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A new species of Hatchet-faced Treefrog *Sphaenorhynchus* Tschudi (Anura: Hylidae) from Quadrilátero Ferrífero, Minas Gerais, southeastern Brazil

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Abstract

A new species of *Sphaenorhynchus* is described from the Municipality of Mariana, State of Minas Gerais, Brazil. It is characterized by the vocal sac moderately developed, single, subgular, with longitudinal folds; white canthal and dorsolateral lines delimited below by a dorsolateral black line from the tip of snout extending beyond the eye to gradually disappearing up to the flanks; and premaxilla and maxilla almost completely edentulous, each bearing 1–5 extremely small teeth. It is most similar with *Sphaenorhynchus orophilus*, from which it can be distinguished by having a less robust forearm in males; glandular subcloacal dermal fold; premaxilla and maxilla almost completely edentulous; and larvae with large marginal papillae in the oral disc. The new species occurs in natural ponds over ironstone outcrops (known as *canga*) on flat terrain, where males call from the floating vegetation.

Key words: *Sphaenorhynchus canga* **sp. nov.**, advertisement call, tadpole, Espinhaço Range, Iron Quadrangle, morphology, taxonomy

Introduction

The Neotropical hylid frog genus *Sphaenorhynchus* Tschudi is a putative monophyletic taxon phylogenetically related to *Dendropsophus*, *Lysapsus*, *Pseudis*, *Scarthyla*, *Scinax* and *Xenohyla* (Faivovich *et al.* 2005; Wiens *et al.* 2006; Wiens *et al.* 2010; Pyron & Wiens 2011). Despite the many morphological synapomorphies that have been suggested for the genus (Duellman & Wiens 1992; Faivovich *et al.* 2005; Araujo-Vieira *et al.* 2015), its monophyly has been tested on the basis of a limited taxon sampling (*S. dorisae*, *S. lacteus* and *S. orophilus*; Faivovich *et al*. 2005; Wiens *et al.* 2006; Pyron & Wiens 2011), and relationships among its species remain uncertain.

Sphaenorhynchus includes 14 small greenish treefrogs (Frost 2015), three of which are widespread throughout the Amazon basin [*S. carneus* (Cope), *S. dorisae* (Goin), and *S. lacteus* (Daudin). *Sphaenorhynchus lacteus* is also present in northeastern Brazil (States of Maranhão and Piauí; Caramaschi *et al.* 2009; Benício *et al*. 2011) and Trinidad (recorded as *Hyla orophila* by Kenny 1969)]. One species, *S. platycephalus* (Werner), is known solely from the poorly preserved holotype originating in "South America" (Harding 1991). The remaining 10 species [*S. botocudo* Caramaschi, Almeida & Gasparini, *S. bromelicola* Bokermann, *S. caramaschii* Toledo, Garcia, Lingnau & Haddad, *S. mirim* Caramaschi, Almeida & Gasparini, *S. orophilus* (Lutz & Lutz), *S. palustris* Bokermann, *S. pauloalvini* Bokermann, *S. planicola* (Lutz & Lutz), *S. prasinus* Bokermann, and *S. surdus* (Cochran)] are distributed in the Atlantic Forest, from the northern portion of the State of Rio Grande do Sul to the State of Pernambuco (Frost 2015).

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In this paper, we describe a new species of *Sphaenorhynchus* collected during fieldwork in the Municipality of Mariana on its limit with the Municipality of Catas Altas, southeastern portion of the Iron Quadrangle (Quadrilátero Ferrífero), Espinhaço Range, State of Minas Gerais, southeastern Brazil.

Material and methods

Adult specimens were fixed in 10% formalin and stored in 70% ethanol. Webbing formula follows Savage & Heyer (1967) as modified by Myers & Duellman (1982). Snout profile terminology follows Duellman (2001). Measurements were (in millimeters) recorded with a digital caliper (0.01 mm). Nine measurements follow Duellman (1970): SVL (snout-vent length), HL (head length), HW (head width), IND (internarial distance), IOD (interorbital distance), ED (eye diameter), END (eye-nostril distance), TL (tibia length), and FL (foot length). Thigh length (THL) and hand length (HAL) follow Heyer *et al.* (1990); and third finger disc diameter (3FD) and fourth toe disc diameter (4TD) Napoli & Caramaschi (1998). ED, END, IND, IOD, HAL, 3FD and 4TD were measured under a stereomicroscope. Sex was determined by examination of secondary sexual characters (nuptial pads, vocal slits, and expansion of the vocal sac). Number and presence of vomerine, premaxillary and maxillary teeth were observed in cleared and double stained specimens with alcian blue and alizarin red (see Appendix 3), prepared following the techniques of Taylor & Van Dyke (1985).

Males of *Sphaenorhynchus canga* **sp. nov.** called among the aquatic vegetation in five ponds (see topic Geographic Distribution) at the locality known as Chapada de Canga (local designation), at the Municipality of Mariana, on its limit with the Municipality of Catas Altas, both at State of Minas Gerais, southeastern Brazil. The advertisement calls were recorded using a digital recorder (Marantz PMD 660) coupled to directional microphone (Sennheiser ME-66), and carried out at 44.1 kHz on 16-bit resolution. Temporal parameters of calls are given as mean ± standard error. Air temperature was obtained using a compact pen-type thermohygrometer. All recordings were analyzed on Raven 1.4 beta software (Cornell Lab of Ornithology Research Program—Bioacoustics Workstation) with FFT = 512 points. A total of eight call parameters were analyzed: note and call duration (s); dominant frequency of calls (Hz); number of notes per call; interval between notes (s); number of pulses per note; interval between pulses (s); and pulse rate (pulses per second). Temporal parameters were obtained directly from the oscillogram. Sound oscillogram and spectrogram were produced with the following parameters: FFT = 256 points, Overlap = 89.8% and hamming window type. Description and terminology of acoustic properties follow Duellman & Trueb (1994).

Tadpoles of the new species were collected with the holotype. They were killed in 5% lidocaine solution, prepared and preserved in 10% formalin. External morphology descriptions, measurements, and proportions were based on six tadpoles in stages 36–39 (Lot UFMG 633a). Variation was analyzed based on further specimens of the series (16 specimens in stages 26–35, Lot UFMG 633b). Terminology for larval morphology follows Altig & McDiarmid (1999), with the exception of the position of the intestinal mass, which follows Faivovich (2002). Measurements and terminology follow Altig & McDiarmid (1999) for TL (total length), BL (body length), TAL (tail length), MTH (maximum tail height), IND (internarial distance), IOD (interorbital distance), TMW (tail muscle width), and TMH (tail muscle height); Lavilla & Scrocchi (1986) for BW (body width), BWN (body width at narial level), BWE (body width at eyes level), BH (body height), ESD (eye-snout distance), END (eye-nostril distance), NSD (nostril-snout distance), ED (eye diameter), ND (narial diameter), SED (snout-spiracular distance), and ODW (oral disc width); Grosjean (2005) for DFH (dorsal fin height) and VFH (ventral fin height); Araujo-Vieira *et al.* (2015) for SL (distance between the insertion of the spiracle and its distal edge). An additional measurement taken was SDEH (spiracle distal margin height, that is, the perpendicular distance between the spiracle distal edge and the ventral surface of the tadpole). All measurements were taken to the nearest 0.1 mm with the aid of ImageTool version 3.00 (Wilcox *et al.* 1996). To obtain high quality photos we used an adjustable platform to support tadpoles immersed in water (Schacht & McBrayer 2009).

Data about the morphological features of adults and known tadpoles of *Sphaenorhynchus* were obtained from preserved specimens (see Appendix 1–3) complemented with descriptions by Lutz & Lutz (1938), Bokermann (1966), Kenny (1969), Bokermann (1973), Cruz (1973), Cruz & Peixoto (1980), Heyer *et al*. (1990), Harding (1991), Nunes *et al.* (2007), Suárez-Mayorga & Lynch (2001), Toledo *et al.* (2007), Caramaschi (2010), Lynch & Suárez-Mayorga (2011), and Araujo-Vieira *et al.* (2015).

Adult specimens and tadpoles are housed in the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Museu de Zoologia João Moojen de Oliveira, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (MZUFV); and Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (UFMG).

Results

***Sphaenorhynchus canga* sp. nov.**
(Figs. 1–2)

Holotype. UFMG-A 5715, adult male, from Chapada de Canga (local designation—1.9 km W MG-129, 20°07'40" S, 43°23'05" W, 915 m elevation), Municipality of Mariana, on its limit with the Municipality of Catas Altas, State of Minas Gerais, southeastern Brazil, collected on February 15, 2009 by Felipe Sá Fortes Leite, Camila R. Rievers, and Michael R. Lindenmann.

Paratypes. Twenty four adult males collected in three localities in the Municipality of Mariana, on its limit with the Municipality of Catas Altas, State of Minas Gerais, southeastern Brazil. Females have not been collected. MNRJ 56337–56346 collected on September 10, 2004 by Felipe Sá Fortes Leite. UFMG-A 5716–5717 collected on February 15, 2009 by Felipe Sá Fortes Leite, Camila R. Rievers, and Michael R. Lindenmann. All specimens collected at the type locality. UFMG-A 7192, 7194, 7205, 7207–7208 collected on November 13, 2010 and January 30, 2011 by Laila P. Mascarenhas and Clara Tiso. MZUFV 11912–11915 collected on February 4, 2012 by Clodoaldo Assis. All specimens collected at 1.5 km W MG-129 (20º7'32" S, 43°23'23" W). UFMG-A 11732, 11738–11739, 5.2 km S MG-129 (20°12' 26"S 43° 25' 03" W), collected on January 25, 2012 by Bruno Teixeira.

Referred specimens. Fourteen adult males: MNRJ 56322–56331, 56334, 56336, 56335 (cleared and double stained specimen), type locality, collected on September 10, 2004 by Felipe Sá Fortes Leite. UFMG-A 7209 (cleared and double stained specimen), 1.5 km W MG-129 (20º7'32"S43°23'23"W), collected on January 30, 2011 by Laila P. Mascarenhas and Clara Tiso.

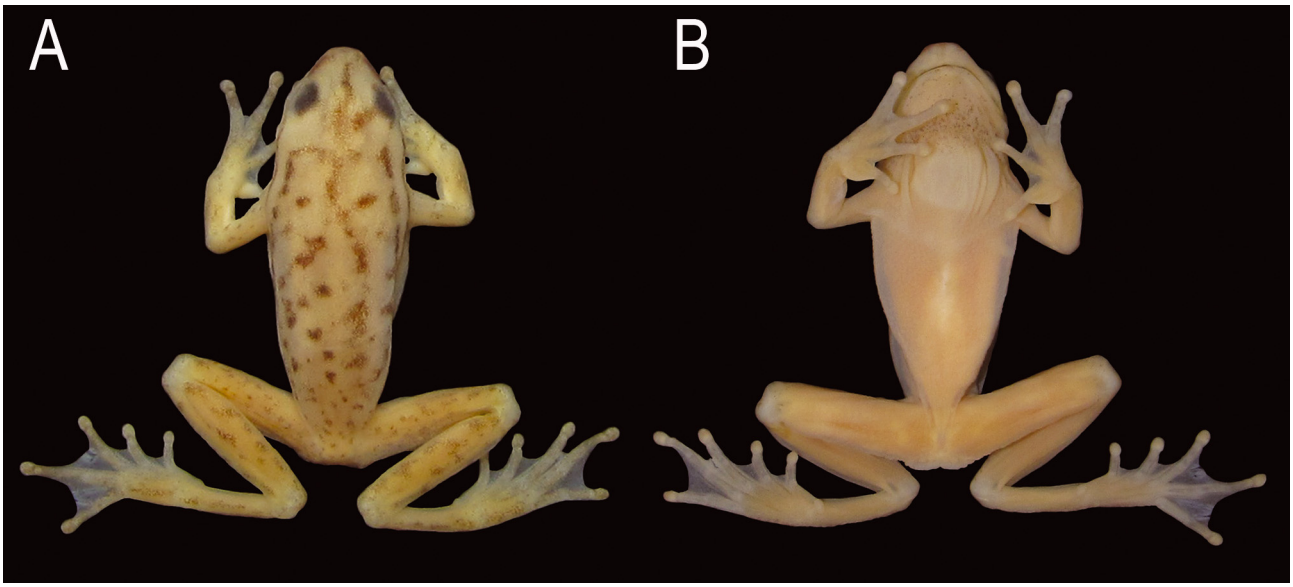

FIGURE 1. (A) Dorsal and (B) ventral views of the holotype of *Sphaenorhynchus canga* **sp. nov.** (UFMG-A 5715). Adult male from Chapada de Canga, Municipality of Mariana, State of Minas Gerais, southeastern Brazil. SVL 27.0 mm.

