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A new species of *Brachycephalus* (Anura: Brachycephalidae) from Serra do Tabuleiro, Southern Brazil

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Abstract

The number of described species of *Brachycephalus* has rapidly increased in the last decade (n = 22, which represents 56% of the total). Species of the genus *Brachycephalus* are mostly distributed in isolated mountaintops from Bahia (northeastern Brazil) to Santa Catarina states (southern Brazil), each one occupying only one or a few adjacent mountaintops. Herein, we described a new species of *Brachycephalus* of the *B. pernix* group, from Serra do Tabuleiro in Santa Catarina state, which also represents the southernmost known species. The new species can be distinguished from its congeners by a combination of characters, including the following: (1) "bufoniform" body; (2) small adult SVL: 9.57–11.10 mm for males and 10.88–12.70 mm for females; (3) head proportionally small (HL/SVL 19–28%) and eye proportionally large (ED/HL 36–56%); (4) dorsum texture rough; (5) snout shape rounded in dorsal and lateral views; (6) general dorsal body color olive green with head, arms and legs yellow-orangish scattered with olive green, and an orangish vertebral stripe spotted with white and brown colors; (7) skull and skeleton without hyperossification; (8) frontoparietal and sphenethmoid not fused; (9) advertisement with one or two high-frequency notes (6,115–6,562 Hz), and 2–4 pulses per note. The type locality is adjacent to Parque Estadual da Serra do Tabuleiro, a protected area, but we observed various agricultural activities in this locality, including the presence of exotic plants, which can change the amount and the quality of leaf litter, somehow compromising the population of the new species. Another aggravating factor is that the municipality of São Bonifácio has conflicts over land use with irregular occupation and unfinished expropriation processes in Parque Estadual da Serra do Tabuleiro. Considering that *Brachycephalus* **sp. nov.** is probably a mountaintop microendemic species, it is paramount that future studies quantifying the new species' full distribution and evaluating population trends to accurately assess its conservation status.

Keywords

Amphibia, Atlantic Forest, *Brachycephalus pernix* group, endemism, integrative taxonomy, pumpkin toadlet, Santa Catarina State

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Introduction

The frog genus *Brachycephalus* currently comprises 39 species with distribution restricted to the Atlantic Rainforest from the southern state of Bahia to the state of Santa Catarina (Bornschein et al. 2016a; Nunes et al. 2021; Condez et al. 2021; Folly et al. 2021; Frost 2023). Species of this genus are leaf-litter inhabitants with direct development (Padial et al. 2014) and are among the smallest vertebrates of the world, with sizes not exceeding 19 mm (Rittmeyer et al. 2012; Condez et al. 2020). This process of miniaturization is related to ecological and morphological features that facilitates or are consequent of this decrease in size (Rittmeyer et al. 2012) as the reduced fecundity, increase in egg size (Pombal 1999; Condez et al. 2014), and the reduction or simplification of bony elements as the loss of skull bones and phalanges and the reduced numbers of functional finger and toes (Yeh 2002; Alves et al. 2006; Silva et al. 2007; Haddad et al. 2010).

Clemente-Carvalho et al. (2011) provided a hypothesis of the phylogenetic relationship between 14 species of *Brachycephalus* described until then. Based on the topology proposed by this study, Ribeiro et al. (2015) named three phenetic groups based on morphology and distribution of the species, namely *Brachycephalus didactylus*, *Brachycephalus ephippium*, and *Brachycephalus pernix* species groups. *Brachycephalus didactylus* species group has not been recovered as a monophyletic in recent studies and their relationships within the genus is not clearly resolved (Reis et al. 2020; Condez et al. 2020; Lyra et al. 2021), while the monophyly of *B. pernix* and *B. ephippium* groups have been consistently corroborated (Clemente-Carvalho et al. 2011; Padial et al. 2014; Condez et al. 2020; Folly et al. 2020; Lyra et al. 2021). Members of the *B. ephippium* and *B. pernix* groups, known as pumpkin toadlets, exhibit "bufoniform" body shape, conspicuous aposematic coloration and distribution restricted to middle to high elevation in mountain ranges of Atlantic Rainforest domains (Ribeiro et al. 2015; Bornschein et al. 2016a), with exception of the lowland *B. actaeus*, of the *B. pernix* group (Monteiro et al. 2018a). Members of *B. ephippium* group have distribution centered in southeastern Brazil and presence of dermal co-ossification (Ribeiro et al. 2015), while members of *B. pernix* group have distribution restricted to southern Brazil (Ribeiro et al. 2015; Condez et al. 2020).

The known diversity of the genus *Brachycephalus* has increased considerably in the last decade with the description of 22 species (Frost et al. 2023). Most of this recently described species (14) are included in the *B. pernix* group which is currently the most species-rich on the genus, comprising 19 species distributed in Paraná and Santa Catarina states, Brazil: *B. actaeus* Monteiro et al., 2018a; *B. albolineatus* Bornschein et al., 2016b; *B. auroguttatus* Ribeiro, Firkowski, Bornschein & Pie, 2015; *B. boticario* Pie et al., 2015; *B. brunneus* Ribeiro, Alves, Haddad & Reis, 2005; *B. coloratus* Ribeiro et al., 2017; *B. curupira* Ribeiro et al., 2017; *B. ferruginus* Alves, Ribeiro, Haddad & Reis, 2006; *B. fuscolineatus* Pie et al., 2015; *B. izecksohni* Ribeiro, Alves, Haddad & Reis, 2005; *B. leopardus* Ribeiro, Firkowski & Pie, 2015; *B. mariaeterezae* Bornschein et al., 2015; *B. mirissimus* Pie et al., 2018; *B. olivaceus* Bornschein et al., 2015; *B. pernix* Pombal, Wistuba & Bornschein, 1998; *B. pombali* Alves, Ribeiro, Haddad & Reis, 2006; *B. quiririensis* Pie & Ribeiro, 2015; *B. tridactylus* Garey, Lima, Hartmann & Haddad, 2012; and *B. verrucosus* Ribeiro, Firkowski, Bornschein & Pie, 2015.

Is consensus that the known taxonomic diversity in *Brachycephalus* tends to increase with additional surveys in mountain ranges of southern and southeastern Brazil (Haddad et al. 2010; Bornschein et al. 2016b). Herein, we contribute to the knowledge of the diversity and distribution of this genus by describing a new species of the *B. pernix* group, which also represents the southernmost known species. The specimens were collected during an anuran survey in Serra do Tabuleiro State Park (Parque Estadual da Serra do Tabuleiro), supported by Biodiversity Research Program (Programa de Pesquisa em Biodiversidade – PPBio – CNPq process 457524/2012-0). Diagnosis of the new species is based on external morphology, color pattern, vocalization, osteology, and molecular evidence (mtDNA).

Methods

Sampling

We sampled individuals of new species during an inventory of the amphibians of the Parque Estadual da Serra do Tabuleiro, a conservation unit in the Serra do Tabuleiro mountain complex, eastern state of Santa Catarina, Southern Brazil. This protected area comprises 84.130 ha covered by a complex of phytogeographic units of Atlantic Rainforest (Socioambiental 2002; IMA 2018). The coastal line in this portion of the state is covered by mangroves and coastal vegetation (restingas) on Quaternary sand plain. The slopes are covered by Ombrophilous Dense Forest up to 1000 meters above sea levels. The higher altitudes of the Serra do Tabuleiro are represented by a mosaic of Campos de Altitude (highland fields) and forest patches, with fragments of 'Araucaria' forest being present in isolated areas of the western part of the Serra do Tabuleiro (Socioambiental 2002; IMA 2018).

We carried out two field expeditions to the western portion of the mountain complex, in November 2015 and in January 2018. Calls of the new species were recorded on an Ombrophilous Dense Forest portion at 900 meters high. On both occasions we camped next to the collection points where we heard the call, in order to collect the species and evaluate the daily cycle frogs' vocalization activity. All specimens were collected by active searches on the leaf litter floor, with a sampling effort of 36.0 hours-person in 2015 and 36.7 hours-person in 2018. In 2018, we also used pitfall-traps (700 ml), totalizing 105 pitfalls-day, but without success in *Brachycephalus* cap-

ture. The collected specimens were killed using 5% lidocaine, had tissue samples (liver) taken, fixed in 10% formalin, and transferred to 70% ethanol for permanent storage (following Conselho Federal de Biologia-CFBio Nº 148/2012).

Osteological, Morphological and Morphometric Assessment

We compared the morphological traits based on the analysis of individuals housed in two herpetological collections: Museu de História Natural Capão da Imbuia and Museu Nacional do Rio de Janeiro (*B. actaeus*, *B. albolineatus*, *B. auroguttatus*, *B. boticario*, *B. brunneus*, *B. coloratus*, *B. curupira*, *B. fuscolineatus*, *B. leopardus*, *B. mariaterezae*, *B. mirissimus*, *B. olivaceus*, *B. pernix*, *B. pombali*, *B. quiririensis*, *B. tridactylus*, and *B. verrucosus*; see Appendix) and on the literature available for the remaining species of the genus.

We analyzed morphological data of the new species of *Brachycephalus* following the terminology for diagnostic features and morphological structures of Monteiro et al. (2018a). For the description of body colors, we follow Ribeiro et al. (2015). As the skin texture is a subjective characteristic and can change due to the specimens' fixation, we prefer to consider only two categories: "smooth" and "rough". Terms like "granular", "rugose", and "very rough" used in other descriptions of *Brachycephalus* species (e.g., Ribeiro et al. 2015; Bornschein et al. 2016b; Pie et al. 2018a) were considered here as "rough". To characterize the vocal slits and choanas, we followed Tyler (1971) and Moura et al. (2021).

A single person (M. Wachlevski) took 14 measurements (in millimeters) of preserved type series specimens with a stereomicroscope Tecnival stereomicroscope, following Condez et al. (2016), in millimeters: SVL (snout-vent length); HL (head length; from tip of snout to angle of jaw); HW (head width; greatest width of head located between angle of jaw); ND (nostril diameter); IND (internostril distance; between inner margins of nostrils); ED (eye diameter); IOD (interorbital distance; between anterior corners of eyes); END (eye-nostril distance; from anterior corner of the eye to posterior margin of nostril); THL (thigh length); TBL (tibia length); FL (foot and tarsus length; from the longest toe to the plantar plus tarsus length); UAL (upper arm length); FAL (forearm length); and HAL (hand length; between the palmar and the longest finger). We determined the sex of each individual by the presence of vocal slits in males and their absence in females; however, we were not able to sex five specimens (CFBH 45452, MNRJ 93856, 93857–58, ZUFMS-AMP14531). We provide measurements of males (n = 8) and females (n = 3) separately, and the measurements of the type series (n = 11).

We cleared and stained the skeleton of three topotypes (CHSA.A 1404, MNRJ 93855 and UNIFESSPA 112, one female and two males, included in the sexed specimens presented in Table 1) for osteological observations, using alizarin red and alcian blue according to Taylor and Van Dyke (1985). The general anatomical terminology follows Trueb (1973; 1993). We use the museum acronyms of Sabaj (2016).

Table 1. Measurements in millimeters of the type series of *Brachycephalus tabuleiro* **sp. nov.**. Measurements abbreviations are listed in Material and Methods. Values are presented as mean ± SD (range).

Measurements	Males (n = 8)	Females (n = 3)
SVL	10.3±0.53 (9.57–11.10)	11.75±0.91 (10.88–12.70)
HL	2.33±0.36 (1.94–2.98)	2.64±0.77 (2.02–3.50)
HW	4.21±0.18 (4.00–4.56)	4.31±0.51 (3.77–4.78)
ND	0.17±0.03 (0.13–0.20)	0.22±0.04 (0.18–0.25)
IND	1.24±0.13 (1.04–1.45)	2.46±0.28 (2.14–2.64)
ED	1.10±0.07 (1.03–1.25)	1.31±0.36 (0.98–1.70)
IOD	2.30±0.17 (1.97–2.48)	2.46±0.28 (2.64–2.14)
END	0.48±0.04 (0.40–0.51)	0.66±0.38 (0.43–1.10)
THL	3.42±0.44 (2.62–3.90)	3.66±0.57 (2.34–4.30)
TBL	3.26±0.14 (3.10–3.49)	3.44±0.05 (3.40–3.50)
FL	4.09±0.17 (3.84–4.35)	3.66±0.23 (3.40–3.85)
UAL	1.75±0.20 (1.46–1.99)	2.11±0.69 (1.63–2.90)
FAL	2.35±0.17 (2.11–2.54)	2.44±0.23 (2.29–2.70)
HAL	1.84±0.05 (1.77–1.91)	2.05±0.24 (1.82–2.30)

Acoustic Analyses

We recorded the advertisement call of three males (unvouchered specimens). Two males were recorded with a Tascam DR-40 recorder with the device's built-in microphones on 12 November 2015, around 10:30 a.m. and one individual was recorded with a Marantz PMD660 professional coupled to a unidirectional microphone Sennheiser ME67 on 7 January 2018, 4:15 p.m, in São Bonifácio municipality, Santa Catarina state. The calls were recorded at a sampling rate of 44.0 kHz and 16 bit resolution. We analyzed calls of four acoustic archives in Raven Pro1.5 for Mac (Bioacoustics Research Program 2012) and constructed audio spectrograms in R software using the package "seewave" (Sueur et al. 2008; R Development Core Team) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat top filter. We analyzed acoustic parameters typically evaluated in anuran taxonomy studies (e.g., Condez et al. 2016; Guimarães et al. 2017; Kohler et al. 2017; Monteiro et al. 2018b): (1) call duration in seconds (CD), (2) interval between calls in seconds (IC), (3) call rate in calls/s (CR),

(4) notes per call (NC), (5) note duration in seconds (ND), (6) interval between notes in seconds (NI), (7) note rate in notes/s (NR), (8) number of pulses per note (PN), (9) pulse rate in pulses/s (PR), (10) pulse duration in seconds (PD), (11) lower frequency (LF), (12) upper frequency (UF), (13) dominant frequency (DF) and, (14) Bandwidth 90% (BD), in Hz. To compare the advertisement call of *Brachycephalus* sp.n. with other *Brachycephalus* species, we used only calls with one note, because were more frequently emitted (see results). All values were presented in mean, standard deviation, values minimum and maximum of each acoustic variable.

