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ECOLOGÍA

Nematodes associated with *Leptodactylus* cf. *mystaceus* (Anura: Leptodactylidae) in agricultural landscapes of Ibiapaba plateau, Ceará state, Brazil

Nematodos asociados con *Leptodactylus* cf. *mystaceus* (Anura: Leptodactylidae) en paisajes agrícolas de la meseta de Ibiapaba, estado de Ceará, Brasil

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RESUMEN

Los paisajes agrícolas impactan la diversidad de anfibios, con pocas especies adaptadas a estos ambientes antropizados y fragmentados. Los leptodactílidos se encuentran comúnmente en los cultivos porque son especies tolerantes. A pesar del aumento de estudios parasitológicos en áreas agrícolas, todavía falta información sobre las interacciones parásito-anuro en estos ambientes. Aquí, nuestro objetivo fue describir los endoparásitos asociados con *Leptodactylus* cf. *mystaceus* en paisajes agrícolas. Capturamos 18 ejemplares de *L.* cf. *mystaceus*, de los cuales catorce estaban infectados por al menos uno de los trece taxones de nematodos (prevalencia general = 78 %). Registramos los siguientes taxones de nematodos: *Aplectana crucifer* Travassos, 1925, *Aplectana meridionalis* Freitas y Lent, 1938, *Aplectana lopesi* Silva, 1954, *Cosmocerca brasiliense* Travassos, 1925, *Cosmocerca paraguayensis* Moravec e Kaiser, 1994, *Cosmocerca parva* Travassos, 1925, *Cosmocerca* sp., *Cosmocerca travassosi* Gomes y Motta, 1967, Cosmocercidae gen. sp., *Multicaecum* sp., *Ochoterenella* sp., *Oxyascaris oxyascaris* Travassos, 1920 y *Rhabdias* sp. De ellos *C. paraguayensis*, *C. travassossi*, *A. meridionalis*, *A. lopesi*, *A. crucifer*, *Multicaecum* sp. y *Ochoterenella* sp. representan nuevos registros para este hospedero. Además, observamos que es poco probable que el tamaño del cuerpo del huésped influya en la abundancia y riqueza de nematodos, al menos desde un punto de vista intraespecífico. Este estudio contribuye al conocimiento de la helmintofauna asociada a leptodactílidos y a la comprensión de la distribución geográfica de las especies de helmintos.

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Palabras clave: anfibios, endoparásitos, nematodos, relictual bosque húmedo

ABSTRACT

Agricultural landscapes impact the diversity of amphibians, with few species adapted to these anthropized and fragmented environments. Leptodactilids are commonly found on crops because they are tolerant species. Despite the increase in parasitological studies in agricultural areas, information on parasite-anuran interactions in these environments is still lacking. Herein, we aim to describe the nematodes associated with *Leptodactylus* cf. *mystaceus* in agricultural landscapes. We captured 18 specimens of *L.* cf. *mystaceus* which fourteen were infected by at least one of the thirteen nematode taxa. The prevalence was 78 % in the host population. We recorded thirteen taxa of nematodes: *Aplectana crucifer* Travassos, 1925, *Aplectana meridionalis* Freitas and Lent, 1938, *Aplectana lopesi* Silva, 1954, *Cosmocerca brasiliense* Travassos, 1925, *Cosmocerca paraguayensis* Moravec and Kaiser, 1994, *Cosmocerca parva* Travassos, 1925, *Cosmocerca* sp., *Cosmocerca travassosi* Gomes and Motta, 1967, Cosmocercidae gen. sp., *Multicaecum* sp., *Ochoterenella* sp., *Oxyascaris oxyascaris* Travassos, 1920, and *Rhabdias* sp., wich *C. paraguayensis*, *C. travassossi*, *A. meridionalis*, *A. lopesi*, *A. crucifer*, *Multicaecum* sp., and *Ochoterenella* sp. represent new records for this host. Furthermore, we note that host body size is unlikely to influence helminth abundance and richness, at least from an intraspecific point of view. This study contributes to the knowledge of the helminth fauna associated with leptodactilids and to the understanding of the geographic distribution of helminth species.

