

A new polymorphic species of egg-brooding frog of the genus *Fritziana* from southeastern Brazil (Anura: Hemiphractidae)

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Abstract. We describe a new species of egg-brooding frog of the genus *Fritziana* from the Atlantic Forest in the state of Espírito Santo, Brazil. The new species was historically considered to represent *Fritziana goeldii*, but is diagnosed by having small snout–vent length for the genus, snout tip mucronate, limbs striped, distal subarticular tubercles simple and round on all fingers and toes, open dorsal pouch in females carrying eggs, and tadpoles deposited in bromeliads. It is also the first species of the genus to exhibit two distinct dorsal patterns: 1) a triangle covering the entire dorsum or 2) an interorbital triangle with a short “V” beginning at its apex. New molecular data is provided and used for comparing the new species with topotypes of three other species of the genus.

Key words. Amphibia, Atlantic Forest, *Fritziana goeldii*, polymorphism, DNA analysis.

Resumo. Descrevemos uma nova espécie de perereca-marsupial para o gênero *Fritziana*, que ocorre na Mata Atlântica no estado do Espírito Santo, Brasil. A nova espécie foi historicamente identificada como *Fritziana goeldii*, mas é diagnosticada por possuir comprimento rostró–cloacal pequeno para o gênero, extremidade do focinho mucronada, membros listrados, tubérculos subarticulares distais simples e redondos em todos os dedos e artelhos, bolsa dorsal aberta em fêmeas carregando ovos e girinos depositados em bromélias. A nova espécie é também a primeira do gênero a apresentar dois padrões de desenhos dorsais distintos: 1) um triângulo cobrindo todo o dorso ou 2) um triângulo interorbital com um “V” curto começando a partir do seu ápice. Novos dados moleculares são usados para comparar a nova espécie com topótipos de três espécies do gênero.

Palavras-Chave. Amphibia, Mata Atlântica, *Fritziana goeldii*, polimorfismo, análises de DNA.

Introduction

The genus *Fritziana* (family Hemiphractidae) was described by MELLO-LEITÃO (1937), and currently contains four species that are endemic to the Brazilian Atlantic Forest in the states of Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (FROST 2015, FRANZ & MELLO 2015). The four species are *Fritziana goeldii* (BOULENGER, 1895), *Fritziana ohausi* (WANDOLLECK, 1907), *Fritziana fissilis* (MIRANDA-RIBEIRO, 1920), and *Fritziana ulei* (MIRANDA-RIBEIRO, 1926). These species are known as egg-brooding frogs because the females carry their eggs on the dorsum until the tadpoles hatch.

The original descriptions of the species of *Fritziana* are not sufficient for distinguishing them because of the brevity of the descriptions used at the time, small sample sizes,

and the precariousness of the type materials or even absence of type series. Furthermore, the four species have extensive overlaps in morphological characters (DUELLMAN & GRAY 1983, FRANZ & MELLO 2015). Therefore, incorrect names were applied in both scientific collections and literature.

Fritziana goeldii was the first species of the genus to be described (BOULENGER, 1895) and the first species discovered with an open dorsal pouch and tadpoles deposited in bromeliad phytotelms. It is the most studied species of the genus, with tadpoles (HAAS 1996), reproductive behaviour, and advertisement call (WEYGOLDT & CARVALHO-E-SILVA 1991) known. *Fritziana goeldii* occurs in the states of São Paulo, Rio de Janeiro, and Espírito Santo (ALMEIDA-GOMES et al. 2014, FROST 2015).

WEYGOLDT & CARVALHO-E-SILVA (1991) tried to breed presumed *Fritziana goeldii* females from the state of Rio

de Janeiro with a presumed *Fritziana goeldii* male from the state of Espírito Santo but obtained nonviable eggs. However, the reproduction between males and females from the state of Rio de Janeiro was successful. We appraise these results as the first published evidence that the populations from Espírito Santo may be reproductively isolated from those in Rio de Janeiro, suggesting that they might represent a different species. There are also unidentified specimens of *Fritziana* from the state of Espírito Santo in taxonomic collections that possess unusual dorsal patterns for the genus and were not previously associated with any known *Fritziana* species.

In order to better diagnose the population of *F. goeldii* in Espírito Santo, we conducted analyses of morphological data of all species and molecular data of three species of the genus *Fritziana*. Herein we describe the population of *Fritziana* from the state of Espírito Santo as a new polymorphic species based on adult external morphology and DNA sequence data.

