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## 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.

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

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14 Running headline: Advertisement call of *B. albolineatus*

15 Abstract

16 *Brachycephalus* are among the smallest terrestrial vertebrates in the world. The genus  
17 encompasses 34 species endemic to the Brazilian Atlantic Rainforest, occurring mostly in  
18 montane forests, with many species showing microendemic distributions to single mountaintops.  
19 It includes diurnal species living in the leaf litter and calling during the day, mainly during the  
20 warmer months of the year. The ecology of the vast majority of the species is unknown,  
21 particularly their advertisement call (AC), which has been described only for seven species of the  
22 genus. In the present study, we describe the AC of *Brachycephalus albolineatus*, a recently  
23 described microendemic species from Santa Catarina, southern Brazil. We analyzed 34  
24 advertisement calls (ACs) and 554 notes from 20 individuals of *B. albolineatus*, recorded  
25 between 5–6 February 2016 in the type locality of the species. We collected five individuals as  
26 vouchers (they are from the type series of the species). Eight individuals we recorded 2–4 times.  
27 *Brachycephalus albolineatus* have a long advertisement call of 40–191 s (mean of 88 s)  
28 composed of 7–26 notes (mean of 14 notes) emitted at a rate of 6–13 notes per minute (mean of 9  
29 notes per minute) and at a dominant frequency of 5–7 kHz (mean of 6 kHz). Advertisement calls

30 are composed of isolated notes and note groups (two notes involved in each particular note  
31 group); the former is composed by one to three pulses and the note groups by two or three pulses  
32 in each note. Most ACs present both isolated notes and note groups, with a few cases showing  
33 only the former. Note groups are emitted invariably in the last third of the AC. Most isolated  
34 notes escalate their number of pulses along the AC (1 to 2, 1 to 3 or 2 to 3). Note duration of  
35 isolated notes varies from 0.002–0.037 s (mean of 0.020 s) and duration of note group vary from  
36 0.360–0.578 s (mean of 0.465 s). We show that there is intra-individual variation in several  
37 parameters [(1) duration and rate of the AC; (2) number of pulses and notes, as well as of isolated  
38 notes and note groups, per AC; and (3) in dominant frequency]. Individuals apparently expend  
39 progressively more energy along their emission of the AC because the AC normally escalates,  
40 incorporating note groups and pulses per note. Individual variation analysis also demonstrated  
41 that less structured ACs (i.e. with notes with fewer pulses) are not fixed individually and can  
42 represent temporal variation. It is possible that isolated notes and note groups could have distinct  
43 purpose, perhaps territorial defense and mating, respectively. Recently, it was demonstrated that  
44 *B. ephippium* and *B. pitanga* are insensitive to the sound of their own calls. However, these  
45 species usually call above the leaf litter and belong to a distinct group that *B. albolineatus*, who  
46 call only under the leaf litter. It is possible that species of the genus that only call underneath the  
47 leaf litter still retain their hearing capacity.

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

## 50 Introduction

51 *Brachycephalus* are among the smallest terrestrial vertebrates in the world (Rittmeyer *et al.*  
52 2012), with most species not exceeding 2.5 mm in body length. The genus includes 34 species  
53 (Frost 2017), occurring from the southern Bahia to northeastern Santa Catarina, Brazil  
54 (Bornschein *et al.* 2016a; see also Pie *et al.* 2013). Most *Brachycephalus* species, particularly in  
55 the *pernix* species group (see below), are microendemic, occurring in one or a few adjacent  
56 mountaintops, with total extents of occurrence comparable to the smallest ranges of species  
57 around world (Bornschein *et al.* 2016a). Species are diurnal, living in the leaf litter in forests of  
58 the Atlantic Rainforest domain (Bornschein *et al.* 2016a and compilation therein). Direct  
59 development, with a reduced number of eggs laid on the soil (Pombal Jr. 1999), was

60 demonstrated for *B. ehippium* (Heyer *et al.* 1990, Pombal Jr. 1999), and this is assumed as the  
61 reproductive pattern for the genus. *Brachycephalus* is characterized by extreme miniaturization,  
62 including a reduced number and size of digits (Hanken & Wake 1993, Yeh 2002, Clemente-  
63 Carvalho *et al.* 2009). Some species are brightly colored, with neurotoxins found in the skin of  
64 two aposematic species (Sebben *et al.* 1986, Pires Jr. *et al.* 2002, 2003, 2005, Schwartz *et al.*  
65 2007), possibly originated from intestinal bacteria (Schwartz *et al.* 2007).

