A remarkable new species of *Brachycephalus* (Anura: Brachycephalidae) from southern Brazil

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Abstract

A new miniaturized frog of the genus *Brachycephalus* (Anura: Brachycephalidae) is described from Morro Santo Anjo in the municipality of Massaranduba, Santa Catarina, southern Brazil. Specimens were collected from the leaf litter between 470 and 540 above sea level. The new species is distinguished from all its congeners by the combination of the following characters: (1) body robust and bufoniform; (2) adult size SVL 10.9 mm ± 0.6 mm (9.9–11.7 mm) for males and 11.4 mm ± 2.0 mm (10.0–12.9 mm) for females mm; (3) smooth dorsum; (4) general color (in life) orange with white dots and stripe in the middle of the head and along its vertebral column; (5) iris completely black; (6) advertisement call composed of note groups; (7) isolated notes with 1–3 pulses; and (8) short isolated notes (0.002–0.027 s). An estimate of the male density of the new species is also presented. Phylogenetic information indicates that the new species is part of the southernmost clade of *Brachycephalus*, which includes *B. fuscolineatus* and *B. boticario*. The severe anthropogenic impacts in and around the type locality indicate that immediate actions should be taken to ensure the long-term preservation of the new species.

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Introduction

Although the first *Brachycephalus* was discovered in the early 19th century, half of its 34 currently recognized species have been described since 2011 (Frost 2018). One of the main reasons for this late burst of new species descriptions is probably the relative inaccessibility of many of its populations, which tend to be restricted to high elevation regions of the Brazilian Atlantic Forest (Pie *et al.* 2013, Bornschein *et al.* 2016a). Many of these species are microendemic, being found only in one or a few adjacent mountaintops (Bornschein *et al.* 2016a), a factor that, together with the destruction of their habitat and their relatively low reproductive rate, makes them particularly vulnerable to extinction. Therefore, there is an urgent need to advance our understanding of the taxonomy and distribution of *Brachycephalus* species to ensure their long-term conservation.

Brachycephalus has been recently divided into three phenetic groups, namely the *B. ephippium*, *B. didactylus*, and *B. pernix* species groups (Ribeiro *et al.* 2015). There are intriguing differences in how species of each group respond to altitude. For instance, species from the *B. didactylus* group occur from sea level to higher altitudes (0–1110 m a.s.l.) and are more environmentally tolerant (Pie *et al.* 2013, Bornschein *et al.* 2016a). On the other hand, species from the remaining groups tend to be found at higher elevations, but can also occur locally at lower altitudes if particular microclimatic conditions are met (*B. ephippium* group occurs between 200 to 1900 m a.s.l. and *B. pernix* group between 455 to 1640 m a.s.l. [Bornschein *et al.* 2016a]). Therefore, it is not surprising that extensive field work in the highlands of the southern Brazilian Atlantic Forest led to the discovery of several new species of *Brachycephalus* of the *B. pernix* group (e.g., Pie & Ribeiro 2015, Ribeiro *et al.* 2015, Bornschein *et al.* 2016b, Ribeiro *et al.* 2017). As part of this continued effort, in the present study we describe a new species of *Brachycephalus* from the state of Santa Catarina, southern Brazil. This species is also a member of the *B. pernix* group and is easily diagnosed by its unique coloration pattern.

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Kommentar [1]: "unique" means that it is the sole and only species that has this coloration pattern. It does not make sense to try to add a comparative to make this superlative even bigger!

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Methods

Procedures with specimens and specimens examined

Collection permits for this study were issued by ICMBIO (10.500, 55918–1). Collected specimens were anaesthetized and euthanized using 2% chloridrate lidocaine, fixed in 10% formalin, stored in 70% ethyl alcohol solution, and deposited in the Museu de História Natural Capão da Imbuia (MHNCI), Curitiba, state of Paraná, Brazil. We also examined specimens deposited in the following Brazilian collections: Célio F. B. Haddad collection, Departamento de Zoologia, Universidade Estadual Paulista, Campus de Rio Claro, state of São Paulo (CFBH); MHNCI; Coleção Herpetológica do Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, state of Paraná (DZUP); Museu Nacional, Rio de Janeiro, state of Rio de Janeiro (MNRJ); Museu de Zoologia da Universidade de São Paulo, São Paulo, state of São Paulo (MZUSP); and Museu de História Natural, Universidade Estadual de Campinas, Campinas, state of São Paulo (ZUEC). A list of the examined specimens is provided in the Appendix 1. All specimens in the type series of the new species are adults.

Morphometric measurements were obtained with a micrometric eyepiece attached to a stereomicroscope. Abbreviations for the 15 measurements used according to Watters *et al.* (2016) were as follows: snout–vent length, distance from tip of snout to opening of the cloaca (SVL); head length, from tip of snout to angle of jaw (HL); head width, greatest width of head located between angles of jaw (HW); eye diameter, from the anterior to posterior corner of the eye (ED); interorbital distance, between anterior corners of the eyes (IOD); internostril distance, between inner margins of nostrils (IND); eye–nostril distance, from anterior corner of the eye to posterior margin of nostril (EN); snout length, distance from the tip of the snout to the anterior corner of the eye (SL); upper eyelid width, greatest width of the upper eyelid margins, measured perpendicular to the anterior-posterior axis (UEW); forearm length, from the flexed elbow to the base of the outer palmar tubercle (FLL); hand length, from the base of the outer palmar tubercle to the tip of finger IV (HL); thigh length, distance from the opening line of the cloaca to the knee (THL); tibia length, distance from the outer surface of the flexed knee to the heel (TL); tarsus length, from the tibiotarsal articulation to the base of the inner metatarsal tubercle (TSL); foot length, from the base of inner metatarsal tubercle to the tip of toe IV (FL). Among the 16 most frequent measurements in the anuran species descriptions, two were omitted because the genus *Brachycephalus* does not have a tympanum and finger disks. With the exception of tarsal length, the measures chosen were observed in 75% of the anuran species descriptions (Watters *et al.* 2016). All measurements are indicated in mm and were obtained by a single researcher (LFR).

