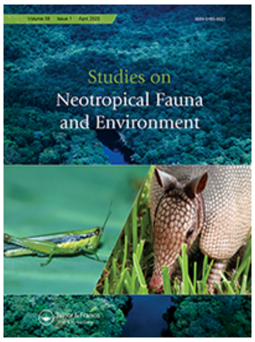

Studies on Neotropical Fauna and Environment

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/nnfe20

Metacercariae of *Clinostomum* Leidy, 1856 (Digenea: Clinostomidae) infecting freshwater fishes throughout Brazil: infection patterns, parasite–host interactions, and geographic distribution

Marcos Tavares-Dias, Luís Mauricio Abdon Silva & Alexandro Cezar Florentino

STUDIES ON NEOTROPICAL FAUNA AND ENVIRONMENT
2023, VOL. 58, NO. 1, 116–129
https://doi.org/10.1080/01650521.2021.1915058

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Metacercariae of *Clinostomum* Leidy, 1856 (Digenea: Clinostomidae) infecting freshwater fishes throughout Brazil: infection patterns, parasite–host interactions, and geographic distribution

Marcos Tavares-Dias [a,b], Luís Mauricio Abdon Silva[c] and Alexandro Cezar Florentino[b]

[a]Laboratório de Aquicultura e Pesca, Embrapa Amapá, Macapá, Brazil; [b]Programa de Pós-Graduação em Biodiversidade Tropical (PPGBio), Universidade Federal do Amapá, Macapá, Brazil; [c]Núcleo de Pesquisas Aquáticas (IEPA/NUPAq), Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (IEPA), Macapá, Brazil

ABSTRACT

This review investigated information about metacercariae of *Clinostomum* Leidy, 1856 published over 91 years (1928 to 2019) to search for infection and geographic distribution patterns of this digenean species in freshwater fish from Brazil. The present study used 101 samples from 60 fish species of different families distributed in Characiformes, Siluriformes, Cichliformes, Gymnotiformes, Cypriniformes, Cyprinodontiformes, and Synbranchiformes. The greatest number of parasite–host associations were found for Cichliformes species of the Cichlidae family. Metacercariae of digeneans that belonged to four species of *Clinostomum* were found, with *Clinostomum marginatum* and *Clinostomum complanatum* (68.7%) the dominant species, whereas 22.2% of parasites were allocated only to the genus *Clinostomum*. There was variation in prevalence, intensity and abundance of *Clinostomum* spp. in host fish, and infection sites included internal and external organs (e.g. intestine, stomach, operculum, muscle, mouth, heart, gills, body cavity, fins). There was similarity in prevalence, intensity, and abundance of *Clinostomum* spp. in detritivorous, omnivorous, and piscivorous host fish. *Clinostomum* metacercariae are distributed in the Amazon, Paraná River, São Francisco, Uruguay, Atlantic North and South, and Southeast basin systems, whereas *C. marginatum* and *C. complanatum* have distribution patterns limited to hydrographic basins of different regions. *Clinostomum* spp. have a cosmopolitan distribution and parasitize a diversity of host fish, and the present study constitutes the most extensive survey regarding these digeneans in fish throughout Brazil.

ARTICLE HISTORY
Received 2 November 2020
Accepted 6 April 2021

KEYWORDS
Digenea; freshwater fish; hosts; infection; parasites

Introduction

Clinostomum spp. of the family Clinostomidae Lühe, 1901 (Trematoda) are digeneans that were established to include *Clinostomum gracile* Leidy, 1856, a species of metacercariae in the intestine of *Esox* sp. (Esocidae), as well as in the gills, fins and muscles of the *Lepomis gibbosus* Linnaeus, 1758 (Centrarchidae). *Clinostomum* spp. occur in freshwater and estuarine systems worldwide and have a complex life cycle (Calhoun et al. 2020). The adult stage is commonly found in the buccal cavity and esophagus of fish-eating birds as the definitive hosts. Snails are first intermediate hosts harboring sporocysts, whereas fish, reptiles and amphibians are second intermediate hosts harboring the metacercarial stage. The life cycle completes when fish-eating birds ingest infected second intermediate hosts. The parasite goes through a migration from deeper tissues of the hosts before becoming adult in the anterior part of the digestive system of fish-eating birds (Dias et al. 2003; Sereno-Uribe et al. 2013, 2018; Shamsi et al. 2013; Pinto et al. 2015; Acosta et al. 2016; Wang et al. 2017; Briosio-Aguilar et al. 2018; Locke et al. 2019; Calhoun et al. 2020). According to Calhoun et al. (2020), several *Clinostomum* species have been reported in over 40 species of birds from six continents. Eggs are released by these definitive hosts into aquatic environments, where they hatch into miracidia and develop into sporocysts upon penetration into mollusks of the genera *Lymnaea*, *Radix*, *Bulinus*, *Biomphalaria*, *Planorbella*, *Helisoma*, etc. (Klass 1963; Dias et al. 2003; Pinto et al. 2015; Wang et al. 2017; Calhoun et al. 2020).

Fish are intermediate hosts for different *Clinostomum* metacercariae, which cause severe damage to hosts as they migrate into fish muscle tissues (Eiras et al. 1999; Malek & Mobedi 2001; Shareef & Abidi 2012; Tansatit et al. 2014; Yasumoto et al. 2018; Calhoun et al. 2020; Shamsi et al. 2021). Infections of *Clinostomum* metacercariae can lead to economic losses in fish farming and the fishing industry,

CONTACT Marcos Tavares-Dias marcos.tavares@embrapa.br

Published online 19 May 2021

where *Clinostomum* infections may be observed through inspection by a sanitation agency or the fish product may be rejected by the consumer (Klass 1963; Kagei et al. 1984; Vianna et al. 2005; Shareef & Abidi 2012; Lima et al. 2014; Aghlmandi et al. 2018; Yasumoto et al. 2018). Parasites can also affect the host fish population by causing physiological and reproductive damage, which can diminish wild fish stocks. However, damage to the host fish depends on the nature of the effects, parasite abundance and environmental factors. Human health can be affected by eating fish infected with *Clinostomum* metacercariae, as this can cause parasitic pharyngitis and laryngitis (Acosta et al. 2016; Wang et al. 2017; Sereno-Uribe et al. 2018; Yasumoto et al. 2018). The practice of eating improperly cooked or raw fish is a public health problem that affects various human populations around the world, particularly impoverished groups in developing countries (Chai et al. 2005).

Clinostomum species are broadly distributed geographically and their metacercariae have a high degree of morphological inconsistency, and hence, the taxonomy of these digeneans has been the subject of discussion. For example, Caffara et al. (2011) affirmed the validity of *Clinostomum marginatum* Rudolphi, 1819 across North America with novel genetic data and morphological analyses of two life-cycle stages. Sereno-Uribe et al. (2013) showed that *C. marginatum* and not *C. complanatum* Rudolphi, 1814 is the species found in Mexico. Furthermore, *C. marginatum* has been proposed as the digenean species of hosts from the Americas, while *C. complanatum* is the species of hosts from Europe and is restricted to the Palearctic region (Caffara et al. 2011; Sereno-Uribe et al. 2013). Several congeneric species of *Clinostomum* have been recognized in the Americas, but uncertainty exists regarding the metacercariae of these species occurring in freshwater fish (Sereno-Uribe et al. 2013), as both *C. complanatum* and *C. marginatum* continue to be found in hosts across North and South America (Sereno-Uribe et al. 2013), including Brazil. Recently, Acosta et al. (2016) demonstrated that *C. complanatum, Clinostomum cutaneum* Paperna, 1964, *Clinostomum phalacrocoracis* Dubois, 1930, and *Clinostomum philippinense* Velasquez, 1960 occur in the Palearctic region, while *C. marginatum, Clinostomum tataxumui* Sereno-Uribe, Pinacho-Pinacho, García-Varela & Pérez-Ponce de León, 2013, and *Clinostomum detruncatum* Braun, 1899 are from Nearctic and Neotropical regions. This latter clade was divided into two subclades, one grouping species from North America and Mexico (*C. marginatum* and *C. tataxumui*), and the other grouping species from Brazil (*C. detruncatum* and *Clinostomum* sp.). *Clinostomum heluans* Braun, 1899 is a New World species and was originally described of *Egretta caerulea* Linnaeus, 1758 from Brazil. There are currently 31 species of *Clinostomum* that are recognized and recorded from all continents other than Antarctica (Briosio-Aguilar et al. 2018).

Some species of *Clinostomum* metacercariae have been reported in Brazil, a vast country with various aquatic ecosystems such as the Amazon River system and a high diversity of approximately 3500 freshwater fish species (Froese & Pauly 2019). Acosta et al. (2016) listed *Clinostomum* spp. for 31 species of freshwater fish in Brazil. However, the distribution of these digeneans throughout Brazil and their infection patterns in host fish are still unknown. Thus, the present study aimed to characterize the distribution patterns of *Clinostomum* spp. associated with Brazilian freshwater fish hosts in the different basins throughout the country. This study is of great interest because the knowledge of the diversity of *Clinostomum* spp. in fish in Brazil may contribute to global estimates of the diversity and distribution of these digeneans in freshwater fish. The present study is also important in regard to biogeography because it records the known distribution of these parasites in different fish species, of which many have been translocated inside Brazil and exported to different places around the world. In addition, understanding the factors that shape regional geographic and infection distribution patterns of parasite species in host fish is a central question in parasite ecology. However, such patterns remain unknown for *Clinostomum* species, impeding the elaboration of global maps of the geographic distributions of these digeneans.