66 The species of the genus have been segregated into three phenetic groups, namely the  
67 *ehippium*, *didactylus*, and *pernix* species groups (Ribeiro *et al.* 2015). The *ehippium* group  
68 includes 12 described species (Bornschein *et al.* 2016a, Guimarães *et al.* 2017) which have  
69 “bufoniform” body shape and dermal ossification (Ribeiro *et al.* 2015). This group has a more  
70 central distribution along the Atlantic Rainforest, from Espírito Santo and Minas Gerais to center-  
71 eastern São Paulo (Bornschein *et al.* 2016a). The *didactylus* group includes only four species  
72 (Bornschein *et al.* [2016a]; including the species described by Condez *et al.* [2016]), which have  
73 “leptodactyliform” body shape and absence of dermal ossification (Ribeiro *et al.* 2015). This  
74 group has a broad geographic distribution, including the species associated with the northernmost  
75 and southernmost limits of the genus (Bornschein *et al.* 2016a). Finally, the *pernix* group includes  
76 17 described species (Bornschein *et al.* 2016a,b, Ribeiro *et al.* 2017) with bufoniform body shape  
77 but lacking dermal ossification (Ribeiro *et al.* 2015). The *pernix* group is distributed in southern  
78 Brazil, in the states of Paraná and Santa Catarina (Bornschein *et al.* 2016a,b). The species of each  
79 group respond differently to altitude, with species from the *didactylus* group occurring from sea  
80 level (0–1110 m a.s.l.) and being much more environmentally tolerant (Pie *et al.* 2013,  
81 Bornschein *et al.* 2016a). Species from the remaining groups depend on high altitude conditions  
82 but can be found locally at lower altitudes, probably in response to particular microclimatic  
83 conditions (*ehippium* group occurs between 200 to 1900 m a.s.l. and *pernix* group between 455  
84 to 1640 m a.s.l.; [Bornschein *et al.* 2016a]).

85 It has been proposed that *Brachycephalus* species resulted from vicariant processes  
86 following upward dispersal of montane forests during past warm/wet climates (Bornschein *et al.*  
87 2016a, Firkowski *et al.* 2016). This diversification is very recent, at least for species of the *pernix*  
88 group, within the past 1 My (Firkowski *et al.* 2016). Possibly due to these historical evolutionary  
89 processes, *Brachycephalus* species are almost exclusively allopatric or parapatric, with few cases  
90 of syntopy (Bornschein *et al.* 2016a). Syntopic species include members of distinct groups and  
91 are in contact only at the altitudinal limits of each other: a higher limit for the “lowland” species

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

94       There is a recent increase in description of new species within *Brachycephalus*, with 20  
95 species described in the last 10 years (Frost 2017). However, the ecology of the vast majority of  
96 their species is unknown (see review of ecological studies in Bornschein *et al.* [2016a]). Call  
97 descriptions of the species are scarce, which is surprising, given that individuals of the species are  
98 usually located by their calls, often emitted at locally high male densities (one person might hear  
99 dozens of males from a single hearing spot). Advertisement calls were described for *B.*  
100 *ephippium* (Pombal Jr. *et al.* 1994, Goutte *et al.* 2017), *B. hermogenesi* (Verdade *et al.* 2008), *B.*  
101 *pitanga* (Araújo *et al.* 2012, Tandel *et al.* 2014, Goutte *et al.* 2017), *B. tridactylus* (Garey *et al.*  
102 2012), *B. crispus* (Condez *et al.* 2014), *B. sulfuratus* (Condez *et al.* 2016), and *B. darkside*  
103 (Guimarães *et al.* 2017).

104       Given that *Brachycephalus* is a group with mostly allopatric species, it is of great interest  
105 to investigate the evolution pattern of their calls. In allopatry, one could expect great similarity  
106 between the call of different species (Bornschein *et al.* 2007, Mauricio *et al.* 2014), due to a lack  
107 of selective pressure to avoid hybridization of closely-related species. However, this needs to be  
108 tested for *Brachycephalus*. Over the course of our studies on mountain frogs from the southern  
109 Atlantic Rainforest, in which we discovered several new species of *Brachycephalus* (Pie &  
110 Ribeiro 2015, Ribeiro *et al.* 2015, Bornschein *et al.* 2016b, Ribeiro *et al.* 2017), we recorded the  
111 advertisement calls of nearly all of them. Given that their diagnoses were easily achieved  
112 through morphological characters, we did not include vocal descriptions in any of those studies.  
113 In the present study, we describe the advertisement call of *B. albolineatus*, a member of the  
114 *pernix* group (Bornschein *et al.* 2016b). *Brachycephalus albolineatus* was recently described  
115 based on a series of eight specimens collected at the type locality, Morro Boa Vista, on the border  
116 between the municipalities of Jaraguá do Sul and Massaranduba, Santa Catarina, southern Brazil  
117 (Bornschein *et al.* 2016b).

## 118 **Methods**

119 We recorded individuals of *Brachycephalus albolineatus* on 25 October 2012 and on 5–6  
120 February 2016; however, the former recordings were discarded due to poor signal-to-noise ratio.  
121 We collected vouchers according to permits issued by ICMBIO - SISBIO (no. 20416–2).  
122 Vouchers belong to the type material of the species, which was deposited in Museu de História  
123 Natural Capão da Imbuia (MHNCI), Curitiba, Paraná state and Museu Nacional (MNRJ), Rio de



