



Morphological variation, advertisement call, and tadpoles of *Bokermannohyla nanuzae* (Bokermann, 1973), and taxonomic status of *B. feioi* (Napoli & Caramaschi, 2004) (Anura, Hylidae, Cophomantini)

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Resumo

Bokermannohyla nanuzae (Bokermann & Sazima 1973) e *B. feioi* (Napoli & Caramaschi 2004) pertencem ao grupo de espécies de *B. circumdata*. A localidade-tipo da primeira espécie é a Serra do Cipó, Serra do Espinhaço, e a da segunda é o Parque Estadual do Ibitipoca, Serra da Mantiqueira, ambas no estado de Minas Gerais, Brasil. O padrão de desenhos dorsais, morfologia oral dos girinos e propriedades temporais dos cantos são indicadas como forma de distinguir essas espécies. Porém, diversos espécimes coletados entre as duas localidades-tipo permanecem sem identificação porque apresentam sobreposição nos caracteres e estados propostos para diagnosticar as duas espécies. Com o objetivo de avaliar a variação desses caracteres, foram realizadas análises de morfologia e morfometria de adultos, vocalizações e morfologia de girinos. Espécimes foram divididos em três unidades taxonômicas operacionais: *B. nanuzae* (Serra do Cipó e localidades ao norte, Serra do Espinhaço), *B. cf. nanuzae* (Quadrilátero Ferrífero, Serra do Espinhaço ao sul da Serra do Cipó), e *B. feioi* (Serra do Ibitipoca, Serra da Mantiqueira). Os padrões de desenhos do dorso e membros apresentam variação clinal e as três unidades são muito similares morfometricamente. As propriedades temporais e espectrais do canto apresentam sobreposição entre essas três unidades. Diferenças diagnósticas originalmente propostas para os girinos são variações intrapopulacionais e ocorrem em espécimes de todas as localidades analisadas. Assim, concluímos que essas três unidades são morfologicamente indistinguíveis. Portanto, *Bokermannohyla feioi* (Napoli & Caramaschi 2004) é designado como um sinônimo júnior de *Bokermannohyla nanuzae* (Bokermann & Sazima 1973), o que estende a distribuição geográfica desta para a Serra da Mantiqueira.

Palavras Chave: Neotrópico, morfometria, taxonomia, distribuição geográfica

Abstract

Bokermannohyla nanuzae (Bokermann & Sazima 1973) and *B. feioi* (Napoli & Caramaschi 2004) belong to the *B. circumdata* species group. The type locality of the former is Serra do Cipó, Espinhaço mountain range, and of the latter is Parque Estadual do Ibitipoca, Mantiqueira mountain range, both in Minas Gerais State, Brazil. Differences on dorsal drawing pattern of adults, oral disc morphology of tadpoles, and temporal properties of calls were proposed to distinguish these two species. However, several specimens found between the two type localities remain unidentified because diagnostic characters and states occur in all of these populations. Thus, in order to assess these characters variations, we performed an analysis of the morphology and morphometry of adults, vocalization, and morphology of tadpoles. Specimens were divided into three operational taxonomic units (OTUs): *B. nanuzae* (Serra do Cipó and northwards, Espinhaço mountain range), *B. cf. nanuzae* (Quadrilátero Ferrífero, Espinhaço mountain range, south of Serra do Cipó), and *B. feioi* (Serra do Ibitipoca, Mantiqueira mountain range). Drawing patterns of the dorsum and limbs show clinal variation and the three units are morphometrically very similar. Temporal and spectral properties of calls overlap in these three units. The diag-

nostic differences originally proposed for tadpoles are intrapopulational variations and occur in specimens from all of the locations analyzed. We found that these three units are morphologically indistinguishable. Therefore, we designate *Bokermannohyla feioi* (Napoli & Caramaschi 2004) as a junior synonym of *Bokermannohyla nanuzae* (Bokermann & Sazima 1973), extending its geographical distribution to the Mantiqueira mountain range.

Key words: Neotropical, morphometry, taxonomy, geographic distribution

Introduction

The hyloid frog genus *Bokermannohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler 2005 currently comprises 33 species distributed in the Brazilian Atlantic rain forest and Cerrado domains (Frost 2014). Faivovich *et al.* (2005) recognized four monophyletic species groups within *Bokermannohyla*: *B. circumdata* group, *B. claresignata* group, *B. martinsi* group, and *B. pseudopseudis* group.

The *B. circumdata* group comprises 20 species and evidence for its monophyly is mostly restricted to molecular data (Faivovich *et al.* 2005; Frost 2014). However, some morphological features are commonly shared among species of the group: no dichotomy of transverse bands on the posterior surface of thighs; large, simple, and sharp prepollex; and hypertrophied forearm in adult males (Heyer 1985; Pombal & Haddad 1993; Caramaschi *et al.* 2001; Napoli & Juncá 2006; Leite *et al.* 2011; Carvalho *et al.* 2012). Bokermann & Sazima (1973) described the adult, tadpole, and advertisement call of *B. nanuzae* based on nine males collected at Serra do Cipó, Municipality of Jaboticatubas (currently Municipality of Santana do Riacho), Minas Gerais State, Brazil. The species was later associated with the *B. circumdata* species group (Faivovich *et al.* 2005) and is currently found in a narrow portion of the Serra do Espinhaço, from the Municipality of Ouro Preto to the Municipality of São Gonçalo do Rio Preto, Minas Gerais, Brazil (Leite *et al.* 2006; Lourenço *et al.* 2006; Canelas & Bertoluci 2007; Lima *et al.* 2014a, b). According to the IUCN Red List, *B. nanuzae* is categorized as “Least Concern” despite its small geographic range (less than 20,000 km²) because it is a common species and does not seem to be in decline (Nascimento *et al.* 2010).

Napoli & Caramaschi (2004) consider *Bokermannohyla feioi* and *B. nanuzae* to be morphologically very similar. They distinguished *B. feioi* from *B. nanuzae* by the presence of a dorsolateral blackish brown stripe from the tip of the snout to the inguinal region (although authors indicated that some specimens of *B. nanuzae* could present this stripe in a vestigial state); presence of transverse dark brown bars on the dorsum (absent in *B. nanuzae*); tadpoles with blackish and light brown areas (homogeneous black in *B. nanuzae*); fewer number of tooth rows on tadpoles oral discs (four posterior tooth rows in *B. feioi* and five in *B. nanuzae*); and advertisement calls with multiple pulsed notes (higher number of note groups in *B. feioi* and 2–4 notes in *B. nanuzae*) and longer notes (note length 0.32–0.35 sec in *B. feioi* and 0.17–0.2 sec in *B. nanuzae*).

Currently, some specimens of the *B. circumdata* group have been collected in other sites within the Espinhaço mountain range, mainly in the “Quadrilátero Ferrífero” region (the southernmost region of the Espinhaço), and remain unidentified. Given that the main reason for these specimens have remained unidentified is that (at a superficial approach) they seem intermediary between *B. feioi* and *B. nanuzae*, our study addresses the variation of adult and tadpole morphological, adult morphometrical, and acoustical data in order to understand the specific limit between these two species and correctly assign the unidentified specimens from “Quadrilátero Ferrífero” to the proper taxon.

Materials and methods

We examined 195 adult individuals (168 males and 27 females) identified as *B. nanuzae*, *B. cf. nanuzae*, or *B. gr. circumdata* from 13 localities within the Espinhaço mountain range and 19 specimens of *B. feioi* (18 males and one female) from the type locality (Parque Estadual do Ibitipoca, Municipality of Lima Duarte, Minas Gerais State, Brazil), including holotypes, paratypes, and topotypes of both species (Fig. 1; Appendix 1). Sex and maturity were determined by the presence of secondary sexual characteristics such as oocytes in females and vocal slits in males. In some cases, gonads were directly examined through a little incision on the flanks.

