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

Marcos R. Bornschein [Corresp., 1, 2], **Mario M. Rollo Jr.** [1], **Marcio R. Pie** [2, 3], **André E. Confetti** [4], **Luiz F. Ribeiro** [2, 5]

[1] Instituto de Biociências, Universidade Estadual Paulista, São Vicente, São Paulo, Brazil

[2] Mater Natura - Instituto de Estudos Ambientais, Curitiba, Paraná, Brazil

[3] Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil

[4] Programa de Pós-Graduação em Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil

[5] Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil

Corresponding Author: Marcos R. Bornschein
Email address: bornschein.marcao@gmail.com

Background. *Brachycephalus* includes miniaturized frogs with restricted geographical distributions throughout the Brazilian Atlantic Forest. Ecological data for most species are still scarce. For instance, advertisement calls have only been described for 12 of the 36 known species, including *B. tridactylus*, a recently described species from southern Brazil. Posteriorly, features of the advertisement call of *B. tridactylus* were compared with congeners and the unique characteristics of its call were highlighted. To confirm these potentially divergent characteristics, we reanalysed an original recording of *B. tridactylus* and analysed our own recordings and verified that the original description of its advertisement call is inaccurate. Thus, we redescribe its advertisement calls.

Methods. We asked the descriptors of *B. tridactylus* the original recordings that they made and requested access to the only original recording deposited in a collection of sounds. We received from André Lima a copy of one recording, the same as the one that had deposited, and obtained permission to re-analyze it. We studied this recording and compared it with our own recordings, made at the type locality of the species on March, 2016. Sound samples were analysed with Raven Pro 1.5.0 and call analyses were made under a note-centered approach.

Results. The original recording was amplified somehow by at least 6 dB and was also clearly low-pass filtered with a cutoff frequency of 10 kHz. Our analyses did not allow us to recognize several of the acoustic parameters normally described in *Brachycephalus*. The sound we heard from the notes overlapped with other signals (noise?), which prevented us from clearly determining the end of the note and other important features, such as the presence of pulses. According to our recordings (n = 15 individuals), *B. tridactylus* emitted a relatively long advertisement call (50.8 s, on average), composed by 10–13 notes emitted in a note rate of 3.7–8.3 notes per minute. Only isolated notes were present. The notes were composed by 1–3 pulses and the note duration varied from 0.002–0.021 s.

Discussion. The original description of the call of *B. tridactylus* is incorrect because it included background noise and amplification artefacts as part of the call parameters. However, we recognize that the original recording and our recordings have captured the same type of call. In our measurements of the species calls, note duration was nearly an order of magnitude shorter as the original description. The existence of notes with 1–3 pulses was not acknowledged in the original description. With few pulses per notes, the advertisement call of *B. tridactylus* is distinct from the notes with several pulses of *B. ephippium*, *B. pitanga*, *B. crispus*, *B. sulfuratus*, and *B. darkside*. The advertisement calls of *B. tridactylus* is also distinct from that of *B. albolineatus* and *B. mirissimus* by having only isolates notes, instead of

isolated notes and note groups.

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Marcos R. Bornschein[1, 2, 6], Mario M. Rollo Jr.[1], Marcio R. Pie[2, 3], André E. Confetti[4] & Luiz F. Ribeiro[2, 5]

[1]*Universidade Estadual Paulista (UNESP), Campus do Litoral Paulista, São Vicente, São Paulo, Brazil*
[2]*Mater Natura – Instituto de Estudos Ambientais, Curitiba, Paraná, Brazil*
[3]*Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil*
[4]*Programa de Pós-Graduação em Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil*
[5]*Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil*
[6]*Corresponding author. E-mail: bornschein.marcao@gmail.com*

Abstract

Background. *Brachycephalus* includes miniaturized frogs with restricted geographical distributions throughout the Brazilian Atlantic Forest. Ecological data for most species are still scarce. For instance, advertisement calls have only been described for 12 of the 36 known species, including *B. tridactylus*, a recently described species from southern Brazil. Posteriorly, features of the advertisement call of *B. tridactylus* were compared with congeners and the unique characteristics of its call were highlighted. To confirm these potentially divergent characteristics, we reanalysed an original recording of *B. tridactylus* and analysed our own recordings and verified that the original description of its advertisement call is inaccurate. Thus, we redescribe its advertisement calls.

