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A New Species of Glassfrog Genus *Vitreorana* (Anura, Centrolenidae) from the Cerrado Domain, Southeastern Brazil

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Abstract: We describe herein a new species of *Vitreorana* from the Cerrado biome at Serra da Canastra National Park, Vargem Bonita, Minas Gerais, Southeastern Brazil. Morphological and molecular data from one nuclear gene (proopiomelanocortin) show that the new species belongs to the genus *Vitreorana*, with a strong support of posterior probability of Bayesian inference. *Vitreorana franciscana* sp. nov. is characterized by its slender body; snout subovoid in dorsal view; outer tarsal and ulnar ridges with low and small, thickened tubercles; cloacal ornamentation composed of an aggregation of small, thickened tubercles, with two elevations on each side of anal opening forming a slight flap, all highlighted by white pigment; and reticulated arrangement of melanophores on dorsum. Furthermore, we describe the calling repertoire and provide data on natural history of the new species.

Key words: Advertisement call; Centroleninae; Phylogeny; Serra da Canastra; Taxonomy

Species belonging to the family Centrolenidae are commonly known as Glassfrogs because of their transparent venters (Cisneros-Heredia and McDiarmid 2007). This family is currently composed of 148 species divided into 12 genera distributed throughout the Neotropics including Central America, northern Andes, Guiana Shield, Amazonia, and southeastern Brazil (Guayasamin et al. 2009; Castroviejo-Fisher et al. 2011; Frost 2014).

The genus *Vitreorana* Guayasamin, Castroviejo-Fisher, Trueb, Ayarzagüena, Rada and Vilà 2009 was recently proposed based on molecular evidence and, at present, comprises eight species distributed along the Cordillera de la Costa in Venezuela, the Precambrian Shields of South America, and the Amazon basin (Guayasamin et al. 2009). Four of those species are known to occur in the Atlantic Forest, from northeastern Brazil to northern Argentina (Heyer 1985): *Vitreorana parvula* (Boulenger 1895), *V. uranoscopa* (Müller 1924), *V. eurygnatha* (Lutz 1925), and *V. baliomma* Pontes, Caramaschi and Pombal 2014. These species occur in ombrophilous formations of the Atlantic Forest, inhabiting riverine canopied vegetation near permanent streams and creeks (Heyer 1985; Heyer et al. 1990; Stetson 2001; Machado et al. 2010). Additional records of Glassfrogs from riparian forests of the Cerrado biome have increased in recent decades. To our knowledge, centrolenid frogs occur in two localities in rocky fields (*campos rupestres*) of riparian forests in the Serra do Cipó National Park (Eterovick and Sazima 2004) and the Serra da Canastra National Park (Haddad et al. 1988), both in the State of Minas Gerais of southeastern Brazil.

Specimens from these populations were not included in the revisions that addressed the taxonomy of Brazilian centrolenid frogs (Taylor and Cochran 1953; Heyer 1978, 1985), and their taxonomic status remains uncertain. Here, we analyze specimens previously identified as *Vitreorana uranoscopa* and describe the first species of *Vitreorana* from the Cerrado biome of southeastern Brazil.

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Materials and Methods

This study was based on analyses of field-collected and museum specimens and field observation of natural history and behavior (Appendix). Additionally, we analyzed photos of the holotype of *Vitreorana parvula* (BMNH 88.2.7.32) and *V. uranoscopa* (ZSM 81/1921). Collected specimens were fixed in 10% formalin and preserved in 70% ethanol. Museum abbreviations follow Sabaj Perez (2014). Sex and maturity were determined by secondary sexual characters (nuptial excrescences, vocal slits, and vocal sacs) and the presence of convoluted oviducts in female specimens. For supraspecific classification we followed the taxonomic arrangement of Guayasamin et al. (2009).

Morphology

Coloration patterns and characteristics were observed in individuals in situ, and photographs of living and well-preserved specimens in ethanol were taken ex situ. Character descriptions followed Lynch and Ruiz-Carranza (1996) for nuptial excrescences; for membrane formula we followed the modifications of Lynch and Duellman (1973), Flores (1985), Ruiz-Carranza and Lynch (1991), Cisneros-Heredia and McDiarmid (2007), and citations therein for definitions of morphological traits. Following Duellman (2001) and Cisneros-Heredia and McDiarmid (2007), we measured the following morphometric characters using a digital caliper (±0.1 mm): (1) snout–vent length (SVL); (2) head width (HW); (3) head length (HL); (4) horizontal eye diameter (ED); (5) interorbital distance (IOD); (6) eye–nostril distance (EN); (7) internarial distance (IN); (8) horizontal tympanum diameter (TD); (9) width of third finger disc (3DW); (10) hand length (HL); (11) thigh length (THL); (12) tibia length (TL); and (13) foot length (FL).

Phylogeny

We obtained partial sequences of the nuclear gene proopiomelanocortin (POMC) from two paratopotypes (MZUFV 9947 and 10046) of the new species. Protocols for DNA extraction, amplification, sequencing, and sequence

TABLE 1.—List of Genbank accession numbers and sequenced individuals, and nucleotide sequences of the nuclear gene, proopiomelanocortin, used in this study.

Species	Locality	GenBank accession numbers	Reference
Allophryne ruthveni	Guyana: Pakatau Creek Camp	AY819077	Wiens et al. (2005)
Cochranella nola	Bolivia: Departamento Cochabamba: Villa Fatima	EU663200	Guayasamin et al. (2008)
Espadarana andina	Venezuela: Estado de Mérida: Quebrada Azul, on the road between La Azulita and El Hato	EU663166	Guayasamin et al. (2008)
Rulyrana spiculata	Peru: Departamento Junin: Provincia Satipo: Distrito Llaylla: Vista Alegre	EU663207	Guayasamin et al. (2008)
Sachatamia punctulata	Colombia: Departamento Antioquia: Municipio de Maceo: Vereda Las Brisas, Hacienda Santa Bárbara	EU663203	Guayasamin et al. (2008)
Teratohyla midas	Ecuador: Provincia Napo: Jatun Sacha	EU663199	Guayasamin et al. (2008)
Vitreorana castroviejoi	Venezuela: Estado Sucre: Península de Paria, near Macuro	EU663189	Guayasamin et al. (2008)
Vitreorana franciscana	Brazil: Minas Gerais: Serra da Canastra National Park	KR921743	This study
Vitreorana franciscana	Brazil: Minas Gerais: Serra da Canastra National Park	KR921744	This study
Vitreorana gorzulae	Guyana: Cuyuni-Mazaru Distrit: Upper Partang River	EU663172	Guayasamin et al. (2008)
Vitreorana helenae	Venezuela: Estado Bolivar: San Ignacio de Yuraní, Quebrada de Jaspe	EU663196	Guayasamin et al. (2008)
Vitreorana oyampiensis	French Guiana: Cayenne: Aya, Trinité	EU663202	Guayasamin et al. (2008)
Vitreorana uranoscopa	Brazil: Corrego do Viadinho, PARNA Caparao ES	JX298142	Fouquet et al. (2012)

editing followed Shepard and Burbrink (2009), except that the primers POMC_DRV_F1 and POMC_DRV_R1 (Vieites et al. 2007) were used for amplification and sequencing.

