ABSTRACT

Brazil is the country with the largest amphibian richness in the world with about 1,136 described species, of which 1,093 are anurans. However, related studies on the knowledge of the associated helminth fauna are scarce, representing only 8% of anurans. This study aimed to identify the parasite communities, and their parasitic parameters in two amphibian species: Chiasmocleis albopunctata (Boettger, 1885) and Dendropsophus nanus (Boulenger, 1889) found in eastern Mato Grosso do Sul State. The hosts were collected through pitfall traps with drift fences (PTDF) and the Active Search method at the breeding sites, euthanized, and the helminths removed and identified. A total of 172 amphibians were collected: 70 individuals from C. albopunctata and 102 individuals from D. nanus. Of this total, 84 were parasitised by at least one taxon. The total number of helminths found was 675. Here we report ten helminth taxa, including two new records for the D. nanus host species, and five records for C. albopunctata host species. Thus, this study represents an important contribution to the knowledge of parasite and host diversity, since the amount of existing studies is insufficient and not representative.

Keywords: Anurans – Diversity Conservation – Helminthology – Parasitology – Wild Animals
Brazil is the country with the largest amphibian diversity in the world with about 1,136 species described, of which 1,093 are anurans (Martins-Sobrinho et al., 2017; Segalla et al., 2019). However, related studies on the knowledge of the associated helminth fauna are scarce, representing only 8% of the anurans. This study had as objective to identify the communities of parasites and its parasitological parameters in two species of amphibians: Chiasmocleis albopunctata (Boettger, 1885) and Dendropsophus nanus (Boulenger, 1889). Anurans are animals widely diverse in their ways of life and can spend their lives close to bodies of water, as well as use them only in the larval stages and breeding periods. They can even live in environments with low water availability. Anurans’ way of life has a great influence on their parasitic composition, just as parasites are major determinants of this host group. However, amphibian helminths constitute a poorly sampled group in taxonomic survey studies (Anjos, 2011; Campião et al., 2014; Graça et al., 2017), faunal surveys are necessary since the vast majority of anurans have not had their helminth fauna described (Graça et al., 2017). Faunal surveys are urgently needed because the number of newly discovered organisms is disappearing faster than new organisms are described (Greene & Losos, 1988; Dobson et al., 2008; Muniz-Pereira et al., 2009). This is especially true in areas such as the Cerrado, which has a very large diversity of species, mainly endemic, but has a high degree of degradation recorded, being constantly impacted by anthropic activities which causes extinction of biodiversity, invasion of exotic species, soil erosion, and fragmentation of habitats (Klink & Machado, 2005; Myers et al., 2000).
fauna, associated parasites, and ecological interactions is an important tool for conservation, recovery, and rational use of the environment (McCallum & Dobson, 1995; Poulin & Morand, 2000; Santos, 2003; Thomas et al., 2005, 2009).

This study aimed to reduce the gap in the helminthological knowledge of anurans by identifying parasite communities and their parasitic parameters in two amphibian species Chiasmocleis albopunctata (Boettger, 1885) and Dendropsophus nanus (Boulenger, 1889) in an area of Cerrado Biome, found in eastern Mato Grosso do Sul state in the Midwest region of Brazil.

MATERIAL AND METHODS

Study Area
The collections were performed in an area of the Gallery Forest adjacent to the Stream of Véstia, at UNESP Research and Extension Teaching Farm (FEPE), Ilha Solteira Campus (20° 21'48" S, 051° 24'17" W), Selvíria Municipality, Mato Grosso do Sul State, Brazil.

Host Collection
The hosts were collected through six sets of Pitfall Traps with Drift Fence (PTDF) (Corn, 1993), consisting of a Y-shaped fence with a centre bucket and a bucket at each “Y” vertex, usually in places near anuran breeding sites. We also used the searching in breeding sites method described by Scott & Woodward (1994), which consists of systematically walking around and through the given collection site and searching for the animals of interest (Halliday, 2006). In this work, the active search collections were always performed by an average of three people for three consecutive hours (from 20:00 to 23:00 hours). The collections took place monthly between September 2013 and November 2015 (SisBio 36667-2; CEUA no. 06/2014).

At UNESP’s Ecology Laboratory of Parasitism (Lecop), the hosts were euthanised with sodium thiopental solution. All internal organs, the cellomatic cavity, and hind limb musculature were evaluated for the presence of helminths.

The hosts were deposited at the Museum of Zoology “Adão José Cardoso” State University of Campinas (ZUEC).

