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Two New Species of *Ischnocnema* (Anura: Brachycephalidae) from Southeastern Brazil and their Phylogenetic Position within the *I. guentheri* Series

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Abstract: We describe two new species of *Ischnocnema* from the states of Minas Gerais and Espírito Santo, southeastern Brazil, based on morphological, bioacoustical, and molecular data. We use three mitochondrial and two nuclear genes in Bayesian inference and maximum likelihood analyses to assess their phylogenetic placement within the *I. guentheri* series. The two new species group with *I. oea* in a well-supported clade in both analyses and have a calcar tubercle that is at least as long as wide. This type of tubercle seems to be a putative synapomorphy for the clade. We provide a revised diagnosis for the *I. guentheri* series, with characters shared by all its members, and discuss the close relationship between the *I. parva* and the *I. guentheri* series.

Key words: Advertisement call**;** External morphology**;** Integrative taxonomy**;** *Ischnocnema* ***feioi*** sp. nov.**;** *Ischnocnema* ***garciai*** sp. nov.**;** *Ischnocnema oea***;** Molecular phylogeny

The genus *Ischnocnema* Reinhardt and Lütken 1862 "1861" comprises 33 species (Frost 2016) and it is currently divided into four series: *I. guentheri, I. lactea, I. parva,* and *I. verrucosa* (Canedo and Haddad 2012, Padial et al. 2014). Ten species are currently recognized in the *I. guentheri* series: *I. epipeda* (Heyer 1984), *I. erythromera* (Heyer 1984), *I. gualteri* (B. Lutz 1974), *I. guentheri* (Steindachner 1867), *I. henselii* (Peters 1870), *I. hoehnei* (B. Lutz 1958), *I. izecksohni* (Caramaschi and Kisteumacher 1989 "1988"), *I. nasuta* (A. Lutz 1925), *I. oea* (Heyer 1984), and *I. venancioi* (B. Lutz 1958). The series occurs throughout the Atlantic Forest in southeastern and southern Brazil and adjacent northern Argentina (Frost 2016). The systematics of the *I. guentheri* series has experienced many changes over the past few decades.

Lynch (1976) divided the former *Eleutherodactylus* Duméril and Bibron 1841 from the Brazilian Atlantic Forest into four species groups based on finger morphology and venter skin texture: the *E. binotatus*, *E. lacteus*, *E. parvus,* and *E. ramagii* groups. The *E. binotatus* (currently *Haddadus binotatus* [Spix 1824]) group contained six species, three of them in the current *Ischnocnema guentheri* series: *I. gualteri, I. guentheri*, and *I. nasuta*. *Ischnocnema henselii* and *I. hoehnei* were not assigned to this group due to lack of data and *I. venancioi* was placed into the *E. lacteus* group. Heyer (1984) studied the variation, systematics, and zoogeography of the former *E. guentheri* (=*I. guentheri*) and created what he called the "*E. guentheri* cluster," based on external morphology. His grouping was a part of the *E. binotatus* group (sensu Lynch 1976), and included *I. gualteri*, *I. guentheri*, *I. nasuta*, and three new species he described at the time: *I. epipeda, I. erythromera,* and *I. oea*. Just over a decade later, Lynch and Duellman (1997) created the *E. binotatus* series to allocate all the Atlantic Forest *Eleutherodactylus* species, including the *E. binotatus*, *E. lacteus*, *E. parvus*, and *E. ramagii* groups (sensu Lynch 1976). Their *E. binotatus* group included the members of the *E. binotatus* group proposed by Lynch (1976), the members of the *E. guentheri* cluster proposed by Heyer (1984), *I. hoehnei*, *I. izecksohni*, and two other species. *Ischnocnema venancioi* was placed in the *E. binotatus* series but was unassigned to any goup. Heinicke et al. (2007), in a molecular study assessing several *Eleutherodactylus* from all over the American continent, reallocated most of the *Eleutherodactylus* from the Brazilian Atlantic Forest to the genus *Ischnocnema*, and Hedges et al. (2008) divided the genus into five series. They placed *I. venancioi* in the *I. lactea* series, and their *I. guentheri* series included 11 species: all the members from the former *E. guentheri* cluster (Heyer 1984) plus *I. henselii, I. hoehnei, I. izecksohni, I. octavioi* (Bokermann 1965), and *I. vinhai* (Bokermann 1975 "1974"). Shortly thereafter, Canedo et al. (2010) examined the external morphology of *I. octavioi* and based on these observations reallocated the species to the *I. verrucosa* series. Canedo and Haddad (2012) made the first phylogenetic study encompassing most species of *Ischnocnema* (more than 80% of the described species at the time), and transferred *I. vinhai* to the genus *Pristimantis* Jiménez de la Espada 1870. They also included *I. venancioi* in the *I. guentheri* series based on its phylogenetic placement. Gehara et al. (2013) did the first attempt in assessing the taxonomy of the *I. guentheri* series using molecular and acoustic data together. Authough they did not make any taxonomic decision, they found four candidate species related to *I. guentheri* and *I. henselii*, showing that the species richness in the *I. guentheri* series is probably underestimated.

Recent field work in the state of Minas Gerais and museum visits resulted in the discovery of two unnamed species of the *Ischnocnema guentheri* series with overall morphology similar to *I. oea*, from the localities of Serra do Brigadeiro, municipalities of Ervália and Muriaé, and Usina

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Table 1.—Primers used in this study.

Primer		Gene	Sequence	Reference
MVZ59	F	12S	ATAGCACGTAAAAYGCTDAGATG	Graybeal 1997
tRNAphe-L	F	tRNA-F–12S	AAAGCATAACACTGAAGATGTTAAGATG	Goebel et al. 1999
12S F-H	R	12S	CTTGGCTCGTAGTTCCCTGGCG	Goebel et al. 1999
12S A-L	F	12S–tRNA-V	AAACTGGGATTAGATACCCCACTAT	Goebel et al. 1999
tRNAval-H	R	12S–tRNA-V	GGTGTAAGCGARAGGCTTTKGTTAAG	Goebel et al. 1999
12SL13	F	tRNA-V–16S	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller and Hedges 1998
16STitus_1	R	tRNA-V–16S	GGTGGCTGCTTTTAGGCC	Titus and Larson 1996
16SL2A	F	16S	CCAAACGAGCCTAGTGATAGCTGGTT	Hedges 1994
16S-H10	R	16S	TGCTTACGCTACCTTTGCACGGT	Hedges 1994
16SAR	F	16S	CGCCTGTTTATCAAAAACAT	Palumbi et al. 1991
16SBR	R	16S	GACCTGGATTACTCCGGTCTGA	Palumbi et al. 1991
Tyr1B	F	Tyrosinase	AGGTCCTCYTRAGGAAGGAATG	Bossuyt and Milinkovitch 2000
Tyr1E	R	Tyrosinase	GAGAAGAAAGAWGCTGGGCTGAG	Bossuyt and Milinkovitch 2000
Tyr1C	F	Tyrosinase	GGCAGAGGAWCRTGCCAAGATGT	Bossuyt and Milinkovitch 2000
Tyr1G	R	Tyrosinase	TGCTGGGCRTCTCTCCARTCCCA	Bossuyt and Milinkovitch 2000
R182	F	RAG1*	GCCATAACTGCTGGAGCATYAT	Heinicke et al. 2007
R270	R	RAG1	AGYAGATGTTGCCTGGGTCTTC	Heinicke et al. 2007
RAG1FF2	F	RAG1	ATGCATCRAAAATTCARCAAT	Heinicke et al. 2007
RAG1FR2	R	RAG1	CCYCCTTTRTTGATAKGGWCATA	Heinicke et al. 2007

* RAG1 indicates nuclear recombination activating gene 1.

da Fumaça, municipality of Muriaé. The aims of this paper are primarily to (1) describe the two new species using morphological, bioacoustical, and molecular data; (2) evaluate the phylogenetic position of the two new species within the *I. guentheri* series; and (3) reevaluate the diagnostic characters proposed in recent literature for the *I. guentheri* series.

Material and Methods

Taxon and Gene Sampling

Aiming to assess the phylogenetic position of the two new species we compiled a molecular dataset with all nominal species of *Ischnocnema* available in GenBank (all terminals and respective Genbank accession numbers are listed in Appendix I) and also the four unnamed candidate species related to *I. guentheri* from the Gehara et al. (2013) study. Outgroup selection was based on previous phylogenetic studies (Canedo and Haddad 2012; Padial et al. 2014) and included members of the superfamily Brachycephaloidea Günther 1858: *Barycholos* Heyer 1969, *Brachycephalus* Fitzinger 1826, *Craugastor* Cope 1862, *Eleutherodactylus*, *Haddadus* Hedges et al. 2008, *Hypodactylus* Hedges et al. 2008, *Lynchius* Hedges et al. 2008, *Pristimantis*, and *Yunganastes* Padial et al. 2007. We selected the mitochondrial 12S rRNA, tRNAVal, and partial-sequence 16S rRNA genes, and partial sequences of the nuclear tyrosinase precursor (Tyr) and recombination activating gene 1 (RAG1) genes because they were available for most *Ischnocnema* species.

Laboratory Procedures

We extracted whole cellular DNA from 100% ethanol-preserved muscle tissues using the standard ammonium precipitation method (Maniatis et al. 1982). Polymerase chain reaction (PCR) amplifications were carried out using Taq DNA Polymerase Master Mix (Ampliqon S/A, Denmark) and Axygen Maxygene thermocyclers. The standard PCR program consisted of a 3-min initial denaturing step at 95°C, followed by 35–36 (nuclear 40–42) cycles of 20 s at 95°C, 20 s at 45–60°C, and 45 or 80 s at 68 or 72°C, followed by a final extension step of 5 min at 68 or 72°C. We carried out PCR product cleaning using enzymatic purifications (shrimp alkaline phosphatase and exonuclease I; Werle et al. 1994). Purified PCR products were sent to Macrogen Inc. (South Korea) where they conducted sequencing in an ABI 3730XL sequencer. Primer pairs are detailed in Table 1 and GenBank accession numbers are given in Appendix II.

Alignment, Partition Schemes, and Nucleotide Substitution Model Selection

We performed alignment using MAFFT v7.273 (Katoh and Standley 2013). For the nuclear gene fragments we used the FFT-NS-2 algorithm and for the 12S-tVal-16S concatenated fragment we used the E-INS-i algorithm, which is adapted for sequences with conserved domains and variable regions rich in gaps.

We conducted a search for the best partition scheme and best-fitting nuclear models with PartitionFinder v1.1.1 (Lanfear et al. 2012) using the corrected Akaike information criterion (AICc; Hurvich and Tsai 1989) and considering each gene and each codon as separate partitions.

Genetic Distance and Phylogenetic Analyses

We computed uncorrected pairwise distances using R v3.2.4 (R Core Team 2016) with the packages APE v3.4 (Paradis et al. 2004) and SPIDER v1.3-0 (Brown et al. 2012). The fragment of the 16S rDNA employed in the genetic distance calculation was the one delimited by the primers 16S AR–BR (ca. 600 bp; Palumbi et al. 1991).

We conducted tree searches using both maximum likelihood and Bayesian inference optimality criteria. We computed maximum likelihood analysis in RAxML v8.2.2 (Stamatakis 2014), searching the most likely tree 100 times and conducting 1000 nonparametric bootstrap replicates. We computed Bayesian inference analysis in MrBayes v3.2.6 (Ronquist et al. 2012) using two independent runs of 1.0×10^7 generations, starting with random trees and four Markov chains (one cold), sampled every 1000 generations. We discarded 25% of generations and trees as burn-in and performed the run with unlinked character state frequencies, substitution rates of general time-reversible (GTR)

model, gamma shape parameters, and proportion of invariable sites between partitions. We used standard deviation of split frequencies (<0.01), estimated sample size (ESS > 100), and potential scale reduction factor (PSRF; Gelman and Rubin 1992; should approach 1.0 as runs converge) to assess run convergence. We used *Eleutherodactylus* as root for both analyses, and we drew phylogenetic trees using FigTree v1.4.2 (Rambaut 2014).

Morphological Analyses

The following measurements were taken to the nearest 0.1 mm with a Mitutoyo® digital caliper under a stereomicroscope: snout–vent length (SVL), head length (from the tip of the snout to the angle of the jaw), head width (between the angles of the jaws), forearm length (from the elbow to the wrist), hand length (from the wrist to the tip of the third finger), thigh length (from the middle of the cloacal opening to the outer edge of the knee), tibia length (from the outer edge of the knee to the outer edge of the heel), tarsal length (from the outer edge of the heel to the inner metatarsal tubercle), and foot length (from the proximal border of the inner metatarsal tubercle to the tip of the fourth toe). Eye diameter (between anterior and posterior margins of the eye), tympanum diameter (between anterior and posterior margins of the tympanum), eye-to-nostril distance (from the anterior margin of the eye to the posterior margin of the nostril), internarial distance (between the two medial margins of the nostrils), eye-to-eye distance (between the anterior margins of the eyes), third finger disk length (maximum width of disk on third finger), and fourth toe disk length (maximum width of disk on fourth toe) were taken with an ocular micrometer coupled to a stereomicroscope. Sex was determined by the observation of nuptial pads and vocal slits in males and gonads of females. Morphological nomenclature follows previous literature on Brachycephaloidea (Heyer 1984; Heyer et al. 1990; Hedges et al. 2008; Duellman and Lehr 2009). Museum acronyms follow Sabaj (2016) and a full list of specimens examined is given in Appendix III.

