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Phenotypic variation of *Leptodactylus cupreus* Caramaschi, São-Pedro and Feio, 2008 (Anura, Leptodactylidae)

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Abstract

This study describes for the first time the female of *Leptodactylus cupreus* and provides new information concerning its geographical distribution, male's morphology and bioacustics. *Leptodactylus cupreus*, a poorly known species from the Brazilian Atlantic Forest, was originally allocated in the *L. mystaceus* complex of the *L. fuscus* species group. Based on morphological observations, we infer that *L. cupreus* should be in fact related to *L. mystacinus*, a species that, although assigned to the *L. fuscus* species group, is not assigned to the *L. mystaceus* complex. Therefore, we comment the phylogenetic relationships concerning *L. cupreus*, *L. mystaceus* and *L. mystacinus*.

Key words: *Leptodactylus cupreus*, Geographic distribution, Morphological variation, Advertisement call, Female description

Resumo

Este estudo descreve pela primeira vez a fêmea de *Leptodactylus cupreus* e fornece novas informações sobre sua distribuição geográfica, morfologia e bioacústica dos machos. *Leptodactylus cupreus*, uma espécie pouco conhecida da Floresta Atlântica brasileira, foi originalmente alocada dentro do complexo *L. mystaceus* do grupo de espécies de *L. fuscus*. Baseados em observações morfológicas, propomos que *L. cupreus* deve ser mais relacionado a *L. mystacinus*, uma espécie que, apesar de pertencer ao grupo de espécies de *L. fuscus*, não está alocada dentro do complexo *L. mystaceus*. Portanto, comentamos o relacionamento filogenético das espécies *L. cupreus*, *L. mystaceus* e *L. mystacinus*.

Introduction

The genus *Leptodactylus* is divided into five species groups. Within the *Leptodactylus fuscus* group, the *L. mystaceus* complex comprises six species: *L. mystaceus*, *L. spixi*, *L. notoaktites*, *L. elenae*, *L. didymus* and *L. cupreus* (Heyer 1996; De Sá *et al.* 2005; Caramaschi *et al.* 2008), distributed in the Amazon basin, Atlantic Forest, Cerrados, Chacos and Caatingas of South America (Frost 2011).

Caramaschi *et al.* (2008) considered *L. cupreus* as a member of *L. fuscus* group and related to the *L. mystaceus* complex. The species was diagnosed and distinguished from the other species within the *L. fuscus* group by the following combination of traits: general color of dorsum copper without spots; presence of a large black stripe extending from the tip of snout to the shoulder passing through the eye and tympanum; outer metacarpal tubercle divided and smaller than the inner metacarpal tubercle. In addition, the advertisement call presents a combination of traits (not pulsed, call rate of 12 calls per second and dominant frequency between 2,800 and 3,058 Hz) that is also diagnostic (Caramaschi *et al.* 2008).

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Until recently, *L. cupreus* was known to occur only at its type locality (Parque Estadual da Serra do Brigadeiro —municipality of Ervália, state of Minas Gerais, Brazil) and at the municipality of Santa Tereza, state of Espírito Santo, Brazil (Caramaschi *et al.* 2008; Peres *et al.* 2010). Furthermore, a compilation of data gathered from fieldwork, literature and specimens housed at scientific collections increased its geographical range up to 160 km north to the municipalities of Cariacica, Linhares and Barra de São Francisco, state of Espírito Santo (Almeida *et al.* 2011).

The recognition and identification of species belonging and/or associated to the *L. mystaceus* complex have been historically troubled (see Heyer *et al.* 1996; Ponssa 2008). Some species are morphologically very similar to each other and the capture of specimens is not always simple, leading to small local samples of widespread species. Although *L. cupreus* is clearly distinct from other species of the *L. fuscus* group, some specimens from newly recorded populations presented different morphologies that led researchers to question their taxonomic status (e.g. Tonini *et al.* 2010).

Although the number of new taxa of amphibian anurans descriptions increases each year (Rodrigues *et al.* 2010, Frost 2011, IUCN 2012), few contributions deal with intraspecific variation. In some cases, only a handful of specimens are deposited in scientific collections and new records of morphologically variant populations can raise doubts about their taxonomic assignation. Morphological and acoustic variation among populations could be erroneously recognized as specific diagnostic traits of different species if they are not clearly stated in descriptions and/or if few specimens are analyzed.

Herein we provide information not mentioned for the types of *L. cupreus* and a more detailed morphological variation. Given that the original description compared *L. cupreus* mostly with *L. mystaceus* and *L. mystacinus* we discuss the morphological variation biased towards these taxa. Our findings include the first record of a female *L. cupreus*.

Material and methods

Studied specimens are housed at the following collections: Collection Célio F. B. Haddad, Departamento de Zoologia, I.B., UNESP, Rio Claro, SP, Brazil (CFBH), Museu Nacional, Rio de Janeiro, RJ, Brazil (MNRJ) and Museu de Zoologia da Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil (MZUESC). Specimens were collected under license number 13708-1 in the municipality of Camacan and 26378-1 in the municipality of Porto Seguro, granted by Instituto Chico Mendes de Conservação da Biodiversidade—ICMBio.

To enhance comparisons we took the same measurements provided in the original description of *L. cupreus* (Caramaschi *et al.* 2008). Measurements used are: SVL (snout-vent length); HL (head length, from posterior margin of the lower jaw to tip of snout); HW (head width, measured at the level of posterior margin of lower jaw); IND (internarial distance); END (eye to nostril distance); ED (eye diameter); TD (tympanum diameter); HAL (hand length, from the posterior margin of the outer metacarpal tubercle to tip of finger IV); THL (thigh length, from knee to cloaca); and TL (tibia length). Although almost any measurement is influenced by fixation artifacts (see comments in Hayek *et al.* 2001) we tried to keep these artifacts to an acceptable minimum. Thus, all measurements were taken by only one of us (Victor G. D. Orrico), except the female MZUESC 9041 (which was taken by Iuri R. Dias). In addition, we did not measure the upper eyelid width, interorbital distance and foot length, used in the description of *L. cupreus*, due to the high influence of preservation artifacts in these measurements. Fingers are numbered according to the homology concept of Fabrezi (1992).