Helminths Collection
Helminths were collected, fixed, and processed following commonly employed methodologies (Amato et al., 1991). The nematodes were clarified with Aman’s lactophenol (Andrade, 2000), and species identification was based on articles and classification keys (Vicente et al., 1990; Gibbons, 2010; Anderson et al., 2009). Trematodes were submitted to the hydrochloric carmine staining technique (Amato et al., 1991), diaphanized with clove oil, and identified according to Fernandes & Kohn (2014). Parasitic parameters such as Prevalence (P) and Mean Infection Intensity (IMI) were calculated according to Bush et al. (1997), with the aid of the SigmaEstat 3.1 program.

The morphometric, morphological, and photomicrograph data of the helminths were obtained using a computerised LAS V4 image analysis system (Leica Application Suite) adapted to the DM 2500-Leica microscopes with phase interferential contrast system.

The parasites were deposited in the Helminthological Collection of the Department of Parasitology, Institute of Biosciences, Paulista State University (UNESP) (CHIBB 8798 - 8818), Botucatu, State of São Paulo, Brazil.

Ethic aspects
All international, national, and institutional guidelines applicable to the care and use of animals were followed.

RESULTS

A total of 172 amphibians, 70 C. albopunctata (Boettger, 1885) individuals and 102 D. nanus (Boulenger, 1889) individuals were collected. Of these, 84 were parasitised by at least one taxon (P = 48.8%). The total number of helminths found was 675, with a mean infection intensity of 5.8 ± 1.4 (1-154).

In the host species C. albopunctata (Boettger, 1885), 214 helminths were found, of which 207
were nematodes, and seven were trematodes. The overall prevalence was 62.8%, and the IMI was 2.8 ± 0.3 (1-14). The nematodes were the most prevalent helminths (97.7%) with an IMI of 2.8 ± 0.3 (1-14), while trematodes had a prevalence of 2.3% and a total of seven individuals collected in a single host.

In the host species *D. nanus* (Boulenger, 1889), 461 helminths were found, of which 452 were trematodes, five were acanthocephalans, and four were nematodes. The total prevalence of helminths was 39.2%, and the IMI was 11 ± 3.7 (1-154). The prevalence of trematode infection in this host species was 90% with an IMI of 12.2 ± 4.2 (1-154); for acanthocephalans, the prevalence was 5% with an IMI of 2.5 ± 1.5 (1-4), and for nematodes the prevalence was 7.5% with an IMI of 1.3 ± 0.3 (1-2). The taxa found in each host species, infection sites, their prevalence (P) and mean infection intensity (IMI), and abundance are described in Table 1.

Table 1. Parasitic parameters of nematodes and trematodes associated with anurans *Chiasmocleis albopunctata* (Boettger, 1885) and *Dendropsophus nanus* (Boulenger, 1889). ID = Small Intestine; Cav = Cavity; GI = Large Intestine; P = Overall Prevalence; IMI = Mean Intensity of Infection; SI = Site of Infection; E = stomach.

| Helminths                  | P (%)  | IMI               | SI               |
|----------------------------|--------|-------------------|------------------|
| *Cosmocercidae*            | 60.5   | 3.2 ± 0.46 (1-12) | ID, IG, Cav      |
| *Aplectana*                | 2.3    | 3                 | ID               |
| *Cosmocerca parva*         | 67.4   | 2.5 ± 0.4 (1-14)  | ID, IG, Cav      |
| *Mesocoelium monas*        | 2.3    | 7                 | ID               |
| Larva of Nematoda          | 2.3    | 1                 | IG               |
| P (%)                      | 58.6   |                   |                  |
| IMI                        | 5.2 ± 2.7 |                |                  |

| Helminths                  | P (%)  | IMI               | SI               |
|----------------------------|--------|-------------------|------------------|
| *Cosmocercidae*            | 7.5    | 1.3 ± 0.33 (1-2)  | ID, IG           |
| *Lophosicyadiplostomum* sp.| 85     | 13.1 ± 4.5 (1-154)| Rim              |
| Cyst of Trematoda digenea  | 2.5    | 1                 | Rim              |
| Metacercaria unidentified  | 2.5    | 3                 | E                |
| *Neohaematoloechus neivai* | 2.5    | 2                 | Pulmão           |
| *Echinorhynchidae*         | 5      | 2.5 ± 1.5 (1-4)   | E, ID            |
| P (%)                      | 41.2   |                   |                  |
| IMI                        | 11.5 ± 24.5 |            |                  |
Table 2. List of taxa found for each host species, highlighting what has been reported in the literature and what is being reported for the first time.

| Taxon                          | Reference                          |
|-------------------------------|------------------------------------|
| **Dendropsophus nanus** (Boulenger, 1889) |                                    |
| Family Cosmocercidae          | Campião et al. (2014, 2016)        |
| Lophosicyadiplostomum sp.     | Hamann & González (2009)           |
| Cyst of Trematoda Digenea     | Unidentified                       |
| Metacercaria unidentified     | Unidentified                       |
| Neohaematolechus neivai       | First report                       |
| Family Echinorhynchidae       | First report                       |
| **Chiasmocleis albopunctata** (Boettger, 1885) |                                    |
| Family Cosmocercidae          | First report                       |
| Aplectana sp.                 | First report                       |
| Cosmocerca parva              | First report                       |
| Mesocoelium monas             | First report                       |
| Larva of Nematoda             | First report                       |

**DISCUSSION**

We report here ten helminth rates and seven new records, in addition to the host species *C. albopunctata* (Boettger, 1885), which had not been studied so far (Table 2).