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Results. The original recording was amplified somehow by at least 6 dB and was also clearly low-pass filtered with a cutoff frequency of 10 kHz. Our analyses did not allow us to recognize several of the acoustic parameters normally described in *Brachycephalus*. The sound we heard from the notes overlapped with other signals (noise?), which prevented us from clearly determining the end of the note and other important features, such as the presence of pulses. According to our recordings (n = 15 individuals), *B. tridactylus* emitted a relatively long advertisement call (50.8 s, on average), composed by 10–13 notes emitted in a note rate of 3.7–

8.3 notes per minute. Only isolated notes were present. The notes were composed by 1–3 pulses and the note duration varied from 0.002–0.021 s.

Discussion. The original description of the call of *B. tridactylus* is incorrect because it included background noise and amplification artefacts as part of the call parameters. However, we recognize that the original recording and our recordings have captured the same type of call. In our measurements of the species calls, note duration was nearly an order of magnitude shorter as the original description. The existence of notes with 1–3 pulses was not acknowledged in the original description. With few pulses per notes, the advertisement call of *B. tridactylus* is distinct from the notes with several pulses of *B. ephippium*, *B. pitanga*, *B. crispus*, *B. sulfuratus*, and *B. darkside*. The advertisement calls of *B. tridactylus* is also distinct from that of *B. albolineatus* and *B. mirissimus* by having only isolates notes, instead of isolated notes and note groups.

Introduction

The genus *Brachycephalus* currently comprises 36 species (Frost 2018, Pie et al. 2018) of small toadlets (< 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 possess not only minute bodies but also a reduction in the number of digits (Hanken & Wake 1993; Yeh 2002; Clemente-Carvalho et al. 2009). Some species present highly restricted geographical distributions, comparable to the animals with smallest extents of occurrence in the world (Bornschein et al. 2016). Many species are brightly colored, which may be related to aposematism in association with neurotoxins in the skin of some species (Sebben et al. 1986; Pires Jr. et al. 2002, 2003, 2005; Schwartz et al. 2007).

Most *Brachycephalus* species occur at high altitudes, in the forest floor of mountaintops of the Serra do Mar (up to 1900 m above sea level; Bornschein et al. [2016]). Although all the species are diurnal and locally abundant (Bornschein et al. 2018), their restricted sites of occurrence and microendemism make difficult to locate them in the field. Therefore, it is not surprising that more extensive fieldwork led to the description of 23 species in the last 10 years (Frost 2018, Pie et al. 2018). Unfortunately, this high level of new descriptions has not been followed by similar rate of ecological and related studies describing their diet, behaviour, and calls, among other topics. Advertisement calls were described for 33% of the species, specifically for *B. ephippium* (Pombal Jr. et al. 1994; Goutte et al. 2017), *B. hermogenesi* (Verdade et al. 2008), *B. pitanga* (Araújo et al. 2012; Tandel et al. 2014; Goutte et al. 2017), *B. tridactylus* (Garey et al. 2012), *B. crispus* (Condez et al. 2014), *B. sulfuratus* (Condez et al. 2016), *B. darkside* (Guimarães et al. 2017), *B. actaeus* (Monteiro et al. 2018a), *B. albolineatus* (Bornschein et al. 2018), *B. quiririensis* and *B. olivaceus* (Monteiro et al. 2018b), and *B. mirissimus* (Pie et al. 2018).

Brachycephalus tridactylus was recently described based on a series of seven specimens (Garey et al. 2012) collected at the type locality. The species is a micro-endemic frog that occurs in an estimated 41 ha of montane forests in the Atlantic Forest of the northern state of Paraná,

southern Brazil (Bornschein et al. 2016). The original description included the characterization of the advertisement call of the new species, based on the calls of 17 individuals (Garey et al. 2012). Posteriorly, the great difference of the note length of *B. tridactylus* (Garey et al. 2012) in comparison with the one of *B. albolineatus* was highlighted (Bornschein et al. 2018). To confirm these potentially divergent characteristics of the *B. tridactylus* note length, we reanalysed one of the original records of this species and analysed our own records and verified that the original description of the advertisement call of *B. tridactylus* is inaccurate. Thus, in the present study, we redescribe the advertisement calls of *B. tridactylus*.

