

A comparative acoustic analysis of species of *Vitreorana* (Anura: Centrolenidae) from the Brazilian Atlantic Forest and Cerrado, with a description of the call of *V. baliomma* and insights into the taxonomic status of Cerrado populations

Davi Lee Bang,^{1,2,3} Barnagleison Silva Lisboa,^{4,5} Bernardo Franco da Veiga Teixeira,² Ariovaldo Antonio Giaretta,² and Thiago Ribeiro de Carvalho⁶

¹ Laboratório de Zoologia de Vertebrados, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (USP). Av. Bandeirantes, 3900, 14040-901, Ribeirão Preto, SP, Brazil. E-mail: davileebang@yahoo.com.br; davileebang@usp.br.

² Laboratório de Taxonomia, Sistemática e Evolução de Anuros Neotropicais, Instituto de Ciências Naturais do Pontal, Universidade Federal de Uberlândia (UFU). Rua 20, 1600, 38304-402, Ituiutaba, MG, Brazil.

³ Programa de Pós-Graduação em Biologia Comparada, Departamento de Biologia/FFCLRP, Universidade de São Paulo (USP). Av. Bandeirantes, 3900, 14040-901, Ribeirão Preto, SP, Brazil.

⁴ Setor de Zoologia, Museu de História Natural, Universidade Federal de Alagoas (UFAL). Praça Afrânio Jorge s/n, 57010-020, Maceió, AL, Brazil.

⁵ Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Centro de Biociências, Universidade Federal de Pernambuco (UFPE). Av. Professor Moraes Rego, 1235, 50670-901, Recife, PE, Brazil.

⁶ Laboratório de Herpetologia, Departamento de Zoologia e Centro de Aquicultura, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP). Rio Claro, SP, Brazil.

Abstract

A comparative acoustic analysis of species of *Vitreorana* (Anura: Centrolenidae) from the Brazilian Atlantic Forest and Cerrado, with a description of the call of *V. baliomma* and insights into the taxonomic status of Cerrado populations. Advertisement calls are the primary signaling strategy of mating in most anurans. Examining major patterns within and across frog taxa may help to delimit species and understand acoustic communication systems. Herein, the vocalization of *Vitreorana baliomma* is described and new acoustic descriptions are provided for *V. franciscana* and *V. eurygnatha*. Calls of syntopic/sympatric or allopatric populations were compared. The vocalization of *V. baliomma* is composed of two call types—A and B, with Call A being the advertisement call. The vocalization of *V. franciscana* is composed of only one call type (Call A), emitted isolated or in series. The vocalization of *V. eurygnatha* has two types of calls; Call A is similar among Cerrado populations, whereas an Atlantic Forest population has a distinct Call A pattern. The calls of each of these species of the present study is pulsed; however,

Received 03 September 2019
Accepted 20 February 2020
Distributed June 2020

the calls differ in call duration, pulse organization, or pulse duration, and these differences seem to reflect the allopatric or sympatric/syntopic occurrences of populations. The unique call pattern of the Atlantic Forest population of *V. eurygnatha* may represent the advertisement call of the nominal species, and the distinct call of the Cerrado population may indicate cryptic diversity in the species.

Keywords: Advertisement call, character displacement, sympatry, syntopy, vocal repertoire, *Vitreorana baliomma*, *Vitreorana eurygnatha*, *Vitreorana franciscana*.

Resumo

Uma análise acústica comparativa de espécies de *Vitreorana* (Anura: Centrolenidae) da Mata Atlântica e do Cerrado brasileiros, com a descrição do canto de *V. baliomma* e considerações sobre o status taxonômico de populações do Cerrado. Cantos de anúncio representam a forma primária de sinalização para reprodução na maioria dos anuros. Avaliar os principais padrões dentro e entre táxons de anuros pode auxiliar na delimitação de espécies e no entendimento de sistemas de comunicação acústica. Nesse trabalho, a vocalização de *Vitreorana baliomma* é descrita e novas descrições acústicas são fornecidas para *V. franciscana* e *V. eurygnatha*. Cantos entre populações em sintopia/simpatria ou alopatria foram comparados. A vocalização de *V. baliomma* é composta de dois tipos de cantos—A e B, e o Canto A considerado como o canto de anúncio. A vocalização de *V. franciscana* é composta por um único tipo de canto (Canto A), que é emitido isoladamente ou em séries. A vocalização de *V. eurygnatha* possui dois tipos de cantos; o Canto A é similar entre populações do Cerrado, enquanto que uma população da Mata Atlântica possui o Canto A com um padrão distinto. Os cantos de cada espécie do presente trabalho são pulsados; no entanto, os cantos diferem em duração do canto, organização dos pulsos, ou duração dos mesmos, e essas diferenças parecem refletir a condição de alopatria ou simpatria/sintopia entre populações. O padrão único observado para a população da Mata Atlântica de *V. eurygnatha* provavelmente representa o canto de anúncio da espécie nominal, e o canto distinto da população do Cerrado pode indicar diversidade críptica na espécie.

Palavras-chave: canto de anúncio, deslocamento de caráter, repertório vocal, simpatria, sintopia, *Vitreorana baliomma*, *Vitreorana eurygnatha*, *Vitreorana franciscana*.

Introduction

Advertisement calls are the primary form of communication in most anuran species. These calls are often conspicuous, stereotyped signals produced by reproductively active male frogs (Wells 2007). Female frogs react to specific call traits of conspecific males. Thus, advertisement calls can act as reproductive barriers between species (Blair 1958, 1964), and can have immediate effect on acoustic signal divergence (Boul *et al.* 2007). Vocalization traits can be used for species delimitations (Köhler *et al.* 2017), particularly when interspecific call differences emerge among frogs sharing

overlapping vocalization sites (i.e., syntopic or sympatric occurrence; Höbel and Gerhardt 2003, Hoskin *et al.* 2005, Jansen *et al.* 2016), or among morphologically cryptic species that have different acoustic traits (Angulo and Reichle 2008, Carvalho *et al.* 2019). Therefore, speciation patterns can be informed by acoustic descriptions that may reveal processes underlying the evolution of mating signals.

