COMMUNITY STRUCTURE OF *DIPLOSTOMUM* SPP. (DIGENEA: DIPLOSTOMIDAE) IN FISH LENSES

Hubert Désilets

A Thesis

in

the Department

of

Biology

Presented in Partial Fulfillment of the Requirements

for the Degree of Master of Science (Biology) at

Concordia University

Montréal, Québec, Canada

March 2013

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

By: Hubert Désilets

Entitled: COMMUNITY STRUCTURE OF *DIPLOSTOMUM* SPP. (DIGENEA: DIPLOSTOMIDAE) IN FISH LENSES

and submitted in partial fulfillment of the requirements for the degree of

Master of Science (Biology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final Examining Committee:

Selvadurai Dayanandan Chair

David Walsh External

Ian Ferguson Examiner

James Grant Examiner

Daniel McLaughlin Co-supervisor

David Marcogliese Co-supervisor

Approved by Patrick Gulick or Selvadurai Dayanandan

Chair of Department or Graduate Program Director

February 27, 2013 Brian Lewis

Dean of Faculty

ABSTRACT

COMMUNITY STRUCTURE OF *DIPLOSTOMUM* SPP. (DIGENEA: DIPLOSTOMIDAE) IN FISH LENSES

Hubert Désilets

Numerous evolutionary and ecological factors can influence parasite community composition and structure in fish. Time of sampling, host phylogeny, length, age, sex, and interspecific associations among parasite species are among them. I assessed the impact of these factors in larval diplostomid infracommunities (*i.e.*, the assemblage of all the individuals of all parasite species in an individual host at a particular time) composed of four different *Diplostomum* species. I used the barcode region of cytochrome *c* oxidase subunit I to distinguish species of *Diplostomum* in 1065 metacercariae from the lenses of 828 fish distributed in 20 different host species collected in 2006, 2010 and 2011. Negative associations among *Diplostomum* species were found and the strength of these associations was a function of parasite abundance. These results suggest competitive interactions among nearly all *Diplostomum* species encountered. Inter-annual variation in *Diplostomum* infracommunities was far greater than inter-seasonal variation. The differences in infracommunity composition and structure could not be explained by host phylogeny. Host length and age, but not sex, had significant effects on *Diplostomum* infracommunity structure. However, a significant amount of variance in the system could not be explained, indicating the potential importance of other factors such as resistance or exposure in determining infracommunity structure. My work provided

insights on factors affecting natural and potentially interactive larval trematode communities in fish.

Keywords: Host specificity, host-parasite interaction, interspecific competition, temporal variation, eye flukes, larval parasites.

CONTRIBUTION OF AUTHORS

The text of this thesis was written entirely by Hubert D. Désilets. David J. Marcogliese, J. Daniel McLaughlin and Sean A. Locke provided editorial suggestions. David J. Marcogliese and J. Daniel McLaughlin designed the study. Sean A. Locke did the sampling in 2006; Hubert D. Désilets did the sampling in 2010 and 2011, with the assistance of David J. Marcogliese and Sean A. Locke. Hubert D. Désilets did all the fish necropsies and all the ecological analyses. Colleagues at the Canadian Centre for DNA Barcoding at the University of Guelph (Ontario, Canada) did the DNA sequencing of all specimens. Sean A. Locke did the sequence analysis and the species discrimination of ambiguous sequences, when possible.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, J. Daniel McLaughlin and David J. Marcogliese, along with Sean A. Locke for help and guidance. Your support, ideas and comments have contributed immensely to the realization of this project. You make a great team, the best a student can hope for. I would like to thank my committee members, Ian Ferguson and Jim Grant, for useful comments and questions. I would like to thank the staff at the St. Lawrence Center (Environment Canada), Andrée Gendron, Sophie Trépanier, Germain Brault, Claude Lessard, Michel Arseneau, Christopher Blanar, Laurine Bandet, Benjamin Marquis, Julie Payet, Myriam Lefebvre for invaluable help on the field and in the lab. Finally, I would like to thank my lab mates and fellow students, Angela Rose-Lapierre, John Forest, Simon Daoust and Veronica Aponte, for help and support. Your dedication to your studies and research is inspiring.

We acknowledge research support through funding to David J. Marcogliese from STAGE (Environment Canada) and the inter-departmental Genomics Research and Development Initiative, as well as funding from Natural Sciences and Engineering Research Council of Canada Discovery Grant A6979 to J. Daniel McLaughlin. DNA sequencing was aided by funding provided by the Government of Canada through Genome Canada and the Ontario Genomics Institute through the International Barcode of Life project. We thank colleagues at the Canadian Centre for DNA Barcoding for carrying out the molecular processing. We also thank the Department of Biology of Concordia University for financial support and teaching assistantship opportunities.

DEDICATION

To Bianka, who accepted to share this project with me.

To my parents, who have always supported me.

To give anything less than your best is to sacrifice the Gift.

– Steve Prefontaine

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

1. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. COMMUNITY THEORY

A community is generally defined as an assemblage of populations of living organisms in a given habitat and the description and interpretation of community structure are subjects of major interest in ecology. Species abundances and their distributions are determined in nature by numerous factors such as habitat quality, resource availability, environmental stochasticity, and associations among sympatric species, among others (Armstrong and McGehee, 1976; Toft, 1985; Schoener, 1986; Holt and Kotler, 1987; Naeem, 1988; Jaeger and Walls, 1989). At the local scale, interactions among species may be the major factor structuring the communities (MacArthur, 1965; Cody, 1974; Huston, 1994; Belyea and Lancaster, 1999; Weiher and Keddy, 1999). However, more and more studies indicate that large-scale regional patterns and processes (*e.g.*, colonization, dispersal, biotic and abiotic gradients) are good predictors of the composition and structure of local natural communities (Hanski, 1982; Cornell, 1985; Ricklefs, 1987; Pulliam, 1988; Stevens, 1989; Kareiva et al., 1990; Cornell and Lawton, 1992; Hanski and Gilpin, 1997; Srivastava, 1999).

1.2. PARASITE COMMUNITIES

Like their hosts, parasites form communities. There are three precise terms used to describe parasite communities in nature: the infracommunity is the assemblage of all

the individuals of all parasite species (*i.e.*, all infrapopulations) in an individual host at a particular time; the component community refers to all infrapopulations (or infracommunities) of parasites associated with some subset of a host species (*e.g.*, the population of a host species in a given habitat); and the supracommunity comprises all developmental stages of all parasite species (*i.e.*, all suprapopulations) in a particular habitat/ecosystem at a given time (Bush et al., 1997).

The use of parasite assemblages in vertebrates provides some clear advantages in the study of community organization. The relative ease of replicating samples, the similar trophic level and ecological niche shared by different parasitic species, and the ubiquitous occurrence of multiple species in a single host are all advantages of parasite systems (Krasnov et al., 2005). In addition, it is feasible to completely census all the individuals in a parasite community within a host or a host sample, and predation has no cofounding influence on interspecific interactions.

Despite these advantages and the fact that parasite community structure has been a focus in parasitology, the extensive number of studies on the subject has produced differing results and few generalizations have emerged (Esch et al., 1990; Kennedy, 1990; Carney and Dick, 1999; Poulin, 2001, 2007). This is not surprising because numerous factors might shape these communities (Locke et al., 2013). Many evolutionary and ecological forces influence the structure of parasite communities, and separating the respective contribution of each represents a complex problem. Janovy et al. (1992) consider the occurrence of a particular parasite species in a particular host to be an evolutionary phenomenon, while the population structure and frequency distribution parasites in the environment is viewed as an ecological phenomenon. Parasite community composition and structure can be influenced by numerous host factors such as phylogeny, size, age and sex, by species associations and by the host

immune systems. Furthermore, parasite communities can greatly vary over short distances (Marcogliese et al., 2006; Faltýnková et al., 2008).

Host specificity is a fundamental characteristic of parasites and it can be evaluated in different ways such as structural specificity, phylogenetic specificity or specificity in geographic space (Poulin et al., 2011a). Parasite species can be restricted to a few closely related host species or exploit unrelated hosts (Poulin et al., 2011a). Depending on the proportion of parasite species of each type (*i.e.*, generalists or specialists), host phylogeny can be an important factor in determining the parasite community structure. However, ecological differences among hosts can sometimes explain the degree of specificity of some parasites (Cooper et al., 2012).

Kuris et al. (1980) suggested the use of island biogeography theory (MacArthur and Wilson, 1967) to determine the influence of host size on parasite species richness. The authors acknowledged theoretical difficulties unique to host-parasite systems (*e.g.*, host immune defences, induced mortality, short lifespan and vagility) in the use of this relationship between host size and parasite richness. Nevertheless, the theory of island biogeography suggests that larger hosts should harbour richer parasite infracommunities. However, other factors, such as host phylogeny, environmental conditions, host geographical range, population density and diet, also seem to affect the relationship with host size (Kuris et al., 1980; Poulin, 1995, 1997; Nunn et al., 2003; Poulin and Morand, 2004; Lindenfors et al., 2007; Bordes et al., 2009).

Host age can be an important factor in structuring parasite communities. Studies have suggested that young hosts should exhibit more inter-individual differences in parasite infracommunities than older hosts because of environmental stochasticity and differences in parasite acquisition rates, while the longer exposure period would tend to homogenize infracommunities among older hosts (Timi et al., 2010; Timi and Lanfranchi, 2013). However, older hosts often tend to harbour richer and more diverse

infracommunities of increasing complexity and dissimilarity (Zelmer and Arai, 1998, 2004; Bajer et al., 2005; Locke et al., 2012). This could be the result of increased mobility and varied foraging in older hosts (Locke et al., 2012) and/or of parasite interactions that could heighten differences in infracommunity structure in older fish directly, or through processes such as sequential immunization (Karvonen et al., 2009; Rellstab et al., 2011). Still, other studies have found an ambiguous impact of host age on parasite community structure, where parasite intensity and species richness were correlated with age only at certain sampling sites and times (Carney and Dick, 2000).

Another potentially important factor that can influence parasite community structure is host sex, but research has led to differing results. Differences in infracommunity structure have been observed between female and male hosts (Dove, 2000; Krasnov et al., 2012), while others failed to find any significant differences between the sexes (Zelmer et al., 2004; Timi and Lanfranchi, 2009; Yamada et al., 2011; Akoll et al., 2012; Bellay et al., 2012; Drago, 2012). Even in systems where no significant differences in infracommunity structure between females and males are found, impacts on the host may still differ (Adjei et al., 1986). The host sex-related discrepancy in the structure of parasite infracommunities appears to be strongly context-dependent, where the type of host involved, sexual size dimorphism, variation in immunocompetence, and even the season can all have an impact (Krasnov et al., 2012).

Parasites of one species can interact with those of another, leading to non-random associations in individual hosts (*i.e.*, within infracommunities). Associations can be positive (synergistic, facilitation of establishment of one species by another) or negative (antagonistic, competitive interactions between species, exclusion). The null scenario consists of neutral, or no effect of cohabitation between species (Behnke et al., 2001). Thus, it can be asked whether negative or positive associations among parasite species occur within a single host. Various experimental studies demonstrate positive as

well as negative associations among parasite species (Petney and Andrews, 1998). For example, Lello et al. (2004) concluded, after showing occurrence of both positive and negative interspecific associations in a community of intestinal helminths in rabbits, that host immune response might play an important role in shaping parasite community structure because of processes such as the development of cross-immunity. Competitive exclusion (*i.e.*, one interacting species excludes another) or coexistence of interacting species in reduced niches are present at the infracommunity level in some parasite-host systems (Holmes, 1961, 1962; Kennedy and MacKinnon, 1994; Mouillot et al., 2005; Begon et al., 2006). However, others have found inconsistent support for these negative associations (see Poulin, 2001).

1.3. PARASITE COMMUNITIES IN FISH

Parasite communities in fish have certain characteristics. As ectotherms with relatively depauperate infracommunities, fish are predicted to sustain unsaturated communities of species that do not interact (Janovy et al., 1992; Poulin, 2001). Positive, neutral and negative associations have been observed among adult parasites in fish, especially in ectoparasites (Combes, 2001; Bagge et al., 2005). Most adult endoparasites are found in the gastrointestinal tract, a relatively simple linear gradient of niche availability from which each parasite selects. Inspired by seminal work by Bush and Holmes (1986) on birds, a number of studies of parasite associations in freshwater fish have focused on gastrointestinal parasites (Kennedy, 1990; Carney and Dick, 2000; Kennedy and Hartvigsen, 2000; Dezfuli et al., 2001; Kennedy, 2001a). However, conflicting results, ranging from positive to neutral to negative associations, led Poulin and Valtonen (2002) to state that local and/or temporal effects mainly determine parasite

infracommunity structure in fish and that interactions among parasite species are comparatively unimportant.

In fish, one limitation of studies of parasite community structure and species interactions is that most have focused on adult parasites, leaving aside the larval stages, even though the latter are often more abundant in these hosts. There are two main reasons for this. Firstly, larval helminths in freshwater fish are typically thought to be inactive, are often encysted and frequently occur in different tissues. Thus, being sequestered in cysts, they ought to consume few, if any host resources, and theoretically have little or no potential for interspecific associations such as competition or facilitation (Poulin, 2001; but see Faltýnková et al., 2011). Secondly, studying larvae is problematic because identification to species is often impossible with traditional methods. Although the metacercariae of many digeneans can be identified to the genus level, it is virtually impossible to identify them to the species level due to the lack of species specific morphological features. (Criscione et al., 2005; Nolan and Cribb, 2005). The inability to detect species diversity has led specimens of a given genus to be treated as a single species. This is now being resolved through use of DNA methods permitting us to distinguish, and in some case identify, larval species.

1.4. *DIPLOSTOMUM* COMMUNITIES

1.4.1. *Diplostomum* species in fish

Recent DNA studies revealed a high diversity of species in the genus *Diplostomum* (Diplostomidae) within a single site, the lens, in fish of the St. Lawrence River (Québec, Canada) and elsewhere (Locke et al., 2010a,b). In some respects, these assemblages represent an good system with which to study parasite community

structure, dynamics and species associations because they are composed of ecologically similar species of energetically active, unencysted larvae occupying a single site within the fish. Using molecular methods for species discrimination, this study examines potential competitive interactions among species of *Diplostomum*, while assessing the impact of host traits such as phylogeny, size, age and sex on community structure within the fish lens.

1.4.2. *Diplostomum* biology

Parasites of the genus *Diplostomum* are digenetic trematodes that use aquatic piscivorous birds such as gulls, mergansers and terns as definitive hosts (Chappell et al., 1994) (Figure 1). The adults reproduce sexually in the intestine of aquatic birds. Eggs are passed with the feces, embryonate in freshwater and hatch in 19 to 33 days (Dubois, 1961). Each egg releases a single miracidium, a small ciliated larva that actively infects lymnaeid snails. Each miracidium then transforms into a mother sporocyst that produces numerous daughter sporocysts. The daughter sporocysts produce cercariae. Cercariae are dispersal stages and emerge continuously from the snail host starting around 45 days post-infection (Dubois, 1961, Yamaguti, 1975). A single infected snail can shed up to 45 000 cercariae per day over a two month span (Davies et al., 1973). Cercariae that successfully infect the second intermediate host, a fish, penetrate the fish skin or gills, migrate to the lens, vitreous humour or brain (depending on the species of *Diplostomum*) within 24 h and transform into metacercariae. Unlike most digeneans, which are sequestered in cysts in host tissues, *Diplostomum* metacercariae do not form protective cysts. They move actively within their specific infection site and can interact with con- or heterospecifics that occupy the same site. The metacercariae are transmitted back to a piscivorous bird when the infected fish is eaten, and develop into sexually reproducing adults.

A fish infected by *Diplostomum* spp. can experience varying effects on its health and fitness depending on the intensity of the infection (Shariff et al., 1980; Bylund and Sumari, 1981; Laitinen et al., 1996; Rintamäki-Kinnunen et al., 2004; Seppälä et al., 2005a,b; Karvonen and Seppälä, 2008; Voutilainen et al., 2008). *Diplostomum* species that are studied here infect the lens and can impair host vision by producing cataracts (Owen et al., 1993; Seppälä et al., 2005b) as a result of consumption of lens cortical fibers and release of metabolic wastes (Ashton et al., 1969). Even if cataracts are not produced, infections can induce a range of behavioural changes such as an increase in reaction time and, consequently, an increase in predation risk (Crowden and Broom, 1980; Brassard et al., 1982; Seppälä et al., 2004, 2005b, 2011).

1.4.3. *Diplostomum* infracommunity

Numerous factors have or are believed to have an impact on *Diplostomum* infracommunity composition and structure. Among other factors, time of sampling, water flow and quality, host phylogeny and immune response, host length, age, and sex, as well as intraspecific and interspecific interactions, are all expected to affect *Diplostomum* infracommunities.

As with many other parasite species living in the Northern Hemisphere, the life cycle and life-history strategy of *Diplostomum* spp. are influenced by seasonal factors. In North America and, more precisely, in the St. Lawrence River, two peaks of infection in fish are observed, one late in May to June and the other in September (Marcogliese and Compagna, 1999; Marcogliese et al., 2001a,b). The first infection peak is believed to be linked to cercariae shed from overwintered snails that die soon after reproducing, while the second is linked to infections in the new cohort of snails which are acquired during the spring and summer (McKeown and Irwin, 1997). The timing of the second wave of infection in fish reflects the time required for the cercariae to develop in the new cohort

of snails. Inter-annual variation can also arise as a consequence of a temporary or permanent change in environmental conditions (Kennedy and Burrough, 1977; Burrough, 1978; Kennedy, 1981; Marcogliese et al., 2006).

Host phylogeny, linked with specificity of the parasites, might also play an important role in structuring *Diplostomum* infracommunities in fish. Recent studies have shown that the *Diplostomum* species residing in the lenses of fish are generalists, and typically infect a greater number of host species than *Diplostomum* species infecting other sites (*e.g.*, the vitreous humour and brain) (Locke et al., 2010a; Rellstab et al., 2011). One explanation for this phenomenon is linked to the host immune response. The lens capsule protects the metacercariae from immune responses of their host (Lester and Huizinga, 1977; Shariff et al., 1980). This presumably limits the interaction between the parasite and the host immune system following a successful establishment, which in turn reduces selection for specialized adaptation for selected host taxa on the part of the parasite. The lower host specificity of the lens-infecting *Diplostomum* spp. could plausibly result in higher transmission and fitness because the avian hosts feed on multiple species of fish (Feunteun and Marion, 1994; Johnson et al., 2002). In that context, a parasite individual able to establish in a wide variety of hosts has an important advantage. Rellstab et al. (2011) showed that although *Diplostomum* species in the lens are generalists, they nonetheless form different infracommunities in different fish species. However, it was not possible for the authors to determine the cause of the observed variations, which could have arisen from differing ecology of the host species, different affinities for phylogenetic groups of hosts, and/or the presence of fish from multiple populations, as well as interspecific interactions.

1.4.4. *Diplostomum* species associations

A few studies have assessed associations among larval parasites in freshwater fish, including species of *Diplostomum*. For example, Kennedy and Burrough (1977), and Burrough (1978) found mixed-species infections of *Diplostomum spathaceum*, infecting the lens, and *Tylodelphys clavata*, in the vitreous humour, in some hosts, but intensity of both species was rarely simultaneously high. The authors considered this systematic absence of high numbers of both parasite species as evidence of negative interspecific interactions. Others have also found indirect support for competition among diplostomid metacercariae. Kennedy (2001a) surveyed a perch population over 29 years and found that a decrease in prevalence and intensity of a lens-infecting *Diplostomum* species coincided with an increase in two introduced vitreous humour-infecting *Tylodelphys* species. Finally, Karvonen et al. (2006) found that two species of *Diplostomum*, one establishing in the lens and one in the vitreous humour, maintained their preferences in infection site in an experimentally infected novel host. The authors contended that the stability of this specialized site-selection is evidence of site segregation over evolutionary time, hence a historical legacy of past competition between the two species. Taken together, these results suggest that negative associations and competitive interactions can indeed occur among diplostomid metacercariae. However, these studies share several limitations. Firstly, they postulate competition in species inhabiting two different tissues within the same organ (*i.e.*, lens and vitreous humour) and the mechanism underlying such a negative interaction is not clear because the niches of such species do not overlap. Secondly, all studies treated *Diplostomum* metacercariae inhabiting the lens as a single species, which is now known to be a highly problematic assumption (Niewiadomska and Laskowski, 2002; Galazzo et al., 2002; Locke et al., 2010b; Rellstab et al., 2011; Georgieva et al., 2013). Multiple

species of *Diplostomum* can coexist in this constrained infection site, leading to potential intra- and/or interspecific competition for the same spatial and energetic resources.

1.4.5. Effects of host immune system

The species of *Diplostomum* studied here infect the lens of fish, which is considered an immunologically privileged infection site (Lester and Huizinga, 1977; Shariff et al., 1980; Sitjà-Bobadilla, 2008). Because of limited blood flow, most immune reactions that a fish can develop against a parasite cannot be manifested in the lens. This protects visual acuity. However, during the migration period (maximum of 24 h) that cercariae require to travel from the penetration site to the lens, the parasites are vulnerable to the rapid innate response, particularly if the host acquired resistance by previous non-lethal infections (Hines and Spira, 1974; Goven et al., 1980; Whyte et al., 1990; Karvonen et al., 2004a,b, 2005, 2009, 2010). In nature, however, older fish are frequently observed to harbour a greater number of metacercariae than would have been acquired in a single cercarial exposure (Marcogliese et al., 2001b). Moreover, a study on microsatellite loci of *Diplostomum pseudospathaceum* showed that very few identical clones of the parasite are found in individual fish (Reusch et al., 2004), which suggests that metacercariae are acquired in small numbers and accumulate over time due to multiple exposures, rather than all at once. These findings suggest that acquired immune resistance developed by the host may be temporary or incomplete. Höglund and Thuvander (1990) experimentally infected rainbow trout (*Oncorhynchus mykiss*) with cercariae of *Diplostomum spathaceum* on numerous occasions over a 12-week period. They found that although fewer and fewer cercariae were able to establish in the lens over the 12-week period, this reduction was due to cell mediated immunity or non-specific mechanisms of protection targeting the migrating cercariae and not to a specific antibody-driven immune response. Karvonen et al. (2010) conducted a similar

experiment and concluded that although the fish developed some level of resistance to the infections, the process was ineffective and would have insignificant consequences in high exposure conditions in nature.

Based on present knowledge of the innate and acquired immune systems, a specific adaptive antibody-driven immune response aimed at migrating cercariae in a fish would take too much time to initiate. Studies suggest that a migrating larva would establish in the host lens well before specific adaptive antibodies could stop it, even in secondary exposures (Janeway and Medzhitov, 2002; Karvonen et al., 2004a,b, 2005, 2009; Wegner et al., 2007). However, alternative systems similar to the processes observed in invertebrates (Kurtz, 2005) could also be involved. The innate immune system of vertebrates is similar to the immune defenses of invertebrates (Magnadóttir, 2006). Lectin receptors or natural antibody could produce a quick specific response and explain the acquired resistance to parasites without the involvement of specific antibody production by the adaptive immune system (Magnadóttir, 2006; Rauch et al., 2006).

1.4.6. Molecular discrimination of *Diplostomum* species

Species-level discrimination of *Diplostomum* metacercariae using traditional morphological methods is impossible (Niewiadomska and Laskowski, 2002; Cavaleiro et al., 2012), and until recently, it was difficult to determine which and/or how many species of *Diplostomum* may be present in an individual fish. Studies showed *Diplostomum* species can be differentiated by DNA sequences of the internal transcribed spacer (ITS) (Galazzo et al., 2002) and the barcode region of cytochrome *c* oxidase subunit I (COI) (Locke et al., 2010a,b; Cavaleiro et al., 2012; Georgieva et al., 2013) or single-nucleotide polymorphisms (Rellstab et al., 2011). While ITS has been commonly used to distinguish among species of trematodes, sequences of the COI provide greater resolution, including, notably, among locally occurring species of *Diplostomum* (Locke et

al., 2010a,b; Behrmann-Godel, 2013). Unfortunately, the complete taxonomy of the genus *Diplostomum* is not resolved, and confusion, misidentifications and imprecision remain despite the new sequencing tools (Georgieva et al., 2013). Clear identification to species level remains problematic mainly because, although numerous species can be distinguished in metacercariae, few larvae and adults specimens have been linked genetically.

1.5. DESCRIPTION AND AIMS OF THE STUDY

The present study uses molecular data to discriminate among field-collected *Diplostomum* species in order to explore factors that might influence infection intensity, species richness, Shannon diversity and composition of lens-infecting *Diplostomum* spp. infracommunities in fish in natural conditions. I assess the potential influence of time of sampling, phylogeny, size, age and sex of the host on infracommunity structure and composition. I predict that infection intensity will vary seasonally. Because lens-infecting *Diplostomum* species are generalists, I do not expect host phylogeny to have a profound impact on infracommunity composition and structure. However, certain fish species still might harbour similar infracommunities. Based on the hosts as islands theory, I predict that larger/older host will harbour larger, richer and more diverse *Diplostomum* infracommunities. I do not expect to see marked differences in infracommunity composition and structure between female and male hosts. I also evaluate potential interspecific interactions among these parasite species using species-association indicators and predict occurrence of negative interspecific associations among them. The work will contribute to the understanding of parasite community structure and parasite interactions within the community inhabiting the lens. The data will provide

insights on factors affecting natural and potentially interactive larval trematode communities in fish.

2. COMMUNITY STRUCTURE OF *DIPLOSTOMUM* SPP. (DIGENEA: DIPLOSTOMIDAE) IN FISH LENSES

COMMUNITY STRUCTURE OF *DIPLOSTOMUM* SPP. (DIGENEA: DIPLOSTOMIDAE) IN FISH LENSES[1]

Hubert D. Désilets[a], Sean A. Locke[b], J. Daniel McLaughlin[a], David J. Marcogliese[b,*]

[a] Department of Biology, Concordia University, 7141 Sherbrooke West, Montréal, Québec, Canada H4B 1R6

[b] Aquatic Biodiversity Section, Watershed Hydrology and Ecology Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment Canada, 105 McGill Street, Montréal, Québec, Canada H2Y 2E7

* Corresponding author. Tel.: +1 514 283 6499; fax: +1 514 496 7398.

E-mail address: david.marcogliese@ec.gc.ca (David J. Marcogliese)

March 2013

[1] Formatting of the citations in the manuscript and reference list follows the International Journal for Parasitology requirements.

2.1. INTRODUCTION

Few generalizations have emerged from the study of parasite community structure (Esch et al., 1990; Poulin, 2001). This is not surprising because numerous factors might shape these communities (Locke et al., 2013) and many evolutionary and ecological forces can influence the structure of parasite communities (Janovy et al., 1992). Parasite communities in fish are no exception (Kennedy, 1990). As ectotherms with relatively depauperate infracommunities, fish are predicted to sustain unsaturated communities of species that do not interact (Janovy et al., 1992; Poulin, 2001). However, conflicting results, ranging from positive to negative interactions, led Poulin and Valtonen (2002) to state that local and/or temporal effects are the main determinant of parasite infracommunities in fish and that interactions among parasite species are comparatively unimportant.