124 Janeiro, Rio de Janeiro state, Brazil. Analyzed recordings were carried out from 9:00–12:00 a.m.  
125 and from 15:00–18:00 p.m. Climatic conditions during recordings were characterized by air  
126 temperature = 20.8–21.4 °C, soil temperature = 19.4–20.0 °C, and relative air humidity = 86–  
127 100%. We made numbered markings close to the recorded individuals in the field to determine  
128 whether new recordings were from the same individuals, in order to build up the dataset both in  
129 terms of more individuals as well as within-individual variation, with more than one recording  
130 from the same individual. Calls were recorded using the digital recorders Sony PCM-D50 and  
131 PCM-M10, both with sampling frequency rate of 44.1 kHz and 16-bit resolution, and Sennheiser  
132 ME 66 microphones. Recordings were deposited in MHNCI. Sound samples were analyzed with  
133 Raven Lite 2.0 and Raven Pro 1.5 (Bioacoustics Research Program 2012). Time domain variables  
134 were measured from oscillograms and frequency domain variables were measured from  
135 spectrograms. Spectrogram parameters were defined with a 128-point (2.9 ms) Fast Fourier  
136 Transform (FFT), a 3-dB Filter bandwidth of 492 Hz, Hann window, 50% overlap, and a  
137 spectrogram color scheme of Standard Gamma II in Raven Pro and Jet in Raven Lite. We chose  
138 not to noise-filter the spectrograms to avoid eliminating potential sound characters. Final  
139 spectrograms, as well as diagnostic plots, were generated using the Seewave package, v. 2.0.5  
140 (Sueur *et al.* 2008) of the R environment, v. 3.2.2 (R Core Team 2015) using the same window  
141 size and overlap settings as in Raven Lite and Raven Pro, but resampling the audio files at 22.05  
142 kHz.

143 To analyze the advertisement calls, we first selected the most conspicuous spectrograms  
144 and compared them with their corresponding oscillograms. We used a note-centered approach,  
145 defining uninterrupted units of sound as notes and their entire collection as calls (Köhler *et al.*  
146 2017). We described the advertisement calls following parameters and criteria of Köhler *et al.*  
147 (2017). We took the liberty of describing the general parameters of Köhler *et al.* (2017) also for  
148 parts of the call, in order to clarify the distinctions observed in particular parts of the  
149 advertisement calls of *B. albolineatus*. We used the following parameters, which can be seen in  
150 Fig. 1: 1) call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of  
151 the call including only note groups (s); 4) note rate (notes per minute); 5) note rate of the call  
152 including only isolated notes (notes per minute); 6) note rate of the call including only note  
153 groups (notes per minute); 7) number of notes per call; 8) number of isolated notes per call; 9)  
154 number of note groups per call; 10) number of pulses per isolated notes; 11) number of pulses in  
155 each note groups; 12) note duration of isolated notes (s); 13) duration of note group (s); 14) inter-  
156 note interval in isolated notes (s), defined as the time from the end of one isolated note to the



157 beginning of the next note isolated note; 15) inter-note group interval (s), defined as the time  
158 from the end of one note group to the beginning of the next note group; 16) inter-note interval  
159 within note groups (s), defined as the time from the end of the first note to the beginning of the  
160 next note of the same note group; and 17) dominant frequency (kHz). We did not focus on  
161 frequency ranges, given that preliminary work indicated that estimated values are highly  
162 dependent on the quality of the recording and the used cut-off values. The note rate was  
163 calculated taking into account the time from the beginning of the first note to the beginning of the  
164 last note of the calls (or call intervals) and the number of notes included in this counted time (the  
165 last note is not included; Köhler *et al.* [2017]; see also Cocroft & Ryan [1995]).

## 166 Results

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

171 *Brachycephalus albolineatus* emitted a relatively long advertisement call, between 39.93–  
172 191.14 s ( $\bar{x} = 88.37 \pm 35.73$  s; Table 1; see parameter 1 in Fig. 1). Thereafter, the individual  
173 remains silent for several minutes, occasionally for more than 35 min, when it emits a new  
174 advertisement call. A graphical representation of the temporal sequence of notes in each call is  
175 shown in Figure 2. The note rate was 5.89–13.00 notes per minute ( $\bar{x} = 9.15 \pm 1.71$  notes per  
176 minute; Table 1; see parameter 4 in Fig. 1). Advertisement calls included 7–26 notes ( $\bar{x} =$   
177  $14.08 \pm 4.70$  notes; Table 2; see parameter 7 in Fig. 1).

178 The advertisement calls of the species included both isolated notes and note groups (in  
179 this case, with two notes involved in each particular note group; Fig. 1 and 3). Advertisement  
180 calls could be composed only by isolated notes (21% of complete recordings of advertisement  
181 calls), but usually included both isolated notes and note groups (Table 2). Every advertisement  
182 call with isolated notes and note groups began with the former and then changed to note groups  
183 (Table 3, Fig. Fig. 2). The part of the advertisement call composed only by note groups contains,  
184 on average, 29% of the notes of the entire advertisement call ( $\pm 15.4\%$ ; range of 10–61%; see  
185 parameter 9 in Fig. 1) and span, on average, 24.44 s ( $\pm 19.85$  s; range of 0.41–76.37 s; see  
186 parameter 3 in Fig. 1) as opposed to a mean of 53.71 s ( $\pm 25.38$  s; range of 18.39–98.90 s; Table  
187 1; see parameter 2 in Fig. 1) of the part of the advertisement calls with only isolated notes. The  
188 part of the advertisement call with only note groups also had a slower note rate, with 7.80 notes

189 issued per minute, on average ( $\pm 1.65$  note per minute; range of 4.74–11.73 notes per minute; see  
190 parameter 6 in Fig. 1), against 10.29 notes per minute on average ( $\pm 1.59$  note per minute; range  
191 of 7.28–13.62 notes per minute; Table 1), in the part of the call with isolated notes (when note  
192 groups occurs; see parameter 5 in Fig. 1).