The sex of the specimens was determined by the presence of the *linea masculinea*, which is only found in males (Ribeiro *et al.* 2017) and consists of bands of fibrous connective tissue located over the entire extension of the oblique muscles (Duellman & Trueb 1986). The *linea masculinea* can be easily seen when the skin of the ventral region is cut (Ribeiro *et al.* 2017), or, also, occasionally by transparency in specimens with light ventral color (see below). This character is present in all *Brachycephalus* of the *B. pernix* group (Ribeiro *et al.* 2017), in species of the *B. didactylus* group (at least in *B. hermogenesi*) and is absent in species of the *B. ephippium* group (at least in *B. vertebralis*, *B. pitanga* and *B. toby*; LFR & MRB per. obs.). We assigned the new species into one of the three phenetic species groups, considering species with bufoniform body shape and with no *linea masculinea* as belonging to the *B. ephippium* species group, species with bufoniform body shape and *linea masculinea* as belonging to the *B. pernix* species group, and species with leptodactyliform body shape and *linea masculinea* as belonging to the *B. didactylus* species group (as above and modified from Ribeiro *et al.* [2017]).

Molecular phylogeny

To determine the phylogenetic position of the new species within the species group, we sequenced one of the paratypes (MHNCI 10798). Whole genomic DNA was extracted using PureLink™ Genomic DNA kit (Invitrogen™, USA), according to the manufacturer's instructions. One mitochondrial locus (16S rRNA) was amplified by polymerase chain reaction (PCR). PCR was performed in a final volume of 25 µL and consisted of 2 U AmpliTaq DNA polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 1.0 µM each primer (16SA-L CGCCTGTTTATCAAAAACAT and 16SB-H CCCGTCTGAACTCAGATCACGT, Vences *et al.* [2000]) and approximately 30 ng of template DNA. Thermocycling conditions involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 56 °C for 50 s and 72 °C for 1 min; and a final extension at 72 °C for 5 min. PCR products were electrophoresed on 1.5% agarose gels, and positive PCR products were purified using PEG 8000. Sequencing reactions were performed in a final volume of 10 µL, consisting of 0.7 µL ABI Prism® BigDye™ v3.1 (Applied Biosystems Inc., Foster City, CA), 1.0 µL 5X buffer and 1 µL each (3.2 pmol) primer and approximately 30 ng of template DNA. Cycle sequencing conditions included an initial denaturation step of 96 °C for 1 min, followed by 35 cycles of 15 s at 96 °C for denaturation, 15 s of annealing at 50 °C and extension of 4 min at 60 °C. Each locus was sequenced in both directions, and sequencing was performed in an ABI 3500 sequencer. The obtained sequences were aligned with all available 16S sequences of species of the *B. pernix* group on GenBank, as well as one sequence of *B. didactylus* as the outgroup (Table S1) using MUSCLE v3.8.31 (Edgar 2004) under default settings. Regions with ambiguous alignments were omitted from the final analyses. A phylogeny was obtained for concatenated data sets using a Bayesian approach with MrBayes 3.2 (Ronquist *et al.* 2012) after the best model of evolution was determined using jModelTest 2.1.7 (Darriba *et al.* 2012) to be HKY+ Γ. Each analysis consisted of two independent runs, each with four chains, run for 5×10^7 generations with sampling every 1000th generation. After ensuring convergence of separate chains, data sets were combined. Stationary distribution and ESS for all parameters were checked using Tracer v1.5 (Rambaut & Drummond 2009). We disregarded the initial 20% of the trees as burn-in, and using the remaining trees we estimated the maximum clade credibility consensus topology in TreeAnnotator v1.7.5 (Drummond & Rambaut 2007, Drummond *et al.* 2012). The used alignment and MrBayes command block are available in Supplemental Files 1.

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Kommentar [2]: You did only one marker, 16S! Thus, which other markers did you use for this combined analysis?

Advertisement call description

We recorded specimens of the new species on January 15 and 18, 2018 at the type locality of the species (see below). Recordings were carried out from 9:00–12:00 a.m. Climatic conditions during recordings were characterized by air temperature = 20.4–25.8 °C, soil temperature = 19.1–23.2 °C, and relative air humidity = 100%. Calls were recorded using the digital recorders Sony PCM-D50 with a Sennheiser ME 66/K6 microphone, Marantz PMD660 with a Sennheiser ME 66/K6 microphone, and Tascam DR44-WL with a Sennheiser ME 67/K6 microphone, all with sampling frequency rate of 44.1 kHz and 16-bit resolution. Recordings were deposited at MHNCI. Sound samples were analyzed with Raven Pro 1.5 (Bioacoustics Research Program 2012). Time domain variables were measured from oscillograms and frequency domain variables were measured from spectrograms. Spectrogram features were defined with a 128-point (2.9 ms) Fast Fourier Transform (FFT), a 3-dB Filter bandwidth of 492 Hz, Hann window, 50% overlap, and a spectrogram color scheme of Standard Gamma II in Raven Pro and Jet in Raven Lite, as in Bornschein *et al.* (2018). Final spectrograms, as well as diagnostic plots, were generated using the Seewave package, v. 2.0.5 (Sueur, Aubin & Simonis 2008) of the R environment, v. 3.2.2 (R Core Team 2015) using the same window size and overlap settings as in Raven Pro, but resampling the audio files at 22.05 kHz.

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We described the advertisement call of the new species as in Bornschein *et al.* (2018). We used the note-centered approach *sensu* Köhler *et al.* (2017) to define the advertisement call of the species. We determined the end of a given call and the beginning of the next one by the long period of silence between them (Köhler *et al.* 2017), which might last for several minutes and thus is considerably longer than the call itself. We described the advertisement calls following features and criteria of Köhler *et al.* (2017) and particularities as in Bornschein *et al.* (2018), except for frequency 5% and frequency 95%, not considered herein. We used the following features (see figure 1 of Bornschein *et al.* [2018]): 1) call duration (s); 2) duration of the call including only isolated notes (s); 3) duration of the call including only note groups (s); 4) note rate (notes per minute); 5) note rate of the call including only isolated notes (notes per minute); 6) note rate of the call including only note groups (notes per minute); 7) number of notes per call; 8) number of isolated notes per call; 9) number of note groups per call; 10) number of pulses per isolated notes; 11) number of pulses per note in note groups; 12) note duration of isolated notes (s); 13) duration of note group (s); 14) inter-note interval in isolated notes (s), defined as the time from the end of one isolated note to the beginning of the next note isolated note; 15) inter-note group interval (s), defined as the time from the end of one note group to the beginning of the next note group; 16) inter-note interval within note groups (s), defined as the time from the end of the first note to the beginning of the next note of the same note group; 17) note dominant frequency (kHz); 18) highest frequency (kHz); and 19) lowest frequency (kHz). The note rate was calculated taking into account the time from the beginning of the first note to the beginning of the last note of the calls (or call intervals) and the number of notes included in this counted time (Bornschein *et al.* 2018). The highest and lowest frequencies we measured from notes. Some calls began with notes with very reduced range of frequency in relation to subsequent notes. These "warming" notes (*sensu* Bornschein *et al.* 2007) were not included in the calculation of the parameters 12 and 17–19, above. We also compared the advertisement calls of the new species with calls deposited in public institutions, namely MHNCI and Fonoteca Neotropical Jacques Vielliard (FNJV) (Appendix 2).

