Bird-associated permanent ectosymbionts examined at coarse and fine scales

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Systematics and Evolution

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University of Alberta

Abstract

All multicellular organisms host one or more species of symbiont. Endosymbionts of homeotherms experience relatively constant environments whereas those of ectotherms and all ectosymbionts experience variation in external environment. For ectosymbionts, both the presence of a host and a compatible external environment are necessary. Permanent symbionts (those with no off-host stages) are typically transferred from parent to offspring or via close contact between host individuals. There has long been an expectation that permanent symbionts should show patterns of genetic diversification similar to the host's. Using the host/symbiont systems of Rock Pigeons (Columbidae: *Columba livia* Gmelin) and Ovenbirds (Parulidae: *Seiurus aurocapilla* [Linnaeus]) and their permanent ectosymbionts (lice and mites), I investigated factors that influence ectosymbiont assemblage and population structure at both broad and fine scales.

To determine diversity of ectosymbionts that infest Rock Pigeons in Canada and whether there are geographic patterns in assemblages (Chapter 2), I sampled pigeons from: Vancouver BC, Calgary AB, Edmonton AB, Saskatoon SK, Winnipeg MB, Southern Ontario, and Halifax NS. I found 13 species of ectosymbionts: three of feather-dwelling mites, three skin mites, two nasal mites, and five feather lice. Only Vancouver and Halifax had all 13 ectosymbiont species, and only five species were found in every sampling location. I statistically assessed relationships between the local environment and the mite and louse assemblages. Annual precipitation, minimum humidity and maximum humidity of the month the pigeon was euthanized explained 10.6% of the variation in mite assemblages; annual maximum temperature, annual minimum humidity, and minimum temperature, and precipitation in the month before the pigeon was euthanized explained 10.7% of the variation in louse assemblages. Based on their more restricted

distributions, I conclude that feather mites are more strongly affected by local environment than other ectosymbiont groups, and that humid coastal environments are suitable for more ectosymbiont species than dry continental regions.

Rock Pigeons are non-migratory and are usually restricted to urban centres, suggesting that they should show strong genetic structure, which should be mirrored by their permanent ectosymbionts. In Chapter 3, I assess population structure of Rock Pigeons across Canada together with that of two of their louse species, *Columbicola columbae* (Linnaeus) and *Campanulotes compar* (Burmeister). ddRAD sequencing was used for pigeons and mtCOI for lice. Based on ΔK values and DAPC there were three genetic clusters for pigeons: one for Halifax, one for Vancouver and one that included all other sampling locations. The two louse species had different haplotype networks and the genetic structure of *C. compar* more closely resembled that of its host, matching predictions based on previous studies of its vagility relative to *C. columbae*. Rock Pigeons may be less sedentary than typically thought, or continuing introductions of Rock Pigeons are obscuring the signal of local differentiation of both bird and symbionts in most Canadian locations.

In Chapter 4, I move from broad- to fine-scale distribution of symbionts on hosts. Infestation parameters of quill mites (Syringophilidae) are rarely examined because this requires dissection of quills. These mites use their long chelicerae to pierce the quill wall to feed on living tissue, and reproduce only inside the quill; therefore, quill wall thickness and quill volume could influence their colonization success. I assessed the distribution of the quill mite *Betasyringophiloidus seiuri* (Clark) from Ovenbirds from Canada to determine if specific feathers have higher mite prevalences or intensities, and if quill-wall thickness and quill volume vary with either of these infestation parameters. I examined the flight feathers of 21 dead

ovenbirds, nine of which proved to be infested with quill mites. Feathers with the highest

prevalence were Primaries 1 and 2, and Secondaries 1, 2, and 5. There was a strong positive

correlation between quill volume and mean mite intensity. Feathers with quill walls thicker in

some areas than the mites' extended chelicerae had lower prevalences than feathers with walls

consistently thinner than the length of the chelicerae.

In summary, I found that aspects of both the ectosymbionts' immediate habitat, the host,

and the habitat of the host can potentially influence their broad- and fine-scale distribution and

assemblage structure. However, much of the variation in my results was not explained by the

parameters I examined; I recommend manipulative experiments in order to resolve some of the

remaining mysteries.

Preface

A version of Chapter 2 will be submitted for publication to Global Ecology and Biogeography. Order of authorship will be Grossi A. A. and Proctor H.C. I was responsible for experimental design, data collection and analysis as well as writing the manuscript. Dr. Heather Proctor was the supervisory author and involved in the experimental design and manuscript composition.

A version of Chapter 3 will be submitted for publication to Molecular Ecology. Order of authorship will be Grossi A. A, Campbell E. O. and Proctor H. C. I was responsible for experimental design, specimen collection, DNA extractions, louse preparation for sequencing, louse data analysis and SNP analysis for Rock Pigeons, as well as writing the manuscript. Sophie Dang was responsible for ddRAD sequencing library preparation. Dr. Erin Campbell was responsible for bioinformatics data processing. Dr. Heather Proctor was the supervisory author and involved in the experimental design and manuscript composition.

A version of Chapter 4 of this thesis has been published as Grossi A.A., and Proctor H. C. (2020) "The Distribution of Quill Mites (*Betasyringophiloidus seiuri*) among Flight Feathers of the Ovenbird (*Seiurus aurocapilla*)," Journal of Parasitology, 106(1): 82-89. I was responsible for experimental design, data collection and analysis, as well as writing the manuscript. Dr. Heather Proctor was the supervisory author and involved in the experimental design and manuscript composition.

Acknowledgments

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1 - General Introduction

1.1 Evolutionary and ecological relationships between hosts and symbionts

The close association between a host species and its symbionts offers an interesting way to study evolutionary histories, invasion dynamics, past and current population structures and cryptic speciation. Symbiosis was originally defined as "living together of dissimilarly named organisms " (de Bary, 1879), and while the meaning has taken a few detours over the years, in its currently most common definition it still simply means " living together" (Martin and Schwab, 2012). The symbiont in this relationship is usually the smaller organism and the larger one is the host. The effect a symbiont has on its host falls along a continuum between two extremes: at one extreme both parties may benefit (mutualism), at the other the symbiont can have a detrimental effect on its host (parasitism), and somewhere in the middle is a neutral effect of symbiont on host (commensalism). If the influence of a particular symbiont on its host falls into one category at one point in time it does not mean it is necessarily fixed there; as under different circumstances its influence can move along this gradient.

1.2 Symbionts as 'tags' of their host populations

Because of their physical association with host organisms and hypothetically higher rate of genetic divergence (Johnson et al., 2014), symbionts have been used as 'biological tags' for assessment of population structure in their hosts. Different populations of hosts may have different assemblages of symbionts, or their symbionts may vary genetically at the intraspecific level in a way that mirrors population structure of their hosts. Because of this, assessment of symbionts has been suggested as part of management strategies to help distinguish host populations. (MacKenzie and Abaunza, 1998). Biological tags have been used to distinguish between resident colonies of Double-crested Cormorants (*Phalacrocorax auratus* [Lesson])

1

which are protected, from colonies made up of migratory individuals that are unprotected, which current molecular studies were unable to separate (Sheehan et al., 2016). There was enough variation in assemblages of intestinal parasites to correctly assign cormorant individuals as migratory or resident 78% of the time. These different parasite assemblages result from migratory individuals encountering a wider diversity of parasites compared to residents. Biological tags can even give clues about past local extinctions. Population genetic structure of the mite *Spinturnix myoti* Kolenati shows co-differentiation with its host, the Maghrebian Bat (*Myotis punicus* Felten), across North Africa and a Mediterranean island (Bruyndonckx et al., 2010). On the Mediterranean island of Corsica, Bruyndonckx et al. (2010) determined that mites originated from continental Europe whereas their hosts had a North African origin. The most likely explanation for this inconsistency in origin is a host switch from the European Mouse-eared Bat (*Myotis myotis* Borkhausen). However, this species of bat is not currently found on Corsica suggesting they once existed there together in the past.

In order to use symbionts successfully as biological tags, one must know where they are found. Is the symbiont of interest found alongside the host across their whole range or is its distribution different then that of its host? In addition, does the symbiont display the "classic" aggregated distribution in which many hosts have few symbionts and few hosts have many symbionts (Gordon, 1982; Poulin, 2007)? This distribution affects how many hosts one needs to sample in order to accurately assess population level presence/absence of a symbiont. Other aspects of scale are important when conducting symbiont surveys. Take vane-dwelling feather mites for example; at a large geographic scale as elevations increase, intensities of proctophyllodid feather mites infesting passerines decrease (Meléndez et al., 2014). At an even finer scale, symbionts vary in abundance on/in the body of a single host. For example, the feather

mite *Proctophyllodes stylifer* (Buckholz) which is found on the wings of blue tits (*Parus caeruleus*, [Linnaeus]) and depending on the season will change its distribution on the wings (Wiles et al., 2000); in winter time it is found exclusively on the tertiary feathers, but in the summer and autumn can be observed all the way to the outermost primary.

1.3 Permanent ectosymbionts of birds

In my thesis I examine the distribution of permanent ectosymbionts associated with birds. Birds have a diverse group of symbionts associated with them, which utilize almost all external surfaces of the bird. Some ectosymbionts, such as ticks, fleas and many mesostigmatid mites, have free-living stages off the body of the host and are considered 'non-permanent'. Permanent ectosymbionts of birds include: feather-dwelling mites, skin mites, nasal mites, quill mites and feather lice. All of these lack free-living stages and rely primarily on direct contact between hosts to disperse (Atyeo and Gaud, 1979; Marshall, 1981).

Feather-dwelling mites (Astigmata: most families of Analgoidea and Pterolichoidea), reside on the surface of feathers. They are dorsoventrally flattened with long lateral setae, allowing them to fit between the barbs of feathers. They are usually considered harmless commensals (Galván et al., 2012), and may even play a role in cleaning the feathers by consuming bacteria and fungus (Doña et al., 2019). Skin mites (Astigmata: Analgoidea: Epidermoptidae, Dermationidae; Prostigmata: Harpyrhynchidae and some Cheyletidae) have round bodies with short legs (Krantz and Walter, 2009). These are skin- and fluid-feeding parasites that either live on top of or burrow within the skin, forming cysts (Moss and Oliver, 1968). Quill mites (Astigmata: some families of Analgoidea and Pterolichoidea; Prostigmata: Syringophilidae) live inside the hallow calamus of the feather and depending on the species will either use their long chelicera to pierce the quill wall to feed or use chewing mouthparts to

3

consume the pith of the feather (Kethley, 1971; Gaud and Atyeo, 1996). Nasal mites (Mesostigmata: Rhinonyssidae; Astigmata: Turbinoptidae) feed on blood and other tissues within the respiratory passages of their hosts (Krantz and Walter, 2009). Feather lice (Phthiraptera: Ischnocera and Amblycera) live on and consume the feathers of their host. If feather louse populations are not regulated through host preening, they can impact the thermoregulatory quality of the feathers (Clayton, 1991). Some species are also known to be opportunistic feeders of blood (Marshall, 1981).

1.4 The Rock Pigeon system

To examine the distribution of ectosymbionts across a large geographical range I chose to examine the ectosymbiont assemblages of Rock Pigeons (Columbiformes: Columbidae: *Columba livia,* Gmelin). This host was chosen for several reasons, both theoretical and pragmatic: it is found across Canada with the exception of the far north; there are many studies of ectosymbionts infesting *C. livia* in both its native and introduced ranges that I could compare my results to; members of the public bring them into rehabilitation centers where many of the birds succumb to their injuries and are available for scientific study.

Rock Pigeons are native to eastern Europe, western Asia and northern Africa, but due to human introductions, are now found on every continent except Antarctica (Goodwin, 1970). Originally found nesting and roosting in caves and on cliffs they have adapted to human built structures and can now be found in barns, under bridges and have substituted ledges found on buildings for cliff ledges. Rock Pigeons have clutches of 2 eggs, which are incubated for $17 - 18$ days. Once hatched, young are fed "pigeon milk", which is a secretion from the crop lining, and fledge within 14-19 days of hatching (Saxena et al., 2008). Though thought of as a sedentary species, dispersal between colonies does take place. Hetmański, (2007) found that 20-30% of

4

non-reproducing young in Słupsk, Poland would leave their natal colonies usually to join a colony in the same city with a lower density of pigeons. There are two foraging strategies for urban pigeons: foraging within their urban environment and leaving their urban environment to forage up to 25 km away in adjacent agricultural areas (Johnston and Janiga, 1995; Rose et al., 2006). Rock Pigeons were introduced to North America in the 1600's (Schorger, 1952), which adds an interesting element when examining their ectosymbiont assemblages. If a particular sampling location is missing an ectosymbiont species found elsewhere in North America there could be two potential reasons for this: (1) none of the pigeons that colonized that location were infested with that symbiont species ('missing the boat'; Macleod et al., 2010); (2) some of the pigeons that colonized the new location were infested with that species but other factors made that location uninhabitable for the ectosymbiont ('lost overboard'; Macleod et al., 2010). Chapters 2 and 3 of my thesis assesses evidence for each of these options. In Chapter 2, I sampled Rock Pigeons from seven locations that span Canada from coast to coast in order to (1) assess what ectosymbionts are found on Pigeons in Canada and how ectosymbiont assemblages vary based on location, and (2) to see if the variation in assemblages is correlated with local climatic factors. Ectosymbionts being on the outside of their host are exposed to the changes in local temperature and humidity, both of which have been shown to affect ectosymbiont assemblages (Bush et al., 2009; Meléndez et al., 2014). Rock pigeons are a non-migratory species typically restricted to human settlements, but how much connectivity do they have between different urban centres throughout Canada? It could be that once settled into a location, pigeons seldom move between urban centres, thereby also restricting movement of symbionts. Or are pigeons moving between urban centres and taking their ectosymbionts with them, but local climatic conditions are not suitable for the survival of particular ectosymbiont species? In

Chapter 3, I examined the population genetic structure of Rock Pigeons in Canada to assess evidence for population structuring associated with sampling locations: strong structuring would suggest little gene flow. I also determined the genetic structure of two louse species, *Columbicola columbae* (Linnaeus) and *Campanulotes compar* (Burmeister), to test if their population structure reflects geographic differentiation in the host populations (i.e., do they act as 'biological tags'?).

1.5 The Ovenbird system

As part of a larger study that was looking at host movement (not included in my thesis), I looked at the population genetic structure of *Betasyringophiloidus seiuri* (Clark) a quill mite in Ovenbirds, *Seiurus aurocapilla* (Linnaeus), to see if it could be used as a biological tag to reveal fine population structuring in its host (Haché et al., 2017). There are three named subspecies of *Seiurus aurocapilla* based on morphological characteristics: *S. a. aurocapilla* found in central and southeastern Canada and throughout the eastern U.S.A., *S. a. furvior* Batchelder found exclusively in Newfoundland, and *S. a. cinereus* Miller found in the Rocky Mountains of southern Alberta and through western and central U.S.A. (Clements, 2007). The Ovenbird is a migratory species, and based on previous work, eastern and western breeding populations of *S. a. aurocapilla* have segregated overwintering areas (Hallworth and Marra, 2015), suggesting that there is separation by flyway. We determined that based on COI variation, *B. seiuri* is not a good candidate for a biological tag (Haché et al., 2017); however, the sampling procedure to collect the quill mites, which was to pull two tail feathers from live birds, inspired my interest in exploring ectosymbiont distribution upon the host. Haché et al. (2017) found that quill mites from the tail feathers had a very low prevalence (5.1%) compared to other syringophiline species with passerine hosts; however, *B. seiuri* has been recorded from the wing feathers in other

studies (Bochkov and Galloway, 2001). This made me question if there were other feathers that had higher prevalences. In Chapter 4 I examine the flight feathers (wing and tail) of 21 dead Ovenbirds to (1) determine which feathers have the highest prevalence, and (2) whether there is a correlation between mite prevalence and intensity and either quill wall thickness or quill volume. Since quill mites feed by piercing the quill wall with their chelicerae, if feathers have walls thicker then their chelicerae this would limit their distribution to particular feathers (Kethley, 1971). I examined quill volume because quill mites do not reproduce outside of the quill and only disperse between feathers when their host is molting; therefore, each quill may have to hold multiple generations of mites between moulting periods.

Chapter 2 - Variation in Ectosymbiont Assemblages infesting Rock Pigeons (*Columba livia*) from Coast to Coast in Canada

2.1 Introduction

The introduction of a foreign species to a new habitat, whether it be on purpose or by accident, has the potential to also introduce new symbionts (Torchin and Mitchell, 2004; Lymbery et al., 2014; Sheath et al., 2015). However, successful colonization of a new area by a host does not automatically mean its symbionts are also successful. 'Sorting events' can result in a symbiont failing to become established in a new habitat, which often results in newly introduced hosts having a lower diversity of symbionts than in their native ranges (Paterson et al., 2003; Torchin et al., 2003; Macleod et al., 2010). Most obviously, for a symbiont to successfully colonize a new habitat it must not "miss the boat", i.e., it needs to be present in or on at least some of the members of the founding host population. But even if the symbiont is present on members of the founding host population, it still may fail to survive in the new area. If there are too few conspecific symbionts or if they are highly aggregated among host individuals, the chance at successful establishment decreases (Poulin, 2007). The life cycle of the symbiont also plays a role in successful colonization. Symbionts with indirect life cycles need both intermediate and final hosts, and may have a lower probability of establishment than those with direct life cycles that require only a single host species (Paterson et al., 2003).

Studies of host-symbiont introductions and sorting events have frequently focused on birds and their ectosymbiotic arthropods (Dabert et al., 2001; Dabert, 2003, 2004; Paterson et al., 2003; Banks et al., 2006; Johnson et al., 2006). Ectosymbionts of birds include feather mites, skin mites, nasal mites and chewing lice (Gaud and Atyeo, 1996; Price et al., 2003). Although not all of these are closely related phylogenetically, they are all permanent ectosymbionts with

direct life cycles and no stages that live off the body of the host. Most of them rely on direct contact between hosts to disperse (Marshall, 1981; Proctor, 2003), although some species of skin mites and chewing lice can disperse by phoresy on hippoboscid flies (da Cunha Amaral et al., 2013). Vane-dwelling feather mites or chewing lice are most often the subjects of these 'sorting-event' studies (Dabert, 2004; Macleod et al., 2010), while to my knowledge there are no studies that have examined the whole ectosymbiont infracommunity of a host.

An avian host that has been incredibly successful expanding its range is the Rock Pigeon (Columbiformes: Columbidae: *Columba livia,* Gmelin). The original range of the Rock Pigeon, also commonly known as the Rock Dove or just 'feral pigeon', is eastern Europe, western Asia and northern Africa, but due to human introductions, it is now found on every continent except Antarctica (Goodwin, 1970). Within Canada, Rock Pigeons are found year-round in most urban centres as far north as Whitehorse in the Yukon (eBird, 2017). They were originally brought with the French to Nova Scotia's Port Royal in the early 1600's (Schorger, 1952), and with them came at least some of their ectosymbionts. Like many birds, a single Rock Pigeon can host several ectosymbiont species at the same time. However, published ectosymbiont-host records for Rock Pigeons within Canada are scarce and are focused on *C. livia* populations in only a few provinces with the most detailed records coming from Manitoba due to the long term research of Terry Galloway on feather lice (Lamb and Galloway, 2018; Galloway, 2019; Galloway and Lamb, 2019). Outside of Manitoba, published records of feather lice and other ectosymbionts of *C. livia* are sparse. This is probably due to a combination of lack of reporting and true absence of particular ectosymbiont species from some regions. For example, there is only one published report of the vane-dwelling feather mite *Falculifer rostratus* (Buchholz) (Astigmata: Pterolichoidea: Falculiferidae) in Canada, from Rock Pigeons in Ontario (Mitchell, 1953);

9

however, H. Proctor (unpublished) has observed this mite species in great numbers on *C. livia* in coastal British Columbia (Vancouver). Conversely, *F. rostratus* has never been found on Rock Pigeons from Edmonton, Alberta (H. Proctor, unpublished). There are several potential explanations for the apparent lack of *F. rostratus* on Rock Pigeons in Edmonton. It could be due to sampling error; if *F. rostratus* is present in low numbers and at low prevalence, it is possible that it has yet to be detected. If so, increased sampling should reveal this and possibly other as-yet unrecorded ectosymbionts. Alternatively, it may truly be absent from *C. livia* in Edmonton. Such an absence could arise in two ways: (1) the symbiont may have "missed the boat" and was never on any of the birds that colonized Edmonton; (2) the symbiont may have made it to Edmonton but failed to survive and reproduce. A drawback to being a permanent ectosymbiont is that there is little control over where you go and also little control over the abiotic environment you are exposed to. Rock Pigeons are sedentary (non-migratory) and overwinter in the same area that they spend the summer. *Falculifer rostratus* may not be found in Edmonton because the cold, dry winter environment of central Alberta is not suitable for its survival. Abiotic factors have been shown to influence ectosymbiont assemblages (Dowling et al., 2001; Moyer et al., 2002; Carrillo et al., 2007). This is seen in the vane-dwelling feather mites of the family Proctophyllodidae on several species of passerine hosts along an elevational gradient in the Cantarain Mountains of Spain (Meléndez et al., 2014). The authors found that as elevation increased, there was a constant decline in the mean number of feather mites on infested hosts (= intensity). This decline strongly correlated with the decline in temperature along the elevation gradient. Temperature is not the only environmental factor known to affect ectosymbiont survival. Humidity has been shown to influence louse abundance. For example, chewing louse prevalence and intensity on two species of doves (Columbidae) were significantly lower in arid

10

environments (Tucson, Arizona) than in a humid environment (Weslaco, Texas) (Moyer et al., 2002). Response to humidity appears to be taxon-specific, as some louse species are scarce in areas with long humid seasons (Fabiyi, 1996) and some can survive arid periods through their more resistant eggs (Carrillo et al., 2007).

