WENDY HERCILIA BOLAÑOS HERNANDEZ

História natural de *Brachycephalus pulex* Napoli, Caramaschi, Cruz, & Dias, 2011 (Anura, Brachycephalidae)

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Orientador: Dr. Mirco Solé

Co-orientador: Dr. Iuri Ribeiro Dias

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"Aunque nada cambie, si yo cambio, todo cambia"

Honoré de Balçac

RESUMO

O gênero *Brachycephalus* está representado por 36 espécies de anfíbios anuros endêmicas da Mata Atlântica, com distribuição conhecida desde o sul do estado da Bahia, no nordeste, até o estado de Santa Catarina, no sul do Brasil. Suas espécies são reconhecidas por apresentarem miniaturização e em sua maioria, distribuição restrita a topos de montanhas entre os 600 a 2000 metros de altitude. A espécie *Brachycephalus pulex* foi descrita no ano 2011 da RPPN Serra Bonita no município de Camacan no Sul da Bahia. Devido ao pequeno tamanho e hábitos discretos desta espécie, nenhuma informação adicional ao trabalho de descrição foi publicada nos últimos anos permanecendo pouco conhecida, pelo qual, nosso trabalho foi focado em descrever aspectos da história natural desta espécie. Estendemos a distribuição geográfica em 40 km NE, com um novo registro realizado dentro do Parque Nacional Serra das Lontras, estado da Bahia. Descrevemos dois tipos de canto: o primeiro "canto de aquecimento" composto por uma única nota pulsada com uma duração de (Tempo em segundos ou Hertz ± desvio padrão (DP) (mínimo – máximo) 0.27s ± 0.03 (0.208 - 0.298) e uma frequência dominante de 8062.53 Hz ± 350.81 (7687.5 - 8343.8); e o segundo: canto de anuncio, composto por apenas uma nota com duração de 1.40 s ± 0.21 (0.79 - 1.621) e uma frequência dominante de 9507.85 Hz ± 1224.21 (7593.8 - 11062.5). De forma "*ex situ*", *B. pulex* foram observados caminhando em três tipos de ritmo sob a serrapilheira e fazendo tanatoses como forma de defesa sem observar comportamentos agonísticos entre diferentes indivíduos e sexos. Alem disso, machos antes de vocalizar, mudaram a posição de corpo para cantar esticando os membros posteriores.

Indivíduos provenientes do Museu de Zoologia da Universidade Estadual de Santa Cruz e da Coleção de anfíbios Célio Fernando Baptista Haddad (CFBH) foram tomados como base para estudar a morfometria e aspectos reprodutivos, resultando num comprimento rostro-cloacal (CRC) dos indivíduos analisados de 8.15mm ± 0.44 (7.38 - 8.87; N= 22) para fêmeas e de 7.18mm ± 0.53 (6.45 - 8.29; N= 24) para machos. Em relação à reprodução foi possível observar, de forma macroscópica, que as gônadas das fêmeas se encontram embaixo do fígado e do sistema digestivo e cobrem completamente a área abdominal apresentando uma coloração amarelada, sendo compostas por dois ovos com um tamanho de 0.52 mm ± 0.22 (0.24 - 1.87 mm), enquanto que as gônadas masculinas possuem aparência ovoide e pigmentação preta com parênquima de cor branco, estando localizadas embaixo do fígado e aparato digestivo e se encontrando um em cada lado do abdômen e um tamanho de 1.44 mm ± 0.45 (0.41 - 2.51 mm). Finalmente, no segundo capitulo, foi analisado o conteúdo estomacal de 33 indivíduos, dos quais 16 foram machos e 17 foram fêmeas, revelando ácaros, colêmbolas, larvas de coleópteros e mosquitos, formigas e Thysanoptera como itens alimentares de *B. pulex*.

ABSTRACT

The genus *Brachycephalus* is represented by 36 species endemic to the Atlantic Forest, distributed from the south of the state of Bahia, northeastern Brazil, to the state of Santa Catarina, southern Brazil. Its species are recognized for being miniaturized and mostly associated with mountaintops between 600 to 2000 meters above sea level. The species *Brachycephalus pulex* was described in 2011 from the RPPN Serra Bonita, from the municipality of Camacan, southern Bahia. Due to the small size and discreet habits of this species, no information additional to the description work has been published in recent years and remains little known, so our work was focused on describing aspects of the natural history of this species. We extended the geographic distribution to 40 km NE, with a new registration carried out within the Serra das Lontras National Park, state of Bahia. We described two types of calls: the first "warm-up", composed of a single pulsed note with a duration of (Time in seconds or Hertz ± standard deviation (SD) (minimum-maximum) 0.27s ± 0.03 (0.208 - 0.298) and a dominant frequency of 8062.53 Hz ± 350.81 (7687.5 - 8343.8); and the second: advertisement call, composed for only one note with a duration of 1.40 s ± 0.21 (0.79 - 1.621) and a dominant frequency of 9507.85 Hz ± 1224.21 (7593.8 - 11062.5). In an "*ex situ*" manner, *B. pulex* were observed walking in three types of rhythm under the leaf litter and making thanatosis as a form of defense without agonistic behavior between different individuals and genders. Besides, males before vocalizing changed their body position stretching the hind limbs to start singing.

Individuals from the Zoology Museum of the State University of Santa Cruz and the Célio Fernando Baptista Haddad amphibian collection (CFBH) were taken as a basis to study the morphometry and reproductive aspects, resulting in a snout-ventral length (SVL)

of the analyzed individuals of 8.15 mm ± 0.44 (7.38 - 8.87; N = 22) for females and 7.18mm ± 0.53 (6.45 - 8.29; N = 24) for males. Regarding reproduction, it was possible to observe macroscopically that, the gonads of the females are found under the liver and digestive system and completely cover the abdominal area with a yellowish color, is composed of two eggs with a size of 0.52 mm ± 0.22 (0.24 - 1.87 mm), whereas, male gonads have an ovoid appearance and black pigmentation with white parenchyma, being located under the liver and digestive apparatus and meeting one on each side of the abdomen and a size of 1.44 mm ± 0.45 (0.41 - 2.51 mm). Finally, in the second chapter, the stomach contents of 33 individuals were analyzed, of which 16 were males and 17 were females, revealing mites, springtails, coleoptera larvae and mosquitoes, ants and Thysanoptera as food items of *B. pulex*.

SUMMARY

INTRODUCTION

Brazil has the greatest diversity of amphibians in the world with 1136 species, of which 1093 species belongs to the order Anura, 38 species to Gymnophiona and five species to Caudata (Segalla *et al*., 2019). Brachycephalidae is a family of the order Anura, which includes two genera, *Ischnocnema* and *Brachycephalus*, this last accounting for 36 species (Frost, 2020). The genus *Brachycephalus* is endemic to the Atlantic Forest of Brazil and is distributed from the state of Bahia, in the northeastern Brazil (Napoli *et al*., 2011), to the state of Santa Catarina, in the southern Brazil (Bornschein *et al*., 2016). Its species are recognized for being miniaturized, with adults smaller than 25 mm in length, loss of phalanges in the anterior and posterior appendages, hyperossification of skull and skeleton bones and absence of other bony elements (Yeh, 2002).

Most species of this genus shows a geographical distribution restricted to mountain tops, occurring between 600m to 2000m above sea level, however, species like *Brachycephalus hermogenesi*, *Brachycephalus sulfuratus and Brachycephalus didactylus* can be found in environments from 0 to 1000 and 40 to 1050 meters asl (Monteiro *et al*., 2018) witch share morphology characteristic with *Brachycephalus pulex* except the altitudinal distribution, being this species in his description work restrict to a top in a mountain >800 asl (Napoli *et al*., 2011). The possibly have low dispersion capacity, being considered micro-endemic animals (Rauen, 2011; Lima *et al*., 2016; Bornschein, *et al*., 2016; Bornschein *et al*., 2019).

In a work done by Clemente-Carvalho *et al*. (2011) was classified this genus in three species group in a molecular hypothesis, and then, based on morphological traits, Ribeiro *et al*. (2015) named and assigned species to one of these three groups following

morphological similarities: the *Brachycephalus ephippium* group with the presence of dermal co-ossification and bufoniform body; the *B. pernix* group with no dermal co-ossification and bufoniform body and the *B. didactylus* group with no dermal co-ossification and leptodactyliform body (Ribeiro *et al*., 2015).

