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The advertisement call of *Pristimantis boulengeri* (Lynch, 1981) from a population in the Central Andes of Colombia (Anura: Craugastoridae)

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One of the most frequent modalities of anuran communication is the use of acoustic signals (Gerhardt and Huber, 2002). In anurans, acoustic signals have been classified according to the context in which they are emitted (Gerhardt and Huber, 2002). The advertisement calls emitted during the breeding season to attract females and to segregate calling males, is the most conspicuous and studied call (Wells, 2007). However, we still do not know the advertisement calls for many anurans (Wells, 2007; Guerra et al., 2018), especially calls of the species that do not exhibit breeding aggregations around specific aquatic microhabitats (i.e. ponds, creeks). Individuals of these species are more dispersed over the habitat, making it harder to record them. That seems to be the case of the species of the family Craugastoridae in the Andes of Colombia (Vargas-Salinas et al., in prep.), a highly diverse clade of anurans with terrestrial oviposition and direct development of offspring (Lynch et al., 1997).

In this manuscript, we described the advertisement call of *Pristimantis boulengeri* (Lynch, 1981) (Fig. 1A), a species of Craugastoridae that lives both inside and outside forested habitats and occurs between 1750–3300 m a.s.l. on Western and Central Andes of Colombia (Stuart et al., 2008; Buitrago-González et al., 2016). We studied a population located at 2,900 m a.s.l., in the village La Luisa, municipality of Cajamarca, department of Tolima (4.4839° N; 75.4900° W; Fig. 1B-C). In the study area, individuals of *P. boulengeri* usually coexist with *P. uranobates* and *P. racemus*. However, the former species is distinguishable from the latter two species by the presence of (1) parietal peritoneum covered with iridophores; (2) small papillae on the tip of the snout, and (3) a double distal subarticular tubercle on finger IV (Lynch, 1981a; González-Durán et al., 2017). Moreover, males of *P. boulengeri* have a moderate body size (mean snout-vent length, SVL = 22.1 mm, range = 18.6–25.6 mm), while males of *P. uranobates* are smaller (mean SVL = 17.9 mm, range = 15.7–19.8 mm; Lynch, 1991) and males of *P. racemus* are larger (mean SVL = 26.9 mm, range = 25.2–30.2 mm; Lynch, 1980).

We performed nocturnal (20:00–22:00 h) visual encounter surveys (Crump and Scott, 1994) between 14 and 23 March 2017, in search of calling males of *P. boulengeri*. When we observed a signaler, we recorded date, time, and type of substrate where the male was located. We obtained recordings of the advertisement calls at a 24-bit resolution and a sampling rate of 44.1 kHz using a digital recorder (Marantz PMD-661) connected to a unidirectional microphone (Sennheiser K6/ME 66). In addition, we registered body temperature and body size (SVL) of the calling male with an Extech Series 42510 infrared thermometer and a digital stainless hardened calliper (precision = 0.01 mm), respectively. Digital copies of the calls (in WAV format) are deposited at the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia (see Table 1).

We analysed the temporal and spectral characteristics of the advertisement call with the software Raven Pro 1.3 (Bioacoustics Research Program, 2008). The spectrograms were constructed with a 256-point fast Fourier transformation window and the Blackman algorithm. For procedures of measuring call features,

