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

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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 ecology of the vast majority of the species is unknown, particularly their advertisement call (AC), which has been described only for seven species of the genus. In the present study, we describe the AC of *Brachycephalus albolineatus*, a recently described microendemic species from Santa Catarina, southern Brazil. We analyzed 34 advertisement calls (ACs) and 554 notes from 20 individuals of *B. albolineatus*, recorded between 5–6 February 2016 in the type locality of the species. We collected five individuals as vouchers (they are from the type series of the species). Eight individuals we recorded 2–4 times. *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 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 and the note groups by two or three pulses in each note. Most ACs 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 AC. Most isolated notes escalate their number of pulses along the AC (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). We show that there is intra-individual variation in several parameters [(1) duration and rate of the AC; (2) number of

pulses and notes, as well as of isolated notes and note groups, per AC; and (3) in dominant frequency]. Individuals apparently expend progressively more energy along their emission of the AC because the AC normally escalates, incorporating note groups and pulses per note. Individual variation analysis also demonstrated that less structured ACs (i.e. with notes with fewer pulses) are not fixed individually and can represent temporal variation. It is possible that isolated notes and note groups could have distinct purpose, perhaps territorial defense and mating, respectively. Recently, it was demonstrated that *B. ephippium* and *B. pitanga* are insensitive to the sound of their own calls. However, these species usually call above the leaf litter and belong to a distinct group that *B. albolineatus*, who call only under the leaf litter. It is possible that species of the genus that only call underneath the leaf litter still retain their hearing capacity.

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

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Running headline: Advertisement call of *B. albolineatus*

Abstract

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 ecology of the vast majority of the species is unknown, particularly their advertisement call (AC), which has been described only for seven species of the genus. In the present study, we describe the AC of *Brachycephalus albolineatus*, a recently described microendemic species from Santa Catarina, southern Brazil. We analyzed 34 advertisement calls (ACs) and 554 notes from 20 individuals of *B. albolineatus*, recorded between 5–6 February 2016 in the type locality of the species. We collected five individuals as vouchers (they are from the type series of the species). Eight individuals we recorded 2–4 times. *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 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 and the note groups by two or three pulses in each note. Most ACs 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 AC. Most isolated notes escalate their number of pulses along the AC (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). We show that there is intra-individual variation in several parameters [(1) duration and rate of the AC; (2) number of pulses and notes, as well as of isolated notes and note groups, per AC; and (3) in dominant frequency]. Individuals apparently expend progressively more energy along their emission of the AC because the AC normally escalates, incorporating note groups and pulses per note. Individual variation analysis also demonstrated that less structured ACs (i.e. with notes with fewer pulses) are not fixed individually and can represent temporal variation. It is possible that isolated notes and note groups could have distinct purpose, perhaps territorial defense and mating, respectively. Recently, it was demonstrated that *B. ephippium* and *B. pitanga* are insensitive to the sound of their own calls. However, these species usually call above the leaf litter and belong to a distinct group that *B. albolineatus*, who call only under the leaf litter. It is possible that species of the genus that only call underneath the leaf litter still retain their hearing capacity.

Key-words: bioacoustics; note-centered approach; individual variation; call purpose; *Brachycephalus tridactylus*; variation inter species groups.

Introduction

Brachycephalus are among the smallest terrestrial vertebrates in the world (Rittmeyer *et al.* 2012), with most species not exceeding 2.5 mm 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 *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, including a reduced number and size of digits (Hanken & Wake 1993, Yeh 2002, Clemente-Carvalho *et al.* 2009). 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 *ephippium*, *didactylus*, and *pernix* species groups (Ribeiro *et al.* 2015). The *ephippium* group includes 12 described species (Bornschein *et al.* 2016a, Guimarães *et al.* 2017) which have "bufoniform" body shape and dermal ossification (Ribeiro *et al.* 2015). This group has a more central distribution along the Atlantic Rainforest, from Espírito Santo and Minas Gerais to center-eastern São Paulo (Bornschein *et al.* 2016a). The *didactylus* group includes only four species (Bornschein *et al.* [2016a]; including the species described by Condez *et al.* [2016]), which have "leptodactyliform" body shape and absence of dermal ossification (Ribeiro *et al.* 2015). This group has a broad geographic distribution, including the species associated with the northernmost and southernmost limits of the genus (Bornschein *et al.* 2016a). Finally, the *pernix* group includes 17 described species (Bornschein *et al.* 2016a,b, Ribeiro *et al*. 2017) with bufoniform body shape but lacking dermal ossification (Ribeiro *et al.* 2015). The *pernix* group is distributed in southern Brazil, in the states of Paraná and Santa Catarina (Bornschein *et al.* 2016a,b). The species of each group respond differently to altitude, with species from the *didactylus* group occurring from sea level (0–1110 m a.s.l.) and being much more environmentally tolerant (Pie *et al.* 2013, Bornschein *et al.* 2016a). Species from the remaining groups depend on high altitude conditions but can be found locally at lower altitudes, probably in response to particular microclimatic conditions (*ephippium* group occurs between 200 to 1900 m a.s.l. and *pernix* group between 455 to 1640 m a.s.l.; [Bornschein *et al.* 2016a]).

