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Re-description of the Advertisement Call of *Vitreorana uranoscopa* (Müller, 1924) (Anura, Centrolenidae) from the Argentinean Atlantic Forest, with Notes on Natural History

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Abstract. *Vitreorana uranoscopa* inhabits small or medium-sized streams with rocky bottoms of the Interior Atlantic Forest of eastern Brazil, northeastern Argentina, and likely southeastern Paraguay. Most of the available information originates from Brazilian populations. Only a few populations from Argentina have been reported and information about their natural history is almost unknown. This work re-describes the advertisement call of *V. uranoscopa* from a new population discovered in the province of Misiones, Argentina, and includes further data about its reproduction, population density and habitat conservation status. On the basis of 11 recorded males, two call types were recorded. Results showed that the typical advertisement call is formed by pulsed notes released singly, in groups, or in combinations of both at an average rate of 13.86 notes/min. Notes lasted 0.013–0.085 s and had 1-5 well-defined pulses lasting 0.003–0.015 s; the pulse repetition rate was 90.9–166.66 pulses/s. The peak of dominant frequency was 4312.5–4875.0 Hz with a slight, ascending frequency modulation. These data agree with those reported in previous studies, although some differences in the note duration, intercall interval, number of pulses and harmonics were identified. These differences might be due to either the technological limitations at the time of those studies or inter-population variation. The second call type (reported for the first time in *V. uranoscopa*) is formed by 1–2 additional pulses at the beginning of some notes and has lower amplitude than typical calls, but the social context of its emission is still unknown. The calling males perched on the leaves of the marginal vegetation, either alone or occasionally in groups of 2–3 individuals, with a average of 5.5 individuals per 100 m transect. Clutches containing up to 36 eggs or embryos were found on the upper surface of fern fronds. In Argentina, *V. uranoscopa* occurs only in *Araucaria* forests of the Interior Atlantic Forest. Thus, the protection of streams with abundant marginal vegetation seems to be essential for the conservation of this species.

Keywords. Habitat; Population density; Reproduction; Vocalization.

Resumen. *Vitreorana uranoscopa* habita arroyos de tamaño pequeño o moderado con fondos rocosos del Bosque Atlántico Interior del Este de Brasil, Nordeste de Argentina y probablemente del área adyacente del Surdeste de Paraguay. La mayor parte de la información disponible para esta especie corresponde a poblaciones de Brasil. Sólo unas pocas poblaciones de Argentina han sido mencionadas y datos acerca de su historia natural son casi desconocidos. Este trabajo re-describe el canto de anuncio de *V. uranoscopa* de una nueva población descubierta en la provincia de Misiones, Argentina, e incluye datos adicionales sobre su reproducción, densidad poblacional y estado de conservación de su hábitat. Sobre la base de 11 machos grabados, dos tipos de cantos fueron registrados. Los resultados mostraron que el canto de anuncio típico está formado por notas pulsadas liberadas solas o en grupos o una combinación de ambas a una tasa promedio de 13.86 notas por minuto. Las notas duran desde 0.013–0.085 s y tienen de 1-5 pulsos bien definidos que duran entre 0.003–0.015 s; la tasa de repetición de pulsos varió entre 90.9–166.66/s. El pico de frecuencia dominante varió desde 4,312.5–4,875.0 Hz. Se observó además una ligera modulación de frecuencia dominante desde el comienzo hasta el final de la nota. Estos valores son similares a los citados en descripciones previas, aunque se encontraron algunas diferencias en la duración de la nota, en el intervalo intercanto, y en el número de pulsos y harmónicos. Estas diferencias pueden estar asociadas con limitaciones tecnológicas al momento de aquellos estudios o variaciones inter-poblacionales. El segundo tipo de canto (reportado por primera vez en *V. uranoscopa*) está formado por uno a dos pulsos adicionales al comienzo de algunas notas, de amplitud más baja que la de los cantos típicos; su contexto social es aún desconocido. Los machos cantaban sobre las hojas de la vegetación marginal, solos u ocasionalmente en grupos de 2–3 individuos, con un promedio de 5.5 individuos por transecta de 100 m. Se encontraron nidos con hasta 36 huevos o embriones sobre la superficie dorsal de frondes de helechos. En Argentina, *V. uranoscopa* habita solamente selvas de *Araucaria* del Bosque Atlántico Interior. La protección de arroyos con abundante vegetación marginal parece ser esencial para la conservación de esta especie.

