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# A New Species of *Fritziana* Mello-Leitão 1937 (Amphibia: Anura: Hemiphractidae) from the Atlantic Forest, Brazil

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Abstract: We describe a new species of the genus *Fritziana*, historically identified as *Fritziana fissilis* or *F.* aff. *fissilis*. Using adult and tadpole morphology, as well as molecular analyses, we found the new species to be characterized by having the following traits: a mucronate snout tip, a black stripe on the wrist, round subarticular tubercles on the fingers and toes, a closed dorsal egg-pouch in females, tadpoles deposited in bromeliads, and a different mitochondrial transfer RNA gene order, when compared to the general orders already recognized for anurans. This new species occurs in the Atlantic Forest of Brazil, in the states of São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul, and has the largest geographic distribution recorded for a species of *Fritziana*.

**Key words:** Egg-brooding frogs; Hemiphractinae; Mitochondrial tRNA; Gene order; Morphometrics; Tadpoles

Integrative taxonomic studies that have included phenotypic or molecular characters have made major advances in our understanding of the biodiversity and phylogenetic relationships of anurans (e.g., Faivovich et al. 2005; Frost et al. 2006; Padial et al. 2010; Castroviejo-Fisher et al. 2015). This is particularly important for species that are difficult to identify, such as cryptic species or those having considerable overlap in phenotypic characters (Bickford et al. 2007). The anuran family Hemiphractidae Peters 1862, known as egg-brooding frogs, includes 112 species (Frost 2018). The last taxonomic review of the family suggested a well-resolved phylogeny in which two subfamilies containing six genera are recognized; but some genera, such as *Fritziana*, are suspected of containing cryptic species (Castroviejo-Fisher et al. 2015).

The genus *Fritziana* is endemic to the Atlantic Forest of southeastern Brazil (Frost 2018), with six recognized species: *Fritziana goeldii* (Boulenger 1895), *F. ohausi* (Wandolleck 1907), *F. fissilis* (Miranda-Ribeiro 1920), *F. ulei* (Miranda-Ribeiro 1926), *F. tonimi* Walker, Gasparini and Haddad 2016, and *F. izecksohni* Folly, Hepp and Carvalho-e-Silva 2018. These species are known to overlap significantly in morphology, making them difficult to identify and diagnose. Therefore, the current known diversity of the genus is likely an underestimate, particularly for species with closed dorsal pouches, among which are suspected to be at least three undescribed taxa (Castroviejo-Fisher et al. 2015; Walker et al. 2018).

Individuals of *Fritziana* have often been reported as unidentified in community studies and inventories of southeastern and southern Brazil, or as being related to or misidentified as *Fritziana fissilis* (e.g., Garey et al. 2014; Wachlevski et al. 2014; Franz and Mello 2015; Ceron et al. 2017; Comitti 2017). Therefore, many specimens remain in scientific collections without proper identification. Based on molecular analyses, these specimens appear in a clade not related to *F. fissilis*, and members of this clade also show a change in transfer RNA (tRNA) gene order that is unique among the anurans (Walker et al. 2018).

Herein we describe a new species with closed dorsal pouch from the Atlantic Forest of Brazil, based on phenotypic and molecular analyses of adults and tadpoles. This species was informally named by Walker et al. (2018) as *Fritziana* CS3.

## Materials and Methods

### Morphology of Adults

We compared the new species with specimens of *Fritziana fissilis*, *F. goeldii*, *F. ohausi*, *F. tonimi*, and *F. ulei* (Appendix I). Information on *F. ulei* was also based on the redescription of the holotype by Folly et al. (2014) and information on *F. izecksohni* was based only on the original description (Folly et al. 2018). We used the museum acronyms of Sabaj (2016), except for CHUFSC (Coleção Herpetológica da Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil). Mean values are reported ± 1 SD.

Measurements of adult specimens included the following: snout–vent length (SVL), head length (HL), head width (HW), arm length (AL), forearm length (FAL), hand length (HAL), thigh length (THL), tibia length (TBL), tarsus length (TL), foot length (FL), eye diameter (ED), tympanum diameter (TD), eye–nostril distance (END), internarial distance (IND), interorbital distance (IOD), disc diameter

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of Finger IV (DFIV), and disc diameter of Toe IV (DTIV). Measurements of SVL, HL, HW, TBL, FL, ED, TD, END, IND, and IOD follow Duellman (1970); AL, FAL, and TL follow Heyer et al. (1990); HAL follows Zweifel (1985); THL follows Heyer (1978); DFIV and DTIV follow Heyer (1984). Measurements of SVL, HL, HW, AL, FAL, HAL, THL, TBL, TL, and FL were made with digital calipers (±0.1 mm), whereas the other measurements were made with an ocular grid on a stereo dissecting microscope.

Fingers are numbered II–V considering that Finger I is lost in anurans (Fabrezi and Alberch 1996). We follow Heyer et al. (1990) and Duellman (1970) for snout shape and hand and foot tubercle standards. The character "sharp vertical keel" in Folly et al. (2014:395) is used here as "mucronate snout shape."

### Tadpoles

We analyzed seven tadpoles of the new species (Lot CFBH 34437), obtained from the female specimen CFBH 32918. It was collected in the municipality of Bertioga, São Paulo State, in November 2011 by A.M. Luiz, and was maintained in captivity until the tadpoles hatched.

The morphometric variables used for the tadpole description, following Altig and McDiarmid (1999), are total length (TL), body length (BL), body width (BW), body height (BH), tail length (TAL), tail height (TAH), dorsal fin height (DFH), ventral fin height (VFH), internostril distance (IND), IOD, ED, nostril diameter (ND), END, nostril–snout distance (NSD), eye–snout distance (ESD), snout–spiracle distance (SSD), spiracle–vent distance (SVD), and oral disc width (ODW). These measurements were taken from tadpoles in Stages 35, 36, 37, and 39 (Gosner 1960) using digital calipers (±0.1 mm).

### Molecular Analyses

To infer the phylogenetic relationships among the species of *Fritziana*, we used partial sequences of 16S ribosomal RNA gene fragments previously used by Walker et al. (2016), Folly et al. (2018), and Walker et al. (2018), and a newly obtained tissue sample from the municipality of Osório, Rio Grande do Sul State (UFMG 17436). We report the localities of collection and GenBank accession numbers of *Fritziana* (Appendix II), as well as the sequences of other genera of Anura as outgroups (Appendix III). The extraction, amplification, sequencing protocols, and phylogenetic analyses using Bayesian inference follow Walker et al. (2018).

In order to determine which molecular clade should be named as *Fritziana ulei*, Walker et al. (2018) considered two samples: they analyzed the DNA of the specimen ZUFRJ 13347, which was used by Folly et al. (2014) to revalidate *F. ulei*, and the DNA and morphology of the specimen MNRJ 56922, collected in close proximity to the type locality and whose morphology matches with the available descriptions, and with *F. ulei*'s holotype morphology. Since Walker et al. (2018), we were informed that no tissue sample was taken from the specimen ZUFRJ 13347, and that the sample sent to us was mislabeled and actually belongs to an unknown specimen (M. Folly, personal communication). Even without the ZUFRJ 13347 sample, the identification of the clade recognized as *F. ulei* in the Walker et al. (2018) tree should be maintained based on specimen MNRJ 56922.

## Species Account

*Fritziana* ***mitus*** sp. nov.
(Figs. 1–3; Table 1)

*Flectonotus fissilis*: Del Pino 1980:11, in part; Dixo and Verdade 2006:4; Condez et al. 2009:161.

*Fritziana fissilis*: Duellman and Gray 1983:359, in part; Heyer et al. 1990:356; Duellman et al. 2011:31; Blackburn and Duellman 2013:712; Folly et al. 2014:399, in part.

*Fritziana* aff. *fissilis*: Wachlevski et al. 2014:100; Franz and Mello 2015:4; Ceron et al. 2017:296; Comitti 2017:94.

*Fritziana ulei*: Folly et al. 2014:399, in part; Menegucci et al. 2017:1.

**Holotype.**—CFBH 39949, an adult female collected in an Atlantic Forest area in the Parque Estadual da Serra do Tabuleiro (27°44′30″S, 48°48′28″W; 420 m above sea level; datum = WGS84), municipality of Santo Amaro da Imperatriz, Santa Catarina State, Brazil, by Milena Wachlevski, on 14 April 2010 (Figs. 1 and 2). The holotype was collected by active search (at 2130 h) in a bromeliad (*Vriesea* sp.) at a height of 60 cm in relation to the soil.

**Paratopotypes.**—Ten specimens consisting of six adult females without eggs on the dorsum (CFBH 39932-39935, CHUFSC 2625, and MNRJ 90352), two adult females incubating eggs on the dorsum (CFBH 39936 and MNRJ 90351), and two adult males (CFBH 39937 and MNRJ 90350). These specimens were collected in the municipality of Santo Amaro da Imperatriz, Santa Catarina state, Brazil, between July 2009 and January 2013.

