Helminth parasite communities of two *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leiuperidae) populations under different conditions of habitat integrity in the Atlantic Rain Forest of Brazil

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Abstract

Adults of *Physalaemus cuvieri* were collected and necropsied between November 2009 and January 2010. This was carried out in order to report and compare the helminth fauna associated with two populations of this anuran species from the Brazilian Atlantic rain forest under different conditions of habitat integrity. The hosts from the disturbed area were parasitized with five helminth taxa: *Cosmocerca parva*, *Aplectana* sp., *Physaloptera* sp., *Rhabdias* sp., *Oswaldocruzia subauricularis* (Nematoda) and *Polystoma cuvieri* (Monogenea) while those from the preserved area had four helminth taxa: *C. parva*, *Aplectana* sp., *Physaloptera* sp., *Rhabdias* sp., and *Acanthocephalus saopaulensis* (Acanthocephala). Prevalence, mean intensity of infection, mean abundance, mean richness, importance index and dominance frequency of helminth component communities were similar in both areas. The helminth community associated with anurans from the disturbed area had higher diversity than that from the preserved area. This study is the first to report on the acanthocephalan parasites of *Ph. cuvieri*, and the similarity between helminth fauna composition of two host populations under different selective pressures.

Keywords: amphibian, Atlantic Rain Forest, component community, helminth fauna, parasites.

Comunidade de helmintos parasitos de duas populações de *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leiuperidae) sob diferentes condições de integridade de habitat da Mata Atlântica, Brasil

Resumo

Adultos de *Physalaemus cuvieri* foram coletados e necropsiados entre Novembro de 2009 e Janeiro de 2010. Este estudo foi realizado com o objetivo de informar e comparar a helmintofauna associada a duas populações desta espécie de anuro proveniente da Mata Atlântica sob duas condições de integridade de hábitat. Os hospedeiros da área perturbada estavam parasitados por cinco *taxa* de helmintos: *Cosmocerca parva*, *Aplectana* sp., *Physaloptera* sp., *Rhabdias* sp., *Oswaldocruzia subauricularis* (Nematoda) e *Polystoma cuvieri* (Monogenea), enquanto aqueles da área preservada apresentaram quatro *taxa* de helmintos: *C. parva*, *Aplectana* sp., *Physaloptera* sp., *Rhabdias* sp., e *Acanthocephalus saopaulensis* (Acanthocephala). Prevalência, intensidade média de infecção, abundância média, riqueza média, índice de importância específica e freqüência de dominância da comunidade componente dos helmintos foram similares em ambas as áreas. A comunidade helmíntica associada aos anuros da área perturbada apresentou-se mais diversa do que a encontrada naqueles da área preservada. Este estudo é o primeiro a relatar parasitas acantocéfalos em *Ph. cuvieri*, e a semelhança entre a composição da helmintofauna de duas populações hospedeiras sob diferentes pressões seletivas.

Palavras-chave: anfíbios, Mata Atlântica, comunidade componente, helminto fauna, parasitas.

1. Introduction

The Atlantic rain forest harbours the greatest diversity of anurans in Brazil, approximately 180 species of amphibians (Haddad, 1998; Haddad and Prado, 2005). However, due to the constant environmental degradation and forest fragmentation this high diversity is threatened (Young et al., 2001; Eterovick et al., 2005; Lips et al., 2005), which makes this biome one of the 10 hotspots of world biodiversity (Myers et al., 2000; Mittermeier et al., 2005). The Atlantic rain forest in São Paulo State has been substantially affected by forest fragmentation because prolonged intense exploitation of natural resources (Myers et al., 2000; Mittermeier et al., 2005). Nevertheless, continuous strips of forest still exist in state parks such as the Parque Estadual da Serra do Mar – Núcleo Santa Virgínia (Fundação SOS Mata Atlântica, 2008).

In this context of habitat fragmentation, parasites and predators are some of the first species to be affected by habitat fragmentation, mainly in relation to their distribution and abundance (Gibb and Hochuli, 2002; Laurance et al., 2002); however, the knowledge of those the dynamics of these changes is not well poorly known understood (McCallum and Dobson, 2002). According to McCallum and Dobson (1995), the parasites, like their hosts, also have great ecological importance and contribute to the maintenance of diversity.

