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The advertisement call of *Pristimantis subsigillatus* (Anura, Craugastoridae)

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Abstract. We describe for the first time the advertisement call of *Pristimantis subsigillatus* from southern Ecuador. Our study provides a detailed quantitative characterization of the advertisement call of *P. subsigillatus*, filling a gap in our knowledge of this genus, the most speciose among vertebrates. Males called perched on vegetation 0.5-2.5 m above ground, always during mild rain. The advertisement call is composed of a single note with a duration of 63-80 ms, with an initial short pulse (3-10 ms) followed by a longer tonal component. Call rates ranged between 4-12 calls/min. The dominant frequency varied between 2.02-2.82 kHz.

Keywords. Advertisement call, Anura, Craugastoridae, acoustics.

Vocalizations play an important role in the biology and evolution of anurans since acoustic parameters may encode information about species, sex, fitness, reproductive availability, territoriality or distress (Gerhardt and Huber, 2002; Wells and Schwartz, 2006). Acoustic parameters can be genetically and environmentally shaped, therefore deciphering the information within vocal repertoires and analysing their variability in time and space is vital to tracing and understanding evolutionary patterns (Duellman and Trueb, 1994; Gerhardt, 1994; Cocroft and Ryan, 1995; Wells and Schwartz, 2006). Four main types of calls are currently described in anurans (Duellman and Trueb, 1994): advertisement, reciprocation, release and distress, and their functionality is relatively well known. Advertisement calls represent one of the most conspicuous and important components of anuran communication, acting as a premating isolating mechanism and thus providing valuable data for phylogenetic studies (Cocroft and Ryan, 1995; Vences and Wake, 2007).

With more than 470 species, direct-developing frogs of the genus *Pristimantis* make up for almost one third of the anuran species of Ecuador (Ron et al., 2011), and the genus is by far the most diverse among terrestrial vertebrates (Hedges et al., 2008; Frost, 2016). Species of the genus *Pristimantis* have a high phenotypic variability within populations and scant morphological differences among species (Crawford and Smith, 2005). This frequently causes misidentifications even in museum specimens (Padial et al., 2008). Advertisement calls can be a valuable tool in describing or distinguishing among species when morphological characters are not sufficient in species identification (e.g., Köhler and Lötters, 1999; Reichle et al., 2001).

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Pristimantis subsigillatus (Fig. 1) was described by Boulenger (1902) from Salidero, Esmeraldas Province, Ecuador and is considered a locally abundant species in western Ecuador and south-western Colombia at elevations of 100-930 m a.s.l. (Lynch, 1980; Lynch and Duellman, 1997; Frenkel et al., 2013). This species is encountered most frequently in forests, at night, perching on vegetation at heights of 0.6-10 m above the ground; males usually call during rainy nights from vegetation at 2-5 m height (Lynch and Duellman, 1997). The advertisement call was described by Lynch (1980) as a single sharp explosive ''tweet" or as "a single, clear, bell-like note". However, to the best of our knowledge, there is no quantitative characterization of these calls. Our paper provides the first quantitative description of the advertisement call in *P. subsigillatus*.

DC recorded the advertisement calls of three *P. subsigillatus* males from Buenaventura Ecological Reserve, EL Oro Province, Ecuador (Table 1). Calls were recorded with an Olympus LS-11 Linear PCM Recorder and a RØDE NTG2 condenser shotgun microphone, at 44.1 kHz sampling frequency and 16-bit resolution, in wav file format. Air temperature and humidity were measured with a data logger (Lascar Electronics, model EL-USB-2-LCD, accuracy: ± 0.5°C; ± 5%). The three focal males were captured following the recording sessions after being photographed. The snout-vent length (SVL) was measured to the nearest 0.1 mm with dial callipers, and then the animals were anaesthetized with benzocaine, fixed with formalin 10% and preserved in 70% alcohol. The specimens were deposited in the amphibian collection of Museum of Zoology of the Pontificia Universidad Católica del Ecuador (specimen ID is provided in Table 1). Following Toledo et al. (2015) recordings were deposited in Fonoteca Zoológica - www.fonozoo.com, at Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain (records ID are provided in Table 1).

Individuals were identified as *P. subsigillatus* based on the characters described by Lynch and Duellman (1997),

Fig. 1. Male *Pristimantis subsigillatus* calling (Photo credit: José Seoane Rodríguez).

especially as having skin on dorsum finely shagreened, that of venter areolate, without dorsolateral folds, discoidal fold prominent, tympanic membrane and annulus evident, snout subacuminate in dorsal view, truncate or protruding in profile, snout bearing a small papilla at tip, canthus rostralis relatively sharp, upper eyelid lacking tubercles, outer fingers bearing broad discs, heel lacking or bearing very small tubercle and fifth toe much longer than the third (Lynch, 1980; Lynch and Duellman, 1997). Despite the fact that identification of *Pristimantis* species based only on morphological characters is challenging, we are confident that the recorded individuals belong to *P. subsigillatus*. We have conducted a thorough inventory of amphibians in the area and are familiar with the species found there (e.g., Székely et al. 2016), and *P. subsigillatus* cannot be confounded with other species of the genus present based on morphological characters.

