Disentangling a freshwater amphipod–acanthocephalan system from ecological and molecular perspectives

by

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Abstract

One of the major goals in ecological research is to understand factors that influence distribution, diversity and prevalence of parasites and their hosts. How hosts are distributed geographically clearly restricts the spatial distribution of associated obligatory parasites. This restriction is more complicated for multi-host parasites, because they are affected by the geographical distribution of both intermediate and final host species. Taxonomic and genetic diversity of parasites in a particular area can be influenced by the history of colonization, with time available for colonizing a new area being particularly relevant. Prevalence of parasites in final hosts is likely to be positively related to their prevalence in intermediate hosts, but what determines the prevalence of parasites in intermediate hosts? In this thesis I explore these questions using a host–parasite system widely distributed in freshwater bodies across the Holarctic: acanthocephalan worms that use aquatic birds and mammals as final hosts and the amphipod *Gammarus lacustris* Sars as an intermediate host. Both the intermediate host and the parasites can be transported long distances to new areas, including newly formed water bodies, by clinging to the feathers of birds (the amphipods) or by being transported inside the bodies of intermediate and final hosts (the acanthocephalans).

I explore the mechanisms influencing acanthocephalan prevalence and the intraspecific genetic diversity of *Polymorphus* species in their intermediate host *G. lacustris* in water bodies in and near Edmonton, Alberta, Canada. Most of these were human-made water bodies with known ages of construction. To identify acanthocephalan larvae to species level, I tested the consistency and accuracy of the traditional method of morphological identification of waterfowl-associated cystacanths, which is based on proboscis hook arrays, using computer-

based statistical simulations together with molecular and morphological techniques. I found high accuracy of species identification for waterfowl-associated acanthocephalans based on hook morphology of real and simulated adult specimens. By using both molecular and morphological approaches, I differentiated four putative species of *Polymorphus* based on larvae.

In field research over three years, I found that waterbody age and the abundance of common final hosts were important factors for acanthocephalan prevalence in 36 water bodies in both 2015 and 2016. In additional sampling in 2017, I found that acanthocephalan prevalence was significantly higher in older water bodies than in young ones. Furthermore, I tested how waterbody age is related to mtDNA genetic diversity of *G. lacustris* and *Polymorphus* species from ten water bodies with various ages. After controlling for the species richness of known hosts and waterbody size, I found that the intraspecific genetic diversity of *G. lacustris* had a hump-shaped relationship with waterbody age, which suggests that certain genotypes might out compete others over time. In contrast, *P.* cf. *paradoxus* Connell & Corner showed a linear relationship between its intraspecific genetic diversity and waterbody age, which is predicted by the 'pure' colonization-time hypothesis. After conducting these studies at a fine geographical scale, I investigated whether mtDNA population structure of *G. lacustris* is related to waterfowl flyways in North America, Europe and Asia and whether the Rocky Mountains acted as a barrier to gene flow. I found that mtDNA population structure of *G. lacustris* is correlated with flyways but was not strongly influenced by the Rocky Mountains.

This thesis research highlights the importance of habitat age and use by final hosts for parasite prevalence in intermediate hosts, and provides empirical evidence for differing relationships between habitat age and intraspecific genetic diversity for the host and parasite

species. Furthermore, I show how waterfowl might influence invertebrate dispersal and genetic connectivity within and among continents. Future research could assess whether population genetic structure of *Polymorphus* species also matches host flyways. My research provides insight into the mechanisms by which host and parasites become distributed at different spatial scales that are broadly relevant for many other host–parasite systems.

Preface

A version of Chapter 2 will be submitted to Parasitology Research. Heather Proctor will be the supervisory author on this manuscript, who helped with project creation, financial support and editing of the manuscript. I was responsible for sampling amphipods and acanthocephalans, morphometric measurement of cystacanth hooks, acanthocephalan identification, data analyses and writing the manuscript.

A version of Chapter 3 will be submitted to Ecology. Heather Proctor will be the supervisory author on this manuscript, who helped with project creation, financial support and editing of the manuscript. I was responsible for collecting amphipods and acanthocephalans in the field, measurements of environmental variables, data analyses and writing the manuscript.

A version of Chapter 4 will be submitted to Parasitology. Heather Proctor will be the supervisory author on this manuscript, who helped with project creation, financial support and editing of the manuscript. I was responsible for collecting amphipods and acanthocephalans in the field, mtDNA amplifications and sequencing of amphipods and acanthocephalans, genetic data analyses and writing the manuscript.

A version of Chapter 5 will be submitted to Freshwater Biology. This will be a sole-authored publication. I was responsible for collecting amphipods and acanthocephalans in the field, mtDNA amplifications and sequencing, genetic data analyses and writing the manuscript.

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Chapter 1 General introduction

1.1. Multi-host parasitism

Parasitism can be defined as a long-term association between two organisms, where one (the parasite) lives inside or on the surface of the other (the host) and has harmful effects on the host (Schmidt and Roberts 1985). Parasites evolved from free-living organisms to exploit hosts as resources for feeding, reproduction and survival (Poulin 2011). Some parasites have evolved to require more than one host species to fulfill their life cycles. In such complex life cycles, larvae occupy 'intermediate' hosts in which sexual reproduction does not occur (though asexual reproduction may take place), while adult parasites occupy 'final' or 'definitive' hosts in which sexual reproduction occurs (Choisy et al. 2003, Parker et al. 2003). Some parasites (e.g., trematodes) may use more than one intermediate host species for their larval stages. Multi-host parasites can impact the survival and reproduction of many host species (Baudoin 1975, Minchella 1985, Amat et al. 1991,), with consequences that influence whole communities and even ecosystems (Hatcher and Dunn 2011). Despite the ecological importance of multi-host parasites, the diversity and natural history of many taxa are poorly known (Poulin and Morand 2005).

One potential reason for the poor understanding of the biology of multi-host parasites is the difficulty of identifying their larval stage and linking larvae to known adults. Larval parasites are small bodied and often lack the adult-only morphological traits that are crucial for species-level identifications. Molecular tools that provide the opportunity for species diagnosis and discovery have been utilized for parasite species identification in many studies (Locke et al. 2010a, Locke et al. 2010b, Oceguera-Figueroa et al. 2010, Nadler and Pérez-Ponce de León 2011). One of the most commonly used genetic markers is mitochondrial cytochrome c oxidase subunit I gene (COI), which has become the standard DNA 'barcoding' region for taxonomic identification (Hebert et al. 2003, Hansen et al. 2007, Ferri et al. 2009, Bergmame et al. 2011). In particular, several studies support the utility of COI marker to identify and discover parasite species (Ferri et al. 2009, Bergmame et al. 2011, Alcántar-Escalera et al. 2013). Together with morphometric analysis, such molecular approaches can link larval parasites to known adults (Caffara et al.

2011, Locke et al. 2011). In Chapter 2, I use a combination of morphological and molecular methods to delimit four multi-host acanthocephalan species.

1.2. Geographical distribution patterns and processes

How hosts and their associated parasites are distributed geographically is one of the most important themes for modern parasitologists and ecologists (Morand and Krasnov 2010). The geographical distribution of hosts can be determined by environmental factors (Stensgaard et al. 2013), as well as the dispersal ability of hosts themselves or the animal vectors that transport hosts (Figuerola and Green 2002, Green and Figuerola 2005, Reynolds et al. 2015, Coughlan et al. 2017). In contrast, the geographical distribution of an obligatory parasite is restricted to that of its hosts. Distribution is even more restricted for parasites with multi-host life cycles where distribution is determined by that of both intermediate and final hosts (Fredensborg et al. 2006). In some cases, these parasites can share nearly identical geographical regions with their hosts (Hoberg 1992, Martínez-Aquino et al. 2013). In contrast, hosts can live without their parasites and not every parasite is successfully transported via its hosts to new areas. Because of this, the geographical distributions of hosts and parasites may not be congruent in every case (Nuismer et al. 2003). One of the best known patterns illustrating the incongruent distribution of parasites and their hosts is that the prevalence and diversity (genetic or taxonomic) of introduced parasites is often lower in invaded areas compared to their hosts' historical ranges (Dlugosch and Parker 2008). Torchin et al. (2003) studied 26 host species which included invertebrates, fishes, birds, mammals, amphibians and reptiles, and found that the number of parasite species and parasite prevalence was higher in their original ranges than in invaded areas. For example, in North America, native snails were infected with ten trematode species whereas invasive snails had only one trematode species (Torchin et al. 2003). Similarly, in Ireland, the native amphipod *Gammarus duebeni* Lilljeborg has five parasite species whereas three introduced amphipod species harbor three parasite species (Dunn and Dick 1998, MacNeil et al. 2003a, MacNeil et al. 2003b).

The incongruence between host and parasite distributions or genetic diversity could be the result of multiple factors. One is that, by chance, certain species or genotypes of parasites present on or in the hosts in the original area are absent from the hosts that found a new population ('missing the boat', Paterson and Gray 1997). Another possibility is sampling error on the part of

biologists: the prevalence of the non-native parasites may be too low in invaded areas to detect (Paterson and Gray 1997, MacLeod et al. 2010). Another potential mechanism is that parasite death induced by mortality of infected hosts ('sinking with the boat' MacLeod et al. 2010) as a result of negative effects of parasites on hosts is exacerbated by stressful environmental conditions, predation or competition during transit or at new location (MacLeod et al. 2010, Hatcher et al. 2012, Telfer and Bown 2012). Even if local environment conditions favour competent hosts, the parasites may fail to establish ('lost overboard', MacLeod et al. 2010) because they may have small population sizes with reduced genetic variation (founder effect), which are likely to suffer from genetic bottlenecks and genetic drift (Barrett and Schluter 2008). A rescue effect may occur if multiple introductions of parasites counteract founder effects (Rius et al. 2015). This also holds at the species level, as Guegan and Kennedy (2009) found that helminth species richness is positively related to the time since fish hosts were introduced in Britain. Similarly, Ebert et al. (2001) showed that endoparasite richness in *Daphnia* increases linearly with duration of continuous inhabitation of *Daphnia*.

1.3. Study system: acanthocephalan–amphipod system the freshwater amphipod *Gammarus lacustris* and acanthocephalan worms

The Acanthocephala, commonly known as thorny-headed worms, are obligatorily endoparasitic invertebrates possessing an eversible proboscis armed with hooks. All known acanthocephalans use an arthropod intermediate host for their larval stage and then are transmitted to the gut of definitive vertebrate hosts for their adult stage (Kennedy 2006). Although this group has been historically treated as a phylum, Sielaff et al. (2016) provide molecular evidence that Acanthocephala is phylogenetically related to the rotifer group Bdelloidea and hence belongs within the phylum Rotifera. Amin (2013) estimated that Acanthocephala includes 1298 named species, 157 genera and 26 families in three primary classes: Archiacanthocephala (28.5% of species), Eoacanthocephala (14.5%) and Palaeacanthocephala (57%). These three classes have different ecological and biological characteristics. All known eoacanthocephalan species are aquatic: they use ostracods, copepods and amphipods as intermediate hosts for their larval stage and reptiles, fish and amphibians as definitive hosts (Schmidt 1985). Similarly, most known species of Palaeacanthocephala are aquatic (Kennedy 2006). Intermediate hosts of aquatic Palaeacanthocephala are isopods and amphipods and their definitive hosts include fish,

mammals, amphibians and waterfowl (Schmidt 1985, Kennedy 2006). In contrast, archiacanthocephalans use terrestrial arthropods (e.g., Dermaptera, Coleoptera, Orthoptera and Myriapoda) as intermediate hosts for the larval stage and adults infect birds and mammals (Schmidt 1985, Kennedy 2006). Some studies recognize a fourth class, Polyacanthocephala, a small group in which species are aquatic and use South American caimans as definitive hosts (Amin 1987, García-Varela et al. 2002), although other aspects of their life histories are not known.

Acanthocephalans generally have four life-history stages: acanthor stage (embryonated eggs), larval acanthella and cystacanth stage in the intermediate host, and the adult stage in the definitive host (Schmidt 1985). Adults exhibit sexual dimorphism with females being larger than males (Kennedy 2006). Sexual reproduction appears to be the norm, and it is unclear whether any form of asexual reproduction occurs (Kennedy 2006). Adults move along the gut of the final host presumably seeking preferred feeding sites. For example, Burlingame and Chandler (1941) showed that adult *Moniliformis dubius* Meyer live in the posterior part during the first couple of weeks and then move anteriorly to locate better sites for nutrient absorption (Crompton 1973). Adults take nutrients from the lumen contents of the host's gut rather than by ingesting gut tissues (Crompton 1973). Besides feeding in their preferred sites in the host's gut, males and females contact each other and mate in relatively precise sites of host's gut where males move toward females to fertilize them (Kennedy 2006). Eggs develop into acanthors in ovarian balls which develop from ovaries in the body of female adult acanthocephalans (Whitfield 2009). Mature shelled acanthors are sorted within the uterine bell of females and released into the final host's gut and finally into the environment in host faeces (Crompton 1985). Sometimes, the body of a pregnant female decays and mature acanthors exit and infect intermediate hosts (Kennedy 2006).

Although mature acanthors can be potentially consumed by any invertebrate species, those eaten by non-competent invertebrates fail to develop completely (Kennedy 2006). If mature acanthors are eaten by competent arthropod intermediate hosts, they hatch in the intestine and penetrate into the hemocoel. There they transform first into an acanthella larvae that cannot infect a definitive host, and finally into a cystacanth that is infective to a definitive host (Kennedy 2006). Cystacanths obtain energy from intermediate hosts for their own development (Gismondi et al.

2012). This can result in decreased ability to withstand chemical stress in intermediate hosts (e.g., Gismondi et al. 2012). Cystacanths infect definitive vertebrate hosts when that host consumes an infected intermediate host, and then develop into adults in the definitive host's gut. Both cystacanths and adult acanthocephalans possess a spiny proboscis, which is inverted in the cystacanth but everted in the adult. The proboscis is used to hook onto the mucosa of intestinal wall of the definitive host where it may cause physical damage, anemia and general debilitation, resulting in weight loss and potentially even death of definitive hosts (Barnes 1963, Itämies et al. 1980, McDonald 1988).

Acanthocephalans are widely distributed in freshwater, marine, and terrestrial habitats. Freshwater acanthocephalans are the dominant helminths in some geographical locations and hosts. For example, the acanthocephalans in freshwater fish are more common in Canada than in the tropics; in Canada, they occur as far north as Baffin Island (Curtis 1979, Choudhury and Dick 2000). Acanthocephalans dominate parasite assemblages of eels (Anguillidae) in the British Isles and inland European water bodies (Conneely and McCarthy 1984, Moravec 1985, Kennedy 1990, Callaghan and McCarthy 1996, Sures et al. 1997, Kennedy 2009), but not in other regions (North America, New Zealand and Australia) (Hine 1978, Cone et al. 1993, Marcogliese and Cone 1993, Kennedy 2009).

In Alberta, Canada, acanthocephalans are common parasites in freshwater habitats, many of which (e.g. *Polymorphus*) use amphipods such as *Gammarus lacustris* Sars as intermediate hosts for their larval stage and are transmitted to the guts of their final hosts (several waterfowl species [e.g., Mallard and grebes]), the muskrat *Ondatra zibethicus* (Linnaeus) and beaver *Castor canadensis* (Kuhl) (Connell and Corner 1957, Denny 1969, Bush 1980, Anteau et al. 2011; Figure 1.1). In Alberta, *G. lacustris* is an omnivorous and opportunistic species feeding on algae, zooplankton, chironomid larvae and caddisflies (Anderson and Raasveldt 1974, Moore 1977, de March 1981). It also eats the soft body parts of snails and dead freshwater invertebrates, based on my own personal observations. These amphipods can be found in the upper layers of sediments, on detritus and in the water column (Wilhelm and Schindler 2000). In Alberta, female *G. lacustris* have one brood in June (Clifford 1969) and probably have one generation per year, with moderate egg production (ca. 20.2 eggs/female in May and 16.2 in June) (Mathias and Papst 1981). Offspring grow rapidly in later summer and autumn, and adults overwinter

without body growth. Amphipods are important prey for many species of waterfowl and shorebirds in western Canada (e.g., Lindeman and Clark 1999). Some, but not all, amphipod-eating waterfowl are known to act as definitive hosts for the acanthocephalans that parasitize *G. lacustris* (Stock 1985, Butterworth 2002). *Gammarus lacustris* is widely distributed across the whole Holarctic region including North America, Europe and Asia (Väinölä et al. 2008). Its wide distribution is likely to be related to waterfowl-mediated dispersal where amphipods use hook-like claws on pereopods 3–7 to cling to the feathers of waterfowl (or fur of aquatic mammals) and can hold on for up to 2 hours out of water (Segerstråle 1953, Gherardi 2007). The chance of transport increases when *G. lacustris* is infected by acanthocephalans that manipulate the infected amphipods to move to the water surface and elevate the possibility of attaching onto moving objects (e.g., *Polymorphus paradoxus* Connell & Corner) (Helluy and Holmes 1990). Positive phototaxis and clinging of *G. lacustris* infected by acanthocephalans can also increase the chance of being eaten by waterfowl.

1.4. Thesis objectives and outline

The overarching purpose of my thesis research is to use this acanthocephalan–amphipod system to elucidate ecological and short-time scale evolutionary mechanisms influencing host and parasite distributions. To fulfill this objective, I identify acanthocephalan cystacanths to species level. In Chapter 2, I test the consistency and accuracy of the traditional method of morphological identification of cystacanths, which is based on proboscis-hook arrays. In addition, I use computer-based statistical simulations together with molecular and morphological techniques to investigate whether the characters of proboscis hooks are sufficient for discriminating different taxonomical levels of waterfowl-associated acanthocephalans collected from Alberta water bodies (Chapter 2). One main objective of my thesis is to identify the ecological mechanisms influencing acanthocephalan prevalence and intraspecific genetic diversity using *Polymorphus* species in the intermediate host *G. lacustris* at a fine geographical scale (Chapter 3). Previous studies have found that time available for host and parasite colonization is important for, and positively correlates with parasite richness (i.e. colonization-time hypothesis in Guégan and Kennedy 1993 and Ebert et al. 2001) and genetic diversity of parasites (Haag et al. 2005, Herborg et al. 2007, Roman and Darling 2007). To test the colonization-time hypothesis from the perspective of population genetics, I link waterbody age

to the mtDNA genetic diversity of *G. lacustris* and intraspecific genetic diversity of *Polymorphus* species from ten water bodies of various ages (3–53 years) (Chapter 4). In addition to testing the genetic-diversity-age relationship, I extended the colonization-time hypothesis to prevalence–age relationship and hypothesized that parasite prevalence should increase as time available for host and parasite colonization. To test this extended colonization-time hypothesis for parasite prevalence, I use a 2-year dataset collected from 36 water bodies in the vicinity of Edmonton from 2015 to 2016. To further test the effect of waterbody age on acanthocephalan prevalence, I did additional sampling from ten of the 36 water bodies (5 young and 5 old) in 2017 (Chapter 3). Besides waterbody age, previous studies have shown that the abundances of intermediate and definitive hosts are related to parasite prevalence (Hechinger and Lafferty 2005, Lagrue and Poulin 2015). Parasites are more likely to encounter locally common final-host taxa are more likely for parasites to encounter and infect than rare final-host taxa (Canard et al. 2014), and therefore common final-host taxa are expected to contribute to parasite prevalence greatly. In contrast, non-host taxa that consume intermediate hosts, and are dead-ends for parasite transmission, are predicted to reduce parasite prevalence in intermediate hosts. To test these ideas, I statistically test the effects of the abundance of locally common, rare, potential dead-end and non-amphipod-eating definitive hosts on parasite prevalence (Chapter 3). In Chapter 5, I scale up my research scope to explore population structure of *G. lacustris* across the Holarctic region and investigate how it is related to waterfowl flyways and the Rocky Mountains by combining the mtDNA sequences of *G. lacustris* collected from central Alberta with the mtDNA sequence from other places in North America, Europe and Asia. Through a multifaceted exploration of the *Gammarus*–acanthocephalan system, this thesis aims to provide broad insights into mechanisms by which hosts and parasites become distributed at different spatial scales.

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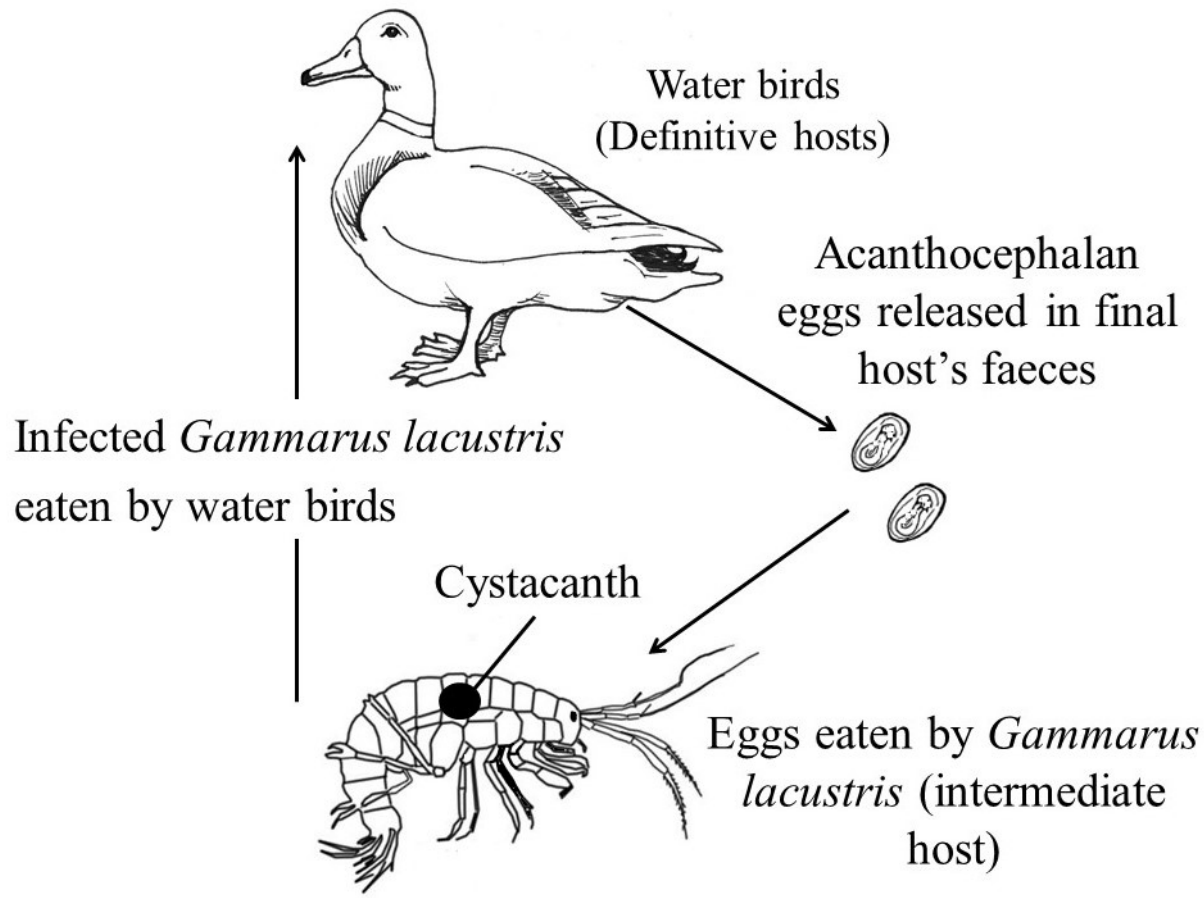

Figure 1.1 Life cycle of freshwater acanthocephalans. Eggs of *Polymorphus* spp. are eaten by *Gammarus lacustris* and develop into cystacanth in haemocoel of *G. lacustris*, and then are transmitted to the guts of vertebrates (e.g., waterfowl) where they develop into the adult stage (Mallard drawn by Dr. Heather Proctor).

Chapter 2 Application of DNA barcoding, morphometric analysis and machine-learning approaches in differentiating cystacanths of four *Polymorphus* species (Acanthocephala: Polymorphidae) in central Alberta, Canada.

2.1. Introduction

How many parasite species there are on Earth is a challenging question for parasitologists (Poulin and Morand 2005). This is especially true for parasites in which larval and adult stages use different hosts. One potential reason for the poorly known diversity of multi-host parasites is that the larval stages of these parasites cannot be easily identified to species level based on their larval morphological traits alone (Nadler and De León 2011, Alcántar-Escalera et al. 2013). Fortunately, molecular tools offer potential approaches for larval stage identification (Nadler and De León 2011). Several studies have supported the validity of using mitochondrial cytochrome oxidase I (COI) sequences for species-level identification to link larvae to known adults in multi-host parasites including digenean flatworms (Caffara et al. 2011, Locke et al. 2011, Gordy et al. 2017) and acanthocephalans (Alcántar-Escalera et al. 2013).

Another factor that impedes the discovery of parasite species is that morphological identification of multi-host parasites (especially larval stages) requires professional systematic expertise, and the differentiation of many morphologically similar parasite species is generally based on multiple subtle morphological traits. For many parasite taxa including acanthocephalans, numerical and statistical morphometric techniques (such as ordination) are commonly used for the taxonomic identification of larval and adult stage of parasites (Wickström et al. 2001). Recently, machine-learning approaches have been applied to taxonomical and phylogenetic problems (Dopazo and Carazo 1997, Zhang et al. 2008). These methods have been used to identify non-linear relationships within data (Breiman et al. 1984, Breiman 2001), and computer systems can be trained using a set of verified morphological data and then generalized to classify taxa based on morphological differences. Machine-learning methods have been adopted for the taxonomic identification of plants (Rossatto et al. 2011, Grinblat et al. 2016) and animals (Haralabous and Georgakarakos 1996, Wang et al. 2012, Santana et al. 2014). Although they

have not yet been applied to parasites, these modelling techniques have the potential to be used for taxonomic identification of multi-host parasites using multiple morphological traits.

Thorny-headed worms (Acanthocephala) are examples of multi-hosts endoparasites that utilize arthropods as intermediate hosts for their larval stages (acanthella and cystacanth) and then can be transmitted via predation to the guts of vertebrates where the adult stage matures in the gut (Kennedy 2006). Adult acanthocephalans mate in their definitive hosts and females produce eggs that are released into water or land with the definitive hosts' feces (Crompton and Nickol 1985, Kennedy 2006). Waterfowl are common definitive hosts for acanthocephalans. The life cycles of waterfowl-associated acanthocephalans typically include freshwater amphipods as intermediate hosts (Kennedy 2006). So far, the known waterfowl-related acanthocephalans belong to the Class Archiacanthocephala, with five families, 13 genera and 51 species (McDonald 1988). The genus *Polymorphus* (Polymorphidae) is one of the most speciose in the Archiacanthocephala, containing 26 described species (McDonald 1988). Although the life cycles and biology of several *Polymorphus* species have been studied (Denny 1969, Bethel and Holmes 1974, Bauer et al. 2005), only Alcántar-Escalera et al. (2013) have applied both morphometric and molecular analysis to differentiate *Polymorphus* species.

In Alberta, Canada, species of *Polymorphus* are common and use freshwater amphipods (e.g., *Gammarus lacustris* Sar) as intermediate hosts and several species of waterfowl and semi-aquatic mammals as definitive hosts (Denny 1969). Three common species that use *G. lacustris* as the intermediate host in central Alberta are: *P. contortus* (Bremser in Westrumb), *P. marilis* Van Cleave and *P. paradoxus* Connell & Corner (Denny 1969, Podesta and Holmes 1970, Bethel and Holmes 1974, Tokeson and Holmes 1982, Helluy and Holmes 1990). Previous studies have used morphological traits of the proboscis and hind-body wall to identify *Polymorphus* larva to species (Denny 1969). However, there is no genetic evidence for the distinctness of any of these three species, and no numerical or statistical tests have been conducted to elucidate the validity of utilizing proboscis hooks to differentiate these three putative *Polymorphus* species.

Herein, I report the first occurrence of larval stage of *Polymorphus* cf. *strumosoides* Lundström in *G. lacustris* in Alberta. I use morphometric analysis to differentiate larvae of four putative *Polymorphus* species: *P.* cf. *contortus*, *P.* cf. *marilis*, *P.* cf. *paradoxus*, and *P.* cf. *strumosoides*

based on morphology of proboscis hooks. Then, I apply machine-learning approaches to see whether they corroborate the results of morphometric analysis. Before this, I develop and test a best machine-learning model that can accurately identify acanthocephalan to species-level. I construct a phylogenetic tree based on mtDNA sequences and assess whether it corroborates the morphological analysis. And finally, I explore whether morphological and molecular variation in these taxa shows any geographical patterns.

2.2. Material and methods

2.2.1. Specimen sampling, preliminary identification and laboratory protocols

A total of 79 cystacanths were collected from *G. lacustris* specimens in 17 water bodies, in Alberta, Canada, in May 2014 and 2015 (Figure 2.1; Table 2.1). One sampling point was haphazardly selected at wadeable depth in each of the 17 water bodies, where *Gammarus lacustris* were collected by sweeping a dip net through the water column above the substrate at wadeable depths. Cystacanths were dissected out with the aid of a dissecting microscope and were placed in tap water overnight to cause them to swell and evert their proboscises. I used these specimens for morphological identification by chopping off proboscises and mounting them on slides in polyvinyl alcohol (PVA) mounting medium, with the rest of the body being preserved in absolute ethanol in a -20 °C freezer for DNA extraction. All acanthocephalans were identified to species level, according to Denny (1969), McDonald (1988) and Amin (1992), based on morphological characters including presence and arrangement of anterior trunk spines, variation in proboscis hook size, proboscis shape, number of longitudinal hook rows (NHR), number of hooks per a longitudinal row (HPR), and the length of the largest hook among all those examined (LLH) (Figure S2 2).

I extracted whole genomic DNA from acanthocephalan specimens using DNeasy 96 Blood and Tissue Kit (QIAGEN). COI universal primers from Alcántar-Escalera et al. 2013 were used to amplify acanthocephalan the barcoding mitochondrial Cytochrome c oxidase subunit I (COI) region and to sequence ca. 702 bp fragments of mitochondrial Cytochrome c oxidase subunit I. I performed polymerase chain reactions (PCR) for acanthocephalans in a total volume of 25 µl containing 1.25 µl 10×PCR reaction buffer, 2.5 µl dNTPs (2 mM), 0.25 µl of each primer (10 µM), 1.0 µl DNA template, 1.25 µl $MgCl_2$ (50 mM), 0.5 µl homemade Taq DNA polymerase and 18 µl dH_2O. PCR conditions were as follows: 120 s at 94 °C, 35 cycles of 60 s at 94 °C, 60 s

at 40 °C, 60 s at 72 °C, then followed by 300 s at 72 °C. I purified all PCR products using ExoSAP (New England Biolabs) and then sent them to Molecular Biology Service Unit in the Department of Biological Sciences, University of Alberta for DNA sequencing. Sequence chromatograms were viewed and checked for accuracy in FinchTV (Geospiza Inc.). All COI sequences of acanthocephalans will be submitted to GenBank.

2.2.2. Sequence alignment, trimming and phylogenetic analysis

Sequence alignments were conducted in ClustalX 2.0 using default parameters (gap opening: 10; gap extension: 0.2; delay divergent sequences: 30%; DNA transition weight: 0.5) and then checked visually and trimmed in DNAMAN 7.0. A total of 79 *Polymorphus* COI sequences were aligned and trimmed to 569 bp in length. Based on results of a previous phylogenetic study (Alcántar-Escalera et al. 2013), I used representatives of two other taxa of Polymorphidae as outgroups (*Hexaglandula corynosoma* [Travassos] and *Southwellina hispida* [Van Cleave]). Before constructing the phylogenetic tree, I used PartitionFinder 2 to select the best nucleotide evolution model for the sequence alignment based on the corrected Akaike information criterion (AICc) values (Lanfear et al. 2017). I conducted Bayesian phylogenetic analysis in MRBAYES with default priors, random starting trees and GTR+G+I evolutionary model. Bayesian analysis included three heated and one cold Markov chains, with sampling frequency of every 500 generations for one million generations. Bayesian analysis discarded the burn-in samples (first 25% of total samples) and summarized the remaining 75% samples to construct the consensus tree with posterior probabilities for all branches being estimated using the 50% majority rule. TRACER was used to assess the run convergence (Drummond and Rambaut 2007). The final consensus tree was visualized in FigTree (version 1.4.3; http://tree.bio.ed.ac.uk/software/figtree/). I also aligned the DNA sequences with the *Polymorphus* mtDNA sequences that are available in Barcode of Life Data System and National Center for Biotechnology Information (*P. trochus* Van Cleave, *P. minutus* [Goeze] and *P. obtusus* Van Cleave). The second Bayesian analysis was conducted based on this DNA sequence alignment with the same parameter settings as the first Bayesian analysis (except for the sampling frequency being every 500 generations for 30 million generations).

2.2.3. Morphometric analysis

Morphometric analysis was conducted in the R environment (https://www.r–project.org/). The purpose of the analysis was to examine the morphological variation between *Polymorphus* spp. larvae from *G. lacustris*. I followed Huffman and Bullock (1975) and Wayland (2010) to detect morphological difference between acanthocephalan specimens. For my analysis, I only considered cystacanths with fully everted proboscis and hooks for the morphometric analysis. In total, 631 hooks from 79 acanthocephalan larvae were measured and retained in the analysis. Specifically, I included five commonly used proboscis-hook-related traits including NHR and HPR, LLH, hook length (HL) and width of the hook base (HB) (Figure S2 2). For the HL and HB measurements, I haphazardly selected one longitudinal row of fully everted and best profiled hooks, and measured each of the hooks (usually 7–8 hooks per row). Hooks were measured using a Leica DMLB compound scope at 40X magnification with differential interference contrast (DIC) lighting. In addition, two derived characters (index of hook area [HA= HL × HB/2] and the ratio of base width and hook length [RHB= HB×100/ HL]) were calculated based on the HL and HB following Huffman and Bullock (1975). I conducted principal component analysis (PCA) on HL, HB, HA and RHB of 631 hooks, separately. Then, PCA loadings for all the hooks of each of the four traits were extracted and retained with NHR, HPR, LLH in another PCA. Prior to the analysis, all morphometric data were standardized to reduce the influence of different units of measurement on the PCA, as recommended by Wayland (2010).

2.2.4. Simulation of hook pattern of acanthocephalans and machine learning approaches

All simulations and modelling were conducted in the R environment (https://www.r–project.org/). The purpose of the simulation of hook pattern and machine-learning approaches was to determine if it corroborated the results of traditional morphometric analysis and molecular analysis. Prior to testing, I first simulated data describing proboscis hooks based on four related traits (NHR, HPR, LLH and whether hooks are similar to each other at middle horizontal level) to test whether these four traits are sufficient for differentiating among waterfowl-associated acanthocephalans. I chose these four characters because they are key for acanthocephalan identification at the species level and are consistently recorded for most waterfowl-related acanthocephalan species in taxonomic keys (Denny 1969, McDonald 1988, Amin 1992). Many of these traits were recorded as “ranges” in keys for some taxa (e.g., NHR of

P. contortus is between 15 and 18) while reported as single values for others (e.g., NHR of *P. corynoides* Skrjabin is 10). In reality, traits reported as single values might not be definitely fixed and may deviate to some extent. To address this possibility, I simulated the traits of proboscis hooks in two scenarios. In the first scenario, for traits described with a range of values, I randomly sampled data points within that range, while keeping each "single value" trait fixed at that value. In the second scenario, I assumed that range data for each trait of each species followed a normal distribution bounded by the range, with the average of the maximum and minimum of the range as the mean for the normal distribution and the mean standard deviation of ranges across all taxa as the standard deviation for the normal distribution. Similarly, I assumed that each of the "single value" traits followed a normal distribution within the mean range of that trait across all species, and the normal distribution had the "single value" as mean and had the mean standard deviation of the range across all taxa as the standard deviation of the normal distribution. For both scenarios, every simulation generated 200 individuals for each species (for a total of 43 species, 9 genera, 4 families and 2 orders; see Table 2.2). Based on the simulated data, I applied random forest (RF) and classification and regression tree (CART) to train two different models for each of the two scenarios with a total of four models (RF-1, CART-1, RF-2, and CART-2) to predict acanthocephalan species identity. Then I ran tenfold cross-validation to evaluate each of the four models based on three criteria: prediction accuracy, Cohen's Kappa Statistic (Kappa) and the area under receiver operating characteristic curve (AUC) for each trained model. These three criteria are commonly used for model evaluation in machine learning approaches (Sor et al. 2017). Specifically, I randomly partitioned the simulated data (200 individuals per species) into ten equal subsamples (20 individuals each), nine of which (a total of 180 individuals) were used as the training dataset to train a model and one of which (a total of 20 individuals) was used for testing the model. I did this process ten times, and for each model I used the aforementioned three criteria to evaluate performance. In addition, I used empirical data of the four proboscis hook-related traits from real adult acanthocephalan specimens (all belong to Polymorphidae including *Polymorphus strumosoides*, *P. trochus, P. marilis, P. obtusus, P. paradoxus and Pseudocorynosoma constrictum* [Van Cleave]) as a test dataset to assess model performance. I also used Friedman tests to determine whether the three criteria of the model performance were significantly different between RF and CART for the two different scenarios.

2.3. Results

2.3.1. Phylogenetic analysis

Bayesian phylogenetic trees showed that *P.* cf. *contortus* clustered as a clade and sister to *P.* cf. *strumosoides* with 100% posterior probability support for each clade (Figure 2.2). The clade of *P.* cf. *contortus* and *P.* cf. *strumosoides* is sister to the clade of *P.* cf. *paradoxus* and *P.* cf. *marilis* with ca. 100% posterior support for each clade. *Polymorphus* cf. *marilis* formed a clade with 97% posterior support, which was deeply nested within *P.* cf. *paradoxus* (Figure 2.2). The outgroup taxon *Hexaglandula corynosoma* is located at the base of the phylogenetic tree. The pairwise genetic distance showed that genetic divergence between *P.* cf. *paradoxus* and *P.* cf. *marilis* (0.017) was lower than that between *P.* cf. *paradoxus* and *P.* cf. *contortus* (0.295) or between *P.* cf. *paradoxus* and *P.* cf. *strumosoides* (0.254) (Table 2.3). Similarly, *P.* cf. *strumosoides* was more similar to *P.* cf. *contortus* (with lower genetic distance: 0.210) than to *P.* cf. *paradoxus* (0.254) and *P.* cf. *marilis* (0.259) (Table 2.3). The genetic distance between my *Polymorphus* specimens and *Southwellina hispida* ranged from 0.340 to 0.344, while the divergence to *Hexaglandula corynosoma* varied from 0.376 to 0.396 (Table 2.3).

2.3.2. Morphometric analysis and machine learning approaches

In the morphological analysis of 79 specimens, HL ranged from 25–87.5 µm, HB from 7.5–35 µm, HA from 103.1–1443.8 µm^2, RHB from 15.2–53.8 µm, NHR from 14–19, HPR from 7–12 and LLH from 42.5–87.5 µm. On average, *Polymorphus* cf. *contortus* possessed shorter HL (25–45 µm), smaller HB (10–17.5 µm) and shorter LLH (42.5–45 µm) compared to *P.* cf. *marilis* and *P.* cf. *paradoxus* (HL: 27.5–65 µm and 37.5–87.5 µm; HB: 7.5–27.5 µm and 7.5–35 µm; LLH: 57.5–65 µm and 75–87.5 µm). *Polymorphus* cf. *marilis* is armed with 15–17 longitudinal hook rows and 7–8 hooks per row, while the proboscis of *P.* cf. *paradoxus* had 14–19 longitudinal hook rows and 7–9 hooks per row. *Polymorphus* cf. *strumosoides* possesses more hooks per row (HPR: 12) compared to *P.* cf. *contortus* (7), *P.* cf. *marilis* (7–8) and *P.* cf. *paradoxus* (7–9), and it is armed with shorter LLH (52.5 µm) hooks than *P.* cf. *marilis* and *P.* cf. *paradoxus*.

The PCA first axis accounted for 19.1 % of total variation for morphometric data and was correlated with LLH, HA, HB and HL (Figure 2.3; Figure 2.4). The second PCA axis explained

an additional 17.1% of the total variation and was related to HA, HB and HL (Figure 2.3). The third PCA axis explained another 14.9 % of the total variation and was associated with HA, HB, HL and HA (Figure 2.4). Principal component analysis showed that the features of proboscis hooks can separate the four putative *Polymorphus* species (F=9.68, p<0.01; Figure 2.4).

I trained models in two different simulation scenarios to predict the classification of acanthocephalan species based on simulated and empirical data using RF and CART. Overall, for the simulated data overall, all models in the two scenarios performed better in classifying order and family levels than genus and species levels (Friedman chi-squared: F=120, df=3, p<0.01; Table 2.4). With regard to empirical data, I did not calculate the accuracy, Cohen's Kappa statistic and AUC for family and order levels because all acanthocephalan specimens used in simulations were from the same order and family. RF outperformed CART in the two different simulation scenarios (RF-1, CART-1, RF-2, and CART-2) at the species level, with high tenfold cross-validated accuracy (simulated data: F=20, df=1, p<0.01; empirical data: F=7.20, df=1, p<0.01), Cohen's Kappa statistic (simulated data: F=20, df=1, p<0.01; empirical data: F=7.20, df=1, p<0.01) and AUC (simulated data: F=20, df=1, p<0.01; empirical data: F=13.24, df=1, p<0.01) (Table 2.4). At the species level, RF for the first simulation scenario (RF-1) had the best performance compared to other combinations of model and scenarios (CART-1, RF-2, and CART-2) with significantly higher mean classification accuracy (simulated data: F=28.92, df=3, p<0.01; empirical data: F=23.63, df=3, p<0.01), Kappa (simulated data: F=28.92, df=3, p<0.01; empirical data: F=22.32, df=3, p<0.01) and AUC (simulated data: F=27.12, df=3, p<0.01; empirical data: F=20.74, df=3, p<0.01). Species-level identification of cystacanths by RF-1 was consistent with the results of ordination.

