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**Taxonomic review of *Scinax fuscomarginatus* (Lutz, 1925) and related species
(Anura; Hylidae)**

Francisco Adolfo Brusquetti Estrada

Dissertação apresentada ao
Instituto de Biociências do Câmpus
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Mestre em Ciências Biológicas (Zoologia)**

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2011**

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RESUMO

Scinax fuscomarginatus, *S. parkeri*, *S. trilineatus* e a recentemente descrita *S. lutzorum* são espécies muito similares morfológicamente que ocorrem em formações abertas da América do Sul ao leste dos Andes. *Scinax parkeri* e *S. trilineatus* tiveram sua validade taxonômica questionada enquanto *S. fuscomarginatus* é considerada por vários autores como um complexo de espécies. Levando em conta a similaridade morfológica e a incerteza taxonômica, nesse trabalho revisamos a taxonomia de *Scinax fuscomarginatus*, *S. trilineatus*, *S. parkeri* e *S. lutzorum* usando dados de morfologia externa, cantos de anúncio e sequências moleculares. Os principais objetivos foram investigar a existência de outras espécies sob o nome *S. fuscomarginatus* e avaliar o "status" taxonômico de *S. parkeri*, *S. trilineatus*, *S. lutzorum* e *Hyla madeirae* (único sinônimo de *S. fuscomarginatus*). Nas análises filogenéticas com dados moleculares encontramos uma profunda e rica estrutura genética tanto intra quanto inter-específica. As amostras incluídas formaram um grupo monofilético bem suportado que está estruturado em vários clados e subclados. *Hyla madeirae* e a população da Serra do Cachimbo correspondem a subclados distintos enquanto as espécies atualmente válidas (*S. fuscomarginatus*, *S. parkeri*, *S. trilineatus* e *S. lutzorum*) se agrupam em um único clado. Este clado apresenta subestrutura marcada, mas a concordância entre subclados e as espécies reconhecidas é limitada. A alta variação intraespecífica e intrapopulacional encontrada nos caracteres morfológicos usados para a diagnose original das espécies não permitiu a diferenciação entre *S. parkeri*, *S. trilineatus*, *S. lutzorum* e *S. fuscomarginatus*. Entretanto, alguns caracteres permitem a distinção de *Hyla madeirae* e dos espécimens da Serra do Cachimbo. De forma semelhante, nenhuma das espécies consideradas válidas foram distinguíveis em análises morfométricas, apenas *Hyla madeirae* e os espécimens da Serra do Cachimbo apresentam uma tendência a apresentar a cabeça mais larga. O canto de anúncio de todas as espécies consideradas apresenta a mesma estrutura: uma nota multi pulsionada de frequência modulada com taxas de repetição variável, e coincide com os resultados encontrados nos dados moleculares e morfológicos. Com base nas múltiplas evidências encontradas, propomos a sinonímia de *S. trilineatus*, *S. parkeri* e *S. lutzorum* com *S. fuscomarginatus*; a revalidação de *Hyla madeirae* sob a nova combinação *Scinax madeirae*, e descrevemos a população da Serra do Cachimbo como uma nova espécie.

ABSTRACT

Scinax fuscomarginatus, *S. parkeri*, *S. trilineatus* and the recently described *S. lutzorum* are morphologically very similar species that occur in open formations of South America east of the Andes. *Scinax parkeri* and *S. trilineatus* had its taxonomic validity questioned, and *S. fuscomarginatus* is considered by many authors as a species complex. Given the morphological similarity and taxonomic uncertainty, we review the taxonomy of *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum* using data from external morphology, advertisement calls and molecular sequences. The main goals were to investigate the existence of other species under the name *S. fuscomarginatus* and assess the taxonomic status of *S. parkeri*, *S. trilineatus*, *S. lutzorum* and *Hyla madeirae* (the only synonym within *S. fuscomarginatus*). In the phylogenetic analysis obtained with the molecular data we found rich and deep genetic structure both within and between species. The samples included formed a well supported monophyletic group that is divided into several clades and subclades. The specimens of *Hyla madeirae* and those from Serra do Cachimbo corresponded to distinct subclades while the currently valid species (*S. fuscomarginatus*, *S. parkeri*, *S.* and *S. trilineatus lutzorum*) were grouped into a single clade. This clade showed marked substructure, but the concordance of the recognized species to subclades were limited. The high intrapopulation and intraspecific variation found in morphological characters used for the original diagnoses did not allow us to differ among *S. parkeri*, *S. trilineatus*, *S. lutzorum* and *S. fuscomarginatus*. However, some characters support the distinction of *Hyla madeirae* and specimens from Serra do Cachimbo. Similarly, none of valid species were distinguishable in morphometric analysis while *Hyla madeirae* and the specimens from Serra do Cachimbo showed a trend in having wider heads. The advertisement call of all species has the same structure: a unique modulated frequency multipulsed note with variable repetition rates, coinciding with the findings in molecular and morphological data. Based on multiple evidence, we propose the synonymy of *S. trilineatus*, *S. parkeri* and *S. lutzorum* with *S. fuscomarginatus*; the revalidation of *Hyla madeirae* under the new combination *Scinax madeirae*, and describe the specimens from Serra do Cachimbo as a new species.

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INTRODUCTION

The genus *Scinax* Wagler, 1830 occurs from eastern and southern Mexico to Argentina and Uruguay, including Trinidad and Tobago, and St. Lucia (FROST, 2011). This widespread distribution along with the ability to occupy several types of habitats resulted in a high diversity. *Scinax* is currently the largest genus in the family Hylidae with 102 recognized species (FROST, 2011), and new species are frequently described (e.g. 12 new species described in the last five years; see FROST, 2011). The last phylogenetic assessment of the relationships in this genus revealed two main large clades, the *S. catharinae* clade, including the *S. catharinae* and *S. perpusillus* groups, and the *S. ruber* clade, including the *S. rostratus* and *S. uruguayus* groups, with 43 species not being assigned to any of the previous groups (FAIVOVICH et al., 2005). *Scinax fuscomarginatus* (A. LUTZ, 1925) is one of the unassigned species, along with other morphologically similar species: *S. parkeri* (GAIGE, 1929), *S. trilineatus* (HOGMOED and GORZULA, 1979), and the recently described *S. lutzorum* Cardoso and Pombal, 2010. *Scinax parkeri* and *S. trilineatus* have had their taxonomic validity questioned. *Scinax parkeri* was considered a junior synonym of *S. fuscomarginatus* by B. Lutz (1973), being posteriorly revalidated by Duellman and Wiens (1992) and De la Riva et al. (1997). *Scinax trilineatus* was suggested as synonym of *S. fuscomarginatus* by Martins (1998) but the explanation for this proposal was not exposed. Currently, only *Hyla madeirae* Bokermann, 1964 is considered a synonym of *S. fuscomarginatus* (B. LUTZ, 1973).

Scinax fuscomarginatus has a widespread distribution across the South American continent east of the Andes. In the original description A. Lutz (1925) did not designate type specimens, citing São Paulo and Belo Horizonte as localities included in the distribution of this species. Latter, B. Lutz (1973) defined Belo Horizonte, in the state of Minas Gerais, Brazil, as the type locality. The name *S. fuscomarginatus* was subsequently used for populations occurring from northern Argentina to north and northeastern Brazil, registered in open formations of the Pantanal, Cerrado, Humid Chaco, and Chiquitanía (sensu DINERSTEIN et al., 1995), and in enclaves of open habitat within the Atlantic Forest and Amazonia (BRASILEIRO et al., 2005; TOLEDO and HADDAD, 2005a; b; HADDAD et al., 2008; FREITAS and SILVA, 2007; LEITE et al., 2008; DE LA RIVA et al., 2000; BRUSQUETTI and LAVILLA, 2006).

While the wide distribution of *Scinax fuscomarginatus* covers open areas across the continent, the related species have more restricted ranges. *Scinax parkeri* was described from Buena Vista in the department of Santa Cruz de la Sierra, Bolivia. The distribution of *S. parkeri* is not well documented, with this uncertainty being probably related to misidentifications regarding *S. fuscomarginatus* (DE LA RIVA et al., 2000). Nevertheless, the minimal distribution expected for *S. parkeri* includes eastern Bolivia and Mato Grosso do Sul, Brazil (FROST, 2011), and probably adjacent Paraguay (BRUSQUETTI and LAVILLA, 2006). *Scinax trilineatus* was described from El Manteco, Estado Bolívar, Venezuela and is known from lowlands of Venezuela, Guyana and Surinam, and adjacent Brazil (FROST, 2011). Finally, the recently described *S. lutzorum* is known only from the type locality in the state of Tocantins, Brazil.

Several authors have noted variation in call among populations of *Scinax fuscomarginatus*. Toledo and Haddad (2005) described differences in dominant frequency and call duration between populations from Estação Ecológica de Itirapina and Ribeirão Branco (POMBAL et al., 1995), both localities in São Paulo state, Brazil, distant approximately 230 kilometers. Bueno (2001) and Bueno et al. (2002) reported variations in the call structure from different populations of *S. fuscomarginatus* of central Brazil. Morphological variation were also reported; Cardoso and Pombal (2010) found the snout, inner and outer metatarsal tubercle and vocal sac shapes to vary throughout their sample of *S. fuscomarginatus*, suggesting that it is a species complex.

Taking into account the morphological similarity and the taxonomic uncertainty of these species, we here review the taxonomy of *Scinax fuscomarginatus* and the related *S. trilineatus*, *S. parkeri*, and *S. lutzorum* using external morphology, advertisement calls, and molecular sequence data. Our specific aims are i) to investigate the existence of other species being misidentified under the name *S. fuscomarginatus*, and ii) to assess the taxonomic status of *S. parkeri*, *S. trilineatus*, *S. lutzorum*, and *Hyla madeirae*.

TAXONOMIC HISTORY

A. Lutz (1925) described *Scinax fuscomarginatus* as *Hyla fuscomarginata* in a short paragraph without reference material, citing the provenance of the specimens as "S. Paulo" (São Paulo, state of São Paulo, Brazil) and "Bello Horizonte" (Belo Horizonte, state of Minas Gerais, Brazil), presumably referring to the species distribution. A year later, the same author (A. LUTZ, 1926) presented english and portuguese versions for this work (the original was written in french) without adding data to the original description and including "Rio" (Rio de Janeiro, state of Rio de Janeiro, Brazil) as the specimens provenance.

Cochran (1955) described an adult male specimen collected in Bello Horizonte (Belo Horizonte), state of Minas Gerais, Brazil, and deposited in the National Museum of Natural History, Washington, United States (USNM 96964) as cotype of *Scinax fuscomarginatus* (Fig. 1: A, B, C). The author also included material from São Paulo (Brazil) and Rio de Janeiro, including a specimen from Amorim (Rio de Janeiro) previously designed by the same author as paratype of *S. similis* (USNM 96213; COCHRAN, 1952). Cochran's description of *S. fuscomarginatus* is well detailed but does not include diagnosis or comparisons with other species, only a comment regarding the similarity of the head and snout shape with *Hyla wernerii* Cochran, 1952 (currently in the genus *Dendropsophus*). Figures of two specimens are included in this work (COCHRAN, 1955; Plate 11, Figures I-K); the first corresponding to a specimen from Recreio dos Bandeirantes, state of Rio de Janeiro, Brazil (USNM 97613) and the second to a cotype (USNM 96964). Judging from the figures and pictures of these exemplars, the specimen from Rio de Janeiro does not correspond to *S. fuscomarginatus*, being apparently related to *S. alter* (Fig. 1: D, E, F).

Bokermann (1966) stated that the real problem with the type locality of *Scinax fuscomarginatus* is related to the correct identification of this species, pointing that *S. fuscomarginatus* is a common species in the Brazilian southeastern coast and that it is not found in Belo Horizonte or in the São Paulo city. However, Bokermann (1966) was referring to a large series of specimens of *S. cf. alter* deposited in the Museu de Zoologia, Universidade São Paulo (MZUSP), determined by himself as *Hyla fuscomarginata* (F. BRUSQUETTI personal observation).

The first formal diagnosis of *Scinax fuscomarginatus* was presented by B. Lutz (1973), who designed the specimens ALMN 845, 846 and USNM 96964 collected in Belo Horizonte, state of Minas Gerais, Brazil, as syntypes. In addition to the diagnosis, a

description was presented based in the two syntypes deposited at the Adolpho Lutz collection (ALMN 845 and 846, currently housed in the Museu Nacional, Rio de Janeiro). Specimens from the states of Minas Gerais (Belo Horizonte, Vespasiano, and Lagoa Santa), Bahia (Salvador) and Goiás (Lagoa Formosa) were also identified as *S. fuscomarginatus*, but without references to vouchers. B. Lutz (1973), in this same work, proposed the species *Hyla parkeri*, *H. lindneri* Miller and E. Ilmich, 1936 and *H. madeirae* as synonyms of *Scinax fuscomarginatus*. Later, *H. parkeri* was revalidated (DUELLMAN and WIENS, 1992; DE LA RIVA et al., 1997) and *H. lindneri* considered as junior synonym of *S. squalirostris* (GALLARDO, 1961; LAVILLA and CEI, 2001), being *H. madeirae* currently the only synonym of *S. fuscomarginatus*.

Hyla madeirae was described by Bokermann (1964) based on specimens from Porto Velho, state of Rondônia, Brazil. With the exception of one paratype deposited in the MNRJ, all the type series is housed at the MZUSP. In its original description the author mentioned the affinities with *Scinax parkeri* (as *H. parkeri*) from which it differs by the wider head and more prominent snout. B. Lutz (1973), arguing that the characters used by Bokermann to diagnose *H. madeirae* from *S. parkeri* are not valid, proposed *H. madeirae* as synonym of *Scinax fuscomarginatus*. B. Lutz (1973) noted, however, that the specimens WCAB 10771 and MNRJ 3963 (paratypes of *H. madeirae*) present differences in the color pattern of dorsolateral stripes. Finally, Duellman and Wiens (1992) examined topotypes of *Hyla madeirae* (KU 92066-68) in their revision of *Scinax*, considering it as a synonym of *S. fuscomarginatus*.

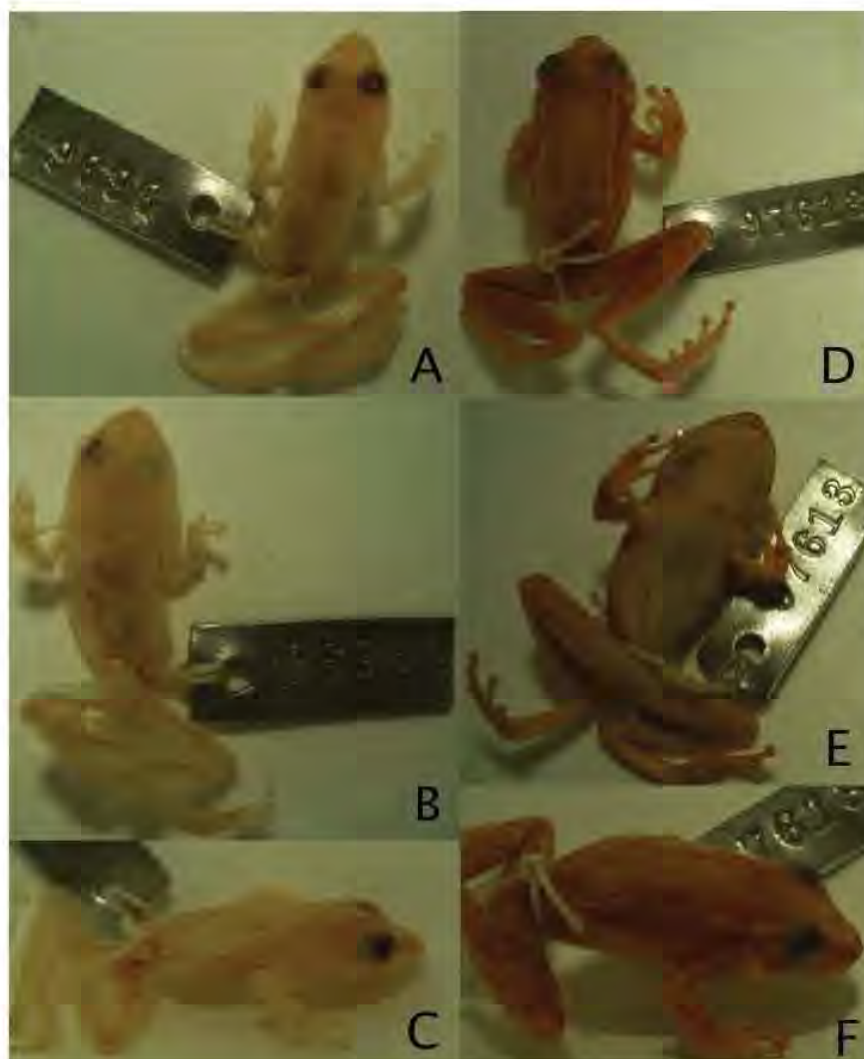


Figure 1. Specimens analyzed by Cochran (1955; Plate 11, Figures I-K). A), B), C): dorsal, ventral, and lateral views of *Scinax fuscomarginatus* (cotype described by Cochran, 1955. USNM 96964. Belo Horizonte, Minas Gerais, Brazil); D), E), F): dorsal, ventral, and lateral views of *Scinax* cf. *alter* (as *Hyla fuscomarginata* in Cochran, 1955, USNM 97613, Recreio dos Bandeirantes, Rio de Janeiro, Brazil). Photograph by Bianca Berneck.

Scinax parkeri was described as *Hyla parkeri*, being the holotype from Buena Vista, department of Santa Cruz de la Sierra, Bolivia and is currently housed at the University of Michigan Museum of Zoology (UMMZ 67460) (GAIGE, 1929). *Scinax parkeri* was considered as junior synonym of *S. fuscomarginatus* by B. Lutz (1973) and revalidated by Duellman and Wiens (1992). De la Riva et al. (1994) considered *S. parkeri* as a valid species following Duellman and Wiens (1992) and described the advertisement call of one specimen from the type locality. Few years later the same authors (DE LA RIVA et al., 1997), based on bioacoustical parameters of the call previously described from Bolivia (DE LA RIVA et al., 1994) and of calls from Belém, state of Pará, Brazil (DUELLMAN and PYLES, 1983) and

Campinas, state of São Paulo, Brazil (CARDOSO, 1981), concluded that *S. parkeri* is a valid species but without any formal proposal.

Scinax trilineatus was described from a locality 12 km SE from El Manteco, state of Bolívar, Venezuela, as *Ololygon trilineata* (HOOGMOED & GORZULA, 1979). The description was based on the holotype, a female specimen deposited in the Naturalis (Nationaal Natuurhistorisch Museum; formerly Rijksmuseum van Natuurlijke Historie) (RMNH 18257). Two male paratypes from the type locality (RMNH 18258-9) and one subadult (RMNH 18260) from Sipalini, Suriname, are also deposited in the RMNH, with the last specimen being the only record known for this country. All paratypes housed in the American Museum of Natural History are from localities of southern Guyana: Isherton (AMNH 43637), Rupununi River (AMNH 46248), and Parabam (97944-48). Martins (1998) cited *S. fuscomarginatus* (as *Hyla fuscomarginata*) for Ilha de Maracá, state of Amapá, Brazil, and commented that the specimens from Venezuela, described as *Ololygon trilineatus* by Hoogmoed and Gorzula (1979), belong to *S. fuscomarginatus* but without explaining this assumption.

Recently, Cardoso and Pombal (2010) described *Scinax lutzorum*, a species closely related to *S. fuscomarginatus*, *S. parkeri*, and *S. trilineatus*. The authors noted that the specimens ALMN 845-50 are recorded as syntypes of *S. fuscomarginatus* in the catalogue of Adolpho Lutz collection and designated the specimen ALMN 845 as lectotype, and part of the specimens until now recognized as syntypes (ALMN 847-850 and USNM 96964) as paralectotypes. The authors stated that the specimen ALMN 846 do not correspond to *S. fuscomarginatus* but probably to *Dendropsophus rubicundulus*.

Fouquette and Delahousaye (1977) based on sperm type and external morphology defined five groups for the genus *Scinax* (as *Ololygon*): *Scinax ruber*, *S. catharinae*, *S. rostratus*, *S. staufferi*, and *S. x-signatus* groups, placing *S. fuscomarginatus* in the *S. staufferi* group along with *S. staufferi*, *S. nasicus*, *S. squalirostris*, *S. parkeri*, *S. baumgardneri*, and *S. wandae*, and *S. berthae* and *S. goinorum* as probable members. Duellman and Wiens (1992) added to the group *S. agilis*, *S. danae*, *S. exiguus*, and *S. trilineatus*. Faivovich (2002) did not recognize the *S. staufferi* group, and Faivovich et al. (2005) considered the species formerly included in this group as members of the *Scinax ruber* clade without assigning their species to particular groups.

MATERIAL AND METHODS

Taxon sampling and material examined

B. Lutz (1973) based on the type material provenance, defined Belo Horizonte, Minas Gerais, Brazil as the type locality of *Scinax fuscomarginatus*. However, this material was collected in 1924, since then, the city of Belo Horizonte has grown significantly and there are no new records of the species in that city. Taking this into account, we consider the material from Lagoa Santa, state of Minas Gerais, Brazil as topotype due to its proximity to the type locality, about 35 km from Belo Horizonte.

For molecular analyses we included tissue samples of 263 individuals from 72 localities (Figure 2, appendix 1), being two from Argentina, six from Paraguay, four from Bolivia, one from Venezuela and 59 from Brazil. Aiming to cover all the described species we analyzed samples of *Scinax fuscomarginatus* (including topotypes), *S. parkeri* (including topotypes), and *S. trilineatus*. We also included topotypical material of *Hyla madeirae* (synonym of *S. fuscomarginatus*). For *Scinax lutzorum* we analyzed material from localities approximately 35 km distant from the type locality. We selected outgroups following morphological and molecular evidence provided in Faivovich (2002) and Faivovich et al. (2005). According to these authors, *S. fuscomarginatus* is in the *S. ruber* clade and present morphological affinities with *S. cruentommus*, *S. staufferi*, *S. squalirostris*, and *S. nasicus*. Therefore, we included *S. nebulosus* from the *S. rostratus* group (*S. ruber* clade), four other species from the *S. ruber* clade not assigned to any groups (*S. acuminatus*, *S. exiguus*, *S. squalirostris*, and *S. nasicus*), and to root the trees we selected *S. berthae* from the *S. catharinae* group in the *S. catharinae* clade.

For morphological analyses, quantitative and qualitative, we included 540 specimens (appendix 2). We analyzed type series and new topotypical material of *Scinax fuscomarginatus* and *Hyla madeirae* and topotypical material of *S. parkeri*. The material of *S. trilineatus* is from 300 km S of its type locality, and those of *S. lutzorum* 35 km from its type locality. We included pictures of the type series of *S. parkeri*, *S. trilineatus*, and *S. lutzorum* in qualitative comparisons.

For call analyses we used call records of 60 males from 26 localities including three of the four valid species and *Hyla madeirae*. We analyzed calls obtained from the type localities

of *Scinax fuscomarginatus*, *S. parkeri*, and *H. madeirae*; the recordings of *S. trilineatus* correspond to a locality 300 km S of the type locality (appendix 3).



Figure 2. Samples localities for molecular analyses. In red types localities sampled: circle *Scinax fuscomarginatus*; square *Hyla madeirae*; triangle *Scinax parkeri*. In blue type localities not sampled: circle *Scinax trilineatus*, square *Scinax lutzorum*.

Vouchers and examined material are deposited in the following collections:

- Amphibian Collection Célio F. B. Haddad, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil (CFBH);
- Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ);
- Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP);
- Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina (MACN);
- Herpetology Collection of the Universidade Nacional de Brasília, Brasília, Brazil (CHUNB);
- Herpetology Collection of the Instituto de Investigación Biológica del Paraguay, Asunción, Paraguay (IIBP-H);
- Museo Nacional de Historia Natural del Paraguay, Asunción, Paraguay (MNHNP);
- Diego Baldo collection housed in the Museo de La Plata, La Plata, Argentina (MLP-DB);
- Zoological Collection of the Universidade Federal de Mato Grosso, Cuiabá, Brazil (UFMT);
- Zoological Collection of the Universidade Federal de Goiás, Goiás, Brazil (ZUFG);
- Zoological Collection of the Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil (ZUFMS);
- Museu de Zoologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil (ZUEC-AMP);
- Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (MCNAM);
- Museo Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia (MNKA).

Call records are deposited in the sound collections of the following institutions:

- Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales de Madrid, Madrid, España;
- Sound collection Célio F. B. Haddad (CFBH sound) housed at Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil;
- Sound collection of Instituto de Investigación Biológica del Paraguay (IIBP), Asunción, Paraguay;
- Sound collection of Museu de Zoologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil (ZUEC-AMP).

Molecular methods

We extracted total genomic DNA from samples conserved in ethanol 100 % (muscle or liver) using the DNeasy extraction kit (Qiagen, Valencia, CA, USA), following the manufacturers protocol. We used specific primers to amplify mitochondrial fragments from the genes Cytochrom C Oxidase subunit 1 (hereafter referred to as CO1), NADH Dehydrogenase subunit 2 (hereafter ND2) and 16S ribosomal RNA gene (hereafter 16S) (Table 1).

Table 1: Primers, sequenced fragments, primer sequences, and bibliographic reference.

Primer	Fragment	Sequence	Reference
ANF1	CO1	ACHAAYCAYAAAGAYATYGG	M. Lyra (unpublished)
ANR1	CO1	CCRAARAATCARAADARRTGTTG	M. Lyra (unpublished)
ND2_L4646	ND2	ATTGAAGCCGCCACAAAATA	Austin (2008)
ND2_H5540	ND2	TTTAGGGCTTTGAAGGC	Macey <i>et al.</i> (1997)
ND2_L4437	ND2	AAGCTTTCGGGCCCATACC	Macey <i>et al.</i> (1997)
ND2intHalbo2	ND2	GTCTAATTTATCCTAAGTTTC	Prado <i>et al.</i> , (submetido)
16S_AR	16S	CGCCTGTTTATCAAAAACAT	Palumbi <i>et al.</i> (1991)
16S_BR	16S	CCGGTCTGAACTCAGATCACGT	Palumbi <i>et al.</i> (1991)

We amplified the fragments with polymerase chain reactions (PCR) using a commercial kit (Master Mix, Fermentas). For CO1 and ND2 we used the following PCR program: initial denaturation step at 94°C (5 min), 35 cycles consisting of 94°C (1 min)

denaturation, 45°C (CO1) and 47-57°C (ND2) (1 min) annealing, 72°C (1 min) extension, and a final extension step at 72°C (5 min). For 16S we applied an initial denaturation step at 94°C (3 min), 35 cycles consisting of 94°C (30 s) denaturation, 50°C (30 s) annealing, 72°C (90 s) extension, and a final extension step at 72°C (7 min).

We assessed the size of the amplified fragments and relative concentration by electrophoresis in 1 % agarose gel using Ethidium Bromide to stain in the initial tests, and “Blue Green” for the following amplifications. PCR products were sent to Macrogen Company (Korea) for sequencing in both directions (forward and reverse) using Bigdye terminator chemistry. Sequencing products were purified by precipitation (ethanol) and sequenced using automatic ABI capillary sequencers 3730XL (<http://www.macrogen.com/eng/sequencing/automatic.jsp>).

Alignment and phylogenetic analyses

We checked chromatograms and edited the sequences using CodonCode Aligner (version 3.5.4). Sequences from each fragment were aligned separately with Clustal X (THOMPSON et al., 1997) in Bioedit (HALL, 1999) and alignments were corrected by eye.

For construction of phylogenetic trees we used Maximum Likelihood (ML) and Bayesian Inference (BI) concatenating the three mitochondrial fragments. We performed ML in RAxML (STAMATAKIS, 2006) using the RAxML-GUI interface (SILVESTRO and MICHALAK, 2010). We partitioned the datasets by gene and by codon positions (in protein coding fragments) resulting in 7 partitions as follows: CO1 codon positions 1, 2, 3; ND2 codons positions 1, 2, 3, and 16S. We performed 10 runs with 1000 thorough bootstrap replicates. We used the “General Time-reversible” nucleotide substitution model with gamma heterogeneity rates and a proportion of invariable sites (GTRGAMMAI) for all partitions.

Prior to BI analyses we partitioned the CO1 and ND2 fragments into codons (as described above) to estimate the evolutionary model that best fit each partition. We used the program JModeltest 0.1.1 (POSADA, 2008) and selected models under the AIC criterion (AKAIKE, 1973).

We constructed BI trees in Mr Bayes 3.1 (RONQUIST and HUELSENBECK, 2003), implementing the selected model of nucleotide substitution for each particular partition. We used two independent runs, each with four chains sampling every 1000 generations for 30 million generations, keeping all priors under default settings. We verified convergence among

runs by examining the standard deviation of split frequencies between independent runs, by checking effective sample sizes and by plotting the $-\ln L$ per generation using Tracer 1.4.1 (Rambaut and Drummond, 2007), and the first 7500 trees were discarded as burn-in.

We considered L Bootstrap values $\geq 70\%$ to indicate strongly supported clades (HILLIS and BULL, 1993). For BI we considered strongly supported the clades with posterior probabilities above 0.95, considering that short internodes with relatively high posterior probabilities (particularly those with low bootstrap values) could result from over-estimates of confidence (ALFARO et al., 2003; ERIXON et al., 2003).

We also calculated the uncorrected p -distance of the ribosomal 16S gene fragment inside and between the main clades and subclades, expressed as the number of nucleotide differences per site (YANG, 2006) using Mega 4 (TAMURA et al., 2007).

Morphological analysis

We qualitatively analyzed the structures most commonly used in taxonomic studies in terms of presence/absence, shape, position, size relative to other structures, and color pattern, following the terminology of Duellman (1970), Heyer et al. (1990), and Cei (1980). The webbing formula follows the notation of Savage and Heyer (1967) as modified by Myers and Duellman (1982). The sex was determined by the examination of secondary sexual characters (vocal sac, vocal slits, and nuptial pads). Drawings were made using a stereomicroscope with a drawing tube.

To quantitatively account for morphological variation we conducted univariate and multivariate morphometric analyses on adult males only, because few female specimens were available. Structures that are commonly influenced by fixation techniques and conservation such as the discs of fingers and toes were not considered. We measured specimens with a digital caliper of 0.01 mm precision using a stereomicroscope. All measures were taken by a single author (FB) to minimize interobserver bias (HAYEK et al., 2001; LEE, 1982). The measures and abbreviations used in this work are: SVL (snout-vent length), HL (head length), HW (head width), TD (tympanum diameter, horizontal), ED (eye diameter, horizontal), IOD (interorbital distance), EN (eye-nasal distance, anterior corner of orbit to posterior margin of external narina), IND (internarial distance, between the interior margins of the nostrils), ESD (eye-snout distance, from tip of the snout to anterior corner of the eye), FAL (forearm length, from elbow to the proximal margin of the thenar tubercle), FL (femur length), TL (tibia

length), and TAL (tarso length, from the tibiotarsal articulation to the proximal margin of the internal tarsal tubercle).