It has been proposed that *Brachycephalus* species resulted from vicariant processes following upward dispersal of montane forests during past warm/wet climates (Bornschein *et al.* 2016a, Firkowski *et al.* 2016). This diversification is very recent, at least for species of the *pernix* group, within the past 1 My (Firkowski *et al.* 2016). Possibly due to these historical evolutionary processes, *Brachycephalus* species are almost exclusively allopatric or parapatric, with few cases of syntopy (Bornschein *et al.* 2016a). Syntopic species include members of distinct groups and are in contact only at the altitudinal limits of each other: a higher limit for the "lowland" species

(*didactylus* group) and a lower limit for the "montane" species (*ephippium* and *pernix* groups; Bornschein *et al.* [2016a]).

There is a recent increase in description of new species within *Brachycephalus*, with 20 species described in the last 10 years (Frost 2017). However, the ecology of the vast majority of their 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. *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), 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*. Over the course of our studies on mountain frogs from the southern Atlantic Rainforest, in which we discovered several new species of *Brachycephalus* (Pie & Ribeiro 2015, Ribeiro *et al.* 2015, Bornschein *et al.* 2016b, Ribeiro *et al*. 2017), we recorded the advertisement calls of nearly all of them. Given that their diagnoses where easily achieved through morphological characters, we did not include vocal descriptions in any of those studies. In the present study, we describe the advertisement call of *B. albolineatus*, a member of the *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, on the border between the municipalities of Jaraguá do Sul and Massaranduba, 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; however, the former recordings were discarded due to poor signal-to-noise ratio. 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 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 close to 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 within-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 Lite 2.0 and Raven Pro 1.5 (Bioacoustics Research Program 2012). Time domain variables were measured from oscillograms and frequency domain variables were measured from spectrograms. Spectrogram parameters 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. We chose not to noise-filter the spectrograms to avoid eliminating potential sound characters. Final spectrograms, as well as diagnostic plots, were generated using the Seewave package, v. 2.0.5 (Sueur *et al.* 2008) of the R environment, v. 3.2.2 (R Core Team 2015) using the same window size and overlap settings as in Raven Lite and Raven Pro, but resampling the audio files at 22.05 kHz.

To analyze the advertisement calls, we first selected the most conspicuous spectrograms and compared them with their corresponding oscillograms. We used a note-centered approach, defining uninterrupted units of sound as notes and their entire collection as calls (Köhler *et al.* 2017). We described the advertisement calls following parameters and criteria of Köhler *et al.* (2017). We took the liberty of describing the general parameters 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 *B. albolineatus*. We used the following parameters, 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; and 17) dominant frequency (kHz). We did not focus on frequency ranges, given that preliminary work indicated that estimated values are highly dependent on the quality of the recording and the used cut-off values. 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]).

Results

We recorded calls from 29 individuals of *Brachycephalus albolineatus* but analyzed 34 advertisement calls from 20 individuals, five of which collected as vouchers (MHNCI 10296–9, MNRJ 90349). We recorded eight individuals 2–4 times ($\acute{x}$ = 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 ($\acute{x}$ = 88.37 ± 35.73 s; Table 1; see parameter 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 Figure 2. The note rate was 5.89–13.00 notes per minute ($\acute{x}$ = 9.15 ± 1.71 notes per minute; Table 1; see parameter 4 in Fig. 1). Advertisement calls included 7–26 notes ($\acute{x}$ = 14.08 ± 4.70 notes; Table 2; see parameter 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. 1 and 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 3, Fig. 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 parameter 9 in Fig. 1) and span, on average, 24.44 s (± 19.85 s; range of 0.41–76.37 s; see parameter 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 parameter 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 parameter 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 parameter 5 in Fig. 1).

The number of pulses per isolated notes varies from 1–3, more commonly two pulses (58%; Table 4; Fig. 3; see parameter 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). 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). The number of pulses in each note groups varied from 2–2 to 3–3, with additional combinations, being more common 3–3 pulses (62%; Table 4; Fig. 3; see parameter 11 in Fig. 1, explaining how we count pulses in note groups).

