

UNIVERSIDADE FEDERAL DO PARANÁ

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Padrões de interação e diversificação dos Monogenoidea parasitos de brânquias de peixes dulcícolas na Região Neotropical

CURITIBA  
2013

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Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Ecologia e Conservação. Curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas da Universidade Federal do Paraná.

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CURITIBA  
2013



## PARECER

Os abaixo-assinados, membros da banca examinadora da defesa da dissertação de mestrado, a que se submeteu **Mariana Pires Braga** para fins de adquirir o título de Mestre em Ecologia e Conservação, são de parecer favorável à **APROVAÇÃO** do trabalho de conclusão da candidata.

Secretaria do Programa de Pós-Graduação em Ecologia e Conservação.

Curitiba, 19 de fevereiro de 2013.

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## AGRADECIMENTOS

Agradeço ao meu querido orientador, Professor Walter Boeger pela confiança, atenção e incentivo desde a nossa primeira conversa na sua sala, pela oportunidade de crescimento profissional e pessoal e principalmente por sempre poder contar com sua ajuda em qualquer dificuldade.

À minha co-orientadora, Sabrina, sempre muito disposta a discutir novas ideias e com muita paciência para me ensinar a programar! Tive muita sorte de encontrar você durante o primeiro ano do mestrado. Você me ensinou uma outra forma de olhar para os mesmos problemas. Ao Professor Maurício Moura pelas várias conversas até encontrarmos a melhor forma de analisar os dados. Ainda, ao Professor Paulo R. Guimarães Jr. pela ajuda e atenção durante a minha visita à USP

À todos do Laboratório de Ecologia Molecular e Parasitologia Evolutiva pela convivência e trocas diárias. Cada um me ensinou um pouco mesmo que sem perceber. Agradeço também àqueles que contribuíram diretamente com esse trabalho, principalmente com a atualização do banco de dados, que foi uma peça fundamental.

Gostaria de agradecer especialmente as pessoas que me acompanharam mais de perto: Renata, Micheli, Raphael e Letícia. Cada um de vocês me inspirou de uma forma diferente e a amizade de vocês tem sido muito valiosa para mim!

Agradeço infinitamente aos meus pais por sempre acreditarem nos meus sonhos, até mais do que eu mesma. Vocês são uma referência de amor e segurança para toda a nossa família! Hoje eu sei que muito do que eu sou é reflexo das escolhas que vocês fizeram e da dedicação à família. Amo muito vocês!

Da mesma forma, agradeço àquele que sempre está ao meu lado, João Felipe. É difícil explicar a segurança e coragem que você me passa. Todo esse tempo juntos e mal posso esperar pelos que vêm pela frente! *'I wish I was the verb to trust and never let you down'*. Te amo pra sempre!

Meus queridos amigos biólogos! André e Marcelo, além dos almoços e cafés no Politécnico, jantares improvisados e a tão esperada viagem para a praia, também me identifico muito com vocês cientificamente (apesar de cada um estar fazendo uma coisa diferente). Sei que qualquer rumo que nossas vidas tomem, vocês sempre serão meus amigos do coração.

Ao curso de Pós Graduação em Ecologia e Conservação, todos os professores que tive contribuíram de alguma forma para a realização deste trabalho. Aos amigos de curso pelas discussões e conversas na cantina que sempre trazem boas ideias. Finalmente, agradeço à CAPES pelo suporte financeiro durante esses dois anos.

*If kindness is your king than heaven will be yours before you meet your end.*

David J. Matthews

## RESUMO EXPANDIDO

Redes de interação são usadas para representar qualquer tipo de relacionamento entre objetos discretos. Análise de redes tem sido utilizada para retratar a complexidade de sistemas em várias áreas da pesquisa. Fluxos de energia caracterizando relacionamento trófico entre espécies são interações (*links*) que também podem ser medidas entre espécies (nós) na rede. As vantagens de tratar interações entre espécies como uma rede não incluem apenas uma representação visual de sistemas ecológicos complexos, mas também representa um mecanismo formal de medir atributos das espécies, propriedades de sistemas inteiros e como eles variam no espaço e no tempo. Alguns atributos biológicos parecem ter influência sobre os padrões de interação em redes ecológicas, e.g. abundância das espécies, tipo e intimidade da interação. Até recentemente considerava-se que redes de interações antagonísticas (parasito-hospedeiro, predador-presa) são caracterizadas por alta modularidade e baixo aninhamento. Contudo, trabalhos sugerem que redes antagonísticas, assim como as mutualísticas, podem ser modulares e/ou aninhadas dependendo da intimidade da interação, ou seja, o grau de integração biológica entre os indivíduos que interagem. Parasitismo, que é a interação consumidor-recurso mais comum na natureza, pode ser visto como uma rede bipartida de interações de alta intimidade. A análise de redes tem sido extensivamente aplicada a interações mutualísticas, como entre plantas e seus polinizadores ou dispersores. Comparativamente, ainda existem poucos estudos de redes parasito-hospedeiro, embora parasitos constituam um componente fundamental de teias tróficas. Monogenoidea é uma classe de Platyhelminthes composta principalmente por ectoparasitos que vivem nas brânquias ou superfície corporal de peixes marinhos e dulcícolas. Esses parasitos possuem ciclo de vida monoxênico, i.e. completam seu ciclo de vida em uma única espécie hospedeira e podem ser capazes de colonizar hospedeiros a partir de um indivíduo. Os Monogenoidea também são conhecidos pela alta especificidade à espécie hospedeira, que não é necessariamente o resultado de coespeciação apenas, mas também de processos não vicariantes como troca de hospedeiro.

O objetivo do primeiro capítulo deste trabalho é descrever padrões gerais de distribuição dos Monogenoidea que parasitam brânquias de peixes dulcícolas na Região Neotropical. A descrição de padrões gerais e únicos das interações parasito-hospedeiro podem apontar associações que podem ser usadas como modelo para o estudo da importância relativa da história evolutiva e de fatores ecológicos recentes sobre as regras de montagem que atuam sobre as redes ecológicas. Este trabalho é baseado em um banco de dados das ocorrências de espécies de Monogenoidea em espécies de peixes hospedeiras. A partir do banco de dados, matrizes de interação foram criadas organizando as espécies em diferentes níveis taxonômicos. Somente parasitos de brânquias foram selecionados porque eles compõem o grupo melhor conhecido de monogenoideos na Região Neotropical. Para avaliar a estrutura das redes foram calculados aninhamento e modularidade para cada rede. Como estamos lidando com uma região biogeográfica, a distribuição espacial foi incluída ao modelo nulo uma vez que espécies que não co-ocorrem não têm chance de interagir. As interações entre as famílias de peixes e gêneros de parasitos foi caracterizada por ser modular, mas não aninhada e evidenciou a estruturação dos gêneros de parasitos entre as ordens de peixes. Por isso, a distribuição dos gêneros e espécies de parasitos entre as espécies hospedeiras foi analisada separadamente

para cada uma das três maiores ordens de peixes (Perciformes, Siluriformes e Characiformes). Em geral, essas redes apresentaram baixa conectividade e organização em módulos, por vezes associados às famílias hospedeiras. A modularidade observada não foi decorrente da estruturação espacial das espécies nas bacias hidrográficas, uma vez que o grau de modularidade foi significativo mesmo quando comparado ao modelo nulo que inclui a distribuição espacial das espécies hospedeiras. A probabilidade de uma interação ocorrer depende da coocorrência das espécies que interagem em uma dada localidade na escala de tempo ecológico. Porém a história evolutiva dessas espécies deve ditar quais interações são possíveis. Apesar da maioria das espécies e gêneros de parasitos serem específicos a sua espécie hospedeira ou a um grupo filogeneticamente próximo de hospedeiros, alguns parasitos são mais generalistas. Os resultados desse capítulo evidenciam a influência da filogenia dos hospedeiros sobre a distribuição dos Monogenoidea que parasitam brânquias de peixes e também sustentam a influência esperada do tipo e intimidade da interação sobre a estrutura de redes parasito-hospedeiro.

Além de olhar para a estrutura das redes, é de fundamental interesse para ecólogos entender os fatores que geram, mantêm e restringem as associações parasito-hospedeiro, com implicações para estudos de doenças emergentes, controle biológico, invasões biológicas e respostas a mudanças climáticas. Estudos filogenéticos têm encontrado evidências para dispersão entre hospedeiros relativamente distantes, podendo estar relacionada a eventos de diversificação, em diversos sistemas parasito-hospedeiro. Entre parasitos monogenoideos, acredita-se que a ocorrência nos hospedeiros não pode ser explicada somente pelo modelo de coespeciação, mas que trocas de hospedeiro e especiação simpátrica devem estar associadas à origem de vários clados. O segundo capítulo deste trabalho objetiva buscar os fatores que moldam os padrões gerais de compartilhamento de parasitos, usando análise de caminhos como uma forma de gerar hipóteses sobre a evolução das interações entre os Monogenoidea e os peixes que ocorrem nos rios da Região Neotropical. O compartilhamento de gêneros de parasitos pelas espécies de peixes foi obtido do mesmo banco de dados utilizado no primeiro capítulo. As variáveis explanatórias incluíram o relacionamento filogenético, preferências ambientais, características biológicas e a distribuição geográfica das espécies hospedeiras. Os dados foram analisados com todas as espécies e gêneros, para cada uma das três maiores ordens de hospedeiros e separando parasitos com distribuição restrita e ampla. De forma geral, a filogenia tem o maior efeito direto sobre o compartilhamento de parasitos. Contudo, os resultados variam de acordo com o grupo de peixes e entre parasitos família-específicos e generalistas. Os resultados reforçam a importância de incluir a história evolutiva em estudos de associações ecológicas, porém outros fatores precisam ser adicionados para explicar os padrões específicos de alguns grupos. Distribuição geográfica das espécies hospedeiras nas bacias dos rios é um fator muito importante que aumenta a chance de colonização de hospedeiros não relacionados filogeneticamente, especialmente para os parasitos generalistas. Finalmente, as preferências ecológicas e características biológicas são fatores adicionais fundamentais para se compreender as interações parasito-hospedeiro.

Palavras-chave: Redes ecológicas, parasitismo, modularidade, conservacionismo filogenético.

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## **Capítulo 1 - Patterns of interactions between Neotropical freshwater fishes and their Monogenoidea gill parasites**

### **Abstract**

Network analysis has been extensively applied to food webs and mutualistic interactions such as pollination and seed dispersal. Proportionally, there are still few studies of host-parasite associations, though parasites are key components of food webs. Previous studies suggest that antagonistic interactions of high intimacy are associated with lower network connectance and to higher modularity. We looked for broad patterns of distribution of parasites on their hosts as a preliminary way to evaluate the relative influence of past history and recent ecological features on the assembly rules shaping ecological networks. We analyzed a database of component communities of Monogenoidea parasitizing fishes from Neotropical rivers, from 23 watersheds, based on species descriptions and records of occurrence published until 2011. The network between host families and parasite genera was significantly modular but not nested, and revealed that each fish order has a unique parasite genera composition. Hence, interactions between lower taxa were analyzed separately for the three larger fish orders (Perciformes, Siluriformes e Characiformes). Networks tended to be loosely connected and organized in modules and the observed modularity was not a byproduct of geographic distribution on river basins. The probability of an interaction to occur depends on the co-occurrence of partner species in a given locality in ecological time, however, their past evolutionary history might limit the range of possible interactions. Despite the general high host specificity of parasites, some have a larger host range. Among hosts, the *piranha* clade of the Serrasalminidae stands out in terms of parasite richness per host species. These hosts not only have more parasite genera but also share them, so parasites have a wider host

range. Our results highlight the strong phylogenetic influence on the distribution of Monogenoidea parasites on their fish hosts in several taxonomic levels.

## **1 Introduction**

Interaction networks can be used to represent any kind of relationship between discrete objects. Network analysis has been used to depict the complexity of systems in various fields of research. On natural ecosystems biotic interactions do not occur in isolation, instead, they are imbedded in a network in which energy flow between trophic levels (e.g. predator-prey, host-parasite, plant-pollinator) are depicted as links between species in a network of ecological interactions. This approach provides both a visual representation of complex ecological systems, and a formal way to measure species and network properties (Poulin 2010). The indices that measure these properties inform about network structural patterns but not about the underlying mechanisms (Junker et al. 2012).

