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Advertisement call of *Brachycephalus albolineatus* (Anura: Brachycephalidae)

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Background. *Brachycephalus* are among the smallest terrestrial vertebrates in the world. The genus encompasses 34 species endemic to the Brazilian Atlantic Rainforest, occurring mostly in montane forests, with many species showing microendemic distributions to single mountaintops. It includes diurnal species living in the leaf litter and calling during the day, mainly during the warmer months of the year. The natural history of the vast majority of the species is unknown, such as their advertisement call, which has been described only for seven species of the genus. In the present study, we describe the advertisement call of *Brachycephalus albolineatus*, a recently described microendemic species from Santa Catarina, southern Brazil. **Methods.** We analyzed 34 advertisement calls from 20 individuals of *B. albolineatus*, recorded between 5–6 February 2016 in the type locality of the species, Morro Boa Vista, on the border between the municipalities of Jaraguá do Sul and Massaranduba, Santa Catarina, southern Brazil. We collected five individuals as vouchers (they are from the type series of the species). We used the note-centered approach *sensu* Köhler *et al.* (2017) to describe the advertisement calls of the species. **Results.** *Brachycephalus albolineatus* have a long advertisement call of 40–191 s (mean of 88 s) composed of 7–26 notes (mean of 14 notes) emitted at a rate of 6–13 notes per minute (mean of 9 notes per minute) and at a note dominant frequency of 5–7 kHz (mean of 6 kHz). Advertisement calls are composed of isolated notes and note groups (two notes involved in each particular note group); the former is composed by one to three pulses (mean of 2.0) and the note groups by two or three pulses in each note (mean of 2.7). Most advertisement calls present both isolated notes and note groups, with a few cases showing only the former. Note groups are emitted invariably in the last third of the advertisement call. Most isolated notes escalate their number of pulses along the advertisement call (1 to 2, 1 to 3 or 2 to 3). Note duration of isolated notes varies from 0.002–0.037 s (mean of 0.020 s) and duration of note group vary from 0.360–0.578 s (mean of 0.465 s). **Discussion.** Individuals increase the complexity of their calls as is proceeds, incorporating note groups and pulses per note. Intra-individual variation analysis also demonstrated that less structured advertisement calls (i.e. with notes with fewer pulses) are not stereotyped. It is possible that isolated notes and note groups could have distinct function, perhaps territorial defense and mating, respectively. We believe that using a note-centered approach facilitates comparisons with calls of congeners, as well as underscores the considerable differences in call structure between species in a single group and among species groups.

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Advertisement call of *Brachycephalus albolineatus* (Anura: Brachycephalidae)

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Running headline: Advertisement call of *B. albolineatus*

Abstract

Background. *Brachycephalus* are among the smallest terrestrial vertebrates in the world. The genus encompasses 34 species endemic to the Brazilian Atlantic Rainforest, occurring mostly in montane forests, with many species showing microendemic distributions to single mountaintops. It includes diurnal species living in the leaf litter and calling during the day, mainly during the warmer months of the year. The natural history of the vast majority of the species is unknown, such as their advertisement call, which has been described only for seven species of the genus. In the present study, we describe the advertisement call of *Brachycephalus albolineatus*, a recently described microendemic species from Santa Catarina, southern Brazil.

Methods. We analyzed 34 advertisement calls from 20 individuals of *B. albolineatus*, recorded between 5–6 February 2016 in the type locality of the species, Morro Boa Vista, on the border between the municipalities of Jaraguá do Sul and Massaranduba, Santa Catarina, southern Brazil. We collected five individuals as vouchers (they are from the type series of the species). We used the note-centered approach *sensu* Köhler *et al.* (2017) to describe the advertisement calls of the species.

Results. *Brachycephalus albolineatus* have a long advertisement call of 40–191 s (mean of 88 s) composed of 7–26 notes (mean of 14 notes) emitted at a rate of 6–13 notes per minute (mean of 9 notes per minute) and at a note dominant frequency of 5–7 kHz (mean of 6 kHz). Advertisement calls are composed of isolated notes and note groups (two notes involved in each particular note group); the former is composed by one to three pulses (mean of 2.0) and the note groups by two or three pulses in each note (mean of 2.7). Most advertisement calls present both isolated notes and note groups, with a few cases showing only the former. Note groups are emitted invariably in the last third of the advertisement call. Most isolated notes escalate their number of pulses along the advertisement call (1 to 2, 1 to 3 or 2 to 3). Note duration of isolated notes varies from 0.002–0.037 s (mean of 0.020 s) and duration of note group vary from 0.360–0.578 s (mean of 0.465 s).

Discussion. Individuals increase the complexity of their calls as is proceeds, incorporating note groups and pulses per note. Intra-individual variation analysis also demonstrated that less structured advertisement calls (i.e. with notes with fewer pulses) are not stereotyped. It is possible that isolated notes and note groups could have distinct function, perhaps territorial defense and mating, respectively. We believe that using a note-centered approach facilitates comparisons with calls of congeners, as well as underscores the considerable differences in call structure between species in a single group and among species groups.

Introduction

Brachycephalus are among the smallest terrestrial vertebrates in the world (Rittmeyer *et al.* 2012), with most species not exceeding 2.5 cm in body length. The genus includes 34 species (Frost 2017), occurring from the southern Bahia to northeastern Santa Catarina, Brazil (Bornschein *et al.* 2016a; see also Pie *et al.* 2013). Most *Brachycephalus* species, particularly in the *B. pernix* species group (see below), are microendemic, occurring in one or a few adjacent mountaintops, with total extents of occurrence comparable to the smallest ranges of species around world (Bornschein *et al.* 2016a). Species are diurnal, living in the leaf litter in forests of the Atlantic Rainforest domain (Bornschein *et al.* 2016a and compilation therein). Direct development, with a reduced number of eggs laid on the soil (Pombal Jr. 1999), was demonstrated for *B. ephippium* (Heyer *et al.* 1990, Pombal Jr. 1999), and this is assumed as the reproductive pattern for the genus. *Brachycephalus* is characterized by extreme miniaturization, with is possible related to a reduced number and size of digits (Hanken & Wake 1993, Yeh 2002, Clemente-Carvalho *et al.* 2009) and loss of some morphological features of the auditory apparatus (Silva, Campos & Sebben 2007). Some species are brightly colored, with neurotoxins found in the skin of two aposematic species (Sebben *et al.* 1986, Pires Jr. *et al.* 2002, 2003, 2005, Schwartz *et al.* 2007), possibly originated from intestinal bacteria (Schwartz *et al.* 2007). The species of the genus have been segregated into three phenetic groups, namely the *B. ephippium*, *B. didactylus*, and *B. pernix* species groups (Ribeiro *et al.* 2015). Possibly due to historical evolutionary processes (Bornschein *et al.* 2016a, Firkowski *et al.* 2016), *Brachycephalus* species are almost exclusively allopatric or parapatric, with few cases of syntopy (Bornschein *et al.* 2016a).

