ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 36: 23–29 (2023)
DOI 10.3897/herpetozoa.36.e95863

Trophic ecology of the Atlantic Forest endemic tree frog *Boana bischoffi* (Boulenger, 1887) (Anura, Hylidae)

Gabriela de Araujo Pereira[1], Célio F. B. Haddad[2], Marcelo José Sturaro[1]

1 *Universidade Federal de São Paulo, Instituto de Ciências Ambientais, Químicas e Farmacêuticas (ICAQF), Departamento de Ecologia e Biologia Evolutiva, Av. Professor Artur Riedel, 275, Jardim Eldorado, Diadema, CEP 09972-270, São Paulo, Brazil*

2 *Departamento de Biodiversidade e Centro de Aquicultura (CAUNESP), Instituto de Biociências, UNESP - Universidade Estadual Paulista, Campus Rio Claro, Av. 24 A, 1515, Bela Vista, CEP 13506-970 Rio Claro, São Paulo, Brazil*

https://zoobank.org/905916FA-FC21-419B-8F9B-02E1C6BE0CF4

Corresponding author: Marcelo José Sturaro (marcelosturaro@gmail.com)

Academic editor: Lukas Landler ♦ **Received** 30 September 2022 ♦ **Accepted** 4 January 2023 ♦ **Published** 9 February 2023

Abstract

Studies of natural history are important to accumulate knowledge about aspects of diet, reproduction, and habitat use, which can assist the conservation biology for endangered groups, such as amphibians. Here we evaluated the trophic ecology and sexual size dimorphism of *Boana bischoffi*, a widely distributed and endemic tree frog species of the Brazilian Atlantic Forest. We analysed 80 individuals, covering the distribution of the species and combined our data-set with data from the literature. Gastrointestinal items were separated, accounted, and identified to the highest possible taxonomic resolution. Subsequently, the size and mass of prey items were measured. Afterwards, we calculated rates of relative importance for each prey category. The items of greatest relative importance were beetles (Coleoptera), termites (Isoptera), crickets (Orthoptera) but also harvestmen (Opiliones). We did not find a relation between female snout-vent length, mouth width and length with prey length. In males, the mouth length and width are related to prey length. We found a sexual dimorphism in size typical for hylid frogs, with females being larger than males. Our data expand the knowledge about the alimentary biology of *B. bischoffi*, but further research focusing on other aspects of the natural history such as possible intersexual dietary divergence and food niche overlapping, environmental prey availability and selection is still needed.

Key Words

amphibians, diet, sexual dimorphism, trophic ecology

Introduction

Data about alimentary biology are essential to understand several aspects of an animal's life history and functional interaction between organisms, including energy flow and food webs as well as the development of conservation strategies (Anderson and Mathis 1999; Anderson 2002). Anurans mostly feed on invertebrates, e.g., arthropods (insects and spiders) but also molluscs and annelids, and occasionally small vertebrates (e.g., fish, birds, and even other frogs). Thus, they have an important role as regulators of the density of prey species (e.g., Duellman and Trueb 1986; Pizzatto and Shine 2008; Solé and Rödder 2009; Paunović et al. 2010; Luza et al. 2018).

Anurans play a fundamental role in the ecosystem since they are a source of food for several other animals, such as spiders (Foerster et al. 2017), snakes (Pombal 2007), fishes, insects (Haddad and Bastos 1997), and birds (Roulin and Dubey 2013), being an important component in trophic network (Toledo et al. 2007). Abundant species are good candidates for studies on the trophic biology, because of their important contribution to the matter and energy transfer between different trophic levels (Moser et al. 2017). *Boana bischoffi* (Boulenger, 1887) is a tree frog

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endemic to Atlantic Forest, that occurs from north of the state of São Paulo to the Rio Grande do Sul state, Brazil, and inhabits forests, open areas, and even degraded environments (e.g., Haddad et al. 2008; Garcia and Kwet 2010). Its body size varies from 40 to 65 mm (Ribeiro et al. 2005) with females being visually larger than males (personal communication).