In this study I had two main goals: (1) to increase our knowledge of the diversity and distribution of arthropod ectosymbionts of *Columba livia* across Canada; (2) to test whether geographic variation in these ectosymbiont assemblages is more suggestive of a history of "missing the boat" or of being influenced by abiotic factors including cold, dry winter weather.

2.2 Materials and Methods

2.2.1 Collection and ectosymbiont identification

Rock Pigeons were salvaged from rehabilitation centres and a monitoring program nspanning Canada from the west to the east coasts: Vancouver, British Columbia; Calgary, Alberta; Edmonton, Alberta; Saskatoon, Saskatchewan; Winnipeg, Manitoba; Kingston, Ontario; Belleville, Ontario; Toronto, Ontario; and Halifax, Nova Scotia (see Appendix 2.1 for latitudes, longitudes and collection dates). Due to the similar latitude and relatively close proximity of Kingston, Belleville and Toronto (~240 km), Rock Pigeons from these locations were combined and are referred to as from Southern Ontario (S. Ontario). A few pigeons came from locations outside of the city the rehabilitation centre is located (Appendix 2.1). Due to how pigeons were acquired, they were not sampled evenly throughout the year; 52% of pigeons examined in this study were collected in four months of the year. Therefore, temporal changes in ectosymbiont assemblages were not examined, see Appendix 2.2 for variation in collection dates.

At the rehabilitation centres the Rock Pigeons were euthanized, individually bagged, then frozen immediately. Depending on the location, I either picked up the frozen pigeons in person

11

or had them shipped overnight to the University of Alberta. Ectosymbionts were removed by washing the pigeons according the procedure outlined in Grossi et al. (2014), with the addition of a nasal rinse (20 ml of water was forced through each nostril using a syringe, with backwash being collected in the general washings bucket). Washings were passed through a 43 µm sieve, a mesh size fine enough to retain small skin mites. Ectosymbionts collected from the pigeon washings were stored in 95% ethanol.

Each washing was examined under a dissecting microscope (Leica MEB126, Leica Microsystems Inc., Concord Canada). All lice (adults and nymphs) were counted and removed and a subset of each morphospecies of mite was removed. Due to the large number of mites present, they were only identified to species but were not counted. Pigeons from Toronto were collected as part of the Fatal Light Awareness Program (FLAP) as already dead birds due to window strikes, and it is therefore not known how long the pigeons were deceased before they were collected. Since lice are known to leave the body of a dead host (Petryszak et al., 1996), louse collection data was only used in prevalence calculations, while mite collection data was used for all analysis. I slide-mounted representatives of each louse and mite morphotype. These specimens were first cleared in 85% lactic acid (Fisher Scientific, Fair Lawn, New Jersey) for 24 hours, and then slide mounted in commercially available phenol-free poly-vinyl alcohol medium (PVA; BioQuip Products, Rancho Dominguez, California). Slides were cured for 4 days at ca. 40 °C on a slide-warmer and then examined using Differential Interference Contrast optics on a Leica DMLB compound microscope. The following literature was used to identify the ectosymbionts: Gaud, (1976), and Gaud and Atyeo, (1996) for feather mites; Smiley, (1970), and Bochkov and Galloway, (2001) for skin mites; Knee et al., (2008) for nasal mites; Emerson, (1957), Price et al., (2003), Adams et al., (2007) and Galloway and Palma, (2008) for lice.

Exemplars of each species have been deposited in the E.H. Strickland Entomological Museum at the University of Alberta (UASM80575 - UASM80587).

2.2.2 Ectosymbiont Assemblages

Prevalence, the proportion of host individuals infested with one or more individuals of a particular symbiont species (Bush et al., 1997) was calculated for each species of ectosymbiont at each sampling location. Mean intensity, the average number of a particular symbiont among infected hosts (Bush et al., 1997) was calculated for each louse species at each sampling location. I used the Quantitative Parasitology software package (Rózsa et al., 2000) to calculate both metrics. In order to provide a comparative context for interpretation of my results, I compiled records of ectosymbionts of *C. livia* from published and unpublished surveys from as far back as I could find published records (1971).

I used non-metric multidimensional scaling (NMDS) to explore differences in ectosymbiont assemblages between sampling locations. NMDS was conducted on the mite and louse assemblages separately. For these analyses, the mite dataset consisted of presence/absence data and the louse dataset consisted of count data. I produced NMDS plots using the "metaMDS" function in the Vegan package v. 2.4-6 (Oksanen et al., 2018) in the R statistical program (R Core Team, 2018). Jaccard distance was used for the mite prevalence data and the louse count data was first ln (x+1) transformed and Bray – Curtis distance was used. To examine observed differences in ectosymbiont assemblages based on sampling location, I performed permutational multivariate analysis of variance (PERMANOVA) on each dissimilarity matrix using the "adonis" function in the Vegan package, with 999 permutations. This was followed by *post hoc* pairwise comparisons between sampling locations, with the p-values adjusted using the Bonferroni correction (Bland and Altman, 1995).

2.2.3 Potential Effects of Local Climate on Ectosymbiont Assemblages

To assess potential effects of meteorological variables on ectosymbiont assemblages I conducted a distance-based redundancy analysis (dbRDA) on the mite data and a redundancy analysis (RDA) on the louse data. I assembled meteorological data from Environment Canada (https://climate.weather.gc.ca/prods_servs/cdn_climate_summary_e.html). These data consisted of records from the collection location of the pigeon, including the following: minimum, maximum, and average temperature and humidity and total precipitation, from the month before each bird was euthanized ('previous monthly'); from the month that the bird was euthanized ('monthly'); and for the month that the bird was euthanized plus the previous 11 months ('annual'). Some pigeons had no collection date associated with them, and therefore meteorological data could not be associated with those individuals. This caused sample sizes of hosts to differ from those in previous analyses (see Table 2.1) for several sites: Calgary n = 28, Edmonton n = 18, Saskatoon n = 0, S. Ontario n = 5 (lice), 7 (mites), Halifax n = 21.

Since meteorological variables were measured in different units, these data were standardized using the "decostand" function in the vegan package in R. To reduce collinearity among meteorological variables, I performed a stepwise reduction based on Variance Inflation Factor (VIF), in which variables with the largest VIF values were removed until only variables with VIF values less then 5 remained (Simon, 2009). This was done for the mite and louse data separately using the "vif" function from the car package in R. For mites the dbRDA was performed using the "capscale" function in the vegan packages using Jaccard distance. For lice the RDA was performed using the "rda" function in the vegan package. In both cases, meteorological variables were selected using forward model choice with the "OrdiR2step" function in the vegan package in R. P-values of selected meteorological variables were corrected

for multiple comparisons using the Holm–Bonferroni method. Variation partitioning was used to determine how much of the variation was explained by the selected meteorological variables verses spatial variation. The spatial component was calculated by transforming the latitude and longitude of pigeon collection locations using distance-based Moran's eigenvector maps spatial eigenfunctions with the package adespatial, and variation partitioning was carried out with the "varpart" function.

2.3 Results

2.3.1 Diversity, prevalence and intensity

  I examined a total of 162 Rock Pigeons from the seven sites in Canada and found 13 ectosymbiont species (Tables 2.1 and 2.2). Eight of these were mites: feather-dwelling mites *F. rostratus*, *Pterophagus columbae* (Sugimoto) and *Diplaegidia columbae* (Buchholz); the skin mites *Harpyrhynchoides gallowayi* Bochkov, OConnor and Klompen, *Harpyrhynchoides columbae* (Fain), *Ornithocheyletia hallae* Smiley; the nasal mites *Tinaminyssus melloi* (Castro) and *Tinaminyssus columbae* (Crossley). A total of 48 025 lice were collected, made up of five species: *Columbicola columbae* (Freire & Duarte), *Campanulotes compar* (Burmeister), *Coloceras tovornikae* Tendeiro, *Hohorstiella lata* Piaget, and *Bonomiella columbae* Emerson (Appendix 2.3). Additionally, two louse species, *Myrsidea* sp. (1 female; 1 male; 1 nymph) and *Machaerilaemus maestus* (Kellogg and Chapman) (2 females) were each found on a single host from Calgary and Nova Scotia, respectively. Due to their occurrence, each on only a single host and the low number of individuals collected, I believe these are 'stragglers', lice found on atypical hosts where they are assumed to be unable to maintain populations (Ròzsa, 1993). Stragglers have been shown to be the precursor for a host switching events, and therefore their presence should be noted.

The feather-dwelling mites *F. rostratus*, *P. columbae* and *D. columbae* had highest prevalences in Vancouver and Halifax and while they all had different distributions across sampling locations, they were all absent from Saskatoon (Figures 2.1, 2.2 and 2.3, Table 2.1). For skin mites, *H. gallowayi* and *O. hallae* were found in all locations and *H. columbae* was found infesting pigeons from Vancouver, Edmonton, Southern Ontario and Halifax (Figure 2.4, 2.5, and 2.6, Table 2.1). All three skin mites had their highest prevalences in Southern Ontario. Both species of *Tinaminyssus* nasal mites were found in every location (Table 2.1). *Tinaminyssus melloi* had the highest prevalence in Vancouver and the lowest prevalence in Edmonton (Figure 2.7), while *T. columbae* had the highest prevalence in Edmonton and the lowest in Southern Ontario (Figure 2.8).

Of the lice, only two species were found in every location: *Colu. columbae* and *Camp. compar* (Figures 2.9A and 2.10A, Table 2.2); both species had high prevalences in all locations. Mean intensity varied based on location for both species with *Colu. columbae* having the highest mean intensity in Vancouver (326.07 lice/infested pigeon) and Halifax (303.13 lice/infested pigeon) (Figure 2.9B, Table 2.3). *Campanulotes compar* also had the highest mean intensities in Vancouver (282.0 lice/infested pigeon) and Halifax (239.17 lice/infested pigeon) (Figure 2.10B). *Coloceras tovornikae* was found in Vancouver, Calgary, Saskatoon, Winnipeg, and Halifax (Figure 2.11A) with the highest prevalence and mean intensity (Figure 2.11B) in Winnipeg (56.7%, 54.24 lice/infested pigeon, respectively). *Hohorstiella lata* was found in Vancouver, Edmonton, Winnipeg, and Halifax (Figure 2.12A) with the highest prevalence in Vancouver (26.7 %), and the highest mean intensity in Edmonton (182.0 lice/infested pigeon); however this is based on only a single heavily infested individual (Figure 2.12B). In all other locations the mean intensities for *H. lata* were less then 20 lice/infested pigeon. *Bonomiella columbae* was

16

found in Vancouver, Saskatoon and Halifax (Figure 2.13A); however only one individual pigeon

was infested in Saskatoon. The mean intensity of *B. columbae* was low with the highest being

7.25 lice/infested pigeon in Vancouver (Figure 2.13B).

2.3.2 Assemblage structure

The NMDS plot for mites (Figure 2.14) shows overlap in the mite assemblages between

the different locations. To examine if there were significant differences in assemblages between

locations, I conducted a PERMANOVA; this showed that there was a significant difference in

assemblage structure between the locations (df = 6, F-value = 7.01, R^2 = 0.284, p = 0.001).

Pairwise comparisons among locations revealed that mite assemblages in Vancouver were

significantly different from every other location except Halifax and that Halifax's mite

assemblages were significantly different from every other location other then Vancouver (Table

2.4). The louse NMDS plot (Figure 2.15) shows even more overlap between the locations with

most overlaid on top of each other with the exception of Winnipeg, which was influenced by the

high intensities of *Colo. tovornikae.* The PERMANOVA for the lice also was significant (df = 6,

F-value = 4.40, R^2 = 0.162, p = 0.001). Pairwise comparisons among locations revealed that

louse assemblages in Winnipeg were significantly different from those in Vancouver, Edmonton

and Halifax, and that Vancouver was significantly different from Calgary (Table 2.4).

2.3.3 Potential Effects of Local Climate on Ectosymbiont Assemblages

The dbRDA model for mite assemblages was significant (p = 0.001), and three

meteorological variables were found to potentially influence mite assemblages: annual

precipitation, monthly minimum humidity and monthly maximum humidity (Figure 2.16). After

Holm –Bonferroni correction, monthly maximum humidity was not significant (Table 2.5).

Through variance partitioning, the meteorological variables alone explained 10.6 % of the

variation, spatial variables alone explained 3.0 % and the meteorological x spatial interaction

explained 9.5 % of assemblage composition, leaving ~77 % of the variance unexplained.

The RDA model for lice was also significant (p = 0.001) and four meteorological

variables were found to potentially influence louse assemblages: previous monthly minimum

temperature, previous monthly precipitation, annual maximum temperature and annual minimum

humidity (Figure 2.17). After Holm – Bonferroni correction, only annual maximum temperature

was significant (Table 2.5). Through variance partitioning, the meteorological variables alone

explained 10.7 % of the variation, spatial variables alone explained 0.2 % and the environmental

x spatial interaction explained 10.5 % of assemblage composition, leaving ~78.6 % of the

variance unexplained.

2.4 Discussion

Of the thirteen ectosymbiont species I found on Rock Pigeons, four (all mites) are new

collections recorded for Canada: *Ornithocheyletia hallae,* which has been recorded from Rock

Pigeons in Brownsville Texas (Smiley, 1970), and *Pterophagus columbae, Diplaegidia*

columbae and *Harpyrhynchoides columbae,* which have not been recorded on Rock Pigeons in

North America. All other mite and louse species have been previously recorded from Rock

Pigeons in Canada (Rayner, 1932; Emerson, 1957; Galloway and Palma, 2008; Knee et al., 2008;

Bochkov et al., 2015). *Passeroptes bispinosa* (Banks), a skin mite, was previously recorded on

Rock Pigeons in Ontario (Banks, 1909), but was not found infesting any of the pigeons used in

this study. There are also an additional fourteen species of permanent ectosymbionts that have

been recorded from Rock Pigeons worldwide that were not found in this survey. Two louse

species *Colpocephalum turbinatum* Denny and *Columbicola tschulyschman* Eichler, have been

recorded from Rock Pigeons in Louisiana and Utah U.S.A., respectably (Hill and Tuff, 1978;

Harbison et al., 2008). The remaining 12 ectosymbionts have never been recorded on Rock Pigeons within North America; four skin mites (*Megninia cubitalis* Megnin, *Myialges anchora* Sergent and Trouessart, *Myialges lophortyx* [Furmann and Tharshis] and *Rivoltasia bifurcata* [Rivolta]), three feather-dwelling mites (*Pterophagoides paradoxus* Gaud and Barré, *Pterophagus strictus* Mégnin and *Pterolichus obtusus* Robin) and five louse species (*Coloceras aegypticum* [Kellogg and Paine], *Coloceras damicorne* [Nitzsch], *Coloceras israelensis* (Tenderio), *Coloceras liviae* (Tenderio), and *Physconelloides zenaidurae* (McGregor)) (Oudemans, 1935; Dubinin, 1947b; Gaud and Petitot, 1948; Schorger, 1952; Dubinin, 1956; Cerny, 1970; Millthorpe and Eves, 1971; Gaud and Barré, 1988; Price et al., 2003; da Cunha Amaral et al., 2013).

The distribution of ectosymbionts was not homogeneous across all sampling locations. There were two locations, Vancouver and Halifax, in which all ectosymbiont species found in this study were present. It should be noted that prevalence was not invariably high at these two locations, as some ectosymbiont species were only found infesting one host. In addition to high ectosymbiont richness, the mite species *Pterophagus columbae* was only found in these locations. It is not surprising that pigeons in Halifax are host to this high diversity of ectosymbiont species since this is a known point of introduction (Schorger, 1952). The equally high diversity in Vancouver, and sharing of the otherwise rare species *P. columbae*, was not expected as these two locations are the most distant from each other. However, both Vancouver and Halifax are port cities, and therefore have coastal elements influencing their climates. It's also possible that *C. livia* and its ectosymbionts could have been introduced independently to Vancouver via importation of pigeons from Europe; however, I know of no such importation

records. Mite assemblages in Vancouver and Halifax were significantly different from those in other sampling locations (Table 2.4); however, this pattern was not seen in lice.

For this study Rock Pigeons were mainly acquired from rehabilitation centres, which presents two main drawbacks. First, I had no control over the number of pigeons that were turned in to the rehabilitation centres, this resulted in an uneven sample size of hosts across sampling locations and season. Therefore, it is possible that ectosymbionts in some locations were not detected, especially in locations that have smaller sample sizes, and for ectosymbiont species that have low prevalence. Second, I had no control over the health of the pigeons sampled. If the reason the pigeon was admitted to the centre (injury, illness) also hindered its ability to preen and scratch, this could cause their ectosymbiont loads to be higher then those found on healthy pigeons. The vane-dwelling feather mite *Falculifer rostratus* had never been observed on pigeons from Edmonton before (H. Proctor, unpublished) and I also did not find it on the 23 pigeons examined from Edmonton. This species was also absent from pigeons from Saskatoon. *Falculifer rostratus* as well as the down-dwelling *Diplaegidia columbae* had highest intensities in Vancouver and Halifax, both of these mite species live on feathers and therefore are more exposed to local climatic changes than mites that live closer to the surface of the host's body. In contrast, skin mites and nasal mites that may be less exposed did not have elevated prevalences in coastal sampling locations and they were found infesting pigeons in every location sampled except for *H. columbae* which had a patchy distribution across Canada. The meteorological variables that were significant were annual precipitation and monthly minimum humidity. Both precipitation and humidity have been shown to influence feather-dwelling mites. In the vane-dwelling mite *Proctophyllodes troncatus* Robin, it has been experimentally shown that water can be taken up from the atmosphere only down to a relative humidity of 55% (Gaede and Knülle,

1987). As vane-dwelling mites do not feed on fluids produced by their host and their sources of food are not likely to provide adequate amounts of water, it is likely that the majority of their water comes from the atmosphere; if true, this would explain why locations like Edmonton and Saskatoon that are very dry in the winter are not suitable for feather dwelling mites.

Columbicola columbae and *Camp. camper* were the only louse species found infesting pigeons in all six sampling locations. Both species generally had high prevalences, with *Colu. columbae* infesting 100% of the pigeons sampled from Vancouver and Halifax. The other louse species *Colo. tovornkiae, H. lata,* and *B. columbae* all had patchy distributions with on average much lower mean intensities then those shown by *Colu. columbae* and *Camp. camper.* The meteorological variable that was significant is annual maximum temperature. Nelson and Murray (1971) experimentally manipulated the temperature that *Colu. columbae, Camp. compar,* and *H. lata* were raised at and found that temperatures between 32 – 40 ˚C were optimal for oviposition and egg development.

While annual changes in ectosymbiont intensities were not examined in this study, Galloway and Lamb (2015) examined 542 Rock Pigeons from Winnipeg Manitoba over a 10 year period; they found that *Colu. columbae* and *Camp. compar* both had annual peaks in abundance in September of approximately 100 lice/bird and the lowest abundances in February to March. *Coloceras tovornikae* and *H. lata* both peak in March with approximately 6 lice/bird and *C. tovornikae* has a second peak in August. Unfortunately a similar study has not been done on the mites infesting Rock Pigeons, however other species of vane-dwelling feather mites also show annual changes in abundance (Blanco and Frías, 2001; Hamstra and Badyaev, 2009; Pap et al., 2010).

Coloceras tovornikae, B. columbae and *H. lata* all have low prevalences usually less then 30% and have low mean intensities usually less then 20 lice per bird. This coupled with the knowledge that *Colo. tovornikae* and *H. lata* peak with approximately 6 lice per bird in Winnipeg (Galloway and Lamb, 2015), perhaps their patchy distribution is due to a naturally low prevalence and intensity, which makes it harder to successfully establish in new areas.

The two straggler species found in this study, *Myrsidea* sp. and *Machaerilaemus maestus* appear to be true stragglers as they have never seen them found on Rock Pigeons before (Table 2.6). While this is probably not a host switching event, host switching has occurred on Rock Pigeons before. *Picobia zumpti* (Lawrence) (Syringophilidae) a quill mite previously only recorded from *Streptopelia capicola* (Sundevall) and *Streptopelia senegalensis* (L.) (Columbiformes) in South Africa was found in 1999 infesting Rock Pigeons in the USA (Bochkov et al., 2006).

While compiling Table 2.6, infestation records of Rock Pigeons in the literature there was one thing that jumped out at me: of the 20 records from 1971 to 2018, *Columbicola columbae* was reported infesting pigeons in every record and 17 of the 20 records reported having a prevalence of greater then 50%. I also found *C. columbae* present in all seven of my sampling locations. Therefore, I am confident in stating that *C. columbae* is the most prevalence ectosymbiont found on Rock Pigeons.

