, Voc, T	T	Voc, T	Voc	Voc	V, Voc	V, Voc, T	V, Voc, T	V, Voc, T	V, Voc, T	1, 2, 3, 4
<i>Dendropsophus seniculus</i>	V, Voc	V, Voc	V, Voc	V, Voc	T	T	T	T	V	V, Voc, T	V, Voc, T	V, Voc, T	T	1, 2, 3
<i>Hypsiboas albopunctatus</i>	V, Voc, T	V, Voc, Am	V, Voc, T	V, Voc	T	T	T	Voc	V, T	V, Voc, Fr	Voc	Voc	Voc	4
<i>Hypsiboas faber</i>	V, Voc	V, Voc	V, Voc, T	V, Voc	T	T	Voc	Voc	V, T	V, Voc, T, E	V, Voc, T	V, Voc, T	V, Voc, T	1, 2, 3, 4
<i>Hypsiboas polytaenus</i>	V, Voc	Voc	Voc	V, Voc	V, Voc	V	V, Voc	Voc	Voc	V, Voc	V, Voc	V, Voc	V, Voc	4
<i>Phyllomedusa burmeisteri</i>														2, 4
<i>Scinax aff. flavoguttatus</i>	Voc		T							V	V, Voc	V, Voc		4*
<i>Scinax aff. perereca</i>	T	T	T	T	T	T	V	V	V	Voc	Voc	Voc	Voc	4*
<i>Scinax gr. ruber</i>	V, Voc	V, Voc	V, Voc	V, Voc, T, Fr	Voc, T, Fr	V, Voc	V	V	V	T	T	T	T	1, 2, 3, 4
<i>Scinax curitaca</i>	V, Voc	V, Voc	V, Voc	V	V	V	V	V	V	Voc	V, Voc	Voc	V, Voc	1, 2, 3, 4
<i>Scinax eurydice</i>	V, Voc, T	V, Voc, Am, T	V, Voc, T	T	V	V	V	V	V	V, Voc	V, Voc, T	V, Voc	V, Voc	1, 2, 3, 4
<i>Scinax fuscomarginatus</i>	V, Voc, T	V, Voc	V, Voc, T	V, Voc, T	V	V	V	V	V	V, Voc	V, Voc, T	V, Voc	V, Voc, T	1, 2, 3, 4
<i>Scinax fuscovarius</i>														1, 3, 4
<i>Scinax luizotavioi</i>	T	V, Voc, T	T	V, Voc, col	V, Voc	V, Voc	V, Voc, T	V, Voc, T	V, Voc, T	V, Voc	V, Voc	T	T	4*
<i>Scinax rogerioi</i>	V, Voc, T	Voc, T	V, Voc, T	V, Voc, T	V, Voc, T, Fr	V, Voc, T	V, Voc, T	Voc, T	Voc, T	V, Voc	Voc, T	V, Voc	Voc, T	1, 2, 3, 4
<i>Sphaenorhynchus aff. surdus</i>														1, 2, 3, 4
Family Leptodactylidae														
<i>Leptodactylus furnarius</i>	Voc	Voc		Voc	Voc	V	V	V, Voc	V	Voc	Voc	V, Voc	Voc	1, 4
<i>Leptodactylus fuscus</i>	Voc	Voc		Voc	Voc	V	V, Voc	V, Voc	V, Voc	V, Voc	V, Voc	Voc	Voc	1, 2, 3, 4
<i>Leptodactylus jolyi</i>	V	V, Voc		V	V	V	V	V	V	Voc	Voc, T	Voc	Voc	1, 3
<i>Leptodactylus labyrinthicus</i>	V	V	V	V	V	V	V	V	V	V, Voc	Voc	V	V	1, 3, 4
<i>Leptodactylus latrans</i>											V, Voc, T	V, Voc	V	1, 2, 3, 4
<i>Leptodactylus mystacinus</i>											Voc	Voc	V	2, 3
<i>Physalaemus crombiei</i>	Fr, T	Voc	Voc	T		V	V	Voc	Voc	Voc	V, Voc	V, Voc	V, T	4*
<i>Physalaemus cuvieri</i>	V, Voc, Fr							Voc	V, Voc, T, E	Voc, T	V, Voc, T	V, Voc, T	T, Fr	1, 2, 3, 4
<i>Physalaemus orophilus</i>								V	V	V, E	V, Voc	V, Voc	V, Voc, T, Fr	4*
Family Microhylidae														
<i>Elachistocleis cesarii</i>	V, Fr, T	Voc		V	V, T	V	V	V, Voc	Voc, T	V, Voc, T	V, Voc, T	Voc, T	V, T, Fr	1, 2, 3, 4
Species total (adults)	16	17	11	13	8	12	5	13	12	23	25	20	17	
Species total (tadpoles)	9	7	11	9	8	5	2	4	4	4	12	9	14	