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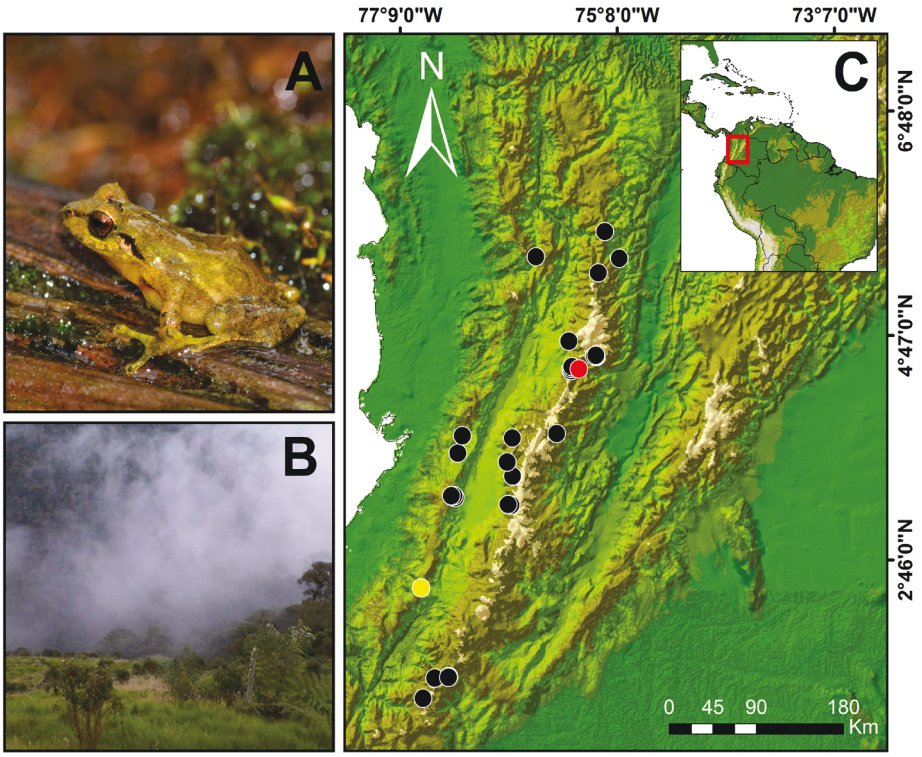

Figure 1. *Pristimantis boulengeri,* male number one in Table 1 (A), and the habitat of the species in the study area (B). Geographic distribution of *P. boulengeri* in Colombia (C): study area at municipality of Cajamarca, department Tolima (red dot), type locality of the species at Cerro Munchique, department of Cauca (yellow dot), and historical records (black dots) obtained from Lynch (1981), Bernal et al. (2005), Cadavid et al. (2005), Duarte-Marín et al. (2018), and GBIF (2018).

we followed terminology by Toledo et al. (2014) and Köhler et al. (2017). Numerical call parameters are given as mean ± standard deviation (SD) followed by range in parenthesis. Although we present here measures based on a given number of calls or pulses (*n*), the sampling unit for the descriptive analyses of the advertisement call is the recorded individual. We did not collect voucher specimens, but we have photographic records of individuals that can be made available upon request.

We obtained 53 advertisement calls belonging to four males found on leaves at a mean height of 1.50 m, in an open area. The advertisement call of *P. boulengeri* usually consisted of one or two pulses (Fig. 2), similar to sharp "tics" very characteristic of other species of *Pristimantis* (Batallas and Brito, 2014; 2016); however, some males emitted calls with up to nine pulses in rapid succession. The duration of the call (i.e. including multiple pulses) was 0.421 ± 0.39 s (0.022–0.959 s) and its amplitude level remained constant throughout the call. The dominant frequency of the call corresponded to the fundamental one and it was 3.77 ± 0.112 kHz (3.62–3.89 kHz). We registered three harmonic bands besides the fundamental frequency. The first, second, and third harmonics had a frequency value of 7.67 ± 0.34 kHz (7.23–7.97 kHz, *n* = 86), 11.47 ± 0.25 kHz (11.29–11.65 kHz, *n* = 62), and 15.37 ± 0.74 kHz (14.85–15.90 kHz, *n* = 37), respectively. Given our small sample size (*n* = 4 males), we could not test possible relationships between temperature and body size with call features (see Table 1).