One limitation of studies of parasite community structure and species interactions in fish is that most have focused on adult parasites primarily in the digestive tract and failed to consider larval stages, even though the latter are often more abundant in these hosts. There are several reasons for this. Larval stages are believed to exhibit lower diversity and specificity than adult parasites (Poulin, 2001). Most larval helminths in freshwater fish are typically thought to be inactive, are often encysted and different larvae frequently occur in different tissues. They ought to consume few, if any host resources, and have little potential for interspecific interactions such as competition or facilitation (Poulin, 2001, but see Faltýnková et al., 2011). In addition, study of larval parasite communities, particularly metacercariae, is problematic because species level identification is often impossible with traditional methods.

Molecular identification methods allow a greater level of species discrimination than previously possible (Hebert et al., 2003). Larval parasite species can now be

distinguished although identification to species level remains problematic (Georgieva et al., 2013). Using these techniques, Locke et al. (2010a,b) showed that there is greater diversity in these larval parasite assemblages in fish than previously thought. Unlike many larval helminths, most metacercariae of the genus *Diplostomum* (Digenea: Diplostomidae) are unencysted and constrained to a restricted infection site, the eyes, and especially the lenses of fish hosts. Therefore they may be in competition for limited spatial and energetic resources. Thus, they constitute a good system with which to study infracommunity composition, structure, and potential interactions among species. Molecular methods provide means of distinguishing among these morphologically indistinguishable metacercariae (Galazzo et al., 2002; Niewiadomska and Laskowski, 2002; Locke et al., 2010a,b; Rellstab et al., 2011; Cavaleiro et al., 2012; Behrmann-Godel, 2013; Georgieva et al., 2013).

Lens-infecting *Diplostomum* species are generalists (Locke et al., 2010a; Rellstab et al., 2011) and their infracommunities might be influenced by numerous evolutionary and ecological factors. Indeed, time of sampling (Marcogliese and Compagna, 1999; Marcogliese et al., 2001a,b), host phylogeny and immune response (Locke et al., 2010a; Rellstab et al., 2011), host length (Kuris et al., 1980), age (Zelmer and Arai, 1998, 2004; Carney and Dick, 2000; Timi et al., 2010; Timi and Lanfranchi, 2013), and sex (Dove, 2000; Timi and Lanfranchi, 2009; Akoll et al., 2012; Drago, 2012) are all potential factors that could shape *Diplostomum* infracommunities. Several studies have examined associations among diplostomids (Kennedy and Burrough, 1977; Burrough, 1978, Kennedy, 2001b; Karvonen et al., 2006). Taken together, these studies suggest that competitive interactions and negative associations can indeed occur among diplostomids. However, these studies share several limitations. Firstly, they postulate competition in species inhabiting two different tissues within the same organ (*i.e.*, lens and vitreous humour) and the mechanism underlying such a negative interaction is not

clear because the niches of such species do not overlap. Secondly, all studies treated *Diplostomum* metacercariae inhabiting the lens as a single species, which is now known to be a highly problematic assumption (Niewiadomska and Laskowski, 2002; Galazzo et al., 2002; Locke et al., 2010b; Rellstab et al., 2011; Georgieva et al., 2013).

In this study, we examine parasite communities in natural fish populations using the barcode region of cytochrome *c* oxidase subunit I (COI) to distinguish among species of *Diplostomum* (Locke et al., 2010a,b), to assess *Diplostomum* infracommunity composition and structure. The effects of host phylogeny, sampling time, host length, age and sex on probability of infection of the host, on variation in infection intensity, and on species richness, Shannon diversity and composition of infracommunities were examined. We predict seasonal variation in *Diplostomum* infection levels, a weak effect of host phylogeny on infracommunity composition and structure, an increase in intensity of infection, species richness and diversity with host size and age, and no marked differences between infracommunities of female and male hosts. We also explore interspecific associations among *Diplostomum* spp. that could influence the composition of the communities. We predict occurrence of negative interspecific associations among these parasite species.

2.2. MATERIALS AND METHODS

2.2.1. Study locality and fish collection

Fish were collected at two localities on Lake Saint-François, along the St. Lawrence River. Most fish (*n* = 706) were collected on the north shore (LSF-1 in Figure 2) near Creg Quay Marina (45.161 °, -74.430 °) in Bainsville (Ontario, Canada) on four occasions: 20 June 2006, 13-14 September 2010, 14-15 June 2011, and 13-14

September 2011. A small sample (*n* = 122) was also collected on the south shore (LSF-2 in Figure 2) near Pointe Dupuis (45.128 °, -74.404 °) in Saint-Anicet (Québec, Canada) on 21 June 2006. The main sampling locality and two focal species were selected based on studies by Locke et al. (2010a, 2013) who found that the diversity and abundance of lens-infecting *Diplostomum* spp. were particularly high in golden shiner (*Notemigonus crysoleucas*) collected in LSF-1 in 2006. In addition to golden shiner, yellow perch (*Perca flavescens*) was also selected for two reasons: in 2006, it was infected by most lens-infecting *Diplostomum* spp. occurring in the St. Lawrence and this host species is phylogenetically independent from golden shiner. Approximately 20 individuals of golden shiner and yellow perch per age group (0+, 1+, 2+, ≥3+) and up to 10 individuals of all other fish species present were collected, representing 6 orders, 9 families and 20 species (Table 1).

Fish were caught in shallow water with a beach seine (22.6 m x 1.15 m, with 3 mm mesh). They were killed immediately in an overdose of MS 222 solution (Sigma Chemical, St. Louis), placed in plastic bags and kept on ice during transport to the laboratory where they were frozen at -80 °C and stored until examination.

2.2.2. Fish examination and ageing

Each fish was thawed, weighed to the nearest 0.01 g, and standard length was measured to the nearest millimeter. Fork length was recorded instead of standard length on fish collected in 2006. As a consequence, standard lengths were estimated using linear regressions calculated with the 2010 and 2011 samples (fork lengths were also measured on these hosts).

In fish collected in September of 2010, the opercular bone and scales were used to estimate the age of each host. The left operculum was removed and placed in hot water until muscles and skin could be removed easily, then cleaned, and dried at room

temperature for a minimum of two weeks, at which time the fish was aged based upon growth bars (Bardach, 1955; Khan and Khan, 2009). Ten scales were removed from the left side of each fish, dorsal to the lateral line, between the anterior and posterior insertions of the dorsal fin(s). Scales were cleaned with fine forceps in soapy water, rinsed, mounted between two microscope slides and dried at room temperature for at least two weeks before scale growth rings were counted (Pierce et al., 1996; Khan and Khan, 2009). The resulting age-length curves for each fish species collected in September 2010 were used to estimate the ages of conspecifics collected on all other occasions. The ages of ten banded killifish (*Fundulus diaphanus*) and eight white suckers (*Catostomus commersonii*) were estimated from age-length relationships in Abraham (1985) and Chalanchuk (1998) because operculum and scales were not collected on these fish caught in 2011.

2.2.3. Fish necropsy

Fish were necropsied and sex was determined upon observation of gonads. Eyes were removed and placed intact in separate petri dishes; the lens of each eye was then removed intact and examined in separate dishes with a stereomicroscope. All parasites observed in each lens were collected. *Diplostomum* metacercariae from each eye were preserved separately in 1.5 mL Eppendorf tubes filled with 95% ethanol and stored at -20 °C or -80 °C until DNA extraction.

2.2.4. Molecular discrimination of *Diplostomum* species

The DNA of each *Diplostomum* specimen was used as a species discrimination tool because *Diplostomum* metacercariae are impossible to identify with traditional morphological methods (Niewiadomska and Laskowski, 2002; Cavaleiro et al., 2012). All *Diplostomum* metacercariae from the lenses of the fish collected were sent to the

Canadian Centre for DNA Barcoding in Guelph, Ontario, for analysis of their cytochrome *c* oxidase subunit I (COI) sequences. DNA from each *Diplostomum* specimen was extracted, amplified and sequenced using the primers Plat-diploCOX1F/R and, in a few cases, MplatCOX1dF/R, and protocols described by Moszczynska et al. (2009). The resulting COI sequences were aligned with the MUSCLE method (Edgar, 2004; Nuin et al., 2006) and evolutionary divergence was calculated within and among *Diplostomum* species using neighbour-joining analysis of Kimura 2-parameter distances (Kimura, 1980) with pairwise deletion of gaps in MEGA 5.1 (Tamura et al., 2011). These calculations insured the preciseness of the species discrimination method: low divergence within species and high divergence among species result in confident species discrimination and reduced risks of errors. The Kimura 2-parameter model allowed precise calculations that take into account differences in chances of transition and transversion occurrence at a homologous site between two sequences.

2.2.5. Data analysis

Some specimens of *Diplostomum* did not yield sequences, which resulted in incomplete species-level information on the parasite communities in some hosts. As a consequence, different subsets of data were used in accordance with different hypotheses and analytical requirements, based on sequencing success and infection level (Table 2). Infracommunity analyses were restricted to lens-infecting *Diplostomum* species only. To assess *Diplostomum* spp. infection levels, prevalence (*i.e.*, proportion of infected fish in a sample) and mean intensity (*i.e.*, mean number of parasites per infected fish in a sample) were calculated in each host species (Bush et al., 1997) using data sets 1 and 2 respectively (Table 2).

Factors distinguishing infected from uninfected fish were explored with a logistic regression using data set 1 (Table 2). Variation in the host infection state (infected or

uninfected) was regressed against the time of collection (year, month and their interaction), phylogeny (order, species), and morpho-physiology (standard length, age, sex) of hosts. The most parsimonious model was obtained by a forward stepwise selection method based on minimizing the Akaike information criterion (AIC) (Bozdogan, 1987; Burnham and Anderson, 2002, 2004). The Nagelkerke/Cragg and Uhler pseudo-R^2 was calculated as a rough indication of model performance and goodness of fit (Nagelkerke, 1991).

The probability of a host being infected with single *versus* multiple *Diplostomum* species was also analyzed by logistic regression using the same explanatory variables and model selection used in the previous procedure, but using data set 3 (Table 2). Although the *Diplostomum* species discrimination was incomplete for some infracommunities, all hosts included in data set 3 could be classified as harbouring single or multiple *Diplostomum* species.

Three generalized linear models (GLMs) were constructed to characterize the influence of host traits on the intensity, species richness and Shannon diversity of lens infections (Paterson and Lello, 2003). Shannon diversity quantifies the uncertainty in predicting the species identity of an individual that is randomly selected from the data (Shannon and Weaver, 1963). The GLM on intensity was run using data set 2, while the GLMs on species richness and diversity were run using data set 4 (Table 2). Intensity, richness and the exponential function of diversity (*i.e.*, $e^{Diversity}$) were regressed against sampling time (year and month of capture, and their interaction), phylogeny (order and species), and morpho-physiology (standard length, age group and sex) of hosts. Intensity and richness were modeled using a negative binomial error distribution (Alexander et al., 2000), while the exponential function of diversity was modeled using a Gamma distribution (for positive non-zero continuous data). The best model was

selected with a forward stepwise selection minimizing AIC. Nagelkerke/Cragg and Uhler pseudo-R^2 were also calculated (Nagelkerke, 1991).

A redundancy analysis (RDA) was conducted using data set 4 (Table 2) to examine the influence of host traits on the composition of *Diplostomum* infracommunities, as well as associations among host species and parasite species, among *Diplostomum* species themselves, and similarities in infracommunity composition among different host species. Parasite abundance was Hellinger-transformed to accommodate problems associated with comparisons with abundances of zero, and to reduce the influence of species with high abundances (Legendre and Legendre, 1998). The initial model included the variables used in logistic regressions, except that a continuous numerical variable (Modified Julian Date) was used to assign a sampling time for each fish in order to improve explanatory power and facilitate interpretation of the resulting RDA graphical triplot. The best model was selected using a forward stepwise selection maximizing the adjusted R^2.

To assess pairwise associations among *Diplostomum* species, a matrix of Spearman correlation coefficients was constructed using data set 5 (Table 2). The partitioned infracommunity data from left and right lenses were used because interactions among metacercariae would most likely occur within lenses, and to avoid trivial cases of single specimen infections in the calculations (*i.e.*, by default, a single specimen in a lens cannot interact with a representative of another *Diplostomum* species). Because there are difficulties (*i.e.*, highly skewed frequency distribution and high frequency of zeros) involved in calculating correlation coefficients on species abundance data (Legendre and Legendre, 1998, p.292), the infracommunity data were Hellinger-transformed before the calculation of the Spearman coefficients (Legendre and Gallagher, 2001; Legendre, 2005). This transformation does not affect the results of the

Spearman correlations (Legendre, 2005). The *P* calculated for each coefficient was adjusted by the Holm's method for multiple inferences (Holm, 1979).

All analyses were conducted using the version 2.15.2 of the *R* software (*R* Core Team, 2012) with the 'ade4', 'glmulti', 'labdsv', 'lme4', 'MASS', 'Rcmdr', 'rgl' and 'vegan' packages. Software and packages are available online through the comprehensive *R* archive network (http://cran.r-project.org).

2.3. RESULTS

2.3.1. Species of *Diplostomum*

Five species of lens-infecting *Diplostomum* (*Diplostomum huronense*, *Diplostomum indistinctum* and *Diplostomum* sp. 1, sp. 3 and sp. 4) were distinguished by comparison of COI sequences with those previously found by Locke et al. (2010a,b) (Figure 3, Appendix 2). All COI sequences, original DNA trace files, and host and sampling information are stored in project *FLUKE* at http://boldsystems.org. Mean evolutionary divergence of DNA sequences was 0.46% (range from 0.22% to 0.76%) within and 11.9% (range from 10.4% to 13.8%) among species (Table 3), indicating that the discrimination method was precise. Only one specimen of *D. indistinctum* was found (in the lens of a golden shiner) and this species was not considered further in ecological analyses. No other parasite species were found in fish lenses.

The number of *Diplostomum* metacercariae collected from the lenses varied among host species and sampling times. Sixty-six percent of these metacercariae were successfully sequenced. There were differences in success of sequencing among sampling times and host species. Infected fish from 2006 represented 45.6% of all infected hosts collected and harboured the majority (71.6%) of the successfully discriminated *Diplostomum* metacercariae. Infected fish from 2010 represented only

14.4% of all infected hosts and harboured 8.5% of the successfully discriminated metacercariae, while fish from 2011 harboured 21.2% of discriminated metacercariae in 40.0% of the infected hosts (Table 4).

Approximately one third of all fish were infected with *Diplostomum*. *Diplostomum* sp. 1 and sp. 4 were more prevalent than *Diplostomum* sp. 3 and *D. huronense* (Table 5). Mean intensity was generally high in cypriniforms. Eleven of 20 host species were infected by more than one species of *Diplostomum* and seven harboured more than two. *Diplostomum huronense* was only found in cypriniforms; white sucker, golden shiner, sand shiner and bluntnose minnow (Table 6). Preliminary analyses showed that sampling locality (*i.e.*, north or south shore) was not a significant factor in determining the probability of infection and in structuring the *Diplostomum* infracommunities. As a consequence, fish collected on the south shore in June 2006 were pooled with those from the north shore in all subsequent analysis.

2.3.2. Probability of infection

A logistic regression revealed the probability of a fish being infected by *Diplostomum* (data set 1, Table 2) was best explained by year of sampling and fish standard length (ΔAIC to full model = -8.12, pseudo-R^2 = 0.460). Hosts collected in 2010 and 2011 had less chance of being infected, while larger fish, regardless of year, had a higher chance of being infected (Table 7). Host species was also included in the model, but was not significant. No other model was within two units of the AIC value of the most parsimonious model, indicating that year of sampling and fish length are the best predictors of whether fish are infected or not (Burnham and Anderson, 2002).

2.3.3. Probability of mixed-species infection

A second logistic regression showed the probability of a host being infected by more than one *Diplostomum* species (data set 3, Table 2) was influenced by fish standard length (ΔAIC to full model = -22.74, pseudo-R^2 = 0.543). According to this most parsimonious model, larger hosts had a higher chance of harbouring mixed-species infections (Table 8). Year of sampling and host species were included in the model, but were not significant. Two other models were within two units of the AIC value of the most parsimonious model. These involved (1) host length and host species (ΔAIC to most parsimonious model = 1.67, pseudo-R^2 = 0.505) and (2) host length and species, year and month of sampling (ΔAIC to most parsimonious model = 2.00, pseudo-R^2 = 0.543). In these two alternative models, larger hosts also had a higher chance of harbouring mixed-species infections (Table 8).

2.3.4. Variation in intensity, richness and diversity

Three GLMs were constructed in an effort to explore in more detail the influence of selected host traits on infection intensity, species richness and Shannon diversity of *Diplostomum* infracommunities. The most parsimonious model for intensity of infection (data set 2, Table 2) included year of sampling, host species and age (ΔAIC to full model = -9, pseudo-R^2 = 0.553). This model showed that infection intensity was lower in hosts collected in 2010 and 2011. Infection intensity was higher in golden shiner, sand shiner and bluntnose minnow (all Cypriniformes, Cyprinidae) compared to other host species. The model also showed a significant progressive increase in infection level with host age (Table 9).

The most parsimonious model for infracommunity richness (data set 4, Table 2) also included year of sampling, taxonomic order and age (ΔAIC to full model = -31.72, pseudo-R^2 = 0.385). Hosts caught in 2006 had significantly richer *Diplostomum*

infections than hosts collected in 2010-2011. Taxonomic order and host age were included in the model, but their effects were not significant (Table 10).

The most parsimonious model for Shannon diversity (data set 4, Table 2) included year of sampling, taxonomic order and age (ΔAIC to full model = -11.29, pseudo-R^2 = 0.642). Year of sampling had a significant positive effect on diversity of infection. In general, perciform hosts had significantly more diverse infections than hosts from other orders. Diversity was marginally (*P* of coefficient = 0.051) low in hosts of age group +2 and was significantly reduced in hosts of age group ≥3+ (Table 11). No other model was within two units of the AIC value of the most parsimonious models presented, indicating that year of sampling and fish age are the best predictors of intensity, richness and diversity of *Diplostomum* infections (Burnham and Anderson, 2002).

2.3.5. Infracommunity composition

In a partial RDA with parasites partitioned into left and right lenses, individual host identity explained almost all of the observed variance (92%), even when all the other host traits (*i.e.*, sampling date, host species, length, sex and age group) were included. In other words, in a given host, the species assemblages from the left and right lenses are similar, although some stochastic variation remains. This similarity between partitioned infracommunities in a given host indicates that individual host identity is important. This result suggests that if host traits affect associations among *Diplostomum* species, they should affect both lenses the same way, even if the potential interactions among species of *Diplostomum* would most likely occur within each respective lens.

In consequence, a RDA was run on infracommunity data in which left and right lenses were pooled to form a single infracommunity for each fish. The RDA model (data set 4, Table 2) that explained the greatest amount of variation included sampling date, host species, length, sex and age group, and explained 25% of the variance (adjusted

R^2 = 0.249; model significant at F = 3.2428, P = 0.0001, 9999 permutations). The first axis, significant at F = 52.4626, P = 0.0001, 9999 permutations, explained 16.0% of variance; the second axis, significant at F = 19.5517, P = 0.0001, 9999 permutations, explained 6.0% of variance; the third axis, significant at F = 8.3249, P = 0.0002, 9999 permutations, explained 2.5% of variance. The RDA graphical triplot (Figure 4; see also additional rotations in Appendix 3) shows that host age and sex had little influence on the composition of *Diplostomum* infracommunities: their centroid values were relatively closely grouped, with the exception of ≥3+ host individuals that were associated with *Diplostomum* sp. 4. Hosts of undetermined sex were more closely associated with *Diplostomum* sp. 1, but this could be the result of the very low sample size (n = 11) of these hosts.

There appeared to be some temporal variation in which the common *Diplostomum* sp. 1 and sp. 4 decreased in abundance between 2006 and 2010-2011 while the more rare *Diplostomum* sp. 3 and *D. huronense* increased (Figure 4). This increase was not in absolute number of metacercariae, but rather resulted from a decrease in their variation, resulting in a detection of an increase in the RDA.

Diplostomum sp. 3 and *Diplostomum* sp. 4 were found in higher numbers in larger hosts as these parasite species were correlated with host standard length (Figure 4). Conversely, *Diplostomum* sp. 1 showed a strong negative correlation with host length, indicating it was more abundant in smaller hosts.

Certain species of *Diplostomum* were associated with certain fish species but these associations did not correspond in any obvious way to host phylogeny (Figure 4). Infracommunities in hosts of the same genus did not form groups and those in fish species from different orders and families were often clustered. For example, *Diplostomum* sp. 4 characterized pumpkinseed (*Lepomis gibbosus*, Perciformes, Centrarchidae), johnny darter (*Etheostoma nigrum*, Perciformes, Percidae) and yellow

perch (Perciformes, Percidae). *Diplostomum* sp. 3 characterized golden shiner (Cypriniformes, Cyprinidae) and brook silverside (*Labidesthes sicculus*, Atheriniformes, Atherinidae). Balanced intensities of *Diplostomum* sp. 1 and sp. 4 were found in rock bass (*Ambloplites rupestris,* Perciformes, Centrachidae) and bluntnose minnows (*Pimephales notatus*, Cypriniformes, Cyprinidae). The centroids of the remaining host species were either strongly overlapping as a result of the small sample size and/or low parasite prevalence, or isolated in the RDA space (Figure 4). Finally, the RDA triplot also suggested negative associations among all *Diplostomum* species because the vectors were directionally opposed in the RDA space (Figure 4, Appendix 3).

2.3.6. *Diplostomum* species associations

The Spearman correlation coefficients (data set 5, Table 2) showed the presence of four significant negative associations. *Diplostomum* sp. 1 was negatively associated with *Diplostomum* spp. 3 and 4. *Diplostomum* sp. 4 was also negatively associated with *Diplostomum* sp. 3 and *D. huronense* (Table 12). There was a negative correlation between intensity of infection and the strength of species associations as measured by Spearman coefficients (Figure 5). All non-significant associations were associated with *D. huronense*, the species with the lowest mean intensity, and the strength of significant negative associations was higher between *Diplostomum* species with higher intensities, *i.e.*, *Diplostomum* sp. 1 and 4 (Tables 6 and 12, Figure 5).

2.4. DISCUSSION

2.4.1. Results summary

To our knowledge, this study is one of the first to use molecular data to analyse community composition and structure of morphologically indistinguishable larval parasites in fish (see also Rellstab et al., 2011; Locke et al., 2013; Behrmann-Godel, 2013; Georgieva et al., 2013). We explored the effects of several variables, including some not previously examined in this context, such as inter-annual and inter-seasonal variation, host phylogeny, size, age and sex. Most importantly, we found negative associations among the four *Diplostomum* species infecting fish in Lake Saint-François and strong inter-annual variation in infracommunity composition and structure.

Our analysis highlighted the importance of individual host identity in the structuring of *Diplostomum* infracommunities in fish. On the one hand, 25% of the variance in *Diplostomum* infracommunity composition was explained with sampling date, host species, length, age group and sex in the best RDA model. On the other hand, individual host identity alone explained 92% of the variance in a RDA on infracommunities partitioned into left and right lenses. The difference in explanatory power and the relatively low adjusted R^2 of the best RDA model by itself (0.249) suggest that unmeasured individual host characteristics, such as genetics, immune resistance and exposure, may be important. The contribution of these factors to the structuring of *Diplostomum* infracommunity remains to be determined. Considering this, the overall impact of the measured host traits on *Diplostomum* infracommunity composition and structure, although often statistically significant, is probably small. A similar conclusion also emerged from analyses of probability of infection and GLMs on intensity, richness and diversity. Indeed, their pseudo-R^2 values, ranging from 0.385 to 0.642, show that a

significant part of the variance in each model could not be explained by the measured host traits.

2.4.2. Negative associations among *Diplostomum* species

Fish have long been considered to harbour relatively depauperate, unsaturated infracommunities composed of non-interactive species (Janovy et al., 1992; Poulin, 2001), particularly for larval helminths, which are thought to be inactive, being often encysted, and displaying little or no potential for interspecific associations such as competition or facilitation (Poulin, 2001). However, the negative associations revealed herein are consistent with those of earlier studies on diplostomid metacercariae by Kennedy and Burrough (1977), Burrough (1978), Kennedy (2001a) and Karvonen et al. (2006), but the molecular method used to discriminate species allowed for a more rigorous analysis. Indeed, numerous results obtained in this study revealed the presence of negative associations among *Diplostomum* species. Firstly, although Fenton et al. (2010) showed that correlation analysis has a low accuracy of identifying negative parasite interactions when they were in fact present in simulated data, our Spearman correlations showed occurrence of strong negative associations among four pairs of *Diplostomum* species. The strength of these negative associations was directly related to the intensity of infection of the interacting *Diplostomum* species. This makes intuitive sense since competitive interactions might be expected to be stronger between more abundant species simply because specimens of these species will come into contact more often. Secondly, the four main species of *Diplostomum* studied were clearly separated in the RDA space in the infracommunity analysis, indicating negative correlations among all of them. Thirdly, although intensity of infection was progressively higher as host age increased, Shannon diversity was lower in older hosts. This indicated that although older fish harboured more *Diplostomum* metacercariae in general, one

species was dominant, resulting in low diversity values. This could be the result of negative interspecific interactions or cross-immunization (Karvonen et al., 2009; Rellstab et al., 2011). All these results tend to confirm presence of negative interspecific associations, underlying potential negative interactions among these species.

2.4.3. Annual and seasonal variation

There was substantial inter-annual variation in infection probability, intensity and species richness of *Diplostomum* infections. In 2006, fish were not only more likely to be infected than in 2010 or 2011, they were also more often infected with communities of higher intensity and greater richness. The generally lower infection intensity in 2010 and 2011 could be attributed to variation in infracommunity composition in which the two most abundant species, *Diplostomum* sp. 1 and sp. 4, experienced a marked population decrease. Conversely, Shannon diversity was slightly higher in 2010 and 2011. However, the data did not reflect this unexpected result. In sharp contrast to 2006 hosts, most of the hosts collected in 2010 and 2011 and included in data set 4 (Table 2) were infected by a single metacercaria, consequently displaying a diversity value of zero. It is unclear why the GLM detected such an effect of year of sampling.

No significant inter-seasonal variation in *Diplostomum* infracommunity composition and structure was found between the June and September samples. This means that the importance of inter-annual variation surpassed any potential variation between late spring and early fall. The magnitude of the significant drop in infection level in 2010 and 2011 can explain this: the ratio of *Diplostomum* found to fish sampled in 2011 was more than three times lower than in 2006. This result agrees with findings of Marcogliese et al. (2006) who showed strong inter-annual differences in parasite communities of spottail shiner and in overall *Diplostomum* spp. abundance in nearby localities on the St. Lawrence River.

