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## A new diploid species belonging to the *Odontophrynus americanus* species group (Anura: Odontophrynidae) from the Espinhaço range, Brazil

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### Abstract

The *Odontophrynus americanus* species group is a complex of diploid and tetraploid species hardly distinguished by morphological characters. It currently consists of three allopatric diploid species (i.e. *O. cordobae*, *O. lavillai*, and *O. maisuma*) and one widely distributed tetraploid species (i.e. *O. americanus*). We herein describe a new diploid allopatric species from *campo rupestre*, a typical phytophysognomy of the Espinhaço Range, Brazil. The new species is distinguishable by the diploid complement of  $2n = 2x = 22$  chromosomes, small to medium-sized dorsal dark brown blotches with low contrast on a light brown background, light mid-dorsal stripe absent or greatly interrupted in most specimens with yellowish coloration as the background of both head and flanks of the body, advertisement call with dominant frequency of 840–1080 Hz, pulse rate of 90.5–106.7 pulses/s, and small tadpoles (TL = 24.30–35.69 mm).

### Resumo

O grupo *Odontophrynus americanus* é um complexo que inclui espécies diplóides e tetraplóides dificilmente distinguíveis por caracteres morfológicos. O grupo atualmente consiste em três espécies diplóides em alopatría (i.e. *O. cordobae*, *O. lavillai* e *O. maisuma*) e uma tetraploide amplamente distribuída (i.e. *O. americanus*). Neste estudo, descrevemos uma nova espécie diplóide em alopatría oriunda do campo rupestre, uma fitofisionomia típica da Serra do Espinhaço, Brasil. A nova espécie é distinguível pelo componente diplóide de  $2n = 2x = 22$  cromossomos, manchas marrom escuras de tamanho pequeno a médio com baixo contraste em relação à coloração marrom clara do dorso, listra clara ausente ou muito interrompida na região médio-dorsal da maioria dos espécimes, com coloração amarelada assim como a cabeça e os flancos do corpo, canto de anúncio com frequência dominante de 840–1080 Hz, taxa de pulsos de 90.5–106.7 pulsos/s, e girinos pequenos (TL = 24.30–35.69 mm).

**Key words:** taxonomy, morphology, karyotype, vocalization, tadpole, endemism, Serra do Cipó, *campo rupestre* (rupesian grassland)

## Introduction

At present, the genus *Odontophrynus* Reinhardt and Lütken, 1862 includes 11 species of stout, warty fossorial frogs widely distributed in eastern and southern South America (Frost 2016). This genus was previously allocated in the tribe Odontophrynini (Family Leptodactylidae) along with the genera *Proceratophrys* Miranda-Ribeiro, 1920 and *Macrogenioglottus* Carvalho, 1946 (as a junior synonym of *Odontophrynus*) by Lynch (1971). Currently, these three genera are placed in a separate family, Odontophrynidae Lynch, 1971 according to Pyron & Wiens (2011).

Although a comprehensive phylogeny is lacking for the genus *Odontophrynus*, some morphological characters support its monophyly (Blotto *et al.* 2017), and three phenetic species groups are recognized: the *O. americanus*, *O. cultripes*, and *O. occidentalis* species groups. In addition, *O. salvatori* Caramaschi, 1996 is currently not associated to any group (Savage & Ceï 1965; Ceï 1987; Rosset 2008; Caramaschi & Napoli 2012). The *O. americanus* species group has been the focus of several cytogenetic studies (Beçak *et al.* 1966; Beçak & Beçak 1974; Barrio & Pistol de Rubel 1972; Ruiz *et al.* 1981; Rosset *et al.* 2006; and literature cited therein). It consists of a complex of cryptic diploid and tetraploid frogs that includes: *O. americanus* (Duméril and Bibron, 1841), a tetraploid species widely distributed in Argentina, Bolivia, Brazil, eastern Paraguay, and Uruguay; *O. lavillai* Ceï, 1985, a Chacoan diploid species found in Argentina, Bolivia, Paraguay, and Mato Grosso do Sul, Brazil; *O. cordobae* Martino and Sinsch, 2002, a diploid species of the central region of Argentina; and *O. maisuma* Rosset, 2008, a diploid species found in the coastal dunes of Brazil and Uruguay (Ceï 1985; Martino & Sinsch 2002; Rosset *et al.* 2006; Rosset 2008; Borteiro *et al.* 2010; Rosset & Baldo 2014; and literature cited therein).

This study describes a new diploid species from *campo rupestre* (rupestrian grassland), a typical phytophysognomy of the Espinhaço Range, Brazil (Magalhães 1966; Olson *et al.* 2001; Alves *et al.* 2014; Silveira *et al.* 2015), belonging to the *Odontophrynus americanus* species group (previously reported as *Odontophrynus* sp. by Rosset *et al.* 2006), based on its morphology, karyotype, vocalization, and tadpoles.

## Materials and methods

**Sampling.** Adults were collected by hand and euthanized via anesthetic overdose (5% lidocaine). Specimens were then fixed in 10% formalin, preserved in 70% ethyl alcohol and housed at the Amphibian Collection of Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais State, Brazil. Adult males were identified by the presence of a pigmented vocal sac and clearly developed nuptial pads. Sex of juveniles was determined by the presence of vocal slits in males.

Comparisons of adult specimens were based on observations of museum material. Specimens used for comparison (listed in Appendix) are deposited at Célio F.B. Haddad Collection, Universidade Estadual Paulista Júlio Mesquita Filho, Rio Claro, São Paulo, Brazil (CFBH); Museu Nacional do Rio do Janeiro, Rio do Janeiro, Brazil (MNRJ); Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (MCP); Fundación Miguel Lillo, Instituto de Herpetología, Tucumán, Argentina (FML); Instituto de Biología Animal, Mendoza, Argentina (IBA); Instituto y Museo de Ciencias Naturales, Universidad Nacional de San Juan, San Juan, Argentina (IMCN-UNSJ); Laboratorio de Genética Evolutiva, Posadas, Misiones, Argentina (LGE); Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina (MACN); Centro Nacional de Investigaciones Iológicas, Buenos Aires, Argentina (CENAI, housed at MACN); Museo de Historia Natural de Asunción, Asunción, Paraguay (MHNP); Museo de Historia Natural de San Rafael, Mendoza, Argentina (MHNSR); Museo de La Plata, La Plata, Buenos Aires, Argentina (MLP); Universidad Nacional del Nordeste, Corrientes, Argentina (UNNEC); Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK, holotype of *Odontophrynus cordobae*, housed at MLP); Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay (ZVCB).

**Morphology.** Measurements of adult specimens were taken with digital calipers to the nearest 0.1 mm. We measured the snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), internarial distance (IND), eye-nostril distance (END), tibia length (TL), and foot length (FL) in agreement with Duellman (2001); arm length (AL) and thigh length (THL) follow Heyer *et al.* (1990); distance between the anterior margins of eyes

(AMD) follow Garcia *et al.* (2003); and inner metatarsal tubercle length (MTL) in agreement with Rosset (2008). Gland terminology (i.e. postorbital, temporal, and parotoid) is based on Savage & Cei (1965) but we prefer named glandular warts instead of gland. Fingers are numbered as I–IV following Heyer *et al.* (1990) and webbing formula follows Savage & Heyer (1967), as modified by Myers & Duellman (1982).

**Karyotype.** We analyzed eight male specimens. Four were captured at Serra do Cipó, Municipality of Santana do Riacho (19°04'13"S, 43°38'16"W, 1290 m a.s.l.; UFMG 13901–4); and four were captured at Serra do Cabral, Municipality of Joaquim Felício (17°51'53"S, 44°17'55"W, 1224 m a.s.l.; UFMG 13945–8); Minas Gerais State, Brazil. Chromosome preparations were obtained from testicles as described by Eicher (1966) and stained with 5% Giemsa in Sorensen buffer. Each specimen was injected with 0.1% solution of colchicine intraperitoneally (0.1 ml per 10 g of body weight) four hours before euthanasia. Homologues were paired, grouped according to centromere position and arranged in order of decreasing size (Levan *et al.* 1964) with modifications proposed by Green & Sessions (1991, 2007).

**Bioacoustics.** Recordings are housed at Coleção Bioacústica UFMG. We analyzed 35 calls from four individuals recorded at Serra do Cipó (19°15'29"S, 43°32'5"W, 1360 m a.s.l., Municipality of Santana do Riacho, 1 November 2010, air temperature 19.2°C, CBUFMG 724–5), 20 calls from four individuals recorded at Serra do Cabral (17°54'45"S, 44°14'46"W, 1090 m a.s.l., Municipality of Buenópolis, 27 and 28 November 2012, air temperature 19.2°C, CBUFMG 704–6), and six calls from two individuals recorded at the Municipality of Diamantina (18°16'27"S, 43°40'53"W, 1391 m a.s.l., 20 October 2008, CBUFMG 702), Minas Gerais State, Brazil.

Each recording site included several individuals in calling activity, which hampered the accurate discrimination between recorded individuals. Moreover, the difficulty of approaching the calling males made voucher specimens uncertain (see also Natural History section). However, every recording had individuals collected from the site shortly after the recordings. Calls were recorded using a Marantz PMD660 digital recorder coupled with a Sennheiser ME66 unidirectional microphone at sampling rate of 44.1 kHz and 16 bits resolution.

Bioacoustical analysis was made in the software Raven Pro 1.5 (Bioacoustics Research Program 2014). Spectrogram settings: window type = Hann; window size = 512 samples; overlap = 80%; DFT size = 1024 samples; grid spacing = 46.9 Hz. Temporal parameters were measured directly from the oscillogram. Spectral parameters were acquired using measurements from Raven Pro 1.5. Sound figures were obtained with Seewave 1.7.3 (Sueur *et al.* 2008), package of R 3.0.2 platform, 64-bit version (R Development Core Team 2008). Seewave settings: window type = Hanning; window length = 1024 samples; overlap = 80%. Note terminology follows Köhler *et al.* (2017). Parameters analyzed follow Heyer *et al.* (1990) and Littlejohn (2001): note duration (ND), pulses/note (PN), pulse duration (PD), interpulse duration (IPD), pulse rate (PR; pulses/s) and dominant frequency (DF). Since we could not accurately identify each individual present in the recordings, it was not possible to measure intervals between notes. Results are given as mean ± standard deviation, range.

**Tadpoles.** Larvae were collected at Parque Estadual Serra do Cabral (17°51'04"S, 44°14'47"W), Municipality of Buenópolis, Minas Gerais State, Brazil, by T. Pezzuti and F. Leal, 30 November 2012. Tadpoles are also housed at the Coleção Herpetológica da UFMG (UFMG 1581 containing six individuals in stages 30–38). No other species belonging to Odontophrynidae were observed in the surveyed sites, assuring the confirmation of tadpole identity.

Terminology for tadpole description follows McDiarmid & Altig (1999). Twenty-three measurements were taken from six specimens between stages 30–38 (Gosner 1960): total length (TL), body length (BL), tail length (TAL), maximum tail height (MTH), internarial distance (IND), interorbital distance (IOD), tail muscle width (TMW), and tail muscle height (TMH) follow Altig & McDiarmid (1999); body width (BW), body width at narial level (BWN), body width at eyes level (BWE), body height (BH), eye-snout distance (ESD), eye-nostril distance (END), nostril-snout distance (NSD), eye diameter (ED), narial diameter (ND), snout-spiracular distance (SED), and oral disc width (ODW) follow Lavilla & Scrocchi (1986); dorsal fin height (DFH) and ventral fin height (VFH) follow Grosjean (2005); and spiracle length (SL; distance between the insertion of the spiracle and its distal edge) and spiracle distal edge height (SDEH; perpendicular distance between the spiracle distal edge and the ventral surface of the tadpole) follow Pinheiro *et al.* (2012). Measurements were taken with ImageTool 3.00 (Wilcox *et al.* 2002). Photos used an adjustable platform to support tadpoles immersed on water (Schacht & McBrayer 2009). Morphological features of tadpoles belonging to the *O. americanus* species group were obtained from Lavilla & Scrocchi (1991), Fabrezi & Vera (1997), Grenat *et al.* (2009), Borteiro *et al.* (2010), and Nascimento *et al.* (2013).