Although patterns of advertisement call evolution in centrolenid frogs have been evaluated at a macroevolutionary scale (Escalona *et al.* 2019), calls of some species of *Vitreorana* (Guayasamin *et al.* 2009) have not been described—viz., *V. baliomma* Pontes, Cara-

maschi, and Pombal, 2014 and *V. parvula* (Boulenger, 1895). The calls of about 80% of centrolenids are either unknown or lack detailed descriptions (Dautel *et al.* 2011). The lack of acoustic data impedes our understanding of the systematics of Glass Frogs such as *Vitreorana* (Castroviejo-Fisher *et al.* 2011), and hinders assessments of intraspecific variation.

We predict that Brazilian species of *Vitreorana* have different advertisement calls because vocalizations usually are species specific in anurans, especially in taxa that co-occur (syntopic species) in the Atlantic Forest and Cerrado. Further, we expect that different populations of a same species will share a basic acoustic pattern, apart from variations related to environmental factors (e.g., temperature) and morphological constraints (e.g., body size). Herein, we describe the advertisement call of *V. baliomma*, and provide new call descriptions for *V. eurygnatha* (Lutz, 1925) and *V. franciscana* Santana, Barros, Pontes, and Feio, 2015 from other regions/biomes. Our analysis enabled us to compare major acoustic patterns combined with morphological traits between species which show allopatric and sympatric / syntopic occurrences.

Materials and Methods

Sampling

The following species of *Vitreorana* were sampled from Brazilian localities, as follow. *Vitreorana baliomma*: Estação Ecológica de Murici (09°12'32" S, 35°51'37" W, 463 m a.s.l.; datum WGS84 in all cases) in Murici, state of Alagoas (AL). *Vitreorana eurygnatha* and *V. franciscana*, which occur syntopically at the following localities: Parque Nacional da Serra da Canastra (20°18'05" S, 46°31'19" W, 850 m a.s.l.) in Vargem Bonita, state of Minas Gerais (MG); and Córrego Borrachudo (19°19'40" S, 46°00'1" W, 1123 m a.s.l.) in São Gotardo (MG). *Vitreorana eurygnatha*: Reserva Biológica Augusto Ruschi (19°53'49" S, 40°32'30" W, 838

m a.s.l.) in Santa Teresa, state of Espírito Santo (ES).

Recordings and specimens of *Vitreorana franciscana* from Vargem Bonita were the same analyzed by Santana *et al.* (2015). Acoustic data for *V. uranoscopa* (Müller, 1924) were obtained from Haga *et al.* (2014) and Zaracho (2014). Appendix I contains detailed information on sound recordings, including the voucher specimens. Calls were recorded in the field with digital recorders (M-audio Microtrack® II and Marantz® PMD 660) and unidirectional microphones (Sennheiser® K6/ME67 and K6/ME66). The recorders were adjusted to sampling rates of 44.1 kHz or 48.0 kHz and bit depth of 16 points. Files were stored as uncompressed wave files. Sound files and specimens were deposited in the following Brazilian collections: Collection of Amphibians of the Universidade Federal de Uberlândia (AAG-UFU), Uberlândia, Minas Gerais state; Museu de Zoologia João Moojen (MZUFV) at Universidade Federal de Viçosa, Viçosa, Minas Gerais state; and Herpetological Collection of the Museu de História Natural (MUFAL) at Universidade Federal de Alagoas, Maceió, Alagoas state.

Species Identification

Specimens from Murici ($N = 8$ males) were identified as *Vitreorana baliomma* based on features from the original description (Pontes *et al.* 2014): snout–vent length (SVL) = 17.8–21.4 mm (mean = 19.4, SD = 1.1); vomerine teeth absent; snout truncate in dorsal view and rounded in lateral view; tympanum non-pigmented with outline barely visible and supratympanic fold poorly developed around the upper portion of the tympanum; dorsal skin texture smooth; poorly defined nuptial pad; Finger I longer than Finger II; in life, green dorsum with minute white punctuations; in preservative, dorsum cream with small and star-shaped melanophores distributed uniformly; and hands and feet yellowish with melanophores, when present, restricted to the dorsal portion of Finger IV and Toes IV and V.

Specimens of *Vitreorana eurygnatha* from Vargem Bonita ($N = 1$ male), São Gotardo ($N = 2$) and Santa Teresa ($N = 2$) were identified based on the following traits provided by Santana *et al.* (2015): SVL = 16.9–20.5 mm (mean = 18.5, SD = 1.4); snout truncate in dorsal and lateral views; dorsal skin texture smooth; rounded melanophores distributed uniformly on the dorsum; tympanum non-pigmented; and tubercles in the cloacal region rounded, with similar size, and poorly pigmented.

Specimens of *Vitreorana franciscana* from São Gotardo ($N = 2$ males) were identified on the basis of Santana *et al.* (2015): SVL = 21.3–21.5 mm (mean = 21.4, SD = 0.1); snout sub-ovoid in dorsal view; sloping in profile; dorsal skin texture shagreen; melanophores clustered on the dorsum; tympanum white; tubercles enameled, forming two prominences on each side of the cloaca; upper lip ridge of tubercles absent; ridges on the outer surfaces of forearms, shanks, and tarsi discrete; iris tan with black encircling reticulations.

Acoustic Analysis

The following settings in Raven Pro 1.5 (Center for Conservation Bioacoustics 2014) were used for the acoustic analysis: window type = Hann, window size = 256 samples, 3dB filter bandwidth = 270 Hz or 248 Hz, window overlap (locked) = 90%, hop size = 0.54 or 0.59 ms, DFT size = 1024 samples, grid spacing = 46.9 or 43.1 Hz. Sound figures were produced using seewave 1.7.6 (Sueur *et al.* 2008) and tune R 1.3.2 (Ligges *et al.* 2018), packages implemented in R 3.6.1 (R Development Core Team 2015), using the following parameters: window type = Hann, FFT overlap = 90%, FFT size = 256 points. Spectrograms were produced with a relative amplitude color scale of 36 dB (red = maximum amplitude).

The following acoustic traits were analyzed: call duration (time from onset to offset of vocal emission), number of pulses per call (amplitude modulations within call, both

complete and incomplete), pulse rate (quotient of number of pulses by each call duration), pulse duration (time comprised from onset to offset of amplitude modulations within calls), interval between pulses (duration of silent gaps between pulses), interval between calls (measured only when calls were emitted in sequence or when a different type of call was emitted shortly after main call type), and the call dominant frequency (point of greatest energy in advertisement call).