Sequences were aligned with sequences downloaded from GenBank (Table 1) in Geneious Pro v6.1.3 using the MUSCLE algorithm with default parameters (Edgar 2004). The matrix of 448 base pairs of aligned sequences of POMC showed no indels. The most appropriate model of nucleotide substitution for the alignment was TnR+G, determined with jModeltest (Posada 2008). We performed a Bayesian phylogenetic analysis using MrBayes v3.2.2 (Ronquist et al. 2012). We performed two analyses for 20 million generations and sampled every 1000 generations with a 25% burn in. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v1.5 (Rambaut and Drummond 2007).

Bioacoustics

Vocalizations were recorded in situ using a Panasonic RR-US450 digital recorder with an internal microphone on 25 August 2009, around 2100 h (air temperature = 19.8°C; call label = ASUFRN 223; voucher specimen under the label MZUFV 9968 or MZUFV 9970), and with a digital recorder M-audio Microtrack II coupled to a directional microphone Sennheiser K6/ME66 on 30 November 2010, around 2220 h (air temperature = 20.8°C; call labels = Vitreorana_sp_1aMGLM_AAGmt_pI; Vitreorana_sp_1aMGLM_AAGmt_pII; Vitreorana_sp_1bMGLM_AAGmt_pI; Vitreorana_sp_1bMGLM_AAGmt_pII; Vitreorana_sp_1cMGLM_AAGmt_pII; voucher specimen under the label MZUFV 10735 or MZUFV 10736). Advertisement calls were digitized at a sampling rate of 44,000 Hz and a sample size of 16 bits. We measured the following parameters: (1) note duration (s); (2) dominant frequency (Hz); (3) number of pulses per note; and (4) pulse rate. Temporal parameters were measured directly from the waveform and frequency parameters were measured directly from the audiospectrogram (with window function Hann, amplitude logarithmic, window size 256 samples, and overlap 99%). Additionally, we compared the call of the new species with recordings of *V. eurygnatha* and *V. uranoscopa* from Boraceia (Heyer et al. 1990). We downloaded these recordings and digitized them at a sampling rate of 14,000 Hz and a sample size of 16 bits. Sound analyses and graphs were made with Raven Pro v1.4 (Charif et al. 2010). Terminology follows Duellman and Trueb (1986). Mean values are reported ±1 SD.

SPECIES DESCRIPTION

Vitreorana ***franciscana*** sp. nov.
(Figs. 1–3; Table 2)

Centronella sp. Haddad, Andrade and Cardoso 1988:11.

Holotype.—MNRJ 81776, adult male collected by A.B. Barros and E. Teixeira, on 25 August 2009, near base of Casca D'Anta waterfall (20°18′05′′S, 46°31′19′′W, 850 m above sea level [m a.s.l.]; datum = SAD69), São Francisco River, Serra da Canastra National Park, Vargem Bonita, State of Minas Gerais in southeastern Brazil.

Paratopotypes.—Ten males: MNRJ 81779 collected on 30 January 2010 by A.B. Barros, V.J. Germano, and D. Gennari; MZUFV 9968, 9970 collected on 25 August 2009 by A.B. Barros and E. Teixeira; MZUFV 9978–79 and MNRJ 81778 collected on 22 September 2009 by A.B. Barros, A.P. Motta, and C.D. Paula; MZUFV 9982 collected on 24 September 2009 by A.B. Barros, A.P. Motta, and C.D. Paula; MZUFV 10046–47 and MNRJ 81776–77 collected on 17 October 2009 by A.B. Barros, L.R. Sueiro, and C.D. Paula; MZUFV 10323 collected on 03 December 2009 by A.B. Barros, T.H. Condez, F.C. Centeno, and C.D. Paula.

Paratype.—MNRJ 78704, collected in 1998, collector not designated, Fazenda Veredas, Presidente Olegário (18°24′S,

TABLE 2.—Mean values (±1 SD; in mm) of measurements of the holotype and 10 males of *Vitreorana franciscana* sp. nov.

Character[A]	Mean ± SD	Range
SVL	23.1 ± 0.7	21.9–24.1
HW	7.9 ± 0.3	7.4–8.2
HL	8.5 ± 0.4	7.9–8.9
ED	3.0 ± 0.2	2.8–3.4
IOD	3.5 ± 0.4	2.9–4.2
EN	2 ± 0.1	1.7–2.1
IN	2.1 ± 0.1	1.8–2.3
TD	1.1 ± 0.1	1.0–1.3
3DW	1.4 ± 0.1	1.2–1.6
HL	7.8 ± 0.1	6.7–8.7
THL	12.5 ± 0.1	11.3–13.1
TL	12.9 ± 0.1	12.0–13.5
FL	17.1 ± 0.6	16.1–18.2

[A] SVL = snout–vent length; HW = head width; HL = head length; ED = horizontal eye diameter; IOD = interorbital distance; EN = eye–nostril distance; IN = internarial distance; TD = horizontal tympanum diameter; 3DW = width of third finger disc; HL = hand length; THL = thigh length; TL = tibia length; FL = foot length.