We followed Köhler et al. (2017) for the call definition, which is considered an acoustic unit of frog vocalization, separated from other calls by periods of silence (longer than the call), known as call-centered approach. However, some advertisement call descriptions of *Brachycephalus* species used a note-centered approach (e.g., Bornschein et al. 2018; Pie et al. 2018a; Folly et al. 2020) that defines an entire coherent unit of sound as call, which is more applicable in cases of complex calls composed of different note types (Toledo et al. 2015; Köhler et al. 2017). We considered the advertisement call of the new species as a simple call, and the call-centered seemed more appropriate. Thus, in order to provide a proper comparison among all species of the genus, we used the values of each note equivalent to a call for *B. darkside* (Guimarães et al. 2017), *B. albolineatus* (Bornschein et al. 2018), *B. mirissimus* (Pie et al. 2018a) and, *B. bufonoides* (Folly et al. 2020). For instance, instead of using the value of the total duration of the call described in the articles with the note-centered approach, we consider the duration of the notes to compare with the duration of the call of the new species. We obtained comparative acoustic data for other species from the available literature (Wistuba 1998; Pombal et al. 1994; Verdade et al. 2008; Araújo et al. 2012; Garey et al. 2012; Condez et al. 2014; Tandel et al. 2014; Condez et al. 2016; Guimarães et al. 2017; Bornschein et al. 2018; Monteiro et al. 2018a; Monteiro et al. 2018b; Pie et al. 2018a; Folly et al. 2020; Nunes et al. 2021). We deposited the sound recordings in the Coleção Audiovisual do Semiárido (CASA) in Universidade Federal Rural do Semi-Árido (CASA 154–157).

Phylogenetic Inference and Genetic Distances

Whole genomic DNA was extracted from liver tissue using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN). PCR amplification and sequencing was performed with the primers 16Sar-L (CGCCTGTTTATCAAAAACAT) and 16Sbr-H (CCGGTCTGAACTCAGATCACGT) of Palumbi et al. (2002) to amplify a section of the mitochondrial 16S ribosomal RNA gene. PCR conditions followed those described by Costa et al. (2016). PCR products were purified with ethanol/sodium acetate and sequenced with an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, California/CA). We edited the sequences by aligning forward and reverse reads using Geneious v. 9.1.8. (https://www.geneious.com). Final sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank). For phylogenetic analyses, we based on 16S sequences from 104 specimens of *Brachycephalus* available in GenBank (Table S1) along with *Ischnocnema lactea* (JX267308) as outgroup. We highlight that we used only sequences that cover the same 16S section from Palumbi et al. (2002), and due to the immense amount of 16S sequences available (>340 sequences), many of them from the same species, we chose, when available, up to three sequences from each species and candidate/non-described species identified in previous studies (e.g., Condez et al. 2021; Folly et al 2021). We performed a Bayesian phylogenetic analysis of 16S using BEAST v.2.6.6 (Bouckaert et al. 2019) for 50 million generations, sampling every 5,000 steps using a Yule Process tree prior. We selected the most appropriate nucleotide substitution model based on the Bayesian information criterion (BIC) with jModelTest (Darriba et al. 2012): HKY. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v1.7.1 (Rambaut et al. 2018). The first 10% of sampled genealogies were discarded as burn-in, and the maximum clade credibility tree with median node ages was calculated with TreeAnnotator v.2.6.3 (Bouckaert et al. 2019). We also calculated sequence divergence (uncorrected p distance) among species/individuals with complete deletion of gaps, using MEGA v10.1.8 (Kumar et al. 2018).

Results

Brachycephalus tabuleiro sp. nov.

https://zoobank.org/6B326FE8-854C-4263-A840-35E60DA588A0

Figs 1–6

Holotype. MNRJ 93859, adult male, collected at Serra do Tabuleiro, São Bonifácio municipality, Santa Catarina state, Brazil (27°53’58”S, 48°53’5”W, 915 m.a.s.l.; datum = WGS84), on 12 November 2015, by L.O. Drummond, L.T. Sabagh, and L. Ugioni.

Paratypes. MHNCI 11498 (adult male), and UNIFESSPA 113 (adult female) collected with the holotype, CFBH 45453, CHSA.A 1403, MNRJ 93854–55, UNIFESSPA 112, ZUFMS-AMP14532 (adult males), CHSA.A 1404, and MHNCI 11499 (adult females) collected at the type locality, on 7 January 2018, by L. Ugioni, M. Wachlevski, B. Cesário, and D.C. Passos.

Non-type material. ZUFMS-AMP14531, MNRJ 93856–58 and, CFBH 45452 (adults not sexed) collected at the type locality, on 7 January 2018, by L. Ugioni, M. Wachlevski, B. Cesário, and D. C. Passos.

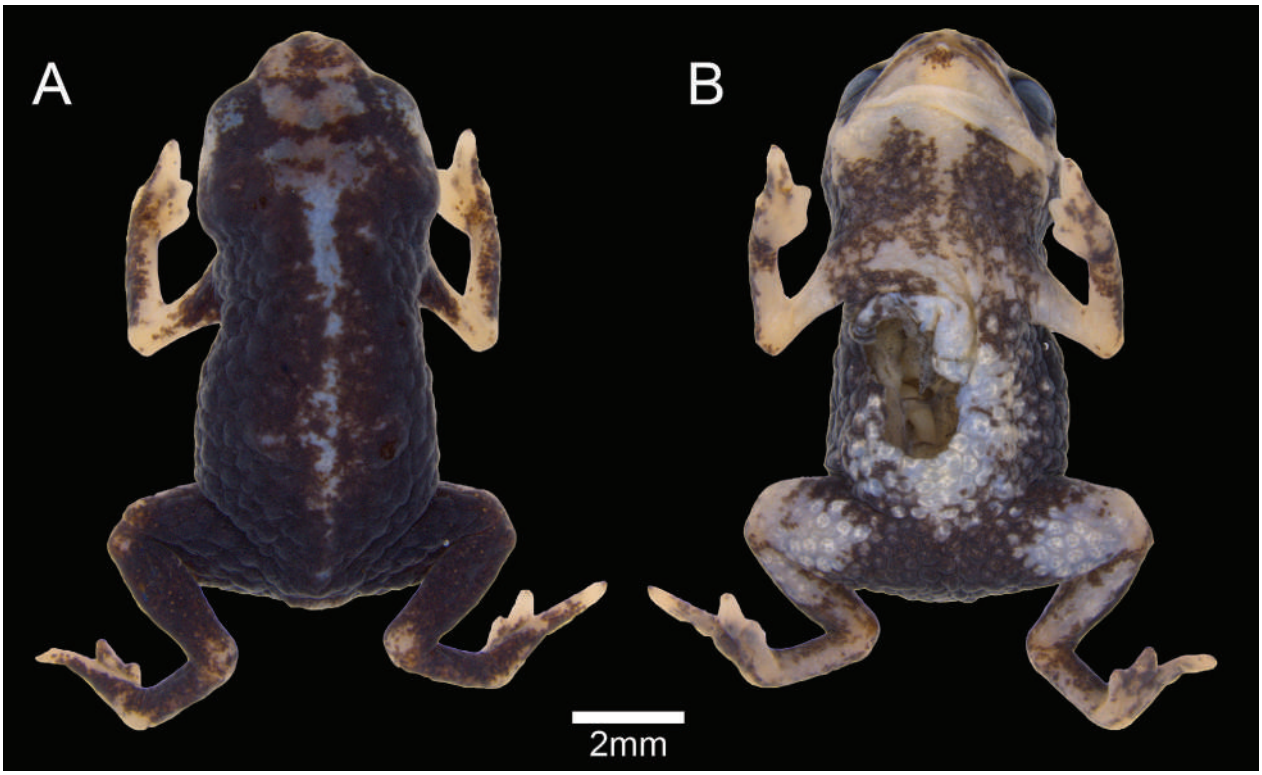

Figure 1. Holotype of *Brachycephalus tabuleiro* (MNRJ 93859, adult male, SVL 10.40 mm) from Serra do Tabuleiro, São Bonifácio municipality, Santa Catarina state, Brazil. (**A**) Dorsal and (**B**) ventral views.

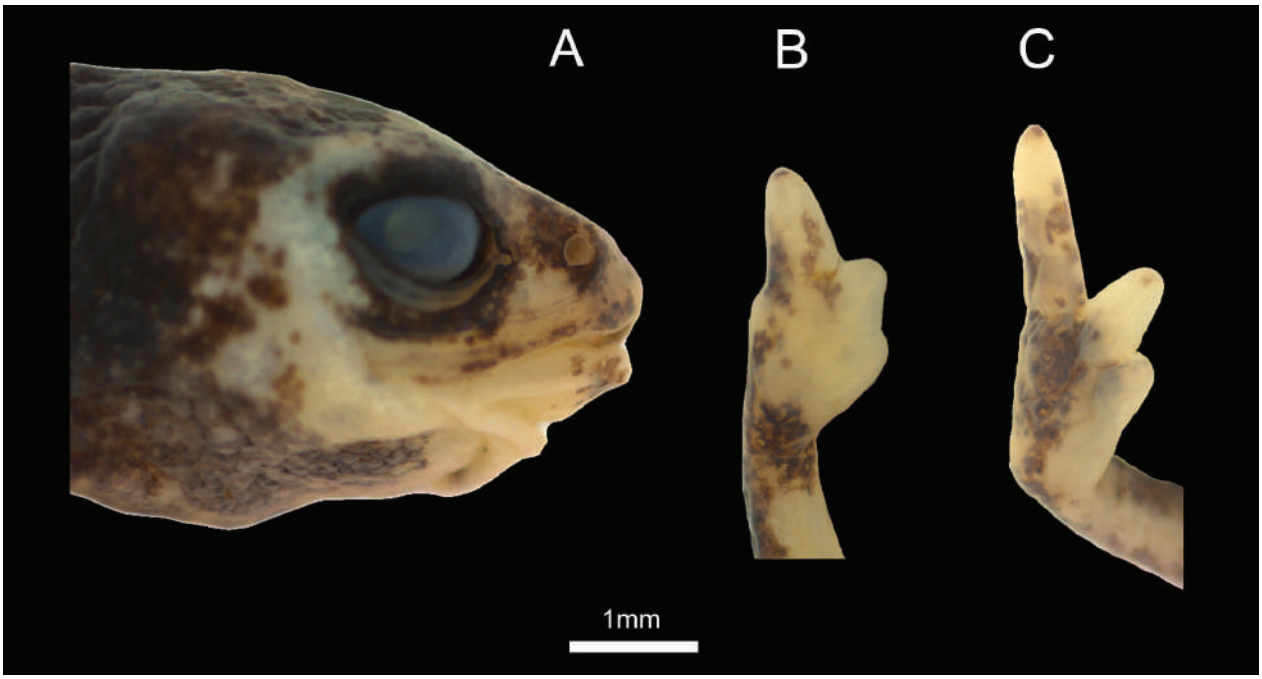

Figure 2. Holotype of *Brachycephalus tabuleiro* (MNRJ 93859, adult male, SVL 10.40 mm) from Serra do Tabuleiro, São Bonifácio municipality, Santa Catarina state, Brazil. (**A**) Lateral view of the head, ventral views of the (**B**) hand and the (**C**) foot.

