



## The advertisement and courtship calls of *Phyllobates bicolor* (Anura: Dendrobatidae) from a natural population in the Colombian pacific cloud forests

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*Phyllobates bicolor* (Bibron in La Sagra, 1840) is the type species of the genus *Phyllobates*, which includes four other species (*sensu* Grant *et al.*, 2017): *P. vittatus* (Cope, 1893), *P. lugubris* (Schmidt, 1857), *P. aurotaenia* (Boulenger, 1913) and *P. terribilis* (Myers *et al.*, 1978). Intense populations reduction and fragmentation (Kahn *et al.*, 2016) coupled to the occurrence of taxonomic misidentifications since its description (Silverstone, 1976; Myers *et al.*, 1978; Maxson & Myers, 1985; Lötters *et al.*, 1997; Widmer *et al.*, 2000; Márquez *et al.*, 2012) have constrained the study of the acoustic communication in *P. bicolor*. Zimmermann & Zimmermann (1985) described *P. bicolor*'s advertisement and courtship calls in captivity, presumably misidentified as *P. terribilis* (Lötters *et al.*, 2007; Kahn *et al.*, 2016). Even so, calling behaviour in frogs in natural conditions may not be mirrored in captivity (Köhler *et al.*, 2017). On the other hand, Erdtmann & Amézquita (2009) summarized the advertisement call of *P. bicolor* using the data of only one individual whose origin is not stated and retrieved from a public database which is currently not accessible. Recently, we visited two populations in Santa Cecilia (Risaralda, Colombia) that match the phenotypic description (Myers *et al.*, 1978; Kahn *et al.*, 2016) and the geographic distribution (Myers & Böhme, 1996; Márquez *et al.*, 2020) of *P. bicolor*. This study aimed to describe the advertisement (AC) and courtship calls (CC) of *P. bicolor* in natural conditions.

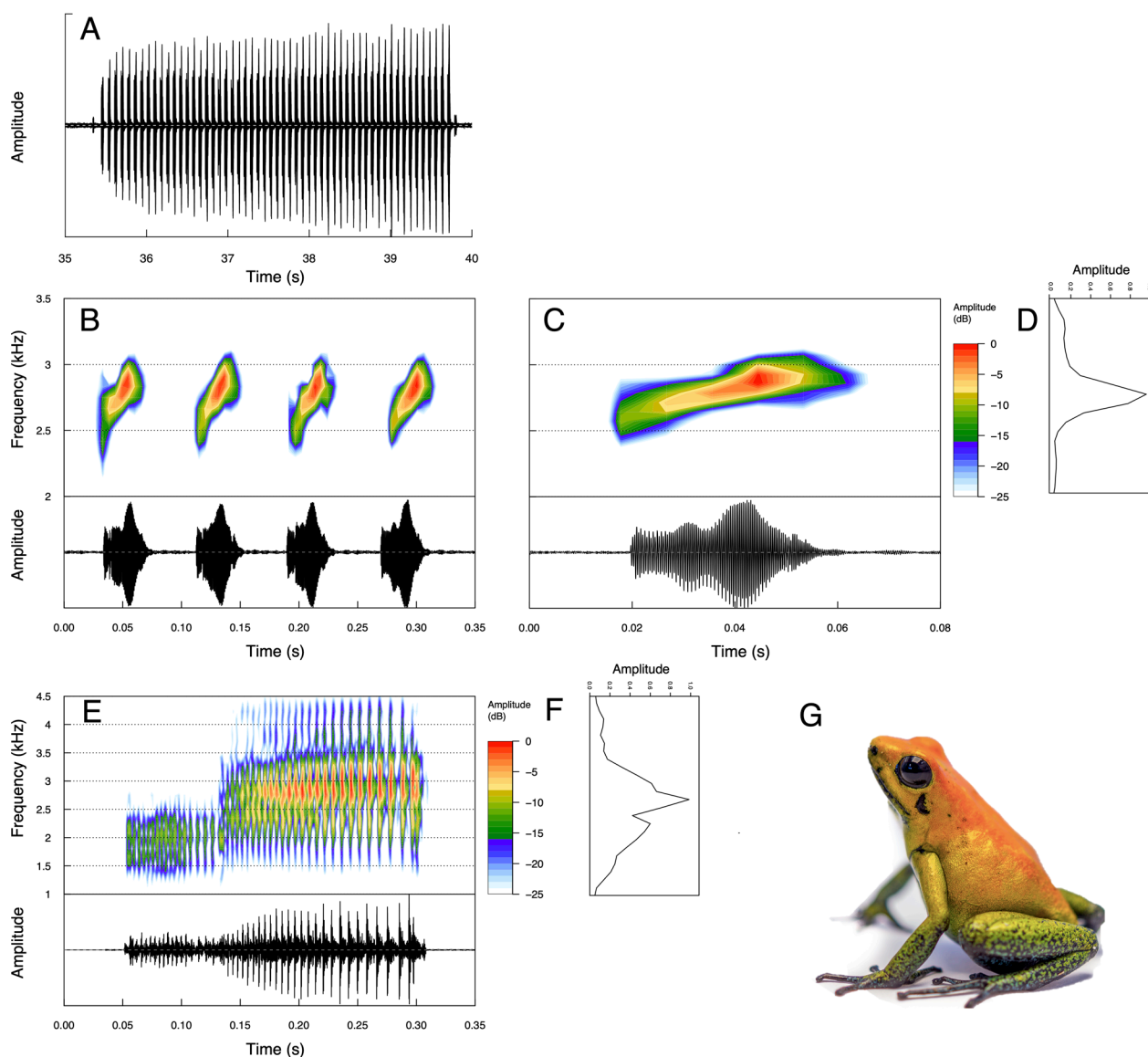
We recorded the AC of six individuals of *P. bicolor* in July 2018, in a secondary forest during early hours in the morning (6–9 am). For each individual, we recorded ~1 min of consecutive and spontaneous advertisement calls with a Zoom H4n pro digital recorder coupled to a directional (Sennheiser ME66/K6P) microphone placed about 2 m in front of the calling male. The CC was recorded for only one individual as the occurrence of both male and female is not common. Immediately after recording, we measured the temperature of the frog back using an infrared thermometer (Oakton model WD-35639) and captured the frog to measure its body size (snout-vent-length, SVL) to the nearest 0.01 mm with a digital calliper (Table 1).

