

Use of fish as intermediate hosts by helminth parasites: A comparative analysis

José L. Luque^{1*} and Robert Poulin²

¹Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Caixa Postal 74.508, CEP 23851-970, Seropédica, RJ, Brazil; ²Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

Abstract

Parasite assemblages of marine fishes include an important number of larval stages of helminth parasite species that use fish as intermediate or as paratenic hosts. In previous comparative studies, larval helminths have typically been lumped with other endoparasites, and there has been therefore no study of the biodiversity and relative abundance of larval helminths and of the factors that may influence them. Here, we performed a comparative analysis across 50 species of teleost fishes from the coast of Brazil; we evaluated the effects of several host traits (body size, social behaviour, feeding habits, preference for benthic or pelagic habitats, depth range, ability to enter brackish waters and geographical distribution) on the richness and abundance of larval helminths. Among all the potential correlates of larval helminth infection investigated in this study, only two were significant when controlling for host phylogenetic influences: Host body length was correlated positively with larval helminth abundance, and fish species with a restricted geographical distribution (Atlantic coast of Brazil mainly) had greater larval helminth abundance than their relatives with a broader (whole Atlantic or cosmopolitan) distribution. Different results were obtained if no correction was made for host phylogeny: Using species values as independent statistical observations, some additional host features also appeared associated with larval helminth species richness or abundance. The results of these analyses indicate that fish phylogeny matters. Apparently, some lineages of fish harbour more larval helminths (more species and/or more individuals) than others merely because of historical reasons (i.e., ancient associations between certain parasite taxa and fish taxa) and not really because of their present ecological characteristics.

Key words

Comparative analysis, host phylogeny, larval helminths, fish parasites, Brazil

Introduction

Several recent studies have focused on the ecological importance of larval helminths in aquatic ecosystems. On the one hand, the use of intermediate hosts by larval helminths provides insights into the evolutionary origins and present status of trophic relationships within an ecosystem (Marcogliese 2001). On the other hand, patterns of intermediate host use can also have major repercussions on the structure of parasite communities in the definitive hosts (Lotz *et al.* 1995, Dezfuli *et al.* 2000, Poulin and Valtonen 2001, Vickery and Poulin 2002).

The parasite assemblages of marine fishes include the larval stages of several groups of parasite helminths that use fish as intermediate hosts. This feature is essentially the outcome of trophic relationships involving fish, and recent studies have focused on how this relates with the structure of the aquatic food web and whether using certain fish as intermediate hosts

favors the transmission of helminth parasites (Marcogliese 2001, 2002). In line with the ideas of Bush *et al.* (1993) and Lotz *et al.* (1995), Poulin and Valtonen (2001) showed that assemblages of larval helminth parasites in fishes are not random collections of locally available species, but rather structured packets of parasites that travel together along common transmission routes. There may thus be recurrent patterns in the use of fish as intermediate hosts by helminths.

General predictions about larval helminth species richness and abundance in different fish species can be made based on the trophic transmission of the larvae. Since larval helminths in fish hosts are transmitted to their definitive hosts (larger predatory fish, birds, or marine mammals) by predation, clearly the best fish species to use as intermediate hosts would be small-bodied enough to serve as prey, and they should not be top predators (they would have to be near the bottom or middle of the food chain, not at the very top: George-Nascimento 1987, Marcogliese 2002). Predatory fish should be exposed to

*Corresponding author: jlluque@ufrj.br

more infective helminth larvae in their diet than planktivores; over evolutionary time, this should translate in higher parasite colonization. Also, benthic fishes (with broader diets) should be harbouring a greater species richness and abundance of larval helminths than pelagic fishes (which tend to have a more specialized diet: Campbell *et al.* 1980, Marcogliese 2002). Additionally, fishes with broader geographical distributions, with access to a greater depth range, and/or with the ability to enter brackish waters should harbour more larval helminths than fishes with more restricted distributions, simply as a result of the greater variety of prey they must feed on.

In spite of the ecological importance of the presence of larval helminths in fish, and the fact that some fish species clearly harbour more larval helminths than others, there has been no study of the biodiversity and relative abundance of larval helminths among different fish species and of the factors that may influence it. In previous comparative studies of parasite biodiversity in fish hosts, the larval helminths were pooled with other endoparasites and not treated as a distinct group (e.g., Sasal *et al.* 1997, Morand *et al.* 2000, Simková *et al.* 2001, Luque *et al.* 2004).

The objectives of our study are to identify features of fish species that make them suitable as intermediate hosts, by determining which features are statistically associated with either more species or more individual larval helminths. Because the influence of host phylogeny on parasite species biodiversity is well documented (e.g., Poulin and Rohde 1997, Morand and Poulin 2003), our study corrected for phylogenetic relationships among fish species, to control for the possibility that some lineages of fish harbour more larval helminths (more species and/or more individuals) than others because of historical reasons and not really because of their ecological characteristics.

