

UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCÊNCIAS, CAMPUS DE BOTUCATU
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

Metazoários endoparasitas de anuros em uma área de transição
entre Cerrado e Mata Atlântica: composição, estrutura e
variáveis relacionadas



Aline Aguiar

Botucatu

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variáveis relacionadas**

Aline Aguiar

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Palavras-chave: Anfíbios; Digenéticos; Helmintos; Nematóides; Oligoqueta.

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"Eu venho de lá, onde o bem é maior. De onde a maldade seca, não brota. De onde é sol mesmo em dia de chuva e chuva chega como benção.

Lá sempre tem uma asa, um abrigo para proteger do vento e das tempestades.

Eu venho de um lugar que tem cheiro de mato, água de rio logo ali e passarinho em todas as estações.

Eu venho de um lugar em que se divide o pão, se divide a dor e se multiplica o amor.

Eu venho de um lugar onde quem parte fica para sempre, porque só deixou boas lembranças.

Eu venho de um lugar onde criança é anjo, jovem é esperança, e os mais velhos são confiança e sabedoria.

Eu venho de um lugar onde irmão é laço de amor e amigo é sempre abraço. Onde lar acolhe para sempre como o coração de mãe.

Eu venho de um lugar que é luz mesmo em noite escura. Que é paz, fé e carinho.

Eu venho de lá, e não estou sozinha, "sou catadora de lindezas", sobrevivo de encantamento, me alimento do que é bom, do bem.

Procuro bonitezas e bem querer, sobrevivo do que tem clareza e só busco o que aprendi a gostar.

Não esqueço de onde venho e vou sempre querer voltar.

Meu lugar se sustenta do bem que encontro pelo caminho, junto à maços de alfazema e alecrim. Assim, sou como passarinho carregando a bagagem de bondade, catando gravetos de cheiro, para esquentar e sustentar o ninho...

Esse lugar é fácil encontrar. Silencie, respire, desarme-se, perceba, é pertinho. Este lugar que pulsa amor é dentro da gente, é essência, está em cada um de nós. Basta a gente buscar."
(Rita Maidana)

*Não me dêem fórmulas certas, porque eu não espero acertar sempre.
Não me mostre o que esperam de mim, porque vou seguir meu coração!*

*Não me façam ser o que não sou, não me convidem a ser igual, porque sinceramente
sou diferente! Não sei amar pela metade, não sei viver de mentiras, não sei voar com os
pés no chão. Sou sempre eu mesma, mas com certeza não serei a mesma para sempre!*

*Gosto dos venenos mais lentos, das bebidas mais amargas, das drogas mais poderosas,
das idéias mais insanas, dos pensamentos mais complexos, dos sentimentos mais fortes.*

*Tenho um apetite voraz e os delírios mais loucos. Você pode até me empurrar de um
penhasco que eu vou dizer: - E daí? Eu adoro voar!*

Clarice Lispector

"Se não sabes, aprende; se já sabes, ensina"

Confúcio

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Resumo

Este estudo primeiramente investigou e descreveu a diversidade de parasitas metazoários associados a 26 espécies de anuros em uma região de transição entre Mata Atlântica e Cerrado no noroeste paulista, Brasil. Em seguida, procuramos analisar os padrões de agregação e distribuição das populações parasitárias nas diferentes espécies de hospedeiros bem como a estrutura e composição da comunidade componente. Também foram reportados novos registros de ocorrência em anuros do Brasil e considerações taxonômicas para algumas espécies de helmintos. As 26 espécies de anuros apresentaram um total de 40 taxa de parasitas metazoários: *Aplectanahylambatis*, *Aplectanamembranosa*, *Brevimulticaecum* sp. (larva), *Cosmocerca* cf. *chilensis*, *Cosmocerca parva*, *Cosmocerca podicipinus*, *Cosmocercidae* gen. sp., *Falcaustramacula*, *Ochoterenelladigiticauda*, *Ochoterenella* sp., *Oswaldocruziamazzai*, *Oxyascariscaudacutus*, *Oxyascaris* sp., *Parapharyngodon* cf. *alvarengai*, *Physaloptera* sp. (larva), *Raillietnemaminor*, *Raillietnema* sp., *Rhabdias* sp.1, *Rhabdias* sp.2, *Rhabdias* sp.3, *Schrankianaformosula*, *Spiroxys* sp. (larva) (Nematoda), *Centrorhynchidae* gen. sp. (cisto), Cistacanto não identificado (*Acanthocephala*), *Cylindrotaenia americana* (Cestoda), *Brachycoelium salamandrae*, *Bursotrema* sp. (metacercária), *Catadiscus marinhoi*, *Catadiscus propinquus*, *Catadiscus* sp., *Clinostomum* sp., *Gorgoderinadiaster*, *Gorgoderina* sp., *Heterodiplostomum* sp. (metacercária), *Lophosicyadiplostomum* sp. (metacercária), metacercária não identificada, *Neohaematoleus neivai*, *Rauschiella* sp. (Digenea), *Polystoma* cf. *lopezromani* (Monogenea) e *Dero* (*Allodero*) *lutzi* (Oligochaeta). Variáveis do hospedeiro como espécie, tamanho e hábito, e outras do ambiente como estação e condições do habitat, foram avaliadas como potenciais preditoras na composição da comunidade parasitária. Observamos que o tamanho e a espécie hospedeira influenciaram na composição e sugerimos que anuros mais próximos filogeneticamente apresentam fauna parasitária mais similar do que outros.

Palavras-chaves: parasitas; anuros; riqueza; Nematoda; Digenea

Abstract

In the present study, we firstly investigated and described the diversity of metazoan parasites associated with 26 anuran species from a transitional area between Mata Atlântica and Cerrado in Northwest of São Paulo State, Brazil. Thereafter, we searched for aggregation and distribution patterns of parasite populations from different host species as well as the structure and composition of component communities. Also, we reported new records of occurrence in Brazilian anurans and taxonomic considerations for some parasite species. The 26 anuran species presented a total of 40 metazoan parasite taxa: *Aplectanahylambatis*, *Aplectanamembranosa*, *Brevimulticaecum* sp. (larvae), *Cosmocerca* cf. *chilensis*, *Cosmocercaparva*, *Cosmocerca podicipinus*, *Cosmocercidae* gen. sp., *Falcaustramascula*, *Ochoterenelladigiticauda*, *Ochoterenella* sp., *Oswaldocruziamazzai*, *Oxyascariscaudacutus*, *Oxyascaris* sp., *Parapharyngodon* cf. *alvarengai*, *Physaloptera* sp. (larvae), *Raillietnema minor*, *Raillietnema* sp., *Rhabdias* sp.1, *Rhabdias* sp.2, *Rhabdias* sp.3, *Schrankianaformosula*, *Spiroxys* sp. (larvae) (Nematoda), *Centrorhynchidae* gen. sp. (cyst), Unidentified cystacanth (Acanthocephala), *Cylindrotaeniaamericana* (Cestoda), *Brachycoeliumsalamandrae*, *Bursotrema* sp. (metacercariae), *Catadiscusmarinholutzi*, *Catadiscuspropinquus*, *Catadiscus* sp., *Clinostomum* sp., *Gorgoderinadiaster*, *Gorgoderina* sp., *Heterodiplostomum* sp. (metacercariae), *Lophosicyadiplostomum* sp. (metacercariae), Unidentified metacercariae, *Neohaematoloechusneivai*, *Rauschiella* sp. (Digenea), *Polystomac* cf. *lopezromani* (Monogenea) and *Dero* (*Allodero*) *lutzi* (Oligochaeta). Host variables such as species, body size and habit, and other variables of environment (season and habitat conditions) were evaluated as potential predictors for composition of parasite communities. We observed body size and species of host influenced in composition and we suggested that anurans closely related presented parasite fauna more similar than others.

Key words: parasites; anurans; richness; Nematoda; Digenea

Introdução geral

Entre muitos desafios da ciência, há mais de um século tentamos quantificar a diversidade biológica existente no planeta, ou ao menos adquirir pistas para inferirmos tal diversidade e abundância de indivíduos em um determinado ambiente (May, 1988). Relacionado a todos os organismos componentes da diversidade que vemos a "olho nú" ainda há muitas formas de vida que constituem uma diversidade "oculta" vivendo dentro ou sobre um organismo (Poulin & Morand, 2000). Ao longo da evolução várias estratégias e formas de vida parasitária foram surgindo como organismos unicelulares (protozoários) e metazoários nos diversos grupos animais (Poulin & Morand, 2004). Nesse contexto, os helmintos constituem um grupo muito diverso que são estudados juntos pois compartilham os mesmos habitats e estão sujeitos as mesmas variações ambientais.

Essa complexa relação parasita-hospedeiro influencia e é influenciada como toda comunidade ecológica em um ecossistema, e sendo uma relação que encontra-se em constante co-evolução é necessário que esses organismos sejam estudados em conjunto levando em conta a biologia e a história evolutiva de cada um (Poulin, 2007).

Dentre os animais mais estudados em relação a sua helmintofauna estão os vertebrados e, dentro desse grupo, os anfíbios tornam-se interessantes por apresentar um estilo de vida que ocorre em meio aquático e terrestre, permitindo sua exposição à diferentes comunidades parasitárias além da interação com fatores ambientais distintos (Goater & Goater, 2001).

Estima-se que a diversidade de anuros no mundo seja em torno de 7.244 espécies, e o Brasil é um dos territórios com maior diversidade, com cerca de 15% (1.080 spp.) da diversidade global (Frost, 2016; Segalla et al., 2016). Essa grande diversidade no Brasil, bem como na região Neotropical, está relacionada às florestas ainda remanescentes como a Mata Atlântica e Cerrado. A disponibilidade de nichos, refúgios e relevo com montanhas das florestas resultou em processos de especiação ao longo de milhares de anos (Haddad et al., 2008). Dada tamanha riqueza e ocupação de diversos microhabitats por esses vertebrados, é esperado que a diversidade parasitária associada à anurofauna seja maior que o número de espécies hospedeiras, visto que um único indivíduo pode abrigar uma comunidade (Bush et al., 1997).

De acordo com Campião et al. (2014), em anfíbios da América do Sul existe uma riqueza de 278 espécies de helmintos relacionadas a 185 espécies de anuros. O Brasil é descrito como o país mais diverso em helmintos associados a anfíbios dentre os demais

países sul-americanos. Os nematoides parasitas de anfíbios da América do Sul são os helmintos com maior riqueza de espécies (aproximadamente 150 espécies reportadas) e com maior número de registros no Brasil e na Argentina. Em seguida estão os digenéticos com cerca de 96 espécies parasitando anfíbios como hospedeiros definitivos ou intermediários. Os monogeneas constituem um grupo menos diverso, porém mais específico em relação aos hospedeiros, sendo reportadas cerca de 15 espécies do parasita em 16 espécies de anfíbios. Os cestoides também são caracterizados pela baixa riqueza em anfíbios no continente sul-americano, com cerca de 13 espécies em apenas 14 espécies de anuros. Acantocéfalos constituem um filo relativamente diverso, porém aproximadamente 15 espécies parasitam anfíbios da América do Sul (Campião et al., 2014). Dentre esses parasitas metazoários, o grupo Oligochaeta talvez seja o menos representativo em anfíbios devido à baixa ocorrência e por se conhecer apenas uma espécie envolvida com o parasitismo até o momento, *Dero (Allodero) lutzi* (Morais et al., 2017).

Os muitos fatores envolvidos nessa relação parasito-hospedeiro, como a fisiologia do hospedeiro, condições ambientais e ciclo biológico do parasita, nos fornece muitas possibilidades de estudos em diversas linhas de pesquisa. Porém, é fato que um dos primeiros passos seja inventariar para então conhecer quem são os componentes dessa diversidade oculta e a que grupo pertencem.

Dessa forma, este estudo tem como primeiro objetivo fornecer o conhecimento acerca da biodiversidade de endoparasitas metazoários relacionados a anfíbios, e em especial anfíbios provenientes de uma área de transição entre Mata Atlântica e Cerrado, caracterizados pela alta riqueza e endemismo. Ainda nesse contexto, informações a respeito de novos registros de ocorrências, ampliação da área de distribuição e algumas considerações taxonômicas são fornecidas e discutidas sobre algumas espécies de helmintos.

O segundo objetivo desse estudo foi investigar e discutir os potenciais preditores da composição de helmintos nessas populações de anfíbios levando-se em conta variáveis do hospedeiro como hábito, tamanho do corpo, espécie hospedeira e do ambiente como sazonalidade e habitat.

Além da ampliação do conhecimento acerca do tema, os resultados aqui apresentados em dois capítulos podem ser chaves para entendimentos ecológicos e biogeográficos posteriores.

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Capítulo 1

Metazoan endoparasites associated with 26 anuran species from a transitional area
between Cerrado and Atlantic Rain Forest, Brazil

**METAZOAN ENDOPARASITES ASSOCIATED WITH 26 ANURAN SPECIES
FROM A TRANSITIONAL AREA BETWEEN CERRADO AND ATLANTIC
RAIN FOREST, BRAZIL¹**

ABSTRACT: In the present study, we described the endoparasite structure of 26 anuran species from a transitional area between Cerrado and Atlantic Rain from Brazil. Nine hundred and seventy-eight anurans of four families (Bufonidae, Hylidae, Microhylidae, and Leptodactylidae) were collected. The metazoan parasites richness was composed by nematodes (22), acantocephalans (2), digenetics (13), monogeneans (1), cestodes (1), and oligochaetes (1), being identified 40 *taxa* and 1.92 of diversity. The most prevalent parasite was metacercarie of *Lophosicyadiplostomum* sp. while *Aplectana membranosa* was the most abundant helminth, and all helminths presented an aggregated distribution. Leptodactylids had high richness of helminth, especially *Leptodactylus podicipinus* that showed the most richness helminth fauna with 16 species. In addition to new parasite records and remarks on their biological cycle, we presented a significant correlation between parasite abundance and the size of the amphibians of ten anuran populations.

The amphibian richness of Brazil is composed of 1,026 species and it represents approximately 14% of the global biodiversity of this vertebrate class (Segalla et al., 2014; Frost, 2015). Important aspects of this high number of species are the biomes such as Mata Atlântica, Cerrado, and also their transitional areas which harbor several habitats, allowing various speciation and endemism (Haddad and Prado, 2005; Mittermeier et al., 2005). Although Brazilian Rain Forest is greatest known for its biologic diversity (approximately 1,600,000 animal species) and Cerrado (approximately 320,000 animal species) (ICMBio, 2016; Instituto Brasileiro de Florestas, 2016) these figures are still underestimated, though. In view of this, the vast number of habitats that amphibian species are able to explore contributes to composition and maintenance of their associated parasite communities (Aho, 1990; Goater and Goater, 2001). It is estimated that probably only about 10 to 20% of global diversity is known (May, 1988; Hammond, 1992), and consequently our understanding of parasites is very scarce (Poulin and Morand, 2004).

¹Manuscrito de acordo com as normas da revista *Journal of Parasitology*.

In South America, the knowledge about helminths associated to amphibians have been reviewed by Campião et al. (2014), that presented a richness of 278 helminth species associated with 185 anuran species. In addition to that, Brazil has the greatest richness followed by Argentine, Paraguay, and Peru. Anurans, with more helminth records are the species from Leptodactylidae and Hylidae (Camião et al., 2014). However, this finding can be related to abundance and relative facility to find and collect these hosts, as well as the number of researchers studying amphibian parasites in each country mentioned. There are species that are not common, due to their difficulty to be found and collected what result in a lack of knowledge about many anuran species.

Another point of helminthological studies is the taxonomic quality, because several helminth species were described a long time ago (e.g. Travassos, 1920, 1930; Freitas and Lent, 1939a). Then, technology has contributed towards advances and improvements of details of morphologic structures in addition to molecular tools.

Thus, studies investigating the biological richness and its geographical distribution are important for the beginning of any other research. We listed and described the structure of helminth communities associated with 26 anuran species as well as the dispersion of these helminths in an anuran community, and the potential relation between host size and helminth abundance were verified.

MATERIALS AND METHODS

Study area

This study was conducted in a particular area referred as Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí (RPPN) (Figure 1) under the responsibility of Companhia Energética do Estado de São Paulo (CESP), and it covers municipalities of Castilho, São João do Pau d'Alho, and Paulicéia, São Paulo State, Brazil. The RPPN is crossed by Aguapeí River, which belongs to Paraná Basin, Bauru Sub-basin, and is characterized with slightly wavy terrain (Sallun and Suguio, 2006). The area is a transition between Mata Atlântica and Cerrado, which is characterized by fragments of Seasonal Forest and plains. Its type of terrain and climatic zone with rainy and dry seasons allow periodical inundations in the region (Comitês das Bacias Hidrográficas dos Rios Aguapeí e Peixe – CBH-AP, 1997; Boin, 2000).

Collection of anurans

The anurans were collected by visual search and by pitfall traps in four expeditions: two in a dry season – July 2012 and July 2013, and two in a rainy season – January 2013 and January 2014 (SISBIO number permission 31716-2). The weight of anurans was measured using Pesola® weighing scale and a caliper (0.01 precision) was used for measuring of snout-vent-length (SVL). They were killed with tiopental sodic and then they were necropsied to searching helminth parasites in all organs and body cavity. We then fixed the anurans with formaldehyde (10%) and preserved in alcohol 70%, and deposited them in the Coleção de Vertebrados da Universidade Estadual de Campinas and Coleção Herpetológica da Universidade Federal do Mato Grosso do Sul. Anurans nomenclature follows Frost (2016) and Segalla et al. (2016).

Collection of helminths and procedures in laboratory

All organs and body cavity were searched for helminth parasites using a stereomicroscopy. Parasites found were counted and registered according to the site of infection. Nematodes were fixed using hot absolute alcohol, while acanthocephalans were firstly kept on cold water for exposition of proboscis and then they were fixed with absolute alcohol; cestodes, digeneans, and monogeneans were fixed using absolute alcohol under slight pressure of cover slip; whereas oligochaets were fixed only in alcohol 70%. Subsequently, all helminths were maintained in labeled bottles with ethanol 70%.

In laboratory, helminths were mounted in temporary slides for observation of taxonomic structures using a computerized system of image analysis (LAS DIC, Leica Microsystems, Wetzlar, Germany). The nematodes and oligochaets were cleared with lactic acid or lactophenol, and the others helminths such as acanthocephalans, cestodes, digeneans, and monogeneans were stained with chloridric carmine, dehydrated with alcoholic series and then cleared with eugenol (Amato et al., 1991; Rey, 2001).

The morphology of some helminths was evaluated by scanning electron microscopy (SEM). First, they were fixed in absolute alcohol, dehydrated in graded alcohol series and dried to critical point with liquid CO₂ in CPD 020 (Balzer Union). Then they were mounted on an aluminum stub using conductive double-sided tape, coated with gold–palladium and examined with the use of a Quanta 200 scanning electron microscope (FEI Company; from Centro de Microscopia Eletrônica de Botucatu, São Paulo, Brazil) (adapted from Allison et al., 1972).

Helminths were deposited in Coleção Helminológica do Instituto de Biociências, Departamento de Parasitologia, Unesp Campus Botucatu.

Data analysis

Parasitism descriptors, such as prevalence (P), mean abundance (MA), mean intensity of infection (MII), mean richness (MR), component community, and supra-community followed Bush et al. (1997). The richness was described as number of helminth *taxa*, and Brillouin index was calculated for diversity (Krebs, 1989).

All values were reported as mean $\pm$ standard error (SE), with exception of size means (SVL, snout-vent length (cm) and weight (g)) which were reported with standard deviation (SD).

We used Morisita dispersion index (Id) for each helminth species to establish their aggregation level in supracommunity of anuran fauna, and it was calculated by the software PASSaGE 2 (version 2.0.11.6.).

Spearman correlation was calculated using Sigma Stat (version 3.5) to verify relations between hosts' size (weight and snout-vent length) and abundance of parasites.

RESULTS AND DISCUSSION

There were several generalist helminths of component communities such as nematodes of Cosmocercidae. Although a few were specific to only one anuran species, such as the monogenean *Polystoma* cf. *lopezromani*, the digenetic *Neohaematoloechus neivai*, and the nematode *Raillietnema minor* which were found exclusively in *Trachycephalus typhonius*, *Pseudis paradoxa*, and *Pithecopus azureus*, respectively. To start with, it was necessary to organize and analyze the results as supracommunity and then, each component communities.

Supracommunity

A total of 978 anuran individuals of 26 species belong to four families (Bufonidae, Hylidae, Leptodactylidae, and Microhylidae) were collected (Table 1). The prevalence of parasitized anuran was 59.4% (581 individuals) and the mean intensity of infection was 36.3 ± 4.1 parasite per host, while the mean abundance was 21.6 ± 2.5 . The metazoan parasite richness from this anuran community was composed by nematodes, acantocephalans, digenetics, monogeneans, cestodes, and oligochaetes, being identified 40 *taxa* infecting these anurans (Table 2, Appendix 1), resulting in 1.6 ± 0.03 of mean

richness (from 1 to 5 parasite species in a single host). According to Brillouin index, the diversity of parasites from anuran community was 1.92.

The total number of helminths recovered was 21,106 and the majority of anurans presented a range from one to seven helminths, while others were infected not later than 1,302 helminths.

Nematodes were the helminths with the highest number of species (22), followed by digenetics with 13 species and the other helminth groups had only one species (Table 2). However, with exception of *Cosmocercidae* gen. sp. the most prevalent species was *Lophosicyadiplostomum* sp. (metacercariae) which were presented in 10% of anurans (in 15 species hosts). Followed by *Catadiscus* sp. associated with 9.6% of anurans (9 species hosts), *Cosmocerca podicipinus* with 9% but associated with only three host species. Finally, *Aplectana membranosa* in 7% of anurans (9 species hosts) (Table 2; Figure 2). Thus, we considered *Lophosicyadiplostomum* sp., *Catadiscus* sp., and *A. membranosa* as the most generalist species, because of its high prevalence and high number of host species associated with them (Figure 3). Moreover, this metacercaria was one of the most abundant helminth, with 5,927 individuals, but *A. membranosa* was the most abundant species with 7,039 individuals (Table 2). According to dispersion index (Morisita), all helminth populations were considered aggregated even though some had presented as more or less aggregated (Table 2). However, the most aggregated helminth was *Aplectana hylambatis* (676.0), *Cosmocerca* cf. *chilensis* (326.0), *Falcaustra mascula* (320.4), *Ochoterenella digiticauda* (978.0), *Oswaldocruzia mazzai* (978.0), *Oxyascaris caudacutus* (586.0), *Parapharingodon* cf. *alvarengai* (483.7), *Rhabdias* sp1 (614.7), *Brachicoelium salamandrae* (978.0), *Clinostomum* sp. (698.6), *Gorgoderina* sp. (978.0), unidentified metacercaria (978.0), *Neohaematolechus neivai* (396.8), and *Dero (Allodero) lutzi* (645.6). These findings can indicate that these helminths are in high abundance and in few hosts.

