

Redescription of the advertisement call of *Brachycephalus tridactylus* (Anura: Brachycephalidae)

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Abstract

Redescription of the advertisement call of *Brachycephalus tridactylus* (Anura: Brachycephalidae). Miniaturized frogs of the genus *Brachycephalus* have restricted geographical distributions throughout the Atlantic Forest. Ecological data for most species are scarce and advertisement calls have been described for only 12 of the 36 known species, including *B. tridactylus*. The considerable duration of note length of the advertisement call of *B. tridactylus* has been mentioned in comparative studies of vocalizations in *Brachycephalus*. The single original recording of *B. tridactylus*, together with recordings made in March 2016 at the type locality, were analyzed to document any variation and redescribe the advertisement call of the species. In the recent recordings of *B. tridactylus*, the average length of the call is 50.8 s and the call is composed of 10–13 notes emitted at a note rate of 3.7–8.3 notes per min. The notes are isolated and composed of 1–3 pulses, with the note duration varying from 0.002–0.021 s. These values are not consistent with the original description of the call of *B. tridactylus*. The note duration is 89% shorter and the original description does not include notes with 1–3 pulses. The notes of *B. tridactylus* have few pulses, whereas those of *B. ephippium* have several. The advertisement call of *B. tridactylus* differs from that of *B. albolineatus* and *B. mirissimus* by having only isolated notes.

Keywords: bioacoustics, Brazilian Atlantic Forest, isolated notes, note-centered approach.

Resumo

Redescrição do canto de anúncio de *Brachycephalus tridactylus* (Anura: Brachycephalidae). Os anuros miniaturizados do gênero *Brachycephalus* apresentam distribuição geográfica restrita ao longo da Floresta Atlântica. Dados ecológicos da maioria das espécies são escassos e cantos de anúncio foram descritos de apenas 12 das 36 espécies conhecidas, incluindo *B. tridactylus*. A considerável duração das notas do canto de anúncio de *B. tridactylus* foi mencionada em um estudo comparativo de vocalizações de *Brachycephalus*. A única gravação original de *B. tridactylus* e

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gravações feitas em março de 2016 na localidade tipo foram analisadas para documentar alguma variação e redescrever o canto de anúncio da espécie. Nas recentes gravações de *B. tridactylus*, o canto de anúncio dura em média 50,8 s e é composto por 10–13 notas emitidas a uma taxa de 3,7–8,3 notas por min. As notas são isoladas, compostas por 1–3 pulsos e duram de 0,002 a 0,021 s. Esses valores não são consistentes com a descrição original do canto de anúncio de *B. tridactylus*. A duração da nota é 89% menor e a descrição original não inclui notas com 1–3 pulsos. As notas de *B. tridactylus* têm poucos pulsos, enquanto que as de *B. ephippium* têm vários. O canto de anúncio de *B. tridactylus* difere daquele de *B. albolineatus* e *B. mirissimus* por ter apenas notas isoladas.

Palavras-chave: abordagem centrada em notas, bioacústica, Floresta Atlântica brasileira, notas isoladas.

Introduction

Brachycephalus currently contains 36 species (Frost 2018) of small toads (< 2.5 cm in body length) endemic to the Atlantic Forest of Brazil. The smallest species of the genus are among the smallest terrestrial vertebrates in the world (Rittmeyer et al. 2012); they have minute bodies and a reduction in the number of digits (Hanken and Wake 1993, Yeh 2002, Clemente-Carvalho et al. 2009). Some species have highly restricted geographical distributions (Bornschein et al. 2016). The bright colors of many species may be related to aposematism in association with neurotoxins in the skin (e.g., Sebben et al. 1986, Pires Jr. et al. 2002, 2003, 2005).

