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The advertisement call, color patterns and distribution of *Ischnocnema izecksohni* (Caramaschi and Kisteumacher, 1989) (Anura, Brachycephalidae)

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ABSTRACT

Ischnocnema izecksohni *inhabits the gallery forests from the Quadrilátero Ferrífero, Southern Espinhaço range, state of Minas Gerais, southeastern Brazil, and it is considered endemic to this region. Its closest related species is* I. nasuta *according to the original description. We describe the advertisement call of* I. izecksohni *based on specimens recorded and collected at the municipality of Nova Lima, state of Minas Gerais, distant about 10 km straight line from its type locality. The advertisement call consists of a group of notes emitted sporadically without a regular interval between the calls. Call duration (n = 36 calls in four individuals) ranged from 1.03 to 1.85 s (= 1.52 ± 0.21 s) and the call rise time from 0.66 to 1.52 s (= 1.16 ± 0.25 s), with 34-57 notes per call (= 47.42 ± 6.03). Peak frequency ranged from 2250 to 2625 Hz, the dominant frequency from 1317.8 to 3128.0 Hz and interval between notes from 22.00 to 41.00 ms (= 28.63 ± 0.03 ms). From the examination of herpetological collections, morphological and bioacoustical data we extended the species known distribution ca. 200 km eastward, to ten new localities, all of them outside the Quadrilátero Ferrífero region, at the Mantiqueira mountain range. We analyzed color patterns and we find some dorsal patterns not described at the original description of* I. izecksohni. *We also make some comments concerning the taxonomic status of* I. izecksohni *and* I. nasuta.

Key-Words: Vocalization; Taxonomy; Polymorphism; *Ischnocnema guentheri* species series.

INTRODUCTION

The genus *Ischnocnema* Reinhardt & Lütken was recently revalidated to include the old eleutherodactylid frogs from the Brazilian Atlantic Forest (Heinicke *et al.,* 2007), except *Haddadus binotatus* (Spix, 1824) and *H. plicifer* (Boulenger, 1888), allocated in the family Craugastoridae Hedges,

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Duellman & Heinicke, 2008. *Ischnocnema* contains 34 species split in the *I. guentheri, I. lactea, I. parva, I. ramagii* and *I. verrucosa* species series (Hedges *et al.* 2008; Frost, 2011). The *I. guentheri* species series comprises eleven species: *I. epipeda* (Heyer, 1984), *I. erythromera* (Heyer, 1984), *I. gualteri* (B. Lutz, 1974), *I. guentheri* (Steindachner, 1864), *I. henselii* (Peters, 1870), *I. hoehnei* (A. Lutz, 1958), *I. izecksohni* (Caramaschi & Kisteumacher, 1989), *I. nasuta* (A. Lutz, 1925), *I. octavioi* (Bokermann, 1965), *I. oea* (Heyer, 1984), and *I. vinhai* (Bokermann, 1975). This species series is widely distributed throughout the Atlantic Forest biome occurring since the southern state of Bahia to the state of Santa Catarina, and *I. henselli* occurs at the Araucaria forests in the state of Rio Grande do Sul, Brazil, and Misiones, Argentina (Hedges *et al.* 2008).