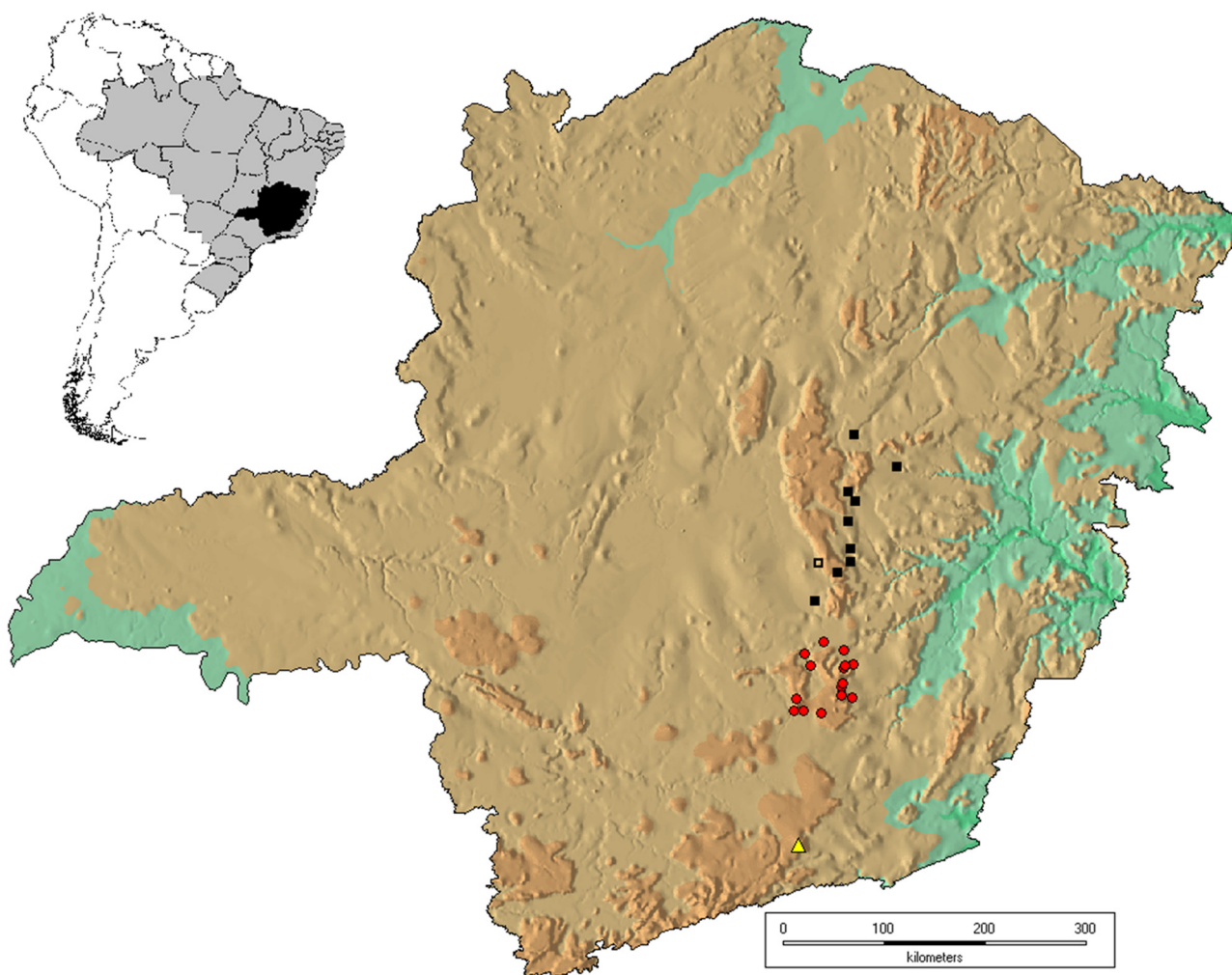


FIGURE 01. Geographic distribution of specimens analyzed, Minas Gerais State, Brazil. Squares, circles and triangles correspond to *B. nanuzae*, *B. cf. nanuzae*, and *B. feioi* OTUs, respectively. The empty square and the triangle represent the type locality of *B. nanuzae* (Serra do Cipó) and *B. feioi* (Parque Estadual do Ibitipoca), respectively.

Examined specimens are housed in the following Brazilian collections: Coleção Célio F. B. Haddad (CFBH, Universidade Estadual Paulista, Rio Claro, SP), Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG, Belo Horizonte, MG), Coleção de Anfíbios do Museu de Ciências Naturais (MCNAM, Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, MG), Museu Nacional (MNRJ, Rio de Janeiro, RJ), Museu de Zoologia (MZUSP, Universidade de São Paulo, São Paulo, SP), Museu de Zoologia “Adão José Cardoso” (ZUEC, Universidade Estadual de Campinas, Campinas, SP), Museu de Zoologia “João Moojen” (UFV, Universidade Federal de Viçosa, Viçosa, MG), Coleção Herpetológica da Universidade Federal de Ouro Preto (UFOP, Universidade Federal de Ouro Preto, Ouro Preto, MG).

Additional material (adults and tadpoles) was collected at the type locality of *B. nanuzae*, at Serra do Cipó, Municipality of Santana do Riacho (19°15'15"S, 43°32'39"W, 1360 m above sea level) and at the type locality of *B. feioi* (adults), at Parque Estadual do Ibitipoca (21°42'48"S, 43°53'55"W, 1,330 m above sea level), Municipality of Lima Duarte, both in Minas Gerais State, Brazil. Collected specimens were deposited in MCNAM. Fieldworks were made under collection permits issued by ICMBIO (number 13328–4) and IEF/MG (number UC 097/10).

Dorsal pattern standards were proposed during the analysis of a large number of individuals and are described below. Fingers are numbered II–V considering that finger I is lost in anurans (Fabrezi & Alberch 1996). Abbreviations used for measurements (in mm) of adult specimens are: SVL (snout-vent length), HL (head length), HW (head width), AL (arm length), FAL (forearm length), HAL (hand length), THL (thigh length), TBL (tibia length), FL (foot length, including tarsus), ED (eye diameter), TD (tympanum diameter), END (eye-nostril

distance), IND (internarial distance), IOD (interorbital distance), UEW (upper eyelid width), DFIV (diameter of finger IV disc), and DTIV (diameter of toe IV disc). Snout-vent length, HL, HW, TBL, ED, TD, IND, IOD, and UEW follow Duellman (1970), AL, FAL, HAL, THL, and TL follow Heyer *et al.* (1990) and FL, END, DFIV, and DTIV follow Napoli & Caramaschi (1999). Snout-vent length, HL, HW, AL, FAL, HAL, THL, TBL, and FL were measured with callipers, whereas the other measurements were made with an ocular grid on a stereo dissecting microscope. We follow Heyer *et al.* (1990) for snout shape standards and hand and foot tubercles and Savage & Heyer (1967) for webbing formula, which was analyzed in 70 specimens from different localities.

We divided adult specimens into three operational taxonomic units (OTU) to perform morphological and morphometric comparisons. Individuals from Serra do Cipó and northern portion of the Espinhaço mountain range composed the *B. nanuzae* unit (61 specimens). Specimens collected at Parque Estadual do Ibitipoca were considered the *B. feioi* unit (19 specimens). Individuals from other localities in the Quadrilátero Ferrífero region formed the *B. cf. nanuzae* unit (134 specimens listed on Appendix 1).

We used the Shapiro-Wilk Test to check if morphometric parameters present normal distribution and the *t*-Test to verify the occurrence of sexual dimorphism. The largest fifteen specimens of each sex independent of the operational taxonomic unit were selected for this test, in order to minimize the effect of differences in number of individuals in each sample. We did it just to verify if we should use males and females in analyses. Test results were considered significant when $p < 0.05$. A Principal Component Analysis (PCA) was performed to understand patterns of variation and covariation of morphometric data of the three operational taxonomic units previously defined, using only specimens for which the 17 measurements were available. This analysis was performed in the package Statistic 7.0 for Windows (STATSOFT 2011) using the covariance matrix rather than a correlation matrix, in order to retain all information about variance and covariance.

We recorded occurrence frequencies of tadpole morphological features and call traits in each operational taxonomic unit to confirm if the diagnoses proposed on original descriptions are maintained with increasing geographic distribution and to check if any other unreported feature could be considered as diagnostic.

We analyzed tadpoles of *B. nanuzae* (15 tadpoles) and *B. feioi* (36 tadpoles) from the type localities, including those described by Napoli & Caramaschi (2004), and tadpoles of the *B. cf. nanuzae* operational taxonomic unit (22 tadpoles from four localities). We paid special attention for tooth row formula and color pattern, currently considered larval features distinguishing *B. feioi* from *B. nanuzae* (see Napoli & Caramaschi, 2004). Tooth row formula follows Altig (1970). All tadpoles analyzed are between stages 26 and 36 according to Gosner (1960).

We analyzed 114 advertisement calls from one specimen (MCNAM 14829) of *B. nanuzae* (Serra do Cipó, 29 October 2010, 7:00 PM, air temperature 18°C) and 41 calls from two specimens (MCNAM 15351 and MCNAM 15353) of *B. feioi* (Parque Estadual do Ibitipoca, 14 and 15 January 2011, 20:00–20:30 PM, air temperature 21.6°C). We also analyzed 70 calls of four specimens (MNRJ 55070, 55071, 60460–61) from Reserva Particular do Patrimônio Natural (RPPN) Serra do Caraça (Municipality of Catas Altas, 20°05'33.44"S, 43°28'51.74"W, 10 November 2008, 21:15–21:30 PM, air temperature 24°C; and 27 September 2009, 21:10 PM, air temperature 17°C) and 39 calls of two specimens (not collected) from Serra do Gandarela (Municipality of Rio Acima, 20°07'22.13"S, 43°39'33.18"W, 28 April 2010), all of which assigned to the *B. cf. nanuzae* operational taxonomic unit. Temperatures were recorded *in loco* with a thermo-hygrometer when available. Moreover, we compare the acoustical data described here with those exposed in Napoli & Caramaschi (2004), Carvalho *et al.* (2012), and Lima *et al.* (2014b).