Even though *B. bischoffi* is a widely distributed species, aspects related to its natural history have been poorly studied, with only a single study describing the diet of a population in the Rio Grande do Sul, Brazil (Moser et al. 2019). Furthermore, there are no data on sexual size dimorphism and trophic biology from other populations of this species. Thus, in this study, we evaluate the alimentary biology of the tree frog *Boana bischoffi*, a widely distributed and endemic species of the Atlantic Forest, to answer the following questions: (1) What is the diet composition of *B. bischoffi*? (2) Which are the most important component preys? (3) Is there sexual dimorphism in size in *B. bischoffi*?

Methods

We examined 80 adults of *Boana bischoffi* from 26 localities in total (Fig. 1, Appendix 1) deposited at the Célio F. B. Haddad Amphibian Collection (**CFBH**), Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil. We selected specimens to have a balance that was representative of the four seasons (spring, summer, fall and winter) and both sexes.

We determined the sexes through direct observation of the gonads and secondary sexual characteristics of males such as the presence of a vocal sac and/or vocal slits. We measured the snout-vent length (**SVL**), mouth width (**MW**), and mouth length (**ML**) following Napoli (2005), with a digital calliper (to the nearest 0.1 mm). We examined the sexual size dimorphism (**SSD**) of SVL, HL and HW carrying out Student's t-testes. We tested for possible deviation from a normal distribution of the data using the Shapiro-Wilk test (Zar 2010). We also calculated the Sexual Size Dimorphism index following (Lovich and Gibbons 1992).

We analysed the composition of the diet examining both stomachs and intestines of each specimen to increase the efficiency to find a respective gut content (following Silva and Britto-Pereira 2006). First, we carefully separated the prey using scissors and forceps, under a stereomicroscope Nikon SMZ 745, and identified the order and family level when possible. Second, we measured the length and width of each prey with a digital calliper (to the nearest 0.1 mm). Third, we measured the mass of each prey after drying the material under 60 °C for about 5 minutes, using a digital scale Toledo XS205 (to the near-

