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Helminth parasites of amphibians: species richness and distribution in South America, and community ecology in Pantanal, Brazil

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Campo Grande, MS

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Dedico este trabalho à minha mãe.
Por ser minha mentora, grande amiga e maior incentivadora.

To my mother.
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Sumário (Contents)

Resumo

Este estudo investiga os padrões de riqueza e distribuição de helmintos parasitas de anfíbios em duas escalas geográficas. Listamos os helmintos associados aos anfíbios da América do Sul (artigo 1) e a onze espécies de anuros provenientes de uma região do Pantanal (artigo 4). Investigando a diversidade e padrão de interação, encontramos uma correlação entre riqueza de helmintos e tamanho do hospedeiro, e um padrão aninhado na rede de interações dos parasitos e anfíbios da América do Sul (artigo 2). Análises com hospedeiros do Pantanal mostraram um padrão semelhante: relação positiva entre tamanho do hospedeiro e riqueza de espécies de helmintos, e um padrão aninhado na rede de interações. Para anuros do Pantanal, descrevemos também a diversidade taxonômica de parasitos, que não foi explicada pelas características do hospedeiro (tamanho e hábito). A similaridade entre as comunidades de helmintos não foi explicada pela história evolutiva dos hospedeiros. Um fator importante para a similaridade entre essas comunidades foi a baixa especificidade, observada na maior parte das espécies de helmintos (artigo 5). O baixo grau de especificidade foi observado também, mas em menor extensão, em anfíbios da América do Sul. Análises combinando características de hospedeiros e parasitas mostraram que a especificidade dos helmintos é o principal determinante do risco de coextinção de helmintos associados a anuros da América do Sul. (artigo 3). Um outro fator importante na determinação da diversidade dos parasitos, é o ambiente em que o hospedeiro está. Observamos no Pantanal, que anfíbios provenientes de uma área mais preservada (reserva ecológica) tinham maior riqueza, prevalência e abundância de helmintos do que os coletados em uma área de pastagem (artigo 6).

Abstract

In this study, we investigate patterns in the richness and distribution of amphibian parasites in two different scales. We list the helminth parasites of amphibians from South America (article1) and from an area in Pantanal, Brazil (article 4). Accessing parasite diversity and distribution, we found a nested pattern in the network of South American anurans and their helminths, and that larger hosts harbour richer parasite faunas (article 2). Analyses with hosts from Pantanal showed similar results: host size is a determinant of parasite species richness and the interaction network is nested. We also described parasite taxonomic diversity in hosts from Pantanal, which was not determined by host´s size or habit. Similarity in parasite communities did not correlate to host phylogeny, but was strongly influenced by the low specificity observed in most helminth species (article 5). Low host specificity was also observed, but to a lesser extent, in parasites of South American anurans. Analysing host and parasite traits, we observed that host specificity is the most crucial trait influencing the coextinction probabilities of helminths associated with anurans from South America (article 3). Another important factor affecting parasite diversity is its host´s habitat. We could observe in anurans from Pantanal that hosts from a protected area (nature reserve) had greater species richness and higher prevalence and abundance of helminth parasites than hosts from an impacted area (article 6).

Apresentação

“Existe, potencialmente, uma infinidade de caminhos que você pode percorrer em sua carreira científica. A escolha certa pra você é, como em qualquer amor verdadeiro, aquela que te desperte interesse e paixão, e prometa o prazer de uma vida toda de devoção”

Essa frase de Edward Wilson em “Cartas para um jovem cientista” descreve bem minha sensação ao escrever os manuscritos a seguir. Olhar para a natureza, perceber sua diversidade, procurar padrões, e depois tentar entendê-los é mesmo um caminho fascinante. Logo nos primeiros passos deste caminho, que ainda está no início, comecei a me interessar pela biologia dos anfíbios. Quanto mais eu aprendia sobre eles, mais pensava nos anfíbios como os organismos mais encantadores. Desde então, muito do meu esforço em entender a natureza tinha, ainda que inconscientemente, os anfíbios como personagens principais, ou organismos modelo. Então, ainda na graduação, fui apresentada a uma estratégia de vida muito comum na natureza, mas que não era – e ainda não é – das mais carismáticas, o parasitismo. Quanta complexidade, inteligência e beleza eu descobri ao observar a biologia dos parasitos, organismos que até então eu imaginava apenas como “primitivos”. Assim, unir esses dois interesses em um único modelo de estudo foi uma consequência natural.

Esse conjunto de manuscritos vai falar a respeito de anfíbios e seus parasitos, em particular, um grupo muito comum de parasitos, os helmintos. Começamos o estudo com uma pergunta clássica: anfíbios são parasitados por helmintos? Se sim, quem são os helmintos que parasitam anfíbios? Para responder essas perguntas utilizamos duas fontes de informação diferentes: uma que nos permitisse investigar padrões em larga escala, e outra que permitisse conhecer nosso sistema de estudo de forma mais minuciosa. Dividimos então a tese em duas partes, na primeira parte pesquisamos a literatura e na segunda investigamos os anfíbios mais comuns em uma região do Pantanal. Cada parte é composta por três artigos, e cada artigo foi formatado de acordo com o periódico no qual temos a intenção de publicar.

O primeiro artigo é uma lista das espécies de helmintos que parasitam anfíbios da América do Sul, um *checklist* recentemente publicado na *Zootaxa* (devido a extensão deste manuscrito, apresentamos aqui o resumo, o artigo completo está anexo). No segundo artigo procuramos compreender melhor a diversidade que observamos nesta lista. Investigamos se o tamanho e a amplitude de distribuição geográfica dos anuros são bons preditores da riqueza de helmintos observada. Escrevemos o segundo artigo de acordo com as normas de publicação da *American Naturalist.* Terminamos a primeira parte imaginando um cenário pessimista: sendo os anfíbios o grupo de vertebrados mais ameaçados de extinção, nos perguntamos o que aconteceria com a fauna de parasitos se sua espécie de hospedeiro fosse extinta. Nossa motivação principal associada a essa pergunta, é identificar as características que fazem uma espécie ser mais ou menos vulnerável ao risco de extinção. Preparamos esse manuscrito de acordo com as normas de publicação da *Biology Letters.*

O primeiro artigo da segunda parte é também uma lista de espécies. Dentre os de anfíbios mais abundantes no Pantanal, inventariamos as comunidades parasitárias de onze espécies. Preparamos este manuscrito de acordo com as normas da *Comparative Parasitology,* na categoria *Faunal inventory.* Em seguida procuramos descrever e compreender melhor a diversidade que observamos. Assim, no segundo artigo, escrito com intenção de publicação na *Parasitology,* descrevemos a estrutura da rede de interações dos anuros e helmintos coletados no Pantanal. Por fim, observando a heterogeneidade espacial do ambiente em que coletávamos os anfíbios, nos perguntamos se a diversidade de parasitos responderia a essa variável extrínseca – o ambiente em que o hospedeiro está. Comparamos então comunidades de helmintos de hospedeiros coletados em dois locais de diferentes níveis de preservação. Esse último manuscrito foi redigido de acordo com as normas de publicação da *Journal of Parasitology.*

Com a expectativa de ter contribuído para a melhor compreensão da ecologia do parasitismo em anfíbios anuros, agradeço o seu interesse em ler os resultados desses últimos quatro anos de estudo, com a expectativa e desejo que seja também uma leitura agradável.

PREFACE

"The potential paths you can follow with a scientific career are vast in number. The subject for you, as in any true love, is one in which you are interested and that stirs passion promises pleasure from a lifetime of devotion."

These lines written by Edward Wilson in "Letters to a Young Scientist" describe well how I felt while I wrote the articles you are about to read. To look at nature, perceive its diversity, search for patterns, and then trying to understand such patterns is a fascinating pathway. In my first steps of this path, which is still in its beginning, I became interested in the biology of amphibians. The more I learned about them the more I would think of amphibians as the most interesting organisms. Since then, most of my efforts to understand nature had, even that unconsciously, amphibians as model organisms. Then, while still in my undergrads, I was introduced to a very common –but not so charismatic - way of life in nature: parasitism. How amazed I was to find such complexity, intelligence and beauty observing parasite biology, which I would formerly think as just "primitive". Thus, to embrace both interests in a single study system was a natural consequence.

This set of manuscripts are about amphibians and their parasites, one of the most common groups of parasites in particular, helminths. We started this study with a very common, yet fundamental, question: Do amphibians have helminth parasites? If so, what are the helminth parasites of amphibians? To answer these questions we searched from two different sources: one that would allow us to investigate large scale patterns, and one that would allow us to understand the particularities of our study system. We thus divided the thesis in two parts, in the first part we searched published reports and in the second we investigated the most common anuran species in an area of the Pantanal region. Each part of the thesis is composed of three articles, and each article is formatted according to the guidelines of the journal we intend to publish at.

The first manuscript is a list of the helminth parasites of South American amphibians, which was recently published at "*Zootaxa"* (we present the abstract here, the complete manuscript is attached). In the second article, we aimed to understand more deeply the diversity we observed in this list. We described parasite biodiversity in South American amphibians, testing the influence of host body size and geographic range in helminth species richness. This manuscript was prepared according to the "*American Naturalist"* publishing guidelines. We then finish the first part, picturing a pessimistic scenario: once amphibians are the most threatened vertebrates, we imagined what would happen with the helminth parasites if their hosts went extinct. Our main motivation associated with this question is to identify which species traits make them more or less vulnerable to extinction. We prepared this article for publication at "*Biology Letters".*

As in part I, the first manuscript of the second part is also a survey. Among the most common anuran species in Pantanal, we surveyed the helminth parasites of eleven species. We prepared this manuscript according to the guidelines of the journal "*Comparative Parasitology",* as a "*Faunal inventory"* paper. Secondly, we aimed to describe and understand better the diversity of parasites in anurans from Pantanal. Thus, in the second manuscript, which we wrote following the guidelines of "*Parasitology",* we access the influence of host traits in parasite diversity and network structure. Lastly, observing the environmental heterogeneity where we collected the anurans, we wondered whether parasites would respond to this extrinsic variable – their host´s habitat. We then compared helminth communities of anurans from an impacted and a protected area. This last manuscript was written according to publishing guidelines of the *Journal of Parasitology.*

Expecting to contribute to a better understanding of anuran parasite ecology, I am grateful for your interest in reading the results of these last four years. I also expect and wish you will have a good time with the reading.

I. Species Richness and Distribution of helminth parasites of South American Anurans.

Checklist of Helminth parasites of Amphibians from South America

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Abstract

Parasitological studies on helminths of amphibians in South America have increased in the past few years. Here, we present a list with summarized data published on helminths of South American amphibians from 1925 to 2012, including a list of helminth parasites, host species, and geographic records. We found 194 reports of helminths parasitizing 185 amphibian species from eleven ountries: Argentina, Brazil, Chile, Colombia, Equador, French Guyana, Guyana, Paraguay, Peru, Uruguay and Venezuela. Helminth biodiversity includes 278 parasite species of the groups Acanthocephala, Nematoda, Cestoda, Monogenea and Trematoda. A list of helminth parasite species per host, and references are also presented. This contribution aims to document the biodiversity of helminth parasites in South American amphibians, as well as identify gaps in our knowledge, which in turn may guide subsequent studies.

Key words: Acanthocephala, Nematoda, Cestoda, Monogenea, Trematoda Amphibia, Helminth, Parasite, South America

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Who Has More Parasites: the best studied, the biggest or the one that is everywhere? Determinants of Parasite Diversity in South American Anurans

Abstract: We describe parasite biodiversity in South American amphibians, testing the influence of host body size and geographic range in helminth fauna richness. We found that nematodes are the most common anuran parasites. Host-parasite networks have a nested pattern, and rare helminth taxa are generally associated with hosts that harbour the richest parasite faunas. Host size is positively correlated with helminth fauna richness, but geographic range is not. These results remained consistent after correcting for uneven study effort and host phylogeny. We thus provide estimates of how parasite fauna richness is expected to increase along with anuran body size.

Key words: Anura, Helminth, parasite, species richness, nestedness, South America

Introduction

What determines the number of different species in a given habitat? The search for general laws remains a core issue in community ecology. Parasite ecology is no exception, and parasitologists have dedicated great effort to unveil the laws structuring parasite assemblages (Poulin 1995, 1997, 2007, Bush et al. 2001). Observing how some host species carry so many parasites while others have so few, to assume parasite species richness as a host trait seems a sensible pathway in this pursuit.

One of the main theoretical basis for the study of parasite species richness is the theory of island biogeography. Because parasite communities are formed by colonization and extinction process just like other communities, and because of the insular nature of hosts as habitats, the theory has become popular and influential in parasite community ecology. In this scenario, the rates of parasite colonization and extinction would vary according to features of the hosts (Poulin and Morand 2004).

In particular, the body size of the host species is a good potential predictor of parasite species richness (PSR). Large-bodied hosts may provide more space and other resources, and possibly a broader diversity of niches for parasites. Larger hosts live longer, representing less ephemeral habitats than smallbodied species. Thus, larger hosts also have longer exposure to parasites (Poulin 1997). Similarly, a wider geographical range of the host may result in encounter with and colonization by a greater number of parasite species. Hosts species ranging over vast areas will overlap with the geographical distribution of several other host species, creating numerous opportunities for host switching (Bush et al. 2001). However, the validity of host body size and geographic range as determinants of PSR is frequently questioned. Unlike islands, hosts can inherit parasites from their ancestors, making it crucial to consider the effect of autocorrelation in comparative analysis across host species (Poulin and Morand 2004). When such corrections are made, the effect of host size and range might lose strength or statistical significance (Poulin 1997, Nunn et al. 2003).

A broad view, including ecological and evolutionary mechanisms is needed to understanding parasite biodiversity, which can be studied at several scales. As defined by Poulin and Morand (2004), “the parasite fauna represent the highest hierarchical level of parasite assemblages; it is composed by all parasite species reported for a given host. Parasite faunas are artificial rather than biological entities, but might be the most relevant scale for macroecological studies”. Here, we investigate the influence of host features relevant to helminth parasite fauna richness in South American amphibians.

Amphibians are very interesting models to study parasite diversity, they comprise a diverse group in terms of taxonomy and life history strategies. Moreover, South America is one of the world’s hotspots of amphibian biodiversity and harbours around 2,599 species (Frost 2013). Nonetheless, when we think about quantitative measures or ecological approaches to understand parasite biodiversity, amphibians are the least studied vertebrate group (Aho 1990, Barton 1999). We use a dataset of published reports of helminth parasites of South American amphibians to: (i) describe parasite biodiversity across hosts lineges; (ii) access the nestedness of host-parasite interaction; (iii) test the influence of host body size and geographic range on PSR, correcting the effect of uneven sampling effort and phylogenetic correlation among the hosts; (iv) estimate the amount of sampling effort required to describe amphibian PSR, and how PSR is expected to change with host body size.

Methods

We compiled data on host-parasite interactions from a recent list of helminth parasites of South American amphibians (Campião et al. 2014). Two different types of studies constitute this list, the ones focusing on the parasite species (where the known hosts are reported for each parasite), and the ones that focus on particular hosts (all parasites of these hosts are reported). We considered the number of published parasite reports per host our measure of study effort. Only reports that identified host and helminth to species were considered. Because of the shortage in data on other amphibian orders, analyses were carried only with anuran hosts. We compile data on anurans body size (mean snout vent length) from papers, field guides and museum assessments; and geographic range from Global Amphibian Assessment database (GAA) (IUCN 2011).

We searched for patterns in species association by evaluating the degree of nestedness in the interaction anurans and their parasites. We adopted the NODF metric (Almeida-Neto *et al*. 2008), and assessed the randomness of matrix nestedness by the analysis of null models. The calculation of the NODF metric and the simulation of the null models (1000 randomizations) were calculated using the program ANINHADO (Guimarães and Guimarães, 2006).

To test our main hypothesis, we constructed a non-linear model assuming host size and geographic range as determinants of amphibian PSR. It is recognized that the effort dedicated in sampling hosts will determine how well we know parasite diversity. Very frequently, the measure of how intensely hosts have been studied is the best predictor of PSR, making the role of ecological variables, if any, very difficult to detect (Poulin and Morand, 2004). To control for such bias, we also considered study effort a determinant of PSR. Nonlinear least squares models relax the requirement of linearity. Then, we first considered an exponential relationship between study effort and PRS, calculated as a Holling type III function (Bolker 2007). This S-shaped curve is quadratic near the origin, but different from a linear model, it will eventually reach an asymptote. The Holling type III function was calculated as:

$$f(x) = a*x^2/(b^2+x^2) \qquad (1)$$

where *f(x)* is the number of parasites per host, *x* is the number of studies per host and *a* e *b* are the constants. Here, *a* representes the greatest PRS a host can have – the asymptote and *b* is the number of studies needed to reach it (Bolker 2007). However, we also expect the PSR to have an exponential relationship with host's body size and geographic range (as in a Possion regression). Thus, we have:

$$a= \exp(c+d*y+e*z) \qquad (2)$$

where *c* is the intercept, *y* is host body size, *z* is host geographic range, and *d* and *e* are the respective coefficients. Combining equations (1) and (2) we have:

$$f(x) =\exp(c+d*y+e*z)*x^2 / (b^2+ x^2) \qquad (3)$$

Using equation 3 we avoid the undesired effect of expecting PRS to increase unlimitedly with study effort. We adjusted this model using the Gauss-Newton algorithm in the nls function in *R.*

Despite having the uneven study effort effect corrected, another important assumption when making a comparative test is that any values for related species are not truly independent, and treating them as such may lead to pseudoreplication and increased chance of Type I error (Poulin 1995). Because we consider parasite species richness a host trait, it is necessary to consider that such trait could be inherited from a common ancestor. Therefore, we tested our main hypothesis with an alternative model, a comparative analysis using generalized estimating equations (GEE) as described by Paradis and Claude (2002). GEE is an extension of generalized linear models (GLMs) for correlated data. The phylogenetic variance-covariance or correlation matrix, expects variances and covariances of a continuous trait assuming it evolves under a Brownian model.

In the GEE we assumed PSR is determined, additively, by the body size, geographic range and study effort of each host. Once there may be a limit to PSR in a given host, a negative interaction between host body size and study effort is expected. This is because we expect that less study effort is needed for a smaller host to reach the PSR asymptote than it is needed to a bigger host. Thus, we have:

PSR = body length + geographic range + study effort+ (body length * study effort)

The correlation among hosts was calculated according to the phylogeny of Amphibia proposed by Pyron and Wiens (2011). Based in this tree, we removed all branches of the species that were not in our database of host-parasite interaction and reconstructed amphibian's phylogenetic tree with the *"ape"* package (Paradis et al. 2004). The package *"igraph"* (Csardi and Nepusz 2006) was used to construct the graph with anuran phylogeny and parasite interections.