Counting calling males

We obtained an approximate estimate of male density following the methods indicated in Bornschein *et al*. (2016a) and Ribeiro *et al.* (2017). We slowly walked along a small trail that crossed the study area as a transect in three days. We than select, in the third day, the point where the species appeared to be abundant and spent approximately 5 h placing markings on the vegetation at the positions where we recorded a calling male. We then measured the extent of the sampling area (= the extent of the auditory sampling) and counted the number of markings, resulting in an estimative of calling males per area. We used the classification of Brazilian vegetation proposed by the RADAMBRASIL project (*in* Veloso, Rangel & Lima 1991) to characterize the habitats of the species. Altitudinal records were obtained after plotting the geographical coordinates of the lowest and highest altitudinal records in the field using Google Earth.

Description style and registration of nomenclatural act

The present description follows the layout of our other recent descriptions of new species of *Brachycephalus* (Pie & Ribeiro 2015, Ribeiro *et al.* 2015). We refer to the manual digits as I–IV rather than II–V, to avoid confusion for most taxonomists. The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new name contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:AAB0DCA9-4587-42B3-812E-7752EB58F726. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Results

Brachycephalus mirissimus sp. nov.

Urn:lsid:zoobank.org:act:urn:lsid:zoobank.org:act: urn:lsid:zoobank.org:act:B1C3F142-E68C-4B4F-9647-F69CA6C25EB9

Figures 1–4

Holotype. MHNCI 10793 (Figures 1–3) adult male, collected at Morro Santo Anjo (26°37'41"S, 48°55'50"W; 535 m a.s.l.), municipality of Massaranduba, state of Santa Catarina, southern Brazil, on 15 January 2018 by Marcos R. Bornschein and Larissa Teixeira.

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Kommentar [3]: How did you determine these GPS data? With a hand-held device in the field or with GoogleEarth? What datum? WGS 1984?

Paratopotypes. MHNCI 10794 adult male, collected on 15 January 2018 by Marcos R. Bornschein and Larissa Teixeira; MHNCI 10795–9 and MHNCI 10802–3 adult males, collected on 18 January 2018 by Marcos R. Bornschein, Marcio R. Pie, Luiz F. Ribeiro, André Confetti, and Mário J. Nadaline; MHNCI 10800–1 adult females, collected on 18 January 2018 by Marcos R. Bornschein, Marcio R. Pie, Luiz F. Ribeiro, André Confetti, and Mário J. Nadaline.

Diagnosis – morphology. *Brachycephalus mirissimus* is a member of the genus *Brachycephalus* based on its position in a phylogenetic tree (Figure 5). *Brachycephalus mirissimus* is a member of the *B. pernix* group, as defined by Ribeiro *et al.* (2015) and modified above, by having a bufoniform body shape and *linea masculinea* (Figure 3C and Figure 4H). Within *Brachycephalus*, *B. mirissimus* is distinguished from all of the species in the genus by the following combination of characters: (1) body robust and bufoniform; (2) adult size SVL 10.9 mm ± 0.6 mm (9.9–11.7 mm) for males and 11.4 mm ± 2.0 mm (10.0–12.9 mm) for females mm (Table 1); (3) smooth dorsum (Figure 3 and 4); (4) general color (in life) orange with white dots and stripe in the middle of the head and along its vertebral column (Figure 3 and 4); and (5) iris completely black (Figure 3A). *Brachycephalus mirissimus* is unique among the species of its genus by its distinctive white dorsal pattern in contrast with an orange body. *Brachycephalus albolineatus* have a very similar white dorsal pattern, but not in all specimens and, when present, they also show a green to dark green dorsum. *Brachycephalus boticario*, *B. auroguttatus*, *B. mariaeterezae*, *B. quiririensis*, *B. guarani* (Clemente-Carvalho *et al.* 2012), some *B. ferruginus*, and some *B. verrucosus* also have mid-dorsal stripes but with different coloration: yellow in *B. boticario*, *B. quiririensis*, *B. auroguttatus*, and *B. verrucosus*, light blue in *B. mariaeterezae*, brown in *B. guarani* (Clemente-Carvalho *et al.* 2012), and reddish-brown in *B. ferruginus*. The dorsum of some of these species is also different: orange in the new species, as opposed to yellow in *B. mariaeterezae*, brown in *B. quiririensis*, light brown in *B. boticario*, light green in *B. verrucosus*, brown in *B. quiririensis*, and pure yellow anteriorly and increasingly mixed with brown instead of yellow toward the posterior region