Carla S. Cassini and Victor G. D. Orrico recorded two specimens of *L. cupreus* on 03 April 2012, air temperature at approximately 24ºC, at the Reserva Particular do Patrimônio Natural (RPPN) Estação Veracel, municipality of Porto Seguro, state of Bahia. These were at a chorus of about five specimens; all emitting advertisement calls only, about 20 to 50 meters from each other, under fallen tree trunks, litter and herbaceous vegetation of a dry temporary pond surrounded by a tropical rain forest. The forest is partially surrounded by cleared farmlands where several specimens of *L. fuscus* were calling.

For recordings, we used a Marantz Professional digital recorder model PMD-660 at a sampling rate of 44.1 kHz and an unidirectional Sennheiser ME-66 microphone. Sound analyses, oscillograms, spectograms and power spectrae were performed using Raven Pro 1.4 following parameters: Bandwidth = 248 Hz; FFT length = 256. Terminology used for call comparisons follows Duellman and Trueb (1994).

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We examined two females: CFBH 23632, from the municipality of Linhares, state of Espírito Santo, Brazil, and MZUESC 9041, from the municipality of Camacan, state of Bahia, Brazil. The specimen CFBH 23632 already presented a dissection at the ventral surface and in the specimen MZUESC 9041 we were able to see oocytes by transparency.

Results

The type localities of *L. cupreus* and *Chiasmocleis mantiqueira* are the same ("Lagoa das Bromélias", at the district of Careço, municipality of Ervália, state of Minas Gerais; Cruz *et al.* 2007; Caramaschi *et al*, 2008; Renato N. Feio pers. comm.). Nevertheless, the coordinates given by each contribution (Cruz *et al.* 2007; Caramaschi *et al*, 2008) are not concordant. Caramaschi *et al.* (2008)'s coordinates corresponds to a location nearby the municipality of Ouro Preto, state of Minas Gerais, which is about 100 km E from the coordinates of the type locality of *C. mantiqueira* (20°53'S, 42°31'W; Cruz *et al.* 2007). Notwithstanding, Motta *et al.* (2010) describe the tadpole of *L. cupreus* from its type locality which is concordant with the above mentioned coordinates of *C. mantiqueira*. Thus, we consider the latter as the correct coordinates of the type locality of *L. cupreus*.

