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On the diagnosis and conservation of the poorly known bromeligenous *Scinax arduous* Peixoto, 2002 (Amphibia; Anura; Hylidae)

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Abstract

Since *Scinax arduous* description, many other populations belonging to the *Scinax perpusillus* group have been recorded for the States of Espírito Santo and Minas Gerais, Southeastern Brazil. Both in collections and publications most of these new specimens are identified as *S. arduous*, *Scinax* cf. *arduous*, *Scinax* cf. *perpusillus*, *Scinax* gr. *perpusillus*, *S. perpusillus* and *S. v-signatus*. Such state of affairs may be due to the lack of information on the original description of *S. arduous*. Only two individuals [the holotype (female) and the paratype (male)] were used in the original description and diagnosis, therefore, information on variation and distribution were not available. Furthermore, in *S. arduous* description, the section on coloration in life was based only on two juveniles raised in laboratory. Herein we redefine *Scinax arduous* based on 44 males and 17 females from the type locality, the Municipality of Santa Teresa, in the State of Espírito Santo. In addition, we provide information on its conservation status, distribution, natural history, vocalization, tadpoles, and detailed photographs of both adults and larvae.

Key words: *Alcantarea*, *Scinax perpusillus* group, tadpole, taxonomy, vocalization

Introduction

The treefrog genus *Scinax* Wagler ranges from Argentina and Uruguay to Mexico and is currently composed of 113 species (Faivovich *et al*. 2005; Frost 2015). Faivovich (2002) suggested that the genus is divided in two clades (*S. ruber* and *S. catharinae*). The second clade is composed by the *S. catharinae* and the *S. perpusillus* groups of species, but monophyly of the latter was not adequately tested due to the inclusion of only one species as representative of the group (Faivovich *et al*. 2005). Earlier, Lutz (1968) commented on the similarities exhibited in bromeliads usage by two of the species, *S. perpusillus* (Lutz & Lutz) and *S. v-signatus* (Lutz). Peixoto (1987) named the group "*perpusillus*", to include close relatives of this bromeligenous group, which is currently composed by 13 species distributed along the Atlantic tropical forest from the State of Santa Catarina to the State of Espírito Santo (Alves-Silva & Silva 2009) and has its most inland record in the State of Minas Gerais (Lacerda *et al.* 2012). The number of species in the *Scinax perpusillus* group is certainly underestimated (Silva & Alves-Silva 2008). Vouchers used to document the occurrence of several specimens are tentatively identified as *Scinax perpusillus*, *Scinax* aff. *perpusillus* or *Scinax* gr. *perpusillus* (see Heyer *et al*. 1990; Pombal & Gordo 2004; Oliveira & Navas 2004; Ramos & Gasparini 2004; Carvalho-e-Silva *et al*. 2008). These uncertainties in properly identifying some of the material is usually related to the poor diagnosis for species of the group, which seems also related to difficulties in defining potential new species.

Scinax arduous Peixoto, 2002 is certainly one of these problematic species. Since its description many other populations of *S. perpusillus* group have been recorded in the State of Espírito Santo and Minas Gerais and referred

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to as *S. arduous*, *Scinax* cf. *arduous*, *Scinax* cf. *perpusillus*, *Scinax* gr. *perpusillus*, *S. perpusillus*, and *S. v-signatus* (see Peixoto 2002; Ramos & Gasparini 2004; Rödder *et al.* 2007; Teixeira *et al.* 2007; Teixeira & Rödder 2007; Feio *et al.* 2008; Lacerda *et al.* 2009; Almeida *et al*. 2011; Montesinos *et al.* 2012; Moura *et al*. 2012). Such taxonomic impediment may be related to the small sample size of *S. arduous* used for the original description. For instance, only two individuals [the holotype (a female) and the paratype (a male)] were used for the diagnosis and descriptive sections. Also, the description of the color in life was based on two juveniles individuals only that were raised in the laboratory.

In order to adequately offer a taxonomic solution to this information gap, herein we present a new description of *Scinax arduous* based on a large sample size collected from the type locality. Our aim is to improve this species characterization and provide additional information on morphometric and color pattern variation. Additionally, we describe its vocal repertoire, a larger sample of tadpoles and provide information on its natural history, distribution and conservation status.

Material and methods

We examined 61 adult specimens of *Scinax arduous* collected in the Municipality of Santa Teresa, State of Espírito Santo, Brazil. These specimens are deposited in the following herpetological collections: MBML (Museu de Biologia Mello Leitão, Santa Teresa, Espírito Santo, Brazil), MNRJ (Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil), MZUFV (Museu de Zoologia João Moojen, Departamento de Biologia Animal, Universidade Federal de Viçosa, Minas Gerais, Brazil) and RU (Coleção Herpetológica da Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil). Thirty-three out of these 61 specimens were collected during our samplings in the Municipality of Santa Teresa. All adult specimens collected during our fieldwork were anesthetized with 5% Lidocaine, fixed in 10% formalin, and transferred to 70% ethanol for permanent storage.

The following measurements were taken under a stereomicroscope with digital caliper (nearest 0.1 mm). These measurements followed Duellman (1970) and Heyer *et al.* (1990): snout-vent length (SVL), head length (HEL), head width (HW), thigh length (THL), tibia length (TBL), foot length (FL), eye diameter (ED), tympanum diameter (TD), interocular distance (IOD), eye-nostril distance (END) and internostril distance (IND). Snout shape follows Heyer *et al.* (1990).

Tadpole measurements were taken under a stereomicroscope with digital caliper (nearest 0.1 mm). These measurements followed Altig & McDiarmid (1999) and Silva & Alves-Silva (2011): total length (LT), body length (BL), body height (BH), body width (BW), tail length (TL), tail height (TH), dorsal fin height (DFH), ventral fin height (VFH), eye diameter (ED), inter-narial distance (IND), interorbital distance (IOD), eye-snout distance (ESD), eye-nostril distance (END), oral disc width (ODW). We measured 27 tadpoles on Stage: 25 (n=4), 26 (n=6), 27 (n=1), 28 (n=2), 29 (n=2), 30 (n=2), 31 (n=4), 35 (n=3), 36 (n=1), 38 (n=1), and 40 (n=1). Only stages 25, 31, and 35 were used for external morphology descriptions. To determine tadpole stages of development we used Gosner (1960).

Calling males were recorded from ground bromeliads located in forested area in the Municipality of Santa Teresa, State of Espírito Santo (air temperature varied 15–25°C). We used a Sony TCM 5000 EV/Microphone Sennheiser M¨66 (16000 Hz and 16 bits resolution). The call oscillogram and spectrogram were analyzed and built using Raven Pro 1.4 with FFT of 256 points, 89.8% overlap and hann window type. Bioacoustical terminology follows Duellman & Trueb (1994). Dominant frequency was tracked using the measurement *Peak frequency* from Raven Pro 1.4.

Vouchers (adults, larvae and vocalization) are listed at the Appendix section.

Results

New diagnosis. (1) medium to large sized species within the *Scinax perpusillus* group (males 19.5–23.6 mm and females 22.4–28.5 mm SVL); (2) head longer than wide; (3) tibia larger than thigh; (4) few tubercles scattered throughout the dorsal surface, including the head; (5) dorsum coloration typical of the *S. perpusillus* group of species, with different tones of gray or cream with transversal dark bars originating on each inguinal region and converging on the third portion of the dorsum in an inverted V-shaped figure and irregular dark pigmentation

spread on the dorsum varying in intensity and coverage area; (6) lateral dark stripe from the region of the eye to the anterior margin of the inguinal region; (7) head with an interocular dark transversal bar; (8) transversal dark bars along the anterior and posterior members; (9) inguinal region with discrete disperse glandular acini; (10) gular region with irregular dark pigmentation; (11) irregular bright colored patches (yellow in most specimens and orange on only one) on hidden area of the thigh, tibia, calf and foot of live specimens; (12) tadpoles lacking any yellow mark; and (13) distinct vocalization with multipulsed notes (4–14 notes/call, call duration 156.8–763.4 ms, and dominant frequency ranging from 3100.8–5168.0 Hz).