Diagnosis. The new species can be diagnosed by the following set of characters: (1) SVL 26.2–30.2 mm in males; (2) tympanic membrane absent; (3) snout protruding in profile; (4) vocal sac single, subgular, moderately developed, extending to the middle of the pectoral region, with longitudinal folds; (5) canthal white line present; (6) dorsolateral white line from the eye to sacral region; (7) dorsolateral black line from the tip of snout extending beyond the eye to gradually disappearing up to the flanks; (8) slender forearm in males; (9) ulnar fold absent or when present poorly developed and/or slightly crenulated; (10) glandular subcloacal dermal fold; (11) vomerine

teeth present; (12) premaxilla and maxilla almost completely edentulous, each bearing 1–5 extremely small teeth; (13) tadpole with a medium-sized spiracle (SL 25–29% of BL); (14) presence of large marginal papillae (about twice the size of the small ones), arranged together or alternating with the small papillae; and (15) advertisement call (0.008–1.23 s of duration), composed of 1–9 pulsed notes, each with duration of 0.005–0.020 s.

Comparison with other species. The SVL (26.2–30.2) distinguishes males of *Sphaenorhynchus canga* **sp. nov.** from those of *S. carneus* (15.1–19.8; Duellman 1974), *S. mirim* (15.7–18.2; Caramaschi *et al.* 2009), *S. pauloalvini* (17.9–22.2, 19.7 ± 1.5, *n* = 22), and *S. planicola* (19.1–24.1, 21.2 ± 1.4, n = 22). The absence of tympanic membrane differentiates the new species from *S. lacteus* and *S. pauloalvini* (tympanic membrane present; Fig. 3B). The protruding snout in lateral view separates *S. canga* **sp. nov.** from *S. pauloalvini* and *S. planicola* (truncated in lateral view). A moderately developed vocal sac, extending approximately to the middle of the pectoral region differentiates males of *S. canga* **sp. nov.** from those of *S. prasinus* (less developed, reaching the anterior region of pectoral region), *S. mirim* and *S. planicola* (well developed, extending laterally and toward the posterior pectoral region). Additionally, the vocal sac of the new species is characterized by the presence of longitudinal lateral folds, which are absent in *S. botocudo*, *S. mirim*, *S. pauloalvini*, and *S. prasinus*.

The presence of a dorsolateral black line from the tip of snout extending beyond the eye to gradually disappearing up to the flanks clearly separates *Sphaenorhynchus canga* **sp. nov.** from *S. dorisae* and *S. mirim* (absent), *S. lacteus* and *S. prasinus* (only canthal black line), *S. botocudo* (from the tip of snout to the sacral region, but with a depression in the middle of the flanks; see also Fig. 3 in Caramaschi *et al.* 2009), and *S. bromelicola* (from the tip of snout to the sacral region, but reddish pigmented; Bokermann 1966). The presence of canthal/dorsolateral white lines delimited below by a dorsolateral black line differentiates the new species from *S. dorisae*, *S. mirim*, *S. pauloalvini* and *S. planicola* (absent in these species).

Ulnar fold absent or when present poorly developed and/or slightly crenulated distinguishes the new species from *Sphaenorhynchus dorisae*, *S. lacteus*, *S. mirim*, *S. planicola* and *S. prasinus* (well developed and smooth ulnar dermal fold). Also, the lack of dermal appendages on elbow and heel separates the new species from *S. dorisae* (dermal fold on elbow and triangular calcar appendage), and *S. mirim*, *S. planicola* and *S. prasinus* (dermal fold on elbow and round calcar appendage). The presence of a glandular dermal fold on the subcloacal region differentiates *S. canga* **sp. nov.** from *S. carneus* and *S. pauloalvini* (absent), *S. dorisae* (dermal flap with triangular lateral margins), and *S. lacteus*, *S. mirim*, *S. planicola* and *S. prasinus* (dermal flap with round lateral margins).

The presence of vomerine teeth distinguishes *Sphaenorhynchus canga* **sp. nov.** from *S. carneus* (absent in this species). In addition, the new species distinguishes from *S. carneus*, *S. dorisae*, *S. mirim* and *S. planicola* by the presence of premaxillary and maxillary teeth (absent in these species). Otherwise, a premaxilla and maxilla almost completely edentulous, each bearing 1–5 extremely small teeth differentiates the new species from *S. botocudo*, *S. bromelicola*, *S. caramaschii*, *S. lacteus*, *S. palustris*, *S. prasinus*, and *S. surdus* (premaxilla and maxilla, each bearing at least ten teeth).

The new species is also distinguished from the others species of the genus by some larval characteristics. External morphological characters that distinguish tadpoles of *Sphaenorhynchus canga* **sp. nov.** are also presented in the Table 1. *Sphaenorhynchus canga* **sp. nov.** is promptly separated from *S. caramaschii*, *S. carneus*, *S. dorisae*, *S. lacteus*, *S. pauloalvini*, *S. planicola*, and *S. prasinus* (short spiracle; Lynch & Suárez-Mayorga 2011; Araujo-Vieira *et al*. 2015), and *S. palustris* (extreme long spiracle; Nunes *et al.* 2007; Araujo-Vieira *et al*. 2015) by having a medium-sized spiracle (SL 25–29% of BL; see also Araujo-Vieira *et al*. 2015). The A-2 gap is larger in *S. canga* **sp. nov.** (Fig. 8D), whereas it is smaller (equivalent to A-2 lateral segment) in *S. carneus*, *S. dorisae*, *S. palustris*, *S. pauloalvini*, *S. planicola*, and *S. prasinus* (Suárez-Mayorga & Lynch 2001). It differs from *S. palustris* by having A-2 larger than A-1 (A-1 > A-2 in *S. palustris*); *S. bromelicola* by having P-1 slightly larger than P-2, (P-1 = P-2 in *S. bromelicola*); *S. dorisae* by the absence of posterior gap in marginal papillae (present in *S. dorisae*; Suárez-Mayorga & Lynch 2001); and *S. carneus* by having the labial tooth row formula 2(2)/3(1) [1/2-3(1) in *S. carneus*; Suárez-Mayorga & Lynch 2001].

The presence of longitudinal dark stripes on the tail musculature distinguishes the new species from *Sphaenorhynchus dorisae*, *S. lacteus*, *S. planicola* and *S. prasinus* (absent in these species), *S. carneus* (on the medial muscle line), and *S. pauloalvini* (on the ventral muscle margin). Tadpoles of *S. canga* **sp. nov.** also differ from those of *S. bromelicola*, *S. dorisae*, *S. pauloalvini*, and *S. surdus* (small and homogeneously sized marginal papillae; see also Suárez-Mayorga & Lynch 2001; Lynch & Suárez-Mayorga 2011; Caramaschi 2010; Araujo-Vieira *et al*. 2015), by having larger marginal papillae (at least about twice the size of the smaller papillae) arranged together or alternating with the smaller ones in the oral disc.

The advertisement call of *Sphaenorhynchus canga* **sp. nov.** further differentiates it from that of *S. caramaschii* (call duration of 5.23–11.0 s, 22–43 notes/call, and note duration of 0.037–0.07 s; Toledo *et al.* 2007; Toledo *et al.* 2014) by its shorter duration, lower number of notes/call, and shorter note duration (call duration of 0.008–1.23 s, 1–9 notes/call, and note duration of 0.005–0.020 s); *S. mirim* (9–25 pulses/note; Lacerda *et al.* 2011) by its lower number of pulses/note (1–5 pulses/note); *S. pauloalvini* and *S. prasinus* (interval between notes of 0.01–0.02 s in *S. pauloalvini*, and 0.014–0.018 s in *S. prasinus*; Toledo *et al.* 2014) by its larger interval between notes (0.05–0.23 s); and *S. planicola* (note duration of 0.08–0.17 s, and interval between notes of 0.24 s; Toledo *et al.* 2014) by its shorter note duration and shorter interval between notes (note duration of 0.005–0.020 s, and interval between notes of 0.05–0.23 s).

The new species can be differentiated from the holotype of *Sphaenorhynchus platycephalus* (SVL 33.0 mm; holotype IZUW 90; female) by the presence of subcloacal ornamentation; and vomerine, premaxillary and maxillary teeth, all of which *S. platycephalus* lacks (Harding 1991).

Sphaenorhynchus canga **sp. nov**. is most similar with *S. orophilus* from which distinguishes by having generally a slender forearm in males (more robust forearm in *S. orophilus*; Fig. 4); glandular subcloacal dermal fold (many swelled tubercles, but not forming a dermal fold in *S. orophilus*; Fig. 5); premaxilla and maxilla almost completely edentulous, each bearing 1–5 extremely small teeth (premaxilla and maxilla, each bearing at least ten teeth in *S. orophilus*); and tadpoles with larger marginal papillae (at least about twice the size of the smaller papillae) arranged together or alternating with the smaller ones in the oral disc (small and homogeneously sized marginal papillae in *S. orophilus*; see Fig. 4 in Cruz & Peixoto 1980, and also Fig. 4A in Araujo-Vieira *et al*. 2015).

Description of holotype. Body moderately slender; head slightly longer than wide, 29.4% (HL/SVL) and 27.4% (HW/SVL) of SVL (Fig. 1). Snout slightly truncated in dorsal view, protruding in profile (Fig. 2A–B). Nostrils lateral, directed forward, distance between nostrils 53% of IOD. *Canthus rostralis* rounded. Loreal region slightly convex. Eyes slightly small, ED 32.3% smaller than IOD. Tympanic membrane absent, unmodified skin on this area; tympanic cavity partially visible by the translucent skin (Fig. 2B). Tympanic annulus rounded. Supratympanic fold barely evident, from the upper portion of the tympanum to the insertion of the arm. Vocal sac single, subgular, moderately developed, extending to the middle of the pectoral region, with longitudinal folds. Vocal slits present, located diagonally to the longitudinal body axis, originating laterally of the tongue and running towards the corner of the mouth. Tongue ovoid, free laterally and posteriorly notched. Vomerine teeth extremely small, irregularly disposed on a dentigerous process located behind the choanae. Premaxillary and maxillary teeth indiscernible under high magnification. Choanae round.

