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PHYLOGENY AND ECOLOGY ILLUMINATE THE EVOLUTION OF ASSOCIATIONS UNDER THE STOCKHOLM PARADIGM: *AGLAIOGYRODACTYLUS* SPP. (PLAYHELMINTHES, MONOGENOIDEA, GYRODACTYLIDAE) AND SPECIES OF LORICARIIDAE (ACTINOPTERYGII, SILURIFORMES)

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STOCKHOLM PARADIGM
HOST-PARASITE ASSOCIATION
ECOLOGICAL FITTING
GYRODACTYLIDAE
ECTOPARASITES

ABSTRACT. – We present a hypothesis of the history of the association of a clade of seven species of *Aglaiogyrodactylus* (a small clade of oviparous Gyrodactylidae), ectoparasites of loricariid catfishes. Ecological parameters (mainly abundance) of the associations were analyzed under a phylogenetic framework based on new and available data for hosts and parasites. Comprehensive sampling of fish and parasites was performed in a stretch of a stream on the coast of Paraná, Brazil (Marumbi River). Distribution of associations was not haphazard but no parasite species was collected only from a single host; correspondingly no ancestral-host reconstruction indicated the existence of a single host species for hypothetical parasite ancestor. Host range was strongly associated with ecological fitting and inheritance. The analysis could not identify any event of cospeciation but one event of historical oscillation in host range was revealed. Abundance was considered an adequate proxy for compatibility and suggest that cases of shift in this parasitological parameter are likely associated with divergence of the parasite following coadaptation to host species. One case of possible allopatric speciation was detected. The results are in accordance with the elements of the Stockholm Paradigm, which supports an alternative for studies of historical associations.

INTRODUCTION

During the last half century, traditional paradigms of coevolution led to paradoxes of dispersion, success, and evolution of interspecific ecological associations (Thompson 2005). The *Parasite Paradox* (Agosta *et al*. 2010) is based on a combination of ecological and phylogenetic observations. Parasites are putative ecological specialists and yet (a) specialists (small host range) and generalists (wide host range) do not occur equally, with specialists predominating while generalists are rare; (b) many emerging diseases occur more rapidly than expected by the chance origin of evolutionary novelties associated with host-use capabilities; and (c) phylogenetic comparisons routinely show high levels of host-switching in the (co) evolutionary diversification of inter-specific associations. The Stockholm paradigm (Brooks *et al*. 2014, Araujo *et al*. 2015, Hoberg & Brooks 2015) is a conceptual foundation that resolves the parasite paradox, explaining how specialists can host-switch easily and how generalists can evolve from specialists.

Empirical studies indicate that host range expands and contracts within clades on macroevolutionary time scales. This is in accordance with the oscillation hypothesis (Janz & Nylin 2008). This hypothesis indicates that not only do specialists evolve from generalists but new generalists evolve from older specialists. Thus, ecological specialization appears not to be an evolutionarily dead end and new specialists need not necessarily be recruited from novel generalist lineages following the extinction of older specialists.

The primary mechanism permitting the transition from specialist to generalist is ecological fitting (Janzen 1985, Brooks & McLennan 2002), which allows host-range expansion prior to the evolution of genetic information related to the associations with new hosts. Phylogenetic conservatism in host resource evolution, coupled with a widespread geographic distribution of species having those host resources produces geographic mosaics of uninfected, yet susceptible, hosts. The phenomena called "parasite spillover" (*i.e*. pathogen introduction by non-native host species; Cleaveland *et al*. 2002, Prenter *et al*. 2004) and "parasite spillback" (*i.e*. non-indigenous host is competent to native pathogens; Kelly *et al*. 2009) are good examples of ecological fitting. Even parasites with complex (multi-host) life cycles are capable of taking advantage of ecological fitting (Brooks *et al*. 2006, Malcicka *et al*. 2015).

Agosta (2006) was the first to recognize that ecological fitting requires parasite fitness space to include not only hosts with resources being utilized successfully at a given time, but also those used in the past (*e.g*. Nylin *et al.* 2015) and those that could be used but are not currently accessible or "apparent" (Feeny 1976, Courtney 1985). That is, in keeping with fundamental Darwinian principles. Survival in changing environmental conditions is based on pre-existing variation in the species that, by definition, was less than optimal in the previous environments. Ecological fitting is possible because Darwinian fitness space is by its nature "sloppy" rather than tightly optimized (see also Agosta & Klemens 2008, Agosta *et al.* 2010). Sloppy Fitness Space in coevolution links the distinction between realized and fundamental host range to the distinction between fundamental and realized niche space in ecology. This is the basic dynamic module of the Stockholm paradigm.

This sets the stage for true coevolutionary episodes. Newly produced generalists, occurring in multiple hosts in different geographic areas, may experience localized coevolutionary responses that produce new true specialists (species with restricted host ranges due to limited capacity to use many host resources) and faux generalists (specialists that may appear generalists because they occur on a diverse but phylogenetically limited host clade), according to the geographic mosaic theory of coevolution (Thompson 2005). The localized coevolutionary responses may, but need not, include the evolution of defense and counter-defense traits, so the Geographic Theory of coevolution subsumes classic coevolutionary Arms Race and Resource Tracking models (Thompson 2005, Agosta *et al.* 2010, Janz 2011).