Comparison with other species. A combination of traits distinguishes *Scinax arduous* from all other species of the *S. perpusillus* group. It differs from *S. alcatraz* by lacking evident inguinal gland (evident inguinal gland present in *S. alcatraz*; Faivovich *et al.* 2010) and by the presence of transversal dark bars originating on each inguinal region and converging toward the third portion of the dorsum in an inverted V-shaped figure (absent in *S. alcatraz*). *Scinax arduous* differs from *S. atratus* by its larger size (maximum SVL 19.2 mm in males and 20.0 mm in females of *S. atratus*; Peixoto 1988) and by the dorsal pattern typical of the *S. perpusillus* group of species (marbled in *S. atratus*; Peixoto 1988). From *Scinax belloni*, it can be easily distinguished by having dorsum of body and limbs densely covered by prominent granules, lacking any trace of markings on dorsum, inguinal region and hidden surfaces of hind limbs, by having evident inguinal glands and by its distinct advertisement call and larval features (see below for precise bioacoustical and larval comparisons). *Scinax arduous* differs from *S. cosenzai* by its distinctive advertisement call. It easily differs from *S. faivovichi* by its larger size (16.2–18.0 mm SVL in males of *S. faivovichi* and 18.6–21.7 in females; Brasileiro *et al.* 2007b). *Scinax arduous* can be distinguished from *S. insperatus* by having yellow markings in the inguinal region and hidden surfaces of limbs (lack of yellow marks on *S. insperatus*; Silva & Alves-Silva 2011) and by larval features. It differs from *S. littoreus* by its male larger size (*S. littoreus* SVL 17.4–19.9 mm; x=18.8; sd=0.8; n=10), by lacking evident inguinal gland (evident inguinal gland present in *S. littoreus*; Faivovich *et al*. 2010), and by its distinctive advertisement call and larval features. It differs from *S. melloi* by its larger size (SVL 15.9–17.9 mm in males and maximum SVL 18.7 mm in females of *S. melloi*; Peixoto 1988 and Brasileiro *et al*. 2007a) and by its dorsal pattern typical of the *S. perpusillus* group of species (marbled in *S. melloi*; Peixoto 1988). *Scinax arduous* can be distinguished from *S. peixotoi* by its larger size (18.8–20.7 mm SVL in males of *S. peixotoi* and 22.4–25.1 in females; Brasileiro *et al.* 2007a), tibia larger than thigh (tibia same size as thigh in *S. peixotoi*; Brasileiro *et al.* 2007a) and by its distinctive advertisement call. It can be distinguished from *S. perpusillus* by its larger size (male of *S. perpusillus* SVL 16.1–20.7 mm; x=18.8; sd=1.4; n=12) and its distinctive advertisement call. *Scinax arduous* differs from *S. tupinamba* by its larger size (SVL 16.1–19.2 mm in males of *S. tupinamba*; Silva & Alves-Silva 2008) and by larval features. Finally, it differs from *S. v-signatus* by its gular region with dark chromatophores randomly dispersed (*S. v-signatus* has a dark pigmentation on the gular region organized in the form of a Y or V; Lutz 1973; Peixoto 2002).

The tadpole of *Scinax arduous* can be promptly distinguished from those of *S. belloni*, *S. insperatus* and *S. tupinamba* by lacking any yellow marks: golden melanophres disperse especially on the head of *S. belloni* (Silva-Soares *et al*. 2010); fins with yellow colored spots in later stages of *S. insperatus* (Silva & Alves-Silva 2011); and yellow stripe on the head between the nostrils and the eyes in *S. tupinamba* (Silva & Alves-Silva 2008). It further differs from *S. belloni* by the absence of a dark band along the dorsal and ventral fins (present in *S. belloni*; Silva-Soares *et al.* 2010). It can be distinguished from *S. littoreus* by its larger total length (LT 16.0–19.9 mm in stages 25–38; Peixoto 1988a) and from *S. perpusillus* by its spiracle posterodorsaly orientation (spiracle posterolaterally oriented; Peixoto 1987). External morphology, morphometric or coloration data were not enough to differentiate *S. arduous* tadpoles from those of *S. cosenzai*, *S. melloi* and *S. v-signatus*.

The advertisement call of *Scinax arduous* has more notes per call than *S. perpusillus* (3–6 notes/call; Pombal & Bastos 2003), *S. peixotoi* (3–5 notes/call; Brasileiro *et al.* 2007b), *S. belloni* (2–3 notes/call; Peres & Simon 2011) and *S. littoreus* (2–5 notes/call; Pontes *et al*. 2013). Comparing calls with the same number of notes, the advertisement call of *S. arduous* is shorter than those from *S. cosenzai* and longer than *S. perpusillus*: *Scinax arduous* calls with 4–6 notes have duration of 156.8–367.5 ms, while *Scinax cosenzai* calls with the same number of notes have duration of 812.2–1018.5 ms (Lacerda *et al*. 2012) and *S. perpusillus* with 3–6 notes lasts 92.0–174.0 (Pombal & Bastos 2003). *Scinax arduous* cannot be distinguished from the other species belonging to the *S. perpusillus* group regarding spectral parameters, except for *S. belloni* (dominant frequency of 3078 Hz; Peres & Simon 2011).

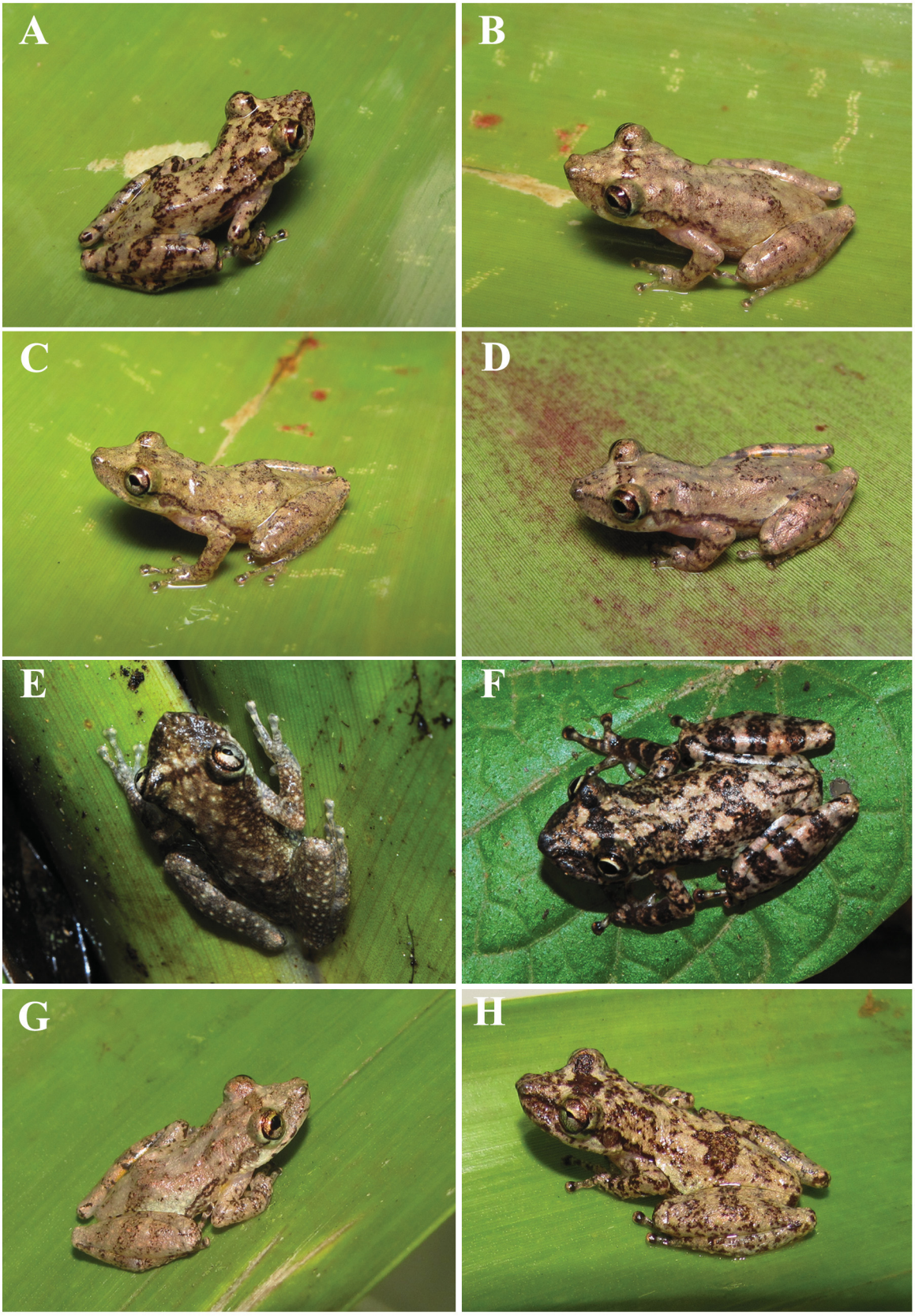

FIGURE 1. Individuals of *Scinax arduous* collected in bromeliads at rocky outcrop-A: A) UFMG 15439 (male SVL 22.5 mm), B) UFMG 15443 (male SVL 21.1mm), C) UFMG 15442 (male SVL 21.3 mm), and D) UFMG 15441 (male SVL 21.6 mm); forested area: E) UFMG 15434 (male 22.9 mm) and F) UFMG 15433 (male 22.9 mm); ornamental bromeliads from MBML: G) MZUFV 12561 (male SVL 20.1 mm) and H) MZUFV 12565 (male 22.9 mm). Photos A–D, G and H by JVAL; and E and F by RBF.

