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Geographic distribution, habitat use and vocalizations of the leaf-litter frog Ischnocnema henselii (Anura: Brachycephalidae) in the subtropical Atlantic Forest

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Abstract. Data on geographic distribution and ecology are essential when defining the conservation status of a species. Herein, we present new information about the geographic distribution, habitat and advertisement call of Ischnocnema henselii, an endemic species of the southern Atlantic Forest. We conducted this study in forest fragments in the state of Santa Catarina, Southern Brazil, from January 2008 to November 2012. We recorded I. henselii in eight fragments over 100 ha in size, both inside and outside conservation units. We often observed less than 20 males in calling activity. The calling activity was daytime and nighttime, and more intense in the rain. We observed individuals perched on vegetation, on leaves or branches, on the leaf litter or under tree trunks. Duration of advertisement calls ranged from 14.71 ± 1.68 s (n = 11 calls) and interval between notes was 0.22 ± 0.13 s (n = 99 notes). We observed that the advertisement calls had a feature that we call "alternating modulation" in the amplitude of the notes. We suggest that further bioacoustic studies should analyze the relevance of this alternating modulation, to understand its role in communication. Although I. henselii is a habitat specialist, it is currently considered a common species, classified as Least Concern by Brazilian and IUCN lists. In the region studied, characterized by small and isolated remnants of the Atlantic Forest, I. henselii is not an abundant species and its spatial distribution is always associated with wet forest fragments. The increasing degradation of forest remnants in this region causes microclimatic and structural changes that may compromise the permanence of strictly forest and moisture-dependent species for reproduction, such as I. henselii.

Keywords: Advertisement calls, Amphibia, natural history, Southern Brazil.

Resumo. Distribuição geográfica, uso do habitat e vocalizações da rãzinha-de-folhiço Ischnocnema henselii (Anura: Brachycephalidae) na Mata Atlântica subtropical. Informações sobre distribuição geográfica e ecologia são essenciais para a definição do estado de conservação de uma espécie. Aqui, nós apresentamos novas informações sobre a distribuição geográfica, habitat e canto de anúncio de I. henselii, um anfíbio endêmico do Sul da Mata Atlântica. Nós conduzimos o estudo em fragmentos florestais no estado de Santa Catarina, sul

do Brasil, de janeiro de 2008 a novembro de 2012. Registramos I. henselii em oito fragmentos com tamanho acima de 100 ha, no interior ou fora de unidades de conservação. Nós frequentemente observamos menos de 20 machos em atividade de vocalização. As atividades de vocalização foram registradas de dia e à noite e eram mais intensas sob chuva. Observamos indivíduos empoleirados na vegetação, sobre folhas ou galhos, no folhiço ou sob troncos de árvores caídas. A duração do canto de anúncio foi 14,71s ± 1,68 (n = 11 cantos) e o intervalo entre as notas foi de 0,22s ± 0,13 (n = 99 notas). Os cantos de anúncio apresentaram o que chamamos de "modulação alternada" na amplitude das notas. Sugerimos que outros estudos analisem a relevância desta "modulação alternada", visando compreender a sua relação com a comunicação nesta espécie. Embora I. henselli seja especialista de habitat, é considerada comum, classificada na categoria "preocupação menor" nas listas brasileira e mundial de espécies ameaçadas de extinção. Na região de estudo, caracterizada por remanescentes pequenos e isolados de Mata Atlântica, I. henselii é pouco abundante e encontrada somente em fragmentos florestais úmidos. A crescente degradação dos remanescentes florestais nesta região ocasiona mudanças microclimáticas e estruturais que podem comprometer a permanência de espécies estritamente florestais e dependentes da umidade para reprodução, como I. henselii.

Palavras-chave: Canto de anúncio, Amphibia, História natural, Sul do Brasil.

Introduction

The genus *Ischnocnema* Reinhardt and Lütken, 1862 is currently composed of 33 species (Frost, 2017), distributed in central and eastern Brazil and adjacent northern Argentina, with most species associated with the Brazilian Atlantic Forest (Canedo & Haddad, 2012; Padial *et al*., 2014). Species of this genus show a specialized reproductive mode, with direct development of terrestrial eggs (Haddad & Prado, 2005). Thus, it is a habitat specialist, requiring forested areas, with the presence of moist leaf litter in which to deposit its terrestrial eggs (Haddad & Prado, 2005).