Diagnosis. *Brachycephalus tabuleiro* is a new species of the *B. pernix* group and can be distinguished from its congeners using the following combination of characters: (1) "bufoniform" body; (2) small adult SVL: 9.57–11.10 mm for males (n = 6), 10.88–12.70 mm for females (n = 3); (3) head proportionally small (HL/SVL 19–28%) and eye proportionally large (ED/HL 36–56%); (4) dorsum texture rough; (5) snout shape rounded in dorsal and lateral views; (6) general dorsal body color olive green with head, arms and legs yellow-orangish scattered with olive green, and an orangish vertebral stripe spotted with white and brown colors; (7) background yellow-orangish in the ventral region with reticulated green stains, mainly concentrated in the peripheral portion; (8) fingers I and

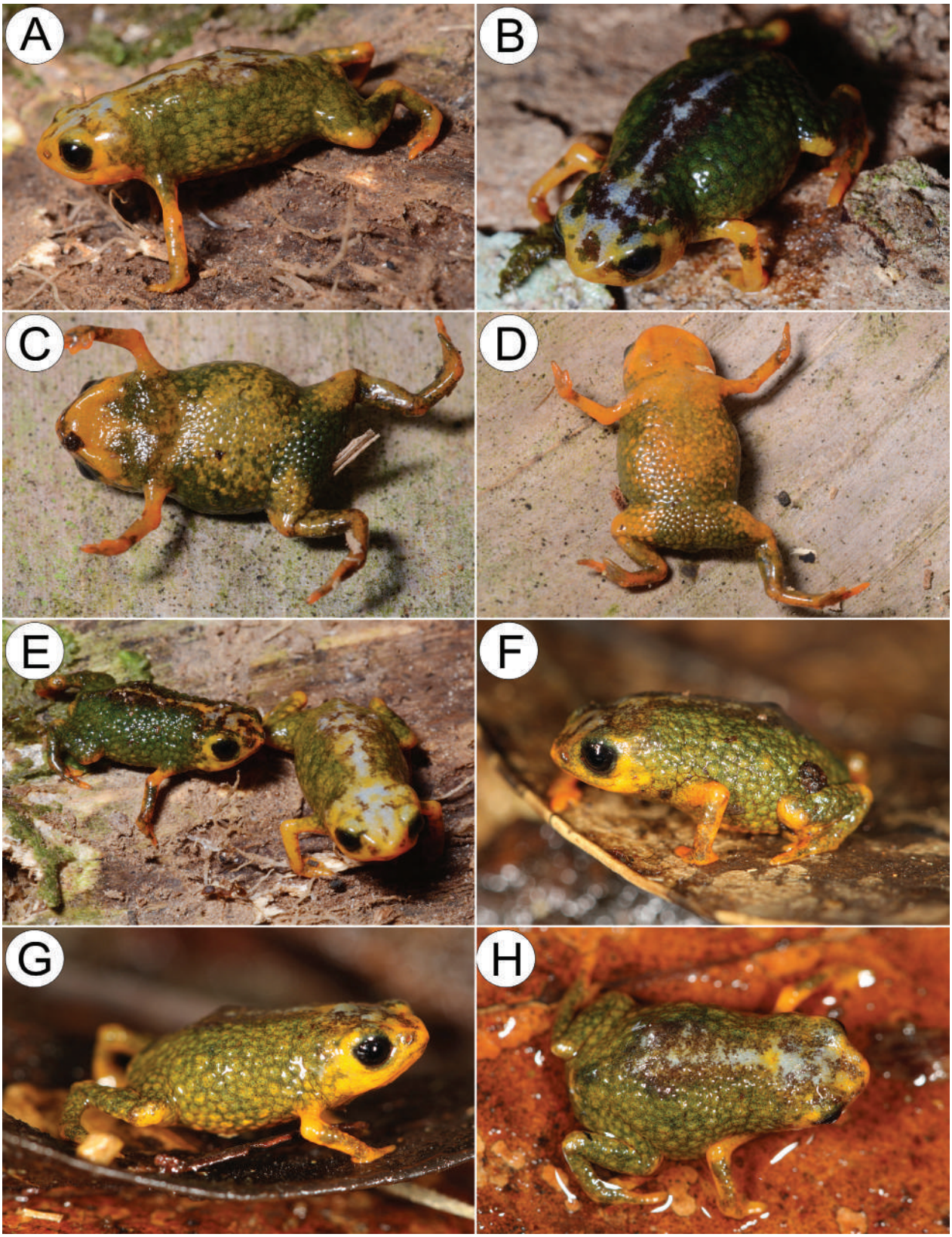

Figure 3. Live specimens of *Brachycephalus tabuleiro*. **A** UNIFESSPA 113 (adult male), **B** MHNCI 11498 (adult female), **C** MNRJ 93859 (adult male, holotype), **D** MHNCI 11498 (adult female), **E** MNRJ 93859 (adult male, holotype) and UNIFESSPA 113 (adult male), **F** CHSA.A 1404 (adult female), **G** and **H** ZUFMS-AMP 14531 (adult not sexed). Photos: Diego José Santana (A, B, C, D, E), Milena Wachlevski (F, G, H).

IV greatly reduced, represented externally by a small lump, finger II reduced but distinct, and finger III larger and robust; (9) tips of the fingers I, II, and III rounded; (10) toes I and V present but externally indistinguishable, toe II greatly reduced, toe III short and distinct, and toe IV larger and robust; (11) tips of the toes II, III, and IV

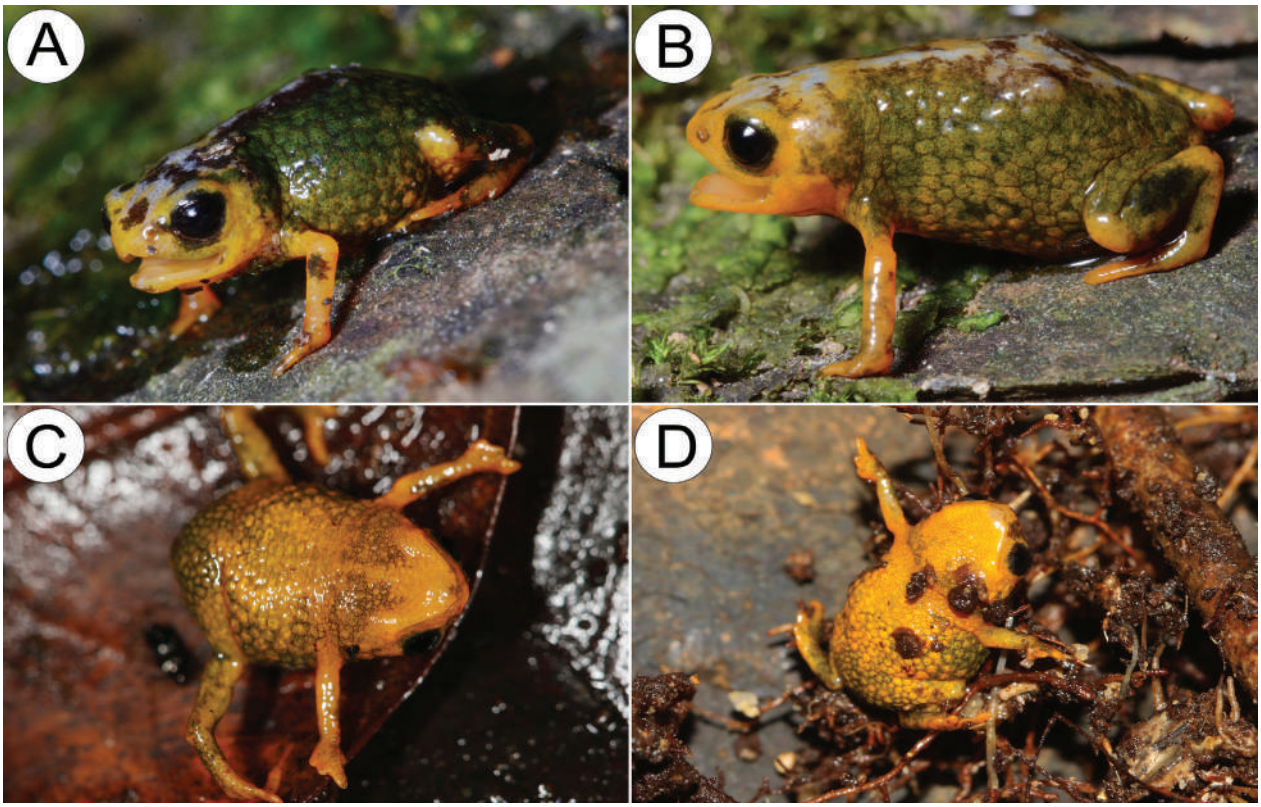

Figure 4. Behavior. **A** MHNCI 11498 (adult female), **B** UNIFESSPA 113 (adult male), **C** ZUFMS-AMP 14531 (adult not sexed), **D** UNIFESSPA 112 (adult male). Photos: Diego José Santana (A, B), Milena Wachlevski (C, D).

rounded; (12) skull and skeleton without hyperossification; (13) frontoparietal and sphenethmoid not fused; (14) premaxillary odontoids presents; (15) relative lengths of the transverse processes in descending order: III > IV > V > II > VI > VII >VIII; (16) radius and ulna fused, (17) phalangeal formula of fingers 1-2-3-1; (18) phalangeal formula of toes 0-2-3-4-0; (19) advertisement with one or two high-frequency notes (6115.4–6562.5 Hz), and 2–4 pulses per note.

Comparisons. Characteristics of compared species are presented in parentheses. The new species presents a "bufoniform" body – term defined by Miranda-Ribeiro (1920) – and yellow-orangish coloration, which distinguished it from *B. didactylus*, *B. hermogenesi*, *B. pulex*, *B. puri*, and *B. sulfuratus* ("leptodactyliform" body – term defined by Ribeiro et al. 2015 – and cryptic coloration). The absence of dermal ossification distinguishes the new species from *B. alipioi*, *B. bufonoides*, *B. crispus*, *B. darkside*, *B. ephippium*, *B. garbeanus*, *B. guarani*, *B. ibitinga*, *B. margaritatus*, *B. nodoterga*, *B. pitanga*, *B. rotenbergae*, *B. toby*, and *B. vertebralis* (some degree of hyperossification of the skull and skeleton in these species; Ribeiro et al. 2015; Condez et al. 2021; Nunes et al. 2021).

From species with "bufoniform" body, some degree of dorsal yellow-orangish coloration (except *B. curupira* and *B. brunneus* that present brown dorsal coloration, *B. olivaceus* and some specimens of *B. actaeus* that present dark-green dorsal coloration), and absence of dermal ossification, *Brachycephalus tabuleiro* differs by the following characteristics: (1) small body size SVL 9.57–11.10 mm in males, 10.88–12.70 mm in females [males SVL 11.6–12.5 mm and females SVL 13.0–14.5 mm in *B. ferruginus* (Alves et al. 2006); males SVL 12.0–13.3 mm and females SVL 14.1–15.8 mm in *B. pernix* (Pombal et al. 1998); males SVL 12.6–13.9 and females SVL 14.6–15.3 mm in *B. pombali* (Alves et al. 2006); and females of the following species: SVL 12.5–13.1 mm in *B. izecksohni* (Ribeiro et al. 2005); 13.5–13.8 in *B. tridactylus* (Garey et al. 2012) (see Table 1 for more measurements)]; (2) proportionally shorter head relative to body length (HL/SVL 19–28%; mean 23 ± 3) for the 16 adult examined specimens [28–34% in *B. albolineatus* (Monteiro et al. 2018); 29–38% in *B. auroguttatus* (Ribeiro et al. 2015); 31–36% in *B. boticario* (Ribeiro et al. 2015); 33–41% in *B. brunneus* (Ribeiro et al. 2005); 35–40% in *B. ferruginus* (Alves et al. 2006); 29–34% in *B. fuscolineatus* (Ribeiro et al. 2015); 31–35% in *B. leopardus* (Ribeiro et al. 2015); 29–36% in *B. mariaeterezae* (Ribeiro et al. 2015); 32–36% in *B. olivaceus* (Ribeiro et al. 2015); 36–39% in *B. pombali* (Alves et al. 2006); 31–36% in *B. quiririensis* (Pie and Ribeiro 2015); 30–36% in *B. verrucosus* (Ribeiro et al. 2015)]; (3) proportionally larger eye diameter related to head length (ED/HL 36–56%, mean 49 ± 5) [20–29% in *B. brunneus* (Ribeiro et al. 2005); 23–28% in *B. ferruginus* (Alves et al. 2006); 26–36% in *B. olivaceus* (Ribeiro et al. 2015); 23–27% in *B. pombali* (Alves et al. 2006); 28–34% in *B. quiririensis* (Pie and Ribeiro 2015)]; (4) dorsum texture rough [smooth in *B. brunneus* (Ribeiro et al. 2005), *B. coloratus* (Ribeiro et al. 2017), *B. curupira* (Ribeiro et al. 2017), *B. ferruginus*