The knowledge about aspects of natural history is limited to few species within the genus, most of which are restricted to comments inserted at the original species description articles, as well as aspects of morphology, geographic distribution, population, reproductive parameters, vocalization and diet (Pombal Jr. *et al*., 1992; Pombal Jr. 1999; Van Sluys *et al*., 2007; Almeida-Santos et a., 2011; Ribeiro *et al*., 2014, de Oliveira & Haddad, 2015; Folly *et al*., 2020); being all members mostly having daytime activity, aposematic coloring (except the *B. didactylus* group) and direct development (Pires *et al*., 2003, Pombal Jr, 1999) associated to leaf litter, a greater volume of eggs and small species size (Almeida-Santos *et al*., 2011).

Vocalizations studies in anurans, constituting an important tool for the identification and delimitation of species that are often confused by morphological similarity (Gambale & Bastos, 2014). Also, represents important communication signals that help to understand the behavior associated with different type of calls (Vielliard & Silva, 2004). This call that frequently is use to mate attraction, can also represent signals of courtship, release and aggressive conduct like territorial behavior in male-male interactions (Toledo *et al*., 2015). Within the 36 species of *Brachycephalus*, 13 species have its advertisement call described: *B. ephippium* (Pombal, 1994), *B. pernix* (Wistuba, 1998), *B. hermogenesi* (Verdade *et al*., 2008), *B. pitanga* (Araujo *et al*., 2012; Oliveira & Haddad, 2017), *B. tridactylus* (Garey *et al*., 2012), *B. crispus* (Condez *et al*., 2014), *B. sulfuratus* (Condez *et al*., 2016), *B. darkside*

(Guimarães *et al*., 2017), *B. albolineatus* (Bornschein *et al*., 2018), *B. actaeus* (Monteiro *et al*., 2018), *B. olivaceus*, *B. quiririensis* (Monteiro *et al*., 2018) and *B. bufonoides* (Folly *et al*., 2020).

For other hand, most anurans are considered opportunistic foragers preying on resources that are normally present at the environment, while other species actively seek for food (Eterovick & Sazima, 2004; Wells, 2007). Information about diet to his genus is available just for six species of *Brachycephalus*: *B. pitanga* (Oliveira & Haddad, 2015), *B. brunneus* (Fontoura *et al*., 2011), *B. didactylus* (Almeida-Santos *et al*., 2011), *B. ephippium* (Pombal, 1992), *B. pernix* (Wistuba, 1998) and *B. garbeanus* (Dorigo *et al*., 2012). All these studies demonstrate that the most frequent, most important or most consumed trophic resources in *Brachycephalus* diet are mites, collembolans and ants.

Brachycephalus pulex was described by Napoli *et al*., (2011), was collected at the RPPN Serra Bonita (Camacan, BA) near the top of the mountain between 800-930 m a.s.l. in an area dense of leaf litter. The description was done in based four adults with a snout-vent length of 8.2 ± 0.1 SVL and was cited as closely allied to *B. didactylus* and *B. hermogenesi* by brown color, small body size and body leptodactyliform. It doesn't have information about reproduction, vocalization or diet and, due to the geographical restrictions discussed in the article by Bornschein *et al*., (2019), these authors propose to classify *Brachycephalus pulex* as VU D-2 (Vulnerable - area of occupation <2000 km2) because until now it's only know by the type locality.

Based on this, our work focused on natural history to understand how and where this species live, doing observations about vocalization and diet, analyzing morphometry and

reproductive aspects and expanding occurrence area. With all this information it can be construct in a future, a tool for design of conservation and management strategies for this species in the areas of occurrence, in addition to being the only representative of the genus registered until today in northeastern Brazil (Napoli *et al*., 2011).

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CHAPTER ONE.

Range extension, advertisement call, morphological and reproductive aspects of the Flea-toad *Brachycephalus pulex* Napoli, Caramaschi, Cruz & Dias, 2011 (Anura, Brachycephalidae) in Bahia state, Brazil

Wendy Hercilia Bolaños Hernandez[1]*, Iuri Ribeiro Días[1] & Mirco Solé[1,3]

[1]*Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz. Campus Soane Nazaré de Andrade, Rodovia Jorge Amado, Km 16, Bairro Salobrinho CEP 45662-900, Ilhéus – Bahia, Brasil.*

[2]*Lab. Sistemática de Vertebrados. Pontificia Universidade Católica do Rio Grande do Sul (PUCRS), Av. Ipiranga 6681, Prédio 40, sala 110, 90619-900, Porto Alegre, Rio Grande do Sul, Brazil.*

[3]*Herpetology Section, Zoologisches Forschungsmuseum, Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany.*

**Corresponding author. E-mail: wendyhbh@gmail.com*

Abstract

Brachycephalus pulex was known only from its type locality at RPPN Serra Bonita, state of Bahia, northeasthern Brazil. We studied aspects of geographic distribution vocalization, morphology and some reproductive parameters to expand the knowledge about this species. We extended the species distribution to 40 km NE in the city of Arataca (Bahia state), representing the second locality registered for this flea-toad. During an *ex-situ* observation, the vocalization was recorded: showing an advertisement call composed of only one note with a duration of 1.40 seconds ± 0.21 SD (0.79 min – 1.62 max), formed by commonly 8.71 ± 2.01 (3 - 10) pulses and dominant frequency of 9507.85 Hz ± 1224.21 (7593.8 - 11062.5; N=24), being completely different from all calls described from other species within the genus. We also found, after analyzing individuals of *B. pulex* from the Museu de Zoologia da Universidade Estadual de Santa Cruz (UESC), that this species shows a variation in the color pattern. Females are one millimeter bigger than males being this the only dimorphism found. Gonad size of males is not correlated with the total length, but there is a positive correlation between the size of the females and the volume of eggs. Finally we found that females of *Brachycephalus pulex* have possibly only two small eggs in every clutch, fewer in comparison with other species in the genus.

Key words*: cryptic color, flea-toad, geographic distribution, gonads, Serra das Lontras, vocalization.*

The genus *Brachycephalus* is composed of 36 described species (Frost, 2020) that are endemic to the Atlantic Forest of Brazil. They are distributed from the south of the state of

Bahia (northeastern Brazil) (Napoli *et al*., 2011), to the state of Santa Catarina, southern Brazil (Bornschein *et al*., 2016). Most species of this genus have a restricted geographical distribution (Bornschein *et al*., 2019) to mountain tops between 600 to 2000 meters above sea level (Rauen, 2011; Lima *et al*., 2016; Bornschein, *et al*., 2016), although, species like *Brachycephalus hermogenesi* and *Brachycephalus sulfuratus,* can be found in environments from 0 to 1000 and 40 to 1050 meters asl (Monteiro *et al*., 2018). Bornschein *et al*. (2019), following IUCN criteria suggested *Brachycephalus pulex* to be classified as a vulnerable species D2, due to the restricted area it occupies, having been registered only at the type locality Serra Bonita RPPN (Napoli *et al*., 2011).

The study of acoustic communication in anurans is important for understanding the behavior associated with calling (Vielliard & Silva, 2004) constituting an essential tool for the identification and delimitation of species (Gambale & Bastos, 2014) as in amphibians advertisement calls are considered a pre-zygotic reproductive barrier (Gerhardt, 1994; Funk *et al*., 2009). Of the 36 species of *Brachycephalus*, 13 have described advertisement calls: *B. ephippium* (Pombal, 1994), *B. pernix* (Wistuba, 1998), *B. hermogenesi* (Verdade *et al*., 2008), *B. pitanga* (Araujo *et al*., 2012; Oliveira & Haddad, 2017), *B. tridactylus* (Garey *et al*., 2012), *B. crispus* (Condez *et al*., 2014), *B. sulfuratus* (Condez *et al*., 2016), *B. darkside* (Guimarães *et al*., 2017), *B. albolineatus* (Bornschein *et al*., 2018), *B. actaeus* (Monteiro *et al*., 2018), *B. olivaceus, B. quiririensis* (Monteiro *et al*., 2018) and *B. bufonoides* (Folly *et al*., 2020).

