CARLA DA SILVA GUIMARÃES

TAXONOMIA E HISTÓRIA NATURAL DE ANFÍBIOS ANUROS DA SERRA DO BRIGADEIRO, MATA ATLÂNTICA, MINAS GERAIS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Animal, para obtenção do título de *Magister Scientiae*.

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APROVADA: 01 de abril de 2016.

P.Santos
Patrícia Silva Santos

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Felipe Sá Fortes Leite

Renato Neves Feio
(Orientador)


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MAS O CACHORRO CUIDA DA CASA! É COMPANHEIRO NOS PASSEIOS, NA ALEGRIA E NA TRISTEZA!
O QUE UM SAPO FAZ POR VOCÊ?!
EU NÃO TENHO AMIGOS POR INTERESSE, MINHA SENHORA!
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RESUMO

GUIMARÃES, Carla da Silva, M. Sc., Universidade Federal de Viçosa, abril de 2016. **Taxonomia e história natural de anfíbios anuros da Serra do Brigadeiro, Mata Atlântica, Minas Gerais**. Orientador Renato Neves Feio.

No presente trabalho são apresentados novos dados sobre taxonomia e história natural de espécies de anfíbios anuros da Serra do Brigadeiro, Mata Atlântica, porção norte do complexo serrano da Mantiqueira, sudeste do Brasil. Entre os capítulos abordados, destaca-se o reconhecimento de duas novas espécies pertencentes aos gêneros *Brachycephalus* e *Scinax.* A primeira, conhecida popularmente como sapinho-pingo-de-ouro, anteriormente identificada como *B. ephippium,* tem sua nova identidade subsidiada por caracteres morfológicos (ex. grande tamanho dos adultos, corpo bufoniforme completamente amarelo–alaranjado em vida), osteológicos (ex. placa paravertebral convexa bem desenvolvida, primeira placa vertebral convexa em forma de trapézio e a segunda de retângulo, respectivamente), histológicos (presença de tecido conjuntivo negro envolvendo a musculatura dorsal) e bioacústicos (ex. canto de anuncio com estruturas harmônicas). A segunda nova espécie, até então reconhecida como *Scinax* sp. (aff. *rizibilis*), pertence ao complexo grupo de espécies *S. catharinae* e tem sua nova identidade sustentada por caracteres morfológicos em adultos (ex. tamanho médio entre 23.03–33.59 mm, focinho subovóide/subelíptico, machos com saco vocal expandido lateralmente, antebraço e calo nupcial hipertrofiados) e girinos (ex. disco oral com pequeno interrupção dorsal, papilas marginais unisseriada alternada, fórmula oral 2(2)/3), de bioacústica (ex. canto de anúncio com duração de 0.8–1.52s, caracterizado por uma série de cinco notas pulsionadas, com 60 pulsos/notas, pico de energia entre 3100.8–4565 Hz), e moleculares (ex. distâncias intraespecífica 0–2.9% e interespecífica 11%–16%). Ambas espécies têm sua distribuição geográfica conhecida apenas para a Serra do Brigadeiro. Além disso, é descrito a fase larval da espécie *Scinax cosenzai*, a qual é descrita com localidade tipo na Serra do Brigadeiro. Os girinos desta espécie bromelígena são caracterizados pela ausência de manchas amarelas ou douradas, comprimento total entre 19.9–31.1 mm, disco oral constituído de uma fileira de papilas marginais na porção anterior e alternada na ventral, e nadadeiras com a mesma altura. Por fim, é apresentada uma lista atualizada das espécies de anfíbios para a Serra do Brigadeiro contento 61 registros.

ABSTRACT

GUIMARÃES, Carla da Silva, M. Sc., Universidade Federal de Viçosa, April, 2016. **Anurans taxonomy and natural history from Serra do Brigadeiro, Atlantic Forest, Minas Gerais.** Advisor: Renato Neves Feio.

This paper presents new data on the taxonomy and natural history of anurans from the Serra do Brigadeiro, Atlantic Forest, northern portion of Serra da Mantiqueira mountain range, southeast Brazil. Among the chapters presented, it is emphasized the recognition of two new species belonging to the genus *Brachycephalus* and *Scinax*. The first, popularly known as pumpkin toadlets and previously classified as *B. epphipium*, has its new identity supported by morphology (e.g. large size of adults, bufoniforme body and completely yellow-orange in life), osteology (e.g. convex central shape and laterally rounded paravertebral plates, first spinal plate convex with trapezium-shaped), histology (e.g. presence of black connective tissue covering all dorsal muscles), and bioacustic (e.g. presence of harmonic structures in its advertisement call). The second new species, so far recognized as *Scinax* sp. (aff. *rizibilis*), belongs to the complex group of species *S. catharinae*. Its new identity is sustained by morphology in adults (e.g. medium size, subovoid/sublliptical snout in dorsal view, males with vocal sac expanded and hypertrophied forearms and nuptial pad) and tadpoles (e.g. oral disc with small dorsal gap in marginal papillae, marginal papillae unisseriated, oral formula 2(2)/3), bioacustics (advertisement call characterized by a series of usually five pulsed notes, call duration 0.8–1.52 s, with about 60 pulses each, and peak of energy between 3100.8–4565 Hz), and molecular (e.g. intraspecific 0–2.9% and interespecific 11%–16% distances). Both species are only known for the Serra do Brigadeiro and their names honor the Serra da Mantiqueira mountain range, from which Serra do Brigadeiro is included. Moreover, we describe the tadpoles *Scinax cosenzai,* species with type locality at the Serra do Brigadeiro. The tadpoles of this bromeligenous species are characterized by the lack of any yellow or golden marks, total length of 19.9–31.1, anterior portion of oral disc with a single row of labial papillae, dorsal and ventral fins equal in height. Finally, it is presented an updated list of amphibians from the Serra do Brigadeiro, with 61 registers.

1. INTRODUÇÃO GERAL

Berço de uma diversidade extraordinária de espécies, o bioma Mata Atlântica destaca-se mundialmente entre as florestas tropicais pela sua elevada riqueza e alto índice de endemismo de suas espécies (Myers *et al.* 2000, Mittermeier *et al.* 2004). Entretanto, na contra mão, devido a intensa e desordenada ação humana sobre o meio ambiente este bioma encontra-se amplamente degradado, restando atualmente 5–8% de sua cobertura original (Fontes *et al.* 2000, SOS Mata Atlântica 2008).

Em Minas Gerais, a Mata Atlântica mantém o padrão de fragmentação e antropização observado no restante do Brasil, sendo que os remanescentes mais significativos se encontram encravados nos altos de serras, mantidos preservados pela própria dificuldade de acesso imposta pelo relevo (Fontes *et al.* 2000; Haddad *et al.* 2013). Coincidentemente ou não, a maioria dos estudos sobre herpetofauna realizados na Mata Atlântica no estado contemplam as regiões serranas, como aquelas que constituem o complexo da Mantiqueira (Nascimento *et al.* 2009, Bérnils *et al.* 2009, Moura *et al.* 2012).

Nesse contexto, destaca-se a Serra do Brigadeiro, um dos maiores remanescentes desse bioma localizada na porção norte do conjunto serrano da Mantiqueira, considerada de “Extrema” importância dentre as “áreas prioritárias para a conservação da biodiversidade de Minas Gerais” (Drumond *et al.* 2005). Apresenta um relevo bastante movimentado, com cota máxima de altitude de 1.985m, coberto predominantemente por Floresta Estacional Semidecidual Montana e campos de altitude nas áreas mais elevadas (Veloso *et al.* 1991; Moura *et al.* 2012). Inserido na serra encontra-se o Parque Estadual da Serra do Brigadeiro (PESB), importante unidade de conservação que abrange os municípios de Araponga, Divino, Ervália, Fervedouro, Miradouro, Muriaé, Pedra Bonita e Sericita.

Em seus quase vinte anos de existência, o PESB já proporcionou importantes contribuições ao conhecimento e conservação da fauna, principalmente dos anfíbios (Moura *et al.* 2012), sendo classificado como área de importância especial para conservação desses animais no estado de Minas Gerais (Drummond *et al.* 2005). Os estudos com esse grupo se iniciaram na década de 1990 através de incursões esporádicas ao longo estações chuvosas (Moura *et al.* 2012), e tiveram continuidade até o presente projeto, subsidiando trabalhos de monografias e dissertações de estudantes da Universidade Federal de Viçosa, Minas Gerais (ex. Santos 2003; Assis 2009; Motta 2010; Moura *et al.* 2012; Guimarães 2013).

A trajetória desses estudos possibilitou a descoberta de novas espécies (*Physalaemus maximus* Feio *et al.* 1999, *Chiasmocleis mantiqueira* Cruz *et al.* 2007, *Leptodactylus cupreus* Caramaschi *et al.* 2008, *Scinax cosenzai* Lacerda *et al.* 2012, e mais recentemente, *Procetatophrys mantiqueira* Mângia *et al.* 2014), e registro de 58 espécies de anfíbios (Moura *et al.* 2012), contendo 9 espécies com identidade inespecífica (*Brachycephalus* aff. *didactylus*, *Ischnocnema* gr. *guentheri*, *Gastrotheca* aff. *ernestoi*, *Phasmahyla* sp., *Scinax* sp. (aff. *perereca*), *Scinax* sp. (aff. *perpusillus*), *Scinax* sp. (aff. *rizibilis*), *Crossodactylus* gr. *gaudichaudii* e *Leptodactylus* sp. (aff. *mystaceus*)). Além desses resultados, vários outros trabalhos voltados para história natural e distribuição geográfica das espécies também foram realizados (Tabela 1**)** possibilitando a complementação do conhecimento acerca da biologia das espécies. Este histórico evidencia a importância biótica da Serra do Brigadeiro e ilustra importantes lacunas de trabalhos sobre anurofauna, tornando fundamental o desenvolvimento de novas pesquisas de cunho taxonômico, de história natural, biogeográfico e de conservação das espécies ali ocorrentes.

Essa perspectiva é observada não só na Serra do Brigadeiro, mas também em âmbito estadual e nacional. Embora o Brasil seja destaque mundial pela alta diversidade de anfíbios, com mais de 1020 espécies (SBH 2014), sua real riqueza é ainda substimada, com dezenas de novas espécies sendo anualmente descobertas e descritas. Para se ter uma idéia, nos últimos 15 anos foram descritas para o Brasil aproximadamente 340 espécies de anfíbios, o que equivale a 33% da diversidade atual desse grupo no país (Frost 2016, SBH 2014). Além disso, a falta de conhecimento sobre a biologia, taxonomia e a distribuição geográfica das espécies dificulta tentativas de determinação do estado de conservação de grande parte dos anfíbios ressaltando a importância de estudos sobre os mesmos (Machado *et al.* 1998, Haddad 2008, Leite *et al.* 2008).

Diante deste panorama, a presente dissertação é apresentada sob as temáticas da Taxonomia e História Natural, as quais são compostas por trabalhos independentes que se propõem a solucionar parte das lacunas no conhecimento da anurofauna da Serra do Brigadeiro, que permaneceram ao longo dos anos de inúmeras pesquisas.

Tabela 1. Trabalhos sobre história natural de anfíbios encontrados na Serra do Brigadeiro, Minas Gerais, Brasil.

Ano	Autores	Título
2009	Lacerda J.V., Assis B., Santana D.J. e Feio R.N.	Anurans in bromeliads, Parque Estadual da Serra do Brigadeiro, state of Minas Gerais, southeastern Brazil
2010	Mângia S., Santana D.J. e Feio R.N.	Advertisement call of the cycloramphid toad *Proceratophrys melanopogon* (Miranda-Ribeiro, 1926)
	Motta A.P., Pirani R.M., Silva E.T., Santana E.T., Mângia S. e Feio R.N.	New record and distribution extension of *Zachaenus carvalhoi* Izecksohn 1983 (Anura, Cycloramphidae) in south-eastern Brazil
	Motta A.P., 2, Silva E.T., Feio, R.F. e Dergam J.A.	The tadpole of *Leptodactylus cupreus* Caramaschi, Feio & São Pedro, 2008 (Anura, Leptodactylidae)
	Moura M.R., Dayrell J.S. e Feio R.N.	*Dendropsophus decipiens* and *Dendropsophus minutus*: Defensive Behaviour
	Moura M.R. e Feio R.N.	*Bokermannohyla caramaschii* (Caramaschi's treefrog): Defensive Behavior
	Moura M.R. e Feio R.N.	Hypsiboas faber (Wied-Neuwied) predation on *Scinax* aff. *perereca* (Anura: Hylidae) in southeastern Brazil
	Moura M.R., Santana D.J., Mângia S. e Feio R.N.	*Proceratophrys melanopogon* (Black-bearded Horned Leaf Toad): Defensive Behavior
	Moura M.R., Feio R.N. & Dixo M.	*Zachaenus carvalhoi* (Carvalho's Bug-eyed Frog): Defensive Behavior
	Pirani R.M, Mângia S., Santana D.J., Assis B. e Feio R.N.	Rediscovery, distribution extension and natural history notes of *Hylodes babax* (Anura, Hylodidae) with comments on southeastern Brazil biogeography
2011	Moura, M.R., Motta A.P. e Feio R.N.	An unusual reproductive mode in *Hypsiboas* (Anura: Hylidae)
	Mott T., Moura M.R., Maciel A.O. e Feio, R.N.	Review of the distribution of *Luetkenotyphlus brasiliensis* tken, 1851) (Amphibia, Gymnophiona, Siphonopidae) with notes on morphological variation
	Pirani R.M., Pezzuti T.L., Motta A.P. e Feio, R.N.	The tadpole of *Hylodes babax* Heyer, 1982 (Amphibia, Anura, Hylodidae)
	Silva E.T., Coelho H.L., Viana R.S. e Santos P.S.	Amphibia, Anura, Leiuperidae, *Physalaemus feioi* Cassini, Cruz and Caramaschii, 2010 distribution extension
2012	Moura M.R., Lacerda J.V. e Feio R.N.	The advertisement call of *Haddadus binotatus* (Spix, 1824) (Anura; Craugastoridae)
	Moura, M.R., Motta A.P., Fernandes V.D. e Feio R.N.	Herpetofauna da Serra do Brigadeiro, um remanescente de Mata Atlântica em Minas Gerais, Sudeste do Brasil
	Santana D.J., Motta A.P., Pirani R.M., Silva E.T. e Feio, R.N.	Advertisement Call and Tadpole of *Chiasmocleis mantiqueira* Cruz, Feio and Cassini, 2007 (Anura, Microhylidae)
	Taucce P.G., Leite F.S., Santos P., Feio R.N. e Garcia P.C.	The advertisement call, color patterns and distribution of *Ischnocnema izecksohni* (Caramaschi and Kisteumacher, 1989)
2013	Coelho C.A., Moura M.R. e Feio, R.N.	*Zachaenus carvalhoi* (Carvalho's Bug-eyed Frog): Reproduction
	.S., Lacerda J.V. e Feio R.N.	Advertisement call of *Zachaenus carvalhoi* Izecksohn, 1982 (Cycloramphidae) from southeastern Brazil
	Silva E.T., Santos P.S., Coelho H.L., Viana R.S., Carrara R. e Garcia P.C.	New records of *Ischnocnema verrucosa* tken, 1862 and I. surda Canedo, Pimenta, Leite and Caramaschi, 2010 (Anura, Brachycephalidae) in Minas Gerais state, Brazil
2014	Guimarães C.S., Peixoto M.A., Lacerda J.V. e Feio R.N.	The tadpole of *Scinax cosenzai* (Anura Hylidae)
2015	Moura, M.R., Coelho-Augusto, C. & Feio, R.N.	Notes on an unexpected reproductive behavior of *Scinax luizotavioi* (Caramaschi & Kisteumacher, 1989) (Anura, Hylidae)

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2. ARTIGOS CIENTÍFICOS

2.1 ARTIGO I – Guimarães, C.S., Luz, S.B., Rocha, P.C. & Feio, R.N. The dark side of pumpkin toadlet: a new species of *Brachycephalus* (Anura: Brachycephalidae) from Serra do Brigadeiro, southeastern Brazil

2.2 ARTIGO II – Guimarães, C.S., Lyra, M. & Feio, R.N. A new species of the *Scinax catharinae* group from Atlantic Forest of southeastern Brazil.

2.3 ARTIGO III – Guimarães, C.S., Peixoto, M.A.A., Lacerda, J.V.A. & Feio, R.N. Feio. 2014. The tadpole of *Scinax cosenzai* (Anura: Hylidae). *Salamandra*, 50 (2): 99–104.

2.1. ARTIGO I – Guimarães, C.S., Luz, S.B., Rocha, P.C. & Feio, R.N. The dark side of pumpkin toadlet: a new species of *Brachycephalus* (Anura: Brachycephalidae) from Serra do Brigadeiro, southeastern Brazil

The dark side of the pumpkin toadlet: a new species of *Brachycephalus* (Anura: Brachycephalidae) from Serra do Brigadeiro, southeastern Brazil

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Abstract: A new species of the pumpkin toadlet (genus *Brachycephalus*) is described from Serra do Brigadeiro, municipality of Ervália, state of Minas Gerais, southeastern Brazil. Specimes were collected amidst the leaf litter, from 1266–1498 m above sea level. *Brachycephalus* **sp. nov.** is mainly characterized by the presence of black connective tissue covering all dorsal muscles, a well developed convex-shaped paravertebral plates, first spinal plate convex trapezium-shaped, second spinal plate convex rectangle-shaped, and by the presence of harmonic structures in its advisement call.

Key words: Amphibia, *Brachycephalus* **sp. nov**., Atlantic Rainforest, bioacoustics, natural history

Introduction

Popularly known as pumpkin toadlets, the frogs of the genus *Brachycephalus* are characterized by the bright yellow-orange aposematic colors and the high degree of miniaturization, which places the genus among the smallest terrestrial vertebrates (Estrada & Hedges 1996, Pires *et al.* 2005). Due to miniaturization, species of *Brachycephalus* lack some bones of the skull (e.g. neopalatine and columella), phalanges, fingers and toes (Hanken 1993, Trueb & Alberch 1985, Yeh 2002). Moreover, the skull and post-cranial region might present degrees of hyperossification that range from absent to the formation of dorsal plates (Clemente-Carvalho *et al*. 2009).

Endemic to the Brazilian Atlantic Forest and mostly distributed in south and southeast Brazil, species of *Brachycephalus* are known for the states of Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Minas Gerais, Espírito Santo and southern Bahia (Dayrell *et al*. 2006, Napoli *et al*. 2011, Ribeiro *et al.* 2005, 2015, Pie *et al*. 2013). Although the genus comprises 30 recognized species, over 30% of those species were described in the past 3 years (Frost 2016). This clearly indicates that the real diversity of the genus is yet to unveiled (Ribeiro *et al*. 2015).

Although the phylogenetic relationship of *Brachycephalus* is still under study, some of the latest works agree on the presence of two sister clades, one of them further divided in two smaller clades (i.e. clade 1 *sensu* Clemente-Carvalho *et al.* 2011 and Padial *et al.* 2014). On the other hand, Pie *et al.* (2013) and Ribeiro *et al.* (2015) agree on the classical morphological arrangement of the genus in three groups: *B. pernix* Pombal, *didactylus* (Izecksohn) and *ephippium* (Spix). The later comprise species such as *B. ephippium*, which is characterized by the bufoniform body shape and dorsal dermic ossification (Clemente-Carvalho *et al.* 2012, Ribeiro *et al.* 2015). Notes on the natural history of this genus are restricted to a only few species (Almeida-Santos *et al*. 2011, Araújo *et al*. 2012, Dorigo *et al.* 2012, Fontoura *et al*. 2011, Lima *et al.* 2013, Pombal Jr. *et al*. 1994, 1999, Verdade *et al*. 2008, Ribeiro *et al*. 2014).

As discussed by Pie *et al.* (2013), the specific habitats observed in high elevation areas within the Atlantic Forest provides the perfect environment for *Brachycephalus* species. The recent discover of further new species isolated in sky islands matched the cluster proposed by Pie *et al.* (2013) and reinforces the microendemism observed in the genus (Clemente-Carvalho *et al*. 2008, Condez *et al*. 2014, Pie & Ribeiro 2015, Ribeiro *et al.* 2015). However, Pie *et al.* (2013) did not mention the reports of *Brachycephalus* species in the Serra do Brigadeiro mountain range (Dayrell *et al.* 2006, Moura *et al.* 2012), one of the largest remains of Atlantic Forest in the north portion of Serra da Mantiqueira mountain range, Minas Gerais state.