Keywords: amphibians, endoparasites, nematodes, relictual humid forest

INTRODUCTION

Amphibians are important for parasitological studies due to being rather susceptible to parasite infection (Koprivnikar *et al.* 2012). Although approaches in this field of knowledge have been neglected for many years (Marcogliese 2004, Poulin and Morand 2004), recently, several works developed in Brazil have been dedicated to comprehending different aspects of parasitism associated with this group of hosts (Campião *et al.* 2014, Araújo-Filho *et al.* 2015, Madelaire *et al.* 2017, Silva *et al.* 2018, Morais *et al.* 2020).

Both anurans and endoparasites are bioindicators of environmental disturbances (Marcogliese 2005, Vidal *et al.* 2010, Koprivnikar *et al.* 2012). Furthermore, anurans inhabit diverse microhabitats and play an important role in food webs (Bernarde 2012). Although increasing of helminth checklists associated with anurans in different environments (e.g., Campião *et al.* 2014, Toledo *et al.* 2017, Silva *et al.* 2018), there are hosts and areas still undersampled, and the helminth richness is underestimated (Campião *et al.* 2014). Therefore, the importance of helminth inventories for the parasitology advance is decisive (Poulin *et al.* 2018).

Agricultural landscapes have an impact on amphibian diversity (Marcogliese 2005, Marcogliese *et al.* 2009, Stuart *et al.* 2004, Gonçalves *et al.* 2017), being few species adapted to these anthropized and fragmented environments. Bufonids and leptodactilids are commonly found in agricultural crops because are more tolerant species (Prado and Rossa-Feres 2014, Díaz-Ricaurte *et al.* 2020). Despite increasing parasitological studies in agricultural areas (e.g., Schotthoefer *et al.* 2011, Campião *et al.* 2016a), and being the parasite life cycles influenced by the environment (McKenzie 2007), there is still insufficient information about parasite-anuran interactions in these landscapes.

Brazil harbors the highest anuran richness, representing about 15 % of the anurans worldwide (Segalla *et al.* 2021, Frost 2023). Nevertheless, most of them do not have parasitological studies or are undersampled (Campião *et al.* 2014). The genus *Leptodactylus* Fitzinger, 1826 is currently formed by 84 species classified into four species groups: *L. fuscus* (Schneider, 1799), *L. latrans* (Steffen, 1815), *L. melanonotus* (Hallowell, 1861), and *L. pentadactylus* (Laurenti, 1768) distributed in Southern North America, South America, and the West Indies (Silva *et al.* 2020, Frost 2023). Within the *L. fuscus* group the *L. mystaceus* complex is composed of five species: *L. didymus* (Heyer, García-Lopez, and Cardoso, 1996), *L. elenae* (Heyer, 1978), *L. mystaceus* (Spix, 1824), *L. notoaktites* (Heyer, 1978), and *L. spixi* (Heyer, 1983) (Silva *et al.* 2020). Species of the *L. mystaceus* complex have poor information about the helminths associated with them (e.g., Goldberg *et al.* 2009, Campião *et al.* 2014). The *L. mystaceus* is a medium-sized frog characterized to have a generalist diet (Camera *et al.* 2014), well distributed in Caatinga, Cerrado, and Atlantic rain forests (Silva *et al.* 2020). However, this species requires a taxonomic review, because it seems to be a candidate for a new species (Silva *et al.* 2020). Here, we aim to identify the parasitic nematodes of *Leptodactylus* cf. *mystaceus* inhabiting agricultural landscapes of the Ibiapaba plateau, Ceará state, northeastern Brazil.

MATERIAL AND METHODS

Study area

Anuran sampling was carried out in four localities in the Ibiapaba plateau: Tianguá (3.718611°S 40.945°W, and 3.723611°S 40.95027°W) and Ubajara (3.872222°S 40.9333°W, and 3.801944°S 40.950833°W) municipalities, Ceará state, Northeastern Brazil (Fig. 1). Both sampled areas are in agricultural crops such as corn, beans, and bananas.