Call Analyses

We recorded advertisement calls from both of the new species using a Marantz PMD 660 or PMD 661 or a Tascam DR-40, coupled to a Sennheiser K6/ME66 unidirectional microphone. Recordings were carried out at 44.1 kHz on a 16-bit sampling size. To analyze the recordings we used the software Raven Pro v1.4 (Bioacoustics Research Program 2011). Spectrograms were produced using window size of 512 samples, 75% overlap, hop size of 128 samples, discrete Fourier transform of 1024 samples, and window type Hann. Resolution, contrast, and brightness were the program defaults. We obtained spectrogram and oscillogram figures using tuneR v1.0 (Ligges et al. 2013) and seewave v2.0.2 (Sueur et al. 2008) packages of R platform v3.2.4 (R Core Team 2016). Spectrogram figures were produced with window length of 512 samples, 75% overlap, hop size of 128 samples, and window name Hanning. Call recordings of P.P.G. Taucce (PPGT 001–008) are deposited in the CFBH collection and remaining analyzed call recordings are deposited in the Bioacoustics Collection of the Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (CBUFMG 916–917) and in the voice collection of the Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil (MNVOC 043:1–3). Voucher specimens are housed at CFBH, MZUFV, and UFMG. Full information for the recordings is listed in Appendix IV.

The following acoustic parameters were taken: call duration (=call length from Cocroft and Ryan 1995), call rise time (Hepp and Canedo 2013), dominant frequency (Cocroft and Ryan 1995), notes per call, note repetition rate (Gehara et al. 2013), and note repetition rate acceleration (Gehara et al. 2013). *Ischnocnema oea* recently had its advertisement call described (Hepp and Canedo 2013). Although we did not reanalyze the recordings used in this description, we measured note repetition rate acceleration for the sake of comparison with our recordings, since this parameter was not used by Hepp and Canedo (2013).

TABLE 2.—Best partition scheme and respective best-fitting molecular models. RAG 1 = nuclear recombination activating gene 1; Tyr = tyrosinase; GTR = general time-reversible; HKY = Hasegawa-Kishino-Yano; K80 = Kimura 1980.

Partition	Model
12S	GTR + Γ + I
tVal	GTR + Γ
16S	GTR + Γ + I
RAG1 1st and 2nd positions	HKY + Γ
RAG 1 3rd position	K80 + Γ
Tyr 1st and 2nd positions	GTR + Γ + I
Tyr 3rd position	GTR + Γ

RESULTS

Alignment, Partition Schemes, and Nucleotide Substitution Model Selection

We obtained a final alignment of 3585 base pairs divided in three mitochondrial and two nuclear genes, respectively: 12S rRNA (1016 bp), tRNAVal (75 bp), partial 16S rRNA (1533 bp), partial RAG1 (417 bp), and partial Tyr (531 bp). The best-fit partition scheme comprised seven partitions, which are shown with respective substitution models used in the Bayesian inference analysis in Table 2. For the maximum likelihood analysis we used the GTR model with γ-distribution for all the partitions because RAxML does not support estimating different models for different partitions.

Genetic Distance and Phylogenetic Analyses

The uncorrected pairwise distance of partial 16S rRNA between *Ischnocnema oea* and *I. garciai* was 10.4 to 10.7% and between *I. oea* and *I. feioi* it was 9.9%. The genetic distance between *I. feioi* and *I. garciai* was 7.0 to 7.8%. Distances among these species and other closely related species within the *I. guentheri* series are summarized in Table 3.

The Bayesian inference and the maximum likelihood analyses resulted in the same topology. Mostly with high support, we recovered all currently recognized *Ischnocnema* series as reciprocally monophyletic groups, as well as the same relationships among series as those recovered by Canedo and Haddad (2012; Fig. 1). However, we recovered the *I. guentheri* and the *I. parva* series with low support (61% of posterior probability and 55% of maximum likelihood bootstrap, 91% of posterior probability and 62% of maximum likelihood bootstrap, respectively). Within the *I.*

Table 3.—Uncorrected pairwise genetic distances within and between members of the *Ischnocnema guentheri* series closely related to *I. oea*. Within-species distances are bolded. Data are shown as range (mean) where appropriate.

	Uncorrected pairwise distance between species							
	I. feioi	*I. garciai*	*I. oea*	*I. guentheri*	*I. henselii*	*I. izecksohni*	*I. nasuta*	*I. erythromera*
I. feioi	**0.0–1.5 (0.9, *n* = 3)**							
I. garciai	7.0–7.8 (7.5)	**0.0 (*n* = 4)**						
I. oea	9.9	10.4–10.7 (10.6)	**0.0 (*n* = 2)**					
I. guentheri	9.7–10.7 (10.3)	13.1–13.6 (13.3)	14.0–14.3 (14.1)	**0.0–0.5 (0.1, *n* = 11)**				
I. henselii	10.2–12.1 (11.3)	12.8–14.3 (13.7)	13.6–14.8 (13.9)	7.5–9.0 (8.2)	**0.0–3.6 (1.8, *n* = 57)**			
I. izecksohni	10.7–11.9 (11.1)	13.6–13.8 (13.7)	13.6	12.8–13.1 (13.1)	13.6–14.5 (13.8)	**0.0 (*n* = 2)**		
I. nasuta	10.9–12.8 (11.7)	13.8–14.8 (14.3)	13.3–14.0 (13.6)	12.3–13.1 (12.8)	13.6–14.8 (13.9)	1.2–1.9 (1.8)	**0.0–3.2 (2.3, *n* = 4)**	
I. erythromera	9.4–10.7 (9.9)	11.6–11.9 (11.8)	13.1–13.6 (13.3)	11.1–11.6 (11.5)	12.4–13.8 (13.1)	12.1–12.6 (12.4)	11.9–12.6 (12.2)	**1.0 (*n* = 2)**

guentheri series, results were mostly consistent with previous hypotheses (Canedo and Haddad 2012; Gehara et al. 2013). *Ischnocnema oea* clustered with *I. garciai* and *I. feioi* in a well-supported clade (100% of posterior probability and maximum likelihood bootstrap) and was the sister species of *I. garciai*.

Morphological Analyses

Morphological characteristics allowed us to distinguish the three new species within the *Ischnocnema oea* cluster from all other members from the *I. guentheri* series. The main character states distinguishing the three species are the calcar tubercle being at least as long as wide in adult males (absent or less long than wide in other species; Fig. 2) and smaller SVL. Among the three species, *I. oea* is morphologically indistinguishable from *I. garciai*, but *I. feioi* has a larger SVL (Table 4) and a straight canthus rostralis in dorsal view (concave in the other two species).

Call Analyses

We analyzed 52 advertisement calls from 12 individuals, and all of them showed the same basic structure: groups of short notes emitted sporadically, with irregular intervals between calls. The advertisement calls begin with low-energy notes, increasing in energy gradually until a peak is reached, which is accordant with the other known calls of the *Ischnocnema guentheri* series. Despite having great genetic distance and being morphologically distinguishable (see above), *I. oea* and *I. feioi* have similar advertisement calls, exhibiting some degree of overlap in all analyzed parameters (Table 5; Fig. 3A,B). However, *I. garciai* has a notably distinct advertisement call (Table 5; Fig. 3C).

Based on the molecular, bioacoustical, and morphological data presented here we consider the three species within the *Ischnocnema oea* cluster as distinct evolving lineages. Here we redescribe *I. oea* and describe the other two new species.

Species Accounts

Ischnocnema oea (Heyer 1984)

Figs. 4A, 5

Eleutherodactylus oeus Heyer 1984: Heyer (1984:iii, 22 [his Table 20], 23, 26, 27 [his Fig. 21], 31–33 [his Fig. 26], 40), species description.

Eleutherodactylus (Eleutherodactylus) oeus: Lynch and Duellman (1997:229 [their Appendix III]).

Ischnocnema oea: Heinicke et al. (2007:by implication); Hedges et al. (2008:25, 27, 151 [their Appendix I]); Canedo et al. (2010:632–633); Canedo and Haddad (2012:611, 619 [their Table 3]), Padial et al. (2014:122 [their Appendix II]).

Holotype.—MNRJ 1244, adult male. Municipality of Santa Teresa, state of Espírito Santo, Brazil. Collected by Augusto Ruschi in December 1942.

Paratypes.—USNM 235612, MZUSP 59684 (not examined).

Diagnosis.—In the *Ischnocnema guentheri* series by phylogenetic placement (Canedo and Haddad 2012; Fig. 1) and the following combination of characters: (1) long legs, tibia length > 60% of SVL; (2) one large, conspicuous, glandular appearing nuptial pad on Finger I; (3) dorsum smooth. *Ischnocnema oea* is distinguished from all other

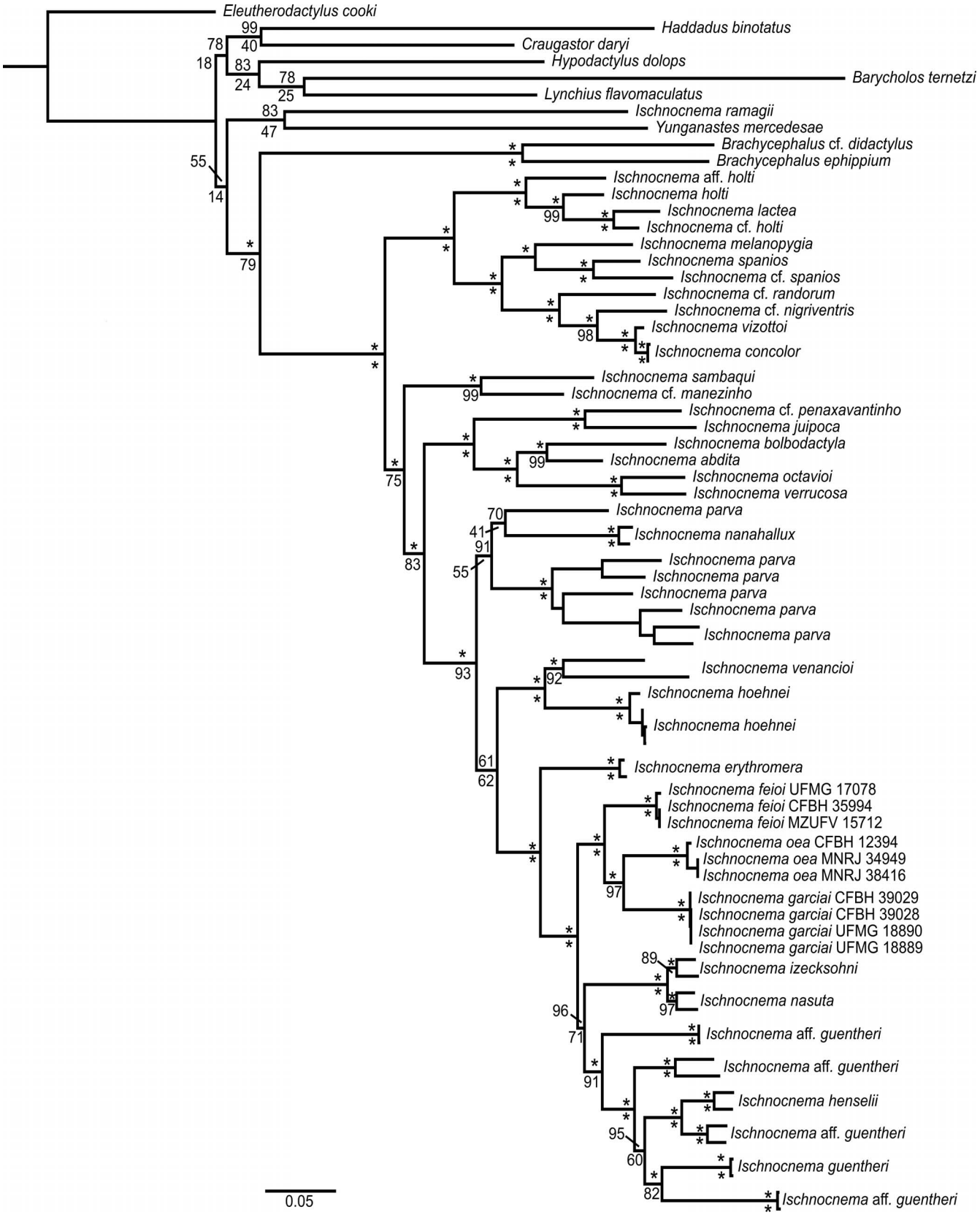

FIG. 1.—The 50% majority rule consensus tree from Bayesian inference analysis of concatenated mitochondrial 12S rRNA, tVal rRNA, 16S rRNA, and nuclear recombination activating gene 1 (RAG1) and tyrosinase precursor (Tyr), showing Bayesian posterior probabilities (above branches) and maximum likelihood nonparametric bootstrap values (below). Asterisks (*) indicate 100% values.