The cause of the inter-annual variation in *Diplostomum* infracommunity composition and structure was not explained by the analyses. However, one notable change in the St. Lawrence River since Marcogliese et al. (2006) conducted their study is the introduction of the round goby (*Neogobius melanostomus*). In 2006, round goby was not observed in Lake Saint-François, but by 2010 and 2011, it was abundant. These invasive fish can significantly reduce gastropod biomass (Kipp and Ricciardi, 2012), thus potentially affecting the early phases of *Diplostomum* spp. life cycles. The round goby, with its high abundance, could also have "diluted" the available pool of cercariae, consequently reducing the exposure of the other sympatric fish species (Keesing et al., 2006; Poulin et al., 2011b). Other biotic and abiotic factors (Dunson and Travis, 1991), such as water flow (Janovy et al., 1997; Marcogliese, 2001), or final host distribution and habitat use (Brown et al., 1988; Marcogliese et al., 2001a; Smith, 2001; Byers et al., 2008), may also be involved in inter-annual variation of *Diplostomum* spp. populations, and could be assessed in future work.

2.4.4. Absence of phylogenetic affiliations

Intensity of infection of *Diplostomum* spp. (as a group) was affected by host species, with high levels in cyprinids. Curiously, however, no fish species was more likely to acquire an initial infection than another. Host phylogeny at the order level also influenced the diversity of infection with perciform hosts having significantly more diverse infections than hosts from other orders. Again, the data did not reflect this unexpected result and discrepancy in sequencing success (Table 4) might be the cause of these results. The "cypriniform effect" might have been reduced or the "perciform effect" might have been inflated by the fact that a significant number of heavily infected cypriniforms were excluded from the diversity analysis because of incomplete *Diplostomum* species determination.

Our results are in accordance with studies on *Diplostomum* that showed that lens-infecting species are generalists, often infecting numerous fish species (Locke et al., 2010a; Rellstab et al., 2011). The lower host specificity of the lens-infecting *Diplostomum* spp., presumably caused by the establishment in an immunologically privileged site (Lester and Huizinga, 1977; Shariff et al., 1980; Sitjà-Bobadilla, 2008), could plausibly result in higher transmission and fitness because the avian hosts feed on multiple fish species (Feunteun and Marion, 1994; Johnson et al., 2002). The degree of specificity varied among *Diplostomum* species, with *D. huronense* being confined to only four cypriniform host species. This represents an example of strong phylogenetic specificity (Poulin et al., 2011a). However, *D. huronense* has been found in perciform hosts (rock bass and yellow perch) in nearby localities on the St. Lawrence River (Locke et al., 2010b), indicating that *D. huronense* might be less specific than predicted by our results or that its geographic specificity varies (Poulin et al., 2011a). *Diplostomum* sp. 4 exhibited specificity to a lesser degree, infecting various phylogenetically diverse host species, but showing a close association with yellow perch. Rellstab et al. (2011) found a similar phenomenon while studying European species of *Diplostomum* and suggested that differences in host immune responses against certain parasite species could be the cause of such patterns. While certain species of hosts harboured similar infracommunities, the fact that hosts of the same genus never formed exclusive groups and that fish species from different orders and families clustered together indicate that host phylogeny was not a major factor in structuring *Diplostomum* infracommunities in fish. However, these host-parasite associations might be artefacts of sequencing success (Table 4), where the exclusion of incompletely discriminated infracommunities might have biased the position of host species centroids in the RDA space. For example, numerous heavily infected bluntnose minnows had to be discarded from the analysis resulting in apparent infracommunity resemblance with rock bass even though this latter

species exhibit half of the prevalence value and a fifth of the mean intensity value of bluntnose minnow (Tables 4-6). Yet, studies on other host-parasite systems have also found that host phylogeny could not explain community structure adequately (Muñoz et al., 2007). Overall, these results on phylogenetic affiliation are in line with recent work that shows that host phylogeny has more impact at larger scales (*e.g.*, presence/absence of species and Sørensen dissimilarity, component communities or at the species level) than at the finer host infracommunity scale (Locke et al., 2013).

2.4.5. Host effects

Although the contribution of the measured host traits to *Diplostomum* infracommunity composition and structure in our system is probably small, certain host traits significantly influenced infection intensity and infracommunity richness, diversity, and composition. Host length and age were strongly correlated and their respective effects were complementary. Host length had a positive effect on probability of infection and of mixed infections. Larger hosts had more chance of being infected and more chance of harbouring mixed-species infections than smaller ones. However, host age influenced intensity of infection and diversity in different ways. There was a progressive increase in infection level with host age, but the opposite was found for diversity. Most of these results fall in line with the predictions made by Kuris et al. (1980) using a modified version of the island biogeography theory of MacArthur and Wilson (1967) in which larger/older hosts are expected to harbour parasite infracommunities of higher intensity and species richness than smaller/younger ones. The one exception would be the decrease of Shannon diversity in older hosts. This result is a consequence of a proportionally higher number of “unbalanced” assemblages in which one *Diplostomum* species is clearly outnumbering the others present in mixed-species infracommunities in ≥3+ hosts compared to younger ones. This results in lower diversity values even if

intensity of infection is high. That could be explained by annual variation in parasite recruitment in which older fish might have experienced high recruitment in the years preceding the arrival of the young fish cohorts. Differences in sample size between young and old hosts might also have obscured the analyses. Nevertheless, as stated earlier, this situation could be the result of direct negative interactions among *Diplostomum* species or an indirect impact of cross-immunization (Karvonen et al., 2009; Rellstab et al., 2011). At the infracommunity composition scale, no obvious variation among the different age groups was detected, with the exception of ≥3+ hosts that appeared to harbour more *Diplostomum* sp. 4. However, immigration of older fish from other localities cannot be ruled out as a possibility that could explain differences in infection levels and infracommunity diversity observed among age groups. Indeed, parasite communities in fish can significantly vary over short distances (Marcogliese et al., 2006) and the older hosts collected in this study could have been immigrants from another locality with different *Diplostomum* infracommunity composition and structure.

Host sex was not an important factor in determining *Diplostomum* infracommunity composition and structure: female and male hosts were very similar. These results are consistent with numerous studies in various host-parasite systems that failed to find any differences in infracommunity composition and structure between host sexes (Zelmer et al., 2004; Timi and Lanfranchi, 2009; Yamada et al., 2011; Akoll et al., 2012; Bellay et al., 2012; Drago, 2012).

2.4.6. Limitations of the study

Our study has certain limitations that could not be avoided. Most (707 out of 1065) metacercariae were successfully sequenced and assigned to particular *Diplostomum* species. However, the sequencing success varied among sampling times and host species. The reasons of this inability to sequence certain specimens and of the

variation among sampling times and host species are unclear, but they are unlikely related to quantity of DNA provided by the specimens or their actual size (Moszczynska et al., 2009). This problem in sequencing success gave rise to some difficulties in interpretation of certain analyses. Nevertheless, the discrimination results that were obtained are consistent with those of Locke et al. (2010a,b).

3. FINAL CONCLUSIONS AND SUMMARY

The main objectives of the present study were to identify factors influencing the composition and the structure of lens-infecting *Diplostomum* infracommunities in fish. I assessed the potential influence of time of sampling, phylogeny, size, age and sex of the host. I also evaluated potential interspecific interactions among these parasite species using species association indicators.

My system involved four *Diplostomum* species establishing in the lenses of their hosts. The DNA of each metacercaria from all the infracommunities of fish belonging to 20 species sampled in a fluvial lake, Lake Saint-François, in 2006, 2010 and 2011, was analyzed and species were distinguished using the barcode region of cytochrome *c* oxidase subunit I. The analyses revealed negative associations among *Diplostomum* species, confirming my initial hypothesis. The strength of these associations was directly proportional to the mean intensity of the parasite species involved. These negative associations suggest the presence of competitive interactions among nearly all *Diplostomum* species. Contrary to my expectations, analyses revealed important inter-annual variations in *Diplostomum* infracommunities that were far greater than seasonal variations. As expected, I found that host phylogeny could not explain the differences observed in infracommunity composition and structure. Meanwhile, host length and age, but not sex, had significant impacts on *Diplostomum* infracommunities. As predicted, infection intensity was higher in larger/older host, but diversity was unexpectedly lower. However, a significant amount of variance in the system could not be explained, indicating the potential importance of other factors such as resistance or exposure in determining infracommunity structure.

The work contributed to the understanding of parasite community structure and species interactions within parasite infracommunities. The data provided insights on factors affecting natural and potentially interactive larval trematode communities in fish. The study system provided an opportunity to study these effects concurrently in the same host in a field situation. A possible next step to get a better understanding of processes affecting the infracommunity composition and structure of these parasite species would be to explore more in depth the involvement and mechanics of the host immune system and host exposure in mixed-species infections of *Diplostomum*. Well-designed laboratory studies are needed to assess this. Also, as my study showed that a large proportion of variance in community structure was unexplained, other studies on *Diplostomum* infracommunities in natural conditions should include other host- and environment-related traits in an attempt to explain more of the variation in community structure. Such studies could also provide information on the causes of the temporal variation that was observed in this host-parasite system.

FIGURES AND TABLES

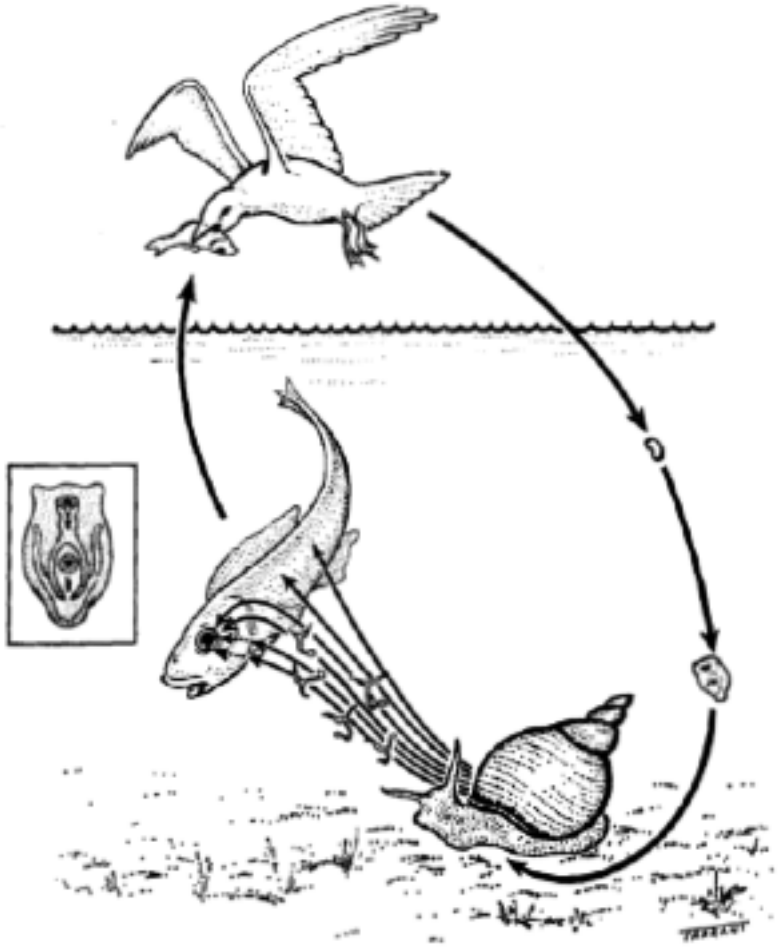


Figure 1. Life cycle of parasites of the genus *Diplostomum*. Adult parasites establish in the intestines of aquatic birds. They reproduce sexually and eggs pass with the host feces. The eggs embryonate and hatch in freshwater, releasing miracidia that infect lymnaeid snails. The miracidia then undergo development into mother sporocysts that produce daughter sporocysts asexually. These in turn produce large numbers of cercariae asexually. Cercariae leave the snail and, following contact with a fish, they penetrate the skin, migrate to the eyes and transform into metacercariae that are infective to birds. These are transmitted to a piscivorous bird when the infected fish is eaten. The parasites can take from 15 to 18 weeks to complete the life cycle. Diagram taken from Ashton et al. (1969).

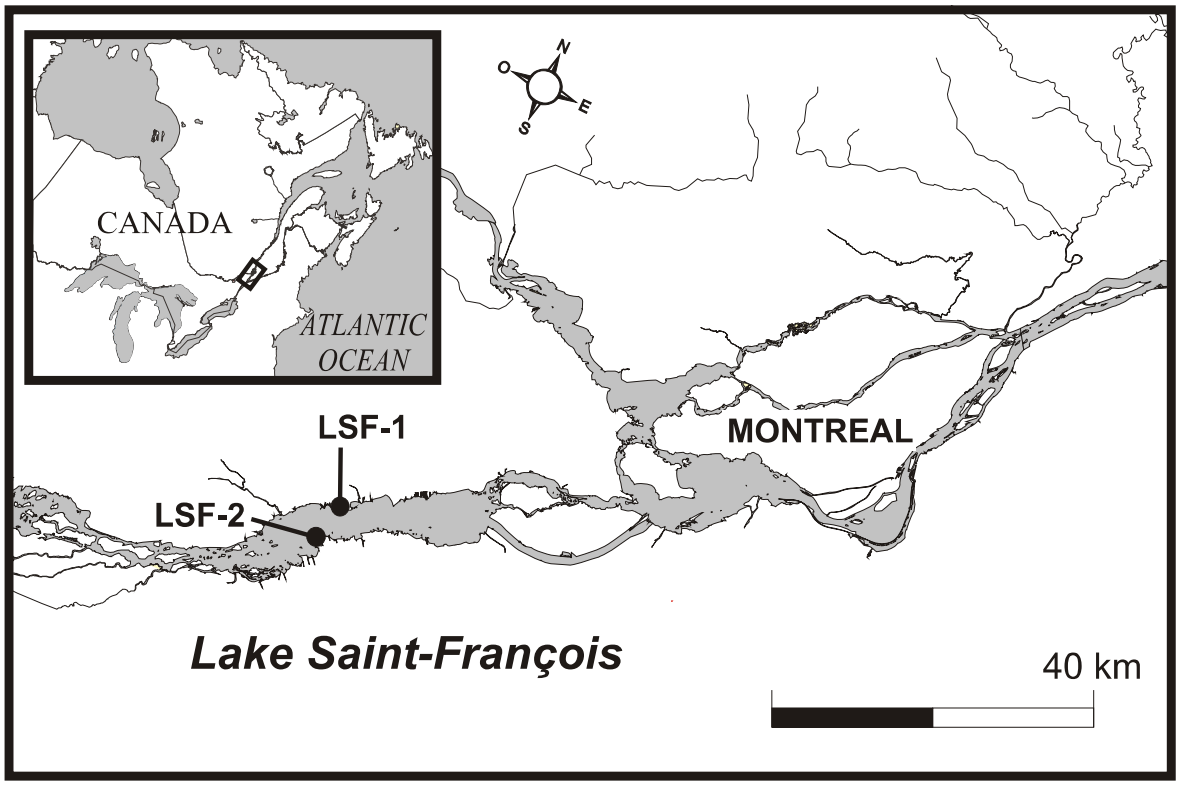


Figure 2. Sampling localities in Lake Saint-François, along the St. Lawrence River, where fish were collected in 2006, 2010 and 2011. Most (*n* = 707) fish were taken at LSF-1 (near Creg Quay Marina, 45.161 °, -74.430 °, in Bainsville, Ontario, Canada) and 122 hosts were collected at LSF-2 (near Pointe Dupuis, 45.128 °, -74.404 °, in Saint-Anicet, Québec, Canada).

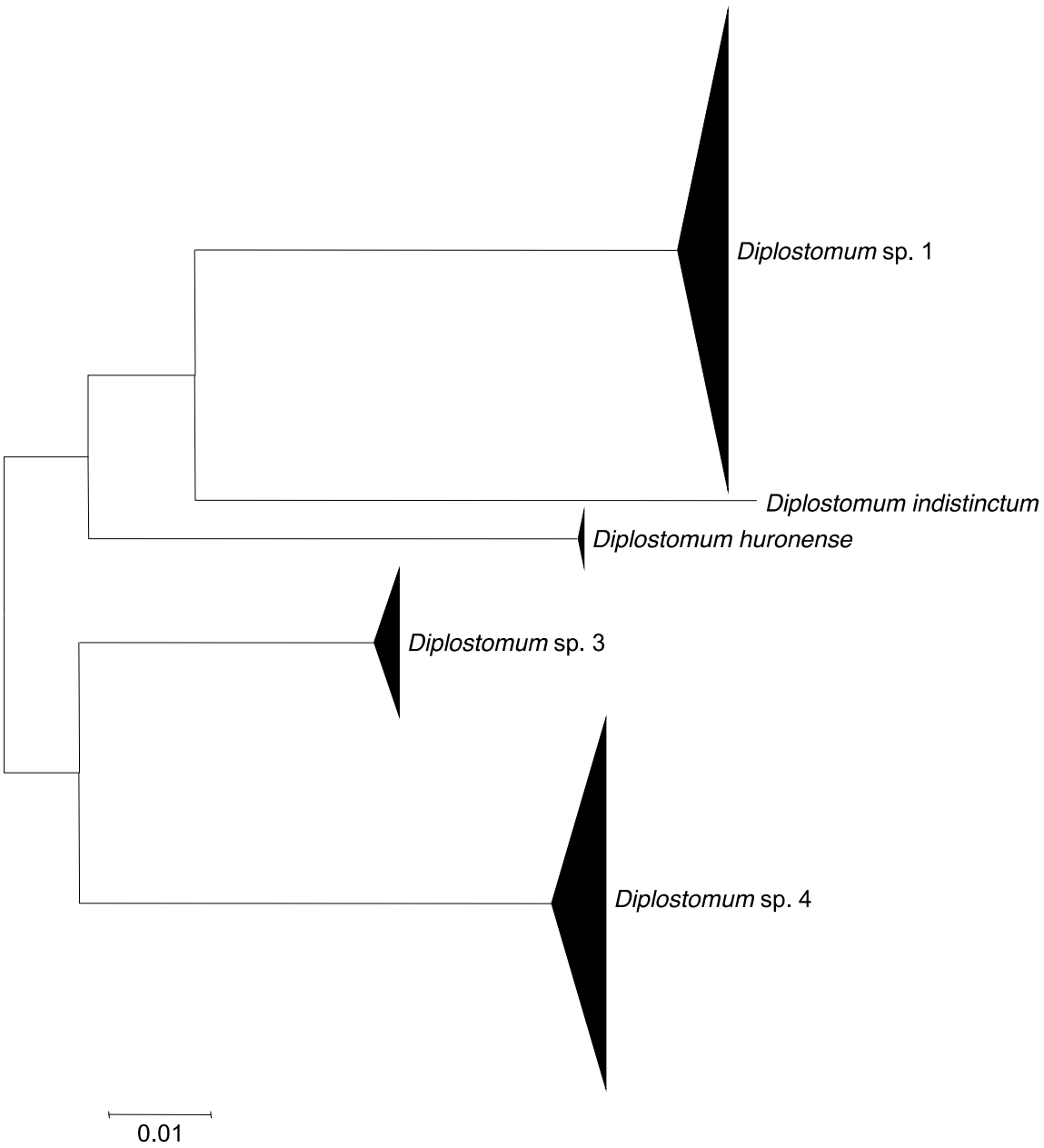


Figure 3. Neighbour-joining phenogram profile of 693 sequences of cytochrome oxidase I obtained from *Diplostomum* spp. metacercariae (neighbour-joining analysis of Kimura 2-parameter distances with pairwise deletion of gaps). The vertical height of black triangles is proportional to the number of specimens sequenced. The width of black triangles, and horizontal branch lengths, are both proportional to sequence dissimilarity.

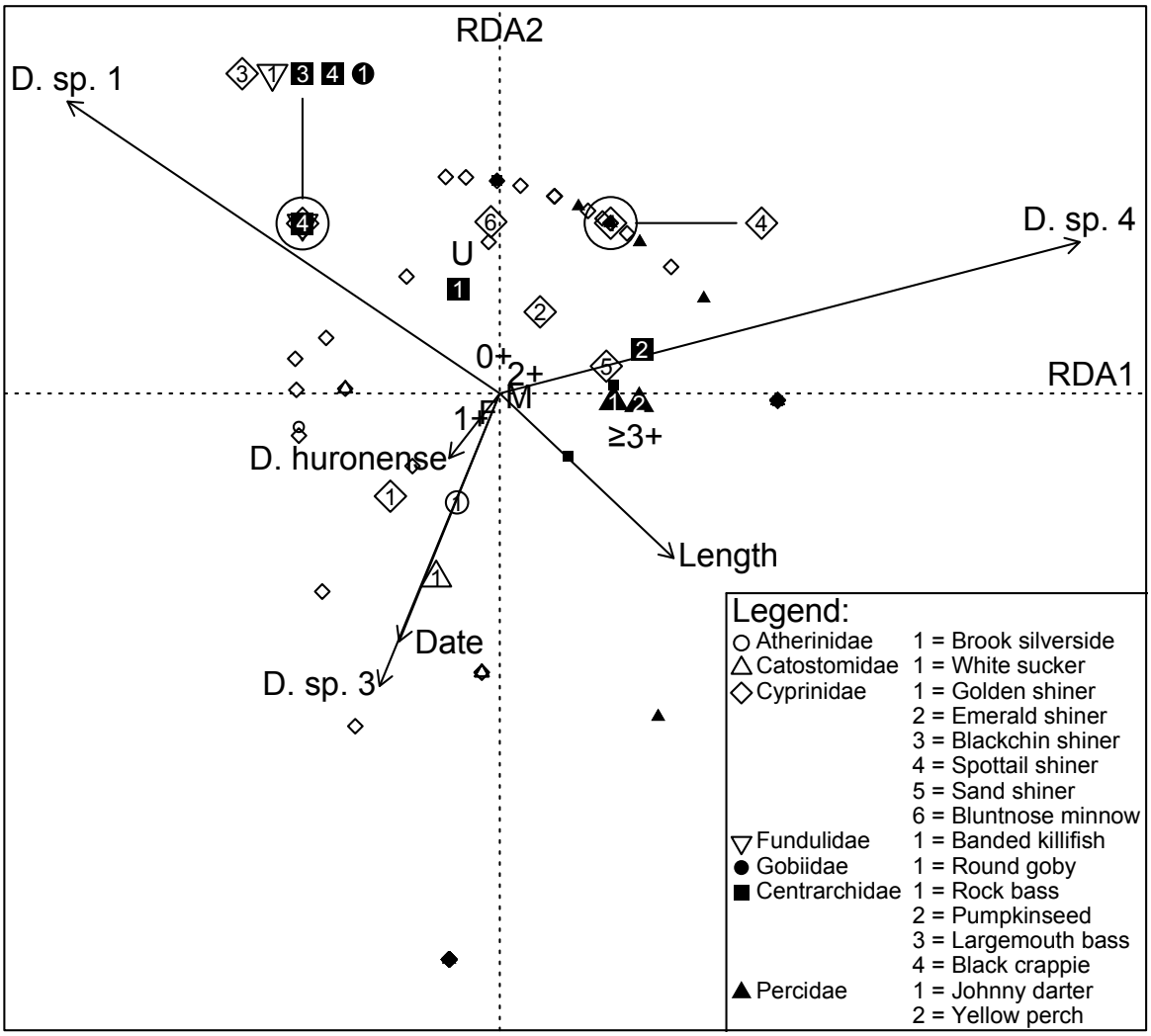


Figure 4. Redundancy analysis triplot of *Diplostomum* spp. lens infracommunities in fish retaining correlations between descriptive variables (*i.e.*, type 2 scaling). The model explains 25% of the variance with axes RDA1 and RDA2 explaining 16.0% and 6.0% respectively. Species of *Diplostomum*, fishing date and host length appear as arrows. Host sex (M = males, F = females, U = undetermined sex) and age (0+, 1+, 2+, ≥3+) are indicated at their centroid values. Small symbols represent infracommunity position of each individual host while larger numbered symbols indicate the position of host species centroids. This triplot was constructed using only infracommunities formed by the 411 successfully sequenced *Diplostomum* specimens in 150 hosts (data set 4, Table 2).

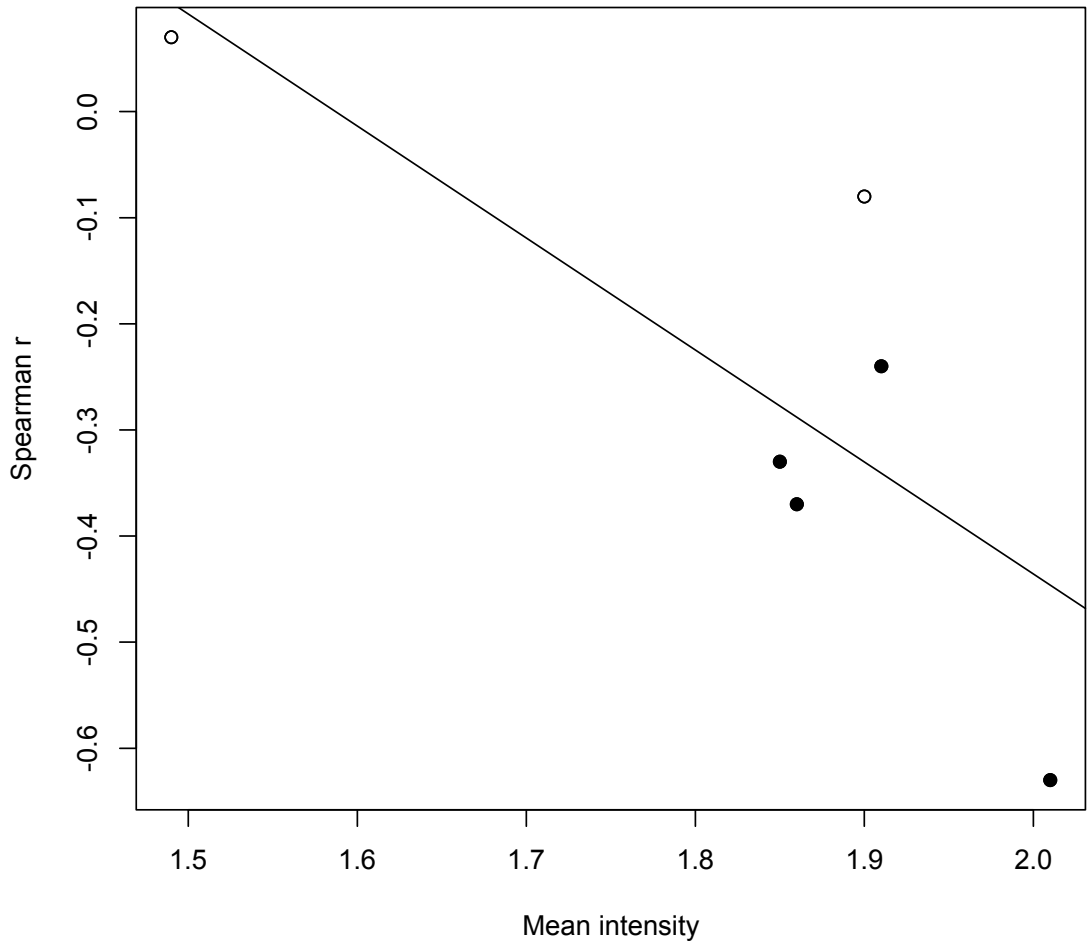


Figure 5. Scatterplot of mean intensity of *Diplostomum* spp. infections and strength of associations in pairs of *Diplostomum* species (linear model marginally significant at F = 6.1, P = 0.069, adjusted R^2 = 0.505). The four significant Spearman r coefficients are identified by closed circles and the two non-significant ones (between *Diplostomum* sp. 1 and *D. huronense*, and between *Diplostomum* sp. 3 and *D. huronense*), by open circles. The mean intensity values were calculated by averaging the infection intensities of each *Diplostomum* species in a pair (data set 5, Table 2).