Component communities

We have studied a total of 26 component communities of metazoan parasites anuran. Prevalence (P), mean intensity of infection (MII), mean abundance (MA), mean richness (RM) and new records of helminths are presented in the following texts and Tables.

The component community of *Rhinella schneideri* (n= 21) had seven helminth taxa, and RM of 1.25 ± 0.18 per host, while diversity was 1.19. Overall prevalence was 57%, and the most prevalent parasite was *Rhabdias* sp.1 (P= 28.6%), while the most abundant was *Oswaldocruzia mazzai* which presented high intensity of infection with 116 individuals in a small intestine (Table 3). In addition to *O. mazzai*, larvae of *Brevimulticaecum* sp. are also reported for the first time in this bufonid. The toads had mean SVL of 10.1 ± 4.0 and mean weight of 164.4 ± 175.0 . Although the weak correlations (low values of rs), the helminth abundance were positive and significant for both SVL (rs= 0.48; p= 0.02) and weight (rs= 0.5; p= 0.01).

The component community of *Dendropsophus minutus* (n= 37) had four helminth taxa and a cyst very immature which identification was not possible. Diversity was 0.05 while mean richness was 1.2 ± 0.1 , and the overall prevalence was 54%. It should also be noted the high intensity of *Lophosicyadiplostomum* sp. (n= 916) infecting the kidneys of 19 frogs (P= 51.3%), and this study describes for the first time the component community of *D. minutus* (Table 4). These hylids had mean SVL of 2.3 ± 0.25 and mean weight of 0.68 ± 0.24 . No correlation was observed between abundance and SVL (rs= 0.06; p= 0.71), but weight presented positive correlation with helminth abundance (rs= 0.42 e p= 0.01).

Among the 35 specimens of *Hypsiboas* aff. *raniceps* 62.8% were parasitized and a component community of eight helminth taxa (Table 5), with a mean richness of 1.5 ± 0.14 and a diversity index of 0.61 were observed. The most prevalent parasite was the digenetic *Gorgoderina diaster* (P= 34.3%) infecting the urinary bladder of anurans while *Lophosicyadiplostomum* sp. was the more abundant (Table 5). The SVL mean of individuals was 6.5 ± 0.55 , and 14.0 ± 4.3 for mean weight. These variables were not correlated with helminth parasite abundance (rs= -0.1 and p= 0.5 for SVL; rs= -0.09 and p= 0.6 for weight).

Hypsiboas raniceps (n= 44) presented ten helminth taxa in its component community resulting in 1.4 ± 0.13 of mean richness while the diversity was 0.59. Overall prevalence was 56.8% of parasitized anurans (Table 6), and the most prevalent and abundant helminth was metacercaria of *Lophosicyadiplostomum* sp. with 729 parasites in 20.4% of hosts (Table 6). We reported several new records, such as *Brevimulticaecum* sp., *Ochoterenella* sp., *Physaloptera* sp., *Rhabdias* sp.2, *Catadiscus* sp. and *Lophosicyadiplostomum* sp., which were not registered for *H. raniceps*. There

were positive correlations between SVL (mean= 6.24 $\pm$ 1.7; rs= 0.33; p= 0.028) and parasite abundance, but not with weight (mean= 12.5 $\pm$ 6.6; rs= 0.14; p= 0.37).

The population of *Pithecopus azureus* (n= 47) had 80.5% of parasitized anurans, and the component community presented nine helminth *taxa* (Table 7) and mean richness of 2.08 $\pm$ 0.12 per host while the diversity was 0.86. Of the 2,561 helminths, 1,804 were metacercariae of *Lophosicyadiplostomum* sp. infecting the kidneys, and 560 were nematodes of *Raillietnema minor* from the large intestine. With the exception of Cosmocercids, which has already been reported for *P. azureus*, the remaining eight helminth *taxa* are new records for this hyliid. The mean of SVL and weight of *P. azureus* were respectively 3.1 $\pm$ 0.7 and 1.8 $\pm$ 0.7, and there were the same values of correlations for both with parasite abundance (rs= 0.36; p= 0.01).

The 38 specimens of *Pseudis paradoxa* presented a component community of seven helminth *taxa* (Table 8), a mean richness of 1.52 $\pm$ 0.14 per host, and a diversity index of 1.38. The overall prevalence was 81.6%, while the more prevalent and abundant helminths were the digenetics *Catadiscus* spp., *Rauschiella* sp., and *Neohaematoloechus neivai*. Some studies have reported several helminth species associated with this frog (e.g. Travassos et al., 1969; Kehr and Hamann, 2003) and its congeneric *P. platensis* (Campi o et al., 2010). However, we found new records such as larvae of *Brevimulticaecum* sp., metacercariae of *Heterodiplostomum* sp., and *Catadiscus propinquus*. The mean of SVL and weight of these frogs were 4.3 $\pm$ 0.97 and 10.7 $\pm$ 4.5 respectively, but neither presented correlation with helminth abundance (rs= 0.1 and p= 0.5 for SVL; rs= 0.1; p= 0.4 for weight).

From the 12 specimens of *Scinax nasicus*, there was only a cystacanth of Centrorthynchidae associated with mesentery of a frog (P= 8.3%; MA= 0.08 $\pm$ 0.08; MII= 1.0). The SLV and weight had mean of 3.5 $\pm$ 0.3 and 2.9 $\pm$ 0.3, respectively. However, the minimal prevalence and abundance of parasites hindered correlative analyses with these variables.

Only five specimens of *Scinax ruber* were collected, and they presented three helminth *taxa* in component community (Table 9), a mean richness of 1.3 $\pm$ 0.3, and diversity of 0.54. Correlation analyze with helminth abundance were not supported due to low sampling of anurans, but the mean of SVL was 3.72 $\pm$ 0.6 and the weight was 4.0 $\pm$ 2.0.

Although the low sampling of *Scinax* cf. *similis* (n= 2), there were five parasite *taxa* (Table 10), and these findings were the first parasite report for this anuran species.

Both anurans presented parasites including the oligochaete *Dero (Allodero) lutzi*, and the mean richness was 2.5 ± 1.5 while diversity was 0.56. These hylids had a mean of SVL and weight of 2.8 ± 0.4 and 1.1 ± 0.2 , respectively. However, low sampling of hosts hindered correlations with parasite abundance.

In the sampling of *Scinax fuscomarginatus*, only one individual presented a cosmocercid in the small intestine and two cystacanth of Centrorthynchidae in mesentery, resulting in a diversity of 0.37. Whereas for *Scinax* sp. (n= 3) no parasite was found associated with them.

The component community of *Scinax fuscovarius* (n= 51) had nine parasite taxa including the oligochaete *Dero (Allodero) lutzi* (Table 11), a mean richness of 1.3 ± 0.1 , and diversity of 0.29. Overall prevalence was 60.8% and the most abundant species was *Lophosicyadiplostomum* sp., while *Rhabdias* sp.2 was the most prevalent parasite (35.3%). Some studies of *S. fuscovarius* reported parasitism with *Cosmocerca* spp. and *Aplectana* spp. (Silva, 1954; Masi-Pallares and Maciel, 1974; Baker and Vaucher, 1984; Vicente et al., 1991; Bursey et al., 2001). However, all parasite species found in the present study were new records for this hylid with exception of *O. caudacutus*. Correlation with SVL (4.0 ± 0.8) and parasite abundance was significant ($r_s = 0.29$; $p = 0.03$), likewise parasites were correlated with weight (4.7 ± 1.9) significantly ($r_s = 0.33$; $p = 0.02$).

The 16 specimens of *Trachycephalus typhonius* presented eight helminth taxa, a mean richness of 2.2 ± 0.4 (Table 12), and 0.69 of diversity. In addition, the overall prevalence was 68.7% while the monogenean *Polystoma* cf. *lopezromani* was the most prevalent (56%) and *Aplectana membranosa* was the most abundant (Table 12). From the total, only *P.cf. lopezromani* was previously reported in this anuran, whereas the others were new records for *T. typhonius*. These hylids had mean SVL of 6.6 ± 1.43 and mean weight of 19.2 ± 11.3 . No correlation was observed between abundance and weight ($r_s = 0.48$; $p = 0.06$), but SVL presented positive correlation with helminth abundance ($r_s = 0.5$; $p = 0.02$).

Of the 19 specimens of *Dermatonotus muelleri* only three were parasitized (P= 15.8%), and the single helminth associated with them was the nematode *Aplectana hylambatis* (n= 678) which presented high intensity of infection (MII= 226.0 ± 165.7 ; MA= 35.7 ± 29.3) in the large intestine. This nematode has already been found in *D. muelleri* from Paraguay, but recently it was recorded in Brazilian anurans (Aguiar et al.,

2015a). The low number of hosts (only three) hindered correlation analyzes of parasite abundance with weight and SVL of anurans.

The 40 specimens of *Elachistocleis bicolor* presented a component community with four helminth *taxa* (Table 13), a mean richness of 1.2 ± 0.2 , and 0.64 of diversity index. Overall prevalence was 30% and cosmocercid nematodes were the most prevalent (27.5%) and abundant helminth. All these helminths were new records because there were only two digenetic species reported for *E. bicolor* from Argentina - *Styphlodora* sp. and *Travtrema* aff. *stenocotyle* (Campião et al., 2014). The mean of SVL was 2.3 ± 0.4 and the weight was 1.4 ± 0.6 , but there were no correlation with these variables with parasite abundance ($r_s = 0.04$ and $p = 0.8$; $r_s = 0.1$ and $p = 0.5$ respectively).

The 23 specimens of *Physalaemus albonotatus* presented three helminth *taxa* (Table 14), a mean richness of 1.2 ± 0.25 and diversity of 0.80. Overall prevalence was 17.4% and cosmocercid nematodes were the most prevalent helminth associated with 13% of frogs. Whereas metacercariae of *Lophosicyadiplostomum* sp. and cystacanths of Centrorhynchidae were new records for *P. albonotatus*. The mean of SVL was 1.9 ± 0.2 and the weight was 0.7 ± 0.2 , but there were no correlation with these variables with parasite abundance ($r_s = -0.25$ and $p = 0.2$; $r_s = -0.14$ and $p = 0.5$, respectively).

The 35 specimens of *Physalaemus centralis* presented a component community with seven helminth *taxa* (Table 15), mean richness of 1.35 ± 0.11 , and diversity index of 0.95. Overall prevalence was 57% and the most abundant and prevalent species was the digenetic *Bursotrema* sp. (metacercariae) with 28.6%. These findings represent the first study of helminth fauna of *P. centralis*. These frogs had 2.49 ± 0.4 of SVL and 1.6 ± 0.9 of weight, but none has correlated with parasite abundance ($r_s = -0.04$ and $p = 0.8$; $r_s = -0.12$ and $p = 0.4$, respectively).

The component community associated with *Physalaemus cuvieri* ($n = 32$) presented three helminth *taxa* (Table 16), a mean richness of 1.0 ± 0.0 , and 0.40 of diversity. Overall prevalence was 43.7%, and cosmocercid nematodes were the most prevalent (34%), while metacercariae of *Bursotrema* sp. were the most abundant helminth (Table 16). The present study is the fifth about *P. cuvieri*, and in contrast to others (e.g. Vaucher, 1990; Santos and Amato, 2012; Aguiar et al., 2015b), we did not verify the monogenean *Polystoma cuvieri* such as these studies. However, metacercariae of *Bursotrema* sp. is the first report for this frog. Mean of SVL and weight of frogs

were, respectively, 2.37 ± 0.36 and 1.4 ± 0.8 . However, no correlation was observed between the abundance of parasites and these variables ($p= 0.34$ and $r_s= -0.17$).

From the total of six individuals of *Physalaemus marmoratus*, only one was parasitized with a single encysted larva of *Brevimulticaecum* sp. in the stomach wall. Due to low prevalence and intensity of infection, no correlation analyze was realized with abundance of parasite and size of host. Besides the present study, the unique record until now about helminths associated with *P. marmoratus* was Walton (1935), which reported *Filaria bufonis* in the body cavity.

From the six individuals of *Physalaemus nattereri* there were five infected with encysted larvae of *Brevimulticaecum* sp. on the digestive tract, resulting in 83% of prevalence and 2.0 ± 0.5 , and 1.7 ± 0.5 of MII and MA, respectively. Due to low sampling, no correlation analyze was realized with abundance of parasite and size of host. In addition to this finding, *Filaria bufonis* (Walton, 1935) and cosmocercid nematodes (Vicente et al., 1991) have never been reported in *P. nattereri* (Campião et al., 2014).

The component community of *Pseudopaludicola mystacalis* ($n= 59$) presented six helminth taxa (Table 17), mean richness of 1.3 ± 0.2 , and diversity of 0.87. Overall prevalence was 10% and they had a low intensity of infection with exception of unidentified metacercariae, which were recovered 45 specimens. These findings are the first for *P. mystacalis*, while for its congeners (*Pseudopaludicola falcipes* and *Pseudopaludicola boliviana*) there were reports of helminths (Duré et al., 2004; González and Hamann 2004, 2009 and 2012), but no one was from Brazil. Mean of SVL and weight of frogs were, respectively, 1.5 ± 0.2 and 0.5 ± 0.3 . However, no correlation was observed between abundance of parasites and these variables ($r_s= 0.08$; $p= 0.53$ for SVL; $r_s= 0.06$; $p= 0.6$ for weight).

The component community of *Leptodactylus chaquensis* ($n= 143$) presented 13 helminth taxa (Table 18), a mean richness of 1.75 ± 0.1 , and diversity index of 1.30. Overall prevalence was 58%, and cosmocercid nematodes was the most prevalent (23%) while *A. membranosa* was the most abundant helminth (Table 18). Although several studies have reported helminths of *L. chaquensis*, we presented new records for this frog such as *Falcaustra mascula*, *Oxyascaris* sp., *Brevimulticaecum* sp., and *Lophosicyadiplostomum* sp. (Table 18). The SVL mean of individuals was 4.6 ± 1.7 , and 11.3 ± 13.6 for mean weight. Abundance of parasites was significantly correlated with these variables: SVL ($r_s= 0.52$; $p= 0.00$) and weight ($r_s= 0.54$; $p= 0.00$).

Fifty specimens of *Leptodactylus fuscus* were collected, and 66% were parasitized. The component community presented nine helminth taxa (Table 19), a mean richness of 1.16 ± 0.06 , and diversity of 0.64. From the 5,119 recovered helminths 3,696 were specimens of *Schrankiana formosula* and 1,373 were *A. membranosa*, presenting high intensity of infection in the intestines. Although there are many studies with this leptodactylid, only *S. formosula* has already been reported, other helminths from the present study were new records for *L. fuscus*. These frogs presented mean of SVL and weight of 3.9 ± 0.76 and 5.9 ± 3.23 , respectively. Both variables correlated with parasite abundance: $r_s = 0.68$ and $p < 0.001$ for SVL, and $r_s = 0.76$ and $p < 0.001$ for weight.

Twenty specimens of *Leptodactylus latrans* were collected, and 90% were parasitized. The component community presented nine helminth taxa (Table 20), a mean richness of 1.9 ± 0.2 , and diversity of 0.48. The most prevalent and abundant was *A. membranosa* associated with 45% of frogs presenting 1,429 individuals (Table 20). There are many studies about helminths of *L. latrans*, but no one has even reported the occurrence of *Lophosicyadiplostomum* sp. in kidneys of these frogs (see Campião et al., 2014). These frogs presented mean of SVL and weight of 6.9 ± 1.7 and 35.2 ± 25.0 , respectively. Neither was correlated with parasite abundance: $r_s = 0.42$ and $p = 0.06$ for SVL, and $r_s = 0.29$ and $p = 0.21$ for weight.

Eight specimens of *Leptodactylus mystacinus* were collected, and 100% were parasitized. The component community presented four helminth taxa (Table 21), a mean richness of 1.7 ± 0.2 , and 0.28 of diversity index. *Aplectana membranosa* was the most prevalent (100%) and abundant (1,271 individuals) helminth in this leptodactylid (Table 21). There are some helminthological studies of *L. mystacinus* (see Campião et al., 2014), but all helminths found in the present study for this frog population represent new records. The SVL mean of these frogs was 5.7 ± 0.3 , and weight was 16.2 ± 4.4 . Parasite abundance correlated positively with weight ($r_s = 0.8$; $p = 0.005$) but not with SVL ($r_s = 0.2$; $p = 0.5$).

From the 225 specimens of *Leptodactylus podicipinus*, 177 were parasitized with at least one helminth ($P = 78.6\%$) presenting a component community with 16 helminth taxa, mean richness of 1.7 ± 0.06 (Table 22), and diversity index 1.84. A total of 1,788 helminths were recovered and *A. membranosa* (471 individuals) was the most abundant while *C. podicipinus* was the most prevalent (37%) (Table 22). Likewise some leptodactylids, *L. podicipinus* has many helminthological studies, but it was founded

eight new records (*A. membranosa*, *Brevimulticaecum* sp., *F. mascula*, *Raillietnema* sp., *Clinostomum* sp., *Heterodiplostomum* sp., *Lophosicyadiplostomum* sp., and *Cylindrotaenia americana*) and a high richness (16 *taxa*) compared with all previous reports which numbered 14 *taxa* (see Campião et al., 2014). The SVL mean of these frogs was 3.1 ± 0.8 , and weight was 3.4 ± 2.3 . These variables correlated positively with parasite abundance resulting in the same values for SVL and weight ($r_s = 0.6$; $p < 0.001$).

From all anuran populations, 12 presented prevalence more than 60%: *S. cf. ruber* (60%), *S. fuscovarius* (60.8%), *H. aff. raniceps* (63%), *L. fuscus* (66%), *T. typhoni* (68.7%), *L. podicipinus* (78.6%), *P. azureus* (80.5%), *P. paradoxa* (81.6%), *P. nattereri* (83%), *L. latrans* (90%), *S. cf. similis* (100%), and *L. mystacinus* (100%) (Figure 4). While the highest values for MA and MII were in *P. azureus* (54.5 ± 12.7 ; 67.4 ± 15.0), *T. typhoni* (99.0 ± 80.5 ; 143.7 ± 116.1), *L. fuscus* (102.4 ± 24.2 ; 155.1 ± 33.2), *L. latrans* (80.3 ± 33.6 ; 89.3 ± 36.8), and *L. mystacinus* (169.6 ± 45.7 ; 169.6 ± 45.7) (Figure 5). *Dermatonotus muelleri* presented the highest MII but a small MA (Figure 5) only because three hosts presented high intensity of infection.

Of the 26 anuran species which were studied, we found 40 *taxa* of metazoan parasites. The component community of *L. podicipinus* presented a high richness with 16 helminth *taxa*, followed by *L. chaquensis* (13), *H. raniceps* (10), *P. azureus*, *L. latrans*, *L. fuscus*, and *S. fuscovarius* with nine helminth *taxa*, and *T. typhoni*, *H. aff. raniceps* with eight helminth *taxa* (Figure 6). As the similar way, the helminths of *L. podicipinus* presented a high diversity (1.84), followed by *P. paradoxa* (1.38), *L. chaquensis* (1.30), and *R. schneideri* (1.19) (Figure 6). On the other hand, *S. cf. similis* presented the highest mean richness (2.5), followed by *T. typhoni* (2.2), *P. azureus* (2.1), *L. latrans* (1.9), and *L. podicipinus*, *L. mystacinus*, *L. chaquensis* (1.7) (Figure 6).

Analyzing the four anuran family and their habit we observed that Leptodactylidae was the more richness in relation to helminths, and hylids presented a high abundance of helminths. These differences can be related to habits of the families as observed in others studies (e.g. Bursey et al., 2001; Bolek and Coggins, 2003). Leptodactylids are frequently found in transitional habitat between pounds and dry land providing encounters with many types of helminths, while hylids and bufonids are more restricted to their arboreal and terrestrial habitat, respectively (Duellman and Trueb, 1986).

Ten anuran populations presented positive correlation between SLV and weight with parasite abundance: *R. schneideri*, *D. minutus*, *H. raniceps*, *P. azureus*, *S. fuscovarius*, *T. typhonius*, *L. chaquensis*, *L. fuscus*, *L. mystacinus*, and *L. podicipinus*. Correlations with frog's body size and parasite abundance have been related in several studies (Hamann and Kehr, 1998; Bolek and Coggins, 2003; Hamann et al., 2006; Santos et al., 2013; Toledo et al., 2015). The majority of these authors lead us that the proposition of the larger surface area of the host may also increase opportunities for infection in addition to providing more space, nutritional substances, and a greater number of niches for parasites (Poulin and Morand 2004; Hamann et al. 2010; Santos et al., 2013). However, it is still unclear for some species that do not present a significant correlation as we observed here mainly in leptodactylids from subfamily Leptodactylinae (*Physalaemus* spp. and *P. mystacalis*) and *L. latrans*. Members of Leptodactylinae are very small in relation to the others leptodactylids of this study and they did not present correlations corroborating with previous studies which small hosts have parasite abundance lesser than the large ones. On the other hand, in our study, large size of *L. latrans* did not correlate with parasite abundance, even in the findings of Toledo et al. (2015) about *L. latrans* the correlation was weak as the authors have concluded. Since parasite abundance was not correlated neither the small nor the large size of anurans, we believe that significant correlation can be better understood in medium-sized species of frogs.