Recordings were made with a Marantz PMD 660 digital recorder at sample rate of 44.100 kHz and sample size of 16 bits, coupled with a Sennheiser ME66/K6 microphone. We used RavenPro 1.3 (Build 32) for Mac OS X from the Cornell Laboratory of Ornithology (Bioacoustics Research Program, 2003–2008). Call analysis and figure editing were low cut filtered in 500 Hz, FFT with 256 points, and 16-bit resolution. Call terminology follows Duellman & Trueb (1986). The following quantitative parameters were measured: call duration, number of pulses, pulse duration, interval between the two structures observed (see results), and dominant frequency (carrier frequency). Temporal parameters were measured directly from the waveform and frequency parameters were measured directly from the audiospectrogram.

Napoli & Caramaschi (2004) pointed out that they used the call of *B. nanuzae* described by Bokermann & Sazima (1973) to compare it with *B. feioi* and that the absence of new recordings impeded a more detailed analysis. We, therefore, use our recent recordings of *B. nanuzae* in the comparisons rather than data provided by Bokermann & Sazima (1973), thus allowing consistent comparisons. Napoli & Caramaschi (2004) did not indicate the call

terminology they used, so we tried to appropriately designate the authors' description to Duellman & Trueb (1986) terminology in order to perform these comparisons.

Results

Some characters analyzed for the operational taxonomic units did not present variation, hence they were used only to redescribe the species below. Those with variations correspond to some drawing patterns, morphometric data, tadpole color and morphology, and vocalization parameters. These variations are described in the sections below.

Drawing pattern. Drawing patterns variation is related to longitudinal lines and transverse bars. They are:

- Dorsolateral line (Fig. 02): a longitudinal black or brown line extending from the tip of the snout to the inguinal region, which may be complete, incomplete, or absent. The complete line may be dashed or not, and well or poorly marked along its length, but always distinguishable. In addition, this line may serve as a border for the dorsum color pattern, typically darker on the dorsum and lighter on the body sides. The incomplete line always starts on the snout, but may end at the anterior edge of the eyes, at the tympanum, or at the middle of the dorsum.
- Transverse bars on dorsum: brown and light brown thick bars that may be present (sometimes as an irregular outline, disrupted or replaced by dark and light spots, not forming a striped pattern) or absent.
- Vertebral line: black longitudinal median line starting on the top of snout and extending to the middle of the dorsum. It may be present or absent.
- Stains on flanks: thick sagittal bars presenting an irregular outline or dark and light spots, not forming a striped pattern. They may also be absent.
- Transverse bars on limbs: dark brown transverse bars on the dorsum of forearms, thighs, legs, and feet. Bars are always present and well defined on thighs. On the other parts, they occasionally do not resemble bars, rather dark and light spots, not forming a striped pattern, but still considered present. They may also be absent.
- Line on lateral surfaces of forearms and legs: a thin black or brown longitudinal line extending along the lateral surface of forearms and legs, which may be present (even being poorly marked or incomplete in some specimens) or absent.

Distribution of the drawing patterns observed among analyzed males and females is summarized in Table 1. The presence and absence of lines (dorsolateral and on lateral surfaces of forearms and legs) and brown transverse bars on dorsum presented a clinal variation among specimens of *B. nanuzae*, *B. cf. nanuzae*, and *B. feioi* OTUs. On most specimens of the *B. nanuzae* OTU, dorsolateral lines are incomplete and lines on forearms and legs are absent. On the *B. cf. nanuzae* unit, these characters/states are more common. On the *B. feioi* unit, all individuals have a complete dorsolateral line; forearms and legs lines are absent in just one specimen.

All specimens analyzed have transverse bars on thighs, a character used to define the *B. circumdata* species group (Heyer 1985; Pombal & Haddad 1993; Caramaschi *et al.* 2001), but bars do not extend to their anterior and posterior surfaces. In some individuals, including one paratype of *B. nanuzae*, bars are faded or even absent probably due to preservation artifacts.

Transverse bars on limbs (except on thighs) are present in all specimens of *B. feioi* and in most individuals of the *B. cf. nanuzae* and *B. nanuzae* units. However, the presence of this trait varies for each limb and gradually decreases in northern populations.

Vertebral line was found only in a few individuals of the *B. nanuzae* OTU. Stains on flanks do not present clinal variation; they were observed on most specimens of *B. feioi* and *B. nanuzae*, but are less common on specimens of *B. cf. nanuzae*, which have a central geographic distribution.

Morphometric analysis. All morphometric parameters present normal distribution. Sexual dimorphism was verified only for SVL ($t=-2.63$ and $p=0.014$), HW ($t=-2.44$ and $p=0.021$), TD ($t=-2.43$ and $p=0.022$), DFIV ($t=-2.10$ and $p=0.045$), and DTIV ($t=-2.14$ and $p=0.041$). Therefore, we used only males data to perform the PCA.

The analysis shows that the three OTUs highly overlap, thus all measurements do not separate them (Fig. 03). First and second components explain 81.33% of all variation. All the scores for the first component are negative, indicating that the variation found in this component is more related to size rather than shape. The largest variation was found on SVL and the smallest on DFIV.

TABLE 1. Drawing patterns of specimens of the *B. nanuzae*, *B. cf. nanuzae*, and *B. feioi* OTUs. *n* = number of specimens analyzed, male or female. Values in parentheses represent percentages. Dashes indicate no specimens showing that state.

Character	State	<i>B. nanuzae</i> n=60	<i>B. cf. nanuzae</i> n=134	<i>B. feioi</i> n=19
Dorsolateral line	Complete	20 (33.33)	97 (72.39)	19 (100)
	Incomplete	30 (50)	32 (23.88)	–
	Absent	10 (16.67)	5 (3.73)	–
Transverse bars on dorsum	Present	10 (16.67)	68 (50.75)	19 (100)
	Absent	50 (83.33)	66 (49.25)	–
Vertebral line	Present	16 (26.6)	–	–
	Absent	44 (73.4)	134 (100)	19 (100)
Stains on flanks	Present	24 (40)	18 (13.43)	14 (74)
	Absent	36 (60)	116 (86.57)	5 (26)
Transverse bars on forearms	Present	45 (75)	123 (91.79)	19 (100)
	Absent	15 (25)	11 (8.21)	–
Transverse bars on thighs	Present	56 (93.33)	133 (99.25)	19 (100)
	Absent	4 (6.67)	1 (0.75)	–
Transverse bars on legs	Present	53 (88.33)	128 (95.52)	19 (100)
	Absent	7 (11.67)	6 (4.48)	–
Transverse bars on feet	Present	43 (71.67)	90 (67.16)	19 (100)
	Absent	17 (28.33)	44 (32.84)	–
Line on lateral surfaces of forearms	Present	12 (20)	58 (43.28)	18 (94.73)
	Absent	48 (80)	76 (56.72)	1 (5.26)
Line on lateral surfaces of legs	Present	15 (25)	65 (48.51)	18 (94.73)
	Absent	45 (75)	69 (51.49)	1 (5.26)

Tadpoles. We observed intrapopulational variation in tadpoles of the *Bokermannohyla nanuzae*, *B. cf. nanuzae*, and *B. feioi* OTUs. The number of tooth rows varies among individuals of the same OTU, which presented 2(2)/4(1) or 2(2)/5(1) formulae (Fig. 04, Table 2). Most tadpoles of the *B. nanuzae* OTU (80%) have five posterior tooth rows whereas the presence of four posterior tooth rows is more common on the *B. cf. nanuzae* (72.7%) and on the *B. feioi* units (86.1%). Furthermore, one tadpole of *B. cf. nanuzae* and two of *B. feioi* have only three posterior tooth rows.

TABLE 2. Tadpoles morphology variation on specimens of the *B. nanuzae*, *B. cf. nanuzae*, and *B. feioi* OTUs. *n* = number of specimens analyzed. Values in parentheses represent percentages.