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in *B. auroguttatus*. Two of those species with relatively similar pattern with a contrastingly colored stripe on dorsum have a similar orange dorsum as found in *B. mirissimus*, i.e. *B. ferruginus* and *B. guarani* (Clemente-Carvalho *et al.* 2012). Specimens of *B. ferruginus* without a reddish-brown stripe on dorsum have dispersed reddish-brown patches on middle dorsum or an entirely orange dorsum. *Brachycephalus mirissimus* is also distinguished from some of those species by its coloration on ventral surface, being orange instead of orange with brownish-green regions in *B. albolineatus*, yellow in *B. verrucosus*, yellow with small brown spots in *B. mariaeterezae*, orange with small green spots in *B. ferruginus*, orange anteriorly and brown with orange spots posteriorly in *B. quiririensis*, and orange mixed with brown in *B. auroguttatus*. *Brachycephalus mirissimus* is also reminiscent of some specimens of *B. fuscolineatus*, but nevertheless can be safely distinguished from that species by its orange body and white stripe in contrast with the yellow flanks and dark-brown to black middle of the dorsum of *B. fuscolineatus*. The new species shares the similar rough dorsum of *B. mariaeterezae*, *B. olivaceus*, *B. auroguttatus*, *B. verrucosus*, *B. fuscolineatus*, *B. leopardus*, *B. boticario*, and *B. quiririensis*, but is distinct in this feature from *B. fuscolineatus*, that have smooth dorsum. *Brachycephalus mirissimus* is also easily distinguished from the remaining species of the *B. pernix* group by the orange body with white dorsal stripe coloration instead of (1) dark brown in *B. brunneus* and *B. curupira*, (2) orange (or orange and yellow), normally with dark spots on the flanks, in *B. izecksohni*, *B. leopardus*, *B. pombali*, and *B. tridactylus*, (3) orange on head and on central dorsum with black remaining parts in *B. pernix*, and (4) pale red from the head to the pelvic region and yellowish green on the lateral body and dorsal thighs in *B. coloratus*. The bufoniform body shape and light color of the body also distinguish the new species from all species of the *B. didactylus* group, namely *B. didactylus* (Izecksohn, 1971), *B. hermogenesi*, *B. pulex*, and *B. sulfuratus*, which have leptodactyliform body shapes and homogeneous dorsal coloration, at times with an “X”-shaped darker mark on their dorsum (Izecksohn 1971; Giaretta & Sawaya 1998; Napoli *et al.* 2011; Condez *et al.* 2016). Apart from the difference in coloration between *B. mirissimus* and all species of the *B. ephippium* group, it also differs in body size of males, which is larger in adults of some species of the *B. ephippium* group, such as *B. alipioi* (SVL = 12.5–16.2 mm; Pombal & Gasparini [2006]), *B. darkside* (SVL = 14.8–18.5 mm; Guimarães *et al.* [2017]), *B. margaritatus* (SVL = 15.0–18.9 mm; Pombal & Izecksohn [2011]), *B. nodoterga*, *B. garbeanus*, and *B. bufonoides* (SVL = 12.4 mm, 17.6 mm, and 13.5 mm, respectively; Pombal [2010]).

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Diagnosis – advertisement calls. The advertisement calls of *Brachycephalus mirissimus* resemble those of species of the *B. pernix* group, as well as *B. hermogenesi*, from the *B. didactylus* group, in that they are composed of relatively short notes, as opposed to the “buzz” structure found in species of the

B. ephippium group (see below; see Table S2). With isolated notes having 1–3 pulses, *B. mirissimus* is easily distinguishable from *B. crispus* (7–12 pulses per note; Condez *et al.* [2014]), *B. darkside* (5–8 pulses per note; Guimarães *et al.* [2017]), *B. ephippium* (5–15 pulses per note; Pombal Jr., Sazima & Haddad [1994]), and from *B. pitanga* (6.90–13.30 pulses per note; Tandel *et al.* [2014]; see also Araújo *et al.* [2012]), of the *B. ephippium* group, and is also easily distinguishable from *B. sulfuratus* (6.90–13.30 pulses per note; Condez *et al.* [2016]), from the *B. didactylus* group. The distinctiveness of the new species from those species above can also be expressed by the duration of the isolated notes, short in *B. mirissimus* (0.002–0.027 s; mean of 0.010 s ± 0.007 s) and long in *B. crispus* (mean of 0.28 s ± 0.02 s; Condez *et al.* [2014]), *B. darkside* (0.083–0.163 s; Guimarães *et al.* [2017]), *B. ephippium* (0.093–0.125 s; Pombal Jr., Sazima & Haddad [1994]), *B. pitanga* (0.15–0.25 s; Tandel *et al.* [2014]; see also Araújo *et al.* [2012]), and *B. sulfuratus* (0.131–0.233 s; Condez *et al.* [2016]). Within species of the *B. pernix* group, the advertisement calls of *B. mirissimus* are easily distinguished from those of *B. tridactylus* by having note groups, whereas the latter presents only isolated notes. On the other hand, we cannot distinguish the advertisement call of the new species from that of *B. albolineatus* (Bornschein *et al.* 2018), due to the general similarity, as least considering the features described to date. Finally, we also cannot distinguish the advertisement call *B. mirissimus* from that of *B. hermogenesi* (Verdade *et al.* 2008), but in this case this is likely due to the simplicity of the description of the call of *B. hermogenesi* rather than by its supposed similarities.

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Description of the holotype. Male with robust bufoniform body; head slightly wider than long; head length 40% of snout–vent length; snout short: its shape semicircular in dorsal view, and rounded in lateral view (Figure 2); nostrils protuberant, directed anterolaterally; canthus rostralis not distinct; lips nearly sigmoid; loreal region weakly concave; eye slightly protruding in dorsal and lateral views; eye diameter 33% of head length; tympanum indistinct; vocal sac not expanded externally; tongue longer than wide, with posterior half not adherent to floor of mouth; choanae relatively small, rounded; vomerine teeth absent. Upper arm and forearm relatively slender, upper arm approximately as long as forearm; tips of Fingers I, II and II rounded, Finger IV greatly reduced; relative lengths of fingers IV<I<II<III; subarticular tubercles and inner and outer metacarpal tubercles absent; legs short, thigh robust; thigh length 39% of SVL, crus length 86% of thigh length; toe II short but distinct, toe III distinct and toe IV long; toes I and V not visible externally; relative length of toes II<III<IV; subarticular tubercles and inner metatarsal tubercles absent; outer metatarsal tubercle distinct, large and ovoid. Skin rough on dorsum of head and central body; skin granular on dorsolateral surfaces of body, flanks, and dorsal surface of thighs, with juxtaposed, large glandular warts; sides of the body granular;

large, round juxtaposed glandular warts on the sides of the body, belly and thighs; chin, arms, and legs smooth.

Coloration of the holotype. In life, almost completely orange, except for a patch on the dorsum of the head, a line in the middle of the dorsum and a pair of small white patches in the distal dorsum, partly surrounded by a thin yellow margin; dorsal part of leg articulations light orange; iris black (Figure 3). In preservative, orange regions turned yellowish pale cream and white region 1) remain white or 2) became pale cream or 3) became pale cream with white edges (Figure 1).