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 a sound collection, specifically at Fonoteca Neotropical Jacques Vielliard (FNJV 0032950). The curator allowed us to access the recording. We also contacted two of the authors of the original description of *B. tridactylus* (Michel V. Garey and André M. X Lima) and we came to know that André M.X. Lima had made the recordings. He sent us one recording, the same one deposited in the Fonoteca Neotropical Jacques Vielliard (A.M.X. Lima pers. comm; 2018). Therefore, we analysed only the recording at FNJV.

Our recordings of B. tridactylus

We conducted a field expedition to the type locality of *B. tridactylus* (*sensu* Bornschein et al. 2015) to make our own recordings. We recorded individuals on 10 March 2016 at the Reserva Particular do Patrimônio Natural Salto Morato (RPPN-SM), municipality of Guaraqueçaba, Paraná, southern Brazil. We collected vouchers according to permits issued by ICMBIO - SISBIO (no. 20416–2) and by the owners of the RPPN-SM. Specimens were deposited in the Museu de História Natural Capão da Imbuia (MHNCI), Curitiba, state of Paraná, Brazil and in the Célio F. B. Haddad collection (CFBH), Departamento de Zoologia, Universidade Estadual Paulista, Campus de Rio Claro, state of São Paulo, Brazil.

We made the recordings between 14:00 h and 16:45 h. During this period, approximate climate conditions were characterized by air temperature = 24.0 °C, soil temperature = 20.9 °C, and relative air humidity = 99%. We made numbered markings close to the recorded individuals in the field to determine whether new recordings were from the same or a new individual. Calls were obtained using digital recorders Sony PCM-D50 and PCM-M10, both with a sampling frequency rate of 44.1 kHz and 16-bit resolution, connected to Sennheiser ME 66 microphones. All the recordings were deposited in MHNCI.

Advertisement call analysis

Sound samples were analysed 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, Hann window, 50% overlap, and spectrogram colour 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 Core Team 2018) using the same window size and overlap settings as in Raven Pro, but resampling the audio files at 22.05 kHz.

We described the advertisement calls following parameters, criteria, and the note-centered approach of Köhler et al. (2017). We also followed the general presentation order and particularities in Köhler's parameters, as presented in Bornschein et al. (2018). We took into account 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*. To check how we did our measurements, please see figure 1 in Bornschein et al. (2018).

Results

Analysis of the original recording of B. tridactylus

The recording of *B. tridactylus* provided by FNJV has a duration of 50 s and included eight short notes (excluding other signals from the background). The spectrogram segment represented in Garey et al. (2012) at p. 270, which contains four notes in an interval of approximately 18 s, corresponds to the last part of that audio. The recording is very noisy throughout its length and presents more signals other than *B. tridactylus* notes. Tests conducted independently with sound editors Sound eXchange (SoX; version 14.4.2; http://sox.sourceforge.net/) and Audacity version 2.2.2 (Audacity Team, 2018) allowed us to conclude that the audio was amplified somehow by at least 6 dB and also clearly low-pass filtered with a cutoff frequency of 10 kHz. Therefore, there is no acoustic information above 10 kHz.

The analysis of the recording does not allow us to recognize the acoustic parameters normally described in *Brachycephalus*, such as note length, number of pulses and upper and lower frequency limits. The sound we hear from the notes overlap with other signals (noise?), which prevents us from clearly determining the end of the note (Figure 1A). There is a concentration of energy at the beginning of the note emission, but this is gradually attenuated and prolonged, preventing us from determining whether there are some pulses or none and making it difficult to clearly discern the frequency limits of each note (Figure 1A).