**Diagnosis.**—Based on 26 males and 24 females collected throughout the known distribution area of the new species (Appendix I), the new species is characterized by the following: (1) TD equal to 89% of the DTIV (females, 55–115% [87 ± 15%, $n = 24$]; males, 59–135% [91 ± 18%, $n = 26$]); (2) HW equal to 93% of the HL (females, 84–109% [95 ± 6%, $n = 24$]; males, 78–102% [91 ± 6%, $n = 26$]); (3) a mucronate snout tip; (4) distal subarticular tubercles which are simple and round on the hands and feet; (5) the most common color pattern characterized by an interorbital triangle followed posteriorly by two parallel lines on the dorsum; (6) a black stripe on the wrist; (7) an egg-brooding pouch on the back of the females with the skin covering the eggs, and with a medial opening (the pouch in the reproductive period is evident even when empty); (8) tadpoles deposited in bromeliads; and (9) a change in the mitochondrial tRNA gene order (instead of having the order proline–phenylalanine–12S, the new species has the order phenylalanine–proline–12S).

**Comparisons with other species.**—On average, *Fritziana mitus* sp. nov. is small when compared to other species within the genus. Therefore, large specimens of *F. mitus* sp. nov. might overlap in size with small specimens of *F. ohausi*, *F. goeldii*, *F. tonimi*, and *F. izecksohni* (SVL: *F. mitus* sp. nov.: males = 16.16–23.67 mm [19.97 ± 1.65 mm, $n = 26$], females = 17.43–28.35 mm [24.28 ± 2.47 mm, $n = 24$]; *F. tonimi*: males = 21.4–26.62 mm [23.67 ± 1.71 mm, $n = 14$], females = 27.04–31.20 mm [28.72 ± 1.90 mm, $n = 4$]; *F. goeldii*: males = 19.52–30.16 mm [27.12 ± 2.69 mm, $n = 21$], females = 30.41–37.03 mm [34.16 ± 3.47 mm, $n = 7$]; *F. ohausi*: males = 18.74–31.57 mm [26.80 ± 3.11 mm, $n = 43$], female = 27.6 mm [$n = 1$]; *F. fissilis*: males = 25.51–

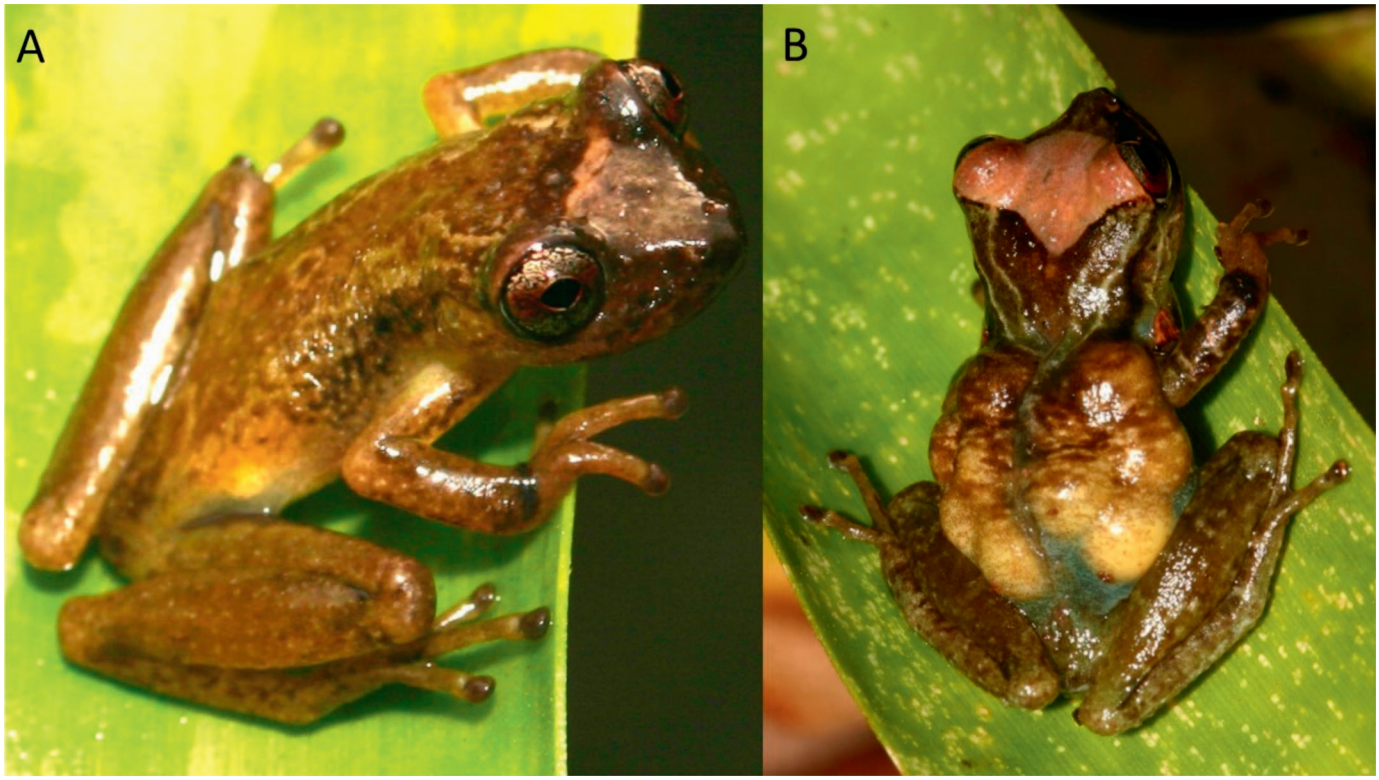


FIG. 1.—Adult females of *Fritziana mitus* sp. nov. (A) without eggs on dorsum (holotype CFBH 39949) and (B) with eggs on dorsum (paratopotype MNRJ 90351). Both from the Parque Estadual da Serra do Tabuleiro, municipality of Santo Amaro da Imperatriz, state of Santa Catarina, Brazil.

29.97 mm [$n = 2$], females = 28.29–31.68 mm [30.13 ± 1.63 mm, $n = 5$]; *F. ulei*: males = 21.68–27.59 mm [23.24 ± 1.56 mm, $n = 14$], females = 23.07–25.07 mm [$n = 2$]; *F. izecksohni*: males = 21.5–29.4 mm [25.9 ± 2.8 mm, $n = 5$], female = 33.5 mm [$n = 1$]).

The TD:DTIV ratio is larger in *Fritziana mitus* sp. nov. than in the other species except in *F. goeldii* (TD/DTIV = 0.89 in *F. mitus* sp. nov., females = 0.55–1.15 [0.87 ± 0.15, $n = 24$], males = 0.59–1.35 [0.91 ± 0.18, $n = 26$]; 0.79 in *F. fissilis*, females = 0.70–0.88 [0.77 ± 0.07, $n = 5$], males = 0.73–0.95 [$n = 2$]; 0.99 in *F. goeldii*, females = 0.92–1.31 [1.09 ± 0.12, $n = 7$], males = 0.61–1.38 [0.95 ± 0.16, $n = 24$]; 0.84 in *F. tonimi*, females = 0.72–1.18 [0.90 ± 0.22, $n = 4$], males = 0.64–0.98 [0.83 ± 0.11, $n = 14$]; 0.70 in *F. ohausi*, female = 0.78 [$n = 1$], males = 0.42–0.96 [0.70 ± 0.14, $n = 43$]; and 0.70 in *F. ulei*, females = 0.75–0.78 [$n = 2$], males = 0.42–0.96 [0.69 ± 0.13, $n = 14$]). *Fritziana mitus* sp. nov. has a proportionately wider head than *F. ohausi* and *F. izecksohni*, and a longer head than the other species (mean HW/HL for males = 0.93 in *F. mitus* sp. nov., females = 0.84–1.09 [0.95 ± 0.06, $n = 24$], males = 0.78–1.02 [0.91 ± 0.06, $n = 26$]; 0.83 in *F. izecksohni*; 0.86 in *F. ohausi* males = 0.79–0.96 [0.86 ± 0.04, $n = 43$]; 0.94 in *F. goeldii*, females = 0.90–1.01 [0.96 ± 0.04, $n = 7$], males = 0.89–1.01 [0.89 ± 0.03, $n = 24$]; 0.91 in *F. tonimi*, females = 0.89–0.94 [0.93 ± 0.02, $n = 4$], males = 0.84–0.98 [0.90 ± 0.04, $n = 14$]; 0.95 in *F. ulei*, females = 0.94–0.99 [$n = 2$], males = 0.91–1.99 [0.95 ± 0.03, $n = 14$]; and 0.93 in *F. fissilis*, females = 0.87–1.00 [0.93 ± 0.05, $n = 5$], males = 0.88–0.97 [$n = 2$]).

