Examination of the diversity and assembly processes of digenean trematode communities in Alberta, and the implications of spatio-temporal community dynamics on swimmer's itch transmission in recreational lakes

by

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Abstract

The preservation of biodiversity on our planet is crucial to our health. However, we cannot preserve what we do not understand. Biodiversity surveys most often forget to include some of the most diverse organisms on our planet, the parasites. Historically given a bad rap because of the diseases they can cause, parasites are often not thought of as organisms we should preserve. What most do not realize is that parasites are engrained in food-webs and ecosystems, playing many different important roles in the processes that regulate populations and more. Parasites can also form their own communities, and the processes that underly their assembly can have important impacts on the transmission of certain diseases. The focus of my doctoral research has been to examine the diversity and assembly processes of digenean trematode communities in Alberta, and the implications of community dynamics on their transmission of in recreational lakes.

Trematodes are parasitic flatworms that have complex life cycles involving two or more host species. Some of the underlying factors that can affect trematode community assembly in their snail hosts are environmental factors that relate to the quality of the ecosystem, and therefore the suitability for their hosts. As well, the ecological interactions, those occurring between species, such as competition and predation, can impact community assembly as well. So, if we have an understanding of how trematode communities are formed, we may predict how changes to the important factors may impact the transmission of diseases they can cause.

I conducted a longitudinal species survey of snails and trematodes across six lakes in central Alberta over three summers. From the survey, we applied morphological and molecular genetic analyses to identify snail and trematode species. Overall, we uncovered an incredible diversity of trematodes, with 67 species and counting. Surprisingly, there were only five snail species needed to host them. From spatio-temporal analyses, we found no clear patterns for species composition in communities, though it does appear as if the ecoregion and the amount of nutrients in the water determining the trophic status may be playing a role. Furthermore, for some very abundant species, dissolved oxygen content in the water is an important predictor for their presence. All communities examined shared a trematode composition pattern of a few highly abundant and common trematode species, and many rare and inconsistent species.

This information has important implications for the local public health issue of swimmer's itch in Alberta. Swimmer's itch is a re-emerging, neglected allergic condition caused by trematodes of the family Schistosomatidae. People are exposed to the larvae of schistosomes when swimming and recreating in natural water bodies. The larvae emerge from their snail first intermediate host and actively penetrate the skin of unsuspecting swimmers, causing a rash that can last up to two weeks. What these larvae are actually attempting to do is to infect a duck, their definitive host. These accidental exposures to humans have many downstream public health impacts, most being indirect effects as a result of discontinued use of lakes for recreation, due to the economic, cultural, and spiritual values associated with them.

My doctoral research has laid the foundation for understanding the aspects of the swimmer's itch issue, the biological side and the human perspective. From our species survey, we found seven schistosome species capable of causing swimmer's itch, and all were rare in each community. This was a surprising re-

sult, given that our swimmer's itch survey, a longitudinal survey of people who had experienced swimmer's itch from across Canada, revealed many reports of swimmer's itch every year, even from the lakes we had directly sampled snails from. In total, in Alberta there were 101 lakes that had reported swimmer's itch over five years. One trend found among both surveys was the occurrence of peaks in trematode species diversity, snail infection prevalence, and swimmer's itch occurrences during the months of July and August, which will be an interesting avenue for future research. Overall, my doctoral research has contributed to a broad understanding of swimmer's itch transmission, as well as the spatio-temporal dynamics of trematode community assembly processes.

Preface

This thesis is an original work from the compilation of my doctoral research. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Human Research Ethics Board, Project Name "Enhancing accessibility and use of Alberta's natural water recreation areas through prevention of swimmer's itch transmission", No. Pro00048511, May 05, 2014.

The general introduction and discussion in Chapters 1 and 7 are my original work. In Chapter 2, my supervisor, Associate Professor Dr. Patrick C. Hanington, and I both designed the study and composed the manuscript. I collected the data, completed all analyses, and wrote the final manuscript. I was assisted in field work and lab processing of snails by the middle authors in the publication:

> Gordy, M. A., Kish, L., Tarrabain, M., & Hanington, P. C. (2016). A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada. *Parasitology Research*, 115(10), 3867-3880.

Some of the research conducted for this thesis was part of an international collaboration led by Dr. Hanington at the University of Alberta. The research conducted and presented in Chapter 3 was in collaboration with Assistant Professor Dr. Sean A. Locke as the lead collaborator at The University of Puerto Rico, Mayagüez, collaborator, Dr. Timothy Rawlings, Associate Professor at Cape Breton University, and collaborator Angela Rose Lapierre, a doctoral student at Concordia University. Dr. Rawlings and Ms. Lapierre provided specimen samples and sequences and provided feedback on manuscript drafts.

Dr. Locke provided adult trematode voucher specimens, sample sequences, and drawings, and conducted some statistical analyses and contributed to writing the manuscript. I provided all cercarial trematode samples and sequences, conducted most of the analyses and wrote the manuscript, published as:

> Gordy, M. A., Locke, S. A., Rawlings, T. A., Lapierre, A. R., & Hanington, P. C. (2017). Molecular and morphological evidence for nine species in North American *Australapatemon* (Sudarikov, 1959): a phylogeny expansion with description of the zygocercous *Australapatemon mclaughlini* n. sp. *Parasitology Research*, *116*(8), 2181-2198.

In Chapter 4, I collected all the data, designed and performed the research, completed all analyses, and wrote the manuscript, published as:

> Gordy, M.A., & Hanington, P.C. (2019). A fine-scale phylogenetic assessment of digenean trematodes in central Alberta reveals we have yet to uncover their total diversity. *Ecology and Evolution.* 2019;00:1–53. https://doi.org/10.1002

In Chapter 5, we collaborated with Associate Professor Dr. Janet Koprivnikar at Ryerson University on the study of trematode community ecology. Dr. Koprivnikar was involved in the conceptual design of the study along with Dr. Hanington and I, and provided guidance on and contributions to the statistical modelling and analyses conducted. She also contributed to the manuscript composition. I collected the data, conducted the community analyses, and contributed to the statistical modelling of environmental variables. I also completed the writing, to be submitted for publication:

> Gordy, M.A., Koprivnikar, J., & Hanington, P.C. (2018) Environmental and ecological factors that drive trematode community assembly in central Alberta.

For the research conducted and presented in Chapter 6, Dr. Hanington led the collaboration with Dr. Tyler P. Cobb of the Royal Alberta Museum and

the Alberta Biodiversity Monitoring Institute. Dr. Cobb provided data and maps for the distributions of species important to swimmer's itch in Alberta and feedback on manuscript drafts. I was responsible for the study design, data collection, all analyses and writing the manuscript, published in Environmental Health as:

> Gordy, M.A., Cobb, T.P., and & Hanington, P.C. (2018). Swimmer's itch in Canada: a look at the past and a survey of the present to plan for the future. *Environmental Health*, 17(1): 73. doi: 10.1186/s12940-018-0417-7

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Contents

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Abbreviations and Symbols

Abbreviations and Full Spelling

28S ..... large subunit ribosomal DNA

AB ..... Alberta

ABGD ..... Automatic Barcode Gap Discovery

ABMI ..... Alberta Biodiversity Monitoring Institute

AIC ..... Akaike Information Criterion

ALMS ..... Alberta Lake Management Society

ANOVA ..... Analysis Of Variance

ASW ..... Artificial spring water

BC ..... British Columbia

BI ..... Bayesian Inference

BIC ..... Bayesian Information Criterion

BLAST ..... Basic Local Alignment Search Tool

BOLD ..... Biodiversity Of Life Datasystems

bp ..... base pair

C ..... Celsius

CBC ..... Canadian Broadcasting Company

CCA ..... Canonical Correspondence Analysis

CI ..... Confidence Interval

corr. coeff. ... correlation coefficient

cox1 ..... Cytochrome c oxidase subunit 1

df ..... degrees of freedom

DNA ..... Deoxyribo Nucleic Acid

exp(H) ..... Shannon entropy

FBTs ..... Food-borne trematodiases

FREPs ..... Fibrinogen related proteins

gDNA ..... genomic DNA

GLM ..... Generalized Linear Model

GPS ..... Global Positioning System

H ..... Shannon diversity index

HMDS ..... hexamethyldisilazane

ITS ..... Internal Transcribed Spacer

JC ..... Jukes-Cantor

K2 ..... Kimura-2

km ..... kilometers

LIN ..... Lineage

LLN1 ..... Lac La Nonne Site #1

LLN2 ..... Lac La Nonne Site #2

max ..... maximum

MB ..... Manitoba

MCMC ..... Markov Chain Monte Carlo

MDGs ..... Millenium Development Goals

MGC ..... "Michelle Gordy Cercaria" sample identifier

min ..... Minute

min ..... minimum

ml ..... milliliter

ML ..... Maximum Likelihood

mm ..... millimeter

MUSCLE .... Multiple Sequence Comparison by Log Expectation

n or N ..... number

nad1 ..... Nictonamide Adenine Dinucleotide dehydrogenase subunit 1

NB ..... New Brunswick

ng ..... nanograms

NL ..... Newfoundland and Labrador

NS ..... Nova Scotia

nt ..... nucleotide

NT ..... Northwest Territories

NZ ..... New Zealand

ON ..... Ontario

PCR ..... Polymerase Chain Reaction

PEI ..... Prince Edward Island

pKa ..... acid disassociation constant

pmol ..... picomole

PP ..... Pelican Point

ppm ..... parts per million

ppt ..... parts per thousand

QC ..... Quebec

qPCR ..... quantitative PCR

RAD ..... Rank Abundance Dominance

rDNA ..... ribosomal DNA

RS ..... Rochon Sands

SDGs ..... Sustainable Development Goals

SEM ..... Scanning Electron Microscopy

SK ..... Saskatchewan

sp ..... species

spp ..... species plural

SPR ..... Subtree Pruning and Regrafting

TN ..... The Narrows

Tukey HSD .. Tukey's Honest Significant Difference

UN ..... United Nations

UNDP ..... United Nations Development Programme

WASH ..... Water, Sanitation, and Hygiene

x g ..... relative centrifugal force

α ..... alpha

β ..... beta

γ ..... gamma

μ**l** ..... microliter

χ^2 ..... Chi-squared

θ ..... theta

Abbreviated Species Names

ApspA ..... *Apatemon sp. A*

ApspB ..... *Apatemon sp. B*

ApspC ..... *Apatemon sp. C*

Aubulin1 ..... *Australapatemon burti LIN1*

Aumc ..... *Australapatemon mclaughlini*

Ausplin10 ..... *Australapatemon sp. LIN10*

Ausplin3 ..... *Australapatemon sp. LIN3*

Ausplin4 ..... *Australapatemon sp. LIN4*

Ausplin5 ..... *Australapatemon sp. LIN5*

Ausplin6 ..... *Australapatemon sp. LIN6*

Ausplin8 ..... *Australapatemon sp. LIN8*

Ausplin9A ..... *Australapatemon sp. LIN9A*

Ausplin9B ..... *Australapatemon sp. LIN9B*

AvscspA ..... *Avian schistosomatid sp. A*

AvscspB ..... *Avian schistosomatid sp. B*

AvscspC ..... *Avian schistosomatid sp. C*

Bosp ..... *Bolbophorus sp.*

Coco ..... *Cotylurus cornutus*

Coga ..... *Cotylurus gallinulae*

CospA ..... *Cotylurus sp. A*

CospB ..... *Cotylurus sp. B*

CospC ..... *Cotylurus sp. C*

CospD ..... *Cotylurus sp. D*

CospE ..... *Cotylurus sp. E*

CospF ..... *Cotylurus sp. F*

CospG ..... *Cotylurus sp. G*

CospH ..... *Cotylurus sp. H*

DigenspO .... *Diplostomidae gen. sp. O*

DigenspX .... *Diplostomidae gen. sp. X*

Dibalin2 ..... *Diplostomum baeri LIN2*

Diin ..... *Diplostomum indistinctum*

Disp1 ..... *Diplostomum sp. 1*

Disp3 ..... *Diplostomum sp. 3*

Disp4 ..... *Diplostomum sp. 4*

DispA ..... *Diplostomum sp. A*

DispB ..... *Diplostomum sp. B*

DispC ..... *Diplostomum sp. C*

Drau ..... *Drepanocephalus auritus*

EcspA ..... *Echinoparyphium sp. A*

EcspA2 ..... *Echinoparyphium sp. A2*

EcspB ..... *Echinoparyphium sp. B*

EcspC ..... *Echinoparyphium sp. C*

EcspD ..... *Echinoparyphium sp. D*

EcspE ..... *Echinoparyphium sp. E*

Ecsplin1A .... *Echinoparyphium sp. Lineage 1 A*

Ecsplin1B .... *Echinoparyphium sp. Lineage 1 B*

Ecsplin2 ..... *Echinoparyphium sp. Lineage 2*

Ecsplin3 ..... *Echinoparyphium sp. Lineage 3*

EcreB ..... *Echinostoma revolutum B*

EctrlinA ..... *Echinostoma trivolvis Lineage A*

Ecgesp ..... *Echinostomatidae gen. sp.*

HagespA ..... *Haematoloechidae gen. sp. A*

Hysplin1 ..... *Hypoderaeum sp. Lineage 1*

Neam ..... *Neodiplostomum americanum*

NospA ..... *Notocotylidae sp. A*

Orsp2 ..... *Ornithodiplostomum sp. 2*

Orsp8 ..... *Ornithodiplostomum sp. 8*

Peis ..... *Petasiger islandicus*

Pesp4 ..... *Petasiger sp. 4*

Plsp ..... *Plagiorchis sp.*

Posp4 ..... *Posthodiplostomum sp. 4*

PsspA ..... *Psilostomatidae gen. sp. A*

Scdo ..... *Schistosomatium douthitti*

Trph ..... *Trichobilharzia physellae*

Trst ..... *Trichobilharzia stagnicolae*

Trsz ..... *Trichobilharzia szidati*

TyspA ..... *Tylodelphys sp. A*

Glossary of Terms

Allogenic- Parasites with complex life cycles that transit between two or more ecosystems during their life.

Alpha diversity - The diversity of species within the local species pool. This is usually measured at each sample site.

Anthropogenic - A resulting effect on nature from the influence of human beings.

Apharyngeate cercaria - Larval digenean trematodes that develop in sporocysts within pulmonate or prosobranch snails.

Arcuate - Shaped like a bow; curved.

Aspinose - Without spines.

Autogenic - Parasites with complex life cycles that spend their whole life in one ecosystem.

Barcoding (Molecular) - A taxonomic method for identifying species based on a unique gene sequence region, typically of a mitochondrial gene.

Bayesian Inference - A statistical method based on Bayes' Theorem for updating the probability for a hypothesis as more information becomes available to generate a posterior probability. In other words, it answers the question "What is the probability of the data, given the hypothesis?" as opposed to maximum likelihood methods that ask "What is the probability of the hypothesis, given the likelihood of the data?".

Beta diversity - Is the ratio between the local species diversity (alpha) and the regional species diversity (gamma).

Biodiversity - The variety of species on Earth. This term is often used interchangeably with diversity.

Bipartite - Made into two parts.

Branchiate - A term to describe gill-bearing snails.

These definitions have been derived from several general sources including Wikipedia, Schell's 1985 Handbook of the trematodes of North America north of Mexico, and the Online dictionary of invertebrate zoology: complete work by the Harold W. Manter Laboratory of Parasitology.

Brevifurcate - Having a small bifurcation in reference to the tail of a trematode cercaria being divided into two parts or a fork-tail.

Cercaria - A larval, free-swimming stage of a digenean trematode that emerges from the snail first-intermediate host to actively seek the next host in its life cycle.

Clade - In taxonomy, a group of organisms that includes a common ancestor and its descendants that make up a singular branch in a phylogeny.

Community assembly - Is the study of the processes that form ecological communities by examining species identities and abundances as they consist within the local species pool. Theory of community assembly assumes a greater regional pool of species from which the local species are filtered through various processes and traits.

Community ecology - The spatio-temporal study of the interactions between species that make up a community.

Community evenness - Evenness is a measure of dominance among all species in the community. A truly even community would be composed of equally abundant species. A community with low evenness has a dominant species, or one with high abundance in comparison to the other species in the community.

Community structure - A characteristic of a community that describes the connectedness of individuals and their comparison within a network of species.

Component community - Herein defined as all of the trematode species that are found among all of their intermediate snail host species within a collection site.

Compound community - A level of community that consists of the compilation of the regional component communities.

Congeneric - Belonging to the same genera.

Copulatory bursa - An anatomical feature of trematode sex organs, involved with sperm delivery.

Delimitation - In species identification, setting a limit or boundary for pairwise nucleotide differences of gene regions used for molecular barcoding to delineate species.

Delineation - Precise identification of species.

Direct and indirect antagonism - Antagonism is the association of organisms in which one benefits at the expense of the other, and can occur directly or by association, indirectly.

Disease ecology - Is a field of ecology that focuses on the interactions of species as they relate to disease transmission. This field often looks specifically at host-pathogen interactions and their relationships to environmental factors in a biogeographical context.

Distomate - A trematode with an oral and ventral sucker.

Ecoregion - A classification of a geographically defined ecological region, typically by climate and vegetation.

Ecosystem engineers - Any organism that modifies or maintains its environment.

Effective species - A number of species derived from a measure of diversity. For instance, using Shannon entropy (H), effective species would be calculated as the exponent of H. Effective species is considered to be "true diversity", especially when calculated this way.

Encapsulate - In snails, a process by which the immune cells, haemocytes, surround and enclose a pathogen to kill it.

Encysting - In trematode larvae, a process by which cercariae transform to a resting stage either in a second intermediate host or externally, on vegetation. The process typically involves the loss of the tail and the formation of a thick protective covering.

Furcae - Each half of the forked tail of certain morphological types of trematode cercaria is a furca.

Furcocercaria - Trematode cercariae that have furcated tails.

Gamma diversity - The regional pool of the diversity of species, usually composing all sample sites together.

Genital cone - The area around the copulatory bursa and ejaculatory or hermaphroditic duct in the trematode reproductive tract.

Guild - The composition of parasite species among just one host species across its entire distribution.

Haemocytes - The immune cells of snails produced by the haematopoeitic organ.

Heterogeneity - Of or being diverse in character.

Hirudunid - Leeches in the subclass Hirudinea of the Phylum Annelida

Holdfast organ - An adhesive organ found among Strigeid trematodes that provides a unique way of feeding through enzyme secretion and external digestion. This organ is thought to be a determinant for host specificity among definitive hosts for this group **(Johnson1971)**.

Hologenophores - A voucher specimen used for genetic analysis.

Holotype - A single type specimen from which a species is described.

Infection prevalence - The ratio of infected hosts among the entire measurable population.

Infracommunity - A level of community defined in parasitological terms by all the parasite species within an individual host.

Intergeneric - Occurring between different genera.

Internal rugae - Ridges or wrinkles found in tissue.

Interspecific - Occurring between different species.

Intrageneric - Occurring within a single genus, usually between different species within the genus.

Intraspecific - Occurring within a single species, usually between different individuals.

Lineage - In molecular phylogenetics, a single line of descent that does not branch or include the common ancestor, like a clade does.

Mechanistic or immunological compatibility - In context of this thesis, the ability of a parasite to infect a host by suppressing the immune function of the host, often through cellular, molecular or chemical inhibition.

Metacercaria - A larval form of some trematode species in which the cercaria encysts within a second intermediate host and waits to be eaten by the definitive host, i.e. Echinostomes.

Monophyletic - A group of organisms that descended from a common ancestor.

Morphometric - Measurements of morphological features of an organism.

Morphotype - A group of organisms defined by a specific morphological characteristic.

Nearest-neighbor interchange - The simple and recursive exchange of neighboring branches or subtrees within a phylogenetic analysis.

Nodal support - Statistical support from recursive iterations of likelihood or probability given for the placement of a particular group in a phylogeny. The node is the place where splits occur among taxa.

Nucleotide Substitution Model - Mathematical models of the evolution of nucleotide substitutions in DNA. Several models are tested among a group of DNA sequences to find the best fit to explain the patterns of sequence differences.

Ordination - Methods for taking multi-dimensional data and configuring it to two-dimensional space for ease of finding patterns and relationships.

Overdispersion - In statistics, the presence of greater variability in the data than ould be expected given a particular model.

Oversaturation - In phylogenetics, the presence of multiple substitutions occurring at the same site in a gene or the occurrence of too many mutations from individual to individual, such that you cannot distinguish patterns. This is a common occurrence among highly evolving genes, which also happen to be good genes for barcoding as well.

Ovo-testes - A reproductive organ in hermaphroditic gastropods composed of both ovaries and testes.

Paragenophore - A museum voucher of the same species as the molecular voucher to which it typically accompanies, but a different individual.

Parenchyma - The cellular tissue between the body wall and organs.

Patent infection - In gastropods infected with trematode larvae, a patent infection is one in which the development of the larvae has completed development to the point of cercariae emerging from the snail tissue.

Phenotypic plasticity - When individuals of the same species (same genotype) have variable morphological or behavioural characteristics (phenotypes). Also referred to in text as morphological plasticity.

Phylogenetics - The taxonomic study of the relationships between species as it relates to their evolution, based on genetics.

Phylogeny - A phylogenetic tree representing the evolutionary relationships between taxa.

Phylogram - A phylogenetic tree in which the distance between branches is representative of character change (nucleotide substitutions).

Proteolytic gland - A gland in trematodes that produces proteases (protein digesting enzymes).

Pulmonate - A group of gastropods that are air-breathing. They either have lungs or lung-like organs.

Rarefaction - A mathematical method in ecology to plot the expected number of species based on the observed species. The expected number of species is based on a recursive sampling of the data. It is meant to estimate species richness.

Redia - A larval trematode stage of certain species of trematodes (i.e. Echinostomes) that develops within the snail first intermediate host, but differs from a sporocyst in that it has mouth parts. There are mother and daughter redia. The mother redia produce daughter redia, and the daughter redia produce cercaria.

Rényi entropy - A measure of diversity that is calculated for different scales. As the scale becomes larger, the more rare species are increasingly downrated.

Richness - The number (count) of species.

Rugose hermaphroditic duct - Refers to the area in hermaphroditic trematode adults in which the female and male reproductive ducts meet. The description of rugose refers to the wrinkly appearance upon description.

Sequence homology - Refers to the similarity among genetic sequences and their relation to ancestry.

Shannon entropy - A diversity index sometimes referred to as Shannon-Weiner or Shannon-Weaver index that is derived from information theory and uses the log of species abundance in its calculations.

Sporocyst - A larval form of certain species of trematodes (i.e. Strigeids) that develops within the snail first intermediate host. There are both mother and daughter sporocysts. The mother sporocyst produces daughter sporocysts and the daughters produce cercariae. Sporocysts are basically reproductive sacs and do not contain mouth parts.

Systematics - The study of the diversity of organisms and their relationships to each other over time.

Transition/Transversion ratio - Refers to the ratio of point mutation changes and takes into account the rates at which a nucleotide changes to the same type of nucleotide by the rate at which it changes to a different type of nucleotide, for instance a purine to purine or pyrimidine to pyrimidine change would be a transition, whereas a purine to pyrimidine would be a transversion.

Vagility - The relative ability of an organism to move across geographical space.

Vitelline follicles - Unfertilized/immature trematode egg cells.

Xiphidiocercariae - A type of cercaria characterized by the presence of a stylet (sharp, pointed, protrusion) at the anterior end, in its oral sucker that allows it to actively penetrate its host. This is found commonly among species that utilize insect larvae as second intermediate hosts (i.e. Plagiorchis spp.).

Zygocercous - A type of trematode cercaria characterized by a unique behavioural adaptation in which individuals join together in formations (rosette or pine-cone like in appearance) by connecting their tails.

Chapter 1

General Introduction

1.1 Overview

Biodiversity loss or gain can have direct effects on human health. For decades, we have known that biodiversity loss has been occurring across the globe at alarming rates. This has fueled much research into the connections between biodiversity and ecosystems, with evidence supporting a relationship between healthier ecosystems and greater biodiversity. Humans gain many benefits from healthy ecosystems, known as ecosystem services. Greater crop yields and healthier fisheries are two examples of ecosystem services enhanced by greater diversity and the downstream effects on changes within the ecosystem that promote better health (Cardinale et al. 2012). For instance, having a greater number of local plant species within an area, otherwise called species richness, is associated with greater nutritional availability for people (Lachat et al. 2017). In urban centers, greater biodiversity and availability of plants as "green roofs" has been associated with reducing air pollution (Getter and Rowe 2006). Restoring biodiversity in ocean communities has been associated with improving water quality and increasing biological productivity (Worm et al. 2006). Greater biodiversity has also been correlated in many circumstances with pathogen reduction, or "dilution" by decreasing the opportunity for transmission to the susceptible population and, therefore, regulating disease (P. T. Johnson, Preston, Hoverman, and LaFonte 2013; P. T. Johnson, Preston, Hoverman, and Richgels 2013; Ostfeld and Keesing 2000). Additionally, there can be a multitude of indirect effects of biodiversity loss or gain

on human health and mental well-being through cultural pathways, in which we place value on cultural goods generated by greater biodiversity (i.e. wild bird diversity for bird-watching) (reviewed in (Clark et al. 2014)). Overall, the ecosystem services gained by humans are abounding; yet, our understanding of the connections between biodiversity and ecosystem services is still developing.

The United Nations (UN) has noted on multiple occasions the importance of biodiversity, not only for health of humans, animals, and the environment, but also for economic well-being, stating in 2010,

> *"Without preserving biodiversity and preserving our natural habitat, the Millennium Development Goals (MDGs) just cannot be achieved... The loss of biodiversity and the degradation of natural resources impact first and foremost the poor and the women and the vulnerable and we should not forget that three quarters of the world's population depend on natural resources for their daily living and their daily survival, from the food, the shelter, the recreation, everything; three quarters of the world population is directly related to biodiversity on this planet."* $\sim$ *UN Development Programme (UNDP) Environment and Energy Group Director Veerle Vandeweerd* ("Preserving World's Biodiversity Vital for Economic Development, UN Official Warns — UN News" 2018)

The UN has since developed a Strategic Plan for Biodiversity 2011-2020, including the Aichi Targets, and considers biodiversity and ecosystems as integral within the Sustainable Development Goals (SDGs) and targets as a part of their 2030 Agenda for Sustainable Development (Secretariat of the Convention on Biological Diversity 2015). While understanding and maintaining biodiversity is pertinent to our futures, parasites suffer from being under the paradigm of pest eradication because of the detrimental harm they cause to human health, crops, and domestic and agricultural animals (Dougherty et al. 2016). Despite conservation efforts for biodiversity and ecosystems, human health takes precedence within the SDGs, thus, the focus of these efforts is highly stratified to the most visible organisms: the plants and vertebrates.

This is not to say human health is unimportant, rather, if we do not consider the full spectrum of the problem and focus only on what we see in front of us (eradication of the parasites that cause disease), we might be missing the greater picture. This human-first approach negates the importance of understanding the biodiversity of parasites, their roles within ecosystems, and how greater parasite diversity might potentially lead to a net positive effect on human health by reducing transmission of the particularly virulent species through antagonistic interactions (P. T. Johnson, Preston, Hoverman, and LaFonte 2013).

Greater diversity of parasites does not equate to greater disease risk (P. T. Johnson, Preston, Hoverman, and LaFonte 2013). Most parasites go undetected because they cause relatively little harm to their hosts. It has been estimated that every animal on Earth hosts at least one parasite (Robert Poulin and Morand 2000). The number of parasite species of medical and veterinary importance make up a very small fraction of the estimated total number of parasitic species. Less than 100 species of parasite are commonly found infecting humans (Cox 2002), though 437 species have been listed (De Vriese et al. 2001). The total number of parasites is estimated to comprise 40% of the known species described on Earth (A. Dobson et al. 2008). It is estimated that the number of parasitic helminth species alone is, conservatively, 50% more ($\sim$75,000) than the vertebrate species (45,000) that host them (A. Dobson et al. 2008; Robert Poulin and Morand 2004). Yet, for most parasites, we know little to nothing of their life cycles, their interactions with other species, and thus, their role in ecosystems and potential to impact or regulate the transmission of those that do cause infections in people.

Unfortunately, our estimates of parasite diversity suffer from a lack of continuous data. Most entries in the literature are the original species descriptions, without work to expand our knowledge of their geographical distributions, life cycles, and host-use. There is a great disconnect between historical records that utilize morphological descriptions and modern records that include additional, molecular characterization. Therefore, making estimates of total species diversity can be difficult. To complicate estimates further, recent

studies have revealed issues with cryptic morphology among trematode species (G. Pérez-Ponce de León and R. Poulin 2018), likely a common problem among parasites that further hinders our estimates and exemplifies the need to adapt more stringent molecular methods to current surveys and species identifications.

There is a crucial need to improve both the quality and quantity of parasite surveys to start filling in the knowledge gaps left behind by the free-living, species surveys that do not include them. We know that parasites play important roles in food webs, and as ecosystem engineers, on top of their roles as disease agents (reviewed in (Hatcher and Dunn 2011)). However, these examples come from a few, well-studied systems. Surely, we cannot expect all parasites to be the same, and need to expand our perspective on these interactions in addition to improving our resolution of biodiversity. We need to understand the interactions that are occurring between parasites and hosts, as well as with non-host species, and what factors determine a suitable environment (both within and outside of the host), so we may better understand what limits parasite distributions. Particularly, when making decisions around whether to invest efforts towards elimination or control of a particularly virulent or prevalent parasite, it is important to have a broad comprehension of the factors that may impede progress and sustainability of those control/elimination efforts. Such factors are ecologically and environmentally based. For instance, the elimination of any species from their environment can make vacant niche space available for a new species to take over (Chelsea L Wood and P. T. Johnson 2015).

From a disease perspective, parasites contributed to over 2.2 billion infections across the world in 2016, of which 90% could be contributed to parasitic helminths (Hotez 2018a). While overall the prevalence of parasitic helminth infections is declining globally, some have seen a rise in prevalence since 1990, specifically, food-borne trematodiases and Cysticercosis, which have risen by 26% and 18% respectively (Hotez 2018a). While projections into 2050 would suggest a greater decline for many parasitic helminth infections due to reduced global poverty, there may be a rise for some, in particular, because of

increased urbanization, specifically Urban Schistosomiasis, of which clusters have already begun to appear in Africa and South America (Hotez 2018a).

Schistosomiasis is a disease caused by digenean trematode flatworms of the genus *Schistosoma*, of which three species, *mansoni, haematobium*, and *japonicum* are regionally responsible for most infections ("WHO — Schistosomiasis" 2017). Schistosomes have complex life cycles and utilize a freshwater, gastropod snail as their first intermediate host, where they undergo larval development, and emerge as infectious, free-swimming larvae that penetrate the skin of humans in contaminated water sources. Once a person is infected, the larvae travel through the vasculature until they reach the hepatic portal vein of the liver or the venous plexus of the bladder (depending on the species) and mature into adult worms. The adults will release eggs through the intestines or bladder, then excreted with the feces or urine, helping them gain access to the environment, where they will search for a snail host and continue the life cycle. The disease, Schistosomiasis, is caused by several inflammatory reactions to different stages of development of the worms within the host, in addition to blood loss, due to adult worms feeding on red blood cells ("WHO — Schistosomiasis" 2017).

Schistosomiasis disproportionately afflicts those living in impoverished regions where suitable gastropod hosts reside ("WHO — Schistosomiasis" 2017). While estimates vary from 190 (Hotez 2018a) to 261 million (Baan et al. 2016) people infected with schistosomes, most infections ($\sim$ 90%) occur in sub-Saharan Africa and most often in rural areas. The urbanization of Schistosomiasis is a recent problem that has begun to emerge due to rapid rates of migration and disordered urbanization in some cities (Dabo et al. 2015; Hotez 2018a). If further migration to urban centers occurs, as projected by 2050, Schistosomiasis may become a greater problem in these areas. Prevalence of Schistosomiasis is strongly correlated with areas of poverty, poor infrastructure, and poor hygiene practices (Hotez 2018a). The primary driver in maintaining schistosome life cycles in these areas is lack of access to clean water. Because schistosomes have two-host life cycles, snails can easily be-

come exposed to new schistosome eggs in areas where human feces and urine contaminates local water sources, continuing the life cycle.

Second to Schistosomiasis, in their impact, are food-borne trematodiases (FBTs) caused by the consumption of (non-schistosome) trematode larval stages in second-intermediate hosts, primarily fish and crustaceans, or encysted stages on vegetables. These infections are largely found in Asia by *Clonorchis* and *Opistorchis* spp.; however, other species like *Paragonimus* spp. reside in central and west Africa, and *Fasciola* spp. have a global distribution ("WHO — Foodborne trematodiases" n.d.). Overall, there are fewer deaths from FBTs ($\sim$ 7,000/year), but there are over 200,000 infections recorded annually from just the four, primary species. Further public health impacts from FBTs are more economic in nature, seen as losses in the agriculture and aquaculture industries, reduced animal productivity, and reductions in exported goods ("WHO — Foodborne trematodiases" n.d.).

For both Schistosomiasis and FBTs, chemotherapy is available to treat infections in people. The most commonly used drug is Praziquantel, which acts in killing adult worms. Unfortunately, this drug does not kill juvenile worms, prevent infection, or eliminate the millions of eggs that can become lodged in other organs and cause granulomas. Schistosomiasis has specifically been targeted by the World Health Organization at the World Health Assembly in 2012, for elimination by 2020, with the primary response being mass chemotherapy ("Sixty-fifth World Health Assembly: Elimination of schistosomiasis" 2012). However, now at 2018, we are nowhere close to elimination (Ross et al. 2017), and need to consider better integrative strategies that examine the determinants of health, tackle the underlying causes of poverty and lack of access to clean water, and develop new ways to prevent transmission.

In contrast, North Americans are rarely affected by parasitic helminths today, as improvements to health and hygiene have greatly decreased the prevalence of common infections in the past century. However, this may be changing, as regions afflicted by high rates of poverty do still suffer from infections that were thought to be eradicated long ago (i.e. Hookworm) (McKenna et al. 2017). Furthermore, examples from the state of Texas recently have connected

poverty, trade, and human migration to a rise in the rates of emerging and neglected tropical diseases within the last five years. The most common disease among these has been Toxocariasis with over 700,000 reported cases (Hotez 2018b).

In terms of trematodes that afflict North Americans today, avian schistosomes, when they are encountered in recreational waters, are the most commonly encountered species. The trematode family Schistosomatidae is composed of approximately 100 species (Sara V Brant and Eric S Loker 2005), of which six species specialize for infecting humans and cause the disease Schistosomiasis. All other schistosome species specialize to infect birds, particularly waterfowl, or mammals, and can have broad geographical distributions, primarily for those infecting migratory species (Sara V Brant and Eric S Loker 2009). With a global distribution, and impacts even in developed countries with advanced infrastructure, cercarial dermatitis—colloquially "swimmer's itch" (now referred)—is a neglected, allergic condition caused by the accidental exposure of humans to non-specific, schistosome cercariae in natural, aquatic ecosystems (Kolárová et al. 2013). Schistosomes that specialize for waterfowl or small mammal hosts produce cercariae, which have the capability to penetrate human skin, but are not successful in causing infections. The penetration of the skin by the cercariae elicits an immune response. The resulting rash is characterized by extreme itching, redness, and swelling around the area of penetration, where a raised, red papule forms. This rash can last anywhere from a few days up to two weeks (Kolárová et al. 2013). The direct health impacts of swimmer's itch are short-lived and the symptoms easily treatable with over-the-counter, topical, anti-itch creams. From a public health perspective, the impact of swimmer's itch should be estimated by impact on the healthcare system, based on doctor's visits to treat symptoms and in diagnosis for those not familiar with swimmer's itch. However, swimmer's itch is not a reportable condition, meaning incidence is not centrally recorded. Therefore, many knowledge gaps exist in our understanding of swimmer's itch prevalence and distribution, and we cannot estimate the downstream costs associated with

the occurrence of outbreaks of swimmer's itch as one could do for a disease like Schistosomiasis.

While the majority of schistosomes are not known to cause true infections within people, swimmer's itch can often result in secondary, bacterial infections, due to scratching, which can be cause for concern. Subsequent exposures to schistosome cercariae have also been known to elevate the degree of the allergic symptoms experienced, often translating into intensified swelling, itching, and discomfort (Petr Horák, Mikeš, et al. 2015). Although not typically thought of as a health risk to healthy individuals, evidence suggests that it may be possible for certain species of schistosome that cause swimmer's itch to go beyond the skin and further develop in small children or immunocompromised individuals. Species, such as *Schistosomatium douthitti*, that normally infect small rodents, have been shown, experimentally, to infect small primates (Malek 1977). An avian schistosome, *Trichobilharzia regent*, that has an affinity for the central nervous system, has been shown, experimentally, to infect both mice and rats (Horák et al. 1999). However, there appear to be no case reports of either species going beyond the dermis in humans.

It has been noted on multiple occasions that the occurrences of swimmer's itch are increasing on a global scale and re-emerging in many areas (Petr Horák and Kolárová 2011; Kolárová et al. 2013; Marszewska, Cichy, Bulantová, et al. 2018). It has been hypothesized that increased cases of swimmer's itch could be due to climate change affecting the host and parasite populations, or the increased use of natural water bodies for recreation. There is great potential for both factors to contribute towards increased transmission (Marszewska, Cichy, Bulantová, et al. 2018). Accurately detecting increases in incidence for a non-reportable condition is difficult enough but determining cause and effect for increases in disease transmission of parasites with complex life cycles can be incredibly difficult to tease apart.

The one common thread among nearly every digenean species is their reliance on aquatic snails as an obligate intermediate host within their life cycle. Snails are required for digenean larval development and transmit the cercarial, free-swimming stage of these parasites. For human exposure, this is the

most important aspect of the life cycle to understand because people are either exposed directly to the cercariae that penetrate the skin, or they ingest the animal of which the cercariae have penetrated and encysted within. Understanding the dynamics of snail communities is a key component to understanding the potential for trematode transmission. Additionally, snails can act as bioindicators, as they are highly sensitive to the presence of pollutants in the environment, and can bioaccumulate toxins, such as microcystins produced by cyanobacteria (Zurawell et al. 2007). Thus, a comprehensive understanding of snail biology, ecology, and their interactions with trematode parasites has the potential to contribute an incredible wealth of knowledge that can be translated into many public health applications.

Specific to the re-emerging issue of swimmer's itch, it has recently been discovered that the presence and abundance of the snail species *Potamopyrgus antipodarum* in lakes in Europe may have a considerable impact on the infection rates of the avian schistosome *T. regent* in its intermediate host *Radix balthica*. In experiments, increased density of *P. antipodarum* greatly reduced infection in *R. balthica* in acting as a decoy. In other words, *T. regent* is unable to successfully establish within *P. antipodarum*, but their miracidia continue to attempt to infect the snail, drawing them away from their natural hosts (Marszewska, Cichy, Bulantová, et al. 2018). While this study is preliminary for field tests, it provides strong evidence for the "decoy-effect", otherwise referred to by ecologists as the "dilution effect" hypothesis and confirms trematode-snail community composition is an important factor for determining infection success of trematodes among their snail hosts (P. T. Johnson, Lund, et al. 2009).

The fundamental core of the dilution effect hypothesis is that greater biodiversity reduces, and therefore regulates, disease transmission. Reduction of transmission opportunities for parasites is more probable if there are greater numbers of non-hosts, or dead-end, species within the environment, as they can act as a barrier, or buffer, between transmitting host species. Biodiversity loss, because of anthropogenic change, can therefore result in selection for the most resilient hosts and pathogens, and leave fewer species available to act as

dead-end/non-transmitting or incompetent hosts. Though a highly debated hypothesis, as to its blanket generality (P. T. Johnson, Preston, Hoverman, Henderson, et al. 2012; P. T. Johnson, Preston, Hoverman, and Richgels 2013; P. T. Johnson and David W Thieltges 2010; Ostfeld and Keesing 2000; Chelsea L. Wood and Kevin D. Lafferty 2013; Chelsea L. Wood, Sandin, et al. 2014), a recent meta-analysis of over 200 effect sizes on 61 parasite species revealed strong support for the dilution effect across a broad ecological context, across broad categories of host-parasite systems, and even across broad study designs (Civitello et al. 2015). While there may be exceptions, this is a strong argument for the importance of maintaining biodiversity.

Considering the global impact of parasites on human health and the underappreciated importance of their contribution to the greater biodiversity on the planet, there is an obvious and necessary reason to more fully understand the diversity and ecology of parasites. There are many potential influences on disease transmission by parasites that we do not fully understand. Fluctuations in parasite populations, regional differences in their community structure and species composition, effects of climate change, rapid urbanization among other anthropogenic impacts, and parasite associations within and among hosts can all affect the rate and probability of disease transmission. In this context, there is a need for interdisciplinary research to connect the pieces and enable a better solution for how we view parasites, both as a natural and important aspect of ecosystems, and as disease-causing agents. Historically, our solutions have been unsustainable, without foresight, and typically utilizing a singular mode of action; combatting Schistosomiasis is a perfect example of this, and one which has recently been criticized as needing to go beyond chemotherapy as a singular mode of action, to incorporate snail control, local health needs, and WASH (WAter, Sanitation, and Hygiene) solutions (Lo et al. 2018).

The aims of my thesis endeavored to provide a better understanding of the underlying connections between trematode diversity and disease transmission potential. I incorporated methods and perspectives of trematode diversity, their associations with intermediate gastropod hosts, insights towards their ecology, community assembly, and community structure, to provide a more

cohesive understanding. I also analyzed how environmental, spatio-temporal, and ecological factors contributed to trematode community structure and composition. Stemming from these analyses, I took a holistic approach towards examining our local public health issue of swimmer's itch in Alberta, with some expansion into greater Canada. I utilized quantitative and qualitative methods to further examine the issue of swimmer's itch, specifically to gain an understanding of the role of schistosomes in trematode communities, and the human perspective of having contracted swimmer's itch to understand where and when it occurs and the effects it might have on future lake use. Furthermore, I discuss the current challenges in the field and areas in need of further research.

1.2 Specific aims and objectives

Broadly, the aim of my thesis was to examine digenean trematode transmission patterns in central Alberta lake ecosystems. The application of this knowledge is meant to inform biodiversity surveys and contribute to our understanding of trematode community ecology. Furthermore, I aimed to apply this information to an analysis on the state of swimmer's itch in Alberta, Canada. The broad aim of my thesis was achieved through two sub-aims discussed below and followed by specific objectives.

1. Examine snail and trematode community composition and patterns over space and time. My broad hypothesis was that snail and trematode communities are structured by environmental and ecological factors that determine presence and persistence of species and maintenance within the community. The broad objectives were to:

    (a) Conduct a comprehensive, longitudinal species survey of digenean trematode larvae from their first-intermediate, freshwater, snail hosts across six lakes in central Alberta over three years from June to September.

(b) Gather relevant metrics of environmental features, including water quality during each collection event.

(c) Identify snails and trematodes to species level using a combination of morphological and molecular barcoding methods.

(d) Examine community structure, diversity, and infection prevalence, and how they fluctuate over time, across samples, lakes, and ecoregions.

(e) Test whether environmental or ecological factors act as important predictors for trematode presence and diversity.

(f) Examine if trends in the transmission of trematode species over time are consistent or predictable.

2. Assess the state of swimmer's itch as a public health risk in Alberta and identify knowledge gaps. The specific objectives within this aim were to:

(a) Develop a feasible method for surveillance of swimmer's itch among lake users across Canada.

(b) Determine whether there are any geographical or temporal patterns that relate to swimmer's itch occurrences.

(c) Identify the etiological agents of swimmer's itch in central Alberta by taxonomic identification of schistosome and snail host species.

(d) Infer potential definitive host species for schistosomes within Alberta and map their distributions.

(e) Identify the knowledge gaps specific to transmission of swimmer's itch in Alberta through assessment of the literature, species surveys and distributions of species relevant to swimmer's itch, public knowledge and opinions, and patterns within trematode community ecology as assessed from the previous aim.

1.3 Outline of the thesis

In this introductory chapter, I have discussed the importance of biodiversity, the neglect of parasites to our understanding of ecosystem services generated by enhanced biodiversity, and the role of parasitic helminths to human health in a global context. I have also discussed our need to have a better understanding of parasite diversity and ecology to comprehend important factors that relate to the complexities of disease transmission. These ideas led to the formation of my broad hypothesis and research questions. The aims and objectives of my thesis were then provided. The following chapters describe specific sections of my thesis work, providing a brief contextual preface, then describing specific background information, methods, results, and conclusions. Each section is followed by conclusions to connect chapters within the broader scope of the thesis.

In chapter 2, I introduce the study region, sampling protocols, and methods used for morphological and molecular analysis for species identification of trematodes and their snail hosts. I further investigate the host-parasite relationships and address hypotheses related to host and parasite richness.

In chapter 3, I investigate a genus of trematode found among the collections described in chapter 2 that exemplifies some current issues in the field, including cryptic species, the need for molecular phylogenetics to delineate species with greater confidence, and limitations in current molecular databases. I describe a new trematode species as a result of improved methods for analysis, compared to those used in the previous chapter. This chapter, therefore, provides an expansion upon the methods from the previous chapter, and provides a proof-of-concept for the work presented in the 4th chapter.

Chapter 4 provides a detailed, species-level molecular phylogeny for each trematode family discovered within my collections, and then describes trematode diversity, host associations, and issues surrounding species delimitation and delineation.

In Chapter 5, I discuss the importance of trematode biodiversity in ecosystems, and summarize the literature to discuss the concepts of community ecol-

ogy and importance of understanding species interactions in disease transmission. I then discuss questions surrounding the processes of trematode community structure and assembly. Furthermore, I examine the effects of environmental and ecological factors on the composition of trematode communities in lakes of central Alberta and how they differ spatio-temporally.

In Chapter 6, I provide an analysis of the state of swimmer's itch in Alberta and greater Canada, providing context for the relationship of the thesis to the local public health issue of concern. This analysis incorporates a historical review of the literature and knowledge of swimmer's itch in Canada prior to my study. This investigation utilizes a mixed-methods approach (quantitative and qualitative analyses) to the surveillance of swimmer's itch in Canada.

In Chapter 7, my thesis is then summarized and placed within the greater philosophical context, with a general discussion of the contributions my work has provided to the field.

Chapter 2

A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada

2.1 Introduction

With global strategic plans in place to both understand and protect natural ecosystems that support our current biodiversity (UNEP 2015), understanding the factors that define species distributions and influence the interactions between species has never been more relevant. By definition, biodiversity studies must consider the entire spectrum of organisms that reflect an ecosystem; however, very often parasites are completely excluded from such large-scale investigations. By leaving out parasites, we not only miss out on true measurements of biodiversity, but neglect their roles within ecosystems; roles demonstrated to significantly affect species interactions, nutrient cycling, food-web topology, and ecosystem stability (reviewed in (Chelsea L Wood and P. T. Johnson 2015)). Therefore, it remains important to continue efforts in surveying the extent of parasite biodiversity, distributions, and interactions within and among

Parts of this chapter were published in Gordy, M.A., Kish, L., Tarrabain, M., and Hanington, P.C.. 2016. "A Comprehensive Survey of Larval Digenean Trematodes and Their Snail Hosts in Central Alberta, Canada." Parasitology Research 115(10): 3867–80. http://www.ncbi.nlm.nih.gov/pubmed/27245072

their hosts, across broad geographic space, to better understand the extent of the roles parasites play across ecosystems (A. Dobson et al. 2008; Whiteman and Parker 2005).

Digenetic trematodes are parasitic Platyhelminthes that have a cosmopolitan distribution and are known, as a group of parasites, to infect the largest diversity of animals (Littlewood and R.A. Bray 2001). There have been $\sim$ 18,000 species of digenean described, yet, very little is known about the species that do not directly impact human health. Nearly all digeneans require a snail as their first intermediate host in order to complete their larval development. Within the snail host, digenean larvae develop and generate a free-swimming stage called a cercaria, which leaves the snail to infect the next host in the life cycle of the parasite (Littlewood and R.A. Bray 2001). Very often, digeneans will display high specificity for their snail host, and this can be driven by both ecological (Donald et al. 2004; Gibson and Bray 1994) and immunological factors (Bayne, Hahn, and Bender 2001; P C Hanington, Forys, and E S Loker 2012; Sapp and E. Loker 2000a; Sapp and E. Loker 2000b). Thus, compatible snails within the environment shape the distribution of digenean trematodes across the landscape, as they are essential for successful transmission of the parasite within an ecosystem.

Often, the focus of digenean studies relates to their effects on, or descriptions within, vertebrate definitive hosts, in which the adult worms reside, or second intermediate hosts, in which larval digeneans encyst in a metacercarial stage to facilitate transmission through food web dynamics. Because digeneans utilize multiple hosts to complete their life cycle, this not only complicates the determination of complete life cycles, for which many are unknown, but can complicate the identification of species as well (Faltýnková, Nasincová, and Kablásková 2008; Gibson 1987). Adult worms have more defined morphological features than their larval counterparts, making them easier to identify within their definitive hosts. However, the use of molecular tools and genetic markers have revealed that cryptic species remain an issue, even at this life cycle stage, and that morphology alone does not reveal complete diversity (reviewed in (Nadler and León 2011)). Genetic barcoding has made linking

digenean larvae to their adult counterparts achievable without the need to experimentally complete the entire life cycle (Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011). The use of these tools, and the current state of the databases that contain this information, though, are arguably in their infancy. As the databases are currently saturated with information relevant to parasites that cause disease in humans and agricultural animals, there is a prevailing need to populate them with information from a more diverse and representative breadth of species. This is relevant to identifications of cryptic or novel species, to delineating digenean life cycles and distributions, and towards answering a plethora of evolutionary questions including those of host-parasite interactions; not to mention, relevance towards parasite conservation as well (Besansky, Severson, and Ferdig 2003; A. Dobson et al. 2008; Kevin D Lafferty et al. 2008; Whiteman and Parker 2005).

To put into perspective the relative prior knowledge of invertebrate biodiversity relevant to this study, the most recent published collections of aquatic invertebrate species surveys of Alberta were gathered in the late 1980s. These efforts resulted in the textbook publication by Hugh F. Clifford in 1991, "Aquatic Invertebrates of Alberta" in which some snail and trematode species were mentioned (Clifford 1991), but their relationships to each other, distributions, and pervasiveness were not explored. In 2004, a survey specific to gastropods within the Central Parkland Subregion of Alberta was conducted by the Fish and Wildlife Division of Alberta Sustainable Resource Development. Unfortunately, snail species were only identified by shell morphology (Prescott and Curteanu 2004), which may not represent true diversity (Gustafson et al. 2014). Several trematode species have been identified previously in Alberta from various second intermediate and definitive host species, and a few from the snail first intermediate host. These reports, however, were by no means comprehensive, nor expansive. A major goal for this study was, therefore, to add to the understanding of the biodiversity of snails and trematodes in Alberta, and to provide a baseline for comparison to other regions.

In Canada, the majority of digenean species descriptions have come from fish, such that there is even a "Guide to the Parasites of Fishes in Canada"

(Cribb 1997), with a special section for the Trematoda. Guides such as this are lacking for other animals. In the few parasite surveys conducted in Alberta, there have been reports of adult digeneans in birds (Albert O Bush and John C Holmes 1986; Hair and J. Holmes 1970; Palmieri 1973; Ramalingam and W. Samuel 1978; Stock and J. Holmes 1988; Vermeer 1969), larval metacercariae in fish (Baldwin and Goater 2003; Leong and J. Holmes 1981; Schleppe and Goater 2004; Zelmer and Arai 1998), and a few adults in frogs (Bursey and Goldberg 1998; Goldberg, Bursey, and Wong 2002) and mammals (Giebelhaus, Kennedy, and Moraiko 1998; Králová-Hromadová et al. 2011; W. M. Samuel, Barrett, and Lynch 1976). Only five studies describe the relationships between digeneans and snail hosts within Alberta (Morris et al. 1982; Sankurathri and John C Holmes 1976; Schleppe and Goater 2004; Shostak, Dharampaul, and Belosevic 1993), and only one has, thus far, extensively examined the breadth of these relationships over a long period of time (Sankurathri and John C Holmes 1976). However, within that study, only one snail species was analyzed for its associations with larval digeneans, within only a single lake.

In order to gain a more comprehensive view of the breadth of digenean-snail relationships within Albertan lake ecosystems, to both understand biodiversity and compatible associations, a longitudinal species survey was conducted over two years, spanning six different lakes, among three major ecoregions. Here, the presence and distribution of 39 digenetic trematode species and their associations among 5 snail species as intermediate hosts are reported from the surveyed lakes. Many of these digeneans were identified by sequencing a region of the mitochondrial gene, cytochrome *c* oxidase subunit 1 (*cox1*), and have not been reported in Alberta prior to this study. A broad analysis is provided to explore how infection prevalence among certain snail species changes over the course of the summer, which has further implications towards understanding the dynamics between these parasites and their definitive hosts. Likewise, as many digeneans are known to be highly specific in their compatibility for their snail intermediate host, the longitudinal nature of the collections for this survey allows for a more extensive view of these associations as opposed to one-sample-point collections that may miss them due to developmental or

definitive host timing. This survey provides new links for digenean trematode life cycles within Alberta and allows for further investigation into the structure of these communities and the dynamic processes that shape and change them over time and space.

2.2 Material and methods

2.2.1 Study sites and collection procedures

Over a two-year period (2013-2014), snails were collected from four lakes on a biweekly basis from June to September, with one collection site at each lake: Isle (site 3), Wabamun (site 4), Gull (site 8), and Buffalo (site 9) Lakes. In each year, one additional lake was added and sampled biweekly: 2013 – Lac la Nonne (2 sites: 1 & 2), 2014 – Pigeon Lake (3 sites: 5-7). In 2014, additional sites (10 & 11) were added for collections at Buffalo Lake to account for its larger size (Table 2.1 & Figure 2.1). Because each of these lakes was surrounded by private properties, collecting sites were chosen based on accessibility. All collecting sites were in shallow water, no more than 1-meter-deep, and went from the shoreline to no more than 3 meters out from shore. The expanse of each collection site differed by length, depending on whether it was an open beach area, in which the beach was sampled as well as the areas of vegetation directly adjacent to them, or a boat launch, which was normally surrounded by dense vegetation on either side and thus provided a narrower collecting area.

The goal was to collect 300 snails from each site at each sampling time, but the final number varied depending on relative snail density at each collection time. Therefore, time was used as a secondary collecting metric in addition to snail numbers, with collecting times not exceeding one hour at each site. Snails were gathered, either by handpicking them with blunt insect forceps, or by scraping around the vegetation, rocks, and lake bottom with a handheld metal sieve. Snails were temporarily placed in plastic containers with lake water and paper towels for transport, and then brought back to the laboratory for processing. Snails were then transferred to larger plastic tanks with artificial

spring water (ASW) (Ulmer 1970) and a piece of red leaf lettuce. The day after collecting, each snail was wiped down with a paper towel and 70% ethanol and then placed in a multi-well culture plate containing ASW and left on the bench top under fluorescent lighting. The laboratory was kept at a 12-hour light/dark cycle. Over a 24-hour period, all snails were examined twice for patent digenean infections by looking for the presence of cercariae in the ASW under a dissecting microscope.

All infected snails were photographed against a gridded background (graph paper with 5mm x 5mm squares) for later measurements and then placed in 15-50ml conical tubes containing 100% ethanol and stored at -20°C. The digenean cercariae were collected in 2ml self-standing, screw-cap tubes with o-rings and filled with 100% ethanol. Because ASW transferred as well, cercariae were allowed to settle to the bottom of the tube, all but 50ul of liquid was removed, and the tube was refilled with 100% ethanol and stored at -20°C. In 2014, cercariae were also collected into 1.5ml tubes with 100ul ASW and 100ul of RNAlater® Stabilization Solution (Life Technologies, cat. No. AM7024) (50% final concentration), stored at 4°C, to improve the DNA extraction results.

2.2.2 Species identification

Snails and digeneans were identified using a combination of morphological and molecular methods. Snails were first identified to family based on shell morphology and past reports in Alberta using the Aquatic Invertebrates of Alberta as a guide (Clifford 1991). Digenean cercaria morphology was categorically typed by referencing the Handbook of Trematodes of North America North of Mexico (Schell 1985). In addition, digeneans that had been previously reported in Alberta were used as a reference point to guide initial identifications. Considering that morphological typing of snails and larval digeneans can be inaccurate (Detwiler, Bos, and Minchella 2010; Gustafson et al. 2014; Morningstar et al. 2014), largely due to the likelihood of cryptic species and inconsistent estimates of species numbers, emphasis was placed on sequencing of the cytochrome *c* oxidase subunit 1 gene (*cox1*) for comparison to specimens in GenBank. *Cox1* was selected because of its wide use as a barcoding

gene for Platyhelminthes in previous studies (Moszczynska et al. 2009; Van Steenkiste et al. 2014) and the availability of sequences within GenBank for comparison with snail specimens as well.

2.2.3 Larval trematode morphometrics

Representative images of the cercariae were taken with a Zeiss Axio Imager.A2 compound microscope and mounted Zeiss AxioCam MRc camera based on general morphology and swimming behaviours to distinguish between them. Several cercariae, representing broad morphological types, were wet mounted onto microscope slides and either photographed while in the resting state, or if they were too active, were heat killed by waving the microscope slide over an electric Bunsen burner briefly, then photographed (Figure 2.2). As most of the cercariae were still alive while photographed, and thus moved and stretched, we took several pictures of different cercariae of the same type to get a measurement range. Cercariae morphological features were measured using the Zen software (Zeiss) and measurement tools. Measurements were taken of length and width of the cercaria body, tail, and if present, furcae, and then ratios were calculated for body length:width, length of tail:body, and length of tail:furcae (Table 2.2).

2.2.4 DNA extraction and sequencing

Snails

Snails were randomly selected for molecular analysis based on their morphological characterization. Because only infected snails were preserved, all molecular data is derived from infected individuals. Uninfected snails were only characterized to family, and in some cases genera, based on morphology. DNA was extracted from infected representatives of Lymnaeid, Planorbid, and Physid snails using a small piece of ethanol-fixed, headfoot tissue using the E.Z.N.A.® Mollusc DNA Kit according to the manufacturer's instructions (Omega Bio-Tek, cat. No. D3373-02). Partial *cox1* fragments were amplified by PCR in 25ul total reaction volumes containing DreamTaq Green PCR Mastermix (2X)

(Thermo Scientific, cat. No. K1081), 25pmol of each primer (Folmer region) (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'- TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994), and >100ng of whole genomic DNA (gDNA). Amplification was achieved using the following thermocycler protocol: initial DNA denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, primer annealing at 50°C for 45 sec., and primer extension at 72°C for 1 min., followed by a final extension at 72°C for 5 min. The 650bp amplicons were then electrophoresed in 2% Agarose and purified using the GeneJet Gel Extraction Kit (Thermo Scientific, cat. No. K0692). Purified amplicons were sent to Macrogen Inc. (Korea) for Sanger sequencing, in which the same PCR primers were used to amplify both strands.

Digenean trematodes

The methods for tissue preparation prior to DNA extraction from digenean cercariae were slightly different, depending on the preservation method. For ethanol-preserved samples, 10 or more cercariae were transferred to a 1.5ml conical tube and placed in a vacuum centrifuge for 10 min. to evaporate the ethanol prior to extraction. For samples preserved in 50% RNAlater (containing a minimum of 10 cercariae), a modified protocol from Webster (2009) was used and samples were diluted further by adding 500ul of water, then vortexed and subsequently centrifuged at 16,000 x g for 5 min., then all liquid was aspirated with a pipettor, leaving a pellet of cercariae at the bottom.

Whole gDNA was extracted using the Webster method (Webster 2009) and the DNeasy® Blood & Tissue Kit (Qiagen, cat. No. 69504) with slight modifications: adding an RNAse step, by adding 1ul of RNAse A (Omega BioTek, cat. No. D3373-02) after tissue digestion was complete, vortexed and incubated samples at room temperature for 2 min. before moving on to the precipitation steps.

Partial *cox1* mitochondrial sequences were generated according to Moszczynska et al. (2009), using the primers MplatCOX1dF and MplatCOX1dR for amplicon generation and shortened regions for sequencing as specified in Moszczyn-

ska et al. (2009) (Table 2.3). PCR was performed in 25ul volumes using the AccuStart^{TM} II PCR SuperMix (Quanta BioSciences, Inc., cat. No. 95137-500), 12.5pmol of each primer (Table 2.3), and >50ng gDNA. Amplicon purification and sequencing was performed in the same manner as for the snail *cox1* amplicons as stated above. These methods did not work well for Schistosomatidae cercariae, nor for certain other digenean samples. Therefore, a second method, as described in Van Steenkiste et al. (2014b), using primers Dice1F and Dice11R as well as the *Trichobilharzia* specific primers CO1F15 and CO1R15 from Brant and Loker (2009) were used to generate amplicons for sequencing when the initial approach was unsuccessful.

For *Petasiger* sp., representative *cox1* sequences had not previously been submitted to the GenBank database, thus 28s and NADH (Nicotinamide Adenine Dinucleotide) dehydrogenase subunit 1 (nad1) gene fragments were sequenced instead and used for all comparison purposes. Primers and thermocycling conditions were derived from Selbach et al. (2014). PCR was performed as previously stated, but using only 10pmol of each primer per reaction.

2.2.5 Alignments and species delineation

All sequences were first trimmed and analyzed for quality using the 4peaks (Nucleobytes) software, then transferred to Geneious version (6.1.6) (http://www.geneious.com, (Kearse et al. 2012)) for alignment of forward and reverse sequences. Consensus sequences were then used to search the NCBI BLASTn database, and the submissions with the highest percent nucleotide identity were retrieved and used to create alignments and percent identity matrices. A 5% nucleotide divergence of *cox1* sequences was used as a cut-off value and metric for species delineation for both snails and digeneans. This threshold was chosen as a conservative standard, based on previous findings in the literature that reveal a maximum intraspecific mtDNA divergence of 0.3-2.2% (Vilas, Criscione, and Blouin 2005) for digeneans (with a range of 3.9-25% divergence between congeners) (Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010; Moszczynska et al. 2009), and the noted 'typical 5% divergence' between mollusc species (Lawton et al. 2015). If more than 5%

divergence was found, the GenBank sequence with the highest percent identity match was used to identify the closest related species available in the database (specified by an asterisk throughout the text in this chapter). A representative sequence from each species of snail and digenean identified in this study was submitted to GenBank under accession numbers KT831342-KT831388.

2.2.6 Species richness and infection prevalence

Cumulative species richness was calculated by recording the number of snail and digenean species at each lake (subsites combined) over the entire course of the survey. Additionally, digenean species richness was evaluated separately among each snail host species across all lakes. To assess the correlation between cumulative host and parasite species richness, pooled across years, a basic regression analysis was completed using the Microsoft Excel 2013 Analysis ToolPak.

A Generalized Linear Model (GLM) with a quasibinomial distribution was used to assess and compare the overall contributions of each snail host type (by family) to digenean infection prevalence. Infection prevalence was evaluated as untransformed, proportional data (the proportion of infected to uninfected snails) pooled by lake (for Buffalo, Gull, Isle, and Wabamun) and year. Snail species were pooled by family to account for species that were found in lower numbers, but contributed to infection prevalence, and because uninfected snails were only characterized to this level. The response variable in the model was prevalence (proportion of infected-to-uninfected) and the explanatory variable was snail family (Lymnaeid, Physid or Planorbid). This model was tested against a null model, where the explanatory variable was set equal to one. An F-test was used to compare the two models and test for a significant difference between them. Using the R package *multcomp* (Hothorn, Bretz, and Westfall 2008), Tukey Contrasts were used as a multi-comparison method to test the main effects of snail type on infection prevalence and to derive associated confidence intervals. An alpha level of 0.05 was used for all statistical tests. All of the above analyses were performed using R (R Core Team 2015).

2.3 Results

2.3.1 Species and combinations

Snail species and familial distributions

Five snail species were identified in this survey from samples of infected individuals: *Lymnaea stagnalis*, *Stagnicola elodes*, *Physella gyrina*, *Helisoma trivolvis*, and *Planorbula armigera** (Table 2.4). Wabamun and Buffalo Lake (site 11) were the only lakes in this study at which all species of snail were found (Figure 2.1). The majority of snails found at most collecting sites were Lymnaeids, with the majority represented by the species *S. elodes*. The second most prominent snail type found were Physids, which were the most abundant snail type found at Wabamun Lake (site 4) and Pigeon Lake (sites 5 and 6). Planorbid snails (combining both *Helisoma* and small, unidentified Planorbid species) were present at most sites and lakes, except at Pigeon Lake and Buffalo Lake (sites 9 and 10). Planorbids only made up large proportions of the overall collected snail populations at two sites, 4 and 11 (summarized in Table 2.5).

Infection prevalence

In 2013, 8,910 snails were collected, of which 1,175 (13%) had patent digenean infections. In 2014, 4,269 snails were collected and 602 (14%) had patent digenean infections. The main effect of snail family on pooled infection prevalence was significant ($F(18,20) = 6.2946$, $P = .0085$). Mean infection prevalence was significantly different between Lymnaeids and Physids (Tukey Post-Hoc, $P = 0.0239$, 95% CI for Physid-Lymnaeid [-3.1863, -0.1777]), but not for other comparisons among the three snail families (Figure 2.3). Lymnaeids had the highest pooled infection prevalence, and *S. elodes* contributed most to overall infections among those sampled (Figure 2.4).

Overall snail infection prevalence varied over the course of the survey. In 2013, peak infection prevalence was observed in July with as high as 64% (Lac La Nonne) of all snails collected harboring at least one patent digenean infection. A second peak observed in late August and early September was

also prominent at each lake. The bimodal infection prevalence of 2013 was contrasted in 2014, when peak infection prevalence was less consistent between lakes and was observed predominantly in late August (maximum of ~74% at Isle Lake) (Table 2.6).

Trematode species and distributions

Based on representative *cox1* sequencing of 384 samples combined from 2013 and 2014 (1777 total), 9 families of digenean trematode were identified, represented by 24 genera, and comprising 39 species. It was difficult to identify many species definitively using morphology alone; thus, DNA sequence information was used as a means to complement morphological assessments. Using this approach revealed much more species diversity than what was identified when considering morphology alone. Therefore, not every species represented by genetic data has corresponding morphometric data available at this time (Table 2.2 and Figure 2.2).

Sequence homologies between the *cox1* sequences of this study and their most similar GenBank entries, as detailed in Table 2.5, ranged from 74.7-100% nucleotide identity. Using this molecular approach, species were only identified with a confidence level equal to that of their nucleotide identity, because only a small fraction of digenean trematode diversity is represented within the current GenBank database at this time. As a result, many samples could only be characterized to their genus because of a lack of a clear definition for species delineation using *cox1* in many digenean families, and because there is not enough genetic information to define a species based solely on *cox1* barcoding in all cases. Moreover, often those samples that could be identified to a species level tended to have more numerous and robust sequence information available in the GenBank database, clarifying the distinction.

Digenean species richness was highest at Wabamun Lake and Buffalo Lake (combined sites PP, RS, and TN) with 19 species, followed by Lake Isle with 16, Gull Lake with 15, Lac La Nonne (combined sites LLN1 and LLN2) with 12, and finally Pigeon Lake with 3 trematode species (Table 2.7). The less frequent collecting at Lac La Nonne and Pigeon Lake may account for the

lower than expected trematode species richness at these lakes. The cumulative species richness at each lake revealed a strong positive correlation between host (snail) and parasite (trematode) species richness (corr. coeff. = 0.908, df = 4, $P = 0.012$) (Figure 2.5).

Evaluating the digenean species shared across lakes revealed that each lake possessed at least one unique species, while several lakes shared species in common. Lake Isle, Wabamun, and Buffalo each possessed 5 unique digenean species, whereas Gull Lake had 4 unique species. There were 2 species shared between the Western lakes, Wabamun and Isle, and these were *Pseudopsilostoma varium** and *Ichthyocotylurus* sp.3*. There was only one shared species between the Southern lakes, Buffalo and Gull, and this was *Trichobilharzia szidati*. Three species were shared between Wabamun and Buffalo, the two lakes with the highest species richness, and these were *Apharyngostrigea pipientis**, *Diplostomum* sp.2*, and *Ornithodiplostomum* sp.4*. One species, *Hypoderaeum* sp., was shared between Gull, Buffalo, and Wabamun, but not found at Isle. One species, *Petasiger* sp.4, was shared between Buffalo, Wabamun, and Isle lakes, but not found at Gull Lake. Seven species were common to all four lakes, and these were *Australapatemon burti**, *Cotylurus gallinulae**, *Diplostomum* sp.4, *Echinostoma caproni**, *Echinostoma trivolvis**, *Notocotylus* sp., and *Plagiorchis* sp. (Figure 2.6A).

Snail-trematode combinations

Cumulative digenean species richness, by snail species, revealed *S. elodes,* as being infected by the greatest number of digeneans; 25 different species were associated with *S. elodes.* This was followed by *P. gyrina,* cumulatively infected with 13 species, *H. trivolvis* with 12, *L. stagnalis* with 7, and finally *P. armigera** was found to harbor only a single digenean species. It is worth noting that 15 of the 25 different species of digenean associated with *S. elodes* were unique to it, never observed in any of the other snail species (Table 2.7 and Figure 2.7).

To date, 60 different combinations of snail and digenean trematode were uncovered through this survey (Figure 2.7). Twenty-nine out of the 39 species

(74%) of the observed digeneans emerged from only a single snail species and were thus characterized as being snail host specialists. The remaining 10 species (26%) emerged from more than one snail host species, and were considered snail host generalists. However, digenean species that infected more than one snail species (generalists) were observed more frequently than those found in only one snail species (specialists). Among the specialists, there were 15 unique to *S. elodes*, 2 to *L. stagnalis*, 6 to *H. trivolvis*, 5 to *P. gyrina,* and 1 unique digenean infection within *P. armigera**, which was by *Neopetasiger islandicus*. Among the digeneans infecting more than one snail species, there was one shared between the Lymnaeids, which was *Schistosomatium douthitti**. One species was shared between *H. trivolvis* and *S. elodes*, which was *Diplostomum* sp.8*. Two echinostomes species, *Echinostoma caproni** and *Echinostoma* sp. were shared between *S. elodes* and *P. gyrina*. Shared between three snail species were *Hypoderaeum* sp., *Notocotylus* sp. (between *S. elodes, P. gyrina,* and *H. trivolvis*), and *Cotylurus gallinulae** (between *L. stagnalis, S. elodes,* and *P. gyrina*), the latter two being common to all lakes. Finally, there were 3 species shared across all snails but *P. armigera**, including *Australapatemon burti*, Echinostoma trivolvis*,* and *Plagiorchis* sp. that were also common to all lakes (Figure 2.6B).

When considered based on genus rather than species, incorporated data from *cox1* sequencing and morphology identifies *Plagiorchis* sp. as the most prevalent digenean within this study, comprising 58% (1,031 infections) of all snail infections. Interestingly, *Plagiorchis* sp. seems to be common among co-infections. Four snails in each year were collected that had patent co-infections, with two different digenean species actively emerging. In all but one case of co-infection, one of the digenean species was a *Plagiorchis* sp. In 2013, an *L. stagnalis* from Gull Lake was co-infected with a *Plagiorchis* sp. and a *Strigeidae* gen., at Lake Isle, an *S. elodes* was co-infected with a *Trichobilharzia stagnicolae* and a *Plagiorchis* sp., at Lac La Nonne, site 2, a *P. gyrina* was co-infected with *Cotylurus gallinulae** and an Echinostomatidae gen., and at Buffalo Lake, an *S. elodes* was co-infected with two morphologically different *Plagiorchis* sp. In 2014, three *S. elodes* from Lake Isle were co-infected with

a *Plagiorchis* sp. and one with *T. stagnicolae*, one with a *Trichobilharzia* sp., and one with a Strigeidae gen. The other snail from 2014 was an *S. elodes* from Gull Lake co-infected with a *Plagiorchis* sp. and *Schistosomatium douthitti**.

2.4 Discussion

The results from this comprehensive survey have revealed an exceptional diversity of digenean trematodes among relatively few snail host species. This survey revealed 39 digenean trematode species to be associated among five snail intermediate hosts within six lakes in central Alberta. For many of the digenean species recovered, there have not been previous reports from any host species in Alberta, adding to the known overall parasite biodiversity in this province (Appendix A). As the majority of digenean species reported in Alberta have been from second intermediate or definitive hosts, the revelation of snail intermediate host associations through this study has provided a crucial connection for digenean life cycles in this region.

The use of both morphological and molecular methods for species identification was important to the success of revealing the level of biodiversity found. Molecular methods, in particular, have proven to be most essential to the process for species identification among larval trematodes in this study. However, there remain challenges in identifying larval specimens to the species level, as a result of a great lack of available sequences to compare among in the databases. Barcoding, in general, suffers from the fact that ~90% of biodiversity comes from undescribed, unknown species (Rubinoff, Cameron, and Will 2006). Therefore, until those species have representative molecular data recorded in databases, we must still rely on other methods for identification, such as morphological characterization. As barcoding gains in popularity, there will be a greater advantage in determining the level of sequence divergence in *cox1* that is appropriate for each family, genera, or even species, a value that is widely unknown for many families within the Digenea, and an ongoing challenge for cryptic species (Simona Georgieva, Faltýnková, et al. 2014; Georgieva et al. 2013). Likewise, having more sequences generated from

adult specimens, from which greater morphological separation is possible, will greatly benefit the creation of links between larval and adult digeneans. Addressing these challenges with further sequence information, sampling, and surveys of adult digeneans will likely improve future measurements of true biodiversity in this region of Canada and elsewhere.

Beyond new location records and new host associations, this survey has also revealed patterns of spatial heterogeneity and host-parasite interactions that may be of importance towards understanding local community dynamics of snail and digenean species. The present study shows that cumulative host and parasite species richness is strongly correlated, further supporting the hypothesis that increased host richness leads to increased parasite richness through greater colonization ability (Hechinger and Kevin D Lafferty 2005; Hudson, A. P. Dobson, and Kevin D Lafferty 2006; P. T. Johnson, Preston, Hoverman, and LaFonte 2013). Lakes supporting a greater diversity of snails, in particular Wabamun and Buffalo Lakes, supported the largest diversity of trematode species (19 each), as compared to all other lakes that had both lower snail and trematode diversity.

However, other evidence suggests that it may not be increased diversity of snail hosts, in general, that drives increased trematode species richness, but rather driven more by the particular composition of snail species present, abundance of those species, and the degree of host specialization among digeneans. Despite the high snail diversity at Gull, Isle, and Buffalo lakes, *S. elodes* was far more likely to be infected than any other snail species and harbored 25 different digenean species (15 specialists and 10 generalists). *P. gyrina* had the lowest overall infection prevalence (pooled across lakes) compared to all other snail hosts in the survey, however, it was able to host the second highest diversity of digenean species (n = 13). *H. trivolvis* was found to host 12 digenean species, but had a higher observed infection prevalence (pooled across lakes) than *P. gyrina*. Though known in other parts of the world to host a large cumulative diversity of digeneans, *L. stagnalis* (Loy and Haas 2001), surprisingly, only hosted 7 different digenean species among all the lakes sampled. In contrast to this trend, the composition of the infected

snail population at Wabamun lake displayed a strong temporal relationship with respect to what species of snail carried the brunt of the patent infections. In late June-early July, *H. trivolvis* was the primary infected snail species, and over the season, Physids became incorporated in July and followed shortly by Lymnaeids, which had the most infections at the end of the season. This trend was very similar in both years, indicating that there may be important temporal definitive host dynamics driving this trend at Wabamun Lake.

The presence of *S. elodes* snails seems to play a large role in the capacity for more diverse assemblages of trematode species. In addition to hosting the largest cumulative diversity of digeneans and comprising the highest infection prevalence within this survey, *S. elodes* was also the most abundant snail at most of the collecting sites (representing majority of Lymnaeids in Table 2.5). The abundance of *S. elodes* and association with a diverse assemblage of digenean trematodes, primarily specialists, may be explained by patterns of local adaptation that have been observed in many others systems (Diego P. Vazquez et al. 2005; D. Vazquez et al. 2007). Simply, that host specialization in species interactions is highly asymmetric, and the general pattern it follows is that hosts with high parasite richness are often parasitized most by specialists, and hosts with low parasite richness are often parasitized by generalists (Diego P. Vazquez et al. 2005). The abundance-asymmetry hypothesis suggests that abundant species have more interactions with rare species that tend to be more specialized than do less abundant species (D. Vazquez et al. 2007). This idea is based on the probability of interactions, such that a more abundant host species has a greater chance of interacting with a rare parasite than a less abundant host species does. While in this survey, the *S. elodes* data support this hypothesis, in stark contrast, the second-most abundant species, *P. gyrina*, does not. Despite *P. gyrina* having high parasite richness (n = 13), the generalists outnumber the specialists 11-to-2. The less abundant snail species, *H. trivolvis* and *L. stagnalis*, expected to have more generalist parasites than specialists, also portray contrasting results, in that *L. stagnalis* fits the expected trend (5 generalists to 2 specialists), but *H. trivolvis* has an equal number of specialists and generalists (6:6). This suggests either that

there is a threshold of abundance to specialization that results in the expected asymmetrical pattern, or that there are other significant interactions at play to drive these observed differences. There are several factors that could alter such a prediction, in that there may be variance in host susceptibility or interactions between host species that alter distributions and chances of interactions with a larger number of rare species. Therefore, while having a broader diversity of available snail hosts in a location may promote overall greater trematode diversity, it is important to consider both the relative susceptibility of the snail hosts and the definitive host diversity in the area, as they are likely playing important roles in driving the host-parasite richness correlation.

From a mechanistic compatibility perspective, very little is known about *S. elodes,* except that this snail has been used in immunological studies to examine the ability of haemocytes to encapsulate larvae of various digenean species (Sapp and E. Loker 2000b), finding no difference between their success and that of other snails. *S. elodes* has not been the focus of any studies, so far, by which particular mechanisms have been revealed that may set them apart from any other snail species in their susceptibility to digenean infection. Clearly, within the context of Alberta, it seems a relevant avenue to further study the host-parasite interactions occurring between this snail species and various digeneans that infect it, to understand the dynamics underlying the transmission of digenean trematodes within Albertan lake ecosystems.

With regard to digenean distribution, the most abundant genus found was *Plagiorchis* Lühe 1899, and because of this abundance, it is likely to have an important role in local trematode community dynamics. A *Plagiorchis* sp. was found to infect all but one of the surveyed snail species, *Planorbula armigera**. Making up 58% of the entire snail infections assessed in this study, *Plagiorchis* sp. is one of the few digeneans found at every site and lake. Interestingly, *Plagiorchis* sp. may be playing a role in co-infections within these digenean communities, as all but one co-infection found includes *Plagiorchis* sp. as one of the two infecting digeneans. Co-infections do not seem to occur very frequently in the communities sampled, and because of such low numbers, it is difficult to assess the importance of *Plagiorchis* sp. to this process, but it is

an intriguing idea in need of further exploration. Without yet knowing the exact species, it is difficult to discern infection dynamics from the perspective of other hosts. Though considerable work has been done to describe some species, particularly *Plagiorchis vespertilionis* that infects European bats (V. Tkach, Pawlowski, and Sharpilo 2000), there is a strong need to further our collective efforts and understanding of the species within this genus. *Plagiorchis* sp. are known to have high morphological variability and low host specificity (V. Tkach, Pawlowski, and Sharpilo 2000), as evidenced by the classic work of Blankespoor (Harvey D. Blankespoor 1974) on adult *Plagiorchis noblei,* which makes them difficult to identify without the use of molecular data. Despite the known association between *Plagiorchis elegans* and *S. elodes*, previously collected from Quebec (Lowenberger and M. Rau 1994), the sequences from specimens in this study did not closely match any *P. elegans cox1* sequences in GenBank. As *Plagiorchis* spp.utilize insect second intermediate hosts and commonly infect birds and mammals (Vasyl V. Tkach 2008), without sampling among these potential other hosts, it will remain difficult to identify this species that exists so commonly in this region of Alberta.

2.5 Conclusions

In summary, this study provides a comprehensive analysis of snails and their compatible digenean trematodes within central Alberta. The number of digenean species identified as part of this study vastly increases our collective understanding of the diversity and abundance of digenean trematodes that likely reflect a majority of the species that are present in Western Canadian freshwater ecosystems. The longitudinal nature of this dataset will facilitate myriad future investigations into the dynamics of how both snail and digenean communities change over time in these ecosystems, and importantly, provides much needed information related to the contribution of digenean parasites to overall ecosystem biodiversity in Alberta wetlands.

Finally, this study has established the groundwork for the investigations to follow in this thesis. Working in natural ecosystems presents many challenges

not experienced in the laboratory. It takes time to establish appropriate locations for sampling, sampling methods, the consideration of the environment and the natural fluctuations experienced because of weather and unforeseen circumstances, among other reasons. The first two years of field work, described in this chapter, presented several challenges, one of the greatest challenges being the rare capture of snails from any of the three major beaches at Pigeon Lake. As the survey continued, sampling locations at Pigeon Lake had to be dropped from the study for feasibility and to focus on those for which snails were consistently found. The continuation of the longitudinal survey and analyses of snail and trematode community composition is further discussed in chapters 4 and 5.

Taking this study from the field to the lab, for the purpose of species identifications, presented another major challenge. As discussed previously, there are many challenges surrounding the use of molecular barcoding for species identifications. After publishing this chapter, it was discovered that cryptic species may be an issue in need of further consideration. The investigation discussed in the next chapter was fueled by this very issue: a morphologically odd and unique cercaria was revealed to be the same species as another very common morphotype by BLAST results. The differences in morphology and behavior between the cercariae required further investigation.

Table 2.1: Collection location descriptions and years sampled.

Lake/ River Basin	**Site Name/Type**	**Year(s) Sampled**	**GPS Coordinates**	**Trophic Status/ Ecoregion**	**Surface Area (km2)/ Mean Depth(m)**
Isle Lake/ N. Saskatchewan	Unknown/ Boat Launch	2013-2014	53°38'4.19"N, 114°39'53.58"W	Hypereutrophic/ Boreal Forest	23/4.1
Wabamun Lake/ N. Saskatchewan	Provincial Park/ Beach	2013-2014	53°33'36.62"N, 114°26'23.74"W	Eutrophic/ Boreal Forest	81.8/6.3
Lac La Nonne/ Athabasca	Site#1: Unknown/ Boat Launch	2013	53°56'55.54"N, 114°21'38.46"W	Hypereutrophic/ Boreal Forest	11.8/7.8
	Site#2: Unknown/ Boat Launch	2013	53°54'40.49"N, 114°16'54.16"W		
Pigeon Lake/ N. Saskatchewan	Provincial Park/ Beach	2014	53° 1'36.73"N, 114° 7'35.26"W	Eutrophic/ Boreal Forest	96.7/6.2
	Silver Beach/ Beach	2014	53° 2'37.29"N, 113°59'38.87"W		
	Ma-Me-O/ Beach	2014	52°58'26.52"N, 113°57'52.42"W		
Gull Lake/ S. Saskatchewan-Red Deer Sub Basin	Aspen Beach Provincial Park/ Beach	2013-2014	52°27'31.50"N, 113°58'21.90"W	Eutrophic/ Mixed:Aspen Parkland & Boreal Mixed Wood	80.6/5.4
Buffalo Lake/ S. Saskatchewan- Red Deer Sub Basin	Pelican Point/ Beach	2013-2014	52°31'19.63"N, 112°49'54.15"W	Eutrophic/ Aspen Parkland	93.5/2.8
	Rochon Sands/ Beach	2014	52°27'49.81"N, 112°53'3.78"W		
	The Narrows Provincial Recreational Area/ Boat Launch	2014	52°27'6.07"N, 113° 3'18.83"W		

Table 2.2: Cercariae morphometrics

Trematode Species (No./sp./host type)	Length Body	Width Body	Length Tail	Width Tail	Length Furcae	Width Furcae	Body Length: Width	Length Tail : Body	Length Tail : Furcae	Accession #	Fig. 3	Snail Intermediate Host
Apatemon sp. 1*	-	-	-	-	-	-	-	-	-	KT831359	-	
*Apharyngostrigea pipientis** (n=1)	180.132	56.821	273.348	38.697	292.123	27.363	3.17	1.52	0.94	KT831377	U	*Helisoma trivolvis*
										-		
*Australapatemon burti** (n=1)	169.112	50.928	142.235	36.908	198.285	17.99	3.32	0.84	0.72	-	T	*Lymnaea stagnalis*
*Australapatemon burti** (n=2)	61.697-	40.639-	89.948-	35.512-	142.336-	18.523-	1.59-	1.46-	0.63-	-	T	*Physella gyrina*
	113.515	71.551	189.057	38.997	213.5	21.316	1.72	1.67	0.89			
*Australapatemon burti** (n=14)	74.417-	39.163-	85.264-	27.154-	133.223-	12.519-	0.9-	1.05-	0.64-	KT831351	T	*Stagnicola elodes*
	158.152	71.277	162.09	44.338	198.169	21.789	221	1.15	0.82			
Bolbophorus sp. (n=1)	212.014	33.205	288.029	39.104	299.187	29.24	6.39	1.36	0.96	KT831373	K	*Helisoma trivolvis*
*Cotylurus gallinulae** (n=1)	277.361	33.533	254.083	38.258	257.226	26.675	8.27	0.92	0.99	-	S	*Lymnaea stagnalis*
*Cotylurus gallinulae** (n=11)	115.852-	38.35-	173.37-	31.667-	210.826-	16.405-	3.02-	0.83-	0.75-	KT831347	S	*Stagnicola elodes*
	286.321	91.299	237.295	41.675	315.488	26.16	3.14	1.5	0.82			
Diplostomum baeri (n=1)	160.05	44.784	260.697	35.301	288.511	18.114	3.57	1.63	0.9	KT831353	G	*Stagnicola elodes*
*Diplostomum huronense**	-	-	-	-	-	-	-	-	-	KT831378	-	
Diplostomum indistinctum	-	-	-	-	-	-	-	-	-	KT831379	-	
										KT831362		
Diplostomum sp. 1	-	-	-	-	-	-	-	-	-	KT831362	-	
Diplostomum sp. 2* (n=2)	85.267-	46.242-	143.366-	30.961-	169.203-	18.216-	1.84-	1.25-	0.84-	KT831382	I	*Stagnicola elodes*
	116.156	48.461	145.371	33.146	173.835	19.297	2.4	1.68	0.85			
Diplostomum sp. 3	-	-	-	-	-	-	-	-	-	KT831358	-	
Diplostomum sp. 4 (n=2)	144.986-	44.204-	199.052-	36.076-	213.181-	17.387-	3.08-	1.31-	0.84-	KT831354	F	*Stagnicola elodes*
	158.08	51.264	206.481	37.506	244.857	21.207	3.28	1.37	0.93			
Diplostomum sp. 8*	-	-	-	-	-	-	-	-	-	KT831360	-	
Drepanocephalus auritus	-	-	-	-	-	-	-	-	-	KT831381	-	
Echinoparyphium sp.(n=2)	330.645-	48.575-	454-	44.949-	-	-	6.04-	0.9-	-	KT831349	E	*Stagnicola elodes*
	580.996	96.236	523.353	46.163			6.8	1.37				
*Echinostoma caproni**	-	-	-	-	-	-	-	-	-	KT831370	-	
Echinostoma sp.	-	-	-	-	-	-	-	-	-	KT831355,	-	
										KT831361		
*Echinostoma trivolvis** (n=1)	138.727	158.859	537.347	36.934	-	-	0.87	3.87		-	C	*Physella gyrina*
*Echinostoma trivolvis** (n=4)	327.713-	101.403-	411.457-	42.503-	-	-	1.33-	1.26-	-	KT831367	C	*Stagnicola elodes*
	428.561	321.026	828.267	94.164			3.23	1.93				
Fibricola sp. (n=1)	152.899	58.398	251.27	34.775	222.725	22.392	2.62	1.64	1.13	KT831357	J	*Stagnicola elodes*
Gorgoderina sp. (n=4)	233.544-	167.501-	306.213-	65.284-	-	-	1.39-	1.31-	-	KT831348	X	*Stagnicola elodes*
	336.567	226.426	801.409	82.094			1.48	2.38				
Haematoloechus sp.	-	-	-	-	-	-	-	-	-	KT831372	-	
Hypoderaeum sp.(n=1)	368.365	180.378	413.473	54.377	-	-	2.04	1.12	-	KT831350	D	*Stagnicola elodes*
Ichthyocotylurus sp. 3* (n=2)	195.688-	48.818-	188.116-	39.704-	215.17-	18.397-	2.59-	0.96-	0.87-	KT831371	R	*Stagnicola elodes*
	229.372	88.596	263.279	41.047	253.461	33.533	4.0	1.15	1.04			
Notocotylus sp.	-	-	-	-	-	-	-	-	-	KT831364	-	
Ornithodiplostomum sp. 2	-	-	-	-	-	-	-	-	-	KT831368	-	
Ornithodiplostomum sp. 4*	-	-	-	-	-	-	-	-	-	KT831363	-	
Ornithodiplostomum sp. 8	-	-	-	-	-	-	-	-	-	KT831383	-	
Neopetasiger islandicus (n=1)	177.019	63.567	1852.714	182.975	-	-	2.78	10.47	-	KT831342,	A	*Planorbula armigera*
										KT831342		
Petasiger sp.(n=2)	128.776-	97.094-	551.371-	180.776-	-	-	1.33-	4.28-	-	-	B	*Helisoma trivolvis*
	147.561	98.395	778.724	219.347			1.50	5.28				
Petasiger sp. 4 (n=4)	90.46-	98.265-	564.38-	101.929-	-	-	0.92-	3.84-	-	KT831343,	B	*Helisoma trivolvis*
	169.706	121.953	651.53	200.964			1.39	6.24		KT831345		

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Table 2.2 – *Continued from previous page*

Trematode Species (No./sp./host type)	Length Body	Width Body	Length Tail	Width Tail	Length Furcae	Width Furcae	Body Length: Width	Length Tail : Body	Length Tail : Furcae	Accession #	Fig. 3	Snail Intermediate Host
Plagiorchis sp.(n=1)	212.254	61.563	104.8	19.008	-	-	3.45	0.49	-	-	W	*Lymnaea stagnalis*
Plagiorchis sp.(n=11)	79.804-302.173	20.111-145.257	77.861-136.195	23.201-37.171	-	-	2.08-3.97	0.45-.0.98	-	KT831380	W	*Stagnicola elodes*
*Pseudopsilostoma varium**	-	-	-	-	-	-	-	-	-	KT831366	-	
Schistosomatidae gen. (n=1)	170.988	63.087	72.621	40.73	124.506	18.092	2.71	0.42	0.58	-	Q	*Stagnicola elodes*
Schistosomatidae gen. (n=1)	289.825	69.814	348.162	48.003	204.625	17.703	4.15	1.2	1.7	KT831369	N	*Physella gyrina*
*Schistosomatium douthitti** (n=1)	221.467	69.72	261.43	27.541	92.57	20.314	3.18	1.18	2.82	KT831376	O	*Stagnicola elodes*
Strigeidae gen. sp. 9* (n=4)	81.032-430	63.334-204	151.445-447.749	34.187-51.7	198.865-206.53	14.997-17.703	1.28-2.1	1.04-1.87	0.76-2.17	KT831346	V	*Stagnicola elodes*
Telorchis sp.	-	-	-	-	-	-	-	-	-	KT831374	-	
Trichobilharzia physellae	-	-	-	-	-	-	-	-	-	KT831365	-	
Trichobilharzia stagnicolae (n=7)	226.841-324.606	49.726-69.749	297.226-391.175	38.27-46.026	186.606-269.682	18.216-39.698	4.56-4.65	1.2-1.31	1.45-1.59	KT831352	M	*Stagnicola elodes*
Trichobilharzia szidati (n=1)	302.662	79.437	405.073	46.482	238.074	27.992	3.81	1.34	1.7	KT831375	P	*Lymnaea stagnalis*
*Tylodelphys scheuringi** (n=1)	183.98	45.64	204.948	34.024	246.05	17.703	4.03	1.11	0.83	KT831356	H	*Helisoma trivolvis*

Table 2.3: PCR and sequencing primers

Primer name	Forward/ Reverse	Sequence 5'- 3'	Usage	Gene	Size (bp)	Ref.
Snail						
LCO1490	F	GGTCAAC AAATCATA AAGATAT TGG	PCR/ Sequencing	*cox1*	650	(Folmer et al. 1994)
HCO2198	R	TAAACTTC AGGGTGA CCAAAAA ATCA	PCR/ Sequencing	*cox1*	650	(Folmer et al. 1994)
Trematode						
MplatCOX1dF	F	TGTAAAA CGACGGC CAGTTTW CITTRGAT CATAAG	PCR/ Sequencing	*cox1*	650	(Moszczynska et al. 2009)
MplatCOX1dR	R	CAGGAAA CAGCTAT GACTGAA AYAAYAII GGATCICC ACC	PCR/ Sequencing	*cox1*	650	(Moszczynska et al. 2009)
CO1F15	F	TTTNTYTC TTTRGATC ATAAGC	PCR/ Sequencing	*cox1*	500	(Brant and Loker 2009)
CO1R15	R	TGAGCWA YHACAAA YCAHGTA TC	PCR/ Sequencing	*cox1*	500	(Brant and Loker 2009)
Dice1F	F	ATTAACCC TCACTAAA TTWCNTT RGATCAT AAG	PCR/ Sequencing	*cox1*	650	(Van Steenkiste et al. 2014)
Dice11R	R	TAATACG ACTCACTA TAGCWGW ACHAAATT THCGATC	PCR/ Sequencing	*cox1*	650	(Van Steenkiste et al. 2014)
NDJ11	F	AGATTCG TAAGGGG CCTAATA	PCR/ Sequencing	*nad1*	500	(Kostadinova and Herniou 2003)
NDJ2a	R	CTTCAGCC TCAGCATA AT	PCR/ Sequencing	*nad1*	500	(Kostadinova and Herniou 2003)
ZX-1	F	ACCCGCT GAATTTA AGCATAT	PCR/ Sequencing	28s	1200	(Bray et al. 2009)
1500R	R	GCTATCCT GAGGGAA ACTTCG	PCR / Sequencing	28s	1200	(Tkach et al. 2003)

Table 2.4: Snail species identification results based on *cox1* sequencing

Snail Species	Accession No.	(n)	% Identity Range	GenBank Accession Matches
Lymnaea stagnalis	KT831385	2	99.7-100%	GU680908, AY227369
Stagnicola elodes	KT831386	44	92.1-99.8%	HQ969867
Physella gyrina	KT831388	7	99.2-100%	AF346741
Helisoma trivolvis	KT831387	5	98.8-99.0%	AY227371
*Planorbula armigera**	KT831384	1	88.60%	EF012176

*closest match to GenBank

Table 2.5: Snail abundance and digenean trematode infection prevalence by collection site and year

		Season 1 (2013)						**Season 2 (2014)**					
Lake/ Site # in Fig. 1	Snail Type	# collected	prop. of total (%)	# infected	%infected	prop. total infected (%)	# trematode species	# collected	prop. of total (%)	# infected	% infected	prop. total infected (%)	# trematode species
Lake Isle / 3	*Physid*	202	0.085	7	0.035	0.026	2	35	0.0534	7	0.2	0.05	4
	Lymnaeid	2016	0.8485	252	0.125	0.926	14(+)	615	0.9375	131	0.213	0.942	10 (+)
	Helisoma	158	0.0665	13	0.082	0.048	4(+)	5	0.0076	1	0.2	0.007	1
	Small Planorbid	0	0	0	0	0	0	1	0.0015	0	0	0	0
Lac La Nonne / 1	*Physid*	138	0.096	35	0.254	0.132	4(+)	-	-	-	-	-	-
	Lymnaeid	1179	0.8199	227	0.193	0.853	10(+)	-	-	-	-	-	-
	Helisoma	121	0.0841	4	0.033	0.015	2(+)	-	-	-	-	-	-
	Small Planorbid	0	0	0	0	0		-	-	-	-	-	-
Lac La Nonne / 2	*Physid*	324	0.4519	83	0.256	0.68	7(+)	-	-	-	-	-	-
	Lymnaeid	370	0.516	38	0.103	0.311	6(+)	-	-	-	-	-	-
	Helisoma	23	0.0321	1	0.043	0.008	1	-	-	-	-	-	-
	Small Planorbid	0	0	0	0	0	0	-	-	-	-	-	-
Wabamun Lake / 4	*Physid*	655	0.497	18	0.027	0.261	6(+)	386	0.4742	15	0.039	0.3	3(+)
	Lymnaeid	496	0.3763	43	0.087	0.623	8(+)	154	0.1892	20	0.13	0.4	5(+)
	Helisoma	126	0.0956	7	0.056	0.101	4(+)	228	0.2801	15	0.066	0.3	4(+)
	Small Planorbid	41	0.0311	1	0.024	0.014	1	46	0.0565	0	0	0	0
Pigeon Lake / 6	*Physid*	-	-	-	-	-	-	80	0.9302	4	0.05	1	3
	Lymnaeid	-	-	-	-	-	-	6	0.0698	0	0	0	0
	Helisoma	-	-	-	-	-	-	0	0	0	0	0	0
	Small Planorbid	-	-	-	-	-	-	0	0	0	0	0	0
Pigeon Lake / 5	*Physid*	-	-	-	-	-	-	5	0.4167	0	0	0	0
	Lymnaeid	-	-	-	-	-	-	7	0.5833	0	0	0	0
	Helisoma	-	-	-	-	-	-	0	0	0	0	0	0
	Small Planorbid	-	-	-	-	-	-	0	0	0	0	0	0

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Table 2.5 – *Continued from previous page*

		Season 1 (2013)						**Season 2 (2014)**					
Pigeon Lake / 7	*Physid*	-	-	-	-	-	-	1	0.027	0	0	0	0
	Lymnaeid	-	-	-	-	-	-	36	0.973	0	0	0	0
	Helisoma	-	-	-	-	-	-	0	0	0	0	0	0
	Small Planorbid	-	-	-	-	-	-	0	0	0	0	0	0
Buffalo Lake/ 9	*Physid*	15	0.0082	1	0.067	0.006	1	22	0.0235	1	0.045	0.007	1
	Lymnaeid	1806	0.9918	173	0.096	0.994	12(+)	914	0.9765	136	0.149	0.993	8(+)
	Helisoma	0	0	0	0	0	0	0	0	0	0	0	0
	Small Planorbid	0	0	0	0	0	0	0	0	0	0	0	0
Buffalo Lake / 10	*Physid*	3	0.1154	0	0	0	0	47	0.125	0	0	0	0
	Lymnaeid	23	0.8846	1	0.043	1	1	329	0.875	85	0.258	1	4(+)
	Helisoma	0	0	0	0	0	0	0	0	0	0	0	0
	Small Planorbid	0	0	0	0	0	0	0	0	0	0	0	0
Buffalo Lake / 11	*Physid*	-	-	-	-	-	-	56	0.1051	0	0	0	0
	Lymnaeid	-	-	-	-	-	-	328	0.6154	47	0.143	0.77	3(+)
	Helisoma	-	-	-	-	-	-	82	0.1538	14	0.171	0.23	7(+)
	Small Planorbid	-	-	-	-	-	-	67	0.1257	0	0	0	0
Gull Lake / 8	*Physid*	36	0.0446	4	0.111	0.015	2(+)	137	0.1702	1	0.007	0.008	1
	Lymnaeid	772	0.9554	271	0.351	0.985	15(+)	667	0.8286	129	0.193	0.992	12(+)
	Helisoma	0	0	0	0	0	0	1	0.0012	0	0	0	0
	Small Planorbid	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.6: Percent infection prevalence of snails at each lake over the course of the collection survey

2013	**June**	**July**			**August**		**Sept.**
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Wabamun	0.0067	0.0145	0.0288	0.1081	0.0545	0.1005	0.0421
Isle	0.05	0.2071	0.4515	0.2036	0.0885	0.063	0.1068
Lac La Nonne	-	0.3065	0.6415	0.2162	0.081	0.2103	0.2404
Pigeon	-	-	-	-	-	-	-
Buffalo	0.0137	0.1304	0.1284	0.0921	0.037	0.2254	0
Gull	0.2581	0.6667	0.4884	0.2692	0.2867	0.4419	0.3

2014	**June**		**July**		**August**		**Sept.**
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Wabamun	0	0.0385	0.0533	0.1212	0.1029	0.0593	0.0652
Isle	0	0.2727	0.2222	0.1795	0.0694	0.7477	0.25
Lac La Nonne	-	-	-	-	-	-	-
Pigeon	0	0	0	0	0	0	0.0615
Buffalo	0.0747	0.2119	0.1101	0.156	0.2183	0.2357	0.1399
Gull	0	0	0.0875	0.125	0.1418	0.3086	0.4348

Table 2.7: Cumulative species richness stratified by lake and snail intermediate host

Cumulative Richness

Lake	**Snail species**	**Trematode species**
Wabamun	5	19
Isle	4	16
Lac La Nonne	3	12
Pigeon	2	3
Buffalo	5	19
Gull	3	15
Snail		
L. stagnalis		7
S. elodes		25
P. gyrina		13
H. trivolvis		12
*P. armigera**		1

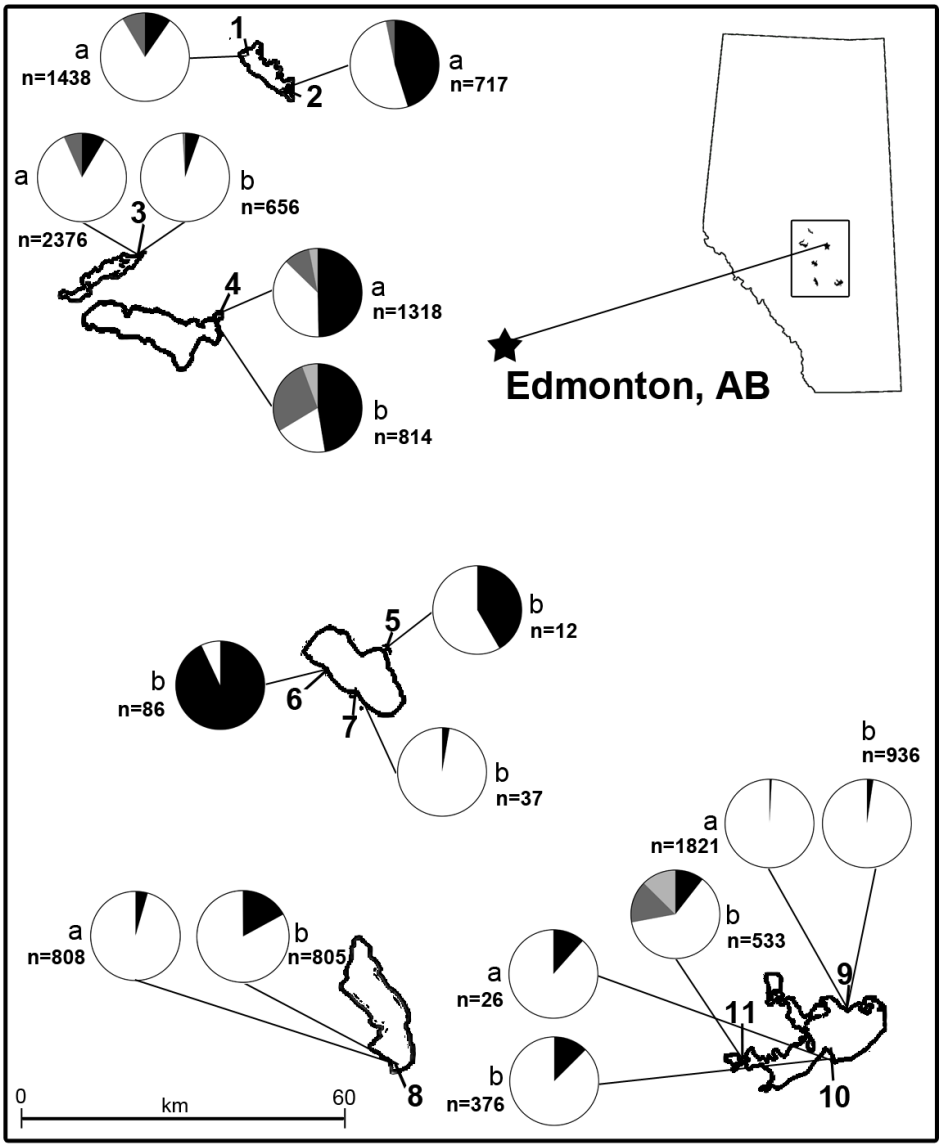

Figure 2.1: Collections map and patterns of snail heterogeneity.
Snails were collected from 11 sites, across six lakes (Lac La Nonne = sites 1 & 2, Lake Isle = site 3, Wabamun Lake = site 4, Pigeon Lake = sites 5-7, Gull Lake = site 8, and Buffalo Lake = sites 9-11), and over two years in central Alberta. Overall, the snails collected could be grouped into four different types (○ = Lymnaeid, ● = Physid, ● = Helisoma, ● = Small Planorbid). The pie charts represent the proportions of the four snail types among all the snails collected from that site by season (n= total snails collected, a = collections in 2013, b = collections in 2014).