For multivariate analyses we log-transformed the raw morphometric measures to linearize variation. We performed a principal components analyses (PCA) to explore variation without a priori grouping by examining the distribution of individuals in multivariate space. We conducted PCA with the software STATISTICA (STATSOFT, 2010).

Call analyses

Toledo and Haddad (2005b) identified four types of calls for *Scinax fuscomarginatus*: advertisement, sporadic, territorial, and fighting calls. We analyzed only advertisement calls, following the call definitions of these authors.

We analyzed records digitized with 16 bit resolution, and 44.100 Hz sampling rate using the program Raven Pro 1.4 (Cornell Ornithology Lab). We produced audioespectrograms and oscilograms using Raven Pro 1.4, and analyzed the data with Fast Fourier Transformation using a window of 256. We followed the definition of McLister *et al.* (1995) considering a note as the main unit of sound, consisting of one or more pulses, produced during a single airflow cycle. This definition is applicable to *S. fuscomarginatus* vocalization because the vocal sac is externally evident, and the trunk and vocal sac increase and decrease during the call activity in a clearly way. We analyzed note duration (in seconds), pulse rate (pulses/seconds), and dominant frequencies.

RESULTS

Alignment and phylogenetic analyses

The concatenated mitochondrial dataset (CO1 + ND2 + 16S) has a total of 2192 bp with 511 parsimony-informative sites. The nucleotide substitution models that best fitted the codon partitions corresponding to the first, second and third positions were TrNef+G (TAMURA and NEI, 1993), TPM3uf (KIMURA, 1981) and GTR+I+G (LANAVE et al., 1984) for the CO1 fragment; HKY+G (HASEGAWA et al., 1985), TVM+G (POSADA, 2003), and TIM3+G (POSADA, 2003) for the ND2 fragment. The nucleotide substitution model that best fitted the 16S fragment was GTR+G (LANAVE et al., 1984).

ML and BI analyses resulted in very similar topologies and only ML trees are shown. *Scinax fuscomarginatus* and related species clustered together forming a monophyletic and well supported group, structured in two major clades, A and B (Fig. 3). Clade A (Fig. 4), with good support, is structured in two supported subclades (A1 and A2). Subclade A1 contains the topotypical specimen of *Hyla madeirae* (Porto Velho, state of Rondônia, Brazil) and part of the samples from eastern Bolivia (Los Lagos and San Sebastián, department of Santa Cruz de la Sierra; and Caparu, department of Beni) while subclade A2 comprises all specimens from Serra do Cachimbo, state of Pará, eastern Amazonia, Brazil.

The well supported clade B includes all specimens representing valid species (*Scinax trilineatus*, *S. fuscomarginatus*, *S. parkeri*, and *S. lutzorum*). This clade is structured in two main clades. In the first, partially supported, three subclades are evident (Fig. 5): B1 representing the only sampled individual of *S. trilineatus* (Venezuela), B2 including the topotypical specimens of *S. fuscomarginatus* from Lagoa Santa, state of Minas Gerais, along with other samples from this state, samples from the states of Goiás, southern state of Bahia, and state of São Paulo (except the locality of Teodoro Sampaio), Brazil, and B3 including samples from southeastern state of Tocantins and western state of Bahia, Brazil.

The second main clade within clade B is well supported and groups three subclades (Figs. 6, 7), B4 including topotypical material of *Scinax parkeri* (Buena Vista, department of Santa Cruz de la Sierra, Bolivia) together with the remaining material from eastern Bolivia (Los Lagos, San Sebastián and Caparu), B5 including the remaining samples from the Brazilian state of São Paulo (locality of Teodoro Sampaio), and B6 containing the remaining samples from localities ranging from northern Argentina to northeastern Brazil, including two

specimens assigned to *S. lutzorum* (CFBH 11431, 11433 from 30 km E from type locality). This large clade shows a shallow structure (comparing to other divergences within the tree) with three well supported subclades that overlap in their geographic distribution at southern state of Mato Grosso (Brazil), center and southern Paraguay (Guairá and Ñeembucú departments). The first subclade includes specimens from northeastern Brazil to Mato Grosso, and part of the specimens of Paraguay (Guairá and Ñeembucú departments), the second subclade contains specimens from south-center state of Mato Grosso (Serra das Araras, Lucas do Rio Verde, Tesouro and Guiratinga) to middle-north state of Mato Grosso do Sul, Brazil, and the third subclade contain the remaining specimens from Paraguay, all specimens from Argentina, southern state of Mato Grosso do Sul, and from the state of Mato Grosso (localities of Dom Aquino and Chapada dos Guimarães).

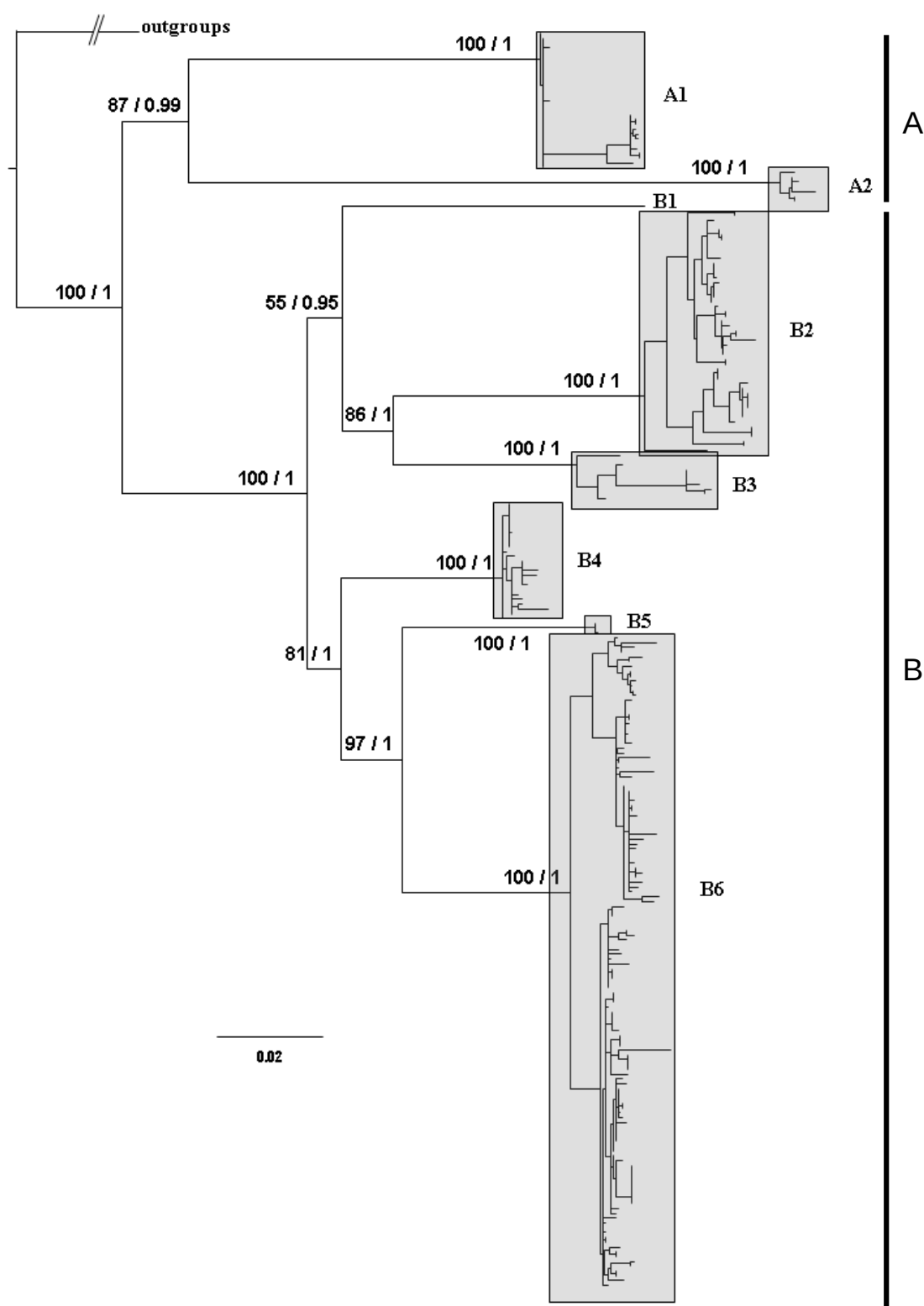


Figure 3. Maximum Likelihood phylogeny of the combined mitochondrial fragments (CO1+ND2+16S). Scale represents the expected changes per site. Node numbers indicate support values of ML and BI respectively. Black bars represent main clades and gray boxes subclades.

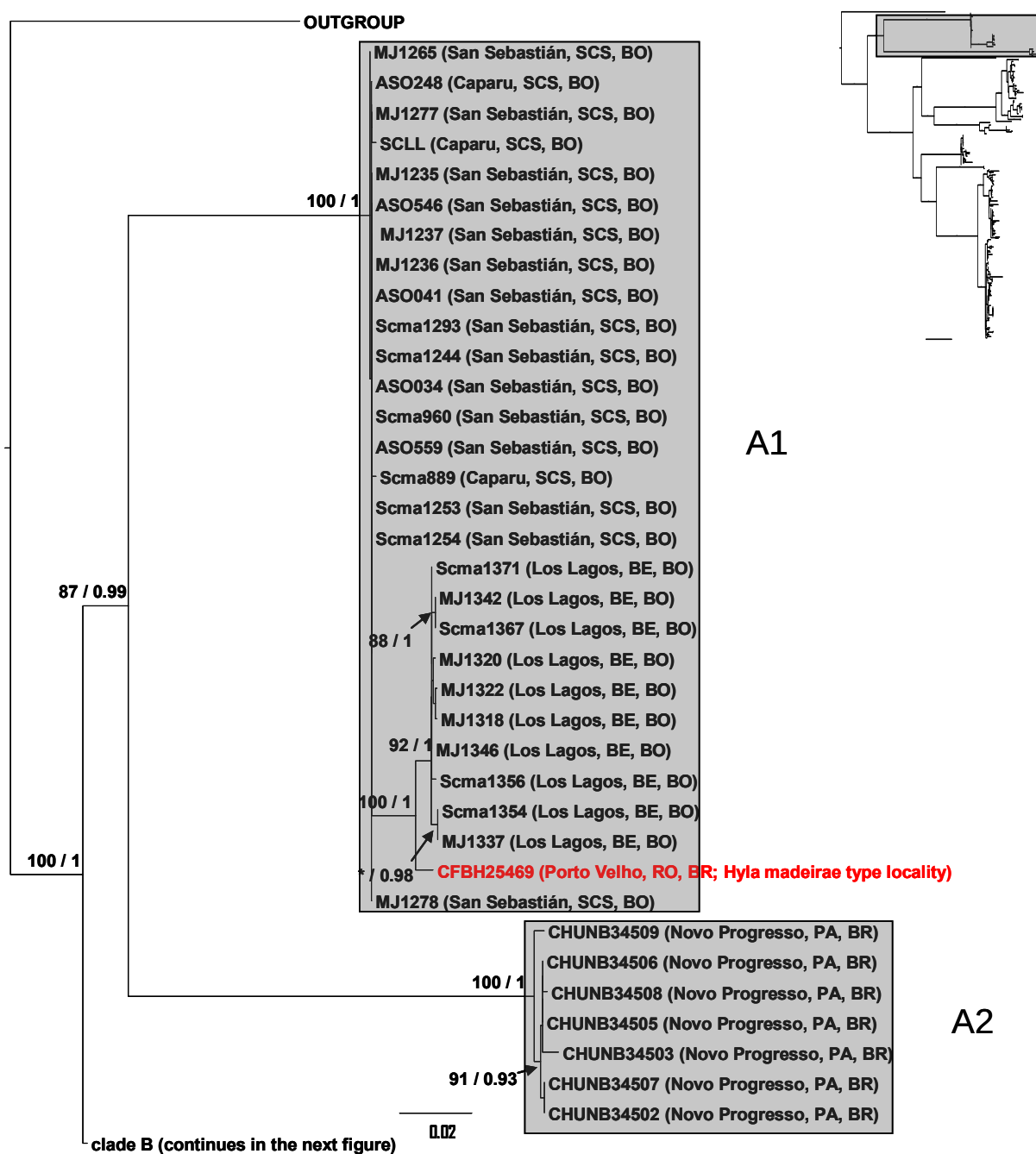


Figure 4. Maximum Likelihood phylogeny of the combined mitochondrial fragments (COI+ND2+16S) showing the clade A in detail. Scale represents the expected changes per site. Node numbers indicate support values of ML and BI respectively. Gray boxes represent subclades (see text for details).

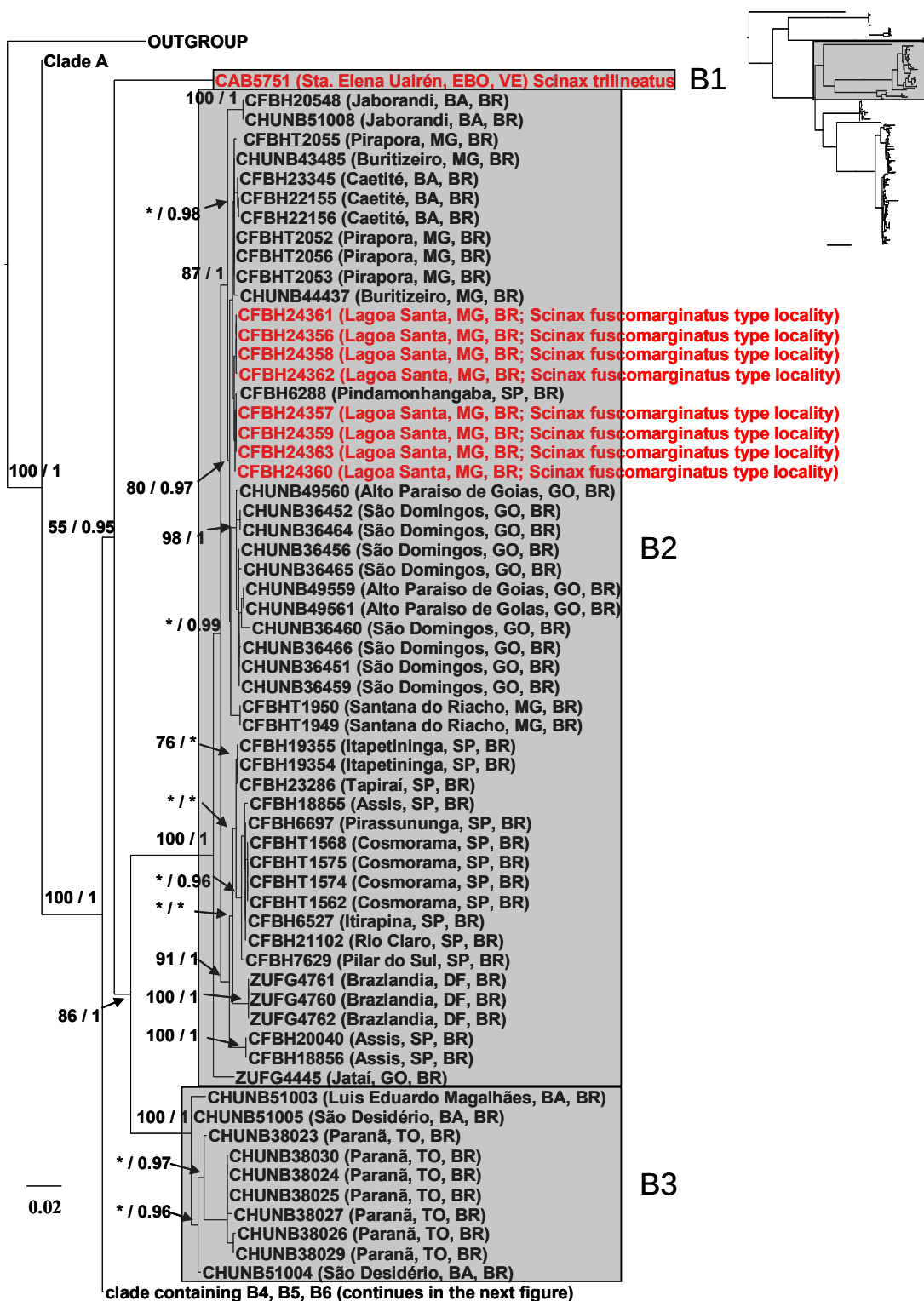


Figure 5. Maximum Likelihood phylogeny of the combined mitochondrial fragments (CO1+ND2+16S) showing the subclades B1, B2, and B3 in detail. Scale represents the expected changes per site. Node numbers indicate support values of ML and BI respectively, values under 75 for L and 0.95 for BI are represented by $\rightarrow^*$. Gray boxes represent subclades (see text for details).

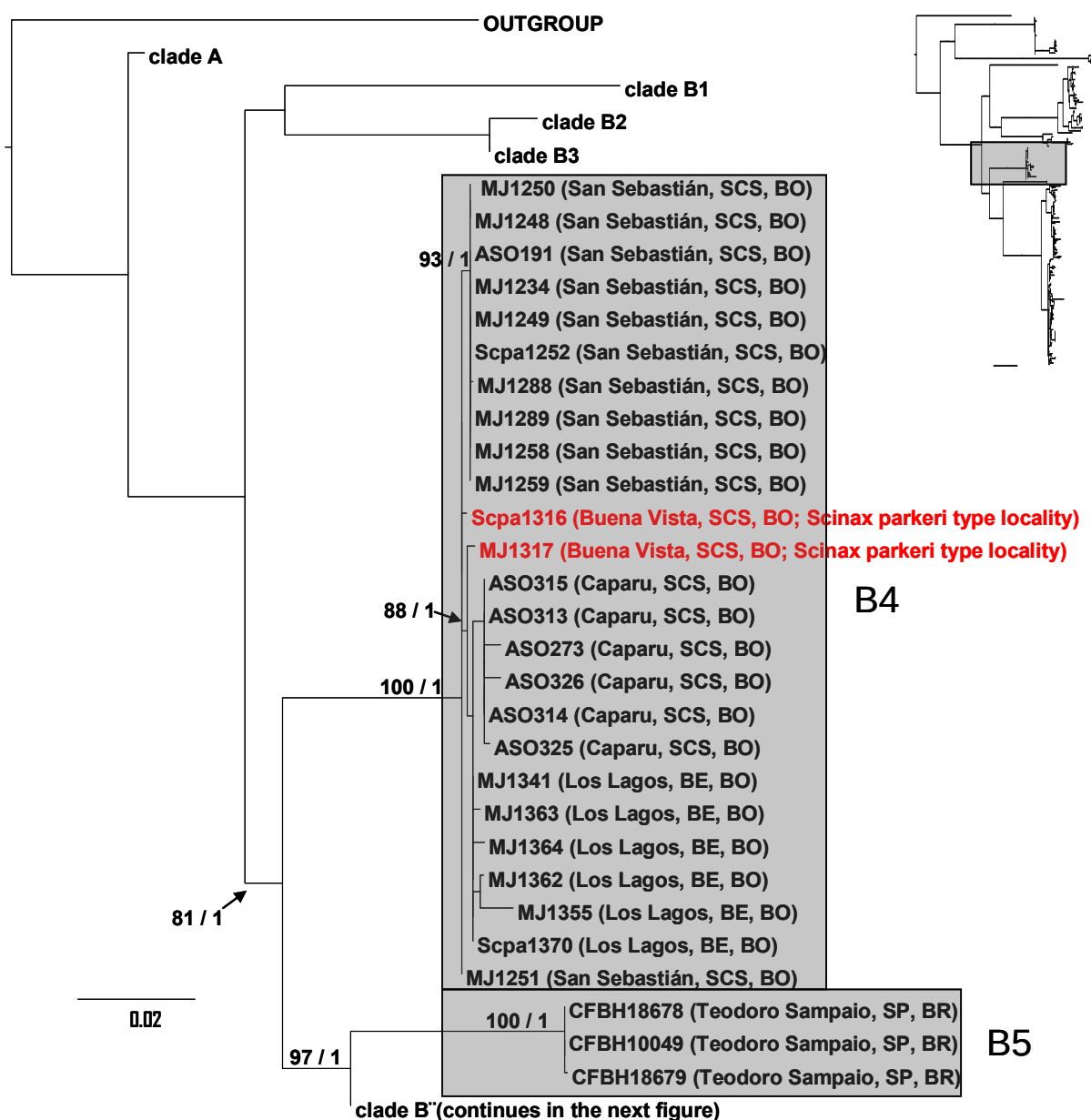
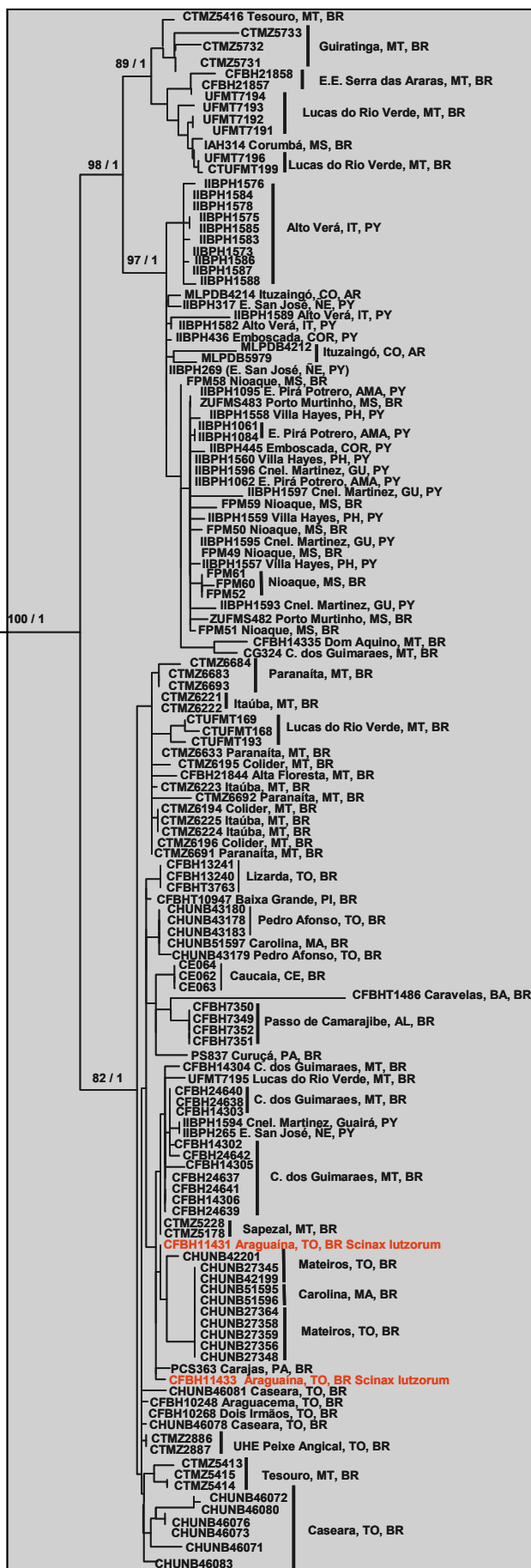


Figure 6. Maximum Likelihood phylogeny of the combined mitochondrial fragments (CO1+ND2+16S) showing the subclades B4 and B5 in detail. Scale represents the expected changes per site. Node numbers indicate support values of ML and BI respectively. Gray boxes represent the subclades (see text for details).

→

Figure 7. Maximum Likelihood phylogeny of the combined mitochondrial fragments (CO1+ND2+16S) showing the subclade B6 in detail. Scale represents the expected changes per site. Node numbers indicate support values of ML and BI respectively, values under 70 for ML and 0.95 for BI are represented by —*—.

Subclade B5



B6

0.02

Genetic Divergence

Genetic divergence (uncorrected *p*-distance) within clades (e.g. between subclades of the same main clade) for the ribosomal 16S fragment ranged from 1.3% to 12.5%, being strikingly large within some subclades. We found divergence to be higher among individuals of subclade B6 reaching the maximum value of 4.3% for individuals from Mato Grosso (Lucas do Rio Verde) and northern Argentina (province of Corrientes); in the subclade B2 we found the maximum divergence between individuals from São Paulo (Tapiraí) and Goiás (São Domingos), reaching 1.2%; and in the subclade A1 the maximum was 1% between individuals from San Sebastián and Los Lagos.

Divergence between subclades is given in Table 2. Taking into account the phylogenetic inference presented here we founded divergences between individuals of clades A and B from 4.9-12.5% and between the two main clades within the clade B (B1+B2+B3 vs. B4+B5+B6) from 2.6 to 9.8% (Fig. 8).

Table 2. Divergence (uncorrected *p*-distance) calculated for the 16S fragment between subclades, given in percentatge. Percentages on the diagonal (bolded) represent intrasubclades divergence.

	subclade B6	subclade B2	subclade B3	subclade B5	subclade A2	subclade A1	subclade B4	subclade B1
subclade B6	0.0-4.3							
subclade B2	2.6-8.1	0.2-1.2						
subclade B3	4.8-9.8	5.3-6.2	0.0					
subclade B5	1.3-7.7	5.8-6.5	7.2	0.0				
subclade A2	4.9-11.3	7.0-8.2	8.5	8.2	0.0			
subclade A1	4.9-12.5	7.0-8.9	8.1-9.6	7.6-8.7	7.3-7.5	0.2-1.0		
subclade B4	1.3-8.3	5.7-6.6	7.2-7.6	4.7-5.0	7.7-8.0	8.3-9.0	0.0-0.5	
subclade B1	2.6-8.7	5.9-7.0	6.7	6.3	8.1	8.9-9.9	6.0-6.4	0.0

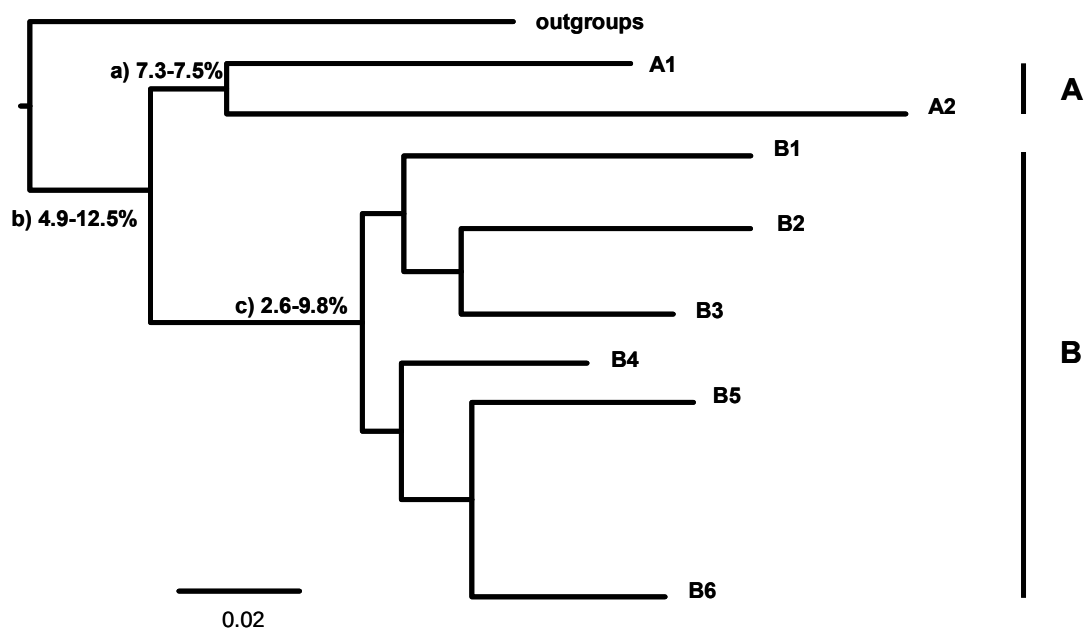


Figure 8. Schematic mitochondrial tree showing the 16S divergence values (uncorrected p -distance). Nodes numbers represents the genetic divergence range given in percentage: a) represents the range divergence between individuals of subclades A1 and A2; b) between individuals of clades A and B; and c) between individuals of subclades B1 + B2 + B3 and B4 + B5 + B6.

Morphological analyses

Morphological variation within species and populations did not allow us to distinguish among any of the currently recognized species (*Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*) using qualitative morphological characters. We identified some character states that distinguished *Hyla madeirae* (currently a synonym of *S. fuscomarginatus*) and specimens from Serra do Cachimbo, mostly corresponding to color patterns like the dorsolateral stripes, stripes in the dorsal and external surfaces of tibia, and upper lip pattern, but we have also identified shape related characters such as snout shape in dorsal view, loreal region and the degree of development of toe webbing. These characters are described below:

- 1) Dorsolateral stripes: we identified two states in this character, convergent dorsolateral stripes and divergent (sometimes parallels) dorsolateral stripes. The most common condition is divergent, present in all the specimens of *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, *S. lutzorum*, and in specimens from Serra do Cachimbo; all the specimens of *Hyla madeirae* have the convergent state (Fig. 9).



Figure 9. Dorsal view showing the dorsolateral stripes. A) convergent dorsolateral stripes (MZUSP 73663; *Hyla madeirae*, holotype), B) divergent dorsolateral stripes (CFBH 24357; *Scinax fuscomarginatus*, topotype).

- 2) Pattern of upper lip: although this character is somewhat variable, we identified two basic states in this character, immaculate or finely spotted, and having more conspicuous spots forming patches. The analyzed specimens of *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, *S. lutzorum*, and those from Serra do Cachimbo typically present the first state; whereas *Hyla madeirae* shows the second state (Fig. 10).



Figure 10. Lateral view of the head showing the upper lip pattern. A) finely spotted (CFBH 25437; *Scinax fuscomarginatus*, topotype) and B) conspicuously spotted forming patches (CFBH 25469; *Hyla madeirae*, topotype).

- 3) Color pattern on the dorsal surfaces of tibia: almost all specimens analyzed have dark stripes crossing the tibia (some specimens have a longitudinal pattern, which is extremely rare and not characteristic of any population). We identified two states in this character: transversal and oblique dark stripes. *Scinax fuscomarginatus*, *S.*

parkeri, *S. trilineatus*, and *S. lutzorum* have oblique stripes and *Hyla madeirae* and specimens of Serra do Cachimbo have transversal stripes (Fig 11).