All the pulses of *P. boulengeri* exhibited an amplitude pattern with attack shorter than the decay (*sensu* Hepp and Carvalho-e-Silva, 2011). The pulse duration was 0.008 ± 0.0016 s (0.0063–0.0099 s, *n* = 109) and the dominant frequency of pulses was 3.78 ± 0.115 kHz (3.62–3.89 kHz, *n* = 109). In calls composed of two or more pulses, the number of pulses per call was 3.20 ± 2.16 (1–9, *n* = 53), the inter-pulse interval duration was

Table 1. Advertisement call features for each individual of *Pristimantis boulengeri* recorded at municipality of Cajamarca, Central Andes of Colombia, followed by a summary. Values are reported as mean ± standard deviation (minimum – maximum). The frequency of harmonics is measured per pulse; for the first and the third male, the second and third harmonics were absent in our recordings. Body size (Snout-vent length, SVL), body temperature (Body T), and BSA Codes assigned at the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia, are shown. Individuals were not collected.

Body/Acoustic measurements	Individual				Summary
	1	2	3	4	
SVL (mm)	23.82	23.45	21.84	24.39	23.38 ± 1.09 (21.84–24.39)
Body T (°C)	11.5	10.8	11.4	8.4	10.52 ± 1.45 (8.4–11.5)
BSA Code	BSA-18008	BSA-18009	BSA-18010	BSA-18011	
n pulses measured / *n* calls measured	14 / 5	34 / 32	30 / 11	31 / 5	109 / 53
Pulses/call	2.8 ± 1.48 (1–5)	1.06 ± 0.25 (1–2)	2.73 ± 3.00 (1–9)	6.2 ± 3.11 (1–9)	3.20 ± 2.16 (1–9)
Call duration (s)	0.363 ± 0.308 (0.007–0.838)	0.022 ± 0.047 (0.008–0.214)	0.341 ± 0.555 (0.006–1.454)	0.959 ± 0.569 (0.008–1.493)	0.421 ± 0.39 (0.022–0.959)
Call Dominant Frequency (kHz)	3.79 ± 0.00	3.89 ± 0.13 (36.17–39.62)	3.62 ± 0.00	3.79± 0.00	3.77 ± 0.112 (3.62–3.89)
Pulse duration (s)	0.006 ± 0.002 (0.005–0.013)	0.009 ± 0.001 (0.007–0.013)	0.008 ± 0.001 (0.006–0.011)	0.006 ± 0.001 (0.006–0.009)	0.008 ± 0.0016 (0.0063–0.009)
Inter-pulse interval duration (s)	0.188 ± 0.025 (0.168–0.253)	0.180 ± 0.023 (0.164–0.197)	0.176 ± 0.027 (0.147–0.272)	0.174 ± 0.014 (0.148–0.215)	0.179 ± 0.006 (0.174–0.188)
Pulse rate (*n* pulses/s)	8.12	10.5	7.2	6.62	7.98 ± 1.82 (6.62–10.50)
Pulse Dominant Frequency (kHz)	3.81 ± 0.09 (3.79–4.13)	3.89 ± 0.13 (3.62–3.96)	3.62 ± 0.00	3.79 ± 0.00 (3.78–3.78)	3.78 ± 0.115 (3.62–3.89)
Harmonic 1 (kHz)	7.97 ± 0.23 (7.58–8.29)	7.89 ± 0.09 (7.58–7.92)	7.23 ± 0.00	7.57 ± 0.06 (7.23–7.58)	7.67 ± 0.34 (7.23–7.97)
Harmonic 2 (kHz)		11.65 ± 0.37 (11.02–12.06)		11.29 ± 0.15 (11.02–11.37)	11.47 ± 0.25 (11.29–11.65)
Harmonic 3 (kHz)		15.90 ± 0.14 (15.81–16.19)		14.85 ± 0.20 (14.47–15.16)	15.37 ± 0.74 (14.85–15.90)

0.179 ± 0.006 s (0.174–0.188 s, n = 56), and the pulse repetition rate was 7.98 ± 1.82 pulses/second (6.62–10.50, n = 14).