The population of *Brachycephalus ephippium* reported by Dayrell *et al.* (2006) is the most continental record for the genus and over 130 kilometers from its nearest conspecific species. Given the characteristic microendemism of the genus, we investigated the taxonomic status of this population and herein we describe this population as a new species, and provide data on its osteology, histology, bioacoustics and natural history.

Materials and Methods

Specimens were collected at the municipalities of Ervália and Miradouro, south portion of the Serra do Brigadeiro, state of Minas Gerais, Brazil, euthanized in 5% lidocaine, fixed in 10% formalin and stored in 70% ethyl alcohol solution. Sex of some individuals was determined by the presence of testicles in dissected males and by visualization of oocytes through skin semi-transparency in females. Specimens are housed at the Museu de Zoologia João Moojen of Universidade Federal de Viçosa (MZUFV), Viçosa, Minas Gerais state, Brazil. Comparison with other species of *Brachycephalus* was based on literature and specimens deposited at Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro state (MNRJ) and MZUFV.

Fourteen measurements were taken with a micrometric ocular in a stereomicroscope (Olympus SZ61): head length (HL), head width (HW), eye diameter (ED), interorbital distance (IOD), eye-snout distance (END), nostril diameter (ND), internostril distance (IND), arm length (AL), forearm length (FAL), hand length (HAL), thigh length (THL), tibia length (TBL), tarsus length (TAL) e foot length (FL). The snout-vent length (SVL) was taken with a digital caliper (0.01 mm). All measurements are in millimeters. Morphological terminology and description of the snout shape follow Cei (1980) and Heyer (1990).

Osteological descriptions and observations were based on the modified technique from Taylor and Van Dyke (1985), we used potassium hydroxide instead of tripsin and borax. Individuals with different sizes were cleared and double-stained with Alizarin red and Alcian blue to distinguish bone and cartilage. Histology analysis: after thoracotomy, fragments of tissue were removed and immediately immersed in freshly prepared histological fixative Karnovsky (Karnovsky 1965) for 24h at room temperature for morphological analysis. Fixed fragments were dehydrated in ethanol, and embedded in methacrylate resin (Historesin®, Leica). Blocks were cut into 3µm thick histological sections and stained with hematoxylin and eosin (H&E). The slides were visualized, and the images were captured using a light microscope (Olympus BX-60, Olympus, Tokyo, Japan) equipped with a digital camera (Olympus QColor-3; Olympus, Tokyo, Japan).

We analyzed the call from six individuals of *Brachycephalus* **sp. nov.** recorded at Parque Estadual da Serra do Brigadeiro, municipality of Ervália, Minas Gerais state, on 20 November 2015 (between 12h00 and 13h00, air temperature 22°C) and 17 December 2015 (between 17h00 and 18h00, air temperature 19°C). Voucher specimens are housed at MZUFV (MZUFV 16627, 16629, 16630, 16636). Recordings were made using a Marantz PMD661 digital recorder coupled with a Sennheiser ME66/K6 unidirectional microphone, sampling rate 48.0 kHz and 24 bits resolution. Spectral and temporal parameters were measured using Raven Pro 1.5 (Cornell Lab of Ornithology Bioacoustics Research Program) directly from the spectrogram and oscillogram respectively. Spectrogram featured window type Hann; window size = 512 samples; overlap = 70%; hop size = 3.49 ms; DFT size =

1024 samples and; grid spacing = 43.1 Hz. Sound graphics were obtained using Seewave (Sueur *et al.* 2008) package of R platform (R Core Team 2014), using Hanning window, FFT = 512 and 70% overlap. Following Rocha *et al.* (2016), the dominant frequency (DF) was considered as the range from the lowest to the higher values acquired through the spectrogram, representing the most energetic part of the call. The peak frequency (PF) is always within the DF and was given by the Raven Pro 1.5. Temporal parameters measured include call duration (CD), interval between calls (CI), call rate (CR), number of notes per call (NN), note duration (ND), interval between notes (NI), note rate (NR), number of pulses per note (PN) and pulse rate (PR). Further call terminology follow Toledo *et al.* (2015).

Results

Brachycephalus sp. nov.

Brachycephalus ephippium – Dayrell, Cassini & Feio, 2006. *Herpetological Review*, 37 (3), 357.

Brachycephalus ephippium – Moura *et al*., 2012. *Biotaneotropica*, vol. 12, no.1.

(Figures 1 – 3 and 5)

Holotype. MZUFV 16636, an adult male (17.9 mm SVL), collected at “Trilha do Cruzeiro” (20^{o}52’40.7” S, 042^{o}31’14.6” W at 1266m a.s.l.), Parque Estadual da Serra do Brigadeiro (PESB), district of Careço, municipality of Ervália, state of Minas Gerais, Brazil, on 17 December 2015 by C.S. Guimarães, P.C. Rocha, C.L. Assis, R.N. Feio, C. Eisemberg, S. Reynolds and W.T. Davies.

Paratypes. Specimens collected at type locality: MZUFV 16634–35 (males) collected with holotype. MZUFV 16627, 16629–30, 16632–33 (males), and MZUFV 16628, 16631 (females) collected on 20 November 2015 by C.S. Guimarães, P.C. Rocha and R.N. Feio. MZUFV 16491, 16579 (adults, histology), 16780 (female) collected on 13 August 2015 by C.S. Guimarães, P.S. Hote and S.A.B. Luz. MZUFV 15716 (male), 15717, 15718 (juvenile, cleared and stained), 15719 (juvenile), and MZUFV 15720–21 (adult specimens) collected on 4 December 2014 by C.S. Guimarães, P.G. Taucce and B. Lisboa. MZUFV 15557–58, 15559 (histology), 15560–61 (adult specimens), 15565 (male), 15567–15571, 15717 (adult specimens) and MZUFV 15566 (juvenile, cleared and stained) collected on 18 September 2014 by C.S. Guimarães, C.L. Assis and R.N. Feio. Specimens collected at “Mata do Pai Inácio”, PESB, Municipality of Miradouro, State of Minas Gerais, Brazil (20^{o}46’42” S, 42^{o}29’ W at 1340 m a.sl.): MZUFV 6658 (adult), 6659–60 (cleared and stained adults) collected on 5 December 2005 by R.N. Feio, E.F. Oliveira and J.S. Dayrell. MZUFV 2897 (adult) collected on 5 September 1996 by R. Harvey.

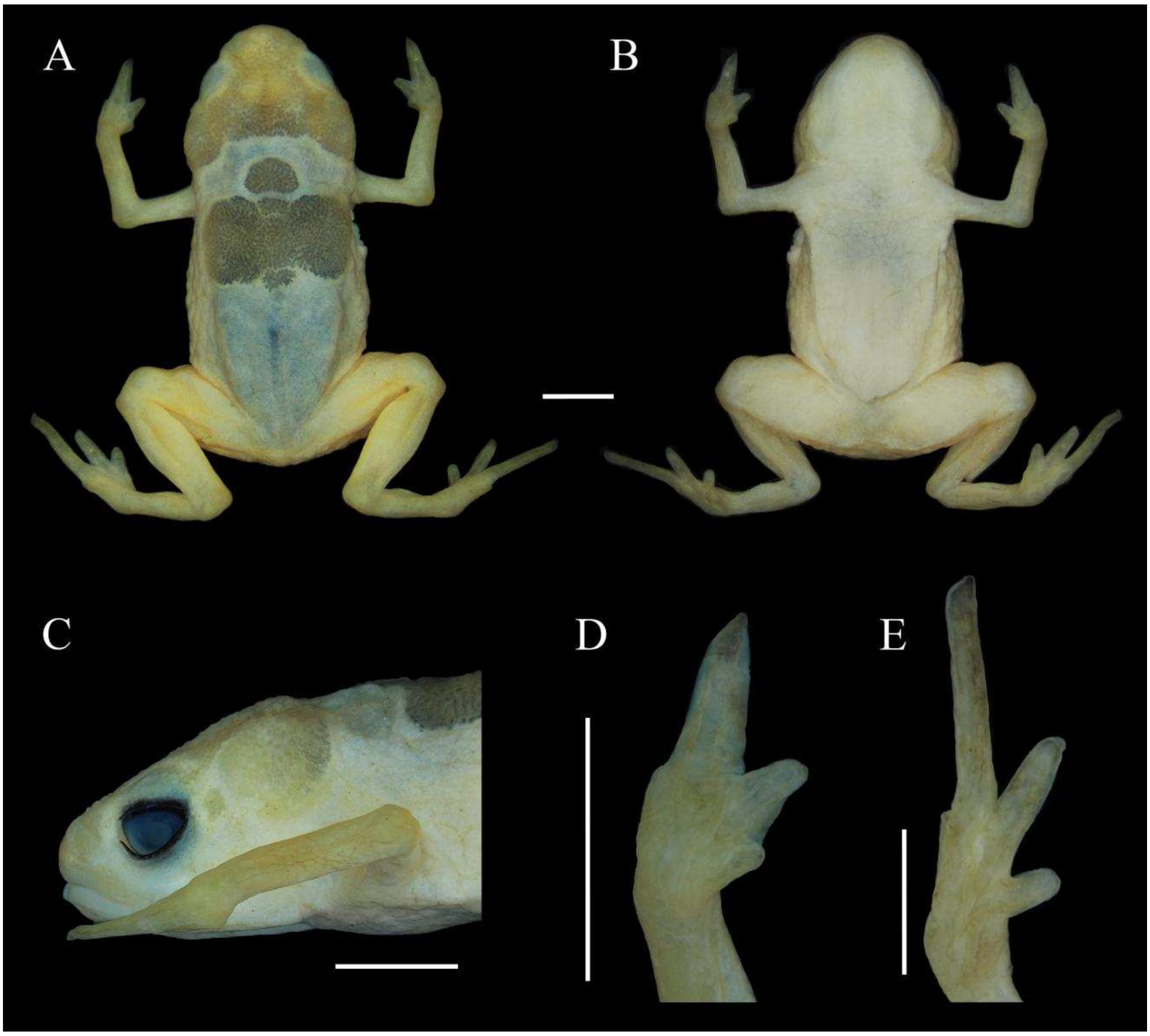

Figure 1. *Brachycephalus* **sp. nov.**, holotype (MZUFV 16636, SVL 17.9 mm). (A) Dorsal and (B) ventral views of the body; (C) lateral view of the head; (D) palmar view of the hand and (E) plantar view of the foot. Scal bar = 3 mm.

Diagnosis. The new species is characterized by (1) larger size (SVL of adult: 14.75–18.52 mm); (2) bufoniforme body; (3) large head; (4) absence of maxillae teeth; (5) absence of metacarpal and metatarsal tubercles; (6) dermal ossification in the skin of head and dorsum; (7) presence of dorsal bony shield; (8) dorsal shield not projected up over the vertebral spine; (9) convex central shape and laterally rounded paravertebral plates; (10) first spinal plate convex trapezium-shaped; (11) parotic plates laterally projected; (12) lack ossified warts; (13) presence of black connective tissue covering all dorsal muscles; (14) body completely yellow-orange in life; (15) presence of harmonic structures in its advertisement call.

Comparison with other species. *Brachycephalus* **sp. nov.** differs from the remaining species of the genus by presence of a well developed convex-shaped paravertebral plate, first spinal plate convex trapezium-shaped, and the presence of black connective tissue covering all dorsal muscles. Additional characters diagnosing the new species are listed below.

Dermal ossification in the skin of head and dorsum of *Brachycephalus* **sp. nov.** distinguishes it from *B. auroguttatus, B. boticario, B. brunneus, B. didactylus, B. ferruginus, B. fuscolineatus, B. hermogenesi, B. izecksohni, B. leopardus, B. mariaeterezae, B. olivaceus, B. pernix, B. pombali, B. pulex, B. quiririensis, B. sulfuratus, B. tridactylus* and *B.verrucosus* (dorsal skin without dermal ossification in the referred species). The new species differs from the remaining species by presence of dorsal bony shield, except *B. ephippium, B. garbeana* and *B. margaritatus* (dorsal bony shield present in those species). The lack of ossified warts distinguishes *Brachycephalus* **sp. nov.** from *B. atelopoide, B. crispus* and *B. margaritatus* (present in those species).

The absence of metacarpal and metatarsal tubercles differs the new species from *Brachycephalus hermogenesi* (metacarpal and out metatarsal presents), *B. auroguttatus, B. boticario, B. brunneus, B. fuscolineatus, B. hermogenesi, B. izecksohni, B. leopardus, B. mariaeterezae, B. olivaceus, B. pitanga, B. pulex, B. quiririensis, B. toby* and *B. verrucosus* (outer metatarsal present). *Brachycephalus* **sp. nov.** differs from *B. brunneus, B. bufonoides, B. ferruginus, B. izecksohni* and *B. pombali* by the absence of maxillae teeth (present in those species). The large head (wider than longer) distinguishes *Brachycephalus* **sp. nov.** from *B. ephippium* and *B. garbeana* (head longer than wider in those species).

Brachycephalus **sp. nov.** is completely orange in life, which distinguishes it from all other species of the genus with colored stripes and/or spots, background greenish, brownish or reddish (exception *B. alipioi, B. atelopoide, B. bufonoides, B. ephippium, B. margaritatus,* also completely orange). In preservative, the new species differs from these exceptions by the darkish background dorsum (pale cream in the referred exceptions).

The new species differs from *Brachycephalus auroguttatus, B. boticario, B. brunneus, B. didactylus, B. fuscolineatus, B. guarani, B. hermogenesi, B. izecksohni, B. leopardus, B. mariaeterezae, B. nodoterga, B. olivaceus, B. pitanga, B. pulex, B. quiririensis* and *B. verrucosus* by larger SVL (combined SVL in referred species 7.0–14.0 < 14.75–18.52). *Brachycephalus* **sp. nov.** also differs from *B. didactylus, B. hermogenesi* and *B. pulex* by the body bufoniform and rounded snout in dorsal view (body leptodactyliform and pointed snout in those species).

The presence of dorsal shield bony in *Brachycephalus* **sp. nov.** resembles the ones observed in *B. ephippium*, *B. garbeana* e *B. margaritatus*. The new species differ from *B. ephippium* by the well-developed parotic plates, laterally projected (not projected in *B. ephippium*), and by the convex paravertebral plates (flat in *B. ephippium*). *Brachycephalus* **sp. nov.** differ from *B. garbeana* by the dorsal shield not projected up over the vertebral spine (projected over the borders over the vertebral spine), by the convex central shape and laterally rounded paravertebral plates (paravertebral plates flat with square edges in *B. garbeana*). It is distinguished from *B. margaritatus* by the absence of protuberances (scattered well-developed protuberances in *B. margaritatus*), by the first spinal plates with lozenge and rectangular shape (triangular in *B. margaritatus*) and by the dorsal plate with rounded edges (nearly square and down curved in *B. margaritatus*).

The advertisement call of *Brachycephalus* **sp. nov.** distinguishes it from all its congeneric species by the presence of harmonic structures. It is distinguished from *B. tridactylu*s by the pulsed notes (Table 3), from *B. pernix* by having more pulses per note, and from *B. ephippium* and *B. pitanga* by having less pulses per note. It is distinguished from *B. crispus*, *B. hermogenesi* and *B. pitanga* by the longer notes. From *B. crispus*, *B. hermogenesi* and *B. pitanga* by the higher note rate. *Brachycephalus* **sp. nov.** has the lowest peak of energy within the genus (PF in this work; Dominant frequency in Condez *et al.* 2014, Verdade *et al.* 2008, Araújo *et al.* 2012 and Garey *et al.* 2012).

Description of holotype. Body robust and bufoniform; head wider than long; head length 32.9% of SVL; snout short, rounded in dorsal and lateral views; nostrils slit-shaped, slightly protuberant, directed anterolaterally; canthus rostralis indistinct; loreal region weakly concave; lips sigmoid; eyes slightly protruding, directed anterolaterally; ED 28.7% of HW and 30% of HL; tympanum indistinct; vocal sac not externally expanded; vocal slit presents; tongue longer than wide, posterior half not adhered to mouth floor; choanae small and round; vomerine odontophores absent.

Arm and forearm slender; arm slightly shorter than forearm (AL 22.3% and FAL 22.3% of SVL); forearm slightly hypertrophied; hands shorter than arm. All finger are distinct; fingers II and III robust; I and IV very small, vestigial; tip of finger I rounded and the others pointed; relative lengths of fingers IV < I < II < III; subarticular, inner and outer metacarpal tubercles absents (Fig. 1D). Tibia as long as thigh (TBL 37.2% and THL 39.5% of SVL); foot longer than tarsus and shorter than tibia; toes II, III e IV relatively distinct with pointed tip, I and V vestigial; relative length of toes I < V < II < III < IV; subarticular, inner and outer metatarsal tubercles absent (Fig. 1E).

Skin on head and dorsum rough with dermal ossifications; posterior region of the body with few scattered warts; granular dorsolateral skin of the body and around cloacal opening; skin on belly and limbs smooth (Figs. 1 and 5).

Measurements of holotype (in mm). SVL 17.9, HL 5.9, HW 6.16, ED 1.77, IOD 2.22, END 1.0, ND 0.44, IND 2.22, AL 4.0, FAL 4.3, HAL 3.3, THL 7.08, TBL 6.66, TAL 4.13, FL 5.6.

Osteology. (Figure 2) The double stained materials revealed that the skull of *Brachycephalus* **sp. nov.** has a well developed parotic plate with ornamented dermal roofing bones. Dorsally, the anterolateral corner of the parotic plate is directed toward the frontoparietal. Posterolaterally, the parotic plate is expanded, causing the posterior region of head to be wider than shoulder girdle. Nasal, sphenethmoid, frontoparietals, prootics, and exoccipital fused, forming a dorsal cranial plate. Premaxillae broad, not fused medially, odontoids absent. Alary process of premaxillae distinct, narrowly separated from the nasal and widely separated from each other, with length approximately twice of height. In ventral view, maxillae arched, odontoids absent. Quadratojugal absent. Pterygoid present. Vomer fused, vomerine odontophores absent. Palatine absent. Parasphenoid robust. Squamosal enlongated in lateral view. Anterior zygmatic ramus short and not ornamented. Tympanic anuulus absent. Mandible edentate.

Pectoral girdle arciferal and robust. Procoracoid and epicoracoid fused and completely ossified. Procoracoid and epicoracoid synostotically united with clavicle, coracoid, and scapula. Supraescapula expanded, with anterior half ossified as cleithrum. Omosternum and sternum absent. Ventral column composed of 8 nonimbricate vertebrae and spinal process of vertebrae hiperossified. Atlas (first pressacral vertebra) lacks transverse process. Presence of paravertebral and spinal plates well developed and ornamented. Spinal plates associated with the spinal process of all presacral and sacral vertebrae. First spinal plate (vertebra II) broad; trapezium convex-shaped. Second spinal plate (vertebra III) thin, almost rectangle convex-shaped and nearly joint with principal group of dorsal plates. Dorsally, the paravertebral plate completely conceal the transverse process of vertebra IV-VII, and partially cover the posterior tip of transverse process of vertebra III. Lateral edge of the paravertebral plates almost rounded with the anterolateral.

Posterolateral corners of each plates slightly reaching above level of transverse process of vertebra II, nearly reaching the sacral diapophysis. Dorsal and proximally, paravertebral plates fused medially to the block consisting of the fusion of spinal plates IV-VIII. Ventrally, transverse process of vertebrae IV and V fused to the paravertebral plates. Dorsally, the centra of each paravertebral plate has an elevation that provide a convex shape.