We used the software Raven Pro 1.3 to measure four temporal (note duration, note interval, call duration and number of notes) and three spectral (note dominant frequency, note minimum frequency and note maximum frequency) features of the AC, and three temporal (call duration, pulse number and pulse duration) and six spectral (call and pulse dominant frequency, call and pulse minimum frequency, and call and pulse maximum frequency) features of the CC, based on Köhler *et al.* (2017). The recordings were analysed using a sampling rate of 44.1 kHz with an FFT of 512 points, allowing 50% of overlap between points, using the Blackman window type. Using identical parameters, the R package Seewave (Sueur *et al.*, 2008) was used to create the spectrograms and sonograms (Fig. 1). Sound recordings were deposited at the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt, Colombia (IAvH-CSA-34251 to IAvH-CSA-34257) and at the Macaulay library at the Cornell Laboratory of Ornithology. We reported measurements as mean  $\pm$  standard deviation (range).

Considering Köhler *et al.* (2017) classification, the AC of *P. bicolor* is best described as a call composed by a series of notes, in which each note is produced by a single expiration (Fig 1A, B, C). Each call has a duration of  $6.6 \pm 1$  s, composed by a series of  $75.4 \pm 6.9$  notes. The latter, in turn, are best described as unpulsed, tonal with frequency modulation (Fig 1C). Each note has an average duration of  $37.3 \pm 2$  ms which is similar to Erdtmann & Amézquita (2009) values

(38 ms) but with only some of the recordings of Zimmermann & Zimmermann (1985) ( $62.9 \pm 22.6$  ms). We argue that the greater variation and higher values in the latter description done in captivity, in comparison to our data, are probably due to reverberation effects, common in enclosures. On the other hand, the AC notes' frequency values of our study are accordant with previous reports (Table 1).

The courtship call of *P. bicolor* is best described as a single pulsatile harmonic note (Fig 1E). Given that it is emitted only in the presence of a female it has been described as a short-range call (Zimmermann, 1989). It is characterized by lower frequency and longer duration values in comparison to the notes of the AC (comparison performed only within the individual that issued the courtship call, CC: 2670 Hz and  $242.8 \pm 4.9$  ms, AC:  $2954 \pm 22$  Hz and  $34.7 \pm 1.6$  ms). Zimmermann & Zimmermann (1985) described longer CC than ours ( $606 \pm 243$  ms), probably due to reverberation effects as it happened for the AC (Table 2). We observed that the CC varied within the temporal domain (Fig 1E), therefore, we analysed the call taking measurements of the whole call but also at the initial, middle, and final parts of the signal (Table 2). Overall, this work expands our knowledge of Colombian endangered poison frogs and highlights the importance to study acoustic communication in natural conditions.



**FIGURE 1.** Advertisement call: sonogram of a series of notes (A), spectrogram and sonogram of four consecutive notes (B), spectrogram and sonogram of a single note (C), and the spectrum of power (D). Courtship call: spectrogram and sonogram of the whole signal (E), and spectrum of power (F). Individual of *P. bicolor*, photo taken by JHR (G).

**TABLE 1.** Spectral and temporal traits of the advertisement call of *Phyllobates bicolor*. Abbreviations: Ind (Individuals), T° (temperature in Celsius), SVL (snout vent-length), CD (call duration), ND (note duration), NI (note interval), NDF (note dominant frequency), NMINF (note minimum frequency), NMAXF (note maximum frequency), NN (number of notes). \* Individual who emitted the courtship call described and represented in Fig 1E and F. \*\*Call represented in Fig 1 A, B, C and D.