During data analyses, we observed that one anuran species, *Leptodactylus latrans,* was always very influential in all models. Once this species had a very high Cook distance (Bollen and Jackman 1990), we checked its validity as a sample and found it may actually be a complex of species that are now all cited as *L. latrans* (Lavilla et al. 2010, Frost 2013). Thus, we removed this species from all analyses. All analyses were carried out in R 2.14.1 (R Development Core Team 2013).

Results

Parasite diversity in anurans

We compiled data of 283 helminth parasites in 180 anuran species, but only 225 helminths and 156 anurans remained after excluding non-specific reports. Nineteen host families are included, Bufonidae, Hylidae and Leptodactylidae are the most representative and account together with almost 60% of the anuran species studied for helminth parasites in South America. Moreover, these are the only host families comprising all major helminth groups (Figure 1). On the other hand, some anuran families seem to have depauperate parasite fauna, as Hemiphractidae, that is parasitized only by Monogenea. However, it is likely that those hosts were studied for specific parasite groups, and were not completely surveyed for other helminths.

We found helminths of the phylum Acanthocephala (two families), Platyhelminthes (two families of Cestoda, one family of Monogenea and of 19 families of Trematoda) and Nematoda (24 families). The most common helminths are nematodes, which occur in practically all host families. Parasites within this group were able to colonize all hosts lineges. Gastrointestinal roundworms of the families Cosmocercidae, Kathlaniidae, Molineidae, Physalopteridae, and lungworms of Rhabdiasidae are the most reported helminths. Trematodes are the second most diverse parasite group and occur in most anuran families, but are more linked to clades of aquatic anurans, such as *Lithobates* and *Pseudis* species (Figure 2). Trematodes are the second most recorded helminth parasites in amphibians, also occurring in most anuran families.

Acanthocephalans, cestodes and monogeneans are less common and more restricted to few anuran species (Figure 2). It is also interesting to note that rare parasites, such as acanthocephalans, cestodes and monogeneans, generally occurred within hosts also parasitized by nematodes and trematodes (Figures 1 and 2). We then tested and found a nested pattern in host-parasite network (NODF=4.46, P>0.01).

On average, helminth host range was 3.2 (± 4.7, min: 1, max: 34). Out of the 225 helminth species, 113 were restricted to a single host, but the degree of host specificity (host range here) seemed to be not random among helminth taxa. Indeed, all monogeneans are specialists, and 57% of the parasites with a host range of 10 or more are nematodes belonging to the same superfamily (Cosmocercoidea) (Figure 2).

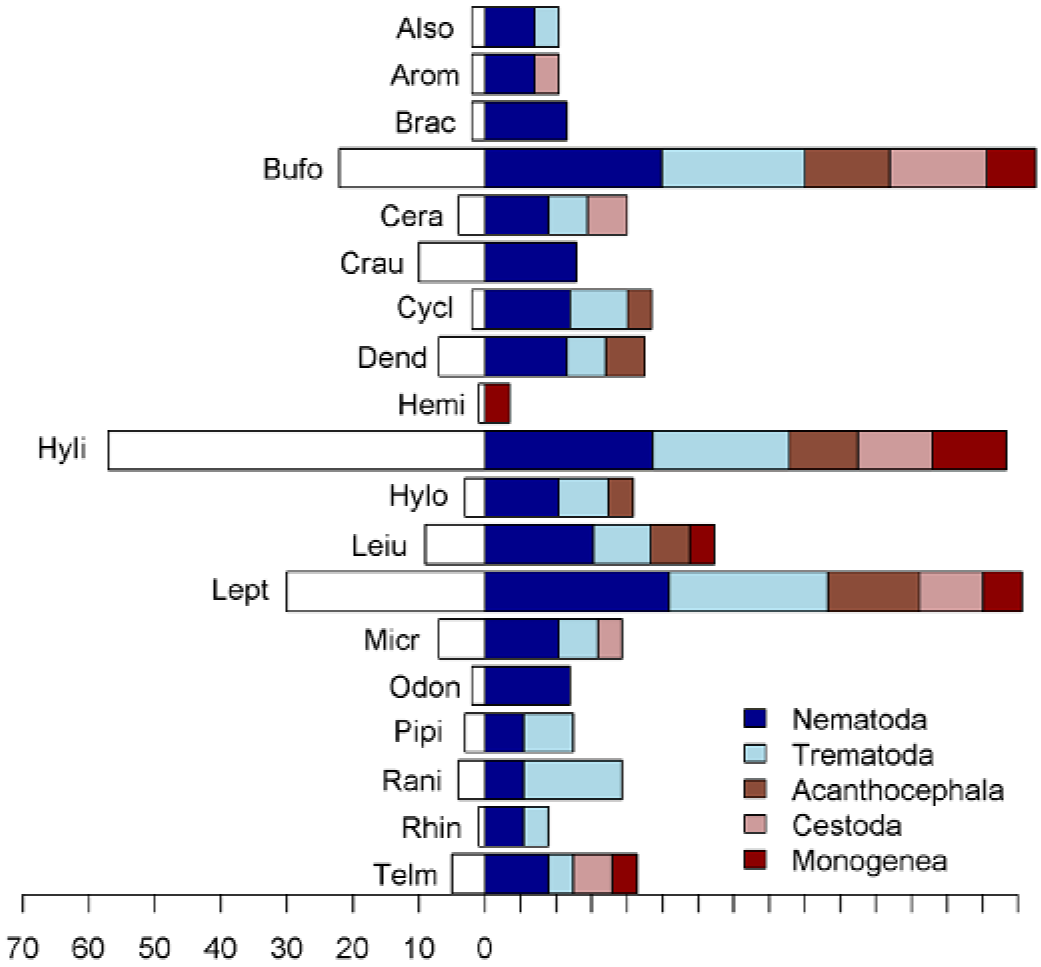

Figure 1. Barplot of helminth species reported to different anuran families. White bars show the number of anuran species surveyed, color bars show the amount (log transformed) of helminth parasites reported for each host family.

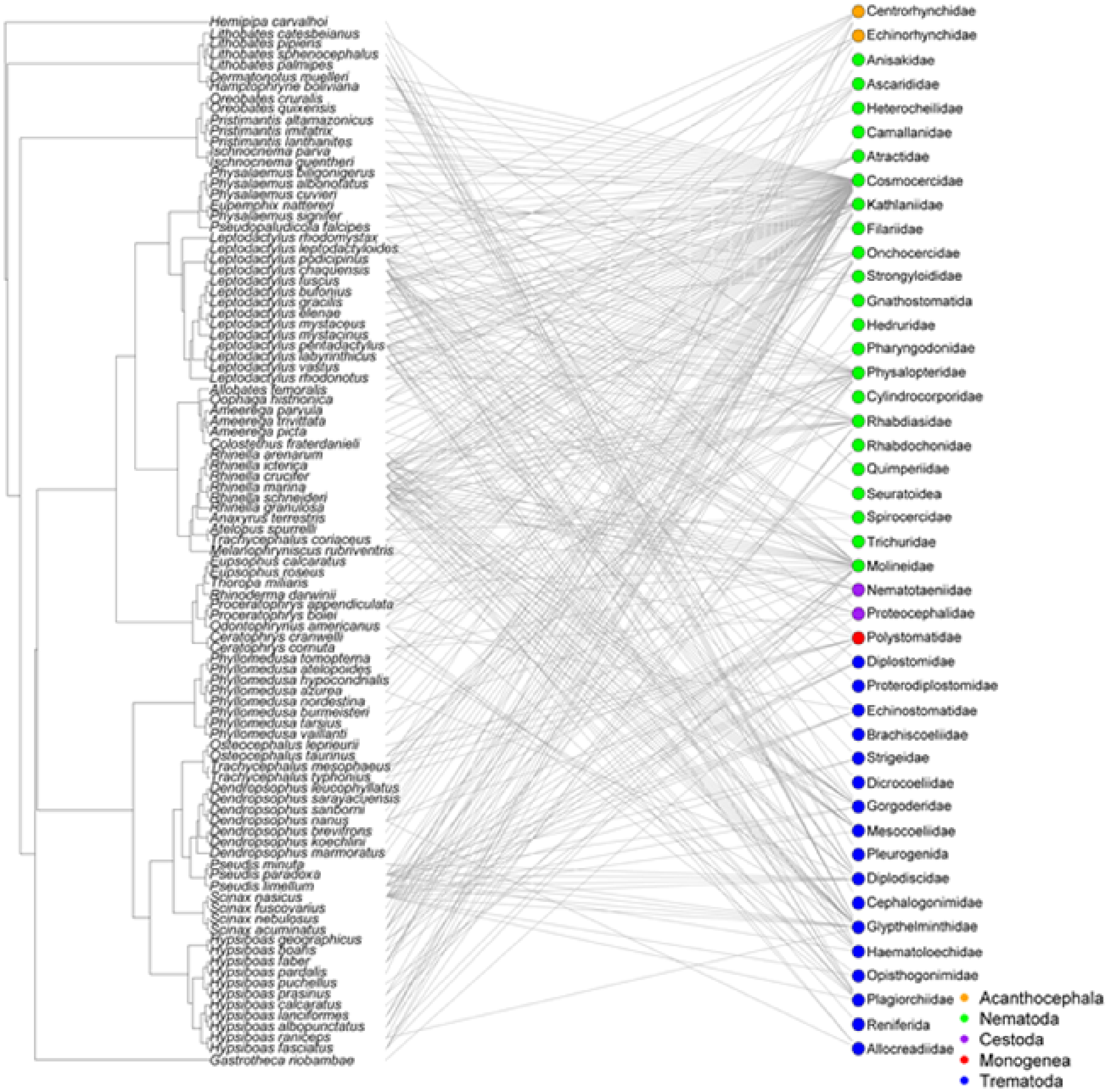

Figure 2. Interacting network of South American anurans and helminth families. Anuran phylogeny is adapted from Pyron and Wiens (2001).

Determinants of parasite richness

We considered host size, geographic range and study effort the predictor variables of helminth PSR in a nonlinear least square model. As expected, study effort is strongly related to PSR (Table 1). Based on the equation (2), we can observe that an average of four studies is needed to reveal 50% of the PSR expected for an anuran host (Table 1). It is important to remember that our dataset, and therefore our predictions, are based in all kinds of studies, including parasite taxonomic reports. Host size is too a good predictor of PSR, and the larger the anuran the richer its parasite fauna is expected to be (Figure 3). Host area, on the other hand, had a very low coefficient (Table 1), indicating that larger geographic range of the host does not imply richer parasite fauna in anurans.

Table 1. Results from the nls model for the relationship between study effort, host size and geographic range and parasite species richness in anurans from South America.

Variable	Estimate	Standart error	t	Pr (>|t|)
Intercept	1.1319159	0.8338761	1.357	0.177
Study effort	4.5727182	0.3569919	12.809	<0.0001
Body size	0.0087551	0.0006565	13.337	<0.0001
Geographic range	0.1147532	0.0855097	1.342	0.182

If we consider for this model, the largest geographic range (once it is irrelevant), we can estimate how helminth PSR can increase in response to stronger study efforts (Figure 3). Similarly, we can assume the greatest study effort (26 studies) and estimate mean parasite richness expected for anurans of different body lengths from our dataset (Table 2).

Table 2. Estimates of helminth species richness to South American anurans of different body sizes.

Anuran body length (mm)	**Expected helminth parasite species richness**
30	12.3
50	14.6
70	17.4
90	20.8
110	24.8
130	29.5
150	35.2
170	49.9
190	59.4
210	50.1

To avoid confounding effects of hosts' phylogeny, we conducted a second model (Generalized Estimating Equation - GEE), correcting the effect of correlation among hosts. In this analysis, we only used host-parasite interactions for 118 anuran species, which are included in amphibian's phylogeny. Despite GEE assuming a linear relation between PSR and all variables, which is not true for study effort, it led us to the same conclusions as the nls. Host geographic range remains statistically irrelevant, and host size and study effort significant. Actually, the effect of host size is slightly stronger when we take host phylogeny into account (Table 3), and it interacts negatively with study effort. This indicates that less study effort is required to describe the PSR of smaller hosts.

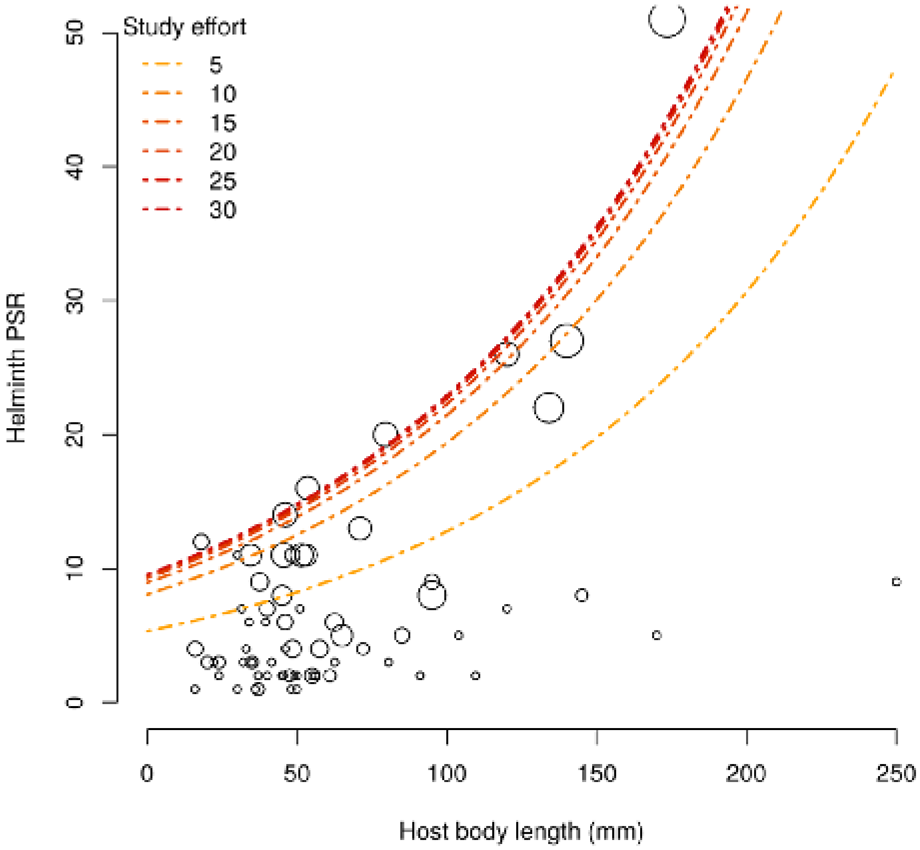

Figure 3. Estimates of helminth parasite species richness (PRS) to South American anurans of different body sizes in response to the number of studies (study effort). Each circle represents an anuran species, the size of each circle shows the real study effort, dashed lines show the estimated PRS in response to different study effort.

Table 3. Results from the Generalized Estimating Equation for the relationship between study effort, host size, geographic range and parasite species richness in anurans from South America. Phylogenetic df (dfP): 19.07968

Variable	**Estimate**	**Standart error**	**T**	**Pr (T > |t|)**
Intercept	2.7047	0.5463	4.9505	<0.001
Study effort	0.1454	0.0250	-1.8071	<0.001
Body length	0.0055	0.0012	4.4886	<0.001
Geographic range	-0.0779	0.04313	-1.8071	0.09
Study effort * Body length	-0.0005	0.0001	-4.5501	<0.001

Discussion

A good amount of information (23%) on host-parasite interaction was lost after excluding non-specific reports. The lack of taxonomy accuracy is very common when studying invertebrates (Pik et al. 1999), including the parasitic ones (Brooks 2001). More specifically, anurans are hosts to a great diversity of larval helminths (Campião et al. 2014). This is probably because of the position such vertebrates occupy in ecosystem foodwebs (Poulin and Leung 2011, Shah et al. 2013). Because amphibians are prey to several reptile, bird and mammal species, they can act as intermediate or paratenic hosts in the life cycle of several parasite taxa. However, the precise identification of most larval helminths is only possible through molecular biology, which has just recently become used more widely by parasitologists (Poulin and Leung 2010, Locke et al. 2010). Therefore, despite the study of parasite diversity having come to a point where there is an amount of data allowing analysis to uncover general patterns, there is still an appealing request for taxonomic studies, especially in the tropics (Dobson et al. 2008, Poulin and Leung 2010, Poulin and Forbes 2012).

Among all parasite species, 54% are restricted to a single host. Nonetheless, most studies with amphibian helminth assemblages agree about the lack of host specificity often found among these parasites (Aho 1990, Barton 1999, Bursey et al. 2001, Goater and Goater 2001). Data on South American anuran parasites indicate that the low host specificity is quite common, but generally restricted to some helminth taxa (Figure 2). Notwithstanding, the distribution of specialist parasites amongst the hosts was not random, exhibiting a nested pattern. Nestedness is a common feature in host-parasite networks, where those species found in species depauperate communities are subsets of those found in communities with greater species richness (Poulin, 1996; 2010).

Study effort is the strongest predictor of parasite species richness. Indeed, the most studied hosts (toads, tree-frogs, and frogs of Bufonidae, Hylidae and Leptodactylidae, respectively) had by far the richest parasite faunas, and some anuran families that seem to have depauperate parasite faunas had actually been poorly studied. We estimated that an average of four studies is needed to describe 50% of the parasite fauna richness in anurans. Only 22% of host species reached this. However, our dataset includes both descriptions and taxonomic reports of particular helminth species as well as complete surveys of helminth communities in host populations. Hosts may reach higher PSR with less study effort if they are more target to complete surveys. Nonetheless, data on South American anurans indicate that PSR is still underestimated for most species.

We found a positive correlation of parasite species richness and host body size for a large dataset of anuran hosts. This result remained consistent after correcting for confounding effects of hosts phylogeny. Poulin and Morand (2004) and Bush et al. (2001) state that host body size play a substantial role in the diversification of some parasite fauna, but agreed that its importance was far from being universal. Nonetheless, Kamiya et al. (2014) later assume, based on a large interspecific dataset, that the relationship between host body size and PSR is universal across host and parasite taxa and across levels or scales of study. The underlying mechanism could be that large-bodied hosts may be easier to colonize because of the greater amounts of food they ingest, their large surface area, greater vagility, and greater niche availability (Poulin 2007). Bush et al. (2001), Poulin and Morand (2004), and Kamiya et al. (2014) all sum a good amount of evidence of the positive correlation between PSR and body size for a variety of host taxa, but none of them report data on amphibian hosts. Here we add another piece of evidence, for a poorly studied group of hosts, of the role of host size in structuring parasite assemblages.