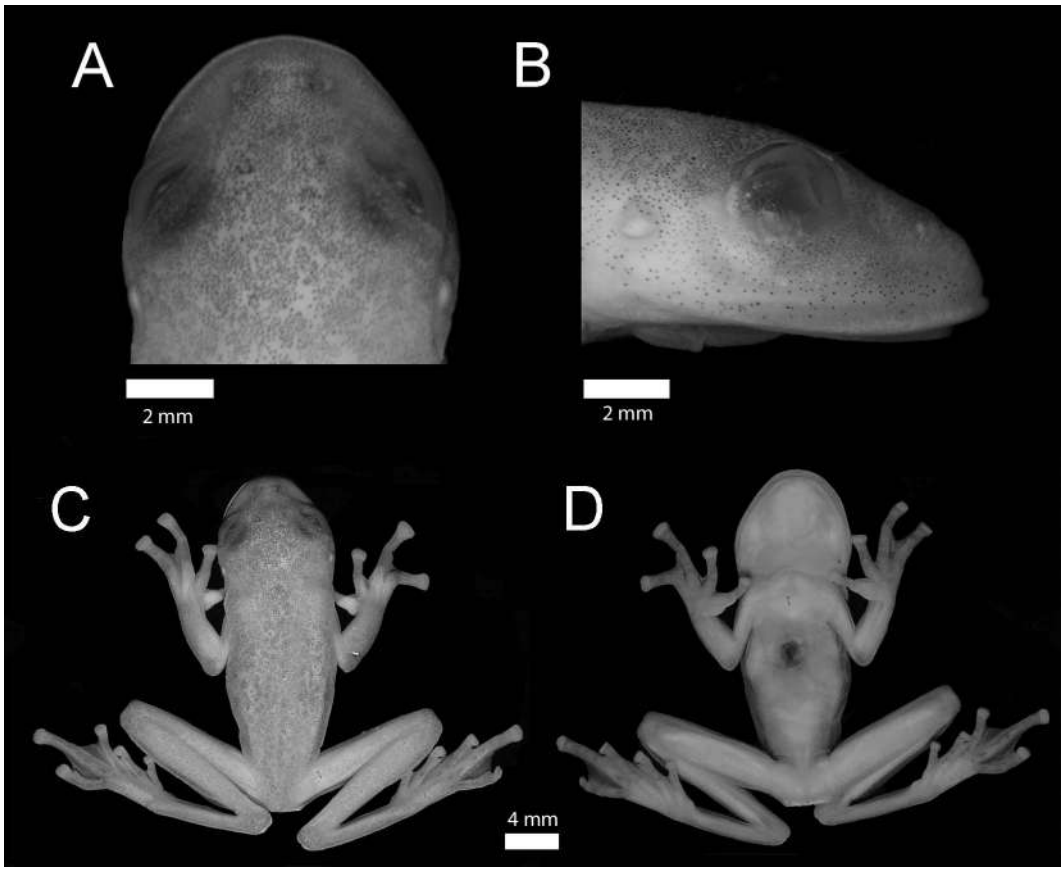

FIG. 1.—*Vitreorana franciscana* sp. nov. holotype (MNRJ 81776, SVL 24.0 mm). Dorsal (A) and lateral (B) views of the head; dorsal (C) and ventral (D) views of the specimen.

46°25′W, 900 m a.s.l.; datum = WGS84), State of Minas Gerais.

Generic placement.—The analysis of nuclear gene POMC recovered *Vitreorana franciscana* sp. nov. as being embedded within *Vitreorana* (Fig. 4) with a high value of posterior probability (0.98). Our tree topology provides evidence that *V. franciscana* sp. nov. belongs in the *Vitreorana* clade but does not resolve relationships within *Vitreorana* species. In addition, species of *Vitreorana* are diagnosed by the presence of a white peritoneum covering the liver, green bones visible by transparency in life, and peritoneum covering the urinary bladder with a layer of iridophores (Guayasamin et al. 2009). The new species presents all the aforementioned characteristics, which reinforces its placement in the genus *Vitreorana* (sensu Guayasamin et al. 2009).

Diagnosis.—*Vitreorana franciscana* sp. nov. is diagnosed by the following combination of characters: (1) absence of vomerine teeth; (2) snout subovoid in dorsal view and sloping in profile; (3) tympanum rounded, evident, directed upwards, and white in life and preservative, with supratympanic fold poorly developed around the upper portion of the tympanum to the shoulder; (4) dorsal skin texture shagreened; (5) cloacal ornamentation consisting of a flap above cloaca and an aggregation of small, thickened, enameled tubercles which are more pronounced in each side of cloacal opening, forming a slight flap in ventral view; (6) parietal peritoneum translucent (P0 condition), peritonea white on heart, liver, urinary bladder, kidneys, and testes (V4 condition); (7) bulbous liver (H2 condition); (8) adult males lacking humeral spine; (9) webbing formula between Fingers III (1^{+}–2^{-}) (2–2^{-}) IV; (10) webbing formula on Toes I (1–1^{+})–(2^{-}–2^{+}), II (1–1^{+})–(2–2^{-}), III (1–1^{+})–(1^{+}–2), IV (1^{-}–2)–(1–$1)^{+}$ V; (11) outer tarsal and ulnar ridges low, composed of small, thickened, enameled tubercles; (12) nuptial excrescences consisting of densely packed glands on dorsomedial surface of thumb, forming a defined pad (Type I); (13) Finger I longer than II; (14) eye diameter larger than width of disc on Finger III; (15) in life, dorsum leaf green reticulated with yellowish shadows; (16) in preservative, background coloration cream, star-shaped, and minute melanophores clustered in arrangement, giving a reticulated aspect; (17) in life, iris tan with encircling black reticulations and in preservative silvery white with black encircling reticulations; (18) hands and feet yellowish, melanophores usually present in dorsal portion of Finger IV and Toes IV and V; (19) translucent gastrointestinal peritoneum; (20) males call from dorsal surface of leaves in riverine understory of permanent rivulets and creeks, advertisement call composed of 6–8 multipulsed notes (7 ± 1.4), call duration of 2.9–3.5 s (3.2 ± 0.4 s), each note is composed of 3–6 pulses (4.2 ± 0.7), dominant frequency ranges from 4136.6–4490 Hz (4333.6 ± 83.8 Hz), and pulses per second from 44.9–81.6 (61 ± 10.9); (21) eggs deposited on dorsal surface of leaves hanging over water; and (22) small to medium size males, SVL 21.9–24.1 mm (23.1 ± 0.7 mm).

