Patterns of interactions of a large fish–parasite network in a tropical floodplain

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Summary

1. Describing and explaining the structure of species interaction networks is of paramount importance for community ecology. Yet much has to be learned about the mechanisms responsible for major patterns, such as nestedness and modularity in different kinds of systems, of which large and diverse networks are a still underrepresented and scarcely studied fraction.

2. We assembled information on fishes and their parasites living in a large floodplain of key ecological importance for freshwater ecosystems in the Paraná River basin in South America. The resulting fish–parasite network containing 72 and 324 species of fishes and parasites, respectively, was analysed to investigate the patterns of nestedness and modularity as related to fish and parasite features.

3. Nestedness was found in the entire network and among endoparasites, multiple-host life cycle parasites and native hosts, but not in networks of ectoparasites, single-host life cycle parasites and non-native fishes. All networks were significantly modular. Taxonomy was the major host's attribute influencing both nestedness and modularity: more closely related host species tended to be associated with more nested parasite compositions and had greater chance of belonging to the same network module. Nevertheless, host abundance had a positive relationship with nestedness when only native host species pairs of the same network module were considered for analysis.

4. These results highlight the importance of evolutionary history of hosts in linking patterns of nestedness and formation of modules in the network. They also show that functional attributes of parasites (i.e. parasitism mode and life cycle) and origin of host populations (i.e. natives versus non-natives) are crucial to define the relative contribution of these two network properties and their dependence on other ecological factors (e.g. host abundance), with potential implications for community dynamics and stability.

Key-words: ecological networks, fish–parasite interactions, modularity, nestedness, upper Paraná River floodplain

Introduction

Nestedness is a widespread pattern in ecological networks (Jordano, Bascompte & Olesen 2003; Vázquez & Aizen 2004; Bascompte & Jordano 2007), often characterized by the existence of a group of generalists, interacting mostly among themselves, and a set of specialists interacting preferentially with generalists and rarely among themselves (see Bascompte *et al.* 2003; Jordano, Bascompte & Olesen 2003). In a perfectly nested network, all interactions of specialists must be subsets of generalists interactions, and this arrangement can be caused by several processes, including abundance-driven interactions among species (Lewinsohn & Prado 2006), higher extinction rates of specialists that interact only among themselves (Ollerton 2006), and convergence or complementarity of traits among a set of related species (Guimarães *et al.* 2006; Rezende, Jordano & Bascompte 2007; Santa-maría & Rodríguez-Gironés 2007). In contrast, the main feature of a modular or compartmented structure is the existence of recognizable subsets (i.e. modules) of species

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interacting significantly more among themselves than with species from other subsets (Lewinsohn & Prado 2006). The presence of modules may be associated with the phylogenetic constraints and/or co-evolutionary dynamics (e.g. Red Queen Hypothesis) that determine interactions, which might lead to high specialization (Poulin 1995, 1997; Lewinsohn & Prado 2006).

In fact, there is a continuum between nested and modular structures that is generally attributed to the type of interaction (i.e. mutualistic versus antagonistic) and the degree of biological association between individuals of interacting species (i.e. 'interaction intimacy' – Ollerton 2006; Guimarães *et al.* 2007; Fontaine *et al.* 2011). Mutualistic networks tend to be more nested and less modular than antagonistic networks (Graham *et al.* 2009). On the other hand, networks characterized by less intimate interactions (e.g. nonsymbiotic mutualists or predator-prey) tend to be nested, while networks with high level of intimacy (e.g. symbiotic mutualists or host-parasite) tend to be modular because of high levels of physiological integration and physical or trophic dependence that lead to evolutionary specialization (Fontaine *et al.* 2011). Nevertheless, these distinctions between type and intimacy of interaction cannot explain differences in nestedness and modularity that may be observed between host–parasite networks or subnetworks. They are all antagonistic and characterized by high intimacy. So other functional distinctions should explain their structural differences. For instance, there is a major distinction between parasites with a single-host life cycle (SHLC) (i.e. those requiring only one host to complete the life cycle) and parasites with a multiple-host life cycle (MHLC) (i.e. those requiring at least two hosts). Evidence suggests that the second type has lower host specificity (Poulin 1998; Sasal, Desdevises & Morand 1998), and then a higher tendency for nestedness and lower tendency for modularity. Many MHLC parasites have fishes as intermediate host during the larval stage (Luque & Poulin 2004; Takemoto *et al.* 2009). Being flexible with respect to intermediate host species enhances the chances of getting into the final host, where sexual reproduction takes place (Combes, Bartoli & Théron 2002; Vickery & Poulin 2002; Luque & Poulin 2004). Differences in host specificity may also arise between endoparasites and ectoparasites, but mostly due to dispersal type. Endoparasites are generally ingested by their host in a passive manner, which may lead to low specificity. In contrast, ectoparasites generally infect their host through active dispersal (Pariselle *et al.* 2011), allowing them to be more specific in their host choices.