For this genus, sexual dimorphism related to the larger size of the females in relation to the males has been reported (Pombal Jr, 1999; Pombal Jr. & Izecksohn, 2011; Pie *et al*., 2018), an aspect that its observed in 90% of anuran species, which can be explained by the

correlation between reproductive success (the amount of eggs that a female can produce) and body size (bigger size, bigger amount of eggs; Wells, 2007).

In this study, we expand the geographical distribution of *Brachycephalus pulex* by providing a new occurrence locality, description of the advertisement call "*ex situ*" and analyzed of morphology and reproductive aspects from specimens collected in two localities.

MATERIALS AND METHODS

Geographic distribution - *Brachycephalus pulex* was found during the night, through active search, during field works undertaken in April, October and December 2018 by Omar Rojas Padilla and Vinicius Queiroz Mendez at Parque Nacional da Serra das Lontras, in a locality called by locals as "Peito de Moça" (S15.16491° W39.34904°, 826 meters asl) and another unnamed adjacent mountain peak (S15.16780° W39.34380°, 760 m elev.), at Arataca municipality, Bahia State (Figure 1). Collected individuals were deposited in the Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC) (Unnamed mountain, 760m: MZUESC 21691- 21694, 21696, 21697; Peito de moça, 826m: MZUESC 21695). Collecting permit was issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio 59889-1).

Vocalization - Due to the difficult of being seen vocalizing in their natural habitat, beneath the leaf-litter, the specimens trapped in different fieldworks at Serra Bonita (October 2019 and February 2020) were held in captivity to continue their observation and subsequent attempt at vocal recording. Two males were observed vocalizing *ex situ*, but only one was recorded. In the first case, unfortunately, the recorder did not make the

recording correctly, losing the data completely. The second specimen was recorded at 5 February 2020 at 00:00 am. Records was taken with a Marantz PMD 660 digital equipment, at a sampling rate of 44.1 kHz with 16-bit resolution, equipped with a Sennheiser ME45 unidirectional external microphone disposed five centimeters distant from the calling individual. The parameters registered during this study were: air temperature (°C) and relative humidity (%). The voucher specimen is deposited at the MZUESC (WB150; SVL= 6.86 mm). The recordings were analyzed using the Raven Pro 1.5.0 software (Cornell Lab of Ornithology, Ithaca, NY), the image of the spectogram and oscillogram were created with a window length of 512 and a "hanning" window, using the Seewave sound analysis package (Suer *et al*., 2008), in the R software environment program (R Core Team, 2020). The following parameters analyzed were: number of notes per call, note duration (s), interval between notes (s), number of pulses per note, pulse duration (s) and dominant, lower and higher frequency of the notes (Hz).

Behavior "ex situ"- individuals captured of *Brachycephalus pulex* were observed in captive breeding to understand more about his behavior in the following cases:

1. Individuals:

1.1. One in front each other (male – male; female – female; male - female) for 15 minutes.

2. Experiment with the mirror (Pombal *et al*., 1994)

2.1. Individuals were put in front a mirror for 15 minutes to see if they reacted to the image the "another" individual in the same place. This experiment was done with both sex (five males and two females)

Color variation: to verify if there is variation in the color in *Brachycephalus pulex*, we photographed exemplars from the MZUESC collection with a Leica S9I Stereozoom microscope. The images were from the body dorsally and ventrally of all individuals in order to compare them (N =46).

Morphometry: 37 individuals of *Brachycephalus pulex* from the Zoological Museum of the Santa Cruz State University (MZUESC), Ilhéus, state of Bahia, Brazil, and 9 from the Célio Fernando Baptista Haddad Amphibian Collection (CFBH), State University of São Paulo, Rio Claro (UNESP/Rio Claro), state of São Paulo, Brazil, were used to do this analysis. The specimens on both collections come from the municipality of Camacan, Serra Bonita, the type locality of this species (MZUESC: ID-13, 5-70, 88-96, 98, WB145-151; N = 30; CFBH –39376-39384; N = 9), or the municipality of Arataca, Serra das Lontras (MZUESC: 21691-21697; N = 7). The sex of the animals was determined by observing the gonads, in the specific case of individuals deposited in the MZUESC collection, and by the presence of vocal slits, in specimens deposited at CFBH collection.

Ten morphometric characters following Duellman (2001) and Heyer *et al*. (1990) were measured: SVL (Snout vent length), HL (head length), HW (head width), ED (eye diameter), DIO (interorbital distance), END (Eye - Narina diameter), DIN (internarial distance), THL (Thigh length), and TL (tibia length). A possible difference between males and females in size (SVL) will be analyzed with a Student test. In additional, we compared the size of all species in the genus *Brachycephalus*.

Description of the gonads: Eggs or testicles were measured with a Leica S9I Stereozoom microscope. The place and position of the gonads, pigmentation, connective

tissue color and the presence of adipose bodies were annotated. In addition, the volume of gonads was calculated using the formula $V = 4 / 3\pi (L / 2) (W / 2)^2$ (formula for ellipsoid bodies: L = length and W = width, according to Griffiths & Mylotte, 1987). To check if a relationship between the size (SVL) of the animal and the volume of the gonads exists we run a Spearman correlation test. This analyzed was done with 33 exemplars from the MZUESC.

RESULTS

The *Brachycephalus pulex* found in Serra das Lontras (Arataca – BA) represent the second record for this species, extending the known species distribution 40 km NE to one of the last remnants of mountain forest in the region. This new population (SVL (mm) 8.3 ± 0.1 (8.16 - 8.43; N= 4 females; 7.2 ± 0.7 (6.52 - 7.84; N= 3 males) was found in the dense leaf litter layer at a very humid place, with great abundance of epiphytes and bromeliads (Figure 1).

During two nightly field works through active search *Brachycephalus pulex* was found in October 2019 in a 7-day field work, with a rainy weather, dense fog, temperature of 21 ° C and 80% humidity, three specimens (2 males and one female) were found in an area of less than 1 m^2 and approximately 20 cm between each one, exposed in the leaf litter, 4 meters away from the main path. In February 2020 during a 4-day field work, with dry weather, after a light rain on the last day at 21 ° C and 92% humidity, 3 individuals (two males and one female) were found in the same way as the previous ones, in an area smaller than $1m^2$ at approx. 20cm from each other. At 6 meter distance a further isolated male was found.

In captive breeding, before individuals were observed vocalizing, we noticed that males of *Brachycephalus pulex* only vocalize when they see a potential partner. Once the female has been detected, the male changes the position of the body extending the hind limbs before starting to call. This behavior was observed in two cases: In the first, the female was resting under a leaf, whilst the male, walking near the same leaf, noticed her, and walked towards the bottom of the space produced by the two leaves where the female was, positioning him behind, then, changed the body posture and began to vocalize. The female ignored the male and after a few minutes the male gave up calling and walked away. In the second case, the male was resting in the middle of a leaf when a female who was walking around passed next to him. The moment he perceived her, he changed the posture of the body and began to vocalize while the female continued on her way until disappearing from the sight of the male. At this point he also stopped calling. In both cases the male was calling behind the back of the female. We noted that *B. pulex* remained motionless for a few seconds when they were with another individual, continuing their path without coming into contact. No agonistic behavior was observed. The experiment with the mirror was also tested without obtaining any response from any individual of *B. pulex.*

We analyzed 31 calls from a single individual. We recorded in captive breeding, two different calls. The “warm-up” call was comprised by a single note with a single pulse and a duration of 0.27s ± 0.03 (0.208 - 0.298), a dominant frequency of 8062.53 Hz ± 350.81 (7687.5 - 8343.8), lower frequency of 1831.17 Hz ± 44.01 (1805.4 - 1895.6) and a highest frequency of 19549.67 Hz ± 1469.33 (16338.7 - 20671.6). The advertisement call of *Brachycephalus pulex* (Table 1) is comprised by a single note with duration of 1.40 s ± 0.21 (0.79 - 1.621) and note interval of 1.02 s ± 0.32 (0.638 - 2). Every note is commonly

formed by 8.71 ± 2.01 (3 - 10) and the pulse duration during 0.11 s ± 0.04 (0.05 - 0.28). The dominant frequency is 9507.85 Hz ± 1224.21 (7593.8 - 11062.5). The lower frequency is 1601.30 Hz ± 196.95 (1173.5 - 1805.4) and the highest 20840.84 Hz ± 1152.42 (18775.9 - 22386.7). The environmental parameters registered at the moment of call were 27°C with 92% humidity (Figure 2).

