A review of the geographical distribution and differentiation of the recently described flea toad *Brachycephalus sulfuratus* in relation to *B. hermogenesi* (Anura: Brachycephalidae)

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Background. The flea toad *Brachycephalus sulfuratus* was recently described from southeastern and southern Brazil. In its description, the authors overlooked previous identifications of flea toads as *Brachycephalus* sp. nov. and *B. hermogenesi* occurring in the same regions, which could suggest the possibly of three flea toads coexisting in southern Brazil. In addition, *B. sulfuratus* is characterized by substantial phenotypic variability, to an extent that compromises its current diagnosis with respect to its congener *B. hermogenesi*. Therefore, the current state-of-affairs regarding the geographical distribution of these two species and the identification of previously known populations is hitherto uncertain. Our goals are to reassess previous records of flea toads attributable to *B. hermogenesi*, *B. sulfuratus*, and *Brachycephalus* sp. nov., considering the description of *B. sulfuratus*, and to review the distinction of *B. sulfuratus*. **Methods.** A critical analysis of the species identity of specimens attributable to *B. hermogenesi*, *B. sulfuratus* or to a similar, potentially undescribed, flea toads from southeastern and southern Brazil was based either on the analysis of morphology or on their advertisement calls. This analysis includes our independent examinations of specimens and, when impossible, critical examinations of published descriptions. **Results.** We found that morphological and call traits originally proposed as diagnostic for *B. sulfuratus* in relation to *B. hermogenesi* vary intraspecifically. Live individuals with ventral yellow spots correspond to *B. sulfuratus*; individuals without yellow spots can be either *B. sulfuratus* or *B. hermogenesi*. In preservative, they are indistinguishable. Previous records of *Brachycephalus* sp. nov. correspond to *B. sulfuratus*. We propose the highest number of pulses per note of the call of *B. sulfuratus* as the only diagnostic trait in relation to *B. hermogenesi*. Regarding their distributions based in our revision, only *B. sulfuratus* occurs

in southern Brazil, without any overlap with *B. hermogenesi*. There is a narrow gap between the distributions of these species around the southeast of the city of São Paulo. Biogeographic events might have led to vicariance are discussed.

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Abstract

28 **Background.** The flea toad *Brachycephalus sulfuratus* was recently described from southeastern

29 and southern Brazil. In its description, the authors overlooked previous identifications of flea

30 toads as *Brachycephalus* sp. nov. and *B. hermogenesi* occurring in the same regions, which could

31 suggest the possibly of three flea toads coexisting in southern Brazil. In addition, *B. sulfuratus* is

32 characterized by substantial phenotypic variability, to an extent that compromises its current

33 diagnosis with respect to its congener *B. hermogenesi*. Therefore, the current state-of-affairs

34 regarding the geographical distribution of these two species and the identification of previously

35 known populations is hitherto uncertain. Our goals are to reassess previous records of flea toads

36 attributable to *B. hermogenesi*, *B. sulfuratus*, and *Brachycephalus* sp. nov., considering the

37 description of *B. sulfuratus*, and to review the distinction of *B. sulfuratus*.

38 **Methods.** A critical analysis of the species identity of specimens attributable to *B. hermogenesi*,

39 *B. sulfuratus* or to a similar, potentially undescribed, flea toads from southeastern and southern

40 Brazil was based either on the analysis of morphology or on their advertisement calls. This

41 analysis includes our independent examinations of specimens and, when impossible, critical

42 examinations of published descriptions.

43 **Results.** We found that morphological and call traits originally proposed as diagnostic for *B.*

44 *sulfuratus* in relation to *B. hermogenesi* vary intraspecifically. Live individuals with ventral

45 yellow spots correspond to *B. sulfuratus*; individuals without yellow spots can be either *B.*

46 *sulfuratus* or *B. hermogenesi*. In preservative, they are indistinguishable. Previous records of

47 *Brachycephalus* sp. nov. correspond to *B. sulfuratus*. We propose the highest number of pulses

48 per note of the call of *B. sulfuratus* as the only diagnostic trait in relation to *B. hermogenesi*.

49 Regarding their distributions based in our revision, only *B. sulfuratus* occurs in southern Brazil,

50 without any overlap with *B. hermogenesi*. There is a narrow gap between the distributions of

51 these species around the southeast of the city of São Paulo. Biogeographic events might have led

52 to vicariance are discussed.

53

54

Introduction

Species of the *Brachycephalus didactylus* species group, commonly known as flea toads, are distributed throughout much the Atlantic Forest of Brazil (Bornschein, Pie & Teixeira 2019a). The first described flea toad was *B. didactylus*, in 1971 (Izecksohn 1971) as the only member of a new genus, *Psyllophryne*. The second flea toad species, *B. hermogenesi*, was described nearly three decades later, in 1998 (Giaretta & Sawaya 1998), at the time as the second species of the genus *Psyllophryne*. Recently, other two flea toads were described, namely *B. pulex* (Napoli et al. 2011) and *B. sulfuratus* (Condez et al. 2016). All members of the *B. didactylus* species group (*sensu* Ribeiro et al. 2015; Bornschein, Pie & Teixeira 2019a) are distinguished by the leptodactyliform body shape and the absence of dermal ossification. The remaining groups within *Brachycephalus* include the *B. ephippium* species group, with bufoniform body shape and presence of dermal ossification, and the *B. pernix* species group, equally with bufoniform body shape but without dermal ossification, as in the *B. didactylus* species group (Ribeiro et al. 2015).

Only recently have flea toads been recorded in southern Brazil. The first records were of *B. hermogenesi* to the Reserva Particular do Patrimônio Natural Salto Morato, municipality of Guaraqueçaba, in the northern coast of Paraná (Pereira et al. 2010, Santos-Pereira et al. 2011), and at Colônia Castelhanos, municipality of Guaratuba, in southern Paraná, initially as "*Brachycephalus* aff. *hermogenesi*" (Cunha et al. 2010) and later as "*B. hermogenesi*" (Oliveira et al. 2011). Just a few years later, Pie et al. (2013) published 14 localities of a flea toad identified as *Brachycephalus* sp. nov. 1, from Paraná and Santa Catarina. These authors also reidentified the record from Colônia Castelhanos as *Brachycephalus* sp. nov. 1. Records from Reserva Particular do Patrimônio Natural Salto Morato of Pereira et al. (2010) and Santos-Pereira et al. (2011) were inadvertently omitted from Pie et al (2013). Later, Bornschein et al. (2016a) compiled 18 localities of a flea toad as *Brachycephalus* sp. 1., including the 14 records of Pie et al. (2013) treated as *Brachycephalus* sp. nov. 1. Bornschein et al. (2016a) also reidentified previous records of the flea toad of the Reserva Particular do Patrimônio Natural Salto Morato and Colônia Castelhanos as *Brachycephalus* sp. 1.

The flea toad *B. sulfuratus* was described based on a series of 28 specimens distributed from southern São Paulo to northern Santa Catarina (Condez et al. 2016). In that study, previous records of *Brachycephalus* sp. nov. 1 from Pie et al. (2013) and *Brachycephalus* sp. 1 from

86 Bornschein et al. (2016a) were omitted and the opinion of Condez et al. (2016) related to this

87 alleged new species of flea toad is unknown. Condez et al. (2016) considered the presence of the

88 flea toad *B. hermogenesi* in Paraná, based on Cunha et al. (2010) and Oliveira et al. (2011).

89 However, the voucher specimen of Cunha et al. (2010) and Oliveira et al. (2011), a single

90 specimen deposited in the Museu de História Natural, Universidade Estadual de Campinas,

91 Campinas (ZUEC 16602), was reidentified by Condez et al. (2016) as *B. sulfuratus*. Remaining

92 records of *B. hermogenesi* in Paraná, from Pereira et al. (2010) and Santos-Pereira et al. (2011),

93 were overlooked by Condez et al. (2016).

94   An additional important aspect is the fact that one occurrence record of a flea toad in

95 Santa Catarina, called Castelo dos Bugres, provide specimens identified as *B. sulfuratus* (Condez

96 et al. (2016), *Brachycephalus* sp. 1. (Bornschein et al. 2016a), and *Brachycephalus* sp. nov. 1

97 (Pie et al. 2013). By overlooking specimens from this locality, Condez et al. (2016) missed the

98 opportunity to determine if it was conspecific with their newly described *B. sulfuratus*. At this

99 moment, the uncertainty in the identification of some important occurrence records seems to

100 indicate three possible scenarios. First, one could envision that there are three similar species of

101 flea toads in southern Brazil, namely *B. hermogenesi* (Pereira et al. 2010, Santos-Pereira et al.

102 2011), *Brachycephalus* sp. nov. (Pie et al. 2013, Bornschein et al. 20016a), and *B. sulfuratus*

103 (Condez et al. 2016). Second, records of *B. hermogenesi* in southern Brazil could be erroneous,

104 given that some of these records (Cunha et al. 2010, Oliveira et al. 2011) were assigned to *B.*

105 *sulfuratus* or *Brachycephalus* sp. nov. (Pie et al. 2013, Condez et al. 2016), leading to an

106 expectation that two species might occur in these regions (*B. sulfuratus* and *Brachycephalus* sp.

107 nov.). Third, if the new unnamed species identified by Pie et al. (2013) and Bornschein et al.

108 (2016a) is conspecific with *B. sulfuratus*, because the situation verified at Castelo dos Bugres,

109 there would be a single species of flea toad in southern Brazil (*B. sulfuratus*).

110   Recently, Bornschein, Pie & Teixeira (2019a) reviewed the available occurrence records

111 of flea toads from southeastern and southern Brazil and reverted most of the records of

112 "*Brachycephalus* sp. nov. 1" (Pie et al. 2013), "*Brachycephalus* sp. 1" (Bornschein et al. 2016a),

113 and *B. hermogenesi* from southern Brazil (Pereira et al. 2010, Santos-Pereira et al. 2011, 2016) in

114 favor of *B. sulfuratus*. Some records that could not be adequately reassessed by Bornschein, Pie

115 & Teixeira (2019a) were reverted to "*Brachycephalus* sp. cf. *B. sulfuratus*", including the

116 records of *B. hermogenesi* from Cunha et al. (2010) and Oliveira et al. (2011). Bornschein, Pie &

117   Teixeira (2019a) disregarded the possibility of a third unnamed species of flea toad in southern

118   Brazil, but one question remains: the proper identification of *B. sulfuratus* and *B. hermogenesi*.

119   In this sense, the identification criteria used by Bornschein, Pie & Teixeira (2019a) to reevaluate

120   the records of flea toads was not indicated. In addition, there may still be uncertainty in the

121   identification of flea frogs by other authors, as records of *B. hermogenesi* in southern Brazil

122   continue to be published (Santos-Pereira et al. 2016, 2018, Leivas et al. 2018). Given this

123   uncertainty, the aim of this study is to reanalyze the diagnostic morphological traits used to

124   distinguish *B. hermogenesi* and *B. sulfuratus* and redefine its geographical distributions and

125   distributional limits.

126

Materials & Methods

128   A critical analysis of the species identity of specimens attributable to *Brachycephalus*

129   *hermogenesi*, *B. sulfuratus* or to a similar, potentially undescribed, flea toad from southeastern

130   and southern Brazil was based either on the analysis of their morphology or on their

131   advertisement calls. Our dataset includes the compilation of Bornschein, Pie & Teixeira (2019a),

132   with a few updates. We began by analyzing the actual diagnosis and parameters of the

133   comparisons between species based on the description of *B. sulfuratus* (Condez et al. 2016). We

134   looked for those features in old museum specimens, in our collected specimens, and in sources

135   provided in the literature, particularly photographs of live specimens. Given that our approach

136   uncovered ambiguity in the proposed diagnostic attributes to separate the new species from *B.*

137   *hermogenesi*, we sought new traits that could be useful to distinguish them. New distinctive

138   features found were then erected as diagnostic attributes. In this procedure, we act in accordance

139   of the Recommendation 13A of the International Code of Zoological Nomenclature

140   (http://www.iczn.org/). Given that the new distinctive features were listed among described

141   characteristics of the new species, it was not necessary for us to re-describe these characteristics.

142   Rather, we simply demonstrate the existence of the corresponding features in specimens that we

143   identified.