2.4.1 Summary and Significance

Ectosymbiont-host records such as the ones I reported, which are based on numerous locations with basic infestation parameters, give us a baseline of information on what is present. This information is essential for recognising host switching events, range extensions and changes in life histories.

While it appears that some ectosymbiont species missed the boat when Rock Pigeons were introduced to, and spread throughout Canada, a fair number have been successful in establishing themselves with their host. In this study I found thirteen ectosymbiont species infesting pigeons in Canada. Both coastal locations had every ectosymbiont species identified in this study present. Since there are few other studies that look at large-scale distributions of multispecies assemblages of ectosymbionts on avian hosts, it's unclear if this is a phenomenon restricted to ectosymbionts of *C. livia* or if this trend of higher ectosymbiont diversity on the coast compared to inland counterparts is true for other widespread host species as well.

The variation seen in ectosymbiont assemblages does not suggest that ectosymbionts are missing the boat with perhaps the exceptions of the lice *Colo. tovornikae, B. columbae* and *H. lata.* Local climatic differences explain statistically significant variation in ectosymbiont assemblages and appear to most strongly affect feather dwelling mites. However, there is still a large portion of variation unexplained (~77% for mites and ~78% for lice). Ideally, one would have a more structured sampling scheme with regular collections from pigeons throughout the year, and manipulative experiments to determine how much influence temperature and humidity have on the ectosymbionts.

Table 2.1: Prevalence of mites found on *Columbae livia* collected from seven locations across Canada. 95% confidence intervals in brackets.

	Vancouver (n = 30)	Calgary (n = 30)	Edmonton (n = 23)	Saskatoon (n = 14)	Winnipeg (n=30)	S. Ontario (n = 11)	Halifax (n = 24)
Falculiferidae							
Falculifer rostratus	93.3 (78.66 – 98.8)	10.0 (2.79 – 26.32)	0	0	6.7 (1.2 – 21.34)	9.1 (0.47 – 40.44)	83.3 (62.76 – 94.09)
Pterophagus columbae	13.3 (4.69 – 29.78)	0	0	0	0	0	41.7 (23.4 – 62.75)
Analgidae							
Diplaegidia columbae	76.7 (58.44 – 88.84)	6.7 (1.2 – 21.34)	8.7 (1.57 – 27.81)	0	6.7 (1.2 – 21.34)	27.3 (7.89 – 59.55)	83.3 (62.76 – 94.09)
Harpirhynchidae							
Harpyrhynchoides gallowayi	10.0 (2.79 – 26.32)	6.7 (1.2 – 21.34)	30.4 (14.52 – 52.21)	21.4 (6.12 – 50.0)	20.0 (9.09 – 38.16)	45.5 (19.96 – 73.54)	20.8 (8.59 – 41.51)
Harpyrhynchoides columbae	10.0 (2.79 – 26.32)	0	17.3 (6.17 – 38.87)	0	0	18.2 (3.34 – 50.0)	16.7 (5.91 – 37.24)
Cheyletidae							
Ornithocheyletia hallae	26.7 (13.09 – 44.94)	20.0 (9.09 – 38.16)	26.1 (12.03 – 47.78)	7.1 (0.37 – 31.71)	10.0 (2.79 – 23.35)	36.4 (13.51 – 66.71)	12.5 (3.5 – 31.0)
Rhinonyssidae							
Tinaminyssus melloi	46.7 (29.79 – 65.24)	36.7 (21.35 – 55.05)	8.7 (1.57 – 27.81)	14.2 (2.6 – 42.56)	33.3 (17.73 – 51.68)	27.3 (7.89 – 59.55)	29.2 (13.92 – 50.0)
Tinaminyssus columbae	16.7 (6.81 – 34.75)	20.0 (9.09 – 38.16)	21.7 (8.99 – 43.34)	7.1 (0.37 – 31.71)	10.0 (2.79 – 23.35)	9.1 (0.47 – 40.44)	16.7 (5.91 – 37.24)

Table 2.2: Prevalence of lice found on *Columbae livia* collected from seven locations across Canada. 95% confidence intervals in brackets.

	Vancouver (n = 30)	Calgary (n = 30)	Edmonton (n = 23)	Saskatoon (n = 14)	Winnipeg (n=30)	S. Ontario (n = 11)	Halifax (n = 24)
Philopteridae							
Columbicola columbae	100 (88.85 – 100)	73.3 (55.06 – 86.91)	91.3 (72.19 – 98.43)	85.7 (57.44 – 97.4)	33.3 (17.73 – 51.68)	90.9 (59.56 – 99.53)	100 (86.09 – 100)
Campanulotes compar	96.7 (82.28 – 99.82)	76.7 (58.44 – 88.84)	91.3 (72.19 – 98.43)	92.9 (68.29 – 99.63)	66.7 (48.32 – 82.27)	81.1 (50.0 – 96.7)	100.0 (86.09 – 100)
Coloceras tovornikae	3.3 (0.18 – 17.72)	16.7 (6.81 – 34.750	0	28.6 (10.41 – 57.43)	56.7 (38.17 – 73.67)	0	4.2 (0.22 – 20.37)
Menoponidae							
Hohorstiella lata	26.7 (13.09 – 44.94)	0	4.3 (0.23 – 21.25)	0	10.0 (2.79 – 26.32)	27.3 (7.89 – 59.55)	4.2 (0.22 – 20.37)
Bonomiella columbae	26.7 (13.09 – 44.94)	0	0	7.1 (0.37 – 31.71)	0	0	12.5 (3.5 – 31.0)

Table 2.3: Mean intensity of lice found on *Columbae livia* collected from seven locations across Canada. 95% confidence intervals in round brackets, ranges in square brackets.

	Vancouver	Calgary	Edmonton	Saskatoon	Winnipeg	S. Ontario	Halifax
Philopteridae							
Columbicola columbae	326.07 (246.73 – 427.70) [22 – 841]	64.23 (33.86 – 111.95) [1 – 2026]	136.19 (95.14 – 219.05) [1 – 640]	173.58 (97.50 – 287.67) [4 – 542]	107.50 (20.30 – 280.60) [1 – 589]	53.75 (16.50 – 96.25) [2 – 118]	303.13 (171.0 – 696.33) [1 – 2669]
Campanulotes compar	282.0 (186.03 – 441.55) [6 – 1428]	47.78 (32.17 – 71.87) [1 – 194]	104.76 (67.29 – 175.10) [1 – 524]	110.08 (66.46 – 199.23) ^ [2 – 318]	102.80 (61.70 – 183.60) [4 – 568]	122.67ˣ [4-195]	239.17 (148.48 – 400.29) [1 – 2669]
Coloceras tovornikae	1.0*	18.60 (2.20 – 44.20) [1 – 65]	-	15.0 (4.25 – 25.50) [4 – 28]	54.24 (22.29 – 117.59) ^ [1 – 404]	-	5.0*
Menoponidae							
Hohorstiella lata	14.50 (6.38 – 23.75) [1 – 31]	-	182.0*	-	3.67 ˣ [1 – 5]	-	20.0*
Bonomiella columbae	7.25 (4.50 – 12.38) [2 – 20]	-	-	3.0*	-	-	6.33 (5.03 – 7.33) [5 – 8]

*only one host infested

ˣ sample size too small to calculate confidence interval

^ 90 % confidence interval, due to small sample size

Table 2.4: Results from pairwise Permutational Analysis of Variance tests examining differences in ectosymbionts assemblages infesting Rock Pigeons (*Columbia livia*) (n = 162) sampled from seven different locations across Canada, p < 0.05 are in bold.

Pairs	Mites			Lice		
	F-value	R^2	Adjusted P-value	F-value	R^2	Adjusted P-value
Vancouver vs. Calgary	12.87	0.23	**0.02**	11.81	0.19	**0.02**
Vancouver vs. Edmonton	15.41	0.27	**0.02**	3.92	0.07	0.08
Vancouver vs. Saskatoon	9.58	0.23	**0.02**	3.32	0.07	0.25
Vancouver vs. Winnipeg	13.70	0.24	**0.02**	14.34	0.21	**0.02**
Vancouver vs. S. Ontario	8.59	0.19	**0.02**	3.08	0.09	0.57
Vancouver vs. Halifax	2.60	0.05	0.31	1.84	0.03	1.00
Halifax vs. Calgary	14.88	0.29	**0.02**	4.29	0.09	0.08
Halifax vs. Edmonton	13.83	0.29	**0.02**	1.11	0.02	1.00
Saskatoon vs. Halifax	9.27	0.27	**0.02**	0.68	0.02	1.00
Winnipeg vs. Halifax	13.81	0.27	**0.02**	6.28	0.12	**0.02**
S. Ontario vs. Halifax	8.28	0.21	**0.02**	1.17	0.04	1.00
Calgary vs. Edmonton	4.14	0.13	0.06	4.03	0.08	0.13
Calgary vs. Saskatoon	1.90	0.09	1.00	1.26	0.03	1.00
Calgary vs. Winnipeg	1.13	0.4	1.00	2.42	0.05	0.71
Calgary vs. S. Ontario	2.33	0.9	1.00	1.01	0.04	1.00
Edmonton vs. Saskatoon	0.93	0.05	1.00	0.85	0.02	1.00
Edmonton vs. Winnipeg	2.91	0.09	0.52	6.95	0.13	**0.02**
Edmonton vs. S. Ontario	0.72	0.03	1.00	1.00	0.04	1.00
Saskatoon vs. Winnipeg	0.48	0.02	1.00	2.78	0.07	0.46
Saskatoon vs. S. Ontario	0.68	0.05	1.00	0.86	0.05	1.00
Winnipeg vs. S. Ontario	1.14	0.05	1.00	1.58	0.05	1.00

Table 2.5: Environmental variables chooses through forward selection. A distance-based redundancy analysis was performed on the mite data and a redundancy analysis was performed on the louse data. P-values have been corrected for multiple comparisons using the Holm-Bonferroni method.

Environmental Variable	R^2 adjusted	F – value	Adjusted P-value
dbRDA – Mites			
annual precipitation	0.08139	9.41	0.011
monthly minimum humidity	0 .01076	2.11	0.011
monthly maximum humidity	0.00591	1.60	0.198
RDA - Lice			
annual maximum temperature	0.2280	39.70	0.001
previous monthly maximum humidity	0.03566	7.30	0.012
previous monthly minimum temperature	0.01925	4.46	0.102

Table 2.6: Infestation parameters of ectosymbionts infesting Rock Pigeons (*Columba livia*) reported from 1971 to 2018. Sample size - number of hosts, P - prevalence, MI - mean intensity.

Source	Brown, 1971		Rózsa, 1990		Rózsa, 1990		Dranzoa et al., 1999	Forond et al., 2004	
Location	Boston, USA		Budapest, Hungary		Kerekegyház, Hungary		Kampala, Uganda	Tenerife, Canary Islands	
Sample size	72		120		120		34	50	
Measurement	P	MI	P	MI	P	MI	P	P	MI
Mites									
Analgidae									
Diplaegidia columbae									
Megninia cubitalis									
Argasidae									
Argas reflexus									
Cheyletidae									
Neocheyletiella heteropalpa									
Dermanyssidae									
Dermanyssus gallinae	8.3	10.3						6	241
Falculiferidae									
Falculifer rostratus									
Harpirhynchidae									
Harpirhynchus sp.	19.4	5.1							
Laminosioptidae									
Laminosioptes cysticola									
Rhinonyssidae									
Tinaminyssus columbae	20.8	4.2							
Tinaminyssus melloi	37.5	8.1	42	10.3	23	7.9		10	218.3
Syringophilidae									
Picobia sp.	1.4	1							
Syringophilus columbae	1.4	1							
Lice									
Menoponidae									
Bonomiella columbae									
Colpocephalum turbinatum									
Hohorstiella lata	41.7	6.3							
Hohorstiella streptopeliae									
Menopon gallinae								55.9		
Menacanthus stramineus	4.2	1.7					64.7		
Philopteridae									
Campanulotes compar			71	4.9	77	5.4		94	48.4
Chelopistes meleagiridis							70		
Coloceras tovornikae									
Columbicola columbae	91.7	153.6	97	16.6	92	17.9	94.1	100	111.4
Goniocotes gallinae									
Goniodes sp.									
Goniodes gigas									
Lipeurus caponis									
Fly									
Hippoboscidae									
Pseudolynchia canariensis	11.1	1.4					100	36	6.2
Flea									
Pulicidae									
Echidnophaga gallinacea									

Table 2.6: Continued.

Source	Williams, 2005 (unpublished)		Adang et al., 2008		Naz et al., 2010		Musa et al., 2011		Radfar et al., 2011
Location	Illinois, USA		Zaria, Nigeria		Karachi, Pakistan		Bangladesh		South Khorasan, Iran
Sample size	21		240		68		24		58
Measurement	P*	MI*	P	MI	P	MI	P	MI	P
Mites									
Analgidae									
Diplaegidia columbae	95.24	211.25							
Megninia cubitalis									
Argasidae									
Argas reflexus									
Cheyletidae									
Neocheyletiella heteropalpa									
Dermanyssidae									
Dermanyssus gallinae	61.90	4.31	2.5	1.5					
Falculiferidae									
Falculifer rostratus	85.71	226.5							
Harpirhynchidae									
Harpirhynchus sp.									
Laminosioptidae									
Laminosioptes cysticola									1.96
Rhinonyssidae									
Tinaminyssus columbae									
Tinaminyssus melloi	28.57	5							
Syringophilidae									
Picobia sp.									
Syringophilus columbae									
Lice									
Menoponidae									
Bonomiella columbae									
Colpocephalum turbinatum					35.3	1437.1	33.33	9.25	
Hohorstiella lata	4.76	4			51.4	230.1			
Hohorstiella streptopeliae					1.47	4			
Menopon gallinae			6.3	5.5			16.66	9	44.82
Menacanthus stramineus							33.33	9.5	
Philopteridae									
Campanulotes compar	100	268.62			58.8	614.3			
Chelopistes meleagiridis									
Coloceras tovornikae									
Columbicola columbae	100	152.76	63.8	17.9	70.4	438.2	50	12.75	74.13
Goniocotes gallinae									
Goniodes sp.			10.8	5.7					
Goniodes gigas									
Lipeurus caponis							25	9.5	
Fly									
Hippoboscidae									
Pseudolynchia canariensis			37.1	3.6					75.86
Flea									
Pulicidae									
Echidnophaga gallinacea									

*Calculated by A. Grossi based on count data from Williams (2005).

Table 2.6: Continued.

Source	Radfar et al., 2012a	Radfar et al., 2012b		Begum and Sehrin, 2012		Galloway and Lamb, 2014		Rezaei et al., 2016
Location	Birjand, Iran	Sistan region, Iran		Dhaka, Bangladesh		Winnipeg, Canada		Kermanshah province, Iran
Sample size	58	46		60		659		700
Measurement	P	P	MI	P	MI	P	MI	P
Mites								
Analgidae								
Diplaegidia columbae								
Megninia cubitalis								
Argasidae								
Argas reflexus		13.04	4					74.14
Cheyletidae								
Neocheyletiella heteropalpa								
Dermanyssidae								
Dermanyssus gallinae				13.33	2.8			8.28
Falculiferidae								
Falculifer rostratus								
Harpirhynchidae								
Harpirhynchus sp.								
Laminosioptidae								
Laminosioptes cysticola	1.96							
Rhinonyssidae								
Tinaminyssus columbae								
Tinaminyssus melloi								
Syringophilidae								
Picobia sp.								
Syringophilus columbae								
Lice								
Menoponidae								
Bonomiella columbae								
Colpocephalum turbinatum				71.66	6.51			
Hohorstiella lata						19.8	7.4	
Hohorstiella streptopeliae								
Menopon gallinae	44.82	32.6	7.13	100	5.52			10.43
Menacanthus stramineus				46.66	3.43			9
Philopteridae								
Campanulotes compar						84.6	66.6	
Chelopistes meleagiridis				46.66	3.32			
Coloceras tovornikae						33.6	8.6	
Columbicola columbae	74.13	41.3	6	100	7.93	66.5	68.5	61.7
Goniocotes gallinae				31.66	8.84			
Goniodes sp.								
Goniodes gigas								
Lipeurus caponis				51.66	73.97			
Fly								
Hippoboscidae								
Pseudolynchia canariensis	75.85			63.33	1.42			27.7
Flea								
Pulicidae								
Echidnophaga gallinacea				8.33	2.6			

Table 2.6: Continued.

Source	Saikia et al., 2017	da Cunha Amaral et al., 2017	Chaechi-Nosrati et al., 2018	Laku et al., 2018		Abdulhakim et al., 2018
Location	Assam, India	Pelotas, Brazil	Lahijan, Iran	Port Harcourt, Nigeria		Tripoli, Libya
Sample size	324	79	180	50		100
Measurement	P	P	P	P	MI	P
Mites						
Analgidae						
Diplaegidia columbae						
Megninia cubitalis			13.3			
Argasidae						
Argas reflexus						
Cheyletidae						
Neocheyletiella heteropalpa						
Dermanyssidae						
Dermanyssus gallinae			3.3			
Falculiferidae						
Falculifer rostratus			31.6			
Harpirhynchidae						
Harpirhynchus sp.						
Laminosioptidae						
Laminosioptes cysticola						
Rhinonyssidae						
Tinaminyssus columbae						
Tinaminyssus melloi						
Syringophilidae						
Picobia sp.						
Syringophilus columbae						
Lice						
Menoponidae						
Bonomiella columbae		8.9				
Colpocephalum turbinatum		55.7				
Hohorstiella lata		33				
Hohorstiella streptopeliae						
Menopon gallinae	4.62		6.6			3
Menacanthus stramineus	2.77		41.6			
Philopteridae						
Campanulotes compar		62				
Chelopistes meleagiridis				8	1	
Coloceras tovornikae						
Columbicola columbae	12.03	97.5	88.3	24	5.3	82
Goniocotes gallinae	1.85					18
Goniodes sp.			53.3			
Goniodes gigas	1.23					
Lipeurus caponis	2.16					
Fly						
Hippoboscidae						
Pseudolynchia canariensis	15.12	58.2	73.3	4	1	1
Flea						
Pulicidae						
Echidnophaga gallinacea						