Host specificity should also depend on the match between the parasites' and hosts' traits, which in turn is mediated by host phylogeny. One could expect that closely related hosts are more likely to share parasites than distantly related hosts because of niche conservatism (Wiens & Graham 2005), and consequently, they have greater chances to present nested parasite compositions. The occurrence and degree of nestedness may depend further on a variety of ecological factors, among which species abundance has received special attention (Krishna *et al.* 2008; Araujo *et al.* 2010; Verdú &

Valiente-Banuet 2011). However, body size or biomass may be also important to nestedness, as they define the total amount of tissue that the individual and the population have available to infection. Thus, understanding the interplay among parasite lifestyle, host population features and taxonomy can provide insights on how host–parasite interactions are configured, and on the mechanisms driving the relative contributions of nestedness and modularity (Bascompte & Jordano 2007; Olesen *et al.* 2007; Graham *et al.* 2009).

In this study, we describe the fish-parasite interaction network in the Paraná River basin floodplain (Brazil), a tropical system of great ecological importance in South America (Hoeinghaus *et al.* 2009). Based on our knowledge of host and parasite biology, we aimed to evaluate the dependence of nestedness and modularity on: (i) host ecological traits, such as abundance, body size and biomass; (ii) host taxonomy (as a proxy for phylogeny), (iii) host origin (native versus non-native species) (iv) type of interactions between parasites and hosts (endoparasites versus ectoparasites) and parasite life cycle (SHLC versus MHLC). We expect endoparasites and MHLC parasites to have a more nested and less modular pattern of interactions because of their lower host specificity. With respect to the ecological traits of host populations, we predict that the parasite fauna of fish species with lower abundance, lower biomass and/or smaller body size should be nested within the parasite fauna of more abundant and larger species, which have a larger amount of available tissue and may serve as an easier target for parasite infection, resulting in increased vulnerability (defined here as the number of parasite species per host, Schoener 1989). We also expect that closely related hosts have higher chance to belong to the same network module and have a more nested parasite composition than distantly related hosts, as nestedness depends positively on parasite sharing (Almeida-Neto *et al.* 2008). Recently, the Paraná River floodplain has experienced several introductions of non-native fish species (Júlio *et al.* 2009). The potential of these species to disrupt interaction patterns is investigated here by evaluating nestedness, modularity and host influences separately for native and non-native fishes. Non-natives should have fewer parasites (Torchin *et al.* 2003) with little overlap among themselves and with native hosts because of their different evolutionary origins, generating as a consequence a highly modular and non-nested network structure.

Materials and methods

STUDY AREA

The Upper Paraná River basin has an area of 880 000 km^2, equivalent to 10% of Brazilian territory (Agostinho *et al.* 2007). This region is severely impacted by dams (Agostinho *et al.* 2004; Agostinho, Pelicice & Gomes 2008) and the longest stretch of river free of dams (230 km), representing less than half the extension of the original floodplain habitat and where this study was conducted, is situated between the Porto Primavera Dam and Itaipu Reservoir (22°00′ and 23°30′S, 53°00′ and 53°30′W; Agostinho *et al.* 2004; Fig. 1). Because this region is the last area with flowing water in the Paraná River, this
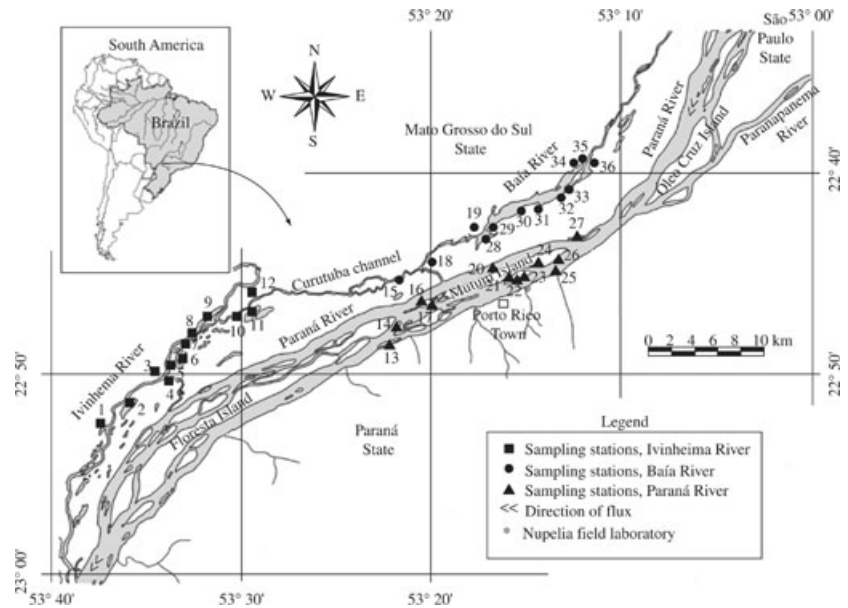
Fig 1. Upper Paraná River floodplain and the locations of sampling sites from 2000 to 2008.