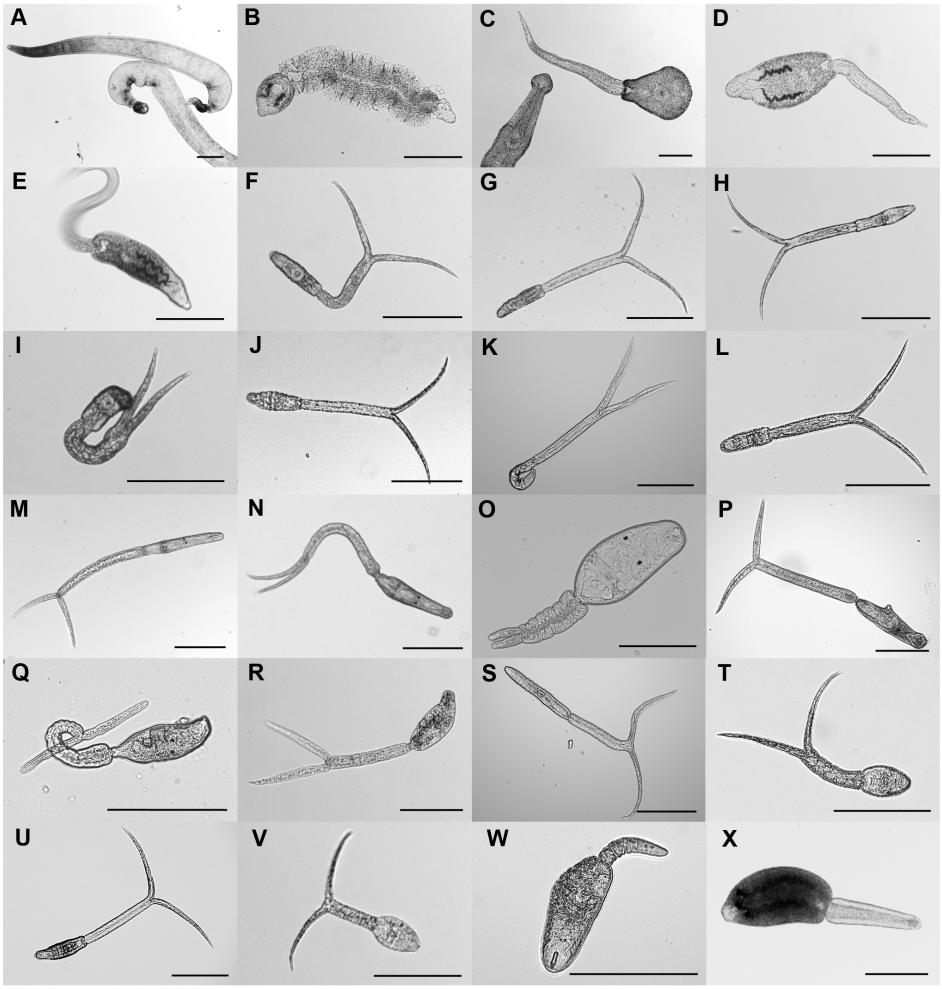

Figure 2.2: Photographs of digenean trematode cercariae from field collections. Scale-bars: 200um. A-E: Echinostomatidae (A) *Neopetasiger islandicus*, (B) *Petasiger* sp. 4, (C) *Echinostoma trivolvis**, (D) *Hypoderaeum* sp., (E) *Echinoparyphium* sp., F-L: Diplostomatidae (F) *Diplostomum* sp. 4, (G) *Diplostomum baeri*, (H) *Tylodelphys scheuringi**, (I) *Diplostomum* sp. 2*, (J) *Fibricola* sp., (K) *Bolbophorous* sp., (L) *Ornithodiplostomum* sp., M-Q: Schistosomatidae (M) *Trichobilharzia stagnicolae*, (N) Schistosomatidae gen., (O) *Schistosomatium douthitti**, (P) *Trichobilharzia* sp., (Q) Schistosomatidae gen., R-V: Strigeidae (R) *Ichthyocotylurus* sp. 3*, (S) *Cotylurus gallinulae**, (T) *Australapatemon burti**, (U) *Apharyngostrigea pipientis**, (V) Strigeidae gen., W: Plagiorchiidae (W) *Plagiorchis* sp., X: Gorgoderidae (X) *Gorgoderina* sp.

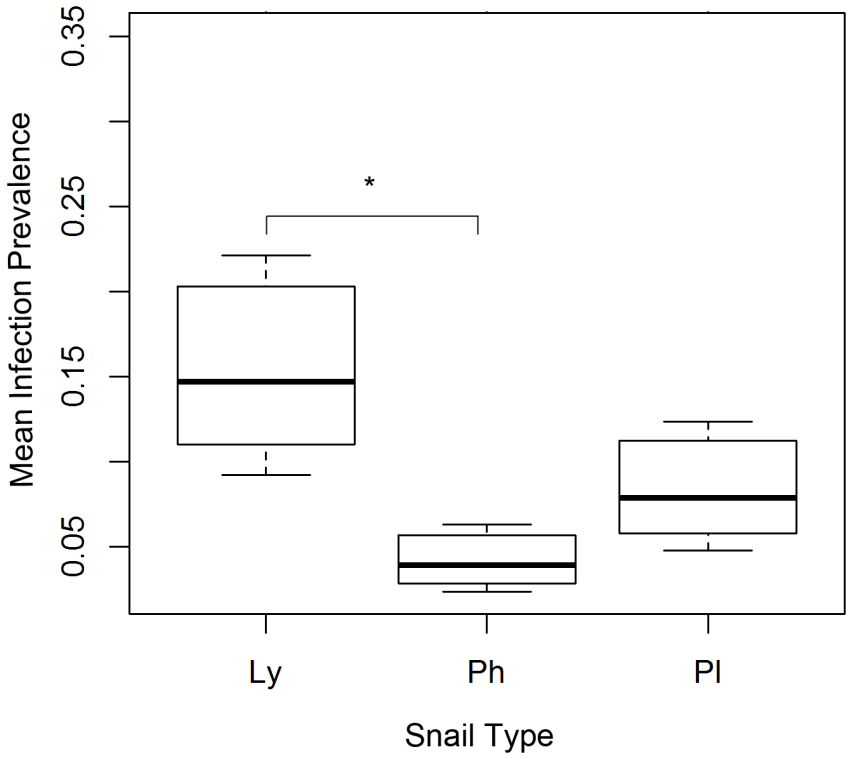

Figure 2.3: Boxplot of mean proportions of infected-to-uninfected snails by snail family.
Multiple comparisons of means across snail types revealed that mean infection prevalence is significantly different between Physids and Lymnaeids (Tukey Post-Hoc, $P = 0.0239$, 95% CI for Ph-Ly [-3.1863, -0.1777]), but not for other comparisons (Ly = Lymnaeid, Ph = Physid, Pl = Planorbid).

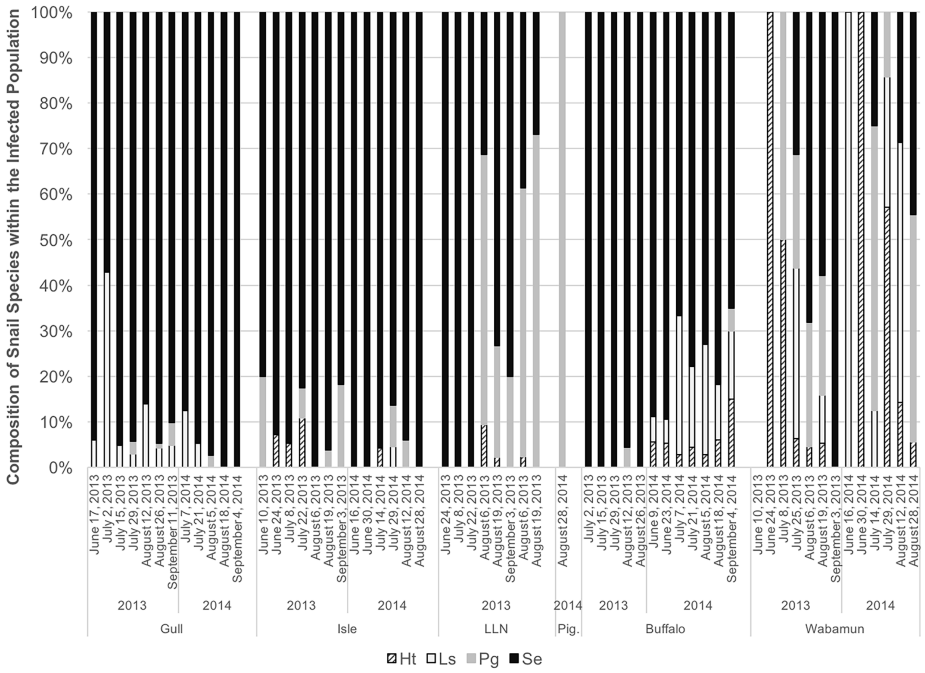

Figure 2.4: Composition of snail species within the infected population, stratified by lake and time.
Bars represent the composition of the infected snail population by collection day at each lake for both collecting years. The shaded sections represent the proportion of the total infected snails, made up by each snail species (Ht = *Helisoma trivolvis,* Ls = *Lymnaea stagnalis*, Pg = *Physella gyrina,* Se = *Stagnicola elodes*) on that day.

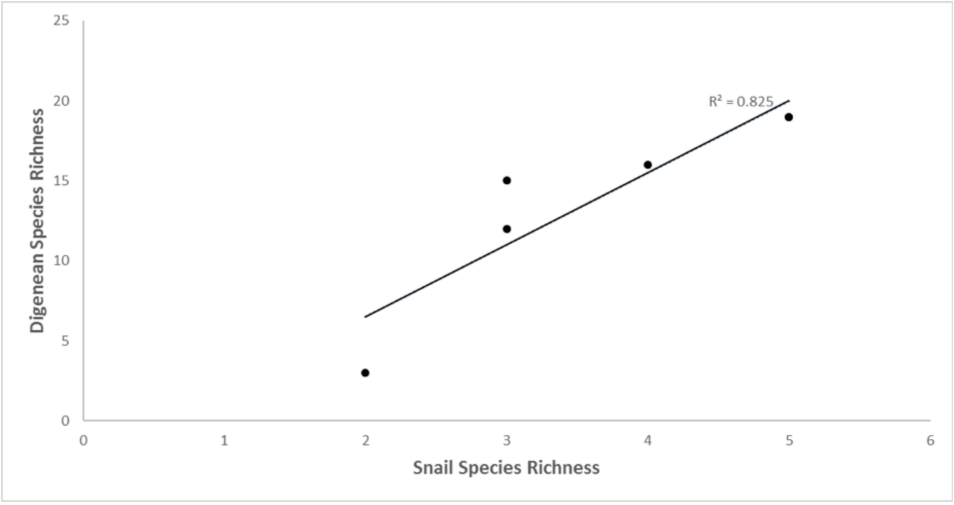

Figure 2.5: Correlation of host and parasite richness.
Plot of snail to digenean species richness as stratified by lake (Table2.7). Richness was strongly, positively correlated between the two groups (corr. coeff. = 0.908, df = 4, p =0.012).

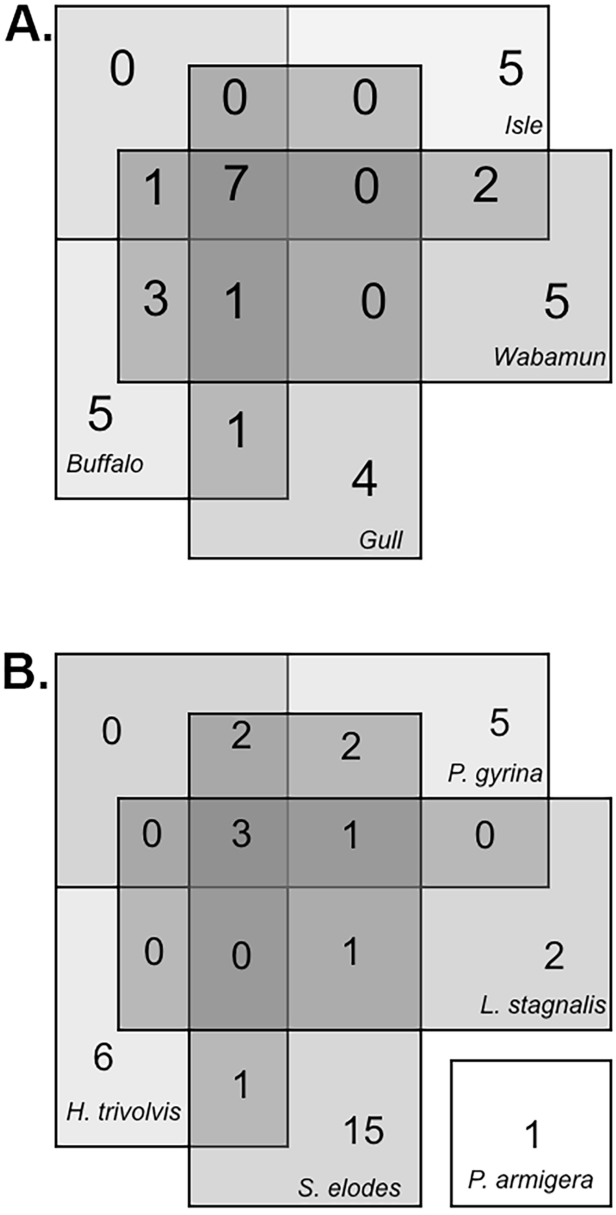

Figure 2.6: Venn diagrams of unique and shared species.
A) Represents the numbers of digenean species unique and shared across lakes, adjusted to only include lakes Wabamun, Isle, Gull, and Buffalo. B) Represents the numbers of unique and shared digenean species across snail species.

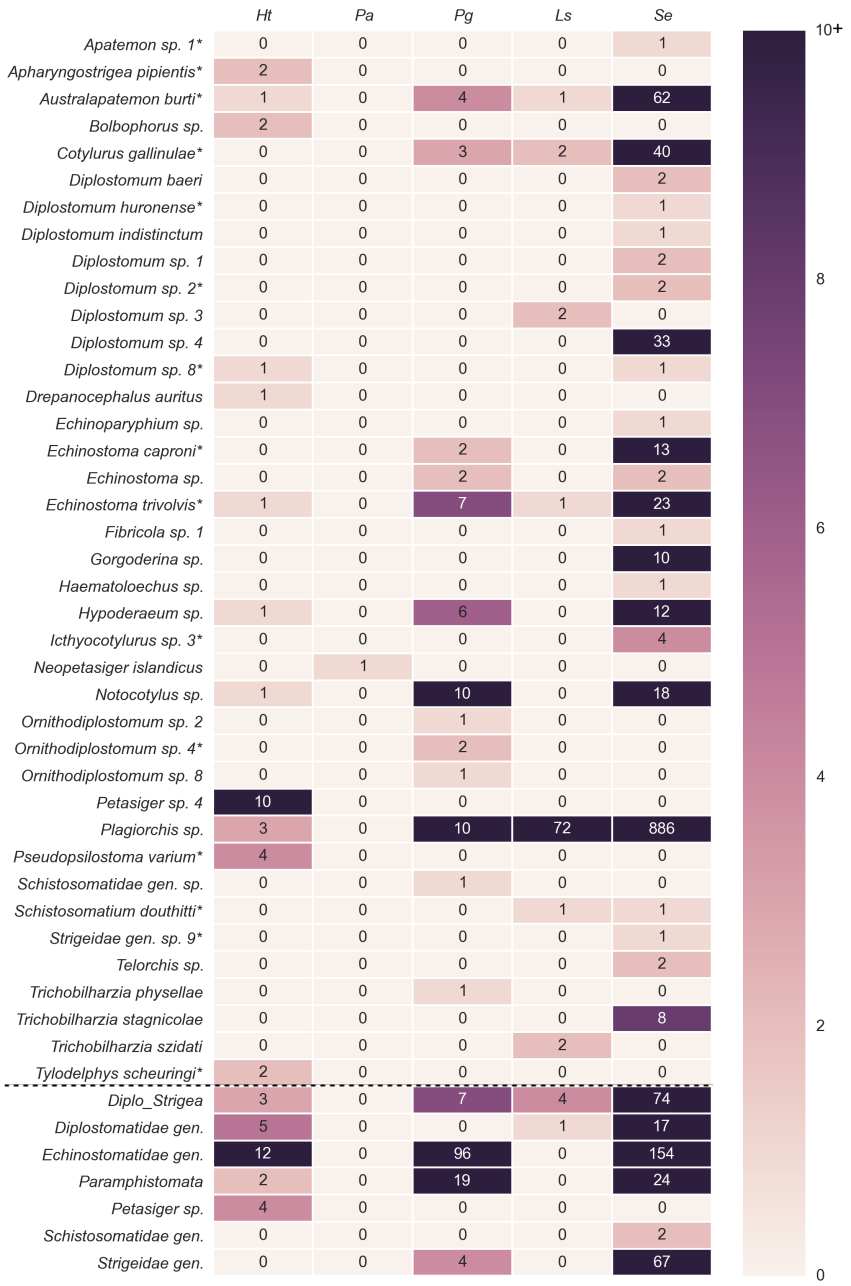

Figure 2.7: Frequency heat-map table of snail and digenean combinations found in central Alberta lakes.
Rows describe digenean species and the number found to be infecting each snail species as distinguished by columns. The dotted line separates the samples from which there is sequence information on the top from those in which descriptions are based on morphology alone on the bottom. Column titles: *Ht* = *Helisoma trivolvis*, *Pa* = *Planorbula armigera**, *Pg* = *Physella gyrina*, *Ls* = *Lymnaea stagnalis*, and *Se* = *Stagnicola elodes*.

Chapter 3

Molecular and morphological evidence for nine species in North American *Australapatemon* (Sudarikov, 1959): a phylogeny expansion with description of the zygocercous *Australapatemon mclaughlini* n. sp.

3.1 Introduction

3.1.1 Preface

Some of the major limitations to incorporating parasites into biodiversity surveys stem from the decline in classically trained parasitologists, as the knowledge needed to know where and how to look for a broad diversity of parasites is by no means trivial. However, even for trained parasitologists, it can be difficult to identify parasites to species, even with the advance of molecular technologies. It requires skills incorporating taxonomic methods that utilize

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multiple lines of evidence and advanced statistical analysis including morphological characterization and molecular phylogenetics. The primary issue being that for most groups of parasites there is little molecular information available, or that which is available is based on the most common species, or those that are model organisms raised in laboratories. So, even if the sequence of a gene from a parasite of interest is discovered, there may not be anything that resembles it in any molecular databases.

Much of this issue, in the case of digenean trematodes, stems from the historical characterization of adult worms from morphological characteristics and the methodological inability to successfully derive quality DNA from formalin-preserved specimens (personal communication with several curators of parasite collections across museums in the U.S.). Because of current limitations in the field surrounding the collection of vertebrates and difficulties in obtaining collection permits, it is becoming harder to attain new specimens of adult trematodes, especially in anything beyond waterfowl and agricultural or aquaculture animals. These limitations create a gap because of the complex nature of trematode life cycles involving multiple hosts and life stages that are very morphologically distinct. While one could argue that experimental life cycles are still an option, this practice is becoming even more rare in the literature and difficult to achieve in the laboratory because of animal-use permits and justification for their use, considering the amount of data retrieved and the breadth of animal hosts required.

Trematodes are, therefore, often collected from their snail hosts for biodiversity surveys, and this stems from several reasons: 1. Snails are easy to collect, as they primarily stay in shallow water around the edges of the lakes; 2. The digenean larvae emerge from the snail and swim freely in the water, making it easy to collect the parasite while keeping the host alive; 3. Adults and other intermediate stages are often difficult to obtain because of the requirements for obtaining collection permits, to kill the hosts and retrieve the worms, often without first knowing if they are infected, and most importantly; 4. Snails stay in the ecosystem, whereas other hosts may come and go (migra-

tory waterfowl), making it more probable to capture the trematode from the snail host, than from the vertebrate host.

There are also disadvantages to collecting trematodes from their snail, first-intermediate hosts. Snails often exhibit a low prevalence of trematode infection within their populations, making it necessary to collect a large number of snails for examination, and lowering the probability of capturing specific species of interest. One of the challenges associated with identifying trematode species by their larval stages is that they lack the development of key morphological characteristics found in their adult stage (Schell 1985). Despite this, putative, new, trematode species have often been described by just their larval stages (RM Cable 1956; Cort and Brooks 1928; Hendrickson and Kingston 1974; Stunkard, Willey, and Rabinowitz 1941). A lack of morphologically distinct characters results in the necessity of molecular methods to delineate species. Likely the greatest challenge of using molecular methods, currently, is the deficiency of species represented within molecular databases like GenBank and the Biodiversity Of Life Database (BOLD). Even with large barcoding projects, it is obvious that not everyone subscribes to the utility of the mitochondrial gene, cytochrome *c* oxidase subunit 1(*cox1*), and this results in some samples/species being represented by one set of genes, while other samples are represented by a different set of genes. Because of issues surrounding substitution saturation, certain genes are not always a legitimate option. Our ability to compare across samples thus becomes hindered, or made more difficult and costlier, by having to sequence more genes and assess the validity of the comparisons. There is still much work to be done in the systematics of digenean trematodes, much of which stems from the need for the community to develop a standard for all to follow.

In making assessments of the diversity of trematodes in Alberta, this chapter lays the foundation for the methodology and the utility of integrative taxonomy and multiple lines of evidence for delineating species. This chapter advances upon the methods utilized in the previous chapter and provides an integrative analysis to characterize a new species with cryptic relatives. This chapter provides a proof-of-concept for the methods used in later chapters

and further discusses the challenges of integrative taxonomy and species delineation among digenean trematodes. This study was borne from finding an odd behavioural and morphological adaptation, rare among digenean larvae, and never before described from Alberta.

3.1.2 Background

Reports of digenetic trematodes with zygocercous cercariae, often referred to as "Rat King", "Rattenkönig", or "Aggregacercaria", are rare. In the past century (since 1888), there have been only 11 descriptions of aggregating cercariae. Aggregation is a unique behavioural adaptation that results in the joining together of several to hundreds of individual cercariae into bundles or nets (Beuret and Pearson 1994; R.M. Cable and McLean 1943; RM Cable 1956; RM Cable 1963; Dronen, N.O. 1973; Hendrickson and Kingston 1974; Komiya 1941; W.E. Martin 1968; WE Martin and V. Gregory 1951; H. Miller 1929; H. Miller 1930; Pintner 1891; Ward 1916; Wardle 1988). Zygocercous cercariae join by the tails to form rosettes, like the "Rat King" phenomenon from which they were originally described, or pine-cone-like structures (reviewed in Table 3.1). This adaptation is believed to assist cercariae in being consumed by their next host, thought to be a fish, based on a few experimental studies (Dronen, N.O. 1973). Little information regarding life cycle progression or host use is available for zygocercous specimens described to date. Moreover, there are no available genetic resources for any of these cercariae, resulting in limited understanding of their phylogenetic affinity, beyond tentative identifications at the family level.

The present study began with a morphological and molecular characterization of zygocercous cercariae emerging from the snail, *Physella gyrina* (Say, 1821) (used interchangeably with *Physa gyrina* throughout text), collected from a lake in central Alberta. The morphology of these cercarial larvae was compared to that of other zygocercous larval trematodes in the literature, and to close relatives identified through mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) DNA sequences. Sequence comparisons suggested the zygocercous cercariae belonged to *Australapatemon burti* (Miller, 1923), based on

an adult trematode sequenced by Hernández-Mena et al. (2014), although the zygocercous forms we collected displayed morphological differences to cercariae of this species (Cort 1928; Miller Jr. 1923; Miller Jr. 1926; Stunkard, Willey, and Rabinowitz 1941). An expanded sampling effort provided additional *cox1* sequences from cercariae collected during a large-scale survey of digeneans in central Alberta (M. Gordy et al. 2016) and from cercariae and adult worms collected across North America. These results placed the zygocercous cercariae in one of nine genetically distinct lineages of *Australapatemon*, matching sequence from adult *A. burti* from ducks sampled by Hernández-Mena et al. (2014) in Mexico.The morphological differences between thezygocercous and non-zygocercous cercariae, along with morphology and host-use of adults in Manitoba and Mexico, further corroborated the distinctions among lineages identified in *cox1* comparisons. These findings indicate hidden species diversity within *Australapatemon*. Among the nine lineage distinguished, one is tentatively identified as *A. burti* (Miller, 1923), and one is a new species of *Australapatemon*.

3.2 Material and methods

3.2.1 Specimen collection

Most data reported here are based on cercariae from snails collected as part of a parasitological survey of several lakes in central Alberta (see methods within Chapter 2, as published in Gordy et al. 2016), from June 2013 to September 2015. On July 13th and August 10th, 2015, collections from Rochon Sands Provincial Park at Buffalo Lake (52.4638361 N, -112.8843833 W) yielded two *Physella gyrina* snails infected with a trematode with zygocercous type cercariae. These snails were placed in small plastic containers with artificial spring water (ASW) (Ulmer 1970), fed Red Leaf lettuce *ad libitum*, and monitored over several days to count and capture emerging cercariae. One individual cercaria of an aggregate was separated in a dilute solution of tricaine mesylate, used to relax the aggregate, before using fine forceps to pull it apart. The aggregates were otherwise impossible to separate. The individual cercaria was

wet mounted and photographed using the Zeiss Axio Imager.A2 compound microscope and mounted Zeiss AxioCam MRc camera. The number of zygocercous aggregates per day were recorded, and finally, both cercariae and snail were preserved in 100% ethanol for later analyses. A permanent mount was prepared for the zygocercous cercariae aggregates using Grenacher's Borax-Carmine stain and mounted in Canada balsam. Drawings were made from photographs of wet-mounted specimens.

Additional material was obtained from gastropod and avian hosts elsewhere in North America. The latter included cercariae from planorbid snails at two localities in California (Santa Clara area, sampled in August 2009, and Pleasanton area, June 2009) and cercariae from *Helisoma campanulatum* (Say, 1821) sampled from a lake in Cape Breton, May 2012. In addition, we included data from adult worms from Anatidae (Anserinae: *Anas acuta* (Linnaeus, 1758) (n = 2), *Aythya collaris* (Donovan, 1809)(n = 1), *Bucephala albeola* (Linnaeus, 1758)(n = 1); Anatinae: *Oxyura jamaicensis* (Gmelin, 1789)(n = 1)) collected from the southern end of Lake Manitoba, Manitoba, Canada in 2008 and 2009. In the latter collections, live adults and cercariae from freshly killed hosts were placed directly into 70-95% ethanol.

Voucher samples of permanent mount slides for the zygocercous cercariae and for several representative adult worms, including type material, were donated to the Royal Alberta Museum, in Edmonton, Alberta, Canada.

3.2.2 Molecular analyses

Cercariae collected in Alberta were initially identified by partial sequencing of the mitochondrial *cox1* gene, using primers Dice1F and Dice11R for amplification, and a shortened version of these primers for Sanger sequencing (Van Steenkiste et al. 2014), as previously described (M. Gordy et al. 2016). Additionally, partial large subunit (28S) and internal transcribed spacer regions (ITS1-5.8S-ITS2) of ribosomal DNA (rDNA) sequences were generated for select specimens to include in phylogenetic analyses. Universal primers BD1 and BD2 were used to amplify ITS1-5.8S-ITS2 sequences as previously described for *Clonorchis sinensis* (Looss, 1907) (Tatonova, Chelomina, and Besprosvan-

nykh 2012). Sequences for 28S rDNA were amplified as previously described for other trematodes (M. Gordy et al. 2016). *Cox1* fragments from cercariae and adults obtained outside Alberta were amplified and sequenced as described by Moszczynska et al. (2009). Sequences of rDNA from non-Albertan specimens were generated using primers and protocols in Littlewood and Olson (2001) (for 28S) and Galazzo et al. (Galazzo et al. 2002) for ITS1-5.8S-ITS2. All newly generated sequences were submitted to GenBank (accession numbers: HM385485-HM385486, HM385535-HM385536, HM385537-HM385538, KY207548- KY207628, KY570946-KY570948, KY587394-KY587403, KY587 405 - KY587406, MF124269-MF124270).

Our molecular analysis built on a recent phylogeny of *Australapatemon* Sudarikov, 1959 and *Apatemon* Szidat, 1928 (Blasco-Costa, Cutmore, et al. 2016). The *cox1,* 28S, and ITS strigeid sequences used and generated by Blasco-Costa et al. (2016) were separately aligned with newly obtained sequences using Multiple Sequence Comparison by Log Expectation (MUSCLE) (Edgar 2004) in Geneious v.10.0.5 (http://www.geneious.com, Kearse et al. 2012). Alignments were trimmed to the shortest available sequence prior to phylogenetic analyses (*cox1*: 408 nt; 28S: 809 nt; ITS: 525 nt). MEGA7 was used for model testing, initial Maximum Likelihood (ML) analyses, and p distance calculations (Kumar, Stecher, and Tamura 2016). Model selection for each dataset was based on ML fits of 24 different nucleotide substitution models, with the best model of evolution being that with the lowest BIC score (Bayesian Information Criterion). The models of nucleotide evolution used for ML trees in MEGA7 were HKY+G+I (*cox1*) and K2+G (28S and ITS) and tree nodes were assessed with 1,000 bootstrap replicates. All ML analyses in MEGA7 employed four discrete gamma categories, used complete deletion if there were gaps/missing data, inferred trees using nearest-neighbor-interchange as the heuristic method, while initial trees were generated automatically with the neighbor-joining method. Tree options in Geneious were slightly different; thus, the second-best models were used, namely HKY85+G (*cox1*) and JC69+G (28S and ITS). The PhyML plugin (Guindon et al. 2010) in Geneious was used to test the robustness of initial ML trees generated in

MEGA7. The following options were selected to run these analyses: bootstrap branch support with 1,000 replicates, transition/transversion ratio estimated, proportion of invariable sites estimated, number of substitution categories was four, gamma distribution parameter estimated, optimized for topology/length/rate, and using an SPR topology search. The MrBayes plugin v. 3.2.6 (Huelsenbeck and Ronquist 2001) in Geneious was used for Bayesian Inference (BI) analyses. All BI trees were constructed from two independent MCMC runs of four chains (temp 0.2) for 107 generations, sub-sample frequencies of 104 generations, and burn-in of 105 generations, per the standard deviation of split frequency values (<0.01). A consensus topology and nodal support, estimated as posterior probability values, were generated from trees remaining beyond the burn-in period (Huelsenbeck and Ronquist 2001). The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.652, as estimated by PhyML).

The web app for Automatic Barcode Gap Discovery (ABGD; wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) was used to test hypotheses of lineage separation (Puillandre et al. 2012), coupled with *a priori* assumptions of species differentiation, using a 5% cut-off value for *cox1* (Vilas, Criscione, and Blouin 2005). Default values were applied to ap distance matrix input into the ABGD program. The ABGD method has been used previously as a supportive tool for delimiting species among trematodes of the families Clinostomidae (Sean A Locke, Caffara, et al. 2015; Gerardo Pérez-Ponce de León et al. 2016), Diplostomidae (Sean A Locke, Caffara, et al. 2015), and Opecoelidae (Lõpez et al. 2015; Oliva et al. 2015).

3.2.3 Morphological analyses of cercariae

The zygocercous cercariae and other cercariae (representative samples from each following intermediate host: *Stagnicola elodes* (Say, 1821), *Helisoma trivolvis* (previously named *Planorbella trivolvis* (Say, 1817)), and *Physella gyrina*) with high nucleotide identity (>95%) to *A. burti* sequences, matched through BLAST (tblastn), were selected for morphological analyses. Samples of cercariae stored in 95-100% ethanol were imaged using a scanning

electron microscope (SEM) (Model XL30 by FEI COMPANY North America NanoPort, 5350 NE Dawson Creek Drive Hillsboro, Oregon 97124 USA). Samples were prepared by first transferring to a 0.2 micron GTTP membrane atop a 25 mm swinnex filter holder with o-ring (Millipore). While the membrane was still wet, the top of the filter holder was tightened into place. Then, using a 1 ml luer lock syringe, the samples were taken to 100% hexamethyldisilazane (HMDS) through the following series: 1 ml of 100% ethanol (twice), 1ml of 75% ethanol:25% HMDS, 1 ml of 50% ethanol:50% HMDS, 1 ml of 25% ethanol:75% HMDS, and 1 ml of 100% HMDS (twice). After the fluid was run through the syringe, there was a five-minute waiting period before the next fluid was run through. After the samples were in 100% HMDS, all remaining fluid was pushed through by filling the syringe with air and pressing through until no further liquid came out the other end. Finally, the membranes were dried completely for several hours before being cut and placed onto the SEM stud for sputtering and subsequent imaging.

Measurements were taken from SEM images using Scandium 5.0 (Olympus Soft Imaging Systems) to capture the following major external morphological features for comparison between specimens and those in the literature: cercarial body length and width, tail stem length and width, furcal length and width, oral sucker to ventral sucker length, ventral sucker to tail stem length, ventral sucker length and width, length and width of spines found on the body, tail stem, and furcae. Additionally, for the zygocercous cercariae, measurements were taken for the papules found on the tail stem. Because the dehydration step during the SEM processing appeared to cause some collapsing of the tissues, creating wrinkles, measurements were also taken from a small number of cercariae preserved for permanent mounting, to test for artefacts or distortion due to different methods of specimen preparation. Because resolution of the permanent-mount material was not as great as in that prepared for SEM, the only measurements taken were of body length and width and tail stem length and width. Light microscope images were taken using a compound Zeiss Axio Scope.A1 and PictureFrame v. 2.3 (Optronics) software, and measurements were made using ZEN (Zeiss) software. Sizes of morphological features were

compared using an independent samples Kruskal-Wallis analysis of variance, and *post hoc* multiple comparisons test, with Bonferroni correction for multiple tests, and an alpha level of 0.05, using IBM SPSS Statistics v. 24.0.

3.2.4 Morphological analyses of adults

In the lineages genetically distinguished herein for which adult specimens were available, DNA was often extracted from a subsample of a worm, and the remainder was stained in acetocarmine and mounted laterally on a slide in Canada balsam (i.e. as hologenophores, *sensu* (Pleijel et al. 2008)). In one lineage (LIN8), DNA was extracted from the entire worm, and the voucher is an intact worm that appeared indistinguishable from the sequenced specimen when it was taken from the same host (i.e. a paragenophore, *sensu* Pleijel et al., 2008). The adult vouchers were compared to published descriptions, with emphasis on accounts originating geographically close or from the same host as in the original description. Measurements were made with both an ocular micrometer and using imaging software, and one specimen was drawn using a camera lucida. Measurements were taken from uncollapsed, laterally oriented eggs along the entire length of the uterus, unless eggs were clearly different in size or shape due to differences in maturation. Unless otherwise stated, measurements (in μm) are reported as a range followed in parentheses by mean $\pm$ standard deviation, and number of specimens in which the structure was measured.

3.3 Results

3.3.1 Molecular phylogenetics

The *cox1* sequence derived from the zygocercous cercariae showed a 99.56% nucleotide similarity to *A. burti* (JX977725) from *Anas americana* in Mexico(isolate 180 from Hernández-Mena et al. 2014). Other matches to other *A. burti* isolates from Hernández-Mena et al. (2014) were attained with *cox1* sequences from non-zygocercous cercariae derived from a previous study (M. Gordy et al. 2016). Therefore, nucleotide alignments and phylogenetic analy-

ses of these combined specimens were used to assess the relationships among these samples to clarify these extreme differences in cercarial behaviour.

Confirming initial tblastn results, both ML and BI trees in all three data sets (*cox1*, 28S, ITS) strongly supported the placement of the zygocercous cercaria (MGC1935) in *Australapatemon,* nested among other *A. burti* samples from Mexico, and separate from *Australapatemon niewiadomski* Blasco-Costa, Poulin, and Preswell, 2016 and, in separate clades, other strigeid genera (Blasco-Costa, Cutmore, et al. 2016) (Figure 3.1-3.2). Both 28S and ITS markers, though generally supportive, and confirming genus-level monophyly seen in Blasco-Costa et al. (2016), were less informative for discriminating between species than *cox1*, possibly because sequences were available from fewer samples and had relatively low intrageneric divergence (mean intrageneric divergence across the Strigeidae: 28S $\leq$ 1.2%, ITS $\leq$ 6%) (Table 3.2). Thus, initial species-level delineation among samples within the genus *Australapatemon* was achieved by analysis of *cox1*.

The *cox1* phylogeny showed nine lineages (LIN1-LIN9) within *A. burti*. Members of each lineage differed by less than 6.8% and by at least 6.7% from those in other lineages. The lineages corresponded to identical clusters of sequences in ABGD (prior maximal distance P = 2.15e-02, MinSlope = 1.5) (Puillandre et al. 2012) (evolutionary divergences as p distances given in Table 3.2). Lineages 2, 3, and 5 were each represented by a single sequence. In the other six lineages, maximum intraspecific divergence ranged from 0.5-6.8% (LIN1 (*A. burti*): 6.5%, LIN4: 5.0%, LIN6: 3.3%, LIN7 (*Australapatemon mclaughlini* n. sp.): 0.5%, LIN8: 1.0%, LIN9: 6.8%), and mean intraspecific divergence from 0.2-3.5% (LIN1 = 1.8%, LIN4 = 3.5%, LIN6 = 2.2%, LIN7= 0.2%, LIN8 = 0.3%, and LIN9 = 3.0%). Between lineages, the greatest interspecific divergence value was found between LIN5 and LIN8 (14.4%), and the smallest interspecific difference between LIN6 and LIN7 (6.7%). Overall, the range of genetic divergence by gene region among each new lineage and congeneric species was 6.7-14.4% (*cox1*), 0.0-1.2% (28S), and 0.4-1.9% (ITS).

Sequences of *cox1* from the zygocercous cercariae grouped phylogenetically with those from six adult worms from North America to form a distinct lineage,

LIN7 (*A. mclaughlini* n. sp.). Five of the adult worms were collected from Northern Pintail (*A. acuta*) in Lake Manitoba, Manitoba, Canada, and one adult worm was from an American widgeon, *Anas americana* (Gmelin, 1789) collected from Baja California Sur, Mexico, i.e., isolate 180, identified as *A. burti* by Hernández-Mena et al. (2014)(JX977725.1). LIN7 was positioned within a monophyletic clade also composed of lineages 4 (LIN4), 5 (LIN5), and 6 (LIN6). With the exception of LIN5, all members of this clade utilize *P. gyrina* as an intermediate host and anatid birds as their definitive host. A single cercarial specimen, representing LIN5, was found emerging from the snail *S. elodes* (Figure 3.1).

3.3.2 Host use and parasite morphology

The genetic distinction between clades was supported by differences in intermediate and definitive-host use in several lineages that were sampled more than once (LIN6 and LIN8 in *P. gyrina*, LIN9 in *S. elodes*; LIN7 in *P. gyrina* and *Anas* spp.; LIN8 in *O. jamaicensis*). However, because host specificity may correlate with sampling effort, the support that host-specific distributions imply for putative species is also likely related to sampling effort. For example, 68/80 sequenced cercarial isolates were from *S. elodes* (Lymnaeidae), and consequently a lineage in our samples might be associated only with *S. elodes* by chance, even if it naturally occurs in other hosts. On the other hand, only 7/80 sequenced cercarial samples were obtained from *P. gyrina* (Physidae), such that lineages found only in this host are more likely truly specific to it. To address this, we did not assess host specificity of three lineages recovered from single individual hosts (LIN2, LIN3, LIN5). In other lineages, we randomized the 80 lineage-snail-host associations 10,000 times. The observed host distribution of LIN9 (nine isolates all from in *S. elodes*) was not different from random ($P = 0.351$), but the likelihoods of recovering LIN6, LIN7 and LIN8 only from *P. gyrina* were very small ($P \leq 0.0099$) if these lineages were equally capable of infecting other snail species sampled. The mixed snail-host associations of LIN1 mirror those of the data as a whole (60/66 LIN1 isolates in *S. elodes*; $P = 0.827$). Neither the wide snail-host spectrum of LIN1, nor

the narrow host ranges of the other lineages seem to be purely an artefact of sampling effort, because the number of cercarial isolates sequenced was not related to the number of snail-host species in each lineage (Spearman's rho = 0.616, $P = 0.11$, n = 8). Permuting the smaller database of adult-parasite avian-host associations (consisting of seven lineages, LIN1, 2, 4, 6, 7, 8, 9, from nine individual birds in seven host species) 10,000 times showed the probability of two LIN8 occurring only in *O. jamaicensis* (one in Manitoba, one in Durango, Mexico (Hernández-Mena, García-Prieto, and Martín García-Varela 2014)) to be 0.0278. The only other potential host specificity among the adult worms is in LIN7, which was only recovered from *Anas* spp. The probability of two LIN7 samples occurring in *Anas* was 0.275. Thus, three *cox1* lineages (LIN6, LIN7, and LIN8) are supported by host-specific distributions that are unlikely to be an artefact of sampling effort.

Cercarial morphometrics (14/16) were significantly different across lineages (Table 3.3). There was no significant difference between measurements in SEM and those of permanently mounted, stained samples, nor from wet-mounts. The only lineage within which there were significant morphometric differences among cercariae was LIN1 (Table 3.4). Notably, there were no differences between genetically divergent samples within LIN9 ($P > 0.05$ for all comparisons between MGC1376 and MGC1360).

Within LIN1, cercariae varied morphometrically. For instance, samples MGC1557 (*S. elodes*) and MGC1179 (*H. trivolvis*) had significantly different body and furcal spine dimensions, as well as different furcal lengths (4/16 morphometrics in Table 3.4-3.5). This lineage was recovered from five different pulmonate species in Western and Eastern Canada and the Southwestern USA (Figure 3.1 and Table 3.6). Despite this broad distribution, the only link to an adult was with the isolate from *Anas diazi* (Ridgway, 1886) in Mexico (sample 138 of Hernández-Mena et al. 2014).

Varying degrees of morphological distinction were observed among adults of LIN2, LIN4, LIN7, LIN8 and LIN9 (Tables 3.7-3.8). Adults of most lineages could be distinguished mainly by their total length, ratio of hindbody to forebody length, and egg size. Dense vitelline follicles prevented visualization of

the ovary in all specimens, and the genital cone characteristic of *Australapatemon* was observed in two specimens of LIN7, as well as in the single specimen of LIN9. No substantial difference was seen among the adults of LIN4 and LIN8, and cercariae of both lineages emerged from *P. gyrina*.

Because these parasites mature in migratory waterfowl, and some species have been recorded in diverse birds from distant localities (e.g. (Drago, Lía I. Lunaschi, et al. 2007; Drago and Lía Inés Lunaschi 2010; M.E. 1974)), we compared the morphology of the adult vouchers of the nine lineages with all species of *Australapatemon* (Table 3.7 and Table 3.9), although we focus on species known in North America. One lineage is newly described herein (LIN7 = *A. mclaughlini* n. sp.), and most others could not be assigned to described species, nor described.

3.3.3 Description

Australapatemon mclaughlini n. sp.

Family Strigeidae Railliet, 1919

Subfamily Strigeinae Railliet, 1919

Genus *Australapatemon* Sudarikov, 1959

Description of adult (Figure 3.3A-B; Table 3.7)

[Measurements from 7 specimens (4 subsampled hologenophores, 2 paragenophores, and holotype), ex *Anas acuta* L. Measurements in micrometres; widths dorso-ventral]

Total length 1484-1851 (1671 ± 143, 5); body distinctly bipartite. Maximum width of forebody at level of ventral sucker. Forebody cup-shaped, 347-473 (386 ± 55, 5) long, 475-630 (527 ± 64, 5) wide. Hindbody arcuate, curved dorsally, widest at level of anterior testis, 1137-1378 (1298 ± 92, 6) long, 535-662 (616 ± 48, 6) wide. Ratio of forebody to hindbody length 1:2.9-3.7 (3.4 ± 0.3, 5). Oral sucker terminal, 78-117 (102 ± 14, 5) x 88-113 (102 ± 9, 5). Ventral sucker in median dorsal wall of forebody, 125-160 (142 ± 15, 4) x 173-185 (177 ± 15, 4). Holdfast organ bilobed; proteolytic gland not observed. Pharynx small, difficult to observe, 39-58 (52 ± 11, 3) long. Testes tandem,

large; anterior testis asymmetrical, bilobed; anterior margin at 17-27 (23 ± 4, 4)% of hindbody; 341 x 390 (1). Posterior testis asymmetrical, bilobed; posterior margin at 72-79 (77 ± 3, 4)% of hindbody; 293(1) x 240-341 (291 ± 72, 3). Seminal vesicle convoluted in dorsal post-testicular region, often distending adjacent tegument. Ovary not observed. Vitellaria follicular, confined to hindbody, densely distributed, extending posteriorly in two ventro-lateral fields to level of copulatory bursa. Vitelline reservoir intertesticular; median. Eggs 115-131 (120 ± 5, 6) x 83-88 (85 ± 2, 6). Copulatory bursa large with dorsally oriented terminal opening. Genital cone delimited from surrounding parenchyma, one eighth to one sixth of hindbody length; 192-218 (202 ± 14, 3) x 166-188 (177 ± 16, 2). Hermaphroditic duct within genital cone lined with internal rugae.

Description of cercaria (Figure 3.3C-D; Table 3.5)

[Measurements in micrometres, based on three aggregates preserved in 100% ethanol and imaged with SEM. Body and tail lengths/widths are composite measurements of SEM and permanent mount cercariae from a total of six aggregates.] Freshwater, apharyngeate, distomate, zygocercous furcocercaria. Body elliptical, 78.69-149.50 (115.56 ± 18.71, 69) long, 29.82-49.00 (39.08 ± 5.07, 71) wide. Tegument spines dense at oral sucker and becoming sparser towards posterior extremity of body. Body spines 1.41-2.53 (2.00 ± 0.36, 12) long, 0.25-0.55 (0.45 ± 0.09, 12) wide. No apparent eye-spots. Ventral sucker post-equatorial, 4.83-7.70 (6.46 ± 1.27, 6) long, 7.36-13.59 (9.44 ± 2.25, 6) wide, and containing 4-5 rows of large spines 1.38-2.26 (1.75 ± 0.46, 3) long, 0.30-0.45 (0.38 ± 0.07, 3) wide. Tail stem thinner or of similar width to body at junction but widens further towards furcae, 101.24-247.40 (179.29 ± 27.37, 50) long, 18.66-82.70 (47.28 ± 11.39, 43) wide, aspinose, and covered in protruding papules 2.36-3.92 (3.0 ± 0.44, 14) long, 1.22-3.03 (1.50 ± 0.19, 14) wide. Furcae 47.33-98.90 (80.30 ± 28.70, 3) long (from base of tail stem to bundle with other furcae), 12.17-34.19 (21.70 ± 8.40, 11) wide, and narrowing to a blunt end. Furcal spines 1.06-1.59 (1.40 ± 0.23, 5) long, 0.62-1.06 (0.80 ± 0.17, 8) wide. Excretory bladder bilobed and roughly triangular in shape, in posterior body

region near base of tail stem. Cercarial aggregation by attachment of distal portion of furcae and specialized furcal muscles, in masses of 4-44 individuals. Aggregates non-swimming, resting on substrate in spherical mass, emerging from snail at rate of 20 per day (based on two captive snails observed over three days), occurring free and between mantle tissue and shell of snail, suggesting aggregation immediately post-emergence.

Details of type material

Type-host: Anas acuta (definitive host)

First intermediate host: Physella gyrina Say

Site of infection: Intestine (definitive host); ovo-testes and digestive gland (first intermediate host).

Prevalence: In 1 out of 2 birds (Lake Manitoba); in 2 of 127 snails collected (Buffalo Lake).

Type-locality: Delta Marsh, Lake Manitoba, Manitoba, Canada (50.183° N, -98.383° W) (definitive host); Rochon Sands Provincial Park, Buffalo Lake, Alberta, Canada (52.464 N, -112.884 W) (first intermediate host).

Other localities: Guerrero Negro, Baja California Sur (27.959° N, -114.056° W) (definitive host: *Anas americana* JX977725, sample 180 (Hernández-Mena, García-Prieto, and Martín García-Varela 2014)).

Type-material: Holotype (adult worm); Holotype (cercariae). Deposited in The Royal Alberta Museum.

Representative DNA sequences: cox1: KY207615, KY587395, KY587402, KY587403, KY587405, KY587406, JX977725; 28S rDNA: KY207627; ITS1-5.8S-ITS2: KY207628.

Etymology: This species is named after J. Daniel Mclaughlin, who collected and generously provided adult worms studied herein, and who has made numerous, important contributions to the systematics and ecology of helminths in aquatic systems in North America.

Remarks

Adults of *Australapatemon mclaughlini* n. sp. possess characters typical of *Australapatemon* Sudarikov, 1959, namely a muscular genital cone delimited from surrounding parenchyma that is traversed by a rugose hermaphroditic duct. The length of the hindbody relative to the forebody (see Figure 3.3B) is greater in adults of *A. mclaughlini* n. sp. compared to all species of *Australapatemon*, as well as the lineages characterized genetically herein, but not *Australapatemon anseris* (Dubois, 1967). *A. mclaughlini* n. sp. is also distinguished from most species and lineages (other than *Australapatemon canadensis* (Dubois and Rausch, 1950), *Australapatemon fuhrmanni* (Dubois, 1937), *Australapatemon minor* (Yamaguti, 1933), and LIN2 and LIN9) by having larger eggs (Table 3.9). Several features distinguish *A. mclaughlini* n. sp. from species of *Australapatemon* reported in North America. Compared with *A. anseris* (a predominantly European species reported in North America by (M.E. 1974)), *A. mclaughlini* n. sp. is characterized by a smaller total length, oral sucker, pharynx, ventral sucker, posterior testis and genital cone, and greater egg length. *A. mclaughlini* n. sp. is distinguished from *A. burti* (as described by (Stunkard, Willey, and Rabinowitz 1941)) and *A. canadensis* by having a long hindbody relative to its forebody, and eggs are longer in *A. mclaughlini* n. sp. than in *A. burti*. *A. mclaughlini* n. sp. has smaller total length and is more robust (wider relative to length in both fore- and hindbody) than *A. canadensis*. The aggregating habit of cercariae of *A. mclaughlini* n. sp. differs from the behavior of cercariae in *Australapatemon bdellocystis* (Lutz, 1921),*A. burti*,*Australapatemon intermedius* (Johnston, 1904),*Australapatemon magnacetabulum* (Dubois, 1988) and *A. minor* (Georges Dubois 1968; Davies and Ostrowski de Nunez 2012) and other genetic lineages herein. The cercariae of *A. mclaughlini* n. sp. are also distinct from cercariae of other lineages genetically characterized herein, as well as from *Cercaria burti* (Miller, 1923), in body, tail, and tegumental spine sizes (Tables 3.5 and 3.10; Figure 3.4). In addition, spines on the body of cercariae of *A. burti* extend only to the ventral sucker (Miller Jr. 1923; Miller Jr. 1926) while those of *A. mclaugh-*

lini n. sp. span the length of the cercarial body (Figure 3.4B). The presence of tegumental spines of cercariae of *A. mclaughlini* n. sp. distinguishes it from *Cercaria laramiensis,* which lacks spines (Hendrickson and Kingston 1974) (Table 3.1, 3.10, and Figure 3.4). The only other described aggregating cercariae possibly belonging to the Diplostomoidea, *Cercaria absurda,* forms chain-like aggregates (H. Miller 1930) rather than the rosette formation seen in *A. mclaughlini* n. sp.

The other four genetically distinguished lineages of *Australapatemon* in which adults were recovered (LIN2, LIN4, LIN8, LIN9) also displayed varying degrees of morphological distinction from species already described in this genus (Table 3.3 and 3.9). The single adult specimen of LIN2 had larger oral and ventral suckers and eggs than *A. burti*,and was smaller in total length, but otherwise similar, to *A. canadensis*. The two adult specimens in LIN4 were smaller in total length and oral sucker width, but with a larger oral sucker, and were otherwise similar to *A. burti*. Compared with *A. canadensis*, adults of LIN4 were smaller in total length and egg size, and had a more spherical forebody. The adult of LIN8 was smaller in total length and oral sucker width, and had a larger pharynx and ventral sucker than *A. burti*, but was otherwise similar to *A. burti*. In comparison to *A. canadensis*, the forebody of LIN8 was more spherical, and the hindbody wider relative to its length. The adult of LIN9 also resembled *A. burti*, although it was smaller in total length, with longer hindbody relative to forebody. It differed from *A. canadensis* in its smaller total length, ventral sucker, genetical and flattened, ovoid forebody shape. However, because most adults from these four lineages were subsampled for DNA extraction, and only 1-2 adults were obtained per lineage (Table 3.7), assessment of morphological variation was not possible and key features for many comparisons were not observed. For example, adults of LIN4, LIN8 resemble both *A. burti* and *Australapatemon congolensis* (Dubois 1956), two species usually discriminated by dimensions of the genital cone (Dubois 1968), a structure obscured by vitelline fields in vouchers of these lineages. Moreover, high divergence in *cox1* within one lineage (LIN9) may indicate the presences of additional species (see discussion). For these reasons,

Lineages 2, 4, 8 and 9 were not identified or described as new species based on this material.

3.4 Discussion

Historically, the status of *A. burti* (Miller, 1923) has been in a state of flux. When still referred to as *Cercaria burti*, it was believed that these cercariae may be the larval form of *Apatemon gracilis* (Rudolphi, 1819), a worm commonly found in palmate birds across Europe, Japan, and North America. It was determined, however, that because *C. burti* had a longer metacercarial developmental period and utilized pulmonate snails in North America, it was likely a different species from *A. gracilis*, known to infect branchiate snails (Stunkard, Willey, and Rabinowitz 1941). However, since then, *A. burti* has been one of the most common cercariae found in Europe among pulmonate snails, despite no reports of adult *A. burti* in this region ((Blasco-Costa, Cutmore, et al. 2016; Faltýnková, Niewiadomska, et al. 2007) and references within).

The distinction between *Apatemon* and *Australapatemon* has also been questioned, with the latter considered a subgenus of *Apatemon* by Dubois (1968). Recent authorities, however, consider *Australapatemon* a valid genus, distinguished from *Apatemon* by a well-defined genital cone in the adult (Niewiadomska 2002). This was supported by the phylogenetic analyses of Blasco-Costa et al. (2016) based on two species of *Australapatemon* and several species of *Apatemon*. Blasco-Costa et al. (2016) noted high variation in *cox1* (6.3-13.1%) within four isolates of *A. burti* from Mexico (Hernández-Mena, García-Prieto, and Martín García-Varela 2014) studied here and predicted that further analyses may reveal cryptic species among this group, and among other strigeid genera. Our results support this prediction, revealing nine strongly supported lineages nested within *A. burti* from Mexico, and separate from *A. niewiadomski* from New Zealand, the only two species from which molecular data were available. With data linking larvae to adults across North America, it is apparent that these nine lineages are supported by statis-

tically significant distinctions in host use, morphological characters that do not overlap with other known North American *Australapatemon* species, and by high *cox1* sequence divergence between monophyletic clades. The molecular, morphological and host-use data gathered here suggests four adults identified as *A. burti* (Hernández-Mena, García-Prieto, and Martín García-Varela 2014) belong to four separate species with ranges extending into the USA and Canada. The prior collections in Mexico include *A. diazi* (JX977727.1-LIN1), *Anas cyanoptera* (Vieillot, 1816) (JX977726.1-LIN6), *A. americana* (JX977725.1-LIN7, *A. mclaughlini* n. sp.), and *O. jamaicensis* (JX977728.1-LIN8) (Hernández-Mena, García-Prieto, and Martín García-Varela 2014).

Among the samples considered here, LIN1 appears the most likely to contain *A. burti* as described by Miller (1923, 1926) and others (Cort 1928; Stunkard, Willey, and Rabinowitz 1941). The wide geographic distribution and diversity of snails infected by LIN1 are consistent with this identification. *Australapatemon* (*Cercaria*)*burti* was described from *Helisoma* (*Planorbis*) *trivolvis* in Burt Lake, Michigan (Miller Jr. 1923; Miller Jr. 1926),and found in *Lymnaea humilis* (*modicella*)(Say, 1822)in the type locality soon after (Cort 1928), suggesting early on that this species is a generalist. Similarly, cercariae of LIN1, the only first-intermediate-host generalist in this report, were found emerging from *H. trivolvis* (MGC1179 in Table 3.5 and 3.7 and Figures 3.1 and 3.3), *H. campanulatum*, *Lymnaea stagnalis* (Linnaeus, 1758), *S. elodes* (MGC1557 in Table 3.5, 3.7 and Figure 3.1), and *P. gyrina* (Figure 3.1), from localities in Nova Scotia, Alberta, and California, which together encompass the type locality. All other lineages reported here infect a single snail species, none of which belong to *Helisoma* or *Lymnaea*, the hosts associated with *A. burti* in the type locality. This line of evidence also suggests members of LIN4, 8 and 9 are not *A. burti,* despite morphological resemblance of adults to those described by Stunkard et al. (1941). Unfortunately, we encountered no adults from LIN1, but the samples from *A. diazi* studied by (Hernández-Mena, García-Prieto, and Martín García-Varela 2014) were also evidently similar to *A. burti*. *Anas diazi* is limited to the lower Southwestern USA and Mexico (Lepage, Vaidya, and Guralnick 2014), implying that another definitive host

species must be transmitting LIN1 to snails in Alberta, where this bird has never been reported (Committee 2015). Previous studieshave reported *A. burti* from at least ten other anatid species (see Blasco-Costa et al. 2016 and reference within). Further sampling is needed to better understand definitive host specificity. However, if LIN1 does truly represent *A. burti*, then it would not be surprising to find a wide variety of anatids can be infected with members of this lineage.

However, several factors suggest an alternative hypothesis for the high genetic diversity within LIN1, namely that it is comprised of multiple, recently derived species. For example, within our molecular phylogeny, the cercariae that utilize *Helisoma* species display greater nucleotide substitutions within *cox1* than those from other snail hosts. Also, while the mean intraspecific *cox1* divergence in LIN1 is under the 5% cut-off, the range extends to 6.5%, suggesting more than one species within this lineage by this measure alone, even if no partition was made in ABGD barcode gap analysis. There is also significant variation in cercarial morphometrics within LIN1 (Table 3.4-3.5). Different snail hosts could account for this phenotypic variability in LIN1, but it is also possible that multiple, closely related, host-specific species lie within it.

Isolates of LIN9 also display high intra-clade *cox1* divergence (1.0-6.8%) that exceeds the 5% cut-off hypothesis for species delimitation, but again, no further distinction was indicated by ABGD. Sample MGC1376 is 6.5-6.8% different from all other representatives in LIN9, yet there was no significant morphometric difference between this and other isolates in LIN9 (MGC1360). In both LIN1 and LIN9, we predict that further sampling, more detailed morphological analysis, and data from other molecular markers may support separate species within these lineages.

The aggregating habit of cercariae of *A. mclaughlini* n. sp. highlights the importance of pairing classical approaches with molecular analyses. By taking such a combined approach, our results can be shown to support the hypothesis of Cable and McLean (1943) that aggregation has evolved independently as a secondary specialization, in this case, within a member of the genus *Aus-*

tralapatemon. Thishas implications for the life cycle of *A. mclaughlini* n. sp. and the taxonomy of the genus *Australapatemon*. One of the characters distinguishing *Australapatemon* from *Apatemon* is that metacercariae of members of the former genus are found in leeches, rather than fish (David Blair 1976; Blasco-Costa, Cutmore, et al. 2016; Dubois 1968; Johnston and Angel 1951; McCarthy 1990; Negm-Eldin and R. W. Davies 2002; Niewiadomska 2002; Stunkard, Willey, and Rabinowitz 1941; Vojtek 1964). The spherical structure and loss of the ability to swim *en masse* of zygocercariae is generally thought to imply ingestion by a fish host, but only Dronen (1973) has successfully infected fish with (echinostomatid) zygocercous cercariae. Notably, Hendrickson and Kingston (1974) were unable to infect fish with zygocercous cercariae closely resembling the isolates we collected. This raises several possibilities for the zygocercous phenotype of *A. mclaughlini* n. sp. and the potential mechanism for infecting its next host, namely 1) external penetration of leech tegument, a counter-intuitive strategy for aggregating cercariae, 2) ingestion by leeches, which generally lack fine-scale visually acuity (Harley, Cienfuegos, and Wagenaar 2011; Harley, Rossi, et al. 2013), 3) ingestion by a fish, rather than a hirudinid second intermediate host, or 4) loss of the second intermediate host altogether, with ingestion of the aggregate by the definitive host. The latter is possible because both bird hosts of *A. mclaughlini* n. sp., *A. acuta* and *A. americana,* are dabbling ducks that mainly eat bits of vegetation in water, and other small items like invertebrates. Davies and Ostrowski de Núñez (2012) noted a similar incongruence in the life cycle of *A. magnacetabulum*, in which infection of leeches was verified experimentally, but which was described from birds (*Rupornis magnirostris* (Gmelin, 1788)*, Strix rufipes* (King, 1828)) (Dubois 1985) not known to feed on leeches.

The encounter of multiple lineages in a strigeid morphotype may not seem surprising, considering the diversity that has emerged in the sister family Diplostomidae (Sean A Locke, Caffara, et al. 2015) and the intraspecific plasticity and genetic diversity among the Strigeidae ((Blasco-Costa, Cutmore, et al. 2016) and references within). However, the diversity encountered here is nonetheless remarkable; we are unaware of a molecular survey revealing nine

candidate species within one nominal digenetic trematode. Most other studies report much less cryptic diversity, and the number of cryptic species encountered is driven by sampling effort, both in the Diplostomoidea (Blasco-Costa and Sean A Locke 2017) and other parasitic and free-living taxa (R. Poulin and G. Pérez-Ponce de León 2017; Robert Poulin and Presswell 2016). Interestingly, in the only study of digenetic trematodes reporting a comparable number of lineages (8) within a single morphotype, sampling effort was much larger than herein (324 isolates sequenced by (Miura, A. Kuris, and Torchin 2005), versus 97 sequenced herein). Our material was collected mainly for the purposes of molecular analysis, and one disappointing consequence is that most of the lineages could not be described or identified. However, morphological and ecological distinctions were nonetheless observed among vouchers of these lineages, several of which were considered a single species by researchers with deep expertise in molecular phylogenetics and morphological taxonomy (Hernández-Mena, García-Prieto, and Martín García-Varela 2014). Further identifications or taxonomic descriptions will require additional collections, but the general finding of (at least) nine species of *Australapatemon* in North America has implications for diversity and distribution of species in this genus. For one, the two-to-three species of *Australapatemon* known in North America (*A. burti*, *A. canadensis* and possibly *A. anseris*) (Dubois 1968; M.E. 1974) clearly do not represent the true diversity of the genus in the Nearctic. Although the migratory ranges of anatids and other definitive hosts make wide distributions in all species in *Australapatemon*, most are known from a single biogeographic region (D. Davies and Ostrowski de Nunez 2012; Dubois 1968; M.E. 1974). Moreover, few digeneans that mature in birds have been confirmed from more than one biogeographic region with DNA sequences. There are two noteworthy exceptions: a recent molecular study of the diplostomid, *Austrodiplostomum ostrowskiae,* infecting double-crested cormorants, revealed its range extends to both the Nearctic and Neotropics (M. García-Varela et al. 2016); and perhaps the most suggestive exception is *Trichobilharzia querquedulae* (McLeod, 1937), a schistosome parasite of anatids that Ebbs et al. (2016) (Ebbs et al. 2016) foundto be globally distributed. Despite these exceptions, it is more

common to find putatively cosmopolitan avian parasites to be made up of geographically isolated, genetically distinct lineages (Caffara et al. 2011; Sean A Locke, Caffara, et al. 2015). This suggests that the lineages reported here likely include undescribed species, rather than new North American records of existing species of *Australapatemon*, and that molecular verification is desirable for species described in North America and reported in South America (Drago, Lía I. Lunaschi, et al. 2007; Drago and Lía Inés Lunaschi 2010).Taken together, these results add to our understanding of the life cycles of these parasites—linking larvae to adults and other larval stages—and extend our knowledge of regional trematode distributions and local biodiversity.

To characterize diversity within the *A. burti* group, and the genus *Australapatemon*,molecular data are needed from the other seven species in this genus: *Australapatemon minor* (Yamaguti, 1933), *A. bdellocystis* (Lutz, 1921), *A. fuhrmanni* (Dubois, 1937), *A. canadensis* (Dubois & Rausch, 1950), *A. congolensis* (Dubois et Fain, 1956), *A. anseris* (Dubois, 1967), and *A. magnacetabulum* (Dubois, 1988). Further studies may reveal even greater species diversity within the genus *Australapatemon*, and likely within other genera of the Strigeidae.

3.5 Conclusions

Though the field of taxonomy has been around for as long as humans have had language (Raven, Berlin, and Breedlove 1971), in only the past few decades have molecules been the focus of taxonomic classifications, confirming previously discovered patterns and opening our eyes to new ones. The utility of DNA barcoding has no doubt revolutionized the field and has allowed us to understand the evolution and relatedness of species in a whole new light, and with relative ease. However, this method is not a cure-all for our species identification woes. In fact, it remains a highly debatable practice (T. R. Gregory 2005; Hickerson, Meyer, and Moritz 2006; Rubinoff, Cameron, and Will 2006), as it cannot be applied to all groups of animals in the same way, because evolutionary rates in mitochondrial genes are known to vary among

metazoan taxa (Saccone et al. 1999). Additionally, in order for new barcode sequences to be informative to molecular phylogenies, they rely on populated databases of the same gene region for closely related species (further discussion of this can be found in Chapter 4). In other words, a strong foundation for species hypotheses is needed before testing their relationships with genetics. A reoccurring theme throughout this thesis, as demonstrated in this chapter and the previous, is that the identification of species must be an integrative process that applies multiple methods and lines of evidence. Specifically, for digenean trematodes, this includes morphological descriptions and measurements, well-supported, molecular phylogenetics that ideally cover more than one gene-region, host species identifications, knowledge of their geographical distributions, and previous descriptions from the literature. This process is far from trivial.

As exemplified in this chapter, it can be very difficult to attain all the pieces of information needed to make an accurate species identification. Specifically, there were many newly discovered lineages within *Australapatemon*, based on the molecular phylogenies presented, but only enough information at this time was available for a single species description. While in the next chapters, as much information as possible is used to describe trematode diversity, communities, and their relationship to the transmission of swimmer's itch, there remains an extensive quantity of information to be attained by future researchers. I have attempted to lay the foundation for this future research in Alberta by being the first to characterize trematode diversity and communities at a fine-scale level, from which future research can build upon.

Table 3.1: Review of zygocercous and aggregating cercariae.

Superfamily	Predicted Family	Trematode	Founder	Location	Snail Host	Number of Aggregates	Phototaxis	Aggregation method
Opisthorchioidea	Heterophyidae	*Heron Island Zygocercaria*	Beuret & Pearson, 1994	Heron Island coral cay in the Capricornia Section of the Great Barrier Reef	*Clypeomorus batillariaeformis*	Few to several hundred (up to 700)	Positive	Tail gripping by posterior half of tail
Opisthorchioidea		*Cercariae W*	Miller, 1929	Tortugas, Loggerhead Key	*Cerithium litteratum*	35	Positive	Adherent properties to slender terminal portion of tail
Opisthorchioidea	Heterophyidae	*Cercaria caribbea LXX*	Cable, 1963	Awa di Oostpunt, Curacao	*Cerithium litteratum*	A few to many	Positive	Slender terminal portion of tail firmly entangled
Opisthorchioidea	Heterophyidae	*Cercaria caribbea XVI*	Cable, 1956	Punta Arenas near Joyuda, Puerto Rico	*Cerithium algicola*	'small numbers as well as many separate larva"	Unknown	Slender terminal portion of tail attaches, not very adherent
Renicoloidea	Renicolidae	*Cercaria buchanani*	Martin & Gregory, 1951	Playa del Ray, California	*Cerithidia californica*	200–300	Unknown	Adherent properties to proximal portion of tail
Diplostomoidea		*Cercaria absurda*	Miller, 1927	San Juan Island, Washington	Several species of *Planorbis*	3–4	Unknown	Chain-like aggregates, not typical zygocercous type
Diplostomoidea	Clinostomatidae, Strigeidae, or Diplostomidae	*Cercaria laramiensis*	Hendrickson & Kingston, 1974	Runoff pond of the Big Laramie River near Interstate 80, Wyoming	*Physa gyrina* (Say)	4–150	Unknown	'muscular 'prongs' on the distal portion of the furcae and¡U+0085¿mucoid secretion"
Diplostomoidea	Strigeidae	*Australapatemon mclaughlini* n. sp.	Gordy et al., 2017	Rochon Sands, Buffalo Lake, Alberta, Canada	*Physella gyrina* (Say)	4–44	Unknown	Furcae firmly entangled
Echinostomatoidea		*Cercaria radiata*	Komiya, 1941	Outskirts of Shanghai in Chinese waters	*Melanoides fortunei*	20–25	Unknown	Slender terminal portion of tail attaches
Echinostomatoidea		*Cercaria gorgonocephala*	Ward, 1916	Lake Erie near Put-in-Bay, Ohio	*Goniobasis sp.* (Williams, 1931)	50–60	Unknown	Unknown
Echinostomatoidea	Psilostomatidae, Echinostomatidae	*Cercaria gorgonocephala*	Ward, 1916	Douglas Lake, Michigan	*Goniobasis livescens*	32–40	Positive	Slender terminal portion of tail firmly entangled
Echinostomatoidea	Psilostomatidae	*Cercaria gorgonocephala*	Ward, 1916	Allsea River, Oregon	*Oxytrema silicula*	50–60	Unknown	Adhesive material used to form aggregates after leaving the snail
Bucephaloidea		*Cercaria pleuromerae*	Wardle, 1988	Gulf of Mexico, southeast of New Orleans, Louisiana	*Pleuromeris armilla*	Unknown	Unknown	Form a cercarial net, not typical zygocercous type
		Cercaria clausii	Monticelli, 1888	Captiva Island, Florida	*Lamellaria leucospaera*	10–20	Unknown	Unknown
		Cercaria clausii	Monticelli, 1888	Rovigno, Adriatic Sea	*Trivia europea*	Unknown	Unknown	Unknown

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Table 3.1 – *Continued from previous page*

Superfamily	Predicted Family	Trematode	Max aggregates/day	Pigmentation	Body Length/ Width (μ m)	Tail Length/ Width (μ m)	Other features	Reference(s)
Opisthorchioidea	Heterophyidae	*Heron Island Zygocercaria*	11	Anterior tail heavily to lightly orange pigmented	53.5–80/23–32.5	579.5–744.5/26–55.5	Each body oriented in same direction; cercariae are oriented in a tight dextral or sinistral spiral; dorsal fin fold on body; up to 4 aggregates joined together; oral sucker modified into a penetration organ with 3 spines on anterior lip; eyespots; 7 pairs prevesicular penetration glands; oval epithelial excretory bladder	Beuret, J. & Pearson, J.C. 1994. Description of a new zygocercous cercaria (Opisthorchioidea: Heterophyidae) from prosobranch gastropods collected at Heron Island (Great Barrier Reef, Australia) and a review of zygocercariae. Syst. Parasitol. 27: 105–125.
Opisthorchioidea		*Cercariae W*	Unknown	Unknown	Unknown	Unknown		Miller, H. M. 1929. Continuation of study on bahvior and reactions of marine cercariae from Tortugas. Carnegie Institute of Washington Yearbook 28 (for 1928–29): 292–294.
Opisthorchioidea	Heterophyidae	*Cercaria caribbea LXX*	Unknown	Reddish-brown in tail	92–97/50–56	660–790/124–127	Entire cuticle covered in fine spines; spherical eye spots; develop in rediae; aggregates not spherical	Cable, R.M. 1963. Marine cercariae from Curacao and Jamaica. Z. Parasitol. 23: 429–469.
Opisthorchioidea	Heterophyidae	*Cercaria caribbea XVI*	Unknown	Primarily in the narrow posterior tail	100–110/50–54	1400/135–140	Spherical eye spots and spines over entire body, develop in rediae	Cable, R.M. 1956. Marine cercariae of Puerto Rico. Scientific Survey of Porto Rico and Virgin Islands. Vol XVI-Part 4. 491–577.
Renicoloidea	Renicolidae	*Cercaria buchanani*	Unknown	Tail light brown	195–290/53–98	204/131	Develop in sporocysts; emerge from snail individually, then clump; moved slowly and haphazardly in water	Martin, W.E. & Gregory, V.L. 1951. *Cercaria buchanani* n.sp., an Aggregating Marine Trematode. Trans. Am. Micro. Soc. 70(4): 359–362

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Table 3.1 – *Continued from previous page*

Superfamily	Predicted Family	Trematode	Max aggregates/day	Pigmentation	Body Length/ Width (μ m)	Tail Length/ Width (μ m)	Other features	Reference(s)
Diplostomoidea		*Cercaria absurda*	Unknown	Unknown	183/55	285/114	Spination on body and on tail stem. Tail spines on distal half of tail stem. Furcae 80um long by 30 μ m wide at base. Does not swim freely.	Miller, H. M. 1930. Formations and behaviour of aggregations of cercariae. J. Parasitol, 17, 111–112; Miller, H.M. 1927. Furcocercous Larval Trematodes from San Juan Island, Washington. Parasitol. 19(01):61–83.
Diplostomoidea	Clinostomatidae, Strigeidae, or Diplostomidae	*Cercaria laramiensis*	Unknown	Unknown	91–150/46–71	230–260/41–60	Develop in sporocysts; furcae do not directly intertwine but are in a mass of mucus in the center; body non-spinose	Hendrickson, G.L. & Kingston, N. 1974. *Cercraia laramiensis* sp. n. a freshwater Zygocercous cercaria from *Physa gyrina* Say, with a discussion of cercarial aggregation. J. Parasitol. 60(5):777-781.
Diplostomoidea	Strigeidae	*Australapatemon mclaughlini* n. sp.	20	None	78.7–148.1/29.8–46.6	101.2–210.3/18.7–26.6	Spines on body and furcae; distinct papules on tail stem	This manuscript
Echinostomatoidea		*Cercaria radiata*	Unknown	Unknown	110/60	450/60	Develop in rediae; form radial aggregates; No spines on body or tail	Komiya, Y. 1941. A new "Zygocercaria", *Cercaria radiata*, and its excretory system (Cercaria from Chinese fresh waters No. 3). J. Shanghai Sci. Inst. N.S. 1(3): 229–232.
Echinostomatoidea		*Cercaria gorgonocephala*	Unknown	Unknown	Unknown	Unknown		Ward, H. B. 1916. Notes on two free-living larval trematodes from North America. J. Parasitol., 3: 10–20; Williams, S.R. 1931. Observations on *Cercaria gorgonocephala* Ward. Ohio J. Sci., 31:115–119.
Echinostomatoidea	Psilostomatidae, Echinostomatidae	*Cercaria gorgonocephala*	4–6 every third day	Light green or white to dark brown	153–168/86–102	Unknown	Develop in rediae; Tail dvided into two regions, of which the smaller distal ends knot up and stick to one side of the aggregate; move about by thrashing motions; metacercaria encyst in buccal cavity of fish and form spines after 8 days.	Dronen, N. O., Jr. 1973. Studies on macrocercous cercariae of the Douglas Lake, Michigan area. Tr. Am. Micr. Soc. 92: 641–648.

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Table 3.1 – *Continued from previous page*

Superfamily	Predicted Family	Trematode	Max aggregates/day	Pigmentation	Body Length/ Width (μ m)	Tail Length/ Width (μ m)	Other features	Reference(s)
Echinostomatoidea	Psilostomatidae	*Cercaria gorgonocephala*	Unknown	Proximal half of tail contains yellow-brown granules	110–160/50–80	460–700/42–70		Martin, W.E. 1968. *Cercaria gorgonocephala* Ward, 1916, a Zygocercous species in Northwestern United States. Trans. Am. Microscop. Soc., 87(4) 472–476.
Bucephaloidea		*Cercaria pleuromerae*	Unknown	Unknown	143–175/30–50	28–31/48–58	Develop in sporocysts; infect a clam	Wardle, W.J. 1988. A Bucephalid larva, *Cercaria pleuromerae* n. sp. (Trematoda: Digenea), parasitizing a deepwater bivalve from the Gulf of Mexico. J. Parsitol 74(4):692–694.
		Cercaria clausii	Unknown	Unknown	Unknown	Unknown	Deveop in rediae; eye spots; hair like structures on tail	Cable, R.M. & McLean, R.A. 1943. The occurrence of *Cercaria clausii* Monticelli, a marine Rattenkonig larval trematode, on the west coast of Florida. Not. Nat., 129:1–7.
		Cercaria clausii	Unknown	Unknown	Unknown	Unknown		Pintner, T. 1891. Ueber *Cercaria clausii* Monticelli, Arbeiten aus dem Zoologischen Instituten der Universitat Wien, 9:285–294.

Table 3.2a: Estimates of evolutionary divergence over sequence pairs between and within groups among three genetic markers (*cox1*, 28S, ITS1-5.8S-ITS2)

cox1

	A.sp. 'jamiesoni'	*A.sp*1	*A*.sp 3	*A*.sp 4	*Ap. cornu*	*Ap.pipientis*	*Au.niew*	LIN1	LIN2	LIN3	LIN4	LIN5	LIN6	LIN7	LIN8	LIN9
Apatemon sp. 'jamiesoni'	**0.003**	0.013	0.015	0.016	0.016	0.017	0.017	0.018	0.018	0.018	0.017	0.019	0.018	0.018	0.018	0.017
Apatemon sp. 1	0.111	**0.057**	0.013	0.013	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.015	0.014
Apatemon sp. 3	0.107	0.097	**0.000**	0.015	0.016	0.017	0.017	0.016	0.017	0.017	0.016	0.017	0.017	0.017	0.016	0.015
Apatemon sp. 4	0.122	0.106	0.099	**0.003**	0.017	0.018	0.017	0.017	0.017	0.017	0.017	0.018	0.017	0.018	0.017	0.017
Apharyngostrigea cornu	0.15	0.159	0.145	0.15	**0.078**	0.007	0.016	0.016	0.017	0.017	0.016	0.017	0.016	0.017	0.016	0.017
Apharyngostrigea pipientis	0.143	0.151	0.143	0.145	0.041	**0.000**	0.016	0.017	0.017	0.017	0.017	0.018	0.017	0.017	0.017	0.017
Australapatemon niewiadomski	0.149	0.153	0.151	0.15	0.156	0.143	**0.014**	0.015	0.016	0.016	0.015	0.017	0.016	0.016	0.016	0.016
Australapatemon burti LIN1	0.152	0.145	0.136	0.149	0.153	0.148	0.126	**0.018**	0.011	0.013	0.013	0.014	0.012	0.013	0.014	0.013
LIN2	0.162	0.157	0.16	0.159	0.173	0.163	0.136	0.074	-	0.013	0.013	0.016	0.013	0.013	0.015	0.013
LIN3	0.167	0.163	0.138	0.159	0.173	0.165	0.131	0.09	0.09	-	0.014	0.015	0.015	0.014	0.016	0.014
LIN4	0.166	0.157	0.143	0.156	0.169	0.165	0.133	0.094	0.097	0.108	**0.035**	0.015	0.012	0.013	0.013	0.014
LIN5	0.177	0.152	0.145	0.173	0.163	0.163	0.14	0.107	0.13	0.12	0.112	-	0.013	0.014	0.017	0.015
LIN6	0.149	0.143	0.14	0.142	0.162	0.16	0.136	0.085	0.091	0.109	0.083	0.093	**0.022**	0.011	0.014	0.014
Australapatemon mclaughlini n. sp. LIN7	0.157	0.147	0.147	0.166	0.17	0.161	0.141	0.096	0.083	0.108	0.103	0.097	**0.067**	**0.002**	0.015	0.015
LIN8	0.147	0.145	0.129	0.14	0.15	0.138	0.131	0.101	0.112	0.119	0.106	**0.144**	0.107	0.125	**0.003**	0.013
LIN9	0.147	0.14	0.122	0.153	0.17	0.16	0.135	0.101	0.094	0.117	0.113	0.119	0.105	0.11	0.092	**0.030**

(a) (b) (c) Each marker is represented by a matrix. Below the diagonal is the number of base pair differences per site from averaging over all sequence pairs between groups (interspecific divergence values), otherwise referred to as *p* distance values. Minimum and maximum mean interspecific divergence values among *A. burti* lineages are in bold and underlined. Standard error estimates are based on 1000 bootstrap replicates and above the diagonal. The number of base pair differences per site from averaging over all sequence pairs within each group (intraspecific divergence values) is shown along the diagonal in bold. Missing values, represented by a minus sign, are present for groups that contain singletons, and therefore estimates cannot be made. For *cox1*, the analysis involved 120 nucleotide sequences with a total of 399 positions in the final dataset. For 28S, the analysis involved 14 nucleotide sequences with a total of 807 positions in the final dataset. Finally, for ITS1-5.8S-ITS2, the analysis involved 26 nucleotide sequences with a total of 482 positions in the final dataset. All positions containing gaps and missing data were eliminated

Table 3.2b: Estimates of evolutionary divergence over sequence pairs between and within groups among three genetic markers (cox1, 28S, ITS1-5.8S-ITS2)

28s

	D. phoxini	*D.* sp.	*Ic. Erraticus*	*C. longicollis*	*A.* sp. 'jamiesoni'	*Ap. pipientis*	*Ap. cornu*	LIN9	LIN7	LIN1	*R. ovata*	LIN8	*Au. niewiad*
Diplostomum phoxini	-	0.004	0.008	0.008	0.009	0.008	0.008	0.01	0.009	0.009	0.009	0.01	0.01
Diplostomum sp.	0.012	-	0.009	0.008	0.009	0.008	0.008	0.01	0.01	0.01	0.01	0.01	0.01
Ichthyocotylurus erraticus	0.061	0.067	-	0.008	0.009	0.009	0.009	0.01	0.009	0.009	0.009	0.01	0.009
Cardiocephaloides longicollis	0.055	0.059	0.053	-	0.009	0.009	0.009	0.01	0.01	0.01	0.01	0.011	0.011
Apatemon sp. 'jamiesoni'	0.068	0.077	0.072	0.078	-	0.005	0.006	0.007	0.007	0.007	0.007	0.007	0.007
Apharyngostrigea pipientis	0.063	0.067	0.072	0.068	0.026	-	0.002	0.008	0.007	0.007	0.007	0.008	0.008
Apharyngostrigea cornu	0.064	0.068	0.076	0.069	0.03	0.004	-	0.008	0.007	0.007	0.007	0.008	0.008
LIN9	0.078	0.082	0.082	0.086	0.046	0.05	0.05	-	0.003	0.003	0.003	0.003	0.004
Australapatemon mclaughlini n. sp. LIN7	0.074	0.078	0.081	0.084	0.047	0.046	0.046	0.006	-	0	0	0.002	0.003
Australapatemon burti LIN1	0.074	0.078	0.081	0.084	0.047	0.046	0.046	0.006	**0.000**	-	0	0.002	0.003
Uncultured organism from *Radix ovata*	0.074	0.078	0.081	0.084	0.047	0.046	0.046	0.006	0	0	-	0.002	0.003
LIN8	0.077	0.081	0.081	0.089	0.047	0.051	0.051	0.009	0.005	0.005	0.005	-	0.003
Australapatemon niewiadomski	0.081	0.084	0.081	0.089	0.047	0.051	0.051	**0.012**	0.009	0.009	0.009	0.006	**0.000**

Table 3.2c: Estimates of evolutionary divergence over sequence pairs between and within groups among three genetic markers (cox1, 28S, ITS1-5.8S-ITS2)

ITS1-5.8s-ITS2

	T. scheuringi	*T. excavata*	*T. clavata*	*C. gallinulae*	*Ic. pilieatus*	*Ca. sp.*	*Ca. medioconiger*	*Ap. pipientis*	*Ap. cornu*	*P. plataleae*	*P. diovadena*	*P. cincta*	*A.* sp. 3
Tylodelphys scheuringi	-	0.01	0.011	0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.015	0.015	0.014
Tylodelphys excavata	0.06	-	0.007	0.016	0.015	0.016	0.015	0.016	0.016	0.017	0.017	0.017	0.015
Tylodelphys clavata	0.06	0.027	-	0.016	0.015	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.015
Cotylurus gallinulae	0.145	0.154	0.156	-	0.011	0.015	0.015	0.016	0.016	0.016	0.016	0.016	0.015
Ichthyocotylurus pilieatus	0.135	0.154	0.156	0.071	-	0.014	0.014	0.015	0.015	0.016	0.016	0.016	0.014
Cardiocephaloides sp.	0.139	0.158	0.154	0.129	0.114	-	0.004	0.015	0.015	0.015	0.015	0.015	0.015
Cardiocephaloides medioconiger	0.139	0.156	0.151	0.131	0.112	0.01	-	0.015	0.015	0.015	0.015	0.015	0.015
Apharyngostrigea pipientis	0.145	0.162	0.164	0.143	0.135	0.131	0.129	-	0	0.011	0.011	0.011	0.011
Apharyngostrigea cornu	0.145	0.162	0.164	0.143	0.135	0.131	0.129	0	-	0.011	0.011	0.011	0.011
Parastrigea plataleae	0.164	0.187	0.18	0.154	0.137	0.137	0.139	0.066	0.066	-	0.005	0.005	0.011
Parastrigea diovadena	0.16	0.183	0.176	0.158	0.145	0.137	0.139	0.071	0.071	0.012	-	0.003	0.011
Parastrigea cincta	0.162	0.185	0.178	0.156	0.143	0.135	0.137	0.068	0.068	0.01	0.006	-	0.011
Apatemon sp. 3	0.124	0.147	0.158	0.122	0.118	0.112	0.114	0.058	0.058	0.064	0.073	0.071	**0.012**
Apatemon sp. 3	0.135	0.156	0.164	0.133	0.129	0.122	0.124	0.064	0.064	0.066	0.075	0.073	0.01
Apatemon sp. 'jamiesoni'	0.129	0.151	0.162	0.127	0.122	0.116	0.118	0.058	0.058	0.068	0.077	0.075	0.004
Apatemon gracilis	0.131	0.154	0.16	0.131	0.118	0.118	0.12	0.062	0.062	0.068	0.077	0.075	0.008
Apatemon gracilis	0.131	0.154	0.162	0.131	0.118	0.118	0.12	0.062	0.062	0.071	0.079	0.077	0.008
Apatemon sp. 1	0.133	0.154	0.162	0.131	0.127	0.12	0.122	0.062	0.062	0.068	0.077	0.075	0.008
Apatemon sp. 2	0.135	0.156	0.164	0.133	0.129	0.122	0.124	0.064	0.064	0.071	0.079	0.073	0.01
Australapatemon niewiadomski	0.135	0.154	0.16	0.135	0.118	0.122	0.12	0.068	0.068	0.085	0.093	0.087	0.033
Australapatemon mclaughlini n. sp. LIN7	0.139	0.154	0.16	0.139	0.127	0.129	0.127	0.075	0.075	0.091	0.1	0.093	0.029
Australapatemon burti LIN1 (CAN)	0.135	0.154	0.16	0.135	0.122	0.124	0.122	0.071	0.071	0.087	0.095	0.089	0.025
Australapatemon burti LIN1 (MEX)	0.135	0.154	0.16	0.135	0.122	0.124	0.122	0.075	0.075	0.087	0.095	0.089	0.029
LIN8	0.137	0.154	0.162	0.139	0.124	0.127	0.124	0.073	0.073	0.091	0.1	0.093	0.035
LIN9	0.133	0.151	0.158	0.139	0.124	0.127	0.124	0.073	0.073	0.091	0.1	0.093	0.031

Table 3.2c: Columns continued...

ITS1-5.8s-ITS2	*A.* sp. 3	*A.* sp. 'jamiesoni'	*A. gracilis*	*A. gracilis*	*A.* sp. 1	*A.* sp. 2	*Au. niewiad*	LIN7	LIN1	LIN1	LIN8	LIN9	
Tylodelphys scheuringi	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	
Tylodelphys excavata	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	
Tylodelphys clavata	0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.015	0.015	0.016	0.016	0.015	
Cotylurus gallinulae	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	
Ichthyocotylurus pilieatus	0.015	0.015	0.014	0.014	0.015	0.015	0.014	0.014	0.014	0.014	0.014	0.014	
Cardiocephaloides sp.	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	
Cardiocephaloides medioconiger	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	
Apharyngostrigea pipientis	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.012	0.012	
Apharyngostrigea cornu	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.012	0.012	
Parastrigea plataleae	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.013	0.013	0.012	0.013	0.013	
Parastrigea diovadena	0.012	0.012	0.012	0.012	0.012	0.012	0.013	0.014	0.013	0.013	0.014	0.014	
Parastrigea cincta	0.011	0.012	0.011	0.012	0.012	0.012	0.013	0.013	0.013	0.013	0.013	0.013	
Apatemon sp. 3	0.004	0.003	0.004	0.004	0.004	0.004	0.008	0.007	0.007	0.007	0.008	0.008	
Apatemon sp. 3	-	0.004	0.004	0.005	0.003	0.004	0.009	0.009	0.008	0.009	0.009	0.009	
Apatemon sp. 'jamiesoni'	0.01	-	0.004	0.004	0.004	0.004	0.008	0.008	0.007	0.008	0.009	0.009	
Apatemon gracilis	0.01	0.008	**0.002**	0.002	0.004	0.004	0.008	0.008	0.008	0.008	0.009	0.009	
Apatemon gracilis	0.012	0.008	0.002	**0.002**	0.004	0.005	0.008	0.008	0.008	0.008	0.009	0.009	
Apatemon sp. 1	0.006	0.008	0.008	0.01	-	0.004	0.009	0.008	0.007	0.008	0.008	0.008	
Apatemon sp. 2	0.008	0.01	0.01	0.012	0.006	-	0.009	0.008	0.008	0.008	0.009	0.009	
Australapatemon niewiadomski	0.044	0.037	0.037	0.037	0.037	0.039	-	0.005	0.005	0.006	0.006	0.006	
Australapatemon mclaughlini n. sp. LIN7	0.039	0.033	0.035	0.035	0.033	0.035	0.015	-	0.003	0.004	0.006	0.005	
Australapatemon burti LIN1 (CAN)	0.035	0.029	0.031	0.031	0.029	0.031	0.015	**0.004**	**0.003**	0.003	0.005	0.004	
Australapatemon burti LIN1 (MEX)	0.039	0.033	0.035	0.035	0.033	0.035	0.019	0.008	**0.004**	**0.003**	0.006	0.005	
LIN8	0.041	0.039	0.041	0.041	0.035	0.037	0.019	**0.019**	0.015	**0.019**	-	0.006	
LIN9	0.039	0.035	0.037	0.037	0.035	0.037	0.017	0.01	0.006	0.01	0.017	-	

Table 3.3: Statistics for morphometric comparisons of cercariae of lineages 1, 6, 7, 8, and 9 with those of *Australapatemon* (Cercaria) *burti* (Miller, 1923), *Cercaria laramiensis* (Hendrickson & Kingston, 1974), and *Cercaria absurda* (Miller, 1927). Statistical tests used were Kruskal-Wallis analysis of variance (H), and post hoc multiple comparisons test, with Bonferroni correction for multiple tests. Statistical significance ($\alpha = 0.05$) is indicated by bold text and an asterisk. Only significant comparisons between lineages/species are listed.

