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 & Nylin 2007). 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.
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 coevolutionary responses that produce new true specialists (species 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 Aglaiohyrodactylus Kritsky et al. 2007 (Platyhelminthes, Monogenea, Gyrodactylidae). Species of Aglaiohyrodactylus 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 Monogenea, 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 Guillermto 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 electromcrosis 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’-GCCCTATCACTTACGATGGTA-3’) and 18SF (5’-CCAGCTTGA TCCTGCGAGGTTCACCCT-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 MgCl2, 1x PCR-Buffer (Invitrogen, Carlsbad, CA, USA), 0.5 pmol 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).

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

<table>
<thead>
<tr>
<th>TAXON</th>
<th>VOUCHER*</th>
<th>GENBANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsalidae (outgroup)</td>
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<td>Encotyllela chinonemi</td>
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<td>Udonellidae (outgroup)</td>
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<td>Udonella caligorum</td>
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<td>Viviparous Gyrodactyliidae (outgroup)</td>
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<td>Gyrodactylus sp.</td>
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<td></td>
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<tr>
<td>Oviparous Gyrodactyliidae (outgroup and ingroup)</td>
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<td></td>
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<tr>
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<td>KX981456</td>
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<td>KX981459</td>
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<tr>
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<td>KX981460</td>
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<td>Aglaiogyrodactylus forficuloides</td>
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<td>Aglaiogyrodactylus guttus</td>
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<td>Aglaiogyrodactylus salebrosus</td>
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<td>Aglaiogyrodactylus pedunculatus</td>
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<td>KX981465</td>
</tr>
<tr>
<td>Onychogyrodactylus hydaticus</td>
<td>CHIOC 38686</td>
<td>KX981457</td>
</tr>
</tbody>
</table>

* 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://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 & Huelsenbeck 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 Gyractylidae (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 with 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 sympathy 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-

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**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 modu-
larity 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) (Hypo-
tominae); Hisonotus leucofrenatus (Miranda Ribeiro, 1908), Pareiorhaphis splendens (Bizerril, 1995), Kronich-
thys lacerta (Nichols, 1919), Schizolecis guntheri (Miranda-
da Ribeiro, 1918) (Hyphoptopomatinae); 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 (Gyrolyactys spp.)
and oviparous (Phanerothecium spp. and Onychogyro-
dactylus 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 spe-
cies 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 Aglaiogyro-
dactylus are shared by 2-5 loricariids (Fig. 1).

In general, all ecological parameters presented rela-
tively low values. Abundance (total number of parasites
collected/total number of hosts examined) was higher
than 1 only for the associations between A. forciculoides
and K. lacerta (6.37); A. pedunculatus and H. leucofre-
natus (1.7); A. ctenistus and P. splendens (1.28); and
A. forciculatus 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 spe-
cies, 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 inten-
sity (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>
<thead>
<tr>
<th>Parasites</th>
<th>Host fishes</th>
<th>Prevalence (%)</th>
<th>Intensity</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aglaiogyrodactylus pedunculatus</td>
<td>Ancistrus multispinis</td>
<td>3.57 (1/28)</td>
<td>1.00 (1/1)</td>
<td>0.04 (1/28)</td>
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<tr>
<td>Hisonotus leucofrenatus</td>
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<tr>
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<td>0.07 (1/14)</td>
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<td>1.00 (1/1)</td>
<td>0.03 (1/32)</td>
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<td>Schizolecis guntheri</td>
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<td>6.00 (6/1)</td>
<td>0.11 (6/53)</td>
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<td>1.50 (3/2)</td>
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cies to host 5 species of parasite genus. Although, on average, abundance values for one host species were approximately two-times higher than values observed for any other species of this parasite species.

4 out of the 6-host species, abundance values for one host species were the only species with the wider range of host species, incorporated *Rineloricaria* sp. and *G. 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.
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(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 eustatic 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

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.
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. guttatus + 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 parasite 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. guttatus, 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 Araújo 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. guttatus 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

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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 Pseudotobothryis 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. guentheri – 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 sympathy; 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

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in developing adequate algorithms to incorporate wide-spread 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 qualitative and quantitative 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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