2.4. Discussion

I found that traits associated with proboscis hooks are sufficient for differentiating among *P.* cf. *contortus*, *P.* cf. *paradoxus* and *P.* cf. *marilis*. These morphometric results corroborate the finding of Denny (1969) that the length of the largest proboscis hook can be used to differentiate *P. contortus*, *P. paradoxus* and *P. marilis*. One difference between my study and Denny (1969) is that my study measured multiple traits of proboscis hooks to differentiate *P.* cf. *contortus*, *P.* cf. *paradoxus* and *P.* cf. *marilis,* versus a single trait. Furthermore, my study found the same acanthocephalan species assemblage that Denny (1969) found in the Edmonton area (except for

the single *P. strumosoides* infection), suggesting that the acanthocephalan assemblage appears to be compositionally stable in this region across the 50 years. No acanthocephalan species other than three *Polymorphus* species (*P. paradoxus*, *P. marilis* and *P. contortus*) and *Pseudocorynosoma constrictum* (*Corynosoma constrictum* Van Cleave, 1918) were found in surveys of water birds in Alberta (e.g., ten waterfowl and four grebe species [Western Grebe, Red-necked Grebe, Horned Grebe and Eared Grebe]; Butterworth 1982, Stock and Holmes 1986). This does not mean these water birds are incompetent hosts for other acanthocephalan species. For example, Storer (2000) showed that the aforementioned four grebes can host several *Pseudocorynosoma* spp. and *Andracantha mergi* (Lundström, 1941). Rather, the absence of other acanthocephalan species in water birds in Edmonton may result from 'missing the boat' (Paterson and Gray 1997). Another reason for the stable acanthocephalan composition in Alberta could be related to incompetence of *G. lacustris* as an intermediate host for acanthocephalan species other than *P. paradoxus*, *P. marilis* and *P. contortus*.

My study showed that the morphological variation of proboscis hooks within *P.* cf. *paradoxus* specimens is larger than the intraspecific variation within each of *P.* cf. *marilis* and *P.* cf. *contortus*. This may be because *P.* cf. *paradoxus* is numerically abundant in my samples (N=65 out of 79). This high abundance of *P.* cf. *paradoxus* might be related to the way of sampling *G. lacustris* in my study. Sampling amphipods at wadeable areas is more likely to get *G. lacustris* infected with *P. paradoxus* cystacanths than those with *P. contortus* because *P. paradoxus*-infected *G. lacustris* show positive phototaxis and tend to cling onto moving objects in the surface of water (Bethel and Holmes 1973; Bethel and Holmes 1974). *Polymorphus* cf. *strumosoides* can be separated well morphologically from *P.* cf. *marilis*, *P.* cf. *contortus* and *P.* cf. *paradoxus* along the first and third PC axes. It has more hooks per row than the other three species, and the largest hook in shorter than in *P.* cf. *marilis* and *P.* cf. *paradoxus* but longer than in *P.* cf. *contortus*. These PCA results are further corroborated by the classification of the four *Polymorphus* species by the best machine learning model.

My morphological and phylogenetic results were generally consistent, supporting the identity of the four *Polymorphus* lineages. Genetically, *P.* cf. *contortus* and *P.* cf. *strumosoides* were particularly well separated from *P.* cf. *paradoxus* and *P.* cf. *marilis* based on COI. In contrast, the Bayesian phylogenetic tree displayed a monophyletic cluster of *P.* cf. *marilis* specimens

nested within the *P.* cf. *paradoxus* specimens. The first possibility is that *P.* cf. *marilis* and *P.* cf. *paradoxus* occasionally hybridize in Alberta, but no strong evidence of hybridization exists for any acanthocephalan species. The second potential reason for the phylogenetic nestedness is that COI alone might not be sufficient to differentiate *P.* cf. *paradoxus* and *P.* cf. *marilis* and more effective genetic markers may be needed (e.g., small [18S] and large-subunit nuclear ribosomal RNA [28S] and cytochrome c oxidase subunit 1 [cox 1]). Thirdly, it is possible that *P.* cf. *marilis* is not a distinct species, but is merely a distinct subclade of *P.* cf. *paradoxus*. Besides the molecular and morphological differences between the two potential species, biologically, *P. marilis* can manipulate *G. lacustris* to become negatively phototactic, which is different from *P. paradoxus* that makes infected *G. lacustris* positively phototactic (Bethel and Holmes 1973). All evidence except for COI suggests that *P.* cf. *marilis* is likely to be a separate species. In addition, the single *P. obtusus* specimen available nested within the *P.* cf. *marilis* clade in my second Bayesian analysis (Figure S2 1). This may suggest that *P. obtusus* and *P. marilis* may be conspecific, or that the COI marker cannot differentiate them. However, this very small sample size of *P. obtusus* cannot give conclusive answer to the identities of *P. obtusus* and *P. marilis*. Future studies are needed that use multiple markers for sequencing more adult and cystacanth specimens of *Polymorphus* to study the phylogenetic relationship between *P. obtusus*, *P.* cf. *paradoxus* and *P.* cf. *marilis* and confirm the identity of the latter species.

Polymorphus cf. *paradoxus* showed large molecular intraspecific variations compared to other *Polymorphus* spp. of this study. This could be due simply to large sample size for *P*. cf. *paradoxus*. Further, the Bayesian phylogenetic tree showed some branches with high Bayesian posterior probability within the clade, suggesting that cryptic diversity might exist within *P.* cf. *paradoxus*. However, I did not find obvious geographical patterns for the intraspecific molecular variation via visual examination of the distribution of the variation, or any consistent pattern between morphological and molecular intraspecific variations for *P.* cf. *paradoxus*. This lack of geographical and biological patterns suggest that future investigation of this species using other morphological traits (e.g., trunk spines) or other genetic markers may be warranted. It is possible that the currently recognized *Polymorphus* species may not represent the actual diversity within this genus.

To my knowledge, my study is the first to assess identification accuracy of parasites across different taxonomical levels using multiple machine-learning models. My RF and CART models performed differently across different taxonomical levels under the two scenarios I implemented. As expected, the classification accuracy of the two models increased as taxonomic resolution was relaxed from species to order level. My results indicated that RF generally performed better than CART based on accuracy, Kappa and AUC in the two scenarios across different taxonomical levels. My findings are consistent with previous studies where RF performed better than CART in addressing a variety of ecological and evolutionary problems (Moisen and Frescino 2002, Gislason et al. 2006). This might be due to the difference in the algorithm used in each model: a random forest usually generates more classification trees to provide more classification accuracy than CART which produces a single tree (Breiman et al. 1984, Breiman 2001).

My study provides the morphological and molecular evidence for differentiating at least four *Polymorphus* species (*P.* cf. *contortus*, *P.* cf. *paradoxus*, and *P.* cf. *strumosoides*) and morphological evidence for the distinctiveness of *P.* cf. *marilis*. Ideally, future studies will include adult specimens to confirm the taxonomic identity of the four *Polymorphus* species. My study supports the utility of features of proboscis hooks to differentiate waterfowl-related acanthocephalan species. My study also shows that machine learning can be helpful for the identification of acanthocephalan species. These approaches might prove useful for species delimitation for other acanthocephalan taxa and other parasites.

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Table 2.1 Geographical coordinates of Albertan water bodies where cystacanths were sampled for *Gammarus lacustris* (Gammaridae)

Waterbody name	Geographical coordinates		Sample size of cystacanths
W01	53.5187	-113.2195	1
W04	53.4982	-113.3959	1
W05	53.4819	-113.3913	5
W06	53.4531	-113.3903	1
W09	53.4450	-113.3899	1
W12	53.6310	-113.4603	6
W14	53.6316	-113.4732	6
W15	53.6326	-113.4861	6
W17	53.6384	-113.5015	6
W20	53.6321	-113.5332	6
W21	53.6214	-113.5498	8
W26	53.4955	-112.9747	7
W31	53.4942	-113.6751	6
W33	53.5330	-113.6696	6
W34	53.4469	-113.3467	1
W41	53.6167	-113.5074	6
Narrow Lake	54.6189	-113.6176	6

Table 2.2 List of waterfowl-associated acanthocephalans used in simulation and morphological study. ca= cystacanths and adults; a= adults only; c= cystacanths only.

Species and authority	Genus	Family	Order	Class	Data type
Acanthocephalus anguillae (Müller)	*Acanthocephalus*	Echinorhynchidae	Echinorhynchida	Palaeacanthocephala	Simulated
Acanthocephalus lucii (Müller)	*Acanthocephalus*	Echinorhynchidae	Echinorhynchida	Palaeacanthocephala	Simulated
Acanthocephalus ranae (Schrank)	*Acanthocephalus*	Echinorhynchidae	Echinorhynchida	Palaeacanthocephala	Simulated
Centrorhynchus aluconis (Müller)	*Centrorhynchus*	Centrorhynchidae	Polymorphida	Palaeacanthocephala	Simulated
Sphaerirostris picae (Rudolphi)	*Sphaerirostris*	Centrorhynchidae	Polymorphida	Palaeacanthocephala	Simulated
Plagiorhynchus cylindraceus (Goeze)	*Plagiorhynchus*	Plagiorhynchidae	Polymorphida	Palaeacanthocephala	Simulated
Plagiorhynchus gracilis (Petrochenko)	*Plagiorhynchus*	Plagiorhynchidae	Polymorphida	Palaeacanthocephala	Simulated
Andracantha tunitae (Weiss)	*Andracantha*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Andracantha mergi (Lundström)	*Andracantha*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Andracantha phalacrocoracis (Yamaguti)	*Andracantha*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Arhythmorhynchus invaginabilis (Linstow)	*Arhythmorhynchus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Arhythmorhynchus teres Van Cleave	*Arhythmorhynchus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Corynosoma semerme (Forssell)	*Corynosoma*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated

Corynosoma sudsuche Belopolskaya	*Corynosoma*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Filicollis anatis (Schrank)	*Filicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Filicollis trophimenkoi (Atrashkevich)	*Filicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus actuganensis Petrochenko	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus acutis Van Cleave and Starrett	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus biziurae Johnston and Edmonds	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus cincli Belopolskaya	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus contortus (Bremser in Westrumb)	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[c]
Polymorphus cucullatus Van Cleave and Starrett	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus diploinflatus Lundström	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus kostylewi Petrochenko	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus marilis Van Cleave	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[ca]

Polymorphus mathevossianae Petrochenko	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus meyeri Lundström	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus minutus Goeze	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[a]
Polymorphus obtusus Van Cleave	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[a]
Polymorphus paradoxus Connell and Corner	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[ca]
Polymorphus phippsi Kostylew	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus pupa (von Linstow)	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus sp.	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus strumosoides Lundström	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[ca]
Polymorphus swartzi Schmidt	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Polymorphus trochus Van Cleave	*Polymorphus*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[a]
Profilicollis altmani (Perry)	*Profilicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Profilicollis arcticus (Van Cleave)	*Profilicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Profilicollis botulus (Van Cleave)	*Profilicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Profilicollis formosus (Schmidt	*Profilicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated

and Kuntz)					
Profilicollis major (Lundström)	*Profilicollis*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Pseudocorynosoma anatarium (Van Cleave)	*Pseudocorynosoma*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Pseudocorynosoma constrictum (Van Cleave)	*Pseudocorynosoma*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated/ empirical[a]
Pseudocorynosoma enrietti (Molfie and Freitas-Fernandez)	*Pseudocorynosoma*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated
Pseudocorynosoma strumosum (Rudolphi)	*Pseudocorynosoma*	Polymorphidae	Polymorphida	Palaeacanthocephala	Simulated

Table 2.3 Genetic distance among *Polymorphus* species (*P.* cf. *contortus* [sample size: N=3], *P.* cf. *marilis* [N=10], *P.* cf. *paradoxus* [N=65] and *P.* cf. *strumosoides* [N=1]) and outgroups (*Southwellina hispida* [N=1] and *Hexaglandula corynosoma* [N=1]) used in this study.

COI	*P.* cf. *contortus*	*P.* cf. *marilis*	*P.* cf. *paradoxus*	*P.* cf. *strumosoides*	*S. hispida*	*H. corynosoma*
P. cf. *contortus*	0.004	0.305	0.295	0.210	0.340	0.396
P. cf. *marilis*	0.305	0.009	0.017	0.259	0.344	0.376
P. cf. *paradoxus*	0.295	0.017	0.006	0.254	0.344	0.376
P. cf. *strumosoides*	0.210	0.259	0.254	NA	0.342	0.376
S. hispida	0.340	0.344	0.344	0.342	NA	0.327
H. corynosoma	0.396	0.376	0.376	0.376	0.327	NA

Table 2.4 Model performances of random forest (RF) and classification and regression tree (CART) at four different taxonomical levels based on three tenfold-cross-validated criteria: mean prediction accuracy, mean Cohen's Kappa Statistic and the mean of Area Under the Curve (AUC). Simulations were based on two scenarios with the best model in bold (see Methods for details).

Method	Test data	Species			Genus			Family			Order		
(Scenario)		Accuracy	Kappa	AUC	Accuracy	Kappa	AUC	Accuracy	Kappa	AUC	Accuracy	Kappa	AUC
RF (1)	Simulated	**0.983**	**0.983**	**0.995**	**0.998**	**0.997**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
	Empirical	**0.929**	**0.909**	**1**	**1**	**1**	**1**	–	–	–	**–**	**–**	**–**
CART (1)	Simulated	0.836	0.833	0.948	0.935	0.913	0.921	0.993	0.974	0.981	0.996	0.964	0.971
	Empirical	0.436	0.377	0.949	0.993	0.977	1	–	–	–	–	–	–
RF (2)	Simulated	0.865	0.862	0.945	0.949	0.933	0.974	0.983	0.931	0.975	0.989	0.914	0.960
	Empirical	0.650	0.596	0.920	0.793	0.536	1	–	–	–	–	–	–
CART (2)	Simulated	0.701	0.694	0.923	0.835	0.770	0.888	0.968	0.867	0.952	0.985	0.877	0.930
	Empirical	0.600	0.538	0.878	1	1	1	–	–	–	–	–	–

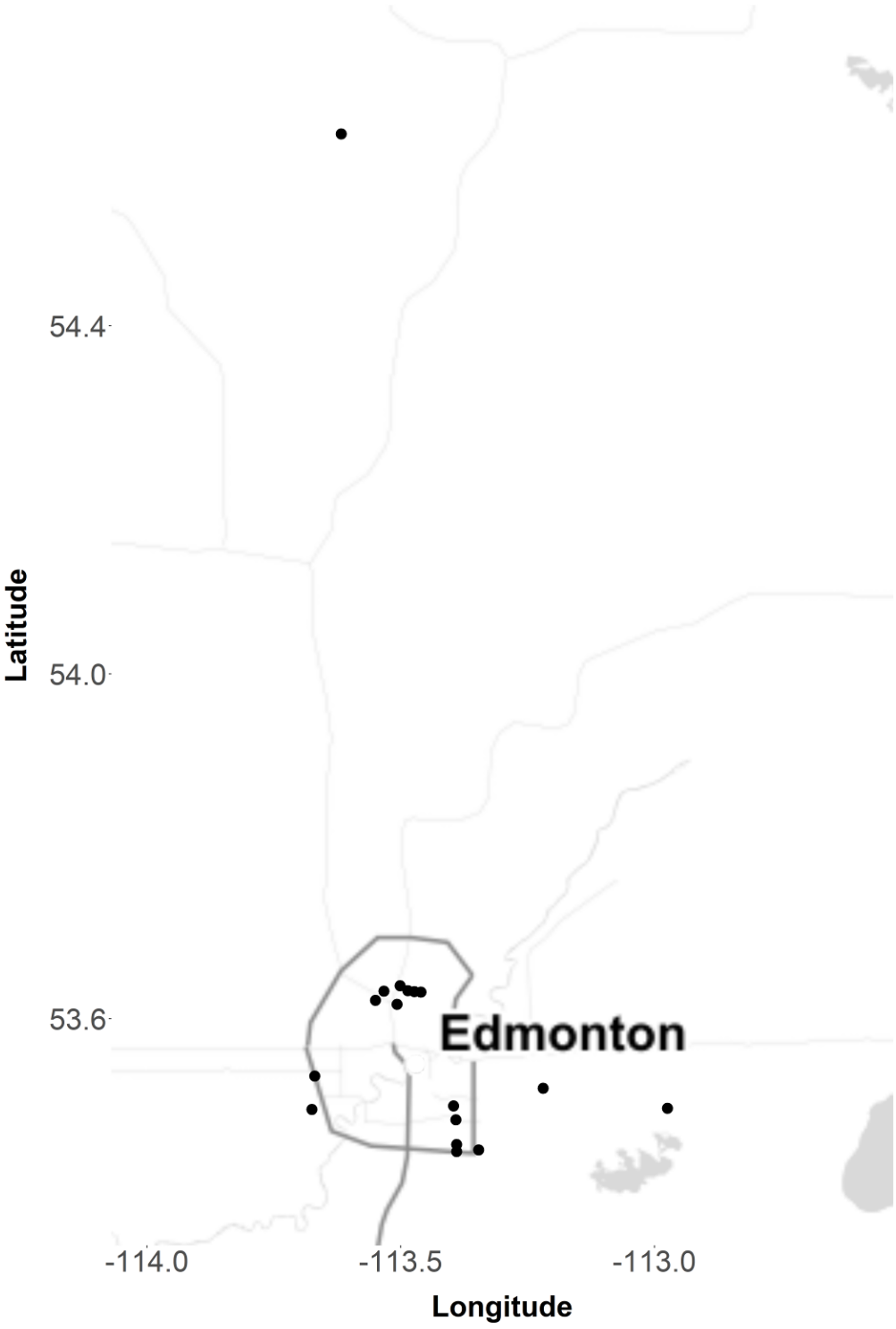

Figure 2.1 Sampling locations of cystacanths from *Gammarus lacustris* (Gammaridae) in Alberta, Canada.

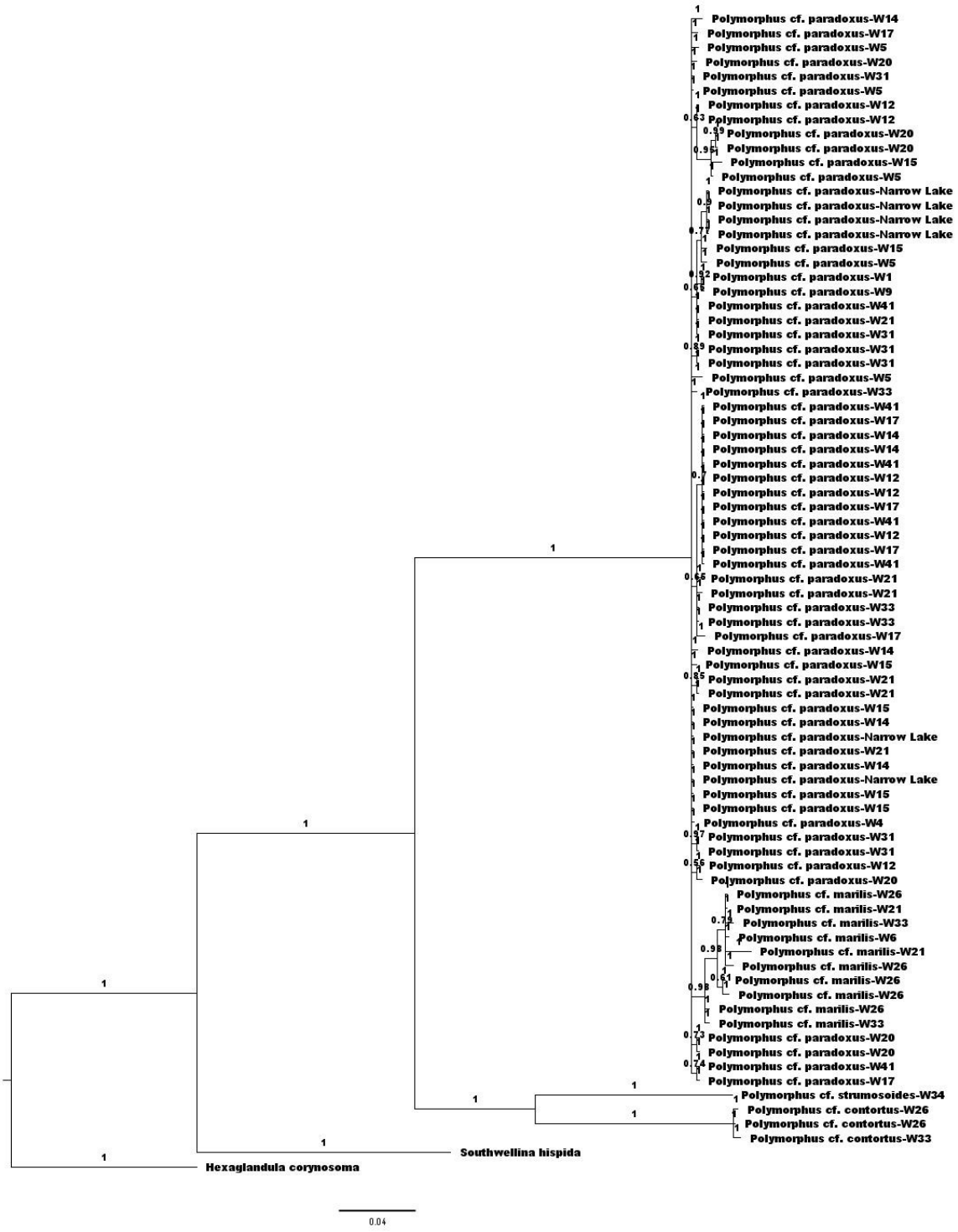

Figure 2.2 Bayesian phylogenetic tree based on COI sequences from 79 cystacanths of four putative *Polymorphus* species from Alberta (*P.* cf. *contortus*, *P.* cf. *marilis*, *P.* cf. *paradoxus* and *P.* cf. *strumosoides*) with 2 outgroup taxa (*Hexaglandula corynosoma and Southwellina hispida*) based on sequence data from GenBank.

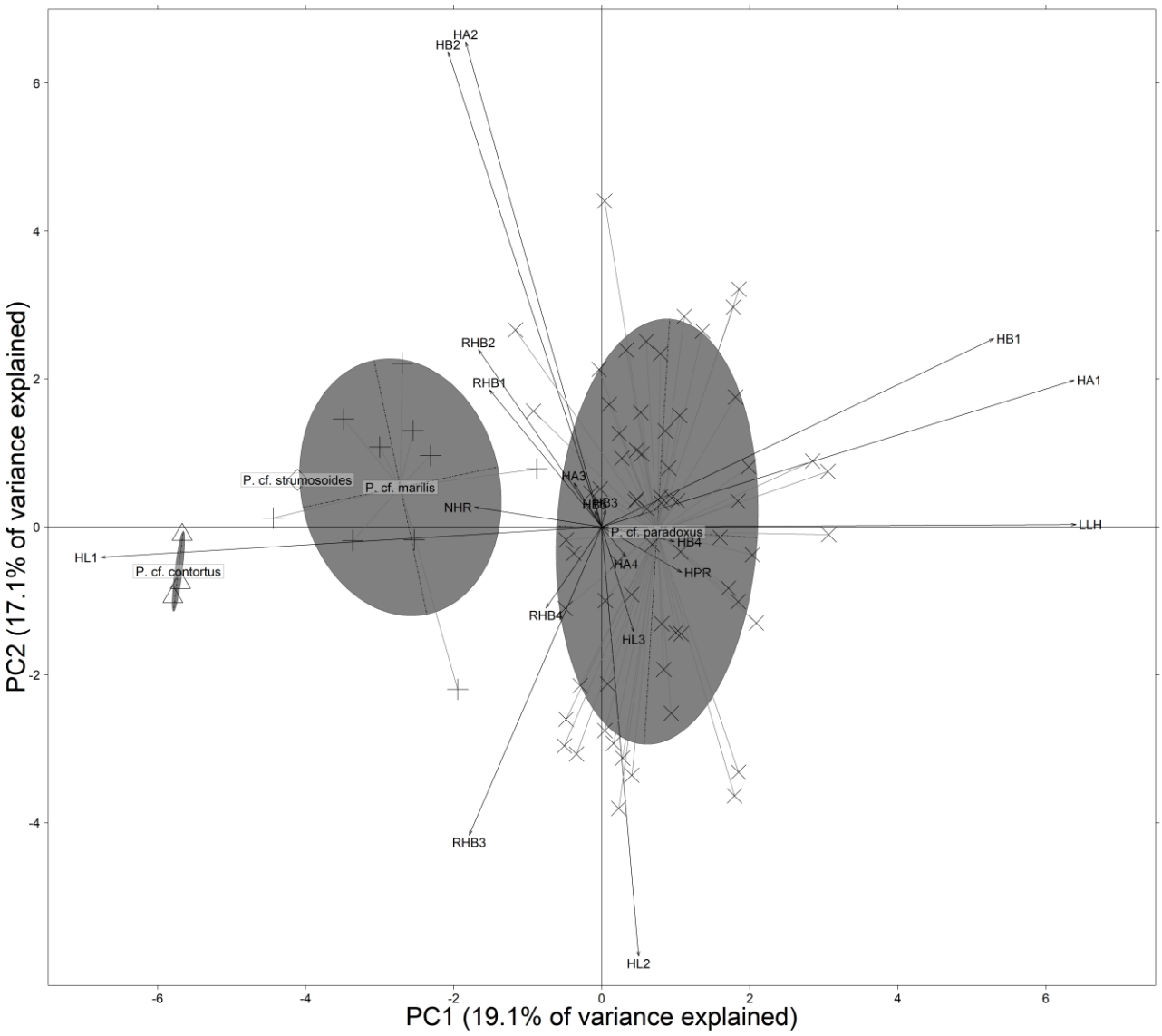

Figure 2.3 Principal component analysis (PCA) plots of four acanthocephalan species (*Polymorphus* cf. *contortus*, *P.* cf. *marilis*, *P.* cf. *paradoxus* and *P.* cf. *strumosoides*) for the first two axes (PC1 and PC2) based on variation in morphological traits of their proboscis hooks: the number of longitudinal hook rows (NHR), number of hooks per a longitudinal row (HPR), the length of largest among all hooks examined (LLH), hook length (HL) and base width (HB), index of hook area (HA) and the ratio of base width and hook length (RHB).

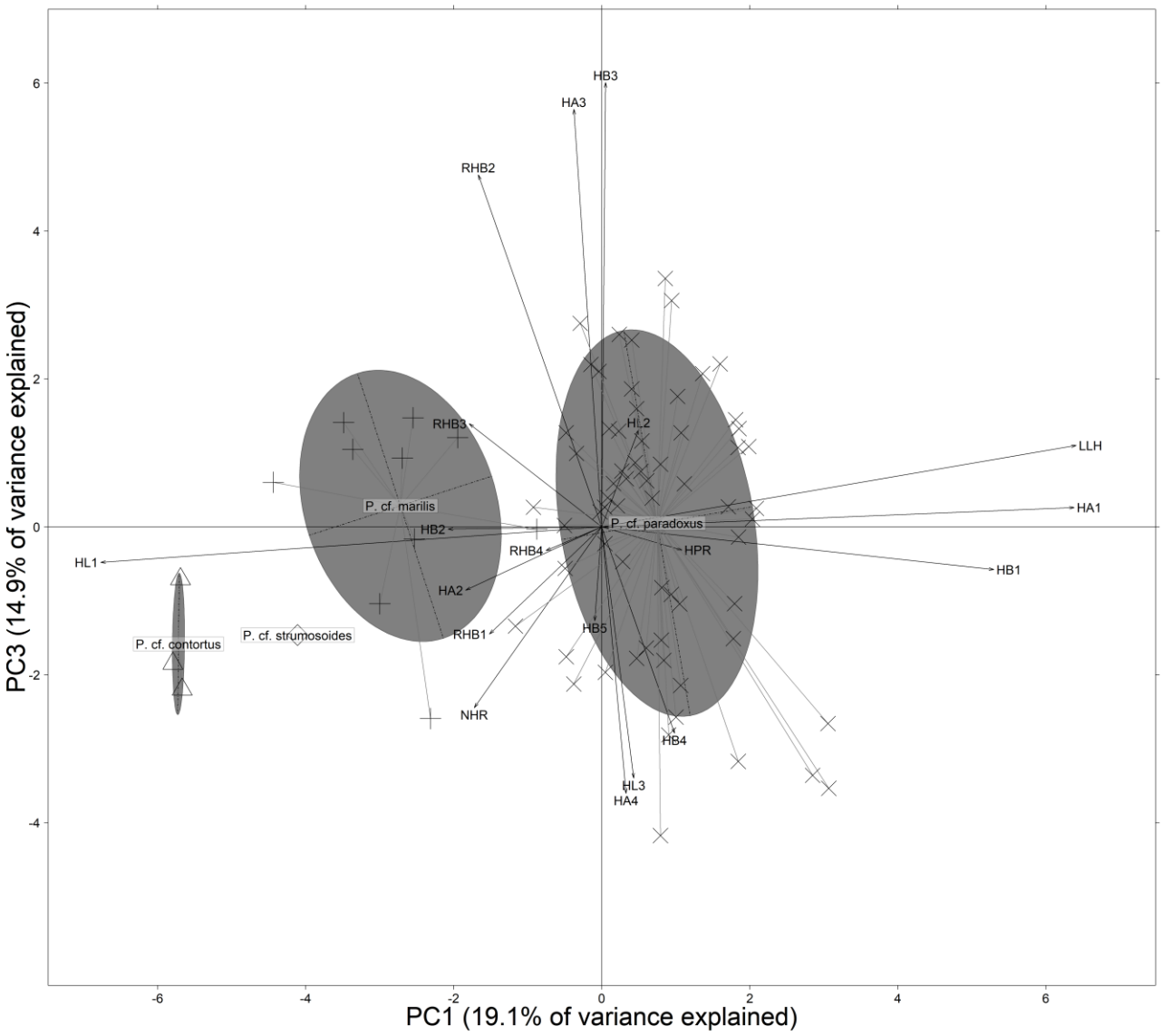

Figure 2.4 Principal component analysis (PCA) plots of four acanthocephalan species (*Polymorphus cf. contortus*, *P. cf. marilis*, *P. cf. paradoxus* and *P. cf. strumosoides*) for the first two axes (PC1 and PC3) based on variation in morphological traits of their proboscis hooks: the number of longitudinal hook rows (NHR), number of hooks per a longitudinal row (HPR), the length of largest among all hooks examined (LLH), hook length (HL) and base width (HB), index of hook area (HA) and the ratio of base width and hook length (RHB).

Chapter 3 Parasite prevalence increases with the abundance of common final hosts and waterbody age in a trophically transmitted parasite system

3.1. Introduction

Parasites are ubiquitous but often underappreciated biological components in terrestrial and aquatic ecosystems, and they comprise more than half of the known species on Earth (Brooks and Hoberg 2006). They play pivotal roles in affecting host reproduction and survival, with consequences that can influence host populations and communities (Lanciani 1975, Gustafsson et al. 1994, Hatcher and Dunn 2011, Lachish et al. 2011). Many parasites utilize one or more intermediate hosts for their larval stages and then are transmitted to final hosts for their adult stage. Such complex life cycles can provide fitness benefits to the parasite via amplification through asexual reproduction in intermediate hosts or increased probability of finding a mate in a final host (Brown et al. 2001, Rauch et al. 2005). In addition the final host providing a food source, parasite expansion to new geographical areas can be facilitated by movements of their final hosts (Kennedy 1976, Esch et al. 2009). New areas may vary in the availability of competent intermediate and final hosts. Some new regions may not have all the hosts necessary for the complex life cycles of parasites, which may reduce the chance of parasite populations establishing in those areas (Torchin and Mitchell 2004). The size of a parasite population in a particular area is also likely to be affected by the length of time since colonization, with very recently established populations being smaller than longer-established ones. Also, if a host population is initiated by a small number of founders and those parasites were highly aggregated with low prevalence in the original area, parasites may be absent in the new area due to chance (‘missing–the–boat’, Patterson and Ruckstuhl 2013). As time passes, there is increasing chance that new host individuals arrive bearing parasites (Guégan and Kennedy 1993). For relatively ephemeral habitats, such as small bodies of standing water, I might expect to see that parasite prevalence (= proportion of the host population that is infected) increases as waterbody age increases (i.e. a positive prevalence–age relationship), until a point of saturation is reached.

In addition to the prevalence–age relationship, parasite prevalence may tend to vary with local intermediate host density, since the intermediate host provides nutrients and habitats for the larval stages of parasites (Lagrue and Poulin 2015). If parasites produce a large number of infective stages that exceed the number of intermediate hosts, parasite prevalence in these hosts tends to be high because the high parasite:host ratio may increase the chance of each individual host being exposed to infective stages (Ewers 1964). In contrast, when the intermediate host population is large compared to the abundance of infective stages, the absolute number of infections can increase but parasite prevalence may be low since high host density may reduce risk of exposure with infective stages to each individual host ('safety in numbers' and 'encounter-dilution effect', Mooring and Hart 1992, Côté and Poulin 1995, Rifkin et al. 2012, Patterson and Ruckstuhl 2013, Buck et al. 2017).

Finally, parasite prevalence might be limited by abundance of final host availability in a particular location (Robson and Williams 1970, Smith 2001, Hechinger and Lafferty 2005). Increasing final host abundance might increase the total number of eggs being shed in host feces in an area, which may increase the probability of susceptible intermediate hosts encountering infective stages and therefore increase parasite prevalence in the area (Byers et al. 2008). However, not all competent final hosts contribute equally to parasite prevalence (Holmes et al. 1977). Parasite prevalence might be most strongly influenced by the "commonness" of final host species. Suitable final hosts that are common enough at a location for parasites to encounter and infect are more likely to contribute to local parasite abundance than are rare host taxa (Canard et al. 2014). Locally common final hosts would exert stronger selection on parasites to be adapted to infecting them than would rare hosts (Dybdahl and Lively 1998, Lively 1999, Lively and Dybdahl 2000), and therefore might contribute more to parasite prevalence in intermediate hosts. In addition, non-host species that consume intermediate hosts are dead-ends from the point of view of the parasite, and therefore might reduce parasite prevalence in intermediate hosts (Mouritsen and Poulin 2003, Kaldonski et al. 2008).

To test these ideas, I studied a water bird/muskrat–amphipod–acanthocephalan system. The acanthocephalan life cycle and ecological interactions with intermediate and final hosts are well known (Connell and Corner 1957, Denny 1969, Bethel and Holmes 1974, Helluy and Holmes 1990), including classic studies carried out in the same geographical area as my own research

(e.g., Bethel and Holmes 1973). The freshwater amphipod *Gammarus lacustris* Sars (Gammaridae) is a common intermediate host for acanthocephalan worms, including several *Polymorphus* spp. (Polymorphidae) such as *P. contortus* (Bremser in Westrumb), *P. marilis* Van Cleave, *P. paradoxus* Connell and Corner, which are consumed as eggs by the amphipods (Denny 1969, Zohar and Holmes 1998). Each egg develops into a cystacanth larva inside the amphipod. Neurological manipulation by certain acanthocephalan species causes the amphipods to display positive phototaxis and to cling to moving objects (Helluy and Holmes 1990). This not only makes infected *G. lacustris* more likely to be consumed by their final hosts (several species of water birds, muskrat *Ondatra zibethicus* [Linnaeus] and Canadian beaver *Castor canadensis* [Kuhl]; Connell and Corner 1957, Bush 1980, Butterworth 1982, Stock 1985), but also increases the probability of both amphipods and acanthocephalans being carried alive to new water bodies in the plumage or fur of the host (Swanson 1984). Once in the gut of the final host, acanthocephalans mature and engage in sexual reproduction. Adults of *Polymorphus* spp. can live for several months in the gut of the final host (Crompton and Whitfield 1968). Acanthocephalan infection intensity (= number of parasite individuals per infected host) and their density (no. parasite/m^2) have been shown to be positively related to both intermediate and final host density in one study in New Zealand (Lagrue and Poulin 2015).

In this study, I focused on acanthocephalan prevalence and not infection intensity in intermediate hosts. Cases of individual *G. lacustris* in the Edmonton area carrying more than one cystacanth are rare (Z. Song, U. Alberta, pers. obs.), and therefore presence/absence is a good approximation of intensity. Although more than one species of *Polymorphus* is present in the Edmonton area (see Chapter 2), they require slide-mounting to differentiate, and so I pooled all *Polymorphus* spp. together for pragmatic purposes in this ecological study. I sampled amphipods at wadeable depths where sunlight can reach and therefore *G. lacustris* infected by *P. paradoxus* cystacanths that show positive phototaxis (Bethel and Holmes 1973) were more likely to be the main component of my samples compared to *P. contortus*- or *P. marilis*-infected ones (see Chapter 2). I predicted that the prevalence of acanthocephalan cystacanths in *G. lacustris* would increase with waterbody age (a proxy of time since colonization time of the parasite and its hosts) and with the abundance of common, documented final-host species. I predicted that acanthocephalan prevalence in *G. lacustris* would have a relatively weak positive relationship with the abundance of rare final host species. I also predicted that acanthocephalan prevalence

would be correlated negatively with abundance of predatory water birds that consume infected *G. lacustris* but are not competent final hosts. Similarly, I expected density of *G. lacustris* to be negatively correlated with acanthocephalan prevalence; the extremely high densities of *G. lacustris* density encountered in the water bodies studied (up to ca. 2445 individuals/m^2, see Results) might increase the total number of infections in an area but decrease risk of exposure to infective stages for each individual host. I tested these hypotheses using a dataset collected from 36 water bodies of various ages across three years with two collections during the open-water period each year. Potential covariates that may influence acanthocephalan prevalence were incorporated into my statistical models to control for variation in other biotic and abiotic factors among water bodies.

3.2. Material and methods

3.2.1. Samplings and materials

I conducted this study in 33 man-made water bodies with construction times that ranged from 1962 to 2012, and three natural water bodies (Antler Lake, Hastings Lake and Boag Lake; prairie pothole lakes, precise ages unknown but definitely >100 years), in the vicinity of Edmonton, Alberta, Canada (53.568601 N, 113.491237 W) (Figure S3 1). In 2015 and 2016 I sampled all 36 sites twice per year, in May and August. In May and August 2017, I selected a subset of the 36 water bodies including five young (waterbody age relative to 2015: 3–7) and five old (26–38) to further test the effects of waterbody age on acanthocephalan prevalence in intermediate host. At each of the 36 water bodies, I haphazardly selected three sampling points restricted to wadeable depths. In each sampling occasion, I collected *G. lacustris* by sweeping a dip net through the water column immediately above the substrate for 1 min (= ~1.1 m^2 sampled), and their density was estimated by dividing number of *G. lacustris* sampled in each site-sampling event by 1.1. All collected *G. lacustris* were taken back to the laboratory and assessed for presence of acanthocephalan cystacanth larvae, which can be seen as red dots beneath the host's integument, with the aid of a dissecting microscope. Cystacanths were dissected out from infected amphipod and were placed in tap water overnight to cause them to swell and evert their proboscises. I used a dissecting scope to identify acanthocephalan larvae to genus level according to McDonald 1988; all acanthocephalans I found belong to the genus *Polymorphus* (Polymorphidae). I did not identify all specimens to species-level because species-

level identification needs a compound scope and slide-mounting for thousands of specimens would not have been feasible.

3.2.2. Bird and muskrat surveys

For water bodies <98 hectares, water birds (mostly Anseriformes, Charadriiformes and Podicipediformes, see Table 3.1) and muskrat were visually surveyed for the entire water body. Each survey was conducted at a single spot well away from water body using binoculars and a spotting scope to avoid disturbing birds and muskrat. All visible water birds were identified to species level (Note: all white terns in this study were grouped as *Sterna hirundo* Linnaeus, the most common tern species in the Edmonton area (http://species.abmi.ca/pages/species/birds/CommonTern.html), and their abundance estimated as the number of birds observed per hectare. For three large water bodies (>98 hectares), I surveyed all visible water birds and muskrat on the water surface and on shore that I was able to identify using a spotting scope at each of the same sampling points as for sampling *G. lacustris*. Distance between sampling points was visually assessed to be more than ~200 m. Each survey was conducted over half an hour during the day time before 3pm. Since the longest distance that I was able to identify birds and muskrat using the spotting scope was ~200 m, I estimated the area for birds and muskrat survey as half of a 200 m radius area centered on the sampling point (the proportion of the surface area that I surveyed ranging from 2.7–19.2%).

Since I want to test the effects of common final hosts on parasite prevalence in intermediate hosts, and not all birds that visit these water bodies are suitable final hosts for acanthocephalans (Vermeer 1969, Hair and Holmes 1970, Bush 1980, Butterworth 1982, Stock 1985, Edwards and Bush 1989, Smith 2007, Gladden and Canaris 2009), I grouped observed species into host and non-host taxa for Polymorphidae based on whether previous studies support the waterbird taxa as host Polymorphidae or not (see Table 3.1 for details). The amphipod-eating water birds that are not supported as host for Polymorphidae by previous studies were grouped as predatory water birds that are not known to be hosts for Polymorphidae (i.e. non-host predatory water birds). Canada Goose is a herbivorous waterfowl and no previous records show that it is a host for Polymorphidae. If a final host was observed at more than half of water bodies in a collection period during a collection event, it was regarded as a common final host for that set of surveys. The presence/absence of fish was checked visually as I sampled at each sampling point.

Although beavers could be a competent host for *Polymorphus*, I did not observe any beavers during the three years of sampling.

3.2.3. Potential environmental covariates

I evaluated emergent vegetation (EV), submersed aquatic vegetation (SAV) and substrate composition because aquatic plants and substrate type affect *G. lacustris* distribution within a water body (Yemelyanova et al. 2002), thereby possibly influencing acanthocephalan prevalence. Overall EV was assessed visually in five ranks from no plant cover to abundant plant cover (>75%) for each of the three sampling points. SAV was assessed visually in the same five ranks for 1 m^2 for each of the three areas per water body. The dominant substrates were visually assessed at each spot into four categories: mud, sand, gravel and stone and their presence/absence was recorded numerically. Because water quality may influence the feeding rate of *G. lacustris* which can further affect its parasite infection (Maltby et al. 2002), eight water quality factors were measured at each sampling point in each water body in 2015 including total nitrogen (TN), total phosphorus (TP), chlorophyll a (Chla), dissolved oxygen (DO), water temperature (Temp), pH, salinity and total dissolved solids (TDS). All factors except TN, TP and Chla were measured again in 2016 and 2017. DO, Temp, pH, salinity and TDS were measured with the Oakton Multi-Parameter Meter (PCD 650 Meter Kit) at the three sampling areas for each water body.