Materials and methods

Morphological analyses

We analysed specimens of *Fritziana fissilis*, *F. ohausi*, *F. ulei*, and *F. goeldii* from several localities throughout their geographical distributions. All specimens examined, including the type series, are deposited in the following collections: Coleção de Anfíbios Célio F. B. Haddad (CFBH, Universidade Estadual Paulista, Rio Claro, SP, Brazil), Museu Nacional (MNRJ, Rio de Janeiro, RJ, Brazil), Museu de Zoologia da Universidade de São Paulo (MZUSP, Universidade de São Paulo, São Paulo, SP, Brazil), Coleção de Anfíbios do Departamento de Zoologia (ZUFJR, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil), and Smithsonian Institution, Museum of Natural History (USNM, Washington, DC, USA). For a complete list of specimens examined, see Appendix 1.

Abbreviations used for measurements (in mm) of adult specimens are: SVL (snout–vent length), HL (head length), HW (head width), AL (arm length), FAL (forearm length), HAL (hand length), THL (thigh length), TBL (tibia length), TL (tarsus length), FL (foot length), ED (eye diameter), TD (tympanum diameter), END (eyenose distance), IND (internarial distance), IOD (interorbital distance), UEW (upper eyelid width), DFIV (diameter of Finger IV disc), and DTIV (diameter of Toe IV disc). For SVL, HL, HW, TBL, FL, ED, TD, IND, IOD, and UEW, we follow DUELLMAN (1970); for AL, FAL, HAL, THL, and TL, we follow HEYER et al. (1990); and for END, DFIV, and DTIV, we follow NAPOLI & CARAMASCHI (1999). Snout–vent length, HL, HW, AL, FAL, HAL, THL, TBL, TL, and FL were measured with digital calipers (0.01 mm precision), whereas the other measurements were taken with an ocular grid on a stereo dissecting microscope.

Fingers are numbered II–V, considering that Finger I is lost in anurans (FABREZI & ALBERCH 1996). We follow

the terminologies of HEYER et al. (1990) and DUELLMAN (1970) for snout shape and hand and foot tubercles.

Molecular analyses

To infer the phylogenetic relationships of the new species, we obtained 14 tissue samples from specimens from the type localities, or their surroundings, of *Fritziana goeldii*, *F. fissilis*, *F. ohausi*, and the new species, which were deposited in the tissue collection Célio F. B. Haddad, Universidade Estadual Paulista, Rio Claro, SP (CFBHT) or in the Museu Nacional, Rio de Janeiro, RJ (MNRJ). We included a sample of *Gastrotheca* sp. from the Smithsonian Institution, Museum of Natural History (USNM) to root our analysis.

Total DNA was extracted using Qiagen Inc. or Pure-Link® Genomic DNA kits by following manufacturers' protocols, and 16S with valina fragment [16sAR and 16sBR – PALUMBI et al. (1991); 16sTitus and H10 – TITUS & LARSON (1996); 12s L13 – FELLER & HEDGES (1998); 16S L2a – HEDGES (1994)] was amplified (initial hold at 94°C for 3–5 min; 30–45 cycles at 94°C for 20–30 s, 48–68°C for 20–30 s, 68–72°C for 1–1.5 min; followed by a final hold at 68–72°C for 7–10 min) and sequenced with BigDye v.3.1® (Applied Biosystems) by Macrogen Inc. (Seoul, South Korea) or in “Centro de Estudos de Insetos Sociais”, Universidade Estadual Paulista “Júlio de Mesquita Filho” in Rio Claro, SP, Brazil. Sequences were aligned with Mafft software version 7 online (KATO & STANDLEY 2013). All sequences are available at GenBank (Appendix 2).

We estimated intraspecific and interspecific uncorrected p-distances with Mega6 (TAMURA et al. 2013). Parsimony analyses were performed in TNT 1.1 (tree analysis using New Technology, GOLOBOFF et al. 2008) using the ‘new technology search’ (Driven search at level 50, sector searches, drift, and tree fusing; random seed 1; replace existing trees, auto-constrain). We also performed 1,000 parsimony jackknife replicates with absolute frequencies. Nodes with jackknife values equal or larger than 0.70 were considered supported.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub: 8D5EC041-C080-4829-B22A-7C07A9CF0057. The electronic edition of this work was published in a journal with an ISSN, has been archived, and is available from the following digital repository: www.salamandra-journal.com.