INTRODUCTION

Centrolenidae comprises 148 Neotropical anuran species commonly known as glass frogs (Frost, 2014). These species are nocturnal, epiphyllous, and arboreal, and deposit their eggs out of the water on vegetation or rocks above streams (Guayasamin et al., 2009). *Vitreorana* occurs below 1900 m from the Cordillera de la Costa of Venezuela and the Guianas to French Guiana, Amazonia of Colombia, Ecuador, Peru, Bolivia, and in the Atlantic Forest of Brazil and Argentina (Muñoz and Aguayo, 2009; Frost, 2014). Eight taxa of the genus are recognized and the advertisement calls of six of these taxa have been described (Barrio, 1968; Heyer, 1985, Heyer et al., 1990; Señaris and Ayazagüena, 2005; Castroviejo-Fisher et al., 2009; Wen et al., 2012).

Vitreorana uranoscopa (Müller, 1924) inhabits near small or moderate-sized streams with rocky bottoms, in the Interior Atlantic Forest of eastern Brazil (from Minas Gerais to northern Rio Grande do Sul), northeastern

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Argentina, and likely southeastern Paraguay (Frost, 2014). This ecosystem is considered a biodiversity hotspot and one of the most threatened in the world. In Argentina, a large and unfragmented area of the Interior Atlantic Forest still remains, but the factors responsible for loss and fragmentation have not been discontinued (Giraudo et al., 2003). In this context, knowledge about the biodiversity of this forest, mainly about its endemic species as *V. uranoscopa,* might have a crucial role in the conservation of this ecosystem.

Most of the available information on *Vitreorana uranoscopa* comes from populations from Brazil. Distribution, acoustic, and biological data of several populations are known (Heyer, 1985, Heyer et al.,1990; Eterovick et al., 2005; Canelas and Bertoluci, 2007; Lucas and Fortes, 2008; Machado et al., 2010; Savaris et al., 2011). In contrast, little is known about the populations of this frog in Argentina (Barrio, 1968; Stetson, 2000; Vaira et al., 2012), except for a brief description of the advertisement call (Barrio, 1968).

This work re-describes the advertisement call of *Vitreorana uranoscopa* from a new population discovered in the province of Misiones, Argentina, and includes further data about its reproduction, population density and habitat conservation status. Although there are some published descriptions of the call of *V. uranoscopa* (Barrio, 1968; Heyer, 1985, Heyer et al., 1990), this re-description is relevant because previous analyses were most likely limited by technological issues. Moreover, describing the advertisement calls of different populations may help to distinguish between intra- and inter-population variations, and thus facilitate the identification of species limits in this widespread taxon.

MATERIALS AND METHODS

Fieldwork was carried out in a relict of the Argentinean Atlantic Forest covered with Paraná pine (*Araucaria angustifolia*) near the town of San Antonio, Dept. General Belgrano, Misiones province, Argentina (26°02'S, 53°47'W; Fig. 1). The local landscape is an undulated plateau, about 527 m elevation, originally covered with *Araucaria* Atlantic Forest (Cabrera, 1976), which currently remains in small scattered patches. The regional climate is Subtropical with annual rainfall of 1900 mm, and a mean monthly temperature of 23°C in January and 14°C in July (Instituto Provincial de Estadística y Censos de Misiones, 2012).

Six streams with rocky bottoms were explored at least once from 16–22 October and 6–11 December 2013, for 10 nights (Fig. 1). Both Perito Moreno and Grande streams are located inside the Reserva Natural Estricta San Antonio (RNESA, Administración de Parques Nacionales, APN); the Pesado stream is on the western border of the reserve, adjacent to an agricultural field; the Rolador and Tigre streams cross a protected area at the Campo Anexo Manuel Belgrano (CAMB, Instituto Nacional de Tecnología Agropecuaria, INTA), and the Monyolito stream is in an agricultural field. The streams were 1–7 m wide and 10–50 cm deep. Nocturnal stream searches (Parris, 1999) were conducted. This involved two people walking slowly along a 500 m transect of each stream from 19:00–23:0 h, spotlighting for frogs with headlamps and counting and

Table 1. Acoustic variables of advertisement calls of *Vitreorana uranoscopa* from San Antonio (Misiones province, Argentina). $\bar{X}$ + SD (minimum–maximum). *n* = number of specimens recorded (number of analyzed calls). Temperature range: 19–27.2°C.