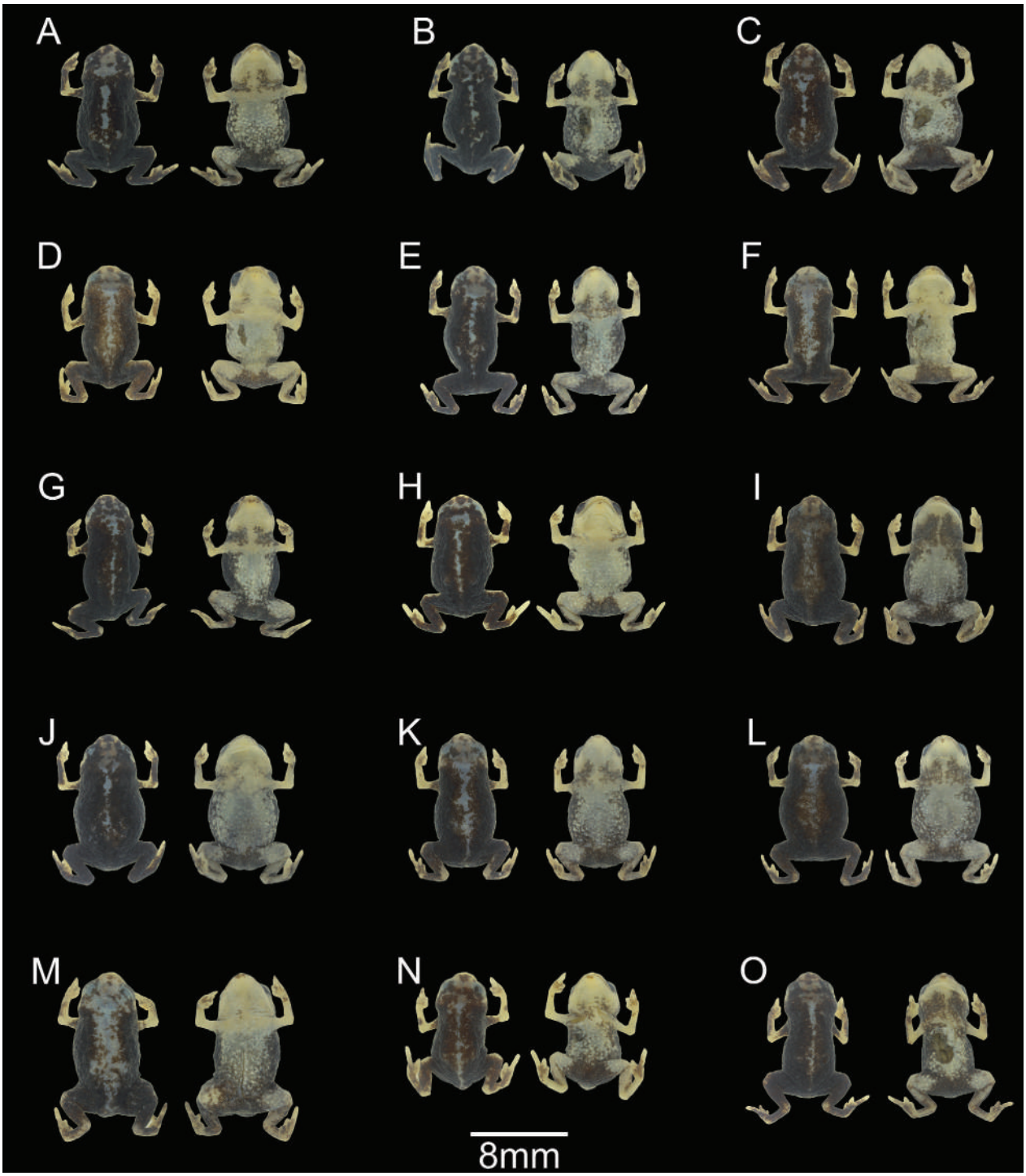

Figure 5. Morphological and color variation of preserved specimens of *Brachycephalus tabuleiro* from Serra do Tabuleiro, São Bonifácio municipality, Santa Catarina state, Brazil. **A** UNIFESSPA 113 (adult male), **B** CFBH 45452 (adult not sexed), **C** MNRJ 93856 (adult not sexed), **D** CHSA.A 1403 (adult male), **E** MNRJ 93857 (adult not sexed), **F** MNRJ 93854 (adult male), **G** MNRJ 93857 (adult not sexed), **H** MNRJ 93855 (adult male), **I** CHSA.A 1404 (adult female), **J** UNIFESSPA 112 (adult male), **K** ZUFMS-AMP 14532 (adult male), **L** ZUFMS-AMP 14531 (adult not sexed), **M** MHNCI 11499 (adult female), **N** MHNCI 11498 (adult female), **O** MNRJ 93859 (adult male, holotype).

(Alves et al. 2006), *B. izecksohni* (Ribeiro et al. 2005), *B. leopardus* (Ribeiro et al. 2015), *B. pernix* (Pombal et al. 1998), *B. pombali* (Alves et al. 2006), and *B. tridactylus* (Garey et al. 2012)]; (5) snout shape rounded in lateral and dorsal views [semicircular in dorsal view in *B. coloratus* (Ribeiro et al. 2017), *B. mirissimus* (Pie et al. 2018), and *B. pernix* (Pombal et al. 1998); mucronate in dorsal view in *B. brunneus* (Ribeiro et al. 2005) and *B. quiririensis* (Pie and Ribeiro 2015); and slightly truncated in dorsal and lateral views in *B. leopardus* (Ribeiro et al. 2015)]; (6) olive green general color with head, arms and legs yellow-orangish scattered with olive green, and an orangish vertebral stripe spotted with grey and brown colors [brown in *B. brunneus* (Ribeiro et al. 2005); bright

yellow or orange general dorsal body color in *B. auroguttatus* (Ribeiro et al. 2015), *B. boticario* (Pie et al. 2015), *B. coloratus* (Ribeiro et al. 2017), *B. ferruginus* (Alves et al. 2006), *B. fuscolineatus* (Pie et al. 2017), *B. izecksohni* (Ribeiro et al. 2005), *B. leopardus* (Ribeiro et al. 2015), *B. mariaeterezae* (Bornschein et al. 2015), *B. mirissimus* (Pie et al. 2018a), *B. quiririensis* (Pie and Ribeiro 2015), *B. pernix* (Pombal et al. 1998), *B. pombali* (Alves et al. 2006), and *B. tridacylus* (Garey et al. 2012)]; (7) finger IV greatly reduced, represented externally by a small lump [finger IV absent in *B. tridactylus* (Garey et al. 2012); (8) tips of the fingers I, II, and III rounded [finger II pointed in *B. brunneus* and *B. leopardus* (Ribeiro et al. 2005; Ribeiro et al. 2015); finger III pointed in *B. auroguttatus* (Ribeiro et al. 2015), *B. brunneus* (Ribeiro et al. 2005), *B. coloratus* (Ribeiro et al. 2017), *B. curupira* (Ribeiro et al. 2017), *B. ferruginus* (Alves et al. 2006), *B. fuscolineatus* (Pie et al. 2015), *B. leopardus* (Ribeiro et al. 2015), *B. olivaceus* (Bornschein et al. 2015), *B. pombali* (Alves et al. 2006), *B. quiririensis* (Pie and Ribeiro 2015), and *B. verrucosus* (Ribeiro et al. 2015); all fingers pointed in *B. pernix* (Pombal et al. 1998) and *B. izecksohni* (Ribeiro et al. 2005)]; (9) toes I and V not visible externally, toe II greatly reduced, toe III short and distinct, and toe IV larger and robust [toe II short and distinct in *B. auroguttatus* (Ribeiro et al. 2015), *B. boticario* (Pie et al. 2015), *B. coloratus* (Ribeiro et al. 2017), *B. curupira* (Ribeiro et al. 2017), *B. fuscolineatus* (Pie et al. 2015), *B. leopardus* (Ribeiro et al. 2015), *B. izecksohni* (Ribeiro et al. 2005), *B. mariaterezae* (Bornschein et al. 2015), *B. mirissimus* (Pie et al. 2018), *B. olivaceus* (Bornschein et al. 2015), *B. pombali* (Alves et al. 2006), *B. quiririensis* (Pie and Ribeiro 2015), *B. tridactylus* (Garey et al. 2012), and *B. verrucosus* (Ribeiro et al. 2015)]; (10) tips of the toes II, III, and IV rounded [II pointed in *B. albolineatus* (Bornschein et al. 2016), *B. brunneus* (Ribeiro et al. 2005), *B. ferruginus* (Alves et al. 2006), *B. izecksohni* (Ribeiro et al. 2005), and *B. quiririensis* (Pie and Ribeiro 2015); III pointed in *B. leopardus* (Ribeiro et al. 2015); IV pointed in *B. actaeus* (Monteiro et al. 2018), *B. ferruginus* (Alves et al. 2006), and *B. quiririensis* (Pie and Ribeiro 2015)]; (11) frontoparietal and sphenethmoid not fused [frontoparietal and sphenethmoid fused in *B. actaeus* (Monteiro et al. 2018) and *B. coloratus* (Ribeiro et al. 2017)]; (12) premaxillary odontoids presents [absent in *B. coloratus* (Ribeiro et al. 2017)]; (13) relative lengths of the transverse processes in descending order: III > IV > V > II > VI > VII>VIII [III > IV > V ≅ VI ≅ VII > VIII ≅ II in *B. brunneus* (Ribeiro et al. 2005); III > IV > II > V ≅ VI ≅ VII ≅ VIII in *B. ferruginus* and *B. pombali* (Alves et al. 2006)]; (14) radius and ulna fused [not fused in *B. brunneus* (Ribeiro et al. 2005), *B. ferruginus* (Alves et al. 2006), *B. izecksohni* (Ribeiro et al. 2005), and *B. pombali* (Alves et al. 2006)] (Fig. 6).

The advertisement call with one note of *Brachycephalus tabuleiro* differs from most of its congeners by a presence of pulsed notes with a lower number of pulses per note (2–4) [(13–17 in *B. bufonoides* (Folly et al. 2020), 7–12 in *B. crispus* (Condez et al. 2014), 5–8 in *B. darkside* (Guimarães et al. 2017), 5–15 in *B. ephippium* (Pombal et al. 1994), 4–5 in *B. hermogenesi* (Verdade et al. 2008), 8–12 in *B. ibitinga* (Condez et al. 2021), 7–14 in *B. pitanga* (Araújo et al. 2012), 8–13 in *B. rotenbergae* (Nunes et al. 2021), 4–7 in *B. sulfuratus* (Condez et al. 2016)]. Based on a call-centered approach, the advertisement call of the new species can be emitted with one or two notes (Fig.7). Considering calls with one note, the advertisement call of *Brachycephalus tabuleiro* **sp. nov.** reaches longer duration (0.028–0.062 s) than *B. albolineatus* (note duration 0.002–0.037 s; Bornschein et al. 2018), and *B. mirissimus* (note duration 0.002–0.027 s; Pie et al. 2018a), and is shorter than *B. bufonoides* (note duration 0.222–0.308 s; Folly et al. 2020), *B. crispus* (note duration 0.280 s; Condez et al. 2014), *B. darkside* (note duration 0.083–0.163 s; Guimarães et al. 2017), *B. ephippium* (note duration 0.093–0.125 s; Pombal et al. 1994), *B. hermogenesi* (note duration 0.200 s; Verdade et al. 2008), *B. ibitinga* (note duration 0.180–0.250 s; Condez et al. 2021), *B. pitanga* (note duration 0.150–0.250 s; Araújo et al. 2012), *B. rotenbergae* (note duration 0.130–0.140 s; Nunes et al. 2021), *B. sulfuratus* (note duration 1.500–2.300 s; Condez et al. 2016), and *B. tridactylus* (note duration 0.110 s; Garey et al. 2012). Regarding the dominant frequency, the advertisement call of the new species (6,115.4–6,546.0 Hz) is higher than *B. bufonoides* (4,130–4,880 Hz, Folly et al. 2020), *B. crispus* (3,500–5,700 Hz, Condez et al. 2014), *B. darkside* (2,856.4–3,796.9 Hz, Guimarães et al. 2017), *B. ibitinga* (4000–4300 Hz, Condez et al. 2021), *B. pitanga* (4,900 Hz, Araújo et al. 2012), *B. rotenbergae* (2,840–4,520 Hz, Nunes et al. 2021), and *B. tridactylus* (4800 Hz, Garey et al. 2012), and lower than *B. actaeus* (6,600–7,300 Hz, Monteiro et al. 2018a), *B. hermogenesi* (6,800 Hz, Verdade et al. 2008), and *B. olivaceus* (6,400–7,000 Hz, Monteiro et al. 2018b). In addition, pulse duration of the advertisement call of the new species (0.003–0.009 s) lasts shorter than *B. crispus* (pulse duration 0.027 s, Condez et al. 2014), *B. olivaceus* (pulse duration 0.015–0.025 s; Monteiro et al. 2018b), *B. quiririensis* (pulse duration 0.012–0.017 s; Monteiro et al. 2018b), and *B. sulfuratus* (pulse duration 0.020–0.030 s; Condez et al. 2016). For more detail and complete comparison, see Table 2.