There has been a recent increase in the description of new species within *Brachycephalus*, with 20 species described in the last 10 years (Frost 2017). However, the natural history of the vast majority of the species is unknown (see review of ecological studies in Bornschein *et al.* [2016a]). Call descriptions of the species are scarce, which is surprising, given that individuals of the species are usually located by their calls, often emitted at locally high male densities (one person might hear dozens of males from a single hearing spot). Advertisement calls were described for *B. ephippium* (Pombal Jr., Sazima & Haddad 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), and *B. darkside* (Guimarães *et al.* 2017).

Given that *Brachycephalus* is a group with mostly allopatric species, it is of great interest to investigate the evolution pattern of their calls. In allopatry, one could expect great similarity between the call of different species (Bornschein *et al.* 2007, Maurício *et al.* 2014), due to a lack of selective pressure to avoid hybridization of closely-related species. However, this needs to be tested for *Brachycephalus*. In the present study, we describe the advertisement call of *B. albolineatus*, a member of the *B. pernix* group (Bornschein *et al.* 2016b). *Brachycephalus albolineatus* was recently described based on a series of eight specimens collected at the type locality, Morro Boa Vista, Santa Catarina, southern Brazil (Bornschein *et al.* 2016b).

Methods

We recorded individuals of *Brachycephalus albolineatus* on 25 October 2012 and on 5–6 February 2016 at the type locality of the species, i.e. Morro Boa Vista (26°30’58” S, 49°03’14” W; 820–835 m above sea level), on the border between the municipalities of Jaraguá do Sul and Massaranduba, state of Santa Catarina, southern Brazil. We collected vouchers according to permits issued by ICMBIO - SISBIO (no. 20416–2). Vouchers belong to the type material of the species, which was deposited in Museu de História Natural Capão da Imbuia (MHNCI), Curitiba, Paraná state and Museu Nacional (MNRJ), Rio de Janeiro, Rio de Janeiro state, Brazil. Analyzed recordings were carried out on 5–6 February 2016 from 9:00–12:00 a.m. and from 15:00–18:00 p.m. Climatic conditions during recordings were characterized by air temperature = 20.8–21.4 °C, soil temperature = 19.4–20.0 °C, and relative air humidity = 86–100%. We made numbered markings on the vegetation above the recorded individuals in the field to determine whether new recordings were from the same individuals, in order to build up the dataset both in terms of more individuals as well as intra-individual variation, with more than one recording from the same individual. Calls were recorded using the digital recorders Sony PCM-D50 and PCM-M10, both with sampling frequency rate of 44.1 kHz and 16-bit resolution, and Sennheiser ME 66 microphones. Recordings were deposited in MHNCI. Sound samples were analyzed with Raven Pro 1.5 (Bioacoustics Research Program 2012). Time domain variables were measured from oscillograms and frequency domain variables were measured from spectrograms. Spectrogram features were defined with a 128-point (2.9 ms) Fast Fourier Transform (FFT), a 3-dB Filter bandwidth of 492 Hz, Hann window, 50% overlap, and a spectrogram color scheme of Standard Gamma II in Raven Pro and Jet in Raven Lite. Final spectrograms, as well as diagnostic plots, were generated using the Seewave package, v. 2.0.5 (Sueur, Aubin & Simonis 2008) of the R environment, v. 3.2.2 (R Core Team 2015) using the same window size and overlap settings as in Raven Pro, but resampling the audio files at 22.05 kHz.

We used the note-centered approach *sensu* Köhler *et al.* (2017) to define the advertisement call of the species. We determined the end of a given call and the beginning of the next one by the long period of silence between them (Köhler *et al.* 2017), which might last for several minutes and thus is considerably longer than the call itself. We described the advertisement calls following features and criteria of Köhler *et al.* (2017). We took the liberty of describing the general features of Köhler *et al.* (2017) also for parts of the call, in order to clarify the distinctions observed in particular parts of the advertisement calls of *Brachycephalus albolineatus*. We used the following features, which can be seen in Fig. 1: 1) call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of the call including only note groups (s); 4) note rate (notes per minute); 5) note rate of the call including only isolated notes (notes per minute); 6) note rate of the call including only note groups (notes per minute); 7) number of notes per call; 8) number of isolated notes per call; 9) number of note groups per call; 10) number of pulses per isolated notes; 11) number of pulses in each note groups; 12) note duration of isolated notes (s); 13) duration of note group (s); 14) inter-note interval in isolated notes (s), defined as the time from the end of one isolated note to the beginning of the next note isolated note; 15) inter-note group interval (s), defined as the time from the end of one note group to the beginning of the next note group; 16) inter-note interval within note groups (s), defined as the time from the end of the first note to the beginning of the next note of the same note group; 17) note dominant frequency (kHz); 18) highest frequency (kHz); and 19) lowest frequency (kHz). The note rate was calculated taking into account the time from the beginning of the first note to the beginning of the last note of the calls (or call intervals) and the number of notes included in this counted time (the last note is not included; Köhler *et al.* [2017]; see also Cocroft & Ryan [1995]). The dominant frequency across all notes in a call sample was calculated with the function dfreq from the R package seewave. This function brings as an output a plot with all dominant frequencies in a specific file or file segment. Alternatively, the output can be a vector of dominant frequency values. All the default arguments of the function were followed, with the exception of the overlap, for which we chose the value of 90% and the amplitude threshold of signal detection, whose value we determined as of 5%. The highest and lowest frequencies represent the frequency range of each pulse and was calculated considering the longest continuous interval of the green color of the "Standard Gamma II" color type from the Color Map of Raven Pro. We measured the highest and lowest frequencies from pulses in notes with one or more pulses, but when they had more than one pulse we considered the measures only from the first and second pulses.

Results

We recorded calls from 29 individuals of *Brachycephalus albolineatus* but analyzed 34 advertisement calls from 20 individuals, five of which were collected as vouchers (MHNCI 10296–9, MNRJ 90349). We recorded eight individuals 2–4 times ($\bar{x}$ = 2.75 times per individual). The calls we deposited resulted in 34 separate recordings (MHNCI 001–34).

Brachycephalus albolineatus emitted a relatively long advertisement call, between 39.93–191.14 s ($\bar{x}$ = 88.37 ± 35.73 s; Table 1; see feature #1 in Fig. 1). Thereafter, the individual remains silent for several minutes, occasionally for more than 35 min, when it emits a new advertisement call. A graphical representation of the temporal sequence of notes in each call is shown in Fig. 2. The note rate was 5.89–13.00 notes per minute ($\bar{x}$ = 9.15 ± 1.71 notes per minute; Table 1; see feature #4 in Fig. 1). Advertisement calls included 7–26 notes ($\bar{x}$ = 14.08 ± 4.70 notes; Table 1; see feature #7 in Fig. 1).

The advertisement calls of the species included both isolated notes and note groups (in this case, with two notes involved in each particular note group; Fig. 3). Advertisement calls could be composed only by isolated notes (21% of complete recordings of advertisement calls), but usually included both isolated notes and note groups (Table 2). Every advertisement call with isolated notes and note groups began with the former and then changed to note groups (Table 2, Fig. 2). The part of the advertisement call composed only by note groups contains, on average, 29% of the notes of the entire advertisement call (± 15.4%; range of 10–61%; see feature #9 in Fig. 1) and span, on average, 24.44 s (± 19.85 s; range of 0.41–76.37 s; see feature #3 in Fig. 1) as opposed to a mean of 53.71 s (± 25.38 s; range of 18.39–98.90 s; Table 1; see feature #2 in Fig. 1) of the part of the advertisement calls with only isolated notes. The part of the advertisement call with only note groups also had a slower note rate, with 7.80 notes issued per minute, on average (± 1.65 note per minute; range of 4.74–11.73 notes per minute; see feature #6 in Fig. 1), against 10.29 notes per minute on average (± 1.59 note per minute; range of 7.28–13.62 notes per minute; Table 1) in the part of the call with isolated notes (when note groups occurs; see feature #5 in Fig. 1).