The municipality of Tianguá has a mean altitude of 775.9 m, a temperature ranging from 22 to 24°C, and a mean rainfall of 1210 mm, with rains concentrated between January and May (Ipece 2018a). The municipality of Ubajara has a higher mean altitude (847.5 m), similar range of temperature (24 to 26°C), and mean rainfall (1483 mm), but the rains are concentrated between January and April (Ipece 2018b). Both municipalities are in the Ibiapaba plateau and the main vegetation types are the Seasonal Tropical Deciduous Forests, Seasonal Tropical Evergreen Forests, and Savannas (Silveira *et al.* 2020).

Sampling

Anuran sampling occurred from January 11th to the 23rd of 2019 using visual and auditory (Heyer *et al.* 1994) searches. The collected specimens were sacrificed with 2 % lidocaine and necropsied to investigate their associated helminths. The anurans were deposited in the Núcleo Regional de Ofiologia (NUROF) of Universidade Federal do Ceará, Fortaleza, Ceará state, northeastern Brazil.

Parasitological procedures

We analyzed the presence of endoparasites in the following organs: digestive tract, lungs, heart, liver, and kidneys (processed fresh). The helminths were cleared, measured, and analyzed according to each taxonomical group and specialized literature (Vidal *et al.* 2001). We clarified them using lactic acid because we found just nematodes (Andrade 2000). For species identification, we used the following literature: Yamaguti (1961), Vicente *et al.* (1991), Anderson (2000), and Gibbons (2010). Nematodes were deposited in the Parasitological collection of Universidade Federal do Ceará (CPUFC), Fortaleza, Ceará state, northeastern Brazil.

Statistical analyses

Through the Quantitative Parasitology 3.0 software (Rózsa *et al.* 2000), and according to Bush *et al.* (1997), we measured the following parasitological descriptors: prevalence, abundance, mean abundance, and mean infection intensity.

To investigate the relationship between anuran body size and helminth abundance and richness we used a linear mixed-models (LMMs) framework (function 'lme') fitted by restricted maximum likelihood with random effects (random = ~1) as implemented in the package nlme (Pinheiro and Bates 2000) for R software (R Core Team 2021). We included "species sex" as a random-effect term to account for the non-independence in the observations. We used the package ggplot2 to produce the graphs (Wickham 2016).

RESULTS

From the 18 hosts necropsied, fourteen were parasitized with at least one nematode taxon (prevalence 78%). A total of 280 nematode specimens were collected, representing thirteen taxa: *Aplectana crucifer* Travassos, 1925, *Aplectana meridionalis* Freitas e Lent, 1938, *Aplectana lopesi* Silva, 1954, *Cosmocerca brasiliense* Travassos, 1925, *Cosmocerca paraguayensis* Moravec e Kaiser, 1994, *Cosmocerca parva* Travassos, 1925, *Cosmocerca* sp., *Cosmocerca travassosi* Gomes e Motta, 1967, Cosmocercidae gen. sp., *Multicaecum* sp., *Ochoterenella* sp., *Oxyascaris oxyascaris* Travassos, 1920, and *Rhabdias* sp. (Table 1).

Table 1. Nematode parasites of *Leptodactylus* cf. *mystaceus* in agricultural landscapes of Ibiapaba plateau, Ceará state, Northeastern Brazil.