Most species of *Brachycephalus* are found on the forest floor at high altitudes in the mountains of the Serra do Mar in the Atlantic Forest Domain (up to 1900 m a.s.l.; Bornschein et al. 2016). Although all species are diurnal and locally abundant (Bornschein et al. 2018), they are difficult to find owing to their restricted ranges and microendemism. In the last decade, intensified fieldwork has led to the description of 23 species (Frost 2018), but data on natural history lag behind the rate of new species descriptions. Advertisement calls have been described for 12 of 36 species (33%): *B. actaeus* Monteiro, Condez, Garcia, Comitti, Amaral, and Haddad, 2018 (Monteiro et al. 2018a); *B. ephippium* (Spix, 1824) (Pombal et al. 1994, Goutte et al. 2017); *B. albolineatus* Bornschein,

Ribeiro, Blackburn, Stanley, and Pie, 2016 (Bornschein et al. 2018); *B. crispus* Condez, Clemente-Carvalho, Haddad, and Reis, 2014 (Condez et al. 2014); *B. darkside* Guimarães, Luz, Rocha, and Feio, 2017 (Guimarães et al. 2017); *B. hermogenesi* (Giaretta and Sawaya, 1998) (Verdade et al. 2008); *B. mirissimus* Pie, Ribeiro, Confetti, Nadaline, and Bornschein, 2018 (Pie et al. 2018); *B. olivaceus* Bornschein, Morato, Firkowski, Ribeiro and Pie, 2015 (Monteiro et al. 2018b); *B. pitanga* Alves, Sawaya, Reis, and Haddad, 2009 (Araújo et al. 2012, Tandel et al. 2014, Goutte et al. 2017); *B. quiririensis* Pie and Ribeiro, 2015 (Monteiro et al. 2018b); *B. sulfuratus* Condez, Monteiro, Comitti, Garcia, Amaral, and Haddad, 2016 (Condez et al. 2016); and *B. tridactylus* Garey, Lima, Hartmann, and Haddad, 2012 (Garey et al. 2012).

Brachycephalus tridactylus was described based on a type series of seven specimens collected at the type locality, Reserva Particular do Patrimônio Natural Salto Morato, Paraná, southern Brazil (Bornschein et al. 2015). The species is a micro-endemic frog that occupies an estimated 41 ha of montane forests in the Atlantic Forest (Bornschein et al. 2016). The description of the advertisement call of the new species was based on the calls of 17 individuals (Garey et al. 2012). Bornschein et al. (2018) reported that the note length of *B. tridactylus* is greater than that of *B. albolineatus*. To confirm this apparent difference in the duration of notes, the original recordings of *B. tridactylus* were reanalyzed and

compared with our recent recordings of the species. A description of the advertisement call of *B. tridactylus* follows.

Materials and Methods

Original Recordings of B. tridactylus from Garey et al. (2012)

Only one of the original recordings of *Brachycephalus tridactylus* described by Garey *et al.* (2012) was deposited in the sound collection of Fonoteca Neotropical Jacques Viellard (FNJV 0032950) and this recording was made by André M.X. Lima. The other recordings were lost with the theft of the personal computer on which the audio files were stored (A. M. X. Lima pers. comm. 2018).

Our Recordings of B. tridactylus

We visited the type locality of *Brachycephalus tridactylus* (*sensu* Bornschein *et al.* 2015) to record the frogs on 10 March 2016 at the Reserva Particular do Patrimônio Natural Salto Morato (RPPN-SM; 25°08'09" S, 48°17'59" W) in the municipality of Guaraqueçaba and state of Paraná in southern Brazil. Voucher specimens are deposited in the Museu de História Natural Capão da Imbuia (MHNCI 10294, 10730), Curitiba, state of Paraná, Brazil, and in the Célio F. B. Haddad collection (CFBH 43887–9), Departamento de Zoologia, Universidade Estadual Paulista, Campus de Rio Claro, state of São Paulo, Brazil.

Calls were recorded between 14:00 and 16:45 h when the air temperature was 24.0°C, the soil temperature was 20.9°C, and the relative humidity was 99%. Numbered ribbons were affixed to the vegetation above the individuals recorded to determine whether new recordings were from the same or a new individual. Calls were recorded with digital recorders (Sony PCM-D50 and PCM-M10) with a sampling frequency rate of 44.1 kHz and 16-bit resolution, connected to Sennheiser ME 66 microphones.

All recordings are deposited in MHNCI (035–051). We shifted the position of the microphone slightly (along 1 m²) and used the level of the input signal of the recording to locate the calling frog. Consequently, the intensity of audio capture during recording varies slightly.