About the color variation in *Brachycephalus pulex*, we found that in dorsal view can be totally dark brown or show clear spots around the head, trunk or limbs. Some individuals have a clear line straight in the middle of the body and others can have straight clear lines on posterior limbs. In a ventral view, we observed a whole white spot just in the middle of the chest and clear spots around the abdomen or a uniform dark brown color (Figure 3). This coloration is maintained even in preserved (alcohol 70%) specimens.

After measured 46 individuals of *Brachycephalus pulex*, 22 females had a SVL of 8.15 ± 0.4 mm (7.38 - 8.87) and 24 males a SVL of 7.10 ± 0.47 mm (6.45 - 7.90). The Student t test (Test t: $p<0.001$; t: 8.99E-10) revealed that females are statistically larger than males (Table 2).

Gonads: from the 18 females and 19 males, were measured gonads of 17 female and 16 males macroscopically. Two eggs with a size of 0.52 mm ± 0.22 (0.24 - 1.87 mm) were found in females, and they were located under the liver and digestive system, covering the entire abdominal area, presenting a yellowish color surrounded by white adipose bodies. In two females, the ovarian granulations had a less yellow color observed by the transparency of the connective tissue layer that surrounds the organ and only one female had gonads with less mass in terms of size compared to the rest of the observed individuals and absent

adipose body. The testicles measured 1.44 mm ± 0.45 (0.41 - 2.51 mm), and have an ovoid appearance and black pigmentation with white parenchyma. They are located under the liver and digestive system, on every side of the abdomen. There was an asymmetry in size between the right and left testicles, the one on the right side being larger than the left, and they were located at different heights and had fat accumulation apically that could be abundant or not (Figure 4).

The Spearman correlation test explains that there is a positive but weak correlation between total length (SVL) and female gonads R^2= 0.238 (p: 0.003), being not significant for males R^2= 0.017 (p: 0.490) (Table 3).

DISCUSSION

We report on the first record of *Brachycephalus pulex* outside its type locality, however we are aware that there is still a lack of information in many places that have not been explored yet, especially mountain tops. Our new record reveals the presence of this frog in the Serra das Lontras National Park, a protected area of 11.343,84 hectares, protecting remnants of Atlantic Forest in the state of Bahia (ICMBio, 2020). At the same time, the altitude range is extended to 760 a 960 m. Even with this new locality, its threat status, according to IUCN as DD (Data Deficient) we reinforced the information published in Bornschein *et al.* (2019) in which this species should be considered as Vulnerable (VU-D2). In the didactylus group (Ribeiro *et al*., 2015) to which *B. pulex* is proposed to belong, this species is the only one that still shows a distribution restricted to altitudes, differing from *B. didactylus* which show an altitudinal range from 35-1400 m, *B. hermogenesi* 0-1140 and *B.* sulfuratus 40 to 1050 m (Bornschein *et al*., 2019).

Brachycephalus pulex was observed only during the night, even when extensive daytime searches were performed (aprox. 63h). No vocalization was heard and they were always fully exposed in the middle of the leaf litter. According to our observations and as registered for other species of this genus, we hypothesize that *B. pulex* has a distribution by patches, with several individuals being found within a small area. In an "*ex situ*" approach, some behaviors similar to those described in the work of Wistuba (1998) were observed, such as: 1*) Displacement*: We observed that individuals walked at two types of rhythms, one slowly, in which the animals were observed bending down the body and / or the head making rapid movements towards the ground as if they were feeding and a fast one in which the toads simply moved from one place to another; *2) Defense*: When an individual felt threatened, it crouched its body and picked up posterior and anterior limbs towards the body, being thus mimetized with his surroundings. *B. pulex* was never observed jumping unless it felt threatened, so we consider the jump in this work as a kind of response to a strong stimulus; and *3) Cleaning*: In which all individuals were observed raising their hands and feet passing them through the body with circular movements

The behavior observed prior to *Brachycephalus pulex* starting to call was the same reported by Oliveira & Haddad (2017) for *B. pitanga*, where the male extended the body with intense movements of the abdomen to produce sound. Also, the fact that females did not correspond to the males calls, could be explained by the possibility that *B. pulex* does not have a fully developed inner ear like already reported for *B. pitanga* and *B. epipphium* (Goutte *et al*., 2017). Nevertheless, this is just a presumption and a complete morphological and behavioral study would be required to determine this. Also, the experiment with the mirror where was observed agonistic behavior in individuals of *B. pernix* with behavior

performs movements with his arms when it encounters another individual, wasn't observed in *B. pulex*. In this case, when two *B. pulex* had visual contact they remained motionless for a few seconds, continuing their path without coming into contact.

The advertisement call of *Brachycephalus pulex* is composed by only one note per call as also reported for *B. pernix, B. tridactylus, B. olivaceus* and *B. quiriensis*. The note duration is considerable much larger compare with the others (Mean= B. crispus: 0.280s; B. bufonoides: 0.271s; B. hermogenesi: 0.2s; B. sulfuratus: 0.195s; B. pitanga: 0.170s; B. tridactylus: 0.110s; B. ephipppium: 0.112s; B. darkside: 0.115s; B. quiririensis: 0.05s; B. olivaceus: 0.038s; B. pernix: 0.031-0.063s, Monteiro *et al*., 2018), and higher/lower frequencies and stronger than reported for others species too of the genus.

Most species in the genus *Brachycephalus*, exhibit brilliants colors like yellow or orange related to aposematic coloration and tetrodotoxin toxicity (Pires *et al*., 2005). However, in the didactylus group, all species have criptic coloration (Giaretta & Sawaya, 1998; Almeida-Santos *et al*., 2011, Napoli *et al*., 2011; Condez *et al*., 2016). Even with its dark coloration, in *Brachycephalus pulex* we found a pattern color like the described by Napoli *et al*. (2011) with dark brown x-shaped mark on dorsum, but also, we found other patterns, such as the big clear spot (almost white) on the dorsum and ventral parts of the body, that extend the variety of coloration patterns for this species. As so, for most anuran species, females of the genus *Brachycephalus* are larger than males (Pombal Jr, 1999; Pombal Jr. & Izecksohn, 2011; Pie *et al*., 2018). Our data confirm this pattern for *B. pulex*, where females are one millimeter bigger in comparison with males, expanding the knowledge about the morphometric variation of the species, which was described based on only four non-sexed specimens. With this data, *B. pulex* can be considered the smallest

species within its genus (see Appendix 1) (Pombal Jr, 1999; Pombal Jr. & Izecksohn, 2011; Pie *et al*., 2018).

After compared color and size of gonads, we found that *Brachycephalus epipphium* in the paper described by Pombal *et al*. (1994) females had similar coloration but eggs larger from that founds to *B. pulex* in our study. We also compared with the observations made by Almeida *et al* (2011) and Oliveira (2013) where females of *B. didactylus* and *B. pitanga* have depigmented eggs (cream color) and diameter of 1.6 and 1.8 mm, which comparatively, the eggs are different from *B. pulex,* that had yellow eggs and present a smaller size. The correlation between bigger females presenting larger gonad´s volume was seen in *B. garbeanus* and *B. didactylus* too (Dorigo *et al*., 2012).

Finally, regarding the number of eggs, *B. ephippium* and *B. pitanga* have an average of five eggs, whereas *B. ferruginus* and *B. didactylus* have an average of three (Alves *et al*., 2006; Almeida-Santos *et al*., 2011). In contrast *B. pulex* showed a maximum of two mature eggs, a possible consequence of the small size of this species.