Nestedness and modularity have emerged as consistent patterns found in bipartite networks of species interactions. In a nested network, interactions of the specialist species are a subset of the interactions among generalist species (Bascompte & Jordano 2003). This structure is more commonly found in mutualistic networks but it was observed also in some antagonistic networks (Vacher et al. 2008; Graham et al. 2009; Bellay et al. 2011; Lima et al. 2012). Modularity is characterized by recognizable subsets of species that are linked more tightly within a module than they are to species of other modules (Olesen et al. 2007). Several processes may promote the emergence of modularity. Some interactions may be more likely than others due to spatial or temporal segregation (Vázquez et al. 2009), as well as divergent selection regimes or phylogenetic constraints (Cattin et al. 2004; Vacher et al. 2008).

Some biological attributes have been suggested to play a key role in shaping patterns of interaction within ecological networks, including abundance (Vázquez et al. 2007),

interaction type (Thébault & Fontaine 2010) and interaction intimacy (Guimarães et al. 2007; Pires & Guimarães 2012). Until recently, antagonistic networks were thought to be characterized by high modularity and low nestedness (Lewinsohn et al. 2006). However, Pires and Guimarães (2012) suggested that antagonistic networks can display a nested structure when interactions have a low degree of intimacy. At the community level, antagonistic interactions with high intimacy are associated with lower network connectance and to higher modularity, probably due to the high level of biological integration between individuals (Pires & Guimarães 2012).

Parasitism, the most common resource-consumer interaction on nature, can be represented as a bipartite network (Mouillot et al. 2008) of interactions with high intimacy. Network analysis has been extensively applied to mutualistic interactions such as pollination and seed dispersal (Bascompte & Jordano 2007) but there are still few studies of host-parasite networks (Mouillot et al. 2008), though parasites are key components of food webs (Lafferty et al. 2006; Marcogliese & Cone 1997; Thompson et al. 2004; Kuris et al. 2008). For instance, parasites can alter community structure through direct and indirect effects on the number of free-living species or their relative abundance (Wood et al. 2007)

Although parasitism is one of the most intimate associations, high intimacy of individual partners does not necessarily imply in high specialization at species level (Pires & Guimarães 2012). In fact, the mechanisms underlying specialization are not fully understood in ecology (Desdevises et al. 2002b). Assuming that parasites are not specialized on particular host species but on resources, Brooks and McLennan (2002) suggested that hidden among the "true" specialists and generalists are 'faux specialists' and 'faux generalists'. Faux specialists are generalists that use a restricted amount of the resources they could use because of ecological factors while faux generalists are specialized on a resource that is phylogenetically widespread.

Parasites may have several hosts during their life cycle, complicating the pattern of interactions in the network. Addressing only parasites with direct life cycle, like the Monogenoidea (Platyhelminthes), avoids these difficulties. Monogenoidea is a species-rich class of Platyhelminthes, strictly parasitic. The group is diverse in terms of number of species and also with respect to their morphology and ecology (Poulin 2002). They have expanded their microhabitat preferences (site of infestation) from the skin of early vertebrates to internal and external organs of a range of living aquatic vertebrates, consequently displaying a variety of designs nowadays (Kearn 1994). Monogenoids are also known to be host specific, since each species infects only one or very few host species (Poulin 1992; Sasal et al. 1998). This specificity is not necessarily the result of strict coespeciation between host and parasite lineages, but also of non-vicariant processes such as host switching (Boeger & Kritsky 1997).

Using network analysis, we intend to look for broad patterns of distribution of Monogenoidea gill parasites on Neotropical freshwater fishes. Our prediction is that the high interaction intimacy and host specificity displayed by monogenoids favor high modularity. We are aware that the number of hosts that a given parasite uses is not sufficient as a predictor of specificity (Agosta et al. 2010; Poulin et al. 2011), however the search for broad and unique patterns of host-parasite interactions can reveal good model associations to evaluate the relative influence of past history and recent ecological features on the assembly rules shaping ecological networks.

## **2 Methods**

A database of component communities of Monogenoidea parasitizing fishes from Neotropical rivers was prepared in the Molecular Ecology and Evolutionary Parasitology Laboratory. The main sources of information on host-parasite interactions were articles of species descriptions and records of occurrence published until 2011. Only gill parasites were

selected because they are the best-known group of monogenoids in the neotropics. Besides, processes like transmission and dispersion may vary between parasites with different reproduction modes or infection sites (Boeger et al. 2003). Such differences would add a source of variation that will not be addressed in this study. This resulted in 451 interaction records of 310 parasite species that occur in 141 host species. From this interaction list, we organized a matrix with parasites in columns and fishes in rows. An element representing a host-parasite interaction received the value of 1; no interaction received a 0.

Host-parasite interactions were organized into five matrices grouping species at different taxonomic levels. Each matrix depicts interactions between: (1) fish orders and parasite families, (2) fish families and parasite subfamilies, (3) fish families and parasites genera, (4) fish species and parasite genera, and (5) fish and parasite species (Supplementary Figures).

Networks for the three larger fish orders (Perciformes, Siluriformes and Characiformes) and their parasites were analyzed separately to evaluate interaction patterns between host species and parasite genera, and between host and parasite species. We did it because these orders do not share any parasite genera. In the networks comprising parasite genera, we included only host species with known phylogenetic position. Additionally, fish species distribution on river basins was gathered from Fishbase (Froese and Pauly 2012) and Reis et al. (2003). According to species geographic distribution, we classified 23 river basins ranging from Mexico to Argentina, where each species could be present or absent.

In order to define interaction patterns, networks were drawn with *plotweb* function in *bipartite* package (Dormann et al. 2009) of R (R Development Core Team, 2011 - <http://www.r-project.org>) and Pajek (de Nooy et al. 2005). Nestedness analysis was performed with the software ANINHADO (Guimarães & Guimarães 2006) using NODF metric (Almeida-Neto et al. 2008). To test if networks are more nested than expected by

species richness and heterogeneity of interactions, NODF recorded values were compared to those of 1000 random matrices generated by Erdős-Rényi (ER) null model and CE null model ('null model 2' of Bascompte & Jordano 2003). The first model generates random networks with same connectance in average, and the second does the same controlling for the number of interactions per species in the network.

Modularity was analyzed with the software MODULAR (Marquiti et al. 2013) using Newman and Girvan's metric (Newman & Girvan 2004) modified for bipartite networks (Barber 2007) and modules were identified by the simulated annealing (SA) optimization method (*i.e.* subgraphs whose nodes are more connected to one another than to the nodes outside the subgraph). For each network we computed the modularity index  $M$  and its level of significance by comparing the recorded  $M$  value to that of 100 random networks generated by the same null models used for the nestedness analysis. Since host and parasite species are distributed across a biogeographic region and only species that co-occur at least in one river basin can interact, species distribution on river basins could affect network modularity. Thus, we generated 100 networks based on a null model that imposes forbidden links between species that do not co-occur, assuming that the potential distribution of parasites on river basins is the same of their hosts (see Supplementary Methods for null model details). Modularity was then computed for each network generated by this model (GAM - Geography Aware Model) and compared to that of the original network.

To build hosts phylogenetic trees, we followed Oliveira et al. (2011) for the relationship among species of Characiformes, and Hubert & Renno (2006) and Ortí et al. (2008) for best resolution within Serrasalminae. For relationships among species of Siluriformes we followed Sullivan et al. (2006), and Lundberg et al. (2011) for resolution within Pimelodidae; and Lopez-Fernandez et al. (2010) for Perciformes. Cladograms were

drawn in Mesquite 2.75 program (Maddison and Maddison, 2011) adding all species whose position was known, not allowing polytomies.

### 3 Results

Fish hosts were classified in seven orders (Fig. 1). Three of these orders are parasitized by two families of parasites and the other four, only by one family (Fig. S1). From the five parasite families, four have been recorded from just one fish order (i.e. Diplectanidae, Monocotylidae, Microcotylidae and Hexabothriidae). On the other hand, species of Dactylogyridae occur in all orders but Rajiformes. Increasing taxonomic resolution, it was noticed that Ancyrocephalinae is the subordinate widespread taxon, for it occurs in 23 of the 24 host families included in this study. The seven other subfamilies parasitize 1-3 host families. Likewise, host families harbor 1-3 parasite subfamilies each (Fig. S2).

The network between host families and parasite genera is more modular ( $M = 0.79$ ,  $p < 0.001$ ) and less nested ( $NODF = 6.20$ ,  $p > 0.05$ ) than the expected by ER and CE null models. This network highlights the separation of parasites by host orders (Fig. 1). Host and parasite species are grouped in 11 modules and each host order is organized in 1-3 modules. The exceptions are Gymnotiformes and Cyprinodontiformes, which share species of *Urocleidoides sensu strictu* Mizelle and Price, 1964 with some families of Characiformes, hence belong to the same module. Most parasite genera are also restricted to one host family. For instance, each perciform family represents a module without connections with other modules. For Siluriformes and Characiformes, modules are composed of 1-6 host families and there are links between them. In these cases, parasite fauna is not as taxonomically structured as in Perciformes. Among hosts, Serrasalminidae, Characidae and Pimelodidae are the families with higher richness of parasite genera, and are also species-rich clades. Among parasites, *Urocleidoides* has the largest host range, parasitizing nine families of three orders.

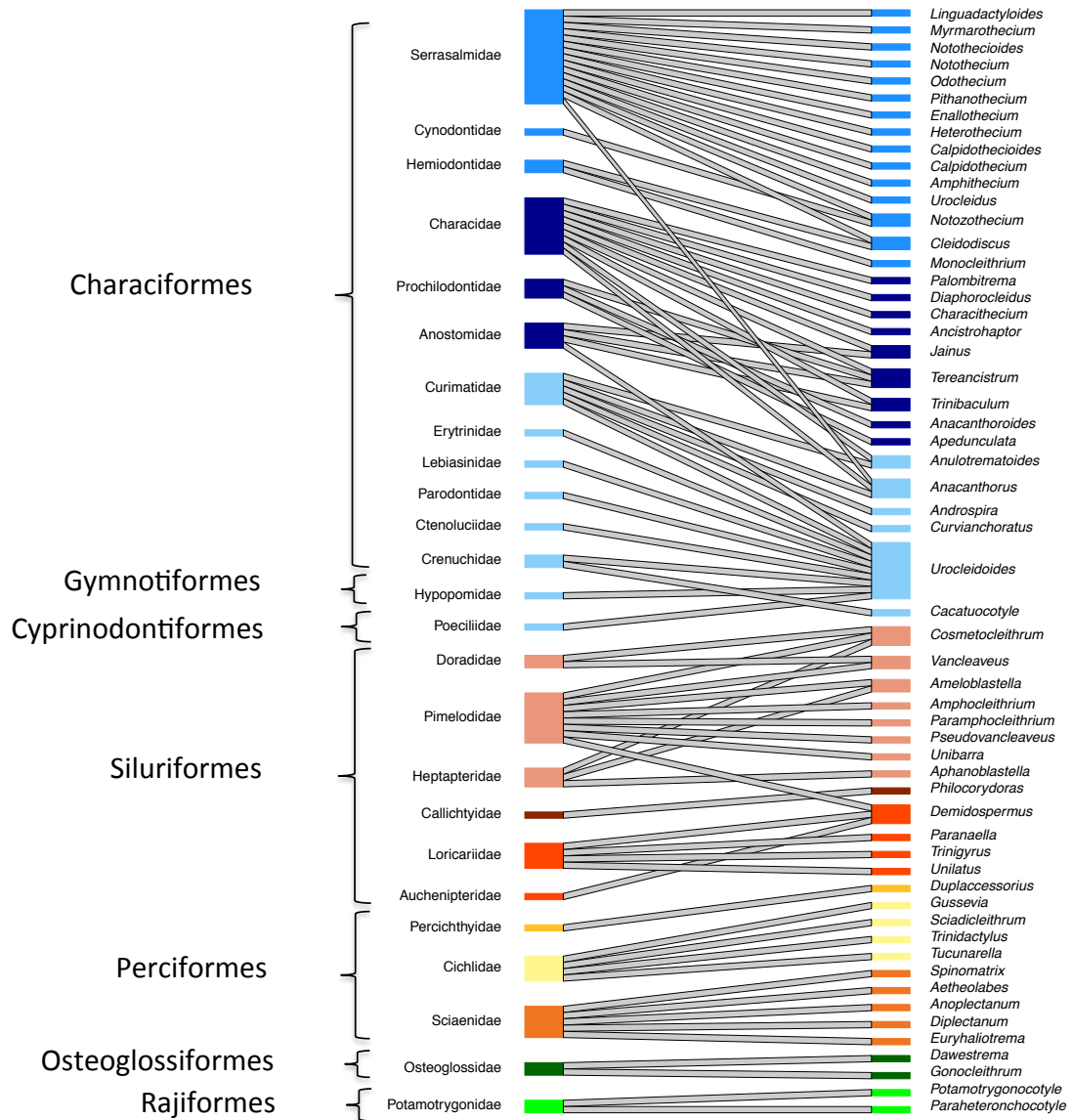


Figure 1. Network of interactions between fish families and parasite genera. Fish families are grouped by orders. Color of nodes represents modules.

