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Patterns of interaction between Neotropical freshwater fishes and their gill Monogenoidea (Platyhelminthes)

Mariana P. Braga · Sabrina B. L. Araújo · Walter A. Boeger

Abstract Using network analysis, we looked for broad patterns of distribution of Monogenoidea gill parasites on Neotropical freshwater fishes within a host phylogenetic framework. We analyzed a database of Monogenoidea parasitizing fishes from Neotropical rivers, from 23 watersheds, based on species descriptions published until 2011. Host–parasite interactions were organized into five matrices grouping species at different taxonomic levels. The network of interactions between host families and parasite genera was significantly modular and revealed that each fish order has a unique composition of parasite genera. Hence, interactions between lower taxa were analyzed separately for the largest fish orders (Perciformes, Siluriformes, and Characiformes). Networks tended to be loosely connected and organized in modules. Despite the putative high host specificity of monogenoids, some have a wider host range that includes distantly related host species. Among the hosts, the clade composed by the piranhas (*Serrasalmus* spp. and related species, Serrasalmidae) stands out in terms of parasite richness per host species, resulting in a more connected network. The history of the lineages of each host order within Neotropical freshwaters seems to have a great influence on the extent of parasite sharing. The observed modularity was influenced by both spatial structure and phylogenetic relatedness of species. In average, 37 % of modules of networks between host species and parasite genera were associated with a particular river basin and 63 % of modules were associated with a host family. Hence, spatial structure determines the co-occurrence of host and parasite species, but their evolutionary history is the main factor defining which interactions are possible.

M. P. Braga (✉) · W. A. Boeger
Laboratório de Ecologia Molecular e Parasitologia Evolutiva, Setor de Ciências Biológicas, Departamento de Zoologia, Universidade Federal do Paraná, Caixa Postal 19073, CEP 81531-980 Curitiba, PR, Brazil
e-mail: mpiresbr@gmail.com

M. P. Braga · S. B. L. Araújo · W. A. Boeger
Programa de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná, Caixa Postal 19031, CEP 81531-980 Curitiba, PR, Brazil

Introduction

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 (Kearn 1994). Monogenoids are also known to be highly host specific, since most 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 can result also from other adaptive and non-adaptive processes, such as speciation by host switch (Boeger and Kritsky 1997). Although the number of hosts that a given parasite uses is not by itself an adequate measure of host specificity (Agosta et al. 2010; Poulin et al. 2011), the search for broad and unique patterns of host–parasite interactions can represent a good model to evaluate the relative influence of past history and recent ecological processes on the assembly rules shaping ecological networks.

Using network analysis, we looked for broad patterns of distribution of Monogenoidea gill parasites on Neotropical freshwater fishes within a host phylogenetic framework. Network analysis explores 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. This approach provides both a visual representation of complex ecological systems as well as a formal way to measure species and network properties (Poulin 2010). Although the indices that measure these properties only inform about network structural patterns (Junker et al. 2012), the underlying mechanisms can be assessed using independent sources of information.

Nestedness and modularity have emerged as consistent patterns found in bipartite networks of species interactions. In a nested network, host-specific parasites interact mainly with hosts with high parasite diversity, while generalist parasites are found on both specialist and generalist hosts (Bascompte et al. 2003). Nestedness is more commonly observed in mutualistic networks but has also been reported in antagonistic networks (e.g., hosts and parasites) (Vacher et al. 2008; Graham et al. 2009; Bellay et al. 2011; Lima et al. 2012). Pires and Guimarães (2012) suggested that antagonistic networks can display a nested structure when interactions have a low degree of intimacy. 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, such as spatial or temporal segregation (Vázquez et al. 2009) and divergent selection regimes or phylogenetic constraints (Cattin et al. 2004; Vacher et al. 2008). Here, we hypothesize that the high interaction intimacy and host specificity displayed by gills monogenoids favor high modularity.

Methods

A database of component communities of Monogenoidea parasitizing fishes from Neotropical rivers was compiled using species descriptions and records of occurrence published until 2011. We deliberately included only gill parasites for several reasons. First, 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), making it difficult to draw general conclusions from the investigated patterns. The resulting list, which included 451 interaction records (310 parasite species occurring in 141 host species), was used to compile several matrices of presence/absence of host–parasite interactions accounting for different taxonomic levels (see Electronic supplementary material).