193 The number of pulses per isolated notes varies from 1–3, more commonly two pulses  
194 (58%; Table 4; Fig. 3; see parameter 10 in Fig. 1). The isolated notes that initiate the  
195 advertisement call do it with one pulse (8 advertisement calls) or two pulses (16 advertisement  
196 calls). However, most of isolated notes along the advertisement call escalated the number of  
197 pulses (1 to 2, 1 to 3 or 2 to 3; 18 advertisement calls), whereas the isolated notes maintained a  
198 constant number of pulses only in six of the advertisement calls (2 to 2). The number of pulses in  
199 each note groups varied from 2–2 to 3–3, with additional combinations, being more common 3–3  
200 pulses (62%; Table 4; Fig. 3; see parameter 11 in Fig. 1, explaining how we count pulses in note  
201 groups).

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

208 Note duration of isolated notes varies from 0.002–0.037 s ( $\bar{x} = 0.020 \pm 0.007$  s) and  
209 duration of note groups varies from 0.360–0.578 s ( $\bar{x} = 0.465 \pm 0.053$  s; Table 5; see also  
210 parameters 12 and 13 in Fig. 1 and Fig. 3). The inter-note interval in isolated notes is, on average,  
211 6.663 s (4.092–12.248  $\pm 1.705$  s) and the inter-note group interval is, on average, 6.871 s (4.322–  
212 10.678  $\pm 1.768$  s; Table 5; see parameters 14 and 15 in Fig. 1). The inter-note interval within note  
213 groups is, on average, 0.412 s (0.319–0.526  $\pm 0.050$  s; Table 5; see parameter 16 in Fig. 1). The  
214 dominant frequency varies from 5.34–7.32 kHz ( $\bar{x} = 6.38 \pm 0.30$  kHz; Table 5; Fig. 4). Two  
215 individuals presented calls with dominant frequency below the mean (MHNCI 026–7) and two  
216 other from the mean upward (MHNCI 001 and 003), while the remaining showed dominant  
217 frequency crossing the mean in both directions (Fig. 4). In Figure 4 we can also observe intra-  
218 individual variations. Finally, regarding the frequency of pulses, when more than one pulse  
219 occurred in a given note, the subsequent pulses had a smaller frequency amplitude, especially  
220 reduced in the highest than the lowest frequency (values not shown, but see Fig. 3).

221 **Discussion**

222 The long advertisement calls of *Brachycephalus albolineatus*, and their variations, allowed us to  
223 see a trend of individuals investing progressively more energy along their emission. There are  
224 three sources of evidence for this: 1) advertisement calls normally escalated, incorporating note  
225 groups at the last third part of the call (76%) and 2) pulses per note increased during the emission  
226 of isolated notes (up to three; 62%); and 3) note groups usually had 3–3 pulses per note (62%),  
227 which is the combination of the groups with highest number of pulses (Table 3). Individual  
228 variation analysis also demonstrated that less structured advertisement calls (i.e. with notes with  
229 less pulses) are not fixed individually and can represent temporal variation. In the only species of  
230 the *pernix* group with its advertisement calls described to date, *B. tridactylus* (Garey *et al.* 2012),  
231 there was no evidence of escalation in structure. It is also possible that the advertisement calls  
232 with isolated notes and note groups could have distinct purposes, perhaps territorial defense when  
233 composed only by the former and territorial defense plus female preference when composed by  
234 isolated notes and note groups. This hypothesis can be tested in the laboratory and the call  
235 purpose also in the field, by recording throughout the year and placing a female and a male close  
236 to a calling male. This can be very revealing to explain effects of individual's arousal and  
237 functions of the calls.

238 There are only a few species of *Brachycephalus* with described advertisement calls  
239 (Pombal Jr. *et al.* 1994, Verdade *et al.* 2008, Araújo *et al.* 2012, Garey *et al.* 2012, Condez *et al.*  
240 2014, 2016, Tandel *et al.* 2014, Goutte *et al.* 2017), and their simplified description and absence  
241 of standardization in their descriptions prevented us to conduct a more detailed comparison with  
242 the call of *B. albolineatus*. Nevertheless, *B. albolineatus* is the only known species with a long  
243 advertisement call that is structurally modified along its emission, i.e. more structured (with notes  
244 with increasingly more pulses).

245 Another striking difference is how much the advertisement call of *Brachycephalus*  
246 *albolineatus* is longer than that of *B. tridactylus* (Garey *et al.* 2012), both of the *pernix* group  
247 (average of 88.37 s and 0.11 s, respectively). We do not rule out the possibility that Garey *et al.*  
248 (2012) could have overlooked differences in the structure of notes in the advertisement of *B.*  
249 *tridactylus* and that this species does indeed exhibits some level of structuring such as that of *B.*  
250 *albolineatus*. We also believe that the description of the advertisement calls by Garey *et al.*  
251 (2012) considered a criterion named as call-centered approach (Köhler *et al.* 2017), while we  
252 adopted the note-centered approach of Köhler *et al.* (2017). In other words, in the comparison of  
253 the advertisement calls of both species, as described, we are comparing one call composed by

254 several notes (*B. albolineatus*) with a single note of the call of *B. tridactylus*. The one-pulse notes  
255 of *Brachycephalus albolineatus* may represent “warming notes” (*sensu* Bornschein *et al.* 2007),  
256 which refers to notes beginning a call and that are attenuated (e.g. less intense [less audible]),  
257 although one-pulse notes also appear along the call in some advertisement calls.