The number of pulses per isolated notes varies from 1–3 ($\bar{x}$ = 2.00 ± 0.60; Table 1; Fig. 3; see feature #10 in Fig. 1). The isolated notes that initiate the advertisement call do it with one pulse (8 advertisement calls) or two pulses (16 advertisement calls; Table 2). However, most of isolated notes along the advertisement call escalated the number of pulses (1 to 2, 1 to 3 or 2 to 3; 18 advertisement calls), whereas the isolated notes maintained a constant number of pulses only in six of the advertisement calls (2 to 2; Table 2). The number of pulses in each note groups varied from 2–3 ($\bar{x}$ = 2.70 ± 0.46; Table 1; Fig. 3; see feature #11 in Fig. 1). The total number of pulses in note groups varied from 4–6 ($\bar{x}$ = 5.40 ± 0.82; Table 1). Occurred four combinations of number of pulses in note groups (2–2 to 3–3), being more common the combination of 3–3 pulses (62%; Table 1 and 2). All pulses both in isolated notes and note groups are interrupted units of the subsequent pulses, isolated by short moment of silence.

Note duration of isolated notes varies from 0.002–0.037 s ($\bar{x}$ = 0.020 ± 0.007 s) and duration of note groups varies from 0.360–0.578 s ($\bar{x}$ = 0.465 ± 0.053 s; Table 1; see features #12 and #13 in Fig. 1). The inter-note interval in isolated notes is, on average, 6.663 s (4.092–12.248 ± 1.705 s; see feature #14 in Fig. 1) and the inter-note group interval is, on average, 6.871 s (4.322–10.678 ± 1.768 s; Table 1; see features #15 in Fig. 1). The inter-note interval within note groups is, on average, 0.412 s (0.319–0.526 ± 0.050 s; Table 1; see feature #16 in Fig. 1). The note dominant frequency varies from 5.34–7.32 kHz ($\bar{x}$ = 6.38 ± 0.30 kHz; Table 1). Two individuals presented calls with note dominant frequency below the mean (MHNCI 026–7) and two other from the mean upward (MHNCI 001 and 003), while the remaining showed note dominant frequency crossing the mean in both directions. Finally, the highest frequency spans from 6.686–10.552 kHz ($\bar{x}$ = 7.98 ± 0.47 kHz) while the lowest frequency span from 3.130–6.087 kHz ($\bar{x}$ = 4.53 ± 0.52 kHz; Table 1).

Discussion

In this study we used a note-centered approach (*sensu* Köhler *et al.* [2017]) to describe the advertisement call of *Brachycephalus albolineatus*. We believe that there are two advantages for a note-centered approach to describe the calls of species of the *B. pernix* group. First, it is consistent with descriptions of calls of the species of the *B. ephippium* and *B. didactylus* groups (Table 3). For instance, in the *B. ephippium* group, the advertisement call of *B. crispus* has been described as “a long and low-intensity buzz with a regular repetition of notes” (Condez *et al.* 2014); the call of *B. darkside* “is characterized by pulsed notes emitted in extremely long sequences” (Guimarães *et al.* 2017), whereas the call of *B. pitanga* “[...] consists of low-intensity pulsed notes uttered in a long series” (Araújo *et al.* [2012]; see Pombal Jr., Sazima & Haddad [1994] for a similar description in the case of *B. ephippium*). Likewise, in the *B. didactylus* group, the call of *B. hermogenesi* “may be simple, constituted by a single note, or complex, composed of groups of two to seven similar notes" (Verdade *et al.* 2008), whereas the call of *B. sulfuratus* is "long, composed of a set of 4–7 high-frequency notes [...] repeated regularly" (Condez *et al.* 2016). In all those cases, the call was considered as the entire sequence of notes. On the other hand, Garey *et al.* (2012) considered single notes as calls and largely overlooked any patterns over longer periods of time. Second, using a note-centered approach facilitates comparisons with calls of congeners, as well as underscores the considerable differences in call structure between species in a single group and among species groups.

There are only a few species of *Brachycephalus* with described advertisement calls. In Table 3 we summarize all data and features used in those descriptions. It is striking the extent to which descriptions vary in the number of features used and in how often they lacked important details, such as methodological procedures and sample size. These issues make it difficult to conduct a more precise comparison with the call of *B. albolineatus*. Nevertheless, *B. albolineatus* is the only known species with an advertisement call that is structurally modified along its emission, i.e. more structured (with notes with increasingly more pulses and with note groups). However, as stated above, we do not rule out the possibility that the advertisement call of *B. tridactylus* indeed exhibits some level of structuring such as that of *B. albolineatus*. Another striking difference is how much the note of *B. albolineatus* is shorter than that of *B. tridactylus* (Garey *et al.* 2012), both of the *B. pernix* group (average of 0.020 s and 0.11 s, respectively). *Brachycephalus albolineatus* have a very reduced number of pulses in isolated notes in comparison with the species of the *B. ephippium* and *B. didactylus* groups, i.e. a mean of two pulses against means of 6.3 pulses in *B. darkside* (Guimarães *et al.* 2017), 10.0 pulses in *B. crispus* (Condez *et al.* 2014), 10.9 and 11.1 pulses in *B. pitanga* (Araújo *et al.* 2012, Tandel *et al.* 2014), and 12 pulses in *B. ephippium* (Pombal Jr., Sazima & Haddad 1994; Table 3), in species of the *B. ephippium* group, and against a mean of 8.8 pulses in *B. sulfuratus*, of the *B. didactylus* group (Condez *et al.* 2016; Table 3). *Brachycephalus albolineatus* has the highest interval in the range of note dominant frequency, that include a variation of 2 kHz, only slightly comparable to the range variation of 1.2 kHz of *B. pitanga* (Tandel *et al.* 2014; Table 3). Meanwhile it is premature to provide a discussion about this variation, given that most of the available data of dominant frequency in *Brachycephalus* only report their average values (Table 3). It should be noted that the large frequency range of the "dominant frequency" for *B. darkside* presented by Guimarães *et al.* (2017), including a variation of 3.3 kHz, is not comparable to the variation in *B.*

albolineatus because the measurement refers to a frequency range (Table 3). The one-pulse notes of *B. albolineatus* may represent “warming notes” (*sensu* Bornschein *et al.* 2007), which refers to notes beginning a call and that are attenuated (e.g. less intense [less audible]), although one-pulse notes also appear along the call in some advertisement calls.