Different from expected, anurans that are widely distributed geographically do not have, necessarily, richer parasite faunas. Besides promoting geographical overlap with more host species, host range often correlates positively to species abundance and niche breadth (Slatyer et al. 2013). All that could potentially provide more opportunities for colonization of parasites trophically and/or directly transmitted. Indeed, host geographic range is positively related to PSR for fishes, birds and mammals (see the review by Poulin and Morand 2004), and has also been pointed as a universal predictor of PSR (Kamiya et al. 2014). However, we found no effect of host geographic range in determining PSR of South American anurans, whatever the analysis corrected or not for phylogeny.

Overall, we found that nematodes are the most common anuran parasites, and rare helminth taxa are generally associated with larger hosts that harbour the richest parasite faunas. Study effort is the most crucial preditor of PSR and it interacts negatively with host size. Anurans body size determines PSR, the larger the anuran the richer the parasite fauna. Considering both the structure and the determinants of PRS in anurans, specialist parasites are more likely to be associated with large hosts.

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Cryptic Biodiversity Loss: How Anuran Parasites Respond to the Extinction of their Hosts?

Summary

Amphibians characterize the current biodiversity crisis being the vertebrate group with the highest number of threatened species and well-documented recent extinctions. One of the outcomes of species extinction is the coextinction of its dependents, which is a poorly understood route to biodiversity loss. Here, we estimate the extinction risk of helminth parasites of South America anurans. We compiled data on 157 amphibians and 194 helminth species. Parasite species associated with few hosts were the most prone to extinction. Extinction probabilities varied amongst helminth groups: monogeneans had the highest extinction probabilities, followed by cestodes, nematodes and trematodes. Acanthocephalans were the least threatened. For most parasites species, host vulnerability decreased extinction probabilities. However, 44% of the specialist parasites were negatively affected by host vulnerability. Overall, we found that the host specificity of parasites and the vulnerability of their hosts combine to determine the coextinction risks of anuran helminth parasites, and the outcome of this interaction varies with the helminth group.

Key words: Anura, parasite, coextinction, specialist, host.

Introduction

Biodiversity is declining at alarming rates, similar to historical mass extinctions [1]. The rapid change in atmospheric conditions, habitat fragmentation, pollution, invasive species and pathogens represent more extreme ecological stressors than most living species have previously experienced [2]. Amphibians characterize the current biodiversity crisis, with well-documented recent extinctions in response to such stressors [3]. At least 32% of existing amphibians are at threat from extinction [4] and numerous populations are facing major population declines, morphological deformities and severe pathogen infections [5]. Thus, amphibians represent an especially sensitive group of organisms to extinction.

One of the outcomes of species extinction is the coextinction of its dependents, which is one of the most common, but least understood routes to biodiversity loss [6, 7]. Despite being generally targeted as drivers of host extinctions, parasites species may be even more prone to and affected by extinction than free-living organisms [8]. Parasite extinction may first seem beneficial to hosts, especially those endangered, but some long-term consequences might be severely disadvantageous, such as loss of genetic diversity of their hosts and increased abundance of other pathogenic parasites [9, 10].

Coextinctions are often difficult to document, and models estimating coextinction rates may therefore be useful to predict and prevent future biodiversity loss under conditions of ongoing global change [11]. These estimates are influenced by both host and parasite traits, and the interactions between these two components. The degree of host specificity is a key factor to coextinction risk, since parasites with restrict host relationships are more likely to go extinct together when their hosts do [9]. Parasite extinction proneness may also vary with their host's extinction vulnerability [12]. Assuming that extinctions are not random in nature and hosts vary in their likelihood of going extinct therefore provides a more realistic scenario of how parasite biodiversity will respond to hosts extinction [13]. Here, we use data on helminth parasite of South America anurans and their helminth parasites to identify how parasite specificity and host vulnerability interact to determine parasite coextinction rates.

Material and Methods

We compiled reports of helminth parasites of amphibians from South America from a recently published list [14]. This list reports 298 helminth taxa in 186 amphibian species. We conducted the analysis with amphibians of the order Anura only, and excluded all reports in which the host or parasite were not identified to species.

We assumed that a parasite species would persist if at least one of its host species persists. Assuming that host extinction events are statistically independent, this means that the probability Q_i that parasite species i persists can be expressed as

$$Q_i = \prod_{j \in H_i} q_j,$$

where q_j is the probability that host species j is extant and the product runs over the set H_i of all hosts of parasite species i. We model host extinction as a Markov process with rate r_j, so that the probability that host species j is extant after time t is $q_j = e^{-r_j t}$. By assuming particular relationships between the host extinction rate r_j and host traits, we are able to study how parasite extinction probabilities varied under different scenarios for host extinction. We do not have any specific information on the time scale for host extinction, so rather than choosing an explicit value for t we instead assumed that a particular fraction of the hosts had gone extinct. The mean number of extant host species after time t is

$$S(t) = \sum_j q_j\,(t),$$

where the sum runs over all host species, so with the use of a nonlinear equation solver we are able to find numerically the value of t corresponding to a particular value of $S(t)$.

We adopted anuran geographic range as the measure of host vulnerability and modeled as r_j, assuming that the smaller geographic range is, the greater chances a species has to go extinct. Several other factors might be important in determining amphibians' vulnerability to extinction, but we focused on geographic range because it is positively correlated to species' niche breadth and abundance [15], is the most important driver of amphibians extinction risk [16], and is known for all host species in the dataset. Geographic range data were compiled from IUCN [17].

We generated bootstrap distributions of the extinction probabilities by randomizing the extinction rates r_j among the host species. This allows us to test the null hypothesis that parasites extinction risk and host geographic range are not related. To access the effect of host vulnerability in the extinction probabilities of each parasite species, we compared the observed estimates to the median of the bootstrap confidence interval. We recorded the proportion of parasites species that were different of the bootstrap median, and tested whether these proportions varied among parasite groups (Acanthocephala, Cestoda, Monogenea, Nematoda, Trematoda) with a chi square test.

Results

We compiled data on 157 anurans and 194 helminth species. Around 60% of the hosts are associated with more than one parasite species. Parasite host range was on average 3.7 (sd= 6.68), and 52% of the helminth species were connected to a single host species. Monogenea were the most specialized parasites, while Nematoda the most generalist (Table 1).

Table 1. Host range of the helminth parasites of South American anurans. A=Acanthocephala, C=Cestoda, M=Monogenea, N=Nematoda, T=Trematoda.

	Number of helminth species				
Host range	**A**	**C**	**M**	**N**	**T**
1	2	6	10	58	35
2	0	1	1	14	12
>2	3	1	0	33	18
Total	5	8	1	105	65

Parasite species associated with few hosts were the most prone to extinction. Seventy-five percent of the extinction probabilities generated by the null model were higher than those generated by the model that accounted with the geographic range of each host species ($\chi^2 = 45.5464$, df = 1, $p < 0.001$). This indicates that host vulnerability decreased helminth extinction probabilities for most helminth species. However, host vulnerability increased the extinction probability of 44% of the specialist parasites ($\chi^2 = 6.0131$, df = 2, p= 0.04946).

Extinction probabilities varied among helminth groups. Monogenea had the highest extinction probabilities, and this pattern was consistent in every percentage of simulated host extinction (Figure 1). Monogenea were also the most negatively affected by host vulnerability (Fisher's Exact Test for Count Data p=0.02). Cestodes were the second most prone to extinction, followed by nematodes and trematodes, which had very similar results. Acanthocephalans were the last threat.

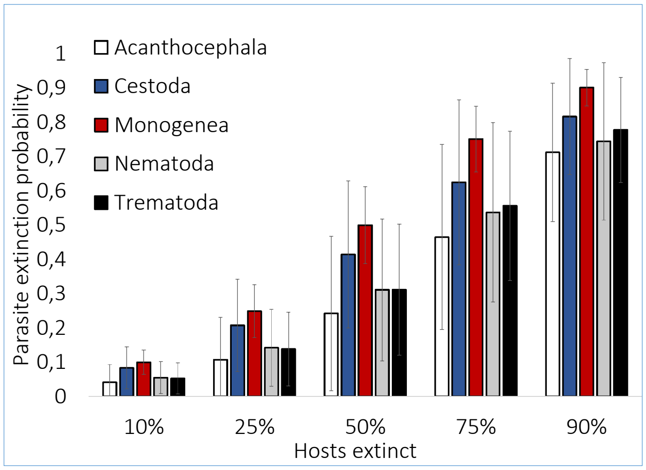

Figure 1. Estimates of coextinction probabilities of helminth parasites of South American anurans.

Discussion

Our estimates confirmed that specialist parasites are the most vulnerable to coextinction. Indeed, because parasites ultimately need their hosts for persistence, the number of host species a parasite is associated to is the most crucial parasite trait determining its chance to extinction [13].

A recent study estimating coextinction of fish parasites found that the most specialized parasites, such as the monogeneans, tended to occur in non-threatened hosts, minimizing their extinction risks [18]. We found different results for anurans. Specialized parasites are as likely to occur in vulnerable hosts as are the generalists, being therefore confirmed as the most prone to extinction. Powell (2011) found similar results in an insect-plant mutualist system, which did not support reduced specialization of dependent species on the threatened hosts. Additionally, the densities of dependents in the threatened hosts were lower, suggesting they might go extinct before, as a response to host's lower abundance [19]. As such, extinction rates of parasite species might exceed the number of free-living extinctions, and parasites may go extinct more rapidly than their hosts [7, 12, 20]. This is of particular concern for amphibians, which is the vertebrate group with the highest number of threatened species [17], implying that a considerable number of parasites might go extinct even before being discovered. Moreover, helminth parasite diversity can contribute to decrease the disease risk in anurans [21]. Therefore, the loss of parasite species implicate in unpredictable threats to ecosystem health.

Host range and extinction probabilities varied amongst different helminth groups. Acanthocephalans are the least threatened helminths. This is probably due to the reduced number of association between anurans and acanthocephalans in the dataset, which reflects the rarity of these parasites in amphibians [22]. Additionally, most of the acanthocephalan species we analysed have a relatively wide host range. Nonetheless, considering the full number of species recorded, Nematoda and Trematoda comprise most of the helminth species that could potentially survive after anurans extinctions. Thus, once coextinction probabilities are strongly influenced by parasite specificity, which varies among taxonomic groups (with Monogenea being the most threatened), helminth coextinction probabilities are likely to be phlylogenetic constrained. Differently, the host's extinction probabilities might not be constrained phylogenetically. A study of the correlates of amphibian extinction risk revealed that the effect of phylogeny is weak, and geographic range is the best predictor [16]. This suggests that in an anuran-helminth system, host vulnerability is mostly affected by extrinsic factors (*i.e.* habitat degradation), while the main driver of parasite risk is intrinsic (specificity).

It is important to note that we did not consider the differences in life cycle complexity observed amongst and within parasite groups, once it is unknown for most helminth species in the dateset. Parasites that require one or more intermediate hosts might go extinct due to the missing of such hosts even their definitive anuran host is not endangered. Thus, the extinction probabilities of complex life-cycle parasites might be underestimated. On the other hand, parasites are able to include, change, or even reduce the number of hosts required to complete their life cycle [23, 24], which could potentially increase their chances of survival in a host extinction scenario.

Our coextinction model, like any model based on empirical data, was influenced by sampling biases. Many parasite species considered restricted to a narrow set of hosts might occur in other unsampled hosts, which leads to the overestimation of their extinction probabilities [25]. Also, our model assumes parasites exploited all host species equally, which is a simplification of what actually occurs. Host-parasite associations are generally assymetrical, and even generalist parasites may have a preferable host, whose extinction could affect parasite fitness in a way that would subsequently lead to its extinction [26].

Overall, we found that the host specificity of parasites and the vulnerability of their hosts combine to determine the coextinction risks of anuran helminth parasites. They interact differently in different parasite taxonomic groups, and the most specialized groups are the most endangered.

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II. Diversity and Community Ecology of Helminth Parasites of Anurans from Pantanal, Brazil.

Helminth Parasites of 11 Anuran Species from the Pantanal Wetland, Brazil

ABSTRACT. We examined the anurans *Dendropsophus nanus, Hypsiboas raniceps, Phyllomedusa azurea, Pseudis limellum, Pseudis paradoxa, Scinax nasicus, Trachycephalus typhonius, Leptodactylus chaquensis, Leptodactylus fuscus, Leptodactylus podicipinus, Physalaemus albonotatus* from southeastern Pantanal, Brazil. These 11 anurans species were associated with 37 helminth taxa: 1 undetermined acanthocephalan, 28 nematodes, 6 digenetic trematodes, 1 undetermined helminth cyst, and 1 petastomid. Helminth species richness varied from 2 in the hylids *D. nanus* and *P. limellum* to 19 species in *L. chaquensis.* Cosmocercids, such as *A. hylambatis, C. podicipinus* and *Parapharygodon* were the most prevalent and abundant nematodes. Trematodes only reached high prevalence and abundance in the aquatic frog *P. paradoxa,* where *Catadiscus* sp. and *G. palmipens* were central species*.* Most helminth species were shared among different host species. We report in total 48 new host records, and provide remarks on the life cycle of the helminth parasites.

KEY WORDS. Anura, Helminth, Nematoda, Cosmocercidae, Trematoda, Acanthocephala, parasite, prevalence, Pantanal, Brazil.

It has been more than three decades since the first efforts to describe and document the world biodiversity began (Dobson et al., 2008). Parasites are generally an overlooked, but yet very important, component of global biodiversity. Despite the increasing number of inventories, we are still far from the complete knowledge of parasite diversity (Poulin and Morand, 2004).

Brazil is a megadiverse country. It harbours the richest anuran fauna in the world (Segalla et al. 2012), but only 8% of Brazilian anurans have been surveyed for helminth parasites. In this study, we list and provide remarks on the life cycle of the helminth parasites of 11 anuran species (*Dendropsophus nanus, Hypsiboas raniceps, Phyllomedusa azurea, Pseudis limellum, Pseudis paradoxa, Scinax nasicus, Trachycephalus typhonius, Leptodactylus chaquensis, Leptodactylus fuscus, Leptodactylus podicipinus, Physalaemus albonotatus*) from southeastern Pantanal, Brazil.

MATERIAL AND METHODS

Anurans were collected in Fazenda Alegria (18º59'S and 56º39'W), southeastern Pantanal, Mato Grosso do Sul State, Brazil. Our field trips to collect the host species were conducted in the rainy seasons of 2011, 2012 and 2013. Anurans were euthanized with an overdose of sodium thiopental solution, their body cavity, digestive tract, accessory organs and musculature were examined for helminth parasites. Nematodes were fixed in hot alcohol-formaldehyde-acetic acid (AFA) solution; cestodes and trematodes were fixed under cover slip pressure also using cold AFA; acanthocephalans were maintained in cold water until their probosces were extruded and then fixed in cold AFA. All helminths were preserved in 70% ethyl alcohol. For identification, acanthocephalans, cestodes and trematodes were stained with carmine and cleared with eugenol while nematodes were cleared with lactophenol.

RESULTS

We collected 229 hosts belonging to 2 anuran families and 11 species. These were associated with 37 helminth taxa: 1 undetermined acanthocephalan, 28 nematodes, 6 digenetic trematodes, 1 undetermined helminth cyst, and 1 petastomid. Helminth species richness varied from 2 in the hylids *D. nanus* and *P. limellum* to 19 species in *L. chaquensis* (Table 1).

Acanthocephala

Undetermined Acanthocephala

*Hosts***:** *H. raniceps, L. chaquensis, L. fuscus, L. podicipinus, P. azurea, P. paradoxa* and *T. typhonius*

Prevalence, mean abundance, and range: H. raniceps, 1 of 36 hosts (2.8%, 0.08 ± 0.5, 3); *L. chaquensis* 3 of 20 hosts (15%, 0.4 ±1.14, 2–4); *L. fuscus,* 2 of 30 hosts (6.7%, 0.1 ±0.40, 1–2); *L. podicipinus* 2 of 35 hosts (5.7%, 1±5.74, 2–34); *P. azurea,* 4 of 29 hosts (13.8%, 1.8 ±8.18, 1–44); *P. paradoxa,* 1 of 37 hosts (2.7%, 0.02 ± 0.16, 1); *T. typhonius,* 4 of 11 hosts (36.4%, 1.6 ± 1.67, 1–9).

Stage: Cystacanth

Site of infection: Body tissues

Remarks: Acanthocephalans have complex life cycles, which certainly include at least an invertebrate intermediate host and a vertebrate final host (Kennedy, 2006). The cystacanth stage found in this study might indicate that anurans are potential paratenic or second intermediate hosts to these parasites. The transmission to the final host occurs through ingestion of the intermediate and/or paratenic host. *H. raniceps, P. azurea, L. fuscus, P. paradoxa* and *T. typhonius* are new host records.

Nematoda

Ascarididae

Brevimulticaecum sp.

*Hosts***:** *D. nanus, H. raniceps, L. chaquensis, L. fuscus*, *L. podicipinus, P. azurea, P. paradoxa, S. nasicus* and *T. typhonius.*

Prevalence, mean abundance, and range: D. nanus, 1 of 5 hosts (20%, 0.2 ± 0.44, 1); *H. raniceps,* 7 of 36 hosts (19.4%, 1.1 ± 2.90, 1–7); *L. chaquensis, 6* of 20 hosts (30%, 7.4 ±20.25, 3–88); *L. fuscus, 8* of 30 hosts (26.7%, 5.3 ±13.34, 1–57), *L. podicipinus, 2* of 35 hosts 5.7%, 0.7±3.23, 11–16); *P. azurea, 2* of 29 hosts (6.9%, 0.5 ±2.42, 2–13); *P. paradoxa,* 2 of 37 hosts (5.4%, 2.4 ±12.91, 12–78); *S. nasicus,* 1 of 10 hosts (10%, 0.1 ±0.31, 1); *T. typhonius, 4* of 11 hosts (36.7%2 ±5.08, 1–17).

Stage: Encysted larvae

Site of infection: body tissues

Remarks: Amphibians act as intermediate or paratenic hosts in the life cycle of these parasites. Crocodilians, freshwater rays and teleosts are considered the main definitive hosts (Anderson, 2000). *D. nanus, H. raniceps, L. chaquensis, L. fuscus*, *L. podicipinus, P. azurea, P. paradoxa, S. nasicus* and *T. typhonius* represent new host records.

Porrocaecum sp.

Hosts: *H. raniceps, L. chaquensis, L. fuscus*, *P. azurea* and *T. typhonius.*

Prevalence, mean abundance, and range: H. raniceps, 2 of 36 hosts (5.5%, 0.3 ±1.47, 4–8); *L. chaquensis,* 1 of 20 hosts (5%, 0.1 ±0.44, 2); *L. fuscus, 3* of 30 hosts (10%, 4.2±18.74, 8–16); *P. azurea, 1* of 29 hosts (3.4%, 0.03 ±0.18, 1); *T. typhonius, 1* of 11 hosts (9%, 5.7 ±18.99, 63).