*Fritziana mitus* sp. nov. differs from *F. fissilis* and *F. ohausi* by having a mucronate snout tip (the tip of the snout is not mucronate in *F. fissilis* and in *F. ohausi*), and differs from *F. fissilis* and *F. izecksohni* by having round subarticular tubercles on all the fingers and toes (subarticular tubercles are bifid in *F. fissilis* and *F. izecksohni*).

Although *Fritziana mitus* sp. nov. can exhibit a uniform color pattern (Fig. 3F) or a bronze blotch covering the entire dorsal surface of the head (similar to *F. ulei*, Folly et al. 2014); the most common pattern (Fig. 3A) is characterized by an interorbital triangle (also present in *F. goeldii*, *F. ohausi*, and *F. tonimi*) followed posteriorly by two parallel lines on the dorsum (a bilobated stain in *F. goeldii* and *F. tonimi*; an inverted V in *F. ohausi*). The type series of *F. fissilis* is discolored, but the dorsal pattern described in the original description seems to be similar to the pattern of the new species. Folly et al. (2014) noted the presence of an hourglass-shaped interorbital mark in other specimens identified by them as *F. fissilis*. *Fritziana izecksohni* exhibits a triangular mark covering the dorsum to the sacral region, but few specimens are known, and the occurrence of other patterns is possible. *Fritziana tonimi* can also have two long lines from the eye to the cloacal region forming a large V. The black stripe on the wrist is present in only the new species.

*Fritziana mitus* sp. nov. differs from *F. goeldii*, *F. ohausi*, and *F. tonimi*, by having a closed dorsal pouch in females during egg incubation (as opposed to an opened pouch in *F. goeldii*, *F. ohausi*, and *F. tonimi*). Although the holotype of *F. izecksohni* shows dorsal folds on its back, indicating a closed dorsal pouch, no recorded observation of a female transporting eggs has occurred; therefore, its pouch morphology remains unknown. The new species further differs from *F.*

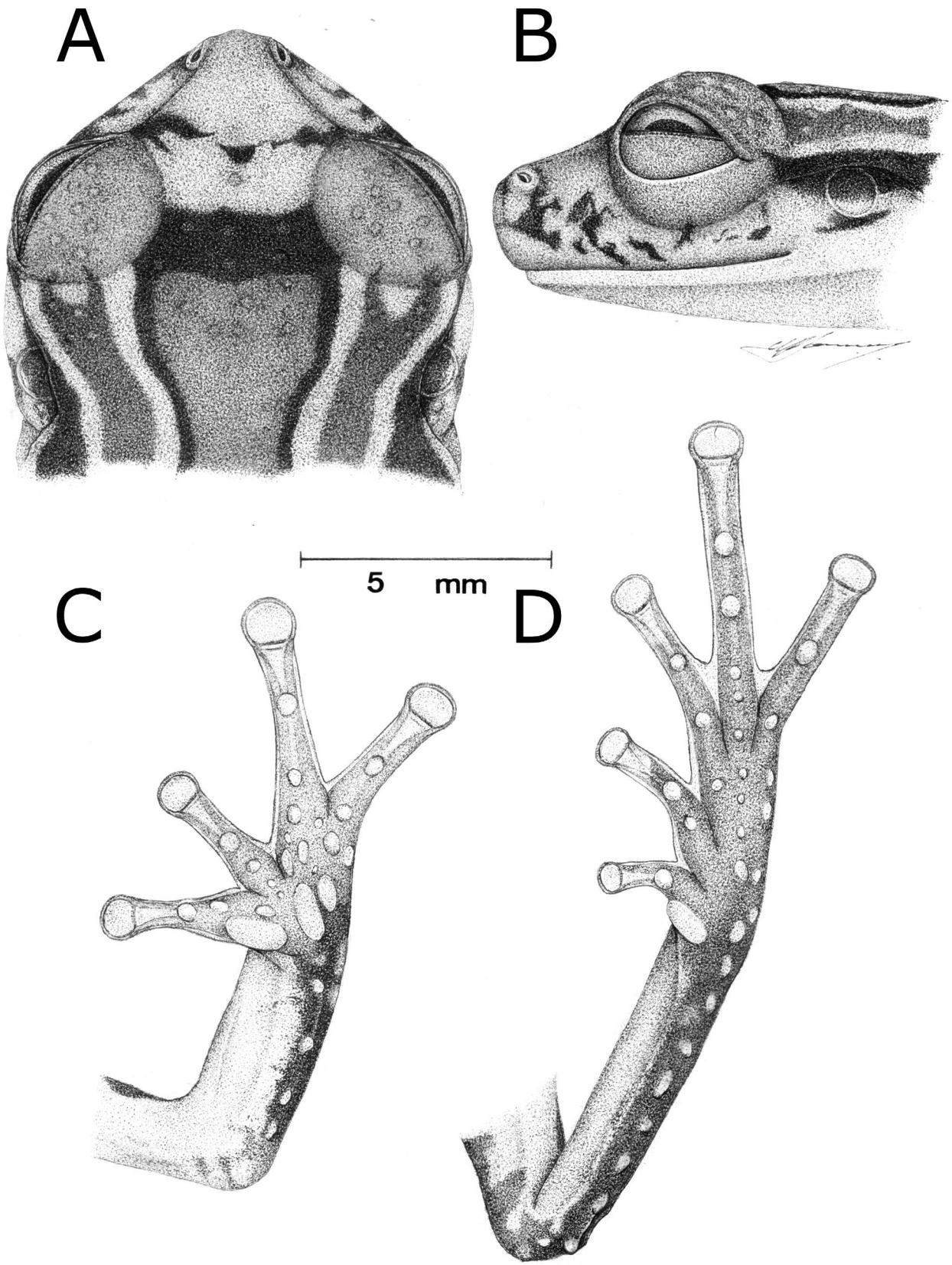


FIG. 2.—*Fritziana mitus* sp. nov., holotype (CFBH 39949) from the Parque Estadual da Serra do Tabuleiro, municipality of Santo Amaro da Imperatriz, state of Santa Catarina, Brazil. (A) Dorsal and (B) lateral views of the head; (C) palmar view of the left hand; and (D) plantar view of the left foot.

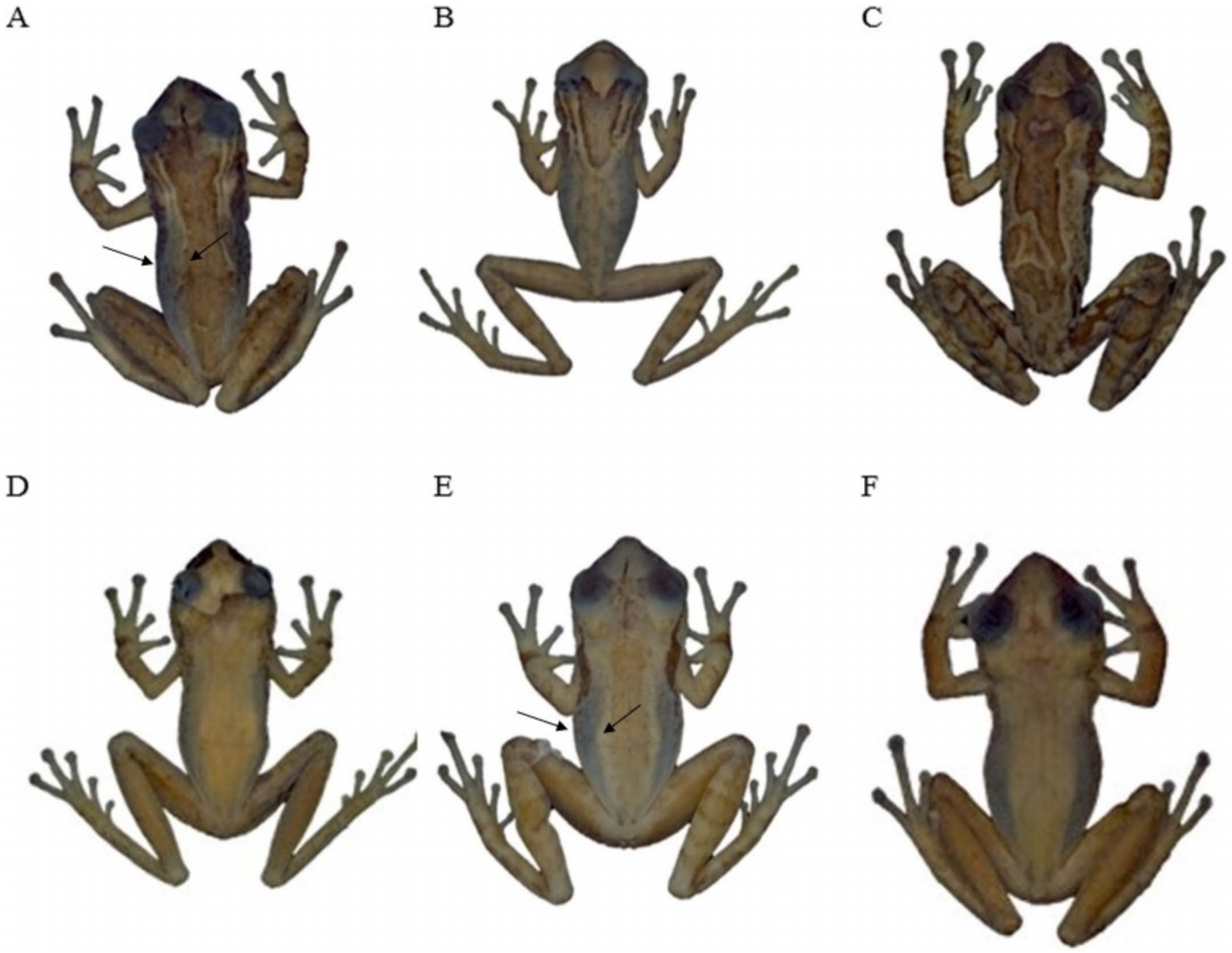


FIG. 3.—Dorsal patterns of *Fritziana mitus* sp. nov. The most common pattern (A) CFBH 25723 and some variations, with an interorbital bar, are in (B) CFBH 12194 and (C) MZUSP 139225. Other possible patterns, without an interorbital bar, are in (D) CFBH 10008, (E) CFBH 19955, and (F) CFBH 24277. Arrows indicate skin crests that delimit the dorsal pouch of females without eggs on the dorsum. A color version of this figure is available online.