258         In a recent study, Goutte *et al.* (2017) suggested that *Brachycephalus ephippium* and *B.*  
259 *pitanga* are insensitive to the sound of their own calls. This raises some questions about the  
260 validity of discussions about the possible reproductive and behavioral use of calls in the case of  
261 *B. albolineatus*, as well as for the use of calls in the taxonomy of the group. Goutte *et al.* (2017)  
262 questioned the fact that the maintenance of calls is energetically costly and favors predation and  
263 parasitism. They suggest that calls may have been maintained in the studied species because of  
264 the call side effects (e.g. vocal sac movement), because they are aposematic and thus less  
265 susceptible to the effect of predation, or by evolutionary inertia, for example. The relevant issue  
266 to be discussed here is that *B. ephippium* and *B. pitanga*, both members of the *ephippium* group,  
267 present vocal and visual behavioral (vocal sac movements) above the leaf litter (Goutte *et al.*  
268 2017), unlike *B. albolineatus* and all other species of the *pernix* group (MRB *et al.*, per. obs.),  
269 which call exclusively under the leaf litter and vocal sac movements are not visible. We do not  
270 abandon the hypothesis that the *Brachycephalus* species of the *pernix* and *didactylus* groups have  
271 a more complete auditory system than *B. ephippium* and *B. pitanga* and the ability to perceive  
272 their own calls. This is an interesting subject brought only now to the fore and open to further  
273 discussion.

## 274 **Conclusions**

275 The advertisement call of *Brachycephalus albolineatus* is long and composed by isolated notes  
276 and note groups, which tend to be emitted during the last third of the call. Intra-individual  
277 variation demonstrates that calls can be composed only by isolated notes or by isolated notes and  
278 note groups in a subsequent call. Number of pulses per notes escalates along the call. The call  
279 variations in number of notes, of pulses and eventual incorporation of note groups may be related  
280 to individual arousal levels. It is also possible that the advertisement calls have distinct purposes,  
281 perhaps territorial defense when composed only by isolated notes and territorial defense plus  
282 female preference when composed by isolated notes and note groups.

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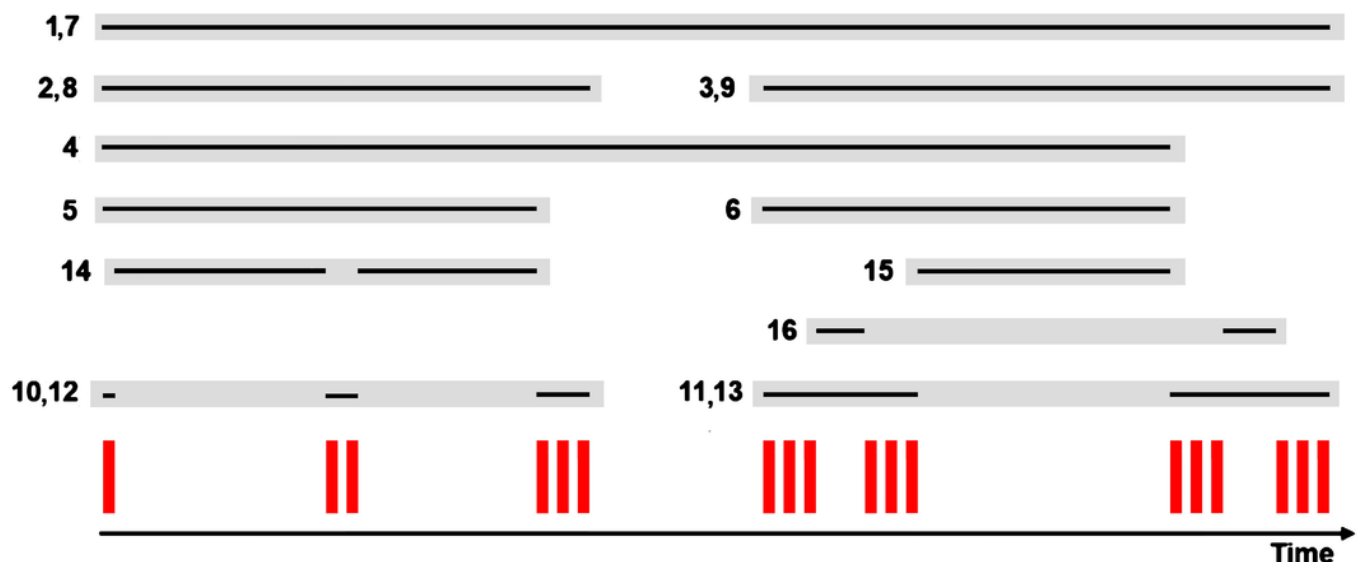


