

A review of the diagnosis and geographical distribution 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 records of flea toads that had been identified as "*Brachycephalus sp. nov.*" and *B. hermogenesi* occurring in the same regions, which could suggest the possibility of up to 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 diagnosis of *B. sulfuratus*.

Methods. A critical analysis of the species identity of flea toad specimens attributable to *B. hermogenesi*, *B. sulfuratus*, or to a potentially undescribed species from southeastern and southern Brazil was based either on the analysis of morphology or on their advertisement calls. These analyses include our independent examinations of specimens and, when not possible, examinations of published descriptions. To allow for a consistent comparison of advertisement calls between *B. hermogenesi* and *B. sulfuratus*, we made recordings of both species, including in the type locality of the former. **Results.** We found that morphological and call characters 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 that the reduced number

of notes per call and the presence of only isolated notes in the call of *B. sulfuratus*, as opposed to a high number of notes per call with isolated notes and note groups in the call of *B. hermogenesi*, as the only diagnostic characters between them. Regarding their distributions and based in our assessment, 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. Our revision also revealed that some records previously attributed to *B. hermogenesi* in Rio de Janeiro and north São Paulo represent a distinct, unidentified flea toad that is not *B. sulfuratus*. Both species occur side by side in Corcovado, São Paulo, a locality from where five paratypes of *B. hermogenesi* were obtained. Biogeographic events that might have led to vicariance between *B. hermogenesi* and *B. sulfuratus* are discussed.

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2 **distribution of the recently described flea toad**
3 ***Brachycephalus sulfuratus* in relation to *B.***
4 ***hermogenesi* (Anura: Brachycephalidae)**

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25 **Abstract**

26 **Background.** The flea toad *Brachycephalus sulfuratus* was recently described from southeastern
27 and southern Brazil. In its description, the authors overlooked previous records of flea toads that
28 had been identified as “*Brachycephalus* sp. nov.” and *B. hermogenesi* occurring in the same
29 regions, which could suggest the possibility of up to three flea toads coexisting in southern
30 Brazil. In addition, *B. sulfuratus* is characterized by substantial phenotypic variability, to an
31 extent that compromises its current diagnosis with respect to its congener *B. hermogenesi*.
32 Therefore, the current state-of-affairs regarding the geographical distribution of these two species
33 and the identification of previously known populations is hitherto uncertain. Our goals are to
34 reassess previous records of flea toads attributable to *B. hermogenesi*, *B. sulfuratus*, and
35 “*Brachycephalus* sp. nov.”, considering the description of *B. sulfuratus*, and to review the
36 diagnosis of *B. sulfuratus*.

37 **Methods.** A critical analysis of the species identity of flea toad specimens attributable to *B.*
38 *hermogenesi*, *B. sulfuratus*, or to a potentially undescribed species from southeastern and
39 southern Brazil was based either on the analysis of morphology or on their advertisement calls.
40 These analyses include our independent examinations of specimens and, when not possible,
41 examinations of published descriptions. To allow for a consistent comparison of advertisement
42 calls between *B. hermogenesi* and *B. sulfuratus*, we made recordings of both species, including
43 in the type locality of the former.

44 **Results.** We found that morphological and call characters originally proposed as diagnostic for
45 *B. sulfuratus* in relation to *B. hermogenesi* vary intraspecifically. Live individuals with ventral
46 yellow spots correspond to *B. sulfuratus*; individuals without yellow spots can be either *B.*
47 *sulfuratus* or *B. hermogenesi*. In preservative, they are indistinguishable. Previous records of
48 *Brachycephalus* sp. nov. correspond to *B. sulfuratus*. We propose that the reduced number of
49 notes per call and the presence of only isolated notes in the call of *B. sulfuratus*, as opposed to a
50 high number of notes per call with isolated notes and note groups in the call of *B. hermogenesi*,
51 as the only diagnostic characters between them. Regarding their distributions and based in our
52 assessment, only *B. sulfuratus* occurs in southern Brazil, without any overlap with *B.*
53 *hermogenesi*. There is a narrow gap between the distributions of these species around the
54 southeast of the city of São Paulo. Our revision also revealed that some records previously
55 attributed to *B. hermogenesi* in Rio de Janeiro and north São Paulo represent a distinct,

56 unidentified flea toad that is not *B. sulfuratus*. Both species occur side by side in Corcovado, São
57 Paulo, a locality from where five paratypes of *B. hermogenesi* were obtained. Biogeographic
58 events that might have led to vicariance between *B. hermogenesi* and *B. sulfuratus* are discussed.

59

60

61 **Introduction**

62 The genus *Brachycephalus* Fitzinger, 1826 includes 36 small diurnal anuran species that
63 live in the leaf litter across the Brazilian Atlantic Rainforest (Bornschein, Pie & Teixeira 2019a).
64 Most species present small geographic distributions, restricted to one or few adjacent
65 mountaintops (Pie et al. 2013, Bornschein et al. 2016a, Bornschein, Pie & Teixeira 2019a).
66 *Brachycephalus* has been divided in three phenetic groups, based on body shape and
67 presence/absence of dermal co-ossification (Ribeiro et al. 2015), and presence/absence of *linea*
68 *masculina* (Pie et al 2018a): the *B. ephippium* group, with 12 species distributed from Espírito
69 Santo and Minas Gerais south to São Paulo, southeastern Brazil (Bornschein, Pie & Teixeira
70 2019a); the *B. pernix* group, with 19 species distributed in Paraná and Santa Catarina, southern
71 Brazil (Bornschein, Pie & Teixeira 2019a); and the *B. didactylus* group, with four species
72 commonly known as flea toads and distributed throughout much the Atlantic Forest of Brazil,
73 from Bahia to Santa Catarina, northeastern, southeastern, and southern Brazil (Bornschein, Pie &
74 Teixeira 2019a). Members of the *B. didactylus* species group (*sensu* Ribeiro et al. 2015;
75 Bornschein, Pie & Teixeira 2019a) are distinguished by their leptodactyliform body shape and
76 the absence of dermal ossification and absence of *linea masculina*. The *B. ephippium* species
77 group includes species with bufoniform body shape, presence of dermal ossification and absence
78 of *linea masculina*, and, finally, the *B. pernix* species group includes species equally with
79 bufoniform body shape but without dermal ossification and with *linea masculina* (Ribeiro et al.
80 2015).

81 The first described flea toad species was *B. didactylus*, in 1971 (Izecksohn 1971) as the
82 only member of a new genus, *Psyllophryne*. The second flea toad species, *B. hermogenesi*, was
83 described nearly three decades later, in 1998 (Giaretta & Sawaya 1998), at the time as the second
84 species of the genus *Psyllophryne*. This genus was then synonymized in favor of *Brachycephalus*
85 when it was discovered that this genus also had an omosternum, whose presence until then
86 exclusive in *Psyllophryne* diagnosed that genus in relation to *Brachycephalus* (Kaplan 2002).

87 Recently, other two flea toads were described, namely *B. pulex* (Napoli et al. 2011) and *B.*
88 *sulfuratus* (Condez et al. 2016). Only recently have flea toads been recorded in southern Brazil.
89 The first records were of *B. hermogenesi* to the Reserva Particular do Patrimônio Natural Salto
90 Morato (RPPNSM), municipality of Guaraqueçaba, in the northern coast of Paraná (Pereira et al.
91 2010, Santos-Pereira et al. 2011), and at Colônia Castelhanos, municipality of Guaratuba, in
92 southern Paraná, initially as “*Brachycephalus aff. hermogenesi*” (Cunha et al. 2010) and later as
93 “*B. hermogenesi*” (Oliveira et al. 2011). Shortly thereafter, Pie et al. (2013) published 14
94 localities of a flea toad identified as “*Brachycephalus sp. nov. 1*”, from Paraná and Santa
95 Catarina. These authors also reidentified the record from Colônia Castelhanos as
96 “*Brachycephalus sp. nov. 1*”. Occurrences from RPPNSM of Pereira et al. (2010) and Santos-
97 Pereira et al. (2011) were overlooked by Pie et al (2013). Later, Bornschein et al. (2016a)
98 compiled 18 localities of a flea toad as *Brachycephalus sp. 1.*, including the 14 localities of Pie et
99 al. (2013) treated as “*Brachycephalus sp. nov. 1*”. Bornschein et al. (2016a) also reidentified
100 previous records of the flea toad of the RPPNSM and Colônia Castelhanos as *Brachycephalus*
101 *sp. 1.*

102 After these discoveries, the flea toad *B. sulfuratus* was described in 2016 based on a
103 series of 28 specimens distributed from southern São Paulo to northern Santa Catarina (Condez
104 et al. 2016). However, these authors did not take into account the information available in Pie et
105 al. (2013) and Bornschein et al. (2016a). Rather, Condez et al. (2016) only considered the
106 presence of the flea toad *B. hermogenesi* in Paraná, based on Oliveira et al. (2011). However, the
107 voucher specimen of Oliveira et al. (2011), a single specimen deposited in the Museu de História
108 Natural, Universidade Estadual de Campinas, Campinas (ZUEC 16602), was reidentified by
109 Condez et al. (2016) as *B. sulfuratus*, whereas the remaining records of *B. hermogenesi* in
110 Paraná, from Pereira et al. (2010) and Santos-Pereira et al. (2011), were not considered by
111 Condez et al. (2016).

112 The absence of a nomenclatural review of records of flea toads in southern Brazil can be
113 evidenced by the fact that a single location in Santa Catarina, called Castelo dos Bugres, was
114 recorded as harboring specimens identified as “*Brachycephalus sp. nov. 1*” (Pie et al. 2013), or
115 *Brachycephalus sp. 1.* (Bornschein et al. 2016a), and *B. sulfuratus* (Condez et al. (2016). No
116 analysis has been carried out to ensure that the unidentified species represents *B. sulfuratus*, so
117 that the uncertainty in the identification of some important occurrence records seems to indicate

118 three possible scenarios. First, one could envision that potentially there are three similar species
119 of flea toads in Paraná and Santa Catarina, southern Brazil, namely *B. hermogenesi* (Pereira et al.
120 2010, Santos-Pereira et al. 2011), *Brachycephalus* sp. (Pie et al. 2013, Bornschein et al. 20016a),
121 and *B. sulfuratus* (Condez et al. 2016). Second, records of *B. hermogenesi* in southern Brazil
122 could be erroneous, given that some of these records (Cunha et al. 2010, Oliveira et al. 2011)
123 were assigned to *B. sulfuratus* or “*Brachycephalus* sp. nov.” (Pie et al. 2013, Condez et al. 2016),
124 leading to an expectation that two species might occur in these regions (*B. sulfuratus* and
125 *Brachycephalus* sp.). Third, if the unidentified species of Pie et al. (2013) and Bornschein et al.
126 (2016a) is conspecific of *B. sulfuratus*, there could be a single species of flea toad in southern
127 Brazil (*B. sulfuratus*).

128 Recently, Bornschein, Pie & Teixeira (2019a) reviewed the available occurrence records
129 of flea toads from southeastern and southern Brazil and reverted most of the records of
130 “*Brachycephalus* sp. nov. 1” (Pie et al. 2013), “*Brachycephalus* sp. 1” (Bornschein et al. 2016a),
131 and *B. hermogenesi* from southern Brazil (Pereira et al. 2010, Santos-Pereira et al. 2011, 2016) in
132 favor of *B. sulfuratus*. Some records that could not be adequately reassessed by Bornschein, Pie
133 & Teixeira (2019a) were reverted to “*Brachycephalus* sp. cf. *B. sulfuratus*”, including the
134 records of *B. hermogenesi* from Cunha et al. (2010) and Oliveira et al. (2011). Bornschein, Pie &
135 Teixeira (2019a) disregarded the possibility of a third unnamed species of flea toad in southern
136 Brazil, but one question remains: the proper identification of *B. sulfuratus* and *B. hermogenesi*.
137 In this sense, the identification criteria used by Bornschein, Pie & Teixeira (2019a) to reevaluate
138 the records of flea toads were not indicated. In addition, there may still be uncertainty in the
139 identification of flea toads by other authors, as records of *B. hermogenesi* in southern Brazil
140 continue to be published (Santos-Pereira et al. 2016, 2018, Leivas et al. 2018). Given this
141 uncertainty, the aim of this study is to reanalyze the diagnostic morphological characters used to
142 distinguish *B. sulfuratus* from *B. hermogenesi* and redefine their geographical distributions and
143 distributional limits.

144

145 **Materials & Methods**

146 The critical analysis of the species identity of specimens attributable to *Brachycephalus*
147 *hermogenesi*, *B. sulfuratus*, and to a potentially undescribed flea toad from southeastern and
148 southern Brazil provided in our study was based either on the analysis of their morphology or on

149 their advertisement calls. We looked for records in museum specimens, in acoustic collections,
150 and in the literature. The analyzed museum collections include Museu de História Natural Capão
151 da Imbuia (MHNCI), Curitiba, Paraná, Brazil, Coleção Herpetológica do Departamento de
152 Zoologia (DZUP), Universidade Federal do Paraná, Curitiba, Paraná, Brazil, and Museu de
153 História Natural (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. The
154 sound collection analyzed include MHCNI, Xeno-Canto sound collection (www.xeno-canto.org),
155 and Fonoteca Neotropical Jacques Vieliard (FNJV; <https://www2.ib.unicamp.br/fnjv/>).

156 The analyses began by the assessment of the original diagnosis of *B. sulfuratus* (Condez
157 et al. 2016). We looked for the proposed diagnostic characters in museum specimens, calls,
158 sources provided in the literature, and our own photographs of live specimens. Given that this
159 procedure uncovered ambiguity in the proposed diagnostic characters to separate *B. sulfuratus*
160 from *B. hermogenesi*, we sought for new characters that could be useful to distinguish them.
161 New distinctive characters were then erected as diagnostic characters, acting in accordance of the
162 Recommendation 13A of the International Code of Zoological Nomenclature
163 (<http://www.iczn.org/>).

164 When comparing the calls between *B. sulfuratus* and *B. hermogenesi*, we noticed that the
165 calls of *B. hermogenesi* described by Verdade et al. (2008) were from a site 112 km distant in a
166 straight line from the type locality of this species (Giaretta & Sawaya 1998). As this distance is
167 considerable in relation to distances between other species of the genus (Pie et al. 2013,
168 Bornschein et al. 2016a), we made additional recordings in the type localities of *B. hermogenesi*
169 (Núcleo Picinguaba and Corcovado; Giaretta & Sawaya [1998]) and in the locality where
170 Verdade et al. (2008) described the calls of this species (Estação Biológica de Boracéia), as well
171 as in other locations of records of *B. hermogenesi* (e.g., Parque Natural Municipal Nascentes de
172 Paranapiacaba; Verdade, Rodrigues & Pavan [2009]).

173 Our recordings, deposited in the MHNCI, were made using analogical (Sony TCM–
174 5000EV) and digital (Marantz PMD660, Sony PCM–D50 and PCM–M10, and Tascam DR44-
175 WL) devices, with Sennheiser ME 66 and ME 67 microphones. Analogical recordings were
176 digitized at 44.1 kHz and 16 bit using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, New
177 York, USA). Digital recordings were made equally with sampling frequency rate of 44.1 kHz
178 and 16-bit resolution. We analyzed calls under note-centered approach (Köhler et al. 2017), as
179 Bornschein et al. (2018, 2019b) and Pie et al. (2018a). The definition of call used by Condez et

180 al. (2016) is the one defined by Köhler et al. (2017) as note-centered approach, in which several
181 notes emitted continuously over a period represent the call of the species, in contrast to the call-
182 centered approach, in which each note represents a call. Remaining call terminology used were
183 those of Bornschein et al. (2018). Spectrograms were produced using Seewave package, version
184 2.1.6 (Suer, Aubin & Simonis 2008), in R. 4.0.3 (R core team 2018). We made adjustments in
185 contrast and brightness with the intention of lightening the images and best highlighting the
186 pulses. We chose not to noise-filter the spectrograms to avoid eliminating sound characters.

187 We also included unpublished records in an analysis of *B. sulfuratus* and *B. hermogenesi*,
188 vouchered with specimens collected and deposited in the MHNCI. Collection permits were
189 issued by ICMBIO (10.500, 22470–2/1911426, and 55918–1). Geographical coordinates are
190 based on the WGS84 datum. Elevations for literature records and author’s records were obtained
191 from Google Earth, after plotting the location point (Bornschein et al. 2016a).

192 Finally, we generated a phylogenetic tree based on a concatenated dataset of all
193 mitochondrial 12S and 16S mitochondrial loci available on GenBank for specimens of the *B.*
194 *didactylus* species group (Table S1). Sequences were aligned using MAFFT (Kato et al. 2002)
195 and analyzed under a single GTRGAMMA model in RAxML 8.2.12 (Stamatakis 2014). Support
196 values were obtained by bootstrapping using the automatic halting option. The final tree was
197 rooted by its midpoint. Whenever possible, the corresponding localities available on their
198 GenBank records were standardized based on the toponyms indicated in Table 1.

199

200 **Results**

201 Our list of specimens and calls analyzed of *B. sulfuratus* and *B. hermogenesi*, per locality, is
202 provided in Table 1 and Appendix 1.