Results

Fritziana tonimi sp. n.
(Figs 1–4)

ZooBank LSID: urn:lsid:zoobank.org:act:DoF34CE0-593D-46A1-A543-B8A917CB1205

Remarks: This species has been previously referred to as *Fritziana goeldii* or *Flectonotus goeldii* by the following authors: DUELLMAN & GRAY 1983 (part.), WEYGOLDT & CARVALHO-E-SILVA 1991 (part.), DUELLMAN et al. 2011, ALMEIDA et al. 2011, GASPARINI 2012, ALMEIDA-GOMES et al. 2014, FOLLY et al. 2014 (part.), and CASTROVIEJO-FISHER et al. 2015.

Holotype: CFBH 24809, adult female, collected in the Reserva Biológica Augusto Ruschi, Município de Santa Teresa, Espírito Santo state, Brazil, (19°54'25" S; 40°30'24" W) between 26–29 October 2009 by C. C. CANEDO and J. L. GASPARINI (Figs 1–3).

Paratypes (13 specimens): Adult males (11 specimens): CFBH 30920, collected with the holotype; CFBH 30710–30712, collected at the same locality as the holotype on 15–17 November 2011 by J. L. GASPARINI and C. F. B. HADDAD; MNRJ

38397–38398, collected on 24–28 January 2005, and MNRJ 56060–56062, collected on 19–23 January 2009, all at the Estação Biológica Santa Lúcia, Município de Santa Teresa, Espírito Santo state, by J. P. POMBAL JR; MNRJ 40700, collected at the Campus do Museu de Biologia Prof. Mello Leitão, Município de Santa Teresa, Espírito Santo state, on 20 January 2006 by J. P. POMBAL JR; ZUFJR 13834, collected on 27 August 2005, next to the “Escola Superior São Francisco de Assis”, Município de Santa Teresa, Espírito Santo state by R. L. TEIXEIRA, W. PERTEL, and J. M. BORLOTE. Adult females with eggs on the dorsum (two specimens): ZUFJR 13832 and 13833, collected with specimen ZUFJR 13834.

Diagnosis: The new species can be diagnosed by the combination of the following characters: 1) small SVL for the genus (range [mean \pm SD, N] for SVL in mm in males 21.40–26.62 [23.67 \pm 1.65, N = 14]; and females 27.04–31.20 [28.72 \pm 1.65, N = 4]); 2) snout with a mucronate tip; 3) skin smooth dorsally; 4) medium-sized tympanum (range [mean \pm SD, N] for TD in mm: males 0.62–1.23 [0.91 \pm 0.22, N = 14]; females 0.93–1.48 [1.16 \pm 0.15, N = 4]); 5) limbs striped; 6) distal subarticular tubercles simple and round on all fingers and toes; 7) dorsal patterns include a triangle covering the entire dorsum or an interorbital triangle with a short “V” beginning at its apex; 8) open dorsal pouch in females carrying eggs; 9) tadpoles deposited in bromeliad phytothelms.

Comparisons with other species: *Fritziana tonimi* sp. n. differs from all other species of *Fritziana* by its two dorsal patterns. The most common dorsal pattern of *F. tonimi* sp. n. is an interorbital triangle and a posteriorly opening “V” or bilobed mark that normally reaches the level of the shoulders (morphotype I, Fig. 4A). The second pattern is exclusive to the new species and consists of two thick dorsolateral lines that start on the snout, extend to the eye and tympanum, and continue posteriorly before converging in the cloacal region, thereby forming a triangle (morphotype II, Fig. 4B). Among the species that may have an interorbital triangle, *F. ohausi* has a long “V” from the apex of the triangle, and *F. goeldii* has a bilobed blotch, similar to morphotype I of the new species (Fig. 4A). *Fritziana ulei* has an interorbital bronze pentagon (FOLLY et al. 2014), and *F. fisilis* has two straight lines from the open triangle.