Species	Ind / calls/ T°/ SVL (cm)	CD (s)	ND (ms)	NI (ms)	NDF (Hz)	NMINF (Hz)	NMAXF (Hz)	NN
<i>P. bicolor</i> (This study)	1/	6.1	34.7 ± 1.6 (32–37)	44.4 ± 0.5 (44–45)	2954 ± 22 (2929–2972)	2355 ± 21 (2326–2369)	3074 ± 162 (3014–3531)	81
	23.7/ 3.5*							
	1/	8.4 ± 1.4 (6.4–9.7)	39.3 ± 2 (37–44)	53.3 ± 3.7 (49–60)	2366 ± 79 (2263–2497)	2274 ± 104 (2110–2411)	2480 ± 72 (2411–2670)	78.8 ± 12.8 (67–93)
	24.6/ 4.2							
	1/	6.5 ± 0.2 (6.4–6.6)	39.1 ± 2.3 (35–45)	60.3 ± 3 (55–66)	2182 ± 87 (1981–2283)	1988 ± 40 (1938–2110)	2275 ± 45 (2196–2369)	67.5 ± 4.9 (64–71)
	2/							
	24.3/ 4.5							
	1/	5.5 ± 0.9 (4.5–6.1)	35.0 ± 1.6 (31–39)	44.5 ± 2.1 (42–48)	2759 ± 42 (2713–2842)	2387 ± 124 (2153–2584)	2866 ± 45 (2799–2929)	66 ± 9.5 (56–75)
	3/							
	24.5/ 3.8**							
	1/	6.7 ± 0.4 (6.5–7)	38.4 ± 2 (34–43)	48.2 ± 2 (44–52)	2682 ± 111 (2584–2885)	2536 ± 29 (2455–2584)	2919 ± 47 (2842–3015)	78 ± 4.2 (75–81)
	2/							
	23.8/ 3.9							
	1/	6.7 ± 0.4 (6.3–7)	37.3 ± 2 (35–43)	43.8 ± 4.7 (38–50)	2762 ± 16 (2756–2799)	2606 ± 28 (2584–2670)	2845 ± 11 (2842–2885)	81.3 ± 3.8 (77–84)
	3/							
24.5/ 3.7								

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TABLE 1. (Continued)

Species	Ind / calls/ T°/ SVL (cm)	CD (s)	ND (ms)	NI (ms)	NDF (Hz)	NMINF (Hz)	NMAXF (Hz)	NN
<i>P. bicolor</i> (This study)	Average/	6.6 ± 1	37.3 ± 2	49.1 ± 6.6	2617 ± 287	2358 ± 218	2743 ± 301	75.4 ± 6.9
	-/	(5.5–8.4)	(34.7–39.3)	(43.8–60.3)	(2182–2954)	(1988–2606)	(2275–3074)	(66–81.3)
	24.2 ± 0.4							
	(23.7–24.6)/							
	3.9 ± 0.4							
	(3.5–4.5)							
<i>P. bicolor</i> (Erdtmann & Amézquita, 2009)	1/	-	38	45	2519	2448	2803	-
	1/							
	-/							
<i>P. bicolor</i> (Zimmermann & Zimmermann, 1985)	3.6							
	-/	3.8 ± 0.8	62.9 ± 22.6	48 ± 16	2450 ± 38.7	1987 ± 160	2749 ± 146	-
	15/							
	22–30/ 4.2							

**TABLE 2.** Spectral and temporal traits of the courtship call of *Phyllobates bicolor*. Abbreviations: Ind (Individuals), CD (call duration), CDF (call dominant frequency), CMINF (call minimum frequency), CMAXF (call maximum frequency), PN (pulse number), PD (pulse duration), PDF (pulse dominant frequency), PMINF (pulse minimum frequency), and PMA XF (pulse maximum frequency).

Species	Ind /calls	CD (ms)	CDF (Hz)	CMINF (Hz)	CMAXF (Hz)	PN
<i>P. bicolor</i> (This study)	1/2	242.8 ± 4.9 (239.3–246.3)	2670 ± 0	1852 ± 61 (1809–1895)	3187 ± 0	46 ± 0.7 (45–46)
<i>P. bicolor</i> (Zimmermann & Zimmermann, 1985)	-/12	606 ± 243	2375 ± 84	1739 ± 162	2875 ± 296	-

**TABLE 2. (Continued)**

Species	Call Section	PD (ms)	PDF (Hz)	PMINF (Hz)	PMA XF (Hz)
<i>P. bicolor</i> (This study)	Initial	4.2 ± 0.8 (3.5–6.4)	1947 ± 83 (1809–2067)	1370 ± 27 (1292–1378)	2248 ± 75 (2153–2412)
	Middle	5.8 ± 1.3 (4.6–8.5)	2607 ± 181 (2239–2842)	2059 ± 72 (1895–2153)	3132 ± 111 (3015–3359)
	Final	10.3 ± 2.4 (7.5–14.8)	2708 ± 63 (2670–2842)	2125 ± 61 (2067–2239)	3283 ± 100 (3187–3445)

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