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Reassessment of the vocal repertoire of a nest-building gladiator frog, *Boana pardalis* (Anura, Hylidae, Cophomantinae): implications for its diagnosis within the *B. faber* species group

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ABSTRACT

In this study, we re-describe the advertisement and territorial calls of *Boana pardalis*, carry out an acoustic comparison between the studied species and the other congeners of the *B. faber* group, and report for the first time the tympanic amplexus for the studied species. The advertisement call of *B. pardalis* can be used to supplement its diagnosis in the *B. faber* group based on temporal call traits, e.g. emission rate and emission pattern, as well as the call envelope.

Introduction

The Neotropical frog genus *Boana* Gray, 1825 (see Dubois 2017) includes 92 species that are distributed throughout Central and South America (Frost 2016; Orrico et al. 2017). *Boana* is recognized as monophyletic (Faivovich et al. 2005; Wiens et al. 2005, 2010; Pyron & Wiens 2011; Duellman et al. 2016), supported by 33 transformations in nuclear and mitochondrial protein and ribosomal genes (Faivovich et al. 2005). The *B. faber* species group, as defined by Faivovich et al. (2005), is one of the monophyletic clades within the genus, which is essentially diagnosed by molecular transformations in mitochondrial and nuclear genes, and comprises nine species: *B. albomarginata* (Spix, 1824), *B. crepitans* (Wied-Neuwied, 1824), *B. xerophylla* (Dumeril & Bibron, 1841), *B. faber* (Wied-Neuwied, 1821), *B. lundii* (Burmeister, 1856), *B. pardalis* (Spix, 1824), *B. exastis* (Caramaschi & Rodrigues, 2003), *B. pugnax* (Schmidt, 1857) and *B. rosenbergi* (Boulenger, 1898). The inclusion of the three latter species in this group was tentatively based only on shared traits of their morphology (e.g. lichenous color pattern) and reproductive biology (construction of egg-laying clay nest by males), without molecular information (Faivovich et al. 2005). In contrast to this, Orrico et al. (2017) did not consider the *B. faber* group as monophyletic due to a weak phylogenetic relationship between *B. albomarginata* and the remaining species of the group (also reported by Wiens et al. 2006; Kolenc et al. 2008), and a nested position of the *B. albopunctatus* group within the *B. faber* group, which was strongly supported. However, Orrico et al. (2017) did not take any formal action on this issue, as their molecular analyses were simply aimed at exploring genetic divergence within *B. crepitans*. For all species of the *B. faber* group the advertisement calls were already described (Breder 1946; Fouquet 1966; Bokermann 1968; Duellman 1970, 2001; Kluge 1981; Martins & Haddad 1988; Heyer et al. 1990; Höbel 2000; Guimarães et al. 2001; Bernal et al. 2004; Giasson & Haddad 2006; Casal & Juncá 2008; Loebmann et al. 2008; Martins et al. 2009).

The leopard tree frog, *Boana pardalis*, is also known as a nest-building gladiator frog due to its pugnacious behavior, the well-developed prepollical spines that the males use in their fights and the construction of clay nests by males (Lutz 1960, 1973; Bokermann 1968; Kluge 1979; Heyer et al.
Material and methods

Sampling of call recordings

We recorded eight males of *B. pardalis* and analyzed 118 of their advertisement calls. Forty-three calls of three males were recorded in the municipality of Salesópolis (23°37’52.65”S, 45°55’17.32”W; 860 m asl, Serra do Mar mountain range), 20 calls by one male in the municipality of Cunha (23°14’7.07”S, 45°1′10.23”W, Serra do Mar mountain range) both in the State of São Paulo (SP). We also recorded 25 calls by two males in the municipality of Chiador, state of Minas Gerais (MG) (21°55′S, 42°51′W; ca. 580 m asl), and 30 calls by two males in the municipality of Duas Barras, state of Rio de Janeiro (RJ) (22°06′S, 42°35′W; ca. 930 m asl) (Figure 1). We also analyzed 14 territorial calls from the eight recorded males. Our sample from Salesópolis is especially important because it is from the locality where the call of *B. pardalis* was previously described (Heyer et al. 1990), but the data of which are not in complete accordance with our newly gathered information on the species calls. In Salesópolis, vocalizations were recorded from 21:20 to 21:24 h on 29 December 2015 by AAG and FSA (air temperature 21°C, water temperature 20°C), in Cunha around 19:40 h on 5 November 2011 by AAG (air and water temperatures 15°C), in Chiador from 19:00 to 19:10 h on 5 September 2011 by TRC and LBM (air temperature