	H	df	*P*	n	Significant comparisons	Adjusted *P* (Bonferroni)
Body Length	21.585	7	**0.003***	151	LIN8:LIN7	**0.043***
					LIN8:LIN1	**0.026***
					LIN8:LIN6	**0.018***
					LIN8: *A. burti*[b]	**0.020***
Body Width	75.778	7	**0.000***	150	LIN6:LIN1	**0.000***
					LIN6:LIN9	**0.022***
					LIN6:LIN8	**0.008***
					LIN7:LIN1	**0.000***
					LIN7:LIN9	**0.006***
					LIN7:LIN8	**0.002***
Tail Length	65.776	7	**0.000***	130	LIN8:LIN7	**0.000***
					LIN8: *C. laramiensis*[c]	**0.005***
					LIN9:LIN7	**0.005***
					LIN9: *C. laramiensis*[c]	**0.042***
					LIN6:LIN7	**0.003***
					LIN1:LIN7	**0.000***
Tail Width	56.539	7	**0.000***	118	LIN6:LIN1	**0.004***
					LIN6:LIN7	**0.000***
					LIN6: *C. laramiensis*[c]	**0.039***
					LIN9:LIN7	**0.033***
					LIN1:LIN7	**0.001***

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Table 3.3 – *Continued from previous page*

	H	df	*P*	n	Significant comparisons	Adjusted *P* (Bonferroni)
Furca Length	37.585	7	**0.000***	105	LIN7:LIN1	**0.017***
					LIN6:LIN1	**0.000***
Furca width	24.785	6	**0.000***	100	LIN6:LIN1	**0.002***
					LIN6: *C. laramiensis*[c]	**0.035***
OS to VS length	13.335	5	**0.020***	61		
VS to tail length	8.083	4	0.089	58		
VS length	25.375	4	**0.000***	51	LIN9:LIN1	**0.049***
					LIN7:LIN1	**0.019***
VS width	25.038	4	**0.000***	51	LIN7:LIN1	**0.030***
VS spine length[a]	7.958	2	**0.019***	20		
VS spine width[a]	8.591	2	**0.014***	26		
Body spine length[a]	25.456	2	**0.000***	134	LIN1:LIN7	**0.000***
					LIN6:LIN7	**0.012***
Body spine width[a]	22.977	2	**0.000***	99	LIN1:LIN7	**0.000***
					LIN6:LIN7	**0.028***
Furca spine length[a]	1.908	2	0.385	26		
Furca spine width[a]	16.322	2	**0.000***	39	LIN1:LIN7	**0.002***

VS = Ventral sucker, OS = Oral sucker
a. Data only available for LIN1, LIN6, and LIN7
b. *Australapatemon burti* (Miller, 1923)
c. *Cercaria laramiensis* (Hendrickson & Kingston, 1974)

Table 3.4: Statistics for significant comparisons between cercaria within LIN1. Statistics reported are from a post hoc multiple comparisons test, with Bonferroni correction for multiple tests. Statistical significance ($\alpha = 0.05$) is indicated by bold text and an asterisk.

	Significant comparisons	Adjusted P (Bonferroni)
Body Length	MGC1179:MGC1423	**0.008***
	MGC1423:MGC1417	**0.001***
Tail Length	MGC1413:MGC1424	0.044*
	MGC1413:MGC1427	0.032*
Furca Length	MGC1413:MGC1557	0.001*
	MGC1413:MGC1427	0.006*
	MGC1557:MGC1428	0.012*
	MGC1557:MGC1417	0.023*
	MGC1427:MGC1428	0.034*
	MGC1557:MGC1179	0.041*
VS to Tail Length	MGC1423:MGC1417	0.012*
	MGC1423:MGC1179	0.043*

VS=Ventral Sucker

Table 3.5: Statistics tests of morphometric comparisons of cercariae in Table 3.1, using Kruskal-Wallis analysis of variance (H) and post-hoc multiple comparisons test, with Bonferroni correction. Statistical significance ($\alpha = 0.05$) is indicated by bold text and an asterisk. Only significant comparisons between and within lineages and species are listed.

	H	df	*P*	n	Significant comparisons	Adjusted *P* (Bonferroni)
Body Length	17.714	6	**0.007***	99	1935(LIN7)–1179(LIN1)	**0.010***
Body Width	10.919	6	0.091	98		
Tail Length	42.085	6	**0.000***	78	1557(LIN1)–1935(LIN7)	**0.007***
					1557(LIN1)–*C. laramiensis*	**0.008***
					1744(LIN6)–1935(LIN7)	**0.000***
					1744(LIN6)–*C. laramiensis*	**0.007***
Tail Width	40.307	6	**0.000***	66	1557(LIN1)–1935(LIN7)	**0.017***
					1744(LIN6)–1935(LIN7)	**0.000***
Furca Length	33.456	6	**0.000***	53	1935(LIN7)–1179(LIN1)	**0.000***
					1557(LIN1)–1179(LIN1)	**0.000***
					1744(LIN6)–1179(LIN1)	**0.002***
Furca width	15.615	5	**0.008***	48		
OS to VS length	2.865	4	0.581	19		
VS length	5.418	3	0.144	16		
VS width	4.984	3	0.173	16		
VS to tail length	6.835	3	0.077	16		
VS spine length	9.705	3	**0.021***	20		
VS spine width	12.497	3	**0.006***	26		
Body spine length	38.495	3	**0.000***	134	1557(LIN1)–1744(LIN6)	**0.033***
					1557(LIN1)–1179(LIN1)	**0.006***
					1557(LIN1)–1935(LIN7)	**0.000***
					1744(LIN6)–1935(LIN7)	**0.009***
Body spine width	25.988	3	**0.000***	99	1179(LIN1)–1935(LIN7)	**0.000***
					1557(LIN1)–1935(LIN7)	**0.003***
					1744(LIN6)–1935(LIN7)	**0.021***
Furca spine length	13.341	3	**0.004***	26	1557(LIN1)–1179(LIN1)	**0.015***
					1744(LIN6)–1179(LIN1)	**0.037***
Furca spine width	28.526	3	**0.000***	39	1557(LIN1)–1179(LIN1)	**0.001***
					1557(LIN1)–1935(LIN7)	**0.000***

VS = Ventral sucker, OS = Oral sucker

Table 3.6: Summary data from samples from which DNA sequences were obtained.

Organism	Seq-ID	Collection-date	Lat-Lon	Haplogroup	Host	Developmental-stage	*cox1*	*ITS1-5.8S-ITS2*	28S
Australapatemon burti	MGC4	10-Jun-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207548		
Australapatemon burti	MGC138	24-Jun-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207549		
Australapatemon sp.	MGC173	02-Jul-13	52.459 N -113.973 W	LIN9	*Stagnicola elodes*	C	KY207550		
Australapatemon burti	MGC367	15-Jul-13	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207551		
Australapatemon burti	MGC425	22-Jul-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207552		
Australapatemon burti	MGC471	25-Jul-13	53.56 N -114.44 W	LIN1	*Stagnicola elodes*	C	KY207553		
Australapatemon burti	MGC473	25-Jul-13	53.56 N -114.44 W	LIN1	*Stagnicola elodes*	C	KY207554		
Australapatemon burti	MGC480	29-Jul-13	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207555		
Australapatemon burti	MGC482	29-Jul-13	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207556		
Australapatemon sp.	MGC576	06-Aug-13	53.634 N -114.665 W	LIN9	*Stagnicola elodes*	C	KY207557		
Australapatemon sp.	MGC579	06-Aug-13	53.634 N -114.665 W	LIN9	*Stagnicola elodes*	C	KY207558		
Australapatemon burti	MGC592	06-Aug-13	53.911 N -114.282 W	LIN1	*Stagnicola elodes*	C	KY207559		
Australapatemon burti	MGC601	06-Aug-13	53.911 N -114.282 W	LIN1	*Stagnicola elodes*	C	KY207560		
Australapatemon burti	MGC605	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207561		
Australapatemon burti	MGC609	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207562		
Australapatemon burti	MGC613	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207563		
Australapatemon burti	MGC617	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207564		
Australapatemon burti	MGC622	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207565		
Australapatemon burti	MGC628	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207566		
Australapatemon burti	MGC631	06-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207567		
Australapatemon burti	MGC646	06-Aug-13	53.949 N -114.361 W	LIN1	*Stagnicola elodes*	C	KY207568		
Australapatemon sp.	MGC793	19-Aug-13	53.911 N -114.282 W	LIN4	*Physella gyrina*	C	KY207569		
Australapatemon burti	MGC843	19-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207570		
Australapatemon burti	MGC845	19-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207571		
Australapatemon burti	MGC884	19-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207572		
Australapatemon burti	MGC892	19-Aug-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207573		
Australapatemon burti	MGC893	19-Aug-13	53.911 N -114.282 W	LIN1	*Stagnicola elodes*	C	KY207574		
Australapatemon burti	MGC944	26-Aug-13	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207575		
Australapatemon burti	MGC956	26-Aug-13	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207576		
Australapatemon sp.	MGC957	26-Aug-13	52.459 N -113.973 W	LIN3	*Stagnicola elodes*	C	KY207577		
Australapatemon burti	MGC1125	03-Sep-13	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207578		
Australapatemon burti	MGC1127	03-Sep-13	53.949 N -114.361 W	LIN1	*Stagnicola elodes*	C	KY207579		
Australapatemon burti	MGC1179	09-Jun-14	52.452 N -113.055 W	LIN1	*Helisoma trivolvis*	C	KY207580	KY207626	KY207625
Australapatemon burti	MGC1331	14-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207581		
Australapatemon sp.	MGC1360	21-Jul-14	52.459 N -113.973 W	LIN9	*Stagnicola elodes*	C	KY207582		
Australapatemon sp.	MGC1376	21-Jul-14	52.522 N -112.832 W	LIN9	*Stagnicola elodes*	C	KY207583		
Australapatemon burti	MGC1410	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207584		
Australapatemon burti	MGC1411	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207585		
Australapatemon burti	MGC1413	29-Jul-14	53.634 N -114.665 W	LIN1	*Physella gyrina*	C	KY207586		
Australapatemon sp.	MGC1414	29-Jul-14	53.634 N -114.665 W	LIN8	*Physella gyrina*	C	KY207587		
Australapatemon burti	MGC1417	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207588		
Australapatemon burti	MGC1421	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207589		
Australapatemon burti	MGC1422	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207590		
Australapatemon burti	MGC1423	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207591		
Australapatemon burti	MGC1424	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207592		
Australapatemon burti	MGC1425	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207593		
Australapatemon burti	MGC1427	29-Jul-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207594		
Australapatemon burti	MGC1428	29-Jul-14	53.634 N -114.665 W	LIN1	*Lymnaea stagnalis*	C	KY207595		
Australapatemon sp.	MGC1451	05-Aug-14	52.464 N -112.884 W	LIN9	*Stagnicola elodes*	C	KY207596		
Australapatemon sp.	MGC1456	05-Aug-14	52.464 N -112.884 W	LIN5	*Stagnicola elodes*	C	KY207597		

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Table 3.6 – *Continued from previous page*

Organism	Seq-ID	Collection-date	Lat-Lon	Haplogroup	Host	Developmental-stage	*cox1*	*ITS1-5.8S-ITS2*	28S
Australapatemon burti	MGC1461	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207598		
Australapatemon burti	MGC1469	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207599		
Australapatemon burti	MGC1522	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207600		
Australapatemon burti	MGC1526	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207601		
Australapatemon burti	MGC1527	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207602		
Australapatemon burti	MGC1528	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207603		
Australapatemon burti	MGC1529	05-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207604		
Australapatemon burti	MGC1557	12-Aug-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207605		
Australapatemon burti	MGC1572	12-Aug-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207606		
Australapatemon burti	MGC1578	12-Aug-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207607		
Australapatemon burti	MGC1581	12-Aug-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207608		
Australapatemon burti	MGC1622	12-Aug-14	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207609		
Australapatemon burti	MGC1667	18-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207610		
Australapatemon burti	MGC1671	18-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207611		
Australapatemon burti	MGC1674	18-Aug-14	52.459 N -113.973 W	LIN1	*Stagnicola elodes*	C	KY207612		
Australapatemon sp.	MGC1744	28-Aug-14	53.027 N -114.126 W	LIN6	*Physella gyrina*	C	KY207613		
Australapatemon burti	MGC1745	28-Aug-14	53.56 N -114.44 W	LIN1	*Stagnicola elodes*	C	KY207614		
Australapatemon sp.	MGC1971	20-Jul-15	53.634 N -114.665 W	LIN6	*Physella gyrina*	C	KY207616		
Australapatemon burti	MGC1983	20-Jul-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207617		
Australapatemon burti	MGC1997	20-Jul-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207618		
Australapatemon burti	MGC2133	04-Aug-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207619		
Australapatemon burti	MGC2137	04-Aug-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207620		
Australapatemon burti	MGC2141	04-Aug-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207621		
Australapatemon sp.	MGC2189	10-Aug-15	52.464 N -112.884 W	LIN8	*Physella gyrina*	C	KY207622		
Australapatemon burti	MGC2285	17-Aug-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207623		
Australapatemon burti	MGC2310	17-Aug-15	53.634 N -114.665 W	LIN1	*Stagnicola elodes*	C	KY207624		
Australapatemon burti	FLUKE2172-12	28-May-12	46.0692 N - 60.308 W	LIN1	*Helisoma campanulatum*	C	KY587399		
Australapatemon burti	FLUKE2173-12	28-May-12	46.0692 N - 60.308 W	LIN1	*Helisoma campanulatum*	C	KY587398		
Australapatemon burti	FLUKE2171-12	28-May-12	46.0692 N - 60.308 W	LIN1	*Helisoma campanulatum*	C	KY587394		
Australapatemon sp.	TREMA2131-09	01-Oct-08	50.183 N -98.383 W	LIN4	*Aythya collaris*	A	KY587397		
Australapatemon sp.	TREMA2134-09	01-Oct-08	50.183 N -98.383 W	LIN4	*Aythya collaris*	A	KY587396		
Australapatemon sp.	TREMA2614-10	01-Sep-09	50.183 N -98.383 W	LIN2	*Bucephala albeola*	A	HM385535		
Australapatemon burti	TREMA2377-10	04-Aug-09	California	LIN1	Planorbidae sp.	C	KY587401		
Australapatemon burti	TREMA2441-10	04-Aug-09	California	LIN1	Planorbidae sp.	C	HM385485	KY570948	
Australapatemon burti	TREMA2379-10	26-Jun-09	37.6709 N -121.956 W	LIN1	Planorbidae sp.	C	KY587400		
Australapatemon burti	TREMA2443-10	26-Jun-09	37.6709 N -121.956 W	LIN1	Planorbidae sp.	C	HM385486	KY570947	
Australapatemon sp.	TREMA2617-10	01-Sep-09	50.183 N -98.383 W	LIN8	*Oxyura jamaicensis*	A	HM385538	KY570946	
Australapatemon sp.	TREMA2616-10	01-Sep-09	50.183 N -98.383 W	LIN8	*Oxyura jamaicensis*	A	HM385537		MF124269
Australapatemon sp.	TREMA2615-10	01-Sep-09	50.183 N -98.383 W	LIN8	*Oxyura jamaicensis*	A	HM385536		
Australapatemon sp.	TREMA2613-10	01-Sep-09	50.183 N -98.383 W	LIN9	*Anas acuta*	A	HM385534		MF124270
Australapatemon mclaughlini n. sp.	MGC1935	13-Jul-15	52.464 N -112.884 W	LIN7	*Physella gyrina*	C	KY207615	KY207628	KY207627
Australapatemon mclaughlini n. sp.	TREMA2143-09	01-Oct-08	50.183 N -98.383 W	LIN7	*Anas acuta*	A	KY587395		
Australapatemon mclaughlini n. sp.	TREMA2142-09	01-Oct-08	50.183 N -98.383 W	LIN7	*Anas acuta*	A	KY587402		

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Table 3.6 – *Continued from previous page*

Organism	Seq-ID	Collection-date	Lat-Lon	Haplogroup	Host	Developmental-stage	*cox1*	*ITS1-5.8S-ITS2*	28S
Australapatemon mclaughlini n. sp.	TREMA2141-09	01-Oct-08	50.183 N -98.383 W	LIN7	*Anas acuta*	A	KY587403		
Australapatemon mclaughlini n. sp.	TREMA2139-09	01-Oct-08	50.183 N -98.383 W	LIN7	*Anas acuta*	A	KY587405		
Australapatemon mclaughlini n. sp.	TREMA2138-09	01-Oct-08	50.183 N -98.383 W	LIN7	*Anas acuta*	A	KY587406		
Apatemon sp.	MGC574-Apatemon_sp.	06-Aug-13	53.634 N -114.665 W		*Stagnicola elodes*	C	KT831359		

Table 3.7: Morphometrics from adults of genetically distinguished lineages of *Australapatemon* and comparison with congeners (μm). Data from the present study are reported as mean (range) and ranges are reported from other studies.

	LIN2 (n=1)	LIN4 (n=2)	LIN7 (n=7)	LIN8 (n=1)	LIN9 (n=1)	*anseris* (Dubois, 1967)	*bdellocystis* (Lutz, 1921)	*burti* (Miller, 1923)
Total length	1865	1558 (1505-1610)	1689 (1484-1851)	1263	1160	3330-4500**	2500	1800-2500
Forebody length	1320	465 (450-479)	396 (347-473)	403	340	600 - 1260	800	300-600
Forebody width	545	450 (450-450)	540 (475-630)	393	445	640 - 860	800	300-450
Hindbody length	484	1093 (1055-1131)	1307 (1137-1378)	860	820	1750 - 3500	1600	700-1300
Hindbody width	417	555 (550-560)	614 (535-662)	400	384	620 - 890	800	350-500
Hindbody/forebody length	2.42	2.35 (2.34-2.36)	3.30 (2.91-3.70)	2.13	2.41	1.9 - 3.0	2	1.2-2.4**
Oral sucker length	152	115 (115-115)	108 (104-117)	113		150 - 220	150	90-125
Oral sucker width	116	132 (132-132)	105 (101-113)	132		120 - 170		65-90
Pharynx length			52 (39-58)			70 - 104	100	36-45
Pharynx width						40 - 100		36-45
Ventral sucker length	153	184 (184-184)	144 (125-160)	175	139	210 - 330	200	90-140
Ventral sucker width	146	181 (181-181)	177 (173-185)		131	245 - 310		90-140
Ovary length						200 - 210	200	70-120
Ovary width						230 - 250		70-120
Anterior testis length						380 - 600	400	200-300
Anterior testis width		280				380 - 660	450	200-300
Posterior testis length						420 - 670	400	200-300
Posterior testis width		300	240 (240-240)			420 - 770	450	200-300
Genital cone length			205 (192-218)		143	440 - 660		145-200**
Genital cone width			188 (188-188)		173	300 - 450		110-155**
Egg length	120-130	91.5 (90-93)	123 (110-131)	95	106-121	90-110		90-100
Egg width	75-80	67 (67-67)	84 (65-86)	57	67-72	65-70		62-70
Other				spines on FB lobes				
Source						Dubois, 1968	Dubois, 1968	Stunkard et al. 1941

Table 1.7 - Columns continued

	canadensis (Dubois & Rausch, 1950)	*congolensis* (Dubois & Fain, 1956)	*fuhrmanni* (Dubois, 1937)	*intermedius* (Johnston, 1904)	*magnacetabulum* (Dubois, 1988)	*minor* (Yamaguti, 1933)	*niewiadomski* (Blasco-Costa, Poulin, & Presswell, 2016)
magnacetabulum (Dubois, 1988)	*minor* (Yamaguti, 1933)	*niewiadomski* (Blasco-Costa, Poulin, & Presswell, 2016)					
Total length	2040-3200*	1530-2400*	2250-3300*	3240-5000*	1080-1400	2500	1350-1999
Forebody length	510-960	490-740	590-960	1000-1500	420-450	250-870	452-712
Forebody width	370-770	560-860	540-1000	860-1250	360-370	280-630	361-536
Hindbody length	870-2270	1004-1630	1260-1960	2200-3000	660-950	540-1730	888-1412
Hindbody width	420-900	490-640	510-780	850-1270	270-310	250-670	348-545
Hindbody/forebody length	1.2-2.8	2.1-2.4	1.7-2.9	2.0-2.5		1.4-2.6	1.6-2.4
Oral sucker length	120-220	145-190	135-200	150-250	92-95	80-145	103-145
Oral sucker width	105-170	115-140	105-170	145-220	70-80	60-135	97-145
Pharynx length	60-85	55-67	78-104	80-130	70-73	40-65	44-64
Pharynx width	60-85	38-42	57-92	50-85	55-68	33-65	41-60
Ventral sucker length	140-245	215-245	180-260	250-340	130-200	92-198	130-217
Ventral sucker width	160-235	175-210	160-260	250-340	105-170	80-198	142-193

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Table 3.7 – *Continued from previous page*

	canadensis (Dubois & Rausch, 1950)	*congolensis* (Dubois & Fain, 1956)	*fuhrmanni* (Dubois, 1937)	*intermedius* (Johnston, 1904)	*magnacetabulum* (Dubois, 1988)	*minor* (Yamaguti, 1933)	*niewiadomski* (Blasco-Costa, Poulin, & Presswell, 2016)
Ovary length	105-190	145-155	90-160	210	63-105	66-135	91-148
Ovary width	125-210	235-240	135-280	330	90-115	105-163	118-194
Anterior testis length	250-470	210-270	235-390	460-490	75-165	99-306	191-361
Anterior testis width	235-440	290-310	190-420	490-650	105-175	10-326	169-333
Posterior testis length	335-640	230-300	270-420	400-490	115-120	130-408	193-327
Posterior testis width	240-475	300-340	200-470	480-650	70-95	99-367	200-385
Genital cone length	235-470	260-280	310-400	640-850	115-165	150-280	154-224
Genital cone width	180-330	210-240	210-320	420-500	115-150	120-190	
Egg length	95-125	87-98	98-122	72-110	100-105	99-132	94-103
Egg width	65-80	53-65	64-81	62-73	60-63	50-77	55-72
Other							
Source	Dubois, 1968	Dubois, 1968	Dubois, 1968	Dubois, 1968	Dubois, 1988	Dubois, 1968	Blasco-Costa et al. 2016

*Lower range of length taken from illustration
**From Dubois, 1968

Table 3.8: Comparison of adult morphology between lineages. Listed are structures that differ among the lineages. TL=total length; OS=oral sucker; PH=pharynx; VS=ventral sucker; FB=forebody; HB=hindbody; HB:FB=hindbody length/forebody length; OV=ovary; AT=anterior testis; PT=posterior testis; GC=genital cone

	***A. mclaughlini* n. sp. LIN7**	**LIN2**	**LIN4**	**LIN8**	**LIN9**
***A. mclaughlini* n. sp. LIN7**					
LIN2	VS, HB:FB				
LIN4	VS, HB:FB, eggs	TL, VS, OS, HB:FB, eggs			
LIN8	TL, FB shape, OS, VS, HB:FB, eggs	TL, VS, OS, HB:FB, eggs	Similar		
LIN9	TL, FB W, HB W, GC, HB:FB	TL, VS, HB:FB, eggs	VS, eggs, FB shape	FB shape, VS, eggs	TL, FB shape, VS, eggs

Table 3.9: Comparison of morphology of adults in four genetically distinguished lineages of *Australapatemon* and *A. mclaughlini* n. sp., with ten species of *Australapatemon*. Listed are differences between the lineage and the species (e.g., adults of *A. mclaughlini* n. sp. are smaller than *A. anseris*, but have larger eggs). TL=total length; OS=oral sucker; PH=pharynx; VS=ventral sucker; FB=forebody; HB=hindbody; HB:FB=hindbody length/forebody length; AT=anterior testis; PT=posterior testis; GC=genital cone.

	***Australapatemon anseris* (Dubois, 1967)**	***Australapatemon bdellocystis* (Lutz, 1921)**	***Australapatemon burti* (Miller, 1923)**
Distribution (type locality)	Holland, south of Rotterdam	Brazil, Bom Successo	Michigan, USA
Disribution (other localities)	Europe, Asia, North America	Venezuela	North America, Europe (cercaraie only)
Type host	*Anser anser*	*Australorbis immunis, Columbia livia* (exp.)	*Helisoma trivolvis*
Other hosts	*Anser fabalis*	*Cairina moschata, Amatona brasiliensis*	*Helisoma anceps, Lymnaea humilis modcella, Lymnaea stagnalis jugularis, Bathyomphalus contortus, Anas Platyrhinchos, Anas americana, Anas crecca, Anas discors, Anas penelope, Anas rubripes, Aythya affinis*
***A. mclaughlini* n. sp. LIN7**	Smaller TL, OS, PH, VS, PT, GC; larger eggs	Smaller OS, PH, VS; larger HB:FB, non-spherical FB	Larger HB:FB, eggs
LIN2	Smaller TL, VS; larger eggs	Smaller TL, VS; larger HB:FB	Larger OS, VS, HB:FB, eggs
LIN4	Smaller TL, OS, VS, AT, PT	Smaller TL, OS, VS, AT, PT; non-spherical FB	Smaller TL, OSW; larger VS. Similar.
LIN8	Smaller TL, PH, VS	Smaller TL, OS, VS; non-spherical FB	Smaller TL, OSW; larger PH, VS. Similar
LIN9	Smaller TL, GC; HB more robust	Smaller TL, VS; larger HB:FB; non-spherical FB	Smaller TL, larger HB:FB. Similar
	***Australapatemon canadensis* (Dubois and Rausch, 1950)**	***Australapatemon congolensis* (Dubois and Fain, 1956)**	***Australapatemon fuhrmanni* (Dubois, 1937)**
Distribution (type locality)			
Disribution (other localities)	Wisconsin, Alaska	Rwanda	Europe
Type host			
Other hosts			
***A. mclaughlini* n. sp. LIN7**	Smaller TL, FB more spherical, larger HB:FB	Larger HB:FB, eggs	Smaller TL, OS, PH, GC; mostly larger HB:FB, eggs
LIN2	Smaller TL	Larger HB:FB, eggs	Smaller TL
LIN4	Smaller TL, eggs, FB more spherical	Similar	Smaller TL, smaller eggs
LIN8	Smaller TL, FB more spherical, HB more robust relative to length	Smaller TL, FB W, HB W, VS. Similar.	Smaller TL, PH, VS, eggs
LIN9	Smaller TL, VS, GC, FB more spherical	Smaller TL, FB W, HB W, VS, GC, eggs	Smaller TL, VS, GC

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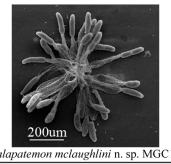
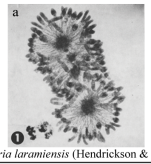
Table 3.9 – *Continued from previous page*

	***Australapatemon intermedius* (Johnston, 1904)**	***Australapatemon magnacetabulum* (Dubois, 1988)**	***Australapatemon minor* (Yamaguti, 1933)**	***Australapatemon niewiadomski* (Blasco-Costa, Poulin and Presswell 2016)**
Distribution (type locality)				
Disribution (other localities)	Australia	Paraguay	Japan, Europe	New Zealand
Type host				
Other hosts				
***A. mclaughlini* n. sp. LIN7**	Smaller TL, OS, VS, AT, PT, GC; larger HB:FB, eggs	Larger TL, OS, AT, PT, GC, eggs, wider FB, HB	Smaller TL; larger HB:FB	Wider GC; larger HB:FB, eggs
LIN2	Smaller TL, VS; larger eggs	Larger TL, eggs	Smaller TL, OS	Larger OS, eggs
LIN4	Smaller TL, OS, VS, AT, PT	Larger TL, OS, AT, PT, smaller eggs	Smaller TL, smaller eggs	Wider FB and HB
LIN8	Smaller TL, OS, PH, VS	Wider FB, HB, larger OS, smaller PH, eggs	Smaller TL, smaller eggs. Similar	Smaller TL, FB and HB more robust
LIN9	Smaller TL, VS GC, larger eggs	FB more robust, HB wider, eggs larger	Smaller TL, GC L. Similar	Smaller TL, GC; larger eggs

Table 3.10: Morphometric comparisons among cercariae. Measurements are given as ranges (in micrometres). a) The zygocercous cercariae of *Australapatemon mclaughlini* n. sp. (LIN7) are compared to two zygocercous diplostomoids, *Cercaria laramiensis* (Hendrickson and Kingston, 1974) and *Cercaria absurda* (Miller, 1927) and b) representative cercariae from LIN1 and LIN6 (see Figure 1) are compared to original descriptions of *Australapatemon* (*Cercaria*)*burti* (Miller, 1923).



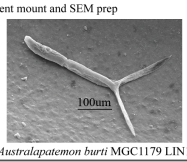


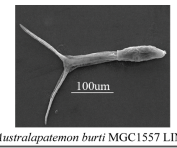
a

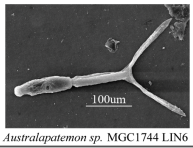
	Australapatemon mclaughlini n. sp. MGC1935 LIN7	*Cercaria laramiensis* (Hendrickson & Kingston, 1974)	*Cercaria absurda* (Miller, 1927)
Number of cercariae per aggregate	4–26	4–150	3–4
Number of aggregates per day	20	–	–
Body length (µm)	79–149§	91–150	183
Body width (µm)	30–49§	46–71	55
Tail Length (µm)	101–247§	230–260	285
Tail width (µm)	19–83§	41–60	114
Furca length (µm)	47–99	95–171	80
Furca width (µm)	12–34	27–40	30
Oral to ventral sucker length (µm)	43–80	49–81	–
Ventral sucker to tail stem (µm)	31–56	–	–
Ventral sucker length (µm)	4.83–7.70	19–23	–
Ventral sucker width (µm)	7.36–13.59	20–23	–
Ventral sucker spine length (µm)	1.38–2.26	–	–
Ventral sucker spine width (µm)	0.30–0.45	–	–
Body spine length (µm)	1.41–2.53	–	–
Body spine width (µm)	0.25–0.55	–	–
Tail spine length (µm)	–	–	11–16
Tail spine width (µm)	–	–	–
Furca spine length (µm)	1.06–1.59	–	–
Furca spine width (µm)	0.62–1.06	–	–
Tail Papillae	Yes	Yes	No
Tail Papilla length (µm)	2.36–3.92	–	NA
Tail Papilla width (µm)	1.22–3.03	–	NA

a. Reuse with permission from Allen Press

§ Measurements derived from both permanent mount and SEM prep







b

	Australapatemon burti MGC1179 LIN1	*Australapatemon burti* MGC1557 LIN1	*Australapatemon sp.* MGC1744 LIN6	*Cercaria burti* (Miller, 1923)
Number of cercariae per aggregate	NA	NA	NA	NA
Number of aggregates per day	NA	NA	NA	NA
Body length (µm)	138–156	106–131	111–135	88–240
Body width (µm)	42–48	37–44	30–43	34–52
Tail Length (µm)	163–178	118–131	110–145	113–165
Tail width (µm)	26–36	26–27	21–32	26
Furca length (µm)	163–194	120–162	129–162	139–181
Furca width (µm)	15–30	13–19	9–18	–
Oral to ventral sucker length (µm)	56–82	55–64	58–68	–
Ventral sucker to tail stem (µm)	54–62	40–51	41–53	–
Ventral sucker length (µm)	12.44–14.39	4.92–11.29	5.61–7.91	–
Ventral sucker width (µm)	13.65–14.67	6.18–11.38	6.97–11.76	–
Ventral sucker spine length (µm)	1.17–1.25	0.74–1.74	0.69–0.98	–
Ventral sucker spine width (µm)	0.28–0.30	0.19–0.26	0.19–0.24	–
Body spine length (µm)	1.06–2.18	0.75–1.93	1.02–2.15	–
Body spine width (µm)	0.18–0.35	0.19–0.42	0.19–0.42	–
Tail spine length (µm)	–	1.04–2.04	0.85–1.49	–
Tail spine width (µm)	–	0.28–0.56	0.24–0.50	–
Furca spine length (µm)	1.83–2.62	0.80–1.49	0.99–1.55	–
Furca spine width (µm)	0.60–0.81	0.14–0.34	0.24–0.37	–
Tail Papillae	No	No	No	No
Tail Papilla length (µm)	NA	NA	NA	NA
Tail Papilla width (µm)	NA	NA	NA	NA

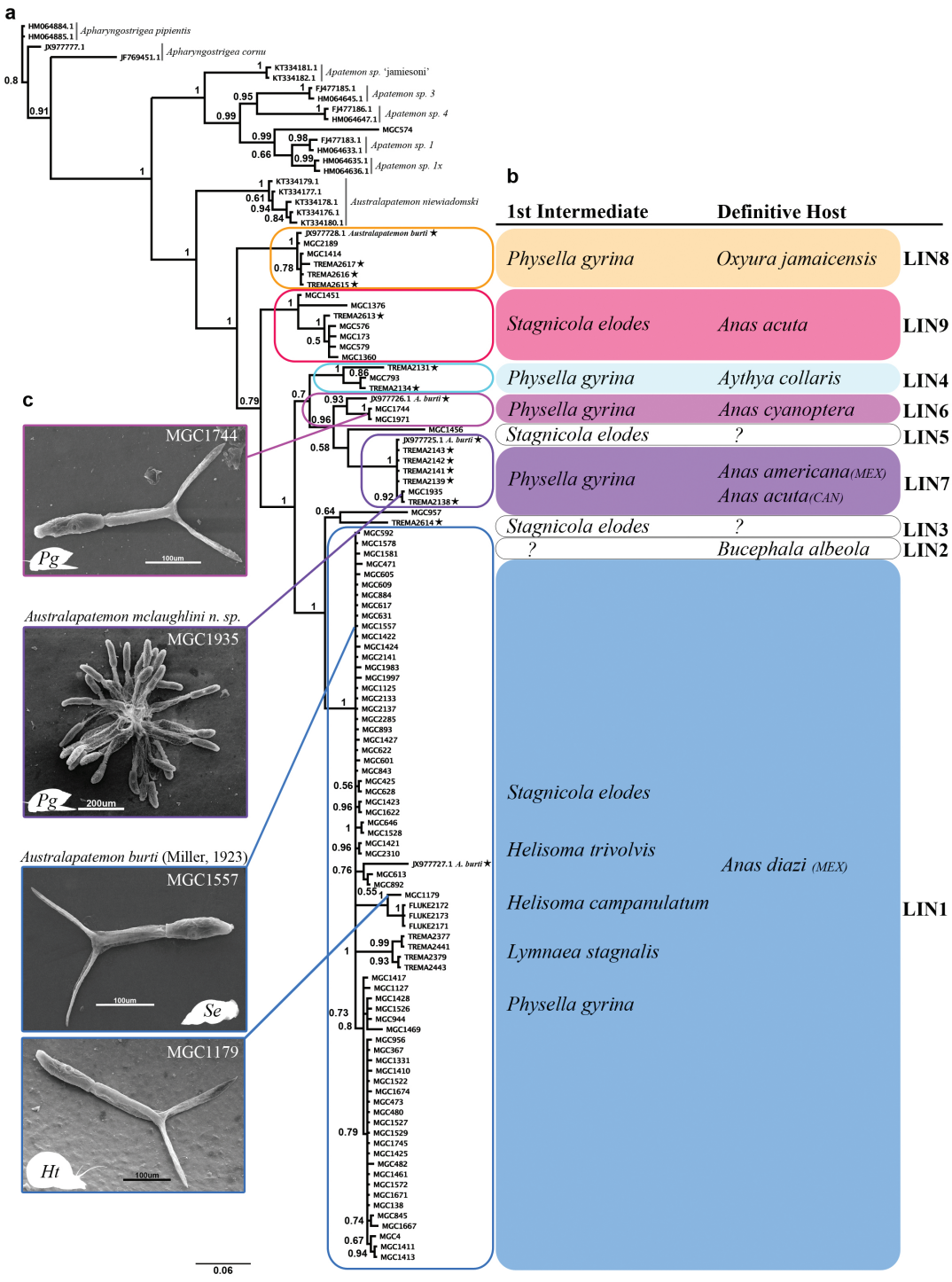


Figure 3.1: Integrative evidence for new lineages within the *Australapatemon*.

Figure 3.1: Integrative evidence for new lineages within the *Australapatemon*. a) Bayesian inference phylogram generated from partial *cox1* gene sequences with posterior probability values >0.50 reported. GenBank (GB) accession numbers are associated with samples derived from the database, while all other sample names represent new sequences. Sample names correspond to new GB accession numbers provided in Table 3.6. Sequences from adult worms are indicated by a black star. Adults collected in Mexico and studied by Hernández-Mena et al. (2014) are labelled *A. burti*. Scale bar denotes number of substitutions per site. Lineages are identified by differently coloured rounded rectangles that correspond to b) same colour-shaded rectangles indicating first intermediate and definitive host use for each lineage. Singletons are not indicated on the tree and are denoted by unshaded rectangles. Each lineage is labelled at the far right of the rectangles as LIN1-LIN9. Question marks denote missing host information. c) Examples of cercarial morphologies from LIN1, LIN6 and LIN7, in SEM. Coloured outlines correspond to lineage colours, and lines indicate placement within each lineage. First intermediate host use is indicated on each image: Pg = *Physella gyrina*, Se = *Stagnicola elodes*, and Ht = *Helisoma trivolvis*. White shapes indicate samples are from type hosts. Triangle represents *Australapatemon mclaughlini* n. sp., and circle indicates *Australapatemon burti*.

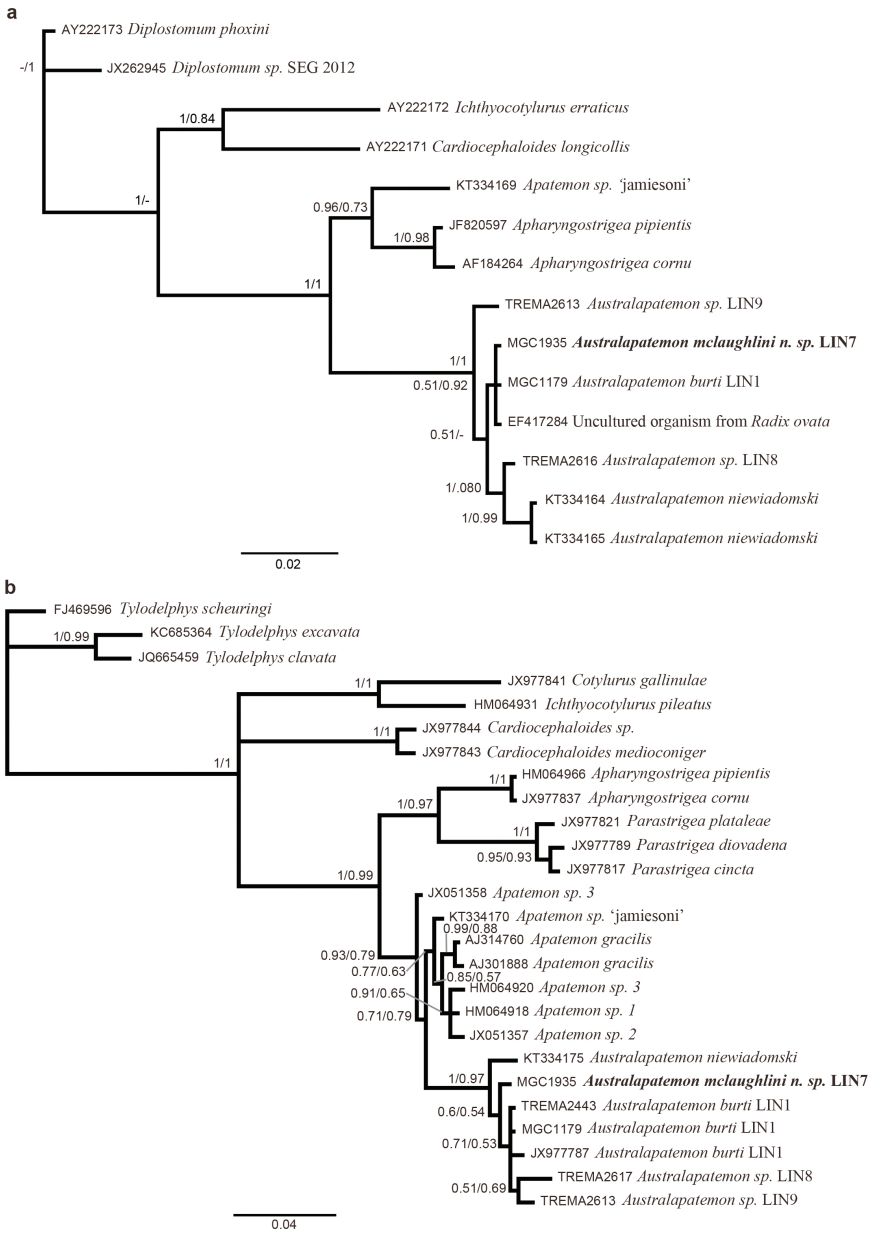


Figure 3.2: Bayesian inference phylograms of the Strigeidae and outgroups (Diplostomidae) derived from a) 28S, and b) ITS1-5.8S-ITS2 rDNA gene sequences with posterior probability values followed by bootstrap proportions given above branches. Values less than 0.5 are not reported. Scale bars denote number of substitutions per site. Figure 3.3 *Australapatemon mclaughlini* n. sp. a) Adult (holotype); scale bar = 500 μm b) Silhouettes of paratypes (paragenophores and hologenophores, based on photographs taken prior to subsampling and DNA extraction); scale bar = 500 μm. c) Outline of cercarial zygocercous aggregate; scale bar = 200μm. d) Individual cercaria, ventral view; scale bar = 50μm.

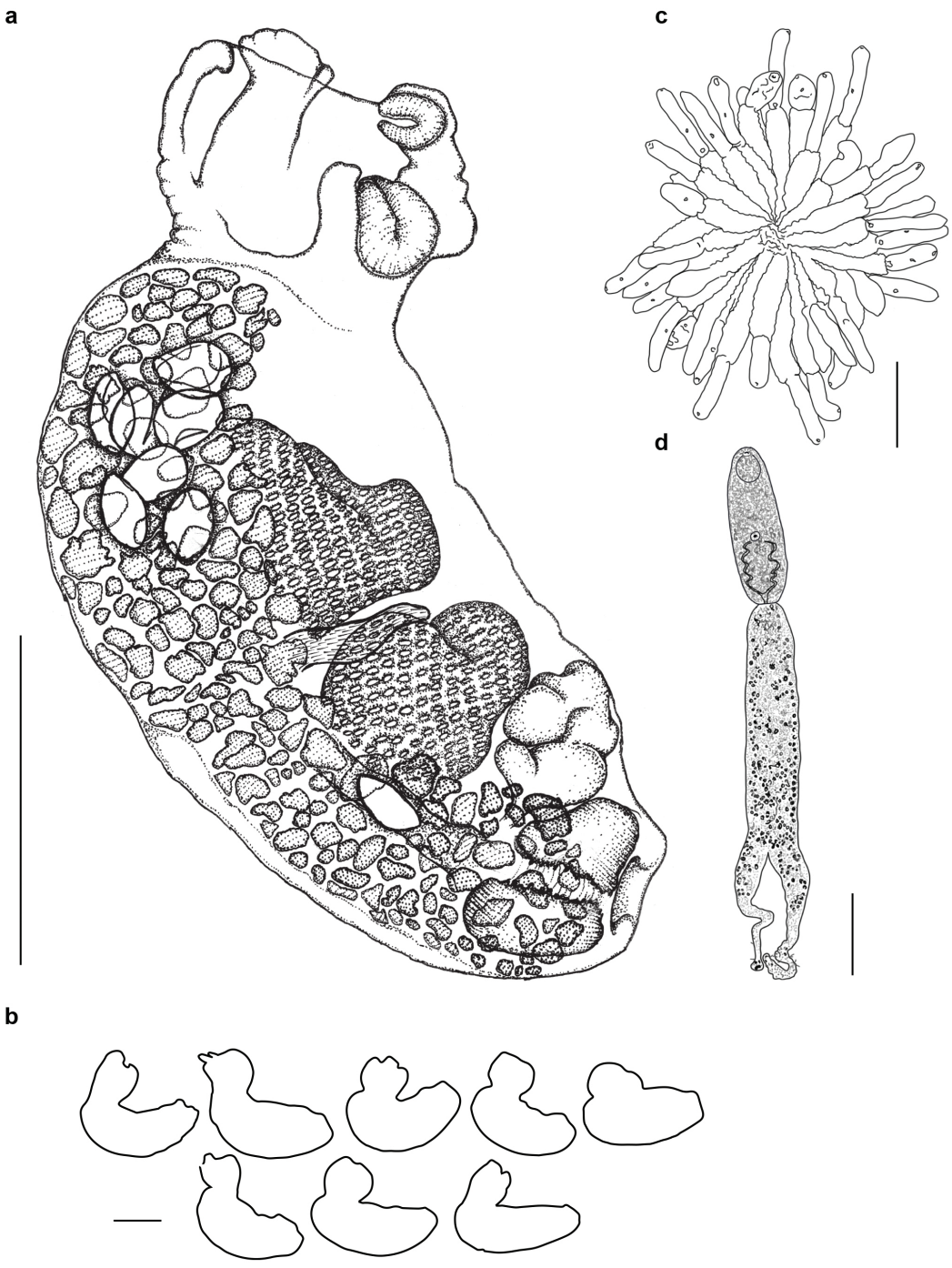


Figure 3.3: *Australapatemon mclaughlini* n. sp. a) Adult (holotype); scale bar = 500 μm b) Silhouettes of paratypes (paragenophores and hologenophores, based on photographs taken prior to subsampling and DNA extraction); scale bar = 500 μm. c) Outline of cercarial zygocercous aggregate; scale bar = 200μm. d) Individual cercaria, ventral view; scale bar = 50μm.

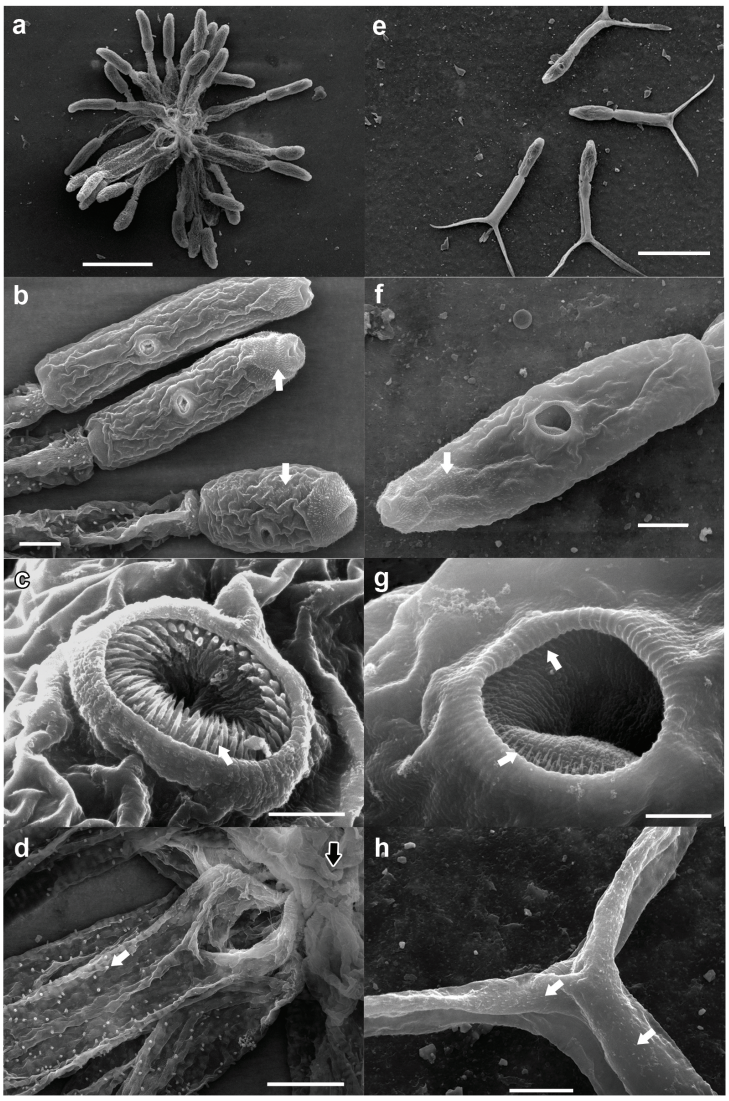


Figure 3.4: Scanning electron micrographs (SEM) of zygocercous (LIN7, MGC1935: a) *Australapatemon mclaughlini* n. sp. and non-zygocercous (LIN1, MGC1179: b) *Australapatemon burti* cercariae. 1a) Zygocercous aggregate; scale bar = 100μm. 2a) Ventral view of three individual zygocercous bodies. White arrows indicate tegumental spination; scale bar = 20μm. 3a) Ventral sucker of zygocercous cercaria. White arrows indicate sucker spines; scale bar = 5μm. 4a) Zoomed-in view of cercaria tail among the aggregate tail bundle. White arrow indicates papules on tail, black arrow indicates narrowing of furcal muscles, specialized for holding on to others; scale bar = 50μm. 1b) Four individual cercariae; scale bar = 100μm. 2b) Ventral view of individual cercaria body. White arrow indicates body spination; scale bar = 20μm. 3b) Zoomed-in view of ventral sucker. White arrows indicate ventral sucker spines; scale bar = 5μm. 4b) Zoomed-in view of cercaria furcae. White arrows indicate tail and furcal spines used for measurements; scale bar = 20μm.

Chapter 4

A fine-scale phylogenetic assessment of digenean trematodes in central Alberta reveals we have yet to uncover their total diversity

4.1 Introduction

4.1.1 Preface

Beyond the challenges associated with delineating species, as I demonstrated in chapters 2 and 3, selecting methods for measuring true diversity has been a hotly debated topic for many years, and there remains to this day no exact solution. Imperfect species detection is a common problem among biodiversity surveys (Jarzyna and Jetz 2016), but our ability to increase the resolution of detection through methods like molecular taxonomy, will help us attain a clearer picture of diversity.

In the past two decades, molecular taxonomy of trematodes has greatly improved our understanding of species relationships, revealed the presence of cryptic species, and helped link larval forms to their adult counterparts. How-

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ever, the focus of these studies is directly related to the interest and expertise of the individual researcher, which has resulted in certain taxa being studied in greater depth than others (Robert Poulin and Jorge 2018). Because of this, there are many knowledge gaps, notably between historical records and modern collections, such that they are not easily connected. Despite over one hundred years of digenean trematode parasite species descriptions, from a wide diversity of vertebrate and invertebrate host species, our ability to recognize the diversity of trematode species within a single lake remains an incredible challenge.

The aims of this chapter were to expand the sampling of trematode communities, as described in chapter 2, for an additional year, for a total of three years of data, and to use molecular taxonomy methods to identify trematode samples to the species level. During this investigation, knowledge gaps specific to Alberta, as well as to molecular databases, are identified and discussed.

4.1.2 Background

Trends in the ecology of pathogens are reliant upon an accurate identification of both pathogen and host species. However, the precise identification of endoparasites can be precarious, due to the lack of hard, morphological structures that arthropod ectoparasites have (Mathison and Pritt 2014). Furthermore, larval and immature endoparasites often lack reproductive structures that identify their adult counterparts. Both of these problems are common among helminths (K. Jensen and Bullard 2010; Roeber, Jex, and Gasser 2013; Schell 1985). Additionally, the revelation of cryptic species is becoming more common, as molecular methods expose diversity not identifiable by traditional, morphological methods (Detwiler, Bos, and Minchella 2010; Detwiler, Zajac, et al. 2012; Georgieva et al. 2013; Herrmann et al. 2014; Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010; Miura, A. Kuris, and Torchin 2005; Nadler and León 2011; G. Pérez-Ponce de León and R. Poulin 2018). Finally, there is a lack of general survey data on parasites, causing gaps in our understanding of diversity and richness for defined geographical locations (R. Adlard and O'Donoghue 1998; Robert D. Adlard, T. L. Miller, and Smit 2015;

Mollaret et al. 1997). Taken together, plastic and cryptic morphology, with a lack of survey data, makes it more difficult to correctly identify a parasite sample from a new location.

Recent meta and spatial analyses have shown that our understanding of parasite diversity is biased toward location, time, and parasite class, correlating with when and where taxonomists are most active during their careers; and it is argued that more taxonomists are needed (Robert Poulin 2014; Robert Poulin and Jorge 2018). Molecular methods have come a long way in allowing faster and more precise species identifications and the ability to make hypotheses about species relationships and evolution considering cryptic morphology. However, even with these methods, regional checklists of host-parasite relationships remain incomplete (Robert Poulin and Presswell 2016). One major issue is depauperate and biased databases, directly related to research and funding interests, expertise, and the natural evolution of improving methodologies over time. So, not only do we need more taxonomists, but we need them to study more broadly to fill in these gaps in our understanding of parasite diversity.

Ecologically speaking, most parasites have incomplete life cycle descriptions. Likewise, our understanding of their distributions and interactions within and among host species are limited due to a lack of surveillance records and repeated or long-term studies. The dispersion of parasite data constrains our knowledge of the finer details of their ecology across broad geographic ranges. Additionally, unreliable morphological assessments in survey data present the caveats that 1) the species identities may not be accurate, and 2) the survey may not represent true diversity within the area, missing cryptic species all together and underestimating overall diversity. Furthermore, the onset of molecular methods for species identifications has widened the knowledge gap through revelations of prior undetected diversity that cannot always be traced to a described species. In fact, the revelation of cryptic species is enhanced with greater sequencing effort, and more so for trematodes than any other group of parasitic helminth (G. Pérez-Ponce de León and R. Poulin 2018). This, overall, can make it incredibly difficult to understand the larger

picture when it comes to parasite ecology because we are lacking long-term, field studies, and precision in data collection.

Digenean trematodes are a very large group of parasitic helminths, with complex life cycles. The adult worms infect vertebrate hosts, in which their eggs are passed into the environment with the feces of the animal. The eggs hatch and infect a snail (or other mollusk), in which their larval development occurs. Larvae will emerge from their obligate, snail, first-intermediate host to then infect either a second-intermediate host or a definitive host, depending on the species. Current estimates for the number of trematode species range from 18,000 (Cribb T H and Warren, Alan Bray, R.A., Littlewood, D.T.J., Pichelin, S.P. and Herniou, E.A. 2001) to 24,000 (Robert Poulin and Morand 2004).

Traditionally, taxonomic descriptions of trematodes are from morphological traits of adult worm stages derived from vertebrate hosts, as their most prominent features are fully developed and measurable, contrasting the less-developed features of the larval stages (Schell 1985). With the onset of molecular barcoding, not only have we realized the problems of cryptic morphology and the need for multiple lines of evidence for species delineation, but that for trematodes, we can now use larval stages to delineate species (Detwiler, Bos, and Minchella 2010; Detwiler, Zajac, et al. 2012; Georgieva et al. 2013; Michelle A Gordy, Sean A Locke, et al. 2017; Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010; Schwelm et al. 2018; Miroslava Soldánová et al. 2017). This is advantageous in that it is considerably easier to collect larvae from snail, first-intermediate hosts. The disadvantage is the lack of a direct connection between adult morphological records and molecular records.

The goal of this study was to capture an accurate identification of the trematode biodiversity among snail first-intermediate hosts to establish a better, ecological understanding of trematode communities and how they differ geographically and change over time. In this study, we use molecular phylogenetic methods to assess species relationships, to identify collected specimens, and account for possible cryptic morphology. Snails and trematodes were collected from six lakes in central Alberta, Canada over three years, from June

to September. This longitudinal dataset provides novel contributions to the species diversity of trematodes, new geographical species records in central Alberta, and snail host association records, to better connect trematode life cycles. Though the data collected for this study was a continuation of our previous long-term dataset (M. Gordy et al. 2016), the use of phylogenetic methods herein both expand and improve upon our understanding of trematode diversity and clarify identification issues we confronted previously.

Several trematode families have previously been given a considerable amount of attention in molecular phylogenies, more than others (e.g. Diplostomidae and Echinostomatidae). Therefore, species delimitation methods and acceptable sequence divergence limits have been tested for specific genes within these, well-studied, trematode families (Blasco-Costa and Sean A Locke 2017; Detwiler, Zajac, et al. 2012; Simona Georgieva, Faltýnková, et al. 2014; Georgieva et al. 2013). Most trematode families have not been given such attention. Though there are general assumptions extrapolated from previous studies, such as 5% sequence divergence of cytochrome *c* oxidase subunit 1 (*cox1*) as an acceptable limit for species delimitation (Vilas, Criscione, and Blouin 2005), this remains to be tested for all trematode families. Herein, we test this 5% assumption for delimitation using *cox1* across 7 trematode families.

The resulting diversity estimates from this study exemplify both the power and utility of molecular phylogenetics for species identification; but this study also identifies gaps and caveats that trematode taxonomists may face in future studies. Therefore, we provide commentary on the current caveats of the field of trematode taxonomy, cryptic species, depauperate databases, and areas in need of further research. We also provide a current record of trematode and host associations within Alberta and encourage the continued effort to better understand trematode diversity from both a regional and global context.

4.2 Material and methods

4.2.1 Trematode and snail sample collection and selection

As a continuation of the two-year survey described in Gordy et al. (2016), snails were collected for an additional year in the same manner from the following sites: Lake Isle, Lake Wabamun, Gull Lake – Aspen Beach, and Buffalo Lake – Pelican Point, Rochon Sands, and The Narrows (Figure 4.1). All methods regarding collection and sample processing, including molecular methods were the same as previously described (M. Gordy et al. 2016; Michelle A Gordy, Sean A Locke, et al. 2017).

Briefly, snails were collected from sites previously established, and brought back to the laboratory for examination of patent infection by larval trematodes. Trematode infections, when patent, resulted in larval cercariae emerging from the snail into the surrounding water. Free-swimming cercariae were detected with a dissecting microscope, collected from the sample well, and preserved for downstream molecular work. Our original aim was to extract DNA and barcode every parasite sample. However, with over 2400 samples, this goal was not feasible in cost and time. Nearly half the trematodes derived from the total collection were xiphidiocercariae, and previous sequencing efforts revealed these samples to be closest to *Plagiorchis* sp. (M. Gordy et al. 2016). Therefore, much of the sequencing efforts went to all other morphotypes for which there were enough cercariae available for sequencing (i.e. >10 cercariae, to keep a voucher stored in ethanol). For cost feasibility, we chose to sequence only 70 haphazardly sampled xiphidiocercariae samples, representative of sites and snail host species from which they were found. The sequencing effort strategy for all other morphotypes was complete coverage.

4.2.2 DNA isolation, sequencing, and analysis

DNA was extracted from cercariae preserved in 50% RNAlater or 95% ethanol, as previously described (M. Gordy et al. 2016). The partial NADH dehydrogenase subunit 1 (*nad1*) mitochondrial gene was sequenced for all cercariae for

which morphological characterization or previous mitochondrial *cox1* sequencing attempts (M. Gordy et al. 2016) placed them in the family Echinostomatidae. Because of high saturation within the *cox1* gene for this family, *nad1* has been the gene of choice in the literature (Detwiler, Bos, and Minchella 2010; Detwiler, Zajac, et al. 2012; Simona Georgieva, Faltýnková, et al. 2014; Morgan and D. Blair 1998), and best represented the samples within GenBank for comparisons. For all other families, partial *cox1* was used (M. Gordy et al. 2016; Michelle A Gordy, Sean A Locke, et al. 2017; Moszczynska et al. 2009; Van Steenkiste et al. 2014). Nucleotide sequence inspection, trimming, alignments, model testing for best-fit substitution models, and Maximum Likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were as described in Gordy et al. (2017). Model testing, utilizing BIC scores for determining best-fit, was implemented in MEGA7 (Kumar, Stecher, and Tamura 2016). All BI analyses were run in the MrBayes (Huelsenbeck and Ronquist 2001) plugin with chain length 10,000,000, subsampling frequency 100,000, 4 heated chains (chain temp 0.2), and burn-in length of 1,000,000. All ML analyses were run with the PhyML plugin (Guindon et al. 2010), estimating parameters, and with 1,000 bootstrap iterations. All molecular analyses were run using Geneious version 11 (http://www.geneious.com, (Kearse et al. 2012)).

Phylogenies were first constructed using a broad sampling of taxa within each family. Sequences of the same gene (either *cox1* or *nad1*) were gathered from each species available in GenBank within that family. Because there are no standard methods yet employed for molecular taxonomic analysis within the Digenea, and much sequencing effort has been based on personal preference, we were unable to consistently attain a good representation of the species or even genera for several families, including Psilostomidae, Notocotylidae, and Plagiorchiidae. Because of issues with substitution saturation at broader taxonomic groupings for some families, their phylogenies were further refined into either genera or groups of closely related genera that were previously published as such (e.g. *Hypoderaeum* is paraphyletic to *Echinoparyphium* within

the family Echinostomatidae (Detwiler, Bos, and Minchella 2010; Kostadinova et al. 2003)).

Phylogenies were constructed at a family level with nonredundant sequences to understand species relationships. These family-based phylogenies were used as a benchmark for later phylogenies, in which redundant sequences were included for identification of individual sequences (specimen samples). Because there were many sequences, some phylogenies were divided to reduce the computation time (i.e. Strigeidae, Diplostomidae, and Echinostomatidae). We only present the information relevant to species identification phylogenies below, as the species relationships were the same as those within the nonredundant family-level phylogenies.

4.2.3 Species delimitation

Trematode samples were first separated by gross morphology, evidenced by previously published larval trematode descriptions (Schell 1985). Then, percent nucleotide identities by tBLASTn (Altschul et al. 1990), phylogenies from the literature where available (Blasco-Costa, Cutmore, et al. 2016; Detwiler, Bos, and Minchella 2010; Michelle A Gordy, Sean A Locke, et al. 2017; Hernández-Mena, Martín García-Varela, and Gerardo Pérez-Ponce de León 2017; Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010), and species names given to the sequences in GenBank to which they most closely matched from BLAST results were used to group samples into trematode families and hypothesized genera.

After phylogenetic analyses, because many of our sequences were not directly within monophyletic groups of previously identified species, we employed additional tools to further distinguish taxa. The web app Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) was used in combination with *a priori* assumptions of a 5% cut-off in sequence divergence for species delimitation using p distances calculated in MEGA7 (Michelle A Gordy, Sean A Locke, et al. 2017; Kumar, Stecher, and Tamura 2016). For ABGD, nucleotide alignments were inserted and tested using all three distance measurements (Jukes-Cantor, Kimura 2.0, and simple distance) to look for agreements on

grouping and prior maximal distance, using the default minimum slope of 1.5. Other specific methods will be described separately for each trematode family below. The one family that was included in downstream diversity analyses, but not described below is the Schistosomatidae because their phylogeny from this dataset was described and previously published (Michelle A Gordy, Cobb, and Patrick C Hanington 2018).

Family Notocotylidae:

A final alignment of 98 *cox1* sequences with a length of 327 bp was used for phylogenetic analyses. *Echinostoma Hortense* (KR062182) was used as an outgroup because of its prior-demonstrated phylogenetic relationship to the notocotylid *Ogmocotyle sikae* (KR006934.1) (Liu et al. 2016), which was one of only two sequences from GenBank we were able to use for comparison. The *E. Hortense* sequence did cause one small gap in the final alignment. Only *O. sikae* and *Notocotylus* sp. BOLD (KM538104) were used for comparison to the 95 sequences from this study, due to a lack of Notocotylid *cox1* sequences available with significant overlap. HKY + G was the best substitution model and was used for phylogenetic analyses.

Family Psilostomidae:

A *cox1* nucleotide alignment was made for 11 sequences, six from this study and five from GenBank, for a final length of 498 bp. *Echinochasmus japonicus* (NC_030518) was used as the outgroup for phylogenetic analyses because of its previously demonstrated relationship outside of Psilostomidae, but within the superfamily Echinostomatoidea (Vasyl V. Tkach, Kudlai, and Aneta Kostadinova 2016). Three other species were used for comparison, namely *Sphaeridiotrema globulus* (GQ890329), *S. pseudoglobulus* (GQ890328 & FJ477222), and *Pseudopsilostoma varium* (JX468064). HKY + G was the best supported nucleotide substitution model and was used for phylogenetic analyses. Because there were so few sequences, and therefore groups of species, ABGD was not utilized for confirmation.

Family Haematoloechidae:

A final nucleotide alignment consisted of seven sequences, one from *Plagiorchis* sp. (FJ477214) as the outgroup, two from GenBank (KM538096-7:

Haematoloechus sp. BOLD) and four from this study. The *Plagiorchis* sequence was used as the outgroup, based on previous use as such for phylogenies of Haematoloechidae sequences (Snyder and Vasyl V. Tkach 2001). The alignment was 469 bp long with a few short gaps due to the outgroup sequence. HKY + G + I was the best supported nucleotide substitution model.

Family Plagiorchiidae:

A final nucleotide alignment of 56 *cox1* sequences was 437 bp in length. A sequence for *Haematoloechus* sp. (KM538096) was used as the outgroup (for reasons previously specified). Model test results showed the best nucleotide substitution model was HKY + I, which was utilized in BI and ML analyses.

Family Echinostomatidae:

Though *nad1* was the primary gene of interest for this family, based on previous work, many samples from this study were first (or only) analyzed using *cox1* sequences. To resolve the issue of having some samples of one gene and some of another, sequencing of both genes for a few samples were done to make the link between gene trees. The only successful sequences from this attempt were from isolates MGC16B, MGC1214, and MGC1665. These sequences allowed the comparison between *nad1* and *cox1* phylogenies.

An alignment was made for all echinostome *cox1* sequences from this study along with those gathered from GenBank to represent as many species as available and that covered the same region of the gene. The final alignment included 113 sequences and was 391 bp long. Two sequences for *Euparyphium capitaneum* (KY636235-6) were used as the outgroup (Vasyl V. Tkach, Kudlai, and Aneta Kostadinova 2016). From GenBank, sequences from the genera *Drepanocephalus, Hypoderaeum,* and *Echinostoma*, were included in the alignment, as those were all that were available. Sequences included in the alignment from this study were from the genera *Echinoparyphium* and *Echinostoma*, and while there were no reference sequences within certain clades, there was overlap from the *nad1* gene tree to confirm the identity of these clades. The best fit model was GTR + G for both genes and for all genera within this family. The *nad1* phylogenies were split into multiple groups as discussed below.

Drepanocephalus:

The final nucleotide alignment (*nad1*) was 390 bp in length and included two *D. auritus* (KP053262 & KP053263) sequences, one *Drepanocephalus* sp. (KP053264), two unknowns from the current study (MGC2147 & MGC2353), and a *Fasciola hepatica* (KT893744) sequence as the outgroup. Minor gaps were present between base pairs 180-190 where *F. hepatica* has a couple base pair differences. Because there were so few sequences, ABGD was not used for confirmation.

Neopetasiger:

The final nucleotide alignment (*nad1*) of 21 sequences was 313 bp long, and minor gaps occurred between base pairs 108-116 due to *F. hepatica* (KT893744), which was used as an outgroup for this alignment.

Echinostoma:

A final nucleotide alignment (*nad1*) of 73 sequences was 386 bp long and included 31 unknown sequences from this study and all available species with significant overlap in the same region from GenBank. As in Georgieva et al. (2017), among others, *Isthmiophora melis* (AY168948) was used as an outgroup.

Echinoparyphium/Hypoderaeum:

The final nucleotide alignment for *nad1* was 304 bp long, with some minor gaps at position 81, 84, and 298, due to the outgroup, and included 262 sequences. Once again, *I. melis* was used as an outgroup. Both *Hypoderaeum* and *Echinoparyphium* sequences were included in this alignment, because previous phylogenies have shown them as paraphyletic (Detwiler, Bos, and Minchella 2010).

Superfamily Diplostomoidea:

Family Diplostomidae:

Based on recent phylogenies by Hernandez-Mena et al. (2017), and to reduce the overall size of the analysis, the Diplostomidae were divided into two groups for phylogenetic analyses utilized for identifications. Diplostomidae-I included the genera *Austrodiplostomum, Tylodelphys*, and *Diplostomum*, and resulted in a final nucleotide alignment of 197 sequences at 347 bp, using

Ornithodiplostomum scardinii (KX931425) as outgroup. Diplostomidae-II included the genera *Bolbophorus, Posthodiplostomum, Ornithodiplostomum, Neodiplostomum*, and *Alaria*, with a final alignment of 104 sequences at 317 bp and using *Crocodillicola pseudostoma* (MF398317-8) as the outgroup. For both groups, GTR + G + I was the best substitution model and used for phylogenetic analyses.

Family Strigeidae:

Like the Diplostomidae, the Strigeidae were divided into two groups for analyses, and named after the ordering found in Hernandez-Mena et al., (2017). Strigeidae-I included the genera *Cardiocephaloides, Cotylurus*, and *Ichthyocotylurus*. The final alignment was 356 bp and included 152 sequences. *Tylodelphys scheuringi* (FJ477223) was used as the outgroup for phylogenetic analyses. Strigeidae-II included genera from *Apatemon* and *Australapatemon*, with *Apharyngostrigea* spp. as the outgroup (HM064884-5, JX977777, & JF769451). The final nucleotide alignment was 392 bp and included 313 sequences. The best nucleotide substitution model for the Strigeidae was HKY + G + I and used in all phylogenetic analyses.

Richness and recovery calculations The following packages were utilized in R version 3.4.3 (R Core Team 2017) to calculate richness and diversity metrics and plot them: *vegan* (Oksanen et al. 2018) and *dplyr* (Wickham et al. 2017). Species richness was derived using the diversityresult (*vegan*) command to add unique species by site as well as pooled species richness for all sites, by snail species, and to view how they were represented by lake. Species accumulation and rarefaction were analyzed using the specaccum (*vegan*) command, utilizing the "collector" method to derive site richness in the order the data were collected and the "rarefaction" method to view an individual-based, rather than site-based, method for species accumulation, respectively. An Arrhenius nonlinear model was fit to a species accumulation curve to view the species-area relationship utilizing the specaccum with "random" method and fitspecaccum (*vegan*) commands. If we assume that morphological identification of larval trematodes gives the greatest confidence at the taxonomic scale of family, we predict that accumulation curves will plateau faster than with information de-

rived from molecular phylogenetic identifications that can provide confidence to the species level. To show this, we repeated the same accumulation and rarefaction analyses at the level of trematode family. This process was repeated for snail species, with exception of the Arrhenius nonlinear model, which would not converge.

4.3 Results

A total of 17,447 snails were collected over the three-year period across all 11 sites (Figure 4.1 and Table 4.1). Snail species abundances are as follows: *Stagnicola elodes* = 9,505 (54.48%), *Lymnaea stagnalis* = 516 (2.96%), unidentified lymnaeid = 2,252 (12.91%), *Helisoma trivolvis* = 1,166 (6.68%), *Planorbula armigera* = 1 (0.01%), unidentified planorbid = 143 (0.82%), and *Physa gyrina* = 3,864 (22.15%). Of these collections, only 2,452 (14%) snails carried patent trematode infections, meaning cercariae were actively emerging from the snail. Unidentified lymnaeids and planorbids mentioned above were all uninfected. Most infections were found among *S. elodes* snails (1,892/77.16%), followed by *P. gyrina* (354/14.44%), *L. stagnalis* (123/5.02%), *H. trivolvis* (82/3.34%), and finally *P. armigera* (1/0.04%). Of these infections, 1,149 (46.8%) were classified as xiphidiocercariae by morphology (by having a clearly defined stylet in the anterior rim of the oral sucker (Schell 1985).

A total of 1,091 trematode cercariae samples were successfully extracted and sequenced for downstream molecular phylogenetic analyses. Less than 200 cercariae samples, excluding xiphidiocercariae, were not included in the final diversity analyses, either because of low quantities of cercariae, low quantity or quality of DNA, or bad sequencing results. Phylogeny results will be discussed in the same order as above, by family, in the sections below.

Several new lineage and singletons have emerged from these phylogenies, and we refer to them below as 'species.' We acknowledge the limitations of using molecular phylogenies for species identifications, without further supporting evidence (e.g. sequences from adult specimens) and that others would refer to them as operational taxonomic units (OTUs). However, we prefer to

use the term species to remain consistent with our previous publications and sequence names.

Family Notocotylidae:

Despite there being 19 different species represented in GenBank from the superfamily Pronocephaloidea, only 5 species had *cox1* sequences available at the time of this analysis, and one of those sequences was from a region other than the typical barcoding region (Folmer). Two of the sequences, Notocotylidae gen. sp. 1 NZ and sp. 2 NZ were eventually removed from analyses because they did not align well. Therefore, the only sequences from GenBank left for phylogenetic comparisons with our sequences were *Ogmocotyle sikae* (mitochondrion, complete genome: NC_027112.1:6904-8460), *Notocotylus* sp. BOLD (KM538104), and Notocotylidae sp. MSB (KX670216).

The former Gorgoderidae:

From BLAST results, several sequences in our dataset matched most closely to the sequence for *Gorgoderina* sp. (FJ477202) in GenBank. When attempting to find other sequences for use in downstream analyses, we found that nearly every species in this family was only represented by 28S or ITS. A *cox1* sequence was available from *Pseudophyllodistomum macrobranchiola* (LC002523); however, the sequence was downstream of the Folmer region and did not overlap with our sequences. Upon further investigation, we found that these sequences matched very close to our other sequences for Notocotylidae sp., despite no BLAST matches from GenBank to Notocotylids. We therefore dissolved the Gorgoderidae sequence group, merging these sequences with the other Notocotylidae sequences, and have updated our previously published sequence (KT831348).

Our phylogenetic analyses have revealed four Notocotylid species from our samples. Both ML and BI trees agreed on topology with strong statistical support (Figure 4.2A). Though all ABGD methods agreed, they only split the groups into three (JC pmax = 0.0215; K2 and simple pmax = 0.0129): *E. hortense, O. sikae* and *Notocotylus* sp. The only GenBank sequence to group with our sequences was *Notocotylus* sp. BOLD (KM538104).

Based on pairwise distances, however, *Notocotylus* sp. as a single group determined by ABGD was not supported based on the 5% cut-off, as several sequences within the group were more than 5% different from others, despite the average intraspecific divergence being 3.0% for all. Two sequences, isolates MGC683 and MGC1730, expressed 6.8-10.2% and 5.0-10.2% intraspecific divergence, respectively. Without including these sequences, the range of intraspecific divergence was 0.0 – 5.6%, which is more reasonable for a single lineage, however, still beyond the cut-off. We suspected further division within the tree topology, as some sequences continued to be closer or above the 5% divergence cut-off. Those that grouped outside of the primary clade (identified as *Notocotylus* sp. A) and closer to MGC683 were then separated further and support by intraspecific divergence was then within the cut-off range. In doing this, the average interspecific divergence between *Notocotylus* sp. A and D is 3.8% with a range of 2.8 – 5.6% (Table 4.2).

In considering the snail host species, *Notocotylus* sp. B (MGC1730) and C (MGC683), utilized *P. gyrina* and *Helisoma trivolvis*, respectively, clearly supporting differentiation. However, the other isolates within *Notocotylus* sp. A and D used both *P. gyrina* and *S. elodes* as hosts; but curiously, *Notocotylus* sp. A was a primary *Physa* infecting species (36 *P. gyrina*/ 3 *S. elodes*), while *Notocotylus* sp. D was a primary *Stagnicola* infecting species (49 *S. elodes*/ 5 *P. gyrina*).

Family Psilostomidae:

Only a few species within the Psilostomidae had representation by *cox1* in GenBank and significant overlap with our sequences. In molecular phylogenies, none of the sequences from this study grouped with any of the GenBank species representing the Psilostomidae family, but created their own monophyletic group, sister to *P. varium*. Both BI and ML trees agreed on topology (Figure 4.2B). The six sequences from this study were 0-0.8% divergent from each other, with an average intraspecific divergence of 0.4%, and interspecific divergence of 14.3-24.6% (Table 4.3). Because of the low identity to any of the available genera from this family, the sequences from this study have therefore

been identified broadly as Psilostomidae gen. sp. A. All six samples were derived from cercariae emerging from *Helisoma trivolvis* snails.

Family Haematoloechidae:

Despite there being 18 *Haematoloechus* spp. with *cox1* sequences available in GenBank at the time, only two sequences from this database overlapped with our sequences because of different choices in sequenced *cox1* regions. In addition, no other genera within the Haematoloechidae were currently represented in GenBank.

The four Haematoloechidae sequences from this study were 100% identical to each other, but 13.4-25.8% divergent from GenBank sequences (Figure 4.3A and Table 4.4). These four sequences were generalized to Haematoloechidae gen. sp. A, because there were no specific species within GenBank or other evidence that could provide more specificity at this time. Both BI and ML trees agreed with strong support for topology, as suspected for such little information. All four sequences were derived from samples that came from *S. elodes* snails collected at Pelican Point at Buffalo Lake.

Family Plagiorchiidae:

Most Plagiorchiidae sequences in GenBank use a different region of the *cox1* gene, downstream from the Folmer region. The only sequence that aligned with ours was one *Plagiorchis* sp. (FJ477214). Phylogenetic analyses of Plagiorchiid sequences resulted in both ML and BI trees agreeing on topology with strong statistical support for external nodes and moderate support for internal nodes (Figure 4.3B). All methods within ABGD supported the differentiation of lineages within the tree to nine groups other than the outgroup (Pmax (All) = 0.004-0.0599). Pairwise and averaged intraspecific divergence values were supported by the 5% cut-off, and the highest value was 2.1% within Lineage 1. The average interspecific divergence had a range from 8.9-18.8% (Table 4.5). Further support for the differentiation of some lineages was found among intermediate host use, as Lineage 6 utilized *H. trivolvis*, Lineage 7 used *L. stagnalis*, and all other lineages were found emerging from *S. elodes*. Because this diversity was greater than we had expected by morphology (indicating possibly two species based on relative size) and prior BLAST results, we were unable

to assign the unsequenced samples to these nine different lineages. Therefore, in downstream diversity analyses that require abundance information, these lineages have been conservatively lumped into one species, called *Plagiorchis* sp.

Family Echinostomatidae:

For each separate alignment, ML and BI phylogenies were compared and found to agree on major topology. In instances where external node topology disagreed between the two methods, this was identified as a separate tree.

Drepanocephalus: Both *nad1* sequences from this study grouped monophyletically with *D. auritus* sequences. *Drepanocephalus* sp. was paraphyletic to the *D. auritus* group and displayed a nucleotide divergence range to the *D. auritus* group of 14.4-15.5% (Figure 4.4A). The intraspecific divergence within the *auritus* group ranged from 0.0-4.4%, with an average of 2.2% (Table 4.6). Both samples from this study came from *H. trivolvis* snails, which match with other records of specimens derived from planorbid snails in different geographical regions, specifically the U.S.A. and Brazil (Table 4.7). Recent work has revealed the synonymy of *Drepanocephalus auritus* with *Drepanocephalus spathans*, with *spathans* as the chosen name (Hernández-Cruz et al. 2018). Therefore, we have identified our sequences according to this.

Neopetasiger:

The ten sequences from this study all grouped within *Neopetasiger* sp. 4 and were less than 1% different in nucleotide identity from *Neopetasiger* sp. 4 (KM191817), with an average intraspecific divergence of 0.2% and an interspecific divergence of 21.1-28.2% (Figure 4.4B and Table 4.8). All *Neopetasiger* sp. samples from this study were derived from *H. trivolvis* snails, further indicating their specialization for planorbid snails, as indicated by other studies (Table 4.7).

Echinoparyphium/Hypoderaeum:

All methods in ABGD agreed on separation of the alignment into 17 groups (Pmax (all) = 0.0129) (Figure 4.5A). This separation was further supported by considering the range of intraspecific divergence values reported previously for several of these same lineages (Detwiler, Bos, and Minchella 2010). Further-

more, most groups supported a clear separation of lineages by first intermediate host use, confirmed from both Indiana and Alberta. For most lineages, the average within group nucleotide divergence was less than 5%. Despite ABGD results, some lineages with greater than 5% divergence, upon further inspection, revealed evidence for further splitting, including *Echinoparyphium* sp. Lineage 3 and *Hypoderaeum* sp. Lineage 1. For example, though ABGD showed *Echinoparyphium* sp. Lineage 3 to be one group made of four sequences, their p distance values were very divergent. The two sequences from GenBank previously identified as Lineage 3 were 2.7% divergent from each other, but 9.7 – 11% divergent from the two sequence from our study that were 3.7% divergent from each other. To us, this was a clear split, and was also highly supported by posterior probabilities and bootstrap values in phylogenetic analyses as well. We therefore derived a new lineage, *Echinoparyphium* sp. Lineage 4.

Within the *Hypoderaeum* sp. Lineage 1 clade, there was an obvious split occurring with three sequences forming their own clade (MGC577, MGC650, & MGC824). This split was not supported by ABGD or by host-use, as all utilized *S. elodes* snails. The nucleotide divergence, however, ranged between 0.3-5.4%. The small clade that was found diverging from the rest was 0.3-0.7% different from each other and 5.0-5.4% different from the others. The split was obvious and well supported within the phylogenies. We have therefore split this lineage into two groups, now including *Hypoderaeum* sp. Lineage 2 (Figure 4.5A).

Several additional new lineages have been added to the genus *Echinoparyphium* because of our sequencing efforts. We have labeled these as *Echinoparyphium* sp. A – E, and for the two that are close to the previously identified *Echinoparyphium* sp. Lineage 1, we have labeled as *Echinoparyphium* sp. Lineage 1 A – B (Figure 4.5A).

One group we could not clearly delineate further, despite divergence higher than the cut-off. *Echinoparyphium* sp. Lineage 2, displayed above 5% intraspecific divergence, with an intraspecific range of 0.0 – 5.7% and an average of 1.2%. The one isolate responsible for this greater divergence value is MGC369

that ranges from 1.7 – 5.7% from all other isolates within this lineage. All other isolates in this lineage have an intraspecific divergence range from 0.0 – 4.3% without MGC369. While this difference would seem a clear divergence, the phylogeny does not support the placement of MGC369 outside of this lineage. From host-use, we find that MGC369 utilized *L. stagnalis*, whereas the majority of lineage 2 isolates used *S. elodes*. While this would also seem to support differentiation, one other member MGC16B also utilized *L. stagnalis*, with very close sequence homology to other lineage 2 members (0.3 – 4.3%). Because neither the phylogenies nor host-use support further differentiation for this group, MGC369 remains in this lineage.

The *cox1* phylogenies for the Echinostomatidae (Figure 4.5B), for the most part, were well supported and matched patterns seen within the *nad1* phylogenies for this family. Because a few samples had both *cox1* and *nad1* sequences available, the lineage identities were informed by *nad1* because there were not many GenBank *cox1* sequences that matched. Overall, there was only one lineage within the *cox1* phylogeny that had no overlapping sequences, and these have been labeled broadly as Echinostomatidae gen. sp.

There were two unexpected patterns found within the *cox1* phylogeny as compared to the *nad1*. The lineage we identified as *Echinoparyphium* sp. Lineage 2 by *nad1* had a split, with very large divergence from isolate MGC16B to the other members of the lineage, upwards of 22.7%. Because there were no clear trends to help us understand this difference between the two genes, we have chosen to continue to include it within this same lineage, with the noted caveat.

The other unexpected pattern was within the lineage *Echinoparyphium* sp. A. Like the previous example, the lineage has split based on the *cox1* gene. The range of pairwise distance within this group, including members of both split lineages, was 0.0 – 21.4%, with an average intraspecific divergence of 10.5%. Without further evidence, one might conclude that this could be due to oversaturation in *cox1*, as previously noted for the echinostomes. We did see that one defining feature also separating these clades was intermediate host use. The clade that includes the isolate MGC1143 utilized *S. elodes* snails,

whereas members of the clade with isolate MGC658 all used *P. gyrina* snails. By *nad1*, MGC1143 diverged from the other members of this clade by 1.0-4.7%. MGC658 diverged by 0.3-3.3%. Both could be considered within an acceptable range, leaving the decision of lump or split nearly impossible based on sequences alone. Host use, especially for the first intermediate snail host, is strong evidence that these are more likely to be two different species. In considering that these snails are members of different families, and that the only other examples of different snail species being used within other genera of this family utilize species within the same snail family, namely *S. elodes* or *L. stagnalis*, both members of the Lymnaeidae, our best judgement is to split this into two species, based on host use (Tables 4.9-4.10).

Echinostoma:

There was strong nodal support by both BI and ML trees for the topology of the *Echinostoma* species (Figure 4.6). All ABGD distance methods supported the separation of the alignment into 15 groups (Pmax (JC & K2) = 0.0359; Pmax (simple dist.) = 0.02154). Intraspecific divergence values, based on the delineation cut-off, did not always support the same groups. For instance, *E. miyagawai, E. robustum*, and *E. revolutum* all exhibited ranges greater than 5%, despite the average being lower, except for *E. robustum* whose average was 5.4%. Placement of one sequence within the tree did not match expectations but had high statistical support; *E. robustum* (GQ463053) grouped within a clade of *E. miyagawai*. Inclusion of the *robustum* sequence did explain the greater intraspecific divergence within this clade, but there was not support for its placement with the other *robustum* sequences that also exhibit high intraspecific divergence. Further inspection of this particular *robustum* sequence has shown previous assessments that have identified this same trend, indeed showing it to be *E. miyagawai* (Simona Georgieva, Faltýnková, et al. 2014).

Sequences labeled/identified as *E. trivolvis* from GenBank resulted in two paraphyletic groups within the tree, the separation of which was confirmed by ABGD and within group divergence values of less than 5%. These observations confirmed previous lineage separation by J. T. Detwiler, et al. (2010).

The *Echinostoma* sequences from the present study all fit within two clades, either *E. revolutum* or *E. trivolvis* Lineage A. The *revolutum* group exhibited higher than expected intraspecific divergence that ranged from 0.0-6.0%. Though not supported by ABGD, there did appear to be two separate groups emerging, one that has been found among *S. elodes* snails (Lineage B), and the other among *Lymnaea* spp. and ducks (Lineage A). By splitting these lineages, we saw more reasonable intraspecific divergence values within Lineage B (0.0-1.6%), yet Lineage A continued to exhibit divergence higher than the cut-off (0.0-5.7%) (Table 4.11). Because Lineage B isolates all utilized the same snail host, we were more confident in the grouping of this lineage, but believe that further sampling will likely show greater differentiation within Lineage A.

Family Diplostomidae:

For both Diplostomidae-I and Diplostomidae-II groups, BI and ML phylogenies agreed on minor topologies, with greater support for external nodes and less support and agreement between the two methods for internal nodes (Figure 4.7). For Diplostomidae-I, all distance methods in ABGD agreed on 41 total groups (Pmax = 0.059), further supported by the 5% cut-off. A result worth noting from this phylogeny is that a sequence we previously identified as *Tylodelphys scheuringi* (KT831356) has now split from this group into a separate, new lineage we are now calling *Tylodelphys* sp. A. Several sequences from this study did not group specifically with any available GenBank sequences and have formed distinct lineages among the *Diplostomum* species. These have been identified as *Diplostomum* spp. A-C (Figure 4.7 and Table 4.12). Other lineage splits seen within *D. baeri* and *Tylodelphys* sp. 2 have previously been described (Miroslava Soldánová et al. 2017) and are further supported with our phylogeny.

Twenty-three groups were identified for Diplostomidae-II, supported by all distance methods of ABGD (Pmax = 0.059), and the 5% cut-off. Two lineages made up of sequences from this study did not group within a specific clade of previously identified sequences, and have thus been identified generally as Diplostomidae gen. sp. O and sp. X. One such sequence was previously identified as being most like *Ornithodiplostomum* sp. 4 (KT831363), however, in this

phylogeny, it grouped far from the other *Ornithodiplostomum* sequences. Of note is that a sequence from GenBank previously identified as *Posthodiplostomum* sp. 3 (FJ477217) grouped with high statistical support with sequences of *Posthodiplostomum centrarchi* (KX931421-3), supporting a very recent report of this same identification (Stoyanov et al. 2017) (Figure 8 and Table 4.13).

Family Strigeidae:

Few species with sequences across the *cox1* barcoding region were available from GenBank for comparison within the Strigeidae-I group. At the start of our analyses, only two species had matched with some of our sequences, *Cotylurus cornutus* and *C. gallinulae*. More recently, more *Cotylurus* species have been added to GenBank (Sean A. Locke et al. 2018), and these additions helped define three previously unidentifiable lineages from phylogenies. Both ML and BI trees agreed with strong statistical support for the division of all aligned sequences into 16 groups, which was further supported by ABGD (Pmax (all) = 0.0077-0.0129). Sequences from the present study were all more closely related to *Cotylurus* as opposed to *Ichthyocotylurus*, based on p distances. Five could be identified to previously named species (*C. cornutus, C. gallinulae, C. flabelliformis, C. marcogliesei*, and *C. strigeoides*) and six other lineages did not match to any GenBank sequences and have been identified as *Cotylurus* sp. A – F. Clade division is further supported by intermediate host use. While intraspecific divergence was within the cut-off for all species, there was lower than expected interspecific divergence between *Cotylurus cornutus* and *Cotylurus flabelliformis* (4.2%) (Figure 4.9A and Table 4.14).

The Strigeidae-II group utilized the previously published phylogenies of Blasco-Costa, et al. (2016) and Gordy, et al. (2017) as a starting point, with new sequence additions. Unfortunately, there were still no additional species in GenBank to add that would help inform this phylogeny further. However, the addition of new sequences from the present study have revealed even greater diversity than found previously and has supported the previously derived lineages. While both ML and BI trees agreed on topology and provided medium to strong node support, ABGD methods did not agree with the number of groups informed by previous phylogenies or across methods (JC: 26 groups

(Pmax = 0.0077), 21 groups (Pmax = 0.0129 – 0.0215), 14 groups (Pmax = 0.0359); K2: 26 groups (Pmax = 0.0129), 21 groups (Pmax = 0.0215 - 0.0359), 13 groups (Pmax = 0.059); Simple: 29 groups (Pmax = 0.007), 28 groups (Pmax = 0.0129), 18 groups (Pmax = 0.0215), 13 groups (Pmax = 0.0359)). Examining divergence based on p distances better supported the phylogenetic results, with 23 groups (including out-group sequences from *Apharyngostrigea* spp.) having been within the 5% intraspecific cut-off and having greater than 5% interspecific divergence, all except for *Australapatemon burti* LIN1, which had an intraspecific divergence range of 0.0-6.4% and an average of 1.1%. There were only a few sequences that reached the highest part of that range, one new sequence, MGC1629 that came from *S. elodes*, and five previously published sequences: four from Gordy et al., (2017) (KY587401, HM385485, KY587400, HM385486), all cercariae derived from *Planorbis* sp. snails in California, and JX977727, an adult from Mexico. Though they differed from some other LIN1 sequences greater than 5%, they were more similar to other LIN1 sequences with divergence less than 5%, which made it difficult to clearly delineate whether there was one monophyletic clade or more. Currently, there is not enough evidence to clearly support more than one clade within Lineage 1.

Therefore, with the best supported information, there appeared to be 23 groups within the Strigeidae-II, which revealed three new species of *Apatemon*: species A, which included our previously published *Apatemon* sp. (KT831859), and species B and C. Though these three species all utilized *S. elodes*, they were molecularly divergent. Within the *Australapatemon* clade, a new lineage appeared from isolate MGC2030 that utilized *P. gyrina*, identified herein as Lineage 10. Lineage 9, with the addition of more sequences, as predicted in Gordy, et al. (2017), has revealed the greater likelihood and separation of this lineage into two, which we have called Lineage 9A and Lineage 9B, both of which were hosted by *S. elodes* snails (Figure 4.9B and Table 4.15).

4.3.1 Species richness and rarefaction

Based on our estimates of species, as described above and evidenced from molecular phylogenies, we have recovered 79 trematode species from 5 snail

host species across six lakes in central Alberta. Richness recoveries were greatest at Isle Lake (38 trematode species/4 snail species), followed by Wabamun Lake (27/5), Gull Lake (24/3), Lac La Nonne Site #1 (18/3), Lac La Nonne Site #2 (16/3), Buffalo Lake – Pelican Point (16/3), Buffalo Lake – Rochon Sands (13/2), Buffalo Lake – The Narrows (13/4), and finally, Pigeon Lake Provincial Park (3/1) (Tables 4.1;4.7;4.16).

Of the 79 total trematode species reported here, 59 are newly identified species in this report that have resulted in 15 updated identifications for previously published sequences (Table 4.7). Thirty-nine of the 59 new identifications represent novel lineages/singletons (represented by "***" in Table 4.7), with another 2 lineages that represent a recent split (*Australapatemon* sp. LIN9A/9B). The remaining 20 species were previously identified, and for 15 of them, we have added further sequenced specimens, confirming their previous identifications and adding to our understanding of species presence and abundances in Alberta lakes (Table 4.7 and Appendix A).

Examining the relationship of trematode species richness and sample size (by sites/area and individuals) through rarefaction and non-linear models revealed a stark contrast between whether confidence for delimitation was at a family level (morphological analysis) or a species level (molecular analysis) (Figure 4.10A-C). Considering the accumulation of trematode families, the curves plateaued (individual-based) or approached one (site-based), suggesting we likely captured the available trematode families within our samples and sample region. However, when looking at the curves based on trematode species accumulation, there was no plateau, suggesting that there was potentially greater trematode species diversity than we captured from our sampling. Snails, on the other hand, plateaued in rarefaction analyses (Figure 4.10D-E). This was not at all surprising, considering that over three years of collections, we had yet to find more than 7 species.

The greatest richness recoveries by snail host species were found among *S. elodes* (40 trematode species), followed by *P. gyrina* (26), *H. trivolvis* (15), *L. stagnalis* (10), and *P. armigera* (1), following the same trend as identified previously (M. Gordy et al. 2016), but with more total species (Table 4.17).

Specificity for snail host species was high (55 specialist trematode species) among all but 15 trematodes, of which were found to infect 2 or more snail species (generalists). Some trematodes were found infecting snails from completely different families, these were: *Australapatemon burti* LIN1 (*S. elodes, L. stagnalis, H. trivolvis, P. gyrina*), *Echinoparyphium* sp. LIN1A (*S. elodes, H. trivolvis, P. gyrina*), and *Notocotylus* sp. A/D (*S. elodes, P. gyrina*).

4.4 Discussion

Fine-scale molecular analyses of trematodes in central Alberta have revealed many new and important insights about their diversity. What is perhaps most surprising is that species accumulation curves would suggest we have yet to capture all the possible trematode species within our sample area. Comparing the species-level to family-level accumulation clearly demonstrates how important the molecular phylogenetic perspective is. Herein, we have used the family-level as a proxy for the type of results achieved by morphological analysis only, in considering trematode larval stages. While morphological identification of trematode larvae can be less costly in terms of materials, it does not afford a very high level of confidence because of the issues surrounding cryptic species and underdeveloped, definable features. Family-level accumulation based on individuals and sites is achieved at a much higher rate than species, as expected, and reaches a plateau earlier. If, for instance, this representation is true of the number of species attained by a typical survey, it is likely that trematode surveys are missing much of the actual diversity present. This is important to note because of the potential impact on how we might interpret community assembly and structure in natural environments, especially in consideration of cryptic species.

Overall, the trematode species richness found by this longitudinal survey exceeded expectations, and the number of snail species needed in a community to maintain a diverse set of trematodes was surprisingly small. In our original morphological assessments, we expected 29 trematode species. With the use of molecular assessments, based on BLAST identities and fewer sequenced

samples, generated from the first two years of the study, we had expanded our view to 39 identified species (M. Gordy et al. 2016). Now, with more available sequence information, and the use of more stringent methods, we have, in total, recovered double the species from previous assessments at a total of 79 trematode species, 55 of which are new records to Alberta from this study alone. This raises the recorded trematode species in Alberta to 114, representing 16 families (Appendix 1).

For an ecoregion that has previously been considered species-poor (Hoberg et al. 2012), sub-Arctic lake ecosystems have presented a surprising amount of trematode diversity from recent surveys. From one lake in Norway, on the 69th parallel, 24 different trematode species were recovered, representing 7 different, common families from lakes in the Northern hemisphere (e.g. Strigeidae, Diplostomidae, Schistosomatidae, Echinostomatidae, Notocotylidae, Plagiorchiidae) (Miroslava Soldánová et al. 2017). Though further South, between the 54 th and 52nd parallel, our study is still considered within the sub-Arctic region and has uncovered a range of 3 – 38 trematode species representing 3 – 8 families, each, among six lakes (the lower end, from Pigeon Lake and Lac La Nonne, were only sampled in one year as opposed to three years for the other lakes). In-between, sampling of fish from the Saint Lawrence River in Quebec (between the 49th and 44th parallel) has revealed 47 species of just diplostomoids (Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010). From these surveys, it is apparent that our perspective of what constitutes incredible or unexpected diversity is changing and will continue to change as we take a closer look with molecular data. In all three of these studies, the unveiling of cryptic diversity has been a large component. From a recent meta-analysis of 110 studies, it has been noted that there is a trend, particularly among trematodes, that sequencing effort positively correlates with more cryptic species as opposed to any other group of helminths. This has been attributed to differences in trematode biology, and our ability as taxonomists to identify them by their morphological characters, or lack thereof (G. Pérez-Ponce de León and R. Poulin 2018).