*ohausi* on account of its reproductive behavior of releasing tadpoles into bromeliads (tadpoles are released into bamboo by *F. ohausi*). Although Folly et al. (2018) suggested that tadpoles of *F. izecksohni* develop in bromeliads, no clutch or tadpoles have been observed, so the microhabitat for this life-history stage remains unknown for *F. izecksohni*.

The new species differs from *Fritziana fissilis*, *F. goeldii*, *F. ohausi*, *F. tonimi*, and *F. ulei* by possessing a unique order of mitochondrial genes. Instead of the most common neobatrachian gene order (proline–phenylalanine–12S; Zhang et al. 2013), the new species has an order of phenylalanine–proline–12S (Walker et al. 2018:Fig. 4). The gene order in *F. izecksohni* remains unknown.

**Description of holotype.**—Adult female without eggs on dorsum; head wider than long; HL 33% of SVL; snout rounded with tip slightly mucronate in dorsal view, protruding in lateral view. Nostril elliptical and protuberant, laterally oriented; IND 17% of the HW. Canthus rostralis distinct and straight; loreal region straight. Tympanum slightly evident; tympanic annulus hidden beneath skin; TD 35% of ED; supratympanic fold slightly developed. Postrictal tubercles absent. Eye oriented anterolaterally, its diameter 35% of HW; IOD 33% of HW; upper eyelid and interorbital region with small tubercles. Tongue ovoid, free on its posterior portion, and lacking a posterior notch; vomerine teeth in two oblique series that are next to each other; choanae large and rounded, well separated from each other. Dorsum smooth, belly areolate; ventral surfaces of thigh and pericloacal area granulated; gular region smooth. Flank homogeneous in color. Cloacal opening directed posteriorly at the upper level of thighs. Row of tubercles present on forearms; adhesive discs of fingers developed, expanded, and not emarginated; interdigital webbing absent on hand; relative length of fingers: III < II < V < IV; subarticular tubercles rounded; thenar tubercle developed and elliptic; two palmar tubercles developed, elliptical, and parallel to each other. FL 41% of SVL; THL 89% of the TBL; very small calcar present; tubercle on tibia absent; row of tubercles present on the tarsus; tibia slightly longer than thigh and together approximately the same length as SVL; adhesive discs of toes moderate in size, expanded, and not emarginated; interdigital webbing absent between Toes I

TABLE 1.—Mean ± 1 SD and range for measurements (mm) of male and female *Fritziana mitus* sp. nov., including the type series and additional specimens (see Appendix I). SVL = snout–vent length, HW = head width, HL = head length, 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 = eye–nostril distance, IND = internarial distance, IOD = interorbital distance, DFIV = disc diameter of Finger IV, DTIV = disc diameter of Toe IV.

| | Males ($n$ = 26) | | Females ($n$ = 24) | |
|---|---|---|---|---|
| Trait | Mean ± SD | Range | Mean ± SD | Range |
| SVL | 19.97 ± 1.65 | 16.16–23.67 | 24.28 ± 2.47 | 17.43–28.35 |
| HW | 6.57 ± 0.63 | 5.26–7.82 | 8.05 ± 0.89 | 5.63–9.33 |
| HL | 7.24 ± 0.58 | 6.22–8.65 | 8.45 ± 0.86 | 6.43–9.92 |
| AL | 5.69 ± 0.37 | 4.88–6.44 | 6.70 ± 0.78 | 4.96–8.45 |
| FAL | 4.08 ± 0.35 | 3.33–4.88 | 4.83 ± 0.59 | 3.54–6.55 |
| HAL | 5.82 ± 0.54 | 4.35–6.89 | 6.75 ± 0.72 | 5.09–8.84 |
| THL | 9.47 ± 0.77 | 7.07–10.80 | 11.11 ± 1.08 | 8.09–13.77 |
| TBL | 10.68 ± 0.90 | 8.64–13.10 | 12.67 ± 1.11 | 9.45–15.27 |
| TL | 5.77 ± 0.48 | 4.66–7.11 | 6.81 ± 0.63 | 5.08–8.08 |
| FL | 8.75 ± 0.78 | 6.89–10.34 | 10.49 ± 1.27 | 7.48–14.80 |
| IND | 1.26 ± 0.13 | 1.05–1.60 | 1.46 ± 0.19 | 1.11–1.91 |
| IOD | 2.41 ± 0.46 | 0.62–3.46 | 2.95 ± 0.30 | 2.28–3.58 |
| ED | 2.67 ± 0.26 | 2.16–3.09 | 3.01 ± 0.34 | 2.34–3.64 |
| END | 2.21 ± 0.28 | 1.85–3.09 | 2.51 ± 0.27 | 1.85–3.02 |
| TD | 0.84 ± 0.13 | 0.62–1.11 | 0.97 ± 0.16 | 0.62–1.23 |
| DFIV | 0.97 ± 0.12 | 0.70–1.16 | 1.16 ± 0.17 | 0.87–1.50 |
| DTIV | 0.93 ± 0.12 | 0.65–1.19 | 1.12 ± 0.16 | 0.82–1.45 |

and II and vestigial between others; relative length of toes: I < II < III < V < IV; subarticular tubercles rounded, few supernumerary tubercles; inner metatarsal tubercle elliptic and well developed; outer metatarsal tubercle slightly evident and rounded.

**Coloration of holotype.**—In life, dorsal coloration of holotype brownish-yellow with a brownish interorbital fleck on the head. Dorsolateral stripe brownish with cream-colored outer edge extending from posterior margin of eye to midbody. Dorsal surface of discs on fingers dark. A dark brown band present on the wrist. In lateral view, maxilla with irregular dark spots. Venter with a uniform cream color. Iris pale bronze; a red horizontal stripe across eye (Fig. 1). In preservative, the specimen has the same general color patterns, but the colors are paler and the dark surface of the finger discs is not visible.

**Measurements of holotype (in mm).**—SVL 24.7, HW 8.42, HL 8.13, AL 6.9, FAL 5.13, HAL 6.81, THL 11.62, TBL 13.01, TL 7.26, FL 10.1, IND 1.42, IOD 2.78, ED 2.96, END 2.47, TD 1.05, DFIV 1.09, and DTIV 1.04.

**Morphological variation (Table 1).**—The snout is usually round in the lateral view (72.5% of the specimens), but sometimes truncate (20.7%) or protruding (6.8%). In the dorsal view, it is round (34.5%) or truncate (31%), but can also be pointed (17.25%) or subovoid (17.25%). The snout tip is slightly mucronate in the majority of specimens (86.25%), but not mucronate in others (13.75%).

The dorsal skin is smooth with tubercles on the upper eyelid present in the minority of preserved specimens (17.3%), but they were apparent in live specimens even though they were not visible in the same specimens when preserved. Although rare, tubercles might also be present on the snout and between the eyes. The belly is grainy and the throat might be slightly grainy or smooth, with the rest of the ventral surface smooth. A dorsolateral stripe is usually present (65.6%) and when present it begins on the snout, passes over the eye, and extends posteriorly until the tympanum, the middle of the body, or the cloacal region. A characteristic pattern is formed by an interorbital bar and two stripes; the two stripes originate on the posterior corner of the eyes and converge in the middle of the head where they extend parallel to the middle of the body (Fig. 3A). A small stripe might be present anteriorly and perpendicularly to the interorbital bar. Some specimens are uniformly beige with no distinct pattern (34.5%). In life, the dorsal coloration can be greenish, yellowish, bronze, or light brown. There might also be a bronze mark on the head, but this color is not visible in preserved specimens (Fig. 1B). Specimens mostly have only one stripe on the wrist, like a bracelet, and other stripes or dots on the forelimb are rare (10.35%). The fingertips might be blackish, but this is normally only observed in live specimens. The hind limbs usually lack any type of pattern, but on rare occasions they might have dots or stripes (20.7%). When large dots are present on the legs they can be brown, bronze, or cream (Fig. 1B). Also, a row of tubercles might be present on the forearms (41.4%) or more often, on the hind limbs (86.25%).