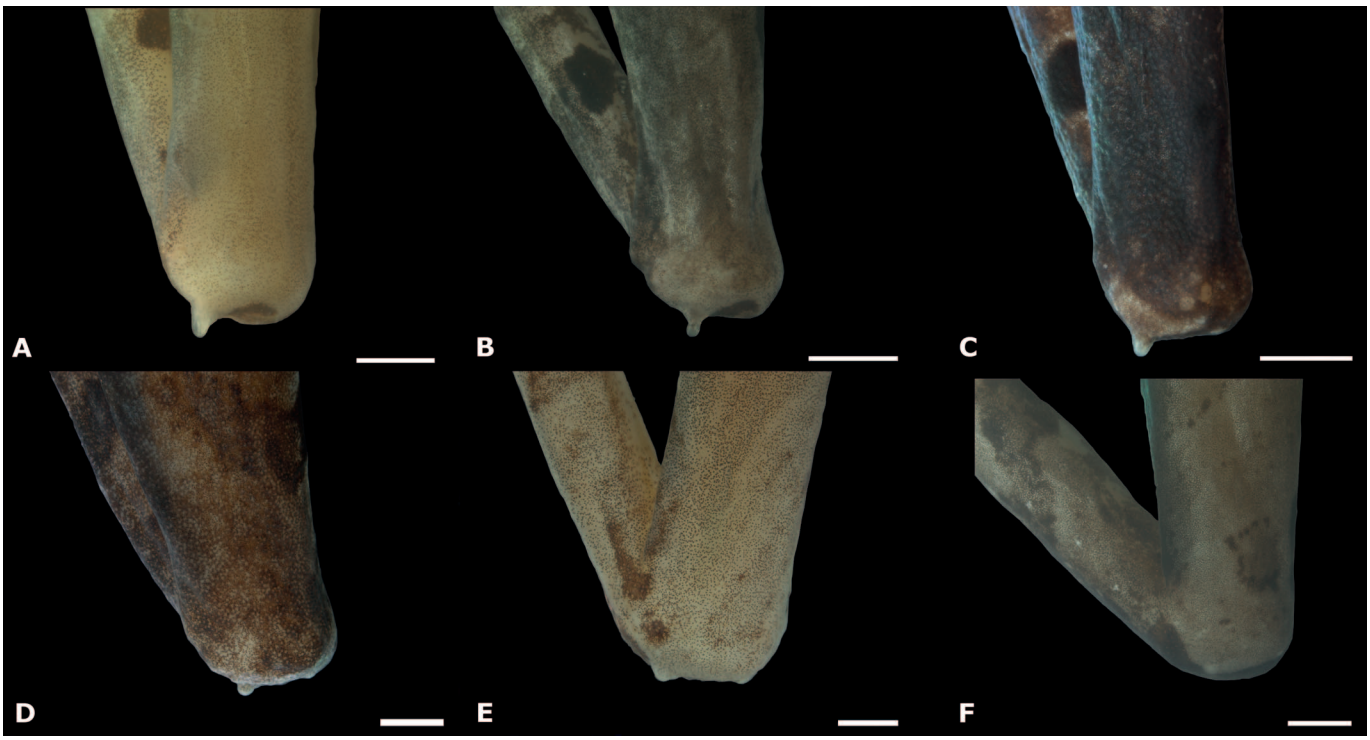

FIG. 2.—Calcar tubercles of members of the *Ischnocnema guentheri* series: (A) *I. feioi* (UFMG 3285), (B) *I. garciai* (CFBH 39029), (C) *I. oea* (CFBH 24778), (D) *I. guentheri* (CFBH 27443), (E) *I. hoehnei* (CFBH 8336), and (F) *I. izecksohni* (CFBH 35793). Scale bars = 1 mm. A color version of this figure is available online.

TABLE 4.—Snout–vent length (SVL) and body proportions of *Ischnocnema oea*, *I. feioi*, and *I. garciai*. Data are given as range (mean ± SD) where appropriate.

	Adult males			Adult females	
Character	*Ischnocnema oea* (n = 13)	*Ischnocnema feioi* (n = 4)	*Ischnocnema garciai* (n = 16)	*Ischnocnema oea* (n = 2)	*Ischnocnema garciai* (n = 2)
SVL (mm)	13.5–17.8 (16.0 ± 1.3)	20.7–23.6 (22.1 ± 1.2)	13.3–18.5 (16.8 ± 1.2)	24.7–25.0	21.9–24.7
Head length/SVL	0.44–0.52 (0.48 ± 0.03)	0.40–0.44 (0.42 ± 0.02)	0.39–0.47 (0.43 ± 0.02)	0.42–0.42	0.40–0.41
Head width/SVL	0.33–0.40 (0.38 ± 0.02)	0.32–0.34 (0.33 ± 0.01)	0.33–0.39 (0.36 ± 0.01)	0.36–0.38	0.34–0.36
Eye diameter/head length	0.20–0.30 (0.26 ± 0.03)	0.25–0.28 (0.26 ± 0.01)	0.26–0.32 (0.29 ± 0.02)	0.26–0.28	0.28–0.30
Tympanum diameter/ eye diameter	0.27–0.66 (0.45 ± 0.12)	0.40–0.53 (0.46 ± 0.07)	0.41–0.55 (0.47 ± 0.04)	0.45–0.55	0.45–0.47
Tibia length/SVL	0.67–0.74 (0.70 ± 0.02)	0.69–0.79 (0.73 ± 0.04)	0.64–0.72 (0.69 ± 0.03)	0.66–0.69	0.66–0.72
Thigh length/SVL	0.57–0.69 (0.64 ± 0.04)	0.61–0.66 (0.63 ± 0.02)	0.56–0.66 (0.61 ± 0.03)	0.60–0.61	0.57–0.64

TABLE 5.—Advertisement call parameters comparing the members of the *Ischnocnema guentheri* series. Data are given as ranges.

Species	Call duration (s)	Call rise time (%)	Dominant frequency (kHz)	Notes per call	Note rate (notes/s)	Note repetition rate acceleration (%)	Source
Ischnocnema feioi	1.54–5.51	79–100	2.53–3.23	10–27	4.13–6.19	–26 to 21	This study
Ischnocnema garciai	14.84–29.11	45–92	3.27–3.88	57–96	3.27–4.47	5–198	This study
Ischnocnema oea	4.56–8.49	90–99	3.09–4.13	25–41	4.80–5.70	–9 to 61	Hepp and Canedo (2013), this study
Ischnocnema gualteri	1.50–1.90	—	2.10–2.70	4–9	—	—	Heyer (1984)
Ischnocnema guentheri	26.30–41.90	—	2.81–3.28	71–146	2.20–3.50	31–121	Gehara et al. (2013)
Ischnocnema henselii	10.00–23.00	—	2.10–3.10	86–170	6.60–7.10	107–125	Kwet and Solé (2005), Gehara et al. (2013)
Ischnocnema izecksohni	1.03–2.15	—	2.25–2.63	34–60	26.91–32.10	—	Taucce et al. (2012)
Ischnocnema nasuta	1.15–1.50	—	2.10–2.60	34–43	—	—	Heyer (1984)

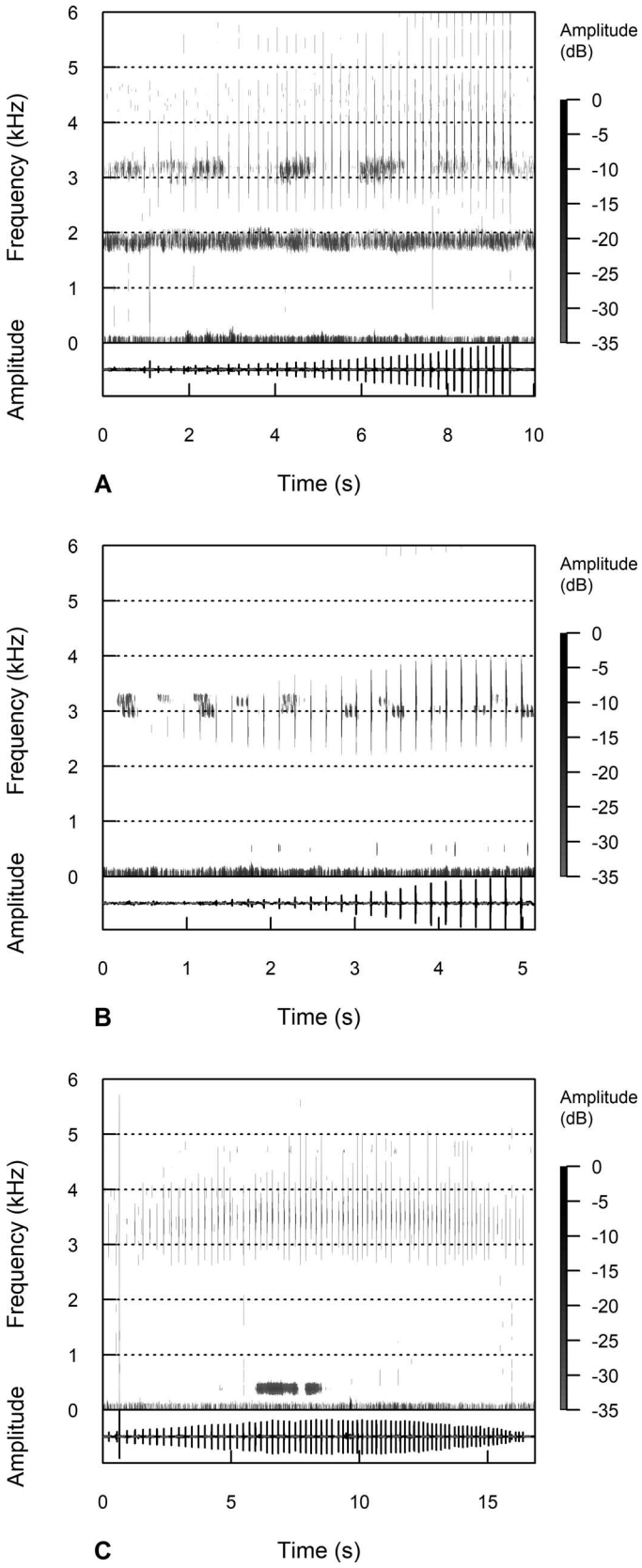

FIG. 3.—Advertisement call of three species of the *Ischnocnema guentheri* series. Oscillogram (below) and spectrogram (above) of (A) *I. oea* (recording MNVOC 043:2), (B) *I. feioi* (recording PPGT 004), and (C) *I. garciai* (recording PPGT 007).

species of the *I. guentheri* series by the following combination of characters: (1) calcar tubercle at least as long as wide in adult specimens; (2) small size (SVL in males 13.5–17.8 mm, $n = 13$; females 24.7–25.0, $n = 2$); (3) posterior face of the thigh uniform or mottled; (4) canthus rostralis concave in dorsal view; (5) Finger I approximately the same size as Finger II; (6) advertisement call duration 4.56–8.49 s; (7) dominant frequency 3.09–4.13 kHz; (8) 25–41 notes per call; (9) note repetition rate 4.80–5.70 notes/s; (10) note repetition rate acceleration –9 to 61%.

Redescription of the holotype.—Small size (SVL 17.1 mm). Head longer than wide; head length 44% of SVL, head width 33% of SVL; snout rounded in dorsal and lateral views; nostrils rounded, oriented laterally, located near the tip of the snout; canthus rostralis moderately distinct, curved; loreal region slightly concave; eyes protuberant and laterally oriented, eye diameter 30% of head length; tympanum distinct, rounded, tympanic membrane undifferentiated, annulus present, visible externally, tympanum diameter 38% of eye diameter; supratympanic fold absent; vocal slits present; vocal sac single, subgular, slightly expanded externally, with a fold of skin on the right side; tongue large, elliptical, posterior notch absent; choanae rounded; dentigerous processes of the vomer located posteromedially to choanae, triangle-shaped, medially separated by a gap approximately the width of one dentigerous process, teeth present, barely distinct.

Forelimbs slender; fingers slender, bearing discrete fringes, with small discs on Fingers I and II, larger discs on Fingers III and IV with a V-shaped median slit in dorsal view; finger lengths I $\approx$ II $<$ IV $<$ III; palmar tubercle barely distinct; thenar tubercle elliptical, barely distinct; single nuptial pad apparently glandular, with the same color as the hand, extending dorsaly from the distal to the proximal portion of the metacarpus on Finger I, divided ventrally on the distal margin of the thenar tubercle, extending all over its caudal third; palm smooth with one barely distinguishable supernumerary tubercle; single subarticular tubercles prominent, rounded, and large.

Hind limbs slender; shank longer than thigh, tibia length 67% of SVL, thigh length 60% of SVL; calcar tubercle well developed, cone-shaped, as long as wide; knees with two pointed tubercles; tarsal fold absent; toes long, slender, fringed, with large discs on Toes II–V, which have a V-shaped median slit in dorsal view; small disc on Toe I; toe lengths I $<$ II $<$ III $<$ V $<$ IV; inner metatarsal tubercle elliptical, much larger than the rounded outer metatarsal tubercle; sole of the foot smooth, with one supernumerary tubercle; single large, prominent, and rounded subarticular tubercles.

Dorsal skin smooth, with a few sparse tubercles; dorsal surface of the snout and upper eyelid with some barely distinguishable, pointed tubercles; venter smooth, with no tubercles; discoidal and thoracic folds present.