Repeated through time, the basic dynamic module produces the macroevolutionary oscillations first documented by Janz & Nylin (2008). Major climatological and geological perturbations comprise one means of revealing such episodes. According to the taxon pulse hypothesis (Erwin 1979, 1981, 1985), biotic diversification occurs through alternating episodes of biotic isolation and expansion, with occasional evolution of novel ecological traits in isolation, leading to (1) general biogeographic patterns resulting from both isolation and expansion, (2) reticulated area relationships, (3) clade-specific patterns of isolation and expansion, and (4) phylogenetic conservatism in niche components relevant to transmission dynamics and microhabitat preference (see Brooks & McLennan 2002 for extensive documentation of niche conservatism; for a recent update, see Wiens *et al.* 2010). Empirical studies examined in light of the taxon pulse hypothesis have found clear support for it (Spironello & Brooks 2003, Bouchard & Brooks 2004, Bouchard *et al.* 2004, Halas *et al.* 2005, Lim 2008, Eckstutt *et al.* 2011). Additionally, the literature of the past decade contains multiple studies reporting alternating episodes of dispersal and isolation, without explicitly invoking the taxon pulse (*e.g.* the discussion on the evolution of the community of the Atlantic Forest by Leite *et al.* 2016 and Raposo do Amaral *et al.* 2016). Hoberg & Brooks (2008, 2010, 2013) summarized evidence that climate change and other geographic/geologic events can catalyze the dynamics encompassed in the dynamic module of the Stockholm Paradigm, producing complex geographic mosaics of specialized host-parasite systems having vast potential for disease emergence. This led Brooks & Ferrao (2005) to characterize the world as a "minefield of evolutionary accidents (emerging infectious diseases) waiting to happen".

With the elements of the Stockholm paradigm, we evaluate the partial phylogeny of the species of *Aglaiogyrodactylus* Kritsky *et al.* 2007 (Platyhelminthes, Monogenoidea, Gyrodactylidae). Species of *Aglaiogyrodactylus* are all oviparous ectoparasites of the body surface of loricariid (Siluriformes) hosts, inhabiting the small coastal streams of southern Brazil. Gyrodactylidae encompasses more than 500 nominal species found worldwide living on the body surface of teleost fishes, some amphibians, and even decapod mollusks (Boeger *et al.* 2003). Features of the reproductive biology and transmission dynamics of gyrodactylids form the basis for theories about their high species richness, wide geographic distribution and high degree of endemism. Like most Monogenoidea, gyrodactylids are monoxenous (lacking intermediate hosts or vectors); however, unlike most monogenoids, most gyrodactylids are viviparous, many exhibiting polyembryony (Boeger *et al.* 2003). Direct and continuous transmission increases the capacity of establishing a viable population in a single generation which has led to suggestions that the high rates of speciation in different populations of the same host species (sister species occurring on the same host species) and by host switching (non-sister species on the same host species) (Zietara & Lumme 2002, Boeger *et al.* 2003, Boeger *et al.* 2005).

At present, there are fewer oviparous than viviparous nominal species of gyrodactylids. According to Boeger *et al.* (2003), these fishes exhibit hard surfaces, ideal for deposition of their adhesive eggs. This putative reduced diversity and restricted host and geographic range, relative to viviparous members of the family, reinforces theories that viviparity was an evolutionary innovation responsible for high diversification rates in the group (Brooks & McLennan 1993, Boeger *et al.* 1994). The combination of high species richness, broad geographic distribution and host range, and specialized but phylogenetically conservative reproductive modes and life history traits all make the Gyrodactylidae an excellent model system for studies of the Stockholm paradigm.

The main host group of the oviparous Gyrodactylidae is species of Loricariidae (Siluriformes). Loricariidae is the most species-rich family within the order (Nelson 2006) even though the distribution is limited to South America and part of Central America. Species of this family are usually found in lotic habitats and have limited tolerance to elevated salinity of marine waters, although they may survive for short periods of time in mesohaline habitats present in upper reaches of estuaries (Brion Guillermo *et al.* 2013, Capps *et al.* 2011).

Our study system is a clade of seven species forming part of the oviparous species of *Aglaiogyrodactylus* inhabiting loricariid catfishes in a single stream on the coast of Paraná, Brazil. In the process of collecting specimens for a molecular phylogenetic analysis of the parasites, data about host range and ecological parameters were also recorded. The aim of this study is to apply the precepts of the Stockholm Paradigm to propose a hypothesis on the evolution of this host-parasite system. When viewed in a phylogenetic framework, ecological data, we postulate, add breadth to studies investigating the evolution of host-parasites associations under the Stockholm paradigm. The rich diversity of Loricariidae in the stream should provide the opportunity for species of *Aglaiogyrodactylus* to explore a range of potential hosts which should be limited solely by the capacity of each individual species of parasite. Hence, we expect, also, that ecological parameters of each association should provide a proxy for the degree of compatibility of the association, which may allow an analysis of the relationship between these parameters and the history of the host-parasite system.

MATERIAL AND METHODS

Host fish were caught in a small stream in the Marumbi River of Paraná state (25°30’32.8”S, 48°52’31.3”W) in March and August 2010, and May 2011. Fish hosts were captured with electronarcosis following the standard methodology. Hosts were immediately individually placed in plastic containers, anesthetized with benzocaine, and subsequently euthanized by sectioning of the medulla. After this procedure, the fish and parasites sampled for molecular procedures were fixed in 95% ethanol, and those sampled for morphological procedures were fixed in 5 % formalin.

In the laboratory, parasites were collected from the sediment using a small probe with the aid of a dissecting microscope. Some specimens fixed in 5 % formalin were stained with Gomori’s trichrome and mounted in Canada balsam (Humason 1979) to study the morphology of “soft” structures. Other specimens were cleared and mounted in Hoyer’s (Humason 1979) for analysis of sclerotized structures. The morphological study was performed with the aid of a microscope with phase contrast and/or DIC (Olympus BX 51) and a digital camera (Olympus QColor 5). Voucher specimens were deposited in the Parasitological Collection of the Instituto Oswaldo Cruz (see Table I). Ecological parameters of each association were calculated following Bush *et al.* (1997).

The total DNA of parasites was extracted using the DNeasy kit (Qiagen, Hilden, Germany) adapted to monogenoids. For each parasite extracted, one voucher slide per specimen was prepared to contain the haptor (with haptoral sclerites) and/or the anterior portion (with the copulatory organ) were mounted in Hoyer’s medium. The total DNA of host species was extracted from the remaining portion of the animal’s body using the DNeasy blood and tissue kit (Qiagen, Qiagen, Valencia, CA).