Upper arms and forearms slender. Ulnar fold poorly developed and slightly crenulated. Dermal appendages on elbow absent. Hand large, HAL 32.2% of SVL. Fingers slightly robust. Relative finger length I<II<IV<III. Discs moderately developed and round. Subarticular tubercles single, rounded. Supernumerary tubercles small, single, and rounded. Inner metacarpal tubercle single, elliptical, medium-sized; outer metacarpal tubercle flat, discrete, medially divided. Webbing formula I 2^{+}–$2^{1/2-}$ II 2^{-}–$2^{1/2+}$ III 2–2 IV; free parts of fingers discretely fringed (Fig. 2C). Thick, wide, light colored nuptial pad covering the dorsal surface of Metacarpal I and covering the external margin of the inner metacarpal tubercle, reaching the antepenultimate subarticular tubercle.

Hind limbs slender; THL about the same size of FL and TL, representing about 46% of SVL. FL 45.5% of SVL. Dermal appendages on heel and on the outer edge of tarsus absent. Toes slender. Relative length of toes I<II<III~V<IV. Discs rounded and slightly expanded. Subarticular tubercles single, rounded. Supernumerary tubercles small, single, and rounded. Inner metatarsal tubercle medium-sized, elliptical; outer metatarsal tubercle absent. Webbing formula I $1^{1/2-}$–2^{-} II $1^{+-}2$ III $1^{+-}2^{+}$ IV 2^{+}–1^{+} V; free parts of toes discretely fringed (Fig. 2D). White glandular subcloacal dermal fold present.

Dorsal surfaces smooth, including arms and legs. Vocal sac and ventral surfaces of arms, tibia and tarsus smooth. All other ventral surfaces densely covered by rounded, low granules.

Coloration in life. Dorsal surfaces are light green with scattered round and irregular dark brown blotches on body, and finely spotted light brown blotches on limbs. Venter light green. Skin translucent, especially on the ventral surface of body and limbs, and at the sides of the body, so that it is possible to see the muscles, veins, green bones, and the white parietal peritoneum covering all organs. Subgular region finely dark brown spotted. Iridescent white canthal and dorsolateral lines present, delimited below by a dark dorsolateral line from the snout to the sacral region. Iris golden with many brown reticulations.

TABLE 1. Main external morphological characteristics that distinguish known tadpoles of *Sphaenorhynchus*.

Species	Tadpole description references	Marginal papillae	Submarginal papillae	LTRF	A-2 gap size	Anterior tooth row sizes	Posterior tooth row sizes	Spiracle length	Narial valve	Posterior gap in marginal papillae	Longitudinal stripes on the tail musculatur
S. canga **sp. nov.**	Present study	Uniserial, elongated and short papillae intercalated	Present	2(2)/3(1)	Equivalent to three times the A-2 lateral segment	A2>A1	P1>P2	Medium-sized	Present	Absent	Median, dorsal, ventral
S. bromelicola	Bokermann (1966); Araujo-Vieira *et al*. (2015)	Uniserial, uniform sized	Absent	2(2)/3(1)	Equivalent to A-2 lateral segment	A2>A1	P1=P2	Medium-sized	Present	Absent	Median and dorsal
S. caramaschii	Araujo-Vieira *et al*. (2015)	Uniserial, elongated and short papillae intercalated	Present	2(2)/3(1).	Equivalent to three times the A-2 lateral segment	A1=A2	P1>P2	Short	Present	Absent	Median, dorsal, ventral
S. carneus	Suarez-Mayorga & Lynch (2001)	Uniserial, elongated and short papillae intercalated	Present	1/2-3(1)	Equivalent to A-2 lateral segment	–	P1>P2	Short	–	Absent	Median
S. dorisae	Suarez-Mayorga & Lynch (2001)	Uniserial, uniform sized	Present	2(2)/3(1)	Equivalent to A-2 lateral segment	A2>A1	P1>P2	Short	–	present	Absent
S. lacteus	Kenny (1969); Suarez-Mayorga & Lynch (2001); Araujo-Vieira *et al*. (2015)	Uniserial, elongated and short papillae intercalated	Present	2(2)/3(1)	Equivalent to three times the A-2 lateral segment	A2>A1	P1>P2	Short	Present	Absent	Absent
S. orophilus	Cruz & Peixoto (1980); Heyer *et al* (1990); Araujo-Vieira *et al*. (2015)	Uniserial, uniform sized	Present	2(2)/3(1)	Equivalent to three times the A-2 lateral segment	A2>A1	P1>P2	Medium-sized	Present	Absent	Median, dorsal, ventral
S. palustris	Nunes *et al.* (2007); Araujo-Vieira *et al*. (2015)	Uniserial, elongated and short papillae intercalated	Present	2(2)/3(1)	Equivalent to A-2 lateral segment *	A1>A2	P1>P2	Long	Present	present	Median, dorsal, ventral
S. pauloalvini	Bokermann (1973); Araujo-Vieira *et al*. (2015)	Biserial, uniform sized	Absent	2(2)/3(1)	Equivalent to A-2 lateral segment	A1=A2	P1>P2	Short	Present	Absent	Ventral
S. planicola	Cruz (1973); Araujo-Vieira *et al*. (2015)	Uniserial, elongated and short papillae intercalated	Present	2(2)/3(1)	Equivalent to A-2 lateral segment	A2>A1	P1>P2	Short	Present	present	Absent
S. prasinus	Bokermann (1973); Araujo-Vieira *et al*. (2015)	Uniserial, elongated and short papillae intercalated	Present	2(2)/3(1)	Equivalent to A-2 lateral segment	A1>A2	P2>P1	Short	Present	Absent	Absent
S. surdus	Caramaschi (2010); Araujo-Vieira *et al*. (2015)	Uniserial, uniform sized	Present	2(2)/3(1)	Equivalent to three times the A-2 lateral segment	A2>A1	P1>P2	Long	Present	Absent	Median, dorsal, ventral

*Caramaschi (2010) stated that *S. palustris* presents A-2 gap size approximately equivalent to three times the A-2 lateral segment. Nevertheless the gap seems to be of the same length of the segment (see Nunes *et al*. 2007)

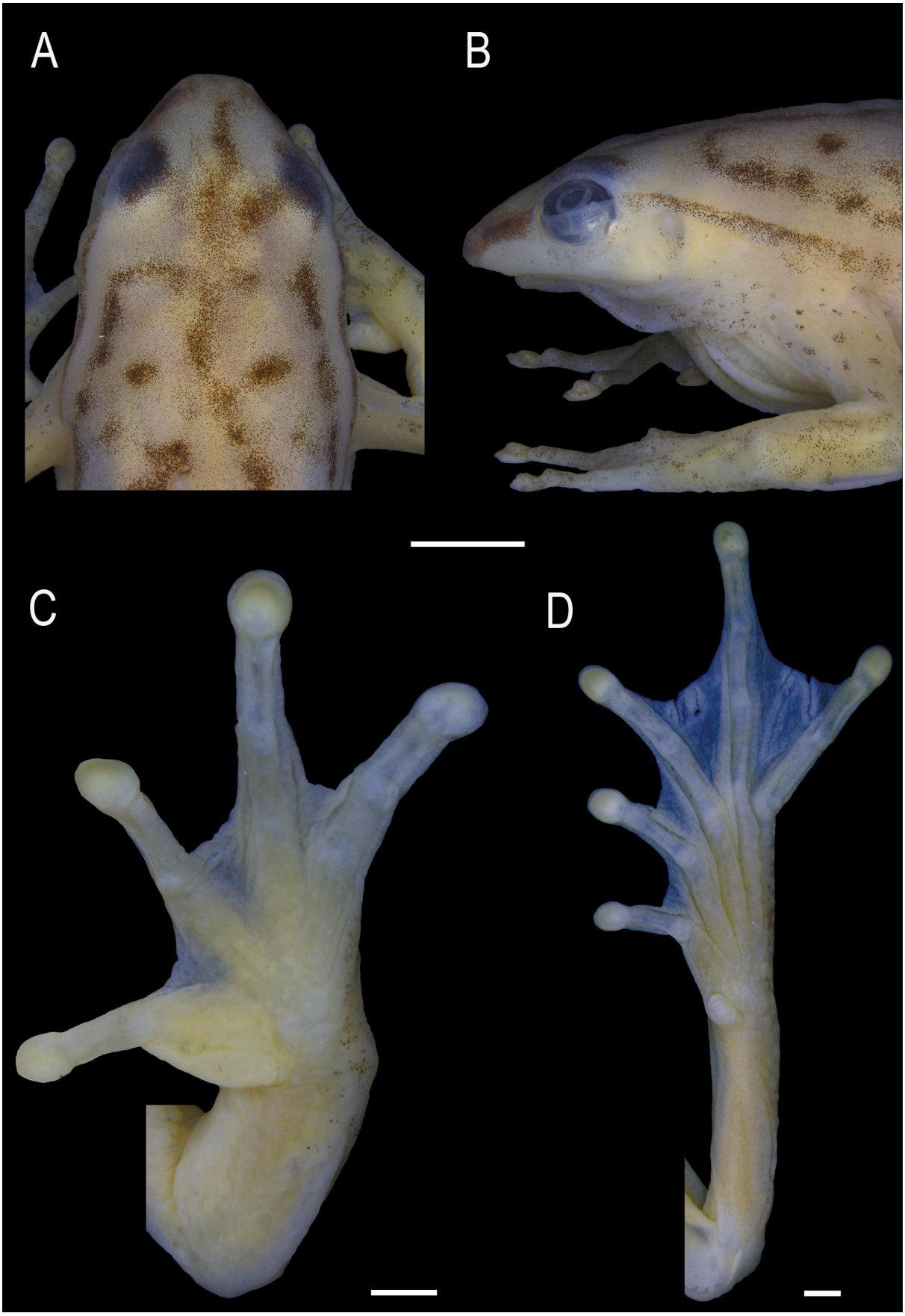

FIGURE 2. *Sphaenorhynchus canga* **sp. nov.**, holotype (UFMG-A 5715). (A) Head in dorsal and (B) lateral views. (C) Left hand and (D) foot in ventral views. Scale bars = 3 mm (upper) and 1 mm (lower).

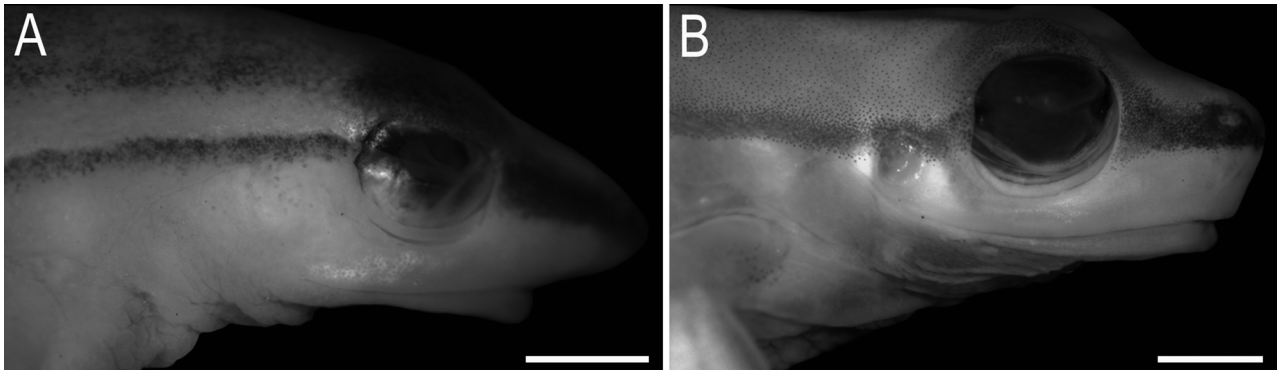

FIGURE 3. Head in lateral view of (A) *Sphaenorhycnhus canga* **sp. nov.** (MNRJ 56334) and (B) *S. pauloalvini* (MNRJ 4329) showing the absence and presence of tympanic membrane, respectively. Scale bars = 2 mm.