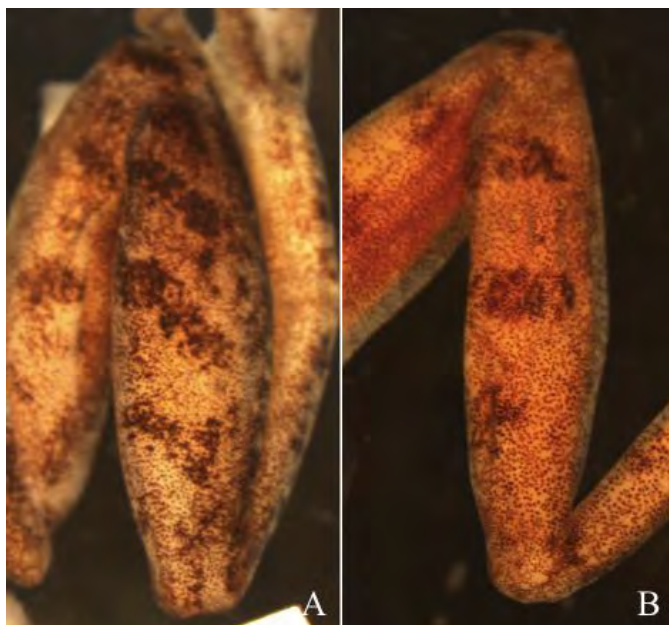


Figure 11. Dorsal view of tibia showing the striped pattern. A) oblique stripes (CFBH 24362; *Scinax fuscomarginatus*, topotype) B) transversal stripes (CHUNB 9939; *Hyla madeirae*).

- 4) Color pattern on the external surface of tibia: we identified two states, presence of a dark longitudinal stripe, or absence. *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum* have a longitudinal stripe; *Hyla madeirae* and specimens of Serra do Cachimbo have a uniform color pattern on the external surface of tibia (Fig. 12).

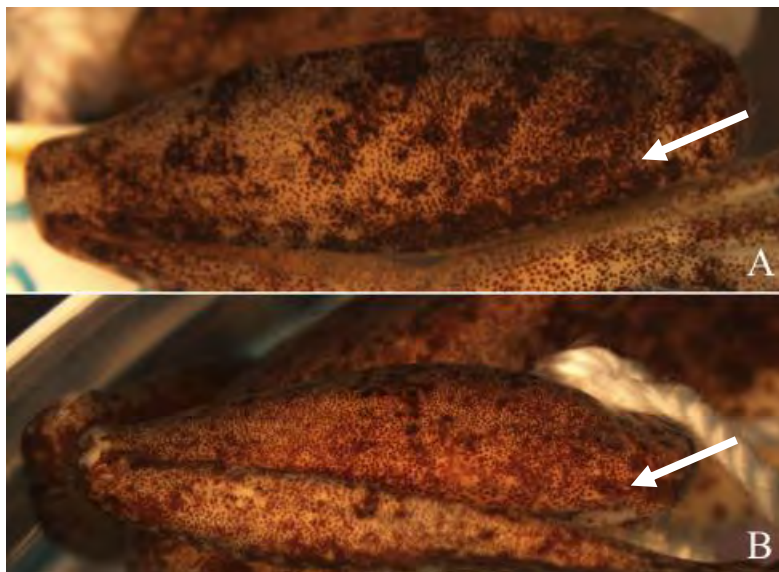


Figure 12. External view of tibia A) presence of longitudinal dark stripe (CFBH 24362; *Scinax fuscomarginatus*, topotype) and B) absence of longitudinal dark stripe (CFBH 25469; *Hyla madeirae*, topotype).

- 5) Snout shape in dorsal view: we identified two states of this character, rounded or sub-elliptical. *Hyla madeirae* and the specimens from Serra do Cachimbo have rounded snout in dorsal view; *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum* have sub-elliptical snout in dorsal view (Fig. 13).

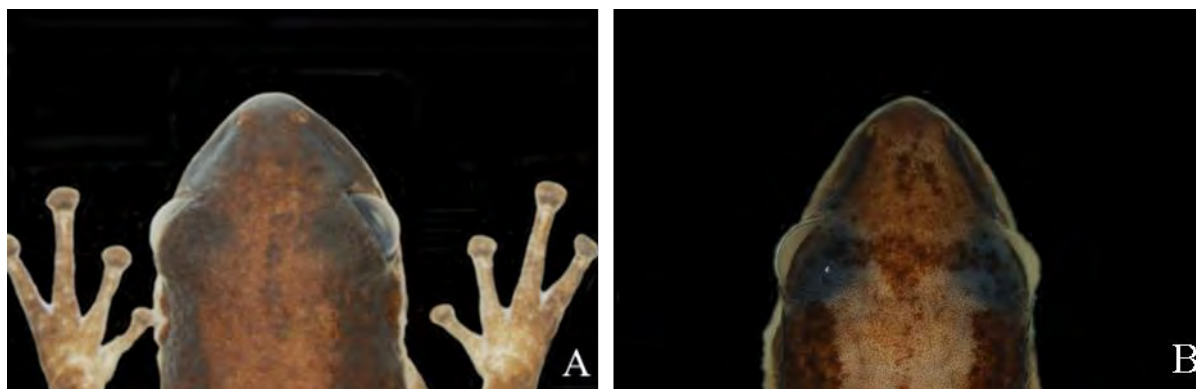


Figure 13. Dorsal view of the head showing snout and loreal region shape. A) rounded snout and loreal region with softly sloping toward lips (CFBH 25470; *Hyla madeirae*, topotype) and B) sub-elliptical snout and loreal region with steep sloping toward lips (CFBH 24357, *Scinax fuscomarginatus*, topotype).

- 6) Loreal region: the loreal region is concave in all the specimens analyzed, but present variation with the inclination angle showing two states; loreal region softly sloping toward lips or steep sloping toward lips. *Hyla madeirae* and specimens from Serra do Cachimbo have the first state (softly sloping); *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum* the second state (steep sloping) (Fig. 13).
- 7) Toe webbing: *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, *S. lutzorum*, and *Hyla madeirae* show a few variations in toe webbing but basically they have well developed webbing. Specimens of Serra do Cachimbo show less development of toe webbing, more evident between Toes II and III where the webbing reaches or slightly passes the sub-articular tubercle of finger II (Fig. 14).

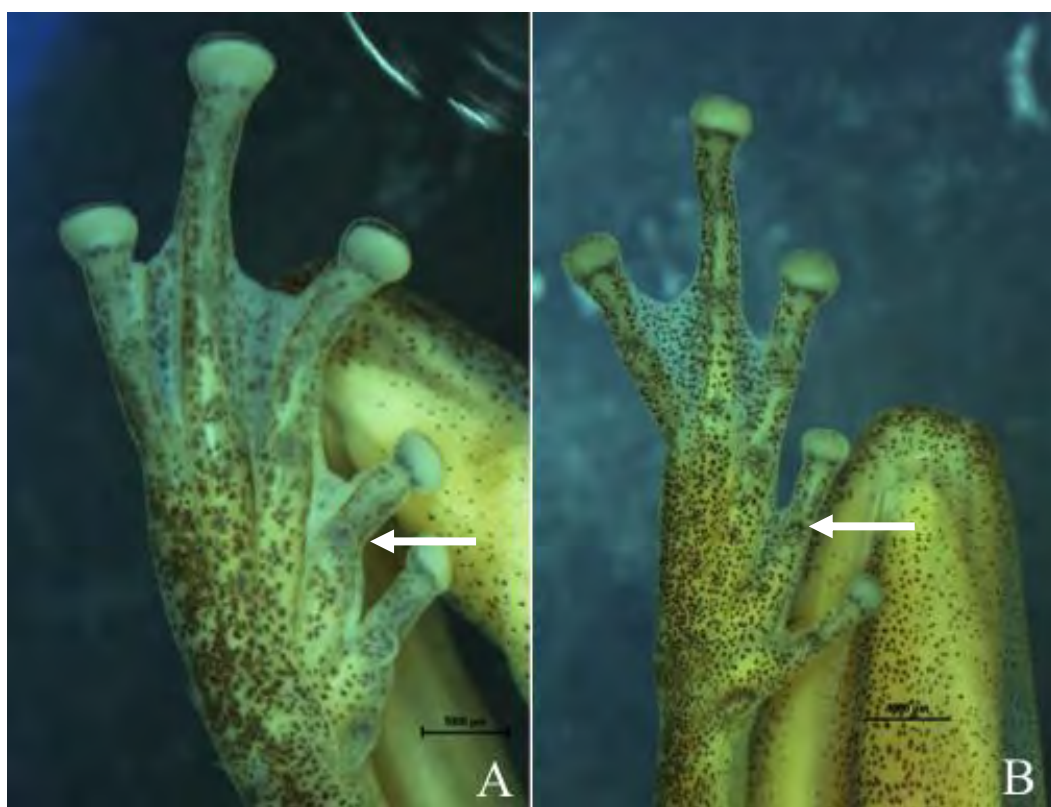


Figure 14. Ventral view of foot showing Toe webbing. A) well developed webbing (CFBH 24363, *Scinax fuscomarginatus*, topotype), B) webbing reaching or slightly passing the distal margin of the subarticular tubercle of Toe II (CHUNB 34505, Serra do Cachimbo, Pará). Arrow indicates the position of the subarticular tubercle of Toe II.

Morphometric analyses

The PCA from log transformed measures resulted in two principal components representing 66.48% and 9.11% of the entire variation (Table 3). None of the currently recognized species (Fig. 15, A.) and main mitochondrial subclades (Fig. 14, B) is distinguishable in the biplot representing the scores of analyzed specimens.

Table 3. PCA scores from log-data for the two first axis for all the variables.

Variables	Axis 1	Axis 2
SVL	0.3117	-0.08878
HL	0.3096	-0.01306
HW	0.2987	0.04524
TD	0.2004	0.5867
ED	0.2407	0.419
IOD	0.2772	0.07573
EN	0.268	0.09894
IND	0.2251	0.3814
ESD	0.2774	-0.03886
FAL	0.2755	-0.2217
FL	0.2962	-0.2681
TL	0.3054	-0.2804
TAL	0.294	-0.3288

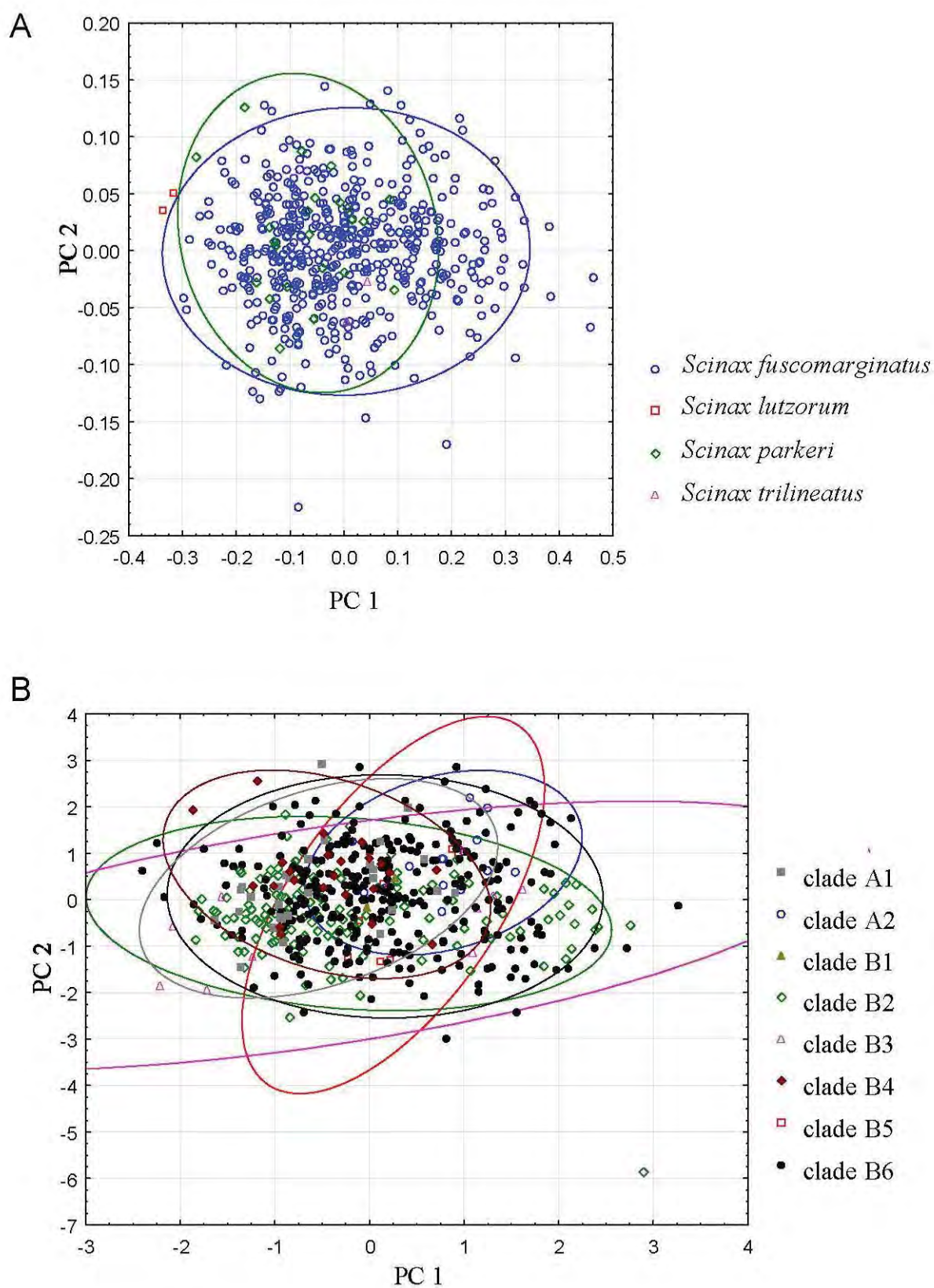


Figure 15. PCA scores biplot from log data showing the distribution of individuals in multivariate space. A) currently recognized species, B) mitochondrial subclades.

The currently recognized species were also not distinguished in univariate analyses, but some body proportions revealed slight differences: the HW/HL and HW/SVL proportions are slightly different in *Hyla madeirae* and in populations of Serra do Cachimbo regarding to remaining sample, but with a high degree of overlap. The SVL showed some differences between specimens of *H. madeirae* and those of Serra do Cachimbo, being *H. madeirae* larger, but also with some overlap (Table 4).

Table 4. Measurements and body proportions for the currently recognized species (*Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*), *Hyla madeirae* and Serra do Cachimbo specimens (as Cachimbo). For each variable is given mean values, standard deviation, and range in parentheses. Measurements abbreviations as in material and methods.

	<i>S.fuscomarginatus</i> n= 467	<i>S. parkeri</i> n= 23	<i>S. trilineatus</i> n= 2	<i>S. lutzorum</i> n= 2	<i>H. madeirae</i> n= 22	Cachimbo n= 15
SVL	20.39±1.99 (15.65-26.69)	21.19±1.31 (18.39-23.47)	20.8±0.68 (20.32-21.28)	24.8±0.42 (24.50-25.10)	20.85±1.41 (18.03-23.05)	18.61±0.93 (16.71-19.97)
HL	6.74±0.61 (4.65-8.21)	7.05±0.38 (6.38-7.67)	7.02±0.21 (6.87-7.16)	8.12±0.04 (8.09-8.15)	6.81±0.39 (6.09-7.66)	6.32±0.35 (5.86-6.91)
HW	5.66±0.61 (4.14-7.33)	5.94±0.37 (5.39-6.56)	5.60±0.25 (5.42-5.77)	7.26±0.24 (7.09-7.43)	6.15±0.48 (5.10-6.88)	5.59±0.25 (5.16-5.99)
TD	0.75±0.11 (0.45-1.10)	0.82±0.11 (0.67-1.10)	0.66±0.02 (0.64-0.67)	1.03±0.01 (1.02-1.03)	0.78±0.08 (0.62-0.94)	0.73±0.07 (0.63-0.82)
ED	2.03±0.20 (1.00-2.49)	2.15±0.24 (1.70-2.56)	2.22±0.09 (2.15-2.28)	2.64±0.10 (2.57-2.71)	2.15±0.20 (1.67-2.49)	2.10±0.11 (1.91-2.26)
IOD	2.03±0.22 (1.51-2.79)	2.18±0.23 (1.85-2.65)	1.95±0.04 (1.92-1.97)	2.57±0.10 (2.50-2.64)	2.15±0.21 (1.68-2.47)	1.96±0.11 (1.74-2.16)
EN	2.04±0.22 (1.44-2.75)	2.16±0.18 (1.85-2.59)	2.02±0.02 (2.00-2.03)	2.52±0.11 (2.44-2.60)	2.07±0.15 (1.83-2.31)	1.86±0.25 (1.58-2.68)
IND	1.45±0.16 (0.27-1.86)	1.59±0.15 (1.23-1.80)	1.51±0.12 (1.42-1.59)	1.63±0.04 (1.60-1.66)	1.53±0.11 (1.30-1.73)	1.30±0.06 (1.19-1.41)
ESD	3.00±0.28 (2.01-3.72)	3.16±0.28 (2.34-3.57)	2.77±0.02 (2.75-2.78)	3.39±0.11 (3.31-3.46)	3.04±0.20 (2.72-3.48)	2.74±0.17 (2.44-3.04)
FAL	3.53±0.40 (2.46-4.61)	3.64±0.35 (3.10-4.39)	3.3±0.11 (3.22-3.38)	4.34±0.13 (4.24-4.43)	3.66±0.39 (2.98-4.35)	3.42±0.23 (3.12-3.79)
FL	8.67±0.95 (6.00-11.14)	8.97±0.61 (7.77-9.91)	8.81±0.03 (8.79-8.83)	10.4±0.24 (10.23-10.57)	8.91±0.64 (7.97-10.37)	7.80±0.48 (7.13-8.55)
TL	9.86±0.94 (7.17-12.36)	10.08±0.53 (8.58-10.77)	9.48±0.13 (9.39-9.57)	11.81±0.11 (11.73-11.88)	9.83±0.66 (8.75-11.41)	8.69±0.52 (7.88-9.83)
TAL	5.61±0.55 (4.23-7.26)	5.69±0.34 (5.17-6.56)	5.45±0.30 (5.23-5.66)	6.94±0.08 (6.88-7.00)	5.65±0.41 (4.97-6.30)	5.02±0.24 (4.64-5.46)
FL/SVL	0.43±0.48 (0.38-0.42)	0.42±0.03 (0.35-0.48)	0.42±0.01 (0.41-0.43)	0.42±0.00 (0.42-0.42)	0.43±0.02 (0.39-0.47)	0.42±0.02 (0.39-0.45)
HW/SVL	0.28±0.31 (0.26-0.27)	0.28±0.02 (0.21-0.30)	0.27 (0.21-0.30)	0.29±0.00 (0.29-0.30)	0.30±0.01 (0.27-0.32)	0.30±0.01 (0.29-0.32)
HW/HL	0.84±0.99 (0.89-0.89)	0.84±0.03 (0.77-0.88)	0.80±0.01 (0.79-0.81)	0.89±0.03 (0.87-0.92)	0.90±0.05 (0.78-0.99)	0.89±0.03 (0.82-0.97)
HL/SVL	0.33±0.31 (0.30-0.31)	0.34±0.01 (0.31-0.35)	0.34 (0.31-0.35)	0.33±0.01 (0.32-0.33)	0.33±0.01 (0.31-0.35)	0.34±0.01 (0.32-0.37)
TL/SVL	0.48±0.47 (0.46-0.46)	0.48±0.02 (0.44-0.51)	0.46±0.01 (0.45-0.46)	0.48±0.00 (0.47-0.48)	0.47±0.02 (0.45-0.52)	0.47±0.02 (0.43-0.50)
TAL/SVL	0.28±0.28 (0.27-0.27)	0.27±0.01 (0.24-0.29)	0.26±0.01 (0.26-0.27)	0.28±0.00 (0.28-0.28)	0.27±0.01 (0.25-0.30)	0.27±0.01 (0.25-0.30)
FAL/SVL	0.17±0.20 (0.16-0.17)	0.17±0.02 (0.15-0.20)	0.16 (0.15-0.20)	0.17±0.00 (0.17-0.18)	0.18±0.01 (0.15-0.20)	0.18±0.01 (0.16-0.21)
TD/SVL	0.04±0.06 (0.03-0.04)	0.04±0.01 (0.03-0.05)	0.03 (0.03-0.05)	0.04±0.00 (0.04-0.04)	0.04±0.00 (0.03-0.05)	0.04±0.00 (0.03-0.05)
ED/SVL	0.10±0.10 (0.06-0.09)	0.10±0.01 (0.08-0.12)	0.11±0.01 (0.10-0.11)	0.11±0.01 (0.10-0.11)	0.10±0.01 (0.08-0.12)	0.11±0.01 (0.10-0.12)
IOD/SVL	0.10±0.11 (0.10-0.10)	0.10±0.01 (0.09-0.11)	0.09 (0.09-0.11)	0.10±0.00 (0.10-0.11)	0.10±0.01 (0.09-0.12)	0.11±0.01 (0.10-0.11)

	0.10±0.11 (0.09-0.10)	0.10±0.01 (0.09-0.12)	0.10±0.00 (0.09-0.10)	0.10±0.00 (0.10-0.10)	0.10±0.00 (0.09-0.11)	0.10±0.01 (0.08-0.13)
EN/SVL	0.07±0.08 (0.02-0.07)	0.07±0.01 (0.06-0.08)	0.07	0.07±0.00 (0.06-0.07)	0.07±0.01 (0.06-0.09)	0.07±0.00 (0.06-0.08)
IND/SVL	0.15±0.14 (0.13-0.14)	0.15±0.01 (0.10-0.17)	0.13±0.01 (0.13-0.14)	0.14±0.01 (0.13-0.14)	0.15±0.01 (0.13-0.17)	0.15±0.01 (0.13-0.17)
ESD/SVL	0.45±0.46 (0.43-0.45)	0.45±0.04 (0.31-0.49)	0.39±0.01 (0.38-0.40)	0.42±0.01 (0.41-0.42)	0.45±0.03 (0.40-0.51)	0.43±0.02 (0.38-0.46)
ESD/HL						

Call analyses

The total values are given in the Table 5. Audiospectrogram and oscilogram of the currently recognized species are given in the Figure 16.

The advertisement call of all currently valid species analyzed here shown the same basic structure: a frequency modulated multipulsed note with variable repetition rate. The dominant frequency was not different between species, being extremely variable even in an intra-population level (e.g. 3164 to 3879 Hz in *Scinax fuscomarginatus* topotypes).

Note duration in *Scinax parkeri* was relatively short (0.30-0.50 seconds) and was clearly distinguishable from *S. fuscomarginatus* topotypes (0.62-0.74 seconds). However, the two ranges are included into the interval obtained for calls from throughout the distribution of *S. fuscomarginatus* (0.32-0.94 seconds). The same occurred for values registered for *S. trilineatus* (0.57-0.89 seconds), while for *Hyla madeirae* the overlap in note duration was only partial (0.72-1.16 seconds).

For pulse rate we also found high degree of overlap between the values registered for *Scinax fuscomarginatus* (113-201 pulses/second), *S. trilineatus* (160-164 pulses/second) and, *Hyla madeirae* (103-144 pulses/second), while *S. parkeri* shown higher pulse rate, not overlapping with the other species (209-271 pulses/second). However, major differences were found among *S. parkeri* individuals from different localities (209-210 vs. 257-271 pulses/second; Buena Vista and Los Lagos, respectively) or between *S. fuscomarginatus* individuals from the same locality (e.g. 165.20-167.66 vs. 194.98-201.19 pulses/seconds; Altamira, state of Pará, Brazil).

Table 5. Call parameters for *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *Hyla madeirae*. For each variable is given mean values, standard deviation, and range in parentheses.

<i>Scinax fuscomarginatus</i>					
ID	locality	notes	notes duration (s)	fundamental freq. (hz)	pulses/second
CFBH24360	Lagoa Santa (MG)	13	0.71±0.02 (0.67-0.74)	3785.99±61.29 (3635.10-3874.30)	155.85±1.61 (154.06-158.43)
CFBH24361	Lagoa Santa (MG)	8	0.56±0.06 (0.49-0.62)	3368.77±102.07 (3164.60-3470.80)	161.31±2.06 (159.23-164.47)
CFBH24362	Lagoa Santa (MG)	7	0.65±0.03 (0.62-0.70)	3526.5±70.18 (3368.80-3572.90)	163.29±0.44 (162.87-163.93)
CFBH24363	Lagoa Santa (MG)	12	0.65±0.03 (0.62-0.71)	3734.56±110.64 (3572.90-3879.20)	158.74±1.24 (157.23-160.26)
CFBH19-02	Ribeirao Branco (SP)	31	0.65±0.02 (0.62-0.69)	3573.35±158.01 (3252.50-3826.50)	168.74±0.60 (168.21-169.43)
CFBH33-06	Ribeirao Branco (SP)	29	0.72±0.04 (0.63-0.78)	3673.4±116.73 (3396.00-3826.50)	137.46±2.21 (133.85-139.78)
CFBH33-09	Ribeirao Branco (SP)	15	0.64±0.04 (0.60-0.71)	3759.5±117.59 (3587.3-3922.1)	164.32±3.36 (160.36-166.95)
fita 11 cut 5	Paulínia (SP)	25	0.60±0.01 (0.57-0.63)	3555.58±78.59 (3444.40-3666.70)	176.13±1.82 (174.28-178.27)
m. diabo1	Morro do Diabo (SP)	10	0.87±0.04 (0.78-0.92)	3756.68±85.42 (3675.00-3879.20)	117.45±2.85 (113.42-120.48)
CFBH53-07	Piracanjuba (GO)	13	0.46±0.03 (0.43-0.52)	3467.72±56.36 (3348.20-3539.50)	188.45±5.45 (185.40-198.15)
CFBH58-06	São João (GO)	22	0.60±0.01 (0.58-0.63)	3443.81±59.63 (3348.20-3539.50)	138.75±1.57 (136.59-140.94)
fita 11 cut 6	Mineiros (GO)	44	0.60±0.02 (0.56-0.66)	4355.52±144.86 (4111.10-4444.40)	122.77±1.92 (121.55-126.09)
AFF-cut 1	Pirenópolis (GO)	10	0.37±0.03 (0.34-0.41)	3600.02±99.41 (3444.40-3666.70)	173.56±4.06 (168.35-177.93)
RPB 5-5	Silvania (GO)	10	0.36±0.02 (0.33-0.40)	3470.86±144.34 (3368.80-3675.00)	169.47±2.95 (166.15-172.62)
RPB 6-3	Silvania (GO)	17	0.63±0.01 (0.61-0.65)	3287.12±85.42 (3164.60-3368.80)	168.47±0.94 (167.78-170.07)
RPB 6-4	Silvania (GO)	12	0.62±0.04 (0.54-0.67)	3287.1±111.80 (3164.60-3470.80)	170.07±1.22 (168.35-171.23)
RPB 6-5	Silvania (GO)	15	0.67±0.02 (0.62-0.70)	3042.08±45.66 (2960.40-3062.50)	174.48±2.24 (171.23-176.05)
RPB 6-6	Silvania (GO)	10	0.60±0.02 (0.55-0.63)	3287.12±45.66 (3266.70-3368.80)	173.38±1.63 (171.82-175.43)
RPB 6-9	Silvania (GO)	11	0.55±0.02 (0.50-0.57)	3205.44±91.32 (3062.50-3266.70)	173.5±1.65 (170.65-174.82)
RPB 6-10	Silvania (GO)	11	0.49±0.04 (0.41-0.54)	3287.12±45.66 (3266.70-3368.80)	174.48±2.24 (171.23-176.05)
AFF-cut 2	F. de Araguaia (TO)	11	0.34±0.02 (0.32-0.36)	3644.46±144.89 (3444.40-3777.80)	184.50±1.08 (183.15-185.87)
AFF-cut 3	F. de Araguaia (TO)	18	0.3±0.02 (0.27-0.35)	3755.58±92.95 (3666.7-3888.9)	193.80±1.06 (193.05-195.31)
Igor Rob	Caucaia (CE)	20	0.58±0.01 (0.56-0.61)	3675	172.13±4.01 (169.49-179.21)
fita 2 cut 1	B. do Bugres (MT)	23	0.6±0.03 (0.54-0.68)	3786.35±71.16 (3666.70-3888.90)	184.35±1.55 (183.36-187.07)
AFF-cut 4	Cáceres (MT)	15	0.79±0.02 (0.74-0.82)	3822.24±60.85 (3777.80-3888.90)	143.46±5.74 (134.05-148.81)
fita 11 cut 7	Altamira (PA)	25	0.84±0.03 (0.78-0.91)	4111.1±111.1 (4000-4222.2)	197.01±2.88 (194.98-201.19)
fita 11 cut 8	Altamira (PA)	17	0.85±0.05 (0.74-0.94)	3066.66±126.67 (2888.90-3222.20)	166.49±0.92 (165.20-167.66)
7965	Ipeán (PA)	10	0.72±0.03 (0.67-0.79)	4209.5±97.6 (4060.7-4373.1)	164.65±2.94 (160.32-168.36)
7966	Ipeán (PA)	7	0.74±0.03 (0.70-0.80)	4112.8±184.6 (3748.4-4269.0)	169.75±3.99 (162.69-172.36)
7967	Ipeán (PA)	8	0.65±0.02 (0.63-0.68)	4269±78.7 (4164.9-4373.1)	168.03±7.01 (160.18-179.44)
7969	Ipeán (PA)	7	0.67±0.04 (0.64-0.75)	4164.87±167.89 (3748.40-4373.10)	174.83±1.73 (172.78-176.66)
7970	Ipeán (PA)	8	0.62±0.03 (0.58-0.66)	4242.98±48.19 (4164.90-4269.00)	178.41±1.82 (176.26-180.73)
7971	Ipeán (PA)	8	0.60±0.03 (0.55-0.64)	3709.34±156.80 (3540.10-3956.60)	176.70±1.09 (175.17-177.78)
AL001	P. de Camarajibe (AL)	4	0.40±0.01 (0.39-0.41)	4457.67±155.96 (4287.50-4593.80)	189.95±17.05 (177.89-202)
AL002	P. de Camarajibe (AL)	12	0.45±0.03 (0.41-0.52)	4125.39±58.14 (4017.80-4209.10)	169.97±2.32 (166.67-173.12)
AL003	P. de Camarajibe (AL)	9	0.40±0.03 (0.37-0.48)	4125.4±45.82 (4065.60-4161.30)	171.88±2.15 (168.54-174.26)
AL004	P. de Camarajibe (AL)	10	0.45±0.05 (0.39-0.54)	4031.46±102.25 (3922.10-4209.10)	166.27±5.85 (160.87-173.00)
AL005	P. de Camarajibe (AL)	4	0.46±0.01 (0.45-0.47)	4149.3±81.68 (4065.60-4256.90)	171.44±2.23 (168.18-173.00)
AL006	P. de Camarajibe (AL)	7	0.45±0.01 (0.44-0.47)	4079.27±59.94 (3970.00-4161.30)	169.31±1.22 (168.18-170.73)
AL007	P. de Camarajibe (AL)	9	0.46±0.02 (0.43-0.48)	4182.52±59.12 (4065.60-4256.90)	167.16±2.35 (164.86-169.76)
IIBP 001	Amambay (PY)	31	0.88±0.04 (0.76-0.94)	3940.4±55.87 (3879.20-3981.20)	152.12±3.18 (148.36-155.76)
IIBP 002	Amambay (PY)	19	0.75±0.03 (0.70-0.80)	3715.84±91.32 (3572.90-3777.10)	164.58±0.97 (163.39-165.56)
IIBP 003	Amambay (PY)	20	0.58±0.03 (0.51-0.62)	3675±144.39 (3470.80-3777.10)	176.98±3.53 (174.82-183.15)
IIBP 004	Amambay (PY)	22	0.81±0.01 (0.78-0.83)	3838.34±91.28 (3777.10-3981.20)	153.58±1.26 (152.00-154.80)
IIBPH 1593	Guairá (PY)	11	0.51±0.02 (0.49-0.53)	3960.8±45.62 (3879.20-3981.20)	168.19±4.02 (164.54-174.47)
IIBPH 1594	Guairá (PY)	16	0.52±0.05 (0.44-0.58)	3817.9±245.84 (3572.90-4083.30)	180.78±2.13 (177.30-182.48)
IIBPH 1557	P. Hayes (PY)	11	0.51±0.01 (0.49-0.54)	4018.34±68.82 (3879.20-4083.30)	186.93±4.33 (179.21-189.39)
IIBPH 1558	P. Hayes (PY)	6	0.49±0.01 (0.48-0.50)	3762.5±137.32 (3572.90-3981.20)	193.70±3.38 (189.40-198.41)
IIBPH 1559	P. Hayes (PY)	19	0.38±0.03 (0.33-0.43)	3842.05±82.57 (3777.10-3981.20)	192.33±2.53 (187.96-194.55)
IIBPH 1560	P. Hayes (PY)	14	0.36±0.02 (0.32-0.38)	4027.62±115.16 (3777.10-4185.40)	196.08±0.63 (195.31-196.85)
DB 001	Corrientes (AR)	30	0.58±0.06 (0.45-0.67)	3955.74±209.55 (3675.00-4287.50)	140.17±2.79 (135.32-142.22)