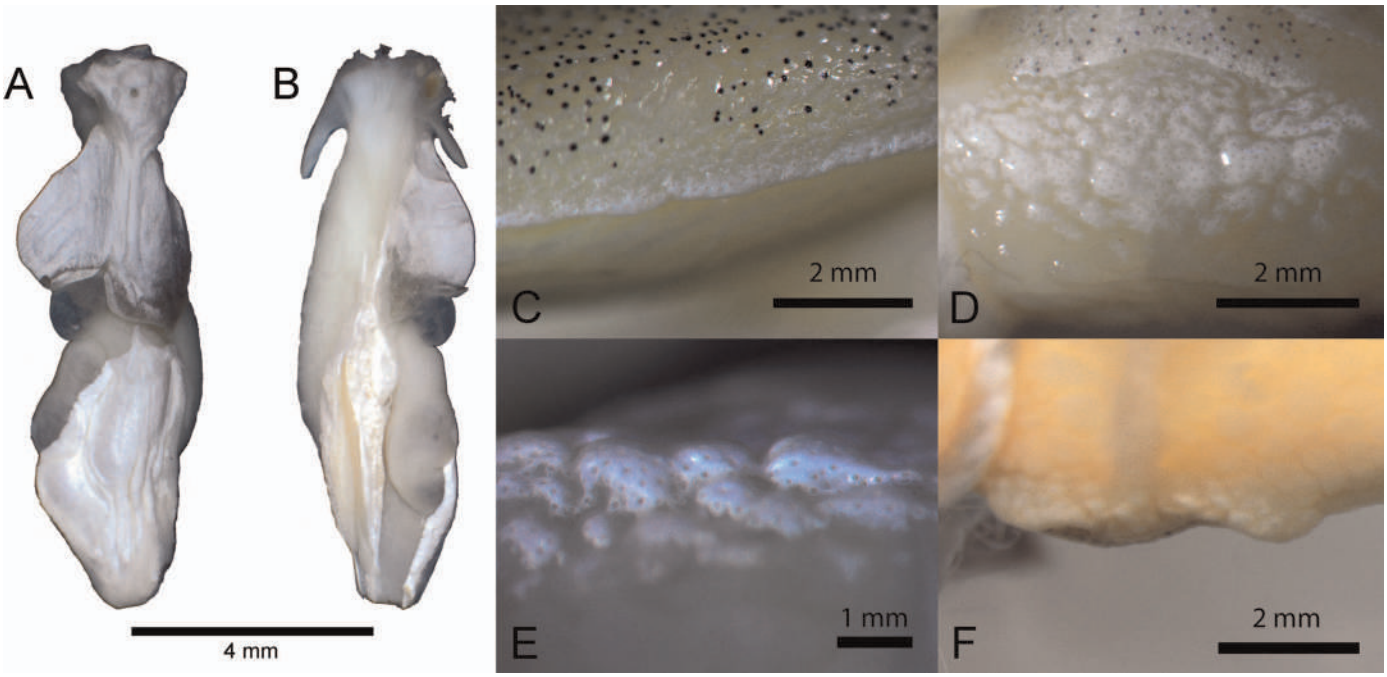

FIG. 2.—*Vitreorana franciscana* sp. nov. paratopotype (MNRJ 81779). Dorsal (A) and ventral (B) views of the viscera; ulnar ridge (C), cloaca (D), close-up view of cloacal tubercles (E), and ventral view of the cloacal ornamentation (F) of *V. franciscana* paratopotype (MNRJ 81776). Dorsal view is to the top in C, D, and E.

Comparisons with other species.—In addition to aforementioned traits, *Vitreorana franciscana* sp. nov. is distinguished from *V. antisthenesi*, *V. castroviejoi*, *V. gorzulae*, *V. helenae*, and *V. oyampiensis* by having translucent visceral peritoneum along the gastrointestinal tract (vs. white peritoneum).

Vitreorana franciscana sp. nov. is distinguished from *V. baliomma* by having snout subovoid in dorsal view and sloping in profile (vs. snout truncate in dorsal view, rounded in profile); dorsal skin texture shagreen (vs. dorsal skin texture smooth); peritonea white on heart, liver, urinary bladder, kidneys, and testes (V4 condition; vs. iridophores covering peritonea of heart, liver, gall bladder, and gastrointestinal tract [V5 condition]); in life, dorsum leaf green reticulated with yellowish shadows (vs. minute white punctuations along dorsum of *V. baliomma*).

Vitreorana franciscana sp. nov. differs from *V. eurygnatha* by having a subovoid snout in dorsal view and sloping in profile (vs. snout truncate in dorsal view and truncate or rounded in profile); star-shaped melanophores clustered on the dorsum (vs. dotted or star-shaped melanophores distributed uniformly on the dorsum); tympanum rounded, directed upwards, and white (vs. tympanum indistinguishable through the skin, annulus evident, rounded, and nonpigmented); cloacal ornamentation consisting of an aggregation of thickened, enameled tubercles forming two prominences on each side of anal opening, giving the appearance of a slight flap (vs. cloacal region composed of rounded tubercles, all similar in size and poorly pigmented); and dorsal skin texture shagreen (vs. smooth).

The new species is distinguishable from *V. parvula* by the presence of two enameled warts on each side of the cloacal opening (vs. cloacal region with granular seat patch); subovoid snout in dorsal view (vs. semicircular or nearly rounded); and snout sloping in profile (vs. snout slightly truncate in profile, but probably an artifact of preservation, likely rounded in profile [Heyer 1985]).

Vitreorana franciscana sp. nov. differs from *V. uranoscopa* by having outer tarsal, ulnar, and foot ridges low and composed of small, thickened, enameled tubercles (vs. well developed and crenulated outer tarsal, ulnar, and foot ridges); upper lip ridge absent (vs. upper lip ridge crenulated and enameled); in life, iris tan with black encircling reticulations (vs. iris silver with black encircling reticulations); and cloacal ornamentation composed of an aggregation of small, thickened, enameled tubercles with two slight flaps in each side of cloacal opening (vs. two fleshy flaps in each side of cloacal opening surrounded by small enameled tubercles).

Vitreorana franciscana sp. nov. is also distinguishable from the aforementioned species on the basis of its advertisement call (Table 3).

Description of the holotype.—Adult male, SVL 24.0 mm. Body slender; head moderately distinct, somewhat longer than wide; HL 34.1% and HW 34.2% of SVL; snout subovoid in dorsal view and sloping in profile; nostrils rounded and protuberant; loreal region concave; upper lips pigmented by white and distinct; moderate sized eyes, ED = 34.4% of HW, not prominent, directed anterolaterally at an angle of 45°; ED narrower than IOD; supertympanic fold hardly visible, extending from the orbit region to shoulder; tympanum externally visible, rounded, directed upwards, and enameled; TD = 37.9% of ED; vomerine teeth absent; choanae medium-sized, rounded; vocal slits extending laterally from the lateral edge of the tongue to the angle of jaws; tongue smooth and ovoid; single median subgular vocal sac.

Humeral spine not visible externally, arm slender; forearm slightly thicker with minute and star shaped melanophores in dorsal portion reaching Finger IV; ulnar ridge poorly

FIG. 3.—Dorsal (upper panel) and ventral (lower panel) views of a topotype for *Vitreorana franciscana* sp. nov., in life (specimen not collected). Photo by T.H. Condez.

developed, noncrenulated, and enameled; relative finger lengths I <II <IV <III; discs expanded, truncate on the tip; 3DW = 6.3% of SVL, membrane formula on Hand I Trace II 2 Trace III 2^{-} 2 IV. Palmar tubercle kidney-shape; thenar tubercle absent; subarticular tubercles simple, round, small; few palmar supernumerary tubercles poorly noticeable; nuptial excrescence consisting of densely packed nonkeratinized glands, forming a defined pad, pale colored, extending from ventrolateral base to dorsal surface of Finger I, covering its proximal half (Type I); prepollex concealed.