For the parasites, the primers 18S7f (5’-GCCCTATCAACTTACGATGGTA-3’) and 18SF (5’-CCAGCTTGATCCTTCTGCAGGTTCACCTAC-3’), of Littlewood & Bray (2001) were used to amplify and sequence partial fragments from 18S rDNA. The polymerase chain reaction (PCR) was performed with the following program: initial denaturation at 95 °C for 5 min followed by 40 cycles as follows: denaturation at 95 °C for 45 s, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and final extension 72 °C for 5 min. PCR was achieved in 25 μl containing 20-40 ng of template DNA, 3 mM $MgCl_2$, 1× PCR-Buffer (Invitrogen, Carlsbad, CA, USA), 0.5 pmoles of each primer, 0.4 mM dNTP, and 1U Platinum Taq polymerase (Invitrogen) in a total volume of 25 μl. Confirmation of the amplification of the fragments by PCR was achieved through electrophoresis in a 1.5% agarose gel, subsequent staining in GelRed (Biotium®) and visualization under UV light. Amplified products were purified with the MinElute kit (Qiagen). The

Table I. – Lists of parasites species included in the ingroup and outgroup for phylogenetic analysis, with respective family, vouchers, and Genbank identification code.

TAXON	VOUCHER*	GENBANK
Capsalidae (outgroup)		
Benedenia sp.		AJ228774
Encotyllabe chironemi Robinson, 1961		AJ228780
Udonellidae (outgroup)		
Udonella caligorum Johnston, 1835		FJ946831
Viviparous Gyrodactylidae (outgroup)		
Gyrodactylus sp.		JX840355
Oviparous Gyrodactylidae (outgroup and ingroup)		
Phanerothecium sp. (outgroup)	CHIOC 38687	KX981456
Aglaiogyrodactylus calamus Kritsky, Vianna & Boeger, 2007	CHIOC 38688	KX981458
Aglaiogyrodactylus conei Kritsky, Vianna & Boeger, 2007	CHIOC 38689	KX981459
Aglaiogyrodactylus ctenistus Kritsky, Vianna & Boeger, 2007	CHIOC 38690	KX981460
Aglaiogyrodactylus forficulatus Kritsky, Vianna & Boeger, 2007	CHIOC 38691	KX981461
Aglaiogyrodactylus forficuloides Kritsky, Vianna & Boeger, 2007	CHIOC 38692	KX981462
Aglaiogyrodactylus guttus Kritsky, Vianna & Boeger, 2007	CHIOC 38693	KX981463
Aglaiogyrodactylus salebrosus Kritsky, Vianna & Boeger, 2007	CHIOC 38695	KX981464
Aglaiogyrodactylus pedunculatus Kritsky, Vianna & Boeger, 2007	CHIOC 38694	KX981465
Onychogyrodactylus hydaticus Kritsky, Vianna & Boeger, 2007	CHIOC 38686	KX981457

* Corresponding collection numbers from a credited collection will be provided prior to publication.

sequencing reactions were analyzed in an automatic sequencer, ABI 3130 (Applied Biosystems). Sequences were edited using BioEdit v.7.0.9 (Hall 1999) and Geneious v.4.5 (created by Biomatters; available from http http://www.geneious.com/, Drummond *et al*. 2011).

Final alignment of the parasite sequences (18S rDNA) was obtained with MAFFT (Katoh *et al*. 2002), available on the GUIDANCE server (http://guidance.tau.ac.il/; Penn *et al*. 2010), used to guide the removal of parts of alignment that showed low reliability. Phylogenetic analyses were performed using MrBayes v3.1.1 (Ronquist & Huelsebeck 2003) in the platform Cipres (Miller *et al*. 2015) using 2 runs, 4 chains, 20 million generations, burnin of 100,000 and with GTR+I+gamma as the initial model (defined with jModelTest; Darriba *et al*. 2012). The choice of the outgroup species was based on their phylogenetic relationships relative to the Gyrodactylidae (Boeger & Kritsky 1993, 2001, Olson & Littlewood 2002) (Table I). Three species not detected in the Marumbi River were included in the analysis for comparative purposes (Table I). More comprehensive preliminary analysis indicated that *O. hydaticus* is a member of *Aglaiogyrodactylus* – pending nomenclatural formalization – hence, it was included in the analysis.

The phylogenetic relationships of the host species were constructed based on the phylogenetic analysis of Lujan *et al*. (2015). Whenever the species studied herein was not present in the hypothesis of Lujan *et al*. (2015), its relative position was based on the position of their congeners. Genetic distances between species of parasites were calculated directly from the trees presented by Lujan *et al*. (2015). The putative correlation of the number of shared parasites and the phylogenetic distance between host species was evaluated by testing the fit of the data to distinct models using the software Past (Hammer *et al*. 2001). Phylogenetic changes in host range of *Aglaiogyrodactylus* spp. were inferred using the biogeographic protocol proposed by Lieberman (2000, 2003a,b) with the software Mesquite v.2.75 (Maddison & Maddison 2011) using the parsimony trace character history analysis on the parasite cladogram.

To evaluate the randomness of the observed bipartite network of host-parasite relationships, we calculated the Shannon Entropy (H) (Shannon 1948) for the observed network and compared it with 1,000 networks simulated in the R environment (R Core Team 2016) using the package *vegan* (Oksanen *et al*. 2016). Z-score was calculated in Microsoft Excel. The null model chosen, *r0_ind*, corresponds to the *R0* model (see Wright *et al*. 1998) – under this model, simulations retain the number of hosts and parasites, but draws randomly individuals from a uniform probability distribution until the sum of abundances of a certain among all host species equaled to the observed value for that parasite species. The protocol and model used to test the randomness of the network assumed that in the present scenario the *opportunity* to utilize hosts in sympatry is maximized and the major limiting process to hinder this to happen are differences in the *compatibility* of the actor species. We postulate that if the observed network is not statistically distinct from randomly generated networks, the associations are not organized by *com-*