3.2.4. Waterbody age and size estimation

The age of the 33 constructed water bodies was obtained directly from the inventory list of their construction year from the City of Edmonton or estimated using historical aerial imagery (1962–2017) from Google Earth (Google Inc. 2018). For the 3 old natural water bodies, I estimated their age using historical aerial imagery (1962–2017) from both Google Earth (Google Inc. 2018) and the University of Alberta map library using the following criteria: if a water body first appeared on a history aerial imagery in a specific year and had not disappeared after that year, that year was designated its year of 'origin'. Note that these natural water bodies almost certainly existed prior to 1962, but pre–1962 imagery was not available. I recorded the origin of these three water bodies as 1962 in my data to avoid potentially inflating the effect of waterbody age on acanthocephalan prevalence. I also removed the three old natural water bodies and reanalyzed the correlation of waterbody age with acanthocephalan prevalence. The general

results regarding the effects of waterbody age on acanthocephalan prevalence were consistent for both analyses (see Table 3.2–Table 3.6). Waterbody size was estimated for each collection event based on historical aerial imagery from software Google Earth (Google Inc. 2018).

3.2.5. Statistical analysis

All analyses were conducted in R language (R Development Core Team 2017). I used generalized linear mixed models (GLMM) to test effects and evaluate the relative importance of the following factors on acanthocephalan prevalence in the intermediate host (= proportion of the host population that is infected with at least 1 individual of any species of *Polymorphus*), accounting for potential environmental covariates: waterbody age, abundance of common final host species, abundance of rare final host species, abundance of non-host waterbird species, and abundance of the intermediate-host amphipods. Specifically, I first analyzed data separately for each of the four collection events from 2015 to 2016 using binomial GLMMs where sampling point was nested within waterbody identity as a nested random variable and waterbody identity as a crossed random variable. Then I constructed an overall model that retained all the data from the six collection events across 2015 to 2017. For 2017 data, instead of modelling data from each of the May and August samples, I modeled all data from 2017 due to relatively small sample size (30 samples per collection event with three samples each of the ten water bodies). For both the overall model and 2017 GLMM where collection event and waterbody identity were crossed random factors and sampling point was nested within waterbody identity as a random factor, I used Beta-binomial distribution instead of binomial distribution with logit link function because these two models with binomial distribution showed overdispersion. To reduce the multicollinearity among potential explanatory variables, I conducted a preliminary analysis for all models and removed the variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) (Fox and Monette 1992, Rogerson 2001). All non-normally distributed independent variables were ln(x+1) or square-root-transformed before being included in models to improve normality, and then were centered and scaled to improve model fitting. Model adequacy was confirmed by inspecting graphs of square-rooted standardized Pearson residuals vs. fitted (Bolker et al. 2009). In order to test whether there was a significant difference in acanthocephalan prevalence between young and old water bodies, I used GLMMs with binomial distribution for May and August 2017, respectively, retaining waterbody group as a categorical

explanatory variable with sampling point nested within waterbody identity as a random variable. GLMMs with Binomial and Beta-Binomial distributions were performed using R package "glmmadmb".

3.3. Results

In total, 83827 *G. lacustris* were examined, and acanthocephalan prevalence varied from 0–1 (mean 0.34) for the 36 water bodies studied from 2015 to 2017 (Table 3.7; Table S3 1). During this study period, *G lacustris* density ranged from 0 to 2445.45/m^2 across the sampling points in my study sites (Table 3.7). A total of 44 water bird species were recorded across the three years (Table 3.7). Species richness of water birds ranges from 0 to 15 per survey (Table 3.7; Table 3.8). The most commonly occurring water birds across my surveys were Mallard, Canada Goose, Red-necked Grebe, Blue-wing Teal, and American Coot (Table 3.1; Table 3.8). Among them, Mallard and Red-necked Grebe are the most common final hosts for acanthocephalans (Table 3.1; Table 3.8). On average, the abundance of common final hosts (mean: 5.65 /ha) was higher than the abundance of rare known final hosts (2.87 /ha), predatory water birds not known to be hosts (2.30 /ha) and herbivorous water birds (3.50 /ha; Canada Goose only; Table 3.7).

Environmental conditions across my studied sites were characterized by mean pH of 8.06 (with a range of 5.91–9.94), mean water temperature of 18.50 °C (7.80–29.90), mean salinity of 519.28 ppm (145.80–980.20), and mean dissolved oxygen of 8.41 mg/L (1.43–18.51) (Table 3.7). The coverage of EV and SAV ranged from 0 to 87.5% (with means of 33.36 and 24.67%, respectively) in my study sites during the three years (Table 3.7). The most common substrate type was mud, followed by stone, gravel and sand. In 2015, TP, Chla and TN varied from 25 to 2845 (with a mean of 173.42), from 1.67 to 4503.89 (104.25) and from 25 to 4840 (800.77) µg/L across my studied sites, respectively (Table 3.7). Water bodies ranged from 3 to 54 years old (with a mean of 23.65) at the beginning of this study, and ranged from 0.22 to 692.39 ha (with a mean of 27.22) in surface area. Detailed information on the measured variables for water body can be found in Table 3.9.

For the overall model, acanthocephalan prevalence increased significantly as both waterbody age and the abundance of common final hosts increased (Figure 3.1 A and B). In contrast, acanthocephalan prevalence decreased as the abundance of non-host predatory water birds and intermediate host (*G. lacustris*) density increased (Figure 3.1 D and F). Abundances of rare final

hosts and herbivorous water birds were not significantly correlated with acanthocephalan prevalence in the overall model (Figure 3.2 F; Table 3.10).

For the individual models for each collection in 2015 and 2016, acanthocephalan prevalence had significant positive correlations with waterbody age for all collections except Aug 2016 (Figure 3.1 A; Figure 3.2 A–E; Table 3.11–Table 3.14). Similarly, the abundance of common final hosts was significantly correlated with acanthocephalan prevalence across three years from 2015 to 2017 (Figure 3.1 B and Figure 3.2 A–E). In contrast, only the individual models for Aug 2016 supported negative correlations between intermediate host density and acanthocephalan prevalence (Figure 3.1 F and Figure 3.2 A–E).

The age-focused collections in 2017 showed that mean acanthocephalan prevalence was significantly higher in old (1977-1989) water bodies than in young (2008 and 2012) ones (May 2017: Chi=33.25, $p<0.01$; Aug 2017: Chi= 7.35, $p<0.01$; Figure 3.3). The model for 2017 showed that acanthocephalan prevalence was correlated positively with waterbody age and abundance of common and rare final hosts (Figure 3.1 A, B and C; Figure 3.2 E; Table 3.15).

All six models (including 4 individual models, the model for 2017 and the overall model) supported the importance of the abundance of common final hosts for acanthocephalan prevalence, and waterbody age was a significant correlate of the infection prevalence across five of six models (Figure 3.2 and Figure 3.4). In contrast, only three models supported the importance of the intermediate host density, and two models showed significant association of acanthocephalan prevalence with predatory water birds that are not known to be host (Figure 3.2 and Figure 3.4). Only one model supported the significance for abundance of rare hosts and herbivorous water birds (Figure 3.2 and Figure 3.4, respectively). Overall, the importance of the abundance of common final hosts and waterbody age in acanthocephalan prevalence was greater than the abundance of intermediate hosts, rare hosts, non-host predatory water birds and herbivorous water birds (Figure 3.2). In six models, the most significant environmental variables were substrate type (gravel), SAV, water temperature and depth where I sampled amphipods (Table 3.10–Table 3.14). The importance of these variables was supported by two or three models (Table 3.10–Table 3.14), while one model supported the statistical significance of salinity, EV, presence of fish and two substrate types (mud and stone) (Table 3.10, Table 3.12, Table 3.13).

3.4. Discussion

My findings showed that the abundance of common final host species and waterbody age were the main ecological correlates of acanthocephalan prevalence in the amphipod intermediate host, while abundance of non-host species and the intermediate hosts themselves were statistically significant in only a few models. These results support my hypotheses that colonization time and the abundance of common final hosts are primary drivers of the prevalence of a trophically-transmitted parasite in intermediate hosts. To my knowledge, this is the first field study to document the importance of both waterbody age and waterbody usage by common final hosts in parasite prevalence, accounting for potential environmental covariates.

The prevalence–age relationship I document corroborates the colonization-time hypothesis, which has been supported by previous studies of natural parasite populations (helminths associated with fish: Guégan and Kennedy 1993); bacteria, protists, fungi and algae associated with *Daphnia*: Ebert et al. 2001). Even after incorporating environmental heterogeneity among water bodies by including 11 environmental variables in my models, my results repeatedly showed the prevalence–age relationship in the majority of my models. Alternative explanations for prevalence–age relationship could be due to other unmeasured ecological factors (e.g., taxon richness in water body, predation on competent hosts and competition between competent hosts and other taxa) that are correlated with waterbody age but independent of time available for host and parasite colonization. Taxon richness is likely to increase as waterbody age increases because old water bodies probably have higher chance of accumulating different taxa than young ones (e.g., Olmo et al. 2012; Olmo et al. 2016). As more taxa are accumulated in a water body, competition between competent hosts and other taxa or predation probably increases and may reduce the abundance of competent hosts, thereby decreasing parasite infections and prevalence (Johnson and Thieltges 2010). Besides, the increased biodiversity in a water body can reduce parasite infections and prevalence by disturbing the effective contact rate between competent hosts and infective stages (‘dilution effect’; Johnson and Thieltges 2010). Thus, the aforementioned scenarios would result in negative prevalence-age relationship, which is opposed to my findings of positive prevalence–age relationship. Based on verbal analysis above, the most likely explanation for the positive prevalence-age relationship I documented is that

acanthocephalan prevalence increases with the increase of time available for host and parasite colonization.

Adaptation by parasites to common hosts can result in higher frequency of locally common hosts being infected than rare hosts (Lively and Dybdahl 2000). Previous mathematical, laboratory and field studies (Dybdahl and Lively 1998, Lively 1999) have demonstrated that locally common hosts were successfully tracked by parasites and were more heavily infected compared to rare hosts. These findings corroborated my results, in which all models showed statistically positive associations between acanthocephalan prevalence and common final host abundance (Figure 3.1). All models also suggested that the abundance of common hosts was more important for parasite prevalence than abundance of rare hosts (Figure 3.2 and Figure 3.4). One evolutionary explanation for my results could be that selection might favour individual acanthocephalan parasites that are better able to infect common hosts (Lively and Dybdahl 2000). The potential ecological reason could be that common hosts might be more available for parasites to infect, encounter parasites more frequently and contribute more to spatial variation of parasite prevalence compared to rare hosts (Canard et al. 2014). Non-host predatory water birds may prey on infected amphipods but might not be competent for acanthocephalans to complete their life cycles (Leung and Poulin 2008). These arguments are consistent with my findings that the abundance of non-host predatory species was negatively correlated with acanthocephalan prevalence across two models, which partially supported the 'dead-end' effect of these species on parasite prevalence. My results also show that the herbivorous bird, Canada Goose, appears to have negative effects on acanthocephalan prevalence. One potential mechanism is that Canada Goose may physically interfere with transmission between intermediate hosts and suitable final hosts by aggressively defending territories and young by displacing other water birds (probably including some species of waterbird hosts [e.g., Mallard]; Z. Song, U. Alberta, pers. obs.). Canada Goose may accidentally consume infected *G. lacustris* when preening clinging amphipods from their feathers, and may serve as dead-end hosts for acanthocephalans. Note that my findings were based on snapshots of water bird abundance at each water body during each survey event, and did not consider water bird movement between water bodies and water-bird-mediated dispersal of parasitized amphipods between water bodies. These factors were outside of the scope of my study.

My findings with respect to the intermediate host, *G. lacustris* were less expected and less intuitive, as the effect of *G. lacustris* density on infection prevalence was statistically significant in only two models (Figure 3.1). Both overall and August 2016 models showed significant and negative correlations between acanthocephalan prevalence and intermediate host density, in accordance with previous studies (Mooring and Hart 1992, Côté and Poulin 1995, Rifkin et al. 2012, Patterson and Ruckstuhl 2013, Buck et al. 2017). This could be attributed to high density of intermediate hosts reducing the infection risk to each individual host. But high density of intermediate hosts should ensure absolute number of infections in a certain area ('benefits-to-parasites', Buck et al. 2017). Another four models showed nonsignificant but negative associations between acanthocephalan prevalence and intermediate host density. These nonsignificant results do not necessarily mean that the density of intermediate host does not matter for parasite prevalence. Instead, it suggests that 'encounter-dilution effect' and 'benefits-to-parasites' may interplay to obscure the significant association between intermediate host density and parasite prevalence.

In addition to the biotic variables discussed above, my results support the role of some environmental factors. The statistical significance of water temperature for acanthocephalan prevalence could result from high water temperature increasing the feeding rate of intermediate hosts and accordingly increasing the chance of infection (Maltby et al. 2002). In addition to water temperature, my results showed a positive regression coefficient of salinity with acanthocephalan prevalence. One mechanism for this positive relationship could be that amphipods parasitized with acanthocephalans have higher salinity tolerance than unparasitized amphipods. Piscart et al. (2007) found a positive association between mortality of unparasitized amphipods and salinity levels (1-12 μg/L). Salinity concentrations used in Piscart et al. (2007) are much higher than those in the water bodies I worked with, and might not reliably extrapolate to my study. Further manipulative studies should be applied to attribute the causation to the correlation of acanthocephalan prevalence with salinity. The negative regression coefficient for water depth in models suggests acanthocephalan prevalence decreases as water gets deeper. This is consistent with previous studies (Campbell 1990, Oliva et al. 2004) and this negative association could be related to positive phototaxis of infected amphipods which would likely cause them to accumulate in shallow, better lit water. The significance of substrate types (e.g., gravel and sand) in models may be related to a difference in substrate preference between

parasitized and unparasitized amphipods. To my knowledge, no published research has documented this, but it would be easily testable. Positive regression coefficients in models indicated that high acanthocephalan prevalence occurred in *G. lacustris* found in relatively low and moderate coverage of submerged and emergent vegetation. This is possibly because unparasitized amphipods prefer high plant coverage to hide and were less abundant at lower coverages, thereby increasing the acanthocephalan prevalence at low and moderate coverage of submerged and emergent vegetation (MacNeil et al. 2003). The significance of fish presence/absence in acanthocephalans is only supported by one model. This may be due to inadequate visual assessment of fish that may miss fish in sites where fish exist. Future manipulative studies are required to test its importance.

Besides all the aforementioned biotic and abiotic factors, other factors may potentially influence acanthocephalan prevalence. First, geographical proximity of water bodies to each other may influence acanthocephalan prevalence by nearby water bodies potentially 'exchanging' more infected amphipods that cling to water birds traversing short distances compared to geographically distant water bodies. Secondly, there was probably more than a single *Polymorphus* species in my samples. Different *Polymorphus* species manipulate *G. lacustris* in different ways (Bethel and Holmes 1973), so that they may have different relationships with biotic and abiotic factors. For instance, in comparison to *P. contortus*, the prevalence of *P. paradoxus* may increase more rapidly with waterbody age (up to a certain point) because *P. paradoxus*-infected *G. lacustris* more likely to be transferred to a newly-established water body by clinging onto features of birds. Third, waterbird behaviors should be considered in the future studies of the prevalence of acanthocephalan (and of other trophically-transmitted parasites that use water birds as final hosts). Host species that stay and breed may contribute more to acanthocephalan prevalence in corresponding water bodies than those that just pass through water bodies (e.g., in Edmonton area water bodies, Red-necked Grebes breed locally whereas scoters just use the water bodies for brief stops during migration).

In summary, my findings showed that acanthocephalan prevalence increases as waterbody age and the abundance of common final hosts increase, and the influence of these two factors overshadowed the abundance of intermediate and rare final hosts, as reflected in my six models. Abundance of non-host water bird species was as important as density of intermediate hosts,

although the mechanism for this is unclear. Although the role of single or multiple hosts in parasite prevalence has been tested in previous studies (e.g., Lagrue and Poulin 2015, Buck et al. 2017), to my knowledge, no field study has explored the effects of multiple hosts, commonness of final hosts and colonization time on parasite prevalence and compared their relative importance accounting for environmental heterogeneity. The generality of these findings should be tested in other regions and in host–parasite systems.

3.5. References

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Table 3.1 Summary table of waterbird groups sorted by family and then by genus. Group codes: known hosts for Polymorphidae (2), predatory water birds that are not known to be hosts for Polymorphidae (1) and herbivorous waterfowl (0). Common final-host species were bolded and rare final hosts are designated with asterisk in the third column 'Common name'.

Family	Scientific name	Common name	Groups	Literature
Anatidae	*Anas acuta* Linnaeus	Northern Pintail*	2	Crompton and Harrison (1965)
Anatidae	*Anas americana* Gmelin	American Wigeon*	2	Butterworth (1982)
Anatidae	*Anas clypeata* Linnaeus	Northern Shoveler*	2	Crompton and Harrison (1965)
Anatidae	*Anas discors* Linnaeus	Blue-winged Teal*	2	Butterworth (1982)
Anatidae	*Anas platyrhynchos* Linnaeus	**Mallard**	2	Butterworth (1982)
Anatidae	*Anas strepera* Linnaeus	Gadwall*	2	Butterworth (1982)
Anatidae	*Aythya affinis* [Eyton]	Lesser Scaup*	2	Bush (1980)
Anatidae	*Aythya americana* [Eyton]	Redhead	1	Schmidt (1969)
Anatidae	*Aythya collaris* [Donovan]	Ring-necked Duck*	2	Butterworth (1982)
Anatidae	*Aythya marila* Linnaeus	Greater Scaup*	2	Butterworth (1982)
Anatidae	*Aythya valisineria* [Wilson]	Canvasback*	2	Butterworth (1982)

Anatidae	*Branta canadensis* [Linnaeus]	Canada Goose	0	No literature supports it as a host for Polymorphidae [Canada Goose is herbivorous].
Anatidae	*Bucephala albeola* [Linnaeus]	Bufflehead*	2	Gladden and Canaris (2009)
Anatidae	*Bucephala clangula* [Linnaeus]	Common Goldeneye*	2	Crompton and Harrison (1965)
Anatidae	*Bucephala islandica* [Gmelin]	Barrow's Goldeneye*	2	Schmidt (1965)
Anatidae	*Lophodytes cucullatus* [Linnaeus]	Hooded Merganser*	2	McDonald (1988)
Anatidae	*Melanitta fusca* [Linnaeus]	White-winged Scote*	2	Butterworth (1982)
Anatidae	*Melanitta perspicillata* [Linnaeus]	Surf Scoter*	2	Skerratt et al. (2005)
Anatidae	*Mergus merganser* Linnaeus	Common Merganser*	2	Lapage (1961)
Anatidae	*Oxyura jamaicensis* [Gmelin]	Ruddy Duck*	2	Butterworth (1982)

Ardeidae	*Ardea Herodias* Linnaeus	Great Blue Heron*	2	Birmani et al. (2011)
Ardeidae	*Nycticorax nycticorax* [Linnaeus]	Black-crowned Night Heron*	2	Crompton and Harrison (1965)
Gaviidae	*Gavia immer* [Brünnich]	Common Loon	1	No literature supports it as a host for Polymorphidae.
Laridae	*Chlidonias niger* [Linnaeus]	Black Tern*	2	Amin and Heckmann (1991)
Laridae	*Chroicocephalus Philadelphia* [Ord]	Bonaparte's Gull*	2	Hair and Holmes (1970)
Laridae	*Larus argentatus* Pontoppidan	Herring Gull	1	No literature supports whether it's a host for Polymorphidae.
Laridae	*Larus californicus* Lawrence	California Gull	1	Vermeer (1969a); Vermeer (1969b)
Laridae	*Larus delawarensis* Ord	Ring-billed Gull	1	Vermeer (1969a)
Laridae	*Leucophaeus pipixcan* [Wagler]	Franklin's Gull	1	No literature supports whether it's a host for Polymorphidae.
Laridae	*Sterna forsteri* Nuttall	Forster's Tern	1	No literature supports whether it's a host for Polymorphidae.
Laridae	*Sterna hirundo* Linnaeus	Common Tern*	2	Crompton and Harrison (1965)

Pelecanidae	*Pelecanus erythrorhynchos* Gmelin	American White Pelican*	2	Kinsella et al. (2004)
Phalacrocoracidae	*Phalacrocorax auritus* [Lesson]	Double-crested Cormorant*	2	Threlfall (1982)
Podicipedidae	*Aechmophorus occidentalis* [Lawrence]	Western Grebe*	2	Stock (1985)
Podicipedidae	*Podiceps auritus* [Linnaeus]	Horned Grebe*	2	Stock (1985); Stock and Holmes (1987)
Podicipedidae	*Podiceps grisegena* [Boddaert]	**Red-necked Grebe**	2	Crichton (1969)
Podicipedidae	*Podiceps nigricollis* Brehm	Eared Grebe*	2	Stock (1985)
Podicipedidae	*Podilymbus podiceps* [Linnaeus]	Pied-billed Grebe*	2	Stock (1985)
Rallidae	*Fulica americana* Gmelin	American Coot*	2	Van Cleave (1945)
Recurvirostridae	*Recurvirostra americana* Gmelin	American Avocet*	2	Edwards and Bush (1989); Crompton and Harrison (1965)
Scolopacidae	*Steganopus tricolor* Viellot	Wilson's Phalarope*	2	Yanez and Canaris (1988)
Scolopacidae	*Tringa melanoleuca* [Gmelin]	Greater Yellowlegs	1	No literature supports whether it's a host for Polymorphidae.

Scolopacidae	*Tringa semipalmata* [Gmelin]	Willet*	2	Smith (2007)
Scolopacidae	*Tringa solitaria* Wilson	Solitary Sandpiper	1	Nickol (1966)

Table 3.2 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in overall model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis. Three old natural water bodies were excluded from analysis.

Model	Estimate	Std. error	z value	P
Intercept	-0.967	0.496	-1.950	0.051
G. lacustris density	-0.071	0.071	-0.990	0.321
Waterbody age	0.374	0.131	2.860	**0.004**
Abundance of common known final host	0.344	0.075	4.560	**<0.001**
Abundance of rare known final host	0.117	0.072	1.620	0.106
Abundance of non-host predatory water birds	-0.170	0.066	-2.580	**0.010**
Abundance of herbivorous waterfowl	-0.015	0.068	-0.220	0.827
Continuous environmental covariates				
pH	-0.042	0.073	-0.580	0.565
Water temperature	0.200	0.066	3.010	0.003

Salinity	0.000	0.097	0.000	0.997
Dissolved oxygen	-0.027	0.068	-0.390	0.693
Water depth	-0.204	0.055	-3.730	**<0.001**
Waterbody size	0.056	0.120	0.470	0.639
Categorical environmental covariates				
Emergent vegetation2	-0.065	0.160	-0.410	0.685
Emergent vegetation3	0.143	0.149	0.960	0.338
Emergent vegetation4	-0.023	0.163	-0.140	0.886
Emergent vegetation5	-0.039	0.159	-0.240	0.808
Submersed aquatic vegetation2	-0.012	0.164	-0.080	0.940
Submersed aquatic vegetation3	-0.191	0.161	-1.180	0.237
Submersed aquatic vegetation4	-0.312	0.189	-1.650	0.099
Submersed aquatic vegetation5	-0.273	0.174	-1.570	0.117
Gravel1	-0.499	0.178	-2.800	0.005
Mud1	-0.036	0.147	-0.240	0.809
Sand1	0.420	0.206	2.040	0.041

Stone1	0.568	0.183	3.110	0.002
Fish1	0.258	0.147	1.760	0.078

Table 3.3 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in May 2015 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis. Three old natural water bodies were excluded from analysis.

Model	Estimate	Std. error	z value	P
Intercept	0.477	0.529	0.900	0.368
G. lacustris density	-0.296	0.161	-1.840	0.066
Waterbody age	0.585	0.189	3.100	**0.002**
Abundance of common known final host	0.346	0.160	2.160	**0.031**
Abundance of rare known final host	0.382	0.158	2.420	**0.016**
Abundance of non-host predatory water birds	0.245	0.167	1.470	0.142
Abundance of herbivorous waterfowl	0.027	0.144	0.190	0.852
Continuous environmental covariates				
Total P	0.194	0.190	1.020	0.307
Chla	0.014	0.197	0.070	0.944

Total N	-0.285	0.215	-1.330	0.184
Water temperature	0.533	0.168	3.170	0.002
Salinity	0.462	0.148	3.120	0.002
Water depth	0.126	0.169	0.750	0.453
Waterbody size	0.034	0.163	0.210	0.836
Categorical environmental covariates				
Emergent vegetation2	-0.373	0.412	-0.900	0.366
Emergent vegetation3	-0.049	0.382	-0.130	0.899
Emergent vegetation4	0.256	0.427	0.600	0.548
Emergent vegetation5	0.027	0.417	0.060	0.949
Submersed aquatic vegetation2	-0.162	0.445	-0.360	0.715
Submersed aquatic vegetation3	-0.694	0.432	-1.610	0.108
Submersed aquatic vegetation4	-0.868	0.410	-2.120	0.034
Submersed aquatic vegetation5	-0.612	0.475	-1.290	0.198
Gravel1	0.490	0.608	0.810	0.421
Mud1	-0.294	0.433	-0.680	0.498

Sand1	-0.320	0.603	-0.530	0.596
Stone1	-0.385	0.361	-1.070	0.286

Table 3.4 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in August 2015 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis. Three old natural water bodies were excluded from analysis.

Model	Estimate	Std. error	z value	P
Intercept	-2.227	0.376	-5.930	<0.001
G. lacustris density	-0.200	0.121	-1.660	0.098
Waterbody age	0.382	0.137	2.780	**0.005**
Abundance of common known final host	0.400	0.107	3.730	**<0.001**
Abundance of rare known final host	0.049	0.131	0.370	0.708
Abundance of non-host predatory water birds	0.237	0.193	1.230	0.220
Abundance of herbivorous waterfowl	-0.326	0.185	-1.760	0.079
Continuous environmental covariates				
Total N	0.059	0.105	0.560	0.575
Salinity	0.639	0.117	5.450	<0.001

Dissolved oxygen	-0.104	0.101	-1.030	0.304
Water depth	-0.238	0.095	-2.510	0.012
Waterbody size	0.033	0.128	0.260	0.796
Categorical environmental covariates				
Emergent vegetation2	0.364	0.312	1.170	0.243
Emergent vegetation3	0.014	0.282	0.050	0.962
Emergent vegetation4	0.756	0.301	2.510	0.012
Emergent vegetation5	-0.071	0.302	-0.240	0.813
Submersed aquatic vegetation2	-0.335	0.334	-1.000	0.316
Submersed aquatic vegetation3	-0.566	0.334	-1.690	0.090
Submersed aquatic vegetation4	-0.648	0.375	-1.730	0.084
Submersed aquatic vegetation5	-0.028	0.356	-0.080	0.937
Gravel1	-0.899	0.282	-3.180	0.001
Mud1	0.447	0.347	1.290	0.198
Stone1	0.170	0.261	0.650	0.516

Table 3.5 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in May 2016 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis. Three old natural water bodies were excluded from analysis.

Model	Estimate	Std. error	z value	P
Intercept	2.106	0.502	4.200	<0.001
G. lacustris density	0.186	0.202	0.920	0.357
Waterbody age	0.822	0.268	3.060	**0.002**
Abundance of common known final host	0.724	0.261	2.770	**0.006**
Abundance of rare known final host	0.338	0.230	1.470	0.142
Abundance of non-host predatory water birds	-0.523	0.207	-2.520	**0.012**
Abundance of herbivorous waterfowl	-0.262	0.290	-0.910	0.365
Continuous environmental covariates				
pH	-0.151	0.249	-0.600	0.546
Water temperature	0.584	0.279	2.090	0.036

Salinity	0.222	0.265	0.840	0.401
Dissolved oxygen	0.100	0.247	0.400	0.686
Water depth	-0.288	0.160	-1.810	0.071
Waterbody size	-0.231	0.259	-0.890	0.372
Categorical environmental covariates				
Emergent vegetation2	-0.708	0.480	-1.470	0.140
Emergent vegetation3	-0.482	0.546	-0.880	0.378
Emergent vegetation4	-0.088	0.560	-0.160	0.875
Emergent vegetation5	-0.637	0.441	-1.440	0.149
Submersed aquatic vegetation2	-0.155	0.469	-0.330	0.742
Submersed aquatic vegetation3	-0.323	0.654	-0.490	0.622
Submersed aquatic vegetation4	0.644	1.301	0.490	0.621
Submersed aquatic vegetation5	-0.930	0.601	-1.550	0.122
Gravel1	-1.935	0.537	-3.610	<0.001
Mud1	-1.157	0.423	-2.740	0.006
Sand1	-1.073	0.743	-1.440	0.149

Fish1	0.931	0.382	2.440	0.015

Table 3.6 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in August 2016 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis. Three old natural water bodies were excluded from analysis.

Model	Estimate	Std. error	z value	P
Intercept	-2.185	0.317	-6.890	<0.001
G. lacustris density	-0.267	0.132	-2.020	**0.043**
Waterbody age	-0.163	0.250	-0.650	0.514
Abundance of common known final host	0.830	0.208	3.990	**<0.001**
Abundance of rare known final host	-0.218	0.213	-1.020	0.306
Abundance of non-host predatory water birds	0.228	0.206	1.110	0.268
Abundance of herbivorous waterfowl	0.335	0.230	1.460	0.145
Continuous environmental covariates				
Water temperature	0.156	0.168	0.930	0.354
Salinity	0.128	0.224	0.570	0.570

Dissolved oxygen	-0.045	0.128	-0.350	0.724
Water depth	-0.158	0.115	-1.380	0.167
Waterbody size	0.414	0.233	1.780	0.075
Categorical environmental covariates				
Emergent vegetation2	-0.425	0.361	-1.180	0.238
Emergent vegetation3	-0.196	0.267	-0.730	0.462
Emergent vegetation4	-0.098	0.258	-0.380	0.704
Emergent vegetation5	-0.318	0.349	-0.910	0.362
Submersed aquatic vegetation2	0.703	0.300	2.340	0.019
Submersed aquatic vegetation3	-0.180	0.253	-0.710	0.478
Submersed aquatic vegetation4	-0.020	0.373	-0.050	0.958
Submersed aquatic vegetation5	-0.378	0.337	-1.120	0.263
Gravel1	-0.607	0.421	-1.440	0.149
Mud1	0.582	0.252	2.310	0.021
Sand1	0.961	0.453	2.120	0.034
Fish1	0.067	0.238	0.280	0.779

Table 3.7 Descriptive statistics of biotic and abiotic factors from 36 water bodies studied in the vicinity of Edmonton, Alberta across 3 years (2015–2017).

Variables	Total	Mean	SD	Min	Max
Biotic variables					
Acanthocephalan prevalence	–	0.34	0.32	0.00	1.00
G. lacustris density (No./m^2)	–	154.89	259.70	0.00	2445.45
Abundance of common known final host (No./hectare)	–	5.65	8.45	0.00	89.17
Abundance of rare known final host (No./hectare)	–	2.87	3.67	0.00	27.14
Abundance of non-host predatory water birds (No./hectare)	–	2.30	13.19	0.00	146.47
Abundance of herbivorous waterfowl (No./hectare)	–	3.50	8.37	0.00	66.79
Number of species of water birds observed	44	4.12	2.37	0	14
Abiotic variables					
pH	–	8.06	0.76	5.91	9.94
Water temperature (°C)	–	18.50	3.85	7.80	29.90
Salinity (ppm)	–	519.28	202.92	145.80	980.20
Dissolved oxygen (mg/L)	–	8.41	2.55	1.43	18.51

Water depth (inch)	–	10.94	4.99	2.60	37.50
Waterbody age (year)	–	23.65	16.84	3.00	54.00
Waterbody size (hectare)	–	27.22	44.91	0.22	692.39
Emergent vegetation (%)	–	33.36	35.16	0.00	87.50
Submersed aquatic vegetation (%)	–	24.67	33.98	0.00	87.50
Total P (ug/L)	–	173.42	233.72	25	2845
Chlorophyll *a* (ug/L)	–	104.25	324.00	1.67	4503.89
Total N (ug/L)	–	800.77	894.28	25	4840

Table 3.8 Presence/absence dataset of muskrat, fish and aquatic birds from 36 water bodies in the vicinity of Edmonton, Alberta across 3 years (2015–2017).

	W01	W02	W03	W04	W05	W06	W07	W08	W09	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W23	W24	W26	W27	W29	W30	W31	W32	W33	W34	W35	W36	W37	W40	W41
Muskrat	0	1	1	0	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1
Fish	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0	0	1	1	1	1	0	1	0	0	1	0	0	0
American Avocet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
American Coot	1	1	1	0	1	1	1	0	1	1	1	1	0	1	0	1	0	0	1	1	0	0	1	1	1	0	0	0	0	1	1	0	1	1	0	1
American White Pelican	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
American Wigeon	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0
Barrow's Goldeneye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Black-crowned Night Heron	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1
Black Tern	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Blue-winged Teal	1	1	1	1	0	0	0	0	1	1	1	1	0	0	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
Bonaparte's Gull	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Bufflehead	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1
California Gull	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Canada Goose	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1
Canvasback	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Common Goldeneye	0	1	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0
Common Loon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Common Merganser	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Common Tern	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1
Double-crested Cormorant	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
Eared Grebe	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0
Franklin's Gull	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Forster's Tern	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Gadwall	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1
Great Blue Heron	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Greater Scaup	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0
Greater Yellowlegs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Herring Gull	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Hooded Merganser	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Horned Grebe	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0
Lesser Scaup	1	1	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	0	1	1	0	0	0	1	0	1
Mallard	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Northern Pintail	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Northern Shoveler	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pied-billed Grebe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red.necked Grebe	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	1
Wilsons Phalarope	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Redhead	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0
Ring-billed Gull	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	0	1	1
Ring-necked Duck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Ruddy Duck	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solitary Sandpiper	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Surf Scoter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Western Grebe	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
White-winged Scoter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Willet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3.9 Mean values of abiotic factors from 36 water bodies in the vicinity of Edmonton, Alberta across 3 years (2015–2017). The names of three natural water bodies were bolded and constructed water bodies were unbolded. Note: waterbody age is not a mean and is estimated relative to 2015. The abbreviations and measurement unit of the abiotic factors: WT: Water temperature (°C); SA: Salinity (ppm); DO: Dissolved oxygen (mg/L); WD: Water depth (cm); WA: Waterbody age (year); WZ: Waterbody size (hectare); EV: Emergent vegetation (%); SAV: Submersed aquatic vegetation (%);TP: Total P (ug/L); Chla: Chlorophyll *a* (ug/L); TN: Total N (ug/L).

	pH	WT	SA	DO	WD	WA	WZ	EV	SAV	TP	Chla	TN
W01	8.83	18.38	430.93	8.80	58.01	53	100.70	30.83	78.33	316.33	125.20	1259.50
W02	7.68	20.88	418.78	8.91	26.49	53	5.98	54.17	60.21	66.33	46.83	411.17
W03	8.72	19.54	399.98	7.71	25.35	53	8.97	59.58	49.38	292.00	68.78	903.50
W04	7.84	19.04	453.79	9.77	25.58	6	0.56	22.50	19.17	185.50	51.46	623.67
W05	7.57	16.81	270.94	7.37	27.20	32	1.91	71.11	0.97	107.00	61.65	522.33
W06	8.12	17.63	604.96	9.09	32.21	8	1.57	59.79	17.08	84.50	47.87	960.17
W07	8.01	17.34	335.99	7.24	45.34	25	1.04	78.13	21.46	104.00	56.00	568.00
W08	8.08	20.64	541.66	10.35	19.76	8	0.22	11.04	58.96	124.83	17.51	569.00
W09	8.34	18.14	462.11	9.67	15.98	4	1.81	42.92	17.36	105.50	57.83	1372.00
W10	8.98	19.87	392.92	12.04	25.86	23	4.95	22.50	0.00	214.67	247.05	484.33
W11	8.42	18.42	444.92	9.54	29.79	28	2.00	20.42	16.67	95.33	52.40	317.00
W12	7.99	18.64	596.54	8.01	25.35	7	2.29	14.03	30.00	125.17	82.05	638.00
W13	8.49	19.48	646.31	8.88	26.80	17	3.07	14.38	11.25	72.50	39.00	312.67
W14	7.98	19.94	666.50	8.17	27.84	26	2.53	57.36	6.94	96.00	88.95	574.50

W15	8.07	20.02	498.23	7.28	33.30	35	1.88	20.97	0.97	118.50	109.46	572.50
W16	8.39	20.12	543.96	8.92	28.40	35	2.70	4.58	21.81	80.33	66.78	497.00
W17	7.87	18.53	625.05	7.61	29.36	16	1.73	27.08	0.00	97.17	79.45	522.00
W18	7.92	17.93	634.08	8.47	22.00	14	0.46	69.17	22.71	256.67	88.10	1415.17
W19	7.80	18.07	706.78	7.34	22.58	14	1.83	12.71	33.96	215.67	106.07	854.83
W20	7.62	20.14	703.23	7.53	23.01	3	2.30	9.44	32.78	37.50	16.12	486.17
W21	7.76	17.11	755.91	7.39	30.91	23	1.97	22.50	0.42	103.50	88.70	804.67
W23	8.19	18.61	641.97	9.40	30.63	22	0.52	26.04	22.92	98.17	33.51	385.33
W24	8.33	16.77	469.41	7.90	23.55	18	11.95	46.67	55.42	318.67	37.97	913.50
W26	8.24	18.69	292.65	8.00	55.04	53	216.36	40.83	34.58	337.00	165.54	1928.67
W27	8.39	16.63	890.48	7.94	20.75	53	690.29	14.79	13.33	172.67	195.80	2077.33
W29	7.34	15.87	198.36	7.04	29.13	13	0.85	38.54	63.96	58.17	13.56	376.17
W30	8.48	17.91	235.34	8.54	26.72	10	0.47	2.50	8.75	70.67	42.28	491.83
W31	7.64	17.88	253.59	8.57	25.73	11	2.36	23.33	20.21	163.83	66.53	591.33
W32	8.23	19.54	323.50	7.78	25.32	53	1.53	0.00	32.50	576.50	812.39	1339.17
W33	7.65	20.38	230.24	9.65	22.86	53	3.11	14.79	49.79	92.50	78.92	740.83
W34	8.10	17.14	774.08	6.54	19.51	7	5.05	63.13	16.04	264.17	104.39	1320.33
W35	8.47	18.45	547.61	8.34	19.69	7	0.65	53.33	47.50	360.33	47.72	1399.67
W36	7.77	18.11	732.81	9.19	27.46	7	1.41	47.22	25.56	299.67	277.52	713.50
W37	7.90	18.06	811.75	8.39	25.48	9	1.80	31.25	5.21	225.33	98.29	933.33
W40	7.15	15.19	393.87	6.75	19.69	8	0.62	48.54	33.75	199.33	48.94	568.83
W41	8.14	18.19	598.02	8.56	33.12	38	13.35	30.97	2.64	107.00	132.29	379.83

Table 3.10 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in overall model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis.

Model	Estimate	Std. error	z value	P
Intercept	-0.988	0.484	-2.040	0.041
G. lacustris density	-0.142	0.067	-2.130	**0.033**
Waterbody age	0.472	0.124	3.820	**<0.001**
Abundance of common known final host	0.324	0.077	4.210	**<0.001**
Abundance of rare known final host	0.077	0.068	1.140	0.256
Abundance of non-host predatory water birds	-0.165	0.063	-2.610	**0.009**
Abundance of herbivorous waterfowl	-0.051	0.065	-0.790	0.431
Continuous environmental covariates				
pH	-0.031	0.067	-0.470	0.638
Water temperature	0.205	0.065	3.160	0.002

Salinity	0.029	0.090	0.320	0.747
Dissolved oxygen	-0.033	0.063	-0.520	0.600
Water depth	-0.281	0.056	-4.990	<0.001
Waterbody size	-0.117	0.115	-1.020	0.310
Categorical environmental covariates				
Emergent vegetation2	-0.086	0.152	-0.570	0.571
Emergent vegetation3	0.166	0.144	1.150	0.250
Emergent vegetation4	0.004	0.155	0.020	0.980
Emergent vegetation5	-0.070	0.149	-0.470	0.637
Submersed aquatic vegetation2	0.030	0.155	0.190	0.846
Submersed aquatic vegetation3	-0.183	0.159	-1.150	0.250
Submersed aquatic vegetation4	-0.236	0.183	-1.290	0.197
Submersed aquatic vegetation5	-0.194	0.166	-1.170	0.241
Gravel1	-0.503	0.171	-2.940	0.003
Mud1	-0.035	0.143	-0.250	0.806
Sand1	0.297	0.193	1.540	0.123

Stone1	0.560	0.178	3.140	0.002
Fish1	0.207	0.144	1.440	0.150

Table 3.11 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in May 2015 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis.

Model	Estimate	Std. error	z value	P
Intercept	0.223	0.551	0.400	0.686
G. lacustris density	-0.328	0.171	-1.910	0.056
Waterbody age	0.441	0.224	1.970	**0.049**
Abundance of common known final host	0.735	0.247	2.980	**0.003**
Abundance of rare known final host	0.110	0.205	0.540	0.592
Abundance of non-host predatory water birds	0.067	0.213	0.320	0.752
Abundance of herbivorous waterfowl	-0.107	0.200	-0.530	0.593
Continuous environmental covariates				
Total P	0.223	0.551	0.400	0.686
Chlorophyll *a*	-0.328	0.171	-1.910	0.056
pH	0.441	0.224	1.970	0.049

Water temperature	0.735	0.247	2.980	0.003
Salinity	0.110	0.205	0.540	0.592
Dissolved oxygen	0.067	0.213	0.320	0.752
Water depth	-0.107	0.200	-0.530	0.593
Categorical environmental covariates				
Emergent vegetation2	-0.112	0.345	-0.320	0.746
Emergent vegetation3	-0.214	0.371	-0.580	0.563
Emergent vegetation4	-0.265	0.441	-0.600	0.547
Emergent vegetation5	0.107	0.396	0.270	0.787
Submersed aquatic vegetation2	-0.235	0.425	-0.550	0.581
Submersed aquatic vegetation3	-0.365	0.451	-0.810	0.419
Submersed aquatic vegetation4	-0.609	0.475	-1.280	0.199
Submersed aquatic vegetation5	-0.082	0.526	-0.160	0.876
Gravel1	-0.626	0.619	-1.010	0.312
Mud1	-0.421	0.441	-0.950	0.340
Sand1	0.140	0.598	0.230	0.815

Stone1	-0.159	0.503	-0.320	0.752

Table 3.12 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in August 2015 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis.