Figure 1. Distribution map of our acoustic sample for *Boana pardalis* and its type locality. Municipalities: (1) Salesópolis – SP; (2) Cunha – SP; (3) Chiador – MG; (4) Duas Barras – RJ; and (5) Magé – RJ (type-locality). Brazilian states: MG – Minas Gerais; SP – São Paulo; and RJ – Rio de Janeiro.
16.6°C, water temperature 19.6°C), and in Duas Barras from 20:33 to 20:50 h on 11 September 2011 (air temperature 18°C, water temperature 21°C) by TRC and LBM. Voucher specimens are deposited in the Collection of Amphibians of the Universidade Federal de Uberlândia (AAG-UFU), municipality of Uberlândia, state of Minas Gerais, Brazil, under the following accession numbers: AAG-UFU 0485–0486 (recorded and collected in Chiador, Minas Gerais, Brazil).

**Sound analyses**

Calls were recorded with a ME67/K6 Sennheiser directional microphone (Sennheiser electronic GmbH & Co. KG, Germany) connected to a Marantz PMD 671 recorder (Marantz, Japan), or with a ME66/K6 Sennheiser directional microphone connected to a M-audio Microtrack II recorder (M-audio, USA). Calls were recorded at a sampling rate of 44.1 kHz and a sample size of 16 bits (see Appendix). We analyzed calls using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program 2014) with the following settings: window type = Hann, window size = 256 samples, 3 dB filter bandwidth = 248 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked), grid spacing (spectral resolution) = 43.1 Hz. Temporal traits were measured on oscillograms and the spectral traits on spectrograms. We measured the dominant frequency through the ‘Peak Frequency (Hz)’ function; the minimum and maximum values for the dominant frequency band were obtained through ‘Frequency 5% (Hz)’ and ‘Frequency 95% (Hz)’ functions, respectively. We also calculated the call rise time through the ‘Peak Time’ function. We generated call figures using Seewave v. 2.0.2 package (Sueur et al. 2008) on R version 3.3.1 (R Core Team 2016). Seewave settings were: Hanning window, 90% overlap and 512 points resolution (FFT).

**Terminology and procedures**

During fieldwork, we heard and recorded two types of calls broadcast by males of *B. pardalis*, which we assumed to be the advertisement and territorial calls (*sensu* Toledo et al. 2015), taking into consideration the reproductive and aggressive contexts observed, respectively. We measured the call traits following the acoustic terminology proposed by Cocroft and Ryan (1995). We calculated means and standard deviations from mean values for each individual, whereas the range encompassed the minimum and maximum values of the whole sample. We analyzed duration and intervals for all pulses from each of the analyzed calls.

We generated sound figures from the two calls recorded by Heyer et al. (1990) to compare with the calls recorded by us for *B. pardalis*. The audio file in wav format is available as supplementary material at Frogs of Boracéia: Online Audio Supplement (http://vertebrates.si.edu/herps/frogs_boraceia/calls/hyla_pardalis.wav to listen and download the audio file).

For qualitative comparison with our samples from *B. pardalis*, we also generated sound figures of the advertisement calls of four species from the *B. faber* group for which we have recordings in our database: *B. albomarginata* from the municipality of Salesópolis (SP), *B. crepitans* from the municipality of Curvelo (MG), *B. faber* from the municipality of Atibaia (SP), and *B. lundii* from the municipality of Uberlândia (MG) (Appendix). Vocalizations of these species were already described, but often the published sound figures did not enable us to assess the fine-scale features of the calls or simply to facilitate a direct comparison of the advertisement call patterns in the group.