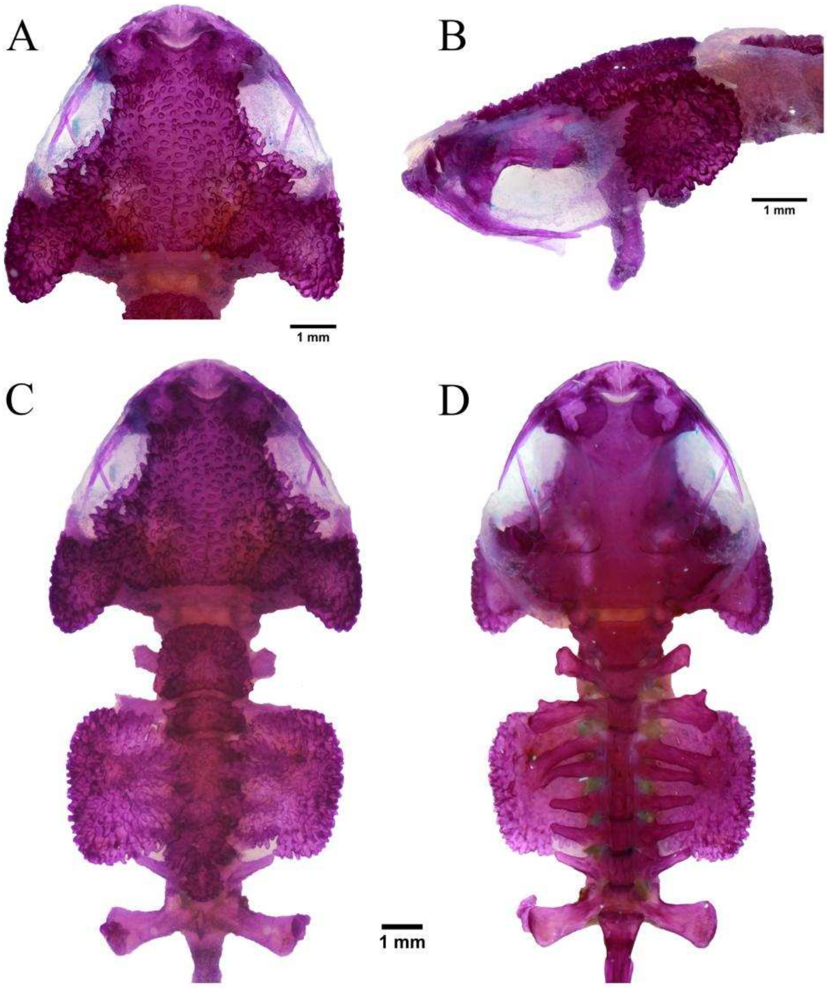

Figure 2. *Brachycephalus* **sp. nov.** (paratype MZUFV 15566, SVL 16.14 mm) cleared and double-stained specimen. Dorsal (A) and lateral (B) view of the head; dorsal (C) and ventral (C) views of the body.

Forearm slightly shorter than humerus. Radius and ulna fused and distinguishable. Manus with distal carpals (I-V) fused, with centrale, radiale and ulnare about the same size. Prepollex very reduced, with one element. Falangeal formula 1-2-3-1. Tips of the terminal phalangeal elements of fingers arrow-shaped. Tibia and fibula fused distinguishable, forming tibiafibula. Tibiafibula and femur of approximately the same length. Tibiale and fibulare fused at their distal and proximal ends, not fused medially. Hindlimbs with tarsal elements I, II, III present, and IV-V absent; centrale present. One very-reduced prehallical element. Phalangeal formula 1-2-3-4-1. Tips of terminal phalangeal elements of toes II, III and IV arrow shaped. Toes I and V reduced with terminal phalangeal element rounded in shape.

Histology. The histological analysis revealed that the darkened tissue that covers the dorsal musculature is a connective tissue (Fig. 3A) with chromophores. The later holds a dark pigment that grants fixed specimens a dark color to the dorsum (Fig. 1A). This same pigment was also observed among the dorsal muscles (Fig. 3B).

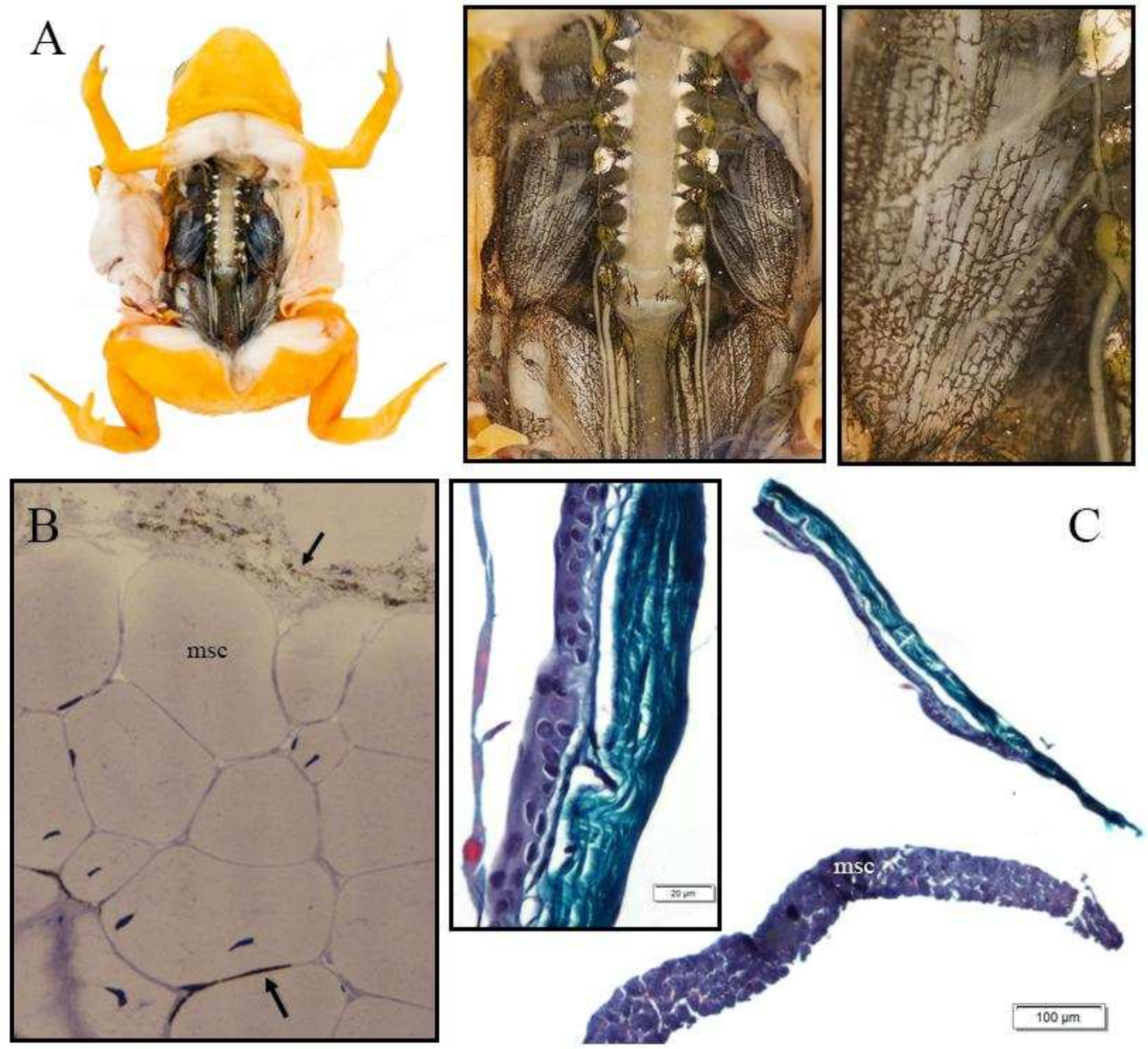

Figure 3. Histological analysis: (A) *Brachycephalus* **sp. nov.** dissected, and zoom in the dark tissue, detail in the provision of tissue that follows the adjacent muscle fibers. Transverse sections (B) inclusion in resin and (C) inclusion in paraffin, connective tissue (zoom left). The arrows indicate the pigments of connective tissue, muscle tissue (msc).

Coloration of Holotype. In life, body bright yellow-orange. Venter yellow-orange, slightly pale compared to remaining of body. Dark background beneath the yellow-orange skin at central region of dorsum, from posterior region of skull until inguinal region. Dorsal plates slightly dark. Eyes completely black (Fig. 5).

Coloration in preservative. General body color cream. Dark background beneath the cream skin from central region of dorsum until inguinal region, including posterior region of skull. Ossified regions such as skull and dorsal plates greyish. Eyes completely black (Fig. 1).

Variation. Measurements of adults and juveniles given in Table 1. Density of warts at posterior region of body and granular regions varies between individuals (i.e. some specimens with body entirely smooth). Dermal ossification of head and dorsum varies ontogenetically; juveniles can lack hyperossification or present skull and post-cranial skeleton less ornamented than in adults. Paravertebral plate can change proportionally in size; may be bigger or smaller in individuals of same size. Second spinal plate may contact spinal plates fused with paravertebral plates. Spinal plate of VIII presacral vertebra and sacral vertebra may be separated from the dorsal shield. Top of scamosal may present ornamented dermal ossification. Some specimens presented 1/3 of tong adhered to floor of mouth, and oval choanae.

Table 1. Measurements in millimeters of *Brachycephalus* **sp. nov.** type series. Character abbreviations are listed in Material and Methods section.

	Adults (n = 31)		**Juveniles (n = 3)**	
Traits	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
SVL	16.70 ± 1.28	14.75 - 18.52	12.99 ± 2.01	11.29 - 15.22
HL	5.50 ± 0.43	4.80 - 6.33	4.53 ± 0.53	3.95 - 5.00
HW	6.32 ± 0.45	5.40 - 7.25	5.22 ± 0.60	4.60 - 5.80
ED	1.83 ± 0.40	1.60 - 3.36	1.50 ± 0.07	1.42 - 1.57
IOD	3.73 ± 0.37	2.50 - 4.10	2.99 ± 0.35	2.74 - 3.40
END	1.01 ± 0.06	0.88 - 1.15	0.82 ± 0.13	0.71 - 0.97
ND	0.47 ± 0.03	0.44 - 0.55	0.37 ± 0.04	0.35 - 0.42
IND	2.17 ± 0.14	1.86 - 2.45	1.84 ± 0.26	1.60 - 2.13
AL	3.39 ± 0.26	2.80 - 3.85	2.83 ± 0.74	2.08 - 3.56
FAL	3.81 ± 0.48	2.83 - 4.50	3.12 ± 0.58	2.76 - 3.80
HAL	3.15 ± 0.36	2.04 - 3.60	3.05 ± 0.53	2.43 - 3.36
THL	7.00 ± 0.70	6.33 - 9.30	4.65 ± 2.09	2.30 - 6.33
TBL	6.48 ± 0.32	5.86 – 7.00	4.23 ± 1.94	2.11 - 5.93
TAL	4.24 ± 0.42	3.46 - 5.00	3.88 ± 0.83	3.00 - 4.65
FL	5.41 ± 0.39	4.75 - 6.16	4.33 ± 0.57	4.00 - 5.00

Call description. We observed two distinct calls of *Brachycephalus* **sp. nov.** herein referred as the advertisement call and an aggressive call (sensu Toledo *et al.* 2015). The advertisement call is the most common type of call (Fig. 4A, Table 2, five individuals) and is characterized by pulsed notes emitted in extremely long sequences (NN > 250 notes per call; not fully recorded). Each note presented a slight decrease in amplitude (Fig. 4A) and average of six pulses (PN = 6.3 ± 0.7, 5 – 8 pulses) emitted at constant rate (PR = 56.9 ± 4.9, 36.8 – 78.4 pulses/s). Note duration (ND = 111.5 ± 13.7, 83 – 163 ms), interval between notes (NI = 159.5 ± 14.5, 122 – 215 ms, n = 783) and note rate (NR = 211.4 ± 25.6, 186.4 – 243.4 notes/min, n = 5 recordings) also presented little variation through the call. Dominant frequency (DF) ranged from 2484.4 to 5765.6 Hz with peak of energy around 3.3 kHz (PF = 3382.1 ± 184.6, 2856.4 – 3796.9 Hz). Up to three harmonics could be present in the advertisement call.

Table 2. Parameters of the advertisement and the aggressive calls of *Brachycephalus* **sp. nov..** Description based on six individuals, recorded at Parque Estadual da Serra do Brigadeiro, 20 November 2015, air temperature 22°C, and 17 December 2015, air temperature 19°C.

	Advertisement call (n = 5 individuals)	**Agressive call (n = 1 individual)**
Number of notes per call	> 250	24.5 ± 7.9 (15 – 41) n = 10
Call duration (s)	> 100	4.1 ± 1.3 (2.4 – 6.9) n = 10
Interval between calls (s)	–	2.8 ± 0.7 (2.0 – 3.8) n = 9
Call rate (calls/min)	–	8.46 n =1
Note duration (ms)	111.5 ± 13.7 (83 – 163) n = 790	31.1 ± 5.7 (18 – 44) n = 245
Interval between notes (ms)	159.5 ± 14.5 (122 – 215) n = 783	143.3 ± 16.7 (96 – 334) n = 235
Note rate (notes/min)	211.4 ± 25.6 (186.4 – 243.4) n = 5	356.6 ± 6.5 (343.7 – 364.2) n = 10
Number of pulses per note	6.3 ± 0.7 (5 – 8) n = 790	2.5 ± 0.5 (2 – 3) n = 245
Pulse rate (pulses/s)	56.9 ± 4.9 (36.8 – 78.4) n = 790	79.9 ± 9.6 (57.1 – 111.1) n = 245
Peak frequency (Hz)	3382.1 ± 184.6 (2856.4 – 3796.9) n = 790	3429.7 ± 146.5 (3046.9 – 3984.4) n = 245
Dominant frequency range (Hz)	2484.4 – 5765.6	2906.2 – 4406.2

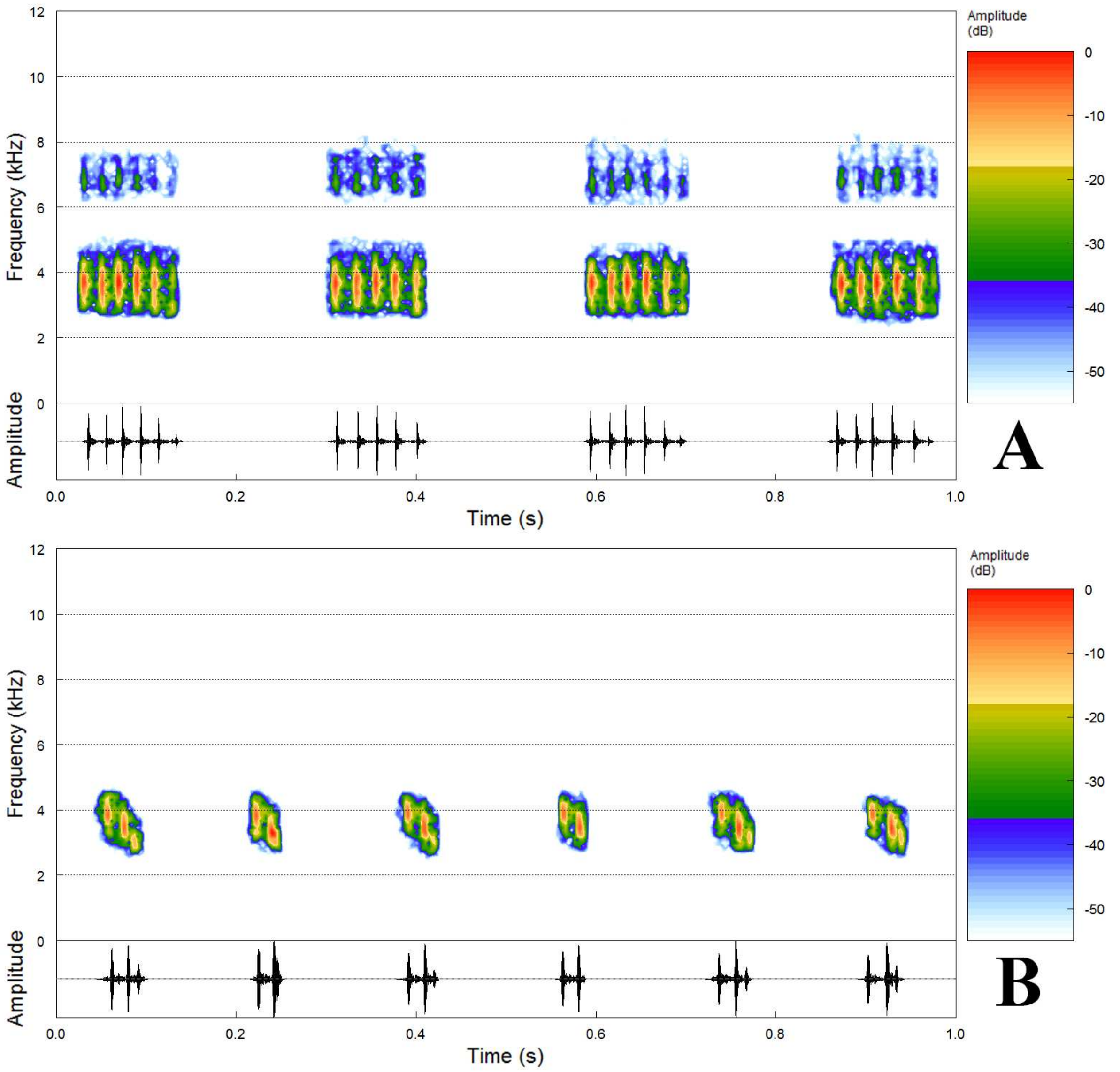

Figure 4. Spectrogram (above) and oscillogram (bellow) of the calls of *Brachycephalus* **sp. nov.** (A) Sequence of four notes of the advertisement call, recorded on 20 November 2015, 12h40, air temperature 22°C, paratype MZUFV 16629. (B) Sequence of six notes of the aggressive call, recorded on 17 December 2015, 19h00, air temperature 19°C, holotype MZUFV 16636.

A second type of call was recorded whilst a male *Brachycephalus* **sp. nov.** called within 20 cm of a conspecific male in an aggressive social context (sensu Toledo *et al.* 2015). The aggressive call (Fig 4B, Table 2, n = 245 notes) is also characterized by pulsed notes emitted in sequences. However, each note had average of 2.5 pulses (PN = 2.5 ± 0.5, 2–3 pulses) emitted at higher rate (PR = 79.9 ± 9.6, 57.1–111.1 pulses/s) than observed in the advertisement call. There is an increase in amplitude from the first to the second pulse, followed by a decline in amplitude from the second to the third pulse. Sometimes the second and third pulses were juxtaposed. The aggressive call was considerably shorter than the advertisement call (CD = 4.1 ± 1.3, 2.4–6.9 s, n = 10 calls), with variable number of notes

(NN = 24.5 ± 7.9, 15–41 notes/call, n = 10 calls) and it was emitted about eight times per minute (CR = 8.46 calls/min, n = 1) with brief intervals (CI = 2.8 ± 0.7, 2.0–3.8 s, n = 9). Each note was shorter than the ones from the advertisement call (ND = 31.1 ± 5.7, 18–44 ms), emitted at higher rate (NR = 356.6 ± 6.5, 343.7–364.2 notes/min, n = 10 calls) and with variable intervals (143.3 ± 16.7, 96–334 ms, n = 235). Dominant frequency (DF) ranged from 2906.2 to 4406.2 Hz with peak of energy around 3.4 kHz (PF = 3429.7 ± 146.5, 3046.9–3984.4 Hz). Notes of the aggressive call presented a descending frequency modulation: the first pulse presented the highest values of dominant frequency (DF^1 = 3421.9 – 4218.8) and peak of energy (PF^1 = 3895.5 ± 73.4, 3703.1–3984.4 Hz); the second pulse had intermediate values (DF^2 = 3046.9–3937.5 Hz; PF^2 = 3481.6 ± 109.8, 3328.1–3656.2 Hz) and the third pulse had the lowest values (DF^3 = 3281.2–3515.6 Hz; PF^3 = 3066.4 ± 71.7, 2953.1–3328.1 Hz).

Table 3. Call parameters in the genus *Brachycephalus*. Note duration in ms (ND); Interval between notes in ms (NI); Note rate in notes/s (NR); Number of pulses per note (PN); Pulse rate in pulses/s (PR); Peak frequency in kHz (PF); Dominant frequency range in kHz (DF). 1: Peak frequency in this work. 2: Emphasized frequencies in Pombal *et al.* 1994. 3: Notes/min in Araújo *et al.* 2012. 4: Calls/s in Garey *et al.* 2012.