The anuran *D. nanus* (Boulenger, 1889) is found from Suriname and French Guiana, to Uruguay, Argentina, Paraguay and Bolivia, and in Brazil it occurs from the Northeast to the South (Frost, 2019). The registration of two new helminth taxa for this species demonstrates the lack of studies in this area.

Existing parasitological studies revealed 11 parasitic helminth taxa of *D. nanus* (Boulenger, 1889), these being Centrorhyncus sp., Unidentified Acanthocephala, Contracaecum sp., Cosmocerca podicipinus Baker & Vaucher, 1984, Cosmocercoida gen. sp., Cylindrotaenia sp., Creptotrema sp., Digenea gen. sp., Plagiorchiata gen. sp., Diplostomidae gen. sp., and Brevimulticaecum sp. (Hamann & Kehr, 1998; González & Hamann, 2011; Aguiar et al., 2014; Campião et al., 2014, 2016; Graça et al., 2017).

The anuran *D. nanus* (Boulenger, 1889) has an aquatic larval phase, remains in the vegetation near the water as an adult, and uses the aquatic environment in reproduction (Uetanabaro et al., 2008). Proximity to water during the anuran life cycle is highly related to the high prevalence of trematode parasites (McAlpine, 1997; Muzzall et al., 2001; Bolek & Coggins, 2001, 2003; Paredes-Calderón et al., 2004; Hamann et al., 2006ab; Schaefer et al., 2006; Todd, 2007; Toledo et al., 2018), which explains the helminth fauna described for *D. nanus* (Boulenger, 1889) in this work. Thus, the use of water bodies during the life cycle of *D. nanus* (Boulenger, 1889) contributed to determining the parasite community of this anuran.

The Cosmocercidae family comprises of several nematode species and have been reported in several South American countries parasitising the intestine, rectum, stomach, and lungs of their hosts. Unidentified individuals of the family have been reported in Dermatotus muelleri (Boettger, 1885), Physalaeus nattereri (Steindachner, 1863) (=Eupemphix nattereri), Boana prasina (Burmeister, 1856) (=Hypsoiboas prasins), Leptodactylus latrans (Steffen, 1815), Leptodactylus pentadactylus (Laurenti, 1768),
Leptodactylus podicipinus (Cope, 1862), Pethecopus azureus (Cope, 1862) (=Phyllomedusa azurea), Rhinella crucifer (Wied-Neuwied, 1821), Rhinella icteric (Spix, 1824) and Rhinella diptycha (Cope, 1862) (=Rhinella schneideri) (Campião et al., 2014). In the present study, for the species D. nanus (Boulenger, 1889), individuals of this family were found in the small and large intestines of the anurans. Acanthocephalans, belonging to the family Echinorhynchidae, have been reported in Paraguay and Brazil parasitising the intestine and body cavity of the hosts, being some species from this family Acanthocephalus acutispinus Machado Filho, 1968, Acanthocephalus caspanensis Fernandez and Ibarra, 1989, Acanthocephalus correalmiai Machado Filho, 1970, Acanthocephalus ula u Holst and Santos, 1989 and Pseudocanthocephalus lutzi (Hamann, 1891) (Campião et al., 2014). However, in this study, individuals of this family were found in the small intestine and the stomach of the anurans. Trematoda Lophosicyadiplostomum is an anuran parasite in its larval phase and has been found in Argentina and Brazil parasitising the kidneys of these hosts. Individuals of the genus have been reported in Scinax nasicus (Cope, 1862), Lysapsus limellum Cope, 1862 (=Pseudis limellum), and D. nanus (Boulenger, 1889) (=Hyla nana) (Hamann & González, 2009; Campião et al., 2014). In the present study, this species was found parasitising the kidneys. The trematode Neohaematoloechus neivai (Travassos & Artigas, 1927), have been found in Brazil and Venezuela parasitising the lungs of their hosts (Campião et al., 2014; Fernandes & Kohn, 2014), having already been documented in several South American countries parasitising the stomach, intestine and body cavity of the hosts. Individuals of the genus have been reported in Leptodactylus latrans (Steffen, 1815), Leptodactylus lauriniceps (Spix, 1824), Leptodactylus pentadactylus (Laurenti, 1768), Pseudis paradoxa (Linnaeus, 1758), and Lithobates palmipes (Spix, 1824) (=Rana palmipes).