Apart from habitat fragmentation (Hartvigsen and Halvorsen, 1994; Poulin and Morand, 1999), factors such as host biology (Aho, 1990; Hamann et al., 2006) and the composition of the host fauna can influence the composition of parasite communities (Poulin and Mouillot, 2003).

In anthropogenic environments, characterized by low biodiversity, parasites with heteroxenic life cycles have little chance of finding the host and completing their life cycle. In this case, it is expected that parasites with monoxenic life cycles are more prevalent. On the other hand, in preserved environments, with high biodiversity, the parasites with heteroxenic life cycles parasite species can be biologically successful because of the presence of hosts that are necessary for their life cycle (Gibb and Hochuli, 2002; Laurance et al., 2002; McKenzie, 2007).

Physalaemus cuvieri, popularly known as "Frog-dog", is an anuran species of small size (approximately 2.75 cm) and widely distribution, ranging from northeastern to mid-western and southern Brazil, and parts of Uruguay, Paraguay and Argentina (Haddad et al., 2008; Frost, 2013). It is mainly nocturnal and reproduces in small water bodies of water within open areas, present in all Brazilian biomes (Dixo and Verdade, 2006; Pombal and Haddad, 2007).

This study aims to characterize the composition of helminth communities associated with populations of *Ph. cuvieri* from two Brazilian Atlantic rain forest sites under different conditions of habitat integrity: a rural area with pastures and forest fragments and a preserved forest area.

2. Material and Methods

2.1. Study area and collection of amphibian hosts

In November 2009 and January 2010, 43 specimens of *Ph. cuvieri* were collected (permission granted through SISBIO, the Brazilian Biodiversity Information System - license no. 18240-1) at two contrasting areas of Atlantic rain forest in São Paulo State, Brazil: 21 specimens in the disturbed area and 22 in the preserved area. The disturbed area is located in the São Luiz do Paraitinga (SLP) municipality (23° 13' S; 45° 18' W) and it is characterized by a mosaic of pastures and forest fragments. The preserved area is located in the Serra do Mar State Park – Núcleo Santa Virgínia (NSV) (23° 24' S; 45° 03' W), belonging to the municipalities São Luís do Paraitinga, Cunha, Ubatuba and Natividade da Serra. Both locations are in the Serra do Mar region known as Alto do Rio Paraíba – between the Paraíba do Sul river valley and the northern coast of São Paulo State. The region has a mean altitude of about 742 m and is characterized by a humid temperate climate with a dry winter and hot summer (CEPAGRI, 2008). The frogs were captured during visual searches at night; afterwards, they were killed with sodium thiopental solution (Thiopentax®), necropsied and all organs were examined in search of helminths.

2.2. Parasitological procedures

The helminths found were collected, fixed in alcohol-formaldehyde-acetic acid solution and preserved in 70% ethyl alcohol. The monogenoids were fixed under coverslips, applying slight pressure. For species identification, nematodes were cleared with lactophenol and monogenoids and acanthocephalans were stained with carmine and cleared with creosote, and analysed in a computerized image analysis system (Qwin Lite 3.1; Leica Microsystems, Wetzlar, Germany). Identification was based on Vaucher (1990), Vicente et al. (1991), Smales (2007), Anderson et al. (2009) and Gibbons (2010). The helminth parasites were deposited in the Helminthological Collection of the Biosciences Institute in Botucatu (CHIBB), Department of Parasitology, Universidade Estadual Paulista.

2.3. Data analysis

The ecological descriptors of parasitism - prevalence, mean abundance (MA) and mean intensity of infection (MII) - were calculated according to Bush et al. (1997). The importance index, an integrated index, was calculated by the sum of the prevalence value and relative abundance followed by multiplication by 100 (Bursey et al., 2001). This index is an overall estimate of the influence of a species within a community.The dominance frequency of each component from parasite infracommunities was calculated according to Rohde et al. (1995) and it is represented by the number of times that a parasite species is dominant in the total of hosts analysed. The Z test was used to compare the prevalences and the Mann–Whitney test was used to compare the abundances and the MII between populations. Both tests were calculated using Sigma-Stat 3.1 software. The Brillouin index was used for helminth fauna diversity where we excluded from the calculation the immature helminths, such as unidentified larvae and cysts, and female specimens of cosmocercid nematodes, since we could not identify all these nematodes at the species level. The diversity index was used calculated for helminth fauna associated with the two populations of anurans with Past software - 2.15 version.