Figure 2.1: Prevalence of the feather mite *Falculifer rostratus* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
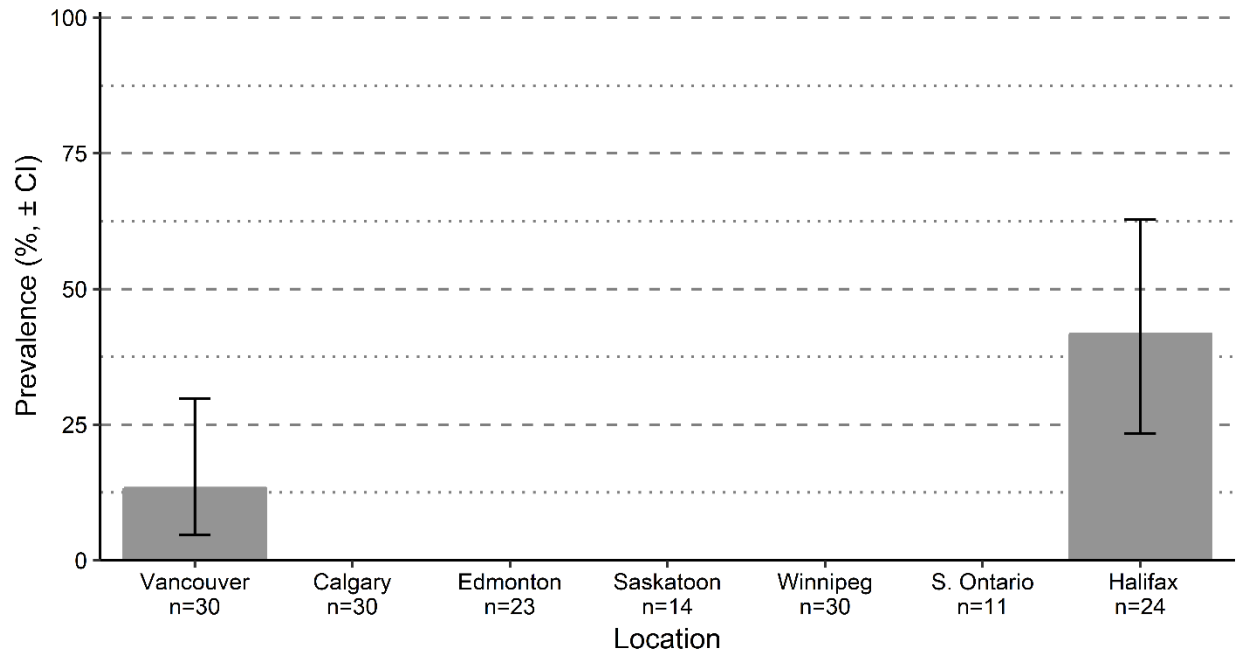
Figure 2.2: Prevalence of the feather mite *Pterophagus columbae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
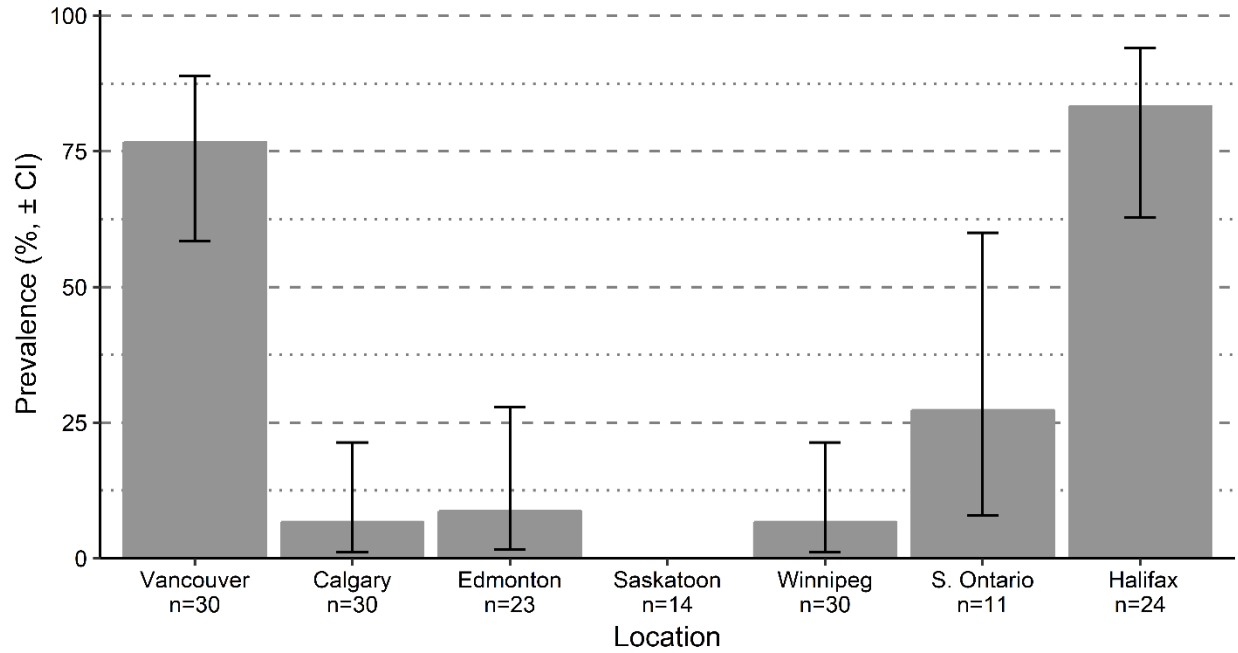
Figure 2.3: Prevalence of the feather mite *Diplaegidia columbae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
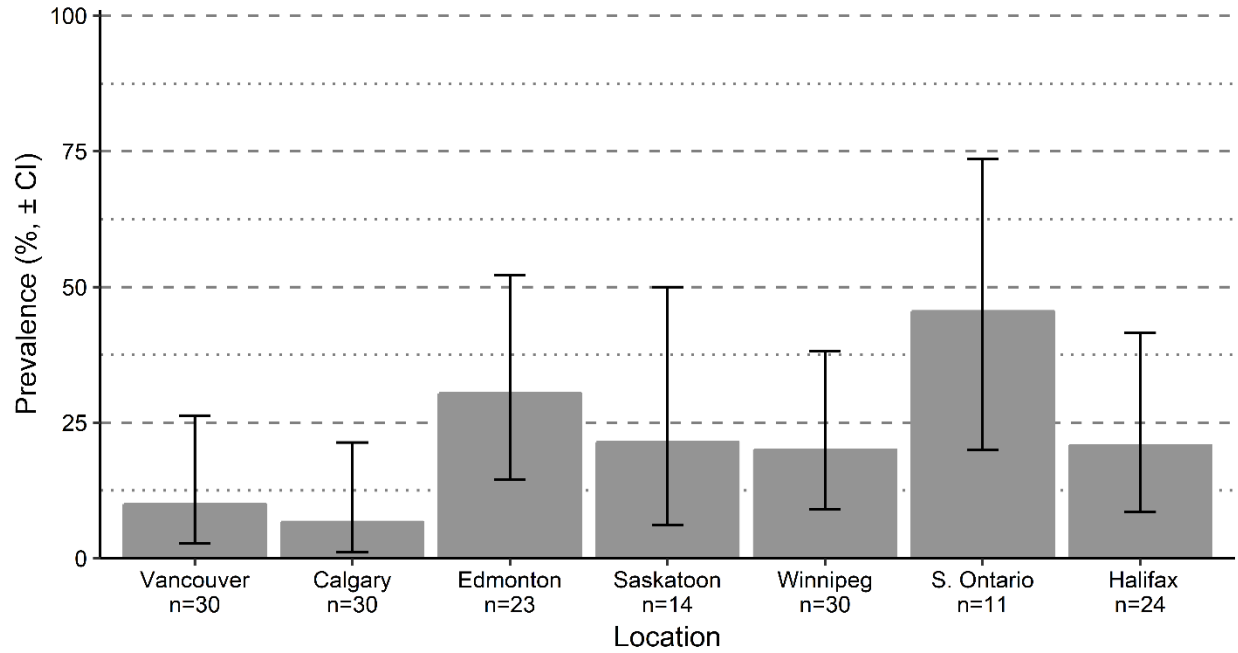
Figure 2.4: Prevalence of the skin mite *Harpyrhynchoides gallowayi* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
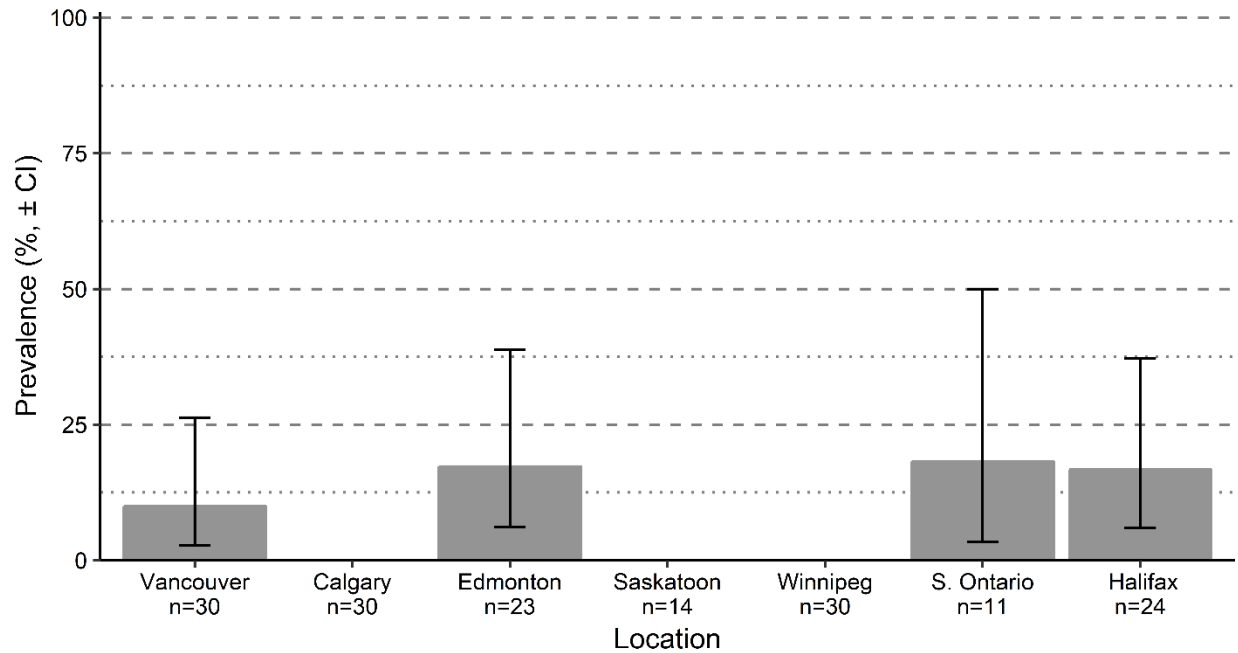
Figure 2.5: Prevalence of the skin mite *Harpyrhynchoides columbae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
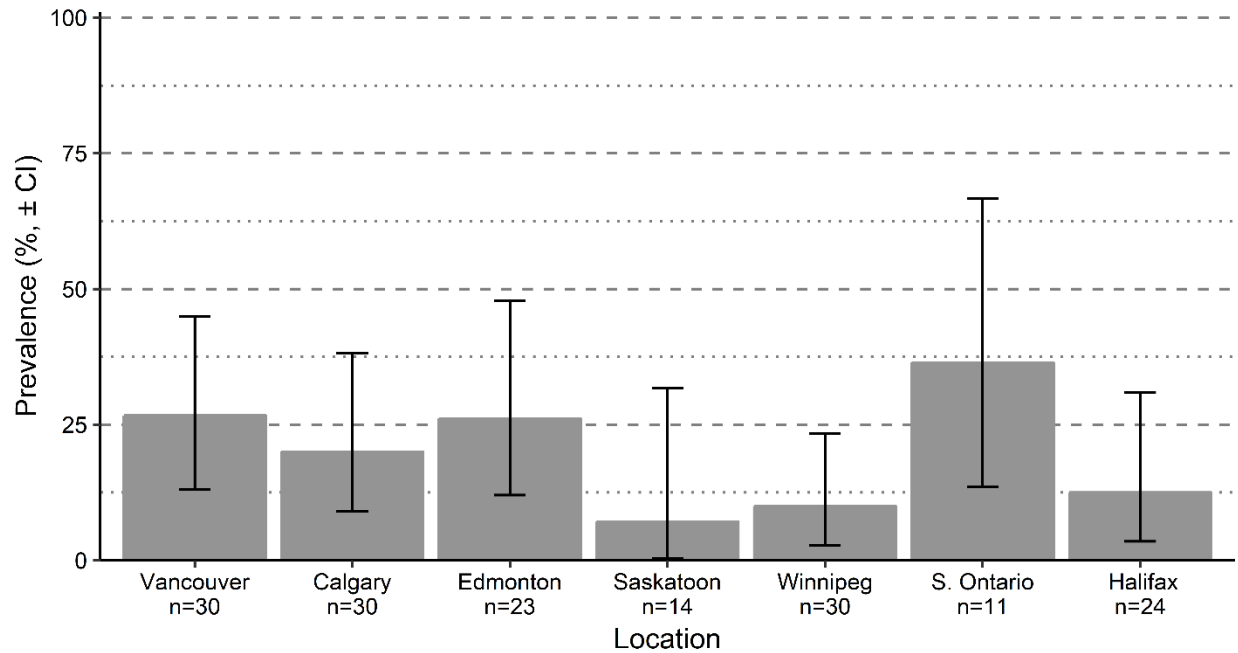
Figure 2.6: Prevalence of the skin mite *Ornithocheyletia hallae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
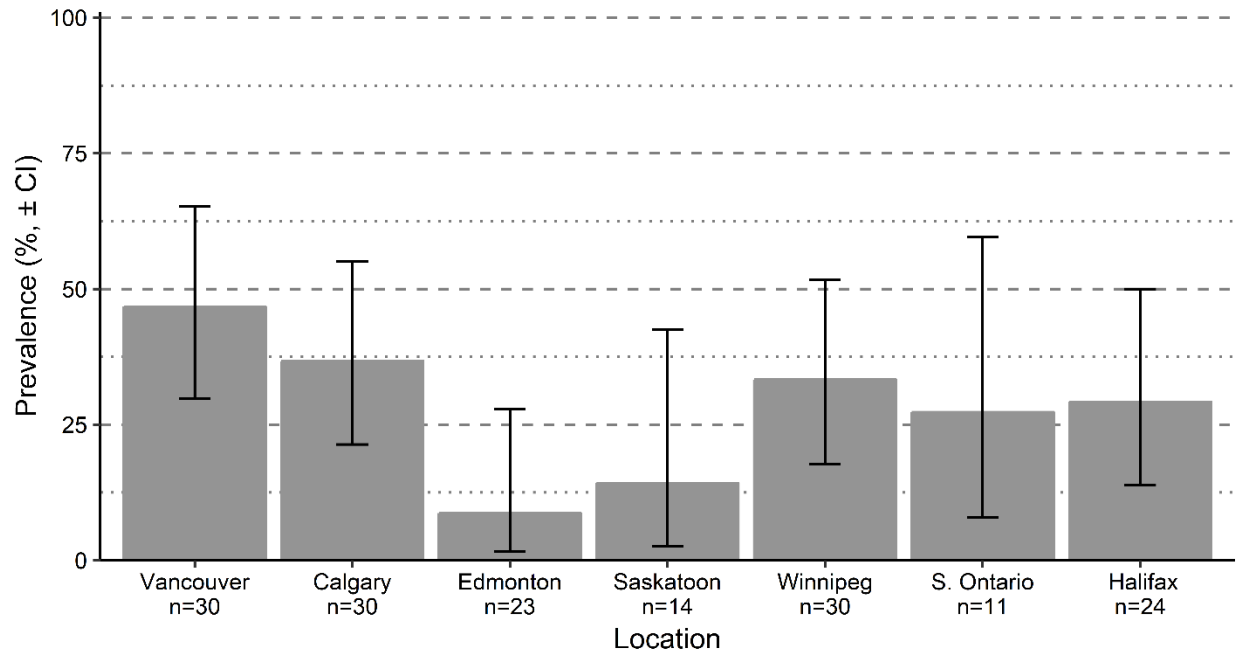
Figure 2.7: Prevalence of the nasal mite *Tinaminyssus melloi* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
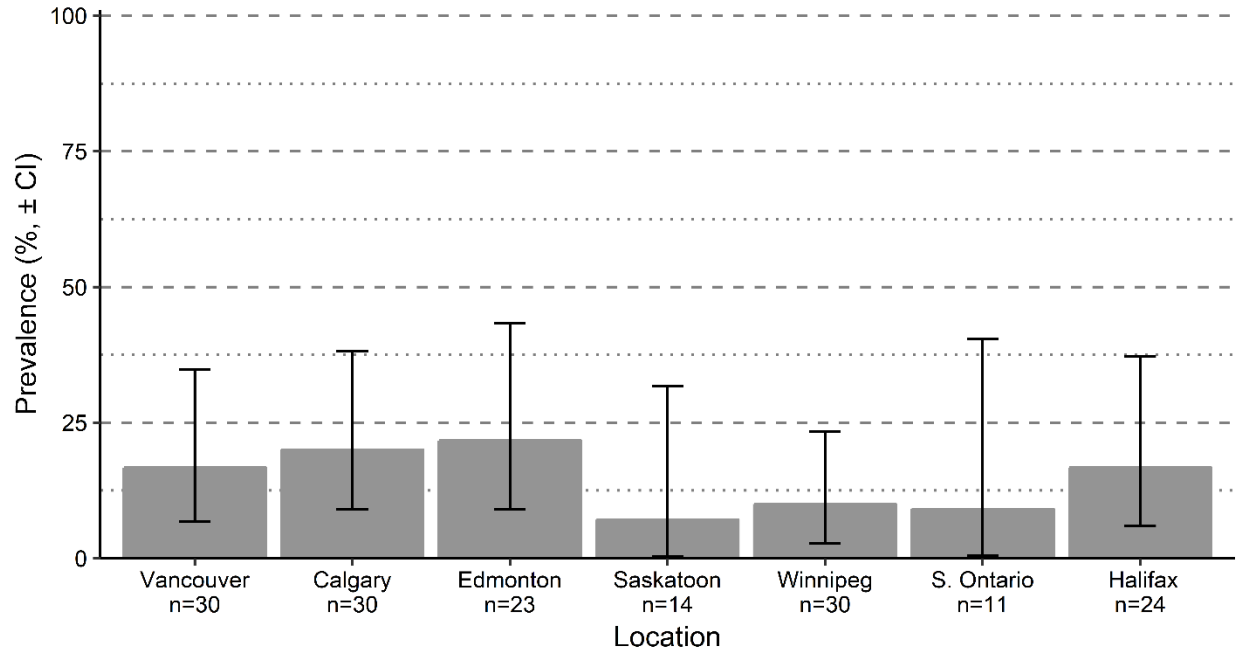
Figure 2.8: Prevalence of the nasal mite *Tinaminyssus columbae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location.
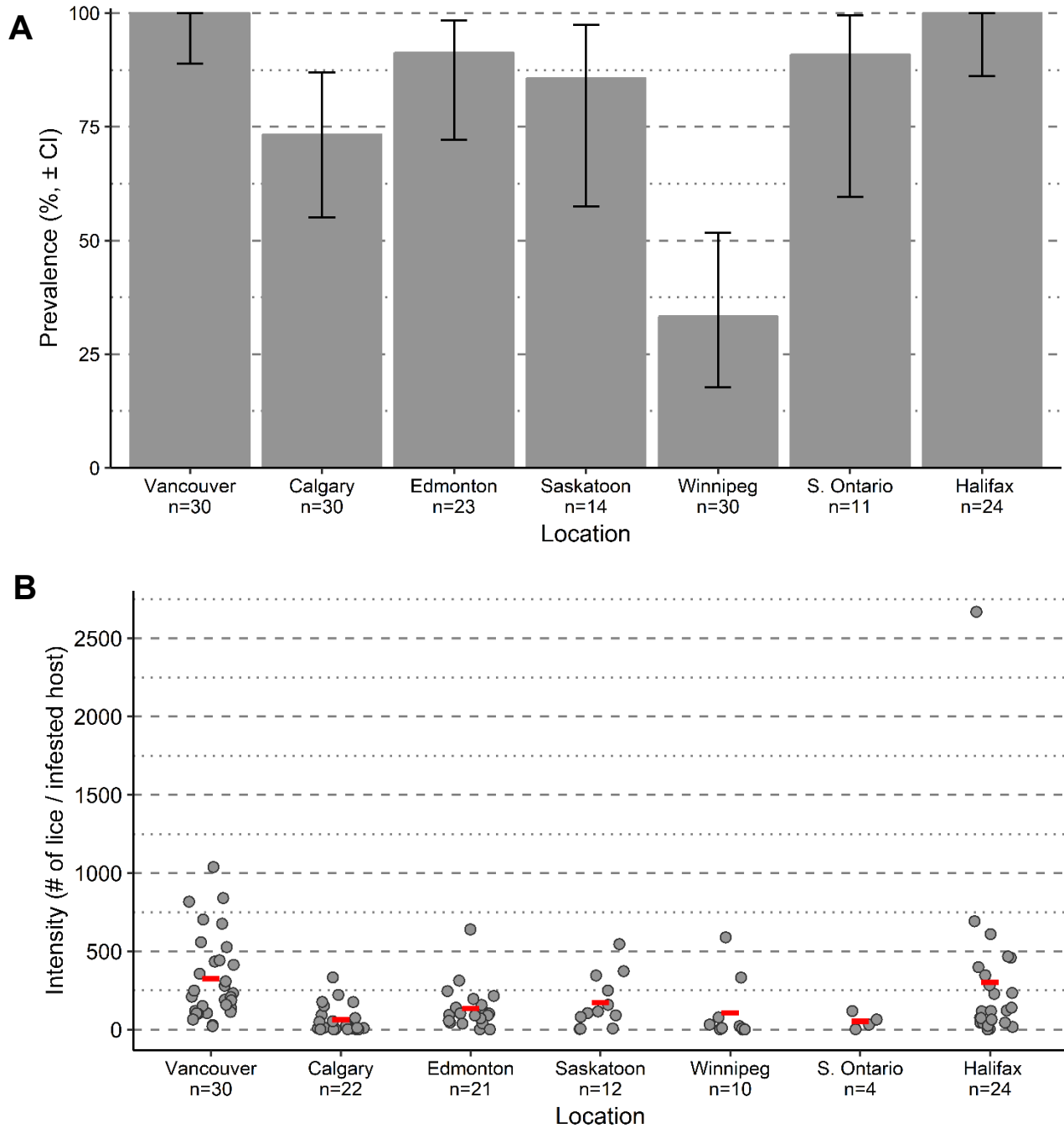
Figure 2.9: (A) Prevalence of the feather louse *Columbicola columbae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location. (B) Mean intensity (red bar), each gray point represents the number of *C. columbae* on an infested host. Sample sizes refer to the number of infested *C. livia* from each location.
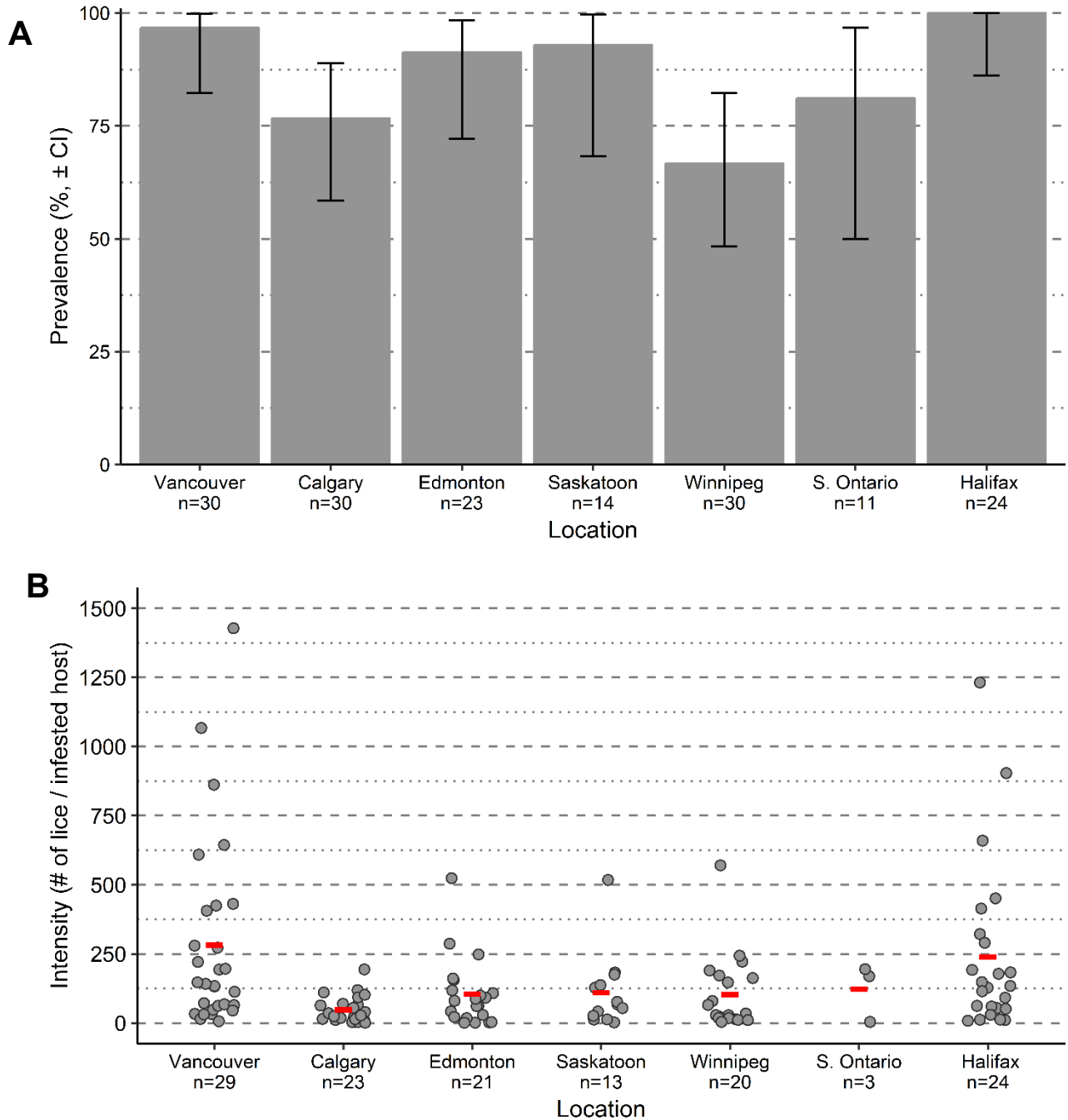
Figure 2.10: (A) Prevalence of the feather louse *Campanulotes compar* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of C. *livia* examined from each location. (B) Mean intensity (red bar), each gray point represents the number of *C. compar* on an infested host. Sample sizes refer to the number of infested *C. livia* from each location
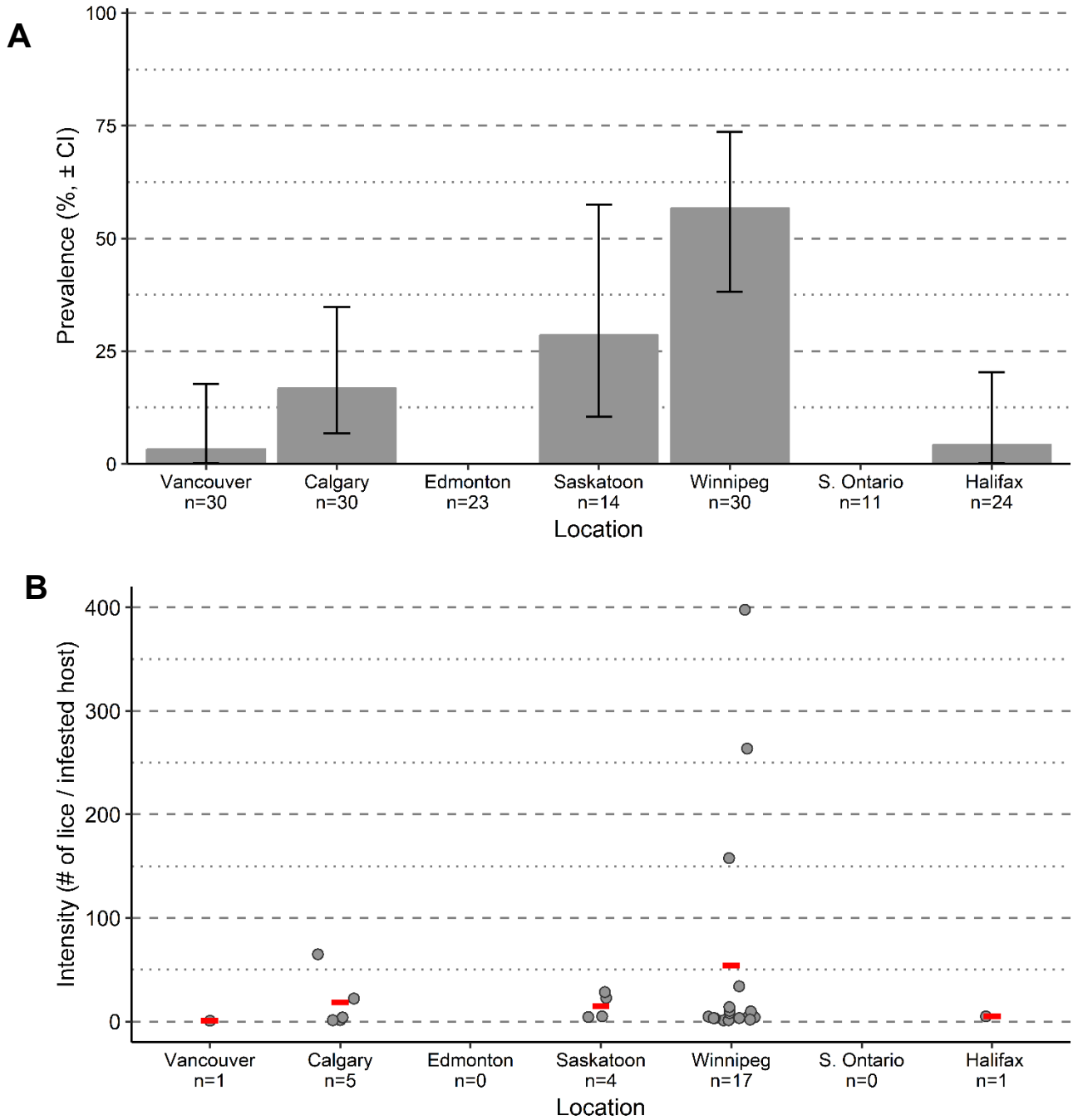
Figure 2.11: (A) Prevalence of the feather louse *Coloceras tovornikae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location. (B) Mean intensity (red bar), each gray point represents the number of *C. tovornikae* on an infested host. Sample sizes refer to the number of infested *C. livia* from each location.
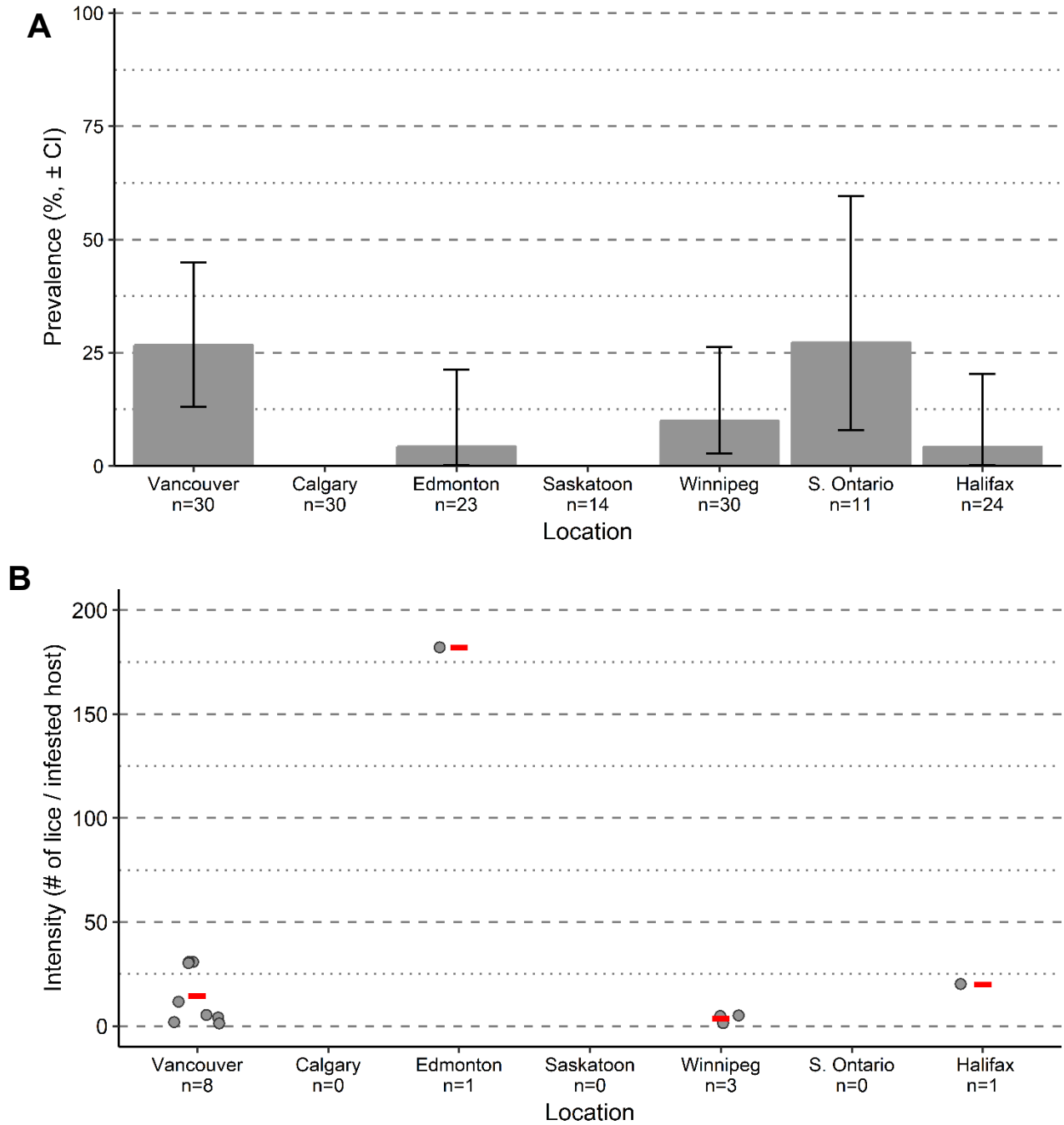