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Gelöscht: become

Measurements of holotype (in mm). SVL = 10.9, HL = 3.7, HW = 4.4, ED = 1.3, IOD = 2.4, IND = 1.3, EN = 0.7, SL = 0.8, UEW = 0.8, FLL = 2.8, HAL = 1.8, THL = 4.3, TL = 4.0, TSL = 2.8, FL = 3.1.

Variation in the type series. Morphometric variation is given in Table 1. There are slight differences in coloration among specimens (Figure 4). The orange coloration has a yellowish hue in some specimens, both in the dorsal and in the ventral regions. In addition, the width of the white stripe and the extent of the white spots on the dorsum of the head and on the distal dorsum also vary (Figure 1). The latter is light yellow instead of white in some individuals.

Gunther Koehler 19.5.2018 20:40
Gelöscht: between

Gunther Koehler 19.5.2018 20:40
Gelöscht: might

Gunther Koehler 19.5.2018 20:41
Gelöscht: might also

Gunther Koehler 19.5.2018 20:41
Gelöscht: be

Phylogenetic relationships. The phylogenetic analysis of species of the *B. pernix* species group places *Brachycephalus mirissimus* as part a clade which includes *B. fuscolineatus* and *B. boticario* (Figure 5), which are the southernmost species of the genus and are distributed in the region of the new species (Figure 6).

Advertisement call. We analyzed 31 advertisement calls from 12 individuals, six of which were collected (MHNCI 10793–8). We recorded eight individuals 2–5 times ($\bar{x}$ = 3.37 times per individual). The calls we deposited resulted in 31 separate recordings (MHNCI 052–82). All the described features are shown in Table 2 and the distribution of the number of pulses per note are provided in Table 3. Some advertisement calls were not recorded from the beginning, with some notes being heard before the recordings started (Table 3). To measure the duration of the call, we also considered the advertisement calls with up to two initial notes missing in the recordings. Below, we describe the call features reporting the mean ± SD, with the range in parentheses.

Gunther Koehler 19.5.2018 20:41
Gelöscht: between

Brachycephalus mirissimus emitted an advertisement call of 111.83 ± 46.60 s (37.70–255.20; Fig. 7A). An individual can remain silent for several minutes after emitting an advertisement call (occasionally for more than 20 min), when it emits a new call. Advertisement calls included 23.55 ± 10.29 notes (6–52), with a note rate of 11.69 ± 2.12 note per minute (7.48–15.93). The advertisement calls included both isolated notes and note groups (in this case, with two notes involved in each particular note group; Table 3, Fig. 7C, E). Advertisement calls could be composed only by isolated

Gunther Koehler 19.5.2018 20:42
Gelöscht: s

Gunther Koehler 19.5.2018 20:42
Gelöscht: s

Gunther Koehler 19.5.2018 20:42
Gelöscht: notes

Gunther Koehler 19.5.2018 20:42
Gelöscht: notes

Gunther Koehler 19.5.2018 20:42
Gelöscht: notes per minute

Gunther Koehler 19.5.2018 20:42
Gelöscht: notes per minute

notes (26.7% of the advertisement calls), but usually included both isolated notes and note groups (Table 3). Each advertisement call with note groups began with isolated notes and then changed to note groups (Table 2). Some advertisement calls begin with warming notes, at least with up to three of this attenuated note (see example of one warming note in the oscillogram of the Figure 7A – a small peak of energy just above zero). The part of the advertisement call composed of isolated notes has a duration of 63.05 s ± 22.83 s (16.26–100.30 s) and include 13.85 notes ± 5.34 notes (6–25 notes), emitted in a rate of 10.54 notes per minute ± 1.59 note per minute (7.48–14.28 notes per minute). The part of the advertisement call composed by note groups has a duration of 47.06 s ± 39.31 s (6.71–182.40 s) and includes 14.00 notes ± 7.58 notes (6–38 notes), emitted in a rate of 17.77 notes per minute ± 4.66 note per minute (12.20–27.43 notes per minute). There are 1.69 pulse per isolated notes ± 0.47 pulse (1–3 pulses; Fig. 7B, D) and 1.96 pulse per note in note groups ± 0.32 pulse (1–3 pulses; Fig. 7C, E). In each note groups (two notes counting in a single value of pulses), there are 3.92 pulses ± 0.60 pulse (2–6 pulses). A total of seven combinations of number of pulses in each note in note groups were recorded, i.e. 1–1 (n = 10 note groups; n = 2 individuals), 2–2 (n = 135 note groups; n = 10 individuals), 3–3 (n = 3 note groups; n = 2 individuals), 1–3 (n= 1 note group), 2–1 (n = 2 note groups; n = 2 individuals), 2–3 (n = 1 note group), and 3–2 (n = 2 note groups; n = 2 individuals). Note duration of isolated notes is 0.01 s ± 0.01 s (0.00–0.03 s) and note duration of note groups is 0.43 s ± 0.04 s (0.36–0.59 s). The inter-note interval in isolated notes is 5.81 s ± 1.33 s (3.92–10.62 s) and the inter-note group interval is 7.02 s ± 1.13 s (5.32–10.93 s). The inter-note interval within note groups is 0.39 s ± 0.03 s (0.35–0.49 s). The note dominant frequency is 6.64 kHz ± 0.27 kHz (6.00–7.23 kHz). Finally, the highest frequency is 8.31 kHz ± 0.52 kHz (7.14–10.06 kHz) while the lowest frequency is 4.37 kHz ± 0.77 kHz (2.67–5.84 kHz).

Gunther Koehler 19.5.2018 20:45
Kommentar [5]: Here and below: Time issues! Be consistent and keep this in the simple past form.

Gunther Koehler 19.5.2018 20:45
Gelöscht: close to the

Gunther Koehler 19.5.2018 20:45
Gelöscht: by

Gunther Koehler 19.5.2018 20:46
Gelöscht: have

Gunther Koehler 19.5.2018 20:43
Kommentar [6]: Here and below: See suggested simplification of format above. I recommend to present the unit only once which results in better readability of the text.

Gunther Koehler 19.5.2018 20:46
Gelöscht: have

Etymology. The specific epithet *mirissimus* is a superlative of the Latin adjective *mirus*, which means wonderful, marvelous.