## Description of the new species

### *Odontophrynus juquinha* sp. nov.

(Figs. 1–4; Table 1)

*Odontophrynus americanus*; Eterovick & Sazima (2000): 443; Eterovick & Fernandes (2001): 687; Eterovick & Fernandes (2002): 34; Eterovick & Barros (2003): 443; Eterovick (2003): 222; Eterovick & Sazima (2004): 99; Nascimento *et al.* (2005): 146; Soma *et al.* (2006): 23; Leite *et al.* (2008): 171; Eterovick *et al.* (2010): 8; Pimenta & Camara (2015): 218.

*Odontophrynus* sp.; Rosset *et al.* (2006): 472.

**Holotype.** UFMG 5516 (Figs. 2–3), an adult male, collected by P. C. A. Garcia and F. Natali on 1 November 2010 at Serra do Cipó, Municipality of Santana do Riacho (19°15'29"S, 43°32'5"W, 1360 m a.s.l.), Minas Gerais State, Brazil.

**Paratypes.** Sixteen adult males: Municipality of Jaboticatubas, Minas Gerais State: UFMG 417 collected by F. T. M. C. Gomes on 3 November 1996; Municipality of Santana de Pirapama, Minas Gerais State: UFMG 11118 collected by H. Thomassen on 14 November 2011; Municipality of Santana do Riacho, Minas Gerais State: UFMG 419 collected by P. C. Eterovick on November 1996, UFMG 420–1 collected by J. Cassimiro on January 2000, UFMG 5511–2 and 5517 collected by P. C. A. Garcia and F. Natali on 1 November 2010 (same as the holotype), UFMG 5597–8 collected by P. P. G. Taucce and F. Leal on 2 November 2010, UFMG 11171–2 collected by H. Thomassen, F. Leal and P. C. Rocha on 30 March 2012, UFMG 13901–4 (Fig. 1, used for karotype) collected by P. C. Rocha and P. H. V. B. P. Silva on 14 October 2012. Two adult females collected at Municipality Santana do Riacho, Minas Gerais State: UFMG 5518 collected by P. C. A. Garcia and F. Natali on 1 November 2010 (same as the holotype), UFMG 5596 collected by P. P. G. Taucce and F. Leal on 2 November 2010. All paratypes were collected at Serra do Cipó, Minas Gerais State.



**FIGURE 1.** *Odontophrynus juquinha* sp. nov. (paratype UFMG 13903; SVL = 46.2 mm); live adult male. Parque Nacional da Serra do Cipó, Municipality of Santana do Riacho, Minas Gerais State, Brazil. Photo by F. Leal.

**Referred specimens.** Pico do Itobira, Municipality of Rio de Contas, Bahia State: one adult female (UFMG 7824) collected by T. L. Pezzuti, L. O. Drummond, B. Imai and L. Rodrigues on 11 January 2010. Pico das Almas,

Municipality of Rio de Contas, Bahia State: two adult males (UFMG 4470 and 4476) and three adult females (UFMG 4469, 4471, and 4488) collected by F. S. F. Leite, M. R. Lindemann and R. B. Mourão on 11 January 2010. Serra Nova, Municipality of Rio Pardo de Minas, Minas Gerais State: three adult males (UFMG 6831, 6833, and 6875) and three adult females (UFMG 6019–20, and 6832) collected by F. S. F. Leite on 16 and 17 December 2007. Serra de Botumirim, Municipality of Botumirim, Minas Gerais State: one adult female (UFMG 12250) collected by F. Leal and H. Thomassen on 8 January 2012. Serra do Cabral, Municipality of Joaquim Felício, Minas Gerais State: four adult males (UFMG 7277–80) and one adult female (UFMG 7275) collected by T. L. Pezzuti and L. O. Drummond on 15 December 2007; four adult males (UFMG 13945–8) used for karyotype; one adult male (UFMG 13949) and one adult female (UFMG 13950) collected by F. Leal and T. L. Pezzuti on 28 November 2012. Serra do Cabral, Municipality of Buenópolis, Minas Gerais State: one adult male (UFMG 7318) collected by T. L. Pezzuti and L. O. Drummond on 15 December 2007; four adult males (UFMG 13927–30) and one adult female (UFMG 13931) collected by F. Leal and T. L. Pezzuti on 27 November 2012. Municipality of Diamantina, Minas Gerais State: three adult males (UFMG 3957–9) collected by F. S. F. Leite and R. B. Mourão on 20 October 2008. Alto Palácio, Municipality of Santana do Riacho, Minas Gerais State: five adult males (CFBH 40116–20) and one adult female (CFBH 40121) collected by C. F. B. Haddad, A. M. Haddad, K. Zamudio, H. Greene, C. P. A. Prado and F. Zara on 10 February 2016; one adult male (CFBH 39947) collected by C. F. B. Haddad, A. Haddad, V. Mallerba, no collection date. Kilometer 113 (Serrotes), Serra do Cipó, Municipality of Santana do Riacho, Minas Gerais State: one adult female (CFBH 00794) collected by C. F. B. Haddad and L. Castanho on 05 October 1989. Serra do Cipó. Kilometer 132, Municipality of Santana do Riacho, Minas Gerais State: one adult male (CFBH 35055) collected by J.P. Pombal Jr, O. C. Oliveira. Crossroad between MG10 and the road to Morro do Pilar, Serra do Cipó, Municipality of Santana do Riacho, Minas Gerais State: one adult male (CFBH 39816) and one adult female (CFBH 39822) C. F. B. Haddad, P. G. Taucce and A. Valencia on 03 February 2015.

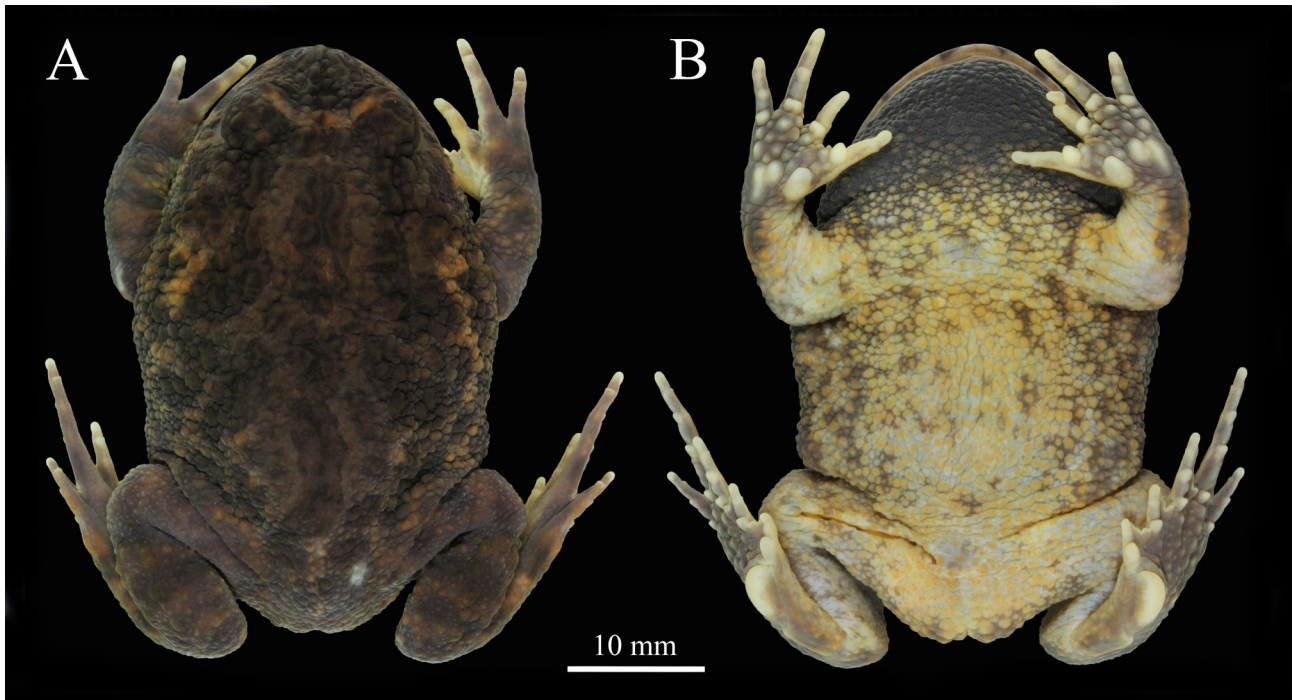
**TABLE 1.** Measurements (in mm, reported as mean  $\pm$  SD, range) of males and females of *Odontophrynus juquinha* sp. nov. type series. See Abbreviations List for definitions of trait acronyms.

Trait	Males (n=16)		Females (n=2)	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
SVL	44.27 $\pm$ 3.83	38.45–52.09	46.05 $\pm$ 1.46	45.01–47.08
HL	13.49 $\pm$ 1.33	11.50–16.00	13.73 $\pm$ 0.26	13.54–13.91
HW	17.46 $\pm$ 1.73	14.41–21.16	17.81 $\pm$ 0.04	17.78–17.84
IND	2.97 $\pm$ 0.35	2.04–3.40	2.65 $\pm$ 0.25	2.47–2.82
AMD	6.76 $\pm$ 0.55	5.90–8.32	6.41 $\pm$ 0.16	6.30–6.52
END	3.13 $\pm$ 0.20	2.55–3.37	3.37 $\pm$ 0.07	3.32–3.42
ED	4.77 $\pm$ 0.46	4.30–5.56	5.31 $\pm$ 0.30	5.09–5.52
AL	22.50 $\pm$ 2.65	18.47–29.89	22.98 $\pm$ 0.12	22.89–23.06
THL	16.40 $\pm$ 12.81	12.81–20.19	16.11 $\pm$ 0.30	15.89–16.32
TL	14.34 $\pm$ 1.19	12.66–16.66	15.33 $\pm$ 0.28	15.13–15.52
FL	26.74 $\pm$ 2.26	22.59–31.34	27.56 $\pm$ 0.27	27.37–27.75
MTL	4.33 $\pm$ 0.39	3.80–5.29	4.44 $\pm$ 0.11	4.36–4.52

**Diagnosis.** *Odontophrynus juquinha* sp. nov. is a medium-sized species belonging to the genus *Odontophrynus* based on a combination of morphological characters (i.e. head wider than long, snout truncate in profile, tympanum hidden, first subarticular tubercle on toe I enlarged, inner metatarsal tubercle large, tarsal fold short, granular skin on the dorsum and venter) (Savage & Ceï 1965; Ceï 1987; Caramaschi 1996; Caramaschi & Napoli 2012). The new species belong to the *Odontophrynus americanus* species group based on the absence of large dorsal, tibial and forearm glandular warts, with postorbital, temporal, and parotoid glandular warts not distinctly developed but with a series of small glandular warts of irregular size and shape, forming glandular ridges longitudinally oriented, on postorbital-parotoid regions (Fig. 2A, 5). *Odontophrynus juquinha* sp. nov. is distinguished from the remaining species belonging to the *O. americanus* species group by the following combination of characters: (1) medium sized (SVL = 38.45–52.09 mm in males in *O. juquinha*); (2) head wider



than long (HL/HW = 0.7–0.8); (3) dorsal dark brown blotches of small to medium-size with low contrast on a light brown background (Fig. 5); (4) light mid-dorsal stripe absent or greatly interrupted in most of the specimens; (5) yellowish coloration of mid-dorsal stripe and background of both the head and the flanks of the body; (6) moderate number of glandular warts on dorsum; (7) diploid complement of  $2n = 2x = 22$  chromosomes with fundamental number  $FN = 44$ ; (8) secondary constriction on the interstitial region of the short arm of the pair 4; (9) advertisement call with dominant frequency of 840–1080 Hz; (10) pulse rate of 90.5–106.7 pulses/s; (11) small tadpoles (mean TL = 24.30–35.69 mm); (12) single lateral emargination on each side of oral disc; (13) caudal end acute; (14) 4–8 submarginal papillae in both sides of lip; and (15) internal wall of spiracle with a small free portion.