Materials and methods

A review on the *Clinostomum* species in freshwater teleost fish of Brazil was performed by searching databases (Scielo, ISI, Scopus, Science Direct, Zoological Records, CAB Abstracts, Lilacs, Capes periodicals and Google Scholar), and available data in 63 scientific articles were systematized and used. A dataset of *Clinostomum* spp. parasitizing freshwater fish populations in Brazil was compiled using taxonomic descriptions of species and surveys on the occurrences of these parasites published between 1928 and 2019. These data comprised surveys on *Clinostomum* species of native fish in rivers, lakes, lagoons and reservoirs distributed throughout Brazil, except for two samples of cyprinids, *Cyprinus carpio* (Linnaeus, 1758) and *Ctenopharyngodon idella* (Valenciennes, 1844), and one sample of the cichlid *Oreochromis niloticus* (Linnaeus, 1758), which are non-native fish present in Brazilian fish farming, as well as three samples of *Rhamdia quelen* (Quoy & Gaimard, 1824), two samples of *Piaractus brachypomus* (Cuvier 1818) and one sample of *Salminus brasiliensis* (Cuvier 1816) from fish farming.

These surveys were chosen because they represent the various ecosystems found in Brazil and may aid in revealing the distribution of *Clinostomum* species in host fish. We used sample data of prevalence, intensity and abundance obtained from published research. In addition, samples of farmed fish were also included, as fish farming is linked to natural water bodies in some regions (rivers, lakes, streams, várzea, and floodplains) and because non-native fish species have been translocated between regions of Brazil. A non-statistical comparison was performed between the parasite samples of wild fish (N = 92) and aquaculture fish (N = 9), since most of the samples were from wild fish populations.

Trophic level was obtained from Froese and Pauly (2019) for each host species and the sampling unit was considered as the number of individuals parasitized by a *Clinostomum* species at a certain location and time. Some of the information used in samples included data on more than one host species. The data were organized in a data frame (extension '.txt') with a list of the following variables: (i) number of fish examined, (ii) number of fish parasitized, (iii) parasite species, (iv) infection site, (v) mean prevalence, (vi) mean intensity, and (vii) mean abundance; along with categorical factors such as: (i) host fish species, (ii) location of sample collection, and (iii) mean length and weight of the hosts. These variables and factors were analyzed with the aim of producing a classification according to groups of parasites, using R with the 'package bipartite' (Dormann et al. 2008; Dormann 2011; R Development Core Team 2017) or similarities for the variables. Spearman's correlation coefficient (*rs*) was also used with log transformed data of correlation analyses of prevalence, abundance, and intensity of *Clinostomum* spp. with the length and weight of hosts. Studies with inconsistent data or outliers related to the host and/or parasite (i.e. parasite infestation site and/or host collection locality) were not included in any of the analyses.

Differences in similarity among the trophic levels (detritivorous, omnivorous and piscivorous) of hosts was based on ranked matrices generated from the Jaccard index (presence/absence) and the Bray–Curtis index (abundance) (Magurran 2004), with 10,000 permutations. Non-metric multidimensional scaling (NMDS) with the Bray–Curtis distances matrix, using *Clinostomum* spp. abundance of hosts according to the trophic levels, was used to visualize the pattern of similarity between host species. In this analysis, we used the R statistical environment (R core team 2017) and the 'Vegan' library (Oksanen et al. 2017).

Brazil has approximately 12% of the world's availability of water resources, i.e. 1.5 million m^3 s^{-1}. The country has a diverse range of main basins: Amazon, Tocantins-Araguaia, North Atlantic, South Atlantic, Southeast Atlantic, São Francisco, Paraná, Paraguay, and Uruguay, which present different hydrodynamic related to differenced factors (e.g. size, urban occupation, exploration of electric power and industries, and water quality). Shapes that encompassed the eight largest continental hydrographic basin systems of Brazil were used to construct a map of the geographical distributions (http://hidroweb.ana.gov.br/HidroWeb.asp?TocItem=4100).

The ecological terms used are those recommended by Rohde et al. (1995) and Bush et al. (1997).

Results

Our search resulted in a total of 101 samples of *Clinostomum* spp. that were found to parasitize 60 fish species of different families distributed into 42 host genera of seven orders. There were four species of digeneans, of which *C. marginatum* and *C. complanatum* were the dominant parasites (68.7%), but other species (22.2%), have been allocated only at the genus level (Table 1 and Figure 1).

The greatest numbers of parasite–host associations were observed for Cichliformes species of the Cichlidae family (Table 2), which is an abundant fish species in Brazil. In farmed *C. carpio, C. idella*, and *O. niloticus*, only *C. complanatum* was found.

Variations in prevalence (0.5–100%), intensity (1.0–-93.8), and abundance (0.006–37.2) of infection were observed for metacercariae of *Clinostomum* spp. in host fish (Figure 2). In addition, *Clinostomum* spp. infection sites occurred in both internal (e.g. liver, heart, stomach, eyes, body cavity, gonads) and external organs (e.g. operculum, gills, mouth, tegument, tongue, fins) (Figure 3), because these are ecto- and endoparasites of fish.

No correlation between the length of hosts and prevalence (*rs* = 0.135, p = 0.353, n = 49), intensity (*rs* = –0.115, p = 0.498, n = 38), and abundance (*rs* = 0.109, p = 0.507, n = 39) of *Clinostomum* spp. was found. No correlation between the weight of hosts and prevalence (*rs* = 0.196, p = 0.217, n = 41), intensity

Table 1. Number of *Clinostomum* spp. of the network in 101 samples of freshwater fish from Brazil.

Parasite species	Parasite samples	Host species	Host family	Host order
Clinostomum marginatum	37	25	10	4
Clinostomum complanatum	32	16	11	5
Clinostomum detruncatum	8	5	4	4
Clinostomum heluans	1	2	1	1
Clinostomum sp.	23	18	11	4

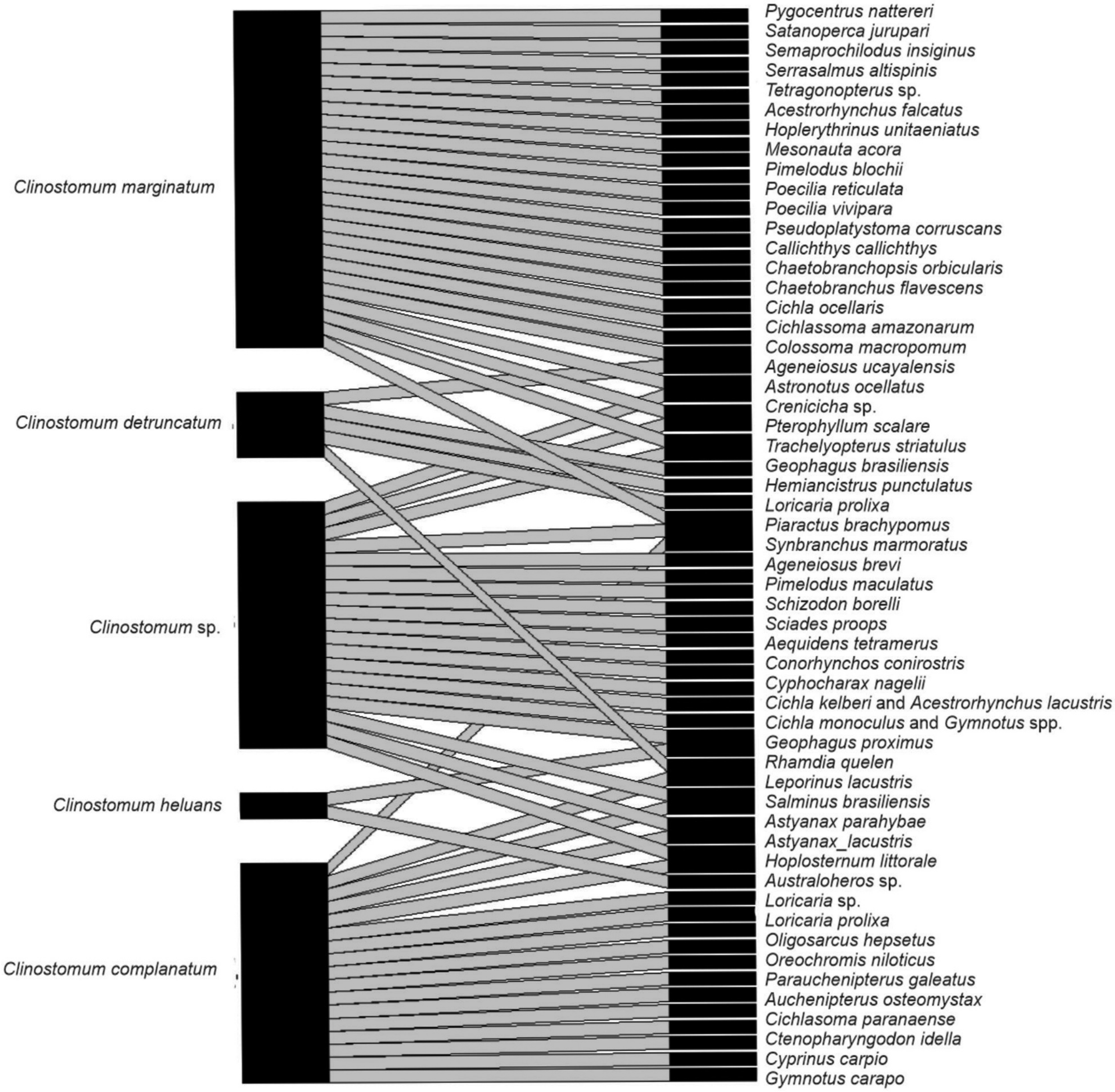

Figure 1. Network of interactions between species of host fish (n = 60) from Brazil and species of *Clinostomum*.