Model	Estimate	Std. error	z value	P
Intercept	-2.171	0.381	-5.700	<0.001
G. lacustris density	-0.155	0.120	-1.290	0.197
Waterbody age	0.347	0.153	2.270	**0.023**
Abundance of common known final host	0.407	0.115	3.530	**<0.001**
Abundance of rare known final host	-0.041	0.108	-0.380	0.703
Abundance of non-host predatory water birds	0.358	0.189	1.900	0.058
Abundance of herbivorous waterfowl	-0.457	0.176	-2.600	**0.009**
Continuous environmental covariates				
Total N	0.156	0.103	1.510	0.130

Salinity	0.525	0.113	4.640	<0.001
Dissolved oxygen	-0.138	0.089	-1.560	0.120
Water depth	-0.141	0.097	-1.460	0.145
Waterbody size	-0.145	0.150	-0.970	0.332
Categorical environmental covariates				
Emergent vegetation2	0.412	0.305	1.350	0.177
Emergent vegetation3	0.076	0.282	0.270	0.787
Emergent vegetation4	0.643	0.299	2.150	0.032
Emergent vegetation5	-0.182	0.275	-0.660	0.510
Submersed aquatic vegetation2	-0.291	0.308	-0.950	0.344
Submersed aquatic vegetation3	-0.655	0.308	-2.120	0.034
Submersed aquatic vegetation4	-0.724	0.341	-2.120	0.034
Submersed aquatic vegetation5	-0.198	0.310	-0.640	0.524
Gravel1	-1.127	0.293	-3.850	<0.001
Mud1	0.450	0.267	1.680	0.093
Sand1	0.136	0.352	0.390	0.699

Stone1	0.178	0.262	0.680	0.496

Table 3.13 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in May 2016 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis.

Model	Estimate	Std. error	z value	P
Intercept	2.085	0.470	4.440	<0.001
G. lacustris density	0.120	0.179	0.670	0.503
Waterbody age	0.722	0.219	3.300	**0.001**
Abundance of common known final host	0.777	0.259	3.010	**0.003**
Abundance of rare known final host	0.124	0.221	0.560	0.574
Abundance of non-host predatory water birds	-0.481	0.209	-2.300	**0.022**
Abundance of herbivorous waterfowl	-0.248	0.268	-0.920	0.356
Continuous environmental covariates				
pH	-0.234	0.225	-1.040	0.298
Water temperature	0.557	0.256	2.180	0.029

Salinity	-0.057	0.241	-0.240	0.813
DO	0.080	0.237	0.340	0.736
Water depth	-0.190	0.146	-1.300	0.192
Categorical environmental covariates				
Emergent vegetation2	-0.718	0.413	-1.740	0.082
Emergent vegetation3	-0.260	0.483	-0.540	0.591
Emergent vegetation4	0.073	0.483	0.150	0.879
Emergent vegetation5	-0.629	0.406	-1.550	0.121
Submersed aquatic vegetation2	-0.152	0.439	-0.350	0.730
Submersed aquatic vegetation3	-0.463	0.636	-0.730	0.467
Submersed aquatic vegetation4	0.382	1.216	0.310	0.754
Submersed aquatic vegetation5	-0.744	0.530	-1.400	0.160
Gravel1	-1.691	0.501	-3.380	0.001
Mud1	-1.160	0.411	-2.820	0.005
Sand1	-1.116	0.740	-1.510	0.131
Fish1	0.757	0.360	2.100	0.036

Table 3.14 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in August 2016 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis.

Model	Estimate	Std. error	z value	P
Intercept	-2.451	0.331	-7.400	<0.001
G. lacustris density	-0.403	0.128	-3.140	**0.002**
Waterbody age	0.284	0.206	1.380	0.168
Abundance of common known final host	0.599	0.210	2.850	**0.004**
Abundance of rare known final host	-0.246	0.219	-1.120	0.261
Abundance of non-host predatory water birds	0.334	0.210	1.590	0.112
Abundance of herbivorous waterfowl	0.050	0.218	0.230	0.817
Continuous environmental covariates				
Water temperature	0.271	0.160	1.690	0.091
Salinity	0.119	0.217	0.550	0.584
Dissolved oxygen	0.072	0.120	0.600	0.549

Water depth	-0.300	0.113	-2.650	0.008
Categorical environmental covariates				
Emergent vegetation2	-0.148	0.345	-0.430	0.668
Emergent vegetation3	0.127	0.265	0.480	0.631
Emergent vegetation4	0.295	0.249	1.190	0.235
Emergent vegetation5	0.000	0.349	0.000	0.999
Submersed aquatic vegetation2	0.674	0.310	2.180	0.030
Submersed aquatic vegetation3	-0.281	0.264	-1.060	0.288
Submersed aquatic vegetation4	-0.096	0.383	-0.250	0.802
Submersed aquatic vegetation5	-0.088	0.334	-0.260	0.792
Gravel1	-0.688	0.444	-1.550	0.121
Mud1	0.506	0.261	1.930	0.053
Sand1	0.926	0.477	1.940	0.052
Fish1	0.154	0.246	0.620	0.533

Table 3.15 Summary output of generalized linear mixed model relating acanthocephalan prevalence as the response variable to waterbody age, *Gammarus lacustris* density, the abundance of common, rare and non-final host as explanatory variables, accounting for environmental covariates in May & August 2017 model. Prior to analysis, non-normally distributed continuous variables were ln(x+1) or square-root-transformed and all continuous variables were centered and scaled. All the explanatory variables with high variance inflation factors (VIFs >5 or GVIFs>2.25) were removed before analysis.

Model	Estimate	Std. error	z value	P
Intercept	-0.737	1.211	-0.610	0.543
G. lacustris density	-0.075	0.199	-0.380	0.706
Waterbody age	2.057	0.478	4.310	**<0.001**
Abundance of common known final host	1.330	0.277	4.810	**<0.001**
Abundance of rare known final host	0.747	0.302	2.470	**0.013**
Abundance of non-host predatory water birds	-0.249	0.245	-1.020	0.310
Abundance of herbivorous waterfowl	0.156	0.217	0.720	0.471

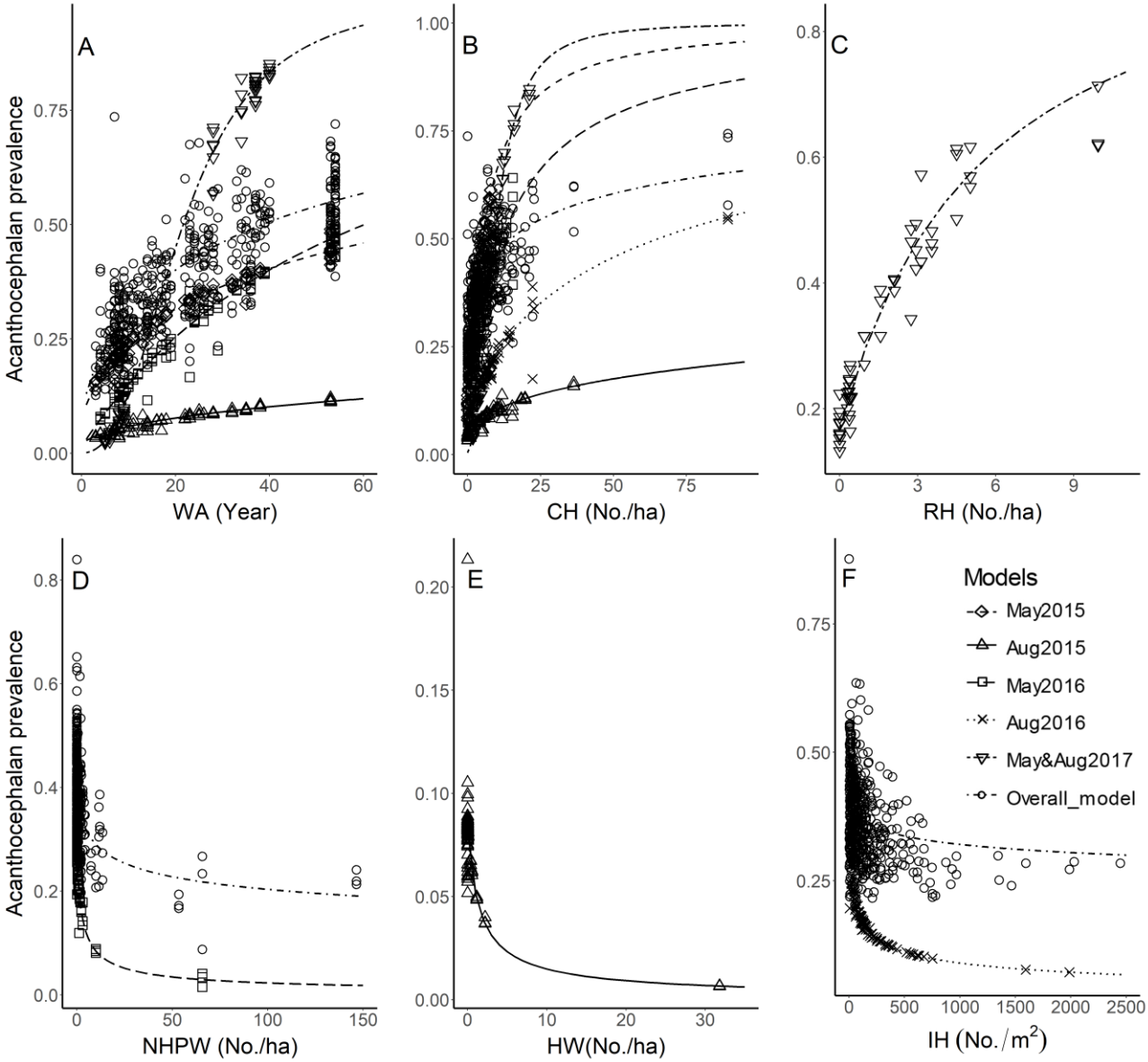

Figure 3.1 Relationships of acanthocephalan prevalence with waterbody age (WA), the abundance of common (CH) and rare (RH) final host species, of non-host predatory water birds (NHPW), herbivorous waterfowl (HW) and of the intermediate host (IH; *Gammarus lacustris*) using statistically significant GLMM models. Each partial residual plot shows the statistically supported changes in acanthocephalan prevalence with one explanatory variable varying with all other continuous explanatory variables held constant at their mean and all categorical variables held at 1. Note that the relationships shown here excluded random effects of waterbody identity and collection event.

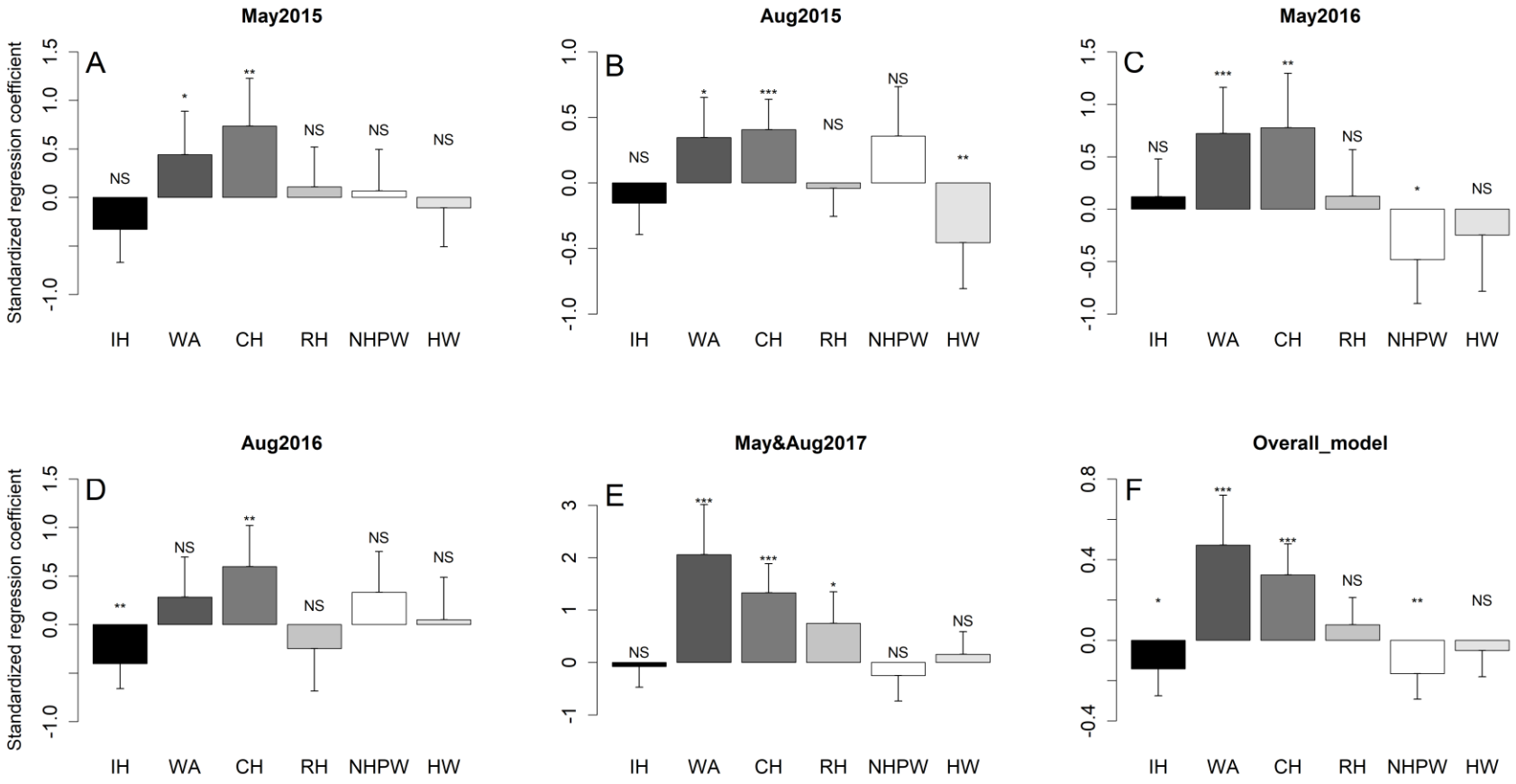

Figure 3.2 Relative importance of waterbody age, host and non-host abundance in acanthocephalan prevalence. Mean ±2*SE is given for standardized GLMM coefficients of the biotic predictors (IH, abundance of intermediate host; WA, waterbody age; CH, abundance of common final hosts; RH, abundance of rare final hosts; NHPW, abundance of non-host predatory water birds; HW: herbivorous waterfowl) across 7 models in A–F. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; NS = nonsignificant

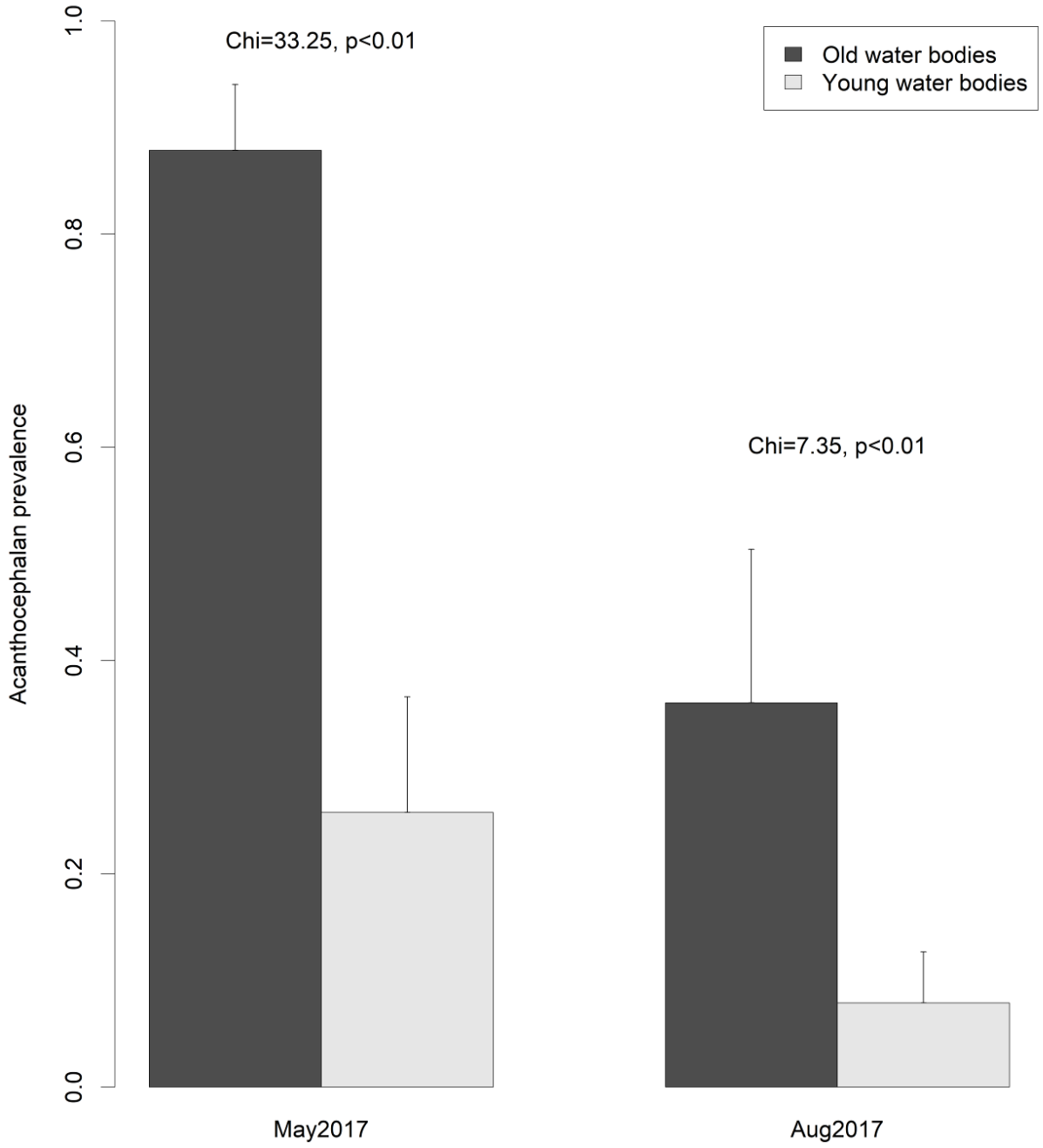

Figure 3.3 Acanthocephalan prevalence (proportion of infected *Gammarus lacustris*) in old (waterbody age relative to 2015: 26–38) and young (3–7) constructed waterbodies in May and August 2017. Error bars show the 2*standard error of the mean.

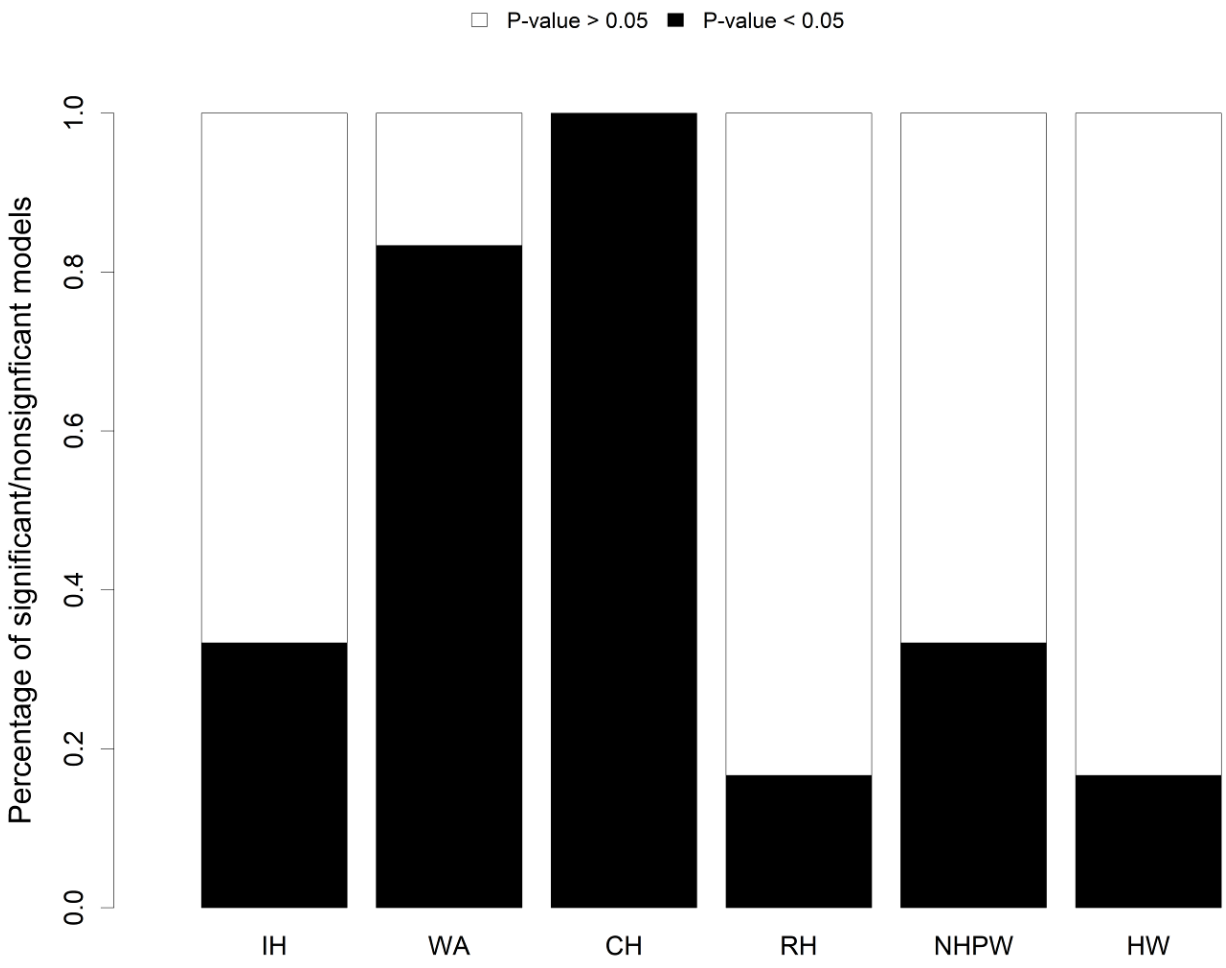

Figure 3.4 The percentage of significant and nonsignificant models among all models tested (six in total) for six factors predicted to be related to the prevalence of acanthocephalan infection in the intermediate host (*Gammarus lacustris*). IH, abundance of intermediate host; WA, waterbody age; CH, abundance of common final hosts; RH, abundance of rare final hosts; NHPW, abundance of non-host predatory water birds; HW: abundance of herbivorous waterfowl.

Chapter 4 Relationship between waterbody age and host and parasite mtDNA diversity.

4.1. Introduction

Intraspecific genetic diversity typically varies over space. This genetic variation is evident even at fine spatial scales for plants (Heywood 1991, Hardy et al. 2005), mammals (Nussey et al. 2005), reptiles (Moore et al. 2008), birds (Garroway et al. 2013), fish (Kovach et al. 2010) and invertebrates (Lynch and Spitze 1994, De Meester 1996). Unsurprisingly, this is also true for hosts and their parasites (Sire et al. 2001, Capelle and Neema 2005, Yin et al. 2012). For example, when a host species expands its range to occupy a new area, the population that eventually develops may include only a subset of the genetic diversity found in their original population (Slade and Moritz 1998, Florentine et al. 2013). During the host range expansion/invasion, their associated parasites can be transported to the new area and their subsequent genetic diversity may also be much lower in the new area than in the original one (Mineur et al. 2015).

One potential reason for low genetic diversity of host populations in invaded areas could be that by chance, the founding hosts contain a subset of the full diversity of genotypes of the source population (Nei et al. 1975, Easteal 1985). Similarly, stochastic loss of parasite genotypes during host invasion (akin to 'missing the boat' at the genetic level; see Torchin et al. 2003) may result in lower genotypic diversity of parasites in the new region. Also, low host genetic diversity in a newly occupied area can reduce the ability of their parasites to colonize the new environment (Forsman 2014) due to smaller number of competent host genotypes for effective transmission. As such, the new area might harbour a reduced number of parasite genotypes that includes only those adapted to a small number of host genotypes.

Although there are clear expectations of lower host and parasite diversity in newly established populations, I know less about how the amount of time available for host and parasite colonization influences their genetic diversity at fine spatial scales. There are two basic scenarios explaining the time–diversity relationship in evolutionary ecology. One is the colonization-time hypothesis, which predicts that genetic diversity should increase as time

available to colonize an area increases (Guégan and Kennedy 1993, Haag et al. 2005, Herborg et al. 2007, Roman and Darling 2007, Kennedy and Guégan 2009). This hypothesis is based on the fundamental assumption that local environmental conditions are always sufficient for supporting host and parasite populations and their associated genotypic diversity. However, another scenario involves the assumption that there is a finite carrying capacity for the genotypic diversity of host and parasites. Once a new area reaches carrying capacity, competition between genotypes intensifies, and some genotypes with higher fitness might be able to out-compete others (Ebert et al. 2002, De Meester et al. 2016). In this second hypothesis, a hump-shaped relationship between colonization time and intraspecific genetic diversity is expected, with an initial increase in diversity as time increases until carrying capacity is reached and overall genetic diversity then declines. Genetic differentiation among populations is potentially high in this scenario because by chance, different populations may end up with different genotypes of colonizers being dominant (Urban and De Meester 2009, Ortells et al. 2014). This is especially true when the effect of competition between genotypes overrides dispersal-induced gene exchange between populations (Boileau et al. 1992, De Meester et al. 2002), resulting in populations that differ genetically even at a fine spatial scale (Haag et al. 2005, Louette et al. 2007, Ortells et al. 2014, De Meester et al. 2016).

To test these ideas, it is ideal to have clearly demarcated habitats of known ages. Man-made water bodies constructed in different years constitute a promising system. *Gammarus lacustris* Sars is a widespread amphipod, which can move from one water body to another by clinging onto the plumage of water birds (Segerstråle 1953). Amphipods can hang onto water birds in flight for up to 2 hours, which is enough time for a bird to fly many kilometers (Segerstråle 1953, Gherardi 2007); such as for example, *Branta canadensis* (Linnaeus) (Canada Goose) can fly at a mean speed of 13.9 m/s (Tucker and Schmidt-Koenig 1971) and could therefore travel 100 km in two hours. This bird-mediated transport of *G. lacustris* is expected to be enhanced when the amphipod is infected by thorny-headed worms (Acanthocephala), some of which can manipulate the nervous systems of their hosts and cause them to swim toward light and grasp onto moving objects (Helluy and Holmes 1990). Water birds, together with muskrats and beavers, serve as the definitive hosts for many species of acanthocephalans that use *G. lacustris* as an intermediate host (Connell and Corner 1957, Bush 1980, Butterworth 1982, Stock 1985).

Here, I hypothesize that the genetic diversity (=genetic variation within water body) of the *G. lacustris* and its acanthocephalan parasites should initially increase as waterbody age increases because more genotypes of both host and parasite should be introduced by water birds over time. If intraspecific competition intensifies over time (as within-pond population density increases), there may be a point at which competitively superior genotypes come to dominate, and genetic diversity decreases, resulting in a hump-shaped relationship with waterbody age. Genetic variation among populations (=genetic divergence among water bodies) might be expected because different water bodies may by chance have had genetically different colonizers. I tested these predictions using constructed water bodies of various ages in Edmonton, Alberta, Canada. These water bodies harbor high densities of *G. lacustris* (up to ca. 2445 /m^2, see Chapter 2) and attract a variety of water birds that can introduce different genotypes of *G. lacustris* and also of genotypes and species of acanthocephalans, both by ferrying infected *G. lacustris*, and by defecating eggs of adult acanthocephalans that they carry in their guts. Because of this, I expected that acanthocephalan genetic diversity should also be positively related to the species richness of the known waterbird hosts to use a water body. To control for this, I included the species richness of known waterbird hosts as a covariate when testing the relationship between waterbody age and acanthocephalan genetic diversity. I also accounted for waterbody size (which approximates carrying capacity of a water body for both amphipod hosts and their parasites) when testing these hypotheses.

4.2. Material and methods

4.2.1. Specimen sampling and laboratory protocols

Gammarus lacustris specimens were sampled in May 2015 from ten constructed water bodies with various ages (3–53 years old) across Edmonton, Alberta, Canada (Table 4.1). The ten constructed water bodies were selected based on the following criteria: (1) *G. lacustris* infected by acanthocephalan should be present; (2) must fall into one of the following age groups: young (3–7 years old in 2015], middle–aged (16–26 years) and old (32–53 years). The age of water bodies and the species richness of water birds known to host acanthocephalans that use *G. lacustris* as an intermediate host are reported in Chapter 3. I collected amphipods by sweeping a dip net. Amphipods were taken alive to the lab and examined for parasites using a dissecting microscope. Cystacanths of acanthocephalans are apparent as red or orange dots in the dorsal

region of the amphipods. I dissected out cystacanths from living or very recently dead amphipods and placed them into tap water to cause the inverted proboscis to evert, thereby displaying the taxonomically important hooks. For molecular studies, I first chose six specimens of acanthocephalans per water body (60 specimens in total) by visually checking the everted proboscis of each acanthocephalan specimen to make my selected specimens as morphologically diverse as possible, and hence potentially capturing genetic diversity. All selected acanthocephalans belonged to the genus *Polymorphus* but may have belonged to more than one species because the two most common 'species' in the Edmonton area are *P.* cf. *marilis* and *P.* cf. *paradoxus*. They are morphologically different but their identities are not fully supported by molecular evidence (Chapter 2). To account for the potential effect of differences in acanthocephalan species composition on genetic diversity, I analyzed data and compared the results before and after removing the relatively less abundant *P.* cf. *marilis* (see below for details). I also used the *G. lacustris* individuals that were the hosts of these 60 specimens for molecular analysis. I only included infected *G. lacustris* for molecular analysis because infected and uninfected amphipods may be genetically divergent (e.g., *Gammarus fossarum* [Koch]; Westram et al. 2011) and they may show different relationships between genetic diversity and waterbody age. All specimens were preserved in absolute ethanol in a -20 °C freezer prior to DNA extraction.

Whole genomic DNA from individual *G. lacustris* and acanthocephalans was extracted using DNeasy 96 Blood and Tissue Kit (QIAGEN). COI universal primers (LCO1490 and HCO2198) were used to amplify the *G. lacustris* COI barcoding region (ca. 702 bp fragments of mitochondrial Cytochrome c oxidase subunit I) (Folmer et al. 1994). I performed polymerase chain reactions (PCR) for *G. lacustris* in a total volume of 25 µl containing 2.5 µl 10×PCR reaction buffer, 4.0 µl dNTPs (2 mM), 1.0 µl of each primer (10 µM), 1.0 µl DNA template, 1.0 µl $MgCl_2$ (50 mM), 0.5 µl in-house Taq DNA polymerase and 14 µl dH_2O. PCR conditions were as follows: 60 s at 94 °C, 5 cycles of 30 s at 94 °C, 90 s at 45 °C, 60 s at 72 °C, then 35 cycles of 30 s at 94 °C, 90 s at 51 °C, 60 s at 72 C, and 300 s at 72 °C (Witt et al. 2006). For acanthocephalans, a partial fragment of barcoding COI region (ca. 702 bp) was amplified and sequenced using primers from a previous study (Alcántar-Escalera et al. 2013). Acanthocephalan PCR reactions were conducted in 25 µl including 1.25 µl 10×PCR reaction buffer, 2.5 µl dNTPs (2 mM), 0.25 µl of each primer (10 µM), 1.0 µl DNA template, 1.25 µl $MgCl_2$ (50 mM), 0.5 µl

homemade Taq DNA polymerase and 18 µl dH_2O. PCR conditions consisted of 120 s at 94 °C, 35 cycles of 60 s at 94 °C, 60 s at 40 °C, 60 s at 72 °C, then followed by 300 s at 72 °C. I purified all PCR products of *G. lacustris* and acanthocephalans using ExoSAP (New England Biolabs) and submitted them to the Molecular Biology Service Unit in the Department of Biological Sciences, University of Alberta for DNA sequencing. Sequence chromatograms were viewed and checked for accuracy in FinchTV (Geospiza Inc.). All COI sequences of *G. lacustris* and acanthocephalans will be submitted to GenBank in association with submission of manuscripts for publishing.

4.2.2. Sequence alignment and trimming

Sequence alignments were conducted for *G. lacustris* and acanthocephalans in ClustalX 2.0 with default parameters (gap opening: 10; gap extension: 0.2; delay divergent sequences: 30%; DNA transition weight: 0.5) and then checked visually and trimmed in DNAMAN 7.0. I excluded five of the *G. lacustris* specimens with high proportion of ambiguous bases (>50%) and ended up including 55 *G. lacustris* sequences with the length of >500 bp for sequence alignment and trimmed into 485 bp. As for acanthocephalan sequences, I excluded one specimen with high proportion of ambiguous bases (ca. 22%) compared to other sequences. I removed another acanthocephalan specimen which was identified as being the sole sequenced representative of *Polymorphus* cf. *contortus* phylogenetically and morphologically (Chapter 2). Because *P.* cf. *marilis* specimens were phylogenetically nested within the clade of *P.* cf. *paradoxus* (Chapter 2), I did the sequence alignments and conducted the analysis in two ways: (1) *P.* cf. *paradoxus*– specimens only (56 in total) and (2) specimens of *P.* cf. *paradoxus* and *P.* cf. *marilis* (58 in total). All *P.* cf. *marilis* and *P.* cf. *contortus* specimens were from one water body (W33; Table 4.1). After removing them, only three specimens were left in that water body and this small sample size is probably unable to capture sufficient genetic diversity for resident *P.* cf. *paradoxus*. Because of this, I deleted water body W33 and retained 53 specimens of *P.* cf. *paradoxus* from the remaining nine water bodies for further analysis (I included the intraspecific genetic diversity of the three *P.* cf. *paradoxus* specimens from water body W33 to the plot of the relationship between waterbody age and genetic diversity of *P.* cf. *paradoxus* across nine water bodies after controlling for waterbody size and species richness of known waterbird hosts; see Figure S4 1). For both scenarios, sequences of acanthocephalans were aligned and trimmed into

525 bp in length. For *G. lacustris*, the high genetic diversity in W33 might be a potential outlier. To avoid its potential bias on analysis, I compared the results before and after removing W33, and both scenarios showed consistent patterns (Figure 4.1; Figure S4 2).

4.2.3. Statistical analysis

All statistical analyses were performed in R (https://www.r–project.org/). I first constructed separate mtDNA genetic distance matrices of the aligned sequences for host and parasite using the generalized Jukes–Cantor model (F81) (Felsenstein 1981). I selected the F81 model for both *G. lacustris* and acanthocephalan alignments because it fit better than other models available in dis.dna by having lower AICc. Then I tested the multivariate homogeneity of dispersions (multivariate analogue of Levene's test for homogeneous variances) among the hosts and parasites from water bodies with different ages to test whether water bodies with different ages had significantly different host or parasite genetic diversity. Multivariate homogeneity of dispersions test was conducted using betadisper function in package vegan (Anderson 2006). I used the nuc.div function from package pegas to calculate nucleotide diversity (i.e., genetic diversity) by summing the number of nucleotide differences between pairs of sequences divided by the number of comparisons (Nei 1987, Doña et al. 2015). After calculating the genetic diversity of hosts and parasites, I conducted linear and polynomial (quadratic and cubic) regressions to determine the best-fit relationships between waterbody age and host/parasite genetic diversity. I ranked the models for host and parasite separately. I adopted polynomial models only when they had significantly better model fit than linear models by explaining more variance (higher adjusted R^2), having $p < 0.05$ in ANOVA diagnosis and low corrected Akaike information criterion (AICc). To further determine the best-fit relationship between waterbody age and *G. lacustris* diversity, I selected the best-fit linear or polynomial models and then added waterbody size to account for its potential effect on host diversity. Similarly, for the parasite genetic diversity, I first conducted linear and polynomial regressions linking its genetic diversity to waterbody age. Then I added waterbody size and species richness of known waterbird hosts to control for their potential effects on parasite genetic diversity. Species richness of known waterbird hosts was retained as a categorical variable because the narrow range of waterbird richness (2–5) cannot guarantee a reliable regression. Prior to analysis, waterbody size was ln(x+1)-transformed to improve normality. I tested spatial autocorrelation and normality on the

residual of the best-fit models for both host and parasite using Moran's I and Shapiro test, respectively. I used principal coordinate analysis (PCoA) to show genetic variation in *G. lacustris* and acanthocephalan populations among three age groups of water bodies [young (3–7 years), middle–aged (16–26 years) and old (32–53 years)]. Analysis of molecular variance (AMOVA) and pairwise AMOVA comparisons were used to test whether genetic differentiation was significantly different among water bodies differing in age. AMOVA was conducted with 1000 permutations to estimate statistical significance, and *p*-values of multiple comparisons were adjusted using Bonferroni method. Haplotype analysis was used to detect if there was any spatial pattern for *G. lacustris* and acanthocephalan genetic diversity.

4.3. Results

The COI diversity of *G. lacustris* was significantly different among the ten constructed water bodies (betadisper: F=3.82, $p<0.01$). I first ranked the models for the relationship between *G. lacustris* genetic diversity and waterbody age after controlling for waterbody size. The quadratic form had the high adjusted R^2 (0.38), low AICc (-66.47) and was significantly better than the linear fitting model (ANOVA: $p<0.01$), with significant regression coefficient of squared item (i.e. quadratic term: WA^2; Table 4.2). Then I removed waterbody size from linear and polynomial models and compared the models. I found that neither quadratic nor cubic models had significant regression coefficients of squared (i.e. WA^2) and cubic items (i.e. WA^3) after controlling waterbody size (Table 4.2). The linear model without waterbody size did not provide a good fit since it had negative adjusted R^2 and the regression coefficient of waterbody size was not significant (Table 4.2). Therefore, I selected the quadratic model with the covariable of waterbody size as the best-fit model for *G. lacustris* (Table 4.2). This best-fit model showed that *G. lacustris* COI diversity peaked at intermediate waterbody age (Figure 4.1).

Similarly, water bodies of different ages had significantly different genetic diversity for *P.* cf. *paradoxus* + *P.* cf. *marilis* (betadisper: F=3.05, $p<0.01$) and for *P.* cf. *paradoxus*–only specimens (betadisper: F=2.44, $p<0.05$). To determine the best-fit relationships between waterbody age and both *P.* cf. *paradoxus* genetic diversity and the genetic diversity for *P.* cf. *paradoxus* + *P.* cf. *marilis*, I first compared linear and polynomial (quadratic and cubic) models without considering any covariate. For *P.* cf. *paradoxus* genetic diversity, the cubic form had a better fit than linear and quadratic forms by having high adjusted R^2 (0.44), low AICc (-64.56)

and significant coefficients for waterbody age (Table 4.3). After I retained waterbody size and species richness for known waterbird hosts, I found that the linear form was better than the other two polynomial models, having high adjusted R^2 (0.63), low AICc (-46.38) and significant regression coefficients of all terms (Table 4.3). The linear form with the two covariates had higher adjusted R^2 than the cubic form without any covariates (Table 4.3). Because of this, I adopted this linear form with waterbody size and species richness of known waterbird hosts as the best-fit model. The best model showed a positive relationship between waterbody age and acanthocephalan genetic diversity (Table 4.2).

For the genetic diversity of *P.* cf. *paradoxus* + *P.* cf. *marilis*, I adopted this linear form with waterbody size and species richness of known waterbird hosts as the best-fit model because the linear model with waterbody age and species richness of known waterbird hosts as covariates had a better fit than the two polynomial models, having the high adjusted R^2 (0.66), low AICc (-67.70) and significant regression coefficients of all terms except the intercept (Table 4.4). The best model showed a positive relationship between waterbody age and acanthocephalan genetic diversity (Figure 4.3). In addition, there was much higher genetic diversity among the sequenced acanthocephalan specimens (*P.* cf. *paradoxus*: 27 haplotypes and *P.* cf. *paradoxus* + *P.* cf. *marilis*: 29 haplotypes) than among the *G. lacustris* specimens (11 haplotypes) (compare y axes of Figure 4.1–Figure 4.3, and haplotype diversity in Figure S4 3 and Figure S4 4).

AMOVA showed that genetic variation among populations of *G. lacustris* from the three age–groups of water bodies was statistically significant ($p<0.05$), although PCoA plot did not show very strong genetic differentiation (Figure 4.4). Multiple AMOVA comparisons showed genetic variation was statistically significant between young and old age-groups (adjusted $p<0.05$) while the other combinations (young vs. middle-aged and middle-aged vs. old) were not significant (adjusted $p>0.05$). Genetic variation of *P.* cf. *paradoxus* and of *P.*cf. *paradoxus* + *P.* cf. *marilis* did not differ significantly among the three age groups ($p>0.05$; Figure 4.5; Figure 4.6). I did not observe any obvious spatial pattern (i.e. spatial differentiation of genetic structure) of haplotypes of *G. lacustris* and acanthocephalans (Figure S4 3 and Figure S4 4) or any spatial autocorrelation in the residuals of their best-fit models. I did not find any statistically significant deviation from normality of residuals of the best-fit models.

4.4. Discussion

My study characterized how genetic diversity of *G. lacustris* and acanthocephalans changes as waterbody age increases to test hypotheses associated with two factors: time available for host and parasite colonization and intraspecific competition. To my knowledge, this is the first to test the relationship between the age of constructed water bodies and genetic diversity of host and parasites in single study. My results showed a humped relationship between mitochondrial COI diversity of the intermediate host amphipod *G. lacustris* and age of the constructed water bodies. The shape of the distribution was consistent with the hypothesis that the genetic diversity of *G. lacustris* peaked at intermediate waterbody age where carrying capacity was possibly reached, after which competition probably eliminated some genotypes, thereby reducing local *G. lacustris* genetic diversity. This idea is further corroborated by the statistically significant genetic differentiation in *G. lacustris* between young and old water bodies, although this genetic differentiation is not obvious in the PCoA plot. Existence of this genetic differentiation between the young and old at such a fine spatial scale (minimum geographical distance between water bodies = 0.95 km, maximum = 20.82 km) might result from the effects of competition between genotypes that overrides the gene flow between populations in different water bodies. This competition may involve late-arriving genotypes being outcompeted by numerically abundant early-arriving genotypes and therefore failing to establish in an already-colonized water body (Waters et al. 2013, De Meester et al. 2016). I suggest that manipulative mesocosm experiments could be helpful to test this idea. Note that all *G. lacustris* that I included in the analysis were clearly susceptible to and infected by *Polymorphus* (mainly *P.* cf. *paradoxus*). In contrast, uninfected amphipods might include those that are not susceptible. Susceptible and unsusceptible amphipods could be genetically different (as was observed in *Gammarus fossarum*; Westram et al. 2011) and therefore, within a given water body, the class 'uninfected amphipod' might be more genetically diverse by having both the susceptible and insusceptible than the class 'infected'. The different levels of genetic diversity might render infected and uninfected amphipods different shaped relationships between genetic diversity and waterbody age. In addition, susceptible amphipods can get infected by *P.* cf. *paradoxus* and therefore are more likely to disperse by water birds to newly-established water bodies compared to insusceptible ones. Because of the relatively high gene exchanges among the populations of *P.*

cf. *paradoxus*-infected amphipods, susceptible amphipods may have smaller genetic differentiation among young, middle-aged and old water bodies than insusceptible ones.