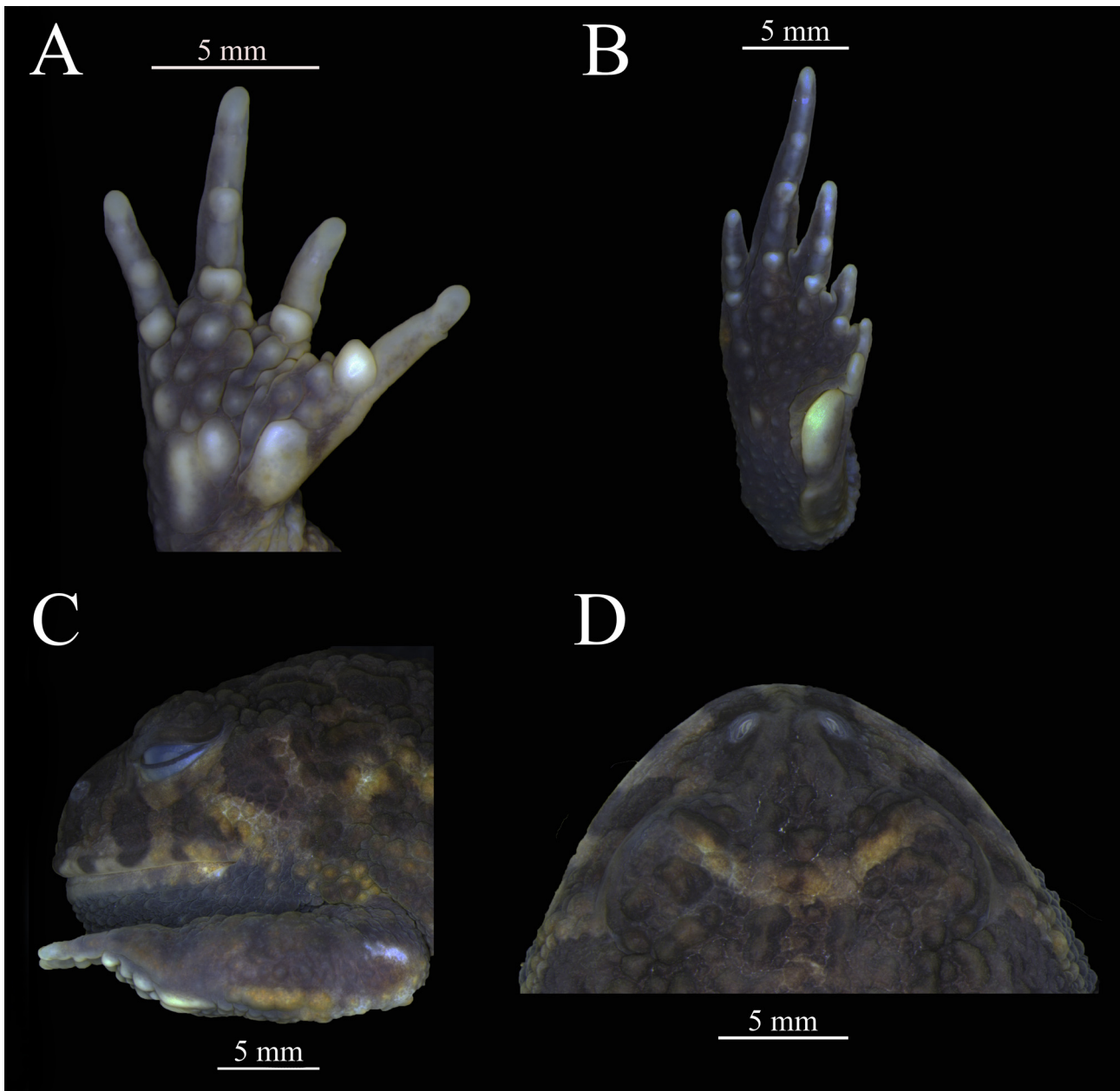
**FIGURE 2.** *Odontophrynus juquinha* sp. nov., UFMG 5516 (holotype), adult male in (A) dorsal and (B) ventral view.

**Comparison with other species.** *Odontophrynus juquinha* sp. nov. is clearly distinguished from the species of *O. cultripes* group (*O. cultripes*, *O. carvalhoi*, and *O. monachus*) and *O. occidentalis* group (*O. occidentalis*, *O. barrioi*, and *O. achalensis*) by the absence of distinctly enlarged postorbital, temporal, and parotoid glandular warts (three or more pairs of enlarged rounded-oval postorbital, temporal, and parotoid glandular warts in *O. cultripes* and *O. occidentalis* species groups) (See Appendix 1 for complete list of examined material). It is distinguished from *O. salvatori* by its larger size (SVL > 38 mm), supernumerary tubercles on hands and feet absent, dorsal surface of fingers and toes smooth, and nuptial pads present (SVL < 32 mm, supernumerary tubercles on hands and feet present, dorsal surface of fingers and toes rugose, and nuptial pads absent in *O. salvatori* (Caramaschi 1996; present work).

Within the *Odontophrynus americanus* species group, *O. juquinha* sp. nov. is distinguished from *O. americanus* (Fig. 5A) by having a diploid complement of  $2N = 2X = 22$  chromosomes (tetraploid complement of 44 chromosomes in *O. americanus*); and a secondary constriction (SC) on the interstitial region of the short arm of pair 4 (SCs on the proximal region of the short arm of chromosomes group 4 and/or on the interstitial region of the long arm of chromosomes group 11). Moreover, the advertisement call of *O. juquinha* sp. nov. distinguishes it from *O. americanus* by the higher pulse rate (*O. juquinha* PR = 90.5–106.7 pulses/s, air temperature = 19.2°C; *O. americanus* PR = 73–77 pulses/s, air temperature = 20°C, Martino & Sinsch, 2002), and its tadpoles distinguish it by being smaller (*O. juquinha* TL = 24.30–35.69 mm, stages 30–38,  $n = 6$ ; *O. americanus* TL = 42 mm, stage 38–39; Nascimento *et al.* 2013 and literature cited therein).

*Odontophrynus juquinha* sp. nov. is distinguished from *O. lavillai* (Fig. 5B) by having dorsal dark brown blotches of small to medium-size with low contrast on a light brown background (two or three large dorsal dark brown blotches, transversally fused, with high contrast in all examined specimens of *O. lavillai*) (Fig. 1, 4 and 5B),

by having a yellowish coloration in the mid-dorsal stripe and in the background of both the head and the flanks of the body (whitish or greyish coloration in *O. lavillai*). Moreover, the advertisement call of *O. juquinha* **sp. nov.** distinguishes it from *O. lavillai* by the higher dominant frequency (*O. juquinha* DF = 840–1080 Hz, SVL = 44.3 ± 3.8; *O. lavillai* DF = 637.1–790.2 Hz, SVL = 58.3 ± 4.3) and by the lower pulse rate (*O. juquinha* PR = 90.5–106.7 pulses/s, air temperature = 19.2°C; *O. lavillai* PR = 107.2–132.5 pulses/s, air temperature = 25°C) (see Table 2 for call comparisons, see also Discussion section for comments on the possible influence of temperature). The tadpoles of the new species can be distinguished from *O. lavillai* by being smaller (*O. juquinha* TL = 24.30–35.69 mm, stages 30–38; *O. lavillai* TL = 55.8 mm, stage 37; Lavilla & Scrocchi 1991), by having a single lateral emargination on each side of the oral disc (ventral and lateral emarginations in *O. lavillai*), and by having 4–8 submarginal papillae (one in *O. lavillai*).



**FIGURE 3.** *Odontophrynus juquinha* **sp. nov.**, UFMG 5516 (holotype), adult male. (A) Left hand, (B) left foot, (C) lateral and (D) dorsal views of head.

*Odontophrynus juquinha* **sp. nov.** is distinguished from *O. cordobae* (Fig. 5C) by having dorsal dark brown blotches of small to medium-size with low contrast on a light brown background (dorsal dark brown blotches with high contrast in all examined specimens of *O. cordobae*) (Fig. 1, 4, and 5C). It is also distinguished by the lack of a



light mid-dorsal stripe, or greatly interrupted in most of the specimens (light mid-dorsal stripe slightly interrupted in *O. cordobae*) (Fig. 5C). Whenever the mid-dorsal stripe is present, its yellowish coloration distinguishes the new species from *O. cordobae* (whitish or greyish coloration in *O. cordobae*). Additionally, the new species is distinguished by the yellowish coloration in the background of both the head and the flanks of the body (whitish or greyish coloration in *O. cordobae*). Moreover, the advertisement call of *O. juquinha* **sp. nov.** distinguishes it from *O. cordobae* by the lower pulse rate (*O. juquinha* PR = 90.5–106.7 pulses/s, air temperature = 19.2°C; *O. cordobae* PR = 111–116 pulses/s, air temperature = 20°C) (see Table 2 for call comparisons, see also Discussion section for comments on the possible influence of temperature). The tadpoles of the new species are distinguished by being smaller than those of *O. cordobae* (*O. juquinha* TL = 24.30–35.69 mm, stages 30–38, n = 6; *O. cordobae* TL = 40 mm, stage 37; Nascimento *et al.* 2013 and literature cited therein) and by having 4–8 submarginal papillae (single or paired in *O. cordobae*).

**TABLE 2.** Advertisement calls of species belonging to the *Odontophrynus americanus* group. Parameters presented as range.

Species	Note duration (ms)	Internote interval (s)	Pulses/Note	Pulse duration (ms)	Interval between pulses (ms)	Pulse rate (pulses/s)	Dominant frequency (Hz)	Reference
<i>O. juquinha</i> <b>sp. nov.</b>	322–610	–	32–62	5–8	3–6	90.5–106.7	840–1080	This work
<i>O. americanus</i>	524–558	–	39–41	5.1–5.5	8.0–8.6	73–77	1025–1075	Martino & Sinsch (2002)
<i>O. cordobae</i>	421–475	–	48–53	3.3–3.7	5.1–5.7	111–116	990–1040	Martino & Sinsch (2002)
<i>O. lavillai</i>	301.5–583.0	1.8–8.4	40.0–62.5	4.5–7.0	1.8–5.0	107.2–132.5	637.1–790.2	Rosset & Baldo (2014)
<i>O. maisuma</i>	570–785	1.6–18.6	43–57	–	–	71.1–77.2	1124–1211	Borteiro <i>et al.</i> (2010)

*Odontophrynus juquinha* **sp. nov.** is distinguished from *O. maisuma* (Fig. 5D) by the head wider than long (HL/HW = 0.7–0.8, n = 16 males of *O. juquinha*; HL/HW = 0.8–0.9, n = 12 males of *O. maisuma*, *sensu* Rosset 2008), by the high density of glandular warts on dorsum (few glandular warts in all examined specimens of *O. maisuma*) (Fig. 5D) (See Appendix 1 for complete list of examined material), by having dorsal dark brown blotches of small to medium-size, with low contrast on a light brown background (one or two pairs of large, nearly symmetrical, longitudinal dorsal dark brown blotches with high contrast in *O. maisuma*) (Fig. 1, 4 and 5D), and by the lack of a light mid-dorsal stripe, or greatly interrupted in most of the specimens (light mid-dorsal stripe uninterrupted in all examined specimens of *O. maisuma*) (Fig. 5D). Whenever the mid-dorsal stripe is present, its yellowish coloration distinguishes the new species from *O. maisuma* (whitish or greyish coloration in *O. maisuma*). Furthermore, *O. juquinha* **sp. nov.** is distinguished by having yellowish coloration in the background of both the head and the flanks of the body (whitish or greyish coloration in *O. maisuma*). The advertisement call of *Odontophrynus juquinha* **sp. nov.** distinguishes it from *O. maisuma* by its lower dominant frequency (*O. juquinha* DF = 840–1080 Hz, SVL = 44.3 ± 3.8; *O. maisuma* DF = 1124–1211 Hz, SVL = 38.4 ± 2.2), and by the higher pulse rate (*O. juquinha* PR = 90.5–106.7 pulses/s, air temperature = 19.2°C; *O. maisuma* PR = 71.1–77.2 pulses/s, air temperature = 6°C) (see Table 2 for call comparisons; see also Discussion section for comments on the possible influence of temperature). Tadpoles of the new species are smaller than those of *O. maisuma* (*O. juquinha* TL = 24.30–35.69 mm, stages 30–38, n = 6; *O. maisuma* TL = 47 mm, stages 33–36; Borteiro *et al.* 2010) and have the caudal end acute (rounded in *O. maisuma*). Additionally, the tadpoles of *O. juquinha* **sp. nov.** are distinguished from those of *O. maisuma* by having 4–8 submarginal papillae (one or few in *O. maisuma*) and by having the internal wall of spiracle with a small free portion (entirely fused to the body in *O. maisuma*).