(rs = 0.049, p = 0.791, n = 31), and abundance (rs = 0.290, p = 0.107, n = 32) of *Clinostomum* spp. was found.

In samples of detritivorous (n = 9), omnivorous (n = 40), and piscivorous (n = 24) hosts, no differences in prevalence, intensity, or abundance of infection for metacercariae of *Clinostomum* spp. were shown (Figure 4). In addition, the clustering analysis according to trophic levels of host fish for *Clinostomum* spp. presented a cophenetic correlation coefficient of 0 with 1000 permutations. This showed that there was a similarity among the trophic levels of the hosts as the detritivorous, piscivorous and omnivorous fish showed no differences (Figure 5). However, all of the results were influenced by a low number of samples in the observed published articles.

The NMDS order indicated that *Clinostomum* spp. presented a moderate dissimilarity between trophic levels of host fish according to stress = 0.030 (Figure 6).

Clinostomum metacercariae presented geographic distribution for host fish from the Amazon, Paraná River, São Francisco, Uruguay, Atlantic North and South, and Southeast basin systems. However, *C. marginatum* and *C. complanatum* had a distribution pattern limited to hydrographic basins of different regions, while undetermined *Clinostomum* species had a wide occurrence (Figure 7).

Table 2. Richness of *Clinostomum* spp. by taxonomic groups in 60 freshwater host fish of Brazil.

Host order	Host family	Host species number	Parasite species richness
Characiformes	Acestrorhynchidae	2	2
	Anostomidae	2	2
	Ariidae	1	1
	Bryconidae	1	1
	Characidae	6	3
	Curimatidae	1	1
	Erythrinidae	2	3
	Prochilodontidae	1	1
	Serrasalmidae	4	2
Siluriformes	Callichthyidae	2	3
	Heptapteridae	1	2
	Auchenipteridae	3	2
	Pimelodidae	4	2
	Loricariidae	4	2
	Ageneiosidae	2	2
Cichliformes	Cichlidae	17	4
Gymnotiformes	Gymnotidae	2	2
Cypriniformes	Cyprinidae	2	1
Cyprinodontiformes	Poeciliidae	2	1
Synbranchiformes	Synbranchidae	1	1

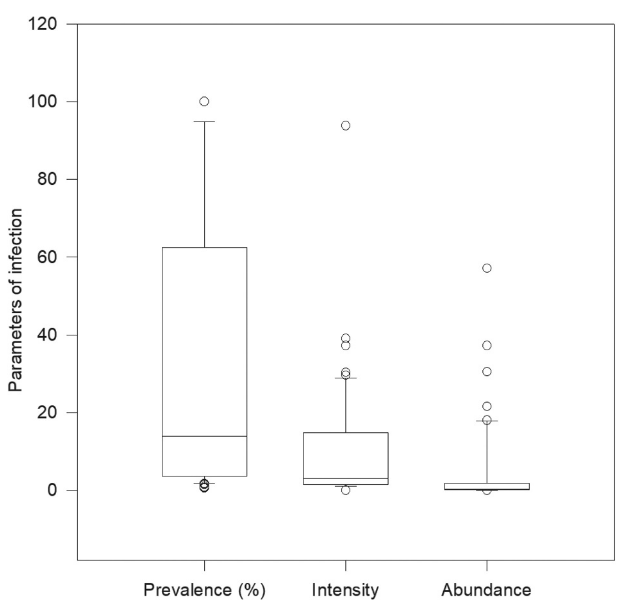

Figure 2. Quantitative descriptors of infection for *Clinostomum* spp. in 73 samples with 43 species of freshwater fish from Brazil (box plots represent medians, interquartile ranges (25–75%), minimum–maximum values and outliers).

Discussion

Distribution pattern in parasite-host interactions

As parasitism is a chance phenomenon, its nature is regulated by several factors that determine the infection levels of the parasites in host fish communities, and thus it influences ecological and evolutionary processes. In host fish, infection patterns with endoparasites may be determined by interactions among local species and the presence of infective stages in the ecosystem (Klass 1963; Shah et al. 2013; Khan et al. 2018; Calhoun et al. 2020). Despite the ubiquity of *Clinostomum* spp. in freshwater fish of diverse basin systems in Brazil, the distribution and infection patterns are currently unknown. In the present study, some patterns were detected in the *Clinostomum* metacercariae community in Brazilian freshwater fish populations: (a) parasites were predominantly of four taxa (*C. complanatum, C. marginatum, C. detruncatum* and *C. heluans*; most of which were *C. marginatum* and *C. complanatum*); (b) there was a predominance of endoparasites rather than ectoparasites, (c) co-infection occurred with other infracommunities of parasites (ecto and endoparasites) in hosts, with prevalence varying from low to moderate, but low in intensity and abundance, and (d) there was a lack of correlation between the abundance of parasites and host body size at the infracommunity level. Ecological relationships of hosts are important in determining the distribution of *Clinostomum* spp. The occurrence and infection patterns of these endohelminths are influenced by food habits and foraging patterns of host fish, and consequently by the variety of prey species selected by the secondary intermediate host fish (Shah et al. 2013), as well as by the presence of final hosts (i.e. fish-eating birds) in the environment and suitable environmental and/or biological factors for the completion of the parasite life cycle. Acosta et al. (2016) reported that *C. complanatum, C. marginatum, C. detruncatum*, and *C. heluans* were found in birds in addition to the 31 species of host fish in Brazil.

Around 3500 different freshwater fish species are found in Brazil (Froese & Pauly 2019), of which less than 2% have been examined for *Clinostomum* metacercariae, making the diversity of these digeneans difficult to predict. The proportion of host fish species examined for parasites varies widely across the Americas, e.g. from 45% in Mexico to less than 5% in South American countries (Choudhury et al. 2016). Metacercariae of *Clinostomum* can live in secondary intermediate hosts for up to four years and develop into adults once a suitable definitive host consumes an infected secondary host (Calhoun et al. 2020).

Clinostomum metacercariae of host fish species in Brazil show little diversity. For trematode species, their biology is shaped by their own evolutionary histories, those of their hosts, and the trophic webs that they are inhabit (Choudhury et al. 2016), as expected also for metacercariae of *Clinostomum* spp. Given that *Clinostomum* spp. metacercariae are mainly endoparasites of fish, they have a life cycle that involves transmission into the trophic web through intermediate and definitive hosts. Consequently, the transmission of these

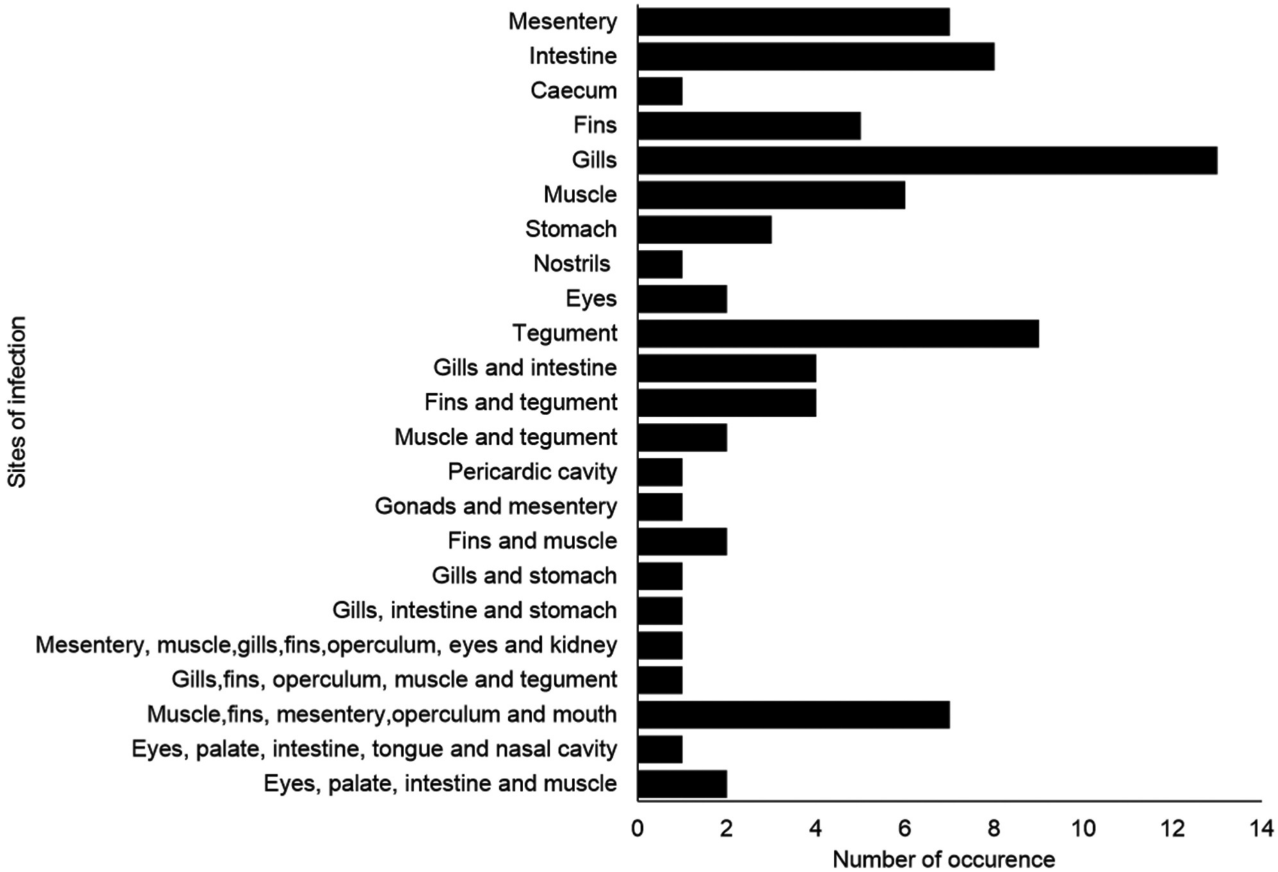

Figure 3. Infection sites of *Clinostomum* spp. in 73 samples with 43 species of freshwater fish from Brazil.