Nematoda	I	P%	MA	MII±SE	IS
Aplectana crucifer Travassos, 1925	19	5.5	1.3	19	SI/LI
Aplectana meridionalis Freitas e Lent, 1938	2	5.5	0.1	2	LI
Aplectana lopesi Silva, 1954	38	5.5	2.1	38	LI
Cosmocerca brasiliense Travassos, 1925	1	5.5	0.07	1	SI
Cosmocerca paraguayensis Moravec e Kaiser, 1994	10	5.5	0.7	10	SI/LI
Cosmocerca parva Travassos, 1925	120	16.6	8.5	40±14.9	SI/LI
Cosmocerca sp.	6	5.5	0.4	6	LI
Cosmocerca travassosi Gomes e Motta, 1967	35	5.5	2.5	35	LI
Cosmocercidae gen.sp.	32	27.7	2.2	6.4±1.6	SI/LI
Multicaecum sp.	4	5.5	0.2	4	SI
Ochoterenella sp.	1	5.5	0.07	1	SI
Oxyascaris oxyascaris Travassos, 1920	10	11.1	0.5	5	LI
Rhabdias sp.	2	11.1	0.1	2	L

Intensity (I), prevalence (P %), mean abundance (MA), mean intensity (MI), standard error (SE), and infection site (IS); large intestine (LI), small intestine (SI), and lungs (L).

The most abundant parasite species recorded in this study were found in the adult stage: *C. parva* (n = 120), *A. lopesi* (n = 38), and *C. travassosi* (n = 35). The Cosmocercidae gen. sp. presented the highest prevalence values. Likewise, *C. parva* presented the highest prevalence and mean infection intensity (Table 1).

Although the regression curve indicated a positive relationship between host body size and helminth richness and abundance (Fig. 2), we found no significant effect of the anuran snout-vent length on these parasitological variables ($p > 0.05$). In addition, the random effect (species sex) explains too little of the total variance for parasite richness (Intercept = $2.171468e^{-06}$) and abundance (Intercept = $7.762439e^{-06}$).

DISCUSSION

According to this study, specimens of *Leptodactylus* cf. *mystaceus* showed a rich fauna of parasitic nematodes compared to other studies for this species, keeping the proper proportions with respect to sampling sizes (Bursey *et al.* 2001, Goldberg *et al.* 2007, 2009). *Leptodactylus* cf. *mystaceus* is a generalist frog with a large niche breadth (Camera *et al.* 2014), and its foraging mode might influence the higher probability of being susceptible to endoparasite infection. In addition, the rich nematode fauna may also be associated with the environment (agricultural crops). Land use increases infection intensity because it suppresses the anurans immunological system and provides suitable habitat for generalist hosts as leptodactilids (Schotthoefer *et al.* 2011, Campião *et al.* 2016a).

High nematode infection, as found in *L.* cf. *mystaceus*, has been reported in other parasitological studies with anurans (e.g. Lambshead 2004, Campião *et al.* 2016a, Toledo *et al.* 2017). The exclusive nematode infection may occur because it is one of the most abundant taxa worldwide (Lambshead 2004, Nielsen *et al.* 2014). In addition, nematodes inhabit distinct environments and microhabitats, being predominantly on the ground, which may favor the nematode infection in terrestrial anurans such as *L.* cf. *mystaceus* (Duellman and Trueb 1986, Lima *et al.* 2012). Additionally, nematodes usually infect hosts as anurans