Table 1. Size, nestedness and modularity of networks including host species and parasite genera and significance for each null model (p values are the proportion of networks generated by null models with equal or higher NODF or M values). Significant results are in bold.

	Network size		Nestedness			Modularity			
	Fish	Parasite	<i>NODF</i>	ER ( <i>p</i> )	CE ( <i>p</i> )	<i>M</i>	ER ( <i>p</i> )	CE ( <i>p</i> )	GAM ( <i>p</i> )
Perciformes	20	6	22.24	0.53	0.7	<b>0.54</b>	0.29	0.28	<b>0.01</b>
Siluriformes	31	13	8.74	0.76	0.87	<b>0.74</b>	0.07	<b>0.02</b>	<b>0</b>
Characiformes	46	29	<b>18.37</b>	<b>0</b>	<b>0</b>	<b>0.57</b>	0.33	0.13	<b>0</b>



Among perciform hosts, parasite genera are restricted to a single fish family, but can occur in many host species (Fig. 2). Modularity and nestedness of this network are not greater than expected by null models ER and CE (Table 1). However, when spatial distribution is considered, modularity is significantly higher than that generated by GAM null model. Cichlidae is the most species-rich perciform family (17 spp.), however has only three parasite genera. *Tucunarella* Mendoza-Franco, Scholz, and Rozkosná, 2010 occurs only in *Cichla monoclus* Spix and Agassiz, 1931; but *Gussevia* Kohn and Paperna, 1964 and *Sciadicleithrum* Kritsky, Thatcher and Boeger, 1989 are widespread in Cichlidae phylogeny. However, when considering the distribution of parasite species on hosts, most parasites are species-specific (Fig. S3). Each *Gussevia* spp. is known from one host species, but hosts can harbor more than one parasite species. Two out of 15 *Sciadicleithrum* species are known from more than one host species, but are restricted to phylogenetically close hosts.

Table 2. Size, nestedness, modularity and connectance of networks including host and parasite species. Significance for modularity is relative to all three null models (p values are the proportion of networks generated by null models with equal or higher NODF or M values). Significant results are in bold.

	Network size		Nestedness		Modularity		N	C	k
	Fish	Parasite	NODF		M				
Perciformes	26	51	1.55	$p_{ER,CE} = 1.0$	<b>0.9</b>	<b><math>p = 0.0</math></b>	20	0.044	1.52
Siluriformes	42	64	1.59	$p_{ER,CE} = 1.0$	<b>0.9</b>	<b><math>p = 0.0</math></b>	31	0.029	1.46
Characiformes	64	176	<b>3.78</b>	<b><math>p_{ER} = 0.00</math></b> $p_{CE} = 0.58$	<b>0.76</b>	<b><math>p = 0.0</math></b>	24	0.026	2.46

\* N, C and k represents number of modules, connectance and mean number of interactions per species, respectively.

Among Sciaenidae, 14 parasite species are known for the three species included in the phylogeny (Fig. 2). Likewise, most parasite species are species-specific, except for *Diplectanum piscinarius* Kritsky and Thatcher, 1984, which occurs in both *Pachyurus*

*bonariensis* Steindachner, 1879 and *Plagioscion squamosissimus* Heckel, 1840, species that belong to different lineages within Sciaenidae. *Plagioscion squamosissimus* is the host with the highest parasite richness, harboring four species of *Diplectanum* Diesing, 1858 and six species of *Euryhaliotrema* Kritsky and Boeger, 2002 (Fig. S3). Given the host species-specificity displayed by congener parasites occurring on both host families, and the low mean number of parasites per host species, the network of interaction between parasite species and perciform hosts is highly modular compared to all null models. On the other hand, nestedness is not significant (Table 2).

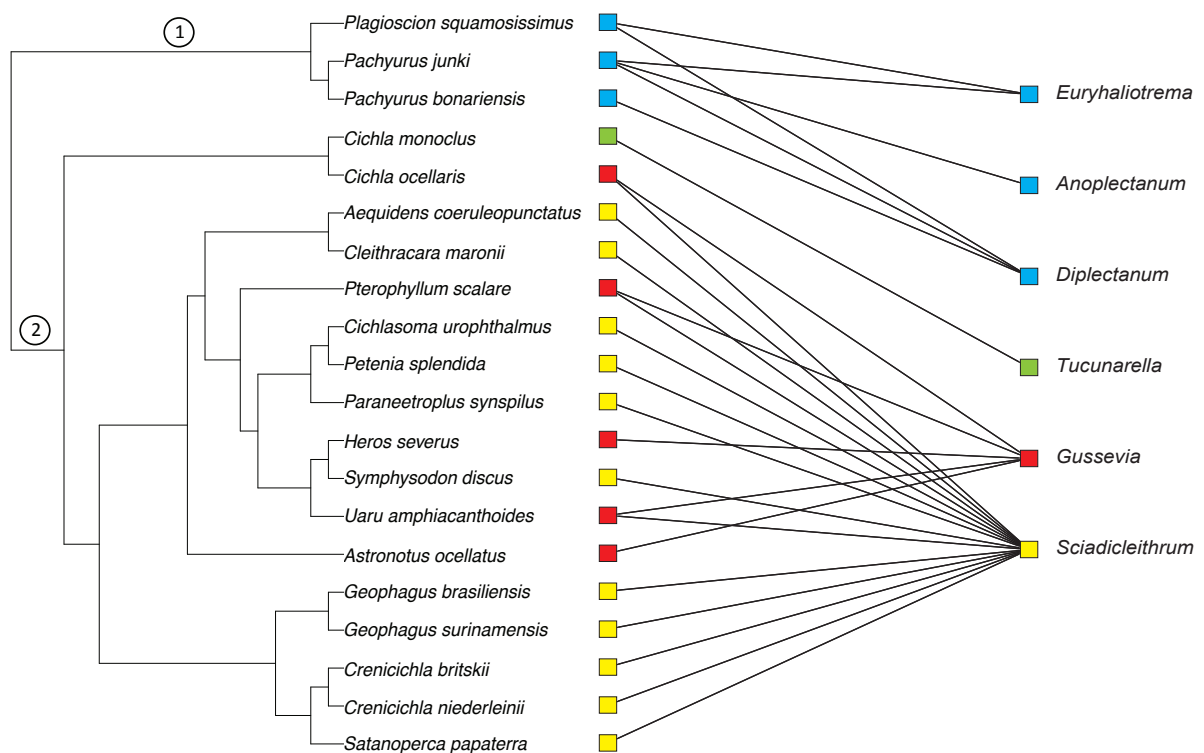


Figure 2. Phylogenetic distribution of parasite genera on Perciformes. The Perciformes phylogeny is on the left and parasite genera are on the right. Links indicate interaction and node colors represent modules. Numbers indicate host families, (1) Sciaenidae and (2) Cichlidae.

The network of interactions among siluriform host species and parasite genera is not significantly nested but it is modular when compared to the null model 2 and GAM (Table 1),

with hosts and parasites grouped in seven modules. Parasite composition of Siluriformes families is not as structured as for Perciformes. Some parasite genera are restricted to one fish family (Fig. 3), but three occur in 2-3 families. *Vancleaveus* Kritsky, Thatcher and Boeger, 1986 is known from Pimelodidae and Doradidae hosts; *Ameloblastella* Kritsky, Mendoza-Franco and Schulz, 2000 is known from Pimelodidae and Heptapteridae hosts; and *Demidospermus* Suriano, 1983 is known from Pimelodidae, Auchenipteridae and Loricariidae hosts.

The network between species of Siluriformes and parasite species is not significantly nested, but is more modular than networks generated by all null models (Table 2). As most parasites are restricted to one host, such as for those occurring on perciform fishes, and most hosts display a small number of partners, the network is highly compartmentalized. From the 65 monogenoid species recorded on the gills of siluriform hosts, only 12 occur in more than one host species (Fig. S4). From those, seven occur only in congener species and four occur in hosts from the same family. The exception is *Demidospermus uncusvalidus* Gutiérrez and Suriano, 1992, the only parasite species reported from two host families, *Pimelodus maculatus* Lacépède, 1803 (Pimelodidae) and *Trachelyopterus galeatus* Linnaeus, 1766 (Auchenipteridae).

The network of interactions among Characiformes and genera of their monogenoid parasites is the only one that is more nested than the expected by null models (Table 1). This network (Fig. 4) is also more modular than GAM networks (Table 1). Although this network is roughly similar to the Siluriformes network (Fig. 3), Serrasalminidae hosts and their parasites interact in a different way. Parasite genera have a larger host range and hosts harbor more parasites. This increased connectance lowers modularity of the entire network, compared to Siluriformes network.

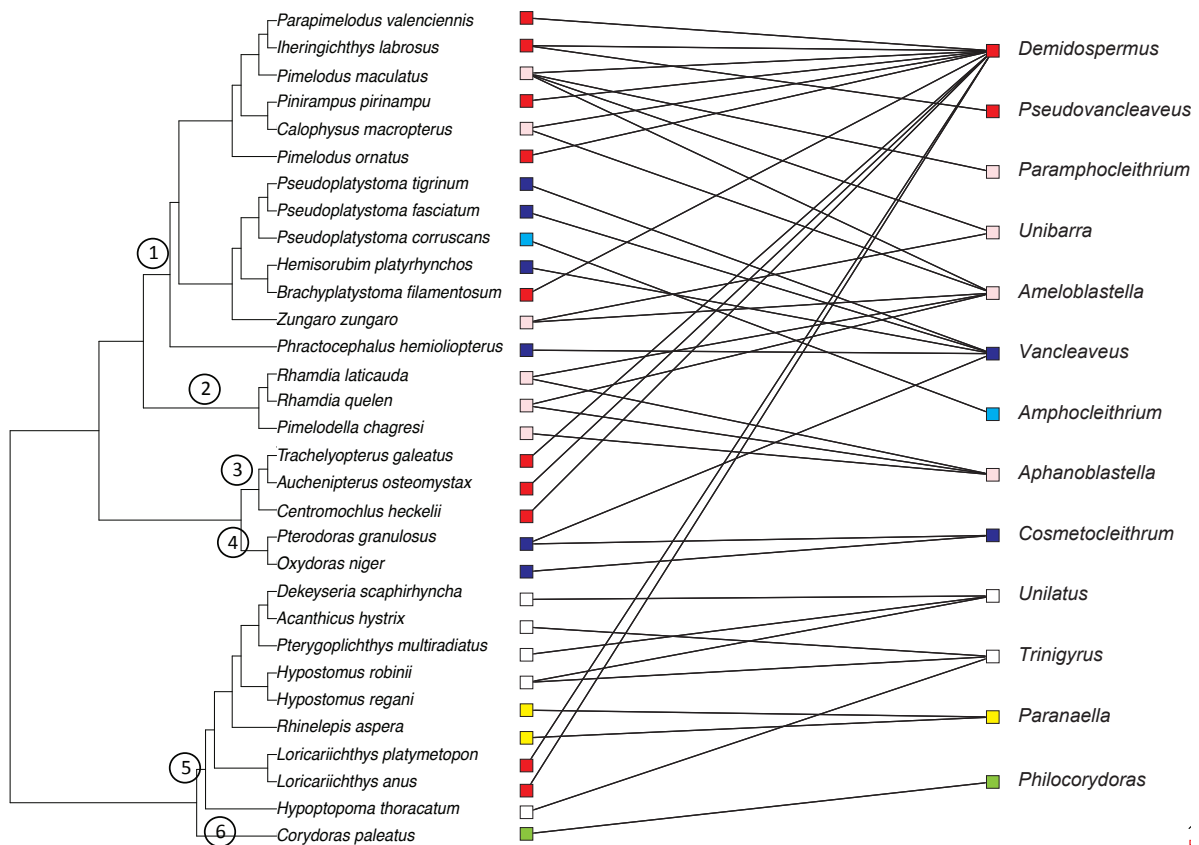


Figure 3. Phylogenetic distribution of parasite genera on Siluriformes hosts. The Siluriformes phylogeny is on the left and parasite genera are on the right. Links indicate interaction and node colors represent modules. Numbers indicate host families, (1) Pimelodidae, (2) Heptapteridae, (3) Auchenipteridae, (4) Doradidae, (5) Loricariidae, and (6) Callichthyidae.