Materials and methods

Data collection

All fish were collected by local fishermen from the coastal waters off the state of Rio de Janeiro, Brazil (latitude 21–23°S), during the period 1991 to 2003. The fishes were identified according to Figueiredo and Menezes (1978, 1980, 2000) and Menezes and Figueiredo (1980, 1985). Each individual fish was measured (total length) and examined for larval helminths, using standard parasitological methods. All internal organs were searched for endoparasites; washings from the lumen of the gut were passed through a sieve (154 µm mesh size) to recover the smallest metazoan parasites. The larval helminths consisted of trematodes (Bucephalidae, Didymozoidae, Hemiuridae, Heterophyidae), cestodes (Pseudophyllidea, Tetracyllidea, Trypanorhyncha), acanthocephalans (Polymorphidae, Rhadinorhynchidae, Serrasentidae) and nematodes (Anisakidae). Within each fish species, all parasites were identified to the level of morphospecies (see Appendix 1). The same parasite species may have occurred in

more than one fish species, but we did not attempt to assess this because our analysis focuses exclusively on parasite species richness within host species. All fish dissections and collection of the parasites were made using the same methods, and all parasite identifications were carried out or confirmed by the same person (JLL). Thus the data do not suffer from the problems associated with data sets compiled from different sources and based on different methods. Quantitative information on the prevalence and intensity of infection by different larval helminth species has been published previously for about half of the fish species (these publications are listed in Luque *et al.* 2004). For each host species, we recorded the total number of fish examined for parasites, as well as four measures of infection by larval helminths: (1) larval helminth richness, or the number of larval helminth species found; (2) relative larval helminth richness, or the ratio of the number of larval helminth species to the total number of endoparasite species found; (3) larval helminth abundance, or the total number of larval helminth individuals found; (4) relative larval helminth abundance, or the ratio of larval helminth abundance to the total abundance of all endoparasite species found. The entire data set is shown in Table I. The number of hosts examined, or sampling effort, is often a key determinant of the number of parasite individuals or species found in a survey (Walther *et al.* 1995), and must therefore be included as a potential confounding variable.

In addition to data on average host body length, obtained from the individual fish examined, we also used in the analysis data on a range of variables compiled previously by Luque *et al.* (2004). The following variables were taken into account for each fish species: (1) whether the fish species forms schools or not, with species adopting schooling only in some parts of the year (e.g., during the reproductive period) classified as schooling; (2) whether its geographic distribution extends to the Atlantic coast of South America (mainly Brazil), the Atlantic coast of both North and South America, or the whole world; (3) whether the fish's habitat is benthic, benthopelagic or pelagic; (4) whether or not it occasionally enters brackish or estuarine waters; (5) whether it is a predator (the majority of species) or a planktivore; and (6) its depth range, measured as the difference between the deepest and shallowest depths at which it occurs. As two of the categorical variables studied were not dichotomous, we reorganized the data to perform the present analyses by changing the categorical variables into dichotomous variables (with only two possible values, 1 and 2, and not 1, 2 and 3). Thus benthic and benthopelagic fish were classified together for comparisons with pelagic fish. With respect to geographical distribution, fish found only along the coast of Brazil formed one group, and fish found throughout the whole Atlantic or worldwide were combined into a second group.

Statistical analyses

We analysed our data in two ways, one that highlighted any existing pattern in the distribution of parasite diversity among

host species, and one that emphasized which factors may have played a role in the evolutionary diversification of parasite assemblages. In the first series of analyses, fish species were

treated as independent observations, using standard parametric tests on log-transformed continuous variables. In analyses using categorical variables (e.g., schooling behaviour or feed-

Table I. Summary of the data on the 50 Brazilian fish species included in the analyses