Ichnocnema henselii (Peters, 1870) was several times confused with *I. guentheri* (Steindachner, 1864), until its revalidation by Kwet & Solé (2005). Both species are morphologically very similar, and are distinguishable mainly by differences in their advertisement call (Kwet & SOLÉ, 2005). Further morphological and call analyses suggest the existence of four undescribed species within the *I. guentheri* and *I. henselii* complex (Gehara *et al*., 2013). Before the study of Gehara *et al.* (2013), I. guentheri was considered to be the most widespread species of the Brachycephalidae (Hedges et al., 2008). Now, the most widespread species of the Brachycephalidade is I. henselii, at least according to our current taxonomic knowledge of this species. Currently, *I. henselii* is known in Misiones, Argentina and from southern Brazil up to São Paulo State (Gehara *et al.,* 2013), and it is one of the few species of the genus with occurrence on the plateaus in the western part of the Atlantic Forest, covered

with remnants of Araucaria and seasonal forests (Hedges *et al*., 2008; Gehara *et al.,* 2013).

For many tropical amphibian species, such as *I. henselii*, there are few data on actual population density and population trends. The most important variables used to assess the threatened status of a species have been the population trends, population size, geographic range (extent of occurrence and area of occupancy which include quality of habitat), and extinction risk (Stuart *et al*., 2004; Becker *et al*., 2007; IUCN, 2017). Herein, we present new information about the geographic distribution, habitat and advertisement call of I. henselii from the southern region of the Atlantic Forest. These new data will support future studies to clarify the taxonomic status and geographic distribution of I. henselii, and also assist in defining priorities for the conservation of amphibians in the southern portion of the Atlantic Forest, as results provide new basic information on the extent of occurrence, area of occupancy (e.g. habitat use), and local population size.

Material And Methods

We conducted this study in Atlantic Forest fragments in the state of Santa Catarina, Southern Brazil, from January 2008 to November 2012. The localities studied have distinct coastal vegetation (municipality of Vidal Ramos) at the easternmost location of our study, Araucaria forest (municipalities of Campos Novos, Palma Sola, Ponte Serrada and São Domingos) and deciduous forest (municipalities of Faxinal dos Guedes, Chapecó and Concórdia) on the inland plateau (Figure 1, Table 1). The vegetation was

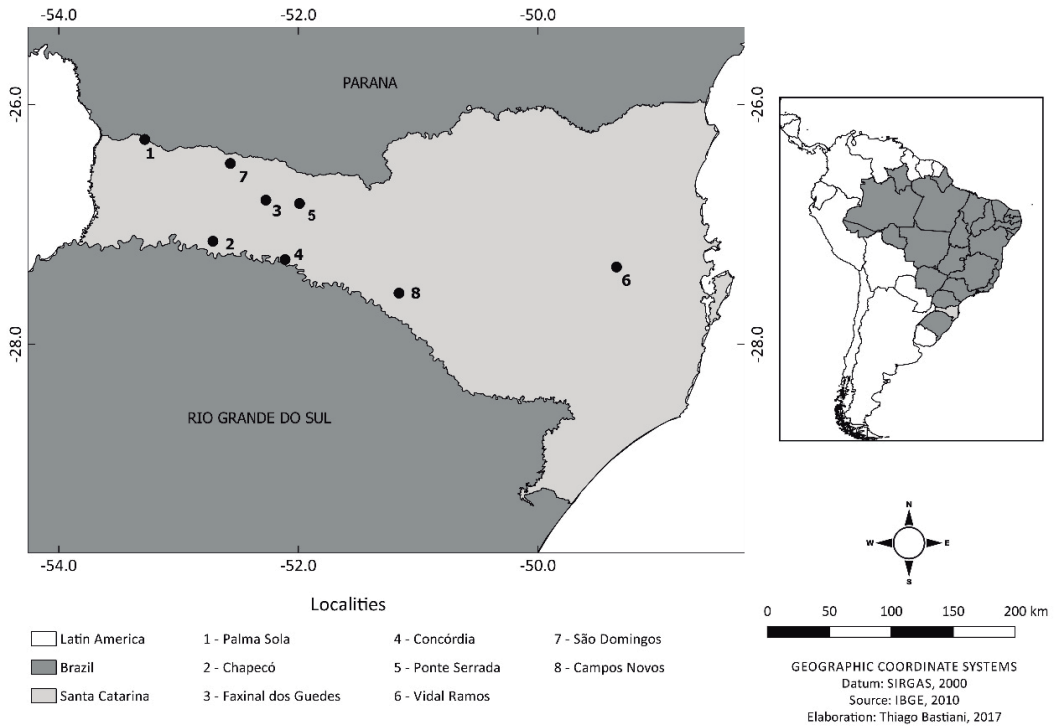

Figure 1. Localities with occurrence of Ischnocnema henselii in the state of Santa Catarina, Southern Brazil.

classified according to Klein (1978). All remnants showed aquatic environments and sites with moist leaf litter.