digeneans depend on predator–prey interactions in ecosystems. Hence, the trophic levels of the host fish can be used as predictors of the trophic web of fish assemblages. In this manner, it can be assumed that host fish species of higher trophic levels are exposed to greater numbers of metacercariae than fish species of low trophic levels, which have less contact with infective stages of *Clinostomum* species in their food than fish of higher trophic levels. In contrast, in the present study the trophic levels of detritivorous, omnivorous and piscivorous fish showed no influence on infection parameters for *Clinostomum* spp. However, the small sampling of host fish available in our study, particularly of detritivorous species, may have influenced these results.

Infection parameters of endoparasites that are used to quantify parasite populations or the severity of infections in fish assemblages are subject to variation because such parameters are derived from complex interactions of phylogeny, ecology and life history of parasites and host fish (Klass 1963; Shah et al. 2013; Fedorčák et al. 2019; Calhoun et al. 2020). As endoparasites can have negative effects on individual and population host fish, to evaluate the extent to which the effects translate to the population and community level depends strongly on the nature of the individual effects, the abundance and biomass of parasites, and other environmental factors, which influence the interactions between hosts and the consequences of the host–parasite interactions. The present study detected infections by metacercariae of *C. marginatum, C. complanatum, C. detruncatum, C. heluans*, and *Clinostomum* spp. in 60 host fish species of Brazil, and the occurrence of infections was more frequent in the viscera>gills>tegument. Metacercariae of *Clinostomum* spp. can inhabit any organ of the host organism (e.g. operculum, liver, heart, gills, body cavity, fin) and may cause severe damage to tissues of infected fish (Klass 1963; Tansatit et al. 2014; Wang et al. 2017; Yasumoto et al. 2018; Fedorčák et al. 2019; Calhoun et al. 2020). Aghlmandi et al. (2018) reported that metacercariae of *C. complanatum* were found mostly on the skin of some host fishes. However, one metacercariae was found in the brain of *Alburnoides bipunctatus* (Bloch, 1782), suggesting that this digenean species is able to migrate to the vital organs of fish, and consequently can affect their functions. Khan et al. (2018) reported infection by *C. complanatum* metacercariae in peritoneal cavity>viscera>gills of *Trichogaster fasciatus* Bloch and Schneider, 1801. In addition, we detected variation in prevalence, intensity, and

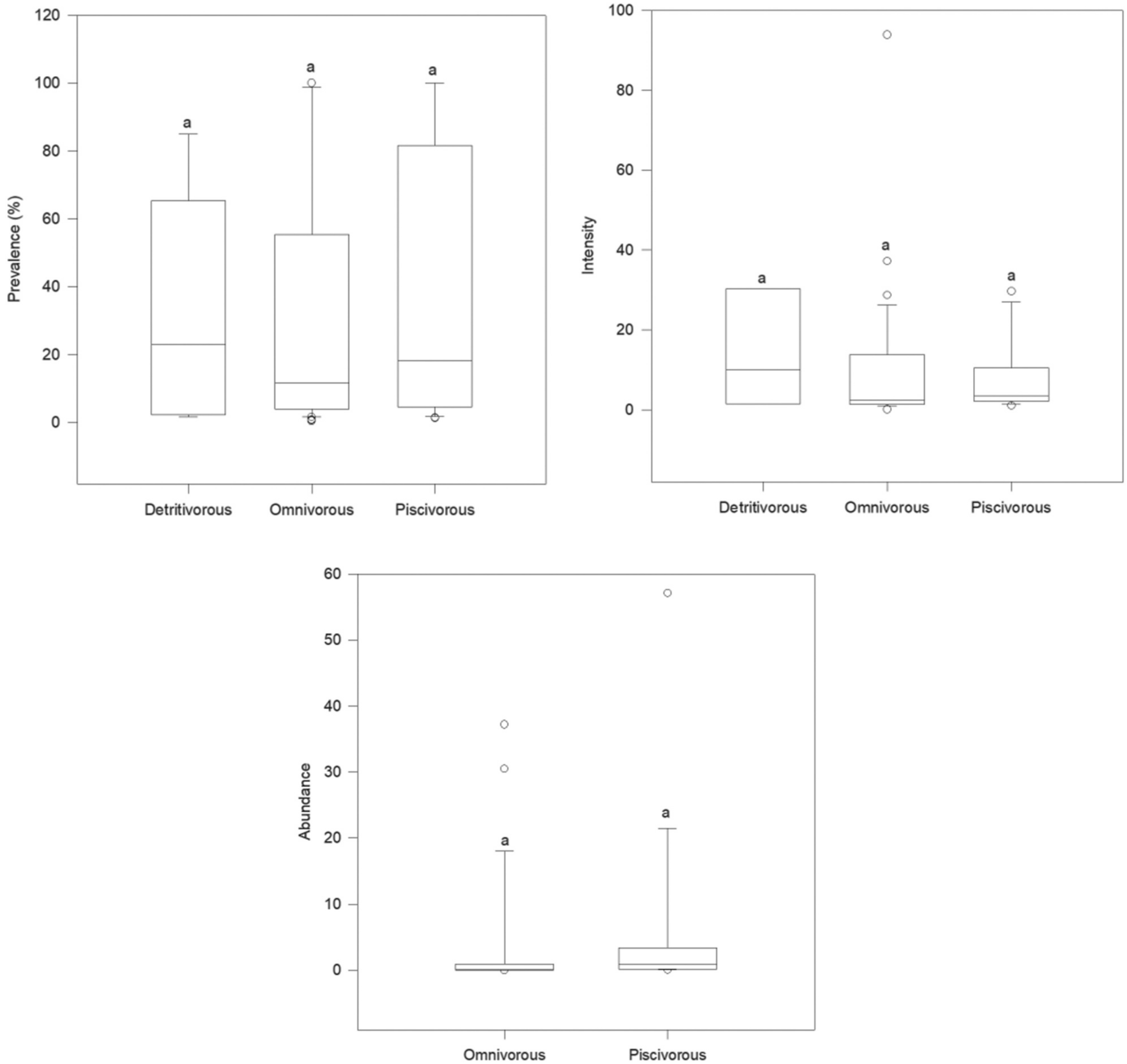

Figure 4. Quantitative descriptors of infection by *Clinostomum* spp. in 73 samples with 43 species of freshwater fish from Brazil (box plots represent medians, interquartile ranges (25–75%), minimum–maximum values and outliers). Different letters indicate no similarity according to Dunn's test ($p > 0.05$).

abundance of infection of *Clinostomum* sp. metacercariae, which may reflect the fluctuations in environmental conditions and in the presence of infected mollusks, the primary intermediate hosts for these digeneans. Such variations in host fish suggest that determinant biotic and abiotic factors of the infection have an influence on the primary and secondary intermediate hosts in ecosystems (Klass 1963; Malek & Mobedi 2001; Shah et al. 2013; Pinto et al. 2015; Wang et al. 2017; Khan et al. 2018; Fedorčák et al. 2019) of the different hydrographic basins investigated here. Since such factors serve as a limitation for the availability of infected snail fauna, they may have a direct effect on the prevalence, intensity and abundance of *Clinostomum* species in the secondary intermediate host fish.