In this present study was also revealed the importance of anurans in the life cycle of some helminth species, such as *Brevimulticaecum* sp., *Physaloptera* sp., *Spiroxys* sp., Cystacanth, *Lophosicyadiplostomum* sp., *Bursotrema* sp., and *Heterodiplostomum* sp., which were larvae or cysts exploring different anuran species as intermediate or paratenic hosts. Adults of these parasites are commonly associated with other vertebrates where they reproduce and complete their life cycle. It is unclear the cycle of *Brevimulticaecum* sp. that was found encapsulated in tissues of the digestive tract of anurans, but according to Anderson (2000), this nematode has to reach an alligator as definitive host, leading us to consider these anurans as prey of this reptile. Larvae of *Physaloptera* sp. infect commonly anurans that ingest infective invertebrates, while vertebrates as birds, reptiles, cats, and dogs can act as definitive hosts (Anderson, 2000). In experimental studies was observed no development for long periods of this larva in *Rana pipiens* concluding the paratenic role of anurans (Anderson, 2000). *Spiroxys* sp. is less frequent, but this nematode also uses anurans as paratenic hosts. Its definitive hosts

can be turtles and other amphibians, while invertebrates as copepods act as first host (Anderson, 2000). Cystacanths have been reported by several studies of helminth fauna in amphibians showing the importance of this relationship (*e.g.* Santos and Amato, 2010; Toledo et al., 2013), and sometimes this helminth can be found in high prevalence, as presented by Aguiar et al. (2014). For acanthocephalans, anurans can be paratenic or intermediate host by ingestion of invertebrates such as isopods (Amato et al., 2003). *Lophosicyadiplostomum* sp. (metacercariae) were found infecting kidneys of almost all anuran species. However, the high prevalence was in *D. minutus* (51.35%) and in *P. azurea* (63.82%) (Table 4 and 7) suggest a preference or specific relation to arboreal anurans. The cycle involves a passeriform bird as definitive host but specific information is unknown (Prudhoe and Bray, 1982; Hamman and González, 2009). The other from Diplostomidae was *Bursotrema* sp. that also occurred in kidneys, but this metacercaria has opossum such as *Didelphis* spp. as definitive host (Dubois, 1976; Prudhoe and Bray, 1982; Hamman and González, 2009). These findings in *P. centralis* and *P. cuvieri* (in the present study), and others leptodactylids previously reported (*e.g.* Hamman et al., 2006; Hamman and González, 2009) made us to believe on a specificity with these anurans. *Heterodiplostomum* sp. is the unique from Proterodiplostomidae reported for anurans from South America (Hamman et al., 2006; Campião et al., 2014), and reptiles act as definitive host for a single species, *Heterodiplostomum lanceolatum* which was reported in several snakes (Travassos et al., 1969; Lunaschi and Drago, 2010). Little is known about the cycle of this digenean. The high prevalence of these immature helminths in this study suggest a great importance of anurans in their biological cycle.

The present list of helminth species provides new records of anurans from South America according to Campião et al. (2014). In addition to the new records of this study, we were able to detect the dominant and generalist helminth species, the host-parasite specificity in some cases (*e.g.* *T. thyphoni* - *P. cf. lopezromani* and *P. paradoxa* - *N. neivai*), the levels of aggregation of helminth populations, the different values of richness, and diversity among 26 host populations. These results expand the knowledge about parasite diversity and community structure of helminths. The findings show a high richness of helminth of anurans from Neotropical region contrasting with some assessment from other regions where the helminth fauna is depauperate (Aho, 1990; Barton, 1999). Host-parasite checklists are essential resources that provide some expectations of the parasites one may find in a particular host species during a

parasitological investigation (Poulin et al., 2016). In Neotropical region, especially from Brazil, studies on helminths of anurans are important due to the high diversity of this vertebrate in this region and their complex life cycle.

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Table 1 – Anuran species and number of parasitized and no parasitized individuals from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil.

Family	Anuran species	Parasitized	No parasitized	Total
Bufonidae	<i>Rhinella schneideri</i>	12	9	21
Hylidae	<i>Dendropsophus minutus</i>	20	17	37
	<i>Pithecopus azureus</i>	38	9	47
	<i>Pseudis paradoxa</i>	31	7	38
	<i>Scinax</i> sp.	0	3	3
	<i>Scinax fuscovarius</i>	31	20	51
	<i>Scinax fuscomarginatus</i>	1	0	1
	<i>Scinaxnasicus</i>	1	11	12
	<i>Scinaxruber</i>	3	2	5
	<i>Scinax</i> cf. <i>similis</i>	2	0	2
	<i>Trachycephalus typhonius</i>	11	5	16
	<i>Hypsiboas raniceps</i>	25	19	44
	<i>Hypsiboas</i> aff. <i>raniceps</i>	22	13	35
Leptodactylidae	<i>Leptodactylus fuscus</i>	33	17	50
	<i>Leptodactylus mystacinus</i>	8	0	8
	<i>Leptodactylus chaquensis</i>	83	60	143
	<i>Leptodactylus latrans</i>	18	2	20
	<i>Leptodactylus podicipinus</i>	177	48	225
	<i>Pseudopaludicola mystacalis</i>	6	53	59
	<i>Physalaemus albonotatus</i>	4	19	23
	<i>Physalaemus centralis</i>	20	15	35
	<i>Physalaemus cuvieri</i>	14	18	32
	<i>Physalaemus marmoratus</i>	1	5	6
	<i>Physalaemus nattereri</i>	5	1	6
Microhylidae	<i>Dermatonotus muelleri</i>	3	16	19
	<i>Elachistocleisbicolor</i>	12	28	40
TOTAL	-	581	397	978

Table 2- Metazoan parasites associated with anurans from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil.

Group	Parasite	Abundance	Prevalence (%)	Dispersion indices
Nematoda	<i>Aplectana hylambatis</i>	678	0.3	676.0
	<i>Aplectana membranosa</i>	7,039	7.3	61.3
	<i>Brevimulticaecum</i> sp.	248	6.0	54.8
	<i>Cosmocerca</i> cf. <i>chilensis</i>	3	0.2	326.0
	<i>Cosmocerca parva</i>	10	0.3	282.5
	<i>Cosmocerca podicipinus</i>	439	9.0	13.1
	<i>Cosmocercidae</i> gen. sp.	870	17.0	22.8
	<i>Falcaustra mascula</i>	29	0.8	320.4
	<i>Ochoterenella digiticauda</i>	4	0.1	978.0
	<i>Ochoterenella</i> sp.	11	0.5	177.8
	<i>Oswaldocruzia mazzai</i>	116	0.1	978.0
	<i>Oxyascaris caudacutus</i>	5	0.2	586.0
	<i>Oxyascaris</i> sp.	9	0.4	190.2
	<i>Parapharyngodon</i> cf. <i>alvarengai</i>	135	0.7	483.7
	<i>Physaloptera</i> sp.	43	1.1	157.0
	<i>Raillietnema minor</i>	560	2.7	79.4
	<i>Raillietnema</i> sp.	1	0.1	0.00
	<i>Rhabdias</i> sp.1	83	0.6	614.7
	<i>Rhabdias</i> sp.2	72	2.2	77.3
	<i>Rhabdias</i> sp.3	55	2.9	79.0
	<i>Schrankiana formosula</i>	3,696	1.3	115.7
	<i>Spiroxys</i> sp.	2	0.2	0.00
Acanthocephala	<i>Centrorhynchidae</i> gen. sp.	32	1.7	47.3
	Unidentified cystacanth	32	1.0	205.1
Cestoda	<i>Cylindrotaenia americana</i>	11	0.7	177.8
Digenea	<i>Brachycoelium salamandrae</i>	58	0.1	978.0
	<i>Bursotrema</i> sp.	180	1.2	304.2
	<i>Catadiscus</i> cf. <i>marinholutzi</i>	11	0.4	248.9
	<i>Catadiscus propinquus</i>	153	4.4	36.7
	<i>Catadiscus</i> sp.	246	9.6	13.5
	<i>Clinostomum</i> sp.	7	0.2	698.6

Table 2- continued

	<i>Gorgoderina diaster</i>	64	1.3	105.3
	<i>Gorgoderina</i> sp.	2	0.1	978.0
	<i>Heterodiplostomum</i> sp.	12	0.5	148.2
	<i>Lophosicyadiplostomum</i> sp.	5,927	10.4	30.2
	Unidentified metacercarie	45	0.1	978.0
	<i>Neohaematoloechus neivai</i>	24	0.5	396.8
	<i>Rauschiella</i> sp.	127	6.2	18.9
Monogenea	<i>Polystoma</i> cf. <i>lopezromani</i>	38	0.9	196.1
Oligochaeta	<i>Dero</i> (<i>Allodero</i>) <i>lutzi</i>	29	0.2	645.6
TOTAL	40 taxa	21106	59.4	-

Table 3- Component community of *Rhinella schneideri* (n=21) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	3	29	1.4 ± 0.9 (0 - 18)	9.7 ± 4.9 (1 - 18)	St, Li
<i>Falcaustra mascula</i>	2	9	0.4 ± 0.4 (0 - 8)	4.5 ± 3.5 (1 - 8)	Si, Li
<i>Ochoterenella digiticauda</i>	1	4	0.2 ± 0.2 (0 - 4)	4.0	Bc
<i>Oswaldocruzia mazzai</i>	1	116	5.5 ± 5.5 (0 - 116)	116.0	Si
<i>Rhabdias</i> sp.1	6	83	3.9 ± 3.1 (0 - 65)	13.8 ± 10.3 (1 - 65)	Lu
<i>Brevimulticaecum</i> sp.	1	3	0.1 ± 0.1 (0 - 3)	3.0	St
Digenea					
<i>Rauschiella</i> sp.	1	1	0.05 ± 0.05 (0 - 1)	1.0	Si
TOTAL	12	245	11.7 ± 6.1 (0 - 116)	20.4 ± 10.2 (1 - 116)	-

*Bc (body cavity); Lu (lung); Si (small intestine), Li (large intestine), St (stomach)

Table 4- Component community of *Dendropsophus minutus* (n=37) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MI ± SE (range)	*Site infection
Nematoda					
<i>Brevimulticaecum</i> sp.	1	1	0.03 ± 0.03 (0 - 1)	1.0	Bc
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	19	916	24.8 ± 7.2 (0 - 163)	48.2 ± 11.8 (1 - 163)	Ki
Cestoda					
<i>Cylindrotaenia americana</i>	1	1	0.03 ± 0.03 (0 - 1)	1.0	Si
Acanthocephala					
Centrorhynchidae (cystacanth)	3	6	0.2 ± 0.1 (0 - 4)	2.0 ± 1.0 (1 - 4)	St
TOTAL	20	924	25.0 ± 7.3 (0 - 167)	46.2 ± 11.6 (1 - 167)	-

*Bc (body cavity); Ki (kidney); Si (small intestine), St (stomach)

Table 5- Component community of *Hypsiboas* aff. *raniceps* (n= 35) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MI ± SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	1	1	0.03 ± 0.03 (0 - 1)	1.0	St
<i>Brevimulticaecum</i> sp. (larvae)	4	13	0.4 ± 0.2 (0 - 8)	3.2 ± 1.6 (1 - 8)	St, Si
Cosmocercidae	2	2	0.06 ± 0.04 (0 - 1)	1.0 ± 0 (1)	Si, Li
<i>Ochoterenella</i> sp.	2	4	0.1 ± 0.1 (0 - 3)	2.0 ± 1.0 (1 - 3)	Bc
Digenea					
<i>Catadiscus</i> sp.	1	3	0.08 ± 0.08 (0 - 3)	3.0 (-)	Li
<i>Gorgoderina disaster</i>	12	58	1.7 ± 0.5 (0 - 15)	4.8 ± 1.2 (1 - 15)	Ub
<i>Lophosicyadiplostomum</i> sp. (metacercarie)	8	419	11.9 ± 7.1 (0 - 237)	52.4 ± 27.7 (1 - 237)	Ki
<i>Rauchiella</i> sp.	3	4	0.1 ± 0.07 (0 - 2)	1.3 ± 0.3 (1 - 2)	Si
TOTAL	22	504	14.4 ± 7.0 (0 - 237)	22.9 ± 10.9 (1 - 237)	-

*Bc (body cavity); Ki (kidney); Si (small intestine), Li (Large intestine), St (stomach), Ub (urinary bladder)

Table 6- Component community of *Hypsiboas raniceps* (n=44) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MI ± SE (range)	*Site infection
Nematoda					
<i>Brevimulticaecum</i> sp. (larvae)	7	73	1.7 ± 0.9 (0 - 38)	10.4 ± 4.9 (1 - 38)	Bc, St, Si, Li
Cosmocercidae	2	3	0.07 ± 0.05 (0 - 2)	1.5 ± 0.5 (1 - 2)	Li
<i>Ochoterenella</i> sp.	3	7	0.16 ± 0.1 (0 - 4)	2.3 ± 0.9 (1 - 4)	Bc, Si
<i>Physaloptera</i> sp.	4	26	0.6 ± 0.4 (0 - 15)	6.5 ± 3.1 (1 - 15)	St
<i>Rhabdias</i> sp2	2	3	0.07 ± 0.05 (0 - 2)	1.5 ± 0.5 (1 - 2)	Lu
Acanthocephala					
Cystacanth	2	2	0.04 ± 0.03 (0 - 1)	1.0 ± 0 (1)	Bc
Digenea					
<i>Catadiscus</i> sp.	3	4	0.1 ± 0.05 (0 - 2)	1.3 ± 0.3 (1 - 2)	Li
<i>Gorgoderina disaster</i>	1	6	0.1 ± 0.1 (0 - 6)	6.0 (6)	Ub
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	9	729	16.6 ± 8.5 (0 - 342)	81.0 ± 35.1 (1 - 342)	Ki
<i>Rauchiella</i> sp.	1	1	0.02 ± 0.02 (0 - 1)	1.0 (1)	Si
TOTAL	25	854	19.4 ± 8.5 (0 - 342)	34.2 ± 14.3 (1 - 342)	-

*Bc (body cavity); Ki (kidney); Si (small intestine), Li (Large intestine), St (stomach), Ub (urinary bladder), Lu (lungs)

Table 7- Component community of *Pithecopus azureus* (n=47) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA $\pm$ SE (range)	MII $\pm$ SE (range)	*Site infection
Nematoda					
Cosmocercidae	4	127	2.7 $\pm$ 2.1 (0 - 96)	31.7 $\pm$ 21.6 (3 - 96)	Bc, Si, Li
<i>Cosmocerca parva</i>	3	10	0.2 $\pm$ 0.1 (0 - 4)	3.3 $\pm$ 1.0 (2 - 4)	Li
<i>Raillietnema minor</i>	26	560	11.9 $\pm$ 3.0 (0 - 103)	21.5 $\pm$ 4.6 (2 - 103)	Li
<i>Physaloptera</i> sp. (larvae)	1	3	0.1 $\pm$ 0.1 (0 - 3)	3.0	St
<i>Brevimulticaecum</i> sp.	1	4	0.1 $\pm$ 0.1 (0 - 4)	4.0	Lv
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	30	1804	38.4 $\pm$ 11.1 (0 - 365)	60.1 $\pm$ 16.2 (1 - 365)	Ki
<i>Catadiscus</i> cf. <i>marinholutzi</i>	1	5	0.1 $\pm$ 0.1 (0 - 5)	5,0	Li
<i>Catadiscus propinquus</i>	3	11	0.2 $\pm$ 0.1 (0 - 6)	3.7 $\pm$ 1.2 (2 - 6)	Li
<i>Catadiscus</i> sp.	10	37	0.8 $\pm$ 0.3 (0 - 8)	3.7 $\pm$ 0.8 (1 - 8)	Li
TOTAL	38	2561	54.5 $\pm$ 12.7 (0 - 365)	67.4 $\pm$ 15.0 (2 - 365)	-

*Bc (body cavity); Ki (Kidney); Si (small intestine), Li (large intestine), St (stomach), Lv (Liver).

Table 8- Component community of *Pseudis paradoxa* (n=38) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
<i>Cosmocerca podicipinus</i>	1	1	0.02 ± 0.02 (0 - 1)	1.0	Si
<i>Brevimulticaecum</i> sp. (larvae)	2	2	0.05 ± 0.04 (0 - 1)	1.0 ± 0.0	He
Digenea					
<i>Catadiscus propinquus</i>	3	30	0.8 ± 0.5 (0 - 14)	10.0 ± 2.6 (5 - 14)	Si, Li
<i>Catadiscus</i> sp.	9	52	1.4 ± 0.5 (0 - 11)	5.8 ± 1.2 (1 - 11)	Li
<i>Rauschiella</i> sp.	26	60	1.6 ± 0.2 (0 - 6)	2.3 ± 0.2 (1 - 6)	Si, Li, Lu
<i>Neohaematoloechus neivai</i>	5	24	0.6 ± 0.4 (0 - 14)	4.8 ± 2.6 (1 - 14)	Lu
<i>Heterodiplostomum</i> sp. (metacercariae)	1	2	0.05 ± 0.05 (0 - 2)	2.0	Bc
TOTAL	31	171	4.5 ± 0.9 (0 - 21)	5.5 ± 1.0 (1 - 21)	-

*Bc (body cavity); He (heart); Si (small intestine), Li (large intestine), Lu (lungs).

Table 9- Component community of *Scinax ruber* (n= 5) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	1	2	0.4 ± 0.4 (0 - 2)	2.0	Li
<i>Rhabdias</i> sp2	1	2	0.4 ± 0.4 (0 - 2)	2.0	Lu
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	2	14	2.8 ± 2.1 (0 - 11)	7.0 ± 4.0 (3 - 11)	Ki
TOTAL	3	18	3.6 ± 2.0 (0 - 11)	6.0 ± 2.5 (3 - 11)	-

* Ki (Kidneys); Li (large intestine), Lu (lungs).

Table 10- Component community of *Scinax* cf. *similis* (n= 2) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA $\pm$ SE (range)	MI $\pm$ SE (range)	*Site infection
Nematoda					
<i>Parapharyngodon</i> cf. <i>alvarengai</i>	1	2	1.0 $\pm$ 1.0 (0 - 2)	2.0	Li
<i>Physaloptera</i> sp. (larvae)	1	1	0.5 $\pm$ 0.5 (0 - 1)	1.0	St
<i>Spiroxys</i> sp. (larvae)	1	1	0.5 $\pm$ 0.5 (0 - 1)	1.0	St
Acanthocephala					
Centrorhynchidae	1	1	0.5 $\pm$ 0.5 (0 - 1)	1.0	St
Oligochaeta					
<i>Dero</i> (<i>Allodero</i>) <i>lutzi</i>	1	23	11.5 $\pm$ 11.5 (0 - 23)	23.0	Ur
TOTAL	2	28	14.0 $\pm$ 12.0 (1 - 28)	14.0 $\pm$ 12.0 (1 - 28)	-

*Li (large intestine), St (stomach), Ur (ureter)

Table 11- Component community of *Scinax fuscovarius* (n= 51) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA $\pm$ SE (range)	MII $\pm$ SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	1	3	0.06 $\pm$ 0.06 (0 - 3)	3.0	Li
<i>Cosmocerca</i> cf. <i>chilensis</i>	2	3	0.06 $\pm$ 0.04 (0 - 2)	1.5 $\pm$ 0.5 (1 - 2)	Si
Cosmocercidae	2	4	0.08 $\pm$ 0.06 (0 - 3)	2.0 $\pm$ 1.0 (1 - 3)	Li
<i>Oxyascaris caudacutus</i>	2	5	0.1 $\pm$ 0.1 (0 - 4)	2.5 $\pm$ 1.5 (1 - 4)	St, Si
<i>Rhabdias</i> sp2	18	58	1.14 $\pm$ 0.3 (0 - 12)	3.2 $\pm$ 0.8 (1 - 12)	Lu
<i>Spiroxys</i> sp.	1	1	0.02 $\pm$ 0.02 (0 - 1)	1.0	Mes
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	10	1322	25.9 $\pm$ 11.1 (0 - 393)	132.2 $\pm$ 43.7 (1 - 393)	Ki
Acanthocephala					
Centrorhynchidae (cystacanth)	3	5	0.1 $\pm$ 0.06 (0 - 3)	1.7 $\pm$ 0.7 (1 - 3)	St, Mes
Oligochaeta					
<i>Dero (Allodero) lutzi</i>	1	6	0.1 $\pm$ 0.1 (0 - 6)	6.0	Ur
TOTAL	31	1407	27.6 $\pm$ 11.1 (0 - 393)	45.4 $\pm$ 17.6 (1 - 393)	-

*Mes (mesentery); Ki (kidneys); Si (small intestine), Li (large intestine), St (stomach), Lu (lungs), Ur (ureter).

Table 12- Component community of *Trachycephalus typhonius* (n= 16) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminth	NHo	NHel	MA $\pm$ SE (range)	MI $\pm$ SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	2	1303	81.4 $\pm$ 81.3 (0 - 1301)	651.5 $\pm$ 649.5 (2 - 1301)	St, Li
Cosmocercidae	1	10	0.6 $\pm$ 0.6 (0 - 10)	10.0	Si
<i>Parapharyngodon</i> cf. <i>alvarengai</i>	6	133	8.3 $\pm$ 5.8 (0 - 93)	22.2 $\pm$ 14.3 (3 - 93)	Si, Li
<i>Rhabdias</i> sp2	1	9	0.5 $\pm$ 0.5 (0 - 9)	9.0	Lu
Digenea					
<i>Catadiscus marinhoi</i>	2	5	0.3 $\pm$ 0.2 (0 - 3)	2.5 $\pm$ 0.5 (2 - 3)	Li
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	2	81	5.0 $\pm$ 4.0 (0 - 63)	40.5 $\pm$ 22.5 (18 - 63)	Ki
Monogenea					
<i>Polystoma</i> cf. <i>lopezromani</i>	9	38	2.4 $\pm$ 1.0 (0 - 15)	4.2 $\pm$ 1.5 (1 - 15)	Ub
Acanthocephala					
Centrorhynchidae (cystacanth)	1	2	0.1 $\pm$ 0.1 (0 - 2)	2.0	St
TOTAL	11	1581	99.0 $\pm$ 80.5 (0 - 1302)	143.7 $\pm$ 116.1 (3 - 1302)	-

*Ub (urinary bladder); ki (kidneys); Si (small intestine), Li (large intestine), St (stomach), Lu (lungs).

Table 13- Component community of *Elachistocleis bicolor* (n= 40) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	11	57	1.4 ± 0.4 (0 - 12)	5.2 ± 1.0 (1 - 12)	Si, Li
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	2	9	0.2 ± 0.1 (0 - 5)	4.5 ± 0.5 (4 - 5)	Ki
<i>Rauschiella</i> sp.	1	3	0.1 ± 0.1 (0 - 3)	3.0	St
Acanthocephala					
Centrorhynchidae	1	3	0.07 ± 0.07 (0 - 3)	3.0	St, Bc
TOTAL	12	72	1.8 ± 0.5 (0 - 15)	6.0 ± 1.2 (0 - 15)	-

*Bc (body cavity); Ki (Kidneys); Si (small intestine), Li (large intestine), St (stomach).

Table 14- Component community of *Physalaemus albonotatus* (n= 23) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	3	4	0,17 ± 0,10 (0 - 2)	1,3 ± 0,3 (1 - 2)	Li
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	1	2	0,09 ± 0,09 (0 - 2)	2,0	Ki
Acanthocephala					
Centrorhynchidae (cystacanth)	1	4	0,17 ± 0,17 (0 - 4)	4,0	St
TOTAL	4	10	0,43 ± 0,24 (0 - 5)	2,50 ± 0,87 (1 - 5)	-

*Ki (kidneys), Li (large intestine), St (stomach).