Interactions between Characiformes hosts and parasite species (Fig. S5) also have a distinct pattern compared to the other fish orders. This network is more nested than the expected by chance and more modular than the expected by all null models (Table 2). From the 15 parasite genera that occur in Serrasalminae hosts, 35 species of 8 genera interact with more than one host species. Most of them are restricted to *Serrasalmus* Lacépède, 1803 spp. Characiformes that belong to the other families do not share parasite species as the serrasalmids. There are only three registered cases of parasite sharing by non-Serrasalminae hosts among Characiformes. Nine parasite species are known in more than one species of

*Triportheus* Cope, 1872 each; two parasite species are shared by *Astyanax* Baird and Girard, 1854 species; and another parasite occurs in two *Characidium* Reinhardt, 1867 species.

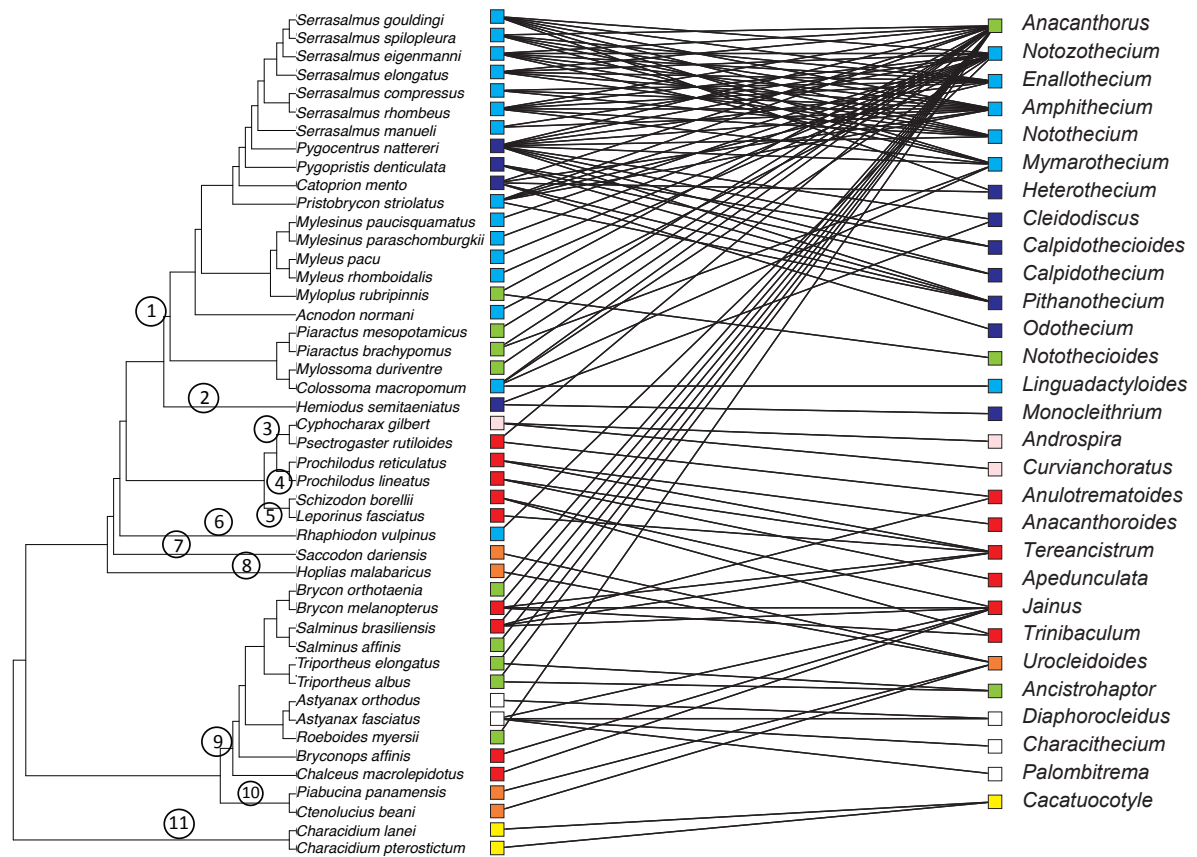


Figure 4. Phylogenetic distribution of parasite genera on Characiformes hosts. The Characiformes phylogeny is on the left and parasite genera are on the right. Links indicate interaction and node colors represent modules. Numbers indicate host families, (1) Serrasalmidae, (2) Hemiodontidae, (3) Curimatidae, (4) Prochilodontidae, (5) Anostomidae, (6) Cynodontidae, (7) Parodontidae, (8) Erythrinidae, (9) Characidae, (10) Ctenoluciidae, (11) Crenuchidae.

#### 4 Discussion

Interaction networks between Monogenoidea gill parasites and their freshwater fish hosts tended to be loosely connected and organized in modules. The observed modularity is not a byproduct of geographic distribution on river basins, on contrary, the addition of spatial structure on GAM null model highlighted the intrinsic modularity of this kind of interaction.

Although nestedness associated with modularity has been found on diverse types of host-parasite networks (Vacher et al. 2008; Graham et al. 2009; Bellay et al. 2011; Lima et al. 2012), interactions between freshwater fish and their parasites were also modular but not nested for networks including only ectoparasites or parasites with direct life cycle, as the Monogenoidea (Lima et al. 2012). Our results also agree with the expectation that higher-intimacy interactions are associated with high specialization and high modularity at the species level (Pires & Guimarães 2012). The organization of species interactions within biological systems may influence community stability (Allesina & Tang 2012), ecosystem functioning (Thébault & Loreau 2003) and coevolution (Guimarães et al. 2011). Numerical simulations (Thébault & Fontaine 2010) and qualitative stability analysis (Allesina & Tang 2012) suggest that low connectance and high modularity in networks of intimate antagonistic interactions are associated with higher stability at the community level.

We found that each fish order has a unique parasite genera composition (Fig. 1). This is probably due to phylogenetic constraints that limit the number of potential hosts for a given parasite genus. The probability of an interaction to occur depends on the co-occurrence of partner species in a given locality in ecological time. However, their past evolutionary history might limit the range of possible interactions (Poulin 2010). These phylogenetic restrictions can also be found at the family level, especially for Perciformes hosts. In fact, each fish order (and some families) should represent a different kind of resource for monogenoid parasites as each one has a unique past history in the Neotropics. The freshwater species of Perciformes included in Cichlidae and Sciaenidae originated from two different events of freshwater colonization, dating back to around 90 Mya and 20 Mya, respectively (Lundberg et al. 2010; Kocher & Stepien 1997; Boeger & Kritsky 2003). Siluriformes is a diverse clade with a worldwide, mostly freshwater distribution. Likewise Perciformes, neotropical Siluriformes are not all descended from a single ancestral species but include four independent

monophyletic clades (Sullivan et al. 2006). Neotropical Characiformes, on the other hand, represent a monophyletic clade (Oliveira et al. 2011). Therefore, broad historical constraints acting at large scales may restrict parasites to host higher taxa with a common evolutionary history (Boeger & Kritsky 1997; Desdevises et al. 2002a; Vacher et al. 2008).

Despite the general high host specificity of monogenoid parasites, some of them have a larger host range. Two unusual distribution patterns were observed among parasite genera. First, within each fish order, one genus had a widespread distribution among host species and a few other genera had an intermediate number of hosts. This is the case of *Sciadicleithrum* on Cichlidae hosts, *Demidospermus* on Siluriformes, and *Anacanthorus* on Characiformes. Second, one genus has been found in hosts from more than one fish order. *Urocleidoides* spp. interact with seven species that belong to six families of three orders (one family of Cyprinodontiformes, one of Gymnotiformes, and four of Characiformes). At the species level, all but one parasite occur in just one host species or in a few hosts of the same fish family. The exception is *Demidospermus uncusvalidus*, which occurs in two distantly related species in Siluriformes phylogeny. Confirmation of the identity of the monogenoid, however, may indicate that this, rather than an exception, represents a case of improper species determination. Indeed, future studies should investigate the mechanisms underlying host range expansion in monogenoid lineages. The ability to use a wider range of hosts may be related to biological and ecological characteristics of both host and parasite lineages but it may also reflect problems in taxonomic resolution of this parasite group.

An important issue is whether patterns of interaction of the studied host-parasite networks could be the result of sampling artifacts. Two main issues originate from the fact that the known species and interaction diversity of the Monogenoidea are primarily known from taxonomic studies. First, as the number of individuals of each host species surveyed is often not available in parasite species descriptions, we could not estimate sampling effort to

use it as a correction factor. Second, host specificity displayed by parasites may be overestimated since most parasite species are known only for their original descriptions. Even though, host specificity may be an intrinsic trait of the Monogenoidea due to its biology. The combination of monoxenic life cycle with the capacity to colonize new hosts from a single parasite individual may have allowed speciation through host switching and adaptive radiations to happen in some Monogenoidea lineages (Brooks & McLennan 1993). Thus, host specificity displayed by these parasites may be the outcome of host shifts followed by adaptation to new conditions (Desdevises et al. 2002b; Bueno-Silva et al. 2011).

From hosts perspective, the *piranha* clade of the Serrasalminae stands out in terms of parasite richness per host species. These hosts not only have more parasite genera but also share them, so their parasites have a wider host range. From the 13 genera occurring on *piranhas*, seven parasitize four or more host species (Fig. 4). It is also true for parasite species, as 32 out of 77 species occur in 2-10 species of the *piranha* clade. While the higher parasite richness of *piranhas* is already known (Boeger & Vianna 2006), the underlying mechanisms and its influence on network structure are now being investigated (M.P. Braga et al., in preparation).

Our results highlight the strong phylogenetic influence on the distribution of Monogenoidea parasites on their fish hosts, as reported in previous studies (Rezende et al. 2007; Cattin et al. 2004; Cooper et al. 2012; Lima et al. 2012). Fish orders and some families seem to represent distinct sets of resources for parasites. Additionally, networks including fish species of each order display different interaction patterns with parasites at genera and species level. These findings encourage a closer look at the internal structure of host-parasite networks, especially those including species from a biogeographical region such as the present study. This work allowed us to evaluate the diversity of interaction patterns among



Monogenoidea parasites and their fish hosts, and it can be used as groundwork for future studies on the community level.

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## Supplementary Figures

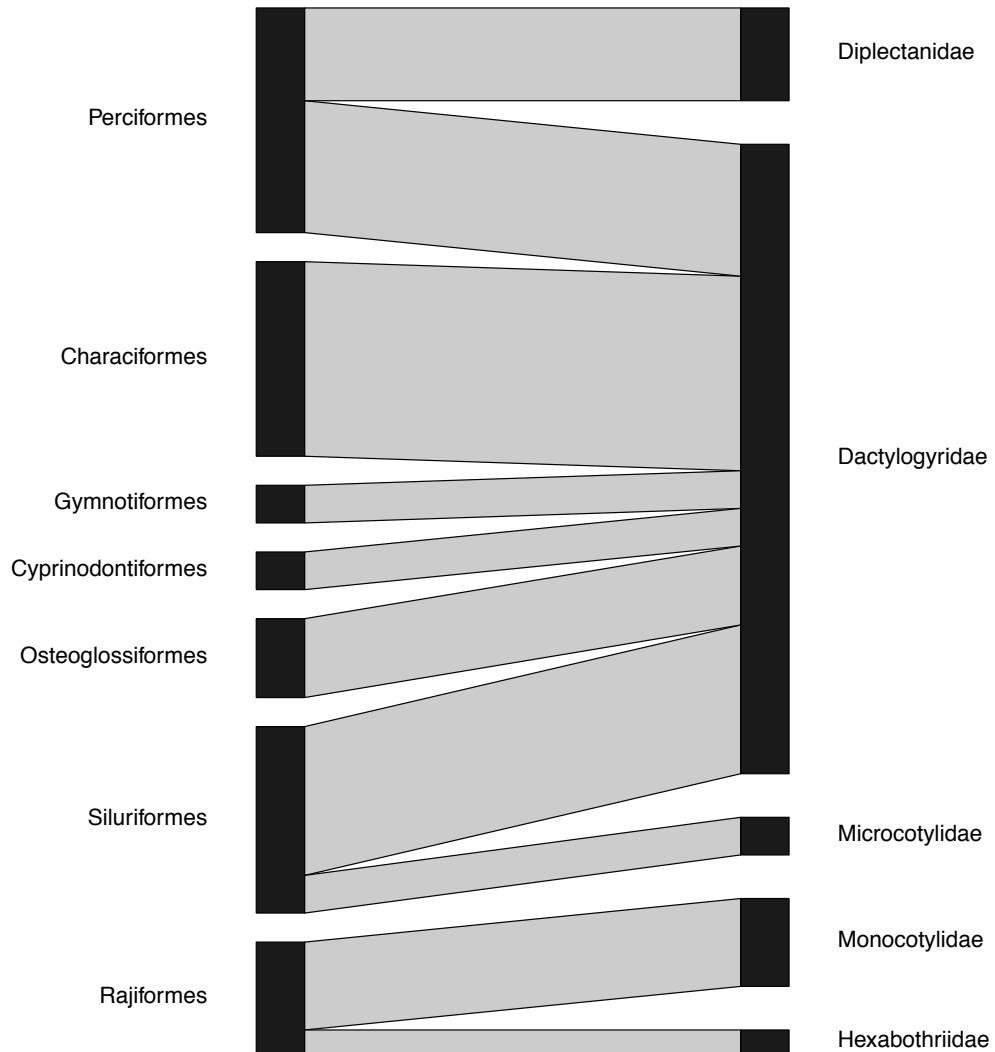


Figure S1 - Network of interactions among fish orders and parasite families. Hosts are on the left and parasites on the right. Lines indicate interaction and thickness of lines are proportional to the number of interactions among species of host and parasite taxa in log scale.