<i>Scinax parkeri</i>					
ID	locality	notes	notes duration (s)	fundamental freq.	pulses/second
MJ 1316	Buena Vista (BO)	17	0.45±0.02 (0.40-0.50)	3697.43±95.68 (3573.10-3825.70)	210.26±0.97 (209.20-210.97)
MJ 1259	San Sebastián (BO)	16	0.44±0.01 (0.42-0.47)	3726.05±64.57 (3675.00-3879.20)	231.06±1.63 (229.36-233.65)
MJ 1252	San Sebastián (BO)	3	0.35±0.03 (0.31-0.37)	3709.03±77.95 (3624.00-3777.10)	215.21±1.07 (214.59-216.45)
MJ 1320	Los Lagos (BO)	11	0.31±0.01 (0.30-0.32)	3733.36±93.69 (3555.60-3888.90)	261.33±5.85 (257.73-271.74)
<i>Scinax trilineatus</i>					
ID	locality	notes	notes duration (s)	fundamental freq.	pulses/second
fita 2 cut 2	S. Elena Uairén (VE)	23	0.73±0.10 (0.57-0.89)	3851.87±57.37 (3777.80-3888.90)	162.31±1.19 (160.78-164.00)
<i>Hyla madeirae</i>					
ID	locality	notes	notes duration (s)	fundamental freq.	pulses/second
CFBH25469	Porto Velho (RO)	12	0.85±0.09 (0.72-0.97)	3452.26±61.53 (3368.80-3572.90)	142.40±2.40 (138.89-144.92)
MJ 1237	San Sebastián (BO)	13	0.96±0.04 (0.88-1.03)	3504.84±66.48 (3368.80-3572.90)	130.82±0.45 (130.54-131.58)
MJ 1265	San Sebastián (BO)	4	1.15±0.01 (1.13-1.16)	3879.2	106.23±4.43 (103.73-112.86)

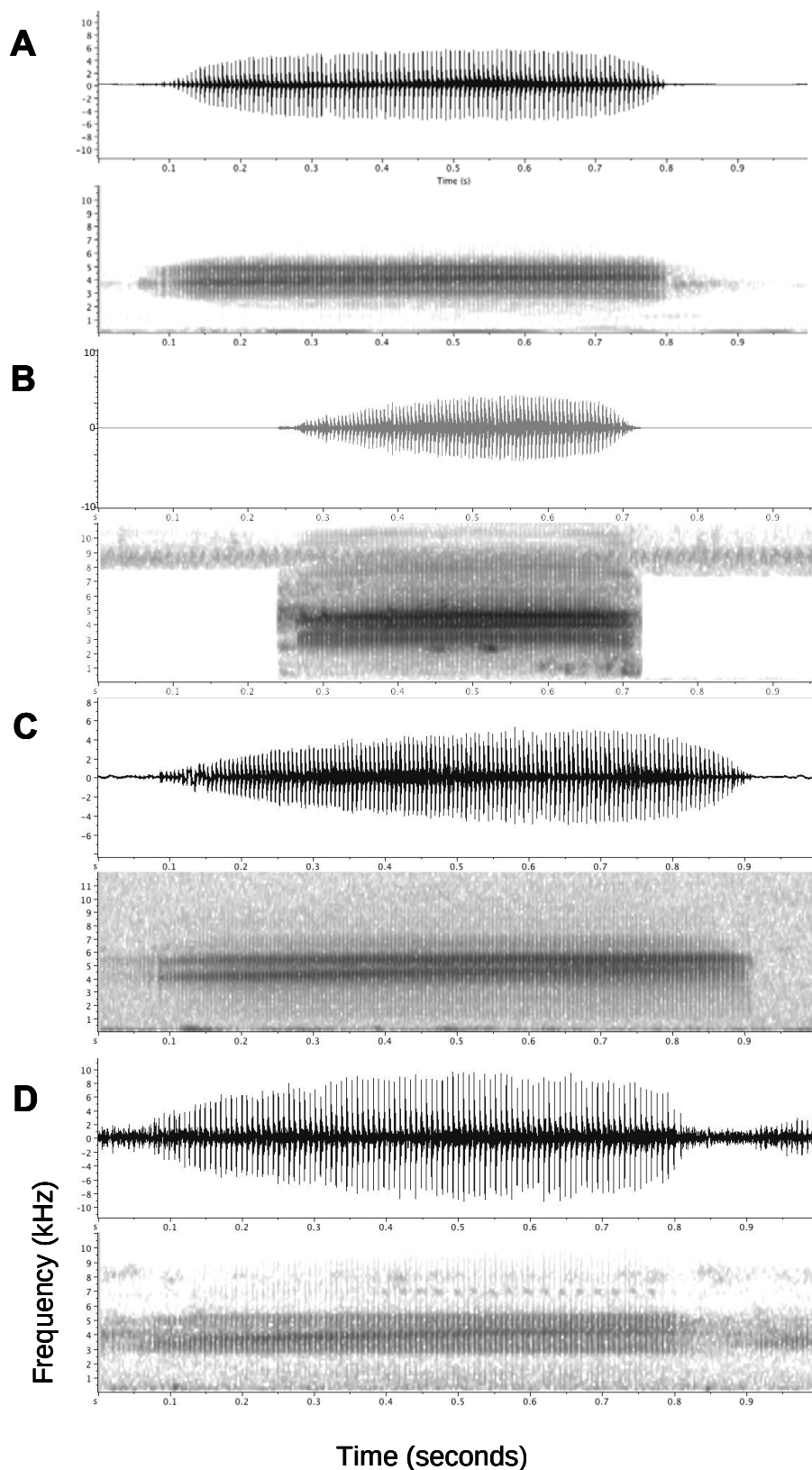


Figure 16. Oscillogram (above) and audiospectrogram (below) of A) *Scinax fuscomarginatus* toptype (CFBH 24360; 22:30 hs; air temperature 25° C), B) *S. parkeri* toptype (MJ 1316), C) *S. trilineatus* (ZUEC 9415 or 9416; 20:00 hs; 23° C), and D) *Hyla madeirae* toptype (CFBH 25469). All intervals correspond to one second.

Correspondence of available names

Scinax fuscomarginatus

In the original description of *Scinax fuscomarginatus*, A. Lutz (1925) did not diagnose nor compare *S. fuscomarginatus* to other species. The description is poorly informative, with the exception of few characters that define *S. fuscomarginatus* like the *canthus rostralis* with a brown stripe in the edge, dorsal pattern with two brown marginal stripes ranging from the eye to the inguinal region, a median (vertebral), and an interocular stripe forming a cross between the eyes; arms and legs with transversal brown bars, ventral region cream, gray and black points in the ventral surfaces of legs (Fig. 17). B. Lutz (1973) diagnosed *S. fuscomarginatus* and added a few characters besides those previously cited by A. Lutz (1925): small size, slender and elongate body, short legs, free fingers, and pustular dorsal surface.



Figure 17. Lectotype of *Scinax fuscomarginatus* ALMN 845. A) dorsal view, B), C), D) dorsal, ventral, and lateral views of the head.

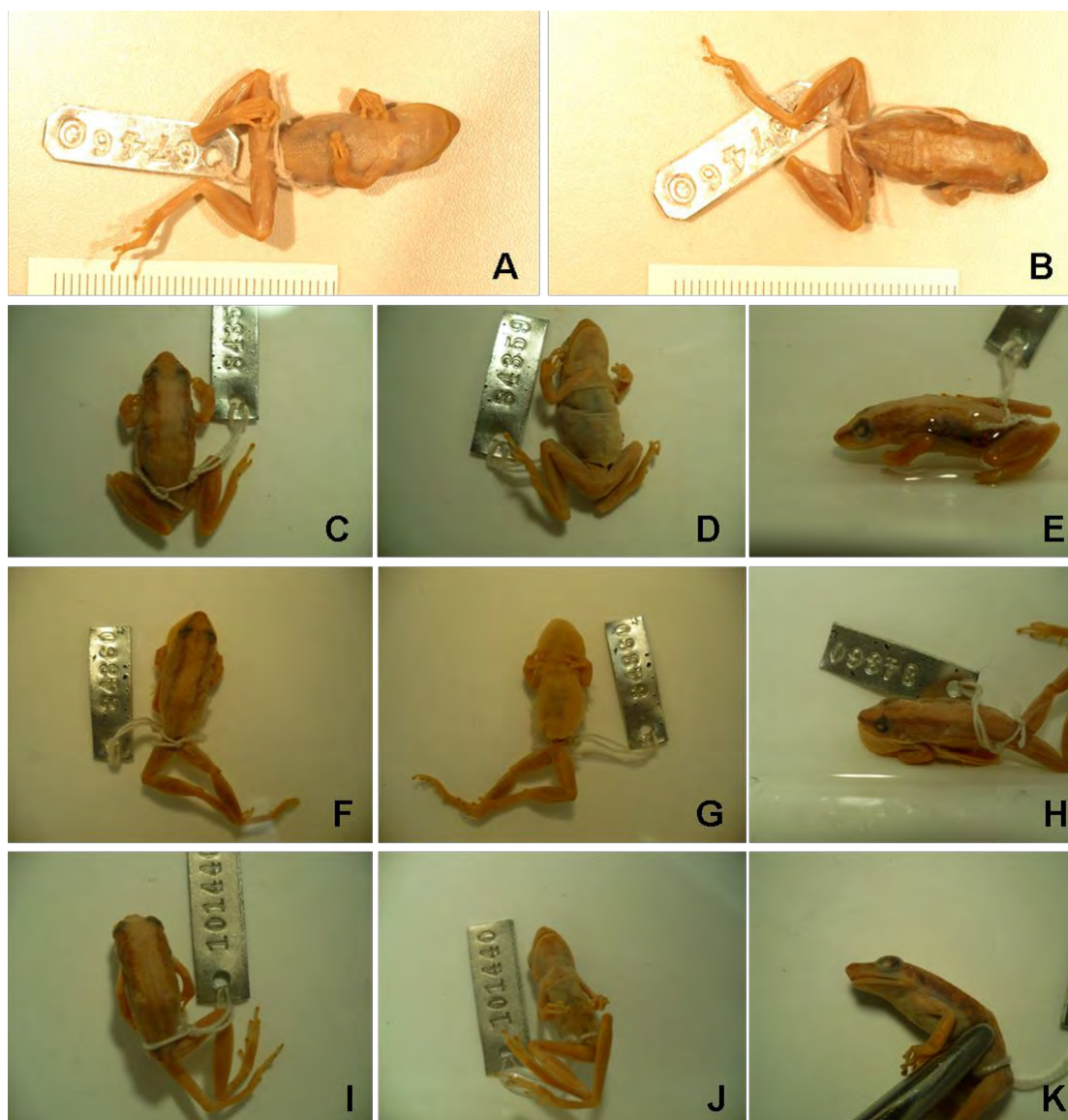


Figure 18. Types of *Scinax parkeri*. A), B) ventral and dorsal views of holotype UMMZ 67460; C), D), E) dorsal, ventral, and lateral views of paratype USNM 84359; F), G), H) dorsal, ventral, and lateral views of paratype USNM 84360; I), J), K) dorsal, ventral, and lateral views of paratype USNM 101440. Photograph by Bianca Berneck.

Scinax parkeri

Gaige (1929) diagnosed *Scinax parkeri* by its small size, long and narrow head and body, proportionally short legs, and free fingers (Fig. 18); all characters matching with *S. fuscumarginatus*, with which it was not compared. This species was synonymized by B. Lutz (1973) and revalidated by Duellman and Wiens (1992) without explanation. De la Riva et al. (1997) concluded that *S. parkeri* is a valid species arguing that the call from the type locality,

described by the same authors in a previous paper (DE LA RIVA et al., 1994) is markedly different from the calls of *S. fuscomarginatus* presented by Duellman & Pyles (1983) from Belém, state of Pará, Brazil, and by Cardoso (1981) from Campinas, state of São Paulo, Brazil. The only diagnostic character between *S. parkeri* and *S. fuscomarginatus* is the advertisement call. However, the call described by De la Riva et al. (1994) do not correspond to *S. parkeri* or any of the related species (see discussion), with new recordings of *S. parkeri* (type locality) examined here being extremely similar to other calls of *S. fuscomarginatus* (see Table 4).

Scinax trilineatus

In the original description Hoogmoed and Gorzula (1979) did not compare *Scinax trilineatus* with *S. fuscomarginatus* or *S. parkeri* (the two being already described at that moment), and the diagnosis and description presented is perfectly applicable to *S. fuscomarginatus*. Only two characters are different in *S. trilineatus* and *S. fuscomarginatus* according to the description, the first being the single palmar tubercle (in accordance with the figure of the holotype) not bifid as in *S. fuscomarginatus*. However, analyses of new material and pictures of the type series (Fig. 19) revealed the bifid condition of the palmar tubercle for *S. trilineatus*. The second character is the absence of an interocular stripe (present in *S. fuscomarginatus*), not mentioned in the text but absent in the holotype figure. However, the analyses of pictures of the holotype (RMNH 18257, Fig. 19, A and B), paratypes (RMNH 18258-60, Fig. 19, C-H), and additional specimens from Venezuela revealed the presence of the interocular stripe.

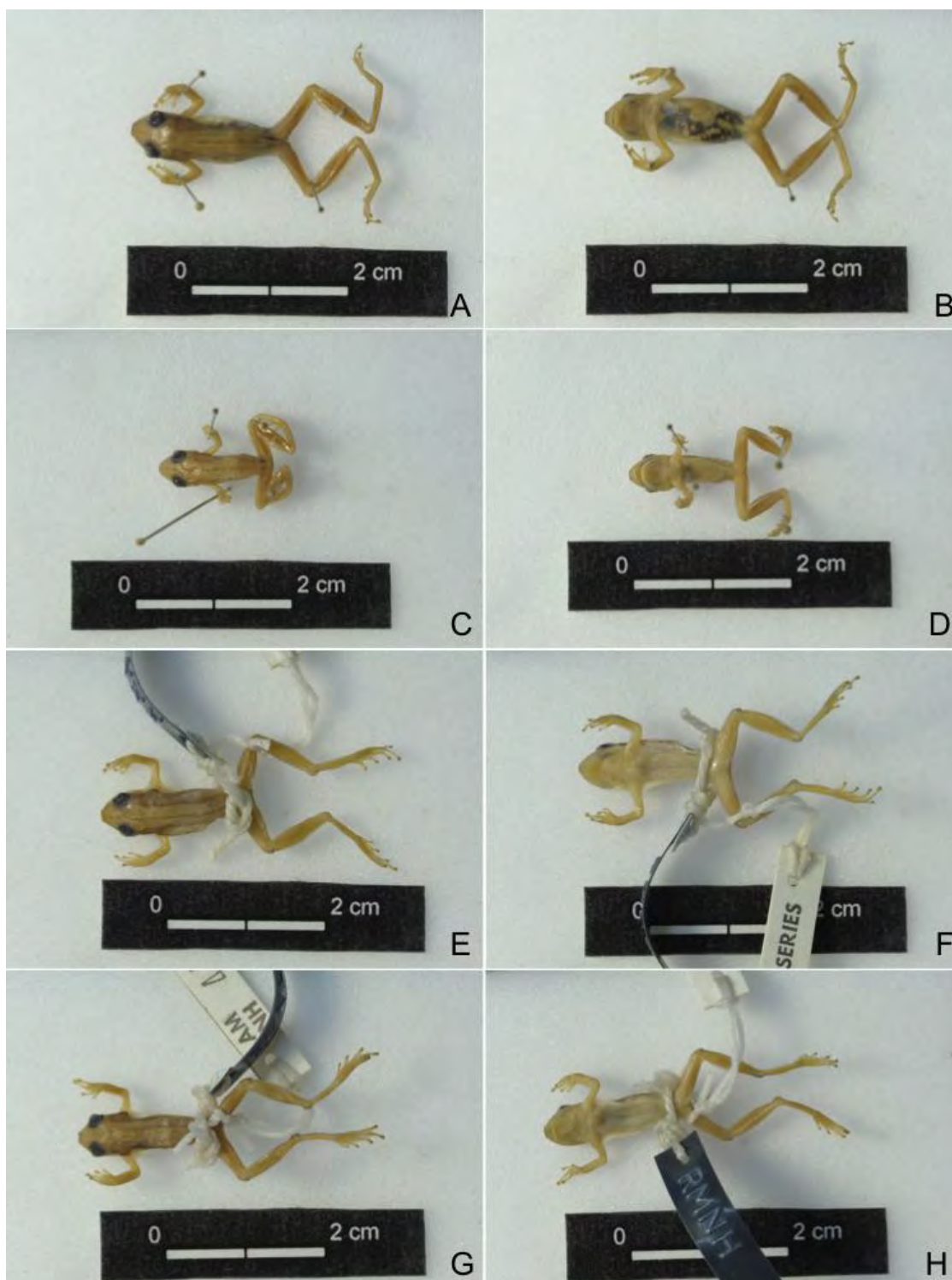


Figure 19. Types of *Scinax trilineatus*. A), B) dorsal and ventral views of holotype RMNH 18257; C), D) dorsal and ventral views of paratype RMNH 18260; E), F) dorsal and ventral views of paratype RMNH 18258; G), H) dorsal and ventral views of paratype RMNH 18259. Photograph by Ronald de Ruiter.



Figure 20. Types of *Scinax lutzorum*. A), B), C), D) dorsal and lateral views of the head, ventral views of hand and foot of the holotype MNRJ 51438; E), F), G), H) dorsal and lateral view of the head, ventral view of the hand and foot of the paratype MNRJ 51439. Photograph by Flavia Netto.

Scinax lutzorum

The description of *Scinax lutzorum* (CARDOSO and POMBAL, 2010) is based on two adult males from the Municipality of Aragominas, state of Tocantins, Brazil (Fig. 20). The authors compared *S. lutzorum* with *S. fuscomarginatus*, *S. parkeri*, and *S. trilineatus*, separating it by its larger size (SVL 23.7 and 25 mm). The maximum size presented by the

authors for the three species is 22.6 mm but we found a great variation in our sample of *S. fuscomarginatus* (SVL 15.65-26.69 mm) precluding to diagnose any of the currently recognized species (see Table 4). Cardoso and Pombal (2010) differs *S. lutzorum* from *S. fuscomarginatus* and *S. parkeri* by its longer snout (ESD/HL 0.43 and 0.51 in *S. lutzorum*). These values are also within the variation of *S. fuscomarginatus* (see Table 4) being very similar to the values presented here obtained from the lectotype and topotypes (ESD/HL 0.42-0.47). The values presented by the authors for *S. parkeri* clearly represent an error judging by the extremely high values presented (ESD/HL 0.8-1.0). We analyzed the same individuals of *S. parkeri* (MACN 14409-11) along with more material from Bolivia including topotypes, with values corresponding to those of *S. lutzorum* presented by Cardoso and Pombal (2010) (ESD/HL 0.41-0.47). Other characters proposed by Cardoso and Pombal (2010) to distinguish *Scinax lutzorum* from the related species are extremely variable in our sample even in an intra-population level, many of them being strongly influenced by the quality of fixation and conservation, and are within the variation presented here. The main diagnostic characters cited by Cardoso and Pombal (2010) to separate *S. lutzorum* and *S. fuscomarginatus* are the supratympanic fold (developed supratympanic fold in *S. lutzorum* and indistinct or slightly developed in *S. fuscomarginatus*; Cardoso and Pombal, 2010) and the degree of development of the webbing of Toe I (Toe I only fringed, with not webbing in *S. lutzorum* and webbing on third phalange of toe I in *S. fuscomarginatus*; Cardoso and Pombal, 2010). These two characters are comparable with that of the topotypes of *S. fuscomarginatus* (Fig. 21).

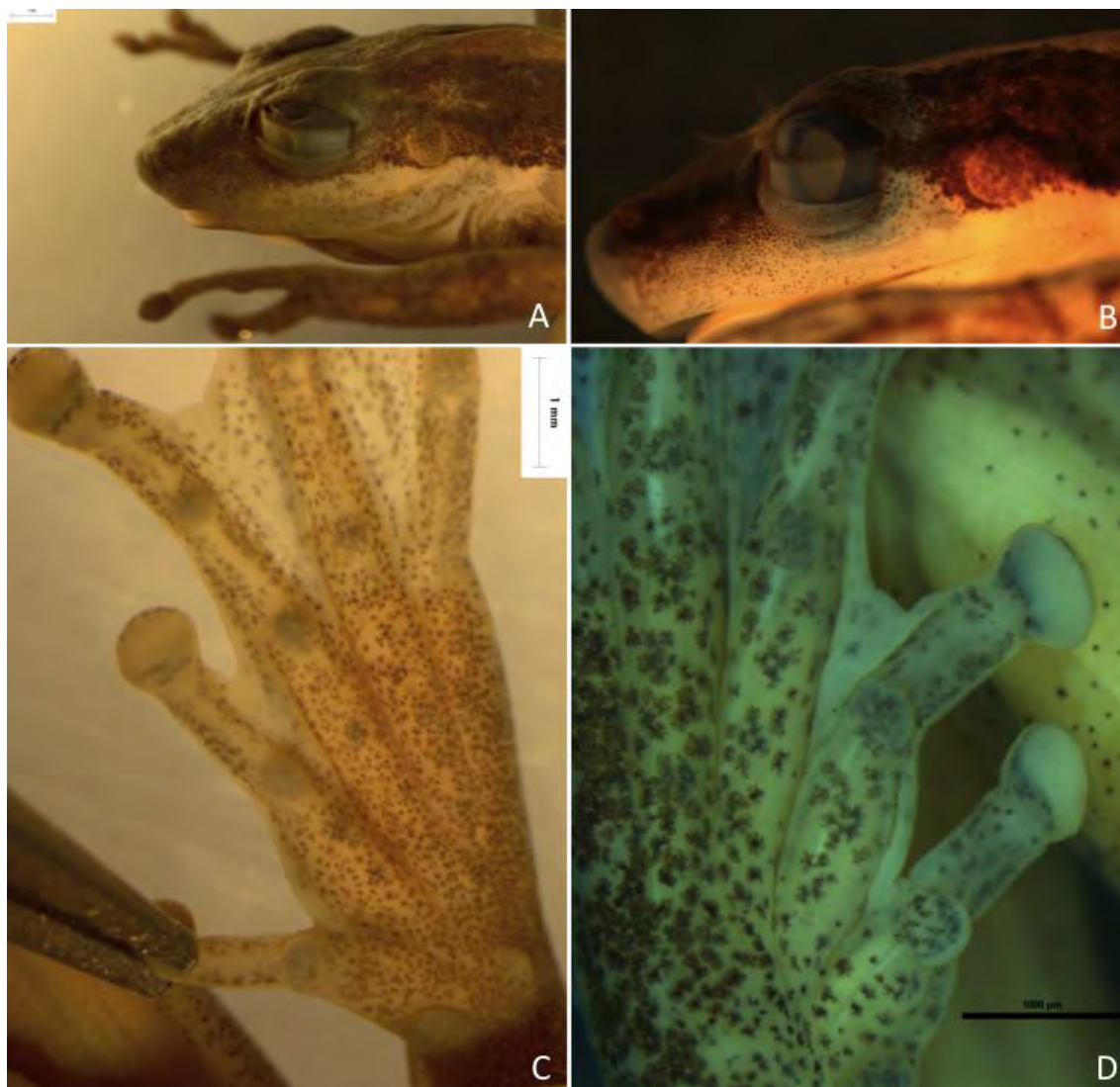


Figure 21. Detailed of supratympanic fold and degree of webbing in Toe I. A), C) Holotype of *Scinax lutzorum* (MNRJ 51438); B), D) Topotype of *Scinax fuscomarginatus* (CFBH 24363).

Hyla madeirae

Bokermann (1964) compared *Hyla madeirae* with *Scinax parkeri* (as *H. parkeri*) from which it differs by the wider head and more prominent snout in profile. The analyses of the type series and new topotypical material of *H. madeirae* show a general wider head but with a high degree of overlap in relation with *S. fuscomarginatus*. Nevertheless, differences in the snout shape are evident, being rounded in *Hyla madeirae* and sub-elliptical in *S. fuscomarginatus*. Other differences, not mentioned by Bokermann, but present in the holotype figure, are the convergent dorsolateral stripes and transversal bars in tibia (divergent or parallels dorsolateral stripes and oblique bars in tibia in *S. fuscomarginatus*) (Fig. 22).

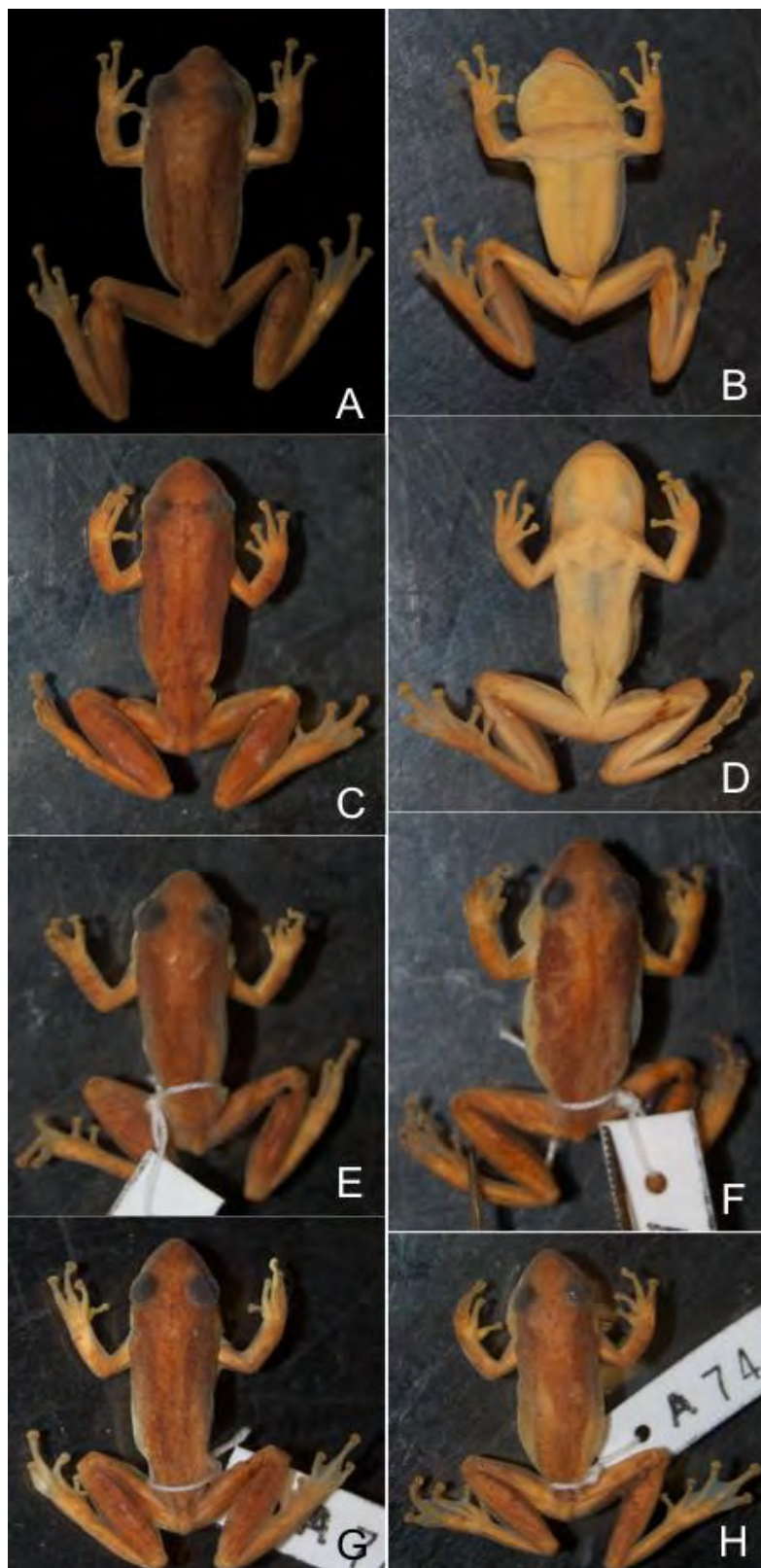


Figure 22. Types of *Hyla madeirae*. A), B) dorsal and ventral views of the holotype MZUSP 73663 (WCAB 10768); C), D) dorsal and ventral views of the allotype MZUSP 73658 (WCAB 10767); E) dorsal view of paratype MZUSP 74489; F) dorsal view of paratype MZUSP 74487; G) dorsal view of paratype MZUSP 74488; H) dorsal view of paratype MZUSP 74490.

Taxonomic conclusions

Based on the variation of molecular, morphologic, and bioacoustic characters we concluded that there is no evidence to support the taxonomic validity of *Scinax parkeri*, *S. trilineatus*, and *S. lutzorum*, because none of these species is distinguishable from *S. fuscomarginatus*. Therefore, we propose the synonymy of *S. parkeri* (GAIGE, 1929), *S. trilineatus* (HOOGMOED and GORZULA, 1979), and *S. lutzorum* Cardoso and Pombal, 2010 with *S. fuscomarginatus* (A. LUTZ, 1925).