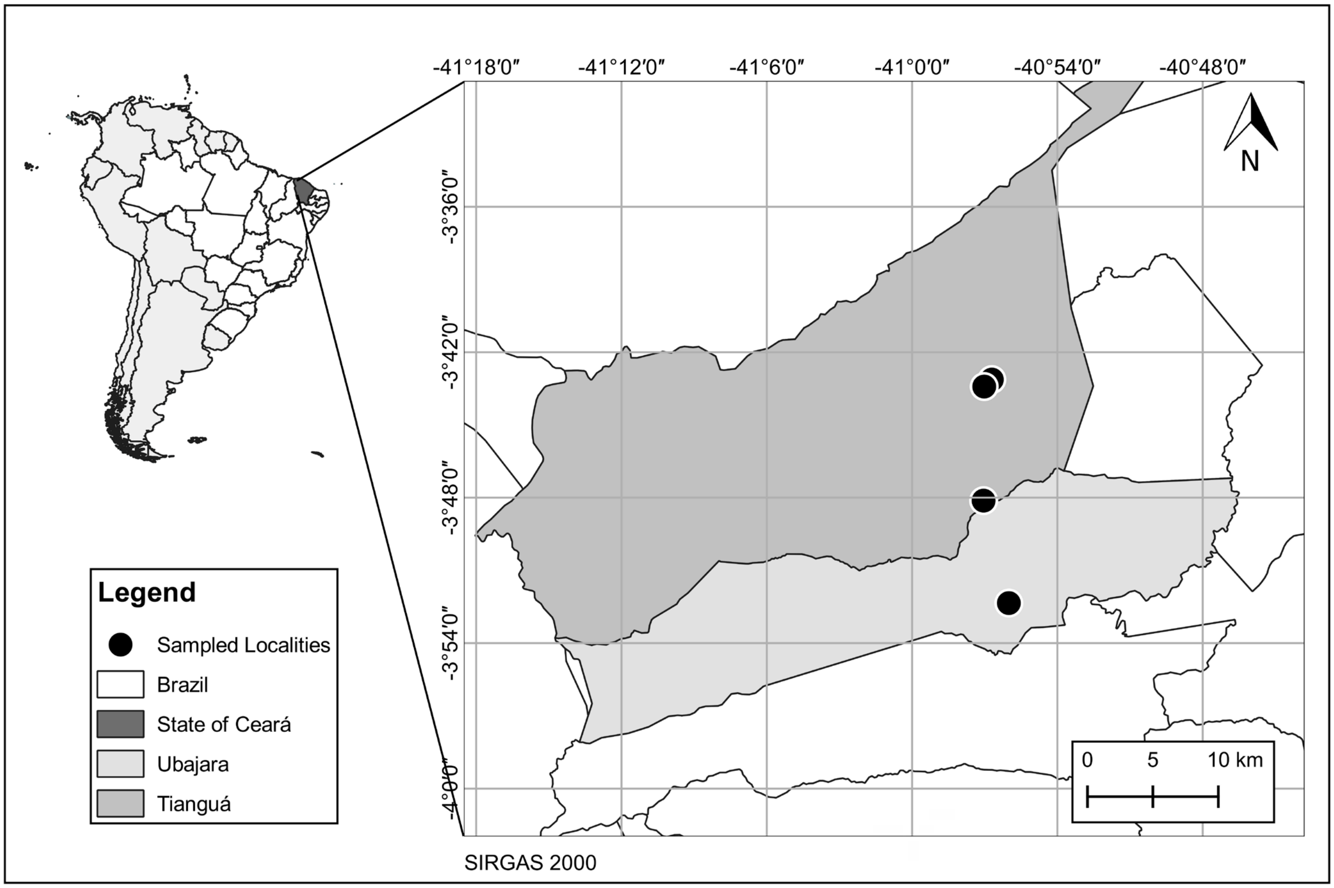

Figure 1. Sampled points in Ibiapaba plateau, municipalities of Ubajara and Tianguá, Ceará state, Northeastern Brazil.

through the ingestion of infected prey (heteroxenic nematodes) or cutaneous penetration (monoxenic nematodes) (Anderson 2000). Most of the helminth species were found in the digestive tract, except for *Rhabdias* sp. whose location were in the lungs.

Perhaps, the Cosmocercidae species prevalence indicates an evolutive significance once this family co-evolved in amphibians and reptiles (Vanderburgh and Anderson 1987). Besides, the genus *Cosmocerca* is widely distributed worldwide, with 37 described species, twelve found in the Neotropical region (Ávila and Silva 2019, Chen et al. 2020, Ni et al. 2020, Alcantara *et al.* 2022, Machado et al. 2022). Species of this genera are soil nematodes with a monoxenic life cycle (Anderson 2000). Thus, we believe that the life habits of the host contributed to the high predominance of Cosmocercidae species in *L.* cf. *mystaceus*.