144   We also included unpublished records, vouchered with specimens collected by the

145   authors and deposited in the Museu de História Natural Capão da Imbuia (MHNCI), Curitiba,

146   state of Paraná, Brazil. Collection permits were issued by ICMBIO (10.500, 22470–2/1911426,

147   and 55918–1). Geographical coordinates are based on the WGS84 datum. Elevations for

148 literature records and author's records were obtained from Google Earth, after plotting the

149 location point.

150   We recorded advertisement calls of flea toads using analogical (Sony TCM–5000EV) and

151 digital (Sony PCM–D50 and PCM–M10) devices, with Sennheiser ME 66 microphones. We

152 digitized analogical recordings at 44.1 kHz and 16 bit using Raven Pro 1.4 (Cornell Lab of

153 Ornithology, Ithaca, New York, USA) without any pre-processing. Spectrograms were produced

154 using the program Raven Pro 1.4 on a personal computer with the following settings: Fast

155 Fourier Transform, 256 points, 50% of overlap, and Hann window type. We made adjustments in

156 contrast and brightness with the intention of lightening the images and best highlighting the

157 pulses. We chose not to noise-filter the spectrograms to avoid eliminating sound characters. All

158 calls were deposited in the MHNCI acoustic collection.

159

160 **Results**

161 *Distinction between* Brachycephalus sulfuratus *and* B. hermogenesi

162   Condez et al. (2016) indicated three morphological characters to distinguish *B. sulfuratus*

163 from the very similar *B. hermogenesi*: 1) "The new species differs from *B. didactylus*, *B.*

164 *hermogenesi*, and *B. pulex* by having (in life) yellow blotches on the ventral surfaces of the

165 throat, chest, arms, and forearms" (Condez et al. 2016: 43, 50); 2) "…the singular inverted v-

166 shaped mark [outlined with white] around the cloacal region in ventral view..., which is not

167 clearly distinguishable in *B. pulex*... and generally rounded and not ornamented in *B. didactylus*...

168 and *B. hermogenesi*..." and usually less distinct in *B. hermogenesi* (Condez et al. 2016: 43, 50);

169 and 3) "the m-shaped mark around the cloacal opening [in dorsal view], which is dark and

170 defined in *B. sulfuratus* sp. nov…, is present but not clearly defined in *B. hermogenesi*" (Condez

171 et al. 2016: 50). However, as we demonstrate below, none of these traits provide an

172 unambiguous identification of *B. sulfuratus* with respect to *B. hermogenesi*. First, some

173 specimens of *B. sulfuratus* that we collected in southern São Paulo, Paraná, and Santa Catarina

174 have revealed that the yellow spots on the ventral surface of this species is still present, on throat,

175 chest, arms, and/or forearms, but not necessarily in all of these body parts. In addition, the

176 amount of yellow is highly variable, being virtually absent in some individuals (Fig. 1).

177 Moreover, three individuals of *B. sulfuratus* collected by us in the state of São Paulo (near the

178 Jurupará dam; Table 1), all collected side by side, have revealed two individuals without any

179 yellow spots on the ventral surface (see one of them in Fig. 1l), yet their advertisement calls
180 confirms that they are indeed *B. sulfuratus* (Fig. 2b, c; see below). Second, the inverted v-shaped
181 mark around the cloacal region can be absent in individuals of *B. sulfuratus* (compare Fig. 6A of
182 Condez et al. [2016] and Fig. 1a). Additionally, this characteristic was mischaracterized as a
183 diagnosis from *B. hermogenesi* on the actual original description: "the ventral inverted v-shaped
184 mark in the chest… are shared among the four species [*B. sulfuratus*, *B. hermogenesi*, *B.*
185 *didactylus*, and *B. pulex*]" (Condez et al. 2016: 50). Also, while describing the variation on the
186 type series, the authors stated that "some individuals present the inverted v-shaped around the
187 cloacal region" (Condez et al. 2016: 46). Finally, the "m-shaped mark around the cloacal
188 opening" was also mischaracterized as a diagnostic trait on the actual original description of the
189 species (Condez et al. 2016: 50): "The m-shaped mark around the cloacal opening in dorsal
190 view… are shared among the four species [*B. sulfuratus*, *B. hermogenesi*, *B. didactylus*, and *B.*
191 *pulex*]."

192   Therefore, there are at the present time no morphological traits that could differentiate
193 either live or preserved specimens (Fig. 3) of *B. sulfuratus* from *B. hermogenesi* (we examined
194 preserved specimens from the type series of *B. hermogenesi*, deposited in ZUEC: ZUEC 9715 –
195 holotype, Fig. 3, and ZUEC 9716–25 - paratypes). However, for identification purposes, we
196 considered individuals with yellow spots on their ventral side as *B. sulfuratus*, whereas
197 individuals without yellow spots could be either *B. sulfuratus* or *B. hermogenesi*. However,
198 specimens with yellow spots of *B. sulfuratus* must be observed in life because in the preservative
199 the change in color prevents separate them in relation to specimens of *B. hermogenesi*.

200   In addition to morphological traits, Condez et al. (2016: 43) included in the diagnosis of
201 *B. sulfuratus* the following parameters of the advertisement call: "advertisement call long,
202 composed of a set of 4–7 high-frequency notes (6.2–7.2 kHz) repeated regularly." The indicated
203 range overlaps with that of *B. hermogenesi*. According to Verdade et al. (2008:545): "The calls
204 [of *B. hermogenesi*] may be simple, constituted by a single note, or complex, composed of
205 groups of two to seven similar notes" with average dominant frequency of 6.8 kHz ± 0.8 (the
206 frequency amplitude was not provided by Verdade et al. [2008]). In summary, the advertisement
207 call of *B. hermogenesi* is composed of 1–7 notes, whereas that of *B. sulfuratus* is composed of 4–
208 7 notes, with the amplitude of the dominant frequency of *B. hermogenesi* overlapping with the
209 amplitude of dominant frequency of *B. sulfuratus* ($\bar{x}$ = 6.7 ± 0.3 kHz; Condez et al. [2016: 46]).

210   In the section "Comparisons with other species", Condez et al. (2016: 50) do not clarify the

211   distinction between these species, by simply stating that "The advertisement call of *B.*

212   *hermogenesi* is the most similar to the new species [*B. sulfuratus*], being quite similar in

213   frequency (dominant frequency = 6.8 kHz), which are the highest recorded for the genus.

214   However, the advertisement call of *B. hermogenesi* can be simple or composed of 2–7 shorter

215   notes with 1–3 pulses (Verdade et al. 2008)."

216   All analyzed advertisement call records (Figs. 2, 4–6) unambiguously allow for the

217   assignment of individuals as *B. sulfuratus* by the number of pulses of single notes, varying

218   between 5–13 in samples shown in the Figs. 2, 4–6 and between 7–11 in the samples of Condez

219   et al. (2016: 46), whereas in *B. hermogenesi* the number of single note pulses is indeed 1–3.

220   Verdade et al. (2008) have not described the number of pulses of notes of *B. hermogenesi*, as

221   stated by Condez et al. (2016:50). However, V. K. Verdade sent us two copies of recordings that

222   she made and used in her study, in which we found two pulses per notes in *B. hermogenesi* from

223   Estação Biológica de Boracéia. Additionally, our recordings of *B. hermogenesi* from Estação

224   Biológica de Boracéia and from it type locality (Corcovado and Núcleo Picinguaba)

225   demonstrated that the number of pulses per note in this species is indeed 1–3.

226   We erect as a diagnosis between *B. sulfuratus* and *B. hermogenesi* the number of pulses

227   of a single note in their advertisement call: 1–3 in *B. hermogenesi* and between 5–13 in *B.*

228   *sulfuratus*. To the best of our knowledge, this is currently the only objective distinction between

229   this species. A more detailed description of call parameters, including specimens across the

230   distribution of each species, might provide additional information to distinguish them (e.g., pace

231   of the advertisement calls, time interval between pulses, and duration of the pulse). We also

232   observed possible population variation in call parameters between recordings of the *B.*

233   *hermogenesi* type localities compared to those located at higher altitudes (e.g., Estação Biológica

234   de Boracéia; see Table 1). This analysis is beyond the scope of this study and will be the focus of

235   a specific study in the future.

236

237   *Reviewed records of* Brachycephalus sulfuratus *and* B. hermogenesi

238   Based on our review of the 14 occurrence records of "*Brachycephalus* sp. nov. 1" from

239   Pie et al. (2013), we conclude that the vouchered records correspond to *B. sulfuratus* (Table 1;

240   Figs. 5–6). Specimens from Pie et al. (2013) have yellow spots on their ventral side and

241 advertisement calls recorded have notes with several pulses (as above). We treated unvouchered

242 records of Pie et al. (2013) as *Brachycephalus* sp. (Table 1). A specimen from one of those

243 unvouchered locations of Pie et al (2013), Castelo dos Bugres, was vouchered by Condez et al.

244 (2016). We also determined that the records of unidentified *Brachycephalus* from "Apiaí",

245 "Caratuval", "Corvo", and "Fazenda Thalia" from Firkowski et al. (2016) correspond to *B.*

246 *sulfuratus* (Table 1) based on the inspection of vouchers (specimens had yellow spots on their

247 ventral region - see Fig. 1; sonograms of some of these records are presented in Figs. 4–6). The

248 records of "*Brachycephalus* sp. 1" from Bornschein et al. (2016a) correspond to *B. sulfuratus*

249 (Table 1): all but one of them are the same records as those presented in Pie et al. (2013) and

250 Firkowski et al. (2016) and were re-identified above. The only exception is the record of

251 "*Brachycephalus* sp. 1" from Reserva Particular do Patrimônio Natural Salto Morato,

252 municipality of Guaraqueçaba, Paraná, identified as *B. sulfuratus* (Table 1) based on their call

253 structure, with notes with several pulses (Fig. 5d). With this proposition we reidentified the

254 previous identification of *B. hermogenesi* for Reserva Particular do Patrimônio Natural Salto

255 Morato (Pereira et al. 2010, Santos-Pereira et al. 2011, 2016, 2018, Leivas et al. 2018; Table 1).

256 Some previous studies reporting "*Brachycephalus hermogenesi*" (Giaretta and Sawaya

257 1998, Dixo and Verdade 2006, Verdade et al. 2008, Condez, Sawaya & Dixo 2009, Verdade,

258 Rodrigues & Pavan 2009) from Rio de Janeiro and São Paulo do not provide enough

259 morphological evidence or other details to allow us to correct their original identification (Table

260 1). Therefore, we propose that these identifications should be reverted as *Brachycephalus* sp.

261 One of these records reverted to *Brachycephalus* sp. involves "*B. hermogenesi*" from the

262 municipality of Piedade, state of São Paulo, of Clemente-Carvalho et al. (2011), whose genetic

263 sequence is deposited in GenBank (HQ435724). The corresponding voucher was obtained by T.

264 H. Condez (pers. comm., 2016) in her study on the same location (Condez, Sawaya & Dixo

265 2009).

266 In the description of *B. sulfuratus* (Condez et al. 2016) there are some specimens, from

267 six different localities, cited as "*B. hermogenesi*" in the appendix. These specimens were used by

268 Condez et al. (2016) only for "morphological analyses". There is the possibility that all of these

269 records are based on preserved material, preventing Condez et al. (2016) to analyze color

270 patterns in life and to unambiguously distinguish them from *B. sulfuratus*, as our statements

271   above. Therefore, we also propose that those identifications should be considered as

272   *Brachycephalus* sp. (Table 1; see also Bornschein et al. 2016a).

273   There is a particular specimen (ZUEC 16602), discussed above and also examined by us,

274   collected in the state of Paraná, that was first identified as "*Brachycephalus* aff. *hermogenesi*"

275   (Cunha et al. (2010), later as "*B. hermogenesi*" (Oliveira et al. (2011), "*Brachycephalus* sp. nov.

276   1" (Pie et al. 2013), "*Brachycephalus* sp. 1" (Bornschein et al. 2016a), and, finally as "*B.*

277   *sulfuratus*" (Condez et al. 2016). We also believe that this specimen may not have been properly

278   analyzed with respect to coloration in life, preventing the precise identification as a specimen in

279   preservative (see above). Therefore, we also propose that this identification should be reverted to

280   *Brachycephalus* sp. (Table 1).