Networks for the three largest fish orders (Perciformes, Siluriformes, and Characiformes) and their parasites were analyzed separately to evaluate patterns of interaction between host species and parasite genera and between host and parasite species. These orders do not share parasite genera. In the networks comprising parasite genera, we included only host species with known phylogenetic position. Additionally, the distribution of fish species on river basins was obtained from Fishbase (Froese and Pauly 2012) and Reis et al. (2003). Based on the geographic distribution of the Neotropical host species of Monogenoidea, we selected 23 river basins (i.e., Amazonas, Tocantins, Orinoco, Parnaiba, São Francisco, Brazil North Coast, Northeast Coast, Southeast Coast, South Coast, Uruguai, Iguaçú, La Plata, Paraná, Paraguay, Essequibo, Guyanas, Trinidad, Cauca, Atrato, Panama, Costa Rica, Nicaragua and Honduras, and Guatemala and Mexico).

In order to define interaction patterns, networks were drawn with *plotweb* function in the package *bipartite* (Dormann et al. 2009) of R 2.15.1 (R Core Team 2012) and Pajek (de Nooy et al. 2005). Nestedness analysis was performed with the software ANINHADO (Guimarães and 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 1,000 random matrices generated by Erdös-Rényi (ER) null model and CE null model ("null model 2" of Bascompte et al. 2003). In the first model, each interaction has a probability to occur equal to the connectance of the network (i.e., the proportion of realized links among all possible links). Random networks generated by this model have, on average, the same connectance of the original network. In the second model (CE), the probability of each link to occur is proportional to the number of interactions of both partner species, so it takes into account both host range and parasite richness per host species.

Modularity was analyzed with the software MODULAR (Marquitti et al. 2013) using Newman and Girvan's metric (Newman and Girvan 2004) modified for bipartite networks (Barber 2007). Modules (i.e., subgraphs whose nodes are more connected to one another than to the nodes outside the subgraph) were identified by the simulated annealing (SA) optimization method. For each network, we computed the modularity index M and its level of significance by comparing the recorded M value to that of 1,000 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 modified CE null model to impose 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 (GAM— Geography Aware Model, see Online resource 2 for null model details). We generated 1,000 networks using GAM, computed modularity for each network, and compared to the

modularity of the original network. In order to compare the modularity among networks, we calculated a standardized measure of modularity $M_{z\text{-}score}=(M-M_{\text{Rand}})/\text{SD}_{\text{Rand}}$, where M is the modularity of the given network and M_{Rand} and SD_{Rand} are mean and standard deviation of the modularity of randomizations (Trøjelsgaard and Olesen 2012) from GAM null model.

We used the program NETCARTO and the algorithm SA to classify nodes into ecological roles according to their within-module degree and participation coefficient among modules, as proposed by Guimerà and Amaral (2005). A species with few interactions is considered (1) a peripheral node if most of its interactions are within the module or (2) a non-hub connector if most of their few interactions are among modules. A species with many interactions is classified as (3) a provincial hub if most of its interactions are within the module or (4) a connector hub if it connects many modules (Guimerà and Amaral 2005).

Binomial distributions were used to test the associations of host families and river basins with particular modules. For each module of networks between host species and parasite genera, we estimated the probability of getting, by chance alone, an equal or higher number of species from the same family or river basin than that observed in the real network. The necessary parameters for this test are the number of successes, the number of trials, and the probability of success. We used, respectively, the number of species that belong to a particular category (fish family or a river basin) that also belongs to a particular module, the number of species in the module, and the proportion of species of the given family or river basin in the whole sample.

To build hosts phylogenetic trees, we followed Oliveira et al. (2011), for the relationship among species of Characiformes, and Hubert and Renno (2006) and Ortí et al. (2008), for the best resolution within Serrasalminae. For relationships among species of Siluriformes, we followed Sullivan et al. (2006), while Lundberg et al. (2011) provided phylogenetic relationships within Pimelodidae. Lopez-Fernandez et al. (2010) determined the relationship within Perciformes. Cladograms were drawn in Mesquite 2.75 (Maddison and Maddison 2011), by adding all host species from the host–parasite dataset with known phylogenetic position, and without allowing polytomies.