**Results**

**Call types in *B. pardalis***

In the field, we observed two different call types for *B. pardalis*. In all cases, the males were isolated at their calling sites, without any close-range encounters. In other words, we only observed long-range interactions among calling males while we recorded the individual calls. The shorter call (hereinafter referred to as ‘advertisement call’) was almost always the only type of call recorded (90% of the 291 calls), while the longer call (hereinafter referred to as ‘territorial call’) was always recorded between shorter call emissions, being far less frequent (10%). In Salesópolis (SP), we also found a couple in tympanic amplexus on the ground (Figure 2), but no clay nest was found around them.

**Redescription of advertisement call**

The advertisement call of *B. pardalis* consists of a pulsed note that varies from 94 to 186 ms in duration, separated by intervals from 1.0 to 18 s (Figures 3a, 4a), and are emitted at rates from 8.2 to 35.3 calls per minute. Call rise time varies from 2 to 91 ms (1–61% of call duration) usually as a slight increase in amplitude between the third and fourth pulse (=midpoint of
call), followed by a more abrupt decrease in amplitude until the last pulse (see Figure 3a). Call (=note) is composed of 6–9 pulses (Figure 3a). Pulses last 8 to 15 ms, separated by intervals from 4 to 23 ms, and emitted at rates of 29 to 74 pulses per second. The dominant frequency varies from 609 to 1547 Hz (Figure 3a), although some values for dominant frequency are more common, such as 609 Hz (n = 28 calls; three males), 656 Hz (n = 29 calls; five males) and 703 Hz (n = 13 calls; four males), which sum 58.3% of the 120 analyzed calls. The dominant frequency at 1547 Hz was the rarest (n = 1 call; one male from Cunha-SP). On average, 90% of total energy of the call is between 439 Hz and 1473 Hz. The minimum call frequency ranges from 112 to 212 Hz, and the maximum from 2137 to 3057 Hz. Quantitative traits are summarized in Table 1.

Redescription of territorial call

The territorial call of *B. pardalis* consists of a long, pulsed note that varies from 258 to 422 ms in duration, and is emitted sporadically (Figures 3b, 4b). Call rise time varies from 196 to 382 ms (74–90% of the overall duration) as a slight increase in amplitude until the second half of the call, followed by a more abrupt decrease in amplitude in the two last pulses (see Figure 3b). Calls are composed of 24–54 pulses (Figure 3b). Pulses last from 3 to 35 ms, separated by intervals from 1 to 21 ms, and emitted at rates from 84 to 162 pulses per second. The dominant frequency varies from 656 to 1453 Hz, although most analyzed calls (86.7%) have a dominant frequency between 1078 and 1453 Hz. On average, 90% of total energy of the call is between 565 Hz and 1566 Hz. The minimum call frequency ranges from 127 to 239 Hz, and the maximum from 2107 to 3084 Hz (Figure 3b). Quantitative traits are summarized in Table 2. An additional short, pulsed note was emitted shortly after (mean = 194 ± 41; 160–253 ms) four territorial calls (Figure 5). This short note has a duration from 31 to 46 ms (mean = 53 ± 20) and is composed of 3 to 4 pulses (mean = 3.5 ± 0.5); which last 6–34 ms (mean = 13 ± 5) and separated by intervals of 2–9 ms (mean = 5 ± 3); emitted at rates of 56–91 pulses per second (mean = 74 ± 17.7). The dominant frequency varies from 1078 to 1266 Hz; minimum frequency ranges from 202 to 205 Hz, and maximum frequency from 2283 to 2478 Hz (Figure 5).
Intra-and interspecific comparisons

The advertisement and territorial calls are easily distinguishable mainly by their temporal traits. The advertisement call has a shorter call duration and call rise time, fewer pulses per call, and lower pulse rate (Figures 3, 4).