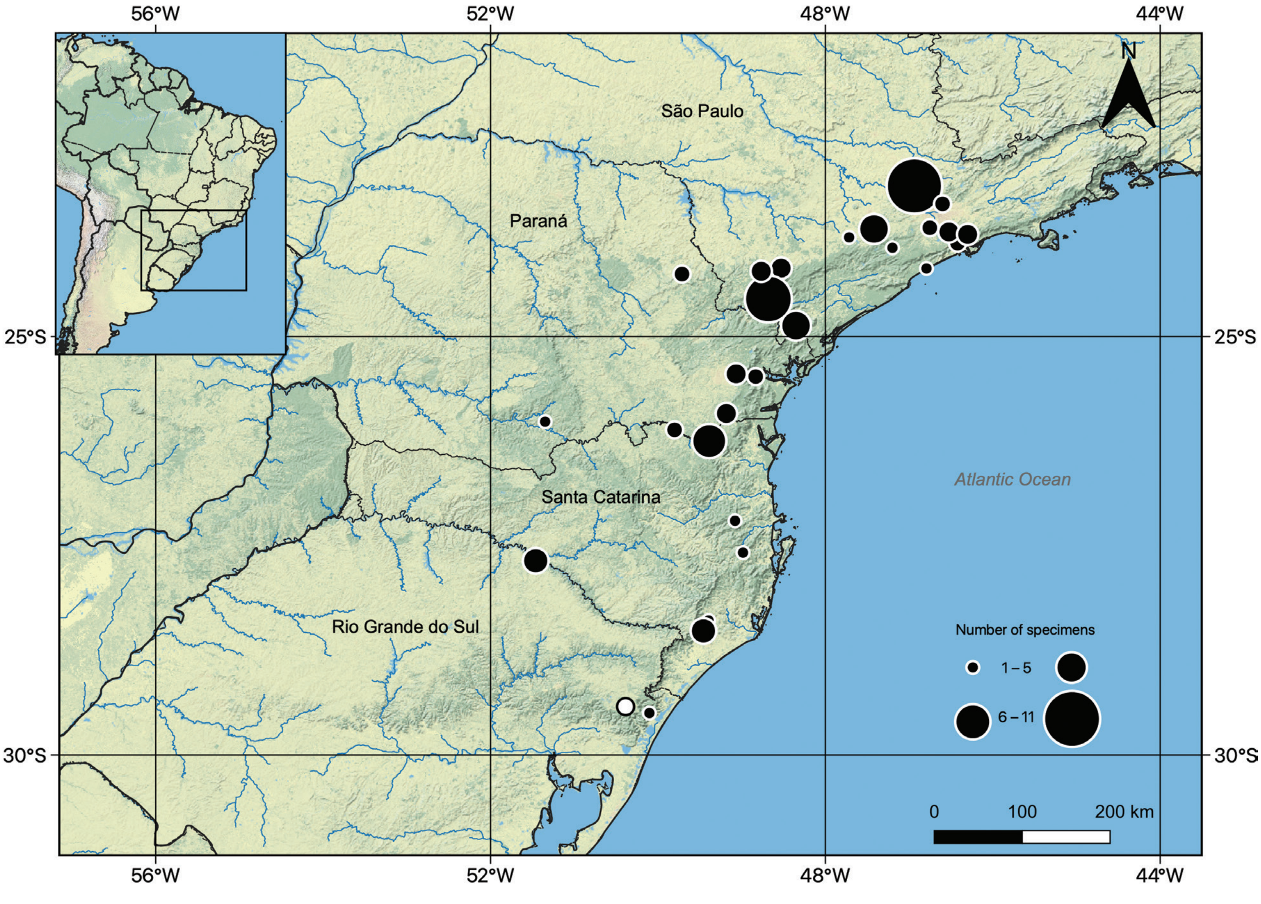

Figure 1. Geographic distribution of *Boana bischoffi* populations used for our trophic biology study. Black circles = examined specimens; white circle = literature data (from Moser et al. 2019).

est 0.00001 g). Then, we identified each prey using Thyssen (2010), Brusca et al. (2016), and Gullan and Cranston (2017), considering mature and immature as different categories. In order to estimate the importance of each prey category in the diet of *B. bischoffi*, we calculated the index of relative importance (IRI) proposed by Pinkas et al. (1971), substituting volume for mass in this formula following Martin et al. (1996): IRI = %O × (%N +%M), where %O is the relative occurrence, %N is the relative abundance, and %M is the relative mass.

To evaluate whether the sampling used was sufficiently representative, we performed two rarefaction curves with prey categoric frequency, one using only our data and a second compiling data set from Moser et al. (2019), both using species diversity estimates in the R package iNEXT (Chao et al. 2014; Hsieh et al. 2020) under the incidence-frequency data option.

We also analysed the possible influence of frog snout-vent length, mouth width, and length in relation to the length of the prey ingested, represented by a simple regression between frog measurements, by sex, and the length of the largest prey item found in each gastrointestinal tract. For this analysis, we only considered intact prey to avoid bias.

All analyses were performed using the R program (R CORE TEAM, 2018).

Results

For the analysis of the sexual dimorphism relative to the SVL, we sampled 80 adult specimens (38 females and 42 males) of *Boana bischoffi*. Females were larger (SVL ranged from 43.9 to 61.1 mm; mean 54.9 ± 3.7 mm) than males (SVL range 34.4–46.2 mm, mean 39.9 ± 2.6 m; t = 20.639, DF = 66.641, p = 0.0001) and had wider (females: 16.2–22.3 mm, mean 19.0 ± 1.4 mm; males: 11.1–16.4 mm, mean 14.1±1.2 mm; t = 16.493, DF = 73.739, p < 0.0001) and longer mouth (females: 14.5–20.1 mm, mean 17.6 ± 1.4 mm; males: 10.7–15.5 mm, mean 12.7±1.1 mm; t = 17.745, DF = 771.752, p < 0.0001). The size dimorphism index also shows that females were larger than males (SDI = 0.376).