Character		<i>B. nanuzae</i> (n=15)	<i>B. cf. nanuzae</i> (n=22)	<i>B. feioi</i> (n=36)
Tooth row formula	2(2)/3(1)	0	1 (4.6)	2 (5.6)
	2(2)/4(1)	3 (20)	16 (72.7)	31 (86.1)
	2(2)/5(1)	12 (80)	5 (22.7)	3 (8.3)
Tail color pattern	brown	9 (60)	5 (22.7)	11 (30.6)
	irregular blotches	6 (40)	17 (77.3)	25 (69.4)
Aggregation of papillae on oral disc	Present	5 (33.3)	9 (40.9)	16 (44.4)
	Absent	10 (66.7)	13 (59.1)	20 (55.6)

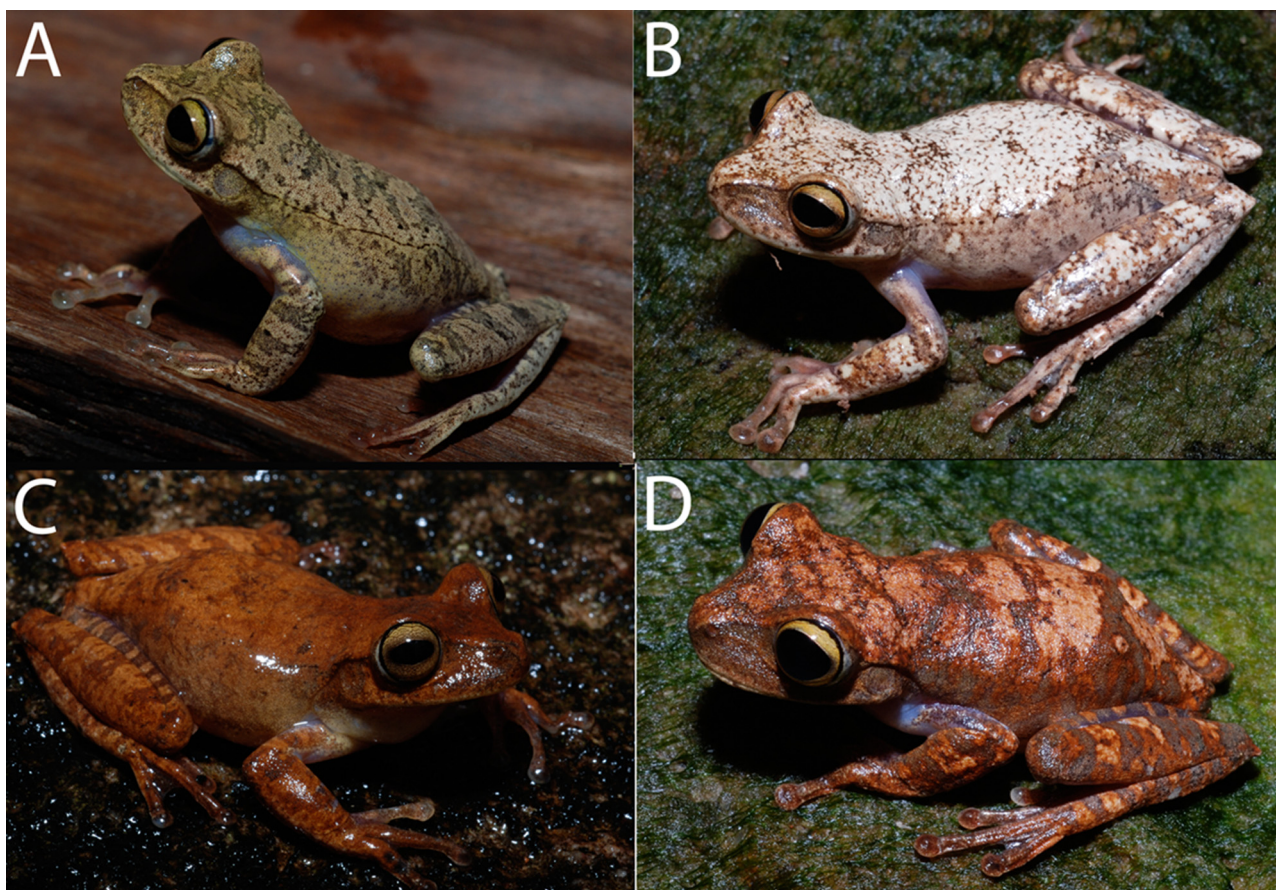


FIGURE 02. Adult males of *Bokermannohyla* cf. *nanuzae* from Catas Altas, Minas Gerais State, with different dorsolateral line patterns: (A) complete, not dashed (MNRJ 55070); (B) complete but dashed (MNRJ 66065); (C) incomplete (MNRJ 51000); (D) absent (MNRJ 66064).

In addition, tail color pattern also varies from homogeneous brown to translucent with irregular brown blotches (Fig. 05). These blotches may vary in number and location, sometimes concentrated on the first third (see figure 4a in Napoli & Caramaschi 2004) or distributed along the whole tail. On most specimens from the *B. nanuzae* OTU, the tail is homogeneous brown (60%) whereas most specimens have irregular brown blotches on tail on the *B. cf. nanuzae* (77.3%) and *B. feioi* (69.4%) units.

The number of labial tooth rows and color pattern have been treated as intraspecific variations in literature and may vary according to the development stage of tadpoles, making it an inappropriate feature to differentiate or identify species in some cases (Kolenc *et al.* 2008; Sánchez 2010). We did not find variations of other external morphological characteristics of tadpoles to distinguish the three OTUs here analyzed. Tadpoles analyzed have body oval in dorsal and ventral views; dorsolateral eyes; rounded and dorsal nostrils; sinistral, short, and posterodorsally directed spiracle, its opening closer to the tip of snout than to anal opening; anal tube short, dextral, attached to ventral fin; tail length 61–64.7% of total length; dorsal fin slightly arched and ventral fin straight; tail tip rounded; anteroventral oral disc, bordered by two rows of rounded papillae, with or without lateral aggregations (found on five specimens of the *B. nanuzae* unit, nine of the *B. cf. nanuzae* unit, and 16 of the *B. feioi* unit); jaw sheaths serrated; anterior jaw sheath slightly arched and posterior jaw V-shaped. For details about internal larval oral anatomy of *B. nanuzae*, see d’Heursel & Haddad (2007).

Call. We recorded two types of calls for *B. nanuzae* from the type locality. Type ‘A’ calls consist of two structures (n=66; Fig. 06a), while type ‘B’ calls have one structure (n=48; Fig. 06b). Means and standard deviations are shown in parentheses. Parameters analyzed for each call type are presented on Table 3.

TABLE 3. Call parameters analyzed for *Bokermannohyla nanuzae* in the present study, Napoli and Caramaschi (2004), and Carvalho *et al.* (2012). * represents means values.

	<i>B. nanuzae</i>			<i>B. cf. nanuzae</i>		<i>B. feioi</i>	
	Serra do Cipó (present study, n=15; one specimen)	Serra do Cipó (Carvalho <i>et al.</i> 2012)	Serra do Caraça (present study, n=70; four specimens)	Serra do Gandarela (present study, n=39; two specimens)	Serra do Caraça (Lima <i>et al.</i> 2014)	Parque Estadual do Ibitipoca (Napoli & Caramaschi, 2004)	Parque Estadual do Ibitipoca (present study, n=36; two specimens)
Call Duration (s)	0.31–0.45 (0.36±0.03, n=66)	0.31–0.49 (0.35±0.028 n=72)	0.21–0.45 (0.36±0.05, n=67)	0.27–0.58 (0.34±0.09, n=24)	0.34*±0.16 (n=100)	0.32–0.35	0.37–0.55 (0.45±0.06, n=21)
Number of Pulses (first structure)	4–27 (12±4, n=1048 pulses in 66 calls)	5–17 (10±0.4)	4–10 (6±1, n=460 pulses in 67 calls)	5–11 (8±1, n=190 pulses in 24 calls)	–	–	3–10 (4±1, n=107 pulses in 21 calls)
Pulse interval	0.001–0.09 (0.022±0.018, n=512)	–	0.001–0.09 (0.021±0.019, n=271)	0.001–0.06 (0.012±0.012, n=753)	–	0.005–0.05	0.001–0.06 (0.023±0.020, n=512)
Pulse duration	0.004–0.023 (0.012±0.004, n=448)	–	0.003–0.016 (0.010±0.002, n=216)	0.002–0.014 (0.006±0.002, n=305)	–	–	0.002–0.022 (0.006±0.003, n=564)
Modulated pulse duration of second structure	0.08–0.20 (0.10±0.02, n=66)	–	0.16–0.48 (0.20±0.07, n=67)	0.10–0.31 (0.13±0.05, n=24)	–	0.15 (overlaid pulses for these authors)	0.19–0.35 (0.27±0.04, n=21)
Interval between the two structure	0.003–0.09 (0.05±0.02, n=66)	0.03–0.15 (0.07±0.006)	0.02–0.07 (0.05±0.01, n=67)	0.012–0.043 (0.02±0.01, n=24)	–	–	0.01–0.05 (0.03±0.01, n=21)
Call Duration (s)	0.31–0.64 (0.45±0.1, n=22)	0.5–0.71 (0.59±0.07)	0.21–0.45 (0.36±0.05, n=5)	0.39–0.80 (0.55±0.12, n=15)	–	–	0.29–0.61 (0.45±0.07, n=20)
Number of pulses	37–60 (49±6, n=1088 pulses in 22 calls)	46–60 (10±2.25)	27–47 (36±9, n=178 pulses in 5 calls)	43–64 (50±7, n=753 pulses in 15 calls)	–	–	43–64 (50±7, n=753 pulses in 20 calls)
Dominant Frequency (kHz)	1.894–2.067 (2.014±0.079, n=114)	2.25–2.63 (2.47±0.06)	1.878–2.412 (2.245±0.129, n=70)	2.067–2.437 (2.359±0.094, n=39)	2.1*±0.74 (n=30) in type A 2.2*±0.31 (n=15) in type B	1.9–2.4	2.067–2.584 (2.379±0.178, n=41)
Second frequency band (kHz)	1.206–1.550 (1.263±0.102, n=114)	–	1.378–1.550 (1.517±0.069, n=70)	1.500–1.687 (1.531±0.071, n=39)	1.1*±0.08 (n=30) in type A 1.1*±0.16 (n=15) in type B	–	1.206–1.550 (1.394±0.153, n=41)

In type 'A' calls, the first structure corresponds to sequential pulses varying in number and interval length. Number of pulses ranges from four to 27 (12 ± 4 , $n=1048$ pulses in 66 calls), and call duration ranges from 0.31 to 0.45 s (0.36 ± 0.03 s, $n=66$). Intervals between pulses range from 0.001 to 0.09 s (0.022 ± 0.018 s, $n=512$) and pulse duration ranges from 0.004 to 0.023 s (0.012 ± 0.004 s, $n=448$). The second structure presents juxtaposed pulses with descending amplitude modulation and duration ranging from 0.08 to 0.20 s (0.10 ± 0.02 s, $n=66$). Although isolated pulses are present at the beginning of this structure, most of them cannot be individually limited, making it difficult to count them. The interval between the two structures ranges from 0.003 to 0.09 s (0.05 ± 0.02 s, $n=66$).