Our results showed the existence of individual variation. For example, we recorded four individuals that emitted one advertisement calls with only isolated notes and others with isolated notes and note groups (Table 3; Fig. 2). Individuals also varied in number of notes emitted per advertisement calls (in up to 10 notes; Table 2; Fig. 2), in number of isolated notes and note groups per advertisement calls (Table 2; Fig. 2), in the number of pulses per notes along the call (Table 3), and in the dominant frequency (Fig. 4; see below).

Note duration of isolated notes varies from 0.002–0.037 s ($\acute{x}$ = 0.020 ± 0.007 s) and duration of note groups varies from 0.360–0.578 s ($\acute{x}$ = 0.465 ± 0.053 s; Table 5; see also parameters 12 and 13 in Fig. 1 and Fig. 3). The inter-note interval in isolated notes is, on average, 6.663 s (4.092–12.248 ± 1.705 s) and the inter-note group interval is, on average, 6.871 s (4.322–10.678 ± 1.768 s; Table 5; see parameters 14 and 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 5; see parameter 16 in Fig. 1). The dominant frequency varies from 5.34–7.32 kHz ($\acute{x}$ = 6.38 ± 0.30 kHz; Table 5; Fig. 4). Two individuals presented calls with dominant frequency below the mean (MHNCI 026–7) and two other from the mean upward (MHNCI 001 and 003), while the remaining showed dominant frequency crossing the mean in both directions (Fig. 4). In Figure 4 we can also observe intra-individual variations. Finally, regarding the frequency of pulses, when more than one pulse occurred in a given note, the subsequent pulses had a smaller frequency amplitude, especially reduced in the highest than the lowest frequency (values not shown, but see Fig. 3).

Discussion

The long advertisement calls of *Brachycephalus albolineatus*, and their variations, allowed us to see a trend of individuals investing progressively more energy along their emission. 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 3). Individual variation analysis also demonstrated that less structured advertisement calls (i.e. with notes with less pulses) are not fixed individually and can represent temporal variation. In the only species of the *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 also possible that the advertisement calls with isolated notes and note groups could have distinct purposes, perhaps territorial defense when composed only by the former and territorial defense plus female preference when composed by isolated notes and note groups. This hypothesis can be tested in the laboratory and the call purpose also in the field, by recording throughout the year and placing a female and a male close to a calling male. This can be very revealing to explain effects of individual's arousal and functions of the calls.

There are only a few species of *Brachycephalus* with described advertisement calls (Pombal Jr. *et al.* 1994, Verdade *et al.* 2008, Araújo *et al.* 2012, Garey *et al.* 2012, Condez *et al.* 2014, 2016, Tandel *et al.* 2014, Goutte *et al.* 2017), and their simplified description and absence of standardization in their descriptions prevented us to conduct a more detailed comparison with the call of *B. albolineatus*. Nevertheless, *B. albolineatus* is the only known species with a long advertisement call that is structurally modified along its emission, i.e. more structured (with notes with increasingly more pulses).

Another striking difference is how much the advertisement call of *Brachycephalus albolineatus* is longer than that of *B. tridactylus* (Garey *et al.* 2012), both of the *pernix* group (average of 88.37 s and 0.11 s, respectively). We do not rule out the possibility that Garey *et al.* (2012) could have overlooked differences in the structure of notes in the advertisement of *B. tridactylus* and that this species does indeed exhibits some level of structuring such as that of *B. albolineatus*. We also believe that the description of the advertisement calls by Garey *et al.* (2012) considered a criterion named as call-centered approach (Köhler *et al.* 2017), while we adopted the note-centered approach of Köhler *et al.* (2017). In other words, in the comparison of the advertisement calls of both species, as described, we are comparing one call composed by

several notes (*B. albolineatus*) with a single note of the call of *B. tridactylus*. The one-pulse notes of *Brachycephalus 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.

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) questioned the fact that the maintenance of calls is energetically costly and favors predation and parasitism. They suggest that calls may have been maintained in the studied species because of the call side effects (e.g. vocal sac movement), because they are aposematic and thus less susceptible to the effect of predation, or by evolutionary inertia, for example. The relevant issue to be discussed here is that *B. ephippium* and *B. pitanga*, both members of the *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 *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 the *Brachycephalus* species of the *pernix* and *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

The advertisement call of *Brachycephalus albolineatus* 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. The call variations in number of notes, of pulses and eventual incorporation of note groups may be related to individual arousal levels. It is also possible that the advertisement calls have distinct purposes, perhaps territorial defense when composed only by isolated notes and territorial defense plus female preference when composed by isolated notes and note groups.

