

Advertisement and release calls of *Rhinella scitula* (Caramaschi and Niemeyer, 2003) (Anura: Bufonidae)

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Recibido: 23 Abril 2020

Revisado: 29 Mayo 2020

Aceptado: 27 Julio 2020

Editor Asociado: D. Baldo

doi: 10.31017/CdH.2020.(2020-072)

ABSTRACT

The anuran calls descriptions have given important taxonomic information in studies within problematic species group. Herein, we describe the advertisement and release calls of *R. scitula*. We analyzed calls recorded in three localities in Mato Grosso do Sul state, Brazil, including the type locality. Males were calling at the margins of permanent streams in forest fragments ca. 21:00 h. The advertisement call of *R. scitula* is multi-pulsed with interior amplitude modulation, resulting in pulse groups. Males emitted non-regular call series with duration of $0.27s \pm 0.03$ (0.23–0.36), note duration of $0.015s \pm 0.004$ (0.007–0.040), pulse duration of $0.008s \pm 0.001$ (0.005–0.015, n=180), pulse group per call of 6.6 ± 0.92 (5–8) and dominant frequency of $1439.7 \text{ Hz} \pm 46.1$ (1378.1–1550.4). The release calls were characterized by a dominant frequency of $1115.8 \text{ Hz} \pm 102.2$ (947.5–1550.4), a frequency bandwidth of $2001.6 \text{ Hz} \pm 527.4$ (861.3–3876). They are formed by pulsed and/or pulsatile notes spaced by non-regular intervals or series of 2–19 calls. From all *R. margaritifera* species group with described advertisement calls, the most different to *R. scitula* and other species in the group was *R. magnussoni*, which has a structurally distinct call. The release calls in *R. granulosa* species group and *R. scitula* has the same pattern of pulsed and/or non-pulsed notes.

Key words: Bioacoustics; Vocalization; True toads; Animal behavior; Taxonomy.

Introduction

Amphibian communication occurs through different sensorial mechanisms, and vocalization is certainly the most important for Anura (frogs, toads, and treefrogs). Their diverse calls have major role in species-specific interactions, like mate attraction and reproduction, but can also be used during aggressive or defensive behaviors (Toledo *et al.*, 2014). The anuran calls descriptions have given important taxonomic information in studies within problematic species group, helping researchers to detect and diagnose cryptic species (Blair, 1972; Guerra *et al.*, 2011; Köhler *et al.*, 2017).

The *Rhinella margaritifera* species group is composed by 19 species of dead-leaf patterned toads, and evidence of cryptic diversity within taxa suggest that species richness in this group is probably higher than currently recognized (Fouquet *et al.*, 2007a; Moravec *et al.*, 2014; Vaz-Silva *et al.*, 2015). Such evidence stems mainly from molecular systematics

approaches (Fouquet *et al.*, 2007a; Moravec *et al.*, 2014), and independent data sources that could complement them for taxonomic purposes, such as the description of bioacoustic parameters, are still scarcely published (Lima *et al.*, 2007; Ávila *et al.*, 2010; Roberto *et al.*, 2012; Vaz-Silva *et al.*, 2015). Among the recognized species in this group (including potential new ones), only *R. dapsilis*, *R. ocellata* and *R. yunga* have the release calls described (Zimmerman and Bogart, 1988; Caldwell and Shepard, 2007; Stynoski *et al.*, 2020) while only ten have their advertisement calls known: *R. dapsilis* (Zimmerman and Bogart, 1988), *R. sp.* from Bolivia (De La Riva *et al.*, 1996); *R. sp.* from French Guiana (clade A *sensu* Fouquet *et al.*, 2007b), *R. castaneotica* (Köhler and Lötters, 1999), *R. martyi* (Fouquet *et al.*, 2007b), *R. lescurei* (Fouquet *et al.*, 2007b), *R. magnussoni* (Lima *et al.*, 2007), *R. ocellata* (Caldwell and Shepard, 2007), *R. paraguayensis* (Ávila *et al.*, 2010), and *R. hoogmoedi* (Roberto *et al.*, 2012).

Rhinella scitula (Caramaschi and Niemeyer,

2003) is a small toad occurs mainly on the margins of temporary or permanent streams near or within gallery forests (Caramaschi and Niemeyer, 2003). In Brazil, its geographic distribution ranges from southwestern to central Mato Grosso do Sul State. The species was also recorded in Paraguay, near the Brazilian border (Sugai *et al.*, 2014). Herein, we describe the advertisement and release calls of *R. scitula* from three localities in Brazil (including specimens from the type locality), and compare bioacoustic parameters of the advertisement calls with those from related species.