Results

Fish hosts were classified in seven orders. Three of these orders harbor species of two families of parasites and, the other four, only one family (Fig. S1). From the five parasite families, species of four of them have been reported from just one fish order (i.e., Diplectanidae, Monocotylidae, Microcotylidae, and Hexabothriidae). On the other hand,

species of Dactylogyridae occur in all orders except for Rajiformes. Increasing taxonomic resolution, it was noticed that Ancyrocephalinae *sensu* Kritsky and Boeger 1989 is the most widespread subordinate taxon, since it occurs in 23 of the 24 host families included in this study. The other seven subfamilies parasitize one to three host families. Likewise, host families harbor one to three 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). The exceptions are Gymnotiformes and Cyprinodontiformes, which share species of *Urocleidoides sensu stricto* Mizelle and Price 1964 (as defined by Kritsky et al. 1986) with some families of Characiformes, hence belonging to the same module. Most parasite genera are also restricted to one host family, although the parasite faunas of Siluriformes and Characiformes are not as taxonomically structured as in Perciformes. Among hosts, Serrasalmidae, Characidae, and Pimelodidae are the families with greater richness of parasite genera and are also species-rich clades. Among parasites, *U. sensu stricto* has the largest host range, parasitizing nine families of three orders. On the network between fish species and parasite genera, *U. sensu stricto* is a provincial hub since it has many interactions but most of them are within module.

Among perciform hosts, parasite genera are restricted to a single fish family but can occur in many host species (Fig. 2). According to both null models, modularity and nestedness of this network are significant (Table 1). Cichlidae is the most species-rich family of Perciformes (17 spp.), despite including only three parasite genera. *Tucunarella* Mendoza-Franco, Scholz, and Rozkosná 2010 is monospecific and occurs only in *Cichla monoculus* Spix and Agassiz 1931; but *Gussevia* Kohn and Paperna 1964 and *Sciadicleithrum* Kritsky, Thatcher, and Boeger 1989 are widespread within the Cichlidae. The latter is the only connector hub of this network. However, when considering the distribution of parasite species on hosts, most parasites are species specific (Fig. S3). Each species of *Gussevia* is known from one host species, but host species can harbor more than one species of this genus. Two out of 15 species of *Sciadicleithrum* are known from more than one host species, but all are restricted to phylogenetically close hosts.

Among Sciaenidae, 14 parasite species are known for the three host species included in the phylogeny (Fig. S3). 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. *P. squamosissimus* is the host with the highest parasite richness,