Coloration of the holotype in preservative.—The specimen is somewhat faded. Background yellowish-brown; dorsum completely variegated; head with cream-colored interorbital bar; brown lateral strip from right below eyes to upper lip; canthus rostralis with brown blotch near nostrils; brown supratympanic stripe starting in tympanum, contouring arm, and reaching abdomen at midbody; inguinal region with brown spot; dorsal portion of forelimbs yellowish-brown

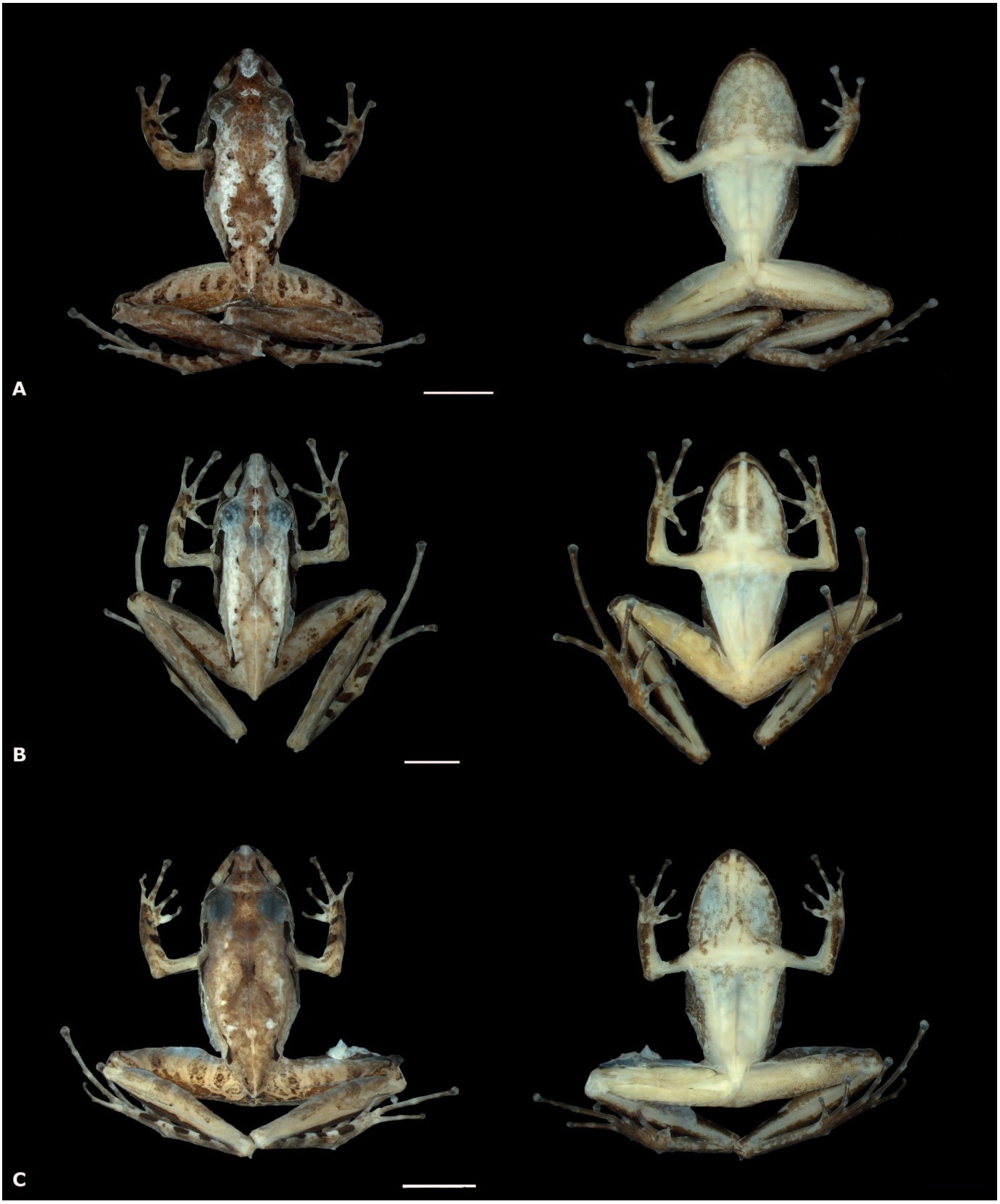

Fig. 4.—Dorsal (left) and ventral (right) views of (A) *Ischnocnema oea* (CFBH 30732), (B) *I. feioi* (CFBH 35994, holotype), and (C) *I. garciai* (CFBH 39028, holotype). Scale bar = 5 mm. A color version of this figure is available online.

FIG. 5.—Dorsal and ventral views of the holotype of *Ischnocnema oea* (MNRJ 1244). Scale bar = 5 mm. A color version of this figure is available online.

with transversal brown stripes; ventral portion of forelimbs yellowish-brown; hidden portion of thigh yellowish-brown; external portion of tibia with brown longitudinal bar; venter yellowish-brown; gular region yellowish-brown with some irregularly spaced brown blotches; margins of jaw brown.

Measurements of holotype (in millimeters).—Snout–vent length 17.1, head length 7.4, head width 5.6, eye diameter 2.2, tympanum diameter 0.8, eye–nostril distance 2.2, internarial distance 1.6, eye-to-eye distance 3.3, forearm length 3.7, hand length 3.8, third finger disk length 0.5, thigh length 10.3, tibia length 11.4, tarsal length 5.8, foot length 9.9, fourth toe disk length 0.6.

Variation.—Additional referred specimens are listed in Appendix III. Some specimens have a subelliptical snout in dorsal view. We found great variation in the shape of the nostrils, which may be triangular, elliptical, and ovoid. The supratympanic stripe may be just a blotch in the upper tympanic region or may reach the midbody without going down to the abdominal region. The shapes of the tongue and choanae openings are highly variable. Some individuals have the tongue and choanae openings rounded, ovoid, and elliptical. Upper eyelid tubercles and finger fringes are absent in some specimens, and postrictal tubercles are present in some. Females were markedly larger than males (SVL in females 24.7–25 mm, $n = 2$; males 13.5–17.8 mm, $n = 13$). In juveniles, the calcar tubercle may be as long as wide, shorter than wide, or absent. Variation in measurements and body proportions are given in Table 4.

Advertisement call.—The advertisement call is described in detail by Hepp and Canedo (2013).

Comparisons with other species.—The long legs (tibia length/SVL = 66–74%) distinguish *Ischnocnema oea* from the species of the *I. lactea* (tibia length/SVL usually < 50%; Hedges et al. 2008), *I. parva* (tibia length/SVL < 60%; Hedges et al. 2008; Brusquetti et al. 2013), and *I. verrucosa* (tibia length/SVL < 55%; Hedges et al. 2008; Canedo et al. 2010, 2012) series, and from *I. sambaqui* (Castanho and Haddad 2000; currently unassigned to any series; tibia length/SVL < 55%; Castanho and Haddad 2000). The large and conspicuous, glandular-appearing nuptial pad on Finger I distinguishes *I. oea* from the species of the *I. lactea* (minute nuptial pad in *I. randorum* [Heyer 1985]; translucent in *I. nigriventris* [A. Lutz 1925] and *I. vizottoi* Martins and Haddad 2010; reduced to some white granules in *I. holti* [Cochran 1948]; absent in *I. melanopygia* Targino et al. 2009 and *I. spanios* [Heyer 1985]; unknown in other species; Heyer 1985; Hedges et al. 2008; Targino and Carvalho-e-Silva 2008; Berneck et al. 2013) and *I. verrucosa* series (except for *I. surda* Canedo et al. 2010, in which the nuptial pad is also large, conspicuous, and glandular-appearing; faint, translucent nuptial pad in *I. karst* Canedo et al. 2012; absent in other species; Hedges et al. 2008; Canedo et al.

2010, 2012) and from *I. manezinho* (Garcia 1996; currently unassigned to any series) and *I. sambaqui* (absent in these last two species; Garcia 1996; Castanho and Haddad 2000). The smooth dorsum distinguishes *I. oea* from the species of the *I. verrucosa* series (dorsum tuberculate in these species; Hedges et al. 2008; Canedo et al. 2010, 2012), from *I. manezinho* (finelly tuberculate; Garcia 1996), and from *I. sambaqui* (slightly rugose to rugose; Castanho and Haddad 2000).

Ischnocnema oea differs from all species of the *I. guentheri* series by having a calcar tubercle that is at least as long as it is wide in adult specimens (calcar tubercle absent or not as long as wide in other species).

By its smaller body size, *Ischnocnema oea* (SVL in males 13.5–17.8 mm; females 24.7–25 mm) differs from *I. erythromera* (SVL in males 22.3–24.4 mm; females 24.3–35.3 mm; Heyer 1984), *I. gualteri* (SVL in males 21.3–34.1 mm; females 33.6–45.7 mm; Heyer 1984), *I. henselii* (SVL in males 21.0–27.5 mm; females 28.4–38.4 mm; Kwet and Solé 2005), *I. izecksohni* (SVL in male 32.4 mm; females 43.5–49.0 mm; Caramaschi and Kisteumacher 1989 "1988"), and *I. nasuta* (SVL in males 24.7–41.5 mm; females 36.1–53.9 mm; Heyer 1984).

By the uniform or mottled posterior surface of its thighs, *Ischnocnema oea* is distinguished from *I. erythromera* (*I. erythromera* with a light area on the posterior surface of the thigh in fixed specimens and red in life; Heyer 1984) and from *I. venancioi* (*I. venancioi* with clear spots surrounded by a dark background in fixed specimens and spots orange or yellow in life; B. Lutz 1958). Finger I approximately the same size as Finger II also distinguishes *I. oea* from *I. venancioi* (Finger I smaller than Finger II in *I. venancioi*). The concave canthus rostralis in dorsal view distinguishes *I. oea* from *I. hoehnei*, *I. izecksohni*, *I. nasuta*, and *I. venancioi* (canthus rostralis straight in dorsal view in these species).

Advertisement call duration (4.56–8.49 s; Hepp and Canedo 2013) distinguishes *Ischnocnema oea* from *I. gualteri* (1.50–1.90 s; Heyer 1984), *I. guentheri* (26.30–41.90 s; Gehara et al. 2013), *I. henselii* (10.00–23.00 s; Gehara et al. 2013), *I. izecksohni* (1.03–2.15 s; Taucce et al. 2012), and *I. nasuta* (1.15–1.50 s; Heyer 1984). *Ischnocnema oea* emits more notes per call (25–41; Hepp and Canedo 2013) than *I. gualteri* (4–9; Heyer 1984) and fewer notes per call than *I. henselii* (86–170; Gehara et al. 2013). The higher dominant frequency (3.09–4.10 kHz; Hepp and Canedo 2013) distinguishes *I. oea* from *I. gualteri* (2.10–2.70 kHz; Heyer 1984), *I. henselii* (2.10–3.10 kHz; Gehara et al. 2013), *I. izecksohni* (2.25–2.63 kHz; Taucce et al. 2012), and *I. nasuta* (2.10–2.60 kHz; Heyer 1984). Note repetition rate distinguishes *I. oea* (4.80–5.70 notes/s; Hepp and Canedo 2013) from *I. henselii* (6.60–7.10 notes/s; Gehara et al. 2013) and *I. izecksohni* (29.91–31.10 notes/s; Taucce et al. 2012), and note repetition rate acceleration distinguishes *I. oea* (–9 to 61%) from *I. henselii* (107–125%; Gehara et al. 2013).

Geographic distribution.—*Ischnocnema oea* is currently known only from the state of Espírito Santo, southeastern Brazil, from the municipalities of Cariacica, Santa Teresa, and Vargem Alta (Fig. 6).

Remarks.—Silva-Soares et al. (2009) expanded the known distribution of *I. oea* to Macaé de Cima, municipality of Nova Friburgo, state of Rio de Janeiro. We examined the referred specimen MBML 212 and concluded that it is probably a juvenile *Ischnocnema nasuta*. Almeida-Gomes et al. (2010) cited *I. oea* from the municipality of Cambuci, state of Rio de Janeiro. We examined the referred specimens (MNRJ 49504–49506) and they are indeed morphologically similar to *I. oea*. But since we are not aware of any morphological differences between *I. oea* and *I. garciai*, and we have no additional data to compare the population from Cambuci with specimens surely belonging to each of these species, the identity of these specimens will remain undetermined.

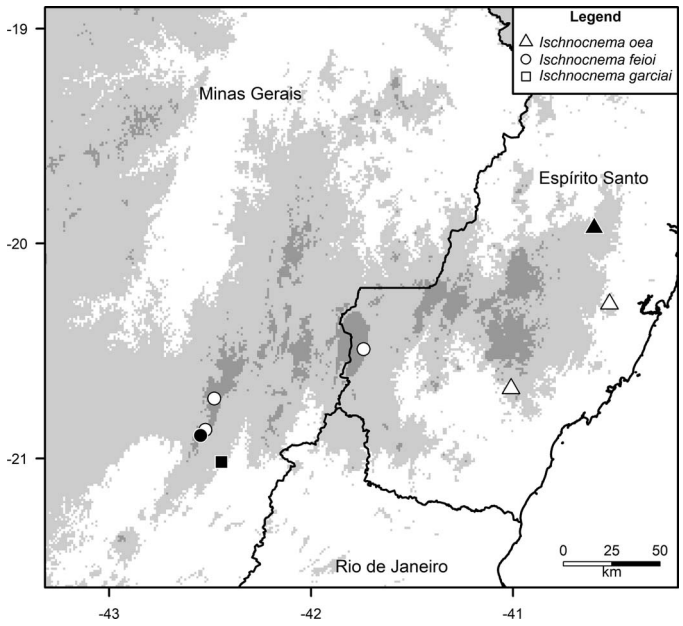

FIG. 6.—Geographic distribution of *Ischnocnema oea*, *I. feioi*, and *I. garciai*. Solid symbols represent type localities of each species. Area above 500 and 1000 m shaded gray.

Ischnocnema ***feioi*** sp. nov.
Figs. 4B, 7

Ischnocnema sp. (aff. *guentheri*): Moura et al. (2012:214 [their Table 2], 216 [their Fig. 2d], 233 [their Appendix 1]), in part; [misidentification]).

Holotype.—CFBH 35994, adult male. Lar dos Muriquis, Serra do Brigadeiro, municipality of Muriaé, state of Minas Gerais, Brazil (20°53′34.7″S, 42°32′48.6″W, 1297 m above sea level [a.s.l.]; datum WGS-84), collected by P.P.G. Taucce, J.V. Lacerda, C.S. Guimarães, L.S. Moreira, and R.N. Feio on 23 January 2014.