Figure 2.12: (A) Prevalence of the feather louse *Hohorstiella lata* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location. (B) Mean intensity (red bar), each gray point represents the number of *H. lata* on an infested host. Sample sizes refer to the number of infested *C. livia* from each location.
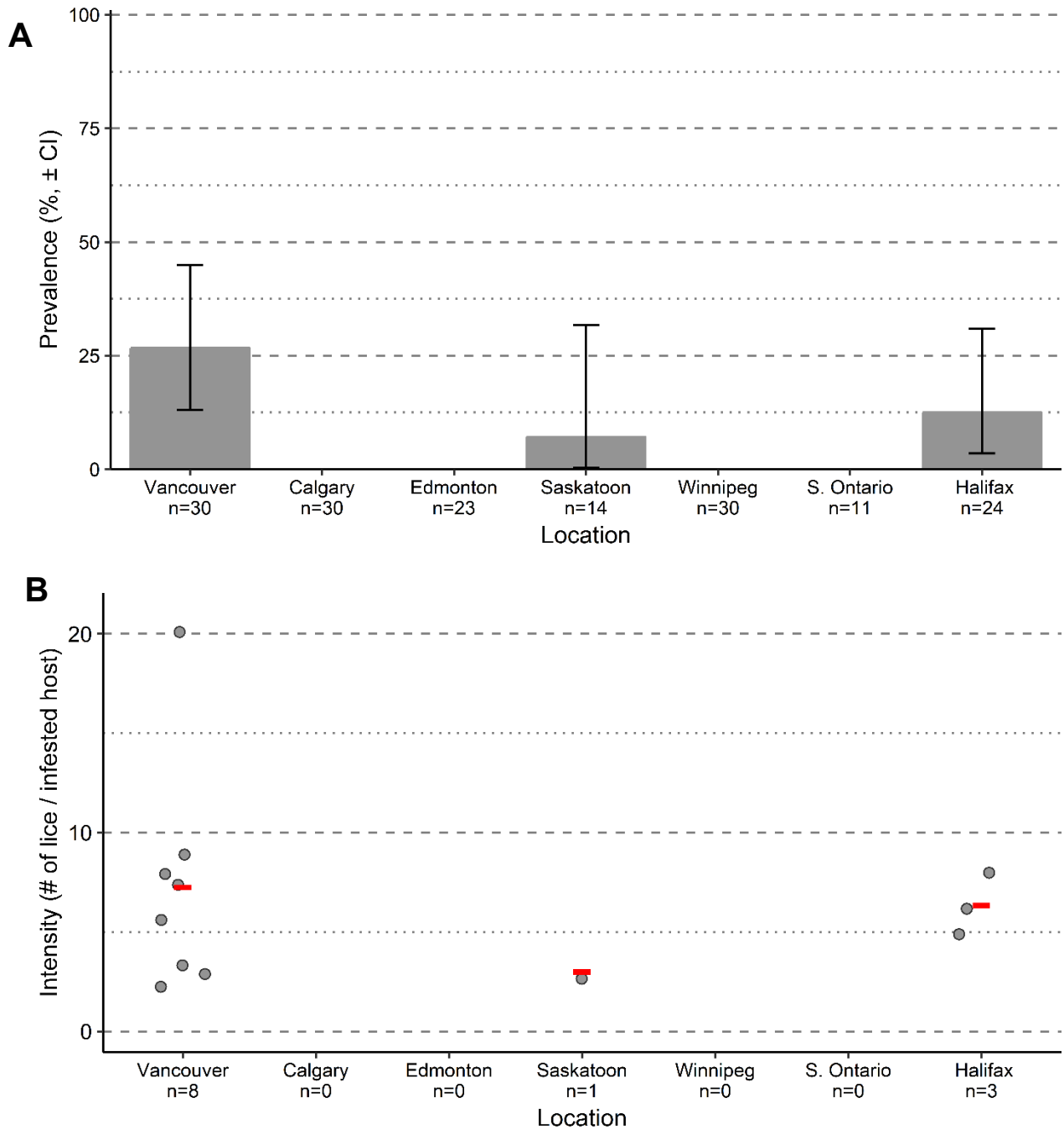
Figure 2.13: Prevalence of the feather louse *Bonomiella columbae* infesting *Columba livia* sampled from seven location across Canada, 95% confidence intervals. Sample sizes refer to number of *C. livia* examined from each location. (B) Mean intensity (red bar), each gray point represents the number of *B. columbae* on an infested host. Sample sizes refer to the number of infested *C. livia* from each location.
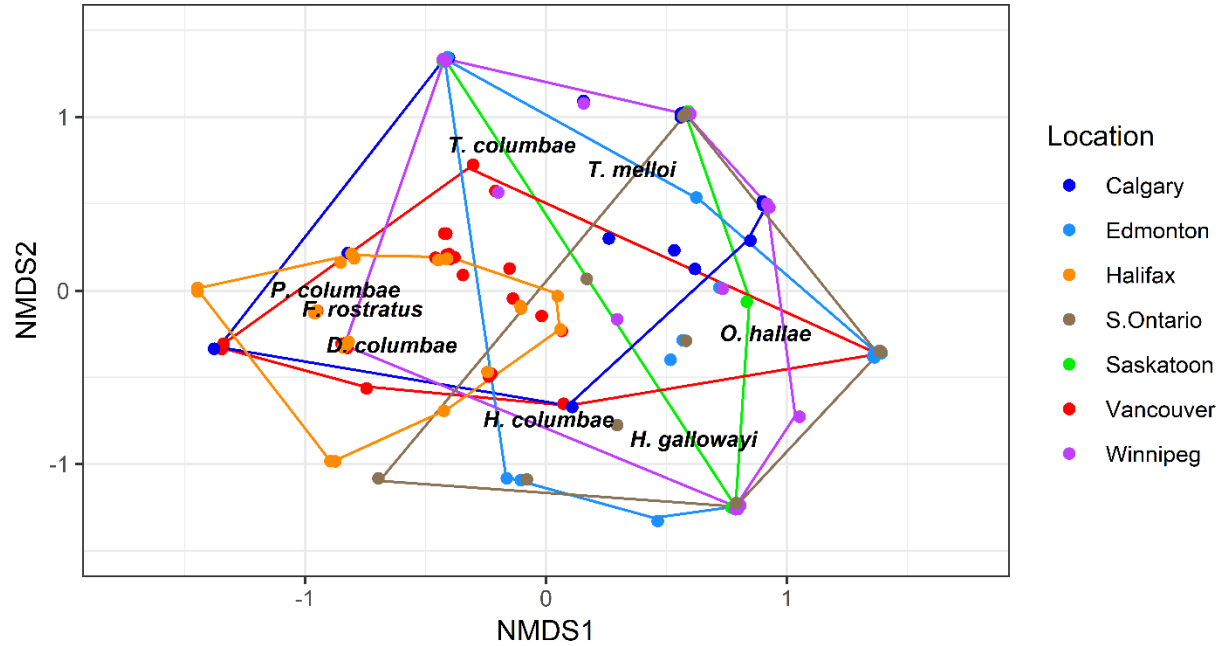
Figure 2.14: Non-metric multidimensional scaling plot of mite assemblages (presence-absence data) infesting *Columba livia* (n = 162) sampled from seven locations across Canada. Locations of mite names indicate the direction of their vectors. Stress = 0.1180, points have been jittered (width = 0.02, height = 0.02), to minimize overlap. Vancouver mite assemblages are significantly different from every other location except Halifax and Halifax was different from every other location other then Vancouver.
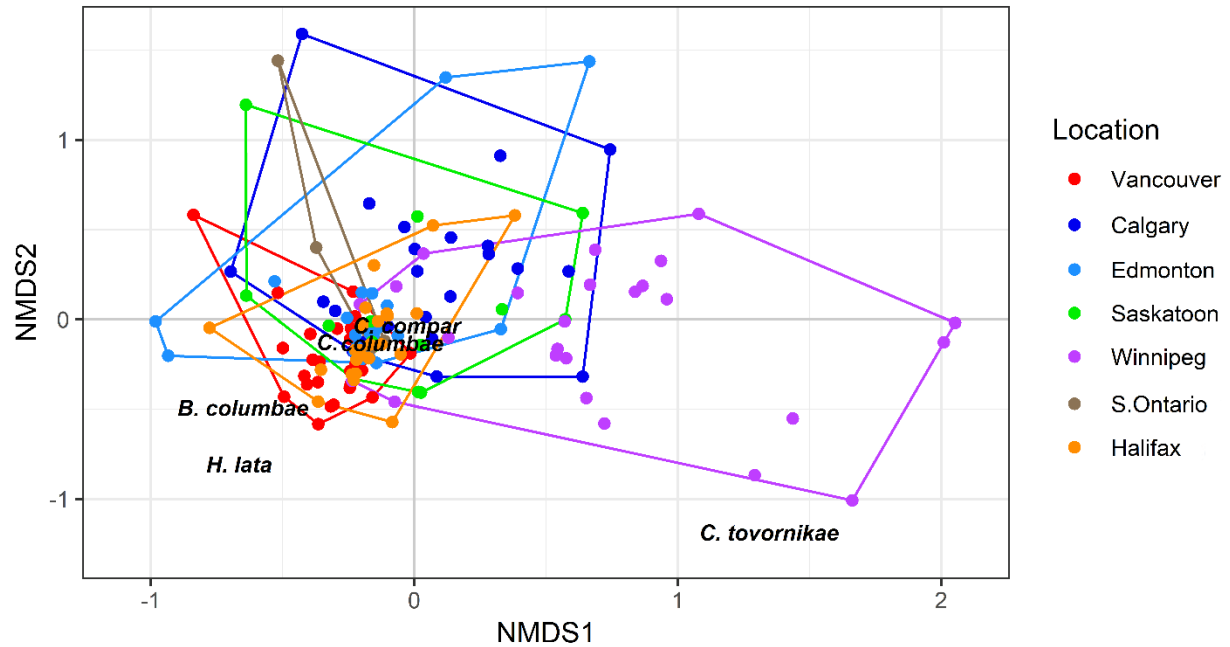
Figure 2.15: Non-metric multidimensional scaling plot of louse assemblages (ln [x+1] abundance data) infesting *Columba livia* (n = 162) sampled from seven locations across Canada. Locations of louse names indicate the direction of their vectors. Stress = 0.1189. Locations that have significantly different louse assemblages are: Vancouver and Calgary, Vancouver and Winnipeg, Edmonton and Winnipeg, Winnipeg and Halifax.
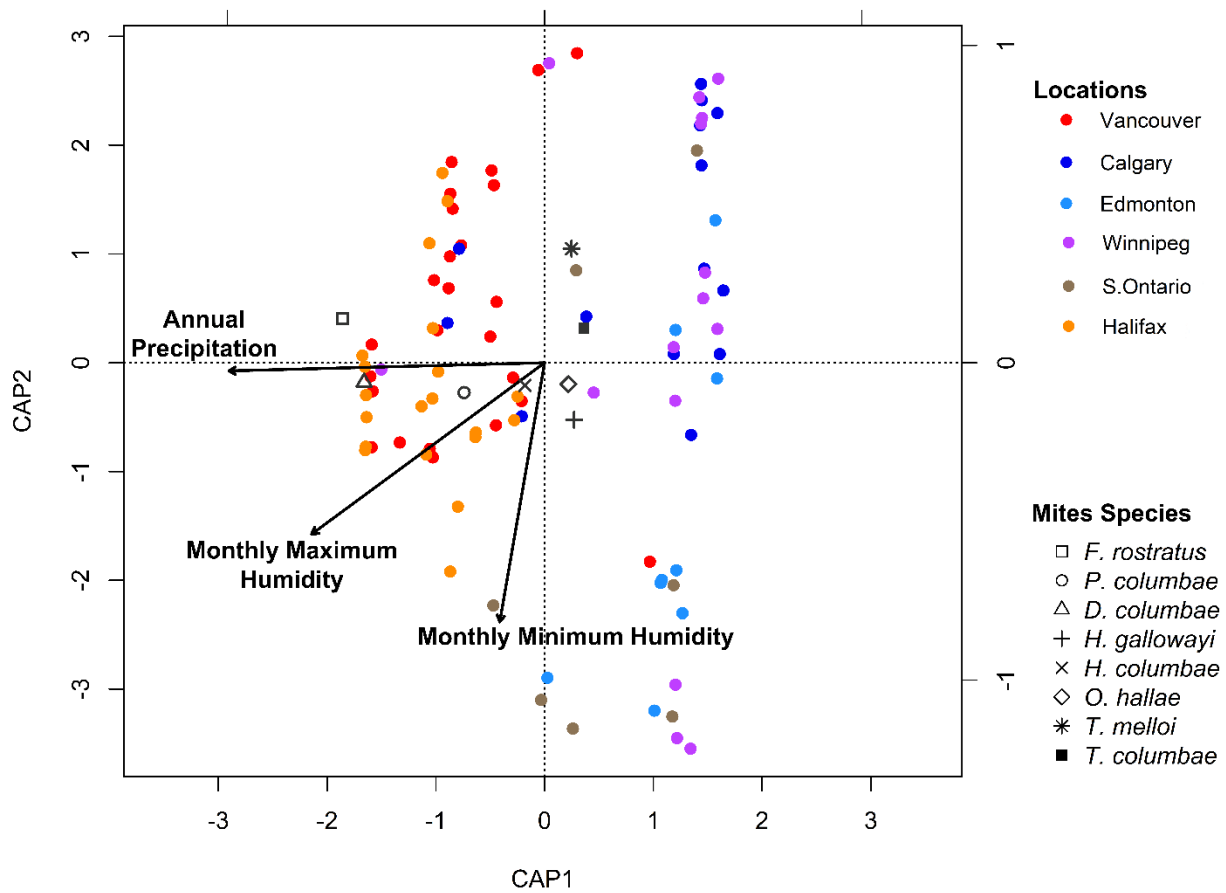
Figure 2.16: Distance based redundancy analysis plot of mite assemblages (presence-absence data) from *Columba livia* sampled from six location across Canada. Vectors are meteorological variables chosen through forward selection. Annual precipitation and monthly minimum humidity were significant ($p < 0.05$) after correction for multiple comparisons.
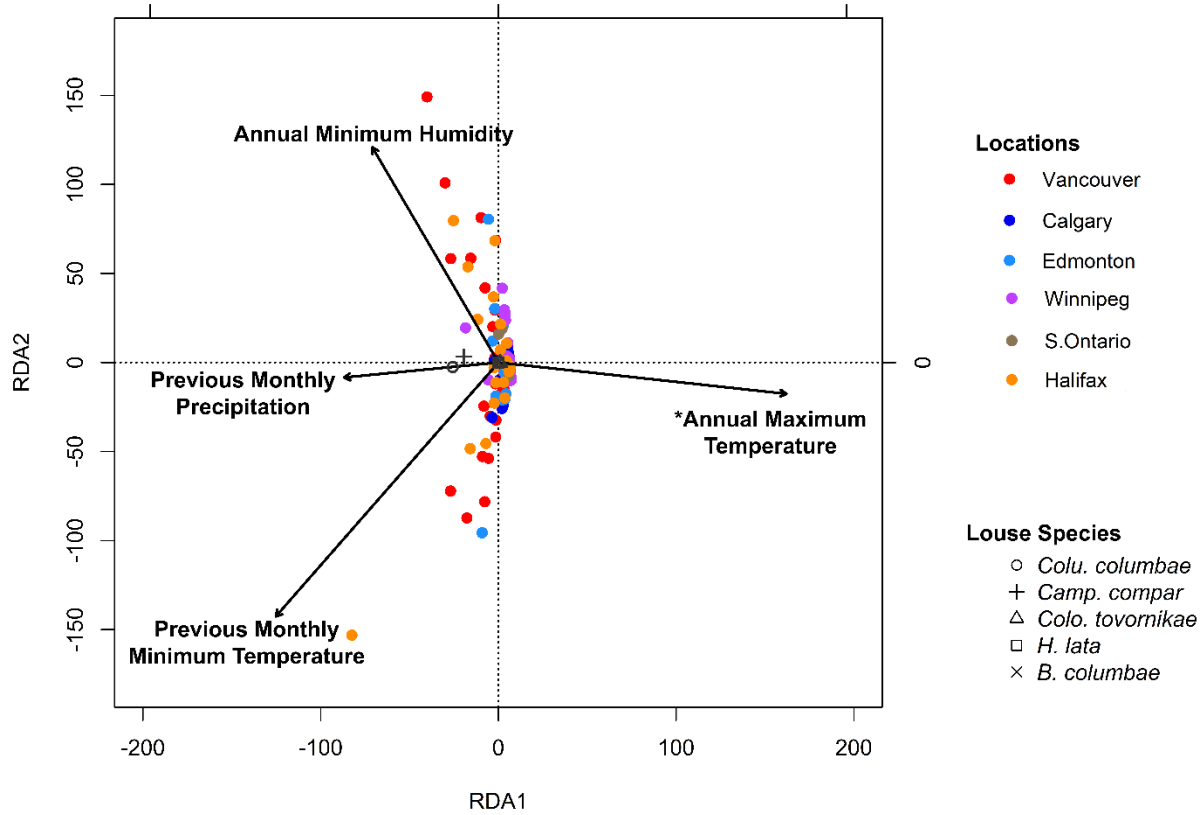
Figure 2.17: Redundancy analysis plot of louse assemblages (abundance data) from *Columba livia* sampled from six location across Canada. Vectors are meteorological variables chosen through forward selection. Annual maximum temperature was significant ($p < 0.05$) after correction for multiple comparisons.