Habitat, abundance, and distribution. We recorded *Brachycephalus mirissimus* calling throughout the day under the leaf litter, but with more intense vocal activity in the morning and later in the day. We did not hear the species throughout the study area, as it showed a patchy distribution, and it is not homogeneously abundant in these patches. In the patch where it appeared particularly abundant, we heard 14 males in 202.58 m^2, resulting in one calling male per 14.5 m^2.

Gunther Koehler 19.5.2018 20:48
Kommentar [7]: Well, I think you can safely round this to 202 m2 without losing any information! Two decimals is a bit rediculous here!

The species is known from the type locality (Figure 6), where it was found between 470–540 m a.s.l. in montane forest (Floresta Ombrófila Densa Montana; Figure 8) that reaches about 18–28 m in height. We did not find the species in montane forest with a lower canopy (< 10 m), which was in a very steep terrain. We estimate its “actual” extent of occurrence (*sensu* IUCN 2012) to be 56.8 ha

Gunther Koehler 19.5.2018 20:48
Gelöscht: in

(calculated considering all areas with 470 m or more of altitude, excluding deforested areas and forested areas in very steep terrains). We considered this estimate as extent of occurrence instead of area of occupancy (*sensu* IUCN 2012) due to the patchy occurrence of the species. That estimate resulted in three in-line polygons, distant from each other by 190 m and 60 m, which became isolated due to replacement of the original vegetation by *Eucalyptus* sp., *Pinus* sp. and palm plantations of *Archontophoenix alexandrae* H. Wendl. & Drude (Figure 9; areas encompassed by polygons are 28.3, 23.1, and 5.4 ha). The historical extent of occurrence (i.e., before the deforestation) taken the above criteria, resulted in a continuous polygon of 111.8 ha.

Gunther Koehler 19.5.2018 20:48
Gelöscht: pattern of

Gunther Koehler 19.5.2018 20:48
Gelöscht: in patches

Gunther Koehler 19.5.2018 20:49
Gelöscht: by deforestations

Gunther Koehler 19.5.2018 20:49
Gelöscht: to

We recorded some species at the type locality that are typical of high altitudes, such as *Quelusia regia* Vell., in the case of plants, and *Piculus aurulentus*, *Attila phoenicurus*, *Carpornis cucullata*, and *Scytalopus speluncae* (taxonomy according to Maurício *et al.* [2010]), in the case of birds. On the other hand, we also recorded some plants and birds at the type locality that are typical of lowland habitats, for example *Bathysa australis* (A.St.-Hil.) K.Schum., *Cecropia* sp., *Bactris setosa* Mart, and *Euterpe edulis* Mart., in the case of plants, and *Myrmotherula unicolor*, *Conopophaga melanops*, *Hemitriccus orbitatus*, and *Phylloscartes kronei*, in the case of birds, showing a mixed flora and fauna from both high and low elevations.

Gunther Koehler 19.5.2018 20:49
Gelöscht: in

Gunther Koehler 19.5.2018 20:49
Gelöscht: in

Gunther Koehler 19.5.2018 20:52
Kommentar [8]: What is “A.St.-Hil.” And “Mart”? If these are the author names of these taxa, then you need to be consistent and either present them for all or none of the mentioned taxa.

Remarks. The type locality of *B. mirissimus* is 17.4 km distant in a straight line from the type locality of *B. albolineatus*, 18.9 km distant from the type locality of *B. fuscolineatus*, and 19.5 km distant from the type locality of *B. boticario*.

Discussion

Our phylogenetic analysis provided clear support for *B. mirissimus* as part of the southernmost clade of *Brachycephalus*, which includes *B. fuscolineatus* and *B. boticario* (Figure 6 – there is no available DNA sequence for *B. albolineatus* to date). It is important to note that the distinctiveness between *B. mirissimus* and *B. fuscolineatus* is higher than that between species in other clades (e.g., between *B. brunneus*, *B. curupira*, and *B. izecksohni*). Despite the close phylogenetic and geographical proximity between the four species indicated in Figure 6, they differ considerably in coloration. On the other hand, the calls of *B. mirissimus* are nearly indistinguishable from the only closely related *Brachycephalus* species whose call has been described to date – *B. albolineatus* (Bornschein *et al.* 2018). Such low rate of evolution in prezygotic isolation mechanisms is not unexpected given that these species are allopatric and the risk of hybridization is minimized. On the other hand, the strong variation in coloration among those species is consistent with aposematism being an important driver in their evolution.

Gunther Koehler 19.5.2018 20:52
Gelöscht: Phylogenetic

Brachycephalus mirissimus is the third species of the *B. pernix* group whose advertisement call has been described to date (see Garey *et al.* 2012, Bornschein *et al.* 2018). The advertisement calls of species of *B. pernix* group share a similar overall resemblance, including structural, temporal, and spectral patterns (see Table S2). This similarity is most apparent when comparing calls of *B. albolineatus* and *B. mirissimus* by the presence of both isolated notes and note groups. On the other hand, note groups are absent from the calls of *B. tridactylus*, according to our analysis (see list of examined recordings in Appendix 2, that include on recording also analyzed by Garey *et al.* [2012]).

Gunther Koehler 19.5.2018 20:53
Kommentar [9]: Meaning unclear – reword!

Although the number of pulses per note in the advertisement call of *Brachycephalus mirissimus* and *B. albolineatus* is not a diagnostic character, given that both species present 1–3 pulses per note, some particularities are striking. In the new species, 20.6% of the notes showed 1 pulse (n = 138 notes), 77.5% showed two pulses (n = 521 notes), and only 1.9% showed three pulses (n = 13 notes), while in *B. albolineatus* there was a similar low percentage of notes with one pulse (4.7%; n = 26 notes), a relatively reduced percentage of notes with two pulses (46.4%; n = 257 notes), but a comparatively very high percentage of notes with three pulses (48.9%; n = 271 notes). The presence of three pulses in these species presents diametrically opposite results, which may reflect a tendency of the new species to be losing the emission of notes with three pulses.

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Gelöscht: s

The altitudinal range of occurrence of *Brachycephalus mirissimus* confirms the tendency of species of *B. pernix* group to occur at lower altitudes at in higher latitudes (Bornschein *et al.* 2016b). The abundance of *Brachycephalus*, estimated using the same methodology, revealed that *B. mirissimus* is much less abundant than *B. curupira* and *B. albolineatus*. We obtained the estimates of one calling individual of *B. mirissimus* per 14.5 m^2 (this study), one calling individual of *B. curupira* per 2–3 m^2 (Ribeiro *et al.* 2017), and one calling individual of *B. albolineatus* per 3–4 m^2 (Bornschein *et al.* 2016b). These results raise concern for the conservation of *B. mirissimus* because all of those estimates were made where the species appeared to be most abundant, yet its abundance is about four to six times lower than its congeners (see below).