From a basic search of the GenBank database, we can see that trematodes are not a neglected group, as there are 877,472 molecular records specific to Digeneans (as of August 2018). However, this is not to say that specific groups of digenean are not neglected nor that representation is not highly skewed to particular gene regions or to those species most important to human or veterinary health. Of the digenean sequences in GenBank, 15,185 were of mitochondrial origin. Considering the two most used mitochondrial genes for barcoding digeneans, we limited our search to *cox1* and *nad1* (ND1), finding that a few families were represented by more than 400 sequences, some having more *nad1* than *cox1* or vice-versa, and this was not consistent with the estimated number of genera or species within the family. For instance, the family Fasciolidae was found to have 463 *cox1* sequences that represented 31 unnamed species (uniquely identified in GenBank) and 4 named species. This family was also represented by 533 *nad1* sequences representing 45 molecular species and 7 named species. Considering that previous assessments have only identified 8 potential species in this family (Cribb T H and Warren, Alan Bray, R.A., Littlewood, D.T.J., Pichelin, S.P. and Herniou, E.A. 2001), this is incredible coverage. Other families, though, have nearly 900 species, like the Opecoelidae (Rodney A. Bray et al. 2016), and have a similar breadth of species and sequences as the Fasciolidae, showing them to be greatly underrepresented (Table 4.18).

In this study, the relevant trematode families with the best *cox1/nad1* coverage from GenBank were the Echinostomatidae, Strigeidae, and Diplostomidae. Despite many genera being represented within these families, there remain many gaps in species identifications. This was apparent through a large variety of unidentified species lineages. Unfortunately, our study has only widened this gap, by identifying even more novel, unidentified species lineages and singletons because we lack molecular evidence from adult worms. However, these efforts are not in vain, as they provide a foundation for further sampling that may create the missing life cycle links between larvae and adults in the future. For instance, the species *Cotylurus marcogliesei* was just described for the first time this year (Sean A. Locke et al. 2018), based on

adult worms derived from a Hooded Merganser in Montreal, QC. The alignment of our sequences to that of Locke et al. has now added a new snail first intermediate host record, *Stagnicola elodes*, in addition to a new geographical record of being in Alberta. Considering that *Cotylurus* spp. have been described as having snails as a second intermediate host, it is possible for them to use the same species, although typically not the exact same snail individual (Graczyk and Shiff 1993b). Meaning that further sampling of *S. elodes* may uncover metacercariae of *C. marcogliesei*. Overall, there is further opportunity for this species' second intermediate host to be discovered to complete our understanding of the life cycle and host-use within.

The trematode families found in Alberta that need greater sampling and effort from both adult worms and molecular barcoding are the Notocotylidae, Psilostomidae, Haematoloechidae, and most importantly, the Plagiorchiidae. The Plagiorchiidae are the most abundant family found in central Alberta lakes, and there is statistical evidence, through phylogenetics presented herein, for the presence of at least nine species. This family is said to be composed of at least 100 species (H. Blankespoor 1977). Furthermore *Plagiorchis* spp. have been indicated as vectors for Potomac Horse Fever (Vaughan, Vasyl V. Tkach, and Greiman 2012), which has been diagnosed among several horses near Edmonton, Alberta (personal communication with horse owners, and positive sequence identifications of *Neorickettsia risticii*, unpublished).

In the Notocotylidae, we identified four species, but all were provisionally named species A – D because, as with many of our samples, there was no clear evidence to connect them to any previously identified species, and the evidence found was quite disparate. From the literature, only two named species have been identified in Canada, including *Notocotylus attenuatus* (Quebec and Manitoba) and *N. urbanensis* (previously *N. filamentis*) (British Columbia and Ontario), and three others have been identified in the Nearctic region, *N. linearis, N. pacifier*, and *N. stagnicolae*. Broadly, these species infect Anatids and aquatic mammals like muskrats (multiple references found in (Gibson, Bray, and Harris 2005)). Prior evidence related to their snail hosts are limited to records from the U.S.A.: *N. attenuatus* has been identified from *Physa*

acuta in the Eastern U.S.A. (Graczyk and Shiff 1993a), and *N. urbanensis* was identified from *Stagnicola emarginata* in Michigan (Keas and Harvey D. Blankespoor 1997). No records to our knowledge have thus far indicated *P. gyrina, S. elodes*, or *H. trivolvis* as intermediate hosts for *Notocotylus* species. The only records of any *Notocotylus* spp. in Alberta previously have been unnamed species found in the shorebirds *Recurvirostra americana* and *Catoptrophorus semipalmatus* (Gibson, Bray, and Harris 2005). Considering that we cannot link these unknown species in shorebirds to our samples, the four species we have identified can be considered new geographical and host records for Notocotylids. A final note about this family is the need for further sampling among *Stagnicola* and *Physa* snail species in Alberta as an effort to further define *Notocotylus* sp. A and D. These two species have lower interspecific divergence between them than between the other species in the family, and exhibit mixed host use, with preference for one host over the other and that happen to be opposite of each other. We speculate that this may be evidence of a current speciation event in which increased host preferences are leading to specialization and resulting in their division, at least on a molecular level.

Both Haematoloechidae and Psilostomidae species were difficult to identify for several reasons. The first reason was that either there were not very many *cox1* sequences available for comparison, or the sequences available for that gene were from an upstream region and did not overlap. The other reason was that there have been no previous records of species from either family in Alberta, nor many records in general from snail hosts, and none from snails within Canada. While lymnaeid snails have previously been indicated as intermediate hosts for *Haematoloechus* spp. (Gibson, Bray, and Harris 2005), to our knowledge, none have been specifically identified from *S. elodes*. Several other snail families (Physidae and Planorbidae) are also hosts for different species of *Haematoloechus*, indicating they do not specialize by snail family, but could specialize for snail species, which may be regionally determined (Gibson, Bray, and Harris 2005). For both families, records within Canada have all come from the Eastern provinces (Quebec, Ontario, New Brunswick, and Nova Scotia) and from definitive hosts (Psilostomidae: Anatid birds and

aquatic mammals; Haematoloechidae: frogs) (Gibson, Bray, and Harris 2005). It is impossible at this point to know whether the presence of species from these families are from recent introductions or not, but they are rare in the fact that we only collected a few from each family over the course of three years. Considering their host species are quite prevalent across Alberta, it is possible that they have been here and remained undetected, but they could also have expanded their distributions westward into Alberta as well.

The gap between morphological and molecular species identities is growing larger, and the effort to find a solution is not growing at the same rate. Without the link between the two, we are missing important information about life cycle dynamics due to host associations and infection processes that could help inform wildlife managers and possibly influence control efforts for human and veterinary diseases caused by trematodes. One possible solution to this, aside from more molecular data from adult worm samples, is the development of methods to derive quality sequence information from historical, adult trematode specimens. As these vouchers have been our historical standard for species identifications, they are our ultimate source for generating molecular libraries by which to further our understanding of trematode diversity, speciation, and evolution with the added benefit of linking life cycles.

Furthermore, we urge the contribution of sequences that represent a broader diversity of digenean trematodes. One current issue is that novel lineages in molecular phylogenies could either represent cryptic species, or they could represent described species for which we have no/limited molecular resources. Therefore, placing emphasis on capturing a broader diversity of trematodes might help bridge knowledge gaps.

4.5 Conclusions

In this chapter, I have significantly built upon the knowledge gained from the longitudinal survey, established in chapter 2, by utilizing the taxonomic methods described in chapter 3. From these analyses, we uncover a diversity of seventy-nine larval trematode species among just five snail host species. Only

fourteen species were identified to a previously described species, while the other sixty-five species are either cryptic or otherwise unrepresented by mitochondrial genes in GenBank. This study currently represents the largest and most diverse singular molecular survey of trematode larval fauna composed of over one thousand mitochondrial sequences. Surprisingly, rarefaction analyses indicate we have yet to capture the complete diversity of trematodes from our sampling area, indicating we may have only begun to scratch the surface. With species identifiers for nearly all the samples in this study, we can now make calculations of diversity and better describe trematode communities and how they change over time within our study area. The following chapter will take the next steps toward describing trematode communities within our sample region, and test hypotheses related to the key drivers behind species presence and persistence within the community over the duration of the study.

Table 4.1: Counts of snail species by collection site

	Pelican Point (BL)	**Rochon Sands (BL)**	**The Narrows (BL)**	**Gull Lake**	**Isle Lake**	**Lac La Nonne**	**Lac La Nonne site #2**	**Pigeon Lake**	**Wabamun Lake**	**Grand Total**
Helisoma trivolvis	–	–	145	4	202	123	23	–	669	**1166**
Lymnaea stagnalis	1	–	462	28	1	–	–	–	24	**516**
Physa gyrina	209	257	329	195	831	138	324	4	1577	**3864**
Planorbula armigera	–	–	–	–	–	–	–	–	1	**1**
Stagnicola elodes	3567	368	36	399	3457	1179	370	–	129	**9505**
Unidentified lymnaeid	–	–	–	1192	–	–	–	–	1060	**2252**
Unidenitifed planorbid	–	–	78	7	1	–	–	–	57	**143**
Grand Total	**3777**	**625**	**1050**	**1825**	**4492**	**1440**	**717**	**4**	**3517**	**17447**

BL = Buffalo Lake

Table 4.2: Average *cox1* divergence within and between groups in the Notocotylidae. The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 98 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 322 positions in the final dataset. Standard error estimates are shown above the diagonal. Average within group divergence is given on the diagonal. Group intraspecific divergence ranges are given as percentages next to species names. Numbers in red are outside the delineation cut-off.

	E. hortens.	*N.* sp. A	*O. sikae*
Echinostoma hortense (out)	-	0.023	0.023
Notocotylidae sp. A (0.0-9.9%)	0.273	**0.030**	0.020
Ogmocotyle sikae	0.258	0.194	-

Table 4.3: Pairwise distance between individual *cox1* sequences in the Psilostomatidae. The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 496 positions in the final dataset.

	MGC342	MGC2085	MGC2089	MGC406	KT831366	MGC1319	*E. japonicus*	*P. varium*	*S. globulus*	*S. pseudoglobulus*	*S. pseudoglobulus*
Psilostomatidae gen. sp. A *** MGC342		0.004	0.004	0.004	0.004	0.004	0.018	0.015	0.019	0.019	0.019
Psilostomatidae gen. sp. A *** MGC2085	0.010		0.000	0.002	0.002	0.002	0.018	0.015	0.019	0.019	0.019
Psilostomatidae gen. sp. A *** MGC2089	0.010	0.000		0.002	0.002	0.002	0.018	0.015	0.019	0.019	0.019
Psilostomatidae gen. sp. A *** MGC406	0.008	0.002	0.002		0.000	0.000	0.018	0.015	0.019	0.019	0.019
Psilostomatidae gen. sp. A *** KT831366	0.008	0.002	0.002	0.000		0.000	0.018	0.015	0.019	0.019	0.019
Psilostomatidae gen. sp. A *** MGC1319	0.008	0.002	0.002	0.000	0.000		0.018	0.015	0.019	0.019	0.019
Echinochasmus japonicus (out)	0.234	0.234	0.234	0.236	0.236	0.236		0.018	0.018	0.018	0.018
Pseudopsilostoma varium	0.143	0.145	0.145	0.147	0.147	0.147	0.230		0.019	0.018	0.018
Sphaeridiotrema globulus	0.242	0.244	0.244	0.246	0.246	0.246	0.228	0.258		0.018	0.018
Sphaeridiotrema pseudoglobulus	0.244	0.246	0.246	0.244	0.244	0.244	0.232	0.254	0.192		0.000
Sphaeridiotrema pseudoglobulus	0.244	0.246	0.246	0.244	0.244	0.244	0.232	0.254	0.192	0.000	

Table 4.4: Pairwise distance between individual *cox1* sequences in the Haematoloechidae. The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 454 positions in the final dataset.

	KT831372	MGC1782	MGC1787	MGC1792	*H.* sp. 9781	*H.* sp. 9782	*Plagiorchis* sp.
Haematoloechidae gen. sp. A ***KT831372		0.000	0.000	0.000	0.016	0.018	0.020
Haematoloechidae gen. sp. A ***MGC1782	0.000		0.000	0.000	0.016	0.018	0.020
Haematoloechidae gen. sp. A ***MGC1787	0.000	0.000		0.000	0.016	0.018	0.020
Haematoloechidae gen. sp. A ***MGC1792	0.000	0.000	0.000		0.016	0.018	0.020
Haematoloechus sp. BOLD:ACK9781	0.134	0.134	0.134	0.134		0.019	0.020
Haematoloechus sp. BOLD:ACK9782	0.207	0.207	0.207	0.207	0.216		0.019
Plagiorchis sp. (out)FJ477214	0.240	0.240	0.240	0.240	0.258	0.218	

Table 4.5: Average *cox1* divergence within and between groups of *Plagiorchis* sp. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. The average within group divergence is given on the diagonal. The range of pairwise distances within each group are given as percentages in the first column after group names. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 55 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 435 positions in the final dataset.

	P. sp. LIN1	*P.* sp. LIN2	*P.* sp. LIN3	*P.* sp. LIN4	*P.* sp. LIN5	*P.* sp. LIN6	*P.* sp. LIN7	*P.* sp. LIN8	*P.* sp. LIN9
Plagiorchis sp. LIN1 (0.0-2.3%)	**0.010**	0.017	0.017	0.015	0.016	0.017	0.017	0.017	0.016
Plagiorchis sp. LIN2	0.165	**-**	0.017	0.017	0.016	0.017	0.016	0.018	0.017
Plagiorchis sp. LIN3 (0.2-0.7%)	0.148	0.178	**0.005**	0.016	0.017	0.017	0.017	0.017	0.018
Plagiorchis sp. LIN4 (0.0-0.7%)	0.123	0.145	0.140	**0.003**	0.015	0.016	0.016	0.016	0.016
Plagiorchis sp. LIN5 (0.2-0.5%)	0.148	0.152	0.173	0.121	**0.003**	0.016	0.015	0.017	0.017
Plagiorchis sp. LIN6	0.169	0.156	0.162	0.143	0.149	**-**	0.016	0.016	0.016
Plagiorchis sp. LIN7 (0.0-0.7%)	0.157	0.158	0.162	0.149	0.125	0.143	**0.004**	0.017	0.017
Plagiorchis sp. LIN8 (0.2-1.8%)	0.166	0.188	0.180	0.140	0.159	0.151	0.154	**0.013**	0.012
Plagiorchis sp. LIN9 (0.0-1.1%)	0.151	0.167	0.171	0.142	0.150	0.155	0.155	0.089	**0.005**

Table 4.6: Pairwise distance between individual *nad1* sequences in the genus *Drepanocephalus*. The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 388 positions in the final dataset.

	F. hepatica	MGC2147	MGC2353	*D. auritus*	*D. auritus*	*D.* sp.
Fasciola hepatica (out)		0.021	0.021	0.021	0.022	0.021
MGC2147 MH368951	0.227		0.000	0.000	0.010	0.018
MGC2353 MH368952	0.227	0.000		0.000	0.010	0.018
Drepanocephalus auritus KP053262	0.227	0.000	0.000		0.010	0.018
Drepanocephalus auritus KP053263	0.235	0.044	0.044	0.044		0.018
Drepanocephalus sp. KP053264	0.245	0.144	0.144	0.144	0.155	

Table 4.7: Host associations, geographical origins, and life stages of specimen sequences used in phylogenies.

					GenBank Accession Number(s)		
Family	**Species**	**Host**	**Host Type**	**Location**	*cox1*	*nad1*	**Reference**
Diplostomidae	*Alaria* sp. 1	*Lithobates pipiens*	2	Canada: Quebec, Saint Lawrence River	JF769439		(Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011)
Diplostomidae	*Alaria* sp. 2	*Pseudacris regilla, Anaxyrus boreas*	2	USA: California, Bart's Pond; San Martin, Weed Pond	JF904535, JF904534, JF904536		(Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011)
Diplostomidae	*Austrodiplostomum ostrowskiae*	*Biomphalaria obstructa, Dorosoma cepedianum*	1, 2	USA: Noxubee County, MS;USA: Dallas County, AL	KT728795, KT728798, KT728799		(Rosser et al. 2016)
Diplostomidae	*Austrodiplostomum* sp. 1	*Pomoxis nigromaculatus*	2	USA: Florida, Tampa, Lake Seminole	KR271029		(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Austrodiplostomum* sp. 2	*Mugil cephalus*	2	USA: Florida, Tampa, Lake Seminole	KR271032		(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Austrodiplostomum* sp. 2	*Menidia beryllina, Ictalurus punctatus*	2	USA: Mississippi	KU707943, KU707945		(Rosser et al. 2016)
Diplostomidae	*Bolbophorus damnificus*	*Menidia beryllina*	2	USA: Mississippi	KU707937		(Rosser et al. 2016)
Diplostomidae	*Bolbophorus* sp.	*Pimephales promelas*	2	Canada: Alberta, Coaldale, McQuillan Lake	KM538081		(Van Steenkiste et al. 2014)
Diplostomidae	*Bolbophorus* sp.	*Menidia beryllina*	2	USA: Mississippi	KU707938, KU707939		(Rosser et al. 2016)
Diplostomidae	*Bolbophorus* sp.	*Helisoma trivolvis*	1	Canada: Alberta, Buffalo Lake	KT831373		(M. Gordy et al. 2016)
Diplostomidae	*Bolbophorus* sp.	*Helisoma trivolvis*	1	Canada: Alberta, Buffalo Lake, Isle Lake, Wabamun Lake	MH368843, MH368847, MH368850, MH368862, MH368871, MH368892, MH368918, MH368919		Present study
Diplostomidae	*Crocodillicola pseudostoma* (out)	*Rhamdia guatemalensis*	2	Mexico: Veracruz, Catemaco Lake	MF398317, MF398318		(Hernández-Mena, Martín García-Varela, and Gerardo Pérez-Ponce de León 2017)
Diplostomidae	Diplostomidae gen. sp. O***	*Physa gyrina*	1	Canada: Alberta, Buffalo Lake	KT831363 §		(M. Gordy et al. 2016)

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					GenBank Accession Number(s)	
Diplostomidae	Diplostomidae gen. sp. O***	*Physa gyrina*	1	Canada: Alberta, Buffalo Lake, Wabamun Lake, Gull Lake, Isle Lake	MH368825, MH368851, MH368854, MH368855, MH368879, MH368880, MH368881, MH368882, MH368883, MH368884, MH368885, MH368886, MH368887, MH368888, MH368889, MH368890, MH368893, MH368903, MH368904, MH368905, MH368906, MH368915, MH368916, MH368917, MH368934, MH368935, MH368936, MH368937, MH368938, MH368939, MH368940, MH368941, MH368942	Present study
Diplostomidae	Diplostomidae gen. sp. X***	*Physa gyrina*	1	Canada: Alberta, Isle Lake	MH368907	Present study
Diplostomidae	*Diplostomum ardeae*	*Ardea herodias*	3	Canada: Quebec, Montreal	KR271033	(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Diplostomum baeri* LIN1	*Perca fluviatilis*	2	Germany: Lake Constance	JQ639181, JQ639182	(Behrmann-Godel 2013)
Diplostomidae	*Diplostomum baeri* LIN2	Not given	3	Canada: Quebec, Montreal	GQ292501	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum baeri* LIN2	*Stagnicola elodes*	1	Canada: Alberta, Wabamun Lake, Isle Lake	MH368863, MH368874, MH368875, MH368928	Present study
Diplostomidae	*Diplostomum huronense*	*Notemigonus crysoleuca, Larus delawarensis*	2, 3	Canada: Ontario	FJ477197	(Moszczynska et al. 2009)
Diplostomidae	*Diplostomum huronense*	*Perca flavescens, Notemigonus crysoleucas*	2	Canada: Quebec, St. Lawrence River, Lake Saint Louis, Beauharnois, Dorval Island	HM064671, HM064672	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum indistinctum*	Catostomidae	3	Canada: Quebec	FJ477196	(Moszczynska et al. 2009)
Diplostomidae	*Diplostomum indistinctum*	*Neogobius melanostomus*	2	Canada: Quebec	GQ292482	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum indistinctum*	*Catostomus commersoni*	2	Canada: Quebec, St. Lawrence River, Lake Saint	HM064673	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum indistinctum*	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake	KT831379	(M. Gordy et al. 2016)

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Table 4.7 – *Continued from previous page*

					GenBank Accession Number(s)		
Diplostomidae	*Diplostomum mergi*	*Radix auricularia*	1	Germany: Hengsteysee	KR149526, KR149527, KR149528		(Selbach, Soldánová, Georgieva, Kostadinova, and Sures 2015)
Diplostomidae	*Diplostomum parviventosum*	*Radix auricularia*	1	Germany: Hengsteysee	KR149510, KR149511, KR149512		(Selbach, Soldánová, Georgieva, Kostadinova, and Sures 2015)
Diplostomidae	*Diplostomum pseudospathaceum*	*Stagnicola palustris*	1	Germany: Hengsteysee	KR149544, KR149545, KR149546		(Selbach2015)
Diplostomidae	*Diplostomum* sp. 1	*Larus delawarensis*	3	Canada: Quebec, Laurentides	GQ292479, GQ292480, GQ292481		(Locke2010)
Diplostomidae	*Diplostomum* sp. 1	*Stagnicola elodes*	1	Canada: Alberta, Wabamun Lake, Isle Lake	MH368857, MH368896, MH368932, MH368943, MH368945		Present study
Diplostomidae	*Diplostomum* sp. 2	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River	GQ292486		(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum* sp. 3	*Micropterus salmoides*	2	Canada: Quebec, St. Lawrence River	GQ292487		(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum* sp. 3	*Lymnaea stagnalis*	1	Canada: Alberta, Wabamun Lake	KT831358		(M. Gordy et al. 2016)
Diplostomidae	*Diplostomum* sp. 3	*Lymnaea stagnalis*	1	Canada: Alberta, Wabamun Lake	MH368837, MH368858		Present study
Diplostomidae	*Diplostomum* sp. 4	*Larus delawarensis*	3	Canada: Quebec, Laurentides	GQ292494, GQ292495		(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Diplostomum* sp. 4	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	KT831354		(M. Gordy et al. 2016)
Diplostomidae	*Diplostomum* sp. 4	*Stagnicola elodes*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH368808, MH368809, MH368813, MH368814, MH368815, MH368816, MH368818, MH368819, MH368820, MH368821, MH368822, MH368823, MH368824, MH368826, MH368827, MH368828, MH368829, MH368830, MH368831, MH368832, MH368833,		Present study

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					GenBank Accession Number(s)		
Diplostomidae	*Diplostomum* sp. 4	*Stagnicola elodes*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH368834, MH368836, MH368839, MH368841, MH368845, MH368848, MH368853, MH368859, MH368861, MH368865, MH368867,	MH368835, MH368838, MH368840, MH368844, MH368846, MH368849, MH368856, MH368860, MH368864, MH368866,	Present study
Diplostomidae	*Diplostomum* sp. 4	*Stagnicola elodes*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH368868, MH368870, MH368873, MH368877, MH368898, MH368900, MH368911, MH368914, MH368925, MH368927, MH368930,	MH368869, MH368872, MH368876, MH368891, MH368899, MH368901, MH368913, MH368924, MH368926, MH368929,	Present study
Diplostomidae	*Diplostomum* sp. 4	*Stagnicola elodes*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH368931, MH368946, MH368948, MH368950	MH368944, MH368947, MH368949,	Present study
Diplostomidae	*Diplostomum* sp. 6	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River	GQ292499		(Sean A Locke, J. Daniel McLaughlin, et al. 2010)
Diplostomidae	*Diplostomum* sp. 7	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River	GQ292500		(Sean A Locke, J. Daniel McLaughlin, et al. 2010)
Diplostomidae	*Diplostomum* sp. 8	*Rana pipiens*	2	Canada: Quebec, Monteregie	GQ292497		(Sean A Locke, J. Daniel McLaughlin, et al. 2010)
Diplostomidae	*Diplostomum* sp. 9	*Percina caprodes*	2	Canada: Quebec, St. Lawrence River	GQ292496		(Sean A Locke, J. Daniel McLaughlin, et al. 2010)
Diplostomidae	*Diplostomum* sp. A***	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	MH368817		Present study
Diplostomidae	*Diplostomum* sp. B***	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	MH368933		Present study
Diplostomidae	*Diplostomum* sp. C***	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Wabamun Lake, Isle Lake	KT831360 §, KT831382 §	KT831378 §,	(M. Gordy et al. 2016)
Diplostomidae	*Diplostomum* sp. C***	*Stagnicola elodes, Helisoma trivolvis* (MGC208)	1	Canada: Alberta, Gull Lake, Wabamun Lake, Isle Lake	MH368810, MH368812, MH368895, MH368921, MH368923	MH368811, MH368852, MH368902, MH368922,	Present study

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					GenBank Accession Number(s)	
Diplostomidae	*Diplostomum* sp. clade Q	*Radix auricularia*	1	Germany: Hengsteysee	KR149554	(Selbach, Soldánová, Georgieva, Kostadinova, and Sures 2015)
Diplostomidae	*Diplostomum* sp. LIN6	*Gasterosteus aculeatus*	2	Norway: Troms, Takvatnet	KM212051, KM212052, KM212053	(Kuhn et al. 2015)
Diplostomidae	*Diplostomum spathaceum*	*Acanthobrama marmid, Perca fluviatilis, Barbus luteus*	2	Iraq: Saladin, Tikreet, Tigris River; Italy: Lecco, Lake Como, Oliveto Lario	KR271467, KR271468, KR271469	(Sean A Locke, J. Daniel McLaughlin, et al. 2010)
Diplostomidae	*Diplostomum spathaceum*	unknown		unknown, likely China	KT736038	Dang, R., et al., 2015, Unpublished
Diplostomidae	*Hysteromorpha triloba*	*Catostomus, Notemigonus crysoleucas*	2	Canada: Nova Scotia, Sackville, Feely Lake; Canada: Quebec, Outaouais, Ottawa River, Wendover	JF769475, JF769476	(Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011)
Diplostomidae	*Neodiplostomum americanum*	*Lithobates aurora*	2	USA: California, HMB 05	JF904537, JF904538, JF769455	(Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011)
Diplostomidae	*Neodiplostomum americanum*	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	KT831357 §	(M. Gordy et al. 2016)
Diplostomidae	*Ornithodiplostomum scardinii*	*Scardinius erythrophthalmus*	2	Czech Republic: Lake Macha	KX931425	(Stoyanov et al. 2017)
Diplostomidae	*Ornithodiplostomum scardinii* (out)	*Scardinius erythrophthalmus*	2	Czech Republic: Lake Macha	KX931425	(Stoyanov et al. 2017)
Diplostomidae	*Ornithodiplostomum* sp. 1	*Etheostoma nigrum*	2	Canada: Ontario, St. Lawrence River	FJ477208	(Moszczynska et al. 2009)
Diplostomidae	*Ornithodiplostomum* sp. 1	*Etheostoma nigrum*	2	Canada: Quebec, St. Lawrence River, Lake Saint Francois, Pointe Dupuis (LSF-2)	HM064742	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Ornithodiplostomum* sp. 2	*Physa gyrina*	1	Canada: Alberta, Wabamun Lake	KT831368	(M. Gordy et al. 2016)
Diplostomidae	*Ornithodiplostomum* sp. 2	*Notemigonus crysoleucas*	2	Canada: Quebec, St. Lawrence River, Lake Saint Louis, Beauharnois	HM064766, HM064768	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Ornithodiplostomum* sp. 2	*Notemigonus crysoleucas*	2	Canada: Quebec, St. Lawrence River, Lake Saint Louis	FJ477210	(Moszczynska et al. 2009)
Diplostomidae	*Ornithodiplostomum* sp. 2	*Physa gyrina*	1	Canada: Alberta, Wabamun Lake	KT831368	(M. Gordy et al. 2016)

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					GenBank Accession Number(s)	
Diplostomidae	*Ornithodiplostomum* sp. 3	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Francois, Pointe Dupuis (LSF-2), Beauharnois	HM064782, HM064780	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Ornithodiplostomum* sp. 3	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Francois, Pointe Dupuis (LSF-2), Beauharnois	FJ477211	(Moszczynska et al. 2009)
Diplostomidae	*Ornithodiplostomum* sp. 4	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Francois, Pointe Dupuis (LSF-2), Beauharnois	HM064786, HM064788	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Ornithodiplostomum* sp. 4	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Francois, Pointe Dupuis (LSF-2), Beauharnois	FJ477212	(Moszczynska et al. 2009)
Diplostomidae	*Ornithodiplostomum* sp. 8	*Pimephales notatus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Ile aux Ours	HM064789	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Ornithodiplostomum* sp. 8	*Physa gyrina*	1	Canada: Alberta, Pigeon Lake	KT831383	(M. Gordy et al. 2016)
Diplostomidae	*Ornithodiplostomum* sp. 8	*Physa gyrina*	1	Canada: Alberta, Isle Lake	MH368908, MH368910, MH368920	Present study
Diplostomidae	*Posthodiplostomum brevicaudatum*	*Perca fluviatilis, Gasterosteus aculeatus*	2	Czech Republic: Lake Macha; Bulgaria: Lake Atanasovsko	KX931418, KX931419, KX931420	(Stoyanov et al. 2017)
Diplostomidae	*Posthodiplostomum centrarchi*	*Lepomis gibbosus, Ardea cinerea*	2, 3	Bulgaria: Lake Atanasovsko; Spain: Lagoon Bassa de les Olles, Ebro Delta; Slovakia: River Danube near Sturovo	KX931421, KX931422, KX931423	(Stoyanov et al. 2017)
Diplostomidae	*Posthodiplostomum cuticola*	*Planorbis planorbis*	1	Lithuania: Curonian Bay near Juodkrante	KX931424	(Stoyanov et al. 2017)
Diplostomidae	*Posthodiplostomum* sp. 1	*Ambloplites rupestris*	2	Canada: Ontario, St. Lawrence River	FJ477215	(Moszczynska et al. 2009)

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Table 4.7 – *Continued from previous page*

					GenBank Accession Number(s)	
Diplostomidae	*Posthodiplostomum* sp. 2	*Lepomis gibbosus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Ile aux Ours	FJ477216	(Moszczynska et al. 2009)
Diplostomidae	*Posthodiplostomum* sp. 3*	*Lepomis gibbosus*	2	Canada: Quebec, St. Lawrence River, Beauharnois	FJ477217	(Moszczynska et al. 2009)
Diplostomidae	*Posthodiplostomum* sp. 4	*Lepomis gibbosus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Ile aux Ours	FJ477218	(Moszczynska et al. 2009)
Diplostomidae	*Posthodiplostomum* sp. 4	*Ardea herodias*	3	Canada: Quebec, Lac Saint-Pierre, Grand Ile	HM064844	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Posthodiplostomum* sp. 4	*Physa gyrina*	1	Canada: Alberta, Isle Lake	MH368909, MH368912	Present study
Diplostomidae	*Posthodiplostomum* sp. 5	*Lepomis gibbosus*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Iles aux Sables	FJ477219	(Moszczynska et al. 2009)
Diplostomidae	*Posthodiplostomum* sp. 7	*Perca flavescens*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Iles aux Sables	FJ477221	(Moszczynska et al. 2009)
Diplostomidae	*Posthodiplostomum* sp. 7	*Perca flavescens*	2	Canada: Quebec, St. Lawrence River, Beauharnois	HM064865, HM064871	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Posthodiplostomum* sp. 8	*Micropterus dolomieu*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Iles aux Sables	HM064873, HM064874, HM064875	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Tylodelphys aztecae*	*Skiffia lermae, Gila conspersa*	2	Mexico	KT175367, KT175368, KT175369	(M. García-Varela et al. 2016)
Diplostomidae	*Tylodelphys clavata*	*Perca fluviatilis*	2	Germany: Lake Constance	JQ639201, JQ639202, JQ639203, JQ639204	(Behrmann-Godel 2013)
Diplostomidae	*Tylodelphys clavata*	*Radix auricularia*	1	Germany: Hengsteysee	JX986908	(Georgieva et al. 2013)
Diplostomidae	*Tylodelphys clavata*	*Perca fluviatilis*	2	Romania: Danube Delta;Italy: Lombardy, Brescia, Oglio River;Italy: Lecco, Lake Como, Oliveto Lario	KR271478, KR271479, KR271480	(Sean A Locke, Caffara, et al. 2015)

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					GenBank Accession Number(s)	
Diplostomidae	*Tylodelphys excavata*	*Planorbarius corneus*	1	Czech Republic: Pond Bohdanec	KC685344	(Chibwana et al. 2013)
Diplostomidae	*Tylodelphys immer*	*Salvelinus fontinalis, Gavia immer*	2, 3	Canada: Quebec, Bas-Saint-Laurent, Central, riviere Bic; Montreal	KR271491, KR271492, KR271493	(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Tylodelphys jenynsiae*	*Cnesterodon decemmaculatus*	2	Argentina: Buenos Aires, La Plata, Urban canal	KR271494, KR271495, KR271496	(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Tylodelphys mashonensis*	*Clarias gariepinus*	2	Tanzania: River Msimbazi, River Ruvu	KC685340, KC685341, KC685342, KC685343	(Chibwana et al. 2013)
Diplostomidae	*Tylodelphys scheuringi*	*Ambloplites rupestris*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Iles aux Sables	FJ477223	(Moszczynska et al. 2009)
Diplostomidae	*Tylodelphys scheuringi*	*Perca flavescens, Ambloplites rupestris*	2	Canada: Ontario, St. Lawrence River, Lake Saint Francois, LSF-1; Lake Saint Louis, Dorval Island	HM064914, HM064915	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Diplostomidae	*Tylodelphys scheuringi*	*Ambloplites rupestris, Perca flavescens*	2	Canada: Ontario, St. Lawrence River, Pointe Dupuis (LSF-2)	KR271508, KR271509	(Chibwana et al. 2013)
Diplostomidae	*Tylodelphys* sp.	*Mystus tengara*	2	India	KU725888, KU725889	Chaudhary, A., et al., 2016, Unpublished
Diplostomidae	*Tylodelphys* sp.	*Gobiomorphus cotidianus*	2	New Zealand	KU588147, KU588148, KU588149	(Blasco-Costa, Cutmore, et al. 2016)
Diplostomidae	*Tylodelphys* sp. 2 LIN1	*Clarias gariepinus*	2	Tanzania: Lake Victoria	KC685358	(Chibwana et al. 2013)
Diplostomidae	*Tylodelphys* sp. 2 LIN2	*Micropterus salmoides, Oreochromis leucostictus*	2	Kenya: Rift Valley, Nakuru District, Lake Naivasha	KF809488, KF809494	(Otachi et al. 2015)
Diplostomidae	*Tylodelphys* sp. 3	*Lepomis microlophus*	2	USA: Mississippi, Ascension Parish	KR271513, KR271514, KR271515	(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Tylodelphys* sp. 4	*Gobiomorus maculatus*	2	Mexico: Oaxaca, Costa Chica, Playa Ventanilla, Laguna Ventanilla	KR271517, KR271518, KR271519	(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Tylodelphys* sp. 5	*Dormitator latifrons, Gobiomorus maculatus*	2	Mexico: Oaxaca, Costa Chica, Playa Ventanilla, Laguna Ventanilla	KR271520, KR271521	(Sean A Locke, Caffara, et al. 2015)
Diplostomidae	*Tylodelphys* sp. 6	*Poecilia latipinna*	2	USA: Mississippi, Ascension Parish	KR271522, KR271523	(Sean A Locke, Caffara, et al. 2015)

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					GenBank Accession Number(s)		
Diplostomidae	*Tylodelphys* sp. A***	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake	KT831356 §		(M. Gordy et al. 2016)
Diplostomidae	*Tylodelphys* sp. A***	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake	MH368842, MH368878, MH368894, MH368897		Present study
Echinostomatidae	*Drepanocephalus auritus*	*Planorbella trivolvis, Biomphalaria straminea*	1	USA; Brazil		KP053262, KP053263	(Pinto et al. 2016)
Echinostomatidae	*Drepanocephalus auritus*	*Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Buffalo Lake		MH368951, MH368952	Present Study
Echinostomatidae	*Drepanocephalus auritus*	*Phalacrocorax auritus*	3	Canada: Ontario, Lake Erie	KM538090		(Van Steenkiste et al. 2014)
Echinostomatidae	*Drepanocephalus auritus*	*Phalacrocorax auritus*	3	USA: lake Near Lakota, Nelson County, North Dakota; Lower Red Lake, Beltrami County, Minnesota; George County, Mississippi	KP683125, KP683126, KP683127, KP638128, KP683129, K638130, KP638131, KP638132		(Kudlai et al. 2015)
Echinostomatidae	*Drepanocephalus auritus*	*Planorbella trivolvis*	1	USA	KR259644		(Pinto et al. 2016)
Echinostomatidae	*Drepanocephalus auritus*	*Helisoma trivolvis*	1	Canada: Alberta, Buffalo Lake	KT831381		(M. Gordy et al. 2016)
Echinostomatidae	*Drepanocephalus auritus*	*Helisoma trivolvis*	1	Canada: Alberta, Buffalo Lake, Isle Lake	MH369294		Present study
Echinostomatidae	*Drepanocephalus mexicanus*	*Phalacrocorax brasilianus*	3	Mexico: Tobasco, Teapa	KY636228, KY636229		(Hernández-Cruz et al. 2018)
Echinostomatidae	*Drepanocephalus* sp.	*Biomphalaria straminea*	1	Brazil		KP05264	(Pinto et al. 2016)
Echinostomatidae	*Drepanocephalus spathans*	*Phalacrocorax brasilianus*	3	Mexico: Durango, Rio Guatimape; Oaxaca, Presa Rio Verde	KY636233, KY636234		(Hernández-Cruz et al. 2018)
Echinostomatidae	*Echinoparyphium aconiatum*	*Lymnaea stagnalis*	1, 2	Finland: Lake Pyykosjarvi		AY168946, AY168947	(Kostadinova et al. 2003)
Echinostomatidae	*Echinoparyphium ellisi*	*Anas platyrhynchos*	3	New Zealand: Clutha River System, Central Otago District, South Island		KY436405, KY436406	(Stoyanov et al. 2017)
Echinostomatidae	*Echinoparyphium poulini*	*Cygnus atratus*	3	New Zealand: Pauerau, Central Otago District, South Island		KY436403, KY436404	(Stoyanov et al. 2017)
Echinostomatidae	*Echinoparyphium recurvatum*	*Lymnaea peregra*	1	UK: Wales, Lake Ceunant		AY168943, AY168944	(Kostadinova et al. 2003)

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					GenBank Accession Number(s)	
Echinostomatidae	*Echinoparyphium recurvatum*	*Pisidium casertanum, Sphaerium* sp.	2	Norway: Lake Takvatn		KY513267, KY513269 (Miroslava Soldánová et al. 2017)
Echinostomatidae	*Echinoparyphium* sp. 1A***	*Physa gyrina, Stagnicola elodes* (MGC1954, MGC2104), *Helisoma trivolvis* (MGC2090)	1	Canada: Alberta, Lac La Nonne, Wabamun Lake, Isle Lake		MH368998, MH368999, MH369001, MH369002, MH369003, MH369004, MH369005, MH369006, MH369007, MH369008, MH369009, MH369010, MH369012, MH369013, MH369014, MH369015, MH369016, MH369017, MH369018, MH369019, MH369022, Present study
Echinostomatidae	*Echinoparyphium* sp. 1A***	*Physa gyrina, Stagnicola elodes* (MGC1954, MGC2104), *Helisoma trivolvis* (MGC2090)	1	Canada: Alberta, Lac La Nonne, Wabamun Lake, Isle Lake		MH369023, MH369024, MH369025, MH369026, MH369028, MH369031, MH369032, MH369033, MH369034, MH369038, MH369042, MH369044, MH369045, MH369046, MH369047, MH369048, MH369049, MH369052, MH369053, MH369054, MH369055, Present study

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					GenBank Accession Number(s)	
Echinostomatidae	*Echinoparyphium* sp. 1A***	*Physa gyrina*, *Stagnicola elodes* (MGC1954, MGC2104), *Helisoma trivolvis* (MGC2090)	1	Canada: Alberta, Lac La Nonne, Wabamun Lake, Isle Lake		MH369056, MH369059, MH369060, MH369062, MH369063, MH369065, MH369066, MH369068, MH369070, MH369075, MH369076, MH369087, MH369089, MH369090, MH369091, MH369093, MH369094, MH369095, MH369096, MH369097, MH369098, Present study
Echinostomatidae	*Echinoparyphium* sp. 1A***	*Physa gyrina*, *Stagnicola elodes* (MGC1954, MGC2104), *Helisoma trivolvis* (MGC2090)	1	Canada: Alberta, Lac La Nonne, Wabamun Lake, Isle Lake		MH369099, MH369100, MH369101, MH369102, MH369121, MH369122, MH369123, MH369125, MH369131, MH369132, MH369133, MH369136, MH369147, MH369155, MH369156, MH369162, MH369163, MH369164, MH369165, MH369166, MH369167, MH369168, MH369178, MH369188, MH369191 Present study
Echinostomatidae	*Echinoparyphium* sp. 1A***	*Physa gyrina*	1	Canada: Alberta, Lac La Nonne	KT831361 §	(M. Gordy et al. 2016)

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					GenBank Accession Number(s)		
Echinostomatidae	*Echinoparyphium* sp. 1A***	*Physa gyrina, Stagnicola elodes* (MGC1954, MGC2104), *Helisoma trivolvis* (MGC2090)	1	Canada: Alberta, Lac La Nonne, Wabamun Lake, Isle Lake	MH369243, MH369245, MH369246, MH369249, MH369250, MH369253, MH369255, MH369272, MH369273, MH369274, MH369277, MH369299, MH369300, MH369301, MH369302, MH369303, MH369304, MH369305		Present study
Echinostomatidae	*Echinoparyphium* sp. 1B***	*Physa gyrina*	1	Canada: Alberta, Isle Lake		MH369181	Present study
Echinostomatidae	*Echinoparyphium* sp. A***	*Physa gyrina, Stagnicola elodes* (MGC1932)	1	Canada: Alberta, Buffalo Lake, Wabamun Lake, Isle Lake, Lac La Nonne, Gull Lake, Pigeon Lake		MH369011, MH369035, MH369043, MH369051, MH369058, MH369061, MH369064, MH369069, MH369081, MH369082, MH369083, MH369084, MH369085, MH369113, MH369120, MH369128, MH369161, MH369169, MH369170, MH369171, MH369172, MH369173, MH369174, MH369175, MH369176, MH369177, MH369179, MH369180, MH369182, MH369183, MH369184, MH369185, MH369187, MH369190	Present study
Echinostomatidae	*Echinoparyphium* sp. A***	*Physa gyrina, Stagnicola elodes* (MGC1932)	1	Canada: Alberta, Buffalo Lake, Wabamun Lake, Isle Lake, Lac La Nonne, Gull Lake, Pigeon Lake	MH369223, MH369247, MH369254, MH369257, MH369266, MH369289, MH369290, MH369291, MH369298, MH369306, MH369307, MH369308, MH369309, MH369310		Present study

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						GenBank Accession Number(s)		
Echinostomatidae	*Echinoparyphium* sp. A2***		*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne		MH369181	Present study
Echinostomatidae	*Echinoparyphium* sp. A2***		*Stagnicola elodes*	1	Canada: Alberta, Lac La Nonne	KT831367 §		(M. Gordy et al. 2016)
Echinostomatidae	*Echinoparyphium* sp. A2***		*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne	MH369232, MH369251, MH369258, MH369260, MH369265, MH369288		Present study
Echinostomatidae	*Echinoparyphium* sp. B***		*Stagnicola elodes*	1	Canada: Alberta, Lac La Nonne		MH368969, MH368970, MH368971, MH368987, MH368988, MH369041, MH369074, MH369086, MH369092	Present study
Echinostomatidae	*Echinoparyphium* sp. C***		*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne		MH369088, MH369152	Present study
Echinostomatidae	*Echinoparyphium* sp. C***		*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne	MH369226, MH369228, MH369233, MH369234, MH369236, MH369237, MH369238, MH369239, MH369240, MH369241, MH369244, MH369252, MH369256, MH369259, MH369261, MH369262, MH369263, MH369264, MH369267, MH369278, MH369280, MH369282, MH369285, MH369296		Present study
Echinostomatidae	*Echinoparyphium* sp. D***		*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake		MH369189	Present study
Echinostomatidae	*Echinoparyphium* sp. E***		*Stagnicola elodes*, *Lymnaea stagnalis* (MGC1878)	1	Canada: Alberta, Gull Lake		MH369109, MH369129, MH369134, MH369135, MH369159	Present study
Echinostomatidae	*Echinoparyphium* sp. E***		*Stagnicola elodes*, *Lymnaea stagnalis* (MGC1878)	1	Canada: Alberta, Gull Lake	MH369275, MH369276		Present study
Echinostomatidae	*Echinoparyphium* sp. Lineage 1		*Ondatra zibethicus*	3	USA: Wisconsin		GQ463103, GQ463104, GQ463105	(Detwiler, Bos, and Minchella 2010)

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					GenBank Accession Number(s)	
Echinostomatidae	*Echinoparyphium* sp. Lineage 2	*Stagnicola elodes*, *Lymnaea stagnalis* (MGC16A/B, MGC369), *Helisoma trivolvis* (MGC219)	1	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake, Wabamun Lake, Lac La Nonne		MH368953, MH368954, MH368955, MH368956, MH368957, MH368959, MH368960, MH368961, MH368962, MH368963, MH368964, MH368965, MH368966, MH368967, MH368968, MH368972, MH368973, MH368974, MH368975, MH368976, MH368977, MH368978, Present study
Echinostomatidae	*Echinoparyphium* sp. Lineage 2	*Stagnicola elodes*, *Lymnaea stagnalis* (MGC16A/B, MGC369), *Helisoma trivolvis* (MGC219)	1	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake, Wabamun Lake, Lac La Nonne		MH368979, MH368980, MH368981, MH368982, MH368983, MH368984, MH368985, MH368986, MH368989, MH368990, MH368991, MH368992, MH368993, MH368994, MH368995, MH368996, MH368997, MH369000, MH369021, MH369027, MH369029, MH369036, Present study

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					GenBank Accession Number(s)	
Echinostomatidae	*Echinoparyphium* sp. Lineage 2	*Stagnicola elodes*, *Lymnaea stagnalis* (MGC16A/B, MGC369), *Helisoma trivolvis* (MGC219)	1	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake, Wabamun Lake, Lac La Nonne		MH369037, MH369039, MH369050, MH369057, MH369067, MH369071, MH369072, MH369073, MH369077, MH369078, MH369079, MH369103, MH369104, MH369105, MH369106, MH369107, MH369111, MH369112, MH369114, MH369115, MH369116, MH369117, MH369118, MH369119, MH369124, MH369126, MH369137, MH369138, MH369139, MH369140, MH369141, MH369142, MH369143, MH369144, MH369146, MH369148, MH369149, MH369150, MH369151, MH369153, MH369154, MH369160, MH369186 Present study
Echinostomatidae	*Echinoparyphium* sp. Lineage 2	*Lymnaea elodes*	1	USA: Indiana, Pond A		GQ463119, GQ463120, GQ463121 (Detwiler, Bos, and Minchella 2010)
Echinostomatidae	*Echinoparyphium* sp. Lineage 2	*Stagnicola elodes*, *Lymnaea stagnalis* (MGC16A/B, MGC369), *Helisoma trivolvis* (MGC219)	1	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake, Wabamun Lake, Lac La Nonne	MH369224, MH369225, MH369283, MH369293	Present study

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					GenBank Accession Number(s)		
Echinostomatidae	*Echinoparyphium* sp. Lineage 2	*Stagnicola elodes*	1	Canada: Alberta, Lac La Nonne	KT831350 §		(M. Gordy et al. 2016)
Echinostomatidae	*Echinoparyphium* sp. Lineage 3	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake, Buffalo Lake		MH369130, MH369158	Present study
Echinostomatidae	*Echinoparyphium* sp. Lineage 3	*Helisoma trivolvis*	1, 2	USA: Indiana, Pond A		GQ463122, GQ463123	(Detwiler, Bos, and Minchella 2010)
Echinostomatidae	*Echinoparyphium* sp. Lineage 3	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake, Buffalo Lake	MH369270		Present study
Echinostomatidae	*Echinostoma bolschewense*	*Viviparus acerosus*	1	Slovakia: Danube at Gabcikovo		KP065608, KP065621	(Simona Georgieva, Faltýnková, et al. 2014)
Echinostomatidae	*Echinostoma caproni*	unknown		Madagascar; Egypt; Cameroon		AF025837, AF025838	(Morgan and D. Blair 1998)
Echinostomatidae	*Echinostoma cf. friedi*	*Planorbis* sp.	1	UK: Wales, Pwll Penarth		AY168937	(Kostadinova et al. 2003)
Echinostomatidae	*Echinostoma desertticum*	unknown		Nigeria: Niger		AF025836	(Morgan and D. Blair 1998)
Echinostomatidae	*Echinostoma hortense* (out)	Dog	3	China	KR062182		(Liu et al. 2016)
Echinostomatidae	*Echinostoma miyagawai*	*Anas platyrhynchos*	3	New Zealand: Clutha River System, Central Otago District, South Island		KY436400	(Stoyanov et al. 2017)
Echinostomatidae	*Echinostoma miyagawai*	*Planorbis planorbis, Aythya fuligula*	1, 3	Czech Republic: Pond Louzek; vicinities of Tovacov		KP065632, KP065640	(Simona Georgieva, Faltýnková, et al. 2014)
Echinostomatidae	*Echinostoma nasincovae*	*Planorbarius corneus*	1	Slovakia: Danube at Gabcikovo; Czech Republic: Pond Hluboky u Hamru		KP065659, KP065674	(Simona Georgieva, Faltýnková, et al. 2014)
Echinostomatidae	*Echinostoma novazealandense*	*Anas platyrhynchos*	3	New Zealand: Clutha River System, Central Otago District, South Island		KY436398, KY436399	(Stoyanov et al. 2017)
Echinostomatidae	*Echinostoma paraensei*	*Glyptophysa*	1	Brazil; Australia: North Queensland, Townsville		AF025834, AF026282	(Morgan and D. Blair 1998)
Echinostomatidae	*Echinostoma paraulum*	*Lymnaea stagnalis, Aythya fuligula*	1, 3	Germany: pond near Poppenwind; Czech Republic: vicinities of Tovacov		KP065677, KP065680	(Simona Georgieva, Faltýnková, et al. 2014)
Echinostomatidae	*Echinostoma revolutum* Lineage A	*Lymnaea peregra*	1	Bulgaria: Grigorevo		AY168934	(Kostadinova et al. 2003)

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					GenBank Accession Number(s)		
Echinostomatidae	*Echinostoma revolutum* Lineage A	*Lymnaea stagnalis, Aythya fuligula*	1, 3	Czech Republic: Pond Vlkovsky; vicinities of Tovacov; Pond Hluboky u Hamru		KP065646, KP065653, KP065658	(Simona Georgieva, Faltýnková, et al. 2014)
Echinostomatidae	*Echinostoma revolutum* Lineage A	Domestic duck	3	Thailand		KP455631, KP455632, KP455633	(**Nagataki2015**)
Echinostomatidae	*Echinostoma revolutum* Lineage A	*Columba livia f. domestica*	3	Poland		KT726380	Ledwon, A., et al., 2015, Unpublished
Echinostomatidae	*Echinostoma revolutum* Lineage B	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake, Gull Lake, Wabamun Lake, Isle Lake, Lac La Nonne		MH369192, MH369193, MH369194, MH369195, MH369196, MH369197, MH369200, MH369201, MH369202, MH369204, MH369206, MH369207, MH369208, MH369209, MH369210, MH369211, MH369213, MH369214, MH369215, MH369216, MH369217, MH369218, MH369219, MH369220, MH369221, MH369222	Present study
Echinostomatidae	*Echinostoma revolutum* Lineage B	*Lymnaea elodes*	1	USA: Indiana, Pond A		GQ463056, GQ463057	(Detwiler, Bos, and Minchella 2010)
Echinostomatidae	*Echinostoma revolutum* Lineage B	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake, Gull Lake, Wabamun Lake, Isle Lake, Lac La Nonne	MH369227, MH369229, MH369230, MH369231, MH369235, MH369242, MH369248, MH369268, MH369279, MH369281, MH369284, MH369286, MH369287, MH369292		Present study
Echinostomatidae	*Echinostoma robustum*	*Lymnaea elodes, Biomphalaria glabrata, Gallus gallus*	1, 1, 3	USA: Indiana, Pond A; Minnesota; Brazil		GQ463053*, GQ463054, GQ463055	(Detwiler, Bos, and Minchella 2010)
Echinostomatidae	*Echinostoma* sp.	*Hydromys chrysogaster*	3	Australia: North Queensland, Townsville		AF026290	(Morgan and D. Blair 1998)
Echinostomatidae	*Echinostoma* sp. IG	*Radix auricularia*	1	Germany: Hengsteysee		KC618449, KC618450	(Georgieva et al. 2013)

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					GenBank Accession Number(s)		
Echinostomatidae	*Echinostoma* sp. NZ-Ad*	*Branta canadensis*	3	New Zealand		AF026289*	(Morgan and D. Blair 1998)
Echinostomatidae	*Echinostoma trivolvis*	*Ondatra zibethicus*	3	Canada: Ontario, Lake Opinicon	KM538091		(Van Steenkiste et al. 2014)
Echinostomatidae	*Echinostoma trivolvis* Lineage A	*Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne		MH369198, MH369199, MH369203, MH369205, MH369212	Present study
Echinostomatidae	*Echinostoma trivolvis* Lineage A	*Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne	MH369271		Present study
Echinostomatidae	*Echinostoma trivolvis* Lineage B	*Ondatra zibethicus, Lymnaea elodes*	3, 1	USA: Wisconsin; Minnesota		GQ463051, GQ463052, GQ463113	(Detwiler, Bos, and Minchella 2010)
Echinostomatidae	*Echinostoma trivolvis* Lineage B	*Ondontra zibethicus*	3	USA: Virginia		JQ670857, JQ670859, JQ670850	(Detwiler, Zajac, et al. 2012)
Echinostomatidae	*Echinostoma trivolvis* Lineage B	unknown		North America		AF025831	(Morgan and D. Blair 1998)
Echinostomatidae	Echinostomatidae gen. sp.***	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	MH369269, MH369295, MH369297		Present study
Echinostomatidae	*Euparyphium capitaneum* (out)	*Anhinga anhinga*	3	Mexico: Veracruz, Tecolutla; Nayarit, La Tovara	KY636235, KY636236		(Hernández-Cruz et al. 2018)
Echinostomatidae	*Fasciola hepatica* (out)	Cattle	3	Iran		KT893744	Akhlaghi, E., et al., 2015, Unpublished
Echinostomatidae	*Hypoderaeum conoideum*	*Lymnaea peregra*	1	Bulgaria: Grigorevo		AY168949	(Kostadinova et al. 2003)
Echinostomatidae	*Hypoderaeum conoideum*	*Anas discors*	3	Canada: Manitoba, Lake Manitoba, South shore, Delta Marsh	KM538101		(Van Steenkiste et al. 2014)
Echinostomatidae	*Hypoderaeum* sp. Lineage 1	*Lymnaea elodes*	1	USA: Indiana, Pond A		GQ463100, GQ463101, GQ463102	(Detwiler, Bos, and Minchella 2010)
Echinostomatidae	*Hypoderaeum* sp. Lineage 1	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne, Wabamun Lake, Isle Lake		MH368958, MH369020, MH369030, MH369040, MH369080, MH369108, MH369110, MH369145, MH369157	Present study
Echinostomatidae	*Isthmiophora melis* (out)	*Planorbis* sp.	1	UK: Wales, Llyn Mawr		AY168948	(Kostadinova et al. 2003)
Echinostomatidae	*Neopetasiger islandicus*	*Planorbula armigera*	1	Canada: Alberta, Wabamun Lake		KT831342	(M. Gordy et al. 2016)

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					GenBank Accession Number(s)		
Echinostomatidae	*Neopetasiger neocomense*	*Podiceps cristatus*	3	Czech Republic		JQ425591	(Simona Georgieva, Aneta Kostadinova, and Skirnisson 2012)
Echinostomatidae	*Neopetasiger* sp. 1	*Gyraulus albus*	1	Germany: Lake Hennetalsperre		KM191808, KM191809	(Selbach, Soldánová, Georgieva, Kostadinova, Kalbe, et al. 2014)
Echinostomatidae	*Neopetasiger* sp. 2	*Gyraulus albus*	1	Germany: Lake Hennetalsperre		KM191810, KM191811	(Selbach, Soldánová, Georgieva, Kostadinova, Kalbe, et al. 2014)
Echinostomatidae	*Neopetasiger* sp. 3	*Planorbis planorbis, Gyraulus albus*	1	Germany: Lake Kleiner Ploener See; Lake Hennetalsperre		KM191814, KM191815, KM191816	(Selbach, Soldánová, Georgieva, Kostadinova, Kalbe, et al. 2014)
Echinostomatidae	*Neopetasiger* sp. 4	*Gasterosteus aculeatus*	2	Canada: Lake Gosling		KM191817	(Selbach, Soldánová, Georgieva, Kostadinova, Kalbe, et al. 2014)
Echinostomatidae	*Neopetasiger* sp. 4	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake		KT831343, KT831345	(M. Gordy et al. 2016)
Echinostomatidae	*Neopetasiger* sp. 4	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Buffalo Lake		MH369311, MH369312, MH369313, MH369314, MH369315, MH369316, MH369317, MH369318	Present study
Haematoloechidae	Haematoloechidae gen. sp. A***	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	KT831372 §		(M. Gordy et al. 2016)
Haematoloechidae	Haematoloechidae gen. sp. A***	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	MH369319, MH369320, MH369321		Present study
Haematoloechidae	*Haematoloechus* sp.	*Rana pipiens*	3	Canada: Quebec, Outaouais, Ottawa River, Wendover; Ontario, Southern Ontario, Chatham-Kent, East of Lake St.Clair and St. Clair National Wildlife Area	KM538096, KM538097		(Van Steenkiste et al. 2014)
Notocotylidae	Notocotylidae gen. sp. A***	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake	KT831348 §, KT831364		(M. Gordy et al. 2016)

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							GenBank Accession Number(s)	
Notocotylidae	Notocotylidae sp. A***	gen.	*Physa gyrina, elodes*	*Stagnicola*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH369323, MH369405, MH369324, MH369406, MH369325, MH369326, MH369327, MH369407, MH369408, MH369328, MH369409, MH369329, MH369330, MH369331, MH369332, MH369333, MH369334, MH369335, MH369336,	Present study
Notocotylidae	Notocotylidae sp. A***	gen.	*Physa gyrina, elodes*	*Stagnicola*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH369337, MH369338, MH369339, MH369340, MH369341, MH369342, MH369343, MH369344, MH369345, MH369410, MH369346, MH369347, MH369348, MH369349, MH369350, MH369351, MH369352, MH369411, MH369353,	Present study
Notocotylidae	Notocotylidae sp. A***	gen.	*Physa gyrina, elodes*	*Stagnicola*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH369354, MH369355, MH369356, MH369357, MH369358, MH369359, MH369360, MH369361, MH369362, MH369363, MH369364, MH369365, MH369366, MH369367, MH369368, MH369412, MH369369, MH369370, MH369371,	Present study
Notocotylidae	Notocotylidae sp. A***	gen.	*Physa gyrina, elodes*	*Stagnicola*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH369372, MH369373, MH369374, MH369375, MH369376, MH369378, MH369413, MH369414, MH369379, MH369380, MH369381, MH369382, MH369383, MH369415, MH369416, MH369384, MH369385, MH369386, MH369387,	Present study
Notocotylidae	Notocotylidae sp. A***	gen.	*Physa gyrina, elodes*	*Stagnicola*	1	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	MH369388, MH369389, MH369390, MH369391, MH369417, MH369392, MH369393, MH369394, MH369395, MH369396, MH369397, MH369398, MH369399, MH369400, MH369401, MH369402, MH369403, MH369404	Present study
Notocotylidae	*Notocotylus* sp.		*Mergus merganser*		3	Canada: Quebec, Hudson, Le Nichoir	KM538104	(Van Steenkiste et al. 2014)

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					GenBank Accession Number(s)	
Notocotylidae	*Ogmocotyle sikae*	unknown		China: Hunan Province, Jishou City	KR006934(NC_027112:6904-8460)	Ma, J., et al., 2015, Unpublished
Plagorchiidae	*Plagiorchis* sp. Lineage 1	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne, Buffalo Lake	MH369420, MH369421, MH369422, MH369433, MH369434, MH369435, MH369441, MH369460, MH369461, MH369463, MH369464	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 2	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	MH369467	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 3	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	MH369442, MH369454, MH369466	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 4	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne, Buffalo Lake, Wabamun Lake, Isle Lake	MH369418, MH369423, MH369425, MH369428, MH369429, MH369431, MH369432, MH369436, MH369437, MH369440, MH369447, MH369452, MH369453, MH369456, MH369462, MH369471	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 5	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake, Lac La Nonne	MH369419, MH369426, MH369427	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 6	*Helisoma trivolvis*	1	Canada: Alberta, Buffalo Lake	MH369470	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 7	*Lymnaea stagnalis*	1	Canada: Alberta, Buffalo Lake, Gull Lake	MH369438, MH369448, MH369455, MH369458, MH369468, MH369469	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 8	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake, Gull Lake	MH369449, MH369450, MH369451, MH369459, MH369465	Present study
Plagorchiidae	*Plagiorchis* sp. Lineage 9	*Stagnicola elodes*	1	Canada: Alberta, Lac La Nonne, Buffalo Lake	MH369424, MH369430, MH369439, MH369443, MH369444, MH369445, MH369446	Present study
Plagorchiidae	*Plagiorchis* sp.	*Larus delawarensis*	3	Canada: Quebec, St. Lawrence River	FJ477214	(Moszczynska et al. 2009)
Psilostomidae	*Echinochasmus japonicus* (out)	*Homo sapiens*	3	Viet Nam: Phu Tho	NC_030518	Le, T.H., et al., 2015, Unpublished
Psilostomidae	*Pseudopsilostoma varium*	*Phalacrocorax auritus*	3	USA: Mississippi	JX468064	(O'Hear et al. 2014)
Psilostomidae	Psilostomatidae gen. sp. A***	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake	MH369477 §	(M. Gordy et al. 2016)
Psilostomidae	Psilostomatidae gen. sp. A***	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake, Isle Lake	MH369473, MH369472, MH369476, MH369474, MH369475	Present study
Psilostomidae	*Sphaeridiotrema globulus*	duck experimentally infected with metacercariae from *Elimia virginica*	2, 3	USA: Lake Musconetcong, New Jersey	GQ890329	(Bergmame, 2011)

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					GenBank Accession Number(s)	
Psilostomidae	*Sphaeridiotrema pseudoglobulus*	duck experimentally infected with metacercariae from *Bithynia tentaculata*	2, 3	Canada: Riviere du Sud, Quebec	GQ890328	(Bergmame, 2011)
Psilostomidae	*Sphaeridiotrema pseudoglobulus*	*Aythya affinis*	3	Canada: Quebec, St. Lawrence River	FJ477222	(Moszczynska et al. 2009)
Strigeidae	*Apatemon* sp. 1	*Etheostoma nigrum*	2	Canada: Quebec, St. Lawrence River, Lake St. Louis	FJ477183	(Moszczynska et al. 2009)
Strigeidae	*Apatemon* sp. 1	*Etheostoma nigrum*	2	Canada: Ontario, St. Lawrence River, Lake Saint Francois	HM064633	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Apatemon* sp. 1x	*Etheostoma nigrum*	2	Canada: Ontario, St. Lawrence River, Lake Saint Francois	HM064635, HM064636	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Apatemon* sp. 3	*Ambloplites rupestris*	2	Canada: Quebec, St. Lawrence River, Lake St. Pierre, Iles aux Sables	FJ477185	(Moszczynska et al. 2009)
Strigeidae	*Apatemon* sp. 3	*Ambloplites rupestris*	2	Canada: Quebec, St. Lawrence River, Lake St. Pierre, Iles aux Sables	HM064645	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Apatemon* sp. 4	*Ambloplites rupestris*	2	Canada: Quebec, St. Lawrence River, Lake Saint Francois	FJ477186	(Moszczynska et al. 2009)
Strigeidae	*Apatemon* sp. 4	*Ambloplites rupestris*	2	Canada: Quebec, St. Lawrence River, Lake St. Pierre, Iles aux Sables	HM064647	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Apatemon* sp. A***	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	MH369603, MH369604, MH369605, MH369606, MH369607, MH369608, MH369609, MH369610, MH369611, MH369612, MH369613, MH369614, MH369615, MH369616, MH369617	Present study
Strigeidae	*Apatemon* sp. B***	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	MH369618	Present study
Strigeidae	*Apatemon* sp. C***	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	MH369619, MH369620, MH369621, MH369622	Present study
Strigeidae	*Apatemon* sp. 'jamiesoni'	*Potamopyrgus antipodarum, Gobiomorphus cotidianus*	1, 2	New Zealand	KT334181, KT334182	(Blasco-Costa, Cutmore, et al. 2016)

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					GenBank Accession Number(s)	
Strigeidae	*Apharyngostrigea pipientis* (out)	*Lithobates pipiens*	2	Canada: Quebec, Monteregie, Boucherville, Etang Saulaie	HM064884, HM064885	(Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011)
Strigeidae	*Apharynogstrigea cornu*	*Ardea alba*	3	Mexico: Veracruz, Panuco	JX977777	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Apharynogstrigea cornu*	*Ardea herodias*	3	Canada: Quebec, St. Lawrence River, Lake St. Louis, Ile aux Herons	JF769451	(Sean A Locke, J Daniel McLaughlin, Lapierre, et al. 2011)
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	KT831346, KT831351	(M. Gordy et al. 2016)
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis, Helisoma campanulatum, Planorbis* sp., *Lymnaea stagnalis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	KY207548, KY207549, KY207551, KY207552, KY207553, KY207554, KY207555, KY207556, KY207559, KY207560, KY207561, KY207562, KY207563, KY207564, KY207565, KY207566, KY207567, KY207568, KY207570,	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis, Helisoma campanulatum, Planorbis* sp., *Lymnaea stagnalis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	KY207571, KY207572, KY207573, KY207574, KY207575, KY207576, KY207578, KY207579, KY207580, KY207581, KY207584, KY207585, KY207586, KY207588, KY207589, KY207590, KY207591, KY207592, KY207593,	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis, Helisoma campanulatum, Planorbis* sp., *Lymnaea stagnalis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	KY207594, KY207595, KY207598, KY207599, KY207600, KY207601, KY207602, KY207603, KY207604, KY207605, KY207606, KY207607, KY207608, KY207609, KY207610, KY207611, KY207612, KY207614, KY207617,	(Michelle A Gordy, Sean A Locke, et al. 2017)

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					GenBank Accession Number(s)	
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis, Helisoma campanulatum, Planorbis* sp., *Lymnaea stagnalis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	KY207618, KY207619, KY207620, KY207621, KY207623, KY207624, KY587399, KY587398, KY587394, KY587401, HM385485, KY587400, HM385486	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369623, MH369624, MH369625, MH369626, MH369627, MH369628, MH369629, MH369630, MH369631, MH369632, MH369633, MH369634, MH369635, MH369636, MH369637, MH369638, MH369639, MH369640,	Present study
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369641, MH369642, MH369643, MH369644, MH369645, MH369646, MH369647, MH369648, MH369649, MH369650, MH369651, MH369652, MH369653, MH369654, MH369655, MH369656, MH369657, MH369658,	Present study
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369659, MH369660, MH369661, MH369662, MH369663, MH369664, MH369665, MH369666, MH369667, MH369668, MH369669, MH369670, MH369671, MH369672, MH369673, MH369674, MH369675, MH369676,	Present study
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369677, MH369678, MH369679, MH369680, MH369681, MH369682, MH369683, MH369684, MH369686, MH369687, MH369688, MH369689, MH369690, MH369691, MH369692, MH369693, MH369694, MH369695,	Present study
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369696, MH369697, MH369698, MH369699, MH369700, MH369701, MH369702, MH369703, MH369704, MH369705, MH369706, MH369707, MH369708, MH369709, MH369710, MH369711, MH369712, MH369713,	Present study

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					GenBank Accession Number(s)	
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369714, MH369715, MH369716, MH369717, MH369718, MH369719, MH369720, MH369721, MH369722, MH369723, MH369724, MH369725, MH369726, MH369727, MH369728, MH369729, MH369730, MH369731,	Present study
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369732, MH369733, MH369734, MH369735, MH369736, MH369737, MH369738, MH369739, MH369740, MH369741, MH369742, MH369743, MH369744, MH369745, MH369746, MH369747, MH369748, MH369749,	Present study
Strigeidae	*Australapatemon burti* LIN1	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	1	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	MH369750, MH369751, MH369752, MH369753, MH369754, MH369755, MH369756, MH369757, MH369758, MH369759, MH369760, MH369761, MH369762, MH369763, MH369685	Present study
Strigeidae	*Australapatemon mclaughlini*	*Anas americana*	3	Mexico: Baja California Sur, Guerrero Negro	JX977725	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Australapatemon mclaughlini*	*Physa gyrina, Anas acuta*	1, 3	Canada: Alberta, Buffalo Lake; Ontario	KY207615, KY207627, KY207628	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon mclaughlini*	*Physa gyrina*	1	Canada: Alberta, Buffalo Lake	MH369764	Present study
Strigeidae	*Australapatemon niewiadomski*	*Barbronia weberi, Anas platyrhynchos*	2, 3	New Zealand	KT334176, KT334177, KT334178, KT334179, KT334180	(Blasco-Costa, Cutmore, et al. 2016)
Strigeidae	*Australapatemon* sp. LIN2	*Bucephala albeola*	3	Canada: Ontario	HM385535	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN3	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake	KY207577	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN4	*Physa gyrina, Aythya collaris*	1, 3	Canada: Alberta, Lac La Nonne; Ontario	KY207569, KY587397, KY587396	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN4	*Physa gyrina*	1	Canada: Alberta, Gull Lake	MH369765	Present study
Strigeidae	*Australapatemon* sp. LIN5	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	KY207597	(Michelle A Gordy, Sean A Locke, et al. 2017)

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					GenBank Accession Number(s)	
Strigeidae	*Australapatemon* sp. LIN6	*Anas cyanoptera*	3	Mexico: Estado de Mexico	JX977726	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Australapatemon* sp. LIN6	*Physa gyrina*	1	Canada: Alberta, Pigeon Lake, Isle Lake	KY207613, KY207616	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN6	*Physa gyrina*	1	Canada: Alberta, Isle Lake, Buffalo Lake, Lac La Nonne	MH369766, MH369767, MH369768, MH369769, MH369770	Present study
Strigeidae	*Australapatemon* sp. LIN8	*Oxyura jamaicensis*	3	Mexico: Durango, Guatimape	JX977728	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Australapatemon* sp. LIN8	*Physa gyrina, Oxyura jamaicensis*	1, 3	Canada: Alberta, Isle Lake, Buffalo Lake; Ontario	KY207587, KY207622, HM385538, HM385537, HM385536	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN8	*Physa gyrina*	1	Canada: Alberta, Isle Lake, Buffalo Lake, Gull Lake	MH369771, MH369772, MH369773, MH369774, MH369775, MH369776, MH369777	Present study
Strigeidae	*Australapatemon* sp. LIN9A	*Stagnicola elodes, Anas acuta*	1, 3	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake; Ontario	KY207550 §, KY207557 §, KY207558 §, KY207582 §, KY207596 §, HM385534 §	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN9A	*Stagnicola elodes, Lymnaea stagnalis* (MGC176B)	1	Canada: Alberta, Lac La None, Gull Lake, Buffalo Lake, Isle Lake	MH369779, MH369780, MH369781, MH369782, MH369783, MH369784, MH369785, MH369786, MH369787, MH369788, MH369789, MH369778	Present study
Strigeidae	*Australapatemon* sp. LIN9B	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	KY207583 §	(Michelle A Gordy, Sean A Locke, et al. 2017)
Strigeidae	*Australapatemon* sp. LIN9B	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake	MH369790, MH369791, MH369792	Present study
Strigeidae	*Australapatemon* sp. LIN10***	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake	MH369793	Present study
Strigeidae	*Cardiocephaloides medioconiger*	*Larus* sp.	3	Mexico: Laguna de T¡e9¿rminos, Campeche	JX977782, JX977783	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Cardiocephaloides* sp.	*Larus occidentalis*	3	Mexico: Baja California Sur, Guerrero Negro	JX977784	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Cotylurus cornutus*	*Radix balthica, Gyraulus acronicus*	1	Norway: Lake Takvatn	KY513231, KY513232, KY513233, KY513234, KY513235, KY513236	(Miroslava Soldánová et al. 2017)
Strigeidae	*Cotylurus cornutus*	*Stagnicola elodes*	1	Canada: Alberta, Gull Lake	KT831347 §	(M. Gordy et al. 2016)

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Table 4.7 – *Continued from previous page*

					GenBank Accession Number(s)	
Strigeidae	*Cotylurus cornutus*	*Stagnicola elodes*, *Helisoma trivolvis* (MGC205)	1	Canada: Alberta, Gull Lake, Isle Lake, Lac La Nonne	MH369478, MH369480, MH369484, MH369485, MH369486, MH369487, MH369488, MH369489, MH369490, MH369491, MH369492, MH369493, MH369494, MH369495, MH369496, MH369497, MH369498, MH369500, MH369501, MH369502, MH369503, MH369504, MH369505, MH369509, MH369510, MH369511, MH369516, MH369532, MH369538, MH369539, MH369544, MH369557, MH369597, MH369601	Present study
Strigeidae	*Cotylurus gallinulae*	*Aythya affinis*	3	Mexico: Sonora, La esperanza	JX977781	(Hernández-Mena, García-Prieto, and Martín García-Varela 2014)
Strigeidae	*Cotylurus gallinulae*	*Physa gyrina*	1	Canada: Alberta, Buffalo Lake, Wabamun Lake, Isle Lake, Lac La Nonne	MH369517, MH369518, MH369525, MH369526, MH369527, MH369528, MH369529, MH369560, MH369571, MH369572, MH369574, MH369575, MH369577, MH369583, MH369584, MH369587, MH369588, MH369590, MH369595, MH369596, MH369599, MH369600	Present study
Strigeidae	*Cotylurus* sp. A***	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	KT831371 §	(M. Gordy et al. 2016)
Strigeidae	*Cotylurus* sp. A***	*Stagnicola elodes*, *Physa gyrina* (MGC1962)	1	Canada: Alberta, Isle Lake, Lac La Nonne, Wabamun	MH369513, MH369520, MH369521, MH369522, MH369523, MH369524, MH369533, MH369537, MH369541, MH369542, MH369543, MH369545, MH369546, MH369547, MH369548, MH369549, MH369550, MH369551, MH369552, MH369554, MH369555, MH369556, MH369558, MH369559, MH369561, MH369562, MH369573, MH369578, MH369579, MH369580, MH369581, MH369582, MH369585, MH369589, MH369591, MH369594, MH369598, MH369602	Present study

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Table 4.7 – *Continued from previous page*

					GenBank Accession Number(s)	
Strigeidae	*Cotylurus* sp. B***	*Physa gyrina*	1	Canada: Alberta, Isle Lake	MH369586	Present study
Strigeidae	*Cotylurus* sp. C***	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake, Gull Lake, Isle Lake, Lac La Nonne	MH369479, MH369481, MH369515, MH369530, MH369531, MH369553, MH369564	Present study
Strigeidae	*Cotylurus* sp. D***	*Stagnicola elodes*	1	Canada: Alberta, Buffalo Lake, Gull Lake, Isle Lake, Lac La Nonne	MH369482, MH369483, MH369499, MH369506, MH369507, MH369508, MH369534, MH369535, MH369536, MH369540, MH369565, MH369593	Present study
Strigeidae	*Cotylurus* sp. E***	*Lymnaea stagnalis*	1	Canada: Alberta, Buffalo Lake, Wabamun Lake	MH369512, MH369514, MH369567, MH369568, MH369569, MH369570	Present study
Strigeidae	*Cotylurus* sp. F***	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	MH369519	Present study
Strigeidae	*Cotylurus* sp. G***	*Lymnaea stagnalis*	1	Canada: Alberta, Buffalo Lake	MH369563, MH369566, MH369576	Present study
Strigeidae	*Cotylurus* sp. H***	*Physa gyrina*	1	Canada: Alberta, Buffalo Lake	MH369592	Present study
Strigeidae	*Ichthyocotylurus pileatus*	*Perca flavescens, Etheostoma nigrum*	2	Canada: Quebec, St. Lawrence River, Lake Saint Louis, Beauharnois	HM064721, HM064726	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Ichthyocotylurus pileatus*	*Perca flavescens*	2	Canada: Ontario, St. Lawrence River, Lake Saint Francois	FJ477204	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Ichthyocotylurus* sp. 2	*Perca flavescens*	2	Canada: Quebec, St. Lawrence River, Lake Saint Louis, Beauharnois	HM064728	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Ichthyocotylurus* sp. 3	*Notropis hudsonius*	2	Canada: Ontario, St. Lawrence River, Lake Saint Francois	HM064729	(Sean A Locke, J Daniel McLaughlin, and David J Marcogliese 2010)
Strigeidae	*Tylodelphys scheuringi* (out)	*Ambloplites rupestris*	2	Canada: Quebec, St. Lawrence River, Lake Saint Pierre, Iles aux Sables	FJ477223	(Moszczynska et al. 2009)

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Table 4.7 – *Continued from previous page*

	GenBank Accession Number(s)
*** Novel by molecular phylogeny; §Record updated in present study; Host Type: 1= First Intermediate, 2=Second Intermediate, 3=Definitive * Most likely *Posthodiplostomum centrarchi* Rows highlighted in gray represent sequences from the present study	

Table 4.8: Average *nad1* divergence within and between genera of *Neopetasiger*. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. On the diagonal are the average within group divergence estimates. The range of pairwise distances within groups is given in parentheses next to the group names. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 20 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 308 positions in the final dataset.

	N. islandic.	*N. necome.*	*P.* sp. 1	*P.* sp. 2	*P.* sp. 3	*P.* sp. 4
Neopetasiger islandicus	**-**	0.025	0.020	0.019	0.021	0.022
Neopetasiger necomense	0.286	**-**	0.026	0.026	0.025	0.024
Petasiger sp. 1	0.172	0.325	**0.000**	0.020	0.023	0.025
Petasiger sp. 2	0.143	0.318	0.149	**0.000**	0.022	0.024
Petasiger sp. 3 (0.3-1.3%)	0.162	0.298	0.231	0.193	**0.900**	0.022
Petasiger sp. 4 (0.0-0.7%)	0.211	0.276	0.282	0.240	0.214	**0.200**

Table 4.9: Average *nad1* divergence within and between genera of *Echinoparyphium/Hypoderaeum.* The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. Intraspecific divergence values are given on the diagonal. The range of p distances is given for each group in parentheses as percentages after species names. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 261 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 299 positions in the final dataset.

	Ec. aconia.	*Ec. ellisi*	*Ec. poulini*	*Ec. recurv.*	*Ec.* sp. A	*Ec.* sp. B	*Ec.* sp. C	*Ec.* sp. D	*Ec.* sp. E	*Ec.* sp. L1	*Ec.* sp. L1A	*Ec.* sp. L1B	*Ec.* sp. L2	*Ec.* sp. L3	*H. conoid.*	*H.* sp. L1
Echinoparyphium aconiatum (0.0%)	**0.000**	0.023	0.023	0.025	0.022	0.024	0.022	0.024	0.022	0.023	0.024	0.023	0.022	0.021	0.022	0.022
Echinoparyphium ellisi (0.0%)	0.204	**0.000**	0.022	0.022	0.022	0.025	0.024	0.024	0.025	0.020	0.021	0.021	0.020	0.020	0.024	0.023
Echinoparyphium poulini (0.0%)	0.207	0.171	**0.000**	0.022	0.021	0.025	0.024	0.024	0.024	0.022	0.022	0.022	0.020	0.020	0.023	0.024
Echinoparyphium recurvatum (0.7-1.3%)	0.239	0.196	0.202	**0.011**	0.022	0.025	0.024	0.025	0.025	0.021	0.023	0.022	0.021	0.021	0.024	0.024
Echinoparyphium sp. A (0.0-5.7%)	0.193	0.176	0.171	0.221	**0.020**	0.023	0.023	0.022	0.024	0.021	0.020	0.021	0.020	0.017	0.022	0.021
Echinoparyphium sp. B (0.0-1.0%)	0.215	0.241	0.268	0.271	0.233	**0.004**	0.021	0.024	0.023	0.024	0.025	0.025	0.025	0.024	0.025	0.024
Echinoparyphium sp. C (3.7%)	0.194	0.221	0.227	0.242	0.220	0.161	**0.037**	0.022	0.022	0.024	0.025	0.024	0.024	0.022	0.024	0.025
Echinoparyphium sp. D	0.217	0.221	0.227	0.231	0.213	0.218	0.187	**-**	0.021	0.024	0.023	0.023	0.023	0.023	0.025	0.025
Echinoparyphium sp. E (0.0-1.3%)	0.179	0.247	0.232	0.258	0.224	0.193	0.171	0.164	**0.008**	0.023	0.024	0.024	0.023	0.023	0.024	0.024
Echinoparyphium sp. LIN1 (2.0-3.0%)	0.202	0.144	0.173	0.200	0.178	0.250	0.241	0.224	0.223	**0.025**	0.015	0.017	0.020	0.019	0.023	0.022
Echinoparyphium sp. LIN1 A (0.0-0.7%)	0.233	0.156	0.188	0.216	0.169	0.251	0.255	0.230	0.227	0.086	**0.002**	0.017	0.020	0.020	0.024	0.023
Echinoparyphium sp. LIN1 B	0.214	0.171	0.171	0.201	0.184	0.255	0.239	0.227	0.235	0.111	0.120	**-**	0.020	0.020	0.022	0.022
Echinoparyphium sp. LIN2 (0.0-5.7%)	0.184	0.145	0.143	0.174	0.164	0.262	0.222	0.210	0.216	0.154	0.157	0.161	**0.012**	0.019	0.023	0.022
Echinoparyphium sp. LIN3 (2.7-11.0%)	0.197	0.179	0.172	0.197	0.149	0.248	0.226	0.223	0.215	0.173	0.182	0.184	0.174	**0.081**	0.022	0.022
Hypoderaeum conoideum	0.204	0.227	0.211	0.227	0.206	0.251	0.232	0.244	0.227	0.215	0.230	0.201	0.195	0.222	**-**	0.013
Hypoderaeum sp. LIN1 (0.0-6.7%)	0.202	0.220	0.221	0.225	0.199	0.246	0.238	0.239	0.239	0.207	0.217	0.191	0.195	0.223	0.071	**0.024**

Table 4.10: Average *cox1* divergence within and between genera of Echinostomatidae.The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. Intraspecific divergence is given on the diagonal. Numbers in red represent groups that exceed the cut-off. The range of p distances is given for each group in parentheses as percentages after species names. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 111 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 383 positions in the final dataset.

	D. auritus	*D. mexican.*	*D. spathans*	*E. trivolvis*	E. gen. sp.	*E. revolu.*B	Ec.gen.sp.	*Ec.*sp.A2	*Ec.*sp.A	*Ec.*sp.C	*Ec.*sp.E	*Ec.*sp.L1A	*Ec.*sp.L2	*Ec.*sp.L3	*H. conoid.*
Drepanocephalus auritus (0.0-2.9%)	**0.006**	0.016	0.009	0.02	0.021	0.021	0.022	0.021	0.019	0.021	0.021	0.02	0.018	0.021	0.021
Drepanocephalus mexicanus (2.3%)	0.129	**0.023**	0.016	0.02	0.02	0.02	0.022	0.02	0.019	0.02	0.02	0.02	0.018	0.019	0.02
Drepanocephalus spathans (0.0%)	0.036	0.132	**0.000**	0.02	0.02	0.021	0.022	0.021	0.02	0.02	0.02	0.021	0.019	0.021	0.021
Echinostoma trivolvis (0.0%)	0.261	0.27	0.256	**-**	0.014	0.017	0.022	0.019	0.019	0.019	0.018	0.018	0.017	0.018	0.018
Echinostomatidae gen. sp. (0.0%)	0.293	0.273	0.279	0.097	**-**	0.016	0.022	0.019	0.018	0.018	0.017	0.018	0.017	0.018	0.018
Echinostoma revolutum Lineage B (0.0-1.8%)	0.265	0.273	0.261	0.15	0.14	**0.010**	0.022	0.019	0.018	0.019	0.018	0.018	0.015	0.018	0.018
Echinostomatidae gen. sp. (0.0-0.5%)	0.309	0.294	0.305	0.275	0.272	0.265	**0.003**	0.021	0.021	0.02	0.021	0.022	0.02	0.021	0.022
Echinoparyphium sp. A2 (0.0-1.6%)	0.253	0.247	0.242	0.199	0.206	0.203	0.261	**0.006**	0.019	0.017	0.018	0.018	0.017	0.019	0.019
Echinoparyphium sp. A (0.0-3.7%)	0.24	0.245	0.249	0.188	0.191	0.187	0.26	0.206	**0.018**	0.019	0.019	0.016	0.014	0.016	0.019
Echinoparyphium sp. C (0.0-1.3%)	0.251	0.241	0.25	0.209	0.184	0.194	0.258	0.167	0.207	**0.007**	0.018	0.019	0.017	0.019	0.018
Echinoparyphium sp. E (0.8%)	0.253	0.255	0.256	0.176	0.158	0.187	0.243	0.185	0.19	0.155	**0.008**	0.018	0.017	0.018	0.019
Echinoparyphium sp. LIN1 A (0.0-1.6%)	0.247	0.247	0.251	0.162	0.17	0.179	0.269	0.186	0.142	0.204	0.182	**0.003**	0.014	0.015	0.019
Echinoparyphium sp. LIN2 (0.3-22.7%)	0.267	0.26	0.272	0.197	0.197	0.184	0.282	0.227	0.164	0.206	0.211	0.141	**0.114**	0.014	0.017
Echinoparyphium sp. LIN3 (0.0%)	0.243	0.24	0.24	0.17	0.175	0.181	0.257	0.187	0.132	0.195	0.172	0.127	0.145	**-**	0.019
Hypoderaeum conoideum (0.0%)	0.261	0.272	0.261	0.188	0.196	0.206	0.269	0.201	0.205	0.187	0.181	0.191	0.209	0.185	**-**

Table 4.11: Average *nad1* divergence within and between groups of *Echinostoma* spp. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. The average within group divergence is given on the diagonal. The range of pairwise distances within each group are given as percentages in the first column after group names. Numbers in red lie outside the delineation cut-off. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 72 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 386 positions in the final dataset.

	E. bolsch.	*E. caproni*	*E. desert.*	*E. friedi*/IG	*E. miyag.*	*E. nas-inc.*	*E. no-vaze.*	*E. paraen.*	*E. pa-raul.*	*E. rev.* A	*E. rev.* B	*E. ro-bust.*	*E.* sp.	*E. triv.* A	*E. triv.* B
Echinostoma bolschwense (0.3%)	**0.003**	0.019	0.018	0.019	0.018	0.018	0.018	0.019	0.018	0.017	0.017	0.017	0.021	0.017	0.017
Echinostoma caproni (2.8%)	0.181	**0.028**	0.017	0.019	0.016	0.018	0.016	0.017	0.016	0.017	0.017	0.016	0.021	0.016	0.017
Echinostoma deserticum	0.152	0.162	-	0.020	0.017	0.018	0.017	0.019	0.017	0.017	0.017	0.017	0.021	0.017	0.018
Echinostoma friedi/IG (0.3-0.5%)	0.191	0.201	0.217	**0.003**	0.019	0.020	0.020	0.020	0.020	0.020	0.020	0.019	0.021	0.020	0.020
Echinostoma miyagawai (0.8-5.2%)	0.164	0.136	0.149	0.194	**0.031**	0.017	0.014	0.017	0.015	0.014	0.014	0.012	0.021	0.016	0.016
Echinostoma nasincovae (0.3%)	0.161	0.154	0.166	0.201	0.148	**0.003**	0.017	0.016	0.018	0.016	0.016	0.016	0.021	0.015	0.014
Echinostoma novazealandense (0.0-0.3%)	0.143	0.120	0.139	0.205	0.092	0.120	**0.002**	0.017	0.015	0.014	0.015	0.014	0.022	0.016	0.016
Echinostoma paraensei (0.3%)	0.174	0.141	0.180	0.195	0.151	0.114	0.135	**0.003**	0.017	0.017	0.018	0.016	0.021	0.017	0.016
Echinostoma paraulum (0.5%)	0.159	0.141	0.145	0.196	0.106	0.150	0.111	0.141	**0.005**	0.015	0.016	0.014	0.020	0.016	0.017
Echinostoma revolutum Lineage A (0.0-5.7%)	0.137	0.139	0.133	0.197	0.107	0.123	0.086	0.148	0.125	**0.010**	0.010	0.014	0.021	0.015	0.016
Echinostoma revolutum Lineage B (0.0-1.6%)	0.147	0.144	0.144	0.196	0.102	0.126	0.098	0.154	0.125	0.052	**0.007**	0.014	0.022	0.015	0.015
Echinostoma robustum (5.4%)	0.154	0.145	0.155	0.200	0.079	0.131	0.098	0.139	0.096	0.106	0.109	**0.054**	0.021	0.015	0.015
Echinostoma sp.	0.237	0.245	0.246	0.262	0.251	0.255	0.261	0.250	0.233	0.250	0.256	0.255	-	0.021	0.021
Echinostoma trivolvis Lineage A (0.0-1.3%)	0.148	0.140	0.152	0.190	0.134	0.097	0.123	0.120	0.136	0.115	0.120	0.126	0.237	**0.006**	0.012
Echinostoma trivolvis Lineage B (0.3-2.8%)	0.140	0.164	0.180	0.207	0.143	0.098	0.125	0.129	0.148	0.127	0.127	0.125	0.250	0.080	**0.018**

Table 4.12: Average *cox1* divergence within and between genera of Diplostomidae-I. The number of base differences per site from averaging over all sequence pairs between groups are shown below the diagonal. Standard error estimate(s) are shown above the diagonal. On the diagonal are the average within group divergence values. Numbers within parentheses after species names represent the range of percent divergence within groups. Species with three asterisks represent novel species by molecular phylogeny. The analysis involved 196 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 334 positions in the final dataset.