Table 1. Distribution and number of fish collected in Lake Saint-François in June 2006, September 2010, and June and September 2011 (*n* = 828).

Order	Family	Species	Common name	Sample size
Atheriniformes	Atherinidae	*Labidesthes sicculus*	Brook silverside	27
Clupeiformes	Clupeidae	*Alosa pseudoharengus*	Alewife	12
Cypriniformes	Catostomidae	*Catostomus commersonii*	White sucker	8
	Cyprinidae	*Notemigonus crysoleucas*	Golden shiner	230
		Notropis atherinoides	Emerald shiner	5
		Notropis heterodon	Blackchin shiner	15
		Notropis hudsonius	Spottail shiner	4
		Notropis stramineus	Sand shiner	13
		Pimephales notatus	Bluntnose minnow	42
Cyprinodontiformes	Fundulidae	*Fundulus diaphanus*	Banded killifish	10
Perciformes	Centrarchidae	*Ambloplites rupestris*	Rock bass	35
		Lepomis gibbosus	Pumpkinseed	48
		Lepomis macrochirus	Bluegill	7
		Micropterus dolomieu	Smallmouth bass	6
		Micropterus salmoides	Largemouth bass	11
		Pomoxis nigromaculatus	Black crappie	5
	Gobiidae	*Neogobius melanostomus*	Round goby	31
	Percidae	*Etheostoma nigrum*	Johnny darter	45
		Perca flavescens	Yellow perch	273
Siluriformes	Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	1

Table 2. Subdivisions of the data used in various statistical analyses. Sequencing success and infection level were the two criteria used to select the appropriate data for each analysis. Partial sequencing success refers to the use of infracommunities in which some *Diplostomum* metacercariae were not successfully sequenced, while complete sequencing success refers to the use of entirely discriminated infracommunities only. The infection level indicates the number of metacercariae present in the selected infracommunities. For most analyses, lens infracommunities were pooled in each host (*i.e.*, the left and right lenses were pooled together) with the exception of data set 5 used for species association analysis.

Data set	Sequencing success	Infection level	Lens infracommunity	Number of hosts	Number of parasites	Analyses
1	Partial	0, 1 and >1	Pooled, all infected and uninfected hosts	828	1065	Prevalence, infection probability (logistic regression)
2	Partial	1 and >1	Pooled, all infected hosts	270	1065	Mean intensity, variation in intensity (GLM)
3	Partial	>1	Pooled, all hosts with single or mixed-species infection determined according to sequencing success	148	943	Mixed-species infection probability (logistic regression)
4	Complete	1 and >1	Pooled, all infected hosts	150	411	Variation in richness and diversity (GLMs), infracommunity structure (RDA)
5	Complete	>1	Partitioned into left and right lenses	94 lenses from 51 hosts	395	Species associations (Spearman correlation)

Table 3. Evolutionary divergence (% of nucleotide substitutions between sequences) within *Diplostomum* species encountered in Lake Saint-François in June 2006, September 2010, and June and September 2011. Evolutionary divergence was calculated using neighbour-joining analysis of Kimura 2-parameter distances (Kimura, 1980) with pairwise deletion of gaps. The average of divergence across all four *Diplostomum* species is equal to 0.46%.

	Mean	Minimum	Maximum
Diplostomum sp. 1	0.76	0.00	2.35
Diplostomum sp. 3	0.22	0.00	2.03
Diplostomum sp. 4	0.61	0.00	3.80
D. huronense	0.26	0.00	1.49

Table 4. Number (N) of fish collected in Lake Saint-François, number of *Diplostomum* metacercariae found in lenses, number of metacercariae from which sequences of cytochrome oxidase I were obtained, and overall sequencing success (%) in June 2006, September 2010, and June and September 2011.

	June 2006				September 2010				June 2011				September 2011			
Host species	N fish	N *Diplostomum*	N discriminated	Sequencing success (%)	N fish	N *Diplostomum*	N discriminated	Sequencing success (%)	N fish	N *Diplostomum*	N discriminated	Sequencing success (%)	N fish	N *Diplostomum*	N discriminated	Sequencing success (%)
Brook silverside					3	7	7	100.0	1	1	1	100.0				
White sucker													3	4	4	100.0
Golden shiner	37	315	199	63.2	15	35	28	80.0	39	110	42	38.2	5	10	9	90.0
Emerald shiner													3	5	4	80.0
Blackchin shiner					1	1	0	0.0					4	4	3	75.0
Spottail shiner	1	3	3	100.0												
Sand shiner									3	10	10	100.0	3	8	8	100.0
Bluntnose minnow	26	289	207	71.6									2	2	2	100.0
Banded killifish									1	3	3	100.0	2	3	3	100.0
Rock bass	11	27	24	88.9												
Pumpkinseed	13	16	10	62.5												
Largemouth bass	1	1	0	0.0									1	1	1	100.0
Black crappie	1	3	1	33.3									1	1	1	100.0
Round goby					1	3	0	0.0					6	13	12	92.3
Johnny darter	9	15	14	93.3									1	1	1	100.0
Yellow perch	24	56	48	85.7	19	51	25	49.0	22	43	18	41.9	11	24	19	79.2
All host species	123	725	506	69.8	39	97	60	61.9	66	167	74	44.3	42	76	67	88.2

Table 5. Prevalence (%) of the four species of lens-infecting *Diplostomum* found in Lake Saint-François in 20 host species. The number of fish examined is indicated in parentheses. Prevalences were calculated using the 1065 metacercariae distributed in 828 infected or uninfected hosts (data set 1, Table 2).

Host species	All species	*Diplostomum* sp. 1	*Diplostomum* sp. 3	*Diplostomum* sp. 4	*D. huronense*
Brook silverside (27)	14.8	7.4	7.4	3.7	0.0
Alewife (12)	0.0	0.0	0.0	0.0	0.0
White sucker (8)	37.5	12.5	0.0	0.0	37.5
Golden shiner (230)	41.7	18.7	15.2	3.9	7.8
Emerald shiner (5)	60.0	40.0	0.0	20.0	0.0
Blackchin shiner (15)	33.3	20.0	0.0	0.0	0.0
Spottail shiner (4)	25.0	25.0	0.0	25.0	0.0
Sand shiner (13)	46.2	23.1	0.0	30.8	7.7
Bluntnose minnow (42)	66.7	50.0	7.1	40.5	2.4
Banded killifish (10)	30.0	30.0	0.0	0.0	0.0
Rock bass (35)	31.4	20.0	5.7	14.3	0.0
Pumpkinseed (48)	27.1	6.3	0.0	12.5	0.0
Bluegill (7)	0.0	0.0	0.0	0.0	0.0
Smallmouth bass (6)	0.0	0.0	0.0	0.0	0.0
Largemouth bass (11)	18.2	9.1	0.0	0.0	0.0
Black crappie (5)	40.0	40.0	0.0	0.0	0.0
Round goby (31)	22.6	19.4	0.0	0.0	0.0
Johnny darter (45)	22.2	6.7	2.2	13.3	0.0
Yellow perch (273)	27.8	8.1	1.8	15.4	0.0
Brown bullhead (1)	0.0	0.0	0.0	0.0	0.0
All host species (828)	32.6	14.9	5.8	11.1	2.8

Table 6. Mean intensity (± standard deviation) of four species of lens-infecting *Diplostomum* found in Lake Saint-François in 16 species of hosts. The number of fish examined is indicated in parentheses. Mean intensities were calculated using the 1065 *Diplostomum* metacercariae distributed in the 270 infected hosts (data set 2, Table 2).

Host species	All species	*Diplostomum* sp. 1	*Diplostomum* sp. 3	*Diplostomum* sp. 4	*D. huronense*
Brook silverside (4)	2.00 (2.00)	2.50 (2.12)	1.00 (0.00)	1.00	
White sucker (3)	1.33 (0.58)	1.00			1.00 (0.00)
Golden shiner (96)	4.90 (4.91)	3.40 (3.18)	2.37 (1.91)	1.56 (1.01)	1.89 (1.23)
Emerald shiner (3)	1.67 (1.15)	1.50 (0.71)		1.00	
Blackchin shiner (5)	1.00 (0.00)	1.00 (0.00)			
Spottail shiner (1)	3.00	1.00		2.00	
Sand shiner (6)	3.00 (2.76)	1.67 (0.58)		3.00 (2.31)	1.00
Bluntnose minnow (28)	10.39 (8.37)	4.24 (3.48)	1.33 (0.58)	6.76 (5.32)	1.00
Banded killifish (3)	2.00 (1.00)	2.00 (1.00)			
Rock bass (11)	2.45 (2.42)	1.57 (0.79)	1.00 (0.00)	2.20 (2.17)	
Pumpkinseed (13)	1.23 (0.83)	1.00 (0.00)		1.17 (0.41)	
Largemouth bass (2)	1.00 (0.00)	1.00			
Black crappie (2)	2.00 (1.41)	1.00 (0.00)			
Round goby (7)	2.29 (0.76)	2.00 (0.89)			
Johnny darter (10)	1.60 (1.90)	1.67 (1.15)	1.00	1.50 (1.22)	
Yellow perch (76)	2.29 (2.01)	1.14 (0.35)	1.20 (0.45)	1.86 (1.83)	
All host species (270)	3.94 (4.89)	2.59 (2.66)	2.04 (1.73)	2.72 (3.31)	1.70 (1.15)

Table 7. Estimated values and significance of coefficients in a logistic regression on the probability of *Diplostomum* infection using the 1065 metacercariae distributed in 828 infected or uninfected hosts (data set 1, Table 2). The model has a percentage of residual deviance of 68.3 on 806 degrees of freedom and a pseudo-R^2 of 0.460. Non-significant (N.S.) host species coefficients were not listed (coefficient significance indicated by symbols: * for $P < 0.05$, *** for $P < 0.001$).

Model	Parameter	Estimate	Standard error	*Z*	*P*	
Most parsimonious						
	Intercept	-1748	701.6	-2.491	0.0127	*
	Year	0.880	0.078	11.31	< 0.0001	***
	Host species	N.S.	N.S.	N.S.	> 0.9795	
	Standard length	-0.060	0.006	-9.826	< 0.0001	***

Table 8. Estimated values and significance of coefficients in a logistic regression on the probability of mixed-species infection using the 943 *Diplostomum* specimens in 148 hosts infected by more than one metacercaria, and in which the host could be classified as infected by single or mixed-species infracommunity (data set 3, Table 2). The most parsimonious model has a percentage of residual deviance of 60.4 on 75 degrees of freedom and a pseudo-R^2 of 0.543, Alternative 1 has a deviance of 63.4 on 76 degrees of freedom and a pseudo-R^2 of 0.505, and Alternative 2 has a deviance of 60.4 on 74 degrees of freedom and a pseudo-R^2 of 0.543. Non-significant (N.S.) host species coefficients were not listed (coefficient significance indicated by symbols: ° for $0.05 < P < 0.1$, * for $P < 0.05$, ** for $P < 0.01$).

Model	Parameter	Estimate	Standard error	*Z*	*P*
Most parsimonious					
	Intercept	-823.6	457.3	-1.801	0.0717 °
	Year	0.412	0.229	1.805	0.0711 °
	Host species	N.S.	N.S.	N.S.	> 0.1373
	Standard length	-0.079	0.029	-2.754	0.0059 **
Alternative 1					
	Intercept	1.888	1.687	1.119	0.2630
	Host species	N.S.	N.S.	N.S.	> 0.1927
	Standard length	-0.047	0.020	-2.420	0.0155 *
Alternative 2					
	Intercept	-827.8	526.8	-1.571	0.1161
	Year	0.414	0.264	1.572	0.1159
	Month	-0.006	0.356	-0.016	0.9873
	Host species	N.S.	N.S.	N.S.	> 0.1373
	Standard length	-0.079	0.029	-2.750	0.0060 **

Table 9. Estimated values and significance of coefficients in a generalized linear model on the intensity of *Diplostomum* infection using the 1065 metacercariae distributed in the 270 infected hosts (data set 2, Table 2). The model has a percentage of residual deviance of 40.6 on 250 degrees of freedom and a pseudo-R^2 of 0.553 (coefficient significance indicated by symbols: ° for $0.05 < P < 0.1$, * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$).

Model	Parameter	Estimate	Standard error	*Z*	*P*	
Most parsimonious						
	Intercept	548.8	54.12	10.14	< 0.0001	***
	Year	-0.274	0.027	-10.13	< 0.0001	***
	Host species					
	Brook silverside	1.649	0.538	3.066	0.0022	**
	White sucker	1.121	0.652	1.718	0.0858	°
	Golden shiner	2.042	0.306	6.671	< 0.0001	***
	Emerald shiner	1.553	0.620	2.504	0.0123	*
	Blackchin shiner	1.664	0.609	2.735	0.0062	**
	Spottail shiner	0.254	0.795	0.320	0.7493	
	Sand shiner	2.370	0.453	5.231	< 0.0001	***
	Bluntnose minnow	2.271	0.302	7.519	< 0.0001	***
	Banded killifish	1.682	0.590	2.852	0.0044	**
	Pumpkinseed	-0.427	0.390	-1.095	0.2737	
	Largemouth bass	-0.310	0.832	-0.372	0.7096	
	Black crappie	0.090	0.676	0.132	0.8946	
	Round goby	1.533	0.434	3.530	0.0004	***
	Johnny darter	0.314	0.404	0.779	0.4362	
	Yellow perch	0.883	0.276	3.195	0.0014	**
	Age					
	1+	0.681	0.117	5.815	< 0.0001	***
	2+	1.199	0.222	5.410	< 0.0001	***
	≥3+	1.667	0.229	7.263	< 0.0001	***

Table 10. Estimated values and significance of coefficients in a generalized linear model on the species richness of *Diplostomum* infection using the 411 successfully sequenced *Diplostomum* specimens in 150 hosts (data set 4, Table 2). The model has a percentage of residual deviance of 64.3 on 142 degrees of freedom and a pseudo-R^2 of 0.385 (coefficient significance indicated by symbols: ° for $0.05 < P < 0.1$, ** for $P < 0.01$).

Model	Parameter	Estimate	Standard error	*Z*	*P*
Most parsimonious					
	Intercept	200.2	71.83	2.787	0.0053 **
	Year	-0.099	0.036	-2.783	0.0054 **
	Host order				
	Cypriniformes	-0.066	0.464	-0.141	0.8877
	Cyprinodontiformes	-0.214	0.737	-0.290	0.7714
	Perciformes	-0.448	0.485	-0.923	0.3561
	Age				
	1+	0.003	0.171	0.017	0.9861
	2+	0.191	0.276	0.690	0.4902
	≥3+	0.587	0.323	1.821	0.0686 °

Table 11. Estimated values and significance of coefficients in a generalized linear model on the Shannon diversity of *Diplostomum* infection using the 411 successfully sequenced *Diplostomum* specimens in 150 hosts (data set 4, Table 2). The model has a percentage of residual deviance of 62.9 on 142 degrees of freedom and a pseudo-R^2 of 0.642 (coefficient significance indicated by symbols: ° for $0.05 < P < 0.1$, * for $P < 0.05$, *** for $P < 0.001$).

Model	Parameter	Estimate	Standard error	*T*	*P*	
Most parsimonious						
	Intercept	-131.1	17.53	-7.477	< 0.0001	***
	Year	0.066	0.009	7.523	< 0.0001	***
	Host order					
	Cypriniformes	0.028	0.122	0.230	0.8182	
	Cyprinodontiformes	0.135	0.198	0.681	0.4973	
	Perciformes	0.268	0.127	2.118	0.0359	*
	Age					
	1+	-0.006	0.042	-0.137	0.8912	
	2+	-0.132	0.067	-1.968	0.0510	°
	≥3+	-0.365	0.077	-4.754	< 0.0001	***

Table 12. Correlations in abundance among species of *Diplostomum* in the lenses of fish (Spearman correlation coefficients on Hellinger-transformed abundance). The coefficients were calculated among 395 successfully sequenced *Diplostomum* specimens found in 94 lenses harbouring more than one metacercaria, from 51 different hosts (data set 5, Table 2). Infracommunities were partitioned into left and right lenses and analysed separately. Spearman *r* coefficients are below the diagonal, and *P* adjusted by Holm's method are above the diagonal. Significant values are printed in bold.

	Diplostomum sp. 1	*Diplostomum* sp. 3	*Diplostomum* sp. 4	*D. huronense*
Diplostomum sp. 1		**< 0.0001**	**< 0.0001**	0.4412
Diplostomum sp. 3	**-0.33**		**< 0.0001**	0.4412
Diplostomum sp. 4	**-0.63**	**-0.37**		**0.0011**
D. huronense	-0.08	0.07	**-0.24**	

REFERENCES

Abraham, B.J., 1985. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) – mummichog and striped killifish. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.40). U.S. Army Corps of Engineers, TR EL-82-4. 23 pp.

Adjei, E.L., Barnes, A., Lester, R.J.G., 1986. A method for estimating possible parasite-related host mortality, illustrated using data from *Callitetrarhynchus gracilis* (Cestoda: Trypanorchyncha) in lizardfish (*Saurida* spp.). Parasitology 92, 227-243.

Akoll, P., Konecny, R., Mwanja, W.W., Schiemer, F., 2012. Infection patterns of Nile tilapia (*Oreochromis niloticus* L.) by two helminth species with contrasting life styles. Parasitol. Res. 110, 1461-1472.

Alexander, N., Moyeed, R., Stander, J., 2000. Spatial modelling of individual-level parasite counts using the negative binomial distribution. Biostatistics 1, 453-463.

Armstrong, R.A., McGehee, R., 1976. Coexistence of species competing for shared resources. Theor. Popul. Biol. 9, 317-328.

Ashton, N., Brown, N., Easty, D., 1969. Trematode cataract in fresh water fish. J. Small Anim. Pract. 10, 471-478.

Bagge, A.M., Sasal, P., Valtonen, E.T., Karvonen, A., 2005. Infracommunity level aggregation in the monogenean communities of crucian carp (*Carassius carassius*). Parasitology 131, 367-372.

Bajer, A., Behnke, J.M., Pawełczyk, A., Kuliś, K., Sereda, M.J., Siński, E., 2005. Medium-term temporal stability of the helminth component community structure in bank voles (*Clethrionomys glareolus*) from the Mazury Lake District region of Poland. Parasitology 130, 213-228.

Bardach, J.E., 1955. The opercular bone of the yellow perch, *Perca flavescens*, as a tool for age and growth studies. Copeia 1955, 107-109.

Begon, M., Townsend, C.R., Harper, J.L., 2006. Ecology: from individuals to ecosystems. Blackwell Publishing, Malden, MA, USA.

Behnke, R.J.M., Bajer, A., Sinski, E., Wakelin, D., 2001. Interactions involving intestinal nematodes of rodents: experimental and field studies. Parasitology 122, S39-S49.

Behrmann-Godel, J., 2013. Parasite identification, succession and infection pathways in perch fry (*Perca fluviatilis*): new insights through a combined morphological and genetic approach. Parasitology 140, 509-520.

Bellay, S., Takemoto, R.M., Oliveira, E.F., 2012. Is the community of fish gill parasites structured in a Neotropical floodplain? Acta Parasitol. 57, 53-60.

Belyea, L.R., Lancaster, J., 1999. Assembly rules within a contingent ecology. Oikos 86, 402-416.

Bordes, F., Morand, S., Kelt, D.A., van Vuren, D.H., 2009. Home range and parasite diversity in mammals. Am. Nat. 173, 467-474.

Bozdogan, H., 1987. Model selection and Akaike's information criterion (AIC): the general theory and its analytical extensions. Psychometrika 52, 345-370.

Brassard, P., Rau, M.E., Curtis, M.A., 1982. Parasite-induced host susceptibility to predation in diplostomiasis. Parasitology 85, 495-501.

Brown, K.M., Leathers, B.K., Minchella, D.J., 1988. Trematode prevalence and the population dynamics of freshwater pond snails. Am. Midl. Nat. 120, 289-301.

Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference: a practical information-theoretic approach (2nd edition). Springer-Verlag, New York, NY, USA.

Burnham, K.P., Anderson, D.R., 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol. Methods Res. 33, 261-304.

Burrough, R.J. 1978. The population biology of two species of eyefluke, *Diplostomum spathaceum* and *Tylodelphys clavata* in roach and rudd. J. Fish Biol. 13, 19-32.

Bush, A.O., Holmes, J.C., 1986. Intestinal helminths of lesser scaup ducks: patterns of association. Can. J. Zool. 64, 132-141.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. J. Parasitol. 83, 575-583.

Byers, J.E., Blakeslee, A.M.H., Linder, E., Cooper, A.B., Maguire, T.J., 2008. Controls of spatial variation in the prevalence of trematode parasites infecting a marine snail. Ecology 89, 439-451.

Bylund, G., Sumari, O., 1981. Laboratory tests with Droncit against diplostomiasis in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 4, 259-264.

Carney, J.P., Dick, T.A., 1999. Enteric helminths of perch (*Perca fluviatilis* L.) and yellow perch (*Perca flavescens* Mitchill): stochastic or predictable assemblages? J. Parasitol. 85, 785-795.

Carney, J.P., Dick, T.A., 2000. Helminth communities of yellow perch (*Perca flavescens* (Mitchill)): determinants of pattern. Can. J. Zool. 78, 538-555.

Cavaleiro, F.I., Pina, S., Russell-Pinto, F., Rodrigues, P., Formigo, N.E., Gibson, D.I., Santos, M.J., 2012. Morphology, ultrastructure, genetics, and morphometrics of *Diplostomum* sp. (Digenea: Diplostomidae) metacercariae infecting the European flounder, *Platichthys flesus* (L.) (Teleostei: Pleuronectidae), off the northwest coast of Portugal. Parasitol. Res. 110, 81-93.

Chalanchuk, S.M., 1998. Growth of white sucker, *Catostomus commersoni*, in thirty-one lakes at the Experimental Lakes Area, Northwestern Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 2207, vi + 65 p.

Chappell, L.H., Hardie, L.J., Secombes, C.J., 1994. Diplostomiasis: the disease and host-parasite interactions. In: Pike, A.W, Lewis, J.W. (Eds.), Parasitic diseases of fish. Samara Publishing Ltd., Tresaith, Dyfed, UK, pp. 59-86.

Cody, M.L., 1974. Competition and the structure of bird communities. Princeton University Press, Princeton, NJ, USA.

Combes, C., 2001. Parasitism: the ecology and evolution of intimate interactions. University of Chicago Press, Chicago, IL, USA.

Cooper, N., Griffin, R., Franz, M., Omotayo, M., Nunn, C.L., 2012. Phylogenetic host specificity and understanding parasite sharing in primates. Ecol. Lett. 15, 1370-1377.

Cornell, H.V., 1985. Local and regional richness of cynipine gall wasps on California oaks. Ecology 66, 1247-1260.

Cornell, H.V., Lawton, J.H., 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. J. Anim. Ecol. 61, 1-12.

Criscione, C.D., Poulin, R., Blouin, M.S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Mol. Ecol. 14, 2247-2257.

Crowden, A.E., Broom, D.M., 1980. Effects of the eyefluke, *Diplostomum spathaceum*, on the behavior of dace (*Leuciscus leuciscus*). Anim. Behav. 28, 287-294.

Davies, R.B., Burkhard, W.T., Hibler, C.P., 1973. Diplostomosis in North Park, Colorado. J. Wildl. Dis. 9, 362-367.

Dezfuli, B.S., Giari, L., De Biaggi, S., Poulin, R., 2001. Associations and interactions among intestinal helminths of the brown trout, *Salmo trutta*, in northern Italy. J. Helminthol. 75, 331-336.

Dove, A.D.M., 2000. Richness patterns in the parasite communities of exotic poeciliid fishes. Parasitology 120, 609-623.

Drago, F.B., 2012. Community structure of metazoan parasites of silverside, *Odontesthes bonariensis* (Pisces, Atherinopsidae) from Argentina. Iheringia Ser. Zool. 102, 26-32.

Dubois, G., 1961. Le genre *Diplostomum* von Nordmann, 1832 (Trematoda: Strigeida). Bull. Soc. Neuchâtel Sci. Nat. 84, 113-124.

Dunson, W.A., Travis, J., 1991. The role of abiotic factors in community organization. Am. Nat. 138, 1067-1091.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792-1797.

Esch, G.W., Shostak, A.W., Marcogliese, D.J., Goater, T.M., 1990. Patterns and processes in helminth parasite communities: an overview. In: Esch, G.W, Bush, A.O., Aho, J.M. (Eds.), Parasite communities: patterns and processes. Chapman and Hall, New York, NY, USA, pp. 1-19.

Faltýnková, A., Karvonen, A., Valtonen, E.T., 2011. Establishment and interspecific associations in two species of *Ichthyocotylurus* (Trematoda) parasites in perch (*Perca fluviatilis*). Parasit. Vectors 4, 85.

Faltýnková, A., Valtonen, E.T., Karvonen, A., 2008. Spatial and temporal structure of the trematode component community in *Valvata macrostoma* (Gastropoda, Prosobranchia). Parasitology 135, 1691-1699.

Fenton A., Viney, M.E., Lello, J., 2010. Detecting interspecific macroparasite interactions from ecological data: patterns and process. Ecol. Lett. 13, 606-615.

Feunteun, E., Marion, L., 1994. Assessment of grey heron predation on fish communities: the case of the largest European colony. Hydrobiologia 279/280, 327-344.

Galazzo, D.E., Dayanandan, S., Marcogliese, D.J., McLaughlin, J.D., 2002. Molecular systematics of some North American species of *Diplostomum* (Digenea) based on sequence data and comparisons with European congeners. Can. J. Zool. 80, 2207-2217.

Georgieva, S., Soldánová, M., Pérez-del-Olmo, A., Dangel, D.R., Sitko, J., Sures, B., Kostadinova, A., 2013. Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity. Int. J. Parasitol. 43, 57-72.