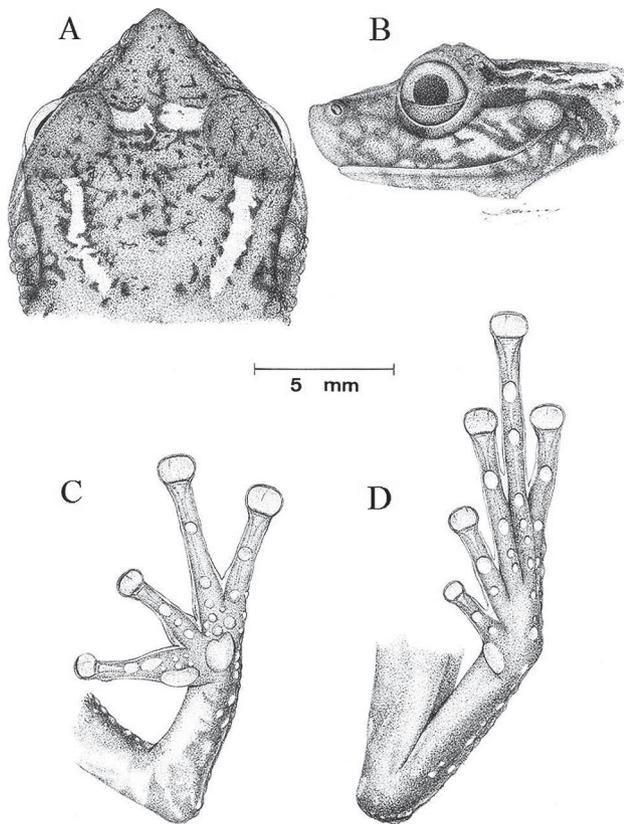


Figure 1. Drawings of the holotype of *Fritziana tonimi* sp. n. (female CFBH 24809). Dorsal (A) and lateral (B) views of the head, palmar view of the left hand (C), and plantar view of the left foot (D).

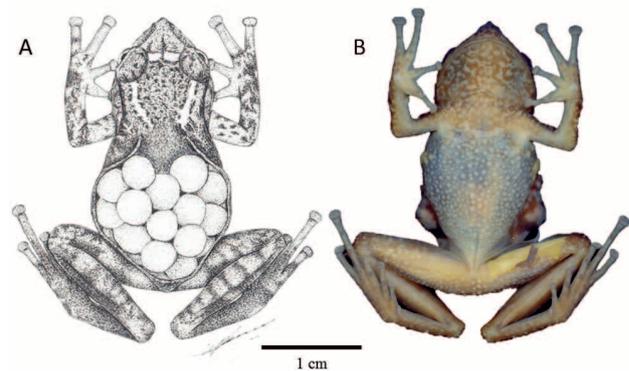


Figure 2. Holotype of *Fritziana tonimi* sp. n. (female CFBH 24809) in dorsal (A, drawing) and ventral (B) views.

Fritziana tonimi sp. n. differs from *Fritziana fissilis* by the tip of the snout being mucronate (not mucronate in *F. fissilis*). From *F. fissilis* and *F. ulei*, the new species differs by having an open dorsal pouch (closed dorsal pouch in *Fritziana fissilis* and *F. ulei*) and round subarticular tubercles (round on Toes I and II, bifid or divided on Toes III, IV, and V in *F. fissilis* and *F. ulei*). On average, *Fritziana tonimi* sp. n. is smaller than all other species of *Fritziana* except *F. ulei*, but overlaps with them in SVL range (SVL range [mean \pm SD, N] in mm of males and females, respectively: *F. tonimi* sp. n.: 21.4–26.62 [23.67 \pm 1.65, N = 14] and 27.04–31.20 [28.72 \pm 1.65, N = 4]; *F. goeldii*: 24.85–30.16 [28.12 \pm 1.41, N = 18] and 30.41–37.03 [33.96 \pm 2.5, N = 6]; *F. ohausi*: 20.87–31.57 [27.09 \pm 2.9, N = 40] and 27.6 [N = 1]; *F. fissilis*: 25.51–29.97 [N = 2] and 25.10–31.68 [29.29 \pm 2.52, N = 6]; and *F. ulei*: 21.68–27.59 [23.24 \pm 1.56, N = 4] and 23.07–25.07 [N = 2]). *Fritziana tonimi* sp. n. differs from *F. ohausi* by its reproductive behaviour of releasing tadpoles into water accumulating in bromeliads (tadpoles released in water accumulating in holes of bamboo in *F. ohausi* – DUELMAN & GRAY 1983) and by its head being less wide than in *F. ohausi* (HW/HL mean for males = 0.86 in *F. ohausi*, and 0.92 in *F. tonimi* sp. n.). The relationship between diameter of the tympanum and the diameter of Finger IV is smaller in the new species than in *F. goeldii* and larger than in *F. ohausi* (TD/ DDFIV mean for males = 0.925 in *F. goeldii*, 0.69 in *F. ohausi*, 0.68 in *F. ulei*, and 0.79 in *F. tonimi* sp. n.).