Types of records: V, visual; Voc, vocalization; T, tadpole; Fr, froglet; E, egg clutch; Am, amplexant pair. 4*, Lake 4 including adjacent forest. **, months when temporary lakes were dry.

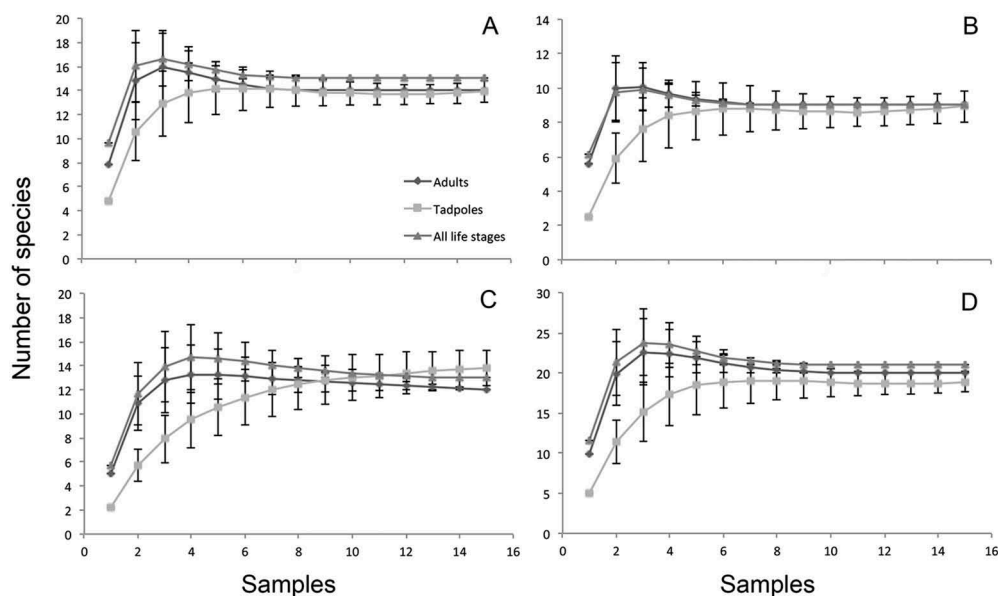


Figure 2. Rarefaction curves based on Jackknife I species-richness estimator for records of adults, tadpoles and all life stages pooled for four *canga* lakes at the Quadrilátero Ferrífero region, south-eastern Brazil.

Discussion

The numbers of anuran species recorded at the sampled lakes are comparable to those reported in studies conducted at other localities in the QFe (see Table 2). The numbers of species recorded as adults were higher than the numbers recorded in the tadpole stage when both life stages were sampled. At the RPPN Caraça, Afonso and Eterovick (2007) recorded 19 species at streams, only five of which were also recorded as tadpoles. Kopp and Eterovick (2006) recorded 22 anuran species at lakes, only half of which were tadpoles. At Rio Acima, Grandinetti and Jacobi (2005) recorded 11 species, two of which were tadpoles and two others were eggs. A higher number of species recorded based on adult individuals compared with tadpoles seems to be the general finding, as also happened in the present study. This is not a reason to overlook the larval stage, as our results show. Although we found adults from more species than tadpoles, we did find tadpoles that represented species that would not have been registered if considering only the records of adult frogs in each of the lakes. This result is reflected in the higher estimate of species richness obtained based on records of adults and tadpoles together. We observed some species as tadpoles before we found them in the adult stage. This has important implications for inventories that need to be conducted in a short period of time. Species-richness estimates showed a higher number of species with data from adult and tadpole records combined for all sample sizes, indicating that no matter how much sampling effort is applied, the results will always be better with the inclusion of tadpole sampling in the species inventory. Silva (2010) also pointed out the better performance of combined methodologies (including tadpole sampling) when inventorying anuran diversity.