In contrast, acanthocephalan genetic diversity (including *P.*cf. *paradoxus* and both *P.*cf. *paradoxus* and *P.* cf. *marilis*) showed a positive relationship with waterbody age after controlling for waterbody size and species richness of known waterbird hosts. These findings support a pure 'colonization-time' scenario, and suggest that the amphipod hosts are not yet saturated by acanthocephalan genotypes (possibly also species) and still have 'room' to harbor more lineages of acanthocephalans. This is consistent with rare cases of multiple acanthocephalan infections in *G. lacustris* (Z. Song, U. Alberta, pers. obs.). I did not find significant genetic differentiation among acanthocephalan populations from different age groups of water bodies. This suggests that competition may not influence parasite genetic structure, or at least its effect does not override the effect of gene flow on structuring acanthocephalan populations. My acanthocephalan results are consistent with patterns reported in other studies concerning colonizing populations. In Europe, the invasive Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853 (Decapoda: Varunidae) showed an increase in genetic diversity over time (Herborg et al. 2007). Similarly, the invasive water flea *Bythotrephes longimanus* Leydig (Cladocera: Cercopagidae) showed increased genetic diversity and a reduced founder effect in North America over a 7 year period (Berg et al. 2002, Roman and Darling 2007). Overall, my findings show that the genetic diversity of *G. lacustris* and their acanthocephalans have different relationships with waterbody age, suggesting that more than one mechanism affects local mtDNA diversity involved in this host–parasite system.

Despite statistical significance of my results, there is still unexplained variation in the genetic diversity of both *G. lacustris* and acanthocephalans. There are several potential mechanisms for this. First, environment could be a factor influencing the survival and coevolution of host and parasite genotypes by selecting the genotypes with high local fitness (Wolinska and King 2009). Second, population size could also be a potential factor affecting genetic diversity. Assuming that the rate of genetic drift is constant, genetic diversity is expected to increase as the effective population size (N_e) increases (Kimura 1984). Many previous studies regarding host–symbiont systems support the positive correlation between genetic diversity and effective population size (e.g. Criscione and Blouin 2005, Criscione et al. 2005, Doña et al. 2015). Third, the frequency of

water birds visiting a water body (data that I do not have) might play a role in structuring the populations of *G. lacustris* and acanthocephalans in that water body by the haphazard transport of different genotypes of *G. lacustris* and acanthocephalans. Fourth, the intraspecific genetic diversity of a parasite can depend on its life cycles and host dispersal. Populations of autogenic trematodes (i.e., cycling only through freshwater hosts and colonizing new freshwater locality by freshwater hosts with limited dispersal) were more highly genetically structured than populations of allogenic ones (i.e., using both freshwater and terrestrial hosts and dispersing between localities by both hosts with high dispersal ability) (Criscione and Blouin 2004). For acanthocephalans, I found that species richness of known waterbird hosts at particular water bodies had a significant and positive regression coefficient in best models (Table 4.3 and Table 4.4) (Figure S4 5 and Figure S4 6). This suggests that different genotypes of *Polymorphus* spp. may utilize different waterbird species as definitive hosts. I retained the species richness of known waterbird hosts in models to control its potential effect rather than to test its importance. Future research is required to test the effect of host species diversity on genetic diversity of *Polymorphus* spp.

Overall, my study showed that waterbody age explains some variation of genetic diversity for *G. lacustris* and acanthocephalans, although host and parasite showed differently shaped functions for diversity with respect to waterbody age. These results suggest that time available for host and parasite colonization influences their genetic diversity at a fine spatial scale. My findings also show that diversity of available waterbird hosts explains a significant amount of variation in acanthocephalan genetic diversity. This supports the importance of host specificity for different genotypes of acanthocephalans. Besides waterbody age, other factors (e.g., effective population size of host and parasite, host–parasite interactions and local environmental factors) are likely to play roles in affecting genetic diversity of host and parasites at fine spatial scales and are excellent subjects for future studies.

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Table 4.1 Features of ten constructed water bodies including locality, waterbody age (WA), waterbody size (WS) and species richness of known waterbird hosts (SW) in Edmonton, Alberta, Canada.

Waterbody name	Geographical coordinates		WA (Year)	WS (hectare)	SW
W05	53.4819 N	113.3913 W	32	1.794	2
W12	53.6310 N	113.4603 W	7	2.237	3
W14	53.6316 N	113.4732 W	26	2.504	3
W15	53.6326 N	113.4861 W	35	1.835	2
W17	53.6384 N	113.5015 W	16	1.675	2
W20	53.6321 N	113.5332 W	3	2.315	5
W21	53.6214 N	113.5498 W	23	1.927	2
W31	53.4942 N	113.6751 W	11	2.381	2
W33	53.5330 N	113.6696 W	53	3.132	5
W41	53.6167 N	113.5074 W	38	13.448	2

Table 4.2 Comparisons of linear, quadratic and cubic regressions regarding host (*Gammarus lacustris*) genetic diversity and waterbody age (WA) with/without considering waterbody size (WS) as a covariate. WS was ln(x+1)-transformed to improve normality. Model comparisons (MC) were conducted between linear and polynomial models (*p*–value from ANOVA). The best model is bolded. * = $p<0.05$; ** = $p<0.01$.

Regression Model	Intercept	WA coefficient	WA^2 coefficient	WA^3 coefficient	WS coefficient	Adjusted R^2	AICc score	MC
Without WS as a covariate								
Linear	4.80×10^{-03}*	2.11×10^{-05}				-0.12	-77.05	
Quadratic	1.84×10^{-03}	4.52×10^{-04}	-1.04×10^{-05}			0.19	-74.27	0.051
Cubic	-1.93×10^{-03}	1.43×10^{-03}	-6.83×10^{-05}	9.36×10^{-07}		0.47	-67.59	<0.01
With WS as a covariate								
Linear	4.71×10^{-03}	1.94×10^{-05}			9.92×10^{-05}	-0.31	-69.85	
Quadratic	**-2.02×10^{-03}**	**6.48×10^{-04}***	**-1.62×10^{-05}***		**2.40×10^{-03}**	**0.38**	**-66.47**	**<0.01**
Cubic	-2.91×10^{-03}	1.25×10^{-03}	-5.44×10^{-05}*	6.62×10^{-07}	1.30×10^{-03}	0.42	-44.82	<0.01

Table 4.3 Comparisons of linear, quadratic and cubic regressions regarding genetic diversity of *Polymorphus* cf. *paradoxus* and waterbody age (WA) with/without considering waterbody size (WS) and species richness of known waterbird hosts (SW) as covariates. WS was ln(x+1)-transformed to improve normality, respectively. Model comparisons (MC) were conducted between linear and polynomial models (*p*–value from ANOVA). The best model is bolded. * = $p<0.05$; ** = $p<0.01$.

Regression Model	Intercept	WA coefficient	WA^2 coefficient	WA^3 coefficient	WS coefficient	SW coefficient	Adjusted R^2	AICc score	MC
Without WS and SW as covariates									
Linear	5.16×10^{-03}*	1.44×10^{-05}					-0.14	-74.39	
Quadratic	6.62×10^{-03}	-1.98×10^{-04}	5.13×10^{-06}				-0.23	-67.84	0.50
Cubic	1.30×10^{-02}*	-1.85×10^{-03}*	1.03×10^{-04}*	-1.58×10^{-06}*			0.44	-64.56	0.01
With WS and SW as covariates									
Linear	**5.14×10^{-03}***	**1.64×10^{-04}***			**-3.07×10^{-03}***	**4.89×10^{-04}/ 6.52×10^{-03}***	**0.63**	**-46.39**	
Quadratic	8.66×10^{-03}	-1.43×10^{-04}	7.17×10^{-06}		-3.89×10^{-03}	4.23×10^{-04}/ 4.83×10^{-03}	0.64	22.77	0.29
Cubic	9.35×10^{-03}	-2.93×10^{-04}	1.49×10^{-05}	-1.20×10^{-07}	-3.75×10^{-03}	2.88×10^{-04}/ 4.35×10^{-03}	0.47	-	0.69

Table 4.4 Comparisons of linear, quadratic and cubic regressions regarding genetic diversity of both *Polymorphus* cf. *paradoxus* and *P.* cf. *marilis* and waterbody age (WA) with/without considering waterbody size (WS) and species richness of known waterbird hosts (SW) as covariates. WS was ln(x+1)-transformed to improve normality, respectively. Model comparisons (MC) were conducted between linear and polynomial models (*p*–value from ANOVA). The best model is bolded. * = $p<0.05$; ** = $p<0.01$.

Regression Model	Intercept	WA coefficient	WA^2 coefficient	WA^3 coefficient	WS coefficient	SW coefficient	Adjusted R^2	AICc score	MC
Without WS and SW as covariates									
Linear	4.11×10^{-03}*	7.72×10^{-05}					0.10	-83.11	
Quadratic	6.66×10^{-03}*	-2.03×10^{-04}	5.28×10^{-06}				0.26	-80.43	0.10
Cubic	8.28×10^{-03}*	-5.35×10^{-04}	1.98×10^{-05}	-1.70×10^{-07}			0.19	-72.10	0.23
With WS and SW as covariates									
Linear	**6.29×10^{-03}**	**9.30×10^{-05}***			**-2.57×10^{-03}**	**-1.09×10^{-04}/ 4.06×10^{-03}***	**0.66**	**-67.70**	
Quadratic	4.60×10^{-03}	2.30×10^{-04}	-2.67×10^{-06}		-2.42×10^{-03}	1.09×10^{-04}/ 5.48×10^{-03}	0.61	-38.35	0.60
Cubic	1.05×10^{-02}	-5.40×10^{-04}	2.78×10^{-05}	-3.22×10^{-07}	-3.49×10^{-03}	5.71×10^{-05}/ 3.54×10^{-03}	0.75	44.29	0.16

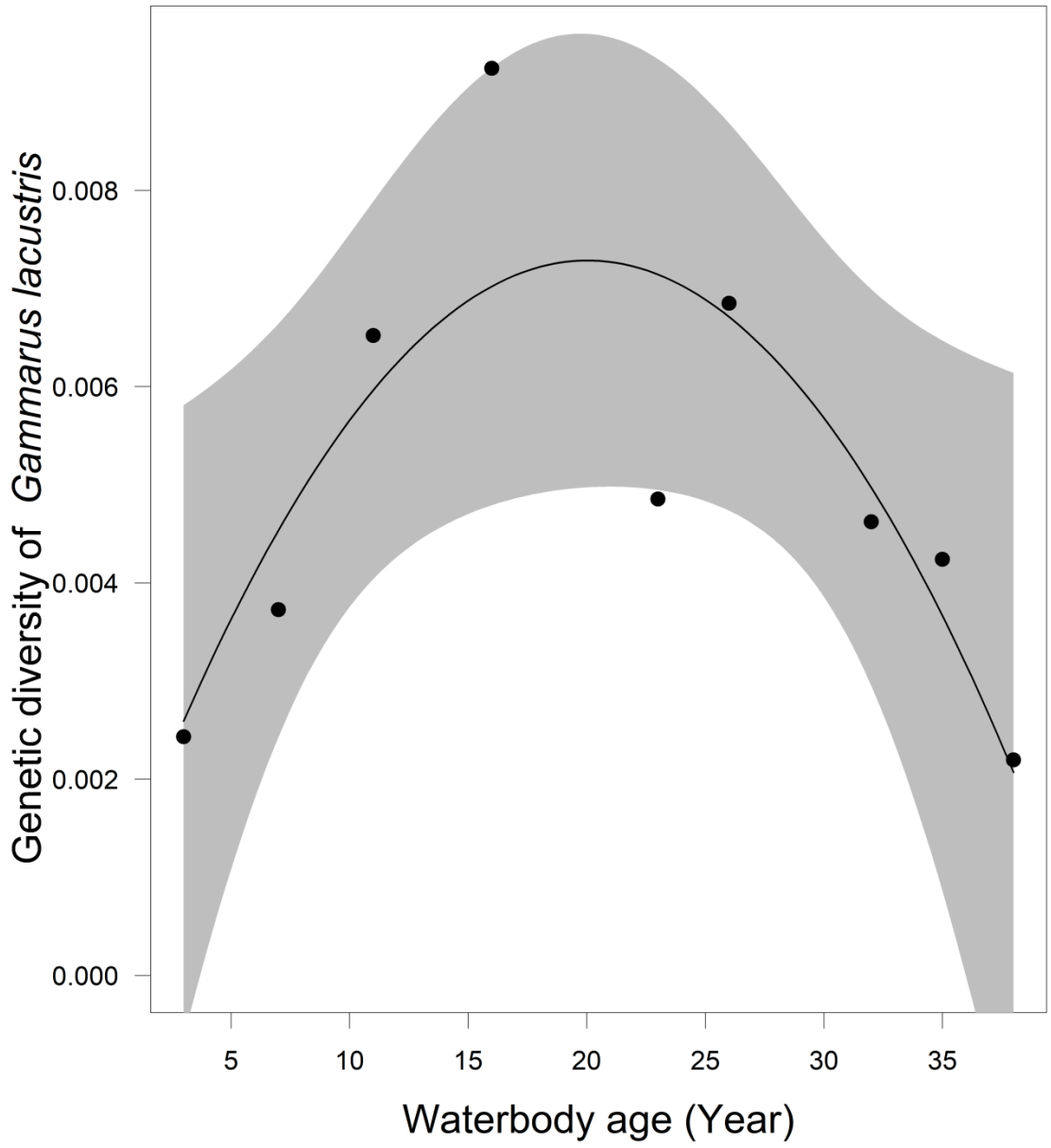

Figure 4.1 The hump-shaped relationship between host (*Gammarus lacustris*) mtDNA genetic diversity and waterbody age across nine water bodies (excluding one water body which is a potential outlier) after controlling for waterbody size. Each dot represents the *G. lacustris* genetic diversity within a water body. The curved line represents the fitted line through dots. The band represents 95% confidence intervals.

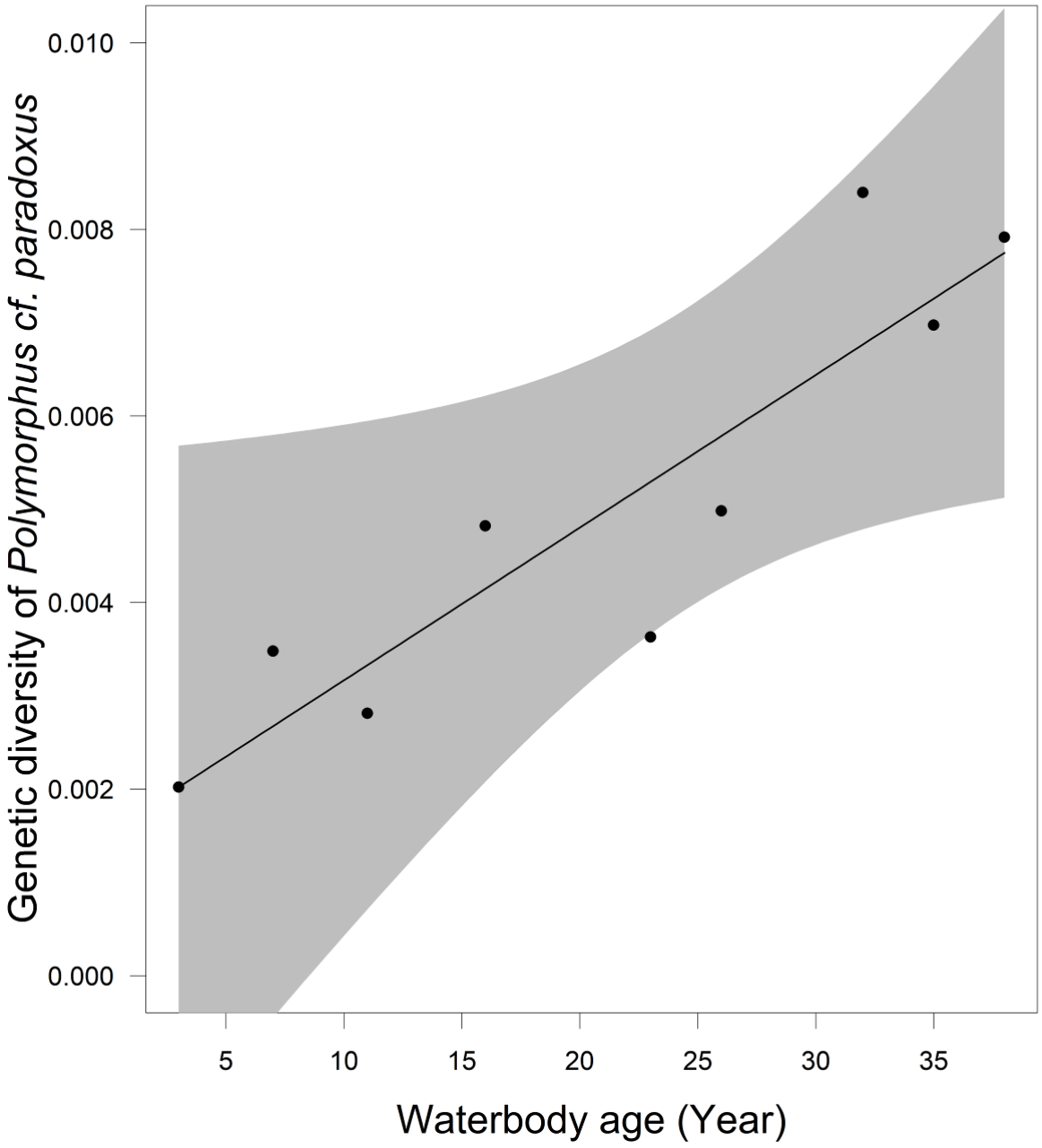

Figure 4.2 The positive relationship between parasite (*Polymorphus* cf. *paradoxus*) mtDNA genetic diversity and waterbody age across nine water bodies (excluding one water body due to small sample size) after controlling for waterbody size and species richness of known waterfowl hosts. Each dot represents the acanthocephalan genetic diversity within a water body. The straight line represents the fitted line through dots. The band represents 95% confidence intervals.

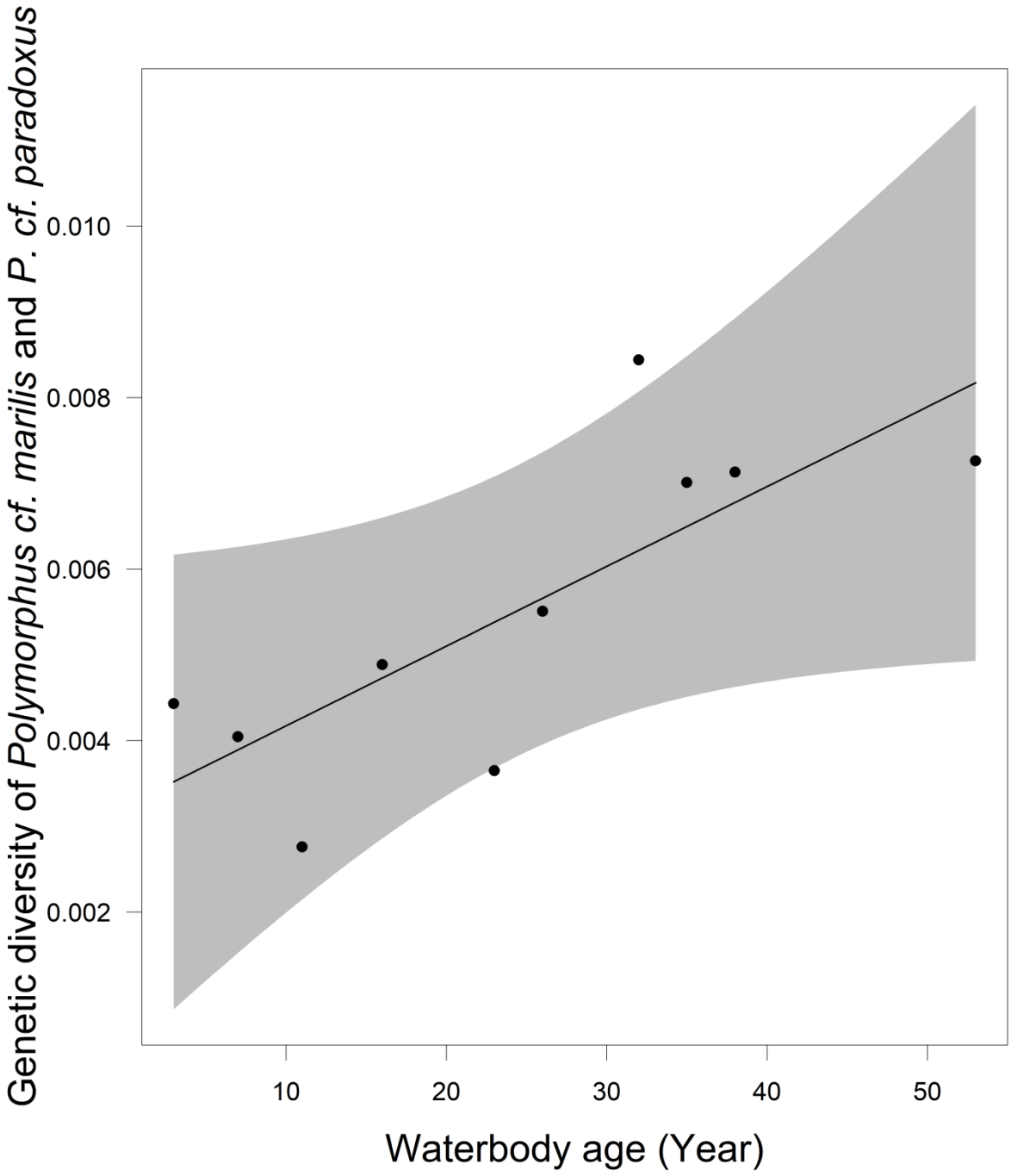

Figure 4.3 The positive relationship between parasite (*Polymorphus* cf. *paradoxus* and *P.* cf. *marilis*) mtDNA genetic diversity and waterbody age across ten water bodies after controlling for waterbody size and species richness of known waterbird hosts. Each dot represents the acanthocephalan genetic diversity within a water body. The straight line represents the fitted line through dots. The band represents 95% confidence intervals.

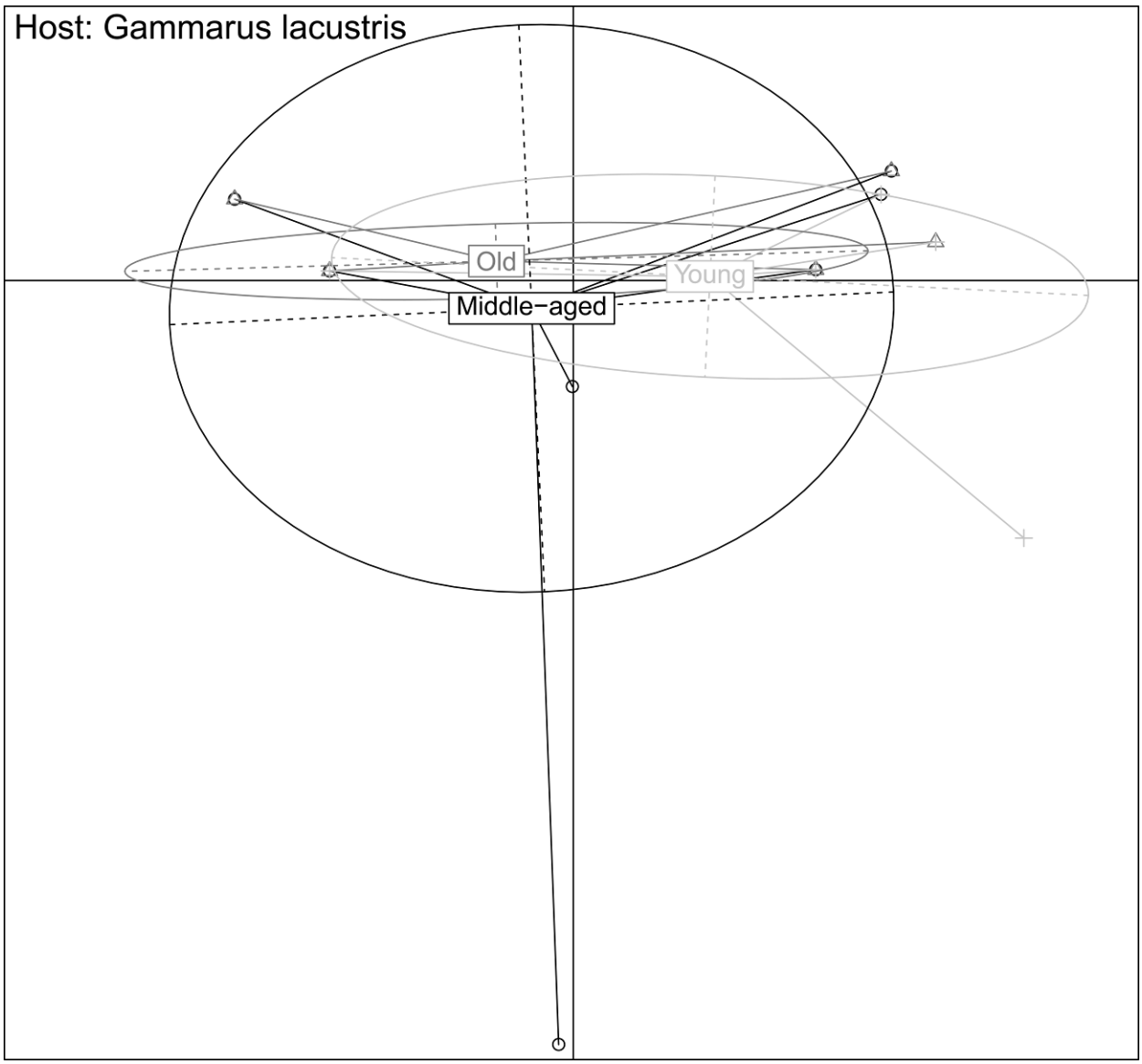

Figure 4.4 Principal coordinate analysis (PCoA) plots showing mtDNA genetic variation of host (*Gammarus lacustris*) categorized by age groups of water bodies [(young (3–7 years; plus symbol), middle-aged (16–26 years; circle symbol) and old (32–53 years; triangle symbol)]. Each dot stands for a specimen and some dots overlay each other due to the small genetic distance between these specimens). Overlap between different age groups suggested the genetic similarity among these groups.

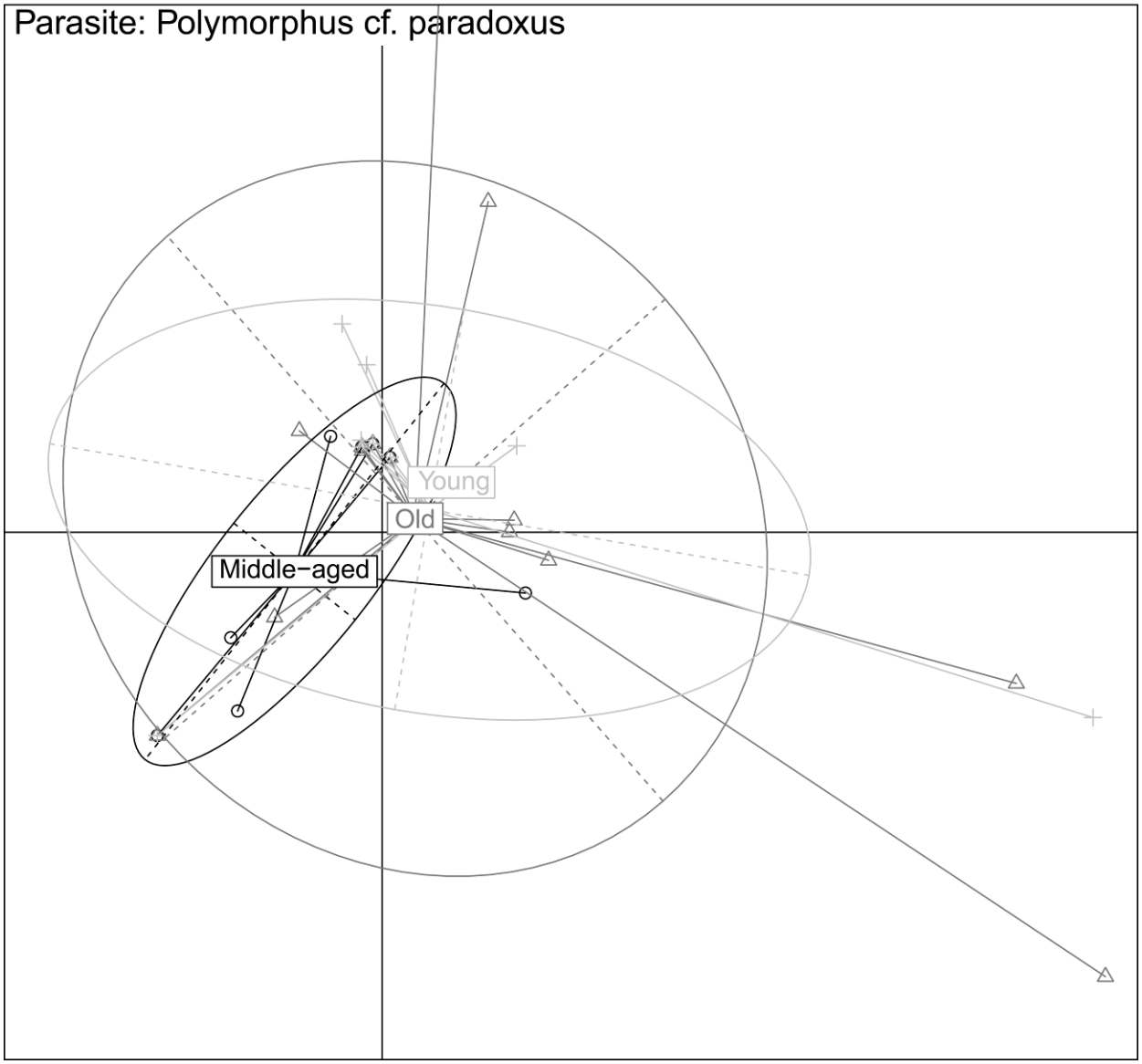

Figure 4.5 Principal coordinate analysis (PCoA) plots showing mtDNA genetic variation of parasite (*P.* cf. *paradoxus*) categorized by age groups of water bodies [(young (3–7 years; plus symbol), middle-aged (16–26 years; circle symbol) and old (32–53 years; triangle symbol)]. Each dot stands for a specimen and some dots overlay each other due to the small genetic distance between these specimens). Overlap between different age groups suggested the genetic similarity among these groups.

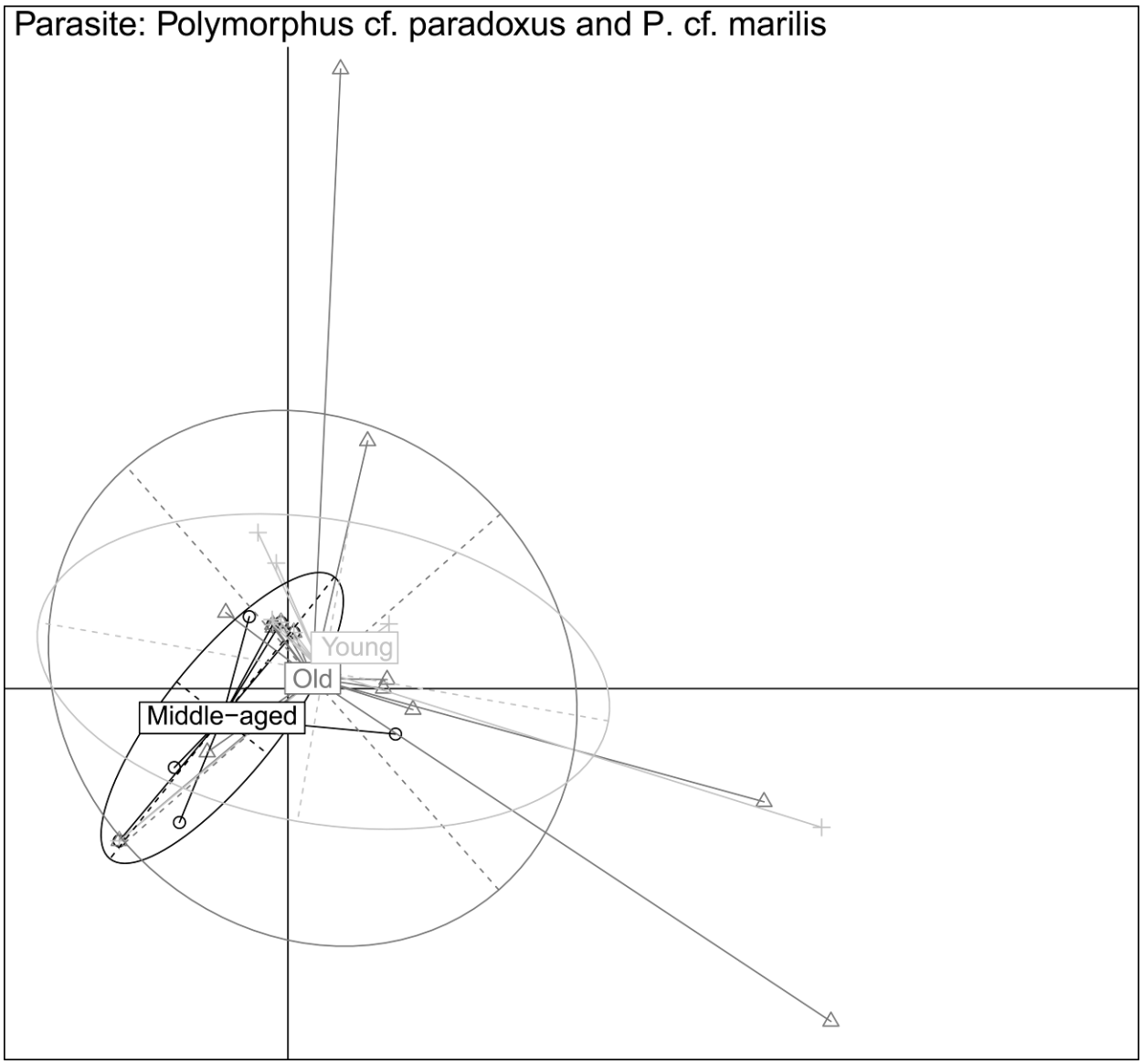

Figure 4.6 Principal coordinate analysis (PCoA) plots showing mtDNA genetic variation of parasite (*P.* cf. *paradoxus* + *P.* cf. *marilis*) categorized by age groups of water bodies [(young (3–7 years), middle-aged (16–26 years) and old (32–53 years)]. Each dot stands for a specimen and some dots overlay each other due to the small genetic distance between these specimens). Overlap between different age groups suggested the genetic similarity among these groups.

Chapter 5 mtDNA genetic structure of a widespread freshwater amphipod *Gammarus lacustris* in relation to waterfowl migration flyways in Holarctic regions

5.1. Introduction

Dispersal is one of the most important processes linking ecology and evolution (Bohonak and Jenkins 2003). It is defined as any movement of individuals between geographical locations, often resulting in gene exchange between populations, modification of phylogeographic structure of species, and influencing dynamics of metacommunities at large spatial scales (Clobert et al. 2012). Dispersal plays a major role in linking geographically separated populations, and is also crucial to successful colonization of new areas. In a newly available area, strong dispersal ability of a colonizing species can reduce founder effects during its early establishment in that area (Roman and Darling 2007). Non-natives with high dispersal ability have high invasive potential and may cause biodiversity loss in the invaded areas (Green and Figuerola 2005). Because of this, examination of how dispersal affects populations can be helpful in understanding the mechanism behind spatial distribution and the spread of species, and could be relevant for evaluation and management of invasions of alien species.

The dispersal of some animal species relies on walking, swimming or flying capacity of the individuals themselves, while other species can spread passively from one location to another via dispersal vectors (Figuerola and Green 2002). One important but often overlooked dispersal vectors is migratory birds (Viana et al. 2016). They have a great capacity for long-distance flight and can disperse many plants and animals that lack significant dispersal ability of their own (Darwin 1859, Coughlan et al. 2017). These passive dispersers move from one location to another by clinging to a bird's feather or feet, or passing through its guts and remaining viable for colonizing new areas (Ridley 1930, Darwin 1859, Boag 1986, Figuerola and Green 2002). Although bird-mediated dispersal has been increasingly recognized as an important ecological driver for the spread of aquatic animals and plants, including invasive species (Reynolds et al. 2015), the role of birds in dispersing species has been assessed at or below continental scales,

and studies assessing its role at both intra- and inter-continental scales is relatively rare (except Mader et al. 1998; Muñoz et al. 2013; Lewis et al. 2014).

Freshwater organisms have particular difficulty traversing land to colonize new water bodies, but waterfowl-mediated transport can both facilitate colonization and reduce genetic differentiation of populations of the transported aquatic organisms (Figuerola et al. 2005). Thus, heterogeneity of the genetic structure of populations of transported species along waterfowl migration routes is expected to be lower than the genetic variation between flyways (=migration routes). Likewise, the population structure of the waterfowl-transported species is expected to mirror the migration flyways of waterfowl. Consistent with these predictions, many studies have found correlations between waterfowl migratory routes and the population genetic structure of transported freshwater invertebrates (Taylor et al. 1998, Freeland et al. 2000, Figuerola et al. 2005, Muñoz et al. 2013).

Waterfowl migration paths might be influenced by geographical characteristics, which may further affect population structure of waterfowl-mediated dispersers. For example, mountain ranges can interrupt waterfowl migrations (Williams et al. 2001), thereby reducing genetic exchanges between the isolated populations of the transported species (Thomas et al. 1998, von Oheimb et al. 2013). In addition to reduced gene flow between populations, transported animal populations may adapt to local environments and evolve, leading to distinct populations or even new species on opposite sides of mountain ranges (von Oheimb et al. 2011, Hou et al. 2014). Isolation-by-distance (IBD) patterns might also be apparent in population structure of waterfowl-transported organisms. This is especially true for the scenario where the chance of surviving passive dispersal decreases as geographical distance increases, e.g., if propagules on feathers are more likely to desiccate or be displaced the longer they are airborne. Under this scenario, populations of the passive dispersers that are distant from each other are expected to be more genetically different than nearby populations, thereby generating a significant correlation between genetic distance of the dispersers' population and geographical distance.

To test these ideas, an ideal candidate would be a widespread species, able to disperse with waterfowl for a long distance, and that has a well-understood biology. The widespread freshwater amphipod species, *Gammarus lacustris* Sars (Gammaridae), meets these criteria. *Gammarus lacustris* is the most widespread freshwater amphipod species in the Holarctic

region, including North America, Europe and central Asia, and is common in permanent standing water bodies of all sizes as well as slowly flowing water (Väinölä et al. 2008). The precise geographical boundaries of its range are unknown. Its current Holarctic distribution is presumably the result of post-glacial expansion from Europe via hitchhiking on waterfowl (Gherardi 2007, Väinölä et al. 2008). *Gammarus lacustris* can use its claw-like hooks on pereopods 3–7 to cling onto fur of aquatic mammals or plumage of waterfowl and can hold on to transport hosts for up to 2 hours out of water (Segerstråle 1953, Gherardi 2007). The probability of clinging to waterfowl can be increased for *G. lacustris* individuals that are infected with endoparasite thorny-headed worms (Acanthocephala). Some acanthocephalans can manipulate the infected amphipods to display positive phototaxis, and also increase their propensity to cling to moving objects (Helluy and Holmes 1990). By this means, *G. lacustris* can move between isolated water bodies, which may act as stepping-stones for traveling long distances and reaching previously uncolonized bodies of water.

Despite its well-known biology (Moore 1977, Helluy and Holmes 1990, Wilhelm and Schindler 2000), only two European studies have addressed how *G. lacustris* population structure is related to waterfowl migration flyways. One of them found relatively lower mitochondrial DNA genetic variation for *G. lacustris* compared to other amphipod species in the Alps (Meyran and Taberlet 1998). The other used multiple allozyme loci and found that the spatial pattern of population differentiation of *G. lacustris* in Northern Europe was consistent with post-glaciation expansion, and could be related to waterfowl migration patterns and spatial connectivity (Vainio and Väinölä 2003). However, to my knowledge, no study has been undertaken to test explicitly the effects of waterfowl flyways and isolation by geographical barriers and distance on the genetic structure of *G. lacustris* across both Palearctic and Nearctic regions.

Herein, I test whether population genetic structure of *G. lacustris* correlates with waterfowl migration flyways. Because previous studies found that waterfowl-transported aquatic invertebrates probably are not free to disperse among continents (Boileau et al. 1992, Gómez et al. 2007, Muñoz et al. 2013), I predicted that waterfowl-mediated dispersal of *G. lacustris* is distance-dependent and therefore IBD is expected to affect *G. lacustris* population structure at inter- and to a lesser extent intra-continental scales. In addition, I expected that the population structure of *G. lacustris* in North America should also be influenced by isolation by the Rocky

Mountains since they form a physical barrier that may make the flyway to the west of the mountains (the Pacific American Flyway) more stringently separated from the flyways to the east than are flyways further east in North America, irrespective of geographical distances between flyways. The Rocky Mountains are not expected to form a complete barrier to the movement of *G. lacustris* and mountains given that waterbird species fly across them regularly (e.g., Houston 1977, Eichhorst 1992). To test these predictions, I used the 710 bp 'barcoding' region of the mitochondrial DNA COI marker which has been previously used to assess population structure of *G. lacustris* and to test the role of waterfowl in influencing population structure of the transported freshwater invertebrates (Figuerola et al. 2005, Hou et al. 2007).

5.2. Material and methods

5.2.1. Specimen sampling and laboratory protocols

I collected 104 *G. lacustris* specimens from 21 water bodies in vicinity of Edmonton, Alberta, Canada (53.5444° N, 113.4909° W) and one water body from Winnipeg, Manitoba, Canada (49.82055° N, 97.22501° W). All specimens were preserved in absolute ethanol in a -20 °C freezer for DNA extraction.