Goven, B.A., Dawe, D.L., Gratzek, J.B., 1980. Protection of channel catfish, *Ictalurus punctatus* Rafinesque, against *Ichthyophthirius multifiliis* Fouquet by immunization. J. Fish Biol. 17, 311-316.

Hanski, I., 1982. Dynamics of regional distribution: the core and satellite hypothesis. Oikos 38, 210-221.

Hanski, I., Gilpin, M.E., 1997. Metapopulation biology: ecology, genetics, and evolution. Academic Press, San Diego, CA, USA.

Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B Biol. Sci. 270, S96-S99.

Hines R.S., Spira, D.T., 1974. Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.) v. acquired immunity. J. Fish Biol. 6, 373-378.

Höglund, J., Thuvander, A., 1990. Indications of nonspecific protective mechanisms in rainbow trout *Oncorhynchus mykiss* with diplostomosis. Dis. Aquat. Org. 8, 91-97.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Statist. 6, 65-70.

Holmes, J.C., 1961. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala). I. General effects and comparison with crowding. J. Parasitol. 47, 209-216.

Holmes, J.C., 1962. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala). II. Effects on growth. J. Parasitol. 48, 87-96.

Holt, R.D., Kotler, B.P., 1987. Short-term apparent competition. Am. Nat. 130, 412-430.

Huston, M.A., 1994. Biological diversity: the coexistence of species on changing landscapes. Cambridge University Press, Cambridge, UK.

Jaeger, R.G., Walls, S.C., 1989. On salamander guilds and ecological methodology. Herpetologica 45, 111-119.

Janeway, C.A.J., Medzhitov, R., 2002. Innate immune recognition. Annu. Rev. Immunol. 20, 197-216.

Janovy, J.J.Jr., Clopton, R.E., Percival, T.J., 1992. The roles of ecological and evolutionary influences in providing structure to parasite species assemblages. J. Parasitol. 78, 630-640.

Janovy, J.J.Jr., Snyder, S.D., Clopton, R.E., 1997. Evolutionary constraints on population structure: the parasites of *Fundulus zebrinus* (Pisces: Cyprinodontidae) in the South Platte River of Nebraska. J. Parasitol. 83, 584-592.

Johnson, J.H., Ross, R.M., McCullough, R.D., 2002. Little Galloo Island, Lake Ontario: a review of nine years of double-crested cormorant diet and fish consumption information. J. Great Lakes Res. 28, 182-192.

Kareiva, P., Mullen, A., Southwood, R., 1990. Population dynamics in spatially complex environments: theory and data [and discussion]. Phil. Trans. R. Soc. Lond. B Biol. Sci. 330, 175-190.

Karvonen, A., Seppälä, O., 2008. Eye fluke infection and lens size reduction in fish: a quantitative analysis. Dis. Aquat. Org. 80, 21-26.

Karvonen, A., Halonen, H., Seppälä, O., 2010. Priming of host resistance to protect cultured rainbow trout *Oncorhynchus mykiss* against eye flukes and parasite-induced cataracts. J. Fish Biol. 76, 1508-1515.

Karvonen, A., Hudson, P.J., Seppälä, O., Valtonen, E.T., 2004a. Transmission dynamics of a trematode parasite: exposure, acquired resistance and parasite aggregation. Parasitol. Res. 92, 183-188.

Karvonen, A., Paukku, S., Seppälä, O., Valtonen, E.T., 2005. Resistance against eye flukes: naive versus previously infected fish. Parasitol. Res. 95, 55-59.

Karvonen, A., Seppälä, O., Valtonen, E.T., 2004b. Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. Parasitology 129, 159-164.

Karvonen, A., Seppälä, O., Valtonen, E.T., 2009. Host immunization shapes interspecific associations in trematode parasites. J. Anim. Ecol. 78, 945-952.

Karvonen, A., Terho, P., Seppälä, O., Jokela, J., Valtonen, E.T., 2006. Ecological divergence of closely related *Diplostomum* (Trematoda) parasites. Parasitology 133, 229-235.

Keesing, F., Holt, R.D., Ostfeld, R.S., 2006. Effects of species diversity on disease risk. Ecol. Lett. 9, 485-498.

Kennedy, C.R., 1981. Long term studies on the population biology of two species of eyefluke, *Diplostomum gasterostei* and *Tylodelphys clavata* (Digenea: Diplostomatidae), concurrently infecting the eyes of perch, *Perca fluviatilis*. J. Fish Biol. 19, 221-236.

Kennedy, C.R., 1990. Helminth communities in freshwater fish: structured communities or stochastic assemblages? In: Esch, G.W, Bush, A.O., Aho, J.M. (Eds.), Parasite communities: patterns and processes. Chapman and Hall, New York, NY, USA, pp.131-156.

Kennedy, C.R., 2001a. Metapopulation and community dynamics of helminth parasites of eels *Anguilla anguilla* in the River Exe system. Parasitology 122, 689-698.

Kennedy, C.R., 2001b. Interspecific interactions between larval digeneans in the eyes of perch, *Perca fluviatilis*. Parasitology 122, S13-S22.

Kennedy, C.R., Burrough, R.J., 1977. The population biology of two species of eyefluke, *Diplostomum gasterostei* and *Tylodelphys clavata*, in perch. J. Fish Biol. 11, 619-633.

Kennedy, C.R., Hartvigsen, R.A., 2000. Richness and diversity of intestinal metazoan communities in brown trout *Salmo trutta* compared to those of eels *Anguilla anguilla* in their European heartlands. Parasitology 121, 55-64.

Kennedy, M.J., MacKinnon, J.D., 1994. Site segregation of *Thelazia skrjabini* and *Thelazia gulosa* (Nematoda: *Thelazioidea*) in the eyes of cattle. J. Parasitol. 80, 501-504.

Khan, M.A., Khan, S., 2009. Comparison of age estimates from scale, opercular bone, otolith, vertebrae and dorsal fin ray in *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Channa marulius* (Hamilton). Fish. Res. 100, 255-259.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111-120.

Kipp, R., Ricciardi, A., 2012. Impacts of the Eurasian round goby (*Neogobius melanostomus*) on benthic communities in the upper St. Lawrence River. Can. J. Fish. Aquat. Sci. 69, 469-486.

Krasnov, B.R., Bordes, F., Khokhlova, I.S., Morand, S., 2012. Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. Mammalia 76, 1-13.

Krasnov, B.R., Mouillot, D., Khokhlova, I.S., Shenbrot, G.I., Poulin, R., 2005. Covariance in species diversity and facilitation among non-interactive parasite taxa: all against the host. Parasitology 131, 557-568.

Kuris, A.M., Blaustein, A.R., Alio, J.J., 1980. Hosts as islands. Am. Nat. 116, 570-586.

Kurtz, J., 2005. Specific memory within innate immune systems. Trends Immunol. 26, 186-192.

Laitinen, M., Siddall, R., Valtonen, E.T., 1996. Bioelectronic monitoring of parasite-induced stress in brown trout and roach. J. Fish Biol. 48, 228-241.

Legendre, P., 2005. Species associations: the Kendall coefficient of concordance revisited. J. Agric. Biol. Envir. S. 10, 226-245.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271-280.

Legendre, P., Legendre, L., 1998. Numerical ecology (2nd English edition). Elsevier Science BV, Amsterdam, Netherlands.

Lello J., Boag, B., Fenton, A., Stevenson, I.R., Hudson, P.J., 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature 428, 840-844.

Lester, R.J., Huizinga, H.W., 1977. *Diplostomum adamsi* sp.: description, life cycle, and pathogenesis in the retina of *Perca flavescens*. Can. J. Zool. 55, 64-73.

Lindenfors, P., Nunn, C.L., Jones, K.E., Cunningham, A.A., Sechrest, W., Gittleman. J.L., 2007. Parasite species richness in carnivores: effects of host body mass, latitude, geographical range and population density. Glob. Ecol. Biogeogr. 16, 496-509.

Locke, S.A., Levy, M.S., Marcogliese, D.J., Ackerman, S., McLaughlin, J.D., 2012. The decay of parasite community similarity in ring-billed gulls *Larus delawarensis* and other hosts. Ecography 35, 530-538.

Locke, S.A., McLaughlin, J.D., Dayanandan, S., Marcogliese, D.J., 2010a. Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome *c* oxidase I and internal transcribed spacer sequences. Int. J. Parasitol. 40, 333-343.

Locke, S.A., McLaughlin, J.D, Marcogliese, D.J., 2010b. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Mol. Ecol. 19, 2813-2827.

Locke, S.A., McLaughlin, J.D., Marcogliese, D.J., 2013. Predicting the similarity of parasite communities in freshwater fishes using the phylogeny, ecology and proximity of hosts. Oikos 122, 73–83.

MacArthur, R.H., 1965. Patterns of species diversity. Biol. Rev. 40, 510-533.

MacArthur, R.H., Wilson, E.O., 1967. The theory of island biogeography. Princeton University Press, Princeton, NJ, USA.

Magnadóttir, B., 2006. Innate immunity of fish (overview). Fish Shellfish Immunol. 20, 137-151.

Marcogliese, D.J., 2001. Implications of climate change for parasitism of animals in the aquatic environment. Can. J. Zool. 79, 1331-1352.

Marcogliese, D.J., Compagna, S., 1999. Diplostomatid eye flukes in young-of-the-year and forage fishes in the St. Lawrence River, Quebec. J. Aquat. Anim. Health 11, 275-282.

Marcogliese, D.J., Compagna, S., Bergeron, E., McLaughlin, J.D., 2001a. Population biology of eyeflukes in fish from a large fluvial ecosystem: the importance of gulls and habitat characteristics. Can. J. Zool. 79, 1102-1113.

Marcogliese, D.J., Dumont, P., Gendron, A.D., Mailhot, Y., Bergeron, E., McLaughlin, J.D., 2001b. Spatial and temporal variation in abundance of *Diplostomum* spp. in walleye (*Stizostedion vitreum*) and white suckers (*Catostomus commersoni*) from the St. Lawrence River. Can. J. Zool. 79, 355-369.

Marcogliese, D.J., Gendron, A.D., Plante, C., Fournier, M., Cyr, D., 2006. Parasites of spottail shiners (*Notropis hudsonius*) in the St. Lawrence River: effects of municipal effluents and habitat. Can. J. Zool. 84, 1461-1481.

McKeown, C.A., Irwin, S.W.B., 1997. Accumulation of *Diplostomum* spp. (Digenea: Diplostomatidae) metacercariae in the eyes of 0+ and 1+ roach (*Rutilus rutilus*). Int. J. Parasitol. 27, 377-380.

Moszczynska, A., Locke, S.A, McLaughlin, J.D., Marcogliese, D.J., Crease, T.J., 2009. Development of primers for the mitochondrial cytochrome *c* oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol. Ecol. Resour. 9, 75-82.

Mouillot, D., Simková, A., Morand, S., Poulin, R., 2005. Parasite species coexistence and limiting similarity: a multiscale look at phylogenetic, functional and reproductive distances. Oecologia 146, 269-278.

Muñoz, G., Grutter, A.S., Cribb, T.H., 2007. Structure of the parasite communities of a coral reef fish assemblage (Labridae): testing ecological and phylogenetic host factors. J. Parasitol. 93, 17-30.

Naeem, S., 1988. Resource heterogeneity fosters coexistence of a mite and midge in pitcher plants. Ecol. Monogr. 58, 215-227.

Nagelkerke, N.J.D., 1991. A note on a general definition of the coefficient of determination. Biometrika 78, 691-692.

Niewiadomska, K., Laskowski, Z., 2002. Systematic relationships among six species of *Diplostomum* Nordmann, 1832 (Digenea) based on morphological and molecular data. Acta Parasitol. 47, 1230-1237.

Nolan, M., Cribb, T.H., 2005. The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. Adv. Parasitol. 60, 101-163.

Nuin, P.A.S., Wang, Z., Tillier, E.R.M., 2006. The accuracy of several multiple sequence alignment programs for proteins. BMC Bioinformatics 7, 471, doi: 10.1186/1471-2105-7-471.

Nunn, C.L., Altizer, S., Jones, K.E., Sechrest, W., 2003. Comparative tests of parasite species richness in primates. Am. Nat. 162, 597-614.

Owen, S. F., Barber, I., Hart, P.J.B., 1993. Low-level infection by eye fluke, *Diplostomum* spp., affects the vision of 3-spined sticklebacks, *Gasterosteus aculeatus*. J. Fish Biol. 42, 803-806.

Paterson, S., Lello, J., 2003. Mixed models: getting the best use of parasitological data. Trends Parasitol. 19, 370-375.

Petney, T.N., Andrews, R.H., 1998. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. Int. J. Parasitol. 28, 377-393.

Pierce, C.L., Rasmussen, J.B., Leggett, W.C., 1996. Back-calculation of fish length from scales: empirical comparison of proportional methods. Trans. Am. Fish. Soc. 125, 889-898.

Poulin, R., 1995. Phylogeny, ecology, and the richness of parasite communities in vertebrates. Ecol. Monogr. 65, 283-302.

Poulin, R., 1997. Species richness of parasite assemblages: evolution and patterns. Annu. Rev. Ecol. Syst. 28, 341-358.

Poulin, R., 2001. Interactions between species and the structure of helminth communities. Parasitology 122, S3-S11.

Poulin, R., 2007. Evolutionary ecology of parasites (2nd edition). Princeton University Press, Princeton, NJ, USA.

Poulin, R., Morand, S., 2004. Parasite biodiversity. Smithsonian Institution Books, Washington D.C., USA.

Poulin, R., Valtonen, E.T., 2002. The predictability of helminth community structure in space: a comparison of fish populations from adjacent lakes. Int. J. Parasitol. 32, 1235-1243.

Poulin, R., Krasnov, B.R., Mouillot, D., 2011a. Host specificity in phylogenetic and geographic space. Trends Parasitol. 27, 355-361.

Poulin, R., Paterson, R.A., Townsend, C.R., Tompkins, D.M., Kelly, D.W., 2011b. Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. Freshw. Biol. 56, 676-688.

Pulliam, H.R., 1988. Sources, sinks, and population regulation. Am. Nat. 132, 652-661.

R Core Team, 2012. *R:* a language and environment for statistical computing. *R* Fundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://R-project.org/.

Rauch, G., Kalbe, M., Reusch, T.B.H., 2006. One day is enough: rapid and specific host-parasite interactions in a stickleback-trematode system. Biol. Lett. 2, 382-384.

Rellstab, C., Louhi, K.-R., Karvonen, A., Jokela, J., 2011. Analysis of trematode parasite communities in fish eye lenses by pyrosequencing of naturally pooled DNA. Infect. Genet. Evol. 11, 1276-1286.

Reusch, T.B.H., Rauch, G., Kalbe, M., 2004. Polymorphic microsatellite loci for the trematode *Diplostomum pseudospathaceum*. Mol. Ecol. Notes 4, 577-579.

Ricklefs, R.E., 1987. Community diversity: relative roles of local and regional processes. Science 235, 167-171.

Rintamäki-Kinnunen, P., Karvonen, A., Anttila, P., Valtonen, E.T., 2004. *Diplostomum spathaceum* metacercarial infection and colour change in salmonid fish. Parasitol. Res. 93, 577-581.

Schoener, T.W., 1986. Mechanistic approaches to community ecology: a new reductionism? Am. Zool. 26, 81-106.

Seppälä, O., Karvonen, A., Valtonen, E.T., 2004. Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke-fish interaction. Anim. Behav. 68, 257-263.

Seppälä, O., Karvonen, A., Valtonen, E.T., 2005a. Impaired crypsis of fish infected with a trophically transmitted parasite. Anim. Behav. 70, 895-900.

Seppälä, O., Karvonen, A., Valtonen, E.T., 2005b. Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. Anim. Behav. 70, 889-894.

Seppälä, O., Karvonen, A., Valtonen, E.T., 2011. Eye fluke-induced cataracts in natural fish populations: is there potential for host manipulation? Parasitology 138, 209-214.

Shannon, C.E., Weaver, W., 1963. The mathematical theory of communication. University of Illinois Press, Urbana, IL, USA.

Shariff, M., Richards, R.H., Sommerville, C., 1980. The histopathology of acute and chronic infections of rainbow trout *Salmo gairdneri* Richardson with eye flukes, *Diplostomum* spp. J. Fish Dis. 3, 455-465.

Sitjà-Bobadilla, A., 2008. Living off a fish: a trade-off between parasites and the immune system. Fish Shellfish Immunol. 25, 358-372.

Smith, N.F., 2001. Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. Oecologia 127, 115-122.

Srivastava, D., 1999. Using local-regional richness plots to test for species saturation: pitfalls and potentials. J. Anim. Ecol. 68, 1-16.

Stevens, G.C., 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. Am. Nat. 133, 240-256.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739.

Timi, J.T., Lanfranchi, A.L., 2009. The metazoan parasite communities of the Argentinean sandperch *Pseudopercis semifasciata* (Pisces: Perciformes) and their use to elucidate the stock structure of the host. Parasitology 136, 1209-1219.

Timi, J.T., Lanfranchi, A.L., 2013. Ontogenetic changes in heterogeneity of parasite communities in fish: disentangling the relative role of compositional versus abundance variability. Parasitology 140, 309-317.

Timi, J.T., Luque, J.L., Poulin, R., 2010. Host ontogeny and the temporal decay of similarity in parasite communities of marine fish. Int. J. Parasitol. 40, 963-968.

Toft, C.A., 1985. Resource partitioning in amphibians and reptiles. Copeia 1985, 1-21.

Voutilainen, A., Figueiredo, K., Huuskonen, H., 2008. Effects of the eye fluke *Diplostomum spathaceum* on the energetics and feeding of arctic charr *Salvelinus alpinus*. J. Fish Biol. 73, 2228-2237.

Wegner, K.M., Kalbe, M., Reusch, T.B.H., 2007. Innate versus adaptive immunity in sticklebacks: evidence for trade-offs from a selection experiment. Evol. Ecol. 21, 473-483.

Weiher, E., Keddy, P.A., 1999. The search for assembly rules in ecological communities. Cambridge University Press, Cambridge, UK.

Whyte, S.K., Chappell, L.H., Secombes, C.J., 1990. Protection of rainbow trout, *Oncorhynchus mykiss* (Richardson), against *Diplostomum spathaceum* (Digenea): the role of specific antibody and activated macrophages. J. Fish Dis. 13, 281-291.

Yamada, F.H., Santos, L.N., Takemoto, R.M., 2011. Gill ectoparasite assemblages of two non-native *Cichla* populations (Perciformes, Cichlidae) in Brazilian reservoirs. J. Helminthol. 85, 185-191.

Yamaguti, S., 1975. A synoptical review of life histories of digenetic trematodes of vertebrates. Keigaku Publishing Co., Tokyo, Japan.

Zelmer, D.A., Arai, H.P., 1998. The contributions of host age and size to the aggregated distribution of parasites in yellow perch, *Perca flavescens*, from Garner Lake, Alberta, Canada. J. Parasitol. 84, 24-28.

Zelmer, D.A., Arai, H.P., 2004. Development of nestedness: host biology as a community process in parasite infracommunities of yellow perch (*Perca flavescens* (Mitchill)) from Garner Lake, Alberta. J. Parasitol. 90, 435-436.

Zelmer, D.A., Paredes-Calderón, L., García-Prieto, L., 2004. Nestedness in colonization-dominated systems: helminth infracommunities of *Rana vaillanti* Brocchi (Anura: Ranidae) in Los Tuxtlas, Veracruz, Mexico. J. Parasitol. 90, 705-710.

APPENDICES

Appendix 1. Definitions.

COI: cytochrome *c* oxidase subunit I; the first 650 bp of this gene form the barcode region that was sequenced and used for *Diplostomum* species discrimination.

Component community: refers to all assemblages of all the individuals of all parasite species (*i.e.*, all infrapopulations) associated with some subset of a host species (*e.g.*, the population of a host species in a given habitat) (Bush et al., 1997).

GLM(s): generalized linear model(s); a generalization of the linear regression that can treat response variables other distributions than the normal distribution (Paterson and Lello, 2003). The linear model is related to the response variable using a link function (negative binomial and Gamma error distributions were used in this study).

Infracommunity: the assemblage of all the individuals of all parasite species (*i.e.*, all infrapopulations) in an individual host at a particular time (Bush et al., 1997).

ITS: internal transcribed spacer; a sequence of non-functional RNA situated between structural ribosomal RNAs (rRNA) on a common precursor transcript. ITS sequences has been used to distinguish among species in various taxa, including *Diplostomum* (Galazzo et al., 2002)

Mean intensity: the mean number of parasites per infected fish in a sample (Bush et al., 1997).

Mixed-species infection: parasite infection in which the host is infected by more than one parasite species.

Parasite associations: the result of interactions among parasites present or trying to establish in a given infracommunity. Associations can be positive (synergistic, facilitation of establishment of one species by another), negative (antagonistic, competitive interactions between species, exclusion), or neutral (no detected effect of cohabitation between species) (Behnke et al., 2001).

Prevalence: the proportion of infected fish in a sample (Bush et al., 1997).

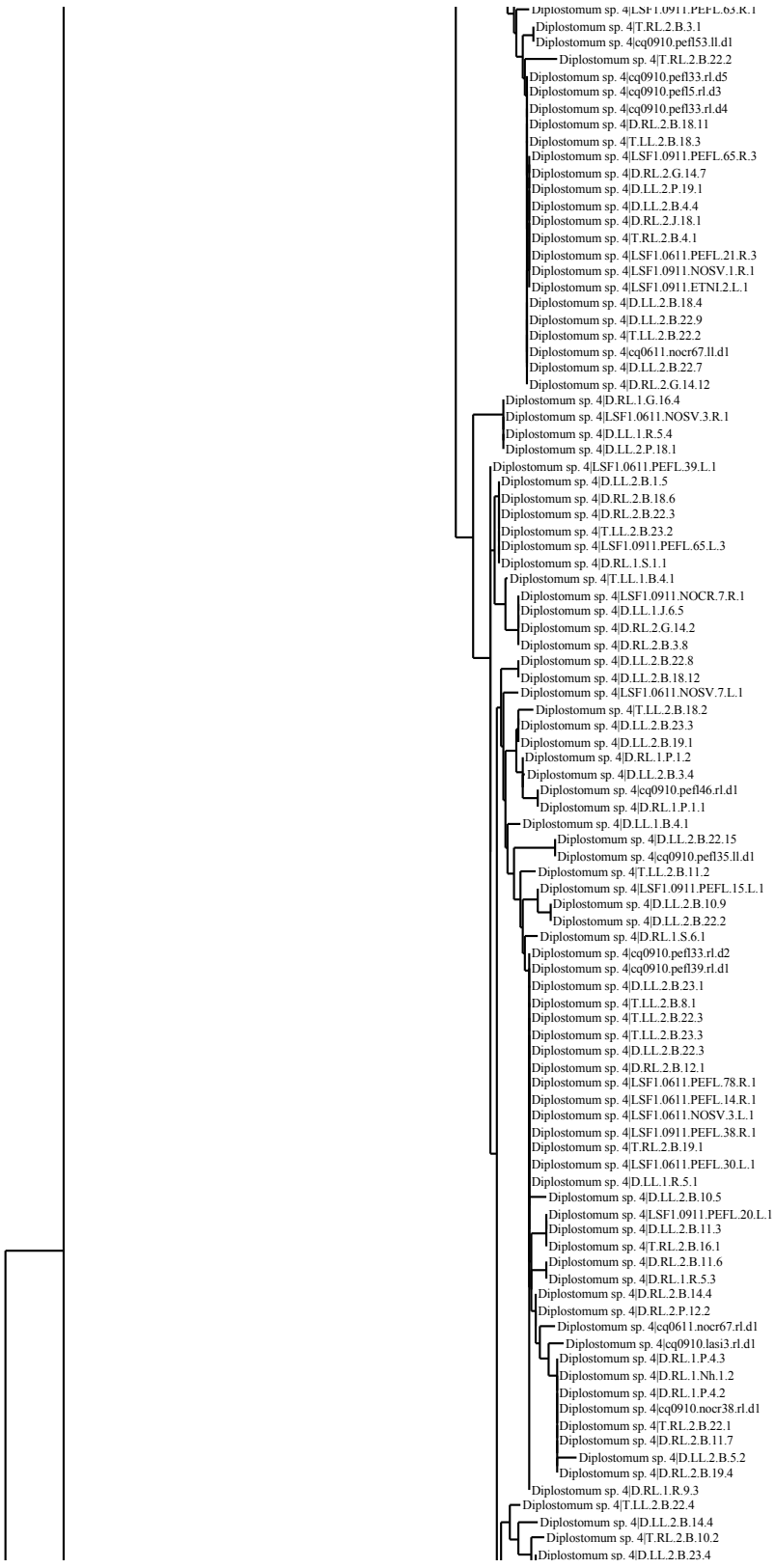
RDA: redundancy analysis; it is a method combining regression and principal component analysis (PCA) used to model multivariate response data. In more details, a RDA is a multivariate multiple linear regression followed by a PCA of the resulting fitted values (Legendre and Legendre, 1998)

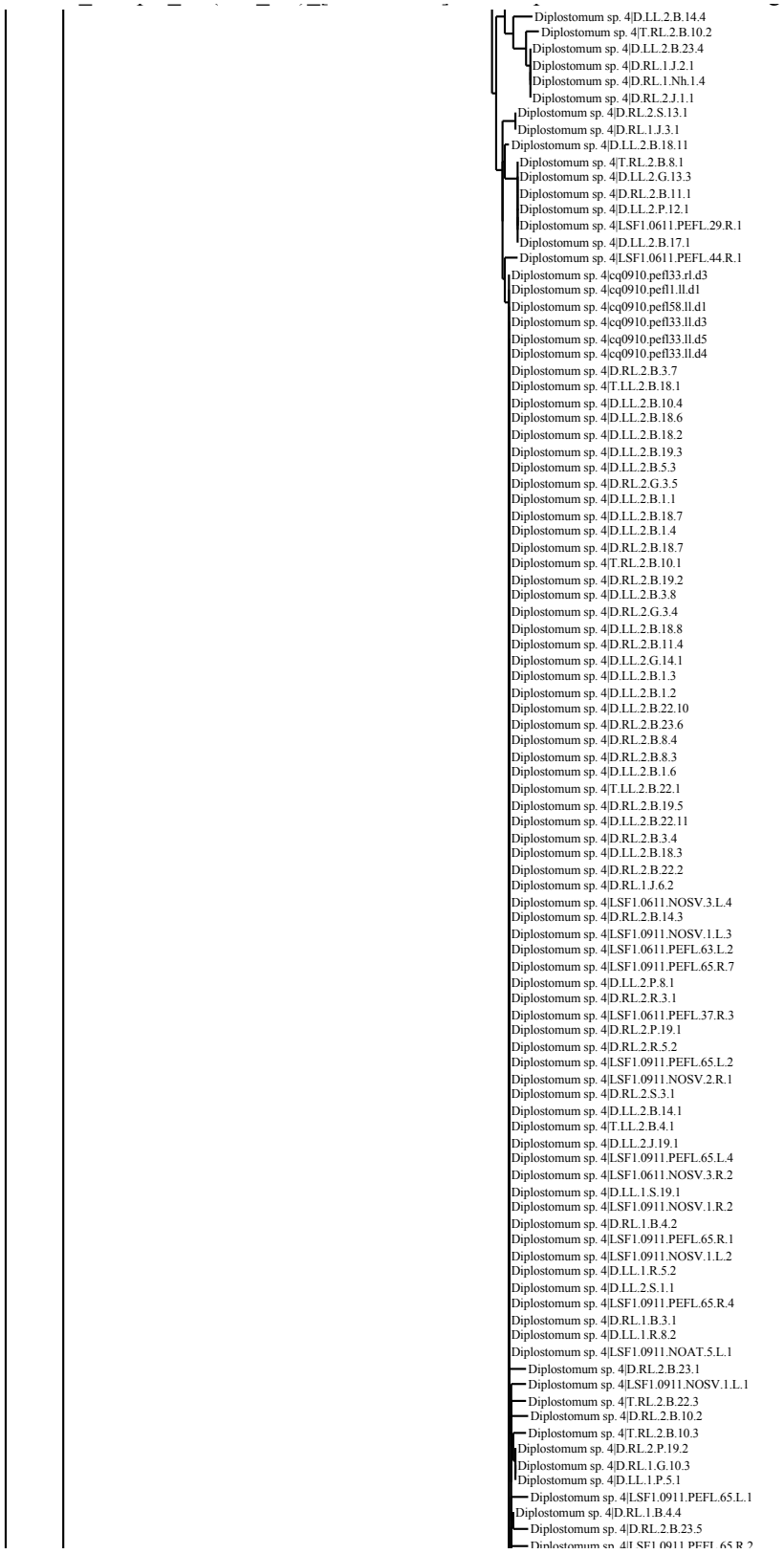
Single-species infection: parasite infection in which the host is infected by only one parasite species.