Description of the holotype: Adult female (Figs 1–3). Head longer than wide, head width 35% of SVL. Snout mucronate in dorsal view and protruding in lateral view. Loreal

region weakly concave. Nostril small, oval, directed laterally. IND 58.2% of IOD. Interorbital distance 1.5 times longer than upper eyelid width. Eye anterolaterally orientated, its diameter 7.4% smaller than END. Tympanum slightly evident and small, its diameter 32% of ED; tympanum round, well marked ventrally and partly covered dorsally by a weak supratympanic fold. Vomerine teeth in two series at a small obtuse angle; vomerine teeth positioned posteriorly and between the choanae; choanae oval and well separated. Tongue ovoid, slightly free, and notched behind. Forearms with isolated tubercles arranged in lines. Fingers with elliptical discs; width of disc of Finger IV 1.4 times larger than tympanum diameter. Relative lengths of fingers: III < II < V < IV. Webbing vestigial between fingers. Subarticular tubercles round and well developed. Supernumerary tubercles present. Inner metacarpal tubercle elliptical and large, outer metacarpal tubercle wide and heart-shaped. Thigh length 44.6% of SVL; foot length 46.5% of SVL. Few tarsal tubercles, arranged in a line. Toes with elliptical discs, the same size as those on fingers. Relative lengths of toes: I < II < III \leq V < IV. Webbing vestigial between Toes I–II and II–III and poorly developed between the others, reaching the middle of the proximal phalanges. Subarticular tubercles round and well developed. Supernumerary tubercles present. Inner metatarsal tubercle large and elliptical; outer metatarsal tubercle small and round, like the subarticular tubercles. Cloacal opening directed posteriorly at upper level of thighs. Dorsal skin smooth, with few small tubercles on upper eyelid and in loreal region. Belly and thighs granular; the rest of ventral face smooth. Dorsum with a widely open dorsal pouch containing 14 pale yellow eggs.



Figure 3. Holotype of *Fritziana tonimi* sp. n. (female CFBH 24809, from the Município de Santa Teresa, Espírito Santo state, Brazil) in life, with eggs in an open dorsal pouch. Note the reduced pouch that is formed by small lateral folds that cover the egg clutch laterally, and the red mites.

Table 1. Ranges, means, and standard deviations (SD) of measurements of males and females of *Fritziana tonimi* sp. n., including the type series and additional material (Appendix 1). All values are in millimetres. For abbreviations of measurements, see Material and methods.

Measurements	Females (N=4)				Males (N=14)				
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
SVL	27.04	31.20	28.72	1.65	21.40	26.62	23.67	1.65	
HW	9.17	9.90	9.64	0.29	6.61	9.17	7.75	0.71	
HL	9.72	10.84	10.41	0.42	7.60	9.78	8.57	0.67	
AL	6.96	8.27	7.51	0.50	5.17	7.39	6.58	0.53	
FAL	5.10	6.28	5.51	0.46	4.16	5.29	4.76	0.30	
HAL	7.20	8.58	7.95	0.61	5.61	7.77	6.65	0.64	
THL	12.24	13.69	12.78	0.55	9.45	11.60	10.50	0.68	
TBL	13.40	14.82	14.10	0.54	10.86	12.87	11.63	0.58	
TL	6.80	7.78	7.28	0.35	5.67	7.05	6.23	0.44	
FL	11.30	12.72	11.84	0.58	8.96	12.09	10.14	0.86	
IND	1.48	2.10	1.85	0.23	1.30	1.91	1.59	0.17	
IOD	3.21	3.52	3.38	0.11	2.28	3.39	2.69	0.28	
UEW	2.22	2.47	2.31	0.10	1.85	2.65	2.23	0.23	
ED	3.09	3.70	3.32	0.23	2.71	3.21	2.94	0.16	
END	2.96	3.33	3.12	0.14	2.41	3.02	2.63	0.21	
TD	0.93	1.48	1.16	0.22	0.62	1.23	0.91	0.15	
DFIV	1.28	1.43	1.34	0.06	0.97	1.45	1.14	0.14	
DTIV	1.26	1.38	1.29	0.05	0.90	1.45	1.10	0.15	

The eggs are covered by skin folds laterally and uncovered dorsally. All the eggs are in contact with the female's dorsum, being distributed as a layer (Figs 2+3). The egg clutch is heart-shaped in dorsal view.