Table 15- Component community of *Physalaemus centralis* (n=35) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	5	6	0.2 ± 0.1 (0 - 2)	1.2 ± 0.2 (1 - 2)	St, Si, Li
<i>Aplectana membranosa</i>	2	8	0.2 ± 0.2 (0 - 6)	4.0 ± 2.0 (2 - 6)	Si, Li
<i>Rhabdias</i> sp3	2	2	0.06 ± 0.04 (0 - 1)	1.0 ± 0.0	Lu
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	2	9	0.2 ± 0.2 (0 - 5)	4.5 ± 0.5 (4 - 5)	Ki
<i>Bursotrema</i> sp.	10	89	2.5 ± 1.4 (0 - 46)	8.9 ± 4.3 (1 - 46)	Ki
<i>Catadiscus</i> sp.	1	1	0.03 ± 0.03 (0 - 1)	1.0	Li
Acanthocephala					
Centrorhynchidae	5	8	0.2 ± 0.1 (0 - 3)	1.6 ± 0.4 (1 - 3)	St, Bc, Si
TOTAL	20	123	3.5 ± 1.3 (0 - 46)	6.1 ± 2.2 (1 - 46)	-

*Bc (body cavity); Ki (kidneys); Si (small intestine), Li (large intestine), St (stomach), Lu (lungs).

Table 16- Component community of *Physalaemus cuvieri* (n= 32) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	11	13	0.4 ± 0.1 (0 - 2)	1.2 ± 0.1 (1 - 2)	Li
<i>Rhabdias</i> sp3	1	1	0.03 ± 0.03 (0 - 1)	1.0	Lu
Digenea					
<i>Bursotrema</i> sp.	2	91	2.8 ± 2.7 (0 - 88)	45.5 ± 42.5 (3 - 88)	Ki
TOTAL	14	105	3.3 ± 2.7 (0 - 88)	7.5 ± 6.2 (1 - 88)	-

*Ki (kidneys); Li (large intestine), Lu (lungs).

Table 17- Component community of *Pseudopaludicola mystacalis* (n= 59) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MI ± SE (range)	*Site infection
Nematoda					
Cosmocercidae	1	1	0.02 ± 0.02 (0 - 1)	1.0	Si
Digenea					
<i>Catadiscuspropinquus</i>	3	3	0.05 ± 0.03 (0 - 1)	1.0 ± 0.0	Li
<i>Catadiscussp.</i>	1	1	0.02 ± 0.02 (0 - 1)	1.0	Li
<i>Lophosicyadiplostomumsp.</i> (metacercarie)	1	8	0.1 ± 0.1 (0 - 8)	8.0	Ki
metacercarie not identified	1	45	0.7 ± 0.7 (0 - 45)	45.0	Mes
Cestoda					
<i>Cylindrotaenia americana</i>	1	5	0.1 ± 0.1 (0 - 5)	5.0	Si
TOTAL	6	63	1.07 ± 0.8 (0 - 45)	10.5 ± 7.2 (1 - 45)	-

*Ki (kidneys); Si (small intestine), Li (large intestine), Mes (mesentery).

Table 18- Component community of *Leptodactylus chaquensis* (n= 143) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA $\pm$ SE (range)	MI $\pm$ SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	21	1180	8.2 $\pm$ 3.1 (0 - 311)	56.2 $\pm$ 18.5 (1 - 311)	Si, Li
<i>Cosmocerca podicipinus</i>	3	13	0.1 $\pm$ 0.05 (0 - 6)	4.3 $\pm$ 0.9 (3 - 6)	Si, Li
Cosmocercidae	33	239	1.6 $\pm$ 0.5 (0 - 47)	7.2 $\pm$ 1.8 (1 - 47)	St, Si, Li
<i>Falcaustra mascula</i>	3	17	0.12 $\pm$ 0.1 (0 - 15)	5.7 $\pm$ 4.7 (1 - 15)	Li
<i>Oxyascaris</i> sp.	3	6	0.04 $\pm$ 0.02 (0 - 3)	2.0 $\pm$ 0.6 (1 - 3)	Si, Li
<i>Brevimulticaecum</i> sp. (larvae)	23	93	0.6 $\pm$ 0.2 (0 - 31)	4.0 $\pm$ 1.3 (1 - 31)	St, Si, Li
<i>Rhabdias</i> sp3	8	10	0.07 $\pm$ 0.02 (0 - 2)	1.2 $\pm$ 0.1 (1 - 2)	Lu
Acanthocephala					
Centrorhynchidae (cystacanth)	3	21	0.15 $\pm$ 0.1 (0 - 13)	7.0 $\pm$ 3.4 (1 - 13)	St
Digenea					
<i>Catadiscus propinquus</i>	2	4	0.03 $\pm$ 0.02 (0 - 2)	2.0 $\pm$ 0.0 (2)	Li
<i>Catadiscus</i> sp.	20	41	0.3 $\pm$ 0.07 (0 - 6)	2.0 $\pm$ 0.3 (1 - 6)	Li
<i>Gorgoderina</i> sp.	1	2	0.01 $\pm$ 0.01 (0 - 2)	2.0 (2)	Ub
<i>Lophosicyadiplostomum</i> sp.	5	202	1.4 $\pm$ 1.3 (0 - 189)	40.0 $\pm$ 37.2 (1 - 189)	Ki
<i>Rauschiella</i> sp.	20	48	0.3 $\pm$ 0.1 (0 - 12)	2.4 $\pm$ 0.5 (1 - 12)	Si
TOTAL	83	1876	13.1 $\pm$ 3.5 (0 - 313)	22.6 $\pm$ 5.8 (1 - 313)	-

*Ki (kidney); Si (small intestine), Li (Large intestine), Lu (lungs), St (stomach), Ub (urinary bladder)

Table 19- Component community of *Leptodactylus fuscus* (n= 50) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MI ± SE (range)	*Site infection
Nematoda					
<i>Schrankiana formosula</i>	13	3696	73.9 ± 23.4 (0 - 794)	284.3 ± 60.4 (51 - 794)	Li
<i>Aplectana membranosa</i>	11	1373	27,5 ± 11.0 (0 - 426)	124.8 ± 38.3 (9 - 426)	Si, Li
Cosmocercidae	4	12	0.2 ± 0.1 (0 - 7)	3.0 ± 1.4 (1 - 7)	Li
<i>Physaloptera</i> sp. (larvae)	1	3	0.06 ± 0.06 (0 - 3)	3.0	St
<i>Brevimulticaecum</i> sp. (encysted larvae)	1	1	0.02 ± 0.02 (0 - 1)	1.0	Si
Digenea					
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	1	26	0.5 ± 0.5 (0 - 26)	26.0	Ki
<i>Rauschiella</i> sp.	3	3	0.06 ± 0.03 (0 - 3)	1.0 ± 0.0	Si
<i>Catadiscus marinhoi</i>	1	1	0.02 ± 0.02 (0 - 1)	1.0	Si
<i>Catadiscus propinquus</i>	3	4	0.08 ± 0.05 (0 - 2)	1.3 ± 0.3 (1 - 2)	Li
TOTAL	33	5119	102.4 ± 24.2 (0 - 794)	155.1 ± 33.2 (1 - 794)	-

*Ki (kidneys); Si (intestine), Li (large intestine), St (stomach).

Table 20- Component community of *Leptodactylus latrans* (n= 20) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA $\pm$ SE (range)	MII $\pm$ SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	9	1429	71.4 $\pm$ 34.0 (0 – 514)	158.8 $\pm$ 65.9 (6 – 514)	Si, Li
<i>Brevimulticaecum</i> sp. (encysted larvae)	4	13	0.6 $\pm$ 0.4 (0 – 8)	3.2 $\pm$ 1.6 (1 – 8)	SI, Li, Bc
<i>Oxyascaris</i> sp.	1	3	0.1 $\pm$ 0.1 (0 - 3)	3.0	Si
Cosmocercidae	9	39	1.9 $\pm$ 0.6 (0 - 7)	4.3 $\pm$ 0.8 (1 - 7)	Si, Li
<i>Falcaustra mascula</i>	2	2	0.1 $\pm$ 0.07 (0 - 1)	1.0 $\pm$ 0.0 (1)	Si, Li
Acanthocephala					
Cystacanth (unidentified)	1	1	0.05 $\pm$ 0.05 (0 - 1)	1.0	Si
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	1	102	5.1 $\pm$ 5.1 (0 - 102)	102.0	Ki
Digenea					
<i>Catadiscus</i> sp.	5	14	0.7 $\pm$ 0.36 (0 – 6)	2.8 $\pm$ 1.0 (1 – 6)	Li
<i>Rauschiella</i> sp.	3	4	0.2 $\pm$ 0.1 (0 - 2)	1.3 $\pm$ 0.3 (1 - 2)	Si
TOTAL	18	1607	80.3 $\pm$ 33.6 (0 - 514)	89.3 $\pm$ 36.8 (1 - 514)	-

*Ki (kidneys); Si (intestine), Li (large intestine), Bc (body cavity).

Table 21- Component community of *Leptodactylus mystacinus* (n= 8) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MI ± SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	8	1271	158.9 ± 45.0 (37 - 447)	158.9 ± 45.0 (37 - 447)	Si, Li, Lu
<i>Rhabdias</i> sp3	4	24	3.0 ± 1.8 (0 - 14)	6.0 ± 3.0 (1 - 14)	Lu
<i>Physaloptera</i> sp. (larvae)	1	4	0.5 ± 0.5 (0 - 4)	4.0	St
Digenea					
<i>Brachycoelium salamandrae</i>	1	58	7.2 ± 7.2 (0 - 58)	58.0	Si
TOTAL	8	1357	169.6 ± 45.7 (37 - 448)	169.6 ± 45.7 (37 - 448)	-

*Lu (lugs); Si (small intestine), Li (large intestine), St (stomach).

Table 22- Component community of *Leptodactylus podicipinus* (n= 225) from RPPN Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil. Number of hosts (NHo), Number of helminths (NHel), Mean abundance (MA) and Mean intensity of infection (MII) with standard error (SE) and range.

Helminths	NHo	NHel	MA ± SE (range)	MII ± SE (range)	*Site infection
Nematoda					
<i>Aplectana membranosa</i>	16	471	2.1 ± 0.6 (0 - 72)	29.4 ± 5.3 (1 - 72)	Si, Li
<i>Cosmocerca podicipinus</i>	84	425	1.9 ± 0.2 (0 - 17)	5.1 ± 0.3 (1 - 17)	Si, Li, Lu
Cosmocercidae	73	321	1.4 ± 0.2 (0 - 16)	4.4 ± 0.4 (1 - 16)	Si
<i>Raillietnema</i> sp.	1	1	0.0 ± 0.0 (0 - 1)	1.0	Si
<i>Falcaustra mascula</i>	1	1	0.0 ± 0.0 (0 - 1)	1.0 (1)	Li
<i>Brevimulticaecum</i> sp. (encysted larvae)	9	34	0.1 ± 0.1 (0 - 19)	3.8 ± 2.0 (1 - 19)	St, Si, Li, Bc
<i>Physaloptera</i> sp.	3	6	0.03 ± 0.02 (0 - 3)	2.0 ± 0.6 (1 - 3)	St
<i>Rhabdias</i> sp3	13	18	0.08 ± 0.02 (0 - 2)	1.4 ± 0.1 (1 - 2)	Lu
Acanthocephala					
Cystacanth (unidentified)	4	8	0.03 ± 0.2 (0 - 3)	2.0 ± 0.4 (1 - 3)	St, Li
Cestoda					
<i>Cylindrotaenia americana</i>	5	5	0.02 ± 0.01 (0 - 1)	1.0 ± 0.0 (1)	Si
Digenea					
<i>Heterodiplostomum</i> sp.	4	10	0.04 ± 0.02 (0 - 4)	2.5 ± 0.5 (2 - 4)	Lm
<i>Lophosicyadiplostomum</i> sp. (metacercariae)	9	284	1.2 ± 1.0 (0 - 225)	31.5 ± 24.5 (1 - 225)	Ki
<i>Clinostomum</i> sp.	2	7	0.03 ± 0.03 (0 - 6)	3.5 ± 2.5 (1 - 6)	Bc
<i>Catadiscus propinquus</i>	29	101	0.4 ± 0.1 (0 - 10)	3.5 ± 0.6 (1 - 10)	Li
<i>Catadiscus</i> sp.	44	93	0.4 ± 0.1 (0 - 7)	2.1 ± 0.2 (1 - 7)	Li
<i>Rauschiella</i> sp.	3	3	0.01 ± 0.01 (0 - 1)	1.0 ± 0.0 (1)	Si
TOTAL	177	1788	7.9 ± 1.3 (0 - 240)	10.1 ± 1.6 (1 - 240)	-

*Lu (lugs); Si (small intestine), Li (large intestine), St (stomach), Ki (kidneys), Bc (body cavity), Lm (leg's musculature).



Figure 1 - Location of mouth of Aguapeí river and surrounding area, Northwest of São Paulo State, Brazil.

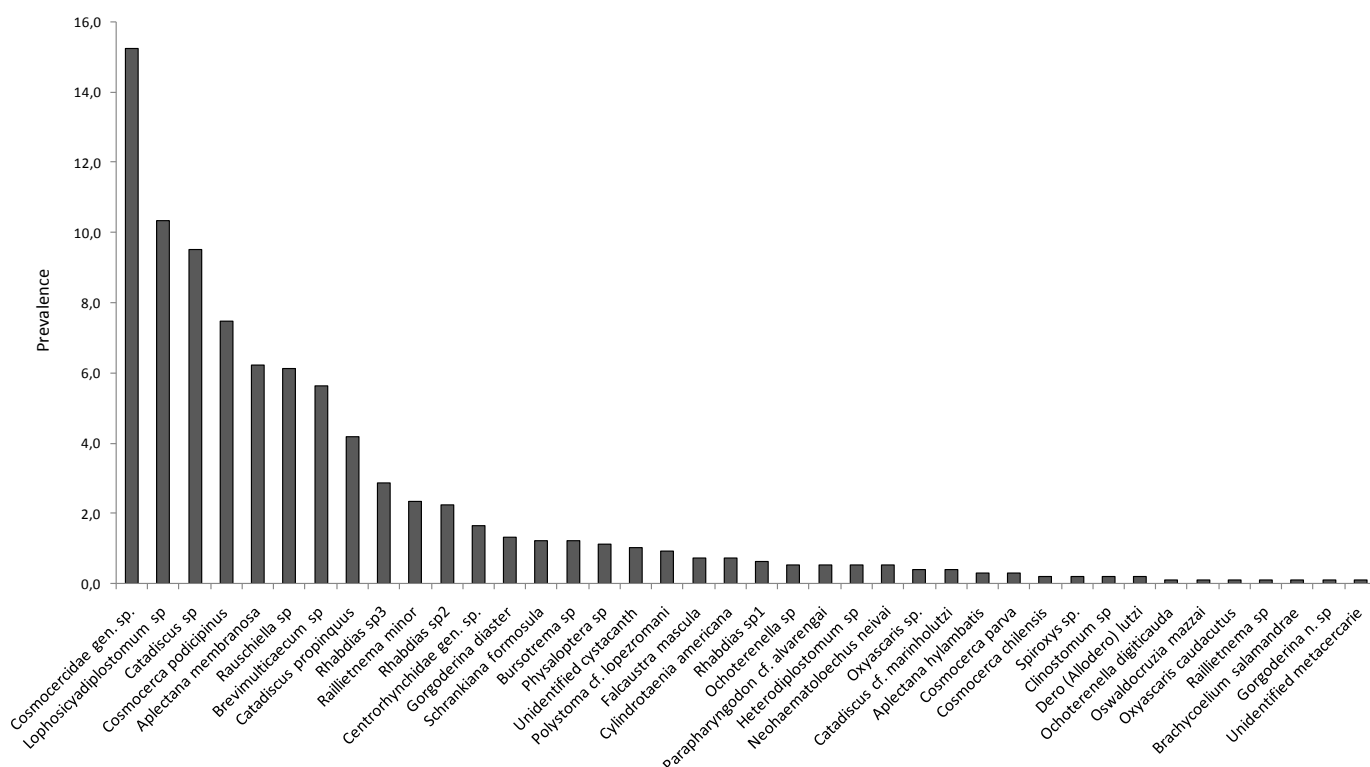


Figure 2 - Prevalence of 40 metazoan parasite species in anuran community

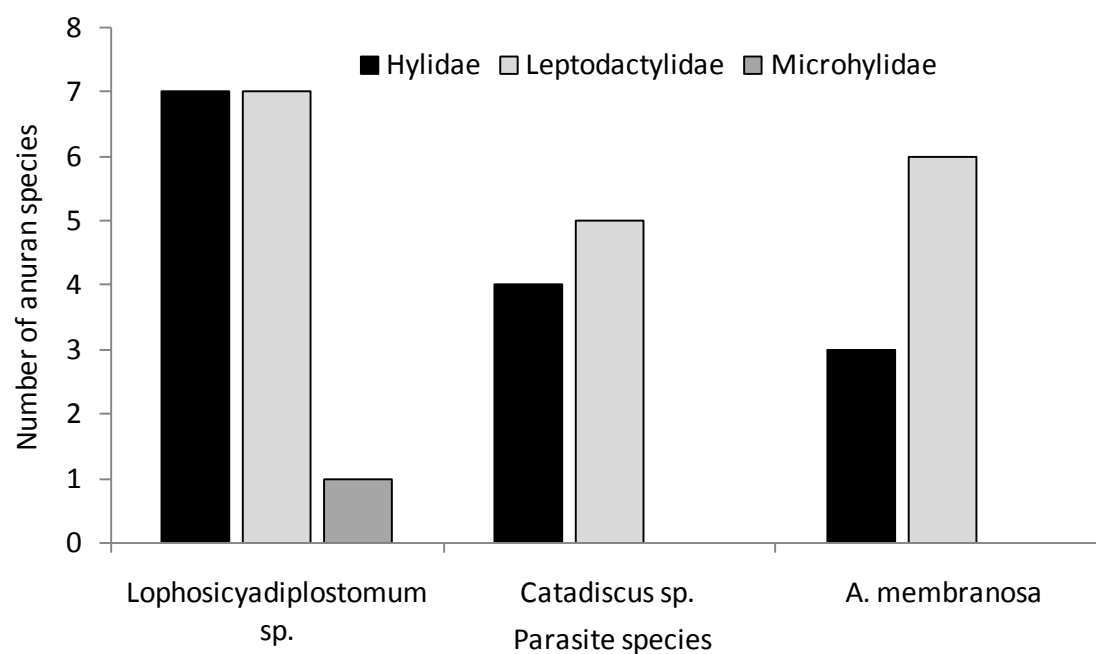


Figure 3- Generalist helminth species (*Lophosicyadiplostomum* sp., *Catadiscus* sp. and *Aplectana membranosa*) associated with anurans from the three families (Microhylidae, Leptodactylidae and Hylidae).

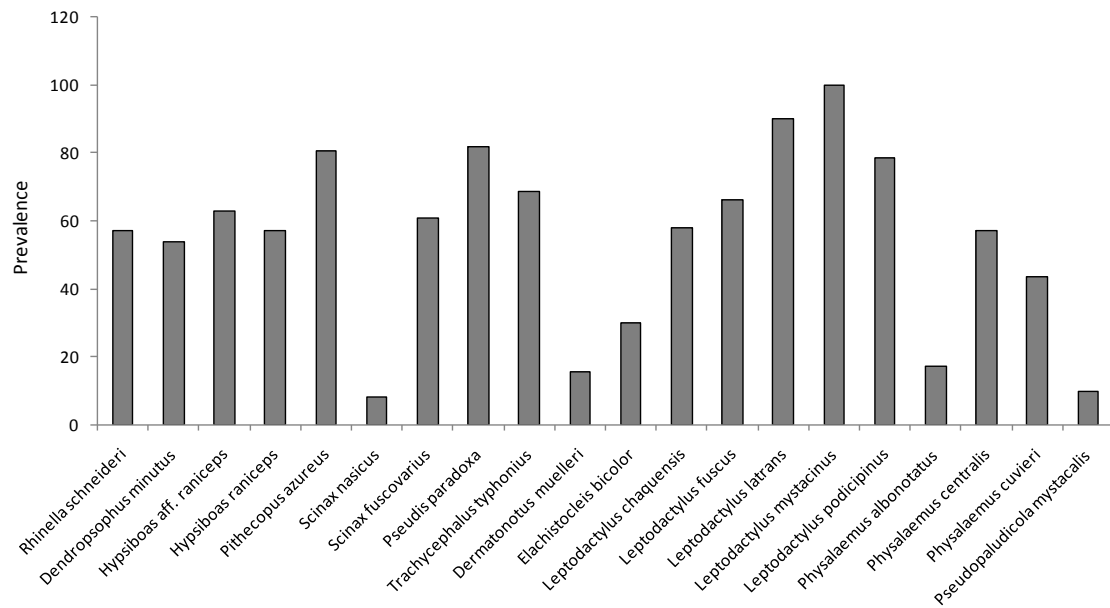


Figure 4 - prevalence of metazoan parasites in 20 anuran species.

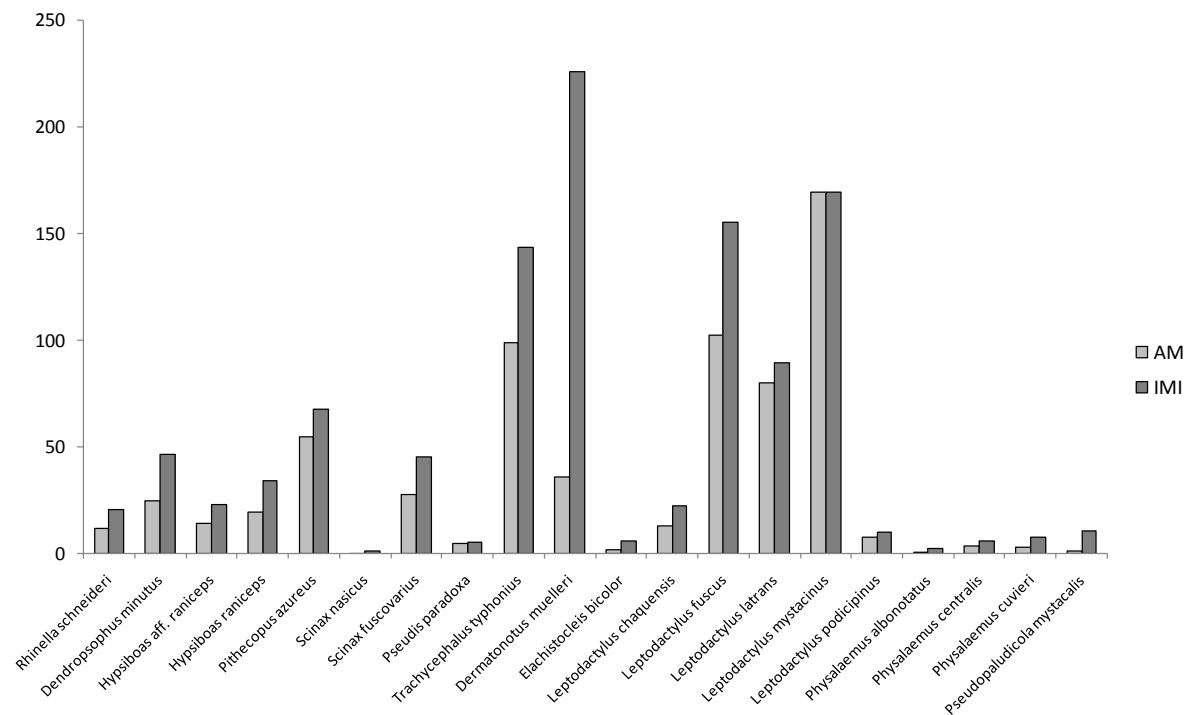


Figure 5 - mean abundance (AM) and mean intensity of infection (IMI) of metazoan parasites in 20 anuran species.