203

204 *Diagnosis between Brachycephalus sulfuratus and B. hermogenesi*

205 Condez et al. (2016) indicated three morphological characters to diagnose *B. sulfuratus*
206 from the very similar *B. hermogenesi*: 1) It “differs from... *B. hermogenesi*... by having (in life)
207 yellow blotches on the ventral surfaces of the throat, chest, arms, and forearms” (Condez et al.
208 2016: 43, 50); 2) a more evident “singular inverted v-shaped mark around the cloacal region in
209 ventral view”, that is “generally rounded and not ornamented in... *B. hermogenesi*...” (Condez et
210 al. 2016: 43, 50); and 3) the presence of an “m-shaped mark around the cloacal opening [in

211 dorsal view], which is... not clearly defined in *B. hermogenesi*” (Condez et al. 2016: 50).
212 Specimens of *B. sulfuratus* collected in southern São Paulo, Paraná, and Santa Catarina (Table 1)
213 have revealed that the yellow spots on the ventral surface of this species might still be present, on
214 the throat, chest, arms, and/or forearms, but not necessarily in all of these body parts. In addition,
215 the amount of yellow is highly variable, being virtually absent in some individuals (Fig. 1).
216 Moreover, in three individuals of *B. sulfuratus* collected by us in the state of São Paulo (near the
217 Jurupará dam; Table 1), two do not present yellow spots on the ventral surface (see one of them
218 in Fig. 11), being identified as *B. sulfuratus* by their advertisement calls (MHNCI 123–5; see
219 below). The inverted v-shaped mark can be absent in individuals of *B. sulfuratus* (compare Fig.
220 6A of Condez et al. [2016] and Fig. 1a). Additionally, the use of this character is inconsistent as
221 a diagnosis from *B. hermogenesi* on the actual original description: “the ventral inverted v-
222 shaped mark... are shared among the four species [*B. sulfuratus*, *B. hermogenesi*, *B. didactylus*,
223 and *B. pulex*]” (Condez et al. 2016: 50). Also, while describing the variation on the type series,
224 the authors stated that “some individuals present the inverted v-shaped around the cloacal
225 region” (Condez et al. 2016: 46). Finally, the “m-shaped mark around the cloacal opening” was
226 also mischaracterized as a diagnostic character on the actual original description of the species
227 (Condez et al. 2016: 50): “The m-shaped mark... are shared among the four species [*B.*
228 *sulfuratus*, *B. hermogenesi*, *B. didactylus*, and *B. pulex*].”

229 Currently, there are no unique morphological character that could differentiate either live
230 or preserved specimens (Fig. 2) for *B. sulfuratus* from *B. hermogenesi*. However, for
231 identification purposes, we considered individuals with yellow spots on their ventral side as *B.*
232 *sulfuratus*, whereas individuals without yellow spots could be either *B. sulfuratus* or *B.*
233 *hermogenesi*. It is important to note that specimens with yellow spots of *B. sulfuratus* must be
234 observed in life because in the preservative the change in color prevents separate them in relation
235 to specimens of *B. hermogenesi*.

236 In addition to morphological characters, Condez et al. (2016: 43) included in the
237 diagnosis of *B. sulfuratus* the following parameters of the advertisement call: “advertisement call
238 long, composed of a set of 4–7 high-frequency notes (6.2–7.2 kHz) repeated regularly.” In the
239 section “Comparisons with other species”, Condez et al. (2016: 50) stating that “The
240 advertisement call of *B. hermogenesi* is the most similar to the new species [*B. sulfuratus*], being
241 quite similar in frequency (dominant frequency = 6.8 kHz), which are the highest recorded for

242 the genus. However, the advertisement call of *B. hermogenesi* can be simple or composed of 2–7
243 shorter notes with 1–3 pulses (Verdade et al. 2008).” In summary, the indicated values overlap
244 with those of *B. hermogenesi*. The advertisement call of *B. hermogenesi* is composed of 1–7
245 notes, whereas that of *B. sulfuratus* is composed of 4–7 notes and the amplitude of the dominant
246 frequency of *B. hermogenesi* (6.8 kHz) is within the range of *B. sulfuratus* (6.2–7.2).

247 These call descriptions do not allow for a reasonable comparison because they are not
248 necessarily considering the same phenomenon. That is, when it was mentioned that *B.*
249 *hermogenesi* call can be simple or composed (Verdade et al. 2008), it was being said, according
250 to the note-centered approach (Köhler et al. 2017), that its call can have isolated notes or note
251 groups, but the total number of notes in the entire *B. hermogenesi* call was not mentioned. In
252 turn, when mentioning that the *B. sulfuratus* call has 4–7 notes (Condez et al. 2016), this
253 represents the total number of notes in the call under note-centered approach (*sensu* Köhler et al.
254 2017) and that all are isolated notes (see Condez et al. 2016). This is one notorious distinctions
255 between the calls of *B. sulfuratus* and *B. hermogenesi*: the former presents only isolated notes
256 (Fig. 3) and the latter presents isolated notes and note groups (Fig. 4), with note groups having
257 2–7 notes, according Verdade et al. (2008), or 2–6 notes, according to our samples (Table 2–3).
258 Other particularities of the call of *B. hermogenesi* in relation to the one of *B. sulfuratus* is the
259 high number of notes per call (≥ 24) and the presence of “attenuated notes” (Fig. 4F), while in
260 the latter the call has few notes per call (≤ 8) without attenuated notes (Table 2–3). We
261 introduced attenuated notes as a new parameter, provisionally named, to describe weak notes
262 issued before the notes along the calls of *B. hermogenesi*, more strongly perceived in
263 spectrograms than in oscillograms (Fig. 4F). Due to this attenuated condition and difficulty in
264 perceiving these notes, we did not include them as being part of note groups. We detect the
265 presence of one attenuated note emitted before notes from both isolated notes and note groups,
266 all of which from only three calls (MHNCI 167, MHNCI 183, MHNCI 215; Table 2).

267 Regarding number of pulses per note, *B. sulfuratus* was described as having 7–11
268 (Condez et al. 2016), but we found 2–14 (Table 1). Verdade et al. (2008) have not described the
269 number of pulses of notes of *B. hermogenesi*, as stated by Condez et al. (2016:50; “with 1–3
270 pulses”). However, as we demonstrated, the number of pulses per note for *B. hermogenesi* is
271 indeed 1–3 (Table 2). We noticed that the calls of individuals of two localities previously
272 attributable of *B. hermogenesi* differs from the descriptions above, by having notes with up to 16

273 pulses and two or rarely three notes in note groups (Fig. 5; Table 1–3). These calls were from
274 Trilha do Corisco, municipality of Paraty, Rio de Janeiro state, and Corcovado, municipality of
275 Ubatuba, São Paulo state (see below; Table 1).

276 We erect as a diagnosis between *B. sulfuratus* and *B. hermogenesi* the few number of
277 notes per call (≤ 8) with only isolated notes of *B. sulfuratus*, while in *B. hermogenesi* the
278 advertisement call has a high number of notes (≥ 24) with the presence of isolated notes and note
279 groups (see Table 3). In depth analysis of spectral and temporal parameters of the calls of *B.*
280 *hermogenesi* will possibly bring other diagnostic parameters, as possibly the note rate, focus of a
281 specific study in the future.

282

283 *Geographical occurrence records of Brachycephalus sulfuratus and B. hermogenesi*

284 Based on our review of the 14 occurrence records of “*Brachycephalus* sp. nov. 1” from
285 Pie et al. (2013), we conclude that the vouchered records correspond to *B. sulfuratus* (Table 1).
286 Specimens from Pie et al. (2013) have yellow spots on their ventral side and advertisement calls
287 with few notes and only isolated notes (as above). We treated unvouchered records of Pie et al.
288 (2013) as *Brachycephalus* sp. (being probably *B. sulfuratus*; Table 1), with the exception of
289 Castelo dos Bugres, due to the fact that, years later, Condez et al. (2016) collected specimens
290 there, confirming the species’ identity as *B. sulfuratus*. We also determined previously
291 unidentified *Brachycephalus* records from “Apiaí”, “Caratuval”, “Corvo”, and “Fazenda Thalia”
292 (Firkowski et al. 2016) as *B. sulfuratus* (Table 1) based on vouchered identification (specimens
293 had yellow spots on their ventral region - see Fig. 1). The records of “*Brachycephalus* sp. 1”
294 from Bornschein et al. (2016a) correspond to *B. sulfuratus* (Table 1): all but one of them are the
295 same records as those records presented in Pie et al. (2013) and Firkowski et al. (2016) and were
296 re-identified above. The only exception is the record of “*Brachycephalus* sp. 1” from RPPNSM,
297 municipality of Guaraqueçaba, Paraná, identified as *B. sulfuratus* (Table 1) based on their call
298 structure, with few notes and only isolated notes (MHNCI 133; Table 2). On the basis of this
299 record, we reverted in favor of *B. sulfuratus* all other records of *B. hermogenesi* at RPPNSM
300 (Pereira et al. 2010, Santos-Pereira et al. 2011, 2016, 2018, Leivas et al. 2018; Table 1).

301 Some previous studies reporting “*Brachycephalus hermogenesi*” (Giaretta and Sawaya
302 1998, Dixo and Verdade 2006, Verdade et al. 2008, Condez, Sawaya & Dixo 2009, Verdade,
303 Rodrigues & Pavan 2009) from São Paulo do not provide enough morphological evidence or

304 other details to allow us to reassess their original identification by us (Table 1; Fig. 6). Therefore,
305 we propose that these identifications should be reverted as *Brachycephalus* sp. (being *B.*
306 *hermogenesi* or *B. sulfuratus*). One of these records reverted to *Brachycephalus* sp. involves “*B.*
307 *hermogenesi*” from the municipality of Piedade, state of São Paulo, of Clemente-Carvalho et al.
308 (2011), whose genetic sequence is deposited in GenBank (HQ435682.1 and HQ435709.1; Table
309 1). The corresponding voucher was obtained by T. H. Condez (pers. comm., 2016) in her study
310 on the same location (Condez, Sawaya & Dixo 2009). Phylogenetic analyses suggest that it
311 might actually be *B. sulfuratus*, which was placed on the tree together with a specimen from the
312 Municipality of Barra do Turvo, in an early-diverging branch of the *B. sulfuratus* clade on the
313 tree (Fig. 7).

314 There are some specimens in the original description of *B. sulfuratus* (Condez et al.
315 2016), from six different localities, cited as “*B. hermogenesi*” in the appendix. It is possible that
316 all of these records were identified based on preserved material, which does not allow for proper
317 identification, as indicated above. Therefore, we also propose that those identifications should be
318 considered as *Brachycephalus* sp. (being *B. hermogenesi* or *B. sulfuratus*; Table 1; see also
319 Bornschein et al. [2016a]).

320 There is a particular specimen, ZUEC 16602 (see introduction), also examined by us,
321 collected in the state of Paraná, that was first identified as “*Brachycephalus* aff. *hermogenesi*”
322 (Cunha et al. (2010), later as “*B. hermogenesi*” (Oliveira et al. (2011), “*Brachycephalus* sp. nov.
323 1” (Pie et al. 2013), “*Brachycephalus* sp. 1” (Bornschein et al. 2016a), and, finally as “*B.*
324 *sulfuratus*” (Condez et al. 2016). There is also the possibility that this specimen may not have
325 been properly analyzed with respect to coloration in life, preventing the precise identification.
326 Therefore, we also propose that this identification should be reverted to *Brachycephalus* sp.
327 (being probably indeed *B. sulfuratus*; Table 1).

328 Advertisement calls analyzed of samples from Trilha do Corisco and Corcovado (in
329 part.), two localities previously considered as occurrence of *B. hermogenesi* (Giaretta and
330 Sawaya 1998, Verdade et al. 2008, Pie et al. 2013, 2018a, Bornschein et al. 2016a, Bornschein,
331 Pie & Teixeira 2019a; Table 1), have reveal substantial differences to made us to considerer that
332 represents other species, unidentified, but not *B. sulfuratus* (Table 2–3). The call from this third
333 species has two notes forming note groups, exceptionally three, and includes notes with a high
334 number of pulses (up to 16; Table 2–3). Specimens we collected at Corcovado (MHNCI 10823–

335 5) confirmed that they belong to the *B. didactylus* species group (*sensu* Pie et al 2018a). Three
336 adjacent locations based on unvouchered records, Morro Cuscuzeiro. Morro do Corcovado, and
337 municipality of Paraty (Table 1), were referred to as *Brachycephalus* sp., perhaps
338 *Brachycephalus* sp. from Trilha do Corisco and Corcovado (Table 1; Fig. 6). This third flea toad
339 *Brachycephalus* sp. occurs in sympatry with *B. hermogenesi* in Corcovado, as proved by our
340 recordings (*B. hermogenesi*: MHNCI 165; *Brachycephalus* sp.: MHNCI 165–205). The
341 phylogenetic analysis revealed that the specimen from Municipality of Paraibuna is indeed *B.*
342 *hermogenesi* (Table 1), being placed with other specimens of the species collected at the type
343 locality (Fig. 7).

344

345 Discussion

346 Based on our analyses, characters previously used as diagnostic for *B. sulfuratus* were
347 quite variable and overlapped with those of *B. hermogenesi*. Moreover, the examination of
348 specimens deposited in the collections MHNCI and ZUEC support this claim. Currently,
349 differences in the call structure – number of notes per call and presence/absence of note groups -
350 is proposed here as the only available sources of evidence supporting the distinction between *B.*
351 *sulfuratus* and *B. hermogenesi*. Even in the field its advertisement calls are very distinct to the
352 human ear and easily distinguishable. The advertisement calls of *B. sulfuratus* sounds like a
353 “trííííí, trííííí, trííííí, trííííí, tríííí”, whereas the calls of *B. hermogenesi* from its type locality
354 sound like a “tíc, tíc, tíc, tíc-tíc, tíc-tíc-tíc, tíc-tíc-tíc, tíc-tíc-tíc, ...”. These transliterations
355 represent isolated notes or note groups (each note separated by comma and note group by
356 hyphen) with distinct durations (= transliteration size) related to the number of notes in the call.
357 This diagnosis between *B. sulfuratus* and *B. hermogenesi* is only feasible under the note-centered
358 approach. Considering their calls under the call-centered approach, there would be no diagnosis
359 to be proposed between them at this moment, because each note would represent a call (Table 3).
360 To the best of our knowledge, this is the first case in which the diagnosis between species of any
361 *Brachycephalus* is made solely by characters of their advertisement call.

362 The first notes emitted from an advertising call by *B. hermogenesi* are usually hardly noticed
363 in the recording and equally difficult to hear in the field. This is the reason why we rarely record
364 the first emissions and many recordings recorded the advertisement call already in progress.
365 These weak starting notes of an advertisement call were called warming notes (Bornschein et al.

366 2018; Table 3), assuming that they would reflect the individual's preparation process to the level
367 of excitement required for the issuance of "typical" strongest notes. Like warming notes,
368 attenuated notes could prepare the individual to issue the immediately subsequent notes at a
369 higher level of arousal.

370 The recognition of the existence of warming notes and attenuated notes, as well as the
371 existence of note groups for understanding the richness of characters in *Brachycephalus* calls
372 (see also above), consolidate the benefit of the note-centered approach over the call-centered
373 approach in describing calls of species of this genus (Bornschein *et al.* 2018). The note-centered
374 approach way for description the calls of *B. hermogenesi* also reinforces the hypothesis of
375 complexity increment along note emissions (Bornschein *et al.* 2018), with the incorporation of
376 note groups during the call emission. These structural particularities would not be perceived
377 under the call-center approach. Under this approach, they would be perceived as a simple
378 intraspecific variation in calls

379 The advertisement calls of *B. hermogenesi* show the same pattern as species from the *B.*
380 *pernix* group (Bornschein *et al.* 2018, 2019b, Pie *et al.* 2018a, Monteiro *et al.* 2018a, 2018b),
381 which includes most species of southern Brazil, whereas the call of *B. sulfuratus* resembles the
382 call of *B. vertebralis* (MRB, unpublished data), for example, from the *B. ephippium* group, which
383 includes most species from the state of São Paulo to the north up to Espírito Santo and Minas
384 Gerais.

385 We now confirm the absence of occurrence records of *B. hermogenesi* in southern Brazil
386 and the presence of *B. sulfuratus* as far north as the east of São Paulo city, only 25 km in straight
387 line from the southernmost site of a confirmed record of *B. hermogenesi* (Parque Natural
388 Municipal Nascentes de Paranapiacaba; Fig.6; Table 1). Most unidentified records (Table 1)
389 represent one or the other of these two species. In fact, it is likely that in southern Brazil only the
390 flea toad *B. sulfuratus* occurs. In this region, our research group has been working with two
391 anuran genera (*Brachycephalus* and *Melanophryniscus*) since 2009, focusing on their
392 distribution, ecology and conservation (e.g., Pie *et al.* 2013, Bornschein *et al.* 2015, 2016a,
393 Bornschein, Pie & Teixeira 2019a), and thus are particularly aware of *Brachycephalus* calls
394 wherever we do field work and yet we never recorded *B. hermogenesi* calls in southern Brazil.

395 In addition, we also underscore the absence of records of *B. sulfuratus* in northern Santa
396 Catarina in some well sampled localities. For example, we obtained no records for *B. sulfuratus*

397 in Morro Boa Vista (26°30'58"S, 49°03'14"W), on the border between the municipalities of
398 Jaraguá do Sul and Massaranduba, where we described *B. albolineatus* (Bornschein et al.
399 2016b), Morro do Baú (26°47'58"S, 48°55'47"W), municipality of Ilhota, and Morro Braço da
400 Onça (26°44'58"S, 48°55'41"W), municipality of Luiz Alves, where we report *B. fuscolineatus*
401 (Ribeiro et al. 2015, Bornschein, Teixeira & Ribeiro 2019c), Morro do Cachorro (26°46'42"S,
402 49°01'57"W), on the border between the municipalities of Blumenau, Gaspar, and Luiz Alves,
403 where we described *B. boticario* (Ribeiro et al. 2015), and Morro Santo Anjo (26°37'41"S,
404 48°55'50"W), municipality of Massaranduba, where we described *B. mirissimus* (Pie et al.
405 2018a). It is possible that the southern limit of the geographical distribution of *B. sulfuratus*
406 occurs at the Morro do Garrafão (Table 1).