	A. ostrow.	*A.* sp. 1	*A.* sp. 2	*D. ardeae*	*D. b* LIN1	*D. b* LIN2	*D. huron.*	*D. indist.*	*D. mergi*	*D. parvi.*	*D. pseudo.*	*D.* sp. 1	*D.* sp. 2	*D.* sp. 4	*D.* sp. 6	*D.* sp. 7
Austrodiplostomum ostrowskiae (0.3-0.9%)	**0.006**	0.017	0.016	0.018	0.020	0.020	0.019	0.020	0.020	0.019	0.018	0.020	0.020	0.020	0.019	0.019
Alaria sp. 1	0.100	-	0.017	0.018	0.020	0.019	0.019	0.020	0.021	0.020	0.019	0.020	0.020	0.020	0.020	0.021
Alaria sp. 2 (0.0%)	0.098	0.099	**0.000**	0.021	0.021	0.021	0.020	0.021	0.020	0.020	0.020	0.020	0.019	0.021	0.021	0.021
Diplostomum ardeae	0.137	0.144	0.162	-	0.019	0.019	0.018	0.017	0.016	0.017	0.017	0.018	0.018	0.017	0.017	0.017
Diplostomum baeri LIN1 (0.9%)	0.146	0.169	0.184	0.136	**0.009**	0.018	0.018	0.019	0.019	0.018	0.017	0.019	0.017	0.018	0.016	0.015
Diplostomum baeri LIN2 (0.0-0.9%)	0.152	0.150	0.170	0.137	0.125	**0.004**	0.017	0.019	0.018	0.018	0.017	0.018	0.019	0.016	0.017	0.017
Diplostomum huronense (0.3-0.6%)	0.147	0.145	0.157	0.128	0.117	0.102	**0.004**	0.016	0.016	0.014	0.017	0.017	0.017	0.017	0.015	0.017
Diplostomum indistinctum (0.3-0.9%)	0.171	0.167	0.176	0.132	0.147	0.148	0.096	**0.005**	0.017	0.016	0.016	0.016	0.017	0.017	0.018	0.019
Diplostomum mergi (0.0-2.4%)	0.158	0.170	0.157	0.113	0.149	0.134	0.101	0.121	**0.016**	0.013	0.016	0.016	0.017	0.016	0.018	0.018
Diplostomum parviventosum (0.6%)	0.151	0.165	0.164	0.107	0.134	0.133	0.086	0.114	0.069	**0.006**	0.017	0.017	0.015	0.016	0.017	0.018
Diplostomum pseudospathaceum (0.0-3.3%)	0.138	0.157	0.163	0.128	0.126	0.119	0.109	0.114	0.117	0.124	**0.018**	0.015	0.017	0.015	0.018	0.017
Diplostomum sp. 1 (0.0-1.5%)	0.160	0.150	0.151	0.139	0.146	0.138	0.102	0.096	0.112	0.116	0.095	**0.007**	0.017	0.018	0.018	0.018
Diplostomum sp. 2	0.146	0.156	0.150	0.123	0.114	0.133	0.109	0.125	0.114	0.092	0.119	0.110	-	0.018	0.016	0.016
Diplostomum sp. 4 (0.0-1.5%)	0.156	0.153	0.166	0.120	0.126	0.098	0.097	0.119	0.106	0.107	0.093	0.120	0.122	**0.004**	0.018	0.018
Diplostomum sp. 6	0.137	0.156	0.177	0.111	0.102	0.104	0.094	0.131	0.138	0.119	0.131	0.133	0.102	0.131	-	0.015
Diplostomum sp. 7	0.135	0.162	0.174	0.117	0.084	0.110	0.115	0.158	0.137	0.140	0.120	0.139	0.099	0.129	0.078	-
Diplostomum sp. 8	0.161	0.156	0.171	0.099	0.141	0.119	0.124	0.137	0.123	0.125	0.112	0.121	0.108	0.114	0.111	0.126
Diplostomum sp. 9	0.170	0.147	0.174	0.135	0.138	0.149	0.128	0.138	0.130	0.124	0.124	0.136	0.123	0.109	0.150	0.138
Diplostomum sp. A***	0.149	0.144	0.165	0.123	0.141	0.137	0.126	0.132	0.118	0.114	0.127	0.109	0.117	0.123	0.141	0.138
Diplostomum sp. B***	0.153	0.168	0.159	0.138	0.120	0.135	0.112	0.143	0.125	0.111	0.122	0.125	0.090	0.135	0.129	0.111
Diplostomum sp. C ***(0.0-2.1%)	0.157	0.149	0.179	0.114	0.141	0.103	0.111	0.125	0.120	0.113	0.107	0.112	0.117	0.111	0.125	0.132
Diplostomum sp. clade Q	0.163	0.180	0.189	0.123	0.130	0.128	0.097	0.129	0.111	0.107	0.123	0.130	0.114	0.117	0.138	0.144
Diplostomum sp. LIN6 (0.3-1.8%)	0.171	0.171	0.180	0.150	0.124	0.136	0.106	0.139	0.123	0.109	0.129	0.121	0.111	0.120	0.120	0.128
Diplostomum spathaceum (0.3-2.1%)	0.155	0.171	0.189	0.115	0.128	0.139	0.089	0.092	0.112	0.105	0.088	0.095	0.115	0.091	0.122	0.127
Tylodelphys aztecae (0.6-1.5%)	0.159	0.138	0.163	0.129	0.157	0.137	0.145	0.159	0.160	0.156	0.131	0.141	0.147	0.132	0.152	0.140
Tylodelphys clavata (0.6-1.2%)	0.156	0.146	0.157	0.131	0.161	0.167	0.145	0.173	0.145	0.163	0.158	0.155	0.149	0.160	0.160	0.144
Tylodelphys excavata	0.123	0.138	0.135	0.120	0.156	0.132	0.109	0.155	0.136	0.137	0.123	0.114	0.144	0.135	0.141	0.144

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Table 4.12 – *Continued from previous page*

	A. ostrow.	*A.* sp. 1	*A.* sp. 2	*D. ardeae*	*D. b* LIN1	*D. b* LIN2	*D. huron.*	*D. indist.*	*D. mergi*	*D. parvi.*	*D. pseudo.*	*D.* sp. 1	*D.* sp. 2	*D.* sp. 4	*D.* sp. 6	*D.* sp. 7
Tylodelphys immer (0.3-0.6%)	0.132	0.121	0.151	0.127	0.168	0.143	0.113	0.133	0.138	0.133	0.153	0.143	0.144	0.126	0.151	0.157
Tylodelphys jenynsiae (0.0-0.9%)	0.112	0.137	0.135	0.135	0.168	0.158	0.142	0.159	0.140	0.138	0.149	0.165	0.158	0.143	0.158	0.167
Tylodelphys mashonensis (0.0-0.3%)	0.146	0.143	0.138	0.121	0.149	0.152	0.141	0.157	0.149	0.144	0.156	0.148	0.152	0.136	0.149	0.149
Tylodelphys scheuringi (0.0-1.2%)	0.129	0.135	0.134	0.120	0.158	0.159	0.145	0.158	0.132	0.141	0.136	0.148	0.146	0.138	0.156	0.165
Tylodelphys sp. 2 LIN1	0.144	0.147	0.156	0.147	0.183	0.165	0.133	0.168	0.161	0.144	0.152	0.170	0.174	0.143	0.165	0.183
Tylodelphys sp. 2 LIN2 (0.9%)	0.132	0.126	0.145	0.112	0.156	0.129	0.128	0.153	0.124	0.133	0.127	0.140	0.153	0.124	0.133	0.147
Tylodelphys sp. 3 (0.3-0.9%)	0.132	0.123	0.134	0.108	0.150	0.147	0.123	0.139	0.134	0.124	0.132	0.135	0.125	0.115	0.132	0.154
Tylodelphys sp. 4 (0.0-0.3%)	0.136	0.146	0.143	0.119	0.153	0.144	0.121	0.148	0.139	0.124	0.140	0.160	0.142	0.127	0.134	0.143
Tylodelphys sp. 5 (0.3%)	0.127	0.133	0.139	0.127	0.147	0.134	0.107	0.147	0.134	0.130	0.143	0.127	0.139	0.122	0.145	0.142
Tylodelphys sp. 6 (0.6%)	0.104	0.130	0.127	0.118	0.149	0.142	0.145	0.161	0.135	0.137	0.112	0.149	0.127	0.140	0.135	0.136
Tylodelphys sp. A*** (0.0-0.9%)	0.122	0.105	0.138	0.108	0.146	0.138	0.125	0.131	0.134	0.124	0.128	0.139	0.117	0.118	0.139	0.139
Tylodelphys sp. IBC-2016 (0.0-0.6%)	0.106	0.121	0.118	0.094	0.143	0.154	0.125	0.141	0.124	0.114	0.125	0.133	0.118	0.133	0.130	0.141
Tylodelphys sp. IND (0.3%)	0.112	0.126	0.138	0.114	0.159	0.164	0.125	0.152	0.126	0.135	0.151	0.157	0.150	0.143	0.147	0.153

Table 4.12 - *Columns continued*

	D. sp. 8	*D.* sp. 9	*D.* sp. A	*D.* sp. B	*D.* sp. C	*D.* clade Q	*D.* sp. LIN6	*D. spath.*	*T. aztecae*	*T.clavata*	*T. excav.*	*T. immer*	*T. jenyns.*	*T. masho.*	*T. scheur.*	*T.*sp. 2LIN1
Austrodiplostomum ostrowskiae (0.3-0.9%)	0.020	0.021	0.020	0.020	0.020	0.020	0.020	0.019	0.020	0.020	0.018	0.018	0.017	0.020	0.018	0.020
Alaria sp. 1	0.020	0.019	0.019	0.021	0.019	0.021	0.020	0.021	0.018	0.020	0.019	0.017	0.019	0.020	0.019	0.020
Alaria sp. 2 (0.0%)	0.021	0.021	0.021	0.020	0.022	0.021	0.021	0.021	0.020	0.020	0.019	0.019	0.018	0.019	0.019	0.020
Diplostomum ardeae	0.016	0.018	0.017	0.018	0.017	0.018	0.019	0.016	0.018	0.018	0.017	0.017	0.019	0.018	0.018	0.020
Diplostomum baeri LIN1 (0.9%)	0.019	0.019	0.019	0.018	0.019	0.018	0.017	0.018	0.020	0.020	0.020	0.020	0.020	0.020	0.019	0.022
Diplostomum baeri LIN2 (0.0-0.9%)	0.018	0.019	0.018	0.019	0.016	0.018	0.019	0.018	0.019	0.020	0.018	0.020	0.020	0.019	0.020	0.020
Diplostomum huronense (0.3-0.6%)	0.019	0.018	0.018	0.017	0.016	0.016	0.016	0.015	0.019	0.019	0.017	0.017	0.019	0.019	0.019	0.018
Diplostomum indistinctum (0.3-0.9%)	0.019	0.018	0.018	0.019	0.017	0.018	0.019	0.014	0.019	0.020	0.019	0.018	0.019	0.020	0.019	0.021
Diplostomum mergi (0.0-2.4%)	0.018	0.018	0.016	0.018	0.017	0.016	0.017	0.016	0.019	0.018	0.018	0.018	0.017	0.019	0.017	0.020
Diplostomum parviventosum (0.6%)	0.018	0.018	0.016	0.017	0.017	0.016	0.016	0.015	0.019	0.019	0.018	0.017	0.017	0.019	0.018	0.018
Diplostomum pseudospathaceum (0.0-3.3%)	0.017	0.017	0.017	0.018	0.016	0.018	0.018	0.013	0.018	0.020	0.017	0.019	0.019	0.019	0.018	0.020
Diplostomum sp. 1 (0.0-1.5%)	0.018	0.019	0.017	0.018	0.017	0.018	0.018	0.015	0.018	0.019	0.017	0.019	0.020	0.019	0.019	0.021
Diplostomum sp. 2	0.018	0.018	0.017	0.016	0.018	0.017	0.017	0.017	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.021
Diplostomum sp. 4 (0.0-1.5%)	0.017	0.016	0.017	0.018	0.017	0.018	0.017	0.015	0.018	0.019	0.018	0.018	0.019	0.018	0.018	0.019
Diplostomum sp. 6	0.017	0.020	0.019	0.018	0.018	0.019	0.017	0.017	0.020	0.020	0.019	0.019	0.019	0.020	0.020	0.020
Diplostomum sp. 7	0.018	0.019	0.019	0.017	0.018	0.019	0.017	0.017	0.019	0.019	0.019	0.020	0.021	0.020	0.020	0.020
Diplostomum sp. 8	-	0.017	0.018	0.018	0.016	0.018	0.015	0.017	0.018	0.019	0.018	0.019	0.019	0.019	0.018	0.020
Diplostomum sp. 9	0.114	-	0.019	0.019	0.016	0.018	0.016	0.017	0.019	0.021	0.020	0.019	0.019	0.020	0.019	0.021
Diplostomum sp. A***	0.132	0.132	-	0.018	0.017	0.017	0.019	0.017	0.020	0.019	0.019	0.019	0.020	0.019	0.019	0.021
Diplostomum sp. B***	0.120	0.135	0.132	-	0.017	0.018	0.017	0.017	0.020	0.018	0.019	0.020	0.020	0.020	0.020	0.021

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Table 4.12 – *Continued from previous page*

Diplostomum sp. C ***(0.0-2.1%)	0.092	0.101	0.108	0.112	**0.005**	0.017	0.017	0.017	0.019	0.019	0.018	0.019	0.019	0.019	0.020	0.021

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Table 4.12 – *Continued from previous page*

	D. sp. 8	*D.* sp. 9	*D.* sp. A	*D.* sp. B	*D.* sp. C	*D.* cladeQ	*D.* sp.LIN6	*D. spath.*	*T. aztecae*	*T.clavata*	*T. excav.*	*T. immer*	*T. jenyns.*	*T. masho.*	*T. scheur.*	*T.*sp. 2LIN1
Diplostomum sp. clade Q	0.129	0.129	0.123	0.132	0.112	**-**	0.017	0.017	0.019	0.019	0.018	0.018	0.019	0.020	0.019	0.019
Diplostomum sp. LIN6 (0.3-1.8%)	0.085	0.108	0.148	0.116	0.107	0.118	**0.012**	0.018	0.018	0.020	0.019	0.018	0.019	0.020	0.019	0.020
Diplostomum spathaceum (0.3-2.1%)	0.117	0.111	0.110	0.128	0.120	0.120	0.128	**0.011**	0.018	0.019	0.018	0.018	0.019	0.019	0.018	0.021
Tylodelphys aztecae (0.6-1.5%)	0.132	0.152	0.166	0.164	0.142	0.158	0.141	0.143	**0.010**	0.020	0.018	0.017	0.020	0.019	0.017	0.020
Tylodelphys clavata (0.6-1.2%)	0.145	0.178	0.149	0.146	0.165	0.156	0.170	0.157	0.154	**0.008**	0.017	0.020	0.019	0.018	0.018	0.018
Tylodelphys excavata	0.129	0.159	0.150	0.141	0.135	0.138	0.139	0.132	0.132	0.111	**-**	0.018	0.018	0.017	0.018	0.018
Tylodelphys immer (0.3-0.6%)	0.147	0.151	0.139	0.162	0.143	0.128	0.132	0.136	0.125	0.147	0.126	**0.004**	0.016	0.017	0.016	0.018
Tylodelphys jenynsiae (0.0-0.9%)	0.146	0.160	0.156	0.166	0.149	0.141	0.148	0.144	0.146	0.152	0.132	0.104	**0.006**	0.018	0.017	0.018
Tylodelphys mashonensis (0.0-0.3%)	0.135	0.146	0.146	0.164	0.144	0.152	0.159	0.158	0.147	0.126	0.116	0.120	0.132	**0.001**	0.017	0.018
Tylodelphys scheuringi (0.0-1.2%)	0.131	0.144	0.146	0.167	0.152	0.146	0.155	0.145	0.118	0.140	0.129	0.118	0.116	0.126	**0.005**	0.018
Tylodelphys sp. 2 LIN1	0.153	0.183	0.171	0.186	0.163	0.162	0.171	0.162	0.161	0.134	0.114	0.127	0.120	0.124	0.126	**-**
Tylodelphys sp. 2 LIN2 (0.9%)	0.135	0.175	0.136	0.156	0.150	0.144	0.162	0.144	0.147	0.123	0.108	0.124	0.125	0.097	0.130	0.097
Tylodelphys sp. 3 (0.3-0.9%)	0.119	0.131	0.141	0.154	0.134	0.137	0.129	0.129	0.122	0.139	0.107	0.079	0.118	0.111	0.081	0.124
Tylodelphys sp. 4 (0.0-0.3%)	0.128	0.159	0.145	0.150	0.157	0.139	0.131	0.138	0.128	0.133	0.131	0.108	0.101	0.130	0.122	0.141
Tylodelphys sp. 5 (0.3%)	0.145	0.166	0.133	0.133	0.133	0.136	0.146	0.126	0.121	0.103	0.091	0.101	0.126	0.126	0.124	0.115
Tylodelphys sp. 6 (0.6%)	0.129	0.171	0.144	0.141	0.147	0.145	0.145	0.151	0.132	0.143	0.124	0.128	0.086	0.122	0.122	0.136
Tylodelphys sp. A*** (0.0-0.9%)	0.133	0.135	0.109	0.149	0.147	0.126	0.146	0.129	0.124	0.143	0.129	0.084	0.111	0.110	0.074	0.125
Tylodelphys sp. IBC-2016 (0.0-0.6%)	0.125	0.143	0.135	0.136	0.143	0.126	0.152	0.129	0.116	0.122	0.093	0.075	0.104	0.109	0.086	0.123
Tylodelphys sp. IND (0.3%)	0.141	0.156	0.142	0.151	0.141	0.139	0.160	0.138	0.152	0.116	0.126	0.118	0.121	0.129	0.127	0.124

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Table 4.12 – *Continued from previous page*

Table 4.12 - *Columns continued*

	*T.*sp. 2LIN2	*T.* sp. 3	*T.* sp. 4	*T.* sp. 5	*T.* sp. 6	*T.* sp. A	*T.* sp. IBC	*T.* sp. IND
Austrodiplostomum ostrowskiae (0.3-0.9%)	0.018	0.019	0.019	0.019	0.017	0.018	0.016	0.017
Alaria sp. 1	0.018	0.018	0.019	0.020	0.019	0.017	0.018	0.018
Alaria sp. 2 (0.0%)	0.019	0.019	0.019	0.020	0.018	0.019	0.017	0.019
Diplostomum ardeae	0.017	0.016	0.018	0.018	0.018	0.016	0.015	0.017
Diplostomum baeri LIN1 (0.9%)	0.020	0.019	0.020	0.020	0.020	0.020	0.019	0.020
Diplostomum baeri LIN2 (0.0-0.9%)	0.018	0.020	0.019	0.019	0.019	0.019	0.020	0.020
Diplostomum huronense (0.3-0.6%)	0.018	0.017	0.017	0.017	0.019	0.018	0.018	0.018
Diplostomum indistinctum (0.3-0.9%)	0.019	0.019	0.019	0.019	0.020	0.018	0.019	0.019
Diplostomum mergi (0.0-2.4%)	0.017	0.018	0.018	0.018	0.017	0.018	0.017	0.018
Diplostomum parviventosum (0.6%)	0.018	0.017	0.017	0.017	0.018	0.017	0.017	0.018
Diplostomum pseudospathaceum (0.0-3.3%)	0.018	0.018	0.018	0.019	0.016	0.017	0.018	0.020
Diplostomum sp. 1 (0.0-1.5%)	0.018	0.019	0.019	0.018	0.019	0.019	0.018	0.020
Diplostomum sp. 2	0.019	0.018	0.020	0.019	0.018	0.018	0.018	0.020
Diplostomum sp. 4 (0.0-1.5%)	0.017	0.017	0.018	0.018	0.019	0.017	0.019	0.020

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Table 4.12 – *Continued from previous page*

	*T.*sp. 2LIN2	*T.* sp. 3	*T.* sp. 4	*T.* sp. 5	*T.* sp. 6	*T.* sp. A	*T.* sp. IBC	*T.* sp. IND
Diplostomum sp. 6	0.018	0.018	0.019	0.020	0.019	0.019	0.018	0.019
Diplostomum sp. 7	0.019	0.020	0.020	0.020	0.019	0.019	0.019	0.020
Diplostomum sp. 8	0.018	0.018	0.018	0.020	0.018	0.019	0.018	0.020
Diplostomum sp. 9	0.020	0.019	0.020	0.020	0.019	0.019	0.019	0.020
Diplostomum sp. A***	0.018	0.019	0.020	0.019	0.020	0.017	0.019	0.019
Diplostomum sp. B***	0.020	0.020	0.020	0.019	0.019	0.019	0.019	0.020
Diplostomum sp. C ***(0.0-2.1%)	0.019	0.019	0.020	0.019	0.019	0.019	0.019	0.019
Diplostomum sp. clade Q	0.019	0.018	0.018	0.018	0.019	0.017	0.018	0.019
Diplostomum sp. LIN6 (0.3-1.8%)	0.020	0.018	0.017	0.019	0.018	0.019	0.019	0.020
Diplostomum spathaceum (0.3-2.1%)	0.019	0.018	0.018	0.017	0.019	0.018	0.017	0.018
Tylodelphys aztecae (0.6-1.5%)	0.019	0.017	0.018	0.018	0.018	0.018	0.017	0.020
Tylodelphys clavata (0.6-1.2%)	0.018	0.019	0.019	0.016	0.019	0.019	0.018	0.018
Tylodelphys excavata	0.017	0.016	0.018	0.015	0.017	0.018	0.015	0.019
Tylodelphys immer (0.3-0.6%)	0.018	0.014	0.017	0.016	0.018	0.015	0.014	0.016
Tylodelphys jenynsiae (0.0-0.9%)	0.018	0.017	0.015	0.018	0.015	0.018	0.016	0.018
Tylodelphys mashonensis (0.0-0.3%)	0.015	0.017	0.018	0.018	0.017	0.017	0.017	0.018
Tylodelphys scheuringi (0.0-1.2%)	0.018	0.014	0.017	0.017	0.018	0.014	0.015	0.018
Tylodelphys sp. 2 LIN1	0.017	0.018	0.018	0.017	0.019	0.018	0.018	0.018
Tylodelphys sp. 2 LIN2 (0.9%)	**0.009**	0.016	0.018	0.017	0.017	0.015	0.017	0.019
Tylodelphys sp. 3 (0.3-0.9%)	0.112	**0.006**	0.015	0.016	0.017	0.014	0.013	0.017
Tylodelphys sp. 4 (0.0-0.3%)	0.127	0.099	**0.004**	0.018	0.014	0.017	0.016	0.017
Tylodelphys sp. 5 (0.3%)	0.115	0.103	0.120	**0.003**	0.018	0.016	0.017	0.017
Tylodelphys sp. 6 (0.6%)	0.114	0.111	0.082	0.117	**0.006**	0.017	0.016	0.018
Tylodelphys sp. A*** (0.0-0.9%)	0.101	0.071	0.108	0.100	0.097	**0.003**	0.016	0.018
Tylodelphys sp. IBC-2016 (0.0-0.6%)	0.119	0.072	0.102	0.106	0.100	0.092	**0.004**	0.017
Tylodelphys sp. IND (0.3%)	0.127	0.114	0.114	0.112	0.124	0.128	0.114	**0.003**

Table 4.13: Average *cox1* divergence within and between genera of Diplostomidae-II. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. On the diagonal are the average within group divergence values. Numbers within parentheses after species names represent the range of percent divergence within groups. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 102 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 307 positions in the final dataset.

	Alaria sp.1	*Alaria* sp.2	*B. damni.*	*B.* sp.	*B.* sp. BOLD	D.gen.sp.O	D.gen.sp.X	*H. triloba*	*N. americ.*	*O.scardinii*	*O.* sp. 1	*O.* sp. 2
Alaria sp. 1	**-**	0.017	0.022	0.022	0.022	0.023	0.022	0.019	0.021	0.022	0.023	0.022
Alaria sp. 2 (0.3-1.0%)	0.109	**0.007**	0.022	0.022	0.023	0.023	0.023	0.019	0.021	0.024	0.023	0.023
Bolbophorus damnificus	0.189	0.189	**-**	0.020	0.019	0.023	0.022	0.021	0.021	0.022	0.023	0.021
Bolbophorus sp. (0.0-3.9%)	0.202	0.210	0.156	**0.012**	0.020	0.022	0.022	0.022	0.022	0.022	0.023	0.021
Bolbophorus sp. BOLD	0.176	0.212	0.121	0.153	**-**	0.023	0.021	0.022	0.022	0.021	0.022	0.020
Diplostomidae gen. sp. O*** (0.0-1.0%)	0.213	0.221	0.203	0.193	0.199	**0.003**	0.018	0.022	0.021	0.018	0.019	0.016
Diplostomidae gen. sp. X***	0.202	0.213	0.189	0.202	0.179	0.115	**-**	0.022	0.022	0.018	0.018	0.014
Hysteromorpha triloba (4.2%)	0.151	0.159	0.184	0.211	0.195	0.208	0.213	**0.042**	0.020	0.020	0.021	0.021
Neodiplostomum americanum (0.3-1.3%)	0.162	0.163	0.179	0.194	0.182	0.199	0.196	0.176	**0.008**	0.021	0.022	0.022
Ornithodiplostomum scardinii	0.202	0.227	0.195	0.199	0.189	0.128	0.121	0.199	0.186	**-**	0.019	0.017
Ornithodiplostomum sp. 1 (0.3%)	0.228	0.232	0.202	0.212	0.189	0.138	0.132	0.195	0.222	0.148	**0.003**	0.018
Ornithodiplostomum sp. 2 (0.0-3.9%)	0.207	0.229	0.174	0.191	0.161	0.105	0.082	0.203	0.200	0.113	0.128	**0.023**
Ornithodiplostomum sp. 3 (2.0-4.2%)	0.219	0.218	0.174	0.203	0.190	0.107	0.090	0.205	0.197	0.118	0.135	0.064
Ornithodiplostomum sp. 4 (0.3-2.9%)	0.224	0.224	0.185	0.184	0.173	0.117	0.116	0.215	0.198	0.143	0.158	0.114
Ornithodiplostomum sp. 8 (0.0-3.9%)	0.212	0.227	0.196	0.196	0.186	0.127	0.129	0.201	0.172	0.128	0.148	0.113
Posthodiplostomum brevicaudatum (0.3-2.0%)	0.227	0.237	0.204	0.213	0.203	0.155	0.153	0.224	0.239	0.172	0.158	0.137
Posthodiplostomum cetrarchi (0.0%)	0.212	0.227	0.189	0.210	0.182	0.185	0.176	0.217	0.216	0.173	0.145	0.160
Posthodiplostomum cuticola	0.205	0.205	0.195	0.196	0.182	0.185	0.182	0.182	0.213	0.186	0.191	0.173
Posthodiplostomum sp. 1	0.215	0.214	0.221	0.202	0.186	0.173	0.166	0.208	0.208	0.195	0.184	0.163
Posthodiplostomum sp. 2	0.189	0.185	0.208	0.196	0.176	0.165	0.160	0.192	0.191	0.189	0.174	0.156
Posthodiplostomum sp. 4 (1.0-4.6%)	0.202	0.215	0.181	0.174	0.159	0.145	0.140	0.189	0.198	0.171	0.145	0.129
Posthodiplostomum sp. 5	0.238	0.255	0.231	0.226	0.199	0.173	0.169	0.226	0.234	0.186	0.145	0.164
Posthodiplostomum sp. 7 (0.3-1.0%)	0.204	0.228	0.181	0.197	0.195	0.182	0.164	0.194	0.203	0.175	0.189	0.162
Posthodiplostomum sp. 8 (0.3-0.7%)	0.231	0.213	0.192	0.199	0.228	0.189	0.191	0.226	0.208	0.208	0.194	0.182

Table 4.13 - *Columns continued*

	O. sp. 3	*O.* sp. 4	*O.* sp. 8	*P. brevic.*	*P.cetrarchi*	*P.cuticola*	*P.* sp. 1	*P.* sp. 2	*P.* sp. 4	*P.* sp. 5	*P.* sp. 7	*P.* sp. 8
Alaria sp. 1	0.022	0.022	0.022	0.022	0.022	0.022	0.023	0.022	0.021	0.023	0.021	0.023
Alaria sp. 2 (0.3-1.0%)	0.023	0.023	0.023	0.023	0.023	0.022	0.023	0.022	0.022	0.024	0.023	0.022
Bolbophorus damnificus	0.020	0.021	0.022	0.022	0.022	0.022	0.024	0.023	0.021	0.024	0.021	0.022
Bolbophorus sp. (0.0-3.9%)	0.021	0.021	0.022	0.022	0.022	0.022	0.023	0.022	0.020	0.023	0.021	0.023
Bolbophorus sp. BOLD	0.021	0.021	0.021	0.022	0.022	0.021	0.022	0.021	0.019	0.022	0.022	0.024
Diplostomidae gen. sp. O*** (0.0-1.0%)	0.016	0.018	0.018	0.021	0.022	0.021	0.021	0.021	0.019	0.020	0.021	0.021
Diplostomidae gen. sp. X***	0.015	0.017	0.018	0.020	0.021	0.021	0.021	0.020	0.018	0.020	0.021	0.022
Hysteromorpha triloba (4.2%)	0.021	0.022	0.021	0.021	0.021	0.021	0.022	0.022	0.021	0.023	0.021	0.023
Neodiplostomum americanum (0.3-1.3%)	0.021	0.021	0.020	0.023	0.022	0.022	0.023	0.022	0.021	0.023	0.022	0.022
Ornithodiplostomum scardinii	0.017	0.019	0.019	0.021	0.021	0.021	0.022	0.021	0.020	0.021	0.020	0.023
Ornithodiplostomum sp. 1 (0.3%)	0.018	0.020	0.019	0.020	0.020	0.022	0.021	0.021	0.019	0.020	0.021	0.022

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Table 4.13 – *Continued from previous page*

	O. sp. 3	*O.* sp. 4	*O.* sp. 8	*P. brevic.*	*P.cetrarchi*	*P.cuticola*	*P.* sp. 1	*P.* sp. 2	*P.* sp. 4	*P.* sp. 5	*P.* sp. 7	*P.* sp. 8
Ornithodiplostomum sp. 2 (0.0-3.9%)	0.012	0.017	0.016	0.018	0.020	0.020	0.021	0.020	0.017	0.019	0.019	0.021
Ornithodiplostomum sp. 3 (2.0-4.2%)	**0.033**	0.016	0.017	0.019	0.019	0.020	0.021	0.021	0.018	0.020	0.020	0.020
Ornithodiplostomum sp. 4 (0.3-2.9%)	0.104	**0.020**	0.017	0.019	0.021	0.021	0.021	0.020	0.018	0.022	0.019	0.022
Ornithodiplostomum sp. 8 (0.0-3.9%)	0.117	0.125	**0.013**	0.021	0.020	0.020	0.020	0.019	0.017	0.020	0.020	0.021
Posthodiplostomum brevicaudatum (0.3-2.0%)	0.146	0.149	0.176	**0.013**	0.020	0.023	0.022	0.022	0.020	0.021	0.021	0.021
Posthodiplostomum cetrarchi (0.0%)	0.159	0.174	0.170	0.155	**0.000**	0.022	0.021	0.020	0.019	0.020	0.020	0.020
Posthodiplostomum cuticola	0.178	0.185	0.171	0.227	0.179	**-**	0.021	0.020	0.019	0.023	0.022	0.022
Posthodiplostomum sp. 1	0.178	0.174	0.154	0.197	0.169	0.169	**-**	0.010	0.021	0.021	0.021	0.020
Posthodiplostomum sp. 2	0.167	0.166	0.144	0.192	0.160	0.153	0.036	**-**	0.020	0.023	0.022	0.022
Posthodiplostomum sp. 4 (1.0-4.6%)	0.143	0.149	0.142	0.176	0.148	0.161	0.173	0.167	**0.031**	0.020	0.020	0.020
Posthodiplostomum sp. 5	0.180	0.197	0.176	0.187	0.160	0.221	0.169	0.192	0.156	**-**	0.021	0.022
Posthodiplostomum sp. 7 (0.3-1.0%)	0.173	0.151	0.167	0.188	0.182	0.201	0.177	0.190	0.179	0.189	**0.007**	0.021
Posthodiplostomum sp. 8 (0.3-0.7%)	0.169	0.182	0.170	0.192	0.168	0.199	0.164	0.177	0.159	0.194	0.183	**0.004**

Table 4.14: Average *cox1* divergence within and between groups of Strigeidae-I. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. Average intraspecific divergence values lie on the diagonal. The range of intraspecific values are given as percentages next to species names. Values in green are below the delineation cut-off for between group divergence. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 142 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 353 positions in the final dataset.

	C. cornutus	*C. gallinulae*	*C.* sp. A	*C.* sp. B	*C.* sp. C	*C.* sp. D	*C.* sp. E	*C.* sp. F	*C.* sp. G	*C.* sp. H	*Ca. mediconiger*	*Ca.* sp.	*Ic. pileatus*	*Ic.* sp. 2	*Ic.* sp. 3
Cotylurus cornutus (0.0-2.8%)	**0.011**	0.014	0.018	0.017	0.013	0.013	0.010	0.009	0.012	0.014	0.019	0.019	0.019	0.018	0.017
Cotylurus gallinulae (0.0-1.7%)	0.093	**0.007**	0.018	0.018	0.015	0.014	0.015	0.015	0.015	0.012	0.020	0.020	0.019	0.018	0.018
Cotylurus sp. A*** (0.0-1.4%)	0.135	0.147	**0.003**	0.018	0.018	0.018	0.018	0.018	0.019	0.018	0.019	0.019	0.018	0.018	0.017
Cotylurus sp. B***	0.130	0.142	0.133	**-**	0.017	0.017	0.016	0.017	0.017	0.018	0.019	0.019	0.019	0.017	0.018
Cotylurus sp. C ***(0.0-1.1%)	0.078	0.093	0.135	0.112	**0.006**	0.013	0.012	0.014	0.014	0.015	0.020	0.020	0.019	0.018	0.017
Cotylurus sp. D*** (0.0-0.6%)	0.078	0.082	0.142	0.118	0.072	**0.002**	0.013	0.013	0.014	0.014	0.020	0.020	0.018	0.016	0.017
Cotylurus sp. E*** (0.0-4.2%)	0.043	0.096	0.143	0.120	0.063	0.067	**0.004**	0.010	0.013	0.014	0.020	0.020	0.019	0.019	0.018
Cotylurus sp. F***	0.043	0.097	0.136	0.125	0.081	0.072	0.041	**-**	0.013	0.014	0.019	0.020	0.019	0.018	0.018
Cotylurus sp. G*** (0.0%)	0.063	0.095	0.150	0.119	0.078	0.081	0.072	0.062	**0.000**	0.014	0.020	0.020	0.020	0.017	0.018
Cotylurus sp. H***	0.082	0.067	0.133	0.139	0.092	0.081	0.088	0.082	0.079	**-**	0.019	0.020	0.019	0.018	0.018
Cardiocephaloides mediconiger (0.0%)	0.169	0.186	0.173	0.167	0.187	0.177	0.177	0.161	0.170	0.156	**0.000**	0.015	0.018	0.018	0.018
Cardiocephaloides sp.	0.172	0.181	0.170	0.159	0.170	0.182	0.180	0.173	0.173	0.173	0.093	**-**	0.020	0.019	0.018
Ichthyocotylurus pileatus (0.0-0.3%)	0.153	0.156	0.160	0.163	0.168	0.139	0.158	0.155	0.172	0.161	0.152	0.180	**0.002**	0.014	0.015
Ichthyocotylurus sp. 2	0.150	0.144	0.144	0.136	0.146	0.114	0.162	0.150	0.144	0.150	0.147	0.147	0.090	**-**	0.014
Ichthyocotylurus sp. 3	0.134	0.145	0.144	0.147	0.139	0.140	0.140	0.142	0.142	0.142	0.142	0.147	0.095	0.079	**-**

Table 4.15: Average *cox1* divergence within and between groups of Strigeidae-II. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. Average intraspecific divergence values lie on the diagonal. The range of intraspecific values are given as percentages next to species names. Values in red are above the delineation cut-off. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 309 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 377 positions in the final dataset.

	A. jamies.	*A.* sp. 1	*A.* sp. 1x	*A.* sp. 3	*A.* sp. 4	*A.* sp. A	*A.* sp. B	*A.* sp. C	*Au.* LIN1	*b. Au. mclaugh.*
Apatemon sp. 'jamiesoni' (0.3%)	**0.003**	0.015	0.014	0.015	0.016	0.016	0.015	0.014	0.018	0.018
Apatemon sp. 1 (0.8%)	0.103	**0.008**	0.009	0.014	0.015	0.014	0.013	0.015	0.018	0.017
Apatemon sp. 1x (0.5%)	0.101	0.038	**0.005**	0.014	0.015	0.014	0.014	0.014	0.017	0.017
Apatemon sp. 3 (0.0%)	0.097	0.092	0.085	**0.000**	0.015	0.016	0.015	0.015	0.017	0.018
Apatemon sp. 4 (0.3%)	0.118	0.095	0.090	0.094	**0.003**	0.018	0.016	0.016	0.018	0.018
Apatemon sp. A ***(0.0-1.0%)	0.124	0.094	0.087	0.114	0.131	**0.004**	0.012	0.015	0.019	0.019
Apatemon sp. B***	0.113	0.084	0.085	0.093	0.107	0.058	**-**	0.015	0.018	0.019
Apatemon sp. C ***(0.0-0.3%)	0.097	0.091	0.092	0.093	0.099	0.101	0.092	**0.001**	0.017	0.018
Australapatemon burti LIN1 (0.0-6.4%)	0.147	0.138	0.136	0.131	0.142	0.158	0.149	0.126	**0.011**	0.014
Australapatemon mclaughlini (0.0-0.5%)	0.150	0.140	0.133	0.142	0.156	0.165	0.162	0.148	0.097	**0.002**
Australapatemon niewiadomski (0.0-2.1*Australapatemon* sp. LIN10***	0.139	0.134	0.117	0.127	0.139	0.142	0.143	0.129	0.084	0.050
Australapatemon sp. LIN2	0.158	0.155	0.149	0.159	0.153	0.170	0.159	0.148	0.075	0.085
Australapatemon sp. LIN3	0.158	0.160	0.154	0.133	0.150	0.156	0.141	0.135	0.087	0.109
Australapatemon sp. LIN4 (0.3-4.8%)	0.161	0.158	0.149	0.139	0.148	0.154	0.163	0.151	0.093	0.101
Australapatemon sp. LIN5	0.174	0.145	0.146	0.143	0.167	0.172	0.167	0.145	0.113	0.099
Australapatemon sp. LIN6 (0.0-4.2%)	0.144	0.146	0.129	0.135	0.134	0.154	0.151	0.138	0.086	0.067
Australapatemon sp. LIN8 (0.0-1.0%)	0.143	0.140	0.136	0.120	0.136	0.147	0.139	0.140	0.100	0.124
Australapatemon sp. LIN9A (0.0-3.2%)	0.141	0.136	0.130	0.116	0.146	0.142	0.132	0.125	0.104	0.112
Australapatemon sp. LIN9B (0.3-1.1%)	0.145	0.145	0.133	0.123	0.149	0.141	0.141	0.111	0.102	0.111

Table 4.15 - *Columns continued*

	Au. niew.	*Au.* LIN10	*Au.* LIN2	*Au.* LIN3	*Au.* LIN4	*Au.* LIN5	*Au.* LIN6	*Au.* LIN8	*Au.* LIN9A	*Au.* LIN9B
Apatemon sp. 'jamiesoni' (0.3%)	0.017	0.018	0.019	0.018	0.018	0.019	0.018	0.018	0.017	0.018
Apatemon sp. 1 (0.8%)	0.018	0.017	0.019	0.019	0.018	0.017	0.018	0.017	0.017	0.018
Apatemon sp. 1x (0.5%)	0.017	0.016	0.019	0.018	0.017	0.018	0.017	0.017	0.017	0.017
Apatemon sp. 3 (0.0%)	0.017	0.017	0.019	0.017	0.017	0.018	0.017	0.016	0.016	0.017
Apatemon sp. 4 (0.3%)	0.017	0.017	0.019	0.019	0.017	0.019	0.017	0.017	0.018	0.019
Apatemon sp. A ***(0.0-1.0%)	0.019	0.018	0.019	0.018	0.018	0.019	0.018	0.018	0.018	0.018
Apatemon sp. B***	0.018	0.018	0.019	0.017	0.018	0.019	0.018	0.017	0.017	0.018
Apatemon sp. C ***(0.0-0.3%)	0.017	0.017	0.018	0.017	0.017	0.018	0.017	0.017	0.016	0.016
Australapatemon burti LIN1 (0.0-6.4%)	0.016	0.013	0.012	0.014	0.013	0.015	0.013	0.015	0.015	0.015
Australapatemon mclaughlini (0.0-0.5%)	0.017	0.011	0.014	0.015	0.014	0.015	0.012	0.017	0.015	0.015
Australapatemon niewiadomski (0.0-2.1%)	**0.013**	0.017	0.017	0.017	0.017	0.018	0.017	0.017	0.017	0.017
Australapatemon sp. LIN10***	0.130	**-**	0.015	0.015	0.013	0.014	0.010	0.016	0.015	0.015

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Table 4.15 – *Continued from previous page*

	Au. niew.	*Au.* LIN10	*Au.* LIN2	*Au.* LIN3	*Au.* LIN4	*Au.* LIN5	*Au.* LIN6	*Au.* LIN8	*Au.* LIN9A	*Au.* LIN9B
Australapatemon sp. LIN2	0.140	0.093	-	0.014	0.014	0.017	0.014	0.015	0.015	0.015
Australapatemon sp. LIN3	0.132	0.098	0.088	-	0.015	0.016	0.014	0.016	0.016	0.015
Australapatemon sp. LIN4 (0.3-4.8%)	0.134	0.086	0.098	0.104	**0.026**	0.015	0.012	0.014	0.015	0.015
Australapatemon sp. LIN5	0.144	0.077	0.138	0.119	0.117	-	0.015	0.017	0.016	0.016
Australapatemon sp. LIN6 (0.0-4.2%)	0.137	0.045	0.094	0.105	0.081	0.097	**0.017**	0.015	0.015	0.014
Australapatemon sp. LIN8 (0.0-1.0%)	0.136	0.107	0.112	0.117	0.105	0.147	0.109	**0.002**	0.014	0.015
Australapatemon sp. LIN9A (0.0-3.2%)	0.136	0.099	0.097	0.117	0.115	0.127	0.107	0.088	**0.009**	0.011
Australapatemon sp. LIN9B (0.3-1.1%)	0.140	0.097	0.100	0.100	0.117	0.117	0.105	0.101	0.061	**0.007**

Table 4.16: Trematode species counts by collection site

	Pelican Point (BL)	**Rochon Sands (BL)**	**The Narrows (BL)**	**Gull Lake**	**Isle Lake**	**Lac La Nonne**	**Lac La Nonne site #2**	**Pigeon Lake**	**Wabamun Lake**	**Grand Total**
Apatemon sp. A	-	-	-	-	16	-	-	-	-	**16**
Apatemon sp. B	-	-	-	-	1	-	-	-	-	**1**
Apatemon sp. C	-	-	-	-	4	-	-	-	-	**4**
Australapatemon burti LIN1	-	-	2	24	165	2	5	-	6	**204**
Australapatemon mclaughlini	-	2	-	-	-	-	-	-	-	**2**
Australapatemon sp. LIN10	-	-	-	1	-	-	-	-	-	**1**
Australapatemon sp. LIN3	-	-	-	1	-	-	-	-	-	**1**
Australapatemon sp. LIN4	-	-	-	1	-	-	1	-	-	**2**
Australapatemon sp. LIN5	-	1	-	-	-	-	-	-	-	**1**
Australapatemon sp. LIN6	-	-	1	-	4	-	1	1	-	**7**
Australapatemon sp. LIN8	1	4	-	1	3	-	-	-	-	**9**
Australapatemon sp. LIN9A	-	2	-	7	6	2	-	-	-	**17**
Australapatemon sp. LIN9B	3	1	-	-	-	-	-	-	-	**4**
Avian schistosomatid sp. A	-	3	2	-	2	-	-	-	-	**7**
Avian schistosomatid sp. B	-	-	-	-	-	-	1	-	-	**1**
Avian schistosomatid sp. C	-	-	-	-	-	-	-	-	1	**1**
Bolbophorus sp.	-	-	6	-	2	-	-	-	2	**10**
Cotylurus cornutus	-	-	-	2	32	-	1	-	-	**35**
Cotylurus flabelliformis	-	-	-	-	1	-	-	-	-	**1**
Cotylurus marcogliesei	1	-	-	3	2	1	-	-	-	**7**
Cotylurus sp. A	-	-	-	-	34	1	1	-	3	**39**
Cotylurus sp. B	-	-	-	-	1	-	-	-	-	**1**
Cotylurus sp. C	-	-	3	-	-	-	-	-	-	**3**
Cotylurus sp. D	-	1	-	-	-	-	-	-	-	**1**
Cotylurus sp. E	2	1	-	2	5	2	-	-	-	**12**
Cotylurus sp. F	-	-	4	-	-	-	-	-	2	**6**
Cotylurus strigeoides	-	1	1	-	11	1	6	-	2	**22**
Diplostomidae gen. sp. O	6	19	-	6	2	-	-	-	1	**34**
Diplostomidae gen. sp. X	-	-	-	-	1	-	-	-	-	**1**
Diplostomum baeri LIN2	-	-	-	-	3	-	-	-	2	**5**
Diplostomum indistinctum	-	-	-	1	-	-	-	-	-	**1**
Diplostomum sp. 1	-	-	-	-	1	-	-	-	5	**6**
Diplostomum sp. 3	-	-	-	-	-	-	-	-	3	**3**
Diplostomum sp. 4	9	-	-	7	24	-	1	-	30	**71**
Diplostomum sp. A	1	-	-	-	-	-	-	-	-	**1**
Diplostomum sp. B	-	-	-	-	1	-	-	-	-	**1**
Diplostomum sp. C	-	-	-	1	10	-	-	-	1	**12**
Drepanocephalus spathans	-	-	3	-	1	-	-	-	-	**4**
Echinoparyphium sp. A	2	-	-	4	22	2	7	2	7	**46**
Echinoparyphium sp. A2	-	-	-	4	-	2	1	-	-	**7**
Echinoparyphium sp. B	-	-	-	-	-	9	-	-	-	**9**
Echinoparyphium sp. C	-	-	-	8	-	16	1	-	-	**25**
Echinoparyphium sp. D	1	-	-	-	-	-	-	-	-	**1**
Echinoparyphium sp. E	-	-	-	6	-	-	-	-	-	**6**
Echinoparyphium sp. Lineage 1 A	-	-	-	-	10	18	46	-	23	**97**
Echinoparyphium sp. Lineage 1 B	-	-	-	-	1	-	-	-	-	**1**

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Table 4.16 – *Continued from previous page*

	Pelican Point (BL)	**Rochon Sands (BL)**	**The Narrows (BL)**	**Gull Lake**	**Isle Lake**	**Lac La Nonne**	**Lac La Nonne site #2**	**Pigeon Lake**	**Wabamun Lake**	**Grand Total**
Echinoparyphium sp. Lineage 2	12	1	-	29	13	27	4	-	3	**89**
Echinoparyphium sp. Lineage 4	-	-	1	-	-	-	-	-	1	**2**
Echinostoma revolutum B	11	-	-	11	2	7	1	-	1	**33**
Echinostoma trivolvis Lineage A	-	-	-	-	2	2	-	-	1	**5**
Echinostomatidae gen. sp.	4	-	-	-	-	-	-	-	-	**4**
Haematoloechidae gen. sp. A	4	-	-	-	-	-	-	-	-	**4**
Hypoderaeum sp. Lineage 1	-	-	-	5	-	-	-	-	1	**6**
Hypoderaeum sp. Lineage 2	-	-	-	-	1	2	-	-	-	**3**
Neodiplostomum americanum	1	-	-	-	-	-	-	-	-	**1**
Neopetasiger islandicus	-	-	-	-	-	-	-	-	1	**1**
Neopetasiger sp. 4	-	-	1	-	2	-	-	-	7	**10**
Notocotylus sp. A	1	1	-	1	12	9	6	-	9	**39**
Notocotylus sp. B	-	-	-	-	-	-	-	-	1	**1**
Notocotylus sp. C	-	-	-	-	-	1	-	-	-	**1**
Notocotylus sp. D	4	1	-	21	23	4	1	-	-	**54**
Ornithodiplostomum sp. 2	-	-	-	-	-	-	-	-	1	**1**
Ornithodiplostomum sp. 8	-	-	-	-	3	-	-	1	-	**4**
Plagiorchis sp.*	343	78	58	257	173	132	26	-	78	**1145**
Posthodiplostomum sp. 4	-	-	-	-	2	-	-	-	-	**2**
Psilostomidae gen. sp. A	-	-	-	-	4	-	-	-	2	**6**
Schistosomatium douthitti	-	-	6	3	-	-	-	-	1	**10**
Trichobilharzia physellae	-	-	-	-	-	1	-	-	-	**1**
Trichobilharzia stagnicolae	-	-	-	-	8	-	-	-	-	**8**
Trichobilharzia szidati	-	-	1	1	-	-	-	-	-	**2**
Tylodelphys sp. A	-	-	-	-	-	-	-	-	5	**5**
Grand Total	**406**	**116**	**89**	**407**	**610**	**241**	**110**	**4**	**200**	**2183**

BL = Buffalo Lake
* includes all lineages

Table 4.17: Snail host-trematode parasite relationships from this study

	Helisoma trivolvis	***Lymnaea stagnalis***	***Physa gyrina***	***Planorbula armigera***	***Stagnicola elodes***	**Grand Total**
Apatemon sp. A	-	-	-	-	16	**16**
Apatemon sp. B	-	-	-	-	1	**1**
Apatemon sp. C	-	-	-	-	4	**4**
Australapatemon burti LIN1	2	1	2	-	199	**204**
Australapatemon mclaughlini	-	-	2	-	-	**2**
Australapatemon sp. LIN10	-	-	1	-	-	**1**
Australapatemon sp. LIN3	-	-	-	-	1	**1**
Australapatemon sp. LIN4	-	-	2	-	-	**2**
Australapatemon sp. LIN5	-	-	-	-	1	**1**
Australapatemon sp. LIN6	-	-	7	-	-	**7**
Australapatemon sp. LIN8	-	-	9	-	-	**9**
Australapatemon sp. LIN9A	-	1	-	-	16	**17**
Australapatemon sp. LIN9B	-	-	-	-	4	**4**
Avian schistosomatid sp. A	-	-	7	-	-	**7**
Avian schistosomatid sp. B	-	-	1	-	-	**1**
Avian schistosomatid sp. C	1	-	-	-	-	**1**
Bolbophorus sp.	10	-	-	-	-	**10**
Cotylurus cornutus	1	-	-	-	32	**33**
Cotylurus flabelliformis	-	-	-	-	1	**1**
Cotylurus marcogliesei	-	-	-	-	5	**5**
Cotylurus sp. A	-	-	1	-	38	**39**
Cotylurus sp. B	-	-	1	-	-	**1**
Cotylurus sp. C	-	3	-	-	-	**3**
Cotylurus sp. D	-	-	1	-	-	**1**
Cotylurus sp. E	-	-	-	-	11	**11**
Cotylurus sp. F	-	6	-	-	-	**6**
Cotylurus strigeoides	-	-	21	-	1	**22**
Diplostomidae gen. sp. O	-	-	34	-	-	**34**
Diplostomidae gen. sp. X	-	-	1	-	-	**1**
Diplostomum baeri LIN2	-	-	-	-	5	**5**
Diplostomum indistinctum	-	-	-	-	1	**1**
Diplostomum sp. 1	-	-	-	-	6	**6**
Diplostomum sp. 3	-	3	-	-	-	**3**
Diplostomum sp. 4	-	-	-	-	71	**71**
Diplostomum sp. A	-	-	-	-	1	**1**
Diplostomum sp. B	-	-	-	-	1	**1**
Diplostomum sp. C	1	-	-	-	11	**12**
Drepanocephalus spathans	4	-	-	-	-	**4**
Echinoparyphium sp. A	-	-	44	-	2	**46**
Echinoparyphium sp. A2	-	-	1	-	6	**7**
Echinoparyphium sp. B	-	-	-	-	9	**9**
Echinoparyphium sp. C	-	-	-	-	25	**25**
Echinoparyphium sp. D	-	-	-	-	1	**1**
Echinoparyphium sp. E	-	1	-	-	5	**6**
Echinoparyphium sp. Lineage 1 A	1	-	94	-	2	**97**
Echinoparyphium sp. Lineage 1 B	-	-	1	-	-	**1**
Echinoparyphium sp. Lineage 2	1	3	-	-	80	**84**

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Table 4.17 – *Continued from previous page*

	Helisoma trivolvis	***Lymnaea stagnalis***	***Physa gyrina***	***Planorbula armigera***	***Stagnicola elodes***	**Grand Total**
Echinoparyphium sp. Lineage 4	2	-	-	-	-	**2**
Echinostoma revolutum B	-	-	-	-	33	**33**
Echinostoma trivolvis Lineage A	5	-	-	-	-	**5**
Echinostomatidae gen. sp.	-	-	-	-	4	**4**
Haematoloechidae gen. sp. A	-	-	-	-	4	**4**
Hypoderaeum sp. Lineage 1	-	-	-	-	5	**5**
Hypoderaeum sp. Lineage 2	-	-	-	-	3	**3**
Neodiplostomum americanum	-	-	-	-	1	**1**
Neopetasiger islandicus	-	-	-	1	-	**1**
Neopetasiger sp. 4	10	-	-	-	-	**10**
Notocotylus sp. A	-	-	36	-	3	**39**
Notocotylus sp. B	-	-	1	-	-	**1**
Notocotylus sp. C	1	-	-	-	-	**1**
Notocotylus sp. D	-	-	5	-	45	**50**
Ornithodiplostomum sp. 2	-	-	1	-	-	**1**
Ornithodiplostomum sp. 8	-	-	4	-	-	**4**
Plagiorchis sp.*	7	89	12	-	1027	**1135**
Posthodiplostomum sp. 4	-	-	2	-	-	**2**
Psilostomidae gen. sp. A	6	-	-	-	-	**6**
Schistosomatium douthitti	-	8	-	-	2	**10**
Trichobilharzia physellae	-	-	1	-	-	**1**
Trichobilharzia stagnicolae	-	-	-	-	8	**8**
Trichobilharzia szidati	-	2	-	-	-	**2**
Tylodelphys sp. A	5	-	-	-	-	**5**
Grand Total	**57**	**117**	**292**	**1**	**1691**	**2158**

* includes all lineages

Table 4.18: Summary of representation for digenean mitochondrial genes in GenBank. Number of mitochondrial sequence records for digenean trematodes (as of August 2018), the number of species they represent, and for those with over 100 sequences available, the number of species with true species names (e.g. *not* "sp. 1", etc.). Search terms used to filter GenBank records were "digenea" and "COX1" and "ND1".

Order	**Family**	**No. seq.** *cox1*	**No. spp.**	**named spp.**	**No. seq.** *nad1*	**No. spp.**	**named spp.**
Strigeidida							
	Schistosomatidae	1434	104	46	523	16	15
	Diplostomidae	1079	110	20	3	3	
	Clinostimidae	390	29	11	1	1	
	Strigeidae	188	46	18	20	7	
	Bucephalidae	66	5				
	Leuchochloridae	31	6		11	4	
	Bolbophoridae	15	5				
	Aporocotylidae	10	10				
	Cyathocotylidae	9	1				
	Spirorchiidae	5	5				
	Fellodistomidae	5	3		12	11	
	Leuchochloridiomorphidae	1	1				
	Panopstidae	1	1				
Plagiorchiida							
	Fasciolidae	463	31	4	533	45	7
	Opecoelidae	416	24	7	203	2	2
	Troglotrematidae	333	24	7	35	6	
	Apocreadiidae	261	5	4			
	Echinostomatidae	228	23	10	344	51	28
	Microphallidae	158	6				
	Allocreadiiidae	143	13				
	Dicrocoeliidae	115	15		33	6	
	Himasthlidae	79	9				
	Monorchiidae	69	17				
	Plagiorchiidae	64	19	16			
	Gorgoderidae	61	17				
	Haematoloechidae	52	23	15			
	Paramphistomidae	49	14		34	5	
	Notocotylidae	42	8	2	2	2	
	Gymnophallidae	41	3				
	Brachycladiidae	39	3				
	Lepocreadiidae	39	1		46	40	
	Philophthalmidae	38	5		20	3	
	Collyriclidae	37	1		8	1	
	Gastrothylacidae	23	6		2	2	
	Renicolidae	21	8		6	4	
	Pleurogenidae	15	2				
	Prosthogonimidae	14	4		16	4	
	Gorgocephalidae	8	1				
	Psilostomidae	5	4	3			
	Callodistomidae	4	4				
	Paramphistomatidae	3	3				
	Telorchiidae	2	2				

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Table 4.18 – *Continued from previous page*

Order	Family	No. seq.	*cox1*	No. spp.	named spp.	No. seq.	*nad1*	No. spp.	named spp.
	Alloglossidiidae	2		1		177		23	17
	Cephalogonimidae	2		1					
	Lissorchiidae	2		2					
	Echinochasmidae	1		1		1		1	
	Olveriidae	1		1					
Opisthorchiida									
	Heterophyidae	680		29	20	3		2	
	Opisthorchiidae	469		13	12	28		8	
	Cryptogonimidae	66		3		1		1	
	Acanthocolpidae	1		1		1		1	
Azygiida									
	Didymozoidae	82		2					
	Derogenidae	14		5					
	Accacoeliidae	5		2					
	Azygiidae	3		3					
	Hirudinellidae	2		2					
	Hemiuridae	1		1					
	Isoparorchiidae	1		1					

Lake	Size (km²)	Depth (m)
Buffalo	93.5	2.8
Gull	80.6	5.4
Isle	23.0	4.1
Lac La Nonne	11.8	7.8
Pigeon	96.7	6.2
Wabamun	81.8	6.3

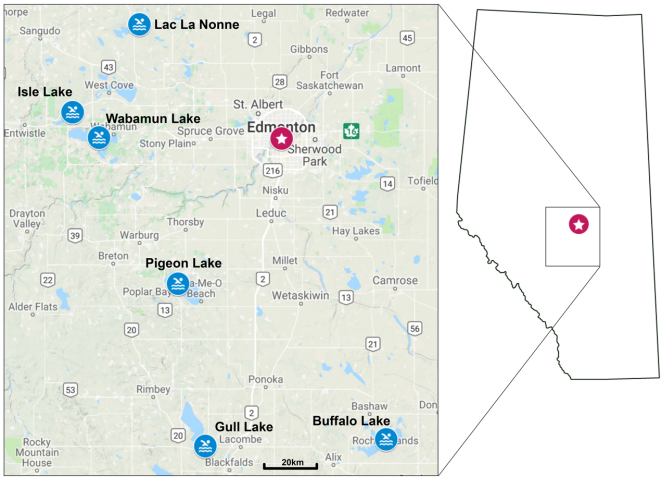

Figure 4.1: Sample collection locations. Map of the six lakes from which snails and trematodes were collected in central Alberta, Canada. Depth of lake is given as a mean depth in meters.

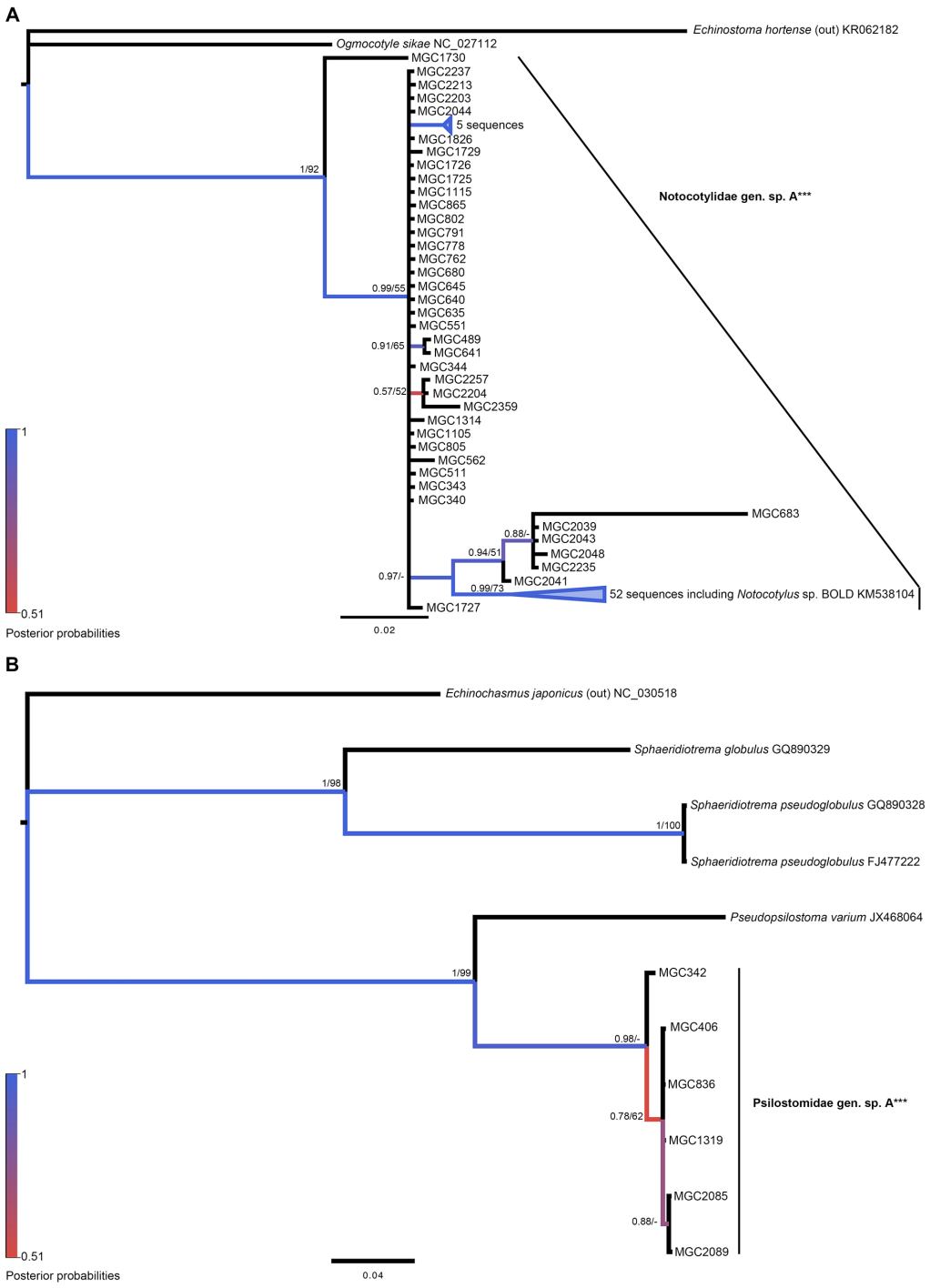

Figure 4.2: Molecular phylogeny of the Notocotylidae and Psilostomatidae based on *cox1*.

Figure 4.2 Molecular phylogeny of the Notocotylidae and Psilostomatidae based on *cox1*. Bayesian inference phylogenies are given. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Posterior probabilities >0.50 and bootstrap values >50 are reported near the nodes respectively. Accession numbers are given after species names. Emboldened taxa with three asterisks represent novel species from molecular analyses. A) Notocotylidae. B) Psilostomidae.

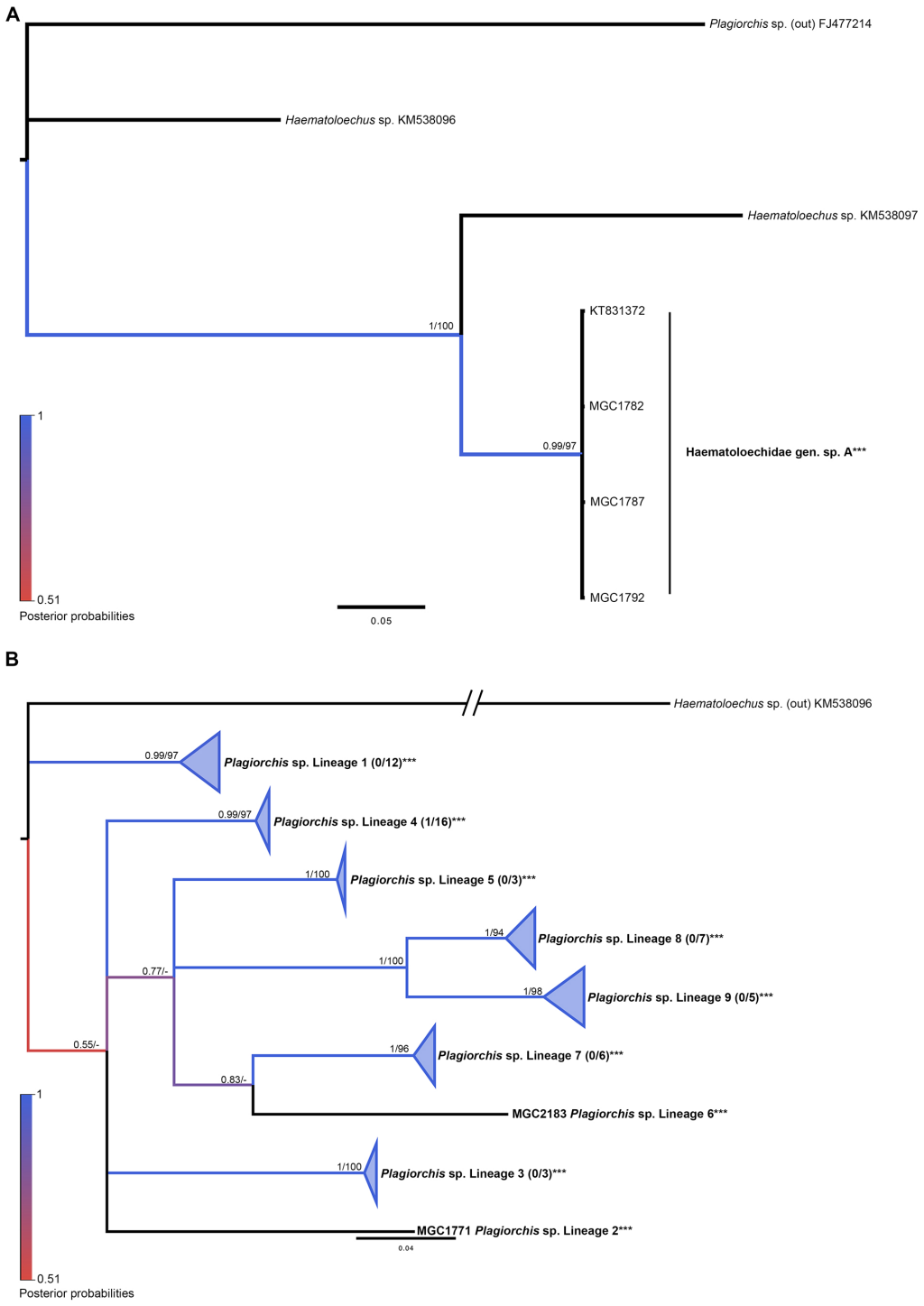

Figure 4.3: Molecular phylogeny of the Haematoloechidae and Plagiorchiidae based on *cox1*.

Figure 4.3 Molecular phylogeny of the Haematoloechidae and Plagiorchiidae based on *cox1*. Bayesian inference phylogenies are given. Clades representing a single species have been condensed for space. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Posterior probabilities >0.50 and bootstrap values >50 are reported near the nodes respectively. Accession numbers are given after species names. Numbers in parentheses after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study. Emboldened taxa with three asterisks represent novel species from molecular analyses. A) Haematoloechidae. B) Plagiorchiidae.

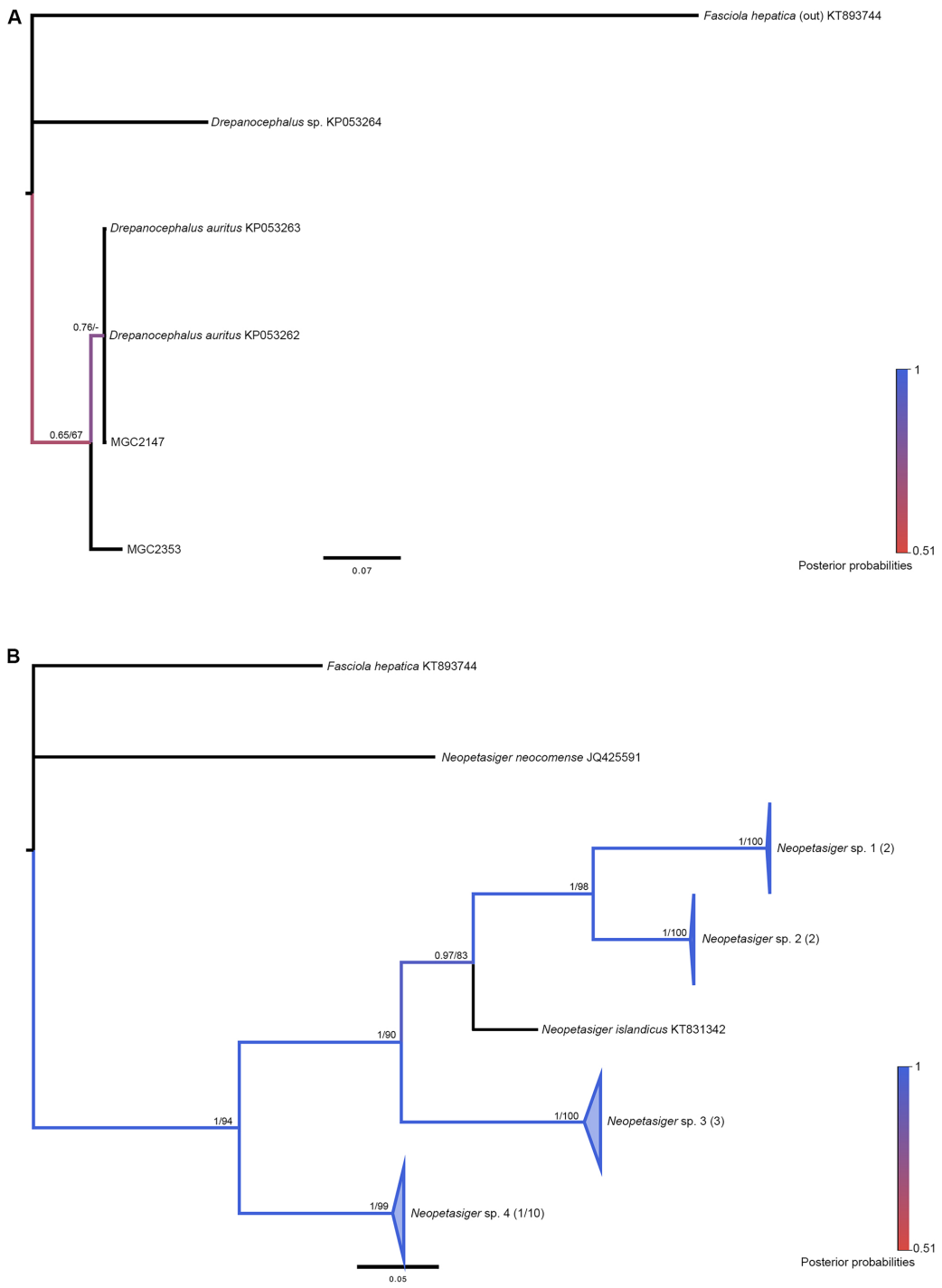

Figure 4.4: Molecular phylogeny of the Echinostomatidae: *Drepanocephalus* and *Neopetasiger* genera based on *nad1*.

Figure 4.4 Molecular phylogeny of the Echinostomatidae: *Drepanocephalus* and *Neopetasiger* genera based on *nad1*. Bayesian inference phylogenies are given. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Posterior probabilities >0.50 and bootstrap values >50 from BI and ML analyses are reported near the nodes respectively. Accession numbers are given after species names. Clades representing a single species have been condensed for space. Numbers in parentheses after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study. A) *Drepanocephalus*. B) *Neopetasiger*.

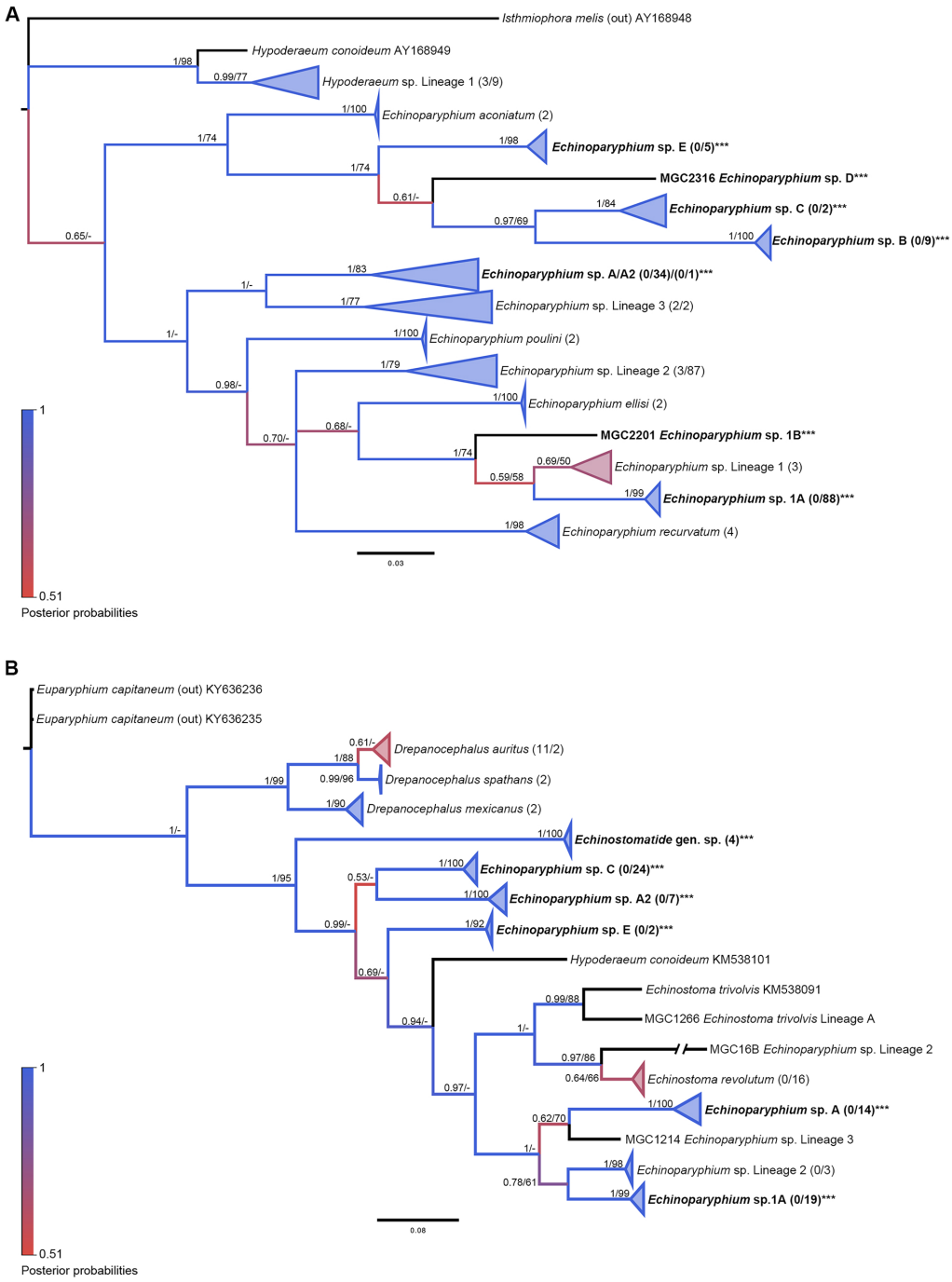

Figure 4.5: Molecular phylogeny of the Echinostomatidae: *Echinoparyphium/ Hypoderaeum* genera based on *nad1* and *cox1*.

Figure 4.5 Molecular phylogeny of the Echinostomatidae: *Echinoparyphium/ Hypoderaeum* genera based on *nad1* and *cox1*. Bayesian inference phylogenies are given. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Posterior probabilities >0.50 and bootstrap values >50 from BI and ML analyses are reported near the nodes respectively. Accession numbers are given after species names. Clades representing a single species have been condensed for space. Numbers in parentheses after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study. Emboldened taxa with three asterisks represent novel species from molecular analysis. A) *nad1*. B) *cox1*.

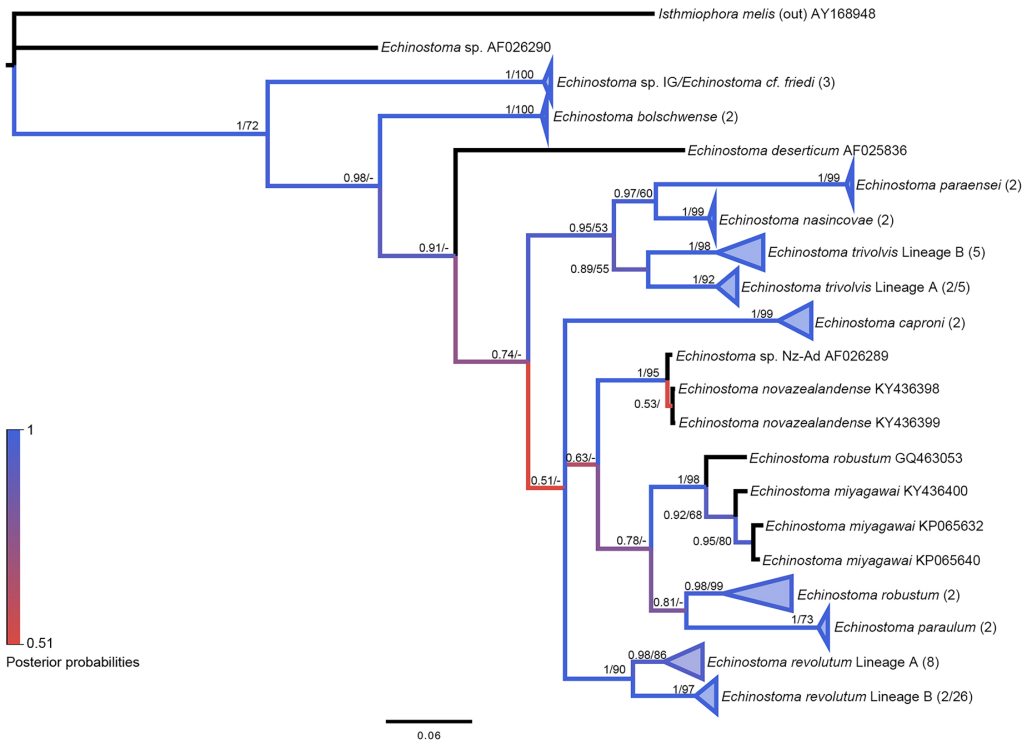

Figure 4.6: Molecular phylogeny of the Echinostomatidae based on *nad1*.Bayesian inference phylogenies are given. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Posterior probabilities >0.50 and bootstrap values >50 from BI and ML analyses are reported near the nodes respectively. Accession numbers are given after species names. Clades representing a single species have been condensed for space. Numbers in parentheses after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study.

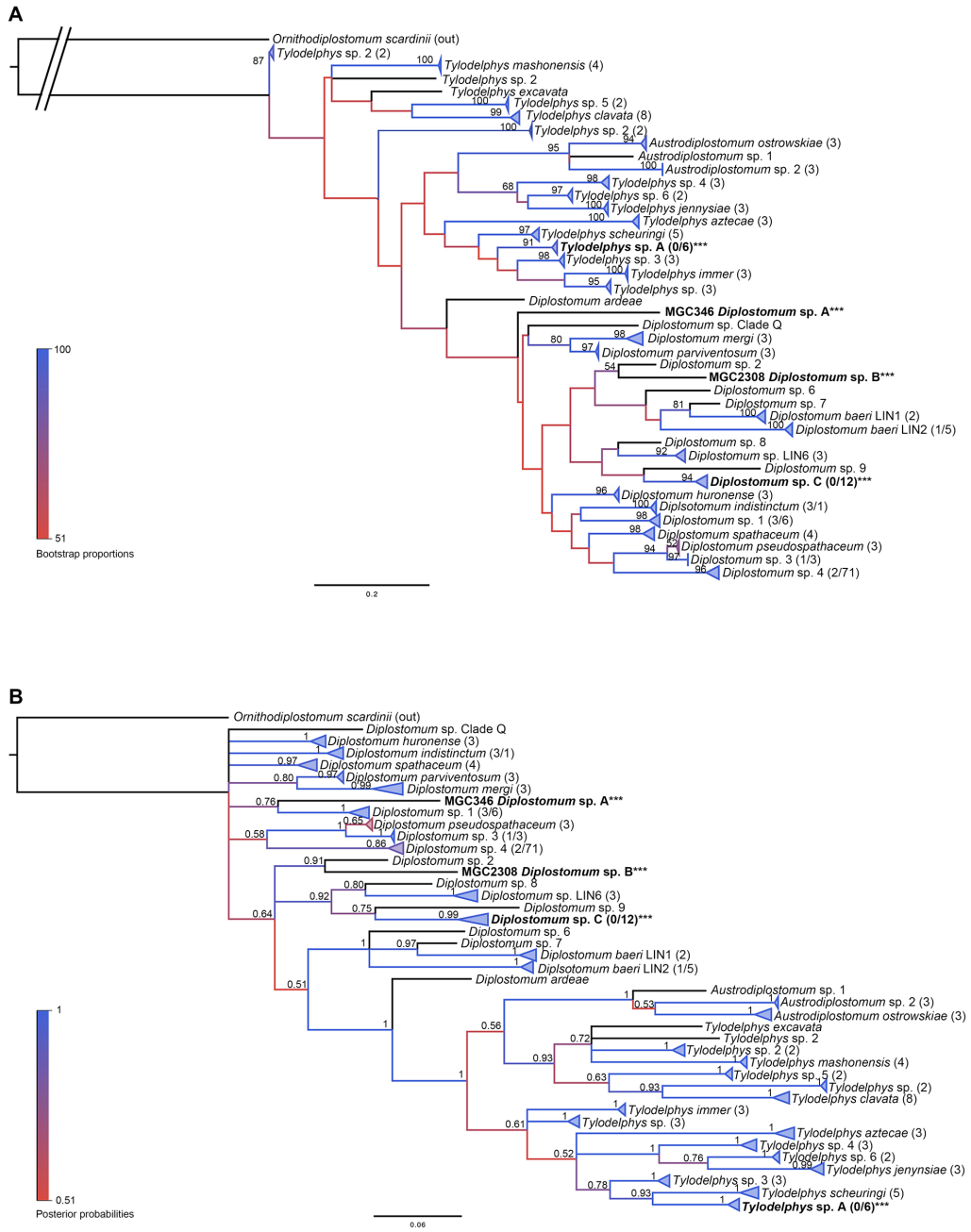

Figure 4.7: Molecular Phylogenies of the Diplostomidae-I Group based on *cox1*.

Figure 4.7 Molecular Phylogenies of the Diplostomidae-I Group based on *cox1*. Clades representing a single species have been condensed for space. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Bootstrap values >50 and posterior probabilities >0.50 are reported near the nodes. Numbers in brackets after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study. Emboldened taxa with three asterisks represent novel species from molecular analyses. A) Maximum-likelihood tree. B) Bayesian inference tree.

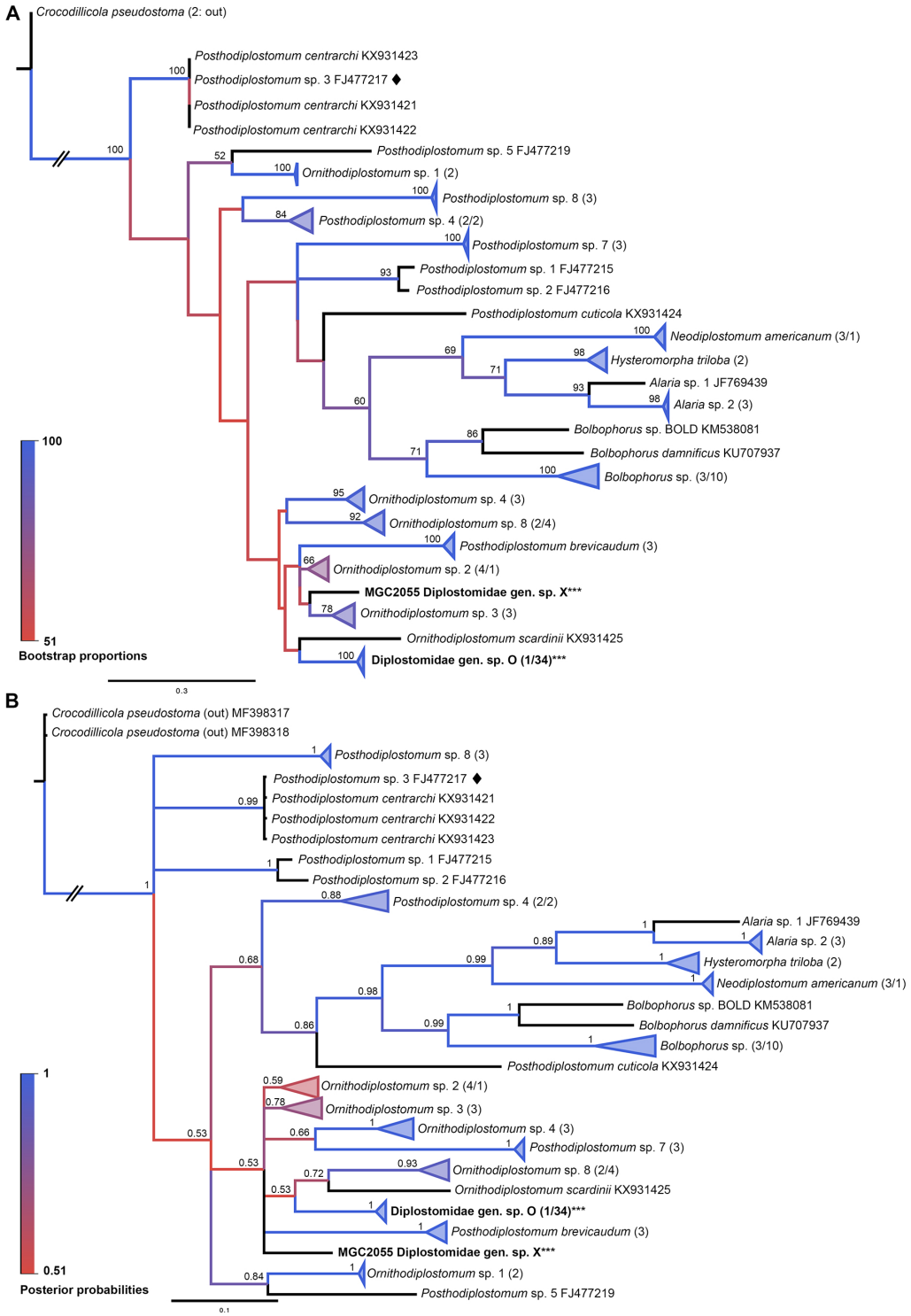

Figure 4.8: Molecular Phylogenies of the Diplostomidae-II Group based on *cox1*.

Figure 4.8 Molecular Phylogenies of the Diplostomidae-II Group based on *cox1*.Clades representing a single species have been condensed for space. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Bootstrap values >50 and posterior probabilities >0.50 are reported near the nodes. Numbers in parentheses after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study. Emboldened taxa with three asterisks represent novel species from molecular analyses. Black diamonds represent sequences identified uniquely in GenBank that have high similarity and likelihood of being the same as a different species. A) Maximum-likelihood tree. B) Bayesian inference tree.

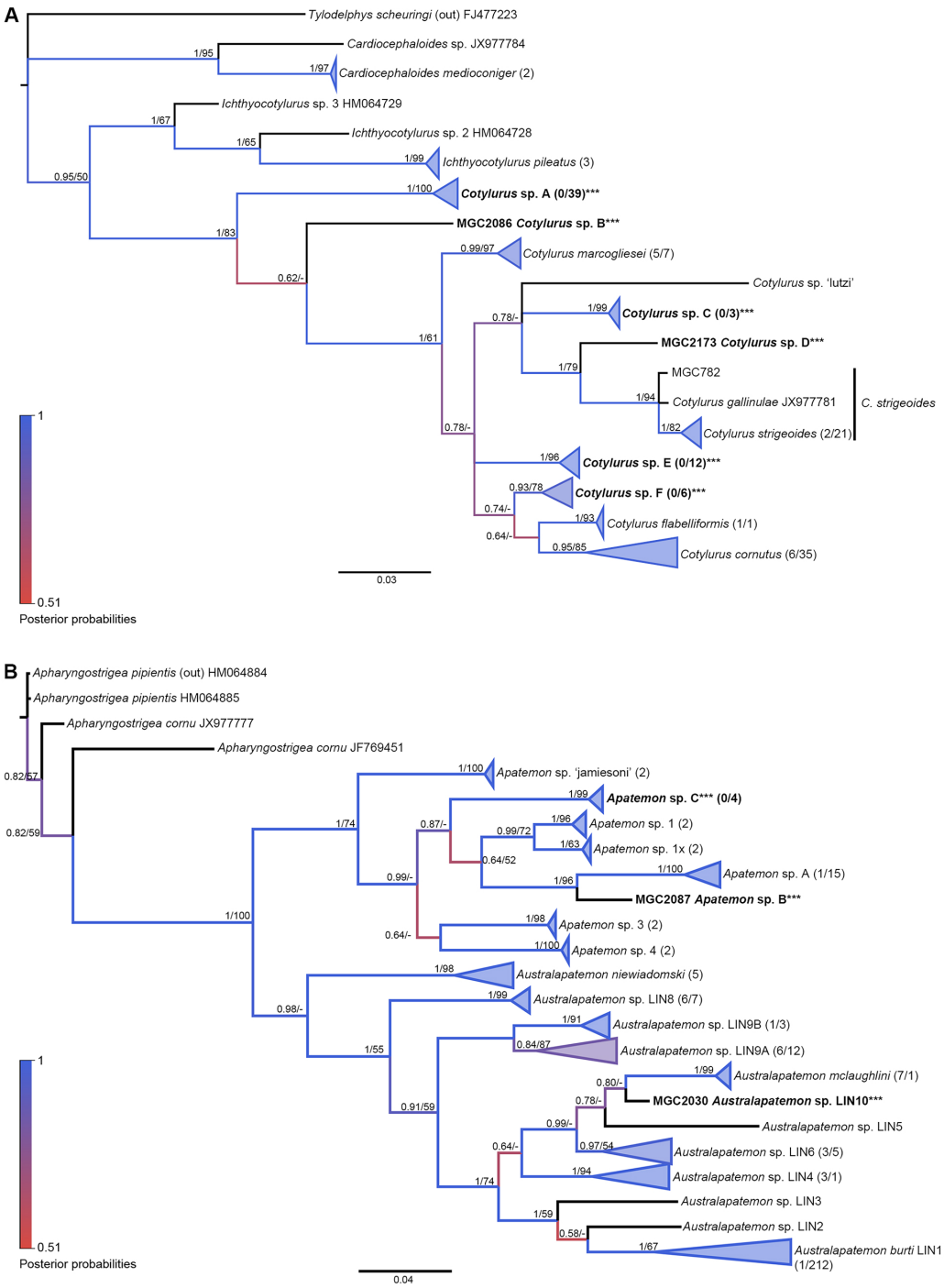

Figure 4.9: Molecular phylogenies of the Strigeidae based on *cox1*.

Figure 4.9 Molecular phylogenies of the Strigeidae based on *cox1*.Bayesian inference phylogenies are given. Clades representing a single species have been condensed for space. Branches are colored by support values from phylogenetic analyses, with blue having the highest support. Posterior probabilities >0.50 and bootstrap values >50 are reported near the nodes respectively. Accession numbers are given after species names. Numbers in parentheses after taxon names correspond to the number of sequences within the clade. The first number is number of GenBank sequences and the second number, if given, represents number of sequences from this study. Emboldened taxa with three asterisks represent novel species from molecular analyses. A) Strigeidae-I. B) Strigeidae-II.

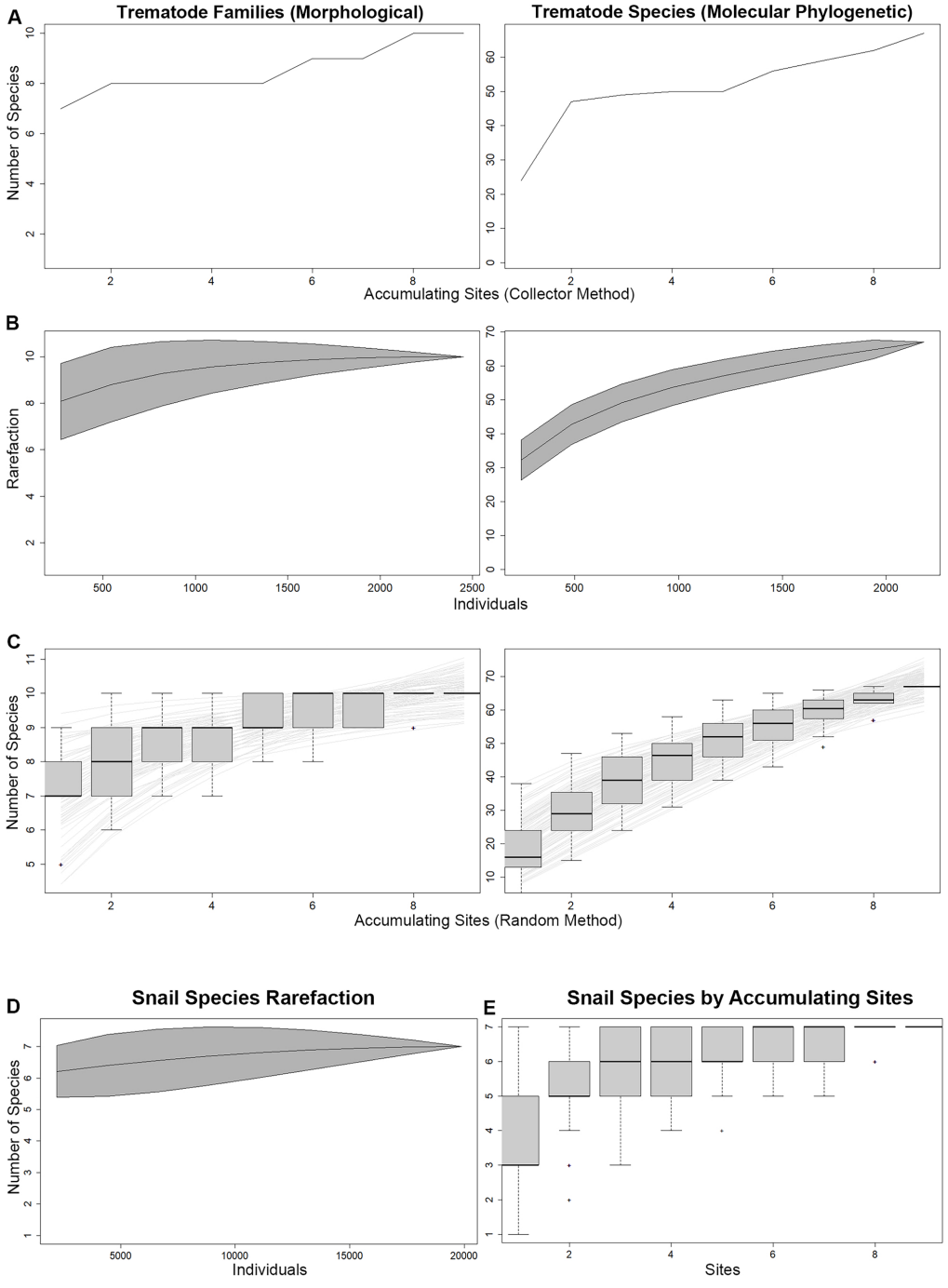

Figure 4.10: Species accumulation.

Figure 4.10 Species accumulation. Several methods confirm that if delineation confidence for species differences is based on morphological identification, a plateau is reached for the maximum number of species within the study area. Whereas if confidence is based on molecular phylogenetic methods, we have yet to attain the true diversity of trematode species within the study area. (a) Collector method for accumulating sites as in the dataset, (b) Rarefaction for number of individuals, (c) Random method for accumulating sites given as a boxplot, with non-linear Arrhenius model results displayed as lines behind the boxplot, (d) Rarefaction for number of individuals by snail species, (e) Random method for accumulating sites given as a boxplot for snail species.

Chapter 5

Environmental and ecological factors that drive trematode community assembly in central Alberta

5.1 Introduction

5.1.1 Preface

> "Biodiversity is the variety of life, including variation among genes, species and functional traits." (Cardinale et al. 2012)

There are several ways in which biodiversity is commonly measured: species richness, a measure of the number of unique species; community evenness, a measure of the equitability among species within a community or region; and heterogeneity, which is a measure of dissimilarity among species in any level of grouping (Cardinale et al. 2012). These measurements are typically given indices to more accurately describe comparisons among communities or populations of organisms. While there are many different indices to describe the three basic levels of diversity (α, β, γ), certain indices can better account for some features of diversity as opposed to others, for instance, the occurrence of rare species, or the heterogeneity of abundances among species (Gardener 2014).

Scale is what defines the three basic levels of diversity. At the lowest level, α diversity describes the local species pool, as the mean species diversity

within a specific habitat or site. The broadest level is γ diversity, as defining the regional pool of species among all habitats or sites. Though both levels provide important information about diversity within and among communities of species, they do not explain the variation among local assemblages. This is where β diversity is important, because it is the ratio of α to γ diversity and describes the change in species identities and abundances among local communities, often referred to as species turnover (Gardener 2014; Mori, Isbell, and Seidl 2018).

Many researchers have tried to best quantitate these scales through the development of diversity indices that incorporate species identities and abundances in a way that one could reliably form predictions. For instance, indices can be based on the level of uncertainty (Shannon index – H) (Shannon 1948) or the probability that two individuals, taken from the same sample at random, belong to the same species (Simpson's index) (Simpson 1949). The choice of scale for diversity and the index chosen to best describe this diversity and its connections to greater processes, like ecosystem functions, are at the core of community ecology research.

The field of community ecology was developed by plant sociologists, and the methods therein have rarely been adapted for use in parasitic systems. The field of parasite ecology has been expanding in scope over the past several decades, especially with "the rise of disease ecology" (Koprivnikar and P. T. J. Johnson 2016), necessitating a different perspective of how parasites function within ecosystems and communities. The methods used in community ecology are very general, meaning they can be applied to plants and animals, including parasites; they describe the interactions between species that make up a community, and how spatio-temporal differences stratify them or alter community structure (Gardener 2014). Spatio-temporal differences can be affected by the environment, including landscape gradients, anthropogenic impacts, and weather, among other variables.

The applications of community ecology to the study of pathogens has been used to better understand the dynamics of disease transmission between animals, and from animals to people, by examining the effects of ecological drift,

selection, and dispersal (reviewed in (Seabloom et al. 2015)). Though the analyses are unexhaustive, the aims of this chapter were to utilize several methods in community ecology to make assessments of trematode and snail diversity, better understand the variables that structure trematode component communities, and describe how their composition differs over time and between communities. This chapter lays the foundation for many future studies to further examine the interplay between host and parasite community dynamics, particularly within freshwater lakes of Alberta.

5.1.2 Background

Trematode larval communities can be analyzed at four levels of community structure: the infracommunity, component community, guild, and by compound community. The larval trematode infracommunity is composed of all species that infect an individual snail intermediate host (i.e. co-infections), and whose structuring forces are internal to the snail. At this community level, factors that drive structure consist of immunological compatibility between snail and trematode, interspecific interactions between trematodes, including direct and indirect antagonism and the resulting hierarchy of species based on competitive ability, and effects on snail host mortality, fecundity and behaviour that can alter further community differences. The next level of structure is the component community. This community type consists of all trematode species infecting all snail host species within a defined geographical space, consisting of a combination of all infracommunities within a host population. At this level of community, the factors acting as primary structuring forces are external to the snail, including various ecological (biotic) and environmental (abiotic) factors on both spatial and temporal scales. A guild, on the other hand, is an assemblage of trematode species among only one host species and can be viewed across the entire geographical distribution of that host species. The final defined community level for larval trematodes is the compound community, in that all local communities are drawn into a regional pool defined by distinct ecosystems (reviewed in (Albert O. Bush et al. 1997; Gerald W.

Esch and Fernandez 1994; G. Esch, Curtis, and Barger 2001; K. D. Lafferty, Sammond, and A. M. Kuris 1994; Sapp and Gerald W Esch 1994)).

Within the current literature, there is significant support for structured trematode communities, and that the forces acting to structure them consist of a combination of immunological, environmental, and ecological factors (Gerald W. Esch and Fernandez 1994; G. Esch, Curtis, and Barger 2001; K. D. Lafferty, Sammond, and A. M. Kuris 1994; Sapp and Gerald W Esch 1994). While the evidence continues to grow for the effects of different, individual factors on community structure, there is no specific set of features that define all communities.

Each major category of factors is engrained with its own set of variables that can range in their phenotypic plasticity. For example, looking at the infracommunity level, immunological factors determined to be important for snail resistance to trematode infection, and therefore compatibility, such as Fibrinogen Related Proteins (FREPs), may be more hard-wired at the genetic level, but can display expression-level plasticity upon challenge by different trematode species (reviewed in (Michelle A. Gordy, Pila, and Patrick C. Hanington 2015)). Some environmental factors, such as temperature, can be highly variable throughout the season, which may not have detrimental effects on snail mortality, as aquatic snails are noted as being incredibly resilient (G. Esch, Curtis, and Barger 2001), but may allow a trematode to establish within a host that is normally resistant, because of temperature-dependent immunological responses (Ittiprasert and Knight 2012).

From an ecological perspective, trematode component communities can portray differing levels of plasticity, depending on the hosts within the life cycles of their respective members, and the abilities of hosts to move across the landscape, termed 'host vagility'. Based on host vagility, there are two categories that trematodes fall within: autogenic trematodes, whom all hosts within their life cycle remain in one defined area, such as a lake, and allogenic trematodes, whom have some hosts within the life cycle that can travel outside of the defined space, such as migratory waterfowl. These host-based dynamics can contribute to spatio-temporal trends of trematode community structure,

showing both local and broad biogeographic patterns, as well as a partial role in the distribution of trematodes across the landscape (Brown et al. 2011; Gerard and Gérard 2001).

To analyze local transmission patterns of trematodes, we must first characterize their communities, to then test the effects of various combined factors on community structure and ultimately develop patterns of local transmission probabilities. Through the above examples, we know that interactions between host and parasite can occur from the molecular to the ecological level. We also know that environmental changes can affect these interactions. We can look at this complex system in a number of ways, but only by understanding local patterns of community structure dynamics, can we begin to assess transmission probabilities, and therefore, risk of human exposure to trematodes within the ecosystems we frequently interact.

Though an ideal community assessment would examine all parasite life cycle stages in all hosts, I focus on the trematode component community within snail intermediate hosts. There are several reasons to begin with the snail host, as discussed in previous chapters. Chiefly, these reasons boil down to feasibility for a short-term study; however, there are many reasons why future biodiversity studies may choose to approach the study of trematodes from their snail hosts as I have done; one being that to preserve biodiversity, sacrificing hosts to find parasites (i.e. adult trematode worms from within vertebrate definitive hosts) is an unideal solution. As molecular approaches to identifying species, and methods for isolating parasite material from hosts in a non-lethal way are further developed, it may be easier to assess infection within vertebrate hosts without the need to harm them in the future.

Parasites are neglected from most biodiversity surveys and ecological studies; yet, they are known as an important component of ecological processes, as organisms that form their own communities, and as effectors on the assembly of other communities within ecosystems. Parasites with complex life cycles, such as digenean trematode flatworms, utilize at least 2-3 species within an ecosystem to complete their development and generate infectious propagules, taking advantage of species networks for the success of their life cycles. De-

spite this knowledge, our understanding of the processes that determine their assembly into communities and that limit their distributions are rudimentary.