Type 'B' calls are composed of a single structure consisting of a great number of sequential pulses ranging from 37 to 60 (49 ± 6 , $n=1088$ pulses in 22 calls). Call duration ranges from 0.31 to 0.64 s (0.45 ± 0.10 s, $n=22$).

Considering both call types, dominant frequency varies from 1.894 to 2.067 kHz (2.014 ± 0.079 kHz, $n=114$). Moreover, all calls also have a second frequency band ranging from 1.206 to 1.550 kHz (1.263 ± 0.102 kHz, $n=114$) and distinct sidebands.

Napoli & Caramaschi (2004) described the advertisement call of *B. feioi* as multiple pulsed notes. In their study, they observed that the first four pulses (figure 3A in Napoli & Caramaschi 2004 present five first pulses) have easily distinguishable intervals between them and are separated from the last group, which comprises several pulses very close to each other. These authors differentiate the advertisement calls of *B. nanuzae* from that of *B. feioi* based on the number of notes and duration. Analyzing the spectrogram they presented (fig. 3A in Napoli & Caramaschi 2004), we found that the pulse groups, described as the first four pulses, and the last group correspond to the two structures of the type 'A' call described herein for *B. nanuzae*. They did not report a second type of call with only one structure (type 'B' call). The calls of *B. feioi* we recorded present the same structure described for *B. nanuzae* in the present study, including the two types of calls ($n=32$ type 'A' calls and $n=9$ type 'B' calls). Thus, call structure in *B. feioi* and *B. nanuzae* is not different, nor is call duration (0.32 to 0.35 s for *B. feioi*, indicated as "note duration" in Napoli & Caramaschi, 2004; 0.37 to 0.55 s for *B. feioi*, present study; and 0.34 to 0.45 s for *B. nanuzae*, present study).

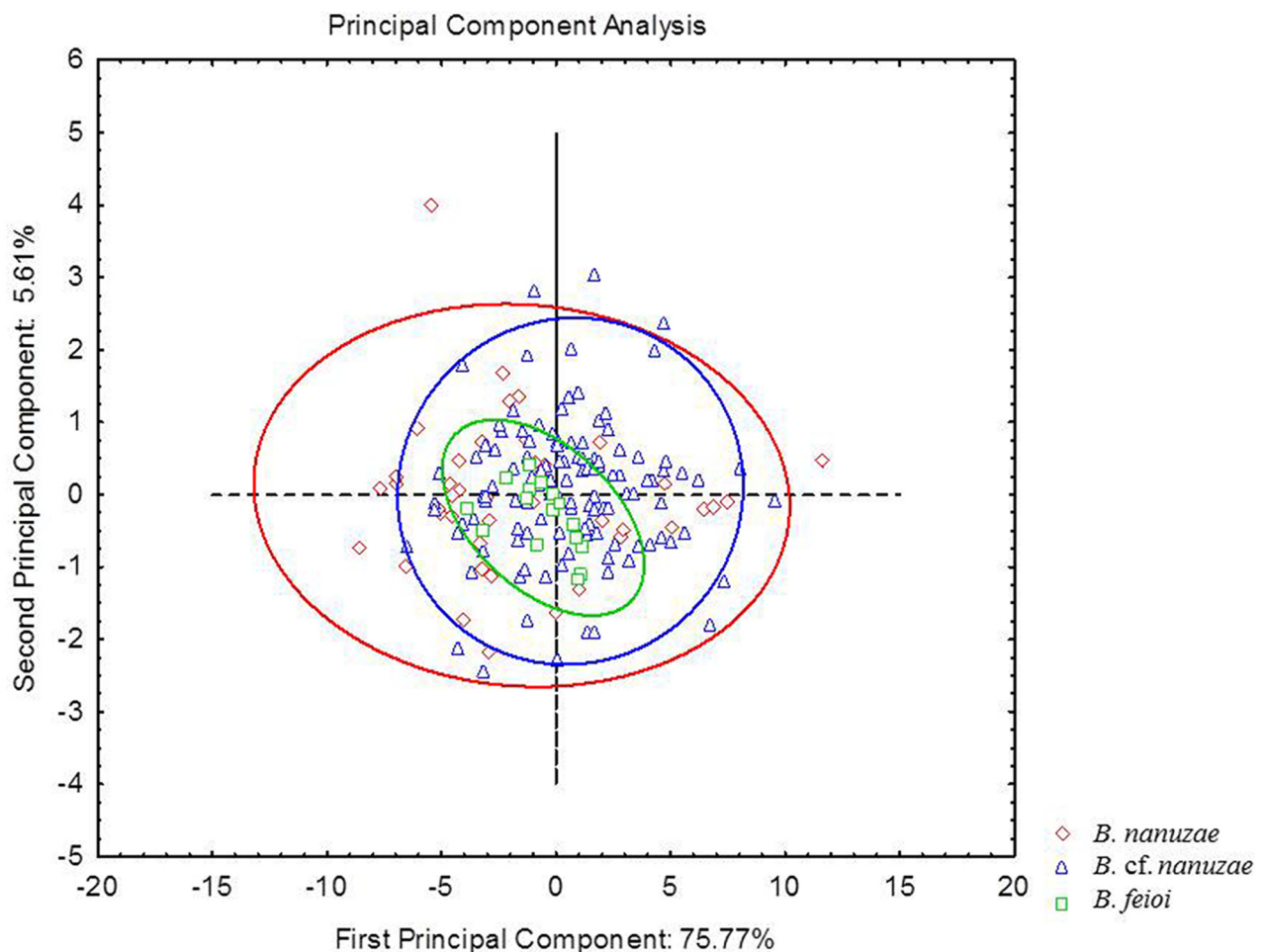


FIGURE 03. Principal Component Analysis (PCA) for 17 morphometric parameters from *B. nanuzae*, *B. cf. nanuzae*, and *B. feioi* OTUs. Ellipses show 95% confidence interval.

The advertisement call of the *B. cf. nanuzae* OTU presented the same structure described above. In fact, vocalizations of the three OTUs shared great similarity (Table 02 and Fig. 07 a–d). Call interval and pulse duration varied within populations of this unit. Dominant frequency ranges overlap in all calls, but is slightly higher on specimens from Serra do Gandarela and Parque Estadual do Ibitipoca.