floodplain is of extreme ecological importance because it maintains the ecological processes and environmental characteristics of the region and can be considered the area most similar to the previous, pristine conditions of this habitat before damming. Further information about this system can be found in Thomaz, Agostinho & Hahn (2004).

FISH AND PARASITE SAMPLING

Fish species were collected quarterly from February 2000 to September 2008 as part of a Long-Term Ecological Research Programme (PELD – SITE 6). We sampled the main aquatic environments of the Upper Paraná River floodplain, including rivers (main and secondary channels), shallow lakes and temporary pools to maximize the number of local habitats sampled, comprising 36 sampling sites (Fig. 1). We used both passive (long lines and gillnets) and active (seine nets) fishing gears. Most of the individuals were caught by gillnets (11 nets; mesh size ranging from 2 to 16 cm opposite knots), which were exposed for a period of 24 h and inspected during three periods (morning, afternoon and night). In still water (shallow and temporary pools), we used one set of 20 m long gillnets, but in lotic environments (channels), we used two sets of 10 m long nets to standardize effort. The long lines with live bait, deployed only in rivers (deeper habitats), were left out overnight and checked the next morning. The 20 m long seine nets with 0·5-cm mesh were used in floodplain lakes during two periods (morning and night). All specimens were measured for standard length (mm) and weight (g). Fish abundance and biomass were expressed as the mean catch per unit effort (CPUE; number of individuals or biomass per 1000 m^2 of gill nets in 24 h) over the period 2000–2007. This period encompassed major yearly changes in fish assemblages, with maximum dissimilarity observed between years 2000 and 2002 (Bray Curtis dissimilarity index = 94% for or CPUE based on fish biomasses). The average dissimilarity between years was 58%, which highlights the importance of including several years to have a good representation of the regional fish assemblage. However, there was no temporal trend of compositional change, as the faunal dissimilarity was not correlated with time difference (Mantel r = 0·07, P = 0·36). Records of

maximum total lengths of fish species were compiled from a study by Graça & Pavanelli (2007). In addition, the definition of species origin (native or non-native) was compiled from Júlio *et al.* (2009). The data on cumulative CPUE, body size and identification of fish species are available in the Supporting Information.

A total of 4875 individuals of 72 fish species were haphazardly selected for parasite analysis, trying to include a broad range of body sizes. More abundant species had more individuals analysed (Spearman's rank correlation between sample size and Catch per Unit of Effort = 0·74; P < 0·001). After taxonomic identification, the fishes were analysed using routine methods of parasitological studies (Eiras, Takemoto & Pavanelli 2002; Eiras *et al.* 2008 for details). Most fishes were analysed in the Field Base of Research in Limnology, Ichthyology and Aquaculture of NUPELIA (Fig. 1). Details about parasitological methods in this study are available in the Supporting Information. Only metazoan parasites were included and were identified through morphological characteristics. A total of 324 taxa were reported, 140 of which were identified to the species level, 120 to the genus level and 64 to the family level. Most of the data used in this study come from a parasitological survey carried out by Takemoto *et al.* (2009) and other basic information on these data (e.g. sample sizes and taxonomic identification) is reported there. The list of parasite taxa and their generality (number of host species) is available in the supporting information.