Description of the Holotype. Body robust, "bufoniform"; head wider than long, head length 20% of SVL; snout short, rounded in dorsal and lateral views; nostrils elliptical and protuberant; canthus rostralis distinct and straight; loreal region slightly concave; eyes oriented anterolaterally; eye diameter 52% (range) of head length; tympanum absent; lips nearly sigmoid; vocal sac not expanded externally; elongated vocal slits; tongue elliptic, longer than wide, with the posterior half not adherent to floor of mouth; vomerine teeth absent; choanae small and rounded, anterior to eyes, separated from each other. Arm and forearm moderately slender; hands with fingers I and IV greatly reduced, represented externally by a small lump; finger II reduced but distinct, and finger III larger and robust; fingertips I, II, and III rounded; finger lengths IV < I < II < III; subarticular tubercles absent; inner and outer metacarpal tubercles absent. Legs relatively short,

Table 2. Acoustic parameter of the advertisement call of the genus *Brachycephalus*. Values are presented as mean ± SD (range). Call duration in seconds (CD); Interval between calls (IC); Notes per call (NC); Note duration in seconds (ND1 for calls with only one note and note one in calls with two notes; ND2 for note two in calls with two notes); Interval between notes in seconds (NI); Note rate in notes/s (NR); Number of pulses per note (PN); Pulse rate in pulses/s (PR); Dominant frequency in Hz (DF). *Note-centered approach (we consider the duration of the notes as call duration, see Material and Methods). **Based on six calls of fig. 7 in Verdade et al. (2008). ***Data from Bornschein et al. (2019).

Species	n	CD	IC	NC	ND1	ND2	NI	NR	PN	PR	DF	Reference
B. tabuleiro (one note)	3 males 34 calls	0.037 ± 0.01 (0.028–0.062) n = 34	6.52 ± 2.0 (2.07–11.25) n = 33	1	0.037 ± 0.01 (0.028–0.062) n = 34	—	—	—	3 ± 0.34 (2–4) n = 34	75.8 ± 13.9 (54.1–107.1) n = 34	6,399 ± 103.4 (6,115-6,546) n = 34	This work
B. tabuleiro (two notes)	1 male 10 calls	0.568 ± 0.20 (0.541–0.628) n = 10	11.31 ± 1.55 (10.03–13.94) n = 8	2 n = 10	0.069 ± 0.006 (0.057–0.075) n = 10	0.058 ± 0.006 (0.050–0.071) n = 10	0.436 ± 0.027 (0.415–0.507) n = 10	3.53 ± 0.14 (3.18–3.70) n = 10	3.50 ± 0.51 (3–4) n = 20	55.10 ± 4.07 (47.62–65.57) n = 20	6,136.9 ± 61.70 (6,115–6,373) n = 10	This work
B. actaeus	6 males	0.04 ± 0.01 (0.030–0.040) n = 110	5.42 ± 1.83 (3.27–7.68) n = 104	1	0.04 ± 0.01 (0.03–0.04) n = 110	—	—	—	(2–3)	0.42 ± 0.15 (0.26–0.63) n = 229	6,900 ± 0.3 (6,600–7,300) n = 110	Monteiro et al. (2018a)
*B. albolineatus**	20 males 34 calls	88.37 ± 35.73 (39.93–191.14)	—	17.26 ± 6.38 (8–29)	0.020 ± 0.007 (0.002–0.037) n = 96	—	—	686.4 ± 3.22 (353.4–1085.4)	2.0 ± 0.59 (1–3) n = 323	—	6,380 ± 0.30 (5,340–7,320)	Bornschein et al. (2018)
*B. bufonoides**	3 males 3 calls	~180.0	—	—	0.271 ± 0.02 (0.222–0.308) n = 352 (0.222–0.308) n = 352	—	0.209 ± 0.02 (0.147–0.265) n = 298	2.15 ± 0.20 (1.98–2.43) n = 3	15.05 ± 0.88 (13–17) n = 352	56.20 ± 4.81 (47.55–71.28) n = 352 (47.55– 71.28) n = 352	4,550 ± 0.001 (4,130–4,880) n = 344	Folly et al. (2020)
B. crispus	5 males 100 calls	0.280 ± 0.02	0.350 ± 20 (0.122–0.215)	1	0.280 ± 0.02	—	0.350 ± 20 (0.122–0.215)	(1.67 ± 0.09)	10 ± 1.19 (7–12)	17.4 ± 2.1	4,600 ± 0.2 (3,500–5,700)	Condez et al. (2014)
*B. darkside**	5 males 1025 calls	30.4 ± 25.3 (2.9–66.2) n = 7	6.2–11.2 n = 2	114 ± 97.1 (9–253) n = 7	0.111 ± 13.7 (0.083–0.163)	—	0.159 ± 14.5 (0.122–0.215) n = 783	0.211 ± 25.6 (0.186–0.243) n = 5	6.3 ± 0.7 (5–8) n = 790	56.9 ± 4.9 (36.8–78.4) n = 790	3,382.1 ± 184.6 (2,856–3,796) n = 790	Guimarães et al. (2017)
*B. ephippium**	—	120 – 360	0.134 ± 6.8	—	0.112 ± 6.5 (0.093–0.125)	—	(0.134 ± 6.8)	—	12 ± 1.96 (5–15)	—	(3,400– 5,300)	Pombal et al. (1994)
*B. hermogenesi**	31 males 1549 calls	(0.200–1.900) n = 27	—	—	—	—	—	1.09	(4–5)**	—	6,800 ± 0.8 n = 5	Verdade et al. (2008)
B. ibitinga	2 males 48 calls	0.22 ± 0.02 (0.18–0.25) n = 48	0.32 ± 0.03 (0.28–0.37) n = 46	1	0.22 ± 0.02 (0.18–0.25) n = 48	—	0.32 ± 0.03 (0.28–0.37) n = 46	1.85 n = 48	10 ± 1 (8–12) n = 48	—	4,100 ± 0.06 (4,000–4,300) n = 48	Condez et al. (2021)
*B. mirissimus**	12 males 31 calls	111.83 ± 46.6 (37.70–255.20)	—	23.55 ± 10.29 (6–52)	0.010 ± 0.007 (0.002–0.027) n = 115	—	—	701.4 ± 2.12 (448.8–95.8)	—	—	6,660 ± 0.28 (6,000–7,230)	Pie et al. (2018a)
B. olivaceus	3 males 27 calls	0.04 ± 0.01 (0.030–0.050)	10.69 ± 4.56 (7.92–19.66)	1	38 ± 7 (0.030–0.050)	—	1.068 ± 456 (0.792–1.966)	0.12 ± 0.03 (0.07–0.16)	2 ± 0 (2–3)	0.26 ± 0.10 (0.14–0.41)	6,800 ± 0.2 (6,400–7,000)	Monteiro et al. (2018b)
B. pernix	—	0.060	0.140	—	0.060	—	0.140	—	3	—	(4,500–6,700)	Wistuba (1998)

Species	n	CD	IC	NC	ND1	ND2	NI	NR	PN	PR	DF	Reference
B. pitanga	14 males 6 calls	0.170 ± 13.0	—	—	0.170 ± 13.0	—	—	2.65 ± 0.2	(11.1 ± 1.2)	(62 ± 8)	4,900 ± 0.2	Araújo et al. (2012)
B. quiririensis	5 males 48 calls	0.05 ± 0.00 (0.040–0.050)	12.58 ± 1.38 (10.90–14.31)	1	0.050 ± 4.0 (0.040–0.050)	—	1.258 ± 138 (1.090–1.431)	0.08 ± 0.01 (0.07–0.11)	3 ± 0 (3–4)	0.30 ± 0.08 (0.20–0.44)	6,300 ± 0.2 (6,200–6,500)	Monteiro et al. (2018b)
*B. rotenbergae**	5 males 204 calls	(120–360) n = 204	0.15 ± 0.03 (0.11–0.25) n = 198	—	(0.09–0.24) n = 204	—	0.15 ± 0.03 (0.11–0.25) n = 198	3.07 ± 0.46 (2.50–3.48)	(5–15) n = 204	60.09 ± 3.71 (50.56–71.82)	3,830 ± 0.35 (3,040–5,300)	Nunes et al. (2021)
B. sulfuratus	11 males 95 calls	1.8 ± 0.2 (1.5–2.3)	4.5 ± 1.7 (3.1–7.4)	(5–6)	0.195 ± 13 (0.131– 0.233)	—	—	—	5.3 ± 0.9 (4–7)	0.2 ± 0.0 (0.1–0.3)	6,700 ± 0.3 (6,200–7,200)	Condez et al. (2016)
B. tridactylus	17 males	0.110 ± 0.0	—	—	0.110 ± 0.0	—	—	0.16 ± 0.03	1–3***	—	4,800 ± 0.2	Garey et al. (2012)

moderately robust; thigh length 34% (range) of SVL, tibia length 92% (range) of thigh length; toes I and V present but externally indistinguishable, toe II greatly reduced, toe III short and distinct, and toe IV larger and robust; toe lengths II < III < IV; tips of the toes II, III, and IV rounded; subarticular tubercles absent; inner and outer metatarsal tubercles absent. Skin on top of the head smooth; dorsal body rough and without dermal co-ossification. Skin on dorsolateral and dorsal surfaces of legs rough; dorsal surface of arms smooth. Ventral surface of the skin is rough, evenly across the body.

Measurements of the holotype (in mm). SVL 10.40; HL 2.09; HW 4.29; ND 0.18; IND 1.12; ED 1.08; IOD 2.48; END 0.48; THL 3.50; TBL 3.22; FL 3.87; UAL 1.63; FAL 2.54; HAL 1.91.

Color of holotype in life. General dorsal body color olive green, with a thin the vertebral stripe white margined of brown blotches; head is orange scattered with olive green, gray, and brown colors; legs are green gradually changing to an orange color on the knees, heels, and toes; arms and hands are orange, spotted brown; fingers are orange. In lateral view, the head is mostly orange, with eye and naris contour, and the upper lip brown. In ventral view, the general background color is orange; dark olive green blotches are present on the throat, cloacal region, and side of the body, extending toward the dorsum (Figs 3, 4).

Color of holotype in preservative. After seven years in preservative, general dorsal body color dark brown, with the head and the vertebral stripe light gray; legs brown gradually fading to a cream color on the knees and heels; arms and hands cream, spotted brown, with elbows cream; fingers and toes are cream. In lateral view, head is mostly cream, with eye and naris contour, and the upper lip brown. In ventral view, general background color is cream; brown blotches are present on the throat, cloacal region, and side of the body, extending toward the dorsum; irregular brown spots are distributed on the belly, legs, fingers, and toes (Fig. 5).