Two types of calls were recognized. Call A, the most frequently emitted signal, is the advertisement call. Call B differs from Call A in temporal structure, such as the envelope, or noticeable differences in duration, pulse number and their temporal organization. We did not use the Call B of *V. franciscana* described by Santana *et al.* (2015) because we noted that it seems to correspond to Call A of this study, although it was emitted as a calling bout with relatively lower amplitude (Results).

Results

Calls (both A and B) of all three *Vitreorana* species analyzed are pulsed. However, they differ in temporal patterns (e.g., call duration, pulse number, and repetition rates; Table 1). Calls are emitted in different patterns as follow: in most cases, (1) a single Call A (i.e., advertisement); (2) a single Call A followed by a sequence of the same call with shorter intervals; (3) a Call B (single or in sequence) shortly after a Call A. Quantitative descriptions of each species calls are presented below.

Vocalization of *Vitreorana baliomma*

The vocalization is composed of two different call types (Table 1; $N = 1$ male recorded from Murici, AL): Call A ($N = 13$ calls; Figure 1) usually followed by a Call B ($N = 10$ cases; $N = 10$ calls; Figure 1); however, Call A may also be emitted alone ($N = 3$ cases). Call A has complete amplitude modulations (always with 7 pulses) and increases slightly in amplitude along the

Table 1. Acoustic features of *Vitreorana baliomma*, *V. eurygnatha*, and *V. franciscana* (this study), and *V. uranoscopa* from Haga *et al.* (2014) and Zaracho (2014). Data are presented as means, standard deviations and ranges. N = number of calls analyzed. * = given as the average of all pulses in Haga *et al.* (2014) and Zaracho (2014). Abbreviations for Brazilian states: AL = Alagoas, ES = Espírito Santo, MG = Minas Gerais, SC = Santa Catarina.

Species/Call traits	Call type	Emission pattern	Call duration (ms)	Number of pulses	Pulse rate/s	1 st pulse duration (ms)	Last pulse duration (ms)	Interval between pulses (ms)	Dominant frequency (Hz)	Interval between calls A-B (ms)
<i>V. baliomma</i> (Murici, AL)	Call A (N = 13)	Single call	192 ± 4 (185–198)	7	36.5 ± 1 (35.3–37.8)	10 ± 2 (8–15)	12 ± 2 (9–14)	19 ± 2 (15–23)	4,979 ± 102 (4,694–5,081)	235 ± 10 (217–249)
	Call B (N = 10)	Single call	107 ± 5 (98–116)	4 ± 0.3 (4–5)	38.4 ± 2.3 (35.4–43.1)	12 ± 2 (9–14)	13 ± 4 (9–21)	18 ± 3 (6–21)	4,948 ± 96 (4,694–5,038)	-
<i>V. eurygnatha</i> (São Cotardo, MG)	Call A (N = 34)	Single call	168 ± 20 (135–226)	3 ± 0.4 (3–4)	19.7 ± 1.9 (15.9–24.4)	14 ± 3 (7–21)	15 ± 4 (8–23)	37 ± 8 (7–61)	4,796 ± 172 (4,453–5,016)	417 ± 100 (332–560)
	Call B (N = 7)	Single call or sequence of 2–3 call	188 ± 16 (154–199)	6 ± 0.8 (5–7)	30.5 ± 3.4 (25.8–36.5)	13 ± 3 (9–15)	12 ± 4 (8–20)	25 ± 12 (8–42)	4,566 ± 97 (4,500–4,781)	-
<i>V. eurygnatha</i> (Vargem Bonita, MG)	Call A (N = 10)	Single call	131 ± 5 (123–143)	3 ± 0.3 (3–4)	23.5 ± 1.7 (22.4–28.0)	10 ± 3 (5–15)	12 ± 2 (9–15)	43 ± 7 (15–49)	4,523 ± 39 (4,500–4,593)	-
<i>V. eurygnatha</i> (Santa Teresa, ES)	Call A (N = 6)	Single call with 2–3 pulse clusters	216 ± 25 (189–249)	18 ± 2.5 (16–22)	84.0 ± 4.5 (78.8–89.7)	4 ± 1 (4–5)	10 ± 5 (4–16)	-	4,830 ± 96 (4,694–4,995)	-
<i>V. franciscana</i> (São Cotardo, MG)	Call A (N = 59)	Single call or in sequence of 3–10 calls	52 ± 10 (33–73)	5 ± 9.0 (4–7)	100.9 ± 8.0 (84.5–121.2)	5 ± 2 (3–14)	9 ± 3 (4–15)	4 ± 1 (1–7)	4,605 ± 91 (4,359–4,734)	-
	Call A (N = 15)	Single call	56 ± 6 (48–69)	6 ± 0.6 (5–7)	102.5 ± 9.0 (90.9–125.0)	4 ± 1 (3–5)	5 ± 2 (3–8)	5 ± 1 (1–6)	4,103 ± 39 (4,031–4,171)	-
<i>V. uranoscopa</i> (Florianópolis, SC) (Haga <i>et al.</i> 2014)	Call A (N = 46)	Single call or in pairs	37 ± 0 (20–84)	3 ± 0.1 (2–4)	77.8 ± 4.4 (35.7–129.0)	10 ± 0* (6–23)	-	-	4,893 ± 57 (4,651–4,996)	-
	Call A (N = 294)	Single call or sequence of 2–7 calls	36 ± 1 (13–57)	4 ± 0.6 (1–5)	97.6 ± 9.1 (41.6–230.7)	10 ± 2* (2–29)	-	-	4,592 ± 346 (3,962–5,063)	-
<i>V. uranoscopa</i> (San Antonio, Misiones Province, Argentina) (Zaracho 2014)	Call A (N = 172)	Single call or sequence of 2–9 calls	38 ± 13 (13–85)	3 ± 0.4 (1–5)	128.5 ± 11.0 (91.0–166.7)	7 ± 2* (3–15)	-	-	4,642 ± 109 (4,313–4,875)	-