Paratypes.—All adult males. MZUFV 15712, Careço, municipality of Ervália, state of Minas Gerais, Brazil, collected by P.P.G. Taucce, B. Lisboa, and C.S. Guimarãe on 3 December 2014. UFMG 3285, Parque Estadual da Serra do Brigadeiro, municipality of Araponga, state of Minas Gerais, Brazil, collected by P.C.A. Garcia, P.S. Santos, and P.P.G. Taucce in December 2009. UFMG 17078, Parque Nacional do Caparaó, municipality of Santa Marta, state of Espírito Santo, Brazil (20°29′25.2″S, 41°44′23.15″W, 1128 m a.s.l.), collected by P.C.A. Garcia on 29 November 2014.

Referred specimens.—MZUFV 15575, juvenile, Trilha do Cruzeiro, Parque Estadual do Brigadeiro, Careço,

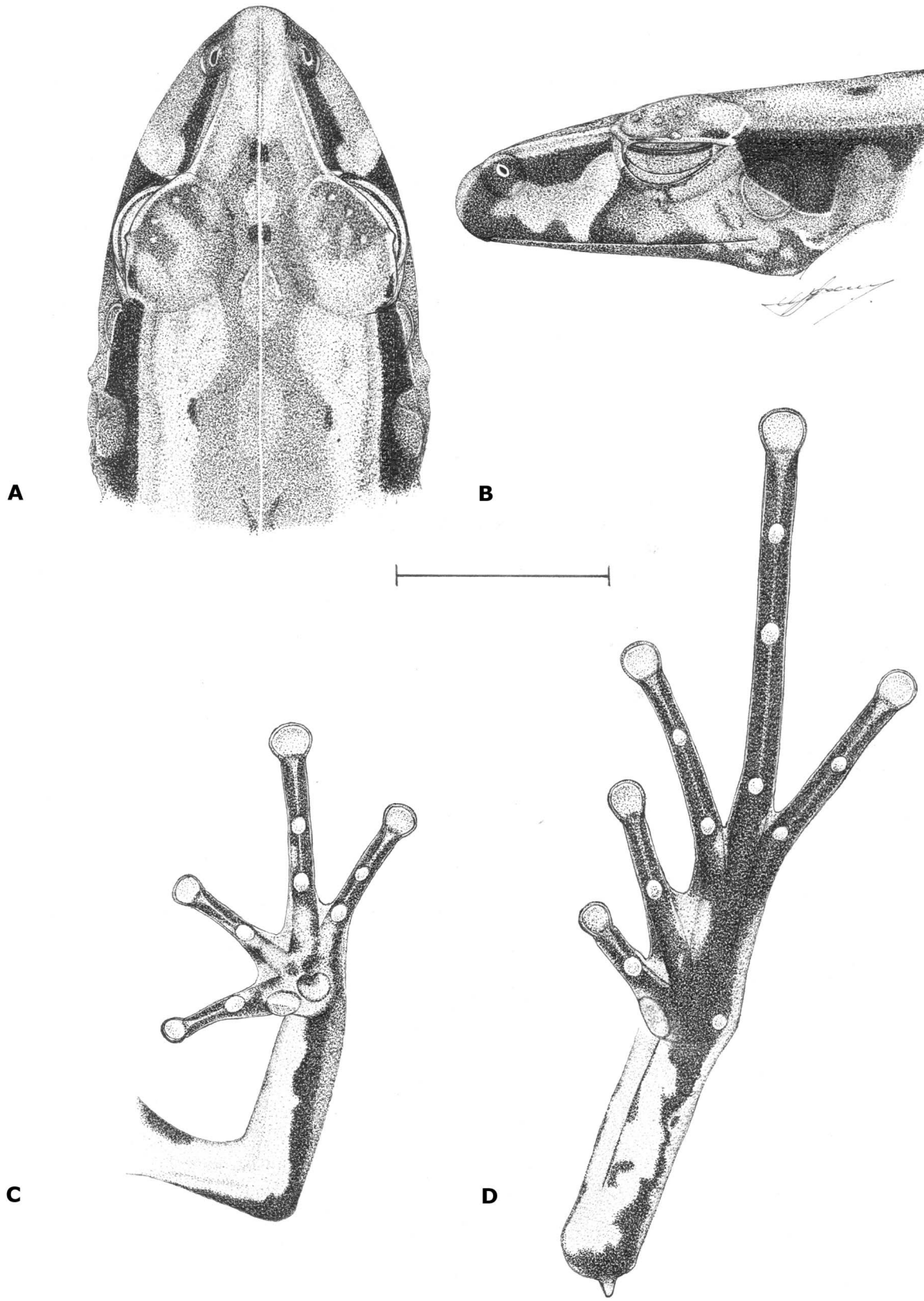

FIG. 7.—Holotype of *Ischnocnema feioi*, CFBH 35994: (A) dorsal and (B) lateral views of the head, (C) ventral view of the left hand, and (D) ventral view of the left foot. Scale bar = 5 mm.

municipality of Ervália, state of Minas Gerais, Brazil, collected by R.N. Feio, C.L. Assis, and C.S. Guimarães on 18 September 2014.

Diagnosis.—In the *Ischnocnema guentheri* series by phylogenetic placement (Canedo and Haddad 2012; Fig. 1) and the following combination of characters: (1) long legs, tibia length > 60% of SVL; (2) one large, conspicuous, glandular appearing nuptial pad on Finger I; (3) dorsum smooth. *Ischnocnema feioi* is distinguished from all other species of the *I. guentheri* series by the following combination of characters: (1) calcar tubercle at least as long as wide in adult specimens; (2) medium size (SVL in males 20.7–23.6 mm, $n = 4$); (3) posterior surface of the thigh mottled; (4) canthus rostralis straight in dorsal view; (5) Finger I approximately the same size as Finger II; (6) advertisement call duration 1.54–5.51 s; (7) dominant frequency 2.53–3.23 kHz; (8) 10–27 notes per call; (9) note repetition rate of 4.13–6.19 notes/second; (10) note repetition rate acceleration of –26 to 21%.

Description of the holotype.—Medium size (SVL = 20.0 mm). Head longer than wide; head length 42% of SVL, head width 33% of SVL; snout subelliptical in dorsal view, rounded in lateral view; nostrils triangular, oriented laterally, located near the tip of the snout; canthus rostralis distinct, straight; loreal region slightly concave; postrictal tubercle present, V-shaped; eyes protuberant, oriented laterally; eye diameter 28% of head length; tympanum distinct, rounded, tympanic membrane undifferentiated, annulus present, visible externally, tympanum diameter 40% of eye diameter; supratympanic fold absent; vocal slits present; vocal sac single, subgular, slightly expanded externally, with two oblique folds of skin on each margin of the throat; tongue large, heart-shaped, posterior notch absent; choanae rounded; dentigerous processes of the vomer located posteromedially to choanae, triangle shaped, medially separated by a gap approximately the width of one dentigerous process, teeth present, six on the right and five on the left dentigerous process.

Forelimbs slender; fingers slender, bearing discrete fringes, with small discs on Fingers I and II, larger discs on Fingers III and IV with a V-shaped median slit in dorsal view; Finger I approximately the same size as Finger II; finger lengths I ≈ II < IV < III; palmar tubercle heart-shaped, its diameter approximately equal to the diameter of the thenar tubercle; thenar tubercle elliptic; single nuptial pad apparently glandular, whitish, extending dorsally from the distal to the proximal portion of the metacarpus on Finger I, divided ventrally on the distal margin of the thenar tubercle, extending all over its caudal third; palm smooth, with one barely distinguishable supernumerary tubercle towards Finger III; single subarticular tubercles prominent, rounded, and large.

Hind limbs slender; shank longer than thigh, tibia length 73% of SVL, thigh length 66% of SVL; calcar tubercle well developed, cone-shaped, as long as wide on left leg, on right leg smashed against the heel; tarsal fold absent; toes long, slender, fringed, with large discs on Toes II–V, which have a V-shaped median slit in dorsal view; small disc on Toe I; toe lengths I < II < III < V < IV; inner metatarsal tubercle elliptical, much larger than rounded outer metatarsal tubercle; sole of foot smooth; single large, prominent, and rounded subarticular tubercles.

Dorsal skin smooth, with a few sparse tubercles; upper eyelid with a few small, barely distinguishable tubercles, one larger distinct tubercle on each side of the eyelids, positioned medially; venter smooth; discoidal fold present; thoracic fold absent.

Coloration of the holotype in preservative.—Background grayish-white; dorsum with medial clear whitish pinstripe from tip of snout to vent over two dark brown spots, one between eyes and other on the posterior fifth of snout, brown X-shaped mark on its second third; yellowish-brown longitudinal middorsal band from posterior fifth of snout to vent, with four grayish-white blotches along it; head with dark brown loreal stripe from tip of snout to eyes, bordering canthus rostralis; lateral strip from right below eyes to upper lip; dark brown supratympanic stripe starting at tympanum, contouring arm, and reaching abdomen at midbody; inguinal region with dark brown spot; forelimbs variegated yellowish-brown to brown with three dark brown blotches dorsally; palm of the hand cream with brown blotches; dorsal portion of hind limbs variegated yellowish-brown to brown and feet with four dark brown blotches; sole of feet brown with cream blotches; ventral portion of forelimbs cream, with some dark brown dots mainly on its posterior margin; hidden portion of thigh cream-colored, mottled dark brown; external portion of tibia with dark brown longitudinal bar; venter cream-colored; gular region cream-colored, with dark brown margins and some small dark brown dot aggregations, and clear cream-colored stripe from tip of snout to end of throat.

Measurements of the holotype (in millimeters).—SVL 22.0, head length 9.2, head width 7.3, eye diameter 2.5, tympanum diameter 1.0, eye–nostril distance 2.6, internarial distance 2.0, eye-to-eye distance 3.9, forearm length 4.6, hand length 6.9, third finger disk length 0.9, thigh length 14.5, tibia length 16.1, tarsal length 7.5, foot length 15.6, fourth toe disk length 0.9.

Variation.—One paratype had an ovoid tympanum. The supratympanic stripe does not reach the abdomen at midbody in some specimens. The postrictal tubercle is elongated or absent in some specimens. Variation of measurements and body proportions are given in Table 4.

Etymology.—The specific epithet honors the Brazilian herpetologist Dr. Renato Neves Feio (Museu de Zoologia João Moojen de Oliveira, Universidade Federal de Viçosa, Minas Gerais, Brazil) for his substantial contributions to the study of the amphibians from Minas Gerais and to the conservation of the "Serra do Brigadeiro" (Brigadeiro Mountain Range) as well as his pleasant company during field work.

Advertisement call.—The advertisement call of *Ischnocnema feioi* ($n = 31$ calls of six males; Table 6; Fig. 3B) was composed of 10 to 27 notes ($\bar{X} = 19.06 \pm 5.09$), emitted sequentially, with the energy increasing in each note throughout the call, until reaching a peak near the end of the call. Call duration ranged from 1.54 to 5.51 s ($\bar{X} = 3.64 \pm 1.24$) and call rise time ranged from 79 to 100% ($\bar{X} = 97 \pm 5$) of the call. Note repetition rate was 4.13–6.19 notes/s ($\bar{X} = 5.20 \pm 0.48$) and note repetition rate acceleration ranged from –26 to 21% ($\bar{X} = -3 \pm 14$). Dominant frequency was 2.53–3.23 kHz ($\bar{X} = 2.94 \pm 0.20$).

Comparison with other species.—The long legs (tibia length/SVL = 69–79%) distinguishes *Ischnocnema feioi* from

TABLE 6.—Advertisement call parameters of five recorded males of *Ischnocnema feioi*. Data are given as a range (mean ± SD) where appropriate.

Call recording	PPGT 001	PPGT 002	PPGT 003	PPGT 004	CBUFMG 916	CBUFMG 917
Number of analyzed calls	2	6	3	4	10	6
Call duration (s)	4.94–5.51	3.70–5.34 (4.84 ± 0.62)	5.07–5.14 (5.12 ± 0.04)	4.18–4.43 (4.28 ± 0.12)	2.49–3.09 (2.82 ± 0.20)	1.54–2.56 (2.11 ± 0.37)
Call rise time (%)	96–99	99–100 (99 ± 0)	98–99 (99 ± 0)	79–100 (91 ± 9)	91–99 (96 ± 4)	91–100 (98 ± 4)
Dominant frequency (kHz)	2.89–2.93	2.71–2.97 (2.90 ± 0.09)	3.06–3.10 (3.09 ± 0.03)	2.76–2.76 (2.76 ± 0)	3.09–3.23 (3.16 ± 0.05)	2.53–2.76 (2.68 ± 0.09)
Notes per call	25.00–27.00	19.00–27.00 (25.00 ± 3.16)	22.00–22.00 (22.00 ± 0)	21.00–22.00 (21.50 ± 0.58)	14.00–18.00 (16.20 ± 1.14)	10.00–15.00 (12.50 ± 1.76)
Note rate (notes/s)	4.90–4.94	4.88–5.10 (4.99 ± 0.08)	4.13–4.18 (4.15 ± 0.03)	4.80–6.19 (5.32 ± 0.62)	5.21–5.82 (5.44 ± 0.21)	5.27–5.90 (5.52 ± 0.22)
Note repetition rate acceleration (%)	2–19	−14 to 7 (1 ± 9)	4–5 (4 ± 1)	18–21 (19 ± 1)	−26 to −9 (−20 ± 5)	−8 to 2 (−3 ± 3)

the species of the *I. lactea* (tibia length/SVL usually < 50%; Hedges et al. 2008), *I. parva* (tibia length/SVL < 60%; Hedges et al. 2008; Brusquetti et al. 2013), and *I. verrucosa* (tibia length/SVL < 55%; Hedges et al. 2008; Canedo et al. 2010, 2012) series and from *I. sambaqui* (tibia length/SVL < 55%; Castanho and Haddad 2000). The large and conspicuous, glandular-appearing nuptial pad on Finger I distinguishes *I. feioi* from the species of the *I. lactea* (minute nuptial pad in *I. randorum;* translucent in *I. nigriventris* and *I. vizottoi*; reduced to some white granules in *I. holti*; absent in *I. melanopygia* and *I. spanios*; unknown in other species; Heyer 1985; Hedges et al. 2008; Targino and Carvalho-e-Silva 2008; Berneck et al. 2013) and *I. verrucosa* series (except for *I. surda*; faint, translucent nuptial pad in *I. karst*; absent in other species; Hedges et al. 2008; Canedo et al. 2010, 2012) and from *I. manezinho* and *I. sambaqui* (absent in these species; Garcia 1996; Castanho and Haddad 2000). The smooth dorsum differentiates *I. feioi* from the species of the *I. verrucosa* series (dorsum tuberculate in these species; Hedges et al. 2008; Canedo et al. 2010, 2012), *I. manezinho* (finely tuberculate; Garcia 1996), and *I. sambaqui* (slightly rugose to rugose; Castanho and Haddad 2000).