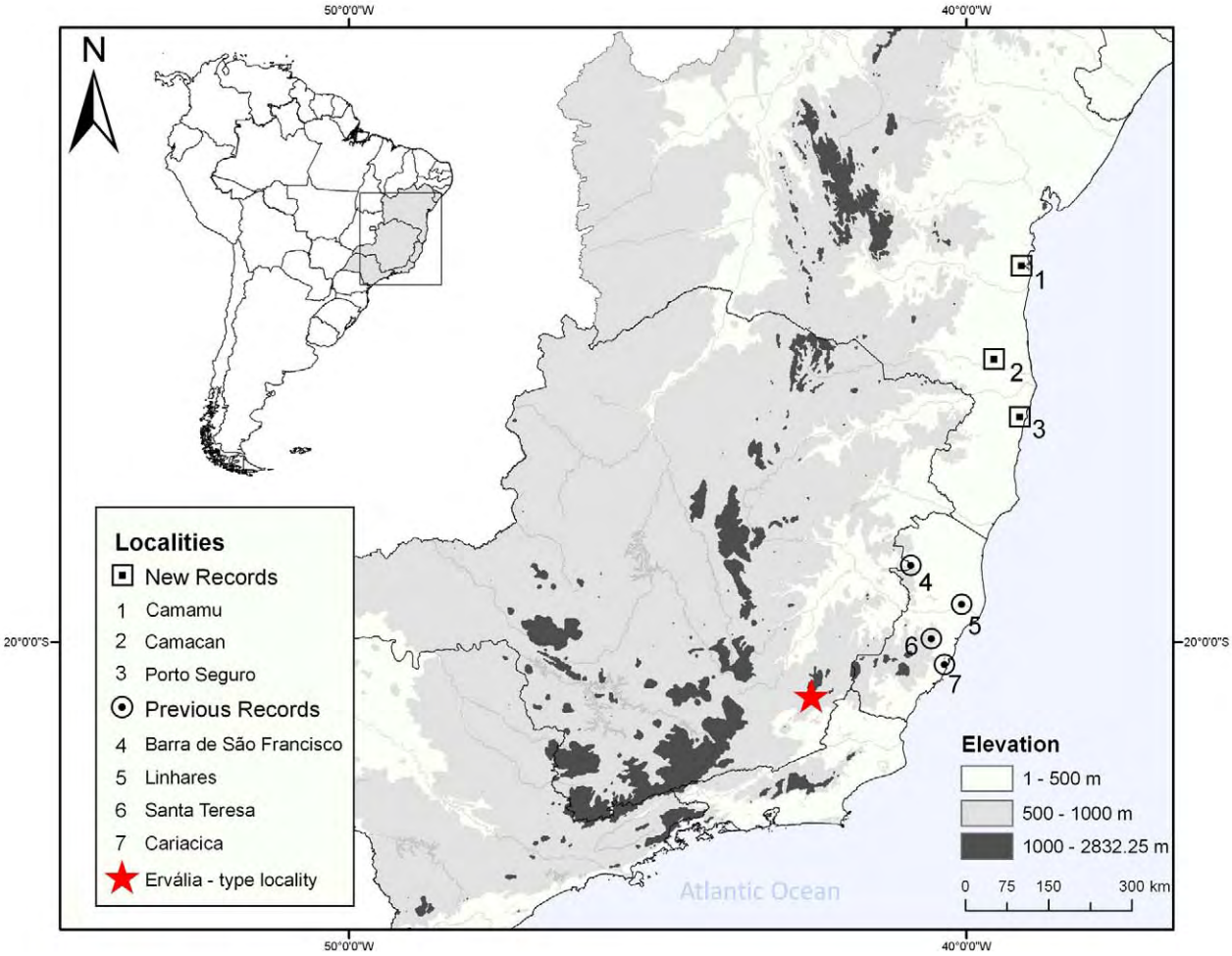

FIGURE 1. Geographical distribution of *Leptodactylus cupreus*.

We report the occurrence of *L. cupreus* at three new municipalities in the state of Bahia, Brazil (Fig. 1), extending its geographic range up to 550 km north. The specimen MNRJ 80244 was collected at the municipality of Camamu (13º56'4"S; 39º07'38"W; no altitudinal record); MZUESC 9041 was collected at RPPN Serra Bonita, municipality of Camacan (15º25'21.9''S; 39º32'52.4''W; 253 m above sea level); MZUESC 7864 was collected at

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the Parque Nacional do Pau Brasil (16º30'43.7''S; 39º18'14.04''W) and the specimens CFBH 32103 - 32104 were collected at the RPPN Estação Veracel (16º21'20,6"S; 39º08'05,4"W; 80 m above sea level), the last two localities are located at the municipality of Porto Seguro. All collection sites are shaded temporary ponds located inside medium-sized fragments of pristine or almost undisturbed forests.

Thus, *L. cupreus* is distributed through fragments of coastal and continental tropical rain forests from about 20 to 1227 m above sea level and about 850 km airline distance from the southernmost record (municipality of Ervália, state of Minas Gerais) to the northernmost record (municipality of Camamu, state of Bahia).

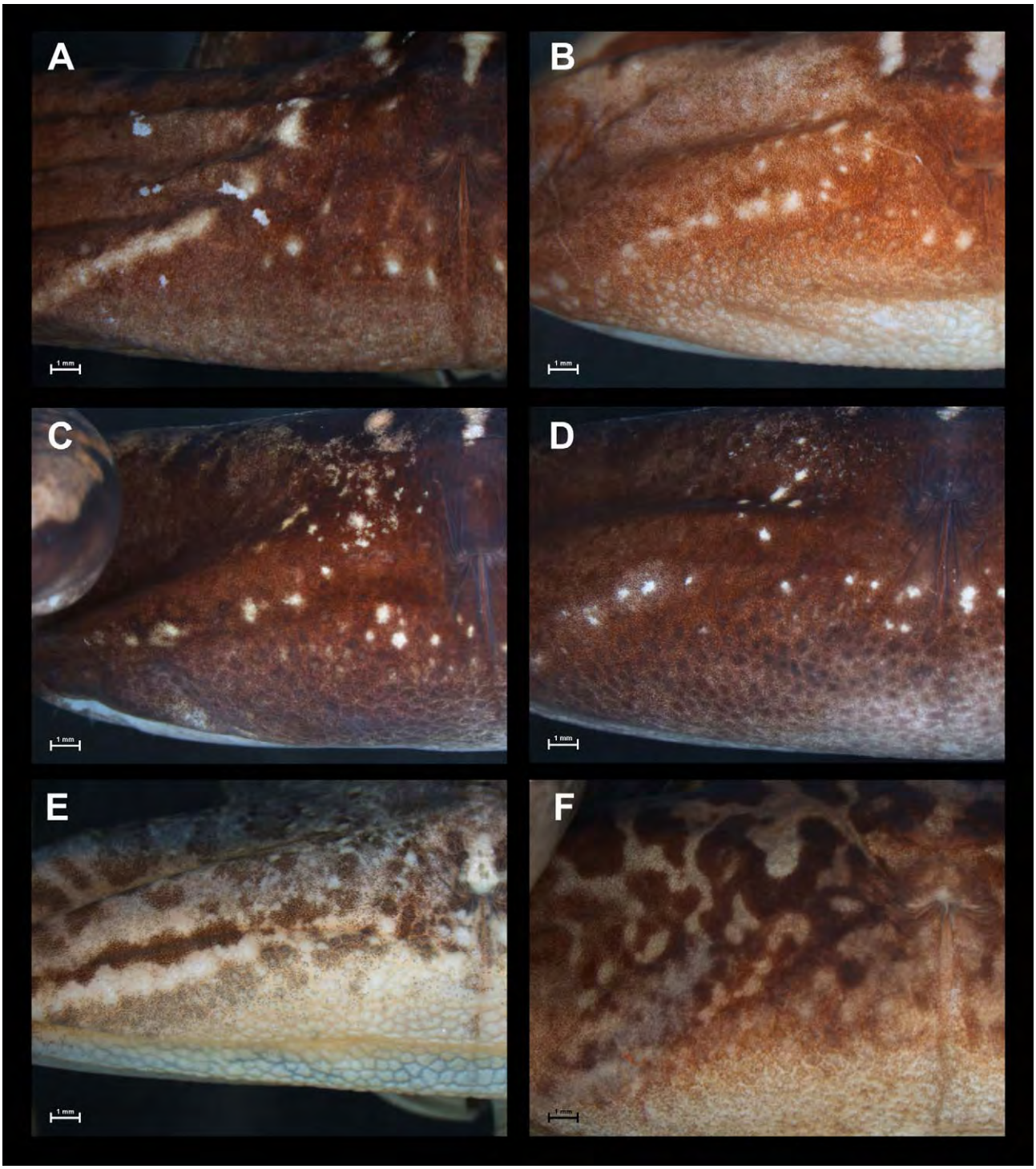

FIGURE 2. Variation of the white line in the posterior region of the thigh. A—*Leptodactylus cupreus*, CFBH 23632, well marked; B, C and D—*L. cupreus*, CFBH 26359, CFBH 32113 and CFBH 32114 respectively, weakly marked; E—*L. mystaceus*, CFBH 17242, well marked; F—*L. mystacinus*, CFBH 18386, absent.