NETWORK BUILDING

The fish–parasite network was defined as a binary (0,1) matrix (**M**), with rows representing hosts/fishes and columns representing parasites. This matrix, together with species names and characteristics, is available in the supporting information. We also analysed six submatrices each containing a given type of parasite (ectoparasite, endoparasite, SHLC or MHLC) or a given type of host (native or non-native) (Fig. 2). For all matrices, we calculated the connectance C, consisting in the proportion of realized interactions with respect to the total possible, where the total possible was calculated as the product between the number of host species and the number of parasite species (Fortuna *et al.* 2010).
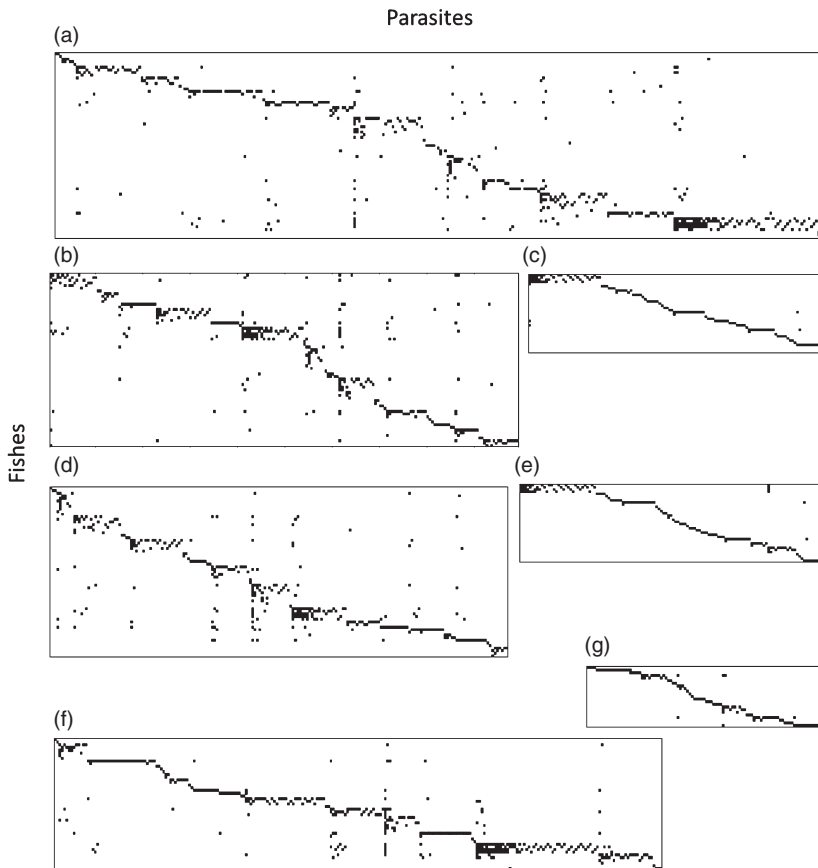
Parasites

Fig 2. Matrices of interactions between fishes (rows) and parasites (columns). The occurrence of an interaction is marked as a dot. Hosts and parasites were ordered to show the modular structure. (a) entire network composed by 72 fish species and 324 parasites; (b) subnetwork containing only endoparasites and their interacting hosts; (c) subnetwork containing only ectoparasites; (d) subnetwork containing only parasites with multiple-host life cycles; (e) subnetwork containing only parasites with single-host life cycles; (f) subnetwork containing only native hosts; (g) subnetwork containing only non-native hosts.

STATISTICAL ANALYSIS

Nestedness investigation

The degree of nestedness was evaluated using the Nestedness metric based on Overlap and Decreasing Fill (NODF) (Almeida-Neto *et al.* 2008), which has the advantage of allowing the calculation of nestedness for each species pair. This is essential for our objective of relating nestedness with host attributes, which requires pairwise comparisons among species. The NODF metric is a type of similarity index, but one which is dependent on the ordering of species. This ordering can reflect any factor hypothesized to contribute to nestedness. For instance, if abundance differences among host species are hypothesized to positively affect nestedness among them, hosts (rows) must be ordered from the most to the least abundant species. However, this procedure does not allow accounting for multiple factors. As we are interested here in the influence of multiple factors, we ordered species according to their number of interactions and related nestedness to ecological or biological factors in a subsequent analysis (see 'Influences of host attributes' below and the Supporting Information for details). To test the significance of nestedness in the entire matrix and in the subnetworks of different parasite and host types, we generated 999 random matrices in each case by reallocating interactions among cells, with allocation probabilities proportional to marginal totals according to the probabilistic model of Bascompte *et al.* (2003) and Guimarães & Guimarães (2006). The calculation of the observed NODF and null model simulations were all carried out using *Aninhado* software (Guimarães & Guimarães 2006).