3. Results

The overall prevalence was 90.9% in the NSV sample (20 individuals were infected by at least one parasite), and 85.7% (18 individuals were parasitized) in the SLP sample, and did not differ between localities ($Z = 0.056$; $p = 0.956$).

The frog population in the preserved area (NSV) had an MII of 4.9 ± 0.8 (range 1-13), an MA of 4.4 ± 0.8 (range 0-13) and mean helminth richness per host (MR) of 1.5 ± 0.1, whereas frogs from the disturbed area (SLP) had an MII of 5.7 ± 1.1 (range 2-19), an MA of 4.9 ± 1.0 (range 0-19) and an MR of 1.4 ± 0.2 (Table 1). There were no differences between the two areas for either in MII (Mann-Whitney U = 367.5; p = 0.64), MA (U = 466.5; p = 0.90) or MR (U = 341.0; p = 0.78) between the two areas.

The *Ph. cuvieri* specimens collected in NSV were parasitized by nematodes and an acanthocephalan: one *Aplectana* sp., 10 *Cosmocerca parva* Travassos, 1925 and 49 unidentified females of cosmocercids (Cosmocercidae), 17 *Physaloptera* sp. (Physalopteridae), three *Rhabdias* sp. (Rhabdiasidae), 17 unidentified cysts and larvae and one *Acanthocephalus saopaulensis* Smales, 2007 (Centrorhynchidae) (Table 2).

Table 1. Prevalence, mean abundance and mean intensity of infection (expressed as means with standard error), total richness and mean richness per host of helminth parasites of *Physalaemus cuvieri* from Núcleo Santa Virgínia (NSV) and from São Luis do Paraitinga (SLP), in São Paulo State, Brazil.

Parameters	NSV (*n* = 22)	SLP (*n* = 21)
Prevalence (%)	90.9	85.7
Mean abundance	4.4 ± 0.8	4.9 ± 1.0
Mean intensity of infection	4.9 ± 0.8	5.7 ± 1.1
Total richness	5	6
Mean richness per host	1.44 ± 0.2	1.38 ± 0.2

The SLP specimens were parasitized by nematodes and a monogenoid: five *Aplectana* sp., seven *C. parva*, 65 unidentified females of cosmocercids, 14 *Physaloptera* sp., four *Rhabdias* sp., three *Oswaldocruzia subauricularis* (Rudolphi, 1819) (Molineidae), and four *Polystoma cuvieri* Vaucher, 1990 (Polystomatidae) (Table 3). Among the samples in which male specimens were present, *C. parva* and *Aplectana* sp. were identified; the remaining cosmocercids were females that could not be identified to species due to the difficulty in distinguishing diagnostic morphological characters in females of this nematode group. *Physaloptera* sp. was found only in larval stages.

Cosmocercid nematodes had the highest importance index (I) values in both host populations studied (I_{NSV} = 142.8 and I_{SLP} = 155.9), followed by *Physaloptera* sp. with I_{NSV} = 58.3 and I_{SLP} = 28.0 (Tables 2 and 3). The cosmocercids were dominant over the other species of the helminth communities, with dominance frequencies of 16 and 15 in NSV and SLP, respectively.

The richness of helminth fauna associated with anurans from NSV was at least five species, and the anurans from SLP presented a richness of at least six helminth species. According to the Brillouin index, the helminth community

Table 2. Prevalence (P), mean intensity of infection (MII) and mean abundance (MA) with standard error (SE), importance index (I), dominance frequency (DF) and site of infection of helminth parasites of *Physalaemus cuvieri* (n = 22) from Núcleo Santa Virgínia, São Paulo State, Brazil.

Parasite	P (%)	MII ± SE	MA ± SE	I	DF	Site of infection
Nematoda						
Cosmocercidae	81.8	3.3 ± 0.5	2.7 ± 0.5	142.8	16	SI, LI, CAV, LUN
Unidentified cysts	9.1	7.5 ± 3.5	0.7 ± 0.5	24.4	1	STO
Unidentified larvae	4.5	2	0.1 ± 0.1	6.6	0	SI, LI
Physaloptera sp.	40.9	1.9 ± 0.3	0.8 ± 0.2	58.3	1	STO
Rhabdias sp.	9.1	1.5 ± 0.5	0.1 ± 0.1	12.1	1	LUN
Acanthocephala						
Acanthocephalus saopaulensis	4.5	1	0.04 ± 0.04	5.6	0	CAV

SI – small intestine; LI – large intestine; CAV – body cavity; LUN – lungs; STO – stomach.

