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Advertisement call and geographical variation in call features of *Dendropsophus berthalutzae* (Anura: Hylidae) from the Atlantic Rainforest of southeastern Brazil

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Acoustic signals are the main mode of communication in anurans (Duellman & Trueb 1994). Calls produced by anurans play an essential role during their reproduction (Wells 1977) and advertisement calls emitted by males are, in many cases, species-specific, representing an important character for species recognition (Abrunhosa et al. 2001; Forti et al. 2010). Thus, the formal description of advertisement calls is relevant for anuran taxonomy (Pombal Jr. et al. 1995).

Dendropsophus berthalutzae (Bokermann, 1962) is a small treefrog belonging to the Dendropsophus microcephalus Group, a clade that also includes D. decipiens, D. haddadi, and D. oliveirai. These four species are characterized by laying eggs on leaves above water (Faivovich et al. 2005). Dendropsophus berthalutzae was described from Paranapiacaba, Santo André municipality, state of São Paulo, southeastern Brazil (Bokermann, 1962), but it is known from other localities in the southern and southeastern Brazilian coast (Frost 2011). The advertisement calls of the other three species in the group have been described elsewhere (Abrunhosa et al. 2001; Santana et al. 2011; Ruas et al. 2012). The vocalization of D. berthalutzae remains unknown and information on its natural history is scarce. We describe here the advertisement call of this species based on recordings from five populations, including that of the type-locality, and provide data on its calling site.

Field work was carried out in the state of São Paulo in five localities of Serra do Mar, a mountain chain that runs along the southeast coast of Brazil. The Serra do Mar is covered by Atlantic Rainforest and the regional climate is hot and wet, without a dry season (Morellato & Haddad 2000). Study sites were: (1) Parque do Zizo, Tapiraí municipality (24°01′ S, 47°48 W; 600 m a.s.l.), (2) Paranapiacaba, Santo André municipality (23°46′ S, 46°22′ W; 750 m a.s.l.), (3) Estação Ecológica Juréia-Itatins, Peruíbe municipality (24°24′ S, 47°05′ W; 140 m a.s.l.), (4) Parque Estadual da Ilha do Cardoso, Cananéia municipality (25°04′ S, 47°55′ W; 9 m a.s.l.) and (5) Parque Estadual da Serra do Mar – Núcleo Picinguaba, Ubatuba municipality (23°21′ S, 44°51′ W; 10 m a.s.l.).

In the vicinities of permanent pools and small swamps 20 males of *Dendropsophus berthalutzae* were recorded. The calling sites of 11 of them were characterized regarding perch nature and distance and height to water surface. Digital recordings were made with 48 KHz and 16 bit of resolution with Marantz PMD660 and PMD670 digital recorders, and Yoga EM 9600 and Sennheiser ME67/K6 microphones positioned about one meter from the calling males. Bioacoustics analysis were made with the software Raven 1.3 for Windows (Cornell Lab of Ornithology), using FFT (Fast Fourier Transformation) = 256 and Overlap = 50. We analyzed the following quantitative call features: (1) frequency band (KHz), (2) dominant frequency (Hz), (3) call duration (s), (4) note duration (s), (5) pulses per note and (6) repetition call rate. Male snout-vent length (SVL) was measured with digital calipers (to the nearest 0.1 mm) and mass was obtained with a Pesola® (to the nearest 0.1 g). Voucher specimens were deposited in the vertebrate collection of Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (ICMBio license number 23799-1), with the accessing codes VESALQ 600, 601, 602, 603, 604, 605, 606, 607, 608, 609 and 610.

Mean male snout-vent length was 22.0 ± 1.5 mm (N = 15) and mean male mass was 0.5 ± 0.1 g (N = 12). The SVL of the holotype is 20 mm (Bokermann 1962). The advertisement call of *Dendropsophus berthalutzae* consists of high frequency trills with multipulsed notes (2–6 pulses per note). We identified two call types: Call 1 has only one note and Call 2 is composed by a sequence of repeated notes that are emitted quickly (Fig. 1c, d). Call 2 seems to be used only in

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choruses. In fact, many frogs change their calls by increasing note repetition rate in dense choruses (Wells 2007). Mean minimum frequency was 3245 ± 358 Hz (N = 20) and mean maximum frequency was 5406 ± 274 Hz (N = 20). Dominanty frequency averaged 4041 ± 81 Hz (N = 16) (Fig. 1a, b). Call 1 duration averaged 0.042 ± 0.012 s (N = 139). Call 2 varied from 2 to 9 notes, and its duration averaged 0.306 ± 0.207 s (N = 48). The rate of call repetition averaged 11 ± 8 calls/min (N = 20).

TABLE 1. Geographical variation in call features (mean, \pm SD, range) of five populations of *Dendropsophus berthalutzae* from the state of São Paulo, southeastern Brazil. * Topotypic population.