407 Contrary to what is found in southern Brazil, the distribution of flea toads in the states of
408 São Paul and southern Rio de Janeiro are poorly known. Our findings indicate the presence of a
409 third flea toad species at the border between São Paulo and Rio de Janeiro states, at least
410 occurring in Corcovado, São Paulo, and Trilha do Corisco, municipality of Paraty, Rio de
411 Janeiro. Corcovado, however, is one locality of paratypes of *B. hermogenesi* and Paraty were
412 also cited as a place of occurrence of *B. hermogenesi* in the original description of this species
413 (Giaretta & Sawaya 1998). The species of the *B. didactylus* group that occurs closest to Rio de
414 Janeiro/São Paulo border, excluding *B. hermogenesi* and *B. sulfuratus*, is *B. didactylus*, in Vila
415 Dois Rios, Ilha Grande, municipality of Angra dos Reis, Rio de Janeiro (Bornschein, Pie &
416 Teixeira 2019a). The Trilha do Corisco is distant from Vila Dois Rios 59 km in a straight line.

417 As we demonstrate in our analyses, there is no confirmed overlap in the distribution of *B.*
418 *hermogenesi* and *B. sulfuratus*, and their geographical replacement occurs in southeastern of São
419 Paulo city, without apparent barriers. There are other examples of discontinuity of the
420 geographical distribution between congeneric species throughout the Atlantic Forest from
421 southeastern to southern Brazil in southeastern São Paulo city, as in the montane bird *Scytalopus*
422 *speluncae* (taxonomy *sensu* Maurício et al. [2010]). Maurício (2005) stated that populations of *S.*
423 *speluncae* from the southeastern of the city of São Paulo to the south of the species distribution
424 represent a distinct species yet to be named, and he treated it as “Southern *Scytalopus speluncae*”
425 (this scenario of southern population of this bird as a new species was supported by other studies
426 [Bornschein et al. 2007, Mata et al. 2009, Maurício et al. 2014, Pulido-Santacruz et al. 2016]). In
427 the region around the southeastern of São Paulo city, cases of hybridization of subspecies or

428 lineages have been reported for at least four species of birds (Pinto 1941, Silva and Stotz 1992,
429 Cabanne, Santos & Miyaki 2007, D’Horta et al. 2011; see also Dantas et al. [2015]). In the state
430 of São Paulo there is another discontinuity which is associated with intraspecific differentiation
431 or even sister species of frogs (Fitzpatrick et al. 2009, Thomé et al. 2010, Amaro et al. 2012) and
432 snakes (Grazziotin et al. 2006).

433 The correspondence between the distribution of the congeneric species in question with
434 the limits of the Serra do Mar is intriguing, given that during the last 20 million years there was
435 no obvious uplift in the region (Gontijo-Pascutti et al. 2012). This time scale is considerably
436 older than the inferred cladogenesis events and therefore geological processes could not have
437 been the primary cause of their divergence, given that *Brachycephalus* toads and *Scytalopus*
438 birds of São Paulo, Paraná, and Santa Catarina originated less than 2–5 million years ago (Pie et
439 al. [2018b] and Pulido-Santacruz et al. [2016], respectively). Likewise, recent neotectonic
440 activities (Late Pleistocene-Holocene) are restricted to the faults and stress regimes (Hasui 1990,
441 Saadi, 1993, Riccomini and Assumpção 1999) and, therefore, also could not have generated the
442 diversification pattern of widely distributed terrestrial species. It is important to note that Thomé
443 et al. (2010), studying the toad *Rhinella crucifer* from the eastern portion of Brazil, associate one
444 genetic break found in eastern São Paulo to neotectonic barriers, specifically the Cubatão shear
445 zone and the Guapiara lineament. However, these are ancient geotectonic activities, from
446 Proterozoic to Cambrian (with Phanerozoic reactivation) and Mesozoic, respectively (Ferreira et
447 al. 1981, Sadowski 1990, Almeida and Carneiro, 1998; see also Riccomini and Assumpção
448 1999). In addition, studies have proposed speciation by vicariance caused by relatively recent
449 events, such as river barriers (e.g., Amaral et al. 2013), sea level variation (Grazziotin et al. 2006,
450 Fitzpatrick et al. 2009), and forest refugia (e.g., Fitzpatrick et al. 2009, Thomé et al. 2010,
451 D’Horta et al. 2011, Amaral et al. 2013). The largest river around the disruption of the
452 geographical distribution of *B. sulfuratus* and *B. hermogenesi* is the Rio Ribeira do Iguape,
453 which intersects the Serra do Mar between São Paulo and Paraná States by continued erosive
454 retreat (Almeida and Carneiro 1998). Alternatively, the disruption of the Serra do Mar in that
455 region originated from a tectonic depression associated with the asymmetric graben of the Sete
456 Barras or Ribeira de Iguape (Melo et al. 1989, Gontijo-Pascutti et al. 2012). However, the
457 formation of the present configuration of the Serra do Mar did not lead to isolation, given that *B.*
458 *sulfuratus* occurs on both banks of the Ribeira do Iguape river. It is plausible that the origin of *B.*

459 *sulfuratus* and *B. hermogenesi*, as well as the other examples mentioned above, might have
460 resulted from climatic variations that promoted vicariance by forest cover disruption followed by
461 the recovery of forest cover, presumably leading to secondary contact.

462 The region in the state of São Paulo, around the southeastern São Paulo city, should be
463 further investigated. Records of flea toads in this region could be obtained as background sound
464 in recordings of birds (e.g., recordings deposited in databases such as www.xeno-canto.org and
465 www.wikiaves.com.br; Table 1). Verdade et al. (2008) made a similar suggestion: to search for
466 records of *B. hermogenesi* in the background of recordings of birds from the Estação Biológica
467 de Boracéia, in the case one wants to seek previous records of this flea toad in this highly
468 sampled locality. As examples, calls of *B. sulfuratus* in Parque Estadual Intervales, municipality
469 of Iporanga, state of São Paulo (Table 1), can be heard in recordings of the birds *Merulaxis ater*
470 (XC80463 and XC18179) and *Eleoscytalopus indigoticus* (XC75544; available at [www.xeno-](http://www.xeno-canto.org)
471 [canto.org](http://www.xeno-canto.org)), and calls of *B. hermogenesi* in Núcleo Santa Virgínea, Parque Estadual da Serra do
472 Mar, municipality of São Luiz do Paraitinga, São Paulo, can be heard in a recording of *E.*
473 *indigoticus* (XC253045; Table 1).

474 We underscore the importance of continuous scrutiny of the distribution and
475 advertisement call analysis of *B. sulfuratus* and *B. hermogenesi*. The advertisement calls of *B.*
476 *hermogenesi* need to be redescribed (see Pie et al. 2018a:12) and a better understanding of the
477 geographical limits between this species and *B. sulfuratus* can elucidate distribution patterns and
478 potentially detect cases of sympatry. To date, the occurrence of *B. hermogenesi* and
479 *Brachycephalus* sp. (other than *B. sulfuratus* and *B. hermogenesi*) at Corcovado, São Paulo, is
480 the only confirmed case of sympatry between species of *Brachycephalus* in the same group.
481 Other cases of sympatry include *Brachycephalus* from distinct groups (*B. pernix* and *B.*
482 *didactylus* groups and *B. ephippium* and *B. didactylus* groups; Bornschein et al. [2016a],
483 Bornschein, Pie & Teixeira [2019a]). Even in sympatry, the differences between the calls of *B.*
484 *hermogenesi* and *Brachycephalus* sp. and between *B. hermogenesi* and *B. sulfuratus* are
485 substantial and could provide pre-zygotic isolation. Although some species in the *B. ephippium*
486 group are additively insensitive to the own advertisement call (Goutte et al. 2017), which would
487 suggest loss of active selection pressure and variation maintained by inertia, it must be
488 considered that this scenario may not apply to the other groups (Monteiro et al. 2018a) and, also,
489 that the species may actively perceive call emissions through vibrations in other body receptors.

490

491 **Conclusions**

492 *Brachycephalus sulfuratus* differs from *B. hermogenesi* only by its advertisement calls;
493 other morphological characters previously suggested to distinguished *B. sulfuratus* from *B.*
494 *hermogenesi* are extremely variable and show overlap between these two species. The
495 advertisement calls of these species differ greatly from each other and can be easily recognized
496 by the human ear in the field. *Brachycephalus sulfuratus* presents few notes per call with only
497 isolated notes and *B. hermogenesi* present high number of notes per call with isolated notes and
498 note groups. The advertisement calls of *B. sulfuratus* resemble those of species of the *B.*
499 *ephippium* species group, whereas the calls of *B. hermogenesi* resemble those of the *B. pernix*
500 species group. Understanding the evolution of these advertisement calls should require a more
501 in-depth investigation.

502 All previous records of *B. hermogenesi* from southern Brazil should instead be
503 considered as *B. sulfuratus*, in a possibly cascading error resulting from the inadequate revision
504 of the records prior to the description of *B. sulfuratus* (Condez et al. 2016). A large region in the
505 south of the state of São Paulo needs to be further investigated to confirm the presence of *B.*
506 *hermogenesi*; the previous records were reverted to *Brachycephalus* sp. *Brachycephalus*
507 *sulfuratus* is distributed much further north than previously thought and it is possible that
508 sympatry with *B. hermogenesi* may occur in the southeast of the city of São Paulo. This region in
509 the southeast of São Paulo is particularly interesting because many species of different taxa have
510 their range limits there. The biogeographic explanation of this pattern seems to be limited to the
511 past distribution of forest patches, which could have been previously isolated and are now
512 distributed continuously, allowing possible secondary contact of species.

513 The *B. hermogenesi* type series possibly includes a second species of flea toad, not yet
514 identified. This situation involves a locality of a *B. hermogenesi* paratype, and probably not the
515 holotype. Therefore, there is no evidence, at this moment, to suspect the name *B. hermogenesi* as
516 a possible synonym for *B. didactylus*. It is necessary to deepen the field studies to identify the
517 local populations and to clarify the limits of the geographic distribution, as well as to review the
518 identification of museum material, including the type series of *B. hermogenesi*.

519 Phylogenetic analysis provided evidence that at least *B. sulfuratus* probably includes
520 more than one species under this name, although this species, as presently defined, has a similar

521 calling pattern in its wide geographical distribution, from southeastern São Paulo to Santa
522 Catarina (Table 1; Fig. 6). In parallel, our *B. hermogenesi* call analyses provided the first
523 association of a call pattern across the geographic distribution of this species (Table 1; Fig. 6),
524 but this does not mean that only one species is necessarily included under this name, because
525 distinct species of *Brachycephalus* may have indistinct calls (Pie et al. 2018a.). Combined with
526 the fact that the *B. didactylus* group includes cryptic species, difficult or even impossible to
527 identify in preservative, that occur or can occur locally in sympathy, we recommend a solid and
528 broad review of the taxonomy of the group based on own analyses of large series of specimens
529 and calls.

530

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535

536 **References**

- 537 Almeida FFM de & Carneiro C dal R. 1998. Origem e evolução da Serra do Mar. Rev Bras Geoc
538 28: 135-150.
- 539 Amaral FR do, Patrick KA, Edwards SV & Miyaki CY. 2013. Multilocus tests of Pleistocene
540 refugia and ancient divergence in a pair of Atlantic Forest antbirds (*Myrmeciza*). Mol Ecol
541 22: 3996-4013.
- 542 Amaro RC, Rodrigues MT, Yonenaga-Yassuda Y & Carnaval AC. 2012. Demographic
543 processes in the montane Atlantic rainforest: molecular and cytogenetic evidence from the
544 endemic frog *Proceratophrys boiei*. Mol Phylogenet Evol 62: 880-888.
- 545 Bornschein MR, Firkowski CR, Baldo D, Ribeiro LF, Belmonte-Lopes R, Corrêa L, Morato
546 SAA & Pie MR. 2015. Three new species of phytotelm-breeding *Melanophryniscus* from
547 the Atlantic Rainforest of southern Brazil (Anura: Bufonidae). PLoS ONE 10: e0142791.
- 548 Bornschein, MR, Firkowski, CR, Belmonte-Lopes, R, Corrêa, L, Ribeiro, LF, Morato, SAA,
549 Antoniazzi-Jr., RL, Reinert, BL, Meyer, ALS, Cini, FA & Pie, MR. 2016a. Geographic and
550 altitudinal distribution of *Brachycephalus* Fitzinger (Anura: Brachycephalidae) endemic to
551 the Brazilian Atlantic Rainforest. PeerJ 4: e2490.

- 552 Bornschein MR, Ribeiro LF, Blackburn DC, Stanley EL & Pie MR. 2016b. A new species of
553 *Brachycephalus* (Anura: Brachycephalidae) from Santa Catarina, southern Brazil. PeerJ 4:
554 e2629.
- 555 Bornschein MR, Maurício GN, Lopes RB, Mata H & Bonatto SL. 2007. Diamantina Tapaculo, a
556 new *Scytalopus* endemic to the Chapada Diamantina, northeastern Brazil (Passeriformes:
557 Rhinocryptidae). Rev Bras Ornitol 15: 151-174.
- 558 Bornschein MR, Pie MR & Teixeira L. 2019a. Conservation status of *Brachycephalus* toadlets
559 (Anura: Brachycephalidae) from the Brazilian Atlantic Rainforest. Diversity 11(150): 1-29.
- 560 Bornschein, MR, Rollo Jr., MM, Pie, MR, Confetti, AE & Ribeiro, LF. 2019b. Redescription of
561 the advertisement call of *Brachycephalus tridactylus* (Anura: Brachycephalidae).
562 Phyllomedusa 18: 3-12.
- 563 Bornschein MR, Teixeira L & Ribeiro LF. 2019c. New record of *Brachycephalus fuscolineatus*
564 Pie, Bornschein, Firkowski, Belmonte-Lopes & Ribeiro, 2015 (Anura: Brachycephalidae)
565 from Santa Catarina state, Brazil. Check List 15: 379-385.
- 566 Bornschein MR, Ribeiro LF, Rollo Jr. MM, Confetti AE & Pie MR. 2018. Advertisement call of
567 *Brachycephalus albolineatus* (Anura: Brachycephalidae). PeerJ 6: e5273.
- 568 Cabanne GS, Santos FR & Miyaki CY. 2007. Phylogeography of *Xiphorhynchus fuscus*
569 (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in
570 southern Atlantic forest. Biol J Linn Soc 91: 73-84.
- 571 Clemente-Carvalho RBG, Klaczko J, Perez SI, Alves ACR, Haddad CFB & Reis SF dos. 2011.
572 Molecular phylogenetic relationships and phenotypic diversity in miniaturized toadlets,
573 genus *Brachycephalus* (Amphibia: Anura: Brachycephalidae). Mol Phylogenet Evol 61: 79-
574 89.
- 575 Condez TH, Sawaya RJ & Dixo M. 2009. Herpetofauna dos remanescentes de Mata Atlântica da
576 região de Tapiraí e Piedade, SP, sudeste do Brasil. Biota Neotrop 9: 157-185.
- 577 Condez TH, Monteiro JP de C, Comitti EJ, Garcia PC de A, Amaral IB & Haddad CFB. 2016. A
578 new species of flea-toad (Anura: Brachycephalidae) from southern Atlantic Forest, Brazil.
579 Zootaxa 4083: 40-56.
- 580 Cunha AK, Oliveira IS de & Hartmann MT. 2010. Anurofauna da Colônia Castelhanos, na Área
581 de Proteção Ambiental de Guaratuba, Serra do Mar paranaense, Brasil. Biotemas 23: 123-
582 134.

- 583 Dantas GPM, Sari EHR, Cabanne GS, Pessoa RO, Marini MA, Miyaki CY & Santos FR. 2015.
584 Population genetic structure of the Atlantic Forest endemic *Conopophaga lineata*
585 (Passeriformes: Conopophagidae) reveals a contact zone in the Atlantic Forest. *J Ornithol*
586 156: 85-99.
- 587 D'horta FM, Cabanne GS, Meyer D & Miyaki CY. 2011. The genetic effects of Late quaternary
588 climatic changes over a tropical latitudinal gradient: diversification of an Atlantic forest
589 passerine. *Mol Ecol* 20: 1923-1935.
- 590 Dixo M & Verdade VK. 2006. Herpetofauna de serrapilheira da Reserva Florestal de Morro
591 Grande, Cotia (SP). *Biota Neotrop* 6: 1-20.
- 592 Ferreira FJF, Moraes RAV, Ferrari MP & Vianna RB. 1981. Contribuição ao estudo do
593 Alinhamento Estrutural de Guapiara. In: 3º Simpósio Regional De Geologia, Curitiba:
594 Sociedade Brasileira de Geologia Núcleo de São Paulo, p. 226-240.
- 595 Firkowski CR, Bornschein MR, Ribeiro LF & Pie MR. 2016. Species delimitation, phylogeny
596 and evolutionary demography of co-distributed, montane frogs in the southern Brazilian
597 Atlantic Forest. *Mol Phylogenet Evol* 100: 345-360.
- 598 Fitzpatrick SW, Brasileiro CA, Haddad CFB & Zamudio KR. 2009. Geographical variation in
599 genetic structure of an Atlantic Coastal Forest frog reveals regional differences in habitat
600 stability. *Mol Ecol* 18: 2877-2896.
- 601 Giaretta AA & Sawaya RJ. 1998. Second species of *Psyllophryne* (Anura: Brachycephalidae).
602 *Copeia* 1998: 985-987.
- 603 Gontijo-Pascutti AHF, Hasui Y, Santos M dos, Soares Jr. AV & Souza IA de. 2012. As Serras do
604 Mar e da Mantiqueira. In: Hasui Y, Carneiro C dal Ré, Almeida FFM de & Bartorelli A.
605 (Eds), *Geologia do Brasil*, Beca, São Paulo, p. 549-571.
- 606 Goutte, S, Mason, MJ, Christensen-Dalsgaard, J, Montealegre-Z, F, Chivers, BD, Sarria-S, FA,
607 Antoniazzi, MM, Jared, C, Sato, LA & Toledo, LF. 2017. Evidence of auditory insensitivity
608 to vocalization frequencies in two frogs. *Scientific Reports* 7: 12121.
- 609 Grazziotin FG, Monzel M, Echeverrigaray S & Bonatto SL. 2006. Phylogeography of the
610 *Botrops jararaca* complex (Serpentes: Viperidae): past fragmentation and island
611 colonization in the Brazilian Atlantic Forest. *Mol Ecol* 15: 3969-3982.