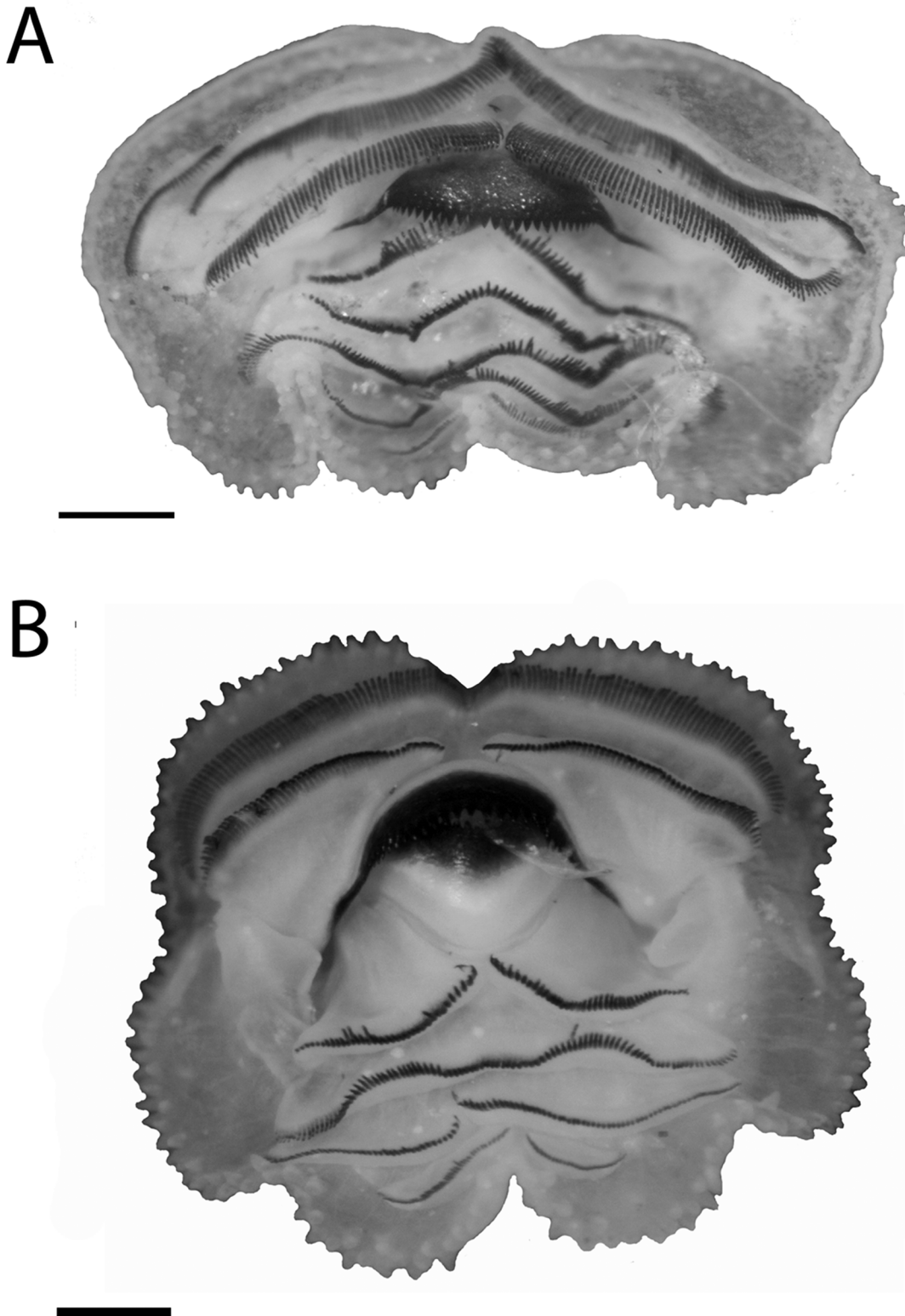


FIGURE 04. (A) Five posterior tooth rows and (B) four posterior tooth rows in the oral disc of tadpoles of *Bokermannohyla nanuzae* from Serra do Cipó, Minas Gerais State, Brazil, stage 37 (MCNAM 16337). Scale bar = 0.25mm.



FIGURE 05. Tadpoles of *Bokermannohyla nanuzae* (MCNAM 16337) from Serra do Cipó, Minas Gerais State, Brazil. Above: tail color pattern homogeneous brown (stage 31). Below: tail color pattern translucent with brown irregular blotches (stage 26). Scale bar = 5mm.

Carvalho *et al.* (2012) recently reassessed the call of *B. nanuzae* based on topotypes. Calls named here as type ‘A’ correspond to the most common call (two notes, A and B) in their study; calls named here as type ‘B’ correspond to the less common call recorded by these authors, which present longer note types with no internote interval. Temporal and spectral properties described by Carvalho *et al.* (2012) are very similar to those described herein except for dominant frequency, which is somewhat lower in our study. However, when all populations we analyzed are considered, dominant frequencies overlap. Several authors have already documented variations in dominant frequency correlated with differences in temperature, male sizes, and social context (Gerhardt 1974; López *et al.* 1988; Hasegawa *et al.* 1999; Castellano *et al.* 2002a; Castellano *et al.* 2002b; Amézquita *et al.* 2009; Brunetti *et al.* 2014).

Carvalho *et al.* (2012) also reported calls with a single structure, named “longer calls”. These authors, as well as Bokermann & Sazima (1973), indicated the presence of additional frequency bands, apparently not harmonics. Although not formally stated by Carvalho *et al.* (2012), this characteristic is also observed in spectrograms of other species of *Bokermannohyla* described in that study. Additional frequency bands have not been observed in any other genera of the tribe Cophomantini. Thus, the taxonomic distribution of the additional frequency bands within Cophomantini must be widely known to test if this characteristic is a synapomorphy of *Bokermannohyla*.

Lima *et al.* (2014b) described three types of calls for *B. cf. nanuzae*: one they considered the advertisement call and two courtship calls. Although it was not mentioned by the authors it is possible to observe in their spectrogram that the advertisement call consisted of two structures (see Fig. 2A, B in Lima *et al.* 2014b) and that acoustic parameters of both structures were described together (see Table 1 in Lima *et al.* 2014b). That is, the advertisement call presented by these authors corresponds to the type A call described herein. In addition, each note described by them corresponds to a full type A call and the fundamental frequency indicated by these authors corresponds to the second frequency band we described. The acoustic parameters presented by these authors are within the ranges of those in our study. The Courtship Call 1 and 2 (*sensu* Lima *et al.* 2014) are described based on a behavior context not observed by us. However, comparing the acoustic parameters presented in both studies we suggest that their Courtship Call 1 is the same we described as the type B call.

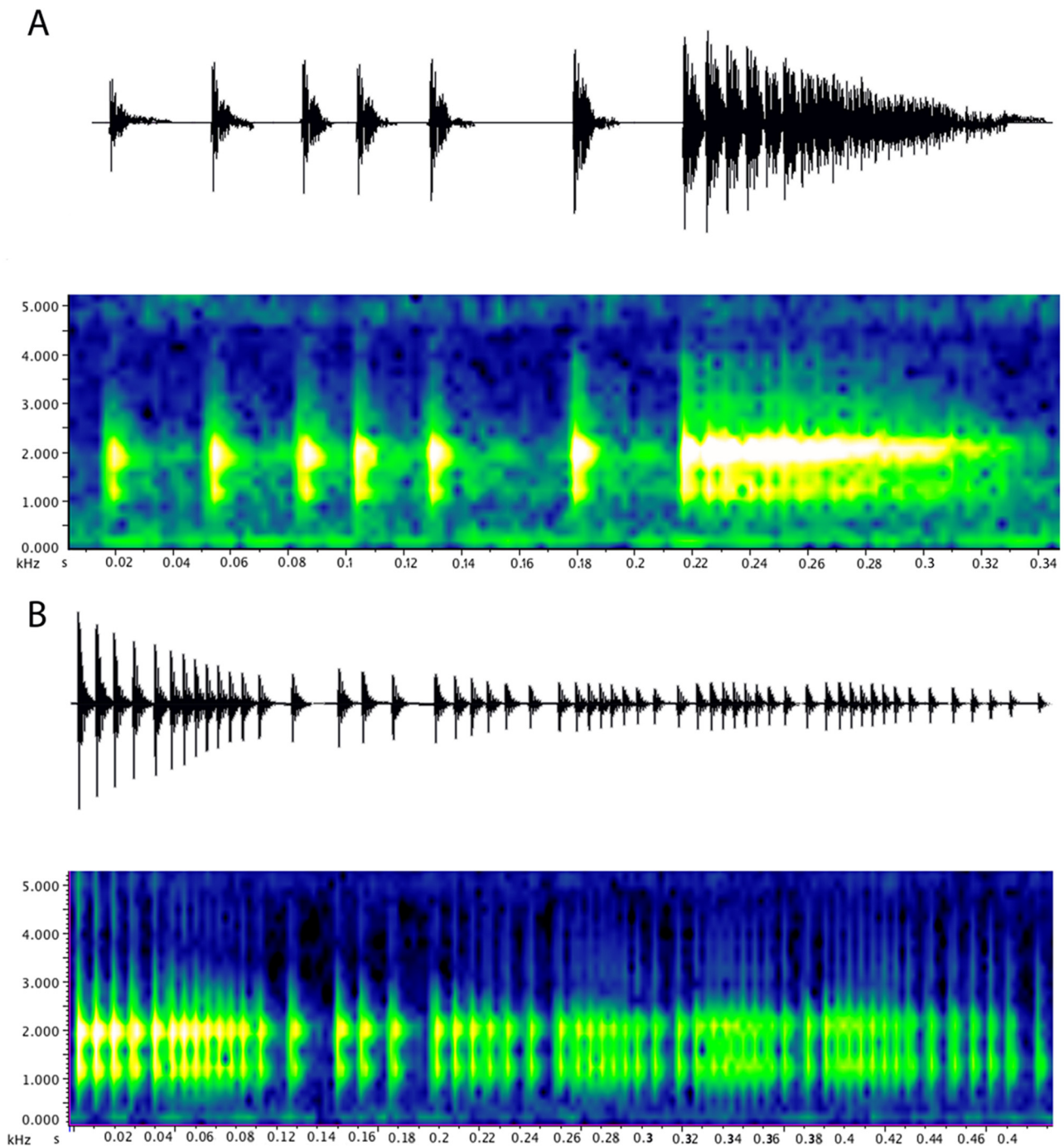


FIGURE 06. Waveform (above) and spectrogram (below) of calls (A) with two structures and (B) with only one structure of *Bokermannohyla nanuzae* (MCNAM 14829), recorded at Serra do Cipó, Municipality of Santana do Riacho, Minas Gerais State, Brazil.