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The morphological variation found between the populations from the state of Bahia and the original description led us to ask if those should be interpreted as interpopulational or interspecific variations. Nevertheless, the advertisement call is nearly identical to the one presented in the original description (see Caramaschi *et al.* 2008).

Morphological description. Color. The white line in the posterior region of the thigh found in *L. cupreus* varies from well to weakly marked (Fig. 2 A–D). When present, well or weakly marked, this line is diagonal to the axis of the thigh. In *L. mystaceus* the white line in the posterior region of the thigh is always present, well marked, and at the same axis of the thigh (Fig. 2 E, Table 2). *Leptodactylus mystacinus* never presents such white line (Fig. 2 F, Table 2). The background color of the posterior region of the thigh is deep brown or black in *L. cupreus*, while in *L. mystaceus* and *L. mystacinus* it is marbled in brown (Fig. 2).

Flanks may vary from bright copper with scattered spots and flecks, with a dark line dorsally (see Caramaschi *et al.* 2008, Figure 3), to dark, nearly solid black, contrasting deeply with the dorsal color pattern (Fig. 3). Additionally, the black stripe of the flanks can be interrupted, reaching only the scapular region (Fig. 3 A–C) or be continuous, fused with the black stripe at the lateral of the head (Fig. 3 D).

The gular region varies from pink (Caramaschi *et al.* 2008) to brownish or blackish gray (scattered with cream dots or not) in life; remaining with this color after fixation. Hands and feet vary from light grey with small yellow and/or copper dorsal flecks (Fig. 3 A, B) to completely light grey (Fig. 3 C, D).

Skin texture. Caramaschi *et al.* (2008) distinguished *L. cupreus* from other species of the *L. mystaceus* complex by the absence in the former of small spines (presumably composed of keratin) in the dorsal surface of tibia. Actually, examined specimens of both *L. cupreus* and *L. mystacinus* do present these small spines (Caramaschi *et al.* 2008, Figure 3; Fig. 4 A, C), while *L. mystaceus* does not (Fig. 4 B, see Table 2).

FIGURE 3. Variation of flanks and hands color. A and B—*Leptodactylus cupreus* from Lagoa das Bromélias, Parque Estadual da Serra do Brigadeiro, municipality of Ervália, state of Minas Gerais (photos: Renato Neves Feio); C—*L. cupreus* from RPPN Estação Veracel, municipality of Porto Seguro, state of Bahia; D—Female of *L. cupreus* from RPPN Serra Bonita, municipality of Camacan, state of Bahia. A and B—copper or darkish brown flanks and hands light grey with yellow and/or copper dorsal flecks; C and D—black flanks and light gray hands.

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FIGURE 4. Spines on dorsal surface of tibia. A—*Leptodactylus cupreus*, CFBH 23632; B—*L. mystaceus*, CFBH 509; C—*L. mystacinus*, CFBH 9804.

FIGURE 5. Spines on inner surface of finger III and IV of *Leptodactylus cupreus* (CFBH 26359).

External morphology. The outer metacarpal tubercle is always divided in *L. cupreus*, and it can be smaller than or as big as the inner metacarpal tubercle. Given that species of the *L. mystaceus* complex present an outer metacarpal tubercle entire and larger than the inner metacarpal tubercle (as do *L. mystaceus*; see Caramaschi *et al.* 2008), this trait distinguishes *L. cupreus* from all other species of the *L. mystaceus* complex. Notwithstanding, *L. mystacinus*, which belongs to the *L. fuscus* group but not to the *L. mystaceus* complex, does present a divided tubercle, that can be smaller or as big as the inner metacarpal tubercle (Cassini, C. S., pers. obs.), just as in *L. cupreus* (Table 2).

Leptodactylus cupreus presents a ridged surface, with small spines, on the inner side of fingers III and IV (Fig. 5). Although this character was neither mentioned nor shown on the holotype's drawings of *L. cupreus* (Caramaschi *et al.* 2008), we were able to examine the type series and confirm its presence.