Variables	*n* = 11 males (172 notes)
Note duration (s)	0.038 ± 0.013 (0.013–0.085)
Notes or notes group/min	13.86 ± 8.88 (7.49–37.3)
Intercall interval (s)	6.06 ± 2.71 (1.117–26.859)
Pulse duration (ms)	0.007 ± 0.002 (0.003–0.015)
Pulses/note	3.32 ± 0.45 (1–5)
Pulses/s	128.46 ± 10.95 (90.9–166.66)
Dominant freq. modulation (Hz)	212.65 ± 35.79 (172.2–562.5)
Peak of dominant freq. (Hz)	4,642.36 ± 108.61 (4,312.5–4,875)
Lower freq. (Hz)	3,630.30 ± 222.28 (2,793.6–4,129.8)
Upper freq. (Hz)	5,690.70 ± 234.50 (5,001.2–6,877.6)
Dominant freq. range (Hz)	2,060.40 ± 203.23 (1,148.1–3,392.0)
Other freq. (Hz)	7,125.0–10,312.5 13,125.0–14,625.0 17,437.5–19,500.0

Figure 1. Study site at San Antonio (General Belgrano, Misiones, Argentina). Sampled transects along streams: 1 = Pesado (Section 1), 2 = Pesado (Section 2), 3 = Grande, 4 = Perito Moreno, 5 = Tigre, 6 = Rolador, 7 = Monyolito.

recording frog advertisement calls. The total sampling effort was 40 person-hours along 5 km of stream. When possible, distances between individuals were recorded using a Garmin eTrex 20 GPS. Distances less than 5 m between calling males were considered group calls.

The calls of 11 males of *Vitreorana uranoscopa* were recorded and analyzed. A voucher calling male is housed at the collection of herpetology of the Universidad Nacional del Nordeste (Corrientes, Argentina): UNNEC 12381. Calls were recorded with an M-audio Microtrack II digital recorder coupled to a Sennheiser ME66/K6 microphone (all set at 44.1 kHz and 16 bits resolution). The microphone was placed 20–50 cm from the calling male. Temperature and humidity were measured at the time of recording. Vocalizations were analyzed using Raven Pro 1.4, 64-bit version (Bioacoustics Research Program, 2012). Figures were generated using the Seewave v.1.6 package (Sueur et al., 2008) on the R (v.2.13.0) platform (R Development Core Team, 2011). Means and standard deviations of temporal and spectral parameters of 172 notes were measured. Temporal acoustic variables were analyzed in oscillograms and spectral variables in spectrograms with the following settings: window size 256 samples, Hann window, contrast 50%, brightness 50%, and time grid overlap 50%. Terminology of the advertisement calls follows that of Heyer et al. (1990). The analyzed sound files are available online as supplementary files (Audio S1).

RESULTS

Two call types were recorded in *Vitreorana uranoscopa*. The typical advertisement call was formed by pulsed notes released alone (n = 7 males) or in groups (n = 2 males) or a mix of both (n = 2 males). Call features are summarized in Table 1 and Figures 2–3. Calls were assumed to be in groups when released at intervals shorter than 500 ms. The mean call rate was 13.86 calls/minute. Notes (Fig. 2, Table 1) lasted 0.013–0.085 s and had 1-5 well-defined pulses of 0.003–0.015 s each; the pulse repetition rate was 90.9–166.66 pulses/s. The dominant frequency was included in the fundamental frequency. The peak of dominant frequency was 4,312.5–4,875.0 Hz. Other frequency bands (harmonics) were detected between 7,125.0–10,312.5 Hz, 13,125.0–14,625.0 Hz, and 17,437.5–19,500.0 Hz. Slight ascending frequency modulation was found in 61% of the notes analyzed, increasing from 172.2–562.5 Hz from the beginning to the end. Note groups (Fig. 3) were formed by sequences of 2–9 notes ($\overline{X}$ = 4.29 ± 2.07 notes) and lasted 0.278–2.575 s ($\overline{X}$ = 0.895 ± 0.554 s). Within each group, the note rate varied between 148–296 notes/min ($\overline{X}$ = 222.57 ± 41.12 notes/min), and the internote interval was 0.128–0.437 s ($\overline{X}$ = 0.231 ± 0.058 s).