Materials and methods

We analyzed calls recorded in three localities in Mato Grosso do Sul state, Brazil: 1) Estância Mimosa Ecotourism, the type locality of the species, in the municipality of Bonito (-20.983160°S, -56.515635°W, 390 m a.s.l.); 2) Vale das Bruxas, Piraputanga district, Aquidauana municipality (-20.455140°S, -55.498411°W, 190 m a.s.l.); and 3) Morro do Paxixi, Camisão district, Aquidauana municipality (-20.451111°S, -55.621111°W, 377 m a.s.l.). We recorded the advertisement calls of one individual at Vale das Bruxas on 29 January 2016 using an Olympus LS 10 recorder. Five individuals to advertisement calls and one to release calls at Estância Mimosa Ecotourism on 14 May 2016 using a Tascam DR-40 recorder. Also, we recorded the release calls of two specimens at Morro do Paxixi on 15 August 2018 using a Tascam DR-40 recorder. The recorders' internal microphones were employed for recordings in all localities. We positioned the recorder about 1 m away from the calling toad and recorded the calls at a sample rate of 44 kHz and 16-bit resolution. Recorded males were collected and are housed in the Zoological Collection of Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP 03740-45 and 11138-39). We analyzed ten bioacoustic parameters generally used for anuran (Köhler *et al.*, 2017), and specifically for species of the *R. margaritifera* group (see Roberto *et al.*, 2012) call description: call duration (s), note duration (s), pulse duration(s), pulse group per call, pulses per pulse group, number of pulses per call, pulse rate (pulse/s), inter-note interval (s), dominant frequency (Hz) and frequency bandwidth (Hz). Call parameters terminology follows the note-centered approach (Köhler *et al.*, 2017). For the advertisement calls, acoustic parameters of *R. scitula* were

compared to those of other species in the *R. margaritifera* group. We analyzed all calls with Raven Pro v1.5 (Bioacoustics Research Program 2017) and built the oscillograms and spectrogram with R 3.4.3 (R Core Team, 2017). We also used the packages tuneR (Ligges *et al.*, 2013) and seewave (Sueur *et al.*, 2008), which analyze, manipulate, display and edit sound recordings. With these packages we process oscillograms and spectral contents (e.g. dominant frequency), and also build spectrograms.

Results

We observed males calling at the margins of permanent streams in forest fragments ca. 21:00 h. Snout vent length of collected males ranged from 38 to 42.5 mm (mean 40.3 mm). The advertisement call of *R. scitula* can be recognized as type II according to Martin (1972) classification, described as multipulsed calls with interior amplitude modulation, resulting in pulse groups (Fig. 1, Table 1). Males from both localities (Aquidauana and Bonito municipalities) emitted non-regular call series, each call with average duration of $0.27\text{s} \pm 0.03$ (0.23–0.36, $n=28$), mean note duration of $0.015\text{s} \pm 0.004$ (0.007–0.040, $n=94$), mean pulse duration(s) of $0.008\text{s} \pm 0.001$ (0.005–0.015, $n=180$), mean pulse group per call of 6.6 ± 0.92 (5–8, $n=28$) and mean dominant frequency of $1439.7\text{ Hz} \pm 46.1$ (1378.1–1550.4, $n=28$). In all calls analyzed, the inter-note interval ($0.027\text{s} \pm 0.007$, 0.013–0.041; $n=81$) decreases from the first to the last (notes are emitted at a faster rate at the end of the call). When analyzing the number of pulses per pulse group (1.89 ± 0.53 , 1–5; $n=152$), we found that most of the values are low (two or one), and only one specimen from Bonito emitted two distinct calls containing the last pulse group with five pulses. We found differences in call parameters between the two localities sampled in this study (Aquidauana and Bonito municipalities) when considering the number of pulses per call (12.7 ± 2.1 , 9–17; $n=28$), pulse rate (46.4 pulses per second ± 8.1 , 27.9–65.2; $n=28$) and frequency bandwidth ($630.6\text{ Hz} \pm 142.7$, 430.7–947.5; $n=28$). The male from Morro do Paxixi, Aquidauana emitted calls with nine or ten pulses, lower pulse rate (27.9–36 pulses per second) and higher frequency bandwidth (861.3–947.5 Hz), while males from Bonito have calls with 11–17 pulses, higher pulse rate (40–65.2 pulses per second) and lower frequency bandwidth (430.7–602.9 Hz).