**Sexual dimorphism.**—During the reproductive period, females exhibit a closed dorsal pouch when transporting eggs (Fig. 1B). The eggs are normally distributed as a layer, without any regular pattern common to all females. Females without eggs might possess, on the dorsum, a skin crest that corresponds to the empty pouch (Fig. 3A,E). Females with eggs removed from their dorsum after preservation possess individual skin chambers on the dorsum. Males possess nuptial pads on the base of Finger II, which can 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.

**External morphology of tadpoles.**—Even when belonging to the same egg clutch and being euthanized simultaneously, the tadpoles show different stages of development. In Stage 35, tadpoles have an elongated body, elliptical in both the dorsal and lateral views (Fig. 4). Their BL is 39.8% of the total length (Table 2). The body is wider than high; BH is 85.7% of its width. The BH is 93.7% of the TAH. The snout is rounded in dorsal view and truncate in lateral view. The eye is dorsally located, anterolaterally

TABLE 2.—Measurements (mm) of tadpoles of *Fritziana mitus* sp. nov. in different developmental stages (stages according to Gosner 1960). TL = total length, BL = body length, BW = body width, BH = body height, TAL = tail length, TAH = tail height, DFH = dorsal fin height, VFH = ventral fin height, IND = internostril distance, IOD = interorbital distance, ED = eye diameter, ND = nostril diameter, END = eye–nostril distance, NSD = nostril–snout distance, ESD = eye–snout distance, SSD = snout–spiracle distance, SVD = spiracle–vent distance, ODW = oral disc width.

| | Stage 35 ($n$ = 2) | | Stage 36 ($n$ = 3) | | Stage 37 ($n$ = 1) | Stage 39 ($n$ = 1) |
|---|---|---|---|---|---|---|
| Trait | Specimen 1 | Specimen 2 | Range | Mean ± 1 SD | Value | Value |
| TL | 14.8 | 15.8 | 13.5–15.3 | 14.67 ± 1.01 | 18.2 | 18.3 |
| BL | 5.9 | 5.4 | 5.0–6.3 | 5.80 ± 0.70 | 6.6 | 6.8 |
| BW | 3.5 | 3.5 | 3.6–3.7 | 3.63 ± 0.06 | 3.7 | 4.0 |
| BH | 3 | 3.1 | 3.2–3.9 | 3.47 ± 0.38 | 3.0 | 3.1 |
| TAL | 10.3 | 11.2 | 8.9–11.0 | 9.90 ± 1.05 | 13.0 | 11.6 |
| TAH | 3.2 | 3.4 | 3.0–3.3 | 3.17 ± 0.15 | 3.6 | 3.5 |
| DFH | 0.9 | 0.9 | 0.9–1.1 | 1.00 ± 0.10 | 1.2 | 1.3 |
| VFH | 1.1 | 1.2 | 0.8–1.0 | 0.93 ± 0.12 | 1.3 | 1.4 |
| IND | 0.9 | 0.8 | 0.7–1.3 | 0.90 ± 0.35 | 1.2 | 1.1 |
| IOD | 1.6 | 1.5 | 1.6–1.8 | 1.70 ± 0.10 | 1.1 | 2.0 |
| ED | 1 | 1 | 1.1–1.2 | 1.17 ± 0.06 | 1.3 | 1.3 |
| ND | 0.3 | 0.4 | 0.3–0.5 | 0.40 ± 0.10 | 0.5 | 0.5 |
| END | 0.7 | 0.8 | 0.8–0.9 | 0.87 ± 0.06 | 0.9 | 1.0 |
| NSD | 0.5 | 0.6 | 0.4–0.7 | 0.60 ± 0.17 | 0.6 | 1.0 |
| ESD | 1.1 | 0.9 | 0.8–1.0 | 0.90 ± 0.10 | 1.1 | 1.2 |
| SSD | 2.7 | 3.1 | 2.6–3.3 | 2.97 ± 0.35 | 3.5 | 3.3 |
| SVD | 4.8 | 4.7 | 4.6–5.0 | 4.77 ± 0.21 | 5.2 | 4.9 |
| ODW | 1 | 1.2 | 0.9–1.0 | 0.97 ± 0.06 | 1.3 | 1.2 |

directed; with an ED equal to 16.9% of the BL; the nostril is closer to the tip of snout than to the eye; the distance between the eye and nostril is 63.6% of the distance between the eye and the tip of snout. The nostril is elliptical in the lateral view and the opening of the nostril is not visible dorsally. The nostril is anteriorly located and directed; the IND is 56.2% of the IOD; the distance between the nostril and the tip of snout is 8.4% of the BL.

The spiracle is sinistral, ventral, short, and with the inner wall connected to the body. The SSD is 45.7% of the BL. The vent tube is as long as it is wide, dextral, with a wide opening, and attached to the ventral fin. The TAL is 69.6% of the total length and tail musculature does not reach the tail tip, which is rounded. In the lateral view, the dorsal fin originates on the tail musculature; the dorsal fin is ascendant in the first third of the tail, then becomes parallel to the tail musculature. The ventral fin is parallel to the tail musculature (Fig. 4). The oral disc is ventral and the ODW is 28.6% of the BW, bordered by a single row of papillae with a large dorsal gap (Fig. 5). Submarginal rows of papillae and labial teeth are absent. Jaw sheaths are developed and completely serrated; the upper is U-shaped and the lower is V-shaped (Fig. 5).


FIG. 4.—Tadpole of *Fritziana mitus* sp. nov., Stage 35 of Gosner (1960), from Bertioga, state of São Paulo, Brazil (lot CFBH 34437). (A) Lateral, (B) ventral, and (C) dorsal views (scale = 1 mm). A color version of this figure is available online.

**Tadpole color.**—Tadpoles preserved in 5% formalin are light brown with very small dark brown blotches; the flanks are lighter than the dorsum. Laterally, the tail is light cream, adorned with small dark brown blotches concentrated in the tail musculature and in the final third of the tail. Ventrally, the body is light brown with a translucent ventral region; the yolk is visible through the skin. The spiracle, the oral disc, and the cloacal tube are translucent beige.

**Molecular analyses.**—Walker et al. (2018) reported the occurrence of a different gene order for mitochondrial tRNA in *Fritziana mitus* sp. nov. (named by them as *F.* CS3) that is seemingly exclusive to this species until now. If the 12S gene is amplified using the primer MVZ59 (Graybeal 1997), the specimens show a fragment with about 200 more base pairs than in other species. We associate this with the proline tRNA gene, indicating a change in the gene order from the common neobatrachian order of proline–phenylalanine–12S, to an order of phenylalanine–proline–12S in the new species (Walker et al. 2018). All the other species of *Fritziana* possess the common neobatrachian size for this fragment of tRNA (Walker et al. 2018); however, this information is missing for *F. izecksohni,* whose gene order is unknown.

Complete Bayesian analyses were made for *Fritziana* by Walker et al. (2018) and by our group (with only part of the 16S fragment, including *Fritziana izecksohni* and outgroups; Fig. 6). Both analyses show that *F. mitus* clusters together,

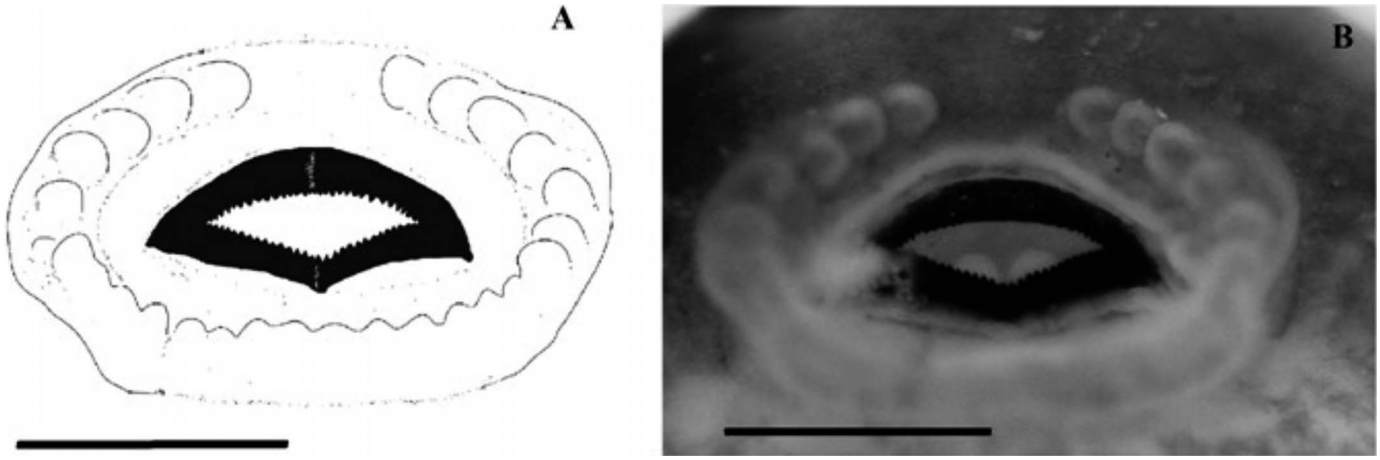


FIG. 5.—Oral disc of the tadpole of *Fritziana mitus* sp. nov., Stage 35 of Gosner (1960), from Bertioga, state of São Paulo, Brazil (lot CFBH 34437). (A) Drawing and (B) photo (scale = 0.5 mm).