- 612 Hasui Y. 1990. Neotectônica e aspectos fundamentais da tectônica ressurgente no Brasil. In: 1^o
613 Workshop sobre Neo-Tectônica e Sedimentação Cenozóica Continental no Sudeste
614 Brasileiro, Belo Horizonte, Sociedade Brasileira de Geologia, Boletim 11: 1-31.
- 615 Izecksohn E. 1971. Novo gênero e nova espécie de Brachycephalidae do Estado do Rio de
616 Janeiro, Brasil. Boletim do Museu Nacional, Zoologia 280: 1-12.
- 617 Kaplan M. 2002. Histology of the anteroventral part of the breast-shoulder apparatus of
618 *Brachycephalus ephippium* (Brachycephalidae) with comments on the validity of the genus
619 *Psyllophryne* (Brachycephalidae). Amphibia-Reptilia 23: 225-227.
- 620 Katoh K, Misawa K, Kuma K & Miyata T. 2002. MAFFT: a novel method for rapid multiple
621 sequence alignment based on fast fourier transform. Nucleic Acids Res. 30: 3059-3066.
- 622 Köhler J, Jansen M, Rodriguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB,
623 Rödel M-O & Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory,
624 terminology, methods and recommendations for best practice. Zootaxa 4251: 1-124.
- 625 Leivas PT, Calixto, P de O, Crivellari, LB, Struett, MM & Moura, MO. 2018 Amphibians of the
626 northern coast of the state of Paraná, Brazil. Herpetology Notes 11: 1029-1045
- 627 Mariotto LR. 2014. Anfíbios de um gradiente altitudinal em mata atlântica. Masters dissertation.
628 Curitiba: Universidade Federal do Paraná.
- 629 Mata H, Fontana CS, Mauricio GN, Bornschein MR, Vasconcelos MF de & Bonatto SL. 2009.
630 Molecular phylogeny and biogeography of the eastern Tapaculos (Aves: Rhinocryptidae:
631 *Scytalopus*, *Eleoscytalopus*): cryptic diversification in Brazilian Atlantic Forest. Mol
632 Phylogenet Evol 53: 450-462.
- 633 Maurício G. 2005. Taxonomy of southern populations in the *Scytalopus speluncae* group, with
634 description of a new species and remarks on the systematics and biogeography of the
635 complex (Passeriformes: Rhinocryptidae). Ararajuba 13: 7-28.
- 636 Maurício GN, Belmonte-Lopes R, Pacheco JF, Silveira LF, Whitney BM & bornschein MR.
637 2014. Taxonomy of “Mouse-colored Tapaculos” (II): an endangered new species from the
638 montane Atlantic Forest of southern Bahia, Brazil (Passeriformes: Rhinocryptidae:
639 *Scytalopus*). The Auk 131: 643-659.
- 640 Maurício GN, Bornschein MR, Vasconcelos MF de, Whitney BM, Pacheco JF & Silveira LF.
641 2010. Taxonomy of “Mouse-colored Tapaculos”. I. On the application of the name

- 642 *Malacorhynchus spelunca* Ménétriés, 1835 (Aves: Passeriformes: Rhinocryptidae).
643 *Zootaxa* 2518: 32-48.
- 644 Melo MS, Fernandes LA, Coimbra AM & Ramos RGN. 1989. O gráben (Terciário?) de Sete
645 Barras, vale do Ribeira do Iguape, SP. *Rev Bras Geoc* 2: 260-262.
- 646 Monteiro JPC, Condez TH, Garcia PCA, Comitti EJ, Amaral IB & Haddad CFB. 2018a. A new
647 species of *Brachycephalus* (Anura, Brachycephalidae) from the coast of Santa Catarina
648 State, southern Atlantic Forest, Brazil. *Zootaxa* 4407: 483-505.
- 649 Monteiro JPC, Condez TH, Garcia PC. de A & Haddad CFB. 2018b. The advertisement calls of
650 two species of *Brachycephalus* (Anura: Brachycephalidae) from southern Atlantic Forest,
651 Brazil. *Zootaxa* 4415: 183-188.
- 652 Napoli MF, Caramaschi U, Cruz CAG & Dias IR. 2011. A new species of flea-toad, genus
653 *Brachycephalus* Fitzinger (Amphibia: Anura: Brachycephalidae), from the Atlantic
654 rainforest of southern Bahia, Brazil. *Zootaxa* 2739: 33-40.
- 655 Oliveira AKC de, Oliveira IS de, Hartmann MT, Silva NR da & Toledo LF. 2011. Amphibia,
656 Anura, Brachycephalidae, *Brachycephalus hermogenesi* (Giaretta and Sawaya, 1998): new
657 species record in the state of Paraná, southern Brazil and geographic distribution map.
658 *Check List* 7: 17-18.
- 659 Pereira M dos S, Candaten A, Milani D, Oliveira FB de, Gardelin J, Rocha CFD & Vrcibradic D.
660 2010. *Brachycephalus hermogenesi*. *Herpetol Review* 41: 506.
- 661 Pie MR, Meyer ALS, Firkowski CR, Ribeiro LF & Bornschein MR. 2013. Understanding the
662 mechanisms underlying the distribution of microendemic montane frogs (*Brachycephalus*
663 spp., Terrarana: Brachycephalidae) in the Brazilian Atlantic Rainforest. *Ecol Model* 250:
664 165-176.
- 665 Pie MR, Ribeiro LF, Confetti AE, Nadaline MJ & Bornschein MR. 2018a. A new species of
666 *Brachycephalus* (Anura: Brachycephalidae) from southern Brazil. *PeerJ* 6: e5683.
- 667 Pie MR, Faircloth BC, Ribeiro LF, Bornschein MR & McCormack JE. 2018b. Phylogenomics of
668 montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands
669 followed by climatic stability. *Biol J Linn Soc* 125: 72-82.
- 670 Pimenta BVS, Bérnils RS & Pombal Jr. JP. 2007. Amphibia, Anura, Brachycephalidae,
671 *Brachycephalus hermogenesi*: filling gap and geographic distribution map. *Check List* 3:
672 277-279.

- 673 Pinto OM de O. 1941. Sobre a variação geográfica das populações de *Cichlocolaptes leucophrus*
674 (Jardine and Selby) com a descrição de uma raça nova. Rev Argent Zoog 1: 165-171.
- 675 Pulido-Santacruz P, Bornschein MR, Belmonte-Lopes R & Bonatto SL. 2016. Multiple
676 evolutionary units and demographic stability during the last glacial maximum in the
677 *Scytalopus speluncae* complex (Aves: Rhinocryptidae). Mol Phylogenet Evol 102: 86-96.
- 678 R Core Team. 2018. R: A language and environment for statistical computing. Version 4.0.3.
679 Vienna: R Foundation for Statistical Computing. Available at <https://www.r-project.org>.
- 680 Ribeiro LF, Bornschein MR, Belmonte-Lopes R, Firkowski CR, Morato SAA & Pie, MR 2015.
681 Seven new microendemic species of *Brachycephalus* (Anura: Brachycephalidae) from
682 southern Brazil. PeerJ 3: e1011.
- 683 Ricommini C & Assumpção M. 1999. Quaternary tectonics in Brazil. Episodes 22: 221-225.
- 684 Saadi A. 1993. Neotectônica da plataforma brasileira: esboço e interpretações preliminares.
685 Geonomos 1: 1-15.
- 686 Sadowski GR. 1991. Megafalha de Cubatão no sudeste brasileiro. Boletim IG-USP, Sér Cient,
687 22: 15-28.
- 688 Santos-Pereira M, Candaten A, Milani D, Oliveira FB, Gardelin J & Rocha CFD da. 2011.
689 Seasonal variation in the leaf-litter frog community (Amphibia: Anura) from an Atlantic
690 Forest area in the Salto Morato Natural Reserve, southern Brazil. Zoologia 28: 755-761.
- 691 Santos-Pereira M, Milani D, Barata-Bittencourt LF, Iapp TM & Rocha CFD. 2016. Anuran
692 species of the Salto Morato Nature Reserve in Paraná, southern Brazil: review of the species
693 list. Check List 12: 1907.
- 694 Santos-Pereira M, Pombal Jr. JP & Rocha CFD. 2018. Anuran amphibians in state of Paraná,
695 southern Brazil. Biota Neotrop 18: e20170322.
- 696 Silva JMC & Stotz DF. 1992. Geographic variation in the Sharp-billed Treehunter *Heliobletus*
697 *contaminatus*. Bull BOC 112: 98-101.
- 698 Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
699 large phylogenies. Bioinformatics 30(9): 1312-1313.
- 700 Sueur J, Aubin T & Simonis C. 2008. Seewave: a free modular tool for sound analysis and
701 synthesis. Bioacoustics 18(2): 213-226.

702 Teixeira L, Ribeiro LF, Corrêa L, Confetti AE, Pie MR & Bornschein MR. 2018. A second
703 record of the recently described *Brachycephalus albolineatus* Bornschein, Ribeiro,
704 Blackburn, Stanley and Pie, 2016 (Anura: Brachycephalidae). Check List 14: 1013-1016.
705 Thomé MTC, Zamudio KR, Giovanelli JGR, Haddad CFB, Baldissera Jr. FA & Alexandrino J.
706 2010. Phylogeography of endemic toads and post-Pliocene persistence of the Brazilian
707 Atlantic Forest. Mol Phylogenet Evol 55: 1018-1031.

708 Verdade VK, Rodrigues MT, Cassimiro J, Pavan D, Liou N & Lange M. 2008. Advertisement
709 call, vocal activity, and geographic distribution of *Brachycephalus hermogenesi* (Giaretta
710 and Sawaya, 1998) (Anura, Brachycephalidae). J Herpetol 42: 542-549.

711 Verdade VK, Rodrigues MT & Pavan D. 2009. Anfíbios anuros da região da Estação Biológica
712 do Alto da Serra de Paranapiacaba. In: Lopes MIMS, Kirizawa M & Melo MM da RF de
713 (Eds), Patrimônio da Reserva Biológica do Alto da Serra de Paranapiacaba: a antiga Estação
714 Biológica do Alto da Serra, São Paulo: Instituto de Botânica, p. 579-603.

715

716

717 FIGURE CAPTIONS

718

719 Figure 1. Ventral view of life specimens of *Brachycephalus sulfuratus* initially deposited in
720 DZUP) and transferred to MHNCI. a) MHNCI 11575 (ex-DZUP 153) (Corvo, Paraná); b)
721 MHNCI 11571 (ex-DZUP 139)(Caratuval, near the Parque Estadual das Lauráceas, Paraná); c)
722 MHNCI 11582 (ex-DZUP 224) (Fazenda Thalia, Paraná); d) MHNCI 11579 (ex-DZUP 221)
723 (Fazenda Thalia); e) MHNCI 11573 (ex-DZUP 151) (Corvo); f) MHNCI 11583 (ex-DZUP 362)
724 (base of the Serra Água Limpa, São Paulo); g) MHNCI 11580 (ex-DZUP 222) (Fazenda Thalia);
725 h) MHNCI 11581 (ex-DZUP 223) (Fazenda Thalia); i) MHNCI 10788 (ex-DZUP 154) (Corvo);
726 j) MHNCI 10790 (near the Jurupará dam, São Paulo); k) MHNCI 10826 (Morro do Garrafão,
727 Santa Catarina); l) MHNCI 10792 (near the Jurupará dam). Notice the variable of yellow spots,
728 absent in specimen “l”, as well as the absence of the dark-brown inverted v-shaped mark on the
729 cloacal region of specimen “a”. Compare sonograms from specimens “j” and “l” in Fig. 2b, c.
730 The presence of yellow spots and v-shaped mark was proposed as diagnostic characteristics to
731 distinguish *B. sulfuratus* from *B. hermogenesi*, but they are variable intraspecifically. For details
732 on geographical localities, see Table 1. Photo credit: Luiz Fernando Ribeiro.

733

734

735 Figure 2. Ventral view of specimens of *Brachycephalus sulfuratus* (a-c) and *B. hermogenesi* (d)
736 in preservative, deposited in MHNCI and ZUEC: a) MHNCI 9800 (Salto do Inferno, Paraná); b)
737 MHNCI 10302 (Mananciais da Serra, Paraná); c) MHNCI 10303 (Corvo, Paraná; ex DZUP
738 589); and d) ZUEC 9715 (Núcleo Picinguaba, São Paulo; holotype of *B. hermogenesi*). Notice
739 the variation in ventral coloration. For details on geographical localities, see Table 1. Photo
740 credit: Luiz Fernando Ribeiro.

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743

744 Figure 3. Oscillograms (above) and spectrograms (below) of *Brachycephalus sulfuratus*. **A.**
745 Example of one entire call with five notes (MHNCI 124; voucher MHNCI 10791 or MHNCI
746 10792; near the Jurupará dam, municipality of Piedade, São Paulo; M. R. Bornschein). **B.**
747 Example of one entire call with two notes (MHNCI 129; voucher MHNCI 11583; Base of the
748 Serra Água Limpa, municipality of Apiaí, São Paulo; M. R. Bornschein). **C.** Example of one
749 note with 10 pulses (MHNCI 124). **D.** Example of one note with three pulses (MHNCI 124).
750 Spectrograms are produced with Hann window, overlap of 50%, and FFT size of 512 points in A
751 and B and 256 points in C and D. For details on geographical localities, see Table 1.

752

753

754 Figure 4. Oscillograms (above) and spectrograms (below) of *Brachycephalus hermogenesi*. **A.**
755 Example of one entire call with 135 notes recorded (MHNCI 165; Corcovado, municipality of
756 Ubatuba, São Paulo; L. F. Ribeiro). **B.** Example of one isolated note with two pulses (MHNCI
757 183; Núcleo Picinguaba, Parque Estadual da Serra do Mar, municipality of Ubatuba, São Paulo;
758 M. R. Bornschein). **C.** Example of one note group with three notes (each with two pulses;
759 MHNCI 180; Núcleo Picinguaba; M. R. Bornschein). **D.** Example of one note group with four
760 notes (each with two pulses; MHNCI 165). **E.** Example of one note group with three notes (the
761 first with three pulses and the remaining with two pulses; MHNCI 166; Estação Biológica de
762 Boracéia, municipality of Salesópolis, São Paulo; M. R. Bornschein). **F.** Example of one note
763 group with three notes, with each note preceded by an attenuated note with one pulse (marked
764 with white arrows; MHNCI 183). Spectrograms are produced with Hann window, overlap of
765 50%, and FFT size of 16,384 points in A, 128 points in B, and 256 points in C–F.

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767

768 Figure 5. Oscillograms (above) and spectrograms (below) of *Brachycephalus* sp. (other than *B.*
769 *sulfuratus* and *B. hermogenesi*). **A.** Example of one entire call with 71 notes recorded (MHNCI
770 200; Corcovado, municipality of Ubatuba, São Paulo; M. R. Bornschein). **B.** Example of one
771 isolated note with seven pulses (MHNCI 198; Corcovado; M. R. Bornschein). **C.** Example of one
772 note group with two notes (with nine and four pulses, respectively; MHNCI 198). **D.** Example of
773 one note group with three notes (the first note with three pulses and the remaining notes with
774 four pulses; MHNCI 211; Trilha do Corisco, municipality of Paraty, Rio de Janeiro; L. F.

775 Ribeiro). Spectrograms are produced with Hann window, overlap of 50%, and FFT size of
776 16,384 points in A and 256 points in B–D.

777

778

779 Figure 6. Current identification of records of flea toads that have been at some point identified as
780 *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species, according to
781 the compilation of localities and review of identifications shown in Table 1. We highlighted the
782 southernmost record of *B. hermogenesi* confirmed (1 – Parque Natural Municipal Nascentes de
783 Paranapiacaba). We also highlight the northernmost confirmed records of *B. sulfuratus* (2 –
784 Núcleo Itutinga-Pilões and 3 – near the Jurupará dam). Abbreviations: RJ = Rio de Janeiro; SP =
785 São Paulo; PR = Paraná; SC = Santa Catarina. Map image is the intellectual property of Esri and
786 is used herein under license. Copyright © 2020 Esri and its licensors. All rights reserved.

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789 Figure 7. Phylogenetic tree based on a concatenated dataset of all mitochondrial 12S and 16S
790 mitochondrial loci available on GenBank for specimens of the *B. didactylus* species group (Table
791 S1). The tree was rooted by its midpoint. Whenever possible, the corresponding localities
792 available on their GenBank records were standardized based on the toponyms indicated in Table
793 1. Notice that the specimen originally identified as *B. hermogenesi* from the Municipality of
794 Piedade (Condez, Sawaya & Dixo 2009, Clemente-Carvalho et al. 2011), was reverted to *B.*
795 *sulfuratus* (Table 1). Branch values correspond to bootstrap support.

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799 **Appendix 1.** Advertisement calls analyzed in the present study. Abbreviation: MHNCI = Museu
800 de História Natural Capão da Imbuia, Curitiba, Paraná.

801

802 *Brachycephalus sulfuratus*. SÃO PAULO: Base of the Serra Água Limpa, municipality of Apiaí
803 MHNCI 129; Biquinha, municipality of Juquiá MHNCI 128; near the Jurupará dam,
804 municipality of Piedade MHNCI 123–5; Núcleo Itutinga-Pilões, Parque Estadual da Serra do
805 Mar, municipality of Cubatão MHNCI 126–7; Serra do Guaraú, on the border of the
806 municipalities of Cajati and Jacupiranga MHNCI 130; Torre Embratel, municipality of Cajati
807 MHNCI 218. PARANÁ: Caratuval, near the Parque Estadual das Lauráceas, municipality of
808 Adrianópolis MHNCI 131; Caratuval, Parque Estadual das Lauráceas, municipality of
809 Adrianópolis MHNCI 132; Entroncamento Teba, Rio Turvo, municipality of Campina Grande
810 do Sul MHNCI 219; Fazenda Thalia, municipality of Balsa Nova MHNCI 134; Morro do Canal,
811 municipality of Piraquara MHNCI 220; Reserva Particular do Patrimônio Natural Salto Morato,
812 municipality of Guaraqueçaba MHNCI 133. SANTA CATARINA: Monte Crista, municipality
813 of Garuva MHNCI 221; Morro do Garrafão, municipality of Corupá MHNCI 137; Morro
814 Garuva, municipality of Garuva MHNCI 136; Serra do Pico, municipality of Joinville MHNCI
815 217; Trucicultura, municipality of Garuva MHNCI 135.