281

282   Discussion

283   Based on our analyses of the characters used in the diagnosis of *B. sulfuratus*, we found

284   that they are variable and overlap with those found in *B. hermogenesi*. Moreover, the

285   examination of specimens deposited in the collections MHNCI and ZUEC support this claim.

286   Differences in number of pulses per note is proposed here as the only available source of

287   evidence supporting the distinction of *B. sulfuratus* and *B. hermogenesi*. We emphasize that the

288   diagnosis between these species based only on the number of pulses cannot be interpreted as a

289   weak diagnosis. Even in the field its advertisement calls are very distinct and easily

290   distinguishable. The advertisement calls of *B. sulfuratus* sounds like a "tríííííí, tríííííí, tríííííí,

291   tríííííí, tríííííí", whereas those of *B. hermogenesi* sound like a "tíc, tíc, tíc, tíc-tíc, tíc-tíc-tíc".

292   These transliterations represent notes (each note separated by comma) with distinct duration

293   related to high number of pulses per note in the first species in opposition of reduced number of

294   pulses in the second. The advertisement calls of *B. hermogenesi* have the same pattern of that of

295   species from the *B. pernix* group (Bornschein et al. 2018, 2019b, Pie et al. 2018a, Monteiro et al.

296   2018a, 2018b), which includes most species of southern Brazil whereas the call of *B. sulfuratus*

297   resembles the one of *B. vertebralis* (MRB, unpublish data), for example, from the *B. ephippium*

298   group, which includes most species from the state of São Paulo to the north. To the best of our

299   knowledge, this is the first case in which the diagnosis between species of *Brachycephalus* is

300   made solely by features of the advertisement call.

301   Our results expand the knowledge on the number of single-note pulses in *B. sulfuratus*,
302   previously described as having between 7–11 pulses (Condez et al. 2016). We underscore a
303   necessity to re-describe the calls of *B. hermogenesi*, particularly given that there is not enough
304   information on how some of the parameters were measured by Verdade et al. (2008).
305   Nevertheless, the objective with our call analysis was to include sonograms showing the
306   structure of the notes (number of pulses) as a "snapshot" to allow for the identification of the
307   species that emitted the calls. A comprehensive description of our recordings would require
308   including the entire sample size of individuals from the same localities, as well as to discard any
309   low-quality calls, even if they were the only available vouchers of certain localities,
310   mischaracterizing the purpose for which calls are included here.

311   We show that there is now confirmed occurrence of *B. hermogenesi* in southern Brazil
312   and that *B. sulfuratus* occurs far north to the east of São Paulo city, only 67 km in straight line
313   from the southernmost site of record of *B. hermogenesi* (Estação Biológica de Boracéia; Fig. 7;
314   Table 1). Although we reversed several records of *B. sulfuratus* and *B. hermogenesi* to
315   *Brachycephalus* sp. (Table 1), this now means that a third species may be involved. The
316   unidentified records in Table 1 represent one or the other of these two species. In fact, it is likely
317   that in southern Brazil only the flea toad *B. sulfuratus* will occur. Only from the south of São
318   Paulo to the north until the city of São Paulo is the distribution of flea toads little known. In
319   southern Brazil, our group has been working with two genera of anurans (*Brachycephalus* and
320   *Melanophryniscus*) since 2009, focusing on their distribution, ecology and conservation (e.g., Pie
321   et al. 2013, Bornschein et al. 2015, 2016a, Bornschein, Pie & Teixeira 2019a), and we do not
322   leave any calls heard like those of the group *B. pernix* without collection and we never found *B.*
323   *hermogenesi*.

324   In addition, we have also absence of records of *B. sulfuratus* in northern Santa Catarina in
325   some well sampled localities. For example, we have made no records for *B. sulfuratus* in Morro
326   Boa Vista (26°30'58"S, 49°03'14"W), on the border between the municipalities of Jaraguá do
327   Sul and Massaranduba, where we described *B. albolineatus* (Bornschein et al. 2016b), Morro do
328   Baú (26°47'58"S, 48°55'47"W), municipality of Ilhota, and Morro Braço da Onça (26°44'58"S,
329   48°55'41"W), municipality of Luiz Alves, where we report *B. fuscolineatus* (Ribeiro et al. 2015,
330   Bornschein, Teixeira & Ribeiro 2019c), Morro do Cachorro (26°46'42"S, 49°01'57"W), on the
331   border between the municipalities of Blumenau, Gaspar, and Luiz Alves, where we described *B.*

332 *boticario* (Ribeiro et al. 2015), and Morro Santo Anjo (26°37'41"S, 48°55'50"W), municipality

333 of Massaranduba, where we described *B. mirissimus* (Pie et al. 2018a). There is a possibly that

334 around Morro do Garrafão (Table 1) is situated the southern limit of the geographic distribution

335 of *B. sulfuratus*.

336   As we demonstrate, there is no confirmed overlap in the distribution of *B. hermogenesi*

337 and *B. sulfuratus* and their replacements occurs in southeastern of São Paulo city, without

338 apparent barriers. There are other examples of discontinuity of the geographical distribution

339 between congeneric species throughout the Atlantic Forest from southeastern to southern Brazil

340 in southeastern São Paulo city, as in the montane bird *Scytalopus speluncae* (taxonomy *sensu*

341 Maurício et al. [2010]). Maurício (2005) stated that populations of *S. speluncae* from the

342 southeastern of the city of São Paulo to the south of the species distribution represent a distinct

343 species yet to be named, and he treated it as "Southern *Scytalopus speluncae*" (this scenario of

344 southern population of this bird as a new species was supported by the result of other studies

345 [Bornschein et al. 2007, Mata et al. 2009, Maurício et al. 2014, Pulido-Santacruz et al. 2016]). In

346 the region around the southeastern of São Paulo city, cases of hybridization of subspecies or

347 lineages of at least four species of birds have been reported (Pinto 1941, Silva and Stotz 1992,

348 Cabanne, Santos & Miyaki 2007, D'Horta et al. 2011; see also Dantas et al. [2015]). In the state

349 of São Paulo there is another discontinuity which is associated with intraspecific differentiation

350 or even sister species of frogs (Fitzpatrick et al. 2009, Thomé et al. 2010, Amaro et al. 2012) and

351 snakes (Grazziotin et al. 2006).

352   The correspondence between the distribution of the congeneric species in question with

353 the limits of the Serra do Mar is intriguing, given that during the last 20 million years there was

354 no obvious uplift in the region (Gontijo-Pascutti et al. 2012). This time scale is considerably

355 older than the inferred cladogenesis events and therefore geological processes could not have

356 been the primary cause of their divergence, given that *Brachycephalus* toads and *Scytalopus*

357 birds of São Paulo, Paraná, and Santa Catarina originated less than 2-5 million years ago (Pie et

358 al. [2018b] and Pulido-Santacruz et al. [2016], respectively). Likewise, recent neotectonic

359 activities (Late Pleistocene-Holocene) are restricted to the faults and stress regimes (Hasui 1990,

360 Saadi, 1993, Riccomini and Assumpção 1999) and, therefore, also could not have generated the

361 diversification pattern of widely distributed terrestrial species. It is important to note that Thomé

362 et al. (2010), studying the toad *Rhinella crucifer* from the eastern portion of Brazil, associate one

363   genetic break found in eastern São Paulo to neotectonic barriers, specifically the Cubatão shear

364   zone and the Guapiara lineament. However, these are ancient geotectonic activities, from

365   Proterozoic to Cambrian (with Phanerozoic reactivation) and Mesozoic, respectively (Ferreira et

366   al. 1981, Sadowski 1990, Almeida and Carneiro, 1998; see also Riccomini and Assumpção

367   1999). In addition, studies have proposed speciation by vicariance caused by relatively recent

368   events, such as river barriers (e.g., Amaral et al. 2013), sea level variation (Grazziotin et al. 2006,

369   Fitzpatrick et al. 2009), and forest refugia (e.g., Fitzpatrick et al. 2009, Thomé et al. 2010,

370   D'Horta et al. 2011, Amaral et al. 2013). The largest river around the disruption of the

371   geographical distribution of *B. sulfuratus* and *B. hermogenesi* is the Rio Ribeira do Iguape,

372   which intersects the Serra do Mar between São Paulo and Paraná States by continued erosive

373   retreat (Almeida and Carneiro 1998) or, alternatively, originated in tectonic depression

374   associated with asymmetric graben of the Sete Barras or Ribeira de Iguape (Melo et al. 1989,

375   Gontijo-Pascutti et al. 2012), which did not lead to isolation, given that *B. sulfuratus* occurs on

376   both of its banks. It is plausible that the origin of *B. sulfuratus* and *B. hermogenesi*, as well as the

377   other examples mentioned above, might have resulted from climatic variations that promoted

378   vicariance by forest cover disruption followed by recovery of forest coverage, presumably

379   leading to secondary contact.

380   The region in the state of São Paulo, around the southeastern São Paulo city, should be

381   further investigated. Records of flea toads in this region could be obtained as background sound

382   in recordings of birds (e.g., those deposited in databases such as www.xeno-canto.org and

383   www.wikiaves.com.br). Verdade et al. (2008) made a similar suggestion: to search for records of

384   *B. hermogenesi* in the background of recordings of birds from the Estação Biológica de Boracéia,

385   in the case one wants to seek previous records of this flea toad in this highly sampled locality. As

386   examples, calls of *B. sulfuratus* in Parque Estadual Intervales, municipality of Iporanga, state of

387   São Paulo (Table 1), can be heard in recordings of the birds *Merulaxis ater* (XC80463 and

388   XC18179) and *Eleoscytalopus indigoticus* (XC75544; available in www.xeno-canto.org).

389   We underscore the importance of continuous scrutiny of the distribution and

390   advertisement call analysis of *B. sulfuratus* and *B. hermogenesi*. Call parameters were poorly

391   described for *B. hermogenesi* and a better knowledge of the geographical limits between these

392   species can elucidate distribution patterns and potentially detect cases of sympatry. To date, there

393   are no confirmed cases of sympatry between species of *Brachycephalus* in the same group, only

394   between species from distinct groups (*B. pernix* and *B. didactylus* groups and *B. ephippium* and

395   *B. didactylus* groups; Bornschein et al. [2016a], Bornschein, Pie & Teixeira [2019a]). The

396   possibility of sympatry between *B. hermogenesi* and *B. sulfuratus* in eastern São Paulo is high,

397   but the distinctions of their advertisement calls could provide pre-zygotic isolation. Although

398   some species in the *B. ephippium* group are additively insensitive to the own advertisement call

399   (Goutte et al. 2017), which would suggest loss of active selection pressure and variation

400   maintained by inertia, it must be considered that this scenario may not apply to the other groups

401   (Monteiro et al. 2018a) and, also, that the species may actively perceive call emissions through

402   vibrations in other body receptors.

403

Conclusions

405   *Brachycephalus sulfuratus* differs from *B. hermogenesi* only by its advertisement calls;

406   other morphological characteristics suggested for its differentiation vary individually and are

407   shared with *B. hermogenesi*. The advertisement calls of these species differ greatly, even in the

408   field, with that of *B. sulfuratus* consisting of many pulses per note and that of *B. hermogenesi* by

409   up to three pulses per note. The advertisement calls of *B. sulfuratus* resemble those of species of

410   the *B. ephippium* species group, whereas the calls of *B. hermogenesi* resemble those of the *B.*

411   *pernix* species group. These similarities are intriguing, given that *B. sulfuratus* and *B.*

412   *hermogenesi* are part of the *B. didactylus* group. Understanding the evolution of these

413   advertisement calls should require a more in depth investigation.

414   All previous records of *B. hermogenesi* from southern Brazil should instead be

415   considered as *B. sulfuratus*, in a possibly cascading error resulting from the inadequate revision

416   of the records prior to the description of *B. sulfuratus* (Condez et al. 2016). A large region in the

417   south of the state of São Paulo needs to be further investigated to confirm the presence of *B.*

418   *hermogenesi*; the previous records were reverted to *Brachycephalus* sp. *Brachycephalus*

419   *sulfuratus* is distributed much further north than previously thought and it is possible that

420   sympatry with *B. hermogenesi* may occur in the southwest of the city of São Paulo. This region

421   in the southwest of São Paulo is particularly interesting because many species of different taxa

422   have their range limits there. The biogeographic explanation of this pattern seems to be limited to

423   the past distribution of forest patches, which could have been previously isolated and are now

424   distributed continuously, allowing possible secondary contact of species.