We found all localities in the QFe with available data on amphibian species composition to be very different from each other, and geographic distance did not explain this dissimilarity. Variability in the types of bodies of water sampled at each locality (see Table 2) is expected to have an influence on species composition, as anurans have specific reproductive modes that determine what bodies of water they choose for reproduction (Duellman and Trueb 1994; Haddad and Prado 2005). However, the types of bodies of water sampled did not explain the groups found either. For instance, Kopp and Eterovick (2006) and the present study included the same habitat types (permanent and temporary lakes, one permanent lake adjacent to a forest and flowing water) and the study sites were close (only 11.7 km apart), but still species composition at the two sites had a dissimilarity of over 60%. The same is true for Brumadinho (I. Inhotim; Linares and Eterovick 2013) and another site at Catas Altas (RPPN Caraça; Canelas and Bertoluci 2007) that are 75 km apart. These results indicate high spatial variability for anuran assemblages in the QFe, which has also been reported for plants (Jacobi et al. 2007). This finding has important conservation implications, meaning that intensive local inventories are necessary to predict the potential impacts of any new enterprise at the QFe.

About 40% of *canga* formations have been destroyed since the 1990s, with mining activities being the main cause (responsible for 85% of the area lost). They also contributed to isolation of habitat patches and a reduction of about 13% in mean patch area (Carmo 2010). In Minas Gerais State, less than 280 ha (2.5%) of *canga* formations are included in legally protected areas (Carmo 2010). Carmo (2010) estimated the current area covered by *canga* as 11,172 ha. Leite (2012) performed a gap analysis on the projected distribution of 47 anuran species endemic to the Espinhaço Mountain Range and found most of them to be far from the ideal level of protection. The ideal level of protection was defined as a species-specific representation target depending on the area occupied by each species. That is, for species with more restricted distribution, a higher percentage of its range should be inside protected areas to achieve its conservation target (see Leite 2012). Some species are not even recorded or expected to occur inside any conservation unit, as is the case for *Sphaenorhynchus* aff. *surdus*, a species endemic to the QFe.

Two described anuran species (*Bokermannohyla martinsi* and *Hylodes uai*) are considered to be endemic to the QFe, whereas many others recorded in the region are distributed up to the central portion of the Espinhaço Mountain Range (Leite et al. 2008, 2012). Many other anurans cannot be assigned to any described species and may represent new species waiting for formal descriptions (e.g. *Adelophryne* sp., *Bokermannohyla* aff. *circumdata*, *Bokermannohyla* aff. *feioi*, *Flectonotus* aff. *fissilis*, *Ischnocnema* gr. *parva*, *Scinax* aff. *flavoguttatus*, *Scinax* aff. *perereca*, *Sphaenorhynchus* aff. *surdus*, *Vitreorana* aff. *eurygnatha*; F.S.F.L., pers. obs.). Additional inventory and taxonomic studies focused on the anurans of the QFe are therefore likely to reveal new species, some of them more than likely endemic.

The QFe is recognized as a centre of diversity and endemism where the distribution of plants is associated with edapho-climatic variations originated by altitudinal gradients (Jacobi et al. 2007). The barriers imposed by altitudinal gradients combined with the limited mobility of amphibians result in many isolated populations and opportunities for speciation, and probably contribute to high beta diversity for anurans (Leite 2012; Nascimento 2013). This scenario, combined with the high levels of threat suffered by

the QFe, indicates a high priority for conservation efforts such as increased investment in species inventories and a better understanding of species distribution patterns. Inventory studies required for the implementation of mining and other destructive activities in the region should be conducted with more effective protocols, including sampling of seasonal variation and all available anuran life stages, and taking into account similarity between the sampled area and other preserved sites.