816

817 *Brachycephalus hermogenesi*. SÃO PAULO: Corcovado, municipality of Ubatuba MHNCI
818 166; Estação Biológica de Boracéia, municipality of Salesópolis MHNCI 166–9; Morro do
819 Cantagalo, municipality of Caraguatatuba MHNCI 222–3; Núcleo Cunha, Parque Estadual da
820 Serra do Mar, municipality of Cunha MHNCI 170–1; Núcleo Picinguaba, Parque Estadual da
821 Serra do Mar, municipality of Ubatuba MHNCI 172–87; Parque Natural Municipal Nascentes de
822 Paranapiacaba, municipality of Santo André MHNCI 213–6; Trilha do Ipiranga 50 m from the
823 Rio Ipiranga, Núcleo Santa Virgínia, Parque Estadual da Serra do Mar, municipality of São Luiz
824 do Paraitinga MHNCI 188–92.

825

826 *Brachycephalus* sp. (other than *B. sulfuratus* and *B. hermogenesi*). RIO DE JANEIRO: Trilha
827 do Corisco, municipality of Paraty MHNCI 206–12. SÃO PAULO: Corcovado, municipality of
828 Ubatuba MHNCI 193–205.

829

Table 1 (on next page)

Current identification of records of flea toads at some point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species, southeastern and southern Brazil

Table 1. Current identification of records of flea toads at some point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species, southeastern and southern Brazil. Our revision resulted in some unidentified records (*B. sulfuratus*, *B. hermogenesi* or a third species); the probable identifications are provided below. Localities are in alphabetical order (accordingly to the respective species).

Abbreviations: FNJV = Fonoteca Neotropical Jacques Viellard; 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; XC = Xeno-Canto sound collection (www.xeno-canto.org).

1 Table 1. Current identification of records of flea toads at some point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an
 2 unidentified related species, southeastern and southern Brazil. Our revision resulted in some unidentified records (*B. sulfuratus*, *B.*
 3 *hermogenesi* or a third species); the probable identifications are provided below. Localities are in alphabetical order (accordingly to the
 4 respective species). Abbreviations: FNJV = Fonoteca Neotropical Jacques Vielliard; MHNCI = Museu de História Natural Capão da
 5 Imbuia, Curitiba, Paraná, Brazil; ZUEC = Museu de História Natural, Universidade Estadual de Campinas, Campinas, state of São
 6 Paulo, Brazil; XC = Xeno-Canto sound collection (www.xeno-canto.org).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>B. sulfuratus</i>					
<i>B. sulfuratus</i>	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).
<i>B. sulfuratus</i>	Base of the Serra Água Limpa, municipality of Apiaí, São Paulo	24°28'52"S, 48°47'12"W; 920 m a.s.l.	Without species identification: Firkowski et al. (2016); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Bornschein et al. (2016b), Ribeiro et al. (2017), Pie et al. (2018a)	Specimen, calls, and genetic sequence on GenBank	Specimen (MHNCI 11583; Fig. 1f) and calls examined (MHNCI 129; Fig. 3B); KX198030.1 analyzed sequence (Fig. 7).
<i>B. sulfuratus</i>	Biquinha, municipality of Juquiá, São Paulo	24°17'43"S, 47°36'26"W; 40 m a.s.l.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 128).
<i>B. sulfuratus</i>	Braço do Norte, municipality of Itapoá, Santa Catarina	26°07'29"S, 48°43'48"W; 240 m a.s.l.	<i>B. sulfuratus</i> : Monteiro et al. (2018a)	Specimen and genetic sequence on GenBank	MG889430.1 analyzed sequence (Fig. 7).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>B. sulfuratus</i>	Caratuval, near the Parque Estadual das Lauráceas, municipality of Adrianópolis, Paraná	24°51'17"S, 48°43'43"W; 900 m a.s.l.	Without species identification: Firkowski et al. (2016); <i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Bornschein et al. (2016b), Ribeiro et al. (2017), Pie et al. (2018a)	Specimen, calls, and genetic sequence on GenBank	Specimen (MHNCI 11571; Fig. 1b) and calls examined (MHNCI 131); KX198031.1 analyzed sequence (Fig. 7).
<i>B. sulfuratus</i>	Caratuval, Parque Estadual das Lauráceas, municipality of Adrianópolis, Paraná	24°51'14"S, 48°42'01"W; 890 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a)	Calls	Calls examined (MHNCI 132).
<i>B. sulfuratus</i>	Castelo dos Bugres, municipality of Joinville, Paraná	26°13'47"S, 49°03'20"W; 790–860 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Condez et al. (2016), Monteiro et al. (2018a)	Specimen, calls, and genetic sequence on GenBank	MK697439.1, MK697487.1, KU321533.1, and MK697390.1 analyzed sequence (Fig. 7).
<i>B. sulfuratus</i>	Centro de Estudos e Pesquisas Ambientais da Univille, Vila da Glória, Distrito do Saí, municipality of São	26°13'39"S, 48°41'31"W; 125 m a.s.l.	<i>B. sulfuratus</i> : Condez et al. (2016)	Specimen, calls, and genetics	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
	Francisco do Sul, Santa Catarina				
<i>B. sulfuratus</i>	Corvo, municipality of Quatro Barras, Paraná	25°20'17"S, 48°54'56"W; 930 m a.s.l.	Without species identification: Firkowski et al. (2016); <i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Bornschein et al. (2016b), Ribeiro et al. (2017), Pie et al. (2018a), Pie et al. (2018b)	Specimen and genetic sequence on GenBank	Specimen examined (MHNCI 10788, MHNCI 11573, MHNCI 11575; Fig. 1a, e, i); KX198033.1 analyzed sequence (Fig. 7).
<i>B. sulfuratus</i>	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 calls	Specimens (MHNCI 11586–7) and calls examined (MHNCI 219).
<i>B. sulfuratus</i>	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.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	Specimen examined (MHNCI 11591).
<i>B. sulfuratus</i>	Fazenda Thalia, municipality of Balsa Nova, Paraná	25°30'58"S, 49°40'12"W; 1,025 m a.s.l.	Without species identification: Firkowski et al. (2016); <i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1:	Specimens, calls, and genetic sequence on GenBank	Specimens (MHNCI 11579–81, MHNCI 11582; Fig. 1c, d, g, h) and calls examined (MHNCI 134); KX198032.1

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
			Bornschein et al. (2016a); <i>B. sulfuratus</i> : Bornschein et al. (2016b), Ribeiro et al. (2017), Pie et al. (2018a)		analyzed sequence (Fig. 7).
<i>B. sulfuratus</i>	near the Jurupará dam, municipality of Piedade, São Paulo	23°56'30"S, 47°23'45"W; 690 m a.s.l.	<i>B. sulfuratus</i> : Pie et al. (2018a)	Specimens and calls	Specimens (MHNCI 10790–2; Fig. 1j, l) and calls examined (MHNCI 123–5; Fig. 3A, C, D).
<i>B. sulfuratus</i>	Mananciais da Serra, municipality of Piraquara, Paraná	25°29'32"S, 48°59'33"W; 970–1,050 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Bornschein et al. (2016b), Ribeiro et al. (2017), Pie et al. (2018a)	Specimen	Specimen examined (MHNCI 10302).
<i>B. sulfuratus</i>	Monte Crista, municipality of Garuva, Santa Catarina	26°04'53"S; 48°55'03"W; 435 m a.s.l.	---	Calls	Calls examined (MHNCI 221).
<i>B. sulfuratus</i>	Morro Anhangava, municipality of Quatro Barras, Paraná	25°22'51"S, 49°01'26"W; 915 m a.s.l.	<i>B. sulfuratus</i> : Condez et al. (2016), Monteiro et al. (2018a)	Specimen and genetic sequence on GenBank	MK697488.1, MK697440.1, KU321534.1, and MG889428.1 analyzed sequences (Fig. 7).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>B. sulfuratus</i>	Morro do Canal, municipality of Piraquara, Paraná	25°30'55"S; 48°58'56"W; 1,315 m	---	Calls	Calls examined (MHNCI 220).
<i>B. sulfuratus</i>	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.	<i>B. sulfuratus</i> : Condez et al. (2016)	Specimen and genetic sequence on GenBank	MK697441.1, MK697489.1, KU321532.1, and MK697392.1 analyzed sequences (Fig. 7).
<i>B. sulfuratus</i>	Morro do Garrafão, municipality of Corupá, Santa Catarina	26°28'23"S, 49°15'57"W; 500–530 m a.s.l.	<i>B. sulfuratus</i> : Pie et al. (2018a), Teixeira et al. (2018)	Specimen and calls	Specimens (MHNCI 10826–8; Fig. 1k) and calls examined (MHNCI 137).
<i>B. sulfuratus</i>	Morro Garuva, municipality of Garuva, Santa Catarina	26°02'29"S, 48°53'14"W; 215–495 m a.s.l.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 136).
<i>B. sulfuratus</i>	Municipality of Barra do Turvo	c. 24°45'S, 48°29'W; altitude?	<i>B. sulfuratus</i> : GenBank	Genetic sequence on GenBank	MK697486.1, MK697438.1, and MK697389.1 analyzed sequences (Fig. 7).
<i>B. sulfuratus</i>	Municipality of Piedade, São Paulo	c. 23°54'S, 47°25'W; altitude?	<i>B. hermogenesi</i> : Condez, Sawaya & Dixo (2009), Clemente-Carvalho et al. (2011); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen and genetic sequence on GenBank	HQ435682.1 and HQ435709.1 analyzed sequences (Fig. 7).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>B. sulfuratus</i>	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.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 126–7).
<i>B. sulfuratus</i>	Parque Estadual da Ilha do Cardoso, municipality of Cananéia, São Paulo	25°06'53"S, 47°55'40"W; 385 m a.s.l.	Possibly <i>B. hermogenesi</i> : Verdade et al. (2008); <i>B. sulfuratus</i> : Condez et al. (2016)	Specimen, calls, and genetic sequence on GenBank	MK697485.1, MK697437.1, KU321535.1, and MK697388.1 analyzed sequences (Fig. 7).
<i>B. sulfuratus</i>	Parque Estadual Intervalles, municipality of Iporanga, São Paulo	24°16'33"S, 48°25'04"W; 820 m a.s.l.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (XC80463 XC18179, XC75544).
<i>B. sulfuratus</i>	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).
<i>B. sulfuratus</i>	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).
<i>B. sulfuratus</i>	Recanto das Hortências, municipality of São José dos Pinhais, Paraná	25°33'24"S, 48°59'38"W; 975 m a.s.l.	<i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Ribeiro et al. (2017), Bornschein et al. (2016b), Pie et al. (2018a)	Specimen	Specimen examined.

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>B. sulfuratus</i>	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.	<i>B. hermogenesi</i> : Pereira et al. (2010), Santos-Pereira et al. (2011, 2016, 2018), Leivas et al. (2018); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a)	Specimen and calls	Calls examined (MHNCI 133).
<i>B. sulfuratus</i>	Salto do Inferno, Rio Capivari, municipality of Bocaiúva do Sul, Paraná	25°00'02"S, 48°37'07"W; 610 m a.s.l.	<i>B. sulfuratus</i> : Ribeiro et al. (2017), Bornschein et al. (2016b), Pie et al. (2018a)	Specimen	Specimen examined.
<i>B. sulfuratus</i>	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.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 130).
<i>B. sulfuratus</i>	Serra do Pico, municipality of Joinville, Santa Catarina	26°08'31"S, 48°57'19"W; 340–720 m a.s.l.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 217).
<i>B. sulfuratus</i>	Torre Embratel, municipality of Cajati, São Paulo	24°52'46"S, 48°15'27"W; 960–990 m a.s.l.	<i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Specimen and calls	Specimen (MHNCI 11588) and calls examined (MHNCI 218).
<i>B. sulfuratus</i>	Trucicultura, municipality of Garuva, Paraná	26°01'33"S, 48°52'02"W; 90 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Bornschein, Pie &	Calls	Calls examined (MHNCI 135).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
Teixeira (2019a)					
<i>B. hermogenesi</i>					
<i>B. hermogenesi</i>	Corcovado, municipality of Ubatuba, São Paulo	23°28'20"S, 45°11'41"W; 30–250 m a.s.l.	<i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a; in part.)	Calls	Calls examined (MHNCI 166; Fig. 4A, D).
<i>B. hermogenesi</i>	Estação Biológica de Boracéia, municipality of Salesópolis, São Paulo	23°39'10"S, 45°53'05"W; 825–900 m a.s.l.	<i>B. hermogenesi</i> : Pimenta et al. (2007), Verdade et al. (2008), Pie et al. (2013), Bornschein et al. (2016a), Condez et al. (2016)	Specimens and calls	Specimens (MHNCI, one uncatalogued specimen) and calls examined (MHNCI 166–9; Fig. 4E), including recordings sent by V. K. Verdade.
<i>B. hermogenesi</i>	Fazenda Capricórnio, municipality of Ubatuba, São Paulo	23°23'27"S, 45°04'26"W; 60 m a.s.l.	<i>B. hermogenesi</i> : Giaretta and Sawaya (1998), Verdade et al. (2008), Pie et al. (2013), Bornschein et al. (2016a), Condez et al. (2016)	Specimens (paratypes)	Specimen examined (ZUEC 9725).
<i>B. hermogenesi</i>	Morro do Cantagalo, municipality of Caraguatatuba, São Paulo	23°36'23"S, 45°23'34"W; 155–195 m a.s.l.	---	Calls	Calls examined (MHNCI 222–3).
<i>B. hermogenesi</i>	Municipality of Paraibuna, São Paulo	c. 23°23'34"S, 45°39'42"W; altitude?	<i>B. hermogenesi</i> : Condez et al. (2016)	Specimen and genetic sequence on GenBank	MK697373.1 analyzed sequence (Fig. 7).
<i>B. hermogenesi</i>	Núcleo Cunha, Parque	23°15'48"S,	<i>B. hermogenesi</i> : Bornschein, Pie &	Specimen and	Specimen (MHNCI, one

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
	Estadual da Serra do Mar, municipality of Cunha, São Paulo	45°02'39"W; 1,045–1,140 m a.s.l.	Teixeira (2019a)	calls	uncatalogued specimen) and calls examined (MHNCI 170–1).
<i>B. hermogenesi</i>	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.	<i>B. hermogenesi</i> : Giaretta and Sawaya (1998), Pimenta et al. (2007), Verdade et al. (2008), Clemente-Carvalho et al. (2009), Pie et al. (2013), Bornschein et al. (2016a), Condez et al. (2016), Pie et al. (2018a)	Specimens (holotype and paratypes), calls, and genetic sequence on GenBank	Specimens (ZUEC 9715–21; Fig. 3d) and calls examined (MHNCI 172–87; Fig. 4B, C, F); MK697472.1, KU321531.1, and MK697374.1 analyzed sequences (Fig. 7).
<i>B. hermogenesi</i>	Núcleo Santa Virgínea, Parque Estadual da Serra do Mar, municipality of São Luiz do Paraitinga, São Paulo	23°19'36"S, 45°07'57"W; 915 m a.s.l.	---	Calls	Calls examined (XC253045).
<i>B. hermogenesi</i>	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.	<i>B. hermogenesi</i> : Verdade, Rodrigues & Pavan (2009); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 213–6).
<i>B. hermogenesi</i>	Sertão da Cutia, municipality of Ubatuba,	not located	<i>B. hermogenesi</i> : Condez et al. (2016)	Specimen	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
	So Paulo				
<i>B. hermogenesi</i>	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.	<i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 188–92).
<i>Brachycephalus</i> sp. (other than <i>B. sulfuratus</i> and <i>B. hermogenesi</i>)					
<i>Brachycephalus</i> sp.	Corcovado, municipality of Ubatuba, São Paulo	23°28'20"S, 45°11'41"W; 30–250 m a.s.l.	<i>B. hermogenesi</i> : Giaretta and Sawaya (1998), Verdade et al. (2008), Pie et al. (2013), Bornschein et al. (2016a), Pie et al. (2018a; collected at “Picinguaba” [= Corcovado]), Bornschein, Pie & Teixeira (2019a; in part.)	Specimens (including paratypes) and calls	Specimens (ZUEC 9722–4, MHNCI 10823–5) and calls examined (MHNCI 193–205; Fig. 5A–C).
<i>Brachycephalus</i> sp.	Trilha do Corisco, municipality of Paraty, Rio de Janeiro	23°16'38"S, 44°46'39"W; 350–725 m a.s.l.	<i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Calls	Calls examined (MHNCI 206–12; Fig. 5D).
<i>Brachycephalus</i> sp. (<i>B. hermogenesi</i> or <i>B. sulfuratus</i>)					
<i>Brachycephalus</i> sp.	Alto Quiriri, municipality of Garuva, Santa Catarina	26°05'34"S, 48°59'41"W; 240 m	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1:	Unvouchered	The calls resemble those of <i>B. sulfuratus</i> (auditory record