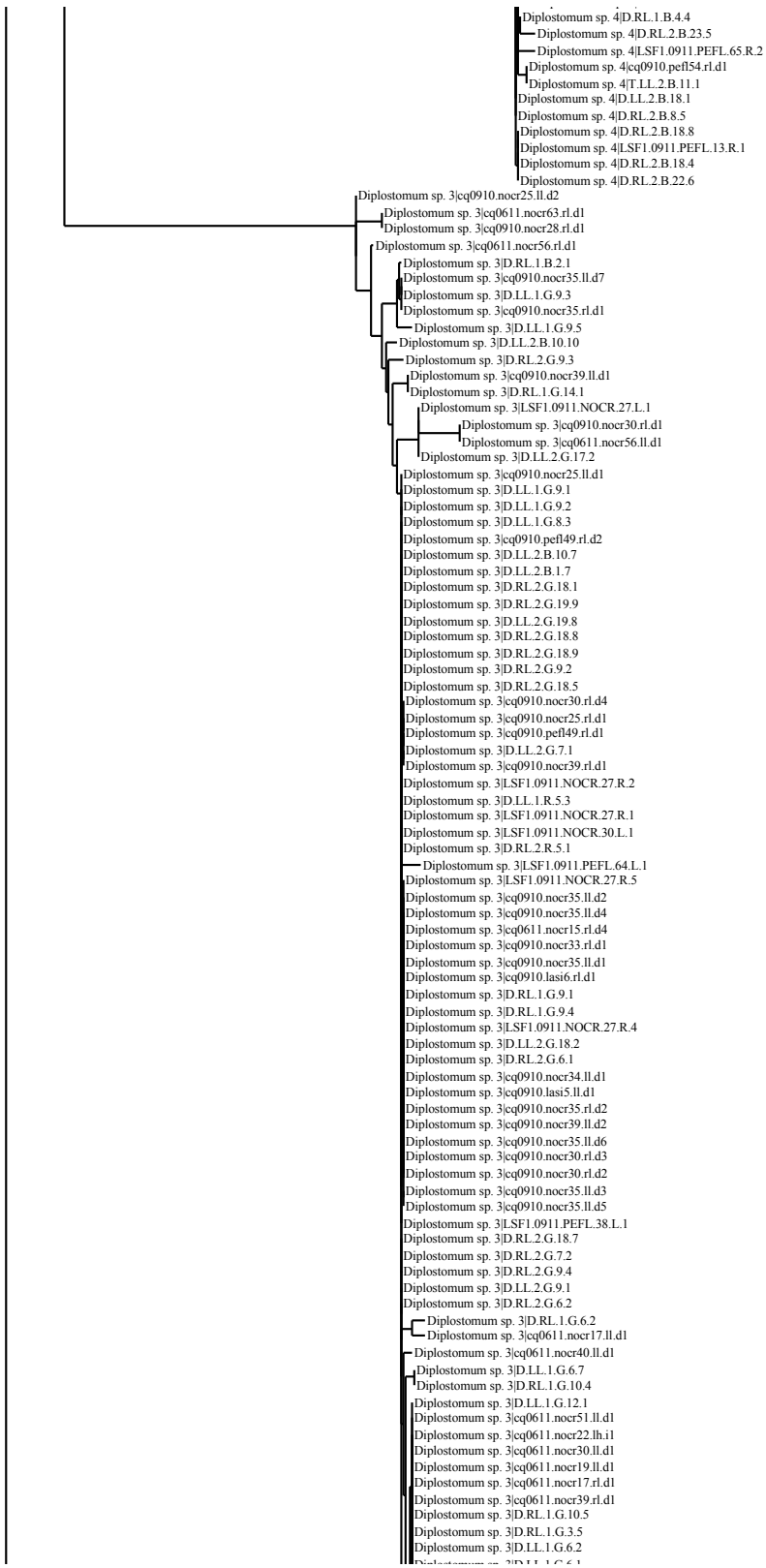
Supracommunity (compound community): comprises all developmental stages of all parasite species (*i.e.*, all suprapopulations) in a particular habitat/ecosystem at a given time (Bush et al., 1997).

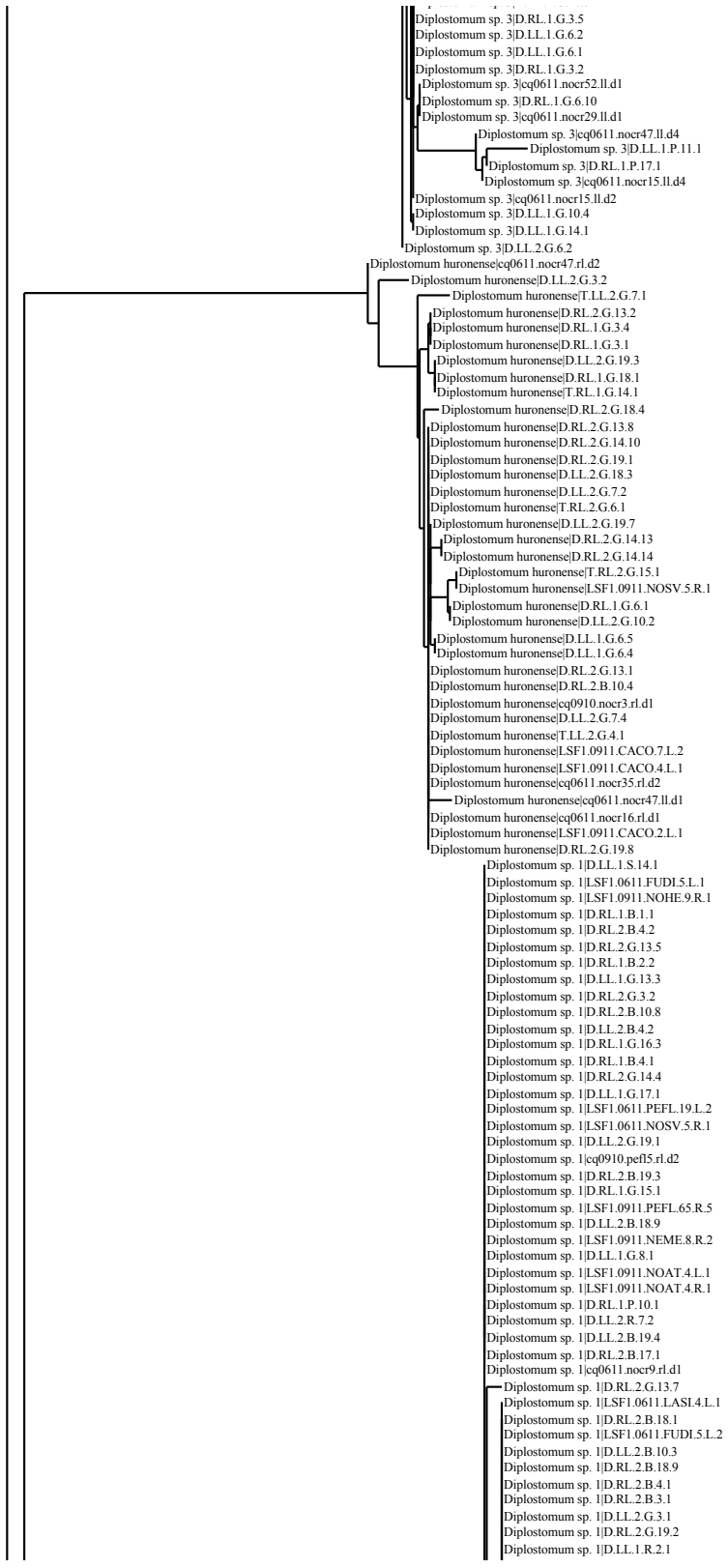
Appendix 2. Neighbour-joining phenogram of 741 sequences of cytochrome oxidase I obtained from *Diplostomum* spp. metacercariae. The Kimura 2-parameter was used in the distance modeling along with pairwise deletion of gaps to construct the tree. Species of *Diplostomum* and sample identification codes are printed at the tip of each branches of the tree. *Diplostomum baeri* is a vitreous humour-infecting species that was also collected during this study, but not included in the data set and the analyses because of its different infection site.

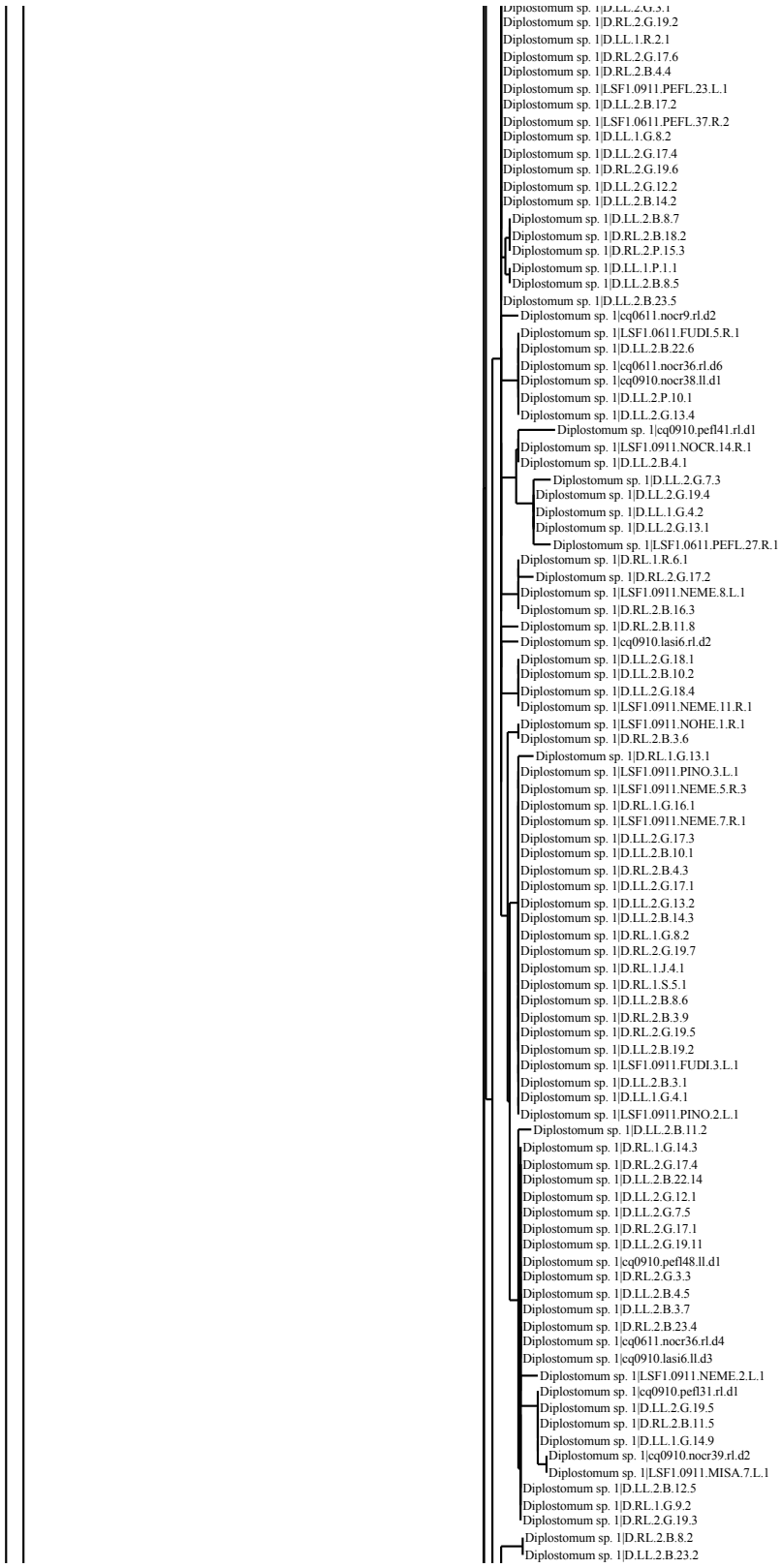

1 %
Diplostomum baeri|D.LH.1.P.10.1
Diplostomum baeri|D.RH.2.P.12.4
Diplostomum baeri|D.RH.2.P.5.1
Diplostomum baeri|D.RH.2.P.15.2
Diplostomum baeri|LSF1.0911.PEFL.2.LH.3
Diplostomum baeri|LSF1.0611.PEFL.22.R.1
Diplostomum baeri|LSF1.0911.PEFL.9.L.1
Diplostomum baeri|D.LH.2.P.8.1
Diplostomum baeri|LSF1.0611.PEFL.22.L.3
Diplostomum baeri|D.RH.1.P.15.1
Diplostomum baeri|D.RH.2.P.5.2
Diplostomum baeri|D.RH.1.P.15.2
Diplostomum baeri|D.RH.2.P.10.1
Diplostomum baeri|D.RH.2.P.8.2
Diplostomum baeri|D.RH.2.P.8.3
Diplostomum baeri|D.RH.2.P.10.2
Diplostomum baeri|D.RH.2.P.8.1
Diplostomum baeri|D.LH.2.P.12.1
Diplostomum baeri|D.LH.2.P.12.3
Diplostomum baeri|D.LH.2.P.12.4
Diplostomum baeri|LSF1.0611.PEFL.38.R.1
Diplostomum baeri|D.LH.2.P.8.3
Diplostomum baeri|D.LH.2.P.12.2
Diplostomum baeri|D.LH.1.P.10.2
Diplostomum baeri|LSF1.0911.PEFL.2.LH.2
Diplostomum baeri|LSF1.0911.PEFL.2.LH.1
Diplostomum baeri|LSF1.0911.PEFL.23.RH.3
Diplostomum baeri|D.LH.2.P.8.2
Diplostomum baeri|D.RH.2.P.12.3
Diplostomum baeri|LSF1.0911.PEFL.20.RH.1
Diplostomum baeri|LSF1.0911.PEFL.23.RH.4
Diplostomum baeri|LSF1.0911.PEFL.11.R.1
Diplostomum baeri|LSF1.0911.PEFL.20.RH.2
Diplostomum baeri|LSF1.0911.PEFL.62.R.1
Diplostomum baeri|LSF1.0611.PEFL.55.L.1
Diplostomum baeri|LSF1.0911.PEFL.16.RH.2
Diplostomum baeri|D.LH.1.P.5.2
Diplostomum baeri|D.RH.2.P.15.1
Diplostomum baeri|LSF1.0911.PEFL.2.LH.5
Diplostomum baeri|cq0910.pefl52.lh.d1
Diplostomum baeri|cq0910.pefl39.rh.d1
Diplostomum baeri|D.RH.2.P.12.5
Diplostomum sp. 4|LSF1.0611.NOSV.3.L.5
Diplostomum sp. 4|T.RL.2.B.11.2
Diplostomum sp. 4|T.LL.2.B.8.5
Diplostomum sp. 4|D.LL.2.B.11.4
Diplostomum sp. 4|LSF1.0911.PEFL.65.R.6
Diplostomum sp. 4|LSF1.0611.PEFL.16.L.1
Diplostomum sp. 4|LSF1.0611.PEFL.19.R.1
Diplostomum sp. 4|D.RL.2.B.18.5
Diplostomum sp. 4|D.LL.2.R.5.3
Diplostomum sp. 4|T.RL.2.B.11.1
Diplostomum sp. 4|T.LL.2.B.23.1
Diplostomum sp. 4|D.LL.2.B.12.3
Diplostomum sp. 4|T.LL.2.B.3.1
Diplostomum sp. 4|D.RL.1.P.1.3
Diplostomum sp. 4|D.LL.2.B.3.2
Diplostomum sp. 4|cq0910.pefl58.rl.d6
Diplostomum sp. 4|D.RL.1.P.15.1
Diplostomum sp. 4|D.LL.1.J.6.1
Diplostomum sp. 4|D.RL.2.P.4.1
Diplostomum sp. 4|D.LL.2.B.4.7
Diplostomum sp. 4|D.RL.2.B.1.3
Diplostomum sp. 4|D.LL.2.S.1.2
Diplostomum sp. 4|T.LL.2.B.8.2
Diplostomum sp. 4|D.LL.1.G.3.2
Diplostomum sp. 4|D.RL.2.P.15.6
Diplostomum sp. 4|D.RL.1.R.5.2
Diplostomum sp. 4|D.LL.1.P.6.1
Diplostomum sp. 4|D.RL.2.P.15.2
Diplostomum sp. 4|D.RL.2.P.15.1
Diplostomum sp. 4|D.RL.2.P.17.2
Diplostomum sp. 4|D.RL.2.P.17.1
Diplostomum sp. 4|D.RL.1.R.5.1
Diplostomum sp. 4|D.RL.1.P.6.2
Diplostomum sp. 4|D.LL.2.P.4.1
Diplostomum sp. 4|D.LL.1.P.8.1
Diplostomum sp. 4|D.LL.2.P.15.2
Diplostomum sp. 4|D.LL.1.P.8.2
Diplostomum sp. 4|D.LL.1.P.14.1
Diplostomum sp. 4|D.LL.1.J.6.2
Diplostomum sp. 4|D.RL.2.B.4.6
Diplostomum sp. 4|D.LL.2.P.15.1
Diplostomum sp. 4|D.RL.1.P.3.1
Diplostomum sp. 4|D.RL.2.P.15.4
Diplostomum sp. 4|D.LL.1.P.14.2
Diplostomum sp. 4|D.RL.1.P.8.1
Diplostomum sp. 4|D.RL.1.P.11.1
Diplostomum sp. 4|D.LL.1.P.3.1
Diplostomum sp. 4|D.RL.2.B.16.1
Diplostomum sp. 4|LSF1.0911.PEFL.63.R.1
Diplostomum sp. 4|T.RL.2.B.3.1
Diplostomum sp. 4|cq0910.pefl53.ll.d1

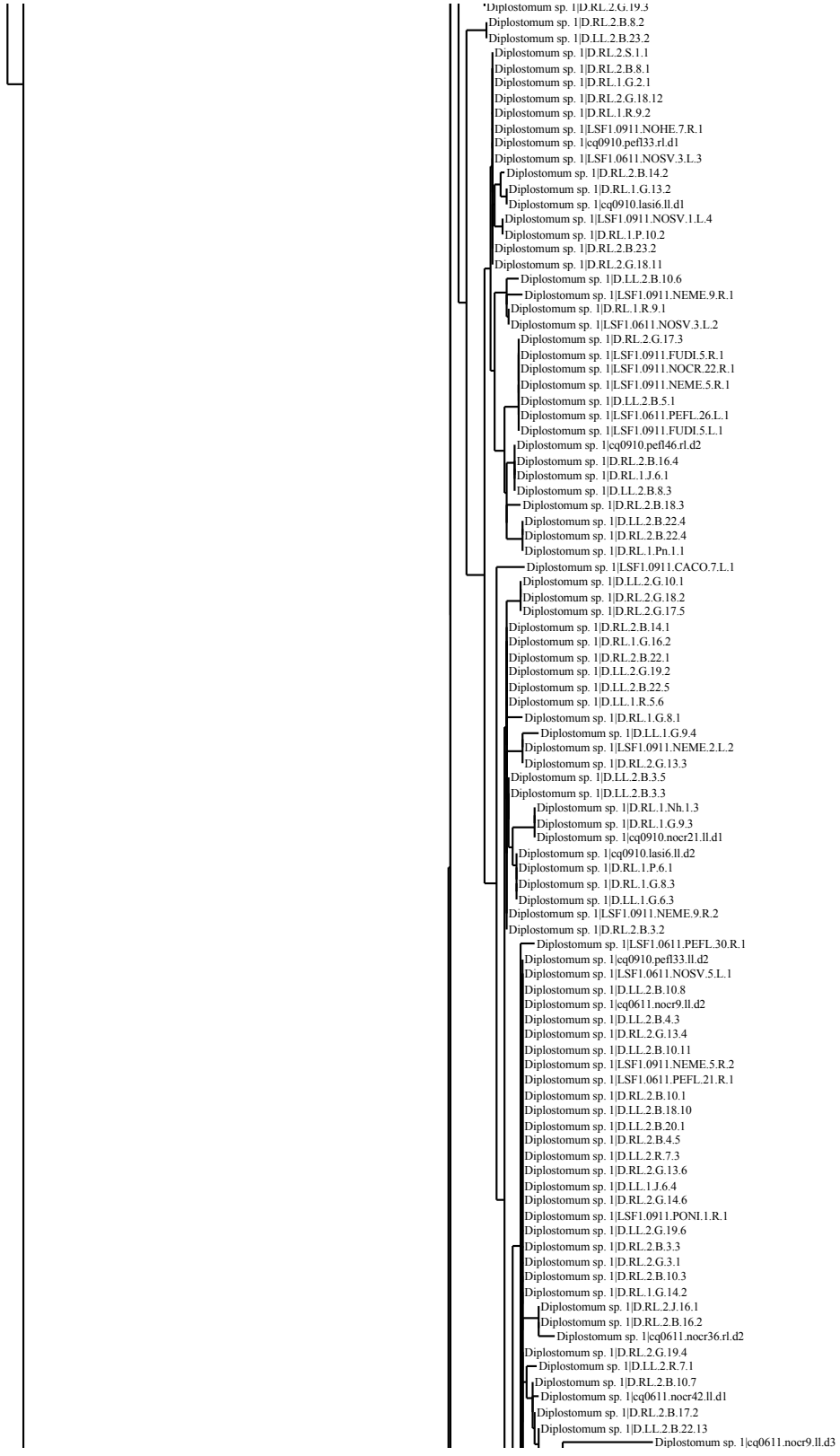

Diplostomum sp. 4|LSF1.0911.PEFL.65.R.1
Diplostomum sp. 4|T.RL.2.B.3.1
Diplostomum sp. 4|cq0910.pefl53.ll.d1
Diplostomum sp. 4|T.RL.2.B.22.2
Diplostomum sp. 4|cq0910.pefl33.rl.d5
Diplostomum sp. 4|cq0910.pefl5.rl.d3
Diplostomum sp. 4|cq0910.pefl33.rl.d4
Diplostomum sp. 4|D.RL.2.B.18.11
Diplostomum sp. 4|T.LL.2.B.18.3
Diplostomum sp. 4|LSF1.0911.PEFL.65.R.3
Diplostomum sp. 4|D.RL.2.G.14.7
Diplostomum sp. 4|D.LL.2.P.19.1
Diplostomum sp. 4|D.LL.2.B.4.4
Diplostomum sp. 4|D.RL.2.J.18.1
Diplostomum sp. 4|T.RL.2.B.4.1
Diplostomum sp. 4|LSF1.0611.PEFL.21.R.3
Diplostomum sp. 4|LSF1.0911.NOSV.1.R.1
Diplostomum sp. 4|LSF1.0911.ETNI.2.L.1
Diplostomum sp. 4|D.LL.2.B.18.4
Diplostomum sp. 4|D.LL.2.B.22.9
Diplostomum sp. 4|T.LL.2.B.22.2
Diplostomum sp. 4|cq0611.nocr67.ll.d1
Diplostomum sp. 4|D.LL.2.B.22.7
Diplostomum sp. 4|D.RL.2.G.14.12
Diplostomum sp. 4|D.RL.1.G.16.4
Diplostomum sp. 4|LSF1.0611.NOSV.3.R.1
Diplostomum sp. 4|D.LL.1.R.5.4
Diplostomum sp. 4|D.LL.2.P.18.1
Diplostomum sp. 4|LSF1.0611.PEFL.39.L.1
Diplostomum sp. 4|D.LL.2.B.1.5
Diplostomum sp. 4|D.RL.2.B.18.6
Diplostomum sp. 4|D.RL.2.B.22.3
Diplostomum sp. 4|T.LL.2.B.23.2
Diplostomum sp. 4|LSF1.0911.PEFL.65.L.3
Diplostomum sp. 4|D.RL.1.S.1.1
Diplostomum sp. 4|T.LL.1.B.4.1
Diplostomum sp. 4|LSF1.0911.NOCR.7.R.1
Diplostomum sp. 4|D.LL.1.J.6.5
Diplostomum sp. 4|D.RL.2.G.14.2
Diplostomum sp. 4|D.RL.2.B.3.8
Diplostomum sp. 4|D.LL.2.B.22.8
Diplostomum sp. 4|D.LL.2.B.18.12
Diplostomum sp. 4|LSF1.0611.NOSV.7.L.1
Diplostomum sp. 4|T.LL.2.B.18.2
Diplostomum sp. 4|D.LL.2.B.23.3
Diplostomum sp. 4|D.LL.2.B.19.1
Diplostomum sp. 4|D.RL.1.P.1.2
Diplostomum sp. 4|D.LL.2.B.3.4
Diplostomum sp. 4|cq0910.pefl46.rl.d1
Diplostomum sp. 4|D.RL.1.P.1.1
Diplostomum sp. 4|D.LL.1.B.4.1
Diplostomum sp. 4|D.LL.2.B.22.15
Diplostomum sp. 4|cq0910.pefl35.ll.d1
Diplostomum sp. 4|T.LL.2.B.11.2
Diplostomum sp. 4|LSF1.0911.PEFL.15.L.1
Diplostomum sp. 4|D.LL.2.B.10.9
Diplostomum sp. 4|D.LL.2.B.22.2
Diplostomum sp. 4|D.RL.1.S.6.1
Diplostomum sp. 4|cq0910.pefl33.rl.d2
Diplostomum sp. 4|cq0910.pefl39.rl.d1
Diplostomum sp. 4|D.LL.2.B.23.1
Diplostomum sp. 4|T.LL.2.B.8.1
Diplostomum sp. 4|T.LL.2.B.22.3
Diplostomum sp. 4|T.LL.2.B.23.3
Diplostomum sp. 4|D.LL.2.B.22.3
Diplostomum sp. 4|D.RL.2.B.12.1
Diplostomum sp. 4|LSF1.0611.PEFL.78.R.1
Diplostomum sp. 4|LSF1.0611.PEFL.14.R.1
Diplostomum sp. 4|LSF1.0611.NOSV.3.L.1
Diplostomum sp. 4|LSF1.0911.PEFL.38.R.1
Diplostomum sp. 4|T.RL.2.B.19.1
Diplostomum sp. 4|LSF1.0611.PEFL.30.L.1
Diplostomum sp. 4|D.LL.1.R.5.1
Diplostomum sp. 4|D.LL.2.B.10.5
Diplostomum sp. 4|LSF1.0911.PEFL.20.L.1
Diplostomum sp. 4|D.LL.2.B.11.3
Diplostomum sp. 4|T.RL.2.B.16.1
Diplostomum sp. 4|D.RL.2.B.11.6
Diplostomum sp. 4|D.RL.1.R.5.3
Diplostomum sp. 4|D.RL.2.B.14.4
Diplostomum sp. 4|D.RL.2.P.12.2
Diplostomum sp. 4|cq0611.nocr67.rl.d1
Diplostomum sp. 4|cq0910.lasi3.rl.d1
Diplostomum sp. 4|D.RL.1.P.4.3
Diplostomum sp. 4|D.RL.1.Nh.1.2
Diplostomum sp. 4|D.RL.1.P.4.2
Diplostomum sp. 4|cq0910.nocr38.rl.d1
Diplostomum sp. 4|T.RL.2.B.22.1
Diplostomum sp. 4|D.RL.2.B.11.7
Diplostomum sp. 4|D.LL.2.B.5.2
Diplostomum sp. 4|D.RL.2.B.19.4
Diplostomum sp. 4|D.RL.1.R.9.3
Diplostomum sp. 4|T.LL.2.B.22.4
Diplostomum sp. 4|D.LL.2.B.14.4
Diplostomum sp. 4|T.RL.2.B.10.2
Diplostomum sp. 4|D.LL.2.B.23.4