The release call of *Rhinella scitula* (Fig. 2) is

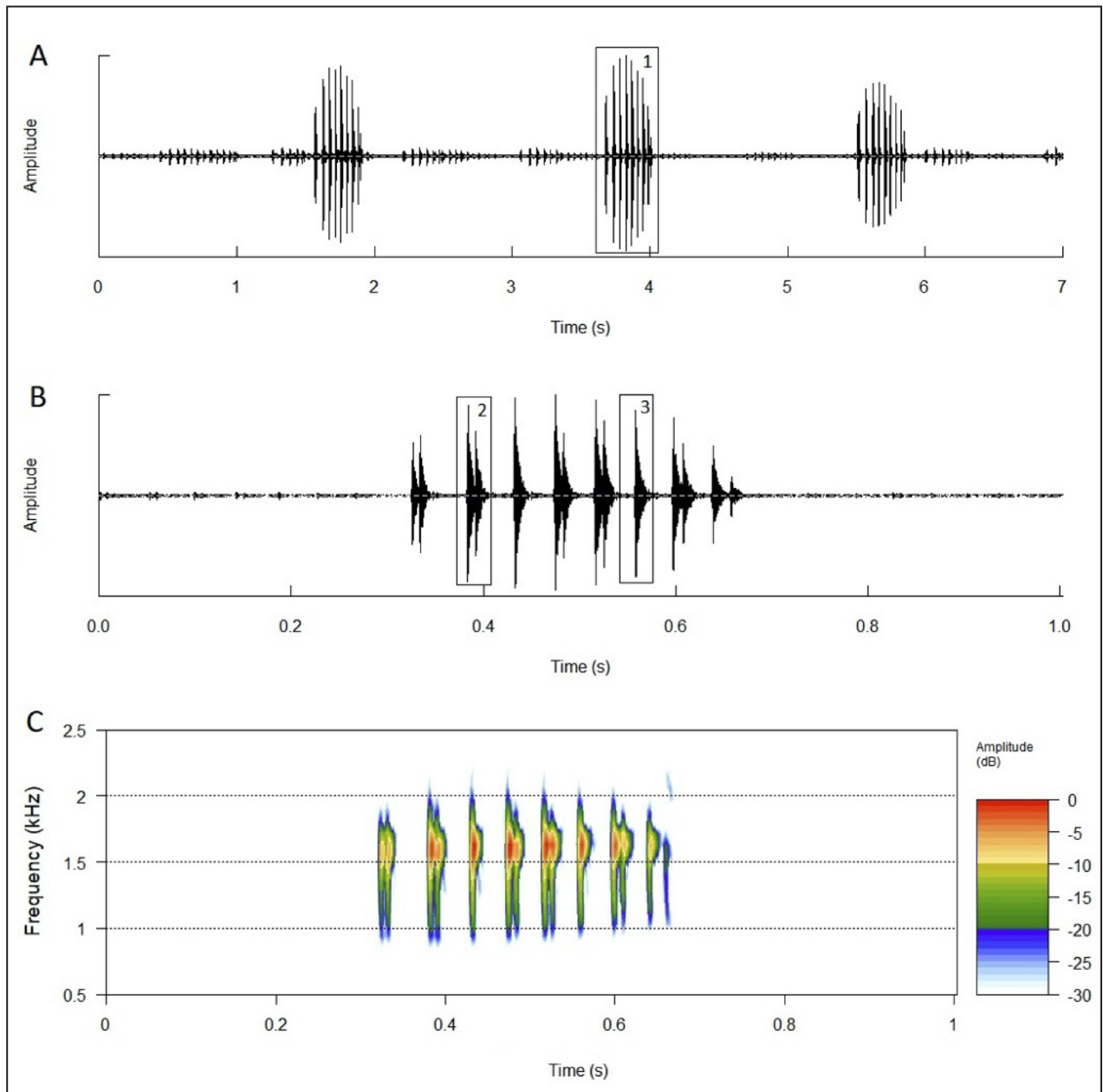


Figure 1. Advertisement call of *Rhinella scitula* (ZUFMS-AMP 03744). A: oscillogram of multiple calls (1 = delimitation of one call); B: oscillogram of single call highlighted at figure 1A (2 = pulse group; 3 = single pulse); C: spectrogram of single call.