Table 1. Localities with occurrence of Ischnocnema henselii in the state of Santa Catarina, Southern Brazil. SF - seasonal forest; MOF - mixed ombrophilous forest; DOF - dense ombrophilous forest. Abundance of calling males: I) 1-2; II) 3-5; III) 6-10; IV) 11-20 and V) 21-50. According to Klein (1978). Activity: N = nocturnal; D = diurnal.

Municipality	Coordinates S/W	Size of the remnant (ha)	Formation	Abundance of calling males	Activity	Total effort (person-hours)
Faxinal dos Guedes	26º48'36.5''/ 52º16'44.3''	100	SF	III	D	8
Ponte Serrada/ Passos Maia (Parque Nacional das Araucárias)	26°49'31.2''/ 51°59'23.4''	12,000	MOF	IV	D/N	16
Vidal Ramos	27º21'20.3''/ 49º20'35.6''	100	DOF	II	N	8
Chapecó	27°08'25.8"/ 52°42'46.7''	150	SF	II	N	12
Palma Sola	26°17'29.3''/ 53°16'54.0'	3,000	MOF	V	D/N	12
Concórdia (Parque Estadual Fritz Plaumann)	27°17'36.0''/ 52°06'38.1''	741	SF	IV	D/N	28
Campos Novos (Parque Estadual Rio Canoas)	27º 34' 24.9''/ 51º 09' 34.5''	1,133	MOF	I	D/N	8
São Domingos (Parque Estadual das Araucárias)	26º 29' 29.1''/ 52º 34' 03.0''	625	MOF	I	N	8

Records of *I. henselii* (Figure 2) were taken through visual and auditory search (sensu Scott & Woodward, 1994), on trails in the forest remnants, during the day and at night. In each area, we performed one to three days of sampling, and each day the effort was two to four hours, totaling approximately 100 person-hours/ total effort (Table 1). The sampling began at the evening and finished between 22 and 23 hours. For each day of sampling we perform one to two hours of effort during the day.

We opted for a conservative measure of the number of males in vocalization activity. Thus, in each area that we surveyed, the abundance of calling males was as follows: (I) 1-2, (II) 3-5, (III) 6-10, (IV) 11-20 and (V) 21-50 (adapted from Canelas & Bertoluci, 2007). For the categorization we consider the total abundance of males in vocalization activity on the day or site with greater abundance for each area, in order to avoid overestimation. Snout-vent-length (SVL, in mm) and mass (in mm) were measured for males and

Figure 2. Habitat used by *Ischnocnema henselii* in the state of Santa Catarina, Southern Brazil. A) male and female in amplexus; B) male perched on branches; C and D) male on leaf litter.

females to characterize two populations, Palma Sola and Concórdia. The number and diameter of the eggs (mm) were obtained for one female. We measured mass with a Pesola® dynamometer (0.1 g), SVL and diameter of eggs with Mytutoyo® digital caliper (0.01 mm). Measurements of SVL and mass are presented as mean ± standard deviation (minimum - maximum). Egg mass volume (mm3) was estimated by multiplying the mean diameter of the eggs by the total number of eggs. To characterize habitat use by males and females, considering the type of substrate (leaf, branch, trunk and foliage), perch height (cm) and nearest distance to water (cm).

Vocalizations were recorded with a Marantz® digital recorder model PMD661 and Sennheiser® directional microphone model K6/ ME66. Recordings were analyzed with Avisoft Sonagraph Light and Cool Edit 96. To characterize the vocalizations by male's information about the dominant frequency was obtained through Fast Fourier Transform (FFT, 1024 points) by Cool Edit 96. Sonograms were obtained by Avisoft Sonagraph Light with FFT in 256 points. A Flat Top window was used for the sonograms due to the pulsed structure of the calls. Acoustic terminology was according to Duellman & Trueb (1994), Gerhardt (1998), and Toledo *et al*. (2014). Distances between the nearest calling neighbors and distances between calling and non-calling males were done with a measuring tape (cm) to characterize the spatial distribution of calling and non-calling males.