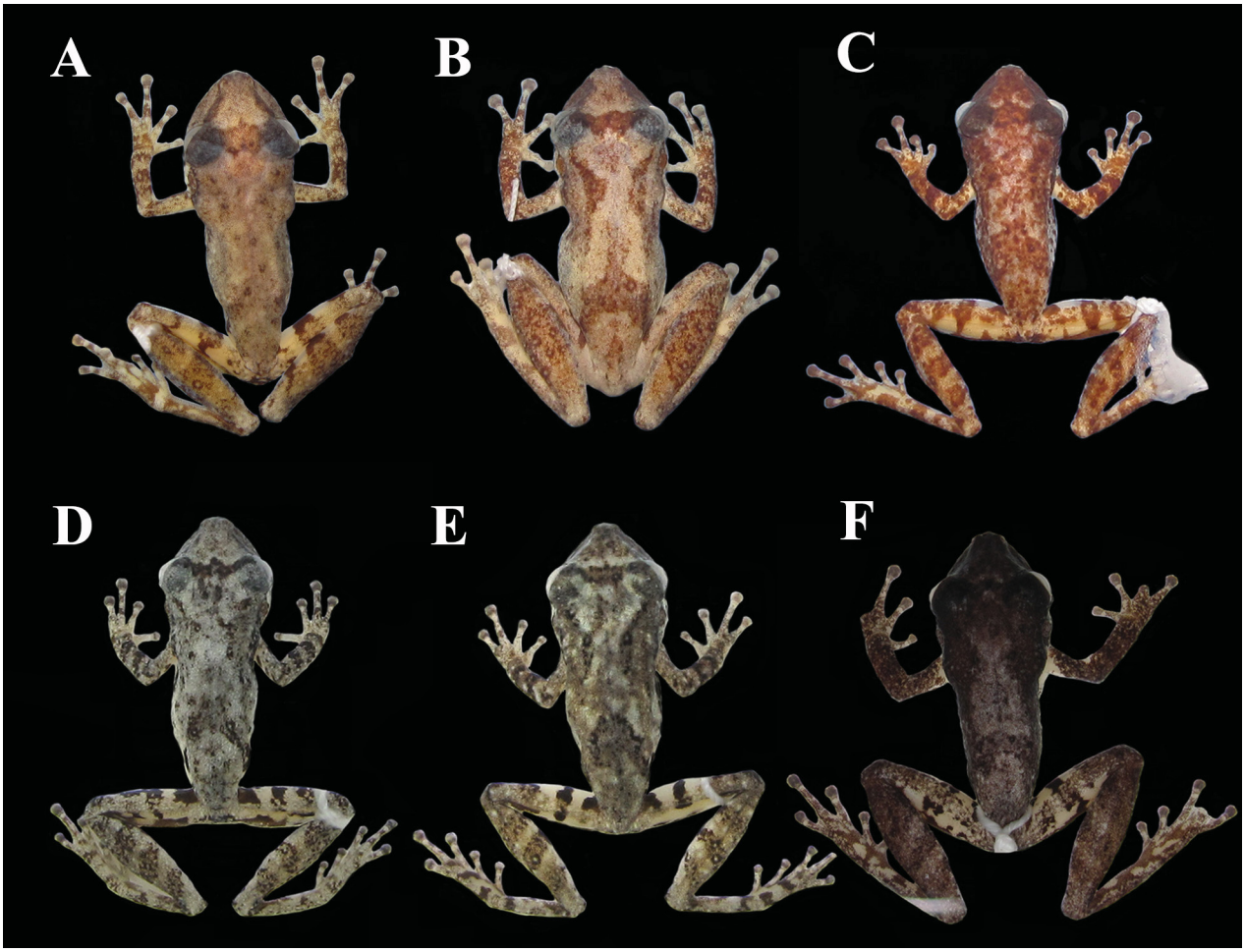

FIGURE 2. Variation in the pigmentation of *Scinax arduous* dorsum: A) MNRJ 38436 (male SVL 21.7 mm); B) MNRJ 40695 (female SVL 26.0 mm); C) MNRJ 28374 (male SVL 21.5 mm); D) UFMG 15444 (male SVL 21.2 mm); E) UFMG 15441 (male SVL 21.6 mm); F) UFMG 15452 (female SVL 22.8 mm).

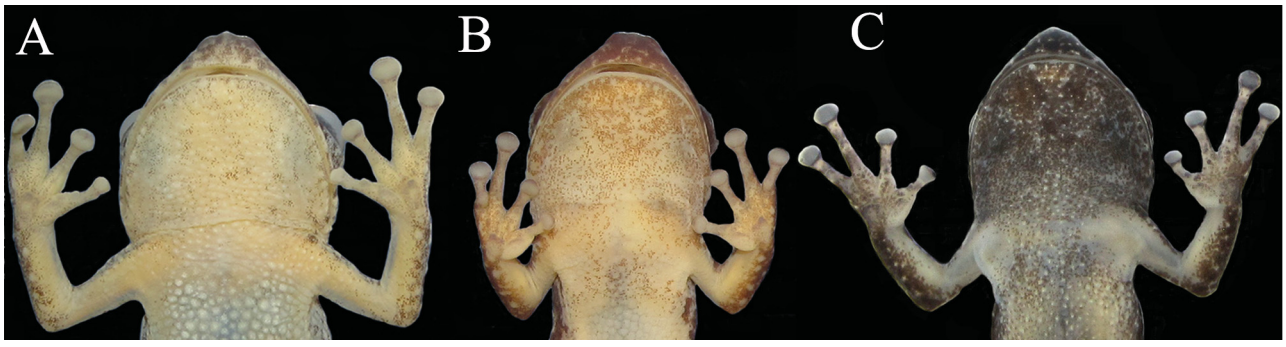

FIGURE 3. Variation in the intensity and area of dark pigmentation on the gular and pectoral region of *Scinax arduous*: A) MNRJ 40696 (female HW = 8.1 mm); B) MNRJ 34928 (male HW = 7.0 mm); C) UFMG 15439 (male HW = 7.7 mm).

Redefinition. Recorded measurements are summarized in table 1. Males 19.5–23.6 mm and females 22.4–28.5 mm SVL; head longer than wide (0.83–0.99 HW/HEL in males and 0.87–0.98 in females); snout protruding in lateral view and mucronate in dorsal view; upper jaw projecting considerably beyond the lower one; loreal region varying from concave to slightly flat; tympanum round; vomerine teeth in two arc shape series between choanae, each containing 3–7 teeth; elliptical choanae laterally located; tongue oval, free laterally and posteriorly; vocal slits at the side of tongue; vocal sac subgular; arms slender; forearms slightly more robust than upper arm; row with 4–6 tubercles on the outer surface of upper arm; fingers slender and medium-sized, relative lengths II<V~III<IV; webbing vestigial between Fingers II and III, basal between III and IV, and IV and V; discs wider than long; disc on

Finger II smaller than the others; palmar tubercle large and bifid; thenar tubercle elliptical; subarticular tubercles single, rounded and more pronounced on the V than others; males with unpigmented nuptial pad on the base of the thumb, proximally from the base of thenar tubercle distally up to the subarticular tubercle; inguinal region with disperse glandular acini; slender legs; tibia longer than thigh (1.03–1.16 TBL/THL in males and 1.03–1.15 in females); sum of thigh and tibia about the same size as SVL; toes slender; toe disks wider than long, about the size of the finger disks; toes with relative lengths I<II<III~V<IV; rounded low tubercles present on the inner surface of the tarsus; feet with round outer metatarsal tubercle and an inner more developed elliptical tubercle; subarticular tubercles evident, rounded and single; ventral surfaces of belly granular; low density of granules randomly distributed through the dorsum.

TABLE 1. Measurements of males (N=44) and females (N=17) of *Scinax arduous.* Range, mean, and standard deviation (SD) are provided in millimeters (mm): snout-vent length (SVL); head length (HEL); head width (HW); thigh length (THL); tibia length (TBL); foot length (FL); eye diameter (ED); tympanum diameter (TD); interorbital distance (IOD); eye-nostril distance (END); and internostril distance (IND).

Character	Male (N=44)			Female (N=17)		
	Mean	SD	Range	Mean	SD	Range
SVL	21.5	0.9	19.5–23.6	25.1	1.7	22.4–28.5
HEL	8.0	0.4	7.3–8.7	9.2	0.7	8.2–10.5
HW	7.4	0.4	6.8–8.8	8.6	0.6	7.6–9.7
THL	10.3	0.5	9.2–11.7	12.4	0.9	11.0–14.0
TBL	11.3	0.5	10.3–12.5	13.4	0.1	11.8–15.8
FL	8.7	0.5	7.8–9.7	10.3	0.8	9.0–12.0
ED	2.9	0.2	2.4–3.3	3.3	0.3	3.0–3.8
TD	1.0	0.1	0.8–1.4	1.3	0.2	1.1–1.8
IOD	2.7	0.2	2.2–3.1	3.0	0.2	2.6–3.4
END	2.7	0.2	2.4–3.0	3.2	0.2	2.7–3.6
IND	1.9	0.1	1.6–2.1	2.1	0.1	1.8–2.4

The dorsum of *Scinax arduous* varies between different tones of cream and gray. It has different densities and areas of irregular dark pigmentation and some dark marks typical of the *S. perpusillus* group which varies in width and distinctness (Figs. 1 and 2), similar to the pattern described by Silva & Alves-Silva (2011) for *S. insperatus*: a interorbital bar; slender stripe from the eye to the tip of the snout through the cantus rostralis appearing to be continuous with the pupil and with a lateral dark stripe that runs from the region of the eye to the anterior margin of the inguinal region; one dark stripe running from each eye towards the vertebral region (not easily visible in some specimens due to its distinctness or to the presence of irregular dark pigmentation on the anterior third of the dorsum); transversal dark bars originating on each inguinal region and converging toward the third portion of the dorsum in an inverted V-shaped figure; transversal dark bars along the anterior and posterior members including fingers and toes; gular region with irregular dark pigmentation varying in intensity, sometimes covering even the chest region (Fig. 3); in life, some irregular yellow colored patches (orange on a single specimen) on hidden area of the thigh, tibia, calf and foot and most individuals with yellow blotch also on the armpit (Figs. 4 and 5); venter is pale whitish and translucent. The dark marks remains in the preserved individuals, but general coloration are more opaque and the hidden areas loses the yellow coloration, becoming white (Fig. 2). Most of the polymorphism observed in *Scinax arduous* corresponds to intra-populational variation regarding the intensity of dark pigmentation and/or the intensity of the yellow aposematic blotches. However, we had in our samples a single male with bright orange aposematic coloration instead of yellow on the hidden surfaces of thigh, tibia, calf, and foot (Fig. 5). Unfortunately, we had only a single specimen collected from this population/locality (UFMG 15432). Although such coloration is considered a diagnostic character for the *S. perpusillus* group, we chose to take a more conservative taxonomic decision naming such specimen as *S. arduous* taking into account morphological and bioacoustical similarities. However, further study with larger samples and/or using molecular tools are necessary to appropriately test this hypothesis.