Recent advancements into the identification of trematode species through molecular barcoding of free-swimming larval stages have allowed us to examine longitudinal trends of community assembly through their presence and abundance. This chapter delves into the longitudinal changes in trematode diversity within component communities among their snail first intermediate hosts, host-parasite relationships, and host diversity influences on community dynamics, as well as the relative role that each species has in the community as a function of their presence and abundance. Concurrently, the role of the environment, in a spatio-temporal context, in structuring communities is also examined. This study illustrates the natural fluctuations of trematode communities over time. Highlighted within is the relative role of each species as a common or rare member of the community. We discuss the impacts their role could play in community dynamics, as indicators of environmental change, as well as our ability to model them.

Digenean trematodes, as with most parasites, are often overlooked in ecological studies; yet, their presence or absence may tell us more about ecosystem processes than by only examining interactions among their hosts, the larger and more visible species (Frainer et al. 2018; Hatcher and Dunn 2011; B. Sures et al. 2017). Digenean trematodes are parasitic flatworms that utilize snails as their first intermediate host for larval development, and a vertebrate as their definitive host. They most often live in aquatic ecosystems and take advantage of food webs, incorporating other vertebrates, invertebrates, and even plants as vehicles for transmission. Trematodes, therefore, have close ties with many other species within the environments they live, as parasites, as competitors, as prey, and as significant proportions of functional biomass in aquatic ecosystems (Hatcher and Dunn 2011; Preston et al. 2013; B. Sures et al. 2017).

Despite their noted importance in ecosystems, we know very little about trematodes in these roles compared to their role in disease transmission processes for species of medical and veterinary importance (i.e. *Schistosoma*

species that cause Schistosomiasis in Africa). Considering that snails in aquatic ecosystems are quite often infected with many different species of trematode (Michelle A Gordy and Patrick C Hanington 2019; Schwelm et al. 2018), it is prudent to recognize that factors affecting the assembly and structure of snail and trematode communities will indeed effect and explain trematode-related disease transmission patterns. However, to study trematodes for hypotheses surrounding the drivers of parasite community diversity, including factors like free-living biodiversity that are hypothesized to reduce transmission (P. T. Johnson, Preston, Hoverman, and LaFonte 2013; Ostfeld and Keesing 2000; Chelsea L. Wood and Kevin D. Lafferty 2013), we first must acquire an accurate representation of their diversity. The challenges associated with accurately representing the diversity of larval trematodes, as well as methods for addressing these challenges, have been discussed in previous chapters (see chapters 2–4).

To understand how trematode component communities fluctuate among their first-intermediate, snail hosts in northern lakes, there are many biotic and abiotic factors that can affect their presence, abundance, and successful continuation of their life cycles within a community that must be examined (D W Thieltges, K. T. Jensen, and Poulin 2008). In Northern latitudes, lake ecosystems are highly dynamic and strongly affected by seasonality. Spring and summer are peak production times for organisms in these ecosystems, and this has effects on growth and reproductive rates, which highly impacts fluctuations in population sizes and generational turnover (Conover 1992). There are both bottom-up and top-down processes affecting host population abundances, such as nutrient availability and food web dynamics (Kevin D Lafferty et al. 2008). There are both direct and indirect anthropogenic impacts that can affect host and trematode presence and abundance, for instance, the introduction of nutrients and pollutants into the ecosystem (Koprivnikar, Baker, and Forbes 2007; Pietrock and David J. Marcogliese 2003), land-use changes that affect species habitats, and recreational uses of the area, like hunting, that directly disturb or alter species populations. Additionally, many definitive hosts, in which the adult worms reside, are migratory species that

travel to northern latitudes for breeding during the spring and summer months. Migratory species behaviour, such as timing of arrival, nesting location, and movement patterns can highly impact community assembly dynamics through the variable input of trematode eggs into the ecosystem. Though not an exhaustive list, these are many of the factors that can possibly determine trends in trematode species richness, abundance, and distribution.

Other factors that may impact trematode component communities are the more specific relationships between trematodes and their hosts and to each other. Immunological compatibility with snail hosts is a key determinant of their distributions; as a highly vagile definitive host may be able to expand the possible distribution, the snail host limits the distribution in terms of continued life cycle success. Competition between trematode species for snail hosts (Keeney et al. 2008) and predation by other organisms (David W. Thieltges et al. 2013) are also key ecological drivers that affect the success of trematode life cycle continuation, and therefore component community richness and abundances. Though there is evidence for the potential of each of these factors to act as a structuring component to trematode communities, there is still much that we do not know in terms of the differences between local and regional versus large-scale processes.

The current study was developed to provide the necessary information for examining how local trematode component communities are structured and change over time in highly dynamic lake ecosystems. We wanted to gain a fine-scale understanding of potential interspecific interactions, timing of arrival, persistence of species in the community, and their roles as either dominant, common, or rare species. Furthermore, we wanted to know if trematode community structure was influenced by environmental factors such as water quality, anthropogenic use of the area, and geographical separation by landscape features.

We hypothesized that variables of water quality that fluctuate over the season, such as dissolved oxygen and temperature, would have the strongest effects on seasonal differences in trematode community composition as a function of their affects on the physiology of the snail intermediate host and the

free-swimming larval stages of different trematode species. We also hypothesized that the more static variables like salinity, pH, and variables of landscape, like anthropogenic use and ecoregion, would be predictors of spatial gradients for community composition as more of a determinant of host presence. The first step towards achieving this goal was to attain an accurate measure of their diversity. This was achieved through the results presented in chapter 4. The aims of this chapter were to examine differences between communities and how their diversity and composition changes over time and as a result of environmental and ecological factors. This study provides a fine-scale perspective of trematode diversity and utilizes multiple methods to test the impact of host relationships and environmental factors on community assembly to then broaden our perspective on their distributions and the factors involved in community structure.

5.2 Material and methods

5.2.1 Trematode and snail host associations

The longitudinal collections of trematode cercariae from snail, first-intermediate hosts in central Alberta were previously described (M. Gordy et al. 2016; Michelle A Gordy and Patrick C Hanington 2019) as were the methods used for species identifications through molecular and morphological assessments (Michelle A Gordy and Patrick C Hanington 2019; Michelle A Gordy, Sean A Locke, et al. 2017). The final dataset included 2,452 trematode samples that represented 79 trematode species identified from among infections found in five freshwater snail species. The collections took place from June to September 2013–2015, on a biweekly basis, across six lakes in central Alberta: Buffalo Lake, Gull Lake, Isle Lake, Lac La Nonne, Pigeon Lake, and Wabamun Lake.

5.2.2 Environmental data

Measurements of water quality were taken at each sample collection using a YSI Professional Plus multiparameter meter (Xylem, Inc.) for the following parameters: water temperature, salinity, pH, dissolved oxygen, and barometric

pressure. The meter was placed in the water at no less than two feet deep, directly in the collection area. Samples were taken at the same time as snail collections, described above.

Habitat-specific measurements included sediment type and anthropogenic use (site type). Sediment type was broadly characterized based on the relative size of the most dominant feature in the sample area (silt, sand, or pebble). Site type was categorized as either a boat launch or a beach for swimming.

Each lake was also characterized by its river basin and ecoregion. This was determined by using the most recent delineations from the Government of Alberta.

5.2.3 Calculations, statistical analyses, and modelling

Trematode component communities are defined here as all the trematode species found among all their snail first intermediate hosts within a defined geographical space (Albert O. Bush et al. 1997), being collection sites. Trematode component communities are derived from the subset of snail samples that contained patent trematode infections, while snail community data was calculated from all snails in each collection, including uninfected snails. Because very few samples came from Pigeon Lake, these were not included in diversity analyses beyond richness calculations.

The following packages were utilized in R v. 3.4.3 (R Core Team 2017) to calculate diversity metrics and plot them: *vegan* (Oksanen et al. 2018), *BiodiversityR* (Kindt and Coe 2005), *dplyr* (Wickham et al. 2017), *ggpubr* (Kassambara 2017), and *ggplot2* (Wilkinson 2011). Species richness was derived using the diversityresult (*vegan*) command to add unique species by site as well as pooled species richness for all sites.

To examine how species diversity of trematode component communities changed over time, each unique combination of collection site and date were considered as separate sample units and assigned a unique number. Both alpha diversity (α) (*vegan*:diversity) and effective species ($\exp(H)$) were calculated from normalized species abundance data for each sample, using the Shannon

index (H). Community evenness was calculated as $\frac{EffectiveSpecies}{SpeciesRichness}$. These metrics were completed for all sites.

For each lake, a Spearman rank correlation was used to test the relationship between snail and trematode richness and snail and trematode effective species. This correlation test was repeated for the three sites within Buffalo Lake for effective species and pooled richness for all sites.

When comparing communities to each other, a shortened dataset was used that only included lakes from which were sampled all three years: Buffalo, Gull, Isle, and Wabamun. Because Buffalo Lake had three sites, these were all included separately rather than pooled so as not to inflate the results. For these six sites, α diversity was based on Shannon entropy ($\exp(H)$) and calculated as described above. Gamma diversity (γ) was calculated as the pooled Shannon entropy for each site by using the diversitycomp (*vegan*) command, of which the exponent was taken for the calculation of effective species. Beta diversity (β) was then calculated based on effective species as $\frac{\exp(\gamma)}{\exp(\alpha)}$. These indices were also calculated in the same manner for different groupings of these sites, based on attributes of landscape features (ecoregion and river basin) and the site type based on anthropogenic use (beach or boat launch).

Multivariate homogeneity of group dispersion was used to explore β diversity among the four groupings (site, site type, ecoregion, and river basin). The vegdist (*vegan*) command was used to generate a Bray-Curtis dissimilarity matrix based on normalized (*vegan*::decostand) abundance data. This dissimilarity matrix was then used in the multivariate dispersion model (*vegan*::betadisper), the results of which were reduced into principle coordinates to assess the differences in two-dimensional space. A permutation test was then run on the dispersion model result using the command permutest (*vegan*) and run for 999 permutations. An ANOVA test was used on the results of the model to look for any significant differences in group dispersion. A post-hoc Tukey's Honest Significant Different (Tukey HSD) test was then used to examine pairwise differences.

In contrast to group dispersion, we also calculated community overlap in species composition among the four grouping levels. Overlap was analyzed in

terms of effective species and using the following formula

$$\frac{\frac{\alpha}{\gamma-\frac{1}{N}}}{1-\frac{1}{N}} \tag{5.1}$$

where N is the number of communities, or separations within each grouping. An analysis of similarity was also conducted by comparing the dissimilarities within and between groups, using a permutation test via anosim (*vegan*) and run for 999 permutations.

Rank-abundance dominance (RAD) models were used to analyze species' dominance in each community. The radfit (*vegan*) command was used to find the best model based on Akaike Criterion Information (AIC) values. The best fit model was then applied to each individual site. Rényi entropy was used (*BiodiversityR*::renyiresult) to analyze the role of rare species in communities.

To test whether environmental variables could be predictors for trematode species composition (relative abundance), we first utilized an ordination method, canonical correspondence analysis (CCA) (*vegan*::cca), to constrain the sample-based species composition data by environmental factors. We used model-building methods within the CCA to find the best-fit model, of which AIC values determined variables to be included, sequentially, via a permutation test (*vegan*:: add1, test = "permutation"). The variables tested were water temperature, month (as a proxy for seasonality, as it can be associated with either relative average air temperature or life cycle timing), salinity (ppt), dissolved oxygen (ppm), pH, barometric pressure (pKa), micro-sediment type (silt, sand, pebble), Latitude, Longitude, trophic status of the lake (Eutrophic or Hypereutrophic), river basin (North Saskatchewan or Red Deer), and ecoregion (Aspen Parkland, Boreal Forrest, or Mixed).

Because CCA is not considered a traditional hypothesis testing method, we then tested the same environmental variables as fixed effects in a Generalized Linear Mixed Model with a multinomial distribution and generalized logit link function in ®IBM ®SPSS Statistics Premium version 24. The response variable used in all models was trematode species, after reducing the dataset to the top 5 most abundant species (*Australapatemon burti* LIN1, *Diplostomum* sp. 4, *Echinoparyphium* sp. Lineage 2, *Notocotylus* sp. A, and *Plagiorchis* sp.).

Random effects in the model were year and collection site. For all predictor variables, at first, each predictor was tested separately for significance and then combined into a full model based on those that were statistically reasonable to include. From there, the full model was paired down by removing insignificant terms, one at a time, until only significant predictors remained. The final model was run with the response variable in both ascending and descending order to retrieve specific effects on the intercept, as an intercept-only model is not an option in SPSS.

Finally, we wanted to determine if any trematode species could be considered as indicator species, given specific environmental variables as determined by the previous analyses. An indicator species would be one that is positively associated with one habitat or environmental feature and negatively associated with another (in the same category), with substantial abundances. For this analysis, we specifically looked at the categorical variables ecoregion, trophic status, and month. The determination of an indicator species is by examining the Pearson residuals of a chi-squared test (*stats*::chisq.test), with significance at or above ± 2.

5.3 Results

5.3.1 Host-Parasite relationships

The relationship between pooled snail host and trematode richness for all sites was positively correlated (r = 0.67, $P = 0.046$) (Figure 5.1A). Sample-based (separate data) richness among five lakes (not including Pigeon Lake) revealed a slightly positive correlation between snail and trematode for Buffalo (r = 0.32, $P < 0.001$), Gull (r = 0.22, $P < 0.001$), and Lac La Nonne (r = 0.37, $P < 0.001$), whereas a negative correlation was found for Isle (r = -0.53, $P < 0.001$) and Wabamun (r = -0.22, $P < 0.001$) lakes (Figure 5.1B). However, effective species, a more accurate measurement of diversity than richness, showed a more neutral relationship (Buffalo: r = 0.084, $P = 0.033$; Lac La Nonne: r = 0.17, $P < 0.001$; Isle: r = -0.27, $P < 0.001$; Wabamun: r = -0.046, $P = 0.48$) or a change in direction (Gull: r = -0.16, $P < 0.001$) (Figure 5.1C). In terms

of effective species, the only strong correlation between snail and trematode diversity was found at The Narrows site at Buffalo Lake (r = 0.71, P <0.001) (Figure 5.1D).

5.3.2 Community metrics

Community evenness, a measure of species' abundance homogeneity, was highest at Lac La Nonne Site #2 (e = 0.402), followed by Wabamun Lake (e = 0.355), Buffalo Lake – The Narrows (e = 0.323), Lac La Nonne Site #1 (e = 0.306), Isle Lake (e = 0.286), Buffalo Lake – Rochon Sands (e = 0.263), Gull Lake (e = 0.208), and finally Buffalo Lake – Pelican Point (e = 0.139). Overall, however, all the communities exhibited generally low evenness. The lack of community evenness is no doubt due to both the high levels of rare species found in all communities (although more so in the southern lakes: Gull and Buffalo) and the presence of a few highly dominant species that stay in the community for longer periods of time (Figure 5.2 & Figures 5.3 – 5.9 Panels B-C).

Viewing these communities from their more natural, dynamic perspective, we can see that diversity fluctuates over time and that there is no strong, consistent pattern from year to year. Although, in each community, there do seem to be peaks in trematode richness (and α diversity) primarily in July and August. Snail richness does fluctuate throughout the season but is less extreme in comparison to trematode richness. Trematode community evenness, or lack thereof, fluctuates with changes in α diversity, as expected because of how it is calculated (effective species / species richness), but very little overall (Figures 5.3 – 5.9 Panels A).

Analysis of homogeneity of variance using Bray-Curtis dissimilarities showed that the effect of site on β diversity was significant ($F(5,93) = 5.631$, P <0.001), specifically, Pelican Point at Buffalo Lake had a significantly lower effect size in all comparisons (Tukey: PP-Gull: $P = 0.050$, PP-Isle: P <0.001, PP-Wab: P <0.001, PP-RS: $P = 0.004$, PP-TN: $P = 0.015$) (Figure 5.10), whereas all other site comparisons were not significantly different.

Grouped by geographical and anthropogenic distinctions tested, there was a significant effect of ecoregion on β diversity (F(2,96) = 4.589, P = 0.012), specifically between Aspen Parkland and Boreal Forest (effect size = 0.165, P = 0.011, CI: [0.0316, 0.298]), a significant effect of river basin (F(1,97) = 8.3205, P = 0.005; effect size = -0.15, CI: [-0.2533, -0.0468]), but no significant effect of site type (beach or boat launch) on β diversity (F(1,97) = 2.9557, P = 0.089) (Table 5.1 & Figure 5.11).

Analysis of similarity by comparing within and between group dissimilarity ranks also confirmed that the species composition is significantly dissimilar by sites (R = 0.2581, P = 0.001). Species overlap among sites was 52.7% (in terms of Shannon diversity) and 54.7% by ecoregion. By river basin, the overlap is 72.2%, and the highest overlap was found between site types at 84.8%.

5.3.3 Effects of environment on community structure and assembly

Of all the environmental variables tested, only trophic status of the lake and landscape variables ecoregion and latitude were significant within the CCA model and together explained 22.9% of the variance in sample-based, trematode community composition (Overall: F(4) = 7.139, P = 0.001; trophic: F(1) = 13.209, P = 0.001; ecoregion: F(2) = 6.299, P = 0.001; latitude: F(1) = 2.748, P = 0.007, permutation test of 999 permutations) (Figure 5.12).

However, when examining the effects of environmental factors as predictors upon the five most abundant trematode species, as opposed to community composition, trophic status is no longer a significant predictor (GLMM: F(4) = 1.742, P = 0.138). As well, in the final significant model (Trematode species $\sim$ Month + Ecoregion + Latitude + Dissolved Oxygen + (1|Year) + (1|Site)), dissolved oxygen and month are additional significant predictors to those also found in the CCA (GLMM (full model): F(28, 945) = 8.006, P <0.001).

Latitude was a specific predictor associated with all most abundant species except *Echinoparyphium* sp. Lineage 2 (*Australapatemon burti* LIN1: coeff. = 42.853, P <0.001; *Diplostomum* sp. 4: coeff. = -10.761, P = 0.013; *Notocotylus* sp. A: coeff. = 11.348, P = 0.034 for reference category *Plagiorchis* sp.

& with reference *A. burti* LIN1, *Plagiorchis* sp.: coeff. = -42.766, P <0.001). On average, *Echinoparyphium* sp. LIN2 and *Plagiorchis* sp. were found at lower latitudes than *A. burti* LIN1, *Diplostomum* sp. 4, and *Notocotylus* sp. A.

Specific effects of ecoregion were found to be associated with all most-abundant species (*A. burti* LIN1: Boreal Forest, coeff. = -48.264, P <0.001; *Diplostomum* sp. 4: Boreal Forest, coeff. = 13.720, P = 0.005; *Echinoparyphium* sp. LIN2: Aspen Parkland, coeff. = -2.646, P <0.001; *Notocotylus* sp. A: Aspen Parkland, coeff. = -1.773, P = 0.020; *Plagiorchis* sp.: Boreal Forest, coeff. = 48.121, P <0.001).

The month of August was a specific, positive predictor for the presence of *A. burti* LIN1 (coeff. = 2.800, P = 0.009) as opposed to the presence of *Plagiorchis* sp. (coeff. = -2.895, P = 0.007) and *Diplostomum* sp. 4 (coeff. = -2.767, P = 0.030). Month had no other specific, significant associations with the other species.

A. burti LIN1 was found at lower dissolved oxygen environments on average (coeff. = -0.149, P <0.001), in comparison to *Plagiorchis* sp. (coeff. = 0.152, P <0.001), *Notocotylus* sp. A (coeff. = 0.129, P = 0.003), *Echinoparyphium* sp. LIN2 (coeff. = 0.198, P = 0.010), and *Diplostomum* sp. 4 (coeff. = 0.164, P <0.001).

As trophic status and ecoregion were the strongest predictors for trematode community composition, we chose these as habitat feature variables for the indicator species analysis. From this analysis, several species were significantly and positively associated with one habitat feature and negatively with the other; but if we limit the abundances for each significant species to at least 50 occurrences, our greatest indicator species is *A. burti* LIN1. For both variables, this species was very positively associated with hypereutrophic lakes in the Boreal Forest and very negatively associated with eutrophic lakes in the Aspen Parkland ecoregion. Considering just one variable, we also see that *Plagiorchis* sp. is very strongly positively associated with eutrophic versus hypereutrophic lakes, and *Notocotylus* sp. A is found positively associated with Mixed and Boreal Forest ecoregions and negatively associated with As-

pen Parkland (Figure 5.13A – B). Considering that month was a significant predictor in the GLMM, and that indicator species are only such if they are present, we ran the same Chi-squared analysis as for the indicator species but using month. *A. burti* LIN1 was the only species to have a strong association from this analysis with month. Confirming GLMM specific effects, *A. burti* LIN1 is highly and positively associated with the month of August and negatively associated with June, July, and September (Figure 5.13C).

5.4 Discussion

In 2016, Gordy, et al., reported a strong, positive relationship between snail host richness and trematode richness, supporting the "host diversity begets parasite diversity" hypothesis (M. Gordy et al. 2016; Hechinger and Kevin D Lafferty 2005). In repeating that correlation, now with more data, we have come across a few interesting findings. First, if we pool the richness data for all sites, we continue to see a positive relationship between host and parasite, though the degree of the relationship is smaller than our previous assessment (M. Gordy et al. 2016). This difference is likely explained by an increase in sampling and more accurate species differentiations (Michelle A Gordy and Patrick C Hanington 2019). However, if we instead use non-pooled data, and allow for within-lake variability, some lakes continue to exhibit a positive, although weaker, correlation, and some lakes have a negative correlation in richness. Specifically, Wabamun and Isle lakes exhibit negative correlations. Despite having relatively high snail and trematode richness, the overall relationship would imply that trematode richness increases with declining snail host richness. This would imply that there are lake-to-lake differences and other factors contributing to the establishment of these host-parasite relationships. One hypothesis that may be derived from this is that greater snail richness may present more decoy hosts in the environment, lowering the success rate for parasite establishment within its correct host.

If instead of richness, we examine the relationship between parasite and host in terms of effective species, that is the number of equally common species,

the correlation becomes neutral, hovering around zero and implying no relationship between host and parasite diversity. Rather, implying species' abundance is impacting the diversity relationship. In other words, these communities are composed of many rare species that are driving the positive correlation in richness. However, at one sampling site, The Narrows at Buffalo Lake, there was a strong, positive correlation between host and parasite effective species. This result suggests strong, site-specific variability across the dataset, and that rare species are having less of an effect, mathematically, on the diversity relationship. This community, of our entire dataset, is the only one to support the host diversity begets parasite diversity hypothesis, at the most basic level.

Our interpretation is that, while it is logical that more host species afford greater colonization ability and therefore greater potential parasite diversity (Hechinger and Kevin D Lafferty 2005), the presence of a few, highly susceptible, host species in a community can also afford greater parasite diversity. Snails are therefore acting as biotic filters for the regional pool of trematode diversity and life cycle success, and therefore, filters of the potential for their colonization into the community (Combes and Théron 2000). For parasites with complex life cycles, we can consider the definitive hosts to bring the initial infectious propagules from the regional pool of trematode species, acting as the first biotic filter. Although we do not have definitive host diversity information at this time, one can hypothesize that a greater diversity of parasites within a greater diversity within the definitive host population would still be limited in their dispersal abilities by the amount of available diversity, but more importantly the susceptibility, of intermediate hosts in a location, because of their important role in maintaining the life cycle. If the filtering species are more susceptible to infection, there will be greater trematode diversity than if they are less susceptible to infection (Figure 5.14). For instance, a couple snail species were found in collections (Unidentified Planorbid and *Valvata tricarinata*) but never patently infected with trematodes, suggesting they may be stronger biotic filters. If a combination of greater host diversity and susceptibility is observed, then we predict there should be greater overall trematode diversity within the local species pool.

This study has revealed that each community is composed of a few highly abundant trematode species that also happen to be common and consistent within the community, while the rest of the community is composed of many rare and inconsistent trematode species. This pattern connects well with the observed, low community evenness, in other words, the heterogeneous nature of species presence and abundance. While community evenness fluctuated very little, the observed diversity of both trematode larvae and snails fluctuated significantly over time, snail hosts less dramatically than trematodes. Among all sites, peaks in diversity were observed typically around July and August. Though not specifically tested within this study, we would hypothesize that these peaks are likely driven by intermediate host population dynamics (birth rates, timing, and time of infectious potential, assuming an age-based immunity profile) and the length of time for trematode development within the snail host, which may be altered by environmental variables.

The most common and abundant species in all the sites sampled was *Plagiorchis* sp. Overall, there was strong statistical support for nine different species of *Plagiorchis* represented among our sequenced samples (Michelle A Gordy and Patrick C Hanington 2019); however, because not all *Plagiorchis* samples were sequenced, we could not assign them to their unique species groups. Further work will no doubt clarify the true diversity of *Plagiorchis* spp. in these ecosystems, and we would not be surprised if there were more species represented. If there are nine or more species of *Plagiorchis*, the dynamic of rare vs. common species in community assembly would likely be very different. Depending on the level of diversity, all species involved in these communities could be considered rare. While their collective dominance is very high, their individual dominance ranks may be more variable.

Although we do not know the specific definitive host species for the trematodes in this study, based on the typical host use of congeners, the majority of definitive hosts are expected to be migratory waterfowl. For *Plagiorchis* sp., however, they are known to infect mammals and birds, including passerines (Harvey D. Blankespoor 1974). It is possible that their life cycles are not dependent on the timing of migratory species arrival, and they are able to es-

tablish within the snail hosts sooner. Another aspect of their life history that could drive their abundance is that after eggs are deposited in the environment by the definitive host, the snail consumes them (Zakikhani and Manfred E. Rau 1999), as opposed to every other genera discovered in these communities, in which swimming miracidia actively penetrate snail tissue (Schell 1985). This difference in the mode of infection may actually increase the chances for snails to become infected by *Plagiorchis*. Based on these differences in life history aspects, and the very low rate of co-infections found in these populations (M. Gordy et al. 2016), it is possible that *Plagiorchis* sp. infections may preclude those relying on migratory species' arrival times, enhancing their success and limiting the success of others. Indeed, this is an important avenue for future research.

In addition to *Plagiorchis* sp., four other trematode species were highly abundant: *Australapatemon burti* LIN1, *Diplostomum* sp. 4, *Echinoparyphium* sp. LIN2, and *Notocotylus* sp. A. The environmental factors latitude, ecoregion, month, and dissolved oxygen were strong overall predictors for the presence of these five species. As specific predictors, there was a contrasting trend between *A. burti* LIN1 and all other species. For instance, we found that dissolved oxygen and the month of August were significant, specific predictors associated with *A. burti* LIN1 presence, although the opposite trend was true of all other highly abundant trematode species. Likewise, indicator analyses consistently indicated *A. burti* LIN1 as being very strongly associated with particular environmental variables. Because of this, *A. burti* LIN1, may provide us with an avenue for specific, future research towards understanding its role in nutrient-rich, hypereutrophic, lakes in the Boreal Forest ecoregion of Alberta. *A. burti* LIN1 was commonly found among these lakes, and in high abundances. Because of this, *A. burti* LIN1 may have a role as an indicator species in Alberta; however, we have much to learn about their life cycle and definitive host associations (Michelle A Gordy, Sean A Locke, et al. 2017) that would more specifically relate to their presence and abundance in Alberta.

Considering *A. burti* LIN1 is mostly found in hypereutrophic lakes, there could be an interaction occurring between temperature during the season

(likely in August), and algal communities that potentially impact the levels of dissolved oxygen in the lake. Increased nutrient levels in lakes can cause algal biomass growth rates to increase, which can both increase food levels (and initial DO) for many aquatic invertebrates, like snails, but can also cause a dramatic decrease in the amount of dissolved oxygen in the water as bacterial decomposition spikes (Hem 1985). Depending on the tolerance of a particular organism to dissolved oxygen availability, this could have significant effects on mortality rates (Misra2016). Anoxic conditions have been indicated as having differential effects on snail mortality in infected snails in salt marsh ecosystems (Sousa and Gleason 1989). Interestingly, the exact same snail host species can react very differently to stress depending on the trematode that it is infected with (Bates et al. 2011; Koprivnikar and Walker 2011). In freshwater ecosystems, it has been noted that most gastropods are unable to withstand anaerobic conditions for more than 48 hours (Pennak 1978). However, pulmonate gastropods will surface for air, perhaps eliminating some of the negative effects of low dissolved oxygen. From the perspective of trematodes, it appears as if *A. burti* LIN1 may be more tolerant to lower dissolved oxygen than the other most highly abundant trematode species. If this is true, it may explain trends in their abundance and persistence.

When considering environmental effects on trematode component community composition, we found that, contrary to our hypothesis, the environmental variables that fluctuated the most over the season, and in comparison, across sites, had no impact on trematode relative abundances. Rather, the greater landscape divisions of ecoregion and the levels of relative nutrients by trophic status were the strongest predictors of community composition. This finding was in line with our hypothesis for greater community compositional differences.

Spatio-temporal differences in species composition, or β diversity, on the other hand were found to significantly differ within divisions of the data by site, river basin, and ecoregion, but there was no significant effect from anthropogenic use of the site (beach or boat launch). Specifically, the site Pelican Point at Buffalo Lake experienced the lowest rate in species turnover com-

pared to all other sites. One possible explanation for this difference could be that the snails found at Pelican Point were almost always found in small, dug-out pools that had collected on the shore of the lake, where contact with the primary lake water was infrequent, except during storms or disturbances of the lake shore. This reduced exposure to the main lake could have multiple effects on the overall assembly process that might improve trematode success in infecting snail hosts, but also be more limiting in exposure to the correct snail host. As well, if the life cycles are dependent upon other hosts, such as fish, the cercariae are likely not reaching these hosts because of the physical barrier to the main lake water, and thus not continuing the life cycles in this area. All of these factors could work to reduce the turnover of species in the community.

Taken together, we can conclude that there is a considerable level of variability within and among trematode component communities, whether examining individual species, their composition into communities over time or geography, or the dynamic nature of their diversity. Having a clearer understanding of their community ecology is important for setting an information baseline for lake managers that may have concerns about greater biodiversity, effects on lake stock, and public health impacts that may affect lake use, like the possibility of contracting cercarial dermatitis, a.k.a. "swimmer's itch" from avian schistosome trematodes. While this study provides some baseline information, there is much work to be done to better connect host-parasite relationships and better understand their distributions.

5.5 Conclusions

While the more common species like *Plagiorchis* sp. and *A. burti* LIN1 may have significant roles in communities simply because of their abundance, there is not much that can be said about the rare species. Rare species were not able to be included in some statistical models because there simply were not enough data points. Thus, at this point, uncovering any trends from which to predict their appearance in the community is not possible. One unfortunate

circumstance was that all schistosome species were rarely present within their communities. This result was unanticipated considering the many swimmer's itch reports received at the lakes from which snails were sampled.

In the next chapter, I will explore the issue of swimmer's itch in Canada, with a special focus on Alberta. Despite being unable to derive any species-specific predictions for their occurrences, we have taken more of a holistic approach to their study, such that we have established a baseline from which we can build upon with future studies. This chapter reveals important insights into both the biological aspect of swimmer's itch transmission, but also the human perspective from swimmers who have contracted the rash.

Table 5.1: Summary of diversity indices over landscape variables.

Lake	**Site**	**Richness**	**(mean ES)**	**(pooled ES)**	β
Buffalo	Pelican Point (PP)	16	1.77	2.43	1.37
	Rochon Sands (RS)	13	1.49	4.49	3.01
	The Narrows (TN)	13	1.93	5.8	3
Gull	Gull	24	2.75	6.56	2.38
Isle	Isle	38	3.82	10.2	2.67
Wabamun	Wabamun (Wab)	27	3.02	10.45	3.46
		River basin			
	PP	Red Deer	1.97	6.42	3.25
	RS	Red Deer	1.97	6.42	3.25
	TN	Red Deer	1.97	6.42	3.25
	Gull	Red Deer	1.97	6.42	3.25
	Isle	North Saskatchewan	3.41	12.67	3.71
	Wab	North Saskatchewan	3.41	12.67	3.71
		Ecoregion			
	PP	Aspen Parkland	1.74	5.41	3.11
	RS	Aspen Parkland	1.74	5.41	3.11
	TN	Aspen Parkland	1.74	5.41	3.11
	Gull	Mixed	2.75	6.56	2.38
	Isle	Boreal Forest	3.42	12.67	3.71
	Wab	Boreal Forest	3.42	12.67	3.71
		Site type			
	PP	Beach	2.24	7.76	3.47
	RS	Beach	2.24	7.76	3.47
	TN	Boat Launch	2.88	10.9	3.78
	Gull	Beach	2.24	7.76	3.47
	Isle	Boat Launch	2.88	10.9	3.78
	Wab	Beach	2.24	7.76	3.47

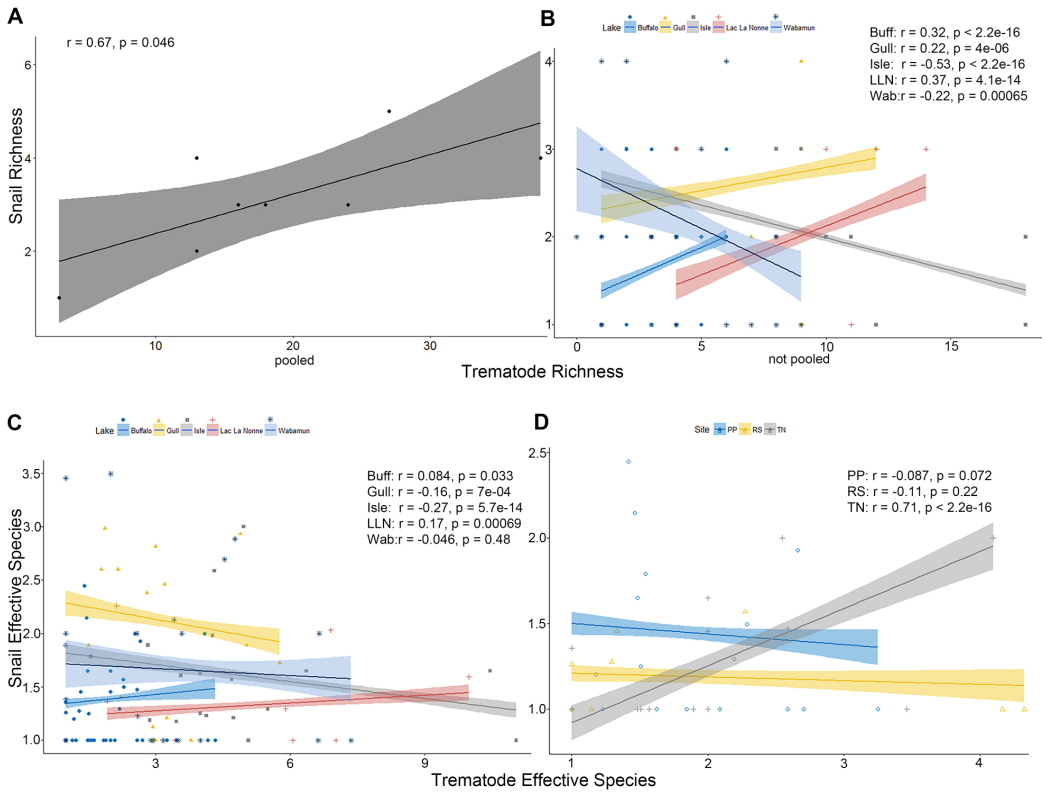

Figure 5.1: Host-Parasite diversity correlations. Spearman rank correlations of A) snail and trematode richness, pooled by site, B) non-pooled, sample-based, snail and trematode richness, C) snail and trematode effective species based on Shannon index for all lakes, and D) effectives species by each site at Buffalo Lake. PP = Pelican Point, RS = Rochon Sands, TN = The Narrows.

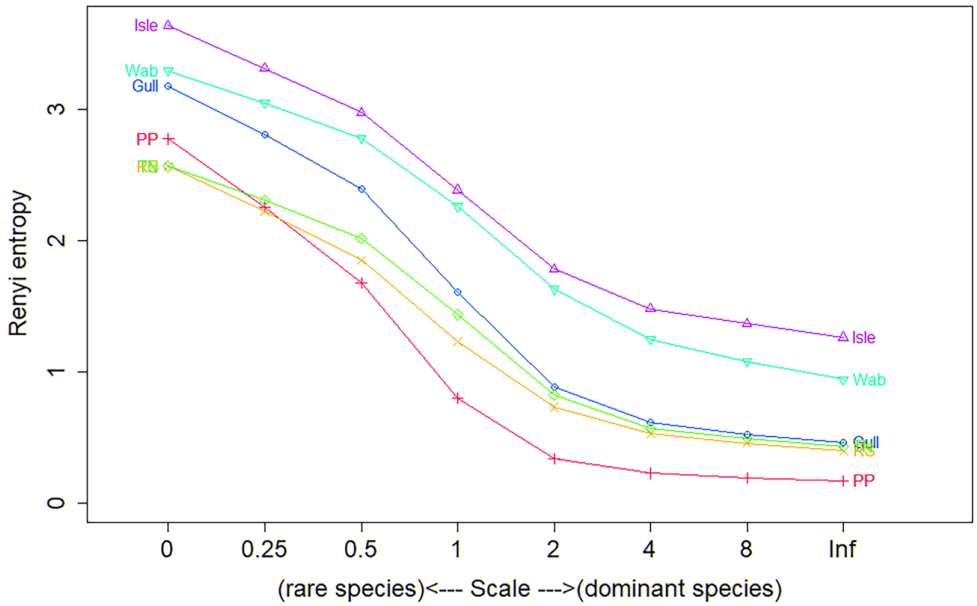

Figure 5.2: Rényi entropy plot for all sites. The Rényi scale represents species richness at 0, effective species ($\exp(H)$) at 1, inverse of Simpson's index at 2, and inverse of the Berger-Parker dominance at infinity. Rare species are given less prominence as the scale advances, and therefore are given a lower value for Rényi entropy.

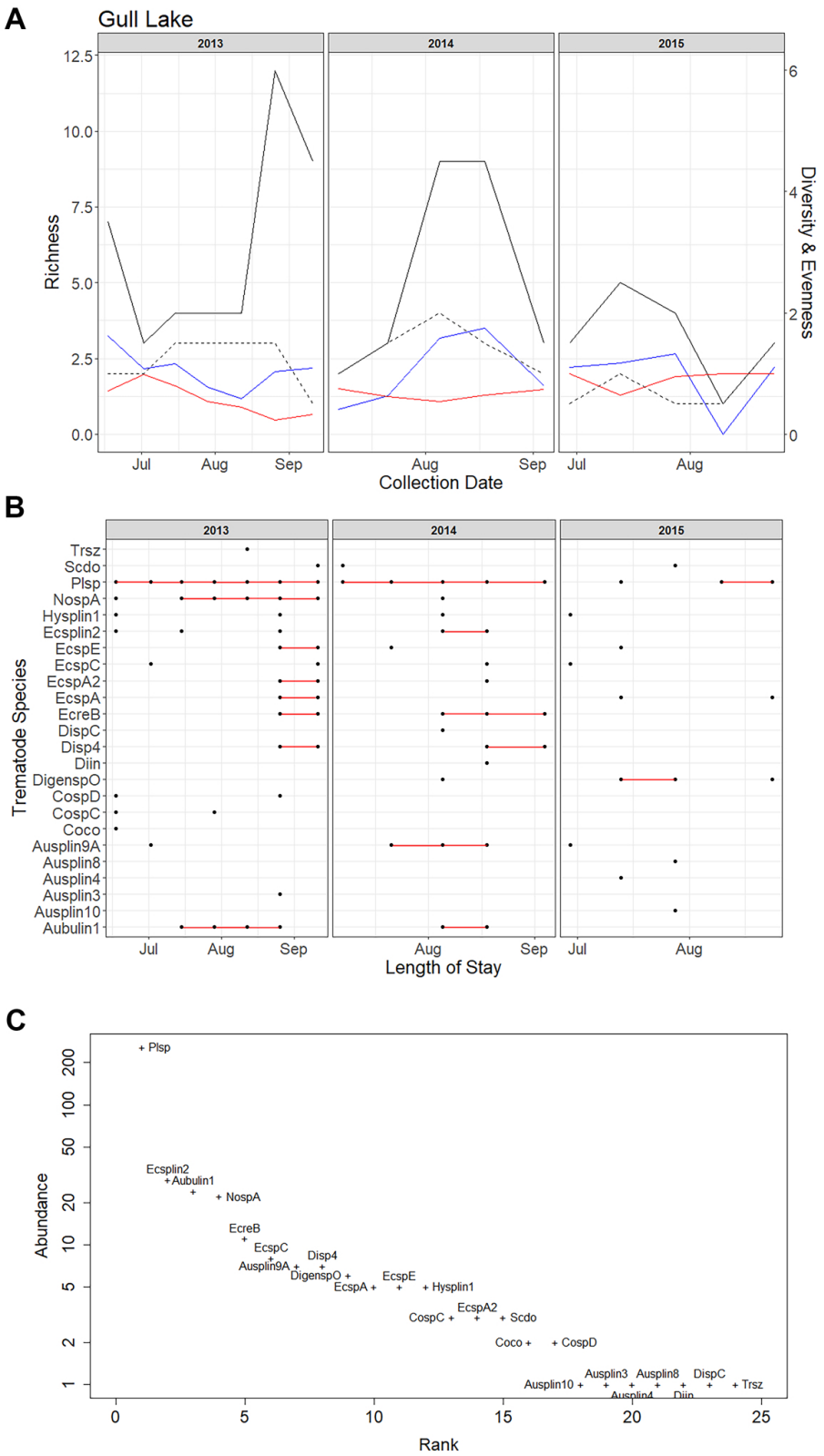

Figure 5.3: Gull Lake Diversity

Figure 5.3 A) Changes in species richness, alpha diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

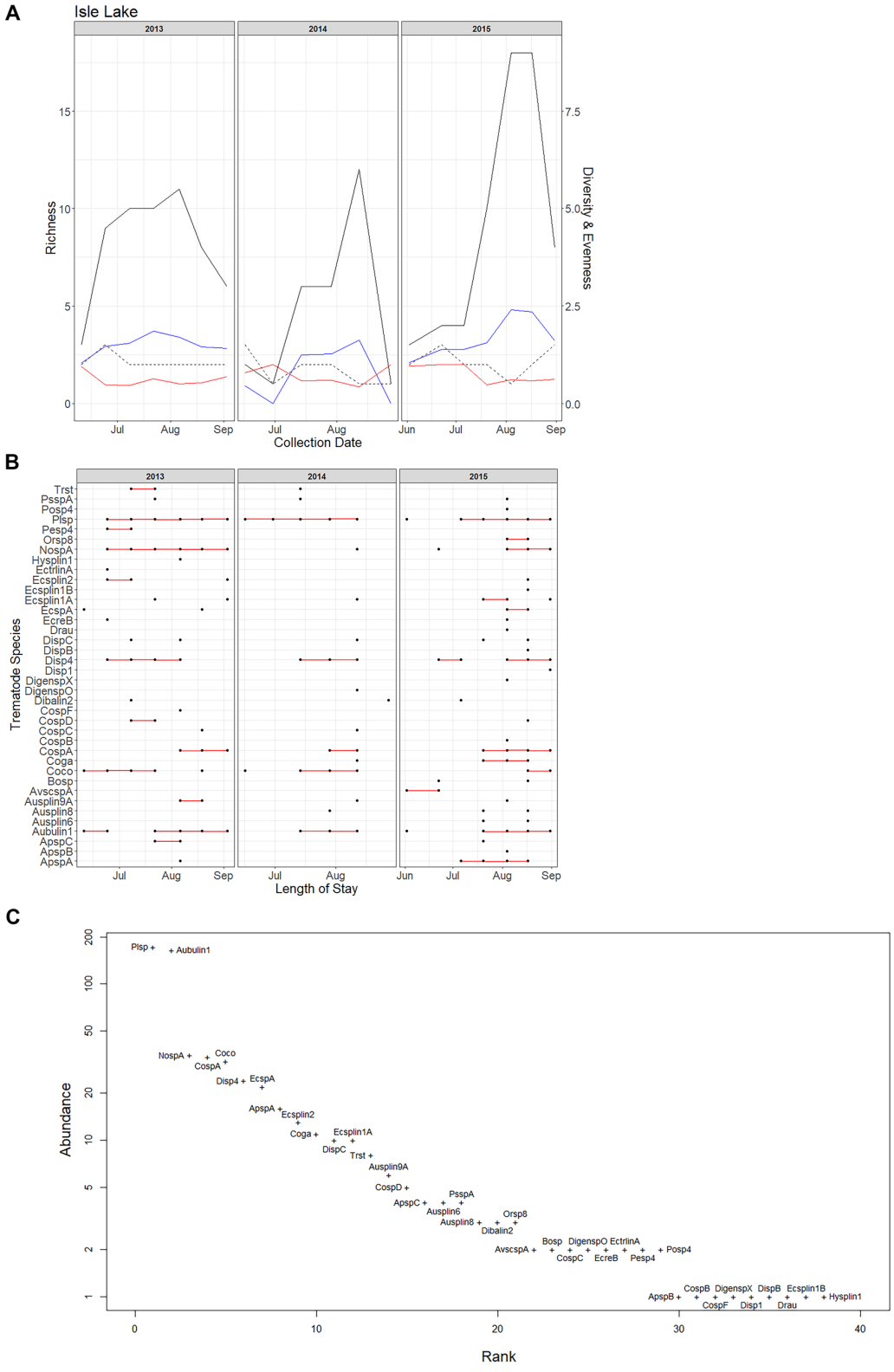

Figure 5.4: Isle Lake Diversity

Figure 5.4 A) Changes in species richness, α diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

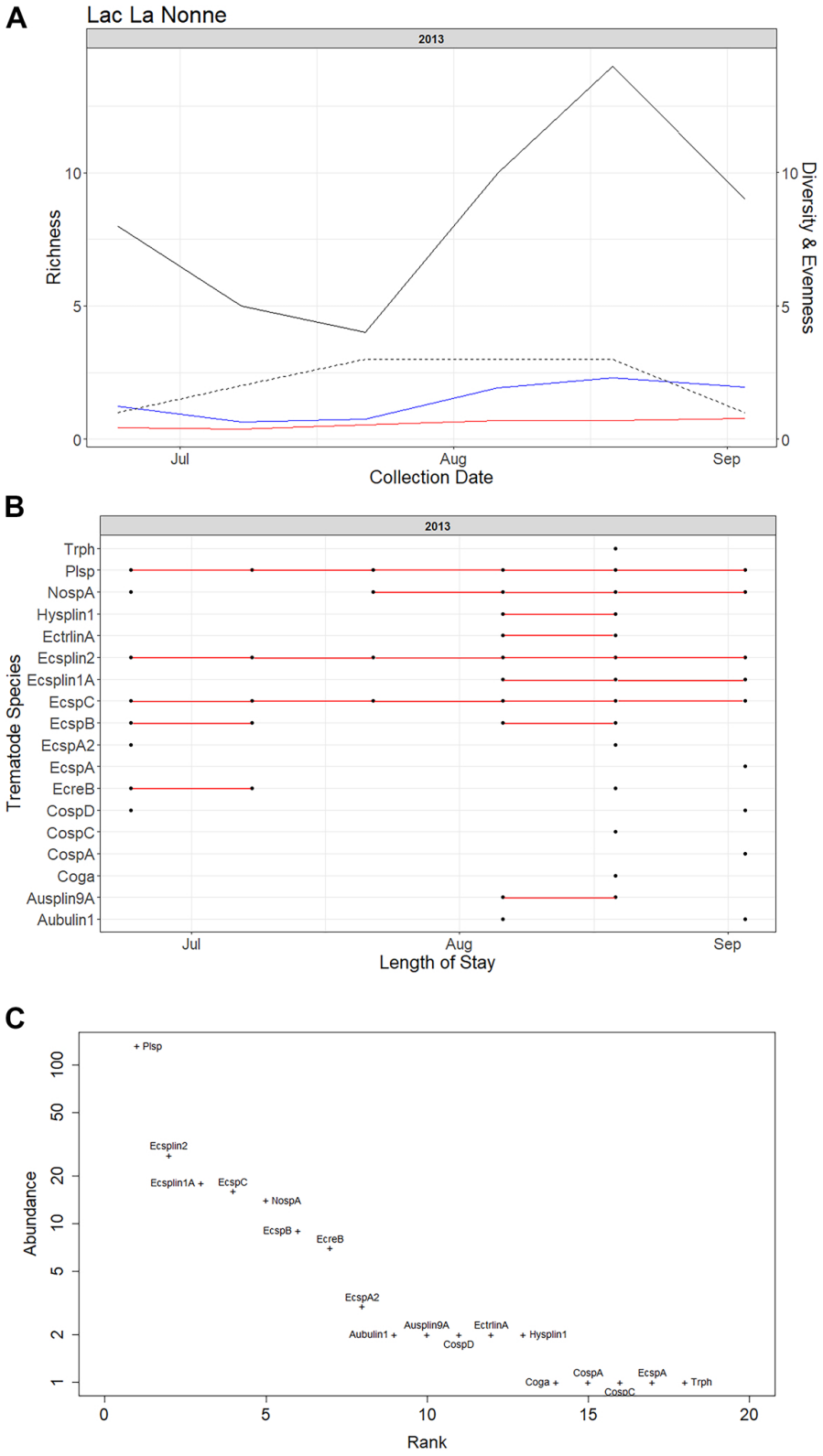

Figure 5.5: Lac La Nonne Diversity

Figure 5.5 A) Changes in species richness, α diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

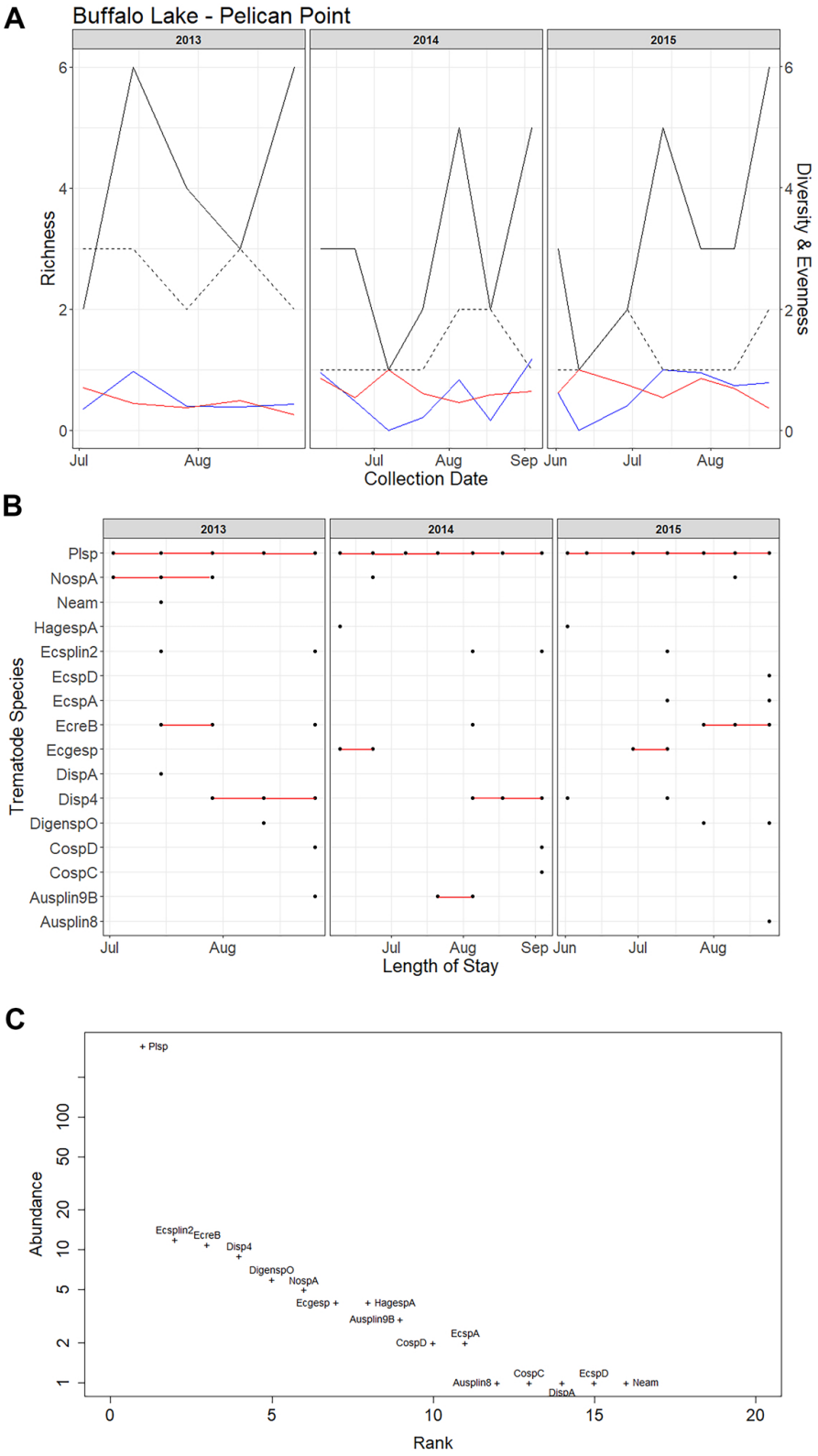

Figure 5.6: Buffalo Lake – Pelican Point Diversity

Figure 5.6 A) Changes in species richness, α diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

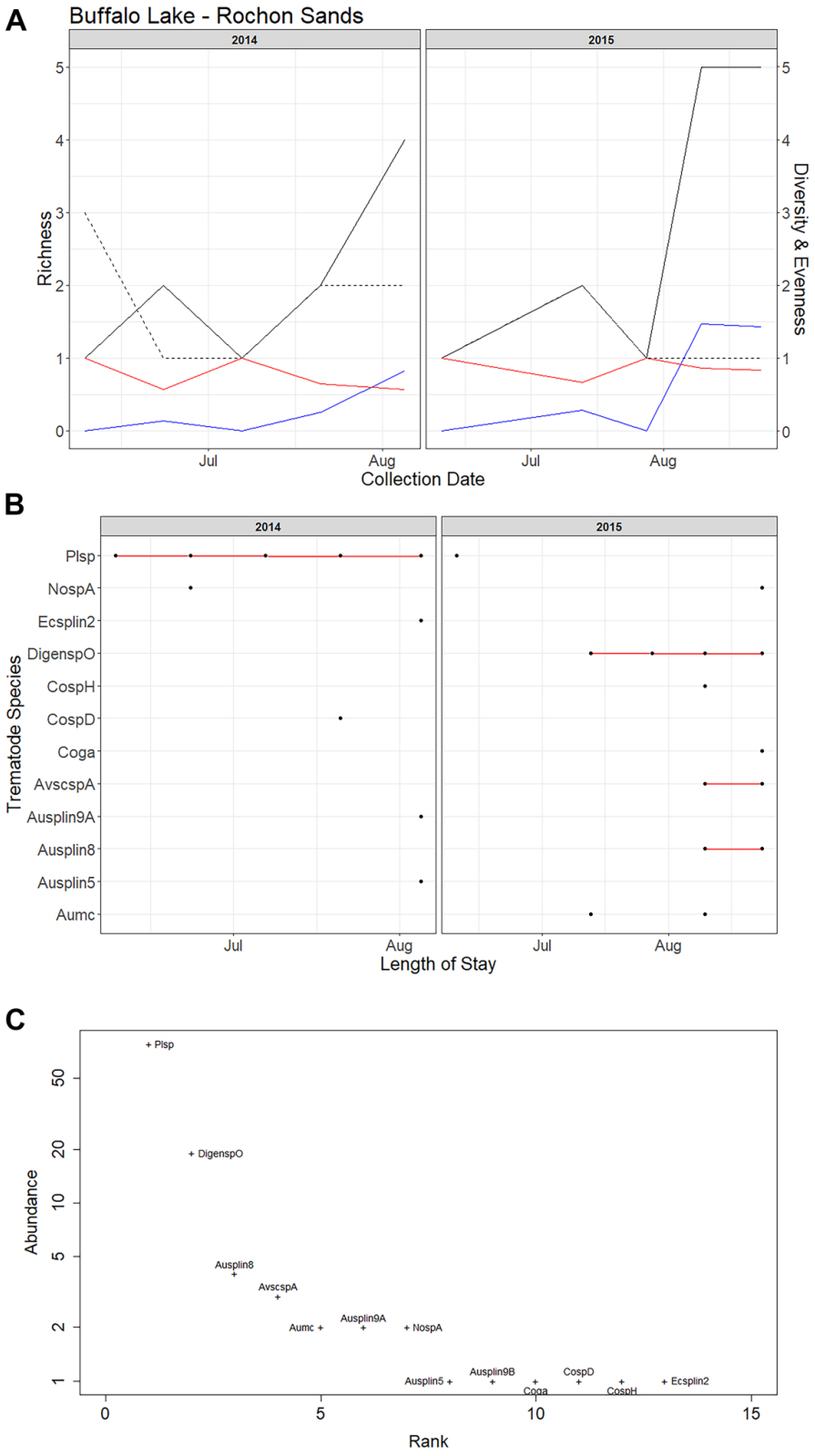

Figure 5.7: Buffalo Lake – Rochon Sands Diversity

Figure 5.7 A) Changes in species richness, α diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

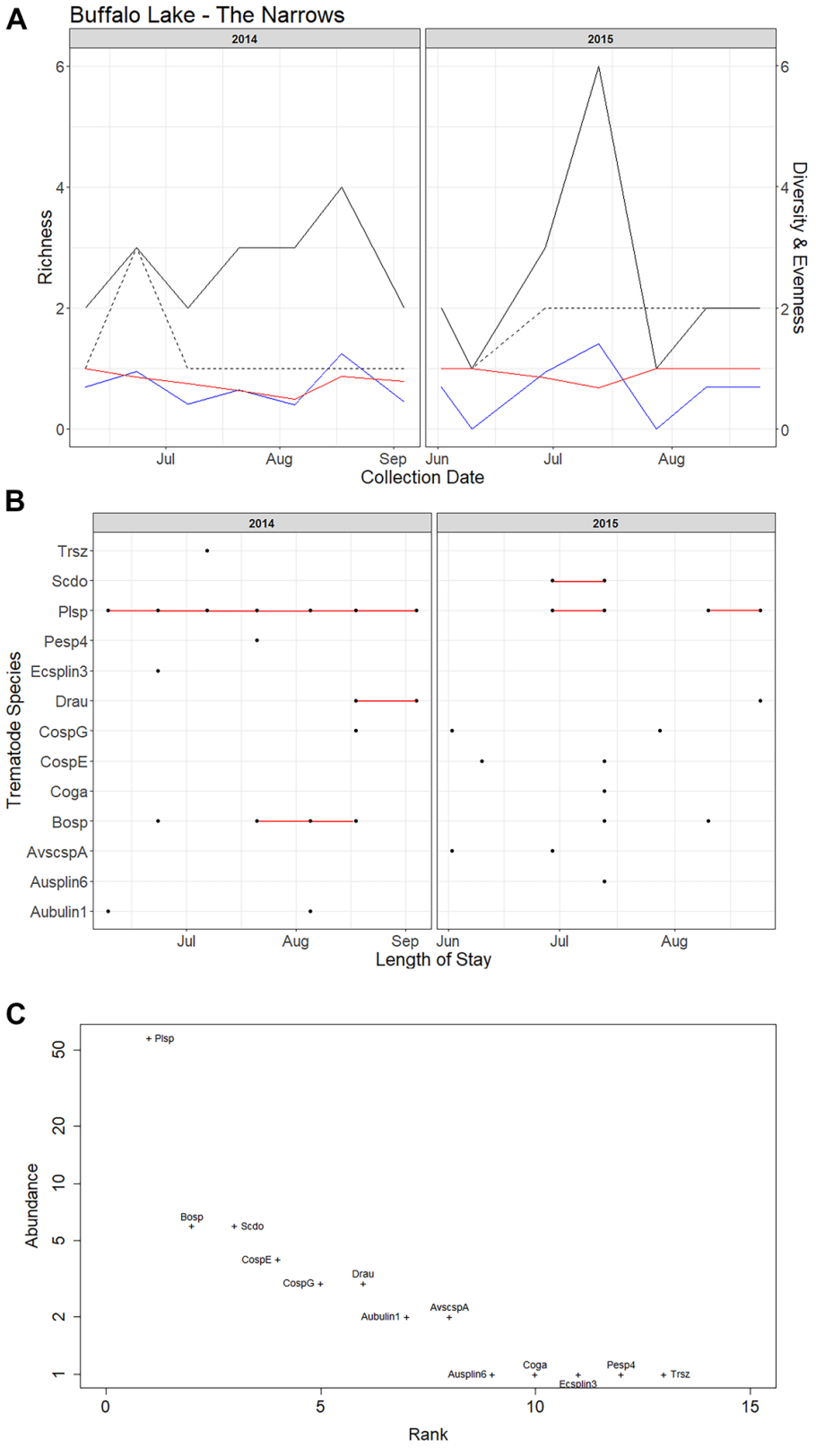

Figure 5.8: Buffalo Lake – The Narrows Diversity

Figure 5.8 A) Changes in species richness, α diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

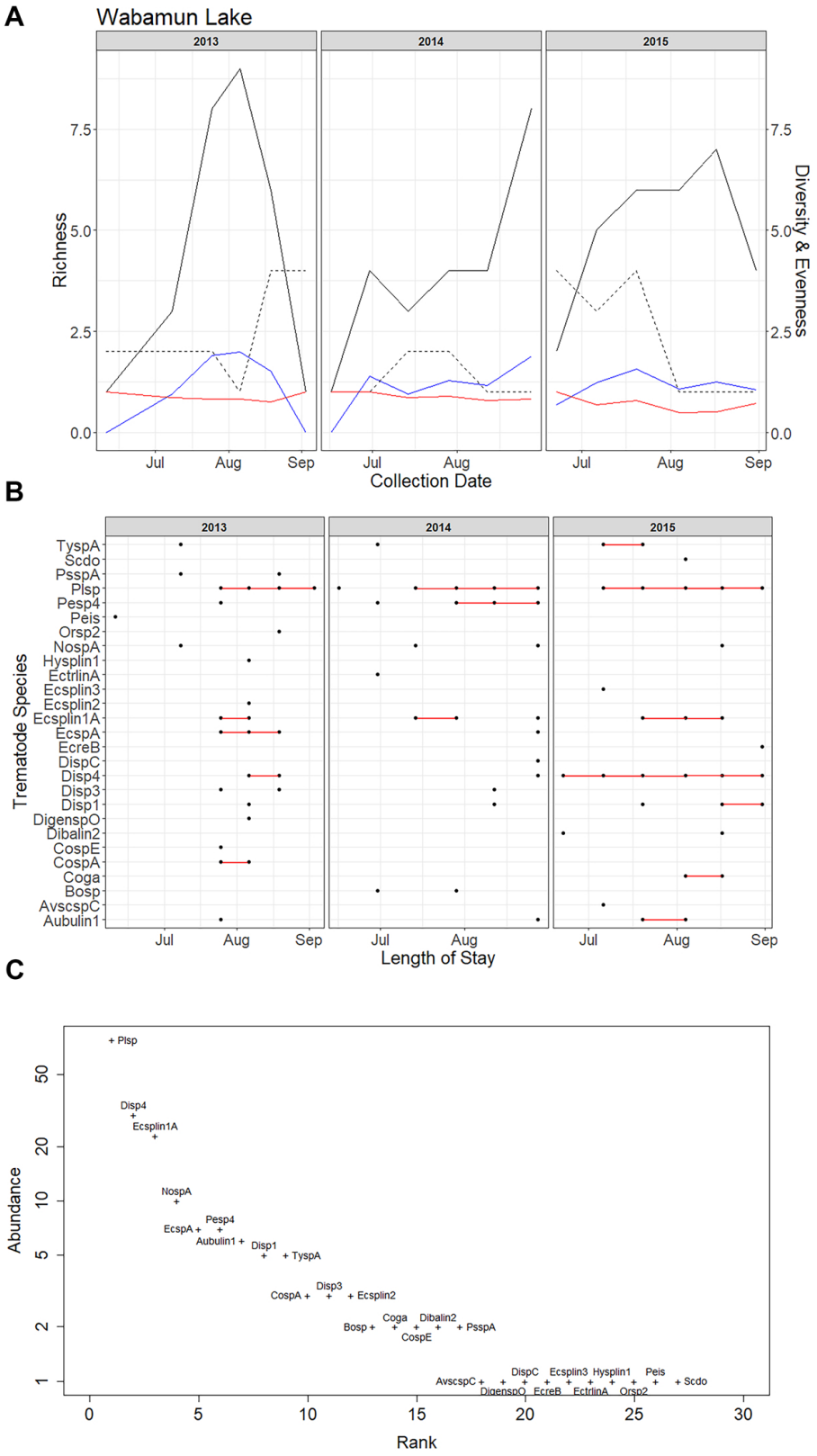

Figure 5.9: Wabamun Lake Diversity

Figure 5.9 A) Changes in species richness, α diversity (H), and community evenness over the length of the study. Solid black line represents trematode richness (left y-axis values), dashed black line represents snail richness including both infected and uninfected snails (left y-axis values), blue line is trematode α diversity (right y-axis values), and the red line represents community evenness (right y-axis). B) Time of arrival and length of stay for trematode species found during the study. Solid black dots represent a capture event, while red lines connecting the dots show continuity in capture events over time. C) Rank-abundance dominance model results. Species with lower ranks have greater abundances. Species abbreviations key in List of Abbreviations.

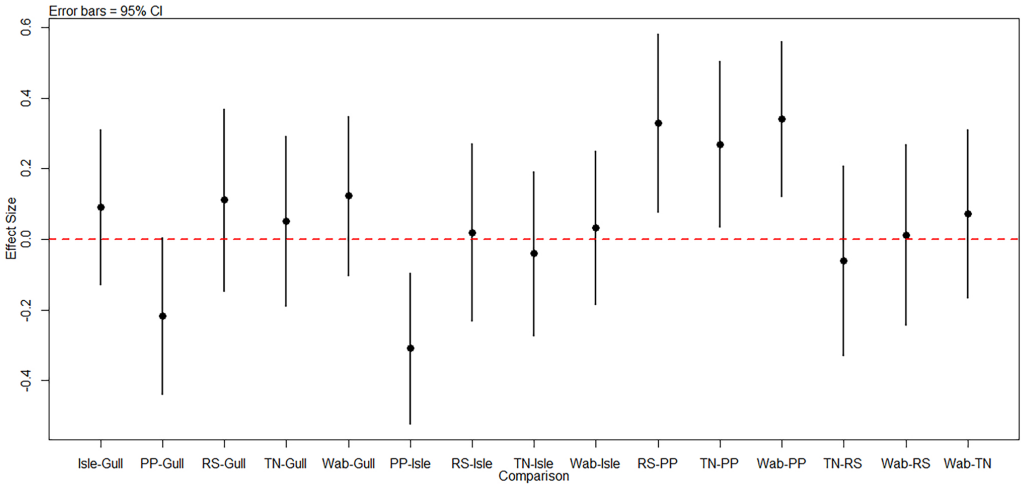

Figure 5.10: TukeyHSD plot for pairwise comparisons of site-based multivariate homogeneity of group variance. The plot gives effect sizes for each comparison and the bars represent 95% confidence intervals.

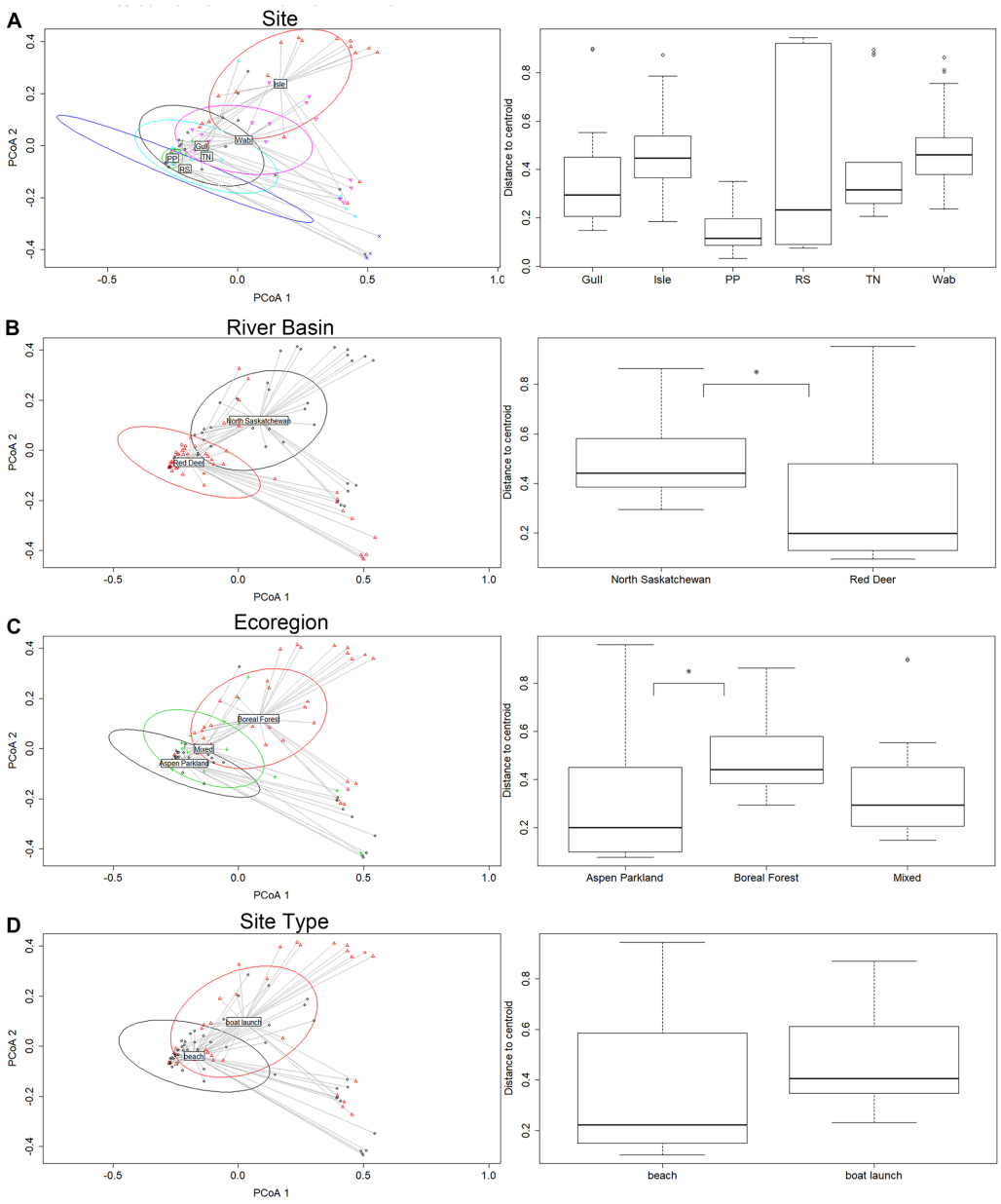

Figure 5.11: Multivariate Homogeneity of Group Dispersion for Trematode Communities. Bray-Curtis dissimilarities were used to examine the homogeneity of variance among samples when grouped by different geographical or anthropogenic-use distinctions. The left panels show the two-dimensional visualizations of the data by Principle Coordinate Analysis plots. Each grouping is labeled in the center, and ellipses represent 95% confidence intervals. The right panels provide a boxplot of the distance to centroid for each group in the multivariate analysis. A) samples grouped by site, B) grouped by river basin, C) grouped by ecoregion, D) group by site-type or anthropogenic use (beach or boat launch). Statistical significance for differences between groups is indicated by an asterisk, except for the site-based boxplot in Panel A (see Figure 5.10).

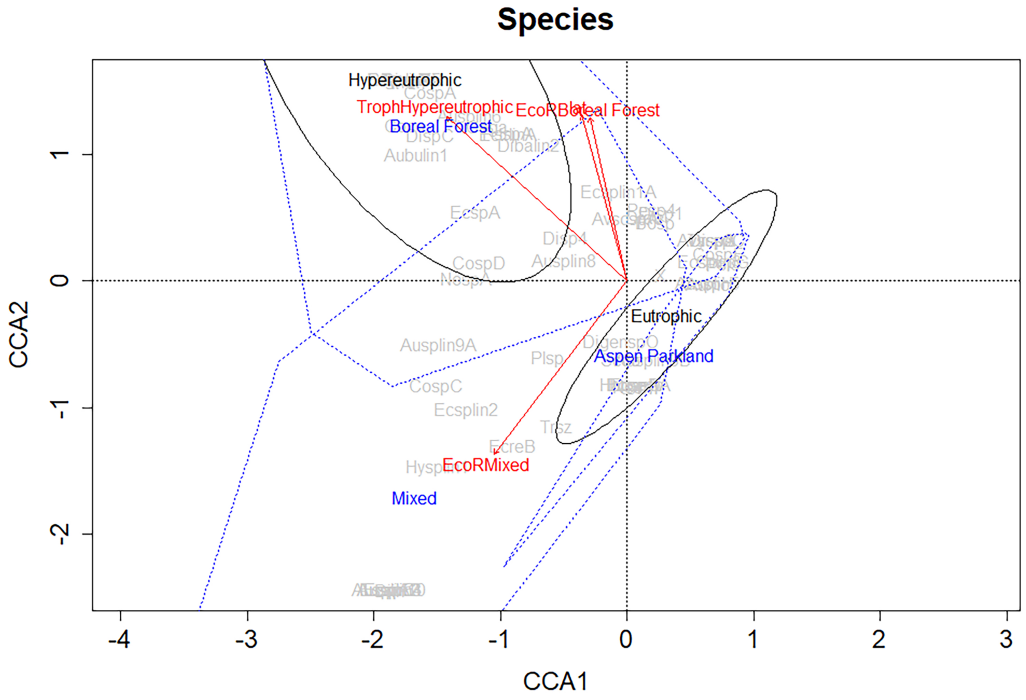

Figure 5.12: Canonical correspondence analysis of trematode component communities. Relative abundances of trematode species by sample are constrained by environmental variables from the best fit model (community ~trophic status ecoregion latitude). Model is significant ($F(4) = 7.139$, $P = 0.001$, permutation test). Trematode species abbreviations are shown in grey. CCA results are in red as eigenvectors. Ecoregions are identified with a blue dotted line. Trophic statuses are identified with ellipses.

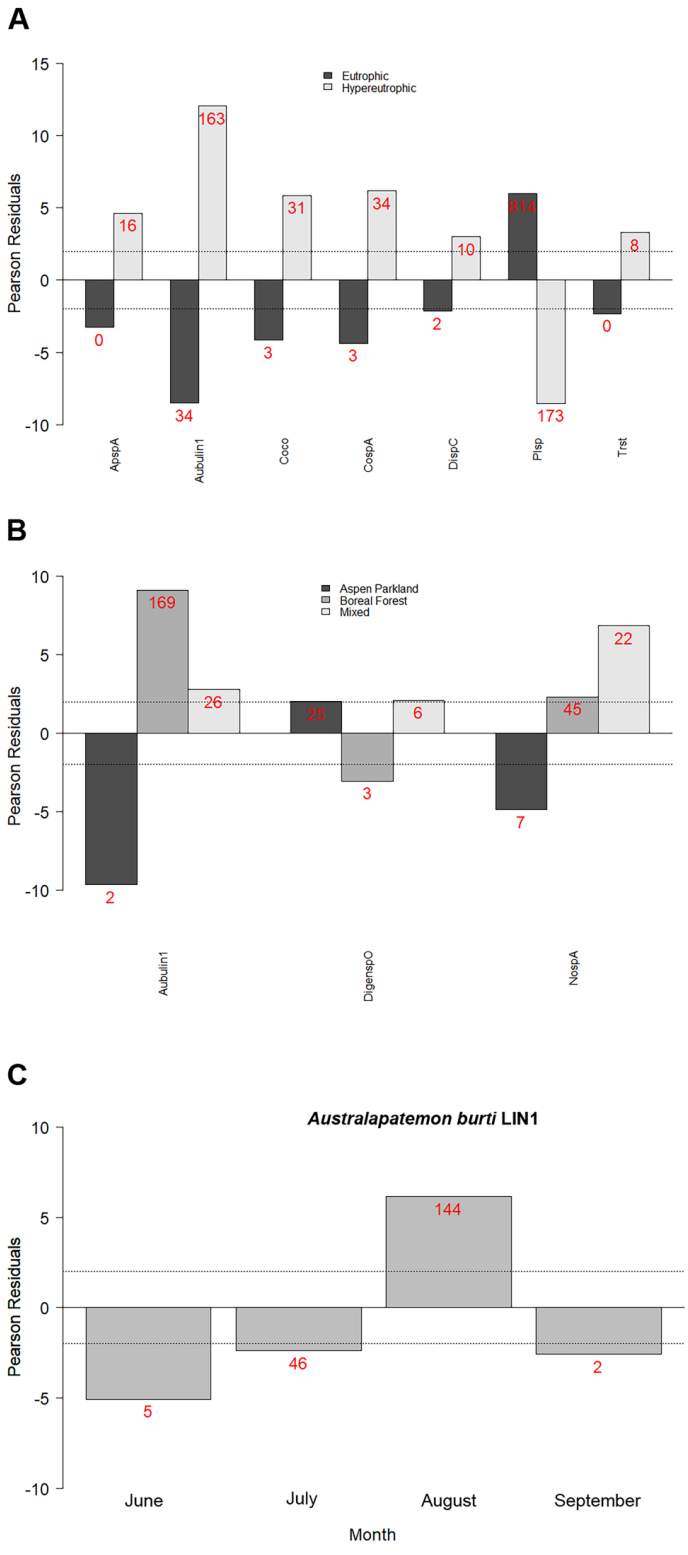

Figure 5.13: Trematode indicator species

Figure 5.13A) Indicator species for lake trophic status. B) Indicator species for three ecoregions in central Alberta. C) *Australapatemon burti* LIN1 as an indicator by month. Species abbreviation key in List of Abbreviations.

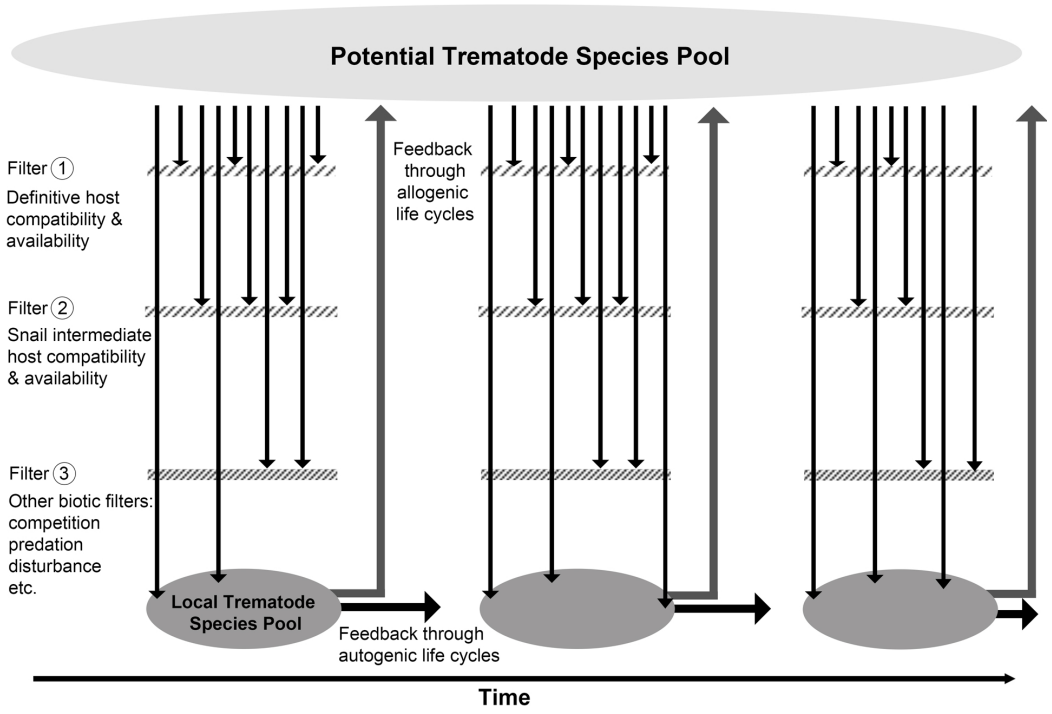

Figure 5.14: Conceptual Model of Trematode Component Community Assembly Based on Biotic Filters. The species composition of a single trematode component community, defined by an isolated geographical feature, such as a lake, is dynamic over time. While there are abiotic factors that contribute to this dynamism, we focus here on the biotic factors known to contribute to the success or failure of trematode infections and life cycle success that also affect community composition. The local trematode species pool of a community, let us say from one particular lake that can be sampled at any time, is provisioned by the regional trematode species pool. Not having a clear scope of just how large the regional pool is, let us imagine it is limited to North America (though we know of the presence of trematodes from Iceland in Alberta). The North American species pool is therefore composed of all the potential trematode species that could contribute to our local species pool. Several biotic interactions with other species act as filters along the path from the regional to the local pool. The first filter we identify is colonization ability from definitive host compatibility and availability. The definitive host must first have a successful infection by a trematode from the regional pool to bring them to the local pool for their potential colonization. There are many immunological and physiological factors that could prevent successful infection, and therefore the chance for colonization. Once a trematode has established a successful infection in a definitive host, and the definitive host has successfully reached the lake, the second filter is then the snail intermediate host in both its presence and compatibility. Trematodes are thought to be more host specific to their snail first intermediate host than to any other host. If this is true, then the filter has a narrower pore size than the first, limiting colonization even more.
(*Continued on next page*)

Figure 5.14(*Continued from previous page*) However, this relationship is one-sided, in that, while a trematode is most often specific for a snail species, a snail species can be susceptible to a wide range of trematode species. If the colonizing trematode has found a susceptible snail host in the lake, then it must go through a few other biotic filters including competition with other colonizers, predation by other species, and other interactions that might still prevent successful establishment. The timing of arrival through the definitive host is based on a multitude of factors but is also important to the success of infection and establishment. Depending on the trematode's life cycle, and the other hosts required, the life cycle may be autogenic and maintained by species that remain in the lake or allogenic, requiring vagile hosts that migrate or otherwise take the trematode out of the lake. Through these two life cycle types, we have feedback loops, where autogenic lifecycles feedback into the local pool, while allogenic lifecycles feedback into the regional pool. This whole process is continuously occurring over time.

Chapter 6

Swimmer's itch in Canada: a look at the past and a survey of the present to plan for the future

6.1 Introduction

6.1.1 Preface

In the previous chapters, I discussed our relative knowledge of trematodes in Alberta and the challenges associated with their species identifications, in addition to the general challenges met in the fields of parasite ecology and taxonomy. I also introduced the collection methods and downstream analyses from which this chapter builds upon. So far, I have shown that there is a much larger diversity of trematodes in central Alberta than we had expected to find, based on previous surveys and the number of snail host species found. Furthermore, molecular analyses have shown that we may have yet to capture all the diversity of trematodes present in these central, Alberta lakes.

In this chapter, I focus on one specific family of trematodes, the Schistosomatidae, because of their distinct role in disease transmission to humans

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and, therefore, their importance to public health. Herein, I examine the local public health issue of swimmer's itch, caused by schistosomes that utilize waterfowl and aquatic mammals as definitive hosts. Swimmer's itch is a globally-distributed, allergic condition, of which we know very little regarding local dynamics of transmission. This study gathers relevant information on swimmer's itch in Canada, from multiple perspectives, including the human experience, parasite and host presence and distributions, and insight from historical perspectives.

6.1.2 Background

Cercarial dermatitis, or 'swimmer's itch', is often referred to as an emerging disease, despite the fact there are no data tracking the number of afflicted people (Kolárová et al. 2013). Swimmer's itch is an allergic rash contracted when swimming in natural water bodies and is by no means a new condition, and reports from the literature suggest a global distribution historically. Nearly 100 years ago, the cause of swimmer's itch was discovered by Dr. William W. Cort at Douglas Lake, Michigan, when handling snails during collections resulted in the development of a rash. Cort found that it was the larval cercarial stage of avian schistosomes (parasitic flatworms) emerging from their snail host that caused the rash by penetrating the skin, and he described the condition as cercarial dermatitis (Cort 1928). Even before Cort's discovery, cercarial dermatitis was informally described using a variety of names. For example, in 1887, skin conditions resembling cercarial dermatitis were described among Japanese rice farmers, referred to locally as 'koganbyo' or 'lakeside disease' (Hunter 1951) (other names reviewed in (Petr Horák, Mikeš, et al. 2015; Kolárová et al. 2013)). Swimmer's itch has been noted to occur in both fresh and saline waters, though it is caused by different schistosome genera cycling through different genera of snail hosts (Petr Horák, Mikeš, et al. 2015).

Although schistosomes in general are noted to cause dermatitis, it is those of the genera *Trichobilharzia* and *Schistosomatium* that are most notably the etiological agents for freshwater ecosystems in North America. *Trichobilharzia* species utilize waterfowl as a definitive host in which they mature into adult

worms, while *Schistosomatium* species utilize small, aquatic mammals (Petr Horák, Mikeš, et al. 2015).

A review of the literature exposes the marked historical presence of swimmer's itch across most of Canada for nearly a century. In Canada, the first report and confirmation of the etiological agent of swimmer's itch, also commonly referred to as 'cercarial dermatitis' or 'schistosome dermatitis' in the literature, was by McLeod in 1934 at Clear Lake, Manitoba (McLeod 1934). Incredibly, this report notes that over 50% of the 55,000 visitors to this lake in the summer of 1933 had contracted swimmer's itch. McLeod relates this outbreak to the designation of the area of Clear Lake as a National Park that increased visitation dramatically, and therefore, exposure of people to the cercariae, which had likely been there long before (McLeod 1934). This first report was by far the largest case report (likely in the history of swimmer's itch) within our literature review and it spurred a series of downstream studies across the nation. Most of these studies focused on identifying the schistosome species and their hosts, sometimes with mention of a swimmer's itch outbreak, but nearly all reports neglecting to specify how many people were affected and what defined an 'outbreak'. A timeline of swimmer's itch across Canada, from the primary literature, shows one to two outbreaks every decade (Table 6.8). These reported outbreaks, however, are not from the same province and show no pattern.

The potential for swimmer's itch to occur via the presence of schistosome cercariae emerging from snail hosts, or adult worms found in definitive hosts has been indicated for most of Canada (Farley 1967; Jarcho and Burkalow 1952). As far back as 1936, W.W. Cort described correspondence with Dr. S.G. Saunders of the University of Saskatchewan, who had reported schistosome positive snails from Paul Lake in British Columbia, the Peace River area of Alberta, Vancouver Island (BC), and Saskatoon, Saskatchewan (Cort 1936). Since then, including the outbreaks listed above, all major provinces, except Prince Edward Island have had either reports of swimmer's itch or the presence of schistosome parasites published (Table 6.8). However, because of the lack of epidemiological data, we have poor estimates of people affected. Beyond

the first report of potentially over 25,000 cases, only 280 cases from across all of Canada for the last 64 years can be accounted for. Analogously, these one-time reports of the schistosome species present as the etiological agent for swimmer's itch in that area leaves a major assumption of a stable and consistent parasite community. Without continuous or even repeated surveillance, we have a poor understanding of these trematode communities and how their populations might fluctuate over time and lead to outbreaks.

Despite the widespread, global distribution of swimmer's itch, there remains limited understanding of many factors that pertain to the life cycles of schistosome species, their hosts, and what might be limiting their distributions. Canada is greatly endowed with freshwater and hosts a large diversity of waterfowl that utilize wetlands as summer breeding grounds; yet, few studies have examined the parasites of these birds, and direct links to swimmer's itch outbreaks within the country. In Alberta, previous reports of swimmer's itch presence have been noted in the literature, but details related to schistosome or host species locality, and impact on people has not been well-demonstrated. In fact, only three accounts of schistosome presence can be accounted for in Alberta. In 1936, Saunders described to Cort authentic accounts of swimmer's itch from the Peace River District of Alberta, but there was no mention of schistosome species or hosts (Cort 1936). In 1940, Hadwen and Fallis described an outbreak at Elk Island National Park, and investigated potential causes, of which they owed the outbreak to *Cercaria elvae* found emerging from *Lymnaea stagnalis* snails. *Cercaria elvae* was first indicated as the causative agent by Cort, and today it is known as *Trichobilharzia ocellate* (D. Blair and Islam 1983; Hunter et al. 1949). The third account was of the presence of *Dendritobilharzia pulverulenta* from an Eared Grebe prior to 1980, of which no further information of location or possible snail host was mentioned (Vusse 1980). The story is quite similar for other Canadian provinces, with past work concentrated in Manitoba, and swimmer's itch reports concentrated around Quebec and in Southern Ontario near the Georgian Bay (Cort 1936; Jarcho and Burkalow 1952; Lévesque, Dewailly, and Boulianne 1990; Lévesque, Giovenazzo, et al. 2002).

To assess whether swimmer's itch is truly an emerging disease, we need a baseline of information for comparison, especially in consideration of the potential for altered risk in association with climate change. It is predicted that warmer temperatures will affect the occurrence rates of swimmer's itch, in that warmer temperatures have been associated with increased schistosome developmental rates (more and faster production of cercarial larvae produced in the snail) and therefore, transmission success. Likewise, lake eutrophication can lead to more nutrient availability for snails, enhancing their success and leading to larger populations that can support more schistosome infections (reviewed in (Petr Horák, Mikeš, et al. 2015)). While schistosomes are not the only the trematodes infecting snails, and there is still much to be learned about their community ecology, and whether prevalence of snail infection would be greater for schistosomes than other species, increased risk is still a possibility in the future. To address this, we need to better understand the dynamics of species interactions, their relationship to the environment, and the same for human interactions and impact on these natural areas.

We predict that swimmer's itch is a more widespread problem in Canada than would be revealed by past reports and coverage within the literature. To test this, we gathered swimmer's itch case reports from individuals through a voluntary, online survey every summer from June 2013 – September 2017. Our aim was to better understand the spread of the issue in Canada, and to determine peaks of transmission. We also endeavoured to identify Alberta schistosome species to better understand the current and potential future spread of swimmer's itch through host species distributions in the province.

6.2 Material and methods

6.2.1 Web-based self-reporting survey

In 2013, a web-based, self-reporting survey was developed as a surveillance tool to gain an understanding of where and when swimmer's itch occurred across Canada. The survey was hosted on the website http://swimmersitch.ca/, which also acted as a general information source for people to reference about

swimmer's itch, including the difference between what a swimmer's itch rash and a cyanobacteria rash look like, the parasite life cycle, and a section for frequently asked questions. The survey was introduced by a brief paragraph to explain the purpose of the survey, followed by an information letter and consent form. The survey entailed 15 questions (Table 6.1), asking respondents for information related to the lake they visited and their swimmer's itch experience. The end of the survey was followed with a general comments section to allow for any other information respondents wanted to provide, including general feelings. The survey also provided a definition of the swimmer's itch rash:

> "An itchy, red, raised rash usually characterized by small reddish pimples that appears after time spent in lakes or ponds. Symptoms can start within a few minutes to 48 hours after being in the water."

People were not actively recruited to fill out the survey, however, the website and associated survey were advertised through social media (Twitter: @swimmersitch_ca & University of Alberta School of Public Health website: https://www.ualberta.ca/public-health/about/this-is-public-health/this-is-public-health-articles/2014/july/swimmers-itch-in-albertas-lakes), official press releases that resulted in interviews with the Canadian Broadcasting Company (CBC), and other media outlets, postcards pinned to community boards and handed out upon personal communication at lakes, through beach outreach by the Alberta Lake Management Society (ALMS), and through personal communication and presentations at conferences.

In 2014, improvements were made to the survey to gain better information for certain questions. For instance, the question "Did anyone you know also contract swimmer's itch on the same day?" changed to "How many people in your party also contracted swimmer's itch on the same day not including yourself?" to change it from a yes/no question to a quantitative response, to better reflect the number of swimmer's itch cases. We also added the question "To your knowledge, was there a Blue-Green Algae warning at this lake the

day you visited?". The latter question was added to gauge whether their rash might be due to a cyanobacteria bloom as opposed to schistosome cercariae.

6.2.2 Web survey statistics

Survey responses and other survey statistics were analyzed using R (R Core Team 2017), with packages *dplyr* (Wickham et al. 2017), *multcomp* (Hothorn, Bretz, and Westfall 2008), *MASS* (Venables 2002), and *car* (Fox et al. 2014). Because most responses were categorical, we used a Chi-Square Goodness of Fit test to compare the expected to observed values within each question. The expected values were based on the null hypothesis of even distributions of possible responses. To test the difference between those who knew whether swimmer's itch was a common problem at the lake, stratified by residency status (own property vs. visitor), a Pearson's chi-squared test with Yates' continuity correction was used.

We wanted to know if the variable month could be a predictor for when peak transmission of swimmer's itch occurs. To determine if there was any significant effect of month on the number of cases, a generalized linear model (glm) was used after removing outliers. The outlierTest from the *car* package was used to test for and identify outliers. We used Analysis of Variance (ANOVA) with Likelihood Ratio Test to compare the models, in addition to comparing the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) estimators, and the ratio of residual deviance to degrees of freedom to test for overdispersion. Initial examination of swimmer's itch case data suggested a negative binomial distribution, as it was highly skewed to the right. However, trying to fit a glm with a negative binomial family resulted in overdispersion (dispersion parameter $\theta = 3.18$ e+26). The models were then re-fit with the Poisson family and log link to adjust for overdispersion. Once the best model was selected, the *lsmeans* package (Lenth 2016) was used for estimating the least-squared means for the rate of swimmer's itch, calculate 95% confidence intervals, and to employ a Tukey Contrasts post-hoc test for pairwise multiple comparisons of means among significant predictors. The package *bbmle* (Bolker and Team 2013) was used for extracting model com-

parison statistics. The package *ggplot2* (Wickham 2009) was used for some graphics.

Several questions allowed for open-ended responses to gain a qualitative understanding of how respondents described the water conditions, the birds they saw in the area, and if they thought information on swimmer's itch was adequate, and where they retrieved this information. For these questions, all words were gathered into a list, and filtered based on frequency and commonality (e.g. the words and, a lot, about, etc.), as well as relevance to the question. Word lists were uploaded to an online word cloud generator (https://worditout.com/word-cloud/create), filtered, and turned into word clouds that reflected the frequency of word use through word size and color. For the water conditions, criteria were set to a minimum frequency of ten uses within the list, while the question about waterfowl type and information resources were set to a minimum frequency of five.

At the end of the survey was a space for general comments, to allow respondents to voice opinions and add extra information if they wished. The comments were coded qualitatively and grouped into themes and subthemes, for a general idea of both what might be missing from the survey, and to gain insight into perceptions and feelings about swimmer's itch.

6.2.3 Map of swimmer's itch cases

ArcGIS Online and ArcGIS Desktop v. 10.6 ArcMap (Esri, 2017) were used to plot swimmer's itch reports received across Canada from 2013-2017. Swimmer's itch cases were associated with GPS locations gathered from lake names given in survey reports. These were mapped as point locations atop the World Light Gray Canvas Base (Esri 2011) and Course_HIST213_North American Rivers/North America (MapServer) layer of North American lakes (http://gis-webfs.bucknell.edu/arcgis/rest/services/Course_HIST213_NorthAmerican Rivers/NorthAmerica/MapServer). The Heat Map tool of ArcGIS Online was used to display the relative amount of swimmer's itch cases at each lake, as individual points were stacked. Thus, the more cases, the redder or whiter the

surrounding area was colored as opposed to the blue area with less overlapping points.

6.2.4 Snail-trematode survey

Snail and schistosome collections were conducted within a larger field-based survey of snail-trematode associations in central Alberta (2013-2014), as reported in Gordy et al. 2016. This study was extended for a third year (2015), using the same methods. Samples described in this study were derived from all three years of the survey.

6.2.5 Molecular phylogenetics of schistosome species

Larval schistosome cercariae were identified initially by the presence of eyespots, upon emerging from their snail intermediate host. Each sample was preserved in either 100% ethanol or 50% RNAlater (Invitrogen) and stored at -20C (ethanol) or 4C (RNAlater). Whole genomic DNA was then extracted from several cercariae of each sample with the DNeasy Blood & Tissue Kit (Qiagen) and used to amplify the Folmer region of the mitochondrial gene, cytochrome *c* oxidase subunit 1 (*cox1*), using primers (dice1F/dice11R and CO1F15/CO1R15) as previously described (M. Gordy et al. 2016). Samples were sent to Macrogen Inc. (Korea) for Sanger sequencing in both forward and reverse directions, using the same primers as for initial amplification.

Nucleotide sequences were trimmed for quality using 4Peaks (Nucleobytes), then imported to Geneious R11.1 (https://www.geneious.com/, (Kearse et al. 2012)) for downstream analyses. Forward and reverse sequences were aligned using the Geneious alignment algorithm and default parameters. Consensus sequences were derived from these alignments, and protein translations were checked for stop codons, using the trematode mitochondrial translation code, and to make sure they were all in the same reading frame. The consensus sequences were then identified to genera by using tBLASTn (National Center for Biotechnology Information, U.S. National Library of Medicine) to find sequence matches with the highest percent nucleotide identity. The sequences were then used in multiple sequence alignments with representative

schistosome sequences derived from GenBank. Sequences generated from this study have been deposited in GenBank under accession numbers (MH168781-MH168796).