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Gelöscht: when

The present extent of occurrence of *Brachycephalus mirissimus* (56.8 ha) is among the smallest ranges of vascular plants and fishes around the world (< 100 ha; Brown, Stevens & Kaufman [1996]) and is similar to other microendemic *Brachycephalus* from southern Brazil (Bornschein *et al.* 2016a). According to the criteria for classification of endangered species of the world (IUCN 2012), *B. mirissimus* corresponds to Critically Endangered based on the following criteria: B2a, b(i). Evidence for adherence to this criterion includes selective harvest of trees, edge effects, and erosion around two roads that go across one of the forest fragments that constitute the extent of occurrence of the new

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Gelöscht: goes

species. We estimate that the present extent of occurrence was reduced by 53% by deforestation (Figure 9).

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Gelöscht: in

Conclusion

In this study we describe *Brachycephalus mirissimus*, a new species of the *B. pernix* species group. The distinctiveness of the *B. mirissimus* is supported by morphological, bioacoustic, and genetic data. The discovery of this new species in a mountain that is relatively small underscores the possibility of new species discoveries at those types of elevation near the southern distribution of *Brachycephalus*, as well as the urgent need for basic biological information to design conservation efforts that ensure their long-term persistence.

Gunther Koehler 19.5.2018 20:55
Kommentar [10]: This is completely redundant. Unless the journal requests this kind of conclusion, I suggest to delete it.

Acknowledgments

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Kommentar [11]: Should be in italics

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Appendix 1. Examined specimens.

Brachycephalus albolineatus. SANTA CATARINA: Morro Boa Vista, boundary of the municipalities of Jaraguá do Sul and Massaranduba MHNCI 10290 (holotype), MHNCI 10295–10300, MNRJ 90349 (all paratypes).

Brachycephalus alipioi. ESPÍRITO SANTO: Alto Castelinho, municipality of Vargem Alta MHNCI 10804–6.

Brachycephalus auroguttatus. SANTA CATARINA: Pedra da Tartaruga, municipality of Garuva DZUP 375 (holotype), DZUP 373–4, 376–85, 387–89 (all paratypes).

Brachycephalus boticario. SANTA CATARINA: Morro do Cachorro, boundary of the municipalities of Blumenau, Gaspar, and Luiz Alves DZUP 440 (holotype), DZUP 414–5, 438–9, 444–5, 459 (all paratypes).

Brachycephalus brunneus. PARANÁ: Camapuã, Serra dos Órgãos, boundary of the municipalities of Campina Grande do Sul and Antonina MHNCI 10165–74; Caratuva, Serra dos Órgãos, municipality of Campina Grande do Sul MHNCI 1919–20, MNRJ 40289–91 (paratypes), MHNCI 10175–84.

Brachycephalus coloratus. PARANÁ: Estância Hidroclimática Recreio da Serra, Serra da Baitaca, municipality of Piraquara MHNCI 10273 (holotype), MHNCI 10274–79, MNRJ 89949–50 (all paratypes).

Brachycephalus curupira. PARANÁ: Serra do Salto, Malhada District, municipality of São José dos Pinhais MHNCI 10280 (holotype), MHNCI 10281–87, 10292 (all paratypes).

Brachycephalus didactylus. RIO DE JANEIRO: municipality of Engenheiro Paulo de Frontin ZUEC 10825; Sacra Família do Tinguá, municipality of Engenheiro Paulo de Frontin ZUEC 1132–3, MZUSP 13613–20, 64810–1, 94621 (topotypes).

Brachycephalus ephippium. RIO DE JANEIRO: Parque Nacional Serra dos Órgãos MZUSP 104140–7; Vale de Revolta MCZ A–108655. SÃO PAULO: municipality of Cotia MHNCI 2611–16.

Brachycephalus ferruginus. PARANÁ: Olimpo, Serra do Marumbi, municipality of Morretes MHNCI 125, 128 (topotypes), MHNCI five specimens uncatalogued.

Brachycephalus fuscolineatus. SANTA CATARINA: Morro do Baú, municipality of Ilhota DZUP 159 (holotype), DZUP 158, 160, 401–5 (all paratypes), MHNCI two specimens uncatalogued.

Brachycephalus hermogenesi. SÃO PAULO: Picinguaba, Parque Estadual da Serra do Mar, municipality of Ubatuba ZUEC 9715 (holotype), ZUEC 9716–25 (paratypes), MHNCI 10823–25.

Brachycephalus izecksohni. PARANÁ: Torre da Prata, Serra da Prata, boundary of the municipalities of Morretes, Paranaguá, and Guaratuba CFBH 7381–2, 7384 (all paratypes), MHNCI 10835.

Brachycephalus leopardus. PARANÁ: Morro dos Perdidos, municipality of Guaratuba DZUP 274–83, MHNCI three specimens uncatalogued; Serra do Araçatuba, municipality of Tijucas do Sul DZUP 490 (holotype), DZUP 478–89, 491–2 (all paratypes).

Brachycephalus mariaeterezae. SANTA CATARINA: Reserva Particular do Patrimônio Natural Caetezal, top of the Serra Queimada, municipality of Joinville MHNCI 9811 (holotype), DZUP 372, 393–9 (all paratypes).

Brachycephalus nodoterga. SÃO PAULO: Reserva Biológica Tamboré, municipality of Santana de Parnaíba MZUSP 147711–6.

Brachycephalus olivaceus. SANTA CATARINA: base of the Serra Queimada, municipality of Joinville MHNCI 9813 (holotype), DZUP 371 (paratype); Castelo dos Bugres, municipality of Joinville MHNCI 9814–8 (paratypes); Morro do Boi, municipality of Corupá MHNCI 10288–9.

Brachycephalus pernix. PARANÁ: Anhangava, Serra da Baitaca, municipality of Quatro Barras MNRJ 17349 (holotype), CFBH 2597–8 (paratypes), MHNCI 1818–9, 3000–4 (all paratypes), MHNCI 1820, ZUEC 9433–7 (paratypes), DZUP 539–55.