In the advertisement calls of *B. albomarginata* from Salesópolis (SP), subunits were barely noticeable (Figure 6a), consisting of a slight increase in amplitude in the first portion of the first subunit, followed by a continuous decrease in amplitude until the last pulse of this portion; this pattern is repeated in the second subunit, but with less sound energy (Figure 6a). *Boana crepitans* has a multi-note call, with the first two notes being shorter and with lower sound energy than the last note, which is longer and contains the peak of amplitude of the

Figure 3. (a) Audiospectrogram (above) and corresponding oscillogram (below) of the advertisement call of *Boana pardalis* from the municipality of Salesópolis, state of São Paulo, Brazil; and (b) audiospectrogram (above) and corresponding oscillogram (below) of the territorial call of *B. pardalis* from the same recording.
call (Figure 6b). Boanalundii has a multi-note call with notes that essentially have the same amplitude modulation pattern, and which are emitted at regular intervals (Figure 6c). Boana faber has a single-note call with an irregular and weak amplitude modulation pattern (Figure 6d) so pulses might not be recognized, whereas B. pardalis possesses amplitude modulation in a relatively regular fashion (Figures 3a, 4a) so that complete pulses, i.e. 100% modulation, can be detected.

### Discussion

#### Acoustic comparisons in the Boana faber species group

In comparison with the most closely related species (Boana faber group), B. pardalis is easily distinguished from B. rosenbergi (mean: 2–3; range: 1–5 notes per call; Duellman 1970; Kluge 1981; Höbel 2000), B. exastis (2–4 notes per call; Loebmann et al. 2008), B.
xerophylla (mean: 4; range: 1–5 notes per call; Fouquette 1966; Duellman 1970; Bernal et al. 2004), B. crepitans (2–5 notes per call; Casal & Juncá 2008; Martins et al. 2009), B. albomarginata (Giasson & Haddad 2006), and B. lundii (mean = 3; range: 1–6 notes per call; in Guimarães et al. 2001 as Hyla biobeba Bokermann & Sazima, 1974) by its advertisement call being composed of a single note, whereas in all abovementioned species the call is formed on average by at least two notes. Accordingly, these six species also have longer advertisement calls in comparison with that of B. pardalis. In addition, B. albomarginata may emit simple or compound advertisement calls, both with two temporal subunits; though sometimes subunit divisions are subtle, particularly in simple calls (Giasson & Haddad 2006).

Figure 4. (a) Audiospectrogram (above) and corresponding oscillogram (below) of the advertisement call of Boana pardalis from Estação Biológica de Boracéia, Salesópolis (SP); and (b) audiospectrogram (above) and corresponding oscillogram (below) of the territorial call of Boana pardalis from the same recording, previously termed as advertisement call by Heyer et al. (1990; see their figure 51). The authors did not distinguish these two types of calls and lumped them together as species' advertisement call. The audio file in wav format is available as supplemental material at Frogs of Boracéia: Online Audio Supplement (http://vertebrates.si.edu/herps/frogs_boraceia/calls/hyla_pardalis.wav).
Boana pardalis differs from the other two species of the B. faber group by the following acoustic traits: B. pugnax has a call that is emitted as a continuous series of notes, with a higher note rate (= call rate: 130 notes per minute) (Duellman 2001); B. faber has a higher call rate (107–124 calls per minute), and the amplitude

Figure 5. Audiospectrogram (above) and corresponding oscillogram (below) of the additional short, pulsed note (indicated by red arrow), which may sporadically be emitted shortly after territorial calls of Boana pardalis from municipality of Chiador, state of Minas Gerais, Brazil; vouchered recording (AAG-UFU 0485).

Figure 6. Audiospectrogram (above) and corresponding oscillogram (below) of the advertisement call of four species of the Boana faber group: (a) single-note call of B. albomarginata with its two temporal subunits; (b) three-note call of B. crepitans; (c) three-note call of B. lundii; and (d) single-note call of B. faber.
modulations are weaker and more irregular than those of *B. pardalis* call, which form complete pulses along the call (Martins & Haddad 1988; Heyer et al. 1990).