Fig. 1 Network of interactions between fish families and parasite genera. Fish families are grouped by orders. Color of nodes represents modules (on the online version)
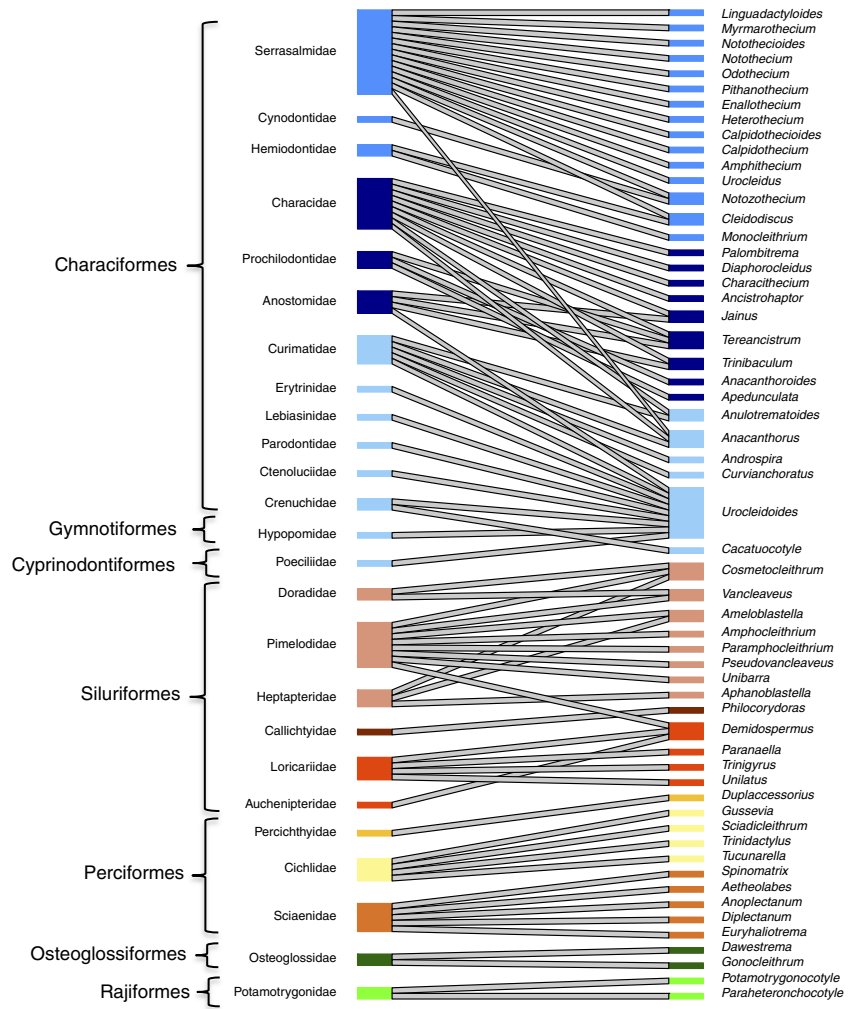
Fig. 2 Phylogenetic distribution of parasite genera on Perciformes hosts. The Perciformes phylogeny is on the *left* and parasite genera are on the *right*. *Links* indicate interaction and *node colors* represent modules (on the online version). *Numbers* indicate host families, *1* Sciaenidae and *2* Cichlidae. *Asterisks* indicate freshwater invasion events
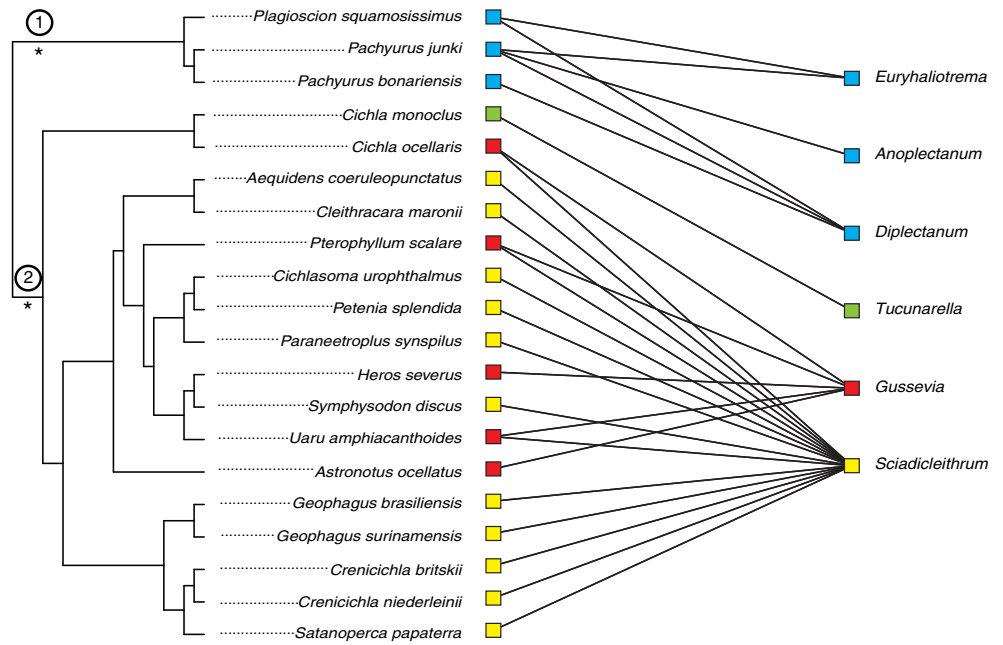
Table 1 Size (number of fish and parasites), nestedness, modularity of networks including host species and parasite genera, significance for each null model (p values are the proportion of networks generated by null models with equal or higher NODF or M values), and connectance (C)

	Network size		Nestedness			Modularity					C
	Fish	Parasite	**NODF**	ER (p)	CE (p)	M	$M_{z\text{-score}}$	ER (p)	CE (p)	GAM (p)	
Perciformes	20	6	22.24	0.53	0.7	0.54	0.03	0.23	0.35	0.48	0.216
Siluriformes	31	13	8.74	0.76	0.87	**0.74**	0.89	**0.05**	**0.02**	0.21	0.102
Characiformes	46	29	**18.37**	**0.0**	**0.0**	0.57	−0.65	0.22	0.07	0.80	0.085

Significant results are in bold

harboring four species of *Diplectanum* Diesing 1858 and six species of *Euryhaliotrema* Boeger and Kritsky 2003. 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).