Stage: Encysted larva

Site of infection: Body tissues

Remarks: Indirect life cylce. Amphibians may act as intermediate or paratenic hosts, they become infect by ingesting infected invertebrates. These helminths complete the cycle when the intermediate host is consumed by the definitive host (which are generally birds) (Anderson, 2000). Indeed, there are reports of adult specimens of *P. reticulatum* and *Porrocaecum* sp. in birds in the Pantanal region (Tavares et al., *in press*), *H. raniceps, L. chaquensis, L. fuscus*, *P. azurea* and *T. typhonius* represent new hosts.

Ascarididae fam. gen. sp.

Hosts: L. chaquensis, L. fuscus, L. podicipinus, P. azurea, P. paradoxa, S. nasicus and *T. typhonius*

Prevalence, mean abundance, and range: L. chaquensis, 2 of 20 hosts (10%9.6 ±42.45, 3–19); *L. fuscus, 1* of 30 hosts (3.3%, 0.1 ±0.54, 3); *L. podicipinus, 4* of 35 hosts (11.4%, 1.2±5.48, 4–32); *P. azurea,* 2 of 29 hosts (6.9%, 0.2 ± 0.94, 1–5); *P. paradoxa,* 3 of 37 hosts (8.1%, 0.2 ± 1.03, 1–6); *S. nasicus, 3* of 10 hosts (30%, 4.9 ±14.45, 1–46); *T. typhonius* 2 of 11 hosts (18.2%, 0.9 ±2.42, 2–8)

Stage: Encysted larva

Site of infection: Body tissues

Remarks: These encysted larvae were in much undifferentiated stage, which made a more accurate identification unfeasible. However, it is very likely these parasites are either *Brevimulticaecum* or *Porrocaecum* species.

Atractidae

Schrankiana formosula Freitas, 1959

Hosts: *L. chaquensis, L. fuscus* and *P. azurea.*

Prevalence, mean abundance, and range: L. chaquensis, 7 of 20 hosts (35%, 6.9 ±14.43, 1–58);

L. fuscus, 9 of 30 hosts (30%, 28.3±62.45, 9–295); *P. azurea,* 5 of 29 hosts (17.2%, 6.4 ±17.10, 3–66)

Stage: Adult

Site of infection: large intestine

Remarks: Direct life cycle. Third stage larvae develop in the uterus of the adult worm and then autoinfect the host. Transmission from host to host is unknown (Anderson, 2000). *L. chaquensis* and *P. azurea* are new host records.

Schrankiana fuscus **Baker and Vaucher, 1988**

Host: *L. fuscus*

Prevalence, mean abundance, and range: 2 of 20 hosts (6.6%, 4.9±24.86, 13–136)

Stage: Adult

Site of infection: Large intestine

Remarks: Pantanal is a new locality record.

Schrankiana **sp.**

Hosts: *L. fuscus* and *P. azurea.*

Prevalence, mean abundance, and range: P. azurea, 1 of 29 hosts (3.4%, 0.9 ± 5.19, 28); *L. fuscus,* 2 of 20 hosts (6.6%, 2±10.94, 2–6).

Stage: Adult

Site of infection: Large intestine.

Remarks: Only female specimens were found, thus it was not possible to assign it to a species. Though, it is probably *Schrankiana formosula* and/or *Schrankiana fuscus.*

Cosmocercidae

Aplectana hylambatis **(Baylis, 1927)**

*Host***:** *T. typhonius*

Prevalence, mean abundance, and range: 9 of 11 hosts (81.9%, 12.4 ±13.11, 1–43)

Stage: adult

Site of infection: Large intestine

Remarks: Direct life cycle. Amphibians are the definitive hosts, transmission occurs through ingestion of the infective larvae (Anderson, 2000). Several anurans are reported as hosts to *A. hylambatis* (Campião et al., 2014), but *T. typhonius* represents a new host and Pantanal a new locality record.

***Aplectana* sp. 1**

Hosts: L. chaquensis and L. podicipinus

Prevalence, mean abundance, and range: L. chaquensis, 3 of 20 hosts (15%, 1.9 ±5.51, 4–21); *L. podicipinus, 4* of 35 hosts (11.4%, 3.1±12.51, 2–22)

Stage: Adult

Site of infection: Large intestine

Remarks: The morphometry does not match with other *Aplectana* species, indicating it is probably as new species.

***Aplectana* sp. 2**

Host: P. albonotatus

Prevalence, mean abundance, and range: 6 of 9 hosts (66.7%, 0.8±0.92, 1–3)

Stage: Adult

Site of infection: Large intestine

Remarks: The morphometry of these specimens does not match with other Aplectana species, indicating it is probably as new species. This is the first report of *Aplectana* in *P. albonotatus.*

***Cosmocerca parva* Travassos, 1925**

Host: P. azurea

Prevalence, mean abundance, and range: 1 of 29 hosts (3.4%, 0.06 ±0.37, 2)

Stage: Adult

Site of infection: Large intestine

Remarks: Cosmocercids have direct lif cycle. Amphibians are the definitive hosts, infective larvae are found in the soil and transmission occurs through skin penetration (Anderson, 2000). *P. azurea* is a new host and Pantanal a new locality record.

Cosmocerca podicipinus **Baker and Vaucher, 1984**

Hosts: D. nanus, H. raniceps, L. chaquensis, L. fuscus, L. podicipinus and *P. azurea.*

Prevalence, mean abundance, and range: D. nanus, 1 of 5 hosts (20%, 0.2 ± 0.44); *H. raniceps,* 4 of 36 hosts (11.1%, 0.86 ±4.66, 1–28); *L. chaquensis,* 11 of 20 hosts (55%, 3.5 ±5.59, 1–18); *L. fuscus,* 7 of 20 hosts (23.3%, 1.2 ±2.53, 2–8); *L. podicipinus,* 20 of 35 hosts (57.1%, 2.3±2.95, 1–11); *P. azurea,* 2 of 29 hosts (6.9%, 0.06 ±0.25, 1).

Stage: Adult

Site of infection: Large intestine

Remarks: H. raniceps a new host record. *C. parva* and *C. podicipinus* are among the helminth species with widest host and locality records in South America (Campião et al., 2014).

Cosmocercella **cf.** ***phyllomedusae*** **Bacher and Vaucher, 1986**

Host: *P. azurea*

Prevalence, mean abundance, and range: 1 of 29 hosts (3.4%, 0.06 ±0.37, 2)

Stage: Adult

Site of infection: Large intestine

Remarks: The life cycle of this species is unknown, but it is likely to be direct with infection occurring through ingestion or skin penetration, as in other cosmocercids (Anderson, 2000). *P. azurea* is a new host and Pantanal a new locality record.

***Oxyascaris oxyascaris* Travassos, 1920**

Hosts: *H. raniceps* and *T. typhonius.*

Prevalence, mean abundance, and range: H. raniceps, 8 of 36 hosts (22.2%, 0.7 ±1.8, 1–8); *T. typhonius,* 5 of 11 hosts (45.4%, 1.4 ±1.96, 1–5).

Stage: Adult

Site of infection: Small intestine

Remarks: Direct life cycle. Amphibians are the final hosts, infection occurs through the ingestion of the infective larvae (Anderson, 2000). *H. raniceps* and *T. typhonius* are new host records.

***Oxyascaris* sp.**

Hosts: L. chaquensis and *L. fuscus.*

Prevalence, mean abundance, and range: L. chaquensis, 5 of 20 hosts (25%, 0.4 ±0.82, 1–3); *L. fuscus Prevalence, mean abundance, and range:* 7 of 20 hosts (23.3%, 0.4 ±1, 1–4).

Stage: Adult

Site of infection: Small intestine

Remarks: Precise identification was not possible because only female specimens were found in these hosts.

***Raillietnema minor* Freitas and Dobbin Jr., 1961**

Host: *P. azurea*

Prevalence, mean abundance, and range: 8 of 29 hosts (27.6%, 26.6 ±50.32, 34–142)

Stage: Adult

Site of infection: Large intestine.

Remarks: Direct life cycle. Transmission might occur through ingestion or skin penetration, as in other members of Cosmocercidae. *P. azurea* is a new host and Pantanal a new locality record.

***Raillietnema* sp.**

Hosts: *L. chaquensis, L. fuscus, L. podicipinus* and *P. azurea.*

Prevalence, mean abundance, and range: L. chaquensis, 8 of 20 hosts (40%, 12.8, ± 33.48, 1–141); *L. fuscus,* 5 of 20 hosts (16.6%, 9.6±34.13, 1–155); *L. podicipinus,* 4 of 35 hosts (11.4%, 1.3±7.25, 1–43); *P. azurea,* 1 of 29 hosts (3.4%, 0.03 ±0.18, 1).

Stage: Adult

Site of infection: Large intestine

Remarks: *L. chaquensis, L. fuscus, L. podicipinus* and *P. azurea* are new host records to the genus.

Cosmocercidae gen. sp.

Hosts: *H. raniceps, L. chaquensis, L. fuscus, L. podicipinus, P. azurea, P. albonotatus, P. limellum, P. paradoxa* and *T. typhonius.*

Prevalence, mean abundance, and range: Hosts: *H. raniceps, 4* of 36 hosts (11.1%, 0.11 ± 0.31, 1); *L. chaquensis,* 7 of 20 hosts (35%, 2.1 ±3.62, 1–12); *L. fuscus,* 9 of 30 hosts (30%, 0.6 ±1.21, 1–4); *L. podicipinus,* 18 of 35 hosts (51.4%, 4.8±15.03, 1–89); *P. azurea,* 4 of 29 hosts (13.8%, 0.5 ± 1.61, 1–6); *P. albonotatus,* 1 of 9 hosts (11.1%, 0.1±0.3, 1); *P. limellum,* 1 of 7 hosts (14.3%, 0.2, 3); *P. paradoxa,* 3 of 37 hosts (8.1%, 0.1 ±0.48, 1–2); *T. typhonius,* 1 of 11 hosts (9%, 3.3 ±11.15, 37).

Stage: Adult

Site of infection: Intestines

*Remarks***:** It is likely that such specimens are either 1 of the *Cosmocerca* or *Aplectana* species that were found in these hosts, once it is not possible, based on the morphometry, to assign Cosmocercidadae to a genus when only female specimens of were found.

Cosmocercoidea fam. gen. sp.

Hosts: *H. raniceps, L. fuscus, L. podicipinus, P. azurea* and *S. nasicus.*

Prevalence, mean abundance, and range: H. raniceps, 2 of 36 hosts (5.5%, 1.1 ± 4.88, 16–25); *L. fuscus,* 2 of 30 hosts (6.7%, 1.7 ±8.27, 7–45); *L. podicipinus,* 1 of 35 hosts (2.9%, 4.4±26.5, 157); *P. azurea,* 2 of 29 hosts (6.9%, 0.7 ±2.88, 9–13); *S. nasicus,* 1 of 10 hosts (10%, 0.3 ±0.94, 3).

Stage: Larvae

Site of infection: Large intestine

Remarks: Direct life cycle. These nematode larvae were found free in the intestines, and might be either 1 of the parasite species of this superfamily found in this study (*Aplectana, Cosmocerca* or *Raillietnema*).

Pharyngodonidae

Parapharyngodon sp.

Host: T. typhonius

Prevalence, mean abundance, and range: 8 of 11 hosts (72.7%, 15.6 ±23.42, 2–27)

Stage: Adult

Site of infection: Intestines

Remarks: Life cycle is unknown. Bursey and Brooks (2004) described *Parapharyngodon*

duniae in *Phrynohyas venulosa* (currently *T. typhonius*) from Costa Rica.

Rhabdiasidae

Rhabdias sp.

Hosts: L. chaquensis, L. podicipinus and *P. paradoxa*

Prevalence, mean abundance, and range: L. chaquensis, 6 of 20 hosts (30%, 0.5 ±1, 1–4); *L. podicipinus,* 13 of 35 hosts (37.1%0.6±1.47, 1–8); *P. paradoxa,* 1 of 37 hosts (2.7%, 0.05 ±0.32, 2).

Stage: Adult

Site of infection: Lungs

Remarks: Direct life cycle. *Rhabdias* spp. alternate between free living and parasite generations. Amphibians are the definitive hosts, and infection occurs through skin penetration of the infective larvae (Anderson, 2000).

Physalopteridae

Physalopteroides venancioi **Wu and Liu, 1940**

Hosts: H. raniceps, L. chaquensis, L. fuscus, L. podicipinus, P. albonotatus, S. nasicus and *T. typhonius*

Prevalence, mean abundance, and range: H. raniceps, 5 of 36 hosts (13.9%, 0.5 ±2.18, 1–13); *L. chaquensis,* 6 of 20 hosts (30%, 4.1 ±9.51, 1–28); *L. fuscus,* 5 of 30 hosts (16.6%, 3.1 ±12.56, 3–68); *L. podicipinus,* 2 of 35 hosts (5.7%, 0.08±0.37, 1–2); *S. nasicus,* 1 of 10 hosts (10%, 0.1 ±0.31, 1); *T. typhonius,* 1 of 11 hosts (45.5%, 3.1 ±6.32, 1–3).

Stage: Adult

Site of infection: Stomach

Remarks: Indirect life cycle. Amphibians are the definitive hosts. They are infected after ingesting the first intermediate host (usually an arthropod) (Anderson, 2000). *H. raniceps, L. chaquensis, L. fuscus, P. albonotatus, S. nasicus* and *T. typhonius* are new host records.

Physaloptera **sp.**

Hosts: H. raniceps, L. chaquensis and *T. typhonius.*

Prevalence, mean abundance, and range: H. raniceps, 1 of 36 hosts (2.7%, 0.1 ±0.83, 5); *L. chaquensis,* 4 of 20 hosts (20%, 0.3 ±0.81, 1–3); *P. albonotatus,* 1 of 9 hosts (11.1%, 0.1±0.3, 1); *T. typhonius,* 2 of 11 hosts (18.2%, 19.3 ±62.52, 5–28).

Stage: Larva

Site of infection: Stomach

Remarks: Indirect life cycle. Amphibians may act as intermediate, paratenic and definitive hosts to *Physaloptera* spp. They are infected after ingesting the first intermediate host (usually an arthropod). When acting as intermediate or paratenic hosts, reptiles are the most common definitive hosts (Anderson, 2000). *H. raniceps* and *L. chaquensis* are new host records.

Spirocercidae

***Physocephalus* sp. 1**

Hosts: *H. raniceps, L. chaquensis, L. fuscus, L. podicipinus, P. albonotatus, P. paradoxa* and *T. typhonius.*

Prevalence, mean abundance, and range: H. raniceps, 1 of 36 hosts (2.8%, 0.05 ±0.33, 2); *L. chaquensis,* 2 of 20 hosts (10%, 8.7 ±27.50, 19–63); *L. fuscus,* 4 of 30 hosts (13.3%, 1.8±6.14, 1–29); *L. podicipinus,* 5 of 35 hosts (14.3%, 1.6±5.29, 1–19); *P. albonotatus,* 1 of 9 hosts (11.1%, 0.1±0.3, 1); *P. paradoxa,* 1 of 37 hosts (2.7%0.1 ±0.98, 6); *T. typhonius,* 1 of 11 hosts (9.1%, 24.4 ±54.07, 2–164).

Stage: Larva

Site of infection: Body tissues

Remarks: Indirect life cycle. Amphibians are the second intermediate or paratenic hosts. They get infected after ingesting the first intermediate host (usually an arthropod). Mammals are the definitive hosts (Anderson, 2000). *H. raniceps, L. chaquensis, L. fuscus, L. podicipinus, P. albonotatus, P. paradoxa* and *T. typhonius* are new host records to the genus.

***Physocephalus* sp. 2**

Hosts: L. podicipinus

Prevalence, mean abundance, and range: 5 of 35 hosts (14.3%, 3±9.48, 4–42)

Stage: Encysted larva

Site of infection: Body tissues

***Physocephalus* sp. 3**

Hosts: L. chaquensis, L. fuscus, P. paradoxa, S. nasicus and *T. typhonius.*

Prevalence, mean abundance, and range: L. chaquensis, 2 of 20 hosts (10%, 4.2 ±17.42, 6–78); *L. fuscus,* 2 of 30 hosts (6.6%, 0.9 ±3.6, 11–17); *P. paradoxa,* 1 of 37 hosts (2.7%, 0.08 ±0.49, 3); *S. nasicus,* 1 of 10 hosts (10%0.2 ±0.63, 2); *T. typhonius,* 4 of 11 hosts (36.4%, 1.9 ±6.33, 21).

Stage: Encysted larva

Site of infection: Body tissues

Rhabdochonidae gen. sp.

Hosts: T. typhonius

Prevalence, mean abundance, and range: 2 of 11 hosts (18.2%, 10 ±28.27, 16–94)

Stage: Encysted larva

Site of infection: Body tissues

Remarks: Indirect life cycle. Amphibians are infected through ingestions of the infective stages and act as intermediate or paratenic hosts. Reptiles are the most common definitive hosts.

Molineidae

Oswaldocruzia **sp. (Trichostrongyloidea: Molineidae)**

Hosts: H. raniceps and *L. chaquensis.*

Prevalence, mean abundance, and range: H. raniceps, 1 of 36 hosts (2.8%, 0.02 ±0.16, 1); *L. chaquensis,* 1 of 20 hosts (5%, 0.05 ±0.22, 1)

Stage: Adult

Site of infection: Small intestine

Remarks: Direct life cycle. Amphibians are the final hosts and get infected throught skin penetration of the infective larva (Anderson, 2000).

Trematoda

Diplostomidae

***Diplostomulum* sp.**

Hosts: L. chaquensis, L. podicipinus, P. azurea, P. paradoxa and *S. nasicus.*

Prevalence, mean abundance, and range: L. chaquensis, 1 of 20 hosts (5%, 1.7 ±7.82, 35); *L. podicipinus,* 1 of 35 hosts (2.9%, 0.4±2.70, 16); *P. azurea,* 2 of 29 hosts (3.4%, 1.6 ±8.72, 47); *P. paradoxa,* 1 of 37 hosts (2.7%, 0.8 ±5.09, 31); *S. nasicus,* 1 of 10 hosts (10%, 0.1 ±0.31, 1).

Stage: Larva (metacercaria)

Site of infection: Large intestine and kidneys.

Remarks: Diplostomidae are parasites of birds and mammals, the hosts reported here may be acting as second intermediate or paratenic hosts (Niewiadomska, 2002).