Much previous work has been done on the systematics of the Schistosomatidae, providing many partial *cox1* sequences in GenBank available for comparison. To avoid problems with substitution saturation within phylogenies, more differently related genera were analyzed in separate alignments. Ultimately, based on BLAST identities, there were two genera, *Trichobilharzia* and *Schistosomatium*, and these were analyzed separately. Multiple sequence alignments were completed using the Geneious algorithm and default parameters. Ends of the alignment were trimmed to the shortest sequence length. The best nucleotide substitution models for rates of DNA evolution were determined using model testing methods with the software MEGA7 (Kumar, Stecher, and Tamura 2016) for both alignments. The model GTR + G + I was found to be the best model for phylogenetic analyses for both genera and used as such in downstream Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Geneious plugins were used for phylogenetic analyses. The following settings were used for BI trees in the MrBayes (Huelsenbeck and Ronquist 2001) plugin: chain length = 10,000,000, subsampling frequency = 100,000, heated chains = 4, burn-in length = 1,000,000, heated chain temp = 0.2, priors using unconstrained branch lengths with GammaDir (1, 0.1,1,1). The following settings were used for ML trees in the PHYML (Guindon et al. 2010) plugin: branch support = bootstrap, number of bootstraps = 10,000, transition/transversion ratio = estimated, proportion of invariable sites = estimated, number of substitution rate categories = 4, gamma distribution parameter = estimated, optimized for topology/length/rate, and topology search used = BEST (best of NNI and SPR search). As a confirmation of distance-based methods, and as additional support for clade distinction, the web app Automatic Barcode Gap Discovery (ABGD; http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) was used in combination with *a priori* assumptions of a 5% cut-off in sequence divergence for species delimitation using p distances calculated in MEGA7. For ABGD, we insert nucleotide alignments and tested all three distance mea-

surements (Jukes-Cantor (JC), Kimura 2.0 (K2), and simple distance) to look for agreements on grouping and prior maximal distance with a minimum slope of 1.

Trichobilharzia

A final alignment of 85 sequences was made using *cox1* genes gathered from 20 sequences from this study and 65 sequences from 17 species of *Trichobilharzia*/Avian Schistosomatids gathered from GenBank. For the phylogenies, *Austrobilharzia variglandis* (AY157196) was used as the outgroup. The final alignment was 432 bp with no gaps.

Schistosomatium

A final alignment of 12 *cox1* sequences (9 from this study) was made, with *Schistosoma bovis* (AY157212) as the outgroup. The final alignment was 509 bp and consisted of a few minor gaps due to *S. bovis*.

6.2.6 Definitive and intermediate host distributions in Alberta

Direct field collections of invertebrates and vertebrate sighting records were derived from the Alberta Biodiversity Monitoring Institute (ABMI) Species and Habitat Raw data depository (http://www.abmi.ca/home/data-analytics/da-top/da-product-overview/Species-Habitat-Data_new.html?scroll=true). The ABMI is a not-for-profit scientific organization that monitors and reports on the status and trend in more than 2500 species and their habitats across the province of Alberta. Data are collected from a systematic grid of 1656 sampling sites spaced 20 km apart across the entire province. Along with data on individual species distributions, detailed sampling protocols for both terrestrial and aquatic ecosystems are publicly available online at www.ambi.ca. Species-level presence (or absence) data collected by ABMI from 2007-2015 were used to estimate the distributions of potential snail intermediate and vertebrate definitive hosts across Alberta likely to be associated with schistosome and swimmer's itch transmission. The species considered as likely to be associated

with schistosome life cycles and retained in the final data set were the following: Vertebrates (Waterfowl): *Anas* spp.: *A. acuta, A. americana, A. clypeata, A. crecca, A. cyanoptera, A. discors, A. penelope, A. platyrhynchos, A. streptera; Aythya* spp.: *Ay. affinis, Ay. americana, Ay. collaris, Ay. marila, Ay. valisineria, Mergus merganser*, and *M. serrator*; Vertebrates (Mammals): *Microtus pennsylvanicus*, and *Microtus richardsoni*; Invertebrates (Gastropods): Lymnaeidae: *Lymnaea* spp., *L. columella, L. stagnalis, L. stagnalis jugularis, Stagnicola* spp., *S. caperata, S. catascopium catascopium, S. elodes, S. exilis*, Physidae: *Physa* spp., *P. skinneri*, and *Physella gyrina*. The assessment of these species as potential hosts is based on previous reports in the literature, according to the Host-Parasite Database of the Natural History Museum of London (Gibson, Bray, and Harris 2005). The GPS coordinates associated with the species within our final dataset were used to map their distributions. We used ArcMap from ArcGIS Desktop v. 10.6 as described above. Within ArcMap on ArcGIS Desktop, we used the Tabulate Intersection tool to get statistics on points that overlapped between vertebrate and invertebrate records as well as swimmer's itch case records, based on GPS for latitude and longitude with an allowance of 10km.

6.3 Results

Over the five years this survey has been active (2013 – 2017), we have received a total of 1316 swimmer's itch reports from across Canada (N = 1302), and a few from the United States (N = 14). Including additional cases reported since 2014, this adds to a sum total of 3,882 cases of swimmer's itch captured by the survey.

We received reports from every province in Canada except Prince Edward Island (Figure 6.1). Most reports, however, were from Alberta (610 reports/1,935 cases), British Columbia (400 reports/1,071 cases), Ontario (159 reports/435 cases), and Saskatchewan (93 reports/311 cases), and were represented every year of the survey. Reports from Manitoba (14 reports/53 cases) occurred every year except 2014. Reports from Quebec (10 reports/19 cases)

were received in 2013, 2014, and 2016. Reports from New Brunswick (3 reports/13 cases), Nova Scotia (4 reports/7 cases), Newfoundland and Labrador (2 reports/7 cases), and the Northwest Territories (1 report/1 case) occurred less frequently, represented in only one or two years.

Because respondents listed a beach name or a lake name, we did not have enough resolution to provide specific GPS locations for each report. Across Canada, there were a total of 268 unique lakes in which swimmer's itch was reported to occur (Figure 6.2). With the inclusion of several beaches at the same lake, there were 323 unique sites in total.

We received swimmer's itch reports from May through September in most years (survey started June 2013) (Table 6.2). The first two years of the survey trended towards August as the peak of the season for swimmer's itch, but the last three years moved towards peak occurrences in July (Figure 6.3A). The greatest number of occurrences over the course of the survey was 540 cases in July of 2015. This, in combination with cumulative cases by month and by year, suggests that July is an important month for swimmer's itch transmission (Figure 6.3B), and that 2015 was a particularly big year for survey reports (Figure 6.3C and D). However, an important interaction between month and year on the rate of swimmer's itch cases was revealed from the best fit of the generalized linear models (cases ˜year*month). Simply, the effect of month on the rate of cases depends on the year and vice versa (Table 6.3 and Figure 6.4). Although July and August both seem important times for swimmer's itch transmission, there is more complexity than can be explained by only month for the number of swimmer's itch cases that may occur, and more importantly, that may be reported.

Survey respondents were asked a series of questions to help us better understand their general awareness of swimmer's itch, their perceptions of the environment, and how their experience might affect future use of lakes for recreation. The majority of respondents were visitors to the lake at which they experienced swimmer's itch, as opposed to owning property on the lake ($\chi^2(1,\ \mathrm{N} = 1315) = 361,\ P < 2.2\text{e-}16$). Whether or not the respondent owned property at the lake had no significant effect on whether they knew

if swimmer's itch was common at that lake ($\chi^2(1,\ N = 1315) = 0.00088$, $P = 0.9763$) (Figure 6.5A). Most respondents said they did not feel like the amount of information available to them about swimmer's itch was adequate ($\chi^2(1,\ N = 1248) = 92.6,\ P < 2.2e\text{-}16$) (Figure 6.6). For those that said there was enough information available, they were asked to provide the resources they commonly consulted in an open-response format. After filtering for frequency and relevance, there were 24 words left, of which the most common was "Internet" (N = 109) (Figure 6.7A). When referencing the comments, the most common websites used by people were Health Link BC (https://www.healthlinkbc.ca/), Alberta Health Services or other Government of Alberta Websites (https://www.albertahealthservices.ca/), and the website used to host the survey (http://swimmersitch.ca/). When asked how people learned of the survey on our website, nearly all respondents answered "Google" ($\chi^2(9,\ N = 1270) = 7267.2,\ P < 2.2e\text{-}16$) (Figure 6.5B). Google was the most frequently accessed source; however, some people did find out about the survey through advertisement methods such as community board postings, and the news.

When asked to rate their case of swimmer's itch, most respondents rated their severity as Medium (N = 866), defined within the question as having a large rash area, very itchy, and having a burning sensation. Much fewer rated their severity as Mild, or a small rash with some itching (N = 342), and even fewer as Severe, and requiring hospitalization or a doctor's visit (N = 100), ($\chi^2(2,\ N = 1308) = 703.28,\ P < 2.2e\text{-}16$) (Figure 6.5C).

A total of 303 respondents provided general comments at the end of the survey. The comments fit into four general codes expressing how the comment section was used: 1. Seeking information about swimmer's itch, 2. Desires swimmer's itch warning, 3. Providing more information, and 4. Providing opinion. These codes were further divided into themes and subthemes to represent generalizations of information, and provide a brief overview of respondents' experiences, opinions, and desires (Table 6.4). While the counts within each theme are not meant to be truly quantitative, the majority of respondents used the comments section to provide more information (N = 232).

Nearly half of those were describing their personal awareness of swimmer's itch, lack thereof, or their personal history of swimmer's itch. The other half talked more about the details within their particular case, including the environment, presence or lack of signs, the severity of their itch, and more. Many respondents believed that there should be some sort of warning system in place, but there was little clarity on who they thought should have that responsibility. Prevention methods were mentioned quite often within the themes as well, whether it was their personal method for trying to avoid or treat the rash, or discussion about common prevention methods that don't work, like showering or towelling off after swimming.

Respondents were asked to describe several variables about the environment in which they were swimming/recreating where they contracted swimmer's itch, including if they noticed the presence of waterfowl and snails, and what the general water conditions were like. They were also asked if they had been aware of any blue-green algae (cyanobacteria) warnings at the lake at that time. Most respondents reported that they had seen waterfowl at the lake ($\chi^2(1, N = 1282) = 350.16$, $P <$ 2.2e-16) (Figure 6.5D). Many also reported the types of waterfowl they had seen, though this was generally "ducks" and "geese". Among these descriptions, 24 words matched the criteria of having a minimum frequency of 5. By far, the word with the highest frequency was "Ducks" (N = 646) (Figure 6.7B). This could be because the question was leading, in providing the examples "ducks, geese, etc.", but it is also likely what most people might be familiar with. In contrast, most respondents reported they had not seen snails. Though the total number of survey responses was quite close to those that had seen snails, their distributions were not even ($\chi^2(1, N = 1285) = 14.606$, $P = 0.0001325$) (Figure 6.5E).

After filtering responses about general water conditions, there were 53 words that met the minimum frequency of ten uses. The word "warm" had the highest frequency (N = 438), followed by the words "clear", "water", and "vegetation" (Figure 6.7C). Respondents were also asked about whether, to their knowledge, there had been a warning about blue-green algae in the area at the time they contracted swimmer's itch. We wanted to get an idea of the

possibility that their rash may have been related to other causes, namely an outbreak of cyanobacteria, which are common in many recreational lakes in Alberta. Nearly all respondents said "No" to this question ($\chi^2(1, N = 998) = 923.45$, $P <$ 2.2e-16) (Figure 6.5F).

We wanted to know how swimmer's itch, and the perception of swimmer's itch after having experienced it, might affect future lake use and the probability of swimming with and without the known risk. More respondents said they use the lake/waterbody less often than they would like because of swimmer's itch ($\chi^2(1, N = 1273) = 98.998$, $P <$ 2.2e-16) (Figure 6.5G), and nearly all respondents said they would visit beaches and lakes in their area more often if they knew that swimmer's itch was not a concern ($\chi^2(2, N = 1293) = 1384.7$, $P <$ 2.2e-16) (Figure 6.5H). While this result is not very surprising, 205 respondents said that their use would be the same either way, suggesting that swimmer's itch is not their biggest concern.

Over the three years of the snail collection survey, 35 out of 15,969 (0.219%) snails collected across lakes Wabamun, Buffalo, Isle, and Gull had patent schistosome infections. From 29 of these samples, we were able to collect enough high-quality DNA for sequencing and species identifications. Twenty of 29 samples came back with high nucleotide identities to *Trichobilharzia* species. Both ML and BI trees agreed on major topologies, and placement of these samples within the avian schistosomes. Within ABGD, both JC and K2 agreed in separation of the alignment into 24 groups (Pmax = 0.0215), while simple distance resulted in 22 groups (Pmax = 0.0215). The two groups that resulted differently from these different methods were for *T. stagnicolae* and *T. regent*. Looking at the pairwise distances, there is not quite enough evidence available to call these different species at this point. For *T. stagnicolae*, there is one sequence that groups separately from the rest (FJ174493). If considered as one group, the average within group divergence is 1.3% with a range of 0–5.5%, which is on the edge of the 5% cut-off in nucleotide divergence for *cox1* we used to delineate species. If the sequence is excluded, the within group divergence is reduced to 0–1.4%. Likewise, for the *T. regent*/cf. *regent*/haplotype *peregra* group, the within group average for the combined set is 1.7% with a range of

0–3.6% nucleotide divergence, which strongly suggests it is one species (Table 6.5).

Some other interesting observations highlighted by the phylogenies is that *T. mergi* (JX456171-2) and that identified as *T.* sp. var. *narochanica* (JQ68153 8 – 40), group together and have a within group divergence of 0.4%, and range of 0–0.7% nucleotide divergence, suggesting they are the same species. Also, sequences FJ174485 and FJ174509 group together, though one is labeled as *T. querquedulae* and the other *T.* sp. D, and they are 100% identical across the sequenced region in this alignment. Because these sequences are not grouping closely to the others identified as *T. querquedulae*, this suggests, they are both *Trichobilharzia* sp. D. Sequences for *T. szidati* also grouped as two separate clades, however, if combined, showed 3.0% within group average divergence with a range of 0–4.8% (Table 6.5).

There is strong evidence that the sequences from this study belong clearly to six different species within the avian schistosomes. Three species did not group closely to other *Trichobilharzia* clades, but rather sister to Avian schistosomatid sp. W2081 (AY829247) and Avian schistosomatid sp. W1285 (AY829246), with average between group nucleotide divergence of 15.2–19.5% between W2081/W1285 and new Avian schistosomatid spp. A, B, and C. Analyses of these sequences among other avian schistosomatid sequences (*Bilharzia polonica*: AY157186, *Dendritobilharzia pulverulenta*: AY157187, *Gigantobilharzia huronensis*: AY157188, *Ornithobilharzia canaliculata*: AY15719 4, *Austrobilharzia terrigalensis*: AY157195, *Allobilharzia visceralis*: EF114219, and *Trichobilharzia ocellata*: AY157189) revealed no strong relationship, other than that previously identified within the *Trichobilharzia* tree. All ABGD methods also supported the groups identified in the *Trichobilharzia* tree (Pmax = 0.007743), and *p* distances supported separation of species based on a 5% cut-off (Avian schistosomatid sp. A within group divergence 0–3.6%; between group divergence for all species 11.5–22.6%) (Figure 6.8). The other species were found to cluster, clearly, within the known groups *T. stagnicolae, T. szidati*, and *T. physellae* (Figure 6.9 & Table 6.5 – 6.6).

Snail intermediate hosts were as expected for the three identifiable schistosome species: *Lymnaea stagnalis* hosting *T. szidati, Stagnicola elodes* hosting *T. stagnicolae*, and *Physella gyrina* hosting *T. physellae* (Sara V Brant and Eric S Loker 2009; Petr Horák, Mikeš, et al. 2015). Of the unknown avian schistosome species (A – C), Physid snails have been noted as a common host for both *Trichobilharzia* and *Gigantobilharzia* spp.; however, *Helisoma trivolvis* snails have only been indicated previously as a possible host for an avian/mammalian schistosome by the identification of emerging cercariae as being brevifurcate-apharyngeate without a finfold (Schmidt and Fried 1997). Unfortunately, we do not have photographs of the one cercariae sample that emerged from *H. trivolvis*, identified here as Avian schistosomatid sp. C, to look for the presence or absence of a finfold. The purpose of looking for this feature is the distinction between schistosome cercariae and cercariae of spirorchid trematodes that also have eye-spots. Spirorchids are trematodes of turtles, that produce cercariae morphologically similar to schistosomes, and utilize *Helisoma* snails for larval development (R.B. Holliman 1971). Despite the lack of morphological characteristics for our sample, the molecular evidence suggests Avian schistosomatid sp. C is not a spirorchid, as nucleotide identity results were highest to *Trichobilharzia* spp., and not *Spirorchis* spp. within GenBank. Even though it is possible this could be an unknown/undescribed species of spirorchid, more molecular similarity to sister species within the same family would be expected. There is strong evidence that this sequence lies within the avian schistosomes, close to *Trichobilharzia* spp.. Further, the only turtle species in Alberta is the Western Painted turtle, that is found in the southernmost part of Alberta, making it unlikely to harbour parasites infecting snails in central Alberta, where the turtles are not present.

High nucleotide identity matches to *Schistosomatium douthitti* were found for nine *cox1* sequences. This identification was confirmed by phylogenetic analysis using an alignment to *S. douthitti* (AY157193), *Heterobilharzia americana* (AY157192) (the other mammalian schistosome species found in North America), and *Schistosoma bovis* (AY157212-out). BI and ML trees agreed on topology. Within group divergence for *S. douthitti* was 0–1.18%, and dif-

ferent from *H. americana* by 22.8–23.27% (Figure 6.10). All methods within ABGD agreed on three groups (JC & K2: Pmax = 0.0077, simple: Pmax = 0.0027). Two snail species were found to host these trematodes, *L. stagnalis* and *S. elodes*. A closely related species to *S. elodes, Lymnaea (Stagnicola) catascopium* and *L. stagnalis* have previously been identified as hosts for *S. douthitti* (Eric S. Loker 1979; Malek 1977). Overall, the evidence is strong that these sequences are representing *S. douthitti* (Table 6.7).

In general, the ABMI datasets are quite extensive, and have a broad range across Alberta for available potential host records for both vertebrates and invertebrates. The overlap of vertebrate and invertebrate records also has excellent coverage (Figures 6.11–19). The greatest concentration of swimmer's itch cases is within central and southern Alberta. Based on Latitude and Longitude values with a 10km buffer, there were 100 points where all three datasets overlapped. These points were mostly distributed between (52°-55°) N (Latitude) and - (115°-112°) W (Longitude) (Figure 6.20). This area is the meeting point of three major watersheds in Alberta: The North Saskatchewan, Battle River, and Red Deer River watersheds, where there is a large concentration of lakes, and so it is no surprise that swimmer's itch is highly concentrated in this area. However, the amount of overlap between vertebrates, invertebrates, and swimmer's itch cases within this region and other parts of Albert is less than ideal. Many gaps remain, where we have swimmer's itch occurrences, but no data on hosts, and vice versa. As most of the vertebrate hosts are migratory waterfowl, their potential to move around the province, and their distribution is therefore relative, and is likely broader than what we have data to support at this time. Invertebrates are less geographically mobile than migratory birds, but many of the snail species of concern in Alberta have broad distributions across the Northern hemisphere.

6.4 Discussion

The current study confirms and significantly expands upon the distribution of swimmer's itch across Canada as revealed in the literature. In fact, many of the

lakes reported in past years continue to serve as areas of consistent swimmer's itch transmission, including Clear Lake (MB), Crescent beach (BC), Cultus Lake (BC), and Lake Nipissing (ON) (Table 6.9). However, this study significantly expands upon our past collective distributions within each province to cover 268 lakes across the country. In Alberta alone, we went from a historical record of one location at Elk Island National Park, to now having case reports from 101 lakes across the province. Considering the size of Canada, the vast number of lakes, and caveats associated with a voluntary survey and localized advertising, it is quite possible we have captured only a small fraction of the actual incidences, albeit far more in a span of 5 years than have been collected over the previous century.

Swimmer's itch, today, remains a non-reportable condition by national health authorities. The unfortunate result of this status is that we cannot compare our surveillance records to any known incidence rates. Likewise, most recreational areas in Canada do not collect demographic information about the number of people at a lake/area at any one time. The best information we could find was in a report by Statistics Canada, in 2013, of a survey of outdoor activities close to the home, reporting that less than 10% of Canadian households engaged in water-related activities such as "swimming, going to the beach, surfing, scuba diving, or snorkeling". They also found that engaging in these activities was associated with higher socioeconomic status (Canada 2013). Unfortunately, this survey does not tell us how many people in Canada live within proximity to water that can be used for recreational purposes, how many people travel for these activities, nor how the data break down regionally. This lack of specific demographic information eliminates the possibility of calculating the true prevalence of swimmer's itch or back-translating the demographics of our survey responses into representative prevalence. While we suspect that, in our study, localized advertising may have impacted the number of reports received to be greater from Alberta, and maybe even British Columbia, by proximity, the voluntary nature of the survey and fact that it did reach every province makes it difficult to know for sure. This issue highlights an important knowledge gap that should be considered in the future for

swimmer's itch surveillance efforts and policy: we need a better understanding of the true prevalence of swimmer's itch and how that translates into effects on people's recreational choices and downstream effects on cultural values that may have important economic impacts.

One of the greatest questions in regard to swimmer's itch research is where efforts (and funding) should be focused. It is a condition that does not leave long-lasting, ill-health effects on people, and so the provinces would be less inclined to spend money from their health budget to fund projects that work towards better understanding swimmer's itch. However, some of the greater effects could come from economic impact as a result of discontinued lake use for recreational purposes. Additionally, how economic impact might change as a result of climate change, greater anthropogenic impact on natural areas, and eutrophication, potentially leading to increased swimmer's itch prevalence.

Despite the brevity of health effects from swimmer's itch, we cannot conclude that there is no impact on the healthcare system. In fact, 100 of our swimmer's itch respondents had rated their itch as severe and having visited a hospital or doctor. Many had described having to go to their family doctor just to find out what it was. Others had described doctors not knowing what the rash was, despite the history of the patient being in the water. There is potential for swimmer's itch to have a greater impact on the healthcare system if swimmer's itch is an emerging disease because there is a general lack of understanding of risk, familiarity with the condition, and knowledge on what to do to prevent or treat it. Therefore, one of the primary areas in need of assessment is that of the economic impact of swimmer's itch on recreational lakes within Canada.

While it is not surprising that our coverage of swimmer's itch cases in Alberta is concentrated where most recreational lakes in the province are located, we would have liked to see more overlap between the swimmer's itch cases and other survey records of invertebrate and vertebrate host species in this area. It is apparent that for many lakes in the province, we need more data related to the potential host species that are correlated with swimmer's itch directly.

The same is true for every province in Canada, considering the small amount of information we have related to schistosomes in general.

The current study has now added seven new schistosome and snail intermediate host records to our understanding of the potential etiological agents responsible for swimmer's itch in Alberta (Figure 6.9, 6.10, and Table 6.8). For a small sampling of lakes within central Alberta, this is substantial diversity for just one family of trematodes. Schistosomes were found at every lake sampled. However, of the total trematode survey completed from 2013-2015, schistosomes had a prevalence rate of 0.2%, suggesting that they are likely rare species in comparison to other digenetic trematodes found in Alberta (M. Gordy et al. 2016; Michelle A Gordy, Sean A Locke, et al. 2017). Alternatively, there could be other factors limiting their ability to infect more snails. This low prevalence rate was surprising to us, especially considering the amount of swimmer's itch reports we were receiving at the same time from the lakes we were sampling. Other reports from the literature tend to find higher prevalence of schistosomes among snails, although in general less snails are collected and examined, and reports are from a single time point (e.g. 1.24 – 1.8% in Poland, 3456 and 299 snails examined, respectively (Marszewska, Cichy, Bulantová, et al. 2018; Marszewska, Cichy, Heese, et al. 2016); 0.9 – 1.3% in Argentina, 402 snails examined (Sara V. Brant et al. 2017); 2.6% in Belgium, 270 snails examined (Caron et al. 2017)). Nevertheless, if the rate of prevalence for schistosomes in snails uncovered through this study is indicative of that for lakes across Alberta, or elsewhere, this holds significance for control measures.

There remain many knowledge gaps across the country on schistosome species presence, hosts and distributions, and life cycle timing. These gaps inhibit our ability to make predictive models, and properly assess risk. It is the basic biology and ecology of these parasites and their hosts that will best inform future risk assessments and potential management strategies. For instance, the discovery that the primary species responsible for swimmer's itch transmission in Michigan is *T. stagnicolae*, which specifically utilizes mergansers as definitive hosts in this area, has led to control initiatives based on

host relocation that has shown successful reduction in snail infection prevalence within control lakes (Rudko et al. 2018). Whether this type of strategy is possible in Alberta, or elsewhere in Canada, is difficult to say because we have more than one species of schistosome utilizing multiple snail hosts, and many definitive host species within the same lake, adding levels of complexity to the problem. By furthering our understanding of the diversity of host-parasite relationships and ecological drivers behind them, we may be able to develop strategies for control and better surveillance.

In the same light, environmental assessments of lakes in which swimmer's itch is a common occurrence may prove to provide important links between species presence, life cycle timing, and rates of swimmer's itch. So far, we know there are higher rates of swimmer's itch generally in July and August in Canada, but because these are the warmest months, it is also when more people are out on the lakes and swimming. This is not generally helpful information to people trying to decide whether or not to swim.

Considering that only one snail infected with a schistosome is necessary to cause swimmer's itch, the concept of control or management of the issue is an arduous task. Past efforts to kill off the snail population, or even treat the birds with anthelminthics have been costly, labor-intensive, and unsuccessful in the long-term (C. L. Blankespoor, R. L. Reimink, and Blankespoort 2001; Ronald L. Reimink, DeGoede, and Harvey D. Blankespoor 1995). These failures necessitate new, innovative solutions.

There are several areas in need of further research and development. First is the need for an accurate and sensitive method to monitor for the presence of schistosome cercariae in the water, as snail collections have proven to be an inefficient and ineffective method for surveillance. Work has already begun to develop and test methods using qPCR-based strategies (Jothikumar et al. 2015; Rudko et al. 2018). Molecular detection of larval cercariae in the water may overall be a better assessment of risk, and this strategy would allow for timely communication of risk to lake users.

Second, and perhaps the most important, is the need for better communication and education for lake users, lake managers, and health authorities on

swimmer's itch as a risk in natural water bodies. While many recreational lakes do use signs near public beaches to warn about swimmer's itch, this is not a standard. Often, these signs will also do a poor job at describing the situation or what a person should be expecting (personal experience). As most people using the lakes for recreation are visitors, there should be no expectation that they should know the history of swimmer's itch at a given site, especially considering that even from published studies, we were unable to determine whether it is a risk at a particular lake. Many survey respondents, after having experienced swimmer's itch, noted that they believed there should have been a warning sign. The development of an effective warning sign is a tricky business. The human information processing model describes multiple steps a person must go through to change their behaviour: first, the warnings must capture the person's attention, then they must comprehend the information, then believe the information and not dismiss it based on beliefs or attitudes, and finally, the person must be motivated to comply (Wogalter 2014). While the first and last steps are easy to accomplish with a health-related sign, the middle two steps are quite challenging in this context. For one, many people believe that swimmer's itch is caused by algae (personal communication), which is not surprising, as often algae or cyanobacterial blooms are quite obvious, visible, and often have a negative stigma attached. Describing that swimmer's itch is caused by a microscopic larval parasite with a complex life cycle that involves both snails and birds/mammals is challenging. The explanation requires adequate comprehension of what a parasite is, what a larval cercaria is, and how a complex life cycle works. Because it cannot be seen without the aid of a microscope, schistosome cercariae as the cause of swimmer's itch is less believable and more abstract than something like algae that a person can see in the water. It is possible that the use of poorly developed swimmer's itch signs might act as a deterrent to recreational use of lakes, which, from an economic perspective, is not ideal. Therefore, it is of utmost importance that if swimmer's itch warning signs are used at lakes, that they are accompanied with educational campaigns and that the information is made accessible. Other challenges arise in defining swimmer's itch from a clinical perspective

(how many papules does a person need to have?), defining an outbreak (how many people must contract it?), and ensuring communication between lake users and health authorities.

From the success of our voluntary survey, it is apparent that people are willing to participate in efforts to address the swimmer's itch problem. There are many avenues for future swimmer's itch research that could benefit from a citizen science approach, considering that surveillance across the entire country is needed. We greatly encourage the development of applications that help towards identifying waterfowl and aquatic snails, as this could provide a strong foundation from which to develop more specific strategies for research and management options. Including people in the research on topics that directly affect them can also be a good strategy for education and communication. A national web-based surveillance system could help us better track swimmer's itch, organize the information, and help us understand where and when it is occurring in a real-time format. This study strongly supports the concept that a citizen science approach can be an effective strategy towards the continued surveillance of swimmer's itch in Canada. Ultimately, this problem would lend well to a systems-thinking approach in that it is too complex for an individual group to conquer. This problem requires a transdisciplinary, collaborative approach that incorporates the public into surveillance programs for improved overall management and communication.

6.5 Conclusions

Swimmer's itch is a greater environmental health hazard across Canada than previous literature would have suggested. This study has provided a proof-of-concept for the utility of a self-reporting surveillance system for swimmer's itch in Canada. However, there are multiple avenues for future research that we should support to better address the problem. While I have discussed many of the knowledge gaps in our collective understanding of schistosomes and swimmer's itch in Canada, one avenue we should focus on in is attaining knowledge of the relationships between schistosomes and their definitive ver-

tebrate hosts. There is no doubt that a large contribution to the swimmer's itch problem is by migratory waterfowl that shed the eggs into, likely, multiple water bodies. If we knew more about their population sizes, their timing of arrival for Spring migration, and had better information on their distributions, we could more specifically analyze how this information may connect to the trends we see in both snail infections and swimmer's itch occurrences. It is especially intriguing that in both examinations of trematode component community diversity (chapter 5) and swimmer's itch occurrences, we find peaks appearing in July and August. It is currently unknown, and not testable with this dataset, whether there is a relationship between higher rates of trematode diversity and more occurrences of swimmer's itch. Considering the previous discussions in this thesis about the diversity-disease relationship, this hypothesis is based on having an increased diversity of hosts that act as a decoy, and thus reduce disease. In this case, it is the diversity of trematodes that is high, which we might infer could be the result of either greater abundances of compatible snail hosts, a reduction in snail hosts that were acting as decoys, or that the time it takes for most trematodes to develop from the time they colonize results in a similar timing of emergence. This would be an excellent avenue for future research.

Table 6.1: Swimmer's itch survey questions and response types and options

Question	**Response Types or Options**
Name of lake/water body	Open Response
Where is the lake/ water body located (city, province, country)?	Open Response
Date you contracted swimmer's itch	Open Response
How many people in your party also contracted swimmer's itch on the same day not including yourself? §	Open Response
How would rate the severity of this case of swimmer's itch?	Mild (small rash, some itching)/ Medium (large rash area, very itchy, burning sensation)/Severe (went to doctor/hospital it was so bad)
To your knowledge, is swimmer's itch a common occurrence at this lake/water body?	Yes/No
Do you own property at this lake/water body, or do you visit?	Own property/Visitor
Do you frequently see waterfowl at this lake/water body?	Yes/No
If yes, please list the types if you know them (ducks, geese, etc.)	Open Response
Have you ever seen snails at this lake/water body?	Yes /No
Describe the water conditions (Temperature, visibility, amount of vegetation, etc.)	Yes/No
To your knowledge, was there a Blue-Green Algae warning at this lake the day you visited?	Yes/No
Do you use this lake/water body less often than you would like because of swimmer's itch	Yes/No
Do you feel there is adequate information available to you about swimmer's itch?	Yes/No/Do not think information is necessary
If you answered yes, which resources do you consult?	Open Response
Would you visit lakes and beaches in your area more often if swimmer's itch wasn't a concern?	Yes/No/The same amount

Continued on next page

Table 6.1 – *Continued from previous page*

Question	**Response Types or Options**
How did you hear about us?	Google search/Twitter/Flyer or handout/Information booth/Word of mouth/Community board posting/News from an internet site/News on the radio/News on Television/Other
If other, please list where you heard of us?	Open Response
Open-ended comments	Open Response

[§] Question added in 2014

Table 6.2: Summary of swimmer's itch survey reports and cases over five years. Reports are individual survey events, while cases are the addition of reports and the additional number of people affected with swimmer's itch at the same time as the person reporting. Brackets after the number of cases represents the maximum number of cases per report at that time.

Month	**May**		**June**		**July**		**August**		**September**				
Year	*Reports*	*Cases*	*Reports*	*Cases*	*Reports*	*Cases*	*Reports*	*Cases*	*Reports*	*Cases*	**Total Reports**	**Additional Cases**	**Total Cases**
2013	NA	NA	19	NA	101	NA	149	NA	25	NA	295	NA	295
2014	0	0	1	2 [1]	64	242[13]	75	281[51]	6	14[2]	144	388	532
2015	3	6[2]	78	283[12]	144	540[15]	102	355[17]	18	48[5]	347	892	1239
2016	7	18[6]	49	169[20]	128	442[12]	56	176[11]	6	31[10]	248	596	844
2017	1	3[3]	16	26[7]	152	432[14]	99	185[12]	13	22[4]	280	665	945
Average	2.75	9	32.6	120	117.8	414	96.2	249.25	13.6	28.75	262.8	635.25	771
Difference	6.25		87.4		296.2		153.05		15.15		508.2		

Table 6.3: Model comparisons for the effect of month and year on the number of swimmer's itch cases

	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	logLik	AIC	BIC	dAIC	df	weight
Model 1: cases ~ year * month	0	-1.49E-14	NA	NA	NA	-58.05524	154.1105	172.0548	0	19	1
Model 2: cases ~ month + year	11	359.2	-11	-359.24	< 2.2e-16	-237.67633	491.3527	498.9082	337.2	8	<0.001
Model 3: cases ~ year	15	3335	-4	-2975.77	< 2.2e-16	-1725.56266	3459.1253	3462.9031	3305	4	<0.001
Model 4: cases ~ month	14	686.3	1	2648.69	< 2.2e-16	-401.217	812.434	817.1562	658.3	5	<0.001

Table 6.4: Swimmer's itch survey comments summary

Codes and Themes	Code and Theme Counts		Subtheme Counts
Seeking information about swimmer's itch	11		
Themes			
wants an effective remedy/prevention	5		
other	6		
Desires swimmer's itch warning	53		
Themes			
generally someone should post warnings	18		
local authorities should post information	14		
signs should be posted at the beach	15		
website should warn people	5		
other	1		
Providing more information	232		
Themes			
clarification on other survey answers	17		
describing personal awareness or lack thereof	42		
Subthemes		*unaware previous to report*	22
		aware but not expecting it	12
		expects to know through warning system	13
		other	2
describing the situation	163		
Subthemes		*description of who got it*	63
		severity	57
		location	45
		timing	40
		signs or lack thereof	27
		environment	4
		prevention methods	9
		area of body affected	14
		animals seen	2
		signs or lack thereof	30
		other	90
giving anecdotal advice	16		
Subthemes		*swimming location and water quality*	8
		methods for prevention and treatment	8
personal history of swimmer's itch	49		
Subthemes		*had it previously*	26
		first time with itch at this lake	21
		sensitive to swimmer's itch	7
		other	2
Providing opinion	66		
Themes			
appreciation for our website	15		
concerned about children	9		
may have to see a doctor	5		
mistrust/fear	6		
wish there were showers/facilities	6		
other	26		

Table 6.5: Nucleotide divergence values for *Trichobilharzia* sequences. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. The average intraspecific divergence is given on the diagonal. Interspecific divergence values are given within parentheses next to species names as a percent range. Values in red represent those that go beyond the delimitation standard. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 85 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 417 positions in the final dataset.

	A. vari.	Avian sp. A	Avian sp. B	Avian sp. C	*T. anseri*	*T. brantae*	*T. franki*	*T. mergi*	*T. phys.*	*T. quer.*
Austrobilharzia variglandis (out)	-	0.018	0.019	0.019	0.018	0.019	0.019	0.019	0.018	0.019
Avian schistosomatid sp. A (0.0-3.6%)	0.187	**0.011**	0.016	0.018	0.017	0.018	0.018	0.017	0.017	0.016
Avian schistosomatid sp. B	0.206	0.141	-	0.018	0.018	0.018	0.017	0.017	0.017	0.017
Avian schistosomatid sp. C	0.204	0.181	0.185	-	0.017	0.019	0.018	0.017	0.017	0.018
Trichobilharzia anseri (0.0-0.7%)	0.181	0.166	0.184	0.157	**0.002**	0.017	0.014	0.015	0.014	0.015
Trichobilharzia brantae (0.0%)	0.187	0.18	0.187	0.187	0.146	**0.000**	0.016	0.017	0.016	0.016
Trichobilharzia franki (0.0-1.7%)	0.197	0.169	0.171	0.175	0.116	0.141	**0.006**	0.015	0.012	0.012
Trichobilharzia mergi (0.0-0.7%)	0.182	0.175	0.176	0.15	0.112	0.153	0.115	**0.004**	0.015	0.014
Trichobilharzia physellae (0.0-1.0%)	0.185	0.175	0.163	0.16	0.116	0.141	0.074	0.119	**0.004**	0.012
Trichobilharzia querquedulae (0.2-2.6%)	0.206	0.158	0.171	0.171	0.125	0.133	0.072	0.11	0.081	**0.011**
Trichobilharzia regenti (0.0-3.6%)	0.185	0.175	0.175	0.154	0.119	0.12	0.11	0.089	0.106	0.109
Trichobilharzia sp. A (0.2-1.4%)	0.195	0.166	0.169	0.173	0.124	0.131	0.083	0.125	0.095	0.081
Trichobilharzia sp. B	0.187	0.173	0.175	0.165	0.129	0.141	0.071	0.104	0.078	0.068
Trichobilharzia sp. C (1.2%)	0.198	0.182	0.173	0.187	0.127	0.129	0.084	0.127	0.086	0.088
Trichobilharzia sp. D (0.0%)	0.177	0.186	0.165	0.149	0.12	0.127	0.114	0.098	0.119	0.111
Trichobilharzia sp. E (0.0-0.5%)	0.171	0.167	0.177	0.153	0.103	0.149	0.119	0.112	0.125	0.124
Trichobilharzia stagnicolae (0.0-5.5%)*	0.175	0.162	0.162	0.153	0.125	0.14	0.127	0.124	0.135	0.121
Trichobilharzia szidati (0.5-4.8%)	0.194	0.158	0.158	0.156	0.106	0.123	0.111	0.102	0.112	0.103
Avian schistosomatid sp. W1285	0.194	0.195	0.189	0.177	0.155	0.161	0.157	0.147	0.157	0.143
Avian schistosomatid sp. W2081	0.218	0.152	0.17	0.175	0.182	0.173	0.157	0.169	0.169	0.158

Table 6.5 - *Columns continued*

	T. regenti	*T.* sp. A	*T.* sp. B	*T.* sp. C	*T.* sp. D	*T.* sp. E	*T. stag.*	*T. szidati*	W1285	W2081
Austrobilharzia variglandis (out)	0.017	0.018	0.018	0.019	0.018	0.018	0.018	0.018	0.019	0.019
Avian schistosomatid sp. A (0.0-3.6%)	0.017	0.018	0.018	0.017	0.018	0.017	0.017	0.017	0.019	0.017
Avian schistosomatid sp. B	0.017	0.017	0.017	0.016	0.017	0.018	0.017	0.016	0.018	0.018
Avian schistosomatid sp. C	0.017	0.018	0.018	0.018	0.017	0.017	0.016	0.017	0.018	0.019
Trichobilharzia anseri (0.0-0.7%)	0.014	0.014	0.015	0.014	0.014	0.014	0.015	0.014	0.017	0.018
Trichobilharzia brantae (0.0%)	0.015	0.016	0.016	0.016	0.016	0.017	0.016	0.015	0.017	0.018
Trichobilharzia franki (0.0-1.7%)	0.014	0.013	0.012	0.012	0.015	0.015	0.015	0.014	0.017	0.017
Trichobilharzia mergi (0.0-0.7%)	0.012	0.016	0.014	0.015	0.014	0.015	0.015	0.013	0.017	0.017
Trichobilharzia physellae (0.0-1.0%)	0.014	0.013	0.012	0.013	0.015	0.016	0.015	0.014	0.018	0.017
Trichobilharzia querquedulae (0.2-2.6%)	0.014	0.013	0.012	0.012	0.014	0.015	0.015	0.013	0.016	0.017
Trichobilharzia regenti (0.0-3.6%)	**0.017**	0.014	0.013	0.014	0.014	0.013	0.014	0.013	0.015	0.017
Trichobilharzia sp. A (0.2-1.4%)	0.1	**0.01**	0.011	0.013	0.014	0.015	0.015	0.013	0.017	0.017
Trichobilharzia sp. B	0.086	0.058	-	0.012	0.014	0.015	0.015	0.013	0.016	0.016
Trichobilharzia sp. C (1.2%)	0.102	0.091	0.079	**0.012**	0.015	0.016	0.015	0.014	0.016	0.017

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Table 6.5 – *Continued from previous page*

	T. regenti	*T.* sp. A	*T.* sp. B	*T.* sp. C	*T.* sp. D	*T.* sp. E	*T. stag.*	*T. szidati*	W1285	W2081
Trichobilharzia sp. D (0.0%)	0.103	0.107	0.103	0.12	**0.000**	0.014	0.014	0.013	0.017	0.017
Trichobilharzia sp. E (0.0-0.5%)	0.102	0.125	0.122	0.132	0.092	**0.003**	0.014	0.013	0.016	0.018
Trichobilharzia stagnicolae (0.0-5.5%)*	0.114	0.121	0.125	0.126	0.109	0.104	**0.013**	0.014	0.017	0.017
Trichobilharzia szidati (0.5-4.8%)	0.109	0.103	0.102	0.12	0.1	0.1	0.118	**0.030**	0.016	0.017
Avian schistosomatid sp. W1285	0.118	0.149	0.132	0.155	0.146	0.14	0.155	0.156	**-**	0.017
Avian schistosomatid sp. W2081	0.165	0.152	0.141	0.167	0.161	0.173	0.144	0.153	0.168	**-**

Table 6.6: Host association and geographical origin of specimens used within the *Trichobilharzia cox1* phylogeny.

Species	Host	Host Type	Location	GenBank Accession Number(s) *cox1*	Reference
Austrobilharzia variglandis (out)	*Larus delawarensis*	3	USA	AY157196	Lockyer, A.E., et al., 2003, Parasitology 126(Part3):203-204
Avian Schistosomatid sp. A	*Physella gyrina*	1	Canada: Alberta, Buffalo Lake, Isle Lake	MH168789, MH168790, MH168795, MH168796	Gordy et al. 2018 Env. Health
Avian Schistosomatid sp. B	*Physella gyrina*	1	Canada: Alberta, Lac La Nonne	MH168785	Gordy et al. 2018 Env. Health
Avian Schistosomatid sp. C	*Helisoma trivolvis*	1	Canada: Alberta, Wabamun Lake	MH168793	Gordy et al. 2018 Env. Health
Avian Schistosomatid sp. W1285	*Biomphalaria sudanica*	1	Lake Victoria, Kenya Fisheries Association landing site, Kisumu, Kenya	AY829246	Brant, S.V., et al., 2006, J. Parasitol., 92(1):77-88.
Avian Schistosomatid sp. W2081	*Ceratophallus natalensis*	1	Lake Victoria, Kenya Fisheries Association landing site, Kisumu, Kenya	AY829247	Brant, S.V., et al., 2006, J. Parasitol., 92(1):77-88.
Trichobilharzia anseri	*Radix balthica, Anser anser*	1, 3	Iceland: Family park, Reykjavik; France: Der-Chantecoq Lake, Marne	KP901380, KP901381, KP901382, KP901383, KP901384, KP901385	Jouet, D., et al., 2015, Infect. Genet. Evol., 34: 298-306.
Trichobilharzia brantae	*Gyraulus parvus, Chen caerulescens*	1, 3	USA; Canada	FJ174482, FJ174484	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia franki	Unidentified	1	Czech Republic	FJ174530	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia franki	*Radix auricularia*	1	France: Der-Chantecoq Lake; Beauvais; Annecy Lake; Strasbourg	HM131198, HM131199, HM131200, HM131201, HM131202	Jouet, D. Unpublished
Trichobilharzia mergi	*Mergus serrator*	3	Iceland: Botsvatn Lake	JX456171, JX456172	Kolarova, L., et al., 2013, Parasitol. Int., 62(3): 300-308.
Trichobilharzia physellae	*Physella gyrina, Mergus merganser, Aythya affinis*	1, 3	USA: Michigan; New Mexico	FJ174519, FJ174520, FJ174521, FJ174522, FJ174523	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia physellae	*Physella gyrina*	1	Canada: Alberta, Lac La Nonne	MH168784	Gordy et al. 2018 Env. Health
Trichobilharzia querquedulae	*Anas clypeata, Anas discors*	3	USA: California; Alaska; Florida	FJ174506, FJ174507, FJ174508, FJ174510, FJ174511	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.

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Table 6.6 – *Continued from previous page*

Species	Host	Host Type	Location	*cox1*	Reference
Trichobilharzia querquedulae	*Anas smithii, Anas rhynchotis, Anas versicolor*	3	South Africa: Free State; New Zealand: South Island; Argentina: Corrientes	KU057180, KU157181, KU057182, KU057183, KU057184	Ebbs, E.T., et al., 2016, Int. J. Parasitol., 46(10): 669-677.
Trichobilharzia regenti	*Cygnus color, Mergus merganser, Anas platyrhynchos, Anas clypeata*	3	France	HM439500, HM439501, HM439502, HM439503, HM439504, HM439505	Jouet, D., et al., 2010, Parasitol. Res. 107(4): 923-930.
Trichobilharzia regenti	*Radix peregra*	1	Czech Republic	AY157190	Lockyer, A.E., et al., 2003, Parasitology 126(Part3):203-204
Trichobilharzia regenti	*Anas clypeata, Anas platyrhynchos*	3	Iran: Fereydoon Kenar	KR108325, KR108326	Fakhar, M., et al., 2016, Parasitol. Int., 65(2):151-8.
Trichobilharzia sp. A	*Anas americana*	3	USA: New Mexico; California; Alaska	FJ174524, FJ174525, FJ174526, FJ174527	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia sp. B	*Anas americana*	3	USA: Alaska	FJ174528	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia sp. C	*Lophodytes cucullatus*	3	USA: Pennsylvania	FJ174529	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia sp. C	*Aix sponsa*	3	USA	KJ855996	Pinto, H.A., et al., 2014, Acta Tropica 138: 38-43.
Trichobilharzia sp. D	*Stagnicola* sp.	1	Canada	FJ174485, FJ174509*	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia sp. E	*Stagnicola* sp., *Anas acuta*	1, 3	Canada	FJ174483, FJ174486, FJ174487	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia sp. var.*narochanica*	*Radix ampla*	1	Belarus: Naroch Lake	JQ681538, JQ681539, JQ681540	Chrisanfova, G.G., et al., 2009, Dokl. Biochem. Biophys. 428: 268-272.
Trichobilharzia stagnicolae	*Stagnicola* sp., *Stagnicola emarginata, Mergus merganser*	1, 3	USA: New Mexico; Michigan	FJ174490, FJ174491, FJ174492, FJ174494	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia stagnicolae	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	KT831352	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
Trichobilharzia stagnicolae	*Stagnicola elodes*	1	Canada: Alberta, Isle Lake	MH168781, MH168782, MH168786, MH168787, MH168788	Gordy et al. 2018 Env. Health
Trichobilharzia szidati	*Lymnaea stagnalis*	1	Czech Republic	AY157191	Lockyer, A.E., et al., 2003, Parasitology 126(Part3):203-204

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Table 6.6 – *Continued from previous page*

Species	Host	Host Type	Location	*cox1*	Reference
Trichobilharzia szidati	*Stagnicola elrodi, Lymnaea stagnalis*	1	USA: Montana, Flathead Lake; Michigan, Blind Sucker Lake	FJ174495, FJ174496	Brant, S.V., and Loker, E.S., 2009, J. Parasitol. 95(4):941-963.
Trichobilharzia szidati	*Lymnaea stagnalis*	1	Canada: Alberta, Gull Lake	MH168783	Gordy et al. 2018 Env. Health
Trichobilharzia szidati	*Lymnaea stagnalis*	1	Canada: Alberta, Buffalo Lake	KT831375	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.

§Sequence updated in present study; *** Novel by molecular phylogeny;
Host Type: 1=First Intermediate, 2=Second Intermediate, 3=Definitive

Table 6.7: Host association and geographical origin of specimens used within the *Schistosomatium cox1* phylogeny.

Species	Host	Host Type	Location	GenBank Accession Number(s) *cox1*	Reference
Schisotosoma bovis (out)	*Mus musculus* (exp.)	3	Tanzania	AY157212	Lockyer, A.E., et al., 2003, Parasitology 126(Part3):203-204
Heterobilharzia americana	*Mesocricetus auratus*	3	USA	AY157192	Lockyer, A.E., et al., 2003, Parasitology 126(Part3):203-204
Schistosomatium douthitti	*Mesocricetus auratus*	3	USA	AY157193	Lockyer, A.E., et al., 2003, Parasitology 126(Part3):203-204
	Stagnicola elodes		Canada: Alberta, Gull Lake	KT831376	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Stagnicola elodes, Lymnaea stagnalis	1	Canada: Alberta, Buffalo Lake , Gull Lake, Wabamun Lake	MH168791, MH168794	Gordy, M.A., et al., 2018 Env. Health

§Sequence updated in present study; *** Novel by molecular phylogeny;
Host Type: 1=First Intermediate, 2=Second Intermediate, 3=Definitive

Table 6.8: Historical literature review of swimmer's itch in Canada

Province	Schistosome spp.	Intermediate Hosts	Definitive Hosts	Location/Outbreaks/Cases	Reference(s)
Alberta				Authentic records of dermatitis at Peace River district[5]	Cort, W.W. (1936) Am. J. Hyg. 24(2):318-333
	Cercaria elvae[2]	*Lymnaea stagnalis*		Summer 1939: Outbreaks in Lakes at Elk Island Park	Hadwen, S. & Fallis, A.M. (1940) Can. Pub. Health Assoc. 31(1):30
	Dendritobilharzia pulverulenta		Eared Grebe (*Podiceps nigricollis*)		Vande Vusse, F.J. (1980) J. Parasitol. 66(5):814-822
	Trichobilharzia stagnicolae	*Stagnicola elodes*		Summer 2013-2014: Isle Lake	This study
	Trichobilharzia szidati	*Lymnaea stagnalis*		Summer 2013-2014: Gull Lake and Buffalo Lake (The Narrows)	
	Trichobilharzia physellae	*Physella gyrina*		August 2013: Lac la Nonne	
	Avian Schistosomatid sp. A	*Physella gyrina*		Summer 2015: Isle Lake and Buffalo Lake	
	Avian Schistosomatid sp. B	*Physella gyrina*		August 2013: Lac la Nonne	
	Avian Schistosomatid sp. C	*Helisoma trivolvis*		July 2015: Wabamun Lake	
	Schistosomatium douthitti	*Lymnaea stagnalis & Stagnicola elodes*		Summer 2013-2015: Gull Lake, Wabamun Lake, Buffalo Lake (The Narrows)	
British Columbia	*Cercaria elvae*[2]	*Lymnaea stagnalis wasatchensis*		Paul Lake	Cort, W.W. (1936) Am. J. Hyg. 24(2):318-333
	Trichobilharzia adamsi	*Physa cf. coniformis*	Peking ducklings*	Summer 1950: Severe outbreak at Cultus Lake	Edwards & Jansch (1955) Can. J. Zool. 33:182-194
	Cercaria columbiensis	*Physa cf. coniformis*			
	Cercaria stagnicolae[1]	*Lymnaea emarginata angulata*		Summer 1963: Two outbreaks at Cultus Lake	Howard, T.E. & Walden, C.C. (1965) J. Appl. Ecol. 2(1):121-135
	Cercaria physellae[3]	*Physa* spp. (*P. ampullacea, P.coniformis, P.occidentalis)*			
	Trichobilharzia stagnicolae	*Stagnicola catascopium*	Common Merganser (*Mergus merganser*)	Cultus Lake	Leighton, B.J., et al. (2000) Parasitol. Int. 49(1):9-17
	Trichobilharzia physellae	*Physa* sp.	Common Merganser (*Mergus merganser*)	Cultus Lake	
	Gigantobilharzia sp.	*Gyraulus parvus*	Unidentified Blackbird	Cultus Lake	
	Austrobilharzia variglandis	*Ilyanassa obsoleta*		Summer 2001: 36 cases; Summer 2002: 44 cases at Crescent Beach	Leighton, B.J. et al. (2004) Env. Health Rev. 48:5-13
Saskatchewan	*Cercaria elvae*[2]	*Lymnaea stagnalis jugularis, L. pallustris nuttalliana, & Physa* sp.		Found widely across the province[2]	Cort, W.W. (1936) Am. J. Hyg. 24(2):318-333
Manitoba	*Cercaria elvae*[2]	*Lymanea stagnalis jugularis*		Clear Lake	Swales, W. (1936) Can. J. Res. 14d(1):6-10
	Cercaria sp.	*Stagnicola emarginata canadensis*			
	Cercaria wardlei	*Limnaea obrussa & Stagnicola emarginata canadensis*		Summer 1933: Over 55,000 visitors to this lake, of which over 50% contracted swimmer's itch at Clear Lake	McLeod, J.A. (1934) Can. J. Res. 10(4):394-403; McLeod, J.A. (1940) Can. J. Res. 18d(1):1-28

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Table 6.8 – *Continued from previous page*

Province	Schistosome spp.	Intermediate Hosts	Definitive Hosts	Location/Outbreaks/Cases	Reference(s)
	Cercaria stagnicolae[1]	*Stagnicola emarginata canadensis*			McLeod, J.A. (1940) Can. J. Res. 18d(1):1-28
	Cercaria dermolestes sp. nov.	*Stagnicola palustris elodes*		Southern Manitoba	
	Ornitohobilharzia aviani sp. nov.		Ring-billed Gulls (*Larus delawarensis*) & Herring Gulls (*Larus argentatus*)	Clear Lake, Lake Winnipeg, Lake Winnipegosis	
	Ornitohobilharzia filamenta sp. nov.		Ring-billed Gulls (*Larus delawarensis*) & Herring Gulls (*Larus argentatus*)		
	Pseudobilharzia querquedulae[3]		Blue-winged Teal (*Anas discors*)		
	Microbilharzia mantobensis[4]		Canvas back duck (*Aythya valisineria*)	Lake Frances	
	Microbilharzia canadensis[4]		Canvas back duck (*Aythya valisineria*)		
	Microbilharzia lari		Ring-billed Gulls (*Larus delawarensis*) & Herring Gulls (*Larus argentatus*)	Lake Winnipeg & Clear Lake	
	Cercaria elvae[2]	*Lymnaea stagnalis*		Summer 1961: Severe outbreaks at several uncontrolled lakes; Schistosomes found in Lake Norris, Lake Winnipeg, and Lake Manitoba	Farley, J. (1962) Can. J. Zool. 40:131-133
	Schistosomatium douthitti	*Lymnaea stagnalis*	Muskrat (*Microtus pennsylvanicus*)	Southern Manitoba	
	Gigantobilharzia lawayi		Ring-billed Gulls (*Larus delawarensis*) & Herring Gulls (*Larus argentatus*)	Twin beaches at Lake Manitoba, Grand beach at Lake Winnipeg, Red River at St. Andrews, and Moose Lake	Farley, J. (1964) Thesis: University of Manitoba
	Gigantobilharzia gyrauli	*Physa gyrina*	Red-winged Blackbird (*Agelaius phoeniceus*), Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*), Brown-headed Cowbird (*Molothrus ater*), Common Grackle (*Quiscalus quiscula*)	Long Lake, Meadows, St. Andrews, Norris Lake, North Shoal Lake, Twin beaches at Lake Manitoba	
	Gigantobilharzia totani		Greater Yellow-legs (Totanus melanoleucus), Lesser Yellow-legs (T. flavipes)	Lake Manitoba, Shoal Lake, Meadows, Moose Lake	
	Trichobilharzia querquedulae		Blue-winged Teal (*Anas discors*), Shoveller (*Spatula clypeata*)	Southern Manitoba	
	Austrobilharzia lari		Bonaparte's Gull (*Larus philadelphia*)	Southern Manitoba	
	Ornithobilharzia canaliculata		Bonaparte's Gull (*Larus philadelphia*)	Southern Manitoba	
				Summer 1977: 6 cases investigated from Clearwater Lake and St. Malo, with additoinal outbreak reports from Gull Lake and Hecla Island	Sekla, L. et al. (1978) Can. J. Pub. Health 69(6):475-480

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Table 6.8 – *Continued from previous page*

Province	Schistosome spp.	Intermediate Hosts	Definitive Hosts	Location/Outbreaks/Cases	Reference(s)
	Trichobilharzia brantae	*Gyraulus parvus*	Snow Goose (*Chen caerulescens*)	Churchill	Brant, S.V. & Loker, E.S. (2009) J. Parasitol. 95(4):941-963
	Trichibilharzia sp. D	*Stagnicola* sp.			
	Trichobilharzia sp. E	*Stagnicola* sp.	Northern Pintail (*Anas acuta*)		
Ontario				Summer 1954: 53 cases at Lake Nipissing	Mitchell, J.C. (1954) A.M.A. Archives of Dermatology and Syphilology 70(6):805-808
	Schistosomatium douthitti	*Lymnaea stagnalis apressa & Lymnaea palustris elodes*		Chaffey's Locks, Crosby, Cameron Lake, Glen Arm, Reaboro, Newcastle, Rondeau Park, Peterboro, Black Lake	Bourns, T.K.R. (1961) Can. J. Zool. 39(1):43-46
Quebec	*Trichobilharzia cameroni* sp. nov.	*Physa gyrina*	Canaries*, Ducklings*, Pigeons*	Summer 1949: Outbreak near Montreal in vicinity of Ste. Anne de Bellevue and along the Ottawa River	Wu, L. (1953) Can. J. Zool. 31:351-373
				Summer 1988: 74 cases from 5 regions - Soit Hull, Portneuf, Lac St-Francois, Rimouski, and Rouyn-Noranda	Levesque, B. (1990) Can. J. Pub. Health 81(4):329-330
	Cercaria ocellata[2]	*Physa gyrina*		Summer 1998: Outbreak at Lac Nairn; Summer 1999: 63 cases from Lac Beauport	Levesque, B. et al. (2002) Epi. Infect. 129(2):379-386
New Brunswick	*Trichobilharzia stagnicolae*	*Lymnaea emarginata*		Lake Magaguadavic & Lake Utopia	Farley, J. (1967) Can J. Zool. 45(6):1300-1302
	Cercaria catascopii n. sp.	*Physa gyrina*		Prior to 1976: 3 cases at Lake Magaguadavic, 2 cases at Lake Utopia, & 1 case at Lake Chamcook; all lakes had snails with identified schistosome species	Scott, M.E. & Burt, M.D. (1976) Can. J. Zool. 54:2200-2207
Nova Scotia	*Trichobilharzia stagnicolae*	*Lymnaea emarginata*		Lake Mush-a-Mush & Lake Ainslie	Farley, J. (1967) Can J. Zool. 45(6):1300-1302
Prince Edward Island[5]	*Austrobilharzia variglandis*	*Nassarius obsoletus*		Bay of Fundy	Farley, J. (1967) Can J. Zool. 45(6):1300-1302

[1] - *Trichobilharzia stagnicolae*
[2] - *Trichobilharzia ocellata*
[3] - *Trichobilharzia physellae*
[4] - *Austrobilharzia variglandis* or *A. terrigalensis* according to Farley, J. (1971) J. Helminthol. XLV:289-320
[5] - as derived from personal communication
*Experimental infections

Table 6.9: Summary of lakes reported throughout the course of the swimmer's itch survey

Province	Lake	Cases	Years Reported
Alberta	Athabasca River at Whispering Hills	2	2015
	Baptiste Lake	1	2016
	Barrier Lake	1	2016
	Battle Lake	1	2015
	Bear Lake	8	2014
	Bear Trap Lake	13	2015
	Bearhills Lake	1	2013
	Bellis Beach Lake	1	2013
	Buck Lake	14	2015, 2016, 2017
	Buffalo Lake	423	2013, 2014, 2015, 2016, 2017
	Burnstick Lake	1	2013
	Calling Lake	2	2013, 2017
	Cameron Lake	29	2015, 2017
	Capt. Ayr Lake	1	2013
	Chestermere Lake	5	2014, 2015
	Chickenhill Lake	7	2016, 2017
	Chump Lake	10	2013, 2015, 2016
	Cold Lake	55	2013, 2014, 2015, 2016
	Cornwall Lake	1	2013
	Cow Lake	2	2013
	Crimson Lake	2	2013
	Dilberry Lake	18	2015, 2017
	Edith Lake	15	2015, 2017
	Elkwater Lake	2	2016
	Fickle Lake	9	2015, 2016
	Floatingstone Lake	72	2013, 2015, 2016, 2017
	Fork Lake	5	2013, 2015
	Fox Creek Trout Pond	12	2014
	Garner Lake	39	2015, 2016
	Gerharts Lake	4	2015
	Ghost Lake	1	2014
	Granum Pond	1	2013
	Gregoire Lake	3	2013, 2015
	Gull Lake	15	2014, 2016, 2017
	Half Moon Lake	39	2013, 2014, 2015, 2016, 2017
	Hanmore Lake	69	2013, 2014, 2015, 2016
	Hasse Lake	1	2017
	Hermitage Pond	1	2013
	Hope Lake	33	2013, 2014, 2015, 2017
	Hubbles Lake	58	2013, 2015, 2016, 2017
	Island Lake	15	2015, 2016, 2017
	Jackfish Lake	49	2013, 2015, 2016, 2017
	Jarvis Lake	2	2014
	Kirk Lake	1	2017
	Lac Bellevue	24	2014, 2015
	Lac La Biche	14	2013, 2014, 2015, 2016
	Lac la Nonne	1	2014
	Lac Sante	45	2015, 2016, 2017
	Lac St Cyr	4	2015
	Lac Ste. Anne	14	2013, 2014, 2015, 2016
	Lake Annette	1	2013
	Lake Bonavista	16	2014, 2015
	Lake Isle	4	2013, 2015
	Lake Newell	1	2017
	Laurier Lake	9	2013, 2015
	Lessard Lake	4	2013
	Lesser Slave Lake	11	2013, 2014, 2015, 2017
	Little Bow Lake	4	2014, 2015
	Long Island Lake	1	2013
	Long Lake	62	2013, 2014, 2015, 2016, 2017
	Lower Therien Lake	2	2014
	Marie Lake	4	2013, 2014
	McKenzie Lake	8	2015
	Millers Lake	3	2017
	Mink Lake	3	2015
	Miquelon Lake	11	2015, 2016
	Mons Lake	3	2015, 2017
	Moose Lake	19	2013, 2014, 2015
	Nakamun Lake	16	2013, 2015, 2016
	North Buck Lake	61	2013, 2014, 2015, 2016, 2017
	Open Creek Dam	2	2016
	Park Lake	7	2015, 2017
	Pigeon Lake	90	2013, 2014, 2015, 2016, 2017
	Pine Lake	7	2017
	Rattlesnake Lake	3	2013, 2017
	Red Deer River	2	2015
	Reesor Lake	4	2015
	Rock Lake	1	2017
	Ross Lake	16	2013, 2014, 2017
	Rundle Park	1	2015
	Saskatoon Lake	1	2013
	Shorncliffe Lake	15	2013, 2015
	Skeleton Lake	8	2016, 2017
	Spring Lake	28	2013, 2014, 2015, 2016, 2017
	Spruce Coulee Reservoir	1	2017

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Table 6.9 – *Continued from previous page*

Province	Lake	Cases	Years Reported
	St Mary Reservoir	1	2015
	Sundance Lake	1	2013
	Sylvan Lake	25	2013, 2015, 2016, 2017
	Three Mile Bend at Red Deer River	5	2016
	Thunder Lake	8	2013, 2015
	Touchwood Lake	4	2014
	Travers Reservoir	2	2013, 2015
	Trestle Creek Golf Resort	31	2015, 2016
	Twin Lake	44	2016, 2017
	Wabamun Lake	201	2013, 2014, 2015, 2016, 2017
	Wasa Lake	1	2015
	Wedge Pond	2	2017
	Whitefish Lake	4	2013, 2016
	Whitney Lake	26	2013, 2014, 2015, 2016, 2017
	Wizard Lake	3	2015, 2016
	Wolf Lake	10	2013, 2015
British Columbia	Adams Lake	24	2013, 2014, 2016, 2017
	Alta Lake	27	2013, 2014, 2015, 2017
	Arrow Lake Park	1	2013
	Arrow Lakes	1	2013
	Babine Lake	2	2013, 2016
	Bear Lake	3	2013, 2017
	Buttle Lake	12	2013, 2014, 2016, 2017
	Centennial Beach at Boundary Bay	1	2017
	Charlie Lake	3	2013
	Chehalis Lake	4	2015
	Chilliwack Lake	3	2013, 2016
	Columbia River	1	2013
	Comox Lake	10	2013, 2014, 2016, 2017
	Cowichan Lake	5	2015
	Crescent Beach	45	2013, 2014, 2015, 2016, 2017
	Cultus Lake	132	2013, 2014, 2015, 2016, 2017
	Cusheon Lake	5	2015
	Dunn Lake	7	2014
	Echo Lake	2	2015
	Enid Lake	1	2013
	Fishblue Lake	6	2016, 2017
	Francois Lake	2	2014
	Harrison Lake	29	2015, 2016, 2017
	Horne Lake	85	2013, 2014, 2015, 2016, 2017
	Inzana Lake	6	2017
	Kalamalka Lake	18	2013, 2014, 2015, 2016
	Kennedy Lake	3	2015
	Kin Beach	1	2013
	Kokanee Lake	1	2013
	Kootenay Lake	20	2013, 2014, 2017
	Langford Lake	4	2017
	Lac La Hache	12	2013, 2016, 2017
	Lake Pinantan	6	2015, 2017
	Lake Windermere	29	2013, 2014, 2016, 2017
	Little Shuswap Lake	17	2013, 2014, 2015
	Long Lake	1	2015
	Loon Lake	1	2014
	Lost Lake	1	2013
	Lost Lake (Whistler)	3	2017
	Mabel Lake	33	2013, 2014, 2015, 2016, 2017
	Madden Lake	1	2013
	Maiden Lake	4	2014
	Mara Lake	10	2013, 2016
	Monte Lake	2	2013, 2016
	Nadsilnich Lake	1	2017
	Nanaimo Lakes #2	1	2013
	Nicola Lake	4	2015
	Nukko Lake	2	2016
	Okanagan Lake	57	2013, 2014, 2015, 2016, 2017
	Osoyoos Lake	3	2013, 2015
	Pacific Ocean near White Rock	7	2013, 2014, 2016
	Paul Lake	20	2014, 2015, 2017
	Peckhams Lake	2	2015
	Premier Lake	1	2013
	Rolley Lake	7	2017
	Sasamat Lake	3	2013, 2016, 2017
	Shuswap Lake	281	2013, 2014, 2015, 2016, 2017
	Shuswap River	1	2014
	Spider Lake	3	2015
	Stuart Lake	1	2013
	Surveyors Lake	8	2013
	Thetis Lake	1	2017
	Tie Lake	2	2017
	Trout Lake	6	2014, 2015
	Wasa Lake	65	2013, 2014, 2015, 2016, 2017
	Weston Lake	4	2015
	Wood Lake	6	2015, 2017
	Woss Lake	2	2014
Manitoba	Clear Lake	23	2013, 2015, 2016
	Dorothy Lake	3	2017
	Kenton Reservoir	4	2015
	Lake Winnipeg	1	2017
	West Hawk Lake	1	2017
	Wild Oaks Campground Beach	21	2017

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Table 6.9 – *Continued from previous page*

Province	Lake	Cases	Years Reported
New Brunswick	Fisher Lakes	4	2015
	St John River	9	2014, 2015
Newfoundland	Northwest Pond	6	2013
Northwest Territories	Great Slave Lake	1	2013
Nova Scotia	Grand Lake	1	2013
	Lake Ainslie	4	2015
	Lake Banook	1	2015
	Mattatall Lake	1	2015
Ontario	Adams Lake	1	2016
	Balsam Lake	1	2017
	Baptiste Lake	1	2015
	Bass Lake	22	2013, 2015, 2016
	Bass Lake near Lombardy	1	2013
	Baxter Lake	3	2015
	Bear Lake	3	2016
	Berford Lake	1	2013
	Big Rideau Lake	3	2016
	Black River	2	2016
	Bobs Lake	1	2013
	Bon Echo Provincial Park at Mazinaw Lake	2	2016
	Boshkung Lake	5	2016, 2017
	Cameron Lake	3	2016
	Centennial Lake	1	2013
	Chesley Lake	2	2015
	Commando Lake	3	2015
	Cranberry Lake	1	2015
	Crotch Lake	4	2015
	Elliot Lake	1	2014
	Farlain Lake	4	2017
	Fitzroy Provincial Park	1	2013
	Golden Lake	30	2013, 2015, 2016, 2017
	Guelph Lake	1	2015
	Gull Lake	5	2015
	Gullivers Lake	9	2016
	Harmony Beach at Lake Superior	2	2016
	Havilland Bay at Lake Superior	3	2016
	Horseshoe Lake	3	2014, 2016
	Jack Lake	11	2013, 2014, 2017
	Kashagawigamog Lake	1	2013
	Kawawaymog Lake	1	2015
	Kennisis Lake	1	2013
	Koshlong Lake	1	2015
	Lake Clear	14	2015, 2016
	Lake Couchiching	2	2015
	Lake Erie	15	2013, 2015, 2017
	Lake Huron at Southampton	2	2015
	Lake Huron Georgian Bay	21	2014, 2015, 2016, 2017
	Lake Huron Sauble Beach	3	2013, 2017
	Lake Kamaniskeg	11	2013
	Lake Louisa	2	2016
	Lake Manitou	1	2013
	Lake Muskoka	5	2015
	Lake Nipissing	30	2013, 2014, 2015, 2016, 2017
	Lake Nosbonsing	6	2017
	Lake Ontario	5	2013, 2016, 2017
	Lake Rosseau	1	2014
	Lake Temagami	2	2016
	Lake Wilcox	1	2017
	Lake Wolsey	2	2015
	Limerick Lake	2	2015
	Little Cranberry Lake	3	2015
	Marl Lake	3	2015
	Mazinaw Lake	16	2013, 2015, 2016
	Mink Lake	3	2016
	Missinaibi Lake	3	2017
	Mississippi River	11	2016
	Moon River at Georgian Bay	1	2016
	Mooneys Bay	1	2013
	Musselman Lake	4	2017
	Napanee River (Camden East)	2	2017
	Orr Lake	9	2015, 2016
	Ottawa River at Harvey Creek	4	2015
	Ottawa River at Haydon Park	1	2015
	Ottawa River Pembroke	2	2015
	Peninsula Lake	2	2016
	Pigeon Lake	1	2013
	Red Cedar Lake	3	2015
	Regina Bay at Glouster Pool at Georgian Bay	3	2016
	Remi Lake	4	2014, 2015
	Rice Lake	2	2013
	Riley Lake	3	2015
	Round Lake	2	2017
	Sandbar Lake	2	2017
	Shabomeka Lake	8	2015, 2016
	Simcoe Lake	15	2013, 2015, 2016
	Six Mile Lake	3	2016
	Spencer Creek	1	2013
	St Marys River	1	2013
	Stoney Lake	1	2017
	Sturgeon Bay Provincial Park at Georgian Bay	2	2016

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Table 6.9 – *Continued from previous page*

Province	Lake	Cases	Years Reported
	Tait Lake	1	2016
	Thunder Bay at Lake Superior	17	2016
	Trout Lake	30	2013, 2014, 2015, 2016
	Upper Canada Campground Pond	10	2014
	Valens Lake	5	2016
	Wasaga Beach Georgian Bay	2	2015
	White Lake	4	2014, 2015
	Wild Goose Beach Lake Superior	3	2015
	Wollaston Lake	1	2016
Quebec	Grand Lac MacDonald/Lake MacDonald	5	2013, 2016
	Lac Cameron	1	2013
	Lac des Seize Iles	1	2013
	Lac Massawippi	1	2016
	Lac Meech	2	2016
	Lac Opasatica	1	2013
	Lac Phillipe	5	2013, 2016
	Riviere des Outaouais	3	2014
Saskatchewan	Blackstrap Lake	3	2015
	Buffalo Pound Lake	6	2013, 2015
	Candle Lake	3	2015
	Chitek Lake	18	2017
	Delaronde Lake	1	2015
	Greenwater Lake	2	2013
	Greig Lake	54	2013, 2014, 2015, 2016, 2017
	Jackfish Lake	22	2016
	Jeannette Lake	1	2013
	Jumbo Lake	1	2013
	Kimball Lake	36	2014, 2015, 2016, 2017
	Lac des Iles	5	2013, 2017
	Lac La Ronge	1	2013
	Last Mountain Lake	2	2017
	Lower Fishing Lake	3	2013
	Madge Lake	8	2013, 2015
	Makwa Lake	1	2013
	Marean Lake	2	2013
	Martins Lake	51	2017
	Matheson Lake	16	2015, 2016
	Meeting Lake	1	2013
	Memorial Lake	7	2013, 2016, 2017
	Murray Lake	1	2017
	Pierce Lake	28	2013, 2015, 2016
	Shell Lake	3	2013, 2014
	Suffern Lake	16	2013, 2014, 2017
	Turtle Lake	10	2014, 2017
	Wakaw Lake	6	2013, 2015
	Waskesiu Lake	3	2016, 2017
United States	Detroit Lake	1	2015
	Frenchman Lake	3	2016
	Higgins Lake	1	2013
	Lake McDonald	2	2013
	Osoyoos Lake	2	2015
	San Diego River Mission Bay	1	2015
	Silverwood Lake	3	2015
	Osoyoos Lake	2	2017
	Big Creek Lake	2	2017
	Truckee River	1	2017

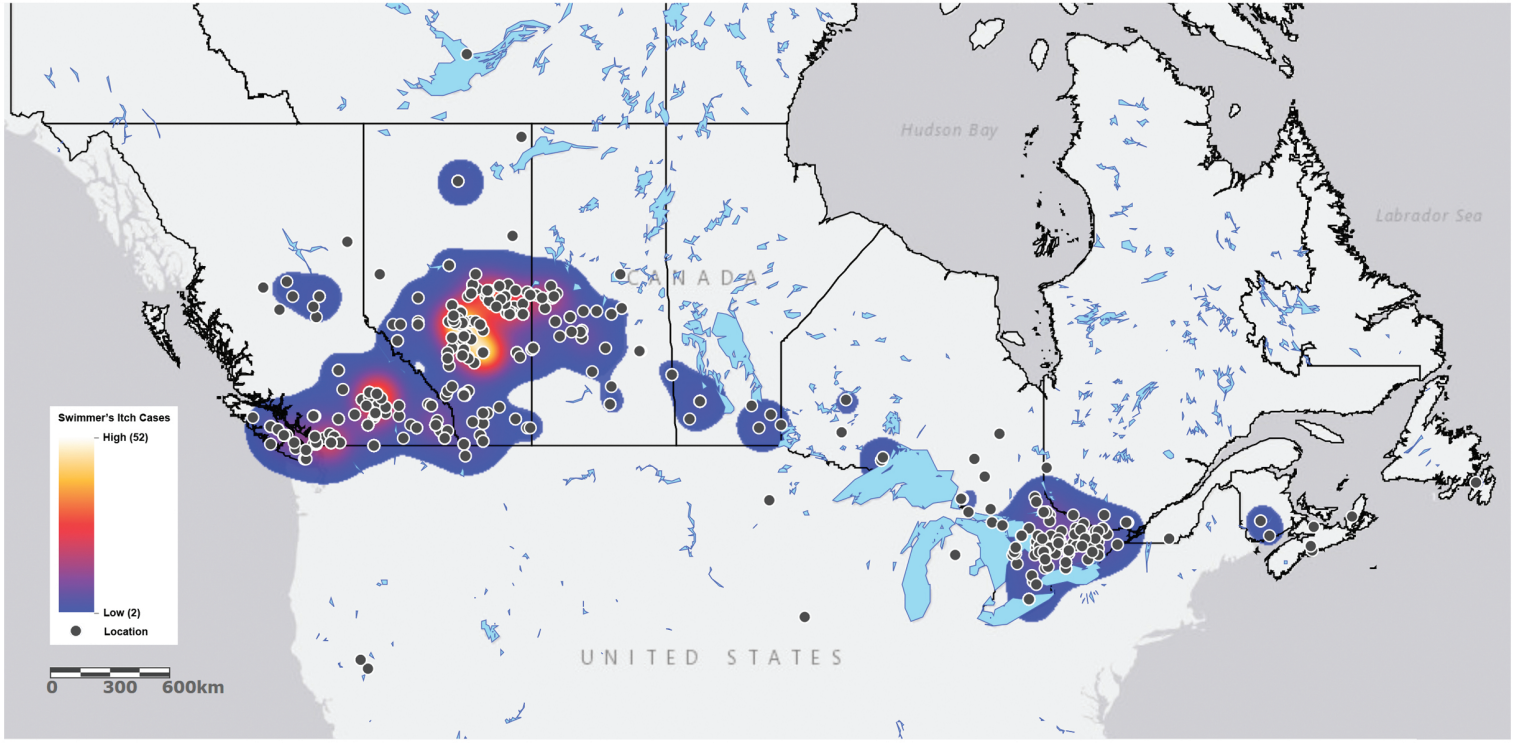

Figure 6.1: Locations of Swimmer's Itch Reports Across Canada and the U.S. Each swimmer's itch report is represented by a black dot on the map. As many reports are from the same location, the heatmap represents the level of stacking of these reports. Red and white areas represent more reports than blue areas. Single reports have no surrounding colour.

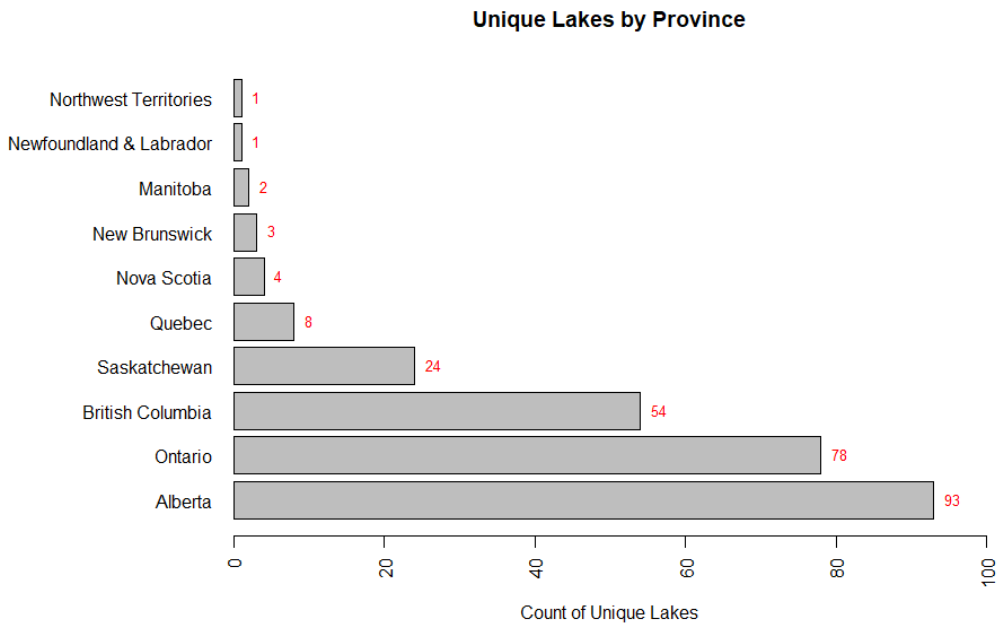

Figure 6.2: Number of Unique Lakes from which Swimmer's Itch Reports were Received by Province.

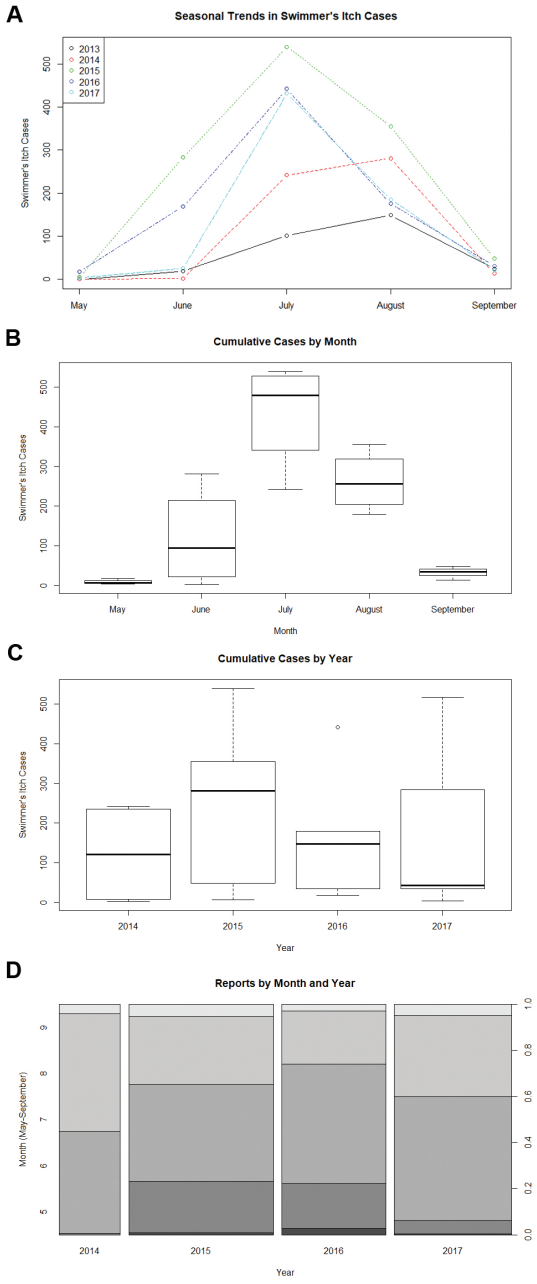

Figure 6.3: Trends in Swimmer's Itch Occurrences. A). The total number of swimmer's itch cases per month, as gathered by survey reports, are plotted for each year. B). Cumulative swimmer's itch cases by month (2014-2017). C). Cumulative swimmer's itch cases by year; cases for 2013 were not included, as this question was not added to the survey until 2014. D). Spine plot of swimmer's itch case proportions by month over four years (2014-2017). Grey-scale partitions within bars are proportions of cases in each month within the year. Width of the bars reflects the total sample size for each year.

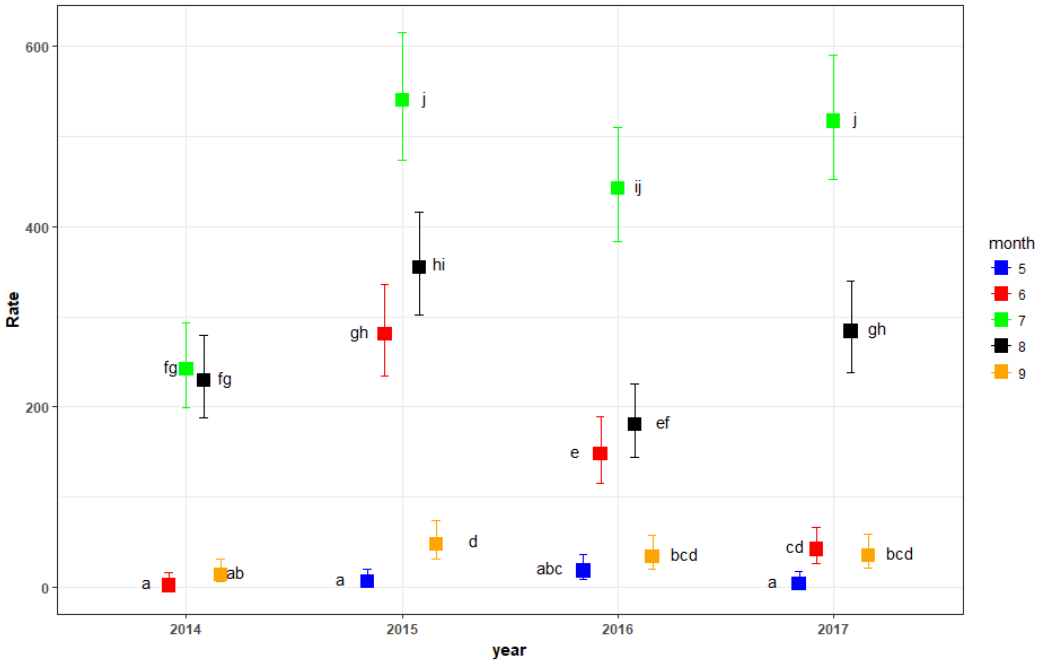

Figure 6.4: Rates of Swimmer's Itch Cases Over Time. Least-square means of swimmer's itch case counts over five months in each year for four years (2014-2017). Boxes indicate the least-square means of the rate. Error bars indicate the 95% confidence intervals around the rate. Rates sharing a letter are not significantly different (Tukey-adjusted comparisons).

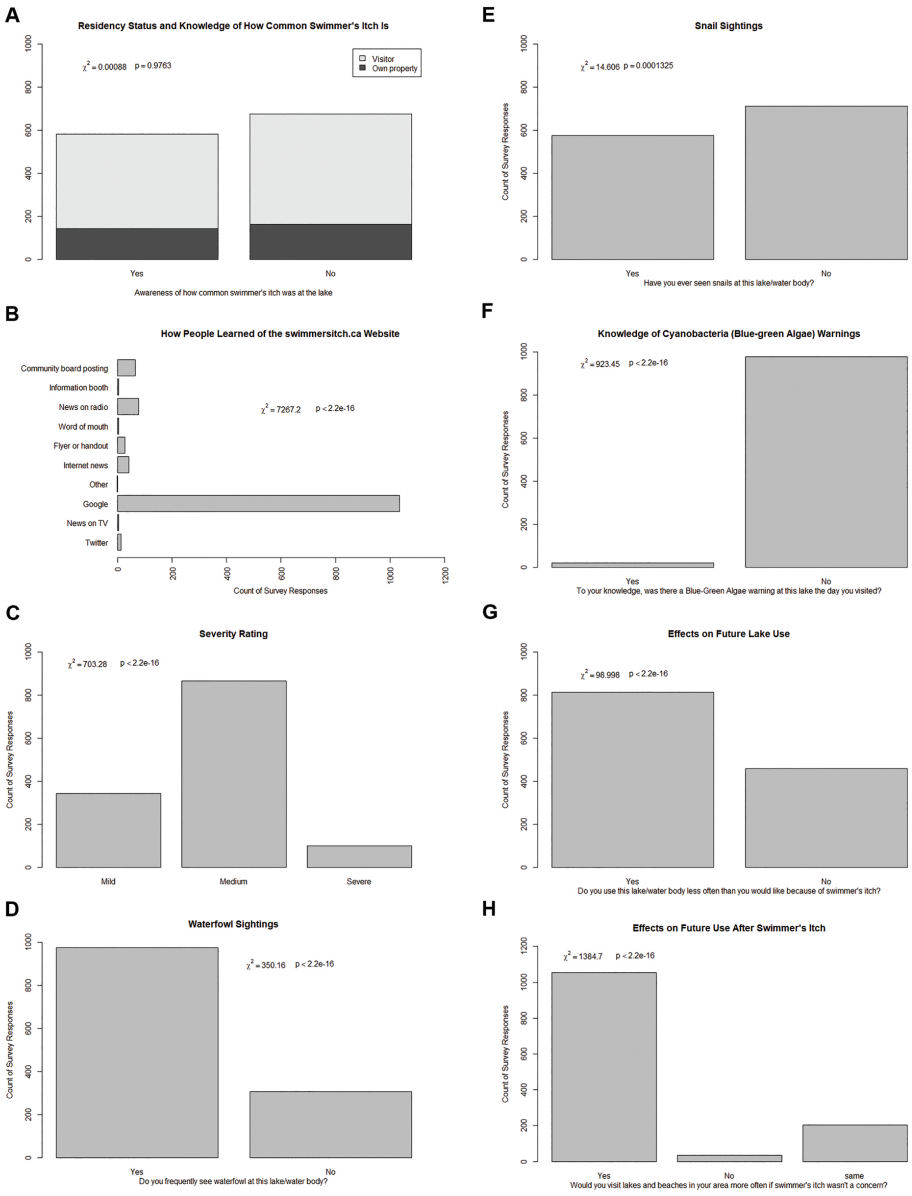

Figure 6.5: Self-Reporting Survey Results. A). Residency status and knowledge of how common swimmer's itch is. B). How respondents heard about or found the survey. C). Swimmer's itch severity rating as identified by respondents. D). Whether or not respondents sighted waterfowl at the lake during the time they contracted swimmer's itch. E). Whether or not respondents sighted snails at the lake. F). Whether or not respondents were aware of a blue-green algae (cyanobacteria) warning sign at the lake. G). Potential effects on future use of the lake for recreation after having had swimmer's itch. H). Whether respondents would visit more, less, or the same amount if they knew whether or not swimmer's itch was a risk.

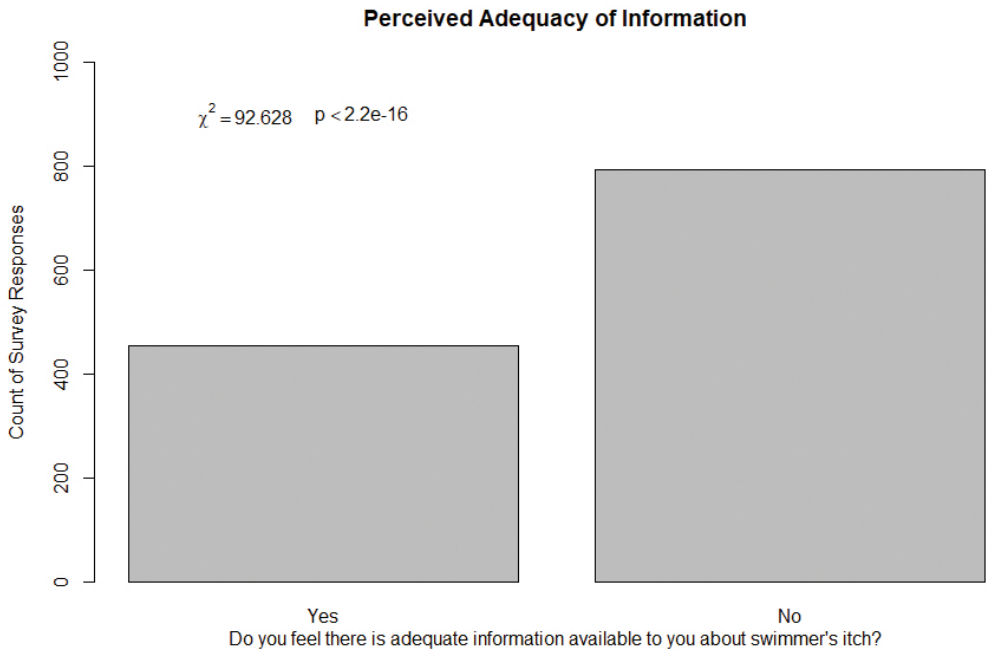

Figure 6.6: Respondents Opinions on Whether or Not the Amount of Swimmer's Itch Information Available To Them Was Adequate.

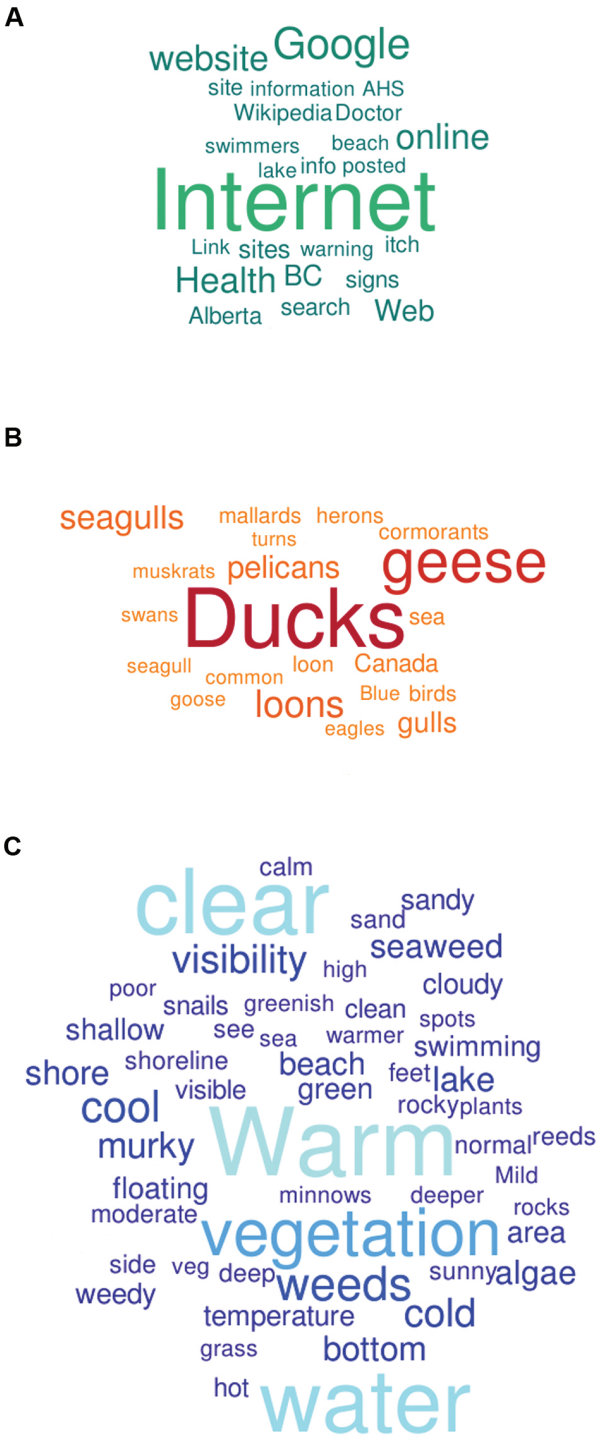

Figure 6.7: Word Clouds of Most Commonly Used Words in Descriptive Text Answers. A). Most common sources sought for swimmer's itch information. B). Most common waterfowl sightings. C). Most common descriptors of water quality.

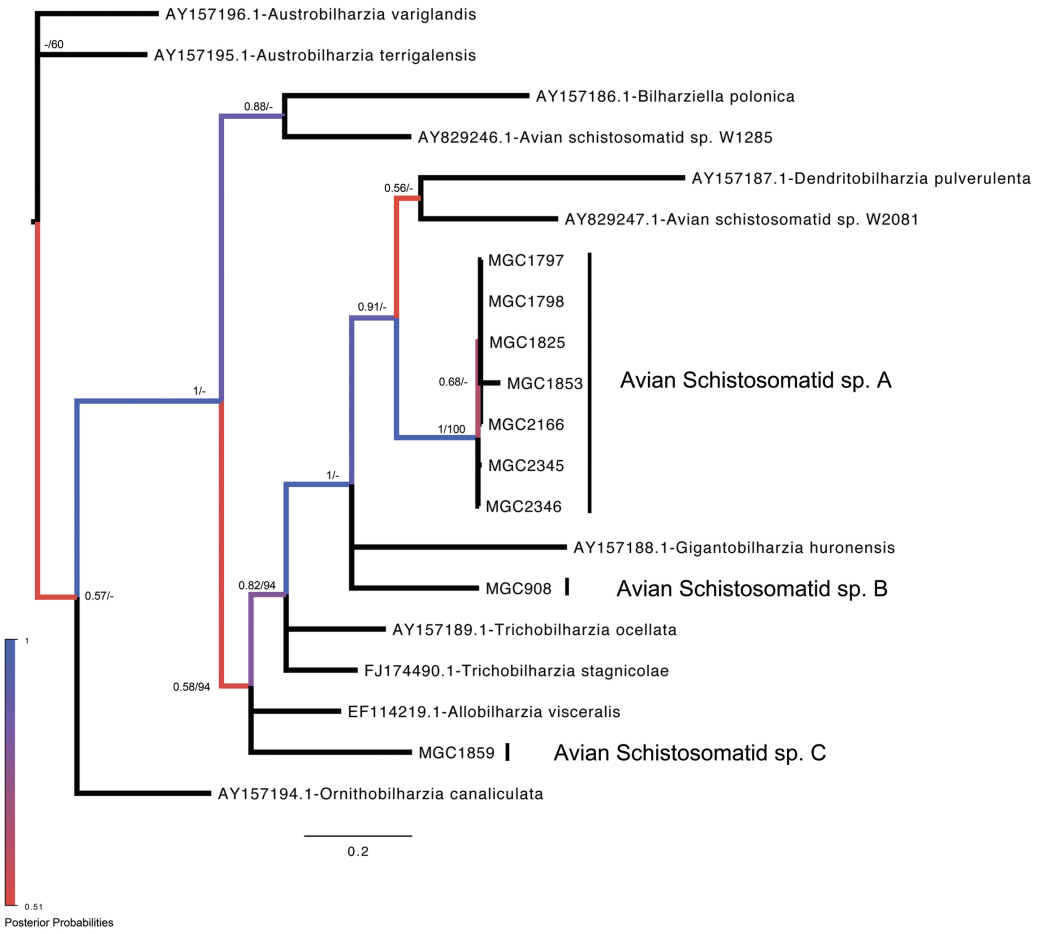

Figure 6.8: Phylogenetic Tree of Avian Schistosomes. Tree topology is based on Bayesian Inference. Nodal support is indicated with posterior probabilities (shown by coloured branches and associated numeric probabilities) followed by bootstrap support from Maximum Likelihood. GenBank accession numbers precede taxon names. All sequences from this study are labeled with 'MGC'.