Ischnocnema izecksohni inhabits the gallery forests from the Quadrilátero Ferrífero, Southern Espinhaço range, state of Minas Gerais, southeastern Brazil, and it is considered endemic to this region (Leite *et al.* 2008). The species is described from Parque das Mangabeiras, Municipality of Belo Horizonte (19°55'S and 43°56'W, approximately 850 m elevation) and the description is based on three specimens (two adult females and an adult male), and its closest related species is *I. nasuta* according to the original description. *Ischnocnema izecksohni* differs from *I. nasuta* because it is slightly more robust, has smaller tympanum, shorter snout, more developed fringes on toes, and different coloration (Caramaschi & Kisteumacher, 1989). Within the series of *I. guentheri,* only four species have their vocalization described: *I. nasuta,* based on a specimen from Parque Nacional do Caparaó, state of Minas Gerais, southeastern Brazil; *I. gualteri,* based on a specimen from the type locality (municipality of Teresópolis, state of Rio de Janeiro, southeastern Brazil) and *I. guentheri,* based on a specimen recorded in the municipality of Pirabeiraba, Santa Catarina, at a distance about 670 km straight line from the type locality of the species (municipality of Rio de Janeiro, state of Rio de Janeiro) (Heyer, 1984). Heyer *et al.* (1990) describe the advertisement call of *I. guentheri* from Estação Biológica da Boracéia, municipality of Salesópolis, SP. Kwet & Solé (2005) revalidated *I. henselii,* and described two different vocalizations for the species. The authors describe and compare calls of some populations of *I. guentheri,* which leads them to conclude that *I. guentheri* comprises a complex of two or three species. The anuran advertisement call is considered species-specific and functions in mate recognition, and vocal differences are important pre-zygotic isolation mechanisms (Kelley *et al.* 2001). With the technology becoming increasingly affordable and easy to use, the bioacoustic parameters have become a taxonomic tool as important as morphological data in the anuran systematics. It helps untie species identity in taxonomically difficult and morphologically cryptic or polymorphic groups (Kwet & Angulo, 2002; Angulo *et al.* 2003; Angulo & Reichle, 2008; Channing *et al.* 2002; Ron *et al.* 2005; Toledo *et al.* 2007). Heyer (1984) separated some exemplars of *I. guentheri* species series in 14 dorsal patterns. All the species analyzed in the paper have more than one dorsal pattern. Caramaschi & Kisteumacher (1989) only describe one type of dorsal pattern for *I. izecksohni,* but this is probably due the few exemplars used in the description (2 females and a male). Herein we describe the advertisement call of *I. izecksohni* and we compare it to some reported and unreported calls of other populations within the *I. guentheri* species series. We also describe different color patterns and extend the geographic distribution for the species in Minas Gerais, Brazil.

MATERIALS AND METHODS

Four specimens of *I. izecksohni* were recorded at RPPN Mata Samuel de Paula, municipality of Nova Lima, in the Quadrilátero Ferrífero region, state of Minas Gerais, southeastern Brazil (20°00'S, 43°52'W, approximately 900 m elevation), approximately 10 km straight-line from the type locality. Two specimens were recorded at Parque Estadual da Serra do Brigadeiro (PESB), municipalities of Araponga and Fervedouro, state of Minas Gerais, in the Mantiqueira mountain range (20°43'S, 42°29'W, approximately 1300 m elevation), approximately 200 km straight-line from the type locality. Voucher specimens are deposited at the Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG) (Appendix I). Records were made with a digital recorder Marantz PMD 660, with a frequency sampling rate of 16 bits, and a Sennheiser ME-66 super-cardioid shotgun condenser microphone and analyzed with the program Raven 1.4 beta for Windows (Cornell Lab of Ornithology Research Program Bioacoustics Workstation). The audiospectrograms were produced with FFT of 256 points, overlap 75% and window Hamming. Resolution, contrast and brightness settings were the program default. Measurements of the following acoustic parameters were taken: call duration, note interval, notes per call, according to Kwet & Solé (2005); call rise time follows Cocroft & Ryan