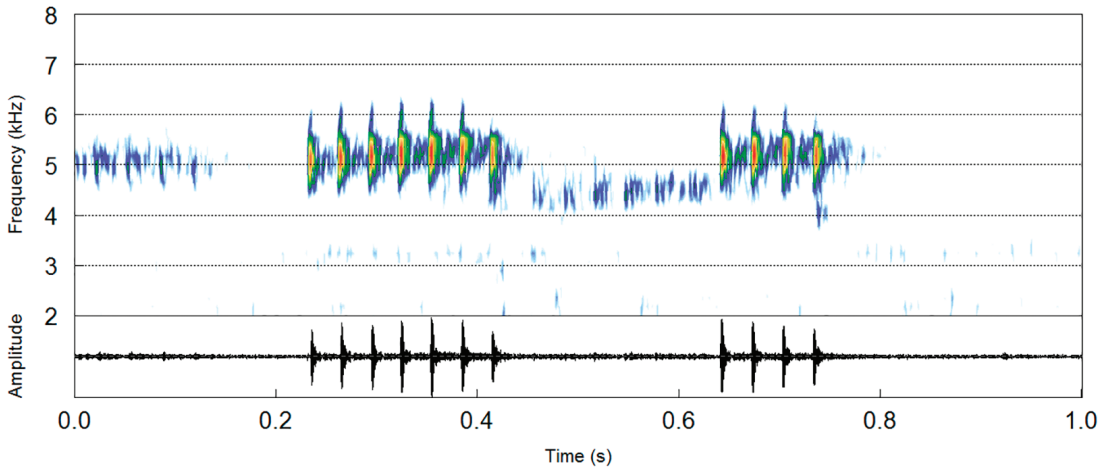


Figure 1. Spectrogram and corresponding oscillogram of the vocalization of *Vitreorana baliomma* from Murici: Call A followed by a Call B. Sound file = *Vitreorana baliomma*_Murici-Alagoas-BSLISBOA-29.IV.13_MZ000032. See further information on recordings in Appendix I.

call. Call A duration varies from 185–198 ms. Pulses are emitted at a rate of 35.3–37.8 pulses/s. The dominant frequency ranges from 4694–5081 Hz. When Call A is followed by a Call B, the interval between them varies from 217–249 ms. Call B also is pulsed, though composed of fewer modulations. Call B duration varies from 98–116 ms and is formed by 4 or 5 pulses, which are emitted at a similar rate compared to Call A. The dominant frequency ranges from 4694–5038 Hz.

Vocalization of *Vitreorana eurygnatha*

The vocalization of the population from São Gotardo is composed of two call types (Table 1; Figures 2A; $N = 3$ males). Call A ($N = 34$ calls) is pulsed and has complete amplitude modulations. It has a slight increase in amplitude along the call. Call A duration varies from 135–226 ms and has 3 or 4 pulses. Pulses are emitted at a rate of 15.8–24.9 pulses/s. In some instances, the first pulse has remarkably lower amplitude. The dominant frequency ranges from 4453–5016 Hz. Call B ($N = 3$ calls; Figure 2A) was emitted by only one male as a single call ($N = 2$ cases) or in sequences ($N = 2$ cases) of two or three calls.

Call B always is emitted shortly after a Call A and has the same overall pulse structure. However, Call B has different and irregular patterns of amplitude modulation, more pulses (5–7) and lower intensity (Figure 2A). The interval between Calls A and B varies from 332–560 ms. Each Call B has a duration of 154–199 ms. Pulses are emitted at a rate of 25.8–36.5 pulses/s. The dominant frequency ranges from 4500–4781 Hz. When Calls B are emitted in sequences, the interval between them varies from 389–649 ms (mean = 518, SD = 130).

Only Call A ($N = 1$ male) was recorded in the population of *Vitreorana eurygnatha* from Vargem Bonita, possibly due to the small sample size. The call (Figure 2B) is mostly consistent with the acoustic description for recordings from São Gotardo; however, slight differences were found in quantitative traits such as call duration, pulse rate, and dominant frequency (Table 1).

Vitreorana eurygnatha from Santa Teresa has a different call from those of the Cerrado populations described above. The vocalization is composed of a single call type, Call A (Figure 2C; Table 1; $N = 2$ males) for comparative purposes. Calls are pulsed with complete or

incomplete amplitude modulations and formed by two distinct portions. The first has a progressive increase in amplitude until it reaches the second part, in which two or three pulse clusters are formed and separated by brief gaps of silence. The duration of Call A is similar to that of the Cerrado populations, ranging from 189–249 ms, but has a remarkably greater pulse number (16–22). Pulses are emitted at a higher rate (78.8–89.7 pulses/s) compared to Cerrado populations. The dominant frequency is quite similar, ranging from 4694–4995 Hz.

Vocalization of *Vitreorana franciscana*

The vocalization of the population of *Vitreorana franciscana* from São Gotardo is composed of Call A (Figures 3A; Table 1; $N = 2$ males; 59 calls), which is pulsed and has complete amplitude modulations. Calls are broadcast in two different ways; the first is a single call, and the second is a sequence of calls with lower amplitude that follow shortly after a first high-amplitude call (Figure 3A). Call A lasts 33–73 ms and has 4–7 pulses (Figure 3A). Pulses are emitted at a rate of 84.5–121.2 pulses/s. In some cases, last pulses can have longer duration than the others, with or without internal and incomplete amplitude modulation. The dominant frequency ranges from 4359–4734 Hz.

Only one specimen of *Vitreorana franciscana* was recorded from Vargem Bonita. The calls (Figure 3B; $N = 1$ male) resemble the overall structure described for calls from São Gotardo; they have similar values for temporal traits (Table 1), but have a lower dominant frequency (4031–4171 Hz).

Discussion

The acoustic data for *Vitreorana baliomma* is informative as additional evidence to the diagnosis of this species from northeastern Brazil; however, owing to the limited sample size, we cannot assess intraspecific variation and

patterns of call emission. Additional sampling should be conducted at the type locality of the species in the southern part of Bahia and from intermediate populations between the type locality and Murici, from which we obtained acoustic data (Figure 4), to assess the taxonomic identity of *Vitreorana* populations across coastal Atlantic forests in northeastern Brazil. Nevertheless, the description of calls of *V. baliomma* enables comprehensive acoustic comparisons with the calls of other Glass Frogs (Escalona *et al.* 2019).

Our study reveals that species of *Vitreorana* with calls already described from the Brazilian Atlantic Forest and Cerrado (*V. eurygnatha*, *V. franciscana*, and *V. uranoscopa*) diverge quantitatively, especially in cases of syntopy/sympatry. In comparing the calls of these species, we noted two trends. First, in the case of syntopic species (e.g., *V. eurygnatha* and *V. franciscana*; Figures 2A, B, 3A, B, and 4), there are pronounced differences in the calls. Second, in the case of allopatric species (e.g., *V. franciscana* and *V. uranoscopa*; Figures 3 and 4), calls are similar. We assume that the presence or absence of selective pressures influence characteristics of acoustic mating signals in sympatric and allopatric populations of these frogs, respectively, and that these pressures are reinforced by reproductive isolation in response to sexual selection (Blair 1964, Hoskin *et al.* 2005). Therefore, in cases of sympatric occurrence, acoustic niche partitioning (Duellman and Pyles 1983, Höbel and Gerhardt 2003) between species of *Vitreorana* may have triggered selective pressures to avoid signal overlapping.