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Figure 1

Representation of some parameters considered in the description of the advertisement call of *Brachycephalus albolineatus*.

Representation of some parameters considered in the description of the advertisement call of *Brachycephalus albolineatus*. Numbers correspond with 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; four notes in the example); 5) note rate of the call including only isolated notes (notes per minute; two notes in the example); 6) note rate of the call including only note groups (notes per minute; one note in the example); 7) number of notes per call (five notes in the example); 8) number of isolated notes per call (three notes in the example); 9) number of note groups per call (two notes in the example); 10) number of pulses per isolated notes (1, 2 and 3 in the example); 11) number of pulses in each note groups (3–3 and 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).

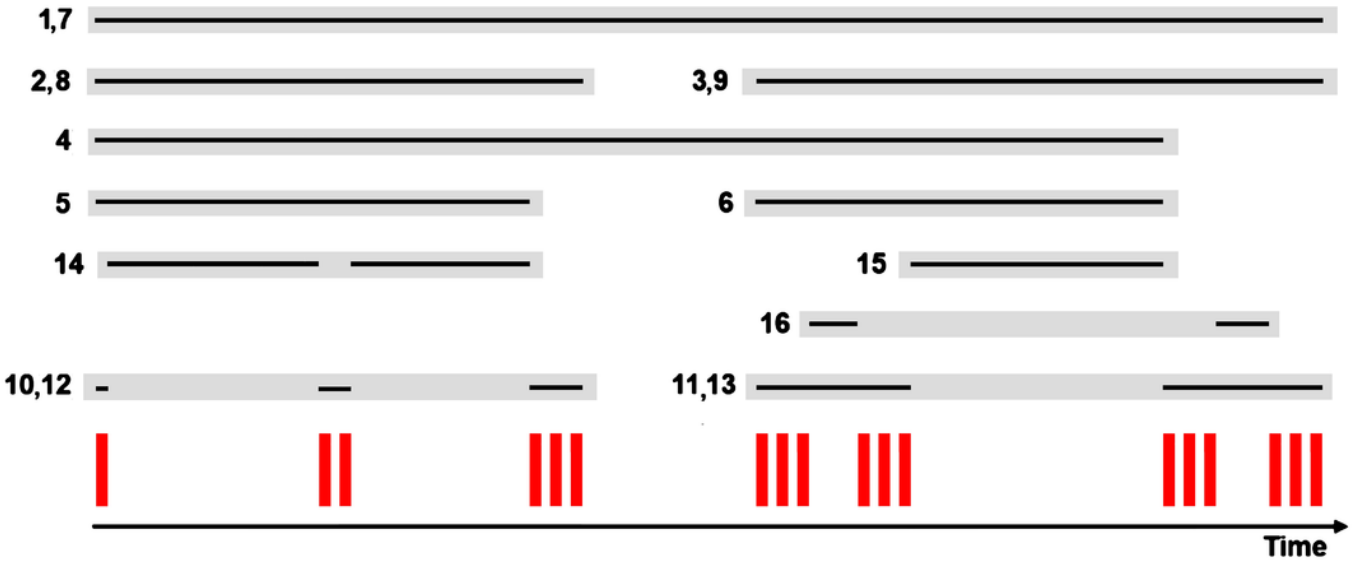

Figure 2

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).