Osteology. The Skull is slightly wider than long in dorsal view, without hyperossification. Premaxillae broad, not fused medially, reduced odontoids present in par dentalis; alary process distinct, taller than wide and separated of nasals; posteromedial process distinct, posteromedial process reduced. Maxillae elongated and arched with reduced odontoids in par dentalis; par facialis reduced with anterior region smaller than the posterior one; preorbital processes distinct, long and anterodorsally oriented; the pars palatine extend from the anterior tip of the maxilla to the base of the posterior process and presents the anterior third larger than the posterior one. The quadratojugals is present, slender, with the maxillary process pointed. Nasals widely separated medially and presents an irregular trapezoidal shape in dorsal view; nasals and sphenethmoid fused, the paraorbital process is absent. Frontoparietals paired, not fused, rectangular shaped, frontoparietal fontanelle roofed; anteriorly the frontoparietals overlap

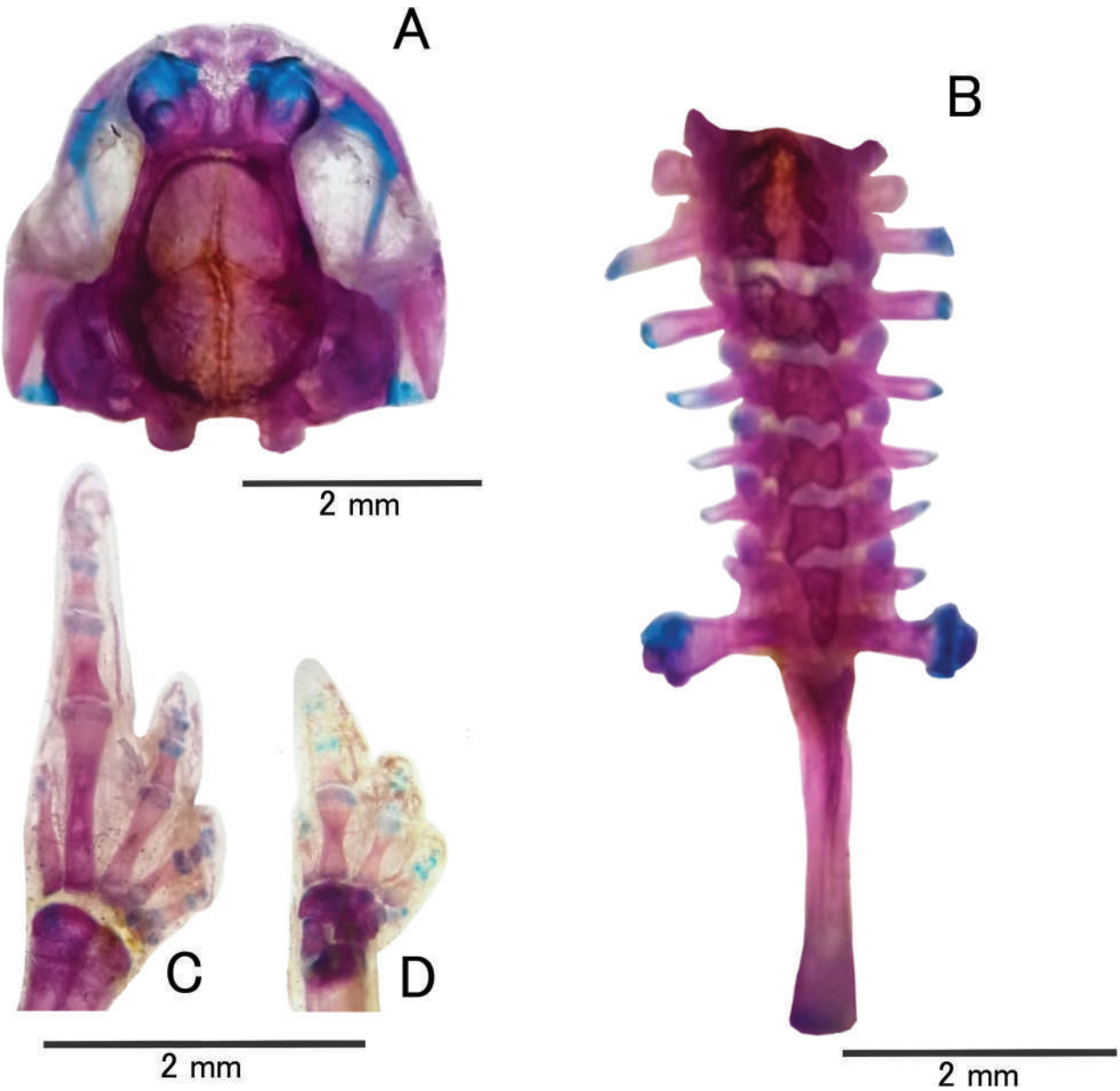

Figure 6. *Brachycephalus tabuleiro*. (**A**) Dorsal views of the skull, (**B**) vertebrae, and (**C**) dorsal views of the left foot and (**D**) hand (UNIFESSPA 112).

the posterior margin of the sphenethmoid. Vomers cartilaginous and reduced with the prechoanal and postchoanal process ossified. Frontoparietals, prootics, and exoccipitals fused. Neopalatines absent. Parasphenoid is cross-shaped without ornamentation; the cultriforme process is broad and touches the sphenethmoid; lateral alary processes are long. Squamosal is T-shaped in lateral view; the anterior zygomatic ramus is short, with approximately 1/4 of posterior otic ramus length and expanded dorsoventrally at its anterior margin; posterior ramus is approximately at same length of the ventral ramus and projected posterodorsally. The ventral ramus is large and slightly expanded ventrally. The lateral surface of the ventral process is flat; the lamina alaris in the bases of the anterior processes is concave. The pterygoids are slender and T-shaped; the tips of the anterior and posterior ramus are acuminated, tips of the medial ramus are truncated; the anterior ramus is the longest and does not articulate with the maxilla; the posterior ramus is shortest and articulating with the ventral ramus of the squamosal; the short and broad medial ramus articulating with the prootic; the anterior and medial ramus form a straight margo orbitalis. The exoccipitals, and prootics are fused, these bones form the posterior part of the skull; each paired occipital condyle has a marked base and a distal, rounded head. Operculum not ossified. Tympanic annulus and columellae absent. Mandible elongated and edentate, compound by a large angulosplenial, by a reduced cartilaginous dentary and a small mentomecklian cartilage. The Hyoid apparatus is formed by the central corpus, by the hyale anteriorly, by the posterlateral process and by an ossified posteromedial processes; the corpus hyoid is cartilaginous, rectangular and longer than wide; the cartilaginous hyale are long and project anteriorly from the anterolateral margins of the corpus hyoid; a long anterior process of hyale is present; the alary processes of the hyoid are

poorly developed; the posterolateral process is short and triangular; the posteromedial processes are long, well ossified, the proximal and distal ends of the posteromedial processes are wider. The pectoral girdle is arciferal, composed by the fusion of the clavicle, coracoid, and scapula; epicoracoid completely ossified and fused with the coracoid; the procoracoid cartilages are continuous with the epicoracoid medially and are partially ossified. Suprascapula fused with coracoid and scapula; sternum and omosternum are not present. Skeleton without hyperossification. Vertebral column composed of eight presacral, procoelous and nonimbricate vertebrae; dorsal ossification of neural arches is complete; the atlas does not present transverse process that is present in all other presacral vertebrae; the relative lengths of the transverse processes in descending order are: III > IV > V > II > VI > VII > VIII; neural spines are not evident. The sacral diapophyses extend posterolaterally and are expanded distally. The distal ends of the sacral diapophyses are cartilaginous and overlie the anterior ends of the ilial shafts. The urostyle is long and slender with approximately 2/3 of the length of the presacral, the urostyle presents a dorsal longitudinal ridge that is higher anteriorly and decreases in height posteriorly. Pelvic girdle V-shape in dorsal view, composed by a fused ilium, pubis, and ischium; each ilial shaft has a low longitudinal crest that decreases in height in the anterior third. The acetabulum is completely ossified with well-defined margins. Forelimbs with humerus slightly curved, with a small crest at the proximal end of the ventral surface; radius and ulna with sulcus intermedius distinct. Manus with distal carpals 3-4-5 and distal carpal 2 presents; radiale and ulnare about the same size; prepollex elements very reduced with one prepollical element; phalangeal formula 1-2-3-1; tips of the terminal phalangeal elements arrow-shaped in fingers I–III, rounded in finger IV. Hindlimbs with tibia and fibula fused; femur and tibiofibula of approximately the same length; fibulare and tibiale fused distal and proximally but separated in the middle portion. Pes with distal tarsal 2–3 and distal tarsal 1 presents; phalangeal formula 1-2-3-4-0. Tips of the terminal phalangeal elements arrow-shaped in toes II–IV, rounded in toes V (Fig. 6).

Advertisement call. The advertisement call of *Brachycephalus tabuleiro* **sp. nov.** is characterized by one multipulsed note (76.2% of all calls, n = 34), or in a sequence of two multipulsed notes (23.8% of all calls, n = 10; Fig. 7, Table 2). Calls with one note present 2 to 4 pulses, a duration of 0.037 ± 0.009 second (0.028–0.062 s, n = 34 calls), in an interval between calls 6.52 ± 2.0 seconds (2.07–11.25 s, n = 31 intervals), the mean of fundamental frequency 3,914 ± 902.9 Hz (2,394–5,264 Hz, n = 34 calls), dominant frequency 6,399 ± 103.4 Hz (6,115–6,546 Hz, n = 34 calls) and bandwidth 90% 583.8 ± 141.8 Hz (430.7–937.5 Hz, n = 34 calls). Calls with two notes were emitted sequentially by one male, but other males regularly vocalized with two notes, recorded in the background. Calls with two notes present a duration of 0.568 ± 0.02 second (0.541–0.628 s, n = 10 calls), in an interval between calls 11.31 ± 1.6 seconds (10.03–13.94 s, n = 9 intervals). The note one present 3 or 4 pulses, duration 0.069 ± 0.01 second (0.057–0.075 s, n = 10 notes), fundamental frequency 3,618 ± 332.0 Hz (3,040–4,090 Hz, n = 10 notes), dominant frequency 6,124 ± 27.2 Hz (6,115–6,202 Hz, n = 10 notes) and Bandwidth 90% 628.8 ± 81.7 Hz (516.8–775.2 Hz, n = 10 notes). The note two also present 3 or 4 pulses, duration 0.058 ± 0.01 second (0.050–0.071 s, n =10 notes), fundamental frequency 3621 ± 241.9 Hz (3,269–4,034 Hz, n = 10 notes), dominant frequency 6,115 ± 0 Hz (n = 10 notes) and Bandwidth 90% 611.5 ± 48.9 Hz (516.8–689.1 Hz, n = 10 notes). The interval between notes of calls with two

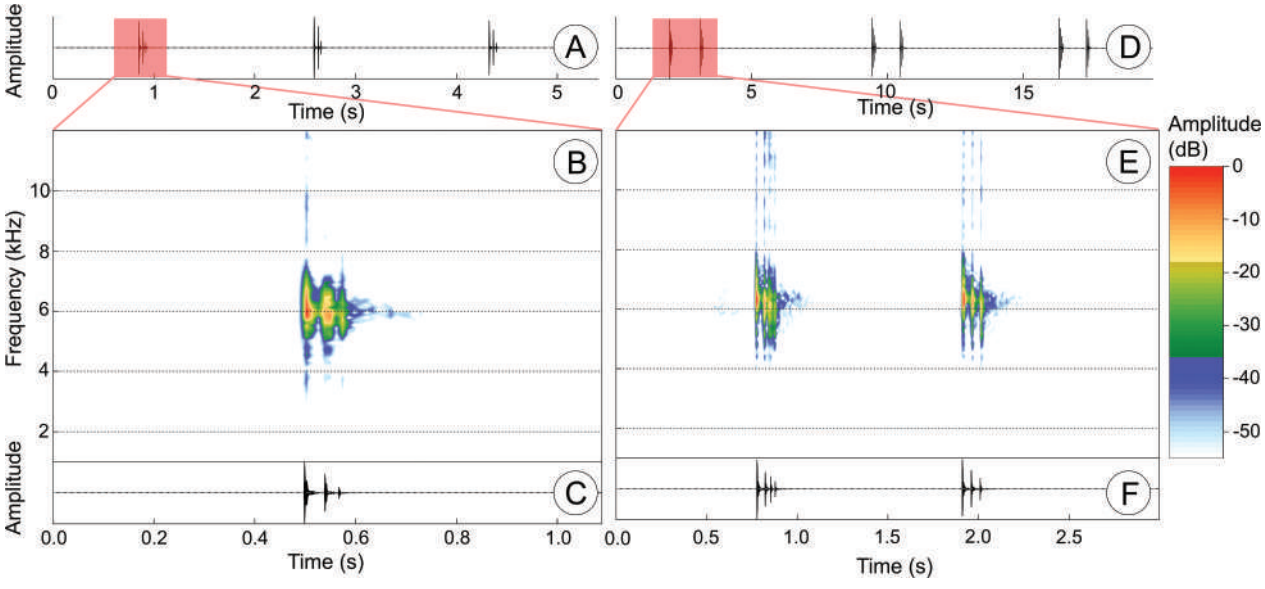

Figure 7. Advertisement calls of *Brachycephalus tabuleiro* recorded at Serra do Tabuleiro, São Bonifácio municipality, Santa Catarina state, Brazil. (A) Call sequence of three calls with only one note each, (B) the spectrogram and (C) oscillogram of one highlighted call (CASA 154). (D) Call sequence of three calls with two notes each, (E) the spectrogram and (F) oscillogram of one highlighted call (with two notes) (CASA 157).