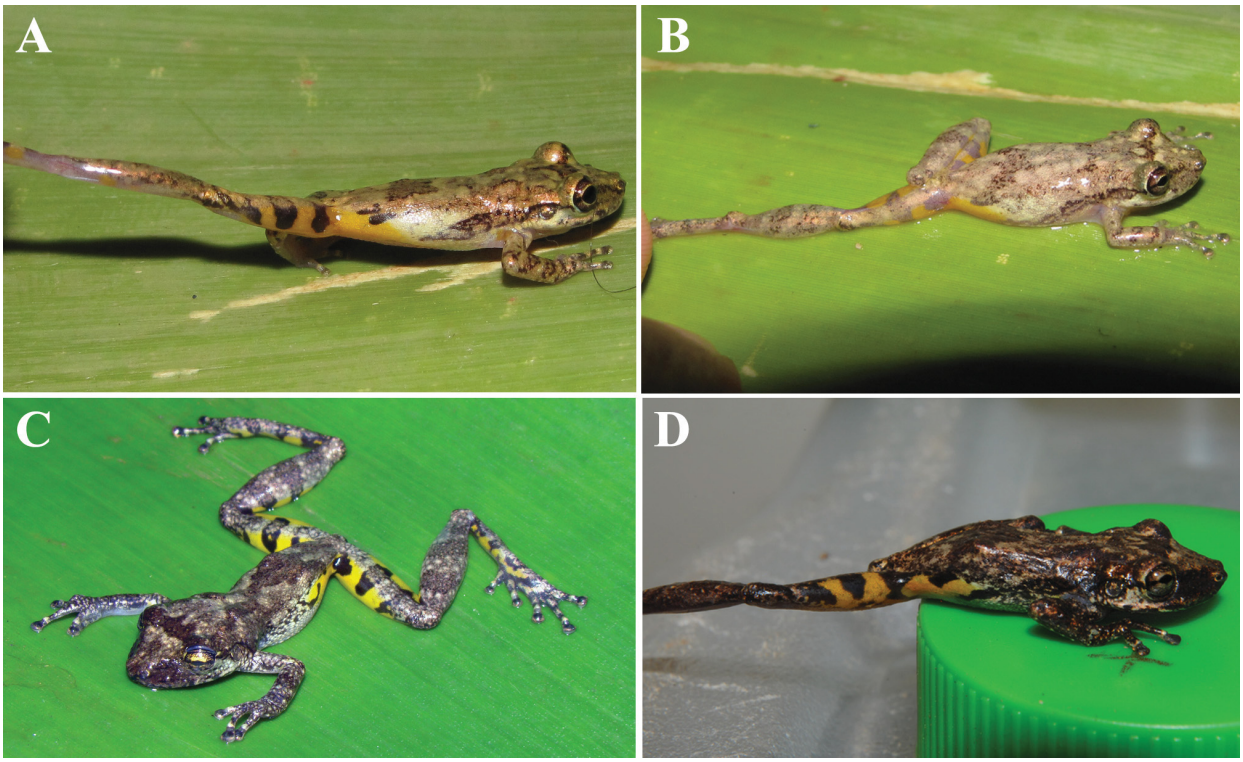

FIGURE 4. Yellow blotches from the hidden area of inguinal region, thigh, tibia, calf and foot of *Scinax arduous*: A) UFMG 15437 (male SVL 20.5 mm); B) UFMG 15443 (male 21.1 mm); C) RU 8394 (female 24.5 mm); and D) UFMG 15455 (female 23.9 mm). Photos A and B by JVAL; C by Gabriela Bittencourt, and D by RBF.

TABLE 2. Number of notes per call, number of analyzed calls, call duration, number of notes per second and peak of frequency of *Scinax arduous* vocalization. *Call type II.

Notes/call (Number of calls)	Call duration (ms)	Note/sec	Peak of frequency (Hz)
1 (3)*	11.0; 17.0; 19.4	-	4125.0; 4125.0; 4687.5
2 (6)*	188.6–239.4 (x=214.9; sd=20.0)	8.3–10.6 (x=9.4; sd=0.8)	3750–4875 (x=4077.3; sd=413.5)
3 (2)*	302.5; 373.6	8.0; 9.9	3789.8; 3789.8
4 (3)*	491.5; 528.0; 663.4	6.0; 7.5; 8.1	3789.8; 4687.5; 4875.0
4 (5)	156.8–238.3 (x=211.0; sd=32.5)	16.8–25.5 (x=19.4; sd=3.5)	3750.0–3962.1 (x=3850.7; sd=102.9)
5 (40)	222.8–308.0 (x=275.8; sd=15.7)	16.2–22.4 (x=18.2; sd=1.1)	3617.6–4687.5 (x=4005.8; sd=200.9)
6 (102)	268.9–367.5 (x=331.1; sd=15.6)	16.3–22.3 (x=18.1; sd=0.9)	3617.6–4875.0 (x=3947.5; sd=183.7)
7 (97)	283.2–451.5 (x=385.5; sd=19.3)	15.5–24.7 (x=18.2; sd=1.0)	3789.8–4687.5 (x=3923.4; sd=139.4)
8 (39)	336.7–465.7 (x=410.9; sd=44.3)	17.2–23.7 (x=19.7; sd=2.3)	3789.8–4687.5 (x=4030.2; sd=213.5)
9 (27)	368.8–527.8 (x=449.0; sd=58.1)	17.0–24.4 (x=20.4; sd=2.7)	3789.8–4875.0 (x=4071.8; sd=216.1)
10 (26)	396.8–574.4 (x=490.0; sd=64.7)	17.4–25.2 (x=20.7; sd=2.7)	3789.8–5168.0 (x=4156.0; sd=330.0)
11 (22)	452.3–636.5 (x=552.4; sd=63.0)	17.3–24.3 (x=20.2; sd=2.4)	3100.8–4875.0 (x=3972.6; sd=462.6)
12 (13)	503.0–706.5 (x=605.1; sd=60.1)	17.0–23.8 (x=20.0; sd=2.0)	3789.8–4312.5 (x=4143.7; sd=132.0)
13 (4)	575.0–720.4 (x=644.4; sd=62.4)	18.0–22.6 (x=20.3; sd=1.9)	4125.0–4134.4 (x=4129.7; sd=5.4)
14 (7)	575.1–742.1 (x=694.7; sd=60.4)	18.8–24.3 (x=20.3; sd=1.9)	3962.1–4687.5 (x=4184.7; sd=230.2)

Vocalization. *Scinax arduous* emits vocalization in call series as observed in other species of *Scinax perpusillus* group (Fig. 6) (Alves-Silva & Silva 2009; Silva & Alves-Silva 2011; Lacerda *et al*. 2012; Pontes *et al*. 2013). We analyzed a total of 382 calls (table 2) emitted by at least eight males (some recordings had another males

calling in the background making its quantification difficult). The combined values of these vocalizations have duration of 156.8–763.4 ms (x=398.7; sd=105.5; n=382 calls); 4–14 notes/call (x=7.5; sd=2.1; n=382 calls); 15.5–25.5 notes/sec (x=18.9; sd=1.9; n=382 calls); 5.6–47.6 ms/note (x=25.3; sd=5.6; n=951 notes); 1–9 pulses/note (x=5.0; sd=1.6; n=101 notes); 157.9–298.7 pulses/sec (x=220.8; sd=30.7; n=101 notes); and peak frequency of 3100.8–5168.0 Hz (x=3993.4; sd=227.8; n=387 calls). A different type of call was sporadically emitted with a lower note rate, herein referred to as call type II (Fig. 6): 11.0–663.4 ms/call (x=243.7; sd=187.2; n=14 calls type II); 1–4 notes/call (x=2.2 notes/call; sd=1.0; n=14 calls type II); 6.0–10.6 notes/sec (x=8.7; sd=1.3; n=11 calls type II); and peak of frequency of 3750.0–4875.0 Hz (x=4112.2; sd=391.1; n= 14 calls type II). Our data includes males calling isolated, males with other ones calling in the recordings background but without any evident interaction, and males calling in duet (antiphonic). Although our data was not conclusive in relating any variation in the call structure to the males' social context, we observed an increase of call rate and with no overlap between vocalizations of males in duet (Fig. 6). No calls type II was emitted during antiphony.