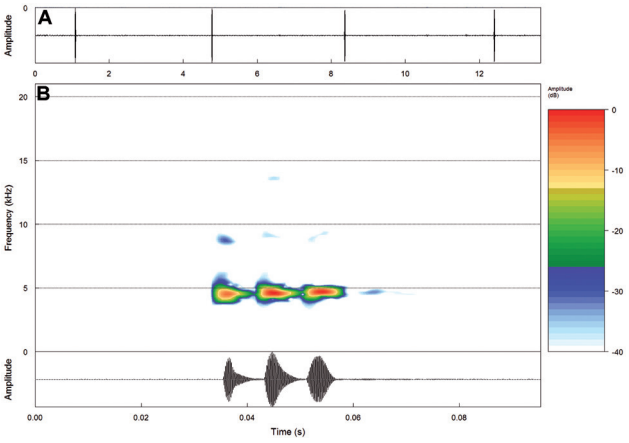

Figure 2. Typical advertisement call of a male *Vitreorana uranoscopa* from Reserva Natural Estricta San Antonio, General Belgrano, Misiones, Argentina. **(A)** Oscillogram with four single calls. **(B)** Audiospectrogram (above) and corresponding oscillogram (below) of the first call. Recording made 09 December 2013, 21:0 h. Air = 21°C, water = 26°C. Recording file (Audio S1): Vitreorana_uranoscopa_VHZ_MT2_227_San_Antonio_file0116.

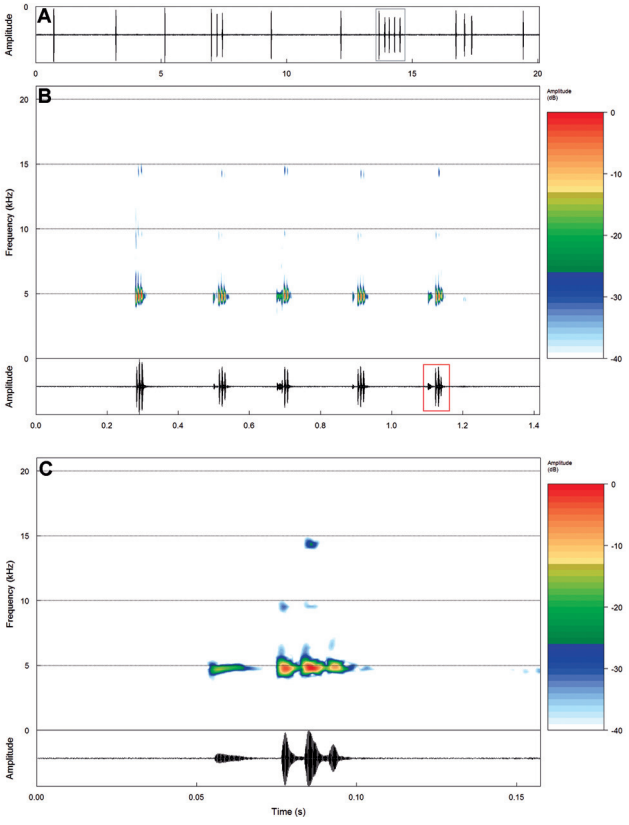

Figure 3. Advertisement calls of a male *Vitreorana uranoscopa* from Tigre stream, Campo Anexo Manuel Belgrano (INTA), General Belgrano, Misiones, Argentina. **(A)** Oscillogram with singles notes combined with note groups. **(B)** Audiospectrogram (above) and corresponding oscillogram (below) of note group outlined in gray. **(C)** Audiospectrogram (above) and corresponding oscillogram (below) of the note outlined in red. Recroding made 08 December 2013, 21:0 h. Air = 21°C, water = 26°C. Recording file (Audio S1): Vitreorana_uranoscopa_VHZ_MT2_225b_San_Antonio_file0111.