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

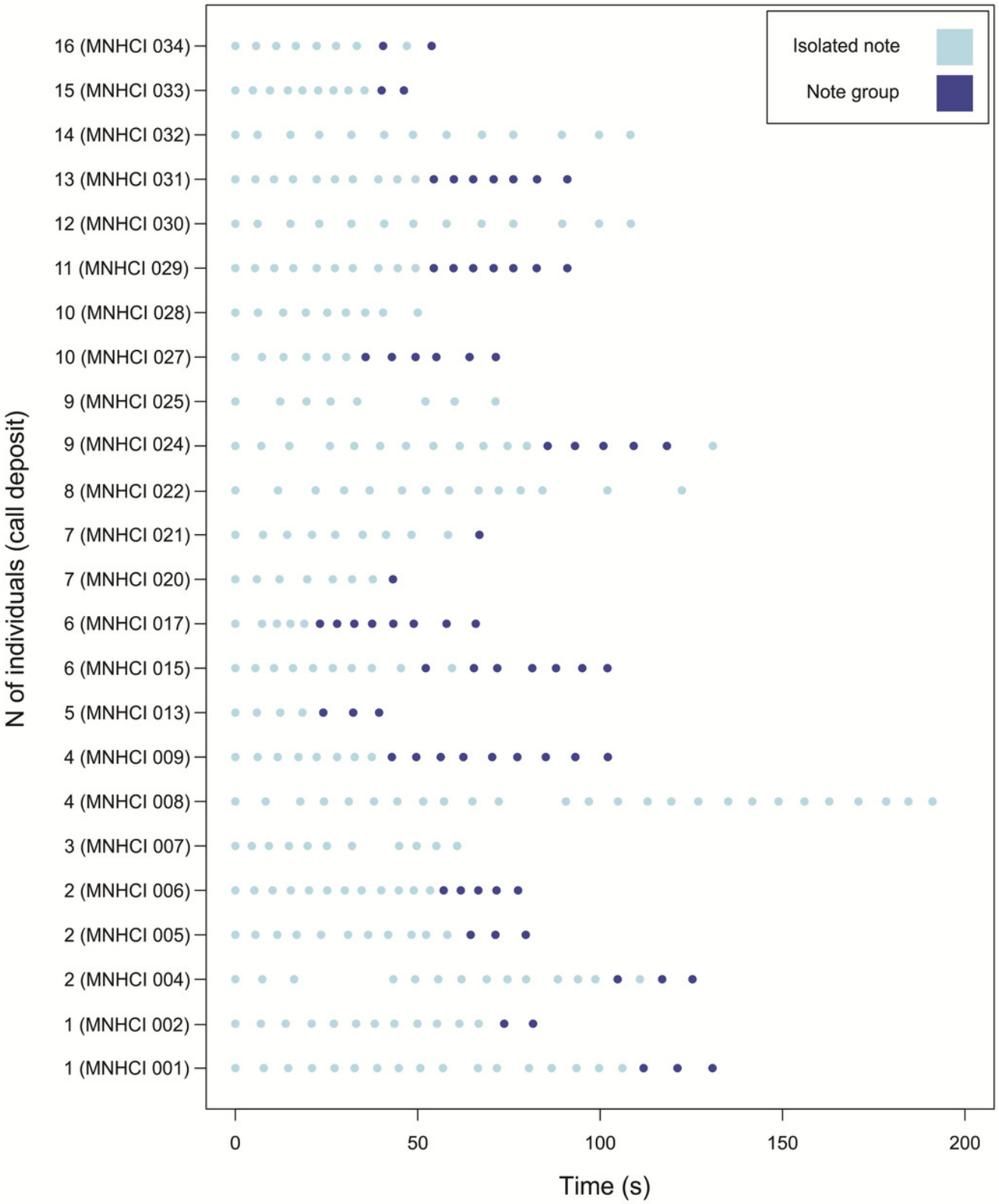
Isolated note
Note group
16 (MNHCI 034)
15 (MNHCI 033)
14 (MNHCI 032)
13 (MNHCI 031)
12 (MNHCI 030)
11 (MNHCI 029)
10 (MNHCI 028)
10 (MNHCI 027)
9 (MNHCI 025)
9 (MNHCI 024)
8 (MNHCI 022)
7 (MNHCI 021)
7 (MNHCI 020)
6 (MNHCI 017)
6 (MNHCI 015)
5 (MNHCI 013)
4 (MNHCI 009)
4 (MNHCI 008)
3 (MNHCI 007)
2 (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 notes of the advertisement call of *Brachycephalus albolineatus*.

Example of notes of the advertisement call of *Brachycephalus albolineatus*. A, C, D) All examples observed of isolated notes, with one pulse (A: MHNCI 008), two pulses (C = MHNCI 022), and three pulses (D = MHNCI 026). B, D, F) Examples of note groups, with 3–3 pulses (B: MHNCI 026), 3–2 pulses (D = MHNCI 027; individual collected and housed at MNRJ 90349), and 2–3 pulses (F = MHNCI 026). Notice the gradual reduction of the amplitude of frequency in subsequent pulses. Abbreviations: MHNCI = Museu de História Natural Capão da Imbuia; MNRJ = Museu Nacional, Rio de Janeiro. Spectrograms produced with FFT size of 128 points, Hann window, and overlap of 88%.