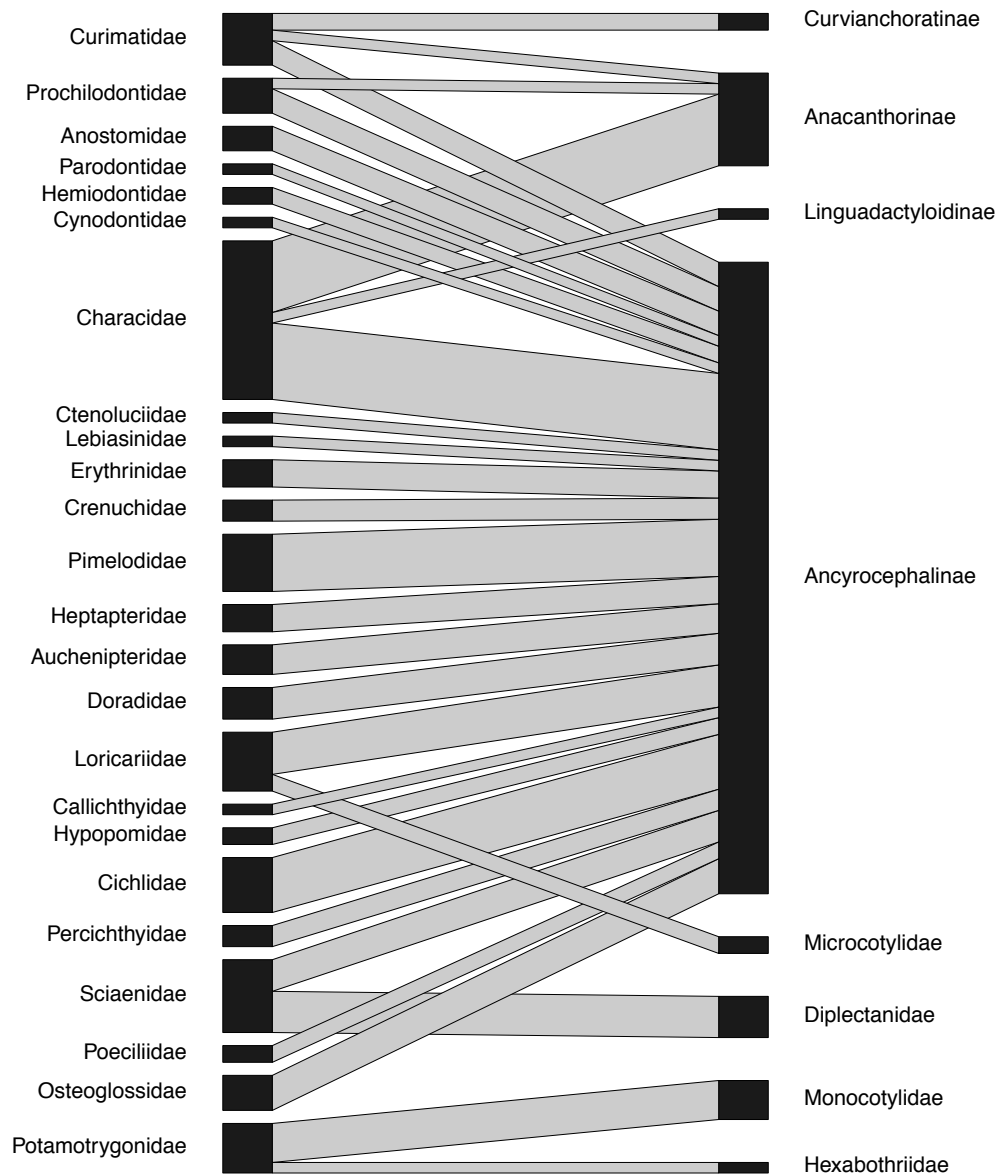


Figure S2 - Network of interactions among fish families and parasite sub-families (for Dactylogyridae) or families. Hosts are on the left and parasites on the right. Lines indicate interaction and thickness of lines are proportional to the number of interactions among species of host and parasite taxa in log scale.



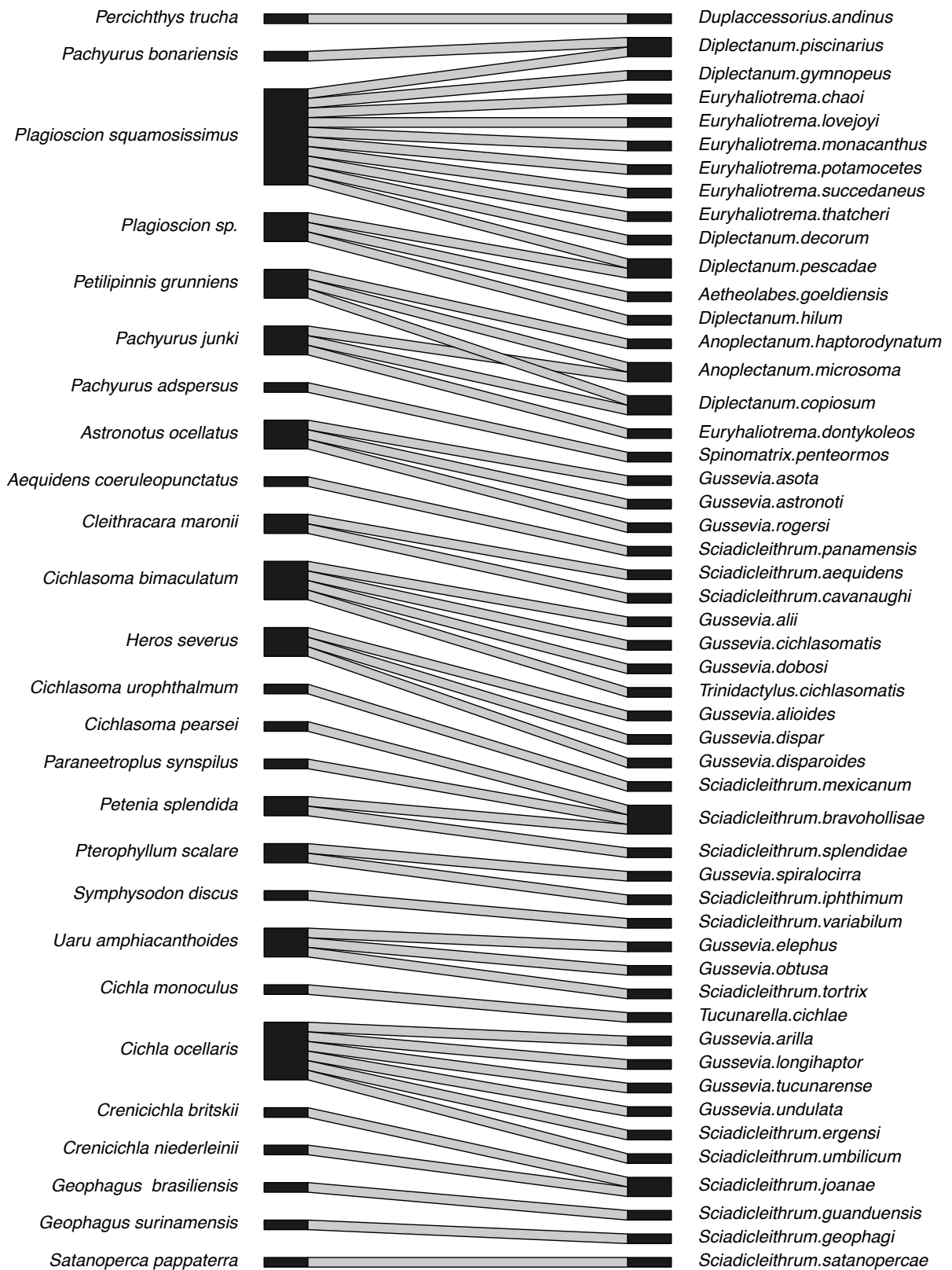


Figure S3 - Network of host-parasite interactions between Perciformes and parasite species. Hosts are on the left and parasites on the right.

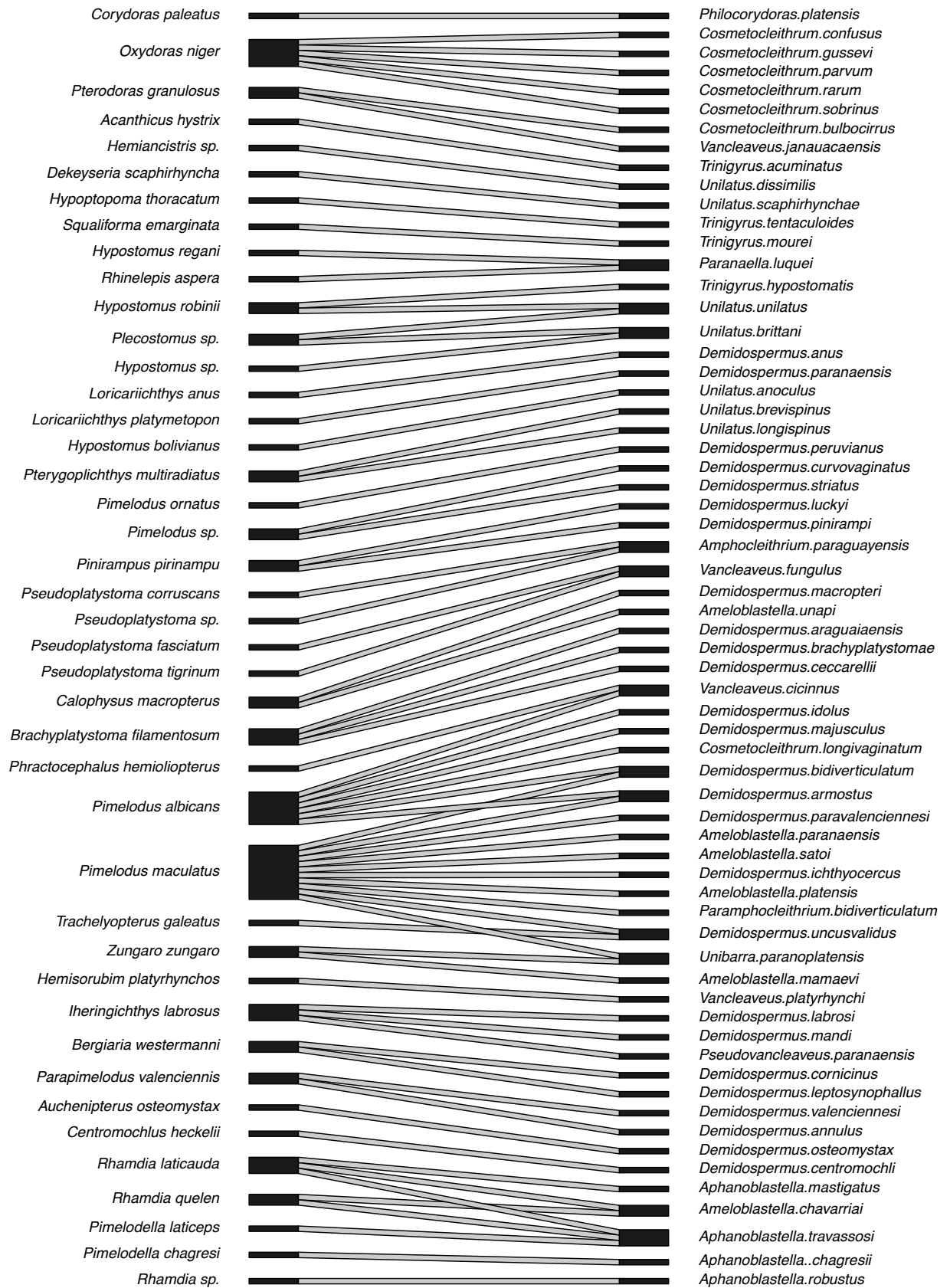


Figure S4 - Network of host-parasite interactions between Siluriformes and parasite species. Hosts are on the left and parasites on the right.