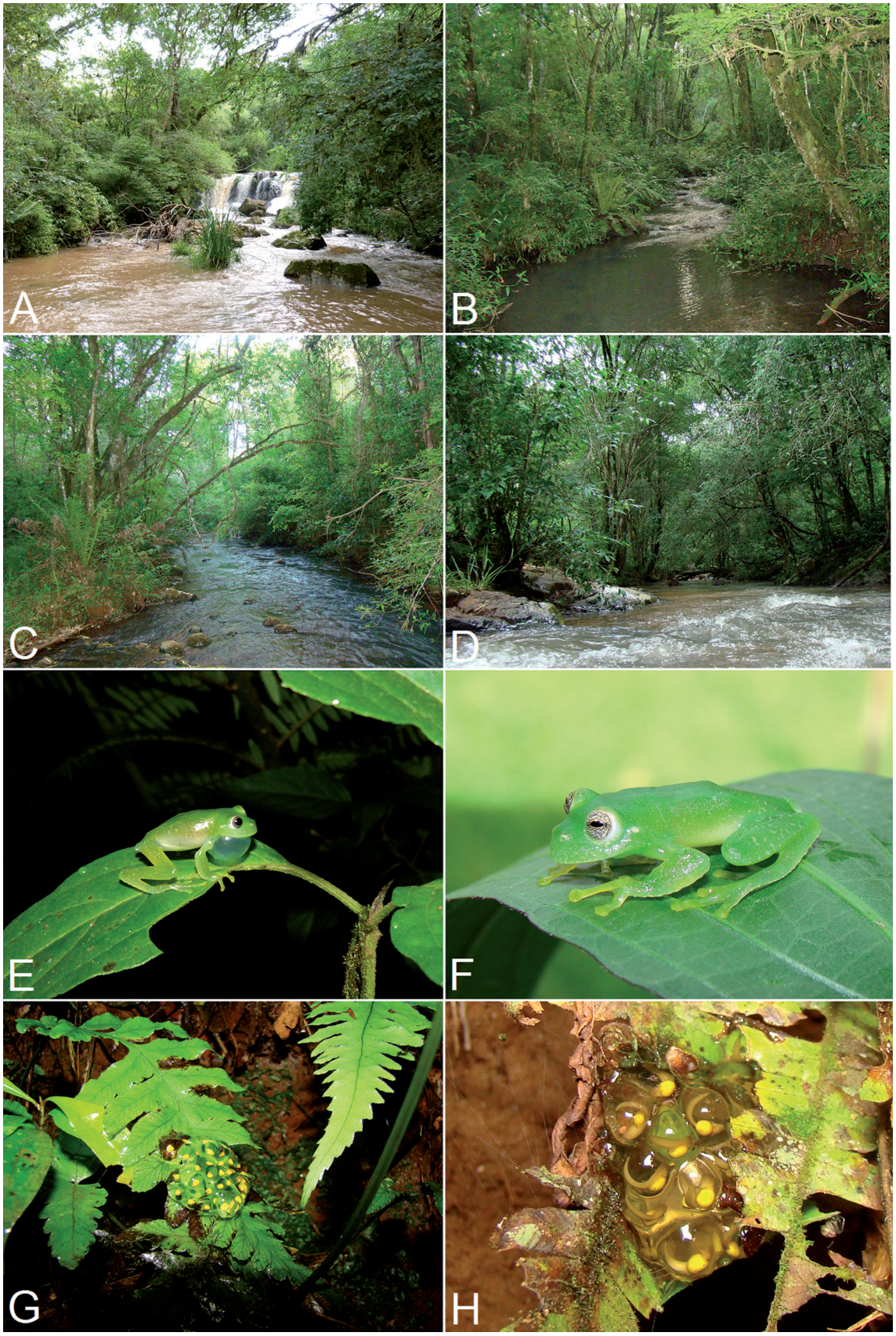

Figure 4. (A) Rolador stream. **(B)** Grande stream. **(C)** Pesado stream. **(D)** Monyolito stream. **(E)** Calling male on marginal vegetation at Tigre stream. **(F)** Voucher UNNEC 12381. **(G–H)** Egg clutches on fern fronds.

Three individuals emitted a different call type. At the beginning of some ordinary notes (9.9% of the notes analyzed), they added 1–2 pulses lasting 0.001–0.019 ms, and of lower amplitude than typical calls (Fig. 3B). These pulses started at 0.007–0.028 s before each typical note and their dominant frequency peak varied from 4500–5062 Hz.

Individuals of *Vitreorana uranoscopa* were found in all streams except Monyolito (Fig. 4A–D). Considering all streams, 19 and 22 calling males were heard in October and December respectively, with an average of 5.5 individuals per transect. A maximum of nine individuals per transect occurred at the Rolador stream. The males called perched on the leaves of the marginal vegetation, 60–300 cm above the water ($\overline{X}$ = 160 cm; n = 21; Fig. 4E–F). They called alone or occasionally in groups of 2–3 individuals. The distance between calling males varied between 10 m and 350 m (n = 9 between 10–50 m; n = 4 between 51–100 m; n = 10 > 100 m). Only two other anurans were found syntopically, *Hypsiboas curupi* (n = 17) and *Scinax aromothyella* (n = 2). In October 2013, five egg clutches were found on the upper surface of fern fronds, all 15–70 cm above the water level ($\overline{X}$ = 46 cm) at Rolador and Perito Moreno streams (Fig. 2G–H). Four of the clutches analyzed had 9, 24, 33, and 36 eggs or embryos at different developmental stages. No males were observed near or in contact with the eggs.