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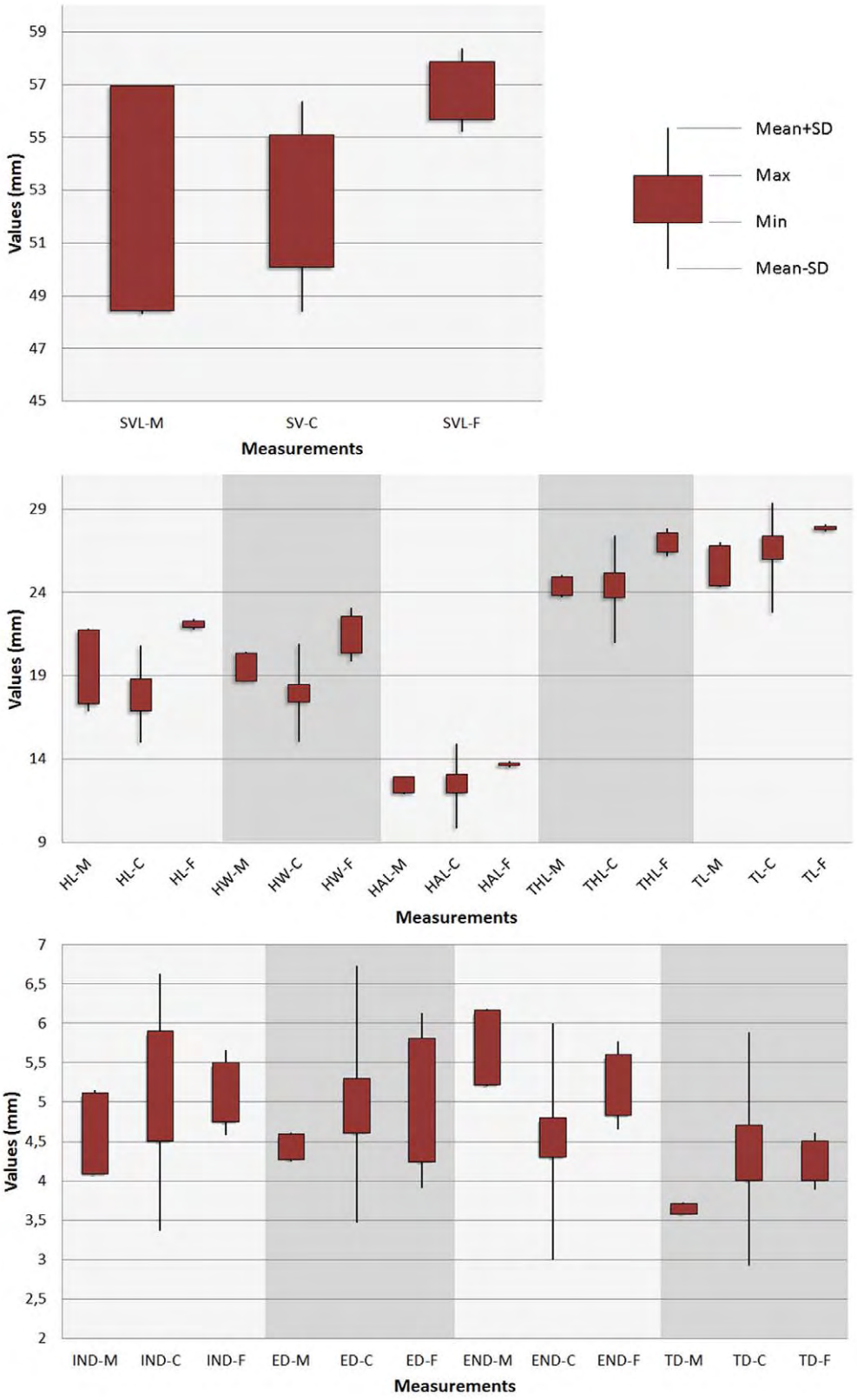

FIGURE 6. Boxplots of morphometric comparisons within *Leptodactylus cupreus* populations. Morphometric parameters followed by: **–M)** measurements of males from this study; **–C**) measurements of males taken by Caramaschi *et al.* (2008); and **–F**) measurements of females. Maximum (Max) and minimum (Min) values found within each population sample are represented by the upper and lower surfaces, respectively, of the quadrangle. The upper and lower lines of each boxplot represent the value of the sum and the subtraction, respectively, of the average of the sample and its correspondent standard deviation.

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Measurements of *L. cupreus* are shown on Table 1. The measurements of the populations from the state of Bahia do not differ from those of the type locality (Figure 6). Males and females do not differ morphometrically (Figure 6). Morphological comparisons between *L. cupreus*, *L. mystaceus* and *L. mystacinus* are shown on Table 2.

TABLE 1. Morphometric variation of *L. cupreus*. Value of parameters: range (mean ± standart deviation).

Morphometric parameter	**Caramaschi** *et al.* **2008** (males, n=8)	This study (males, n=4)	This study (females, n=2)
SVL	50.1–55.1 (52.4 ± 3.96)	48.5 – 57.0 (52.2 ± 3.9)	55,7 – 57.9 (56.8 ± 1.6)
HL	16.9–18.8 (17.9 ± 2.88)	17.3 – 21.8 (19.0 ± 2.0)	21.9 – 22.1 (22.3 ± 0.3)
HW	17.4–18.5 (18.0 ± 2.89)	18.7 – 20.4 (19.6 ± 0.8)	20.4 – 22.6 (21.5 ± 1.6)
IND	4.5–5.9 (5.0 ± 1.62)	4.1 – 5.1 (4.6 ± 0.5)	5.5 – 4.8 (5.1 ± 0.5)
END	4.3–4.8 (4.5 ± 1.49)	5.2 – 6.2 (5.6 ± 0.4)	4.8 – 5.6 (5.2 ± 0.5)
ED	4.6–5.3 (5.1 ± 1.62)	4.3 – 4.6 (4.4 ± 0.1)	4.2 – 5.8 (5.0 ± 1.1)
TD	4.0–4.7 (4.4 ± 1.47)	3.6 – 3.7 (3.6 ± 0.1)	4.0 – 4.5 (4.3 ± 0.4)
HAL	12.0–13.1 (12.4 ± 2.52)	12.0 – 12.9 (12.4 ± 0.4)	13.6 – 13.8 (13.7 ± 0.1)
THL	23.7–25.2 (24.2 ± 3.19)	23.8 – 25.0 (24.4 ± 0.6)	26.5 – 27.6 (27.0 ± 0.8)
TL	26.0–27.4 (26.1 ± 3.26)	24.4 – 26.8 (26.0 ± 1.1)	27.8 – 28.0 (27.9 ± 0.2)

TABLE 2. External morphology traits of *Leptodactylus cupreus*, *L. mystaceus* and *L. mystacinus*.