Discussion

The drawing patterns used by Napoli & Caramaschi (2004) to diagnose *B. feioi* are found in some specimens of all units analyzed in this study, including topotypes and type specimens of *B. nanuzae*. Morphometric parameters do not distinguish OTUs, as shown by the PCA (Fig. 3). The number of labial tooth rows and tail color pattern used as diagnostic characters in Napoli & Caramaschi (2004) is not sustained by our data. Finally, the advertisement calls of *B. feioi* and *B. nanuzae* are not different although Napoli & Caramaschi (2004) considered the call of the former as composed of a single structure and our interpretation led to the recognition of two call types.

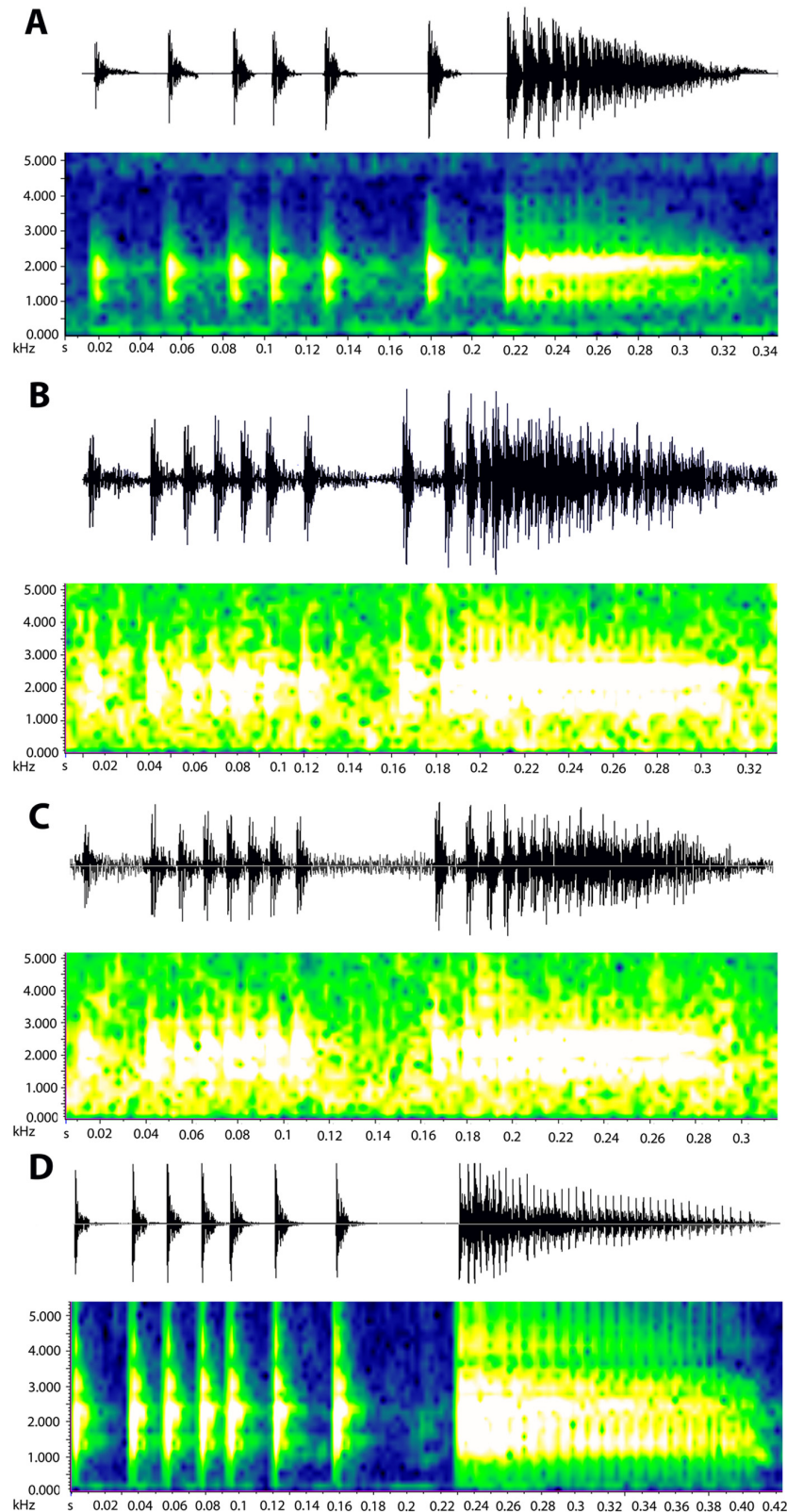


FIGURE 07. Waveform (above) and spectrogram (below) of calls of (A) *Bokermannohyla nanuzae* (MCNAM 14829) recorded at Serra do Cipó, Municipality of Santana do Riacho, Minas Gerais State, Brazil, (B) *B. cf. nanuzae* (unvouchered) recorded at Serra do Gandarela, Municipality of Rio Acima, Minas Gerais State, Brazil, (C) *B. cf. nanuzae* (MNRJ 60460) recorded at Serra do Caraça, Municipality of Catas Altas, Minas Gerais State, Brazil, and (D) *B. feioi* (MCNAM 15352) recorded at Serra do Ibitipoca, Municipality of Lima Duarte, Minas Gerais State, Brazil.

The analysis of morphometric parameters, morphology of adults and tadpoles, and call properties of *B. nanuzae* and *B. feioi* lead us to conclude that our OTUs have morphological differences among them; however, these differences are not sufficient to distinguish two or more species. For this reason and according to the Phylogenetic Species Concept (Davis & Nixon, 1992), we consider *Bokermannohyla feioi* (Napoli & Caramaschi 2004) as a junior synonym of *Bokermannohyla nanuzae* (Bokermann & Sazima 1973) and all previously unidentified specimens herein analyzed as *B. nanuzae*.

Taxonomic account

Bokermannohyla nanuzae (Bokermann & Sazima 1973)

Hyla nanuzae Bokermann & Sazima 1973. Holotype: WCAB 45808 (now MZUSP 73648). Type locality: “riacho de montanha, a altura do km 126 da Serra do Cipó, Jaboticatubas, Minas Gerais, Brasil” (mountain creek, ca. km 126 of the road of Serra do Cipó, municipality of Jaboticatubas, in a literal translation to the English).

Hyla feioi Napoli & Caramaschi 2004. Holotype: MNRJ 21356. Type locality: Parque Estadual do Ibitipoca (21°84'39" S, 43°85'59" W; 1,180m altitude), Conceição do Ibitipoca, Municipality of Lima Duarte, Minas Gerais State, Brazil. **New synonymy.**

Bokermannohyla feioi—Faivovich, Haddad, Garcia, Frost, Campbell & Wheeler 2005.

Bokermannohyla nanuzae—Faivovich, Haddad, Garcia, Frost, Campbell & Wheeler 2005.

Redescription of *Bokermannohyla nanuzae* (Bokermann & Sazima 1973)

Diagnosis. A small-sized species of the *Bokermannohyla circumdata* group (SVL males 37.9 ± 0.25 mm; females 40.2 ± 0.35 mm) diagnosed by the following characters and/or states: (1) head longer than wide, approximately 1/3 of SVL; (2) tympanum diameter ca. 50% of eye diameter (males 2.44 ± 0.25 mm; females 2.58 ± 0.31); (3) flanks and ventral surfaces immaculate; (4) hidden parts of the body, including anterior/posterior surfaces of thighs, reddish or purple on live specimens; (5) completely regular dark brown transverse bars on the dorsal surfaces of thighs; (6) at least two call types: one with pulsed structures, varying in number and interval between pulses, and the other formed by a single long sequence of pulses.

Measurements and morphology. Snout rounded in dorsal and lateral views; canthus rostralis distinct; loreal region oblique. Tympanum rounded, partially covered by the supratympanic fold, which extends from the posterior edge of eyes to the origin of arms or nearly before. Vomerine teeth in two separate series, posterior and between the choanae; vocal sac single, subgular, poorly developed; males with two vocal slits, lateral to the tongue; tongue cordiform.

Dermal crenulated fringe on forearm present or absent; when present, may reach finger V. Inner metacarpal tubercle absent; outer metacarpal tubercle poorly developed, cordiform, sometimes divided. Subarticular tubercles rounded, well developed; supernumerary tubercles rounded, small, abundant. No webbing between fingers II and III. Eighteen different web formulae found on hands on the combination III 2 ($2\frac{1}{2}$) – 3 ($2, 2\frac{1}{2}, 3\frac{1}{2}$) IV $2\frac{1}{2}$ ($2, 3$) – 2 ($2\frac{1}{2}, 3$) V. The most common was II – III 2 – 3 IV $2\frac{1}{2}$ – 2 V, present in 25 specimens (35.7%).

Skin of belly, chest, and ventral surfaces of thighs granular; dorsal, flank, and gular surfaces smooth.