Ischnocnema feioi differs from all species of the *I. guentheri* series, except for *I. oea*, by having a calcar tubercle that is at least as long as it is wide in adult specimens (absent or not as long as wide in other species).

By its smaller body size, *Ischnocnema feioi* (SVL in males 20.7–23.6 mm) differs from *I. izecksohni* (SVL in male 32.4 mm; Caramaschi and Kisteumacher 1989 "1988") and *I. nasuta* (SVL in males 24.7–41.5 mm; Heyer 1984). By its larger body size, *I. feioi* differs from *I. oea* (SVL in males 13.5–17.8 mm).

By the mottled posterior surface of the thighs *Ischnocnema feioi* is distinguished from *I. erythromera* (*I. erythromera* with a light area on the posterior surface of the thigh in fixed specimens and red in life; Heyer 1984) and from *I. venancioi* (*I. venancioi* with clear spots surrounded by a dark background in fixed specimens and spots orange or yellow in life; B. Lutz 1958). Finger I being approximately the same size as Finger II also distinguishes *I. feioi* from *I. venancioi* (Finger I about half of the size of Finger II in *I. venancioi*). The straight canthus rostralis in dorsal view distinguishes *I. feioi* from *I. oea* (canthus rostralis curved in dorsal view in this species).

Advertisement call duration (1.54–5.51 s) distinguishes *Ischnocnema feioi* from *I. guentheri* (26.30–41.90 s; Gehara et al. 2013), *I. henselii* (10.00–23.00 s; Gehara et al. 2013), and *I. nasuta* (1.15–1.50 s; Heyer 1984). *Ischnocnema feioi* emits more notes per call (10–27) than *I. gualteri* (4–9; Heyer 1984) and fewer notes per call than *I. guentheri* (71–146; Gehara et al. 2013), *I. henselii* (86–170; Gehara et al. 2013), *I. izecksohni* (34–60; Taucce et al. 2012), and *I. nasuta* (34–43; Heyer 1984). Note repetition rate distinguishes *I. feioi* (4.13–6.19 notes/s) from *I. guentheri* (2.20–3.50 notes/s; Gehara et al. 2013), *I. henselii* (6.60–7.10 notes/s; Gehara et al. 2013), and *I. izecksohni* (29.91–31.10 notes/s; Taucce et al. 2012) and note repetition rate acceleration distinguishes *I. feioi* (–26 to 21%) from *I. guentheri* (31–121%; Gehara et al. 2013) and *I. henselii* (107–125%; Gehara et al. 2013).

Geographic distribution.—*Ischnocnema feioi* is known only from the Serra do Brigadeiro, in the municipalities of Araponga, Muriaé, and Ervália, state of Minas Gerais, Brazil,

and from the Caparaó National Park, municipality of Santa Marta, state of Espírito Santo, Brazil (Fig. 6), at elevations over 1000 m a.s.l.

Remarks.—Figure 2d from Moura et al. (2012) corresponds to paratype UFMG 3285 of *Ischnocnema feioi*, although the specimen is not in their examined material list. All examined specimens have a clear cream-colored ventral stripe from the tip of the snout to the end of the throat on a dark brown background. Although it is not a common trait in the *I. guentheri* series, we did not use it as a diagnostic character because some *I. oea* and *I. izecksohni* exemplars possess the same pattern.

Ischnocnema ***garciai*** sp. nov.
Figs. 4C, 8

Ischnocnema sp.: (Santana et al. 2010:2 [their Table 1], 3 [their Fig. 2C], 4, 10 [their Appendix 1]).
Ischnocnema oea (Heyer 1984): Mângia et al. (2011:164 [their Fig. 1], 165), [misidentification]).

Holotype.—CFBH 39028, adult male. Usina da Fumaça, municipality of Muriaé, state of Minas Gerais, Brazil (21°0′57.6″S, 42°26′36.6″W, 430 m a.s.l.), collected by P.P.G. Taucce and B. Lisboa on 30 November 2014.

Paratopotypes.—CFBH 39026–39027, 39029–39033, MNRJ 90703–90704 (adult males), all collected with the holotype. UFMG 18889 (adult male), collected by P.P.G. Taucce, F.F. Pezzini, E.K.O. Hatori, and D.M. Neves, on 18 January 2014. UFMG 18890 (adult male), collected by P.P.G. Taucce and B. Lisboa on 29 November 2014. MZUFV 8894–8895 (adult females) and MZUFV 8896–8899 (adult males) collected by D.J. Santana and E.T. Silva on 13 September 2008.

Referred specimens.—MZUFV 8900, juvenile, Usina da Fumaça, municipality of Muriaé, state of Minas Gerais, Brazil, collected by D.J. Santana and E.T. Silva on 13 September 2008.

Diagnosis.—In the *Ischnocnema guentheri* series by phylogenetic placement (Canedo and Haddad 2012; Fig. 1) and the following combination of characters: (1) long legs, tibia length > 60% of SVL; (2) one large, conspicuous, glandular appearing nuptial pad on Finger I; (3) dorsum smooth. *Ischnocnema garciai* is distinguished from all other species of the *I. guentheri* series by the following combination of characters: (1) calcar tubercle at least as long as wide in adult specimens; (2) small size (SVL in males 13.3–18.5 mm, $n = 16$; SVL in females 21.9–24.7 mm, $n = 2$); (3) posterior surface of thigh mottled; (4) canthus rostralis concave in dorsal view; (5) Finger I approximately the same size as Finger II; (6) advertisement call duration 14.84–29.11 s; (7) dominant frequency 3.27–3.88 kHz; (8) 57–96 notes per call; (9) note repetition rate of 3.27–4.47 notes/s; (10) note repetition rate acceleration of 5–198%.

Description of the holotype.—Small size (SVL = 17.1 mm). Head longer than wide; head length 43% of SVL, head width 37% of SVL; snout rounded in dorsal and lateral views; nostrils rounded, oriented laterally, located near the tip of the snout; canthus rostralis moderately distinct, curved; loreal region slightly concave; postrictal tubercle present, slightly distinct; eyes protuberant and laterally oriented, eye diameter 28% of head length; tympanum distinct, rounded, tympanic membrane undifferentiated, annulus present, visible externally, tympanum diameter 48% of eye diameter; supratympanic fold absent; vocal slits present; vocal sac single, subgular, slightly expanded externally, with a longitudinal fold of skin from the posterior part to half of the throat on both sides; tongue large, heart-shaped, posterior notch absent; choanae elliptical; dentigerous processes of the vomer located posteromedially to choanae, triangle shaped, medially separated by a gap approximately the width of one dentigerous process, teeth present, six on the right an seven on the left dentigerous process.

Forelimbs slender; fingers slender, bearing discrete fringes, with small discs on Fingers I and II, larger discs on Fingers III and IV with a V-shaped median slit in dorsal view; finger lengths I ≈ II < IV < III; palmar tubercle heart-shaped, its diameter approximately equal to thenar tubercle; thenar tubercle elliptic; single nuptial pad apparently glandular, conspicuous, extending dorsally from the distal to the proximal portion of the metacarpus on Finger I, divided ventrally on the distal margin of the thenar tubercle, extending all over its caudal third; palm smooth, with one barely distinguishable supernumerary tubercle; single subarticular tubercles prominent, rounded, and large.

Hind limbs slender; shank longer than thigh, tibia length 70% of the SVL, thigh length 60% of SVL; calcar tubercle well developed, cone-shaped, as long as wide; tarsal fold absent; toes long, slender, fringed, with large discs on Toes II–V, which have a V-shaped median slit in dorsal view; small disc on Toe I; toe lengths I < II < III = V < IV; inner metatarsal tubercle elliptical, much larger than the rounded outer metatarsal tubercle; sole of the foot smooth, with one supernumerary tubercle; single large, prominent, and rounded subarticular tubercles.

Dorsal skin smooth; upper eyelid with a few barely distinguishable pointed tubercles and one distinct tubercle on each eyelid margin, positioned medially; venter smooth, with no tubercles; discoidal fold present; thoracic fold absent.

Coloration of the holotype in preservative.—Background variegated, predominantly light brown, with brown and grayish-white details; dorsum with medial clear whitish pinstripe from tip of snout to vent, with barely distinguishable X-shaped brown mark on its second third; head brown with light brown interocular bar and light brown spot bordered by two dark brown spots on tip of snout; dark brown loreal stripe from tip of snout to eyes, bordering canthus rostralis; dark brown lateral strip from right below eyes to upper lip; dark brown supratympanic stripe starting at tympanum, contouring arm, and reaching abdomen at midbody; inguinal region with dark brown spot; forelimbs variegated of brown with light brown with two dark brown blotches dorsally; palm of the hand brown and cream-colored; dorsal portion of hind limbs striped with brown and light brown alternately; dorsal surface of feet with three dark brown blotches; sole of feet brown; ventral portion of forelimbs cream-colored, with some dark brown dots mainly on posterior margin; hidden portion of the thigh cream-colored, mottled dark brown; external portion of tibia with dark brown longitudinal bar; venter cream-colored with some aggregations of brown dots on thorax; gular region cream-colored with brown dots spread throughout.

Measurements of the holotype (in millimeters).—Snout–vent length 17.1, head length 7.4, head width 6.3, eye

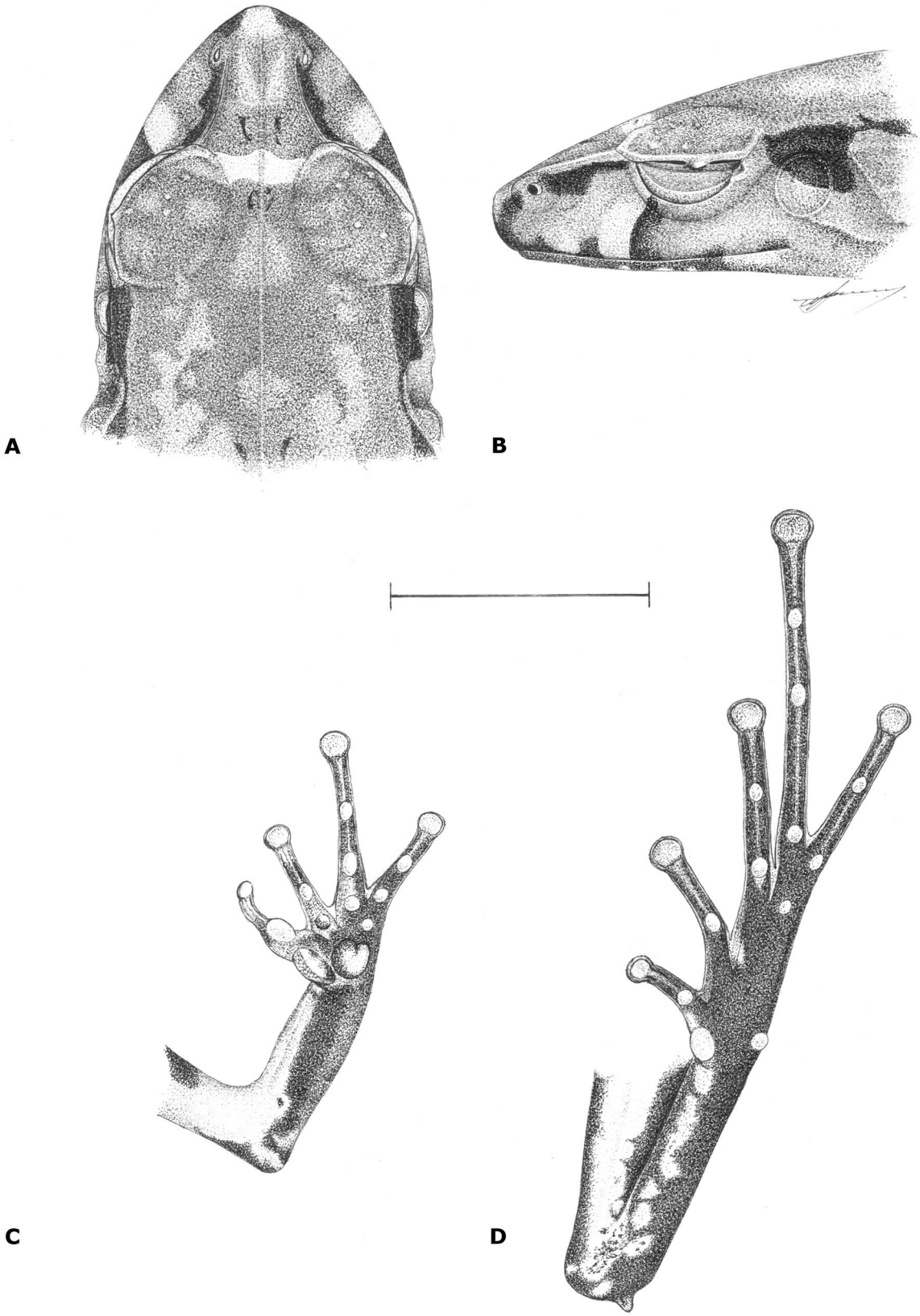

FIG. 8.—Holotype of *Ischnocnema garciai*, CFBH 39028: (A) dorsal and (B) lateral views of the head, (C) ventral view of the left hand, and (D) ventral view of the left foot. Scale bar = 5 mm.