Slender hind limbs, FL = 75.6% and TL = 54.2% of SVL; outer tarsal ridges noncrenulated, poorly developed, highlighted by white; relative toe length I > II > III ≈ IV > V; adhesive discs expanded, truncated on the tip; webbing formula of Toes I (1^{+}–2^{+}), II (1–2), III (1^{+}–2), IV (2–1^{+}) V; outer metatarsal tubercle absent; inner metatarsal tubercle present, elliptical, near the base of Toe I; subarticular tubercles simple, rounded; supernumerary tubercles slightly visible on plantar surface. Cloacal decoration composed of an aggregation of thickened, small tubercles around, and a pair of pronounced warts in each side of, cloacal opening, all highlighted by white pigment.

Dorsal surfaces of head, body, and limbs, and lateral of head shagreen; throat and ventral surface of upper limbs smooth; belly and ventral surface of the hind limbs slightly granular with small and round granules, all of similar size.

Measurements of the holotype (mm).—SVL 24.0; HW 8.2; HL 8.7; ED 2.9; IOD 4.3; EN 2.1; IN 2.3; TD 1.1; 3DW 1.5; HL 8.6; THL 13.0; TL 13.1; FL 18.2.

Color of the holotype in preservative.—Dorsal background cream, surfaces of body, head, and limbs greyish with star-shaped and minute melanophores clustered in arrangement, giving a reticulated pattern. Few and sparse white dots distributed on dorsal surfaces and small spicules in dorsal surfaces, forearms, and loreal region visible under magnification. Ventral portion and flanks cream, immaculate. In freshly preserved specimens outer tarsal, ulnar, and upper lip highlighted by white pigment. Iris background silvery gray with black encircling reticulations. Parietal peritoneum translucent, visceral peritonea covered by iridophores in the pericardium, liver, and urogenital tract (kidneys, urinary bladder, and tests); peritonea of stomach and intestines translucent.

Color in life.—Dorsal surfaces light green with fine yellowish reticulations; white dots dispersed across dorsal surfaces; flanks, hands, and feet yellowish-green. Venter light green, belly translucent, allowing the visualization of internal organs (heart, liver, and urinary bladder) covered by a thin layer of white iridophores. Iris background tan, with concentric black reticulations.

Variation.—Examined specimens are congruent with the diagnostic characters and color of the holotype (Table 3).

Advertisement call.—We recorded two distinct calls (Fig. 5A) for *Vitreorana franciscana*, named Call "A" (possibly an advertisement call) and Call "B" (possibly a territorial call). Call A (Fig. 5B,C) is emitted more frequently and consists of one, multipulsed note in which each note duration ranges from 0.05–0.07 s (0.06 ± 0.01 s) and the dominant frequency ranges from 4306.2–4824.4 Hz (4561.2 ± 258 Hz), with one harmonic band visible (~8500 Hz); frequency modulation was not observed. The number of pulses per note varied from 3–6 pulses (4.2 ± 0.7) and the pulse rate per second from 40.5–113.2 pulses (85.2 ± 24.7; Table 3).

Call B (Fig. 5D,E) is emitted sporadically, usually in-between expressions of Call A. This type of call was emitted when other males were calling nearby. This call is formed by a long, multipulsed sequence of 6–8 notes (7 ± 1.4), duration of 2.9–3.5 s (3.2 ± 0.4 s). Each note was composed of 3–6 pulses (4.2 ± 0.7). The dominant frequency ranged from 4136.6–4490 Hz (4333.6 ± 83.8 Hz; no frequency of modulation was observed) and pulse rate per second from 44.9–81.6 pulses (61 ± 10.9; Table 2).

Etymology.—The São Francisco River is one of the most important rivers in Brazil. This river crosses 2863 km of the Brazilian territory passing through the states of Minas Gerais, Bahia, Pernambuco, Alagoas, and Sergipe. The headwaters of this river are located in Medeiros, Serra da Canastra. The specific epithet "*franciscana*" is a noun in apposition referring to the São Francisco River, because the species habitat is in the São Francisco and its tributaries.

Geographic distribution.—The type locality is near the Casca D'Anta waterfall at Serra da Canastra National Park, Vargem Bonita, and Fazenda Vereda, Presidente Olegário. Both localities are in the Cerrado domain in State of Minas Gerais, Brazil (Fig. 6A). However, this species potentially occurs in Semidecidual Forest in contact zones with Atlantic Forest.