Osborn (1911) attempted to understand the distribution and occurrence of *C. marginatum* in fish across USA and Canada, and observed infections on the gills, mouth, fins, musculature, abdominal cavity, and intestine of host fish. In the Paraná River system of Brazil, the prevalence of infection by *C. complanatum* metacercariae was evaluated in *Loricariichthys platymetopon* (Isbrücker & Nijssen, 1979), *Hoplosternum littorale* (Hancock, 1828), *Parauchenipterus galeatus* (Linnaeus, 1766), *Hoplias*

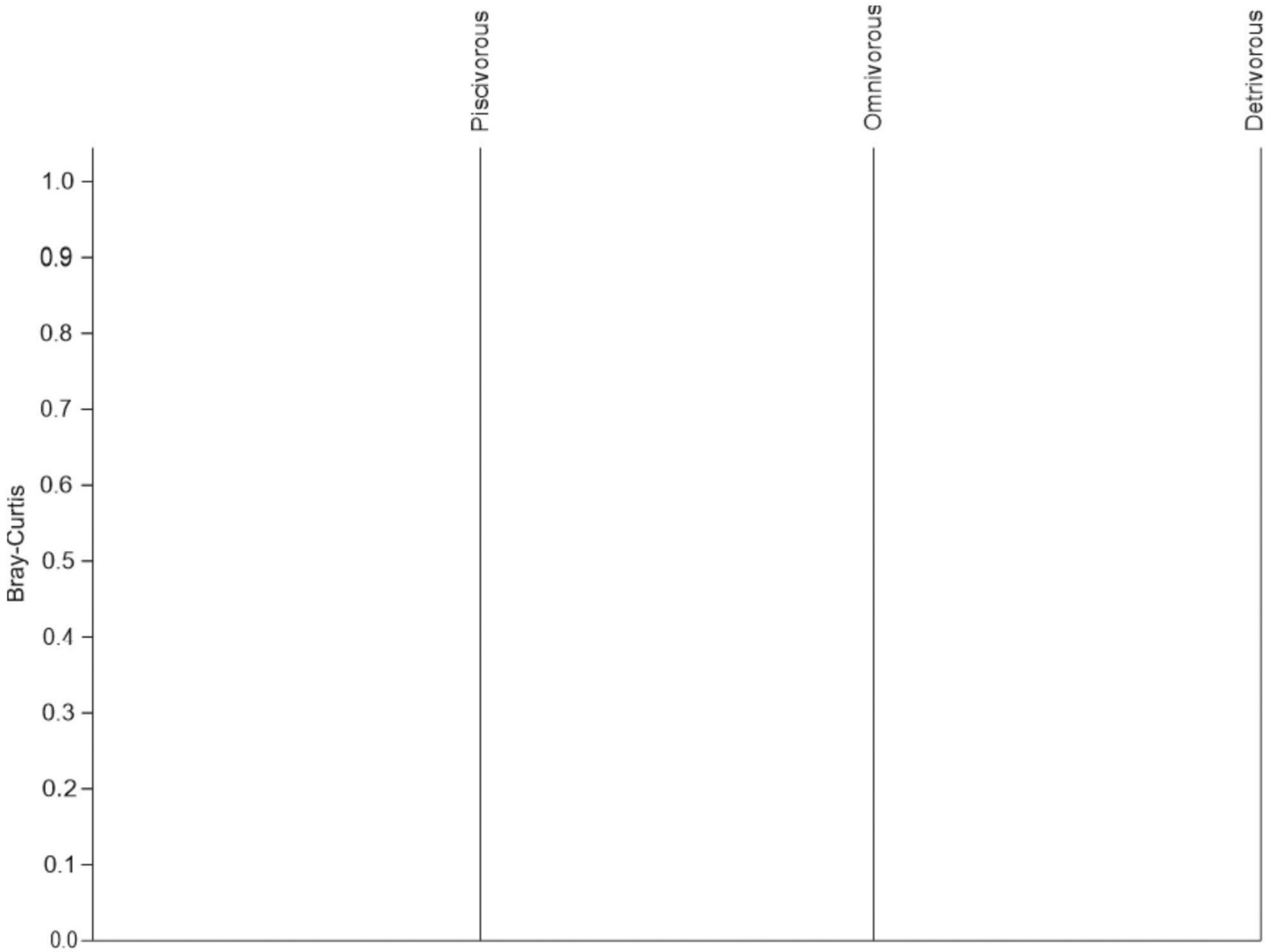

Figure 5. Dendrogram for similarity of Bray–Curtis index for community of *Clinostomum* spp. in host fish of different trophic levels from Brazil.

malabaricus (Bloch, 1794) and *Loricaria* sp. and varied from 2.4% to 60.8% (Dias et al. 2003). Infection by *C. complanatum* has also been reported in the operculum, mandible, muscle and mouth of 23 species of Cyprinidae, Bagridae, Balitoridae, Gobiidae and Cichlidae of five rivers in northwestern Taiwan, with a total prevalence of 9.4% and with a mean intensity of 9.3 per host (Wang et al. 2017). Pérez-Ponce de León et al. (2016) reported the occurrence of *C. complanatum* metacercariae in 76 fish species belonging to 13 families, in two localities in Nicaragua, six species of Costa Rica and in 101 fish species of Mexico. Maleki et al. (2018) reported infection by *C. complanatum* metacercariae in 14 fish species of the Gheshlagh River basin in western Iran. In addition, these authors cited that global estimates for such metacercariae in host fish exceeded 100 species. In 23 fish species of the Nakdong-gang River systems in Korea, the prevalence of infection by *C. complanatum* metacercariae was low, varying from 15.4 to 19.3% and with an intensity of 5.8 to 9.8 per host (Sohn et al. 2019). Recently, Calhoun et al. (2020) analyzed the patterns of infection by *C. marginatum* metacercariae in seven fish species from the Bay Area of California (USA) and found a prevalence that varied from 0 to 4.2% and an intensity of 0 to 7.7 per host. The infection pattern by *C. complanatum* in host fish populations has been well addressed due to their broad geographic distribution. Given the wide distribution and mobility of the main definitive hosts of *C. complanatum* (Ardeidae birds), the broad geographic range of this parasite is plausible, although many records of this digenean species have been questioned (Locke et al. 2019). Nevertheless, studies using molecular biology for this species across its geographic distribution may resolve questions related to its taxonomy.

In Brazil, the first intermediate hosts for *Clinostomum* metacercariae in the different regions are still unknown, except for *C. complanatum* from the upper Paraná River; its cercariae have been reported in *Biomphalaria peregrina* d'Orbigny 1835. In addition, the highest prevalence among fish was found in *L. platymetopon* (60.8%), and among birds was in *Ardea cocoi* (95%) (Dias et al. 2003). Pinto et al. (2015) reported *Clinostomum* sp. cercariae in