Additionally, there are some inconsistencies with respect to recording devices used. Metadata of the recording provided by FNJV inform the use of Tascam DR-03 portable recorder and a built-in microphone. However, in the original description the authors inform that the recording was made with "Tascam DR-08 digital recorder" and "an external microphone" (Garey et al. 2012). André M.X. Lima informed (pers. com. 2018) that the recording was made with Tascam DR-03 and external microphone Sennheiser ME66/k6. Both Tascam DR-03 and Tascam DR-08 were only released in 2010, while the recording was made in 2008. Garey et al (2012) informed that they recorded at 96 kHz, but the only 96 kHz capable recorder Tascam had at the time of the recordings was the not-so-portable HD-P2 model.

Analysis of our recordings of B. tridactylus

We recorded calls of 15 individuals of *Brachycephalus tridactylus*; five of which were collected as vouchers (MHNCI 10294, 10730; CFBH, three uncatalogued specimens). However, the number of individuals analysed for each call parameter varied from 14 to five individuals (Table 1). We recorded one individual twice and a second individual three times. The calls we deposited resulted in 17 separate recordings (MHNCI 035–51).

According to our recordings, *Brachycephalus tridactylus* emitted a relatively long advertisement call. We recorded only one complete advertisement call, which lasted for 55.9 s (Figure 2A), but we recorded fragments of other advertisement calls. Duration of the recorded advertisement calls (complete and incompletely recorded) is, on average, 50.793 s (± 11.708 s; Table 1). Thereafter, an individual could remain silent for several minutes, occasionally for more than 30 min, when it emitted a new advertisement call. The note rate was 3.745–8.330 notes per minute ($\grave{x}$ = 5.159 ± 1.347 notes per minute; Table 1). Advertisement calls of *B. tridactylus* were composed by 10–13 notes ($\grave{x}$ = 11.333 ± 0.976 notes; Table 1; see Figure 1). Only isolated notes were present, note groups being absent. The notes were composed by 1–3 pulses ($\grave{x}$ = 1.968 ± 0.542 pulse; Figures 1B and 2B-D; Table 1) and the note duration varied from 0.002–0.021 s ($\grave{x}$ = 0.012 ± 0.005 s; Table 1). The inter-note interval was, on average, 5.325 s (3.269–9.806 s ± 1.447 s; Table 1). The note dominant frequency varied from 4.134–5.426 kHz ($\grave{x}$ = 5.062 ± 0.303 kHz; Table 1). Finally, the highest frequency varied from 5.682–7.488 kHz ($\grave{x}$ = 6.445 ± 0.447 kHz) while the lowest frequency varied from 2.718–4.605 kHz ($\grave{x}$ = 3.579 ± 0.474 kHz; Table 1).

The number of pulses per note tended to be constant throughout the advertisement calls, including only one or two pulses, but in some advertisement calls they varied, with notes having two or three pulses (Table 2). In Table 2 it is also possible to observe intra-individual variation in the number of pulses per note.

Discussion

We were unable to describe the call of *Brachycephalus tridactylus* from FNJV 0032950. On the other hand, we recognize that the original recording and our recordings captured the same type of call. Thus, we treat our recordings as a redescription of the advertisement call of *B. tridactylus*. We did not have the opportunity of analysing all of the original recordings, but it is quite probable they were all made with the same low quality as FNJV 0032950, given that they were obtained in a single day, by the same person, with the same equipment and, lastly, because the spectrogram of the entire call represented in Garey et al. (2012) came from the very same recording.

In the original description, the authors misinterpreted noise and amplification artefacts as part of the call. The “notes” described in Figures 3A and B of Garey et al. (2012) represent energy of notes overlapped with noise / artefacts. The absence of any signals between those “notes” were probably the result of manual cleaning (a common practice in the vocal descriptions of birds, for example).

It is intriguing that the original records probably made at a sampling frequency of 96 kHz were cut to 10 kHz and the result of recordings with external microphone have produced audios of such low quality. We do not know the reasoning behind these procedures, but the quality of the recording was severely impaired, although still audible. The cut-off procedure is lacking in the original description, as well as the mention of any other analysis parameters (e.g., as sampling rate, fast fourier transform size, overlap, and filter bandwidth).