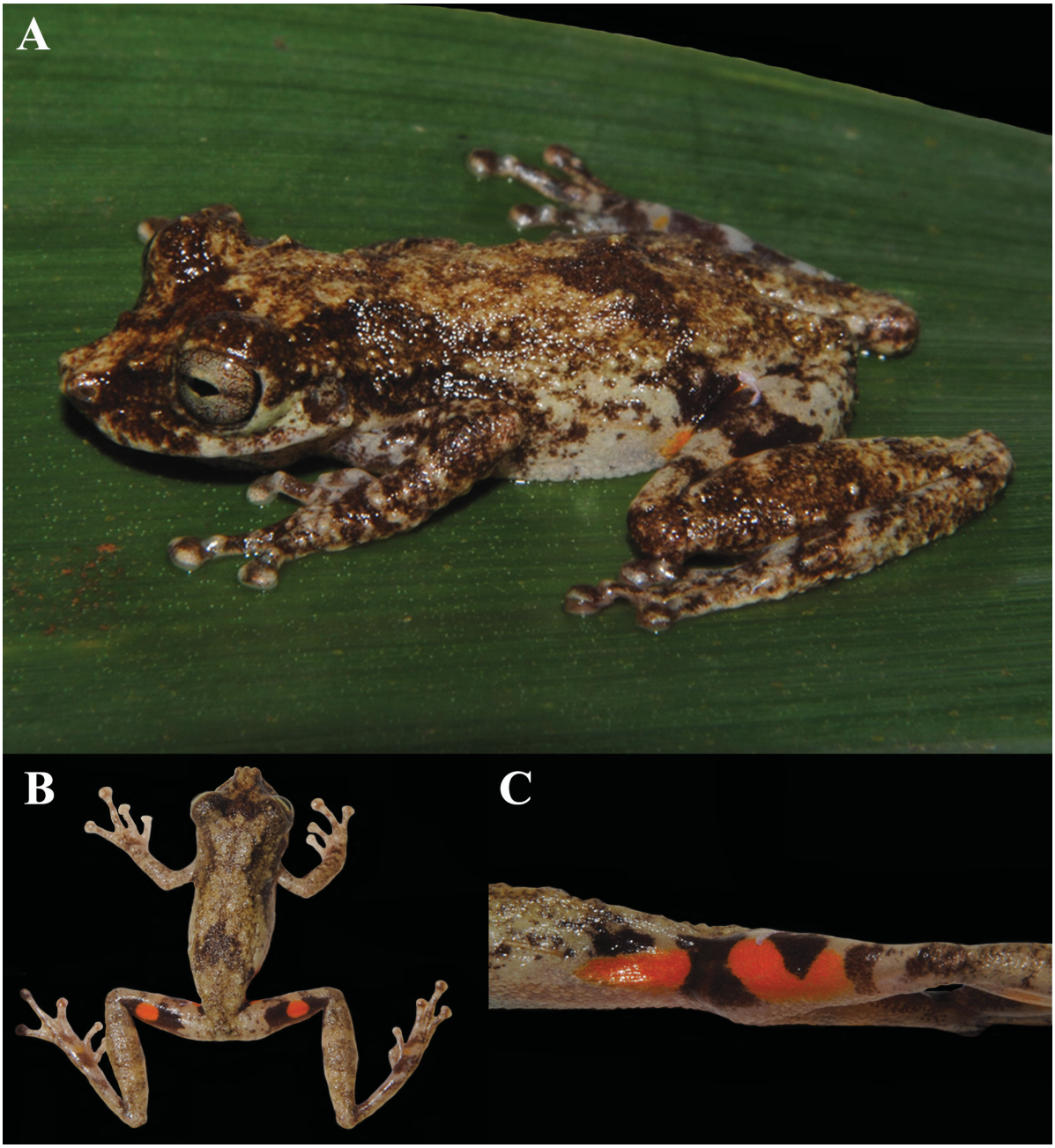

FIGURE 5. Different pattern of coloration with orange instead of yellow blotches on the hidden area of inguinal region, thigh, tibia, calf, and foot of *Scinax arduous* (male UFMG 15432; 22.3 mm SVL): A) alive; B) dorsal view of recently euthanized; C) hidden surface of inguinal and thigh.

TABLE 3. Measurements (in mm) of tadpoles of *Scinax arduous*: total length (LT), body length (BL), body height (BH), body width (BW), tail length (TL), tail height (TH), dorsal fin height (DFH), ventral fin height (VFH), eye diameter (ED), inter-narial distance (IND), interorbital distance (IOD), eye-snout distance (ESD), eye-nostril distance (END), oral disc width (ODW).

Stage (N)	LT	BL	BH	BW	TL	TH	DFH	VFH	ED	IND	IOD	ESD	END	ODW
25 (4)	15.1–18.6 x=16.8 sd=1.7	5.2–7.2 x=6.1 sd=0.9	2.0–2.9 x=2.4 sd=0.4	3.3–4.2 x=3.7 sd=0.4	9.4–11.6 x=10.7 sd=0.9	2.0–3.6 x=2.7 sd=0.6	0.7–1.0 x=0.8 sd=0.1	0.5–0.9 x=0.7 sd=0.2	0.4–0.7 x=0.5 sd=0.1	1.0–1.4 x=1.3 sd=0.2	1.2–2.0 x=1.7 sd=0.3	1.5–2.4 x=2.0 sd=0.4	0.5–0.8 x=0.6 sd=0.1	1.6–2.1 x=1.7 sd=0.2
26 (6)	18.7–23.1 x=21.6 sd=1.5	6.6–8.4 x=7.7 sd=0.7	2.6–3.7 x=3.3 sd=0.4	3.9–4.9 x=4.3 sd=0.4	11.9–15.5 x=14.2 sd=1.3	2.8–3.8 x=3.3 sd=0.4	0.6–1.2 x=1.0 sd=0.2	0.5–1.0 x=0.8 sd=0.2	0.8–1.1 x=0.9 sd=0.1	1.2–1.5 x=1.3 sd=0.1	2.0–2.7 x=2.4 sd=0.2	2.1–3.0 x=2.6 sd=0.3	0.5–1.0 x=0.8 sd=0.2	1.7–2.3 x=2.1 sd=0.2
27 (1)	17.7	7.0	3.3	4.8	10.7	4.8	1.5	1.3	0.8	1.7	2.1	2.0	0.9	2.1
28 (2)	18.3; 19.0	6.2; 6.7	2.9; 3.0	4.3; 4.6	12.4; 12.1	3.2; 2.8	0.8; 0.9	0.6; 0.8	0.7; 0.7	1.3; 1.4	1.6; 1.6	2.0; 1.9	0.8; 0.7	2.0; 2.1
29 (2)	20.7; 21.6	7.0; 7.8	3.1; 3.4	4.7; 5.0	13.8; 13.9	3.5; 4.5	1.0; 1.3	0.9; 1.2	0.8; 1.0	1.5; 1.6	1.7; 2.3	1.9; 2.3	0.6; 0.8	2.0; 2.1
30 (2)	21.4; 21.4	7.6; 7.8	3.7; 3.8	5.0; 5.0	13.8; 13.9	4.4; 4.5	1.3; 1.2	1.0; 1.2	0.9; 0.8	1.6; 1.6	2.2; 2.3	2.4; 2.3	0.8; 0.8	2.6; 2.3
31 (4)	20.4–22.3 x=21.6 sd=0.9	7.4–9.1 x=8.1 sd=0.7	3.5–4.0 x=3.8 sd=0.3	5.1–5.7 x=5.2 sd=0.3	12.3–15.3 x=13.7 sd=1.2	3.6–4.1 x=3.8 sd=0.2	1.0–1.2 x=1.1 sd=0.1	0.9–1.1 x=1.0 sd=0.1	0.9–1.1 x=0.9 sd=0.1	1.3–1.7 x=1.5 sd=0.2	2.2–2.5 x=2.4 sd=0.1	2.0–2.8 x=2.4 sd=0.3	0.7–1.1 x=0.9 sd=0.2	2.1–2.5 x=2.3 sd=0.2
35 (3)	22.4–25.1 x=24.0 sd=1.4	8.6–9.0 x=8.8 sd=0.2	3.9–4.2 x=4.1 sd=0.1	5.2–5.5 x=5.3 sd=0.1	14.6–16.6 x=15.4 sd=1.0	3.6–3.8 x=3.7 sd=0.1	1.0–1.2 x=1.1 sd=0.1	0.8–1.1 x=0.9 sd=0.2	0.9–1.1 x=1.0 sd=0.1	1.5–1.6 x=1.5 sd=0.1	2.2–2.4 x=2.3 sd=0.1	2.7–2.9 x=2.8 sd=2.30.1	0.8–1.0 x=0.9 sd=0.1	2.4–2.7 x=2.5 sd=0.1
36 (1)	22.3	8.9	3.8	5.2	14.1	3.9	1.3	1.0	1.2	1.5	2.6	2.6	0.9	2.3
38 (1)	23.3	8.1	4.3	5.7	15.2	4.3	1.2	1.1	1.1	1.9	2.3	2.3	1.0	2.5
40 (1)	27.3	10.1	4.8	6.0	18.5	4.2	1.4	1.2	1.1	1.7	2.3	3.0	0.9	2.5