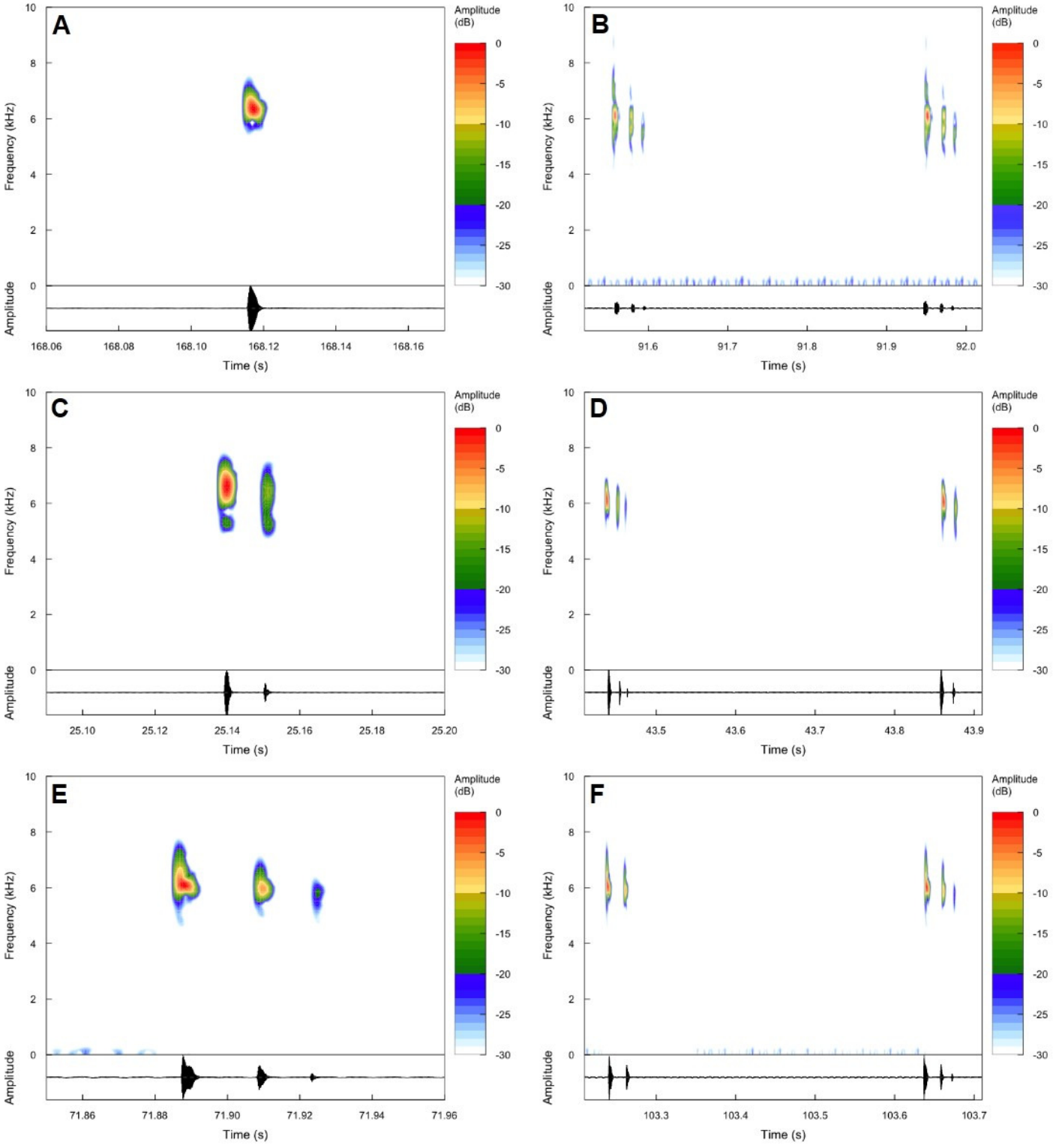
A
B
C
D
E
F
Frequency (kHz)
Amplitude
Amplitude (dB)
Time (s)
0
-5
-10
-15
-20
-25
-30
168.06
168.08
168.10
168.12
168.14
168.16
91.6
91.7
91.8
91.9
92.0
25.10
25.12
25.14
25.16
25.18
25.20
43.5
43.6
43.7
43.8
43.9
71.86
71.88
71.90
71.92
71.94
71.96
103.3
103.4
103.5
103.6
103.7

Figure 4

Distribution of dominant frequencies of the advertisement calls from 10 individuals of *Brachycephalus albolineatus* recorded with a good signal to noise ratio (S/N) conditions.

Distribution of dominant frequencies of the advertisement calls from 10 individuals of *Brachycephalus albolineatus* recorded with a good signal to noise ratio (S/N) conditions. The boxes represent the range, median, and the 1st and 3rd quartile; points represent outliers. In addition, each box color represents distinct individuals (so there are three individuals with more than one advertisement call included). Abbreviations: MHNCI = Museu de História Natural Capão da Imbuia; ID = identification.