Modularity investigation

Modularity (M) was evaluated using *Netcarto* software (Guimerà & Amaral 2005). This index assumes values between 0, when interactions are randomly distributed without forming clear modules (i.e. all species are in the same large cluster), and 1, when there is strong delimitation among modules and all interactions occur between species of the same module. For each network, the statistical significance of M was evaluated with a null model based on 1000 random networks. We also calculated the average of null model values (M_{null}), as proposed by Olesen *et al.* (2007).

Influences of host attributes

We tested the influence of host phylogeny, abundance, body size and biomass on nestedness and modularity by means of multiple regressions on distance matrices (Legendre & Legendre 1998). To control for sampling effort, the number of fish individuals analysed for parasites was included as a covariate. For nestedness, we used the matrix **N** (containing the nestedness degree for each pair of hosts or N_{paired} – see Fig. 1 of Almeida-Neto *et al.* 2008) as a response matrix. Taxonomic distances were used here as a proxy for phylogeny, as in previous studies (Bersier & Kehrli 2008; Krasnov *et al.* 2010). The distance matrices for abundance, body size, biomass and sampling effort were calculated by subtracting the value of the host species with fewer interactions from that of species with more interactions, because of our a priori expectation that the parasite composition of hosts with lower abundance, body size, biomass and/or sampling effort should be nested within the parasite composition of hosts with higher values for these variables. See the Supporting Information for

details on the calculation of distance matrices and the multiple regression for nestedness. A similar approach was used to test for influences of the explanatory variables on network modularity. In this case, the response variable was a matrix **C** with elements $C_{ij} = 1$ if host species i and j belong to the same network module and $C_{ij} = 0$ otherwise. As it is a binary variable, we applied logistic regression. The explanatory matrices of abundance, biomass, body length and sampling effort distances were calculated by the absolute differences, as in this case the species ordering is not important to define the modules as it is for nestedness.

The existence of modules could confound the effects of explanatory variables on nestedness, for the following reason. The expected effect of abundance, biomass or body size on nestedness lies on the hypothesis that more abundant and/or larger host species provide easier targets for infection, so having a parasite composition which contains the composition found in less abundant/smaller species. But this 'target' effect cannot be evaluated if the two hosts are from different modules and support completely different parasite assemblages, which may arise due to other reasons such as phylogenetic divergence. So we repeated the analysis for nestedness, but excluding host species pairs belonging to different modules. Another potential source of bias is the presence of non-native species, which may not follow the same patterns as native species. To account for this, we also excluded non-native hosts from the previous analysis and performed an additional regression with only native species pairs belonging to the same module.

Results

Overall network connectance was 2·18%, and significant nestedness and modularity were detected (Table 1). The subnetworks had contrasting patterns. Networks containing endoparasites or MHLC parasites were significantly nested, while those with ectoparasites or SHLC parasites were not (Table 1). The ectoparasite network was in fact less nested than expected by chance ($P > 0.98$, indicating that less than 2% of the NODFs in null distribution was lower than the observed NODF). Results from native and non-native hosts also differed: native fishes' network was significantly nested, while non-natives' network was less nested than expected by chance (Table 1). The average vulnerability of native hosts was higher than that of non-natives, 8·14 against 4·83 interactions, respectively, and the variance was also greater for natives: 89·08 against 15·24 for non-natives. Natives and non-natives shared only 32 of 324 parasite species. This was significantly lower than expected by chance ($P = 0.028$)

from a permutation test where the order of species origin was randomly assigned in the interaction matrix.

All networks were significantly modular (Table 1). Figure 2 illustrates the interaction matrices with species ordered to show the modular structure (a similar figure, with species ordered by decreasing number of interactions can be find in the Supporting Information). The entire network contained 32 modules. The subnetworks with ectoparasites and SHLC parasites had 31 and 29 modules, respectively, whereas the subnetworks with endoparasites and MHLC parasites had 26 modules, even having many more species (Fig. 2). The non-native and native fish networks had 18 and 26 modules, respectively, although the number of hosts per module is lower for non-natives (1·28) than for natives (1·88).