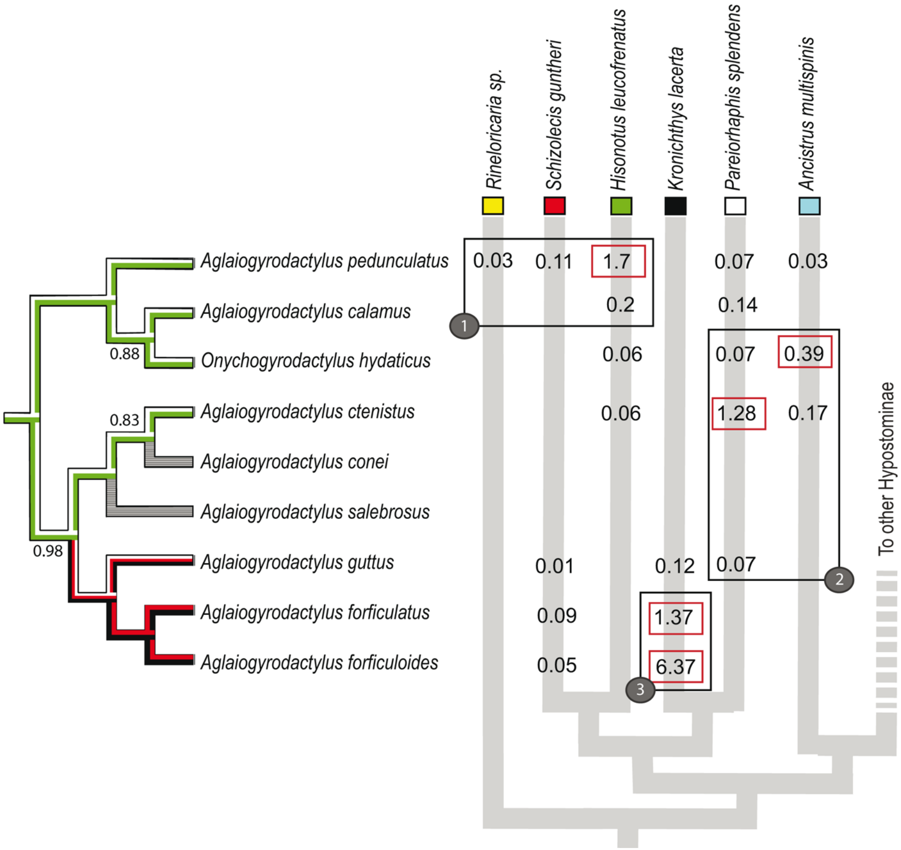

Fig. 1. – Phylogenetic and ecological data for *Aglaiogyrodactylus* spp. and their hosts, loricariid catfishes, in the Marumbi River, Paraná, Brazil. The phylogenetic relationships of the clade of *Aglaiogyrodactylus* spp. (left) is presented with the phylogeny of their hosts based on the phylogeny of Loricariidae of Lujan *et al*. (2015) (right); the observed abundance of each parasite and host species is presented on the intersection of both phylogenies. Posterior probability values for the parasite phylogeny are presented above each branch except when equal to 1. Host range evolution is optimized over the parasite tree – colored branches indicate putative ancestral host-range (colors correspond to the colors indicated by the host name). *Aglaiogyrodactylus conei* and *A. salebrosus* do not occur in that river but were included in the analysis to provide a more complete evolutionary context. Network modules are indicated by rectangles on the host phylogeny (identified by number on a grey circle). Red rectangles indicate the values of abundance that are more than twice the value observed in the remaining associations.

patibility but solely by *opportunity*. The function *computeModules* of the R package *bipartite* (Dormann *et al*. 2009) was used to reconstruct modules of weighted interactions (using respective abundance values). The significance of the observed modularity was defined with the software MODULAR (Marquitti *et al*. 2013) by comparison with 1,000 simulated networks under the model of Bascompte *et al*. (2003) – in this null model "each cell in the interaction matrix has the same probability of being occupied."

RESULTS

The Loricariidae species registered in the Marumbi River were: *Ancistrus multispinis* (Regan, 1912) (Hypostominae); *Hisonotus leucofrenatus* (Miranda Ribeiro, 1908), *Pareiorhaphis splendens* (Bizerril, 1995), *Kronichthys lacerta* (Nichols, 1919), *Schizolecis guntheri* (Miranda Ribeiro, 1918) (Hypoptopomatinae); and *Rineloricaria* sp. (Loricariinae). In general, specimens of these species have limited dimensions, being shorter than 10-15 cm. All these species host species of *Aglaiogyrodactylus*. Species of other genera of both viviparous (*Gyrodactylus* spp.) and oviparous (*Phanerothecium* spp. and *Onychogyrodactylus sudis* Kritsky *et al*., 2007) were also detected on these host species, but they are not the subject of the present study. The network of hosts and their respective species of *Aglaiogyrodactylus* is presented in Fig. 1; Table II presents ecological parameters for each association observed. No parasite species occurs only on a single host species in the Marumbi River; all species of *Aglaiogyrodactylus* are shared by 2-5 loricariids (Fig. 1).

In general, all ecological parameters presented relatively low values. Abundance (total number of parasites collected/total number of hosts examined) was higher than 1 only for the associations between *A. forficuloides* and *K. lacerta* (6.37); *A. pedunculatus* and *H. leucofrenatus* (1.7); *A. ctenistus* and *P. splendens* (1.28); and *A. forficulatus* and *K. lacerta* (1.37) (Table II, Fig. 1). The remaining associations presented abundance below 0.5. Prevalence (percentage of infested host in the sample) was higher than 50 % solely for the same 4 associations with high values of abundance. Mean intensity (mean number of parasites per infested host specimens) was usually low (1-3.7 parasites per fish) and only 2 associations presented values above 5 (Table II). For each parasite species, usually a single host species – among all observed host species – presents higher values of abundance and prevalence, except for the association of *A. calamus* with *H. leucofrenatus* and *P. splendens*. For the scenario of this study, abundance (= mean number of parasites per host in the sample) was considered an adequate ecological parameter to analyze since it incorporates both the other two important measurements, prevalence and mean intensity (Table II).

The network composed of the observed associations of species of *Aglaiogyrodactylus* and their loricariid host fishes in the Marumbi River comprises three mod-

Table II. – Ecological parameters observed for the host-parasite associations in the Marumbi River, Brazil.