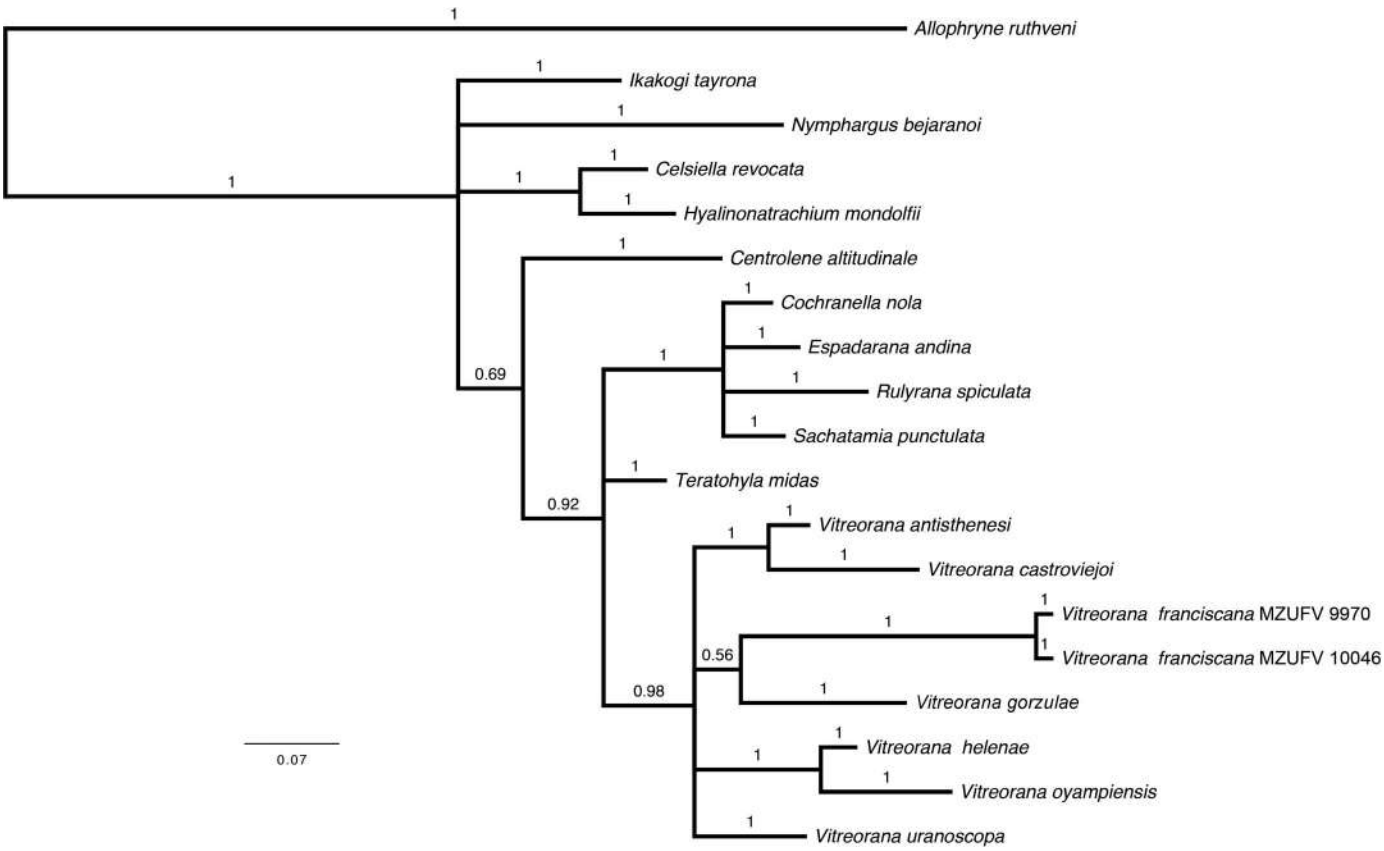

FIG. 4.—Phylogenetic relationship among Centrolenidae genera and *Vitreorana* species, inferred through a Bayesian analysis, based on the proopiomelanocortin (POMC) gene. Values above branches indicate posterior probabilities. Scale bar represents number of substitutions per site.

Environment and habitat.—The Serra da Canastra National Park is a large (71,525 ha), protected remnant of Cerrado vegetation. Despite being largely an open landscape, the Cerrado biome also contains some wet grasslands (*campo úmido*) and riparian forests (*mata de galeria*). These communities are either associated with riparian zones or isolated patches of mesophytic forests. The region near the Casca D'Anta waterfall (Fig. 6D) has a fragment of dense forest with epiphytic flora, containing many permanent streams with rocky and sandy riverbeds.

Males of *Vitreorana franciscana* were observed calling soon after sunset (1800 h) and ceased this activity around 0200 h. During field expeditions, about 25 individuals were observed per night at heights from 0.5 to 1.5 m above ground level. During the peak of the rainy season (August–December), males called from perches of shrubby vegetation on dorsal surfaces of leaves, 0.5–2.0 m distant from a watercourse. Egg clutches (Fig. 6C,D) were laid on dorsal surfaces of leaves hanging over the watercourse (1–1.5 m high). We found five egg clutches, from January to March, containing 42 to 53 embryos.

Following International Union for Conservation of Nature (IUCN) criteria, we suggest that *Vitreorana franciscana* should be assigned the category of "Data Deficient" (DD) until new studies could assess information on its ecology, biology, and geographic distribution.

Discussion

Glassfrogs of the genus *Vitreorana* from Southeastern Brazil are associated with forested streams along the Atlantic Rain Forest (Heyer 1985; Heyer et al. 1990; Machado et al. 2010). In recent decades, however, Glassfrogs have been reported from riparian forests and rocky fields in the Cerrado biome (Haddad et al. 1988; Eterovick and Sazima 2004). Maxson and Heyer (1982) suggested that the colonization of rocky fields by anurans from the Atlantic Forest domain was relictual and dated to the Late Oligocene.

TABLE 3.—Values for parameters of advertisement calls of *Vitreorana* species from Southeastern Brazil. Temperatures in parentheses are those at the time the calls were recorded.

	Vitreorana eurygnatha (18.6°C)		*Vitreorana uranoscopa* (15.5°C)		*Vitreorana franciscana*[A] (19.8°C)		*Vitreorana franciscana*[B] (20.8°C)	
Parameter	$\bar{X}$ ± SD (n = 7)	Range	$\bar{X}$ ± SD (n = 6)	Range	$\bar{X}$ ± SD (n = 15)	Range	$\bar{X}$ ± SD (n = 3)	Range
D. Frequency (Hz)	4897.2 ± 92.1	4823.4–4995.7	4478.9	—	4561.15 ± 257.99	4306.15–4824.36	4333.64 ± 83.8	4136.60–4489.95
Note duration (s)	0.158 ± 0.014	0.138–0.175	0.031 ± 0.003	0.027–0.034	0.06 ± 0.01	0.05–0.07	3.19 ± 0.41	2.90–3.48
Number of pulses	4.9 ± 0.7	4–6	2	—	4.73 ± 1.28	3–6	4.18 ± 0.73	3–6
Pulse rate (pulse/s)	30.6 ± 2.8	28.0–35.0	66.1 ± 6.5	58.8–74.1	85.19 ± 24.67	40.54–113.21	60.98 ± 10.93	44.94–81.63

[A] Call "A."
[B] Call "B."