Calls of Atlantic Forest and Cerrado populations assigned to *Vitreorana eurygnatha* differ categorically (call envelope) and quantitatively in some temporal traits. The type locality of *V. eurygnatha* is located in a region of Atlantic Forest (Figure 4), which might be an indication that the calls described in our study from the Atlantic forest of Santa Teresa may belong to the call pattern of the nominal species. This is supported by a previous call description

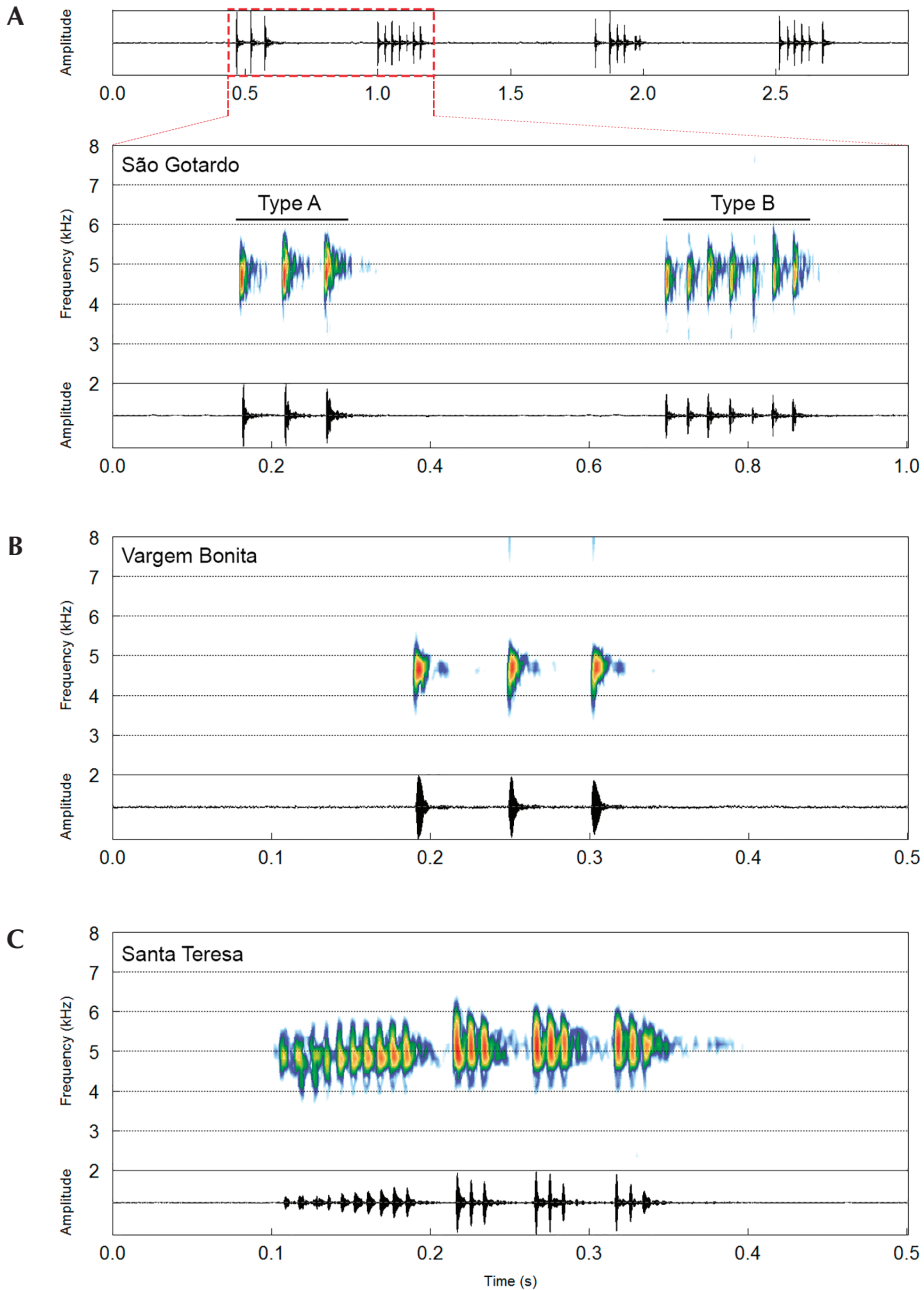


Figure 2. Vocalizations of different populations of *Vitreorana eurygnatha*. (A): a cut (São Gotardo population) containing a Call A followed by a sequence of three Calls B. Lower panel detailing the section outlined: spectrogram and corresponding oscillogram of a Call A and the first Call B of the sequence (sound file = *Vitreor_eurygnatha*SGotardoMG3dTRC_AAGmt); (B) a Call A from Vargem Bonita (sound file = *Vitreor_eurygnatha*CanastraMG1aTRC_AAGmt); (C) a Call A from Santa Teresa (sound file = *Vitreorana_eurygnata*_SantaTeresa-ES_2_BFVT_AAG_m). See further information on recordings in Appendix I.