composed by two types of notes. The first one, a more frequent emitted, is characterized by a pulsed structure with a dominant frequency of $1192.0 \text{ Hz} \pm 54.9$ ($1119.7\text{--}1292.0$, $n=32$) and frequency bandwidth of $2097.8 \text{ Hz} \pm 485.5$ ($1205.9\text{--}3876.0$, $n=32$). This note has a duration of $0.025\text{s} \pm 0.014$ ($0.006\text{--}0.078$, $n=120$) and has 2.9 ± 1.6 (1–8, $n=120$) pulses. The pulses present a duration of $0.006\text{s} \pm 0.002$ ($0.002\text{--}0.021$, $n=120$), emitted in a pulse-rate of $122.7 \text{ pulses/s} \pm 29.9$ ($41.67\text{--}230.77$, $n=120$) separates by silent intervals. The second note type emitted in the release call present a non-

pulsed structured (harmonics) and has a dominant frequency of $1033.6 \text{ Hz} \pm 258.4$ ($947.5\text{--}1722.7$, $n=9$) and frequency bandwidth of $4038.7 \text{ Hz} \pm 4053.8$ ($1033.6\text{--}11369.5$, $n=9$). Most of the dominant frequency are in the fundamental harmonic and this note is composed by 16.7 harmonics (± 4.8 , 5–22, $n=120$). When both notes are present in the same call, the second note is always the last one, and they can also be emitted alone (call formed by only one these notes types). The inter-note interval is $0.022\text{s} \pm 0.012$ ($0.003\text{--}0.068$, $n=120$).

Table 1. Acoustic parameters for *R. gr. margaritifera* species advertisement call. Values are presented as mean \pm standard deviation (range).

Species	Call duration (s)	Note duration (s)	Pulse duration (s)	Pulse group per call	Pulses per pulse group	Number of pulses per call	Pulse rate (pulse/s)	Inter-note interval (s)	Dominant frequency (Hz)	Frequency bandwidth (Hz)	Reference
<i>R. dapsilis</i>	0.12 \pm 0.01 (0.08–0.14)	-	-	-	-	-	89.68 \pm 5.3 (79.88–97.02)	-	-	1560–3360	Zimmerman and Bogart, 1988
<i>R. gr. margaritifera</i> (Bolivia population)	0.238 \pm 0.02 (0.197–0.291)	-	0.005 (0.001–0.009)	6.3 \pm 0.6 (5–7)	2.0 \pm 0.5 (1–4)	-	102.1 \pm 17.4 (52.2–149.2)	-	1332.3 \pm 107 (1211.5–1544.7)	-	De La Riva <i>et al.</i> , 1996
<i>R. castaneotica</i>	0.2 \pm 0.03 (0.18–0.27)	0.01 \pm 0.004 (0.005–0.012)	-	-	1–4	9.1 \pm 1.1 (7–12)	-	-	1650	900–2600	Köhler and Lötters, 1999
<i>R. marlyi</i>	0.3 \pm 0.01	-	0.01 \pm 0.001	6 \pm 0	4.75 \pm 0.96 2 (last pulse group)	-	-	0.026 \pm 0.004	1169 \pm 0.04	-	Fouquet <i>et al.</i> , 2007b
<i>R. lescuriei</i>	0.03	-	0.004 \pm 0.0002	480/min	4.83 \pm 0.79	-	-	0.097 \pm 0.018	1161 \pm 0.015	-	Fouquet <i>et al.</i> , 2007b
<i>R. gr. margaritifera</i> (French Guiana population)	0.29 \pm 0.05	-	0.008 \pm 0.0007	6.75 \pm 0.957	3.25 \pm 0.5 2 (last pulse group)	-	-	0.026 \pm 0.007	1265 \pm 0.035	-	Fouquet <i>et al.</i> , 2007b
<i>R. magnussoni</i>	9.9 \pm 4.5 (3.3–23.1)	0.06 \pm 0.01 (0.02–0.09)	-	-	-	-	-	0.12 \pm 0.02 (0.07–0.26)	2260 (1890–2550)	-	Lima <i>et al.</i> , 2007
<i>R. ocellata</i>	0.28 \pm 0.01 (0.23–0.31)	-	0.017	-	-	11.4 \pm 1 (7–16)	27.9 \pm 0.9	-	1352.9 \pm 54.7 (1185–1501)	-	Caldwell and Shepard, 2007
<i>R. paraguayensis</i>	0.3 \pm 0.04 (0.20–0.42)	-	-	-	-	6.5 \pm 0.65 (5–8)	-	0.02 \pm 0.01 (0.01–0.04)	1438.7 \pm 70.5 (1113.7–1568.5)	-	Ávila <i>et al.</i> , 2010
<i>R. hoogmoedi</i>	0.23 \pm 0.02 (0.2–0.27)	0.028 \pm 0.01 (0.01–0.05)	-	5.44 \pm 0.54 (5–7)	2.4 \pm 1.15 (1–6)	13.12 \pm 1.154 (11–16)	26.47 \pm 3.52 (12.6–33.3)	0.015 \pm 0.008 (0.007–0.063)	1343.4 \pm 42.55 (1292–1378)	495.3–4474.2	Roberto <i>et al.</i> , 2012
<i>R. scitula</i>	0.27 \pm 0.03 (0.23–0.36)	0.015 \pm 0.004 (0.007–0.040)	0.008 \pm 0.001 (0.005–0.015)	6.6 \pm 0.92 (5–8)	1.89 \pm 0.53 (1–5)	12.7 \pm 2.1 (9–17)	46.4 \pm 8.1 (27.9–65.2)	0.027 \pm 0.007 (0.013–0.041)	1439.7 \pm 46.1 (1378.1–1550.4)	630.6 \pm 142.7 (430.7–947.5)	Present study
<i>R. yunga</i>	-	-	2–2.5	-	-	10 \pm 1.2 (6–13)	-	-	863 (689–947)	-	Szynowski <i>et al.</i> , 2020