Taxonomic distance was the only predictor significantly related to nestedness and modularity of the entire network (Table 2). There was a general decrease in both the average nestedness and the chance of belonging to the same module as the taxonomic distance between hosts increases (Fig. 3). This relationship was especially strong at the genus level when compared to other degrees of relatedness. However, the taxonomic influences remained significant even after excluding host pairs of the same genus from analysis ($P = 0.008$ for nestedness and $P = 0.016$ for modularity), indicating that the overall phylogenetic signal persists at higher taxonomic levels. An exception is the effect of taxonomic distance 5, which shows the opposite effect, mainly on the probability of belonging to the same module (Fig. 3b). It represents host pairs of different classes, owing to the occurrence of two ray species: *Potamotrygon falkneri* and *Potamotrygon motoro*. The reason for this possible convergence in parasite fauna is still unknown. Considering only host species pairs of the same module, no relationship of any explanatory variable with nestedness could be detected (Table 2). After excluding non-native species, nestedness was positively and significantly correlated with abundance (Table 2).

The results of regressions on each subnetwork indicated that there is a general confirmation for the negative relationship between taxonomic distance and both nestedness and the chance of belonging to the same module (Supporting Information). The relationship between abundance and nestedness remained significant only for endoparasites and MHLC parasites (besides for natives, whose result is already

Table 1. Statistics of connectance (C), nestedness (Nestedness metric based on Overlap and Decreasing Fill (NODF)), mean nestedness from null model simulations ($NODF_{null}$), probability of $NODF_{null} > NODF$ (p_{NODF}), index of modularity (M), mean modularity from null model simulations (M_{null}) and probability of $M_{null} > M$ (p_M)

Networks	C (%)	NODF	$NODF_{null}$	p_{NODF}	M	M_{null}	p_M
Whole	2·18	4·12	3·24	< 0·01	0·73	0·66	< 0·01
Endoparasites	2·72	4·80	3·90	< 0·01	0·70	0·64	< 0·01
Ectoparasites	3·13	2·91	4·03	0·98	0·84	0·77	< 0·01
Single-host life cycle	3·24	3·18	4·17	0·97	0·83	0·76	< 0·01
Multiple-host life cycle	2·74	4·78	3·95	< 0·01	0·69	0·63	< 0·01
Native fish	3·14	5·72	3·51	0·01	0·70	0·64	< 0·01
Non-native fish	4·92	2·60	5·70	> 0·99	0·84	0·76	< 0·01

Table 2. Results from multiple regression on distance matrices

Response variable	Effect	Coefficient	P
Nestedness	Abundance	−0·39	0·361
	Biomass	−0·28	0·502
	Body length	−0·43	0·209
	Sampling effort	0·14	0·651
	Taxonomic distance	−1·48	< 0·001
Modularity	Abundance	0·14	0·461
	Biomass	−0·23	0·202
	Body length	0·13	0·304
	Sampling effort	−0·01	0·968
	Taxonomic distance	−0·43	0·002
Nestedness (same module)	Abundance	2·19	0·605
	Biomass	−0·31	0·941
	Body length	−1·95	0·561
	Sampling effort	2·76	0·462
	Taxonomic distance	−3·77	0·257
Nestedness (natives in the same module)	Abundance	10·43	0·045
	Biomass	2·38	0·651
	Body length	−4·65	0·324
	Sampling effort	−2·88	0·631
	Taxonomic distance	1·46	0·767

'Nestedness (same module)' means that only host species pairs belonging to the same module were included for analysis; 'Nestedness (natives in same module)' means that only native host species pairs belonging to the same module were included. 'Modularity' in this context means the chance of a host pair belonging to the same network module. Shown are the standardized partial regression coefficients and the associated *P*-values.

presented in Table 2). For the network of ectoparasites, taxonomic distance was positively related to nestedness of hosts belonging to the same module. Nevertheless, this result in particular is sensitive to the exclusion of sample size as a covariate, after which the taxonomic effect looses its significance. For all other analyses, including or excluding sample size does not change qualitatively the conclusions (results not shown).

Discussion

It has been shown elsewhere that nestedness decreases resilience and persistence of food webs, whereas modularity has an opposite effect (Thébault & Fontaine 2010; Stouffer & Bascompte 2011). Several studies with host-parasite networks have detected a nested pattern (González & Poulin 2005; Vázquez et al. 2005, 2007; Graham et al. 2009), and our study is not an exception. A possible explanation is related to network connectance and its effect on the mixed occurrence of nestedness and modules. Networks with low connectance tend to have positive relationships between nestedness and modularity (Fortuna et al. 2010). The connectance found in the present study (2%) is lower than all host–parasite networks investigated by Fortuna et al. (2010), so the simultaneous occurrence of nestedness and modules follows the theoretical expectation.