Advertisement Call Analysis

Sound samples were analyzed with Raven Pro 1.5.0 Build 40 (Bioacoustics Research Program 2017). Time domain variables were measured from oscillograms and frequency domain variables were measured from spectrograms. Spectrogram parameters were defined with a 256-point Fast Fourier Transform (FFT), a 3-dB Filter bandwidth of 492 Hz, DFT 256 samples, Hann window, 50% overlap, and spectrogram color schemes of Standard Gamma II and Jet Black. Final spectrograms, as well as diagnostic plots, were generated using the seewave package, v. 2.1.0 (Sueur 2018) in R version 3.5.1 (R Development Core Team 2018) with the same window size and overlap settings as in Raven Pro.

The advertisement calls were described per the parameters, criteria, and the note-centered approach of Köhler *et al.* (2017). Likewise, we adopted the general presentation order and particularities in Köhler's parameters, as presented in Bornschein *et al.* (2018). We considered all parameters used in describing the call of *Brachycephalus albolineatus* (Bornschein *et al.* 2018), but we used only those applicable to the call characteristics of *B. tridactylus*, as follow: (1) call duration (s); (2) note rate (notes per min); (3) number of notes per call; (4) number of pulses per notes; (5) note duration (s); (6) inter-note interval (s); (7) note dominant frequency (kHz); (8) highest frequency (kHz); and (9) lowest frequency (kHz). Call duration is defined as the time span from the beginning of the first note to the end of the last note emitted in a call. Note rate is the number of notes emitted from the first note to the beginning of the last note (not including the last note). The inter-note

interval is the time interval between two consecutive notes. The highest and lowest frequencies were measured from notes. All measurements were made from the fundamental frequency. Additional information on measurements is available in Figure 1 of Bornschein *et al.* (2018).

Results

Analysis of the Original Recording of Brachycephalus tridactylus

In the original recording of the call of *Brachycephalus tridactylus* (FNJV 0032950), the call lasted 50 s and included eight notes. The entire recording is noisy (signal-to-noise ratio of -6 dB) and has more signals that are not associated with the notes of *B. tridactylus*. There is no acoustic information above 10 kHz. The sampling rate of this recording is set to 48 kHz; thus, it seems that the audio was low-pass filtered with a cutoff frequency of 10 kHz.

The notes of the recording that we heard overlap with other signals, probably reverberation and/or environmental noise (e.g., rain noise; Figure 1A). This prevented us from determining the note length and recognizing and measuring other acoustic parameters, such as the number of pulses and upper and lower frequency limits. There is a concentration of energy at the beginning of the note emission, coincident with the auditory recognition of the note, but the energy is prolonged and attenuates gradually (Figure 1A).

Analysis of Our Recordings of Brachycephalus tridactylus

We recorded calls of 15 individual *Brachycephalus tridactylus*; five of the frogs were collected as vouchers. However, the number of individuals analyzed for each call parameter varied from 5–14 individuals (Table 1). A total of 17 separate recordings (MHNCI 035–051) were deposited in the collection.

The calls that we recorded are of the same type as that of the original recording (FNJV 0032950). The energy signals match our recorded sounds (Figure 1B) but they have a different pattern than those of the original recording (Figure 1A). Consequently, we present a redescription of the advertisement call of the species. The average length of the advertisement call of *Brachycephalus tridactylus* is 50.793 s (± 11.708 s; Table 1; Figure 2A). After emitting a call, the frog might remain silent for several minutes—sometimes for more than 30 min. The note rate is 3.745–8.330 notes per min ($= 5.159 \pm 1.347$ notes per min; Table 1). Advertisement calls of *B. tridactylus* are composed of 10–13 notes ($= 11.333 \pm 0.976$ notes; Table 1; Figure 1). The call consists only of isolated notes. The notes have 1–3 pulses ($= 1.968 \pm 0.542$ pulse; Figures 1B and 2B–D; Table 1) and the note duration varies from 0.002–0.021 s ($= 0.012 \pm 0.005$ s; Table 1). The average inter-note interval is 5.325 s (3.269–9.806 s ± 1.447 s; Table 1). The dominant frequency varies from 4.134–5.426 kHz ($= 5.062 \pm 0.303$ kHz; Table 1). The highest frequency of the notes varies from 5.682–7.488 kHz ($= 6.445 \pm 0.447$ kHz), whereas the lowest frequency ranges from 2.718–4.605 kHz ($= 3.579 \pm 0.474$ kHz; Table 1). There are no harmonics; therefore, the measurements are of fundamental frequencies.