DISCUSSION

Although the typical advertisement call of *Vitreorana uranoscopa* was formed by pulsed notes emitted singly, in groups, or in combination, all had similar temporal and spectral parameters. Barrio (1968) described the call of *V. uranoscopa* from Monyolito stream, a site near Site 1 where the calls for the present study were recorded. Although he collected seven individuals, Barrio (1968) did not mention the sample size for records or the number of calls analyzed. According to Barrio (1968), the advertisement call is formed by sequences of three notes lasting 0.25 s each, with the first note separated from the second note by 0.8 s, and the second note separated from the third note by 0.5 s. Barrio (1968) reported that these sequences were emitted in an irregular way, with an interval range from 5–10 s. He also mentioned a fundamental frequency of 450 Hz (not observed in this study) and a dominant frequency near 5,000 Hz. The temporal pattern agrees with what I refer to as a "group call". However, variables such as note duration and intercall interval differ. The differences are possibly due to the technological limitations at the time of Barrio's (1968) description.

Heyer (1985) summarized the features of the advertisement calls of *Vitreorana uranoscopa* from four Brazilian localities. Later, Heyer et al. (1990) studied the advertisement call of a population from Boracéia, Brazil. Heyer et al. (1990) reported that the advertisement call of this population was formed by 1–5 pulsed notes/call (0.03–0.05 s call duration), with a dominant frequency range between 4,100–5,400 Hz, and with only a first apparent harmonic. Some notes presented frequency modulation, and when this occurred, frequency increased throughout notes. Each note comprised 2–3 pulses with a pulse rate of 80–100 pulses/second. These data are similar to the results presented here, but the Argentinean population shows a wider range of pulse number and additional harmonics. These differences are probably due to inter-population variations or technological issues.

The addition of low amplitude pulses before the well-defined pulsed calls has not been previously reported in the advertisement call of *Vitreorana uranoscopa* (Barrio, 1968; Heyer et al., 1990). A similar variation has been reported in *Centrolene lynchi* and called pre-tonal note (Dautel et al., 2011). This temporal modification has been suggested to increase the information content of the advertisement calls of the frog *Engystomops pustulosus* (Ryan, 2001). In the presence of a female, *Hyalinobatrachium fleischmanni* males can add mews to their call in order to attract them (Greer and Wells, 1980). Here, since these mews were rare, the social context of their emission could not be assessed.

The calls of *Vitreorana* seem to be more conserved in temporal and spectral features than those of other centrolenids. They share basic structural features such as short pulses, harmonics, and frequency modulation (Wen et al., 2012). However, differences between species can be found. The call of *V. uranoscopa* differs from that of *V. antisthenesi* and *V. castroviejoi* in its lower dominant frequency (< 5,000 Hz in *V. uranoscopa*) and from that of *V. helenae* in its shorter note duration (< 0.313 s in *V. uranoscopa*).

The value of population density found (5.5 individuals/500 m) is intermediate relative to other Brazilian populations. A minimum of one individual per 500 m of stream, with a peak abundance of five calling males, was reported for populations of Serra do Caraça, Minas Gerais (Canelas and Bertoluci, 2007), while 2–5 individuals along 100 m of a forest stream were recorded in the Floresta Nacional de Chapecó, Santa Catarina (Lucas and Fortes, 2008).

According to the IUCN, the conservation status of *Vitreorana uranoscopa* is Least Concern (Garcia et al., 2010). Nevertheless, in Argentina, it was recently categorized as Data Deficient since it occurs only in a portion of the Interior Atlantic Forest (*Araucaria* forest) and any other type of relevant information is still unknown (Vaira et al., 2012). The *Araucaria* forest has been decimated because of the economic value of the *Araucaria* (Paraná pine) and today only a few hundred highly fragmented and isolated hectares remain (Giraudo et al., 2003).

The specimens of this study were found in a small area (approximately 150 ha) with forests and streams in

good conservation conditions. Outside this site, searches were conducted only at a section of the Monyolito stream, which is highly modified by human activities. According to Barrio (1968), this species was common at this site. However, no individuals were recorded in this study, probably due to the scarce marginal vegetation on the stream (Fig. 4D). Considering that habitat loss as result of the deforestation has been reported as the main threat for the species (Garcia et al., 2010), the protection of streams with abundant marginal vegetation seems to be essential for the conservation of the Argentinean populations of *V. uranoscopa*.

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ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

Audio S1. A compressed file (in ZIP format) containing the audio files (in WAV format) of the analyzed vocalizations of *Vitreorana uranoscopum*.