Voucher specimens were collected, fixed according to standard methods (Heyer *et al*., 1994) and deposited at the Amphibian Collection of Universidade Comunitária da Região de Chapecó (CAUC0895; CAUC0939; CAUC0989, CAUC2174-2175, CAUC2178-2182).

Results

We recorded *I. henselii* in eight forest fragments over 100 ha in size, one in dense ombrophilous forest, four in mixed ombrophilous forest and three in decidual seasonal forest (Figure 1). It is important to emphasize that four recordings were done in conservation units at Parque Estadual Fritz Plaumann (Concórdia), Parque Nacional das Araucárias (Ponte Serrada/ Passos Maia), Parque Estadual Rio Canoas (Campos Novos), and in Parque Estadual das Araucárias (São Domingos).

The largest number of calling males (>21) was recorded in only one area, in Palma Sola (Table 1). In the fragments of Palma Sola, Concórdia, Ponte Serrada/Passos Maia and Faxinal dos Guedes, we observed calling activity during day and night, and more intense during rain (categories IV and V).

The mean SVL of 15 adult calling males in Palma Sola was 25.80 ± 1.08 mm (23.94 – 27.80 mm) and the mass was 1.19 ± 0.44 g (0.50 – 1.90 g). One female with a SVL of 36.45 mm and mass of 5.10 g had 65 eggs, which weighted 0.60 g. The diameter of 10 eggs was 2.80 ± 0.70 mm (1.00 – 3.70 mm) and the egg mass volume was 182 mm3. In the population of Concórdia, the mean SVL of three females was 39.09 ± 5.77 mm

(32.60 – 43.67 mm) and the mass was 7.38 ± 1.68 g (5.50 – 8.75), while in one male, SVL was 25.61 mm and mass 1.20 g.

In the population of Palma Sola, we observed individuals perched on vegetation, leaves (N = 7) or branches (N = 3), but also on the leaf litter (N = 5) or under tree trunks (N = 2). For the ones that were perched, the height of the perch ranged from 20 to 80 cm (39.00 ± 17.91 cm; N = 10) and the water distance ranged from 110 to 700 cm (427.64 ± 133.86 cm; N = 17). For the population in Concórdia, we observed males perched at 10 cm on vegetation (N = 2) and females on the leaf litter (N = 3). Males were recorded more distant from water (450.00 ± 212.13 cm; 300 – 600 cm; N = 2) than females (68.33 ± 42.52 cm; 20 – 100 cm; N = 3). In all remnants, I. henselii was found exclusively in wet sites, close to aquatic environments.

The d istance between neighboring calling males was 190.0 ± 17.3 cm (160 – 200 cm; N = 2) and 133.3 ± 117.8 cm (50 – 300 cm; N = 3) between calling and non-calling males.

Duration of advertisement calls ranged from 14.71s ± 1.68 (12.97 - 17.82; N = 11 calls/3 males) and interval between notes was 0.22s ± 0.13 (0.10 - 0.55; N = 99 notes/11 calls/3 males). The dominant frequency was 2.25 ± 0.15 kHz (2.01 - 2.63; 44 notes/11 calls/3 males). The dominant frequency showed a slight ascending modulation from the beginning to the end of the call, and also a weak ascending modulation in call amplitude, clearly visible on the oscillogram (Figure 3). The number of notes was 88.00 ± 14.17 (75.00 – 119.00; 11 calls/3 males). We observed that the advertisement calls had a feature that we called “alternating modulation” in the amplitude of the notes. This alternating modulation was perceptible mainly in the oscillogram of the middle third of the advertisement call (Figure 4), where a sequence of alternating low- and high--amplitude notes was clearly evident. The advertisement call begin with a sequence of low--amplitude notes, which are more spaced (Figure 4A), and by the middle third, the notes become closer and display this alternating modulation (Figure 4B), which disappears at the arrival of the last third of the call (Figure 4C).