425

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431

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Records of flea toads *Brachycephalus hermogenesi*, *B. sulfuratus*, and *Brachycephalus* sp.

Table 1. Records of flea toads *Brachycephalus hermogenesi*, *B. sulfuratus*, and *Brachycephalus* sp. (*B. hermogenesi* or *B. sulfuratus*), southeastern and southern Brazil. Localities are in alphabetical order (accordingly to the respective species). Abbreviations: MHNCI = Museu de História Natural Capão da Imbuia, Curitiba, Paraná, Brazil; ZUEC = Museu de História Natural, Universidade Estadual de Campinas, Campinas, state of São Paulo, Brazil.

1 Table 1. Records of flea toads *Brachycephalus hermogenesi*, *B. sulfuratus*, and *Brachycephalus* sp. (*B. hermogenesi* or *B. sulfuratus*),
2 southeastern and southern Brazil. Localities are in alphabetical order (accordingly to the respective species). Abbreviations: MHNCI =
3 Museu de História Natural Capão da Imbuia, Curitiba, Paraná, Brazil; ZUEC = Museu de História Natural, Universidade Estadual de
4 Campinas, Campinas, state of São Paulo, Brazil.

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
B. hermogenesi	Corcovado, municipality of Ubatuba, São Paulo	23°28'20"S, 45°11'41"W; 30–250 m a.s.l.	Giaretta and Sawaya (1998; as *B. hermogenesi*), Verdade et al. (2008; as *B. hermogenesi*), Pie et al. (2013; as *B. hermogenesi*), Bornschein et al. (2016a; as *B. hermogenesi*), Pie et al. (2018a; as *B. hermogenesi* collected at Picinguaba [= Corcovado]), Bornschein, Pie & Teixeira, 2019a (as *B. hermogenesi*)	Specimens (including paratypes) and recordings	Specimens (ZUEC 9722–4, MHNCI 10823–25) and calls examined.
B. hermogenesi	Estação Biológica de Boracéia, municipality of Salesópolis, São Paulo	23°39'10"S, 45°53'05"W; 825–900 m a.s.l.	Pimenta et al. (2007; as *B. hermogenesi*), Verdade et al. (2008; as *B. hermogenesi*), Pie et al. (2013; as *B. hermogenesi*), Bornschein et al. (2016a; as *B. hermogenesi*), Condez et al. (2016; as *B. hermogenesi*)	Specimens and recordings	Specimens (MHNCI, one uncatalogued specimen) and calls examined (including recordings sent by V. K. Verdade; Fig. 2a).
B. hermogenesi	Fazenda Capricórnio, municipality of Ubatuba, São Paulo	23°23'27"S, 45°04'26"W; 60 m a.s.l.	Giaretta and Sawaya (1998;), Verdade et al. (2008; as *B. hermogenesi*), Pie et al. (2013; as *B. hermogenesi*), Bornschein et al. (2016a; as *B. hermogenesi*), Condez et al. (2016; as *B. hermogenesi*)	Specimens (paratypes)	Specimen examined (ZUEC 9725).
B. hermogenesi	Morro Cuscuzeiro, on the border of municipalities of	23°17'50"S, 44°47'21"W; 730–1,090 a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. hermogenesi*)	Recordings	Calls examined.

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
B. hermogenesi	Paraty, Rio de Janeiro, and Ubatuba, São Paulo Morro do Corcovado, Parque Estadual da Serra do Mar, municipality of Ubatuba, São Paulo	23°27'06"S, 45°12'03"W; 250–1,060 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. hermogenesi*)	Recordings	Calls examined.
B. hermogenesi	Municipality of Paraibuna, São Paulo	c. 23°23'34"S, 45°39'42"W; altitude?	Condez et al. 2016 (as *B. hermogenesi*)	Specimen	---
B. hermogenesi	Núcleo Cunha, Parque Estadual da Serra do Mar, municipality of Cunha, São Paulo	23°15'48"S, 45°02'39"W; 1,045–1,140 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. hermogenesi*)	Specimen and recordings	Specimen (MHNCI, one uncatalogued specimen) and recordings examined.
B. hermogenesi	Núcleo Picinguaba, Parque Estadual da Serra do Mar, municipality of Ubatuba, São Paulo	23°22'21"S, 44°49'53"W; 0–700 m a.s.l.	Giaretta and Sawaya (1998), Pimenta et al. (2007; as *B. hermogenesi*), Verdade et al. (2008; as *B. hermogenesi*), Clemente-Carvalho et al. (2009; as *B. hermogenesi*), Pie et al. (2013; as *B. hermogenesi*), Bornschein et al. (2016a; as *B. hermogenesi*), Condez et al. (2016; as *B. hermogenesi*), Pie et al. (2018a; as *B. hermogenesi*)	Specimens (holotype and paratypes) and recordings	Specimens (ZUEC 9715–21; Fig. 3d) and calls examined.
B. hermogenesi	Sertão da Cutia, municipality of Ubatuba, So Paulo	not located	Condez et al. (2016; as *B. hermogenesi*)	Specimen	---
B. hermogenesi	Trilha do Corisco, municipality of Paraty,	23°16'38"S, 44°46'39"W; 350–725 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. hermogenesi*)	Recordings	Calls examined.

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
B. hermogenesi	Rio de Janeiro Trilha do Ipiranga 50 m from the Rio Ipiranga, Núcleo Santa Virgínia, Parque Estadual da Serra do Mar, municipality of São Luiz do Paraitinga, São Paulo	23°20'41"S, 45°08'21"W; 920–940 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. hermogenesi*)	Recordings	Calls examined.
B. sulfuratus	Bairro Rio Vermelho, municipality of Barra do Turvo, São Paulo	24°59'25"S, 48°32'26"W; 790 m a.s.l.	---	Specimen	Specimen examined (MHNCI 11584).
B. sulfuratus	Base of the Serra Água Limpa, municipality of Apiaí, São Paulo	24°28'52"S, 48°47'12"W; 920 m a.s.l.	Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein et al. (2016b; as *B. sulfuratus*), Firkowski et al. (2016; without species identification), Ribeiro et al. (2017; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*)	Specimen and recordings	Specimen (MHNCI 11583; Fig. 1f) and calls examined (MHNCI 129; Fig. 4d).
B. sulfuratus	Biquinha, municipality of Juquiá, São Paulo	24°17'43"S, 47°36'26"W; 40 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Recordings	Calls examined (MHNCI 128; Fig. 4c).
B. sulfuratus	Braço do Norte, municipality of Itapoá, Santa Catarina	26°07'29"S, 48°43'48"W; 240 m a.s.l.	Monteiro et al. (2018a; as *B. sulfuratus*)	Specimen and genetic	---
B. sulfuratus	Caratuval, near the Parque Estadual das Lauráceas, municipality of Adrianópolis, Paraná	24°51'17"S, 48°43'43"W; 900 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein et al. (2016b; as *B. sulfuratus*), Firkowski et al.	Specimen and recordings	Specimen (MHNCI 11571; Fig. 1b) and calls examined (MHNCI 131; Fig. 5b).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
B. sulfuratus	Caratuval, Parque Estadual das Lauráceas, municipality of Adrianópolis, Paraná	24°51'14"S, 48°42'01"W; 890 m a.s.l.	(2016; without species identification), Ribeiro et al. (2017; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*) Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1)	Recordings	Calls examined (MHNCI 132; Fig. 5c).
B. sulfuratus	Castelo dos Bugres, municipality of Joinville, Paraná	26°13'47"S, 49°03'20"W; 790–860 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Condez et al. (2016; as *B. sulfuratus*), Monteiro et al. (2018a; as *B. sulfuratus*)	Specimen, recordings, and genetic	Calls examined.
B. sulfuratus	Centro de Estudos e Pesquisas Ambientais da Univille, Vila da Glória, Distrito do Saí, municipality of São Francisco do Sul, Santa Catarina	26°13'39"S, 48°41'31"W; 125 m a.s.l.	Condez et al. (2016; as *B. sulfuratus*)	Specimen, recordings, and genetics	---
B. sulfuratus	Corvo, municipality of Quatro Barras, Paraná	25°20'17"S, 48°54'56"W; 930 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein et al. (2016b; as *B. sulfuratus*), Firkowski et al. (2016; without species identification), Ribeiro et al.	Specimen	Specimen examined (MHNCI 10788, MHNCI 11573, MHNCI 11575; Fig. 1a, e, i).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
			(2017; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*), Pie et al. (2018b; as *B. sulfuratus*)		
B. sulfuratus	Entroncamento Teba, Rio Turvo, municipality of Campina Grande do Sul, Paraná	25°01'28"S, 48°37'12"W; 785 m a.s.l.	---	Specimens and recordings	Specimens (MHNCI 11586–7) and calls examined.
B. sulfuratus	Estância Hidroclimática Recreio da Serra, Serra da Baitaca, municipality of Piraquara, Paraná	25°27'14"S, 49°00'28"W; 1,150–1,205 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Specimen	Specimen examined (MHNCI 11591).
B. sulfuratus	Fazenda Thalia, municipality of Balsa Nova, Paraná	25°30'58"S, 49°40'12"W; 1,025 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein et al. (2016b; as *B. sulfuratus*), Firkowski et al. (2016; without species identification), Ribeiro et al. (2017; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*)	Specimens and recordings	Specimens (MHNCI 11579–81, MHNCI 11582; Fig. 1c, d, g, h) and calls examined (MHNCI 134; Fig. 6a).
B. sulfuratus	near the Jurupará dam, municipality of Piedade, São Paulo	23°56'30"S, 47°23'45"W; 690 m a.s.l.	Pie et al. (2018a; as *B. sulfuratus*)	Specimens and recordings	Specimens (MHNCI 10790–2; Fig. 1j, l) and calls examined (MHNCI 123–5; Fig. 2b, c, d).
B. sulfuratus	Mananciais da Serra, municipality of Piraquara, Paraná	25°29'32"S, 48°59'33"W; 970–1,050 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1),	Specimen	Specimen examined (MHNCI 10302).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
B. sulfuratus	Monte Crista, municipality of Garuva, Santa Catarina	26°04'53"S; 48°55'03"W; 435 m a.s.l.	Bornschein et al. (2016b; as *B. sulfuratus*), Ribeiro et al. (2017; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*) ---	Recordings	Calls examined.
B. sulfuratus	Morro Anhangava, municipality of Quatro Barras, Paraná	25°22'51"S, 49°01'26"W; 915 m a.s.l.	Condez et al. (2016; as *B. sulfuratus*), Monteiro et al. (2018a; as *B. sulfuratus*)	Specimen and genetics	---
B. sulfuratus	Morro do Canal, municipality of Piraquara, Paraná	25°30'55"S; 48°58'56"W; 1,315 m	---	Recordings	Calls examined.
B. sulfuratus	Morro do Cantagalo, Vila da Glória, Distrito do Saí, municipality of São Francisco do Sul, Santa Catarina	26°10'31"S, 48°42'44"W; 160 m a.s.l.	Condez et al. (2016; as *B. sulfuratus*)	Specimen and genetics	---
B. sulfuratus	Morro do Garrafão, municipality of Corupá, Santa Catarina	26°28'23"S, 49°15'57"W; 500–530 m a.s.l.	Pie et al. (2018a; as *B. sulfuratus*), Teixeira et al. (2018; as *B. sulfuratus*)	Specimen and recordings	Specimens (MHNCI 10826–8; Fig. 1k) and calls examined (MHNCI 137; Fig. 6d).
B. sulfuratus	Morro Garuva, municipality of Garuva, Santa Catarina	26°02'29"S, 48°53'14"W; 215–495 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Recordings	Calls examined (MHNCI 136; Fig. 6c).
B. sulfuratus	Núcleo Itutinga-Pilões, Parque Estadual da Serra do Mar, municipality of Cubatão, São Paulo	23°54'17"S, 46°29'22"W; 55 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Recordings	Calls examined (MHNCI 126–7; Fig. 4a, b).
B. sulfuratus	Parque Estadual da	25°06'53"S, 47°55'40"W;	Verdade et al. (2008; as	Specimen,	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
	Ilha do Cardoso, municipality of Cananéia, São Paulo	385 m a.s.l.	possibly *B. hermogenesi*), Condez et al. (2016; as *B. sulfuratus*)	recordings, and genetics	
B. sulfuratus	Parque Estadual Intervales, municipality of Iporanga, São Paulo	24°16'33"S, 48°25'04"W; 820 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Recordings (www.xeno-canto.org; see text)	Calls examined (low quality for publication).
B. sulfuratus	Pedra da Tartaruga, municipality of Garuva, Santa Catarina	25°59'42"S, 48°54'23"W; 465 m a.s.l.	---	Specimen	Specimen examined (MHNCI 11585).
B. sulfuratus	Pico Marumbi, Parque Estadual do Pico Marumbi, municipality of Morretes, Paraná	25°27'03"S; 48°54'59"W; 1180 m a.s.l.	---	Specimen	Specimen examined (MHNCI 10302)
B. sulfuratus	Recanto das Hortências, municipality of São José dos Pinhais, Paraná	25°33'24"S, 48°59'38"W; 975 m a.s.l.	Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Ribeiro et al. (2017; as *B. sulfuratus*), Bornschein et al. (2016b; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*)	Specimen	Specimen examined.
B. sulfuratus	Reserva Particular do Patrimônio Natural Salto Morato, municipality of Guaraqueçaba, Paraná	25°09'14"S, 48°18'06"W; 40–880 m a.s.l.	Pereira et al. (2010; as *B. hermogenesi*), Santos-Pereira et al. (2011, 2016, 2018; all as *B. hermogenesi*), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Leivas et al. 2018 (as *B. hermogenesi*)	Specimen and recordings	Calls examined (MHNCI 133; Fig. 5d).
B. sulfuratus	Salto do Inferno, Rio Capivari, municipality of Bocaiúva do Sul, Paraná	25°00'02"S, 48°37'07"W; 610 m a.s.l.	Ribeiro et al. (2017; as *B. sulfuratus*), Bornschein et al. (2016b; as *B. sulfuratus*), Pie et al. (2018a; as *B. sulfuratus*)	Specimen	Specimen examined.