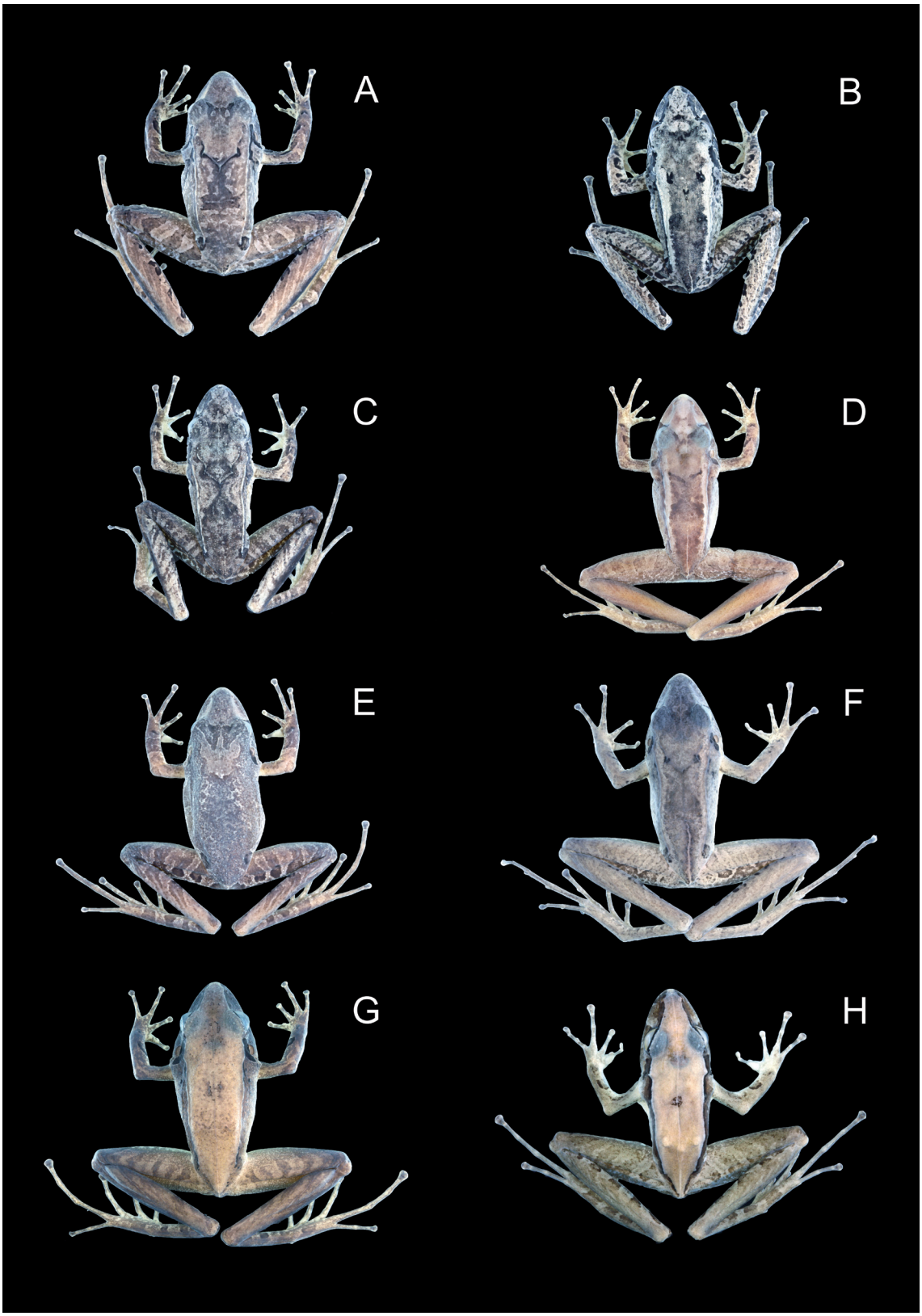

FIGURE 1: Dorsal pattern states of *Ischnocnema izecksohni,* most of them follows Heyer, (1984). **(A)** A-1, adult female; **(B)** A-4, adult male; **(C)** A-5, adult male; **(D)** A-6, adult female; **(E)** A-8, adult male; **(F)** A-8*, adult female; **(G)** B-1, adult female; **(H)** B-3, adult male.

(1995). Peak frequency was obtained directly from the software. Temporal parameters were measured in seconds (s) or milliseconds (ms) and spectral parameters in Hertz (Hz).

For the description of the color patterns and distribution extension we examined specimens (Appendix I) from the Museu de Zoologia João Moojen, Universidade Federal de Viçosa **(MZUFV),** Viçosa, Minas Gerais, Brazil; the Coleção Herpetológica da Universidade Federal de Minas Gerais **(UFMG)** and the Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais **(MCNAM),** both in Belo Horizonte, Minas Gerais, Brazil. Color patterns follow Heyer (1984), except