	B. crispus	***B.* sp. nov.**	***B. ephippium***	***B. hermogenesi***	***B. pernix***	***B. pitanga***	***B. tridactylus***
ND	280 ± 20	111.5 ± 13.7	112 ± 6.5	200	60	170 ± 13	110 ± 20
NI	350 ± 20	159.5 ± 14.5	134 ± 6.8	–	140	–	–
NR	1.67 ± 0.09	3.52 ± 0.42	–	1.09	–	2.65 ± 0.18^3	0.16 ± 0.03^4
PN	7 – 12	6.3 ± 0.7	12 ± 1.96	–	3	11.1 ± 1.2	Unpulsed
PR	17.4 ± 2.12	56.9 ± 4.9	–	–	–	62 ± 8	–
PF	4.6 ± 0.19	3.38 ± 0.18^1	–	6.8 ± 0.8	–	4.9 ± 0.2	4.8 ± 0.2
DF	3.5 – 5.7	2.4 – 5.7	3.4 – 5.3^2	–	4.5 – 6.7	–	3.2 – 6.4
Ref	Condez *et al.* 2014	This work	Pombal *et al.* 1994	Verdade *et al.* 2008	Wistuba 1998	Araújo *et al.* 2012	Garey *et al.* 2012

Natural history. Specimens of *Brachycephalus* **sp. nov.** were found amidst leaf litter of “Trilha do Cruzeiro”, at the habitat Floresta Estacional Semidecidual Montana, between 1266 and 1498 m a.s.l. We observed active individuals from October to December, between 12h and 19h. Males were found calling exposed over leafs or beneath them, and females walking through the leaf litter. During the dry months (from July until September), individuals of *Brachycephalus* **sp. nov.** were found hidden deep into the layers of leaf litter, buried and within roots underground (Fig. 5). The new species is abundant in the area where it occur and reproduce syntopicaly with species of the genus *Ischnocnema*.

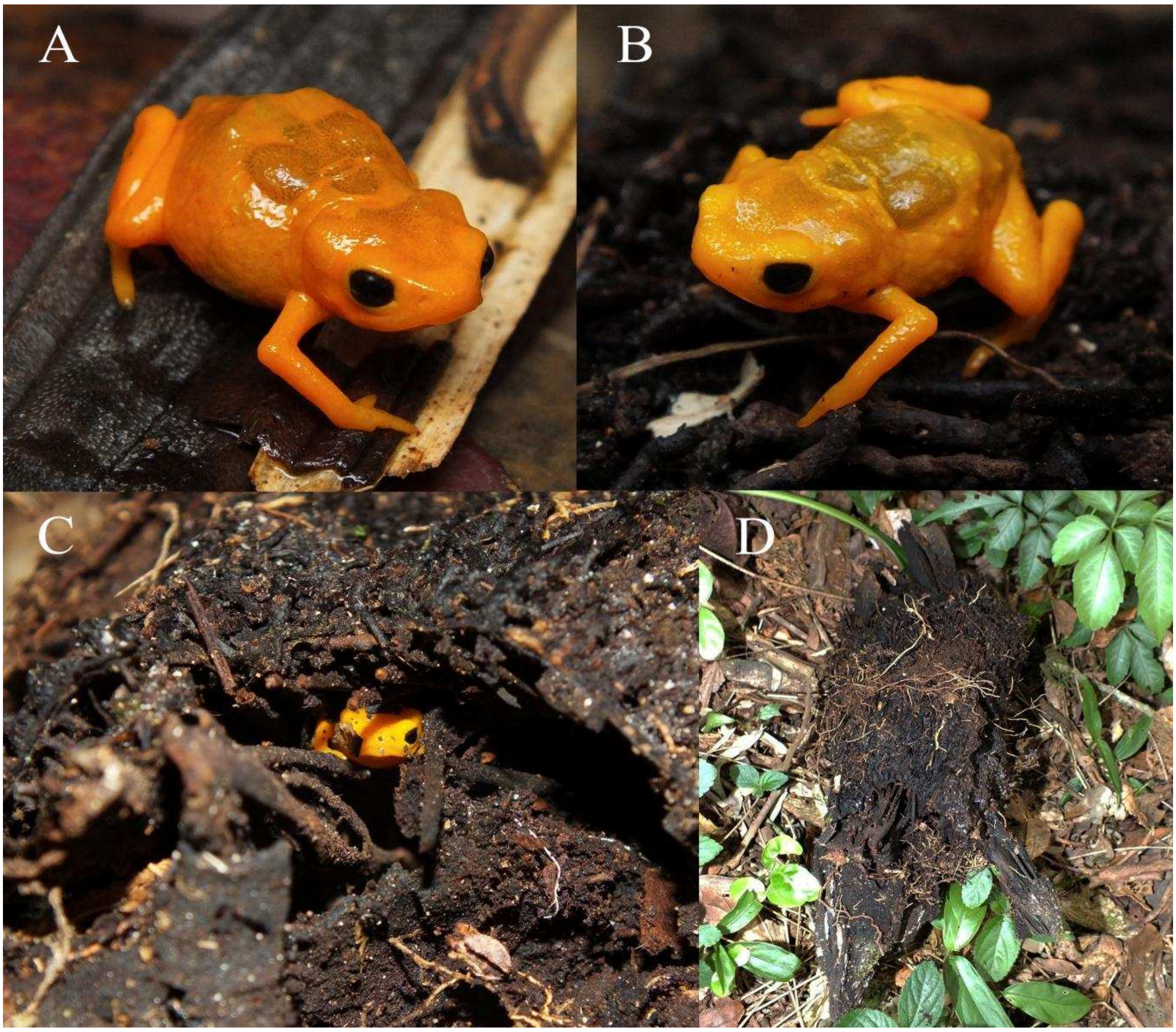

Figure 5. Photos of *Brachycephalus* **sp. nov.** in life: (A) female, (B) male, (C) same male hidden beneath roots, (D) roots where the male was found.

Distribution. *Brachycephalus* **sp. nov.** is known from municipalities of Ervália and Miradouro, both at Serra do Brigadeiro, north portion of Mantiqueira mountain range, Minas Gerais State, southeastern Brazil (Fig. 6). Considering the distances between locations, vegetation and altitude range (1266–1498 m a.s.l.), it is likely that this new species can be found in more locations within the Serra do Brigadeiro mountain range.

Comments. It was not possible to determine the sex of all specimens since females (identified by the presence of oocytes) also presented vocal slips. Several works also mentioned the presence of vocal slits in females of *Brachycephalus* (i.e. Alves *et al.* 2009; Clemente-Carvalho *et al*. 2012; Haddad *et al.* 2010; Pie & Ribeiro 2015; Ribeiro *et al.* 2015), however those authors do not specify how the sex was determined. Therefore, we discourage the use of vocal slits for sex determination in *Brachycephalus* species.

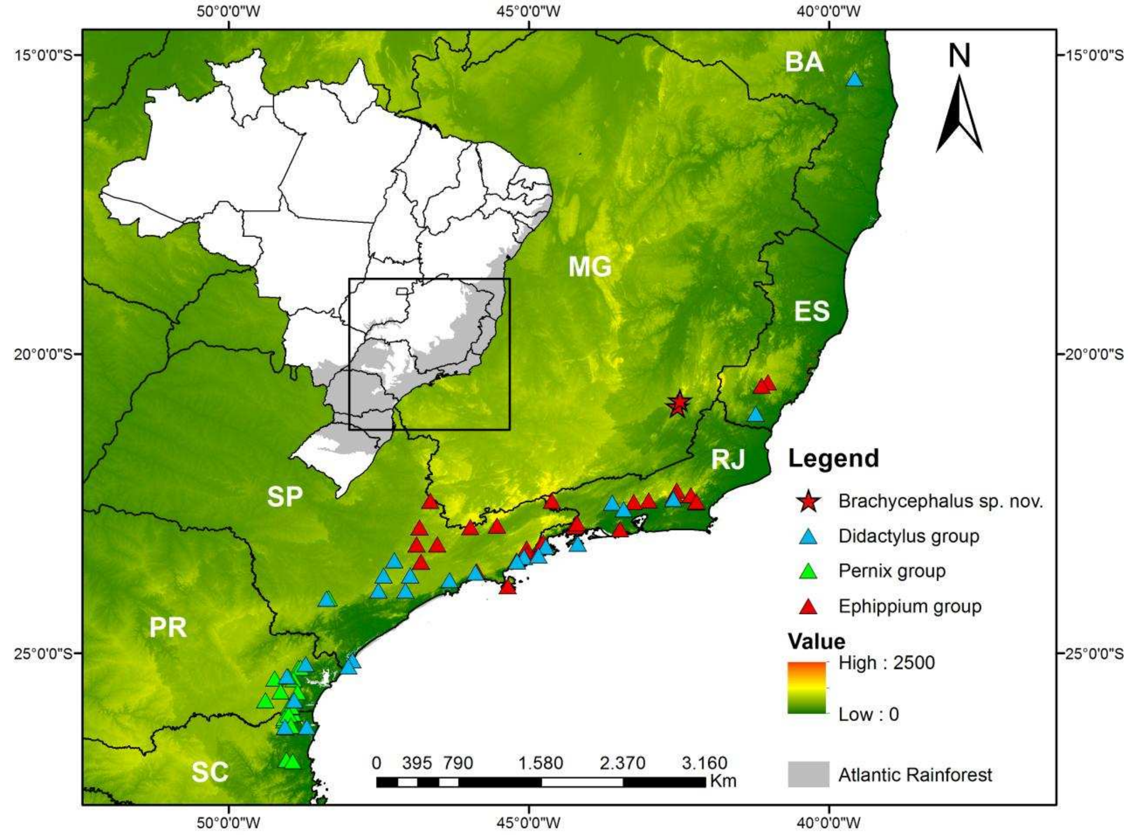

Figure 6. Geographical distribution of the new species, and of *Brachycephalus didactylus*, *B. pernix* and *B. ephippium* groups.

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Appendix

Specimens Examined

Brachycephalus alipioi: Brazil, **Espítio Santo**, Castelo (MZUFV 5877–5882).

Brachycephalus bruneus: Brazil, **Paraná**, Campina Grande do Sul (MNRJ 40289–91, paratypes).

Brachycephalus didactylus: Brazil, **Rio de Janeiro**, Paulo de Frontim (MNRJ 4067–73, paratypes).

Brachycephalus ephippium: Brazil, **Rio de Janeiro**, Maciço da Tijuca (MNRJ 40782–93); Itatiaia (MZUFV 4161).

Brachycephalus garbeanus: Brazil, **Rio de Janeiro**, Nova Friburgo (MNRJ 17440–41, 25390–400, 67498).

Brachycephalus hermogenesi: Brazil, **Paraná**, Guaraqueçaba (MNRJ 87912).

Brachycephalus izecksohni: Brazil, **Paraná**, Quaratuba (MNRJ 76259–60, paratypes).

Brachycephalus margaritatus: Brazil, **Rio de Janeiro**, Petrópolis (MNRJ 85300–396).

Brachycephalus pernix: Brazil, **Paraná**, Quatro Barras (MNRJ 17343, holotyp) and (MNRJ 17328–42, 17427–28, paratypes).

Brachycephalus pitanga: Brazil, **São Paulo**, São Luís do Paraitinga (MNRJ 60790–93, paratypes).

Brachycephalus toby: Brazil, **São Paulo**, Ubatuba (MNRJ 76382–83, paratypes).

Brachycephalus tridactylus: Brazil, **Paraná**, Guaraqueçaba (MNRJ 87908–910).

Brachycephalus vertebralis: Brazil, **Rio de Janeiro**, Paraty (MNRJ 11098, holotype).

2.2. **ARTIGO II** – Guimarães, C.S., Lyra, M. & Feio, R.N. 2016. A new species of the *Scinax catharinae* group (Anura: Hylidae) from Serra da Mantiqueira, Minas Gerais, Brazil

A new species of the *Scinax catharinae* group (Anura: Hylidae) from Serra da Mantiqueira, Minas Gerais, Brazil

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Abstract. We describe a new species of *Scinax* from Serra do Brigadeiro, north portion of the Serra da Mantiqueira mountain range, Atlantic Forest, southeastern Brazil. Morphological and molecular data from COI show that the new species belongs to the *Scinax catharinae* group. *Scinax* **sp. nov.** is mainly characterized by its medium size (23.03–33.53 mm), snout subovoid/subelliptical in dorsal view, *canthus rostralis* marked, males with vocal sac expanded and hypertrophied forearms and nuptial pad, inguinal region and hidden surfaces of thigh with irregular brown blotches on yellow/pale background. We also provide the tadpole description and advertisement calls and natural history. The new species in known only from type locality.

Key words: *Scinax* **sp. nov.**, Atlantic Forest, Serra do Brigadeiro, advertisement call, tadpole

Introduction

Scinax Wagler, 1830 is the largest genus within the family Hylidae, with 115 species distributed from east and south of México until Argentina and Uruguay, including the islands of Trindad, Tobago and St. Lucia (Frost 2016). Duellman & Wiens (1992) and Faivovich (2002) recognized the genus as monophyletic, with this last author dividing it into the clades of *Scinax catharinae* (subdivided into *catharinae* and *perpusillus* groups) and *Scinax ruber*.

Currently, the *Scinax catharinae* group comprises 33 species distributed in Argentina, Brazil, Paraguay and Uruguay (Faivovich 2002, Frost 2016). The majority of those species occur in gallery forests of the Atlantic Forest and Cerrado Biomes of Brazil

(Caramaschi & Kisteumacher 1989; Pombal & Bastos 1996; Pombal *et al.* 2010). Overall, the group's taxonomy is complex and new species are frequently described (Lourenço *et al.* 2014) – average of three species per year in the past five years (Frost 2016). Moreover, only 12 of the described species had their tadpoles described (Lourenço *et al.* 2013), 22 had their vocalization described (Peixoto *et al.* in press) and an updated phylogeny of the genus is lacking.

In 2012 Moura and colleagues presented a list of 57 species of amphibians that included 10 species of *Scinax* from Serra do Brigadeiro, an important remnant of the Atlantic Forest in northern Mantiqueira mountain range, state of Minas Gerais, southeastern Brazil. Among those, a population of *Scinax* sp. considered very similar to *Scinax rizibilis* was diagnosed. In order to clarify the taxonomic identity of this population, we conducted were several field expeditions in recent years. The set of accumulated data were analyzed and allowed us to recognize this population as a new species that is described herein.

Material and methods

Adults and tadpoles were collected at the municipality of Ervália, District Careço, in the southern portion of Serra do Brigadeiro mountain range, state of Minas Gerais, southeastern Brazil. Type specimen is housed at the Museu de Zoologia João Moojen of Universidade Federal de Viçosa (MZUFV), Minas Gerais, Brazil.

Adult specimens were fixed in 10% formalin and stored in 70% ethyl alcohol solution. Tadpoles were prepared and preserved in 10% formalin. Sex was determined by the presence of nuptial pads, extended vocal sacs and vocal slits in males and oocytes in females (by visualization through transparency of the skin). Measurements of adults were taken with a digital caliper (0.01 mm): snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), thigh length (THL), tibia length (TL), foot length (FL), upper arm length (AL), forearm length (FAL) and hand length (HAL). With a micrometric ocular in a stereomicroscope (Olympus SZ61): tympanum diameter (TD), interorbital distance (IOD), eye-nostril distance (END), internarial distance (IND) and eye-snout distance (ESD). All measurements are shown in millimeters. Morphological terminology and description of the snout shape follow Cei (1980) and Heyer *et al.* (1990). Webbing formula notation followed Savage & Heyer (1997) with observation of Fabrezi & Alberch (1996) that the first digit is lost in anurans, thus digits are numbered II–V.

Comparisons of adult specimens was based on observations of museum material and on literature information (Spix 1824; Boulenger 1888; Miranda-Ribeiro 1926; De Witte

1930; Lutz & Lutz 1939; Lutz 1954, 1968, 1973; Barrio 1962; Bokermann 1964, 1967; Cardoso & Haddad 1982; Cruz & Peixoto 1983; Peixoto & Weygoldt 1986; Andrade & Cardoso 1987; Haddad & Pombal 1987; Caramaschi & Kisteumacher 1989; Carvalho-e-Silva & Peixoto 1991; Pombal & Gordo 1991; Pombal & Bastos 1996; Faivovich 2005; Lourenço *et al.* 2009b, 2013, 2014; Pombal *et al.* 2010; Cruz *et al.* 2011; Lima *et al.* 2011). Specimens for comparisons (listed in Appendix 1) are deposited at Célio F.B. Haddad Collection, Universidade Estadual Paulista Júlio de Mesquita Filho, Rio Claro, SP (CFBH); Museu de História Natural da Universidade de Campinas-UNICAMP, Campinas, SP (ZUEC); and MZUFV.

We analyzed 17 calls from eight individuals recorded at type locality on 29 November 2014, between 18h and 21h40, air temperature 18°C. Recordings were made with a TASCAM DR-40 digital recorder; sampling rate 44.1 kHz and 24 bits resolution. Spectral and temporal parameters were measured using Raven Pro 1.5 (Cornell Lab of Ornithology Bioacoustics Research Program) directly from the spectrogram and oscillogram respectively. Spectrogram featured window type Hann; window size = 512 samples; overlap = 70%; hop size = 3.49 ms; DFT size = 1024 samples and; grid spacing = 43.1 Hz. Sound graphics were obtained using Seewave (Sueur *et al.* 2008) package of R platform (R Core Team 2014) with spectrogram parameters: Hanning window; FFT = 512 and 70% overlap. Following Rocha *et al.* (2016), the dominant frequency (DF) represents the most energetic band of the call and consist of the range from the lowest to the highest frequency values acquired in the spectrogram.

The peak frequency (PF) is always within the DF and was given by the software Raven Pro 1.5. Temporal parameters include call duration (CD), number of notes per call (NN), note duration (ND), interval between notes (NI), note rate (NR), number of pulses per note (PN) and pulse rate (PR). Data is presented as mean ± SD; range; n. Further call terminology follow Toledo *et al.* (2015). Comparison of advertisement calls of species belonging to the *S. catharinae* group was based on available literature (Bokermann & Sazima 1973; Heyer 1990; Cardoso & Haddad 1982; Pombal *et al.* 1995, 2010; Nunes *et al.* 2007; Garey *et al.* 2012).

Tadpoles were collected in several developmental stages and species identity was later confirmed in laboratory by raising it until metamorphic stage (Fig. 4E). The individual raised exhibited the same dorsal pattern and morphology as observed in adult specimens. Voucher lots are housed in the MZUFV. Thirteen measurements were taken from 14 specimens at stages 31–36 (sensu Gosner 1960): total length (TL), body length (BL), tail length (TAL), tail height (TH), internarial distance (IND), interorbital distance (IOD), and tail muscle height (TMH) – Altig & McDiarmid (1999); body width (BW), body height (BH), eye–nostril distance (END), eye diameter (ED), and oral disc width (ODW) – Lavilla & Scroccho (1986); and spiracle length (SL). TL and TAL were measured using analogue caliper to the nearest 0.01 mm. Other measurements were made in a stereomicroscope with micrometric ocular (Olympus SZ61). Description of lateral line system follows Lannoo (1987).

Comparison with other described tadpoles of the *Scinax catharinae* group was based on literature available (Bokermann 1964, 1967; Bokermann & Sazima 1973; Peixoto & Weygoldt 1986; Andrade & Cardoso 1987, 1991; Haddad & Pombal 1987; Heyer *et al.* 1990; Pombal & Gordo 1991; Carvalho-e-Silva & Carvalho-e-Silva 1994, 1998; Carvalho-e-Silva *et al.* 1995; Carvalho-e-Silva & Carnaval 1997; Bertoluci *et al.* 2007; Conte *et al.* 2007; Kolenc *et al.* 2007; Lourenço *et al.* 2009b, 2013; Abreu *et al.* 2015).

Genomic DNA was extracted from ethanol-preserved tissues using a standard ammonium acetate DNA extraction protocol. Samples analyzed are in Appendix 2. For comparison purpose we includes other species of *Scinax catharinae* group in analysis. A fragment of the *Citochrome oxidase c subunit 1* (COI) gene was amplified and sequenced using the primers AnfR1 and AnR1 (Lyra *et al.*, *in press*) using the standard protocol described in Lyra *et al.* (*in press*). Reaction products were purified using Exonuclease I and FastAP and sent to Macrogen Inc. for sequencing. Data from two complementary strands were compared to generate a consensus sequence for each DNA fragment using Geneiuos software V6 (Biomatter). A static alignment was obtained using the web version of the software Mafft version 6.5 (Katoh *et al.*, 2005). Maximum likelihood (ML) analyses were performed in PhyML (Guindon *et al.*, 2010) under default parameters and a HKY model of evolution. Bootstrap support was evaluated through 1000 replicates. Tree was rooted with *Scinax fuscovarius*, a member of the *S. ruber* clade (Faivovich *et al.* 2005). Uncorrected p-distances were calculated in MEGA (Tamura *et al.* 2013) for a dataset with all sequences having the same length and no missing data (586 bp).

Results

***Scinax* sp. nov.**

Scinax sp. (aff. *rizibilis*) – Moura *et al*., 2012. *Biotaneotropica*, vol. 12, no.1.