Chapter 3 - Population genetic structure of Rock Pigeons (*Columba livia,* Gmelin) and two of its chewing lice, *Columbicola columbae* (Freire & Duarte) and *Campanulotes compar* (Burmeister), from across Canada.

3.1 Introduction

The expectation that hosts and parasites diversify in parallel (e.g., that speciation in the host lineage should be mirrored by speciation in the parasite's) is one of the three 'rules' of parasitology formulated almost 60 years ago as Fahrenholtz's Rule (Klassen, 1992), which has two predictions (bold numbering added by author):

> "Among numerous (mainly permanent) parasites, **(1)** the historical
> development and splitting of the hosts is paralleled by a corresponding
> development and splitting of the parasites. Therefore, **(2)** the resulting
> phylogenetic relationships of the parasites can be used to draw conclusions
> about the (often obscured) phylogenetic relationships of the hosts."
> (Eichler, 1942: 77).

With the development of molecular phylogenetic analyses, the first prediction of Fahrenholz's Rule was tested mostly at the species level and above (Page, 2002). More recently, this expectation has been extended to the population level, where in addition to assessing whether patterns of diversification in host and parasite are mirrored, many studies have assessed whether the population structure of the (presumably) more rapidly evolving parasite can act as 'biological tags' to demarcate the (presumably) more slowly evolving host populations (Hafner et al., 1994; Ricklefs and Outlaw, 2010; Johnson et al., 2014). But the degree to which population genetic structure of hosts and parasites covaries is confounded by how each member of the system disperses (e.g., mussel larvae and host fish, Vaughn and Taylor, (2000); trematodes

and snails, Miller and Poulin, (2001); ticks and seabirds, McCoy et al., (2005); hippoboscid flies and seabirds, Levin and Parker, (2013)).

A system that offers many opportunities to assess how host population structure impacts parasite dispersal is chewing lice and their avian hosts. Chewing lice are permanent ectoparasites that live on, feed on and attach their eggs to the feathers of their host, thereby completing their entire life cycle upon their host (Marshall, 1981). Chewing lice are wingless and rely mainly on contact between hosts to disperse, both vertically between parents and offspring and horizontally between individuals such as while mating or sharing roosts (Clayton and Tompkins, 1994; Darolova et al., 2001). However, some louse species have the ability to be phoretic, and will hitch rides on blood-feeding louse flies (Diptera: Hippoboscidae) to move longer distances between hosts (da Cunha Amaral et al., 2013). As they have no free-living stages, one would expect that, in general, chewing lice should have a similar population genetic structure to that of their hosts, and that lice exhibiting phoresy should have less strongly structured populations because of greater gene flow.

In this study I investigate the population genetic structure of Rock Pigeons (Columbiformes: Columbidae: *Columba livia,* Gmelin) and two of their chewing lice, *Columbicola columbae* (Freire & Duarte) a "wing louse" and *Campanulotes compar* (Burmeister) a "body louse," within Canada. Rock Pigeons, also commonly known as the Rock Dove or just 'feral pigeon', are not native to Canada. The first recorded introduction was to Nova Scotia in the early 1600's (Schorger, 1952). There have likely been many other introductions to Canada since then, but to my knowledge there are no published records of deliberate or accidental releases. Now, Rock Pigeons are found in most urban centres across the country, and with them are their lice *C. columbae* and *C. compar* (see Chapter 2)*. Columbicola columbae* is a

long, slender and agile 'wing' louse that is found mainly on the wings. When disturbed it will run rapidly to the base of the feather and hide under the coverts or insert itself between the barbs of the wing feathers (Nelson and Murray, 1971; Clayton, 1991). *Campanulotes compar* is a more rounded and slow-moving 'body' louse found on the abdominal feathers. It escapes preening by burrowing into the downy matrix of the feathers (Nelson and Murray, 1971; Clayton, 1991). Within North America both louse species are only found on *C. livia* (Price et al., 2003).

The population structure of wing and body lice on Columbiformes has been previously studied. Johnson et al., (2002) examined the genetic structure using the mitochondrial cytochrome oxidase I (COI) gene from four species of *Columbicola* and five species of *Physconelloides* (another genus of body lice) from nine species of doves (study did not include *C. livia*) from the southern U.S.A. and Mexico. They found that body lice showed significantly more population genetic structure than wing lice, both between localities and between hosts within localities. Similarly, DiBlasi et al., (2018) sampled lice from 3 flocks of Rock Pigeons in Salt Lake City, Utah that were all within 10.2 km of each other. Using microsatellites, they found that *Camp. compar* had greater population structure (both among flocks and among host individuals within flocks) than *Colu. columbae*. In both papers the authors attribute at least part of the genetic difference between the species to phoresy. The genus *Columbicola* has been observed in nature as well as experimentally proven to transfer between hosts by attaching to the Pigeon Louse Fly *Pseudolynchia canariensis* (Macquart), which was introduced to North America along with their hosts (Ansari, 1947; Harbison et al., 2008). *Pseudolynchia canariensis* is most abundant in warm climates and does not normally occur in Canada (Mullen and Durden, 2018); to my knowledge, there is only a single Canadian record of *P. canariensis* in the Canadian

National Collection of Insects, Arachnids and Nematodes (from Windsor in southern Ontario: pers. comm., Dr. Jeffrey Skevington, CNC).

In this study I had three main questions. **(1)** How much connectivity do pigeon populations across Canada have with each other? Since Rock Pigeons are a sedentary (i.e., non-migratory) species (Johnston and Janiga, 1995), one would predict relatively strong population structure across Canada; however, since their 'official' introduction in 1600, there have been many imports of *C. livia* to Canada by pigeon fanciers; According to the Canadian Food Inspection Agency 15435 rock pigeons were imported between 2016-2018, of these pigeons 93.4% came from the United States of American and 6.6% were from the Netherlands (Appendix 3.1). Although these pigeons are unlikely to be deliberately released by their importers, some may occasionally escape and join local feral flocks. To assess whether there is any remaining signal of the original introduction to the east coast of Canada or if subsequent escapees have homogenized pigeon population structure, I quantified genetic diversity from 7 sites ranging from the east to the west coast (a maximum of ~4400 km) using genome-wide single nucleotide polymorphisms (SNP) generated with double digest restriction site associated DNA (ddRAD) sequencing. This method is capable of detecting subtle differences among recently diverged bird populations (e.g., Lavretsky et al., (2019). **(2)** Does the population structure in each of the two louse species reflect geographic differentiation in the host populations? This was assessed by constructing haplotype networks based on COI sequences for the lice and examining whether the degree of differentiation among populations of lice paralleled that of the pigeons. Ideally, I would have used the ddRAD method for the lice as well as the pigeons, but in preliminary extractions, a single louse did not yield sufficient DNA for this method. **(3)** In the absence of the hippoboscid *Pseudolynchia canariensis,* do the phoretic louse species *Columbicola columbae*

and the non-phoretic *Campanulotes compar* have more similar population genetic structure, than has been found in areas where this fly occurs?

3.2 Materials and Methods

3.2.1 Sample collection and DNA extraction

Rock pigeons were salvaged from rehabilitation centres from across Canada: Vancouver, British Columbia; Calgary, Alberta; Edmonton, Alberta; Saskatoon, Saskatchewan; Winnipeg, Manitoba; Kingston, Ontario; Belleville, Ontario; and Halifax, Nova Scotia (for sampling dates and rehabilitation centres refer to Appendix 2.1). Due to their close geographic proximity (~72 km), pigeons from Belleville and Kingston were grouped together and are referred to as Southern Ontario (S. Ontario). Pigeons were frozen as soon as possible after being euthanized and were either picked up or shipped to the University of Alberta using an overnight courier service.

Lice were removed from pigeons following the washing protocol outlined in Grossi et al., (2014) and stored in 95% ethanol. After washing, a sample of tissue was taken from the breast of each pigeon and stored at -20˚C. Total genomic DNA from both louse and pigeon tissue samples was extracted using the DNeasy® Blood and tissue kit (QIAGEN, Valencia, California, U.S.A.). Extraction from pigeon tissue samples followed the manufacture's protocols. Lice were removed from the washing and were cut lengthwise down their thorax and abdomen with a scalpel, then allowed to incubate in the Buffer ATL and proteinase K mixture for 3 days. During this time tubes were vortexed multiple times a day and then centrifuged to collect contents at the bottom. The manufacture's protocols were then followed, except that DNA was eluted with 100 µl of Buffer AE.

To confirm louse identity and retain morphological vouchers, I recovered louse exoskeletons after DNA extraction and slide-mounted them in commercially available poly-vinyl

alcohol medium (PVA) (BioQuip Products, Rancho Dominguez, California). Slides were cured for 4 days at ca. 40 °C on a slide-warmer and then examined using Differential Interference Contrast (DIC) optics on a Leica DMLB compound microscope. I used Price et al., (2003) and Emerson, (1957) to confirm the species identifications. Vouchers are deposited in the University of Alberta E. H. Strickland Entomological Museum (accession numbers UASM80588 – UASM80684).

3.2.2 Rock Pigeon ddRAD sequencing

Double digest restriction site-associated DNA sequencing is a reduced representation sequencing method that is a cost-effective way for surveying genome-wide SNPs. Libraries were constructed following the protocol of MacDonald, Dupuis, Davis, Acorn, Nielsen and Sperling (in review; pers. comm., C. Davis, University of Alberta), which combines a modified bench protocol from Poland et al., (2012) and adaptors modified from Peterson et al., (2012). Restriction enzyme digests were carried out with 200 ng of genomic pigeon DNA and 10 µL restriction master mix (2.5 µL NEB CutSmart buffer (10x), 8 units Sbfl (CCTGCA/GG), 8 units EcoRI (G/AATTC) and 7.2 µL dH$_2$O). Digestion was carried out with the thermo regime: 37°C for 2 hours, followed by 65°C for 20 minutes. To ensure that only fragments that have been cut by both restriction enzymes are amplified and sequenced, adaptors are ligated onto the sticky ends as follows. The forward adaptor has an inline unique index sequence with at least 2 base differences relative to other indices. This allows for sample identification after sequencing. The common reverse adaptor is "Y" shaped, with the distal part of the tail being unpaired. Ligation reactions consisted of: 20 µL restriction digest (from previous step), 2.5 µL forward adapter (0.02 µM), 2.5 µL reverse adapter (3 µM), 15 µL ligation master mix (2 µL NEB CutSmart buffer, 4 µL ATP (10 mM), 0.5 µL T4 DNA ligase (400,000 U/mL), 8.5 µL dH$_2$O). Ligation

reactions were carried out with the following thermo regime: 22°C for 80 minutes, followed by 65°C for 20 minutes. Subpools with 16 unique forward adaptors were made with 5 µL from each sample ligation and purified using the QIAquick PCR Purification kit (QIAGEN, Valencia, California, U.S.A.), following standard protocols and eluted in 60 µL dH$_2$O. Amplification of each purified subpool was carried out with polymerase chain reactions (PCR) and a uniquely indexed reverse primer. During the first PCR cycle, only the forward primer will anneal to the forward adaptor. As DNA is being synthesized, the complementary strand to the unpaired part of the y-shaped tail will also be synthesized. During the second PCR cycle the reverse primer will anneal to the newly synthesized complement of the "y" adaptor tail. At the end of the PCR each sample will have a unique dual index that allows demultiplexing to the individual sample. PCR were carried out with: 10 µL cleaned subpool (from previous step), 5 µL reverse primer (0.2 µM) and 10 µL amplification master mix (1 µL common forward primer (2 µM) , 5 µL Phusion HF buffer (5x), 2.5 µL dNTPs (2 mM), 0.25 µL Phusion High-fidelity DNA polymerase, 1.25 µL dH$_2$O). PCR was carried out with the following thermo regime: 98°C for 30 seconds, 12 cycles (98°C for 10 seconds, 54°C for 20 seconds and 72°C for 1 minute), followed by 72°C for 10 minutes. PCR products were then pooled together and purified using a MinElute PCR Purification kit (QIAGEN, Valencia, California, U.S.A.), following standard protocols. Quantification of library concentration was done with a Qubit™ dsDNA High Sensitivity assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) and verification of library size was carried out using an Agilent HS DNA kit on the Bioanalyzer system (Agilent Technologies, Santa Clara, California, U.S.A.). Libraries were sequenced on an Illumina NextSeq at the Molecular Biology Service Unit (Department of Biological Sciences, University of Alberta) using a Next Seq 500 High Output 1x75 bp kit which produced 400M reads.

3.2.3 Rock Pigeon ddRAD data processing

Demultiplexed ddRAD reads for 192 *C. livia* specimens were aligned to the *Columba livia* genome (Genbank assembly accession GCA_001887795.1) using the BWA-MEM alignment algorithm with default parameters in Burrows-Wheeler Aligner v. 0.7.17 (Li and Durbin, 2009). Genotyping was conducted in Stacks version 2.0 (Rochette et al., 2019) using the refmap.pl pipeline. All parameters were run using default values with the following exceptions: (1) the -r parameter in the populations program was set to 80, which requires loci to be present in at least 80% of the individuals within a given population for it to be retained in the dataset; and (2) in order to reduce genomic linkage in population genomic analyses, a single SNP per locus was outputted. The dataset was then filtered using Vcftools version 0.1.14 (Danecek et al., 2011) by specifying a minimum genotyping quality score of 30, a minimum minor allele frequency of 3%, and a maximum amount of missing data per locus of 20%. To limit the amount of missing data, pigeons that were missing more than 20% of the loci contained in the filtered dataset were removed. This reduced sample size from 192 to 179. All data processing was conducted on the Cedar cluster hosted by Compute Canada.

Total observed and expected heterozygosity, F_{ST} and F_{IS} values as well as pair-wise F_{ST} values between sampling locations were calculated using the adegenet v. 2.1.1 (Jombart et al., 2011) and hierfstat v. 0.04.22 (Goudet, 2005) packages in R v. 1.1.442 (R Core Team, 2018). Observed and expected heterozygosity, and inbreeding coefficients (G_{IS}) for each sampling location were also calculated using GenoDive (Meirmans et al., 2004).

To infer population structure, I performed Bayesian-based clustering analysis using STRUCTURE v. 2.3.4 (Pritchard et al., 2000), which assigns individuals to genetic clusters or populations (K) based on allele frequencies without any prior knowledge of sampling location.