***Neascus* sp.**

Host: L. chaquensis

Prevalence, mean abundance, and range: 1 of 20 hosts (5%, 0.3 ±1.56, 7)

Stage: Larva (metacercaria)

Site of infection: Large intestine

Remarks: *L. chaquensis* is a new host record for metacercaria type *Neascus* sp.

Diplostomoidea fam. gen. sp.

Host: S. nasicus

Prevalence, mean abundance, and range: 1 of 10 hosts (10%, 0.7 ±2.21, 7)

Stage: Larva (metacercaria)

Site of *infection*: Kidneys

Diplodiscidae

***Catadiscus pygmaeus* (Lutz, 1928)**

Host: P. limellum

Prevalence, mean abundance, and range: 1 of 7 hosts (14.3%, 0.4 ± 1.13, 1)

Stage: Adult

Site of infection: Large intestine

Remarks: Indirect life cycle. Amphibians are the finl hosts. Infection occurs through the ingestion of infective metacercariae, when the hosts forage (Hamann 2004). *P. limellum* is a new host record.

***Catadiscus* sp.**

Hosts: H. raniceps, L. chaquensis, L. fuscus, L. podicipinus, P. azurea and *P. paradoxa.*

Prevalence, mean abundance, and range: H. raniceps, 1 of 36 hosts (2.8%, 0.1 ± 0.66, 4); *L. chaquensis,* 8 of 20 hosts (40%, 2.5 ±4.0, 1–19); *L. fuscus,* 1 of 30 hosts (3.3%, 0.03 ±0.18, 1); *L. podicipinus,* 14 of 35 hosts (40%, 1.5±2.80, 1–12); *P. azurea,* 2 of 29 hosts (13.8%, 0.2 ±0.84, 1–4); *P. paradoxa,* 19 of 37 hosts (51.3%, 4.9 ±8.16, 2–33).

Stage: Adult

Site of infection: Large intestine

Remarks: Amphibians are the final hosts. Infection occurs through the ingestion of infective metacercariae, when the hosts forage (Hamann, 2004). *H. raniceps, L. fuscus,* and *P. azurea* are new host records.

Glypthelminthidae

***Glypthelmins palmipedis* (Lutz, 1928)**

Host: P. paradoxa

Prevalence, mean abundance, and range: 19 of 37 hosts (51.3%, 1.1 ±1.39, 1–6)

Stage: Adult

Site of infection: Small intestine

Remarks: Indirect life cycle, which always require a mollusc as intermediate hosts. Amphibians are the final hosts to *Glypthelmins* spp., and are infected through skin penetration of the infective cercariae (Hamann, 2006).

Pentastomida

Undetermined Pentastomida

Host: S. nasicus

Prevalence, mean abundance, and range: 2 of 10 hosts (20%, 4.9 ±14.47, 3–46)

Stage: Nymph

Site of infection: Kidneys

Remarks: Pentastomids comprise a small, entirely parasitic group of animals that inhabit the respiratory tracts of vertebrates, mostly reptiles. Larval development generally occurs in vertebrate and invertebrate intermediate hosts (Lavrov et al., 2004). *S. nasicus* is a new host record.

Undertermined cyst

Hosts: L. chaquensis, L. fuscus, L. podicipinus, P. azurea, P. paradoxa and *T. typhonius.*

Prevalence, mean abundance, and range: L. chaquensis, 14 of 20 hosts (70%, 105.3 ±191.12, 1–159); *L. fuscus,* 5 of 30 hosts (16.7%, 2.5 ±7.36, 6–37); *L. podicipinus,* 13 of 35 hosts (37.1%, 39±130.20, 1–315); *P. azurea,* 1 of 29 hosts (*Stage:* 3.4%, 1.4 ±7.61, 41); *P. paradoxa,* 15 of 37 hosts (40.5%, 18.2 ±56.25, 2–318); *T. typhonius,* 1 of 11 hosts (9%, 0.2 ±0.60, 1).

Stage: Encysted larva

Site of infection: Body tissues

Table 1. Helminth parasites associated with 11 anurans species from Pantanal, Brazil.

Host Family/species	**Helminth species**
Hylidae	

Dendropsophus nanus	*Brevimulticaecum* sp.
	Cosmocerca podicipinus
Hypsiboas raniceps	Acanthocephala (undetermined)
	Brevimulticaecum sp.
	Catadiscus sp.
	Cosmocerca podicipinus
	Cosmocercidae gen. sp.
	Cosmocercoidea fam. gen. sp.
	Oswaldocruzia sp.
	Oxyascaris oxyascaris
	Physaloptera sp.
	Physalopteroides venancioi
	Physocephalus sp. 1
	Porrocaecum sp.
Phyllomedusa azurea	Acanthocephala (undetermined)
	Ascarididae gen. sp.
	Brevimulticaecum sp.
	Catadiscus sp.
	Cosmocerca parva
	Cosmocerca podicipinus
	Cosmocercella cf. *phyllomedusae*

	Cosmocercidae gen. sp.
	Cosmocercoidea fam. gen. sp.
	Diplostomulum sp.
	Porrocaecum sp.
	Raillietnema minor
	Raillietnema sp.
	Schrankiana formosula
	Schrankiana sp.
	Undertermined cyst
Pseudis limellum	*Catadiscus pygmaeus*
	Cosmocercidae gen. sp.
Pseudis platensis	Acanthocephala (undetermined)
	Ascarididae gen. sp.
	Brevimulticaecum sp.
	Catadiscus sp.
	Cosmocercidae gen. sp.
	Diplostomulum sp.
	Glypthelmins palmipedis
	Physocephalus sp. 1
	Physocephalus sp. 3
	Rhabdias sp.

	Undertermined cyst
Scinax nasicus	Ascarididae gen. sp.
	Brevimulticaecum sp.
	Undertermined cyst
	Cosmocercoidea fam. gen. sp.
	Diplostomoidea fam. gen. sp.
	Diplostomulum sp.
	Pentastomida (undetermined)
	Physalopteroides venancioi
	Physocephalus sp. 3
Trachycephalus typhonius	Acanthocephala (undetermined)
	Aplectana hylambatis
	Ascarididae gen. sp.
	Parapharyngodon sp.
	Brevimulticaecum sp.
	Cosmocercidae gen. sp.
	Oxyascaris oxyascaris
	Parapharyngodon sp.
	Physaloptera sp.
	Physalopteroides venancioi
	Physocephalus sp. 3

	Physocephalus sp.1
	Porrocaecum sp.
	Rhabdochonidae gen. sp.
	Undertermined cyst
Leptodactylidae	
Leptodactylus chaquensis	Acanthocephala (undetermined)
	Aplectana sp. 1
	Ascarididae gen. sp.
	Brevimulticaecum sp.
	Catadiscus sp.
	Undertermined cyst
	Cosmocerca podicipinus
	Cosmocercidae gen. sp.
	Diplostomulum sp.
	Neascus sp.
	Oswaldocruzia sp.
	Oxyascaris sp.
	Physaloptera sp.
	Physalopteroides venancioi
	Physocephalus sp. 1
	Physocephalus sp. 3

	Porrocaecum sp.
	Raillietnema sp.
	Rhabdias sp.
	Schrankiana formosula
Leptodactylus fuscus	Acanthocephala (undetermined)
	Ascarididae gen. sp.
	Brevimulticaecum sp.
	Catadiscus sp.
	Cosmocerca podicipinus
	Cosmocercidae gen. sp.
	Cosmocercoidea fam. gen. sp.
	Oxyascaris sp.
	Physalopteroides venancioi
	Physocephalus sp. 1
	Physocephalus sp. 3
	Porrocaecum sp.
	Raillietnema sp.
	Schrankiana formosula
	Schrankiana fuscus
	Schrankiana sp.
	Undertermined cyst

Leptodactylus podicipinus	Acanthocephala (undetermined)
	Aplectana sp. 1
	Ascarididae gen. sp.
	Brevimulticaecum sp.
	Catadiscus sp.
	Cosmocerca podicipinus
	Cosmocercidae gen. sp.
	Cosmocercoidea fam. gen. sp.
	Diplostomulum sp.
	Physalopteroides venancioi
	Physocephalus sp. 1
	Physocephalus sp. 2
	Raillietnema sp.
	Rhabdias sp.
	Undertermined cyst
Physalaemus albonotatus	Aplectana sp. 2
	Cosmocercidae gen. sp.
	Physaloptera sp.
	Physocephalus sp. 1

DISCUSSION

We found 37 helminth taxa, 13 of them in larval stages. Helminth species found in larval stages lack several features used to distinguish helminth species morphologically. In these cases, the number of parasites species might be underestimated, and each of these taxa can include more than one species. Nematodes accounted with most (73%) of the helminth species we found. Indeed, Nematoda are the most frequent helminth parasites in South American amphibians (Campião et al., 2014).

Cosmocercids, such as *A. hylambatis, C. podicipinus* and *Parapharygodon* were the most prevalent and abundant nematodes. Indeed, anurans have low vagility, and this may increase the transmission success of these direct life–cycle nematodes (McAlpine 1997). Trematodes only reached high prevalence and abundance in *P. paradoxa,* where *Catadiscus* sp. and *G. palmipens* were central species*.* This is not surprising once *P. paradoxa* is an aquatic frog, which might favour the transmission of trematodes (Kehr et al. 2000, Kehr and Hamann2003, Campião et al. 2010). Most helminth species were shared among different host species, and even those we found in a single host in this study, as *A. hylambatis*, *C. parva*, *Cosmocercella* cf. *phyllomedusae*, *Glypthelmins palmipedis,* and *Raillietnema minor*, are known to infect other host species (Campião et al., 2014).

The present study is a contribution to the knowledge of the helminth fauna of Neotropical amphibians. Pantanal wetlands harbour a rich and abundant anuran fauna, but little is known about its parasite diversity. Besides unvealing the crypitic biodiversity, inventories of parasite species are important to subsidize ecological and evolutionary studies.

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Diversity and Patterns of Interaction of an Anuran-Parasite Network in the Pantanal Wetland

SUMMARY

We describe the diversity and structure of a host-parasite network of eleven anuran species and their helminth parasites in the Pantanal wetland. Specifically, we investigate how the heterogeneous use of space by hosts changes parasite transmission success and diversity, and how the local pool of parasites exploits sympatric host species. We examined 229 specimens, interacting with 37 parasite taxa. Mixed effect models indicated the effect of anuran body size, but not the habit, as a determinant of parasite species richness. Variation on taxonomic diversity on the other hand, was not significantly correlated to host size or habit. Similarity in parasite communities did not correlate to host phylogeny, indicating no effect of the evolutionary relationship among anurans on the similarities on their parasite communities. We found a nested, but not modular pattern in the host-parasite network, which is probably a result of low host specificity observed amongst most helminths in this study. Overall, we found that host attributes, such as body size, were important in determining parasite community richness, whereas parasite attributes (specificity) were important to network structure.

KEY WORDS: network, nestedness, helminth community, parasite, Amphibian.

KEY FINDINGS

The diversity of helminth parasite communities was determined by host body size, but not by host habit.

Evolutionary relationships amongst anurans were not determinants to the similarities of their parasite communities. Host-parasite network is nested, but not modular.

INTRODUCTION

Identifying which factors affect the diversity of parasite communities across hosts is one of the major quests in parasite ecology. The most common approach to untangle the process behind the patterns, is inferring which factors correlate to what we observe. For example, which host traits correlate to parasite diversity? When we observe different hosts exploring a given habit, how do we expect these hosts to be explored as habitats for the local pool of parasite species? Which host species would be the most parasitized, and which traits would favour high parasite exploitation? These questions have been studied extensively, and major advances in this field have occurred in the past years, unveiling some mechanisms underlying the patterns long observed (Poulin, 2007).

Body size is the best-studied host trait explaining parasite biodiversity, being positively related to parasite species richness (Kamya *et al.* 2014, see also Article 2). Large-bodied hosts may be easier to colonize because of the greater amounts of food they ingest, their large surface area, greater mobility, wider niche breadth, and longer time of exposure to parasites (Poulin, 2007). Additionally, other host features (such as diet, behaviour and habit) might be equally important in determining parasite diversity and composition (Poulin and Morand, 2004). Host habit may play an import role in parasite assembling because, all else being the same, variations in habitat use by hosts would imply in varying exposure to parasite infective stages. Nonetheless, few studies have examined the influence of host habit in parasite communities (Aho, 1990; Hamann *et al.* 2013).

One promising way of studying parasite biodiversity is under the concepts of network theory (Proulx *et al.* 2005). Ecological networks have been considered the building blocks of biodiversity, and the understanding of their structure is important to the understanding of the whole ecosystem functioning (Joppa and Williams, 2013). Network analysis provides a useful framework to identifying, understanding and predicting how parasites and hosts interact (Poulin, 2010; Lima *et al.* 2012; Krasnov *et al.* 2012). The architecture and patterns of connectivity in the network is determined by the adaptation between the interacting species, which for us are hosts and their parasites. These adaptations include a variety of host traits (such as body size, immune response, feeding habit and behaviour), and parasite traits (abundance, dispersal ability and the degree of specificity) (Krasnov *et al.* 2012). Because the structure of ecological networks can affect the resilience and fragility of the whole ecosystems, identifying which host traits are influential to network structuring is a relevant conservation issue (Dunne *et al.* 2002).

In this study, we investigate how the heterogeneous use of space by hosts changes parasite colonization success and diversity, and how the local pool of parasites exploits sympatric host species. Specifically, we report helminth prevalence, species richness and taxonomic diversity of eleven anuran species, examining how parasite diversity varies across hosts of different size and habit. We further investigate the interaction proprieties of this anuran-parasite network.

METHODS

This study was carried out with anurans collected in the farmland Fazenda Alegria (18°59'S e 56°39'W), southeastern Pantanal, Brazil. Our field trips to collect the host species were conducted in the rainy seasons of 2011, 2012 and 2013. Anurans were hand-captured and taken to the lab, where they were euthanized with an overdose of sodium thiopental solution. We recorded their snout-vent-length and examined their body cavity, digestive tract, accessory organs and musculature for helminth parasites. Parasites were collected and processed according to standard procedures (described in more details on article 4).

We examined 229 host specimens, interacting with 37 parasite taxa (one acanthocephalan cystacanth, 28 nematodes, six trematodes, an undetermined helminth cyst, and one pentastomid nymph). Three nematode taxa were excluded when they could confound the analyses, such helminths were either females or larvae (lacking morphological features that allow the identification to lower taxonomic levels). Thus, to avoid data pseudoreplication, we removed from analysis nematode larval specimens assigned to Ascarididae gen. sp. in hosts that were associated with *Brevimulticaecum* or *Porrocaecum* species (because these belong to Ascarididae). Similarly, specimens of Cosmocercidae gen. sp. and Cosmocercoidea fam. gen. sp. were removed from analyses in hosts associated with *Aplectana, Cosmocerca* or *Cosmocercella* species. *Schrankiana* sp. was excluded from analyses when associated with hosts that had *S. formosula* or *S. fuscus.* The term infracommunity refers to the helminth community in a single host. Parasite prevalence was analyzed as defined by Bush *et al.* (1997). All analyses were performed in R (R Development Core Team, 2013).

We adopted two measures of parasite biodiversity: the number of helminth taxa per host (species richness) and taxonomic diversity. This last accounts for the variety of taxa to which the species belong, and thus, it captures some of the phylogenetic diversity in the sample. The taxonomic diversity index takes both the abundance and phylogenetic relatedness (based on the distance of a classification tree) amongst species into account. We used parasite phylum, class, superfamily, family and genus to build the classification tree, and the functions "*tax2dist"* and "*taxondive"* of the vegan package (Oksanen *et al.* 2013) to calculate the taxonomic diversity for each infracommunity. We tested the relation of host traits (mean body size and habit) and parasite diversity (species richness and taxonomic diversity) with mixed effect models using the "*lme4*" package (Bates *et al*., 2014). The acanthocephalan cystacanth, unidetermined cyst, the pentastomid nymph, and Rhabdochonidae gen. sp. were exluded from this analysis.

To test whether closely related hosts had more similar helminth communities, we compared distance matrices of host´s phylogeny and parasite communities. We first reconstructed amphibian's phylogenetic tree from Pyron and Wiens (2001) to our 11 anuran species with the *ape* package, and used the function "*cophenetic.phylo"* to compute the pairwise distances between the pairs of tips from the phylogenetic tree using its branch lengths (Paradis *et al.* 2004). Pairwise distance measures among hosts based on the dissimilarity of their parasite communities (considering data on parasite prevalence) were calculated with Bray-Curtis distance. We then tested if the two distance matrices were correlated with a mantel test with 1000 permutations.

The degree of nestedness of the network was evaluated using the NODF metric (Almeida-Neto *et al*. 2008). The randomness of matrix nestedness was assessed by the analysis of null models. The calculation of the NODF metric and the simulation of the null models (1000 randomizations) were calculated using the program ANINHADO (Guimarães and Guimarães, 2006). The detection of a modular pattern in network interactions was assessed with the program MODULAR (Marquitti *et al.* 2014). The program generates a value of modularity (M) for the interaction matrix and verifies if the degree of modularity differs from those generated by random networks (based on 1000 randomizations). Network graphs were constructed with the package "*igraph"* in R (Csardi and Nepusz 2006).

RESULTS

Helminth species richness and prevalence varied across hosts (Figure 1). Prevalence was generally low; only eight helminth taxa reached prevalences higher than 50% (Table 1).

Among eleven host species, *L. chaquensis* had the highest values of helminth diversity, followed by the treefrog *T. typhonius*. Leptodactylids had higher parasite biodiversity than hylids of similar size. Among the median-sized hylids, the aquatic frog *P. platensis* had the highest taxonomic diversity. In general, small anurans had low parasite diversity, despite their habit and taxonomy (Table 2). The Mixed effect model indicated anuran body size as a determinant of parasite species richness (Table 3). Variation on taxonomic diversity on the other hand, was not significantly correlated to host size or habit (Table 3). Similarity in parasite communities did not correlate to host phylogeny (Mantel statistic r: 0.1223, P= 0.30869).

We found a nested (NODF= 44.93, P (CE) = 0.02), but not modular (M=0.25, P=0.99) pattern in the host-parasite network (Figure 3, 4). Among all parasite species, 14 were associated with a single host and 11 were associated with five or more. The larval nematode *Brevimulticaecum* sp. was the most generalist helminth, occurring in nine host species, but this taxa might e composed of more than one species. Among adult worms, the nematode *Cosmocerca podicipinus* was the most generalist, associated with six host species (Table 1).