We analysed the gastrointestinal contents of 38 females and 42 males of *B. bischoffi*. Of these, 43 (six only with fragments of plants) had their digestive tracts with prey (53.8%), of which 18 were females and 25 males. The percentages of gastrointestinal tracts with some contents were relatively higher in the summer (54%) and spring (64%) than in the fall (45%) and winter (42%). We identified 112 alimentary items in total distributed into 13 prey categories (Table 1). *B. bischoffi* consumed exclusively arthropods, totalling the 13 prey categories, with possible accidental ingestion of plants only. The most abundant prey items in the diet of *B. bischoffi* were termites (Isoptera) (56.3%), beetles (Coleoptera) (12.5%), and crickets (Orthoptera) (7.1%). beetles (26.1%), crickets (17.4%), and spiders (Araneae) (13.0%) were the most frequent prey in the gastrointestinal tracts. The relatively most massive prey items were harvestmen (Opiliones) (24.1%), crickets (16.2), and beetles (14.1%). Considering the index of relative importance, beetles (36%), termites (18.9%), crickets (17.2%), and harvestmen (13.4%) were the most important prey items consumed by female and male *B. bischoffi*.

Table 1. Diet composition of the frog *Boana bischoffi* (n = 43). O = total number of each prey category in the gastrointestinal tract; N = frequency of gastrointestinal tracts containing a given category; M = total mass (g) of each prey category; IRI = Index of Relative Importance; % = percentage value over total.

Prey Categories	O	O%	N	N%	M	M%	IRI%
Araneae	6	5.4	6	13	29.63	2.1	5.8
Opiliones	6	5.4	5	10.9	339.39	24.1	13.4
Blattaria	1	0.9	1	2.2	2	0.1	0.1
Coleoptera	14	12.5	12	26.1	199.32	14.1	36
Diptera	1	0.9	1	2.2	0.81	0.1	0.1
Hymenoptera (ants)	3	2.7	3	6.5	0.69	0	1.3
Hemiptera	1	0.9	1	2.2	58.21	4.1	0.4
Isoptera	63	56.3	2	4.3	4.93	0.3	18.9
Lepidoptera	1	0.9	1	2.2	516.2	36.6	2.5
Larvae of Coleoptera	1	0.9	1	2.2	1.3	0.1	0.1
Larvae of Lepidoptera	6	5.4	4	8.7	25.84	1.8	4
Neuroptera	1	0.9	1	2.2	4.42	0.3	0.2
Orthoptera	8	7.1	8	17.4	228.42	16.2	17.2
Plant matter	27	–	16	–	37.87	–	–

In the gastrointestinal tracts of *B. bischoffi*, we observed 13 prey categories increasing to 20 when we included data from references. In the richness of prey estimative, we found 30 categories for our data and 21 compiling data from Moser et al. (Fig. 2). Female and male snout-vent length (females: r = -0.1195; F = 0.03963; p = 0.8472; n = 10; males: r = 0.01984; F = 1.304; p = 0.2727; n = 16) were not related to prey length. The female mouth length (r = -0.0372; F = 0.6772; p = 0.4344; n = 10) and width (r = -0.0262; F = 0.77702; p = 0.4057; n = 10) did not have a relation to prey length. However, the male mouth length (r = 0.3124; F = 7.816; p = 0.0143; n = 16) and width (r = 0.2388; F = 5.705; p = 0.03155; n = 16) were related to prey length.

Discussion

Female *Boana bischoffi* were larger than males in agreement with the findings of other congeneric species such as *B. albopunctata*, *B. cordobae*, and *B. curupi* (Guimarães et al. 2011; Bastiani et al. 2016; Otero et al. 2017). Shine (1979) observed that, in most anuran species he analysed, females were the larger-sized sex. This pattern is probably related to fecundity because larger females can produce and store a larger number of eggs (Kupfer 2007; Nali et al. 2014; Maerker et al. 2016). Another hypothesis about this pronounced sexual dimorphism in size can