At present, *P. subsigillatus* is not assigned to any species group after it was removed from the former, non-monophyletic, *Pristimantis unistrigatus* group (Padial et al., 2014). *Pristimantis subsigillatus* is most similar to *P. nyctophylax* from which it is distinguished by the absence

Table 1. Information regarding the recordings used in this study. Air temperature = Temp; humidity = H; distance from the tip of the microphone and the focal male = Dist.

Specimen ID/ FonoZoo ID	SVL (mm)	Coordinates	Altitude (m)	Date	Time (h)	Temp (°C)	H (%)	Dist (cm)
QCAZ47284/ 9863-64	28.2	3°39'21.4"S 79°46'08.0"W	464	12 September 2014	23:00	-	-	100
QCAZ62538/ 9865-66	26.1	3°39'16.9"S 79°46'34.8"W	453	08 September 2015	21:00	21	98	25
QCAZ62543/ 9867-69	-	3°39'07.0"S 79°45'43.6"W	600	14 September 2015	18:45	21	98	50

of observable tubercles on the eyelids and heels, presence of small terminal papilla at the tip of the snout, presence of numerous supernumerary plantar tubercles and of the inner tarsal tubercle. Additionally, the eye of *P. nyctophylax* is very distinctive in having orange or red sclera (Lynch and Duellman, 1997). Also, this species occurs usually at higher elevations (1140-2100 m a.s.l.) compared to *P. subsigillatus*.

We used Raven Pro 1.4 software (http://www.birds.cornell.edu/raven) to analyse 54 advertisement calls. We measured the temporal parameters from the oscillograms and the spectral parameters from spectrograms obtained through Hanning function, at a window size of 1024 samples, and 50% overlap. We measured the duration, rise time proportion, dominant frequency (Df), and aggregate entropy (Entropy) of the calls and component parts. We computed rise time proportion, as the ratio between the period from the onset of the sound and the moment of maximum amplitude within the analysed call, and the call duration (Márquez et al., 2005). We also computed the dominant frequency modulation within a call (DfM) as the difference between the dominant frequencies of the two component parts (Márquez et al,. 1996). The aggregate entropy provides a measure of the overall disorder in the sound, by analysing the distribution of energy in the spectrogram; zero entropy values characterize single pure tones, while higher entropy values correspond to greater disorder in the acoustic spectrum (Charif et al., 2010). Following Gerhardt (1991), we computed a coefficient of within-individual variation (CV% = SD/mean X 100) in order to differentiate between static (CV% < 5) and dynamic (CV% > 12) call parameters.

The three *P. subsigillatus* males were calling after sunset perched on leaves or branches up to 2.5 m above ground, during mild rain. In each case, several other males were calling in the background. The advertisement call was composed of a single note with two consecutive parts: an initial short pulsed part, followed by a longer tonal part with harmonics (Fig. 2). Call rates ranged

Table 2. Quantitative description of *P. subsigillatus* advertisement calls. Mean ± SD (above the line) and min-Max values (below the line) are provided for all acoustic parameters analysed. n = call sample size, Df = Dominant frequency, DfM = Dominant frequency modulation.