I extracted whole genomic DNA from individual *G. lacustris* using DNeasy 96 Blood and Tissue Kit (QIAGEN). I used the COI universal primers LCO1490 and HCO2198; (Folmer et al. 1994) to amplify and sequence 710 bp fragments of mitochondrial Cytochrome *c* Oxidase subunit I. Polymerase chain reactions (PCR) were performed in a total of 25 µl containing 2.5 µl 10×PCR reaction buffer, 4.0 µl dNTPs (2 mM), 1.0 µl of each primer (10 µM), 1.0 µl DNA template, 1.0 µl $MgCl_2$ (50 mM), 0.5 µl homemade *Taq* DNA polymerase and 14 µl dH_2O. PCR conditions were as follows: 60 s at 94 °C, 5 cycles of 30 s at 94 °C, 90 s at 45 °C, 60 s at 72 °C, then 35 cycles of 30 s at 94 °C, 90 s at 51 °C, 60 s at 72 C, and 300 s at 72 °C (Witt et al. 2006). PCR products were purified using ExoSAP (New England Biolabs) and then sent to Molecular Biology Service Unit in the Department of Biological Sciences, University of Alberta for Sanger DNA sequencing. Sequence chromatograms were viewed and checked for accuracy in FinchTV (Geospiza Inc.).

5.2.2. Analysis of population genetic structure

I retrieved 198 COI sequences from the Barcode of Life Data System (http://www.boldsystems.org/). Together with the 104 sequences from Edmonton and Winnipeg, I had a total of 302 sequences from 26 localities (including cities and territories; see Table S5 1) across North America, Europe and Asia (Figure 5.1; Appendix A: Table S5 1). All sequences had <50% ambiguous bases, sequence lengths (excluding gaps) > 403 bp, and relatively precise geographic locality data. Six amphipod species closely genetically related to the genus *Gammarus* and that were used previously in studies of *Gammarus* phylogeny were selected as outgroup taxa including two species from *Dikerogammarus* (Gammaridae), two from *Jesogammarus* (Anisogammaridae), one from *Crangonyx* (Crangonyctidae) and one from *Platorchestia* (Talitridae) (Hou et al. 2007). Their sequences were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). I aligned the 302 *G. lacustris* sequences plus the six outgroup sequences in ClustalX 2.0 with default parameters (gap opening: 10; gap extension: 0.2; delay divergent sequences: 30%; DNA transition weight: 0.5) and then checked visually and trimmed to the same length (308 bp) in DNAMAN 7.0.

I analyzed the sequence alignment using Bayesian phylogenetic methods. Bayesian analysis based on COI genetic marker in my study was used to explore the population structure of *G. lacustris* rather than to construct its precise phylogeny. Previous studies supported the usefulness of applying COI gene with phylogenetic analysis to exploring population structure/cryptic diversity (Rius et al. 2008, Pilgrim and Darling 2010, Heger et al. 2013). Prior to constructing the tree, I used PartitionFinder 2 to select the model of nucleotide evolution that best fit the sequence data based on the corrected Akaike information criterion (AICc) values (Lanfear et al. 2017). Bayesian phylogenetic analysis was conducted in MRBAYES with default priors, random starting trees and GTR+G+I evolutionary model. Bayesian analysis included two runs, each of which consisted of one cold and four heated Markov chains, with sampling frequency of every 500 generations for 14 million generations. The first 25% samples were discarded as burn-in, and the remaining trees were summarized to construct the consensus tree and to estimate posterior probabilities for all branches using the 50% majority rule. Run convergence was evaluated in TRACER (Rambaut and Drummond 2007). The resultant consensus tree was visualized in FigTree (version 1.4.3; http://tree.bio.ed.ac.uk/software/figtree/). The parsimony

haplotype network was constructed and illustrated using the haplotypes package in R environment (https://www.r–project.org/).

In addition to Bayesian analysis, I assessed population structure of *G. lacustris* using the clustering analysis of STRUCTURE (Pritchard et al 2010). The number of potential genetic clusters (K) was set to range from one to 26 (= number of localities where *G. lacustris* were collected and COI sequences obtained), with 10 independent runs of 100 000 iterations during "burn-in" period and 100 000 Monte Carlo Markov Chain steps for each of the 26 Ks using LOCPRIOR = 1 to increase the power of detecting weak genetic differentiation. STRUCTURE provided the log likelihood (Ln(P(D))) for each K and the K with highest Ln(P(D)) was retained as the optimal K (Pritchard et al 2010). Estimation of optimal K using Pritchard et al (2010) is sensitive to the existence of IBD patterns in genetic data. Because of this, the optimal K was also identified based on the second order rate of change of likelihood ΔK using the method of Evanno et al. (2005) to avoid any potential bias. I used CLUMPAK 1.1 to cluster and average runs with default settings, and to display STRUCTURE results graphically (Kopelman et al. 2015). I also tested whether IBD patterns confound the results of clustering analysis in STRUCTURE (Meirmans 2012), by using a partial Mantel test in which genetic distance was retained as a dependent matrix and a clustering similarity matrix as an explanatory matrix containing binary code of whether each pair of sequences is in the same genetic cluster (= 0) or not (= 1) while partialing out geographical distance (Drummond et al. 2007). The partial Mantel test was conducted based on all specimens that were assigned with high CLUMPAK-averaged Q (Q>0.7).

5.2.3. Assignment of specimens to waterfowl flyways

I assigned *G. lacustris* specimens to waterfowl flyways according to Boere and Stroud (2006) and Choi et al. (2012). The North American flyways are the Pacific American Flyway, Mississippi American Flyway and Atlantic American Flyway. Relevant European flyways are the East Atlantic Flyway, Black Sea/Mediterranean Flyway. The Asian flyways are West Asian Flyway, Central Asian Flyway and East Asian Flyway. West Pacific Flyway covers eaten Asian and Alaska. Details can be seen in Table S5 2.

5.2.4. Statistical analysis

All statistical analyses were conducted in the R environment (https://www.r–project.org/). Neutrality tests based on Tajima's D statistic (Tajima 1989) were conducted across the entire Holarctic region and for North America, Europe and Asia separately using the R function tajima.test in pegas package. To test for isolation-by-distance (IBD) patterns and examine the contributions of waterfowl flyways and isolation by the Rocky Mountains on the population genetic structure of *G. lacustris*, I constructed a mtDNA genetic distance matrix of the aligned 302 sequences (excluding six outgroup taxa) using the Jukes–Cantor 69 model (JC 69) of the R function dist.dna in the ape package (Jukes and Cantor 1969). The Jukes–Cantor 69 model was selected because it had better fit (and lower AICc) for my *G. lacustris* sequence alignments (without outgroups) than the other models available in dis.dna. The model test was conducted using PartitionFinder 2 (Lanfear et al. 2017). I also tried the commonly-used Kimura 2-Parameter (K2P) model to construct the genetic distance matrix (Kimura 1980). The general patterns were consistent across JC 69 and K2P models.

I conducted principal coordinate analysis (PCoA) on the mtDNA genetic distance using the Cailliez correction to account for negative eigenvectors (Cailliez 1983). The eigenvectors derived from PCoA were retained as response variables in both distance-based redundancy analysis (db-RDA) and partitioning analysis (PCoA and RDA are commonly used in studies of population structure of invertebrates, e.g., Muñoz et al. 2013, Keyse et al. 2018, Matthews et al. 2018). To determine the spatial population genetic structure, I created spatial variables using principal coordinates of neighbour matrices (PCNM) with default setting in vegan package (Borcard and Legendre 2002, Griffith and Peres-Neto 2006). Only the eigenvectors with positive eigenvalues were retained for further analyses. Waterfowl flyways, isolation by the Rocky Mountain and spatial distance (spatial variables [eigenvectors]) were included as explanatory variables in both db-RDA and partitioning analysis to evaluate their relative contributions to the *G. lacustris* population structure. I did overall db-RDA analysis and partitioning analysis at the Holarctic scale and did individual RDA analysis in North America, Europe and Asia, respectively. Mantel tests were used to detect IBD patterns in the whole Holarctic, and in North America, Europe and Asia separately.

5.3. Results

I found 64 polymorph5.5ic loci (18.1%) and 58 haplotypes in my mtDNA alignment of the entire Holarctic dataset (excluding outgroups). Neutrality tests were not statistically significant either across the whole Holarctic region (Tajima's D= -1.78, P= 0.07) or within Europe (Tajima's D= -1.45, P= 0.15). However, the test showed significantly negative Tajima's D values in North America (Tajima's D= -2.33, P= 0.02) and Asia (Tajima's D= -2.12, P= 0.03). The significant negative Tajima's D values suggest an excess of low frequency haplotypes in my sequence alignment. The AMOVA showed that most genetic variation was among continents (66.4%), followed by among countries within continents (17.0%) and among localities within countries (7.2%) (Table 5.1). Variation among continents and among localities was statistically significant, while that among countries was not (Table 5.1). When I grouped *G. lacustris* sequences according to flyways, flyways was statistically significant and explained more genetic variation among flyways (81.4%) than within flyways (18.6%) across the whole Holarctic region (Table 5.1). Population genetic variation of North American *G. lacustris* was explained by between flyway (49.4%) and within flyway (50.6%) differences (Table 5.1). European and Asian *G. lacustris* had more genetic variation within flyways (53.4% and 83.0%) than between (46.6% and 17.3%) (Table 5.1).

The Bayesian phylogenetic tree and statistical parsimony haplotype network showed clustering partly reflective of the broad geographic regions of North America, Europe and Asia (Figure 5.2 and Figure 5.3). The Bayesian phylogenetic tree showed that one European lineage (Finland/Norway) is more similar to Asian *G. lacustris* than to the other two European groups, one of which falls within North American *G. lacustris* (Belarus/Ukraine) and the other is near the base of the tree (Slovenia) (Figure 5.2). Within the North American lineage, the Bayesian tree showed a genetic differentiation gradient from the Pacific American Flyway to the Mississippi American Flyway, and to a greater extent to the Atlantic American Flyway (Figure 5.2).

These patterns were corroborated by the PCoA plot (Figure 5.4). I excluded the single specimen from Bled, Slovenia (GenBank accession number: GBCMA4442-13) for the PCoA visualization because it is very different genetically from all other specimens and its inclusion made the other specimens cluster so closely that I were not able to visually assess the patterns in the plot. After

removing the outlier, my PCoA results showed that Asian *G. lacustris* are different from European and North American *G. lacustris*. European *G. lacustris* are genetically most related to those from the West Coast of North America, followed by Central North American *G. lacustris*. Most specimens from Churchill, Manitoba are genetically different from European and other North American *G. lacustris* (with the exception of some specimens from the Edmonton area that are genetically similar to those from Churchill). Only one specimen from Churchill falls within the Central North American group.

The STRUCTURE analysis showed that Ln(P(D)) peaked at K of 3, while the Evanno method indicated that the optimal K is 5. Because of this inconsistency of optimal K values, I plotted the CLUMPAK-averaged admixture plots for both K = 3 and 5. Both of the admixture plots (K = 3 and 5) showed that the clusters are roughly correlated with regions from Asia, Europe and North America, with apparent genetic admixture between Europe and Asia, between Atlantic American Flyway (Churchill only) and Europe, and between Pacific American Flyway and East Asia (Figure 5.5). Within the North American group, clustering analysis detected some genetic differentiation between the Atlantic American Flyway and both the Mississippi American Flyway and the Pacific American Flyway, although obvious genetic admixture was found among them (Figure 5.5). The partial Mantel test showed genetic clustering was significant after controlling IBD at both K=3 ($R=0.22$, $p<0.01$) and K=5 ($R=0.61$, $p<0.01$).

RDA results are shown in Table 5.2. At the intercontinental scale, the overall RDA model showed that waterfowl flyways explained a greater proportion of genetic variation (30.9%) than geographical distance (PCNM spatial variables) (29.0%) and isolation by the Rocky Mountains (1.7%). Waterfowl flyways and spatial variables interacted to account for more of the genetic variation (27%) than the interaction effect of waterfowl flyways and isolation by the Rocky Mountains (1.4%). Spatial variables and isolation by the Rocky Mountains had quite low interaction influence on the genetic variation (0.4%). Similarly, the genetic variation of North American *G. lacustris* was explained more by waterfowl flyways (13.5%) than by spatial variables (11.9%) and isolation by the Rocky Mountains (7.4%). The interaction of waterfowl flyways and spatial variables accounted for more genetic variance (10.1%) than the interactions of isolation by the Rocky Mountains with either waterfowl flyways (6.7%) or spatial variables (5.8%). In contrast, more genetic variation among European and Asian *G. lacustris* were

attributed to spatial variables (88.5% and 7.2%) compared to waterfowl flyways (37.3% and 4.7%). The pure influence of spatial variables explained a greater proportion of genetic variance of *G. lacustris* from Europe (88.5% - 32.2%=56.3%) and Asia (7.2% - –1.5%=5.7%) than the pure effect of waterfowl flyways (Europe: 37.3% - 32.2%=5.1% and Asia: 4.7% - 1.5%=3.2%) or interactions of spatial variables and waterfowl flyways (Europe: 32.2% and Asia: 1.5%). Mantel tests on relationships between genetic and geographic distance showed that there was significant IBD across all study regions (r= 0.81; *p*= 0.01; Figure 5.6 top left) and within each continent (North America: r= 0.43, *p*= 0.01; Europe: r= 0.88, *p*= 0.01; Asia: r= 0.41, *p*= 0.01; Figure 5.6).

5.4. Discussion

The Bayesian tree, PCoA plot, haplotype analysis and AMOVA reveal genetic differences in *G. lacustris* population structure among North American, European and Asian specimens. Similarly, STRUCTURE results detected these genetic differences at the intercontinental scale, although there is obvious genetic admixture between and within North American, European and Asian regions. These results suggest that *G. lacustris* can traverse between waterfowl flyways. This is especially true for the overlapping flyways between continents (Pacific American Flyway and East Asian Flyway; the Asian flyways and Black Sea/Mediterranean Flyway) and within continents (Pacific American Flyway and Mississippi American Flyway; Mississippi American Flyway and Atlantic American Flyway; Asian flyways). The partial Mantel tests further confirmed that STRUCTURE results are not confounded by IBD pattern. Corroborated by RDA results, the findings show that population structure of *G. lacustris* correlates with waterfowl flyways, suggesting that the historical flyways promoted the past spread of *G. lacustris*. Within North America, together with isolation by the Rocky Mountains, waterfowl flyways appear to have shaped the separation of *G. lacustris* from the West Coast from those of Central North America and Churchill. This division is not complete, supporting that waterfowl occasionally move across the migration flyways (Bellrose 1980), possibly carrying the amphipods during movement. Interestingly, Edmonton *G. lacustris* partially overlap with those from Churchill, Manitoba and also with the other North American *G. lacustris* (Figure 5.4). *Gammarus lacustris* from Churchill and the West Coast of North America are clearly separated genetically, as one would predict by waterfowl flyways, except for one Churchill specimen which falls in the group

from the West Coast of North America group (Figure 5.4). North American *G. lacustris* are overall genetically more similar to European *G. lacustris* than to Asian *G. lacustris* (Figure 5.2, Figure 5.3 and Figure 5.4). This is particularly true for the *G. lacustris* from the Northern Pacific coast (e.g., Alaska), which are more closely related to European *G. lacustris* than to other North American specimens. This indicates that waterfowl may transport *G. lacustris* between Europe and Alaska frequently, as (Alerstam et al. 2007). One specimen from Bled, Slovenia is quite different from other *G. lacustris* and is basal in the Bayesian phylogenetic tree. This suggests that either region might be near the origin of *G. lacustris*, or that there was some error in sequencing of this specimen. To test this rigorously, more specimens from the region are needed.

My findings show that waterfowl flyways are significantly correlated with population mtDNA structure of *G. lacustris* in North America, Europe and Asia. For *G. lacustris* populations from North America and the entire Holarctic, the correlation of population structure with waterfowl flyways is higher than that with pure geographical distance. These results support the roles of waterfowl flyways in shaping the spatial population structure of *G. lacustris* within North America and between North America and Europe plus Asia. Within Europe and Asia, waterfowl flyways explain a lower proportion of genetic variation than spatial variables. Most spatial genetic variation of European and Asian *G. lacustris* occurs within flyways (Table 5.1). This small genetic variation among flyways might be related to the limited number of flyways covering the European specimens (two flyways) and Asia (three flyways) versus North America (four flyways). These results suggest that other factors influenced *G. lacustris* population structure within flyways in Europe and Asia. Because the genetic variation in COI marker could be attributed to historical instead of ongoing events (Figuerola et al. 2005), the correlation between waterfowl flyways and mtDNA genetic structure of *G. lacustris* probably results from past waterfowl-mediated expansion and colonization of new habitats that is reinforced by gene exchange between extant populations. Compared to waterfowl flyways, the Rocky Mountains explain a relatively lower proportion of genetic variation in North America (7.4%) and overall RDAs (1.7%), supporting my hypothesis that these mountains do not form a complete barrier to waterfowl-mediated dispersal of *G. lacustris*.

The statistically significant IBD patterns within and across North America, Europe and Asia indicate that the *G. lacustris* populations studied are not fully connected by high gene flow

within each of these regions and probably have even lower gene flow across all regions. This could be especially true for the *G. lacustris* populations from different continents along different non-overlapping migration routes, which probably reduces the chance of the gene exchange between them. This also suggests that *G. lacustris* past colonization events were distance-dependent and sequential, as *G. lacustris* populations were likely to first colonize nearby newly available habitats with high gene exchange between nearby populations.

My results are consistent with some previous studies focusing on other freshwater crustacean taxa. For instance, Figuerola et al. (2005) provided evidence for the role of waterfowl in mediating the ongoing and past gene flow among populations of three cladoceran species from isolated water bodies across North America. Similarly, Muñoz et al. (2013) found that populations of the fairy shrimp *Artemia franciscana* Kellogg in North, Central and South America were genetically structured by bird migratory flyways. In contrast, not all previous studies supported the congruency between waterfowl-mediated dispersal and population structure of the transported invertebrates. Instead, some studies found high dispersal rates but also high genetic differentiation between invertebrate populations (De Meester et al. 2002). For example, Boileau (1992) found that passively dispersing freshwater invertebrates (such as *Cyprinotus glaucus* Furtos [Ostracoda: Cyprididae]) showed high genetic differentiations among populations, although there are evidence of dispersal of *Cyprinotus* spp. by waterfowl (Proctor 1964). This paradox can be explained by the "Monopolization Hypothesis" that the early arriving genotypes can outcompete the late-arriving genotypes and dominate a newly available area by monopolizing resources (priority effect) (De Meester et al. 2002, De Meester et al. 2016). These processes can increase genetic divergence between populations and could render them resistant to genetic homogenization by gene flow (Boileau et al. 1992). However, this paradox between high dispersal and high genetic differentiation does not eliminate roles for waterfowl-mediated dispersal in influencing the population genetic structure of transported animals. Instead, it suggests that alternative factors (e.g., local adaption and rapid population growth rate) are also important for genetically structuring the populations of waterfowl-transported animals.

The unexplained variation in RDA likely results from a failure to include other important variables affecting *G. lacustris* population genetic structure, such as water quality and presence

of particular predators differentially affecting different genotypes. Similarly, adaptation to local environment might intensify the differentiation of *G. lacustris* population structure. Also, *G. lacustris* might be able to colonize newly available areas through running water (Meyran and Taberlet 1998), even if the connections are only temporary. It is also possible that *G. lacustris* population structure may be subject to the priority effect (De Meester et al. 2002). Finally, in North America, dispersal of *G. lacustris* may have been promoted by the formation of connected water bodies at the margin of retreating glaciers, given that its distribution extends to central North America. This post-glacial expansion may be related to North American *G. lacustris* population structure at a within-continent spatial scale.

Despite the differentiation of *G. lacustris* population structure in North America, its population structure is not as divergent as *Hyalella* '*azteca'*, a common freshwater amphipod lineage in North America with high cryptic species diversity (Witt et al. 2006). One potential reason for this phenomenon could be related to *G. lacustris* and *H.* 'azteca' hosting different acanthocephalan species. *Hyalella* '*azteca'* are commonly infected by *Polymorphus contortus* or *Pseudocorynosoma constrictum* (Van Cleave, 1918; synonym: *Corynosoma constrictum*; Podesta et al. 1970). *P. contortus*- and *P. constrictum*-infected *H.* '*azteca'* do not show strong positive phototaxis (Bethel and Holmes 1977). Thus, they are less likely to be transported for a long distance by water birds, compared to *P. paradoxus*-infected *G. lacustris* which are positively phototactic and tend to cling to the feather of water birds (Bethel and Holmes 1973, Helluy and Holmes 1990). The relatively high common dispersal of *G. lacustris* by water birds may reduce genetic differentiation of *G. lacustris* among localities in North America, while *H.* '*azteca*' may be less frequently transported by water birds, resulting in high genetic divergence among *H.* '*azteca*' populations.

Overall, my study found that population genetic structure of *G. lacustris* is highly correlated with waterfowl migration flyways within and across North America, Europe and Asia. Significant relationships between mtDNA genetic distance and geographical distance suggest historical waterfowl-mediated stepwise colonization of *G. lacustris* within the three continents. Isolation by the Rocky Mountains explained only a small proportion of total genetic variance within North America. My results corroborate many previous studies on the role of waterfowl in transporting freshwater animals over long distances and provide correlational evidence for the

role of waterfowl migration in shaping population structure of transported animals at inter- and intra-continental scales. These findings could improve understanding of mechanisms of the dispersal of aquatic organisms across a broad range of spatial scales and also could be relevant for predicting the spread of invasive aquatic species, including gammarid amphipods in parts of Europe (Jażdżewski 1980).

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Table 5.1 Analysis of molecular variance (AMOVA) for *Gammarus lacustris* populations based on five geographic groupings. Sum of squares (SS), mean square (MS), degree of freedom (df), and percentage of total variance (%V) are given for each grouping. Significant level was tested by 1000 permutations and indicated as a subscript: *=p<0.05 and ^{NS}=p>0.05.

	Source of variation	SS	MS	df	%V
1	Among continents	2.42×10^{-2}	1.21×10^{-2}	2	66.4^{*}
	Among countries within continents	6.21×10^{-3}	7.77×10^{-4}	8	17.0^{NS}
	Among localities within countries	2.62×10^{-3}	1.75×10^{-4}	15	7.2^{*}
	Within localities	3.45×10^{-3}	1.25×10^{-5}	276	9.5
	Total	3.65×10^{-2}	1.21×10^{-4}	301	
2	Among flyways across whole Holarctic region	2.97×10^{-2}	2.97×10^{-3}	10	81.4^{*}
	Within flyways	6.78×10^{-3}	2.33×10^{-5}	291	18.6
	Total	3.65×10^{-2}	1.21×10^{-4}	301	
3	Among flyways across North America	2.68×10^{-3}	5.36×10^{-4}	5	49.4^{*}
	Within flyways	2.74×10^{-3}	1.63×10^{-5}	236	50.6
	Total	5.42×10^{-3}	2.25×10^{-5}	241	
4	Among flyways across Europe	3.05×10^{-3}	3.05×10^{-3}	1	46.6^{*}
	Within flyways	3.49×10^{-3}	3.88×10^{-4}	9	53.4
	Total	6.54×10^{-3}	6.54×10^{-4}	10	
5	Among flyways across Asia	3.15×10^{-4}	1.57×10^{-4}	2	17.3^{*}
	Within flyways	1.51×10^{-3}	3.27×10^{-5}	46	83.0
	Total	1.82×10^{-3}	3.79×10^{-5}	48	

Table 5.2 Relative contributions of waterfowl flyways, spatial variables (PCNM eigenvectors) and isolation by the Rocky Mountains using distance-based redundancy analysis (db-RDA) and partitioning analysis. The proportion of variance explained by each variable based on adjusted R^2 was included.

Source of variation (%)	Continental scale	North America	Europe	Asia
Waterfowl flyways (WF)	30.9	13.5	37.3	4.7
Spatial variables (SV)	29.0	11.9	88.5	7.2
Isolation by Rocky Mountains (IRM)	1.7	7.4	–	–
WF–SV interaction	27	10.1	32.2	1.5
IRM –SV interaction	0.4	5.8	–	–
WF– IRM interaction	1.4	6.7	–	–
WF–SV– IRM interaction	0.2	5.3	–	–

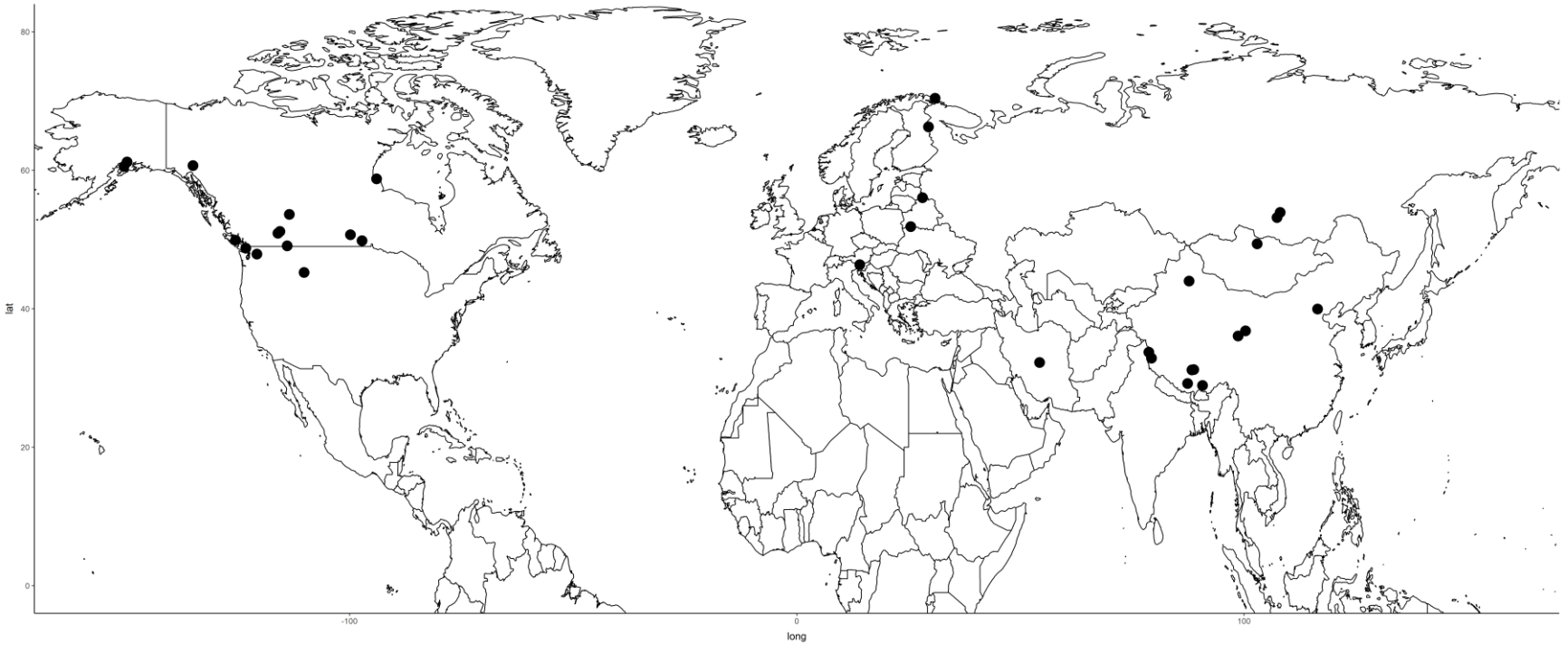
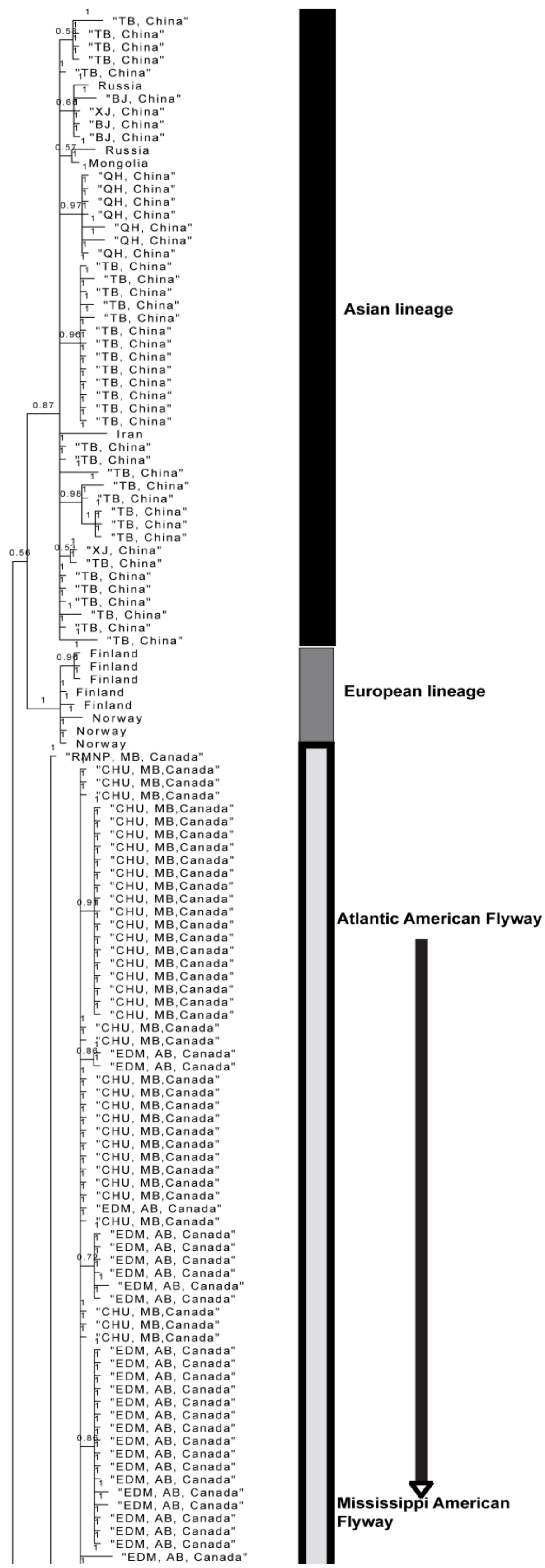
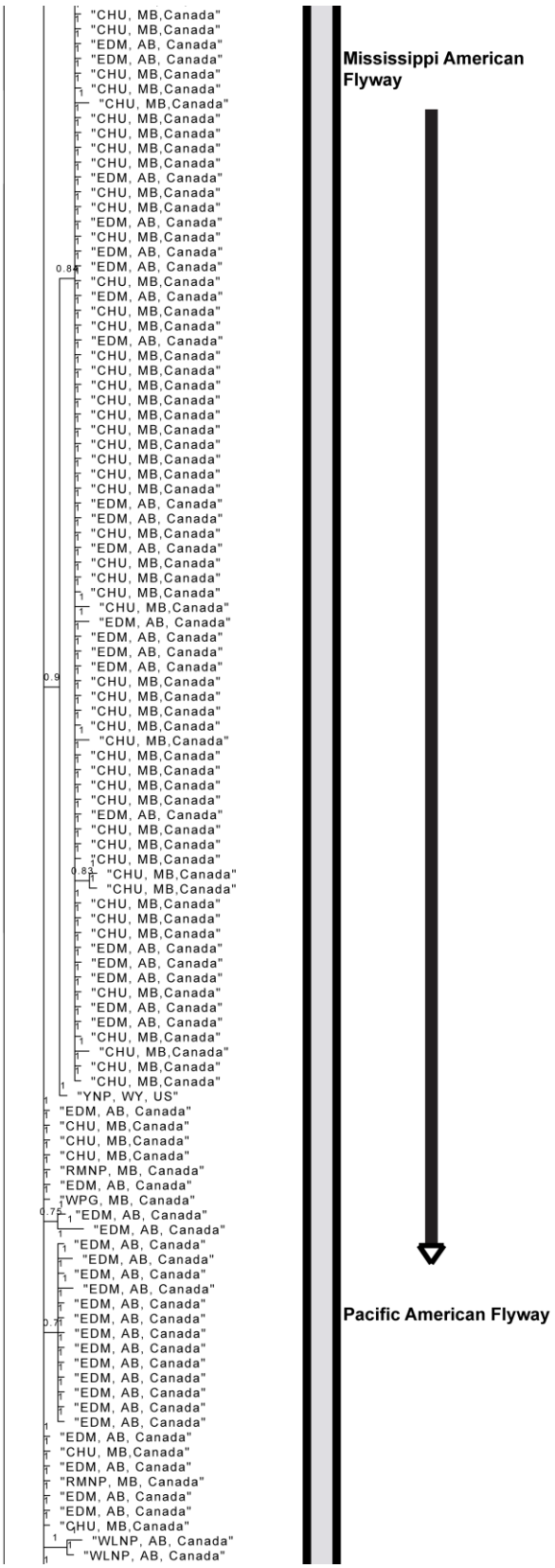


Figure 5.1 Map of locality of *Gammarus lacustris* used in my study. Dots represent sampling locations.


Asian lineage
European lineage
Atlantic American Flyway
Mississippi American Flyway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"RMNP, MB, Canada"
"EDM, AB, Canada"
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"WLNP, AB, Canada"
"WLNP, AB, Canada"
Mississippi American Flyway
Pacific American Flyway

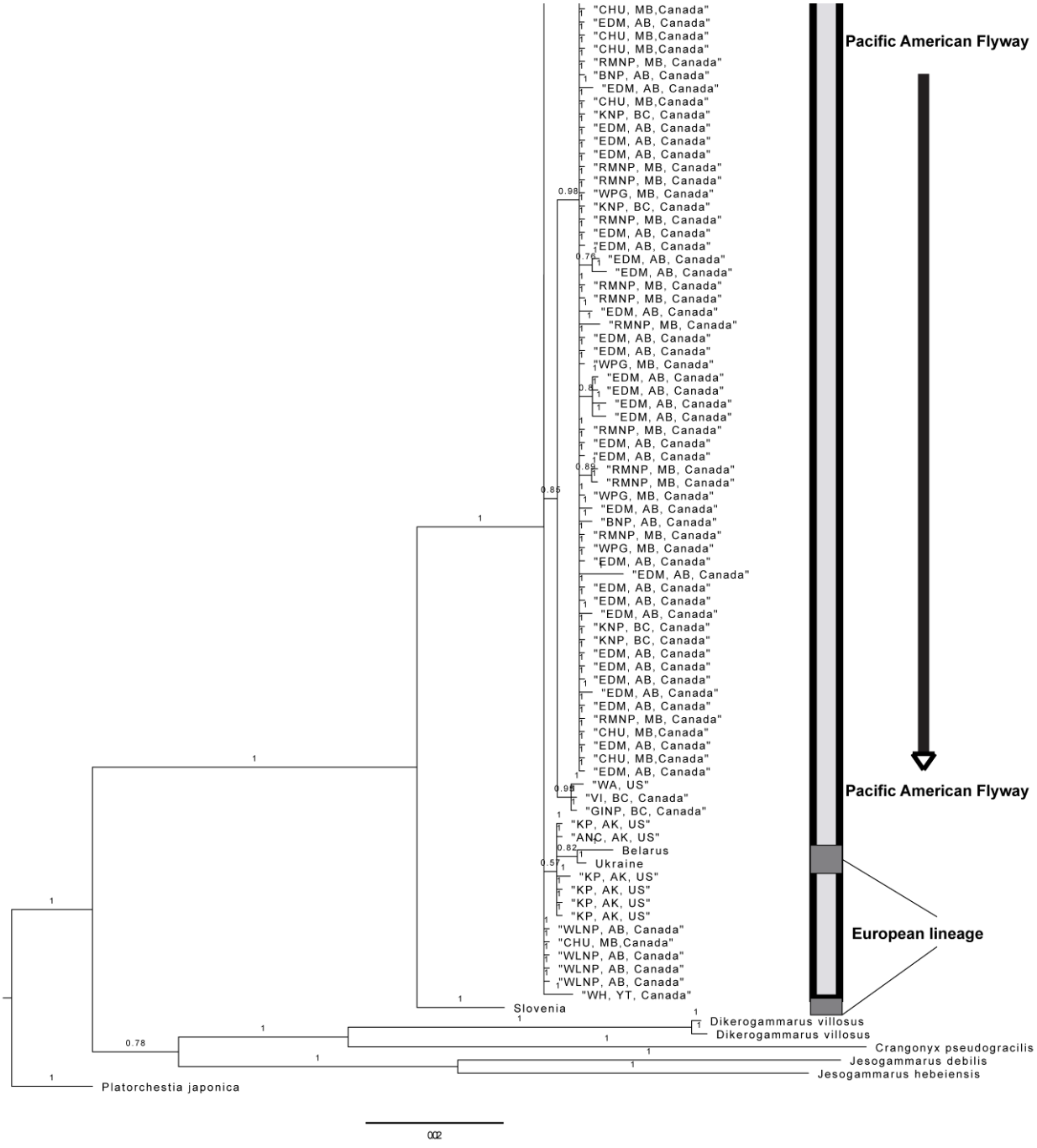

Figure 5.2 Consensus Bayesian phylogenetic tree of *Gammarus lacustris* constructed based on COI gene using 50% majority rule. Numbers associated with the lines are Bayesian posterior probability. Descriptions of abbreviations of location names are in Table S5 1.

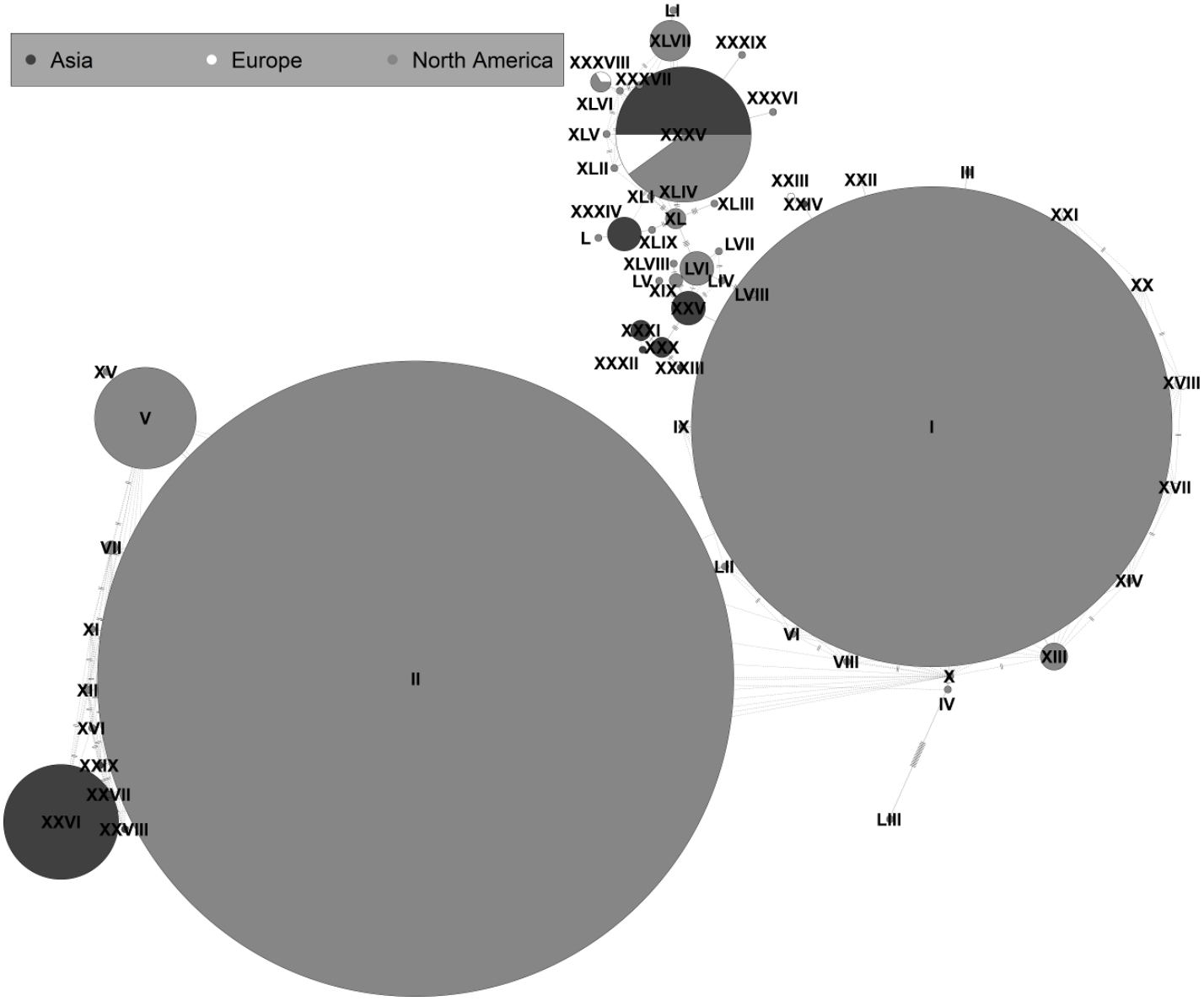

Figure 5.3 Haplotype network of *Gammarus lacustris* across the entire Holarctic region including North America, Europe and Asia.