Parasites	***Host fishes***	***Prevalence (%)***	***Intensity***	***Abundance***
Aglaiogyrodactylus pedunculatus	*Ancistrus multispinis*	**3.57** (1 / 28)	**1.00** (1 / 1)	**0.04** (1 / 28)
	Hisonotus leucofrenatus	**60.00** (9 / 15)	**2.89** (26 / 9)	**1.73** (26 / 15)
	Pareiorhaphis splendens	**7.14** (1 / 14)	**1.00** (1 / 1)	**0.07** (1 / 14)
	Rineloricaria sp.	**3.13** (1 / 32)	**1.00** (1 / 1)	**0.03** (1 / 32)
	Schizolecis guntheri	**1.89** (1 / 53)	**6.00** (6 / 1)	**0.11** (6 / 53)
Aglaiogyrodactylus calamus	*Hisonotus leucofrenatus*	**13.33** (2 / 15)	**1.50** (3 / 2)	**0.20** (3 / 15)
	Pareiorhaphis splendens	**14.29** (2 / 14)	**1.00** (2 / 2)	**0.14** (2 / 14)
Onychogyrodactylus hydaticus	*Ancistrus multispinis*	**10.71** (3 / 28)	**3.67** (11 / 3)	**0.39** (11 / 28)
	Hisonotus leucofrenatus	**6.67** (1 / 15)	**1.00** (1 / 1)	**0.07** (1 / 15)
	Pareiorhaphis splendens	**7.14** (1 / 14)	**1.00** (1 / 1)	**0.07** (1 / 14)
Aglaiogyrodactylus ctenistus	*Ancistrus multispinis*	**7.14** (2 / 28)	**2.50** (5 / 2)	**0.18** (5 / 28)
	Hisonotus leucofrenatus	**6.67** (1 / 15)	**1.00** (1 / 1)	**0.07** (1 / 15)
	Pareiorhaphis splendens	**50.00** (7 / 14)	**2.57** (18 / 7)	**1.29** (18 / 14)
Aglaiogyrodactylus guttus	*Kronichthys lacerta*	**12.50** (1 / 8)	**1.00** (1 / 1)	**0.13** (1 / 8)
	Pareiorhaphis splendens	**7.14** (1 / 14)	**1.00** (1 / 1)	**0.07** (1 / 14)
	Schizolecis guntheri	**1.89** (1 / 53)	**1.00** (1 / 1)	**0.02** (1 / 53)
Aglaiogyrodactylus forficulatus	*Kronichthys lacerta*	**50.00** (4 / 8)	**2.75** (11 / 4)	**1.38** (11 / 8)
	Schizolecis guntheri	**5.66** (3 / 53)	**1.67** (5 / 3)	**0.09** (5 / 53)
Aglaiogyrodactylus forficuloides	*Kronichthys lacerta*	**87.50** (7 / 8)	**7.29** (51 / 7)	**6.38** (51 / 8)
	Schizolecis guntheri	**1.89** (1 / 53)	**3.00** (3 / 1)	**0.06** (3 / 53)

Table III. – Z-scores for observed entropy values for the observed and simulated networks with binary (presence/absence) or abundance values using the algorithm "r0_ind". This algorithm preserves row sums and individuals are shuffled among cells of each row of the matrix.

	With abundance	**Binary**
Observed H	0.508	0.998
Simulated mean H	1.747	1.782
Simulated max H	1.775	1.788
Simulated min H	1.683	1.773
Standard deviation	0.016	0.002
z-score	–79.582	–334.604
p	< 0.00001	< 0.00001

ules (modularity value = 0.375; p = 0.043), which are not linked to phylogenetic clades of hosts nor parasites (Fig. 1). Simulation in the package vegan strongly supports the non-randomness of the observed network (Table III).

At this locality (independent of the ecological indices), *Aglaiogyrodactylus* spp. exhibit two patterns of host range (Fig. 1). The first includes *A. pedunculatus, A. calamus, O. hydaticus*, and *A. ctenistus*. Only the first three species form a clade, and all four are related to each other only at the level of the basal node in the phylogeny. Yet they all parasitize *H. leucofrenatus* and *P. splendens*, and all but not *A. calamus* parasitize *A. multispinis*. *Aglaiogyrodactylus pedunculatus* also infests *Rineloricaria* sp. and *S. guntheri*. The second host-range pattern includes the clade of *A. guttus*, *A. forficulatus*, and *A. forficuloides*, which exhibits considerable distinct host range. All three members of this group parasitize *S. guntheri* and *K. lacerta*. The oldest member of the clade, *A. guttus*, also parasitizes *P. splendens*, a host also shared by parasite species that compose the first pattern in host range described above.

Individual loricariid species can host between one to 5 species of *Aglaiogyrodactylus*. *Rineloricaria* sp. is the least parasitized species and *P. splendens* is the only species to host 5 species of *Aglaiogyrodactylus* (Fig. 1). In 4 out of the 6-host species, abundance values for one parasite species were approximately two-times higher than values observed for any other species of this parasite genus. Although, on *K. lacerta*, the values of abundance were relatively high when compared to the remaining associations within the river. *Schizolecis guntheri* and *Rineloricaria* sp. were the only two host species for which no species of parasite showed any clear "preference" (*i.e.* similar values of abundance across parasites).

Phylogenetic analysis of the members of *Aglaiogyrodactylus* spp. (Fig. 1) has generally high support; all branches have posterior probability values greater than 80%. The correlation between genetic distances and parasite species sharing was considered non-significant under the linear model (r2 = 0.08; p = 0.28) (Fig. 2) or any other model. Despite the lack of significant correlation, sharing initially increases with increasing genetic distance and subsequently subsides.