The number of pulses per note tends to be constant in the advertisement calls and includes only one or two pulses; however, in some advertisement calls, notes might have two or three pulses (Table 2). Intra-individual variation in the number of pulses per note is presented in Table 2.

Discussion

We have determined that the acoustic energy of the notes in the original recording (FNJV 0032950) overlaps with reverberation and/or environmental noise, thereby preventing us, as well as Garey *et al.* (2012), to provide a complete description of the advertisement call of

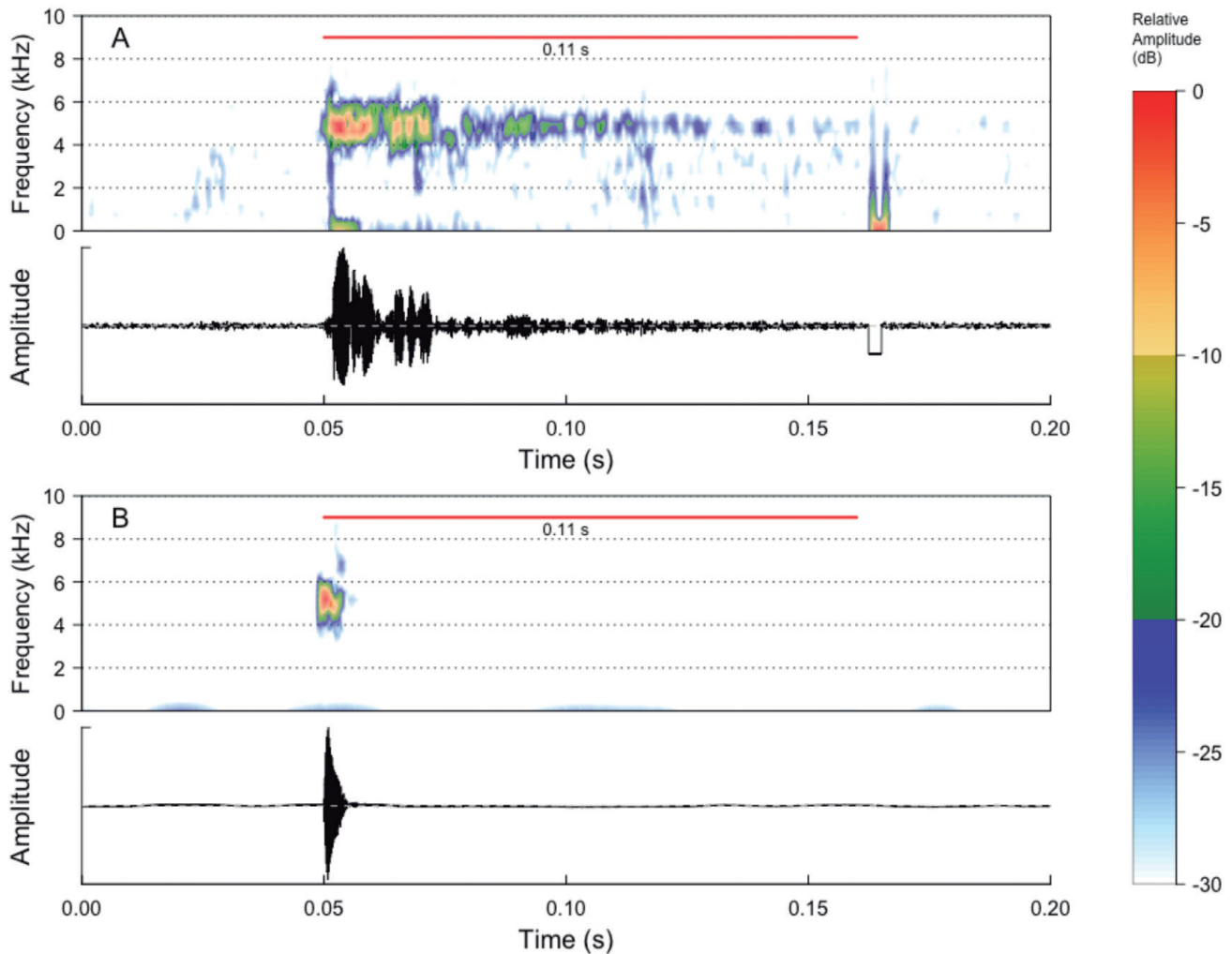


Figure 1. Spectrograms of *Brachycephalus tridactylus* produced for comparison between the audio recording in the FNJV (0032950) and one of our own recordings, built with the same parameters. **A.** Example of one note from the original recording (FNJV 0032950). Notice that it overlaps with other signals. **B.** Example of a note with one pulse obtained from our recordings (MHNCI 043; individual collected and housed at CFBH 43887). The horizontal red bar represents the mean of note duration (0.11 s) of the species according to the description in Garey *et al.* (2012). FFT size of 128 points, Hann window, overlap of 88%.

Brachycephalus tridactylus. Thus, the notes described in Figures 3A and B of Garey *et al.* (2012) and shown in Figure 1A are overlapped by the energy of notes representing reverberation and/or noise.

The original description of advertisement call of *Brachycephalus tridactylus* stated that the call had “one single short note (0.11 ± 0.02 s), with a dominant frequency of 4.8 ± 0.2 kHz. The frequency range is 3.2–6.4 kHz at the beginning

of the note, whereas at the end it decreases to 4.3–5.3 kHz (Figures 3A,B)” (Garey *et al.* 2012; Table 1). The note duration is 89% shorter (Figure 1) in our recordings of *B. tridactylus* and ranges from 0.002–0.021 s ($\bar{x} = 0.012$ s \pm 0.005 s). We also have documented the existence of notes with 1–3 pulses, a feature not observed in the original description (Garey *et al.* 2012). Condez *et al.* (2016) mentioned that *B. tridactylus* has notes with 1–3 pulses based on Garey *et al.*