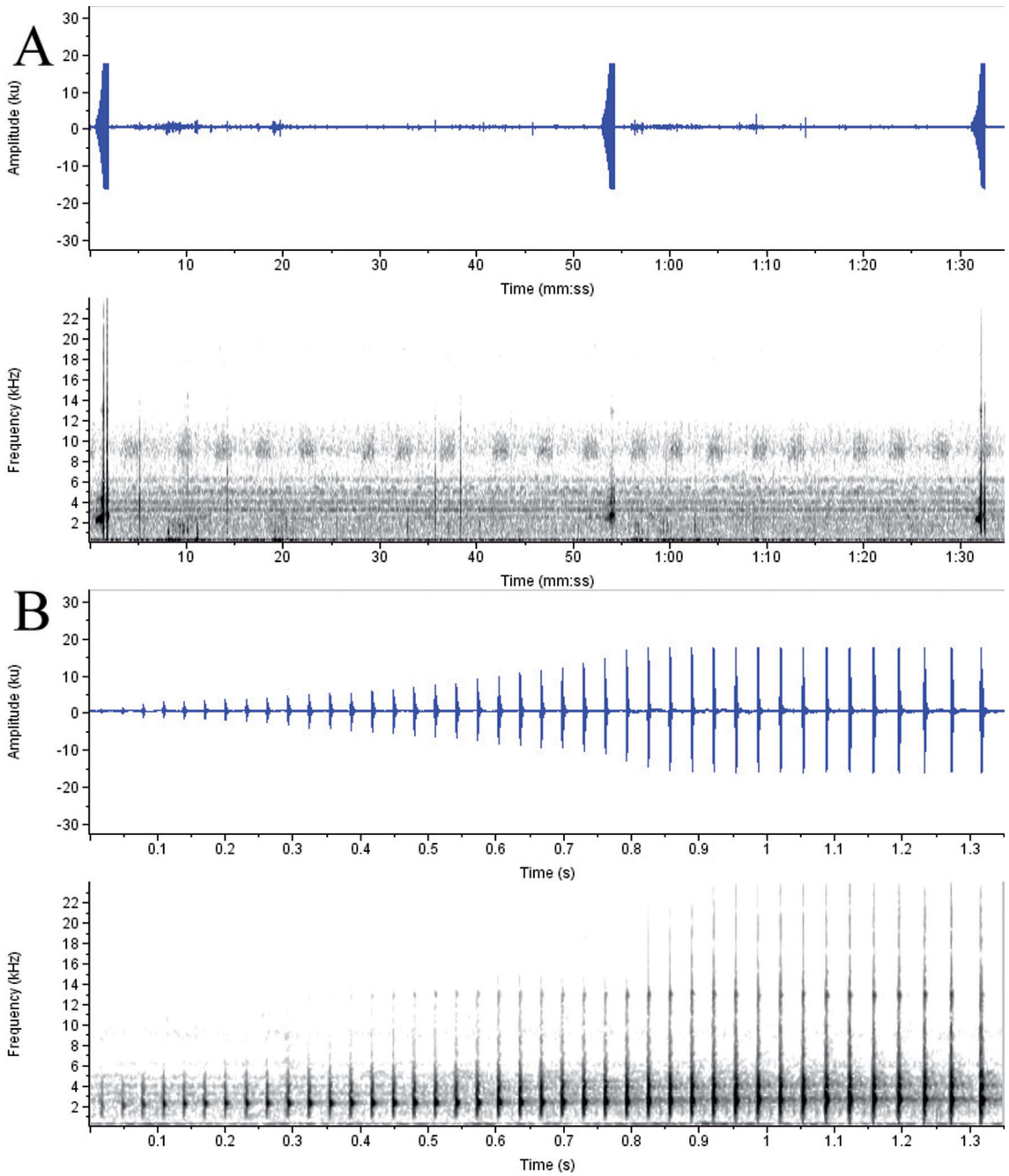

FIGURE 2: Advertisement call of *Ischnocnema izecksohni.* **(A)** Waveform (above) and audiospectrogram (below) of a session of three calls and **(B)** waveform (above) and audiospectrogram (below) of one call. Recording PPGT 11/10/2009, air temperature 20°C. Specimen UFMG0039.

TABLE 1: Advertisement calls of individuals of *Ischnocnema izecksohni* from Nova Lima, MG and Parque Estadual da Serra do Brigadeiro (PESB) and other advertisement calls described for the *I. guentheri* species series. PNC = Parque Nacional do Caparaó. Data are presented like this: Min-max; (mean ± standard deviation); (mean).