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TABLES AND FIGURES

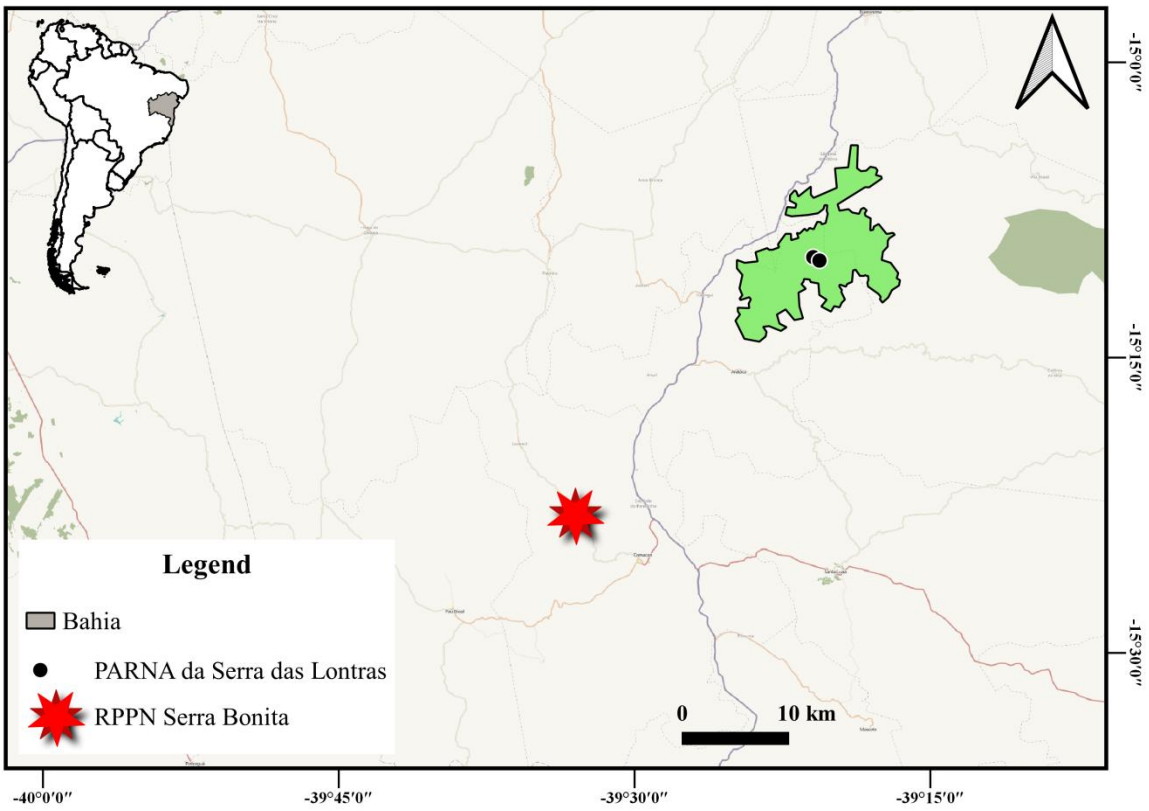

Figure 1. Two new records for *Brachycephalus pulex* from Serra das Lontras National Park, Arataca, Bahia, Brazil.

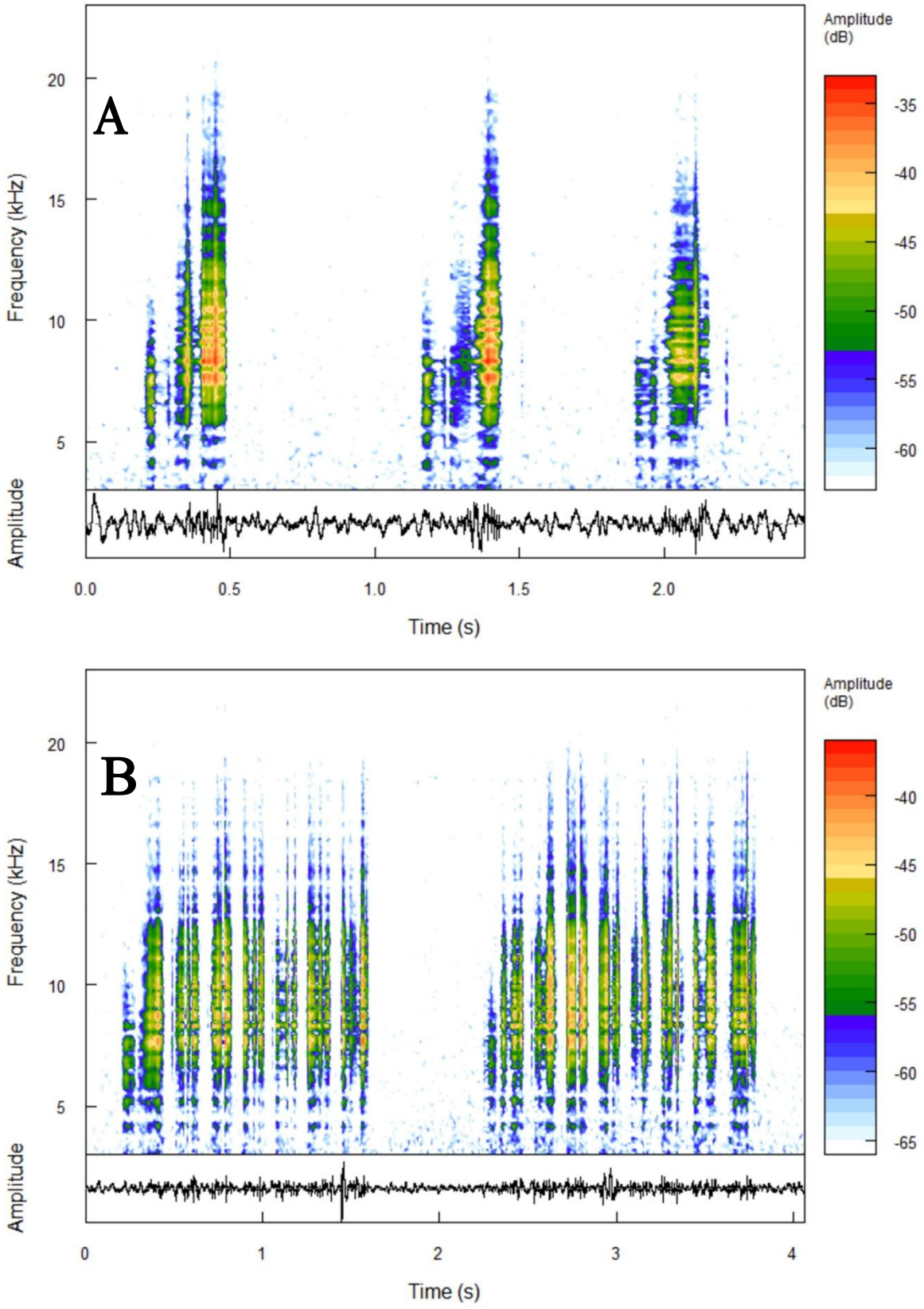

Figure 2. Variation call to *Brachycephalus pulex*: (A) Spectrogram and oscillogram of three consecutive calls "warm-up"; and (B) spectrogram and oscillogram of two consecutive advertisement calls. Voucher WB150, recorded "*ex situ*", on 4 February 2020, air temperature 27°C and air relative humidity 92%.

Figure 3. Color variation in dorsal and ventral view A) in males and B) females of *Brachycephalus pulex.* The numbers represents of individuals photographed, see in Appendix B.

Table 1. Call measurements of the *Brachycephalus pulex.*

Parameters	Warm-up	Advertisement
N° of notes per call	1	1
Note duration (s)	0.27 ± 0.03 (0.208 - 0.298)	1.40 ± 0.21 (0.79 - 1.621)
N° of pulses per note	1	8.71 ± 2.01 (3 - 10)
Pulse duration (s)	Mismo que la nota	0.11 ± 0.04 (0.05 - 0.28)
Inter-notes interval (s)	0.70 ± 0.28 (0.454 - 1.278)	1.02 ± 0.32 (0.638 - 2)
Lower frequency (Hz)	1831.17 ± 44.01 (1805.4 - 1895.6)	1601.30 ± 196.95 (1173.5 - 1805.4)
Upper frequency (Hz)	19549.671 ± 1469.33 (16338.7 - 20671.6)	20840.84 ± 1152.42 (18775.9 - 22386.7)
Dominant frequency (Hz)	8062.53 ± 350.81 (7687.5 - 8343.8)	9507.85 ± 1224.21 (7593.8 - 11062.5)
N	7	24

Table 2. Comparative morphometry among adults measured in mm by Napoli *et al*. (2011) and individuals analyzed in this study.