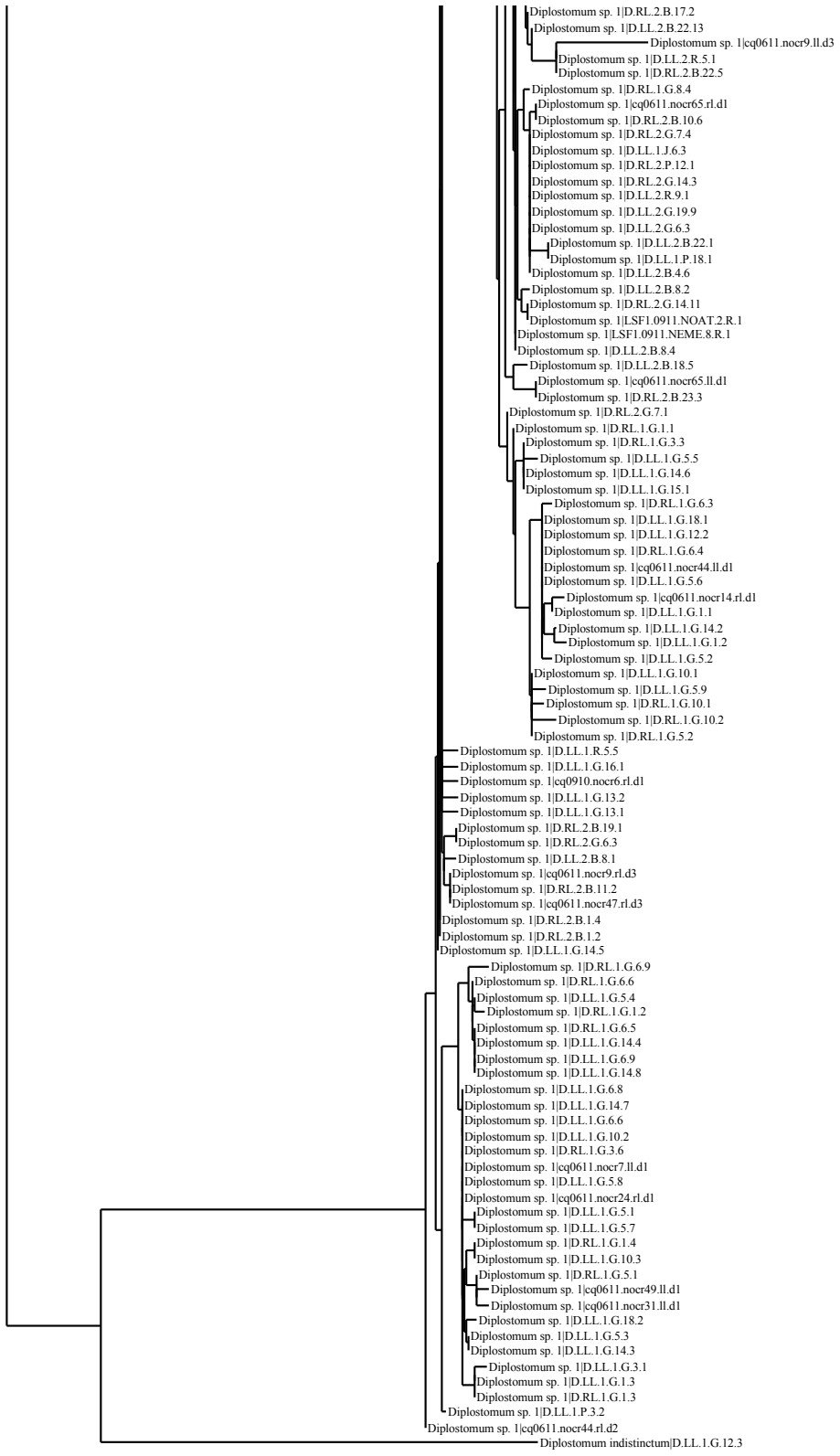

Diplostomum sp. 4|D.LL.2.B.14.4
Diplostomum sp. 4|T.RL.2.B.10.2
Diplostomum sp. 4|D.LL.2.B.23.4
Diplostomum sp. 4|D.RL.1.J.2.1
Diplostomum sp. 4|D.RL.1.Nh.1.4
Diplostomum sp. 4|D.RL.2.J.1.1
Diplostomum sp. 4|D.RL.2.S.13.1
Diplostomum sp. 4|D.RL.1.J.3.1
Diplostomum sp. 4|D.LL.2.B.18.11
Diplostomum sp. 4|T.RL.2.B.8.1
Diplostomum sp. 4|D.LL.2.G.13.3
Diplostomum sp. 4|D.RL.2.B.11.1
Diplostomum sp. 4|D.LL.2.P.12.1
Diplostomum sp. 4|LSF1.0611.PEFL.29.R.1
Diplostomum sp. 4|D.LL.2.B.17.1
Diplostomum sp. 4|LSF1.0611.PEFL.44.R.1
Diplostomum sp. 4|cq0910.pef133.rl.d3
Diplostomum sp. 4|cq0910.pef11.ll.d1
Diplostomum sp. 4|cq0910.pef158.ll.d1
Diplostomum sp. 4|cq0910.pef133.ll.d3
Diplostomum sp. 4|cq0910.pef133.ll.d5
Diplostomum sp. 4|cq0910.pef133.ll.d4
Diplostomum sp. 4|D.RL.2.B.3.7
Diplostomum sp. 4|T.LL.2.B.18.1
Diplostomum sp. 4|D.LL.2.B.10.4
Diplostomum sp. 4|D.LL.2.B.18.6
Diplostomum sp. 4|D.LL.2.B.18.2
Diplostomum sp. 4|D.LL.2.B.19.3
Diplostomum sp. 4|D.LL.2.B.5.3
Diplostomum sp. 4|D.RL.2.G.3.5
Diplostomum sp. 4|D.LL.2.B.1.1
Diplostomum sp. 4|D.LL.2.B.18.7
Diplostomum sp. 4|D.LL.2.B.1.4
Diplostomum sp. 4|D.RL.2.B.18.7
Diplostomum sp. 4|T.RL.2.B.10.1
Diplostomum sp. 4|D.RL.2.B.19.2
Diplostomum sp. 4|D.LL.2.B.3.8
Diplostomum sp. 4|D.RL.2.G.3.4
Diplostomum sp. 4|D.LL.2.B.18.8
Diplostomum sp. 4|D.RL.2.B.11.4
Diplostomum sp. 4|D.LL.2.G.14.1
Diplostomum sp. 4|D.LL.2.B.1.3
Diplostomum sp. 4|D.LL.2.B.1.2
Diplostomum sp. 4|D.LL.2.B.22.10
Diplostomum sp. 4|D.RL.2.B.23.6
Diplostomum sp. 4|D.RL.2.B.8.4
Diplostomum sp. 4|D.RL.2.B.8.3
Diplostomum sp. 4|D.LL.2.B.1.6
Diplostomum sp. 4|T.LL.2.B.22.1
Diplostomum sp. 4|D.RL.2.B.19.5
Diplostomum sp. 4|D.LL.2.B.22.11
Diplostomum sp. 4|D.RL.2.B.3.4
Diplostomum sp. 4|D.LL.2.B.18.3
Diplostomum sp. 4|D.RL.2.B.22.2
Diplostomum sp. 4|D.RL.1.J.6.2
Diplostomum sp. 4|LSF1.0611.NOSV.3.L.4
Diplostomum sp. 4|D.RL.2.B.14.3
Diplostomum sp. 4|LSF1.0911.NOSV.1.L.3
Diplostomum sp. 4|LSF1.0611.PEFL.63.L.2
Diplostomum sp. 4|LSF1.0911.PEFL.65.R.7
Diplostomum sp. 4|D.LL.2.P.8.1
Diplostomum sp. 4|D.RL.2.R.3.1
Diplostomum sp. 4|LSF1.0611.PEFL.37.R.3
Diplostomum sp. 4|D.RL.2.P.19.1
Diplostomum sp. 4|D.RL.2.R.5.2
Diplostomum sp. 4|LSF1.0911.PEFL.65.L.2
Diplostomum sp. 4|LSF1.0911.NOSV.2.R.1
Diplostomum sp. 4|D.RL.2.S.3.1
Diplostomum sp. 4|D.LL.2.B.14.1
Diplostomum sp. 4|T.LL.2.B.4.1
Diplostomum sp. 4|D.LL.2.J.19.1
Diplostomum sp. 4|LSF1.0911.PEFL.65.L.4
Diplostomum sp. 4|LSF1.0611.NOSV.3.R.2
Diplostomum sp. 4|D.LL.1.S.19.1
Diplostomum sp. 4|LSF1.0911.NOSV.1.R.2
Diplostomum sp. 4|D.RL.1.B.4.2
Diplostomum sp. 4|LSF1.0911.PEFL.65.R.1
Diplostomum sp. 4|LSF1.0911.NOSV.1.L.2
Diplostomum sp. 4|D.LL.1.R.5.2
Diplostomum sp. 4|D.LL.2.S.1.1
Diplostomum sp. 4|LSF1.0911.PEFL.65.R.4
Diplostomum sp. 4|D.RL.1.B.3.1
Diplostomum sp. 4|D.LL.1.R.8.2
Diplostomum sp. 4|LSF1.0911.NOAT.5.L.1
Diplostomum sp. 4|D.RL.2.B.23.1
Diplostomum sp. 4|LSF1.0911.NOSV.1.L.1
Diplostomum sp. 4|T.RL.2.B.22.3
Diplostomum sp. 4|D.RL.2.B.10.2
Diplostomum sp. 4|T.RL.2.B.10.3
Diplostomum sp. 4|D.RL.2.P.19.2
Diplostomum sp. 4|D.RL.1.G.10.3
Diplostomum sp. 4|D.LL.1.P.5.1
Diplostomum sp. 4|LSF1.0911.PEFL.65.L.1
Diplostomum sp. 4|D.RL.1.B.4.4
Diplostomum sp. 4|D.RL.2.B.23.5


Diplostomum sp. 4|D.RL.1.B.4.4
Diplostomum sp. 4|D.RL.2.B.23.5
Diplostomum sp. 4|LSF1.0911.PEFL.65.R.2
Diplostomum sp. 4|cq0910.pefl54.rl.d1
Diplostomum sp. 4|T.LL.2.B.11.1
Diplostomum sp. 4|D.LL.2.B.18.1
Diplostomum sp. 4|D.RL.2.B.8.5
Diplostomum sp. 4|D.RL.2.B.18.8
Diplostomum sp. 4|LSF1.0911.PEFL.13.R.1
Diplostomum sp. 4|D.RL.2.B.18.4
Diplostomum sp. 4|D.RL.2.B.22.6
Diplostomum sp. 3|cq0910.nocr25.ll.d2
Diplostomum sp. 3|cq0611.nocr63.rl.d1
Diplostomum sp. 3|cq0910.nocr28.rl.d1
Diplostomum sp. 3|cq0611.nocr56.rl.d1
Diplostomum sp. 3|D.RL.1.B.2.1
Diplostomum sp. 3|cq0910.nocr35.ll.d7
Diplostomum sp. 3|D.LL.1.G.9.3
Diplostomum sp. 3|cq0910.nocr35.rl.d1
Diplostomum sp. 3|D.LL.1.G.9.5
Diplostomum sp. 3|D.LL.2.B.10.10
Diplostomum sp. 3|D.RL.2.G.9.3
Diplostomum sp. 3|cq0910.nocr39.ll.d1
Diplostomum sp. 3|D.RL.1.G.14.1
Diplostomum sp. 3|LSF1.0911.NOCR.27.L.1
Diplostomum sp. 3|cq0910.nocr30.rl.d1
Diplostomum sp. 3|cq0611.nocr56.ll.d1
Diplostomum sp. 3|D.LL.2.G.17.2
Diplostomum sp. 3|cq0910.nocr25.ll.d1
Diplostomum sp. 3|D.LL.1.G.9.1
Diplostomum sp. 3|D.LL.1.G.9.2
Diplostomum sp. 3|D.LL.1.G.8.3
Diplostomum sp. 3|cq0910.pefl49.rl.d2
Diplostomum sp. 3|D.LL.2.B.10.7
Diplostomum sp. 3|D.LL.2.B.1.7
Diplostomum sp. 3|D.RL.2.G.18.1
Diplostomum sp. 3|D.RL.2.G.19.9
Diplostomum sp. 3|D.LL.2.G.19.8
Diplostomum sp. 3|D.RL.2.G.18.8
Diplostomum sp. 3|D.RL.2.G.18.9
Diplostomum sp. 3|D.RL.2.G.9.2
Diplostomum sp. 3|D.RL.2.G.18.5
Diplostomum sp. 3|cq0910.nocr30.rl.d4
Diplostomum sp. 3|cq0910.nocr25.rl.d1
Diplostomum sp. 3|cq0910.pefl49.rl.d1
Diplostomum sp. 3|D.LL.2.G.7.1
Diplostomum sp. 3|cq0910.nocr39.rl.d1
Diplostomum sp. 3|LSF1.0911.NOCR.27.R.2
Diplostomum sp. 3|D.LL.1.R.5.3
Diplostomum sp. 3|LSF1.0911.NOCR.27.R.1
Diplostomum sp. 3|LSF1.0911.NOCR.30.L.1
Diplostomum sp. 3|D.RL.2.R.5.1
Diplostomum sp. 3|LSF1.0911.PEFL.64.L.1
Diplostomum sp. 3|LSF1.0911.NOCR.27.R.5
Diplostomum sp. 3|cq0910.nocr35.ll.d2
Diplostomum sp. 3|cq0910.nocr35.ll.d4
Diplostomum sp. 3|cq0611.nocr15.rl.d4
Diplostomum sp. 3|cq0910.nocr33.rl.d1
Diplostomum sp. 3|cq0910.nocr35.ll.d1
Diplostomum sp. 3|cq0910.lasi6.rl.d1
Diplostomum sp. 3|D.RL.1.G.9.1
Diplostomum sp. 3|D.RL.1.G.9.4
Diplostomum sp. 3|LSF1.0911.NOCR.27.R.4
Diplostomum sp. 3|D.LL.2.G.18.2
Diplostomum sp. 3|D.RL.2.G.6.1
Diplostomum sp. 3|cq0910.nocr34.ll.d1
Diplostomum sp. 3|cq0910.lasi5.ll.d1
Diplostomum sp. 3|cq0910.nocr35.rl.d2
Diplostomum sp. 3|cq0910.nocr39.ll.d2
Diplostomum sp. 3|cq0910.nocr35.ll.d6
Diplostomum sp. 3|cq0910.nocr30.rl.d3
Diplostomum sp. 3|cq0910.nocr30.rl.d2
Diplostomum sp. 3|cq0910.nocr35.ll.d3
Diplostomum sp. 3|cq0910.nocr35.ll.d5
Diplostomum sp. 3|LSF1.0911.PEFL.38.L.1
Diplostomum sp. 3|D.RL.2.G.18.7
Diplostomum sp. 3|D.RL.2.G.7.2
Diplostomum sp. 3|D.RL.2.G.9.4
Diplostomum sp. 3|D.LL.2.G.9.1
Diplostomum sp. 3|D.RL.2.G.6.2
Diplostomum sp. 3|D.RL.1.G.6.2
Diplostomum sp. 3|cq0611.nocr17.ll.d1
Diplostomum sp. 3|cq0611.nocr40.ll.d1
Diplostomum sp. 3|D.LL.1.G.6.7
Diplostomum sp. 3|D.RL.1.G.10.4
Diplostomum sp. 3|D.LL.1.G.12.1
Diplostomum sp. 3|cq0611.nocr51.ll.d1
Diplostomum sp. 3|cq0611.nocr22.lh.i1
Diplostomum sp. 3|cq0611.nocr30.ll.d1
Diplostomum sp. 3|cq0611.nocr19.ll.d1
Diplostomum sp. 3|cq0611.nocr17.rl.d1
Diplostomum sp. 3|cq0611.nocr39.rl.d1
Diplostomum sp. 3|D.RL.1.G.10.5
Diplostomum sp. 3|D.RL.1.G.3.5
Diplostomum sp. 3|D.LL.1.G.6.2


Diplostomum sp. 3|D.RL.1.G.3.5
Diplostomum sp. 3|D.LL.1.G.6.2
Diplostomum sp. 3|D.LL.1.G.6.1
Diplostomum sp. 3|D.RL.1.G.3.2
Diplostomum sp. 3|cq0611.nocr52.ll.d1
Diplostomum sp. 3|D.RL.1.G.6.10
Diplostomum sp. 3|cq0611.nocr29.ll.d1
Diplostomum sp. 3|cq0611.nocr47.ll.d4
Diplostomum sp. 3|D.LL.1.P.11.1
Diplostomum sp. 3|D.RL.1.P.17.1
Diplostomum sp. 3|cq0611.nocr15.ll.d4
Diplostomum sp. 3|cq0611.nocr15.ll.d2
Diplostomum sp. 3|D.LL.1.G.10.4
Diplostomum sp. 3|D.LL.1.G.14.1
Diplostomum sp. 3|D.LL.2.G.6.2
Diplostomum huronense|cq0611.nocr47.rl.d2
Diplostomum huronense|D.LL.2.G.3.2
Diplostomum huronense|T.LL.2.G.7.1
Diplostomum huronense|D.RL.2.G.13.2
Diplostomum huronense|D.RL.1.G.3.4
Diplostomum huronense|D.RL.1.G.3.1
Diplostomum huronense|D.LL.2.G.19.3
Diplostomum huronense|D.RL.1.G.18.1
Diplostomum huronense|T.RL.1.G.14.1
Diplostomum huronense|D.RL.2.G.18.4
Diplostomum huronense|D.RL.2.G.13.8
Diplostomum huronense|D.RL.2.G.14.10
Diplostomum huronense|D.RL.2.G.19.1
Diplostomum huronense|D.LL.2.G.18.3
Diplostomum huronense|D.LL.2.G.7.2
Diplostomum huronense|T.RL.2.G.6.1
Diplostomum huronense|D.LL.2.G.19.7
Diplostomum huronense|D.RL.2.G.14.13
Diplostomum huronense|D.RL.2.G.14.14
Diplostomum huronense|T.RL.2.G.15.1
Diplostomum huronense|LSF1.0911.NOSV.5.R.1
Diplostomum huronense|D.RL.1.G.6.1
Diplostomum huronense|D.LL.2.G.10.2
Diplostomum huronense|D.LL.1.G.6.5
Diplostomum huronense|D.LL.1.G.6.4
Diplostomum huronense|D.RL.2.G.13.1
Diplostomum huronense|D.RL.2.B.10.4
Diplostomum huronense|cq0910.nocr3.rl.d1
Diplostomum huronense|D.LL.2.G.7.4
Diplostomum huronense|T.LL.2.G.4.1
Diplostomum huronense|LSF1.0911.CACO.7.L.2
Diplostomum huronense|LSF1.0911.CACO.4.L.1
Diplostomum huronense|cq0611.nocr35.rl.d2
Diplostomum huronense|cq0611.nocr47.ll.d1
Diplostomum huronense|cq0611.nocr16.rl.d1
Diplostomum huronense|LSF1.0911.CACO.2.L.1
Diplostomum huronense|D.RL.2.G.19.8
Diplostomum sp. 1|D.LL.1.S.14.1
Diplostomum sp. 1|LSF1.0611.FUDI.5.L.1
Diplostomum sp. 1|LSF1.0911.NOHE.9.R.1
Diplostomum sp. 1|D.RL.1.B.1.1
Diplostomum sp. 1|D.RL.2.B.4.2
Diplostomum sp. 1|D.RL.2.G.13.5
Diplostomum sp. 1|D.RL.1.B.2.2
Diplostomum sp. 1|D.LL.1.G.13.3
Diplostomum sp. 1|D.RL.2.G.3.2
Diplostomum sp. 1|D.RL.2.B.10.8
Diplostomum sp. 1|D.LL.2.B.4.2
Diplostomum sp. 1|D.RL.1.G.16.3
Diplostomum sp. 1|D.RL.1.B.4.1
Diplostomum sp. 1|D.RL.2.G.14.4
Diplostomum sp. 1|D.LL.1.G.17.1
Diplostomum sp. 1|LSF1.0611.PEFL.19.L.2
Diplostomum sp. 1|LSF1.0611.NOSV.5.R.1
Diplostomum sp. 1|D.LL.2.G.19.1
Diplostomum sp. 1|cq0910.pefl5.rl.d2
Diplostomum sp. 1|D.RL.2.B.19.3
Diplostomum sp. 1|D.RL.1.G.15.1
Diplostomum sp. 1|LSF1.0911.PEFL.65.R.5
Diplostomum sp. 1|D.LL.2.B.18.9
Diplostomum sp. 1|LSF1.0911.NEME.8.R.2
Diplostomum sp. 1|D.LL.1.G.8.1
Diplostomum sp. 1|LSF1.0911.NOAT.4.L.1
Diplostomum sp. 1|LSF1.0911.NOAT.4.R.1
Diplostomum sp. 1|D.RL.1.P.10.1
Diplostomum sp. 1|D.LL.2.R.7.2
Diplostomum sp. 1|D.LL.2.B.19.4
Diplostomum sp. 1|D.RL.2.B.17.1
Diplostomum sp. 1|cq0611.nocr9.rl.d1
Diplostomum sp. 1|D.RL.2.G.13.7
Diplostomum sp. 1|LSF1.0611.LASI.4.L.1
Diplostomum sp. 1|D.RL.2.B.18.1
Diplostomum sp. 1|LSF1.0611.FUDI.5.L.2
Diplostomum sp. 1|D.LL.2.B.10.3
Diplostomum sp. 1|D.RL.2.B.18.9
Diplostomum sp. 1|D.RL.2.B.4.1
Diplostomum sp. 1|D.RL.2.B.3.1
Diplostomum sp. 1|D.LL.2.G.3.1
Diplostomum sp. 1|D.RL.2.G.19.2
Diplostomum sp. 1|D.LL.1.R.2.1