(Figures 1, 2 and 4)

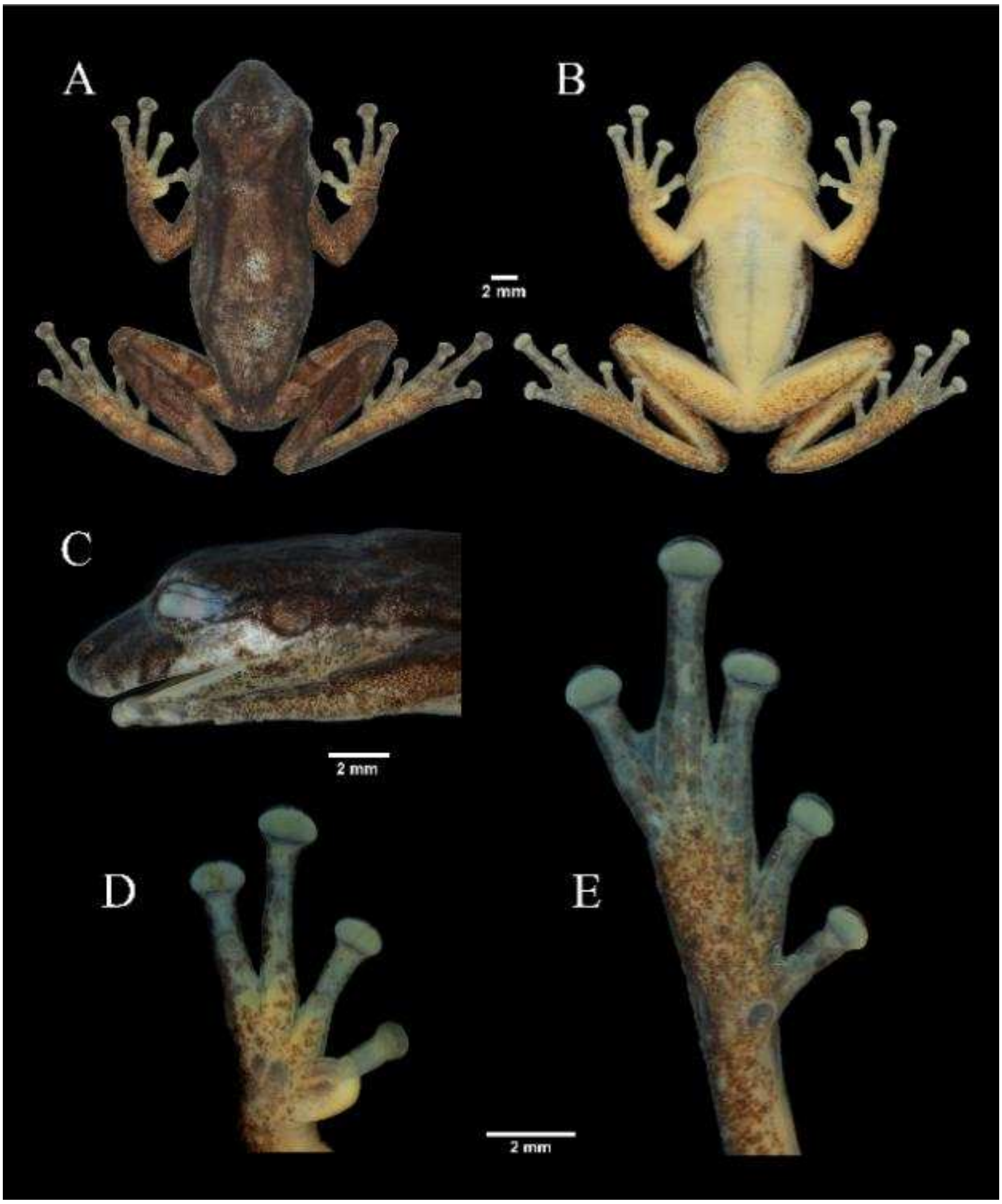

Figure 1. *Scinax* **sp. nov.** holotype (MZUFV15965, SVL 27.79 mm). Dorsal (A) and ventral (B) views of the body, lateral view of the head (C), palmar view of hand (D) and plantar view of the foot (E).

Holotype. MZUFV 15965, an adult male (27.79 mm SVL), collected at Lagoa das Bromélias (21°42'39" S, 43°54'41" O, 1227m a.s.l.), Parque Estadual da Serra do Brigadeiro, District Careço, municipality of Ervália, state of Minas Gerais, Brazil, on 29 November 2014 by C.S. Guimarães and L.M. Cordeiro.

Paratypes. MZUFV 15673, 15676, 15678–15679, 15681, 15956–15960, 15962–15963 (adult males) and MZUFV 15674–15675, 15677, 15680, 15682–15683, 15961, 15964 (adult females) collected with the holotype. MZUFV 15684 (adult female) and MZUFV 15685–15700, 15950–15955 (adult males) collected at the type locality on 28 November 2014, by C.S. Guimarães, M.A.A. Peixoto and L. Alves.

Diagnosis. The new species is characterized by its (1) medium size (SVL male 23.03–33.39, n= 35, females 28.89–33.53, n=9), (2) snout subovoid/subelliptical in dorsal view, (3) *canthus rostralis* marked, (4) vocal slit present in males, (5) males with vocal sac expanded, (6) males with hypertrophied forearms, (7) males with hypertrophied nuptial pad, (8) absence of macroscopic glandular acini on the medial region of the forearm and on the dorsal region of the finger II and III, (9) dorsum covered by scattered tubercle, (10) pectoral fold absent, (11) iguinial region and hidden surfaces of thigh with irregular brown blotches on yellow/pale background, (12) cream venter with few brown spots, (13) advertisement call characterized by a series of usually five pulsed notes (CD = 0.8–1.52 s), with about 60 pulses each, (14) peak of energy between 3100.8–4565 Hz, (15) tadpoles with small dorsal gap in marginal papillae, (16) marginal papillae unisseriated, and (17) LTRF 2(2)/3.

Comparison with other species. *Scinax* **sp. nov.** differs from all species of the *S. catharinae* species group by the hypertrophied nuptial pad (except *S. rizibilis*), and by the vocal sac notably expanded in males (except *S. aromothyella*, *S. berthae* and *S. rizibilis*). The new species differs from *S. agilis*, *S. centralis*, *S. machadoi*, *S. melanodactylus* and *S. skuki* by its bigger size (SVL of males in *Scinax* **sp. nov.** 23.03–33.39, combined SVL of males in these species 12.0–21.2, SVL of females in *Scinax* **sp. nov.** 28.89–33.53, combined SVL of females in these species 13.2–26.6), from *S. ranki* by bigger size of females only (SVL of females in this species 27.0–28.7), and from *S. albicans*, *S. ariadne*, *S. kautskyi*, *S. littoralis*, *S. obtriangulatus* and *S. tripui* by its smaller size of females only (combined SVL of females of these species 35.0–45.0).

The subovoid/subelliptical snout differs *Scinax* **sp. nov.** from *S. albicans*, *S. angrensis*, *S. flavogutattus*, *S. hiemalis*, *S. heyeri*, *S. humilis*, *S. littoralis*, *S. muriciensis*, *S. strigilatus*, *S. trapicheroi* and *S. tripui* (rounded with mucronate tip in these species), *S. ariadne*, *S. brieni*, *S. catharinae*, *S. obtriangulatus* and *S. ranki* (rounded), *S. carnevallii* and *S. kautskyi* (mucronate), *S. jureia* and *S. luizotavioi* (sub-elliptical with acute tip). *Scinax* **sp. nov.** differs from *S. agilis*, *S. albicans*, *S. argyreornatus*, *S. aromothyella*, *S. berthae*, *S. machadoi*, *S. melanodactylus*, *S. ranki*, *S. rizibilis*, and *S. skuki* by its well-marked *canthus rostralis* (poorly marked in these species).

The presence of vocal slit differs the new species from *S. ariadne* and *S. skaios* (absent in these species). *Scinax* **sp. nov.** differs from *S. agilis*, *S. albicans*, *S. angrensis*, *S. argyreornatus*, *S. berthae*, *S. canastrensis*, *S. carnevallii*, *S. centralis*, *S. heyeri*, *S. flavoguttatus*, *S. heyeri*, *S. hiemalis*, *S. humilis*, *S. kautskyi*, *S. littoralis*, *S. machadoi*, *S. muriciensis*, *S. pombali*, *S. ranki*, *S. skuki*, *S. strigilatus*, *S. trapicheroi*, and *S. tripui* in having hypertrophied forearms in males. The absence of glandular acini on the medial region of forearm and on the dorsal region of the fingers II and III differs *Scinax* **sp. nov.** from *S. canastrensis*, *S. carnevallii*, *S. flavoguttatus*, *S. kautskyi*, *S. longilineus*, *S. machadoi*, and *S. tripui* (presente in these species). Skin on dorsum covered by scattered tubercle in *Scinax* **sp. nov.** differs it from *S. aromothyella*, *S. berthae*, *S. melanodactylus* (smooth in these species), *S. ariadne*, *S. canastrensis*, *S. longilineus*, *S. pombali* and *S. skaios* (rough in these species). Absence of pectoral fold differs the new species from *S. agilis* and *S. melanodactylus* (pectoral fold in these species).

The inguinal region and hidden surfaces of thigh with irregular brown blotches on yellow/pale background in *Scinax* **sp. nov.** differs from *S. agilis*, *S. melanodactylus* and *S. rizibilis* (without flash color), *S. ariadne* (irregular light brown blotches on violet or pink background), *S. brieni*, (pale bluish color on the concealed areas), *S. carnevallii* (black spots scattered on whitish background), *S. catharinae* (irregular dark brown blotches on light blue background), *S. flavoguttatus* and *S. heyeri* (irregular brown blotches on orange background), *S. hiemalis* (black blotches on a greenish background), *S. kautskyii* (whitish spots on blackish brown background), *S. luizotavioi* (irregular light brown blotches on pale background), *S. littoralis* (black bars on a whitish or greenish background), *S. obtriangulatus* (dull grayish violet), *S. ranki* (dark brown blotches on greenish background), *S. skaios* (irregular dark brown stripes on brown or pale green background), *S. strigilatus* (greenish), and *S. tripui* (irregular dark brown blotches on greenish background). Cream enter with brown dots in *Scinax* **sp. nov.** differs from *S. ariadne* and *S. tripui* (cream blotches on brown background).

The call of *Scinax* **sp. nov.** is distinguished from *S. rizibilis* and *S. skaios* by the lower number of notes per call (NN = 5.35 ± 0.78, 4–7 in *Scinax* **sp. nov.**, NN = 12.39 ± 3.91, 7–23 in *S. rizibilis*, NN = 42–73 in *S. skaios*). It is distinguished from *S. agilis* and *S. albicans* by the higher number of notes per call (NN = 2 in *S. agilis*, NN = 1 in *S. albicans*). It is distinguished from *S. angrensis* and *S. littoralis* by the higher number of pulses per note (PN = 63.09 ± 12.79, 39–85 in *Scinax* **sp. nov.**, PN = 17.88 ± 5.18, 7–28 in *S. angrensis*, PN = 23.68 ± 2.73, 8–30 in *S. littoralis*). It is distinguished from *S. argyreornatus* by the lower peak of energy (PF = 3.1–4.5 kHz in *Scinax* **sp. nov.**, Dominant frequency = 5.0–6.5 kHz in *S. argyreornatus*). It is distinguished from *S. machadoi* by the longer notes (ND = 128–210 ms in *Scinax* **sp. nov.**, ND = 50 ms in *S. machadoi*). It is distinguished from *S. catharinae* by the shorter duration of the advertisement call (CD = 0.8–1.52 s in *Scinax* **sp. nov.**, CD > 2 s in *S. catharinae*). It is distinguished from *S. hiemalis* by having a single type of note. Unfortunately, the information provided for the call of *S. canastrensis* (Cardoso & Haddad 1982) does not allow its distinction from our species. On the other hand, the sonograms provided for *S. canastrensis* show shorter notes without the amplitude modulation observed in *Scinax* **sp. nov.**.

Description of holotype. Body slender and medium size. Head longer than wide (HL 32.96% of SVL, HW 29.61% of SVL). Snout subovoid in dorsal view and protruding in profile (Figure 1). Nostril elliptical and protruding, located laterally, immediately before the tip of snout, opening directed dorsum-laterally. Canthus rostralis marked and nearly concave. Loreal region slightly concave. Eye large (ED 18.83% of HW) protruding laterally. Interorbital and internostril distance 45.56% and 38.88% of head width, respectively.

Tympanum rounded, *annulus timpanicus* well defined, with diameter measuring 84.51% of ED. Supratympanic fold marked and well evident, extending from posterior corner of eye to just anterior to shoulder. Tongue large, elongated, unattached in the posterior and lateral borders. Vocal slits present, diagonals, in the posterolateral region of the tongue. Vomerine teeth in two contiguous small series of three teeth each, between choanae, though slightly posteriorly. Choanae elliptical. Vocal sac laterally sub-gular, expanded externally.

Members slender, with hypertrophied forearms longer than arms. Outer margins of forearms and tarsus with row of small and rounded tubercles. Hands 26.19% of SVL. Inner metacarpal tubercle single and elliptical, outer metacarpal double, subarticular tubercles single and rounded, supernumerary tubercles small and rounded. Macroscopic glandular acini on the medial region of forearm, and the dorsal region of the finger II and III absent. Webbing absent between fingers II and III, basal between other fingers. Discs on fingers elliptical, wider than long. Length of fingers II<III≅V<IV. Glandular area on medial margin of finger II developed to form a hypertrophied nuptial pad.

Foot 41.45% of SVL. Outer metatarsal tubercle single and elliptical, inner metatarsal single and oval, subarticular tubercles are single and rounded, few supernumerary single and rounded. Length of toes: I<II<V<III<IV. Toes with webbing formula I – II1 – 2III$1^{1/3}$ –

2IV2 – $1^{1/2}$V. Discs elliptical, wider than long. Externally differentiated inguinal gland absent. Pectoral fold absent. Cloacal opening at upper level of thighs. Skin on dorsum covered by scattered tubercles. Granular skin on throat, belly, and undersurfaces of thigh.

Measurements of holotype (in millimeters). SVL 27.79, HL 9.16, HW 8.23, TD 1.31, ED 1.55, IOD 3.75, END 3.2, NW 0.33, IND 2.06, ESD 4.6, THL 12.81, SHL 13.39, TAL 7.67, FL 11.52, AL 7.46, FAL 5.61, HAL 7.28, FW 1.57, and TW 1.55.

Color of holotype in preservative. Dorsal coloration brown, slightly metalic over cream background (Fig. 1A). Interocular region with a W shaped dark brown blot. Dark stripes at edge of upper lip. Iris gray. Dorsolateral region with a dark brown stripe extending from pre-ocular region to mid-body. Lateral region with a dark brown stripe over cream background extending from the edge of eye until inguinal region. Anterior and posterior dorsal surfaces of the arms and legs with dark brown stripes, wrist with a thin dark strip. Cream venter with light brown dots on throat. Inguinal region white with irregular brown blotches.

Color of holotype in life. During the night, completely metallic yellow without blots or stripes, with belly lighter than the dorsum. During the day, light brown with same pattern of stripes and blots observed in preservative. Edge of orbital region yellow. Region between eye and the edge of upper lip yellow. Venter also yellow. Iris metalic greenish yellow.

Variation of adult specimens. Measurements of adults given in Table 1. During the night, live males are like holotype (completely metallic yellow). During the day, some individuals are like holotype, whilst others might be completely metallic cream. In preservative, males are like holotype; variation in the pattern of lines and blotches is shown in Figure 2 (D, E, F). Live females have brown dorsum and pale venter during the night, with same pattern of lines and blotches observed in Figure 2 (A, B, C). During the day, females dorsum is dark brown with same pattern of lines and blotches, venter remain pale. Females in preservative have the same pattern observed in live specimens, although with lighter colors. Females have snout predominantly subovoid. Males might have snout subovoid or subelliptical. Males and females have variable toe webbing formalua: I – II1 – 2III1($1^{2/3}$) – 2IV2 ($2^{2/3}$) – 1($1^{1/3}$)V. Vomerine teeth in two contiguous small series of 3–5 teeth each, between choanae. Some individuals have outer metacarpal bilobed.

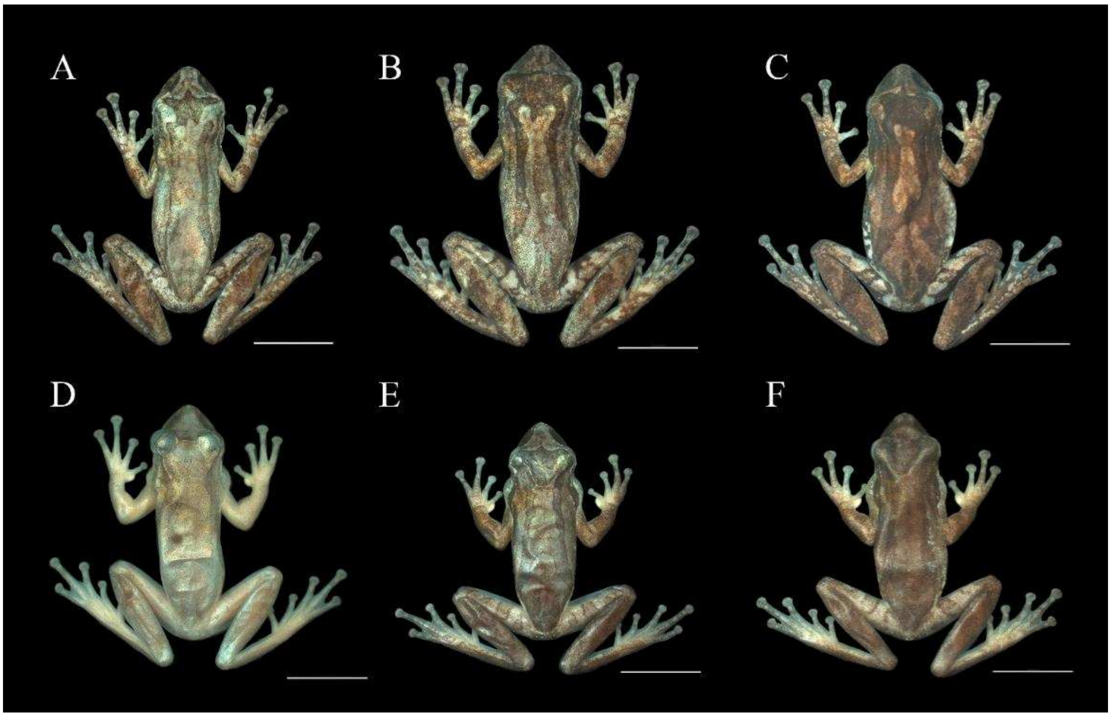

Figure 2. Dorsal color patterns of six paratypes of *Scinax* **sp. nov.** from Ervália, Minas Gerais, Brazil. Females (A) MZUFV 15675, (B) MZUFV 15680, and (C) MZUFV 15682. Males (D) MZUFV 15688, (E) MZUFV 15960, and (F) MZUFV 15962. Scale bar = 10 mm.

Table 1. Range, mean and standard deviation (SD) of the measurements (in mm) of the type series of *Scinax* **sp. nov.** Character abbreviations are listed in Material and Methods section.