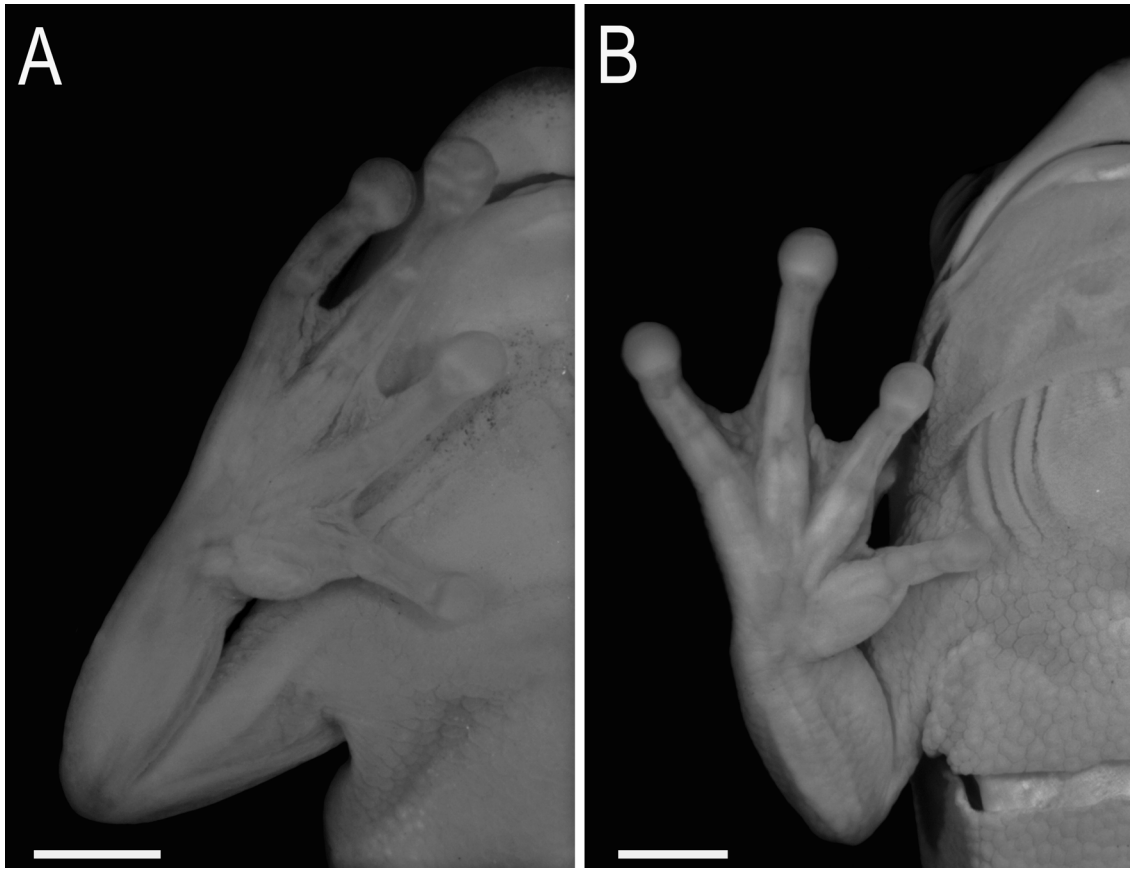

FIGURE 4. Ventral view of the right forearm of males of (A) *Sphaenorhynchus canga* **sp. nov.** (MNRJ 56334) and (B) *S. orophilus* (MNRJ 31735). Scale bars = 2 mm.

Coloration in preservative. The green becomes cream and the color pattern fades and loses its iridescent tones; the iris becomes black. The white of granules on the chest, belly, and ventral surface just below the cloaca almost completely disappear. The white canthal and dorsolateral lines disappear (Figs. 1–2). Dorsolateral black line maintained.

Measurements of the holotype (mm). SVL 27.0; HL 7.9; HW 7.4; IND 1.8; IOD 3.4; ED 2.3; END 2.6; HAL 8.8; THL 12.4; TL 12.3; FL 12.5; 3FD 0.9; 4TD 0.9.

Variation in the type series. The variation is related to males, since that we did not collect females. Snout shape varies between rounded, truncated and slightly mucronated in dorsal view. Ulnar folds are absent, when present are poorly developed, and/or slightly crenulated. Dermal ornamentations on the outer surface of tarsus vary from small tubercles arranged in line to a slightly crenulated dermal fold. The glandular subcloacal dermal fold

varies from discrete to evident. In most individuals, the dark line from the snout extends to the sacral region, in some individuals ends in the middle of the flanks. Dorsal pigmentation varies in intensity and coverage of the dark blotches, from totally uniform green to a strongly brownish blotched dorsum (Fig. 6B–D). Some individuals present a second dorsolateral dark line that is above the white line and extends from the posterior corner of eye to the groin (Fig. 6B). A white longitudinal blotch under the eye is present in most of individuals (Fig. 6B and D). Subgular region varies from finely and conspicuously dark spotted.

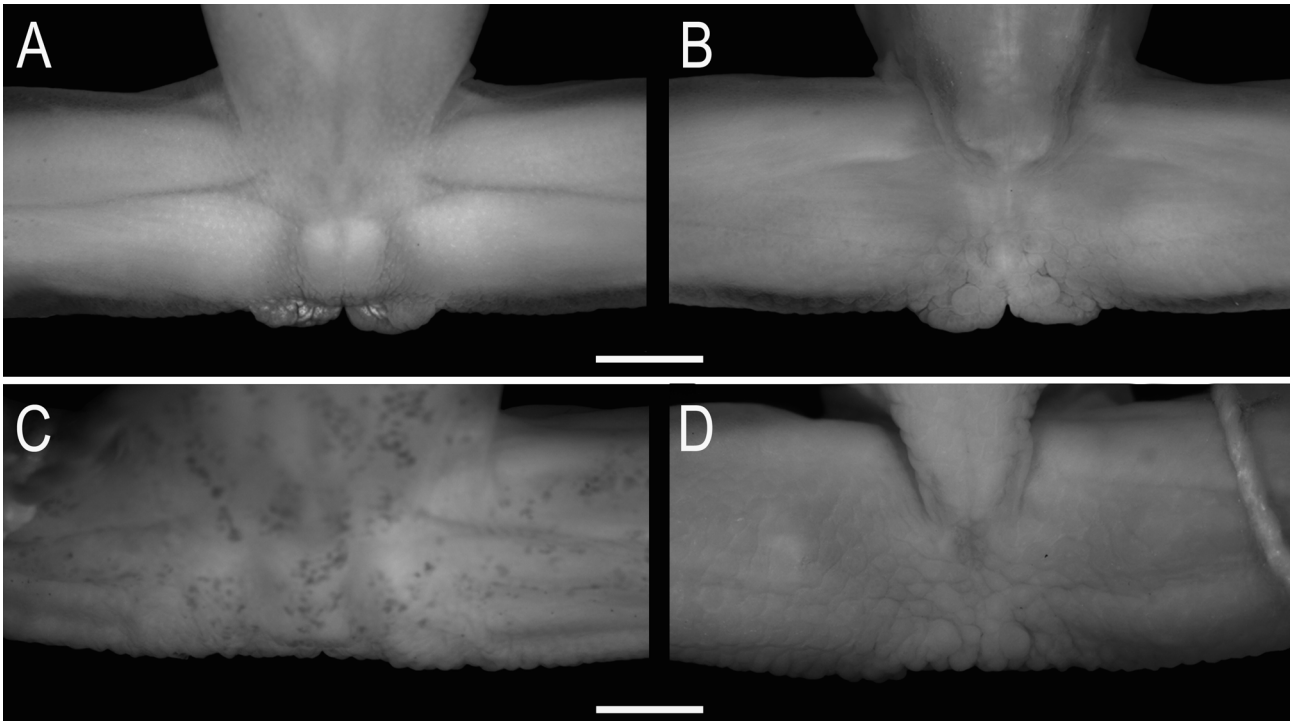

FIGURE 5. Subcloacal ornamentations of *Sphaenorhynchus canga* **sp. nov.**, male, MNRJ 56334 (A) dorsal and (B) ventral views; and *S. orophilus*, male, MNRJ 31735 (C) dorsal and (D) ventral views. Scale bars = 2 mm.

Measurements of the type series (*n* = 25, mean ± standard error, range into parenthesis). SVL 28.1 ± 1.1 (26.2–30.2); HL 7.9 ± 0.4 (6.8–8.5); HW 8.3 ± 0.5 (7.4–9.3); IND 1.9 ± 0.1 (1.7–2.2); IOD 3.6 ± 0.3 (3.0–4.3); ED 2.6 ± 0.2 (2.2–3.7); END 2.6 ± 0.2 (2.2–3.0); HAL 8.7 ± 0.4 (7.9–9.2; *n* = 20); THL 12.9 ± 1.0 (11.2–15.3); TL 13.2 ± 1.0 (10.4–15.2); FL 12.8 ± 0.5 (11.6–14.0; *n* = 21); 3FD 1.1 ± 0.1 (0.9–1.3; *n* = 24); 4TD 1.1 ± 0.1 (0.8–1.3).

Call. Air temperature during recordings: 24–25°C. A total of 119 calls emitted by six males were recorded. We recognized two different call types: type I and type II.

The call type I corresponds to the advertisement call [Fig. 7A–B; *n* = 6 males (UFMG-A 7192, 7194, 7205; 7207–7209); *n* = 116 calls], which consist of 1–9 pulsed notes (2.05 ± 1.43; *n* = 116 calls) with duration of 0.008–1.23 s (0.20 ± 0.25; *n* = 116 calls), the interval between calls is 2.74–20.19 s (8.47 ± 3.79; *n* = 107 intervals). Note duration is 0.005–0.020 s (0.010 ± 0.003; *n* = 237 notes) and the interval between notes is 0.05–0.23 s (0.16 ± 0.02; *n* = 119 intervals). The notes contain 1–5 pulses (2.5 ± 0.8; *n* = 190 notes), which with duration of 0.001–0.017 s (0.004 ± 0.002; *n* = 467 pulses); pulse rate of 58.8–444.4 pulses/s (247.0 ± 73.8; *n* = 190 notes). A total of 47 notes are composed by compacted pulses, for which is difficult to count the number of pulses per note. The dominant frequency is 2067.2–4550.0 Hz (2841.7 ± 537.9; *n* = 237 notes). A first note with smaller amplitude and dominant frequency of 1550.4–2584.0 Hz (2080.0±371; *n* = 14 notes) are sometimes at the beginning of the call (Fig. 7A).