	Napoli *et al*., 2011	This Study	
SVL	8.2 ± 0.1	8.15 ± 0.4 (7.38 - 8.87)	7.10 ± 0.47 (6.45 - 7.90)
HL	3.3 ± 0.2	2.06 ± 0.39 (1.56 - 3.05)	1.87 ± 0.36 (1.33 - 2.74)
HW	3.3 ± 0.2	2.96 ± 0.24 (2.61 - 3.59)	2.59 ± 0.27 (1.87 - 2.94)
THL	4.1 ± 0.1	3.72 ± 0.30 (3.13 - 4.21)	3.50 ± 0.29 (3.11 - 4.32)
TL	3.4 ± 0.1	3.41 ± 0.29 (2.65 - 3.78)	3.18 ± 0.28 (2.43 - 3.55)
ED	1.2 ± 0.1	1.05 ± 0.16 (0.68 - 1.42)	1.02 ± 0.09 (0.85 - 1.25)
END	0.5 ± 0.1	0.51 ± 0.12 (0.33 - 0.76)	0.46 ± 0.09 (0.31 - 0.70)
ND	-	0.19 ± 0.03 (0.10 - 0.25)	0.21 ± 0.16 (0.10 - 0.96)
IOD	1.2 ± 0.3	1.27 ± 0.14 (1.06 - 1.54)	1.19 ± 0.18 (0.93 - 1.74)
IND	1.1 ± 0	1.01 ± 0.07 (0.84 - 1.12)	0.96 ± 0.07 (0.87 - 1.15)
	4?	22♀	24♂

Table 3. Spearman correlation test between total length in individuals of *Brachycephalus pulex* and volume of gonads.

	N° gonads	SVL (min-max)	Egg size (mm)	Vol. gonads (min-max)	Test Spearman (p) Vol gonadas vs SVL
Males	16	6.46 - 7.84	0.52 ± 0.22 (0.24 - 1.87)	0.01 - 0.16	R^2= 0.017 (p: 0.490)
Females	17	7.28 - 8.78	1.44 ± 0.45 (0.41 - 2.51)	0.05 - 3.90	R^2= 0.238 (p: 0.003)

APPENDIX

Appendix A. Snout-vent length (SVL) measurements of the 36 species of the genus *Brachycephalus.*

Sp	Group	SVL (mm)			Literature cited
		Males	**Females**	**All specimens**	
B. atelopoide *	?	?	?	?	Miranda-Ribeiro, 1920
B. didactylus **	didactylus	8.2 ± 0.7 (6.7–9.5; N=16) 6.7 ± 0.1 (6.6; 6.8; N=2)	10.1 ± 0.4 (9.5–10.7; N=9) 8.8 ± 0.7 (7.5–9.6; N=9)		Almeida-Santos *et al*., 2011
B. hermogenesi		8.7	10.5		Giaretta & Sawaya, 1998
B. pulex		**7.10 ± 0.47 (6.45 - 7.90; N=24)**	**8.15 ± 0.4 (7.38 - 8.87; N=22)**		**THIS STUDY**
B. sulfuratus		8 ± 0.4 (7.4 - 8.5; N=13)	9.9 ± 0.4 (9.0 - 10.8; N= 13)		Condez *et al*., 2016
B. alipioi	epipphium			13.9 + 1.3 (12.5 - 16.2; N=14)	Pombal & Gasparini, 2006
B. bufonoides				13,5 - 16,4	Pombal Jr, 2010
B. crispus		12.0 + 0.3 (11.5–12.9; N=22)	14.0 + 0.8 (11.9 -15.6; N=25)		Condez *et al*., 2014
B. darkside				16.7 + 1.3 (14.8-18.5; N=31)	Guimaraes *et al*., 2017
B. ephipphium		14.33 + 0.58 (13.2-15.4; N = 15)	16.66 + 0.9 (16.0-17.9; N = 12)		Pombal *et al*., 1994
B. garbeana			17,6 (N=1)	14,3-14,4 (N=2)	Pombal Jr, 2010
B. guarani				12.1 + 1.7 (8.7 - 13.4; N=8)	Clemente-Carvalho *et al*., 2012
B. margaritatus				16.9, 1.26 (15.0-18.9; N=9)	Pombal Jr, & Izecksohn, 2011
B. nodoterga		11.8; N=1	13. 4 (12.7 - 14.5; N=7)		Heyer *et al*., 1990
B. pitanga		11.5 + 0.4 (10.8 - 12.1; N=9)	13.4 + 04 (12.6 - 14.0; N=10)		Alves *et al*., 2009
B. toby		11.8 + 0.3 (11.3 - 12.1; N=8)	13.9 + (13.4 - 14.2)		Haddad *et al*., 2010
B. vertebralis				12.9 + 1.4 (10.5-15.1; N=27)	Pombal Jr, & Izecksohn, 2011
B. actaeus	pernix	10.1 + 0.4 (9.2 - 10.8; N=17)	11.7 + 0.3 (11.1-12.4;N=15)		Monteiro *et al*., 2018
B. albolineatus				10.74 ± 0.52 (9.9–11.4; N=8)	Bornschein *et al*., 2016
B. auroguttatus				11.49 + 1.27 (9.0 - 13.6: N=17)	Ribeiro *et al*., 2015
B. boticario				11.06 + 0.85 (10.0 - 12.7; N=8)	Ribeiro *et al*., 2015

B. brunneus	10.2 + 0.7 (9.3 - 11.3; N=15)	11.7 + 0.4 (10.9 - 12.0; N=9)		Ribeiro *et al*., 2005
B. coloratus	10.4 ± 0.1 (10.3–10.6; N=5)	12.9 ± 0.5 (12.2–13.3; N=4)		Ribeiro *et al*., 2017
B. curupira	9.9 ± 0.7 (8.9–10.7; N=10)	10.1 ± 1.7 (8.3–12.3; N=4)		Ribeiro *et al*., 2017
B. ferruginus	12.2 + 0.3 (11.6 - 12.5; N=9)	13.8 + 0.6 (13.0 - 14.5; N=4)		Alves *et al*., 2006
B. fuscolineatus			10.97 + 0.91 (9.7 - 12.4; N=7)	Ribeiro *et al*., 2015
B. izecksohni	11.1 + 0.5 (10.3 - 12.1; N=11)	12.8 + 0.2 (12.5 - 13.1; N=4)		Ribeiro *et al*., 2005
B. leopardus			10.82 0.62 (9.7 - 11.9: N=14)	Ribeiro *et al*., 2015
B. mariaeterezae			11.27 + 0.97 (10.4 - 13.4; N=10)	Ribeiro *et al*., 2015
B. mirissimus	10.9 + 0.6 (9.9-11.7)	11.4 + 2.0 (10.0 - 12.9)		Pie *et al*., 2018
B. olivaceus			10.50 + 1.32 (9.4 - 12.9: N=17)	Ribeiro *et al*., 2015
B. pernix	12.53 + 0.44 (12.0 - 13.3; N=23)	14.90 + 0.60 (14.1 - 15.8; N=5)		Pombal *et al*., 1998
B. pombali	13.3 + 0.5 (12.6 - 13.9; N= 4)	15.0 + 0.3 (14.6 - 15.3; N=4)		Alves *et al*., 2006
B. quiririensis			11.12 ± 1.03 (9.9–13.1; N=10)	Pie & Ribeiro, 2015
B. tridactylus	11.63 + 0.83 (10.6 - 11.67; N=4)	13.67 + 0.21 (13.52 - 13.82; N=2)		Garey *et al*., 2012
B. verrucosus			11.35 + 0.99 (9.6 - 13.2; N=14)	Ribeiro *et al*., 2015

? unknown; *Holotype lost; ** Two locality

Appendix B. List of voucher specimens photographed to *Brachycephalus pulex*.