Table 3. Prevalence (P), mean intensity of infection (MII) and mean abundance (MA) with standard error (SE), importance index (I), dominance frequency (DF) and site of infection of helminth parasites of *Physalaemus cuvieri* (n = 21) from São Luis do Paraitinga, São Paulo State, Brazil.

Parasite	P (%)	MII ± SE	MA ± SE	I	DF	Site of infection
Nematoda						
Cosmocercidae	80.9	4.5 ± 1	3.7 ± 0.9	155.9	15	STO, SI, LI
Physaloptera sp.	14.3	4.7 ± 3.2	0.7 ± 0.5	28.0	2	STO
Rhabdias sp.	9.5	2 ± 0	0.2 ± 0.1	13.4	0	LUN
Oswaldocruzia subauricularis	4.8	3	0.1 ± 0.1	7.7	0	SI
Monogenea						
Polystoma cuvieri	14.3	1.3 ± 0.3	0.2 ± 0.1	18.3	1	GB, UB

STO – stomach; SI – small intestine; LI – large intestine; LUN – lungs; GB – gall bladder; UB – urinary bladder.

Table 4. Comparison of mean abundance (MA) and mean intensity of infection (MII) (presented with standard error; SE) and prevalence of helminths of *Physalaemus cuvieri* from Núcleo Santa Virgínia (NSV) and São Luis do Paraitinga (SLP).

	NSV	SLP	Test	p
Cosmocercidae				
MA ± SE	2.7 ± 0.5	3.7 ± 0.9	*U* = 489.5	0.51
MII ± SE	3.3 ± 0.5	4.5 ± 1.0	*U* = 335.5	0.34
Prevalence (%)	81.8	80.9	*Z* = –0.3	0.75
Physaloptera sp.				
MA ± SE	0.8 ± 0.2	0.7 ± 0.5	*U* = 403.5	0.16
MII ± SE	1.9 ± 0.3	4.7 ± 3.2	*U* = 25.5	0.50
Prevalence (%)	40.9	14.3	*Z* = 1.6	0.11
Rhabdias sp.				
MA ± SE	0.1 ± 0.1	0.2 ± 0.1	*U* = 464.0	0.97
MII ± SE	1.5 ± 0.5	2.0 ± 0	*U* = 8.0	0.40
Prevalence (%)	9.1	9.5	*Z* = –0.5	0.64

diversity (H) from NSV frogs (H = 0.97) was lower than that from SLP frogs (H = 1.42).

The helminths shared by the two *Ph. cuvieri* populations were cosmocercid nematodes, *Physaloptera* sp. larvae and *Rhabdias* sp. So, we applied the Z test and the Mann–Whitney test to compare the ecological parameters of shared parasites between localities, and we did not find significant differences between prevalence, MII and MA of parasites associated with these *Ph. cuvieri* populations (Table 4).

4. Discussion

The *Ph. cuvieri* populations studied presented a relatively high parasite richness (four taxa from the preserved area and five taxa from the disturbed area) when compared with the helminth faunas associated with other species of *Physalaemus*, such as *Physalaemus signifer* (Girard, 1853), with three nematode species, and *Physalaemus soaresi* Izecksohn, 1965, with two nematode species (Fabio, 1982). On the other hand, Hamann and González (2009) and González and Hamann (2010) studied the helminths associated with *Physalaemus santafecinus* Barrio, 1965 and found three digenean taxa (in tadpoles) and four nematode taxa (in adults), respectively. For *Physalaemus albonotatus* (Steindachner, 1864), González and Hamann (2012) reported six nematode taxa.