Also based on the evidence presented here, we propose the revalidation of *Hyla madeirae* Bokermann, 1964 under the new combination *Scinax madeirae*. Finally, considering that the specimens from Serra do Cachimbo, state of Pará, Brazil are divergent under all analyzed criteria, not corresponding to any available name, we describe it as a new species (described below).

SPECIES ACCOUNTS

Scinax fuscomarginatus (A. LUTZ, 1925) redescription

Hyla fuscomarginata A. Lutz, 1925

Hyla parkeri Gaige, 1929 **New synonymy**

Ololygon fuscomarginata — Fouquette and Delahoussaye, 1977

Ololygon parkeri — Fouquette and Delahoussaye, 1977 **New synonymy**

Ololygon trilineata Hoogmoed and Gorzula, 1979 **New synonymy**

Scinax fuscomarginata — Duellman and Wiens, 1992

Scinax trilineata — Duellman and Wiens, 1992 **New synonymy**

Scinax parkeri — Duellman and Wiens, 1992 **New synonymy**

Scinax fuscomarginatus — Köhler and Böhme, 1996

Scinax trilineatus — Köhler and Böhme, 1996 **New synonymy**

Scinax lutzorum — Cardoso and Pombal, 2010 **New synonymy**

Lectotype: AL-MN 845 designated by Cardoso & Pombal (2010). Belo Horizonte, Minas Gerais, Brazil, collected by A. Lutz and J. Venâncio in November 1924 (specimen examined).

Paralectotypes: AL-MN 847-850 and USNM 96964 designated by Cardoso & Pombal, 2010. Belo Horizonte, Minas Gerais, Brazil, collected by A. Lutz in November 1924 (specimens examined, USNM 96964 pictures).

Diagnosis

A small size species (males SVL 15.65-26.69 mm) of slender body characterized by dorsal pattern with continuous diverging or parallels brown dorsolateral stripes in a light brown background, uniform light brown color on the posterior surfaces of the thighs, immaculate or finely pointed chest and belly, longitudinal dark stripes on the forearm and tibia external surface, loreal region steep sloping toward lip, and diagonal stripes in dorsal surface of tibia.

Comparison with other species

We compared *Scinax fuscomarginatus* with species of the *S. ruber* clade that correspond geographically, excluding the *S. rostratus* and *S. uruguayus* species group (sensu FAIVOVICH et al., 2005).

Males of *Scinax fuscomarginatus* have smaller body size (SVL 15.65-26.69 mm) compared to *S. acuminatus* (SVL 39-45 mm; B. LUTZ, 1973), *S. boesemani* (28.4-31.8 mm; DUELLMAN, 1986), *S. camposseabrai* (SVL 28.9-33.5 mm; CARAMASCHI and CARDOSO, 2006), *S. castroviejoi* (SVL 41.9 mm; DE LA RIVA, 1993), *S. chiquitanus* (SVL 33.3 mm; DE LA RIVA, 1990), *S. dolloi* (SVL 34.9 mm; PUGLIESE et al., 2009), *S. eurydice* (SVL 44-52 mm; BOKERMANN, 1968), *S. funereus* (SVL 28.7-35.1 mm; DUELLMAN, 1971), *S. fuscovarius* (SVL 41-44 mm; CEI, 1980), *S. hayii* (SVL 39-43 mm; HEYER et al., 1990), *S. iquitum* (35 mm; MORAVEC et al., 2009), *S. manriquei* (SVL 27.7 mm; BARRIO-AMORÓS et al., 2004), *S. perereca* (SVL 34–38.5 mm; POMBAL et al., 1995a), and *S. similis* (SVL 29.4–34.7 mm; CARAMASCHI and CARDOSO, 2006).

By its uniform light brown color on the posterior surfaces of the thighs, *Scinax fuscomarginatus* can be differentiated from *S. caldarum*, *S. cardosoi*, *S. curicica*, *S. duartei*, *S. granulatus*, *S. hayii*, *S. maracaya*, *S. nasicus*, *S. similis* (all with flash yellow color on the posterior surface of the thigh), and *S. rogerioi* (brown spots on the posterior surface of the thigh; PUGLIESE et al., 2009).

By its dorsal pattern with continuous brown dorsolateral stripes from the upper eyelids to the inguinal region *Scinax fuscomarginatus* differs from *S. acuminatus*, *S. baumgardneri* (DUELLMAN, 1986), *S. boesemani* (DUELLMAN, 1986), *S. cabralensis* (DRUMMOND et al., 2007), *S. camposseabrai*, *S. crospedospilus*, *S. danae* (DUELLMAN, 1986), *S. eurydice*, *S. lindsayi* (PYBURN, 1992) and *S. maracaya* (all without dorsolateral stripes); from *S. alter*,

S. auratus, *S. cretatus* (NUNES and POMBAL, 2011) and *S. squalirostris* (all with dorsolateral white stripes); from *S. caldarum* and *S. duartei* (the two with dorsal stripes from between the eyes and connected with a interocular blotch); from *S. pachycrus* (with a lateral dark subcantal stripe from corner of the eye to the inguinal region; B. LUTZ, 1973); from *S. cuspidatus* (with irregular stripes or inverted parenthesis on thoracic region; see B. LUTZ, 1973); and from *S. cruentommus* (with irregular longitudinal marks; DUELLMAN, 1972).

Scinax fuscomarginatus differs from *S. exiguus* by its immaculate or finely spotted chest and belly (all ventral spotted in *S. exiguus*); tympanum slightly distinct (distinct in *S. exiguus*); more developed toes webbing; dark longitudinal stripe in forearm and tibia external surface (lacking in *S. exiguus*); dark brown dorsolateral stripes in a light brown background (light brown dorsolateral stripes in *S. exiguus*).

Scinax fuscomarginatus differs from *S. madeirae* by its sub-elliptical snout in dorsal view (rounded in *S. madeirae*); loreal region steep sloping toward lips (softly in *S. madeirae*); a white, unpigmented or finelly spotted area in upper lip (strongly spotted forming patches in *S. madeirae*); lateral of the body immaculate or finely spotted (strongly spotted in *S. madeirae*); longitudinal dark stripe on the external surface of tibia (absent in *S. madeirae*); dorsolateral stripes divergent or parallels (convergent in *S. madeirae*); oblique stripes on the dorsal surface of tibia (transversal in *S. madeirae*).

Scinax fuscomarginatus differs from *Scinax -eachimbo*” sp. nov. by the dark stripe on the external surface of tibia (absent in *S. -eachimbo*” sp. nov.); oblique stripes on the dorsal surface of tibia (transversal in *S. -eachimbo*” sp. nov.); loreal region steep slope toward the lip (softly sloping in *S. -eachimbo*” sp. nov.); sub-elliptical head in dorsal view (rounded in *S. -eachimbo*” sp. nov.); and by the more developed interdigital webbing, I 2-2 II 1^{1/2}-2 III 1 or 1^{1/2}-2 IV 2-1 or 1^{1/2} V (II 3 – 2 III 3 – 1^{1/2} or 2 IV in *S. -eachimbo*” sp. nov.).

Redescription

Based on the lectotype (ALMN 845) and new topotypical material (CFBH 24356-63 and 24364, Fig. 23). A slender small size frog (males SVL 20.19-21.75 mm), body wide equal to the head. Snout sub-elliptical in dorsal view and protrude in profile. Head longer than wide (HW/HL 83%). Nostrils protuberant, dorsolaterally located, IND 68% of the IOD. *Canthus rostralis* rounded. Loreal region concave and steep sloping toward to lip. Eye large, protuberant, 99% of IOD and slightly larger of EN. Tympanum slightly distinct, small, 81%

of disc diameter of Finger III; tympanic annulus rounded with the superior portion hidden by the supratympanic fold; tympanic membrane of the same color that adjacent region (sometimes with a few less chromatophores density). Supratympanic fold distinct from posterior corner of the eye to the arm insertion. In the mouth corner a row of variable number of tubercles with the first one bigger than others. Vocal sac large, single, subgular, expanded externally, evident by the presence of loose skin with longitudinal folds, laterally reaching the posterior region of the mouth corner, and ventrally reaching the level of the pectoral fold. Tongue ovoid, with lateral and posterior borders free, shallow notch in the posterior region. Vocal slit longitudinal, from the mediolateral portion of the tongue to the jaw angle. Vomerine dentigerous processes between the medial and posterior region of the choanas, without contact between them, 4 to 9 teeth each; choanae oval, slightly diagonal to the sagittal axis.

Arm slender than forearm. Row of unpigmented flat tubercles in the external surface of the forearm. Fingers short; finger discs elliptical, wider than long, disc of Finger I markedly smaller than the others; webbing reduced to a fringed in the proximal and distal surfaces of the Fingers II and III, absent in the others. Ulnar ridge present. Palmar tubercle bifid. Subarticular tubercles of the Fingers I and II conical, of Finger III rounded, and of Finger IV with the distal margin truncated but not bifid. Thenar tubercle large, occupying much of the base of Finger I; a pad between the subarticular tubercle of Finger I and the Palmar tubercle. Unpigmented nuptial pads occupying the posterior region of the base of the Finger I and the posterodorsal region until the distal margin of the subarticular tubercle. Supernumerary tubercles rounded. Hind limbs slender, tibia 51% and femur 43% of SVL. Row of unpigmented flat tubercles in the external surface of the tarsus. Toes slender. Elliptical discs, wider than long, that of the Toes I and II smaller than the others. Oval inner metatarsal tubercle. External metatarsal tubercle rounded, half size of the inner, located with the proximal margin at the level of the distal margin of the Inner metatarsal tubercle or slightly passing. A cutaneous ridge in the proximal surface of Toe I from inner metatarsal tubercle to subarticular tubercle. Cutaneous ridge in the external margin of the base of Toe V. Supernumerary tubercles rounded in the base of the toes. Webbing formula I 2-2 II $1^{1/2}$ -2 III 1 or $1^{1/2}$ -2 IV 2-1 or $1^{1/2}$ V. Tarsal fold absent.

Pre-axilar pectoral fold. Dorsolateral region of the body, dorsal region of the head, upper eyelid, and dorsal region of hind limbs covered with rounded pustules; gular region smooth; chest and belly granular; ventral surface of the thighs shagreened.

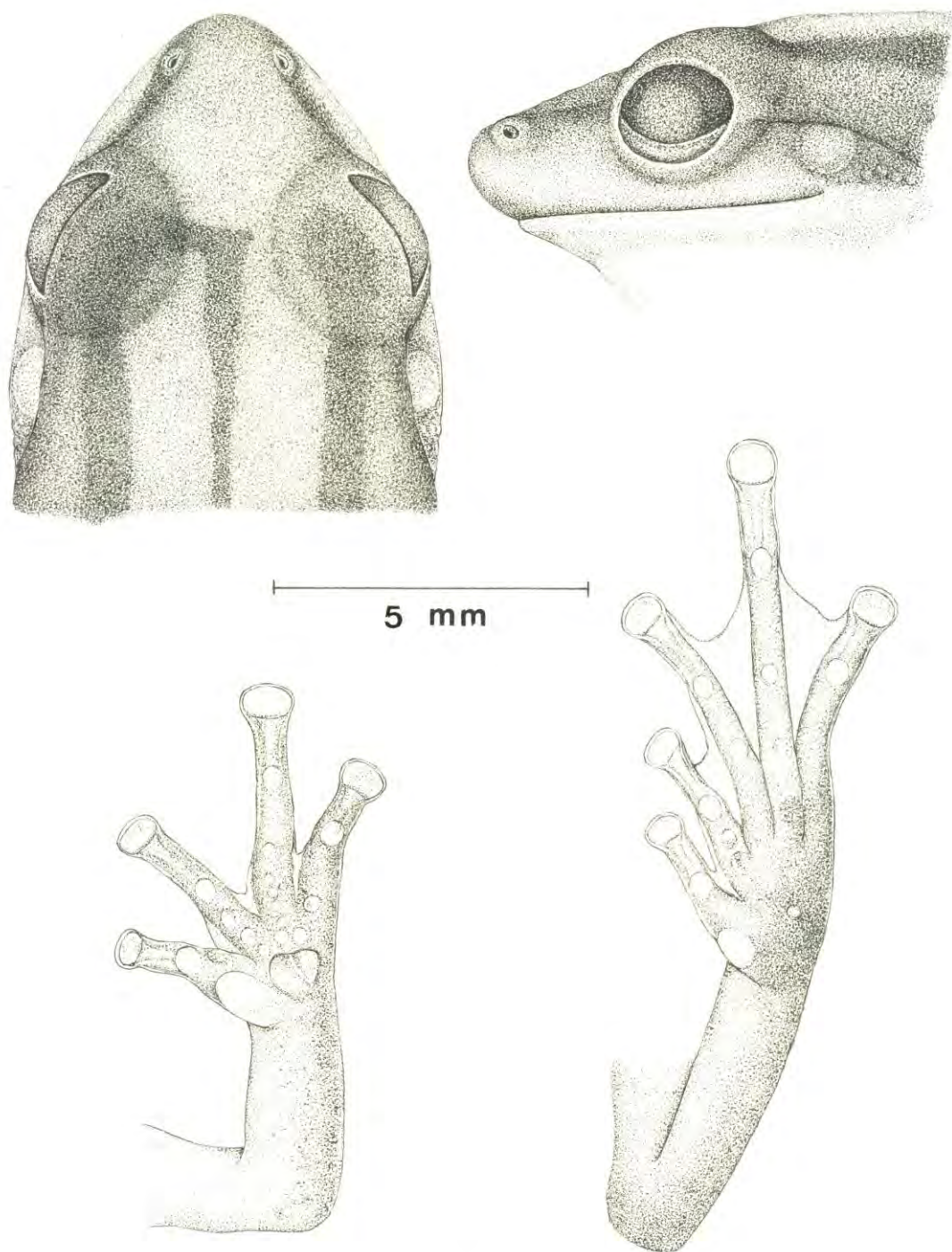


Figure 23. *Scinax fuscomarginatus* (CFBH 24364, topotype). Dorsal and lateral views of the head, ventral views of hand and foot.

Color in preservative

General light brown with a pair of continuous divergent dark brown dorsolateral stripes from upper eyelids to the inguinal region where is more diffused; the supratympanic fold present the same coloration of the dorsolateral stripes; below the dorsolateral stripes and to the level of inferior border of the tympanum a wide brown stripe (with less chromatophores density than dorsolateral stripes) from the supratympanic fold to the inguinal region; dark brown stripe on the *canthus rostralis*; loreal region with sparked chromatophores loosing density to the upper lip; between the eye and the upper lip only a few chromatophores or absent of them; vertebral line from the internostril region to the sacral region wider between the eyes and nostrils, in the portion between the eyes, median region of the dorsum, and in the sacral region; a wide dark brown interocular stripe; finger discs pigmented, dorsal surface of hands with dark brown dots and transversals stripes in Fingers III and IV, like in the forearms. A longitudinal dark brown stripe on the external surface of the Finger IV to the elbow; dorsal surfaces of leg with diagonal dark brown stripes, being one on the heel and three on the tibia; external surfaces of tibia and tarso with a longitudinal dark brown stripe; ventrally, external borders of the gula, shoulders and borders of the belly finely spotted, immaculate in the rest of belly and chest; occult and posterior parts of the thighs and inguinal region uniform light brown.

Variation

Complete variation on measurements and body proportion is given in Table 4. Some specimens show a more pointed snout in dorsal view (Fig. 24, A and B) and others have less developed toe webbing, mainly between Toes II-III (Fig. 24, C and D).

The dorsal pattern presents variation related to the vertebral line, interocular stripe, and dorsolateral stripes (see intrapopulational variation in Fig. 25). Some individuals have longitudinal marble stripe in dorsal tibia surface unlike of diagonal stripes. In general darker individuals have the lateral of body more spotted, finely doted in the chest and in some cases with dark brown pigmentation between the glands that formed the nuptial pad.

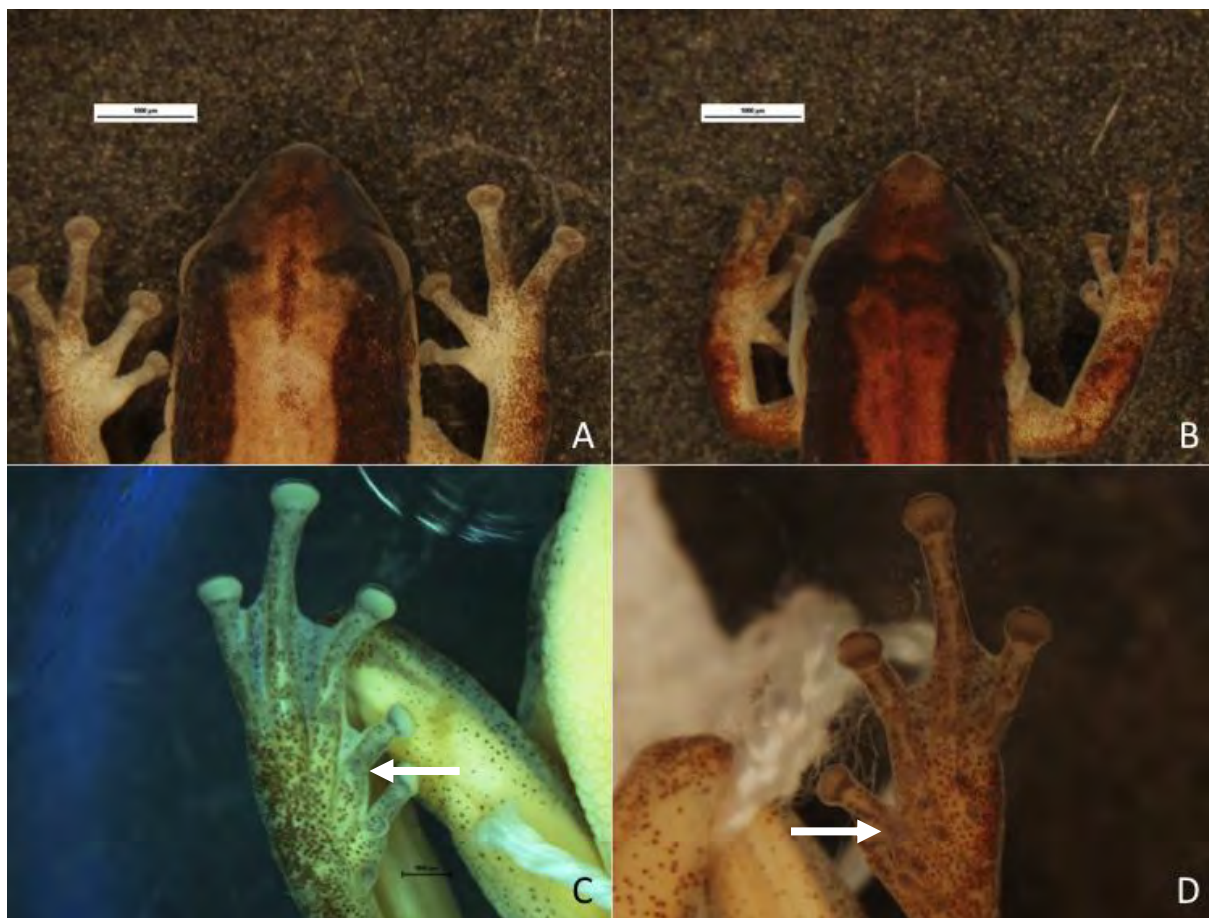


Figure 24. Dorsal views of head of *Scinax fuscomarginatus* showing the variation in snout shape; A) rounded snout (CFBH 24363, Lagoa Santa, MG), B) pointed snout (CFBH 20548, Jaborandi, BA); ventral views of foot of *Scinax fuscomarginatus* showing the webbing variation; C) webbing reaching the median portion between the subarticular tubercle and the terminal disc of the Toe II (CFBH 24363), D) webbing slightly passing the subarticular tubercle of the Toe II (CFBH 20548). Arrow indicates the position of the subarticular tubercle of Toe II.

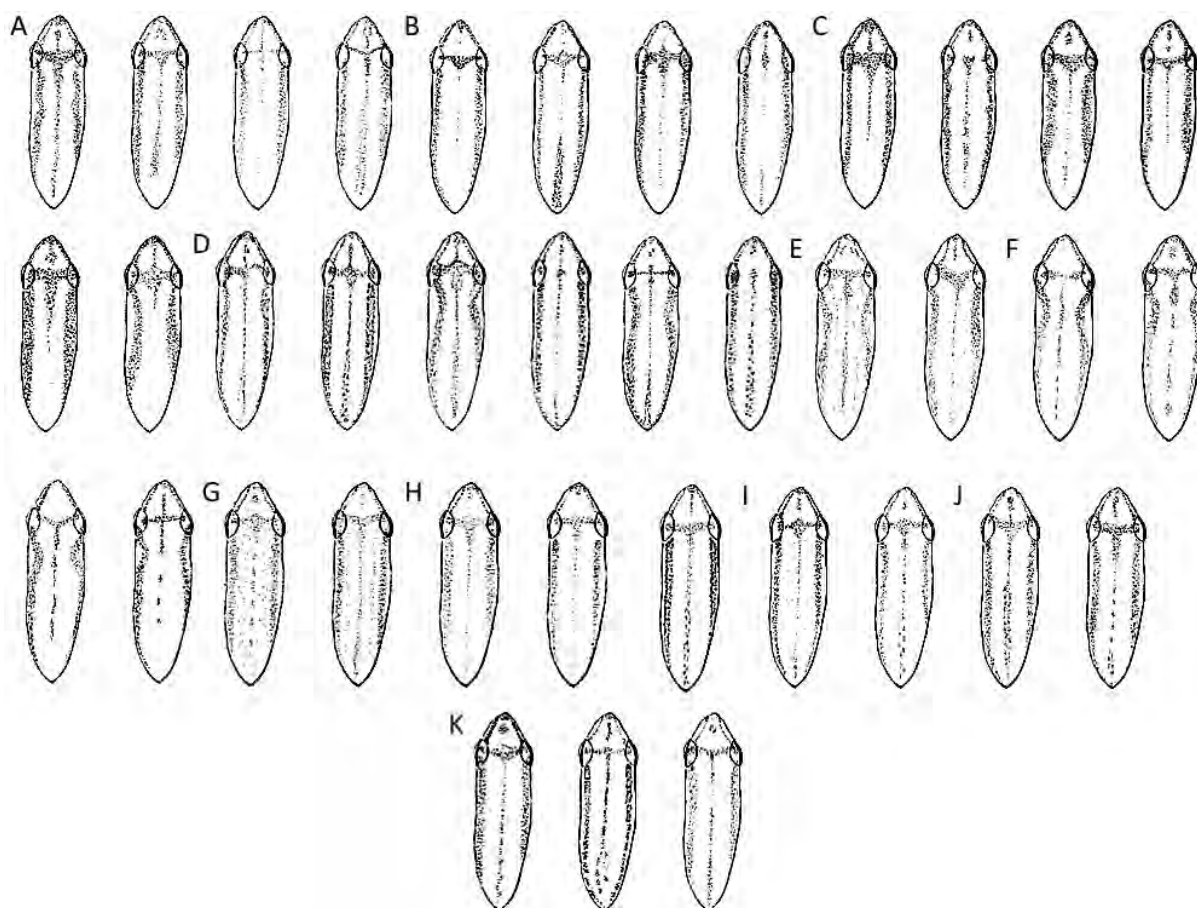


Figure 25. *Scinax fuscomarginatus* intrapopulation dorsal variation (vertebral line, interocular stripe and dorsolateral stripes). Letters represent different populations: A) Lagoa Santa, Minas Gerais, Brazil; B) Mabarakajú Reserve, Canindeyú, Paraguay; C) Cerro Corá National Park, Amambay, Paraguay; D) Chapada dos Guimarães, Mato Grosso, Brazil; E) Altamira, Pará, Brazil; F) Passo de Camarajibe, Alagoas, Brazil; G) Formoso de Araguaia, Tocantins, Brazil; H) Porto Nacional, Tocantins, Brazil; I) Isla Yasyretá, Itapúa, Paraguay; J) Estancia Pirá Potrero, Amambay, Paraguay; K) Fazenda Lazer, Porto Nacional, Tocantins, Brazil.

Advertisement call

The advertisement call of *Scinax fuscomarginatus* were described in several works and from different localities (DUELLMAN and PYLES, 1983; DE LA RIVA et al., 1994; POMBAL et al., 1995; TOLEDO and HADDAD, 2005). All the variation is given in Table 4. The advertisement call of *Scinax fuscomarginatus* (n=56 males) is constituted by a single multipulsed note with modulated frequency. The average note duration is 0.54 seconds long (std= 0.20; amplitude= 0.295-0.831), the average dominant frequency is 3776.10 Hz (std=

325.04; amplitude=3042.08-4457.67), and the average pulses rate is 172.49 pulses/seconds (std= 23.22; amplitude=113.42-271.74).

Distribution

Scinax fuscomarginatus is a widely distributed species, occurs from northern Argentina to north and northeastern Brazil, occupying open areas of Chaco, Cerrado, Pantanal, Chiquitania and lowlands of Venezuela, Guyana and Suriname, and in transition zones of that with Brazilian Atlantic Forest and Amazonia (sensu DINERSTEIN et al., 1995). The more austral record is from Corrientes province (Argentina); in Paraguay is present in Alto Paraná, Amambay, Canindeyú, Concepción, Cordillera, Guairá, Itapúa, Misiones, Ñeembucú, Presidente Hayes, and San Pedro departments. In Bolivia was registered in Santa Cruz de la Sierra and Beni departments. In Brazil is found in the states of Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, São Paulo, Tocantins, Pará, southern Bahia, and in single localities of Maranhão, Ceará, Piauí and Alagoas. We only include samples from one locality from Venezuela, in the state of Bolívar (Santa Elena de Uairén), but Hoogmoed and Gorzula (1979) described *Oloolygon trilineatus* (herein synonymized with *Scinax fuscomarginatus*) from El Manteco, state of Bolívar and also include as paratypes specimens from southern Guyana (Rupununi River) and southern Suriname (Sipaliwini savannas).

Remarks

Alvarez et al. (1996) cited *Scinax* aff. *fuscomarginatus* for the provinces of Corrientes and Formosa, Argentina, considered by Lavilla et al. (2002) as *S. fuscomarginatus*. In Bolivia De la Riva et al. (2002) and Reichle (2006) cited *S. parkeri* and *S. cf. fuscomarginatus*, the first corresponding to *S. fuscomarginatus* and the second to *S. madeirae*, according with our analysis.

Scinax madeirae (BOKERMANN, 1964) new combination, redescription

Hyla madeirae Bokermann, 1964

Holotype: MZUSP-A 73663 (WCAB 10768) (male) Porto Velho, Rondônia, Brazil (specimen examined).

Alotipo: MZUSP-A 73658 (WCAB 10767) (female). Porto Velho, Rondônia, Brazil (specimen examined).

Paratypes: WCAB 10766 (female), WCAB 10769-92 (24 males), MNRJ 3963 (male). Porto Velho, Rondônia, Brazil. November 1962 (five specimens examined, MZUSP-A 74487-90, and MNRJ 3963).

Diagnosis

A small species (males SVL 18-23 mm) of slender body characterized by the dorsal pattern with continuous convergent dark brown dorsolateral stripes in a light brown background, uniform light brown color on the posterior surfaces of the thighs, loreal region softly sloping toward the lips, and transversal stripes on the dorsal surface of tibia.

Comparison with other species

We compared *Scinax madeirae* with species of the *S. ruber* clade that correspond geographically, excluding the *S. rostratus* and *S. uruguayus* species group (sensu FAIVOVICH et al., 2005).

By its smaller size in males (SVL 18.03-23.05 mm) *Scinax madeirae* differs from *S. acuminatus* (SVL 39-45 mm; B. LUTZ, 1973), *S. boesemani* (28.4-31.8 mm; DUELLMAN, 1986), *S. camposseabrai* (SVL 28.9-33.5 mm; CARAMASCHI and CARDOSO, 2006), *S. castroviejoi* (SVL 41.9 mm; DE LA RIVA, 1993), *S. chiquitanus* (SVL 33.3 mm; DE LA RIVA, 1990), *S. cretatus* (25.8-34.7 mm; NUNES and POMBAL, 2011), *S. dolloi* (SVL 34.9 mm; PUGLIESE et al., 2009), *S. eurydice* (SVL 44-52 mm; Bokermann, 1968), *S. funereus* (SVL 28.7-35.1 mm; DUELLMAN, 1971), *S. fuscovarius* (SVL 41-44 mm; CEI, 1980), *S. hayii* (SVL 39-43 mm; HEYER et al., 1990), *S. iquitum* (35 mm; MORAVEC et al., 2009), *S. manriquei* (SVL 27.7 mm; BARRIO-AMORÓS et al., 2004), *S. nasicus* (SVL 27-35 mm; CEI, 1980), *S. perereca* (SVL 34-38.5 mm; POMBAL et al., 1995a), and *S. similis* (SVL 29.4-34.7 mm; CARAMASCHI and CARDOSO, 2006).

By its uniform light brown color on the posterior surfaces of the thighs *Scinax madeirae* can be differentiated from *S. caldarum*, *S. cardosoi*, *S. curicica*, *S. duartei*, *S. granulatus*, *S. hayii*, *S. maracaya*, *S. nasicus*, *S. similis* (flash yellow color on the posterior surface of the thigh), and *S. rogerioi* (brown spots on the posterior surface of the thigh; PUGLIESE et al., 2009).

By its dorsal pattern with continuous dark brown dorsolateral stripes from the upper eyelids to the inguinal region *Scinax madeirae* differs from *S. acuminatus*, *S. baumgardneri* (DUELLMAN, 1986), *S. boesemani* (DUELLMAN, 1986), *S. cabralensis* (DRUMMOND et al., 2007), *S. camposseabrai*, *S. crospedospilus*, *S. danae* (DUELLMAN, 1986), *S. eurydice*, *S. lindsayi* (PYBURN, 1992), and *S. maracaya* (without dorsolateral stripes); from *S. alter*, *S. auratus*, and *S. cretatus* (NUNES and POMBAL, 2011), *S. squalirostris* (dorsolateral white stripes); from *S. caldarum* and *S. duartei* (dorsal stripes from between the eyes and connected with a interocular blotch); from *S. pachycrus* (a lateral dark subcantal stripe from corner of the eye to the inguinal region; B. LUTZ, 1973); from *S. cuspidatus* (irregular stripes or inverted parenthesis on the thoracic region; see B. LUTZ, 1973); and from *S. cruentommus* (irregular longitudinal marks; DUELLMAN, 1972).

Scinax madeirae differs from *S. exiguus* by its finely pointed chest, belly, and borders of gula (spotted in *S. exiguus*); more developed toes webbing; brown dorsolateral stripes in a light brown background (light brown dorsolateral stripes in *S. exiguus*).

Scinax madeirae differs from *S. fuscomarginatus* by its rounded snout in dorsal view (sub-elliptical in *S. fuscomarginatus*); loreal region softly sloping toward the lips (steep sloping in *S. fuscomarginatus*); upper lip markedly spotted (finely spotted, unpigmented, or white stripe in *S. fuscomarginatus*); lateral body markedly spotted (immaculate or finely spotted in *S. fuscomarginatus*); convergent dorsolateral stripes (divergent or parallel in *S. fuscomarginatus*); transversal stripes on the dorsal surface of tibia (oblique in *S. fuscomarginatus*).