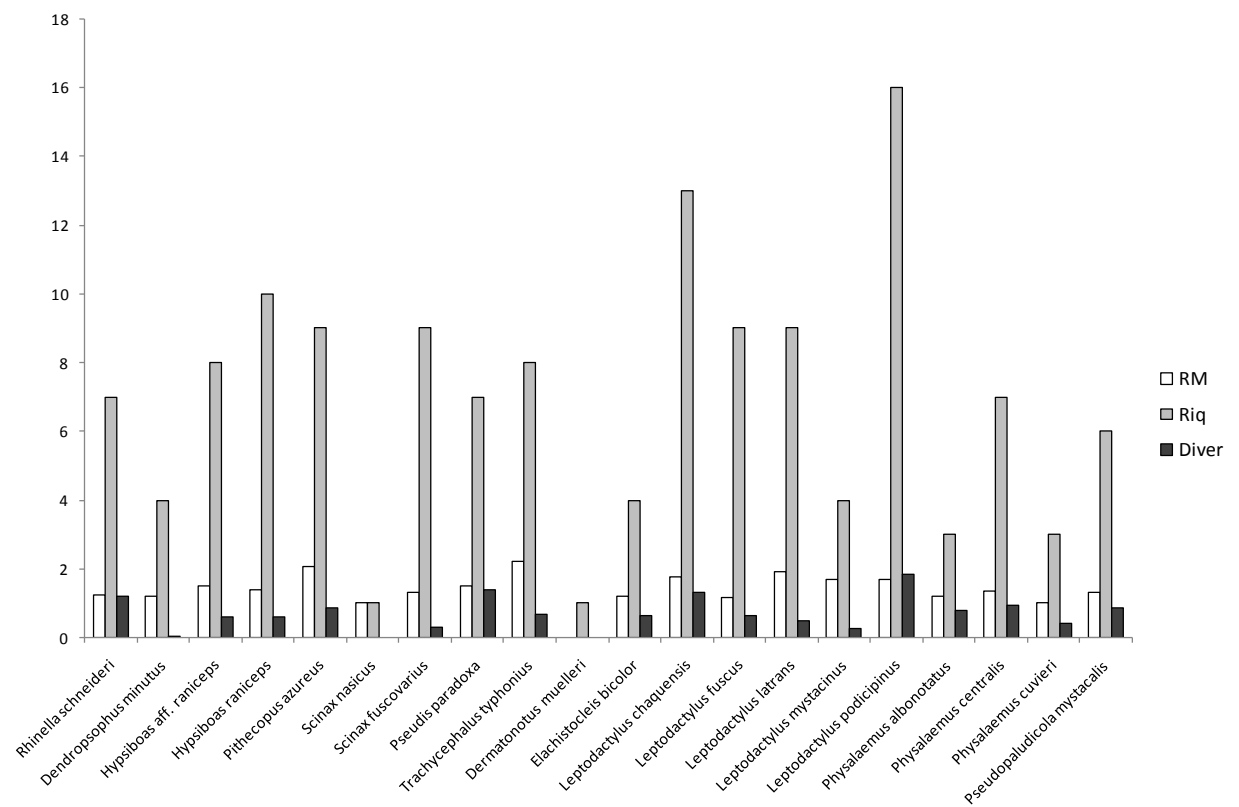


Figure 6 - Mean richness (RM), richness (Riq) and diversity (Diver) of metazoan parasites in 20 anuran species

Photographic appendix
Metazoan parasites of amphibians

1. Digenea

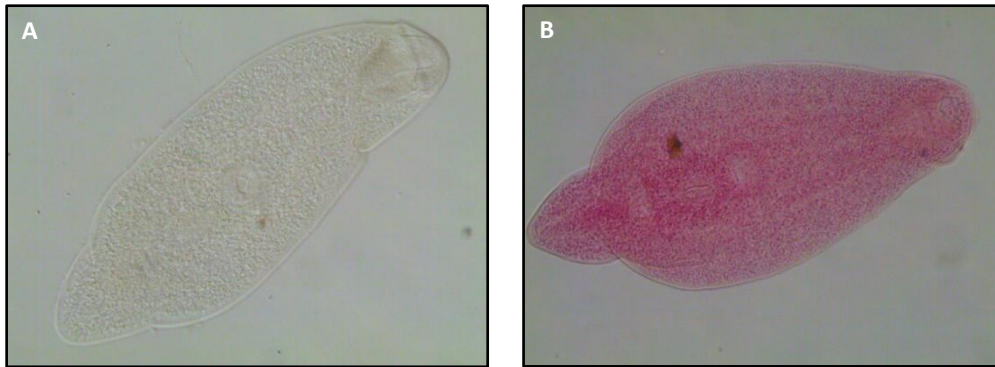


Figure 1.1. Metacercaria of *Lophosicyadiplostomum* sp. associated with kidneys of *Dendropsophus minutus*. A) mounted with phenol (20x); B) specimen stained with cloridric carmine and cleared with eugenol (20x).



Figure 1.3. Metacercaria of *Bursotrema* sp. (20x) associated with kidneys of *Physalaemus centralis*.



Figure 1.2. Unidentified metacercaria associated with the mesenterium of *Pseudopaludicolamystacalis*.



Figure 1.4. Metacercaria of *Heterodiplostomum* sp. (2.5x) encysted in muscles of body cavity of *Pseudis paradoxa*.



Figure 1.5. Metacercaria of *Clinostomum* sp. (1.6x) associated with body cavity of *Leptodactylus podicipinus*.

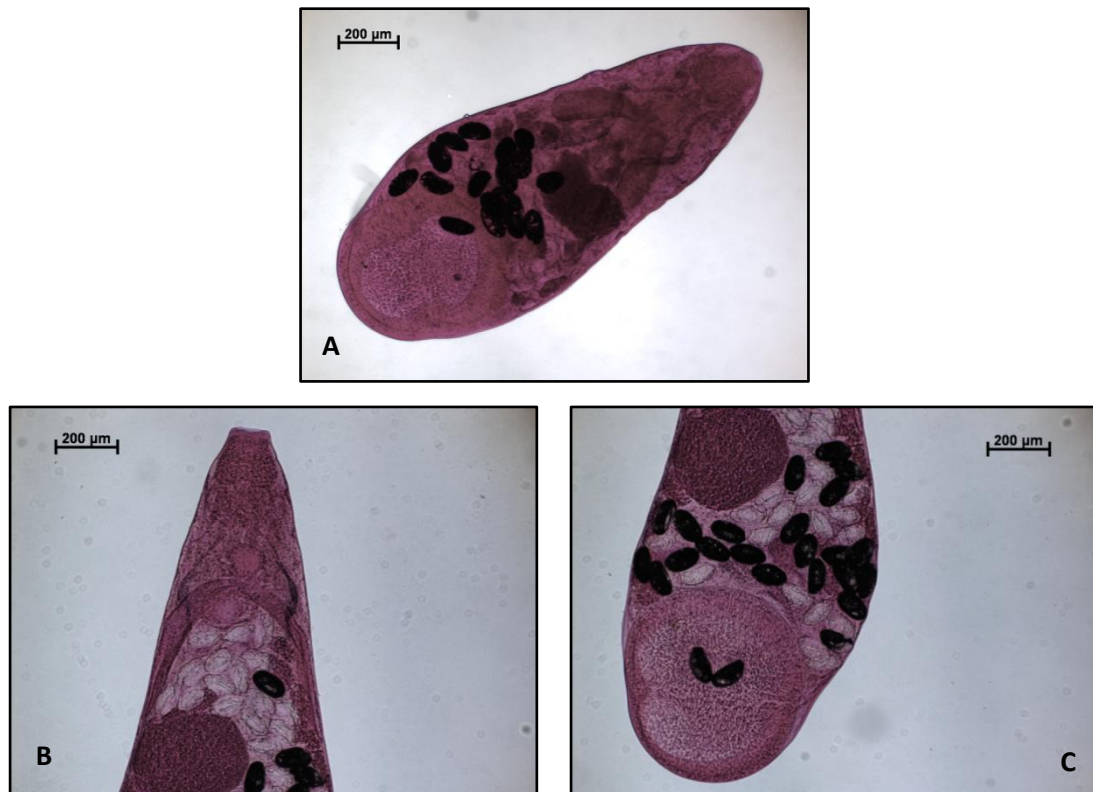


Figure 1.6. *Catadiscus propinquus* (5x) associated with large intestine of *Pseudis paradoxa*. A) total; B) anterior region; C) posterior region showing acetabulum.



Figure 1.7. *Catadiscus marinholutzi* (5x) associated with small intestine of *Leptodactylus fuscus*.

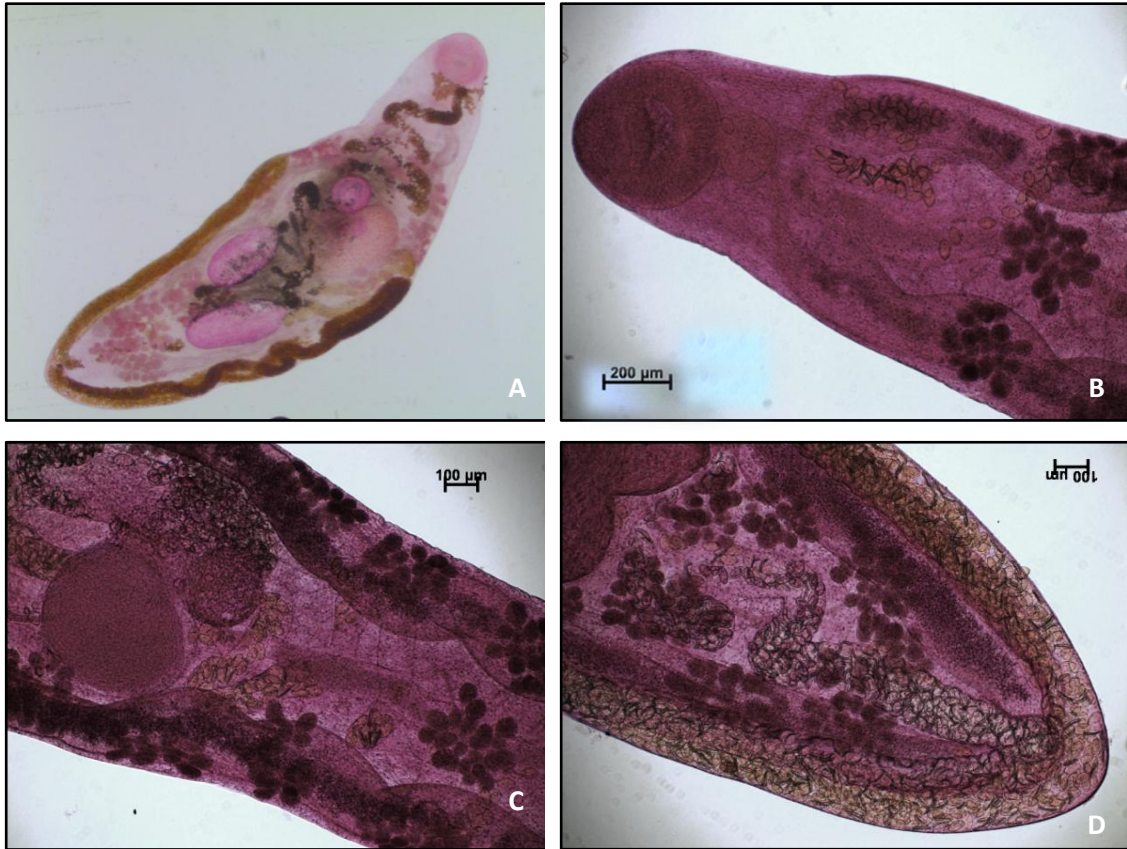


Figure 1.8. *Neohaematoloechus neivai* associated with lungs of *Pseudis paradoxa*. A) total (5x); B) anterior region (5x); C) equatorial region (5x), showing vitelarians in acinus, ovary e branches of intestines; D) posterior region (5x), showing yellow-brown eggs



Figure 1.9. *Rauschiella* sp. associated with digestive tract of *Leptodactylus podicipinus*. A) anterior region (10x); B) posterior region (10x).

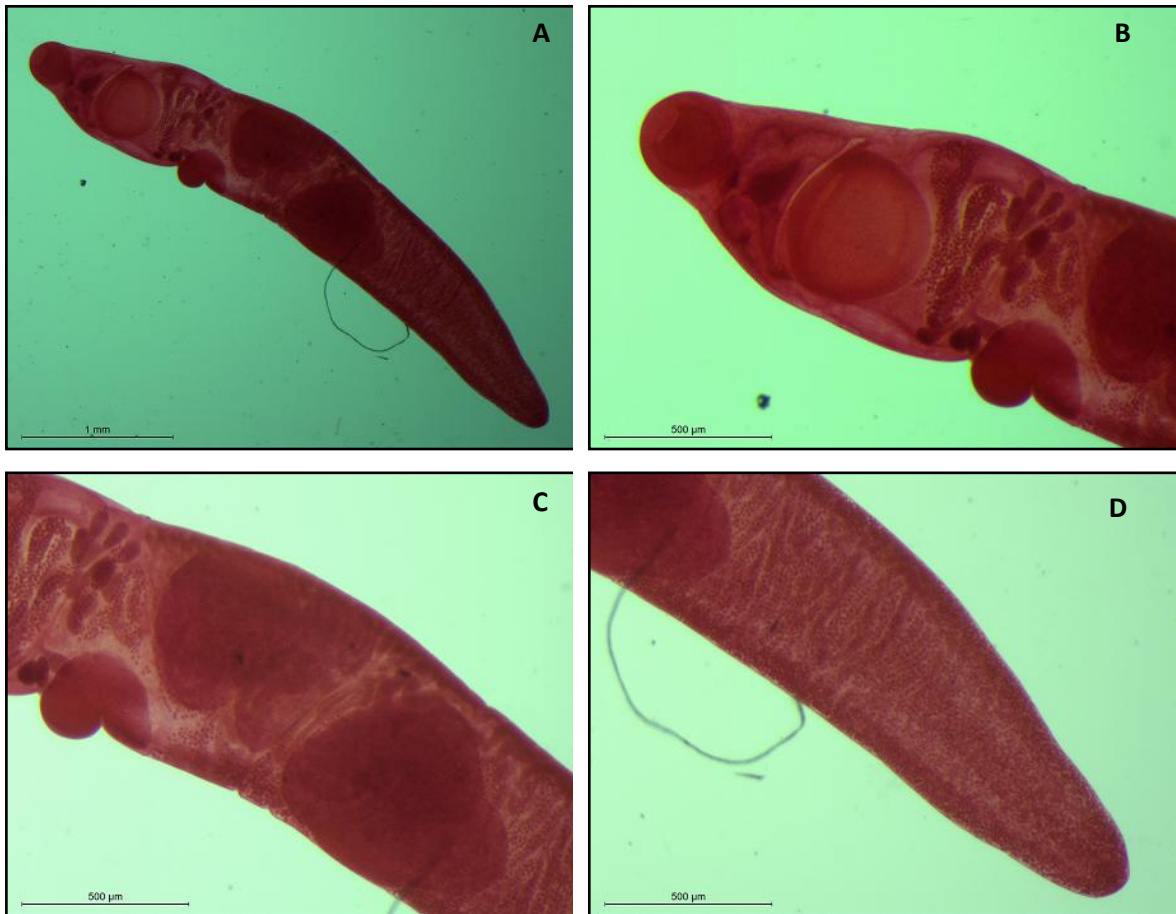


Figure 1.10. *Gorgoderina diaster* associated with urinary bladder of *Hypsiboas* aff. *raniceps*. A) total view; B) anterior region showing acetabulum; C) equatorial region, showing test and ovary; D) posterior region.

2. Monogenea

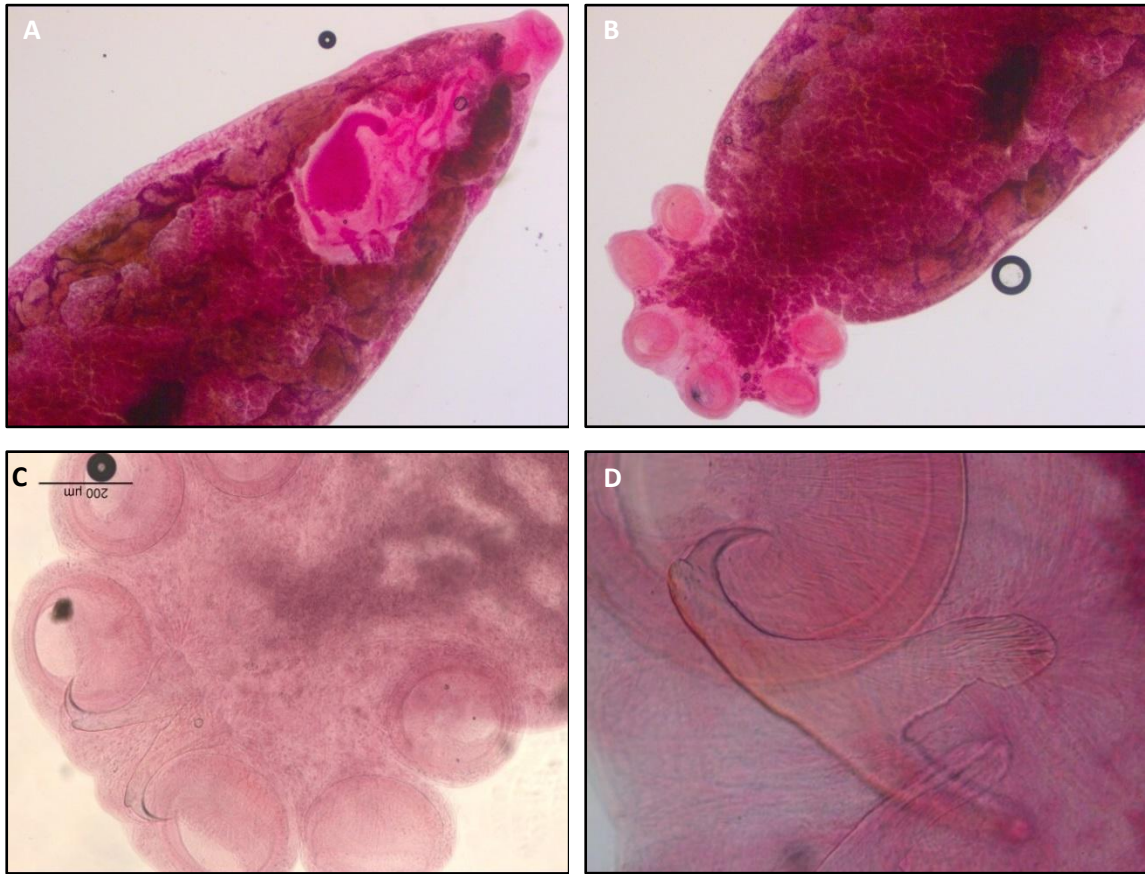


Figure 2.1. *Polystoma* cf. *lopezromani* associated with urinary bladder of *Trachycephalus typhonius*. A) anterior region (2.5x); B) posterior region (2.5x); C) haptor and hamule (10x); D) detail of hamule (20x).

3. Nematoda

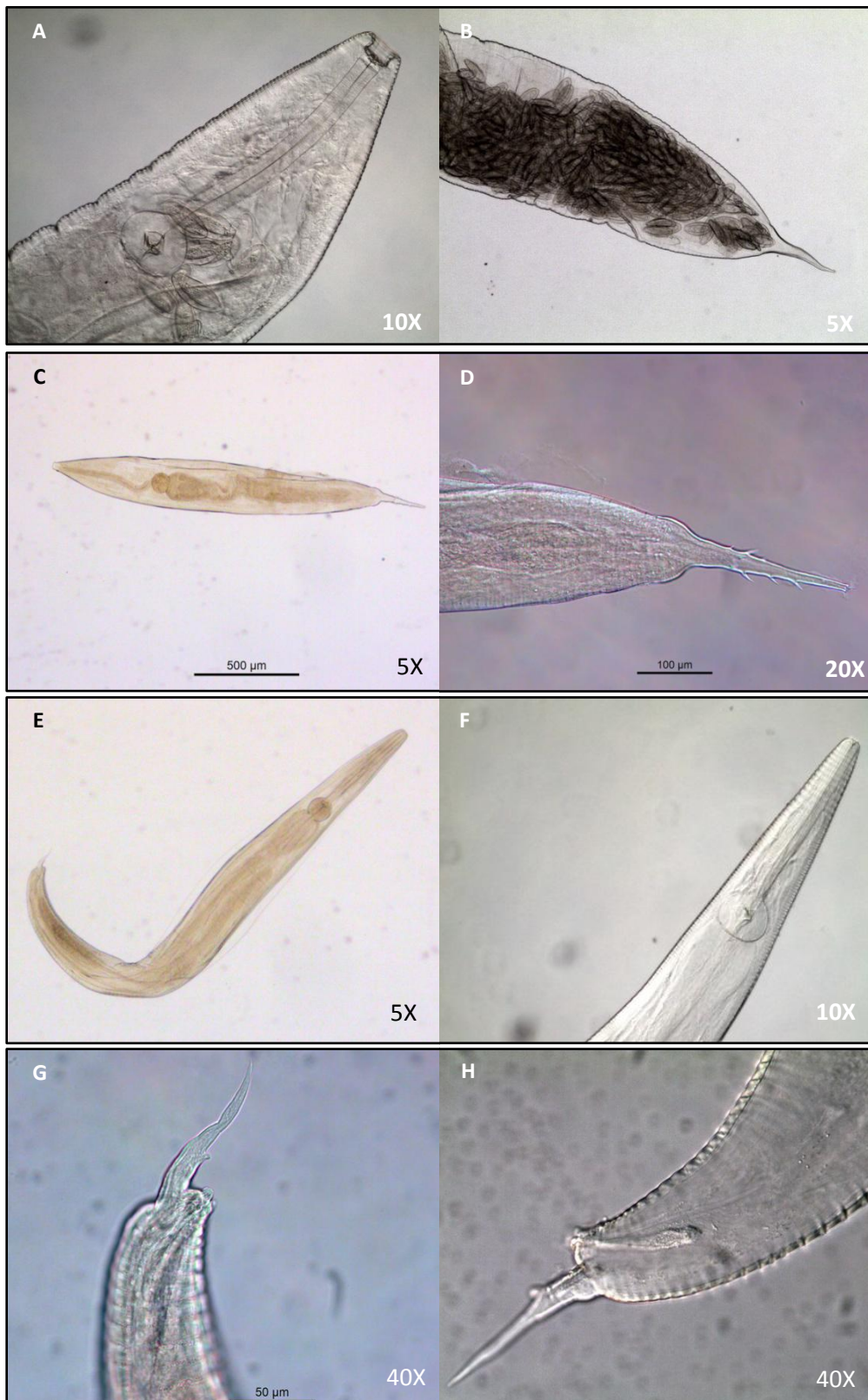


Figure 3.1. *Parapharyngodon* cf. *alvarengai* associated with small and large intestines of *Trachycephalus typhonius*. A) anterior region of female with eggs; B) posterior region of female; C) total view of larvae; D) posterior region of larvae, showing spines of body and tail; E) total view of male; F) anterior region of male, showing transversal grooves; G) tail of male showing conic papillae; H) tail of male showing spicules.