**Tympanic amplexus**

We report for the first time the tympanic amplexus for *B. pardalis*. This type of amplexus was also reported for other two species in the *B. faber* group: *B. faber* (Martins & Haddad 1988; Martins 1993) and *B. rosenbergi* (Kluge 1981). Martins and Haddad (1988) and Martins (1993) discussed that this type of amplexus is probably performed by males as a means to avoid wounds in females, given that the males of these species have well-developed prepollical spines.

**Territorial call**

Wells (2007) stated that the term ‘territorial call’ had been applied to vocalizations of many species that clearly were not territorial, and in these cases, it would be preferable to use the more general term ‘aggressive call’ to describe vocalizations given in any kind of aggressive encounter. Toledo et al. (2015) also restricted the term territorial call to territorial species. Thus, given that *B. pardalis* is clearly a territorial species (Lutz 1960, 1973), and the long calls were recorded during long-range interactions (without any indication of close-range encounters), we apply the term ‘territorial call’ in this case.

In most anuran species, aggressive (or territorial in some cases) and advertisement calls have similar dominant frequencies, but differ in temporal traits (Wells 2007). The advertisement call and territorial calls of *B. pardalis* are quite distinct in the temporal domain, mostly in their durations, number of pulses per call and pulse rate. In addition to *B. pardalis*, the territorial calls of *B. lundii*, *B. faber* and *B. rosenbergi* (as aggressive call type I in Höbel 2000) are also longer than the advertisement call of these species (Martins & Haddad 1988; Höbel 2000; Guimarães et al. 2001).

**Calls previously described**

Bokermann (1968) described two notes for the vocal repertoire of *B. pardalis*: a shorter note with duration of ca. 200 ms, 8–10 pulses per note and dominant frequency ranging between 500 and 2000 Hz; and a longer note with duration of 300 ms; but the other traits were not provided for this note type. We believe that the short note corresponds to the note we assigned to the advertisement call of *B. pardalis*, whereas the longer corresponds to the note we assigned to the species’ territorial call based on similarities mainly in temporal traits.

Heyer et al. (1990) described the advertisement call of *B. pardalis* as a note with duration from 150 to 350 ms, and pulse rate at 50–175 pulses per second. In addition, the authors stated: ‘at slowest pulse rate call pulses are completely separated and appear as notes’ (Heyer et al. 1990, p. 271). These data overlapped with those described by us for both advertisement and territorial notes of *B. pardalis*. Furthermore, the audio file available in the Online Audio Supplement of this article contains five short calls and four long calls, which we classified as ‘advertisement’ and ‘territorial’ calls, respectively. Lastly, the oscillogram and audiospectrogram provided by those authors are in fact a figure of the territorial call of *B. pardalis* (see figure 51 in Heyer et al. 1990). Therefore, the advertisement call of *B. pardalis* can be used to supplement its diagnosis in the *B. faber* group based on the temporal call traits (call duration and pulses per call), emission rate (low call rate/min) and emission pattern (single-note call) of the call, as well as the call envelope (patterns of amplitude modulation).

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References


Appendix

Sound files (wav) of analyzed calls. All archives are deposited in AAG’s acoustic collection and will be also available (only excerpts) at AmphibiaWeb (http://amphibiaweb.org/).

Boana_pardalisSalesopolis1aAAGm671
Boana_pardalisSalesopolis1bAAGm671
Boana_pardalisSalesopolis2aAAGm671
Boana_pardalisSalesopolis3aAAGm671
Boana_pardalisChiadorMG1aLBM_AAGmt
Boana_pardalisChiadorMG2bLBM_AAGmt
Boana_pardalisCunhaSP1aAAGm671
Boana_pardalisDuasBarrasRJ1aLBM_AAGmt
Boana_pardalisDuasBarrasRJ2aTRC_AAGmt
Boana_albomargSalesopolisSP1aFSA_AAGm671
Boana_faberAtibaiaSP5aAAGm671
Boana_crepitansCurveloMG2TRC_AAGmt
Boana_lundiiUberlandiaMG1TRC_AAGmt