Specimen	Measurement	Duration (ms)	Rise time (%)	Df (Hz)	DfM (Hz)	Entropy (u)
47284 (n = 9)	Call	80 ± 2.9	0.141 ± 0.177	2651.0 ± 19.9	435.5 ± 260.8	2.9 ± 0.3
		77 - 85	0.01 - 0.43	2627.1 - 2691.7	-21.5 - 624.5	2.5 - 3.4
	Part 1	7 ± 1.4	0.346 ± 0.186	2215.5 ± 273.9		5.0 ± 0.1
		6 - 10	0.10 - 0.71	2024.1 - 2670.1		4.9 - 5.2
	Part 2	73 ± 3.0	0.298 ± 0.097	2651.0 ± 19.9		2.6 ± 0.1
		69 - 78	0.09 - 0.43	2627.1 - 2691.7		2.4 - 2.8
62538 (n = 15)	Call	63 ± 0.9	0.025 ± 0.005	2584 ± 0	140.7 ± 124.5	2.4 ± 0.5
		61 – 65	0.02 - 0.03	2584	0 - 430.7	1.5 - 3.1
	Part 1	3 ± 0.5	0.443 ± 0.123	2433.3 ± 124.4		4.0 ± 0.1
		3 - 4	0.33 - 0.67	2153.3 - 2584.0		3.6 - 4.2
	Part 2	60 ± 0.8	0.073 ± 0.006	2584 ± 0		2.2 ± 0.5
		58 - 61	0.07 - 0.09	2584		1.4 - 2.9
62543 (n = 30)	Call	70 ± 3.0	0.016 ± 0.004	2822.3 ± 33.4	476.6 ± 202.3	2.6 ± 0.4
		61 - 74	0.01 - 0.03	2756.2 - 2885.4	0 - 645.9	1.9 - 3.2
	Part 1	7 ± 0.7	0.152 ± 0.046	2345.7 ± 201.9		3.8 ± 0.2
		6 - 8	0.12 - 0.29	2239.5 - 2842.4		3.3 - 4.1
	Part 2	63 ± 3.1	0.226 ± 0.163	2822.3 ± 33.4		1.9 ± 0.2
		53 - 67	0.02 - 0.48	2756.2 - 2885.4		1.5 - 2.6
All (n = 54)	Call	70 ± 6.1	0.378 ± 0.083	2727.5 ± 112.2	376.4 ± 242.4	2.6 ± 0.4
		61 - 85	0.01 - 0.43	2584.0 - 2885.4	-21.5 - 645.9	1.5 - 3.4
	Part 1	6 ± 1.9	0.265 ± 0.166	2351.1 ± 208.0		4.1 ± 0.5
		3 - 10	0.10 - 0.71	2024.1 - 2842.4		3.3 - 5.2
	Part 2	64 ± 5.2	0.198 ± 0.150	2727.5 ± 112.2		2.1 ± 0.4
		53 - 78	0.02 - 0.48	2584.0 - 2885.4		1.4 - 2.9

Table 3. Within-individual coefficient of variation of the analyzed acoustic parameters. n = call sample size, Df = Dominant frequency, DfM = Dominant frequency modulation. CV values < 5% are highlighted in grey (static parameters *sensu* Gerhardt, 1991).

Specimen	Measurement	Duration	Rise time	Df	DfM	Entropy
47284 (n = 9)	Call	3.6	125.5	0.8	59.9	10.3
	Part 1	20.0	53.8	12.4		2.0
	Part 2	4.9	32.6	0.8		3.8
62538 (n = 15)	Call	1.4	20.0	0.0	88.5	20.8
	Part 1	16.7	27.8	5.1		2.5
	Part 2	1.3	8.2	0.0		22.7
62543 (n = 30)	Call	4.3	26.6	1.2	42.4	15.4
	Part 1	10.0	30.3	8.6		5.3
	Part 2	4.9	72.1	1.2		10.5

between 4-6 calls/min (individual 47284) to 9-12 calls/min (individuals 62538 and 62543). Descriptive statistics of the analysed acoustic parameters and their intra-individual variation are presented in detail in Tables 2 and 3.

The first part of the note was composed of 2 to 4 pulse groups in individual 47284, while in the other two individuals it consisted of a single pulse group. In individual 62543, the pulse was bell-shaped (Fig. 2C). The second part of the note was always longer than the first, and had a harmonic structure, with the fundamental harmonic corresponding to the dominant frequency. The dominant frequency was higher in the second part and therefore the calls presented an upward frequency modulation pattern. Call duration, part 2 duration and dominant frequency showed low within individual variability (CV < 5%), and therefore can be considered static acoustic parameters, useful in distinguishing among individuals of the population. The first part of the call presented the highest acoustic variability.

Our study provides the first detailed characterization of the advertisement call of *P. subsigillatus*, filling a gap in our knowledge of this poorly studied group. The calls described in this study are markedly different from the calls of *Pristimantis eugeniae*, *P. nyctophylax* and *P. phoxocephalus* which are considered similar species by Lynch and Duellman (1997). *P. eugeniae* has calls with a much higher dominant frequency (3400-3800 Hz) and lack the pulsed initial part of the call, *P. nyctophylax* has calls with lower dominant frequencies, around 2100 Hz. As for *P. phoxocephalus*, the recordings posted in AmphibiaWebEcuador show a high variation of dominant frequencies, from 1900 Hz to more than 3000 Hz, suggesting that maybe more than one taxon was included in these recordings.