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
		a.s.l.	Bornschein et al. (2016a); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)		made by MRB).
<i>Brachycephalus</i> sp.	Colônia Castelhanos, municipality of Guaratuba, Paraná	25°47'58"S, 48°54'40"W; 290 m a.s.l.	<i>Brachycephalus</i> aff. <i>hermogenesi</i> : Cunha et al. (2010); <i>B. hermogenesi</i> Oliveira et al. (2011); <i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>B. sulfuratus</i> : Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	Specimen examined (ZUEC 16602).
<i>Brachycephalus</i> sp.	Dona Francisca, municipality of Joinville, Santa Catarina	26°09'52"S, 48°59'23"W; 150 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	The calls resemble those of <i>B. sulfuratus</i> (auditory record made by MRB).
<i>Brachycephalus</i> sp.	Estação Ecológica Juréia-Itatins, municipality of Iguape, São Paulo	c. 24°27'S, 47°24'W; altitude?	<i>B. hermogenesi</i> : Verdade et al. (2008); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i>	Estrada do Rio do Júlio,	26°17'02"S,	<i>Brachycephalus</i> sp.: Mariotto	Specimen	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
sp.	municipality of Joinville, Santa Catarina	49°06'08"W; 650 m a.s.l.	(2014); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)		
<i>Brachycephalus</i> 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.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	The calls resemble those of <i>B. sulfuratus</i> (auditory records made by MRB and LFR).
<i>Brachycephalus</i> sp.	Fazenda Primavera, municipality of Tunas do Paraná, Paraná	24°53'08"S, 48°45'51"W; 1,060 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	The calls resemble those of <i>B. sulfuratus</i> (auditory record made by MRB).
<i>Brachycephalus</i> sp.	Municipality of Ibiúna, São Paulo	c. 23°39'S, 47°13'W; altitude?	<i>B. hermogenesi</i> : Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B.</i> <i>sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i> sp.	Municipality of Juquitiba, São Paulo	c. 23°56'S, 47°04'W; altitude?	<i>B. hermogenesi</i> : Verdade et al. (2008), Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie	Specimen	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>Brachycephalus</i> sp.	Municipality of Peruíbe, São Paulo	24°18'S, 46°59'W; altitude?	& Teixeira (2019a) <i>B. hermogenesi</i> : Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i> sp.	Municipality of Registro, São Paulo	c. 24°30'S, 47°51'W; altitude?	<i>B. hermogenesi</i> : Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i> sp.	Municipality of Ribeirão Grande, São Paulo	c. 24°06'S, 48°22'W; altitude?	<i>B. hermogenesi</i> : Verdade et al. (2008); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i> sp.	Municipality of Tapiraí, São Paulo	c. 23°57'55"S, 47°30'19"W; 870 m a.s.l.	<i>B. hermogenesi</i> : Verdade et al. (2008), Condez, Sawaya & Dixo (2009); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i> sp.	Parque Estadual de Jacupiranga, municipality of Eldorado, São Paulo	c. 24°38'S, 48°24'W; altitude?	<i>B. hermogenesi</i> : Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>Brachycephalus</i> sp.	Pico Agudinho, Serra da Prata, municipality of Morretes, Paraná	25°36'24"S, 48°43'33"W; 385 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	The calls resemble those of <i>B. sulfuratus</i> (auditory record made by MRB).
<i>Brachycephalus</i> sp.	Reserva Betary, municipality of Iporanga, São Paulo	24°33'08"S, 48°40'49"W; 190 m a.s.l.	<i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	Specimen examined (ZUEC 19931).
<i>Brachycephalus</i> 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.	<i>B. hermogenesi</i> : Verdade et al. (2008), Verdade, Rodrigues & Pavan (2009); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	---
<i>Brachycephalus</i> 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.	<i>B. hermogenesi</i> : Dixó and Verdade (2006), Verdade et al. (2008), Condez et al. (2016); <i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> or <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Specimen	---
<i>Brachycephalus</i> sp.	Sítio Ananias, municipality of Guaratuba, Paraná	25°47'08"S, 48°43'03"W; 25 m a.s.l.	<i>Brachycephalus</i> sp. nov. 1: Pie et al. (2013); <i>Brachycephalus</i> sp. 1: Bornschein et al. (2016a);	Unvouchered	The calls resemble those of <i>B. sulfuratus</i> (auditory record made by MRB).

Species	Locality and state	Geographical coordinates and altitude	Previous identification	Voucher	Our analysis of the record
<i>Brachycephalus</i> sp. cf. <i>B. sulfuratus</i> : Bornschein, Pie & Teixeira (2019a)					
<i>Brachycephalus</i> sp. (<i>B. hermogenesi</i> or <i>Brachycephalus</i> sp. from Corcovado and Trilha do Corisco)					
<i>Brachycephalus</i> sp.	Morro Cuscuzeiro, on the border of municipalities of Paraty, Rio de Janeiro, and Ubatuba, São Paulo	23°17'50"S, 44°47'21"W; 730–1,090 a.s.l.	<i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	The calls resemble those of <i>Brachycephalus</i> sp. of Trilha do Corisco (auditory record made by MRB and LFR).
<i>Brachycephalus</i> sp.	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.	<i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	The calls resemble those of <i>Brachycephalus</i> sp. of Trilha do Corisco (auditory record made by MRB and LFR).
<i>Brachycephalus</i> sp.	Municipality of Paraty, Rio de Janeiro	c. 23°13'07"S, 44°43'15"W; altitude?	<i>B. hermogenesi</i> : Giaretta and Sawaya (1998); <i>Brachycephalus</i> sp. cf. <i>B. hermogenesi</i> : Bornschein, Pie & Teixeira (2019a)	Unvouchered	---

Table 2 (on next page)

Structure of the advertisements calls recording between the geographical distribution of flea toads at some point identified as *B. sulfuratus*, *B. hermogenesi*, and as an unidentified related species

Table 2. Structure of the advertisements calls (AC) recording by the author between the geographical distribution of flea toads at some point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species, southeastern and southern Brazil. Each number represents a note, while the numerical value indicates the number of pulses for each note. Numbers in normal font outside parentheses represent isolated notes and those in normal font between parentheses represents note groups. Numbers in subscript represents attenuated notes (see text for reasons why we do not consider it as forming note groups). Question marks (“?”) represents a note issued whose number of pulses could not be counted. Abbreviations: A = number of isolated notes we hear being emitted before recording the AC; B = AC emission probably interrupted due to the researcher movement in the field.

1 Table 2. Structure of the advertisements calls (AC) recording by the author between the geographical distribution of flea toads at some
 2 point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species, southeastern and southern Brazil.
 3 Each number represents a note, while the numerical value indicates the number of pulses for each note. Numbers in normal font outside
 4 parentheses represent isolated notes and those in normal font between parentheses represents note groups. Numbers in subscript
 5 represents attenuated notes (see text for reasons why we do not consider it as forming note groups). Question marks (“?”) represents a
 6 note issued whose number of pulses could not be counted. Abbreviations: A = number of isolated notes we hear being emitted before
 7 recording the AC; B = AC emission probably interrupted due to the researcher movement in the field.

Individuals (Ind) and call deposit number	Call structure	A	B
<i>B. sulfuratus</i>			
Ind 01 (MHNCI 123), ex 01	14, 11, 11, 11, 10, 9, 8	0	
Ind 01 (MHNCI 123), ex 02	12, 10, 11, 10, 10, 9, 8	0	
Ind 01 (MHNCI 123), ex 03	12, 11, 10, 9, 10, 9, 8	0	
Ind 01 (MHNCI 123), ex 04	14, 11, 10, 10, 10, 10, 8	0	
Ind 02 (MHNCI 124), ex 01	10, 7, 6	0	
Ind 02 (MHNCI 124), ex 02	6, 6, 6, 6	0	
Ind 02 (MHNCI 124), ex 03	9, 7, 7, 7	0	
Ind 02 (MHNCI 124), ex 04	10, 7, 8, 7, 3	0	
Ind 02 (MHNCI 124), ex 05	6, 6, 7, 9, 7, 4	0	
Ind 02 (MHNCI 124), ex 06	10, 9, 8, 8, 8, 7	0	
Ind 02 (MHNCI 124), ex 07	10, 9, 8, 9, 9, 8, 7	0	
Ind 02 (MHNCI 124), ex 08	10, 7, 10, 8, 9, 8	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 02 (MHNCI 124), ex 09	9, 7, 8, 8, 8, 7	0	
Ind 02 (MHNCI 124), ex 10	10, 8, 7, 7, 8	0	
Ind 03 (MHNCI 125), ex 01	12, 10, 9, 9, 9, 8	0	
Ind 03 (MHNCI 125), ex 02	13, 9, 10, 10, 9, 8	0	
Ind 03 (MHNCI 125), ex 03	10, 9, 9, 9, 9, 9	0	
Ind 03 (MHNCI 125), ex 04	13, 9, 10, 9, 10, 8	0	
Ind 03 (MHNCI 125), ex 05	13, 10, 10, 10, 9, 9	0	
Ind 03 (MHNCI 125), ex 06	11, 9, 10, 10, 9, 8	0	
Ind 03 (MHNCI 125), ex 07	11, 9, 9, 9, 8	0	
Ind 03 (MHNCI 125), ex 08	12, 9, 9, 9, 9, 8	0	
Ind 04 (MHNCI 126), ex 01	?, ?, 9, 8, 8	0	
Ind 04 (MHNCI 126), ex 02	7, 8, 8, 8, 7	0	
Ind 04 (MHNCI 126), ex 03	6, 8, 7, 7, 7	0	
Ind 04 (MHNCI 126), ex 04	6, 8, 8, 8, 8	0	
Ind 04 (MHNCI 126), ex 05	6, 7, 7, 7, 7	0	
Ind 04 (MHNCI 126), ex 06	5, 7, 7, 8, 7, 6	0	
Ind 05 (MHNCI 127), ex 01	?, ?, ?, ?	0	
Ind 05 (MHNCI 127), ex 02	?, ?, ?, ?	0	
Ind 05 (MHNCI 127), ex 03	5, 6, 6, 6, 5	0	
Ind 05 (MHNCI 127), ex 04	?, ?, ?, ?, ?	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 05 (MHNCI 127), ex 05	?, ?, ?, ?, ?, ?	0	
Ind 05 (MHNCI 127), ex 06	?, ?, ?, ?, ?	0	
Ind 05 (MHNCI 127), ex 07	7, 8, 8, 8, 7	0	
Ind 06 (MHNCI 128), ex 01	11, 10, 10, 9, 8	0	
Ind 06 (MHNCI 128), ex 02	11, 10, 10, 9, 8	0	
Ind 06 (MHNCI 128), ex 03	11, 10, 9, 10, 8	0	
Ind 06 (MHNCI 128), ex 04	12, 10, 9, 9, 8	0	
Ind 06 (MHNCI 128), ex 05	11, ?, ?, ?	0	
Ind 06 (MHNCI 128), ex 06	11, 10, 9, 8, 7	0	
Ind 06 (MHNCI 128), ex 07	11, 10, 9, 9, 9	0	
Ind 07 (MHNCI 129), ex 01	10, 8	0	
Ind 07 (MHNCI 129), ex 02	12, 8	0	
Ind 07 (MHNCI 129), ex 03	10, 8	0	
Ind 07 (MHNCI 129), ex 04	10, 8, 8	0	
Ind 07 (MHNCI 129), ex 05	10, 8, 7	0	
Ind 08 (MHNCI 129), ex 01	6, 5, 4, 4	0	
Ind 08 (MHNCI 129), ex 02	9, 9, 9, 9	0	
Ind 08 (MHNCI 129), ex 03	11, 8, 9, 9, 9, 9, 9	0	
Ind 08 (MHNCI 129), ex 04	9, 9, 7, 7, 9, 9	0	
Ind 09 (MHNCI 129)	10, 9, 9, 9, ?, 9, 8	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 10 (MHNCI 130), ex 01	10, 7, 7, 6	0	
Ind 10 (MHNCI 130), ex 02	8, 9, 7	0	
Ind 11 (MHNCI 130), ex 01	?, ?, ?, ?, ?, ?	0	
Ind 11 (MHNCI 130), ex 02	?, ?, ?, ?, ?, ?, ?	0	
Ind 11 (MHNCI 130), ex 03	?, ?, ?, ?, ?, ?	0	
Ind 11 (MHNCI 130), ex 04	?, ?, ?, ?, ?	0	
Ind 11 (MHNCI 130), ex 05	11, 10, 9, 9, 9, 9, 8	0	
Ind 11 (MHNCI 130), ex 06	12, 9, 9, 9, 9, 9, 8	0	
Ind 11 (MHNCI 130), ex 07	11, 10, 9, 9, 9, 8	0	
Ind 11 (MHNCI 130), ex 08	11, 9, 8, 9, 8, 8,	0	
Ind 11 (MHNCI 130), ex 09	?, ?, 9, 9, ?, 8	0	
Ind 11 (MHNCI 130), ex 10	?, 9, 8, ?, 8, 8	0	
Ind 12 (MHNCI 131), ex 01	7, 6, 6, 5, 5, 4	0	
Ind 12 (MHNCI 131), ex 02	7, 6, 5, 6, 7, 5	0	
Ind 12 (MHNCI 131), ex 03	8, 6, 6, 6, 6, 5	0	
Ind 13 (MHNCI 132), ex 01	10, 7, 7, 7	0	
Ind 13 (MHNCI 132), ex 02	9, 8, 8, 8, 8	0	
Ind 13 (MHNCI 132), ex 03	10, 8, 8, 8, 8	0	
Ind 13 (MHNCI 132), ex 04	10, 9, 9, 9, 8	0	
Ind 13 (MHNCI 132), ex 05	10, 9, 9, 9, 9	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 13 (MHNCI 132), ex 06	10, 9, 9, 9, 8	0	
Ind 13 (MHNCI 132), ex 07	10, 9, 9, 9, 9	0	
Ind 13 (MHNCI 132), ex 08	11, 9, 9, 9, 9	0	
Ind 13 (MHNCI 132), ex 09	10, 9, 8, 9, 9	0	
Ind 13 (MHNCI 132), ex 10	11, 9, 8, 9, 8	0	
Ind 13 (MHNCI 132), ex 11	10, 9, 10, 8	0	
Ind 13 (MHNCI 132), ex 12	10, 8, 8, 8	0	
Ind 14 (MHNCI 133), ex 01	?, ?, ?, ?	0	
Ind 14 (MHNCI 133), ex 02	?, ?, ?, ?	0	
Ind 14 (MHNCI 133), ex 03	?, ?, ?, ?, ?, ?	0	
Ind 14 (MHNCI 133), ex 04	?, ?, ?, ?, ?, ?	0	
Ind 14 (MHNCI 133), ex 05	?, ?, ?, ?, ?, ?	0	
Ind 14 (MHNCI 133), ex 06	11, 10, 9, 11, 9	0	
Ind 14 (MHNCI 133), ex 07	?, ?, 10, 9, 8	0	
Ind 14 (MHNCI 133), ex 08	8, 9, 9, 9, ?	0	
Ind 14 (MHNCI 133), ex 09	?, ?, ?, ?	0	
Ind 15 (MHNCI 134)	9, 7, 7, 7, 6, 6	0	
Ind 16 (MHNCI 135), ex 01	5, 5, 5, 5	0	
Ind 16 (MHNCI 135), ex 02	?, ?, ?, ?, ?	0	
Ind 17 (MHNCI 136), ex 01	11, 8, 7, 8, 7	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 17 (MHNCI 136), ex 02	12, 9, 8, 8, 8	0	
Ind 17 (MHNCI 136), ex 03	12, 9, 8, 8, 8	0	
Ind 17 (MHNCI 136), ex 04	12, 9, 8, 7	0	
Ind 17 (MHNCI 136), ex 04	10, 9, 8, 5	0	
Ind 17 (MHNCI 136), ex 06	10, 8, 5, 3	0	
Ind 17 (MHNCI 136), ex 07	10, 8, 5	0	
Ind 17 (MHNCI 136), ex 08	9, 8, 6	0	
Ind 17 (MHNCI 136), ex 09	8, 8, 7	0	
Ind 17 (MHNCI 136), ex 10	9, 8, 7, 5	0	
Ind 18 (MHNCI 137), ex 01	6, 7, 6, 2	0	
Ind 18 (MHNCI 137), ex 02	6, 7, 6, 2	0	
Ind 18 (MHNCI 137), ex 03	?, 7, 7, 6	0	
Ind 18 (MHNCI 137), ex 04	8, 7, 8, 7	0	
Ind 19 (MHNCI 217), ex. 01	?, ?, 10, 10, 9	0	
Ind 19 (MHNCI 217), ex. 02	9, 10, 10, 9, 10	0	
Ind 20 (MHNCI 218), ex 01	?, 10, 10, ?, ?, ?	0	
Ind 20 (MHNCI 218), ex 02	?, ?, ?, ?, ?, ?	0	
Ind 21 (MHNCI 219), ex 01	9, 7, 7	0	
Ind 21 (MHNCI 219), ex 02	9, 7, 7, 6	0	
Ind 21 (MHNCI 219), ex 03	9, 7, 7, 7	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 21 (MHNCI 219), ex 04	9, 9, 8, 8, 8	0	
Ind 21 (MHNCI 219), ex 05	10, 8, 8, 8, 8, 8, 8	0	
Ind 21 (MHNCI 219), ex 06	10, 9, 9, 8, 8, 8	0	
Ind 21 (MHNCI 219), ex 07	10, 9, 9, 8, 8, 8	0	
Ind 21 (MHNCI 219), ex 08	10, 9, 9, 9, 8	0	
Ind 21 (MHNCI 219), ex 09	10, 9, 9, 9, 9, 9, 9, 8	0	
Ind 21 (MHNCI 219), ex 10	9, 9, 8, 8	0	
Ind 21 (MHNCI 219), ex 11	10, 8, 7	0	
Ind 21 (MHNCI 219), ex 12	10, 8, 6	0	
Ind 21 (MHNCI 219), ex 13	9, 7, 6	0	
Ind 21 (MHNCI 219), ex 14	9, 8, 7	0	
Ind 21 (MHNCI 219), ex 15	10, 8, 7	0	
Ind 21 (MHNCI 219), ex 16	10, 8, 7	0	
Ind 21 (MHNCI 219), ex 17	10, 8, 7	0	
Ind 21 (MHNCI 219), ex 18	10, 8, 7	0	
Ind 21 (MHNCI 219), ex 19	10, 9, 8	0	
Ind 21 (MHNCI 219), ex 20	10, 9, 8	0	
Ind 21 (MHNCI 219), ex 21	10, 9, 8, 8	0	
Ind 21 (MHNCI 219), ex 22	10, 9, 9, 8	0	
Ind 21 (MHNCI 219), ex 23	10, 9, 8	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 21 (MHNCI 219), ex 24	10, 9, 8	0	
Ind 21 (MHNCI 219), ex 25	10, 9, 8	0	
Ind 22 (MHNCI 220), ex 01	11, 8, 7, 7, 7, 7	0	
Ind 22 (MHNCI 220), ex 02	10, 8, 7, 7, 8, 8	0	
Ind 22 (MHNCI 220), ex 03	9, 8, 7, 7, 8, 7	0	
Ind 22 (MHNCI 220), ex 04	9, 8, 7, 8, 7, 7	0	
Ind 22 (MHNCI 220), ex 05	10, 8, 8, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 06	9, 8, 8, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 07	10, 8, 8, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 08	10, 8, 8, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 09	10, 8, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 10	10, 8, 9, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 11	10, 8, 7, 8, 8, 7	0	
Ind 22 (MHNCI 220), ex 12	10, 8, 8, 8, 8, 8	0	
Ind 22 (MHNCI 220), ex 13	10, 8, 8, 8, 7, 7	0	
Ind 22 (MHNCI 220), ex 14	10, 8, 8, 8, 8, 6	0	
Ind 22 (MHNCI 220), ex 15	10, 8, 8, 8, 8, 7	0	
Ind 22 (MHNCI 220), ex 16	9, 8, 7, 8, 7	0	
Ind 22 (MHNCI 220), ex 17	10, 9, 7, 8, 7	0	
Ind 23 (MHNCI 221), ex 01	8, 7, 6, 7, 6, 5	0	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 23 (MHNCI 221), ex 02	8, 7, 7, 7, 7, 4	0	
Ind 23 (MHNCI 221), ex 03	8, 7, 7, 7, 6	0	
Ind 23 (MHNCI 221), ex 04	8, 6, 7, 7, 6	0	
Ind 23 (MHNCI 221), ex 05	8, 7, 7, 7, 6	0	
Ind 23 (MHNCI 221), ex 06	8, 7, 8, 7, 7	0	
Ind 23 (MHNCI 221), ex 07	8, 8, 7, 7, 7	0	
Ind 23 (MHNCI 221), ex 08	8, 8, 8, 7, 7	0	
Ind 23 (MHNCI 221), ex 09	9, 8, 7, 8, 7	0	
Ind 23 (MHNCI 221), ex 10	9, 8, 8, 8, 7	0	
Ind 23 (MHNCI 221), ex 11	9, 8, 7, 7	0	
Ind 23 (MHNCI 221), ex 12	8, 7, 6	0	
Ind 23 (MHNCI 221), ex 13	9, 7, 7, 6	0	
Ind 23 (MHNCI 221), ex 14	8, 7, 7, 7, 7	0	
Ind 23 (MHNCI 221), ex 15	9, 7, 7, 7, 7	0	
Ind 23 (MHNCI 221), ex 16	8, 7, 8, 7, 6	0	
Ind 23 (MHNCI 221), ex 17	8, 8, 8, 7, 7	0	
Ind 23 (MHNCI 221), ex 18	9, 8, 7, 8	0	
Ind 23 (MHNCI 221), ex 19	9, 8, 8, 7, 7	0	
Ind 23 (MHNCI 221), ex 20	9, 8, 7, 7, 7	0	
Ind 23 (MHNCI 221), ex 21	8, 7, 7, 7, 7	0	