Species	N	Average size (cm)	Larval helminth richness	Trematoda	Cestoda	Acanthocephala	Nematoda	Relative larval helminth richness	Larval helminth abundance	Relative larval helminth abundance
<i>Aluterus monoceros</i>	39	31.2	2	1	0	0	1	0.286	2	0.002
<i>Anchoa tricolor</i>	103	11.1	7	3	1	1	2	0.875	144	0.608
<i>Archosargus rhomboidalis</i>	30	31.3	1	0	0	0	1	0.167	1	0.004
<i>Balistes capriscus</i>	66	35	8	0	4	1	3	0.444	526	0.267
<i>Balistes vetula</i>	30	47.8	3	0	2	0	1	0.333	295	0.510
<i>Brevoortia aurea</i>	42	29.6	3	0	0	0	3	0.750	33	0.892
<i>Caranx hippos</i>	60	43.9	5	0	2	0	3	0.417	97	0.065
<i>Caranx latus</i>	55	33.3	7	0	3	0	4	0.538	545	0.580
<i>Centropomus undecimalis</i>	79	35.2	2	0	0	1	1	0.667	40	0.006
<i>Cephalopholis fulva</i>	30	20.6	3	0	0	1	2	0.600	6	0.097
<i>Chaetodipterus faber</i>	110	27.7	0	0	0	0	0	0.000	0	0.000
<i>Cynoscion guatucupa</i>	73	32.5	5	0	2	1	2	0.357	2383	0.796
<i>Dactylopterus volitans</i>	78	28.5	7	1	2	1	3	0.304	115	0.150
<i>Diapterus rhombeus</i>	32	17.4	4	0	1	1	2	0.667	49	0.681
<i>Euthynnus alleteratus</i>	46	43.9	6	0	2	1	3	0.500	200	0.069
<i>Genypterus brasiliensis</i>	55	42.7	8	0	3	2	3	0.727	1450	0.399
<i>Gymnothorax moringa</i>	30	70.4	5	1	1	0	3	0.625	420	0.435
<i>Haemulon steindachneri</i>	80	19.6	3	1	1	1	0	0.300	53	0.126
<i>Harengula clupeiola</i>	35	20	1	0	0	0	1	0.333	1	0.003
<i>Macrodon ancylodon</i>	31	30	5	0	3	0	2	0.357	223	0.635
<i>Menticirrhus americanus</i>	115	28.4	4	1	1	1	1	0.308	254	0.233
<i>Micropogonias furnieri</i>	100	33.2	7	1	3	1	2	0.412	373	0.334
<i>Mugil platanus</i>	150	54.8	3	1	2	0	0	0.200	2539	0.474
<i>Mullus argentinae</i>	100	17.8	7	1	2	1	3	0.636	873	0.655
<i>Netuma barba</i>	63	43.9	3	0	1	1	1	0.429	36	0.095
<i>Oligoplites palometa</i>	84	38.2	5	0	3	1	1	0.500	114	0.018
<i>Oligoplites saliens</i>	36	36.8	2	0	1	0	1	0.333	23	0.025
<i>Oligoplites saurus</i>	37	29	3	0	2	0	1	0.375	16	0.020
<i>Orthopristis ruber</i>	162	21.6	2	0	1	1	0	0.200	1866	0.308
<i>Pagrus pagrus</i>	90	29.5	7	0	1	2	4	0.778	1182	0.885
<i>Paralichthys isosceles</i>	36	31.2	9	1	2	2	4	0.643	685	0.730
<i>Paralonchurus brasiliensis</i>	93	21.1	5	1	2	1	1	0.455	384	0.500
<i>Peprilus paru</i>	30	23.7	2	0	0	0	2	0.333	76	0.068
<i>Percophis brasiliensis</i>	60	43.6	7	0	2	0	5	0.778	1627	0.908
<i>Pomatomus saltator</i>	55	46.6	8	1	3	1	3	0.667	809	0.453
<i>Priacanthus arenatus</i>	58	37.7	5	0	1	1	3	0.625	421	0.317
<i>Prionotus punctatus</i>	47	29.3	10	2	2	2	4	0.625	871	0.809
<i>Sardinella brasiliensis</i>	35	18.2	0	0	0	0	0	0.000	0	0.000
<i>Sciadeichthys luniscutis</i>	30	35.5	2	0	2	0	0	0.333	6	0.273
<i>Scomber japonicus</i>	100	25.8	6	0	1	2	3	0.600	1635	0.246
<i>Scomber scombrus</i>	43	23.4	3	0	0	0	3	1.000	149	1.000
<i>Scomberomorus brasiliensis</i>	37	46.4	3	0	2	0	1	0.600	16	0.033
<i>Selene setapinnis</i>	89	29.4	7	0	2	0	5	0.467	466	0.535
<i>Sphyræna guachancho</i>	36	36.4	3	1	0	0	2	0.500	65	0.184
<i>Trachurus lathami</i>	51	20.6	3	0	1	0	2	0.600	559	0.936
<i>Trichiurus lepturus</i>	55	122.8	9	1	2	1	5	0.643	15676	0.251
<i>Tylosurus acus acus</i>	31	72.5	4	1	1	0	2	0.667	72	0.143
<i>Umbrina canosai</i>	81	29.8	6	0	2	1	3	0.429	767	0.397
<i>Urophycis brasiliensis</i>	75	28.5	6	0	4	0	2	0.429	505	0.242
<i>Urophycis mystaceus</i>	55	26.4	8	1	4	1	2	0.667	95	0.330

ing habits), it was not possible to use multifactorial ANOVAs because there were too few species in some categories and thus too many empty cells in the factorial matrix. Nevertheless, our analyses allowed us to determine which features of host species are associated with high parasite species richness. In the second round of analyses, we took host phylogeny into account. Closely related host species are likely to harbour similar number of parasite species, and possibly taxonomically related parasite species, because these were inherited from a recent common ancestor; this means that they do not represent truly independent statistical observations. We must therefore control for phylogenetic influences when evaluating the effects of host features (body size, schooling or feeding habits, etc.) on the evolution of parasite assemblages. To achieve this, we used the phylogenetically independent contrasts method (Felsenstein 1985, Harvey and Pagel 1991), implemented with the CAIC version 2.0 program (Purvis and Rambaut 1994). Contrasts were derived from a host tree constructed from published studies on the phylogenetic relationships of fish (Nelson 1994, Carpenter *et al.* 1995, Reed *et al.* 2002, Chen *et al.* 2003, Miya *et al.* 2003). Contrasts were computed on log-transformed data and all regression analyses were forced through the origin (Garland *et al.* 1992). We obtained contrasts corrected for the influence of one or more confounding variables (e.g., sampling effort) by taking the residuals of regressions of a selected variable against the potential confounding variables. For dichotomous variables (schooling behaviour, feeding habit, tolerance of brackish waters), contrasts were computed following Burt (1989). The mean value of these contrasts was compared with zero, as expected from the null hypothesis, using one-group two-tailed *t*-tests.