Specimen	Locality	Air Temperature (°C)	Number of analyzed calls	Call duration (s)	Call Rise Time (s)	Peak frequency (Hz)	Dominant frequency range (Hz)	Interval between notes (ms)	Notes per call
UFMG0039	Nova Lima, MG	20	11	1.16-1.57 (1.42 ± 0.12)	0.66-1.10 (0.91 ± 0.14)	2437.5 and 2625	1553.3-3045.7	24.00-41.00 (28.75 ± 3.31)	36-49 (44.09 ± 3.75)
UFMG0040	Nova Lima, MG	18	11	1.03-1.68 (1.42 ± 0.26)	1.00-1.51 (1.29 ± 0.20)	2250 and 2437.5	1327.2-3124.4	22.00-35.00 (27.35 ± 2.22)	34-53 (45.64 ± 7.49)
UFMG0041	Nova Lima, MG	18	11	1.44-1.85 (1.70 ± 0.11)	1.20-1.52 (1.35 ± 0.10)	2437.5	1317.8-3128.0	25.00-35.00 (29.35 ± 2.13)	45-57 (52.36 ± 3.14)
Not collected	Nova Lima, MG	21	3	1.44-1.82 (1.60 ± 0.19)	0.70-1.08 (0.91 ± 0.19)	2625	1684.7-3103.4	26.00-38.00 (30.23 ± 2.91)	44-53 (48.00 ± 4.58)
UFMG3288	PESB, MG	17.4	10	1.68-2.15 (1.92 ± 0.14)	1.08-1.50 (1.23 ± 0.13)	2437.5	1522.4-3104.5	27.00-40.00 (31.24 ± 2.90)	47-60 (53.40 ± 3.75)
UFMG3290	PESB, MG	17.4	2	1.18-1.27	0.80-0.82	2250	1428.6-3085.7	25.00-36.00	34-36
Ischnocnema nasuta (Heyer, 1984)	PNC, MG	—	4	1.15-1.50	—	—	2100-2600	—	34-43
I. gualteri (Heyer, 1984)	Teresópolis, RJ	15-16	3	1.50-1.90	—	—	2100-2700	—	4-9
I. guentheri (Heyer, 1984)	Pirabeiraba, SC	—	3	1.10-1.75	—	—	1900-2900	—	19-28
I. henselii (Kwet & Solé, 2005)	Southern Brazil	19-20.5	31	10.00-23.00	—	—	2100-3100	70-230	86-170
I. guentheri (Kwet & Solé, 2005)	Bocaina, SP	19-20	3	0.80-1.80 (1.20)	—	—	1800-2500	40-44 (42)	11-36
I. guentheri (Kwet & Solé, 2005)	Morro do Baú, SC	17.5	3	2.10-2.70 (2.40)	—	—	2200-3000	78-91 (83)	22-28
I. guentheri (Kwet & Solé, 2005)	Guamirím, SC	—	1	1.00	—	—	2100-3100	62-74 (69)	14
I. guentheri (Kwet & Solé, 2005)	Pirabeiraba, SC	—	3	1.10-1.80 (1.60)	—	—	1900-2900	57-67 (61)	19-28
I. guentheri (Kwet & Solé, 2005)	Boracéia, SP	19.2	6	0.44-0.64 (0.54)	—	—	2500-3200	48-76 (61)	7-10