	Species		
External morphology	*Leptodactylus cupreus*	*L. mystaceus*	*L. mystacinus*
White line in posterior region of thigh	Present (well or weakly marked)	Present, well marked	Absent
Posterior region of thigh background color	Uniform black or deep brown	Brown, marbled	Brown, marbled
Spines in dorsal surface of tibia	Present	Absent	Present
Inner metacarpal tubercle	Divided, smaller or as big as outer metacarpal tubercle	Entire, larger than outer metacarpal tubercle	Divided, smaller or as big as outer metacarpal tubercle
Ridged surface of the inner side of fingers III and IV	Present	Absent	Present

Female. The specimen CFBH 23632 presents seven large unpigmented oocytes (average diameter = 24 mm; SD= 2.8). Oviduct structure was damaged during tissue sampling by the original collector and internal anatomy observations are thus hindered. However, oviduct shows no pigmentation.

Morphological variation among females from distinct populations was observed. The specimen CFBH 23632 (from the municipality of Linhares, state of Espírito Santo) presents bars on the dorsal surface of the tibia (Fig. 7), while MZUESC 9041 (from the municipality of Camacan, state of Bahia, Brazil) does not (Fig. 3D). In addition, CFBH 23632 presents a well marked white line in the posterior region of the thigh (Fig. 2A), while MZUESC 9041 presents the same line weakly marked. No relevant differences were found among males and females besides the usual sexual dimorphism present in other species of the *Leptodactylus fuscus* group as a chisel-like snout and vocal slits in males (Ponssa and Barrionuevo, 2012). Nevertheless, we found that in females, the gular region is marbled (Fig. 7), while it is brownish or blackish gray (this study) or pink (Caramaschi *et al.* 2008) in males. As more females are collected, we will understand whether the gular coloration of *L. cupreus* is indeed a sexually dimorphic feature or if it shows intrasexual variation.

Advertisement call. Despite the morphological variation found in specimens collected in the state of Bahia, the advertisement calls of this population do not differ significantly from the advertisement call presented in the original description of *L. cupreus* (Fig. 8). The advertisement calls of two individuals of *L. cupreus* recorded at the Estação Ecológica Veracel, municipality of Porto Seguro are composed of slightly modulated notes with duration of about 0.02 seconds; the rate of emission is about 14 notes per seconds; the dominant (and fundamental)

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frequency corresponds to the first harmonic and its frequency is around 2800 Hz (see Table 3). A second harmonic is found around 5600 Hz (range = 4000–6000 Hz) and a third around 8300 Hz (range = 7700-8900 Hz).

TABLE 3. Comparison between advertisement calls of *Leptodactylus cupreus* from the type locality (Caramaschi *et al.* 2008) and two individuals recorded in the present study (RPPN Veracel, municipality of Porto Seguro, state of Bahia). Amplitude (mean ± standard deviation).

	Caramaschi *et al.* 2008	Indiv. 1	Indiv. 2
N of notes	–	500	276
Duration (s)	–	0.01–0.03 (0.02±0.002)	0.01–0.03 (0.02±0.003)
Note rate (note/s)	≈ 12	13.57–14.85 (13.87±0.28)	13.63–14.82 (14.11±0.44)
Dominant frequency (Hz)	2800 – 3058	2584–2928.5 (2754.8±18.8)	2584–2928.5 (2824.6±67.2)
Modulated	Yes, ascendant	Yes, ascendant	Yes, ascendant
Amplitude of modulation (Hz)	–	1887–3430	2217–3220
Harmonics	3	3–5	?

FIGURE 7. Female of *Leptodactylus cupreus*, dorsal and ventral views (CFBH 23632 SVL = 55.5 mm). Notice the absence of the chisel-like snout and the general similarity with males (Fig. 3).

Discussion

We report specimens of *L. cupreus* from the municipalities of Camacan, Camamu and Porto Seguro, state of Bahia, which although recognizable as *L. cupreus*, presented morphological variation in those traits described as diagnostic in the original description of the species. Despite this variation, we concluded that these populations belong to the same species due to the nearly identical advertisement calls. As did Caramaschi *et al.* (2008) we too found (usually) three harmonics; notes with fewer harmonics were usually recorded with lower intensity and that may affect harmonic recording (see Orrico *et al.* 2006).

Although allopatric speciation does not imply distinct advertisement call between species, molecular data would be necessary to test this hypothesis. To date—and based on the data available—we chose to take a more conservative taxonomic decision. However, as more data is collected, different taxonomic conclusions can arise.