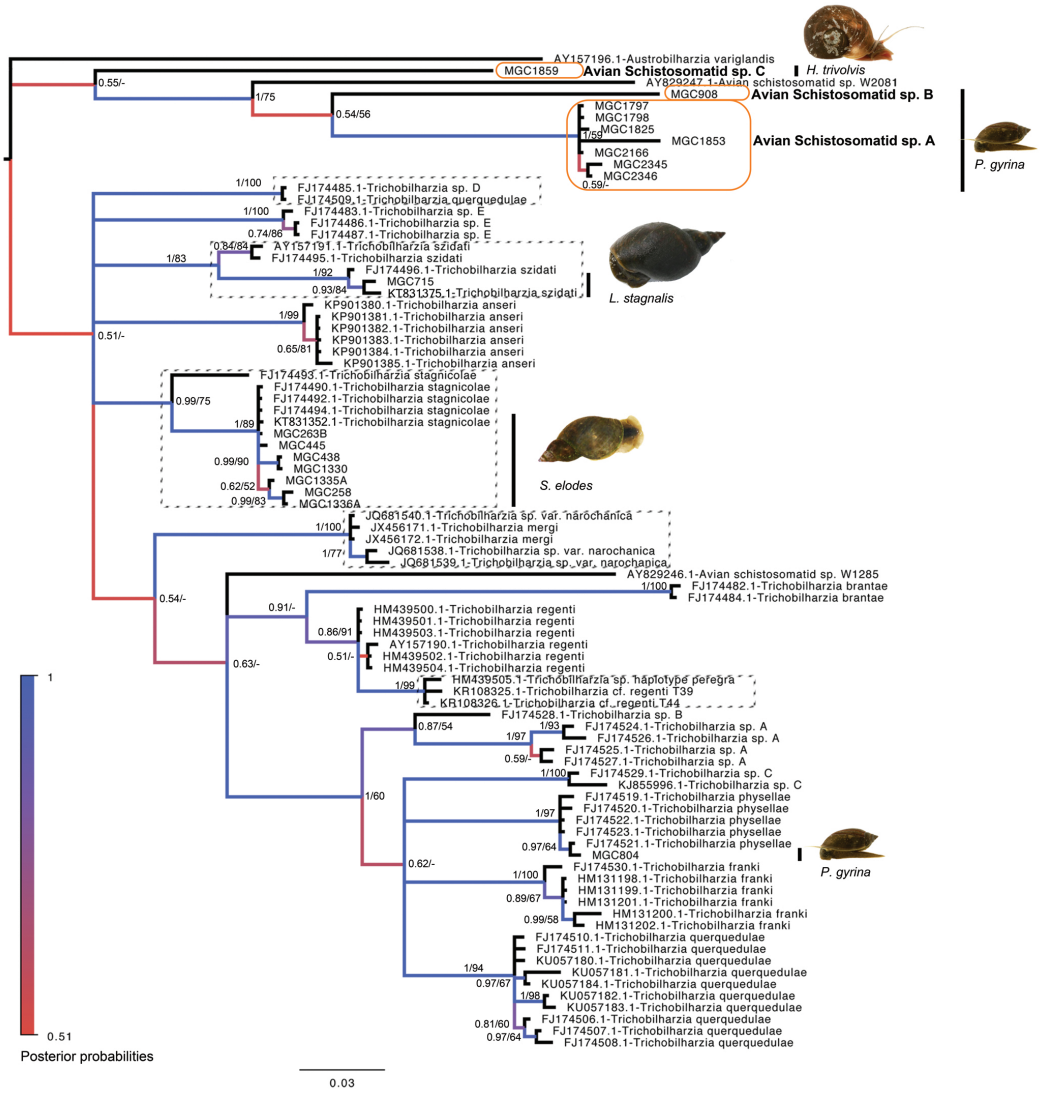

Figure 6.9: Phylogenetic Tree of Avian Schistosome Genera *Trichobilharzia*. Tree topology is based on Bayesian Inference. Nodal support is indicated with posterior probabilities (shown by coloured branches and associated numeric probabilities) followed by bootstrap support from Maximum Likelihood. GenBank accession numbers precede taxon names. All sequences from this study are labeled with 'MGC', and placement is indicated by a black bar to the right of tree. Snail intermediate hosts associated with samples from this study are indicated by a picture to the right.

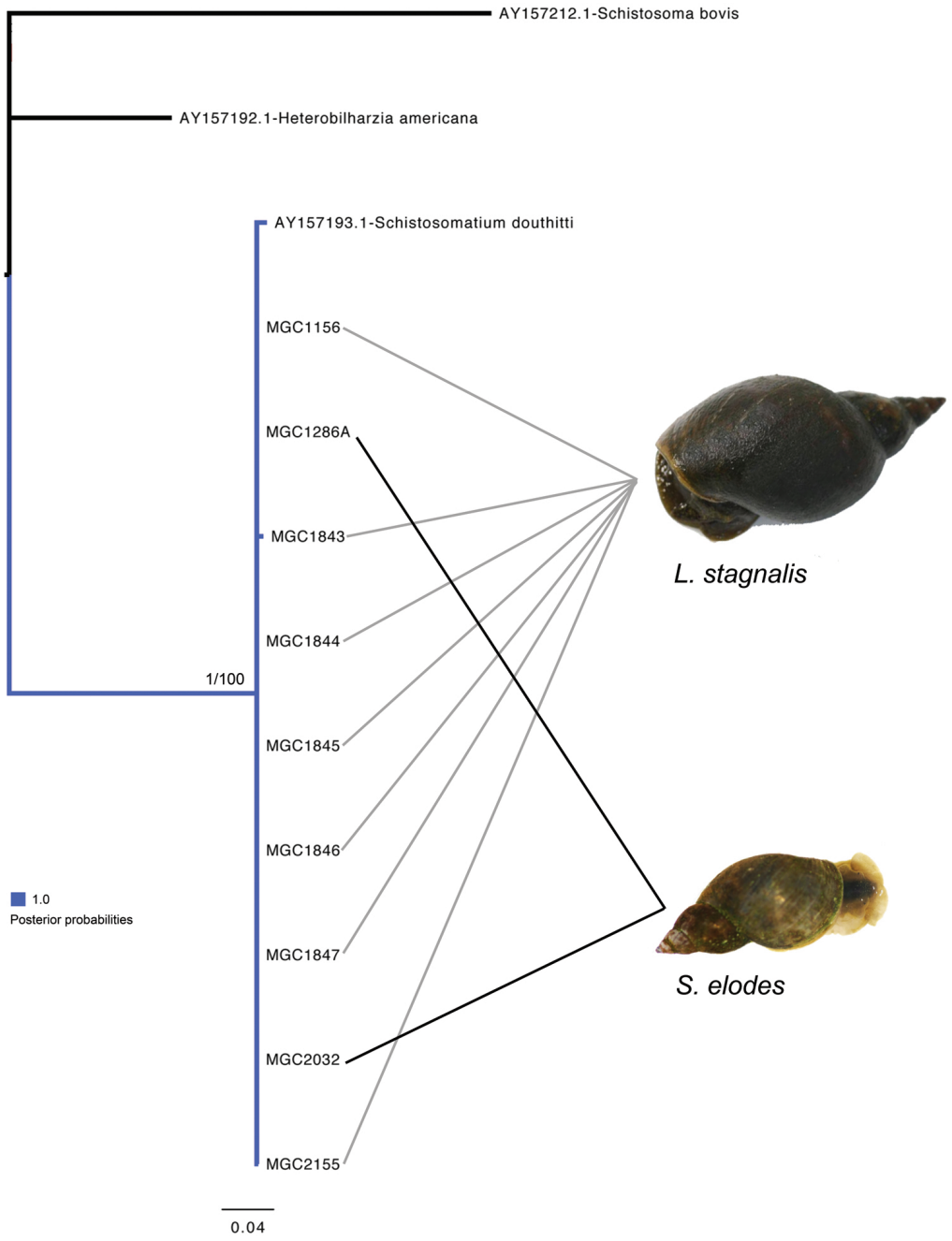

Figure 6.10: Phylogenetic Tree of Mammalian Schistosomes from Alberta. Tree is based on Bayesian Inference. Nodal support is indicated with posterior probability, by both coloured branches and numbers. GenBank accession numbers precede taxon names. All sequences from this study are labeled with 'MGC'. Snail intermediate hosts are associated by lines connecting them to specific taxa.

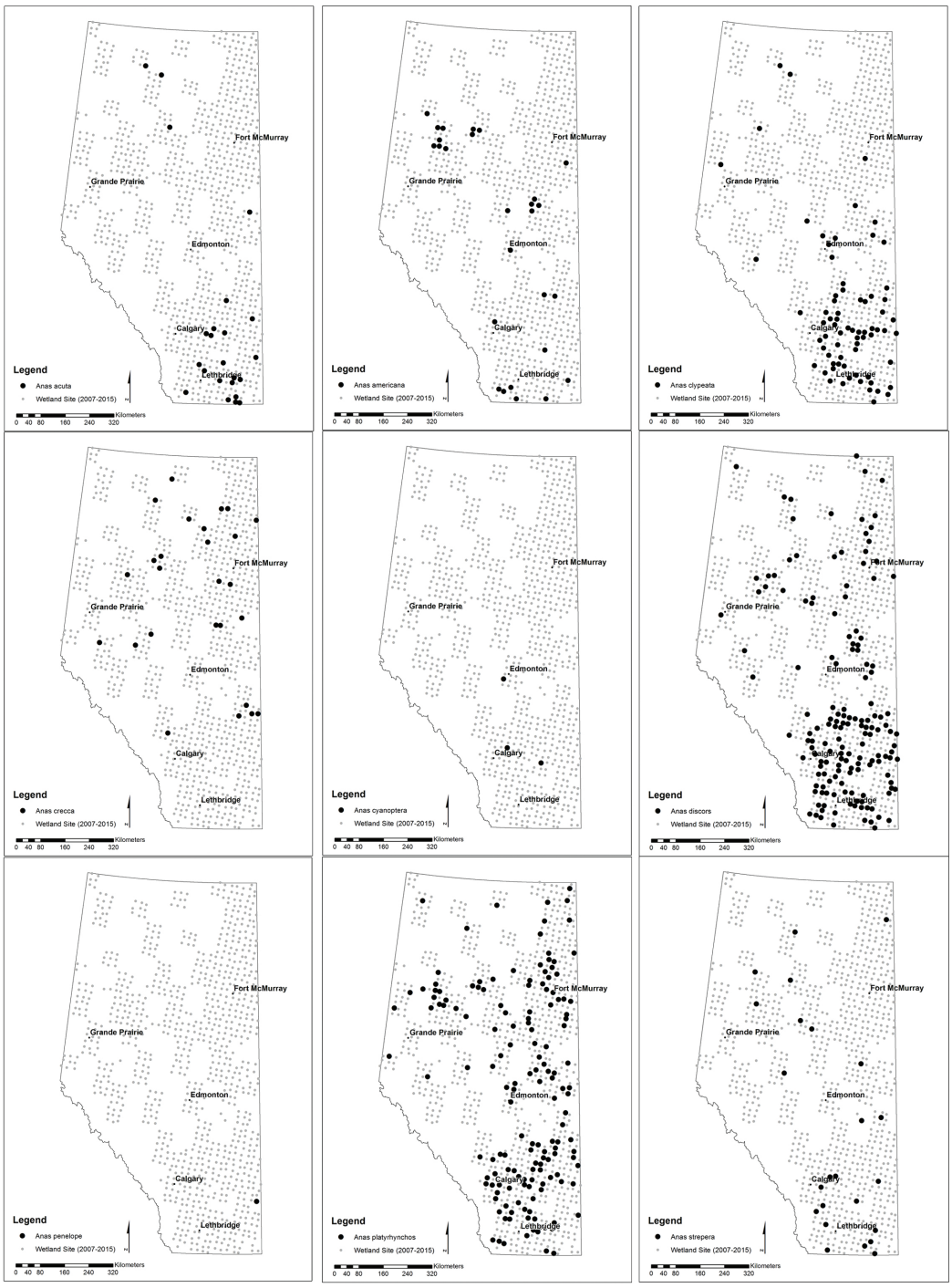

Figure 6.11: *Anas* spp. Distributions Across Alberta Wetlands. Distributions of 9 *Anas* spp. (waterfowl/ducks) as collected by ABMI.

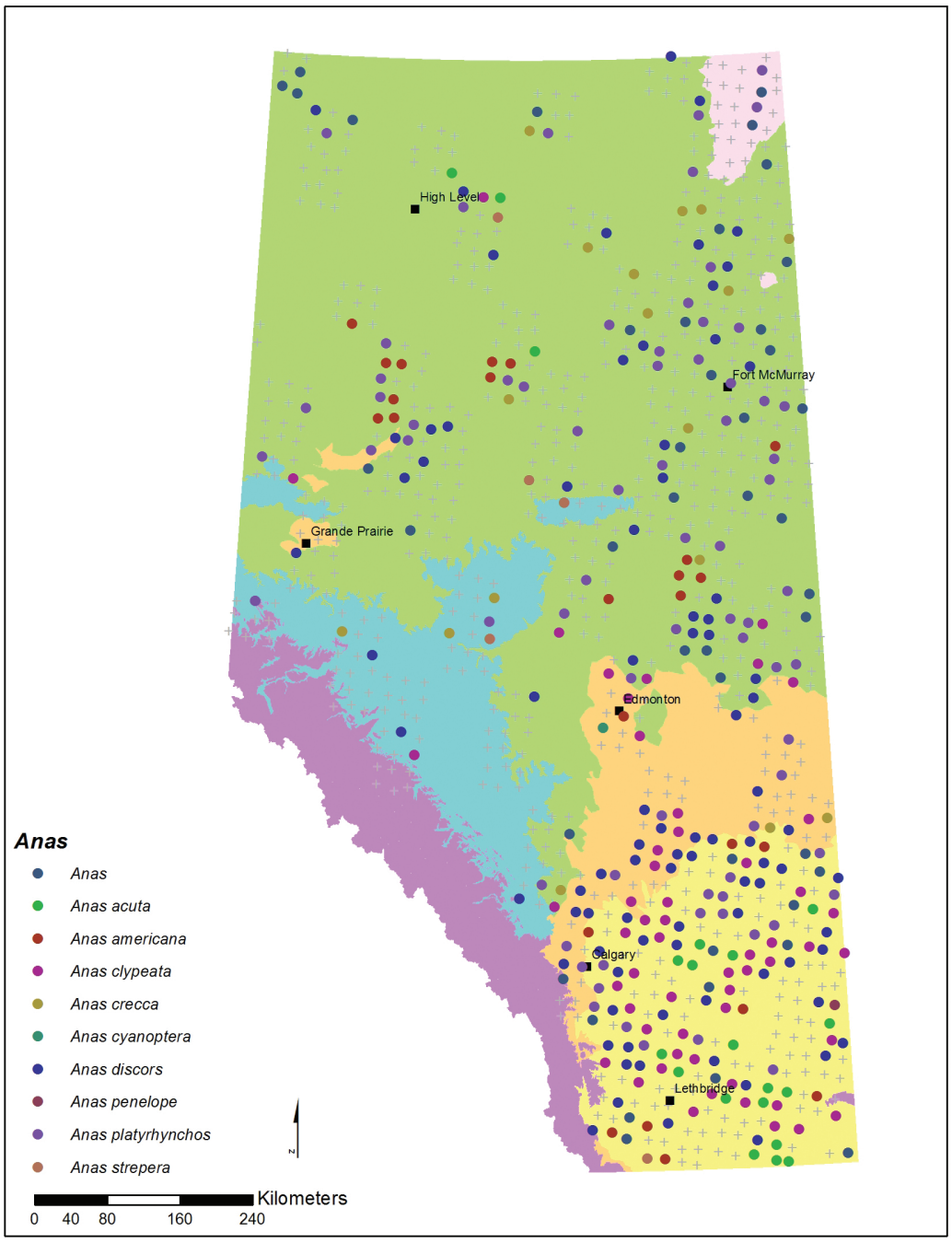

Figure 6.12: Combined Distributions of *Anas* spp. Across Alberta Wetlands.

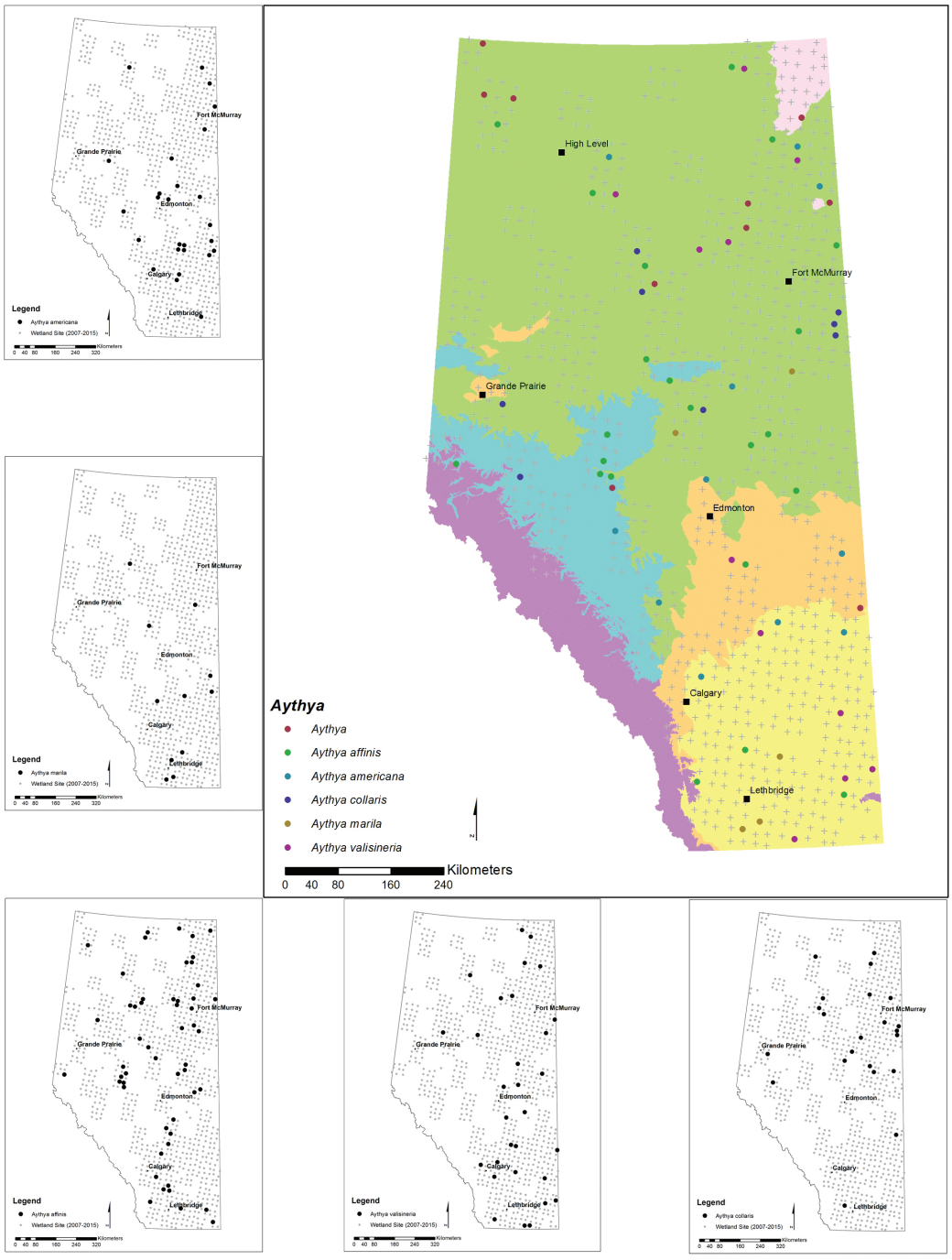

Figure 6.13: *Aythya* spp. Distributions Across Alberta Wetlands. Distributions of 5 *Aythya* spp. (waterfowl/ducks) as collected by ABMI, both individual and in combination.

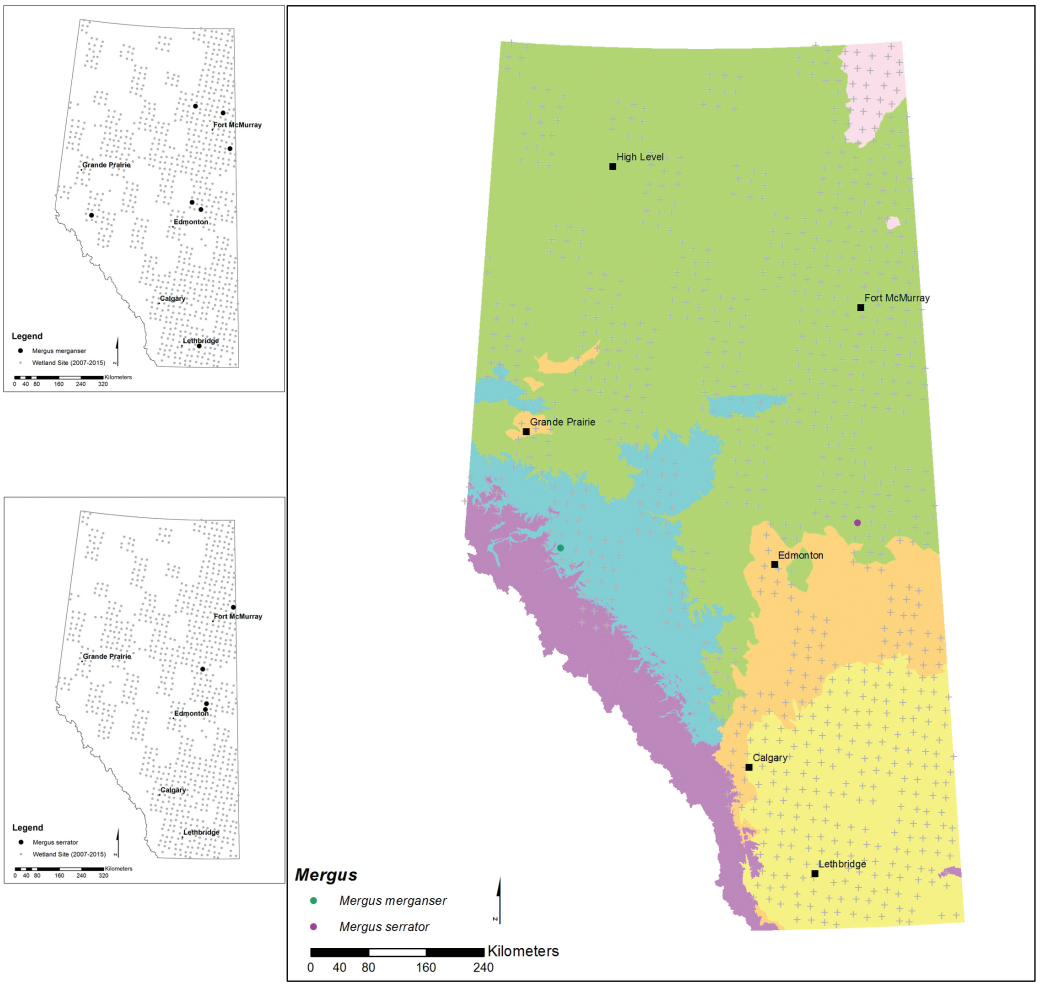

Figure 6.14: *Merganus* spp. Distributions Across Alberta Wetlands. Distributions of 2 species of diving ducks as collected by ABMI. Both individual and combined distributions are reported.

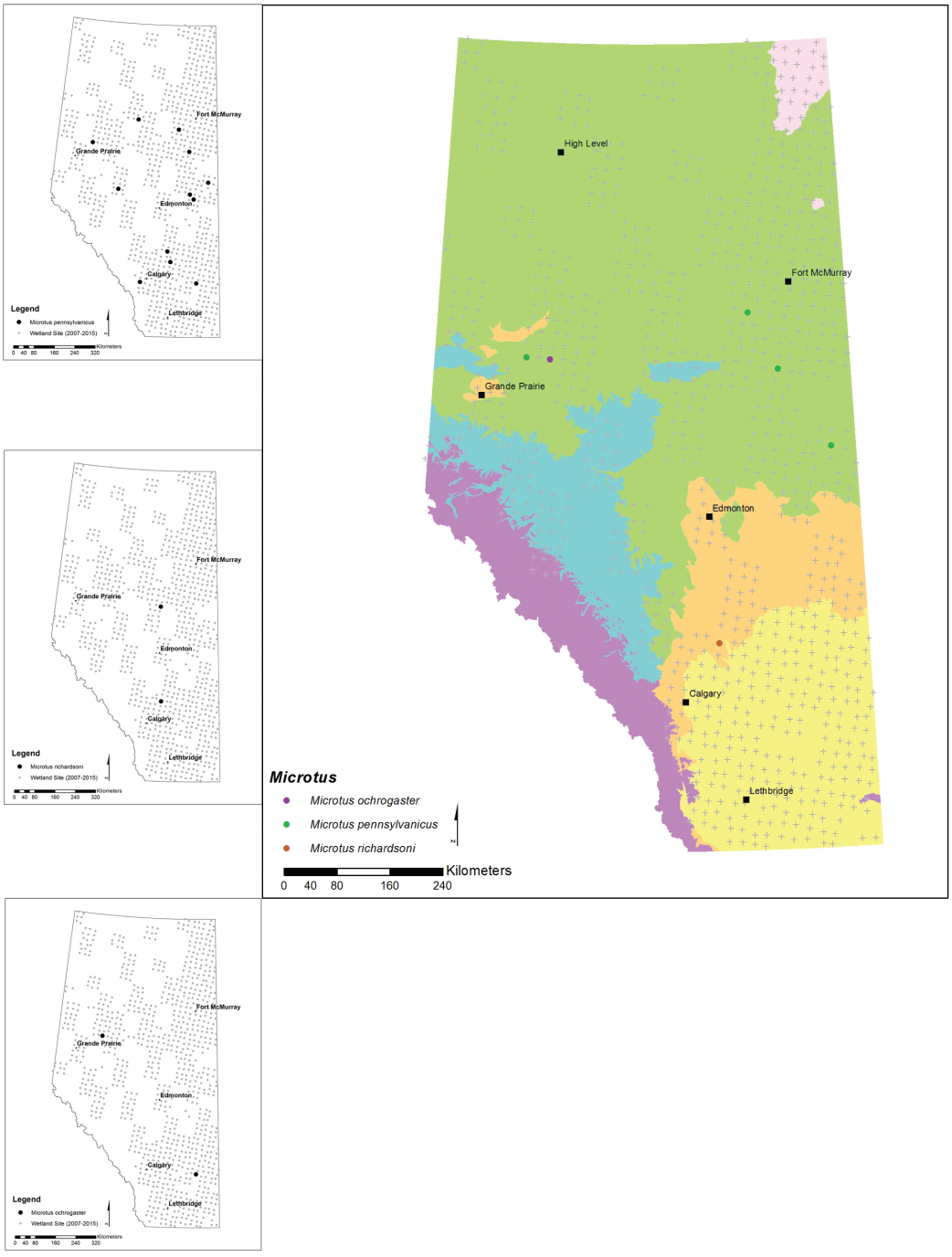

Figure 6.15: *Microtus* spp. Distributions Across Alberta Wetlands. Distributions of 3 species of Muskrat as collected by ABMI. Both individual and combined distributions are reported.

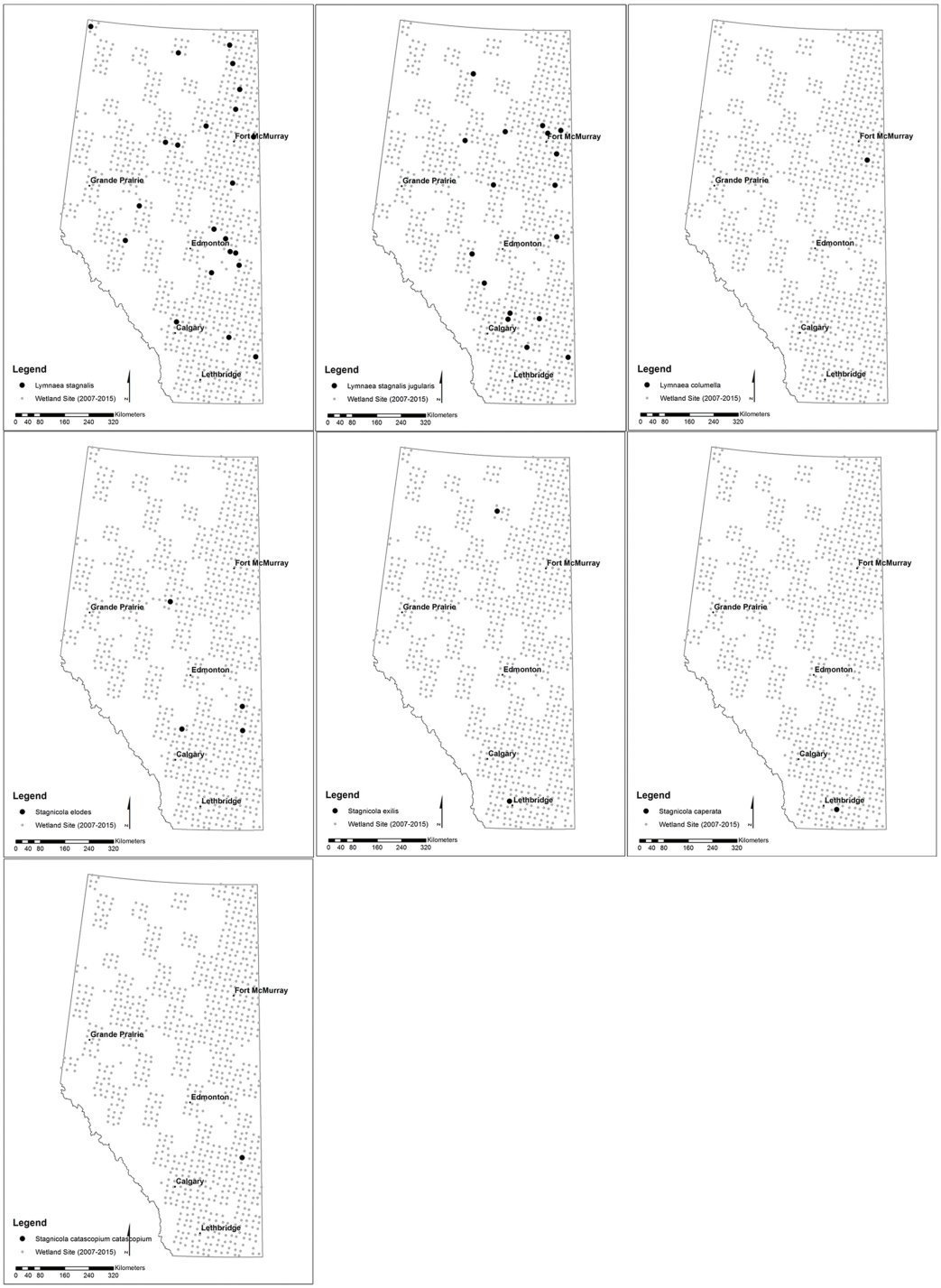

Figure 6.16: Lymnaeid Snail Distributions Across Alberta Wetlands.

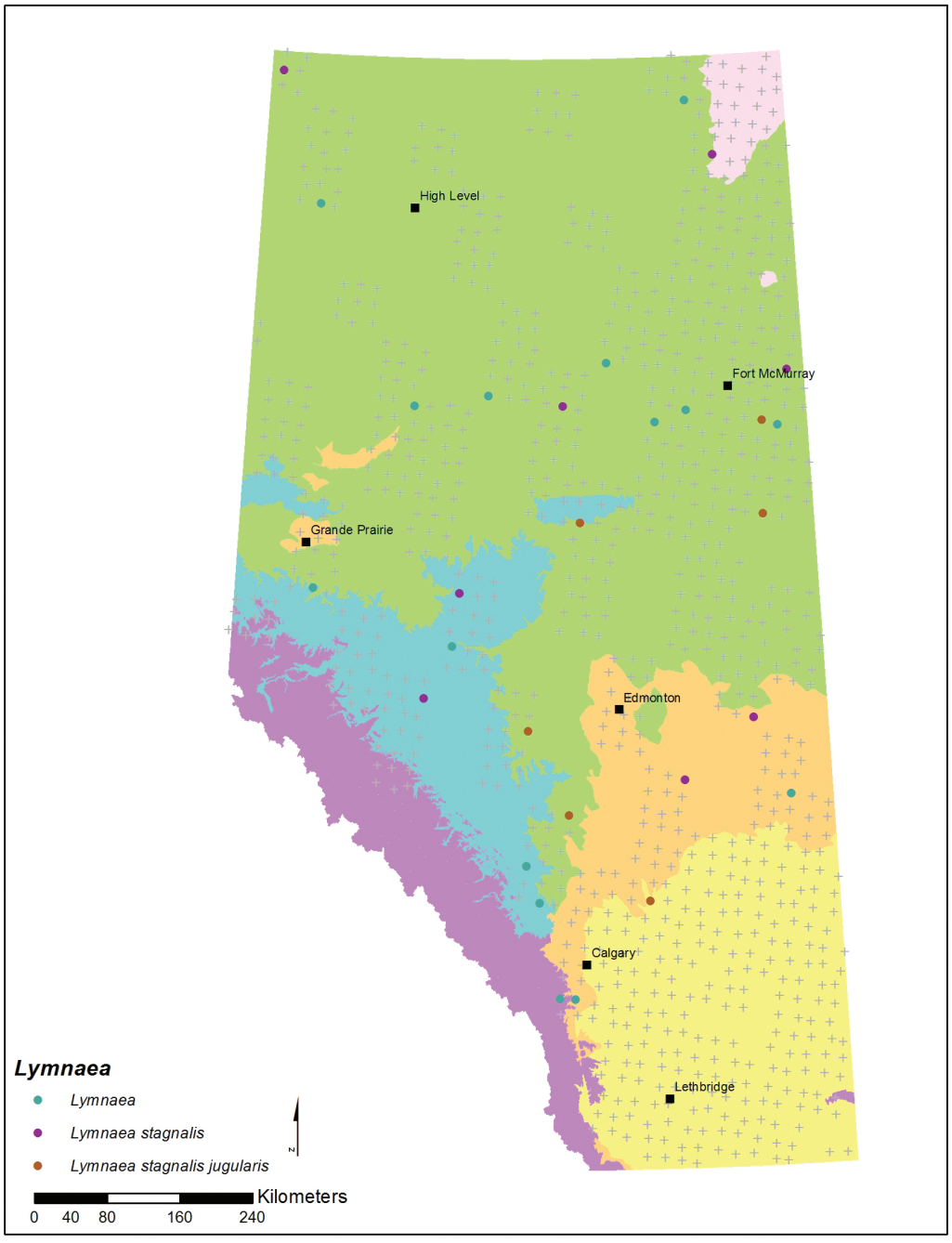

Figure 6.17: Combined Lymnaeid Snail Distributions Across Alberta Wetlands.

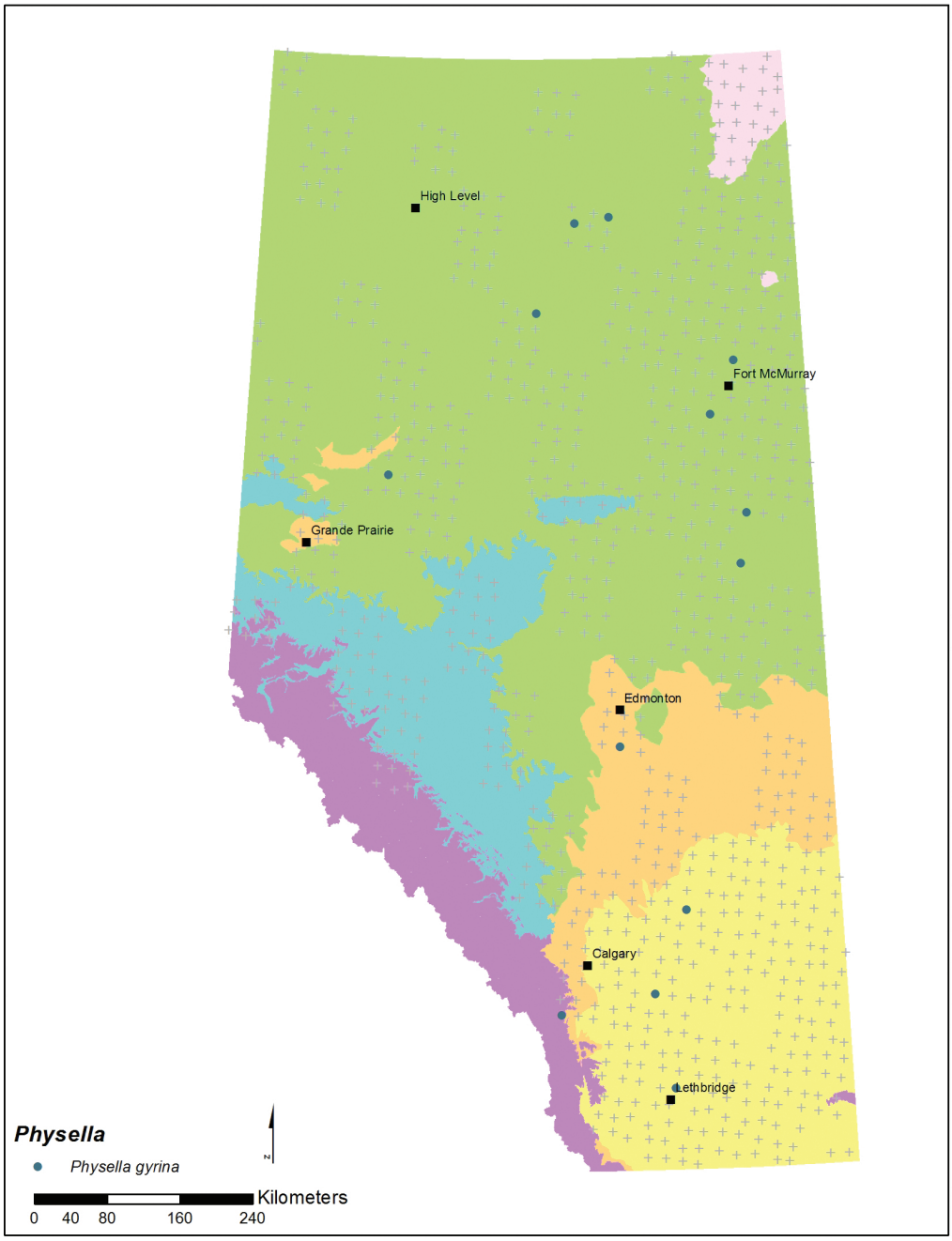

Figure 6.18: Physid Snail Distributions Across Alberta Wetlands.

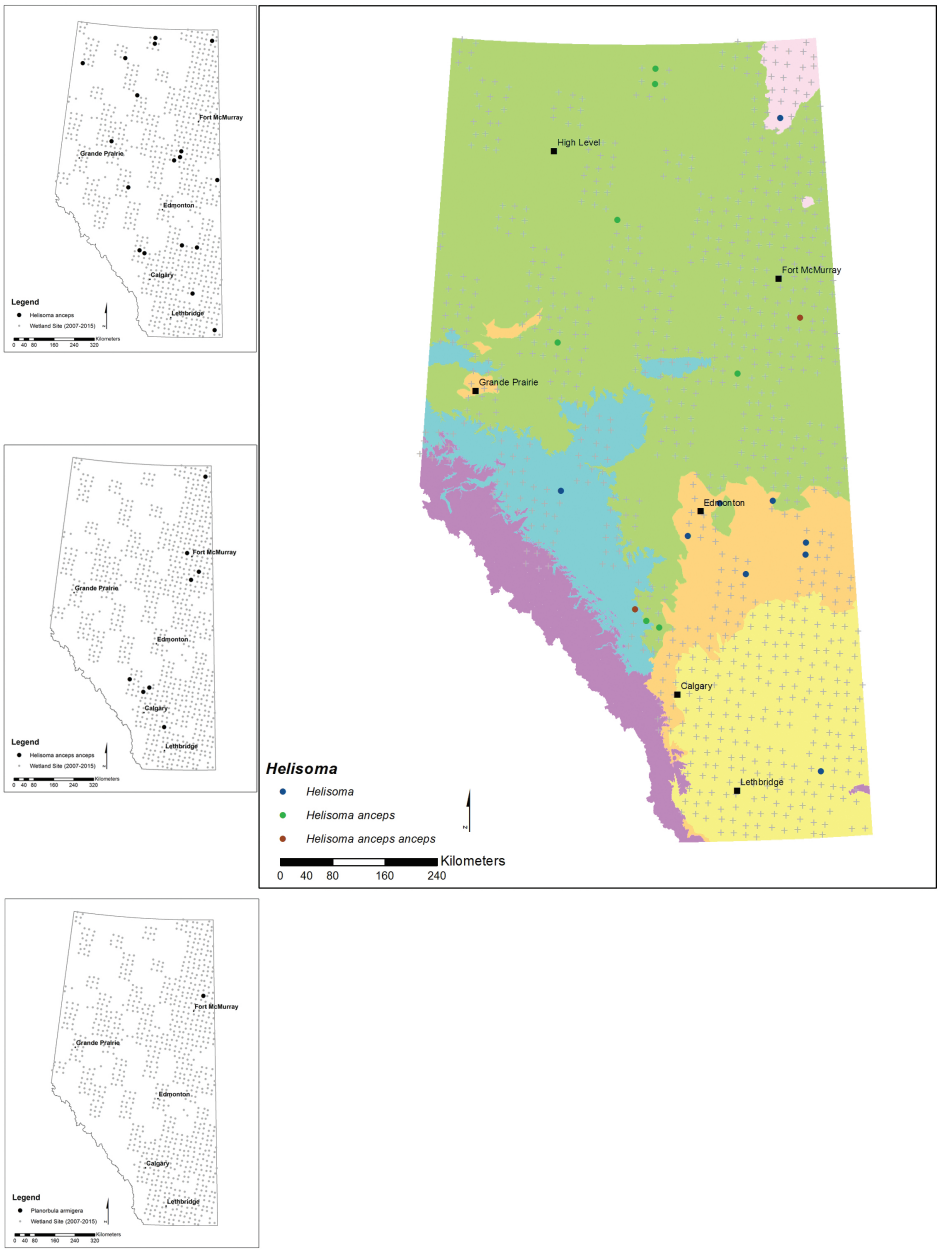

Figure 6.19: Planorbidae Snail Distributions Across Alberta Wetlands.

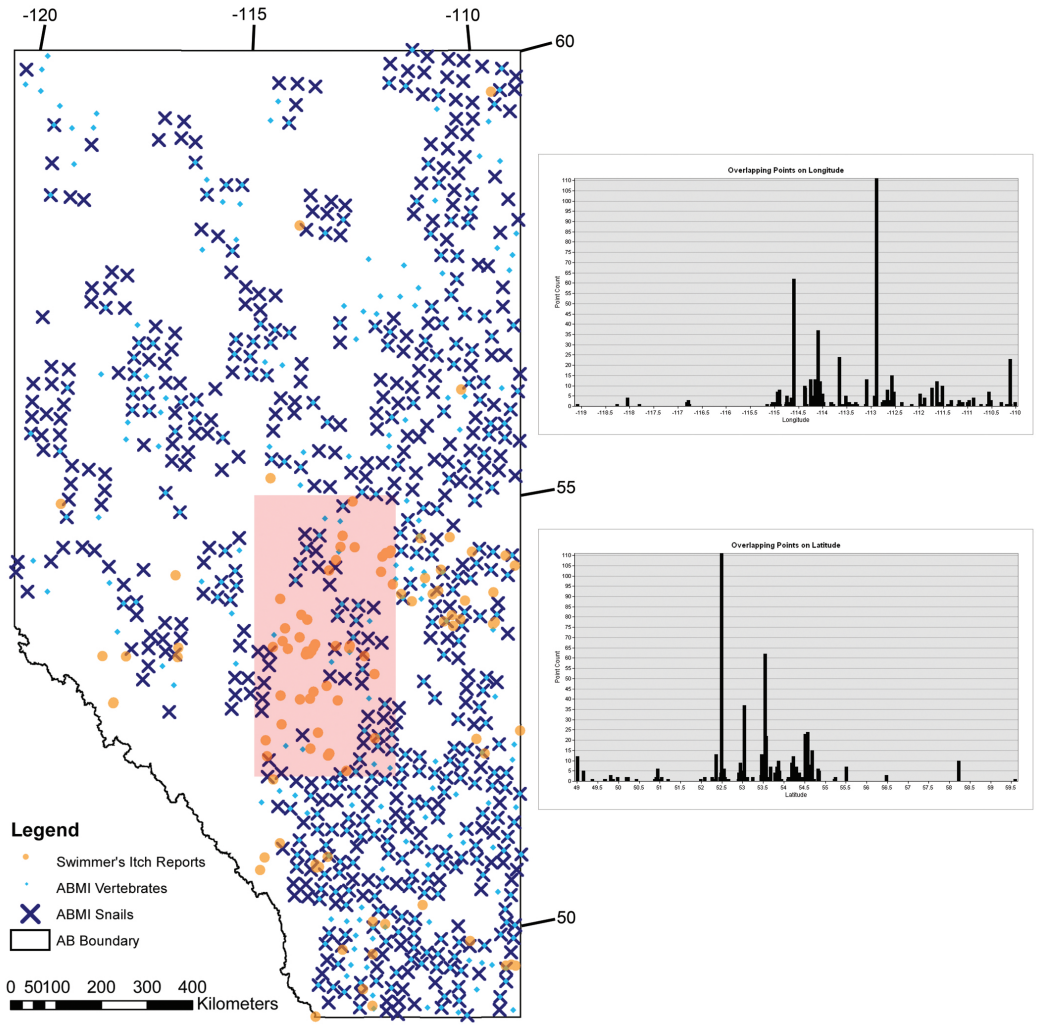

Figure 6.20: Distribution Map of Our Relative Knowledge of Swimmer's Itch in Alberta. This map depicts the distributions of where swimmer's itch cases have occurred (orange circles), and the potential for where swimmer's itch could occur based on the presence of host species. Vertebrate potential host species are depicted by blue diamonds and invertebrate, gastropod potential host species are depicted by a blue 'x'. Where the vertebrates and invertebrates overlap is where there is potential for swimmer's itch transmission. Latitude and Longitude are depicted by tick marks on the outer edge of the map. The graphs on the right-hand side are showing the distribution of overlapping points of latitude and longitude for all three data sets (vertebrate, invertebrate, and swimmer's itch cases). The x-axis being either points of Latitude or Longitude, and the y-axis describing frequency of overlap, as points are stacked (Min = 1, Max = 111, Sum = 607, Mean = 6.07, SD = 13.3). Most overlapping points, and those with greatest frequency, lie within the red bounded box.

Chapter 7

Conclusions

7.1 Overview

Neglected from most biodiversity surveys, parasites are often not considered as important components of ecological processes, as organisms that form their own communities, or as effectors on the assembly of other communities within ecosystems. Parasites with complex life cycles, such as digenean trematode, utilize at least 2-3 species within an ecosystem to complete their development and generate infectious propagules. Despite descriptions of trematodes occurring across the globe, from a wide diversity of vertebrate and invertebrate host species, for hundreds of years (Gibson, Bray, and Harris 2005), our ability to recognize the diversity of trematode species within a single lake remains an incredible challenge.

Digenean trematode distributions, compatibility profiles with their snail hosts, and complete life cycles remain mysteries in many parts of the world. Surveys of digenean biology and ecology provide further insight and perspective into just how incredibly diverse and important helminth parasites are in shaping local ecosystems. Past surveys have provided substantial characterizations of adult digeneans within their definitive hosts, and many now have contributed towards furthering our understanding of larval digeneans within

Parts of this chapter were published in Gordy, M.A., Kish, L., Tarrabain, M., and Hanington, P.C. 2016. "A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada." *Parasitology Research* 115(10): 3867–80. http://www.ncbi.nlm.nih.gov/pubmed/27245072 (September 22, 2016).

their intermediate host communities. However, much information about the diversity of digeneans and their relationships with their snail intermediate hosts are lacking in many locations. This is certainly true in Canada, where few records related to digenean-snail relationships existed before the start of my Ph.D. research.

Currently, there is a need for more information about the presence and distribution of digeneans across Canada, and how this compares to other parts of North America and beyond. To address this diversity gap in Western Canada, six lakes within central Alberta were surveyed for the presence of snails and larval digenean species and their associations over three consecutive summers. This investigation into the diversity of digenean trematodes utilized integrative taxonomic methods to best delineate trematode (and snail) species with the highest level of confidence, given the available evidence.

Ideally, the evidence that should be acquired in order to allow for comparisons among closely related species (or genera) includes the following: a morphological assessment of external and internal features and measurements; a molecular phylogenetic assessment, based on multiple gene sequences, and containing a suitable level of representative congeners to be informative; an understanding of the geographical distributions for all closely related species; and host-association information for all closely related species. I state this as ideal, because we often do not have these luxurious amounts of data available, stemming from the lack of a standard set of methods in the field. Thus, there are many gaps in molecular databases, gaps in trematode species and host records – pertaining to the completeness of life cycles – and a lack of connectivity in a biogeographical context.

Only one of the seventy-nine trematode species uncovered through my thesis work had all the pieces in place to describe it as a new species, *Australapatemon mclaughlini*. Though the story is still incomplete for *A. mclaughlini*, in that we still do not know if there is a second intermediate host or not, we have at least gained enough information to know where to start looking. For most species found in my study, we were limited to molecular assessments, and often in those assessments, limited by the contributions from other re-

searchers in the field. Therefore, many of the digenean trematodes we found are considered putative, cryptic species, until more evidence can be collected.

Some of the important lessons learned from the fine-scale phylogenetic, taxonomic assessments presented in previous chapters were that: morphological measurements, despite their ability to display plasticity, are still important to informing diversity – as demonstrated through the description of *A. mclaughlini* – and that adult worms, because of their historical connections to taxonomic identifications, are still needed for confirmation. Additionally, we found that, although seemingly arbitrary, a 5% cut-off for intraspecific divergence in *cox1* worked well for delimiting most species, especially when there was a good representation of species available for the genus, supporting previous reports in the literature (Vilas, Criscione, and Blouin 2005).

While much of this thesis is very taxonomically heavy, it was absolutely necessary for understanding trematode community assembly processes within central Alberta lakes. For instance, only a few trematode species were both common and highly abundant, while the majority were rare and inconsistent in their appearances from year to year. If we had only identified trematodes by morphology, we would have made very different assessments of their presence and abundance and how that interprets to diversity. Likewise, because of the high rate of rare species found, it is very likely we have not uncovered all the possible diversity of trematodes even within the lakes we have sampled, let alone the rest of Alberta.

The presence of highly diverse communities of rare species is an important revelation, not only for biodiversity surveyors and conservationists, but particularly for the study of schistosomes that cause swimmer's itch. Our survey revealed that the seven species of schistosome found were all rare species within their communities. However, their impact was not at all rare. Our swimmer's itch survey revealed 101 lakes from Alberta that had swimmer's itch reports over the five years of that study, and among those, 1,835 cases of swimmer's itch, many who reported having had gone to the doctor. If causing an allergic condition in humans is considered as a functional trait of schistosomes, they demonstrate a disproportionate effect of impact as compared to the most

common and abundant trematode species. What we have yet to determine is how this may relate to their functions in other ecosystem processes. One can imagine that if high levels of swimmer's itch in a pond/lake led to major efforts for snail control (draining and bleaching the pond/lake), schistosomes could have a very large negative, functional role in the ecosystem by causing anthropogenic impact that led to the disappearance of other species in the ecosystem.

7.2 Significance and applications

The significance of the research I have presented in this thesis lies in the downstream translations of the research to the scientific community and applications of my work in both future research and surveillance efforts. Some of the key contributions I have made to the field are:

- The development of an online swimmer's itch surveillance system (www.swimmersitch.ca).
  - This survey has led to discovery of the distribution and impact of swimmer's across most of Canada. It has captured more cases in the past five years than could be derived from the literature in the past century.
  - Collaboration with Alberta Health Services (AHS) has resulted in the use of our reporting system as a way to inform AHS when to post warning signs at beaches in Alberta.
  - The survey has now been expanded to include swimmer's itch reports from the United States of America and will continue to serve an important role for both swimmer's itch research that is ongoing, as well as a warning system for lake users.
- The derivation of the first description of trematode component communities among their snail first intermediate hosts within Alberta.

  - This has generated insight towards to environmental and ecological factors important to the assembly processes and structure of trematode component communities.
  - Has identified the presence of at least 79 trematode species among just 5 snail host species.To my knowledge, this is the largest diversity of trematodes from one survey.
  - Has identified 7 schistosome species capable of causing swimmer's itch and their snail hosts involved in transmission.
  - Has described a new, rare, species and nine new candidate species from one nominal trematode species, which has confirmed both cryptic morphology and the utility of maintaining morphological assessments in surveys.

- The compilation of literature reviews for:
  - The trematode species of Alberta (Appendix A).
  - The state of knowledge for *Australapatemon* species, with particular review of the global distribution and reports of zygocercous cercariae morphotypes (Chapter 3).
  - The history of swimmer's itch in Canada (Chapter 6).

The work presented in this thesis has, so far, contributed to the publication of two manuscripts, with (at least) two more in review (see Preface).

7.3 Future research

While I have contributed a significant and broad understanding of some of the trematode species and relationships within central Alberta lake ecosystems, with the application towards swimmer's itch and public health, there are several avenues for future research to expand upon the findings presented in this thesis. I have included below some areas in which knowledge gaps can be bridged by testing specific hypotheses that logically stem from my thesis work, and also from collective efforts of the scientific community.

Public health and swimmer's itch in Alberta

Some of the key reasons we study swimmer's itch impacts on public health are not based on their direct health impacts caused by the rash, but their indirect health impacts. Lakes provide many different ecosystem services, one of those being the provision of cultural, spiritual, and recreational benefits. Alberta is enriched with over one thousand lakes, which are highly valuable to residents because of these benefits and their contributions to the local economy (Alberta Environment and Sustainable Resource Development). Tourism is one of Alberta's leading industries and resonates highly around lakes, making any threats to public use of these areas a possible detriment to the local economy. A serious threat towards the enjoyment of Alberta's lakes by residents and visitors are negative impacts on water quality, like the contraction of swimmer's itch. While we know lakes are important, we do not have a good estimate of their economic value, nor a way of quantitatively measuring the economic impact of swimmer's itch.

My doctoral research was the first to provide species-specific analyses of the schistosomes present in Alberta, and to describe their snail hosts involved in transmission. The major gap that still remains in the life cycles of these schistosomes is their specific definitive host species. If we were able to acquire infected waterfowl and aquatic mammals from around Alberta, we may be able to better understand the specifics of their population dynamics that we could not predict due to low sample numbers. For instance, if we knew the definitive hosts, we could better track their activities and distributions through GPS tracking devices and collect information related to their migration timing, and therefore, when eggs may be deposited into Alberta lake ecosystems. We could understand how long they remain at a specific site versus move around from lake to lake. We could even track their migratory routes and answer questions related to where their infections originate, where they take them, and if migration has an effect on their immunological responses to schistosome infections.

Another avenue of research related to swimmer's itch that needs to be explored is how to most effectively communicate swimmer's itch warnings. This was previously discussed in detail in chapter 6, but should be reiterated as a specific area in which particularly public health researchers could play a significant role.

Finally, there is always the need for the development of products or methods that may prevent swimmer's itch transmission, either directly while people are in the water, or indirectly through the prevention of snail infection. Several studies out of Michigan have attempted to treat (C. L. Blankespoor, R. L. Reimink, and Blankespoort 2001; Ronald L. Reimink, DeGoede, and Harvey D. Blankespoor 1995) or relocate birds in order to prevent transmission to the snail host, with treatment using Praziquantel being an ineffective long-term solution. Otherwise, the only marketed product is ®Swimmer's Itch Guard (www.swimmersitchguard.com), a cream that has not had great reviews by lake users (personal communication with Michigan lake association members).

Trematode community ecology

Some specific avenues for further research in trematode community ecology would be to test hypotheses related to specific environmental effects on highly abundant members of the community. For instance, we found that among the five most abundant trematode species that *A. burti* LIN1 appeared more tolerant to lower levels of dissolved oxygen (DO) and found at lower DO levels consistently. Because most Alberta lakes are either eutrophic or hypereutrophic, there are seasonal components to DO levels in these lakes that are related to temperature and the production of algal blooms (discussed in Chapter 5). Also, in Alberta, many lakes experience blooms of cyanobacteria, affecting water quality, in both reducing DO and use of the lakes for recreational purposes, due to toxin production (https://www.albertahealthservices.ca/news/bga.aspx). Laboratory experiments could test the tolerances of snails and highly abundant trematode species to DO content. The differential impact of their survival could be an indicator for effects on trematode community dynamics following algal blooms. Likewise, predictions could be formed re-

garding how snail and trematode communities may be impacted by climate change.

Another area of research that would be of interest is in testing how trematode community dynamics are altered by trematode infection strategy in their snail first intermediate hosts. As described in chapter 5, *Plagiorchis* sp. eggs are consumed by snails, rather than via miracidial penetration of snail head-foot tissue. This could play a significant role in their success as a species in these communities because they may have a higher probability of infection in comparison to miracidia swimming to find a suitable host. This could be tested in the laboratory and could provide important insight towards competition between species that use different strategies. Likewise, in three of the four co-infections found (as described in Chapter 2), *Plagiorchis* sp. was one of the two infecting species. I predict that if *Plagiorchis* sp. infects the snail first, then they would have the dominant advantage, and would prevent infection by other trematodes, but that if the rarer species infects first, that a co-infection would be possible, as a function of the establishment of the prior species having an inhibitory, but not eliminatory effect on *Plagiorchis* in all cases. My reasoning for this is based on the evidence for priority effects impacting infections and co-infections in snails (reviewed in (Armand M. Kuris and Kevin D. Lafferty 1994)).

Trematode taxonomy

The greatest effort needed in the field of trematode taxonomy, in addition to the development of a standard by which the community follows for the collection and descriptions of trematode species, is the extraction of molecular data from historical voucher specimens (paratypes). This effort is crucial to connect the historical descriptions and morphology of adult trematodes to their molecular profiles, therefore providing a way to link larval specimens to their adult counterparts and better understand trematode evolutionary history.

Because of preservation methods and time, old or ancient DNA becomes highly sheared. One of the primary issues is when tissues are preserved in formalin. Formalin causes DNA-protein binding, and can cause base modifi-

cation, in addition to fragmentation (Hykin, Bi, and McGuire 2015). Methods have been developed to address DNA-protein binding problems and increase DNA yield from formalin-fixed museum specimens (Campos and Gilbert 2012). These methods have been used in combination with kit-based, DNA extraction protocols and used on soft tissues from amphibians (Hykin, Bi, and McGuire 2015) and reptiles (Ruane and Austin 2017) for preparation with next-gen sequencing protocols. Next-gen sequencing is perfect for highly fragmented DNA, because DNA-shearing is an initial step in the process of making DNA libraries to run on many platforms, including ®Illumina (Illumina, Inc.). These platforms utilize fragmented DNA to produce short sequence reads, later pieced together with software, and often compared to a reference genome.

Although these techniques have not yet been published in soft-bodied invertebrates, like worms, there has been similar success in even smaller tissue amounts within the forensics and biomedical literature. Successful DNA extraction, PCR, and sequencing has been achieved from formalin-fixed and paraffin embedded human tissue (Shibata, N., and W. J. Martin 1988) and melanoma sections (Volkenandt, McNutt, and Albino 1991), and from 5-year-old slides of cells from human pap smears, preserved and stained under various conditions (Millsaps 2002), to list a few examples. From a combination of techniques described in the literature, particularly to those previously successful on old, soft-tissues, I believe that application of these techniques to preserved trematode worms is achievable.

7.4 Summary

There is still much to learn about the relationships between trematode species within communities, their relationships with their hosts, and with non-host species, as well as the overall impacts that these complex networks have on whole ecosystem health. Particularly, we need a better understanding of the connection between ecosystem health and human health, and how diversity impacts disease. Considering the public health impact of swimmer's itch in Alberta, how widespread the issue is across Canada, and that biodiversity surveys

would suggest the potential for swimmer's itch to occur throughout most lakes (at least in Alberta), further research should take a collaborative, one-health approach that incorporates citizens into the research. It should be collaborative, because of how wide-spread the problem is, but also because it involves many complex questions of species identity and biology, biogeography, health, recreational water use, policy, human-animal/parasite conflict, and more. A one-health approach would require the collaboration of multiple disciplines of researchers towards addressing the goals of better health outcomes. Citizens should also be involved in the research because their direct involvement and buy-in would facilitate solutions that would best fit their interests and needs, while promoting awareness among lake users and contributing to a better overall understanding of the underlying ecological processes. Furthermore, an important lesson learned from the study of trematode communities and the fact that schistosomes are rarely found in snail collections, is that we need a new approach, methodologically, for the collection and study of schistosomes. Particularly, for better understanding of risk factors and the development of tools to measure schistosome impact on humans in the environment, we need innovative approaches that go beyond the snail.

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Appendix A

Trematode Species of Alberta

Table A.1: Digenean trematode diversity of Alberta. A review of the literature is supplemented with species records gathered from this study and previous molecular studies to account for the known trematodes of Alberta, Canada. Host and location records are provided herein.

Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
Allocreadiidae	*Crepidostomum farionis*	Adult	Cold Lake (54.30'N, 110W)	Unidentified	Cisco, Whitefish, Coho Salmon		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
		Adult	Caribou Lake, Eva Lake, Fleming Lake, Margaret Lake, Pitchimi Lake, Semo Lake, Sucker Lake, Wentzel Lake	Unidentified	Various fish species		Baldwin, R.E. and Goater, C.P., 2003, JP, 89(2):215-225
	Crepidostomum isotomum	Adult	Garner Lake, Alberta	Unidentified	Yellow Perch		Zelmer, D.A. and Arai, H.P., 1998, JP, 84(1):24.28
Bolbophoridae	*Bolbophorus* sp.	Cercaria	Canada: Alberta, Buffalo Lake	*Helisoma trivolvis*	Unidentified	KT831373	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Buffalo Lake, Isle Lake, Wabamun Lake	*Helisoma trivolvis*	Unidentified	MH368843, MH368847, MH368850, MH368862, MH368871, MH368892, MH368918, MH368919	Present study
Bunoderidae	*Bunodera luciopercae*	Adult	Cold Lake (54.30'N, 110W)	Unidentified	Unidentified		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
Diplostomidae	Diplostomidae gen. sp. O	Cercaria	Canada: Alberta, Buffalo Lake	*Physa gyrina*	Unidentified	KT831363*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Buffalo Lake, Wabamun Lake, Gull Lake, Isle Lake	*Physa gyrina*	Unidentified	MH368825, MH368851, MH368854, MH368855, MH368879, MH368880, MH368881, MH368882, MH368883, MH368884, MH368885, MH368886, MH368887, MH368888, MH368889, MH368890, MH368893, MH368903, MH368904, MH368905, MH368906, MH368915, MH368916, MH368917, MH368934, MH368935, MH368936, MH368937, MH368938, MH368939, MH368940, MH368941, MH368942	Present study
	Diplostomidae gen. sp. X	Cercaria	Canada: Alberta, Isle Lake	*Physa gyrina*	Unidentified	MH368907	Present study
	Diplostomum adamsi	Metacercaria	Garner Lake, Alberta	Unidentified	Yellow Perch		Zelmer, D.A. and Arai, H.P., 1998, JP, 84(1):24.28
	Diplostomum baeri bucculentum	Metacercaria	NW Territories	Unidentified	Least Cisco		Shostak, et al, 1987, Can. J. Zool., 65

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Diplostomum baeri LIN2	Cercaria	Canada: Alberta, Wabamun Lake, Isle Lake	*Stagnicola elodes*	Unidentified	MH368863, MH368874, MH368875, MH368928	Present study
	Diplostomum indistinctum	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	KT831379	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Diplostomum sp. 1	Cercaria	Canada: Alberta, Wabamun Lake, Isle Lake	*Stagnicola elodes*	Unidentified	MH368857, MH368896, MH368932, MH368943, MH368945	Present study
	Diplostomum sp. 2	unknown	Margaret Lake, AB	Unidentified	Trout Perch		Baldwin, R.E. and Goater, C.P., 2003, JP, 89(2):215-225
	Diplostomum sp. 3	Cercaria	Canada: Alberta, Wabamun Lake	*Lymnaea stagnalis*	Unidentified	KT831358	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake	*Lymnaea stagnalis*	Unidentified	MH368837, MH368858	Present study
	Diplostomum sp. 4	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	KT831354	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH368808, MH368809, MH368813, MH368814, MH368815, MH368816, MH368818, MH368819, MH368820, MH368821, MH368822, MH368823, MH368824, MH368826, MH368827, MH368828, MH368829, MH368830, MH368831, MH368832, MH368833, MH368834, MH368835, MH368836, MH368838, MH368839, MH368840, MH368841, MH368844, MH368845, MH368846, MH368848, MH368849, MH368853, MH368856, MH368859, MH368860, MH368861, MH368864, MH368865, MH368866, MH368867, MH368868, MH368869, MH368870, MH368872, MH368873, MH368876, MH368877, MH368891, MH368898, MH368899, MH368900, MH368901, MH368911, MH368913, MH368914, MH368924, MH368925, MH368926, MH368927, MH368929, MH368930, MH368931, MH368944, MH368946, MH368947, MH368948, MH368949, MH368950	Present study
	Diplostomum sp. A	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH368817	Present study
	Diplostomum sp. B	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	MH368933	Present study

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Table A.1 – *Continued from previous page*

Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Diplostomum sp. C	Cercaria	Canada: Alberta, Gull Lake, Wabamun Lake, Isle Lake	*Stagnicola elodes*	Unidentified	KT831360*, KT831378*, KT831382*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Gull Lake, Wabamun Lake, Isle Lake	*Stagnicola elodes*, *Helisoma trivolvis* (MGC208)	Unidentified	MH368810, MH368811, MH368812, MH368852, MH368895, MH368902, MH368921, MH368922, MH368923	Present study
	Diplostomum spathaceum	Adult	Cooking Lake	Unidentified	Bonaparte's Gulls		Hair, J.D. and Holmes, J.C., 1970, Can. J. Zool. 48:1129-1131
		Larval	Cold Lake (54.30'N, 110W)	Unidentified	Whitefish, Lake Trout, 9-Spine stickleback		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
		Larval	Big Fish Lake, Caribou Lake, Margaret Lake, Wentzel Lake	Unidentified	Various fish species		Baldwin, R.E. and Goater, C.P., 2003, JP, 89(2):215-225
		Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	California Gull, Ring-billed Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270
	Neodiplostomum americanum	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	KT831357*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Ornithodiplostomum ptychocheilus	Cercaria	Lake Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753
		Cercaria/Metacercaria	Central Alberta unnamed lake (54.22'N, 113.27'W)	*Physa gyrina*	Fathead minnows, chickens(experimental)		Schleppe, J.L. and Goater, C.P., 2004, JP, 90(6):1387-1390
	Ornithodiplostomum sp. 2	Cercaria	Canada: Alberta, Wabamun Lake	*Physa gyrina*	Unidentified	KT831368	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake	*Physa gyrina*	Unidentified	KT831368	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Ornithodiplostomum sp. 8	Cercaria	Canada: Alberta, Pigeon Lake	*Physa gyrina*	Unidentified	KT831383	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Isle Lake	*Physa gyrina*	Unidentified	MH368908, MH368910, MH368920	Present study

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Posthodiplostomum minimum	Cercaria/Metacercaria	Central Alberta unnamed lake (54.22'N, 113.27'W)	*Physa gyrina*	Fathead minnows, chickens(experimental)		Schleppe, J.L. and Goater, C.P., 2004, JP, 90(6):1387-1390
	Posthodiplostomum sp. 4	Cercaria	Canada: Alberta, Isle Lake	*Physa gyrina*	Unidentified	MH368909, MH368912	Present study
	Tylodelphys podicipina	Adult	9 lakes in Alberta	Unidentified	*Aechmophorus occidentalis, Podiceps grisegena, Podiceps nigricollis*		Stock, T.M. and Holmes, J.C., 1988, JP, 74(2): 214-227
	Tylodelphys scheuringi	Metacercaria	Garner Lake, Alberta	Unidentified	Yellow perch		Zelmer, D.A. and Arai, H.P., 1998, JP, 84(1):24.28
	Tylodelphys sp. A	Cercaria	Canada: Alberta, Wabamun Lake	*Helisoma trivolvis*	Unidentified	KT831356*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake	*Helisoma trivolvis*	Unidentified	MH368842, MH368878, MH368894, MH368897	Present study
Echinostomatidae	*Drepanocephalus spathans*	Cercaria	Canada: Alberta, Isle Lake, Buffalo Lake	*Helisoma trivolvis*	Unidentified	MH368951, MH368952, MH369294	Present study
		Cercaria	Canada: Alberta, Buffalo Lake	*Helisoma trivolvis*	Unidentified	KT831381	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Echinoparyphium recurvatum	Cercaria/Metacercaria	Lake Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753
		Redia	Isolated pond near Clyde, AB (54.09'N, 113.39'W)	*Helisoma trivolvis*	Unidentified		Morris and Boag, 1982, Can. J. Zool.
		Adult	13 Lakes in Alberta	Unidentified	Lesser Scaup		Bush, A.O. and Holmes, J.C., 1986, Can. J. Zool. 64:132-141
		Adult	Alberta	Unidentified	Great horned owls		Ramalingam, S. and Samuel, W.M., 1978, Can. J. Zool. 56:2454-2456
	Echinoparyphium recurvatum flexum	Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	California Gull, Ringbilled Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Echinoparyphium sp. 1A	Cercaria	Canada: Alberta, Lac La Nonne, Wabamun Lake, Isle Lake	*Physa gyrina, Stagnicola elodes* (MGC1954, MGC2104), *Helisoma trivolvis* (MGC2090)	Unidentified	MH368998, MH368999, MH369001, MH369002, MH369003, MH369004, MH369005, MH369006, MH369007, MH369008, MH369009, MH369010, MH369012, MH369013, MH369014, MH369015, MH369016, MH369017, MH369018, MH369019, MH369022, MH369023, MH369024, MH369025, MH369026, MH369028, MH369031, MH369032, MH369033, MH369034, MH369038, MH369042, MH369044, MH369045, MH369046, MH369047, MH369048, MH369049, MH369052, MH369053, MH369054, MH369055, MH369056, MH369059, MH369060, MH369062, MH369063, MH369065, MH369066, MH369068, MH369070, MH369075, MH369076, MH369087, MH369089, MH369090, MH369091, MH369093, MH369094, MH369095, MH369096, MH369097, MH369098, MH369099, MH369100, MH369101, MH369102, MH369121, MH369122, MH369123, MH369125, MH369131, MH369132, MH369133, MH369136, MH369147, MH369155, MH369156, MH369162, MH369163, MH369164, MH369165, MH369166, MH369167, MH369168, MH369178, MH369188, MH369191	Present study
		Cercaria	Canada: Alberta, Lac La Nonne	*Physa gyrina*	Unidentified	KT831361*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Echinoparyphium sp. 1B	Cercaria	Canada: Alberta, Isle Lake	*Physa gyrina*	Unidentified	MH369181	Present study
	Echinoparyphium sp. A	Cercaria	Canada: Alberta, Buffalo Lake, Wabamun Lake, Isle Lake, Lac La Nonne, Gull Lake, Pigeon Lake	*Physa gyrina, Stagnicola elodes* (MGC1932)	Unidentified	MH369011, MH369035, MH369043, MH369051, MH369058, MH369061, MH369064, MH369069, MH369081, MH369082, MH369083, MH369084, MH369085, MH369113, MH369120, MH369128, MH369161, MH369169, MH369170, MH369171, MH369172, MH369173, MH369174, MH369175, MH369176, MH369177, MH369179, MH369180, MH369182, MH369183, MH369184, MH369185, MH369187	Present study
	Echinoparyphium sp. A2	Cercaria	Canada: Alberta, Gull Lake	*Physa gyrina*	Unidentified	MH369190, MH369127	Present study
		Cercaria	Canada: Alberta, Lac La Nonne	*Stagnicola elodes*	Unidentified	KT831367*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Echinoparyphium sp. B	Cercaria	Canada: Alberta, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH368969, MH368970, MH368971, MH368987, MH368988, MH369041, MH369074, MH369086, MH369092	Present study
	Echinoparyphium sp. C	Cercaria	Canada: Alberta, Gull Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369088, MH369152	Present study
	Echinoparyphium sp. D	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369189	Present study
	Echinoparyphium sp. E	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*, *Lymnaea stagnalis* (MGC1878)	Unidentified	MH369109, MH369129, MH369134, MH369135, MH369159	Present study
	Echinoparyphium sp. Lineage 2	Cercaria	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake, Wabamun Lake, Lac La Nonne	*Stagnicola elodea*, *Lymnaea stagnalis* (MGC16A/B, MGC369), *Helisoma trivolvis* (MGC219)	Unidentified	MH368953, MH368954, MH368955, MH368956, MH368957, MH368959, MH368960, MH368961, MH368962, MH368963, MH368964, MH368965, MH368966, MH368967, MH368968, MH368972, MH368973, MH368974, MH368975, MH368976, MH368977, MH368978, MH368979, MH368980, MH368981, MH368982, MH368983, MH368984, MH368985, MH368986, MH368989, MH368990, MH368991, MH368992, MH368993, MH368994, MH368995, MH368996, MH368997, MH369000, MH369021, MH369027, MH369029, MH369036, MH369037, MH369039, MH369050, MH369057, MH369067, MH369071, MH369072, MH369073, MH369077, MH369078, MH369079, MH369103, MH369104, MH369105, MH369106, MH369107, MH369111, MH369112, MH369114, MH369115, MH369116, MH369117, MH369118, MH369119, MH369124, MH369126, MH369137, MH369138, MH369139, MH369140, MH369141, MH369142, MH369143, MH369144, MH369146, MH369148, MH369149, MH369150, MH369151, MH369153, MH369154, MH369160, MH369186	Present study
		Cercaria	Canada: Alberta, Lac La Nonne	*Stagnicola elodes*	Unidentified	KT831350*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Echinoparyphium sp. Lineage 4	Cercaria	Canada: Alberta, Wabamun Lake, Buffalo Lake	*Helisoma trivolvis*	Unidentified	MH369130, MH369158	Present study
	Echinostoma revolutum	Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	California Gull, Ring-billed Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270
		Adult	Alberta	Unidentified	Great horned owls		Ramalingam, S. and Samuel, W.M., 1978, Can. J. Zool. 56:2454-2456

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Echinostoma revolutum Lineage B	Cercaria	Canada: Alberta, Buffalo Lake, Gull Lake, Wabamun Lake, Isle Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369227, MH369229, MH369230, MH369231, MH369235, MH369242, MH369248, MH369268, MH369279, MH369281, MH369284, MH369286, MH369287, MH369292	Present study
		Cercaria	Canada: Alberta, Buffalo Lake, Gull Lake, Wabamun Lake, Isle Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369192, MH369193, MH369194, MH369195, MH369196, MH369197, MH369200, MH369201, MH369202, MH369204, MH369206, MH369207, MH369208, MH369209, MH369210, MH369211, MH369213, MH369214, MH369215, MH369216, MH369217, MH369218, MH369219, MH369220, MH369221, MH369222	Present study
	Echinostoma trivolvis Lineage A	Cercaria	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne	*Helisoma trivolvis*	Unidentified	MH369271	Present study
	Echinostomatidae gen. sp.	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369269, MH369295, MH369297	Present study
	Hypoderaeum sp. Lineage 1	Cercaria	Canada: Alberta, Gull Lake, Lac La Nonne, Wabamun Lake, Isle Lake	*Stagnicola elodes*	Unidentified	MH368958, MH369040, MH369108, MH369110, MH369145, MH369157	Present study
	Hypoderaeum sp. Lineage 2	Cercaria	Canada: Alberta, Isle Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369020, MH369030, MH369080	Present study
	Neopetasiger islandicus	Cercaria	Canada: Alberta, Wabamun Lake	*Planorbula armigera*	Unidentified	KT831342	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Petasiger nitidius	Cercaria	Amisk Lake (54.35'N, 112.37'W) Baptiste Lake (54.45'N, 113.33'W)	*Heliosoma trivolvis*	Unidentified		Shostak, A.W., 1992, Can. J. Zool. 71:431-434
		Adult	9 lakes in Alberta	Unidentified	*Aechmophorus occidentalis, Podiceps grisegena, Podiceps nigricollis, Podiceps auritus*		Stock, T.M. and Holmes, J.C., 1988, JP, 74(2): 214-227
	Neopetasiger sp. 4	Cercaria	Canada: Alberta, Wabamun Lake	*Helisoma trivolvis*	Unidentified	KT831343, KT831345	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake, Isle Lake, Buffalo Lake	*Helisoma trivolvis*	Unidentified	MH369311, MH369312, MH369313, MH369314, MH369315, MH369316, MH369317, MH369318	Present study
Fasciolidae	*Fasciola hepatica*	Adult	Rimbey, Alberta	Unidentified	Cattle (Holstein steers)		Giebelhaus, I.T. 1998, Can. Vet. J. 39:433
	Fascioloides magna	Adult	Banff National Park (51.12'N, 115.35'W)	Unidentified	*Cervus elaphus canadensis*		Kralova-Hromadova, et al., 2010, IJP, 41:373-383

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
		Adult	Cypress Hills, Elk Island and other parts of Alberta	Unidentified	Moose		Samuel, W.M., 1976, Can J Zool, 54(3)
Gorgoderidae	*Gorgoderina simplex*	Adult	Eastern Alberta (50.35'-56.44'N, 110.40'-114.05'W)	Unidentified	*Bufo hemiophrys* (Canadian Toad)		Bursey, C.R. and Goldberg, S.R. 1998, JP, 84(3):617-618
	Phyllodistomum coregoni	Adult	Cold Lake (54.30'N, 110W)	Unidentified	Whitefish		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
Haematoloechidae	Haematoloechidae gen. sp. A	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	KT831372*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369319, MH369320, MH369321	Present study
Lissorchiidae	*Lissorchis attenuatum*	Adult	Cold Lake (54.30'N, 110W)	Unidentified	9-spine stickleback		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
Notocotylidae	*Notocotylus* sp. A	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	KT831348*, KT831364	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	*Physa gyrina, Stagnicola elodes*	Unidentified	MH369326, MH369333, MH369334, MH369335, MH369345, MH369346, MH369349, MH369350, MH369352, MH369411, MH369353, MH369354, MH369355, MH369362, MH369363, MH369364, MH369365, MH369366, MH369367, MH369372, MH369373, MH369378, MH369381, MH369382, MH369383, MH369415, MH369384, MH369387, MH369390, MH369391, MH369417, MH369393, MH369395, MH369396, MH369397, MH369398, MH369400, MH369401, MH369402	Present study
	Notocotylus sp. B	Cercaria	Canada: Alberta, Wabamun Lake	*Physa gyrina*	Unidentified	MH369416	Present study
	Notocotylus sp. C	Cercaria	Canada: Alberta, Lac La Nonne	*Helisoma trivolvis*	Unidentified	MH369356	Present study
	Notocotylus sp. D	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	KT831348*	Gordy, M.A., et al., 2016, Parasitol. Res.

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
		Cercaria	Canada: Alberta, Isle Lake, Gull Lake, Buffalo Lake, Lac La Nonne	*Stagnicola elodes*, *Physa gyrina*	Unidentified	MH369386, MH369389, MH369392, MH369399, MH369323, MH369405, MH369324, MH369406, MH369325, MH369327, MH369407, MH369408, MH369328, MH369409, MH369329, MH369330, MH369331, MH369332, MH369336, MH369337, MH369338, MH369339, MH369340, MH369341, MH369342, MH369343, MH369344, MH369410, MH369347, MH369348, MH369351, MH369357, MH369358, MH369359, MH369360, MH369361, MH369368, MH369412, MH369369, MH369370, MH369371, MH369374, MH369375, MH369376, MH369413, MH369414, MH369379, MH369380, MH369385, MH369388, MH369394, MH369403, MH369404	Present study
	Notocotylus attenuatus	Adult	Breeding resident in Alberta, but found in SW Texas	Unidentified	Green-winged Teal		Canaris, A.G., Mena, A.C. and Bristol, J.R., 1981, J. Wildlife Dis. 17(1)
		Adult	Alberta	Unidentified	Great horned owls		Ramalingam, S. and Samuel, W.M., 1978, Can. J. Zool. 56:2454-2456
	Notocotylus urbanensis	Cercaria/Metacercaria	Lake Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753
Paramphistomatidae	*Zygocotyle lunata*	Cercaria/Metacercaria	Lysia Lake (55.12'N, 112.29W) Beaver impoundment near Fort McMurray (56.31'N, 111.19'W)	*Helisoma trivolvis*	CD1 Mice (experimental)		Shostak, A.W., Dharampaul, S., and Belosevic, M., 1993, J. Parasitol. 79(6):922-929
Plagiorchiidae	*Plagiorchis elegans*	Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	California Gull, Ring-billed Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270
	Plagiorchis sp.	Adult	Alberta	Unidentified	Great horned owls		Ramalingam, S. and Samuel, W.M., 1978, Can. J. Zool. 56:2454-2456
	Plagiorchis sp. Lineage 1	Cercaria	Canada: Alberta, Gull Lake, Lac La Nonne, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369420, MH369421, MH369422, MH369433, MH369434, MH369435, MH369441, MH369460, MH369461, MH369463, MH369464	Present study
	Plagiorchis sp. Lineage 2	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369467	Present study

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Plagiorchis sp. Lineage 3	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369442, MH369454, MH369466	Present study
	Plagiorchis sp. Lineage 4	Cercaria	Canada: Alberta, Gull Lake, Lac La Nonne, Buffalo Lake, Wabamun Lake, Isle Lake	*Stagnicola elodes*	Unidentified	MH369418, MH369423, MH369425, MH369428, MH369429, MH369431, MH369432, MH369436, MH369437, MH369440, MH369447, MH369452, MH369453, MH369456, MH369462, MH369471	Present study
	Plagiorchis sp. Lineage 5	Cercaria	Canada: Alberta, Gull Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369419, MH369426, MH369427	Present study
	Plagiorchis sp. Lineage 6	Cercaria	Canada: Alberta, Buffalo Lake	*Helisoma trivolvis*	Unidentified	MH369470	Present study
	Plagiorchis sp. Lineage 7	Cercaria	Canada: Alberta, Buffalo Lake, Gull Lake	*Lymnaea stagnalis*	Unidentified	MH369438, MH369448, MH369455, MH369458, MH369468, MH369469	Present study
	Plagiorchis sp. Lineage 8	Cercaria	Canada: Alberta, Buffalo Lake, Gull Lake	*Stagnicola elodes*	Unidentified	MH369449, MH369450, MH369451, MH369459, MH369465	Present study
	Plagiorchis sp. Lineage 9	Cercaria	Canada: Alberta, Lac La Nonne, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369424, MH369430, MH369439, MH369443, MH369444, MH369445, MH369446	Present study
Psilostomidae	Psilostomidae gen. sp. A	Cercaria	Canada: Alberta, Wabamun Lake	*Helisoma trivolvis*	Unidentified	MH369477*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Wabamun Lake, Isle Lake	*Helisoma trivolvis*	Unidentified	MH369473, MH369472, MH369476, MH369474, MH369475	Present study
Renicolidae	*Renicola* sp.	Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	California Gull, Ring-billed Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270
Schistosomatidae	*Austrobilharzia* sp.	Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	Ring-billed Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270
	Avian Schistosomatid sp. A	Cercaria	Canada: Alberta, Buffalo Lake, Isle Lake	*Physa gyrina*	Unidentified	MH168789, MH168790, MH168795, MH168796	Gordy, M.A., et al., 2018, Env. Health. 17(1):73
	Avian Schistosomatid sp. B	Cercaria	Canada: Alberta, Lac La Nonne	*Physa gyrina*	Unidentified	MH168785	Gordy, M.A., et al., 2018, Env. Health. 17(1):73
	Avian Schistosomatid sp. C	Cercaria	Canada: Alberta, Wabamun Lake	*Helisoma trivolvis*	Unidentified	MH168793	Gordy, M.A., et al., 2018, Env. Health. 17(1):73
	Schistosomatium douthitti	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	KT831376	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Buffalo Lake , Gull Lake, Wabamun Lake	*Stagnicola elodes*, *Lymnaea stagnalis* (MGC1878)	Unidentified	MH168791, MH168794	Present study
	Trichobilharzia cameroni	Cercaria	Lake Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Trichobilharzia physellae		Canada: Alberta, Lac La Nonne	*Physa gyrina*	Unidentified	MH168784	Gordy, M.A., et al., 2018, Env. Health. 17(1):73
		Cercaria	Lake Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753
	Trichobilharzia stagnicolae	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	MH168781, MH168782, MH168786, MH168787, MH168788	Gordy, M.A., et al., 2018, Env. Health. 17(1):73
		Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	KT831352	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
	Trichobilharzia szidati	Cercaria	Canada: Alberta, Gull Lake	*Lymnaea stagnalis*	Unidentified	MH168783	Gordy, M.A., et al., 2018, Env. Health. 17(1):73
		Cercaria	Canada: Alberta, Buffalo Lake	*Lymnaea stagnalis*	Unidentified	KT831375	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
Strigeidae	*Apatemon gracilis*	Cercaria	Lake Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753
		Adult	13 Lakes in Alberta	Unidentified	Lesser Scaup		Bush, A.O. and Holmes, J.C., 1986, Can. J. Zool. 64:132-141
		Larval	Cold Lake (54.30'N, 110W)	Unidentified	9-spine stickleback		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
		Adult	9 lakes in Alberta	Unidentified	*Aechmophorus occidentalis, Podiceps grisegena, Podiceps nigricollis, Podiceps auritus*		Stock, T.M. and Holmes, J.C., 1988, JP, 74(2): 214-227
		Adult	Alberta	Unidentified	*Chen hyperborea* and *Mareca americana*		Palmieri, J.R., 1973, JP, 59(6):1063
	Apatemon sp. A	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	MH369603, MH369604, MH369605, MH369606, MH369607, MH369608, MH369609, MH369610, MH369611, MH369612, MH369613, MH369614, MH369615, MH369616, MH369617	Present study

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Apatemon sp. B	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	MH369618	Present study
	Apatemon sp. C	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	MH369619, MH369620, MH369621, MH369622	Present study
	Apharyngostrigea pipientis	Adult	Eastern Alberta	Unidentified	Western Chorus Frog		Goldberg, S.R., Bursey, C.R., and Wong, C., 2002, Northwest Science, 76(1)
	Australapatemon burti LIN1	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	KT831346, KT831351	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis, Helisoma campanulatum, Planorbis* sp., *Lymnaea stagnalis*	Unidentified	KY207548, KY207549, KY207551, KY207552, KY207553, KY207554, KY207555, KY207556, KY207559, KY207560, KY207561, KY207562, KY207563, KY207564, KY207565, KY207566, KY207567, KY207568, KY207570, KY207571, KY207572, KY207573, KY207574, KY207575, KY207576, KY207578, KY207579, KY207580, KY207581, KY207584, KY207585, KY207586, KY207588, KY207589, KY207590, KY207591, KY207592, KY207593, KY207594, KY207595, KY207598, KY207599, KY207600, KY207601, KY207602, KY207603, KY207604, KY207605, KY207606, KY207607, KY207608, KY207609, KY207610, KY207611, KY207612, KY207614, KY207617, KY207618, KY207619, KY207620, KY207621, KY207623, KY207624, KY587399, KY587398, KY587394, KY587401, HM385485, KY587400, HM385486	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
		Cercaria	Canada: Alberta, Isle Lake, Wabamun Lake, Lac La Nonne, Gull Lake, Buffalo Lake	*Stagnicola elodes, Physa gyrina, Helisoma trivolvis*	Unidentified	MH369623, MH369624, MH369625, MH369626, MH369627, MH369628, MH369629, MH369630, MH369631, MH369632, MH369633, MH369634, MH369635, MH369636, MH369637, MH369638, MH369639, MH369640, MH369641, MH369642, MH369643, MH369644, MH369645, MH369646, MH369647, MH369648, MH369649, MH369650, MH369651, MH369652, MH369653, MH369654, MH369655, MH369656, MH369657, MH369658, MH369659, MH369660, MH369661, MH369662, MH369663, MH369664, MH369665, MH369666, MH369667, MH369668, MH369669, MH369670, MH369671, M H369672, MH369673, MH369674, MH369675, MH369676, MH369677, MH369678, MH369679, MH369680, MH369681, MH369682, MH369683, MH369684, MH369686, MH369687, MH369688, MH369689, MH369690, MH369691, MH369692, MH369693, MH369694, MH369695, MH369696, MH369697, MH369698, MH369699, MH369700, MH369701, MH369702, MH369703, MH369704, MH369705, MH369706, MH369707, MH369708, MH369709, MH369710, MH369711, MH369712, MH369713, MH369714, MH369715, MH369716, MH369717, MH369718, MH369719, MH369720, MH369721, MH369722, MH369723, MH369724, MH369725, MH369726, MH369727, MH369728, MH369729, MH369730, MH369731, MH369732, MH369733, MH369734, MH369735, MH369736, MH369737, MH369738, MH369739, MH369740, MH369741, MH369742, MH369743, MH369744, MH369745, MH369746, MH369747, MH369748, MH369749, MH369750, MH369751, MH369752, MH369753, MH369754, MH369755, MH369756, MH369757, MH369758, MH369759, MH369760, MH369761, MH369762, MH369763, MH369685	Present study
	Australapatemon mclaughlini	Cercaria, Adult	Canada: Alberta, Buffalo Lake; Ontario	*Physa gyrina*	*Anas acuta*	KY207615, KY207627, KY207628	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
		Cercaria	Canada: Alberta, Buffalo Lake	*Physa gyrina*		MH369764	Present study

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Australapatemon sp. LIN2	Adult	Canada: Ontario	Unidentified	*Bucephala albeola*	HM385535	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
	Australapatemon sp. LIN3	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	KY207577	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
	Australapatemon sp. LIN4	Cercaria, Adult	Canada: Alberta, Lac La Nonne; Ontario	*Physa gyrina*	*Aythya collaris*	KY207569, KY587397, KY587396	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
		Cercaria	Canada: Alberta, Gull Lake	*Physa gyrina*	Unidentified	MH369765	Present study
	Australapatemon sp. LIN5	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	KY207597	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
	Australapatemon sp. LIN6	Cercaria	Canada: Alberta, Pigeon Lake, Isle Lake	*Physa gyrina*	Unidentified	KY207613, KY207616	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
		Cercaria	Canada: Alberta, Isle Lake, Buffalo Lake, Lac La Nonne	*Physa gyrina*	Unidentified	MH369766, MH369767, MH369768, MH369769, MH369770	Present study
	Australapatemon sp. LIN8	Cercaria, Adult	Canada: Alberta, Isle Lake, Buffalo Lake; Ontario	*Physa gyrina*	*Oxyura jamaicensis*	KY207587, KY207622, HM385538, HM385537, HM385536	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
		Cercaria	Canada: Alberta, Isle Lake, Buffalo Lake, Gull Lake	*Physa gyrina*	Unidentified	MH369771, MH369772, MH369773, MH369774, MH369775, MH369776, MH369777	Present study
	Australapatemon sp. LIN9A	Cercaria, Adult	Canada: Alberta, Gull Lake, Isle Lake, Buffalo Lake; Ontario	*Stagnicola elodes*	*Anas acuta*	KY207550*, KY207557*, KY207558*, KY207582*, KY207596*, HM385534*	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
		Cercaria	Canada: Alberta, Lac La None, Gull Lake, Buffalo Lake, Isle Lake	*Stagnicola elodes, Lymnaea stagnalis* (MGC176B)	Unidentified	MH369779, MH369780, MH369781, MH369782, MH369783, MH369784, MH369785, MH369786, MH369787, MH369788, MH369789, MH369778	Present study
	Australapatemon sp. LIN9B	Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	KY207583*	Gordy, M.A., et al., 2017, Parasitol. Res. 116(8): 2181-98.
		Cercaria	Canada: Alberta, Buffalo Lake	*Stagnicola elodes*	Unidentified	MH369790, MH369791, MH369792	Present study
	Australapatemon sp. LIN10	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	MH369793	Present study
	Cercariae douglasi	Cercaria/Metacercaria	Canada: Alberta, Lac Wabamun (114.35'W, 53.32'N)	*Physa gyrina*	Unidentified		Sankurathri, C.S. and Holmes, J.C., 1976, Can. J. Zool. 54:1742-1753

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
	Cotylurus cornutus	Cercaria	Canada: Alberta, Gull Lake	*Stagnicola elodes*	Unidentified	KT831347*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.
		Cercaria	Canada: Alberta, Gull Lake, Isle Lake, Lac La Nonne	*Stagnicola elodes*, *Helisoma trivolvis* (MGC205)	Unidentified	MH369478, MH369480, MH369484, MH369485, MH369486, MH369487, MH369488, MH369489, MH369490, MH369491, MH369492, MH369493, MH369494, MH369495, MH369496, MH369497, MH369498, MH369500, MH369501, MH369502, MH369503, MH369504, MH369505, MH369509, MH369510, MH369511, MH369516, MH369532, MH369538, MH369539, MH369544, MH369557, MH369597, MH369601	Present study
	Cotylurus erraticus	Larval	Cold Lake (54.30'N, 110W)	Unidentified	White sucker, Whitefish, Cisco		Leong, T.S. and Holmes, J.C., 1981, J. Fish Biol. 18:693-713
		Larval	Big Fish Lake, Caribou Lake, Eva Lake, Fleming Lake, Margaret Lake, Pitchimi Lake, Semo Lake, Sucker Lake, Wentzel Lake	Unidentified	Various fish species		Baldwin, R.E. and Goater, C.P., 2003, JP, 89(2):215-225
		Adult	Beaverhill Lake (53.30'N, 112.30'W) and Miquelon Lake (53.15'N, 112.55'W)	Unidentified	Ring-billed Gulls		Vermeer, K. 1969, Can. J. Zool. 47:267-270
	Cotylurus flabelliformis	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	MH369519	Present study
	Cotylurus hebraicus	Adult	13 Lakes in Alberta	Unidentified	Lesser Scaup		Bush, A.O. and Holmes, J.C., 1986, Can. J. Zool. 64:132-141
	Cotylurus marcogliesei	Cercaria	Canada: Alberta, Buffalo Lake, Gull Lake, Isle Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369479, MH369481, MH369515, MH369530, MH369531, MH369553, MH369564	Present study
	Cotylurus strigeoides	Cercaria	Canada: Alberta, Buffalo Lake, Wabamun Lake, Isle Lake, Lac La Nonne	*Physa gyrina*	Unidentified	MH369517, MH369518, MH369525, MH369526, MH369527, MH369528, MH369529, MH369560, MH369571, MH369572, MH369574, MH369575, MH369577, MH369583, MH369584, MH369587, MH369588, MH369590, MH369595, MH369596, MH369599, MH369600	Present study
	Cotylurus sp. A	Cercaria	Canada: Alberta, Isle Lake	*Stagnicola elodes*	Unidentified	KT831371*	Gordy, M.A., et al., 2016, Parasitol. Res. 115(10): 3867-80.

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Family	Trematode Species	Life Cycle Stage	Locations	Snail Host Species	Definitive /Other Host	GenBank Accession Number(s)	Reference
		Cercaria	Canada: Alberta, Isle Lake, Lac La Nonne, Wabamun	*Stagnicola elodes, Physa gyrina* (MGC1962)	Unidentified	MH369513, MH369520, MH369521, MH369522, MH369523, MH369524, MH369533, MH369537, MH369541, MH369542, MH369543, MH369545, MH369546, MH369547, MH369548, MH369549, MH369550, MH369551, MH369552, MH369554, MH369555, MH369556, MH369558, MH369559, MH369561, MH369562, MH369573, MH369578, MH369579, MH369580, MH369581, MH369582, MH369585, MH369589, MH369591, MH369594, MH369598, MH369602	Present study
	Cotylurus sp. B	Cercaria	Canada: Alberta, Isle Lake	*Physa gyrina*	Unidentified	MH369586	Present study
	Cotylurus sp. C	Cercaria	Canada: Alberta, Buffalo Lake	*Lymnaea stagnalis*	Unidentified	MH369563, MH369566, MH369576	Present study
	Cotylurus sp. D	Cercaria	Canada: Alberta, Buffalo Lake	*Physa gyrina*	Unidentified	MH369592	Present study
	Cotylurus sp. E	Cercaria	Canada: Alberta, Buffalo Lake, Gull Lake, Isle Lake, Lac La Nonne	*Stagnicola elodes*	Unidentified	MH369482, MH369483, MH369499, MH369506, MH369507, MH369508, MH369534, MH369535, MH369536, MH369540, MH369565, MH369593	Present study
	Cotylurus sp. F	Cercaria	Canada: Alberta, Buffalo Lake, Wabamun Lake	*Lymnaea stagnalis*	Unidentified	MH369512, MH369514, MH369567, MH369568, MH369569, MH369570	Present study
Unknown	*Choledocystus pennsylvaniensis*	Adult	Eastern Alberta	Unidentified	Western Chorus Frog		Goldberg, S.R., Bursey, C.R., and Wong, C., 2002, Northwest Science, 76(1)

* Sequence updated in present study