Advertisement calls are an important tool in describing and characterizing anuran species, since they act as a premating isolating mechanism (Ryan, 1988). Based on detailed acoustic analyses, taxonomic relationships can be elucidated (e.g., Padial et al., 2008). As the most speciose vertebrate genus, *Pristimantis* species have been subject to taxonomic debates for a long time (e.g., Hedges et al., 2008; Padial et al., 2014). In August 2016, the total number of *Pristimantis* species recognized was 480, of which 121 new species were described during the last decade only (Frost, 2016). Most descriptions do not include calls and are often based only on museum specimens. Recent reviews based only on molecular data could not shed light on the systematics of this genus (e.g., Hedges et al., 2008; Padial et al., 2014). In order to facilitate taxonomic work, this large genus has been subdivided into several species groups (Lynch and Duellman, 1997). There is a huge variation in the call parameters of the frogs within the genus *Pristimantis* (Padial et al., 2007), and acoustic parameters could prove to be an important tool in the revision of this genus.

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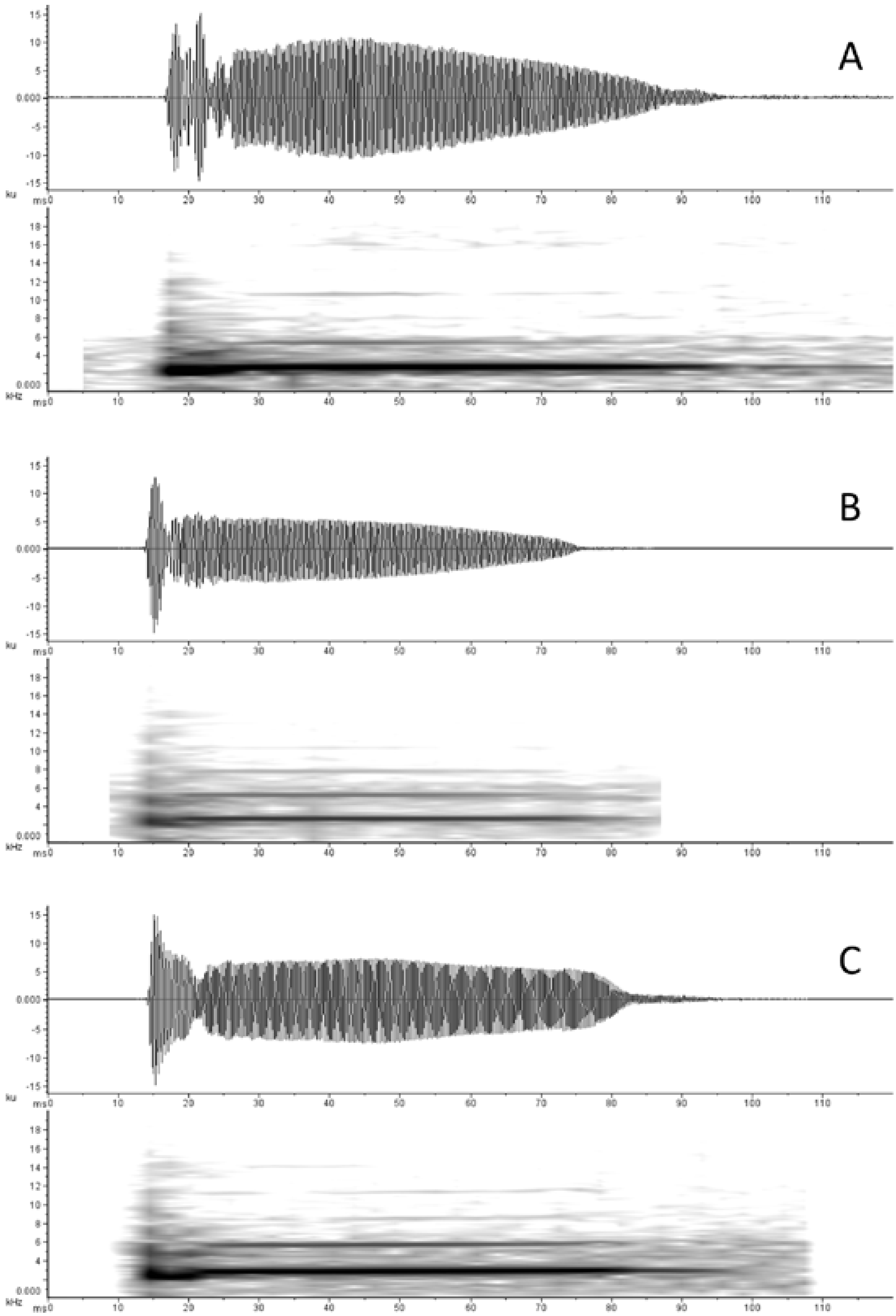

Fig. 2. Oscillogram and spectrogram views of the advertisement calls of the three males of *Pristimantis subsigillatus*: specimen 47284 (A), 62538 (B), and 62543 (C). Spectrogram window size: 256 samples, 50% overlap, hop size 128 samples.

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