and is a sister group to only one sample from the state of Minas Gerais (*F.* CS2 in Walker et al. 2018). Morphologically, this specimen is similar to *F. mitus*, but differs from the new species by the presence of an additional fragment in its tRNA (Walker et al. 2018:Fig. 4). The few specimens collected of *F.* CS2 preclude proper comparisons. *Fritziana* CS2 (Walker et al. 2018) + *F. mitus* sp. nov. form the sister group of another new species, informally named by Walker et al. (2018) as *F.* CS1.

The two samples of *Fritziana izecksohni* used by Folly et al. (2018) might have been contaminated because they cluster with *Euparkerella* and Cophomantini (Hylidae) in the present analyses (Fig. 6). This also explains the high inter- and intraspecific divergence and high length of branches found for *F. izecksohni* by Folly et al. (2018).

**Geographical distribution.**—*Fritziana mitus* sp. nov. occurs in the Brazilian states of São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (Appendix I). This species possesses the largest and southernmost known distribution for the genus *Fritziana* (Fig. 7).

**Conservation status.**—According to the International Union for Conservation of Nature (IUCN) Red List, *Fritziana fissilis* is listed as Least Concern "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 threatened category" (Carvalho-e-Silva and Telles 2010:1). With the description of *F. mitus* sp. nov., the known distribution of *F. fissilis* becomes much smaller and the amount of information about the species is insufficient to place it in one of the categories proposed by IUCN. Therefore, we believe *F. fissilis* should be considered Data Deficient. On the other hand, *F. mitus* has a wide distribution and is the more common species of the genus, and thus qualifying as Least Concern.

**Etymology.**—The specific epithet, *mitus,* is a noun derived from the Greek word μίτος, which means "thread." It is a reference to the mitochondrial DNA, which possesses a gene order exclusive to this species.

**Natural history.**—Individuals of *Fritziana mitus* sp. nov. are found in epiphytic bromeliads, generally of the genus *Vriesea* Lindl, 1–3 m above the ground in forests, but might also be found in bushes. In the Parque Estadual da Serra do Tabuleiro, Santa Catarina State, *F. mitus* sp. nov. can be active at dusk and at night, and is more abundant during the rainy season (September to March). On two occasions, individuals of *F. mitus* sp. nov. were found moving on the forest floor and two other specimens were observed on nonbromeliad leaves. Females may carry 7–10 eggs in the egg-brooding dorsal pouch. The advertisement call was described by Franz and Mello (2015), who identified the species as *F.* aff. *fissilis*.

## DISCUSSION

There are many records of the new species in collections and in literature, but most are incorrectly identified as one of the following names: *Fritziana fissilis* (Duellman and Gray 1983; Heyer et al. 1990), *F.* aff. *fissilis* (Franz and Mello 2015), *F.* gr. *fissilis* (a name with no prior mention in the literature; Santos-Pereira et al. 2016), or *Fritziana* sp. Despite the many morphotypes under the name of *F. fissilis* in Brazilian collections (M. Walker, P.N. Costa, P.C.A. Garcia, and C.F.B. Haddad, personal observations), the lack of additional data on the morphology of all the species of the genus complicates the recognition and description of a new taxa. The overlap in the morphology among several species of *Fritziana* and the incomplete nature of the diagnoses for some of the species in the genus have made it difficult to accurately identify and describe new species.

Folly et al. (2014) used specimens from different localities to revalidate *Fritziana ulei*, but some specimens of *F. mitus* sp. nov. were included in their Appendix 1 under the name *F. ulei*. Based on specimens from Ubatuba, Menegucci et al. (2017) proposed a geographical expansion for *F. ulei*, also based on specimens of *F. mitus* sp. nov. These two species—*F. mitus* sp. nov. and *F. ulei*—are difficult to distinguish using only adult morphology and, until now, were not recognized as being sympatric. As observed by Walker et al. (2018), *F. ulei* is restricted to its type locality and surroundings in the Nova Friburgo municipality and Serra dos Órgãos mountain range, both in Rio de Janeiro State.

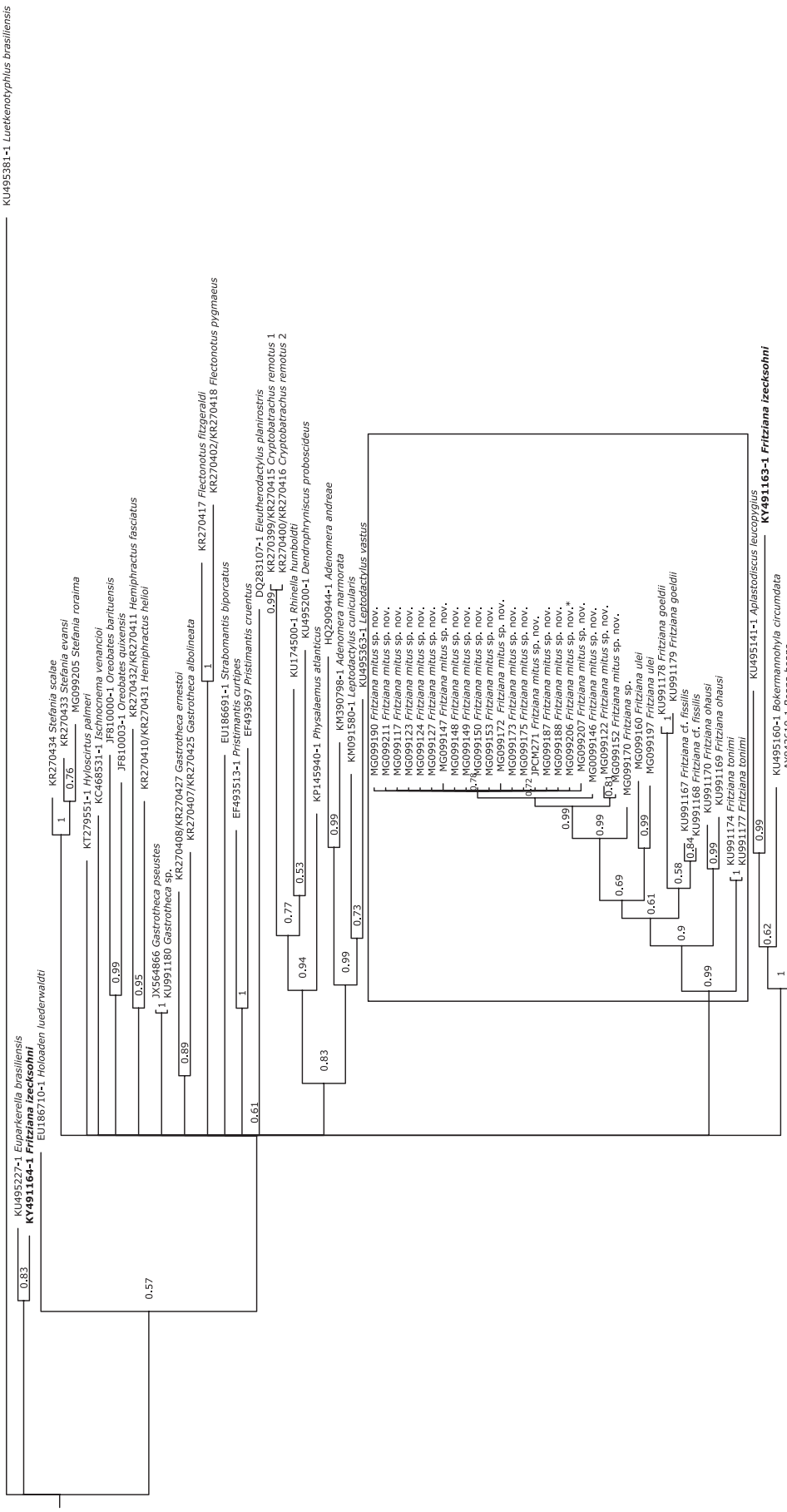


FIG. 6.—Bayesian tree for species in the genus *Fritziana* inferred from 16S ribosomal RNA gene fragments. Samples of *Fritziana izecksohni* used in the original description of that species are indicated in bold. Specimens bound by the square are those that served as the basis for the description of *F. mitus* sp. nov.; the asterisk (*) indicates a paratype included in the analyses. Scale indicates rate of base substitutions per site. See Appendices II and III for sample details.