Because our aim was to expose associations between host features and measures of parasite diversity, we did not apply a Bonferroni correction to our results. Applying the correction

could possibly mask interesting trends worthy of further investigation (Moran 2003).

Results

Overall, we obtained data from 50 fish host species (see Table I). The data were derived from the examination of 3138 individual fish, for an average of 63 hosts per species (range 30 to 162). The phylogeny we used provided a maximum of 40 sets of independent contrasts among the 50 species; the actual number available for the various analyses depends on the variables included.

The number of fish examined per host species correlated significantly with larval helminth abundance (across all 50 fish species, using log-transformed data: $r = 0.446$, $p = 0.0012$), but not with any of the other three measures of larval helminth infection. Not surprisingly, the more fish are examined, the more larval helminths are found. To correct for sampling effort, we used the residuals from the linear regression of larval helminth abundance against sampling effort instead of the actual abundance values in all subsequent analyses; this way, the measure of larval helminth abundance is independent of how many fish were examined for each species.

The number of larval helminth species per host species ranged from 0 to 10. The relative larval helminth richness ranged among fish species from 0 to 1, as did the relative larval helminth abundance. Twenty-five (50%) and 16 (32%) of the fish species showed values of relative larval helminth richness and abundance equal to or higher than 0.5, respectively. Among all the potential correlates of larval helminth infection investigated in this study, and using phylogenetically independent contrasts, only two were significantly, though weak-

Table II. Summary of the statistical associations between seven ecological variables and four measures of larval helminth infection, using phylogenetically independent contrasts computed across 50 species of fish hosts (N = sets of contrasts)

Variable	Larval helminth richness	Relative larval helminth richness	Larval helminth abundance	Relative larval helminth abundance
Host body length (N = 40)	$r = 0.137$	$r = -0.063$	$r = 0.311^*$	$r = -0.072$
Depth range (N = 39)	$r = 0.104$	$r = -0.038$	$r = 0.202$	$r = 0.057$
Schooling behaviour (N = 8)	$t = 0.042$	$t = 0.960$	$t = 0.474$	$t = 0.358$
Benthic vs. pelagic (N = 9)	$t = 0.038$	$t = 0.421$	$t = 1.125$	$t = 0.587$
Use of brackish water (N = 15)	$t = 0.190$	$t = 0.221$	$t = 0.760$	$t = 0.595$
Feeding habits (N = 2)	–	–	–	–
Geographical distribution (N = 9)	$t = 1.836$	$t = 1.017$	$t = 2.986^*$	$t = 2.114$

* $p < 0.05$, (–) – insufficient data for analysis.

Table III. Summary of the statistical associations between seven ecological variables and four measures of larval helminth infection, using species values as independent statistical observations computed across 50 species of fish hosts

Variable	Larval helminth richness	Relative larval helminth richness	Larval helminth abundance	Relative larval helminth abundance
Host body length	$r = 0.210$	$r = -0.055$	$r = 0.419^*$	$r = -0.089$
Depth range (N = 49)	$r = 0.436^*$	$r = 0.205$	$r = 0.475^*$	$r = 0.333^*$
Schooling behaviour	$F_{(1,48)} = 2.760$	$F_{(1,48)} = 1.013$	$F_{(1,48)} = 1.426$	$F_{(1,48)} = 0.001$
Benthic vs. pelagic	$F_{(1,48)} = 0.314$	$F_{(1,48)} = 6.304^*$	$F_{(1,48)} = 0.562$	$F_{(1,48)} = 0.022$
Use of brackish water	$F_{(1,48)} = 0.357$	$F_{(1,48)} = 6.092^*$	$F_{(1,48)} = 0.301$	$F_{(1,48)} = 3.774$
Feeding habits	$F_{(1,48)} = 4.788^*$	$F_{(1,48)} = 0.260$	$F_{(1,48)} = 4.719^*$	$F_{(1,48)} = 0.049$
Geographical distribution	$F_{(1,48)} = 2.821$	$F_{(1,48)} = 0.030$	$F_{(1,48)} = 2.015$	$F_{(1,48)} = 6.149^*$

* $p < 0.05$.

ly, associated with one infection measure (Table II). Host body length correlated positively with larval helminth abundance ($p = 0.048$), and fish species with a restricted geographical distribution (Atlantic coast of Brazil mainly) had higher larval helminth abundance than their relatives with a broader (whole Atlantic or cosmopolitan) distribution ($p = 0.017$) (Table II).