Tadpoles. Measurements of 27 tadpoles are presented on table 3. Body is oval in dorsal view and wider than deep in lateral view. Snout is rounded in dorsal and lateral profiles. Eyes are dorsolateral. Nostrils are rounded (nearly circular), directed laterally, with margins a bit elevated (forming a short tube), and delineated by dark pigmentation situated halfway between the tip of snout and the anterior edge of the eye. Spiracle sinistral, short, tubular, and with the inner wall attached to the body, opening directed to the posterior of the body and to the dorsum at about the middle of the body. Intestines spiral with the center at the center of the abdomen. The vent tube is longer than wide, opens dextrally, is enlarged near the body, and the lower wall is attached to the ventral fin. Tail longer than body. Both fins are pigmented, but the individual chromatophores are more densely distributed on the tail muscle than on the fins. Fins tip rounded, with taller edge near their mid length, and dorsal one slightly taller than the ventral; dorsal fin originating at the junction of the body and tail; both fins tapering to blunt tip at end of the tail, a bit away from the tail musculature ending. Ventrally, the anterior part of the body is darker; dorsally it is possible to observe at least two layers of pigments; one on the skin, the other on the surface of muscles and cartilages of the skull. Mouth anteroventral; oral disc ventral (Fig. 7), large, not emarginated, bordered by one row of papillae interrupted in the anterior region, on the mouth corner the papillae form multiple series, and not organized in rows. Labial teeth organized in two upper and three lower rows of keratodonts, with the second upper row interrupted medially (labial tooth row formula 2(2)/3); on the superior rows, the inner one is slightly larger than the external ones, and on the lower rows the internal ones are also slightly larger than the middle one, that is larger than the external ones; jaw sheaths strongly developed and serrated, the lower one is semicircular, the upper one with a middle blunt projection. Lateral line system present, consisting of round non pigmented stitches forming eleven lines: preorbital; infraorbital, oral, angular (visible only laterally), dorsal (represented by a few stitches, and the line stops near the insertion point of the dorsal fin), medial (runs only to half of the tail musculature), and ventral (only laterally); no lines, or stitches, present ventrally. It is not possible to visualize the stitches along the entire length of any of the lines, and near the posterior edge of the eye, the stitches lump together, not forming a line.

Distribution. Populations of *Scinax arduous* are known from five different localities in the Municipality of Santa Teresa (Fig. 8): 1) ornamental bromeliads from MBML (19°56'8.41"S / 40°35'59.67"W); 2) rocky outcrop-A covered with bromeliad *Alcantarea* sp. exposed to sunlight (19°57'43.34"S / 40°42'48.23"O; Fig. 9A); 3) rocky outcrop-B with different species of partially shaded bromeliads (19°54'27.31"S / 40°31'5.84"O; Fig. 9B); 4) an aggregation of ground bromeliads in forest interior (19°51'54.98"S / 40°34'41.26"O; Fig. 9C); and 5) Estação Biológica de Santa Lucia (19°57'10"S / 40°31'30"W; coordinates reported by Pombal & Bastos 2003).

Natural history. JVAL and HRS visited MBML on April 2012 and both adults and tadpoles were observed inside ornamental bromeliads from different species and sizes. However, no vocal activity was observed during the night. On November 2013 JVAL sampled a rocky outcrop covered by *Alcantarea* sp., herein referred to as rocky outcrop-A (Fig. 9A). Dozens of males were calling, but no tadpoles were found. Although there was plenty of water in the bromeliads, no rain was observed during that day/night sampling.

RBF and team surveyed eight times the rocky outcrop-B (Fig. 9B) from October to December 2012 and June and July 2013. Many males aggregate in this rocky outcrop during rainy nights. Calling males were only heard in December 2012 (rainy season). They were calling in a head down position in the interior of the bromeliad as also observed for other species belonging to the *S. perpusillus* group (Alves-Silva & Silva 2009, Silva & Alves-Silva 2011). *Scinax arduous* seems to select terrestrial bromeliads as only three individuals used epiphyte bromeliads up to 1.8 m above ground. The recorded calling males were in *Vriesea ruschi* possessing similar morphological traits (diameter= 60–74 cm, height= 56–75, number of leaves= 28–33). Adults were also found in another three bromeliad species (*Vriesea bituminosa*, *V. morrenii*, and *V. vagans*). Tadpoles were found in *V. ruschii*, *Quesnelia quesneliana*, and a *V.* aff. *atra*. No individual of *Scinax arduous* was found in the same bromeliad with either congener or cospecifics. This suggests that *S. arduous* may be territorialist which is not unusual for bromeligenous frogs (RBF pers. obs.). During these surveys another 13 species were found occupying bromeliads at rocky outcrop-B; four of them are bromeligenous [(*Dendropsophus* sp. nov. (Ferreira, Pombal, & Faivovich, in description), *Fritziana fissilis* (Miranda-Ribeiro), *F. goeldii* (Boulenger), and *Ischnocnema epipeda* (Heyer)], and nine are bromelicolous [*Bokermannohyla caramaschii* (Napoli), *Gastrotheca megacephala* Izecksohn, Carvalho-e-Silva & Peixoto, *Hypsiboas pardalis* (Spix), *H. semilineatus* (Spix), *I. abdita* Canedo & Pimenta, *Ischnocnema* sp. nov. (aff. *parva*) (RBF pers. obs.), *I. hoenei*, and *S. alter* (Lutz)].

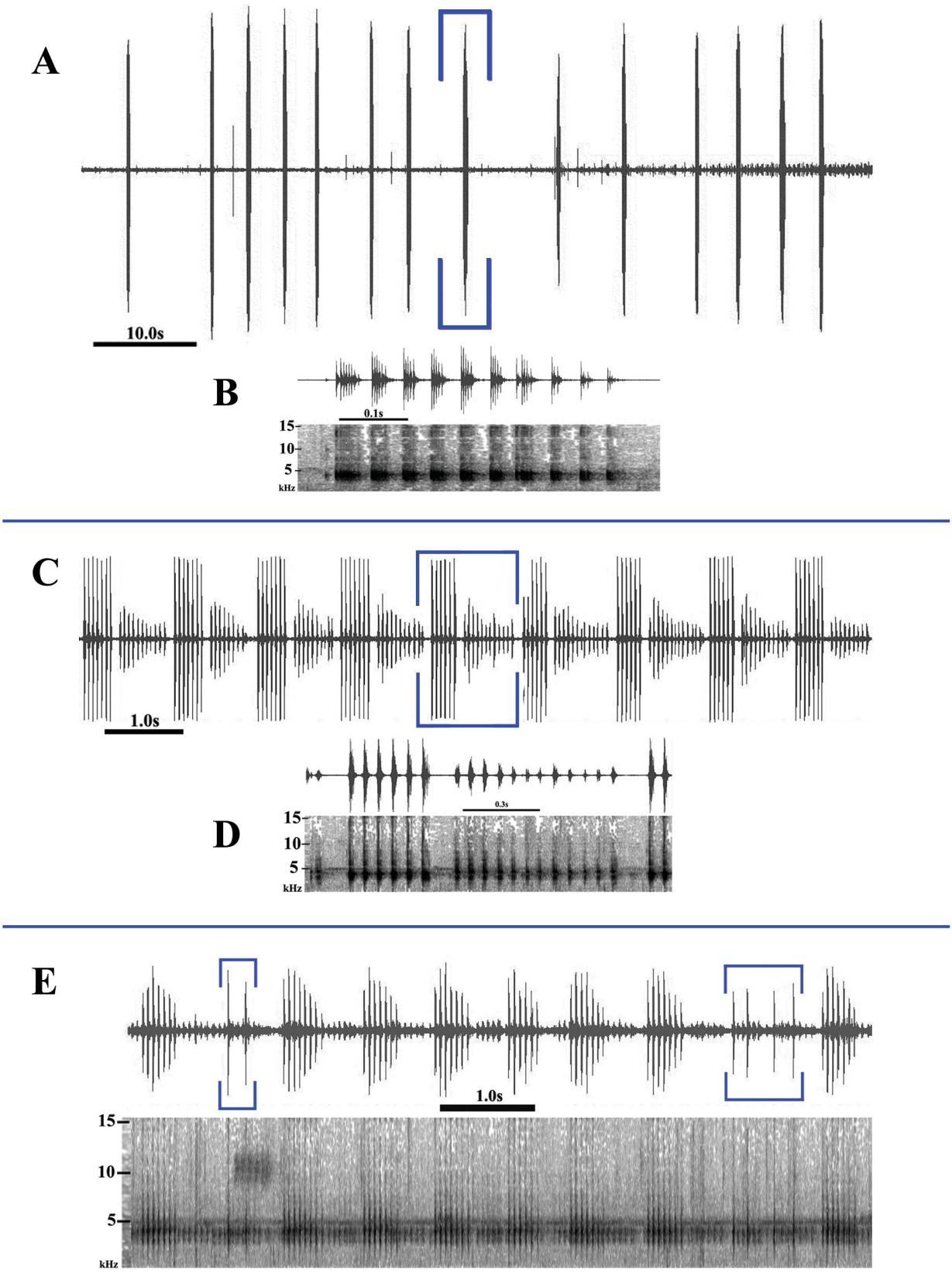

FIGURE 6. Vocalization of *Scinax arduous*: A) oscilogram of a call series emitted by a not interacting male; B) oscilogram and spectrogram of a single call - highlighted in blue; C) oscilogram of portion of the call series emitted by two males in duet; D) emphasis on the high call rate and no overlap between the calls; E) sonogram and spectrogram of portion of a call series emitted by a single male not evidently interacting with others ones calling in the recording background, emphasis on two calls type II highlighted in blue.