The call type II is probably a territorial/aggressive call (Wells 2007), and apparently is given before the advertisement call. The call type II [Fig. 7C–D; *n* = 1 male (UFMG-A 7192); *n* = 3 calls] consists of 3–6 pulsed notes (4.33 ± 1.25; *n* = 3 calls) with duration of 0.66–1.02 s (0.83 ± 0.14; *n* = 3 calls); the interval between notes is 0.14–0.23 s (0.18 ± 0.03; *n* = 10 intervals). The call type II contains two different kinds of notes: the first note is longer (0.19–0.20 s; 0.19 ± 0.004; *n* = 3 notes) with 13 pulses, each with duration of 0.004–0.011 s (0.006 ± 0.001; *n* = 39 pulses); pulse rate of 64.7–68.1 pulses/s (65.9 ± 1.5; *n* = 3 notes), and interval between pulses of 0.002–0.037 s (0.009 ± 0.007; *n* = 37 intervals). The dominant frequency of the first note is 2584.0 Hz (*n* = 3 notes). The

following notes (from second to sixth notes) have durations of 0.005–0.009 s (0.008 ± 0.001; *n* = 10 notes) with 1–2 pulses (1.8 ± 0.4; *n* = 10 notes), each with duration of 0.003–0.006 s (0.004 ± 0.001; *n* = 16 pulses); pulse rate of 111.1–333.3 pulses/s (229.3 ± 60.5; *n* = 10 notes). The dominant frequency is 1722.7–2584.0 Hz (2446.2 ± 264.6; *n* =10 notes).

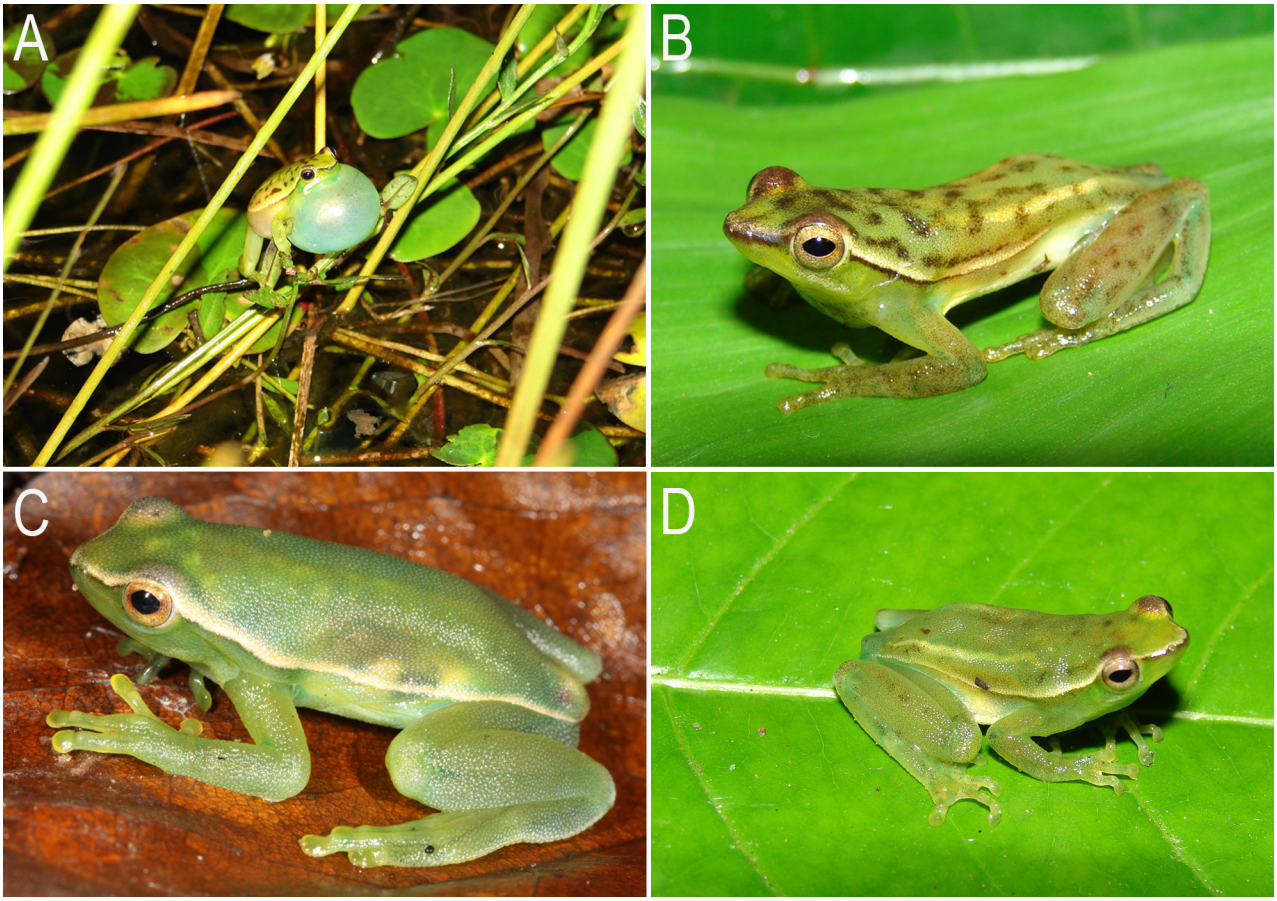

FIGURE 6. (A) Male of *Sphaenorhynchus canga* **sp. nov.** calling from the floating vegetation. (B), (C) and (D) Dorsal color pattern of *S. canga* **sp. nov.** in life.

Tadpole description. Body depressed (BH/BW = 0.73–0.97), ovoid, or elongated elliptical in dorsal view, triangular/depressed in lateral view, from 0.31 to 0.35 times the TL (Fig. 8A–C). Snout rounded in dorsal (BWN/BWE = 0.59–0.66), and lateral views. Eyes medium-sized (ED/BL = 0.18–0.24), laterally located (IOD/BWE = 1.00), visible in ventral view. Nostrils elliptical, medium-sized (ND/BL = 0.035–0.047) laterally located, anterolaterally directed, nearer the tip of snout than to eyes (NSD/ESD = 0.41–0.48); well-developed fleshy flanges on the marginal rim (valved nostrils *sensu* Cruz 1973; Araujo-Vieira *et al.* 2015). Spiracle sinistral, lateral (SDEH/BH = 0.48–0.68), posterodorsally directed, medium-sized (SL/BL = 0.25–0.29), opening on the end of posterior third of the body (SED/BL = 0.91–1.0); centripetal wall longer than the external wall. Intestinal tube circularly coiled, positioned at a right angle to the longitudinal body axis, with the switchback point dislocated from the center of abdominal region. Vent tube medial, posteroventraly directed, short, entirely fused to the ventral fin, positioned above the inferior margin of the ventral fin; ventral and dorsal walls of the same length. Oral disc small-sized (ODW/BW = 0.23–0.30, measured with oral disc folded), anteroventrally positioned, not emarginated; a single row of elongated and short intercalated marginal papillae (large papillae about twice larger than the small papillae) with a wide anterior gap; few (1–3) submarginal papillae randomly distributed laterally in the oral disc (Fig. 8D); LTRF 2(2)/3(1); A-2 with a wide gap (approximately equivalent to three times the lateral segment of A-2) and slightly larger than A-1, P-1 slightly larger than P-2, which is larger than P-3; jaw sheaths narrow, finely serrated on the margins, anterior jaw sheath M-shaped and posterior jaw sheath V-shaped, posterior jaw-sheath wider than anterior. Tail of intermediate height (MTH/TAL = 0.28–0.34) with a slightly robust musculature (TMH/BH = 0.49–0.60); dorsal fin higher than ventral fin, or of the same height (DFH/VFH = 0.84–1.00); tail tip acute.

Dorsal and ventral fins of intermediate height (DFH/TAL = 0.08–0.11; VFH/TAL = 0.07–0.09), with slightly convex free margins; dorsal fin emerging on the posterior third of the body at a median slope; maximum height at the middle third of the tail. Ventral fin origin concealed by the vent tube.

The lateral line is distinct in life and in preserved specimens. All the lines present transversally oriented elliptical whitish stitches. Supraorbital line with 17–20 stitches that converge anteriorly on the head medially to the nares and continues anteroventrally where it encounters a small irregular prenasal series of 3−4 stitches. Poorly developed infraorbital line (5–7 dispersed stitches) begins behind the eyes, extends slightly curved in the lateral profile of the snout. Oral and angular lines indistinct. Posterior infraorbital and supraorbital lines consisting of small aggregate series of four to five stitches, each. Two series of lines extend from the middle of the body throughout the tail: the dorsal line, medially located, consists anteriorly in a short row of 3−4 stitches, which after a gap, continues with 22–28 stitches, until half of tail length, in the dorsal fin base; the second, laterally located, consisting of 12–15 stitches joins the middle caudal series, which presents 13–15 stitches until the half of tail. The ventral body line is poorly defined, with 5–7 stitches anterodorsally to the spiracle.

Tadpole coloration. In life, general pattern greenish-brown (Fig. 9A–B). Body marbled with regularly-scattered dark spots and irregular dark brown spots; presence of dorsal, wide, light, longitudinal stripes from the eyes to the tail-body junction; spiracle light green; iris black with a wide golden ring around the pupil; ventral surface of the body marbled with golden large spots, including the vent tube. Tail musculature and fins (specially along its external margins) with large, round, dark and golden smudged spots; presence of continuous longitudinal dark stripes starting from the end of the body, until the final third of the tail: one on the medial muscle line, and the other two in the dorsal and ventral margins of the caudal musculature; fins equally pigmented; in life, a light blotch in the margin of the dorsal fin, in the region of its anterior origin.

In formalin 10%, general pattern light-brown. The body stripes become faded, remaining only lighter areas on the region anteriorly to the emergence of the dorsal fin. The abdomen and caudal coloration also fades and loses its golden spots.

Measurements (*n* = 6, mean ± standard error, range into parenthesis). TL 51.9 ± 1.9 (49.0–54.3); BL 17.0 ± 0.4 (16.2–17.3); TAL 34.9 ± 2.0 (31.7–37.0); MTH 10.6 ± 0.7 (9.8–11.6); TMH 5.8± 0.3 (5.8–6.1); BH 10.6 ± 0.3 (10.1–10.8); BW 12.0 ± 0.6 (10.9–12.5); BWN 5.5 ± 0.2 (5.3–5.8); BWE 9.1 ± 0.4 (8.5–9.7); ESD 6.3 ± 0.1 (6.1–6.6); ED 2.0 ± 0.1 (1.8–2.2); ODW 3.1 ± 0.3 (2.9–3.5); END 3.6 ± 0.2 (3.3–3.8); NSD 2.9 ± 0.1 (2.8–3.1); ND 0.8 ± 0.1 (0.6–0.9); TMW 5.8 ± 0.3 (5.3–6.1); IND 4.7 ± 0.5 (4.3–5.6); IOD 8.4 ± 0.3 (8.2–9.0); DFH 3.2 ± 0.5 (2.7–4.2); VFH 2.8 ± 0.3 (2.5–3.1); SED 16.4 ± 1.0 (15.1–17.5); SL 4.6 ± 0.3 (4.2–5.1); SDEH 6.0 ± 0.7 (5.1–6.9).

Variation. Tadpoles did not present large morphological variation. In four specimens, the intestinal tube is located more centrally in the abdominal region. Two specimens in stages 27 and 29 have a single row of aligned marginal papillae anteriorly, tending to be biserial laterally and posteriorly.

Natural History. *Sphaenorhynchus canga* **sp. nov.** occurs in permanent, semi-permanent, and less frequently in temporary natural ponds over ironstone outcrops (known as *canga*) on flat terrain (Fig. 9C). These ponds are surrounded by *campos rupestres* (rupestrian grasslands) vegetation, a mosaic of habitat types including exposed grasslands, rock surfaces, cerrado, gallery forests, and small isolated areas of arborescent vegetation. For a review of the ecology and evolution of plant diversity in *campo rupestres*, and a characterization of the vegetation and conservation of the *canga* see Silveira *et al*. (2015) and Jacobi *et al.* (2007; 2011), respectively.