Apparently there is a trend of individuals to invest progressively more energy along the emission of each particular advertisement calls. There are three sources of evidence for this: 1) advertisement calls normally escalated, incorporating note groups at the last third part of the call (76%) and 2) pulses per note increased during the emission of isolated notes (up to three; 62%); and 3) note groups usually had 3–3 pulses per note (62%), which is the combination of the groups with highest number of pulses (Table 2). Intra-individual variation analysis also demonstrated that less structured advertisement calls (i.e. with notes with less pulses) are not fixed individually and can vary in the course of an hour. In the only species of the *Brachycephalus pernix* group with its advertisement calls described to date, *B. tridactylus* (Garey *et al.* 2012), there was no evidence of escalation in structure. It is possible that the advertisement calls with isolated notes and note groups could have distinct functions, perhaps territorial defense when composed only by the former and territorial defense plus mating when composed by isolated notes and note groups. There is a parallel between the differences of isolates notes versus note groups of *B. albolineatus* and the “territorial call” / “aggressive call” versus advertisement call of *B. pitanga* (Araújo *et al.* 2012) and *B. darkside* (Guimarães *et al.* 2017). In both of these territorial / aggressive calls there are shorter notes with reduced number of pulses that in the advertisement calls, like the isolated notes of *B. albolineatus* that span 0.002–0.037 s ($\bar{x}$ = 0.020 s) and have 1–3 pulses ($\bar{x}$ = 2.0 pulses) whereas note groups span 0.360–0.578 s ($\bar{x}$ = 0.465 s) and have 4–6 pulses ($\bar{x}$ = 5.4 pulses).

In a recent study, Goutte *et al.* (2017) suggested that *Brachycephalus ephippium* and *B. pitanga* are insensitive to the sound of their own calls. This raises some questions about the validity of discussions about the possible reproductive and behavioral use of calls in the case of *B. albolineatus*, as well as for the use of calls in the taxonomy of the group. Goutte *et al.* (2017) suggest that calls may have been maintained in the studied species because of the call side effects (e.g. vocal sac movement) or by evolutionary inertia, for example. The relevant issue to be discussed here is that *B. ephippium* and *B. pitanga*, both members of the *B. ephippium* group, present vocal and visual behavioral (vocal sac movements) above the leaf litter (Goutte *et al.*

2017), unlike *B. albolineatus* and all other species of the *B. pernix* group (MRB *et al.*, per. obs.), which call exclusively under the leaf litter and vocal sac movements are not visible. We do not abandon the hypothesis that species of the *B. pernix* and *B. didactylus* groups have a more complete auditory system than *B. ephippium* and *B. pitanga* and the ability to perceive their own calls. This is an interesting subject brought only now to the fore and open to further discussion.

Conclusions

Brachycephalus albolineatus is the first species in the genus whose advertisement call has been recognized as increasing in complexity over the course of its emission. Its advertisement call is long and composed by isolated notes and note groups, which tend to be emitted during the last third of the call. Intra-individual variation demonstrates that calls can be composed only by isolated notes or by isolated notes and note groups in a subsequent call. Number of pulses per notes escalates along the call. These results underscore how a note-centered approach is able to reveal important aspects of the temporal dynamics of the advertisement call of the studied species

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Table 1. Measurements of advertisement call (AC) features of *Brachycephalus albolineatus* and some parameters. Number between brackets represent the number of the feature in Fig. 1.

Table 2. Distribution of the number of pulses per note (separated by ",") along the advertisement calls (AC) of *Brachycephalus albolineatus* (see features 10 and 11 in Fig. 1). Pulses per note groups are indicated between parenthesis, but indicating separately by "–" the number of pulses in each particular note of the group (see Figs. 1 and 3).

Table 3. Comparison of the features used to describe the advertisement call of *Brachycephalus*. Values are expressed by: range (mean ± SD) [sample/individuals].

FIGURES

Figure 1. Representation of some features considered in the description of the advertisement call of *Brachycephalus albolineatus* on a schematic call. Numbers correspond with the order of descriptions in the methods. 1) Call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of the call including only note groups (s); 4) note rate (notes per minute); 5) note rate of the call including only isolated notes (notes per minute); 6) note rate of the call including only note groups (notes per minute); 7) number of notes per call (10 notes in the example); 8) number of isolated notes per call (seven notes in the example); 9) number of note groups per call (three notes in the example); 10) number of pulses per isolated notes (three in the example); 11) number of pulses in each note groups (3–3 in the example); 12) note duration of isolated notes (s); 13) duration of note group (s); 14) inter-note interval in isolated notes (s); 15) inter-note group interval (s); and 16) inter-note interval within note groups (s).

Figure 2. Graphical representation of the emission of isolated notes and note groups of the advertisement calls (AC) of *Brachycephalus albolineatus* (only AC recorded from the beginning were considered). Note the individual variation. The number of pulses of each note can be observed in Table 2. Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia.

Figure 3. Example of an entire advertisement call and also notes of other advertisement calls of *Brachycephalus albolineatus*. A) Entire advertisement call (MHNCI 006; individual collected and housed at MHNCI 10296). B, D, F) All examples observed of isolated notes, with one pulse (B: MHNCI 008), two pulses (D = MHNCI 022), and three pulses (F = MHNCI 026). C, E, G) Examples of note groups, with 3–3 pulses (C: MHNCI 026), 3–2 pulses (E = MHNCI 027; individual collected and housed at MNRJ 90349), and 2–3 pulses (G = MHNCI 026). Abbreviations: MHNCI = Museu de História Natural Capão da Imbuia; MNRJ = Museu Nacional, Rio de Janeiro. Spectrograms produced with a FFT size of 4096 points, Hann window, and overlap of 90% in A and FFT 128 points, Hann window, and overlap of 90% in B–G.