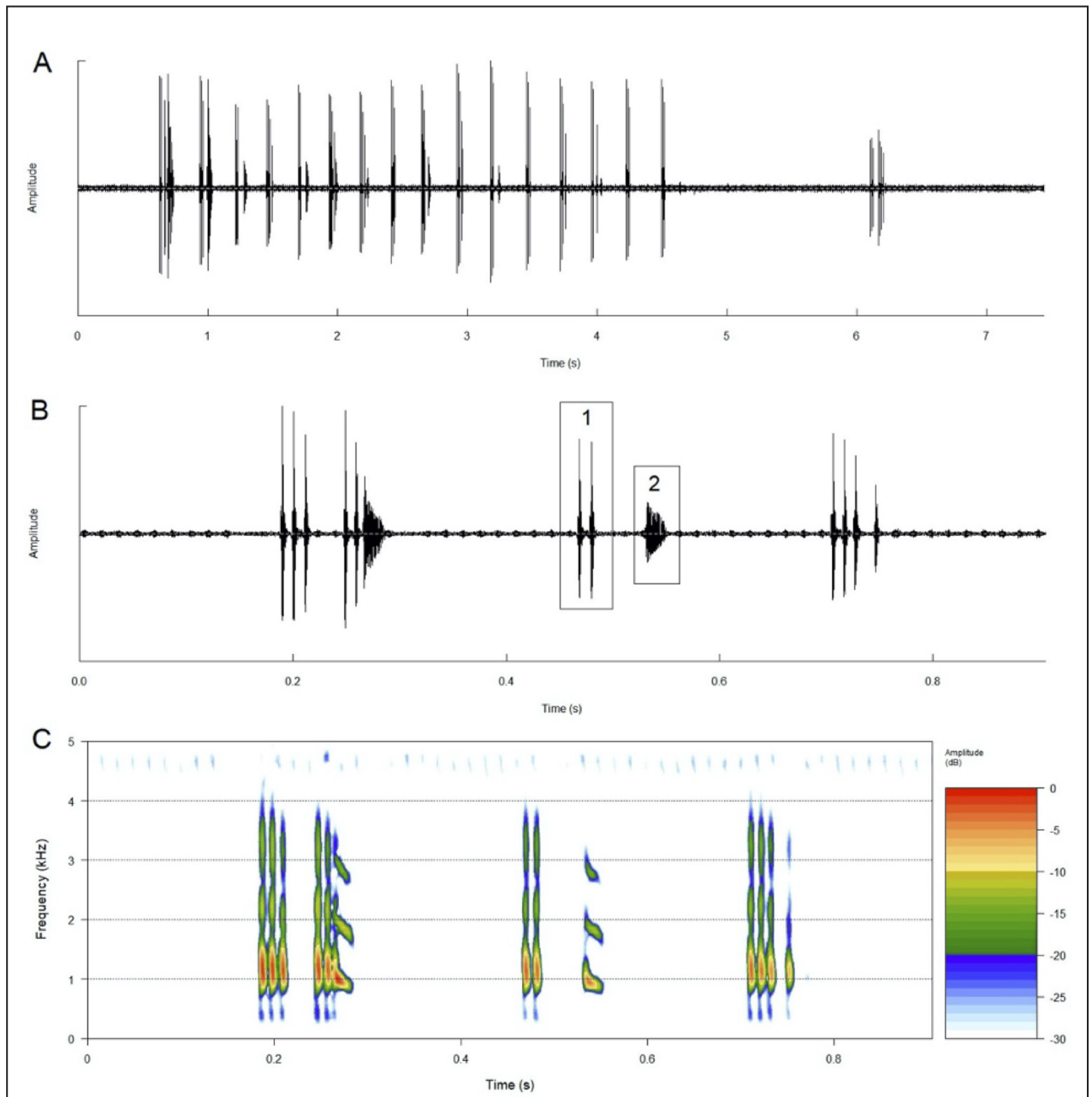


Figure 2. Release call of *Rhinella scitula* (ZUFMS-AMP 11139). A: oscillogram of a sequence of release calls; B: oscillogram of few release calls showing the different note types (1 = pulsed note; 2 = non-pulsed note); C: spectrogram of figure 2B.

Discussion

The advertisement call parameters and structure of *Rhinella scitula* follow the general pattern of *R. margaritifera* species group calls (except *R. magnussoni*), which composed of short pulsed notes, where pulses appear alone or in groups. *Rhinella scitula* values overlap with most of the species, except *R. magnussoni* (9.9 ± 4.5 , 3.3–23.1), which has a longer call duration, and *R. dapsilis* (0.12 ± 0.01 , 0.08–0.14), which is shorter. For *R. lescurei*, the duration of pulse group (mean = 0.003) was informed instead of call

duration. Although there are differences in mean values, note duration overlaps between *R. scitula* and *R. castaneotica* (0.01 ± 0.004 , 0.005–0.012), *R. magnussoni* (0.06 ± 0.01 , 0.02–0.09), and *R. hoogmoedi* (0.028 ± 0.01 , 0.01–0.05). Pulse duration is similar between *R. scitula* and *R. ocellata* (0.017 ± 0), *R. sp.* from French Guiana (0.008 ± 0.0007) and *R. martyi* (0.01 ± 0.001), and lower values are found in *R. lescurei* (0.004 ± 0.0002) and *R. sp.* from Bolivia (mean=0.005, 0.001–0.009), although the range values of the last species overlap with *R. scitula*. The pulse group per call values are similar or overlap bet-

ween *R. scitula*, *R. hoogmoedi* (5.44 ± 0.54 ; 5–7), *R. sp.* from French Guiana (6.75 ± 0.957) and *R. martyi* (6 ± 0) and *R. sp.* from Bolivia (6.3 ± 0.6 , 5–7), while for *R. lescurei* only the pulse group per minutes (480) was informed due to the different call type. Pronounced overlap also occurs in pulses per pulse group between *R. scitula* and *R. hoogmoedi* (2.4 ± 1.5 ; 1–6), *R. sp.