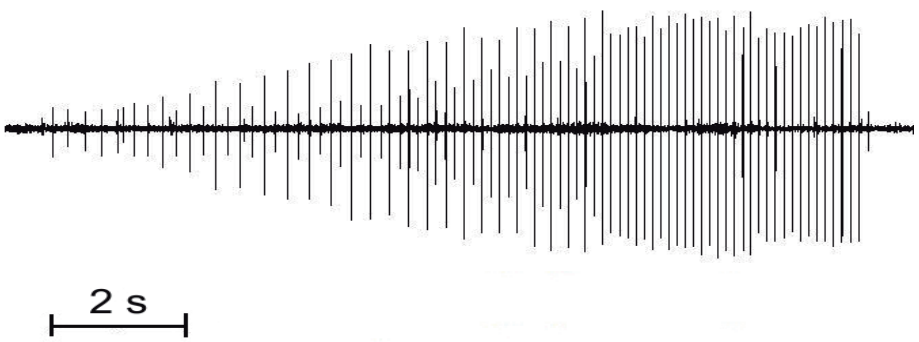

Figure 3. Oscillogram of an advertisement call of *Ischnocnema henselii*, from Palma Sola, in the state of Santa Catarina, Southern Brazil. Recorded on October 12, 2011. Air temperature: 17.5ºC.

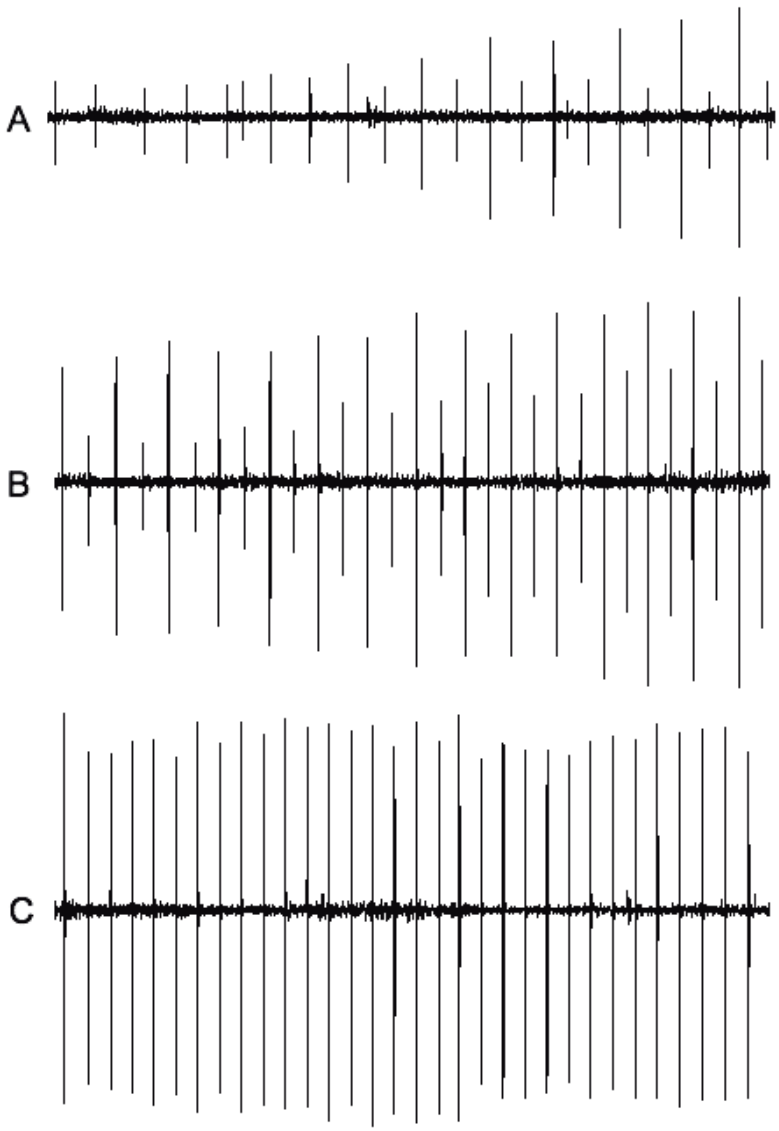

Figure 4. Oscillograms of the same call as in Figure 3, split into the initial third (A), middle third (B), and last third (C). Note the "alternating modulation" in note amplitude, very clear in the middle third.