	Males (n = 35)		**Females (n = 9)**		**Total (n = 44)**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
SVL	33.39 – 23.03	28.00 ± 1.97	33.53 – 28.89	31.40 ± 1.59	33.50 – 23.03	28.70 ± 2.34
HL	10.17 – 8.19	9.38 ± 0.46	11.11 – 9.74	10.47 ± 0.47	11.10 – 8.19	9.60 ± 0.64
HW	9.10 – 7.08	8.05 ± 0.43	10.01 – 8.77	9.25 ± 0.40	10.00 – 7.08	8.30 ± 0.64
TD	4.30 – 1.11	1.47 ± 0.71	1.75 – 1.30	1.51 ± 0.17	4.30 – 1.11	1.47 ± 0.63
ED	3.99 – 1.55	3.30 ± 0.41	4.23 – 3.42	3.65 ± 0.26	4.23 – 1.55	3.37 ± 0.41
IOD	3.75 – 0.32	3.11 ± 0.60	4.25 – 2.81	3.29 ± 0.47	4.25 – 0.32	3.15 ± 0.58
END	3.35 – 1.49	3.00 ± 0.32	4.33 – 2.00	3.26 ± 0.60	4.33 – 1.40	3.05 ± 0.40
IND	2.43 – 1.76	2.12 ± 0.13	2.50 – 2.22	2.34 ± 0.10	2.50 – 1.76	2.17 ± 0.15
ESD	5.00 – 3.00	4.46 ± 0.42	5.33 – 3.83	4.80 ± 0.48	5.33 – 3.00	4.53 ± 0.45
THL	13.63 – 11.39	12.82 ± 0.50	15.17 – 13.23	14.07 ± 0.59	15.20 – 11.39	13.07 ± 0.72
TL	14.84 – 11.84	13.67 ± 0.59	16.55 – 14.83	15.43 ± 0.56	16.60 – 11.84	14.03 ± 0.92
FL	12.69 – 9.87	11.36 ± 0.55	13.94 – 12.34	12.87 ± 0.49	13.90 – 9.87	11.67 ± 0.81
AL	8.21 – 5.98	7.04 ± 0.50	7.82 – 6.61	7.21 ± 0.47	8.21 – 5.98	7.08 ± 0.49
FAL	6.22 – 4.96	5.83 ± 0.25	6.54 – 4.82	5.97 ± 0.49	6.54 – 4.82	5.86 ± 0.31
HAL	7.98 – 6.62	7.34 ± 0.34	8.95 – 7.56	8.28 ± 0.47	8.95 – 6.62	7.53 ± 0.53

Call description. The advertisement call of *Scinax* **sp. nov.** is characterized by a series of pulsed notes (Fig. 3, Table 2, n = 17). Each call has variable duration (CD = 1.04 ± 0.18, 0.8–1.52 s) and is usually composed of about five notes (NN = 5.35 ± 0.78, 4–7 notes/call) with ascending amplitude through the call. Notes had similar duration (ND = 169.1 ± 22.7, 128–210 ms, n = 82), were emitted at regular intervals (NI = 47.4 ± 9.1, 36–100 ms, n = 74) and with stable rate (NR = 309.01 ± 19.18, 175.9–338.9 notes/s). Pulses have a tendency to be juxtaposed and are irregularly spaced through the note. Each note had about 60 pulses (PN = 63.09 ± 12.79, 39–85 pulses/note) emitted at variable rate (PR = 396.87 ± 48.92, 251.4–456.5 pulses/s).

Each note presented a marked amplitude modulation in which the first quarter of the note (23.1 ± 3.01, 18.3–31.9 %) displayed a rise in amplitude, followed by a descend that nearly separates it from the remaining of the note. The following three quarters presents a gradual rise in amplitude that reaches a peak in the final quarter (Fig. 3). The unevenly spaced pulses have a greater tendency to be juxtaposed in the first and in the fourth quarters of the note. Sometimes the last note of an advertisement call is short and similar to the first quarter of a regular note (ND = 39.0 ± 4.8, 26–49 ms, PN = 16.9 ± 2.5, 12–22 pulses/note, PR = 441.7 ± 57.9, 318.2–600 pulses/s, n = 9) (Fig. 3). Peak of energy did not vary through the note nor through the advertisement call (PF = 3930.12 ± 295.0, 3100.8–4565 Hz, n = 82). Dominant frequency ranged from 2110.3 to 5714.0 Hz.

Table 2. Parameters of the advertisement call of *Scinax* **sp. nov.** based on 17 calls from eight individuals recorded at Lagoa das Bromélias, Parque Estadual da Serra do Brigadeiro, municipality of Ervália, Minas Gerais state, on 29 November 2014, air temperature 18°C.

	Advertisement call (n = 17 calls)
Call duration (s)	1.04 ± 0.18 (0.8 – 1.52)
Number of notes per call	5.35 ± 0.78 (4 – 7)
Note duration (ms)	169.1 ± 22.7 (128 – 210) n = 82
Interval between notes (ms)	47.4 ± 9.1 (36 – 100) n = 74
Note rate (notes/min)	309.01 ± 19.18 (175.9 – 338.9) n = 17
Pulses per note	63.09 ± 12.79 (39 – 85)
Pulse rate (pulses/s)	396.87 ± 48.92 (251.4 – 456.5)
Peak frequency (Hz)	3930.12 ± 295.0 (3100.8 – 4565) n = 173
Dominant frequency range (Hz)	2110.3 – 5714.0

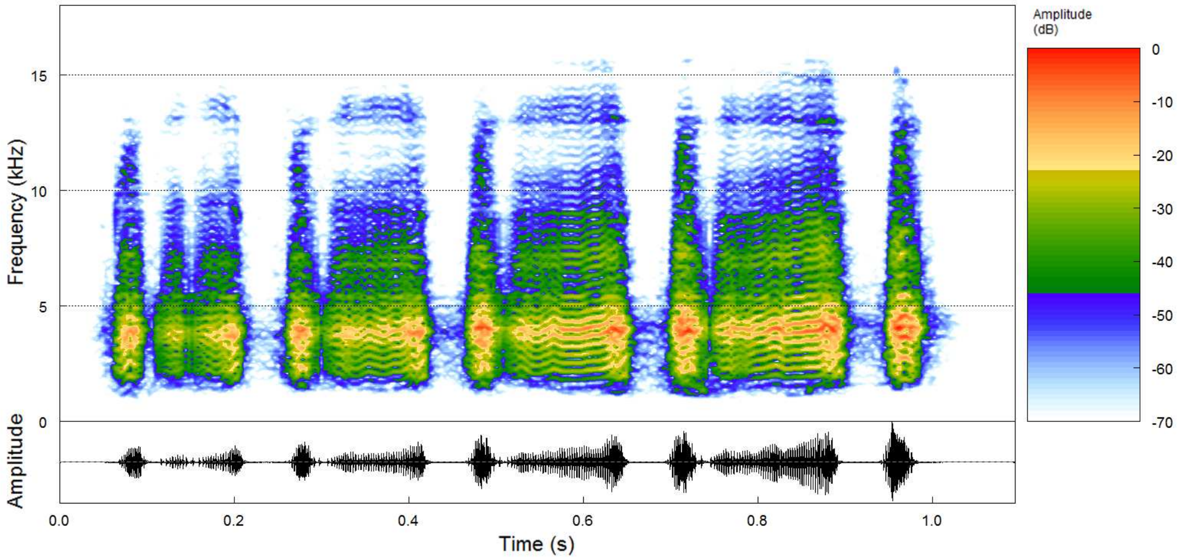

Figure 3. Spectrogram (above) and oscillogram (bellow) of the advisement call of *Scinax* **sp. nov.**, recorded on 29 November 2014 (20h20, air temperature 18°C) holotype MZUFV15965.

Tadpole description. Body is depressed (BH/BW = 0.78–0.79) (Fig. 4), slightly longer than one third of total length (BL/TL = 0.33–0.35), rounded in dorsal view and oval in lateral view. Ventral contour of body flat in the gular region, convex in the abdominal region. Snout rounded in dorsal and lateral views. Nostrils rounded without projections at inner edge, dorsally located, closer to the snout than to eyes, dorsolaterally directed. Eyes large (ED/BW = 0.15–0.18), dorsolaterally located (IOD/BW = 0.50–0.66) and directed dorsolaterally, not visible in ventral view.

Spiracle single, sinistral, lateroventral, posterodorsally projected, its inner wall attached to the body with free end and larger than the external wall. Opening located at the posterior third of the body, below body midline. Intestinal switchback point located at the center of the abdominal region. Vent tube small with dextral opening, entirely fused to ventral fin. Neuromasts are almost indistinguishable. However, we could delimit the following lines: supraorbital, infraorbital, infraorbital, posterior infraorbital, and ventral.

Tail slightly higher than body (TH/BH = 1.14–1.20), with little developed musculature (TMH/TH = 0.27–0.30) that reach the rounded tip of the tail. Dorsal fin emerges on posterior third of the body at a low slope, and origin of ventral fin emerges concealed by vent tube. Both fins are well-developed and with margin slightly convex.

Oral disc (Fig. 4D) positioned ventrally, small-sized (ODW/BW = 0.13–0.22), not emarginated. One row of marginal papillae in alternate disposition, with a small dorsal gap, lateral portion with many submarginal papillae. Tooth row formula (LTRF) 2(2)/3, A1 and A2 with same length, A2 has a small gap, P1, P2 and P3 with same length. Upper jaw sheath

“M” shaped and lower sheath “V” shaped. Both jaws with narrow keratinisation and slightly serrated. Measurements for all the available developmental stages are shown in Table 3.

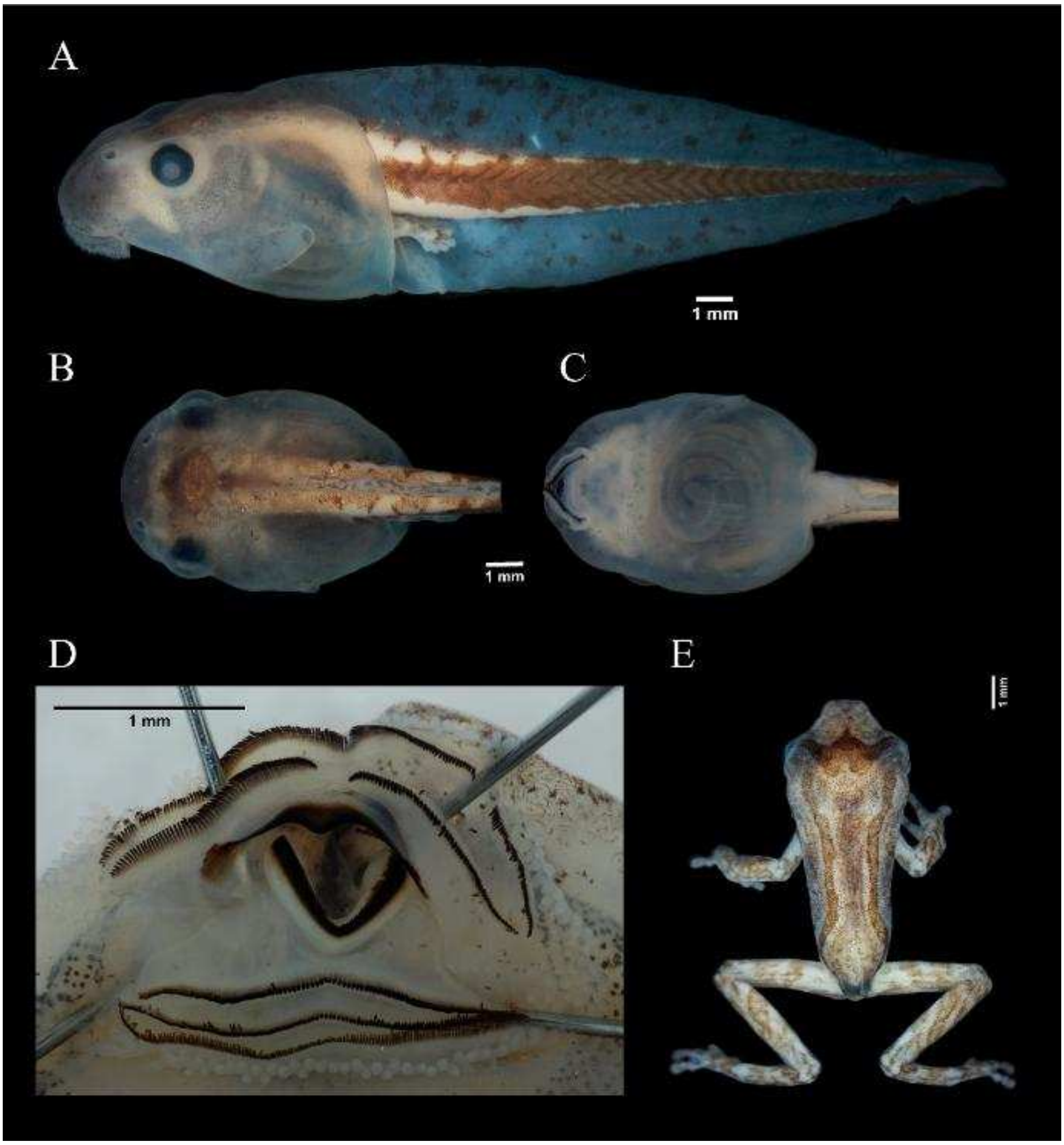

Figure 4. Tadpole of *Scinax* **sp. nov.** (MZUFV 148) at stage 36 according to Gosner (1960): (A) lateral, (B) dorsal and (C) ventral view, (D) oral disc. (E) Juvenile of *Scinax* **sp. nov.** metamorphosed in the laboratory (MZUFV 213, SVL 8.9 mm)

Tadpole coloration in preservative. Body brownish grey with scattered small blotches that cover the whole skin and the spiracle. Venter grey. Brown diamond shaped blotch between the eyes. Iri dark grey. Fins with irregular brown blotches scattered, being the ventral fin with less and smaller blotches. Musculature with a brown stripe from covering the whole length of the tail and two unpigmented stripes that gradually shrink through the first half. This pattern was observed in all tadpoles with slight variation in the density of blotches.

Table 3. Measurements (in mm) of tadpoles of *Scinax* **sp. nov.** from Ervália, state of Minas Gerais, Brazil (MZUFV 132, 148, 207, 213), developmental stages 31–36 (n=14) according to Gosner (1960). Character abbreviations are listed in Material and Methods section.

Characters	Range	Mean ± SD
TL	18.55 - 23.92	21.62 ± 2.38
BL	6.57 - 7.96	7.42 ± 0.62
TAL	12.17 - 15.86	14.23 ± 1.64
TH	4.84 - 5.77	5.29 ± 0.38
IND	1.85 - 2.94	2.36 ± 0.48
IOD	2.68 - 4.22	3.30 ± 0.69
TMH	1.31 - 1.78	1.55 ± 0.19
BW	5.36 - 6.36	5.95 ± 0.45
BH	4.01 - 5.04	4.68 ± 0.46
END	0.76 - 1.00	0.85 ± 0.11
ED	0.83 - 1.19	1.01 ± 0.17
ODW	2.34 - 2.52	2.41 ± 0.09
SL	0.75 - 1.46	1.02 ± 0.31

Comparison with other tadpoles of the *Scinax catharinae* group. Tadpoles of *Scinax* **sp. nov.** differs from *S. ariadne*, *S. flavogutattus*, *S. kautskyi*, *S. ranki* by its rounded snout in dorsal and lateral view (truncate in dorsal view in these species), *S. argyreornatus*, *S. hiemalis*, *S. humilis*, *S. machadoi* (truncated in lateral view in these species), *S. obtriangulatus* (sloped in lateral view in this species), and *S. pombali* (sloped to truncated in lateral view in this species). Tadpoles of the new species differs from *Scinax angrensis* and *S. ranki* by its dorsal nostril (anterolateral and lateral in these species, respectively); from *S. catharinae* and *S. tripui* by its spiracle opening below body midline (opens at the midline in these species); from *S. kautski* by its dextral vent tube (medial in this species), from *S. berthae* by the absence of flagellum at tip of tail (present only in this species).

The oral disc of *Scinax* **sp. nov.** differs by its small dorsal gap in marginal papillae from *S. albicans*, *S. angrensis*, *S. ariadne*, *S. flavoguttatus* and *S. pombali* (dorsal gap absent in these species), *S. argyreornatus*, *S. berthae*, *S. hiemalis*, *S. humilis*, *S. melanodactylus*, *S. litoralis* and *S. obtriangulatus* (wide dorsal gap in these species). Also differs by its marginal papillae uniseriated from *S. hiemalis* (anterior marginal papillae bisseriated), *S. albicans* (posterior marginal papillae bisseriated), *S. agrensis*, *S. aromothyella*, *S. flavoguttatus*, *S. kautskyi* (anterior and posterior marginal papillae bisseriated in these species), *S. ariadne* and *S. pombali* (anterior and posterior marginal papillae multisseriated in these species). The presence of many submarginal papillae in the lateral portion differs the tadpoles of the new species from *S. albicans*, *S. angrensis*, *S. argyreornatus*, *S. catharinae*, *S. humilis*, *S. machadoi*, *S. melanodactylus*, *S. obtriangulatus*, *S. trapicheroi* (few/sparse in these species), *S. luizotavioi*, *S. rizibilis* (forming rows), and *S. berthae* (absent). *Scinax* **sp. nov.** differs from *S. ariadne*, *S. machadoi* and *S.* rizibilis by the LTRF (LTRF = 2/3, 2(2)/3(1), 2(2)/(3), respectively in these species). From *S. aromothyella* and *S. berthae* by the upper jaw sheath "M" shaped (jaw sheath arch shaped in these species), from *S. argyreornatus*, *S. aromothyella*, *S. ariadne*, *S. flavoguttatus*, *S. kautskyi*, *S. machadoi*, *S. obtriangulatus* and *S. trapicheroi* by narrow jaw sheath (wide in these in these species).

Genetic analysis. Intraspecific and Interspecific distances uncorrected p distances are shown in Table 4. All intraspecific distances are considerably low (range from 0 to 2.9%) and interspecific distances (range from 11%–16%) support the distinction of *Scinax* **sp. nov.** as a new species. ML analysis presents a phylogenetic tree with a clear clustering pattern wherein each species can be recognized (Fig. 8), with *Scinax* **sp. nov.** as the sister species of the *S. rizibilis* in this preliminary analysis.

Table 4. Intraspecific and mean interspecific uncorrected p distances for COI among selected species of the *Scinax catharinae* species group.

		Inter (%)				
	Species (intra %)	1	2	3	4	5
1	*Scinax* **sp. nov.** (0.1%)					
2	*Scinax ariadne* (0.6%)	14%				
3	*Scinax aromothyella* (0.02%)	13%	13%			
4	*Scinax catharinae* (2.9%)	14%	13%	15%		
5	*Scinax hiemalis* (0.0%)	14%	11%	12%	15%	
6	*Scinax rizibilis* (2.7%)	11%	15%	14%	16%	15%

Natural history. Lagoa das Bromélias is a temporary pond that completely dries during the dry season (April to September) and reaches up to 80 cm depth and 600 m² of water surface during the rainy season (October to March). The pond is located 1227 m a.s.l. at Parque Estadual da Serra do Brigadeiro (PESB), a conservation unit run by the Instituto Estadual de Florestas do Estado de Minas Gerais. The unit consists of an important remnant of the Brazilian Atlantic Forest within the Serra da Mantiqueira mountain range and with an rich epiphyte flora mainly represented by the families Bromeliaceae and Orchidaceae (Cruz 2007, Caramaschi *et al*., 2008). PESB has an area of about 13000ha encompassing the municipalities of Araponga, Fervedouro, Miradouro, Ervália, Sericita, Pedra Bonita, Muriaé e Divino.

Figure 5. Explosive breeding of *Scinax* **sp. nov.** at Lagoa das Bromélias, Parque Estadual da Serra do Brigadeiro, Minas Gerais.

Scinax **sp. nov.** was first registered at Lagoa das Bromélias on 21 October 2009. Since then, the species has been observed yearly during the months of October and November as an explosive breeder (sensu Wells 1977). During the reproductive period, several males can be found calling on different substrates around and within the pond (e.g. herbs, trees, bushes, rocks, water; Figure 5). Although some amplectant couples can be found in branches and leaves above the water, most of them are found within the pond with the body partially submersed. The high density of eggs laid in the water cover most of pond's surface (Figure 5). At a given occasion, we observed axillar and cranial amplexus (Figure 6), fights between males for a females and eggs being predated by spiders and beetles.

The pond is also the type locality of *Leptodactylus cupreus* and *Chiasmocleis mantiqueira*, the later also being characterized by the explosive breeding behaviour. On 29 November 2014, we registered an explosive breeding of both *Scinax* **sp. nov.** and *C. mantiqueira* in syntopy. Several males of *Chiasmocleis* could be observed swimming, calling and in amplexus within the water (Figure 6F). Moreover, we also registered the interspecific amplexus between both species (Figure 6E). In addition, the species *Aplastodiscus arildae, Dendropsophus minutus, Ischnocnema izecksohni*, *Scinax* aff. *perereca* and *S. eurydice* were also calling around Lagoa das Bromélias.

Figure 6. Natural history notes during the explosive breeding of *Scinax* **sp. nov.**: (A) axillary amplexus; (B) cranial amplexus; (C) male “shifter”; (D) amplectant couples; (E) interspecific amplexos between *Chiasmocleis mantiqueira* and *Scinax* **sp. nov.**; (F) amplectant couples of *C. mantiqueira*.