The network of interactions among siluriform host species and parasite genera is not significantly nested, but it is more modular than the networks generated by ER and CE null models (Table 1), with hosts and parasites grouped in seven modules. This scenario is partially a consequence of spatial structuring, since the observed modularity is not significantly higher than that generated by GAM and two of the seven modules are associated with river basins. The dark blue module (Fig. 3) includes host species that predominantly occur at the Amazon River basin ($p < 0.05$, $n = 6$), and the pink module includes species mainly from Panama and Costa Rica ($p < 0.05$, $n = 6$). Parasite distribution within Siluriformes families is not as delimited as for Perciformes. Some parasite genera are restricted to one fish family (Fig. 3), but three occur in two to three families. *Vancleaveus* Kritsky et al. 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. *Vancleaveus* and *Demidospermus* are provincial hubs in this network, and all the remaining nodes are peripheral.

The network between species of Siluriformes and species of parasites is not significantly nested but is more modular than the networks generated by all null models (Table 2). As most parasites are restricted to one host—similar to those occurring on perciform fishes—and most hosts display a small number of parasite species, the network is highly compartmentalized. From the 65 species of monogenoids found on the gills of siluriform hosts, only 12 occur in more than one host species (Fig. S4). Of those, five occur only in congeneric species and six 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 monogenoidean parasites is the only one that is more nested than the expected by null models (Table 1). However, this network (Fig. 4) is not more modular than null models networks (Table 1). This network is roughly similar to the network of species of Siluriformes (Fig. 3), but one portion of the network of species of Characiformes, composed by the piranha clade (within Serrasalmidae) and their parasites, presents a distinct pattern of interaction. Parasite genera have a

Table 2 Size (number of fish and parasite), nestedness, modularity, number of modules (N), connectance (C), and mean number of interactions per species (k) of networks including host and parasite species

	Network size		Nestedness		Modularity			N	C	k
	Fish	Parasite	**NODF**		M	$M_{z\text{-score}}$				
Perciformes	26	51	1.55	$p_{\text{ER,CE}}=1.0$	**0.90**	2.10	$p=0.0^{a}$	20	0.044	1.52
Siluriformes	42	64	1.59	$p_{\text{ER,CE}}=1.0$	**0.90**	1.72	$p=0.0^{\ a}$	31	0.029	1.46
Characiformes	64	176	**3.78**	$p_{\text{ER}}=0.0$	0.76	1.25	$p_{\text{ER,CE}}=0.0$	24	0.026	2.46
				$p_{\text{CE}}=0.58$			$p_{\text{GAM}}=0.13$			

. Significant results are in bold (p values are the proportion of networks generated by null models with equal or higher NODF or M values)