Number	Locality	N° campo	SEX
1	SB	IURI - 3	M
2	SB	IURI - 88	M
3	SB	IURI - 90	M
4	SB	IURI - 91	M
5	SB	IURI - 95	M
6	SB	WB145	M
7	SB	WB147	M
8	SB	WB148	M
9	SB	WB151	M
10	SB	IURI - 1	F
11	SB	IURI - 2	F
12	SB	IURI - 5	F
13	SB	WB146	F

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CHAPTER TWO

Diet of *Brachycephalus pulex* Napoli, Caramaschi, Cruz, & Dias, 2011 (Anura, Brachycephalidae)

Wendy Hercilia BOLAÑOS HERNANDEZ[1], Iuri RIBEIRO DIAS[1] and Mirco SOLÉ[1,2]

1. Department of Biological Sciences, Universidade Estadual de Santa Cruz, Rodovia Ilhéus-Itabuna, km 16, 45662-900 Ilhéus, Bahia,

Brazil

2. Herpetology Section, Zoologisches Forschungsmuseum, Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany

* Corresponding authors name and email address: Wendy Hercilia Bolaños Hernandez – wendyhbh@gmail.com

Abstract. The genus *Brachycephalus* is represented by 36 species, but information about diet is only available for six, showing that the most frequent, most important or most consumed trophic resources are mites, collembolans and ants. Thirty-three stomach contents of *B. pulex* extracted from individuals housed at the Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC) were analyzed and identified. We estimated the availability of trophic resources in the environment and tested the differences between number of food items ingested and snout-vent length (SVL), and the largest prey per stomach and jaw width (JW). Our results show that the mean number of food items was 5 ± 3.50; the volume of the largest food items ingested was ants with 122.15 mm^3, and the smallest item was Thysanoptera with 0.01 mm^3. Mites represented the largest number of

food items, being present in 87% of the extracted stomachs. We did not find a significant relationship between the number of food items ingested and SVL, neither between the volume of the largest prey per stomach and frog jaw width. These two results about correlation between SVL and number of food items ingested and Volume prey and jaw width were similar to those found for *B. pitanga*, *B. brunneus*, and *B. pernix*. We conclude that *B. pulex* can be regarded as a nocturnal active predator.

Key Words: *Foraging, Flea-toad, Mites, Serra Bonita, Serra das Lontras.*

Running title: Dieta of *Brachycephalus pulex*

Introduction

Studying diet in anurans helps to understand how this group fits into a given ecosystem or habitat (Norval et al. 2014, Marques-Pinto et al. 2019) and how they obtain enough energy to grow and reproduce, among other natural aspects (Zug et al. 2001). While invertebrates are the most common food consumed by most frogs, some species can also feed on small vertebrates (Solé et al. 2009). Most anurans are considered generalist and/or opportunistic species (Duellman & Trueb 1994, Pough et al. 2004, Solé et al. 2019), but several species have been found to actively search for food (Borges et al. 2019, Sah et al. 2019)

According to Toft (1985) there are two adaptive strategies in foraging mode that these animals use: “sit-and-wait” which is the best chance to capture large and more mobile preys and “active foragers” that have more chances to capture small preys that often are abundant

or live in aggregations. Besides that, Toft (1980, 1981 & 1985) also classifies frogs as being specialist in ants (diet rich in this insect, using an active foraging strategy) or being non-ant-specialists (a few or no ants with a sit-and-wait foraging behavior). Recent studies have shown that these two strategies represent extremes and that several species can be found somewhere within a generalist-specialist continuum (Caldart et al. 2012, Protázio et al. 2019). Because an organism can vary its diet according to the place it occupies and the season, studying feeding habits can be a complex task (Zug et al. 2001, Norval et al. 2014, Velasco et al. 2019).

The genus *Brachycephalus* is composed by 36 species that are distributed throughout the Atlantic forest, from the south of the state of Bahia to the south of the state of Santa Catarina in Brazil (Frost 2020). It´s a genus with scarce available information about natural life aspects as the diet, and until now, feeding habits have only been described for six species within the genus: *B. ephippium* (Pombal 1992) *B. pernix* (Wistuba 1998), *B. brunneus* (Fontoura et al. 2011), *B. didactylus* (Almeida-Santos et al. 2011), *B. garbeanus* (Dorigo et al. 2012) and *B. pitanga* (De Oliveira & Haddad 2015).All these studies show that the most frequent, most important or most consumed trophic resources are mites, collembola and ants. In this study, we evaluated the feeding habits of *Brachycephalus pulex*.

Material and Methods

Thirty-three stomach contents extracted from individuals housed in the Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC) were analyzed and its contents identified under a microscope to the minimum possible taxonomic level with help

of the keys by Johnson et al. (2004). Within these 33 individuals, seven came from Serra das Lontras (municipality of Arataca, Bahia) and 26 from the RPPN Serra Bonita (municipality of Camacan, Bahia, type locality of this species).

For every food item, we measured length and width using a Leica S9I Stereozoom microscope and to estimate the volume (mm^3) of every item we used the formula for an ovoid sphereoid: $V=4/3p\ (length/2)\ (width/2)^2$ (Dunham 1983). To calculate the importance index (Ix) by category, we used the formula by Biavati (2004): Ix = (F% + N% + V%) / 3, where: F% = percentage in the frequency of occurrence, N% = percentage of stomachs where item "x" was found, and V% = volumetric percentage. To estimate the original length of prey, such as ants in which we only recovered the heads, the regression formula proposed by Hirai & Matsui (2001) was applied.

In order to estimate the availability of trophic resources in the environment, two 30x30 cm litter samples were collected in the same place where the *Brachycephalus pulex* specimens were previously found (we did this just for specimens from RPPN Serra Bonita); the litter samples were taken to the laboratory and processed using the Berlese-Tüllgren funnel technique (Juncá & Eterovick 2007). Afterwards, the material was sorted and the organisms separated, classified and placed in eppendorf tubes with 70% alcohol. All frogs had their snout-vent length (SVL) and jaw width (JW) measured under a Leica S9I Stereozoom microscope. We used a regression to test differences between: numbers of food items ingested vs SVL and the largest prey per stomach vs JW. To calculated an electivity index we used the equation proposed by Jacobs (1974) "D= Rk - Pk / (Rk + Pk) - (2Rk*Pk)", in which "Rk" is the proportion of prey category k in stomach contents and "Pk" is the proportion of prey category k in the environment This electivity index (D)

varies from -1 when prey k is absent from the diet, but present in the environment, zero (0) when the prey is consumed in the same proportion as it is found in the environment and +1 representing a complete selection or preference for prey k. To test the correlation between categories of prey recovered from frogs captured at RPPN Serra Bonita and Serra das Lontras we used an ANOVA.

Results

Thirty-three individuals of *Brachycephalus pulex* were analyzed, of which seven did not reveal stomach contents. SVL varied from 6.46 mm to 8.78 mm (mean: 7.49 mm; SD: 0.69 mm) and JW from 1.7 mm to 2.82 mm (mean: 2.13 mm; SD: 0.26 mm). The mean number of food items was 5 (SD: 3.50); range (min –max): 1-13; (N=26). Of the 15 categories available in the litter, seven were present in the stomachs of the individuals analyzed (Figure 1). The largest ingested food item was ants with a volume of 122.15 mm^3, and the smallest item was Thysanoptera with 0.01 mm^3. The largest number of food was represented by mites in 87% of the extracted stomachs (Oribatida = 73%; Uropodina = 14%), followed by Collembola (6%) and Coleoptera larvae (3%). We did not find a significant relationship between the number of food items ingested and snout–vent length (r^2=0.032969; p=0.31188); neither between the volume of the largest prey per stomach and frog jaw width (r^2=0.17646; p=0.032634). Regarding the Electivity index (Jacobs, 1974) Diptera (larvae) and Collembola were the categories with largest values (0.78 and 0.71 respectively) (Table 1). Between localities of Serra Bonita and Serra das Lontras Diet did not differ significantly between the localities of Serra Bonita and Serra das Lontras (F=0.08911, p=0.7714) (Table 2).