**Description of the holotype.** Body stout; head wider than longer, its length 77% of its width, 33% of SVL (Fig. 2). Snout rounded in dorsal profile, between sloping and truncate in lateral view (Fig. 3C, D). Canthus rostralis slightly distinct, concave in dorsal view; loreal region slightly concave. Nostrils elliptic elongated, directed dorsolaterally, and situated at the tip of the snout in lateral profile; internarial distance 44% of distance

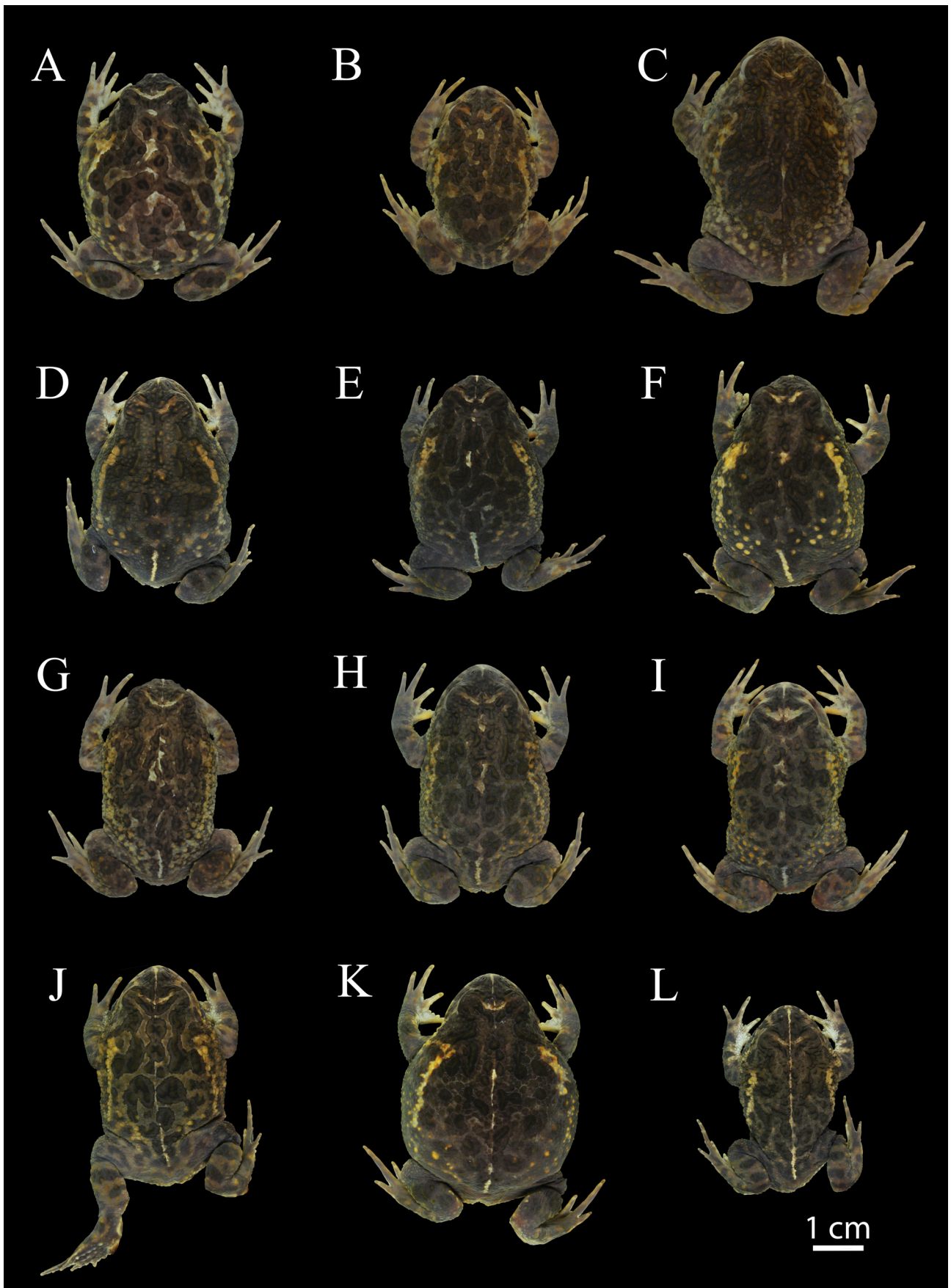
between the anterior margins of eyes, 77% of eye-nostril distance. Eye large, prominent, 32% of head length, its diameter 40% longer than eye-nostril distance. Upper eyelid with distinct warts varying from rounded to elongated, external border of the upper eyelid with a glandular ridge. Tympanum hidden, not visible externally (Fig. 3C, D). Premaxillary and maxillary teeth present; tongue round, approximately half free and notched posteriorly; vomerine teeth in two patches slightly posteromedian to choanae. Vocal sac median, subgular; vocal slits present, longitudinal. Skin of dorsum granular, consisting of glandular warts with variable size, distributed scattered throughout the dorsum, and a series of distinct glandular warts, with irregular size and shape, forming small ridges longitudinally oriented, on postorbital-parotoid region. No distinct temporal glandular wart. Venter uniformly granular. Forelimbs relatively short, stout; glandular longitudinal ridge in the external part of the forearm (Fig. 3C). Lengths of fingers: III < V < II < IV, with a deformation in the tip of left finger III; fringes absent, webbing absent. Inner and outer metacarpal tubercles well developed; inner oval; outer divided, with the inner oval and the outer elongated; subarticular tubercles well developed, oval, slightly bilobated on fingers III and IV; supernumerary tubercles rounded, scattered distributed in the palm. Nuptial pad present on thumb. Hind limbs relatively short, stout; lengths of toes: I < II < V < III < IV; tarsal fold present; subarticular tubercles present, rounded, oval and enlarged on finger I. Inner metatarsal tubercle strong, shovel-like, nonkeratinized; outer metatarsal tubercle slightly distinct, rounded; supernumerary tubercles rounded, scattered distributed in the foot palm. Webbing formula: I 1–2 II 1<sup>1/2</sup>–3 III 2<sup>1/2</sup>–4 IV 4–2<sup>+</sup> V, with dermal fringes developed (Fig. 3B).

**Measurements of the holotype (mm).** SVL 44.3, HL 14.6, HW 18.9, IND 2.6, AMD 6.3, END 3.4, ED 4.8, AL 23.15, THL 17.2, TL 15.0, FL 27.9, MTL 5.1.

**Coloration of the holotype.** Unknown in life. In preservative, dorsal color predominantly dark brown (Fig. 2). Light brown stripe between the eyes, resembling a “U” shape. Head and extremities dark brown in dorsal view. Light brown stripe dorsolaterally, starting at the medial third of the body toward the hind limb. Upper lip light brown; two dark brown blotches connecting each nostril to the upper lip; two dark brown blotches connecting the eyes to the upper lip; four dark brown small blotches in the upper lip. Light brown stripe from the posterior part of the eye towards the forelimb insertion. Venter yellowish-colored, with scattered dark brown blotches gradually increasing on the sides of the body. Gular area with dark grey pigmentation.

**Coloration in life.** Description based on adult male paratypes UFMG 13901, 13903–4, Municipality of Santana do Riacho, Minas Gerais State, Brazil (Fig. 1). Dorsal surface of head, body and extremities dark brown. Yellowish stripe between the eyes, resembling a “U” shape. Yellowish stripe dorsolaterally, starting at the medial third of the body toward the hindlimb. Nut-brown blotches distributed below the dorsolateral yellowish stripe. Upper lip yellowish; two dark brown blotches connecting each nostril to the upper lip; two dark brown blotches connecting the eyes to the upper lip; four dark brown small blotches in the upper lip. Yellowish stripe from the posterior part of the eye towards the forelimb insertion. Venter whitish, with scattered grey blotches gradually increasing on the side of the body. Gular area whitish, crowded with dark grey blotches. Pupil horizontally elliptical; iris golden marbled with dark stripes; dark edges of eyes. The size and shape of the yellowish blotches varied in the analyzed individuals. Dark brown blotch below the eye well defined in UFMG 13901 and 13903, whereas blurred in UFMG 13904. A yellow mid-dorsal stripe well defined in UFMG 13904, interrupted in UFMG 13901 and 13903. Dorsum predominantly light brown in UFMG 13901, dark brown blotches surrounding the longitudinal glandular ridges.

**Variation.** Adult males can be distinguished from adult females by the presence of clearly developed nuptial pads on thumb, and a dark grey-pigmented gular region at the vocal sac. Adult females are usually about the same size as males (SVL = 44.3 ± 3.8, 38.4–52.1 mm in males, n = 16; SVL = 46.1 ± 1.5, 45.0–47.1 mm in females, n = 2). Full mid-dorsal stripe was present in only one individual (i.e. UFMG 13904; Fig. 4L); an interrupted mid-dorsal stripe was present in some individuals (e.g. UFMG 417, 5511–2, 5596, 5598, and 13901) whilst others presented the mid-dorsal stripe only in the supra-cloacal region (e.g. UFMG 419, 420–1, 5518, 5597, 11118, 11171, and 13902–3) (see Fig. 4 for dorsal color variation). Some individuals presented the dorsum light brown in preservative, with dark brown blotches surrounding the longitudinal glandular ridges (e.g. UFMG 417, 5511–2, 5518, 11171, 13901). Some variation was observed in the outer metacarpal tubercle division, with individuals presenting it only partially divided (e.g. UFMG 5512, 5517), and others with a merely distinguishable division (e.g. UFMG 5518, 11171). Although the level of keratinization varied, all paratypes presented the inner metatarsal tubercle keratinized.



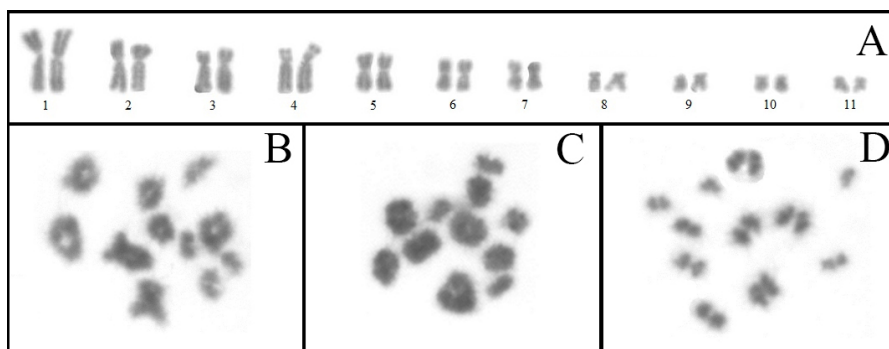
**FIGURE 4.** *Odontophrynus juquinha* sp. nov. dorsal color variation. Paratypes UFMG (A) 5518 (female), (B) 419, (C) 420, (D) 11118, (E) 5598, (F) 5597, (G) 5512, (H) 5511, (I) 11172, (J) 13901, (K) 5596 (female), (L) 13904.