Individuals (Ind) and call deposit number	Call structure	A	B
	(2-?-?), (2-2-2), (2-2), (2-2-2), (2-2-2), (2-?-2), (2-?-2), (2-2-2-1), (2-2-1), (2-2-2-2), (?-?-?-?), (2-?-?-?), (2-1-1), (2-2-2), (2-2-2), (?-2-?), (2-1-2), (2-1-2)		
<i>B. hermogenesi</i> (Morro do Cantagalo)			
Ind 01 (MHNCI 222)	?, ?, ?, ?, 2, 2, ?, 2, 2, 2	?	X
Ind 02 (MHNCI 223)	2, (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2-2), (2-2-2-2), (2-2-2-2), (2-2-2-2)	?	X
<i>B. hermogenesi</i> (Núcleo Cunha)			
Ind 01 (MHNCI 170), ex 01	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	
Ind 01 (MHNCI 171), ex 02	1, 1, 1, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2), 2, (2-2), (2-?), 2, (2-2), (2-2), (2-2), (2-2)	0	
<i>B. hermogenesi</i> (Núcleo Picinguaba)			
Ind 01 (MHNCI 172), ex 01	2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-1), (2-2), (2-2), (2-2), (2-2)	?	
Ind 01 (MHNCI 175), ex 02	1, 2, 1, 1, 2, (2-1), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2)	0	
Ind 02 (MHNCI 173), ex 01	2, 2, 2, 2, 2, 2, 2, (2-1), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-1), (2-1-1), (2-2-2), (2-1-2), (2-2), (2-2-1), (2-2-1), (2-1), (2-1), (1-1), (1-1), (1-1), 1	4	
Ind 02 (MHNCI 177), ex 02	1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2), 2, (2-2), (2-2), (2-1), (2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-1), (2-1), (2-2), (1-1), (1-1)	0	
Ind 02 (MHNCI 182), ex 03	(2-2), (2-2), (2-2), (2-2), (2-2), (2-2)	?	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 03 (MHNCI 174), ex 01	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2), (2-2-2), (2-2-1), (2-2)	6	
Ind 03 (MHNCI 178), ex 02	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2), (2-2-2)	5	
Ind 03 (MHNCI 180), ex 03	2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-1), (2-2-2), (2-2-1), (2-2-1), (2-2-1), (2-2-1)	?	
Ind 03 (MHNCI 181), ex 04	?, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-2), (2-2), (2-2), (2-2-2), (2-2), (2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-1)	1	
Ind 04 (MHNCI 176), ex 01	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, (2-2-1), (2-2-2), (2-2-1), (2-2-2), (2-2-1), (2-2-2), (2-2-1), (2-2-1), (2-2-1), (2-2-1), (2-2-1), (2-2-1), (2-2), (2-2-1), (2-2-1), (2-2), (2-2), (2-2)	3	
Ind 04 (MHNCI 179), ex 02	(2-2), (2-2-2), (2-2-1), (2-2-1), (2-2-1), (2-2-2), (2-2-1), (2-2), (2-1)	?	
Ind 04 (MHNCI 183), ex 03	1, 1, 1, 1, 1, 2, 2, 2, 2, 2, 2, 2, 2, (2-1), (2-1), (2-1), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-2-2), (2-2), (2-2), (2-2-2), (2-2-2), (2-2-1), (2-2-1), (2-1-2-1-2), (2-1-2-1-2), (2-1-2-1-2), (1-2-1-2-1-2), (1-2-1-2-1-2), (1-2-1-2-1-1), (2-1-2-1), (2-1)	0	
Ind 05 (MHNCI 184)	(2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-2), (2-2-1), (2-2-2), (2-2-1)	?	
Ind 06 (MHNCI 185)	1, 1, 2, 2, 1, 1, 2, 2, 2, 2, 2, 2, (2-1), (2-1), 2, (2-1), 2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-1), (2-1), (2-1), (2-2), (2-2), (2-2)	2	
Ind 07 (MHNCI 186)	1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 1, 2, 2, 2, 2, 1, 2, 2, 2, 2, 2, 2, (2-2), (2-2), (2-2), (2-2), (2-2), (2-2), (2-	1	

Individuals (Ind) and call deposit number	Call structure	A	B
Ind 01 (MHNCI 206)	(?-?),(?-?),(?-?),(?-?),(5-?),(?-?),(?-?),(4-?),(?-?),(?-?),(?-?)	?	
Ind 02 (MHNCI 207)	3, (4-3), (4-3), (4-3), (4-3), (4-3), (4-3), (4-3), (4-3), (3-3), (3-3), (3-3), (4-3), (4-3), (3-3)	?	
Ind 03 (MHNCI 208)	4, 3, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5, (4-4), 5, (4-3), 4, (4-4), (5-4), (4-4), (4-3), (4-4), 4, 5, 5, 5, (5-3), 4	?	
Ind 04 (MHNCI 209)	2, 3, 3, ?, 3, 3, 4, (3-3), (4-3), (4-3), (4-3), (3-3), (4-3), (4-3), (4-3), (3-3), (4-3), (4-3), (4-3), (3-3), (3-2), 3, (3-2)	?	
Ind 05 (MHNCI 210)	4, 4, 4, 4, 4, 4, 4, 4, (4-3), 4, (4-3), (4-3), 4, (4-3), (4-3), (4-3)	5	X
Ind 06 (MHNCI 211)	4, (4-3), (3-3), (4-3), (4-4), (4-3), (4-4), (3-3), (4-4), (4-4), (4-4), (4-4), (4-3), (4-3), (4-4), (4-4), (3-4-4), (3-4-3), (4-4), (4-4), (3-3-3), (4-3-3), (3-3-3), (3-2-3), (3-2-3), (3-3), (3-2)	?	
Ind 07 (MHNCI 212)	3, (3-2), (3-3), (3-3), (3-3), (3-3), (3-3), (3-3), (3-3), (3-3), (3-3)	?	

8 ¹ Only the final part of the advertisement call was recorded.

9

Table 3 (on next page)

Parameters distinguishing the advertisement calls of flea toads at some point identified as *B. sulfuratus* and *B. hermogenesi*, including call comparisons of a third flea toad (*Brachycephalus* sp.)

Table 3. Parameters distinguishing the advertisement calls of flea toads at some point identified as *Brachycephalus sulfuratus* and *B. hermogenesi*, including call comparisons of a third flea toad (*Brachycephalus* sp.), originally identified as *B. hermogenesi*.

- 1 Table 3. Parameters distinguishing the advertisement calls of flea toads at some point identified as
- 2 *Brachycephalus sulfuratus* and *B. hermogenesi*, including call comparisons of a third flea toad
- 3 (*Brachycephalus* sp.), originally identified as *B. hermogenesi*.

Parameter	<i>B. sulfuratus</i>	<i>B. hermogenesi</i>	<i>Brachycephalus</i> . sp. from Corcovado and Trilha do Corisco
Note-centered approach			
Number of notes per call	≤ 8	≥ 24	≥ 38
Calls composed only by isolated notes	x		
Calls present note groups		x	x
Presence of warming notes		x	x
Presence of attenuated notes		x	
Maximum number of pulses in isolated notes	14	2	12
Maximum number of pulses per note in note groups	---	3	16
Maximum number of notes in note groups	---	6 ¹	3
Call-centered approach			
Number of notes per call	1	1	1
Calls composed only by isolated notes	---	---	---
Calls present note groups	---	---	---
Presence of warming notes	---	---	---
Presence of attenuated notes	---	---	---
Maximum number of pulses in isolated notes	---	---	---
Maximum number of pulses per note in note groups	---	---	---
Maximum number of pulses per	14	3	16

Parameter	<i>B. sulfuratus</i>	<i>B. hermogenesi</i>	<i>Brachycephalus</i> . sp. from Corcovado and Trilha do Corisco
Note-centered approach			
note			
Maximum number of notes in note groups	---	---	---

4 ¹ Up to seven, according Verdade et al. (2008).

Figure 1

Ventral view of life specimens of *Brachycephalus sulfuratus*

Figure 1. Ventral view of life specimens of *Brachycephalus sulfuratus* initially deposited in DZUP) and transferred to MHNCI. a) MHNCI 11575 (ex-DZUP 153) (Corvo, Paraná); b) MHNCI 11571 (ex-DZUP 139)(Caratuval, near the Parque Estadual das Lauráceas, Paraná); c) MHNCI 11582 (ex-DZUP 224) (Fazenda Thalia, Paraná); 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, São Paulo); 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, São Paulo); k) MHNCI 10826 (Morro do Garrafão, Santa Catarina); 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.

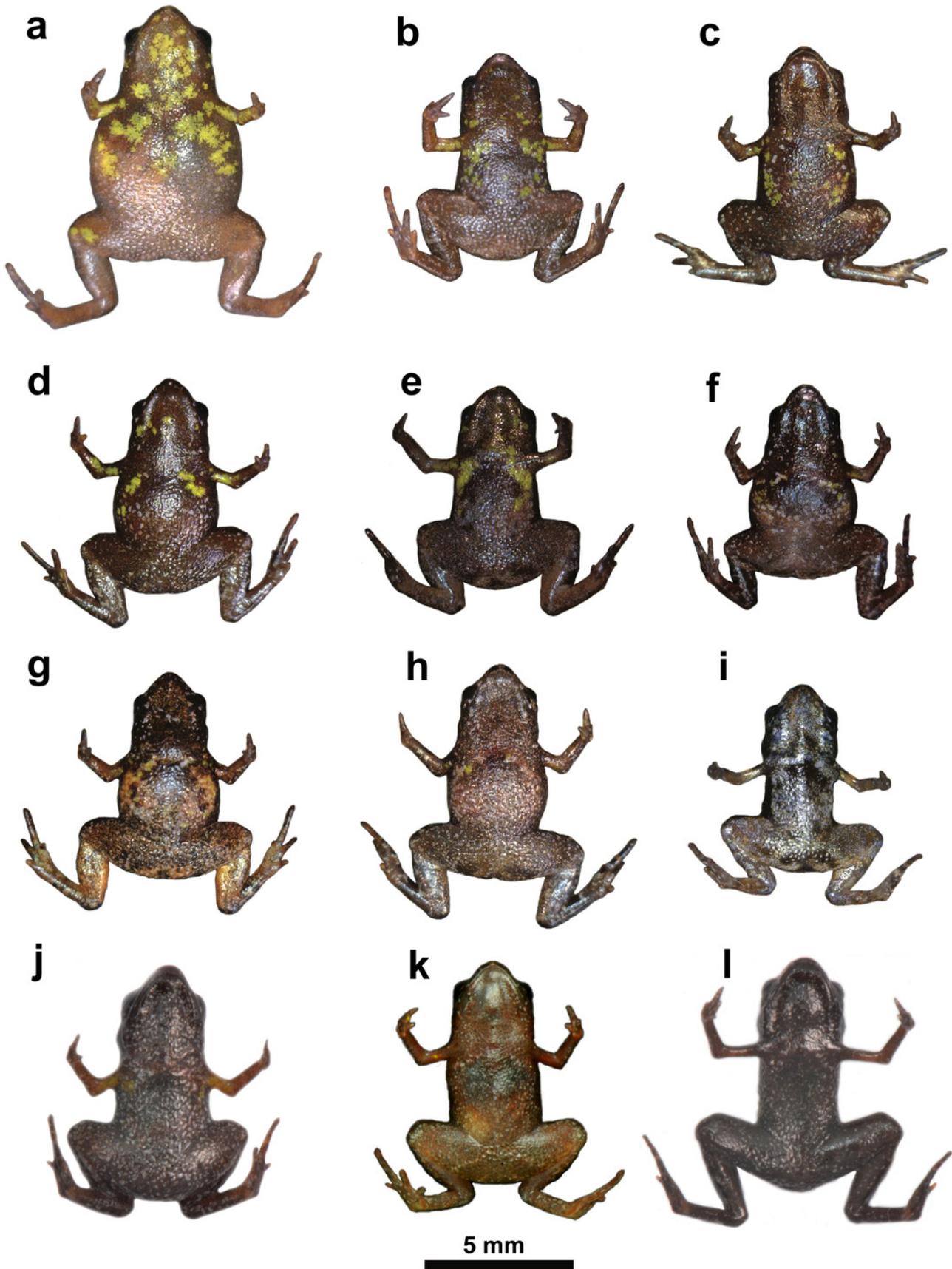


Figure 2

Ventral view of specimens of *Brachycephalus sulfuratus* and *B. hermogenesi*

Figure 2. Ventral view of specimens of *Brachycephalus sulfuratus* (a-c) and *B. hermogenesi* (d) in preservative, deposited in MHNCI and ZUEC: a) MHNCI 9800 (Salto do Inferno, Paraná); b) MHNCI 10302 (Mananciais da Serra, Paraná); c) MHNCI 10303 (Corvo, Paraná; ex DZUP 589); and d) ZUEC 9715 (Núcleo Picinguaba, São Paulo; 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 3

Oscillograms and spectrograms of *Brachycephalus sulfuratus*

Figure 3. Oscillograms (above) and spectrograms (below) of *Brachycephalus sulfuratus*. **A.** Example of one entire call with five notes (MHNCI 124; voucher MHNCI 10791 or MHNCI 10792; near the Jurupará dam, municipality of Piedade, São Paulo; M. R. Bornschein). **B.** Example of one entire call with two notes (MHNCI 129; voucher MHNCI 11583; Base of the Serra Água Limpa, municipality of Apiaí, São Paulo; M. R. Bornschein). **C.** Example of one note with 10 pulses (MHNCI 124). **D.** Example of one note with three pulses (MHNCI 124). Spectrograms are produced with Hann window, overlap of 50%, and FFT size of 512 points in A and B and 256 points in C and D. For details on geographical localities, see Table 1.