The results of the analysis of the measures of larval infection using species values as independent statistical observations were very different from those using phylogenetic contrasts and showed some patterns (Table III). Pelagic fish showed a greater diversity of larval helminths than benthic fish ($p = 0.015$). Fish species that do not enter brackish waters also showed higher values of relative larval helminth richness than those that enter brackish waters ($p = 0.017$). In relation to feeding habits (with insufficient number of contrasts in the previous analysis), the predatory fish species showed greater richness ($p = 0.033$) and abundance ($p = 0.035$) of larval helminths than the herbivorous and planktivorous fishes. Richness ($p = 0.002$) and relative abundance ($p = 0.019$) of larval helminths were correlated with the depth range of fish species. Finally, correlations with host body length and geographical distribution were similar to those obtained from analyses using phylogenetic contrasts (Table III).

Discussion

Recent investigations have attempted to find the key determinants of parasite biodiversity (see reviews in Poulin 1997, Morand 2000, Poulin and Morand 2000). However, the importance or "weight" of larval helminths in the composition of the parasite assemblages of vertebrate hosts has scarcely been studied. With respect to larval helminths, here we have addressed some features long thought to be associated with parasite acquisition in ecological time by individual fish, and over evolutionary time by fish species (see Dogiel *et al.* 1961). When we used species values as independent statistical observations, some host features (body size, feeding behavior, habitat depth, use of brackish waters and geographical distribu-

tion) appeared to influence the richness and abundance of larval helminths, highlighting the relevance of ecological factors in these patterns. When we used phylogenetically independent contrasts, a substantially different picture emerged: only two host features, host body size and geographical distribution, appeared to influence larval helminth abundance, and none of the host features studied showed any relationship with larval helminth richness. These results are not too surprising because body size is a known predictor of total parasite species richness in the marine fish from Rio de Janeiro, as pointed out by Luque *et al.* (2004), as well as in other sets of fish species (see Poulin 1997, Morand 2000). Furthermore, the higher abundance of larval helminths in fish species with a restricted geographical distribution (Atlantic coast of Brazil mainly) than their relatives with a broader (whole Atlantic or cosmopolitan) distribution, may be explained by the characteristics of the local ecosystem and its trophic web. The coastal area of Rio de Janeiro is strongly influenced by upwelling systems and by the subtropical convergence. Moreover, salinity levels, which drop near the Amazon River, may also limit the dispersal of fish and their parasites (see Luque *et al.* 2004). We expected fish species with broad geographical distributions to harbour richer communities of larval helminths, simply because they may be exposed to a wider variety of prey, but not necessarily greater numbers of individual worms.

The possibility that the feeding patterns of fish influence the biodiversity and abundance of their larval helminths remains, because data for our analysis using phylogenetical contrasts were insufficient to test this hypothesis, and, in the analysis using species as statistical independent observations, we found that predatory fish species harboured a greater diversity and abundance of larval helminths than herbivorous and planktivorous fishes. This pattern might be the inevitable outcome of predatory fish being exposed to more infective helminth larvae in their diet than planktivores; over evolutionary time, this should translate into higher parasite colonization rates in predatory fish than in planktivorous fish, pushing up the equilibrium parasite species richness (Luque *et al.* 2004).

Our study is the first to investigate the biodiversity and abundance of larval helminths in a representative dataset of

Neotropical marine fishes using modern comparative approaches. Admittedly, the dataset involved samples pooled across seasons and years, and temporal variation in infection levels may have added an element of variability. Still, this is unlikely to have obscured any of the effects we looked for as these all involve species characteristics that do not vary on these time scales. The results of our two analyses (using species values, and using phylogenetic contrasts) are different because of the apparent influence of fish phylogeny. Apparently, some lineages of fish harbour more larval helminths (more species and/or more individuals) than others because of historical reasons (i.e., ancient associations between certain parasite taxa and fish taxa) and not really because of their present ecological characteristics. These results confirm the importance of the phylogeny of the hosts as a confounding factor in any analysis of the influence of host features on parasite species richness and abundance (Poulin and Rohde 1997, Poulin 2001). Nevertheless, our results also suggest that use of fish as intermediate hosts is not random within an ecosystem, extending to a larger scale the results of Poulin and Valtonen (2001).

Acknowledgements. J.L. Luque was supported by a Fellowship from CNPq (Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico) and by two grants from FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro). R. Poulin was supported by a James Cook Research Fellowship from the Royal Society of New Zealand during this study.