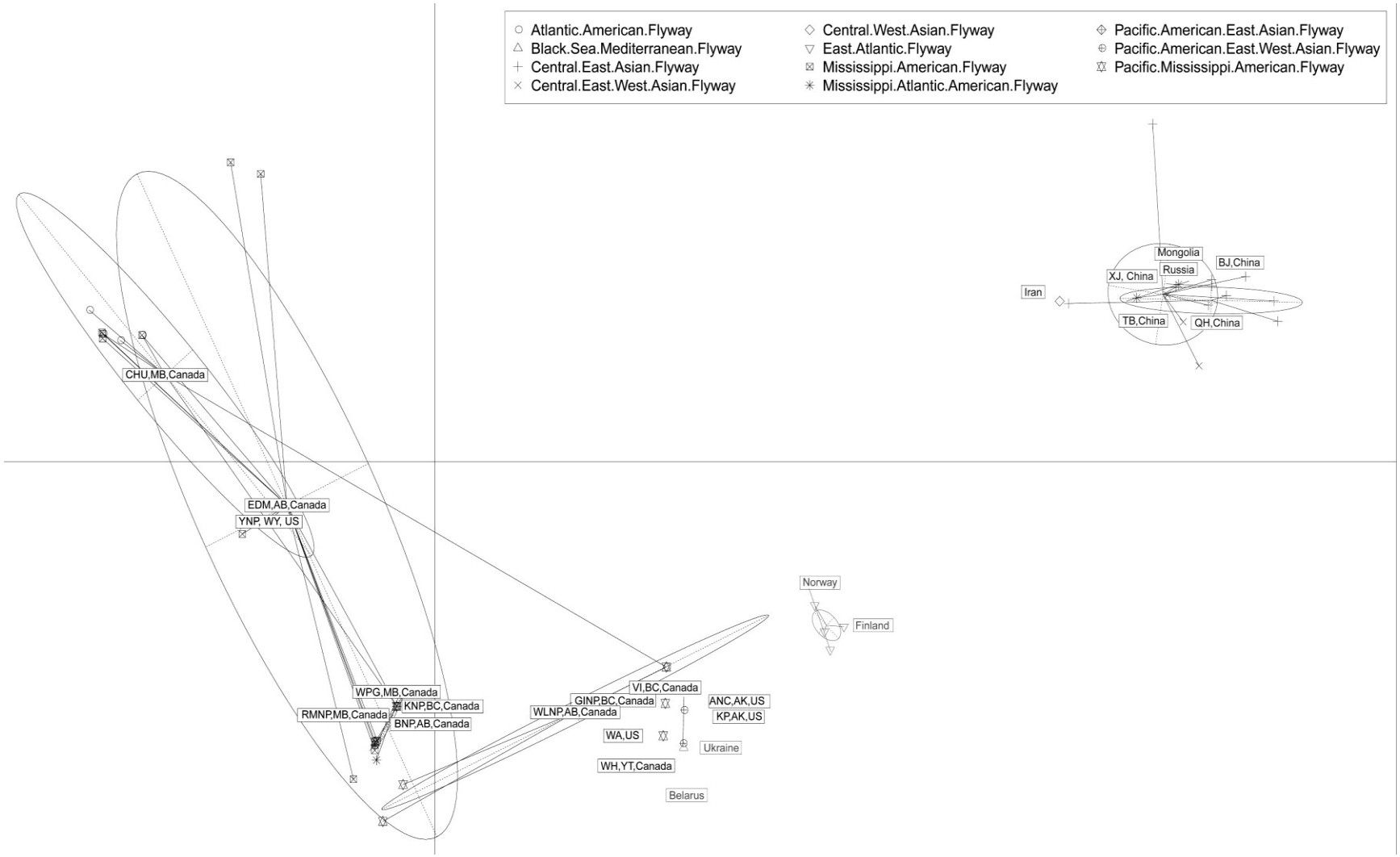

Figure 5.4 Plot of principal coordinate analysis based on COI gene from 301 *Gammarus lacustris* sequences (excluding one outlier species from Bled, Slovenia; see text for explanation) across the entire Holarctic region including North America, Europe and Asia. Details of abbreviations of location names are in Table S5 1.

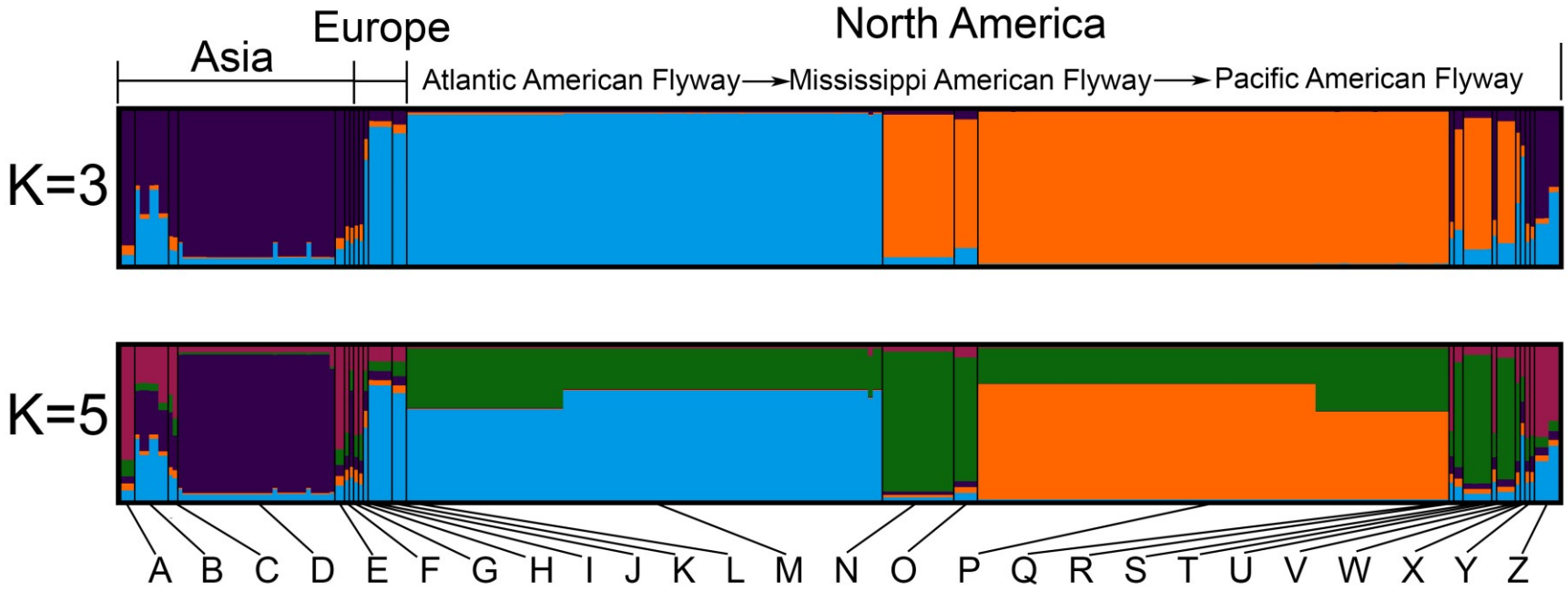

Figure 5.5 CLUMPAK–averaged STRUCTURE admixture plots across 10 independent runs of K=3 and 5 using the LOCPRIOR=1. Label Abbreviations are as followed: A: Beijing, China; B: Qinghai, China; C: Russia; D: Tibet, China; E: Xinjiang, China; F: Mongolia; G: Iran; H: Slovenia; I: Ukraine; J: Belarus; K: Finland; L: Norway; M: Churchill, Manitoba, Canada; N: Riding Mountain National Park, Manitoba, Canada; O: Winnipeg, Manitoba, Canada; P: Edmonton, Alberta, Canada; Q: Yellowstone National Park, Wyoming, United States; R: Banff National Park, Alberta, Canada; S: Waterton Lakes National Park, Alberta, Canada; T: Washington, Washington, United States; U: Kootenay National Park, British Columbia, Canada; V: Gulf Islands National Park, British Columbia, Canada; W: Vancouver Island, British Columbia, Canada; X: Whitehorse, Yukon Territory, Canada; Y: Anchorage, Alaska, United States; Z: Kenai Peninsula, Alaska, United States.

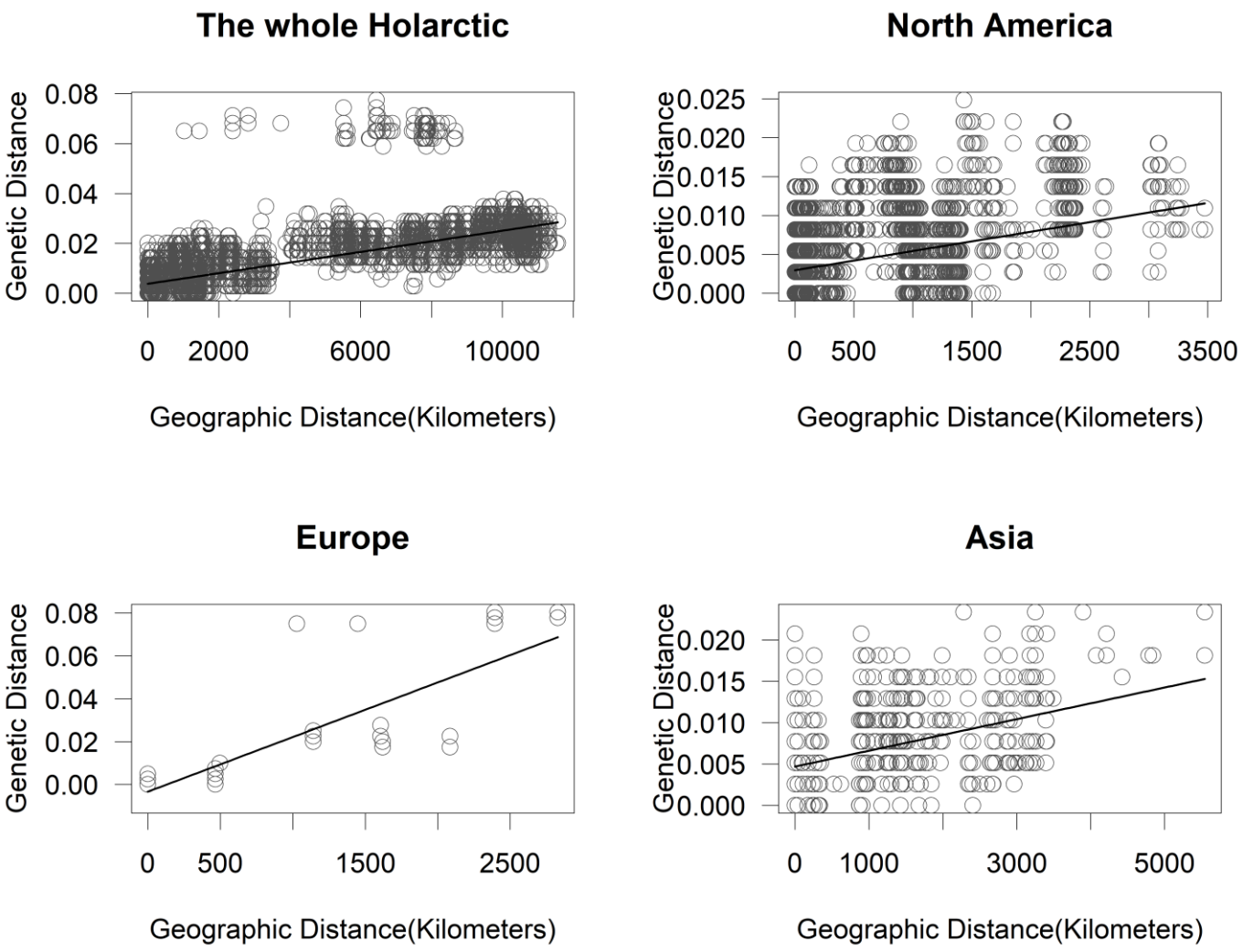

Figure 5.6 Relationships between COI genetic distance (Jukes–Cantor 69 distance) and geographical distance in *Gammarus lacustris* population across and within North America, Europe and Asia.

Chapter 6 Conclusions

6.1. Research summary and implication of current thesis research

The goals of my thesis were to: (1) apply molecular diagnostics, morphometric analysis and machine learning approaches to differentiate cystacanths of *Polymorphus* species in central Alberta, Canada (Chapter 2); (2) test the effects of biotic and abiotic factors on acanthocephalan prevalence in the intermediate host *Gammarus lacustris* (Chapter 3); (3) assess correlation between waterbody age and intraspecific mtDNA diversity for *G. lacustris* and acanthocephalan species (Chapter 4); and (4) explore how mtDNA genetic structure of *G. lacustris* is related to waterfowl flyway across the Holarctic (Chapter 5).

Prior to my study, Denny (1969) found that largest proboscis hook could be used to differentiate *Polymorphus contortus*, *P. marilis* and *P. paradoxus*. This finding is in accordance with morphometric results of my study, supporting that the morphology of the proboscis hook is useful for differentiating the larvae of waterfowl-associated acanthocephalan species. Furthermore, my study showed that machine learning can differentiate the larvae of waterfowl-associated acanthocephalan species based on the four traits of proboscis hooks from simulated data and real adult specimens (*Polymorphus* spp. and *Pseudocorynosoma* spp.). This technique could be applied to the identification of other waterfowl-associated acanthocephalans and perhaps non-waterfowl-associated acanthocephalans. Prior to its further application, more adult specimens of different acanthocephalan species should be tested for the utility of features of proboscis hooks in differentiating acanthocephalan species. My study also suggests that machine-learning approaches offer a potential tool for the identification of other parasitic and nonparasitic organisms. Wang et al. (2012) and Santana et al. (2014) also found that machine-learning methods are useful for insect identification to the order level and identification of bee species, respectively. In addition, my study showed molecular approach using COI marker can differentiate all above species except *P.* cf. *paradoxus* and *P.* cf. *marilis*. Bayesian phylogenetic method showed *P.* cf. *marilis* were nested within *P.* cf. *paradoxus*. I think them likely to be two different species because *P. paradoxus* and *P. marilis* manipuate *G. lacustris* in two different ways: *P. paradoxus*-infected *G. lacustris* are positively phototactic while *P. marilis*-infected *G. lacustris* show negative phototaxis. Furthermore, Bayesian tree and pairwise genetic distances

revealed that *P.* cf. *paradoxus* exhibited high levels of molecular variation, suggesting the existence of cryptic diversity within *P.* cf. *paradoxus*. To further study this cryptic diversity, multiple genetic markers should be used, including mitochondrial gene cytochrome c oxidase (cox 1) and 18S ribosomal RNA, as these genetic markers have been shown to differentiate specimens of acanthocephalans successfully (García-Varela et al. 2002a; García-Varela et al. 2002b).

Denny (1969) found three acanthocephalan species (*Polymorphus contortus*, *P. marilis* and *P. paradoxus*) in *G. lacustris*. After 50 years, I basically confirmed what Denny (1969) observed except a newly found potential acanthocephalan species (*P.* cf. *strumosoides*), suggesting that acanthocephalan assemblage in Edmonton appears to be quite temporally stable across the 50 years. Acanthocephalan prevalence showed a little different picture by increasing as waterbody age increases (Chapter 3). This pattern is further corroborated by age-focused collection in 2017 which showed acanthocephalan prevalence was higher in old water bodies (waterbody age relative to 2015: 26–38) than the young (3–7; Chapter 3). Similarly, genetic diversity of *Polymorphus* cf. *paradoxus* and both *P.* cf. *paradoxus* and *P.* cf. *marilis* continually increased as waterbody age increased, after I controlled for waterbody size and the species richness of known waterbird hosts and waterbody size (Chapter 4). The findings of both acanthocephalan prevalence and genetic diversity support the importance of time available for host and parasite colonization, and molecular results suggest that carrying capacity has not been reached and that a high abundance of uninfected *G. lacustris* is available in the environment. Genetic differentiation in acanthocephalans was not statistically significant between young, intermediate and old water bodies. In contrast, *G. lacustris* showed a hump-shaped relationship between its genetic diversity and waterbody age, suggesting that populations have reached carrying capacity in middle-aged and old water bodies and some genotypes now dominate and have eliminated other genotypes in older water bodies. I also found that the degree of genetic differentiation in *G. lacustris* differed significantly among populations from young (3–7 years), middle-aged (16–26 years) and old water bodies (32–53 years), although principal coordinate analysis does not show very strong genetic differentiation among age groups (Chapter 4). This suggested that the effect of competition on increasing the genetic differentiation among these populations might override the effect of gene flow on homogenizing among-population genetic divergence. Although these findings statistically support the role of competition in influencing genetic

diversity of hosts, experiments should be conducted to investigate whether competition influences the survival rate of genotypes of *G. lacustris*.

In addition to waterbody age, my results support the importance of the abundance of common avian final-hosts on acanthocephalan prevalence in *G. lacustris*, and but showed that the density of this intermediate-host amphipod was correlated negatively (in some cases significantly) with acanthocephalan prevalence in *G. lacustris*. This relationship might reflect an interaction between the density of intermediate amphipod hosts and the abundance of infective acanthocephalan eggs available in environment. When eggs are abundant and amphipods occur at low to moderate densities, acanthocephalan prevalence in the intermediate host is expected to be stable at high levels. In contrast, when intermediate hosts reach high densities and outstrip the occurrence of infective stages, acanthocephalan prevalence should decrease. My results suggest a high abundance of the intermediate amphipod host in my study systems relative to the abundance of infective acanthocephalan eggs. Assessment of this explanation requires investigation of how many of the infective eggs of acanthocephalans occur in the environment, are viable, and are available for consumption by *G. lacustris*. Crompton and Whitfield (1968) showed that *Polymorphus* in mallards could produce very large numbers of eggs (1700 eggs per day per worm), which appears to be sufficient to infect all *G. lacustris* in the environment (up to 2445 individuals /m^2 with the mean of 155 /m^2 with mean based on my study) if all eggs are viable, infective and available for consumption. The negative correlation observed between the density of amphipods and acanthocephalan prevalence might result from low consumption rate of acanthocephalan eggs by amphipods so that not all amphipods have chance to get infected. Factors limiting the infection of acanthocephalans in amphipods may be related to the amphipod foraging behavior.

Amphipods can move between water bodies by clinging onto feathers of water birds. I found that *G. lacustris* populations differed genetically among continents more greatly than among countries within continents, and more greatly than among localities (including cities and territories) within countries. Genetic variation was significantly different among flyways across the entire Holarctic region and among flyways within North America, Europe and Asia. Bayesian phylogenetic analysis, haplotype network, STRUCTURE results and principal coordinate analysis showed that the population structure of *G. lacustris* correlates with

waterfowl migration flyways. A Mantel test supported patterns of isolation by distance across North America, Europe and Asia, suggesting that *G. lacustris* are not fully free to disperse within or between continents. Distance-based redundancy analysis showed that the variation in population genetic structure of *G. lacustris* was primarily explained by waterfowl migration flyways, followed by spatial distance (spatial eigenvectors) and isolation by the Rocky Mountains in the Holarctic and within North America. In contrast, the population genetic structure of *G. lacustris* from Europe and Asia is mainly attributable to spatial distance, followed by waterfowl migration flyways. Swanson (1984) showed that infected amphipods are more likely to be transported by waterfowl than uninfected amphipods. Because of this, population structure of waterfowl-associated acanthocephalans might correlate with waterfowl flyways at continental and intercontinental scales.

6.2. Future directions of research

One potential future direction of my thesis research would be to test how species richness and assemblage structure of *Gammarus*-associated acanthocephalans are correlated with waterbody age, the abundance of intermediate and final hosts, and other environmental factors. I expect that acanthocephalan species richness and assemblage structure are correlated with waterbody age and species richness of known waterfowl hosts. In the future study, local researchers could take advantage of the acanthocephalan specimens that I collected across three years to test these ideas. I would also suggest using newly-constructed water bodies to track how acanthocephalan prevalence/richness changes over time. These ideas can be also tested using the other common amphipod in Alberta, *Hyalella azteca* Saussure, and its associated acanthocephalans (Podesta and Holmes 1970). For testing the relationship between species richness/composition and waterbody age, constructed water bodies with various ages are a useful system, and other endosymbiotic organisms in *G. lacustris* and *Hyalella,* or even other freshwater invertebrates, could be used to see whether positive species–age relationship is true for different freshwater invertebrates and their associated symbionts. To test this idea, genetic diversity of both host and symbionts should be first explored by amplification of multiple genetic markers (e.g., COI, 16S and 18S rDNA). These genetic markers can provide a subset of information from genetic materials for us to estimate genetic diversity of host and symbionts. If more detailed genetic information (e.g., whole genome and transcriptome) is needed to estimate genetic diversity of

host and symbionts, metagenomic or transcriptomic approaches might need to be applied (Srivathsan et al. 2016; Santos et al. 2018).

To my knowledge, no DNA sequences of the four *Polymorphus* species I tentatively identify in my thesis (*P. marilis*, *P. paradoxus*, *P. contortus* and *P. strumosoides*) are in NCBI or BOLD systems at the present time. Thus it would be ideal to obtain DNA from adult specimens of *Polymorphus* species to confirm the taxonomic identity of the four putative species that I identified in Chapter 2. Multiple genetic markers probably should be applied, and this is especially true for assessing whether *P. marilis* and *P. paradoxus* are separate species. In addition, the causes of high intraspecific genetic diversity of *P.* cf. *paradoxus* should be investigated in future studies.

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Appendix

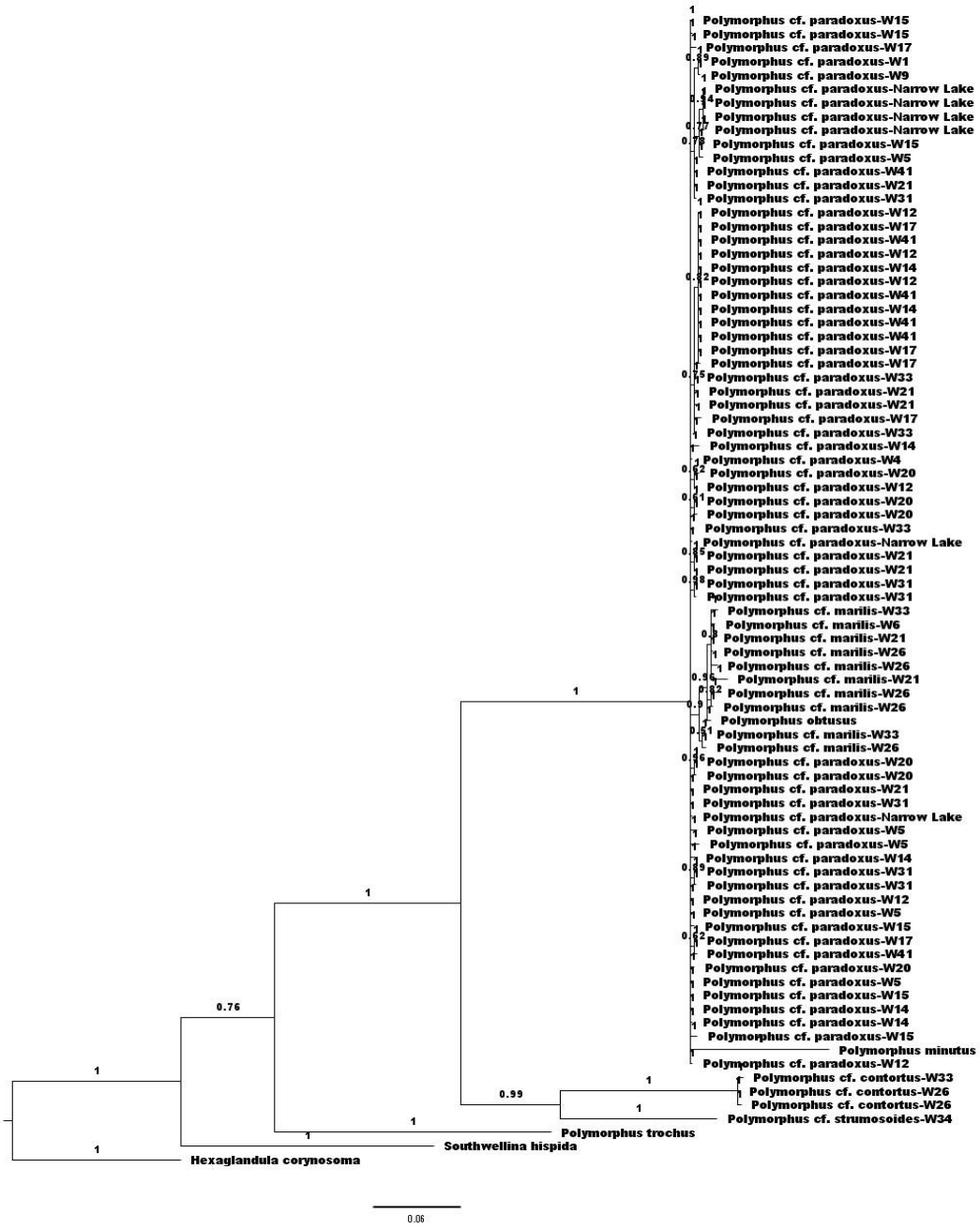

Figure S2 1 Bayesian phylogenetic tree based on COI sequences from 79 cystacanths of four putative *Polymorphus* species from Alberta (*P.* cf. *contortus*, *P.* cf. *marilis*, *P.* cf. *paradoxus* and *P.* cf. *strumosoides*) and three specimens of adult acanthocephalan species (*Polymorphus trochus*, *P. obtusus* and *P. minute*) with two outgroup taxa (*Hexaglandula corynosoma* and *Southwellina hispida*).

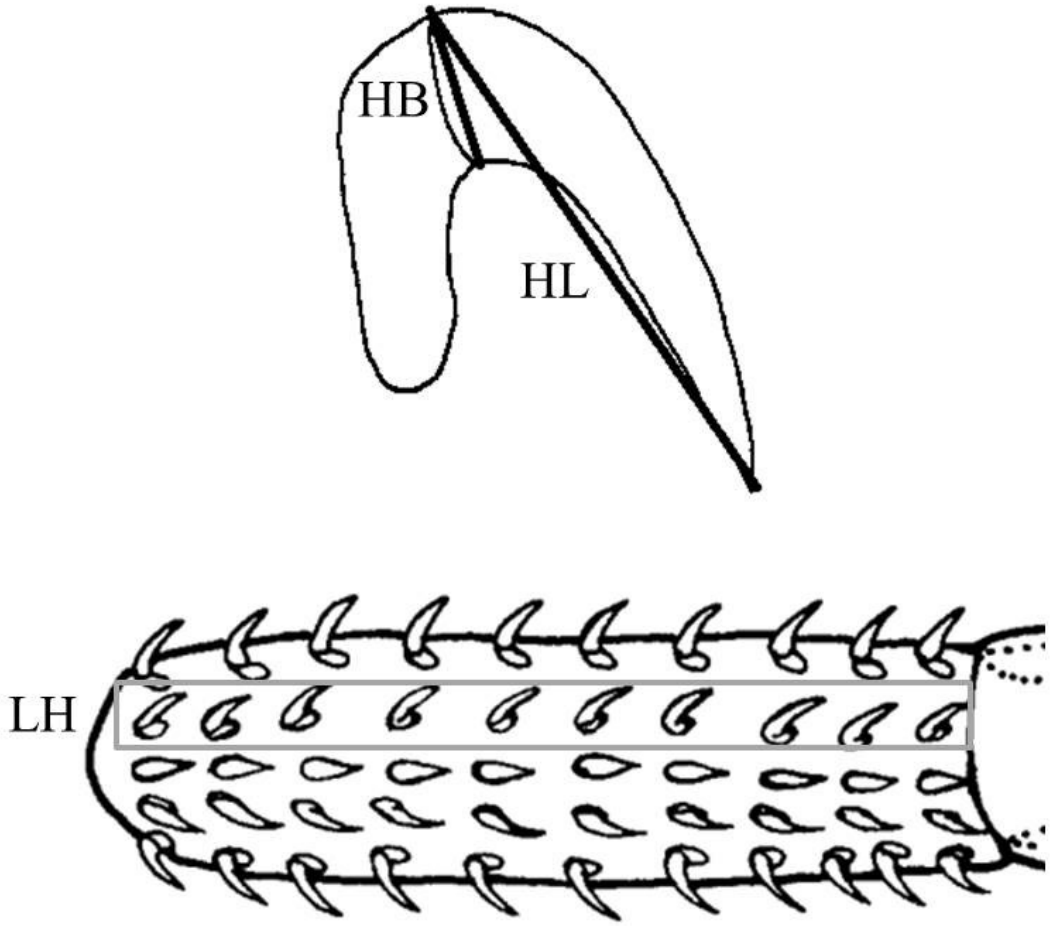

Figure S2 2 Illustration of an acanthocephalan proboscis (at bottom) and a proboscis hook (at top) showing the way of measuring hook length (HL), base wide (HB) and longitudinal hooks (LH). In this case, the number of longitudinal hooks (HPR) is 10 and 5 rows of longitudinal hooks (NHR) are shown. Proboscis drawing modified from McDonald (1988).

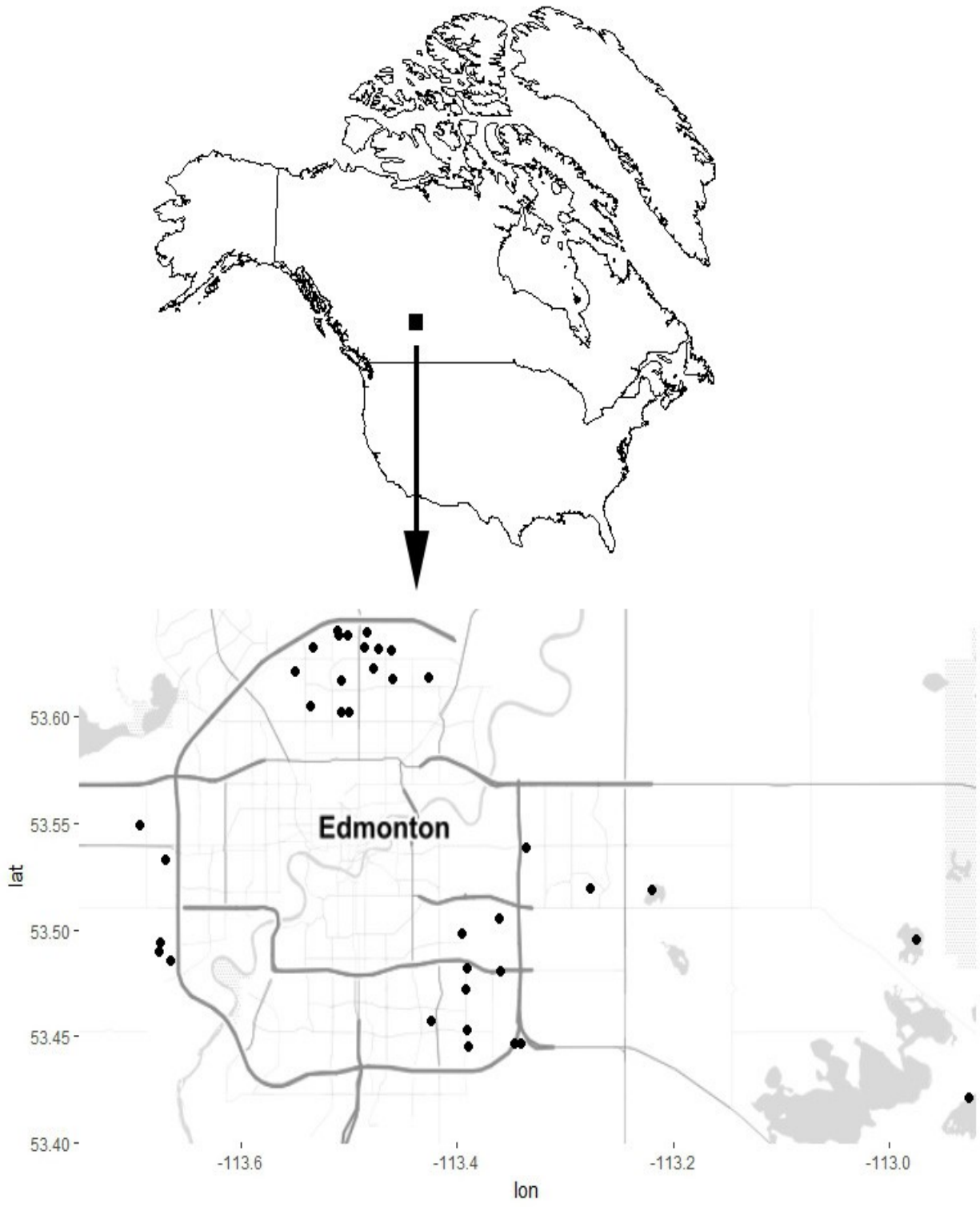

Figure S3 1 Geographic location of 36 water bodies in Edmonton, Alberta, Canada. Each dot represents the location of each water body.

Table S3 1 Dataset of number of infected *G. lacustris* (NI) and number of *G. lacustris* examined (NE) from each of three sampling points in each of 36 water bodies in the vicinity of Edmonton, Alberta across 3 years (2015–2017). Note: sampling area at each sampling point = ~1.1 m^2; NA = not surveyed

	2015				2016				2017			
Waterbody name	May		August		May		August		May		August	
Subsite	NI	NE	NI	NE	NI	NE	NI	NE	NI	NE	NI	NE
W01												
1	31	827	27	745	81	110	67	87	NA	NA	NA	NA
2	32	362	17	360	120	136	51	141	NA	NA	NA	NA
3	64	1009	101	729	83	112	11	208	NA	NA	NA	NA
W02												
1	5	23	9	112	13	14	0	0	NA	NA	NA	NA
2	10	36	5	49	14	16	1	126	NA	NA	NA	NA
3	4	5	12	128	34	38	6	102	NA	NA	NA	NA
4	0	0	0	0	0	0	8	134	NA	NA	NA	NA
W03												
1	2	8	121	306	6	8	1	60	NA	NA	NA	NA
2	8	39	4	11	11	13	3	108	NA	NA	NA	NA
3	17	86	29	76	3	7	3	100	NA	NA	NA	NA
W04												
1	11	159	17	330	6	22	3	29	6	68	8	189
2	18	99	12	297	4	8	4	64	7	67	2	179

3	25	240	7	199	16	19	2	9	3	21	22	432
W05												
1	25	79	8	83	181	190	19	142	135	185	80	187
2	19	246	2	297	50	70	6	69	57	101	31	151
3	9	231	7	111	164	174	7	124	84	114	16	239
W06												
1	10	43	0	7	5	34	4	60	NA	NA	NA	NA
2	11	18	0	4	2	46	2	24	NA	NA	NA	NA
3	8	18	2	26	8	129	1	22	NA	NA	NA	NA
W07												
1	12	14	12	527	17	22	6	166	NA	NA	NA	NA
2	5	51	3	314	27	45	9	206	NA	NA	NA	NA
3	70	71	1	9	13	42	0	66	NA	NA	NA	NA
W08												
1	2	4	138	427	9	34	55	161	NA	NA	NA	NA
2	5	25	164	824	6	18	73	317	NA	NA	NA	NA
3	13	80	22	48	11	28	110	476	NA	NA	NA	NA
W09												
1	13	229	12	280	37	191	11	178	24	187	5	43
2	20	275	64	2233	83	805	7	131	32	256	17	125
3	21	436	11	426	5	75	3	122	18	43	0	17
W10												

1	19	52	72	678	158	214	76	222	NA	NA	NA	NA
2	262	441	52	857	206	348	44	138	NA	NA	NA	NA
3	28	54	140	1612	102	170	167	382	NA	NA	NA	NA
W11												
1	33	39	9	99	7	31	21	63	NA	NA	NA	NA
2	13	27	18	119	7	15	25	276	NA	NA	NA	NA
3	10	23	100	195	0	8	14	24	NA	NA	NA	NA
W12												
1	6	23	20	63	4	12	1	25	0	17	0	33
2	7	25	16	108	8	13	1	24	0	7	0	83
3	7	12	36	213	3	19	12	37	1	11	0	12
W13												
1	2	17	5	27	18	26	49	229	NA	NA	NA	NA
2	7	28	0	21	4	14	9	84	NA	NA	NA	NA
3	4	8	12	73	60	67	26	61	NA	NA	NA	NA
W14												
1	35	59	70	168	36	37	64	120	227	233	21	199
2	23	37	56	117	31	32	96	264	113	117	60	138
3	24	30	93	256	26	29	100	277	75	87	69	169
W15												
1	34	53	48	294	57	58	5	187	92	98	28	80
2	69	70	26	237	40	43	45	827	93	100	29	139

3	41	58	15	167	21	90	58	744	78	90	20	50
W16												
1	51	54	12	172	23	62	49	677	271	275	51	742
2	3	14	33	195	11	26	24	148	89	100	24	409
3	27	48	5	156	11	25	11	120	111	117	90	488
W17												
1	22	25	39	250	93	124	68	394	NA	NA	NA	NA
2	30	36	30	100	143	159	24	393	NA	NA	NA	NA
3	62	77	21	96	37	44	16	303	NA	NA	NA	NA
W18												
1	3	6	2	30	6	17	13	65	NA	NA	NA	NA
2	5	7	1	12	4	26	8	11	NA	NA	NA	NA
3	5	8	1	9	21	42	86	162	NA	NA	NA	NA
W19												
1	9	13	108	1022	58	60	16	363	NA	NA	NA	NA
2	6	23	18	95	204	214	87	423	NA	NA	NA	NA
3	9	31	23	116	69	70	102	637	NA	NA	NA	NA
W20												
1	4	28	2	23	42	63	83	115	22	42	12	40
2	14	46	14	299	23	23	163	191	16	48	2	9
3	17	28	6	84	24	37	61	139	6	14	5	28
W21												

1	69	70	97	107	113	115	54	317	NA	NA	NA	NA
2	73	114	14	48	49	53	11	96	NA	NA	NA	NA
3	33	37	8	165	45	46	13	138	NA	NA	NA	NA
W23												
1	15	16	1	42	5	7	16	21	NA	NA	NA	NA
2	12	18	3	110	0	6	3	5	NA	NA	NA	NA
3	26	29	23	125	3	5	0	6	NA	NA	NA	NA
W24												
1	24	64	29	961	36	54	19	319	NA	NA	NA	NA
2	12	60	38	606	1	30	11	165	NA	NA	NA	NA
3	17	108	35	518	26	117	8	151	NA	NA	NA	NA
W26												
1	92	638	37	347	18	22	8	120	NA	NA	NA	NA
2	2	8	19	312	5	8	4	73	NA	NA	NA	NA
3	55	553	22	536	6	6	6	75	NA	NA	NA	NA
W27												
1	2	135	32	108	10	19	27	358	NA	NA	NA	NA
2	2	75	27	605	7	19	15	313	NA	NA	NA	NA
3	3	80	10	403	25	109	7	54	NA	NA	NA	NA
W29												
1	6	36	2	29	3	14	0	8	NA	NA	NA	NA
2	2	13	1	5	2	6	0	23	NA	NA	NA	NA

3	1	43	0	3	0	3	0	22	NA	NA	NA	NA
W30												
1	18	48	2	82	18	19	18	279	NA	NA	NA	NA
2	3	22	1	19	41	42	17	195	NA	NA	NA	NA
3	1	14	0	11	14	15	6	51	NA	NA	NA	NA
W31												
1	34	56	10	139	33	44	9	139	NA	NA	NA	NA
2	23	51	0	4	11	16	2	12	NA	NA	NA	NA
3	22	37	2	11	18	21	5	46	NA	NA	NA	NA
W32												
1	73	166	16	115	21	21	118	695	NA	NA	NA	NA
2	106	186	10	245	15	16	100	571	NA	NA	NA	NA
3	144	215	17	223	45	46	79	616	NA	NA	NA	NA
W33												
1	34	48	63	482	23	28	10	159	NA	NA	NA	NA
2	7	7	21	443	3	4	54	692	NA	NA	NA	NA
3	24	46	50	399	4	9	8	101	NA	NA	NA	NA
W34												
1	27	1064	27	1064	2	49	16	1753	NA	NA	NA	NA
2	65	1488	45	1479	6	56	10	320	NA	NA	NA	NA
3	14	276	76	2690	3	38	45	2186	NA	NA	NA	NA
W35												

1	0	3	1	54	1	6	0	139	NA	NA	NA	NA
2	0	3	0	22	2	5	1	86	NA	NA	NA	NA
3	0	0	1	1	1	5	1	225	NA	NA	NA	NA
W36												
1	39	86	71	357	44	138	45	601	25	41	3	136
2	43	144	18	81	30	121	37	131	31	105	3	69
3	62	156	41	214	69	116	2	69	43	75	8	130
W37												
1	27	56	311	431	302	312	9	111	NA	NA	NA	NA
2	10	11	151	373	27	42	95	432	NA	NA	NA	NA
3	112	161	30	79	135	136	52	126	NA	NA	NA	NA
W40												
1	27	61	11	205	9	55	12	49	NA	NA	NA	NA
2	23	45	11	174	15	70	13	241	NA	NA	NA	NA
3	56	92	45	534	13	34	19	227	NA	NA	NA	NA
W41												
1	37	40	15	29	121	130	11	25	75	89	185	201
2	39	44	11	19	54	56	33	51	33	35	29	37
3	136	148	24	37	58	58	37	68	4	4	68	87

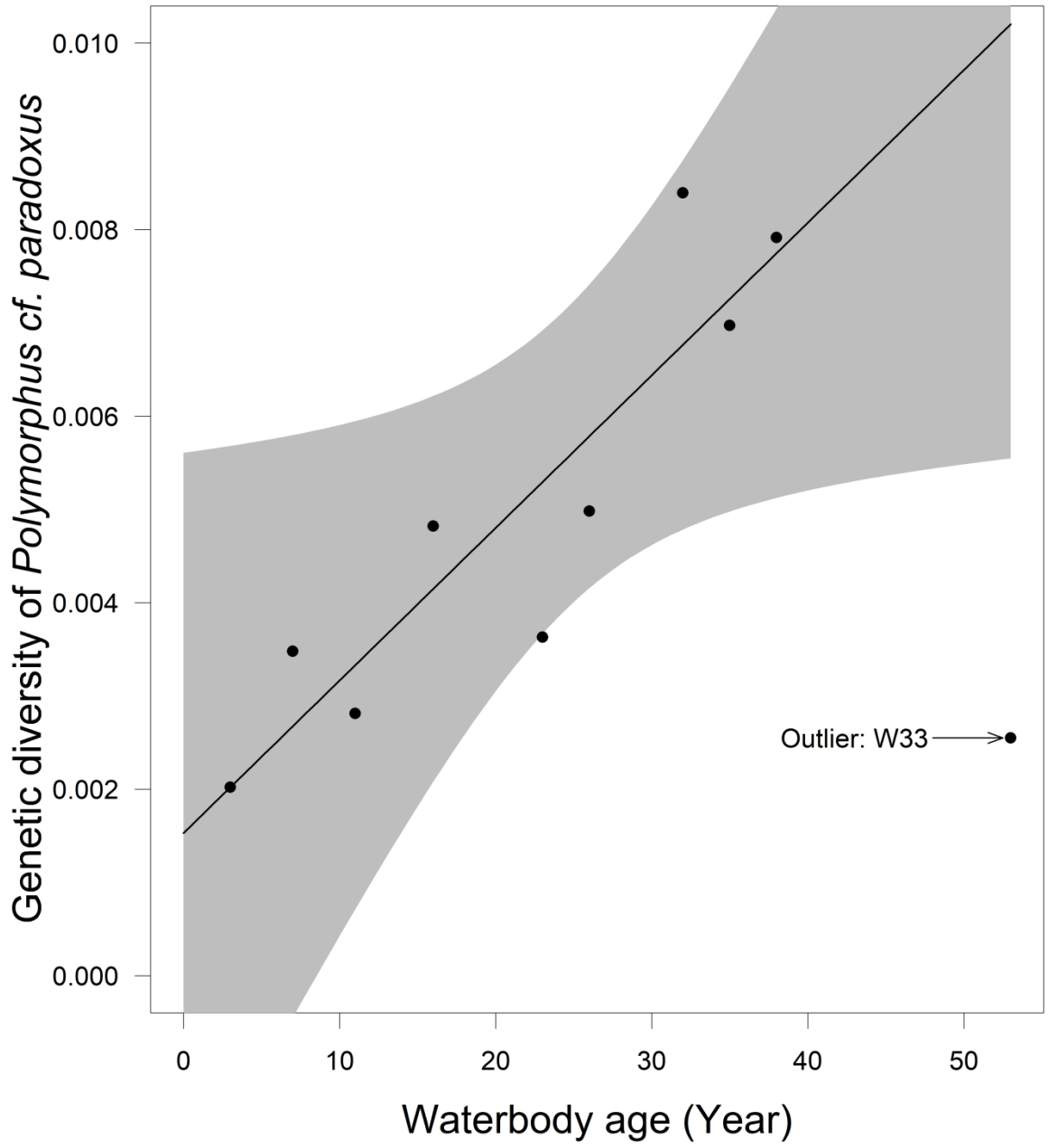

Figure S4 1 The positive relationship between parasite (*Polymorphus* cf. *paradoxus*) mtDNA genetic diversity and waterbody age across ten water bodies [including the outlier (the water body with small sample size)] after controlling for waterbody size and species richness of known waterbird hosts. Each dot represents the acanthocephalan genetic diversity within a water body. The straight line represents the fitted line through dots. The band represents 95% confidence intervals.