Table 1. Prevalence (%) of helminth parasites amongst 11 sympatric anuran species from Pantanal wetland, Brazil. Host species are Dn = *Dendropsophus nanus*, Hr = *Hypsiboas raniceps*, Lc = *Leptodactylus chaquensis*, Lf = *Leptodactylus fuscus*, Lp = *Leptodactylus podicipinus*, Pa = *Phyllomedusa azurea*, Py = *Physalaemus albonotatus*, Pl = *Pseudis limellum*, Pp = *Pseudis platensis*, Sn = *Scinax nasicus* and Tt = *Trachycephalus typhonius.*

[a]Parasite species	**Host species**										
	Dn	**Hr**	**Lc**	**Lf**	**Lp**	**Pa**	**Py**	**Pl**	**Pp**	**Sn**	**Tt**
1. Acanthocephala cystacanth	-	2.8	15	6.7	5.7	13.8	-	-	2.7	-	36.4
2. Aplectana hylambatis	-	-	-	-	-	-	-	-	-	-	81.8
3. *Aplectana* sp. 1	-	-	15	-	11.4	-	-	-	-	-	-
4. *Aplectana* sp. 2	-	-	-	-	-	-	66.7	-	-	-	-
5. Ascarididae gen. sp.	-	-	10	3.3	11.4	6.9	-	-	8.1	30	18.2
6. *Brevimulticaecum* sp.	20	19.4	30	26.7	5.7	6.9	-	-	5.4	10	36.4
7. Catadiscus pygmaeus	-	-	-	-	-	-	-	14.3	-	-	-
8. *Catadiscus* sp.	-	2.8	40	3.3	40	13.8	-	-	51.4	-	-
9. Undertermined cyst	-	-	70	16.7	37.1	3.4	-	-	40.5	-	9.1
10. *Cosmocerca parva*	-	-	-	-	-	3.4	-	-	-	-	-
11. *Cosmocerca podicipinus*	20	11.1	55	23.3	57.1	6.9	-	-	-	-	-
12. Cosmocercella phyllomedusae	-	-	-	-	-	3.4	-	-	-	-	-
13. Cosmocercidae gen. sp.	-	-	-	-	-	-	11.1	14.3	8.1	-	-

[a] Parasite species: numbers listing parasite species correspond to those in Figure 1 and Figure 2.

Table 1. cont.

[a]**Parasite species**	**Host species**[b]										
	Dn	**Hr**	**Lc**	**Lf**	**Lp**	**Pa**	**Py**	**Pl**	**Pp**	**Sn**	**Tt**
14. Diplostomoidea fam. gen. sp.	-	-	-	-	-	-	-	-	-	10	-
15. *Diplostomulum* sp.	-	-	5	-	2.9	3.4	-	-	2.7	10	-
16. *Glypthelmins palmipedis*	-	-	-	-	-	-	-	-	51.4	-	-
17. *Neascus* sp.	-	-	5	-	-	-	-	-	-	-	-
18. *Oswaldocruzia* sp.	-	2.8	5	-	-	-	-	-	-	-	-
19. *Oxyascaris oxyascaris*	-	22.2	-	-	-	-	-	-	-	-	45.5
20. *Oxyascaris* sp.	-	-	25	23.3	-	-	-	-	-	-	-
21. *Parapharyngodon* sp.	-	-	-	-	-	-	-	-	-	-	72.7
22. Pentastomida nymph	-	-	-	-	-	-	-	-	-	20	-
23. *Physaloptera* sp.	-	2.8	20	-	-	-	11.1	-	-	-	18.2
24. *Physalopteroides venancioi*	-	13.9	30	16.7	5.7	-	-	-	-	10	45.5
25. *Physocephalus* sp. 1	-	2.8	10	13.3	14.3	-	11.1	-	2.7	-	9.1
26. *Physocephalus* sp. 2	-	-	-	-	14.3	-	-	-	-	-	-
27. *Physocephalus* sp. 3	-	-	10	6.7	-	-	-	-	2.7	10	36.4
28. *Porrocaecum* sp.	-	5.6	5	10	-	3.4	-	-	-	-	9.1
29. *Raillietnema minor*	-	-	-	-	-	27.6	-	-	-	-	-
30. *Raillietnema* sp.	-	-	40	16.7	11.4	3.4	-	-	-	-	-

[a] Parasite species: numbers listing parasite species correspond to those in Figure 1 and Figure 2.

Table 1. cont.

[a]Parasite species	Host species[b]										
	Dn	Hr	Lc	Lf	Lp	Pa	Py	Pl	Pp	Sn	Tt
31. *Rhabdias* sp.	-	-	30	-	37.1	-	-	-	2.7	-	-
32. Rhabdochonidae gen. sp.	-	-	-	-	-	-	-	-	-	-	18.2
33. *Schrankiana formosula*	-	-	35	30	-	17.2	-	-	-	-	-
34. *Schrankiana fuscus*	-	-	-	6.7	-	-	-	-	-	-	-

[a] Parasite species: numbers listing parasite species correspond to those in Figure 1 and Figure 4.

Table 2: Number of specimens (N), mean body size (mm), habit, total species richness (THR), mean species richness (MHR) and taxonomic diversity (Δ+) of the helminth parasites of eleven anuran species.

	Host traits			Parasite Diversity		
Host species	**N**	**Size (mm)**	**Habit**[a]	**THR**	**MHR**	**Δ+**
Hylidae						
Dendropsophus nanus	5	21.4	Ar	2	0.4 ± 0.49	0
Hypsiboas raniceps	36	57.6	Ar	11	0.9 ± 0.93	12.7±28.9
Phyllomedusa azurea	29	37.2	Ar	12	0.9 ± 1.40	16.7±33.9
Pseudis limellum	7	17.7	Aq	2	0 ± 0.45	0
Pseudis paradoxa	37	36.7	Aq	11	1.7 ± 1.25	34±42.9
Scinax nasicus	11	31.1	Ar	6	1.0 ± 1.14	19±40.5
Trachycephalus typhonius	10	69.5	Ar	12	4.3 ± 1.30	76±0.8
Leptodactylidae						
Leptodactylus chaquensis	20	63.8	ST	19	4.6 ± 1.89	74±11.9
Leptodactylus fuscus	30	41.1	T	13	2.0 ± 1.20	39±36.8
Leptodactylus podicipinus	35	32.1	ST	14	2.6 ± 2.02	43±42.6
Physalaemus albonotatus	9	26.3	T	3	0.1 ± 0.82	6.9±20.8

[a]Habit: Ar-Arboreal, Aq-Aquatic, ST-Semi-terrestrial, T-Terrestrial.

Table 3. Mixed effect models of host traits on helminth infracommunity diversity

Response variable	**Random effect variable**			**Fixed effect variable**				
	Parameter	V[a]	SE[b]	Parameter	Estimate	SE[b]	z	P
Infracommunity species richness	Host species	1.99	1.41	Intercept	0.3374	1.02	0.33	0.74
				Body size	0.033	0.00	28.90	0.001
				Habit Ar	0.2539	1.20	0.211	0.833
				Habit St	2.403	1.42	1.682	0.09
Infracommunity taxonomic diversity	Host species	114.8	10.7	Intercept	7.6725	9.53	0.805	0.454
				Body size	0.0953	0.15	0.629	0.533
				Habit Ar	-0.9726	10.2	-0.095	0.929
				Habit St	18.855	11.9	1.583	0.194
				Habit T	-0.723	11.8	-0.06	0.954

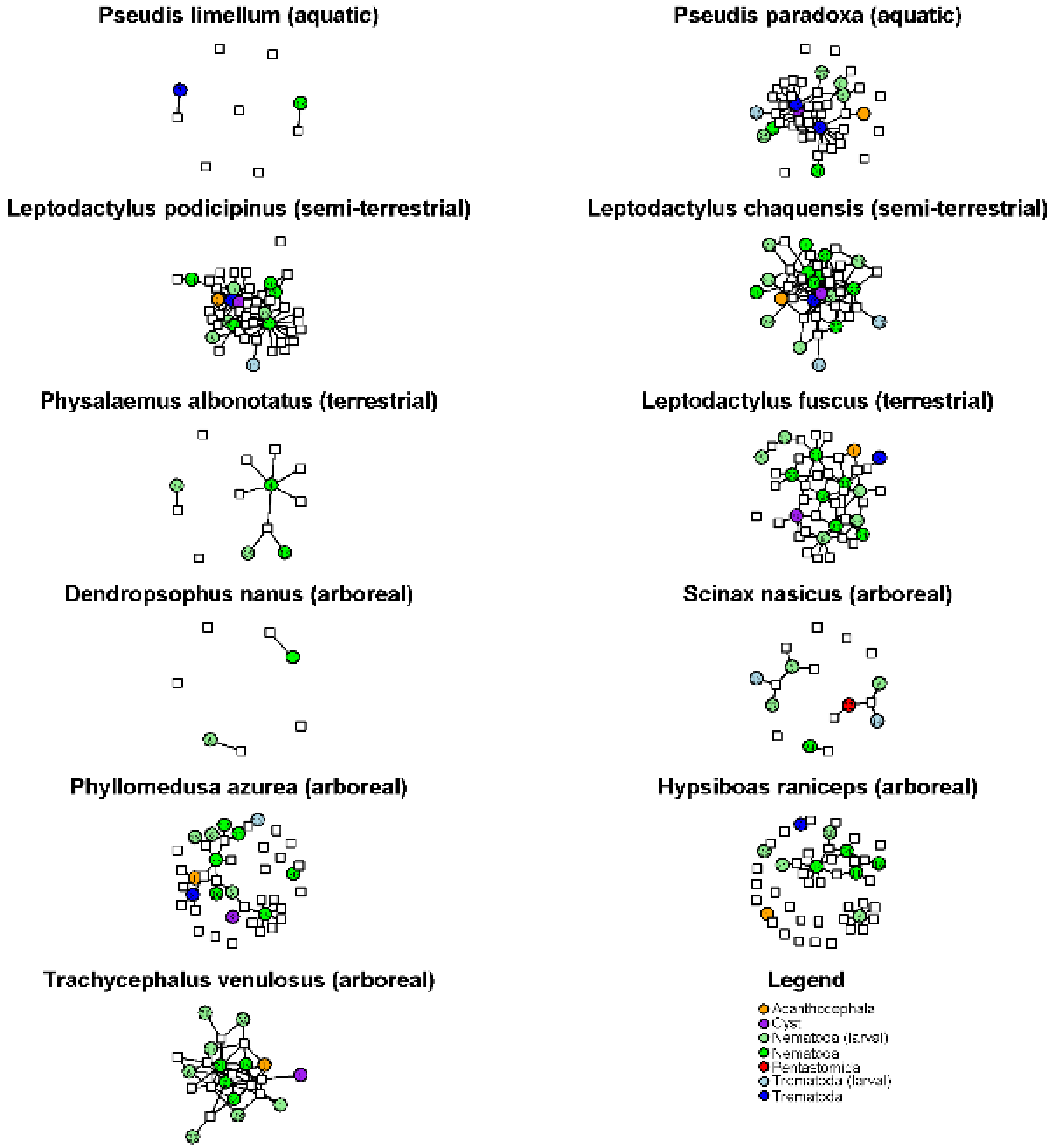


Figure 1. Interaction of host individuals of eleven anuran species (squares) of different habits and their helminth parasites (circles). Host and parasite names are in Table 1.

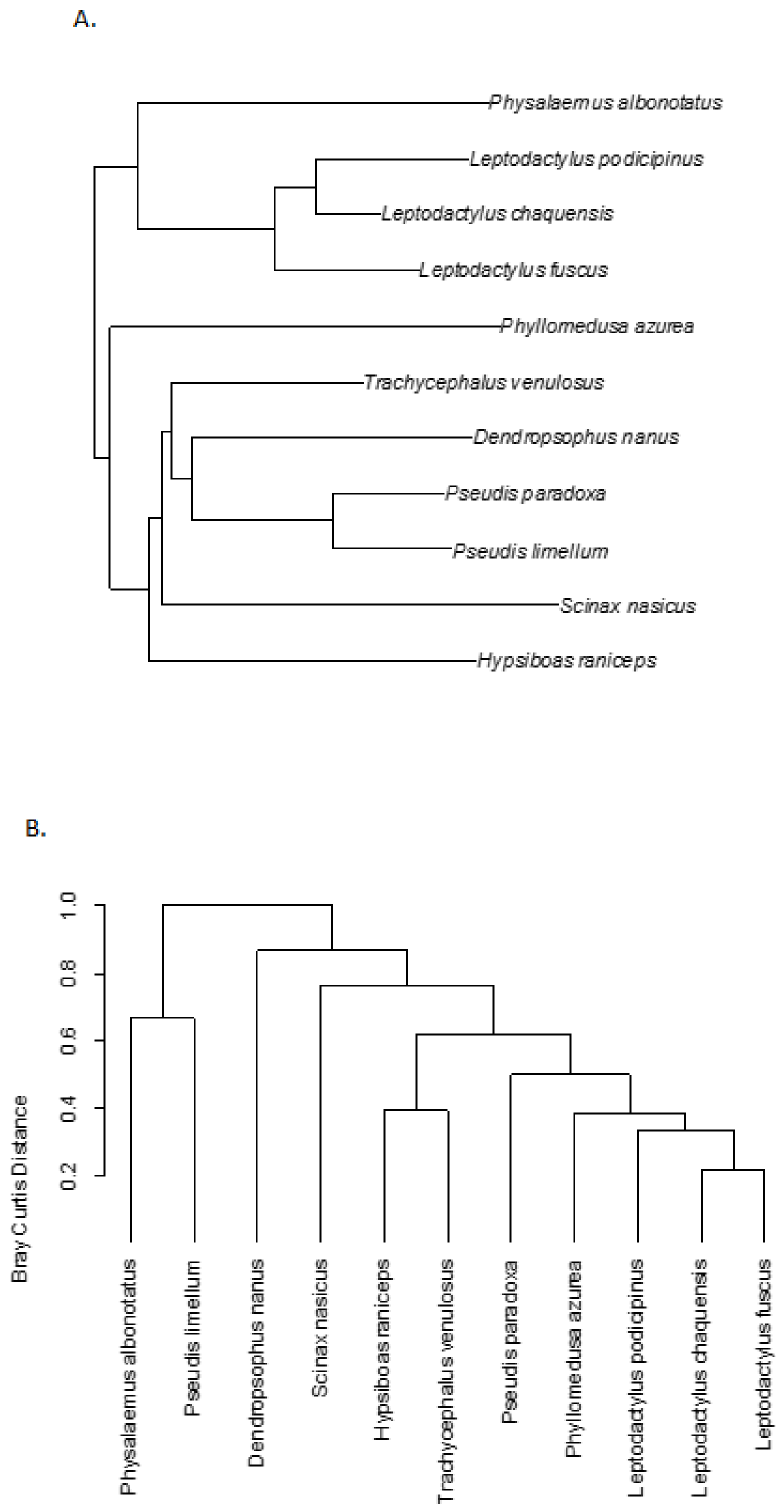
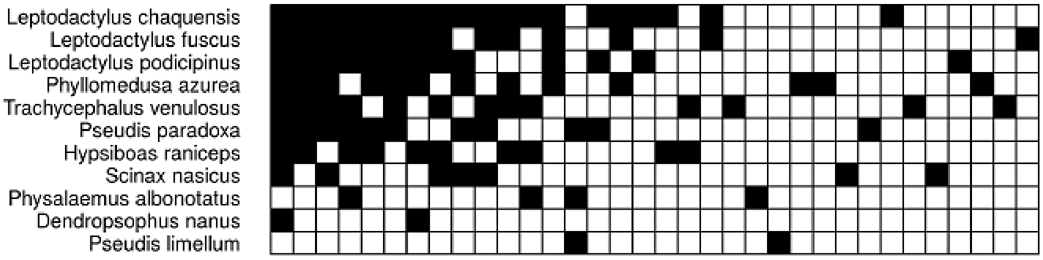


Figure 2. A. Phylogeny of eleven anuran species adapted from Pyron and Wiens (2001).

B. Dendrogram of the similarities amongst eleven anuran species based on the Bray Curtis distance of their helminth communities.



Leptodactylus chaquensis	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	■	■	■	□	■	□	□	□	□	□	□	□	■	□	□	□	□	□	□
Leptodactylus fuscus	■	■	■	■	■	■	■	■	□	■	■	□	■	□	□	■	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	■
Leptodactylus podicipinus	■	■	■	■	■	■	■	■	■	□	□	□	■	□	■	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□
Phyllomedusa azurea	■	■	■	□	■	■	■	□	■	□	■	□	■	□	□	■	□	□	□	□	□	□	□	■	■	□	□	□	□	□	□	■	□	□
Trachycephalus venulosus	■	■	■	■	□	■	□	■	□	■	■	■	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	□	■	□
Pseudis paradoxa	■	■	■	■	■	■	□	□	■	■	□	□	□	■	■	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□
Hypsiboas raniceps	■	■	□	■	■	□	■	■	□	□	■	■	□	□	□	□	□	■	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Scinax nasicus	■	□	■	□	□	□	□	■	■	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	■	□	□	□	□
Physalaemus albonotatus	□	□	□	■	□	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□
Dendropsophus nanus	■	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Pseudis limellum	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□

Figure 3. Incidence matrix of the network of 11 anuran species (rows) and 34 helminth parasites (columns). A filled square represents interactions, and an empty square indicates that no interactions occur.

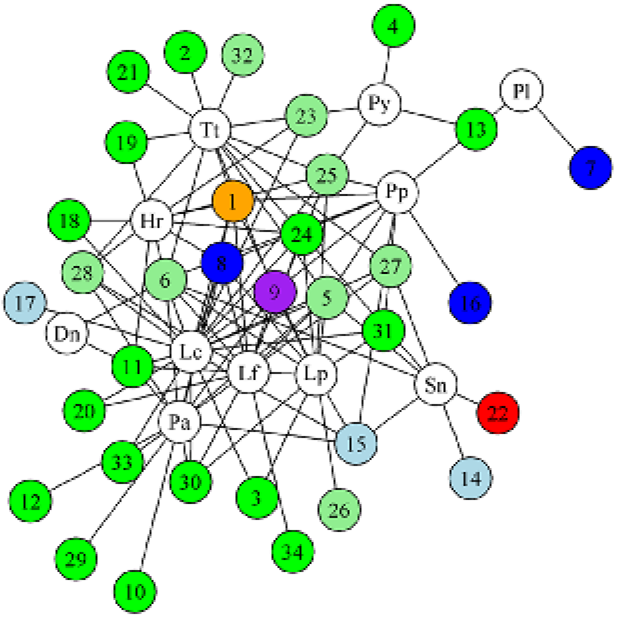


Figure 4. Network of 11 anuran species (white circles) and 34 helminth parasites (coloured circles). Different colours represent different parasite groups: orange – Acanthocephla, light green – Nematoda (larval), dark green – Nematoda (adult), light blue – Trematoda (larval), dark blue – Trematoda (adult), purple – Undetermined helminth cyst, red – Pentastomida. Host and parasite names are in Table 1.