Calling activity is concentrated in the hot and rainy season (October–March) although few males can occasionally be found calling in permanent ponds in the dry season (April–September). Males were found in relatively high densities during the beginning of rainy season (November–January). Males start calling at evening extending through the night. In rainy or foggy days, they can also call during daytime. Most males called from the floating vegetation (Fig. 6A), usually several species of grasses, sedges and *Nymphoides* sp. (Menyanthaceae), above the deepest area of the pond (ca. 1.5 m).

The reproductive mode is number 1 (*sensu* Haddad & Prado 2005): eggs and exotrophic tadpoles in lentic water. Tadpoles occur in lentic, temporary, or permanent water bodies, in open areas. They are nektonic and remain associated with macrophytes at deeper regions of the water bodies. Tadpoles in early development stages were observed in January, and froglets were seen in May. This suggests a developmental period of about 5 months. Tadpoles can be found until September. Tadpoles in earlier stages were easily found on the shallow margins of the pond whereas later stages were harder to find as they stayed on the on the middle of the water column at greater depths.

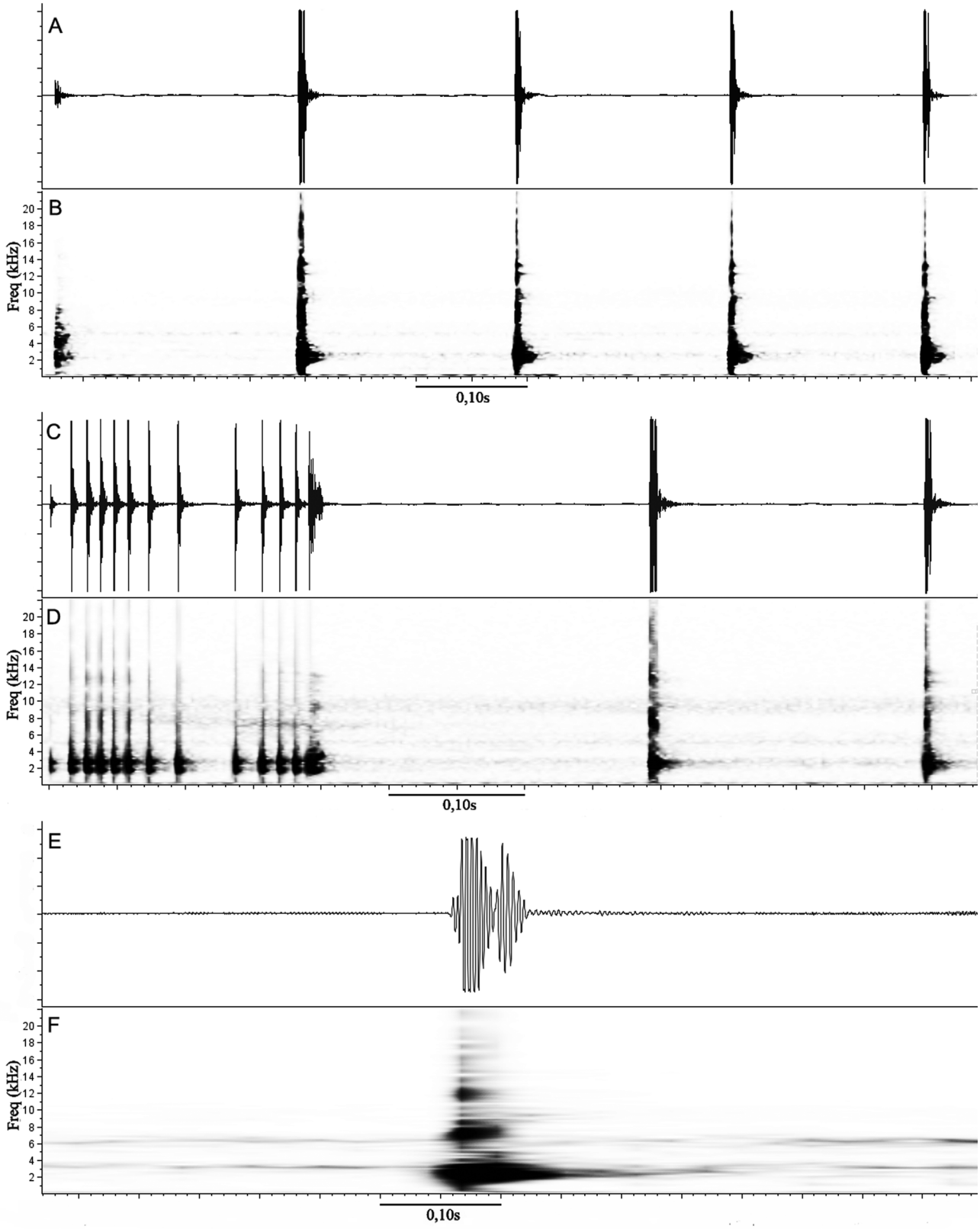

FIGURE 7. Calls of *Sphaenorhynchus canga* **sp. nov.** (A) Oscillogram and (B) spectrogram of the advertisement call (call type I; UFMG-A 7209). Notice a less common note with lower amplitude frequency at the beginning of the call. (C) Oscillogram and (D) spectrogram of the call with probably territorial/aggressive function (call type II; UFMG-A 7192). The call type II precedes the advertisement call. (E) Oscillogram and (F) spectrogram showing the pulse structure of the advertisement call note.

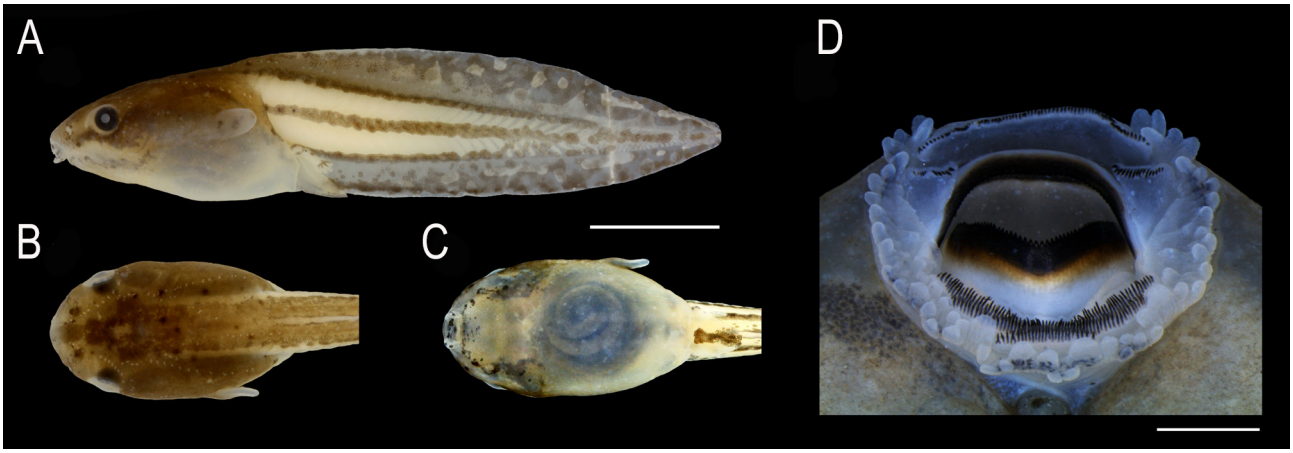

FIGURE 8. *Sphaenorhynchus canga* **sp. nov.**, tadpole (UFMG 950) at stage 36 according to Gosner (1960). (A) Lateral, dorsal (B), and (C) ventral views. (D) Oral disc. Scale bars = 10 mm (left) and 0.5 mm (right).

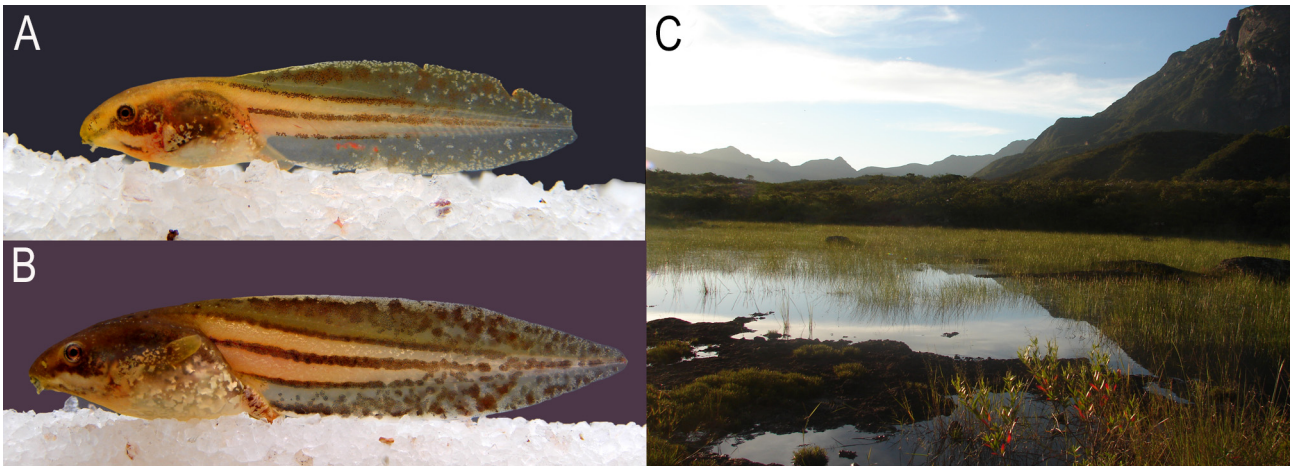

FIGURE 9. Tadpoles of *Sphaenorhynchus canga* **sp. nov.** in life. (A) UFMG 655, stage 26, TL 28.1 mm. (B) UFMG 655, stage 36, TL 52.0 mm. Notice the different spiracle length at the two stages. (C) Typical habitat of *S. canga* **sp. nov.**, at Chapada de Canga (type locality), Municipality of Mariana, State of Minas Gerais, southeastern Brazil.

FIGURE 10. Known geographic distribution of *Sphaenorhynchus canga* **sp. nov.**. The red triangle indicates the type locality (Chapada de Canga, 1.9 km W MG-129, 20°07'40" S, 43°23'05" W). **1:** 0.09 km N MG-129 (20º9'44" S, 43º25'58" W). **2:** 1.3 km NW MG-129. **3:** 1.5 km W MG-129 (20º7'32" S, 43º23'23" W). **4:** 5.2 km S MG-129 (20°12'26" S, 43°25'03" W). The red circle indicates the Serra da Mantiqueira's population of *S. orophilus* (Serra do Ibitipoca, close to Parque Estadual do Ibitipoca, State of Minas Gerais, Brazil).