Scinax madeirae differs from *S. -eachimbo* sp. nov. by its larger size in males [*S. madeirae* SVL 20.85 ± 1.41 (18.03-23.05 mm); *S. -eachimbo* sp. nov. SVL 18.61 ± 0.93 (16.71-19.97)], convergent dorsolateral stripes (divergent in *S. -eachimbo* sp. nov.), and by the more developed interdigital webbing (II 2 – 1 III 2 – 1 IV) (II 3 – 2 III 3 – 1 $\frac{1}{2}$ or 2 IV in *S. -eachimbo* sp. nov.).

Redescription

The description is based on types specimens (MZUSP-A 73663 holotype, MZUSP-A 74487-90, MNRJ 3963 paratypes) and new topotypes (CFBH 25469, Fig. 26; CFBH 25470). A slender small size frog (SVL in males 18.99-22.59 mm) with the posterior region of the body slightly wider than the head. Head dorsally rounded, slightly wider than long (HW/HL 92%). Snout protruding in profile. Nostrils oriented dorsolaterally, upon elevations, IND 69% of IOD. *Canthus rostralis* straight. Loreal region concave, softly sloping toward the lips. Eye large and protruding, approximately equal to IOD and slightly larger than END. Tympanum slightly evident and small, 92% of the diameter of disc of the Finger III; oval tympanic annulus with the upper margin occult by the supratympanic fold; tympanic membrane with the same coloration of the adjacent region. Supratympanic fold evident, from the posterior corner of the eye to the arm insertion. A group of rounded tubercles between the corner of the mouth and the terminal margin of the supratympanic fold. Vocal sac large, single, subgular, evident by the presence of loose skin with longitudinal folds; ventrally reach the shoulders. Ovoid tongue, with lateral and posterior borders free, with a shallow notch in the posterior region. Longitudinal vocal slits from the mediolateral portion of the tongue to the jaw angle. Vomerine dentigerous processes between the posterior margin of the choanae, without contact between them, four (CFBH 25469) or three teeth (CFBH 25470) in each process; oval choanae diagonal to the sagittal axis.

Arm slender than forearm. Fingers short. Subelliptical finger discs, wider than long, disc of Finger I slightly smaller than the others; webbing reduced to fringe in the proximal and distal surfaces of the Fingers II and III, absent in the others. Ulnar ridge present from the distal margin of the palmar tubercle to the subarticular tubercle. Bifid pigmented palmar tubercle. Pigmented subarticular tubercles; that of the Fingers I and II conical, Finger III rounded, Finger IV with the distal margin truncated but not bifid. Thenar tubercle large, occupying much of the base of the Finger I; between the subarticular tubercle of the Finger I and the palmar tubercle there is one flat rounded pigmented tubercle. The nuptial pads are pigmented between the glands, occupying the posterior region of the base of Finger I and its posterodorsal region reaching the level of the subarticular tubercle. Supernumerary tubercles pigmented and rounded. A row of flat unpigmented tubercles in the ventro-lateral and external surfaces of the forearm. Hind limbs slender; tibia 47% and thigh 43% of the SVL. Toes slender. Elliptical discs wider than long; discs of Toes I and II slightly smaller than the others. Inner metatarsal tubercle oval; outer metatarsal tubercle rounded, its size half of the size of

the internal metatarsal tubercle; the outer metatarsal tubercle is located with the proximal margin at the level of the distal margin of the Inner metatarsal tubercle. Subarticular tubercles of Toes I and II conical, the others are rounded. A flat rounded tubercle between the internal metatarsal tubercle and the subarticular tubercle of Toe I. A slightly evident cutaneous ridge in the proximal surface of Toe I. Supernumerary tubercles rounded. Webbing formula I 2-2 II 1^{1/2}-2 III 1^{1/2}-2 IV 2-1 V. Tarsal fold absent. Pectoral fold pre-axilar. Dorsolateral region of the body, dorsal head, and upper eyelids with rounded pustules; gular region smooth; thighs ventral surface shagreen, belly and chest granular.

Color in preservative

Light brown in general with two convergent dark wide stripes from the upper eyelids to the sacral region where they are more diffuse; the supratympanic fold is black; below the dorsolateral stripes and to the level of inferior border of the tympanum a wide brown stripe (less chromatophores density than dorsolateral stripes) from the supratympanic fold to the inguinal region; *canthus rostralis* dark brown, loreal region with the same coloration of the dorsum; dark patches in upper lip; vertebral line poorly evident, wider and more evident between the eyes; interocular stripe wide; dorsal surfaces of hand and foot pigmented with small rounded unpigmented areas; hidden surface of thigh uniform light brown (finely and dense spotted); dorsal surface of leg with four perpendicular stripes, one on the heel and three on the tibia; lateral region of the body with dark blotches, like the external border of the gula and shoulder; the remaining ventral surface immaculate; ventral surface of thigh spotted with blotches in the cloacal region; tympanic membrane with the same color that the adjacent region.

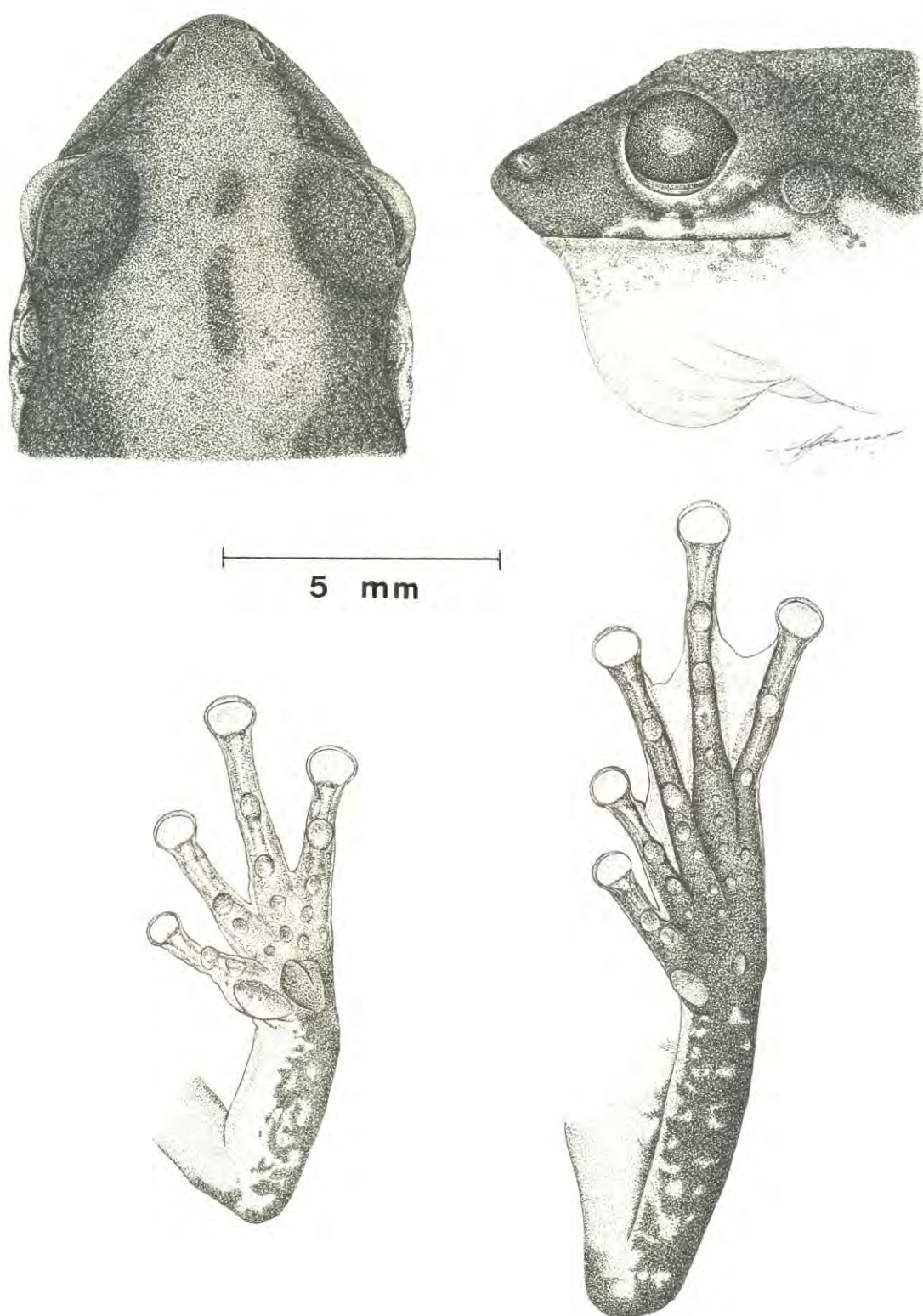


Figure 26. *Scinax madeirae* new combination (CFBH 25469, topotype). Dorsal and lateral views of head, ventral views of hand and foot.

Variation

Complete variation on measurements and body proportions is given in Table 4. Besides the different degrees of color conservation, the general color pattern is very similar in the entire sample. Only one individual (CHUNB 9906) has the dorsal tibia stripes slightly in diagonal orientation, but the color pattern is not well preserved and is almost imperceptible. One individual (CHUNB 9932) present two ulnar tubercles not forming a ridge as in other specimens.

Advertisement call

We analyze calls from four different localities including the type locality (Porto Velho, RO). The advertisement call of *Scinax madeirae* (n=3 males) is constituted by one multipulsed note with modulated frequency, the average note duration is 0.99 seconds (std= 0.15; amplitude= 0.72-1.16), the average dominant frequency is 3612.03 Hz (std= 232.69; amplitude= 3368.8-3879.2), and the average pulses rate is 126.48 pulses/seconds (std= 18.47; amplitude= 103.73-144.92).

Distribution

Scinax madeirae is known from Porto Velho, Rondônia, Brazil (type locality), and three localities of eastern Bolivia: Los Lagos, San Sebastián (department of Santa Cruz de la Sierra), and Caparu (department of Beni).

***Scinax* “cachimbo” sp. nov.**

Holotype: CHUNB 34509 (Fig. 27). Adult male. Serra do Cachimbo, Novo Progresso, state of Pará, Brazil.

Paratypes: CHUNB 34498-99, 34502, 34505-08, 34510. Serra do Cachimbo, Novo Progresso, state of Pará, Brazil.

Diagnosis

A small species (males SVL 18.03-23.05 mm) characterized by the dorsal pattern with continuous brown divergent dorsolateral stripes in a light brown background; uniform light brown color on the posterior surfaces of the thighs; loreal region softly sloping toward the lips; perpendicular stripes on the dorsal surface of tibia; and poorly developed toe webbing.

Comparison with other species

We compared *Scinax* “cachimbo” sp. nov. with species of the *S. ruber* clade that correspond geographically, excluding the *S. rostratus* and *S. uruguayus* species group (sensu FAIVOVICH et al., 2005).

By its smaller size in males (SVL 18.03-23.05 mm) *Scinax* “cachimbo” sp. nov. differs from *S. acuminatus* (SVL 39-45 mm; B. LUTZ, 1973), *S. boesemani* (28.4-31.8 mm; DUELLMAN, 1986), *S. camposseabrai* (SVL 28.9-33.5 mm; CARAMASCHI and CARDOSO, 2006), *S. castroviejo* (SVL 41.9 mm; DE LA RIVA, 1993), *S. chiquitanus* (SVL 33.3 mm; DE LA RIVA, 1990), *S. cretatus* (25.8-34.7 mm; NUNES and POMBAL, 2011), *S. cruentommus* (24.8-27.7 mm; DUELLMAN, 1972), *S. dolloi* (SVL 34.9 mm; PUGLIESE et al., 2009), *S. eurydice* (SVL 44-52 mm; BOKERMANN, 1968), *S. funereus* (SVL 28.7-35.1 mm; DUELLMAN, 1971), *S. fuscovarius* (SVL 41-44 mm; CEI, 1980), *S. hayii* (SVL 39-43 mm; HEYER et al., 1990), *S. iquitum* (35 mm; MORAVEC et al., 2009), *S. manriquei* (SVL 27.7 mm; BARRIO-AMORÓS et al., 2004), *S. perereca* (SVL 34-38.5 mm; POMBAL et al., 1995a), *S. rogerioi* (25-35.6 mm; PUGLIESE et al., 2009), and *S. similis* (SVL 29.4-34.7 mm; CARAMASCHI and CARDOSO, 2006).

By its uniform light brown color on the posterior surface of the thigh *Scinax* “cachimbo” sp. nov. can be differentiated from *S. caldarum*, *S. cardosoi*, *S. curicica*, *S. duartei*, *S. granulatus*, *S. hayii*, *S. maracaya*, *S. nasicus*, *S. similis* (flash yellow color on the

posterior surface of the thigh), and *S. rogerioi* (brown spots on the posterior surface of the thigh; PUGLIESE et al., 2009).

By its dorsal pattern with continuous brown dorsolateral stripes from upper eyelids until the inguinal region *Scinax* “eachimbo” sp. nov. differs from *S. acuminatus*, *S. baumgardneri* (DUELLMAN, 1986), *S. boesemani* (DUELLMAN, 1986), *S. cabralensis* (DRUMMOND et al., 2007), *S. camposseabrai*, *S. crospedospilus*, *S. danae* (DUELLMAN, 1986), *S. eurydice*, *S. lindsayi* (PYBURN, 1992), *S. maracaya* (without dorsolateral stripes), *S. alter*, *S. auratus*, *S. cretatus* (NUNES and POMBAL, 2011), and *S. squalirostris* (all with dorsolateral white stripes), *S. caldarum*, *S. duartei* (dorsal stripes from between the eyes and connected with a interocular blotch), *S. pachycrus* (a lateral dark subcantal stripe from corner of the eye to the inguinal region; B. LUTZ, 1973), *S. cuspidatus* (irregular stripes or inverted parenthesis on the thoracic region; see B. LUTZ, 1973), and by *S. cruentommus* (irregular longitudinal marks; DUELLMAN, 1972).

Scinax “eachimbo” sp. nov. differs from *S. exiguus* by its immaculate or finely dotted chest, belly, and borders of the gula (spotted in *S. exiguus*); brown dorsolateral stripes in a light brown background (light brown dorsolateral stripes in *S. exiguus*).

Scinax “eachimbo” sp. nov. differs from *S. fuscomarginatus* by lacking a dark stripe on the external surface of the tibia (present in *S. fuscomarginatus*), loreal region softly sloping toward the lips (steep sloping in *S. fuscomarginatus*), wider head (HW/HL 92%) (head much longer than wide, HW/HL 83% in *S. fuscomarginatus*); and by the less developed interdigital webbing (I 2-2 II 3 – 2 III 3 – 1 ¹/₂ or 2 IV 2-1/2 V) (I 2-2 II 1 ¹/₂-2 III 1 or 1 ¹/₂-2 IV 2-1 or 1 ¹/₂ V in *S. fuscomarginatus*).

Scinax “eachimbo” sp. nov. differs from *S. madeirae* by its smaller size [*S. eachimbo*” sp. nov. SVL 18.61+0.93 (16.71-19.97); *S. madeirae* SVL 20.85+1.41 (18.03-23.05)], by the dorsal pattern with continuous brown divergent dorsolateral stripes in a light brown background (convergent in *S. madeirae*), and by its less developed interdigital webbing (II 3 – 2 III 3 – 1 ¹/₂ or 2 IV) (II 2 – 1 III 2 – 1 IV in *S. madeirae*).

Description of the holotype

A slender small size frog (SVL 16.71 mm), posterior region slightly wider than the head. Head dorsally rounded, closely longer than wider (HW/HL 88%). Snout acuminate in profile. Nostrils dorsolaterally oriented, slightly protuberant, IND 63% of IOD. *Canthus*

rostralis straight. Loreal region concave with softly sloping toward the lip. Eye large and protruding, slightly larger than the IOD and 89% of the EN. Supratympanic fold evident from the posterior corner of the eye to the arm insertion. Tympanum slightly evident, larger than disc of Finger III (disc of Finger III/DT 92%); rounded tympanic annulus with the upper portion occult by the supratympanic fold; tympanic membrane with the same coloration of the adjacent region. A group of unpigmented rounded tubercles between the mouth corner and the terminal margin of the supratympanic fold. Vocal sac large, single, subgular, externally evident. Tongue ovoid with lateral and posterior borders free. Longitudinal vocal slits extend laterally from the medio-lateral portion of the tongue to the jaw angle. Transverse and reduced oval vomerine dentigerous process between the posterior margin of the choanae; the processes do not contact each other; two teeth in each process; nearly rounded choanae located in diagonal to the sagittal axis.

Arm slightly slender than forearm. Fingers short. Elliptical finger discs, wider than long; disc of Finger I smaller than the others; webbing reduced to fringe in the proximal and distal margins of the Fingers II, III and proximal of IV, absent in I. Unpigmented ulnar ridge extend from the distal margin of the first supernumerary tubercle of Finger IV to the insertion of the same finger. Bifid palmar tubercle. Subarticular tubercles of the Fingers I and II conical and with the same size; of the Fingers III and IV rounded but that of the Finger IV bigger and with the distal margin a slightly free. Thenar tubercle large, occupying the base of the Finger I; a rounded tubercle is present between the subarticular tubercle of the Finger I and the thenar tubercle. Unpigmented nuptial pad occupies the base of the thumb from the distal margin of the subarticular tubercle to the base of the palmar tubercle. Supernumerary tubercles rounded. Hind limbs slender, TL 50% and FL 45% of the SVL. Toes slender. Elliptical discs wider than long; discs of Fingers I and II slightly smaller than the others. Inner metatarsal tubercle oval. Outer metatarsal tubercle rounded, 1/4 of the inner metatarsal tubercle size, proximal margin up to the distal of the inner metatarsal tubercle. Subarticular tubercles of Toes I and II conical, the others rounded. A flat rounded tubercle between the inner metatarsal tubercle and the subarticular tubercle of Toe I. A cutaneous ridge in the proximal surface of Toe I from the subarticular to the inner metatarsal tubercle. Supernumerary tubercles rounded. Webbing formula I 2-2 II 3 – 2 III 3 – 1 ¹/₂ IV 2-1/2 V. Tarsal fold absent.

Pre-axilar pectoral fold. Dorsal surface with uniformly distributed rounded pustules; gula smooth; ventral surface of thigh shagreen; belly and chest granular.

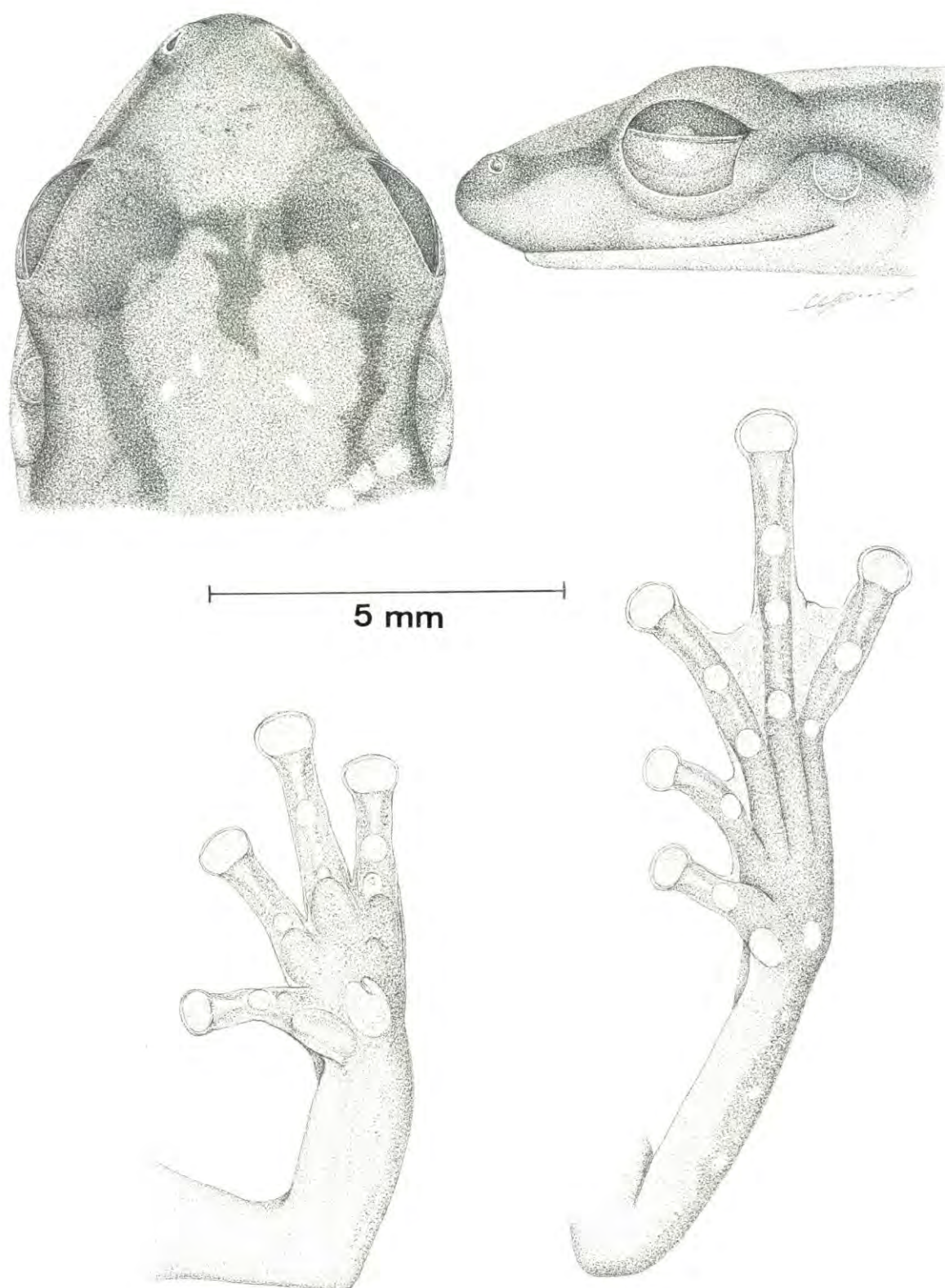


Figure 27. *Scinax eachimbo* sp. nov. holotype (CHUNB 34509). Dorsal and lateral views of the head, ventral views of hand and foot.

Color in preservative

General light brown with a pair of continuous divergent dark brown dorsolateral stripes from upper eyelids to the inguinal region where is more diffused; the supratympanic fold present the same coloration of the dorsolateral stripes; below the dorsolateral stripes and to the level of inferior border of the tympanum a wide brown stripe (with less chromatophores density than dorsolateral stripes) from the supratympanic fold to the inguinal region; dark brown stripe on the *canthus rostralis* reaching the anterior margin of the nares; loreal region with sparked chromatophores loosing density to the upper lip; between the eye and the upper lip only a few chromatophores; vertebral line only evident between the eyes, few dots in the sacral region; a wide dark brown interocular stripe posteriorly oriented; dorsal surface of finger discs dotted; dorsal surface of hands with dark brown dots and transversals stripes in Fingers III and IV; three transversal stripes in the dorsal surface of forearm. A longitudinal dark brown stripe on the external surface of the forearm from the elbow to the insertion of the hand; dorsal surfaces of leg with transversal dark brown stripes, being one on the heel and three on the tibia; external surfaces of tarso and foot with a intermittent longitudinal dark brown stripe; ventrally, external borders of the gula finely spotted, belly and chest immaculate; posterior surface of the thigh uniform light brown.

Variation

Complete variation on measurements and body proportions is given in Table 4. Some paratypes have a inverted triangle blotch between eyes (see variation in Fig. 28); some variation in Toe webbing development, especialy evident between the Toes II and III, with some specimens reaching the distal margin of the subarticular tubercle of Toe II while others slightly exceed the distal margin (see variation in Fig. 29).

Distribution

The species is known from the type locality in the Serra do Cachimbo, state of Pará, western Brazil, an open formation enclave in Amazonian biome.

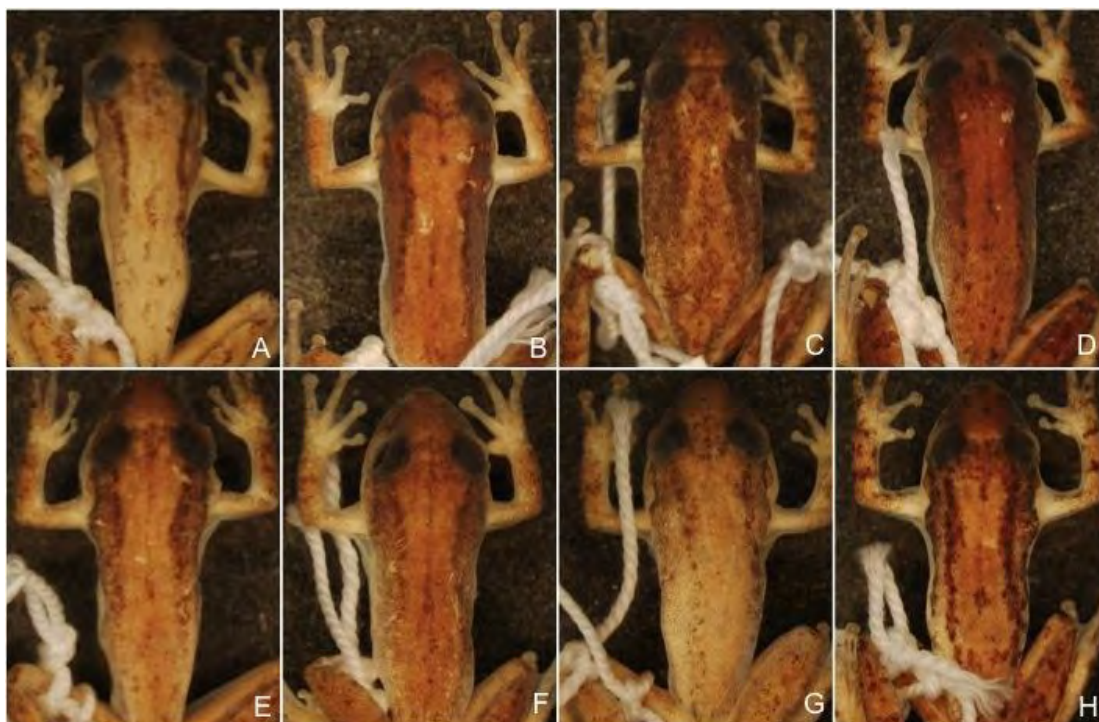


Figure 28. Dorsal view of paratypes of *Scinax eachimbo* sp. nov. showing the dorsal pattern variation. A-H) CHUNB 34498-99, 34502, 34505-06, 34508, and 34510.

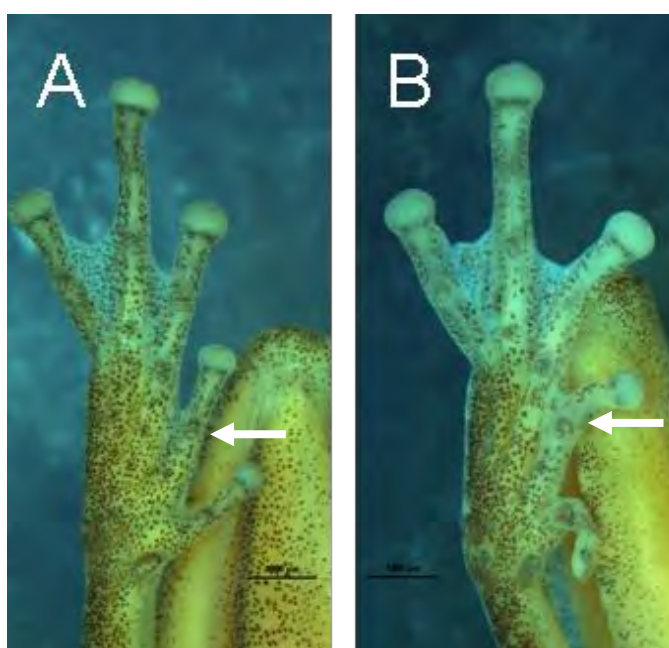


Figure 29. Ventral views of feet showing toe webbing variation in *Scinax eachimbo* sp. nov. A) paratype CHUNB 34505, and B) holotype CHUNB 34509. Arrow indicates the position of the subarticular tubercle of Toe II.

DISCUSSION

In this work we review the taxonomy of *Scinax fuscomarginatus* and the related species *S. parkeri*, *S. trilineatus* and *S. lutzorum*, and the taxonomic status of *Hyla madeirae*, the only current synonym of *S. fuscomarginatus*. We propose the synonymy of *S. parkeri*, *S. trilineatus*, and *S. lutzorum* with *S. fuscomarginatus*, taking into account the absence of morphological and call diagnostic characters, the low degree of genetic divergence, and phylogenetic inference. We consider *Hyla madeirae* as a valid species with the new combination *Scinax madeirae*, according to the morphological and genetic differences in relation to *S. fuscomarginatus*. Finally, we consider the populations of Serra do Cachimbo, Pará, Brazil as a new species based on morphological and genetic differences in relation to the two valid species, *S. fuscomarginatus* and *S. madeirae*.

Phylogenetic analyses

In the phylogenetic analyses we included more than 260 individuals from 72 localities covering the known distribution of most of the species included. Our sample represents particularly well the species *S. fuscomarginatus*, *S. parkeri*, and *S. madeirae* (many localities and topotypes included), which is not the case for *S. trilineatus* and *S. lutzorum*. For *S. trilineatus* we included one sample from approximately 300 km from the type locality (Santa Elena de Uairén, state of Bolívar, Venezuela). Because tissue samples from types or topotypes of *Scinax lutzorum* were not available, we included several samples from many localities surrounding the type locality.

We found deep and rich genetic structure both within and among species. The samples included in the analyses formed a well supported monophyletic group that is structured in several supported clades and subclades. *Scinax madeirae* and *S. -cachimbo* sp. nov. appear as well supported subclades (A1 and A2, respectively), a result that is supported by other lines of evidence (e.g. call variation and morphology, see discussion below). The formerly recognized species (*S. fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*) clustered in a well supported clade (clade B) that, although also structured in several subclades, showed only partial concordance with taxonomy. This is particularly clear for *S. fuscomarginatus*, which is present in several of these subclades (B2, B3, B5, and B6), and for *S. lutzorum*, that is clustered together with several samples of *S. fuscomarginatus* in subclade B6.

On the other hand, genetic clustering seems to reflect the geographic distribution of samples. A rich phylogeographic structure is evident with deeper divergences separating the samples from southeastern Brazil (SP and MG), central Brazil (GO), and southern Bahia (B2); the populations from southeastern Tocantins and southwestern Bahia (B3); the populations from eastern Bolivia (B4); the population of Morro do Diabo (SP; B5), and the samples ranging from Argentina to north and northeastern Brazil (B6). This extensive distribution and the rich structure observed in most of these clades makes *S. fuscomarginatus* an interesting model to study evolutionary processes of biological diversification of South American open formations.