Weak dermal fringe on feet and calcar flap present or absent; distinct ovoid inner metatarsal tubercles; outer metatarsal tubercles absent; subarticular and supernumerary tubercles rounded; the latter small and abundant. Fifty-two webbing formulae were found on foot on the combination I $1\frac{1}{2}$ ($1, 2$) – 2 ($0, 1\frac{1}{2}, 2\frac{1}{2}$) II $1\frac{1}{2}$ ($1, 2$) – $2\frac{1}{2}$ ($2, 3$) III $1\frac{1}{2}$ (1) – $2\frac{1}{2}$ ($2, 3, 3\frac{1}{2}$) IV 2 ($0, 1, 2\frac{1}{2}, 3\frac{1}{2}$) – $1\frac{1}{2}$ ($0, 1$) V. The most common was I $1\frac{1}{2}$ – 2 II $1\frac{1}{2}$ – $2\frac{1}{2}$ III $1\frac{1}{2}$ – $2\frac{1}{2}$ IV 2 – $1\frac{1}{2}$ V, observed in four specimens (5.7%).

Measurements (range, mean, and standard deviation) of the 17 morphometric parameters analyzed for *B. nanuzae* are presented in Table 04.

Color of live specimens. Dorsum reddish brown; hidden surfaces of body reddish or purple. Some individuals with white spots on dorsum, normally on heels, elbows, and knees, sometimes on the cloacal region and dorsum, covering bars or other drawings.

Color of preserved specimens. Same as that of live specimens except for the light to medium brown dorsum (depending on the number of spots and stains) and loss of the reddish or purple pigmentation on the hidden surfaces of body.

TABLE 4. Measurements (in mm) of 17 morphometric parameters obtained for *Bokermannohyla nanuzae*. SD = standard deviation; n = sample number.

	Females				Males			
	Mean	SD	Range	n	Mean	SD	Range	n
SVL	40.42	3.60	32.53–45.9	28	37.95	2.54	26.5–43.03	186
HW	12.75	1.35	10.40–16.17		12.08	1.14	8.1–20.55	
HL	13.54	1.21	11.00–15.77		12.90	0.82	9.37–14.72	
AL	10.66	1.08	8.40–12.93		10.83	7.73	7.3–115.1	
FAL	7.71	0.62	6.77–8.80		7.40	0.77	5.23–12.33	
HAL	11.93	0.98	9.63–13.72		11.88	0.97	7.83–14.83	
THL	19.17	1.55	16.07–21.93		18.21	1.30	12.8–21	
TBL	19.90	1.22	17.53–22.11		18.95	1.34	13.3–22.03	
FL	28.30	1.86	24.20–31.13		26.83	1.80	18.97–31.37	
IND	2.25	0.30	1.66–3.23		2.17	0.30	1.44–5.19	176
IOD	3.97	0.48	3.10–5.70		3.86	0.50	3.10–8.82	
ED	3.37	0.30	2.74–3.89		3.21	0.52	2.51–8.92	
UEW	2.58	0.56	1.93–5.03		2.43	0.40	1.76–6.37	
END	2.95	0.31	2.55–4.08		2.79	0.41	2.19–7.35	
TD	1.81	0.29	1.11–2.85		1.70	0.25	1.21–3.92	
DFIV	1.44	0.24	1.12–2.32		1.37	0.14	0.99–2.06	
DTIV	1.72	0.20	1.38–2.15		1.65	0.19	1.14–3.00	

Sexual dimorphism. As previously shown, morphometric differences between males and females were found only in five of the 17 measurements obtained. Forearms are not hypertrophied on males as usually seen on other species of the *B. circumdata* group. Males with a single, curved, hypertrophied, protruding, spine-shaped prepollex and no nuptial pads on the inner edge of finger II; smaller and not protruding prepollex present in females.

Natural history. *Bokermannohyla nanuzae* occurs at the riparian vegetation of streams on the Espinhaço and Mantiqueira mountain ranges, usually higher than 1,000 m above sea level. Males call alone or mostly in pairs (approximately 1 m from each other) and normally answer to calls of other males or playbacks. Call sites include branches, rocks, inside crevices on rocks, and among roots of the creek bank. These sites are situated more than 1 m from the creek and between 0.3 and 1.0 m from the ground. Other species of the *B. circumdata* group have been observed calling from inside holes or hollow surfaces (Caramaschi & Feio 1990; Heyer *et al.* 1990; Napoli & Caramaschi 2004; Pombal & Gordo 2004). The highest density of calling males was observed in Serra do Caraça, where about 10 males were split into pairs, and each pair was more than 5 m from another. Reproductive activity was observed between October and March, the wet season in Southeastern Brazil. Tadpoles have nocturnal activity and were found in streams hidden among leaves and submerged branches, or swimming over rocky bottoms. During the day, they form clusters under rocks or roots in the stream bed. In order to understand the context of calls emission, breeding behavior, and patterns of microhabitat use by adults of *B. nanuzae* at Serra do Caraça, see Lima *et al.* (2014a, b).

Geographic distribution. The type series of *B. nanuzae* was collected in a stream near km 126 of the road crossing Serra do Cipó, Jaboticatubas, Minas Gerais State (Bokermann & Sazima 1973). This site now corresponds to km 116 of the road MG-010 (1,265 meters above sea level) located in the Municipality of Santana do Riacho, between the district of Serra do Cipó and the Municipality of Conceição do Mato Dentro, at the southern region of the Espinhaço mountain range.

Bokermannohyla nanuzae occurs in streams near the road and inside the boundaries of the Parque Nacional da Serra do Cipó. The species is also found in other protected areas in Minas Gerais, such as Reserva Particular do Patrimônio Natural (RPPN) Santuário do Caraça, Municipality of Catas Altas; RPPN Capitão do Mato, Municipality of Nova Lima; Parque Estadual (PE) Serra do Intendente and Parque Municipal Ribeirão do Campo, both in the Municipality of Conceição do Mato Dentro; PE do Rio Preto, Municipality of São Gonçalo do Rio

Preto; Área de Proteção Ambiental Serra da Brígida and PE do Itacolomi, Municipality of Ouro Preto. Its occurrence was also confirmed in the municipalities of Caeté, Rio Acima, Barão de Cocais, Ouro Branco, Serro, Congonhas, Augusto de Lima, Itabira, Mariana, and Rio Vermelho. In addition, *B. nanuzae* occurs in the Mantiqueira mountain range at the PE do Ibitipoca, Conceição do Ibitipoca, Municipality of Lima Duarte.

The municipalities of São Gonçalo do Rio Preto and Lima Duarte represent the northern and southern limits of the distribution of *B. nanuzae*, respectively. The distribution gap currently observed among the watersheds of the Doce, Paraíba do Sul, and Grande Rivers may be due to lack of sampling, since several Brazilian regions remain unexplored regarding the amphibian fauna.

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APPENDIX 1.

Adults and tadpoles of *Bokermannohyla nanuzae* examined. For collections abbreviations see Materials and Methods:

Adults: **BRAZIL, MINAS GERAIS:** *Bokermannohyla nanuzae* OTU: Conceição do Mato Dentro MCNAM 3093, 3095, 3096, 3367, 3368, 9611, 9612, 10884, 10887, 10894, 10897, 10906, 13682, 13683, 14414, 15019, CHUFMG 4094–4098; Santana do Riacho, Serra do Cipó CFBH 22300, MNRJ 4583, MZUSP 73648, 74157–74159, MCNAM 2943, 2944, 3197, 14828–14830, CHUFMG 771, 772, 788, 789, ZUEC–AMP 1552, 1679, 2216, 2555, 3026–3029; Rio Vermelho CHUFMG 4085–4093; São Gonçalo do Rio Preto, PE Rio Preto MCNAM 3663, 8592–8594, 11689, 11834. *Bokermannohyla cf. nanuzae* OTU: Barão de Cocais MCNAM 949; Caeté MCNAM 775–779, 859, 880, 881, 1350, 1743, 9981, 9982, 10486–10493, 17907, 18126–18127, 18274, 18480, UFV 428–430; Catas Altas CHUFMG 4099–4100, RPPN Santuário do Caraça MNRJ 50998–51003, 55062–55064, 55067–55071, 56410, 56411, 56414–56418, 66052, 66053, 66055, 66057–66061, 66063–66065, 70370–70373, 70375, 70380, MCNAM 3350–3352, 3465, 3608, CHUFMG 791, 792, 795, 797, 798, 800–802; Congonhas do Campo CFBH 18426, CHUFMG 773–778, 781–787, 4065–4075, 4077–4084, UFOP 799A–804A; Mariana MCNAM 13976; Nova Lima MNRJ 36986, 36988, 36990; Ouro Branco MCNAM 6680, 6698; Ouro Preto UFOP 441A, 445A, 694A–700A; Serro MCNAM 11376, 11377. *Bokermannohyla feioi* OTU: Lima Duarte, PE Ibitipoca MNRJ 26285–26295, 46351, 46352, 46354, MCNAM 15351–15353, ZUEC–AMP 7156, 7157.

Tadpoles: **BRAZIL, MINAS GERAIS:** Caeté MNRJ 25422; Congonhas do Campo MNRJ 80891; Catas Altas MNRJ 49734; Lima Duarte, PE do Ibitipoca MNRJ 25167, 26320, 26504, 80892; Mariana MNRJ 54792; Santana do Riacho, Serra do Cipó MCNAM 14764, 16324, 16337, 16349, 16351, 16549.