TABLE 7.—Advertisement call parameters of four recorded males of *Ischnocnema garciai*. Data are given as a range (mean ± SD) where appropriate.

Call recording	PPGT 005	PPGT 006	PPGT 007	PPGT 008
Number of analyzed calls	1	1	5	5
Call duration (s)	29.11	20.89	14.84–19.14 (17.60 ± 1.64)	16.90–20.80 (19.20 ± 1.49)
Call rise time (%)	69	81	45–74 (61 ± 13)	62–92 (80 ± 12)
Dominant frequency (kHz)	3.88	3.45	3.27–3.36 (3.29 ± 0.04)	3.36–3.40 (3.396 ± 0.02)
Notes per call	96.00	79.00	57.00–83.00 (76.60 ± 11.08)	71.00–84.00 (78.60 ± 4.98)
Note rate (notes/s)	3.27	3.74	3.79–4.47 (4.29 ± 0.29)	3.90–4.15 (4.05 ± 0.10)
Note repetition rate acceleration (%)	108	19	85–198 (114 ± 47)	5–61 (40 ± 22)

diameter 2.0, tympanum diameter 1.0, eye–nostril distance 1.7, internarial distance 1.6, eye-to-eye distance 3.2, forearm length 3.7, hand length 5.0, third finger disk length 0.4, thigh length 10.3, tibia length 12.0, tarsal length 5.6, foot length 10.7, fourth toe disk length 0.7.

Variation.—One male specimen and the two female specimens had a subelliptical snout in dorsal view. Nostril shape was also triangular, elliptical, and ovoid. Tympanum was elliptic in two specimens and the postrictal tubercle could also be absent. The supratympanic stripe does not reach the abdomen at midbody in some specimens. Shape of the choanae varied between rounded and elliptical. Toe III could be slightly smaller or slightly larger than Toe V. Female specimens (SVL 21.9–24.7 mm, $n = 2$) were considerably larger than male specimens (SVL 13.3–18.5 mm, $n = 16$). Variation of measurements and body proportions are given in Table 4.

Etymology.—The specific epithet honors the Brazilian herpetologist Dr. Paulo C.A. Garcia (Laboratório de Herpetologia, Departamento de Zoologia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil) for his important contributions to the knowledge of the genus *Ischnocnema* and the amphibians of the Atlantic Forest and in gratitude for his substantial contribution to the academic education of the first author of this paper.

Advertisement call.—The advertisement call of *Ischnocnema garciai* ($n = 12$ calls of four males; Table 7; Fig. 3C) is composed of 57 to 96 notes ($\bar{X} = 79.25 \pm 9.09$), emitted sequentially, with the energy increasing in each note throughout the call, until reaching a peak typically at the beginning of the last third of the call. Most calls (ca. 80%) gradually decreased the energy until the end of the call after reaching the peak. Call duration ranged from 14.84 to 29.11 s ($\bar{X} = 19.50 \pm 3.47$) and call rise time ranged from 45 to 92% ($\bar{X} = 71 \pm 14$) of the call. Note repetition rate was 3.27–4.47 notes/s ($\bar{X} = 4.06 \pm 0.35$) and note repetition rate acceleration ranged 5–198% ($\bar{X} = 75 \pm 51$). Dominant frequency was 3.27–3.88 kHz ($\bar{X} = 3.40 \pm 0.16$).

Comparison with other species.—The long legs (tibia length/SVL = 64–72%) distinguish *Ischnocnema garciai* from the species of the *I. lactea* (tibia length/SVL usually < 50%; Hedges et al. 2008), *I. parva* (tibia length/SVL < 60%; Hedges et al. 2008; Brusquetti et al. 2013), and *I. verrucosa* (tibia length/SVL < 55%; Hedges et al. 2008; Canedo et al. 2010, 2012) series and from *I. sambaqui* (tibia length/SVL < 55%; Castanho and Haddad 2000). The large and conspicuous, glandular-appearing nuptial pad on Finger I distinguishes *I. feioi* from the species of the *I. lactea* (minute nuptial pad in *I. randorum*; translucent in *I. nigriventris* and *I. vizottoi*; reduced to some white granules in *I. holti*; absent in *I. melanopygia* and *I. spanios*; unknown in other species; Heyer 1985; Hedges et al. 2008; Targino and Carvalho-e-Silva 2008; Berneck et al. 2013) and *I. verrucosa* series (except for *I. surda*; faint, translucent nuptial pad in *I. karst*; absent in other species; Hedges et al. 2008; Canedo et al. 2010, 2012) and from *I. manezinho* and *I. sambaqui* (absent in these species; Garcia 1996; Castanho and Haddad 2000). The smooth dorsum distinguishes *I. garciai* from the species of the *I. verrucosa* series (dorsum tuberculate in these species; Hedges et al. 2008; Canedo et al. 2010, 2012), *I. manezinho* (finely tuberculate; Garcia 1996), and *I. sambaqui* (slightly rugose to rugose; Castanho and Haddad 2000).

Ischnocnema garciai differs from all species of the *I. guentheri* series, except for *I. oea* and *I. feioi*, by its calcar tubercle being at least as long as it is wide in adult specimens (absent or not as long as wide in other species).

By its smaller body size, *Ischnocnema garciai* (SVL in males 13.3–18.5 mm; females 21.9–24.7 mm) differs from *I. erythromera* (SVL in males 22.3–24.4 mm; females 24.3–35.3 mm; Heyer 1984), *I. feioi* (SVL in males 20.7–23.6 mm), *I. gualteri* (SVL in males 21.3–34.1 mm; females 33.6–45.7 mm; Heyer 1984), *I. henselii* (SVL in males 21.0–27.5 mm; females 28.4–38.4 mm; Kwet and Solé 2005), *I. izecksohni* (SVL in male 32.4 mm; females 43.5–49.0 mm; Caramaschi and Kisteumacher 1989 "1988") and *I. nasuta* (SVL in males 24.7–41.5 mm; females 36.1–53.9 mm; Heyer 1984).

By the mottled posterior surface of the thighs *Ischnocnema garciai* is distinguished from *I. erythromera* (*I. erythromera* with a light area on the posterior surface of the thigh in fixed specimens and red in life; Heyer 1984) and from *I. venancioi* (*I. venancioi* with clear spots surrounded by a dark background in fixed specimens and spots orange or yellow in life; B. Lutz 1958). Finger I being approximately the same size as Finger II also distinguishes *I. garciai* from *I. venancioi* (Finger I smaller than Finger II in *I. venancioi*). The concave canthus rostralis in dorsal view distinguishes *I. garciai* from *I. feioi*, *I. hoehnei, I. izecksohni*, *I. nasuta*, and *I. venancioi* (canthus rostralis straight in dorsal view in these species).

Advertisement call duration (14.84–29.11 s) distinguishes *Ischnocnema garciai* from *I. feioi* (1.54–5.51 s), *I. izecksohni* (1.03–2.15 s; Taucce et al. 2012), *I. nasuta* (1.15–1.50 s; Heyer 1984), and *I. oea* (4.56–8.49 s; Hepp and Canedo 2013). *Ischnocnema garciai* emits more notes per call (57–96) than *I. feioi* (10–27), *I. gualteri* (4–9; Heyer 1984), *I. oea* (25–42; Hepp and Canedo 2013), and *I. nasuta* (34–43; Heyer 1984). The higher dominant frequency (3.27–3.88 kHz) distinguishes *I. garciai* from *I. feioi* (2.53–3.23 kHz), *I. gualteri* (2.10–2.70 kHz; Heyer 1984), *I. henselii* (2.10–3.10 kHz; Gehara et al. 2013), *I. izecksohni* (2.25–2.63 kHz; Taucce et al. 2012), and *I. nasuta* (2.10–2.60 kHz; Heyer 1984). Note repetition rate distinguishes *I. garciai* (3.27–4.47

notes/s) from *I. henselii* (6.60–7.10 notes/s; Gehara et al. 2013), *I. izecksohni* (29.91–31.10 notes/s; Taucce et al. 2012), and *I. oea* (4.80–5.70 notes/s; Hepp and Canedo 2013).

Geographic distribution.—*Ischnocnema garciai* is known only from the type locality at Usina da Fumaça, municipality of Muriaé, state of Minas Gerais, Brazil (Fig. 6).

Remarks.—Except for advertisement call characters, we are not aware of any phenotypical difference between *Ischnocnema garciai* and *I. oea*, its sister species.

Discussion

Tree Topology and Genetic Distance

Unlike Canedo and Haddad (2012), we recovered the *Ischnocnema guentheri* series as poorly supported (61% of posterior probability and 55% of maximum likelihood bootstrap). This may be a result of the addition of *I. nanahallux* Brusquetti et al. 2013, because the two terminals representing this species in our tree had only the final portion of the 16S rRNA (600 bp) available, which represented only 16.7% of our final alignment. On the other hand, other *Ischnocnema* series and their phylogenetic relationships were recovered with high support, including the *I. guentheri* + *I. parva* series (100% of posterior probability and 93% of maximum likelihood bootstrap).

Fouquet et al. (2007) suggested a mean distance of 3% for 16S rDNA to identify neotropical anuran species. Our results show a genetic distance well above this threshold among almost all examined specimens, including those of *Ischnocnema oea, I. feioi*, and *I. garciai* (Table 3). The only exception is low distance between *I. nasuta* and *I. izecksohni* (1.2–1.9%). Although some authors have discussed the difficulties associated with using genetic distance thresholds to identify species (Padial et al. 2009), arguing that in some cases two distinct species may have a genetic distance as low as 0.0% in partial 16S rDNA (Blotto et al. 2013), the status of *I. nasuta* and *I. izecksohni* is remarkable, because the distance between them is less than the distance within *I. nasuta* itself. Since there are no known morphological characters distinguishing *I. izecksohni* and *I. nasuta* (Taucce et al. 2012), they may indeed be a single species. However, a study taking into account molecular and bioacoustical data from the type locality of *I. nasuta* (in Nova Friburgo, state of Rio de Janeiro, Brazil; A. Lutz 1925) and from throughout a greater part of the known distribution of the two species is necessary to make any taxonomic decision about their validity.

The *Ischnocnema guentheri* Series

Heyer (1984) proposed some diagnostic characters for what he called the *Ischnocnema guentheri* cluster, including a smooth dorsum, white glandular-appearing nuptial pads, and a noticeable calcar tubercle. Hedges et al. (2008) excluded the presence of a calcar tubercle and the nuptial pads, which they said were absent from *I. hoehnei* and unknown in other species of the *I. guentheri* series, and proposed a few other characters, such as an acuminate snout in dorsal view and Finger I approximately the same length as Finger II. Canedo et al. (2010) maintained this diagnosis and reincluded the presence of a nuptial pad. Canedo and Haddad (2012) excluded *I. vinhai* (=*Pristimantis vinhai*) and included *I. venancioi* in the *I. guentheri* series, and even with the inclusion of the latter (which was in the *I. lactea* series) they retained the character of having long legs (tibia length > 60%). Herein, we reformulate the diagnosis to include only characters shared by all members of the current *I. guentheri* series, including *I. epipeda*, *I. erythromera, I. feioi, I. garciai, I. gualteri, I. guentheri, I. henselii, I. hoehnei, I. izecksohni, I. nasuta, I. oea,* and *I. venancioi*: (1) long legs, tibia length > 60% of SVL; (2) large, whitish, glandular-appearing nuptial pads; and (3) dorsum smooth.

Heyer (1984) was the first to propose a group including the former *Eleutherodactylus guentheri* (=*Ischnocnema guentheri*) similar to the current *I. guentheri* series (see Introduction). Among the characters shared by all species in his cluster was a calcar tubercle on the heel and white glandular-appearing nuptial pads. At the time, Heyer (1984) considered only presence/absence character states, and although we have not noticed any remarkable difference in the nuptial pads of members of the *I. guentheri* series, we have found that the calcar tubercle is more developed in the clade containing *I. oea, I. feioi,* and *I. garciai*. Thus, we consider the character of having a calcar tubercle that is at least as long as it is wide a putative synapomorphy for this clade. Even though the development of the calcar tubercle is somewhat variable within the other species of the series, we also noted it is variable among species (Fig. 2), and is worthy of further investigation among members of the *I. guentheri* series. The only species lacking the calcar tubercle is *I. venancioi*.