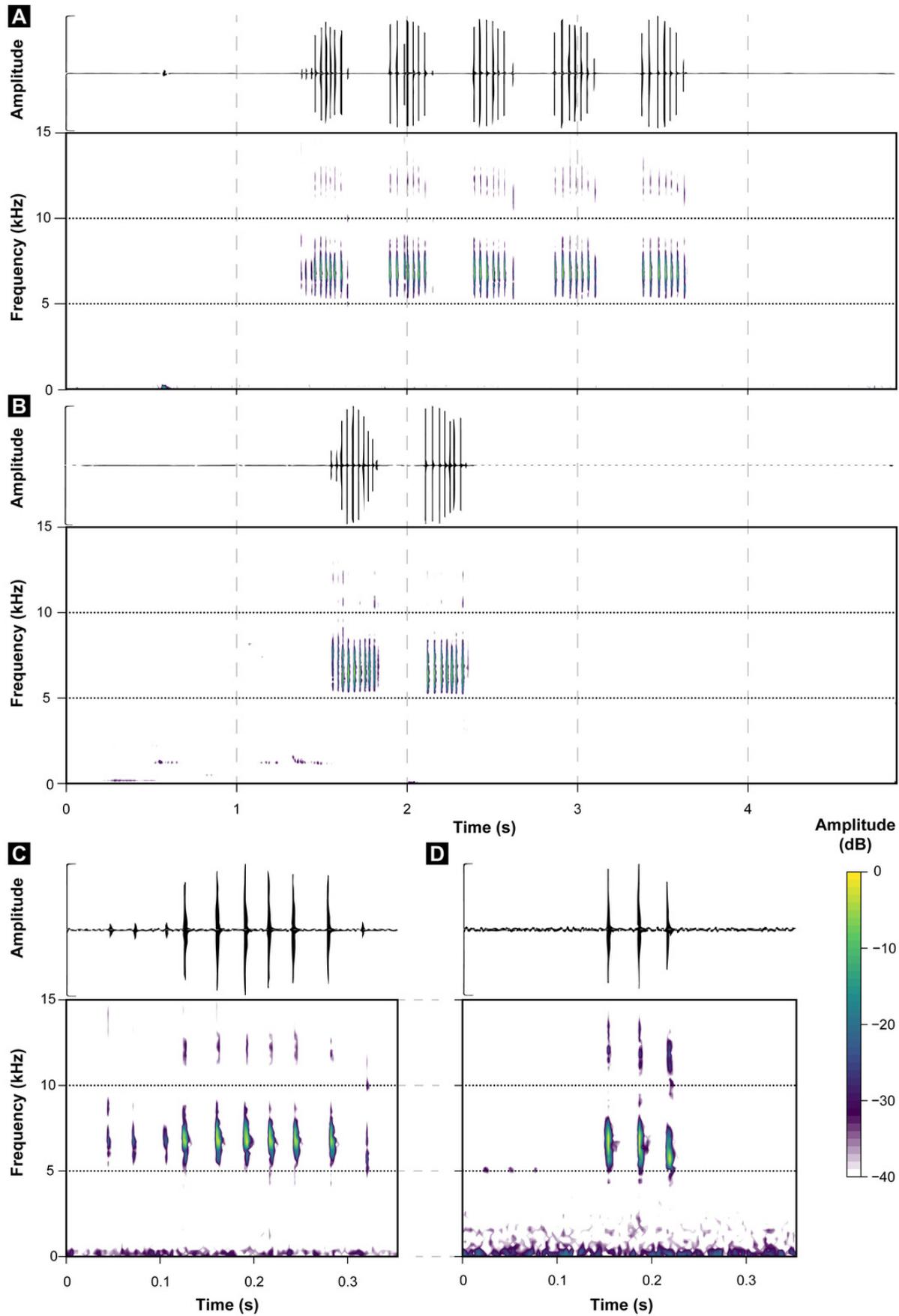


Figure 4

Oscillograms and spectrograms of *Brachycephalus hermogenesi*

Figure 4. Oscillograms (above) and spectrograms (below) of *Brachycephalus hermogenesi*. **A.** Example of one entire call with 135 notes recorded (MHNCI 165; Corcovado, municipality of Ubatuba, São Paulo; L. F. Ribeiro). **B.** Example of one isolated note with two pulses (MHNCI 183; Núcleo Pinguaba, Parque Estadual da Serra do Mar, municipality of Ubatuba, São Paulo; M. R. Bornschein). **C.** Example of one note group with three notes (each with two pulses; MHNCI 180; Núcleo Pinguaba; M. R. Bornschein). **D.** Example of one note group with four notes (each with two pulses; MHNCI 165). **E.** Example of one note group with three notes (the first with three pulses and the remaining with two pulses; MHNCI 166; Estação Biológica de Boracéia, municipality of Salesópolis, São Paulo; M. R. Bornschein). **F.** Example of one note group with three notes, with each note preceded by an attenuated note with one pulse (marked with white arrows; MHNCI 183). Spectrograms are produced with Hann window, overlap of 50%, and FFT size of 16,384 points in A, 128 points in B, and 256 points in C-F.

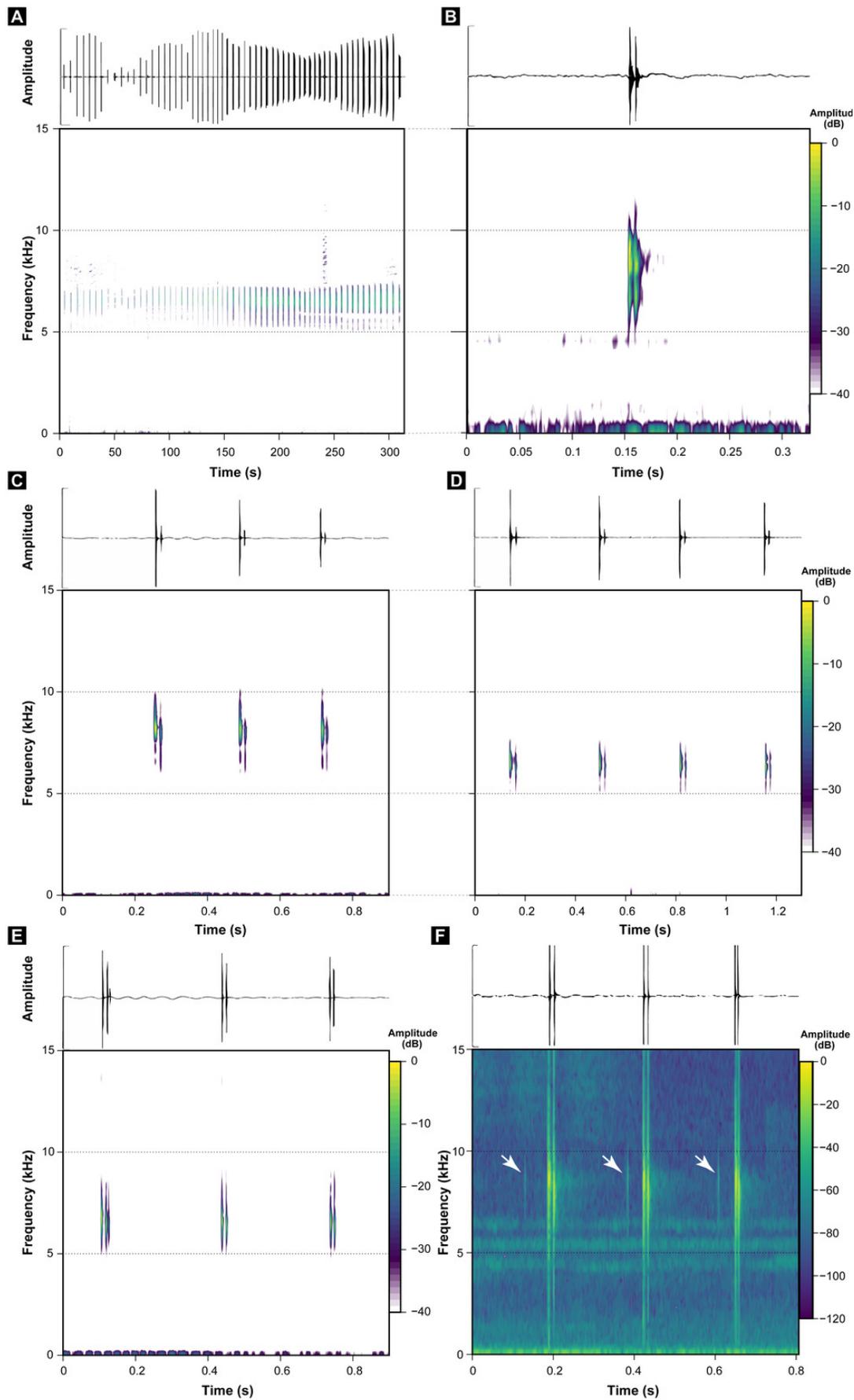


Figure 5

Oscillograms and spectrograms of *Brachycephalus* sp. (other than *B. sulfuratus* and *B. hermogenesi*)

Figure 5. Oscillograms (above) and spectrograms (below) of *Brachycephalus* sp. (other than *B. sulfuratus* and *B. hermogenesi*). **A.** Example of one entire call with 71 notes recorded (MHNCI 200; Corcovado, municipality of Ubatuba, São Paulo; M. R. Bornschein). **B.** Example of one isolated note with seven pulses (MHNCI 198; Corcovado; M. R. Bornschein). **C.** Example of one note group with two notes (with nine and four pulses, respectively; MHNCI 198). **D.** Example of one note group with three notes (the first note with three pulses and the remaining notes with four pulses; MHNCI 211; Trilha do Corisco, municipality of Paraty, Rio de Janeiro; L. F. Ribeiro). Spectrograms are produced with Hann window, overlap of 50%, and FFT size of 16,384 points in A and 256 points in B-D.

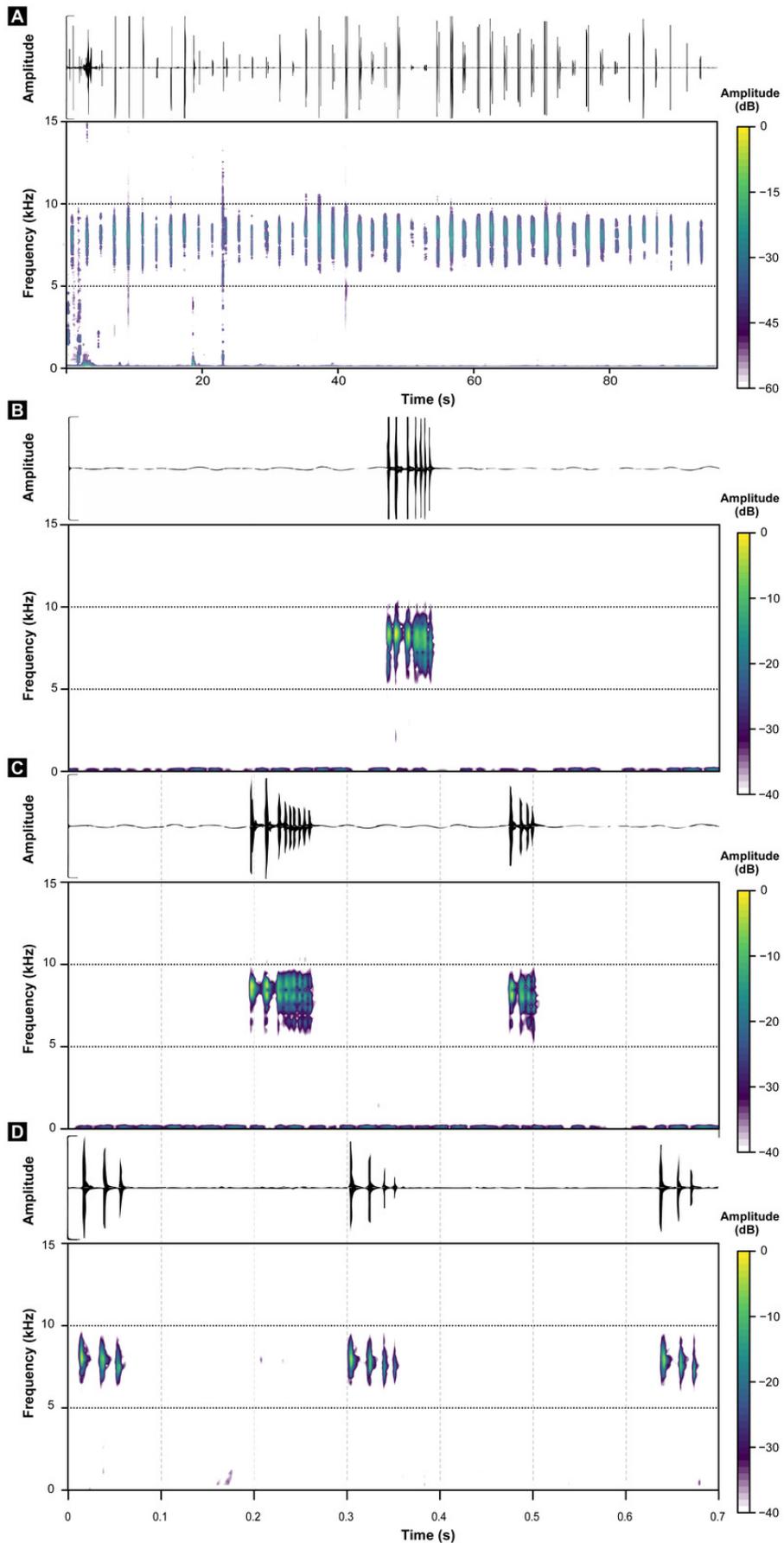


Figure 6

Current identification of records of flea toads that have been at some point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species

Figure 6. Current identification of records of flea toads that have been at some point identified as *Brachycephalus sulfuratus*, *B. hermogenesi*, and as an unidentified related species, according to the compilation of localities and review of identifications shown in Table 1. We highlighted the southernmost record of *B. hermogenesi* confirmed (1 - Parque Natural Municipal Nascentes de Paranapiacaba). 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. Map image is the intellectual property of Esri and is used herein under license. Copyright © 2020 Esri and its licensors. All rights reserved.

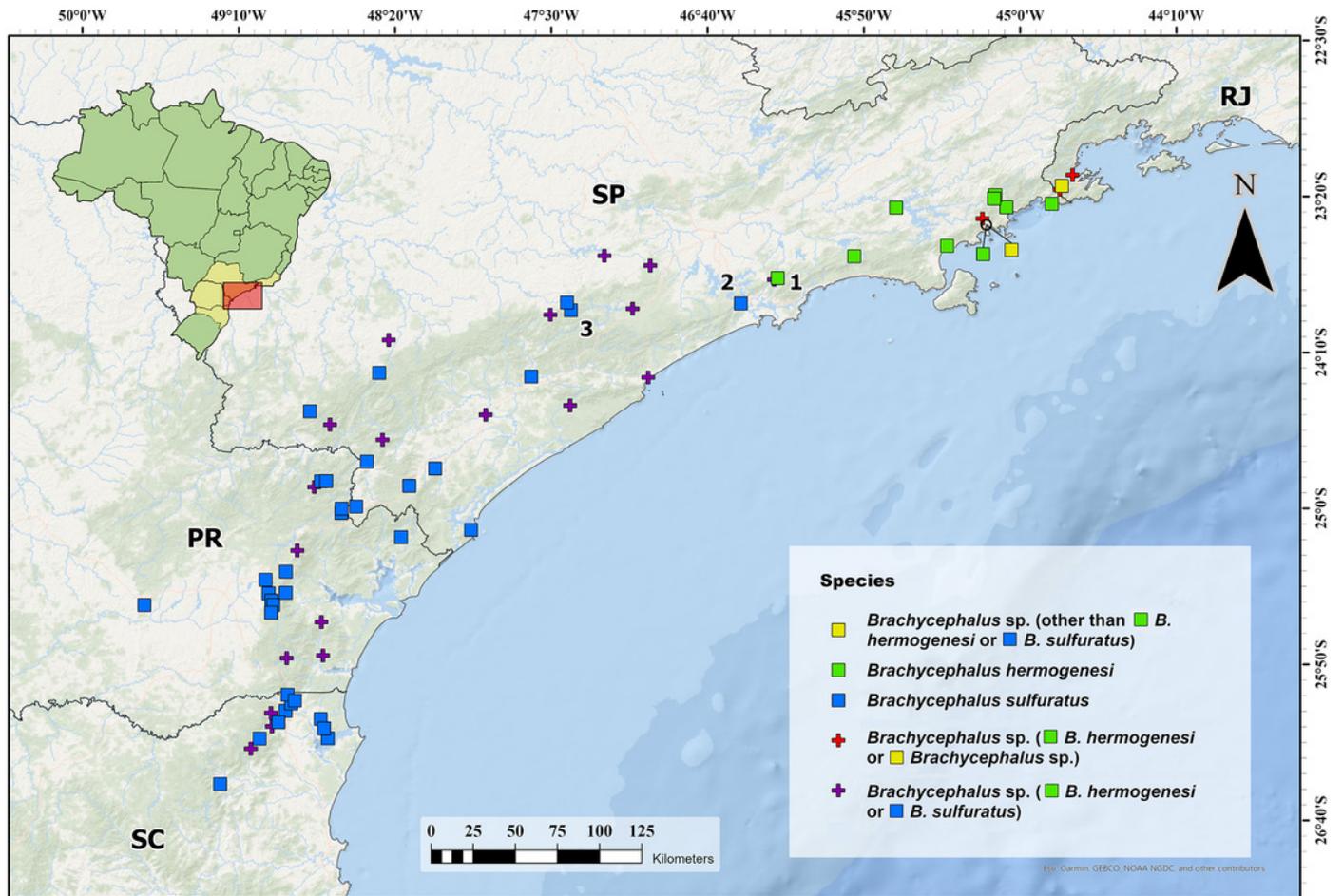


Figure 7

Phylogenetic tree based on a concatenated dataset of all mitochondrial 12S and 16S mitochondrial loci available on GenBank for specimens of the *B. didactylus* species group

Figure 7. Phylogenetic tree based on a concatenated dataset of all mitochondrial 12S and 16S mitochondrial loci available on GenBank for specimens of the *B. didactylus* species group (Table S1). The tree was rooted by its midpoint. Whenever possible, the corresponding localities available on their GenBank records were standardized based on the toponyms indicated in Table 1. Notice that the specimen originally identified as *B. hermogenesi* from the Municipality of Piedade (Condez, Sawaya & Dixo 2009, Clemente-Carvalho et al. 2011), was reverted to *B. sulfuratus* (Table 1). Branch values correspond to bootstrap support.