Support was tested for 1 to 7 genetic clusters (K = 1 to 7). Each value of K was run 10 times with a burn-in period of 250 000 and a total of 1 000 000 Markov Chain Monte Carlo replicates. To determine the most likely number of genetic clusters, Structure Harvester web server v. 0.6.94 (Earl and Vonholdt, 2012) was used to calculate ΔK (Evanno et al., 2005), and membership probabilities were averaged among runs using C_{LUMPAK} (Kopelman et al., 2015). Structure plots were created using C_{LUMPAK} output files with ggplot2 v. 3.1.1 (Wickham, 2016) in R v. 1.1.442 (R Core Team, 2018). In addition, discriminant analysis of principle components (DAPC), was carried out to determine population structure. DAPC partitions genetic variation into between-group and within-group components and tries to maximize the genetic variation of the between-group component while minimizing the within-group component (Jombart and Ahmed, 2011) This was done by using groupings from k-means clustering in the "find.clusters" function to identify the optimal number of clusters based on Bayesian Information Criterion (BIC) and then a DAPC was run using the adegenet package in R. The cross-validation function "Xval.dapc" was used to confirm the correct number of principal components is retained in the DAPC. A principal component analysis (PCA) was also carried out using the adegenet v. 2.1.1 (Jombart and Ahmed, 2011) package in R v. 1.1.442 (R Core Team, 2018), to examine the variation between samples. I also conducted a Mantel test with GenoDive (Meirmans and Tienderen, 2004) to see if there was evidence for isolation by distance. Genetic distances were taken from F_{ST} values that were transformed to $F_{ST}/(1- F_{ST})$ and geographic distances were based on the longitude and latitude of the major city the pigeon was associated with (Appendix 2.1, with the exception of the S. Ontario population which used the longitude and latitude of Kingston, Ontario) taken from log-transformed coordinate distances; these tests were run with 10 000 permutations.

3.2.4 Louse COI Sequencing

A portion of the mitochondrial COI gene was amplified with PCR using the primers L6625 and H7005 (Hafner et al., 1994). Each PCR reaction contained 5.0 ul of DNA template, 1X One *Taq* Standard Reaction Buffer (New England BioLabs® Inc), 0.2 mM dNTP, 1.0 µM of each primer and 0.625 units of One *Taq* DNA polymerase (New England BioLabs® Inc), and enough purified water to reach a final volume of 25 µL. Amplification was carried out using a Eppendorf® Mastercycler (Eppendorf, Humburg, Germany). The thermal regime consisted of an initial denaturation for 2 minutes at 94 °C followed by 40 cycles of 30 seconds at 94 °C, 30 seconds at 46 °C, and 1 minute at 72 °C, with a final extension of 7 minutes at 72 °C. PCR products were purified using ExoSAP (New England BioLabs® Inc) and sequenced in both directions using the original amplification primers and BIGDYE 3.1 chemistry on an ABI3730 sequencer (Applied Biosystems) at the Molecular Biology Service Unit (Department of Biological Sciences, University of Alberta).

3.2.5 Louse COI data processing

Forward and reverse sequences for each individual were assembled into consensus sequences using Geneious v. 11.1.4 (www.geneious.com), which were then manually aligned using BioEdit v. 7.0.5.3 (Hall, 1999) and ends were trimmed to minimize missing data.

The number of haplotypes (H), haplotype diversity (h) and nucleotide diversity (π) were calculated in DnaSP v. 6.12.03 (Rozas et al., 2017). Haplotype networks were built using TCS network method (Clement et al., 2002) in POPART (Leigh and Bryant, 2015). The mean p-distance between sampling locations for each louse species was calculated in Mega7 (Kumar et al., 2016).

3.2.6 Genetic distance comparison between Rock Pigeons and Lice

The pairwise F_{ST} values generated from the pigeon data SNPs was plotted against the p-distance between sampling locations for each louse species.

3.3 Results

3.3.1 Rock Pigeon Genome-Wide SNPs and Population Structure

A total of 161 086 979 reads were generated, of these 79.5% were kept; 4.0% were skipped due to insufficient mapping qualities, 6.4% were skipped due to excessive soft-clipping and 10.1% were skipped due to being unmappable. In the end a total of 1984 SNPs were retained after filtering. Loci had a mean locus depth that ranged from 8 to 1288, with the average depth of 148.59. Thirteen individual pigeons were removed due to high levels of missing data, and so subsequent analyses were run with 179 individuals: 43 from Vancouver, 34 from Calgary, 23 from Edmonton, 13 from Saskatoon, 36 from Winnipeg, 5 from S. Ontario and 25 from Halifax. These populations showed an observed heterozygosity of 0.2544, expected heterozygosity of 0.2667, F_{ST} of 0.0173 and F_{IS} of 0.0461. Pairwise F_{ST} values between the seven sampling locations were low ranging from 0.0060 - 0.0395 (Table 3.1). Genetic diversity measurements by sampling location are shown in Table 3.3.

The results from STRUCTURE suggested two genetic clusters of *C. livia* in the sampled pigeons (K=2, Figure 3.1; Appendix 3.2). One of these clusters is comprised of individuals from Halifax, and the other cluster contains all other sampling locations (Figure 3.2; Structure plots for every value of K tested can be found in Appendix 3.3). The PCA also shows two distinct clusters with Halifax being its own cluster however, very little variance is explained by the axes; 3.7% for the first two principle components (Figure 3.5). The "find.clusters" function produced BIC values that also support 2 genetic clusters (Figure 3.3). The DAPC had three discriminant

eigenvalues retained and shows Halifax as its own cluster, and everything else as the second cluster (Figure 3.4). However, within the second cluster Vancouver is slightly removed from the other location that all overlap with each other. Therefore, I ran a second STRUCTURE analysis following the same protocol as before but this time without individuals from Halifax. The results from this STRUCTURE analysis suggests that there are two genetic clusters for the non-Halifax specimens (Figure 3.6, Appendix 3.4). One cluster is predominantly made up of individuals from Vancouver and the other is made of individuals from the other locations (Figure 3.7, Appendix 3.5). There was weak positive correlation between genetic distance and geographic distance, however this relationship was not statistically significant (r = 0.069, p = 0.374), and therefore there is no evidence of isolation by distance.

3.3.2 Louse Mitochondrial Gene COI and Population Structure

Although I endeavoured to extract and sequence COI from at least 192 *Colu. columbae* and at least 192 *Camp. compar*, DNA quality of the lice was poor overall, possibly because the lice would have thawed and been infiltrated with water during the pigeon-washing process. COI was successfully sequenced from 46 individuals of the wing louse *Columbicola columbae* each from a different host: 7 from Vancouver, 5 from Calgary, 12 from Edmonton, 5 from Saskatoon, 4 from Winnipeg, 1 from S. Ontario and 12 from Halifax. The final alignment of COI was 400 bp in length of which 7 sites were variable, 1 was parsimony informative. There was a total of seven haplotypes, with one main haplotype found in all sampling locations and six other that differ by one nucleotide, forming a 'star-burst shape' surrounding the predominant haplotype (Figure 3.8). All locations had very low nucleotide diversity (range: 0.00077-0.00203, Table 3.3), and mean p-distance between sampling locations can be found in Table 3.4.

COI was successfully sequenced from 36 individuals of the body louse *Campanulotes compar* each from a different host: 2 from Vancouver, 4 from Calgary, 7 from Edmonton, 3 from Saskatoon, 6 from Winnipeg, 1 from S. Ontario and 13 from Halifax. The final alignment was 384 bp in length of which 15 sites were variable, 10 of these being parsimony informative. There was a total of seven haplotypes, again with one main haplotype found in all sampling locations but also a second haplotype found at 4 of the locations (Figure 3.9). While nuclear diversity in *Camp. compar* was higher than that of *Colu. columbae* it was still low, ranging from 0.00175-0.01075 (Table 3.3), and mean p-distance between sampling locations can be found in Table 3.4.

3.3.3 Genetic distance comparison between Rock Pigeons and Lice

The plot that compares genetic distances of *Colu. columbae* to Rock Pigeons shows no correlation (Figure 3.10A). However, the plots that compare genetic distances of *Camp. compar* to Rock Pigeons show that *Camp. compar* from Halifax show differentiation from the lice from other locations, just like their pigeon hosts (Figure 3.10B).

3.4 Discussion

Within the sampled Rock Pigeons there appears to be three genetic clusters: Cluster 1 consisting of individuals from Halifax, Cluster 2 of individuals from Vancouver and Cluster 3 of individuals from the other five locations (Calgary, AB to Southern Ontario).. This is supported by two STRUCTURE analysis; within both STRUCTURE plots admixture was observed. Within the first STRUCTURE plot (Figure 3.4) which includes pigeons from all seven locations sampled, the most admixture seen outside of the Halifax cluster is in Southern Ontario, which is the closest geographically to Halifax. Only a small number of specimens (n = 5) were examined from Southern Ontario and none from Northern Ontario or Québec, which leaves a gap of ~1000 km between Halifax and Southern Ontario; perhaps if individuals had been sampled from this

range, there would be less support for the Halifax cluster. In the second STRUCTURE plot (Figure 3.7) which excludes pigeons from Halifax, there is high levels of admixture seen outside of the Vancouver cluster in all five locations sampled; suggesting that more pigeons are moving west to east then moving east to west.

Most populations in this study are close to the northern edge of the Rock Pigeons range, however they are found throughout North America including southern Northwestern Territories in Canada (~62° N) and as far north as Prudhoe Bay (~70° N) in coastal Alaska (eBird, 2017). DiBlasi et al. (2018) used microsatellites to examine relatively small-scale population structure of three flocks of pigeons within Salt Lake City, Utah. They found that there was low genetic variation among flocks and that 98% of the genetic variation was accounted for by sampling within flocks. Carlen and Munshi-South, (2020) also examined the genetics of pigeons in the USA; however, they focused on the north-to-south distribution among cities along the Northeastern coast (Boston, Providence, New York City, Philadelphia, Baltimore, and Washington, DC). Using ddRAD-Seq they found two genetic clusters: a northern cluster made up of individuals from Boston and Providence and a southern cluster containing all other individuals. They also found that pairs of pigeons that were at least 50 km apart were no more related than they would be at random; in contrast, those within 25 km were highly related. Therefore there is localized gene flow along the northeastern coast of the USA. Jacob et al., (2014) worked at a larger scale and sampled pigeons from locations in France, Spain and Switzerland. To examine within regional differences, samples were taken from six locations within Paris, as well as three locations along the Rhone valley in France. They found that pigeons sampled within the same region had low levels of genetic variation (based on microsatellites) while those from distinct geographic locations had high levels of genetic differentiation. Jacob et al. (2014) also found evidence for isolation by

distance at this larger geographic scale. In contrast, even though the maximum geographic separation between sampling locations in this study (~4400 km) was much greater than the maximum in the European study (~850 km), I found no evidence of isolation by distance. This is probably due to a founder effect caused by the relatively recent human introduction of Rock Pigeons to North America. The first recorded introduction of Rock Pigeons to North America is by the French to Port Royal, Nova Scotia, in 1606 (Schorger, 1952). This is not the only documented introduction: the English sent pigeons to Virginia in 1621 and to Massachusetts in 1642 (Schorger, 1952). It would not be unreasonable to assume that there were many more introductions with settlers that were not recorded. With the introduction of pigeons came the construction of dovecotes, therefore encouraging their reproduction and human-mediated long-distance dispersal. Introductions of *C. livia* to North America continue today with the importation of birds by pigeon fanciers (e.g., several 1000s of European pigeons have been imported since 1959 by one breeder in Florida: http://www.donhartspigeons.com/import-1.html). Some of these imported pigeons no doubt escape and join the general free-living populace.

Even in the absence of the hippoboscid fly *Pseudolynchia canariensis*, the known phoretic host of the wing louse *Columbicola columbae*, *Colu. columbae* from Canada still show less genetic structure than the body louse *Campanulotes compar*. There is clearly more that is driving the structural differences in these louse populations then just the ability to disperse phoretically. It may be as simple as microhabitat preferences. *Columbicola columbae* resides primarily on the wing feathers, which may offer more opportunities to move on to a new host when contact between birds happens. In contrast, *Camp. compar* spends more time in the abdominal feathers of their host (Nelson and Murray, 1971; Clayton, 1991). Dispersal of *Colu. columbae* could also be driven by competition for resources. While *Colu. columbae* and *Camp.*

64

compar reside mainly in different locations on the hosts' body, they both consume the barbules of downy feathers (Nelson and Murray, 1971). Bush and Malenke (2008) found that when pigeons were experimentally infested with either 100 *Colu. columbae,* 100 *Camp. compar* or a combination of 50 of each species*,* after approximately 10 louse generations, pigeons with only *Colu. columbae* had 2-8 fold more *Colu. columbae* then those that were coinfected. They also observed that *Colu. columbae* were found on abdominal feathers significantly less often in the presence of *Camp. compar,* however the converse did not hold; *Colu. columbae* had no significant effect on populations of *Camp. compar* and the presence of *Colu. columbae* did not impact the distribution of *Camp. compar* on the host. Additional factors that might increase gene flow are that *Colu. columbae* has been observed leaving dead hosts faster than *Camp. compar* (Petryszak et al., 1996) and *Colu. columbae* can survive longer then *Camp. compar* off the host (Rem and Zlotorzycka, 1981). It is also possible that hippoboscid species other than *P. canariensis* occasionally visit Rock Pigeons and act as phoretic hosts for *Colu. columbae.* Skvarla and Machtinger (2019) present museum records of hippoboscids from the genus *Lipoptena* (*L. depressa* [Say], which also occurs in Canada) collected from *C. livia* in the United States.

In addition to *Colu. columbae* differing in population-genetic structure from *Camp. compar*, the comparison of genetic distances between *Colu. columbae* and its host showed that they too differ in structure. In contrast, Rock Pigeons and the body louse *Camp. compar* both had highly differentiated Halifax populations. Therefore, the population structure of *Camp. compar* at least partially reflects geographic differences seen in their host. This greater similarity between pigeons and body lice than pigeons and wing lice has been reported by other researchers (Johnson et al., 2002; DiBlasi et al., 2018). The lack of genetic structure in *Colu. columbae*

compared to their host may be because of their larger effective population size (Criscione, 2008), therefore genetic drift will take longer in lice. There might have been stronger evidence of differentiation in the louse species if a more sensitive method, such as ddRDA could have been implemented.

To conclude, even though Rock Pigeons are a non-migratory species, there is either enough movement of individuals between sampling locations that they are not isolated from each other, or continued importation (and accidental release) of pigeons homogenizes most populations. Contrary to my expectation, *Columbicola columbae* showed less population structuring than *Campanulotes compar* , even though the usual explanatory variable for this difference (the phoretic host hippoboscid of *Colu. columbae*, *P. canariensis*) is absent from the landscape. As in previous studies of pigeon louse populations genetics, I found that the genetic structure of the body louse *Campanulotes compar* more closely mirrors that of its host.

Table 3.1: Pairwise F$_{ST}$ values for *Columba livia* based on 1984 SNPs sampled from seven locations across Canada.

Number of *C. livia*		Vancouver	Calgary	Edmonton	Saskatoon	Winnipeg	S. Ontario
43	Vancouver						
34	Calgary	0.0101					
23	Edmonton	0.0121	0.0062				
13	Saskatoon	0.0138	0.0060	0.0102			
36	Winnipeg	0.0126	0.0095	0.0114	0.0115		
5	S. Ontario	0.0206	0.0085	0.0130	0.0125	0.0175	
28	Halifax	0.0412	0.0369	0.0395	0.0412	0.0453	0.0364

Table 3.2: Genetic diversity of *Columba livia* based on 1984 SNPs sampled from seven locations from across Canada.

Sampling location	Number of *Columba livia*	Observed Heterozygosity (Ho)	Expected Heterozygosity (Hs)	Inbreeding coefficient (G_{IS})
Vancouver	43	0.255	0.264	0.034
Calgary	34	0.253	0.269	0.062
Edmonton	23	0.251	0.264	0.048
Saskatoon	13	0.251	0.266	0.055
Winnipeg	36	0.251	0.267	0.060
S. Ontario	5	0.280	0.275	-0.019
Halifax	28	0.239	0.259	0.079

Table 3.3: Genetic diversity of *Columbicola columbae* and *Campanulotes compar* based on a 400 bp and 384 fragment of Cytochrome oxidase subunit I, respectively. n: sample size, H: number of haplotypes, *h:* haplotype diversity, π: nucleotide diversity.

	Columbicola columbae				*Campanulotes compar*			
	n	H	*h*	π	n	H	*h*	π
Vancouver	7	1	-	-	2	1	-	-
Calgary	5	2	0.400	0.00101	4	2	0.500	0.00263
Edmonton	12	3	0.318	0.00084	7	3	0.667	0.00280
Saskatoon	5	2	0.700	0.00203	3	2	0.667	0.00175
Winnipeg	4	2	0.500	0.00138	6	2	0.333	0.00262
S. Ontario	1	1	-	-	1	1	-	-
Halifax	12	2	0.303	0.00077	13	5	0.846	0.01075

Table 3.4: P-distances for *Columbicola columbae* (bottom), and *Campanulotes compar* (top)*,* based on a 400 bp and 384 fragment of Cytochrome oxidase subunit I, respectively.

		Campanulotes compar						
		Vancouver	Calgary	Edmonton	Saskatoon	Winnipeg	Southern Ontario	Halifax
Columbicola columbae	Vancouver		0.001312	0.002254	0.0006544	0.001308	0.000000	0.008774
	Calgary	0.0005031		0.002631	0.001970	0.002076	0.001312	0.008325
	Edmonton	0.0004171	0.0009199		0.002915	0.002753	0.002254	0.008386
	Saskatoon	0.001004	0.0014080	0.001422		0.001965	0.0006544	0.009442
	Winnipeg	0.0006319	0.001135	0.001049	0.001639		0.001308	0.008843
	S. Ontario	0.0000000	0.0005038	0.0004177	0.001005	0.0006329		0.008774
	Halifax	0.0004171	0.0009205	0.0008343	0.001423	0.001049	0.0004177	
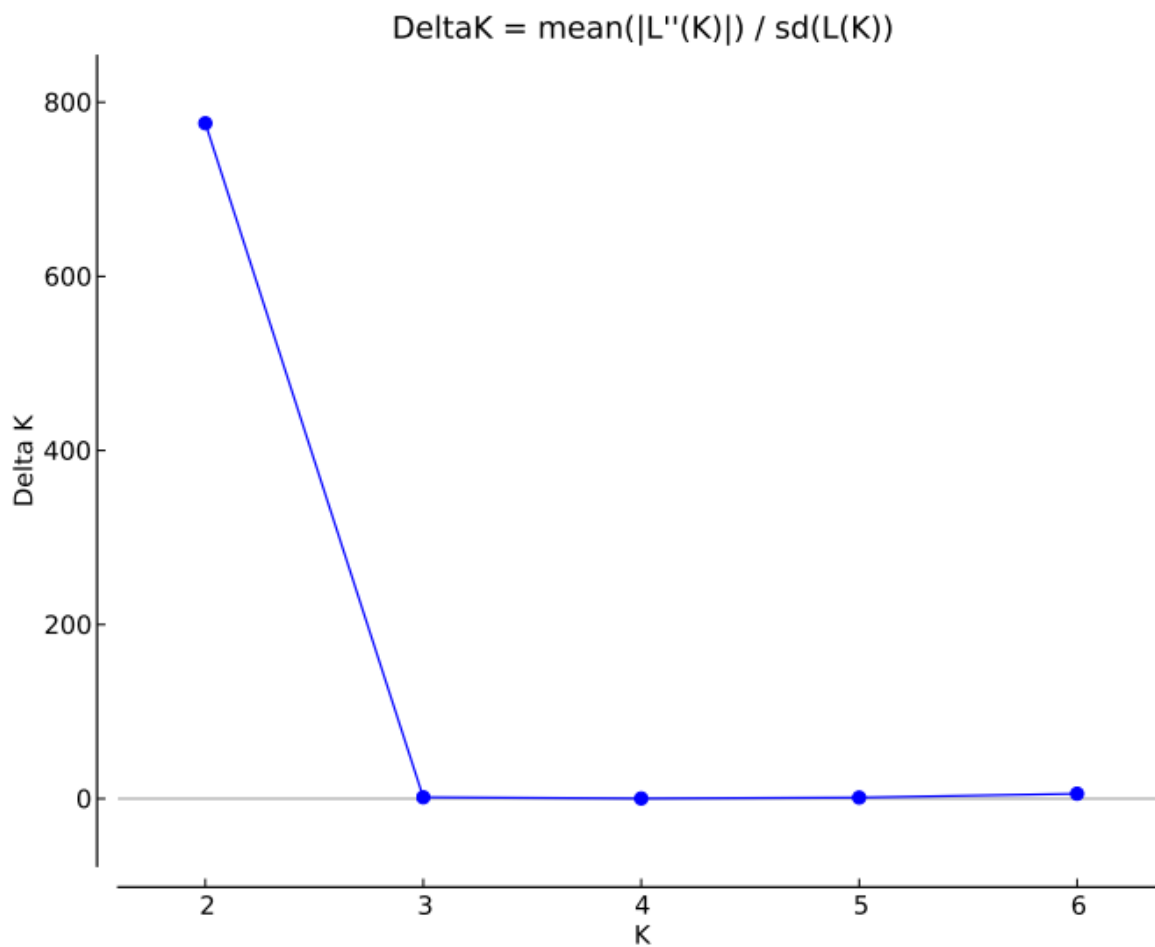
Figure 3.1: ΔK plot for *Columba livia*, showing the most likely number of genetic clusters calculated by STRUCTURE HARVESTER.
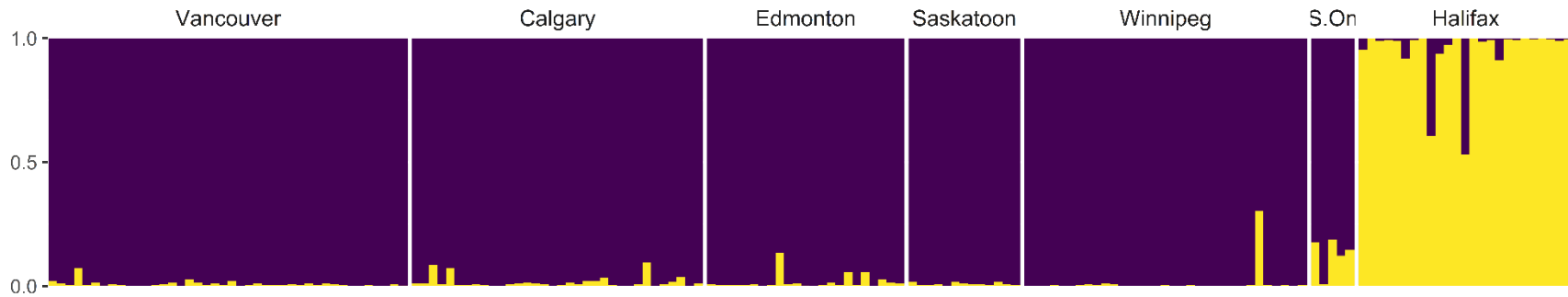
Figure 3.2: Structure plot for *Columba livia* of probabilities of assignment of individuals to different genetic clusters for K=2, based on analysis of SNP using STRUCTURE.
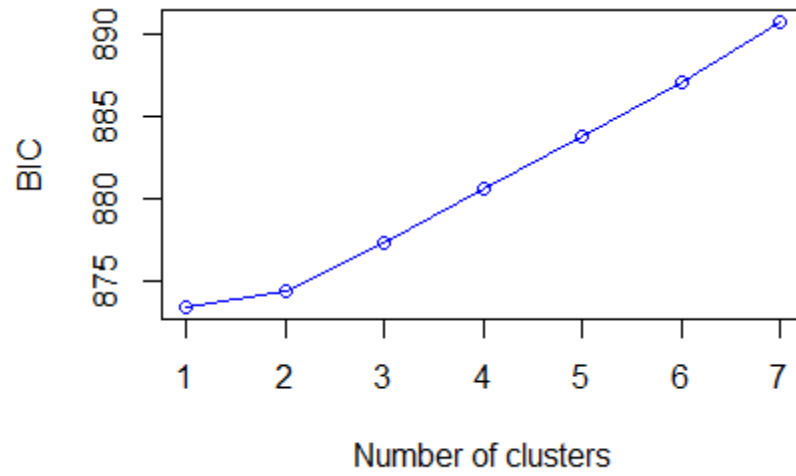
Figure 3.3: Bayesian Information Criterion, showing the most likely number of genetic clusters