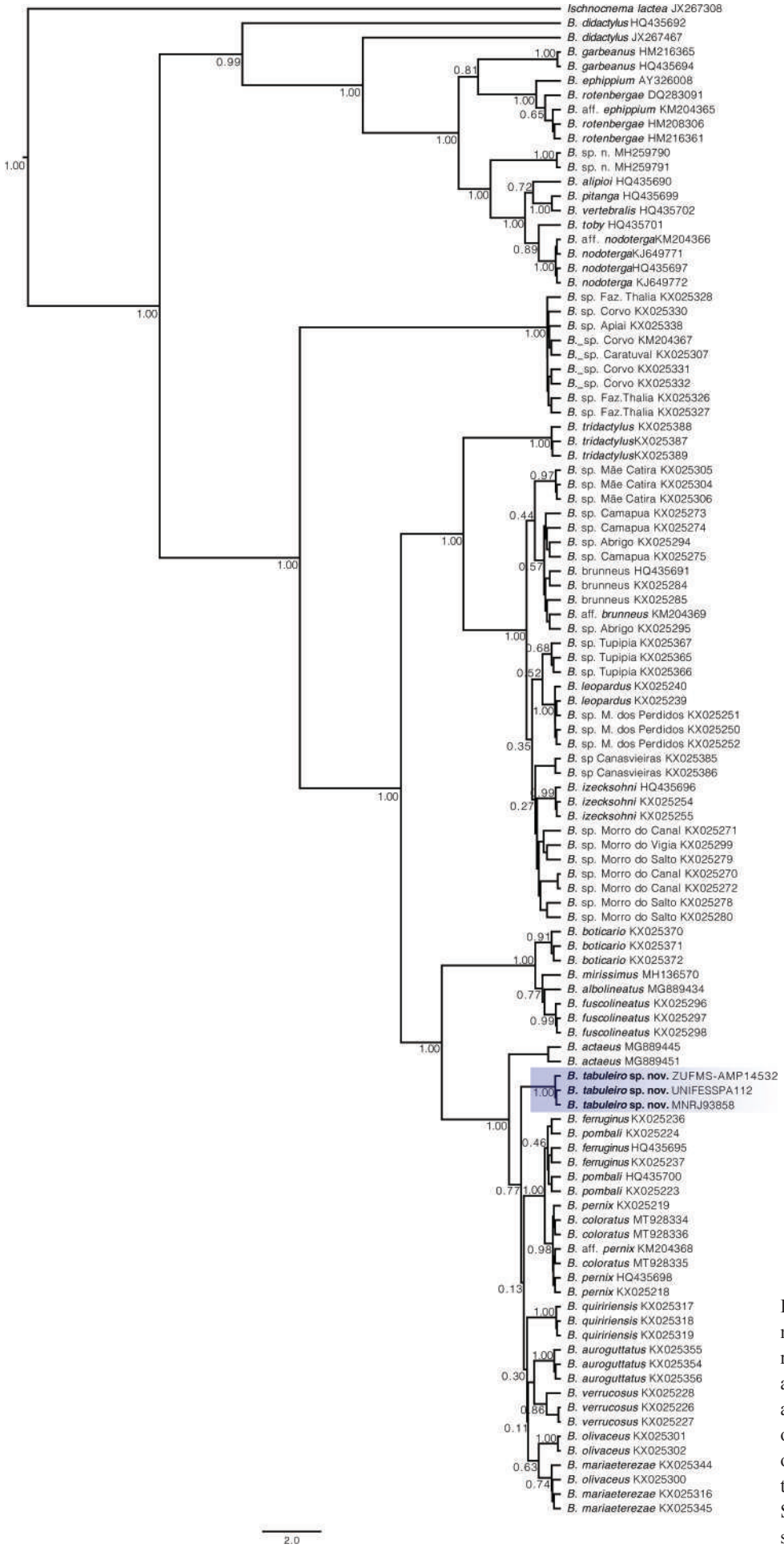

Figure 8. Inset of the maximum clade credibility 16S rRNA mitochondrial gene tree as inferred from a Bayesian analysis in BEAST. Section of the phylogenetic analysis of the 16S mtDNA gene for the genus *Brachycephalus*. Scale indicates rate of base substitutions per site.

notes was in mean 0.436 ± 0.03 second (0.415–0.507 s, n = 10 intervals), note rate of 3.53 ± 0.14 notes/second (3.18–3.70 notes/s, n = 10 calls). All the notes emitted, both in the calls with one and in the calls with two notes, had an average of 3 pulses/note ± 0.49 (2–4 pulses/note, n = 54 notes), and the two first pulses are the most energetics, followed by a decrease in amplitude in the third and fourth pulses.

Variation. The general dorsal body color varies from a dark olive green to a lighter green with some shades of yellow; the vertebral stripe can be well marked with an orangish line spotted with gray and brown colors, with some individuals exhibiting an irregular gray vertebral stripe (not well marked as a continuous and thin line). The head is orange scattered with olive green and brown colors, and some individuals present the head almost completely orange with few gray blotches and without the green color. The ventral background is orange, with some individuals presenting this region with green reticulated spots (Fig. 5). Descriptive statistics of measurement variables from adults are presented in Table 1.

Molecular analysis. Our tree topology (Fig. 8), based only on the 16S mDNA, recovered the *B. pernix* group monophyletic (pp = 1.00). The new species belongs to one of the clades (pp = 1.00) within the *B. pernix* group formed by *B. actaeus*, *B. albolineatus*, *B. auroguttatus*, *B. boticario*, *B. coloratus*, *B. ferruginus*, *B. fuscolineatus*, *B. mariaterezae*, *B. mirissimus*, *B. olivaceus*, *B. pernix*, *B.*

Table 3. Mean uncorrected (p distance) of *Brachycephalus tabuleiro* **sp. nov.** to other species of the genus.

Species	p distance	Species	p distance	Species	p distance
B. actaeus	0.030	*B. garbeanus*	0.164	*B.* sp. Apiai	0.075
B. aff. *brunneus*	0.066	*B. izecksohni*	0.060	*B.* sp. Camapua	0.054
B. aff. *ephippium*	0.153	*B. leopardus*	0.063	*B.* sp. Caratuval	0.075
B. aff. *nodortega*	0.161	*B. mariaterezae*	0.011	*B.* sp. Faz. Thalia	0.075
B. aff. *pernix*	0.015	*B. mirissimus*	0.051	*B.* sp. Mae Catira	0.056
B. albolineatus	0.046	*B. nodoterga*	0.161	*B.* sp. Morro do Canal	0.056
B. alipioi	0.155	*B. olivaceus*	0.011	*B.* sp. Morro do Vigia	0.054
B. auroguttatus	0.011	*B. pernix*	0.013	*B.* sp. Morro dos Perdidos	0.063
B. boticario	0.043	*B. pitanga*	0.157	*B.* sp. Serra das Canasvieias	0.057
B. brunneus	0.058	*B. pombali*	0.010	*B.* sp. Serra do Salto	0.054
B. coloratus	0.011	*B. quiririensis*	0.018	*B.* sp. Tupipia	0.056
B. didactylus	0.163	*B. rotenbergae*	0.154	*B. toby*	0.161
B. ephippium	0.157	*B.* sp.n.	0.155	*B. tridactylus*	0.062
B. ferruginus	0.010	*B.* sp. Corvo	0.078	*B. verrucosus*	0.014
B. fuscolineatus	0.047	*B.* sp. Abrigo	0.054	*B. vertebralis*	0.159

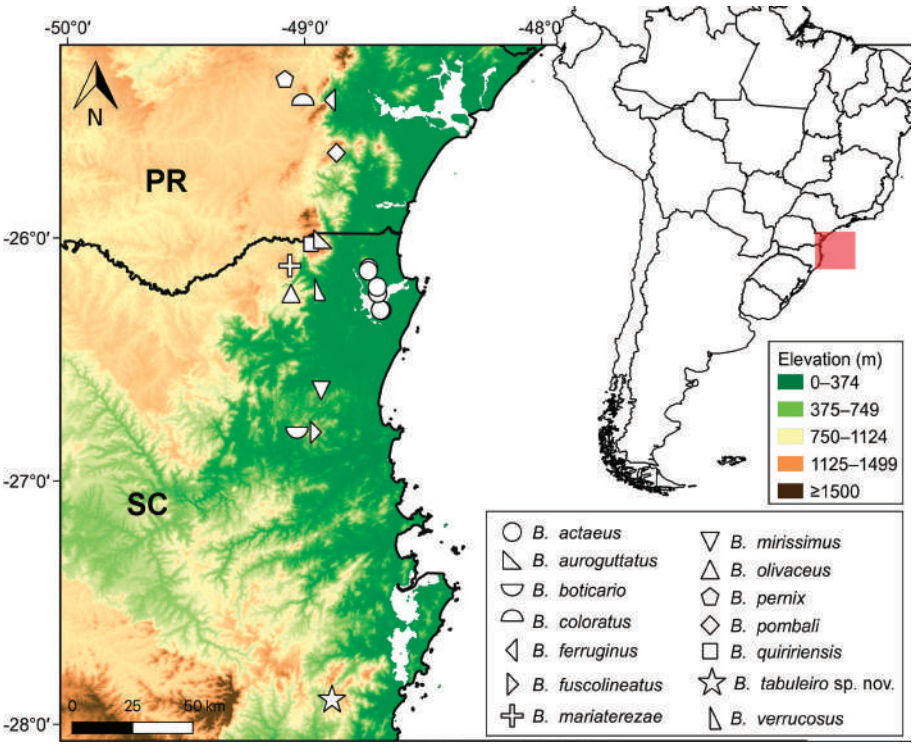

Figure 9. Geographic distribution of *Brachycephalus tabuleiro* and of the more closely related species within the *B. pernix* group, formed by: *B. actaeus*, *B. albolineatus*, *B. auroguttatus*, *B. boticario*, *B. coloratus*, *B. ferruginus*, *B. fuscolineatus*, *B. mariaterezae*, *B. mirissimus*, *B. olivaceus*, *B. pernix*, *B. pombali*, *B. quiririensis*, and *B. verrucosus*.

Figure 10. Type locality environment of *Brachycephalus tabuleiro* at Serra do Tabuleiro, São Bonifácio municipality, Santa Catarina state, Brazil.

pombali, *B. quiririensis*, and *B. verrucosus*. We refrain to further discussion about species relationships based on the small fragment of mtDNA used for this analysis. For a better overview on *Brachycephalus* phylogeny, see Condez et al. (2020), in which three mitochondrial genes were used to recover the species relationships. Average sequence divergences between the new species and congeners in the clade where it is nested ranged from 1.1% (*B. auroguttatus*, *B. coloratus*, *B. mariaeterezae*, *B. olivaceus*) to 5.1% (*B. mirissimus*) (Table 3; Table S2).

Natural History. *Brachycephalus tabuleiro* is diurnal. We found all specimens on the forest floor amidst leaf litter (0–70 mm under surface, n = 9 individuals). Males calling on the leaf litter, below one or more dry leaves (n = 3 individuals) or upside down (n = 1 individual). One

female was found on leaf litter nearby and turned towards a male calling. We observed greater intensity and abundance of vocalization at the beginning of the day (7 a.m.) and at the end of the afternoon (18 p.m.) (dawn/dusk). On days with rain or intense cloudiness, males appear to be active throughout the day. However, on sunny days, at warmer times (10 a.m. to 4 p.m.), the vocal activity and abundance of vocalizing males decreases or neither occurs. We recorded two potentially defensive behaviors for males and females: the mouth-gaping (Fig. 4A–B) and, remaining motionless with arms and legs extended, enabling individuals to be rolled during leaf litter removal (Fig. 4C–D). The mouth-gaping behavior has already been reported for other species of *Brachycephalus* (Toledo et al. 2011).

Distribution. *Brachycephalus tabuleiro* is known from one locality in São Bonifácio municipality, to the Parque Estadual da Serra do Tabuleiro, a protected area in Santa Catarina state, Brazil (Fig. 9). Although the type series was collected outside the Park boundaries, we also recorded males calling inside the protected area.

Etymology. The species is named for the type locality in the Serra do Tabuleiro. The Parque Estadual da Serra do Tabuleiro is the largest remnant of the Atlantic Rainforest in southern Brazil.

Conservation remarks. There is significant anthropogenic interference around the type locality of the new species, both in and around the Parque Estadual da Serra do Tabuleiro. The type locality is beside a dirt road, at the park boundaries (Fig. 10). In this area we observe various agricultural activities such as: (i) *Apis mellifera* apiculture; (ii) bovine cattle; and (iii) forestry of *Pinus* sp. and *Eucalyptus* sp. All these animals and plants are introduced exotic species. The presence of exotic plants can change the amount and the quality of leaf litter (e.g., Liao et al. 2007; Sausen et al. 2014), somehow compromising the population of *B. tabuleiro* on type locality. Another aggravating factor is that the municipality of São Bonifácio has conflicts over land use with irregular occupation and unfinished expropriation processes in Parque Estadual da Serra do Tabuleiro (IMA 2018). In addition, criminal fires and the lack of land regulation have compromised the conservation of this protected area. *Brachycephalus tabuleiro* is probably a mountaintop microendemic species like most species of the *B. pernix* group (e.g., Pie and Ribeiro 2015; Bornschein et al. 2016a; Ribeiro et al. 2017; Condez et al. 2020).