Coloration of the holotype: In life (Fig. 3), the dorsum is grey to silver with black markings and has a dark interorbital triangle bordered by two white stripes that converge from the eyes to the end of the head, from where they continue parallel posteriorly. Laterally, there are two diagonal white streaks from the eyes to the upper lip. The colour of the remainder of the dorsum cannot be identified because of the presence of eggs. The legs are striped, with transversal bars; arms and rest of body marbled. Belly and limbs

pale beige, gular region marbled. Red mites are evident on the skin on the sides of the head and body, near the arms. In preservative, the specimen has the same coloration, but the mites have faded to pale beige.

Morphological variation: Means, standard deviations, and ranges of measurements of *Fritziana tonimi* sp. n. are presented in Table 1. Morphological variation is based on 14 males and four females. In lateral view, the snout is protruding to round and in dorsal view, it is mucronate. The lateral face of the head shows no pattern or has two diagonal white streaks extending from the eyes to the mouth (Fig. 1). A short supratympanic fold is present in three specimens. The venter and the throat region are pale beige, the latter rarely marbled.

Two morphotypes of the new species are recognized. Ten paratypes (CFBH 30710, 30711, 30920, MNRJ 38397, 38398, 40700, 56061, and ZUFRJ 13832–13834) and the holotype represent morphotype I (Fig. 4A). Frogs of morphotype I have an open interorbital triangle (sometimes bordered by two thin white stripes). At the apex of the triangle, there is a posteriorly opening "V" or a bilobed mark that normally reaches the level of the shoulders. Dorsally, the arms and legs are striped and the rest of the dorsum is marbled.

Morphotype II is represented in three paratypes (CFBH 30712, MNRJ 56060 and 56062). Specimens of morphotype II have a thick, black dorsolateral line that starts on the snout, extends to the eye and tympanum, and then continues posteriorly before converging in the cloacal region, thereby forming a triangle on the uniformly beige dorsum (Fig. 4B). Irregular stripes and spots are present on the arms and legs and in areas outside of the triangle.

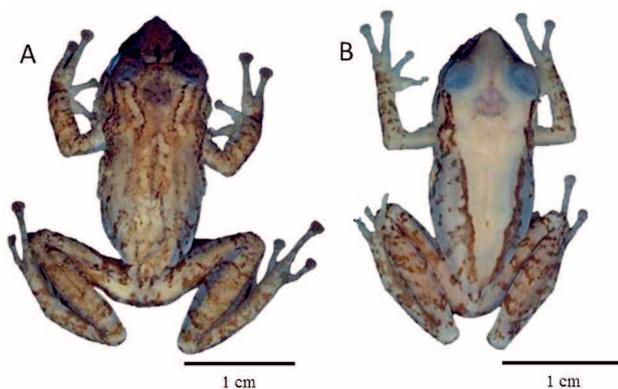


Figure 4. The two recognized dorsal patterns of *Fritziana tonimi* sp. n. in preserved specimens: A) Male (CFBH 30710) representing morphotype I; B) male (MNRJ 56060) representing morphotype II. See text for details.

In life, specimens of the new species may be beige with brown markings or with grey to silver and black markings. Red mites are frequently present on the skin, normally on the sides of the head and near the arms. In preserved specimens, the patterns and colours are slightly faded, and the colour of mites will degrade to pale beige.

Sexual dimorphism: Females are larger than males (females range from 27.04 to 31.20 and males from 21.4 to 26.62 mm, Table 1). They also have an open dorsal pouch for carrying eggs during the reproductive period. Males have nuptial pads on the base of Finger II, which may be pale beige or brown, and vocal slits on the floor of the mouth lateral to the tongue. The vocal sac is indistinct in preserved males.

Geographical distribution: *Fritziana tonimi* sp. n. occurs at the type locality, Reserva Biológica Augusto Ruschi, Município de Santa Teresa, in Município de Domingos Martins, and in the Área de Proteção Ambiental do Goiapaba-Açu, Município de Fundão, all in the Atlantic Forest of the state of Espírito Santo, Brazil (Fig. 5).

Threat category: *Fritziana goeldii* appear as “Least Concern” in the IUCN Red List, because “in view of its wide distribution, tolerance of a broad range of habitats, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more



Figure 5. Known distribution of *Fritziana tonimi* sp. n. The small map represents South America, with Brazil in grey and Espírito Santo state in black. In the coloured map, the star indicates the type locality in Município de Santa Teresa, and the circle another known locality for the species in Domingos Martins, both in the state of Espírito Santo.

Table 2. Intra- and interspecific p-distances between *Fritziana* species for gene 16S.