Brachycephalus pitanga. SÃO PAULO: SP 125, municipality of São Luís do Paraitinga MHNCI 10733–34; Trilha do Ipiranga 50 m from the Rio Ipiranga, Núcleo Santa Virgínia, Parque Estadual da Serra do Mar, municipality of São Luis do Paraitinga MHNCI 10821–22.

Brachycephalus pombali. PARANÁ: Morro dos Padres, Serra da Igreja, municipality of Morretes CFBH 8042 (holotype), 8043–53 (paratypes), MHNCI 11 specimens uncatalogued.

Brachycephalus quiririensis. SANTA CATARINA: Serra do Quiriri, municipality of Campo Alegre DZUP 172 (holotype), DZUP 171, 173–6, 524–30 (all paratypes), MHNCI five specimens uncatalogued.

Brachycephalus sulfuratus. SÃO PAULO: base of the Serra Água Limpa, municipality of Apiaí DZUP 362; near Jurupará dam, municipality of Piedade MHNCI 10829–31. PARANÁ: Caratuval, near the Parque Estadual das Lauráceas, municipality of Adrianópolis DZUP 139; Corvo, municipality of Quatro Barras DZUP 150–7; Fazenda Thalia, municipality of Balsa Nova DZUP 221–4; Mananciais da Serra, municipality of Piraquara MHNCI 10302; Recanto das Hortências, municipality of São José dos Pinhais DZUP 463; Salto do Inferno, Rio Capivari, municipality of Bocaiúva do Sul MHNCI 9800. SANTA CATARINA: Morro do Garrafão, municipality of Corupá MHNCI 10826–28.

Brachycephalus toby. SÃO PAULO: Morro do Corcovado, Parque Estadual da Serra do Mar, municipality of Ubatuba MHNCI 10807–09.

Brachycephalus verrucosus. SANTA CATARINA: Morro da Tromba, municipality of Joinville MHNCI 9819 (holotype), MHNCI 9820, DZUP 464–78 (all paratypes).

Brachycephalus tridactylus. PARANÁ: Serra do Morato, Reserva Natural Salto Morato, municipality of Guaraqueçaba MHNCI 10185–89, MHNCI 10294, 10729–30 (topotypes).

Brachycephalus vertebralis. RIO DE JANEIRO / SÃO PAULO: Morro Cuscuzeiro, Núcleo Picinguaba of the Parque Estadual da Serra do Mar and Parque Nacional da Serra da Bocaina, boundary of the municipalities of Parati, Rio de Janeiro state, and Ubatuba, São Paulo state MHNCI 10810–20.

Appendix 2. Examined recordings.

Brachycephalus albolineatus. SANTA CATARINA: Morro Boa Vista, boundary of the municipalities of Jaraguá do Sul and Massaranduba MHNCI 001–34.
Brachycephalus tridactylus. PARANÁ: Serra do Morato, Reserva Natural Salto Morato, municipality of Guaraqueçaba MHNCI 035–51, FNJV 0032950.

FIGURE CAPTIONS

Figure 1. Holotype of *Brachycephalus mirissimus* (MHNCI 10793), adult male, in preservative in dorsal (A) and (B) ventral view of the body. Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia. White bar = 5 mm.

Figure 2. Holotype of *Brachycephalus mirissimus* (MHNCI 10793), adult male: A = dorsal view of the body; B = lateral view of the head; D = ventral view of right hand; E = ventral view of right foot. Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia. Drawing by Verônica R. Apolônio.

Figure 3. Holotype of *Brachycephalus mirissimus* (MHNCI 10793), adult male, in life in lateral (A), dorsal (B) and ventral view (C). Notice in C, from the left to right, two white arrows indicated the presence of the *linea masculinea*, a pair of bands of fibrous connective tissue present only in males of the species and the vocal sac (photographs by LFR). Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia. Black bar in C = 5 mm.

Figure 4. Variation in coloration of paratypes of *Brachycephalus mirissimus*. A–F = dorsal view; G and H = ventral view. A = MHNCI 10802; B = MHNCI 10799; C = MHNCI 10803; D = MHNCI 10794; E = MHNCI 10801; F = MHNCI 10800; G = MHNCI 10796; H = MHNCI 10803. Notice in H the white arrow indicated the *linea masculinea* (photographs by LFR). Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia. Black bar in H = 5 mm.

Figure 5. Relationships between species of the *Brachycephalus pernix* species group based on a partial sequence of the 16S mitochondrial gene. Phylogenetic analysis was carried out using Bayesian inference and values above branches correspond to node posterior probabilities. Nodes with posterior probabilities lower than 50% were collapsed.

Figure 6. Map indicating the location of the type locality of *Brachycephalus mirissimus* (orange dot), as well as other species of the genus that are found in the region (state of Santa Catarina, southern Brazil) (indicated clockwise): *B. mirissimus*, *B. fuscolineatus*, *B. boticario* and *B. albolineatus*.

Figure 7. Example of an entire advertisement call and also notes of other advertisement calls of *Brachycephalus mirissimus*. A) Entire advertisement call (MHNCI 059, voucher MHNCI 10795). B, D) All examples observed of isolated notes, with one pulse in B (MHNCI 067) and two pulses in D (MHNCI 073, voucher MNHCI 10794). C, E) Examples of note groups, with 1–1 pulses in C (MHNCI 067) and 2–2 pulses in E (MHNCI 082, voucher MHNCI 10797). Spectrograms are produced with a FFT size of 8192 points, Hann window, and overlap of 90% in A and FFT 512 points, Hann window, and overlap of 90% in B-E.

Figure 8. Vegetation at the type locality of *Brachycephalus mirissimus*, at 535 m above sea level, characterized by high-elevation forest (Floresta Ombrófila Densa Montana). A = The middle and low strata of the forest, evidencing the habitat of *Brachycephalus mirissimus* in the leaf litter. B = General view of forest in the type locality.

Figure 9. Impacts at the type locality of *Brachycephalus mirissimus*. A. Morro Santo Anjo with *Eucalyptus* plantations along the slope. B. Forest boundary in half a slope severe affected by edge effects (in the case, with invasion of *Chusquea oxylepis* (Hackel) Ekman) and with plantations of palm *Archontophoenix alexandrae* (first plane) and *Eucalyptus* (background).