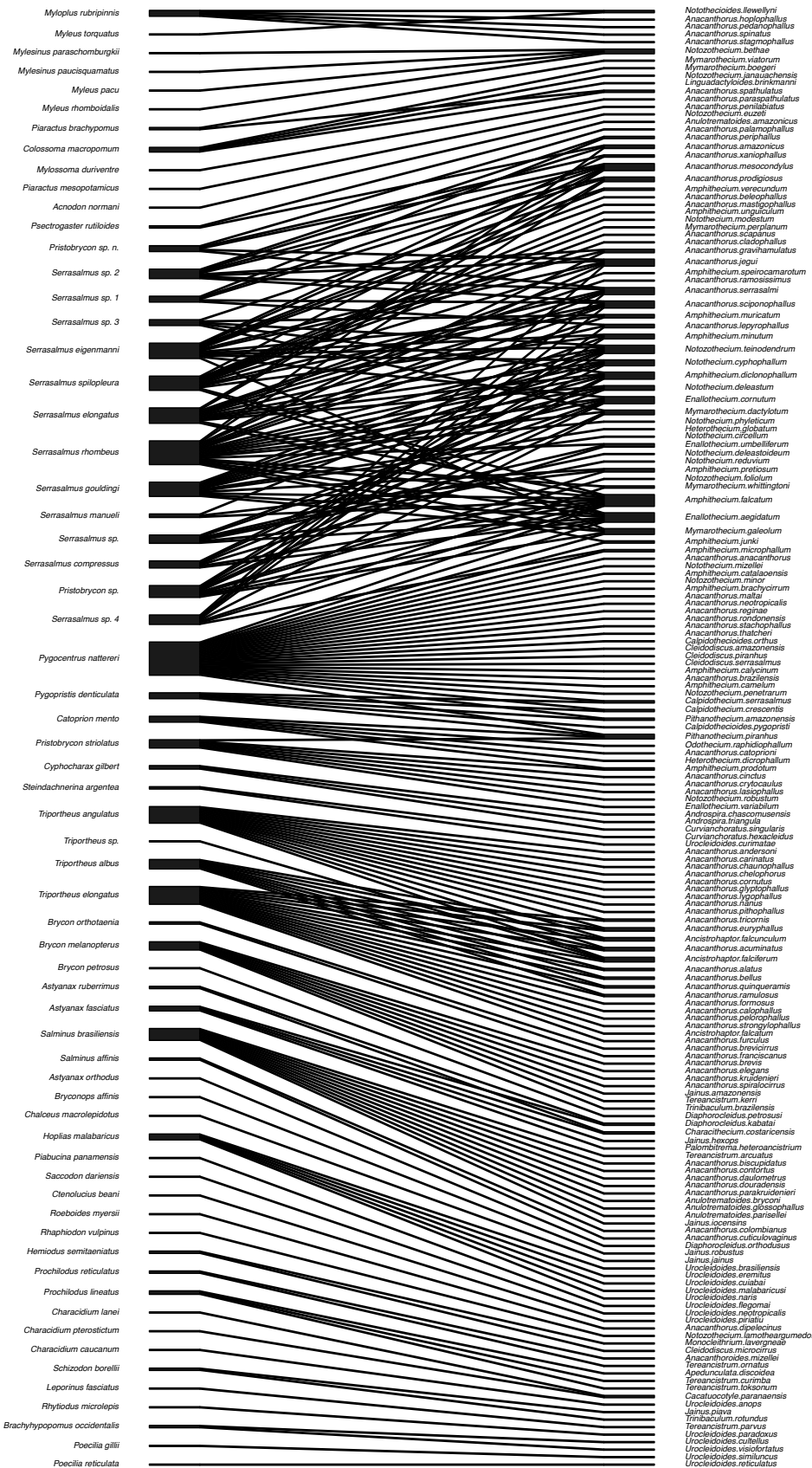


Figure S5 - Network of host-parasite interactions between Characiformes and parasite species. Hosts are on the left and parasites on the right.

## Supplementary Methods

The geographic aware model (GAM) needs two initial matrices: the interaction matrix and the matrix of hosts distribution on river basins. From these two matrices the program generates a co-occurrence matrix that informs whether a given parasite co-occurs in at least one river basin with a given host. Interactions between species that do not overlap their geographic distribution are considered "forbidden links". The remaining possible interactions have a 50% probability to occur. Random networks are generated constrained by the "forbidden links" and with the same number of interactions as the original interaction matrix, but with variable size. We generated and calculated modularity of 100 networks for each network analyzed.

## Capítulo 2 - Drivers of parasite sharing among Neotropical freshwater fishes

### Abstract

Since host-parasite interactions are so ubiquitous, it is of primary interest for ecologists to understand the factors that generate, maintain, and constrain these associations, with implications for studies on emerging infectious diseases, biological control, biological invasions, and biotic responses to climate change. Phylogenetic comparative studies have found abundant evidence for host switching onto relatively unrelated hosts, sometimes related to diversification events, in a variety of host-parasite systems. For monogenoid parasites, it has been suggested that host occurrences cannot be explained solely by the coespeciation model, hence host switching and/or sympatric speciation should be associated with the origins of several monogenoid taxa. We searched for the factors that shape broad patterns of parasite sharing, using path modeling as a way to generate hypotheses about the evolution of host-parasite interactions between Monogeneoidea gill parasites and Neotropical freshwater fishes. Parasite sharing was assessed from an interaction matrix, and explanatory variables included phylogenetic relationship, environmental preferences, biological traits and geographic distribution for each host species. Phylogeny had the strongest overall direct effect on parasite sharing. However, results varied between fish orders, and when host family-specific and generalist parasites were analyzed separately. Phylogenetic relatedness of hosts is the most important factor influencing the distribution of specialist parasite, while geographic distribution is the most important for generalist parasites sharing. Our results reinforce the importance of including evolutionary history into the study of ecological associations. Geographic distribution of hosts emerged as a key factor increasing the chance of colonization of phylogenetically unrelated hosts. Finally, host ecology and biology are important additional factors to understand host-parasite interactions.

## 1 Introduction

Parasitism may be the most common mode of life on nature (Price 1980). Organisms that spend most of their lifetime feeding in or on a single individual of another species can be found in diverse groups of organisms. Monogenoidea is a class of Platyhelminthes strictly parasitic, mostly composed of ectoparasites on gills or body surface of freshwater and marine fishes (Boeger & Vianna 2006). Several species, however, are endoparasitic and found in a variety of host groups, such as crustaceans, molluscs and even one species of mammal. In the Neotropics, the diversity of the Monogenoidea from fishes is still largely unknown, with more than 300 species described in approximately 70 genera, from 144 species of hosts (Boeger & Vianna 2006). Dactylogyridae is the most abundant family in continental waters of South America and species of Gyrodactylidae (*sensu* Boeger & Kritsky, 1997; Boeger et al. 2003) are being systematically described more recently. On the other hand, Diplectanidae, Monocotylidae, and Hexabothriidae appear to be poorly represented in the neotropical fauna (Boeger & Vianna 2006).

Since host-parasite interactions are so ubiquitous, it is of primary interest for ecologists to understand the factors that generate, maintain, and constrain these associations (Agosta et al. 2010), with implications for studies on emerging infectious diseases (Brooks & Ferrao 2005; Brooks & Hoberg 2007), biological control, biological invasions, and biotic responses to climate change (Brooks & McLennan 2002).

### 1.1 The "Parasite Paradox"

One of the remarkable features of parasitism is the conservatism in the range of hosts used, both on ecological and evolutionary time-scales (Thompson 1994, 2005; Brooks & McLennan 1993, 2002; Futuyma & Mitter 1996). For this reason, the prevailing perspective of the evolution of host-parasite associations was, until recently, centered on host specificity.

Speciation among parasitic groups was thought to be promoted by selection for increased specialization of parasites to their hosts, constraining host use (Brooks & McLennan 2002). Although coevolution can be diversifying through local selection in different parts of a geographic range, promoting populations locally adapted (Thompson 1994, 2005), this perspective does not incorporate the origins of new associations. Indeed, there is little evidence for co-speciation as a dominant process in host-parasite evolution (Hoberg & Brooks 2008, 2010). Furthermore, the term 'co-speciation' was first proposed by Brooks (1979) to describe cases in which hosts and parasites underwent concomitant speciation events, not as an evolutionary mechanism.

On the other hand, phylogenetic comparative studies have found abundant evidence for host switching onto relatively unrelated hosts, sometimes related to diversification events, in a variety of host-parasite systems (Boeger & Kritsky 1997; Agosta 2006; Janz et al. 2006; Hoberg & Brooks 2008; Nyman 2010). For less intimate associations, as between plants and plant-feeding insects, there are examples of rapid shifts to new hosts in ecological time-scale among introduced plants (Agosta 2006; Strauss et al. 2006), suggesting that host shifts are common among parasites. The conflict between resource specialization and the ability to host switch is called the 'parasite paradox' (Agosta et al. 2010).

### *1.2 The "Parasite Paradox" Resolution - Ecological Fitting*

Host shifts must start with a host range expansion, even if additional hosts are inferior adaptive alternatives to the original host. The most likely conclusion is that a parasite has the ability to utilize a new host before a successful shift starts (Agosta et al. 2010). Janzen (1985) recognized that despite the place where a given species evolved, its inherited functional abilities may allow it to survive in a variety of conditions. For parasites, it means that even if a group of parasites can utilize a certain environment (including its host) in a way that

suggests a shared evolutionary history, the relevant traits to the interaction may have evolved elsewhere, in distinct evolutionary time, under different conditions and/or with different host species. In this case, the interaction observed is the result of a relatively recent ecological "fit", possibly followed by rapid adaptation. Janzen (1985) called this link between the past history of the species and their present day associations as **ecological fitting**. He also suggested that ecological fitting plays a major role in shaping communities. Indeed, there are many evidences for ecologically fit associations among hosts and parasites (Hoberg & Brooks 2008, 2010).

Agosta and Klemens (2008) proposed three factors that can give rise to the ability of organisms to achieve realized fitness under new conditions, *i.e.* phenotypic plasticity, correlated trait evolution and phylogenetic conservatism in traits related to resource use. With these capacities, organisms possess potential fitness outside the range of conditions in which they have evolved. This region of fitness space is called 'sloppy fitness space' (Agosta & Klemens 2008). According to the authors, parasites are able to ecologically fit with new hosts in at least two ways. First, parasites may shift to a new host species that possesses the same, or highly similar resources as the old host, *i.e.* ecological fitting via resource tracking. Second, parasites may achieve realized fitness in hosts representing a new resource, *i.e.* ecological fitting via sloppy fitness space. These two ways are not mutually exclusive and may represent the extremes of a continuum (Agosta & Klemens 2008).

### *1.3 Evolution of interactions in Monogenoidea*

For monogenoid parasites, it has been suggested that host occurrences cannot be explained solely by the coespeciation model, hence host switching and/or sympatric speciation should be associated with the origins of several monogenoid taxa (Boeger & Kritsky 1997). This is specially apparent for the evolution of the speciose Gyrodactylidae



(Zietara & Lumme 2002; Bakke et al. 2007; Blazek et al. 2008; Bueno-Silva et al. 2011; for exceptions see Sasal & Desdevises 1998; Poisot & Desdevises 2010). Boeger et al. (2003) suggested that the Gyrodactylidae originated after a host-switch from a marine to a demersal freshwater catfish host within South America, and that the ability for transmission as adults is one of the keys for the diversification of the viviparous Gyrodactylidae (Boeger & Kritsky 2003; Boeger et al. 2005), enhancing the ability of the group to speciate by host switching.