Genetic divergence

Several authors proposed divergences of the 16S fragment between 2-3 % as being sufficient for the recognition of different species in Neotropical anurans (CHEK et al., 2001; SYMULA et al., 2003; CAMARGO et al., 2006; FOUQUET et al., 2007; FUNK et al., 2007). This proposal is clearly not applicable for the species considered in this study, taking into account that divergence within subclades of the same species reaches up to 4.3 % (e.g. subclade B6). Genetic divergence within subclades is sometimes larger than between subclades, as in B6. A large divergence amplitude between subclades within the clade B (e.g. 1.3-8.3 between B4/B5+B6 subclades) could be caused by the wide geographic distribution of B6, comprising populations from northern Argentina to north and northeastern Brazil with contact zones with all the remaining clades.

The divergence between subclades A1 and A2 (corresponding to *Scinax madeirae* and *S. "eachimbo"* sp. nov., respectively) coincides with a large sampling gap; samples from both clades are geographically distant in at least 900 km in straight line. However, we find unlikely that this genetic pattern and degree of divergence is caused by the lack of samples between clades, considering that geographic distances three times larger (~3000 km) are associated with smaller divergences (4%) (e.g. subclade B6). It is interesting to note that this gap could reflect a lack of suitable habitat (open formations) because both species seem restricted to different patches of natural Amazonian savanna enclaves embedded in the Amazonian rainforests.

Morphological analyses

We identified characters related to color pattern, snout shape, and toe webbing that allow the diagnose of *Scinax madeirae* and *S. -eachimbo*” sp. nov. On the other hand, careful examination of a fairly comprehensive sampling showed that variation in all characters used to identify the formerly recognized species (*Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*) and other potential characters are not informative, meaning that these species are not diagnosable using qualitative morphological characters. For example, in *Scinax fuscomarginatus*, as defined here, dorsolateral stripes can be divergent or parallel while *S. madeirae* presents convergent dorsolateral stripes, a state that is evident in the holotype figure showed in the original description (BOKERMANN, 1964) and in the material examined here. Bokermann's figure also revealed that stripes crossing the dorsal surface of tibia are diagonal and not perpendicular as in *S. fuscomarginatus*. Bokermann (1964) stated that *S. madeirae* can be distinguishable from *S. parkeri* by its wider head. Using body proportions related to the head width (HW/HL and HW/SVL) we identified some differences but with a high degree of overlap; nevertheless, differences in head shape are evident, since *S. madeirae* has rounded head in dorsal view and in *S. fuscomarginatus* it is sub-elliptical.

Morphometric analyses

In the PCAs all score values for the first component are positive, indicating a strong influence of size, while the other components showing positive and negative correlations indicate shape variation (HUMPHRIES et al., 1981). The PCAs do not separate any of the currently recognized species neither the species as considered here (*Scinax fuscomarginatus*, *S. madeirae*, *S. -eachimbo*” sp. nov.). However, some body proportions show trends that could be useful in helping diagnose the species we considered valid. For instance, proportions related to head width (HW/HL; HW/SVL), are higher in *Scinax madeirae* and in *S. -eachimbo*” sp. nov. than in *S. fuscomarginatus*.

In our sample of *Scinax fuscomarginatus* we found a striking variation in the body size of reproductive active males (showing evident vocal sacs), with the smaller individuals measuring 16 mm and the largest individuals exceeding 25 mm. *Scinax fuscomarginatus* is a widely distributed species with a high latitudinal range (from 4° to 27°) which would involve populations under different climatic conditions such as temperature and rainfall. These factors

are known to influence size in ectothermic vertebrates (ASHTON et al., 2000). Olalla-Tarraga et al. (2009) analyzed the variation in body size of anurans in the Cerrado, finding these animals tend to be smaller in the southwest of the biome (areas surrounding the Pantanal) and larger in the northeast of the biome (areas surrounding the Caatinga); the authors found positive correlations of the body size with water deficit.

Another factor that could explain the large body size variation in our sample of *Scinax fuscomarginatus* relates to reproduction. *Scinax fuscomarginatus* reproduces in permanent or temporary water bodies (TOLEDO and HADDAD, 2005b) with the degree of seasonality depending on the environment. Temporary ponds in arid regions like the Chaco would provide water for shorter periods than in regions with lower water deficit. Several species show an acceleration in metamorphosis influenced by deteriorating environmental conditions, such as pond drying (NEWMAN, 1992), resulting in small body size in froglets and consequently in mature individuals (CRUMP, 1989). The season of collection of specimens could also influence this variation because recruitment in species with prolonged reproductive activity, such as *S. fuscomarginatus*, occurs at different times of the year following the rainfall regime.

Call analyses

We analyzed a total of 64 records from different males obtained at 26 localities covering much of the known distribution of the species analyzed in this study, including topotypes of *Scinax parkeri*, *S. madeirae*, and *S. fuscomarginatus*. The calls showed the same basic structure: a unique modulated frequency multipulsed note. Dominant frequency, pulse rate, and note duration cannot be used to discern any of the species. Air temperature, ranging from 20 to 28°C, and body size, ranging from 17.43 to 23.22 mm, may be causing some variation in temporal parameters (e.g. Blair, 1958). However, Toledo and Haddad (2005b) found no correlation between air temperature or body size with note duration and pulse rate in *S. fuscomarginatus*, but body size was negatively correlated with the dominant frequency of advertisement calls. Another variable that potentially affects the temporal parameters of the advertisement calls in anurans is the social context; it is known that anuran males calling in large chorus, as is usually in the case of *Scinax fuscomarginatus*, modify call duration for reducing overlaps with neighbors (see WELLS, 2007). Toledo and Haddad (2005b) did not

report temporal variation when two males of *S. fuscomarginatus* call in antiphony; nevertheless, records obtained during such interactions were not included in the analysis.

The *Scinax madeirae* advertisement calls have a tendency to be longer and with fewer pulses per second but with certain degree of overlap with those of *S. fuscomarginatus*. However, the two species have been recorded in sympatry in three localities of Bolivia (Los Lagos, San Sebastian, and Yucuma), where differences related to the temporal parameters of the call are more obvious. In San Sebastian the note duration of *S. madeirae* ranges between 0.88-1.06 seconds (n=2) and the pulse rate ranges between 103-131 pulses/second (n=2). In *S. fuscomarginatus* these parameters range between 0.31-0.47 seconds and 214-233 pulses/second (n=2). The calls of both species are more similar in allopatry than in sympatry, which may evidence character displacement. This phenomenon was reported for several species of frogs (e.g. BLAIR, 1974; GERHARDT and SCHWARTZ, 1995; LITTELJOHN, 1999; GERHARDT and HUBER, 2002), and is related to a positive selection on males with calls less similar in relation to the calls of closely related species (with similar calls). This selection is attributed to the lower fitness of the hybrid offspring (WELLS, 2007). Further data will allow us to test for variation in calls recorded in allopatry and sympatry and evaluate if reproductive character displacement occurs in this system.

Correspondence of available names

In the diagnosis and in the description of *Scinax parkeri*, Gaige (1929) do not provide characters or character states that allow to distinguish this species from *S. fuscomarginatus*. Duellman and Wiens (1992) consider *S. parkeri* valid and include among the analyzed material specimens of *S. parkeri* and *S. fuscomarginatus* that, judging by the field numbers (WCAB), were collected and identified by Bokermann. Considering the provenance of the material, Bokermann clearly missidentified these specimens; those identified as *S. parkeri* [KU 92117 (WCAB 14685) and KU 92118 (WCAB 14686)] were collected 15 km E of the type locality of *S. fuscomarginatus*, while the specimens identified as *S. fuscomarginatus* [KU 92029 (WCAB 30117), KU 92030 (WCAB 30178), KU 92031 (WCAB 30179), KU 92034 (WCAB 31282)] were collected at Barra do São João, state of Rio de Janeiro (Brazilian coast). According to Bokermann (1966), *S. fuscomarginatus* is a common species in the Brazilian southeastern coast, not being found in Belo Horizonte or São Paulo. Apparently Bokermann was missidentifying specimens of *Scinax* cf. *alter* as *S. fuscomarginatus*.

(deposited in the MUZUSP). These specimens were also collected at Barra de São João as well as at several localities of the southeastern coast of the states of São Paulo, Rio de Janeiro, and Espírito Santo and apparently are *S. alter* or a close allied species.

De la Riva et al. (1997) concluded that *S. parkeri* is a valid species based on call differences. These authors compared a call record obtained from the *S. parkeri* type locality (DE LA RIVA et al., 1994) with calls from Pará and São Paulo states (DUELLMAN and PYLES, 1983 and CARDOSO, 1981 respectively). However, the call described by De la Riva et al. (1994) do not correspond to *S. parkeri* or any related species; the numerical parameters, the oscilogram, and the spectrogram presented in the description are almost identical to the call of *Dendropsophus leucophyllatus* presented by Marquez et al. (1993) (as *Hyla leucophyllata*). The record used to describe the call of *S. parkeri* is apparently the same presented by De la Riva et al. (2002) in the CD “Sounds of frogs and toads of Bolivia”, where three species are calling synchronously: *D. leucophyllatus*, *D. nanus*, and almost imperceptible *S. parkeri*. The description of the call of *D. leucophyllatus* by De la Riva et al. (1994) (as *S. parkeri*) together with the misidentified material used by Duellman and Wiens (1992), clearly indicate that the revalidation of *S. parkeri* is incorrect. At the same time the multiple lines of evidence shown in this work support that this species should not remain valid.

Like *Scinax parkeri*, *S. trilineatus* was never compared with *S. fuscomarginatus*. According with the holotype figure in the original description (HOOGMOED and GORZULA, 1979) only two characters differs these species, the single thenar tubercle and the lack of interocular stripe. Analysis of photographs of the types and new material confirm the bifid condition of the thenar tubercle and the presence of the interocular stripe. Martins (1998) had already questioned the validity of *S. trilineatus* proposing it as a synonym of *S. fuscomarginatus* but without explanation. This proposal was rejected by Barrio-Amorós (2004) which also did not provide any argument.

The description of *Scinax lutzorum* (CARDOSO and POMBAL, 2010) is based in only two specimens from a single locality (Municipality of Aragominas, state of Tocantins, Brazil). The authors compared this species with *S. fuscomarginatus*, *S. parkeri*, and *S. trilineatus* and proposed a diagnose using a combination of characters that proved extremely variable in our sample, even at intra-population level. Many of these characters are strongly

influenced by the quality of fixation and conservation (F. BRUSQUETTI, personal observation), and their condition lies within the variation of *S. fuscomarginatus*.

As stated above, no morphological character distinguish any of the currently recognized species *Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*. However, characters related to color pattern, head shape, and toe webbing development can separate the three species considered as valid here: *S. fuscomarginatus*, *S. madeirae* and *S. -eachimbo*” sp. nov.

Distribution

Several authors cited material from the Brazilian Atlantic coast as *Scinax fuscomarginatus* (COCHRAN, 1955; BOKERMANN, 1964). B. Lutz (1973) defined Belo Horizonte, Minas Gerais as the type locality of *S. fuscomarginatus* and stated that the species distributed in the Atlantic coast correspond to *Hyla altera* (= *Scinax alter*), but Duellman and Wiens (1992) continued with the mistake indicating material from the Atlantic coast as *S. fuscomarginatus* and from the interior Brazil (Minas Gerais) as *S. parkeri*. *Scinax fuscomarginatus*, as defined here, is one of the most widely distributed Neotropical species. The species occur from northern Argentina to northern and northeastern Brazil, occupying part of Paraguay, eastern Bolivia, eastern Venezuela, Guyanas, and Suriname.

Scinax madeirae is known from the type locality (Porto Velho, Rondônia) and three localities of eastern Bolívia, Los Lagos, San Sebastián, and Caparu. The localities of Porto Velho and Yucuma are connected by afluentes of the Madeira River in Amazonian open formations, and San Sebastian and Caparu are in open areas of the Bolivian Chiquitania.

Scinax -eachimbo” sp. nov. currently is known only from one locality in the Serra do Cachimbo, state of Pará, Brazil. Serra do Cachimbo is considered an Amazonian savanna enclave (FRANÇA et al., 2006). Amazonian savannas are “islands” of open areas within the rainforest and covers approximately 150,000 km² of Brazil (PIRES, 1973); other anuran species, *Dendropsophus cachimbo*, is also only known of this savanna enclave (NAPOLI and CARAMASCHI, 1999; FROST, 2011).

Conclusions

1. We found deep and rich genetic structure both within and among species, with the analyzed species forming a well supported monophyletic group. *Scinax madeirae* and *S. cachimbo* sp. nov. appears as well supported subclades while the formerly recognized species (*S. fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*) are clustered in a single clade. The correspondence among subclades and the taxonomic valid species is limited.
2. We identified characters related to color pattern, head shape, and toe webbing that allow the diagnose of *Scinax madeirae* and *S. cachimbo* sp. nov. On the other hand, careful examination of a fairly comprehensive sampling showed that variation in all characters used to identify the formerly recognized species (*Scinax fuscomarginatus*, *S. parkeri*, *S. trilineatus*, and *S. lutzorum*) and other potential characters are not informative, meaning that these species could not be distinguishable using qualitative morphological characters.
3. The PCAs do not separate any of the currently recognized species neither the species as considered here (*Scinax fuscomarginatus*, *S. madeirae*, *S. cachimbo* sp. nov.). However, some body proportions show trends that could be useful in helping diagnose the species we considered valid. For instance, proportions related to head width are higher in *Scinax madeirae* and in *S. cachimbo* sp. nov. than in *S. fuscomarginatus*.
4. The calls of all the species included here showed the same basic structure: a unique modulated frequency multipulsed note. Dominant frequency, pulse rate, and note duration cannot be used to discern any of the species.
5. We propose the synonymy of *Scinax parkeri*, *S. trilineatus*, and *S. lutzorum* with *S. fuscomarginatus*, taking into account the absence of morphological and call diagnostic characters, the low degree of genetic divergence, and phylogenetic inference. We consider *Hyla madeirae* as a valid species with the new combination *Scinax madeirae*, according to the morphological and genetic differences in relation to *S. fuscomarginatus*. Finally, we consider the populations of Serra do Cachimbo, Pará, Brazil as a new species based on morphological and genetic differences in relation to the two valid species, *S. fuscomarginatus* and *S. madeirae*.

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APPENDIX 1. MATERIAL USED IN MOLECULAR ANALYSES. Columns correspond to vouchers or tissue number, sequenced mtDNA fragments, locality, state or department, country, and geographical coordinates.

ID	mtDNA fragments	Locality	State/Department	Country	lat	long
MLP DB 4212	CO1, ND2, 16S	R P Rincón Santa María, Ituzaingó	Corrientes	Argentina	-27.517	-56.600
MLP DB 4214	CO1, ND2	R P Rincón Santa María, Ituzaingó	Corrientes	Argentina	-27.517	-56.600
MLP DB 5979	CO1, ND2, 16S	R P Rincón Santa María, Ituzaingó	Corrientes	Argentina	-27.539	-56.582
ASO034	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
ASO041	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
ASO191	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
ASO546	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
ASO559	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1234	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1235	CO1, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1236	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1237	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1248	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1249	CO1, ND2	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1250	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1251	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1258	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1259	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1265	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1277	CO1, ND2	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1278	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1288	CO1, ND2, 16S	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1289	CO1, ND2	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
Scma1244SS	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
Scma1253SS	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
Scma1254SS	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
Scma1293SS	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
Scma960SS	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
Scpa1252SS	CO1	Ñuflo de Chavez, San Sebastián	S Cruz de la Sierra	Bolivia	-16.362	-62.021
MJ 1317	CO1, ND2	BuenaVista	S Cruz de la Sierra	Bolivia	-17.458	-63.659
Scpa1316BV	CO1, 16S	BuenaVista	S Cruz de la Sierra	Bolivia	-17.458	-63.659
ASO248	CO1, ND2	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
ASO273	CO1, ND2	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
ASO313	CO1, ND2	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
ASO314	CO1, ND2	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
ASO315	CO1, ND2, 16S	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
ASO325	CO1, ND2, 16S	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
ASO326	CO1	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
SCLL	CO1, ND2	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
Scma889Cap	CO1	Velasco, Caparu	S Cruz de la Sierra	Bolivia	-14.910	-61.072
MJ 1318	CO1, ND2, 16S	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1320	CO1, ND2, 16S	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1322	CO1, ND2, 16S	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1337	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1341	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1342	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1346	CO1, ND2, 16S	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1355	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1362	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1363	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
MJ 1364	CO1, ND2	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815

ID	mtDNA fragments	Locality	State/Department	Country	lat	long
Scma1354LL	CO1	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
Scma1356LL	CO1	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
Scma1367LL	CO1	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
Scma1371LL	CO1	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
Scpa1370LL	CO1	Yucuma, Los Lagos	Beni	Bolivia	-12.769	-65.815
CFBH 7349	CO1, ND2, 16S	Passo de Camarajibe	Alagoas	Brazil	-9.238	-35.493
CFBH 7350	CO1, ND2	Passo de Camarajibe	Alagoas	Brazil	-9.238	-35.493
CFBH 7351	CO1, ND2, 16S	Passo de Camarajibe	Alagoas	Brazil	-9.238	-35.493
CFBH 7352	CO1, ND2, 16S	Passo de Camarajibe	Alagoas	Brazil	-9.238	-35.493
CFBH 22155	CO1	Caetité	Bahia	Brazil	-14.130	-42.509
CFBH 22156	CO1, ND2, 16S	Caetité	Bahia	Brazil	-14.130	-42.509
CFBH 23345	CO1, ND2, 16S	Caetité	Bahia	Brazil	-14.130	-42.509
CFBH-T1486	CO1, ND2	Caravelas	Bahia	Brazil	-17.711	-39.237
CHUNB51008	CO1	Jaborandí	Bahia	Brazil	-14.423	-45.889
CHUNB51003	CO1, ND2, 16S	Luis Eduardo Magalhães	Bahia	Brazil	-12.124	-45.814
CFBH 20548	CO1, ND2, 16S	Rio Pratudinho, Jaborandi	Bahia	Brazil	-14.430	-45.890
CHUNB51004	CO1, ND2	São Desidério	Bahia	Brazil	-12.706	-45.087
CHUNB51005	CO1	São Desidério	Bahia	Brazil	-12.706	-45.087
CE 062	CO1, ND2	E Ecológica do Pecém, Caucaia	Ceará	Brazil	-3.736	-38.653
CE 063	CO1, ND2, 16S	E Ecológica do Pecém, Caucaia	Ceará	Brazil	-3.736	-38.653
CE 064	CO1	E Ecológica do Pecém, Caucaia	Ceará	Brazil	-3.736	-38.653
ZUFG 4760	CO1, ND2, 16S	Brazlandia	Distrito Federal	Brazil	-15.678	-48.199
ZUFG 4761	CO1	Brazlandia	Distrito Federal	Brazil	-15.678	-48.199
ZUFG 4762	CO1	Brazlandia	Distrito Federal	Brazil	-15.678	-48.199
ZUFG 4445	CO1, ND2	Jataí	Goiás	Brazil	-17.885	-51.723
CHUNB36451	CO1	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36452	CO1, ND2, 16S	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36456	CO1, ND2	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36459	CO1, ND2	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36460	CO1, ND2	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36464	CO1	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36465	CO1, ND2	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB36466	CO1, ND2, 16S	São Domingos	Goiás	Brazil	-13.398	-46.318
CHUNB49559	CO1	PNC dos Veadeiros, Alto Paraíso	Goiás	Brazil	-14.004	-47.685
CHUNB49560	CO1, ND2, 16S	PNC dos Veadeiros, Alto Paraíso	Goiás	Brazil	-14.004	-47.685
CHUNB49561	CO1	PNC dos Veadeiros, Alto Paraíso	Goiás	Brazil	-14.004	-47.685
CHUNB51595	CO1, ND2	Brejo do Castelo Velho, Carolina	Maranhao	Brazil	-7.055	-47.130
CHUNB51596	CO1, ND2, 16S	Brejo do Castelo Velho, Carolina	Maranhao	Brazil	-7.055	-47.130
CHUNB51597	CO1, ND2	Brejo do Castelo Velho, Carolina	Maranhao	Brazil	-7.055	-47.130
CFBH 21844	CO1, ND2	Alta Floresta	Mato Grosso	Brazil	-9.876	-56.086
CFBH 14302	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.133	-55.702
CFBH 14303	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.133	-55.702
CFBH 14304	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.133	-55.702
CFBH 14305	CO1, ND2	Chapada dos Guimarães	Mato Grosso	Brazil	-15.133	-55.702
CFBH 14306	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.133	-55.702
CFBH 24637	CO1, ND2	Chapada dos Guimarães	Mato Grosso	Brazil	-15.313	-55.757
CFBH 24638	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.313	-55.757
CFBH 24639	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.313	-55.757
CFBH 24640	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.313	-55.757
CFBH 24641	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.313	-55.757
CFBH 24642	CO1, ND2, 16S	Chapada dos Guimarães	Mato Grosso	Brazil	-15.313	-55.757
CG 324	CO1	Chapada dos Guimarães	Mato Grosso	Brazil	-15.453	-55.739
CTMZ-06194	CO1, ND2	Colider, Ana Lima	Mato Grosso	Brazil	-10.813	-55.456
CTMZ-06195	CO1, ND2	Colider, Ana Lima	Mato Grosso	Brazil	-10.813	-55.456
CTMZ-06196	CO1, ND2	Colider, Ana Lima	Mato Grosso	Brazil	-10.813	-55.456
CFBH 14335	CO1, ND2, 16S	Dom Aquino	Mato Grosso	Brazil	-15.646	-54.917
CFBH 21857	CO1, ND2, 16S	E Ecológica Serra das Araras	Mato Grosso	Brazil	-15.434	-57.187

ID	mtDNA fragments	Locality	State/Department	Country	lat	long
CFBH 21858	CO1, ND2, 16S	E Ecológica Serra das Araras	Mato Grosso	Brazil	-15.434	-57.187
CTMZ-05731	CO1, ND2, 16S	Guiratinga	Mato Grosso	Brazil	-16.336	-53.765
CTMZ-05732	CO1, ND2, 16S	Guiratinga	Mato Grosso	Brazil	-16.336	-53.765
CTMZ-05733	CO1, ND2	Guiratinga	Mato Grosso	Brazil	-16.336	-53.765
CTMZ-06221	CO1, ND2, 16S	Itaúba	Mato Grosso	Brazil	-11.095	-55.304
CTMZ-06222	CO1, ND2, 16S	Itaúba	Mato Grosso	Brazil	-11.095	-55.304
CTMZ-06223	CO1, ND2, 16S	Itaúba	Mato Grosso	Brazil	-11.095	-55.304
CTMZ-06224	CO1	Itaúba	Mato Grosso	Brazil	-11.095	-55.304
CTMZ-06225	CO1, ND2	Itaúba	Mato Grosso	Brazil	-11.095	-55.304
CTMZ-06633	CO1, ND2	Itaúba	Mato Grosso	Brazil	-9.067	-56.779
CTUFMT 168	CO1, ND2, 16S	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
CTUFMT 169	CO1, ND2	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
CTUFMT 193	CO1, ND2, 16S	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
CTUFMT 199	CO1, ND2, 16S	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
UFMT 7191	CO1, ND2, 16S	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
UFMT 7192	CO1, ND2	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
UFMT 7193	CO1, ND2, 16S	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
UFMT 7194	CO1, ND2, 16S	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
UFMT 7195	CO1, ND2	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
UFMT 7196	CO1, ND2	Lucas do Rio Verde	Mato Grosso	Brazil	-13.061	-55.921
CTMZ-06683	CO1, ND2	Paranaita	Mato Grosso	Brazil	-9.067	-56.779
CTMZ-06684	CO1, ND2, 16S	Paranaita	Mato Grosso	Brazil	-9.067	-56.779
CTMZ-06691	CO1, ND2	Paranaita	Mato Grosso	Brazil	-9.067	-56.779
CTMZ-06692	ND2	Paranaita	Mato Grosso	Brazil	-9.067	-56.779
CTMZ-06693	CO1, ND2	Paranaita	Mato Grosso	Brazil	-9.067	-56.779
CTMZ-05178	CO1, ND2, 16S	Sapezal	Mato Grosso	Brazil	-12.985	-58.760
CTMZ-05228	CO1, ND2	Sapezal	Mato Grosso	Brazil	-12.985	-58.760
CTMZ-05413	CO1, ND2, 16S	Tesouro	Mato Grosso	Brazil	-16.077	-53.556
CTMZ-05414	CO1, ND2	Tesouro	Mato Grosso	Brazil	-16.077	-53.556
CTMZ-05415	CO1, ND2	Tesouro	Mato Grosso	Brazil	-16.077	-53.556
CTMZ-05416	CO1, ND2, 16S	Tesouro	Mato Grosso	Brazil	-16.077	-53.556
IAH 314	CO1, ND2	Corumbá	M Grosso do Sul	Brazil	-19.010	-57.657
ZUFMS 482	CO1, ND2, 16S	Fz Patolá, Porto Murtinho	M Grosso do Sul	Brazil	-21.713	-57.726
ZUFMS 483	CO1, ND2, 16S	Fz Patolá, Porto Murtinho	M Grosso do Sul	Brazil	-21.713	-57.726
FPM 49	CO1, ND2	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 50	CO1	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 51	CO1, ND2, 16S	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 52	CO1, ND2, 16S	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 58	CO1, ND2, 16S	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 59	CO1, ND2	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 60	CO1, ND2, 16S	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
FPM 61	CO1, ND2, 16S	Fz Santo Antonio, Nioaque	M Grosso do Sul	Brazil	-21.126	-55.833
CHUNB43485	CO1	Buritizeiro	Minas Gerais	Brazil	-17.187	-44.645
CHUNB44437	CO1	Buritizeiro	Minas Gerais	Brazil	-17.187	-44.645
CFBH 24356	CO1, ND2, 16S	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24357	CO1	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24358	CO1, ND2	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24359	CO1, ND2, 16S	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24360	CO1, ND2, 16S	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24361	CO1, ND2, 16S	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24362	CO1, ND2, 16S	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH 24363	CO1	Lagoa Santa	Minas Gerais	Brazil	-19.535	-43.959
CFBH-T2052	CO1, ND2, 16S	Pirapora	Minas Gerais	Brazil	-17.336	-44.898
CFBH-T2053	CO1	Pirapora	Minas Gerais	Brazil	-17.336	-44.898
CFBH-T2055	CO1	Pirapora	Minas Gerais	Brazil	-17.336	-44.898
CFBH-T2056	CO1, ND2, 16S	Pirapora	Minas Gerais	Brazil	-17.336	-44.898
CFBH-T1949	CO1	S do Cipó, Santana do Riacho	Minas Gerais	Brazil	-19.115	-43.680

ID	mtDNA fragments	Locality	State/Department	Country	lat	long
CFBH-T1950	CO1, ND2, 16S	S do Cipó, Santana do Riacho	Minas Gerais	Brazil	-19.115	-43.680
PS 837	CO1, ND2	Arapiranga, Curuçá	Pará	Brazil	-0.738	-47.853
PCS 363	CO1, ND2, 16S	Carajas	Pará	Brazil	-2.926	-51.861
CHUNB34502	CO1, ND2, 16S	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CHUNB34503	CO1, ND2, 16S	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CHUNB34505	CO1	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CHUNB34506	CO1	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CHUNB34507	CO1	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CHUNB34508	CO1, ND2	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CHUNB34509	CO1, ND2	S do Cachimbo, Novo Progresso	Pará	Brazil	-8.493	55.502
CFBHT10947	CO1, ND2, 16S	Baixa Grande	Piauí	Brazil	-5.865	-42.184
CFBH 25469	CO1, ND2, 16S	Porto Velho	Rondonia	Brazil	-8.858	-63.799
CFBH-T1562	CO1	Cosmorama	São Paulo	Brazil	-20.477	-49.775
CFBH-T1568	CO1	Cosmorama	São Paulo	Brazil	-20.477	-49.775
CFBH-T1574	CO1	Cosmorama	São Paulo	Brazil	-20.477	-49.775
CFBH-T1575	CO1	Cosmorama	São Paulo	Brazil	-20.477	-49.775
CFBH 18855	CO1, ND2, 16S	E Ecológica de Assis, Assis	São Paulo	Brazil	-22.585	-50.378
CFBH 18856	CO1	E Ecológica de Assis, Assis	São Paulo	Brazil	-22.585	-50.378
CFBH 20040	CO1	E Ecológica de Assis, Assis	São Paulo	Brazil	-22.585	-50.378
CFBH 6527	CO1, ND2, 16S	E Ecológica de Itirapina	São Paulo	Brazil	-22.264	-47.834
CFBH 19354	CO1, ND2, 16S	Itapetininga	São Paulo	Brazil	-23.595	-48.054
CFBH 19355	CO1, ND2	Itapetininga	São Paulo	Brazil	-23.595	-48.054
CFBH 10049	CO1, ND2, 16S	P E. Morro do Diabo, Teodoro Sampaio	São Paulo	Brazil	-22.616	-52.169
CFBH 18678	CO1, ND2, 16S	P E. Morro do Diabo, Teodoro Sampaio	São Paulo	Brazil	-22.616	-52.336
CFBH 18679	CO1, ND2, 16S	P E. Morro do Diabo, Teodoro Sampaio	São Paulo	Brazil	-22.616	-52.336
CFBH 7629	CO1, ND2	Pilar do Sul	São Paulo	Brazil	-23.810	-47.714
CFBH 6288	CO1	Pindamonhangaba	São Paulo	Brazil	-22.924	-45.463
CFBH 6697	CO1	Pirassununga	São Paulo	Brazil	-21.997	-47.427
CFBH 21102	CO1	Sítio Cantaclaro, Itapé, Rio Claro	São Paulo	Brazil	-22.273	-47.700
CFBH 23286	CO1, ND2, 16S	Tapirai	São Paulo	Brazil	-23.899	-47.515
CFBH 11431	CO1, ND2, 16S	Araguaina	Tocantins	Brazil	-7.243	-48.026
CFBH 11433	CO1, ND2, 16S	Araguaina	Tocantins	Brazil	-7.243	-48.026
CFBH 10268	CO1, ND2	Fz Vera Cruz, Dois Irmãos,	Tocantins	Brazil	-6.787	-48.083
CHUNB27345	CO1	Jalapão, Mateiros	Tocantins	Brazil	-10.583	-46.417
CHUNB27348	CO1	Jalapão, Mateiros	Tocantins	Brazil	-10.583	-46.417
CHUNB27356	CO1	Jalapão, Mateiros	Tocantins	Brazil	-10.583	-46.417
CHUNB27358	CO1	Jalapão, Mateiros	Tocantins	Brazil	-10.583	-46.417
CHUNB27359	CO1	Jalapão, Mateiros	Tocantins	Brazil	-10.583	-46.417
CHUNB27364	CO1	Jalapão, Mateiros	Tocantins	Brazil	-10.583	-46.417
CFBH-T3763	CO1, ND2, 16S	Lizarda	Tocantins	Brazil	-9.488	-46.609
CFBH 13240	CO1, ND2, 16S	Lizarda	Tocantins	Brazil	-9.488	-46.609
CFBH 13241	CO1, ND2, 16S	Lizarda	Tocantins	Brazil	-9.488	-46.609
CHUNB38023	CO1, ND2	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB38024	CO1, ND2	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB38025	CO1	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB38026	CO1	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB38027	CO1	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB38029	CO1	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB38030	CO1	Paraná	Tocantins	Brazil	-12.615	-47.883
CHUNB46071	CO1, ND2	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46072	CO1, ND2	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46073	CO1, ND2	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46076	CO1, ND2, 16S	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46078	CO1, ND2, 16S	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46080	CO1, ND2, 16S	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46081	CO1, ND2	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957
CHUNB46083	CO1, ND2, 16S	P Estadual do Cantão, Caseara	Tocantins	Brazil	-9.316	-49.957