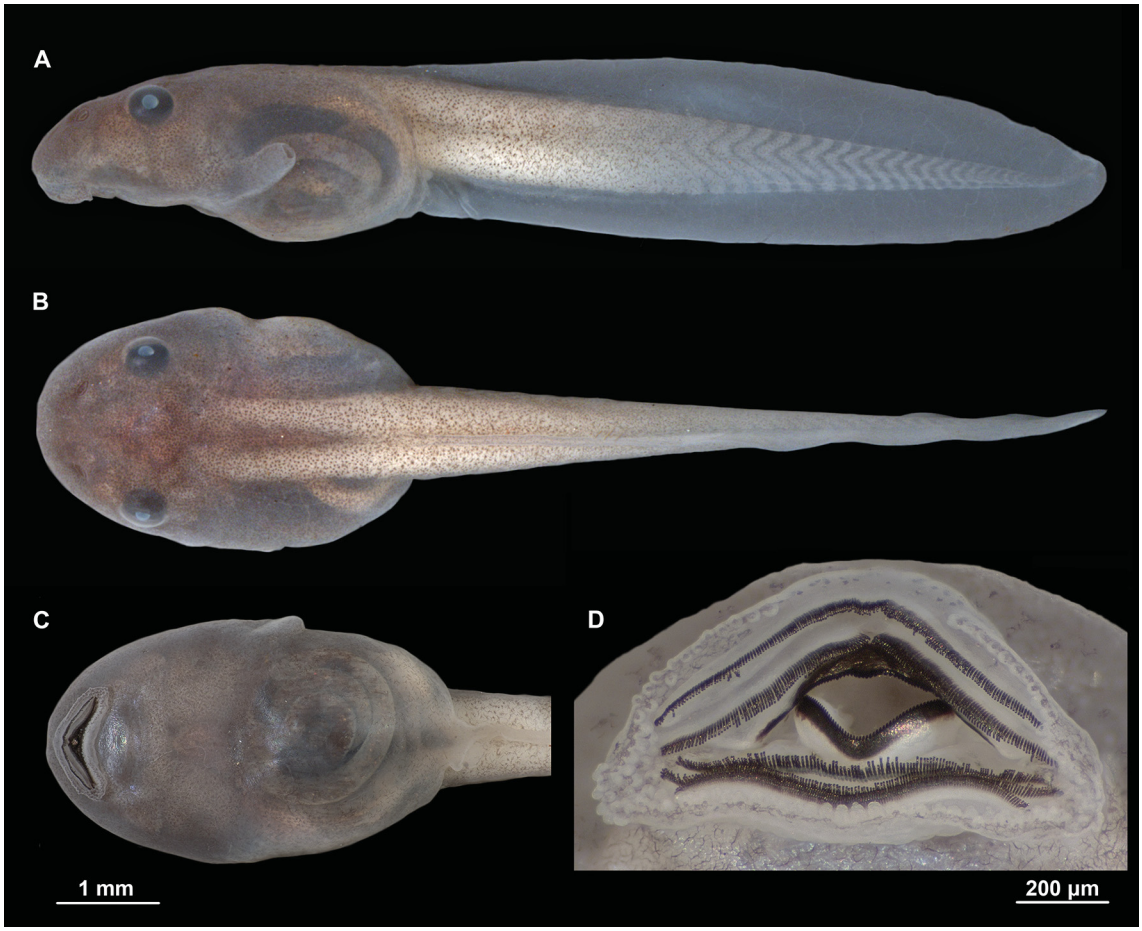

FIGURE 7. Tadpole of *Scinax arduous* (RU 8851): A) Lateral view; B) Dorsal view; C) Ventral view; and D) detail of mouth.

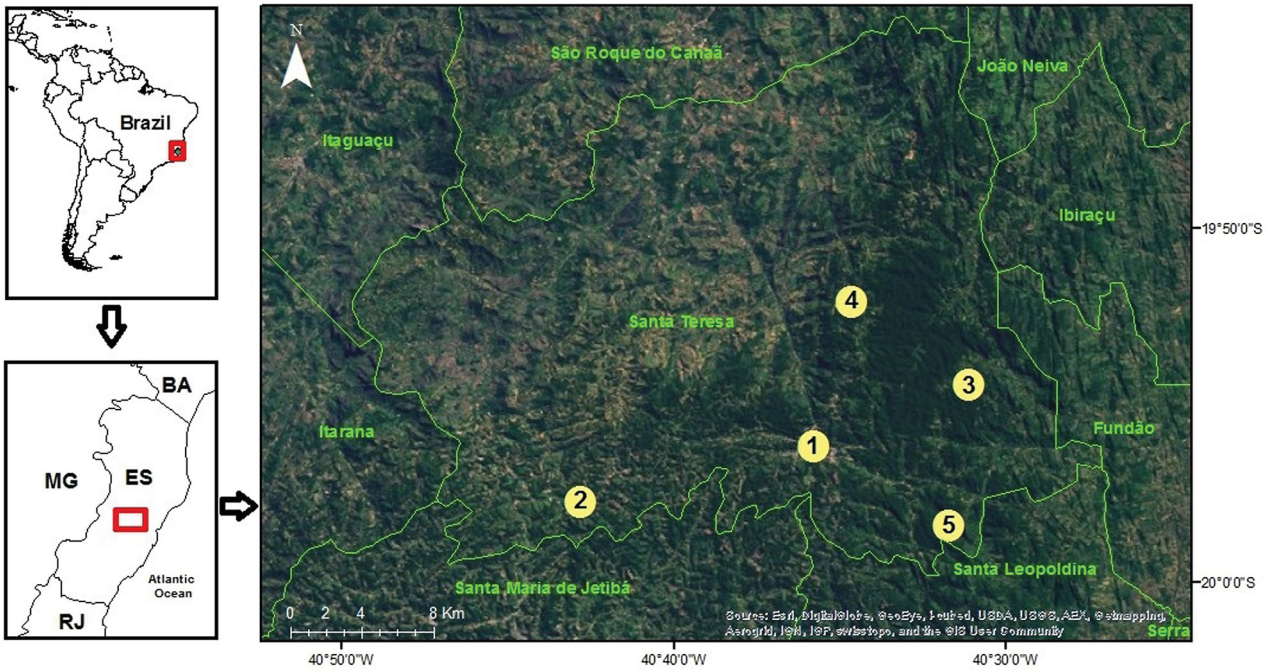

FIGURE 8. Map showing the five localities where *S. arduous* is known to occur at its type locality, Municipality of Santa Teresa (red square), southeastern Brazil: 1) MBML; 2) rocky outcrop-A; 3) rocky outcrop-B; 4) an aggregation of ground bromeliads in forest interior; and 5) Estação Biológica de Santa Lucia. BA= State of Bahia, ES= State of Espírito Santo, MG= State of Minas Gerais, and RJ= State of Rio de Janeiro.