Phylogenetic optimization of the observed host range data (Fig. 1) provides no evidence for co-speciation. Seldom does a species of *Aglaiogyrodactylus* parasitize sister groups of hosts (exceptions are *A. pedunculatus* and *A. guttus*). The ancestral host range in the Marumbi River reconstructed for the species of *Aglaiogyrodactylus* (Fig. 1) is *H. leucofrenatus* + *P. splendens*. In the ancestor of *A. guttus*, *A. forficulatus,* and *A. forficuloides*, the host range changes by losing *H. leucofrenatus* and incorporating *S. guntheri* and *K. lacerta*. Subsequently, within this latter clade, the ancestor of *A. forficulatus* and *A. forficuloides* has reduced the host range by losing *P. splendens*. This reconstruction suggests that several host range changes occurred in the recent times, by some species of *Aglaiogyrodactylus*. For instance, *A. multispinis* was independently incorporated into the host repertoire to the ancestral host range by *A. pedunculatus, O. hydaticus*, and *A. ctenistus*. *Aglaiogyrodactylus pedunculatus*, the species with the wider range of host species, incorporated *Rineloricaria* sp. and *S. guntheri*. Other reconstructed changes in host range are putative historical changes, with losses (*e.g.* the loss of *H. leucofrenatus* by the ancestral of *A. guttus*, *A. forficulatus,* and *A. forficuloides*) and gains (*i.e.* the incorporation of *S. guntheri* and *K. lacerta* by the same ancestral species).

DISCUSSION

The scenario analyzed here represents a cross-sectional study of the long and short-term dynamics of host-parasite associations that are played out on a geographic, geologic, and climatological background. These factors promote isolation and expansion of communities, hence providing variation in the opportunity of contact between species (host and parasites) in ecological and evolutionary times (see Hoberg & Brooks 2015). This study was performed in a single river system, one of many small hydrographic basins in the region and, consequently, opportunity for the different parasite populations to access the different host species within this stream is maximized but it is minimized when species absent in the stream are considered. Araujo *et al.* (2015) suggested that compatibility (*i.e.* the capacity of the parasite species to form a population on a certain host species associated with the ability of that host to cope with the pressure imposed by the parasite) and opportunity were more important than conflict resolution (*i.e.* coadaptation) in establishing new host-parasite associations. Thus, host range tells us something about the compatibility of parasites for various host species in their environments. However, the geographic scale of this study may be too small to observe the influence of possible historical cycles of isolation and expansion events

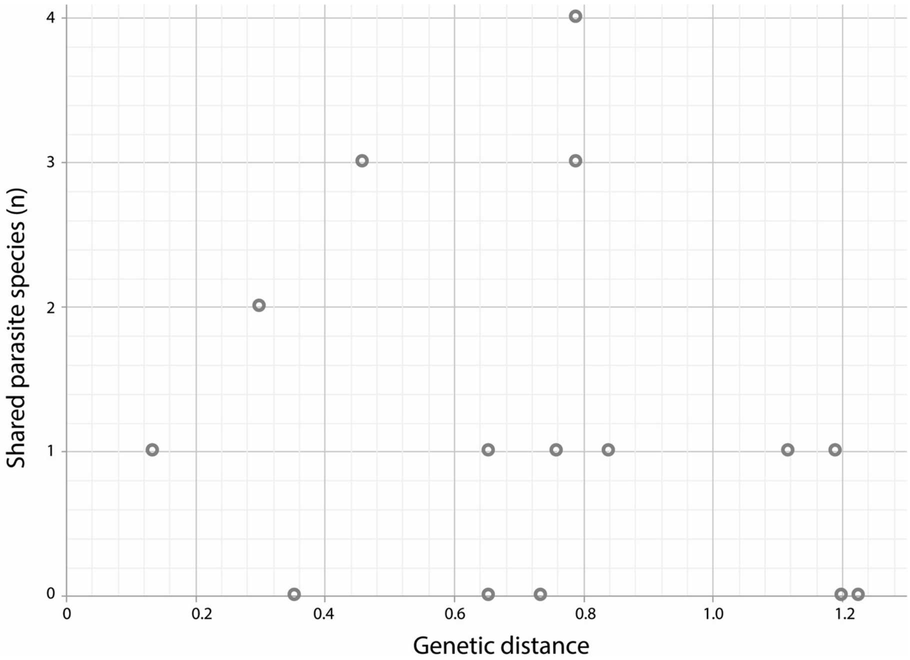

Fig. 2. – Correlation between genetic distance (calculated from the Bayesian Hypothesis of Lujan *et al.* 2015) and number of shared species of *Aglaiogyrodactylus* in the Marumbi River, Brazil.

(Taxon pulse dynamics) in the production of new species. The general area of this study was strongly affected by paleoclimatological events in the last thousands of years, especially associated with eustasic events but also with more recent climatological incidents (*e.g.* heavy storms), which haphazardly promote exchange of aquatic fauna between otherwise isolated watersheds (Tschá *et al.* 2017, Tschá *et al.* 2016).

Despite of these apparent limitations, the reconstructed history of the focal host-parasite system suggests large agreement with most elements of the Paradigm of Stockholm (Hoberg & Brooks 2015). First, the quantitative distribution of host for the parasites observed in this study is expected when host-range expansions result from ecological fitting (see Araujo *et al.* 2015). The non-uniform distribution of host-parasites strongly indicates that each parasite species has distinct information space (see Brooks & Agosta 2012). Consequently, parasite species differ in the capacity of colonizing sympatric species because they present quantitative and qualitative differences in sloppy fitness space, something predicted in theory (Agosta *et al.* 2010) and by modeling (Araujo *et al.* 2015).

The observed values of the ecological parameters for each respective association putatively represent recent adaptation processes, and no unambiguous inference about the history of each observed association can be obtained from their analyses. However, these parameters – in this specific case, the abundance of parasite species on each host species – represent an adequate proxy of compatibility. This conclusion is supported by the fact that, for most cases, abundance values (which represents the combined values of prevalence and intensity) of a parasite species is not uniformly distributed among host species. Indeed, for 5 out of 7 parasite species, the abundance of observed associations is more than twice the value of the remaining associations. Only in two cases is this not so: for *A. guttus* and *A. calamus*. In these cases, the differences between values of parasitological parameters do not appear significantly distinct among hosts (Table II) and may reflect solely the present demographic dynamics of the evolution of the association. Infrapopulations of these parasite species are equally compatible with the range of hosts they inhabit.