Like other parasitological studies on leptodactilids (e.g., Campião *et al.* 2014, Aguiar *et al.* 2015, Silva *et al.* 2018), the most prevalent and abundant species was *Cosmocerca parva*. This species uses anuran species as definitive hosts, and it is considered a generalist and widespread endoparasite (Anderson 2000, Santos and Amato 2013). However, some *Cosmocerca* species are still undersampled as *C. paraguayensis*, being the second record in Brazil registered in the present study (Machado *et al.* 2022). Likewise, *C. travassosi* was reported just for *Boana faber* Wied-Neuwied in 1821, in Rio de Janeiro, Brazil (Vicente *et al.* 1991). Therefore, it is the first record for the Northeastern region and, consequently, for *L.* cf. *mystaceus*.

Aplectana are helminths usually found in amphibians and rarely occurring in reptiles (Vicente *et al.* 1993, Chen *et al.* 2021), with approximately 50 described species (see Ávila and Silva 2010, Campião *et al.* 2014, Amorim *et al.* 2017, Chen *et al.* 2021), of which we found three (*A. meridionalis*, *A. crucifer*, and *A. lopesi*) parasitizing *L.* cf. *mystaceus*. In Brazil, *A. meridionalis* was reported for *Rhinella fernandezae* Gallardo 1957, and *Odontophrynus americanus* Duméril and Bibron 1841, (Santos and Amato 2010, Silva *et al.* 2018), but it is the first record for Ceará state. In ad-

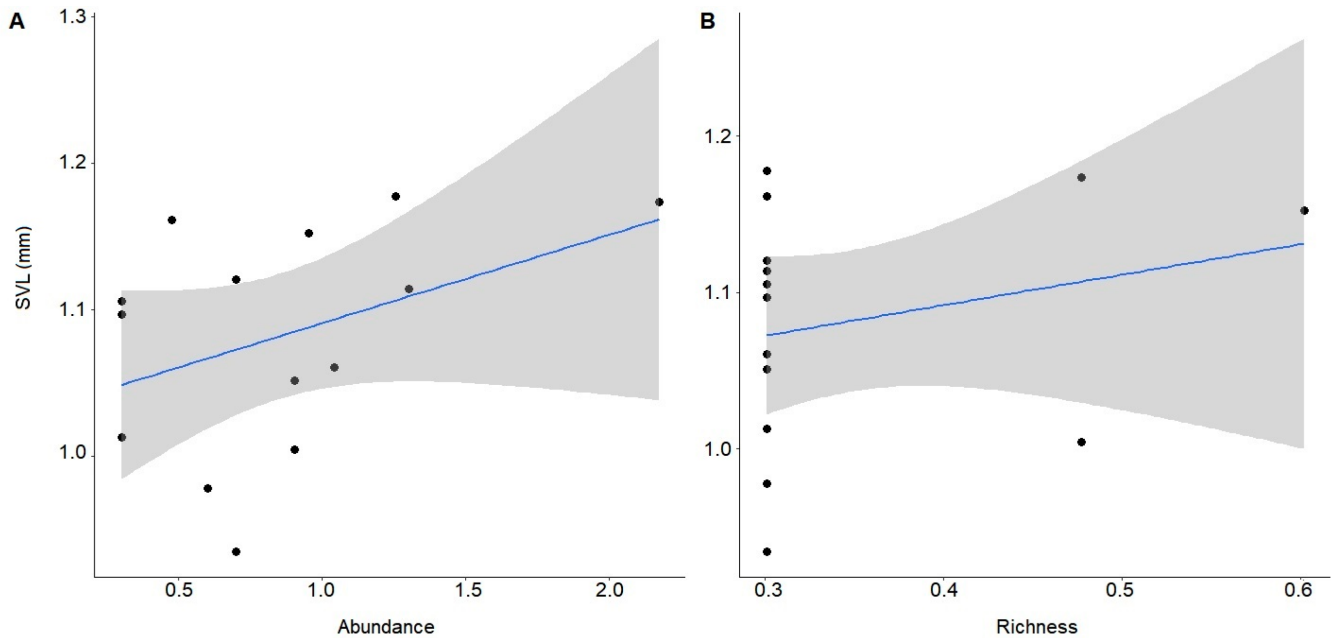

Figure 2. Relationship between anuran body size (SVL – Snout-Vent-Length) and nematodes abundance and richness.

dition, we reported the first record of *A. crucifer* for leptodactilids. Conversely, *A. lopesi* was already found in other leptodactilids (Aguiar *et al.* 2014).