[a] p value for ER, CE, and GAM

Fig. 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 (on the online version). *Numbers* indicate host families, *1* Pimelodidae, *2* Heptapteridae, *3* Auchenipteridae, *4* Doradidae, *5* Loricariidae, and *6* Callichthyidae. *Asterisks* indicate freshwater invasion events
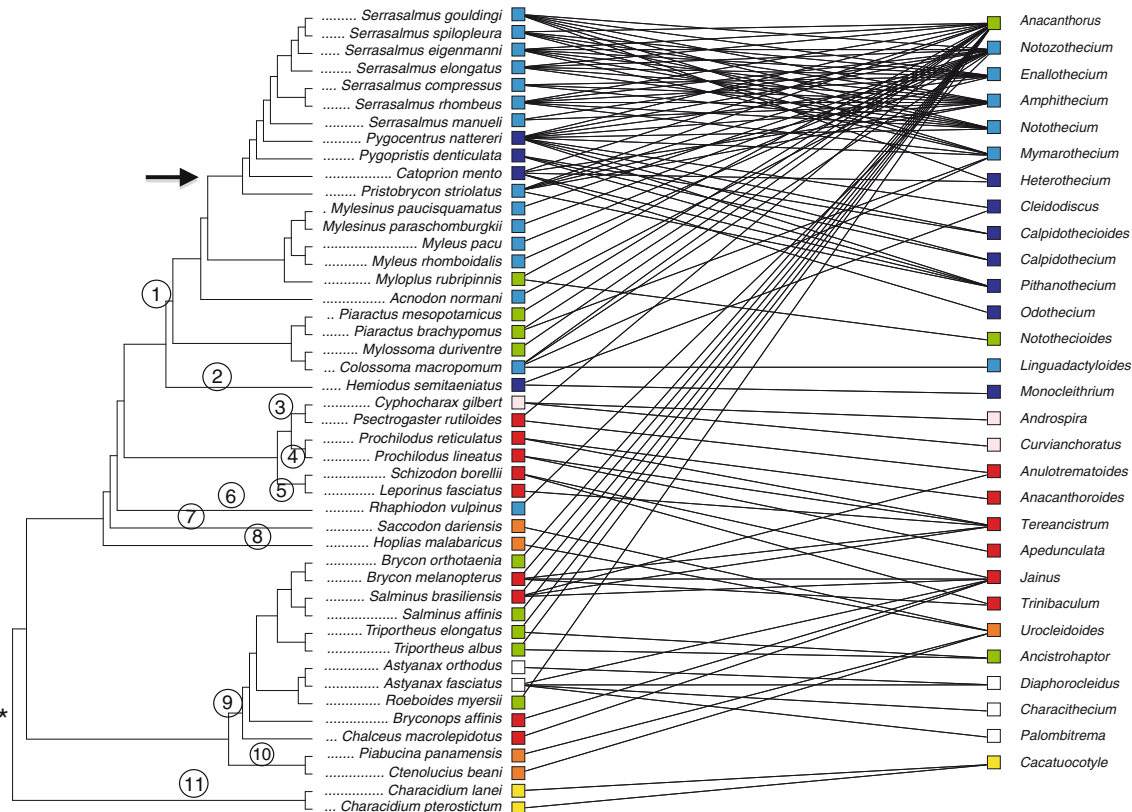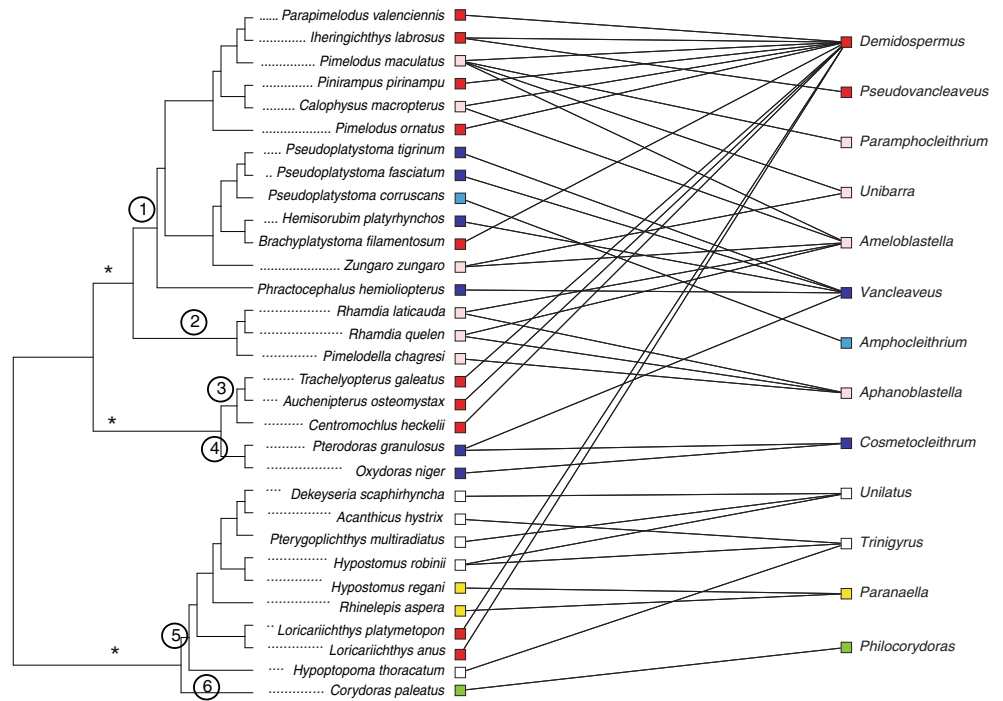

Fig. 4 Phylogenetic distribution of parasite genera on Characiformes hosts. The Characiformes phylogeny is on the *left* and parasite genera are on the *right*. *Links* indicate interaction and *node colors* represent modules (on the online version). *Numbers* indicate host families, *1* Serrasalmidae,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

larger host range and hosts harbor more parasites. This increased local connectance lowers modularity of the entire network, so this network is less modular than the network of Siluriformes (Table 1). The presence of the piranha clade also affects nestedness. When the clade is removed, the nestedness of the network is not greater than the expected by both null models (NODF=11.15, $p > 0.05$). Correspondingly, nestedness is not significant when only *Anacanthorus* and *Notozothecium* are removed (NODF=9.82, $p > 0.05$).

Interactions between Characiformes and parasite species (Fig. S5) also have a distinct structural pattern compared to the other fish orders. This network is more nested than expected by chance and more modular than expected by chance according to ER and CE null models. However, the network of Characiformes and their gill monogenoids is not more modular than the networks generated by GAM (Table 2); hence, observed modularity might be a consequence of spatial structure.