Furthermore, the observed network is also significantly different from the simulated randomized networks (Table III), suggesting that although opportunity is expectably maximized by the sympatry of the host species, compatibility is likely the main process responsible for the structure of the local host-parasite system. If opportunity was the most important determining factor, we would expect to see all parasites utilizing all host species in the river. Indeed, as for all Gyrodactylidae and likely for species of related families (see Bakke *et al.* 1992, Boeger *et al.* 2003, 2005), the monogenoid species in the present study – *Aglaiogyrodactylus* spp. – are continuously colonizing various host species. Thus, in this riverine system, associations are strongly a matter of compatibility (Combes 2001, Araujo *et al.* 2015) and parasite "preference" (see Boeger *et al.* 2005).

As suggested by the correlation analysis between genetic distance of hosts species and the sharing of parasite species (Fig. 2), phylogeny is probably only an approximate proxy for compatibility; sharing is small between species of closely related clades (*i.e.* parasites are minimally shared by the host species studied that are members of a single subfamily) (Fig. 1). Sharing of parasites is apparently more intense for species of closely related but not sister clades while the most distant host species in this study presented the smallest sharing observed. Hence, although not a significant correlation, the results are somewhat in accordance to Braga *et al*. (2014), which regards phylogeny as one, but not the only, variable that drives parasite sharing among host species and groups.

Second, oscillation was observed once in the history of the *Aglaiogyrodactylus* from the Marumbi River. As reconstructed above, the lineage composed by *A. ctenistus* and related species initially retained part of the ancestral host range (*P. splendens*) – reduction in host range – and incorporated new host species (*K. lacerta & S. guntheri*) – increase in host range – before losing the oldest component of its ancestral host range – new reduction of host-range. This fluctuation in host range through time characterizes the process of oscillation as proposed by Janz & Nylin (2008).

The reconstruction of the evolution of host ranges and the modules postulated in network analysis (Fig. 1) suggest yet another historical transition in these associations. The species of the clade composed by *A. pedunculatus*+*A. calamus*+*O. hydaticus* and *A. ctenistus* (included in module 1 and in module 2, respectively) retain the ancestral host range and incorporate new hosts more recently, probably by traits associated with phylogenetic conservatism and resource tracking. The change in the ancestral host range of the remaining clade (*A. guttus*+ *A. forficulatus*+ *A. forficuloides*) is associated with the incorporation of two host species, *S. guntheri* and *K. lacerta*, and a partial loss of the ancestral host range (*H. leucofrenatus*). *Aglaiogyrodactylus forficulatus* and *A. forficuloides* on *K. lacerta* compose module 3 while the module 2 includes associations that apparently represent the "stepping stone" between the associations of species of the module 1 and 3. Though the parasites of module 1 parasitize *P. splendens* only marginally, *A. ctenistus* – a member of the other clade of *Aglaiogyrodactylus* – specialized on this host species. Part of module 2 is composed by *A. guttus*, that retained *P. splendens* in its host range but incorporated *K. lacerta* and *S. guntheri* while showing no clear evidence for specialization to any of its host species. Hence, it is apparent that the specialization of *A. ctenistus* (and maybe, of its ancestor) to *P. splendens*, provided the capacity for the later ancestor lineage to colonize the host sister group *H. leucofrenatus* and, to a less extent, *S. guntheri*. Although not present in the focal study area, *A. salebrosus*, and *A. conei* are also reported from other species of *Pareiorhaphis* from distinct watersheds (Kritsky *et al*. 2007, pers obs) and supports the putative evidence of ancestral association of *Aglaiogyrodactylus* spp. with species of this host genus. The reconstruction and the modular organization further suggest that the common ancestor of *A. forficulatus* and *A. forficuloides* lost *P. splendens* and specialized to *K. lacerta* but retaining *S. guntheri* as host, although at low compatibility.

Third, the Geographic Mosaic Theory of Coevolution (Thompson 2005) suggests that if a parasite becomes widespread, either geographically or among hosts via ecological fitting, new localized specialists may emerge. This process is clearly a subordinate element of the Oscillation Hypothesis (Janz & Nylin 2008). Despite the low abundance of some, all associations revealed in this study likely reflect some degree of compatibility between species of hosts and parasites. Indeed, the incorporation of all associations into a historical reconstruction of the evolution of species of *Aglaiogyrodactylus* and their host species within a stream provided the opportunity to reconstruct, in more detail, events putatively linked with the diversification of the parasite species.

Parasite ecologists commonly find that, for cases in which there are multiple hosts for a parasite species, abundance differs among those hosts. In addition, different parasite species inhabiting the same or different host species within an ecosystem often exhibit different abundances. There is no direct connection between host range and maximum abundance in the macroevolutionary context and there is no clear correlation between ecological metrics and history of the association. However, shifts in compatibility should provide evidence for specialization and speciation by host preference.

Hence, during speciation associated with specialization, we may expect a shift in abundance away from the original host and in the direction of one of the hosts added (or member of the ancestral host range) because of ecological fitting (Araújo *et al*. 2015). These patterns may become visible when both ecological data and historical reconstructions are analyzed together. For instance, although *A. multispinis* was not reconstructed as an ancestral host species of *Aglaiogyrodactylus* spp., an entire clade (*e.g. A. pedunculatus* + *A. ctenistus)* share the ability to use this host species when it is present. Therefore, the speciation of *O. hydaticus* is apparently correlated with the adaptation to *A. multispinis* since this association shows comparatively higher abundance value (0.39) than on the remaining host species.

The persistence of parasites at low ecological values on some host species supports the results of the mathematical model of Araujo *et al*. (2015). During simulations, these authors observed that modeled parasites could maintain a population on a new host species for several generations without adapting to the new resource. In the model, probably likely as in real life, evolution has not produced phenotypes more fit to the resource (= host species) being used and that could be potentially selected positively (see also Fenton *et al*. 2015 for additional modeling support, Boeger *et al*. 2005, Duffus *et al*. 2014 for experimental cases). This scenario is compatible with the values of abundance observed for *A. guttus* and *A. calamus*.