Our data indicate that anuran inventories are likely to provide better results when both larval and adult life stages are sampled, especially in the short term. Improvement of inventory quality is of great importance in regions with high levels of threat like the QFe. The available information for the QFe shows that anuran assemblages vary spatially and that even geographically close sites can present distinct faunas, stressing the importance of good local inventories before the implementation of new impacting enterprises.

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Disclosure statement

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Appendix 1. Voucher specimens housed at the Amphibian (post-metamorphic) and tadpole collections of the Herpetological Collection of the Centre of Taxonomic Collections of the Universidade Federal de Minas Gerais (UFMG).

Amphibia: *Bokermannohyla circumdata* UFMG 7235; *Dendropsophus decipiens* UFMG 7214; *Dendropsophus elegans* UFMG 5196, 5202, 5212, 5222, 5229, 7189, 7190, 7212; *Dendropsophus minutus* UFMG 5203–5208, 5210, 5217–5221, 7182, 7183; *Dendropsophus seniculus* UFMG 7191, 7202, 7213, 7216, 7250, 7251; *Hypsiboas albopunctatus* UFMG 7203, 7217; *Hypsiboas faber* UFMG 5192, 5193, 5195, 5198, 5199, 5237, 7196, 7197; *Hypsiboas polytaenius* UFMG 7229, 7230; *Phyllomedusa burmeisteri* UFMG 7195; *Scinax* aff. *flavoguttatus* UFMG 7254, 7258–7260, 7263–7265; *Scinax* aff. *perereca* UFMG 7257, 7261, 7262, 7266; *Scinax curicica* UFMG 5231, 7201, 7218, 7223, 7232, 7244; *Scinax eurydice* UFMG 5197, 5235, 5236, 7186, 7187, 7224, 7227, 7236; *Scinax fuscomarginatus* UFMG 5211, 7181, 7184, 7185, 7198–7200, 7210, 7211; *Scinax fuscovarius* UFMG 7249; *Scinax luizotavioi* UFMG 7225, 7233, 7234; *Scinax rogerioi* UFMG 5209, 5223–5227, 5230, 5233, 5238, 5239, 7215, 7252, 7255, 7256; *Sphaenorhynchus* aff. *surdus* UFMG 5213–5215, 7192–7194, 7205, 7207–7209, 7219–7221, 7237–7240; *Physalaemus crombiei* UFMG 7241, 7242, 7246, 7247; *Physalaemus cuvieri* UFMG 5240–5243, 7188; *Physalaemus orophilus* UFMG 7231, 7243; *Leptodactylus furnarius* UFMG 7248; *Leptodactylus fuscus* UFMG 5232, 5234, 5245; *Leptodactylus labyrinthicus* UFMG-A 5216; *Leptodactylus latrans* UFMG 7204, 7206; *Leptodactylus mystacinus* UFMG 7253; *Elachistocleis cesarii* UFMG 5194, 5200, 5201, 5228, 5244, 7222. **Tadpoles:** *Bokermannohyla circumdata* UFMG 1294, 1311, 1313, 1333; *Dendropsophus decipiens* UFMG 1310, 1453; *Dendropsophus elegans* UFMG 1300, 1437, 1441, 1444; *Dendropsophus minutus* UFMG 1289, 1297, 1299, 1306, 1307, 1312, 1316, 1317, 1322, 1329, 1330; *Dendropsophus seniculus* UFMG 1319; *Hypsiboas albopunctatus* UFMG 1334, 1454; *Hypsiboas faber* UFMG 1293, 1298, 1327, 1332, 1445; *Scinax* gr. *ruber* UFMG 1284, 1286, 1287, 1303, 1308, 1315, 1320, 1324, 1447, 1449; *Scinax curicica* UFMG 1452; *Scinax* aff. *flavoguttatus* UFMG 1314; *Scinax fuscomarginatus* UFMG 1292, 1295, 1301, 1305, 1323, 1439, 1440, 1443; *Scinax rogerioi* UFMG 1285, 1288, 1302, 1321, 1326, 1335, 1442, 1446, 1450, 1455, 1456; *Sphaenorhynchus* aff. *surdus* UFMG 1296, 1304, 1318, 1325, 1328, 1331, 1448, 1451, 1457; *Physalaemus cuvieri* UFMG 1290, 1436, 1438; *Physalaemus orophilus* UFMG 1309; *Elachistocleis cesarii* UFMG 1291.