In agreement with previous phylogenetic studies (Hedges et al. 2008; Canedo and Haddad 2012; Padial et al. 2014 [except by the tree-alignment + parsimony tree]), we recovered a clade including the *Ischnocnema guentheri* and the *I. parva* series. Despite important differences between the two series (see Results); there are a few important morphological features they share that may reinforce their close relationship.

Brusquetti et al. (2013) noted a well-developed calcar tubercle in *Ischnocnema nanahallux,* and stated that this feature is absent in *I. pusilla* and may be present or absent in *I. parva*. With exception of *I. venancioi*, all other members of the *I. guentheri* series possess the calcar tubercle. Also, *I. parva* and *I. pusilla* possess a large, whitish glandular-appearing nuptial pad, just like that of the members of the *I. guentheri* series. Nuptial pads are also present in *I. surda* (Canedo et al. 2010) and *I. karst* (faint, translucent in this species; Canedo et al. 2012) from the *I. verrucosa* series and in *I. randorum* (minute in this species; Hedges et al. 2008) from the *I. lactea* series. Further study of the morphology and the evolution of these characters within *Ischnocnema* is necessary in order to evaluate the homology of these characters between the *I. guentheri* and the *I. parva* series.

As a result of the present work, we have raised the number of species of the *Ischnocnema guentheri* series to 12. Although *I. feioi* is easily distinguishable from all other closely related species, *I. garciai* and *I. oea* seem to be morphologically cryptic species (see Bickford et al. 2007 for a cryptic species concept). The last *Ischnocnema* from the *I. guentheri* series described based only on morphological characters was *I. izecksohni* (Caramaschi and Kisteumacher 1989 "1988"). A few years later, Kwet and Solé (2005) resurrected *I. henselii* from the synonym of *I. guentheri*, based mainly on bioacoustical characters, and later on some

species had their advertisement calls described (Taucce et al. 2012; Gehara et al. 2013; Hepp and Canedo 2013). Gehara et al. (2013) also assessed molecular data throughout the geographic distribution of *I. guentheri* and *I. henselii* and concluded that *I. guentheri* is a species complex. In agreement with these recent studies involving the *I. guentheri* series, our results show that integrating different datasets is of paramount importance for evaluating the species limits within the series.

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Appendix I.—List of terminals and accession numbers of sequences taken from GenBank. Species names followed by an asterisk are reidentified taxa. RAG1 indicates nuclear recombination activating gene 1.

Species	RAG1 GenBank ID	Tyrosinase GenBank ID	12S–tVal–16S GenBank ID
Barycholos ternetzi	JX267543	JX267680	JX267466
Brachycephalus cf. *didactylus*	JX267544	JX267681	JX267389, JX267467
Brachycephalus ephippium	EU186761	—	AF375484
Craugastor daryi	EF493452	EF493480	EF493531
Eleutherodactylus cooki	EF493413	EF493455	EF493539
Haddadus binotatus	JX267548	JX267685	JX267391, JX267469
Hypodactylus dolops	EF493414	EF493483	EF493394
Ischnocnema abdita	JX267551	JX267687	JX267326, JX267472
Ischnocnema aff. *holti*	JX267554	JX267690	JX267336, JX267475
Ischnocnema bolbodactyla	JX267557	JX267692	JX267327, JX267476
*Ischnocnema henselii**	JX267563	JX267698	JX267328, JX267478
*Ischnocnema henselii**	JX267599	JX267734	JX267303
Ischnocnema cf. *holti*	JX267564	JX267699	JX267329, JX267479
Ischnocnema cf. *manezinho*	JX267566	JX267701	JX267335, JX267481
Ischnocnema cf. *nigriventris*	JX267568	JX267704	JX267398, JX267483
Ischnocnema cf. *penaxavantinho*	JX267574	JX267708	JX267298
Ischnocnema cf. *randorum*	JX267578	JX267799	JX267401, JX267361
Ischnocnema cf. *spanios*	JX267665	JX267805	JX267453, JX267536
Ischnocnema concolor	JX267594	JX267727	JX267413, JX267366
Ischnocnema concolor	JX267595	JX267728	JX267414, JX267493
Ischnocnema erythromera	—	JX267729	JX267340
Ischnocnema erythromera	JX267596	JX267730	JX267341
Ischnocnema aff. *guentheri**	JX267597	JX267731	JX267339, JX267494
Ischnocnema aff. *guentheri**	JX267602	JX267737	JX267417, JX267368
Ischnocnema aff. *guentheri**	JX267605	JX267740	JX267420, JX267370
Ischnocnema aff. *guentheri**	JX267606	JX267741	JX267421, JX267371
Ischnocnema guentheri	JX267611	JX267746	JX267331, JX267501, JX267502
Ischnocnema guentheri	JX267612	JX267747	JX267332, JX267503
Ischnocnema hoehnei	—	JX267749	JX267347
Ischnocnema hoehnei	JX267614	JX267750	JX267372
Ischnocnema hoehnei	JX267615	JX267751	JX267506
Ischnocnema hoehnei	JX267616	JX267752	JX267345, JX267507
Ischnocnema holti	JX267617	JX267754	JX267306
Ischnocnema izecksohni	JX267618	JX267755	JX267307
*Ischnocnema izecksohni**	JX267636	JX267774	JX267433, JX267375
Ischnocnema juipoca	JX267620	JX267757	JX267349
Ischnocnema lactea	JX267632	JX267769	JX267310, JX267518
Ischnocnema melanopygia	JX267634	JX267771	JX267431, JX267292
Ischnocnema nasuta	—	JX267772	JX267311
Ischnocnema nasuta	JX267637	JX267775	JX267434, JX267291, JX267520
Ischnocnema nanahallux	—	—	KC569985
Ischnocnema nanahallux	—	—	KC569986
Ischnocnema octavioi	JX267639	JX267777	JX267334, JX267521
Ischnocnema oea	JX267640	JX267778	JX267338
Ischnocnema oea	JX267641	JX267779	JX267313
Ischnocnema parva	JX267645	JX267783	JX267317
Ischnocnema parva	JX267646	JX267784	JX267435, JX267376
Ischnocnema parva	JX267649	JX267787	JX267438, JX267379
Ischnocnema parva	JX267650	JX267788	JX267439, JX267523
Ischnocnema parva	JX267653	JX267790	JX267442, JX267526
Ischnocnema parva	JX267656	JX267795	JX267445, JX267529
Ischnocnema parva	JX267657	JX267796	JX267446, JX267344, JX267530
Ischnocnema sambaqui	JX267661	JX267801	JX267449, JX267531
Ischnocnema spanios	JX267584	JX267717	JX267407, JX267490
Ischnocnema venancioi	JX267666	JX267806	JX267321
Ischnocnema venancioi	JX267667	JX267807	JX267454, JX267382
Ischnocnema verrucosa	JX267670	JX267810	JX267457, JX267538
Ischnocnema vizottoi	JX267672	JX267812	JX267350
Lynchius flavomaculatus	EU186745	EU186766	EU186667
Pristimantis ramagii	JX267658	JX267797	JX267318
Yunganastes mercedesae	—	—	FJ539071, FJ539066

APPENDIX II.—List of terminals and GenBank accession numbers for sequences generated in this study. Museum acronyms follow Sabaj (2016). RAG1 indicates nuclear recombination activating gene 1.

Species	Voucher no.	RAG1 Genbank ID	Tyrosinase Genbank ID	12S–tVal–16S Genbank ID
Ischnocnema feioi (holotype)	CFBH 35994	MF957146	MF957157	MF957167
Ischnocnema feioi	UFMG 17078	MF957147	MF957156	MF957165
Ischnocnema feioi	MZUFV 15712	MF957150	MF957160	MF957166
Ischnocnema garciai (holotype)	CFBH 39028	MF957148	MF957158	MF957170
Ischnocnema garciai	CFBH 39029	MF957149	MF957159	MF952878, MF957163
Ischnocnema garciai	UFMG 18889	—	—	MF957168
Ischnocnema garciai	UFMG 18890	—	—	MF957169
Ischnocnema aff. *guentheri*	UFMG 13906	MF957144	MF957154	MF952879, MF952883
Ischnocnema aff. *guentheri*	UFMG 13908	MF957145	MF957155	MF952880, MF952884
Ischnocnema aff. *guentheri*	CFBH 41853	MF957141	MF957151	MF957164
Ischnocnema aff. *guentheri*	CFBH 39282	MF957143	MF957153	MF952877, MF957162, MF952881
Ischnocnema oea	CFBH 12394	MF957142	MF957152	MF952876, MF957161, MF952882

APPENDIX III

Specimens Examined

Ischnocnema epipeda.—BRAZIL: ESPÍRITO SANTO: Santa Teresa (MNRJ 1874 *Eleutherodactylus epipedus* paratype).

Ischnocnema erythromera.—BRAZIL: RIO DE JANEIRO: Santa Maria Magdalena: Parque Estadual do Desengano (CFBH 28111–28115); Teresópolis (CFBH 27349, 40985).

Ischnocnema guentheri.—BRAZIL: RIO DE JANEIRO: Rio de Janeiro: Floresta da Tijuca (CFBH 26989–26994, 27440, 27442–27444, MNRJ 31666, 36483, 87540–87541, 87544–87545, 87548).

Ischnocnema henselii.—BRAZIL: PARANÁ: Arianópolis (CFBH 27470–27471); Piraquara (CFBH 11039–11040). SANTA CATARINA: Anitápolis (CFBH 9367–9368); São Bonifácio (CFBH 27549–27554). SÃO PAULO: São Bernardo do Campo (CFBH 12298); Tapiraí (CFBH 23298).

Ischnocnema hoehnei.—BRAZIL: SÃO PAULO: Pilar do Sul (CFBH 8336); Santo André: Paranapiacaba (CFBH 29043).

Ischnocnema izecksohni.—BRAZIL: MINAS GERAIS: Aiuruoca (CFBH 36919–36920); Alto Caparaó: Parque Nacional do Caparaó (CFBH 40977–40980); Belo Horizonte (MNRJ 4217 *Eleutherodactylus izecksohni* holotype, MNRJ 4218–4219 *Eleutherodactylus izecksohni* paratypes); Conceição do Ouro (CFBH 39908–39910); Muriaé (CFBH 35990–35991, 39016, 39020–39021, 39039); Ouro Preto: Rodrigo Silva (CFBH 35793, 35796–35799).

Ischnocnema nasuta.—BRAZIL: RIO DE JANEIRO: Nova Friburgo (CFBH 40981–40984); Macaé de Cima (MBML 212).

Ischnocnema oea.—BRAZIL: ESPÍRITO SANTO: Cariacica: Reserva Biológica de Duas Bocas (CFBH 22517–22518, 22520); Santa Teresa (MNRJ 1244 *Eleutherodactylus oeus* holotype, UFMG 13735–13738, USNM 235612 *Eleutherodactylus oeus* paratype); Santa Teresa: Reserva Biológica Augusto Ruschi (CFBH 24778–24779, 30732, 40987); Santa Teresa: São Lourenço (CFBH 10815–10816, 10876–10877, 27090–27091, 37242); Vargem Alta (CFBH 25050, 27013).

Ischnocnema cf. *oea*.—BRAZIL: RIO DE JANEIRO: Cambuci (MNRJ 49504–49506).

Ischnocnema venancioi.—BRAZIL: RIO DE JANEIRO: Nova Friburgo (CFBH 27435); Teresópolis (CFBH 40986).

APPENDIX IV.—Call records analyzed.

Call ID	Voucher	Species	Locality	Recorder
PPGT 001	CFBH 35994	*Ischnocnema feioi*	Lar dos Muriquis, Muriaé, Minas Gerais, Brazil	Marantz PMD-661
PPGT 002	CFBH 35994	*I. feioi*	Lar dos Muriquis, Muriaé, Minas Gerais, Brazil	Marantz PMD-661
PPGT 003	MZUFV 15712	*I. feioi*	Careço, Ervália, Minas Gerais, Brazil	Marantz PMD-660
PPGT 004	unvouchered	*I. feioi*	Careço, Ervália, Minas Gerais, Brazil	Marantz PMD-660
CBUFMG 916	UFMG 3285	*I. feioi*	Parque Estadual da Serra do Brigadeiro, Araponga, Minas Gerais, Brazil	Marantz PMD-660
CBUFMG 917	UFMG 17028	*I. feioi*	Parque Nacional do Caparaó, Santa Marta, Espírito Santo, Brazil	Tascam DR-40
PPGT 005	CFBH 39028	*I. garciai*	Usina da Fumaça, Muriaé, Minas Gerais, Brazil	Marantz PMD-660
PPGT 006	CFBH 39029	*I. garciai*	Usina da Fumaça, Muriaé, Minas Gerais, Brazil	Marantz PMD-661
PPGT 007	unvouchered	*I. garciai*	Usina da Fumaça, Muriaé, Minas Gerais, Brazil	Marantz PMD-661
PPGT 008	CFBH 39031	*I. garciai*	Usina da Fumaça, Muriaé, Minas Gerais, Brazil	Marantz PMD-661
MNVOC 043:1	unvouchered	*I. oea*	Reserva Biológica Augusto Ruschi, Santa Teresa, Espírito Santo, Brazil	Marantz PMD-660
MNVOC 043:2	CFBH 24778	*I. oea*	Reserva Biológica Augusto Ruschi, Santa Teresa, Espírito Santo, Brazil	Marantz PMD-660
MNVOC 043:3	unvouchered	*I. oea*	Reserva Biológica Augusto Ruschi, Santa Teresa, Espírito Santo, Brazil	Marantz PMD-660