In this study, with the registration of the trematode Neohaematoloechus neivai (Travassos & Artigas, 1927) and acanthocephalans of the family Echinorhynchidae, we increased the number of helminth taxa of D. nanus (Boulenger, 1889) to 13.

The frog C. albopunctata (Boettger, 1885) is found in Bolivia, Argentina, Paraguay, and the Midwest and Southeast Regions of Brazil (Frost, 2019). Although their distribution covers four different countries, the associated helminth fauna of this anuran has never been studied.

The species C. albopunctata (Boettger, 1885) is considered terrestrial and uses the aquatic environment only in the larval phase and during breeding periods (Uetanabaro et al., 2008; Giaretta et al., 2008). Contact with soil may favour nematode infection (Barton, 1999; Bolek & Coggins, 2000, 2003; Iannaccone, 2003; Luque et al., 2005; Sena et al., 2018; Toledo et al., 2018), which explains this marked prevalence of these helminths for this host species. Despite this prevalence of nematodes, the richness of parasite species for this host was low with only three species identified.

For the species C. albopunctata (Boettger, 1885), nematodes of the family Cosmocercidae were found parasitising the body cavity of the hosts. For the Cosmocerca parva Travassos, 1925 helminth, there are studies indicating its presence in Argentina, Brazil, Colombia, Paraguay, Guyana, and Peru (Campião et al., 2014). These parasites are found mainly in the intestine and rectum of their hosts, and have been reported in several species of various genera such as Ameerega, Colostethus, Dendropsophus, Edalorhina, Elachistocleis, Hamptophryne, Hyspobia, Hylodes, Leptodactylus, Odontophrynus, Oreobates, Phyllomedusa, Physalaemus, Pristimantis, Rhaeboophion, Scilla, Procella, Rhinella, Procratophrys, Scarthyla, and Scinax (Campião et al., 2014). In this study, we found Cosmocerca parva Travassos, 1925 in the body cavity of the hosts. Individuals of the genus Aplectana have been documented in several South American countries parasitising the stomach, intestine and rectum of their hosts. Unidentified individuals of the genus have been found parasitising Ceratophrys Cranwelli Barrio, 1980, Dendropsophus microps (Peters, 1872), Dermatophyton muelleri (Boettger, 1885), Haddadus biotatus (Spix, 1824), Boana albopunctata (Spix, 1824) (=Hyspobia albopunctata), Boana pardinis (Spix, 1824) (=Hyspobia pardinis), Leptodactylus bufonius Boulenger, 1894, Leptodactylus chauensis Cei, 1950, Leptodactylus elenae Heyer, 1978, Leptodactylus fuscus (Schneider, 1799), Adenomera marmorata Steindachner, 1867.
Leptodactylus marmoratus (Burmeister, 1861), Leptodactylus mystacinus (Burmeister, 1861), Leptodactylus podicipinus (Cope, 1862), Leptodactylus syphax Bokermann, 1969, Physalaemus signifer (Girard, 1853), Rhinella granulosa (Spix, 1824), Rhinella icterica (Spix, 1824), Rhinella marina (Linnaeus, 1758), Scinax acuminatus (Cope, 1862), Thoropa miliaris (Spix, 1824), and Trachycephalus mesophaeus (Hensel, 1867) (Campião et al., 2014). In this study, Aplectana sp. was found in the small intestine of the host. The Mesocoelium monas (Rudolphi, 1819) Trematoda, according to Campião et al. (2014) and Fernandes & Kohn (2014), have been found in Argentina, Brazil, Colombia, Paraguay, Peru and Venezuela parasitising the small intestine of their hosts, and have been reported in Siphonops annulatus (Mikan, 1822), Leptodactylus fuscus (Schneider, 1799), Leptodactylus mystaceus (Burmeister, 1861), Leptodactylus mystacinus (Burmeister, 1861), Leptodactylus latrans (Steffen, 1815), Leptodactylus pentadactylus (Laurenti, 1768), Rhinella arenarum (Hensel, 1867), Rhinella crucifer (Wied-Neuwied, 1821), Rhinella icterica (Spix, 1824), Rhinella marina (Linnaeus, 1758), Rhinella diptycha (Cope, 1862) (=Rhinella schneideri), Incilius nebulifer (Girard, 1854) and Scinax nasicus (Cope, 1862). In the present study it was also found in the small intestine of the host.

All taxa found in C. albopunctata (Boettger, 1885) have never been reported for this host species, as this is the first study using this approach. Here we highlight the five taxa record for this host species. Additional studies with populations from other locations may show different patterns from those found here.

This study represents an important contribution to the knowledge of the diversity of parasites and hosts since the number of studies is insufficient and not representative. It also helps to complement information for ecosystem studies with a view to facilitating conservation projects.

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