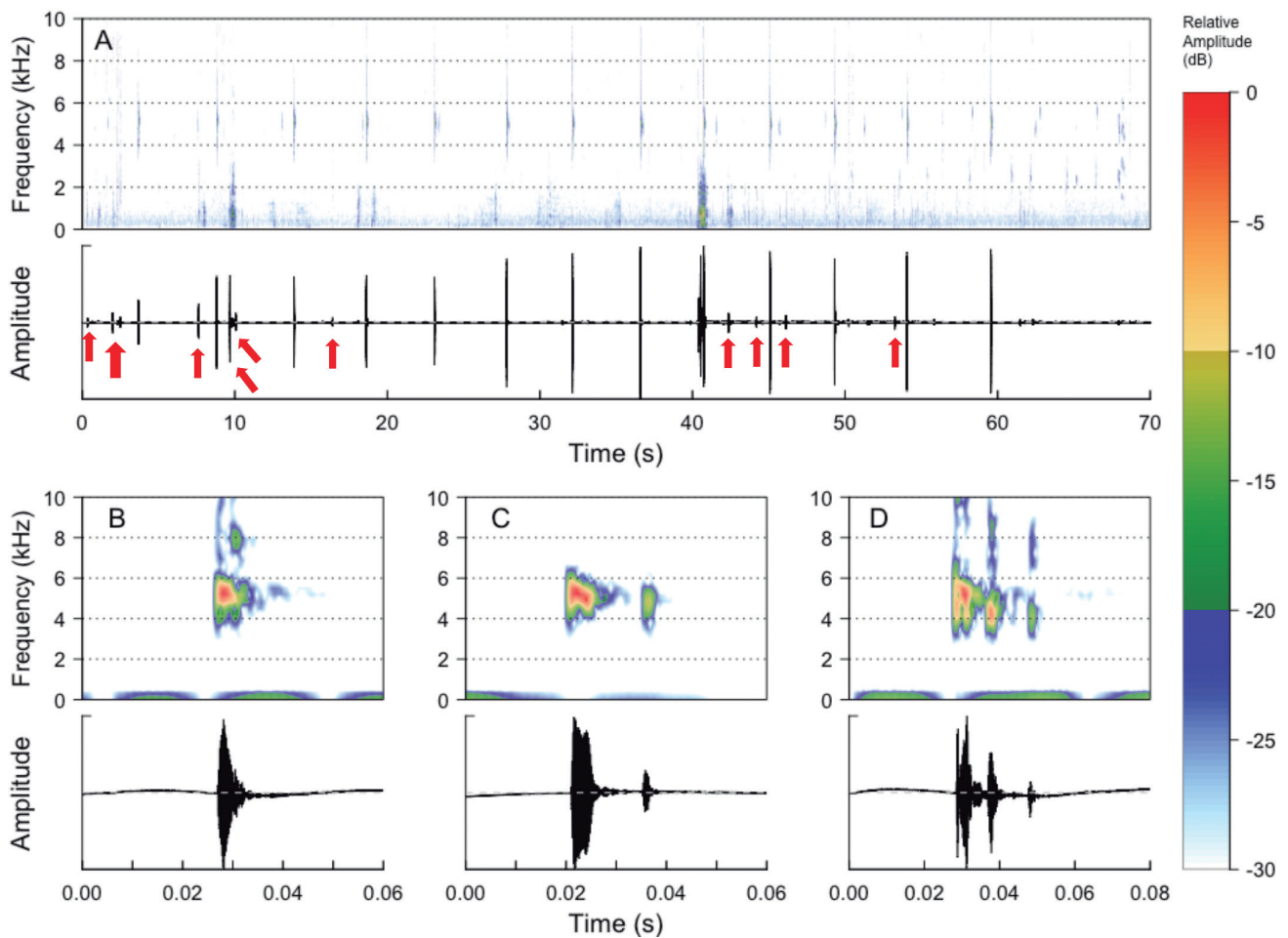


Figure 2. Example of an entire advertisement call and also notes of other advertisement calls of *Brachycephalus tridactylus*. **A.** Entire advertisement call (MHNCI 049; individual not collected), with 13 notes; arrows indicate noise signals. **B.** Note with one pulse (MHNCI 043; individual collected and housed at CFBH 43887). **C.** Note with two pulses (MHNCI 046; individual collected and housed at MHNCI 10730). **D.** Note with three pulses (MHNCI 040; individual collected and housed at CFBH 43889). Spectrograms produced with FFT size of 128 points, Hann window, and overlap of 88%.

(2012), but this information does not appear in Garey *et al.* (2012). Last, we did not record the gradual decrease in frequency described by Garey *et al.* (2012).

Short notes are characteristic of all species of the *Brachycephalus pernix* Group, which includes species from southern Brazil. The mean note lengths of this group are, as follow: 0.010 s for *B. mirissimus* (Pie *et al.* 2018); 0.012 s for *B. tridactylus* (this study); 0.020 s for *B. albolineatus* (Bornschein *et al.* 2018); 0.04 s for *B. actaeus* (Monteiro *et al.* 2018a); 0.04 s for *B. olivaceus*