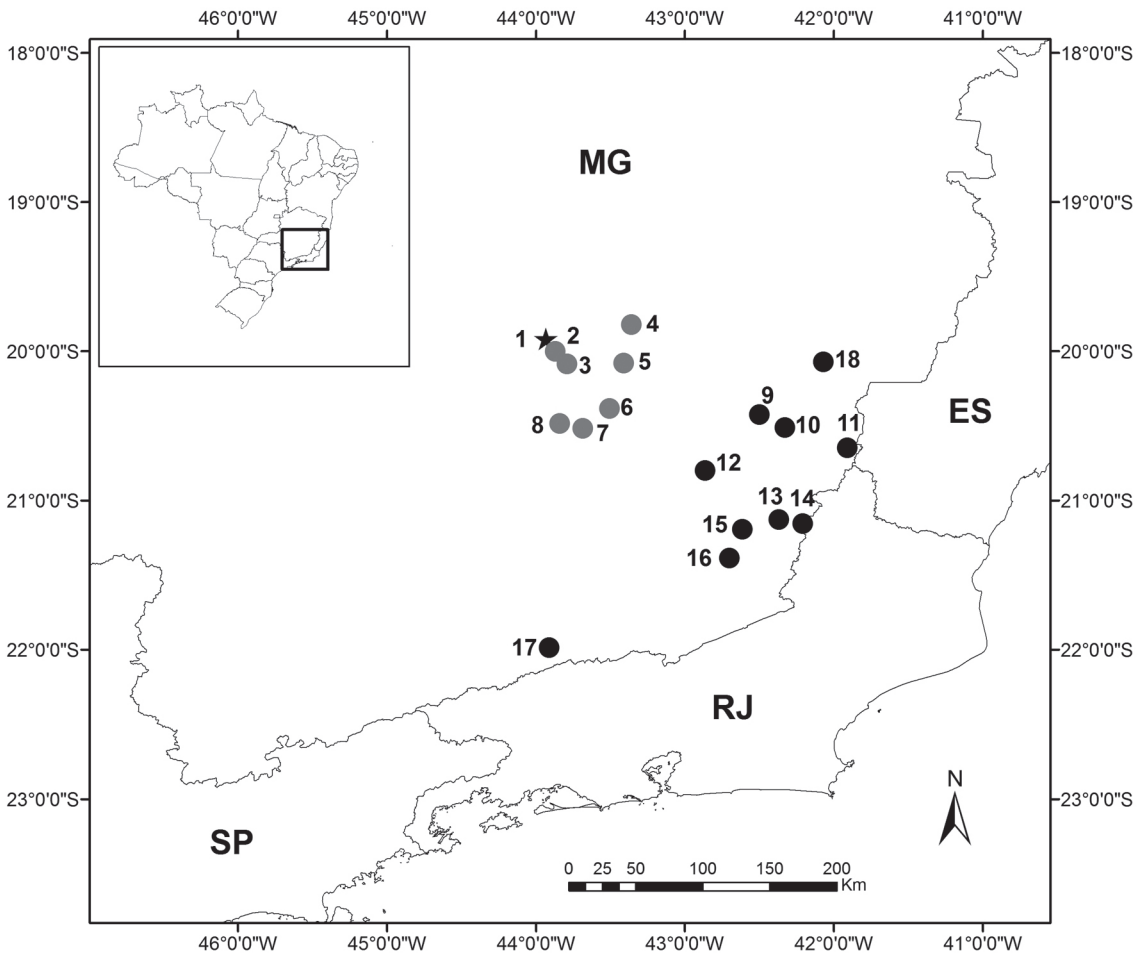

FIGURE 3: Geographic distribution of *Ischnocnema izecksohni*. Star is the type locality, clear spots are literature data and dark spots are the new records for the species. **1** = Belo Horizonte, **2** =Nova Lima, **3** =Rio Acima, **4** =São Gonçalo do Rio Abaixo, **5** =Catas Altas, **6** =Ouro Preto, **7** =Ouro Branco, **8** =Congonhas, **9** =Araponga, **10** =Pedra Bonita, **11** =Espera Feliz, **12** =Viçosa, **13** =Muriaé, **14** =Patrocínio do Muriaé, **15** =Miraí, **16** =Cataguases, **17** =Rio Preto, **18** =Simonésia. MG, state of Minas Gerais, RJ, state of Rio de Janeiro, ES, state of Espírito Santo. Brazil.

for two new general dorsal patterns (Fig. 1) included here: A-8*, which is the variegated pattern with two dark spots in the back; and B-3, which is the same as Heyer's B-1 pattern but with just one dark spot in the back.

RESULTS AND DISCUSSION

Males were found calling at night inside gallery forests. Recorded individuals (UFMG0039, UFMG0040 and UFMG0041) were found on vegetation at a height ranging from 50 cm to about 2 m from the ground. The advertisement call consists of a group of notes emitted sporadically without a regular interval between the calls. Like the other species from the series (*see* Kwet & Solé, 2005 and Heyer, 1984), it increases energy over time until it reaches a peak. Call duration (n = 36 calls in four individuals) ranged from 1.03 to 1.85 s (mean = 1.52 ± 0.21 s) and the call rise time from 0.66 to 1.52 s (mean = 1.16 ± 0.25 s), with 34-57 notes per call (mean = 47.42 ± 6.03) (Fig. 2 and Table 1). Peak frequency varied from 2250 to 2625 Hz, the dominant frequency from 1317.8 to 3128.0 Hz and interval between notes from 22.00 to 41.00 ms (= 28.63 ± 0.03 ms).