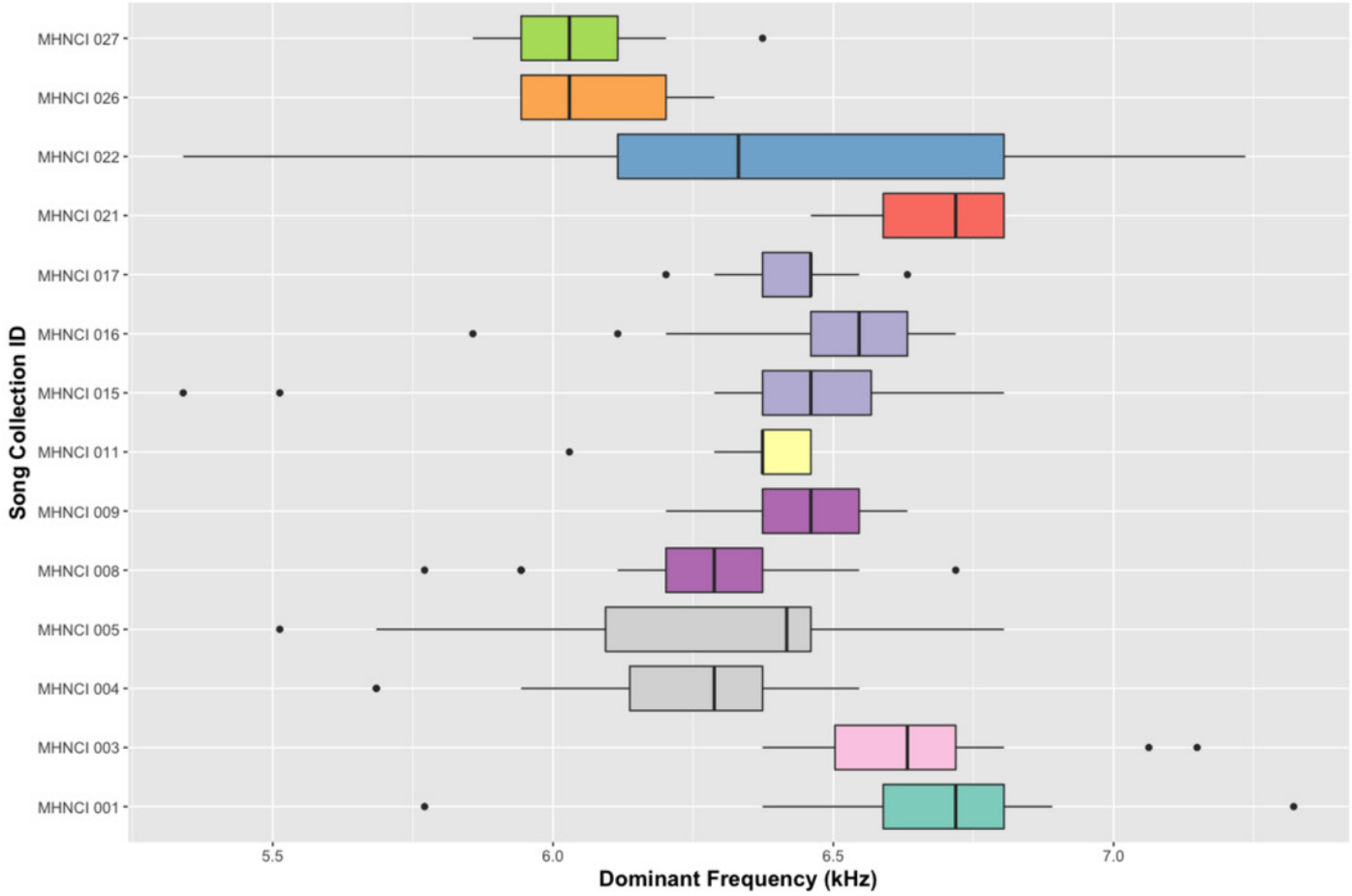

Table 1(on next page)

Call duration (s) and note rate of the advertisement call of *Brachycephalus albolineatus*.

Call duration (s) and note rate of the advertisement call of *Brachycephalus albolineatus*. Note rate = counted notes / duration (= duration from the beginning of the first note until the beginning of the last note divided by the notes counted in this time and is expressed by notes per minute; see text and Fig. 1). Number between brackets represent the number of the parameter in Fig. 1.

Table 1. Call duration (s) and note rate of the advertisement call of *Brachycephalus albolineatus*. Note rate = counted notes / duration (= duration from the beginning of the first note until the beginning of the last note divided by the notes counted in this time and is expressed by notes per minute; see text and Fig. 1). Number between brackets represent the number of the parameter in Fig. 1.