Discussion

Montane frogs generally exhibit considerable levels of endemism (Guedes et al. 2020). This scenario results from the discrepant environmental conditions at high and low elevations, creating the so-called sky islands (He and Jiang 2014). Some groups of amphibians occurring in montane areas present such pattern of distribution (Salerno et al. 2015; Firkowski et al. 2016), including species of the genus *Brachycephalus* that are mostly distributed in isolated mountaintops from Bahia (northeastern Brazil) to Santa Catarina states (southern Brazil), each one occupying only one or a few adjacent mountaintops (Ribeiro et al. 2015; Nunes et al. 2021). Studies using mitochondrial and nuclear data have shown the existence of a great number of highly endemic species for *Brachycephalus* in altitudinal areas in the southern Brazilian Atlantic Forest (Firkowski et al. 2016; Pie et al. 2018b). Despite the recent discoveries of the expanded distribution of mountain *Brachycephalus* species (Bornschein et al. 2021), the discovery of *B. tabuleiro* on a mountain top, where there are no congeneric species, supports and emphasizes the diversity and pattern of endemism within the genus, reinforcing the potential of the sky-island scenario for its diversification (Condez et al. 2020).

In the last decade, the number of described species of *Brachycephalus* has rapidly increased (n =23, which represents 61% of the total species of the genus). This has been accompanied by the use of several lines of evidence, providing molecular support for delimiting species (e.g., Folly et al. 2020; Condez et al. 2021; Lyra et al. 2021; Nunes et al. 2021). Still, several descriptions are based solely on one molecular marker, usually the 16S rRNA (e.g., Monteiro et al. 2018a; Pie et al. 2018a; Condez et al. 2021). Although phylogenies based merely on mitochondrial markers may fail to recover the proper relationship among highly diverse genus, the utility of using 16S as DNA barcoding have been successfully proved for species delimitation (e.g., Vences et al. 2012). Nonetheless, our tree topology, solely based on the barcode DNA 16S rRNA, recovered well supported nodes for major clades, with high posterior probabilities values (>0.95). It is worth to note that the morphological species groups (i.e., the pumpkin toadlets and the flea-toads), historically used for taxonomic proposals, are consistently recovered as paraphyletic (Clemente-Carvalho et al. 2011; Padial et al. 2014; Condez et al. 2020; Lyra et al. 2021). We found similar topologies as these previous studies and, as highlighted by Condez et al. (2020), distinct selective pressures probably drove the variation associated with traits in the genus, which lead to the presence of different traits in the same clade. However, we strongly suggest that for a better overview on *Brachycephalus* phylogeny and evolution of the genus, see Condez et al. (2020), in which a multi-loci approach was used to recover the species relationships.

Brachycephalus tabuleiro has the skull completely roofed, however, the suture of the frontoparietals is present. Among the species of the *B. pernix* group with some osteological study, the suture of frontoparietals can be observed in *B. brunneus* (Ribeiro et al. 2005; Haddad et al. 2010; Reis et al 2020); *B. curupira* (Ribeiro et al. 2017); *B. ferruginus* (Alves et al. 2006); *B. izecksohni* (Ribeiro et al. 2005; Haddad et al. 2010), *B. pombali* (Alves et al. 2006) and *B. pernix* (Haddad et al. 2010). Besides in some species of the *B. didactylus* group, such as *B. her-*

mogenesi (Condez et al. 2020; Clemente-Carvalho et al. 2012; Haddad et al. 2010; Reis et al. 2020) and *B. pulex* (Reis et al. 2020).

Campos et al. (2010) describe the ontogeny of the skeleton of *B. ephippium*, a species with hyperossification. In this species, the roof of the skull is open in young individuals and the frontoparietals continue to develop until the skull is completely roofed. In adult individuals, even the frontoparietals suture is not present (Campos et al. 2010). However, in the morphological phylogeny of the genus *Brachycephalus*, Campos (2011) indicate that *B. brunneus* presents the anterior margin of the frontoparietal fontanela open [characters 37 (Campos 2011)] and the medial portion of frontoparietals juxtaposed but not fused [characters 38 (Campos 2011)] suggesting variation in the genus. Reis et al. (2020) identified that one of the main variations in the skull of the genus *Brachycephalus* is related to the shape of the frontoparietal and indicates that species of the *B. ephippium* group have a more derived and hyperossified skull. The development and degree of the ossification of bones can be related to heterochronic processes (Alberch et al. 1997; Wild 1997), although Trueb and Alberch (1985) suggests that size and the degree of ossification can be variables independent of heterochrony. In this way, the evolution and development of hyperossification and fusion of bone elements (including the frontoparietal) in the skull of species of the *Brachycephalus* genus is not completely elucidated and more studies related to osteology in the genus, mainly related to the ontogeny of the skeleton, are necessary.

Advertisement calls of *Brachycephalus tabuleiro* is very similar to *B. actaeus*, differing basically by lower dominant frequency and for generally to emit calls with three pulses while in *B. actaeus* is more common with two pulses (Monteiro et al. 2018a). Based on a call-centered approach, the advertisement call of *B. tabuleiro* can be emitted with one or two notes. Despite the acoustic communication of *B. tabuleiro* has not yet been investigated, we suggest that calls with two notes may represent the advertisement call of the new species, since calls with two notes were emitted sequentially by one male, but other males regularly vocalized with two notes, recorded in the background. However, the behavioral context of these vocalizations requires studies with this objective.

Compare the morphology and advertisement call among *Brachycephalus* species is difficult, once the studies used nonstandard features (e.g., different terms for the same category, such as "rough", "granular" and "rugose", for skin texture) and different terminologies of the acoustic parameters or even different parameters to describe the calls (for instance, Verdade et al. 2008 used notes instead pulses to describe the calls of *B. hermogenesi*). Condez et al. (2016), comparing the advertisement call of *B. sulfuratus* to its congeners, cited that the note calls of *B. hermogenesi* are composed of 1–3 pulses. However, in the original description (Verdade et al. 2008), the authors have not presented this parameter and, by analyzing the figures, we verify that each call is composed of 4–5 pulses, which was classified as notes by Verdade et al. (2008). Such de-standardization highlights the urgent need for a rigorous review of *Brachycephalus* acoustic traits (as mentioned in Condez et al. 2017), which will certainly promote the taxonomic stability of the genus.

The Atlantic Forest is an important hotspot of biodiversity, currently with less than 12% of forest cover remaining, with the greatest extent area located in its southern distribution (Ribeiro et al. 2011). Species of the pumpkin toadlets lineage are endemic to the Atlantic Forest, occurring in a narrow elevational range, from 500 m up to the limit of occurrence of montane forests (except for *B. actaeus*, which occur at sea level; Bornschein et al. 2016; Condez et al. 2020, 2021). However, all 39 species of the genus have a small distribution area, related with miniaturization evolution process, lower dispersal capacity, and specific environmental requirements (Condez et al. 2020; Bornschein et al. 2021). Therefore, the discovery of *Brachycephalus tabuleiro* extends the distribution limits of pumpkin toadlets' within the Atlantic Forest, making it the southernmost known species for this genus and likely endemic to Serra do Tabuleiro. This mountain range is part of the Serra do Mar, which is one of the five biogeographic subdivisions proposed for the Atlantic Forest (Silva and Castele 2003) and, the one with the largest forest cover (Ribeiro et al. 2011). Further, the Parque Estadual da Serra do Tabuleiro is in the top seven largest protected areas of Serra do Mar subregion, contributing for 2% of the total area of this biogeographic unit (Ribeiro et al. 2011), therefore highlighting its importance for conservation and the need to reduce threats to this protected area (Hassemer et al. 2015). Considering a more refined framework of biogeographical units within the Atlantic Forest (Ribeiro et al. 2011), Serra do Tabuleiro is a distinct formation from the continuous region of Serra do Mar, to the north, and Serra Geral, to the west, and holds several endemisms (Socioambiental 2002) of plants (Hassemer et al. 2015; Rabuske and Külkamp 2018), and frogs (Garcia et al. 2008; Santos et al. 2020), and from now on also *Brachycephalus tabuleiro*, a likely microendemic species.

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Appendix

Examined Specimens

***Brachycephalus actaeus*.** BRAZIL — **Santa Catarina** • 3; São Francisco do Sul; Topotype; MHNCI 10832–34.

***Brachycephalus albolineatus*.** BRAZIL — **Santa Catarina** • 1; Jaraguá do Sul/Massaranduba; Holotype; MHNCI 10290 • 2; same collection data as for preceding; Paratopotypes; MHNCI 10297/10299.

***Brachycephalus auroguttatus*.** BRAZIL — **Santa Catarina** • 1; Garuva; Holotype; MHNCI 10200 (DZUP 375) • 9; same collection data as for preceding; Paratypes; MHNCI 10198 to199 (DZUP 373 to 74)/10202 (DZUP 377)/10204 (DZUP 379)/10207 to 208 (DZUP 382 to 83)/10212 to 214 (DZUP 387 to 89).

***Brachycephalus boticario*.** BRAZIL — **Santa Catarina** • 1; Blumenau; Holotype; MHNCI 10257 (DZUP 440) • 2; same collection data as for preceding; Paratypes; MHNCI 10253 (DZUP 414)/10731 (DZUP 458).

***Brachycephalus brunneus*.** BRAZIL — **Santa Catarina** • 3; Campina Grande do Sul; MNRJ 40289 to 40291.

***Brachycephalus coloratus*.** BRAZIL — **Paraná** • 1; Piraquara; Holotype; MHNCI 10273 • 2; same collection data as for preceding; Paratypes; MHNCI 10276/10278 • 2; same collection data as for preceding; MNRJ 89949 to 89950 (DZUP 505 to 506).

***Brachycephalus curupira*.** BRAZIL — **Paraná** • 1; São José dos Pinhais; Holotype MHNCI 10280 • **2;** same collection data as for preceding; Paratypes MHNCI 10282/10287.

***Brachycephalus fuscolineatus*.** BRAZIL — **Santa Catarina** • 1; Ilhota; Holotype; MHCNI 10231 (DZUP 159) • 2; same collection data as for preceding; Paratypes; 10234 (DZUP 402)/10236 (DZUP 404).

***Brachycephalus leopardus*.** BRAZIL — **Paraná** • 3; Tijucas do Sul; Paratypes; MHCNI 10245 to 10246 (DZUP 485 to 486)/10251 (DZUP 491).

***Brachycephalus mariaterezae*.** BRAZIL — **Santa Catarina** • 1; Joinville; Holotype; MHNCI 9811 (DZUP 168) • 5; same collection data as for preceding; Paratypes; MHNCI 10190 (DZUP 372)/10192 (DZUP 394)/10194 (DZUP 396)/10196 to 10197 (DZUP 398 to 399).

***Brachycephalus mirissimus*.** BRAZIL — **Santa Catarina** • 1; Massaranduba; Holotype; MHNCI 10793.

***Brachycephalus olivaceus*.** BRAZIL — **Santa Catarina** • 1; Joinville; Holotype; MHNCI 9813 (DZUP 170) • 5; same collection data as for preceding; Paratypes; MHNCI 9815 to 9818 (DZUP 164 to 167) • 1; same collection data as for preceding; Paratopotype; MHNCI 10238 (DZUP 371).

***Brachycephalus pernix*.** BRAZIL — **Paraná** • 1; Quatro Barras; Holotype; MNRJ 17349 • 3; same collection data as for preceding; MHNCI 10231/10234/10236.

***Brachycephalus pombali*.** BRAZIL — **Paraná** • 3; Morretes; DZUP 213–215.

***Brachycephalus quiririensis*.** BRAZIL — **Santa Catarina** • 3; Campo Alegre; Paratopotypes; MHNCI 10264 to 10265 (DZUP 175 to 176)/10267 (DZUP 525).

Brachycephalus tabuleiro. BRAZIL — **Santa Catarina** • 1; São Bonifácio; Holotype; MNRJ 93859 • 13, Paratypes; MHNCI 11498, CFBH 45453, CHSA.A 1403, MNRJ 93854–55, UNIFESSPA 112, ZUFMS-AMP 14532, CHSA.A 1404, MHNCI 11499; • 5 adults not sexed, collected at the type locality; CFBH 45452, MNRJ 93856–58, ZUFMS-AMP 14531.

***Brachycephalus tridactylus*.** BRAZIL — **Paraná** • 3; Guaraqueçaba; MHNCI 10187/10294/10729.

Brachycephalus verrucosus. BRAZIL — **Santa Catarina** • 1; Joinville; Holotype; MHNCI 9819 (DZUP 161) • 4; same collection data as for preceding; MHNCI 10216 (DZUP 465)/10222 to 10224 (DZUP 471 to 473).

Supplementary Material 1

Table S1

Authors: Mângia S, Santana DS, de Oliveira Drummond L, Sabagh LT, Ugioni L, Nogueira Costa P, Wachlevski M (2023)

Data type: .xlsx

Explanation note: GenBank accession numbers for 16S gene of specimens included in the molecular analyses.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/vz.73.e102098.suppl1

Supplementary Material 2

Table S2

Authors: Mângia S, Santana DS, de Oliveira Drummond L, Sabagh LT, Ugioni L, Nogueira Costa P, Wachlevski M (2023)

Data type: .docx

Explanation note: Uncorrected p distances for a 523-bp aligned sequence of the 16S gene of the new species and 45 other *Brachycephalus* species (or candidate species) taken from GenBank.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/vz.73.e102098.suppl2