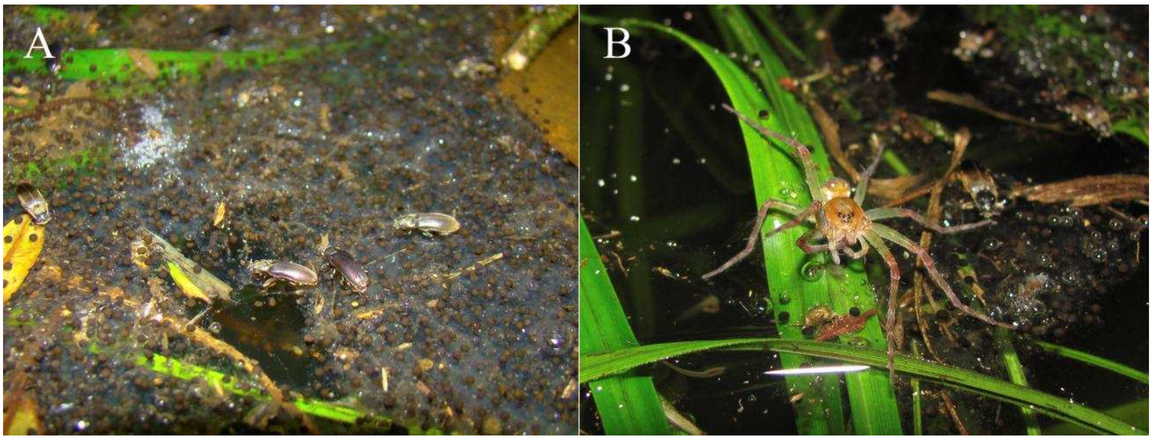

Figure 7. Predation events of eggs during the explosive breeding of *Scinax* **sp. nov.**: (A) predation by beetles and (B) spider.

Distribution. *Scinax* **sp. nov.** is only known for the type locality (Figure 8). Previous studies covering different areas of the Serra do Brigadeiro did not registered the new species (Feio *et al.* 2000, Moura *et al.* 2012). This result may indicate the high specificity of the new species to environmental conditions provided by the Lagoa das Bromélias.

Discussion. Anurans are among the groups more likely to the appearance of cryptic species (Bickford *et al.*, 2007). Overall, those are organisms in which conspecifics recognition and choice of sexual partners is not directly based on morphological features (Bickford *et al.*, 2007). This could be the case of this new species, in which the explosive breeding behavior and the choosing of sexual partners does not seem to be related to the visual recognition of conspecifics. On the other hand, Bastos and Haddad (1999) studied a population of *Scinax rizibilis* from Ribeirão Branco, São Paulo state, in which amplectant couples were significantly correlated with body mass and SVL. However, those characters are often negatively correlated with the dominant frequency (peak frequency in this work) of the advertisement call (see Bastos and Haddad 1999 and references within). Therefore, we agree with Bastos and Haddad (1999) that the body mass or SVL are indirectly chosen through the advertisement call, which corroborates the premise from Bickford *et al.* (2007).

In the light of all the data presented in this work, we observed that *Scinax* **sp. nov.** is sister species to *S. rizibilis* and morphologically very similar. Despite the great resemblance between both species, the set of data analyzed (i.e. morphology, tadpoles, bioacoustics, natural history and molecular data) successfully diagnosed the new species. Moreover, the only location from which *Scinax* **sp. nov.** is known is over XX kilometers from the closest population of *S. rizibilis* (Fig. 8).

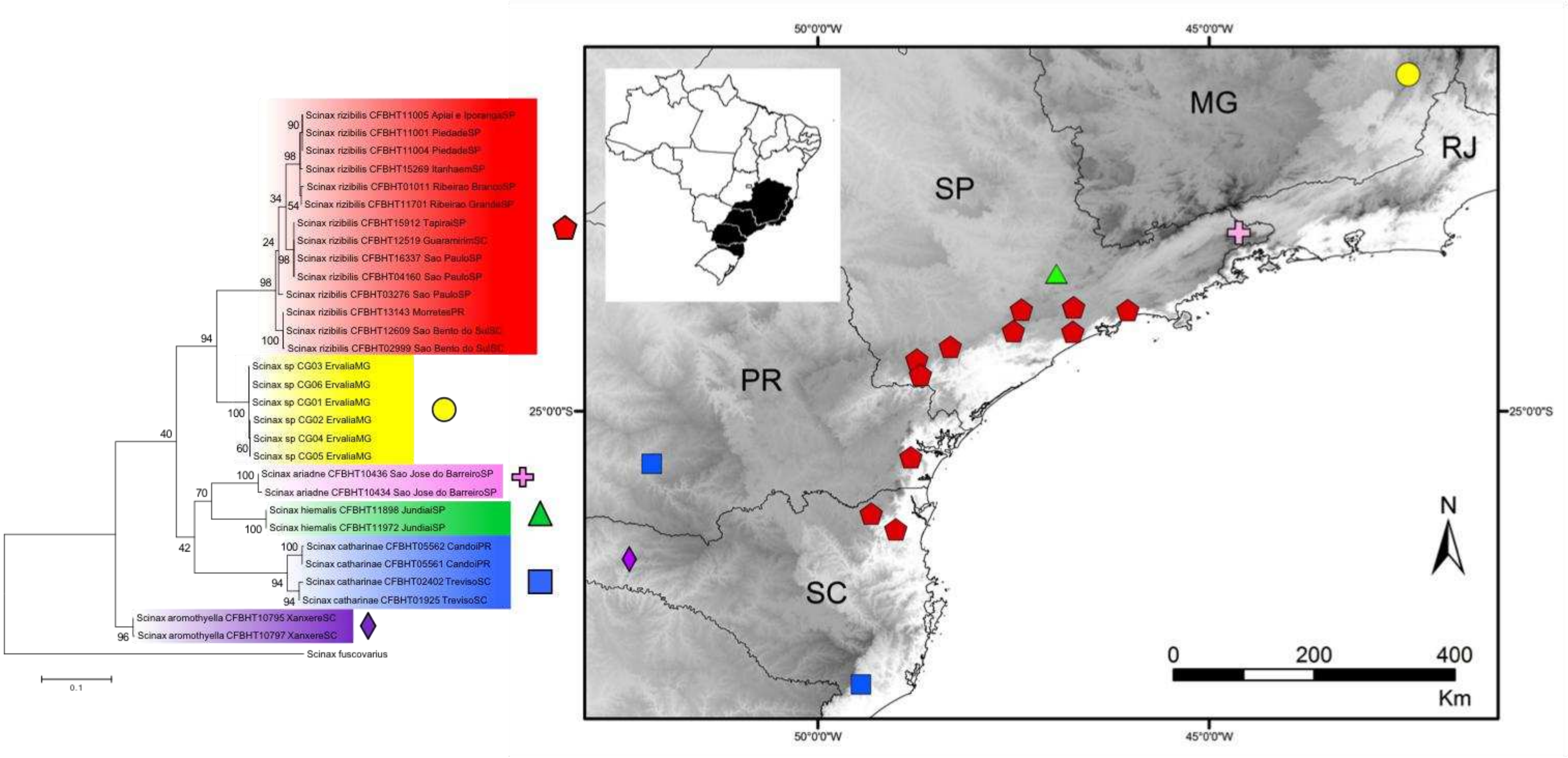

Figure 8. (A) Phylogenetic tree inferred with Maximum likelihood analysis including *Scinax* **sp. nov.** (yellow), and some related species (*S. rizibilis* – red; *S. ariadne* – pink; *S. hiemalis* – green; *S. catharinae* – blue; *S. aromothyella* –purple). **(B)** Type locality (indicated by a yellow dot) of *Scinax* **sp. nov.** in Lagoa das Bromélias, Serra do Brigadeiro, municipality of Ervália, Minas Gerais, southeastern Brazil and geographic distribution of related species analyzed with molecular marker.

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Appendix 1

Specimens Examined

Scinax canastrensis: Brazil, **Minas Gerais**: São Roque de Minas (MZUFV 9960–62, 9983, 10324/28), Antônio Dias (1354–55).

Scinax carnevalli: Brazil, **Minas Gerais**: Caratinga (MZUFV 10516–17, 10583), Marliéria (MZUFV 752).

Scinax centralis: Brazil, **Goiás**: Campo Alegre (MZUFV 6472–74, 6479), Orizona (MZUFV 6509–11).

Scinax flavoguttatus: Brazil, **Minas Gerais**: Lima Duarte (MZUFV 6616, 6856), Passa Quatro (MZUFV 7745), Serra do Sacramento (MZUFV 8373–77).

Scinax longilineus: Brazil, **Minas Gerais**: Luminárias (MZUFV 10708–12), São Roque de Minas (MZUFV 10723, 10726).

Scinax luizotavioi: Brazil, **Minas Gerais**: Ervália (MZUFV 12402, 12457, 12698–99, 12703), Pedra Dourada (MZUFV 12708–09, 12712).

Scinax ranki: Brazil, **Minas Gerais**: Poços de Caldas (MZUFV 8222, 8586)

Scinax rizibilis: Brazil, **São Paulo**: Santo André (CFBH 29050), Pilar do Sul (CFBH 9315), Piedade (CFBH 22281–82), Jequitiba (CFBH 38610), Bertioga (CFBH 38987), Apiaí e Iporanga (CBH 26754), Ribeirão Branco (CFBH 1789, 1791, 1793–94, 1799–1801, 1803–07, 1851, 2346, 2350, 2358–65, 35115, 35311–325) (ZUEC-AMP 7013, 7018–24, 7026–28, 9785–89).

Scinax trapicheiroi: Brazil, **Rio de Janeiro**: Rio de Janeiro (MZUFV 1202–03).

Scinax tripui: Brazil, **Minas Gerais**: Ouro Branco (MZUFV 10512–15), Ouro Preto (MZUFV 12447–50).

Appendix 2

Specimens of Scinax included in the molecular analysis, GenBank accession numbers and collection localities.

Species	**Tissue ID**	**Catalog Num**	**Genbak N**	**Locality**	**State**	**Lat**	**Lon**
Scinax sp nov.	CSG01			Ervalia	MG	-20.696	-42.457
Scinax sp nov.	CSG02			Ervalia	MG	-20.696	-42.457
Scinax sp nov.	CSG03			Ervalia	MG	-20.696	-42.457
Scinax sp nov.	CSG04			Ervalia	MG	-20.696	-42.457
Scinax sp nov.	CSG05			Ervalia	MG	-20.696	-42.457
Scinax sp nov.	CSG06			Ervalia	MG	-20.696	-42.457
Scinax ariadne	CFBHT10436	CFBH-18105	to be provided	Sao Jose do Barreiro	SP	-22.719	-44.618
Scinax ariadne	CFBHT10434	CFBH18106	to be provided	Sao Jose do Barreiro	SP	-22.719	-44.618
Scinax aromothyella	CFBHT10795	CFBH-22759	to be provided	Xanxere	SC	-26.890	-52.408
Scinax aromothyella	CFBHT10797	CFBH-22761	to be provided	Xanxere	SC	-26.890	-52.408
Scinax catharinae	CFBHT05562	CFBHT5562	to be provided	Candoi	PR	-25.672	-52.122
Scinax catharinae	CFBHT05561	CFBHT05561	to be provided	Candoi	PR	-25.672	-52.122
Scinax catharinae	CFBHT02402	CFBH-10319	to be provided	Treviso	SC	-28.490	-49.450
Scinax catharinae	CFBHT01925	CFBH-8498	to be provided	Treviso	SC	-28.490	-49.450
Scinax hiemalis	CFBHT11898	CFBH 24835	to be provided	Jundiai	SP	-23.243	-46.951
Scinax hiemalis	CFBHT11972	CFBH 25404	to be provided	Jundiai	SP	-23.243	-46.951
Scinax rizibilis	CFBHT16337	CFBH-32317	to be provided	Sao Paulo	SP	-23.986	-46.742
Scinax rizibilis	CFBHT11004	CFBH-23274	to be provided	Piedade	SP	-23.704	-47.399
Scinax rizibilis	CFBHT01011	CFBH 6872	to be provided	Ribeirao Branco	SP	-24.340	-48.737
Scinax rizibilis	CFBHT11005	CFBH-23284	to be provided	Apiai e Iporanga	SP	-24.536	-48.688
Scinax rizibilis	CFBHT15912	CFBH-31080	to be provided	Tapirai	SP	-23.976	-47.500
Scinax rizibilis	CFBHT03276	CFBH-12382	to be provided	Sao Paulo	SP	-23.675	-46.732
Scinax rizibilis	CFBHT13143	CFBH-27487	to be provided	Morretes	PR	-25.588	-48.812
Scinax rizibilis	CFBHT12519	CFBH 26680	to be provided	Guaramirim	SC	-26.510	-49.003
Scinax rizibilis	CFBHT04160	CFBH-13567	to be provided	Sao Paulo	SP	-23.675	-46.732
Scinax rizibilis	CFBHT15269	CFBH-31047	to be provided	Itanhaem	SP	-23.986	-46.742
Scinax rizibilis	CFBHT12609	CFBH-22004	to be provided	Sao Bento do Sul	SC	-26.308	-49.317
Scinax rizibilis	CFBHT02999	CFBH-10971	to be provided	Sao Bento do Sul	SC	-26.308	-49.317
Scinax rizibilis	CFBHT11701	CFBH24547	to be provided	Ribeirao Grande	SP	-24.177	-48.308
Scinax rizibilis	CFBHT11001	CFBH-23275	to be provided	Piedade	SP	-23.704	-47.399

2.3 ARTIGO III – Guimarães, C.S., Lyra, M. & Feio, R.N. 2016. A new species of the *Scinax catharinae* group (Anura: Hylidae) from Serra da Mantiqueira, Minas Gerais, Brazil

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2.

The tadpole of *Scinax cosenzai* (Anura: Hylidae)

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Abstract. The bromeligenic *Scinax perpusillus* group is currently composed of 13 species endemic to the Atlantic rainforest, occurring north from the Brazilian state of Espírito Santo to Santa Catarina in the south. *Scinax cosenzai* was recently described from the state of Minas Gerais. It is the species with the most inland record of the group. As its original description lacks information on its larvae, we herein describe the tadpole of *S. cosenzai* and provide a comparison to the other known tadpoles of the group. Thirty-one tadpoles were collected from bromeliads at the Parque Estadual da Serra do Brigadeiro, then analysed and compared to information on tadpoles of the *S. perpusillus* group available in the literature. The tadpole of *S. cosenzai* may be diagnosed by the following set of characters: 1) lack of any yellow or golden marks; 2) a total length of 19.9–31.1 mm (stages 25–38); 3) lateral sections of mouth with labial papillae arranged in 3–4 rows; 4) anterior portion of mouth with a single row of labial papillae; 5) dorsal and ventral fins equal in height.

Key words. Amphibia, bromeliad, larvae, taxonomy, Serra do Brigadeiro, Minas Gerais

Introduction

The hylid genus *Scinax* Wagler, 1830, is currently composed of 111 species of treefrogs, occurring south from Argentina and Uruguay north to Mexico (Frost 2013). Peixoto (1987) was the first to propose the existence of a *Scinax perpusillus* group, currently characterized by species of *Scinax* with bromeligenic habits, interdigital webbing absent between toes I and II, and absent or vestigial between II and III (Faivovich 2002). Although the monophyly of the *Scinax perpusillus* group has not as yet been adequately tested it is continued to be recognized. Its assumed monophyly was also supported by Pombal & Bastos (2003) and Alves-Silva & Silva (2009) with vocalization data and reproductive behaviour characteristics, respectively.

The group is currently composed of 13 species: *Scinax alcatraz* (Lutz, 1973), *S. arduous* Peixoto, 2002, *S. atratus* (Peixoto, 1989), *S. belloni* Faivovich, Gasparini & Haddad, 2010, *S. cosenzai* Lacerda, Peixoto & Feio, 2012, *S. faivovichi* Brasileiro, Oyamaguchi & Haddad, 2007, *S. insperatus* Silva & Alves-Silva, 2011, *S. littoreus* (Peixoto, 1988a), *S. melloi* (Peixoto, 1989), *S. perpusillus* (Lutz & Lutz, 1939), *S. peixotoi* Brasileiro, Haddad, Sawaya & Martins, 2007, *S. tupinamba* Silva & Alves-Silva, 2008, and *S. v-signatus* (Lutz 1968). These species are all endemic to the Brazilian Atlantic tropical coastal forest, distributed north from the state of Espírito Santo south to the state of Santa Catarina (Alves-Silva & Silva 2009), inhabiting bromeliads in both mountain regions and coastal areas (Silva-Soares et al. 2010).

Scinax cosenzai was recently described from the Parque Estadual da Serra do Brigadeiro situated in the municipalities of Araponga and Ervália, state of Minas Gerais, being the most inland record of the group (Lacerda et al. 2012). Herein we describe the tadpole of *S. cosenzai* and provide a detailed comparison to the other known tadpoles of the *S. perpusillus* group.

Material and methods

Tadpoles of *Scinax cosenzai* were collected from bromeliads in the municipality of Araponga, in January of 2008 (20°43'13" S and 42°28'48" W; MZUFV 252), and in the municipality of Ervália, in January of 2012 (20°51'52" S and 42°31'17" W; MZUFV 253), both sites are located within the Parque Estadual da Serra do Brigadeiro, the type locality of the species.

All tadpoles were preserved in formalin 10% and deposited in the herpetological collection of the Museu de Zoologia João Moojen (MZUFV), Universidade Federal de Viçosa, municipality of Viçosa, state of Minas Gerais, southeastern Brazil. The species identity was confirmed by our raising in the laboratory one specimen to metamorph stage (Fig. 1). This individual exhibited the same dorsal pattern and morphology as observed in *Scinax cosenzai* (see Lacerda 2012).

Fourteen measurements were taken from 31 specimens at stages 24–40 sensu Gosner (1960): total length (TL), body length (BL), tail length (TAL), tail height (TH), internarial distance (IND), interorbital distance (IOD), and tail muscle height (TMH) – all following Altig & McDiarmid (1999); body width (BW), body height (BH), eye–nostril distance (END), eye diameter (ED), and oral disc width (ODW) – all following Lavilla & Scroccho (1986); and spiracle length (SL) and cloacal tube length (CTL). TL and TAL were taken using analogue callipers to the nearest 0.1 mm, while the other measurements were taken using a stereomicroscope with a micrometric ocular (Olympus SZ61).

Our comparison to the other described tadpoles of the *Scinax perpusillus* group was based on literature information: *S. arduous* (Peixoto 2002), *S. belloni* (Silva-Soares et al. 2010), *S. insperatus* (Silva & Alves-Silva 2011), *S. littoreus* (Peixoto 1987), *S. melloi* (Peixoto 1988b), *S. perpusillus* (Peixoto 1987), *S. tupinamba* (Silva & Alves-Silva 2008), and *S. v-signatus* (Peixoto 1987).

Results

Description. All 14 measurements from each tadpole of *Scinax cosenzai* are presented in Table 1. Body dorsoventrally compressed (BW > BH), oval in dorsal view (Fig. 2A) and slightly longer than wide, with rounded ends. These ends may have a width difference between them, with the anterior end being less wide than the posterior counterpart. Some tadpoles that were raised in an aquarium and analysed at an advanced stage (near 39) had a piriformous body shape in dorsal view with the posterior end of the body being much wider than the anterior portion (Fig. 2D).

Figure 1. Juvenile of *Scinax cosenzai* metamorphosed in the laboratory (MZUFV 253, SVL 10 mm).

Snout rounded in lateral and dorsal views. Eyes dorsolaterally located. Nostrils rounded and dorsally positioned. Spiracle sinistral with a posterodorsal opening. Terminal portion of spiracle tube with a free end. The arrangement of the intestinal tube is circular and spiralled in the centre of the abdomen during the early stages and shifted laterally (to the right in ventral view) at stages around 37. Dextral ventral tube with a free end a little above the bottom margin of the ventral fin.

Tail height corresponding to 92.7% of the body height (68.0–113.4%, sd = 10.7, n = 31) with a rounded or obtuse (intermediate between rounded and pointed) termination (Figs. 2C and 2E). Tail musculature height corresponding to 49.9% of the tail height (31.8–62.0%, sd = 7.8, n = 31). Dorsal fin from slightly convex to rectilinear, starting in the posterior third of the body with the insertion of the tail. Ventral fin slightly convex, starting at the level of the ventral tube. Dorsal and ventral fins equal in height.