	Intraspecific p-distance	Interspecific p-distance		
		<i>F. fissilis</i>	<i>F. ohausi</i>	<i>F. tonimi</i> sp. n.
<i>F. fissilis</i>	1.6%			
<i>F. ohausi</i>	4.2%	8.4%		
<i>F. tonimi</i> sp. n.	0.1%	8.2%	10.3%	
<i>F. goeldii</i>	1.1%	8.6%	10.3%	10.7%

threatened category” (CARVALHO-E-SILVA & TELLES 2004). With the description of *F. tonimi* sp. n., the distribution of *F. goeldii* has become smaller, and we believe there is not enough information about *Fritziana goeldii* and *F. tonimi* sp. n. at this stage to include them in one of the categories proposed by the IUCN. It should therefore be classified as “Data Deficient” until it can be updated.

Etymology: The specific epithet *tonimi* pays homage to ANTÔNIO DE PÁDUA ALMEIDA (Tonim) for his contributions to the knowledge and conservation of the herpetofauna of the state of Espírito Santo.

Molecular analysis: The smallest intraspecific uncorrected p-distance was found for *Fritziana tonimi* sp. n. (0.1%), and the greatest for *F. ohausi* (4.2%). Interspecific uncorrected p-distances for 16S between *F. tonimi* sp. n. and the other species are 8.2% for *F. fissilis*, 10.3% for *F. ohausi*, and 10.7% for *F. goeldii* (Table 2).

Our parsimony analysis recovered one most-parsimonious tree with a clear clustering pattern wherein each species is recognized and well supported. Both morphotypes of *Fritziana tonimi* sp. n. cluster together, comprising a single clade that is the sister group of the remaining species.

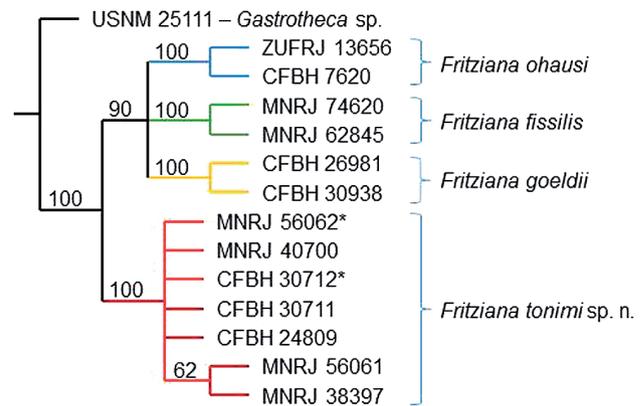


Figure 6. Phylogenetic relationships of *Fritziana* based on the most parsimonious tree. Each colour represents one species. The asterisk (*) indicates specimens of *Fritziana tonimi* sp. n. of morphotype II, whereas the others are of morphotype I.

Remarks

The species of *Fritziana* exhibit considerable morphological similarity, which historically rendered them difficult to identify. Generally, characters that occur in the majority of the specimens of one species may be absent in some individuals of that species and/or present at a lower percentage in individuals of another species (DUELLMAN & GRAY 1983, FRANZ & MELLO 2015).

Fritziana tonimi sp. n. was frequently misidentified as *F. goeldii* in the past because of the similarity in the morphology of the dorsal pouch of females. Some authors reported *F. goeldii*, *F. ohausi*, and *F. fissilis* from the state of Espírito Santo (e.g., DUELLMAN & GRAY 1983, FOLLY et al. 2014, FROST 2015); however, our examination of specimens in collections indicates that only the species described herein occurs in this state. Specimens listed as *F. goeldii* in the molecular analyses by DUELLMAN et al. (2011) and CASTROVIEJO-FISHER et al. (2015) are in fact referable to *Fritziana tonimi* sp. n.

According to FORD (1937), “polymorphism is the existence, in the same habitat, of two or more forms of a species in stable equilibrium”. Colour polymorphism in anurans is characterized by the presence of fixed chromatic phenotypes within or between populations, and it occurs in a variety of anuran species, with some of them being known to comprise more than 15 morphotypes (TOLEDO & HADDAD 2009). *Fritziana tonimi* sp. n. is, to date, the only known species of the genus that clearly presents polychromatism, with two different patterns of dorsal markings that are independent of sex or life stage.