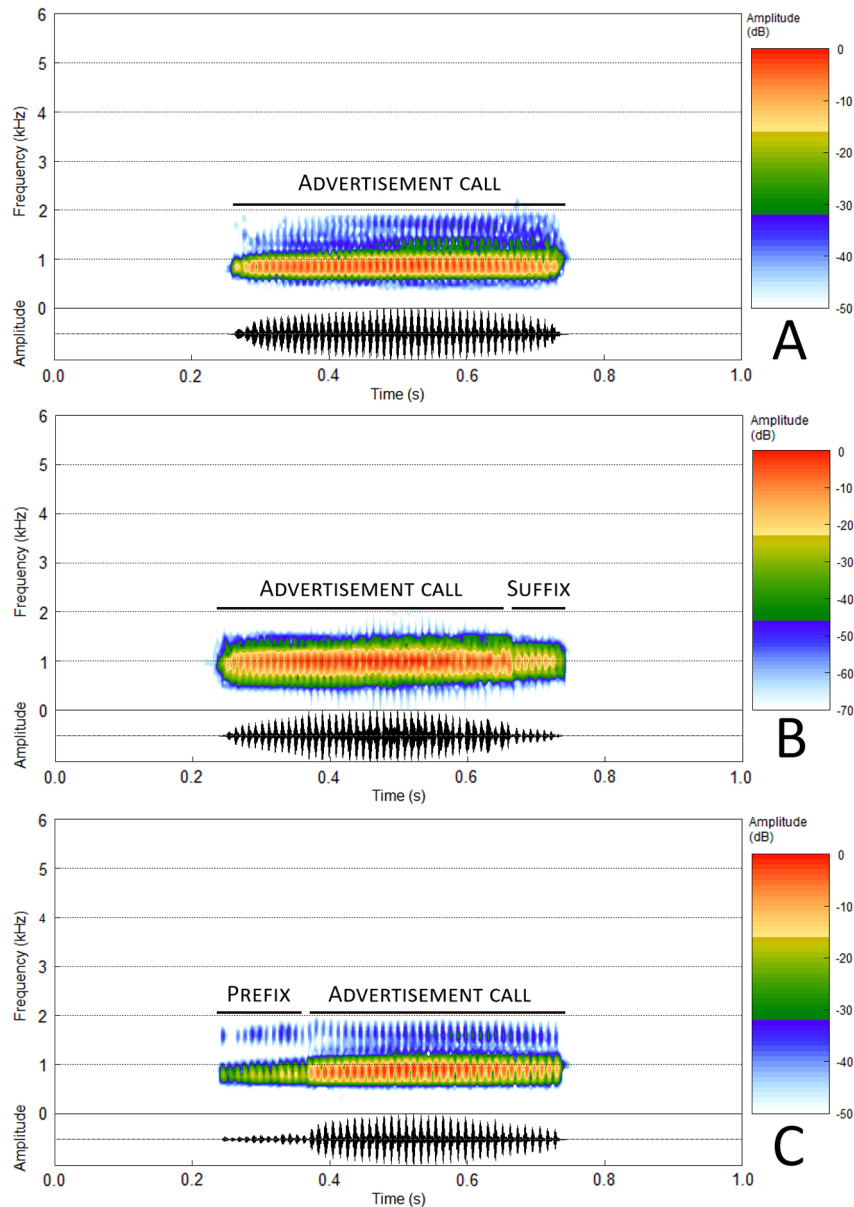
**FIGURE 5.** Live specimens of (A) *Odontophrynus americanus*, (B) *O. lavillai*, (C) *O. cordobae*, and (D) *O. maisuma*.

**Karyotype.** All examined specimens of *Odontophrynus juquinha* **sp. nov.** presented a diploid number of  $2n = 2x = 22$  chromosomes. All chromosomes were biarmed (fundamental number  $FN = 44$ ) and the karyotype consisted of four large (1–4), three medium (5–7), and four small pairs (8–11) (Fig. 6A). Chromosome pairs 1, 5–7, and 11 were metacentric and chromosome pairs 2–4 and 8–10 were submetacentric. Pair 4 had an evident secondary constriction (SC) in the interstitial region of its short arm, which probably bears the nucleolus organizer region (NOR) as it has been observed in the other diploid species of the genus *Odontophrynus* (Barrio & Pistol de Rubel 1972; Ruiz *et al.* 1981; Rosset *et al.* 2006; Borteiro *et al.* 2010). Meiotic analysis revealed 11 ring bivalents with terminal chiasmata in diplotene, diakinesis, and metaphase I; and 11 dyads in metaphase II (Fig. 6B–D).



**FIGURE 6.** (A) Karyotype of a male *Odontophrynus juquinha* **sp. nov.** ( $2n = 2x = 22$ ;  $FN = 44$ ) after conventional Giemsa staining. Meiotic stages: (B) Diakinesis, (C) Metaphase I, (D) Metaphase II.

**Vocalizations.** Advertisement call of *Odontophrynus juquinha* **sp. nov.** is characterized by a single pulsed note (n = 10 individuals), without frequency modulation and with variable duration (ND =  $446.8 \pm 61.0$ , 322–610 ms, n = 61 calls) and number of pulses (PN =  $44.5 \pm 5.7$ , 32–62 pulses/note, n = 61 calls) (Fig. 7A, Table 3). Pulses had mean duration of  $6 \pm 0.8$  ms (PD = 5–8 ms, n = 92 pulses), at regular intervals and constant rate (IPD =  $4.3 \pm 0.9$ , 3–6 ms, n = 85 intervals; PR =  $99.4 \pm 4.1$ , 90.5–106.7 pulses/s, n = 58 notes).



**FIGURE 7.** (A) Advertisement call of *Odontophrynus juquinha* **sp. nov.** and (B) call including a suffix, unvouchered male from Serra do Cipó, municipality of Morro do Pilar, MG, 1 November 2010, air temperature 19.2°C. (C) Call including a prefix, unvouchered male from Serra do Cabral, municipality of Buenópolis, MG, 27 November 2012, air temperature 19.2°C.

The calls from Serra do Cabral and Diamantina had a slightly higher dominant frequency than the one from Serra do Cipó. Other variables presented small variations that overlapped. We observed an affix in 18% of the analyzed notes (n = 11: six calls from two individuals recorded at Serra do Cipó, and five calls from one individual recorded at Serra do Cabral) (Fig. 7B, C). This affix was always part of the same note described as the advertisement call. Males displayed it either after, as a suffix (45.5%, n = 5; Fig. 6B), or before, as prefix (54.5%, n = 6; Fig. 7C). The affix presented short duration (Affix Duration =  $99.8 \pm 29.7$ , 58–149 ms, n = 11; Table 3) and less pulses than the advertisement call (PN =  $10.1 \pm 3.0$ , 6–16 pulses/note, n = 11). Affix' pulses presented the



same characteristics of the advertisement call (PD =  $6.4 \pm 0.9$ , 5–8 ms, n = 63; IPD =  $4.3 \pm 0.9$ , 3–6 ms, n = 55; PR =  $101.7 \pm 3.6$ , 97.3–107.3 pulses/s, n = 11). Dominant frequency is slightly lower than the advertisement call (DF =  $890 \pm 94$ , 796–984 Hz, n = 11). Despite the similarity between the affix and the advertisement call, they are clearly distinguishable in the spectrogram (Fig. 7B, C). Moreover, calls with an affix are audibly distinct than the common advertisement call. Although the affix seems to be related to the presence of other calling males, we did not test its social context.

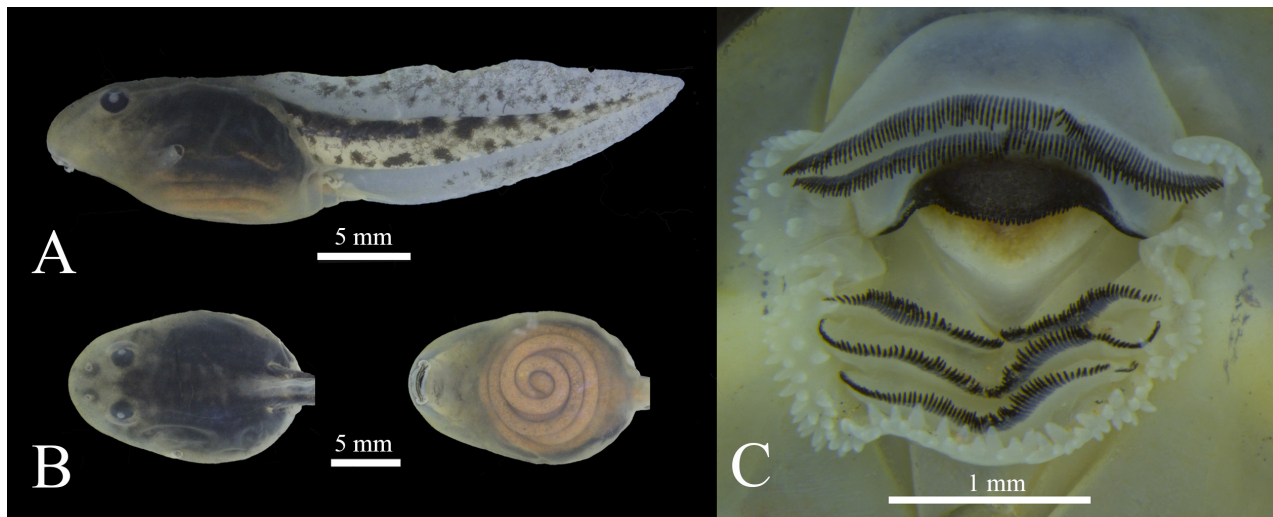
**TABLE 3.** Call parameters of *Odontophrynus juquinha* sp. nov. based on 61 calls from Serra do Cipó, Serra do Cabral, and from the municipality of Diamantina. Data are presented as mean  $\pm$  SD, range.