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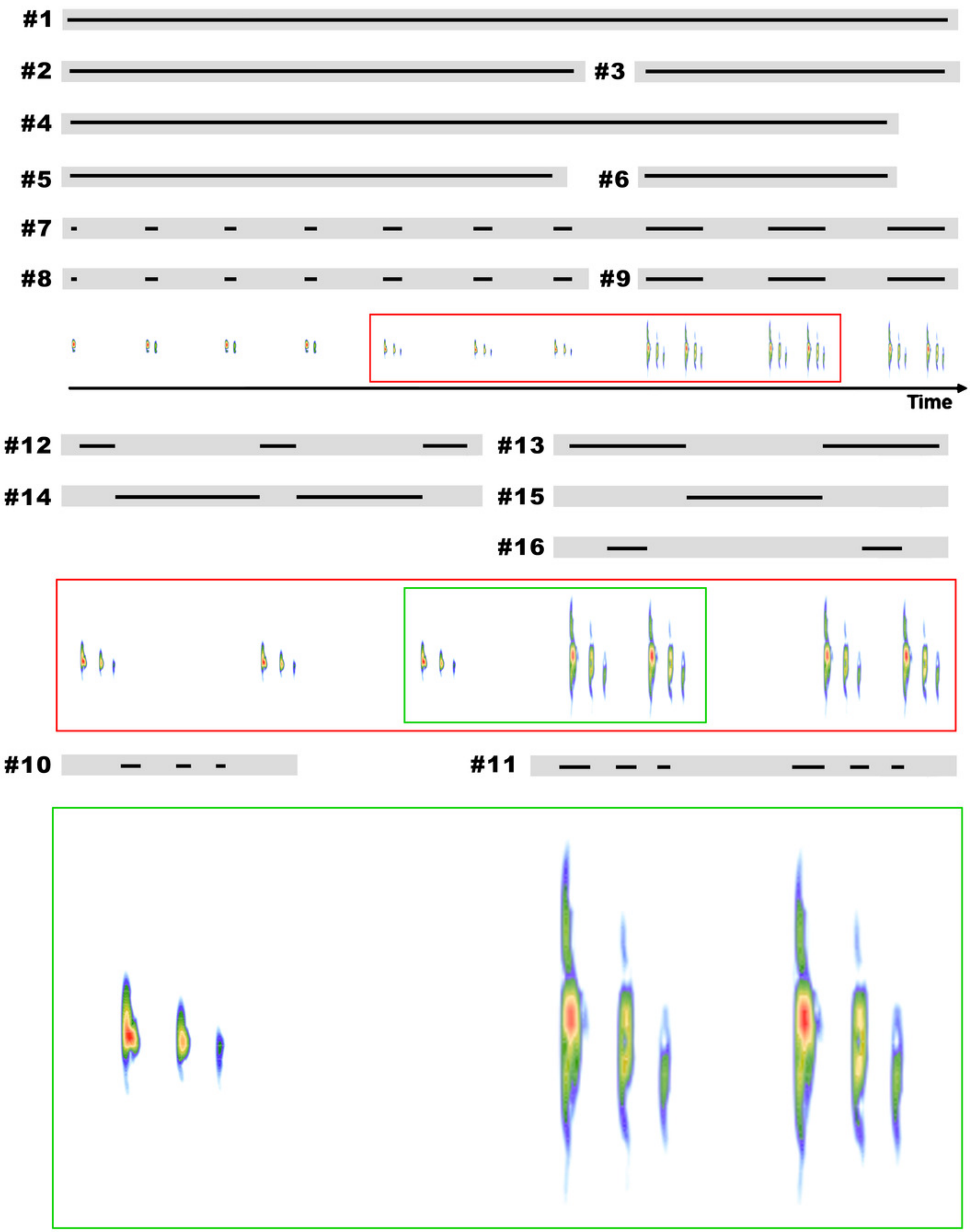
Figure 1

Representation of some features considered in the description of the advertisement call of *Brachycephalus albolineatus* on a schematic call.

Numbers correspond with the order of descriptions in the methods. 1) Call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of the call including only note groups (s); 4) note rate (notes per minute); 5) note rate of the call including only isolated notes (notes per minute); 6) note rate of the call including only note groups (notes per minute); 7) number of notes per call (10 notes in the example); 8) number of isolated notes per call (seven notes in the example); 9) number of note groups per call (three notes in the example); 10) number of pulses per isolated notes (three in the example); 11) number of pulses in each note groups (3–3 in the example); 12) note duration of isolated notes (s); 13) duration of note group (s); 14) inter-note interval in isolated notes (s); 15) inter-note group interval (s); and 16) inter-note interval within note groups (s).


#1
#2
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#8
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Time
#12
#13
#14
#15
#16
#10
#11

Figure 2(on next page)

Graphical representation of the emission of isolated notes and note groups of the advertisement calls (AC) of *Brachycephalus albolineatus* (only AC recorded from the beginning were considered).

Note the individual variation. The number of pulses of each note can be observed in Table 2.

Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia.

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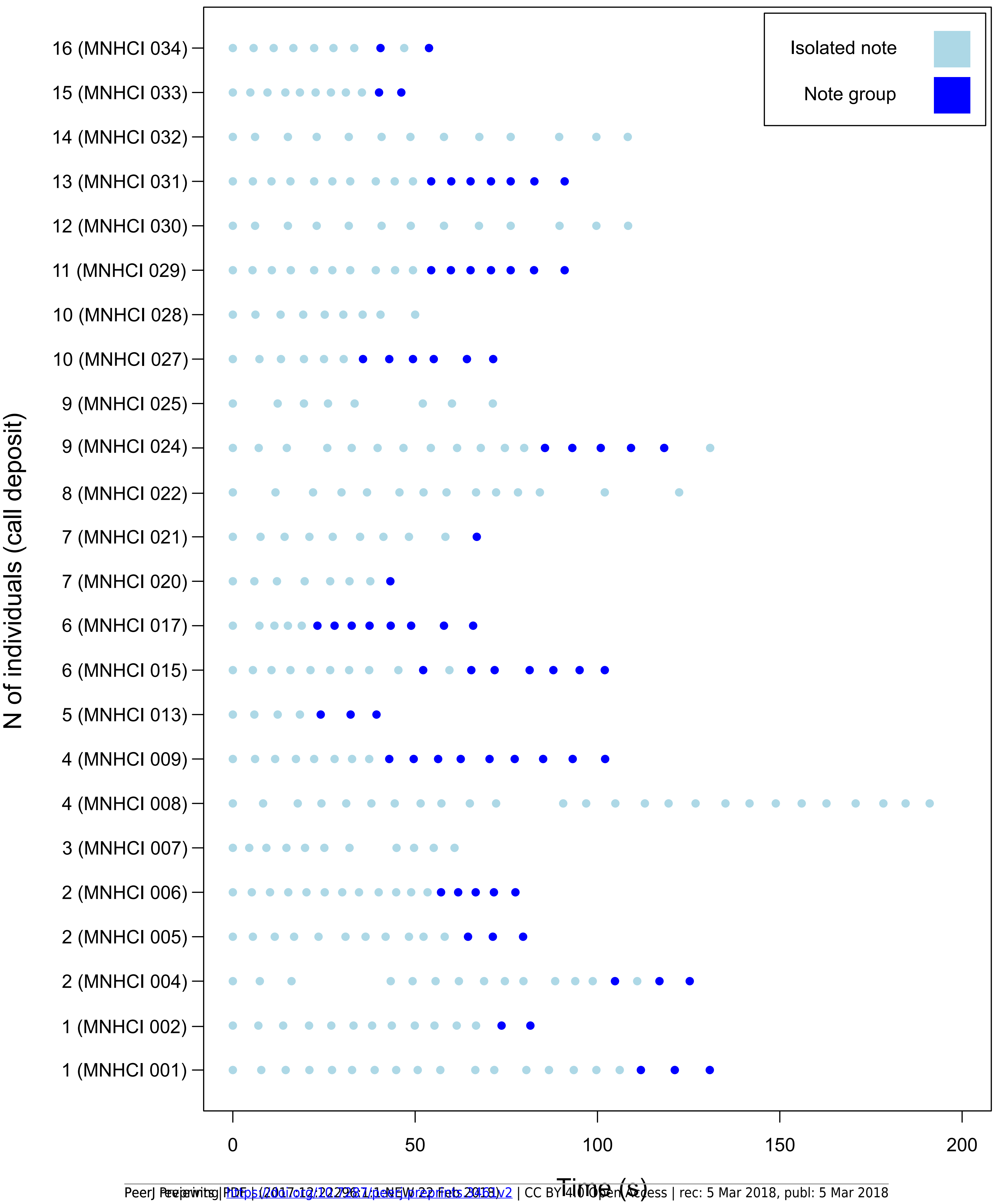
Isolated note
Note group
16 (MNHCI 034)
15 (MNHCI 033)
14 (MNHCI 032)
13 (MNHCI 031)
12 (MNHCI 030)
11 (MNHCI 029)
10 (MNHCI 028)
10 (MNHCI 027)
9 (MNHCI 025)
9 (MNHCI 024)
8 (MNHCI 022)
7 (MNHCI 021)
7 (MNHCI 020)
6 (MNHCI 017)
6 (MNHCI 015)
5 (MNHCI 013)
4 (MNHCI 009)
4 (MNHCI 008)
3 (MNHCI 007)
2 (MNHCI 006)
2 (MNHCI 005)
2 (MNHCI 004)
1 (MNHCI 002)
1 (MNHCI 001)
N of individuals (call deposit)
0
50
100
150
200
Time (s)