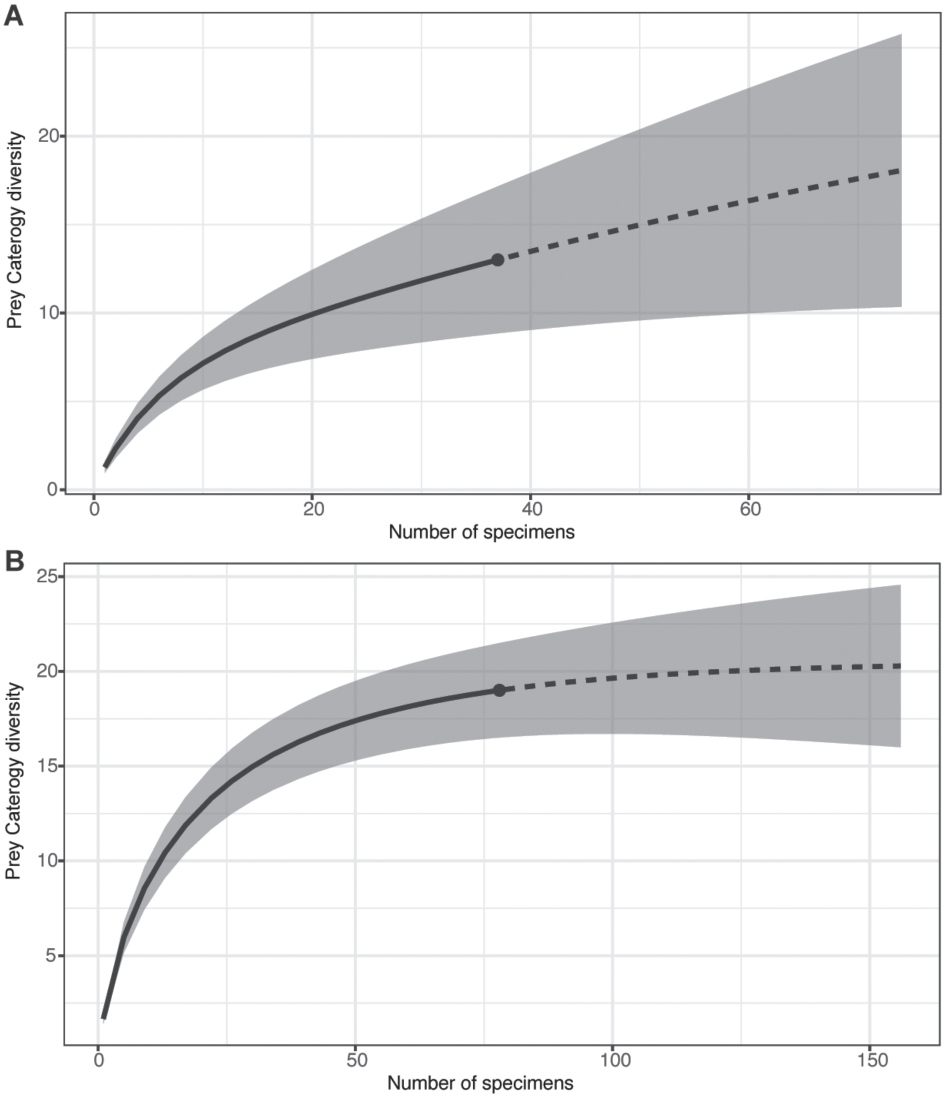

Figure 2. Rarefaction curve of prey categoric frequency in gastrointestinal tracts of *Boana bischoffi* using the data from (**A**) this study (**A**) and (**B**) compiling data of Moser et al. (2019). Continuous line = interpolated; Dashed line = extrapolated.

be related to the arboreal habitat, which is the case with the *B. bischoffi* females that must carry males during the amplexus through the vegetation, leading to larger female body sizes (Silva et al. 2020). More detailed analyses focusing on the sexual dimorphism of arboreal *Boana* species are important to evaluate not only the difference in size but also other body characters and shapes using a geometric morphometric approach.

The absence of prey in about half of the gastrointestinal tracts may be due to the use of specimens deposited in collections of natural history museums, which were collected for many other purposes but were not specifically collected for diet biology studies. Specimens to be used in alimentary biology studies must be euthanized and fixed immediately after being collected (following e.g., Solé et al. 2005; Magalhães et al. 2016; Machado et al. 2019), which is not necessarily the case with specimens found in natural history collections. Also, most of the frogs may have been collected during the reproductive season, when individuals are focused on reproductive success and are not feeding (Duellman and Trueb 1986; Solé and Pelz 2007). After Ceron et al. (2020) *B. bischoffi* is continuously breeding and males keep calling during a prolonged period of nine months, which also may influence its foraging activity. On the contrary, our study specimens with digestive contents were captured during the reproductive period in summer or spring. Therefore, complementary studies comparing the feeding and reproductive activities are necessary to further elucidate the high proportion of empty gastrointestinal tracts of *B. bischoffi*.