	Note duration (ms)		Pulses per note		Dominant frequency (Hz)	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
All localities	446.8 $\pm$ 61.0	322–610	44.5 $\pm$ 5.7	32–62	941.1 $\pm$ 71.8	843.8–1078.1
Type locality (n = 35 calls; 19.2°C)	460.6 $\pm$ 50	360–610	45.5 $\pm$ 5.8	34–62	887.8 $\pm$ 38.7	843.8–937.5
Serra do Cabral (n = 20 calls)	396.5 $\pm$ 39.1	322–443	41.3 $\pm$ 4.5	32–47	1007.8 $\pm$ 35	984.4–1078.1
Diamantina (n = 6 calls)	533.5 $\pm$ 28.7	492–567	49.3 $\pm$ 2.5	46–52	1012.1 $\pm$ 52.7	947.5–1076.7
Affix (all localities)	99.8 $\pm$ 29.7	58–149	10.1 $\pm$ 3.0	6–16	890 $\pm$ 94	796–984
	Pulse duration (ms)		Interpulse interval (ms)		Pulse rate	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
All localities	6.0 $\pm$ 0.8	5–8	4.3 $\pm$ 0.9	3–6	99.4 $\pm$ 4.1	90.5–106.7
Type locality (n = 35 calls; 19.2°C)	6.0 $\pm$ 0.7	5–8	4.3 $\pm$ 0.8	3–6	98.6 $\pm$ 2.8	92.1–102.5
Serra do Cabral (n = 20 calls)	5.8 $\pm$ 0.7	5–8	4.1 $\pm$ 0.9	3–6	103.5 $\pm$ 1.9	99.3–106.7
Diamantina (n = 6 calls)	6.5 $\pm$ 0.9	5–8	4.3 $\pm$ 0.9	3–6	92.5 $\pm$ 1.6	90.5–94.9
Affix (all localities)	6.4 $\pm$ 0.9	5–8	4.3 $\pm$ 0.9	3–6	101.7 $\pm$ 3.6	97.3–107.3

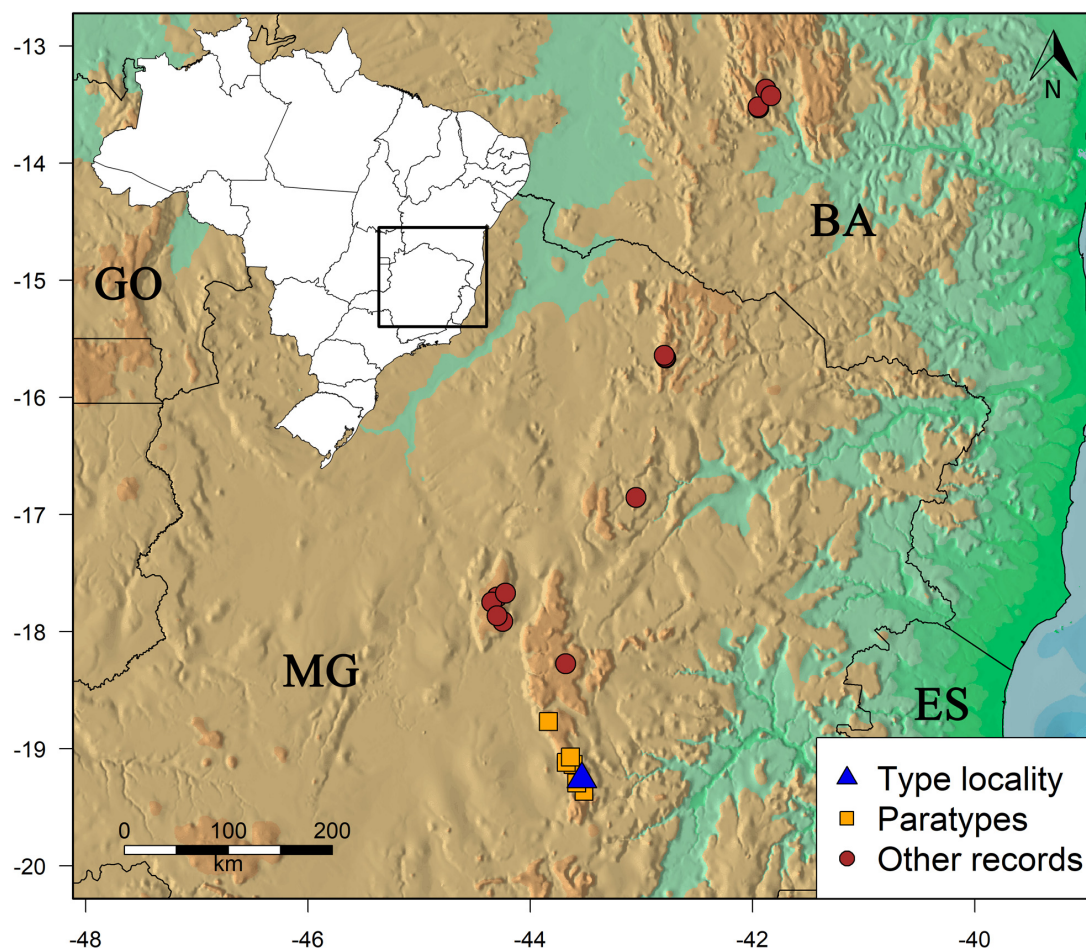
**Tadpole description.** Tadpoles in stage 30–38 have 24.3–35.7 mm of TL (n = 6 individuals). Body depressed (BH/BW = 0.74–0.78) (Fig. 8A, B), longer than one third of total length (BL/TL = 0.40–0.46); oval in dorsal view and globular depressed in lateral view. Snout ovoid in dorsal view (BWN/BWE = 0.69–0.75) and rounded in lateral view. Nostrils elliptic (only one individual has shown rounded nostrils), dorsally located (IND/BWN = 0.28–0.32), with small medial apophysis; closer to snout than to eyes (NSD/END = 0.55–0.65). Eyes large (ED/BWE = 0.25–0.29), dorsally located (IOD/BWE = 0.55–0.61), dorsolaterally directed. Spiracle sinistral, and lateroventral (SDEH/BH = 0.47–0.51); posterodorsally directed, visible in lateral and dorsal view; its inner wall fused to the body wall, with posterior portion free and longer than external wall (one individual has the inner and external edge of spiracle of same size); opening located approximately at half of the body (SED/BL = 0.51–0.57). Intestinal switchback point located at the center of the abdominal region (Fig. 8B). Vent tube long, dextral, with ventral wall fused to bottom edge of ventral fin. Tail and body with approximately same height (MTH/BH = 0.94–1.02); tail musculature slightly developed (TMH/MTH = 0.36–0.38); caudal end acute. Dorsal fin low (DFH/TMH = 0.39–0.43), with margin slightly convex and maximum height nearly half of the tail; emerges on posterior third of the body. Ventral fin with margin slightly convex; origin concealed by vent tube; lower than dorsal fin (DFH/VFH = 1.35–1.55). Oral disc (Fig. 8C) anteroventrally positioned, small (ODW/BW = 0.28–0.32), laterally emarginated; one row of marginal papillae in alternate disposition throughout the oral disc; wide anterior gap present; four to eight submarginal papillae present in both sides of oral disc. Labial tooth row formula (LTRF) 2(2)/3(1); A-1 and A-2 of the same length; P-1 and P-2 of the same length, longer than P-3. Jaw sheaths wide, finely serrated on the margins; upper jaw sheath M-shaped and lower V-shaped.

**Natural History.** Adult males of *Odontophrynus juquinha* sp. nov. can be found calling with the body partially submersed in the shallow water of temporary ponds or streamlets within *campo rupestre* (rupestrian grasslands) vegetation. They often call at the beginning of the raining season (i.e. from September to January) after heavy rains. Males usually prefer to call hidden beneath grasses and bushes, and promptly cease calling when approached. Chorus can be heard from long distances. We also witnessed *O. juquinha* sp. nov. calling during daylight after heavy rains. Tadpoles are exotrophic, benthic, inhabiting mainly lentic environments such as streamlets backwater, swamps and temporary ponds (ecomorphological guild II: A:1; *sensu* McDiarmid & Altig

1999). In addition, species such as *Scinax curicica* Pugliese *et al.*, 2004, *S. pinima* (Bokermann and Sazima, 1973), *S. squalirostris* (Lutz, 1925), *Boana cipoensis* (Lutz, 1968), *Leptodactylus camaquara* Sazima and Bokermann, 1978, *L. cunicularius* Sazima and Bokermann, 1978, *L. furnarius* Sazima and Bokermann, 1978, *L. fuscus* (Schneider, 1799), *L. jolyi* Sazima and Bokermann, 1978, *L. syphax* Bokermann, 1969, and *Physalaemus cuvieri* Fitzinger, 1826, can also be found calling at the same streamlets and ponds that the new species inhabit (Eterovick & Fernandes, 2002; Eterovick & Barros 2003).



**FIGURE 8.** Tadpole of *Odontophrynus juquinha* sp. nov. collected at Serra do Cabral, lot UFMG 1581, stage 34: (A) Lateral view, (B) dorsal and ventral view of body, (C) oral disc.



**FIGURE 9.** Geographic distribution in Brazil of *Odontophrynus juquinha* sp. nov. related to the altitude. MG = Minas Gerais State, ES = Espírito Santo State, BA = Bahia State, GO = Goiás State.

**TABLE 4.** Tadpole measurements (in mm) of *Odontophrynus juquinha* sp. nov. from Serra do Cabral, municipality of Buenópolis, Minas Gerais State, Brazil. Developmental stages 30–38 (Gosner 1960) (n = 6). See Abbreviations List for definitions of trait acronyms.

Characters	Serra do Cabral (UFMG 1581; n = 6)	
	Mean ± SD	Range
TL	30.6 ± 3.9	24.3–35.7
BL	12.9 ± 0.9	11.4–14.3
TAL	17.6 ± 3.1	12.8–21.4
MTH	7.7 ± 0.5	6.2–7.6
DFH	2.9 ± 0.3	2.5–3.4
VFH	2.0 ± 0.1	1.9–2.0
TMH	2.6 ± 0.2	2.3–2.9
BH	7.3 ± 0.7	6.2–8.2
SL	1.1 ± 0.1	1.0–1.2
SDEH	3.6 ± 0.3	3.1–3.9
SED	7.0 ± 0.5	6.5–7.6
ED	2.0 ± 0.1	1.8–2.2
BW	9.6 ± 0.9	8.5–10.8
BWN	5.4 ± 0.4	4.8–5.8
BWE	7.5 ± 0.4	6.8–7.9
TMW	2.4 ± 0.1	2.3–2.5
END	2.6 ± 0.1	2.3–2.7
ESD	4.1 ± 0.2	3.8–4.4
NSD	1.5 ± 0.2	1.4–1.8
ND	0.5 ± 0.05	0.4–0.5
IND	1.6 ± 0.15	1.4–1.8
IOD	4.3 ± 0.2	3.9–4.6
ODW	2.8 ± 0.2	2.4–3.0

**Distribution and conservation.** *Odontophrynus juquinha* sp. nov. occurs in the Espinhaço range, Brazil (Fig. 9), in altitudes ranging from 900 to 1540 m a.s.l. The southernmost record is at Serra do Cipó, municipality of Santana do Riacho, Minas Gerais State; the northernmost record is at Serra do Itobira, municipality of Rio de Contas, Bahia State.

The species extent of occurrence measured by a minimum convex polygon (EO, *sensu* IUCN 2001) has 30,117 km<sup>2</sup>. Within its EO, there are two Federal and six State strictly protected areas (equivalent to IUCN category II, IUCN 1994), namely from south to north Parque Nacional (PARNA) Serra do Cipó \* (338 km<sup>2</sup>), PARNA Sempre-Vivas (1241.5 km<sup>2</sup>), Parque Estadual (PE) Serra do Intendente \* (135.1 km<sup>2</sup>), PE Pico do Itambé (46.9 km<sup>2</sup>), PE Biribiri (169.9 km<sup>2</sup>), PE Rio Preto (121.8 km<sup>2</sup>), PE Serra do Cabral \* (224.9 km<sup>2</sup>), and PE Serra Nova \* (126.5 km<sup>2</sup>), all in the state of Minas Gerais. PE Grão Mogol (284 km<sup>2</sup>) and PE Caminho das Gerais (562.3 km<sup>2</sup>), at the northern state of Minas Gerais, are really close to the species EO border but not within it. There is no Federal or State strictly protected areas within its EO in the state of Bahia. Nonetheless, *Odontophrynus juquinha* sp. nov. has vouchered records only for the four reserves above highlighted by an asterisk.

The Espinhaço range is known for its extremely high species richness and large number of microendemisms, both for amphibian (Leite *et al.* 2011, 2012; Barata *et al.* 2013; Carvalho *et al.* 2013; Araujo-Vieira *et al.* 2015) and plant species (Silveira *et al.* 2015). Nevertheless, it has currently been subject of many antropogenic threats. Among major disturbances frequently associated to the Espinhaço, opencast mining, uncontrolled urbanization, especially linked to tourism expansion, and eucalypt and pine plantations are probably the most likely negatives

impacts to *Odontophrynus juquinha* **sp. nov.** populations. However, due to the relatively large distribution of the new species along the Espinhaço, and the fact it occurs in some reserves, these impacts may be geographically restricted. At Serra do Cipó (type locality), where authors usually make field trips at least once every two years, on average, since 1999, it is unlikely that *Odontophrynus juquinha* **sp. nov.** populations are declining. At the beginning of the rainy season, the species can be found (or heard) easily, in suitable habitats, being not a rare one. Therefore, we suggest that the new species does not meet the criteria and does not qualify for listing in a threatened category, been evaluated as Least Concern (LC), according to IUCN (2001).