Variable	Localities				
	Santo André*	Peruíbe	Cananéia	Tapiraí	Ubatuba
Mean note duration (s)	0.027 ± 0.009 (0.01-0.04) (N = 23)	0.049 ± 0.014 (0.027-0.096) (N = 49)	0.041 ± 0.009 (0.023-0.056) (N = 35)	0.046 ± 0.005 (0.039-0.055) (N = 7)	0.043 ± 0.015 (0.018-0.079) (N = 25)
Pulses per note	2–4	3–5	3–5	2–6	3–5
Mean dominant frequency (Hz)	4043 ± 106 (3881-4294) (N = 25)	$-4103 \pm 81 (3948 - 4281)$ (N = 25)	4042 ± 80 (3913–4175) (N = 36)	3925 ± 108 (3748-4107) (N = 30)	- 4053 ± 99 (3939- 4121) (N = 3)
Mean minimum frequency (Hz)	2781 ± 154 (2463-3031) (N = 25)	$-3380 \pm 262 (2761-3771)$ (N = 56)	- 3254 ± 189 (2899- 3556) (N = 46)	$-2919 \pm 145 (2655-3252)$ (N = 30)	- 3792 ± 226 (3414– 4315) (N = 30)
Mean maximum frequency (Hz)	5516 ± 184 (5224- 5944) (N = 25)	- 5279 ± 269 (4861- 5973) (N = 56)	- 5296 ± 272 (4857- 5837) (N = 46)	- 5298 ± 183 (5015- 5610) (N = 30)	- 5882 ± 262 (5183- 6296) (N = 30)
Call rate (calls/minute)	3–19	3.3-8	5.7–11.3	26–31	9–11.1
Mean SVL (mm)	-	$21.9 \pm 1.4 (20.2 - 23.7)$ (N = 6)	$22.3 \pm 1.0 (21.0 - 23.8)$ (N = 5)	23.3 ± 0.7 (22.6–23.9) (N = 3)	$19.9 \pm 0.2 (19.7 - 20.1)$ (N = 3)
Number of males analyzed	3	6	5	3	3

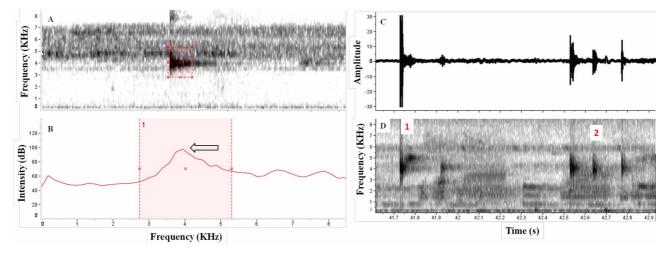


FIGURE 1. Sonogram (A) and intensity spectrum (B) of the advertisement call of a male of *Dendropsophus berthalutzae* from Peruíbe municipality, state of São Paulo, southeastern Brazil. The arrow indicates the dominant frequency. SVL = 23.4 mm; mass = 0.6 g; air temperature = 24°C; relative humidity = 93%. Call recorded on12 December 2010. Oscilogram (C) and sonogram (D) of the vocalization of a male of *Dendropsophus berthalutzae* from Paranapiacaba, Santo André municipality, state of São Paulo, southeastern Brazil, showing Call 1 and Call 2. Air temperature = 23°C; call recorded on 29 January 2011. Data on SVL and mass of this individual and on air relative humidity are not available.

The advertisement call of *D. berthalutzae* differs from that of *D. decipiens* in its lower dominant frequency (4770–5230 KHz in *D. decipiens*) and shorter call duration $(1.10 \pm 0.30 \text{ s in } D. decipiens)$ (Abrunhosa et al. 2001). Like *Dendropsophus berthalutzae*, *D. oliveirai* and *D. haddadi* also emit multipulsed notes, but the call of these species have higher dominant frequency (6038 \pm 118 Hz, 6144 \pm 417 Hz, and 4644 \pm 155 Hz, respectively) (Santana et al. 2011; Ruas et al. 2012). However, the call of *D. oliverai* is longer (0.062–0.074 s) and may attain a higher number of pulses per note

(5–14) (Santana et al. 2011). Additionally, the call of *D. haddadi* is shorter than that of *D. berthalutzae* (Ruas et al. 2012). The different populations of *D. berthalutzae* are similar regarding call features, except by the shorter note duration of the population from Santo André and the higher frequency call of the males from Ubatuba (Table 1). However, the relatively low number of males recorded at each locality does not permit robust statistical inferences. We suggest that the small differences detected in the Ubatuba population could be considered intraspecific variation (an "accent"), which could be explained by the geographical isolation of this population due to the mountainous relief of the northern portion of the coast of the state of São Paulo.

Like other species in the *Dendropsophus microcephalus* Group, males of *D. berthalutzae* called perched on herbs, grasses, and shrubs of the margins of lentic water bodies. The mean height of the perch was 44.2 ± 18.5 cm (N = 11) and the mean distance from perch to water varied from 0 to 25 cm (N = 11). The characteristics of *D. berthalutzae* calling sites are similar to those found to the other species of the Group: *D. bipunctatus*, *D. decipiens* (Abrunhosa et al. 2001), *D. nanus*, *D. sanborni* (Menin et al. 2005), and *D. haddadi* (Ruas et al. 2012).

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