We found 13 prey categories in the diet of *B. bischoffi*, two less than that reported earlier by Moser et al. (2019). However, four invertebrate prey groups were newly recorded i.e., Opiliones, Isoptera, Neuroptera, and Formicidae in the diet, thus increasing the prey richness up to 20 groups, almost matching the prediction of 21 prey groups. Usually, alimentary biology studies have used only their own sampling to estimate the richness of prey items, but they did not reach the plateau, as an indicative of the need to increase sampling efforts (Telles et al.

2013; Magalhães et al. 2016). Including literature data in our analyses proofed an important tool to infer the diet of the Neotropical anurans, which are still poorly studied (Anjos et al. 2020).

Coleoptera, Orthoptera, Isoptera and Opiliones were the most important prey categories in the diet of *B. bischoffi* in accordance with congeners, where Araneae and Diptera also have high representativity (López et al. 2009; Sabagh et al. 2010; Rosa et al. 2011; Barbosa et al. 2014; Moser et al. 2019, 2022; Tupy et al. 2021). In the previous study on the trophic ecology of *B. bischoffi*, Moser et al. (2019) reported Araneae and Coleoptera as the most relative important items. However, we found Coleoptera, Orthoptera, Isoptera, and Opiliones as the main prey items. The difference might be explained by the prey availability in the environment because we examined specimens from several localities (see Fig. 1) but Moser et al. (2019) from only one locality (Floresta Nacional São Francisco de Paula, Rio Grande do Sul, Brazil). Nevertheless, future works focusing on prey availability and food selectivity are necessary to further elucidate this finding.

We found plant material (seed and fragments of leaves) in the gastrointestinal tract of *B. bischoffi*, which was previously reported for other species of *Boana* (López et al. 2009; Sabagh et al. 2010; Barbosa et al. 2014; Moser et al. 2019; Tupy et al. 2021). This ingestion is likely accidental when frogs are capturing prey, as plants are not considered an important part of the anuran diet (e.g., Korschgen and Moyle 1955; Solé and Pelz 2007; Solé and Rödder 2009). However, other authors (e.g., Das 1996; Anderson and Mathis 1999; Silva and Britto-Pereira 2006) have argued that plant ingestion could be intentional, serving as a source of water and nutrients but also constitute a method to eliminate parasites. Future field ethological studies are still necessary to better clear up the controversy about an herbivorous diet of anurans.

We did not find a relation between snout-vent length, mouth width and length to prey length in females of *B. bischoffi*. However, in the males, the mouth length and width are related to prey length, which was also reported earlier for the congener *B. albomarginata* (Tupy et al. 2021). However, Guimarães et al. (2011) and Tupy et al. (2021) showed that frog and prey size were correlated in *B. albopunctata* and *B. pombali*, indicating that predator size also determines prey size (see also Caldwell and Vitt 1999).

Conclusions

Boana bischoffi is a generalist feeder; i.e., its diet is mainly based on several major arthropod groups such as Coleoptera, Isoptera and Orthoptera but also Opiliones. It has an accentuated sexual size dimorphism with females being larger than males.

Future studies comparing alimentary biology are necessary to elucidate possible intersexual dietary divergence or food niche overlapping. Furthermore, environmental prey availability and selection are other important aspects to be evaluated in the future. Our study contributes to understanding the trophic ecology of anurans in the Atlantic Forest and reinforces the importance of including literature data in the analysis.

Acknowledgments

We thank Fabiana E. Casarin dos Santos and Cibele Bragagnolo for helping in the identification of some preys. Also, Adriano Oliveira Maciel, Cinthia Aguirre Brasileiro, Katia Pellegrino and Isabel Bonatelli for helpful feedback and suggestions to improve the manuscript. MJS and the study were financially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process # 434362/2018–2).