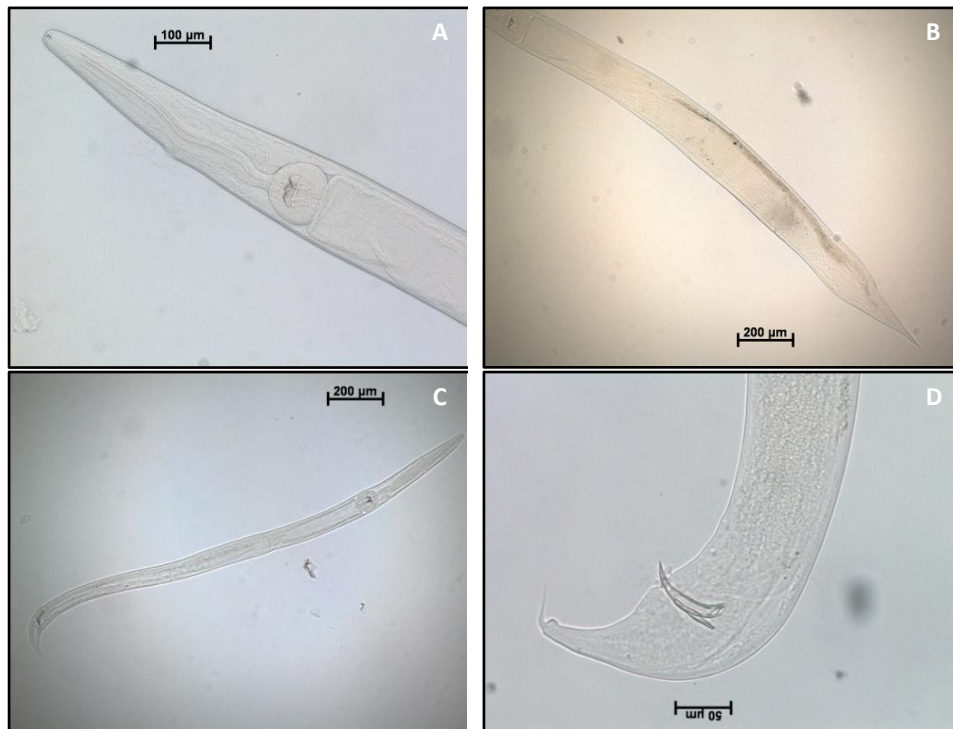


Figure 3.2. *Schrankiana formosula* associated with large intestine of *Leptodactylus fuscus*. A) anterior region of female (10x); B) posterior region of female with eggs (5x); C) total view of male (5x); D) tail of male showing spicules (20x).

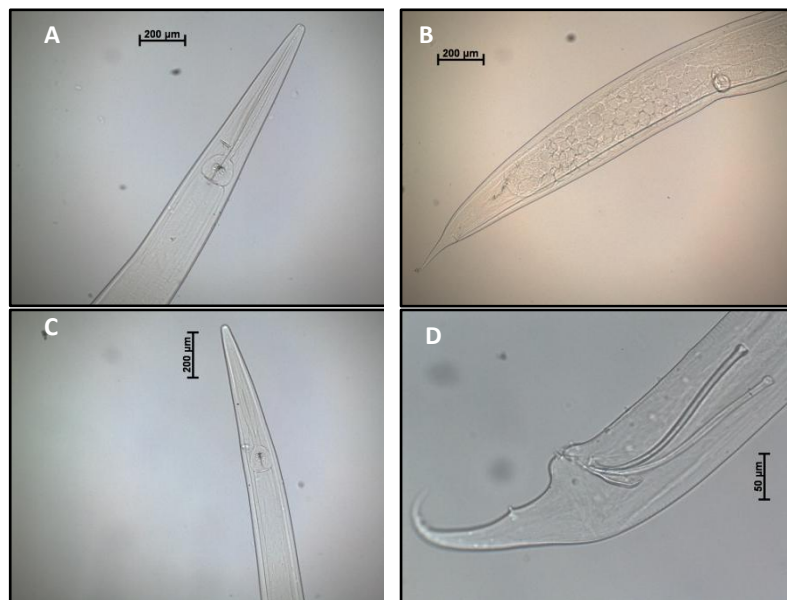


Figure 3.3. *Aplectana membranosa* associated with digestive tract of *Leptodactylus fuscus*. A) anterior region of female (5x); B) posterior region of female with eggs and vulva (5x); C) anterior region of male (5x); D) tail of male showing spicules, gubernaculum and papillae (20x).

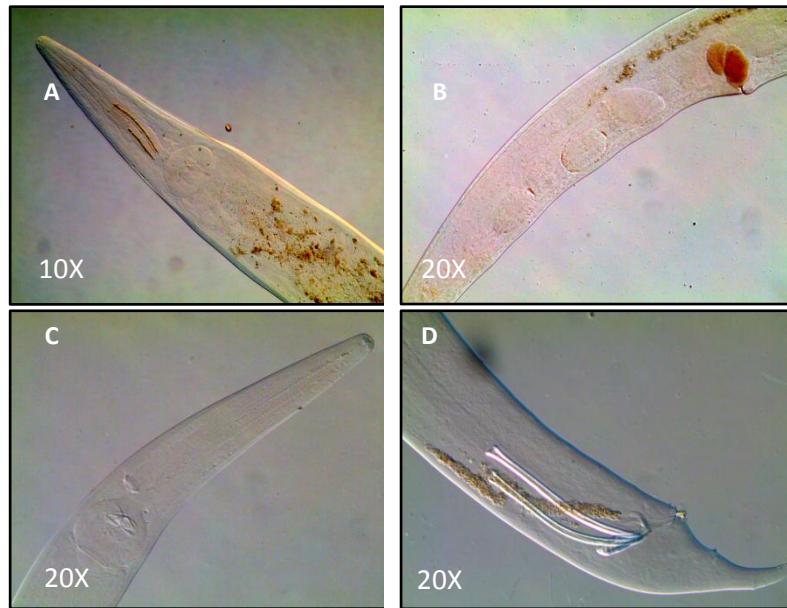


Figure 3.4. *Aplectana hylambatis* associated with large intestine of *Dermatonotus muelleri*. A) anterior region of female; B) equatorial region of female showing eggs and vulva; C) anterior region of male; D) tail of male showing spicules and its hooks and gubernaculum.



Figure 3.5. *Cosmocerca* cf. *chilensis* (male) associated with small intestine of *Scinax fuscovarius*. A) total view (10x); B) anterior region (40x); C) posterior region showing spicules and plectanes (40x).



Figure 3.6. *Oxyascaris caudacutus* (female) associated with stomach and small intestine of *Scinax fuscovarius*. A) anterior region showing lateral enlargement (10x); B) posterior region (5x).



Figure 3.7. *Falcaustra mascula* associated with large intestine of *Rhinella schneideri*. A) anterior region of female (5x); B) detail of bulb and pre-bulb (10x); C) tail of female (5x); D) tail showing spicules of male (10x).



Figure 3.8. *Ochoterenella digicauda* associated with body cavity of *Rhinella schneideri*. A) anterior region of female (5x); B) anterior region of male (5x); C) tail showing spicule of male (40x); D) tail of female (20x).

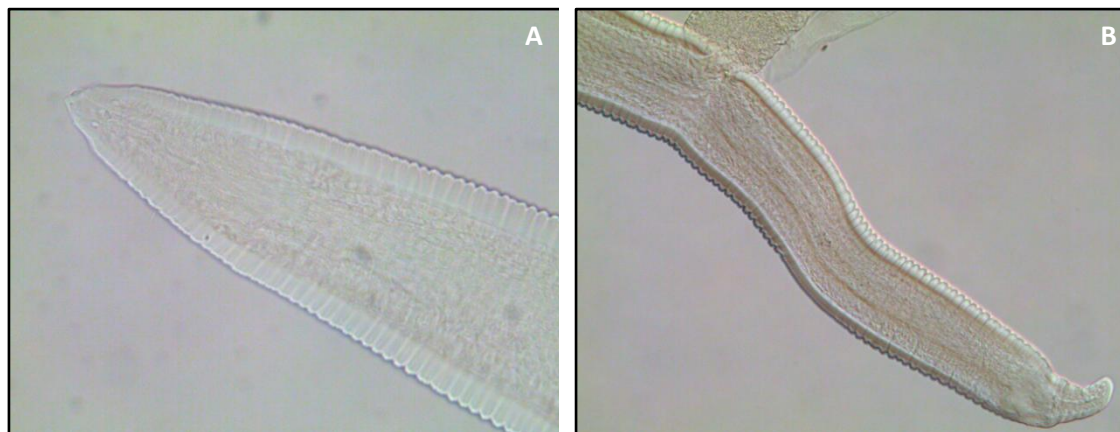


Figure 3.9. Larvae of *Spiroxys* sp. encysted in stomach of *Scinax* cf. *similis* and in mesentery of *S. fuscovarius*. A) anterior region (20x); B) posterior region (10x).

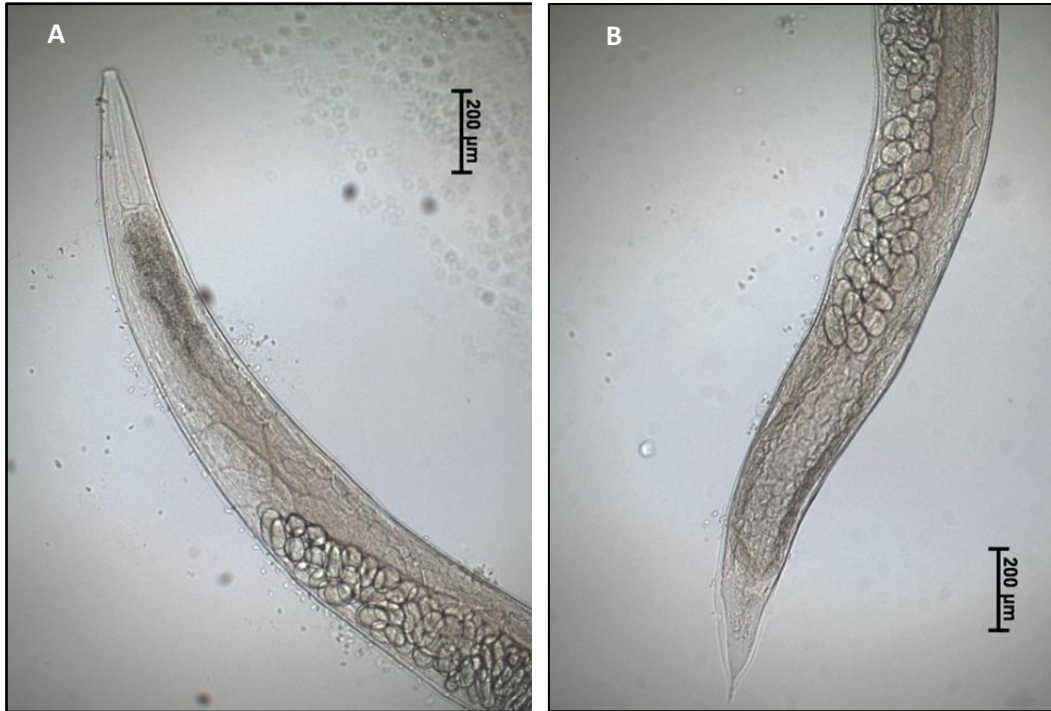


Figure 3.10. *Rhabdias* sp3 associated with lungs of *Leptodactylus mystacinus*.
A) anterior region; B) posterior region

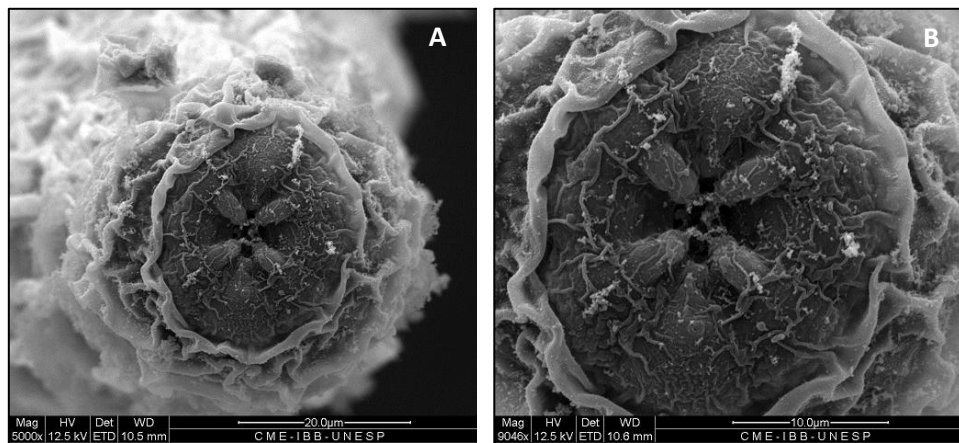


Figure 3.11. Scanning electron microscopy of *Rhabdias* sp2 associated with lungs of *Scinax fuscovarius*. A) mouth (in face); B) detail of lips (in face).

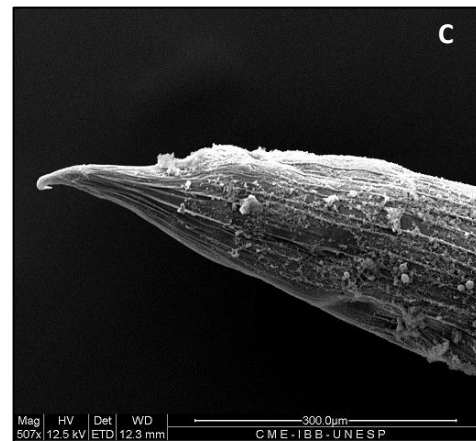
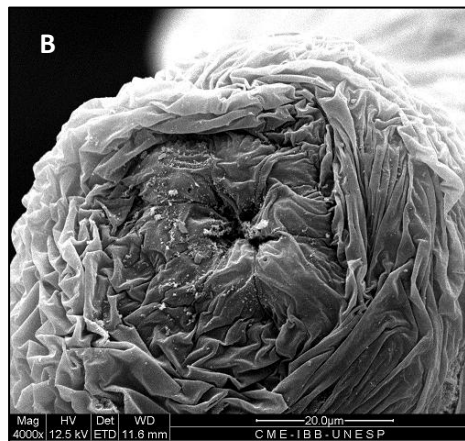
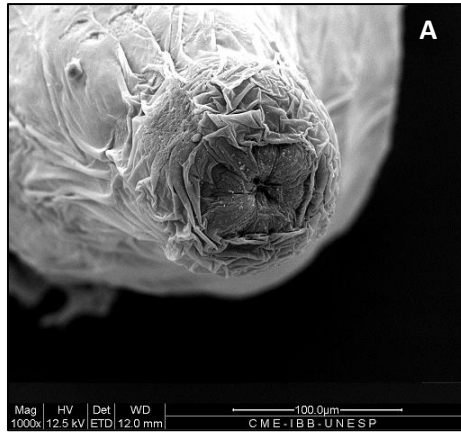


Figure 3.12. Scanning electron microscopy of *Rhabdias* sp1 associated with lungs of *Rhinella schneideri*. A) mouth (in *face*); B) detail of lips (in *face*); C) posterior region, detail of longitudinal grooves of cuticle.

4. Acanthocephala



Figure 4.1. Cystacanths of Centrorhynchidae. A) larvae after cyst's rupture stained with cloridric carmine and cleared with eugenol (10x); B) Cystacanth not ruptured and cleared with phenol (10x).

5. Oligochaeta

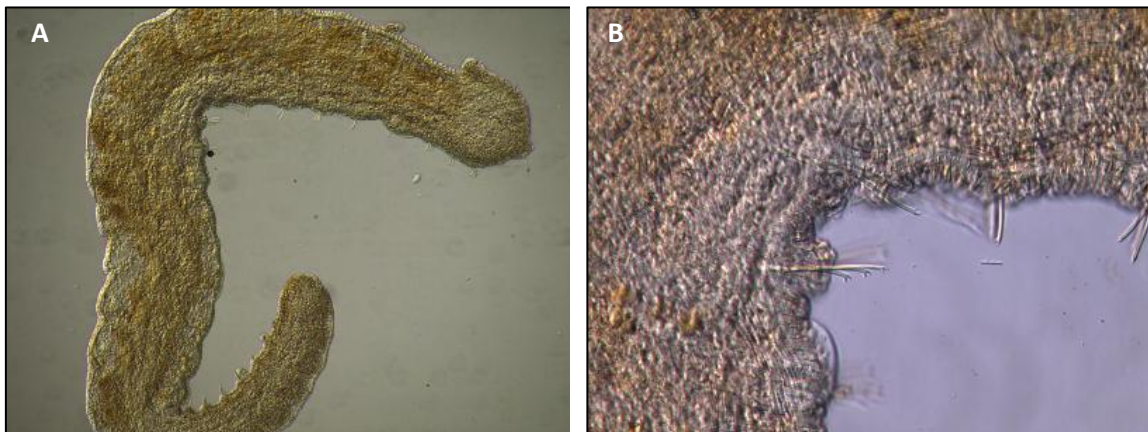


Figure 5.1. *Dero (Allodero) lutzi* (Oligochaeta). A) total view (5x); B) detail of bristles (40x).

Capítulo 2

Environmental or host variables? Which determines parasite composition of anurans
from fragments of transitional areas of Neotropical region in Brazil?

Environmental or host variables? Which determines parasite composition of anurans from fragments of transitional areas of Neotropical region in Brazil?²

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²*Manuscrito preparado de acordo com as normas da revista Parasitology Research.*

Abstract

Like every other community, parasites are under several variables from the environment or evolutionary history with their hosts. We tested variables such as habit, body size, host species, season and environmental features, in parasite composition of anurans from a transitional area between Mata Atlântica and Cerrado. Twenty-six anuran species (978 individuals) were collected in different habitats and seasons at forest fragments in São Paulo State, Brazil. We recovered 40 *taxa* of metazoan parasites associated with 59.4% of anurans, mean abundance of 21.6 ± 2.5 and intensity of infection of 36.3 ± 4.1 parasites per individual. Twenty-two parasite *taxa* occurred in both rainy and dry seasons, and anthropogenic and ponds presented high abundance and richness of anurans and parasites. *Aplectana membranosa* and *Brevimulticaecum* sp. were found in anurans from all environments. Cluster analysis demonstrated that related hosts with overlap of parasite composition signal a phylogenetic influence as well as observed by Permutational Multivariate Analysis of Variance, which showed host species and body size were the main predictors for parasite composition. These findings suggested that regardless the host habit, season or environmental conditions, the anurans of the present study, which have similar body size and species are closely related, presented more similarities in their parasite fauna. Similarities in parasite composition can be related to historical effects as ancestral inheritance in a host lineage.

Keywords

Amphibian, nematode, digenea, Atlantic Forest, Cerrado

Introduction

Determinants of parasite species richness and composition patterns across host species are still poorly understood for amphibians, although some studies on other vertebrates have contributed significantly for these questions (Tavares and Luque 2008; Krasnov et al. 2012; Strona and Lafferty 2013). Several studies on food webs, determinants of parasite species richness, and others are related with vertebrates such as fishes, birds, and mammals (Poulin et al. 2013; Kamiya et al. 2014, Bellay et al. 2015), but reptiles and amphibians are scarce (Brito et al. 2014; Campião et al. 2015a, b). Ecologists seek patterns that explain what we see in nature environments, and in the

past few years, improved statistical methods have contributed to elucidate some questions concerning diversity, composition of communities as well as their distribution in a specific local or in the world (Poulin 1997; Poulin and Morand 2004; Morand 2015).

Like every other community, parasites are under several variables such as those from the environment or evolutionary history with their hosts (Poulin and Morand 2004; Poulin 2007a). An important aspect of the parasite life style is the dependence of an organism in relation to other, and that is why several aspects have to be considered when we are studying the host-parasite relationship (Nunn et al. 2003; Poulin and Morand 2004; Morand 2015). Studies have showed a robust relation with some host variables as determinants for richness, composition, and structure of parasite fauna (Kuris et al. 1980; Poulin 1995; McAlpine 1997; Yoder and Coggins 2007), and the host size appears in numerous studies as the mainly variable affecting the community of parasites (Poulin 1995; Kamiya et al. 2014; Campião et al. 2015a). For instance, there is a tendency for host species with larger body size to present higher values of parasitism (Yoder and Coggins 2007). Hosts with larger bodies present a larger surface area increasing penetration by some parasites, providing more space and niches to parasites. They ingest greater amounts of food allowing several trophic transmission, and it is expected that larger hosts have lived more resulting in longer time of exposure to parasites (Guegan et al. 1992; Poulin 1995; Poulin 2007a). However, other host features such as geographic distribution, diet, behavior and habit have been suggested as an important fact in diversity and in composition of parasite fauna (Aho 1990; Muzzall 1991; Brito et al. 2014).