From the parasites that occur in Serrasalmidae hosts, 35 species of 8 genera interact with more than one host species. Most of them are restricted to species of *Serrasalmus* Lacépède 1803. 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-Serrasalmidae among Characiformes. Nine parasite species are known from 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. Similarly to what happened for the network at parasite generic level, no significant nestedness was detected when the piranhas clade was excluded from the analysis (NODF=2.32, $p > 0.05$).

The binomial distributions (Table S1) showed that, in average, 37 % of modules of networks between host species and parasite genera are associated with hosts that occur in a particular river basin, and that 63 % of modules are associated with host species that belong to a particular family ($p < 0.05$). These proportions vary between fish orders, but all three have more modules associated with host families than with river basins. Additionally, only three modules (16 %) are not associated with a host family or a river basin.

Discussion

Networks of interactions between gill Monogenoidea and their freshwater fish hosts tended to be loosely connected and organized in modules. The observed modularity seems to be mostly influenced by geographic distribution and phylogenetic relatedness of host species. Several studies has shown that, in general, nested host–parasite networks are often also significantly modular (Vacher et al. 2008; Graham et al. 2009; Bellay et al. 2011; Lima et al. 2012), especially at low

connectivities (Fortuna et al. 2010). However, networks including only ectoparasites or parasites with direct life cycle, such as the Monogenoidea, have been observed to be significantly modular and weakly nested (Lima et al. 2012), which is consistent with our results, as well as the idea that high interaction intimacy (i.e., high degree of biological integration among interacting individuals of different species) is associated with high specialization and high modularity at the species level (Pires and Guimarães 2012).

Phylogenetic relatedness of host species has a strong influence on the distribution of Monogenoidea parasites, as reported for other organisms (Rezende et al. 2007; Cattin et al. 2004; Cooper et al. 2012; Lima et al. 2012). We found that most fish orders have a unique composition of parasite genera (Fig. 1). These phylogenetic restrictions can also be found at the family level, such as those observed for Perciformes, and the association between modules and fish families highlighted by binomial distribution test. Previous studies suggest that broad historical constraints act at large scales, restricting parasites to host higher taxa, e.g., orders and families (Boeger and Kritsky 1997; Desdevises et al. 2002a). While the probability of occurrence of an interaction depends on the co-occurrence of partner species in a given locality in ecological time, evolutionary history might limit the range of possible interactions (Poulin 2010) depending on the degree of divergence of host features related to resource use by the parasite (see Agosta et al. 2010).

Each fish order (and some families) apparently represents a different kind of resource for monogenoidean parasites and this may influence the structure of networks between host species and parasite genera, as well as those between host and parasite species. These differences in resource quality likely reflect the unique history of each host lineage in the neotropics. For instance, members of a same order (Perciformes), Neotropical Cichlidae and Sciaenidae clearly originated from two different events of freshwater colonization, dating back to around 90 and 20 Mya, respectively (Lundberg et al. 2010; Kocher and Stepien 1997; Boeger and Kritsky 2003). While it is clear that the Sciaenidae did not lose their marine monogenoids during the freshwater colonization (see Boeger and Kritsky 2003; Fehlauer and Boeger 2005; Boeger et al. 2006), the origin of the symbiosis between monogenoids and Cichlidae is unclear (see Pariselle et al. 2011). In turn, Siluriformes is a diverse clade with a worldwide, mostly freshwater distribution. As well as the Neotropical Perciformes, the Neotropical Siluriformes do not represent a monophyletic assemblage but include descendants of four independent monophyletic clades (Sullivan et al. 2006), three of which were investigated in this study (Fig. 3). The origin of their monogenoid fauna is unclear but likely reflects the differences of the fish lineages, as in Perciformes. Indeed, monogenoid clades unique to the Loricariidae and Callichthyidae (e.g., *Trinigyrus* and

Phylocorydoras, respectively) suggest that the monogenoid fauna of the remaining lineages may share an older history with their hosts with recent events of host switching—such as those apparent between the recognized lineages of Siluriformes (Fig. 3; see *Vancleaveus* and *Demidospermus*). The Neotropical Characiformes, on the other hand, represent a monophyletic clade with an older history of diversification in the neotropics (Oliveira et al. 2011), and it appears that at least part of its Monogenoidea fauna is older than the Gondwana, as suggested by putative sister-group relationship between African and South American genera (e.g., *Annulotrema* and *Annulotrematoides* (Kritsky and Boeger 1995) and *Characidotrema* and *Jainus* (Kritsky et al. 1986)).