For most monogenoids, however, transmission is limited to the free-swimming larval stage. Despite of that, the distribution of *Lamellodiscus* Johnston e Tiegs, 1922 (Diplectanidae, Monogenoidea) species on Sparidae (Teleostei) hosts at Mediterranean Sea cannot be explained by the coespeciation model (Desdevises et al. 2002). The authors noticed that parasites were shared among hosts that displayed social behavior and shared ecological similarities, rather than phylogenetic proximity.

The present study comprises only gill parasites, for it is the best known group of monogenoids in continental waters of the Neotropical region. Furthermore, these species present similar processes of transmission and dispersion, which reduces the inclusion of new sources of variation in the analysis. Since few parasite species occur in more than one host species in our database (probably due to sampling biases) (Chapter 1), we analyzed parasite genera sharing by hosts, with two implied assumptions. First, parasite genera likely represent monophyletic groups, composed by species that descend from a single common ancestor, and, secondly, resource requirements are probably phylogenetically conservative. Members of the same genus should theoretically share a number of ecological, morphological, and behavioral characters, solely because they are descended from a relatively recent common ancestor (Brooks & McLennan 2002).

In this study, we search for the factors that shape broad patterns of parasite sharing, using comparative analysis as a way to generate hypotheses about the evolution of host-

parasite interactions between Monogeneoidea and Neotropical freshwater fishes. These hypotheses can then be tested in specific systems in subsequent studies as phylogenies for parasite clades become available.

## 2 Methods

Parasite sharing was assessed from an interaction matrix between 105 fish species (with known phylogenetic position) of seven fish orders and 55 genera of gill parasites of five families (for database information see Chapter 1). We gathered information on environmental variables, biological traits and geographic distribution for each host species, mainly from FishBase (Froese and Pauly, 2012), but also from Reis et al. (2003). Information available for most host species were used as putative predictors of parasite sharing. Environmental variables included (1) salinity - fish tolerance to brackish water, (2) climate - tropical or subtropical, and (3) habitat - pelagic, benthopelagic or demersal. As biological traits, we used (1) maximum male length, (2) trophic level and (3) vulnerability to fishing. As vulnerability strongly covary with maximum length, it was removed from analysis. From occurrence data, we determined 23 watersheds where fish species could be present or absent.

To build hosts phylogenetic tree, we followed Albert & Reis (2011) for relationship among orders as well as among Siluriform families; Oliveira et al. (2011) for the relationship among Characiform species and, for best resolution within Serrasalminidae, Hubert & Renno (2006) and Ortí et al. (2008). For relationship among Siluriform species, we followed Sullivan et al. (2006), and Lundberg et al. (2011) for resolution within Pimelodidae; and Lopez-Fernandez et al. (2010) for Perciformes. Cladogram was drawn in Mesquite 2.75 program (Maddison and Maddison, 2011) adding all species whose position was known, not allowing polytomies. In cases where the polytomy could not be solved, we chose the pair of

species in the clade with more available information about geographic distribution, ecology and biology.

We assessed causal relationships among variables using structural equation modeling by partial least squares approach (path modeling), through *plspm* package of R (<http://cran.r-project.org/web/packages/plspm/index.html>), since it allows the decomposition and interpretation of linear relationships among descriptors (Legendre & Legendre 1998). In order to use information on phylogeny and environment, pairwise distances between host species were used as descriptors (Rezende et al. 2007; Cooper et al. 2012). Parasite composition distance was calculated based on the interaction matrix, using Jaccard's index through R package *vegan* (<http://cran.r-project.org/web/packages/vegan/index.html>). Phylogenetic distances were calculated using *phydist* function of *picante* package of R (Kembel et al. 2010). Geographical, environmental and biological distances were calculated using Gower's method on *daisy* function of *cluster* package (<http://cran.r-project.org/web/packages/cluster/index.html>). This method calculates latent variables (parasite sharing, phylogeny, geographic distribution, environment and biology) as linear combinations of their indicators. Preliminary analysis showed that trophic level and vulnerability covaried with body size, hence only size was used as indicator for host biology. For the geographic distribution variable, the indicator was occurrence in river basins. The environmental variable was the linear combination of salinity, climate, and habitat indicators. Significance of path coefficients was assessed by bootstrap validation with 500 resamples. In each analysis, we evaluated the outer model and the bootstrap validation of the indicators weight. Valid environmental indicators for each analysis are depicted in figures.

As each order of fish interacts with its own subset of parasites, the analysis was also carried out using submatrices of the three main fish orders, *i.e.* Perciformes, Siluriformes and Characiformes, in order to evaluate possible differences in parasite sharing between fish

orders. Parasites were classified in two groups of host range: parasites with hosts from the same family, and parasites that occur in more than one host family. These groups were also analyzed separately because the factors influencing host "choice" may be different between groups.

### 3 Results

Most parasite genera are restricted to one species or to closely related hosts, which produces the diagonal shape of the interaction chart (Figure 1). Some genera are more dispersed (vertically) within and among fish orders. *Anacanthorus* Mizelle and Price, 1965 and *Notozothecium* Boeger and Kritsky, 1988 have a wide host range among characiforms; *Demidospermus* Suriano, 1983 is widely spread among siluriforms; and *Sciadicleithrum* Kritsky, Thatcher and Boeger, 1989 among perciforms (strict to Cichlidae). The only genus that occurs in more than one order is *Urocleidoides sensu strictu* Mizelle and Price, 1964 (Chapter 1), which parasitizes species of Characiformes, Gymnotiformes and Cyprinodontiformes. Despite the fact that the genus is likely monophyletic (supported by the sharing of a hook-like vaginal sclerite), it is probably an old lineage maybe equivalent to other widespread taxa, *i.e.* Ancyrocephalinae.

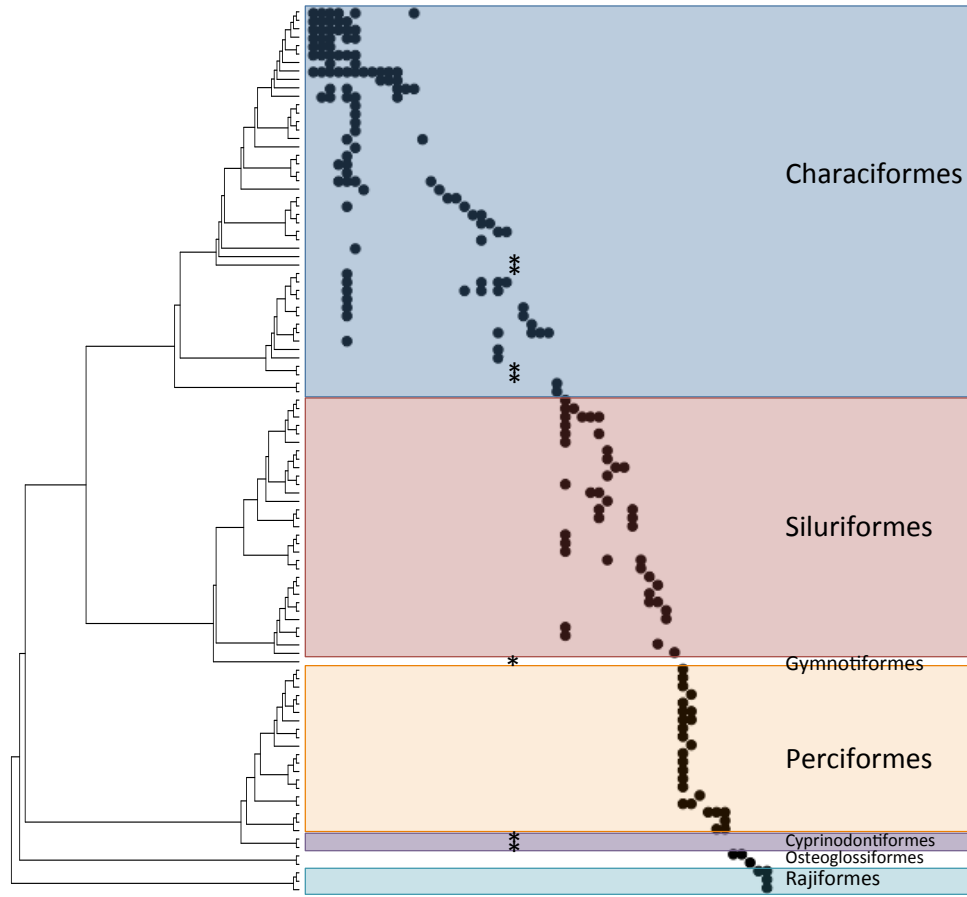


Figure 1 - Phylogenetic distribution of parasite genera. Host phylogeny is on the left; host species are on lines and parasite genera in columns. Interactions are indicated by black dots, and asterisk (\*) for *Urocleidoides*.

According to the path analysis, phylogeny has the strongest direct effect on parasite sharing, followed by geographic distribution, when all fish orders are considered together (Figure 2A). For parasite sharing among Perciformes (6 genera on 20 host species), host geographic distribution has a stronger effect than phylogenetic relatedness (Figure 2B). Yet, host biology also influence parasite sharing, directly and indirectly through geographic distribution. Among Siluriformes (14 genera on 31 host species), a small part of the variation in parasite sharing can be explained by the distribution of the hosts on river basins and their habitat (Figure 2C). This is the only group where phylogenetic relatedness is not associated to parasite sharing. Habitat display a negative direct effect on parasite sharing, yet has positive

indirect effect, so that the total effect is non significant. Total effect of host biology on parasite sharing is also non significant. For Characiformes (30 genera on 46 host species), geographic distribution and the environment (salinity and climate) have some effect on parasite sharing but it is most influenced by phylogenetic relatedness between hosts (Figure 2D).

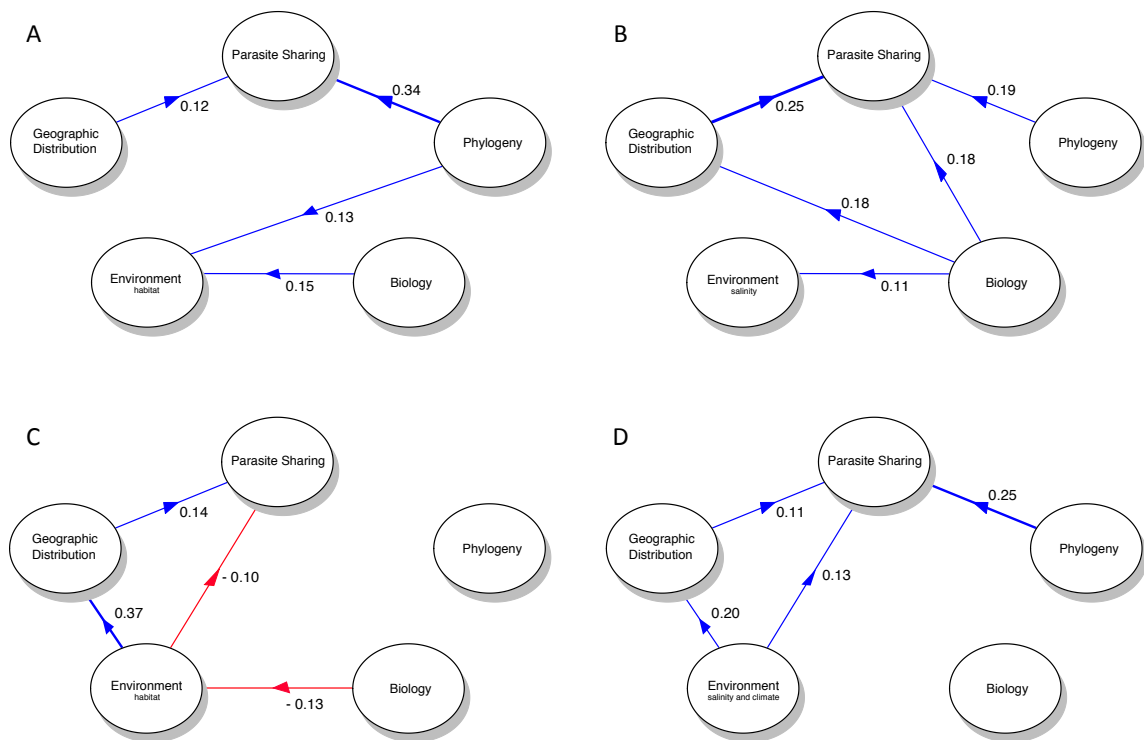


Figure 2 - Path diagrams of the effects of all factors on parasite sharing including all hosts (A), only Perciformes (B), only Siluriformes (C), and only Characiformes (D). Arrows represent effect paths from an effect variable to a response variable. The thickness of the arrows is scaled to standardized coefficients from path analysis and illustrates the relative effect strength. Negative effects are represented in red and positive effects in blue. Only effects  $> 0.1$  and statistically significant ( $p < 0.05$ ) are presented.