We found that closely related hosts have both greater chances of having nested parasite compositions and of
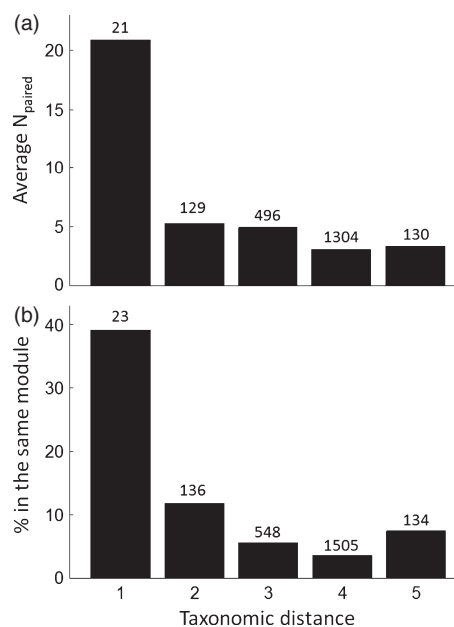

Fig 3. Influence of taxonomic distance between host species on (a) average nestedness between species pairs (N_{paired}); (b) percentage of species pairs belonging to the same network module. Presented above the bars are the numbers of host pairs associated with each taxonomic distance and used for the regression analyses.

belonging to the same network module. This probably comes from the fact that they tend to have similar niches, behaviours and morphology (Wiens & Graham 2005), which, in turn, increases their chance of sharing parasites (Krasnov et al. 2010; Poulin 2010). Sharing parasites is a precondition for any pair of species to show nestedness and also to belong to the same module. It explains why these two features have almost the same pattern of dependence on taxonomic distances (Fig. 3). A corollary is that nestedness is expected to occur more within than between modules, as previously noted by Graham et al. (2009). We can also say that the major effect of taxonomic distance was to define network modules, as its effect on nestedness was no longer significant after excluding species pairs from different modules (i.e. nestedness within modules was not explained by taxonomy). One interesting consequence from this scenario is that the relative contribution of nestedness and modularity for the network structure might be mediated by the phylogenetic diversity of host assemblage. Extrapolating to multiple assemblages of hosts, we expect those with higher phylogenetic diversity to have higher degree of modularity in parasite composition, with larger number of modules, fewer species per module and a lower overall nestedness. In the Paraná River basin, the proportion of species of the same genus seems to be the most important type of phylogenetic redundancy to define the relative importance of nestedness versus modularity. Comparing networks from systems with different host diversities is a mean to test these predictions, which will be feasible in a near future given the increasing amount of available data.

Parasites' biological features are also important for defining interactions, as shown here in the separate analyses of

networks according to parasite type and life cycle. It should be noted that the two classification schemes are strongly related in our data set: most endoparasites are also MHLC parasites, and most ectoparasites are SHLC parasites. Only five parasite species are exceptions to this pattern (Supporting Information). The ectoparasites and SHLC parasites are mainly monogeans, which are known for their relatively high host specificity (Lambert & El Gharbi 1995; Cribb, Chisholm & Bray 2002). This is reinforced by their lower nestedness values and larger number of modules, which presumably result from high levels of specialization (Joppa *et al.* 2010). In contrast, endoparasites and MHLC parasites are mainly nematodes and digeneans, both showing low host specificity (McDonald & Margolis 1995; Poulin 2010), leading to fewer modules and a larger richness of parasites per module. These parasites are generalists at the stage level, as most of them use fish as intermediate host during their life cycle. This generalism is probably important for the parasite to increase its likelihood of infecting the final host and completing the life cycle. Besides, it may have community wide consequences, as stage-structured networks tend to be more stable when consumers or parasites are stage-generalists, as opposed to parasites which appear as generalists at the species level because of ontogenetic host shifts, but are in fact specialists at the stage level (Rudolf & Lafferty 2011).