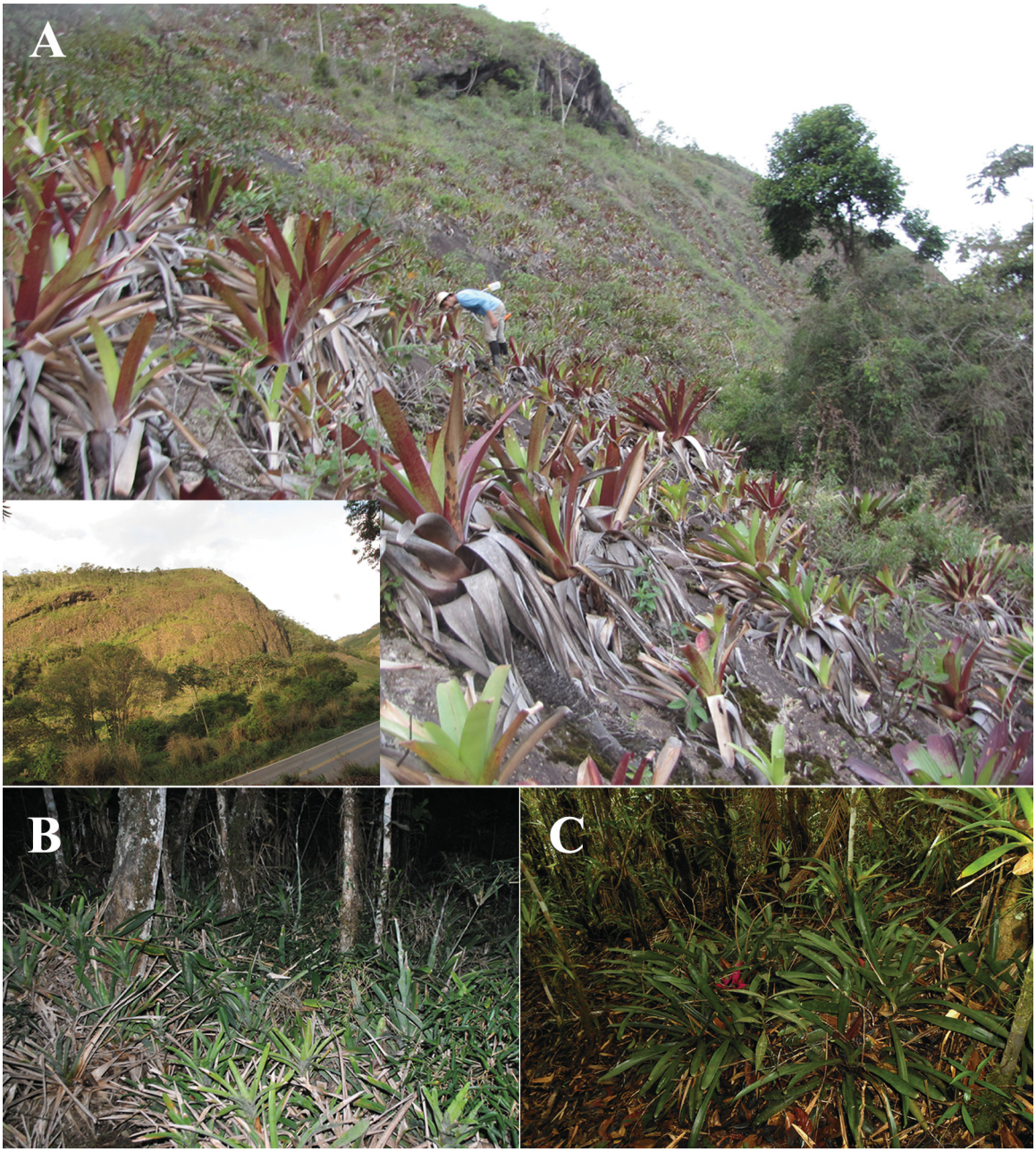

FIGURE 9. A) Rocky outcrop-A covered by bromeliads *Alcantarea* sp. exposed to sunlight. B) rocky outcrop-B with partially shaded bromeliads of different species. C) aggregation of ground bromeliads inside the forest.

Discussion

New diagnosis. Only two individuals [the holotype (female) and the paratype (male)] were available by the time *Scinax arduous* was described (Peixoto 2002), therefore, its original description lacks information on variation and distribution. The present study improves the species diagnosis by providing a redefinition of *Scinax arduous* based on a large sample of topotypes. Unfortunately, the type series was not available for us to examine. Thus, our sample was identified based on the species original description and comparison to the Pombal & Bastos (2003) topotypes used as bioacoustical vouchers.

The unique male measured by Peixoto (2002) did not adequately represent the type locality population as it was based on a small sized male (paratype SVL 19.5 mm), same size as the shortest male measured during the present study. Therefore, in future comparisons this species should be considered as composed of medium to large

sized individuals, contrary to its original description that considered it a small species. Although *Scinax arduous* was described as having head slightly wider than long, we suggest that such character should no longer be used as diagnostic as it was not confirmed in our samples (table 1). For the same reason, we suggest that the sum of THL and TBL lengths being longer than SVL in males and shorter in females should no longer be used as a diagnostic character neither as it varied sexually independently in our samples [(THL+TBL)/SVL 0.9–1.1 in both, males and females).

Although the yellow blotches have been considered an important taxonomic character within the group (Lacerda *et al.* 2012), such characteristic was not mentioned on the species original diagnosis, probably because the color (in life) descriptions was based on only two juvenile individuals, collected as tadpoles and raised in laboratory. Peixoto (2002) reported such blotches only on the inguinal region, hidden surfaces of thigh and tibia, but did not report the ones on the calf, feet and armpits, present in most of our sample. On the new diagnosis, instead of using *yellow blotches*, we preferred *bright colored blotches*, as an orange coloration was also observed on a single specimen during the present study: on the hidden surfaces of inguinal region, thigh, tibia, calf, and foot. Although this coloration approximates it to *S. v-signatus*, we identified this individual as *S. arduous* based on bioacoustical similarities and lack of dark pigmentation on the mental region organized in the form of a Y or V also considered a diagnostic character for *S. v-signatus* (Lutz 1968; Peixoto 2002). Finally, the presence of a discrete dark pigmentation at the throat was also considered a diagnostic character at the species original description. However, we do not agree with that statement as we also observed individuals with intense dark pigmentation among our sample (Fig. 3).

Tadpoles tail length (TL) inferior to 2/3 of the total length (LT) and tail high (TH) inferior to body high (BH) also should no longer be considered diagnostic characters as they were not confirmed in all our sample (TL/LT 0.5–0.7 and TH/BH 0.8–1.4). Instead, we suggest the lack of any yellow mark should be considered diagnostic. Besides, we complemented the tadpole original description by providing detailed photographs and bringing new data, such as descriptive information on the tadpole's lateral lines and measurements from many stages.

Males of *S. arduous* emit long call series as reported for other species of *Scinax perpusillus* group (eg. *S. cosenzai, S. insperatus, S. perpusillus* and *S. v-signatus*) (Alves-Silva & Silva 2009, Silva & Alves-Silva 2011; Lacerda *et al.* 2012). The duration of such call series, number of calls per call series, rate of calls per minute, number of notes per call and consequently the duration of the calls are probably related to the social context. The advertisement call of *S. arduous* was first described by Pombal & Bastos (2003) that found 4–6 notes/call. The vocalization herein reported can reach longer durations and higher number of notes (4–14 notes/call). Pombal & Bastos (2003) also described a second type of vocalization, probably with a territorial function, constituted by a single note with duration of 53–64 ms. We did not record such type of call. As such bioacoustical information is important to differentiate *S. arduous* from many species, it was also considered in the new diagnosis.

Conservation. Although collected specimens of *Scinax arduous* are known from only five areas in Santa Teresa, we strongly believe that such data deficiency is a sampling issue. The Municipality has a lot of rocky outcrops covered by *Alcantarea* sp. similar to the one herein referred to as rocky outcrop-A (Fig. 9A). In addition, we presume that *S. arduous* occurs in most of these sites and in many epiphyte or ground bromeliads in forested areas connecting these rocky outcrops. Such strong relation is also observed for other montane species belonging to the *S. perpusillus* group (see Peres & Simon 2011, Lacerda *et al*. 2012, Silva & Alves-Silva 2011, 2013).

The *Scinax perpusillus* group is currently composed of 13 species: *Scinax alcatraz* (Lutz); *S. arduous* Peixoto; *S. atratus* (Peixoto); *S. belloni* Faivovich, Gasparini & Haddad; *S. cosenzai* Lacerda, Peixoto & Feio; *S. faivovichi* Brasileiro, Oyamaguchi & Haddad; *S. insperatus* Silva & Alves-Silva; *S. littoreus* (Peixoto); *S. melloi* (Peixoto); *S. peixotoi* Brasilerio, Haddad, Sawaya & Martins; *S. perpusillus* (Lutz & Lutz); *S. tupinamba* Silva & Alves-Silva; and *S. v-signatus* (Lutz). Eight of them deserve special attention regarding the conservation status (IUCN 2015): *S. alcatraz*, *S. faivovichi* and *S. peixotoi* are classified as critically endangered (Rodrigues & Cruz 2004; Brasileiro 2008ab); *S. belloni* is considered endangered (Angulo *et al.* 2011); while *S. arduous*, *S. atratus, S. insperatus* and *S. melloi* are classified as data deficient (Peixoto & Pimenta 2004; Cruz & Telles 2004; Peixoto & Telles 2004). Peixoto & Pimenta (2004) states that there is a potential risk for the species regarding the expanding agricultural activity, wood plantations, human settlements, and bromeliad collections for gardening. However deforestation does not seem to be a threat for *Scinax arduous* because Santa Teresa harbors a substantial area of forest and preserved rocky outcrops. As reported for *S. insperatus* by Silva & Alves-Silva (2011), it seems that bromeliad collection does not represent a risk for *S. arduous* as it commonly inhabits bromeliads growing on granitic outcrops

that are hard to reach due to its steepness. Thus, *S. arduous* does not seem to be threatened by extinction. However, wild fires during long draughts may affect bromeliad aggregates growing on these outcrops and prevention strategies may be indicated for outcrops near farms and roads.

The present study brings new data on the morphology and coloration of adults and larvae, vocal repertoire, geographical distribution and conservation status of *S. arduous*, thus improving the knowledge on the taxonomy of the *S. perpusillus* group of species.

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APPENDIX

Referred adult specimens. Topotypes: BRAZIL: State of Espírito Santo: Municipality of Santa Teresa: Museu de Biologia Mello Leitão (RU 7367, 7369, 7371, 8394; MNRJ 28373–28375, 30441, 34926–34930, 40695–40699; MBML 630, 635, 1113–15, 4630; MZUFV 12561–65); Estação Biológica Santa Lucia (MNRJ 30387, 38436–38439); rocky outcrop-A (UFMG 15436–15446); rocky outcrop-B (UFMG 15433–15435, 15447–15458); and forest interior (UFMG 15432).

Referred tadpoles. Topotypes: BRAZIL: State of Espírito Santo: Municipality of Santa Teresa: RU 7476 (two individuals), RU 8851 (14 individuals), MNRJ 86596 (one individual), MNRJ 86597 (three), MNRJ 86598 (three), MNRJ 86601 (one), MNRJ 86602 (three).

Referred vocalization. Recorded from topotypes: BRAZIL: State of Espírito Santo: Municipality of Santa Teresa: UFMG-V541–546.