The advertisement call of *Brachycephalus tridactylus* was originally described as having "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 (Fig. 3A,B)" (Garey et al. 2012; Table 1). In our measurements of *B. tridactylus* calls, note duration was nearly an order of magnitude shorter (Figure 1), ranging between 0.002–0.021 s ($\grave{x}$ = 0.012 s ± 0.005 s). In addition, we demonstrated the existence of notes with 1–3 pulses, a feature that was not acknowledged by Garey et al. (2012). Indeed, although Condez et al. (2016) mentioned that *B. tridactylus* presents notes with 1–3 pulses, referring to Garey et al. (2012), this information does not appear in Garey et al. (2012). Moreover, we did not record the gradual decrease in frequency described by Garey et al. (2012).

The inter- and intra-individual variation in the number of notes and pulses per note in the advertisement calls of *Brachycephalus tridactylus* that we described could potentially be the result of an individual's arousal level. Similar variation in the number of notes and pulses was reported for *B. albolineatus* (Bornschein et al. 2018) and a possible relationship with individual arousal was also suggested. In *B. 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 in accordance with the call-centered approach of Köhler et al. (2017), instead of the note-centered approach of the present description, by which a single note represents the call. Thus, the possibility that the calls of these species include isolated notes and note groups cannot be assessed, preventing comparisons of the macrostructural features with the call of *B. tridactylus*. *Brachycephalus ephippium* (Pombal Jr. et al. 1994), *B. pitanga* (Tandel et al. 2014), *B. crispus* (Condez et al. 2014), *B. sulfuratus* (Condez et al. 2016), and *B. darkside* (Guimarães et al. 2017) present notes with several pulses, being macrostructurally distinct from *B. tridactylus*. This species presents 1–3 pulses per note, instead of 5–15 in *B. ephippium*, 7–14 in *B. pitanga*, 7–12 in *B. crispus*, 7–11 in *B. sulfuratus*, and 5–8 in *B. darkside*. The simplified description of the call of *B. hermogenesi* (Verdade et al. 2008) prevent us from comparing macrostructural features with the call of *B. tridactylus*.

Conclusions

The original description of the call of *Brachycephalus tridactylus* is incorrect because it included background noise and amplification artefacts as part of the call parameters. The original description would be better interpreted and replicable if some recording procedures (e.g.,

frequency cut-off) and analysis procedures had been provided. Our advertisement call redescription demonstrates that the species emits only isolated notes with 1–3 pulses. Macrostructural features of the advertisement call of *B. tridactylus* distinguish them from other species by having only isolated notes (instead of isolated notes and note groups in *B. albolineatus* and *B. mirissimus*) and by having few pulses (up to 3) instead of several pulses (*B. crispus*, *B. darkside*, *B. ephippium*, *B. pitanga*, *B. sulfuratus*).

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FIGURE CAPTIONS

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, uncatalogued individual #1). 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%.

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). B. Note with one pulse (MHNCI 043; individual collected and housed at CFBH, uncatalogued individual #1). 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, uncatalogued individual #2). Spectrograms produced with FFT size of 128 points, Hann window, and overlap of 88%.

Figure 1(on next page)

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.