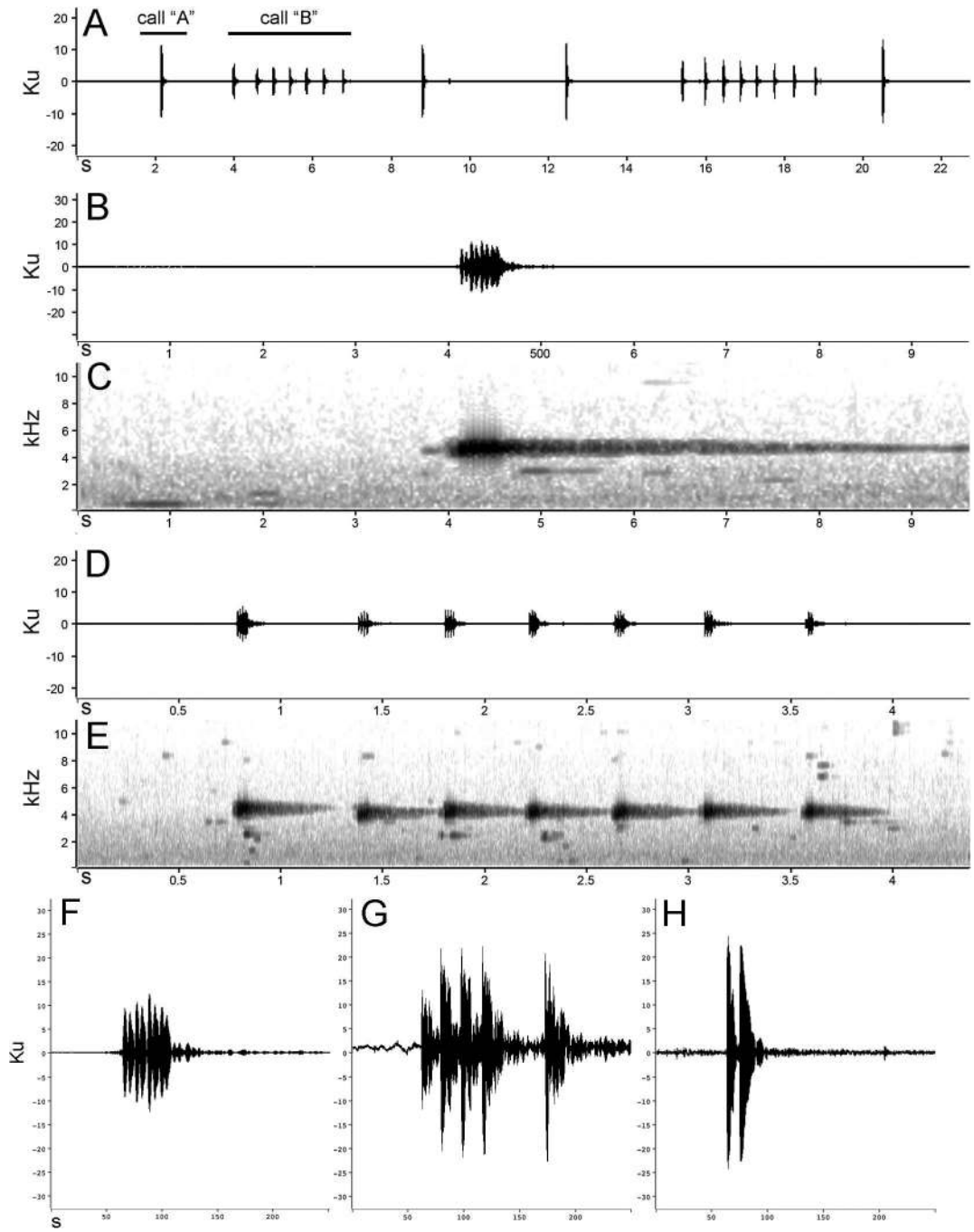

FIG. 5.—*Vitreorana franciscana* sp. nov: (A) audiospectrogram of a call series with "A" and "B" calls emitted by a single male; (B) oscillogram and (C) audiospectrogram of the "A" call; (D) oscillogram and (E) audiospectrogram of the "B" call. Calls recorded around 2100 h at Casca D'Anta waterfall, Municipality of Vargem Bonita, state of Minas Gerais (air temperature = 19.8°C). Oscillogram of a single advertisement call of (F) *V. franciscana* sp. nov. (G) *V. eurygnatha*, and (H) *V. uranoscopa*.

This geological period is characterized by higher humidity when Atlantic Forest areas expanded into the continent. In drier periods later, such forested habitats become restricted to areas around water surrounded by open vegetation. Such a successional process could have isolated populations and reduced gene flow, allowing speciation.

Shared morphological and meristic features indicate that *Vitreorana franciscana* is more similar to *V. uranoscopa* than to other taxa. Based on molecular data, the new species appears allied with *V. gorzulae*. The relationship among these species is weakly supported, however, having low values of posterior probability (pp < 0.42). Additional molecular data for other loci and taxon sampling are needed to correctly infer the relationships among these species. Also, the relationships within the clade composed of *V. castroviejoi* and *V. uranoscopa* with *V. gorzulae* and *V. franciscana* are unresolved. However, even the most-comprehensive Glassfrog phylogenies (Guayasamin et al. 2008, 2009) have several nodes with low support within genera. Despite that, the relationship among genera has a high posterior probability

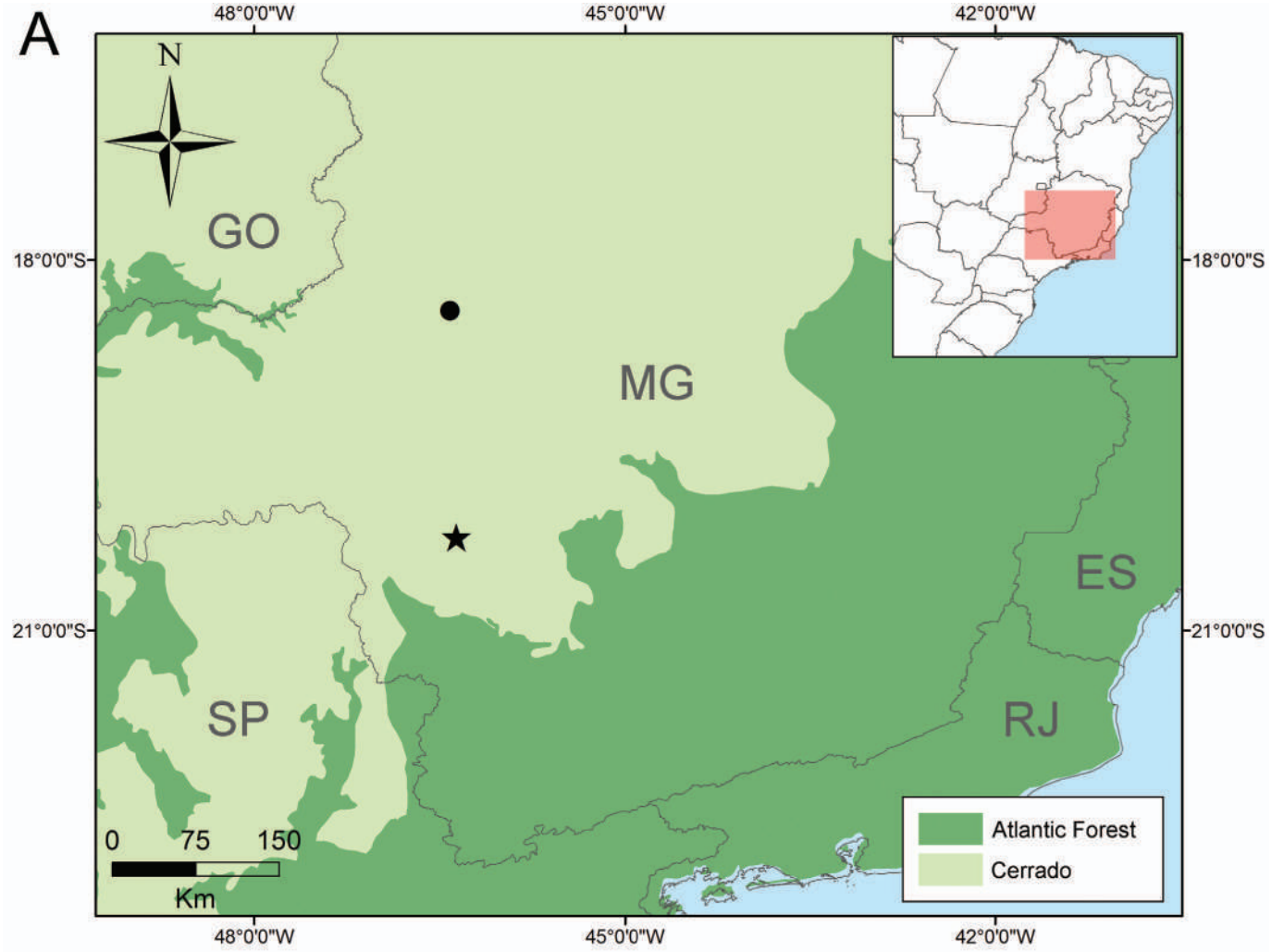

FIG. 6.—(A) Geographic distribution map of *Vitreorana franciscana* sp. nov. Legend: Black star = type locality, within Municipality of Vargem Bonita; black circle = record for Municipality of Presidente Olegário. (B) Stream where *V. franciscana* sp. nov. can be found at Parque Nacional da Serra da Canastra. (C, D) Egg clutches of *V. franciscana* sp. nov. Photos by A.B. Barros.