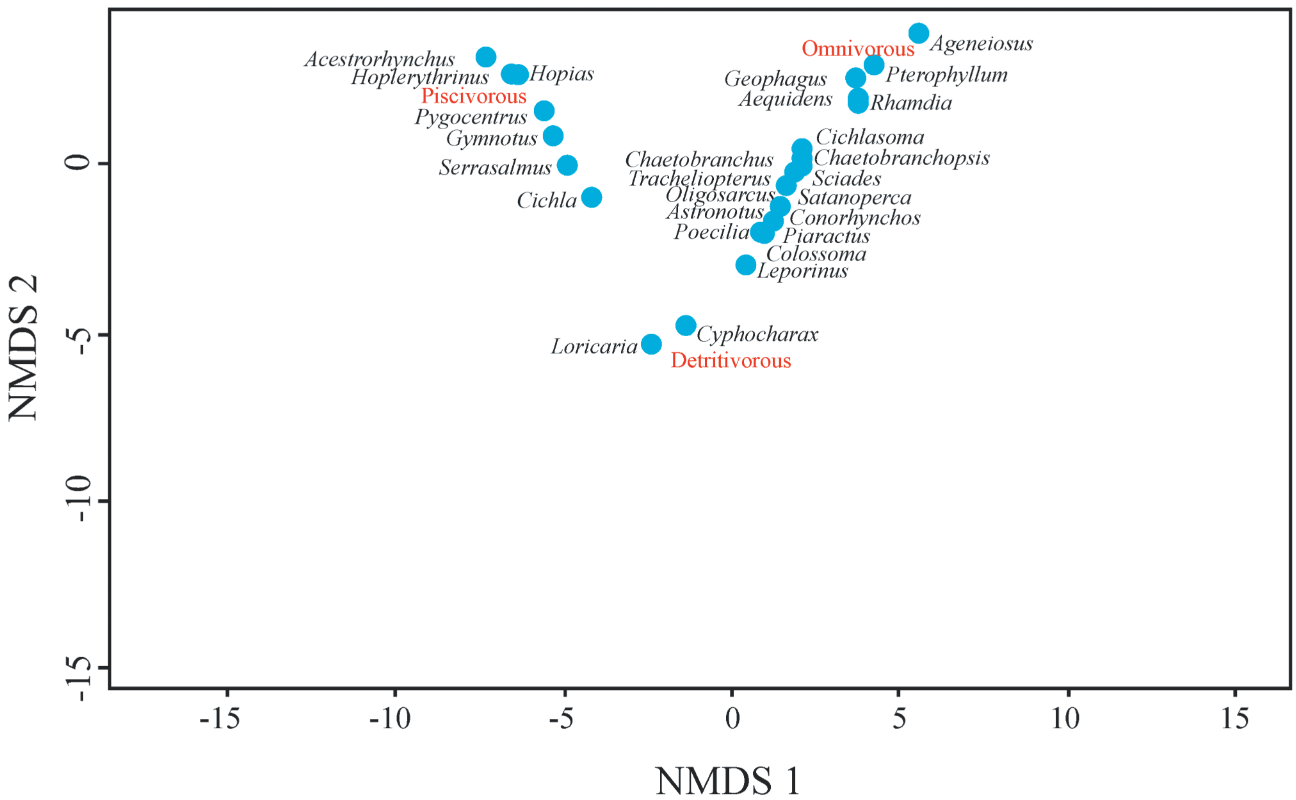

Figure 6. Non-metric multidimensional scaling (NMDS) for *Clinostomum* spp. in host fish of different trophic levels from Brazil. Acestrorhynchus: *Acestrorhynchus falcatus*, Hoplerythrinus: *Hoplerythrinus unitaeniatus, Pygocentrus: Pygocentrus nattereri*, Gymnotus: *Gymnotus* spp. and *Gymnotus carapo*, Serrasalmus: *Serrasalmus altispinis*, Cichla: *Cichla monoculus*, Loricaria: *Loricaria prolixa*, Ageneiosus: *Ageneiosus ucayalensis*, Pterophyllum: *Pterophyllum scalare*, Geophagus: *Geophagus brasiliensis* and *Geophagus proximus*, Rhamdia: *Rhamdia quelen*, Conorhynchos: *Conorhynchos conirostris*, Chaetobranchopsis: *Chaetobranchopsis orbicularis*, Chaetobranchus: *Chaetobranchus flavescens*, Astronotus: *Astronotus ocellatus*, Satanoperca: *Satanoperca jurupari*, Cichlasoma: *Cichlasoma paranaense*, Hoplias: *Hoplias malabaricus*, Aequidens: *Aequidens tetramerus, Oligosarcus: Oligosarcus hepsetus*, Trachelyopterus: *Trachelyopterus striatulus*, Ageneiosus: *Ageneiosus ucayalensis*, Colossoma: *Colossoma macropomum*, Piaractus: *Piaractus brachypomus*, Loricaria: *Loricaria prolixa*, Poecilia: *Poecilia reticulata*, Auchenipterus: *Auchenipterus osteomystax*, Leporinus: *Leporinus lacustris*, Sciades: *Sciades props*, Cyphocharax: *Cyphocharax nagelii.*

Biomphalaria glabrata Say, 1818; *Biomphalaria straminea* Dunker, 1848 and *Biomphalaria tenagophilla* Orbigny, 1835 at the Pampulha Reservoir, in Belo Horizonte, Minas Gerais state in Brazil. Hence, an important aim for future studies would be to determine and identify the natural first intermediate hosts for the *Clinostomum* spp. in other regions of Brazil. In *Carassius carassius* populations, the populations of *Clinostomum schizothoraxi* Kaw, 1950 differed in abundance between three lakes. The prevalence, abundance and intensity of infection had a seasonal influence based on water temperature. Lake environments showed a high degree of variability in the density of mollusks, the first intermediate hosts, and thus the differences in the infection levels were caused by the density of these intermediate hosts in the environment (Shah et al. 2013).

In samples of host fish in the present study, no correlation was detected for the abundance of *Clinostomum* spp. with the body size of host fish. Similar findings were reported by Daly et al. (2002) for *Micropterus dolomieu* Lacepède, 1802 infected by *C. marginatum*. In contrast, Wang et al. (2017) analyzed the correlation of body size with the prevalence and intensity of *C. complanatum* metacercariae for four fish species and found correlations only for *Zacco pachycephalus* Günther, 1868. For fish populations, the pattern of infection by *C. marginatum* metacercariae suggested that hosts with a longer lifespan and larger body size supported a higher prevalence of the parasites (Calhoun et al. 2020; Mohammed et al. 2020), presumably because larger fish are older and may accumulate parasites over time.

Geographic distribution pattern in Brazilian fish

The establishment of geographic distribution patterns of parasites is currently one of the main goals in fish

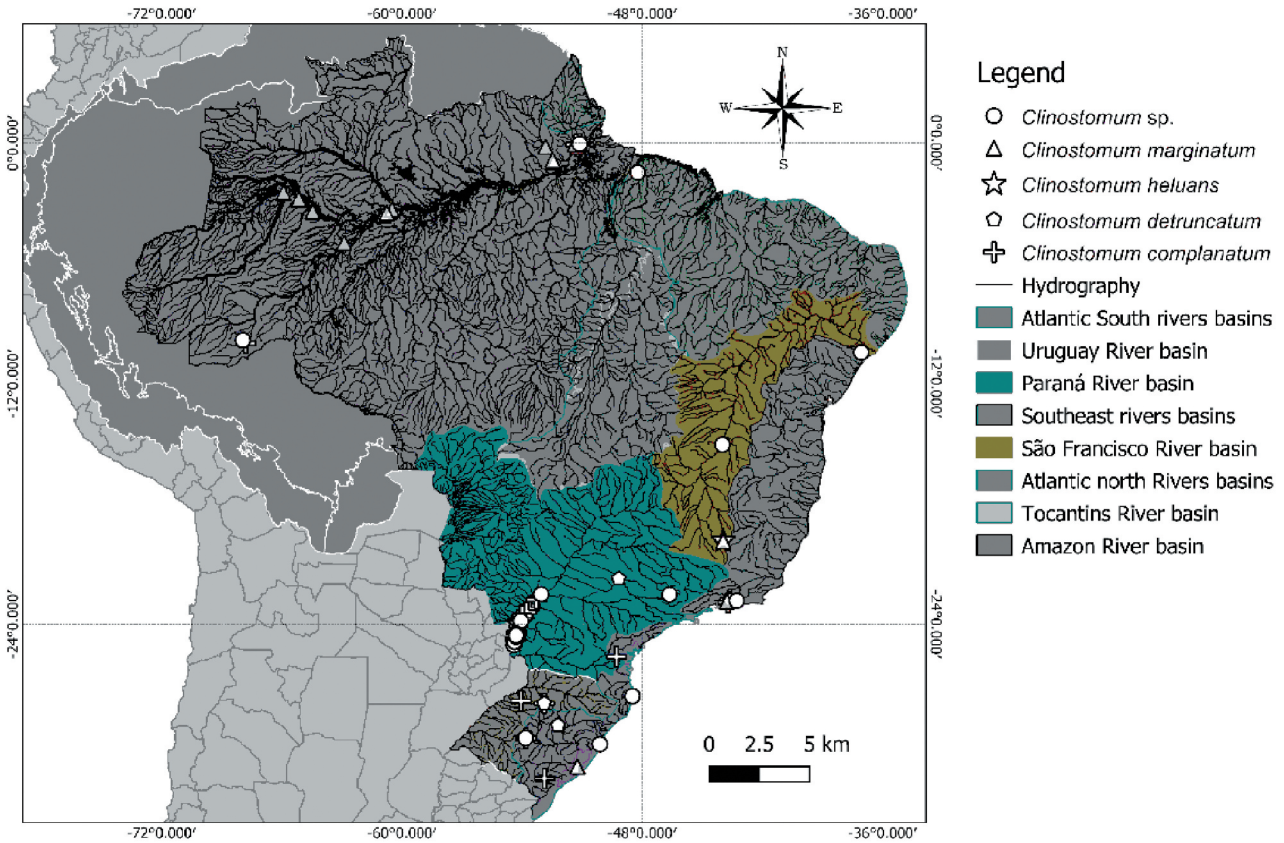

Figure 7. Geographic distribution of *Clinostomum* species in freshwater host fish across hydrographic basin systems in Brazil.

parasitology. Hence, efforts to characterize parasite species in fish hosts are crucial for monitoring and mitigating disease threats in fisheries and aquaculture in the face of global climate changes (see Poulin et al. 2020). Despite the rich diversity of fish fauna in South America, there is little knowledge about the distribution of digenean species across biomes of this Neotropical region. Digenea is the second richest taxon of helminths in fish species in South America, with around 662 known species (Luque et al. 2017), but the geographic distribution of digenean species has been little addressed (Negreiros et al. 2020). Fedorčák et al. (2019) reported the distribution of *C. complanatum* in fish species throughout the Danube River basin across Europe. In contrast, the present study is the first to report information regarding the geographic distribution of *Clinostomum* species throughout Brazilian river basins.

Regional patterns of parasites in fish assemblages can provide insights into the ecological and environmental mechanisms that promote diversification. For many taxa, high levels of diversity typically occur in biogeographic hotspots, which are found in Tropical and Neotropical regions in low latitudes. In addition to climate and latitude, aquatic environments also play a relevant role in regulating the diversity of parasite species in fish assemblages. However, these patterns may be inconsistent across taxa, suggesting that different mechanisms may be important for maintaining different characteristics of parasitic diversity (Shah et al. 2013). Host specificity was not an important factor in the geographic distribution of *Clinostomum* species across Brazilian river basins, since the distribution of these helminths does not reflect that of the host fish families.

Brazilian basins present differenced hydrodynamic related different to factors (e.g. size, urban occupation, exploration of electric power and industries, water quality), which can affect the presence of some parasite species. In the present study, an important factor was observed related to geographic distribution of *Clinostomum* metacercariae across the Brazilian basin systems. Metacercariae of *Clinostomum* sp. are widely distributed in Brazilian river basins, particularly the Amazon, Paraná, São Francisco, Uruguay, North and South, and Southeast Atlantic river basins. However, in the Brazilian Amazon river basin, such metacercariae have been identified as *C. marginatum*, whereas in Paraná, Uruguay, and the Southeast river basins, they have been identified as *C. complanatum*. Many metacercariae of *Clinostomum* species have not been determined, particularly because the two aforementioned species have been the subject of discussion for nearly 200 years regarding the validity of being distinguished by geographical distribution and morphological differences (see Caffara et al. 2011; Sereno-Uribe et al. 2013; Acosta et al. 2016). Pérez-Ponce de León et al. (2016) reported that metacercariae of *C. complanatum* and *Clinostomum* sp. are widely distributed across Middle-America in at least 76 fish species belonging to 13 families across Nicaragua, Costa Rica, and Mexico. Britz et al. (1985) studied the distribution of metacercariae of *Clinostomum tilapiae* Ukoli, 1966 in fish of 11 localities in South Africa and found that only one fish species was infected in nine localities. These authors suggested that the water quality may have affected the presence of snails, the intermediate hosts or the cercariae released for such hosts.