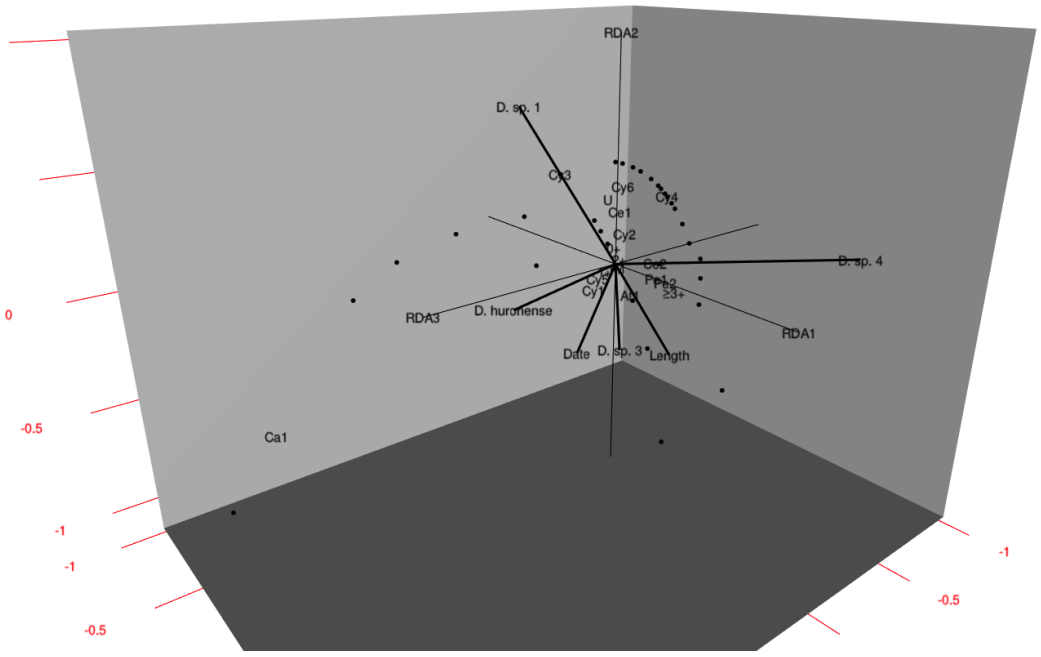

Diplostomum sp. 1|D.RL.2.G.19.2
Diplostomum sp. 1|D.LL.1.R.2.1
Diplostomum sp. 1|D.RL.2.G.17.6
Diplostomum sp. 1|D.RL.2.B.4.4
Diplostomum sp. 1|LSF1.0911.PEFL.23.L.1
Diplostomum sp. 1|D.LL.2.B.17.2
Diplostomum sp. 1|LSF1.0611.PEFL.37.R.2
Diplostomum sp. 1|D.LL.1.G.8.2
Diplostomum sp. 1|D.LL.2.G.17.4
Diplostomum sp. 1|D.RL.2.G.19.6
Diplostomum sp. 1|D.LL.2.G.12.2
Diplostomum sp. 1|D.LL.2.B.14.2
Diplostomum sp. 1|D.LL.2.B.8.7
Diplostomum sp. 1|D.RL.2.B.18.2
Diplostomum sp. 1|D.RL.2.P.15.3
Diplostomum sp. 1|D.LL.1.P.1.1
Diplostomum sp. 1|D.LL.2.B.8.5
Diplostomum sp. 1|D.LL.2.B.23.5
Diplostomum sp. 1|cq0611.nocr9.rl.d2
Diplostomum sp. 1|LSF1.0611.FUDI.5.R.1
Diplostomum sp. 1|D.LL.2.B.22.6
Diplostomum sp. 1|cq0611.nocr36.rl.d6
Diplostomum sp. 1|cq0910.nocr38.ll.d1
Diplostomum sp. 1|D.LL.2.P.10.1
Diplostomum sp. 1|D.LL.2.G.13.4
Diplostomum sp. 1|cq0910.pefl41.rl.d1
Diplostomum sp. 1|LSF1.0911.NOCR.14.R.1
Diplostomum sp. 1|D.LL.2.B.4.1
Diplostomum sp. 1|D.LL.2.G.7.3
Diplostomum sp. 1|D.LL.2.G.19.4
Diplostomum sp. 1|D.LL.1.G.4.2
Diplostomum sp. 1|D.LL.2.G.13.1
Diplostomum sp. 1|LSF1.0611.PEFL.27.R.1
Diplostomum sp. 1|D.RL.1.R.6.1
Diplostomum sp. 1|D.RL.2.G.17.2
Diplostomum sp. 1|LSF1.0911.NEME.8.L.1
Diplostomum sp. 1|D.RL.2.B.16.3
Diplostomum sp. 1|D.RL.2.B.11.8
Diplostomum sp. 1|cq0910.lasi6.rl.d2
Diplostomum sp. 1|D.LL.2.G.18.1
Diplostomum sp. 1|D.LL.2.B.10.2
Diplostomum sp. 1|D.LL.2.G.18.4
Diplostomum sp. 1|LSF1.0911.NEME.11.R.1
Diplostomum sp. 1|LSF1.0911.NOHE.1.R.1
Diplostomum sp. 1|D.RL.2.B.3.6
Diplostomum sp. 1|D.RL.1.G.13.1
Diplostomum sp. 1|LSF1.0911.PINO.3.L.1
Diplostomum sp. 1|LSF1.0911.NEME.5.R.3
Diplostomum sp. 1|D.RL.1.G.16.1
Diplostomum sp. 1|LSF1.0911.NEME.7.R.1
Diplostomum sp. 1|D.LL.2.G.17.3
Diplostomum sp. 1|D.LL.2.B.10.1
Diplostomum sp. 1|D.RL.2.B.4.3
Diplostomum sp. 1|D.LL.2.G.17.1
Diplostomum sp. 1|D.LL.2.G.13.2
Diplostomum sp. 1|D.LL.2.B.14.3
Diplostomum sp. 1|D.RL.1.G.8.2
Diplostomum sp. 1|D.RL.2.G.19.7
Diplostomum sp. 1|D.RL.1.J.4.1
Diplostomum sp. 1|D.RL.1.S.5.1
Diplostomum sp. 1|D.LL.2.B.8.6
Diplostomum sp. 1|D.RL.2.B.3.9
Diplostomum sp. 1|D.RL.2.G.19.5
Diplostomum sp. 1|D.LL.2.B.19.2
Diplostomum sp. 1|LSF1.0911.FUDI.3.L.1
Diplostomum sp. 1|D.LL.2.B.3.1
Diplostomum sp. 1|D.LL.1.G.4.1
Diplostomum sp. 1|LSF1.0911.PINO.2.L.1
Diplostomum sp. 1|D.LL.2.B.11.2
Diplostomum sp. 1|D.RL.1.G.14.3
Diplostomum sp. 1|D.RL.2.G.17.4
Diplostomum sp. 1|D.LL.2.B.22.14
Diplostomum sp. 1|D.LL.2.G.12.1
Diplostomum sp. 1|D.LL.2.G.7.5
Diplostomum sp. 1|D.RL.2.G.17.1
Diplostomum sp. 1|D.LL.2.G.19.11
Diplostomum sp. 1|cq0910.pefl48.ll.d1
Diplostomum sp. 1|D.RL.2.G.3.3
Diplostomum sp. 1|D.LL.2.B.4.5
Diplostomum sp. 1|D.LL.2.B.3.7
Diplostomum sp. 1|D.RL.2.B.23.4
Diplostomum sp. 1|cq0611.nocr36.rl.d4
Diplostomum sp. 1|cq0910.lasi6.ll.d3
Diplostomum sp. 1|LSF1.0911.NEME.2.L.1
Diplostomum sp. 1|cq0910.pefl31.rl.d1
Diplostomum sp. 1|D.LL.2.G.19.5
Diplostomum sp. 1|D.RL.2.B.11.5
Diplostomum sp. 1|D.LL.1.G.14.9
Diplostomum sp. 1|cq0910.nocr39.rl.d2
Diplostomum sp. 1|LSF1.0911.MISA.7.L.1
Diplostomum sp. 1|D.LL.2.B.12.5
Diplostomum sp. 1|D.RL.1.G.9.2
Diplostomum sp. 1|D.RL.2.G.19.3
Diplostomum sp. 1|D.RL.2.B.8.2
Diplostomum sp. 1|D.LL.2.B.23.2


Diplostomum sp. 1|D.RL.2.G.19.3
Diplostomum sp. 1|D.RL.2.B.8.2
Diplostomum sp. 1|D.LL.2.B.23.2
Diplostomum sp. 1|D.RL.2.S.1.1
Diplostomum sp. 1|D.RL.2.B.8.1
Diplostomum sp. 1|D.RL.1.G.2.1
Diplostomum sp. 1|D.RL.2.G.18.12
Diplostomum sp. 1|D.RL.1.R.9.2
Diplostomum sp. 1|LSF1.0911.NOHE.7.R.1
Diplostomum sp. 1|cq0910.pefl33.rl.d1
Diplostomum sp. 1|LSF1.0611.NOSV.3.L.3
Diplostomum sp. 1|D.RL.2.B.14.2
Diplostomum sp. 1|D.RL.1.G.13.2
Diplostomum sp. 1|cq0910.lasi6.ll.d1
Diplostomum sp. 1|LSF1.0911.NOSV.1.L.4
Diplostomum sp. 1|D.RL.1.P.10.2
Diplostomum sp. 1|D.RL.2.B.23.2
Diplostomum sp. 1|D.RL.2.G.18.11
Diplostomum sp. 1|D.LL.2.B.10.6
Diplostomum sp. 1|LSF1.0911.NEME.9.R.1
Diplostomum sp. 1|D.RL.1.R.9.1
Diplostomum sp. 1|LSF1.0611.NOSV.3.L.2
Diplostomum sp. 1|D.RL.2.G.17.3
Diplostomum sp. 1|LSF1.0911.FUDI.5.R.1
Diplostomum sp. 1|LSF1.0911.NOCR.22.R.1
Diplostomum sp. 1|LSF1.0911.NEME.5.R.1
Diplostomum sp. 1|D.LL.2.B.5.1
Diplostomum sp. 1|LSF1.0611.PEFL.26.L.1
Diplostomum sp. 1|LSF1.0911.FUDI.5.L.1
Diplostomum sp. 1|cq0910.pefl46.rl.d2
Diplostomum sp. 1|D.RL.2.B.16.4
Diplostomum sp. 1|D.RL.1.J.6.1
Diplostomum sp. 1|D.LL.2.B.8.3
Diplostomum sp. 1|D.RL.2.B.18.3
Diplostomum sp. 1|D.LL.2.B.22.4
Diplostomum sp. 1|D.RL.2.B.22.4
Diplostomum sp. 1|D.RL.1.Pn.1.1
Diplostomum sp. 1|LSF1.0911.CACO.7.L.1
Diplostomum sp. 1|D.LL.2.G.10.1
Diplostomum sp. 1|D.RL.2.G.18.2
Diplostomum sp. 1|D.RL.2.G.17.5
Diplostomum sp. 1|D.RL.2.B.14.1
Diplostomum sp. 1|D.RL.1.G.16.2
Diplostomum sp. 1|D.RL.2.B.22.1
Diplostomum sp. 1|D.LL.2.G.19.2
Diplostomum sp. 1|D.LL.2.B.22.5
Diplostomum sp. 1|D.LL.1.R.5.6
Diplostomum sp. 1|D.RL.1.G.8.1
Diplostomum sp. 1|D.LL.1.G.9.4
Diplostomum sp. 1|LSF1.0911.NEME.2.L.2
Diplostomum sp. 1|D.RL.2.G.13.3
Diplostomum sp. 1|D.LL.2.B.3.5
Diplostomum sp. 1|D.LL.2.B.3.3
Diplostomum sp. 1|D.RL.1.Nh.1.3
Diplostomum sp. 1|D.RL.1.G.9.3
Diplostomum sp. 1|cq0910.nocr21.ll.d1
Diplostomum sp. 1|cq0910.lasi6.ll.d2
Diplostomum sp. 1|D.RL.1.P.6.1
Diplostomum sp. 1|D.RL.1.G.8.3
Diplostomum sp. 1|D.LL.1.G.6.3
Diplostomum sp. 1|LSF1.0911.NEME.9.R.2
Diplostomum sp. 1|D.RL.2.B.3.2
Diplostomum sp. 1|LSF1.0611.PEFL.30.R.1
Diplostomum sp. 1|cq0910.pefl33.ll.d2
Diplostomum sp. 1|LSF1.0611.NOSV.5.L.1
Diplostomum sp. 1|D.LL.2.B.10.8
Diplostomum sp. 1|cq0611.nocr9.ll.d2
Diplostomum sp. 1|D.LL.2.B.4.3
Diplostomum sp. 1|D.RL.2.G.13.4
Diplostomum sp. 1|D.LL.2.B.10.11
Diplostomum sp. 1|LSF1.0911.NEME.5.R.2
Diplostomum sp. 1|LSF1.0611.PEFL.21.R.1
Diplostomum sp. 1|D.RL.2.B.10.1
Diplostomum sp. 1|D.LL.2.B.18.10
Diplostomum sp. 1|D.LL.2.B.20.1
Diplostomum sp. 1|D.RL.2.B.4.5
Diplostomum sp. 1|D.LL.2.R.7.3
Diplostomum sp. 1|D.RL.2.G.13.6
Diplostomum sp. 1|D.LL.1.J.6.4
Diplostomum sp. 1|D.RL.2.G.14.6
Diplostomum sp. 1|LSF1.0911.PONI.1.R.1
Diplostomum sp. 1|D.LL.2.G.19.6
Diplostomum sp. 1|D.RL.2.B.3.3
Diplostomum sp. 1|D.RL.2.G.3.1
Diplostomum sp. 1|D.RL.2.B.10.3
Diplostomum sp. 1|D.RL.1.G.14.2
Diplostomum sp. 1|D.RL.2.J.16.1
Diplostomum sp. 1|D.RL.2.B.16.2
Diplostomum sp. 1|cq0611.nocr36.rl.d2
Diplostomum sp. 1|D.RL.2.G.19.4
Diplostomum sp. 1|D.LL.2.R.7.1
Diplostomum sp. 1|D.RL.2.B.10.7
Diplostomum sp. 1|cq0611.nocr42.ll.d1
Diplostomum sp. 1|D.RL.2.B.17.2
Diplostomum sp. 1|D.LL.2.B.22.13
Diplostomum sp. 1|cq0611.nocr9.ll.d3

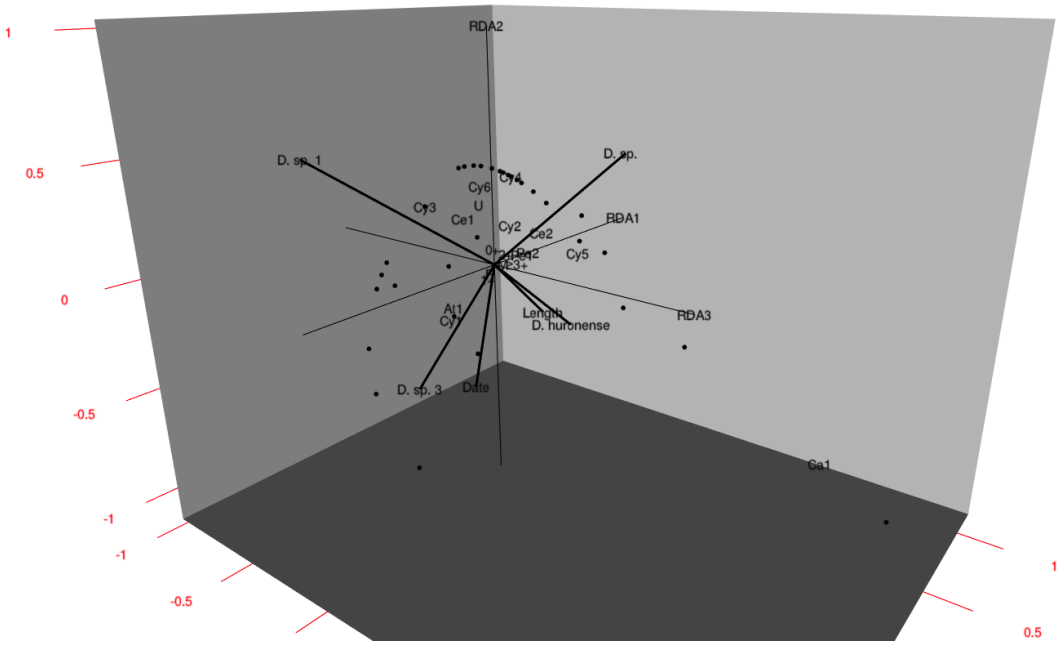

Diplostomum sp. 1|D.RL.2.B.17.2
Diplostomum sp. 1|D.LL.2.B.22.13
Diplostomum sp. 1|cq0611.nocr9.ll.d3
Diplostomum sp. 1|D.LL.2.R.5.1
Diplostomum sp. 1|D.RL.2.B.22.5
Diplostomum sp. 1|D.RL.1.G.8.4
Diplostomum sp. 1|cq0611.nocr65.rl.d1
Diplostomum sp. 1|D.RL.2.B.10.6
Diplostomum sp. 1|D.RL.2.G.7.4
Diplostomum sp. 1|D.LL.1.J.6.3
Diplostomum sp. 1|D.RL.2.P.12.1
Diplostomum sp. 1|D.RL.2.G.14.3
Diplostomum sp. 1|D.LL.2.R.9.1
Diplostomum sp. 1|D.LL.2.G.19.9
Diplostomum sp. 1|D.LL.2.G.6.3
Diplostomum sp. 1|D.LL.2.B.22.1
Diplostomum sp. 1|D.LL.1.P.18.1
Diplostomum sp. 1|D.LL.2.B.4.6
Diplostomum sp. 1|D.LL.2.B.8.2
Diplostomum sp. 1|D.RL.2.G.14.11
Diplostomum sp. 1|LSF1.0911.NOAT.2.R.1
Diplostomum sp. 1|LSF1.0911.NEME.8.R.1
Diplostomum sp. 1|D.LL.2.B.8.4
Diplostomum sp. 1|D.LL.2.B.18.5
Diplostomum sp. 1|cq0611.nocr65.ll.d1
Diplostomum sp. 1|D.RL.2.B.23.3
Diplostomum sp. 1|D.RL.2.G.7.1
Diplostomum sp. 1|D.RL.1.G.1.1
Diplostomum sp. 1|D.RL.1.G.3.3
Diplostomum sp. 1|D.LL.1.G.5.5
Diplostomum sp. 1|D.LL.1.G.14.6
Diplostomum sp. 1|D.LL.1.G.15.1
Diplostomum sp. 1|D.RL.1.G.6.3
Diplostomum sp. 1|D.LL.1.G.18.1
Diplostomum sp. 1|D.LL.1.G.12.2
Diplostomum sp. 1|D.RL.1.G.6.4
Diplostomum sp. 1|cq0611.nocr44.ll.d1
Diplostomum sp. 1|D.LL.1.G.5.6
Diplostomum sp. 1|cq0611.nocr14.rl.d1
Diplostomum sp. 1|D.LL.1.G.1.1
Diplostomum sp. 1|D.LL.1.G.14.2
Diplostomum sp. 1|D.LL.1.G.1.2
Diplostomum sp. 1|D.LL.1.G.5.2
Diplostomum sp. 1|D.LL.1.G.10.1
Diplostomum sp. 1|D.LL.1.G.5.9
Diplostomum sp. 1|D.RL.1.G.10.1
Diplostomum sp. 1|D.RL.1.G.10.2
Diplostomum sp. 1|D.RL.1.G.5.2
Diplostomum sp. 1|D.LL.1.R.5.5
Diplostomum sp. 1|D.LL.1.G.16.1
Diplostomum sp. 1|cq0910.nocr6.rl.d1
Diplostomum sp. 1|D.LL.1.G.13.2
Diplostomum sp. 1|D.LL.1.G.13.1
Diplostomum sp. 1|D.RL.2.B.19.1
Diplostomum sp. 1|D.RL.2.G.6.3
Diplostomum sp. 1|D.LL.2.B.8.1
Diplostomum sp. 1|cq0611.nocr9.rl.d3
Diplostomum sp. 1|D.RL.2.B.11.2
Diplostomum sp. 1|cq0611.nocr47.rl.d3
Diplostomum sp. 1|D.RL.2.B.1.4
Diplostomum sp. 1|D.RL.2.B.1.2
Diplostomum sp. 1|D.LL.1.G.14.5
Diplostomum sp. 1|D.RL.1.G.6.9
Diplostomum sp. 1|D.RL.1.G.6.6
Diplostomum sp. 1|D.LL.1.G.5.4
Diplostomum sp. 1|D.RL.1.G.1.2
Diplostomum sp. 1|D.RL.1.G.6.5
Diplostomum sp. 1|D.LL.1.G.14.4
Diplostomum sp. 1|D.LL.1.G.6.9
Diplostomum sp. 1|D.LL.1.G.14.8
Diplostomum sp. 1|D.LL.1.G.6.8
Diplostomum sp. 1|D.LL.1.G.14.7
Diplostomum sp. 1|D.LL.1.G.6.6
Diplostomum sp. 1|D.LL.1.G.10.2
Diplostomum sp. 1|D.RL.1.G.3.6
Diplostomum sp. 1|cq0611.nocr7.ll.d1
Diplostomum sp. 1|D.LL.1.G.5.8
Diplostomum sp. 1|cq0611.nocr24.rl.d1
Diplostomum sp. 1|D.LL.1.G.5.1
Diplostomum sp. 1|D.LL.1.G.5.7
Diplostomum sp. 1|D.RL.1.G.1.4
Diplostomum sp. 1|D.LL.1.G.10.3
Diplostomum sp. 1|D.RL.1.G.5.1
Diplostomum sp. 1|cq0611.nocr49.ll.d1
Diplostomum sp. 1|cq0611.nocr31.ll.d1
Diplostomum sp. 1|D.LL.1.G.18.2
Diplostomum sp. 1|D.LL.1.G.5.3
Diplostomum sp. 1|D.LL.1.G.14.3
Diplostomum sp. 1|D.LL.1.G.3.1
Diplostomum sp. 1|D.LL.1.G.1.3
Diplostomum sp. 1|D.RL.1.G.1.3
Diplostomum sp. 1|D.LL.1.P.3.2
Diplostomum sp. 1|cq0611.nocr44.rl.d2
Diplostomum indistinctum|D.LL.1.G.12.3

Appendix 3. Redundancy analysis triplot of *Diplostomum* spp. lens infracommunities in fish (additional rotations to Figure 4) with type 2 scaling, retaining correlations between descriptive variables. Species of *Diplostomum*, and fishing date and host length appear as lines. Host sex (M = males, F = females, U = undetermined sex) and age (0+, 1+, 2+, ≥3+) are printed at their centroid values. Closed circles represent the position of the infracommunity of each host individual. Alpha-numerical codes indicate the position of host species centroids: At = Atherinidae (1 = Brook silverside), Ca = Catostomidae (1 = White sucker), Cy = Cyprinidae (1 = golden shiner, 2 = emerald shiner, 3 = blackchin shiner, 4 = spottail shiner, 5 = sand shiner, 6 = bluntnose minnow), Fu = Fundulidae (1 = banded killifish), Go = Gobiidae (1 = round goby), Ce = Centrarchidae (1 = rock bass, 2 = pumpkinseed, 3 = largemouth bass, 4 = black crappie), Pe = Percidae (1 = johnny darter, 2 = yellow perch). Rotation A displays the plot from octant I; rotation B, from octant II; rotation C, from octant VI; rotation D, from octant V. This triplot was constructed using the 411 successfully sequenced *Diplostomum* specimens in 150 hosts (data set 4, Table 2).

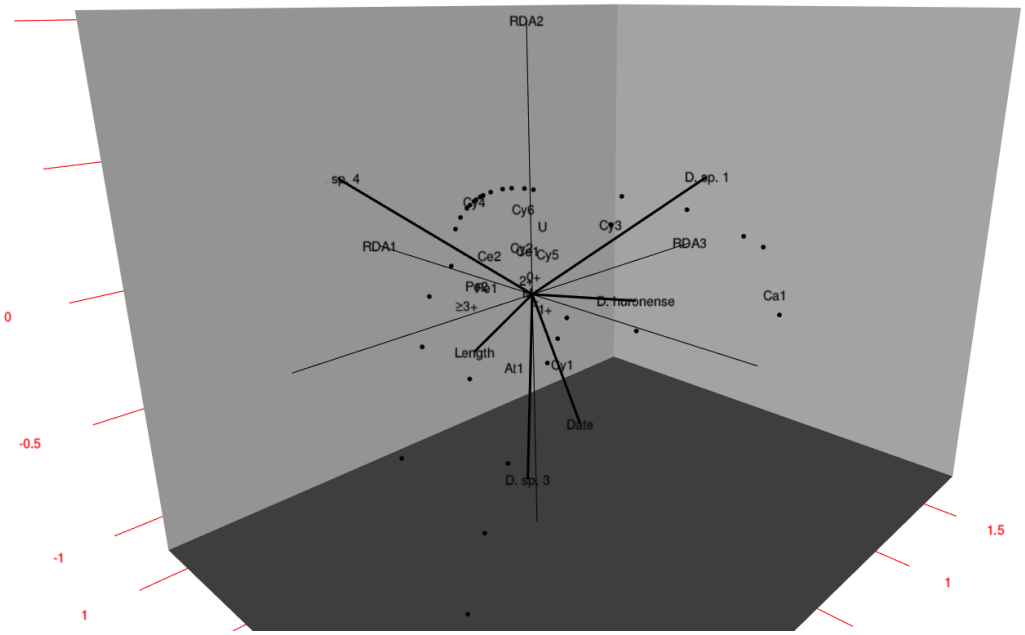
A


RDA2
D. sp. 1
Cy3
Cy6
U
Ce1
Cy2
Cy4
D. sp. 4
Ce2
Pe1-3
23+
Cy5
Cy1
At1
RDA3
D. huronense
RDA1
Date
D. sp. 3
Length
Ca1
0
-0.5
-1
-1
-0.5
-1
-0.5

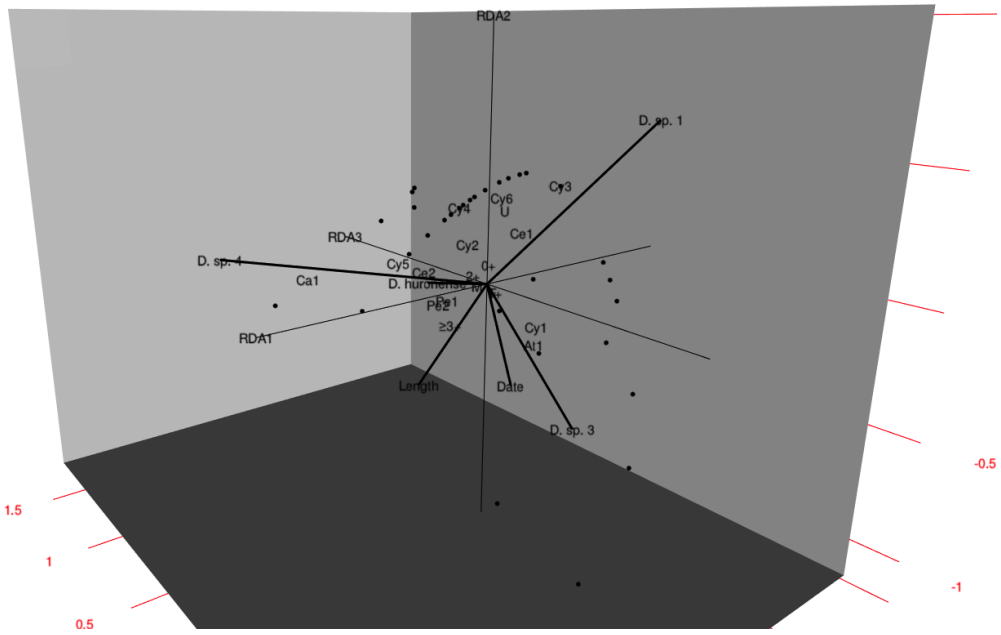
B


RDA2
1
0.5
0
-0.5
-1
-1
-0.5
D. sp. 1
D. sp.
Cy4
Cy6
Cy3
U
Ce1
Cy2
Ce2
RDA1
Cy5
At1
Cy1
Length
RDA3
D. huronense
D. sp. 3
Date
Ca1
1
0.5

C


RDA2
sp. 4
D. sp. 1
Cy4
Cy6
U
Cy3
RDA1
Ce2
Cy2
Ce1
Cy5
RDA3
2+
Pe1
≥3+
0+
D. huronense
Ca1
Length
At1
Cy1
Date
D. sp. 3
0
-0.5
-1
1
1.5
1

D


RDA2
D. sp. 1
Cy3
Cy4
Cy6
U
Ce1
RDA3
Cy2
D. sp. 4
Cy5
Ce2
2+
Ca1
D. huronense
0+
Pe2
Pe1
≥3+
Cy1
At1
RDA1
Length
Date
D. sp. 3
1.5
1
0.5
-0.5
-1