Figure 3

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

A) Entire advertisement call (MHNCI 006; individual collected and housed at MHNCI 10296). B, D, F) All examples observed of isolated notes, with one pulse (B: MHNCI 008), two pulses (D = MHNCI 022), and three pulses (F = MHNCI 026). C, E, G) Examples of note groups, with 3–3 pulses (C: MHNCI 026), 3–2 pulses (E = MHNCI 027; individual collected and housed at MNRJ 90349), and 2–3 pulses (G = MHNCI 026). Abbreviations: MHNCI = Museu de História Natural Capão da Imbuia; MNRJ = Museu Nacional, Rio de Janeiro. Spectrograms produced with a FFT size of 4096 points, Hann window, and overlap of 90% in A and FFT 128 points, Hann window, and overlap of 90% in B–G.

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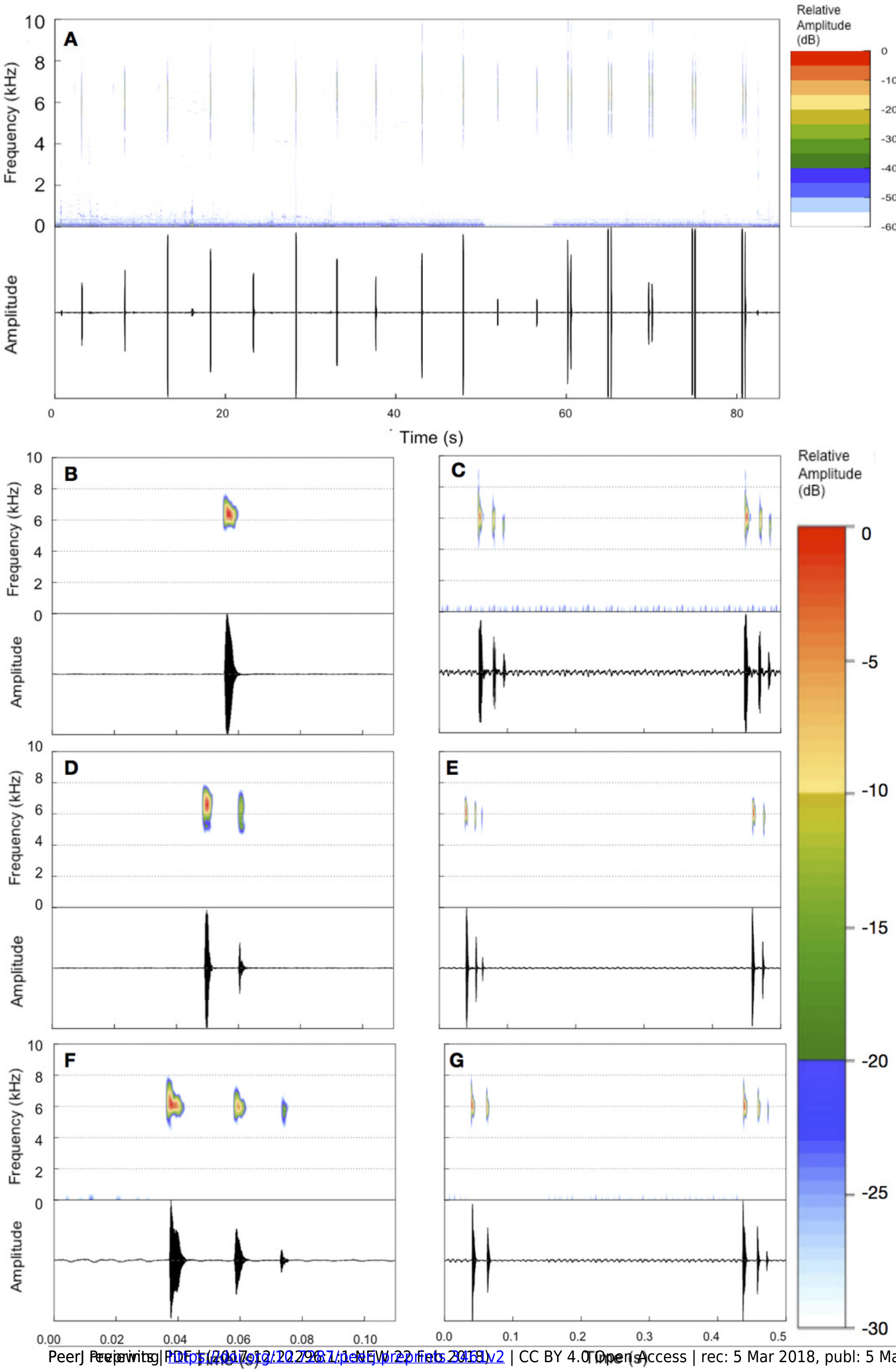
A
B
C
D
E
F
G
Frequency (kHz)
Amplitude
Time (s)
Relative Amplitude (dB)
0
-10
-20
-30
-40
-50
-60
-5
-15
-25
0 20 40 60 80
0.00 0.02 0.04 0.06 0.08 0.10
0.0 0.1 0.2 0.3 0.4 0.5
0 2 4 6 8 10

Table 1 (on next page)

Measurements of advertisement call (AC) features of *Brachycephalus albolineatus* and some parameters.

Number between brackets represent the number of the feature in Fig. 1.

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Table 1. Measurements of advertisement call (AC) features of *Brachycephalus albolineatus* and some parameters. Number between brackets represent the number of the feature in Fig. 1.

Feature / [Analysis]	Range	Mean	SD	N	
				Sample	Individuals
Call duration (s) (1) (entire call)	39.933–191.141	88.368	35.733	24	16
Duration of the call including only isolated notes (s) (2) when note groups is absent	49.971–191.141	100.675	52.423	6	6
Duration of the call including only isolated notes (s) (2) when note groups occurs	18.387–98.896	53.709	25.380	18	13
Duration of the call including only note groups (s) (3)	0.408–76.375	24.438	19.846	25	16
Note rate (notes per minute) (4) (entire call)	5.891–12.997	9.146	1.714	24	16
Note rate of the call including only isolated notes (notes per minute) (5) when note groups is absent	5.891–9.879	7.707	1.707	6	6
Note rate of the call including only isolated notes (notes per minute) (5) when note groups occurs	7.282–13.619	10.288	1.593	18	13
Note rate of the call including only note groups (notes per minute) (6)	4.741–11.727	7.804	1.655	20	14
Number of notes per call (7)	7.00–26.00	14.08	4.70	24	16
Number of isolated notes per call (8)	4.00–26.00	10.96	4.70	24	16
Number of note groups per call (9)	0.00–9.00	3.13	2.77	24	16
[Percentage of number of notes of the entire AC that is composed by note groups in each AC]	0.00–61.54	21.87	18.58	24	16