(pp = 1.00) in our Bayesian topology, suggesting the correct placement of the new species in the genus *Vitreorana*.

Note the call duration of *Vitreorana franciscana* (0.048–0.074 s) is much shorter than that of *V. eurygnatha* (0.138–0.175 s), and the pulse rate in *V. franciscana* (40.5–113.2 pulse/s) is higher than in *V. eurygnatha* (28.0–35.9 pulse/s). Additionally, the pattern of the call in *V. eurygnatha* (Fig. 5F) readily differs from *V. franciscana* (Fig. 5G). *Vitreorana eurygnatha* emitted a call formed by a set of concatenated pulses (usually 3–5 pulses) with the power concentrated in the last pulse. Note duration of *V. franciscana* (0.048–0.074 s) is longer than that of *V. uranoscopa* (0.027–0.034 s; Fig. 5H), and the number of pulses in *V. franciscana* (3–6 pulses) is greater than in *V. uranoscopa* (2 pulses). As far as we know, *V. parvula* is only known from the lectotype (Heyer 1985) and its advertisement call remains unknown.

Since the studies of Taylor and Cochran (1953) and Heyer (1978, 1985), no other work has been conducted about the taxonomy and distribution of Glassfrogs in the Atlantic Forest domain. Those studies were based on a few specimens from Southeastern and Southern Brazilian Atlantic Forest and cover only a portion of their present-day geographic distribution. The description of *Vitreorana franciscana* demonstrates the lack of knowledge on taxonomy of centrolenids from eastern Brazil and confirms that a re-examination is long overdue.

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Appendix
Specimens Examined

Vitreorana baliomma.—BRAZIL: State of Bahia: MNRJ 45857 (Mata de Cabruca, Fazenda Novo Pau Brasil, Municipality of Itamaraju, holotype); MNRJ 35557 (Fazenda Palmeiras, Municipality of Itapebi paratype). State of Sergipe: MNRJ 37904, 37905 (Parque Nacional Serra de Itabaiana, Municipality of Areia Branca, paratypes).

Vitreorana eurygnatha.—BRAZIL: State of Minas Gerais: MNRJ 73421 (Brejo da Lapa, PARNA Itatiaia, Municipality of Itamonte); MZUSP 53178–53180 (Brejo da Lapa, PARNA Itatiaia, Municipality of Itamonte); MZUFV 4590 (Parque Nacional da Serra da Canastra, municipality of São Roque de Minas); MZUFV 7530–7532, 8208 (Parque Estadual da Serra do Brigadeiro, Municipality of Araponga); MZUFV 8074, 8076 (Parque Estadual da Serra do Papagaio, Municipality of Alagoa); MZUFV 8231–8233, 8411 (Parque Estadual da Serra do Papagaio, Municipality of Airuoca). State of São Paulo: MNRJ 73167–85 (Fazenda do Bonito, Parque Nacional da Serra da Bocaina, Municipality of São José do Barreiro–topotypes); MNRJ 73167–73185 (Fazenda do Bonito, PARNA Serra da Bocaina–topotypes); MZUSP 105224–105231 (Campo de Fruticultura, Parque Nacional da Serra da Bocaina, Municipality of São José do Barreiro); MZUSP 105399–402 (Fazenda Lagoinha da Serra, Municipality of Campos do Jordão); MNRJ 17649 (Fazendinha São Luís Mattedi, Municipality of Ribeirão Branco); MNRJ 34758 (Estação Biológica de Paranapiacaba, Municipality of Santo André); MZUSP 53052–59 (Fazenda do Veado, Municipality of Bananal); MZUSP 15153–80 (Cidade Azul, Municipality of Joanópolis). State of Rio de Janeiro: MNRJ 73128–73134 (Parque Nacional da Tijuca, Tijuca, Municipality of Rio de Janeiro); MZUSP 53350–57 (Municipality of Teresópolis); MNRJ 73119–73126 (Planalto do Itatiaia, PARNA Itatiaia, Municipality of Itatiaia).

Vitreorana parvula.—BRAZIL: State of Santa Catarina: BMNH 1947.2.12.67 (lectotype; Municipality of Lages).

Vitreorana uranoscopa.—BRAZIL: State of Santa Catarina: MZUSP 35429–36 (Municipality of Novo Horizonte); CFBH 5280–5282, 5284 (Bairro Lençol, Municipality of São Bento do Sul). State of Minas Gerais: MNRJ 77778–80 (Ponte para o Belchior, Reserva Particular do Patrimonio Natural da Serra do Caraça, Municipality of Catas Altas); MZUSP 145472 (Municipality of Conceição do Mato Dentro); MNRJ 23125 (Mata da Mutuca, Municipality of Nova Lima); MNRJ 24688 (Estação Biológica Mata do Sossego, Municipality of Simonésia); MNRJ 30606–07 (Serrinha, Municipality of Mariana); MZUFV 2744, 6637–6638, 6804, 8278 (Parque Estadual da Serra do Brigadeiro, Municipality of Araponga); MZUFV 3945–3947 (Parque Estadual da Serra do Papagaio, Municipality of Airuoca); MZUFV 6613 (Parque Estadual do Ibitipoca, Municipality Lima Duarte). State of São Paulo: MZUSP 136535–37 (Parque Estadual Carlos Botelho, Municipality of São Miguel Arcanjo); MNRJ 32510–11 (Estrada de Botucatu, Municipality of Botucatu; CFBH 6318–19 PETAR, Núcleo Ouro Grosso, Municipality of Iporanga). State of Rio de Janeiro: MNRJ 78040–43 (Lídice, Municipality of Rio Claro); MNRJ 59546–48 (APA Mantiqueira, Municipality of Resende), MNRJ 77352–62 (Represa Mayrink, Parque Nacional da Tijuca, Tijuca, Municipality of Rio de Janeiro); MNRJ 79412–18 (Reserva Biológica do Tínguá, Municipality of Petrópolis).