We must not assume that low-abundance parasites have limited evolutionary potential (a notion that could be reinforced by calling them "accidental" or "incidental" infections, or "satellite" community members), or that the numerically dominant species are the repository of the evolutionary future of a parasite group. The presently numerically rare associations, accounting (80 % of the host-parasite associations in this study) may become abundant in the future, should environmental stressors, such as climate change and anthropogenic changes, alter the conditions under which abundant species dominate numerically, providing non-abundant species with opportunities to enhance their abundance. Furthermore, such species, surrounded by more optimal hosts (*i.e.* host species on the neighbor watersheds) and awaiting only a change in conditions to provide the opportunity to switch, would possess substantial potential for host-switching and thus emerging disease because the newly-colonized hosts would not have had the opportunity for resistance to evolve (Hoberg & Brooks 2015).

A cursory examination of recently published studies of parasite abundance patterns across a range of parasites and hosts indicates that most host-parasite associations are low-abundance (*e.g.* Olsson-Pons *et al.* 2015, Vhora & Bolek 2015, Werneck *et al.* 2015), in agreement with earlier studies (*e.g.* Canaris & Kinsella 1998, Morand *et al.* 1999, Pérez-del-Olmo *et al.* 2011). This may well account for the observation that only 3/6 component communities in this study exhibit core-satellite structure (see Gaston & Lawton 1989 for discussions of exceptions to core-satellite structure).

Given the complex mosaic of temporal and spatial scaling effects in parasite evolution, however, we must also consider the possibility that rare species of parasites occur in higher abundances on the same or different hosts in other areas (as embodied in both the geographic mosaic theory of coevolution and the taxon pulse). Indeed, this study intensively collected loricariid in a short stretch of the Marumbi River (about 400 meters) for two years, during distinct seasons of the year, and sampling of *A. guttus* and *A. calamus* was consistently small. This assertion supports the estimate that these parasite species do not present a large suprapopulation in the stream or in this portion of the stream. It is, however, possible that the host species with greater ecological indices are not a resident of the lower stretches of the stream sampled and the local population is maintained by the input from suprapopulations from elsewhere in the river bed. However, *A. guttus* was originally reported from *Pseudotothyris obtusa* (Miranda Ribeiro, 1911) in the Dois de Fevereiro River – a stream of another watershed in the region and a host not detected in the Marumbi River – and *A. calamus* from *S. guntheri* – a host well sampled in the area but that did not host this species of parasite (Kritsky *et al.* 2007). A more geographically extensive sampling design within the river should answer this question.

Finally (fourth), some components of the host-parasite system studied also present patterns of associations that suggest events of expansion and isolation. This is the case for *A. forficulatus* and *A. forficuloides*, both highly compatible with *K. lacerta*. This is the expected scenario from another element of the Stockholm Paradigm, the Taxon Pulse Hypothesis (Erwin 1979, 1981, 1985; Hoberg & Brooks 2010). These sister-species of parasites may have diversified and adapted in allopatry followed by secondary contact on undifferentiated host populations, resulting in the observed pattern of relationship. A more geographically comprehensive analysis of the streams located in the coastal plain of the State of Paraná should provide further support for the proposed influence of Taxon-Pulse dynamics in the diversification of parasite species, as suggested by Tschá (2016) for fish species in the area. As suggested by these authors for fish hosts, parasite species likely expanded distribution among previously isolated watersheds during periods of low sea-level and isolation during periods of elevated sea-level, which promoted isolation and facilitated allopatric differentiation and speciation. Sweepstake-like stochastic processes likely resulted in changes in the composition of the host community in the isolated watersheds and may also have influenced subsequent specialization in allopatry of parasites to already compatible host species.

We predict that diversification of parasites under the above temporal dynamics of community composition will be a combination of speciation by 1) Specialization to different host species in sympatry; 2) Specialization to new host species in allopatry; 3) Specialization to one or more host species previously part of the parasite's host range in the absence of the ancestral host of high compatibility; 4) Simple vicariance on the original hosts. Studies of host-parasite systems of several watersheds in the region are already under way to evaluate these predictions.

This study underscores the observation that parasite biodiversity comprises complex mosaics of host relationships and geographic distributions on a variety of spatial and temporal scales (reviewed by Hoberg & Brooks 2008, 2010, 2013, also Ellis *et al.* 2015, Olsson-Pons *et al.* 2015). Thus, robust studies of parasite diversity and diversification emerge require integrative studies combining phylogenetic and ecological data as recommended by Brooks & McLennan (1993, 2002) and Hoberg *et al.* (2015). But even that is not enough; such data must be assessed in light of a robust theoretical framework. The Stockholm Paradigm is such a framework. Examining basic phylogenetic and ecological information under the Paradigm provided direct explanations about this system of monogenoids and their hosts. Thus, the present study strongly indicates that the Stockholm Paradigm represents an alternative and a more realistic framework that provides the basis for theoretically-robust protocols of historical reconstructions of species associations. The criterion of maximum co-speciation (see Brooks 2003), widely spread in the literature in the area is unrealistic, especially faced with the empirical evidence accumulated in the recent years that host switches are historically and evolutionarily common and that species are involved in associations that are both complex and dynamics. Efforts in developing adequate algorithms to incorporate widespread extant species in maximum co-speciation methods already exist (Drinkwater *et al*. 2016) but they lack the ability to recognize that ancestral parasite species may utilize more than one host species. The procedure used in the present study, the modified algorithm described by Lieberman (2000, 2003a, b), an important element of the algorithm PACT (Wojcicki & Brooks 2005), is capable of recognizing these scenarios. In the present study, all ancestral parasite species were postulated to have utilized multiple host species and recent colonizations of host species were also detected. This ability is an important step to recognize speciation by ecological fitting and coadaptation, something fundamental to understand the potential for a pathogen to represent an Emergent Infection Disease to humans or other species of animal and plants.

The study also suggests that, ideally, historical reconstructions of associations should incorporate geographical information spanning many communities to recognize quantitative and qualitative variations in the composition of the focal associations. Such studies may provide crucial information about changes affecting the ecological and evolutionary opportunities available to the parasites resulting from environmental and climate change, or anthropogenic alterations. This is a central tenet of the DAMA protocol (Brooks *et al*. 2014) proposed to help make studies of emerging diseases more anticipatory and cost-effective than crisis-response.

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