DISCUSSION

In general, frog species of *Leptodactylus* had the richest parasite communities, with higher taxonomic diversity. *Leptodactylus chaquensis* was the host with greatest parasite biodiversity. Indeed, semi-terrestrial anurans, such as *L. chaquensis* and *L. podicipinus,* are susceptible to acquiring parasites whose infective stages are both in the water (such as trematodes) and soil (direct life-cycle nematodes). Among the tree frogs, *T. typhonius* harboured the richest helminth community and had the highest value of taxonomic diversity. The parasite communities of these anurans are composed mostly by parasites transmitted through the ingestion of the infective stages. This is probably due to the arboreal habit of *T. typhonius*, which might reduce the chances of acquiring trematodes and direct life cycle nematodes transmitted, respectively, through the water and soil. Notwithstanding, these tree anurans had a high taxonomic diversity (Table 2). It can be explained by the wide range of prey they consume (including Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera, Pseudoescorpionida and Aranae) (Sabagh *et al.* 2010), once several invertebrate species act as intermediate hosts for different parasite taxa (Anderson, 2000).

Differences in foraging strategy may also underlie some of the differences we observed among hosts. For example, leptodactilids are active forragers while most hylids are sit-and-wait predators. Such differences in foraging behaviour may explain why leptodactilids had higher parasite diversity. Among hylids, the aquatic *P. paradoxa* had more diverse parasite communities than the arboreal anurans of similar size. This is not surprising though, once aquatic hosts generally have more diverse parasite fauna than their terrestrial counterparts (Poulin and Morand, 2004).

Our results confirmed host size as a determinant of helminth species richness in anuran hosts (see Article 2). Large anurans always had the most diverse while the small ones had species poor parasite communities (see Table 1). Despite the differences we observed in parasite diversity across hosts of different habits, it was not significantly related to helminth species richness. Similarly, parasite taxonomic diversity did not correlate to host size or habit. This is different from what we expected, once the taxonomic diversity of parasite assemblages can be more sensitive to the influence of host traits than parasite species richness (Luque and Poulin, 2008).

Similarities on parasite communities were not explained by the evolutionary relationship among anurans. Hosts that are closely related phylogenetically may have more similar parasite communities than unrelated hosts (Lima *et al.* 2012; Krasnov *et al.* 2012). We expect that because host switching is probably more frequent among closely related hosts. Assuming phylogenetic trait conservatism, related hosts probably offer the same set of resources to parasites, and are expected to share physiological and behavioural constraints, thus they may have the same chances to be exposed to the same risk factors to acquiring parasites (Poulin, 2007). Notwithstanding, closely related anurans not necessarily had more similar parasite communities, which might be due to the low specificity observed in most parasite species.

We found a nested pattern of interaction between anuran and their helminth parasites. This indicates that specialist parasites tend to interact more often with generalists than to other specialists (Poulin, 1996; 2010). Thus, specialist helminth species generally occurred in anurans with the richest parasite communities, and species poor parasite communities were subsets of those. This result is consistent with several other studies that investigated nestedness in host-parasite networks (Vázquez et al., 2005; Graham *et al.* 2009; Joppa *et al.* 2010, Bellay *et al.* 2011, Lima *et al.*,2012). The mechanisms underlying nestedness in interacting networks are not well understood, but are probably related to species abundance and coevolutionary constrains (McQuaid & Britton, 2013). Moreover, such structural pattern may decrease competition and increase species coexistence, and contribute to network robustness (Fortuna *et al.* 2010).

Anuran-parasite network did not show a modular pattern. Modularity is somewhat expected in host-parasite networks because parasitism generally involve a high degree of intimacy and adaptation between species (Guimarães *et al.* 2007; Olesen *et al.* 2007; Fortuna *et al.* 2010). Indeed, several host-parasite networks were found to have a modular structure (Fortuna *et al.* 2010, Bellay *et al.* 2011, 13; Lima *et al.* 2012; Krasnov *et al.* 2012). The formation of modules indicates that groups of species interact more with one another than with species in the network. Divergent selection and phylogenetic groups of related species could promote modularity, and similarity (either phylogenetic, ecological or functional) is higher amongst species within the same module (Guimarães *et al.*, 2007, Olesen *et al.*,2007; Bellay *et al.*,2011, 13; Lima *et al.*,2012; Krasnov *et al.*, 2012).

The lack of modularity and nested pattern of interaction observed in the network of sympatric anurans and their parasites are probably the result of low host specificity observed amongst most helminths in this study. No host species had a unique parasite community, and several parasite species were shared among different hosts. Even some helminth species that were associated to a single host in this study (*A. hylambatis*, *C. parva*, *C.* cf. *phyllomedusae*, *G. palmipedis,* and *R. minor*) were reported as parasites of a wide range of hosts (Campião *et al.* 2014). Notwithstanding, parasites may be specialists to a particular resource and not to a particular host taxon. If this resource is either widespread amongst hosts or is a result of hosts convergent evolution, then parasites could track this resource despite host´s taxonomic boundaries (Brooks *et al.* 2006). Low host specificity was especially evident among larval nematodes. Indeed, parasites in larval stages may increase the connectivity in host parasite networks, because they tend to be more generalist (Bellay *et al.* 2013). Our results agree with that, as we observed parasites in larval stages interacting with host species of different habits and long phylogenetic distances.

Overall, we found that host attributes, such as body size, were important in determining parasite community richness, whereas parasite attributes (specificity) were important to network structure.

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Land Use Alteration Decreases Species Richness, Prevalence and Abundance of Anuran Helminth Communities from a Tropical Wetland Area

ABSTRACT: Amphibian macroparasites are consistently relevant in the study of environmental changes. Here, we describe the parasite communities of five anuran species (*Hypsiboas raniceps, Phyllomeduza azurea, Pseudis paradoxa, Leptodactylus fuscus* and *Leptodactylus podicipinus*) from two habitats with different levels of preservation (pasture *vs* nature reserve). Specifically, we test whether helminth infracommunities, prevalence and abundance differ according to host collection site. We collected 120 anuran specimens and 25 helminth parasite taxa: Acanthocephala cystacanth; 21 Nematoda and three Trematoda. We found that the helminth communities differed across host collection sites. The response was assymetrical among different parasites in a host, and within the same parasite in different host species, but in general, helminth species richness, prevalence and abundance were higher in hosts from the preserved area.

Biologists frequently search for surrogates (Caro and O´Doherty, 1999; Mellin et al., 2011). Species that can, potentially, represent other species and/or indicate environmental change. Parasitic organisms have been proven good surrogates of both their hosts' biology and environment. Parasites can be biological tags of host population structure (Catalano et al., 2013), phylogeography (Nieberding et al., 2004), diet and trophic interactions (Marcogliese, 2004), and environmental impact (Sures, 2004). However, all those findings are relatively recent, and their validity strengthened as more empirical evidence is added and knowledge gaps filled.

Lafferty (1997) reviewed the potential of parasites as indicators of environmental health. Despite the knowledge gaps and lack of information to support general predictions conclusively by that time, some existing evidence suggested parasites as indicators of this kind of impact. Later, Vidal-Martínez et al. (2010) revisited the issue with meta-analytical procedures of the data published in the last decade and was led to similar conclusions. Parasites are good indicators of environmental impact. They interact in complex ways with stressors and the direction of parasite response, either increase or decrease, varies among taxonomic groups, parasite's life strategy, and stress source (Lafferty and Kuris, 1999; Vidal-Matínez et al., 2010).

Amphibian macroparasites are consistently relevant in the study of amphibian conservation, wildlife disease ecology, and environmental change (Kopivnikar et al., 2012). A few studies have examined the effect of environmental impact on amphibian parasites. Most of these compared amphibian helminth parasite communities from impacted and non-impacted areas (Hamman et al., 2006; Kopivnikar et al., 2006; King et al. 2007, 2010; McKenzie, 2007; Schotthoefer et al., 2011). All these studies found that human land use activities somehow affected parasites. In general, parasite species richness and abundance seem to be negatively affected by environmental alterations (Kopivnikar et al., 2006; Hamman et al., 2006; McKenzie, 2007; Hartson et al., 2011). However, land use alteration may enhance parasite intensities when it facilitates the transmission of certain parasite taxa or affects host immune-competence (Johnson and Chase, 2004; Rohr et al., 2008; Schotthoefer et al., 2011)

Here, we investigate whether parasites respond to impact by comparing helminth communities of five anuran species from two habitats with different levels of preservation. To have stronger sampling effort in the two habitats, we chose the folowing host species from two different habits: the aquatic frog *Pseudis paradoxa,* the tree frogs *Hypsiboas raniceps* and *Phyllomeduza azurea,* the semi terrestrial *Leptodactylus podicipinus* and the terrestrial *Leptodactylus fuscus.* All these anuran species inhabits forested and cattle grazing areas, and we collected them from ponds within a protected nature reserve and from a pasture area. Specifically, we describe the parasite communities of these anurans and test whether helminth infracommunities, prevalence and abundance differ according to host collection site.

MATERIAL AND METHODS

Study sites

This study was carried out in the farmland Fazenda Alegria (18°59'S 56°39'W), southeastern Pantanal, Brazil. Pantanal is one of the world's largest continuous flood plains, surrounded by the Amazon Forest, the Atlantic Forest, Chaco, and Cerrado (Brazilian savanna). Despite being relatively pristine, the Pantanal area accounts for 15% of the cattle management in Brazil. Fazenda Alegria has areas of native pasture with extensive livestock management and a forested protected area consisting of a legal nature reserve (600 ha) that has no cattle or cattle management. Within the farmland, we selected two different study sites: one pond in the pasture area (19°03.885'S 56°45.000'W) and one pond in the nature reserve (19°03.397'S 56°47.011'W). Livestock management often leads water bodies to eutrophication due to more organic matter deposition from the accumulation of cattle excreta. Additionally, cattle trampling clears the vegetation around the pond, making the two ponds very different visually. For a quantitative measure to describe the two ponds, we sent water samples to an environmental quality lab for analyses of total phosphorus and total nitrogen.

Host and parasite collections

The field trips to collect the host species were conducted in the rainy seasons of 2011 and 2013. *L. podicipinus* and *P. paradoxa* were collected in 2011, *L. fuscus* and *P. azurea* in 2013, and *H. raniceps* in both years (16 in 2011 and 10 in 2010). Anurans were hand-captured, transported alive to the laboratory, and then euthanasied with an overdose sodium thiopental solution. Frogs had their body cavity, digestive tract, accessory organs and musculature examined for helminth parasites. Nematodes were fixed in hot alcohol-formaldehyde-acetic acid (AFA) solution; cestodes and trematodes were fixed under cover slip pressure also using cold AFA; acanthocephalans were maintained in cold water until their probosces were extruded and then fixed in cold AFA. All helminths were preserved in 70% ethyl alcohol. For identification, acanthocephalans, cestodes and trematodes were stained with carmine and cleared with eugenol while nematodes were cleared with Amman`s lactophenol.

Analysis

To test whether the study site (pasture vs reserve) was affecting parasite species we considered three types of parasite response: helminth prevalence and abundance (as defined by Bush et al., 1997) and infracommunity species richness. We used generalized mixed effects models (GLMM), which allow us to test for general study site influences, but still considers for variations among hosts and parasites.

Firstly, we modeled the effect of study site (fixed effect variable) on helminth infracommunity richness (response variable), using Poisson distribution. This effect may vary among host species; we thus considered host species a random variable. In the second model we tested for differences in helminth species prevalence across sites using binomial distribution. Here, in addition to variation among host species, we also considered variations among parasite species and among host specimens (random variables). This last random variable was considered because a single host specimen might be infected by more than one parasite species, being repeated in the response variable. The third model was similar to the second, considering the same sources of variation, but the influence of study site on helminth species abundance was calculated using a Poisson distribution.

All analysis were performed in R (R Development Core Team, 2013), using lme4 package (Bates et al., 2014).

RESULTS

The water sample collected in the nature reserve had lower total phosphorus and total nitrogen (P= 0.11 mg/L; N = 3.9 mg/L) than the water collected from the pond in the pasture area (P= 0.27 mg/L; N = 9.5 mg/L). The water bodies of the reserve and pasture are, respectively, mesotrophic and eutrophic (Von Sperling, 1996).

We collected 120 anuran specimens (Table 1) and 28 helminth parasite taxa: one cystacanth of Acanthocephala; 23 Nematoda, three Trematoda, and an undetermined cyst. Among the nematodes, *Aplectana* sp, *Cosmocerca parva, Cosmocerca podicipinus, Cosmocercella* cf. *phyllomedusae,* Cosmocercidae gen. sp.*,* Cosmocercoidea fam. gen. sp.*, Oswaldocruzia* sp., *Oxyascaris oxyascaris, Oxyascaris* sp., *Physalopteroides venancioi, Raillietnema minor, Raillietnema sp., Rhabdias* sp., *Schrankiana formosula, S. fuscus, Schrankiana* sp. were found in adult stages infecting the gastrointestinal tract (except for *Rhabdias* sp. that is a lung parasite), and larvae of Ascarididae gen. sp., *Brevimulticaecum* sp., *Physaloptera* sp., *Porrocaecum* sp., and three morphospecies of *Physocephalus* that were encysted in the body tissues. The nematode specimens assigned to Cosmocercidae, Cosmocercoidea and *Schrankiana* sp. were either females or larvae, and for this reason could not be identified further. However, cosmocercids are probably one of the *Aplectana, Cosmocerca* or *Cosmocercella* species we found, and *Schrankiana* sp. might be either *S. formosula* or *S. fuscus.* Similarly, the larvae of Ascarididae gen. sp. were in initial stages of development, but are likely *Brevimulticaecum* sp. or *Porrocaecum* sp. We thus removed Ascarididae gen. sp., Cosmocercidae gen. sp., Cosmocercoidea fam. gen. sp. and *Schrankiana* sp. from analysis to avoid data pseudoreplication. Trematodes were found in the intestines, *Catadiscus* sp. and *Glypthelmins palmipens* in adult stages, and

Diplostomulum as metacercaria. We also found an encysted helminth that parasitized three of the five host species, but this was undifferentiated and could not be identified morphologically.

Only 18 frog specimens were not parasitized: seven specimens of *H. raniceps,* one *L. fuscus*, four *P. azurea,* and six *P. paradoxa,* 13 out these 18 were from the pasture pond. We found in total 9 helminth species in *P. paradoxa* and *H. raniceps,* 10 helminth species in *P. azurea,* and 12 helminth species in the leptodactylid hosts. However, each parasitized frog harboured on average two or three helminth species, and this did not vary much among the different host species. Infracommunity species richness was higher in frogs specimens of *P. paradoxa* and *L. podicipinus* from the reserve pond (Figure 1).

Most parasite species had higher prevalence in anurans collected in the preserved area (Figure 2, Table 2). The first random variable we considered in model 2, host individuals, had the lowest variance. On the other hand, variance among host species was high, indicating that in terms of parasite prevalence, hosts are similar within, but different across each anuran species. The greatest variation observed in the random predictors was within parasite species. This is because even most helminth species had higher prevalence in hosts from the reserve, but some were more prevalent in hosts from the pasture pond. Additionally, a few helminth species varied across hosts and collection sites. For example, the acanthocephalan cystacanth only occurred in *P. platensis* and *L .fuscus* from the reserve; but in *L. podicipinus* only hosts from the pasture were infected, and in *P. azurea* hosts from the different study sites had similar prevalence. However, it is interesting to observe that all such cases, when the prevalence of parasite species in each study site varied across hosts, it just occurred in helminths with lower prevalences (Figure 2).

The third model showed a similar pattern, indicating that land use also affected parasite abundance (Table 2). Different from the model considering parasite prevalence though, the random predictor that accounted for host individuals was highly variable for helminth abundance, which is expected due to the aggregate pattern of parasite distribution among hosts. There was also a lot of variation in the abundance across parasite species within a host, and within a parasite species across hosts (Table 2). However, except for rare species that occurred in low prevalence, most helminths had higher mean abundance in anurans collected in the reserve (Figure 3).

Table 1. Number of hosts collected from two different ponds, in a farmland area in Pantanal, Brazil.

Anuran species	**Pasture**	**Reserve**
Hypsiboas raniceps	19	7
Leptodactylus fuscus	10	11
Leptodactylus podicipinus	12	13
Phyllomedusa azurea	9	12
Pseudis paradoxa	10	17

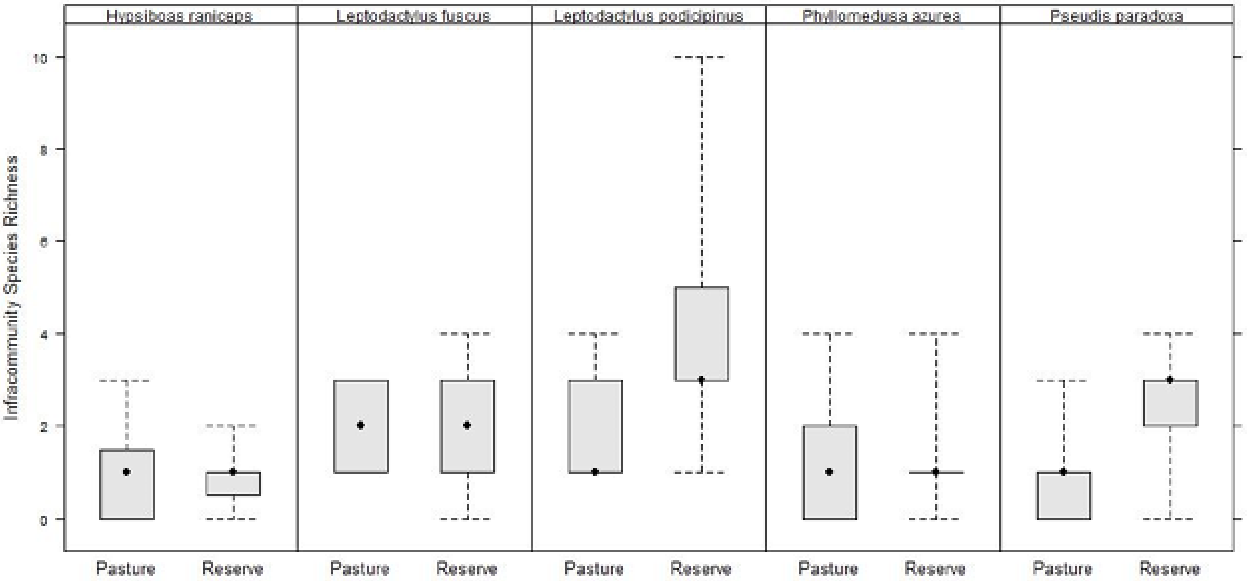

Figure. 1. Boxplot of infracommunity species richness in five anuran species collected from two different ponds, in a farmland area in Pantanal, Brazil.