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Feature / [Analysis]	Range	Mean	SD	N	
				Sample	Individuals
Number of pulses per isolated notes (10)	1.00–3.00	2.00	0.601	324	20
[Number of isolated notes with one pulse]	26.00	---	---	324	20
[Number of isolated notes with two pulses]	188.00	---	---	324	20
[Number of isolated notes with three pulses]	110.00	---	---	324	20
Number of pulses in each note groups (11)	2.00–3.00	2.70	0.459	230	16
[Number of notes of note groups with 2–2 pules]	25.00	---	---	115	16
[Number of notes of note groups with 2–3 pules]	5.00	---	---	115	16
[Number of notes of note groups with 3–3 pules]	71.00	---	---	115	16
[Number of notes of note groups with 3–2 pules]	14.00	---	---	115	16
[Total number of pulses in note groups]	4.00–6.00	5.40	0.825	115	16
Note duration of isolated notes (s) (12)	0.002–0.037	0.020	0.007	96	19
Duration of note groups (s) (13)	0.360–0.578	0.465	0.053	62	16
Inter-note interval in isolated notes (s) (14)	4.092–12.248	6.663	1.705	62	15
Inter-note group interval (s) (15)	4.322–10.678	6.871	1.768	32	13
Inter-note interval within note groups (s) (16)	0.319–0.526	0.412	0.050	55	16
Note dominant frequency (kHz)	5.340–7.321	6.376	0.304	256	10
Highest frequency (kHz)	6.686–10.552	7.980	0.468	326	19
Lowest frequency (kHz)	3.130–6.087	4.531	0.517	326	19

3

Table 2 (on next page)

Distribution of the number of pulses per note (separated by “,”) along the advertisement calls (AC) of *Brachycephalus albolineatus* (see features 10 and 11 in Fig. 1).

Pulses per note groups are indicated between parenthesis, but indicating separately by “–” the number of pulses in each particular note of the group (see Figs. 1 and 3).

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1 Table 2. Distribution of the number of pulses per note (separated by ",") along the advertisement calls (AC) of *Brachycephalus*
2 *albolineatus* (see features 10 and 11 in Fig. 1). Pulses per note groups are indicated between parenthesis, but indicating separately by
3 "–" the number of pulses in each particular note of the group (see Figs. 1 and 3).

N of individuals (call deposit)	Number of pulses per note	Number of notes we hear being emitted before recording the AC
1 (MHNCI 001)	1, 2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, (3–3), (3–3), (3–3)	0
1 (MHNCI 002)	1, 1, 2, 2, 3, 2, 3, 3, 3, 3, 3, 3, (3–3), (3–3)	0
2 (MHNCI 003)	3, 3, 3, 3, 3, 3, 3, 3, 3, (3–3)	?
3 (MHNCI 004)	2, 2, 2, 1, 1, 2, 2, 2, 3, 3, 2, 2, 3, (3–2), 3, (3–3), (2–2)	0
3 (MHNCI 005)	1, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, (2–2), (3–3), (3–3)	0
3 (MHNCI 006)	2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3, (3–3), (3–3), (3–3), (3–3), (3–3)	0
4 (MHNCI 007)	1, 1, 2, 2, 2, 2, 2, 3, 3, 3, 3	0
5 (MHNCI 008)	1, 1, 1, 2, 2, 2, 2, 2, 2, 2, 1, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 1, 2, 2, 2, 2	0
5 (MHNCI 009)	2, 2, 3, 3, 3, 3, 3, 3, (3–3), (3–3), (3–3), (3–3), (3–3), (3–3), (3–3), (3–3), (2–3)	0
5 (MHNCI 010)	2, 3, 3, 3, 3, 3, 3, (3–3), (3–3), (3–3), (3–3), (3–3), (3–3)	?
6 (MHNCI 011)	2, 1, 1, 2, 1, 2, 2, 1, 2, 1, 2, 2, 1	3
6 (MHNCI 012)	2, 2, 2, 2, 2, (3–2)	2
6 (MHNCI 013)	2, 2, 2, 2, (2–2), (2–2), (2–2)	0
7 (MHNCI 014)	2, (3–2), (3–2), (2–2)	?
8 (MHNCI 015)	2, 3, 3, 3, 3, 3, 3, 3, 2, (3–3), 2, (3–3), (3–3), (3–3), (3–2), (3–3), (3–2)	0
8 (MHNCI 016)	2, 2, 2, 2, 3, 3, 3, 3, 3, (3–3), 3, (3–3), (3–3), (3–3), (3–3), (3–3), (3–3), (3–2)	3
8 (MHNCI 017)	2, 2, 3, 2, 3, (3–2), (3–3), (3–3), (3–2), (3–3), (3–3), (3–3), (2–3)	0

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N of individuals (call deposit)	Number of pulses per note	Number of notes we hear being emitted before recording the AC
8 (MHNCI 018)	(3–2), (3–3), (3–3), (3–2), (3–3), (3–3), (3–3), (3–3)	?
9 (MHNCI 019)	2, 2, 2, (2–2), 2, (2–2), (2–2), (2–2, (2–2)	?
9 (MHNCI 020)	2, 2, 2, 2, 2, 2, 2, (2–2)	0
9 (MHNCI 021)	2, 2, 2, 2, 2, 2, 2, 2, 3, (2–2)	0
10 (MHNCI 022)	2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3, 2, 2	0
11 (MHNCI 023)	2, 2, 2, 2, 2, 2	?
12 (MHNCI 024)	2, 2, 2, 2, 2, 2, 2, 2, 2, 3, 2, 2, (3–3), (3–3), (2–3), (3–3), (3–3), 2	0
12 (MHNCI 025)	2, 2, 3, 3, 3, 2, 3, 3	0
13 (MHNCI 026)	2, 3, 3, 3, 2, 3, (3–2), (3–3), (2–3), (3–3), (3–3), (3–3), (2–3)	?
14 (MHNCI 027)	2, 2, 2, 3, 3, 3, (3–2), (3–3), (3–3), (3–3), (3–3), (3–3)	0
14 (MHNCI 028)	1, 2, 2, 2, 2, 3, 3, 3, 2	0
15 (MHNCI 029)	2, 2, 2, 2, 2, 2, 2, 2, 2, (2–2), (2–2), (2–2), (2–2)	0
16 (MHNCI 030)	1, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2–2), (2–2), (2–2), (2–2)	0
17 (MHNCI 031)	2, 2, 3, 3, 3, 3, 3, 3, 3, 3, (3–3), (3–3), (3–3), (3–3), (3–3), (3–3), (3–3)	0
18 (MHNCI 032)	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2	0
19 (MHNCI 033)	1, 1, 2, 1, 2, 2, 2, 2, 2, (2–2), (2–2)	0
20 (MHNCI 034)	2, 2, 2, 2, 2, 2, 2, (2–2), 2, (2–2)	0

4

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Table 3 (on next page)

Comparison of the features used to describe the advertisement call of *Brachycephalus*.

Values are expressed by: range (mean ± SD) [sample/individuals].