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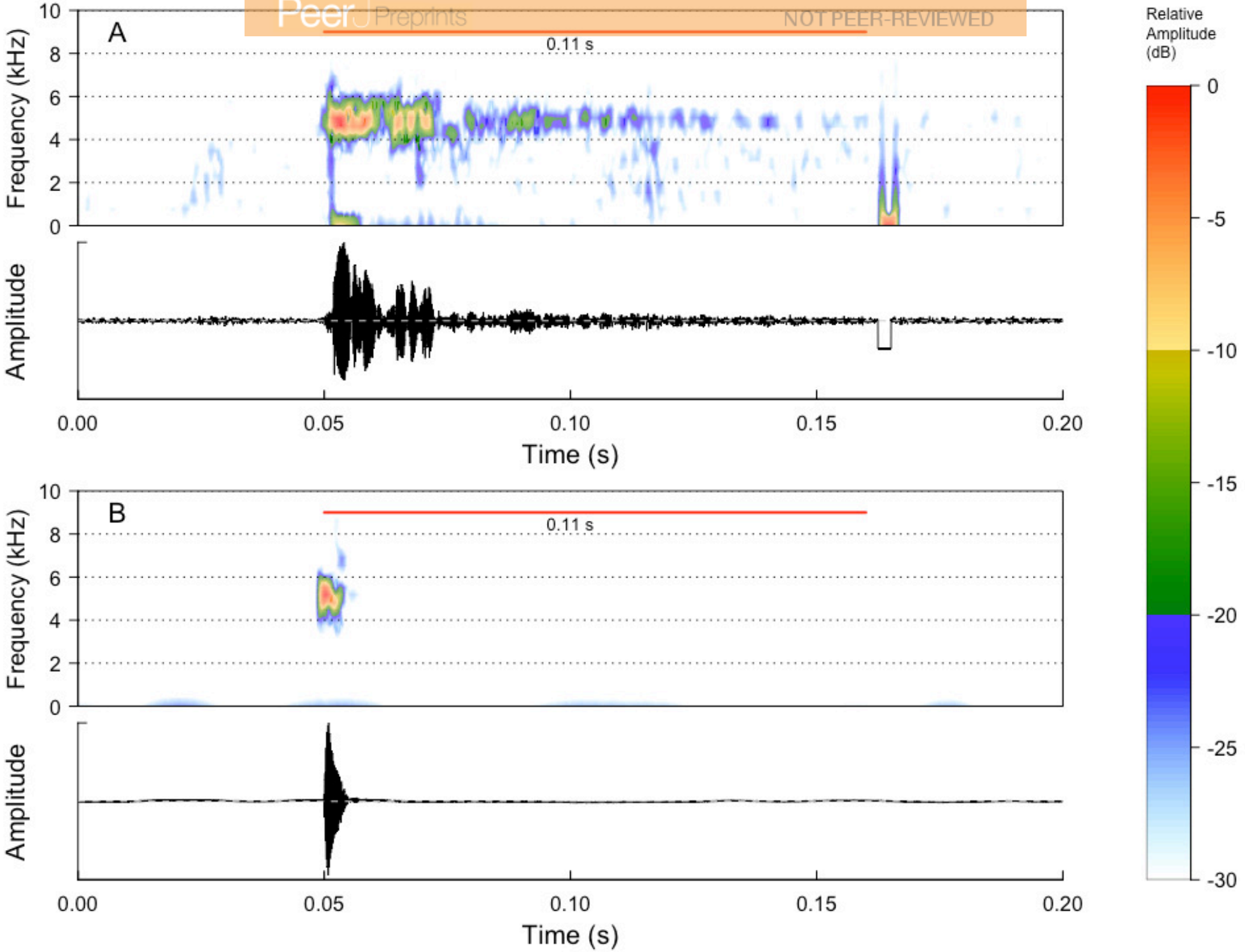

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Figure 2(on next page)

Example of an entire advertisement call and also notes of other advertisement calls of *Brachycephalus tridactylus*.

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). B. Note with one pulse (MHNCI 043; individual collected and housed at CFBH, uncatalogued individual #1). 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, uncatalogued individual #2). Spectrograms produced with FFT size of 128 points, Hann window, and overlap of 88%.