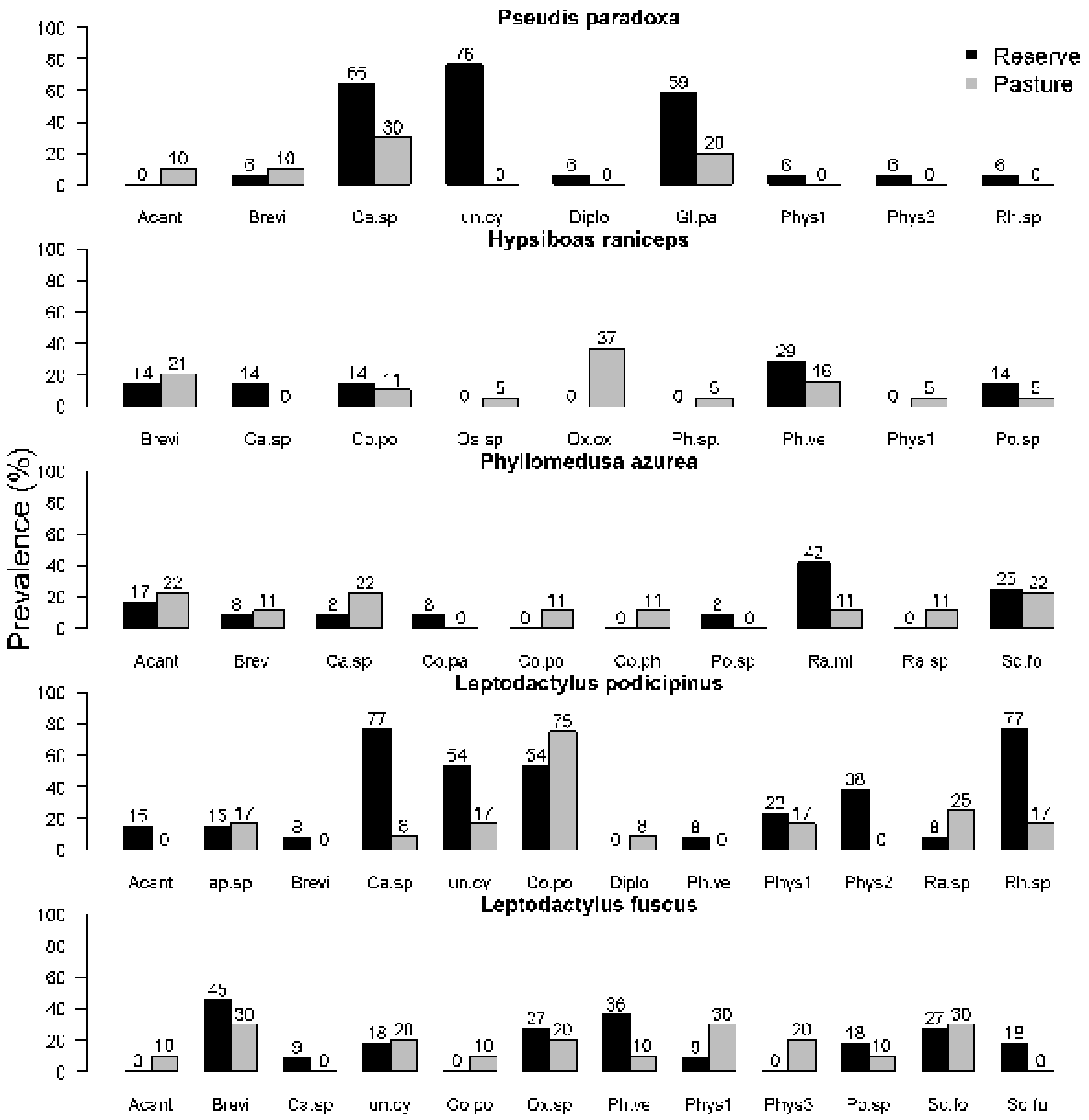

Figure 2. Prevalence (%) of helminth parasites in five anuran species from two contrasting habitats in Pantanal, Brazil. Abbreviated parasite names are as follow: Acant: Acanthocephala, Ap.sp: *Aplectana* sp, Brevi*: Brevimulticaecum* sp, Ca.sp: *Catadiscus* sp., Co.pa: *Cosmocerca parva,* Co.po: *Cosmocerca podicipinus,* Co.ph: *Cosmocercella* cf *phyllomedusae,* Diplo: *Diplostomulum sp,* Gl.pa: *Glypthelmins palmipens,* Ow.sp: *Oswaldocruzia* sp., Ox.ox: *Oxyascaris oxyascaris,* Ox.sp: *Oxyascaris* sp., Ph.ve: *Physalopteroides venancioi,* Ph.sp: *Physaloptera* sp, Phys1: *Physocephalus* sp1, Phys2: *Physocephalus* sp2, Phys3: *Physocephalus* sp3,

Ra.mi: *Raillietnema minor,* Ra.sp: *Raillietnema sp.,* Rh.sp: *Rhabdias* sp., Sc.fo: *Schrankiana formosula,* Sc.fu: *Schrankiana fuscus.*

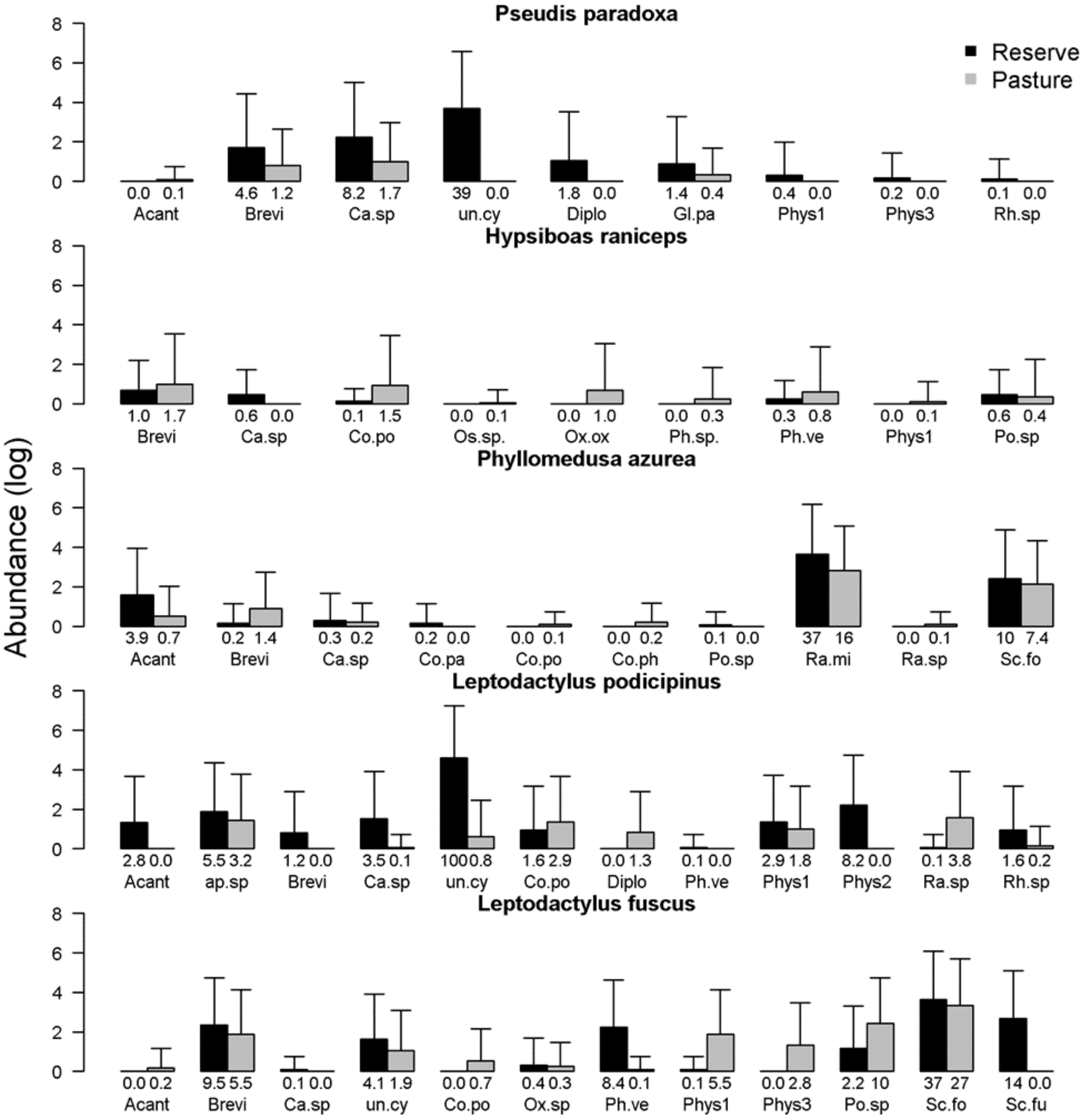

Figure 3. Mean abundance and standard deviation of helminth parasites in five anuran species from two contrasting habitats in Pantanal, Brazil. Abbreviated parasite names are as follow: Acant: Acanthocephala, Ap.sp: *Aplectana* sp, Brevi*: Brevimulticaecum* sp, Ca.sp: *Catadiscus* sp., Co.pa: *Cosmocerca parva,* Co.po: *Cosmocerca podicipinus,* Co.ph: *Cosmocercella* cf *phyllomedusae,* Diplo: *Diplostomulum sp,* Gl.pa: *Glypthelmins palmipens,* Ow.sp: *Oswaldocruzia* sp., Ox.ox: *Oxyascaris oxyascaris,* Ox.sp: *Oxyascaris* sp., Ph.ve: *Physalopteroides venancioi,* Ph.sp: *Physaloptera* sp, Phys1: *Physocephalus* sp1, Phys2: *Physocephalus* sp2, Phys3: *Physocephalus* sp3, Ra.mi: *Raillietnema minor,* Ra.sp: *Raillietnema sp.,* Rh.sp: *Rhabdias* sp., Sc.fo: *Schrankiana formosula,* Sc.fu: *Schrankiana fuscus.*

Table 2. Mixed effects models of the effect of host collection site (pasture vs reserve) on helminth infracommunity species richness, prevalence and abundance of five anuran species.

Response	**Random effect variable**			**Fixed effect variable**				
	Parameter	V^{a}	SE^{b}	Parameter	Estimate	SE^{b}	z	P
Infracommunity species richness (Model 1)	Host species	0.11	0.33	Intercept	0.29	0.18	1.57	0.116
				Study site	0.44	0.14	3.16	0.001
Helminth Prevalence (Model 2)								
	Host species	0.16	0.41	Intercept	-3.30	0.31	-10.7	0.0001
	Parasite species	1.04	1.02	Study site	0.51	0.15	3.28	0.001
	Host specimen	0.05	0.23					
Helminth Abundance (Model 3)								
	Host species	0.50	0.71	Intercept	-3.07	0.55	-5.51	<0.0001
	Parasite species	2.47	1.86	Study site	1.28	0.35	3.59	0.0003
	Host specimen	3.27	1.80					

[a] Variance; [b] Standard error

DISCUSSION

Helminth species richness did not vary much among different anuran species (Figure 1). Apparently, most parasites were able to colonize hosts in both impacted and preserved habitats, but few helminth species infected hosts from one of the localities only. These parasites occurred in low prevalence, making it difficult to assert whether they are restricted to one of the habitats or just not collected in the other. Nevertheless, hosts from the reserve area had on average higher helminth prevalence and abundance.

Parasite prevalence, abundance and transmission in the two study sites

The response to stress is expected to vary according to parasite life strategy (Vidal-Martínez et al., 2010), which explains the variance found in parasite prevalence across the impacted and protected areas within different hosts. Despite the variance, helminth prevalence was usually lower in hosts from the impacted area (Fig. 2). This is especially evident among the trematodes *Catadiscus* sp. and *G. palmipens*, the nematodes *P. venancioi*, *R. minor* and *Rhabdias* sp., and the unindentified cyst. For a better understanding of the mechanisms likely to underlie changes in parasite response, it is important to consider the transmission strategy of these parasites separately (see Marcogliese, 2005).

Trematodes have complex life cycles, which are mostly aquatic, and require a mollusc as intermediate host. The importance of environmental variables in the transmission success of trematodes has been well studied. Shifts in trematode prevalence may follow changes in water quality through the increase or decrease in intermediate host abundance, or through direct effects on the survival of parasite infective stages (Koprivnikar et al., 2006, Poulin, 2006; King et al., 2007, 2008, 2010; Koprivnikar and Poulin, 2009). More productive water bodies are expected to have more snails and, thus, higher trematode prevalence (Schotthoefer et al., 2011). This is different from what we observed for *Catadiscus* sp. and *G. palmipens*. It is possible though, that cattle grazing changes water quality in a way that could potentially decrease the survival of infective stages of these parasites.

The nematodes *P. venancioi, R. minor* and *Rhabdias* sp. have different life strategies. *Physalopteroides venancioi* has an indirect life cycle, it is trophically transmitted to its final host through the ingestion of an invertebrate intermediate host. On the other hand, *Raillietnema minor* and *Rhabdias* sp. have direct life cycle, probably infecting their hosts through skin penetration. The life cycle of these nematodes have not been totally elucidated, except for *Rhabdias* species (Anderson, 2000). Infective stages of *Rhabdias* penetrate host skin and migrate to the lungs, where adult individuals mature and produce eggs that eventually reach the intestines to be released with host's feces. Studies on *Rhabdias* species life cycle show that larval development varies according to soil conditions (Anderson, 2000). Here again, the low prevalence of these three nematodes in the pasture may indicate that the soil conditions in this area decrease the survival and/or infectivity of free-living stages, or yet, reduces the availability of intermediate hosts for *P. venancioi*.

The prevalence of *C. podicipinus* and *O. oxyascaris* followed a contrasting pathway; it seemed to be positively affected in the pasture area. The transmission of these species probably occurs through skin penetration of the infective stages in *Cosmocerca* species. *Oxyascaris* species might infect their hosts through this same way or through the ingestion of the infective larvae. The infective larvae of both species are released with host's feces and develop in the soil (Anderson 2000). The soil is exposed in most of the area surrounding the pond in the pasture, while the reserve pond is mostly covered by herbs. If cattle grazing activities is not decreasing the success of infective stages of these parasites, the lack of vegetation cover in this environment may enhance host exposure to these infective stages in the soil, increasing their transmission success.

We can also observe that some helminth species prevalence varied among hosts and across collection sites (Fig.2). However, it always happened when prevalences were low. Even *Catadiscus* sp. and the unidentified cyst that seemed to be consistently more prevalent in the reserve pond, had similar prevalences in both study sites within hosts they were rare. This might be due to several reasons. One is that the rarity of such parasites may confound whether there is any influence of land use on their prevalences. It also possible that there is a threshold from which parasites species would respond to impact, and effects on parasite prevalence would just be noticeable when this threshold is reached. If this is true, different species would have different thresholds, resulting in great variation in response among different parasites (Vida-Martínez et al., 2010). Lastly, it is also possible that habitat influences parasite prevalence through indirect effects in hosts immune competence. Host Immune influence on parasite communities would also result in great variation in parasite prevalence among hosts and across study sites (Blaustein et al., 2012).

Parasite abundance was too negatively affected in the impacted area. Mixed effects model on parasite abundance was similar to the model predicting parasite prevalence, indicating that helminth abundance was higher in hosts from the protected area. The same mechanisms underlying lower parasite prevalence in hosts from the impacted area may lead to the decreased parasite abundance. The mechanisms beneath our findings are not clear, but some studies have showed that residual anti-helminthics present in clattle feces can decrease the diversity and abundance of insects in pastures (Spratt, 1997; Jensen et al., 2009; Lumaret et al., 2012). This could reduce parasite transmission success because several helminth groups require insect species as intermediate hosts to complete their life cycle (Anderson, 2000). Additionally, anti-helminthic residues might also reduce the survival of helminth free-living infective stages. We thus believe that anti-helminthic residues in cattle feces might be one of the causes of lower parasite biodiversity in hosts from the pasture area.

Linking shifts in parasite communities and host ecology in the two study sites

Differences in helminth species richness, prevalence and abundance across the two sites were more evident in the parasite communities of *P. paradoxa* and *L. podicipinus*. *Pseudis paradoxa* are aquatic frogs, they are the most closely related to the water among the anuran hosts we studied. Indeed, aquatic frogs may be particularly prone to experience changes in their parasite communities due to changes in water quality (McKenzie, 2007). *Leptodactylus podicipinus* are semi-terrestrial frogs, but are too closely related to the water. They live in the interface between aquatic and terrestrial habitats, which allows the infection with parasites from both habitats. On the other hand, *L. fuscus* tends to terrestrially, and most parasites we found in these frogs are trophycally transmitted. Parasite communities of *L. fuscus* from the different habitats were similar in terms of prevalence and abundance, with few larval nematodes having higher abundances in the pasture (Fig. 3). It is interesting to observe how parasite communities of *L. podicipinus* and

L. fuscus responded to land use differently, despite these hosts being congeneric. Such differences highlight the asymmetry in parasite response we observed in this study, which may be jointly influenced by features of the parasite (e.g. life cycle strategy) and host species (e.g. ecology and phylogeny).

Considering all parasites in the tree frogs *H. raniceps* and *P. azurea*, the number of helminth species found in these hosts was slightly higher in the pasture area. We believe this is due to the lack of herb vegetation in the pasture, which causes these frogs to stay on the ground more often. In the reserve pond, those tree frogs were always found perched in aquatic and semi aquatic herbs. However, in the pasture pond, aquatic herbs are scarce and we found tree frogs foraging on the ground. This contact with soil may enhance the chances of acquiring direct-life cycle nematodes, such as the species of *Cosmocerca*, *Cosmocercella*, *Oswaldocruzia* and *Oxyascaris* that were found only in tree frogs from the pasture.

Overall, we found that the helminth communities of five anuran species responded to changes in land use. The response was assymetrical among different parasites in a host, and within the same parasite in different host species. Nevertheless, helminth species richness, prevalence and abundance was generally higher in hosts from the preserved area. These results agree with several other studies on amphibian macroparasites (Kopivnikar et al., 2006; Hamman et al., 2006; McKenzie, 2007; Hartson et al., 2011), and we thus join Hudson et al. (2006) when they state that a healthy ecosystem is one rich in parasites.

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