The populations of host fish distributed throughout a hydrographic basin constitute patches of resources for parasite species. Each fish within a population constitutes a habitable environment for parasites. Fish movement can potentially affect the distribution and abundance of parasites in the river systems. Hence, the parasite communities in wild fish populations of hydrographic basins can vary depending on the continuity or separation of the river systems (Salgado-Maldonado et al. 2019). Studies on the spatial variation of fish parasites reported that the composition of parasite communities was persistent among habitats with connectivity, whereas differences in the communities among rivers with isolated fish populations was observed (Mohammed et al. 2020). In addition, local parasite species richness strongly depends on local host species richness, such that host richness and parasite richness are correlated across sampling localities (Poulin et al. 2020). Nevertheless, the lack of geographical similarities between occurrence sample efforts of *Clinostomum* spp. and host fish diversity, as well as problematic taxa, have not allowed the extrapolation of the biodiversity of these parasite species across Brazil. Hence, it is still unknown if the distribution of the *Clinostomum* species is limited when the population of host fish are separated throughout Brazilian river basins.

The geographic ranges of *Clinostomum* spp. around the world have not been determined due to scarcity of regional databases. However, the zoogeographical patterns for endoparasites have been suggested to be especially influenced by the environmental conditions and host population density, and perhaps the feeding habits of host fish and changes in the prey items are determining factors as well (Daly et al. 2002; Dias et al. 2003; González et al. 2006; Shah et al. 2013; Aghlmandi et al. 2018; Maleki et al. 2018). Biogeographical patterns of prey with infective stages of *Clinostomum* species are considered key determinants of the endoparasite community structure in host fish. Endoparasites such as *Clinostomum* spp. can be used to track pathways within food webs and to elucidate their spatial and temporal patterns (González et al. 2006; Shah et al. 2013). However, studies investigating the causes of *Clinostomum* species distribution in host fish populations have focused on other factors such as environmental quality, temperature, latitude and seasonality (Dias et al. 2003; Daly 2013; Shah et al. 2013; Wang et al. 2017; Khan et al. 2018; Maleki et al. 2018; Yasumoto et al. 2018). Biotic interactions such as the distribution of suitable first intermediate hosts (i.e. snails), infective stages of parasites and the presence of aquatic birds that are definitive hosts (i.e. mostly herons), are also known to play a role in the distribution of *Clinostomum* sp. (Osborn 1911; Daly et al. 2002; Daly 2013; Aghlmandi et al. 2018; Maleki et al. 2018). Therefore, the results of the present study indicate that metacercariae of *Clinostomum* species are distributed across different hydrographic basins in Brazil through infected piscivorous birds, and thus can reach mollusks and fish, which are the intermediate hosts. Nevertheless, it is known that geographic limits of *Clinostomum* species in host fish are produced by the combined influence of abiotic and biotic factors, but the influence of multiple factors and their interactions on the population dynamics at the distribution boundaries of these parasites has been little investigated.

In conclusion, revealing the distribution patterns of parasite species such as *Clinostomum* spp. is increasingly recognized as key to mapping and improving knowledge on the dynamics of parasites in different ecosystems. Such knowledge may lead to more precise mapping of zoogeographical patterns of these parasites in host fish of endemic regions and geographic hotspots, enabling the estimation of parasite species and improving the understanding of infection patterns in host fish with wide geographic distribution, in addition to determining global geographical range limits of *Clinostomum* spp. Biogeographical patterns of *Clinostomum* spp. diversity may also be useful for determining how host–parasite interactions can influence speciation. The variation in the distribution patterns of host–*Clinostomum* interactions was not very evident, due to limitations of our database in connection with infection of *Clinostomum* spp. in fish populations in hydrographic basins of Brazil. Nevertheless, the results have shown several relevant insights regarding the distribution patterns of these parasites in host fish and across the major hydrographic basins of Brazil, and the present study constitutes the most extensive survey regarding species of *Clinostomum* in host fish of these basins. However, the understanding of the biology and distribution of *Clinostomum* species requires sampling efforts from all hosts, i.e. snails as first intermediate host, freshwater fish as second intermediate hosts and piscivorous birds as final/definitive hosts across geographic regions of Brazil. Although had not occurred differences among trophic level of the host fish studied, has been known that composition of the fish diet and the availability of infected prey are factors affecting the distribution of *Clinostomum* species; thus, this hypothesis could be tested in future studies focusing in the first intermediate hosts (mollusks) of these digeneans to extend our knowledge. We conclude that *Clinostomum* species are found only in areas where fish have been sampled and examined extensively, thus, more information may be available about a particular fish species without the fish being truly the most infected fish in the region. Similarly, some regions that were found to be more suitable habitat for these parasites could be because these were of most interest and hence more research were conducted in those regions. These helminths are useful as direct indicators of the diet of the host fish, including aspects of prey selection. Furthermore, we have identified areas with high deficits in sampling efforts for the description of the occurrence of *Clinostomum* species in host fish. Hence, such information provides a clear guide for a better allocation of future research efforts toward mapping these digeneans across Brazilian river basin systems. As human populations are not informed about potential zoonosis of *Clinostomum* species and the consequences of consuming infected fish, it is suggested that fisheries and aquaculture authorities take the initiative to inform local fish farmers about preventing farmed fish from becoming infected with these parasites by controlling snail and bird populations. Medical authorities should also take the initiative to educate medical practitioners and the public about the risk *Clinostomum* spp. may pose to public health. Lastly, this study was based on a literature review of research done by various researchers in different years and using different methods of collection of parasites. The number of parasites found in the host fishes can be dependent on the level of expertise of the examiner or the prevalence and abundance of the parasites in the examined fish, which can be different depending on the seasonality or/and average temperature of the year in different ecosystems. Therefore, all these factors may impact the findings of this present study on the parasitic infection patterns.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Marcos Tavares-Dias was supported by a research fellowship from the Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq, Brazil) [Grant 303013/2015-0].

ORCID

Marcos Tavares-Dias http://orcid.org/0000-0002-8376-1846

References

Acosta AA, Caffara M, Fioravanti ML, Utsunomia R, Zago AC, Franceschini L, da Silva RJ. 2016. Morphological and molecular characterization of *Clinostomum detruncatum* (Trematoda: Clinostomidae) metacercariae infecting *Synbranchus marmoratus*. J Parasitol. 102(1):151–156.

Aghlmandi F, Habibi F, Afraii MA, Abdoli A, ShamsI S. 2018. Infection with metacercaria of *Clinostomum complan.atum* (Trematoda: Clinostomidae) in freshwater fishes from Southern Caspian Sea basin. Rev Méd Vét. 169:147–151.

Briosio-Aguilar R, Pinto HA, Rodríguez-Santiago MA, López-García K, García-Varela M, de León GPP. 2018. Link between the adult and the metacercaria of *Clinostomum heluans* Braun, 1899 (Trematoda: Clinostomidae) through DNA sequences, and its phylogenetic position within the genus *Clinostomum* Leidy, 1856. J Parasitol. 104(3):292–296.

Britz J, Van As JG, Saayman AJE. 1985. Occurrence and distribution of *Clinostomum tilapiae* Ukoli, 1966 and *Euclinostomum heterostomum* (Rudolphi, 1809) metacercarial infections of freshwater fish in Venda and Lebowa, Southern Africa. J Fish Biol. 26:21–28.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol. 83:575–583.

Caffara M, Locke SA, Gustinelli A, Marcogliese DJ, Fioravanti ML. 2011. Morphological and molecular differentiation of *Clinostomum complanatum* and *Clinostomum marginatum* (Digenea: Clinostomidae) metacercariae and adults. J Parasitol. 97:884–891.

Calhoun DM, Leslie KL, Riepe TB, Achatz TJ, McDevitt-Galles T, Tkach VV, Johnson PTJ. 2020. Patterns of *Clinostomum marginatum* infection in fishes and amphibians: integration of field, genetic, and experimental approaches. J Helminthol. 94:e44, 1–12.

Chai JY, Murrell DK, Lymbery AJ. 2005. Fish-borne parasitic zoonoses: status and issues. Inter J Parasitol. 35:1233–1254. doi:10.1016/j.ijpara.2005.07.013.

Choudhury A, Aguirre-Macedo ML, Curran SS, Ostrowski de Núñez M, Overstreet RM, Pérez-Ponce de León G, Portes Santos C. 2016. Trematode diversity in freshwater fishes of the globe II: 'New World'. Syst Parasitol. 93:271–282.

Daly JJ. 2013. Distribution of yellow grub (*Clinostomum marginatum*) metacercariae in black bass (*Micropterus* spp.) from Arkansas Ozark and Ouachita reservoir Lakes. J Arkansas Acad Sci. 67:173–176.

Daly JJ, DeYoung B, Hostetler TL. 2002. Distribution of *Clinostomum marginatum* (Yellow grub) metacercaria in smallmouth bass populations from Crooked Creek in North Central Arkansas. J Arkansas Acad Sci. 56:42–46.