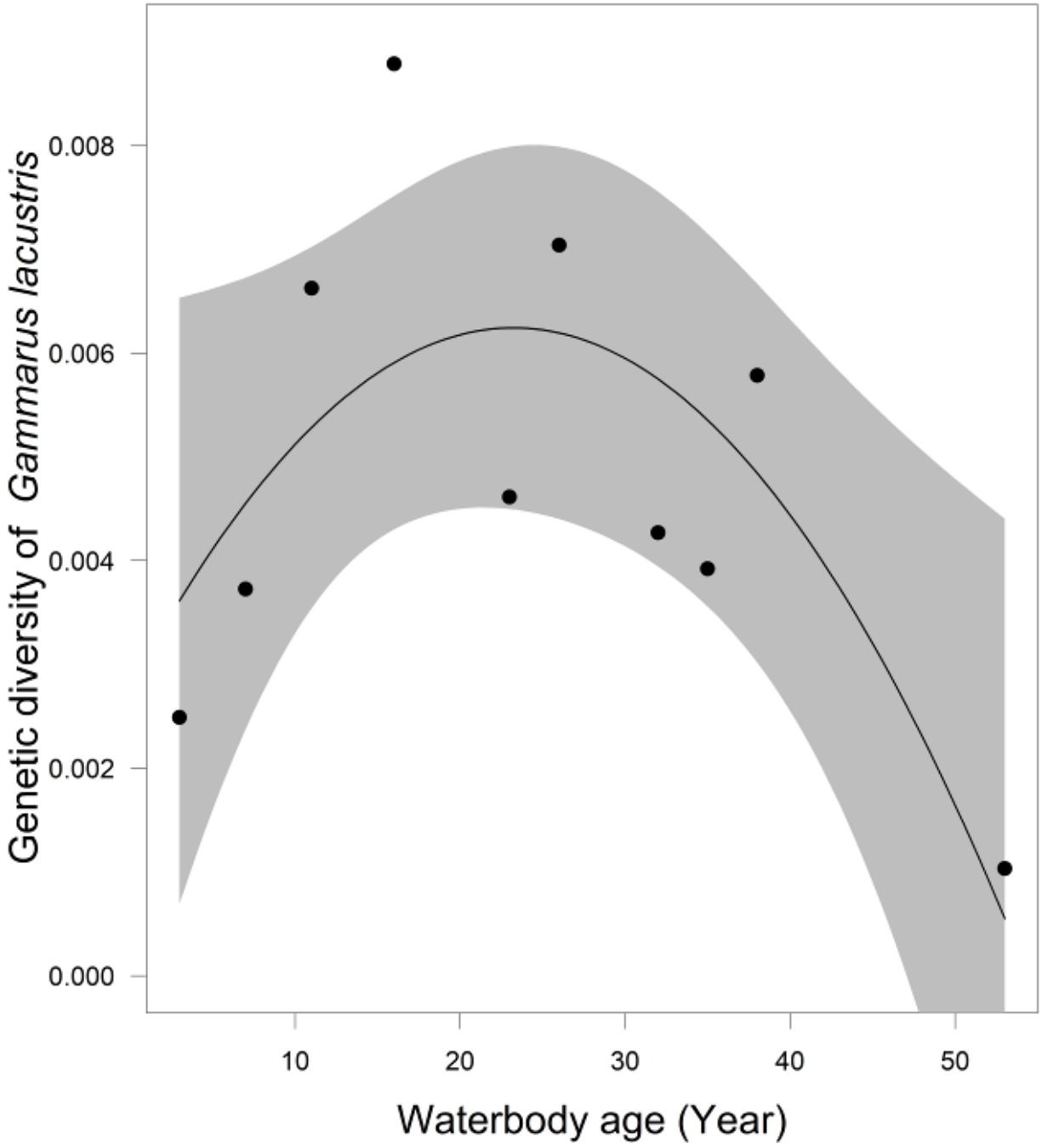

Figure S4 2 The hump–shaped relationship between host (*Gammarus lacustris*) mtDNA genetic diversity and waterbody age across ten water bodies. Each dot represents the *G. lacustris* genetic diversity within a water body. The curved line represents the fitted line through dots. The band represents 95% confidence intervals.

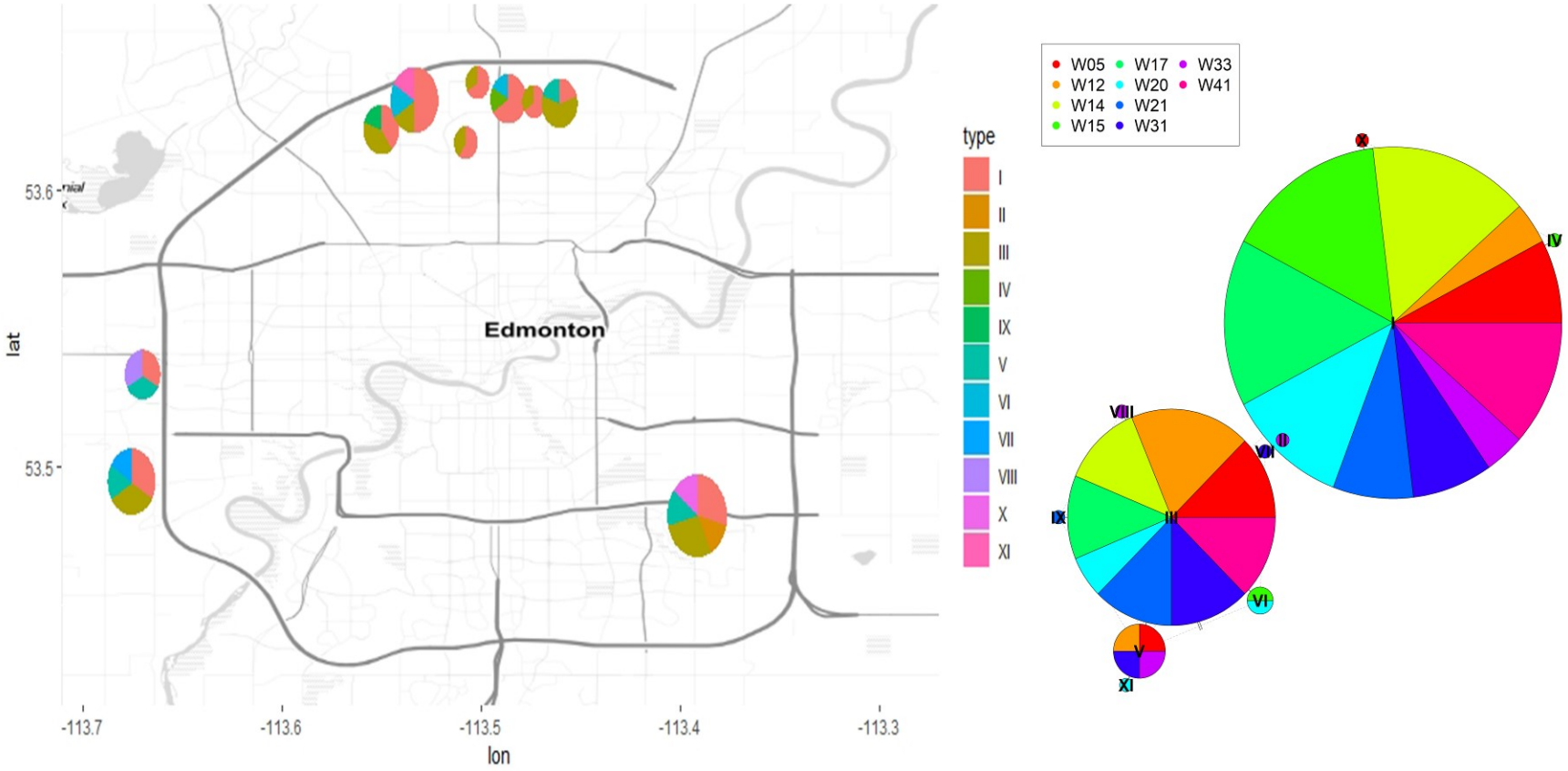

Figure S4 3 Spatial pattern of host (*Gammarus lacustris*) haplotypes (left plot) and its haplotype network (right plot) across my sampling sites. Left plot: each color in the pie chart represents a haplotype of the mtDNA. Right plot: each color in the pie chart represents a water body.

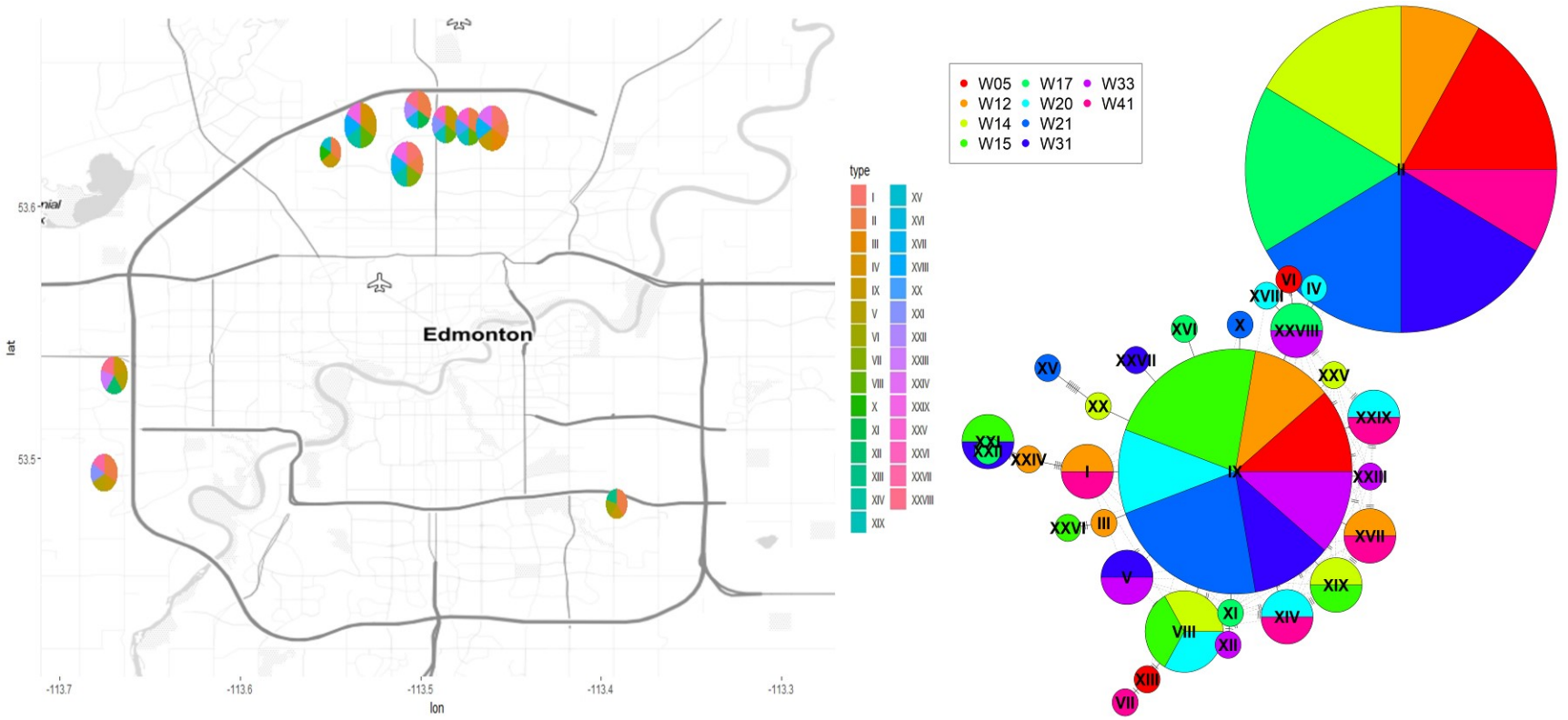

Figure S4 4 Spatial pattern of parasite (*Polymorphus* cf. *paradoxus* and *P.* cf. *marilis*) haplotypes (left plot) and its haplotype network (right plot) across my sampling sites. Left plot: each color in the pie chart represents a haplotype of the mtDNA. Right plot: each color in the pie chart represents a water body.

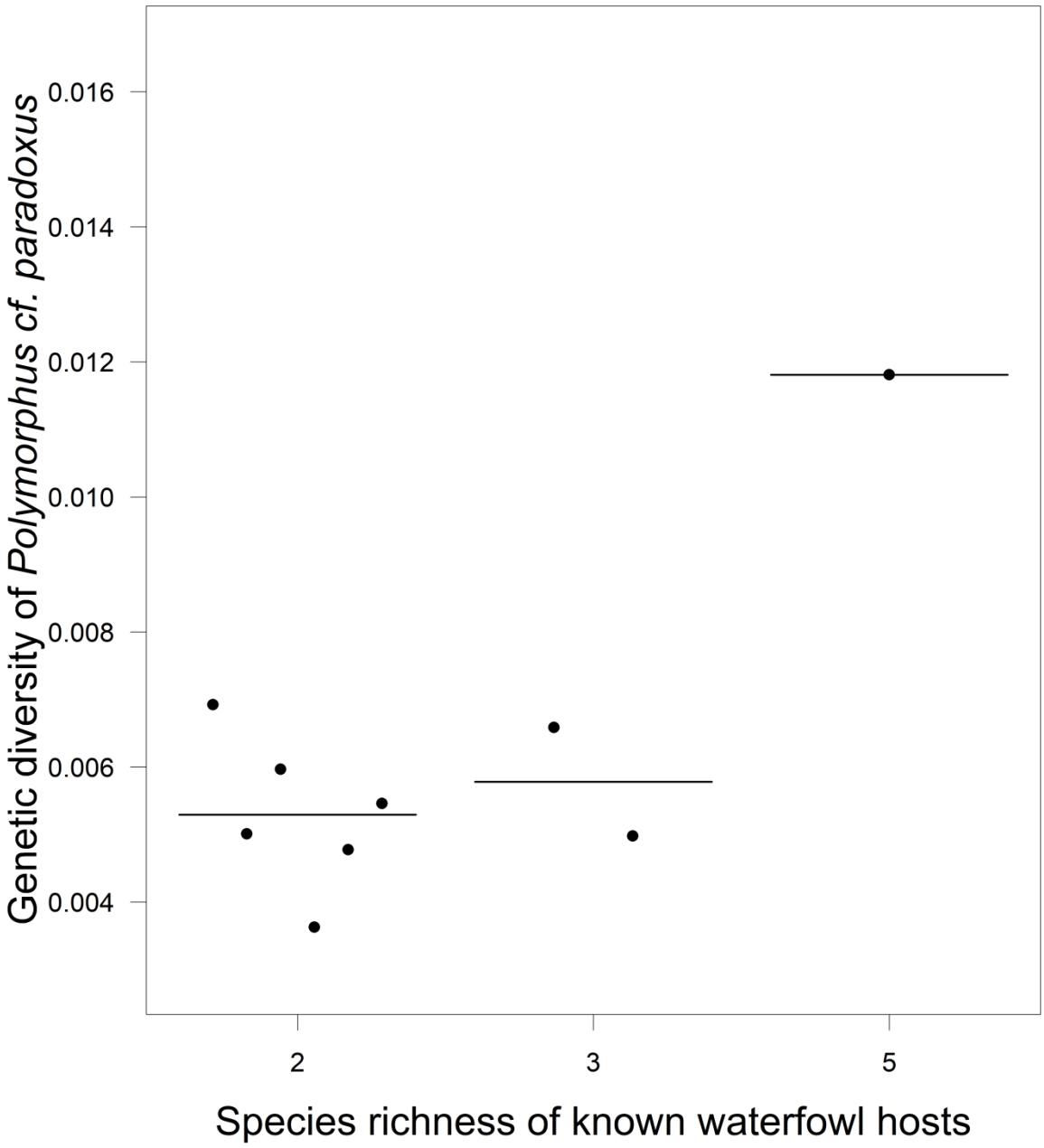

Figure S4 5 The relationship between parasite (*Polymorphus* cf. *paradoxus*) genetic diversity and species richness of known waterfowl hosts across nine water bodies (excluding one water body due to small sample size) after controlling for waterbody size and waterbody age. Each dot represents the acanthocephalan genetic diversity within a water body. The straight lines represent medians for each category.

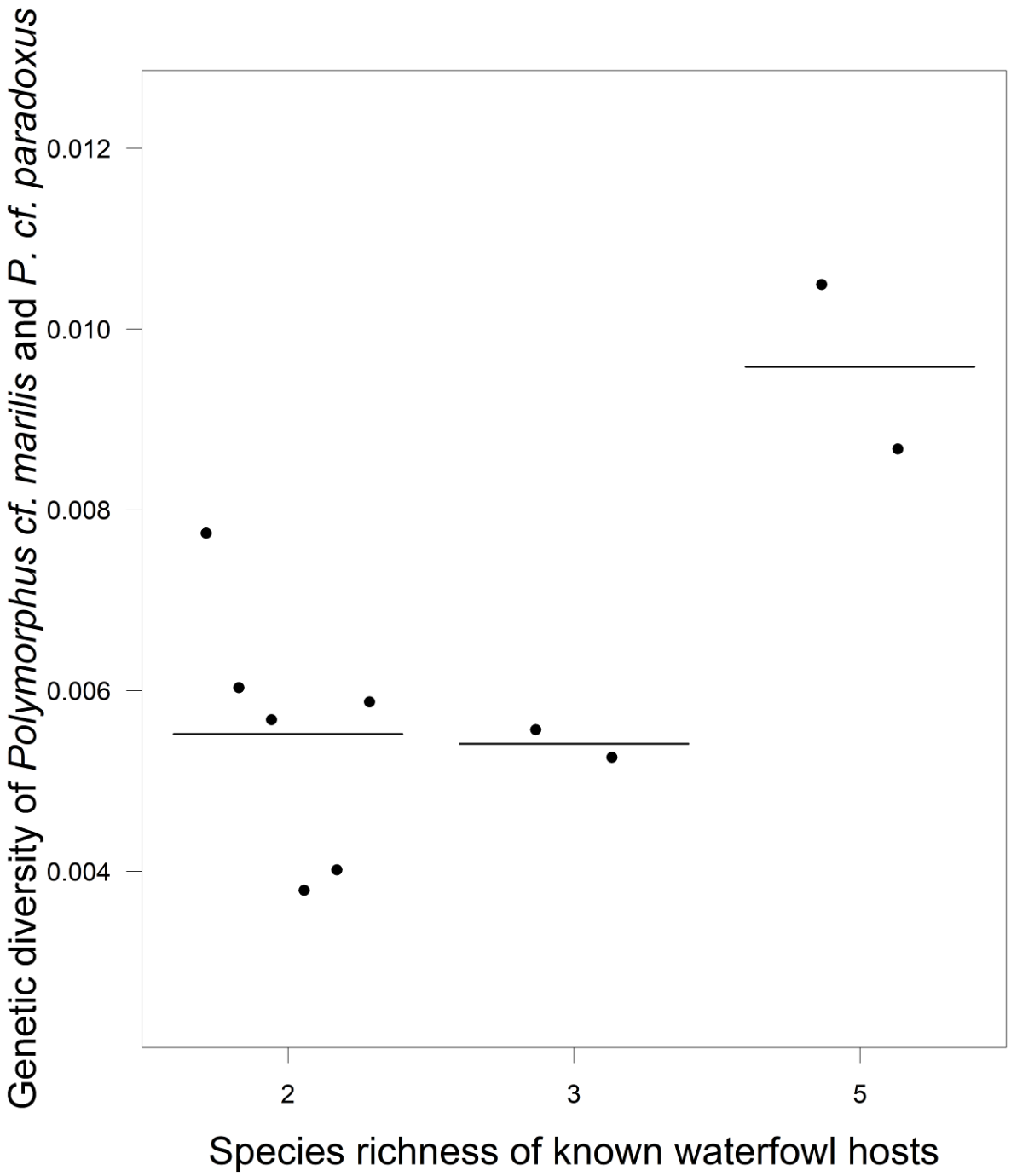

Figure S4 6 The relationship between parasite (*Polymorphus* cf. *paradoxus* and *P.* cf. *marilis*) genetic diversity and species richness of known waterfowl hosts across ten water bodies after controlling for waterbody size and waterbody age. Each dot represents the acanthocephalan genetic diversity within a water body. The straight lines represent medians for each category.

Table S5 1 *Gammarus lacustris* location information and Barcode of Life Data (BOLD) system accession numbers/sample IDs used in my study.

Sample ID/BOLD Accession Nos	locality names	Abbreviations	Longitude	Latitude
CRCN087-09	Banff National Park, Alberta, Canada	BNP, AB, Canada	51.171000	-115.586000
CRCN088-09	Banff National Park, Alberta, Canada	BNP, AB, Canada	51.171000	-115.586000
CCMAL174-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL175-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL176-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL177-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL178-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL179-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL180-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL181-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
CCMAL182-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000

CCMAL183-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.761000	-93.952000
DSMAL001-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
DSMAL002-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
DSMAL004-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
DSMAL005-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
DSMAL006-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
DSMAL007-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
DSMAL013-07	Churchill, Manitoba, Canada	CHU, MB, Canada	58.676000	-94.167000
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1A8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632120	-113.533157
1A9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.618750	-113.617579
1B1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631578	-113.473172
1B10.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.495707	-112.979427
1B11.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.543477	-114.517501
1B12.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.657978	-112.758899
1B2.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632578	-113.486115
1B3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.621447	-113.549829
1B4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.638396	-113.501474
1B6.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.481928	-113.391342
1B7.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.616696	-113.507408
1B8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632120	-113.533157

1B9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.618750	-113.617579
1C1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631578	-113.473172
1C10.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.495707	-112.979427
1C11.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.543477	-114.517501
1C12.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.657978	-112.758899
1C2.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632578	-113.486115
1C3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.494228	-113.675137
1C4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.638396	-113.501474
1C5.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.532953	-113.669601
1C6.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631025	-113.460323
1C7.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.616696	-113.507408
1C8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632120	-113.533157
1C9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.635340	-114.707261
1D1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631578	-113.473172
1D10.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.495707	-112.979427
1D11.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.543477	-114.517501

1D12.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.657978	-112.758899
1D2.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632578	-113.486115
1D3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.494228	-113.675137
1D4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.638396	-113.501474
1D5.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.481928	-113.391342
1D6.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631025	-113.460323
1D7.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.616696	-113.507408
1D8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632120	-113.533157
1D9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.635340	-114.707261
1E1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631578	-113.473172
1E10.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.495707	-112.979427
1E11.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	52.517717	-113.986904
1E12.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.657978	-112.758899
1E2.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.621447	-113.549829
1E3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.494228	-113.675137
1E4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.638396	-113.501474

1E5.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.481928	-113.391342
1E6.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631025	-113.460323
1E8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.618750	-113.617579
1E9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.635340	-114.707261
1F1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631578	-113.473172
1F12.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.657978	-112.758899
1F2.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.621447	-113.549829
1F3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.494228	-113.675137
1F4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.638396	-113.501474
1F5.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.481928	-113.391342
1F6.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631025	-113.460323
1F7.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.616696	-113.507408
1F8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.618750	-113.617579
1F9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.635340	-114.707261
1G1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632578	-113.486115
1G10.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.543477	-114.517501

1G11.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	52.481819	-112.889841
1G12.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.997378	-114.385869
1G2.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.621447	-113.549829
1G3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.494228	-113.675137
1G4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.532953	-113.669601
1G5.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.481928	-113.391342
1G6.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.631025	-113.460323
1G7.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632120	-113.533157
1G8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.618750	-113.617579
1G9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.635340	-114.707261
1H1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632578	-113.486115
1H10.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.543477	-114.517501
1H3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.494228	-113.675137
1H4.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.532953	-113.669601
1H5.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.481928	-113.391342
1H7.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.632120	-113.533157

1H8.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.618750	-113.617579
1H9.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.635340	-114.707261
2A1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.997378	-114.385869
2A3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.441480	-112.758704
2B1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.997378	-114.385869
2C1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.997378	-114.385869
2C3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.441480	-112.758704
2D1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.997378	-114.385869
2D3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.441480	-112.758704
2E1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.585356	-114.473628
2F1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.585356	-114.473628
2F3.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	54.441480	-112.758704
2G1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.585356	-114.473628
2H1.seq	Edmonton, Alberta, Canada	EDM, AB, Canada	53.585356	-114.473628
BBGCO1245-15	Gulf Islands National Park, British Columbia, Canada	GINP, BC, Canada	48.743000	-123.208000
BBGCO1248-15	Kootenay National Park, British Columbia, Canada	KNP, BC, Canada	50.892000	-116.041000

BBGCO1250-15	Kootenay National Park, British Columbia, Canada	KNP, BC, Canada	50.892000	-116.041000
BBGCO1251-15	Kootenay National Park, British Columbia, Canada	KNP, BC, Canada	50.892000	-116.041000
BBGCO1252-15	Kootenay National Park, British Columbia, Canada	KNP, BC, Canada	50.892000	-116.041000
CRCN068-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN078-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN079-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN080-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN083-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN084-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN109-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000
CRCN110-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000
CRCN111-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000
CRCN112-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000
CRCN113-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000
CRCN114-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000
CRCN115-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.882000	-100.044000

CRCN139-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
CRCN141-09	Riding Mountain National Park, Manitoba, Canada	RMNP, MB, Canada	50.678000	-99.803000
GBCMA0153-06	Vancouver Island, British Columbia, Canada	VI, BC, Canada	49.890661	-125.487872
CRCN116-09	Waterton Lakes National Park, Alberta, Canada	WLNP, AB, Canada	49.088000	-113.967000
CRCN121-09	Waterton Lakes National Park, Alberta, Canada	WLNP, AB, Canada	49.088000	-113.967000
CRCN148-09	Waterton Lakes National Park, Alberta, Canada	WLNP, AB, Canada	49.055000	-114.053000
CRCN149-09	Waterton Lakes National Park, Alberta, Canada	WLNP, AB, Canada	49.055000	-114.053000
CRCN150-09	Waterton Lakes National Park, Alberta, Canada	WLNP, AB, Canada	49.055000	-114.053000
CRCN151-09	Waterton Lakes National Park, Alberta, Canada	WLNP, AB, Canada	49.055000	-114.053000
NJCGS1053-11	Whitehorse, Yukon Territory, Canada	WH, YT, Canada	60.672800	-135.025000
2D2.seq	Winnipeg, Manitoba, Canada	WPG, MB, Canada	49.820546	-97.225011
2E2.seq	Winnipeg, Manitoba, Canada	WPG, MB, Canada	49.820546	-97.225011
2F2.seq	Winnipeg, Manitoba, Canada	WPG, MB, Canada	49.820546	-97.225011
2G2.seq	Winnipeg, Manitoba, Canada	WPG, MB, Canada	49.820546	-97.225011
2H2.seq	Winnipeg, Manitoba, Canada	WPG, MB, Canada	49.820546	-97.225011
NJCGS1176-11	Anchorage, Alaska, United States	ANC, AK, US	61.200400	-149.763000

NJCGS1177-11	Kenai Peninsula, Alaska, United States	KP, AK, US	60.536100	-150.462000
NJCGS1178-11	Kenai Peninsula, Alaska, United States	KP, AK, US	60.536100	-150.462000
NJCGS1179-11	Kenai Peninsula, Alaska, United States	KP, AK, US	60.536100	-150.462000
NJCGS1180-11	Kenai Peninsula, Alaska, United States	KP, AK, US	60.536100	-150.462000
NJCGS1181-11	Kenai Peninsula, Alaska, United States	KP, AK, US	60.536100	-150.462000
GBCMA0154-06	Washington, Washington, United States	WA, US	47.893819	-120.726159
GBCMA3487-12	Yellowstone National Park, Wyoming, United States	YNP, WY, US	45.241869	-110.169452
TLAMP059-15	Verchnedvinsk districkt, Vitsyebskaya Voblasts', Belarus	Belarus	56.033300	28.116700
GALP048-16	Finland	Finland	66.261000	29.452000
GALP049-16	Finland	Finland	66.261000	29.452000
GALP050-16	Finland	Finland	66.261000	29.452000
GALP051-16	Finland	Finland	66.261000	29.452000
GALP052-16	Finland	Finland	66.261000	29.452000
GALP055-16	Norway	Norway	70.411000	30.930000
GALP056-16	Norway	Norway	70.411000	30.930000
GALP057-16	Norway	Norway	70.411000	30.930000

GBCMA4442-13	Bled, Slovenia, Slovenia	Slovenia	46.361147	14.093715
GBCMA4951-13	Liubliaz, Ukraine, Ukraine	Ukraine	51.848200	25.472300
GBCM5804-17	Bangong Co, Tibet, China	TB, China	33.715619	78.720262
GBCM5953-17	Bangong Co, Tibet, China	TB, China	33.715619	78.720262
GBCM6312-17	Bangong Co, Tibet, China	TB, China	33.715619	78.720262
GBCM6883-17	Bangong Co, Tibet, China	TB, China	33.715619	78.720262
GBCM9184-17	Bangong Co, Tibet, China	TB, China	33.715619	78.720262
GBCMA1230-08	Beijing, China	BJ, China	39.957620	116.437370
GBCMA1231-08	Beijing, China	BJ, China	39.957620	116.437370
GBCMA1233-08	Beijing, China	BJ, China	39.957620	116.437370
GBCM10088-17	Donggi Cona, Qinghai, China	QH, China	36.072438	98.664323
GBCM11120-17	Donggi Cona, Qinghai, China	QH, China	36.072438	98.664323
GBCM9186-17	Donggi Cona, Qinghai, China	QH, China	36.072438	98.664323
GBCM11342-17	Indus River, Tibet, China	TB, China	32.863131	79.312474
GBCM6172-17	Indus River, Tibet, China	TB, China	32.863131	79.312474
GBCM10732-17	Kotra Co, Tibet, China	TB, China	31.202102	88.758029

GBCM6230-17	Kyaring Co, Tibet, China	TB, China	31.154510	88.345544
GBCM6442-17	Kyaring Co, Tibet, China	TB, China	31.154510	88.345544
GBCM8767-17	Kyaring Co, Tibet, China	TB, China	31.154510	88.345544
GBCM9856-17	Kyaring Co, Tibet, China	TB, China	31.154510	88.345544
GBCM10494-17	Long Co, Tibet, China	TB, China	29.203755	87.393840
GBCM10641-17	Long Co, Tibet, China	TB, China	29.203755	87.393840
GBCM7979-17	Long Co, Tibet, China	TB, China	29.203755	87.393840
GBCM8372-17	Long Co, Tibet, China	TB, China	29.203755	87.393840
GBCM9857-17	Nama Chu, Tibet, China	TB, China	31.202102	88.758029
GBCM7640-17	Qinghai, China	QH, China	36.810395	100.354431
GBCM8102-17	Qinghai, China	QH, China	36.810395	100.354431
GBCMA1229-08	Qinghai, China	QH, China	36.810395	100.354431
GBCMA1226-08	Qinghai, China	QH, China	35.856319	96.644377
GBCM10733-17	Tibet, China	TB, China	31.202102	88.758029
GBCM10967-17	Tibet, China	TB, China	31.202102	88.758029
GBCM11037-17	Tibet, China	TB, China	31.202102	88.758029

GBCM11453-17	Tibet, China	TB, China	31.202102	88.758029
GBCM6489-17	Tibet, China	TB, China	31.202102	88.758029
GBCM6564-17	Tibet, China	TB, China	31.202102	88.758029
GBCM7293-17	Tibet, China	TB, China	31.202102	88.758029
GBCM8373-17	Tibet, China	TB, China	31.202102	88.758029
GBCM8484-17	Tibet, China	TB, China	31.202102	88.758029
GBCM9185-17	Tibet, China	TB, China	31.202102	88.758029
GBCM9657-17	Tibet, China	TB, China	31.202102	88.758029
GBCM9774-17	Tibet, China	TB, China	31.202102	88.758029
GBCM9943-17	Tibet, China	TB, China	31.202102	88.758029
GBCMA1227-08	Tibet, China	TB, China	31.202102	88.758029
GBCMA1228-08	Xinjiang, China	XJ, China	43.989796	87.728930
GBCMA4441-13	Xinjiang, China	XJ, China	43.989796	87.728930
GBCM10167-17	Yamzho Yum Co, Tibet, China	TB, China	28.923342	90.725632
GBCM6997-17	Yamzho Yum Co, Tibet, China	TB, China	28.923342	90.725632
GBCM1622-14	Iran	Iran	32.234140	54.291365

GBCMA4440-13	Selenge River, Bulgan Aimag, Selenge, Mongolia	Mongolia	49.360973	102.929881
GBCMA2689-10	Lake Baikal, Russia	Russia	53.921001	108.078196
GBCMA0152-06	Olkhon Island, Russia	Russia	53.179638	107.376859

Table S5 2 Assignment of localities to waterfowl flyways: Pacific American Flyway (PAF), Mississippi American Flyway (MAF), Atlantic American Flyway (AAF), East Atlantic Flyway (EAF), Black Sea/Mediterranean Flyway (BSMF), Central Asian Flyway (CAF), West Asian Flyway (WAAF), East Asian Flyway (EAsF), and West Pacific Flyway (WPF).

Sample ID/BOLD Accession Nos	Abbreviations of locality names	PAF	MAF	AAF	EAF	BSMF	CAF	WAAF	EAsF	WPF
CRCN087-09	BNP, AB, Canada	1	1	0	0	0	0	0	0	0
CRCN088-09	BNP, AB, Canada	1	1	0	0	0	0	0	0	0
CCMAL174-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL175-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL176-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL177-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL178-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL179-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL180-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL181-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
CCMAL182-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0

CCMAL183-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL001-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL002-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL004-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL005-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL006-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL007-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL013-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL015-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL016-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL017-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL018-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL019-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL021-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL028-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL029-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0

DSMAL030-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL031-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL033-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL040-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL042-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL044-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL052-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL053-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL054-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL057-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL064-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL065-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL066-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL074-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL075-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL076-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0

DSMAL077-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL078-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL080-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL081-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL087-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL088-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL089-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL093-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL099-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL100-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL103-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL104-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL106-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL107-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL110-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL111-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0

DSMAL113-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL114-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL115-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL116-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL117-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL121-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL122-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL124-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL125-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL126-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL132-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL133-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL135-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL136-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL137-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL139-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0

DSMAL142-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL143-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL145-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL146-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL147-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL149-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL152-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL153-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL155-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL156-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL157-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL159-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL162-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL163-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL165-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL166-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0

DSMAL167-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL168-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL172-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL173-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL175-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL176-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL178-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
DSMAL179-07	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
NJCGS103-10	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
NJCGS129-10	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
NJCGS130-10	CHU, MB, Canada	0	0	1	0	0	0	0	0	0
1A1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A11.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0

1A3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1A9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B11.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1B8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0

1B9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C11.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C5.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1C9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D11.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0

1D12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D5.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1D9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E11.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0

1E5.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1E9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F5.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1F9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0

1G11.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G12.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G2.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G5.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G6.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1G9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H10.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H4.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H5.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H7.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0

1H8.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
1H9.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2A1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2A3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2B1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2C1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2C3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2D1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2D3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2E1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2F1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2F3.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2G1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
2H1.seq	EDM, AB, Canada	0	1	0	0	0	0	0	0	0
BBGCO1245-15	GINP, BC, Canada	1	0	0	0	0	0	0	0	0
BBGCO1248-15	KNP, BC, Canada	1	1	0	0	0	0	0	0	0

BBGCO1250-15	KNP, BC, Canada	1	1	0	0	0	0	0	0	0
BBGCO1251-15	KNP, BC, Canada	1	1	0	0	0	0	0	0	0
BBGCO1252-15	KNP, BC, Canada	1	1	0	0	0	0	0	0	0
CRCN068-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN078-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN079-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN080-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN083-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN084-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN109-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN110-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN111-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN112-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN113-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN114-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN115-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0

CRCN139-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
CRCN141-09	RMNP, MB, Canada	0	1	1	0	0	0	0	0	0
GBCMA0153-06	VI, BC, Canada	1	1	0	0	0	0	0	0	0
CRCN116-09	WLNP, AB, Canada	1	1	0	0	0	0	0	0	0
CRCN121-09	WLNP, AB, Canada	1	1	0	0	0	0	0	0	0
CRCN148-09	WLNP, AB, Canada	1	1	0	0	0	0	0	0	0
CRCN149-09	WLNP, AB, Canada	1	1	0	0	0	0	0	0	0
CRCN150-09	WLNP, AB, Canada	1	1	0	0	0	0	0	0	0
CRCN151-09	WLNP, AB, Canada	1	1	0	0	0	0	0	0	0
NJCGS1053-11	WH, YT, Canada	1	0	0	0	0	0	0	1	0
2D2.seq	WPG, MB, Canada	0	1	1	0	0	0	0	0	0
2E2.seq	WPG, MB, Canada	0	1	1	0	0	0	0	0	0
2F2.seq	WPG, MB, Canada	0	1	1	0	0	0	0	0	0
2G2.seq	WPG, MB, Canada	0	1	1	0	0	0	0	0	0
2H2.seq	WPG, MB, Canada	0	1	1	0	0	0	0	0	0
NJCGS1176-11	ANC, AK, US	1	0	0	0	0	0	0	1	1

NJCGS1177-11	KP, AK, US	1	0	0	0	0	0	0	1	1
NJCGS1178-11	KP, AK, US	1	0	0	0	0	0	0	1	1
NJCGS1179-11	KP, AK, US	1	0	0	0	0	0	0	1	1
NJCGS1180-11	KP, AK, US	1	0	0	0	0	0	0	1	1
NJCGS1181-11	KP, AK, US	1	0	0	0	0	0	0	1	1
GBCMA0154-06	WA, US	1	1	0	0	0	0	0	0	0
GBCMA3487-12	YNP, WY, US	0	1	0	0	0	0	0	0	0
TLAMP059-15	Belarus	0	0	0	1	0	0	0	0	0
GALP048-16	Finland	0	0	0	1	0	0	0	0	0
GALP049-16	Finland	0	0	0	1	0	0	0	0	0
GALP050-16	Finland	0	0	0	1	0	0	0	0	0
GALP051-16	Finland	0	0	0	1	0	0	0	0	0
GALP052-16	Finland	0	0	0	1	0	0	0	0	0
GALP055-16	Norway	0	0	0	1	0	0	0	0	0
GALP056-16	Norway	0	0	0	1	0	0	0	0	0
GALP057-16	Norway	0	0	0	1	0	0	0	0	0

GBCMA4442-13	Slovenia	0	0	0	0	1	0	0	0	0
GBCMA4951-13	Ukraine	0	0	0	0	1	0	0	0	0
GBCM5804-17	TB, China	0	0	0	0	0	1	1	1	0
GBCM5953-17	TB, China	0	0	0	0	0	1	1	1	0
GBCM6312-17	TB, China	0	0	0	0	0	1	1	1	0
GBCM6883-17	TB, China	0	0	0	0	0	1	1	1	0
GBCM9184-17	TB, China	0	0	0	0	0	1	1	1	0
GBCMA1230-08	BJ, China	0	0	0	0	0	1	0	1	0
GBCMA1231-08	BJ, China	0	0	0	0	0	1	0	1	0
GBCMA1233-08	BJ, China	0	0	0	0	0	1	0	1	0
GBCM10088-17	QH, China	0	0	0	0	0	1	0	1	0
GBCM11120-17	QH, China	0	0	0	0	0	1	0	1	0
GBCM9186-17	QH, China	0	0	0	0	0	1	0	1	0
GBCM11342-17	TB, China	0	0	0	0	0	1	1	1	0
GBCM6172-17	TB, China	0	0	0	0	0	1	1	1	0
GBCM10732-17	TB, China	0	0	0	0	0	1	0	1	0

GBCM6230-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM6442-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM8767-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM9856-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM10494-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM10641-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM7979-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM8372-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM9857-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM7640-17	QH, China	0	0	0	0	0	1	0	1	0
GBCM8102-17	QH, China	0	0	0	0	0	1	0	1	0
GBCMA1229-08	QH, China	0	0	0	0	0	1	0	1	0
GBCMA1226-08	QH, China	0	0	0	0	0	1	0	1	0
GBCM10733-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM10967-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM11037-17	TB, China	0	0	0	0	0	1	0	1	0

GBCM11453-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM6489-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM6564-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM7293-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM8373-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM8484-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM9185-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM9657-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM9774-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM9943-17	TB, China	0	0	0	0	0	1	0	1	0
GBCMA1227-08	TB, China	0	0	0	0	0	1	0	1	0
GBCMA1228-08	XJ, China	0	0	0	0	0	1	1	1	0
GBCMA4441-13	XJ, China	0	0	0	0	0	1	1	1	0
GBCM10167-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM6997-17	TB, China	0	0	0	0	0	1	0	1	0
GBCM1622-14	Iran	0	0	0	0	0	1	1	0	0

GBCMA4440-13	Mongolia	0	0	0	0	0	1	0	1	0
GBCMA2689-10	Russia	0	0	0	0	0	1	0	1	0
GBCMA0152-06	Russia	0	0	0	0	0	1	0	1	0