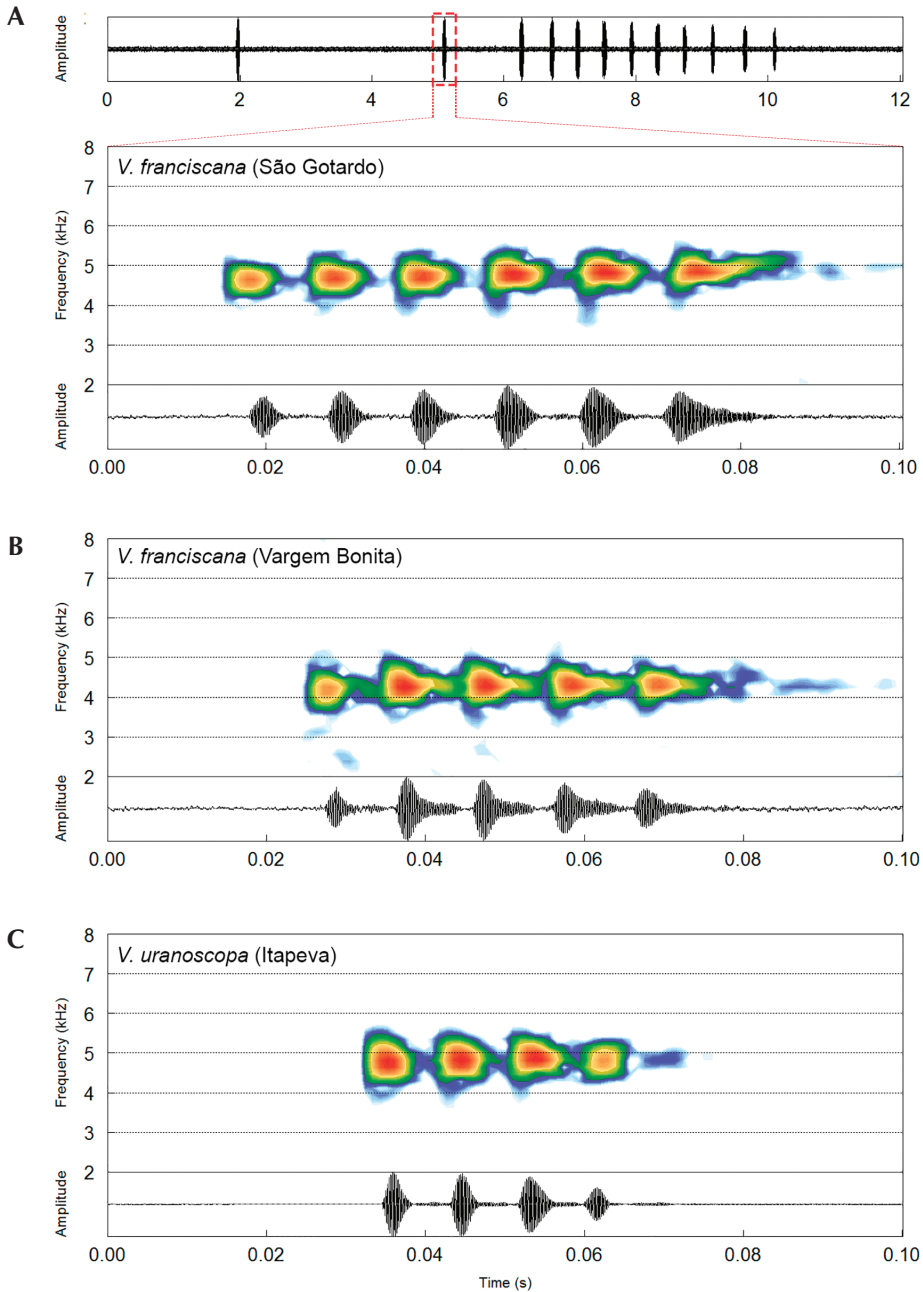


Figure 3. (A) Two Calls A of *Vitreorana franciscana* (São Gotardo); the second one (highlighted) is followed by a sequence of Call A. Highlighted panel corresponds to the spectrogram and oscillogram of the Call A in detail (sound file = Vitreor_franciscanaSGotardMG1aTRC_AAGmt); (B) a Call A of *V. franciscana* from Vargem Bonita (sound file = Vitreor_franciscanaCanastraMG1cLM_AAGmt); (C) a comparative Call A of *V. uranoscopa* from Itapeva, Minas Gerais, southeastern Brazil (sound file = Vitreor_uranoscltapevaMG3aAAGm671). See further information on recordings in Appendix I.

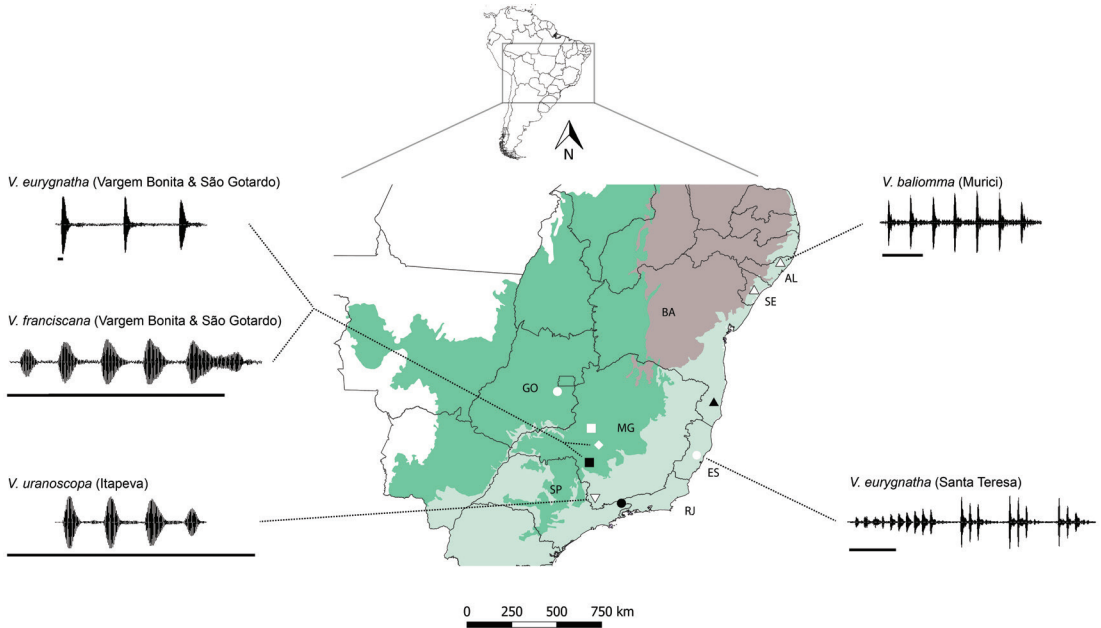


Figure 4. A map of *Vitreorana* species from Cerrado or Atlantic Forest of Brazil depicting the advertisement call of each species or population from localities sampled in the present study: *Vitreorana baliomma* in black triangle = Itamaraju, type locality; white triangles = previous northernmost record, Areia Branca (SE), and northernmost record in Murici (AL) of *V. baliomma*; further occurrence records in Lisboa *et al.* (2019). *Vitreorana eurygnatha* in black circle = Serra da Bocaina, type locality; white circles = in the REBIO Augusto Ruschi, municipality of Santa Teresa (ES); and Luziânia (GO) (Cintra *et al.* 2013). *Vitreorana franciscana* in black square = PARNA da Serra da Canatra, Vargem Bonita, MG, type locality; white square = Presidente Olegário (MG). Rhomboid symbol represents São Gotardo (MG), a common occurrence point for both *V. eurygnatha* and *V. franciscana*. Inverted triangle = one point of occurrence for *V. uranoscopa* from Haga *et al.* (2014). Abbreviations for Brazilian states: AL = Alagoas; BA = Bahia; ES = Espírito Santo; GO = Goiás; MG = Minas Gerais; RJ = Rio de Janeiro; SE = Sergipe; SP = São Paulo. Scale bars for oscillograms = 0.05 ms.