From the 55 parasite genera included in the study, 43 occur only in one host family. The path analysis with these parasites (43 genera on 64 host species) showed that all factors have some influence on parasite sharing (Figure 3A). Phylogenetic proximity of hosts has the strongest effect, followed by environment (climate and habitat), biology (size) and geographic distribution on river basins. The analysis including parasites that exploit more than one host family (now on referred to as "generalists") had a similar result. Parasite distribution of these 12 genera on 64 host species is also most influenced by phylogenetic proximity of hosts, followed by geographic distribution and habitat preference (Figure 3B).

Although results for family-specific and generalists parasites were qualitatively similar, when the analysis was carried out for Characiformes and Siluriformes separately, parasite host range (one or more families) influenced the results. For family-specific parasites on Siluriformes hosts (10 genera on 15 host species), phylogeny is the main driver of parasite sharing, followed by size of host (Figure 3C). On the other hand, the distribution of generalist parasites on Siluriformes (4 genera on 20 host species) is only influenced by geographic distribution of host species (Figure 3D). A similar result was found for Characiformes hosts. The distribution of 22 family-specific genera on 24 Characiformes host species is strongly affected by phylogenetic relatedness of hosts (Figure 3E), but also by host body size and environmental preferences (salinity and climate). For generalist parasites (8 genera on 40 host species), geographic distribution on river basins overcomes phylogenetic influence on parasite sharing (Figure 3F).

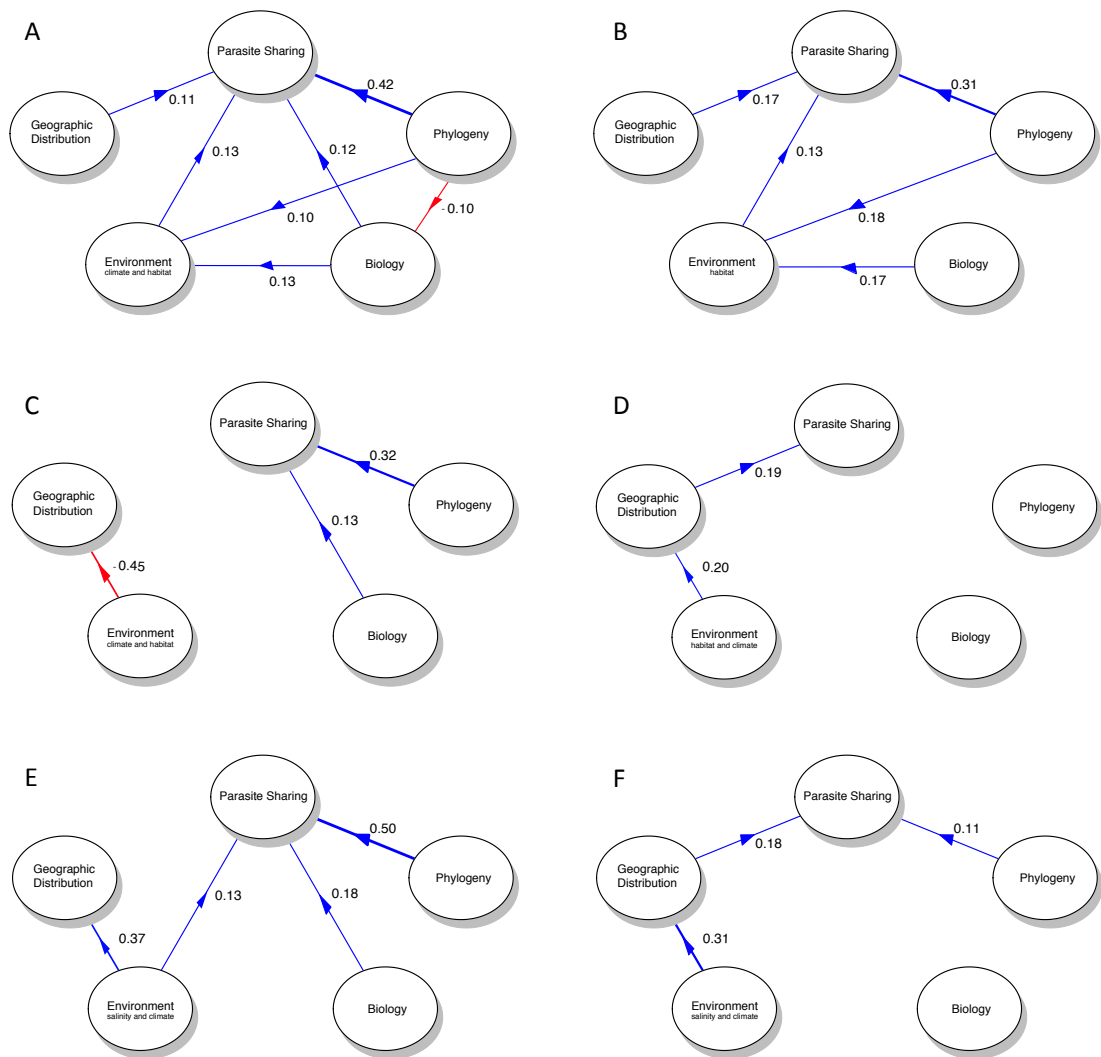


Figure 3 - Path diagrams of the effects of all factors on parasite sharing including all hosts (A-B), only Siluriformes (C-D), and only Characiformes (E-F). A, C and E include only family-specific parasites, while B, D and F include generalist parasites. Arrows represent effect paths from an effect variable to a response variable. The thickness of the arrows is scaled to standardized coefficients from path analysis and illustrates the relative effect strength. Negative effects are represented in red and positive effects in blue. Only effects > 0.1 and statistically significant ( $p < 0.05$ ) are presented.

#### 4 Discussion

Our results suggest that interactions between Monogenea gill parasites and their fish hosts are best predicted by phylogenetic relationship of the hosts, followed by geographic distribution. Environmental and biological attributes (*i.e.* salinity tolerance, climate, habitat,



and body size) had a lower explanation power. A similar pattern was recently found using parasite co-occurrence modeling for the distribution of helminth parasites on fish hosts (Strona & Lafferty 2012). Phylogenetic relatedness could also predict, for species in the same trophic level, the identity of the species with which they interact in a number of mutualistic networks (Rezende et al. 2007). All these findings reinforce the importance of including evolutionary history into mechanistic models of network formation and maintenance (Cattin et al. 2004) for both mutualistic and antagonistic interactions.

Although there is a general individualization between parasite genera occurring on different orders of fish, this cannot be considered an evidence for coespeciation, even if phylogenetic relationships among parasite genera were known (this is not the case in this study). There is no macroevolutionary pattern that, on its own, can distinguish between coespeciation, evolutionary arms race, and colonization scenarios (Brooks & McLennan 2002). For Monogeneoidea, most parasite lineages seem to be restricted to higher taxonomic levels, *i.e.* family or above, probably due to broad historical constraints acting at large scale (Boeger & Kritsky 1997; Desdevises et al. 2002).

In fact, each fish order has a unique past history in the Neotropics. In the case of Perciformes, Cichlidae and Sciaenidae originated from two different events of freshwater colonization. Neotropical Cichlidae (Cichlinae) are a monophyletic clade that is sister to an African cichlid clade (Sparks & Smith 2004). They have a long history of diversification that seems to date back to the Late Cretaceous (> 90 Mya, *e.g.* Lundberg, 2010; Kocher & Stepien 1997). In contrast, Sciaenidae includes mainly primary marine fishes. Freshwater sciaenids in South America are marine derivatives that secondarily colonized continental waters (Sasaki 1989; Casatti 2000). For example, phylogenetic hypotheses suggest that the origin of *Plagioscion* spp. and their parasites occurred after freshwater colonization around 20 Mya, via marine transgressions (Boeger & Kritsky 2003).

It is reasonable to think that cichlid and sciaenid fishes do not represent a similar resource for their parasites as they are distantly related within the phylogeny of the order, hence it is less likely they share parasites. However, most cichlids (14 of 17 species) are parasitized by species of *Sciadicleithrum* Kritsky, Thatcher and Boeger, 1989, and *Gussevia* Kohn and Paperna, 1964 is recorded from 5 cichlid species, spread across distantly related species within the family. Therefore, phylogeny explains only the difference between cichlid and sciaenid parasites. Parasite sharing among cichlids (17 out of 20 perciform species in this study) is best explained by host co-occurrences in river basins.

Siluriformes, the second order with more representatives in the database (31 out of 105 species), is a diverse clade with a worldwide, mostly freshwater distribution. Neotropical siluriforms do not compose a monophyletic group but include four monophyletic clades (Sullivan et al. 2006). Three of them are represented in our analysis and at least two species of each are parasitized by *Demidospermus* spp., the genus of Monogenoidea with the largest host range within the siluriforms. Even looking at the other genera, there are no clear phylogenetic boundaries for the distribution of siluriform parasites. Only geographic distribution and habitat preference of hosts could account for a fraction of parasite sharing. In this case, a more detailed investigation on the factors that underlie parasite sharing is needed.

Characiformes are one of the largest components of the freshwater fish fauna worldwide and are distributed across the New World and Africa (Nelson 2006). Neotropical characiforms form a monophyletic clade, grouped in 14 families (Oliveira et al. 2011). Their parasites displayed a variety of distribution patterns in host phylogeny, e.g. host species specificity, restriction to phylogenetically close related hosts, and distribution across host phylogeny. Even so, phylogeny had the greatest influence on parasite sharing, but environmental preferences and geographic distribution are also important factors to explain parasite sharing among characiforms.

Despite of the observed host order specificity by parasite genera, *Urocleidoidea* spp. occur in 7 species of 6 families (one Cyprinodontiformes, one Gymnotiformes, and four Characiformes families). These species are all tropical and most of them are intolerant of seawater, as most host species in this study. What differentiates them from others is their geographical distribution. With the exception of *Hoplias malabaricus* Bloch, 1794 that is a widespread species, *Urocleidoidea* host species addressed here are restricted to the region between Central America and Orinoco River basin. However this genus is also found in other river basins, like the Paraná River basin (Boeger, personal communication). This may be explained by Brooks & McLennan (2002) concept of 'faux specialists' and 'faux generalists'. Faux specialists are generalists that use a restricted amount of the resources they could use because of ecological factors and faux generalists are specialized on a resource that is phylogenetically widespread. In these cases, the host range of a parasite may be mostly influenced by contact rates, rather than phylogenetic proximity of host species. Although there is not enough information to reconstruct the history of *Urocleidoidea* species and their hosts, this can be seen as evidence that lineages of monogenoid gill parasites can use a diverse range of hosts. Once hosts coexist in Panama rivers, host shifts either by resource tracking or sloppy fitness space may have occurred throughout *Urocleidoidea* diversification in Panama.

Although there is a lack of information on parasite phylogenetic relationship, and on host-parasite interaction diversity and geographical distribution, our results match those found for different kinds of associations, using various methods. Broad historical constraints act at large scales, restricting parasites to host higher taxa, e.g. orders and families (Boeger & Kritsky 1997; Desdevises et al. 2002). Within host lineages that share a relatively recent evolutionary history, ecological aspects may play an important role, providing more opportunity for a host switch. This has also been reported both for monogenoid parasites that are able to exploit ecologically similar hosts (Desdevises et al. 2002) and for a diverse group

of primate parasites (Cooper et al. 2012) whose distribution is influenced by ecology and geographical distribution of hosts. Biological traits accounted for a small part of the variation in parasite sharing, however hosts body size could predict part of parasite distribution among hosts from the same family. Again, it agrees with previous findings for both mutualistic (Donatti et al. 2011) and antagonistic networks (Strona & Lafferty 2012).

Parasite host range in terms of host families is another important factor to understand parasite sharing. While phylogenetic relatedness of hosts is the best predictor of the distribution of parasites that occur in one host family, for generalist parasites within fish orders, geographic distribution plays a major role. In this case, host range seems to be more influenced by contact rates than by phylogenetic proximity of host species.

In the present study we analyzed emerging distribution patterns of host-parasite interactions. These patterns can then be used as groundwork for future studies searching for the specific mechanisms underlying parasite sharing and host switching within each subset of host-parasite interactions. Our results reinforce the importance of including evolutionary history into the study of ecological associations. Geographic distribution of hosts emerged as a key factor increasing the chance of colonization of phylogenetically unrelated hosts. Finally, host ecology and biology are important additional factors to understand host-parasite interactions.

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