References

- Burt A. 1989. Comparative methods using phylogenetically independent contrasts. *Oxford Surveys in Evolutionary Biology*, 6, 33–53.
- Bush A.O., Heard R.W., Overstreet R.M. 1993. Intermediate hosts as source communities. *Canadian Journal of Zoology*, 71, 1358–1363.
- Campbell R.A., Haedrich R.L., Munroe T.A. 1980. Parasitism and ecological relationships among deep-sea benthic fishes. *Marine Biology*, 57, 301–313.
- Carpenter K.E., Collette B.B., Russo J.L. 1995. Unstable and stable classifications of scombroid fishes. *Bulletin of Marine Sciences*, 56, 379–405.
- Chen W.J., Bonillo C., Lecointre G. 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. *Molecular Phylogenetics Evolution*, 26, 262–288.
- Dezfuli B.S., Giari L., Poulin R. 2000. Species associations among larval helminths in an amphipod intermediate host. *International Journal for Parasitology*, 30, 1143–1146.
- Dogiel V.A., Petrushevski G.K., Polyanski Y.I. 1961. Parasitology of fishes. Oliver and Boyd, Edinburgh.
- Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist*, 125, 1–15.
- Figueiredo J.L., Menezes N.A. 1978. Manual de peixes marinhos do sudeste do Brasil II. Teleostei (1). Museu de Zoologia, Universidade de São Paulo, Brazil.
- Figueiredo J.L., Menezes N.A. 1980. Manual de peixes marinhos do sudeste do Brasil III. Teleostei (2). Museu de Zoologia, Universidade de São Paulo, Brazil.
- Figueiredo J.L., Menezes N.A. 2000. Manual de peixes marinhos do sudeste do Brasil VI. Teleostei (5). Museu de Zoologia, Universidade de São Paulo, Brazil.
- Garland T.J.R., Harvey P.H., Ives A.R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology*, 41, 18–32.
- George-Nascimento M. 1987. Ecological helminthology of wildlife animal hosts from South America: a literature review and a search for patterns in marine food webs. *Revista Chilena de Historia Natural*, 60, 181–202.
- Harvey P.H., Pagel M.D. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford.
- Lotz J.M., Bush A.O., Font W.F. 1995. Recruitment-driven, spatially discontinuous communities: a null model for transferred patterns in target communities of intestinal helminths. *Journal of Parasitology*, 81, 12–24.
- Luque J.L., Mouillot D., Poulin R. 2004. Parasite biodiversity and its determinants in coastal marine teleost fishes of Brazil. *Parasitology*, 128, 671–682.
- Marcogliese D.J. 2001. Pursuing parasites up the food chain: Implications of food web structure and function on parasite communities in aquatic systems. *Acta Parasitologica*, 46, 82–93.
- Marcogliese D.J. 2002. Food webs and the transmission of parasites to marine fish. *Parasitology*, 124, S83–S99.
- Menezes N.A., Figueiredo J.L. 1980. Manual de peixes marinhos do sudeste do Brasil IV. Teleostei (3). Museu de Zoologia, Universidade de São Paulo, Brazil.
- Menezes N.A., Figueiredo J.L. 1985. Manual de peixes marinhos do sudeste do Brasil V. Teleostei (4). Museu de Zoologia, Universidade de São Paulo, Brazil.
- Miya M., Takeshima H., Endo H., Ishiguro N.B., Inoue J.G., Mukai T., Satoh T.P., Yamaguchi M., Kawaguchi A., Mabuchi K., Shirai S.M., Nishida M. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Molecular Phylogenetics Evolution*, 26, 121–138.
- Moran M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, 100, 403–405.
- Morand S. 2000. Wormy world: comparative tests of theoretical hypotheses on parasite species richness. In: *Evolutionary biology of host-parasite relationships theory meets reality* (Eds. R. Poulin, S. Morand and A. Skorping). Elsevier Science, Amsterdam, 63–79.
- Morand S., Poulin R. 2003. Phylogenies, the comparative method and parasite evolutionary ecology. *Advances in Parasitology*, 54, 281–302.
- Morand S., Cribb T.H., Kulbicki M., Chauvet C., Dufour V., Faliex E., Galzin R., Lo C., Lo-Yat A., Pichelin S.P., Rigby M.C., Sasal P. 2000. Determinants of endoparasite species richness of New Caledonian Chaetodontidae. *Parasitology*, 121, 65–73.
- Nelson J.S. 1994. Fishes of the world. Wiley, New York.
- Poulin R. 1997. Species richness of parasite assemblages: evolution and patterns. *Annual Review of Ecology and Systematics*, 28, 341–358.
- Poulin R. 2001. Another look at the richness of helminth communities in tropical freshwater fish. *Journal of Biogeography*, 28, 737–743.
- Poulin R., Morand S. 2000. The diversity of parasites. *Quarterly Review of Biology*, 75, 277–293.
- Poulin R., Rohde K. 1997. Comparing the richness of metazoan communities of marine fishes: controlling for host phylogeny. *Oecologia*, 110, 278–283.
- Poulin R., Valtonen E.T. 2001. Interspecific associations among larval helminths in fish. *International Journal for Parasitology*, 31, 1589–1596.