Of the 33 frogs collected, 16 were males and 17 were female. Three males and four females had empty stomachs. Were found 52 items in males and 74 in females. Of the 15 categories available in the litter, six were present in the stomachs of the females (Acariformes, Mesostigmata, Collembola, Formicidae, Coleoptera and Diptera) and five in the stomachs of males (Acariformes, Mesostigmata, Collembola, Coleoptera and Thysanoptera). After comparing the diet between both sexes, we found that males had among one and two categories in their stomachs, while females t presented among one and five categories per stomach. The mean number of items per stomachs was 2.98 (SD: 3.19; min=1; Max=12) in males and 4.51 (SD: 3.71; min=1; Max=13) in females. No significant differences were obtained when comparing the volume of the items preyed by males and females (Test t: $p<0.005$; t: 0.23193)

Discussion

The habitat where frogs live can greatly influence how and on what they feed. Frogs living on the soil and leaf litter usually reveal a large number of mites (Arachnid Class, Order Acari) in their stomachs (Simon & Toft 1991).

For the genus *Brachycephalus* mites have already been reported as important components of their diets. In this study we show that this is also the case for the diet of *B. pulex*. The preferences for mites, followed by collembolans has been reported for *B. pitanga* (De Oliveira & Haddad 2015), *B. brunneus* (Fontoura et al. 2011), *B. pernix* (Wistuba 1998) and is now also being reported for *B. pulex* (this study). Otherwise, *B. didactylus* (Almeida-Santos et al. 2011) and *B. eppiphium* (Pombal 1992) prefer

collembolans over termites and *B. garbeanus* termites followed by ants (Dorigo et al. 2012).

In *Brachycephalus pulex*, the consumption of preys was in the same proportion as the availability in the leaf litter for mites, Collembola, Coleoptera and Diptera larvae. In the diet of *B. brunneus*, items with a high availability in the habitat like mites were also found in the same proportion as in our results. The same was observed for *B. garbeanus* with ants (Fontoura et al. 2011; Dorigo et al. 2012). Soil and litter mites are mostly smaller than 2 mm, thus only representing a valuable energy source for tiny frogs (Simon & Toft 1991).

Differences in the diet between males and females has been reported for other anuran species that can be associated with seasonal abundance of food, ecological tolerances (Duellman & Trueb 1986), size, and behavioral dimorphism (Miranda et al. 2006). In the case of *Brachycephalus pulex* our data suggest that both sexes had a similar diet regarding prey diversity and proportion of prey, like also reported for *B. garbeanus* and *B. pitanga* (Dorigo et al. 2012; De Oliveira & Haddad 2015). We only analyzed potential prey availability in the leaf litter of Serra Bonita (SB), however, the stomach contents of individuals from Serra das Lontras tell us that the microdiversity (leaf litter) of this forest could be pretty similar with that of SB, and for that, we could say that the prey consumption is almost the same.

Regarding the foraging strategies (Toft 1985), *Brachycephalus pulex* can be considered a nocturnal active predator. This type of foraging is the best way to depredate small, slow-moving and often abundant preys, like the mites found in the stomach contents in this work. In addition to this, individuals were observed foraging while walking in the

leaf litter and hunting prey near them was often impossible due to the slow displacement capacity of the toad (W. H. Bolaños pers. Obs). A similar observation was made by De Oliveira & Haddad (2015) for *Brachycephalus pitanga.*

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Table captions

Table 1. Stomach content of individuals of *Brachycephalus pulex* obtained in the collection of the Zoology Museum of the State University of Santa Cruz (MZUESC). Item frequency (F), volume (V), Importance Index (Ix) and Electivity Index (D) for each category presented in net value and percentage.

Table 2. Categories of prey found in stomach contents of 33 individuals of *Brachycephalus pulex* from two locations.

Figure captions

Figure 1. Proportion (%) of categories found in stomachs and leaf litter.

Table 1. Stomach content of individuals of *Brachycephalus pulex* obtained in the collection of the Zoology Museum of the State University of Santa Cruz (MZUESC). Item frequency (F), volume (V), Importance Index (Ix) and Electivity Index (D) for each category presented in net value and percentage

Food item	Availability	%	Stomach contents	%	F	%	V	%	Ix	D
Arachnida										
Acariformes (Oribatida)	1199	79	92	73	22	85	1.065	0.85	53	-0.174
Mesostigmata (Uropodina	226	15	18	14	11	42	0.536	0.43	19	-0.027
Mesostigmata (Gamasina)	10	1	0	0	0	0		0.00	0	-1.000
Acariformes (Prostigmata)	9	1	0	0	0	0		0.00	0	-1.000
Araneae	2	0	0	0	0	0		0.00	0	-1.000
Pseudoscorpiones	1	0	0	0	0	0		0.00	0	-1.000
Hexapoda				0	0	0		0.00	0	
Collembola	15	1	7	6	6	23	0.076	0.06	10	0.709
Hymenoptera	1	0	0	0	0	0		0.00	0	-1.000
Formicidae	5	0	2	2	2	8	122.157	97.28	36	0.659
Neuroptera	1	0	0	0	0	0		0.00	0	-1.000
Thysanoptera	9	1	1	1	1	4	0.014	0.01	2	0.144
Coleoptera (larvae)	20	1	4	3	4	15	0.629	0.50	6	0.419
Diptera (Adult)	5	0	0	0	0	0		0.00	0	-1.000
Diptera (larvae)	3	0	2	2	2	8	1.101	0.88	3	0.780
Myriapoda		0		0	0	0		0.00	0	
Diplopoda	5	0	0	0	0	0		0.00	0	-1.000

Table 2. Categories of prey found in stomach contents of 33 individuals of *Brachycephalus pulex* from two locations.

Category of items	Serra Bonita	Serra das Lontras
Acari	66	44
Diptera	1	1
Coleoptera	2	2
Collembola	3	4
Formicidae	1	1
Thysanoptera	1	0

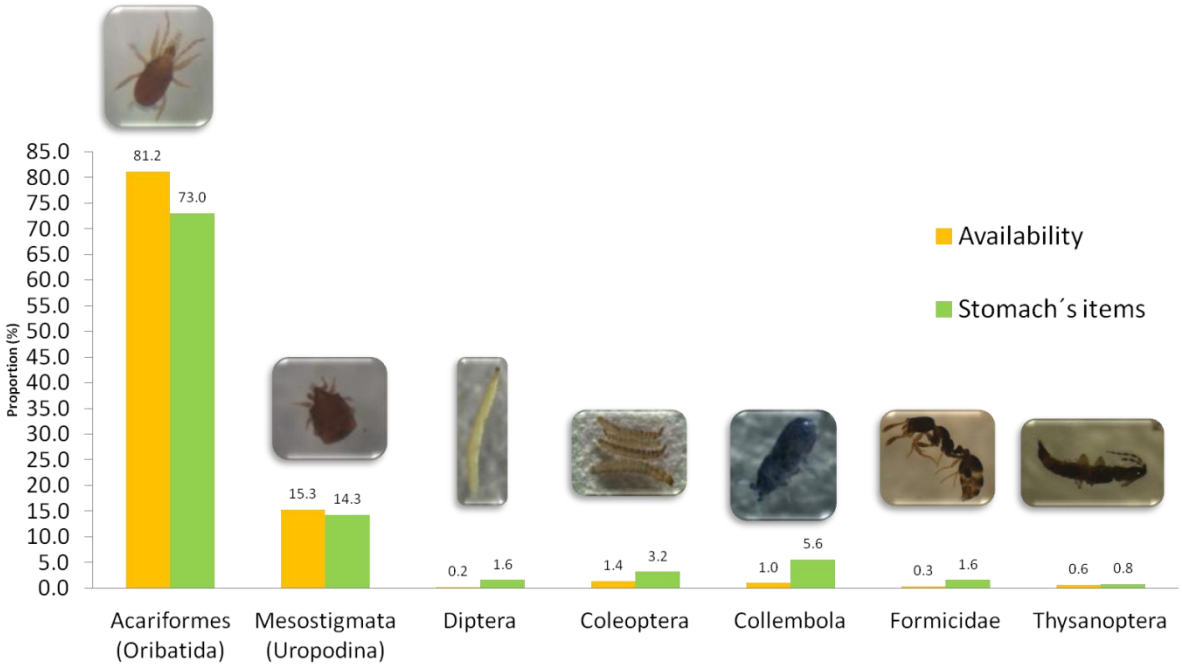

Figure 1. Proportion (%) of categories found in stomachs and leaf litter.