In spite of the small number of species discovered so far in the genus *Fritziana*, the difficulty in identifying species using only morphology and the existence of populations that do not fit the descriptions of the known species suggests that there may be additional cryptic species to be discovered. The use of integrative taxonomy (PADIAL et al. 2010), exploring a variety of evidence-harboring characteristics in addition to adult morphology, may be a fruitful approach to studying the diversity of these frogs. Indeed, such an approach has proven successful in identifying new species, revalidating previously described taxa, and resolving taxonomic problems in other genera of frogs such as *Boophis* (GLAW et al. 2010), *Pristimantis* (PADIAL & DE LA RIVA 2009), and *Chiasmocleis* and *Syncope* (PELOSO et al. 2014). An extensive review of the genus *Fritziana* based on morphological and molecular data is needed in order to better describe known species and recognize new, perhaps cryptic, species if they do indeed exist.

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Appendix 1

Examined specimens

Fritziana ohausi: Brazil: Rio de Janeiro state: Teresópolis (Serra dos Órgãos): MZUSP 77702–77704; CFBH 32572–32573; São Paulo state: São Luiz do Paraitinga (Parque Estadual da Serra do Mar – Núcleo Santa Virgínia): CFBH 7611, 14817–14830, 16276–16282; Pilar do Sul: CFBH 7552, 7620; Santos: CFBH 15950, 23920; Tapiraí: CFBH 16545; Apiaí and Iporanga: CFBH 25601; São José do Barreiro (Serra da Bocaina): MZUSP 31241, 34607–34611, 57731–57732; Santo André (Parque Estadual Municipal Nascentes de Paranapiacaba): CFBH 28974, 28982; MZUSP 77000–77001; Ribeirão Grande (Estação Ecológica de Xitué): MZUSP 139225. Salesópolis (Reserva Biológica de Boracéia): MZUSP 137418–137419.

Fritziana goeldii: Brazil: Rio de Janeiro state: Teresópolis (Serra dos Órgãos): MZUSP 103959–103965; Petrópolis: MNRJ 76201; Rio de Janeiro: CFBH 26981; USNM 318126; Rio de Janeiro (Tijuca): MZUSP 103426–103448; Guapimirim: MNRJ 24631; Cachoeira de Macacu: MNRJ 46537, 53758; Duque de Caxias: MNRJ 54715; São Paulo state: Ubatuba: CFBH 9540; 10909–10910.

Fritziana fissilis: Brazil: Rio de Janeiro state: Teresópolis (Serra dos Órgãos): MZUSP 20611–20612; MZUSP 20614; Rio de Janeiro (Sumaré): MZUSP 128096. Mangaratiba: MNRJ 44551; Nova Friburgo: MNRJ 56942; MZUSP 30, 55691–55692.

Fritziana ulei: Brazil: Rio de Janeiro state: Teresópolis (Serra dos Órgãos): MZUSP 103966–103973; 103976–103978; 103980–103985. Cachoeira de Macacu: MNRJ 44622, 56922.

Fritziana tonimi sp. n.: Brazil: Espírito Santo state: Santa Teresa: CFBH 24809; CFBH 30710–30712; CFBH 30920; MNRJ 28376, 38397–38398, 40700, 56060–56062; ZUF RJ 13832–13834. Domingos Martins: MNRJ 46719–46720; ZUF RJ 6533.

New species of *Fritziana*

Appendix 2

Vouchers, species, locality, and GenBank accession numbers of samples used in molecular analyses. See Material and methods for acronyms. Brazilian states: ES – Espírito Santo; RJ – Rio de Janeiro; SP – São Paulo.

Voucher	Species	Locality	GenBank numbers
MNRJ 62845	<i>Fritziana fissilis</i>	Nova Friburgo, RJ	KU991167
MNRJ 74620	<i>Fritziana fissilis</i>	Teresópolis, RJ	KU991168
ZUFRJ 13656	<i>Fritziana ohausi</i>	Teresópolis, RJ	KU991169
CFBH 07620	<i>Fritziana ohausi</i>	Pilar do Sul, SP	KU991170
CFBH 24809	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991171
CFBH 30711	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991172
CFBH 30712	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991173
MNRJ 40700	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991174
MNRJ 38397	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991175
MNRJ 56061	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991176
MNRJ 56062	<i>Fritziana tonimi</i> sp. n.	Santa Teresa, ES	KU991177
CFBH 30938	<i>Fritziana goeldii</i>	Teresópolis, RJ	KU991178
CFBH 26981	<i>Fritziana goeldii</i>	Rio de Janeiro, RJ	KU991179
USNM 25111	<i>Gastrotheca</i> sp.	Tulcan, Sucumbios (Ecuador)	KU991180