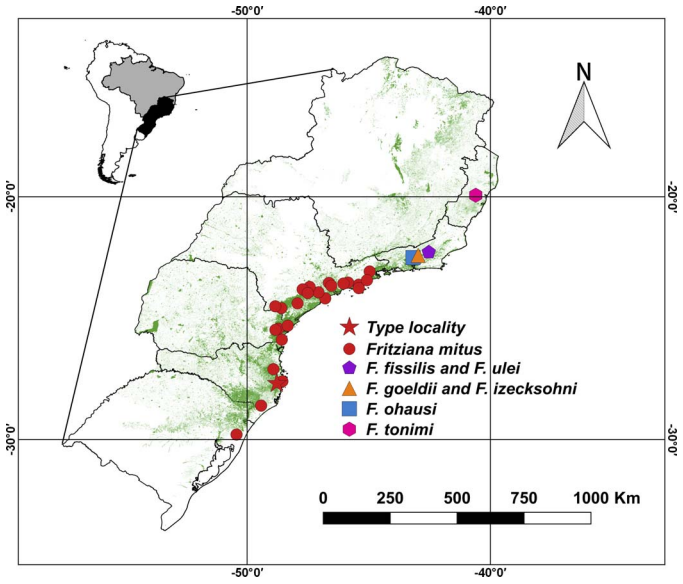


FIG. 7.—Geographical distribution of *Fritziana mitus* sp. nov. The star and circles show the distribution of analyzed specimens (star = type locality in municipality of Santo Amaro da Imperatriz, in the state of Santa Catarina, Brazil [shaded area of inset]). The other markers show the type localities of *F. fissilis* and *F. ulei* (pentagon), Nova Friburgo; *F. goeldii* and *F. izecksohni* (triangle), Teresópolis; *F. ohausi* (square), Petrópolis, all in the state of Rio de Janeiro, and *F. tonimi* (hexagon), Santa Tereza, in the state of Espírito Santo. Green shading represents the remnants of the Atlantic Forest, Brazil. A color version of this figure is available online.

New characters have been found for the genus with the use of different taxonomic tools. For *Fritziana mitus* sp. nov., a new order of mitochondrial genes has been reported (Walker et al. 2018), presenting size variations in the amplified fragments among the species with same primers. Few papers rely on gene order, but here it was useful in distinguishing this species. This is the only known species of Anura with a different order in leucine-tyrosine-proline-phenylalanine a region that normally does not vary (Zhang et al. 2013). A combination of data sources—including advertisement call, tadpole morphology, karyotype, and DNA sequences—are clearly warranted for elucidating the taxonomy of *Fritziana*.

Concerning subarticular tubercles, Franz and Mello (2015) stated that a specimen from the municipality of Caraá in the state of Rio Grande do Sul (until now, referred to as *Fritziana* aff. *fissilis*, UFRGS 7068) has bifid subarticular tubercles. We analyzed the hands and feet photos from the same specimen, however, and found that it has rounded subarticular tubercles, thus being in accordance with *F. mitus* sp. nov.

Franz and Mello (2015) also described the advertisement call of the population of *Fritziana mitus* sp. nov. from southern Brazil (under the name *Fritziana* aff. *fissilis*). Even though the terminology of acoustic parameters for *Fritziana* calls still needs standardization, the information currently available allows us to make useful comparisons. Folly et al. (2018) analyzed the advertisement calls available for *Fritziana* species and demonstrated that the advertisement call of *F. mitus* sp. nov. (therein named *Fritziana* aff. *fissilis* sp. 1) differs from both *F. fissilis* and *F. izecksohni* because of its higher dominant frequency: *F. mitus* sp. nov. with 3166 ± 112.7 Hz, 3000–3375 Hz (Franz and Mello 2015); *F. fissilis* with 2400 Hz (Duellman and Gray 1983); and *F. izecksohni* with 2258.4 ± 107.4 Hz, 2062.5–2437.5 Hz (Folly et al. 2018). Because it is difficult to get close enough to record good-quality calls and collect appropriate voucher material from epiphytic bromeliads, high-quality recordings of the *Fritziana* species are lacking. The data on advertisement calls contributes to the diagnoses, however, reinforcing how important obtaining good vocalization records is to the understanding of this group.

The new species uses the water accumulated in bromeliads to deposit and raise tadpoles, as do members of *Fritziana fissilis*, *F. goeldii*, *F. tonimi*, *F. ulei*, and probably *F. izecksohni*. This observation emphasizes the relationship between these plants and the population health of several species of *Fritziana*. The large quantity of yolk and the reduced buccal apparatus indicate that the tadpoles of *F. mitus* sp. nov. are endotrophic. This is already known for other species in the genus that also exhibit the reproductive mode Number 36: "Eggs carried on dorsum or in dorsal pouch of female; endotrophic tadpoles in bromeliads or bamboo" (Haddad and Prado 2005:210).

*Fritziana mitus* sp. nov. is the seventh species described from this genus and has the largest and southernmost distribution. The description of *F. mitus* sp. nov. implies the use of this name for several populations previously identified as *F. fissilis*, making *F. fissilis* an almost endemic species restricted to its type locality (Nova Friburgo, Rio de Janeiro State) and its surroundings in the Serra dos Órgãos mountain range. Walker et al. (2018) discussed the complexity of the definition of *F. fissilis*, because there are at least two clades that occur at this type locality whose morphology fits the original description and the type series. A proper definition of *F. fissilis* is necessary to facilitate future descriptions of species with closed dorsal pouches, mainly from the Serra dos Órgãos and surrounding areas. The high diversity of *Fritziana* in the Serra dos Órgãos mountain range (containing five of the seven species currently described), makes essential the collection of specimens and tissue samples of *Fritziana* in this mountain range.

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authorizing specimen collection in the Parque Estadual da Serra do Tabuleiro.

RESUMO: Descrevemos uma nova espécie para o gênero *Fritziana*, historicamente identificada como *F. fissilis* ou *F.* aff. *fissilis*. Nos baseamos em morfologia de adultos, girinos e análises moleculares. A nova espécie é caracterizada pelo focinho mucronado, listra negra no pulso, tubérculos subarticulares redondos nos dedos e artelhos, fêmeas com bolsa dorsal fechada para carregar ovos, girinos depositados em bromélias e mudança na ordem dos tRNAs mitocondriais, se comparada com as ordens gerais reconhecidas para anuros. Essa nova espécie ocorre na Mata Atlântica dos estados de São Paulo, Paraná, Santa Catarina e Rio Grande do Sul, sendo esta a maior distribuição geográfica registrada para uma espécie no gênero *Fritziana*.

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## Appendix I

### Specimens Examined

See Sabaj (2016) for the complete names of the collections except for CHUFSC (Coleção Herpetológica da Universidade Federal de Santa Catarina) and Fig. 7 for the distribution map of *Fritziana mitus* sp. nov.

*Fritziana ohausi*.—Brazil: state of Rio de Janeiro: Teresópolis (Serra dos Órgãos): MZUSP 77702–77704; CFBH 32572–32573. State of São Paulo: 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 (Boracéia): MZUSP 137418–137419.

*Fritziana goeldii*.—Brazil: state of Rio de Janeiro: 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. State of São Paulo: Ubatuba: CFBH 9540, 10909–10910.

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

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

*Fritziana tonimi*: Brazil: state of Espírito Santo: Santa Teresa: CFBH 24809; CFBH 30710–30712; CFBH 30920; MNRJ 28376, 38397–38398, 40700, 56060–56062; Domingos Martins: MNRJ 46719–46720.

*Fritziana mitus*.—Brazil: state of São Paulo: between Apiaí e Iporanga (PETAR): CFBH 25632–25634; Biritiba Mirim: CFBH 12240; Caraguatatuba: CFBH 8273; Cunha: CFBH 12194; Iporanga: CFBH 6301; Itanhaém: CFBH 8333, 11177, 12205, 22113; Juquitiba: MZUSP 134762, 134764–134767; Piedade: CFBH 23316; Pilar do Sul: CFBH 8290, 8614, 10008; Salesópolis (Boracéia): MZUSP 137443; CFBH 1557, 1558; Santo André (Paranapiacaba): CFBH 28980; São Paulo: CFBH 13572; Ilhabela: CFBH 17496; Sete Barras: MZUSP 136570; Tapiraí: CFBH 10315; Ubatuba: CFBH 4542, 19955. State of Paraná: Antonina: CFBH 11106; Garuva: UFMG 18553; Guaraqueçaba: MZUSP 137966–137967; Guaratuba: CFBH 5482; Morretes: DZSJPR 5251; Tijucas do Sul: UFMG 18554. State of Santa Catarina: Brusque: MZUSP 9806–9809; Florianópolis: MCP-PUCRS 8284; Santo Amaro da Imperatriz: CFBH 39932–39937, 39949; CHUFSC 2625; MNRJ 90350-90352; São Francisco do Sul: UFMG 17384; Siderópolis: CFBH 24277, 25723. State of Rio Grande do Sul: Osório: UFMG 17436.