(Monteiro *et al.* 2018b); and 0.05 s for *B. quiririensis* (Monteiro *et al.* 2018b). Pulsed notes are characteristic of all species of *Brachycephalus* (compilations in Monteiro *et al.* 2018a,b, Pie *et al.* 2018, and this study). Members of the *B. ephippium* and *B. hermogenesi* Groups have notes with several pulses, as follow: 5–8 in *B. darkside* (Guimarães *et al.* 2017); 5–15 in *B. ephippium* (Pombal Jr. *et al.* 1994); 7–11 in *B. sulfuratus* (Condez *et al.* 2016); 7–12 in *B. crispus* (Condez *et al.* 2014); and 7–14 in *B. pitanga* (Tandel *et al.* 2014). These values are

Table 1. Measurements of acoustic parameters of the advertisement call of *Brachycephalus tridactylus*. ¹*N* = 17 individuals. ²*N* = 5 call durations are fragments of the entire call (Table 2). ³Same as note duration if applicable the call-centered approach of Köhler *et al.* (2017). ⁴Includes notes counted before the beginning of the recording (Table 2). ⁵Not applicable under note-centered approach used here. ⁶The original description stated that “frequency range is 3.2–6.4 kHz at the beginning of the note, whereas at the end it decreases to 4.3–5.3 kHz”.