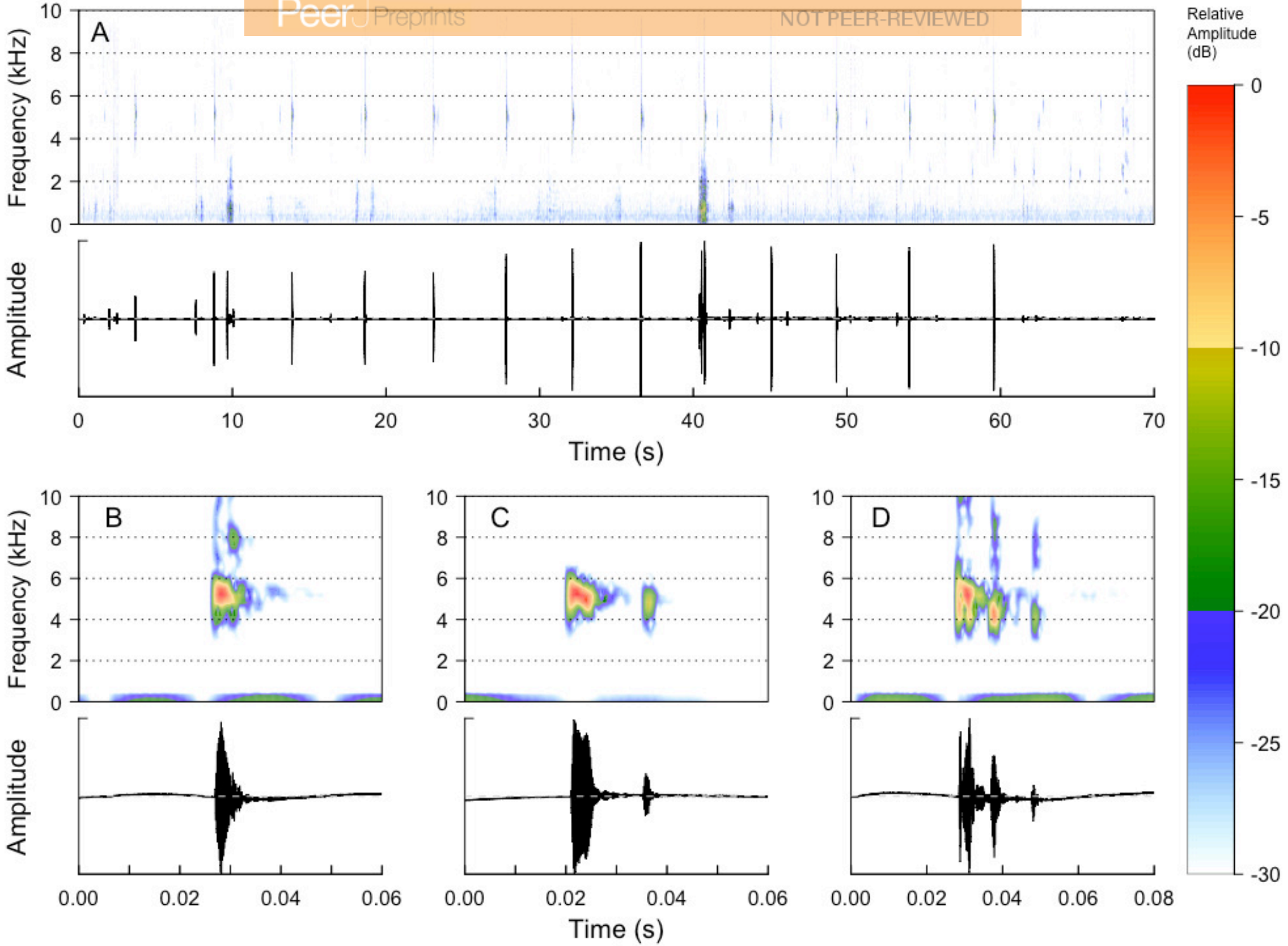

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Table 1(on next page)

Measurements of acoustic parameters of the advertisement call of *Brachycephalus tridactylus*.

Table 1. Measurements of acoustic parameters of the advertisement call of *Brachycephalus tridactylus*.

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Variable	Present description					Original description (Garey et al. 2012)[1]		
	Range	Mean	SD	n		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]	-	-

[1]n = 17 individuals.

[2]n = 5 call durations are fragments of the entire call (see Table 2).

[3]Same as note duration if applicable the call-centered approach of Köhler et al. (2017).

[4]Includes notes counted before the beginning of the recording (see Table 2).

[5]Not applicable under note-centered approach, as in "present description".

[6]In the original description was informed 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".

Table 2(on next page)

Number of pulses per notes (separated by ‘,’) of the advertisement calls of *Brachycephalus tridactylus*.

Table 2. Number of pulses per notes (separated by ‘,’) of the advertisement calls of *Brachycephalus tridactylus*.

Table 2. Number of pulses per notes (separated by ‘,’) 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