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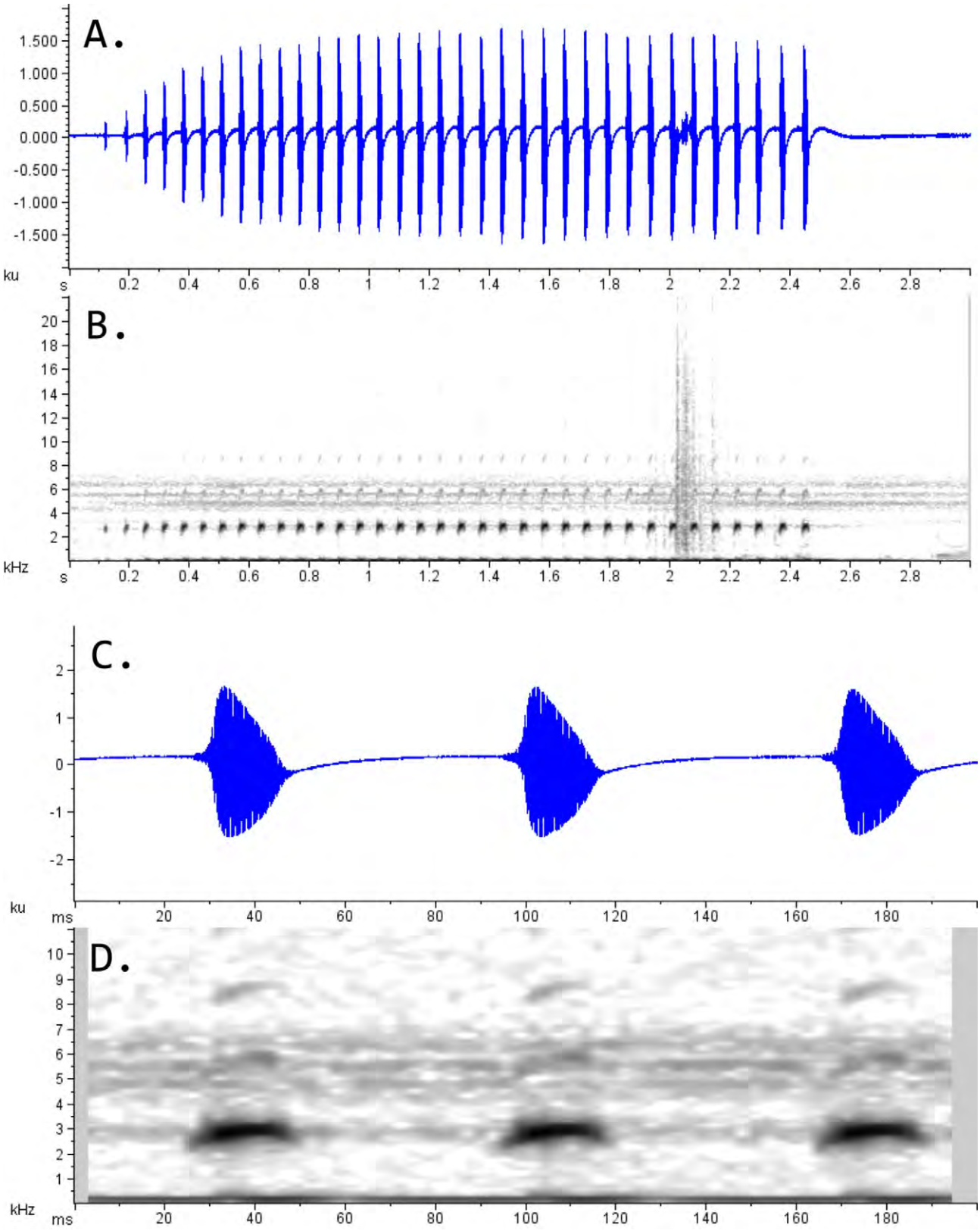

FIGURE 8. Advertisement call of *Leptodactylus cupreus* (Porto Seguro, state of Bahia). A—Detailed oscillogram of one call; B—audiospectrogram of the same call; C—oscillogram of three notes emitted in one second; D—audiospectrogram of the three notes shown in C.

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These new records show that *L. cupreus* is not a high-altitude endemic species like *Physalaemus maximus* and *Chiasmocleis mantiqueira* as predicted by Caramaschi *et al.* (2008). The last two species have been reported to occur in other high-altitude areas (see Baêta *et al.* 2005 for *P. maximus* and Forlani *et al.* 2011 for *C. mantiqueira*). In fact, other forest species of this region in the state of Bahia present geographical distributions somewhat similar to that of *L. cupreus* (e.g., *Ceratophrys aurita* and *Proceratophrys laticeps*; Almeida *et al.* 2011; Frost 2011).

The phylogenetic position of *L. cupreus* remains uncertain. Although it was allocated in the *L. mystaceus* complex of the *L. fuscus* group in its original description (Caramaschi *et al.* 2008), some morphological traits (e.g. presence of spines on the dorsal surface of the tibia and on inner surface of fingers III and IV) show that *L. cupreus* is more similar to *L. mystacinus* than to *L. mystaceus*.

In fact, the monophyly of the *L. mystaceus* complex within the *L. fuscus* species group is doubtful. Ponssa (2008) recovered it as polyphyletic; on the other hand, Pyron & Wiens (2011) recovered it as monophyletic (albeit only marginally supported), and *L. mystacinus* as the basal species of the *L. fuscus* species group. The relationship between *L. cupreus* and the *L. mystaceus* complex (according to Caramaschi *et al.* 2008) is only tentative. It seems clear that external morphology is not straightforward to solve relationships within the *L. fuscus* species group (notice the high levels of homoplasy in Ponssa 2008) and a total evidence analysis should be conducted to understand the evolutionary history within the group.

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Literature cited

Almeida, A.P., Gasparini, J.L. & Peloso, P.L.V. (2011) Frogs of the state of Espírito Santo, southeastern Brazil – The need of looking for 'coldspots'. *Check-List*, 7(4), 542–560.

Baeta, D., Assis, B.d., Bernardo, P.H., São Pedro, V.A., Drummond, L.d.O & Pires, M.R.S. (2005) Geographic distribution: *Physalaemus maximus*. *Herpetological Review*, 36, 200.

Caramaschi, U., Feio, R.N. & São-Pedro, V.A. (2008) A new species of *Leptodactylus* Fitzinger (Anura, Leptodactylidae) from Serra do Brigadeiro, state of Minas Gerais, southeastern Brazil.. *Zootaxa*, 1861, 44–54.

Charif, R.A., Waack, A.M. & Strickman, L.M. (2010) *Raven Pro 1.4 User's Manual*. Cornell Lab of Ornithology, Ithaca, NY. 367pp.