Measure	Entire call		Part of the advertisement call with only isolated notes				Part of the advertisement call with only note groups	
			When note groups is absent		When note groups occurs			
	Duration (1)	Rate (4)	Duration (2)	Rate (5)	Duration (2)	Rate (5)	Duration (3)	Rate (6)
Minimum	39.933	5.891	49.971	5.891	18.387	7.282	0.408	4.741
Maximum	191.141	12.997	191.141	9.879	98.896	13.619	76.375	11.727
Mean	88.368	9.146	100.675	7.707	53.709	10.288	24.438	7.804
SD	35.733	1.714	52.423	1.707	25.380	1.593	19.846	1.655
N of calls	24	24	6	6	18	18	25	20
N of individuals	16	16	6	6	13	13	16	14

Table 2(on next page)

Number of notes per advertisement call (AC) of *Brachycephalus albolineatus* that were recorded from the beginning.

Number of notes per advertisement call (AC) of *Brachycephalus albolineatus* that were recorded from the beginning. Number between brackets represent the number of the parameter in Fig. 1.

Table 2. Number of notes per advertisement call (AC) of *Brachycephalus albolineatus* that were recorded from the beginning. Number between brackets represent the number of the parameter in Fig. 1.

N of individuals (call deposit)	Number of isolated notes per call (8)	Number of note groups per call		Number of notes per call (7)
		Number of notes (9)	% of AC	
1 (MHNCI 001)	17	3	15.0	20
1 (MHNCI 002)	12	2	14.3	14
2 (MHNCI 004)	14	3	17.6	17
2 (MHNCI 005)	11	3	21.4	14
2 (MHNCI 006)	12	5	29.4	17
3 (MHNCI 007)	11	0	0.0	11
4 (MHNCI 008)	26	0	0.0	26
4 (MHNCI 009)	8	9	52.9	17
5 (MHNCI 013)	4	3	42.9	7
6 (MHNCI 015)	10	7	41.2	17
6 (MHNCI 017)	5	8	61.5	13
7 (MHNCI 020)	7	1	12.5	8
7 (MHNCI 021)	9	1	10.0	10
8 (MHNCI 022)	14	0	0.0	14
9 (MHNCI 024)	13	5	27.8	18
9 (MHNCI 025)	8	0	0.0	8
10 (MHNCI 027)	6	6	50.0	12
10 (MHNCI 028)	9	0	0.0	9
11 (MHNCI 029)	9	4	30.8	13
12 (MHNCI 030)	18	4	18.2	22
13 (MHNCI 031)	10	7	41.2	17
14 (MHNCI 032)	13	0	0.0	13
15 (MHNCI 033)	9	2	18.2	11
16 (MHNCI 034)	8	2	20.0	10
Minimum	4.00	0.00	0.00	7.00
Maximum	26.00	9.00	61.54	26.00
Mean	10.96	3.13	21.87	14.08
SD	4.70	2.77	18.58	4.70

Table 3(on next page)

Number of pulses per note (separated by ",") of the advertisement calls (AC) of *Brachycephalus albolineatus* (see parameters 10 and 11 in Fig. 1).

Number of pulses per note (separated by ",") of the advertisement calls (AC) of *Brachycephalus albolineatus* (see parameters 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. Number of pulses per note (separated by ",") of the advertisement calls (AC) of *Brachycephalus albolineatus* (see parameters 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).

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), (3–3)	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), (3–3)	?
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
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, 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

N of individuals (call deposit)	Number of pulses per note	Number of notes we hear being emitted before recording the AC
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), (2–2)	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, 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

Table 4(on next page)

Number of pulses in notes of the advertisement call of *Brachycephalus albolineatus*.

Number of pulses in notes of the advertisement call of *Brachycephalus albolineatus*. Number between brackets represent the number of the parameter in Fig. 1.

Table 4. Number of pulses in notes of the advertisement call of *Brachycephalus alboguttatus*.

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

Number of pulses	Isolated notes (10)	Note groups (11)
1	26 (8%)	
2	188 (58%)	
3	110 (34%)	
2–2		25 (22%)
2–3		5 (4%)
3–3		71 (62%)
3–2		14 (12%)
Total number of notes	324	115

Table 5(on next page)

Measurements of some advertisement call parameters of *Brachycephalus albolineatus*.

Measurements of some advertisement call parameters of *Brachycephalus albolineatus*.

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

Table 5. Measurements of some advertisement call parameters of *Brachycephalus albolineatus*. Number between brackets represent the number of the parameter in Fig. 1.

Parameter	Range	Mean	SD	N	
				Sample	Individuals
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
Dominant frequency (kHz)	5.340–7.321	6.376	0.304	256	10