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
B. sulfuratus	Serra do Guaraú, on the border of the municipalities of Cajati and Jacupiranga, São Paulo	24°47'12"S, 48°07'11"W; 680–835 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Recordings	Calls examined (MHNCI 130; Fig. 5a).
B. sulfuratus	Serra do Pico, municipality of Joinville, Santa Catarina	26°08'31"S, 48°57'19"W; 340–720 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Recordings	Calls examined.
B. sulfuratus	Torre Embratel, municipality of Cajati, São Paulo	24°52'46"S, 48°15'27"W; 960–990 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *B. sulfuratus*)	Specimen and recordings	Specimen (MHNCI 11588) and calls examined.
B. sulfuratus	Truticultura, municipality of Garuva, Paraná	26°01'33"S, 48°52'02"W; 90 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1)	Recordings	Calls examined (MHNCI 135; Fig. 6b).
Brachycephalus sp.	Alto Quiriri, municipality of Garuva, Santa Catarina	26°05'34"S, 48°59'41"W; 240 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)	Unvouchered	The calls resemble those of *B. sulfuratus* (auditory record made by MRB).
Brachycephalus sp.	Colônia Castelhanos, municipality of Guaratuba, Paraná	25°47'58"S, 48°54'40"W; 290 m a.s.l.	Cunha et al. (2010; as *Brachycephalus* aff. *hermogenesi*), Oliveira et al. (2011; as *B. hermogenesi*), Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Condez et al. 2016 (as *B.*	Specimen	Specimen examined (ZUEC 16602).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
			sulfuratus), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)		
Brachycephalus sp.	Dona Francisca, municipality of Joinville, Santa Catarina	26°09'52"S, 48°59'23"W; 150 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)	Unvouchered	The calls resemble those of *B. sulfuratus* (auditory record made by MRB).
Brachycephalus sp.	Estação Ecológica Juréia-Itatins, municipality of Iguape, São Paulo	c. 24°27'S, 47°24'W; altitude?	Verdade et al. (2008; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Estrada do Rio do Júlio, municipality of Joinville, Santa Catarina	26°17'02"S, 49°06'08"W; 650 m a.s.l.	Mariotto (2014; as *Brachycephalus* sp.), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)	Specimen	---
Brachycephalus sp.	Fazenda Pico Paraná, municipality of Campina Grande do Sul, Paraná	25°13'29"S, 48°51'17"W; 1,050–1,085 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)	Unvouchered	The calls resemble those of *B. sulfuratus* (auditory records made by MRB and LFR).
Brachycephalus	Fazenda Primavera,	24°53'08"S, 48°45'51"W;	Pie et al. (2013; as	Unvouchered	The calls resemble those

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
sp.	municipality of Tunas do Paraná, Paraná	1,060 m a.s.l.	*Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)		of *B. sulfuratus* (auditory record made by MRB).
Brachycephalus sp.	Municipality of Ibiúna, São Paulo	c. 23°39'S, 47°13'W; altitude?	Condez et al. (2016; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Municipality of Juquitiba, São Paulo	c. 23°56'S, 47°04'W; altitude?	Verdade et al. (2008; as *B. hermogenesi*), Condez et al. (2016; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Municipality of Paraty, Rio de Janeiro	c. 23°13'07"S, 44°43'15"W; altitude?	Giaretta and Sawaya (1998; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. hermogenesi*)	Unvouchered	---
Brachycephalus sp.	Municipality of Peruíbe, São Paulo	24°18'S, 46°59'W; altitude?	Condez et al. (2016; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Municipality of Piedade, São Paulo	c. 23°54'S, 47°25'W; altitude?	Condez, Sawaya & Dixo (2009; as *B. hermogenesi*); Clemente-Carvalho et al. (2011; as *B. hermogenesi*), Bornschein, Pie	Specimen and genetic sequence on GenBank	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
			& Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)		
Brachycephalus sp.	Municipality of Registro, São Paulo	c. 24°30'S, 47°51'W; altitude?	Condez et al. (2016; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Municipality of Ribeirão Grande, São Paulo	c. 24°06'S, 48°22'W; altitude?	Verdade et al. (2008; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Municipality of Tapiraí, São Paulo	c. 23°57'55"S, 47°30'19"W; 870 m a.s.l.	Verdade et al. (2008; as *B. hermogenesi*), Condez, Sawaya & Dixo (2009; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Parque Estadual de Jacupiranga, municipality of Eldorado, São Paulo	c. 24°38'S, 48°24'W; altitude?	Condez et al. (2016; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Parque Natural Municipal Nascentes de Paranapiacaba, municipality of Santo André, São Paulo	23°46'10"S, 46°17'36"W; 840 m a.s.l.	Verdade, Rodrigues & Pavan (2009; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Unvouchered	---
Brachycephalus	Pico Agudinho, Serra	25°36'24"S, 48°43'33"W;	Pie et al. (2013; as	Unvouchered	The calls resemble those

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
sp.	da Prata, municipality of Morretes, Paraná	385 m a.s.l.	*Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)		of *B. sulfuratus* (auditory record made by MRB).
Brachycephalus sp.	Reserva Betary, municipality of Iporanga, São Paulo	24°33'08"S, 48°40'49"W; 190 m a.s.l.	Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	Specimen examined (ZUEC 19931).
Brachycephalus sp.	Reserva Biológica do Alto da Serra de Paranapiacaba, municipality of Santo André, São Paulo	23°46'40"S, 46°18'45"W; 800–850 m a.s.l.	Verdade et al. (2008; as *B. hermogenesi*), Verdade, Rodrigues & Pavan (2009; as *B. hermogenesi*), Bornschein et al. (2016a; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Unvouchered	---
Brachycephalus sp.	Reserva Florestal de Morro Grande, municipality of Cotia, São Paulo	23°42'08"S, 46°58'22"W; cf. 990 m a.s.l.	Dixo and Verdade (2006; as *B. hermogenesi*), Verdade et al. (2008; as *B. hermogenesi*), Bornschein et al. (201a6; as *B. hermogenesi*), Condez et al. (2016; as *B. hermogenesi*), Bornschein, Pie & Teixeira, 2019a (as *Brachycephalus* sp. - *B. sulfuratus* or *B. hermogenesi*)	Specimen	---
Brachycephalus sp.	Sítio Ananias, municipality of Guaratuba, Paraná	25°47'08"S, 48°43'03"W; 25 m a.s.l.	Pie et al. (2013; as *Brachycephalus* sp. nov. 1), Bornschein et al. (2016a; as *Brachycephalus* sp. 1), Bornschein, Pie & Teixeira,	Unvouchered	The calls resemble those of *B. sulfuratus* (auditory record made by MRB).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
			2019a (as *Brachycephalus* sp. cf. *B. sulfuratus*)		

Figure 1

Ventral view of life specimens of *Brachycephalus sulfuratus*

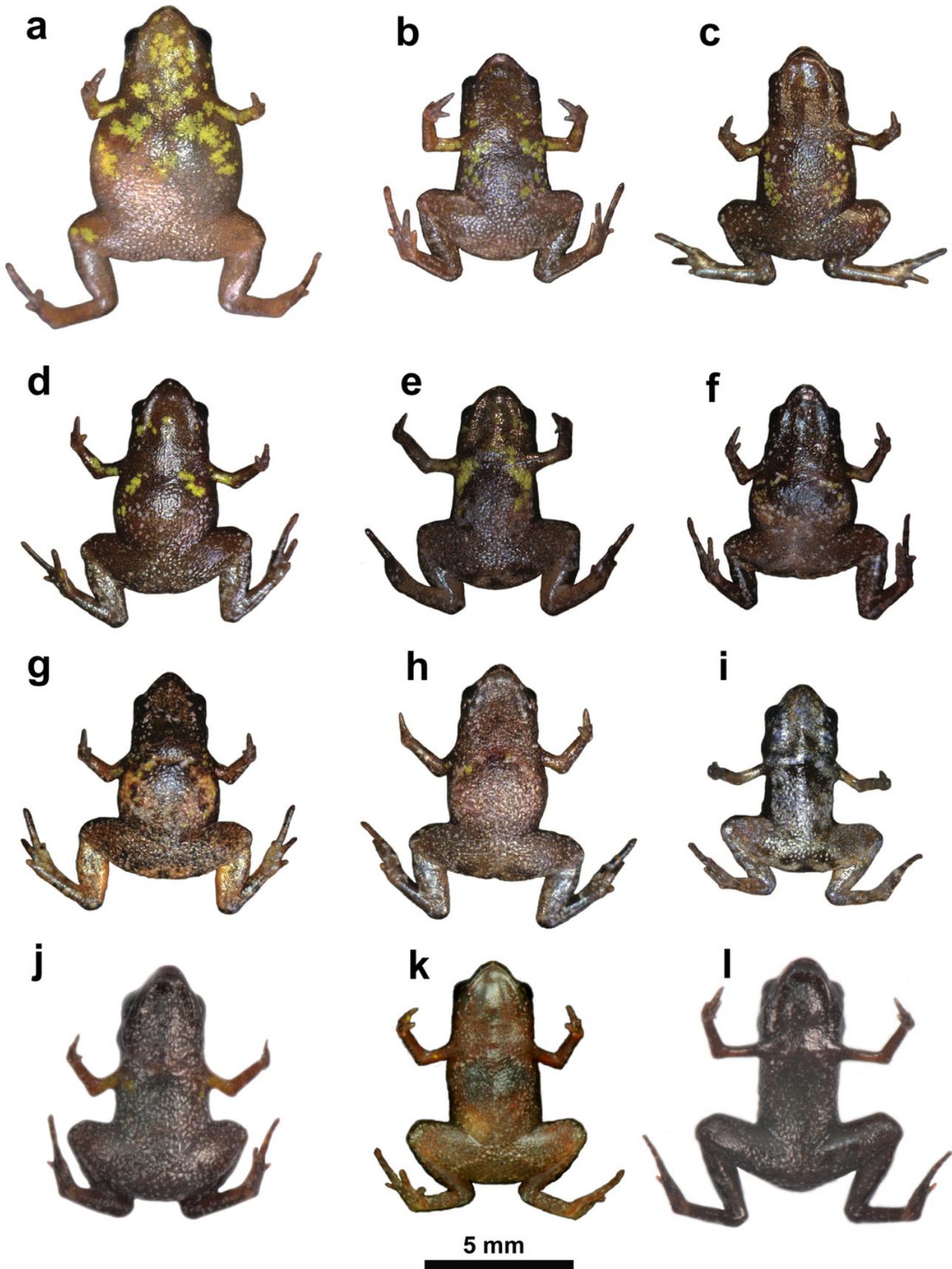
Figure 1. Ventral view of life specimens of *Brachycephalus sulfuratus* initially deposited in the Coleção Herpetológica do Departamento de Zoologia (DZUP), Universidade Federal do Paraná, Curitiba, Paraná, Brazil, and transferred to Museu de História Natural Capão da Imbuia (MHNCI), Curitiba, Paraná, Brazil. a) MHNCI 11575 (ex-DZUP 153) (Corvo); b) MHNCI 11571 (ex-DZUP 139)(Caratuval, near the Parque Estadual das Lauráceas); c) MHNCI 11582 (ex-DZUP 224) (Fazenda Thalia); d) MHNCI 11579 (ex-DZUP 221) (Fazenda Thalia); e) MHNCI 11573 (ex-DZUP 151) (Corvo); f) MHNCI 11583 (ex-DZUP 362) (base of the Serra Água Limpa); g) MHNCI 11580 (ex-DZUP 222) (Fazenda Thalia); h) MHNCI 11581 (ex-DZUP 223) (Fazenda Thalia); i) MHNCI 10788 (ex-DZUP 154) (Corvo); j) MHNCI 10790 (near the Jurupará dam); k) MHNCI 10826 (Morro do Garrafão); l) MHNCI 10792 (near the Jurupará dam). Notice the variable of yellow spots, absent in specimen "l", as well as the absence of the dark-brown inverted v-shaped mark on the cloacal region of specimen "a". Compare sonograms from specimens "j" and "l" in Fig. 2b, c. The presence of yellow spots and v-shaped mark was proposed as diagnostic characteristics to distinguish *B. sulfuratus* from *B. hermogenesi*, but they are variable intraspecifically. For details on geographical localities, see Table 1. Photo credit: Luiz Fernando Ribeiro.