Variable	Present description					Original description (Garey <i>et al.</i> 2012) ¹		
	Range	Mean	SD	<i>N</i>		Range	Mean	SD
				Samples	Individuals			
Call duration (s)	33.713–65.937	50.793	11.708	7 ²	6	- ³	- ³	- ³
Call rate (call per second)	-	-	-	-	-	?	0.16	0.03
Note rate (notes per minute)	3.745–8.330	5.159	1.347	17	14	-	-	-
Number of notes per call	10–13 ⁴	11.333	0.976	7	6	- ⁵	- ⁵	- ⁵
Number of pulses per notes	1–3	1.968	0.542	62	8	0	-	-
Note duration (s)	0.002–0.021	0.012	0.005	49	7	?	0.11	0.02
Inter-note interval (s)	3.269–9.806	5.325	1.447	51	10	- ⁵	- ⁵	- ⁵
Note dominant frequency (kHz)	4.134–5.426	5.062	0.303	44	5	?	4.8	0.2
Highest frequency (kHz)	5.682–7.488	6.445	0.447	54	12	- ⁶	-	-
Lowest frequency (kHz)	2.718–4.605	3.579	0.474	54	12	- ⁶	-	-

Table 2. Number of pulses per note (separated by commas) of the advertisement calls of *Brachycephalus tridactylus*.

Individual (call voucher number)	Number of pulses per notes	Number of notes heard being emitted before recording the advertisement call
1 (MHNCI 035)	2, 3, 2, 2, 2, 2	4
2 (MHNCI 040)	2, 2, 2, 2, 3, 3, 3, 2	3
2 (MHNCI 041)	2, 2, 2, 2, 2, 2, 2	4
3 (MHNCI 042)	3, 2, 2	?
4 (MHNCI 043)	1, 1, 1, 1, 1, 1, 1, 1, 1, 1	2
5 (MHNCI 044)	3	?
6 (MHNCI 045)	2, 2, 2, 2, 2, 2, 2, 2, 2, 2	2
7 (MHNCI 046)	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2	1
8 (MHNCI 049)	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2	0


much higher than those of the *B. pernix* Group: 1–3 in *B. albolineatus* (Bornschein et al. 2018), *B. mirissimus* (Pie et al. 2018), and *B. tridactylus* (this study); 2–3 in *B. actaeus* (Monteiro et al. 2018a) and *B. olivaceus* (Monteiro et al. 2018b), and 3–4 in *B. quiririensis* (Monteiro et al. 2018b). Although Verdade et al. (2008) did not report the pulses in the notes of the call of *B. hermogenesi* of the *B. didactylus* Group, their figures 6B and C seem to depict at least two pulses.

The inter- and intra-individual variation in the number of notes and pulses per note in the advertisement calls of *Brachycephalus tridactylus* described here might reflect individual's arousal level. Similar variation in the numbers of notes and pulses was reported for *B. albolineatus* (Bornschein et al. 2018) and a possible relationship with individual arousal was also suggested.

In *Brachycephalus albolineatus* and *B. mirissimus* (Pie et al. 2018), the advertisement calls are composed of isolated notes and note groups (*sensu* Köhler et al. 2017), in contrast with the advertisement call of *B. tridactylus*, for which we recorded only isolated notes. The advertisement calls of *B. actaeus* (Monteiro et al. 2018a), *B. quiririensis*, and *B. olivaceus* (Monteiro et al. 2018b) were described with the call-centered approach of Köhler et al. (2017) by which a single note represents the call, instead of the note-centered approach of the present description. Thus, the possibility that the calls of these species include isolated notes and note groups cannot be assessed. A note group emitted by a *Brachycephalus* would invariably be treated as two notes (or more, if the case) under the call-centered approach.

Brachycephalus communication is a topic of increasing interest and we are still far from a comprehensive understanding of their evolution. Much still can be found in terms of structural and temporal variation in the advertisement calls of the species of the genus, as well as possible the phylogenetic distribution of this variation.

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