Recently studies have discussed phylogenetic comparative methods in evolutionary history of hosts and their parasites (BrITO et al. 2014; Verneau et al. 2002). This historical factor is important in the determination of parasite community composition of vertebrates (Lima Jr et al. 2012; Brito et al. 2014) since phylogenetic proximity can be understood as an outcome from the use of similar niche and life in similar environments (Lewinsohn et al. 2006; Rezende et al. 2009).

The physical boundaries of parasite (body of host) are known as well as the dependency of parasite in relation to its hosts, but as an ecological system, temporal and spatial variability might be considered for hosts and parasite (Kuris et al. 1980; Poulin and Morand 2004). For instance, recent studies regarding this topic have unveiled this

influence that, habitats present a strong relation with composition of parasites. (Marcogliese 2002; Brito et al. 2014).

Amphibians are good models for these ecological studies because they present a great diversity of species and habitat use (Goater and Goater 2001), especially in Neotropical region. Brazil harbors one of the most rich anuran fauna with approximately of 14% of global diversity of this vertebrate (Segalla et al. 2016; Frost 2016) making this region a great decoy for researches. Moreover, advances in the analysis of multivariate using non-parametric methods have helped us to understand these questions related to communities and their variables (*e.g.* Anderson 2001).

The goal of this study was to verify similarities among composition of parasite species of 26 anuran species. Thereafter, we analyzed the influence of host variables (size, habit, and host species) and environmental variables (season and vegetal formation) as potential determinants of composition of parasite species in anuran fauna.

Material and Methods

Study area and collection of anurans

This study was conducted in a particular area referred as Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí (RPPN) under responsibility of Companhia Energética do Estado de São Paulo (CESP), and it comprises the municipalities of Castilho, São João do Pau d'Alho, and Paulicéia, São Paulo State, Brazil. The RPPN is crossed by Aguapeí River, which belongs to Paraná Basin, Bauru Sub-basin, and is characterized with slightly wavy terrain (Sallun and Suguio 2006). It is a transitional area between Mata Atlântica and Cerrado with fragments of seasonal forest and plains. This transitional area has a variety of environments such as permanent and temporary ponds, native forest fragments, *Brachiaria* grass, pastures, open fields, anthropogenic areas, reforested fragments of five years, and others of three years.

The terrain and climatic zone with rainy and dry season allow periodical inundations in the region. In addition to lowlands of the region, which contributes for temporary ponds, the periodical inundation allows communication among several ponds in rainy season and their separation during the dry season (Comitês das Bacias Hidrográficas dos Rios Aguapeí e Peixe – CBH-AP 1997; Boin 2000). All these features contribute for a mosaic formation with high potential for biological richness.

The anurans were collected by visual search and by pitfall traps in four expeditions: two in a dry season – July 2012 and July 2013, and two in a rainy season –

January 2013 and January 2014 (SISBIO number permission 31716-2). Pitfall traps were mounted with replicates in three vegetation types: *Brachiaria* pastures, reforested fragments of five years, and reforested fragments of three years; only native semi deciduous forest ("Angico") and open fields did not present replicates.

The weight of anurans were measured by Pesola® weighing scale and a caliper (0.01 precision) was used for measuring of snout-vent-length (SVL). They were killed with over dosage of Tiopental sodic and then necropsied searching for helminths parasites in all organs and body cavity. Later, anurans were fixed with formaldehyde (10%) and preserved in alcohol 70% for sending to Coleção de Vertebrados da Universidade Estadual de Campinas and Coleção Herpetológica da Universidade Federal do Mato Grosso do Sul. Anurans nomenclature follows Frost (2016) and Segalla et al. (2016), and their habit nomenclature (arboreal, terrestrial, cryptozoic, and aquatic) follows Haddad et al.(2008).

Collection of helminths and procedures in laboratory

Under a stereomicroscopy, all organs and coelomic cavity were searched for helminth parasites, which were counted and registered in respect to site of infection. Nematodes were fixed by hot absolute alcohol, while acanthocephalans were firstly kept on cold water for exposition of proboscis, and then they were fixed with absolute ethanol. Cestodes, digeneans, and monogeneans were fixed using absolute ethanol with slight compression between slide glasses, whereas oligochaets were fixed only in absolute ethanol. Subsequently, all helminths were kept in labelled bottles with ethanol 70%.

In laboratory, helminths were mounted in temporary slides for observation of taxonomic structures using a computerized system of image analysis (LAS DIC, Leica Microsystems, Wetzlar, Germany). The nematodes and oligochaets were cleared with lactic acid or lactophenol, and the others helminths such as acanthocephalans, cestodes, digeneans, and monogeneans were stained with chloridric carmine, dehydrated with alcoholic series and then cleared with eugenol (Amato et al. 1991; Rey 2001).

The morphologies of some helminths were evaluated by scanning electron microscopy (SEM). Therefore, they were fixed in absolute ethanol, dehydrated in graded alcohol series and dried by critical point with liquid CO₂ in CPD 020 (Balzer Union). Then they were mounted on an aluminum stub using conductive double-sided tape, coated with gold–palladium and examined with the use of a Quanta 200 scanning

electron microscope (FEI Company; from Centro de Microscopia Eletrônica de Botucatu, São Paulo, Brazil) (adapted from Allison et al. 1972).

Helminths were deposited in Coleção Helminológica do Instituto de Biociências, Departamento de Parasitologia, Unesp Campus Botucatu.

Data analysis

Parasitism descriptors, such as prevalence (P), mean abundance (MA), mean intensity of infection (MII), mean richness (MR) followed Bush et al. (1997). The richness was described as number of metazoan parasite *taxa*, and all means were reported with standard error ($\pm$ SE) (Bush et al. 1997).

Cluster analysis using Bray-Curtis index (Krebs 1989) was performed to evaluate similarities in parasite composition among anurans species with assumption of closely related host species presenting more similar helminth fauna. This analysis was performed using PAST (Paleontological Statistics) 2.15 (Hammer et al. 2001).

Host and environmental variables such as habit (arboreal, terrestrial, cryptozoic, and aquatic), size (SVL), host species, season (dry and rainy), and local features (*Brachiaria* pastures, reforested fragments of five years, reforested fragments of three years; native forest - "Angico" and "Buriti", open fields, ponds, dirt tracks, and anthropogenic areas) were measured to predict potential determinants for parasite composition (response variable). We constructed a binomial matrix for parasite composition with individual infected hosts, and in other matrix random variables for each anuran. Therefore, we employed Permutational Multivariate Analysis of Variance (PERMANOVA), which is meant to test differences between groups like an ANOVA test, but with many variables (Anderson 2001; Anderson and Walsh 2013). We use "adonis" function of *vegan* package (Oksanen et al. 2013) in R 2.14.1 (R Development Core Team 2013).

Results

During the four expeditions in dry and rainy season 26 anuran species (978 individuals) were collected from different types of environments (Table 1). We found 59.4% of anurans infected with at least one parasite species presenting MII = 36.3 ± 4.1 which ranged from 1 to 1,301 parasites. Mean abundance was 21.6 ± 2.5 , and the parasite fauna was composed by 40 *taxa* of metazoan parasites (Table 2).

Nematodes and digeneans were the most representative parasites with 22 and 13 species, respectively, while monogenean, acantocephalan, cestode, and oligochaet had only one taxon (*Polystoma* cf. *lopezromani* Combes & Laurent, 1979, Centrorhynchidae, *Cylindrotaenia Americana* Jewell, 1916, and *Dero*(*Allodero*) *lutzi* Michaelsen, 1926, respectively). The majority of nematodes (14 taxa) are directly transmitted to anurans while the other eight have heteroxenous cycle and only three (*Brevimulticaecum* sp., *Physaloptera* sp., and *Spiroxys* sp.) are using anurans as intermediate or paratenic hosts. Thirteen digenean species were found, eight in adult stage and five as metacercariae (*Bursotrema* sp., *Clisnostomum* sp., *Heterodiplostomum* sp., *Lophosyciadiplostomum* sp., and an unknown metacercaria).

In the dry season, we collected 19 anuran species, three were exclusive from this season (*P. albonotatus*, *S. cf. similis*, and *Scinax* sp.). Also in the rainy season a total of 23 anuran species were collected, seven were exclusive species (*D. muelleri*, *L. fuscus*, *L. mystacinus*, *P. azureus*, *P. marmoratus*, *P. nattereri*, and *S. fuscomarginatus*) (Table 1). Regarding the 40 parasite taxa, 34 occurred during the rainy and 28 in the dry season (Table 3), while the occurrence of 22 species were not affected by seasonality, in other words they were in both seasons (Table 3). On the other hand, *Aplectana hylambatis* (Baylis, 1927), *Cosmocerca parva* Travassos, 1925, *Oxyascaris caudacutus* Freitas, 1958 (Baker and Vaucher 1985), *Oxyascaris* sp., *Raillietnema minor* Freitas & Dobbin Jr., 1961, *Schrankiana formosula* Freitas, 1959, *Ochoterenella* sp., *Oswaldocruzia mazzai* Travassos, 1935, *Rhabdias* sp.1, *Brachycoelium salamandrae* Hardy, 1972, *Catadiscus marinhoi* Lutz & Freitas, 1939, and an unknown metacercariae occurred only in the rainy season. Although in the dry season, it presented six exclusive parasites: *Cosmocerca* cf. *chilensis* Lent & Freitas, 1948, *Ochoterenella digiticauda* Caballero, 1944, *Raillietnema* sp., *Spiroxys* sp., *Clinostomum* sp., and *Gorgoderina* sp. (Table 3).

Of the total anurans sampled, 26 individuals did not have their sites recorded, resulting in 952 anurans and 26 species distributed in nine types of environments (Table 4). Anthropogenic and ponds presented high abundance and richness of anurans and parasites (Table 4 and 5). *Brachiaria* pastures, dirt tracks and Buritis had low abundance of anurans and a few parasite species (Tables 4 and 5). *Aplectana membranosa* (Schneider, 1866) and *Brevimulticaecum* sp. were found in anurans from all environments. Others such as Cosmocercidae, Centrorhynchidae, *Lophosyciadiplostomum* sp., and *Catadiscus* sp. occurred in most of them. In general,

the sampled sites shared several parasite species but *Raillietnema* sp., *O. mazzai*, *O. caudacutus*, *O. digiticauda*, *Gorgoderina* sp., *Heterodiplostomum* sp., *F. mascula*, *B. salamandrae*, *Clinostomum* sp., *C. cf. chilensis*, *C. parva*, and unknown metacercariae occurred in only one type of environment (Table 5).

Cluster analysis showed a similarity (*B*) of parasite composition among some anurans, which were closely related such as *P. cuvieri* and *P. centralis* (0.6), *H. aff. raniceps* and *H. raniceps* (0.78), *L. chaquensis* and *L. latrans* (0.82), and *P. marmoratus* and *P. nattereri* (1.0) (Figure 1; Table 6). In other clades of anurans without close affinity (*E. bicolor* and *P. albonotatus*; *S. fuscovarius* and *T. typhonius*) the similarity can be related to similar habits and use of the same type of habitat (Figure 1; Table 6).

Permutational Multivariate Analysis of Variance (PERMANOVA) using host species, host size (SVL), season, and type of environment as potential predictors for parasite composition shows us that host variables, species and size, are the most significant determinant factors (Table 7), indicating that these variables are good predictors for parasite communities of anurans. While differences among dry and rainy seasons, and environment features of anuran hosts did not affect the composition of their parasites (Table 7). In other words, differences in composition of parasite communities were not significantly related to changes in environment.

Discussion

Forty parasite taxa infecting 26 anuran species were evaluated and different composition and mode of infection were found in these parasite communities. Numerous studies on parasite ecology discuss richness and abundance patterns of parasites in relation to their hosts (Nunn et al. 2003; Kamiya et al. 2014; Campião et al. 2015a), and a few have considerable data and present findings related to parasite composition. Studies on parasite composition may demonstrate possible historical effects among hosts and parasites with assumption of hosts more related to present similarities in their parasite composition (Poulin et al. 2010; Brito et al. 2014).

Host variables such as species and size influenced significantly on parasite composition across the anuran fauna of the present study. Therefore, parasite composition varied according to the different host species, indicating a certain specificity, which may be the outcome of phylogenetic component or historical effects among these organisms (Lima Jr. et al. 2012; Brito et al. 2014). In addition, this finding supports the groups formed by similarities of parasite composition in Cluster analysis,

which showed related hosts with overlap of parasite composition. New host lineages can inherit parasites species by the ancestral host, and these daughter lineages offer similar resources to parasites (Poulin 1997). However, they may diverge in relation to ecological traits such as exposition to new parasite species and extinction of others (Poulin 1997). Nonetheless, these new lineages with almost identical parasite faunas will have more similarities in their parasite composition than to those of other host species (Poulin 1997).

We also observed some anurans from the same family or with similar habits, presented common parasite species. Leptodactylids are terrestrial and very active foragers contacting many types of environment (aquatic and terrestrial) (Duellman and Trueb 1986), acquiring high number of parasites species as well as a similar composition among parasite fauna of the family's members. The parasites *A. membranosa* and other cosmocercids were commonly found in terrestrial environment and they were associated with nine leptodactylid species. Digenetics commonly need aquatic invertebrates in their cycle and in the present study, adults and metacercariae were associated with almost all frog species. These facts confirm the vagility and transition of these anurans by various habitats including the aquatic one (Duellman and Trueb 1986; Hamann et al. 2006).

Cryptozoic anurans as *E. bicolor*, *D. muelleri* (Microhylidae), and *P. marmoratus* (Leptodactylidae) were infected by different species of terrestrial nematodes and digenetics. However, this habit did not seem to influence on parasite composition. Differences may be related to low phylogenetic proximity among these anurans since they belong to two families and three genus.

Among hylids, *P. paradoxa* is the unique with aquatic habit and its parasite fauna had five species of digenetics and two nematodes (*Brevimulticaecum* sp. and *Cosmocerca podicipinus*). Larvae of *Brevimulticaecum* sp. uses anurans as intermediate host and other aquatic vertebrates as alligator to complete its life cycle (Anderson 2000). *Cosmocerca podicipinus* is a generalist nematode, which its infective larvae is found in soil and penetrates into the host's skin (Anderson 2000). However, the digenetic *Neohaematoloechus neivai* Travassos & Artigas, 1927 was found only in this aquatic hylid. Tree frogs, such as *Scinax* spp. were the unique infected with the oligochaete *D. allodero lutzi* suggesting a certain specificity with this parasite. *Trachycephalus typhonius* was the unique anuran species infected with *P. cf. lopezromani* and *Parapharyngodon cf. alvarengai* Freitas, 1957; and *Gorgoderina diaster* Lutz, 1926 was

found only in *Hypsiboas* spp.. Some studies have suggested that specialist species are found in communities with high richness (Campião et al. 2015a), but in the present study the restriction of some parasite species in specific hosts were not related to community richness since these specialist parasites were not found in the most rich community.

The influence of host's body size on parasite composition was significant for anurans in the present study. Since body size is an intrinsic feature of species we may consider it as a determined phylogenetically feature which varies among anuran groups. Other studies also found the importance of host's body size in determining parasite richness or composition (Tavares and Luque 2008; Brito et al. 2014), and almost all discussed the fact that larger hosts provide great amount of niches for parasites and large surface area increasing the chances of colonization by parasites (Poulin 2007b).

Other variables as seasonality and environment features have not been related with parasite composition of these anurans. Some studies such as Bolek and Coggins (2000) and Hamann et al. (2006) have showed that these variables are more related to parameters of parasite abundance. Moreover, richness of parasites can be related to the chances of a species to colonize and stay in a new host species (MacArthur and Wilson 1967; Poulin 1997).

The fact that host features as species and body size are significantly predictors in parasite composition agreed with intimate and dependent relationship between parasite and host (Poulin 2007b). The nine immature stages of parasites found here (*Brevimulticaecum* sp., *Bursotrema* sp., cystacanth, *Clinostomum* sp., *Heterodiplostomum* sp., *Lophosicyadiplostomum* sp., unknow metacercariae, *Physaloptera* sp., and *Spiroxys* sp.) also reflect this complex relationship, because they need to reach the definitive host. Then, the intermediate host has to be preyed by an appropriate species or group of vertebrate (Poulin et al. 2013). For instance, in aquatic environment, parasites can use birds as definitive host and fish as intermediate since birds generally occupy higher trophic levels than fish (some birds eat fish, though no fish eat birds) (Poulin et al. 2013). Studies on trophic networks are able to explain better the importance of immature stages in interactions among different trophic levels due to generalist nature of these parasites that allow high number of connections (Bellay et al. 2013; Campiã et al. 2015a).

Therefore, these findings suggested that regardless of host habit, season or environmental conditions, the anurans of the present study with similar body size and

species closely related, presented more similarities in their parasite fauna. In other words, hosts belonging to the same species in different environment or season presented parasite fauna more similar than the other sympatric species. Similarities were resulted from historical effects as ancestral inheritance and phylogenetic position of a species can be related to its ecological (habitat, diet, etc.) and immunological characteristics as well as past history (biogeographic area of origin, etc.) (Poulin et al. 2010). However, some congeneric host species may present different parasite species, because changes in the composition of parasite fauna can occur rapidly, for instance following range expansion (Poulin and Morand 2004). According to Poulin et al. (2010), "The combined information conveyed by a species' phylogenetic position possibly makes it a much better predictor of how many parasite species have been acquired over evolutionary time by a particular host lineage than any ecological variable on its own".

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Table 1 – Anuran species collected in dry and rainy seasons from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil.

Family	habit	Anuran species	Dry	Rainy	Total	
Bufonidae	terrestrial	<i>Rhinella schneideri</i> (Werner, 1894)	20	1	21	
Hylidae	arboreal	<i>Dendropsophus minutus</i> (Peters, 1872)	12	25	37	
		<i>Pithecopus azureus</i> (Cope, 1862)	47	0	47	
		<i>Scinax</i> sp.	0	3	3	
		<i>Scinax fuscovarius</i> (A. Lutz, 1925)	35	16	51	
		<i>Scinax fuscomarginatus</i> (A. Lutz, 1925)	1	0	1	
		<i>Scinax</i> cf. <i>nasicus</i> (Cope, 1862)	11	1	12	
		<i>Scinax</i> cf. <i>ruber</i> (Laurenti, 1768)	4	1	5	
		<i>Scinax</i> cf. <i>similis</i> (Cochran, 1952)	0	2	2	
		<i>Trachycephalus typhonius</i> (Linnaeus, 1758)	13	3	16	
		<i>Hypsiboas raniceps</i> Cope, 1862	32	12	44	
		<i>Hypsiboas</i> aff. <i>raniceps</i>	27	8	35	
		aquatic	<i>Pseudis paradoxa</i> (Linnaeus, 1758)	15	23	38
	Leptodactylidae	terrestrial	<i>Leptodactylus fuscus</i> (Schneider, 1799)	50	0	50
<i>Leptodactylus mystacinus</i> (Burmeister, 1861)			8	0	8	
<i>Leptodactylus chaquensis</i> Cei, 1950			106	37	143	
<i>Leptodactylus latrans</i> (Steffen, 1815)			14	6	20	
<i>Leptodactylus podicipinus</i> (Cope, 1862)			124	101	225	
<i>Pseudopaludicola mystacalis</i> (Cope, 1887)			42	17	59	
<i>Physalaemus albonotatus</i> (Steindachner, 1864)			0	23	23	
<i>Physalaemus centralis</i> Bokermann, 1962			15	20	35	
<i>Physalaemus cuvieri</i> Fitzinger, 1826			9	23	32	
<i>Physalaemus nattereri</i> (Steindachner, 1863)			6	0	6	
Cryptozoic			<i>Physalaemus marmoratus</i> (Reinhardt & Lütken, 1862)	6	0	6
Microhylidae			Cryptozoic	<i>Dermatonotus muelleri</i> (Boettger, 1885)	19	0
		<i>Elachistocleis bicolor</i> (Valenciennes in Guérin-Menéville,1838)		26	14	40
Total			642	336	978	

Table 2 - Composition of metazoan parasites associated with anurans from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil.