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# Figure 1

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

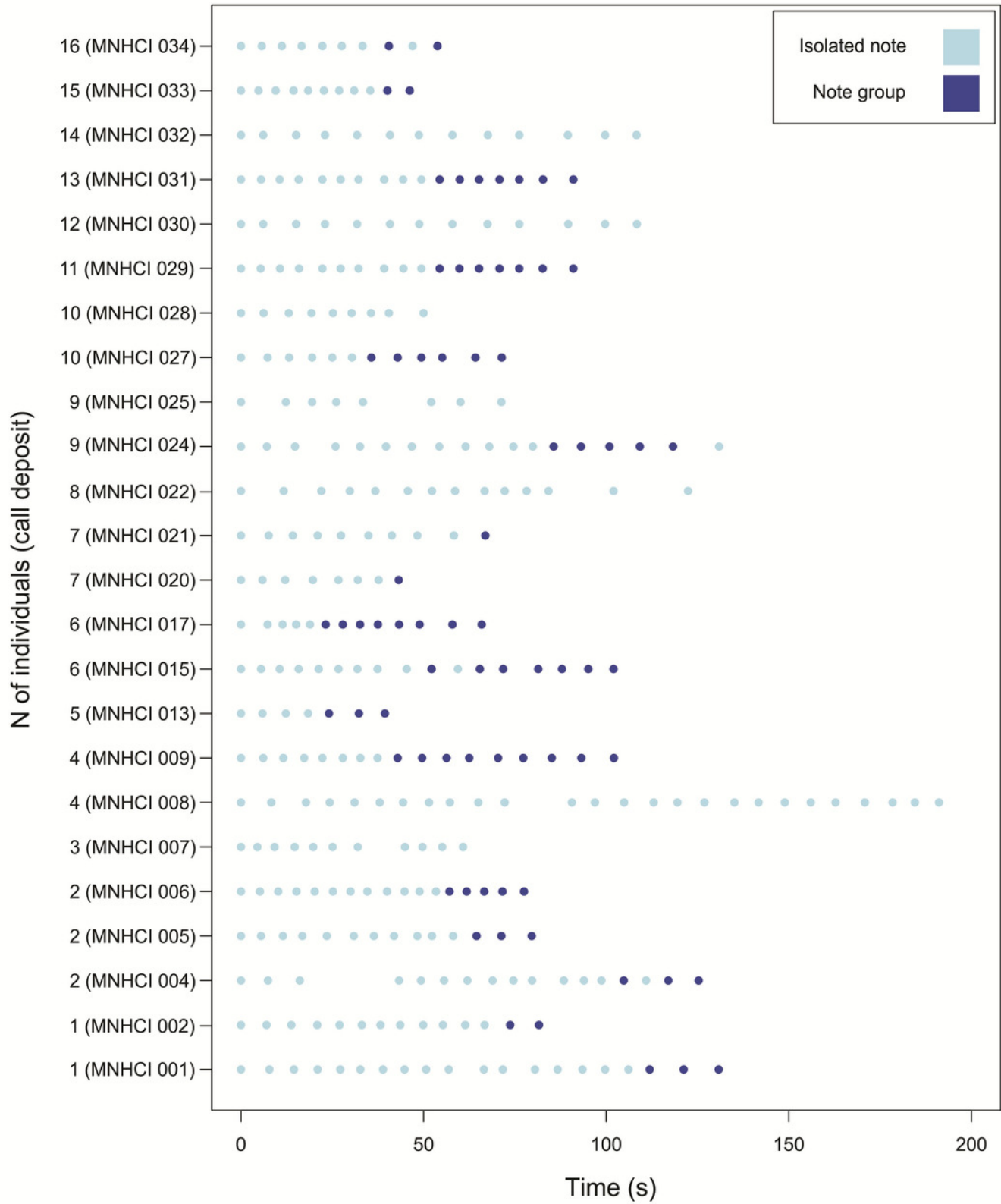
Representation of some parameters considered in the description of the advertisement call of *Brachycephalus albolineatus*. Numbers correspond with descriptions in the methods. 1) Call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of the call including only note 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).