Mouth ventrally located, without emarginations. The anterior portion is surrounded by one row of papillae with a large gap in the centre, 1–3 rows in the posterior section, and 3–4 rows laterally (Fig. 3). Labial tooth row formula 2(2)/3: two anterior rows of labial teeth, the second one with a small gap; three rows of labial teeth in the posterior portion. Jaw sheaths with moderate keratinisation, upper jaw v-shaped (cuspate) and serrated, and lower jaw also serrated.

Colouration in life. Body with translucent skin, allowing to see the internal organs through it. Body loosely speckled with some darker pigment, more concentrated in the anterior third. The fins are also transparent with scattered darker pigment, likewise more concentrated in the anterior portion. The tail musculature is light beige in colour. Some individuals exhibited black blotches on the tail (fins and musculature), varying in density (Fig. 2F).

Colouration in formalin. The body skin turns a little darker, but remains translucent. The fins become less transparent, and take on a light beige colour, emphasizing the dark speckling.

Discussion

Prior to the present study, eight of the 13 described species belonging to the *Scinax perpusillus* group had their tadpoles described; missing were descriptions of those of *S. alcatraz, S. atratus, S. cosenzai, S. faivovichi* and *S. peixotoi*. Although the known tadpoles of the members of the *S. perpusillus* group are evidently very similar to each other, they may differ in the shape of lower jaw, numbers of rows of labial papillae, size of fins, height of tail, height of body, shape of tail termination, or whether the caudal muscles reach the end of the tail (Silva-Soares et al. 2010). Herein, we raise the number of species with described tadpoles within the group to nine by describing the tadpole of *S. cosenzai*, which can be distinguished from the other

Table 1. Fourteen measurements to the nearest of 0.1 mm of 31 tadpoles of *Scinax cosenzai*. Staging follows Gosner (1960); total length (TL), body length (BL), body width (BW), body height (BH), tail length (TAL), tail height (TH), tail muscle height (TMH), eye diameter (ED), interorbital distance (IOD), eye–nostril distance (END), internarial distance (IND), oral disc width (ODW), spiracle length (SL), and cloacal tube length (CTL). Repeated stages represent the number of specimens measured at that stage.

Stage	TL	BL	BW	BH	TAL	TH	TMH	ED	IOD	END	IND	ODW	SL	CTL
24	22.1	8.5	5.0	3.5	13.6	3.5	1.9	0.7	2.5	1.2	1.9	2.1	0.7	1.0
25	23.0	9.3	6.4	4.8	13.7	3.8	2.3	1.0	3.6	1.5	1.9	2.0	0.8	1.0
25	23.7	9.9	7.3	5.8	13.8	5.9	2.5	1.1	4.6	1.9	2.3	2.7	1.2	1.3
26	24.9	9.6	6.7	4.8	15.3	4.0	2.1	1.0	3.7	1.8	2.1	1.7	0.8	1.1
26	22.1	8.5	6.4	4.7	13.6	3.2	1.7	0.9	3.5	1.6	1.9	2.0	0.1	1.1
26	23.8	9.1	5.7	4.5	14.7	4.5	1.9	0.9	3.4	1.5	2.3	2.0	0.8	0.9
26	19.9	8.3	5.7	4.2	11.6	4.4	1.4	0.9	3.3	1.6	1.9	1.6	0.9	0.9
27	22.0	8.0	5.0	4.9	14.0	4.9	2.1	1.0	3.2	1.4	1.8	1.8	1.1	0.6
27	23.4	9.1	5.7	4.9	14.3	4.0	1.4	0.7	3.4	1.4	2.0	2.1	1.1	0.8
28	24.6	8.8	6.0	5.2	15.8	5.9	1.9	1.0	3.8	1.7	2.0	2.4	0.7	1.4
29	21.6	8.4	5.6	4.2	13.2	3.8	1.7	0.9	3.5	1.5	1.9	1.8	0.6	0.9
34	24.5	8.9	6.1	4.5	15.6	4.1	2.4	1.2	3.5	1.7	1.9	2.0	0.8	1.2
34	22.4	9.1	5.6	4.4	13.3	3.9	1.8	0.9	3.4	1.5	1.8	2.2	0.9	1.1
36	23.4	8.9	6.5	4.4	14.5	3.7	2.2	1.2	3.9	1.7	2.1	2.7	1.3	1.1
36	24.5	10.4	7.3	5.9	14.1	5.0	2.4	1.2	3.8	1.9	2.0	2.3	1.3	0.8
36	28.1	10.5	7.8	4.7	17.6	4.9	3.0	1.4	4.2	1.9	2.2	2.3	0.9	1.5
36	30.9	10.7	8.0	5.8	20.2	5.9	2.8	1.4	4.4	2.2	2.2	2.7	0.9	2.1
37	27.1	10.5	7.2	5.3	16.6	5.3	2.5	1.5	4.5	1.8	2.1	2.3	1.5	2.1
37	27.3	10.5	7.2	5.5	16.8	4.7	2.5	1.3	4.2	1.8	2.2	2.4	1.3	1.2
37	27.4	10.5	7.4	5.9	16.9	6.1	2.8	1.5	4.3	2.1	1.9	2.2	1.3	1.9
37	31.1	10.8	8.2	5.2	20.3	5.0	3.1	1.5	4.7	2.0	2.3	2.7	1.2	2.2
38	26.7	10.6	8.9	6.4	16.1	5.5	2.9	1.4	4.4	2.1	2.2	2.7	0.8	0.9
38	29.1	10.6	8.1	5.6	18.5	5.4	2.7	1.5	4.3	2.0	2.3	2.7	1.1	1.0
38	30.8	11.0	7.9	5.6	19.8	5.4	2.4	1.4	4.5	2.1	2.2	2.5	0.5	2.0
39	24.8	10.0	6.8	4.9	14.8	5.5	2.8	1.3	4.5	2.0	2.2	3.1	1.5	1.6
39	27.8	10.5	7.5	6.5	17.3	4.7	2.5	1.3	4.2	1.9	2.2	2.4	1.7	1.1
39	29.7	10.5	8.4	6.1	19.2	5.7	3.2	1.4	4.2	2.2	2.2	2.9	0.4	1.5
40	28.0	11.3	8.2	5.7	17.7	5.5	2.6	1.4	4.5	2.0	2.2	2.5	1.0	0.9
40	28.6	9.9	7.5	5.3	18.7	4.8	2.6	1.5	4.1	2.1	2.1	2.1	1.0	1.5
40	28.6	10.5	7.3	5.6	18.1	4.6	2.3	1.4	4.4	1.9	2.2	2.5	1.2	1.3
40	29.9	11.0	8.3	5.7	18.9	4.9	2.8	1.4	4.5	2.0	2.1	2.6	0.6	1.9

known tadpoles of the group by the combination of the following characters:

The discrete colouration of the tadpoles of *Scinax cosenzai* readily distinguishes it from those of *S. belloni* and *S. insperatus*. The tadpole of *S. tupinamba* has a bright yellow stripe between the eyes and nostrils that differentiates it from all other known tadpoles of the group (Silva & Alves-Silva 2008). The tadpole of *S. belloni* has a golden pigmentation throughout the body and a dark band along the dorsal and ventral fins (Silva-Soares et al. 2010). *Scinax insperatus* differs from all other known tadpoles of the group by having fins with yellow spots at the later stages of its development (Silva & Alves-Silva 2011). The tadpole of *S. cosenzai* furthermore differs from *S. belloni* and *S. insperatus* by having only 3–4 rows of lateral labial papillae (4–6 in *S. belloni* and *S. insperatus*).

The tadpoles of *Scinax arduous*, *S. littoreus*, *S. melloi*, *S. perpusillus* and *S. v-signatus* are similar to *S. cosenzai* in that they lack any trace of yellow or golden markings. However, tadpoles of *S. cosenzai* may have dark pigmentation on the tail (as does *S. tupinamba*) and they are larger than those of *S. arduous* and *S. littoreus* described by Peixoto (1988a, 2002): TL of 16.7–23.0 mm (x = 19.9, n = 13, stages 26–37) in *S. arduous*, and 19.9–31.1 mm (x = 24.9, sd = 3.1, n = 18, stages 26–37) in *S. cosenzai*; TL of 16.0–19.9 mm (stages 25–38) in *S. littoreus* and 19.9–31.1 mm (x = 25.3, sd = 3.1, n = 23, stages 25–38) in *S. cosenzai*.

Regarding colour pattern and total length, the tadpole of *Scinax cosenzai* is most similar to those of *S. melloi*, *S. perpusillus* and *S. v-signatus*. Having a tail with dorsal and ventral fins of equal heights differentiates *S. cosenzai* from all these species (dorsal fin higher than ventral fin

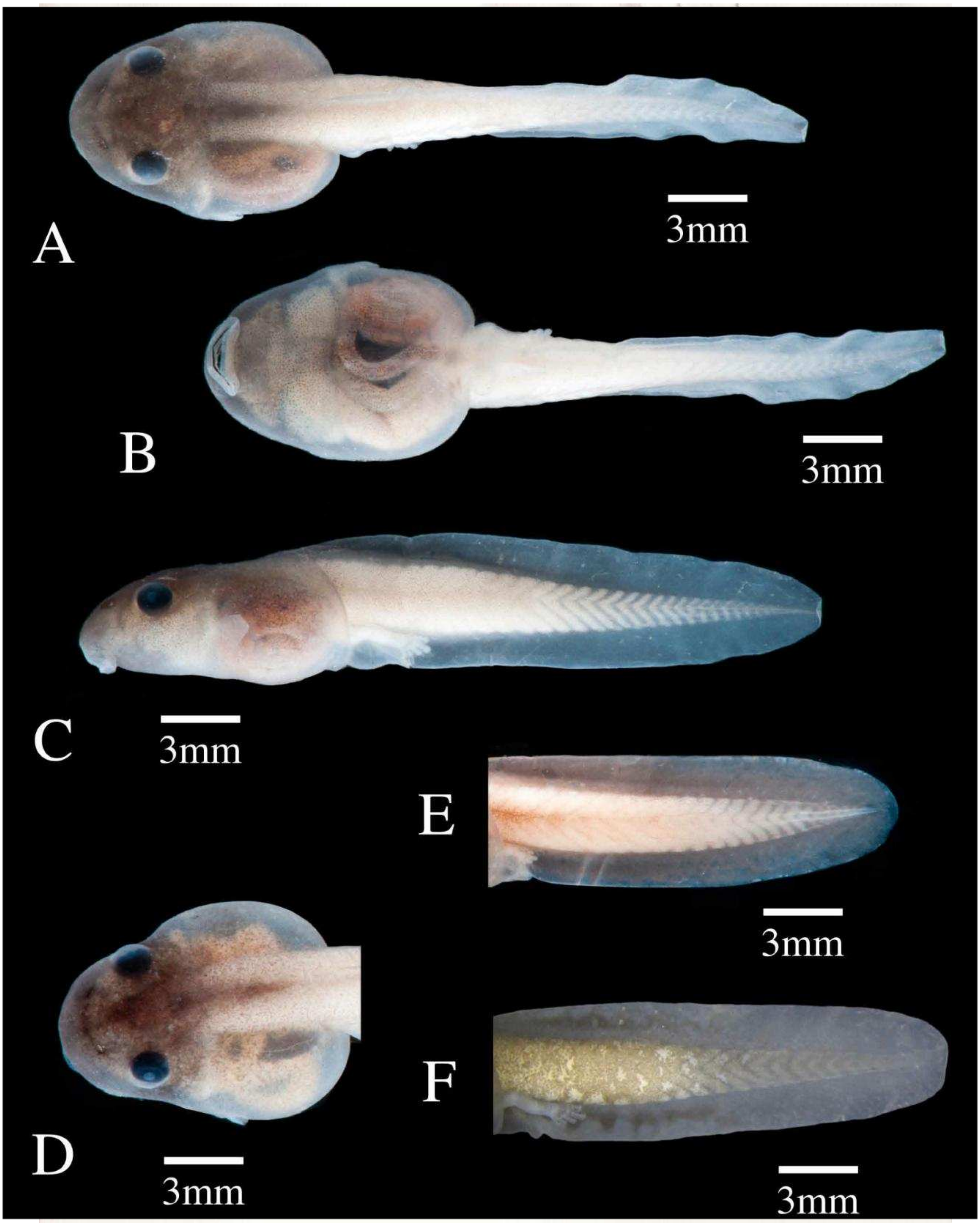

Figure 2. Tadpole of *Scinax cosenzai*: A) Dorsal view with emphasis on the ovoid body shape (stage 36); B) Ventral view (stage 36); C) Lateral view with emphasis on the obtuse tail ending (stage 36); D) Body dorsal view with emphasis on the piriform body shape (stage 38); E) Tail lateral view with emphasis on the rounded tail ending (stage 34); and F) Tail lateral view with black blotches (stage 38).

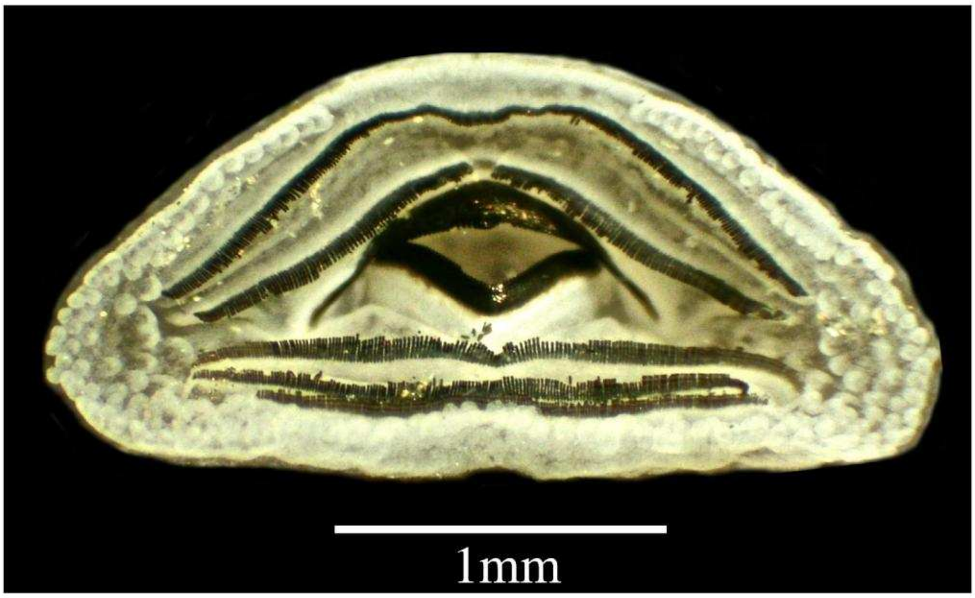

Figure 3. Detail of mouth of tadpole of *Scinax cosenzai* at Stage 36.

in *S. melloi*, *S. perpusillus* and *S. v-signatus*; Peixoto 1987, 1988b), though. The tadpole herein analysed furthermore differs from *S. perpusillus* in having its labial papillae arranged in a single row on the anterior margin of the mouth (two rows in *S. perpusillus*) and from *S. v-signatus* in having fewer lateral rows of labial papillae (four or more rows in *S. v-signatus versus* three or four rows in *S. cosenzai*).

Morphological studies on tadpoles may contribute to the ecology, taxonomy, phylogeny, and other studies on the evolution of anurans (Conte et al. 2007, Kolenc et al. 2008). Recently, tadpole features have come to play an important role in the *Scinax perpusillus* group taxonomy, especially after the descriptions of *S. belloni*, *S. insperatus* and *S. tupinamba*, which have synapomorphies associated to their larvae. Nevertheless, comparisons between morphological characteristics of their larvae are still very difficult to perform within the group, in particular due to the lack of important information such as detailed morphometric data morphological nomenclature standardization, and detailed photographs. Thus, detailed descriptions and identification keys may greatly improve the knowledge on the taxonomy of the *S. perpusillus* group.

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3. RESULTADO GERAL

Tabela 1. Lista de espécies de anfíbios registradas para a Serra do Brigadeiro, Minas Gerais, sudeste do Brasil (atualizada e adaptada de Moura *et al.* 2012).

Família / Espécie

ANURA

Brachycephalidae

1. ~~*Brachycephalus ephippium* (Spix 1824)~~
   Brachyecphalus **sp. nov.** (Guimarães, Luz, Rocha & Feio 2016)
2. *Brachycephalus* aff. *didactylus*
3. *Ischnocnema parva* (Girard 1853)
4. *Ischnocnema verrucosa* tken 1862)
5. *Ischnocnema izecksohni*
6. *Ischnocnema* aff. *guentheri*
7. *Ischnocenam* gr. *lactea*

Bufonidae

1. *Rhinella pombali* (Baldissera-Jr, Caramaschi & Haddad 2004)

Centrolenidae

1. *Vitreorana eurygnatha* (A. Lutz 1925)
2. *Vitreorana uranoscopa* ller 1924)

Ceratophryidae

1. *Ceratophrys aurita* (Raddi, 1823)

Craugastoridae

1. *Haddadus binotatus* (Spix 1824)

Cycloramphidae

1. *Odontophrynus cultripes* tken 1862
2. *Proceratophrys boiei* (Wied-Neuwied 1825)
3. ~~*Proceratophrys melanopogon* (Miranda-Ribeiro 1926)~~
   Proceratophrys mantiqueira Mângia, Santana, Cruz & Feio 2014
4. *Thoropa miliaris* (Spix 1824)
5. *Zachaenus carvalhoi* Izecksohn 1983

Hemiphractidae

1. *Gastrotheca* aff. *ernestoi* Miranda Ribeiro 1920
2. *Gastrotheca* aff. *abolineata*
3. *Fritziana* aff. fissilis

Hylidae

1. *Aplastodiscus arildae* (Cruz & Peixoto 1987)
2. *Aplastodiscus* aff. *leucopygius*
3. *Bokermannohyla caramaschii* (Napoli 2005)
4. *Bokermannohyla ibitipoca* (Caramaschi & Feio 1990)
5. *Dendropsophus decipiens* (A. Lutz 1925)
6. *Dendropsophus elegans (*Wied-Neuwied 1824)
7. *Dendropsophus minutus* (Peters 1872)
8. *Hypsiboas albomarginatus* (Spix 1824)
9. *Hypsiboas albopunctatus* (Spix 1824)

10 *Hypsiboas faber* (Wied-Neuwied 1821)

11. *Hypsiboas pardalis* (Spix 1824)
12. *Hypsiboas polytaenius* (Cope 1870)
13. *Hypsiboas semilineatus* (Spix 1824)
14. *Phasmahyla* sp.
15. *Phyllomedusa burmeisteri* Boulenger 1882
16. *Scinax alter* (B. Lutz 1973)
17. *Scinax crospedospilus* (A. Lutz 1925)
18. *Scinax eurydice* (Bokermann 1968)
19. *Scinax flavoguttatus* (Lutz & Lutz 1939)
20. *Scinax fuscovarius* (A. Lutz 1925)

21 *Scinax luizotavioi* (Caramaschi & Kisteumacher 1989)

22. *Scinax tripui* (Lourenço, Nascimento & Pires 2009)
23. *Scinax* aff. *perereca*
24. ~~*Scinax* aff. *perpusillus*~~
    Scinax cosenzai Lacerda, Peixoto & Feio 2012 *b*
25. ~~*Scinax* aff. *rizibilis*~~
    Scinax **sp. nov.** (Guimarães, Lyra & Feio 2016)

Hylodidae

1. *Crossodactylus* gr. *gaudichaudii*
2. *Hylodes babax* Heyer 1982
3. *Hylodes lateristrigatus* (Baumann 1912)
4. *Megaelosia apuana* Pombal, Prado & Canedo 2003

Leiuperidae

1. *Physalaemus cuvieri* Fitzinger 1826
2. *Physalaemus feioi* Cassini, Cruz & Caramaschi 2010
3. *Physalaemus maximus* Feio, Pombal & Caramaschi 1999

Leptodactylidae

1. *Leptodactylus cupreus* Caramaschi, Feio & São-Pedro 2008
2. *Leptodactylus fuscus* (Schneider 1799)
3. *Leptodactylus labyrinthicus* (Spix 1824)
4. *Leptodactylus latrans* (Steffen 1815)
5. *Leptodactylus mystacinus* (Burmeister 1861)
6. *Leptodactylus* aff. *mystaceus*

Microhylidae

1. *Chiasmocleis mantiqueira* Cruz, Feio & Cassini 2007
2. *Elachistocleis cesarii* Miranda Ribeiro 1920

GYMNOPHIONA

Siphonopidae

1. *Luetkenotyphlus brasiliensis* tken 1852)