* from French Guiana (2 ± 0), *R. martyi* (2 ± 0), *R. castaneotica* (1–4) and *R. sp.* from Bolivia (2 ± 0.5 , 1–4), while for *R. lescurei* the values are higher (4.83 ± 0.79). The number of pulses did not overlap only between *R. scitula* and *R. paraguayensis* (6.5 ± 0.65 , 5–8), which was lower. Overlap occurs between *R. scitula* and *R. hoogmoedi* (13.12 ± 1.154 , 11–16), *R. ocellata* (11.4 ± 1 , 7–16), and *R. castaneotica* (9.1 ± 1.1 , 7–12). Pulse rate overlaps between *R. scitula*, *R. ocellata* (27.9 ± 0.9), and *R. hoogmoedi* (26.47 ± 3.52 , 12.6–33.3), although the mean value was higher in *R. scitula*. Even higher values are found in *R. sp.* from Bolivia (102.1 ± 17.4 , 52.2–149.2) and *R. dapsilis* (89.68 ± 5.3 , 79.88–97.02). Inter-note interval overlaps between *R. scitula* and *R. hoogmoedi* (0.015 ± 0.008 , 0.007–0.063), *R. paraguayensis* (0.02 ± 0.01 , 0.01–0.04), *R. sp.* from French Guiana (0.026 ± 0.007) and *R. martyi* (0.026 ± 0.004), while it is higher in *R. lescurei* (0.097 ± 0.018) and *R. magnussoni* (0.12 ± 0.02 , 0.07–0.26). Dominant frequency overlaps between *R. scitula* and *R. hoogmoedi* (1343.4 ± 42.55 , 1292–1378), *R. paraguayensis* (1438.7 ± 70.5 , 1113.7–1568.5), *R. ocellata* (1352.9 ± 54.7 , 1185–1501), *R. castaneotica* (mean=1650, 900–2600) and *R. sp.* from Bolivia (1332.3 ± 107 , 1211.5–1544.7). Lower dominant frequency is found in *R. sp.* from French Guiana (1265 ± 0.035), *R. lescurei* (1161 ± 0.015), and *R. martyi* (1169 ± 0.04), while higher values are found in *R. magnussoni* (mean=2260, 1890–2550). Frequency bandwidth also overlaps between the species analyzed (table 1).