Discussion And Conclusion

The distribution of *I. henselii* in the Santa Catarina plateau is evermore related to wet and consequently larger forest remnants. In this region, the Atlantic Forest is intensely fragmented, with remnants almost always smaller than 100 ha (Ribeiro *et al*., 2009). Field surveys in other smaller or more anthropized areas did not result in records of *I. henselii* in this region (personal observation). Although *I. henselii* has a wide distribution area, along the subtropical Atlantic rainforest and parts of the Araucaria forest in southern Brazil and reaching São Paulo and Misiones, Argentina (Gehara *et al*., 2013; Frost,

2017), the inland plateau was found to be a distributional gap of about 500 km from the nearest known locality in eastern Santa Catarina until Misiones, Argentina. In this wide area of the plateau, *I. henseli* was found only in two larger protected areas, the Parque Nacional das Araucárias and Parque Estadual Fritz Plaumann.

Our measurement data on SVL from males and females agree with KWET & SOLÉ (2005), who found a distinct sexual dimorphism in size. With our data this sexual dimorphism is even more evident, since we found substantially larger females. The previously reported range of SVL for females was 28.4 – 38.4 mm (Kwet & Solé, 2005), and at Concórdia, we found two females with higher SVL values (41 and 43.67 mm).

Ischnocnema henselii is a common, ground-living species with diurnal and nocturnal activity. Eggs are deposited terrestrially, under trunks or stones (Kwet & Solé, 2005, Conte *et al*., 2010). The only previous report on eggs is for one female that had 26 large (3 mm diameter) and 45 small (1 mm diameter) yellowish eggs (Kwet & Solé, 2005). The only female with eggs found in our study had a similar number of eggs (N = 65). Males of *I. henselii* are reported to call from the ground or from fallen trees and stones (KWET & SOLÉ, 2005). In our study, calling males were found perched on leaves and branches, up to 80 cm, and also on the ground.

The call parameters of *I. henselii* analyzed were similar to the description made previously by Kwet & Solé (2005). Nevertheless, it is striking that there seems to be a slight ascendant modulation of the dominant frequency unlike that reported by Kwet & Solé (2005), who did not mention any modulation. The call analysis by Gehara *et al.* (2013) on recordings of several populations identified as *I. henselii* and *I. guentheri* showed that all calls had a weakly ascendant modulation of frequency. The duration of the calls of *I. henselii* recorded by us was also similar to that reported by Gehara *et al*. (2013), where the call duration of *I. henselii* has intermediate values compared to the long advertisement call of *I. guentheri* from the type locality, and four other lineages of *I. guentheri* that are candidate new species.

A feature apparently previously overlooked in the vocalizations of *I. henselii* is an "alternating modulation" in the energy of the notes. We do not know of other cases of this alternating modulation in the intensity of notes in an amphibian's vocalizations. This feature that we call "alternating modulation" was discernible in all analyzed calls. Similarly, Hepp & Canedo (2013) reported that in *I. oea* (Heyer, 1984) there was an ascending amplitude modulation, with amplitude peak at the end of the call, but this increase was not regular, because sometimes the amount of energy decreases between consecutive notes. We suggest that further bioacoustic studies should analyze the relevance of this "alternating modulation" in the call amplitude, to understand its relation in the communication of *Ischnocnema* species or even to determine if calls of other *Ischnocnema* species also display this feature.

Although it is a habitat specialist, *I. henselii* is currently considered a common species, classified as Least Concern by international (Iucn, 2017) and Brazilian (Mma, 2014) lists. In the areas studied, *I. henselii* is not an abundant species when compared to other sympatric species (Lucas & Marocco 2011; Bastiani & Lucas, 2013), and the area of occupancy is always associated with wetlands inside the forest. The habitat loss and modification cause microclimatic and spatial configuration changes, such as edge effect and reduction in litter humidity, essential for larval development and permanence of amphibians with direct development of terrestrial eggs (Watling & Braga, 2015; Da Silva et al., 2013). Thus, forest fragmentation in this region could eliminate or isolate populations of *I. henselii*, leading to a regional extinction of the species in the future, mainly in dry fragments. Ongoing habitat loss and susceptibility to emerging pathogens imperils the long-term persistence of this species. Furthermore, *I. henselii* is the only species of the family Brachycephalidade with occurrence in the region studied, which would also reduce the phylogenetic diversity.

Our study provides new localities of occurrence of a habitat specialist, in a highly impacted, deforested area. Furthermore, we do not discard the possibility that future studies, mainly molecular, could find new areas of endemism with new species, within *I. henselii* populations. Incomplete taxonomic and distributional gaps may obscure conservation assessment, particularly in species that are considered to be of Least Concern in Red List assessments.

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