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Table 3. Comparison of the features used to describe the advertisement call of *Brachycephalus*. Values are expressed by: range (mean ± SD) [sample/individuals].

Feature	*B. pernix* group		*B. ephippium* group							*B. didactylus* group	
	B. albolineatus	*B. tridactylus*	*B. crispus*	*B. darkside*	*B. ephippium*	*B. ephippium*	*B. pitanga*	*B. pitanga*	*B. pitanga*	*B. hermogenesi*	*B. sulfuratus*
Call duration (s)	39.933–191.141 (88.368 ± 35.733 [24/16]	?–? (0.11 ± 0.02) [?/17][1]	?–300 (? ± ?) [5/?]	2.9–66.2 (30.4 ± 25.3) [7/5]	120–360 (? ± ?) [?/?]					0.2–1.9 (? ± ?) [?/?][2]	1.5–2.3 (1.8 ± 0.2) [95/11]
Call rate (calls per second)										?–? (0.19 ± ?) [?/?][2]	
Interval between calls (s)				6.2, 11.2 [2/?]							3.1–7.4 (5.1 ± 1.4) [95/11]
Note rate (notes per minute)	5.891–12.997 (9.146 ± 1.714 [24/16]			186.4–243.4 (211.4 ± 25.6) [5/?]			?–? (159 ± 11) [?/2]				
Note rate (notes per second)		?–? (0.16 ± 0.03) [11/?]	?–? (1.67 ± 0.09) [5/?]							?–? (1.09 ± ?) [?/?][2]	0.1–0.3 (0.2 ± 0.0) [485/11]
Pulse rate (pulses per second)			?–? (17.4 ± 2.12) [5/?]	36.8–78.4 (56.9 ± 4.9) [790/5]			?–? (62 ± 8) [?/2][3]				6.1–12.3 (9.3 ± 1.8) [?/11]
Number of notes per call	7–26 (14.08 ± 4.70) [24/16]	1		9–253 (114 ± 97.1) [7/5]						1–7 (? ± ?) [?/?][2]	4–7 (5.3 ± 0.9) [485/11]
Number of pulses per isolated notes	1–3 (2.00 ± 0.601) [324/20]	0	7–12 (10 ± 1.19) [100/5]	5–8 (6.3 ± 0.7) [790/5]	5–15 (12 ± 1.96) [57/?]		?–? (11.1 ± 1.2) [?/2]	6.90–14.30 (10.86 ± 1.62) [?/?]			7–11 (8.8 ± 1.3) [?/11]
Number of pulses in each note groups	2–3 (2.70 ± 0.459) [230/16]										

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Feature	*B. pernix* group		*B. ephippium* group							*B. didactylus* group	
	B. albolineatus	*B. tridactylus*	*B. crispus*	*B. darkside*	*B. ephippium*	*B. ephippium*	*B. pitanga*	*B. pitanga*	*B. pitanga*	*B. hermogenesi*	*B. sulfuratus*
Note duration of isolated notes (s)	0.002–0.037 (0.020 ± 0.007) [96/19]	?–? (0.11 ± 0.02) [?/17]	?–? (0.28 ± 0.02) [100/5]	0.083–0.163 (0.111 ± 0.014) [790/5]	0.093–0.125 (0.112 ± 0.006) [19/?]		?–? (0.170 ± 0.013) [?/2]	0.15–0.25 (0.19 ± 0.03) [400/40]			0.131–0.233 (0.195 ± 0.013) [485/11]
Duration of note groups (s)	0.360–0.578 (0.465 ± 0.053) [62/16]										
Pulse duration (s)			?–? (0.027 ± 0.004) [517/5]								0.02–0.03 (0.024 ± 0.005) [?/11]
Inter-note interval in isolated notes (s)	4.092–12.248 (6.663 ± 1.705) [62/15]		?–? (0.35 ± 0.02) [100/5]	0.122–0.215 (0.159 ± 0.014) [783/5]	0.123–0.149 (0.134 ± 0.007) [18/?]			0,20–0.43 (0.28 ± 0.05) [400/40]			
Inter-note group interval (s) (15)	4.322–10.678 (6.871 ± 1.768) [32/13]										
Inter-note interval within note groups (s)	0.319–0.526 (0.412 ± 0.050) [55/16]										
Note dominant frequency (kHz)	5.340–7.321 (6.376 ± 0.304) [256/10]	?–? (4.8 ± 0.2) [?/17]	?–? (4.6 ± 0.19) [100/5]	2.856–3.797 (3.382 ± 0.185) [790/5][4]		?–? (3.94 ± 0.24) [?/5]	?–? (4.9 ± 0.2) [?/2]	4.311–5.550 (4.816 ± 0.414) [400/40]	?–? (5.43 ± 0.30) [?/8]		6.2–7.2 (6.7 ± 0.3) [?/11]
Call dominant frequency (kHz)										?–? (6.8 ± 0.8) [5/?][5]	
Highest frequency	6.686–10.552	6.4 (? ± ?)	?–? (5.7 ±		5.3 (? ± ?)						8.2–10.3 (9.3

Feature	*B. pernix* group		*B. ephippium* group							*B. didactylus* group	
	B. albolineatus	*B. tridactylus*	*B. crispus*	*B. darkside*	*B. ephippium*	*B. ephippium*	*B. pitanga*	*B. pitanga*	*B. pitanga*	*B. hermogenesi*	*B. sulfuratus*
(kHz)	(7.980 ± 0.468)	[?/17][6]	0.17) [100/5][6]		[?/?][6]						± 0.3) [?/11][6]
Lowest frequency (kHz)	3.130–6.087 (4.531 ± 0.517)	3.2 (? ± ?) [?/17][6]	?–? (3.5 ± 0.19) [100/5][6]		3.4 (? ± ?) [?/?][6]						4.5–5.5 (4.9 ± 0.3) [?/11][6]
5%–95% frequency[7]				2.484–5.766 (? ± ?) [?/?]							
"Highest sound pressure" (dB)		?–? (110 ± 5.6) [?/17]				?–? (47.0 ± 5.7) [3/?]	56–66 (? ± ?) [4/?]		?–? (57.6 ± 1.8) [8/?]		
Approach (*sensu* Köhler *et al.* 2017)	note-centered	call-centered	note-centered	note-centered	note-centered	not applicable	note-centered[2]	note-centered	not applicable	note-centered[2]	note-centered
Source	This study	Garey *et al.* (2012)	Condez *et al.* (2014)	Guimarães *et al.* (2017)	Pombal Jr., Sazima & Haddad (1994)	Goutte *et al.* (2017)	Araújo *et al.* (2012)	Tandel *et al.* (2014)	Goutte *et al.* (2017)	Verdade *et al.* (2008)	Condez *et al.* (2016)

[1] Represents note duration under note-centered approach.

[2] Note-centered approach and call-centered approach probably mixed in this measurement.

[3] The unit of measure was erroneously cited as Hz.

[4] Feature cited as "peak frequency" by Guimarães *et al.* (2017) but refers to our dominant frequency.

[5] We are not sure if in the measurement was not mixed with note dominant frequency.

[6] The measurement procedure has not been explained and data may be not comparable.

[7] Feature cited as "dominant frequency" by Guimarães *et al.* (2017).