We found overlap between *R. scitula* and other species from the *R. margaritifera* species group in most of the acoustic parameters measured, indicating that they are (in general) not very useful as species diagnostic characteristic. Furthermore, most of the call description lack important parameters (e.g. pulse group per call and pulses per pulse group) for correctly describe *R. margaritifera* species group call. We recommend that future studies on bioacoustics in this species group consider greater number of parameters based on recent publications (Roberto *et al.*, 2012; Köhler *et al.*, 2017) and describe more release calls within the group in order to identify

which ones can be used in species diagnosis (Grenat and Martino, 2013).

From all *R. margaritifera* species group with described advertisement calls, the most different to *R. scitula* and other species in the group was *R. magnussoni*, which has a structurally distinct call (simple pulse notes with no amplitude modulation; Lima *et al.*, 2007); it has much longer call duration, longer inter-note interval, and higher dominant frequency. The high similarity in advertisement call parameters and morphology between most of the *R. margaritifera* species group species reinforces the need of integrative taxonomy to understand the systematics of complex groups correctly. Descriptions of release calls from other species are needed, once they can also be used as diagnostic characters between cryptic species in anuran (Grenat and Martino, 2013).

There are only three release call described for species within the *R. margaritifera* species group (*R. dapsilis*, *R. ocellata* and *R. yunga*) (Zimmerman and Bogart, 1988; Caldwell and Shepard, 2007; Stynoski *et al.*, 2020). However, Roberto *et al.* (2011) informed that *R. hoogmoedi* emitted release calls when manipulated, and this seemed to trigger other males to start calling. We did not record such behavior for *R. scitula*. Both species within *R. margaritifera* group with release call described have pulsed call (Zimmerman and Bogart, 1988; Guerra *et al.*, 2020), however, *R. scitula* present both pulsed and non-pulsed structures, showing a more complex release call. Pulsed and non-pulsed notes or calls (depending on the approach used for description) also occur in release calls from *R. granulosa*, *R. marina* and *R. spinulosa* species groups (Guerra *et al.*, 2011; Vieira *et al.*, 2014). Comparing the call within the *R. margaritifera* group, the release call of *R. scitula* distinguished from the call of *R. dapsilis* by having a lower dominant frequency (4220–5250 Hz in *R. dapsilis*) and shorter duration (0.07–0.11 s in *R. dapsilis*). From the release call of *R. ocellata*, it distinguishes by the shorter duration (0.086–1.105s in *R. ocellata*). Lastly, from the release call of *R. yunga*, distinguishes by the shorter pulse duration (2–2.5 s in *R. yunga*) and higher dominant frequency (689–947 Hz in *R. yunga*). For a comparison among described release calls of the *Rhinella* species, Guerra *et al.* (2010) summarized data of all described release calls of genus.

Acknowledgments

Collecting permit was provided by Sisbio/ICMBio

(#45889-1). The staff and management of Estância Mimosa for allowing us to conduct the field surveys in their area. We would like to thank the CAPES which provides a PhD Scholarship to MON. FLS and DJS thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for their research fellowship (303006/2014-5 and 311492/2017-7).

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