Although the genus *Ochoterenella* Caballero 1944 comprises 16 species, some of them already reported for anurans (Bain *et al.* 2013) including leptodactilids (Goldberg *et al.* 2009, Teles *et al.* 2014), it is the first record for *L.* cf. *mystaceus*. This helminth was not identified at the species level because it was an immature female. Likewise, *Rhabdias* sp. was also not identified at the species level because it belongs to a complex of cryptic species (Müller *et al.* 2018). Rhabditid nematodes are lung-specific endoparasites that have monoxenic life cycle (Anderson 2000), but just two individuals were registered in the present study.

Regarding the genus *Multicaecum* Baylis 1923, there are reports also for amphibians and reptiles (Sprent *et al.* 1979, Campião *et al.* 2014). Nevertheless, these reports are taken of crocodiles from the Palearctic realm (Sprent *et al.* 1979, Mašová *et al.* 2010), fishes from Africa (Mašová *et al.* 2010), and specimens of *Leptodactylus latrans* Steffen 1815, from Mato Grosso State, Central-Wester Brazil (Travassos *et al.* 1939, Vicente *et al.* 1993). Thus, our record is the third report for Brazil and, the first for the Northeastern region.

Campião *et al.* (2014) listed twelve endoparasites associated with *L. mystaceus* from Norther, Central-Wester, Southeaster, and Southern Brazil regions, besides other countries such as Peru and Ecuador. However, a recent taxonomical review based on morphology, coloration, acoustics, and DNA sequences shows that the species previously recognized as *L. mystaceus* in Ecuador is likely *L. didymus*, there are two populations of *L. mystaceus* complex in Tocantins, Central-Western, other two in Rio de Janeiro state, and other three in Minas Gerais state, both in Southeaster Brazil. In addition, the populations living in Santa Catarina state, Southern Brazil, are *L. notoaktites*, and just the populations of Norther Brazil and Ecuador are recognized as *L. mystaceus* (Silva *et al.* 2020). Thus, although this above-cited taxonomical inaccuracy of the population studied here, all identified helminths are new records for the *L. mystaceus* complex.

Although the general hypothesis that large-bodied hosts may provide more space and other resources, and possibly a broader diversity of niches for parasites (Campião *et al.* 2015, 2016b), we found no support for this hypothesis in the present study. The lack of support for this hypothesis was already observed in other studies, including leptodactilids and hylids hosts (Oliveira *et al.* 2019, Cardoso *et al.* 2021, Machado *et al.* 2022). It seems that this hypothesis is more evident from an interspecific view; however, other key factors need to be more studied to elucidate the main determinants of endoparasite abundance and richness from an intraspecific view.

In conclusion, we observed an exclusively nematode infection in *L.* cf. *mystaceus*, likely due to the study being conducted in agricultural crops. In addition, we presented new helminth occurrence records in Brazil, being all endoparasite's new records in *L. mystaceus* complex. It is important to point out that generalist species such as leptodactylids might be useful species to understand the host-parasite relationships in anthropized environments. Overall, our study contributed to increasing the knowledge of the helminth fauna associated with leptodactilids and to understanding the geographic distribution of helminth species.

PARTICIPATION OF AUTHORS

CS field assistance, writing, and biological analysis; KAC field assistance, writing, statistical, and biological analysis; SSO field assistance, writing, and biological analysis; HTM field assistance, writing and biological analysis; RWA writing and text correction.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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