Dias MLGG, Eiras JC, Machado MH, Souza GTR, Pavanelli GC. 2003. The life cycle of *Clinostomum complanatum* Rudolphi, 1814 (Digenea, Clinostomidae) on the floodplain of the high Paraná River, Brazil. Parasitol Res. 89:506–508.

Dormann CF. 2011. How to be a specialist? Quantifying specialisation in pollination networks. Network Biol. 1:1–20.

Dormann CF, Gruber B, Fruend J. 2008. Introducing the bipartite package: analysing ecological networks. R News. l8 (2):8–11.

Eiras JC, Dias MLGG, Pavanelli GC, Machado MH. 1999. Histological studies on the effects of *Clinostomum marginatum* (Digenea, Clinostomidae) in its second intermediate host *Loricariichthys platymetopon* (Osteichthyes, Loricariidae) of the upper Paraná River, Brazil. Acta Sci Biol Sci. 21:237–241.

Fedorčák J, Šmiga L, Kutsokon I, Kolarčik V, Koščová L, Oros M, Koščo J. 2019. Parasitic infection of *Cobitis elongatoides* Băcescu and Mayer, 1969 by zoonotic metacercariae *Clinostomum complanatum* (Rudolphi, 1814). J Fish Dis. 42:1677–1685.

Froese R, Pauly D. 2019. FishBase. version (12/2019). World Wide Web electronic publication. Available from: www.fishbase.org.

González MT, Barrientos C, Moreno CA. 2006. Biogeographical patterns in endoparasite communities of a marine fish (*Sebastes capensis* Gmelin) with extended range in the Southern Hemisphere. J Biogeog. 33:1086–1095.

Kagei N, Yanohara Y, Uchikawa R, Sato A. 1984. On the yellow grubs, metacercariae of *Clinostomum complanatum* (Rudolphi, 1819), found in the cultured loach. Jpn J Parasitol. 33:59–62.

Khan S, Ahmed S, Serajuddin M, Saifullah MK. 2018. Variation in seasonal prevalence and intensity of progenetic metacercariae of *Clinostomum complanatum* infection in *Trichogaster fasciatus fish*. Beni-Suef Univ J Basic Appl Sci. 7:310–316.

Klass EE. 1963. Ecology of the trematode, *Clinostomum marginatum*, and its hosts in eastern Kansas. Trans Kansas Acad Sci. 66:519–538.

Lima HL, Stefani LM, Pedron FA, Baldissera MD, da Silva AS. 2014. Pro-inflammatory cytokines in the serum of silver catfish (*Rhamdia quelen*) naturally infected by *Clinostomum complanatum*: a preliminary study. J Parasitol. 100:142–143.

Locke SA, Caffara M, Barčák DP, Sonko P, Tedesco P, Fioravanti ML, Li W. 2019. A new species of *Clinostomum* Leidy, 1856 in East Asia based on genomic and morphological data. Parasitol Res. 118:3253–3265.

Luque JL, Pereira FB, Alves PV, Oliva ME, Timi JT. 2017. Helminth parasites of South American fishes: current status and characterization as a model for studies of biodiversity. J Helminthol. 91:150–164.

Magurran AE. 2004. Measuring biological diversity. 1st ed. Oxford (UK): Blackwell Science.

Malek M, Mobedi I. 2001. Occurrence of *Clinostomum complanatum* (Rudolphi, 1819) (Digenea: Clinostomatidae) in *Capoeta capoeta gracilis* (Osteichthys: Cyprinidae) from Shiroud River, Iran. Iran J Pub Health. 30:95–98.

Maleki L, Heidari H, Ghaderi E, Rostamzadeh J. 2018. Occurrence and description of *Clinostomum complanatum* (Rudolphi, 1819) metacercariae in freshwater fishes from Gheshlagh basin, West of Iran. Iran J Anim Biosys. 14:91–103.

Mohammed RS, King SD, Bentzen P, Marcogliese D, van Oosterhout C, Lighten J. 2020. Parasite diversity and ecology in a model species, the guppy (*Poecilia reticulata*) in Trinidad. R Soc Open Sci. 7:191112. doi:10.1098/rsos.191112.

Negreiros LP, Pereira FB, Tavares-Dias M. 2020. *Dadaytrema oxycephala* (Digenea: Cladorchiidae) in definitive host *Pimelodus blochii* (Pisces: Pimelodidae), with morphological and geographic distribution data in fishes from the South America. J Parasit Dis. 44:62–68.

Oksanen JF, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, . Simpson GL, Solymos P, Stevens HH, Szoecs E, et al. 2017. Vegan: community ecology package. R Package version 2.4–3. Available from: https://CRAN.R-project.org/package=vegan.

Osborn HL. 1911. On the distribution and mode of occurrence in the United States and Canada of *Clinostomum marginatum*, a trematode parasitic in fish, frogs and birds. Biol Bull. 20:350–366.

Pérez-Ponce de León G, García-Varela M, Pinacho-Pinacho CD, Sereno-Uribe AL, Poulin R. 2016. Species delimitation in trematodes using DNA sequences: Middle-American *Clinostomum* as a case study. Parasitology. 143:1773–1789.

Pinto HA, Caffara M, Fioravanti ML, Melo AL. 2015. Experimental and molecular study of cercariae of *Clinostomum* sp. (Trematoda: Clinostomidae) from *Biomphalaria* spp. (Mollusca: Planorbidae) in Brazil. J Parasitol. 101:108–113.

Poulin R, Presswell B, Jorge F. 2020. The state of fish parasite discovery and taxonomy: a critical assessment and a look forward. Inter J Parasitol. 50:733–742. In press. doi:10.1016/j.ijpara.2019.12.009.

R Development Core Team. 2017. R: a language and environment for statistical computing. Vienna: R Foundation for statistical computing; [cited 2019 Sept 10]. Available from: http://www.R-project.org/.

Rohde K, Hayward C, Heap M. 1995. Aspects of the ecology of metazoan ectoparasites of marine fishes. Inter J Parasitol. 25:945–970.

Salgado-Maldonado G, Mendoza-Franco EF, Caspeta-Mandujano JM, Ramírez-Martínez C. 2019. Aggregation and negative interactions in low-diversity and unsaturated monogenean (Platyhelminthes) communities in *Astyanax aeneus* (Teleostei) populations in a Neotropical river of Mexico. Int J Parasitol-Par. 8:203–215.

Sereno-Uribe AL, García-Varela M, Pinacho-Pinacho CD, Pérez-Ponce de León G. 2018. Three new species of *Clinostomum* Leidy, 1856 (Trematoda) from Middle American fish-eating birds. Parasitol Res. 117:2171–2185.

Sereno-Uribe AL, Pinacho-Pinacho CD, García-Varela M. 2013. Using mitochondrial and ribosomal DNA sequences to test the taxonomic validity of *Clinostomum complanatum* Rudolphi, 1814 in fish-eating birds and freshwater fishes in Mexico, with the description of a new species. Parasitol Res. 112:2855–2870.

Shah HB, Yousuf AR, Chishti MZ, Ahmad F. 2013. Metacercariae of *Clinostomum schizothoraxi* Kaw, 1950 (Digenea: Clinostomatidae) in *Carassius carassius* (Linnaeus) under different environmental conditions. Folia Parasitol. 60:163–168.

Shamsi S, Day S, Zhu X, McLellan M, Barton DP, Dang M, Nowak BF. 2021. Wild fish as reservoirs of parasites on Australian Murray cod farms. Aquaculture. 539:736584. doi:10.1016/j.aquaculture.2021.736584.

Shamsi S, Halajian A, Tavakol S, Mortazavi P, Boulton J. 2013. Pathogenicity of *Clinostomum complanatum* (Digenea: Clinostomidae) in piscivorous birds. Res Vet Sci. 95:537–539. doi:10.1016/j.rvsc.2013.06.018.

Shareef PAA, Abidi SMA. 2012. Incidence and histopathology of encysted progenetic metacercaria of *Clinostomum complanatum* (Digenea: Clinostomidae) in *Channa punctatus* and its development in experimental host. Asian Pac J Trop Biomed. 2:421–426.

Sohn WM, Na BK, Cho SH. 2019. Infection status with *Clinostomum complanatum* metacercariae in fish from water systems of Nakdong-gang (River) in Korea. Korean J Parasitol. 57:389–397.

Tansatit T, Sobhon P, Sahaphong S, Sangsuriya P, Klinsrithong S. 2014. Prevalence and histopathology of Trichogaster pectoralis harbouring metacercaria of *Clinostomum piscidium* (Southwell and Prashad, 1918) in Central Thailand. Thai J Vet Med. 44:223–230.

Vianna RT, Pereira-Júnior J, Brandão DA. 2005. *Clinostomum complanatum* (Digenea, Clinostomidae) density in *Rhamdia quelen* (Siluriformes, Pimelodidae) from South Brazil. Braz Arch Biol Technol. 48:635–642.

Wang ML, Chen HY, Shih HH. 2017. Occurrence and distribution of yellow grub trematodes (*Clinostomum complanatum*) infection in Taiwan. Parasitol Res. 116:1761–1771. doi:10.1007/s00436-017-5457-3.

Yasumoto S, Kabayama T, Kondo M, Takahashi Y. 2018. Mass mortalities of goldfish *Carassius auratus* infected *Clinostomum* metacercariae, associated with elevated water temperature. Fish Pathol. 53:44–47.