In this short note we describe for the first time the advertisement call of a species belonging to the *P. boulengeri* group, which includes *P. angustilineatus*, *P. baiotis*, *P. boulengeri*, *P. brevifrons*, *P. dorsopictus*, *P. myops*, *P. quantus*, and *P. urani* (González-Durán et al., 2017). Moreover, from the groups closely related to the *P. boulengeri* group (i.e. *P. leptolophus*, *P. devillei*, and *P. myersi* groups; González-Durán et al., 2017; Rivera-Correa et al., 2017), only *P. uranobates* (*P. leptolophus* group; Romero-Garcia et al., 2015) has its call described so far. Although the advertisement call of *P. boulengeri* is relatively similar to that of *P. uranobates*, which also occurs in our study area, the advertisement call of the latter has a non-pulsed note longer than single pulses in *P. boulengeri* (mean duration: 0.022 ± 0.006 s), and emitted at a higher dominant frequency (4.13 ± 0.074 kHz; see Romero-Garcia et al., 2015).

During our recordings, males of *P. boulengeri* tended to emit calls composed of three pulses or more while being in presence of surrounding calling males. This behaviour has been documented in other congeners (e.g. *P. munozi*, Rojas-Runjaic et al., 2014; *P. pulchridormientes*, Chávez and Catenazzi, 2016; *P. ganonotus*, Batallas and Brito, 2016; *P. eremitus*, Hutter et al., 2016) and in anurans of other families (e.g. Centrolenidae, Rios-Soto et al., 2017; Hylidae, Tuttle and Ryan, 1982; Schwartz, 1987; Leptodactylidae, Rand and Ryan, 1981). The occasional emission of complex calls can be interpreted as a competitive response of individuals to conspecific males to defend territory or to increase the chances of attracting females

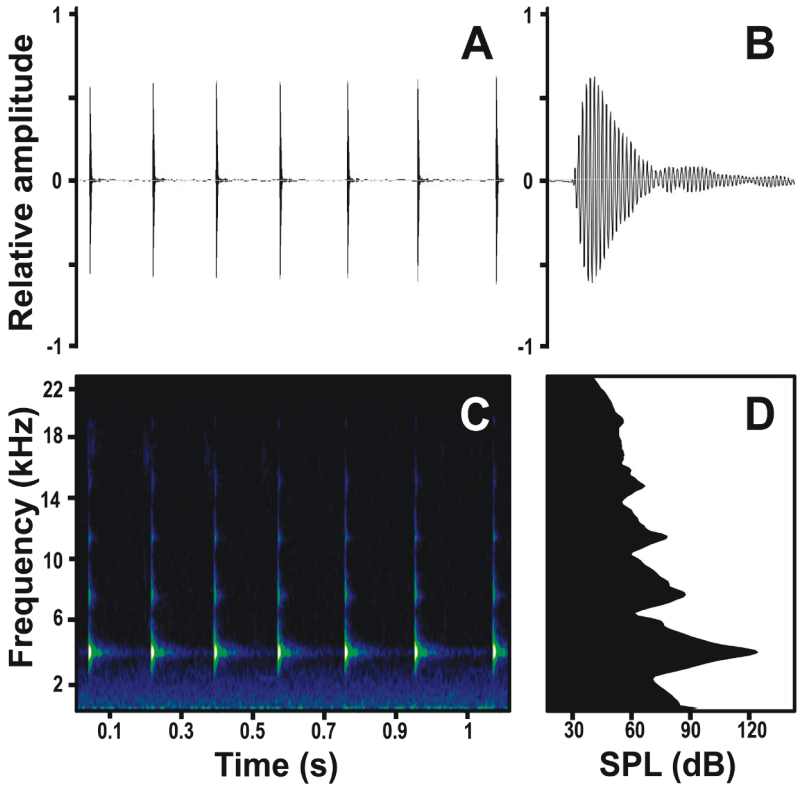

Figure 2. Advertisement call of *Pristimantis boulengeri*. Oscillogram of one call with seven pulses (A), oscillogram showing detail of one pulse (B), spectrogram (C) and power spectrum (D). Recording of male number 4 (BSA-18011, see Table 1), no voucher available. SPL= sound pressure level.

(see Wells, 2007); yet, this hypothesis still needs to be experimentally tested for *P. boulengeri*.

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