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Appendix 1

Adults of the frog *Boana bischoffi* in this study

BRAZIL: **Paraná:** Cruz Machado: Estrada para Usina Hidrelétrica Salto do Vau (♂, CFBH 18261); Jaguariaíva: Fazenda do Edilson (Dono do Hotel Conde Alemão) (2♂♂: CFBH 24725, CFBH 24727); Morretes: Parque Estadual do Pico do Marumbi (♀, CFBH 21926; ♂, CFBH 21927); Piraquara: Brejo na Estrada da casa do Senhor Airton (♀,CFBH 31048), Mananciais da Serra, região Barragem Piraguara (♀,CFBH 11047), Poça na casa do Senhor Airton, estrada para a Pousada Tempero da Serra (♀,CFBH 31044); Tijucas do Sul: Estrada entre Tijucas do Sul e BR 376 (♂, CFBH 8432; 2♀♀,CFBH 8436, CFBH 8438). **Rio Grande do Sul:** Barracão: Parque Estadual do Espigão Alto (4 ♂♂, CFBH 21913 to 21914, CFBH 21916, CFBH 21922); Itati: Reserva Biológica Estadual da Paludosa (♂, CFBH 14592). **Santa Catarina:** Angelina (♂, CFBH 8479); Botuverá: Comunidade Lajeada Baixa; riacho Vicinity (♀, CFBH 10959); Lauro Muller: Brejo próximo a estrada da Serra do Rio do Rastro (♀, CFBH 30324); Mafra: Ponto I (♂, CFBH 8585; ♀, CFBH 8590); São Bento do Sul: Estrada e brejo na "Fazenda 26", estrada velha pra Rio Vermelho (2 ♀♀, CFBH 10975 to10976; 2 ♂♂, CFBH 10986 to 10987), Estrada Saraiva (2 ♀♀, CFBH 3009 to 3010); Treviso: Mina do Carvão, TRV4 (♂, CFBH 12401), próximo a Criciuma (♀, CFBH 8489; ♂ CFBH 8490), Treviso (♂, CFBH 10329). **São Paulo:** Apiaí: Parque Estadual Turístico do Alto Ribeira (♀, CFBH 38662), Base Bulha d'água e capinzal (2 ♀♀, CFBH 26768 to 26769), Núcleo Caboclos (2 ♀♀, CFBH 26805, CFBH 26807; ♂ CFBH 26806), Núcleo Ouro Grosso (3 ♂♂, CFBH 6303 to 6304, CFBH 6324); Barra do Turvo: Parque Jacupiranga (5 ♂♂, CFBH 6339 to 6340, CFBH 6344 to 6345, CFBH 6347); Cubatão: COPEBRAS (♂, CFBH 9243; ♀ CFBH 25828); Guapiara (3 ♀♀, CFBH 14691 to 14692; CFBH 14721); Iporanga (♀, CFBH 14582); Itanhaém, Parque Estadual da Serra do Mar, Núcleo Curucutu, Trilha Mambu (♂, CFBH 12210); Jundiaí, Serra do Japi (2 ♀♀, CFBH 718, CFBH 802; 9 ♂♂, CFBH 8374 to 8376, CFBH 8618 to 8621, CFBH 14433, CFBH 14440), Juquitiba: Parque Estadual de Jurupará (♀, CFBH 38600), Piedade: Parque Estadual do Jurupará (♀, CFBH 23266), Vila Elvio (♀, CFBH 15989; 3 ♀♀, CFBH 15991 to 15992, CFBH 22260); Pilar do Sul (♀, CFBH 8346); Ribeirão Branco, Fazenda São Luiz (Mathedi) (2 ♀♀, CFBH 255 to 256); Ribeirão Branco (♂, CFBH 11290); Santo André: Parque do Pedroso (♂, CFBH 11627), Parque Estadual Municipal Nascentes de Paranapiacaba, próximo a Cachoeira do Pedro Lisa (2 ♀♀, CFBH 28966–28967); São Paulo, Parque Ecológico da Guarapiranga (♂, CFBH 26677), Sítio Roda D'Água (♀, CFBH 31086); Serra da Cantareira (♀, CFBH 5689; ♂, CFBH 5690).