5 mm

Figure 2

Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus hermogenesi* and *B. sulfuratus*

Figure 2. Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus hermogenesi* and *B. sulfuratus*. Calls of *B. sulfuratus* were obtained by the authors for this study and are shown from north to the south. Each figure was produced in Raven Pro 1.4, with the scales of both axes edited in CorelDRAW. a) *B. hermogenesi*: Estação Biológica de Boracéia (recorded by V. K. Verdade, without details). b) *B. sulfuratus*: near the Jurupará dam (MHNCI 123; specimen MHNCI 10790). c) *B. sulfuratus*: near the Jurupará dam (MHNCI 124; specimen MHNCI 10791 or MHNCI 10792). d) *B. sulfuratus*: near the Jurupará dam (MHNCI 125; specimen not collected). Notice two pulses for *B. hermogenesi* and several for *B. sulfuratus*. For details on geographical localities, see Table 1.

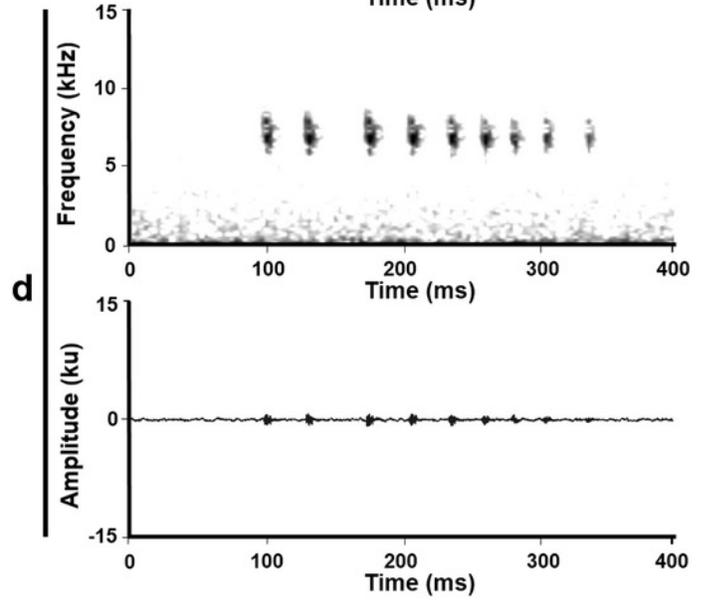
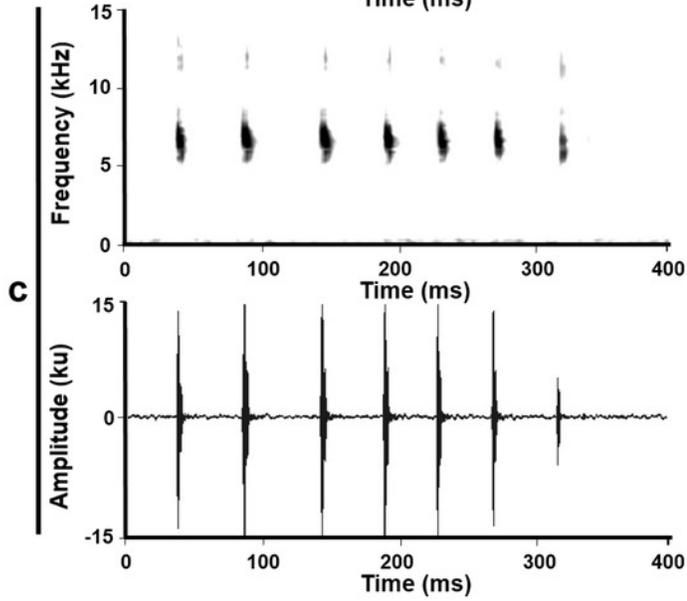
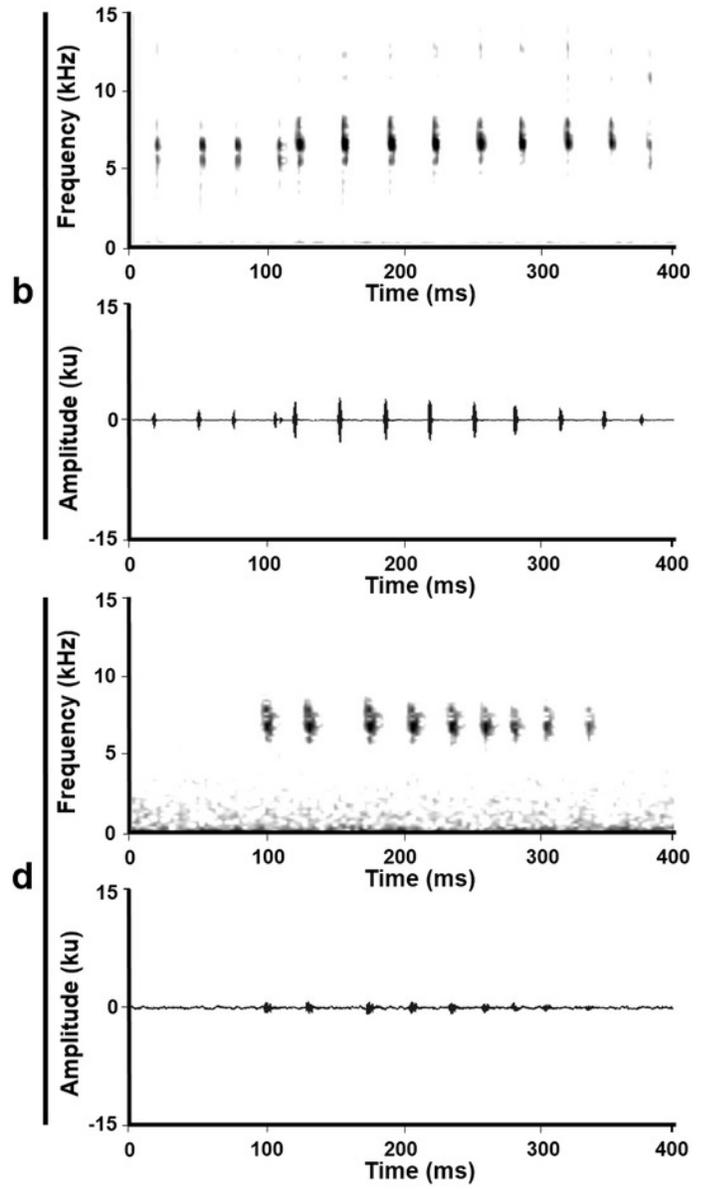
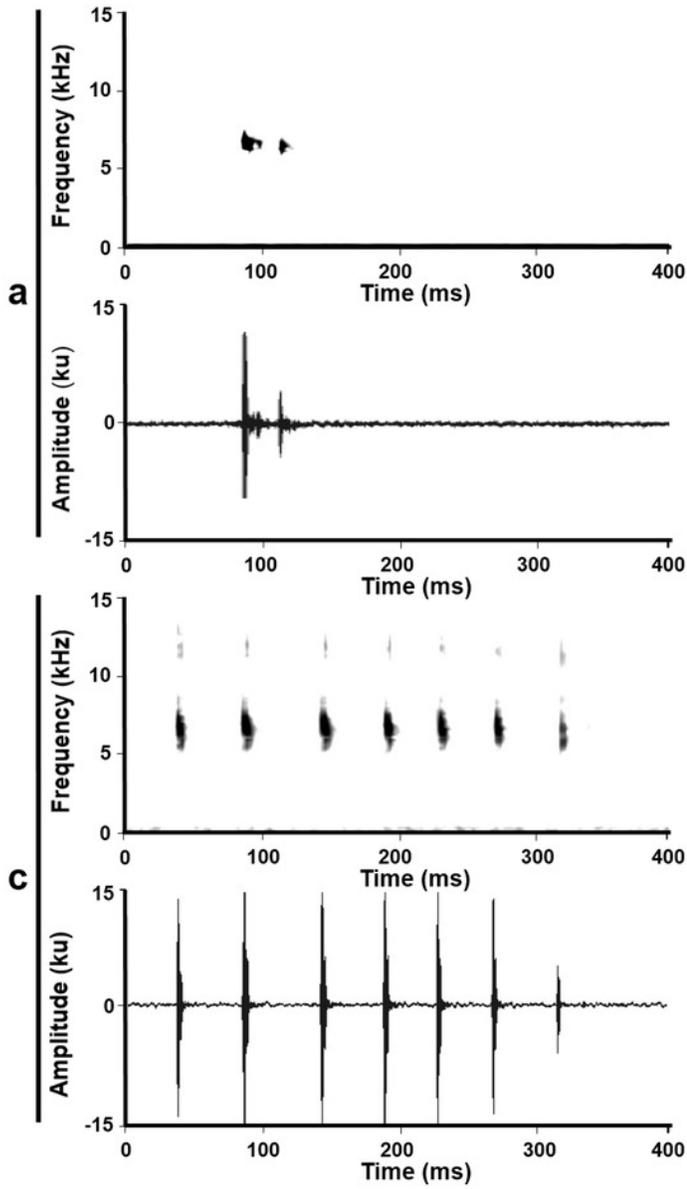



Figure 3

Ventral view of specimens of *Brachycephalus sulfuratus* (a-c) and *B. hermogenesi* (d) in preservative

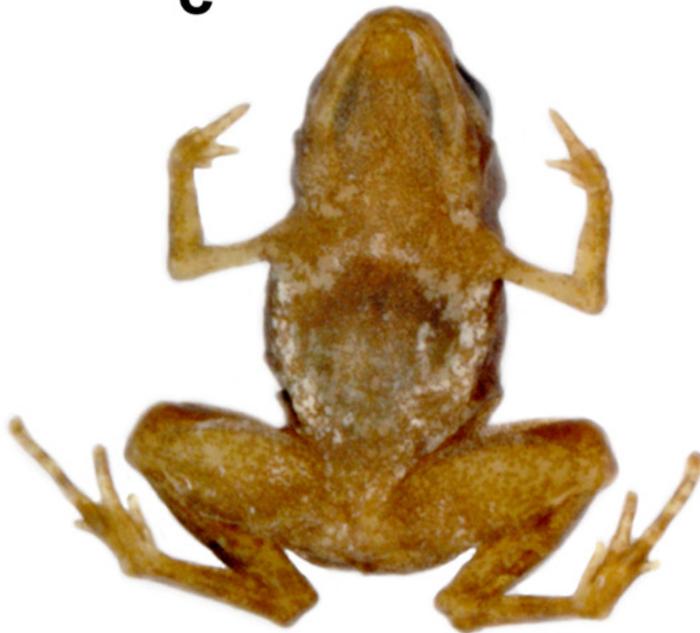
Figure 3. Ventral view of specimens of *Brachycephalus sulfuratus* (a-c) and *B. hermogenesi* (d) in preservative, deposited in the Coleção Herpetológica do Museu de História Natural Capão da Imbuia (MHNCI), Curitiba, Paraná, Brazil, and Museu de História Natural (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil: a) MHNCI 9800 (Salto do Inferno); b) MHNCI 10302 (Mananciais da Serra); c) MHNCI 10303 (Corvo; ex Coleção Herpetológica do Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil, DZUP 589); and d) ZUEC 9715 (holotype of *B. hermogenesi*). Notice the variation in ventral coloration. For details on geographical localities, see Table 1. Photo credit: Luiz Fernando Ribeiro.

a

5 mm

b

5 mm

c

5 mm

d

5 mm

Figure 4

Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus sulfuratus*.