**Etymology.** The specific epithet is a noun in apposition and honors “Juquinha das Flores”, a hermit whose simplicity and gentleness made him a folkloric figure at Serra do Cipó, type locality of the new species. According to the legend, Juquinha nursed from a wolf, ate scorpions, was bitten by over a hundred snakes and his age exceeded a hundred years. He used to collect flowers and roots and trade them for general items or food. His love for the mountains and the joy he provided to those who met him made him a very popular and loved figure.

Juquinha lived and died twice. The first time, he was found dead by his brother and woke up during his own funeral, strengthening the belief that he was immortal. In fact, he suffered from catalepsy, a rare disease that causes the heart to stop, as if the person had died. The second and actual death happened in 1983, at unknown age. In 1987, a homage statue to Juquinha was erected at the Serra do Cipó and became a tourist spot.

## Discussion

Since the discovery of the first case of polyploidy among vertebrates in the 1960s (Beçak *et al.* 1966; Bogart 1967), the tetraploid *Odontophrynus americanus* has been subject of numerous cytogenetic and gene expression studies (Barrio & Pistol de Rubel 1972; Beçak & Beçak 1974; Schmidtke *et al.* 1976; Ruiz *et al.* 1981; Rosset *et al.* 2006; and literature cited therein). In the meantime, morphologically similar diploid specimens ( $2n = 2x = 22$ ) have been found in allopatry, sympatry, and syntopy, composing a complex geographic distribution that constitutes the currently known *O. americanus* species group (Ceï 1985; Beçak *et al.* 1970; Barrio & Pistol de Rubel 1972; Rosset *et al.* 2006).

The difficulty to find any significant morphological difference between diploid and tetraploid specimens has delayed the taxonomic changes in the *Odontophrynus americanus* group. Traditionally, this struggle to diagnose cryptic diploid-polyploid species is also observed in other genera (e.g. *Dryophytes chrysosecelis* (Cope 1880) (2x) / *D. versicolor* (LeConte 1825) (4x) (Hylidae), Wasserman 1970; *Bufo viridis* (Laurenti 1768) complex (2x, 3x, 4x) (Bufonidae) Pisanetz 1978; Stöck *et al.* 2001). However, differences in ploidy are usually associated with differences in geographical distribution and mating behavior (e.g. advertisement calls), which reinforce the reproductive isolation between lineages (Wasserman 1970; Stöck 1998; Martino & Sinsch 2002; Holloway *et al.* 2006). The exception is currently the pair *Phyllomedusa distincta* Lutz, 1950 (2x) / *P. tetraploidea* Pombal and Haddad, 1992 (4x), (Pombal & Haddad 1992; Haddad *et al.* 1994) whose advertisement calls are very similar, geographical distribution overlaps, and natural hybrids are commonly found. In other cases, diploid-tetraploid similar species can be separated by a combination of behavioral, cytogenetic, morphological features, and geographic distribution, as observed in the genus *Neobatrachus* Peters, 1863 (Limnodynastidae) (Mahony & Roberts 1986; Mable & Roberts 1987).

Within the *Odontophrynus americanus* species group, in addition to the differences in the chromosome number, some morphological characters (e.g. adult size, coloration, pattern of dorsal glandular warts) allowed the description of three diploid species, despite some degree of variability: *O. lavillai* from the “Gran Chaco” (Barrio & Pistol de Rubel 1972; Ceï 1985), *O. maisuma* from coastal dunes of Uruguay and southern Brazil (Rosset 2008), and *O. juquinha* **sp. nov.** from the Espinhaço range in central-eastern Brazil (present work). On the other hand, the diploid *O. cordobae*, from Pampas Mountains of Argentina, and the widely distributed tetraploid *O. americanus* are morphologically very similar and can be diagnosed mainly by differences in the structure of their advertisement calls (Martino & Sinsch 2002). In addition, characters such as tadpole morphology, advertisement and release calls can help differentiate *O. americanus*, *O. lavillai*, *O. cordobae*, and *O. maisuma* (Grenat *et al.* 2009; Borteiro *et al.* 2010; Grenat & Martino 2013; Rosset & Baldo 2014).

In the present work, some parameters of the advertisement call (i.e. dominant frequency and pulse rate) also diagnosed the new species from the remaining species of the *Odontophrynus americanus* group. The pulse rate is often associated with the ploidy level in some anurans (e.g. *Dryophytes versicolor* complex, Johnson 1966; Ralin

1968). This is also the case in the *O. americanus* group (Bogart & Wasserman 1972, Martino & Sinsch 2002; Borteiro *et al.* 2010), and have also allowed to differentiate *O. juquinha* **sp. nov.** from *O. americanus* (tetraploid). On the other hand, the dominant frequency of *O. juquinha* **sp. nov.** is similar to the ones of *O. americanus* and *O. cordobae*, higher than *O. lavillai* and lower than *O. maisuma* (see Comparison with other species for details). Interestingly, *O. juquinha* **sp. nov.** has a similar SVL to the ones from *O. cordobae* and *O. americanus* (SVL = 38.45–52.09 mm in males of *O. juquinha*; SVL = 34.5–53.2 mm in males *O. americanus*; SVL = 39.2–51.6 mm in males of *O. cordobae*, Rosset 2008), lower than *O. lavillai* (SVL = 49.4–64.5 in males of *O. lavillai*, Rosset 2008) and higher than *O. maisuma* (SVL = 34.4–40.9 in males of *O. maisuma*, Rosset 2008). As already shown for several anurans, the dominant frequency is often inversely correlated with the SVL (Gerhardt & Huber 2002; Köhler *et al.* 2017; and references therein) and this also appears to be the trend in the *O. americanus* group.

In contrast, the pulse rate of *O. juquinha* is lower than the ones of *O. cordobae* and *O. lavillai*, and higher than the ones of *O. americanus* and *O. maisuma*. Air and water temperature are known to have a positive correlation with temporal features of the advertisement call, such as the pulse rate (Bogart & Wasserman 1972; Gerhardt & Huber 2002). The recordings of *Odontophrynus juquinha* **sp. nov.** made at both Serra do Cipó and Serra do Cabral (there is no information on the temperature of the recordings made at Diamantina) had similar temperature than most of the recordings used in the call description of the remaining species of the *O. americanus* group, allowing for the rightful comparison between them. On the other hand, the pulse rate of *O. maisuma* was based on recordings in which the temperature was considerably lower, which might be related to the lower pulse rate observed (Borteiro *et al.* 2010). Although further studies might reveal an overlap in the pulse rate of *O. juquinha* **sp. nov.** and *O. maisuma* at the same temperature, those species are also diagnosable by the dominant frequency of their advertisement calls and by several morphological features of adults and tadpoles. In addition, these species are separated by over 1400 km (Rosset *et al.* 2006), which possibly consists in a reproductive barrier between them.

As observed in the other diploid species of the *Odontophrynus americanus* group, the secondary constrictions (SCs) of *O. juquinha* **sp. nov.** are on the interstitial region of the short arm of the fourth pair of chromosomes. However, in the tetraploid *O. americanus*, the position of the SCs (and NORs) is different and polymorphic, being located on the proximal region of the short arm of the chromosomes of the group 4, and/or on the interstitial region of the long arm of the chromosomes of the group 11, plus additional SCs and NORs on other chromosomes (Beçak *et al.* 1966, 1970; Ruiz *et al.* 1984; Rosset *et al.* 2006; Salas & Martino 2007; and literature cited therein).

Currently, the tadpoles of all *Odontophrynus* species are known (Nascimento *et al.* 2013; Menegucci *et al.* 2016; and literature cited therein). The tadpole of the new species is a typical pond dwelling anuran larvae and it shows similar characters to those of the remaining species of the *O. americanus* group. Its smaller size distinguished it from the other species of the group and, together with *O. monachus* Caramaschi & Napoli, 2012, is characterized by the smaller size when compared to those of the genus *Odontophrynus* (Nascimento *et al.* 2013; Menegucci *et al.* 2016; and literature cited therein).

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## APPENDIX. Specimens examined.

*Odontophrynus achalensis*.—**ARGENTINA**: CÓRDOBA PROVINCE: Pampa de Achala, Hostería El Cóndor (CENAI 2792, 2970–3, 2975–9); Pampa de Achala (CENAI 3542–3, 3546, 3795–7, 3862–4, 4303, 4352, 4390–1, 4484, MACN 24937, 33438–9, MLP A. 694); Provincial Road 14, 7 km Paraje El Cóndor, Estancia Santo Tomás (MLP A. 3926–8).

*Odontophrynus americanus*.—**ARGENTINA**: BUENOS AIRES PROVINCE: 20 km S Magdalena (MLP A. 3980); Bahía Blanca (CENAI 9637); Bella Vista (MACN 11194); Benavidez (CENAI 5700); Cristiano Muerto-Energía, Cortaderas stream (CENAI 10910); Vicente Pereda-Ariel (MACN 21731); Carlos Casares (CENAI 9682); Casey (MACN 16520); Cazón, 10 km NW Saladillo (MACN 11720); City Bell (MLP A. 3714); D'Orbigny, Estancia San Pablo (MACN 21864); Estancia La Esperanza (MACN 29688); El Triunfo, Estancia San Enrique (MACN 23308); Energía, Estancia Los Médanos (MACN 21092); Estación Timote (MACN 25377); Ezpeleta (CENAI 3942); José C. Paz (MACN 20672); Libres del Sur (MACN 23725); Pehuén-Co (CENAI 93); Provincial Road 76, near Pourtalé (MACN 30110); Punta Indio (MLP A. 3713); San Clemente del Tuyú (MACN 30250); Santa Inés, Estancia El Guanaco (MACN 25236); Sierra de Curamalal, Cerro Pan de Azúcar (MACN 28271); Sierra de la Ventana, Cerro Tres Picos (MACN 25889); Tandil (MACN 21695); LA PAMPA PROVINCE: General Pico (MACN 19436–7); Lihuel Calel (MACN 34336); Río Salado and National Road 143 (CENAI 9778); Santa Rosa (CENAI 6663); CÓRDOBA PROVINCE: Bell Ville (CENAI 10907); James Craig (CENAI 8043); CORRIENTES: Isla San Martín (MLP A. 3720–1); 15 km NO San Carlos (MACN 29577); Colonia Carlos Pellegrini (MACN 23173); General Alvear (= Alvear) (MACN 22941); Felipe Yofré (CENAI 2399); Solari (= Estación Justino Solari) (CENAI 4733); Curuzú Cuatiá (UNNEC 2256); ENTRE RÍOS PROVINCE: 10 km NE Los Conquistadores, Estancia Buena Esperanza (MACN 23549); Concepción del Uruguay (MACN 25103); Médanos (CENAI 9793–4); Mojones Sur (MACN 38103, MLP A. 3715); old National Road 12 (MACN 38101); Paranacito (MACN 23604); Parque Nacional El Palmar (MLP A. 560); Pronunciamento (MACN 27902); Tres Bocas (MLP A. 3712, 3891); SAN LUÍS PROVINCE: Alto Pencoso (IBA 2100); Justo Daract (CENAI 6560); La Florida (IMCN-UNSJ 4050); Santa Rosa del Conlara (MACN 33915); Volcán, El Volcán stream (CENAI 1661); SANTA FE PROVINCE: 12 km SE Villa Eloisa (MACN 24580); Romag-Alejandra, Gusano stream (MACN 25835); Las Rosas (MACN 24223); Roldán (CENAI 10870); Tostado (MACN 8359–60); Venado Tuerto (MACN 28177); SANTIAGO DEL ESTERO PROVINCE: Colonia Dora (MACN 11452, 11455); Pinto (= Estación Pinto) (CENAI 10885); Villa General Mitre (MACN 26195); TUCUMÁN: San Javier (= Villa San Javier) (MACN 25853); Yerba Buena (FML 2158). **URUGUAY**: ARTIGAS DEPARTAMENT: Yuquery (ZVCB 991); CANELONES: Canelones (CENAI 3928); CERRO LARGO DEPARTAMENT: Paso Durazno, 8 km SE Aceguá (MACN 36299); de la Mina stream, 8 km SE Aceguá (ZVCB 2260); COLONIA DEPARTAMENT: Las Limetas stream (ZVCB 884); RIVERA DEPARTAMENT: Establecimiento Trinidad (ZVCB 5327); SALTO DEPARTAMENT: near Salto, Algarrobo stream (ZVCB 1596); Salto Grande (= Salto) (ZVCB 1015); TREINTA Y TRES DEPARTAMENT: Santa Clara de Olimar (ZVCB 111). **PARAGUAY**: CENTRAL DEPARTAMENT: Villeta (MHNP 235); ITAPÚA: Isla Yasyretá, Estancia Melgarejo (MHNP 4083, 4103). **BRAZIL**: MINAS GERAIS STATE: Conceição do Ibitipoca, municipality of Lima Duarte (MNRJ 43499–503); Juiz de Fora (MNRJ 18511); Morro do Ferro, municipality of Poços de Caldas (MNRJ 87820–1); PARANA STATE: municipality of Figueira (MNRJ 48509); SANTA CATARINA STATE: Três Barras (MNRJ 1531); RIO DE JANEIRO STATE: Fazenda Marimondo, municipality of Resende (MNRJ 77491); RIO GRANDE DO SUL STATE: Augusto (MNRJ 3835); Caxias do Sul (MCP 1614); Derrubadas (MCP 1576); General Câmara (MCP 4065); São Borja (MCP 7535); São Francisco de Paula (MCP 3664); Viamão (CENAI 7002); Candiota (MCP 4203); SANTA CATARINA STATE: municipality of Palhoça (MNRJ 74318–22). SÃO PAULO STATE: Eugenio Lefevre (MACN 17189, 17191); Campos do Jordão (MNRJ 26167, 26185, 40069); Colônia Santa Maria, municipality of Botucatu (MNRJ 7414); Fazenda Regina, municipality of Itatinga (MNRJ 70991); Lageado, municipality of Botucatu (MNRJ 81121–5, 69418–27); municipality of Pirajú (MNRJ 21604–6); Rodovia Castelo Branco KM20, municipality of Osasco (MNRJ 98937).