Besides phylogenetic restrictions, geographic structure can also influence the distribution of gill monogenoids on their fish hosts. This is best evidenced by the networks between Siluriformes and monogenoid genera and between Characiformes and monogenoid species. The modularity of these networks is significantly higher than those indicated by ER and CE null models, but it is not higher than that indicated by GAM, which suggests that the spatial structure of interactions on river basins influences the observed modularity. Additionally, 37 % of all modules between hosts and parasite genera are associated with host species that occur in a particular river basin (Table S1).

Despite the high host specificity generally associated to species of Monogenoidea, our results indicate that some genera present an unexpected wide host range. Two particularly unique patterns of host distribution were observed among parasite genera. First, within each fish order, few genera present a widespread distribution among host species, i.e., are network hubs (*Sciadicleithrum* on Perciformes, *Demidospermus* on Siluriformes, and *Anacanthorus* on Characiformes). Second, one genus has species on hosts of more than one fish order. This is the case of *U. sensu stricto*, with species that interact with host species from six families distributed among three orders (one family of Cyprinodontiformes, one of Gymnotiformes, and four of Characiformes).

At the species level, all but one parasite species was reported from hosts of the same fish family. *D. uncusvalidus* occurs in two distantly related species of Siluriformes (Auchenipteridae and Pimelodidae). Confirmation of the identity of this monogenoid, however, is fundamental to determine if this is an exceptional host distribution or if it represents a case of improper species (host or parasite) determination. 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 (Agosta et al. 2010), but it may also simply be an artifact due to problems in taxonomic resolution.

An important issue is whether patterns of interaction of the studied host–parasite networks could be affected by unequal sampling. Three main issues originate from the fact that 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 monogenoid species descriptions, we could not use sampling effort as a correction factor. Second, the species geographic distribution is biased by the heterogeneity in sampling effort. Much of the diversity of Monogenoidea is known from the Amazon watershed, not only because it is a species rich region but also because the parasite sampling effort was strongly concentrated in this region of the Neotropics. Third, host specificity of Neotropical Monogenoidea may be overestimated since most parasite species are known only from their original descriptions. Even though, host specificity may be an intrinsic trait of monogenoidean biology. The combination of monoxenic life cycle and the capacity of individual parasites to colonize new hosts may have promoted speciation by host switching and adaptive radiations in some Monogenoidea lineages (Brooks and McLennan 1993). Thus, the host specificity displayed by species of Monogenoidea may be the outcome of host shifts followed by adaptation to new conditions (Desdevises et al. 2002b; Bueno-Silva et al. 2011).

From the host perspective, the piranha clade (within Serrasalmidae) stands out in terms of parasite richness per host species. These hosts not only harbor many parasite genera but also tend to share their parasites with confamiliar species, so that their monogenoids have a wider host range than observed in other Neotropical host groups. Of the 13 genera of Monogenoidea occurring on piranhas, seven have species that parasitize four or more host species (Fig. 4). For parasite species, 32 out of 77 species occur in 2–10 host species within the piranha clade. While the higher richness of Monogenoidea in piranhas is already recognized (Boeger and Vianna 2006), the underlying mechanisms and its influence on the network structure are now being investigated (M.P. Braga et al., in preparation). Interactions between Characiformes and their parasites have a distinct structural pattern compared to the other investigated fish orders. Networks at both genera and species level show a significant nested pattern, that seems to result from (a) the presence of "generalist" hosts and parasites (the piranha clade mainly), (b) high connectance between "generalists," and (c) the presence of parasites such as *Anacanthorus* and *Notozothecium* that occur in both generalist and specialist hosts (only at genera level).

Our results suggest that the distribution of Monogenoidea parasites on their fish hosts is strongly influenced by evolutionary history, both between and within fish orders. This is likely the result of a combination of processes such as cospeciation and ecological fitting (Janzen 1985). Besides

the influence of host traits on parasite distribution, there is a variation in the width of resource usage by parasites. Although most parasite genera are restricted to a particular host lineage, some have a wider host range that includes distantly related host species. Finally, the history of the lineages of each host order within Neotropical freshwaters also appears to determine the extent of parasite sharing. Hopefully, further understanding of the mechanisms underlying host range expansions and parasite diversity will be achieved as more parasite phylogenies become available.

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