- Purvis A., Rambaut A. 1994. Comparative analysis by independent contrasts, CAIC version 2.0. Oxford University, Oxford.
- Reed D.L., Carpenter K.E., Degraeve M.J. 2002. Molecular systematics of the jacks (Perciformes: Carangidae) based on mitochondrial cytochrome *b* sequences using parsimony, likelihood, and Bayesian approaches. *Molecular Phylogenetics and Evolution*, 23, 513–524.
- Sasal P., Morand S., Guégan J.F. 1997. Determinants of parasite species richness in Mediterranean marine fish. *Marine Ecology Progress Series*, 149, 61–71.
- Simková A., Morand S., Matejusová I., Jurajda P., Gelnar M. 2001. Local and regional influences on patterns of parasite species richness of central European fishes. *Biodiversity Conservation*, 10, 511–525.
- Vickery W.L., Poulin R. 2002. Can helminth community patterns be amplified when transferred by predation from intermediate to definitive hosts? *Journal of Parasitology*, 88, 650–656.
- Walther B.A., Cotgreave P., Price R.D., Gregory R.D., Clayton D.H. 1995. Sampling effort and parasite species richness. *Parasitology Today*, 11, 306–310.

(Accepted August 26, 2004)

Appendix 1. List of larval helminth species on the 50 Brazilian fish species included in the analyses