The call of *I. izecksohni* differs from the call of *I. gualteri* and *I. guentheri* from the municipality of Pirabeiraba, state of Santa Catarina (Heyer, 1984), Boracéia and Bocaina, state of São Paulo, Morro do Baú, Guaramirim and Pirabeiraba, state of Santa Catarina (Kwet & Solé, 2005), because it has a larger number of notes per call. Regarding the call duration, *I. izecksohni* presents a shorter call than that of *I. henselii* (10.00 to 23.00 s) and *I. guentheri* from Morro do Baú (2.10 to 2.70 s). The advertisement call

TABLE 2: Occurrence of pattern states (Fig. 1 and Heyer, 1984) among individuals of *Ischnocnema izecksohni*. Values are percentages.

Type of pattern (n = 49)	Pattern Standards							
General dorsal pattern	A-1	A-4	A-5	A-6	A-8	A-8*	B-1	B-3
	24.49	6.12	16.33	4.08	20.41	20.41	4.08	4.08
Mid-dorsal pin stripe	Absent	A	E					
	59.18	36.74	4.08					
Dorsolateral stripes	Absent	A						
	61.22	38.78						
Snout pattern	Uniform	A	B					
	81.63	16.33	2.04					
Interocular bar	Uniform	B						
	65.31	34.69						
Pelvic spots	Absent	A	B					
	12.25	83.67	4.08					
Supratympanic stripe or spot	A	B						
	95.92	4.08						
Loreal stripe	Uniform	A	B	C				
	48.98	8.16	22.45	20.41				
Upper lip	A	B	C	D				
	10.20	12.25	12.25	65.31				
Outer front aspect of the tibia	Uniform	A	B	C				
	14.29	6.12	14.29	65.31				
Posterior surface of the thigh	A-1							
	100.00							

of *I. izecksohni* has the smallest interval between notes from all the calls analyzed by Kwet & Solé (2005). This parameter could not be compared to the calls in Heyer (1984), since it was not analyzed in the paper.

The calls from the municipality of Nova Lima are very similar to that from the PESB, and the two populations are definitively conspecific. One specimen from the PESB has the call with the largest duration (1.68-2.15 s), but this is probably due to population variations.

The call of *Ischnocnema nasuta* was described from Parque Nacional do Caparaó (PNC), state of Minas Gerais (Heyer, 1984), distant about 200 km straight line from the type locality of the species (municipality of Nova Friburgo, state of Rio de Janeiro). This call is really similar to *I. izecksohni* (table 1, fig. 1 and fig. 24 Heyer, 1984), suggesting that the two populations are conspecifics. We also examined some *I. nasuta* specimens provenient from PNC housed at Museu de Zoologia da Universidade de São Paulo (MZUSP), and the differences mentioned by Caramaschi & Kisteumacher (1989) do not apply to specimens examined. The taxonomic status of *I. nasuta* from PNC or even *I. izecksohni* in relation to *I. nasuta* is depending on the analysis of the call of *I. nasuta* from the type locality.

Literature data reports *I. izecksohni* for the municipalities of Belo Horizonte (Caramaschi & Kisteumacher, 1989), Nova lima (Nascimento *et al.* 1994), Ouro Preto (Pedralli *et al.* 2001), Rio Acima (Grandinetti & Jacobi, 2005), Catas Altas (Canelas & Bertoluci, 2007), São Gonçalo do Rio Abaixo (Bertoluci *et al.* 2009), Congonhas, and Ouro Branco (Leite *et al.* 2008), all of them at the Quadrilátero Ferrífero region. Based on recently collected and recorded specimens we report for the first time the occurrence of *I. izecksohni* for the PESB. The examination of herpetological collections allowed us to record the species also for the municipalities of Cataguases, Espera Feliz, Miraí, Muriaé, Patrocínio do Muriaé Pedra Dourada, Rio Preto, and Viçosa, at the Mantiqueira mountain range, state of Minas Gerais, outside the Quadrilátero Ferrífero, extending its distribution ca. 200 km eastward. We also collected specimens at the municipality of Simonésia, also at the Mantiqueira mountain range. Literature reports *I. guentheri* for the municipalities of Muriaé (Santana *et al.* 2010) and Ouro Branco (São-Pedro & Feio, 2010). We examined the vouchers from both localities and it corresponds to *I. izecksohni.* Silva *et al.* 2011 reported *I.* cf. *izecksohni* for the municipality of Viçosa based on vocalization records. It is probably *I. izecksohni.* For a complete distribution map see Fig. 3.