Anurans	<i>Aplectana hylambatis</i>	<i>Aplectana membranosa</i>	<i>Cosmocerca</i> cf. <i>chilensis</i>	<i>Cosmocerca parva</i>	<i>Cosmocerca podicipinus</i>	<i>Raillietnema minor</i>	<i>Raillietnema</i> sp.	<i>Schrankiana formosula</i>	Cosmocercidae	<i>Parapharyngodon</i> cf. <i>alvarengai</i>	<i>Falcaustra mascula</i>	<i>Oxyascaris caudacutus</i>	<i>Oxyascaris</i> sp.	<i>Ochoterella digiticauda</i>	<i>Ochoterella</i> sp.	<i>Oswaldocruzia mazzai</i>	<i>Rhabdias</i> sp1	<i>Rhabdias</i> sp2	<i>Rhabdias</i> sp3	<i>Physaloptera</i> sp.	<i>Spiroxys</i> sp.	<i>Brevimulticaecum</i> sp.	<i>Brachycoelium salamandrae</i>	<i>Catadiscus marinhoi</i> <i>lutzi</i>	<i>Catadiscus propinquus</i>	<i>Catadiscus</i> sp.	<i>Gorgoderina diaster</i>	<i>Gorgoderina</i> sp.	<i>Neohaematoloechus neivai</i>	<i>Rauschiella</i> sp.	<i>Bursotrema</i> sp.	<i>Clinostomum</i> sp.	<i>Heterodiplostomum</i> sp.	<i>Lophosicyadiplostomum</i> sp.	Unknow metacercaria	<i>Polystoma</i> cf. <i>lopezromani</i>	<i>Cylindrotaenia americana</i>	Centrorhynchidae	cystacanth	<i>Dero (Allodero) lutzi</i>				
<i>Rhinella schneideri</i>								X		X				X		X	X					X								X														
<i>Dendropsophus minutus</i>																						X											X			X	X							
<i>Hypsiboas</i> aff. <i>raniceps</i>		X						X						X								X				X	X			X			X											
<i>Hypsiboas raniceps</i>								X						X			X		X		X					X	X			X				X							X			
<i>Scinax</i> cf. <i>nasicus</i>																																							X					
<i>Scinax</i> cf. <i>ruber</i>								X									X																	X										
<i>Scinax</i> cf. <i>similis</i>									X											X	X																		X		X			
<i>Scinax fuscomarginatus</i>								X																															X					
<i>Scinax fuscovarius</i>	X	X						X			X						X				X														X			X			X			
<i>Scinax</i> sp.																																												

Table 2 - continue

Anurans	<i>Aplectana hylambatis</i>	<i>Aplectana membranosa</i>	<i>Cosmocerca</i> cf. <i>chilensis</i>	<i>Cosmocerca parva</i>	<i>Cosmocerca podicipinus</i>	<i>Raillietnema minor</i>	<i>Raillietnema</i> sp.	<i>Schrankiana formosula</i>	Cosmocercidae	<i>Parapharyngodon</i> cf. <i>alvarengai</i>	<i>Falcaustra mascula</i>	<i>Oxyascaris caudacutus</i>	<i>Oxyascaris</i> sp.	<i>Ochoterenella digiticauda</i>	<i>Ochoterenella</i> sp.	<i>Oswaldocruzia mazzai</i>	<i>Rhabdias</i> sp1	<i>Rhabdias</i> sp2	<i>Rhabdias</i> sp3	<i>Physaloptera</i> sp.	<i>Spiroxys</i> sp.	<i>Brevimulticaecum</i> sp.	<i>Brachycoelium salamandrae</i>	<i>Catadiscus marinhoi</i> lutzi	<i>Catadiscus propinquus</i>	<i>Catadiscus</i> sp.	<i>Gorgoderina diaster</i>	<i>Gorgoderina</i> sp.	<i>Neohaematoloechus neivai</i>	<i>Rauschiella</i> sp.	<i>Bursotrema</i> sp.	<i>Clinostomum</i> sp.	<i>Heterodiplostomum</i> sp.	<i>Lophosicyadiplostomum</i> sp.	Unknow metacercaria	<i>Polystoma</i> cf. <i>lopezromani</i>	<i>Cylindrotaenia americana</i>	Centrorhynchidae	cystacanth	<i>Dero (Allodero) lutzi</i>			
<i>Pithecopus azureus</i>			X	X			X													X	X	X		X	X	X								X									
<i>Trachycephalus typhonius</i>	X							X	X								X						X											X		X		X					
<i>Pseudis paradoxa</i>				X																	X			X	X				X	X													
<i>Leptodactylus chaquensis</i>	X			X				X		X	X	X						X			X			X	X			X		X				X						X			
<i>Leptodactylus fuscus</i>	X						X	X												X	X		X	X						X				X									
<i>Leptodactylus latrans</i>	X							X		X		X										X				X				X					X						X		
<i>Leptodactylus mystacinus</i>	X																	X	X			X																					
<i>Leptodactylus podicipinus</i>	X			X	X	X	X	X		X								X	X	X	X			X	X					X		X	X	X	X			X		X			

Table 2 - continue

Anurans	<i>Aplectana hylambatis</i>	<i>Aplectana membranosa</i>	<i>Cosmocerca cf. chilensis</i>	<i>Cosmocerca parva</i>	<i>Cosmocerca podicipinus</i>	<i>Raillietnema minor</i>	<i>Raillietnema</i> sp.	<i>Schrankiana formosula</i>	Cosmocercidae	<i>Parapharyngodon</i> cf. <i>alvarengai</i>	<i>Falcaustra mascula</i>	<i>Oxyascaris caudacutus</i>	<i>Oxyascaris</i> sp.	<i>Ochoterenella digiticauda</i>	<i>Ochoterenella</i> sp.	<i>Oswaldocruzia mazzai</i>	<i>Rhabdias</i> sp1	<i>Rhabdias</i> sp2	<i>Rhabdias</i> sp3	<i>Physaloptera</i> sp.	<i>Spiroxys</i> sp.	<i>Brevimulticaecum</i> sp.	<i>Brachycoelium salamandrae</i>	<i>Catadiscus marinhoi</i> <i>lutzi</i>	<i>Catadiscus propinquus</i>	<i>Catadiscus</i> sp.	<i>Gorgoderina diaster</i>	<i>Gorgoderina</i> sp.	<i>Neohaematoloechus neivai</i>	<i>Rauschiella</i> sp.	<i>Bursotrema</i> sp.	<i>Clinostomum</i> sp.	<i>Heterodiplostomum</i> sp.	<i>Lophosicyadiplostomum</i> sp.	Unknow metacercaria	<i>Polystoma</i> cf. <i>lopezromani</i>	<i>Cylindrotaenia americana</i>	Centrorhynchidae	cystacanth	<i>Dero (Allodero) lutzi</i>				
<i>Pseudopaludicola mystacalis</i>								X																X	X								X	X		X								
<i>Physalaemus albonotatus</i>								X																										X					X					
<i>Physalaemus centralis</i>	X							X										X							X				X				X					X						
<i>Physalaemus cuvieri</i>								X										X												X														
<i>Physalaemus marmoratus</i>																					X																							
<i>Physalaemus nattereri</i>																					X																							
<i>Dermatonotus muelleri</i>	X																																											
<i>Elachistocleis bicolor</i>								X																						X				X					X					

Table 3 - Parasite species of arboreal, aquatic, cryptozoic, and terrestrial anurans in rainy and dry seasons from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil.

	<i>Aplectana hylambatis</i>	<i>Brachycoelium salamandrae</i>	<i>Catadiscus marinhoi</i>	<i>Cosmocerca parva</i>	<i>Schrankiana formosula</i>	<i>Unknow metacercaria</i>	<i>Ochoterella</i> sp.	<i>Oswaldocruzia mazzai</i>	<i>Oxyascaris caudatus</i>	<i>Oxyascaris</i> sp.	<i>Raillietnema minor</i>	<i>Rhabdias</i> sp1	<i>Aplectana membranosa</i>	<i>Brevimulticaecum</i> sp.	<i>Bursotrema</i> sp.	<i>Catadiscus propinquus</i>	<i>Catadiscus</i> sp.	<i>Centrorhynchidae</i>	<i>cystacanth</i>	<i>Cosmocerca podicipinus</i>	<i>Cosmocercidae</i>	<i>Cylindrotaenia americana</i>	<i>Dero (Allodero) luzi</i>	<i>Falcaustra mascula</i>	<i>Gorgoderina diaster</i>	<i>Heterodiplostomum</i> sp.	<i>Lophosicyadiplostomum</i> sp.	<i>Neohaematoloechus neivai</i>	<i>Parapharyngodon</i> cf. <i>alvarengai</i>	<i>Physaloptera</i> sp.	<i>Polystoma</i> cf. <i>lopezromani</i>	<i>Rauschiella</i> sp.	<i>Rhabdias</i> sp2	<i>Rhabdias</i> sp3	<i>Spiroxys</i> sp.	<i>Clinostomum</i> sp.	<i>Cosmocerca</i> cf. <i>chilensis</i>	<i>Gorgoderina</i> sp.	<i>Ochoterella digiticauda</i>	<i>Raillietnema</i> sp.			
Rainy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
terrestrial		X	X		X	X		X		X		X	X	X	X	X	X	X	X	X	X	X		X		X	X			X		X		X									
arboreal			X	X			X		X		X		X	X		X	X	X	X		X	X	X		X		X		X	X	X	X	X	X									
aquatic														X		X												X					X										
criptozoic	X													X				X									X						X										
Dry													X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
terrestrial													X	X	X	X	X	X	X	X	X	X		X			X				X		X		X			X		X	X	X	X
arboreal														X			X	X					X		X		X		X	X	X		X		X			X					
aquatic														X		X	X			X					X		X						X										
criptozoic																				X								X															

Table 4- Anuran species collected in nine areas from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil

Anurans	anthropogenic	ponds	Angico	reforestation III	reforestation V	Brachiaría	Open field	Buriti	dirt track	Total
<i>Rhinella schneideri</i>	5	7	2	6	0	1	0	0	0	21
<i>Dendropsophus minutus</i>	15	22	0	0	0	0	0	0	0	37
<i>Hypsiboas aff. raniceps</i>	0	35	0	0	0	0	0	0	0	35
<i>Hypsiboas raniceps</i>	19	25	0	0	0	0	0	0	0	44
<i>Pithecopus azureus</i>	21	26	0	0	0	0	0	0	0	47
<i>Scinax cf. nasicus</i>	12	0	0	0	0	0	0	0	0	12
<i>Scinax cf. ruber</i>	4	0	1	0	0	0	0	0	0	5
<i>Scinax cf. similis</i>	0	0	0	0	0	0	0	2	0	2
<i>Scinax fuscomarginatus</i>	1	0	0	0	0	0	0	0	0	1
<i>Scinax fuscovarius</i>	41	0	4	5	0	0	0	1	0	51
<i>Scinax sp.</i>	1	0	2	0	0	0	0	0	0	3
<i>Trachycephalus typhonius</i>	12	1	1	1	0	0	0	1	0	16
<i>Pseudis paradoxa</i>	3	35	0	0	0	0	0	0	0	38
<i>Leptodactylus chaquensis</i>	3	73	6	21	2	4	22	3	5	139
<i>Leptodactylus fuscus</i>	10	9	1	5	6	5	12	0	1	49
<i>Leptodactylus latrans</i>	2	13	0	0	0	0	2	0	3	20
<i>Leptodactylus mystacinus</i>	0	0	3	3	0	1	1	0	0	8
<i>Leptodactylus podicipinus</i>	10	172	16	1	1	0	6	0	0	206
<i>Pseudopaludicola mystacalis</i>	14	45	0	0	0	0	0	0	0	59
<i>Physalaemus albonotatus</i>	0	1	12	4	1	5	0	0	0	23
<i>Physalaemus centralis</i>	4	1	10	1	10	2	7	0	0	35
<i>Physalaemus cuvieri</i>	1	1	7	5	18	0	0	0	0	32
<i>Physalaemus nattereri</i>	6	0	0	0	0	0	0	0	0	6
<i>Physalaemus marmoratus</i>	5	0	0	1	0	0	0	0	0	6
<i>Dermatonotus muelleri</i>	12	0	0	7	0	0	0	0	0	19
<i>Elachistocleis bicolor</i>	8	1	6	3	6	1	7	0	6	38
Total	209	467	71	63	44	19	57	7	15	952

Table 5- Parasite species of anurans from nine areas of Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil.

Areas	<i>Aplectana membranosa</i>	<i>Brevimulticaecum</i> sp.	<i>Cosmocercidae</i>	<i>Centrorhynchidae</i>	<i>Lophosicyadiplostomum</i> sp.	<i>Catadiscus</i> sp.	<i>Catadiscus propinquus</i>	<i>Rauschiella</i> sp.	<i>Rhabdias</i> sp3	<i>Cosmocerca podicipinus</i>	<i>Physaloptera</i> sp.	<i>Polystoma</i> cf. <i>lopezromani</i>	<i>Rhabdias</i> sp1	<i>Bursotrema</i> sp.	<i>Schrankiana formosula</i>	<i>Rhabdias</i> sp2	<i>cystacanth</i>	<i>Catadiscus marinholutzi</i>	<i>Spiroxys</i> sp.	<i>Aplectana hylambatis</i>	<i>Cylindrotaenia americana</i>	<i>Dero (Allobero)</i> <i>lutzi</i>	<i>Gorgoderina diaster</i>	<i>Neohaematoloechus neivai</i>	<i>Ochoterella</i> sp.	<i>Oxyascaris</i> sp.	<i>Parapharyngodon</i> cf. <i>alvarengai</i>	<i>Raillietnema minor</i>	<i>Raillietnema</i> sp.	<i>Oswaldocruzia mazzai</i>	<i>Oxyascaris caudacutus</i>	<i>Ochoterella digiticauda</i>	<i>Gorgoderina</i> sp.	<i>Heterodiplostomum</i> sp.	Unknown <i>mmetacercaria</i>	<i>Falcaustra mascula</i>	<i>Brachycoelium salamandrae</i>	<i>Clinostomum</i> sp.	<i>Cosmocerca</i> cf. <i>chilensis</i>	<i>Cosmocerca parva</i>		
Anthropogenic	X	X	X	X	X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X		X	X											X	X	X	
Ponds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X		X	X	X	X					X			X								
Open field	X	X	X	X	X	X	X	X	X					X			X	X																								
Angico	X	X	X	X	X	X	X	X	X	X		X		X		X			X																			X				
Reforestation III	X	X	X		X	X			X		X		X		X	X				X						X					X											
Reforestation V	X	X	X	X	X				X	X				X	X												X															
Brachiaria	X	X	X					X					X																													
Buriti	X	X		X							X	X							X			X					X							X								
Dirt track	X	X	X			X	X																																			

Table 6- Bray-curtis similarity index (*B*) of parasite composition among anurans from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil. (as = anuran species).

as	Dmi	Dmu	Ebi	Haff	Hran	Lcha	Lfus	Llatr	Lmys	Lpod	Pazu	Palbo	Pcent	Pcuv	Pmar	Pnat	Ppara	Pmys	Rsch	Snas	Srub	Sfus	Ttyph
Dmi	1,000	0,000	0,500	0,333	0,286	0,235	0,308	0,308	0,000	0,300	0,308	0,571	0,364	0,000	0,400	0,400	0,182	0,400	0,182	0,400	0,286	0,308	0,333
Dmu		1,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Ebi			1,000	0,500	0,429	0,353	0,462	0,462	0,000	0,300	0,308	0,857	0,545	0,286	0,000	0,000	0,182	0,400	0,364	0,400	0,571	0,462	0,500
Haff				1,000	0,778	0,571	0,588	0,706	0,167	0,500	0,471	0,364	0,533	0,182	0,222	0,222	0,400	0,429	0,400	0,000	0,364	0,353	0,375
Hran					1,000	0,522	0,526	0,632	0,143	0,538	0,526	0,308	0,353	0,154	0,182	0,182	0,353	0,375	0,353	0,000	0,462	0,316	0,333
Lcha						1,000	0,545	0,818	0,235	0,759	0,455	0,250	0,500	0,250	0,143	0,143	0,500	0,421	0,400	0,000	0,250	0,273	0,286
Lfus							1,000	0,556	0,308	0,560	0,667	0,333	0,375	0,167	0,200	0,200	0,375	0,400	0,375	0,000	0,333	0,333	0,471
Llatr								1,000	0,154	0,640	0,444	0,333	0,500	0,167	0,200	0,200	0,375	0,400	0,500	0,000	0,333	0,333	0,353
Lmys									1,000	0,300	0,154	0,000	0,364	0,286	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,154	0,167
Lpod										1,000	0,480	0,211	0,435	0,211	0,118	0,118	0,522	0,455	0,348	0,000	0,211	0,240	0,250
Pazu											1,000	0,333	0,375	0,167	0,200	0,200	0,375	0,533	0,250	0,000	0,333	0,222	0,353
Palbo												1,000	0,600	0,333	0,000	0,000	0,000	0,444	0,200	0,500	0,667	0,500	0,545
Pcent													1,000	0,600	0,000	0,000	0,143	0,462	0,143	0,250	0,400	0,500	0,533
Pcuv														1,000	0,000	0,000	0,000	0,222	0,200	0,000	0,333	0,167	0,182
Pmar															1,000	1,000	0,250	0,000	0,250	0,000	0,000	0,000	0,000
Pnat																1,000	0,250	0,000	0,250	0,000	0,000	0,000	0,000
Ppara																	1,000	0,308	0,286	0,000	0,000	0,000	0,000
Pmys																		1,000	0,154	0,000	0,444	0,267	0,286
Rsch																			1,000	0,000	0,200	0,125	0,133
Snas																				1,000	0,000	0,200	0,222
Srub																					1,000	0,500	0,545
Sfus																						1,000	0,588
Ttyph																							1,000

^{as}Dmi (*Dendropsophus minutus*), Dmu (*Dermatonotus muelleri*), Ebi (*Elachistocleis bicolor*), Haff (*Hypsiboas aff. raniceps*), Hran (*Hypsiboas raniceps*), Lcha (*Leptodactylus chaquensis*), Lfus (*Leptodactylus fuscus*), Llatr (*Leptodactylus latrans*), Lpod (*Leptodactylus podicipinus*), Lmys (*Leptodactylus mystacinus*), Palbo (*Physalaemus albonotatus*), Pazu (*Pithecopus azureus*), Pcent (*Physalaemus centralis*), Pcuv (*Physalaemus cuvieri*), Pmar (*Physalaemus marmoratus*), Pmys (*Pseudopaludicola mystacalis*), Pnat (*Physalaemus nattereri*), Ppara (*Pseudis paradoxa*), Rsch (*Rhinella schneideri*), Sfus (*Scinax fuscovarius*), Srub (*Scinax cf. ruber*), Snas (*Scinax cf. nasicus*), Ttyph (*Trachycephalus typhonius*).

Table 7- Permutational Multivariate Analysis of Variance (PERMANOVA) using parasite composition of anurans from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo State, Brazil.

Variables	Df	Sums of squares	Mean squares	F. Model	R ²	p
host species	24	85.448	3.5603	13.1130	0.35227	0.001***
season	1	0.348	0.3480	1.2817	0.00143	0.226
SVL	133	41.644	0.3131	1.1532	0.17168	0.001***
environment	9	2.987	0.3318	1.2222	0.01231	0.085
residuals	413	112.135	0.2715	0.46229	0.46229	-
Total	580	242.561	1.00000	-	-	-

*** Significant values

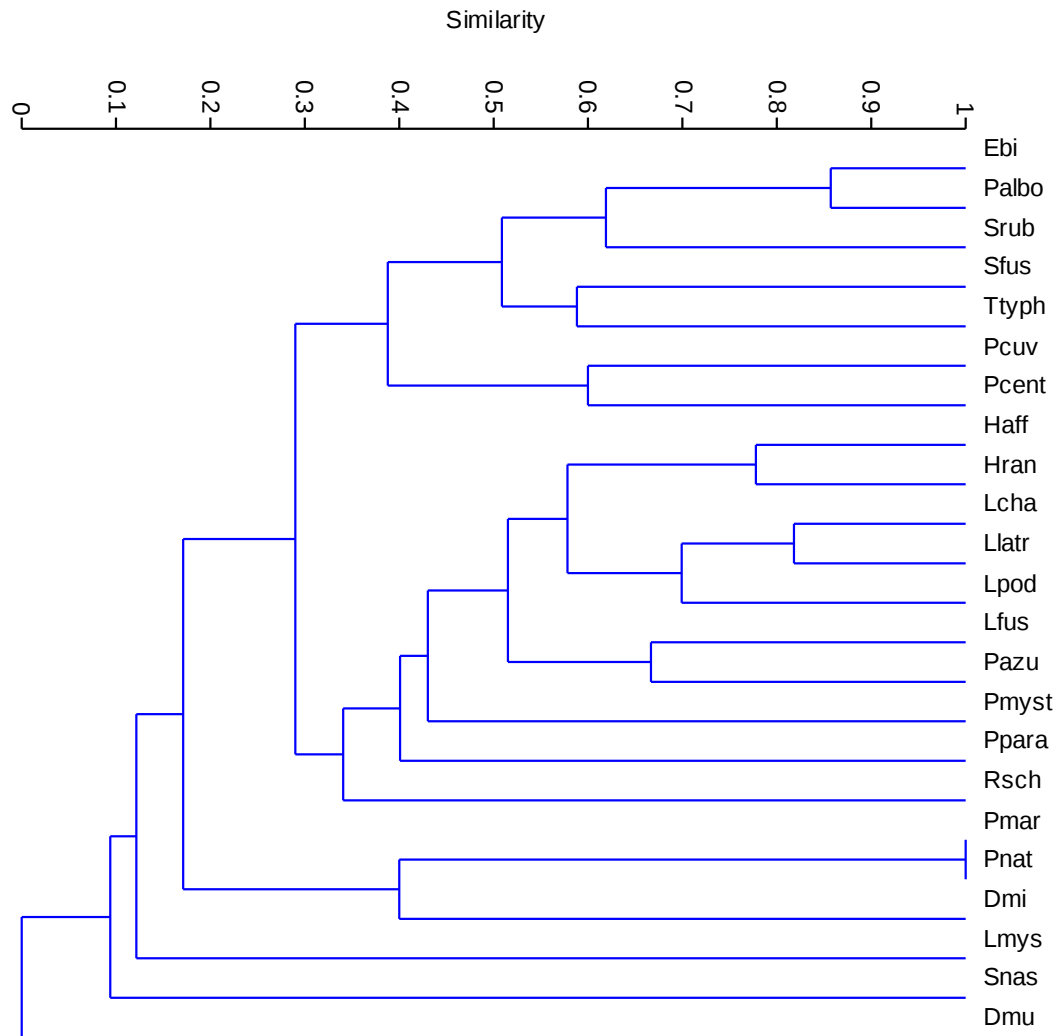


Fig. 1 - Cluster analysis with Bray-curtis index based on presence and absence of parasite composition of anurans from Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí, municipality of Castilho, São Paulo, Brazil, (cofenetic coefficient: 0.6932). Dmi (*Dendropsophus minutus*), Dmu (*Dermatonotus muelleri*), Ebi (*Elachistocleis bicolor*), Haff (*Hypsiboas* aff. *raniceps*), Hran (*Hypsiboas raniceps*), Lcha (*Leptodactylus chaquensis*), Lfus (*Leptodactylus fuscus*), Llatr (*Leptodactylus latrans*), Lpod (*Leptodactylus podicipinus*), Lmys (*Leptodactylus mystacinus*), Palbo (*Physalaemus albonotatus*), Pazu (*Pithecopus azureus*), Pcent (*Physalaemus centralis*), Pcuv (*Physalaemus cuvieri*), Pmar (*Physalaemus marmoratus*), Pmyst (*Pseudopaludicola mystacalis*), Pnat (*Physalaemus nattereri*), Ppara (*Pseudis paradoxa*), Rsch (*Rhinella schneideri*), Sfus (*Scinax fuscovarius*), Srub (*Scinax* cf. *ruber*), Snas (*Scinax* cf. *nasicus*), Ttyph (*Trachycephalus typhonius*).

Conclusões



Conclusões

Considerando o delineamento experimental proposto e os resultados obtidos no presente estudo podemos concluir que:

- 1) A comunidade de anuros estudada (26 espécies), pertencentes as famílias Bufonidae, Hylidae, Leptodactylidae e Microhylidae, apresentou elevada riqueza de endoparasitas metazoários;
- 2) A riqueza, a diversidade e composição de endoparasitos metazoários difere entre as espécies de anuros das famílias estudadas;
- 3) Variáveis ambientais como seca e cheia, bem como as diferentes condições de habitat não influenciaram a estruturação da composição das comunidades de endoparasitas metazoários.
- 4) A espécie hospedeira e o tamanho corporal dos indivíduos foram variáveis preditoras na composição das comunidades de endoparasitas metazoários.