*Odontophrynus barrioi*.—**ARGENTINA**: CATAMARCA PROVINCE: Andalgalá: Aconquija, Río Agua de las Palomas (FML 3245); Km 51 Provincial Road 47, between Andalgalá and Capillitas (FML 2182); Belén: Condor Huasi, Río El Carrizal (FML 3699, 7 specimens); Pomán: Mutquín (CENAI 1368–71); LA RIOJA PROVINCE: Arauco: Aimogasta, Finca Aschá (MACN 24999); Castro Barros: Chuquis (MACN 37502–3); Chilecito: Cachiyuyal (CENAI 0642–7, 0649, 1016–8, 9823–5, IBA 1645); Cuesta de Miranda (CENAI 255, 5 specimens); Famatina: Aguadita Springs, Cuesta La Aguadita, 30km N Famatina (MZUSP 57635, 57637–9, MACN 38691, holotype and paratypes); MLP A. 3871–80, 3883–5; Independencia: Parque Nacional Talampaya (MLP A. 3718); SAN JUAN PROVINCE: Jachal: Ischigualasto (MACN 12979–83, 21433); Valle Fértil: Astica stream (MACN 35361); Río Las Tumanas (IMCN-UNSJ 545); San Agustín del Valle Fértil (CENAI 0348).

*Odontophrynus carvalhoi*.—**BRAZIL**: BAHIA STATE: Lagoa da Rabicha, municipality of Caetité (MNRJ 32988); municipality of Maracás (MNRJ 03981, 15356–7, 35783, 86508); MINAS GERAIS: municipality of Cristália (MNRJ 50199–200); PERNAMBUCO STATE: Poção (MNRJ 00313, holotype).

*Odontophrynus cordobae*.—**ARGENTINA**: CÓRDOBA PROVINCE: Calamuchita, Villa General Belgrano, El Sauce (ZFMK 73460, holotype now in MLP); Cruz Grande (MACN 25749); Dean Funes (CENAI 4522, 4524); Jesús María, Barrio La Florida (LGE 07415, 07420–1, 07437); National Road N° 9, km 771 (LGE 00324–5); San José de la Dormida (CENAI 6756); Santa Rosa de Calamuchita (CENAI 0400); Tanti (MLP A. 3887, 3930); SANTIAGO DEL ESTERO PROVINCE: Villa Ojo de Agua (MACN 26199–200).

*Odontophrynus cultripes*.—**BRAZIL**: MINAS GERAIS STATE: municipality of Belo Horizonte (MNRJ 00315, 51045–7, 51049–51); municipality of São Gonçalo do Rio Abaixo (MNRJ 21401, 34498); Fazenda do Rio Verde, municipality of João Pinheiro (MNRJ 42092); municipality of Codisburgo (MNRJ 51048); municipality of Araxá (MNRJ 51052); Povoado de Gagé,

municipality of Conselheiro Lafaiete (MNRJ 60818, 65100); Serra do Caraça, municipality of Mariana (MNRJ 60337–8, 83219–22); Fazenda do Engenho, municipality of Campos Gerais (MNRJ 66205); municipality of Viçosa (MNRJ 80652); municipality of Grão Mogol (MNRJ 88979–80).

*Odontophrynus juquinha* **sp. nov.** (not cited in the text).—**BRAZIL:** MINAS GERAIS STATE: Serra do Cipó, Jaboticatubas (MNRJ 38017, 45334).

*Odontophrynus lavillai*.—**ARGENTINA:** CHACO PROVINCE: 40 km SE Santa Sylvina (MACN 36796); Juan José Castelli (CENAI 6063); Pampa Grande (MACN 37022–3); CÓRDOBA PROVINCE: Totoralejos (CENAI 2633); FORMOSA PROVINCE: Estancia Don Teo (UNNEC 2084); El Colorado (IBA 1472); Florencia (= La Florencia) (IBA 1469); Formosa (MACN 15526); National Road 81, between Pirané and Palo Santo (MACN 35576); JUJUY PROVINCE: San Salvador de Jujuy, Km 7 (CENAI 9783); SALTA PROVINCE: Finca San Javier, 8 km S Joaquín V. González (FML 4911, 4915, 4922); Gaona (FML 11159); SANTA FE PROVINCE: Margarita (CENAI 6567, 6569); Vera y Pintado, Estancia La Cabaña (CENAI 9771–3); SANTIAGO DEL ESTERO PROVINCE: Casa del Tigre stream, 2 km Villa La Punta (FML 3701–2, holotype and paratype); Choya (CENAI 9752, 9754); Frías (CENAI 5794); El Caburé (MACN 26933); Monte Quemado (FML 3580); Río Saladillo (CENAI 9760–1); Villa La Punta (CENAI 239). **BRAZIL:** MATO GROSSO DO SUL STATE: Fazenda Fronteira, Porto Murinho (CFBH 02496). **PARAGUAY:** ALTO PARAGUAY DEPARTAMENT: Parque Nacional Defensores del Chaco (MHNP 1084); Puerto Casado (MACN 8298); BOQUERÓN DEPARTAMENT: Estancia Pozo Azul (MHNP 1077); PRESIDENTE HAYES DEPARTAMENT: Estancia Juan de Salazar (MHNP 3433); Estancia La Victoria, Km 234 Ruta Trans-Chaco (MHNP 1089); Km 347 Ruta Trans-Chaco, 24 km NW Río Verde (MHNP 1075); Rancho Carandá, 14 km W Km 323 Ruta Trans-Chaco (MHNP 10418).

*Odontophrynus maisuma*.—**BRAZIL:** SANTA CATARINA STATE: Balneario Gaiivotas (MNRJ 31356); RIO GRANDE DO SUL STATE: Tavares, Lagoa do Peixe (MZUSP 65312–3); Torres (MZUSP 122183–4; MNRJ 31420); Tramandaí (MZUSP 26825). **URUGUAY:** CANELONES DEPARTAMENT: Ruta Interbalnearia, km 63 (ZVCB 1024); MONTEVIDEO DEPARTAMENT: Carrasco (CENAI 3492); ROCHA DEPARTAMENT: Cabo Polonio (ZVCB 1517, 3361–3, 3365–8, 11465, MLP A. 4926–8, holotype and paratypes); La Coronilla (ZVCB 15076, 15078).

*Odontophrynus monachus*.—**BRAZIL:** MINAS GERAIS STATE: Parque Nacional da Serra da Canastra, headwaters of the São Francisco River, Municipality of São Roque de Minas (ZUEC 4440, holotype).

*Odontophrynus occidentalis*.—**ARGENTINA:** BUENOS AIRES PROVINCE: Balneario Orense (CENAI 1652–5, 1657–9, 1663, MLP A. 1680); Claromecó (MACN 31951); Monte Hermoso (CENAI 1660, MACN 28310); CÓRDOBA PROVINCE: 2km N Dean Funes (MACN 33914); Anisacate (IBA 1184); Capilla del Monte (IBA 483, MACN 20671); Cruz Chica (MACN 18337–42); La Cumbre (MACN 24642–4, 26228–31); Las Águilas stream (MLP A. 734); Mina Clavero (CENAI 3445, 3557, 4046, 5926–7); Observatorio (MACN 34175–6); Río Ceballos (CENAI 7856); Río Cruz Grande (MACN 20601–2, 20604–5); Santa Rosa de Calamuchita (CENAI 0091, 3 specimens); Valle Hermoso (CENAI 74, 4 specimens); CHUBUT PROVINCE: Telsen (MLP A. 3717); Verde stream and National Road 3 (MACN 30346–50); MENDOZA PROVINCE: 5km from Mendoza (IBA 2204, 15 specimens); Eugenio Bustos (IBA 2104); El Nihuil (IBA 126, 3 specimens; MACN 29158); La Primavera, 23 km E Mendoza (IBA 1346, 3 specimens); Los Toldos stream, 4km W Los Toldos (IBA 2079, 2 specimens); Llancanelo (MLP A. 596); Malargue (IBA 1571, 4 specimens; MLP A. 2142); San Rafael (MHNSR 50–1, 391, MLP A. 4382); Villa Atuel (MLP A. 3916); NEUQUÉN PROVINCE: Río Agrio (CENAI 112); Agrio stream (MACN 380, holotype); Bajada del Agrio (IBA 1314, 7 specimens); RÍO NEGRO PROVINCE: El Rincón (MLP A. 4384–5); General Conesa (MLP A. 2144); General Roca (CENAI 360–1); Meseta de Somuncurá (MACN 38689–90); Nahuel Niyeu stream (MACN 28405); Valcheta stream (IBA 2036, 8 specimens); Viedma (MACN 29029); Villa Regina (MACN 31385); SAN LUIS PROVINCE: Cerro Retana (MACN 38685–7); El Trapiche (IBA 1315, 6 specimens); La Carolina (MACN 38688, 29866); Papagayo (MACN 29367–8).

*Odontophrynus salvatori*.—**BRAZIL:** DISTRITO FEDERAL: Poço Azul, APA Cafuringa, municipality of Brasília (MNRJ 23631); GOIÁS STATE: Parque Nacional Chapada dos Veadeiros, municipality of Alto Paraíso (MNRJ 15870, holotype, 15871 paratype, 27782–6); Parque Estadual Pirineus, municipality of Pirenópolis (MNRJ 23632).