Geographic distribution and conservation. The Espinhaço Range is characterized by high endemic anuran species richness (Leite *et al.* 2008; Carvalho *et al.* 2013), where usually anuran species are narrowly distributed restricted to single or few localities (e.g. Bokermann & Sazima 1973; Leite *et al*. 2011; Leite *et al*. 2012; Barata *et al.* 2013). *Sphaenorhynchus canga* **sp. nov.** is known from its type locality at Chapada de Canga, at the Municipality of Mariana, close to its limit with the Municipality of Catas Altas, southeastern portion of the Iron Quadrangle, southernmost portion of the Espinhaço Range, State of Minas Gerais, southeastern Brazil (Fig. 10), where it was observed in five ponds (type locality—20°07'40" S, 43°23'05" W; 1.5 km W MG-129—20º7'32" S, 43º23'23" W; 1.3 km NW MG-129—20º9'48" S, 43º24'27" W; 0.09 km N MG-129—20º9'44" S, 43º25'58" W; and 5.2 km S MG-129—20°12'26" S, 43°25'03" W). The species extent of occurrence measured by a minimum convex polygon (EO, *sensu* IUCN 2001) has only 17.2 km^2, whereas considering the entire local *canga* environment it would be ca. 50 km^2, the new species does not occur within reserves. A previous anuran inventory (i.e. Canelas & Bertolucci 2007) did not record the species on the Reserva Particular do Patrimônio (RPPN) Natural Santuário do Caraça, Serra do Caraça, Municipality of Catas Altas, a relatively well sampled locality situated approximately 13 km (straight line) from Chapada de Canga. The Iron Quadrangle is the best sampled region in the State of Minas Gerais due to its proximity to the Municipality of Belo Horizonte (the state capital, where the two most important and representatives anuran collections are located, i.e. UFMG and MCNAM) and research centers. Moreover, in the last ten years the region has become target of many inventories for environmental licensing purposes as the *cangas* are in the spotlight of most mining companies.

About 40% of the ironstone outcrops *canga* were destroyed in less than 40 years, remaining around 11.172 ha of these iron rich formations in the state of Minas Gerais, less than 280 ha (2.5%) of which are included in reserves (Carmo 2010). Natural ponds on *canga* are rare and unique fresh water habitats within the Iron Quadrangle. We mapped only nine other ponds with characteristics similar to the ponds where the new species is found, all located at Chapada de Canga, reinforcing its rarity and relevance for conservation of these threatened environment.

Etymology. The specific epithet is an allusion to the occurrence of this species in natural ponds located over ironstone outcrops, known as *canga*.

Remarks. Males of *Sphaenorhynchus canga* **sp. nov.** distinguish from those of *S. orophilus* by the robustness of the forearm (more robust forearm in *S. orophilus*). This character is related to the degree of development of the *m. flexor carpi radialis* in terms of volume and width of its origin (Faivovich 2002). This muscle shows various degrees of development in some breeding males of *Scinax*, from a rectangular, almost flat, strip to a broad-based, triangular, and thick, bulbous belly (Faivovich 2002). Although the taxonomic distribution of this character in *Sphaenorhynchus* is unknown, the difference between *S. canga* **sp. nov.** (slender forearm) and *S. orophilus* (more robust forearm) is sufficient to differ them (see Fig. 4). Similar character states have been used by Faivovich (2005) to differentiate males of *Scinax aromothyella* from those of *S. berthae.*

The acoustic parameters of the advertisement call of the new species (call duration of 0.008–1.23 s; 1–9 notes, note duration of 0.005–0.020 s, interval between notes of 0.05–0.23 s, and dominant frequency of 2067.2–4550.0 Hz) are similar to those of *Sphaenorhynchus orophilus* (call duration of 0.30–2.33, 2–12 notes, note duration of 0.01–0.03 s, interval between notes of 0.16–0.27 s dominant frequency of 1609–2700 Hz; Heyer *et al.* 1990; Toledo *et al.* 2014). Nevertheless, the call type II (territorial/aggressive call) of *S. canga* **sp. nov.** is different from that of *S. orophilus* (call duration of 0.25 s and 20–25 notes or pulses; Heyer *et al.* 1990) by its longer duration and lower number of notes and pulses (call duration of 0.66–1.02 s, 3–6 notes, with 1–13 pulses). This kind of call was also reported for *S. palustris* (call duration of 0.47–0.83 s, 4–6 notes, interval between notes of 0.048–0.17 s, and two kinds of notes; see Lacerda & Moura 2013), and it is similar to the pattern described for the new species (call duration of 0.66–1.02 s, 3–6 notes, interval between notes of 0.14–0.23 s, and two kinds of notes).

Sphaenorhynchus orophilus is known from Serra do Mar in Rio de Janeiro and São Paulo States, and Serra da Mantiqueira in Minas Gerais State, southeastern Brazil (Heyer *et al.* 1990; Carvalho-e-Silva & Caramaschi 2004; Cruz *et al*. 2009; Frost 2015). The records from Serra da Mantiqueira are about only 180 km SSW from the southernmost locality where *S. canga* **sp. nov.** occurs, at Serra do Ibitipoca (close to Parque Estadual do Ibitipoca; Cruz *et al.* 2009; Fig. 10). Unfortunately, we were unable to examine collected material from this population. However, due to the overall similarity between *S. orophilus* and *S. canga* **sp. nov.**, and considering that the type locality of *S. orophilus* is in Serra do Mar (a distinct mountain range; Lutz & Lutz 1938), the taxonomic status of Serra da Mantiqueira's population should be revised.

Larvae of *Sphaenorhynchus canga* **sp. nov.** corroborate Faivovich *et al.* (2005) and Araujo-Vieira's *et al.*

(2015) suggestions that the nostrils with fleshy flanges and anteriorly directed are putative synapomorphies of *Sphaenorhynchus*. The new species also share enlarged marginal papillae on the oral disc with *S. caramaschii*, *S. palustris*, *S. planicola*, and *S. prasinus* (Araujo-Vieira *et al.* 2015). Such character state might be a synapomorphy of less inclusive clade within *Sphaenorhynchus*, as first observed by Cruz & Peixoto (1980) and suggested by Faivovich *et al.* (2005) and Araujo-Vieira *et al.* (2015).

Araujo-Vieira *et al.* (2015) have also defined three character states for the spiracle length (SL) in relation to the body total length (BL) of larvae in *Sphaenorhynchus*: short spiracle, SL 3−11% of BL (*S. caramaschii*, *S. pauloalvini*, *S. planicola*, and *S. prasinus*); medium-sized spiracle, SL 16–24% of BL (*S. bromelicola* and *S. orophilus*), and elongate spiracle, SL reaching at least 28% of BL (*S. palustris*).We observed that larvae of *S. canga* **sp. nov.** show a spiracle with 25–29% of BL, this measure breaks that character state delimitation and place the new species between the species that have medium and elongate spiracles. Although it was not possible to delimit the character states, we tentatively consider that larvae of *S. canga* **sp. nov.** share a medium-sized spiracle with those of *S. bromelicola*, *S. orophilus* and *S. surdus*, being the mean length of its spiracle more similar to that of *S. bromelicola* (see Figs. 5C–D in Araujo-Vieira *et al*. 2015) until those character states be redefined.

Acknowledgments

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APPENDIX 1. Adult specimens examined.

Collection abbreviations are as follows: **CFBH**, Célio F. B. Haddad Amphibian collection, Rio Claro, São Paulo, Brazil; **URCA-H**, Coleção de Herpetologia da Universidade Regional do Cariri, Crato, Ceará, Brazil; **DZSJRP**, Departamento de Zoologia e Botânica, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto, São Paulo, Brazil; **MACN**, Museo Argentino de Ciencias Naturales "Bernadino Rivadavia", Buenos Aires, Argentina; **MCP**, Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil; **MHNCI**, Museu de História Natural Capão da Imbuia, Curitiba, Paraná, Brazil; **MNRJ**, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; **MZUESC**, Museu de Zoologia da Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil; **MZUFBA**, Museu de Zoologia, Universidade Federal da Bahia, Salvador, Brazil; **MZUFV**, Museu de Zoologia João Moojen de Oliveira, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil; **MZUSP**, Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; **UFMG**, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; **UFRGS**, Universidade Federal do Rio Grande do Sul, Departamento de Zoologia, Porto Alegre, Rio Grande do Sul, Brazil; **ZUFG**, Coleção Zoológica da Universidade Federal de Goiás, Goiânia, Brazil; **ZUEC**, Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso", Campinas, São Paulo, Brazil.

***Sphaenorhynchus botocudo*: BRAZIL: Espírito Santo**, Mucurici, Fazenda Matutina, MACN 46458–46459; MNRJ 50625–50626, 50629–50631, 50635–50636, 50639 (paratypes). ***Sphaenorhynchus bromelicola*: BRAZIL: Bahia**, Maracás, Fazenda Canabrava, MNRJ 4289–4290, 4292. Maracás, MZUSP 99478, 99481–99483, 99485–99486, 99488, 99490, 99493, 99497–99499, 101508–101509, 101514–101515, 126109; ZUEC 2789. ***Sphaenorhynchus caramaschii*: BRAZIL: São Paulo**, Ribeirão Branco, Fazenda São Luiz, CFBH 2285–2294, 6933–6937 (paratypes), MNRJ 19373–19377. Iporanga, PETAR-Núcleo Ouro Grosso, CFBH 6320–6323. Ribeirão Grande, CFBH 15581, 15583. Pilar do Sul, CFBH 8289. **Santa**