Figure 4. Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus sulfuratus*. Call were obtained by the authors for this study and are shown from north to the south. Each figure was produced in Raven Pro 1.4, with the scales of both axes edited in CorelDRAW. a) Núcleo Itutinga-Pilões (MHNCI 126; specimen not collected; first specimen). b) Núcleo Itutinga-Pilões (MHNCI 127; specimen not collected; second specimen). c) Biquinha (MHNCI 128; specimen not collected). d) base of the Serra Água Limpa (MHNCI 129; specimen MHNCI 11583). For details on geographical localities, see Table 1.

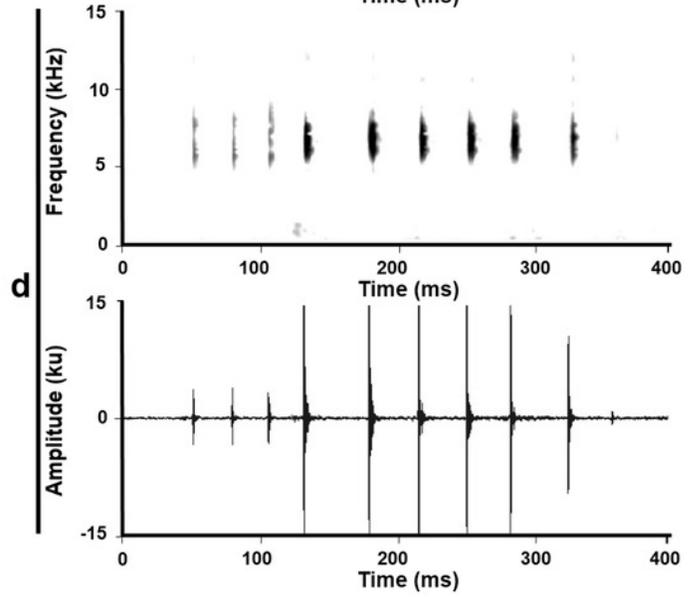
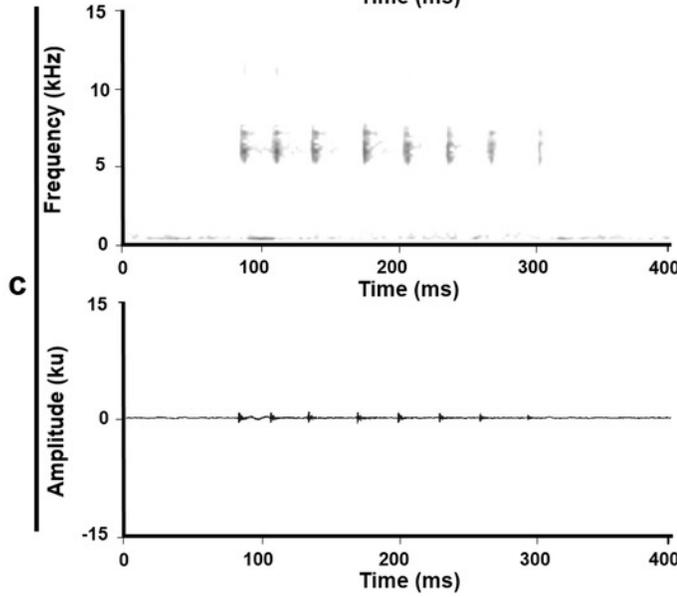
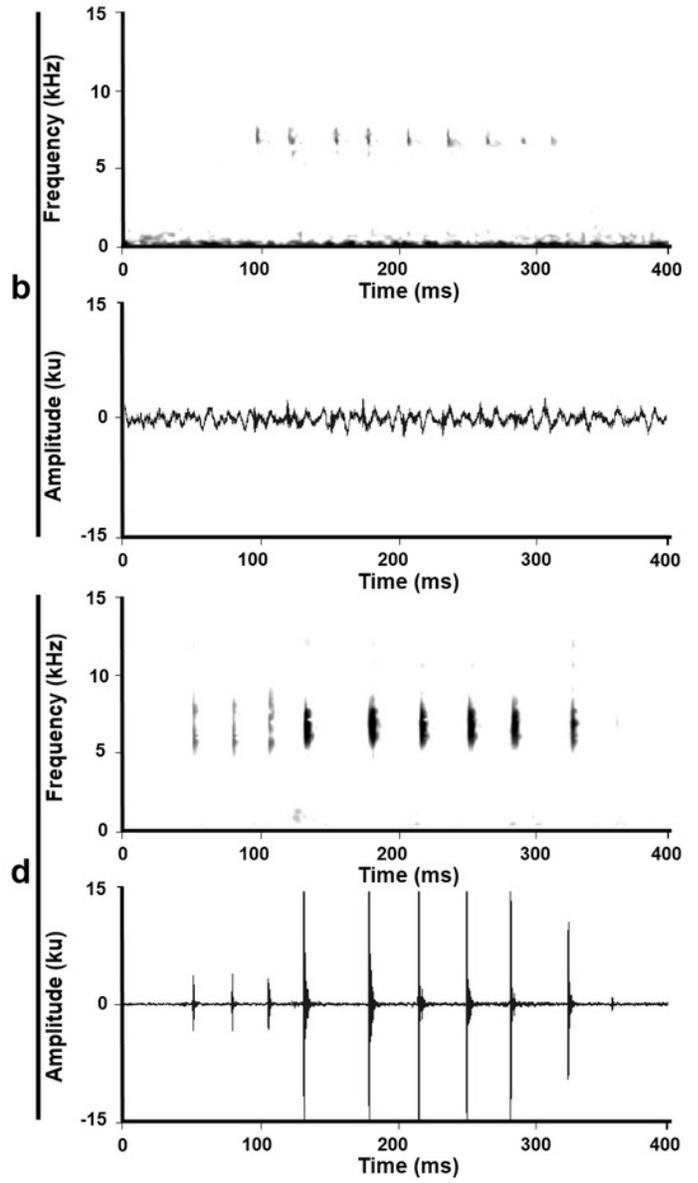
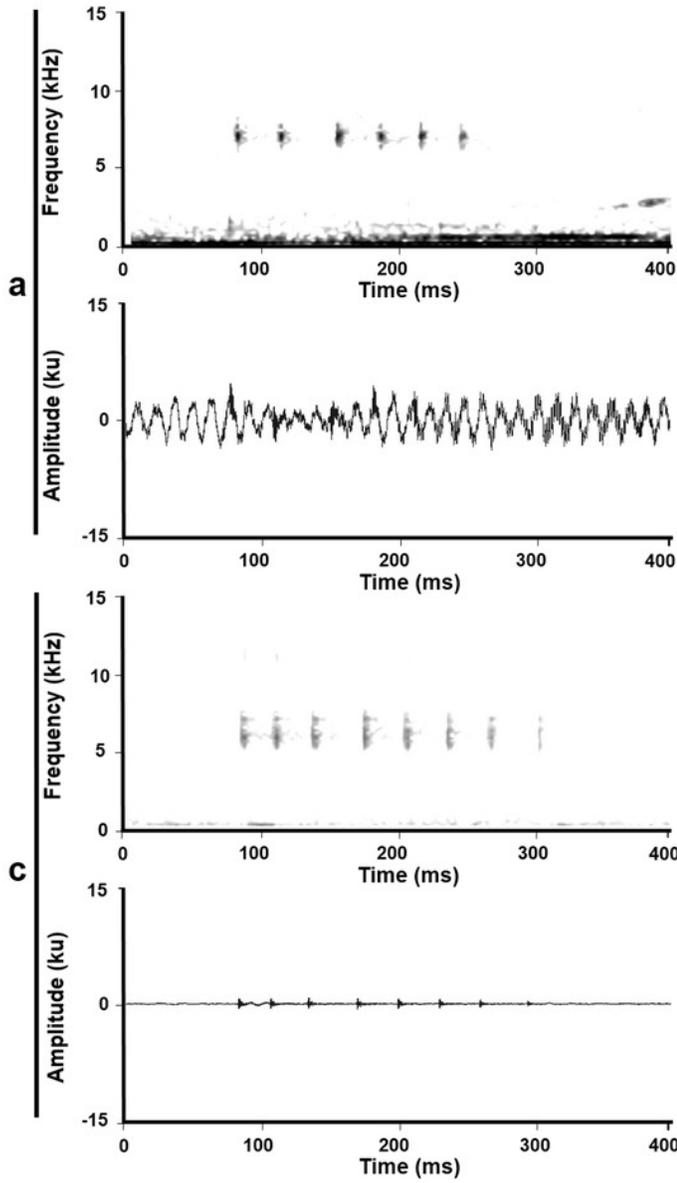



Figure 5

Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus sulfuratus*.

Figure 5. Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus sulfuratus*. Call were obtained by the authors for this study and are shown from north to the south. Each figure was produced in Raven Pro 1.4, with the scales of both axes edited in CorelDRAW. a) Serra do Guaraú (MHNCI 130; specimen not collected). b) Caratuval, near the Parque Estadual das Lauráceas (MHNCI 131; specimen MHNCI 11571). c) Caratuval, Parque Estadual das Lauráceas (MHNCI 132; specimen not collected). d) Reserva Particular do Patrimônio Natural Salto Morato (MHNCI 133; specimen not collected). For details on geographical localities, see Table 1.