ID	mtDNA fragments	Locality	State/Department	Country	lat	long
CHUNB43178	CO1	Pedro Afonso	Tocantins	Brazil	-9.081	-48.239
CHUNB43179	CO1	Pedro Afonso	Tocantins	Brazil	-9.081	-48.239
CHUNB43180	CO1	Pedro Afonso	Tocantins	Brazil	-9.081	-48.239
CHUNB43183	CO1	Pedro Afonso	Tocantins	Brazil	-9.081	-48.239
CFBH 10248	CO1, ND2	Rio Caiapó, Araguacema	Tocantins	Brazil	-8.872	-49.600
CHUNB42199	CO1	Rio Novo, Mateiros	Tocantins	Brazil	-10.562	-46.758
CHUNB42201	CO1	Rio Novo, Mateiros	Tocantins	Brazil	-10.562	-46.758
CTMZ-02886	CO1, ND2, 16S	UHE-Peixe Angical	Tocantins	Brazil	-12.242	-48.404
CTMZ-02887	CO1, ND2, 16S	UHE-Peixe Angical	Tocantins	Brazil	-12.242	-48.404
IIBP-H 1593	CO1, ND2, 16S	Coronel Martínez	Guairá	Paraguay	-25.677	-56.688
IIBP-H 1594	CO1, ND2, 16S	Coronel Martínez	Guairá	Paraguay	-25.677	-56.688
IIBP-H 1595	CO1, ND2	Coronel Martínez	Guairá	Paraguay	-25.677	-56.688
IIBP-H 1596	CO1, ND2	Coronel Martínez	Guairá	Paraguay	-25.677	-56.688
IIBP-H 1597	CO1, ND2	Coronel Martínez	Guairá	Paraguay	-25.677	-56.688
IIBP-H 1573	ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1575	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1576	CO1, ND2, 16S	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1578	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1582	CO1, ND2, 16S	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1583	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1584	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1585	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1586	CO1, ND2, 16S	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1587	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1588	CO1, ND2	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 1589	CO1, ND2, 16S	Alto Verá, Reserva San Rafael	Itapúa	Paraguay	-26.497	-55.786
IIBP-H 436	CO1, ND2, 16S	Emboscada	Cordillera	Paraguay	-25.140	-57.358
IIBP-H 445	CO1, ND2, 16S	Emboscada	Cordillera	Paraguay	-25.140	-57.358
IIBP-H 1557	CO1, ND2, 16S	Estancia La Golondrina	Presidente Hayes	Paraguay	-24.933	-57.700
IIBP-H 1558	CO1, ND2, 16S	Estancia La Golondrina	Presidente Hayes	Paraguay	-24.933	-57.700
IIBP-H 1559	CO1, ND2, 16S	Estancia La Golondrina	Presidente Hayes	Paraguay	-24.933	-57.700
IIBP-H 1560	CO1, ND2	Estancia La Golondrina	Presidente Hayes	Paraguay	-24.933	-57.700
IIBP-H 1061	CO1, ND2	Estancia Pirá Potrero	Amambay	Paraguay	-22.672	-56.258
IIBP-H 1062	CO1, ND2	Estancia Pirá Potrero	Amambay	Paraguay	-22.672	-56.258
IIBP-H 1084	CO1, ND2, 16S	Estancia Pirá Potrero	Amambay	Paraguay	-22.672	-56.258
IIBP-H 1095	CO1, ND2, 16S	Estancia Pirá Potrero	Amambay	Paraguay	-22.672	-56.258
IIBP-H 265	CO1, ND2, 16S	Estancia San José	Ñeembucú	Paraguay	-27.202	-58.449
IIBP-H 269	CO1, ND2, 16S	Estancia San José	Ñeembucú	Paraguay	-27.202	-58.449
IIBP-H 317	CO1, ND2, 16S	Estancia San José	Ñeembucú	Paraguay	-27.202	-58.449
CAB 5751	CO1, ND2, 16S	Santa Elena Uairén	Bolivar	Venezuela	4.603	-61.111
OUTGROUP						
ID	mtDNA fragments	species				
IIBP-H 1396	CO1, ND2, 16S	<i>Scinax berthae</i>				
CAB 5750	CO1, 16S	<i>Scinax exiguus</i>				
CAB 5753	CO1, ND2, 16S	<i>Scinax exiguus</i>				
IIBP-H 262	CO1, ND2, 16S	<i>Scinax nasicus</i>				
IIBP-H 277	CO1, ND2, 16S	<i>Scinax acuminatus</i>				
CFBH 21975	CO1, ND2, 16S	<i>Scinax squalirostris</i>				
CFBHT10951	CO1, ND2, 16S	<i>Scinax nebulosus</i>				

APPENDIX 2. ANALYZED MATERIAL.

Scinax fuscomarginatus

ARGENTINA:

CORRIENTES: Ituzaingó, Proximities of the Reserva Santa Maria (MLP-DB 5982, 5993, 5996, 6006, 6011, 6016, 6017).

BOLIVIA:

SANTA CRUZ DE LA SIERRA: Ichilo, Buena Vista (MNKA 479, 492 *Scinax parkeri* topotypes); Velasco, Flor de Oro (MNKA 980, 981); Andrés Ibañez, Las Cruces 2,5 km W from the Rio Pirai (MNKA 1973); Andrés Ibañez, Las Cruces (MNKA 2179, 3001); Andrés Ibañez, Las Cruces 2 km W from the Rio Pirai (MNKA 2190-93, 2196); Angel Sandoval, San Fernando Community (MNKA 3395-96); Andrés Ibañez, La Belgica (MNKA 4525-26); Andrés Ibañez, Lomas de Arena (MNKA 7544); Andrés Ibañez, Road from Santa Cruz to Porongo (MNKA 7586); Velasco, Estancia Caparú (MNKA 8254-55); Ñuflo de Chávez, Estancia San Sebastián (MNKA 9128-29, 9131).

BENI: Bella Vista (MNKA 7625); Ballivián, Estancia Media Luna (MNKA 7667).

BRAZIL:

ALAGOAS: Passo de Camarajibe (CFBH 7324, 7327-28, 7349-52, 7402-05).

BAHIA: Barreiras (CHUNB 51001-02); Caetitê (CFBH 22155-56); Caravelas (MNRJ 34979-81); Cocos (CHUNB 37325, 42552-53, 42255, 50181); Correntina: Agrovila II (MCNAM 7878-80); Entre Rios (MNRJ 37911); Jaborandi (CHUNB 51007-08); Jaborandi, Pratudinho river (CFBH 20548-49); Luis Eduardo Magalhães (CHUNB 51003); São Desidério (CHUNB 51004-06); São Desidério, Povoado de Almas (CFBH 20537-38).

CEARÁ: Viçosa do Ceará, Santo Mano (CFBH 19386).

DISTRITO FEDERAL: Brasilia (CHUNB 16840-41, 16845-47, 16849, 16853, 16855); Planaltina, Lagoa Bonita (MNRJ 18328-29).

GOIÁS: Alto Paraíso de Goiás (CHUNB 49559-60, 58766); Alvorada do Norte (CHUNB 37939); Aruanã (CHUNB 42732); Britânia (CHUNB 30364, 42711); Cáceres, Baia da Caiçara (ZUEC-AMP 10255-57); Campo Limpo de Goiás, Fazenda Boa Vista (CFBH

15635); Colinas do Sul, LT Serra da Mesa (CHUNB 44701); Goiânia (MNRJ 36026-30); Mineiros (CHUNB 13749, 13967, 13970-71); Paraúna (CHUNB 53123-24); Pirenópolis (CHUNB 52344, ZUEC-AMP 10271-72); São Domingos (CHUNB 36451-52, 36456, 36459, 36460); São João D'Aliação (CFBH 6654-55, 6745); Serranópolis (CHUNB 16883); Silvânia, EFLEX (CFBH 9512); Mossâmedes, Fazenda Poça D'água (MNRJ 32845-49, 32851); Mamboi (MNRJ 27811-13).

MARANHÃO: Carolina (CHUNB 51592, 51595-97, 51602, 51604); São Luiz, UTE Ponta da Madeira (MCNAM 10086, MNRJ 53222).

MATO GROSSO: Alta Floresta (CFBH 21844); Alta Floresta, PCH Apiacás (UFMT 9132, 9146); Alto Araguaia (CHUNB 58720-23, 58726); Barão de Melgaço, Fazenda São Perigara (UFMT 853); Barra do Bugres, Road to Cuiabá km 4 (ZUEC-AMP 7091-92); Barra do Garça (CHUNB 16871, 47555); Brásnorte, PCH Bocaiuva (UFMT 10122-24); Cáceres, Sepotuba river (UFMT 2229, 2274, 2280, 2295); Chapada dos Guimarães, APM Manso (UFMT 1426-28, 2921-27); Chapada dos Guimarães, Serra da Esperança (ZUEC-AMP 6118); Chapada dos Guimarães, NE Água Fria, ca. 7 km of Água Fria district (CFBH 14302-08); Chapada dos Guimarães, Escola Evangélica Buriti (CFBH 14395, 14397-99); Chapada Gaúcha, Serra das Araras State Park (CHUNB 34076-78); Cuiabá, PARNA Chapada dos Guimarães (UFMT 8161); Cuiabá, Road MT-251 (UFMT 8223); Dom Aquino, Fazenda Barreirão, Road MT 344, between Campo Verde and Dom Aquino (CFBH 14334-35); Serra das Araras Ecological Station (CFBH 21857-58); Nobres (CHUNB 40770); Paranaíta, PCH Teles Pires (UFMT 9887); Poconé, Fazenda Santa Inês (UFMT 625-26); Porto Alegre do Norte (CHUNB 47817-18, 47839-40); Primavera do Leste (UFMT 8811, 8816, 8826); Guiratinga, Corrientes river (MNRJ 30999-002); Santa Terezinha, Ilha do Bananal (CHUNB 10854-55, 10857).

MATO GROSSO DO SUL: Alcínópolis, Fazenda Vista Bonita (CHUNB 25964-65); Ribas do Rio Pardo (ZUEC-AMP 6697, 6700-07, 6738); Corumbá, Passo do Lontra (CFBH 4408-10, 3430, 3432-34, 4333); Dourados, PNM of Paragrem (MNRJ 50854-55); Três Lagoas (CFBH 19620-21). Corumbá, RPPN Acurizal, Serra do Amolar (UFMT 2630).

MINAS GERAIS: AHE Queimado (MCNAM 8857); Barão de Cocais (MCNAM 708-12, 10765-66, CHUNB 16842, 16872); Barão de Cocais, 2 Irmãos mine (MCNAM 11268-69); Belo Horizonte (ALMN 845 *Scinax fuscomarginatus* lectotype; 847, 848-50 *Scinax fuscomarginatus* paralectotypes); Buritizeiro, Fazenda do Jatobá (CHUNB 43485, 44632-33, 44436-37); Buritis (CHUNB 16885); Conselheiro Mata (MCNAM 591, 594); Sindrolândia,

Road to Campo Grande, km 25 (MNRJ 58022-23, 58025, 58027, 58029); Fazenda de Casiata, Patos de Minas (MNRJ 58003-04); Guada Mor (MCNAM 10667); Lagoa Grande (CHUNB 52343); Lagoa Santa (CHUNB 52342; CFBH 24356-63; MNRJ 3082, 13289-96, 33672); Mariana/Catas Altas (MCNAM 6441-42); Burucutu Mine CVRD (MCNAM 6606); Lagoa da Prata, Francisco de Assis Rezende Ecological Park (MCNAM 9501); Paracatu, Jazida de Ambrósia (MCNAM 8062-63); Paracatu (CHUNB 25888-90, 25892-93); Periquito (MCNAM 11082); Pirapora (CFBH 9286-90); Pirapora, Paracatu transmission line (MCNAM 11010-11); Serra do Cipó (CFBH 9269); Serra do Cipó, PARNA (MCNAM 3195); Unai (MCNAM 10691, CHUNB 47553).

PARÁ: Altamira, front to the Airport (ZUEC-AMP 7412-16); Altamira, Transamazônica road Km 09-19 to Marabá (ZUEC-AMP 7257-60); Novo Progresso, Serra do Cachimbo (CHUNB 34498, 34499-500, 34502, 34505-10); Novo Progresso, CPBV (CHUNB 40156-57, 40159-61).

PARAÍBA: Guaribas Biological Reserve, Mamanguapé (MNRJ 18042-45);

SÃO PAULO: Assis, Assis Ecological Station (CFBH 18855-56, 20040, 20051); Brotas, Horta Municipal (CFBH 398, 417); Campinas, Taubaté marsh (ZUEC-AMP 4185, 8038-39, 8044-45, 8062-65, 8070-71), Campus UNICAMP (ZUEC-AMP 4206, 7781-82), University city Barão Geraldo (ZUEC-AMP 2921-22), Fazenda Argentina, Barão Geraldo (CFBH 180); Cananéia, Airport (ZUEC-AMP 2898); Ibiúna (CFBH 4561); Itapetininga, Monte Alegre plant (CFBH 19354-55); Itirapina (CFBH 5706-07, 5710, 5714, 5842-46, 6006, 6008, 6622); Itirapina, Itirapina Ecological Station (CFBH 5935, 5938-44, 6057-59, 6527, 6636; ZUEC-AMP 12037-38); Itú, Fazenda São Miguel, Vrejão-Taquaral (APA) (CFBH 597); Jaguariuna, Fazenda Holambra (ZUEC-AMP 8162-63, 8179-81, 10887, 11292-93); Mogi Guaçu, Fazenda Campininha (ZUEC-AMP 8388); Nova Itapirema (ZUEC-AMP 13268); Paulina, João Aranha district (ZUEC-AMP 3173-87); Pilar do Sul (CFBH 7629); Pirajú (MNRJ 19408); Pirajú, Pedreira (MNRJ 31621), Pousada das Araras (MNRJ 50935); Pirassununga (CFBH 6697); Pirassununga, CEPTAS - Cachoeira de Emas (CFBH 21331-33); Ribeirão Branco, Fazendinha São Luiz (CFBH 354, 1849, 2300-01; ZUEC-AMP 6577); Rio Claro, Floresta Estadual Edmundo Navarro Filho (CFBH 19634); Rio Claro, Itapé, Sítio Cantaclaro (CFBH 7132, 21102); Rio Claro, Mata São José (CFBH 7155); Teodoro Sampaio, Morro do Diabo State Park (CFBH 10049, 10074-75, 18286-89, 18678-79); Teodoro Sampaio, Morro do Diabo State Park, Roda D'água dam (CFBH 12064, 12075).

SERJIPE: Santa Luzia do Itanhhy, Mata do Cristo (MNRJ 17933-34).

TOCANTINS. Aguiarnópolis, North-South railroad (CFBH 8160); Araguacema, Caiapó river (CFBH 10248); Araguaína (CFBH 11431, 11433 *Scinax lutzorum*); Caseara, Cantão State Park (CHUNB 46072-73, 46078, 46080, 46083); Colinas do Tocantins (CFBH 19890, CHUNB 52813-14, 57149, 57151-52); Dianópolis (CHUNB 33425); Dois Irmãos, Fazenda Vera Cruz (CFBH 10268); Formoso de Araguaia (ZUEC-AMP 10159-60, 10179-84, CHUNB 38977); Goiatins, near to Tocantins river (MNRJ 43921); Guaraí (CHUNB 50853); Lizarda, Jatobá (CFBH 13240-41); Mateiros, Jalapão (CHUNB 27356, 27359, 27364); Mateiros, 37,2 km W of Mateiros, Novo river (CHUNB 42201, 42206); Palmas (CHUNB 11330, 11332-34, 22074); Paraíso de Tocantins (CHUNB 52815-17); Paranã (CHUNB 38024-26, 38029-30); Cantão State Park, Confusão lagoon (CHUNB 58113); Pedro Afonso (CHUNB 36633, 36635-37, 43178); Porto Alegre de Tocantins (MNRJ 41472); Porto Nacional, road to Fazenda Lazer (CFBH 16491-95); Porto Nacional (ZUEC-AMP 13476-82).

PARAGUAY:

ALTO PARANÁ: 12 km W of Ciudad del Este, Escuela Técnica Forestal (MNHNP 2196); Hernandarias, Vivero Forestal de Itaipú (MNHNP 8158, 6041).

AMAMBAY: 2 km W of Bella Vista, Hacienda Félix Ocariz (MNHNP 2160-78); Estancia Paicuará, 3 km S of Cerro Guazú de Arroyo Blanco (MNHNP 5439), Estancia Paicuará, 45 km W of Capitán Bado, Zapallo stream (MNHNP 4415, 5434-37); Cerro Corá National Park, Administration (MNHNP 2179-81, 2183-93, 2195, 4379, 4390, 4459, 4478, 5259, 5398, 5440-46, 5559).

CANINDEYÚ: 11 km SE of Katueté (MNHNP 8215, 8556); Itanara'í, 7 km N of Itanará, 25 km N of Ypejhú (MNHNP 5438); Mbaracayú Reserve, Puesto Lagunita (IIBP-H 20-21, 37; MNHNP 3649-56, 6840, 7725, 7728-29, 7740-41, 7764-66, 7739, 9851, 10845-48).

CONCEPCIÓN: Concepción (MNHNP 7006, 7008, 7019); 85 km NE of Concepción, Capitigo stream (MNHNP 5313, 5447); Serranía San Luis National Park (MNHNP 6443); Serranía San Luis National Park, Retiro Laurel, 30 km N of Estancia San Fernando (MNHNP 9877-78).

CORDILLERA: Emboscada district, Cabaña Las Marías, Cerro Vy (IIBP-H 436, 445); Compañía Matiauda, 15 km N of San Bernardino, Salado river (MNHNP 4459).

ITAPÚA: Carmen del Paraná (MNHNP 104, 107, 109); Centro de Desarrollo Forestal, Pirapó (MNHNP 139); Alto Verá district, Ditore family property (MNHNP 8799, 8839); Estancia San Isidro, 50 km NW of Pirapó (MNHNP 9110, 9677); Isla Yasyretá, Air Base (MNHNP 211, 215); Isla Yasyretá, Dunes (MNHNP 11090-92); Isla Yasyretá, Estancia Melgarejo, near the Dunes (MNHNP 4108-09, 6062, 6064-70, 8135, 8155-56); Hacienda Martín Leiva, 24 km S of Santiago (MNHNP 180-81).

ÑEEMBUCÚ: 2.7 Km NE of Pilar (MNHNP 10049); Estancia San José, 11 km W of Humaitá (IIBP-H 265, 269, 317; MNHNP 6083-88).

PRESIDENTE HAYES: Estancia Santa María de la Doce, Fundación La Piedad, Retiro San Juan, Km 61.5 of the Transchaco Road, 3 km W from administration (MNHNP 10693); Estancia La Golondrina, 30 km NW of Presidente Hayes (MNHNP 5432-33); General Bruguez Road Km 38 (MNHNP 7106).

SAN PEDRO: Aguaray Guazú River, 2 km NW of Lima (MNHNP 5448, 4477).

VENEZUELA:

BOLIVAR: Km 296 of the road 10 state of Bolívar (ZUEC-AMP 9415-16 *Scinax trilineatus*).

Scinax madeirae

BRAZIL

RONDÔNIA: Porto Velho (MZUSP 73663 (WCAB 10768) *Scinax madeirae* holotype, MZUSP 74487-90, MZUSP 73658 (WCAB 10767), MNRJ 3963 *Scinax madeirae* paratypes, CFBH 25469-70 *Scinax madeirae* topotypes); Vilhena (CHUNB 9902, 9906-07, 9919, 9931).

BOLIVIA

SANTA CRUZ DE LA SIERRA: Velasco, Flor de Oro (3008, 3010, 3014, 3130, 3148-49); Velasco, Los Fierros (MNKA 3016, 3147).

BENI: Bella Vista (MNKA 7658)

Scinax exiguus

VENEZUELA

BOLÍVAR: Rápidos de Kamoirán (ZUEC-AMP 9420-21).

*Scinax acuminatus***BRAZIL**

MATO GROSSO DO SUL: Corumbá, Passo do Lontra (CFBH 6423); Corumbá, Base de Estudos do Pantanal (CFBH 8643, 8645).

*Scinax alter***BRAZIL**

ESPIRITO SANTO: Muniz Freire (CFBH 4108); Serra (CFBH 10838); Mimoso do Sul (CFBH 11103-04); Marataízes (CFBH 18471; 19482-3, 19485-7); Linhares (CFBH 19439, 19441).

*Scinax auratus***BRAZIL**

BAHIA: Salvador (CFBH 14628); Uruçuca (CFBH 14629); Jequié (CFBH 23662).

*Scinax camposseabrai***BRAZIL**

BAHIA: Contendas do Sincorá (CFBH 23482-4).

*Scinax caldarum***BRAZIL**

MINAS GERAIS: Poços de Caldas (CFBH 14, 6385-90 topotypes).

*Scinax curicica***BRAZIL**

MINAS GERAIS: Ouro Preto (CFBH 24372-3, 24379).

*Scinax crospedospilus***BRAZIL**

RIO DE JANEIRO: Itatiaia (CFBH 5737 topotype).

SÃO PAULO: Queluz (CFBH 7525-6).

Scinax duartei

BRAZIL

RIO DE JANEIRO: Itatiaia, Brejo da Lapa (CFBH 872, 9896 topotypes); Petrópolis (CFBH 10187-8)

Scinax eurydice

BRAZIL

BAHIA: Ilhéus (CFBH 2884); Uruçuca (CFBH 23369); Maracás (CFBH 23660 topotype).

Scinax granulatus

BRAZIL

RIO GRANDE DO SUL: São Francisco de Paula (CFBH 3380-1).

SANTA CATARINA: Treviso (CFBH 9849).

Scinax hayii

BRAZIL

RIO DE JANEIRO: Petrópolis (CFBH 10182-6 topotypes).

Scinax maracaya

BRAZIL

MINAS GERAIS: Alpinópolis (CFBH 6621, 22301 topotypes); Itabirito (CFBH 18424-25).

Scinax nasicus

BRAZIL

CORUMBÁ: Passo do Lontra (CFBH 4330, 4386-90).

Scinax similis

BRAZIL

ESPIRITO SANTO: Conceição da Barra (CFBH 4156-7).

RIO DE JANEIRO: São João da Barra (CFBH 5018-9).

Scinax squalirostris

BRAZIL

SÃO PAULO: São José do Barreiro (CFBH 21975-9 topotypes).

APPENDIX 3. CALL RECORDS ANALYZED. Columns represent identification of the call record, voucher, species, locality, record, and microphone used.

ID	Voucher	species	locality	recorder	microphone
MJ 1316	MJ 1316	Scinax parkeri	Bolivia, S. Cruz de la Sierra, Buena Vista	Olympus DM-550	Sennheiser ME66
MJ 1259	MJ 1259	Scinax parkeri	Bolivia, S. Cruz de la Sierra, Ñuflo de Chavez, San Sebastian	Olympus DM-550	Sennheiser ME66
MJ 1252	MJ 1252	Scinax parkeri	Bolivia, S. Cruz de la Sierra, Ñuflo de Chavez, San Sebastian	Olympus DM-550	Sennheiser ME66
MJ 1320	MJ 1320	Scinax parkeri	Bolivia, Beni, Yucuma, Los Lagos	Olympus DM-550	Sennheiser ME66
fita 2 cut 2		Scinax trilineatus	Venezuela, Bolivar, Santa Elena Uairén		
CFBH25469	CFBH25469	Scinax madeirae	Brazil, Rondonia, Porto Velho, Porto Velho		
MJ 1237	MJ 1237	Scinax madeirae	Bolivia, S. Cruz de la Sierra, Ñuflo de Chavez, San Sebastián	Olympus DM-550	Sennheiser ME66
MJ 1265	MJ 1265	Scinax madeirae	Bolivia, S. Cruz de la Sierra, Ñuflo de Chavez, San Sebastián	Olympus DM-550	Sennheiser ME66
CFBH24360	CFBH24360	S. fuscomarginatus	Brazil, Minas Gerais, Lagoa Santa	Marantz PMD 222	Sennheiser ME66
CFBH24361	CFBH24361	S. fuscomarginatus	Brazil, Minas Gerais, Lagoa Santa	Marantz PMD 222	Sennheiser ME66
CFBH24362	CFBH24362	S. fuscomarginatus	Brazil, Minas Gerais, Lagoa Santa	Marantz PMD 222	Sennheiser ME66
CFBH24363	CFBH24363	S. fuscomarginatus	Brazil, Minas Gerais, Lagoa Santa	Marantz PMD 222	Sennheiser ME66
m. diabo1		S. fuscomarginatus	Brazil, São Paulo, Teodoro Sampaio, P. E. Morro do Diabo		
CFBH 19-02		S. fuscomarginatus	Brazil, São Paulo, Ribeirao Branco, Fazenda Mathedi		
CFBH 33-06		S. fuscomarginatus	Brazil, São Paulo, Ribeirao Branco, Fazenda Sao Luiz	Nagra E	Sennheiser ME80
CFBH 33-09		S. fuscomarginatus	Brazil, São Paulo, Ribeirao Branco, Fazenda Sao Luiz	Nagra E	Sennheiser ME80
CFBH 53-07		S. fuscomarginatus	Brazil, Goiás, Piracanjuba, Fz. Recanto Sonhado	UHER 4000	UHER M518A
CFBH 58-06		S. fuscomarginatus	Brazil, Goiás, São João		
fita 11 cut 5		S. fuscomarginatus	Brazil, São Paulo, Paulínia, João Aranha		
fita 11 cut 6		S. fuscomarginatus	Brazil, Goiás, Mineiros, PARNA das Emas	UHER monitor	M538
AFF-cut 1		S. fuscomarginatus	Brazil, Goiás, Pirenópolis	UHER 4200	DB88
RPB 5-5		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	UHER 4000	UHER M518A
RPB 6-3		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	Nagra E	
RPB 6-4		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	Nagra E	
RPB 6-5		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	Nagra E	
RPB 6-6		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	Nagra E	
RPB 6-9		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	Nagra E	
RPB 6-10		S. fuscomarginatus	Brazil, Goiás, Silvania, Lixao	Nagra E	
fita 2 cut 1	ZUEC 7089	S. fuscomarginatus	Brazil, Mato Grosso, Barra do Bugres	UHER R-S 4000	DB88
AFF-cut 4		S. fuscomarginatus	Brazil, Mato Grosso, Cáceres	UHER IC	M634-P9999
AFF-cut 2		S. fuscomarginatus	Brazil, Tocantins, Formoso de Araguaia		M88-PO619
AFF-cut 3		S. fuscomarginatus	Brazil, Tocantins, Formoso de Araguaia		M534-P9999
fita11cut7	ZUEC 7259	S. fuscomarginatus	Brazil, Pará, Altamira	UHER monitor	M538
fita11cut 8	ZUEC 7414	S. fuscomarginatus	Brazil, Pará, Altamira, in front to airoport	UHER monitor	M538
7965	KU 128542	S. fuscomarginatus	Brazil, Pará, Ipean, 3 km E of Belém		
7966	KU 128543	S. fuscomarginatus	Brazil, Pará, Ipean, 3 km E of Belém		
7967	KU 128545	S. fuscomarginatus	Brazil, Pará, Ipean, 3 km E of Belém		
7969	KU 128549	S. fuscomarginatus	Brazil, Pará, Ipean, 3 km E of Belém		
7970	KU 128550	S. fuscomarginatus	Brazil, Pará, Ipean, 3 km E de Belém		
7971	KU 128551	S. fuscomarginatus	Brazil, Pará, Ipean, 3 km E de Belém		
AL001		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
AL002		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
AL003		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
AL004		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
AL005		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
AL006		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
AL007		S. fuscomarginatus	Brazil, Alagoas, Passo de Camarajibe		
IgorRoberto		S. fuscomarginatus	Brazil, Ceará, Caucaia, E. Ecol. do Pecém	Olympus LS-11	

ID	Voucher	species	locality	recorder	Microphone
IIBP 001	IIBPH 1075	<i>S. fuscomarginatus</i>	Paraguay, Amambay, Estancia Pirá Potrero	Marantz PMD 222	Sennheiser ME66
IIBP 002		<i>S. fuscomarginatus</i>	Paraguay, Amambay, Estancia Pirá Potrero	Marantz PMD 222	Sennheiser ME66
IIBP 003	IIBPH 1074	<i>S. fuscomarginatus</i>	Paraguay, Amambay, Estancia Pirá Potrero	Marantz PMD 222	Sennheiser ME66
IIBP 004	IIBPH 1109	<i>S. fuscomarginatus</i>	Paraguay, Amambay, Estancia Pirá Potrero	Marantz PMD 222	Sennheiser ME66
IIBPH 1593	IIBPH 1593	<i>S. fuscomarginatus</i>	Paraguay, Guairá, Coronel Martínez, Estancia Yaca	Marantz PMD 222	Sennheiser ME66
IIBP-H 1594	IIBPH 1594	<i>S. fuscomarginatus</i>	Paraguay, Guairá, Coronel Martínez, Estancia Yaca	Marantz PMD 222	Sennheiser ME66
IIBP-H 1557	IIBPH 1557	<i>S. fuscomarginatus</i>	Paraguay, P. Hayes, La Golondrina	Marantz PMD 222	Sennheiser ME66
IIBP-H 1558	IIBPH 1558	<i>S. fuscomarginatus</i>	Paraguay, P. Hayes, La Golondrina	Marantz PMD 222	Sennheiser ME66
IIBP-H 1559	IIBPH 1559	<i>S. fuscomarginatus</i>	Paraguay, P. Hayes, La Golondrina	Marantz PMD 222	Sennheiser ME66
IIBP-H 1560	IIBPH 1560	<i>S. fuscomarginatus</i>	Paraguay, P. Hayes, La Golondrina	Marantz PMD 222	Sennheiser ME66
DB 001		<i>S. fuscomarginatus</i>	Argentina, Corrientes, Ituzaingó, Reserva Santa Maria	Sony TCM 359	Aiwa JS 445