Appendix 2. List of anuran species gathered from available inventories of sites in the Quadrilátero Ferrífero, southeastern Brazil, with corresponding references.

Taxa	References
Family Siphonopidae	
<i>Luetkenotyphlus brasiliensis</i>	7*
<i>Siphonops annulatus</i>	5
<i>Siphonops paulensis</i>	7
Family Brachycephalidae	
<i>Ischnocnema guentheri</i>	3
<i>Ischnocnema izecksohni</i>	2, 4, 5, 6, 8, 9, 12
<i>Ischnocnema juipoca</i>	3, 4, 7, 8, 12
<i>Ischnocnema surda</i>	12
<i>Ischnocnema</i> gr. <i>lacteus</i>	4
<i>Ischnocnema</i> aff. <i>juipoca</i>	5
Family Bufonidae	
<i>Rhinella pombali</i>	2, 3, 4, 5, 6, 7, 8, 9, 10, 12
<i>Rhinella rubescens</i>	4, 7, 8, 10, 12
<i>Rhinella schneideri</i>	7
Family Centrolenidae	
<i>Vitreorana uranoscopa</i>	3, 4, 8, 9, 12
<i>Vitreorana eurygnatha</i>	6, 11, 12
<i>Vitreorana</i> aff. <i>eurygnatha</i>	4

(Continued)

Appendix 2. (Continued).

Taxa	References
Family Craugastoridae	
<i>Haddadus binotatus</i>	5, 7, 8, 9, 12
Family Cycloramphidae	
<i>Thoropa megatympanum</i>	4
<i>Thoropa miliaris</i>	4, 5
Family Hylidae	
<i>Aplastodiscus arildae</i>	4, 8, 9, 12
<i>Aplastodiscus cavicola</i>	3, 5, 8, 12
<i>Bokermannohyla alverangai</i>	3, 4, 12
<i>Bokermannohyla circumdata</i>	1, 3, 4, 5, 6, 7, 8, 12
<i>Bokermannohyla gr. circumdata</i>	5, 8, 9, 10, 11
<i>Bokermannohyla nanuzae</i>	4, 8, 11
<i>Bokermannohyla martinsi</i>	4, 8, 11, 12
<i>Dendropsophus decipiens</i>	1, 5
<i>Dendropsophus elegans</i>	1, 3, 4, 5, 7, 8, 10, 12
<i>Dendropsophus giesleri</i>	12
<i>Dendropsophus minutus</i>	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12
<i>Dendropsophus seniculus</i>	1, 4, 10
<i>Dendropsophus gr. parviceps</i>	3, 9
<i>Dendropsophus rubicundulus</i>	5, 7
<i>Sphaenorhynchus aff. surdus</i>	1
<i>Hypsiboas albopunctatus</i>	1, 2, 3, 4, 5, 7, 8, 10, 11, 12
<i>Hypsiboas faber</i>	1, 2, 3, 4, 5, 7, 8, 10, 11, 12
<i>Hypsiboas lundii</i>	2, 7
<i>Hypsiboas pardalis</i>	3, 8, 12
<i>Hypsiboas polytaenius</i>	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
<i>Phasmahyla jandaia</i>	4, 8, 9, 12
<i>Phyllomedusa burmeisteri</i>	1, 2, 3, 4, 5, 7, 8, 10, 11, 12
<i>Phyllomedusa ayeaye</i>	3, 12
<i>Scinax aff. similis</i>	7
<i>Scinax aff. flavoguttatus</i>	1, 3, 12
<i>Scinax aff. perereca</i>	1, 4, 5, 8, 9, 10, 11
<i>Scinax gr. catharinae</i>	4
<i>Scinax curicica</i>	1, 5, 8, 10, 12
<i>Scinax duartei</i>	4
<i>Scinax eurydice</i>	1, 4, 5, 10
<i>Scinax fuscomarginatus</i>	1, 5, 7
<i>Scinax fuscovarius</i>	1, 2, 3, 4, 5, 7, 8, 9, 10, 12
<i>Scinax longilineus</i>	2, 6, 8, 9, 12
<i>Scinax luizotavioi</i>	1, 3, 4, 5, 7, 8, 9, 10, 11, 12
<i>Scinax machadoi</i>	4, 11
<i>Scinax rogerioi</i>	1, 12
<i>Scinax cf. alter</i>	5
<i>Scinax cf. tripui</i>	12
<i>Scinax gr. rizibilis</i>	6
<i>Scinax gr. ruber</i>	1, 11
<i>Scinax squalirostris</i>	4, 10, 12
<i>Scinax x-signatus</i>	3, 12
Family Hylodidae	
<i>Crossodactylus trachystomus</i>	4
<i>Crossodactylus bokermanni</i>	11, 12
<i>Hylodes babax</i>	8
<i>Hylodes uai</i>	4, 11
Family Leptodactylidae	
<i>Adenomera bokermanni</i>	4
<i>Leptodactylus cunicularius</i>	3, 8, 12
<i>Leptodactylus furnarius</i>	1, 8, 12
<i>Leptodactylus fuscus</i>	1, 2, 4, 7, 8, 9, 12
<i>Leptodactylus jolyi</i>	1, 4, 9, 10, 12
<i>Leptodactylus labyrinthicus</i>	1, 2, 5, 7, 12