Catarina, Treviso, CFBH 9854, 10325. MZUSP 84589, 134045, 134047. ***Sphaenorhynchus carneus*: BRAZIL: Acre**, Cruzeiro do Sul, Fazenda São Geraldo, ZUEC 3527. Cruzeiro do Sul, Porto Walter, ZUEC 8429, 8431. Tarauacá, Flooded places near the church, ZUEC 5555. **Amazonas**, Capim Flutuante-Rio Solimões, CFBH 4984–4985. Seringal América-Rio Purus MZUSP 50408, 504010. Lago Pacatuba, MZUSP 53710, 53712, 53714–53715. Lago Amaná, MZUSP 58469, 58471–58472, 58474. Tabatinga, MZUSP 111240. **COLÔMBIA**: **Caquetá**, Alingaros, MZUSP 99431–99432, 99436, 99440, 99446, 99448, 99453, 99456, 99458–99459, 99461–99462, 99464–99466, 99468–99469, 99471, 99472. ***Sphaenorhynchus dorisae*: BRAZIL: Acre**, Cruzeiro do Sul, Porto Walter, ZUEC 8426–8427. Rio Tejo, ZUEC 11091, 11095–11098, 11100, 11103, 11106. Cruzeiro do Sul, MCP 10591–10595. Rodrigues Alves, Igarapé Croa-Alto do Juruá, CFBH 15721, 15723. **Amazonas**, Rio Solimões, Igarapé Belém, MZUSP 34669, 34677, 34676, 34680, 34672, 34674. Boca do Paraná do Catito, MZUSP 33190. Beruri, MZUSP 50552. Seringal América, Rio Purus, MZUSP 50413, 50415. Lago Januari, MZUSP 53723, 53720. **PERU: Loreto**, Estirón, Rio Ampiyacu, MZUSP 32808, 32810. ***Sphaenorhynchus lacteus*: BRAZIL: Acre**, Cruzeiro do Sul, Vila Militar, ZUEC 4689, Humaitá do Moa, ZUEC 5429. Cruzeiro do Sul, MCP 10570–10590. Rio Branco, Sítio Engenheiro Ramon, ZUEC 5590. Rio Branco, Parque Zoobotânico UFAC, ZUEC 5570. Xapuri, route to Vila Boa Vista, ZUEC 5705. Mâncio Lima, Lagoa da Cobra, ZUEC 5853. Tarauacá, MZUSP 99340, 99335, 99339, 99337. **Amazonas**, Manaus, URCA-H 3495–3499, Manaus, Lago do Castanho-Rodovia Manaus, ZUEC 3929, 7041. Humaitá, MNRJ 4284–4287. Lago Amaná, MZUSP 58500, 58494, 58497, 58490, 58491. Igarapé Belém, Rio Solimões, MZUSP 32846, 32841, 32845, 32814, 32817, 32835, 32837, 32821, Puruzinho, Rio Madeira, MZUSP 51487, 51493, 51492. Boca do Acre, MZUSP 50310, 50311. São José (Jacaré), Rio Solimões, MZUSP 40367, 40365. Açaituba, Rio Purus, MZUSP 50465. Lago Januari, MZUSP 53726, 53730. Reserva Ducke, MZUSP 75715. Beruri, MZUSP 50581. Borba, MZUSP 51196. Tabatinga, MZUSP 11238. **Pará**, Surinam, MZUSP 84625. Tucuruí, MZUSP 76464. Belém, MZUSP 1505, 1507. Oriximiná, MZUSP 22526. Tapirapé Biological Reserve, MZUSP 140061, 140064, 140068, 140065. Belém, Pasto de Búfalos, EMBRAPA, MNRJ 4288. **Rondônia**, Príncipe da Beira, MZUSP 99426. Porto Velho, MZUSP 99367, 99376, 99375, 99373, 99374, 99370, 99348, 99366, 99354, 99356, 99357, 99347. ZUEC 2707. **Maranhão**, Anajatuba, MNRJ 18270–18275, 18277–18279. São Luiz Gonzaga do Maranhão, MNRJ 36635. **COLÔMBIA: Caquetá**, Los Alicangaros, MZUSP 99409, 99411, 99421, 99419, 99425, 99414**. Isla Santa Sofia**, Amazonas, MZUSP 39168, 39170, 39169, 39172, 39175, 39174. **PERU: Loreto**, Estirón, Rio Ampiyacu, MZUSP 32801, 32802, 32804–32807. ***Sphaenorhynchus mirim*: BRAZIL: Espírito Santo**, Mucurici, Fazenda Matutina, MACN 46460–46462; MNRJ 50648–50650, 50652–50653 (paratypes). ***Sphaenorhynchus orophilus*: BRAZIL: Rio de Janeiro**, Petrópolis, MZUSP 680, 681. Teresópolis, Alto do Soberbo, MZUSP 53464, 53465. Teresópolis, Represa do Guinle, MNRJ 31731, 31732, 31734, 31735, 31737, ZUEC 4096. Rio de Janeiro, MNRJ126. **São Paulo**, Bairro Alto, CFBH 10573. Boráceia, MZUSP 60228–60230, 37668. ***Sphaenorhynchus palustris*: BRAZIL: Bahia**, Porto Seguro, RPPN Estação Vera Céu, MZUSP 127834, 127831, 127835. MNRJ 42649–42657. **Espírito Santo:** Conceição da Barra, Vila de Itaúnas, MNRJ 54979, 54980. Conceição da Barra, Rio Preto National Forest, MNRJ 54981–54983. ***Sphaenorhynchus pauloalvini*: BRAZIL: Bahia**: Una, MZUFBA 7621. **Espírito Santo**, Linhares, MZUSP 101500. Linhares, Estação Experimental Linhares, MNRJ 4303–4306, 4308, 4310, 4312, 4314, 4316, 4318, 4320–4329. ***Sphaenorhynchus planicola*: BRAZIL: Espírito Santo**, Fundão, CFBH 1586. Linhares, CFBH 1575, MNRJ 4331, 4332. Serra, CFBH 1439, 1440. São Mateus, MNRJ 18417, 18418. Marataízes, Distrito de Gomes, Fazenda Sr. Roberto Da Roseira, Marsh near Guarnanoi lake, MNRJ 35025–35027. Anchieta, MNRJ 25335. **Rio de Janeiro**, Magé, Campos dos Escoteiros, Citrolândia, MNRJ 54803–54811. Magé, Vila das Pedrinhas, MNRJ 36265, 4361, 4364. São João da Barra, MNRJ 6716, 6718–6725, 6728. Maricá, MNRJ 39704. Barra da Tijuca, MNRJ 26880. Sernambetiba, Recreio dos Bandeirantes, MNRJ 3520. Recreio dos Bandeirantes, MNRJ 2084. Campos, Fazenda Barra Seca, MNRJ 41573–41583. Campos dos Goytacazes, Lagoas de Cima, Marsh near the lake, MNRJ 54353–54359. Itaguaí, Old route Rio-São Paulo, Km 39, ZUEC 3808. Macaé, Lake near the city access, ZUEC 8572. **Minas Gerais**, Iperó, Fazenda Ipanema, MNRJ 32824–32827. **Bahia**, Trancoso, MNRJ 47811, 47812. Between Barras de Caravelas and Ponta de Areia, MNRJ 4366–4368, 4370, 4372–4374, 4377, 4378. ***Sphaenorhynchus prasinus*: BRAZIL: Espírito Santo**, Linhares, MZUSP 75641, 75643. **Minas Gerais**, Teófilo Otoni, MZUSP 99512, 99513. Almenara, Fazenda Limoeiro, MZUFV, 4152, 5939, 5938. Marliéria, Rio Doce State Park, MZUFV, 2631, 2633, MNRJ 20874. Aimorés, MNRJ 56347. Nanuque, MNRJ 4517. **Pernambuco**, Recife, Dois Irmãos, MZUSP 99503. **Bahia**, Ilhéus, MZUESC 6533, 6534, 6861, 6862, 6863. Mata de São João, MZUFBA 7357, 4344–4346, 2962, 2969–2973. Itagibá, Fazenda Pedra Branca, MNRJ 4295–4297, 56348, 56349. Teixeira de Freitas, Fazenda Alcopadro, MNRJ 29664–29668. **Alagoas**, Rio largo, MNRJ 38680–38683. Quebrangulo, URCA–H 9295. ***Sphaenorhynchus surdus***: **BRAZIL: Santa Catarina**, Rio Vermelho, MZUSP 99510. São Bento do Sul, MZUSP 99508. Perto de São Bento, Rio Vermelho, MNRJ 4402–4404, 4406, 4407, 4410, 4412, 4415. Campo Belo do Sul, UFGRS 2787, 2895–2897, 2899, 2901, 2911, 3089, 3137, MCP 8422. Ponte Serrada, CFBH 15752. Lages, CFBH 8546. Lontras, MCP 1300–1305. Lébon Régis, MCP 8811. Campos Novos, MCP 9324. **Rio Grande do Sul**, Vacaria, UFRGS 2488–2491, 2507, 2788. Bom Jesus, UFRGS 2797, 2893, 2894, 2898, 2900, 2902–2910, 3075, 3076, 3082, 3100, 3102–3104, 3108, 3109, 3112, 3121, 3135, 3136, 3138, 3139, 3145. São José dos Ausentes, MCP 4618–4622. **São Paulo**, Conchas, MZUSP 99521, MNRJ 4333. Apiaí, MZUSP 101466. Guapiara, MNRJ 4335. Fazenda Iperó, MNRJ 18249. **Paraná**, Castro, Caxambú Forest Park, MHNCI 199, 221, 315–317, 319. São José dos Pinhais, Cambuí Forest Reserve, MHNCI 852. Quatro Barras, Estrada Graciosa. Corvo, MHNCI 1738–1747. Quatro Barras, Pinheiros Gralha Azul, Chácara São Francisco de Assis, MHNCI 3657, 3658. Quatro Barras, Estrada Graciosa, Alto da Serra, Rio Taquari, MNRJ 4744–4747, 4751, 5750. Piraquara, Mananciais da Serra, MHNCI 1855, 2858, 2947, 2973, 2983, 5402, 5403. Piraquara, MCP 8324, 8325. Campina Grande do Sul, Cedro, MHNCI 4603–4607. Telêmeco-Borba, Ribeirão Anta Brava, MHNCI 4896. Telêmaco-Borba, Taboal da Vila Preta, MHNCI 4965. Telêmaco–Borba, Lagoa do Gaúcho, MHNCI 4979. Adrianópolis, Rocha Church´s Dike, MHNCI 5401. Tijucas do Sul, DZSJR 8788, 8789, 9049, 8656. Piraí do Sul, CFBH 8223.

APPENDIX 2. Larvae specimens examined.

***Sphaenorhynchus bromelicola*: BRAZIL: Bahia**, Maracás, Fazenda Canabrava, MZUSP 79559. ***Sphaenorhynchus orophilus*: BRAZIL: Between São Paulo and Rio de Janeiro**, Serra da Bocaina, Bonito, ALMN 4324. **Santa Catarina**, between Porto União and Concórdia, Rio Roseira, MZUSP 57915. ***Sphaenorhynchus palustris*: BRAZIL: Bahia**, Porto Seguro, Estação Vera Cruz, RPPN, MNRJ 42616. ***Sphaenorhynchus pauloalvini*: BRAZIL: Bahia**, Ilhéus, MZUSP 79580, 79568. ***Sphaenorhynchus planicola*: BRAZIL: Rio de Janeiro**, Itaguaí, MZUSP 79552. ***Sphaenorhynchus prasinus*: BRAZIL: Bahia**, Ilhéus, MZUSP 79571, 79553.

APPENDIX 3. Cleared and double stained specimens examined.

Sphaenorhynchus botocudo*: BRAZIL: Espírito Santo**, Mucurici, Fazenda Matutina, MACN 46459 (female). ***Sphaenorhynchus bromelicola*: BRAZIL: Bahia**, Maracás, Fazenda Canabrava, MZUSP 101518 (male). ***Sphaenorhynchus caramaschii: **BRAZIL: São Paulo**, Ribeirão Branco, Fazenda São Luiz, CFBH 6937 (male; paratype), CFBH 6933 (female; paratype). ***Sphaenorhynchus carneus*: BRAZIL: Acre**, Tarauacá, Flooded places near the church, ZUEC 5555 (male). ***Sphaenorhynchus dorisae*: BRAZIL, Acre**, Cruzeiro do Sul, Rio Tejo, ZUEC 11096 (female). ***Sphaenorhynchus lacteus*: BRAZIL: Acre**, Cruzeiro do Sul, Humaitá do Moa, ZUEC 5429 (male). ***Sphaenorhynchus mirim***: **BRAZIL: Espírito Santo**, Mucurici, Fazenda Matutina, MACN 46462 (female). ***Sphaenorhynchus orophilus***: **BRAZIL: Rio de Janeiro**, Teresópolis, Alto do Soberbo, MZUSP 53465 (male). Teresópolis, Represa do Guinle, MNRJ 31731 (male). ***Sphaenorhynchus palustris*: BRAZIL: Espírito Santo**, Conceição da Barra, Floresta Nacional de Rio Preto, MNRJ 54982 (male). ***Sphaenorhynchus pauloalvini*: BRAZIL: Espírito Santo**, Linhares, MNRJ4323 (female). ***Sphaenorhynchus planicola*: BRAZIL: Rio de Janeiro**, São João da Barra, MNRJ 6728 (female); Magé, Campo dos Escoteiros, Citrolândia, MNRJ 54808 (male). ***Sphaenorhynchus prasinus*: BRAZIL: Bahia**, Ilhéus, Ponta da Tulha, MZUESC 6861 (female). ***Sphaenorhynchus surdus*: BRAZIL: Santa Catarina**, near São Bento, Rio Vermelho, MNRJ 4412 (male). Lontras, MCP 1302(male). **Paraná**, Piraquara, MCP 8324 (male).