of *V. eurygnatha* from Boracéia (Heyer *et al.* 1990) that agrees with the calls described by us. The striking differences between calls from the Atlantic Forest and the Cerrado emphasize the need of a taxonomic review of populations assigned to *V. eurygnatha* from Cerrado. Moreover, additional Atlantic Forest populations should have their calls evaluated to determine the extent to which acoustic variation across the range of the species coincides with genetic structure within *V. eurygnatha* (see Paz *et al.* 2018).

Although members of *Vitreorana* distributed in the Brazilian Atlantic Forest and Cerrado have a similar overall call structure, the calls differ in quantitative traits; thus, they are species specific in some cases, especially between sympatric species. This study highlights the relevance of acoustic data to inform the taxonomic status of members of the genus. This is particularly relevant to the acoustically divergent populations of *V. eurygnatha*, which are assigned to the same species based on their assumed lack of morphological differentiation. Our findings

suggest that future research should investigate possible mechanisms related to acoustic niche partitioning in sympatric species of *Vitreorana*. To that end, acoustic divergences between sympatric populations of *Vitreorana* should be evaluated to determine whether the differences in calls is explained by short-term adjustments in their vocalization (behavioral plasticity) in response to acoustic niche overlap (Höbel and Gerhardt 2003) or, alternately, by fixed geographical variation in call patterns that may match variations in genetic structure (Velásquez *et al.* 2013) resulting from historical factors such as genetic drift (Irwin *et al.* 2008), natural selection (Velásquez *et al.* 2018), or landscape configuration (Paz *et al.* 2018) in sympatric populations of *Vitreorana*.

Acknowledgments

We thank L. B. Martins, L. Magrini, and A. B. Barros for their help at PARNA da Serra da Canastra. We thank R. N. Feio and C. L. de Assis for providing information on MZUFV specimens. We are grateful to the management staff of REBIO de Santa Teresa and PARNA da Serra da Canastra for allowing us to conduct fieldwork and for providing infrastructure. L. M. Borges, J. S. Junior, R. L. S. Cazarotti, and I. B. Guarda provided insightful comments on drafts of this manuscript. Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) currently provides a doctoral fellowship to DLB (process #2017/27137-7) and a postdoctoral fellowship to TRC (process #2017/08489-0). Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided a Master's fellowship to BSL (process #132436/2012-4). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) provided a doctoral fellowship to BFVT (Finance Code 001). CNPq provided financial support to AAG's lab. CNPq provided a research grant to AAG (#305261/2010-0). Collection permits were conceded by SISBio/ICMBio (#30059; #33507-1). 🐸

References

- Angulo, A. and S. Reichle. 2008. Acoustic signals, species diagnosis, and species concepts: the case of a new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia. *Zoological Journal of the Linnean Society* 152: 59–77.
- Blair, F. W. 1958. Mating call in the speciation of anuran amphibians. *American Naturalist* 92: 27–51.
- Blair, F. W. 1964. Isolating mechanisms and interspecies interactions in anuran amphibians. *Quarterly Review of Biology* 39: 334–344.
- Boul, K. E., W. C. Funk, C. R. Darst, D. C. Cannatella, and M. J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society B* 274: 399–406.
- Carvalho, T. R., A. A. Giaretta, A. Angulo, C. F. B. Haddad, and P. L. V. Peloso. 2019. A new Amazonian species of *Adenomera* (Anura: Leptodactylidae) from the Brazilian state of Pará: a tody-tyrant voice in a frog. *American Museum Novitates* 3919: 1–21.
- Castroviejo-Fisher, S., C. Vilà, J. Ayarzagüena, M. Blanc, and R. Ernst. 2011. Species diversity of *Hyalinobatrachium* glassfrogs (Amphibia: Centrolenidae) from the Guiana Shield, with the description of two new species. *Zootaxa* 3132: 1–55.
- Center for Conservation Bioacoustics. 2014. Raven Pro: Interactive Sound Analysis Software. Version 1.5. URL: <http://www.birds.cornell.edu/raven>.
- Cintra, C. E. D., H. L. R. Silva, and N. J. S. Junior. 2013. New state record of *Vitreorana eurygnatha* (Lutz 1925) (Anura: Centrolenidae) in Brazil. *Herpetology Notes* 6: 587–590.
- Dautel, N., A. L. S. Maldonado, R. Abuza, H. Imba, K. Griffin, and J. M. Guayasamin. 2011. Advertisement and combat calls of the glass frog *Centrolene lynchi* (Anura: Centrolenidae), with notes on combat and reproductive behaviors. *Phyllomedusa* 10: 31–43.
- Duellman, W. E. and R. A. Pyles. 1983. Acoustic resource partitioning in anuran communities. *Copeia* 1983: 639–649.
- Escalona, M. D., P. I. Simões, A. Gonzales-Voyer, and S. Castroviejo-Fisher. 2019. Neotropical frogs and mating songs: the evolution of advertisement calls in glassfrogs. *Journal of Evolutionary Biology* 32: 163–176.
- Guayasamin, J. M., S. Castroviejo-Fisher, L. Trueb, J. Ayarzagüena, M. Rada, and C. Vilà. 2009. Phylogenetic systematics of Glassfrogs (Amphibia: Centrolenidae)