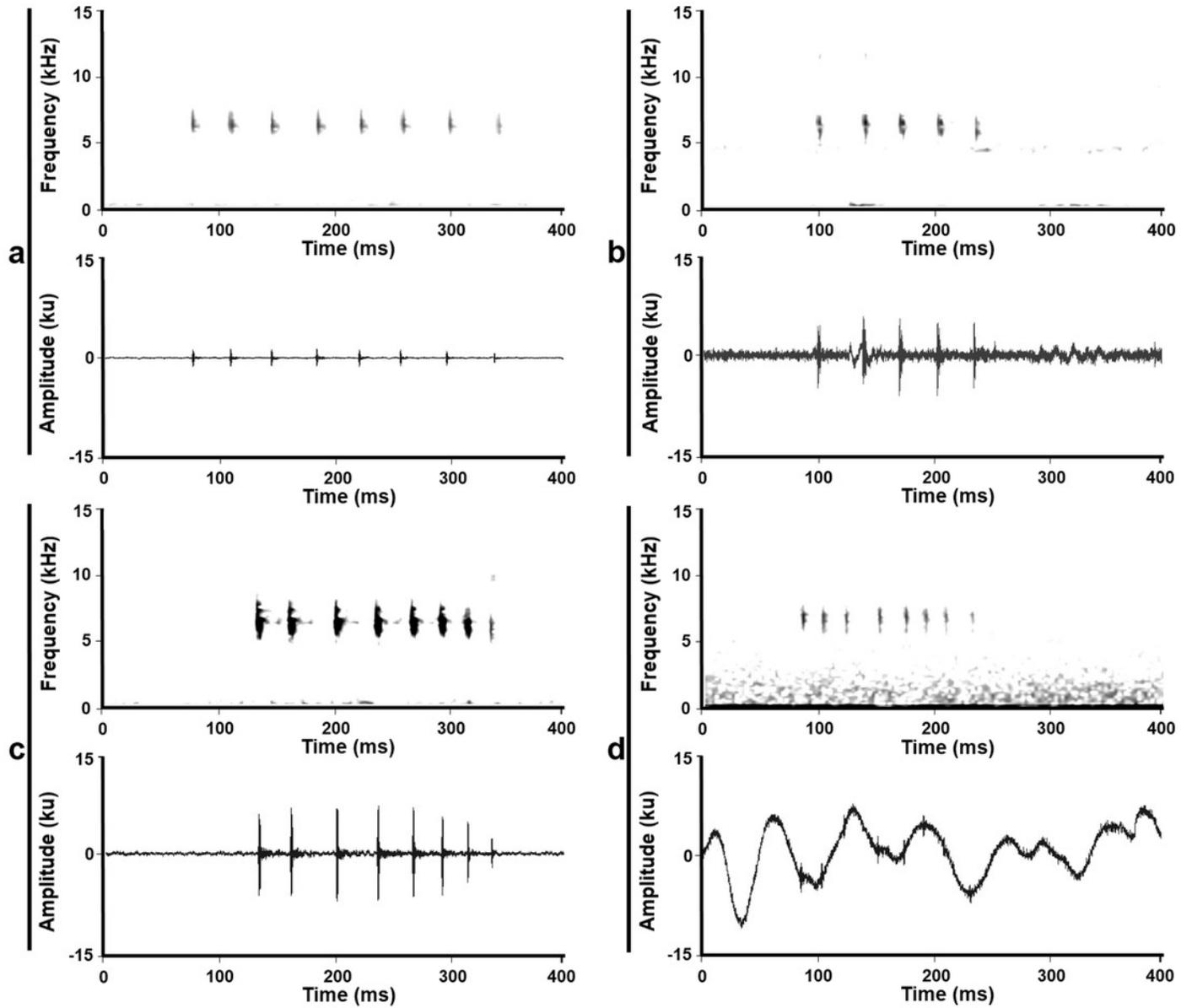



Figure 6

Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus sulfuratus*.

Figure 6. Spectrograms (above) and oscillograms (below) of a single note of the advertisement call of *Brachycephalus sulfuratus*. Call were obtained by the authors for this study and are shown from north to the south. Each figure was produced in Raven Pro 1.4, with the scales of both axes edited in CorelDRAW. a) Fazenda Thalia (MHNCI 134; specimen not collected). b) Truticultura (MHNCI 135; specimen not collected). c) Morro Garuva (MHNCI 136; specimen not collected). d) Morro do Garrafão (MHNCI 137; specimen not collected). For details on geographical localities, see Table 1.

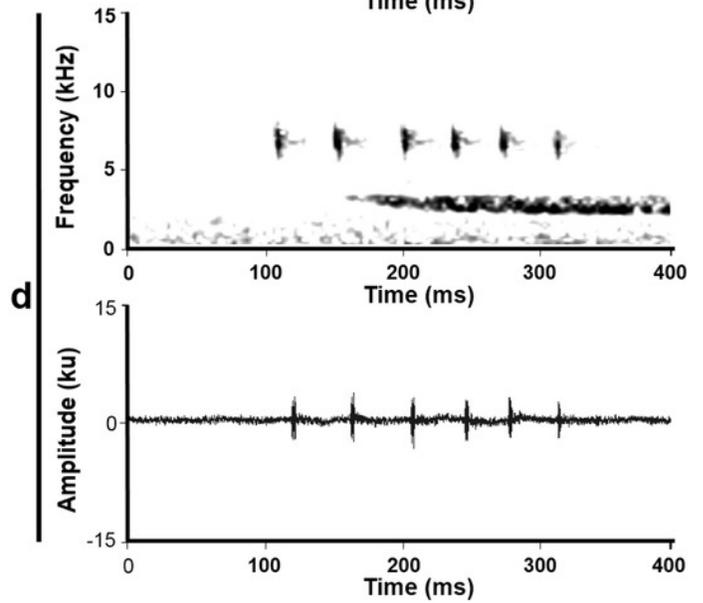
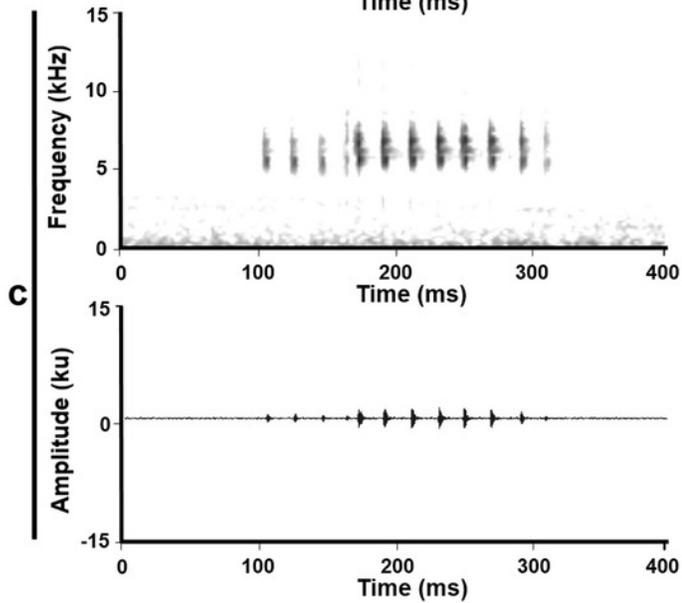
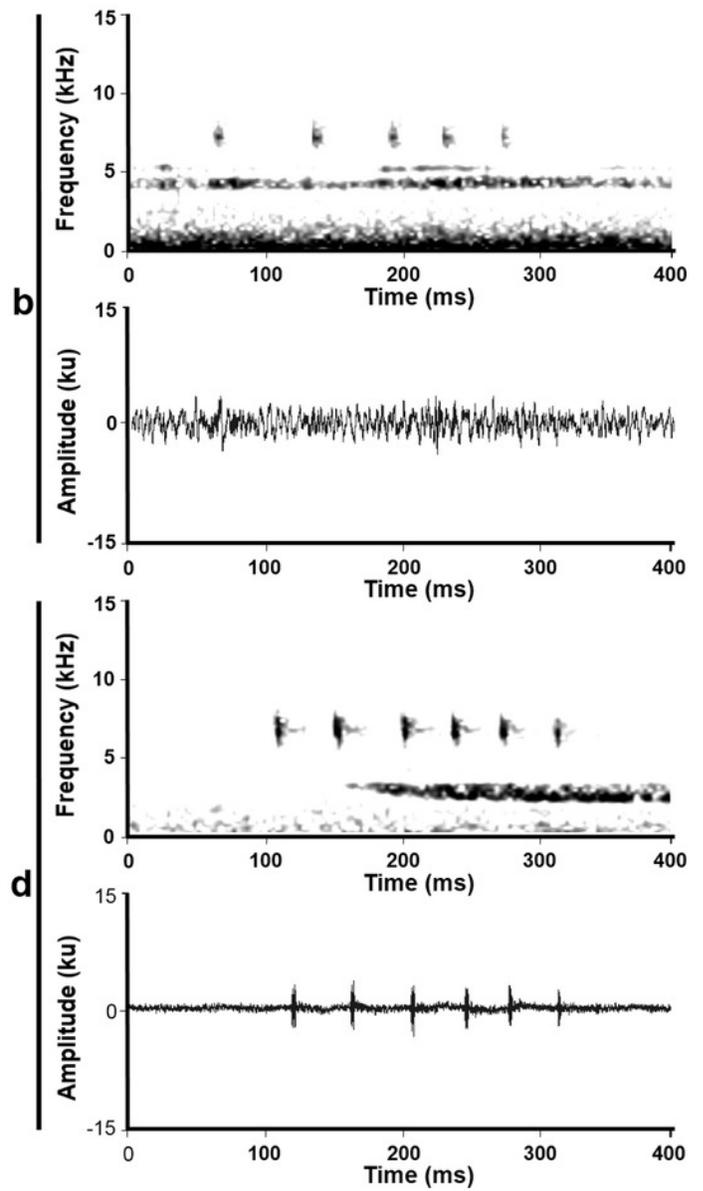
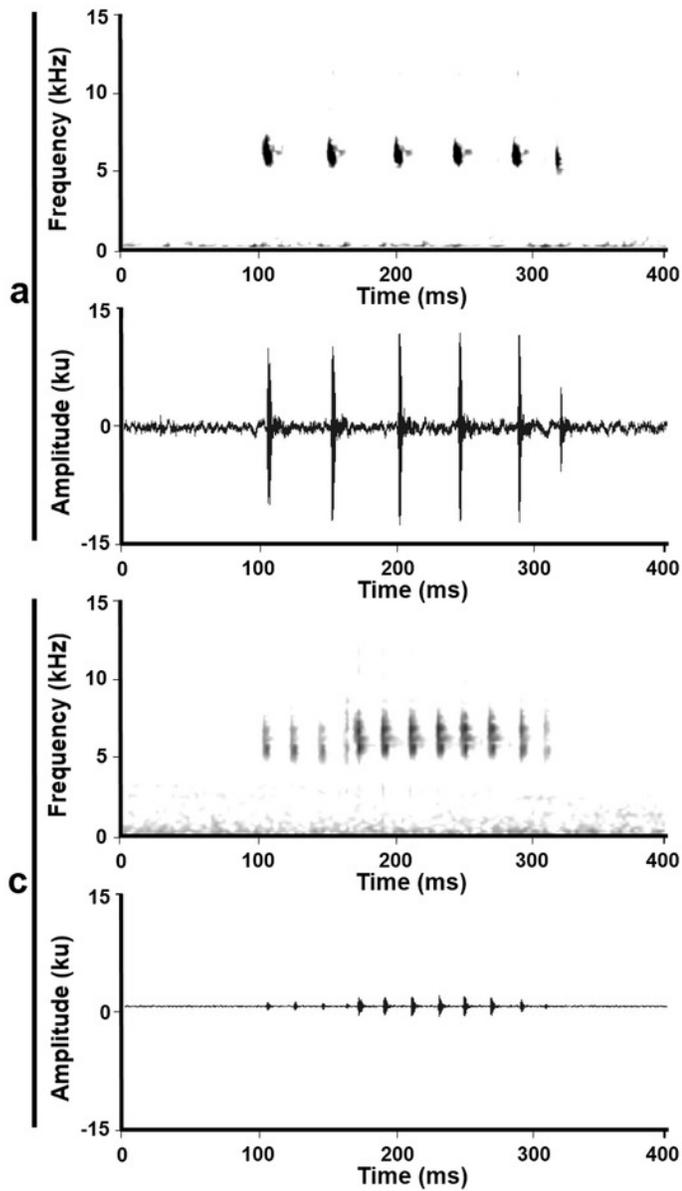



Figure 7

Records of flea toads *Brachycephalus hermogenesi*, *B. sulfuratus*, and of *Brachycephalus* sp.

Figure 7. Records of flea toads *Brachycephalus hermogenesi*, *B. sulfuratus*, and of *Brachycephalus* sp. (*B. hermogenesi* or *B. sulfuratus*), according to the compilation of localities and review of identifications shown in Table 1. We highlighted the southernmost record of *B. hermogenesi* confirmed (1 – Estação Biológica de Boracéia). We also highlight the northernmost confirmed records of *B. sulfuratus* (2 – Núcleo Itutinga-Pilões and 3 – near the Jurupará dam). Abbreviations: RJ = Rio de Janeiro; SP = São Paulo; PR = Paraná; SC = Santa Catarina.