The general dorsal patterns observed were: A-1, A-4, A-5, A-6, A-8, A-8*, B-1 and B-3 (Fig. 1). The mid-dorsal pin stripe was absent or like A and E. The dorsolateral stripe was absent or like the A pattern. The snout pattern was mainly uniform, but we also observed A and B patterns. The interocular bar was

absent or like B. The pelvic and supratympanic spots were mainly like A, but a few individuals showed the B pattern. Pelvic spots could be also absent in a few individuals. The loreal stripe was uniform or like A, B and C. The upper lip pattern was like A, B, C and D. The outer front aspect of the tibia was mainly like C, but the patterns A, B and uniform were also observed. The posterior surface of the thigh was A-1 for all the specimens analyzed. Detailed information about the patterns is showed in Table 2.

Caramaschi & Kisteumacher (1989) described the dorsal pattern of *Ischnocnema izecksohni* as "… variegated-mottled tan and a chevron-like mark on middle third of the body…" It looks like Heyer's A-1 pattern, but we have seen seven more dorsal patterns. They were not conclusive as a putative diagnosis for the species, as well as almost all the other color patterns. Heyer (1984) says that a boldly mottled posterior surface of the thigh pattern distinguishes *I. nasuta* from the other species of the *I. guentheri* species group and mostly *I. guentheri* individuals, which have a light area near the knee or uniform and indistinctly mottled posterior thigh patterns. All *I. izecksohni* specimens we analyzed have the same boldly mottled posterior surface of the thigh pattern as that of *I. nasuta* and we are not aware of any morphologic features distinguishing them. The diagnosis between the two species in Caramaschi & Kisteumacher (1989) is subjective.

As *I. izecksohni* and *I. nasuta* seems to be indistinguishable, two situations are possible. *I. izecksohni* occurs at the Espinhaço mountain range and at the Mantiqueira mountain range, and *I. nasuta* is a very close species occurring at Serra do Mar. Or, *I. izecksohni* and *I. nasuta* are the same species, with the first one being a junior synonym of the second one. Examination of both type series and the analysis of the call of *I. nasuta* from its type locality are necessary to make clear the taxonomic status of the two species, besides genetic and behavioral information.

RESUMO

Ischnocnema izecksohni *habita as matas de galeria do Quadrilátero Ferrífero, sul da Cadeia do Espinhaço, estado de Minas Gerais, sudeste do Brasil e é considerada endêmica desta região. Sua espécie mais próxima, de acordo com a descrição original, é* I. nasuta. *Descreve-se o canto de* I. izecksohni *baseado em espécimes gravados e coletados em Nova Lima, MG, que dista 10 km em linha reta da localidade tipo da espécie. O canto de anúncio consiste em um grupo de notas emitidas esporadicamente sem um intervalo regular entre os cantos. A duração do canto (n = 36 cantos em quatro indivíduos) variou de 1,03 até 1,85 s (= 1,52 ± 0,21 s) e o tempo até a amplitude máxima do canto de 0,66 to 1,52 s (= 1,16 ± 0,25 s), com 34-57 notas por canto (= 47.42 ± 6.03). A freqüência de pico variou de 2250 a 2625 Hz, a freqüência dominante de 1317,8 a 3128,0 Hz e o intervalo entre as notas de 22,00 to 41,00 ms (= 28,63 ± 0,03 ms). A partir do exame de coleções herpetológicas, dados morfológicos e bioacústicos estendeu-se a distribuição da espécie para cerca de 200 km a leste, para mais dez localidades, todas elas fora do Quadrilátero Ferrífero, no complexo da Mantiqueira. Foram analisados padrões de coloração e alguns padrões dorsais além daquele presente na descrição original foram encontrados. São feitos comentários acerca do status taxonômico de* I. izecksohni *e* I. nasuta.

Palavras-Chave: Vocalização; Taxonomia; Polimorfismo; Série de *Ischnocnema guentheri.*

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APPENDIX I

Specimens Examined

MCNAM 509, 5215, 5216, 5573, 5574; MZUFV 1280, 1285, 1344, 2142, 4033, 6309-6312, 6787, 7083, 7106, 7107, 7112, 7393, 7394, 7564, 7565, 7632, 7688, 8022, 8730, 8884, 8926, 9043, 9765, 9766, 9951; MZUSP 57947, 133862, 135691; UFMG 0037-0041, 3286-3292.

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