- and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100: 1–97.
- Haga, I. A., F. S. Andrade, N. P. Toscano, A. Kwet, and A. A. Giaretta. 2014. Advertisement call and habitat of *Vitreorana uranoscopa* (Anura: Centrolenidae) in Brazil. *Salamandra* 50: 236–240.
- Heyer, W. R., A. S. Rand, C. A. G. Cruz, O. L. Peixoto, and C. E. Nelson. 1990. Frogs of Boracéia. *Arquivos de Zoologia* 31: 235–410.
- Höbel, G. and H. C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of Green Tree Frog (*Hyla cinerea*). *Evolution* 57: 894–904.
- Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz. 2005. Reinforcement drives rapid allopatric speciation. *Nature* 437: 1353–1356.
- Irwin, D. E., M. P. Thimman, and J. H. Irwin. 2008. Call divergence is correlated with geographic and genetic distance in greenish warblers (*Phylloscopus trochiloides*): a strong role for stochasticity in signal evolution? *Journal of Evolutionary Biology* 21: 435–448.
- Jansen, M., M. Plath, F. Bruquetti, and M. J. Ryan. 2016. Asymmetric frequency shift in advertisement calls of sympatric frogs. *Amphibia-Reptilia* 37: 1–16.
- Köhler, J., M. Jansen, A. Rodríguez, P. J. R. Kok, L. F. Toledo, M. Emmrich, F. Glaw, C. F. B. Haddad, M.-O. Rödel, and M. Vences. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251: 1–124.
- Ligges, U., S. Krey, O. Mersmann, and S. Schnackenberg. 2018. TuneR: Analysis of Music and Speech. URL: <https://CRAN.R-project.org/package=tuneR>.
- Lisboa, B. S., W. F. S. Santos, S. T. Silva, M. C. Guarnieri, and T. Mott. 2019. A new state record of the glassfrog *Vitreorana baliomma* (Anura: Centrolenidae), with notes on its reproductive biology. *Herpetology Notes* 12: 957–960.
- Paz, A., Z. Spanos, J. L. Brown, M. Lyra, C. F. B. Haddad, M. T. Rodrigues, and A. Carnaval. 2018. Phylogeography of Atlantic Forest glassfrogs (*Vitreorana*): when geography, climate dynamics and rivers matter. *Heredity* 122: 545–557.
- Pontes, R., U. Caramaschi, and J. P. Pombal Jr.. 2014. A remarkable new Glass Frog (Centrolenidae: *Vitreorana*) from the northeast Atlantic Forest, Brazil. *Herpetologica* 70: 298–308.
- R Development Core Team. 2015. R: A Language and Environment for Statistical Computing. Version 3.2.3. URL: <http://www.R-project.org>.
- Santana, D. J., A. B. Barros, R. Pontes, and R. N. Feio. 2015. A new species of Glassfrog genus *Vitreorana* (Anura, Centrolenidae) from the Cerrado domain, southeastern Brazil. *Herpetologica* 71: 289–298.
- Sueur, J., T. Aubin, and C. Simonis. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18: 213–226.
- Velásquez, N. A., J. Marambio, E. Brunetti, M. A. Méndez, R. A. Vásquez, and M. Penna. 2013. Bioacoustic and genetic divergence in a frog with a wide geographical distribution. *Zoological Journal of the Linnean Society* 110: 142–155.
- Velásquez, N. A., F. N. Gómez-Moreno, E. Brunetti, and M. Penna. 2018. The acoustic adaptation hypothesis in a widely distributed South American frog: southernmost signals propagate better. *Scientific Reports* 8: 6990.
- Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. Chicago and London. University of Chicago Press. 1148 pp.
- Zaracho, V. H. 2014. Redescription of the advertisement call of *Vitreorana uranoscopa* (Müller, 1924) (Anura, Centrolenidae) from the Argentina Atlantic Forest, with notes on natural history. *South American Journal of Herpetology* 9: 1–7.

Editor: Jaime Bertoluci

Appendix I. Sound archives analyzed for each species/population of *Vitreorana*, with their respective recording labels; specimens voucher numbers; date, time and temperature at recording. Abbreviations for Brazilian states: AL = Alagoas, ES = Espírito Santo, MG = Minas Gerais.

Sound file	Species	Voucher (SVL mm)	Locality	Date (time at recording)	Air (°C)
Vitreorana_baliomma_Murici-Alagoas-BSLISBOA-29.IV.13_MZ000032	<i>V. baliomma</i>	MUFAL 11067 (17.8)	Murici, AL	29 Apr 2013 (23:29h)	24.7
Vitreor_eurygnathaCanastraMG1aTRC_AAGmt	<i>V. eurygnatha</i>	MZUFV 10735	Vargem Bonita, MG	30 Nov 2010 (22:05h)	20.8
Vitreor_eurygnathaSGotardoMG1bTRC_AAAGmt	<i>V. eurygnatha</i>	AAG-UFU 1022 (16.9)	São Gotardo, MG	28 Jan 2012 (~00:00h)	19.0
Vitreor_eurygnathaSGotardoMG2a,bTRC_AAAGmt	<i>V. eurygnatha</i>	-	São Gotardo, MG	28 Jan 2012 (~01:00h)	19.0
Vitreor_eurygnathaSGotardoMG3a,d,cTRC_AAAGmt	<i>V. eurygnatha</i>	AAG-UFU 1023 (17.8)	São Gotardo, MG	28 Jan 2012 (~02:00h)	19.0
Vitreorana_eurygnata_Santa_Teresa-ES_1_BFVT_AAAG_m	<i>V. eurygnatha</i>	AAG-UFU 6180 (20.5)	Santa Teresa, ES	04 Dec 2017 (20:22h)	22.8
Vitreorana_eurygnata_Santa_Teresa-ES_2_BFVT_AAAG_m	<i>V. eurygnatha</i>	AAG-UFU 6081 (19.8)	Santa Teresa, ES	04 Dec 2017 (20:27h)	22.8
Vitreor_franciscanaCanastraMG1b,cLM_AAAGmt	<i>V. franciscana</i>	MZUFV 10736	Vargem Bonita, MG	30 Nov 2010 (~22:00h)	20.8
Vitreor_franciscanaSGotardMG1aTRC_AAAGmt	<i>V. franciscana</i>	AAG-UFU 1024 (21.3)	São Gotardo, MG	28 Jan 2012 (00:34h)	19.0
Vitreor_franciscanaSGotardMG2aTRC_AAAGmt	<i>V. franciscana</i>	AAG-UFU 1025 (21.5)	São Gotardo, MG	28 Jan 2012 (00:43h)	19.0
Vitreor_uranoscItapevaMG3aAAAGmt671	<i>V. uranoscopa</i>	AAG-UFU 980 (21.5)	Itapeva, MG	01 Jan 2012 (19:47h)	20.0