Of all helminths recorded in the present study, only the monogenoid *Polystoma cuvieri* and the nematode *Cosmocerca parva* were previously reported in *Ph. cuvieri* (Vaucher, 1990; Santos and Amato, 2012, 2013). *Aplectana* sp. and *Physaloptera* sp. were previously reported in other species of this genus (Fábio, 1982; González and Hamann, 2010, 2012), corroborating the generalist pattern of the helminth communities associated with anurans (Aho, 1990; Bursey et al., 2001). There are no previous records of the occurrence of *Rhabdias* sp., *O. subauricularis* and *A. saopaulensis* parasitizing anurans of the genus *Physalaemus* in the South American continent. *Physalaemus cuvieri* is thus a new host for all helminth taxa found in the present study, excluding the monogenoid *Po. cuvieri* and the nematode *C. parva*.

Among the helminths found in both *Ph. cuvieri* populations, the acanthocephalan *A. saopaulensis* and the monogenoid *Po. cuvieri* are the least frequently reported in amphibians (Vaucher, 1990; Smales, 2007). The acanthocephalan transmission to anurans occurs by ingestion of invertebrates parasitized with cystacants that infect the gastrointestinal tract (Kennedy, 2006), making the anuran a definitive or paratenic host (Smales, 2007; Pinhão et al., 2009; Santos and Amato, 2010). The monogenoids, parasites of amphibian urinary bladders, have monoxenic cycles and high specificity with the host. They infect the individual by the gills when they are still in the tadpole phase. After the host's metamorphosis, they migrate to the urinary bladder and reproduce (Smyth, 1994).

Cosmocerca parva and *O. subauricularis* are transmitted directly via infective larvae that penetrate actively in the host's tegument, and adults later can reach the gastrointestinal tract (Anderson, 2000). The lung infection by *Rhabdias* spp. occurs similarly, but only by parthenogenetic females that alternate between free-living and parasitic life cycles in anurans (Anderson, 2000). *Aplectana* spp. are also transmitted directly, but the infection occurs by the ingestion of eggs containing infective larvae that reach the gastrointestinal tract and then mature (Anderson, 2000). *Physaloptera* sp. larvae are found in the stomach mucosa of anurans that ingest insects infected by larvae; the anuran can be a definitive or paratenic host (Anderson, 2000; González and Hamann, 2006).

In the helminth communities associated with the two *Ph. cuvieri* populations, cosmocercid nematodes had the highest prevalence, MA and dominance frequency (Tables 1 and 2), corroborating the generalist pattern of these anuran helminths (Bursey et al., 2001). This is associated with the fact that these species can easily infect a host in a direct way, not requiring intermediate hosts to complete their biological cycle (Anderson, 2000; Hamann et al., 2006).

The high prevalence and abundance of Cosmocercidae, *Physaloptera* sp. and *Rhabdias* sp. may have been responsible for the observed similarity between the two parasite communities (Poulin, 2007), which presented no statistical difference between the overall prevalence, MAs and MIIs of component communities. Among the helminths found in anurans from SLP and NSV, only *Physaloptera* sp. and *A. saopaulensis* have heteroxenic life cycles, in which parasites complete their biological cycle by trophic transmission, requiring an invertebrate as an intermediate host (Anderson, 2000; Kennedy, 2006). The importance of parasites as indicators of environmental quality has been shown in some studies (Gibb and Hochuli, 2002; Laurance et al., 2002; Hamann et al., 2006; McKenzie, 2007) and only richness is not a decisive indicator of environmental conditions (Aho, 1990). The component community and the biology of helminths allow the understanding of the dynamics among parasites, hosts and environment (Poulin, 2007). The frogs from the preserved area (NSV) had a lower helminth richness than those from the disturbed area (SLP), but were parasitized with two species that require invertebrates as the intermediate host. Preserved environments may have a more diverse invertebrate fauna, increasing the chances of the parasite finding its host.

There were no significant differences between the descriptors of parasitism and the helminth fauna composition associated with the two populations of *Ph. cuvieri*; however, according to the Brillouin diversity index, the helminth community associated with frogs from the disturbed area (SLP) had higher diversity than that of the preserved area (NSV). This can be related to the low immune system of the anurans from the disturbed area (SLP), making them more susceptible to parasitism, or the long exposure of the host to the parasites, or the different selection pressures that regulate the helminth community (Aho, 1990; Rollins-Smith, 2001; Todd, 2007; Poulin, 2007).

This research is the first report concerning helminth community associated with *Ph. cuvieri*, an anuran with wide distribution in South America, living in the Atlantic rain forest and other biomes such as Brazilian savanna (Cerrado), representing an important study about this anuran species.

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