(Continued)

Appendix 2. (Continued).

Taxa	References
<i>Leptodactylus latrans</i>	1, 3, 4, 5, 6, 7, 8, 10, 12
<i>Leptodactylus mystaceus</i>	7
<i>Leptodactylus mystacinus</i>	1, 7
<i>Leptodactylus sertanejo</i>	8
<i>Physalaemus crombiei</i>	1, 12
<i>Physalaemus cuvieri</i>	1, 2, 3, 4, 5, 7, 9, 10, 12
<i>Physalaemus evangelistai</i>	4, 10, 12
<i>Physalaemus maximus</i>	3, 12
<i>Physalaemus marmoratus</i>	5
<i>Physalaemus</i> aff. <i>obtectus</i>	5
<i>Physalaemus</i> aff. <i>olfersii</i>	10, 11
<i>Physalaemus orophilus</i>	1
<i>Pseudopaludicola serrana</i>	8, 12
Family Odontophrynidae	
<i>Odontophrynus cultripes</i>	2, 3, 4, 5, 6, 7, 8, 12
<i>Proceratophrys boiei</i>	3, 4, 5, 7, 8, 9, 11, 12
Family Microhylidae	
<i>Elachistocleis cesarii</i>	1, 3, 4, 7, 8, 9, 10, 12
<i>Chiasmocleis</i> sp.	5

References: 1. *Canga* lakes (present study), 2. C. Canto das Águas (Grandinetti and Jacobi 2005), 3. Serra de Ouro Branco¹ (São-Pedro and Feio 2010); 4. RPPN Caraça¹ (Canelas and Bertoluci 2007); 5. E. A. Peti (Bertoluci et al. 2009); 6. A. P. Mutuca (Nascimento et al. 1994); 7. I. Inhotim (Linares and Eterovick 2013) 8. FLOE Uamií (Pirani 2011), 9. E. E. Fechos (Leite 2007) 10. RPPN Caraça³ (Afonso and Eterovick 2007) 11. RPPN Caraça² (Kopp and Eterovick 2006); 12. Serra de Ouro Branco² (São-Pedro and Feio 2011).

*Specimen referred to as sp. 1 in Linares and Eterovick (2013) and posteriorly identified. Superscript numbers represent different studies at the same locality as in Table 2.