Another important functional distinction is between native and non-native host species. For native hosts, the distribution of parasite interactions among closely related species seems to be controlled by differences in abundances, as expected in theory (Krishna *et al.* *2008*; Araujo *et al.* 2010) and observed in other empirical studies (Vázquez *et al.* 2005, 2007; Verdú & Valiente-Banuet 2011). Nevertheless the inclusion of non-native species disrupts this pattern, and no signal of abundance effect was detected. The non-native species have a much lower heterogeneity in vulnerability, which is associated with lower nestedness (Fortuna *et al.* 2010; Table 1), but greater heterogeneity in abundance, and this mismatch could explain the disruption of the pattern when they were included in the abundance-nestedness analysis of the whole network. Some non-native species stand among the most abundant in our study system (Supporting Information), yet their numbers of interactions are not large when compared with natives. These two patterns may be even related, as the relatively lower richness of parasites infecting non-native fishes can represent a case of enemy release, which is a potential explanation to their success in the region (Torchin *et al.* 2003; Júlio *et al.* 2009).

Our results are in line with those of Torchin *et al.* (2003), who reported a conspicuous reduction in the parasitization of introduced species and attributed that pattern to a variety of reasons, 'including reduced probability of the introduction of parasites with exotic species (or early extinction after host establishment), absence of other required hosts in the new location and the host-specific limitations of native parasites adapting to new hosts'. One question that may be addressed by additional studies is whether the distinct parasite composition of non-native species is because of actual functional dif-

ferences with natives (e.g. different trophic behaviour or physiology) or whether they reflect transient dynamics (i.e. the parasite assemblage has not have time to respond ecologically or evolutionarily to the recent community changes imposed by introduced species). Most non-native species were introduced in the floodplain after 1982, when the impoundment to form Itaipu reservoir allowed a fraction of downstream fish fauna to expand its range to the upstream floodplain by the removal of a natural barrier (Júlio *et al.* 2009). Given the prior geographic proximity of downstream and upstream fish fauna, the small overlap in parasite composition between natives and non-natives is remarkable, suggesting that the previous barrier was effective at preventing the dispersal of both fishes and parasite species. It adds to the literature on the subject, which also shows a pattern of low vulnerability in non-native hosts, but which relies mostly on instances where non-natives come from disjunct and geographically distant communities (Torchin *et al.* 2003). Although there is scarce information about the origin of parasites, the low proportion of parasites shared by native and non-native fishes is a strong indication that, while a small set of non-native parasites may have been introduced with fish propagules, most native parasites were not still able to infect non-native hosts. Current evidence on temporal dynamics shows the opposite trend: a parasite (*Austrodiplostomum compactum*) originally observed in only one non-native host species (*Plagioscion squamosissimus*) has spread over native fishes as this host became abundant in the region (Yamada *et al.* 2008).

Finally, levels of nestedness and modularity were independent of host body size and biomass. The absence of a size relationship is consistent with previous studies (Takemoto *et al.* 2005; Poulin *et al.* 2011). Poulin *et al.* (2011) showed that body size is a week predictor of vulnerability (a factor contributing to nestedness) and suggest '(…) *it may be time to abandon it as a general explanation for interspecific variation in parasite diversity'* (page 746). Takemoto *et al.* (2005) using a similar data set for this same community, but analysing only endoparasites, concluded that abundance was the only factor explaining variation in vulnerability among fishes. It is interesting that biomass has not generated the same pattern observed here for abundance. It suggests that the processes of parasite infection are not so dependent on the total amount of available tissue, but rather by the number of discrete entities (individuals) comprising the populations. Also interesting is that the effect of abundance on nestedness was observed only for endoparasites and MHLC parasites, but not for ectoparasites and SHLC parasites. It suggests that differences in host abundance are less important when parasites are constrained to complete the life cycle in a single host and/or they are more able to actively infect a host, both situations leading to increasing specialization.

Conclusion

We found a strong phylogenetic signal in the studied host–parasite network, similarly to studies on mutualistic

networks (Rezende *et al.* 2007) and food webs (Bersier & Kehrli 2008). Besides serving as additional evidence for the importance of phylogeny, our study goes beyond by relating it with two widespread network properties, nestedness and modularity, which may have opposite effects on ecosystem dynamics and resilience (Thébault & Fontaine 2010; Stouffer & Bascompte 2011). The relative contribution of nestedness and modularity also depended on the composition of parasites with respect to parasitism mode and life cycle, as they are linked to the degree of host specificity. Finally, we showed that non-native species have the potential to disrupt patterns of interactions expected to occur in natural communities, such as abundance-driven nestedness among similar hosts. These findings argue in favour of a closer look at the internal structure of ecological networks. It may reveal hidden patterns not detectable by whole-network analyses, but which are assessable by taking into account functional differences among interacting species.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Data S1. Interaction matrix and species information.

Data S2. Regressions on distance matrices.

Data S3. Parasite sampling.

Data S4. Additional results.

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