Host species	Larval helminth species
<i>Aluterus monoceros</i>	didymozoid, <i>Contracaecum</i> sp.
<i>Anchoa tricolor</i>	bucephalid, hemiurid, <i>Rhipidocotyle</i> sp., <i>Polymorphus</i> sp., <i>Hysterothylacium</i> sp., <i>Terranova</i> sp.
<i>Archosargus rhomboidalis</i>	<i>Contracaecum</i> sp.
<i>Balistes capriscus</i>	<i>Callitetrarhynchus speciosus</i> , <i>Nybelinia</i> sp., <i>Scolex pleuronectis</i> , pseudophyllidean, <i>Serrasentis</i> sp., <i>Contracaecum</i> sp., <i>Terranova</i> sp., <i>Raphidascaris</i> sp.
<i>Balistes vetula</i>	<i>Callitetrarhynchus speciosus</i> , <i>Scolex pleuronectis</i> , <i>Contracaecum</i> sp.
<i>Brevoortia aurea</i>	<i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Terranova</i> sp.
<i>Caranx hippos</i>	<i>Callitetrarhynchus gracilis</i> , <i>Nybelinia</i> sp., <i>Contracaecum</i> sp., <i>Terranova</i> sp., <i>Raphidascaris</i> sp.
<i>Caranx latus</i>	<i>Callitetrarhynchus gracilis</i> , <i>Nybelinia</i> sp., <i>Scolex pleuronectis</i> , <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Hysterothylacium</i> sp., <i>Terranova</i> sp.
<i>Centropomus undecimalis</i>	<i>Corynosoma australe</i> , <i>Contracaecum</i> sp.
<i>Cephalopholis fulva</i>	<i>Corynosoma australe</i> , <i>Contracaecum</i> sp., <i>Raphidascaris</i> sp.
<i>Chaetodipterus faber</i>	–
<i>Cynoscion guatucupa</i>	<i>Nybelinia</i> sp., <i>Progrillotia dollfusi</i> , <i>Corynosoma australe</i> , <i>Hysterothylacium</i> sp., <i>Terranova</i> sp.
<i>Dactylopterus volitans</i>	didymozoid, <i>Scolex pleuronectis</i> , <i>Nybelinia</i> sp., <i>Rhadinorhynchus</i> sp., <i>Contracaecum</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp.
<i>Diapterus rhombeus</i>	<i>Nybelinia</i> sp., <i>Corynosoma australe</i> , <i>Contracaecum</i> sp., <i>Raphidascaris</i> sp.
<i>Euthynnus alleteratus</i>	<i>Callitetrarhynchus gracilis</i> , <i>Scolex pleuronectis</i> , <i>Corynosoma australe</i> , <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Raphidascaris</i> sp.
<i>Genypterus brasiliensis</i>	<i>Progrillotia dollfusi</i> , <i>Nybelinia</i> sp., <i>Scolex pleuronectis</i> , <i>Corynosoma australis</i> , <i>Corynosoma</i> sp., <i>Contracaecum</i> sp., <i>Terranova</i> sp., <i>Hysterothylacium</i> sp.
<i>Gymnothorax moringa</i>	didymozoid, <i>Scolex pleuronectis</i> , <i>Hysterothylacium</i> sp., <i>Terranova</i> sp., <i>Raphidascaris</i> sp.
<i>Haemulon steindachneri</i>	didymozoid, <i>Scolex pleuronectis</i> , <i>Serrasentis</i> sp.
<i>Harengula clupeola</i>	<i>Raphidascaris</i> sp.
<i>Macrodon ancylodon</i>	<i>Callitetrarhynchus gracilis</i> , <i>Nybelinia</i> sp., <i>Progrillotia dollfusi</i> , <i>Hysterothylacium</i> sp., <i>Terranova</i> sp.
<i>Menticirrhus americanus</i>	didymozoid, <i>Scolex pleuronectis</i> , <i>Corynosoma australe</i> , <i>Hysterothylacium</i> sp.
<i>Micropogonias furnieri</i>	didymozoid, <i>Scolex pleuronectis</i> , <i>Callitetrarhynchus gracilis</i> , <i>Pterobothrium heteracanthum</i> , <i>Corynosoma australe</i> , <i>Hysterothylacium</i> sp., <i>Terranova</i> sp.
<i>Mugil platanus</i>	<i>Ascocotyle longa</i> , <i>Scolex pleuronectis</i> , phyllobothriid
<i>Mullus argentinae</i>	didymozoid, <i>Nybelinia</i> sp., <i>Heteronybelinia rougetcampanae</i> , <i>Corynosoma australe</i> , <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Raphidascaris</i> sp.
<i>Netuma barba</i>	tetraphyllidean, <i>Polymorphus</i> sp., <i>Contracaecum</i> sp.
<i>Oligoplites palometa</i>	<i>Scolex pleuronectis</i> , <i>Callitetrarhynchus gracilis</i> , <i>Pterobothrium crasicolle</i> , <i>Serrasentis</i> sp., <i>Contracaecum</i> sp.
<i>Oligoplites saliens</i>	<i>Scolex pleuronectis</i> , <i>Contracaecum</i> sp.
<i>Oligoplites saurus</i>	<i>Scolex pleuronectis</i> , <i>Callitetrarhynchus gracilis</i> , <i>Contracaecum</i> sp.
<i>Orthopristis ruber</i>	<i>Scolex pleuronectis</i> , <i>Serrasentis</i> sp.
<i>Pagrus pagrus</i>	<i>Scolex pleuronectis</i> , <i>Corynosoma australe</i> , <i>Corynosoma</i> sp., <i>Anisakis</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Paralichthys isosceles</i>	didymozoid, <i>Nybelinia</i> sp., <i>Pterobothrium</i> sp., <i>Corynosoma australe</i> , <i>Corynosoma</i> sp., <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Paralonchurus brasiliensis</i>	didymozoid, <i>Nybelinia</i> sp., <i>Scolex pleuronectis</i> , <i>Serrasentis</i> sp., <i>Contracaecum</i> sp.
<i>Peprilus paru</i>	<i>Contracaecum</i> sp., <i>Raphidascaris</i> sp.
<i>Percophis brasiliensis</i>	<i>Nybelinia</i> sp., <i>Grillotia</i> sp., <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Pomatomus saltator</i>	didymozoid, <i>Nybelinia</i> sp. 1, <i>Nybelinia</i> sp. 2, <i>Scolex pleuronectis</i> , <i>Corynosoma australe</i> , <i>Anisakis</i> sp., <i>Pseudoterranova</i> sp.
<i>Priacanthus arenatus</i>	<i>Scolex pleuronectis</i> , <i>Corynosoma</i> sp., <i>Contracaecum</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Prionotus punctatus</i>	didymozoid 1, didymozoid 2, <i>Nybelinia</i> sp. 1, <i>Nybelinia</i> sp. 2, <i>Corynosoma australe</i> , <i>Corynosoma</i> sp., <i>Anisakis</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Sardinella brasiliensis</i>	–
<i>Sciaenops ocellatus</i>	<i>Scolex pleuronectis</i> , <i>Callitetrarhynchus gracilis</i>
<i>Scomber japonicus</i>	<i>Scolex pleuronectis</i> , <i>Corynosoma australe</i> , <i>Corynosoma</i> sp., <i>Anisakis</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp.
<i>Scomber scombrus</i>	<i>Anisakis</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp.
<i>Scomberomorus brasiliensis</i>	<i>Scolex pleuronectis</i> , <i>Nybelinia</i> sp., <i>Contracaecum</i> sp.
<i>Selene setapinnis</i>	<i>Callitetrarhynchus gracilis</i> , <i>Nybelinia</i> sp., <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Sphyrna guachancho</i>	didymozoid, <i>Anisakis</i> sp., <i>Raphidascaris</i> sp.
<i>Trachurus lathami</i>	<i>Scolex pleuronectis</i> , <i>Contracaecum</i> sp., <i>Terranova</i> sp.
<i>Trichiurus lepturus</i>	didymozoid, <i>Callitetrarhynchus gracilis</i> , <i>Scolex pleuronectis</i> , <i>Corynosoma</i> sp., <i>Anisakis</i> sp., <i>Contracaecum</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Tylosurus acus acus</i>	didymozoid, <i>Scolex pleuronectis</i> , <i>Hysterothylacium</i> sp., <i>Terranova</i> sp.

<i>Umbrina canosai</i>	<i>Callitetrarhynchus gracilis</i> , <i>Nybelinia</i> sp., <i>Corynosoma australe</i> , <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp., <i>Terranova</i> sp.
<i>Urophycis brasiliensis</i>	<i>Scolex pleuronectis</i> , phyllobothriid, <i>Nybelinia</i> sp., <i>Heteronybelinia</i> sp., <i>Hysterothylacium</i> sp., <i>Raphidascaris</i> sp.
<i>Urophycis mystaceus</i>	didymozoid, <i>Scolex pleuronectis</i> , phyllobothriid, <i>Lacistorhynchus</i> sp., <i>Nybelinia</i> sp., <i>Corynosoma</i> sp., <i>Anisakis</i> sp., <i>Hysterothylacium</i> sp.