Appendix II.—Collection number, species name, locality, and GenBank accession number of the samples of *Fritziana* used in molecular analyses. See Sabaj (2016) for the complete names of collections except for CHUFSC (Coleção Herpetológica da Universidade Federal de Santa Catarina). Brazilian states: ES = Espírito Santo, MG = Minas Gerais, PR = Paraná, RJ = Rio de Janeiro, SP = São Paulo, SC = Santa Catarina, RS = Rio Grande do Sul.

| Collection number | Voucher number | Species | Localities | 16S GenBank |
|---|---|---|---|---|
| CFBHT 84 | CFBH 5726 | *Fritziana mitus* sp. n. | Santo Amaro da Imperatriz, SC | MG099117 |
| CFBHT 1461 | | *Fritziana mitus* sp. n. | Caraguatatuba, SP | MG099122 |
| CFBHT 1625 | CFBH 7620 | *Fritziana ohausi* | Pilar do Sul, SP | KU991170 |
| CFBHT 2359 | CFBH 10008 | *Fritziana mitus* sp. n. | Pilar do Sul, SP | MG099123 |
| CFBHT 2432 | CFBH 10315 | *Fritziana mitus* sp. n. | Tapiraí, SP | MG099124 |
| CFBHT 2862 | CFBH 11106 | *Fritziana mitus* sp. n. | Antonina, PR | MG099127 |
| CFBHT 7392 | CFBH 17496 | *Fritziana mitus* sp. n. | Ilhabela, SP | MG099146 |
| CFBHT 10526 | CFBH 22113 | *Fritziana mitus* sp. n. | Parque Estadual da Serra do Mar–Itanhaém, SP | MG099147 |
| CFBHT 10989 | CFBH 23316 | *Fritziana mitus* sp. n. | Piedade, SP | MG099148 |
| CFBHT 11451 | CFBH 23726 | *Fritziana mitus* sp. n. | Siderópolis, SC | MG099149 |
| CFBHT 11453 | | *Fritziana mitus* sp. n. | Siderópolis, SC | MG099150 |
| CFBHT 11609 | CFBH 19955 | *Fritziana mitus* sp. n. | Ubatuba, SP | MG099152 |
| CFBHT 12325 | CFBH 25723 | *Fritziana mitus* sp. n. | Siderópolis, SC | MG099153 |
| CFBHT 12889 | CFBH 26981 | *Fritziana goeldii* | Rio de Janeiro, RJ | KU991179 |
| MNRJ 40700 | MNRJ 40700 | *Fritziana tonimi* | Santa Teresa, ES | KU991174 |
| MNRJ 44622 | MNRJ 44622 | *Fritziana ulei* | Cachoeiras de Macacu, RJ | MG099160 |
| MNRJ 56062 | MNRJ 56062 | *Fritziana tonimi* | Santa Teresa, ES | KU991177 |
| MNRJ 62845 | MNRJ 62845 | *Fritziana* cf. *fissilis* | Nova Friburgo, RJ | KU991167 |
| MNRJ 74620 | MNRJ 74620 | *Fritziana* cf. *fissilis* | Parque Nacional da Serra dos Órgãos–Teresópolis, RJ | KU991168 |
| CTMZ 2119 | MZUSP 135461 | *Fritziana mitus* sp. n. | Eldorado Paulista, SP | MG099172 |
| CTMZ 2618 | MZUSP 136570 | *Fritziana mitus* sp. n. | Parque Estadual Carlos Botelho–São Miguel Arcanjo, SP | MG099173 |
| CTMZ 7295 | MZUSP 143718 | *Fritziana mitus* sp. n. | Parque Natural Municipal Nascentes de Paranapiacaba–Paranapiacaba, Santo André, SP | MG099175 |
| MCP 10920 | MCP 10920 | *Fritziana mitus* sp. n. | Florianópolis, SC | MG099187 |
| MCP 10921 | MCP 10921 | *Fritziana mitus* sp. n. | Florianópolis, SC | MG099188 |
| CFBH 30938 | CFBH 30938 | *Fritziana goeldii* | Parque Nacional da Serra dos Órgãos–Teresópolis, RJ | KU991178 |
| CFBH 39057 | CFBH 39057 | *Fritziana mitus* sp. n. | Parque das Neblinas–Mogi das Cruzes, SP | MG099190 |
| ZUFRJ | ZUFRJ | *Fritziana ulei* | Nova Friburgo, RJ | MG099197 |
| ZUFRJ 13656 | ZUFRJ 13656 | *Fritziana ohausi* | Parque Nacional da Serra dos Órgãos–Teresópolis, RJ | KU991169 |
| CHUFSC 2625 | CHUFSC 2625 | *Fritziana mitus* sp. n. | Santo Amaro da Imperatriz, SC | MG099206 |
| CHUFSC 2626 | CHUFSC 2626 | *Fritziana mitus* sp. n. | Bombinhas, SC | MG099207 |
| CFBH 15968 | CFBH 15968 | *Fritziana mitus* sp. n. | Parque Estadual Turístico do Alto Ribeira–Iporanga, SP | MG099211 |
| UFMG 17436 | | *Fritziana mitus* sp. n. | Osório, RS | MH795452 |
| ZUFRJ 13978 | | *Fritziana izecksohni* | Serra dos Órgãos, Teresópolis, RJ | KY491164 |
| ZUFRJ 13317 | | *Fritziana izecksohni* | Serra dos Órgãos, Teresópolis, RJ | KY491163 |
| MCNT 154 | MCNAM 12341 | *Fritziana* CS2 | Bom Jardim de Minas, MG | MG099170 |

APPENDIX III.—GenBank accession numbers, species name, voucher collection and number of outgroups used in molecular analyzes. See Sabaj (2016) for the complete names of the collections.

| GenBank accession numbers | Species name | Voucher collection and number |
|---|---|---|
| AY843610-1 | *Boana boans* | RWM 17746 |
| KR270399; KR270415 | *Cryptobatrachus remotus* 1 | MHNLS17664 |
| KR270400; KR270416 | *Cryptobatrachus remotus* 2 | MHNLS18853 |
| DQ283107-1 | *Eleutherodactylus planirostris* | USNM 547959 |
| EF493513-1 | *Pristimantis curtipes* | KU217871 |
| EU186691-1 | *Strabomantis biporcatus* | CVULA7073 |
| EU186710-1 | *Holoaden luederwaldti* | MZUSP131872 |
| KR270417 | *Flectonotus fitzgeraldi* | ZSM16102006 |
| KR270402, KR270418 | *Flectonotus pygmaeus* | MHNLS 17478 |
| KR270408; KR270427 | *Gastrotheca ernestoi* | MNRJ57129 |
| JX564866 | *Gastrotheca pseustes* | TNHC62492 |
| HQ290944-1 | *Adenomera andreae* | QCAZ15998 |
| KR270432, KR270411 | *Hemiphractus scutatus/fasciatus* | JMP2150 / EVACC031 |
| KR270410, KR270431 | *Hemiphractus helioi* | MHNCP9063 |
| JF810000-1 | *Oreobates barituensis* | MCN1360 |
| JF810003-1 | *Oreobates quixensis* | QZ31186 |
| KC468531-1 | *Ischnocnema venancioi* | MNRJ 53932 |
| KM091580-1 | *Leptodactylus cunicularius* | MNRJ 24863 |
| KM390798-1 | *Adenomera marmorata* | |
| KP145940-1 | *Physalaemus atlanticus* | CFBH 7918 |
| KT279551-1 | *Hyloscirtus palmeri* | MZUTI 3083 |
| KU174500-1 | *Rhinella humboldti* | CZUT-A 1717 |
| KU495141-1 | *Aplastodiscus leucopygius* | CFBHT13076 |
| KU495160-1 | *Bokermannohyla circumdata* | CFBHT14585 |
| KU495200-1 | *Dendrophryniscus proboscideus* | MTR ALCX168P68 |
| KU495227-1 | *Euparkerella brasiliensis* | CFBHT12892 |
| KU991180 | *Gastrotheca* sp. | USNM 25111 |
| KU495280-1 | *Boana polytaenia* | CFBHT07917 |
| KU495363-1 | *Leptodactylus vastus* | TG423 |
| KU495381-1 | *Luetkenotyphlus brasiliensis* | CFBHT12316 |
| KR270434 | *Stefania scalae* | MHNLS17152 |
| KU991180 | *Gastrotheca* sp. | USNM 25111 |
| KR270407, KR270425 | *Gastrotheca albolineata* | MNRJ54401 |
| KR270433 | *Stefania evansi* | IRSNB14586 |
| MG099205 | *Stefania roraimae* | USNM49319 |
| EF493697 | *Pristimantis cruentus* | AMNH A12444-A12448 |