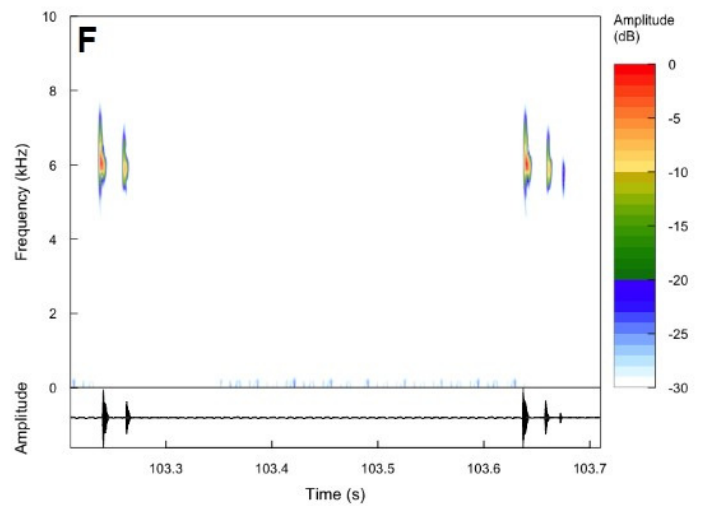
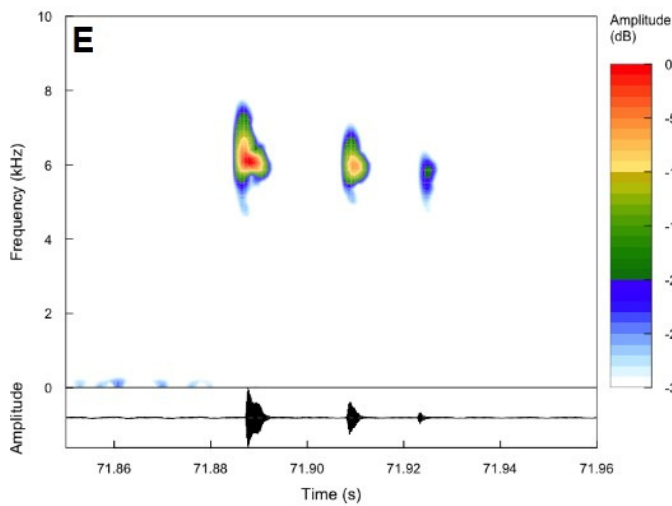
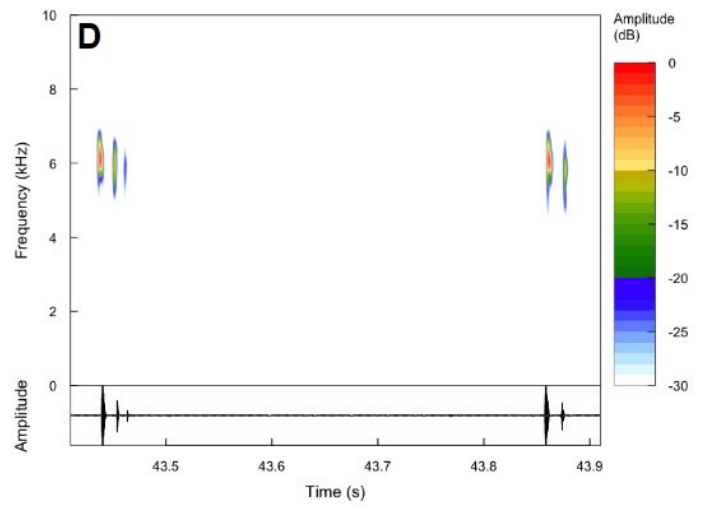
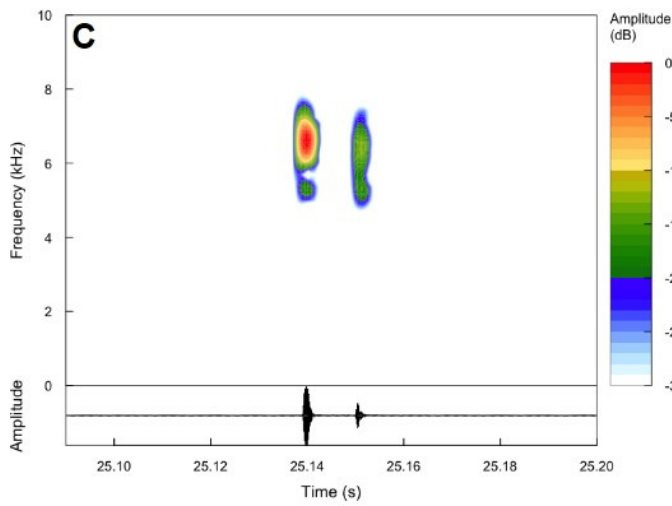
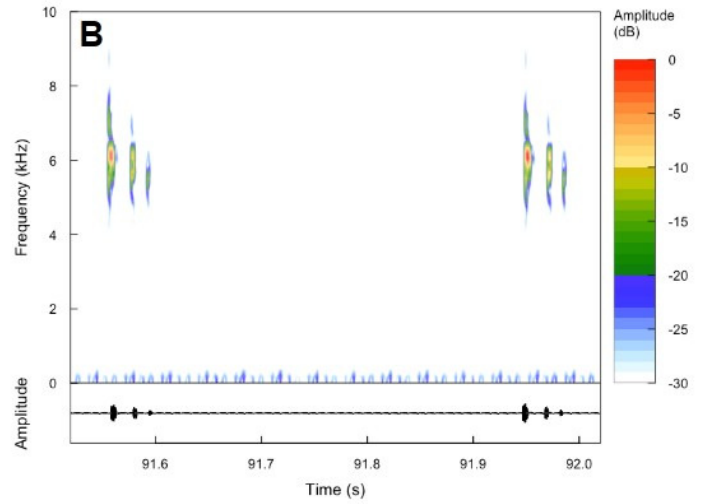
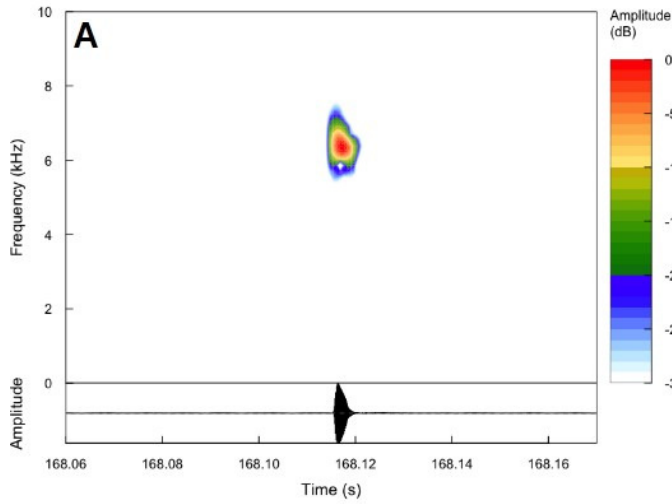
Graphical representation of the emission of isolated notes and note groups of the advertisement calls (AC) of *Brachycephalus albolineatus* (only AC recorded from the beginning were considered). Note the individual variation. The number of pulses of each note can be observed in table 3. Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia.