Costa, L.P. (2003) The historical bridge between the Amazon and the Atlantic forests of Brazil: A study of molecular phylogeography with small mammals. *Journal of Biogeography*, 30, 71–86. http://dx.doi.org/10.1046/j.1365-2699.2003.00792.x

Cruz, C.A.G, Feio, R.N. & Cassini, C.S.C. (2007) Nova espécie de *Chiasmocleis* Méhely, 1904 (Amphibia, Anura, Microhylidae) da Serra da Mantiqueira, Estado de Minas Gerais, Brasil. *Arquivos do Museu Nacional* 65(1), 63–68.

De Sá, R.O., Camargo, A. & Heyer, W.R. (2005) *Leptodactylus dydimus* and *L. mystaceus* phylogenetically sibling species (Amphibia, Anura, Leptodactylidae)? Pp. 90–92. In: Ananajeva, A. & Tsinenko, O. (Eds.) *Herpetologia Petropolitana*. Proceeding of the 12th Ordinary General Meeting of the Societas Europaea Herpetologica, St. Petersburg, Russia.

Duellman, W.E. & Trueb, L. (1994) *Biology of Amphibians*. The Johns Hopkins University Press, Baltimore. xxiv+670pp.

Fabrezi M. (1992) El carpo de los anuros. *Alytes*, 10, 1–29.

Fernandes, D.S., Franco, F.L. & Fernandes, R. (2004) Systematic revision of the genus *Lachesis* Daudin, 1983 (Serpentes, Viperidae). *Herpetologica*, 60(2), 245–260. http://dx.doi.org/10.1655/02-85

Forlani, M.C., A. Valdujo, A., Pavan, D., Maciel, A.O. & Peloso, P.L.V. (2011) Review of the geographical distribution of the white-spotted frog *Chiasmocleis albopunctata* (Boettger, 1885) (Anura, Microhylidae). *Biota Neotropica*, 11(3), 415–419. http://dx.doi.org/10.1590/S1676-06032011000300035

Frost, D.R. (2011) *Amphibian Species of the World: An online reference.* Version 5.5 (31 January, 2011). Electronic Database accessible at http://research.amnh.org/vz/herpetology/amphibia/. American Museum of Natural History, New York, USA.

Hayek, L.A.C., Heyer, W.R. & Gascon, G. (2001) Frog morphometrics: A cautionary tale. *Alytes*, 18(3–4), 153–177.

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Heyer, W.R., García-Lopez, J.M. & Cardoso, A.J. (1996) Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species. *Amphibia-Reptilia*, 17, 7–31. http://dx.doi.org/10.1163/156853896X00252

IUCN. (2012) *The IUCN Red List of Threatened Species*. Version 2012.2. (06 December 2012). Eletronic Database accesile at http://www.iucnredlist.org.

Motta, A.P., Silva, E.T., Feio, R.N. & Dergam, J.A. (2010) The tadpole of *Leptodactylus cupreus* Caramaschi, Feio & São Pedro, 2008 (Anura, Leptodactylidae). *Zootaxa*, 2640, 65–68.

Orrico, V.G.D., Carvalho-e-Silva, A.M.P.T & Carvalho-e-Silva, S.P. (2006) Redescription of the advertisement call of *Aplastodiscus arildae* (Cruz & Peixoto) and description of the call of *Aplastodiscus weygoldti* (Cruz & Peixoto) with general notes about the genus in Southeastern Brazil (Anura, Hylidae). *Revista Brasileira de Zoologia*, 23(4), 994–1001. http://dx.doi.org/10.1590/S0101-81752006000400003

Peixoto, A.L. & Gentry, A. (1990) Diversidade e composição florística da mata de tabuleiro na Reserva Florestal de Linhares (Espírito Santo, Brasil). *Revista Brasileira de Botânica*, 13(1), 19–25.

Peres, J., Simon, J.E., Nascimento, D.S. & Feio, R.N. (2010) Amphibia, Anura, Leptodactylidae, *Leptodactylus cupreus* Caramaschi, Feio and São-Pedro 2008: Distribution extension. *Check-List*, 6(4), 481–482.

Ponssa, L. & Barrionuevo, J.S. (2012) Sexual dimorphismin *Leptodactylus latinasus* (Anura, Leptodactylidae): Nasal capsule anatomy, morphometric characters and performance associated with burrowing behavior. *Acta Zoologia*, 93, 57–67. http://dx.doi.org/10.1111/j.1463-6395.2010.00479.x

Rodrigues, A.S.L., Gray, C.L., Crowter, B.J., Ewers, R.M., Stuart, S.N., Whitten, T. & Manica, A. (2010) A global assessment of amphibian taxonomic effort and expertise. *BioSciense*, 60, 798–806. http://dx.doi.org/10.1525/bio.2010.60.10.6

Tonini, J.F.R., Carão, L.M., Pinta, I.S., Gasparini, J.L., Leite, Y.L.R & Costa, L.P. (2010) Non-volant tetrapods from Reserva Biológica de Duas Bocas, State of Espírito Santo, Southeastern Brazil. *Biota Neotropica*, 10(3), 339–351. http://dx.doi.org/10.1590/S1676-06032010000300032

APPENDIX. Material examined.

Leptodactylus cupreus: CFBH 23632, CFBH 23650, CFBH 23659, CFBH 26430, CFBH 32113, CFBH 32114, MZUESC 7864, MZUECS 9041, MNRJ 47752 (holotype), MNRJ 47753, MNRJ 47754, MNRJ 50436, MNRJ 50437, MNRJ 50438 (paratypes), MNRJ 80244.

Leptodactylus mystaceus: CFBH 509, CFBH 16103, CFBH 17242, CFBH 18672, CFBH 26124, CFBH 31957, CFBH 31959.

Leptodactylus mystacinus: CFBH 999, CFBH 5540, CFBH 9535, CFBH 9790, CFBH 9792, CFBH 9795, CFBH 9804, CFBH 18386.