## Figure 3

Example of notes of the advertisement call of *Brachycephalus albolineatus*.

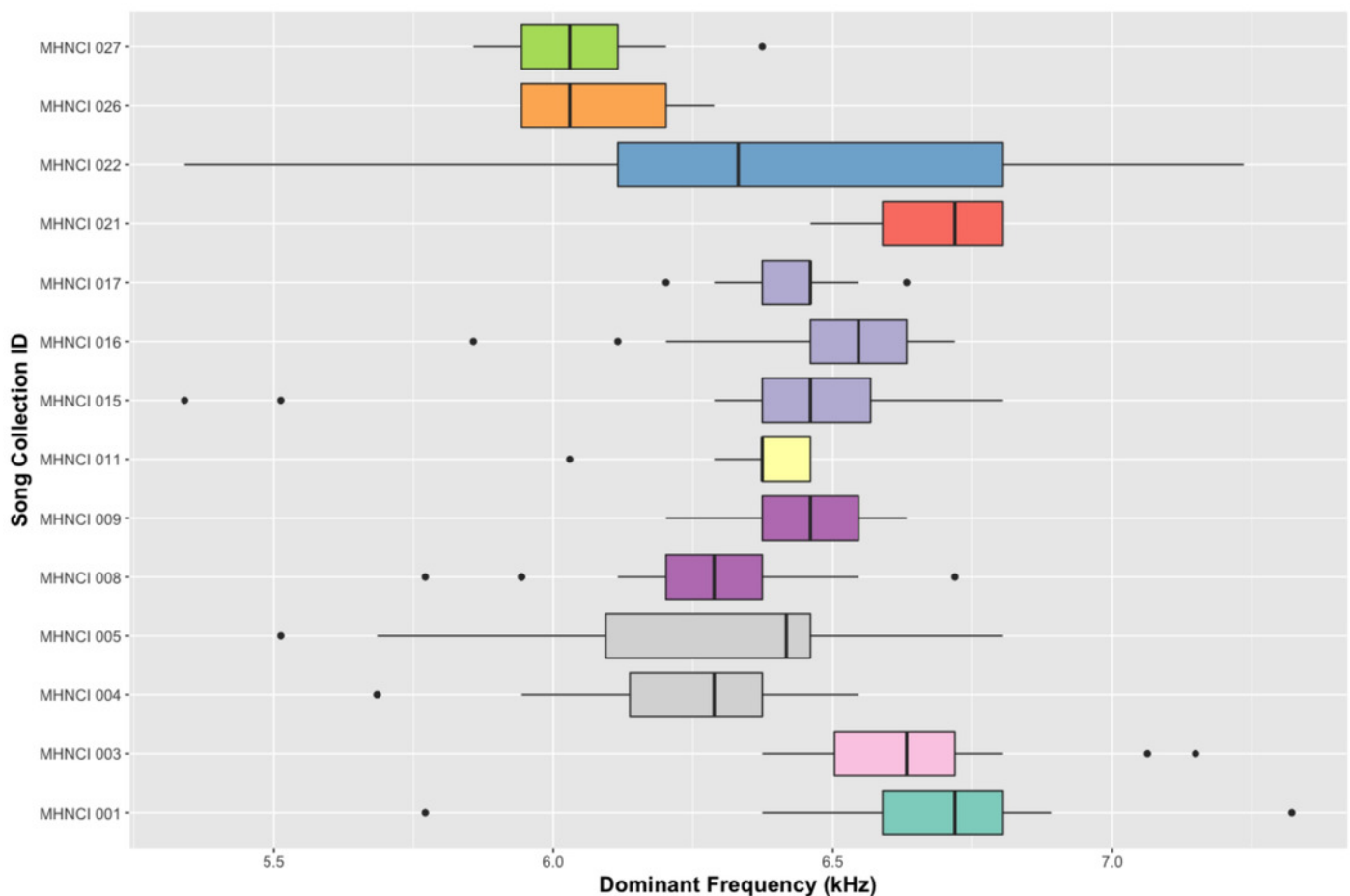
Example of notes of the advertisement call of *Brachycephalus albolineatus*. A, C, D) All examples observed of isolated notes, with one pulse (A: MHNCI 008), two pulses (C = MHNCI 022), and three pulses (D = MHNCI 026). B, D, F) Examples of note groups, with 3-3 pulses (B: MHNCI 026), 3-2 pulses (D = 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%.



## 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 1<sup>st</sup> and 3<sup>rd</sup> 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	
	Duration (1)	Rate (4)	When note groups is absent		When note groups occurs		Duration (3)	Rate (6)
			Duration (2)	Rate (5)	Duration (2)	Rate (5)		
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, 1, 1, 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, 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, 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)	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 albolineatus*.

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