based on K-means calculated by "find.clusters" in adegenet.
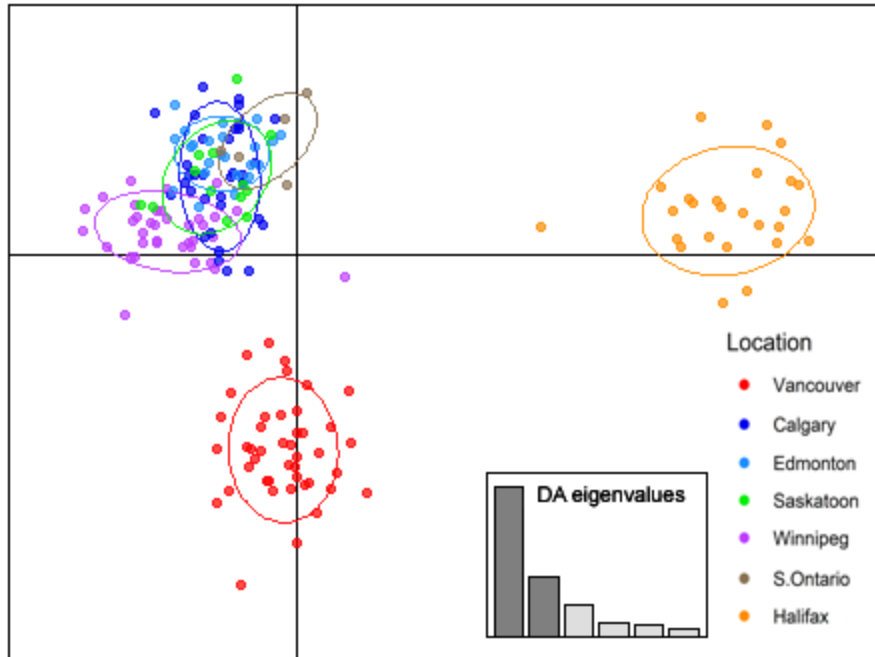
Figure 3.4: Discriminant Analysis of Principle Components of Rock Pigeon (*Columba livia*) SNP data, grouped by sampling location across Canada.
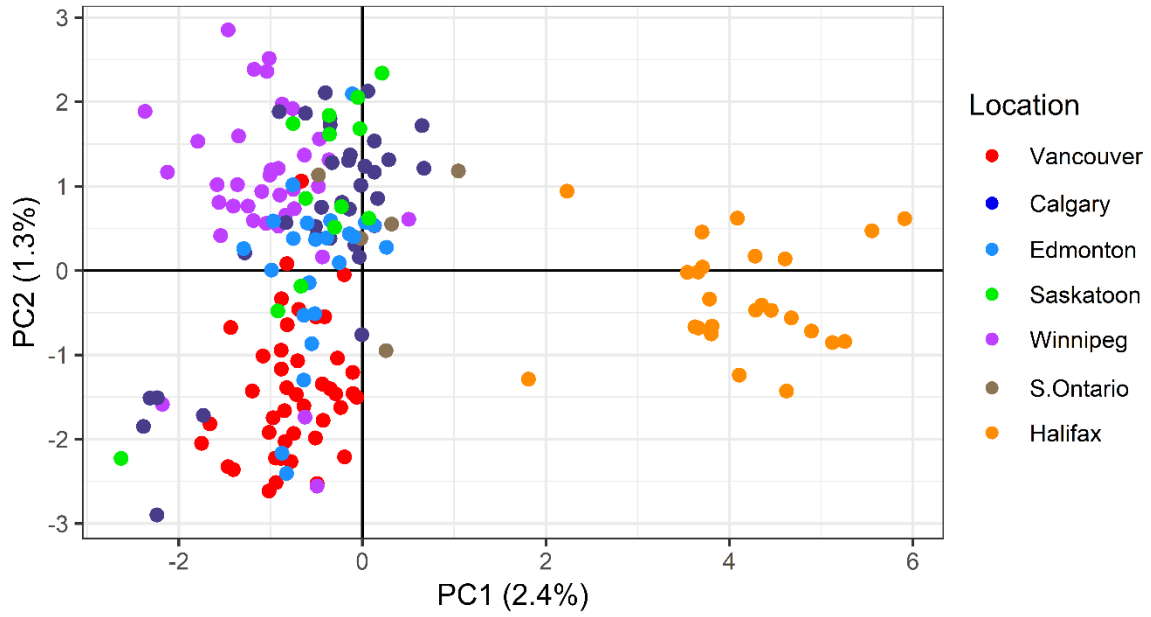
Figure 3.5: Principle Component Analysis of SNP data from Rock Pigeon (*Columba livia*) sampled from seven locations across Canada.
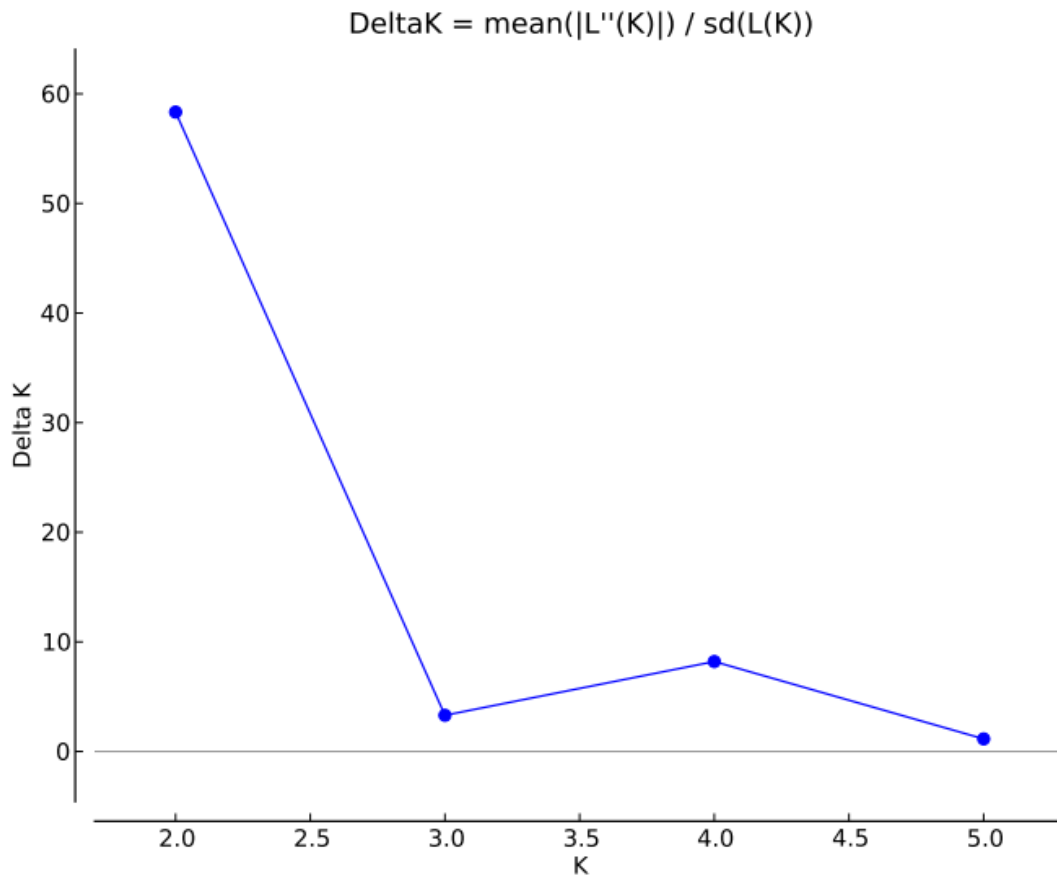
Figure 3.6: ΔK plot for *Columba livia* (sampled from everywhere but Halifax NS.) showing the most likely number of genetic clusters calculated by STRUCTURE HARVESTER.
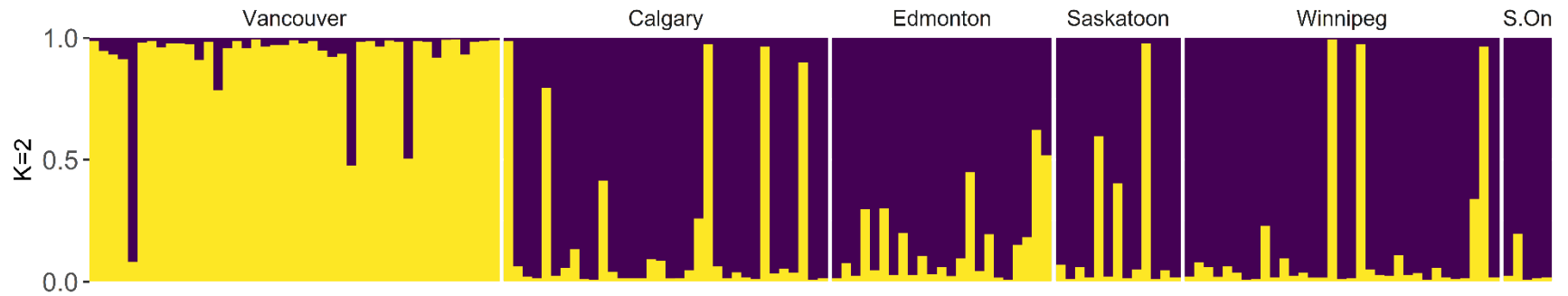
Figure 3.7: Structure plot for Columba livia (sampled from everywhere but Halifax NS.) of probabilities of assignment of individuals to different genetic clusters for K=2, based on analysis of SNP using STRUCTURE.
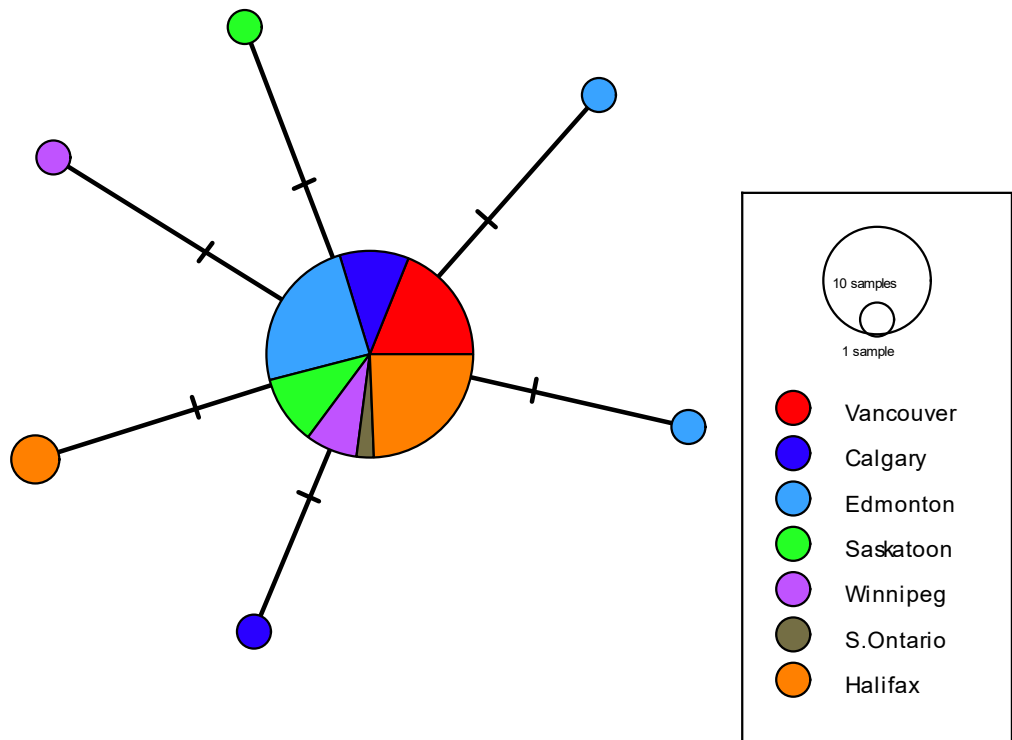
Figure 3.8: TCS haplotype network of *Columbicola columbae* generated from COI (400 bp). *Columbicola columbae* are from Rock Pigeon (*Columba livia*) collected from seven locations across Canada. Hash marks indicated nucleotide substitutions.
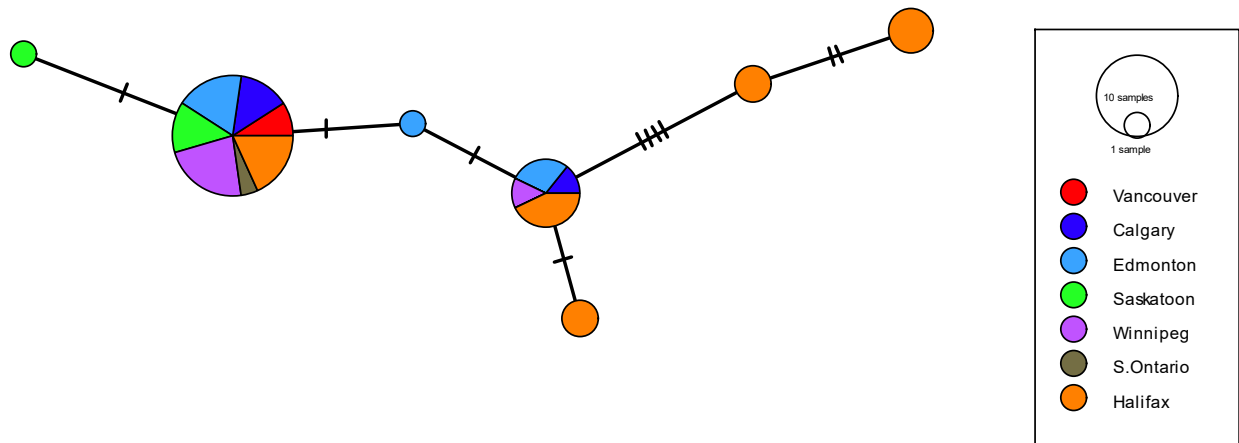
Figure 3.9: TCS haplotype network of *Campanulotes compar* generated from COI (384 bp). *Campanulotes compar* are from Rock Pigeon (*Columba livia*) collected from seven locations across Canada. Hash marks indicated nucleotide substitutions.
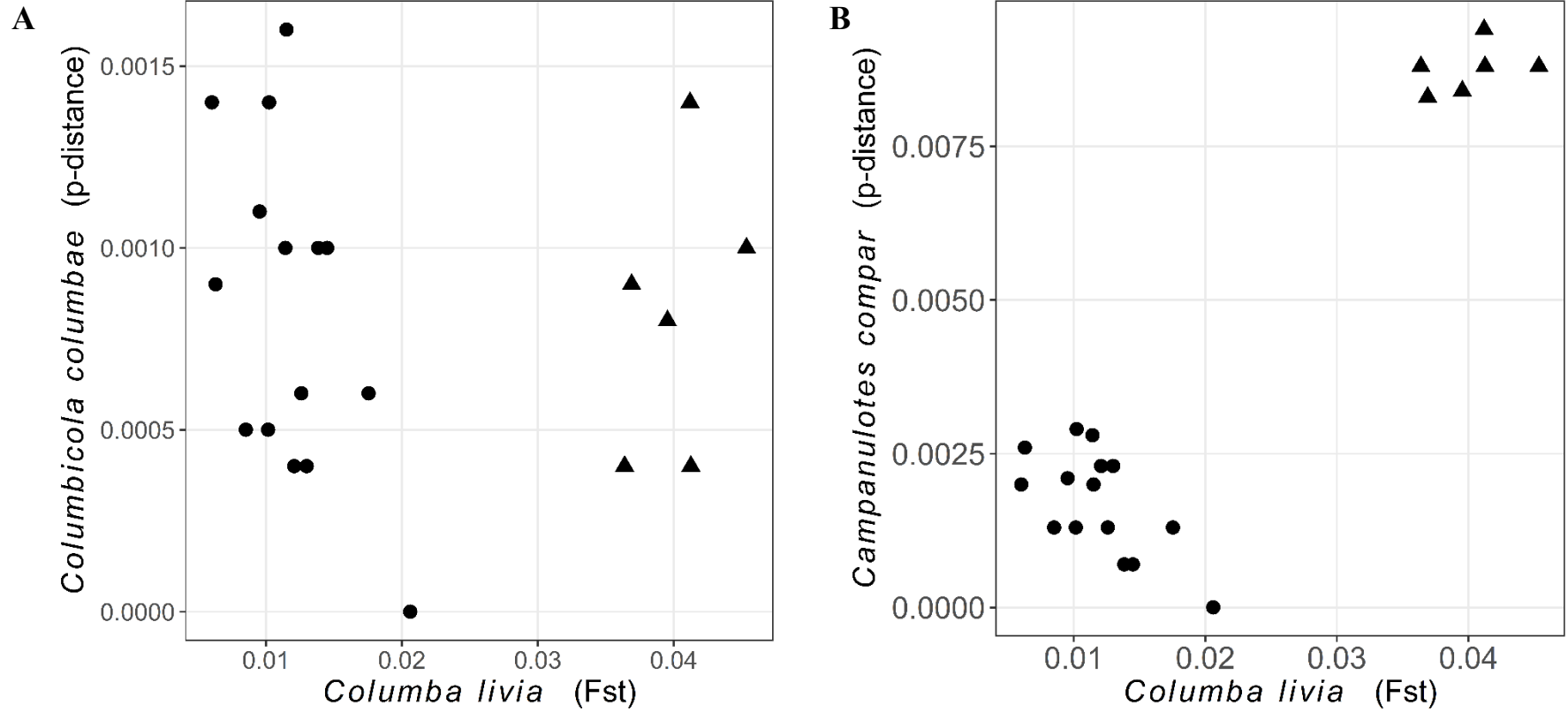
Figure 3.10: Comparison of genetic distance between Columba livia and (A) Columbicola columbae and (B) Campanulotes compar.

Triangles indicate Halifax distances.

Chapter 4 - The distribution of quill mites (*Betaysringophiloidus seiuri*) among the flight

feathers of the Ovenbird (*Seiurus aurocapilla)*

4.1 Introduction

Birds are covered in hundreds to thousands of feathers (Wetmore, 1936) that vary in size
and form, and provide habitat for a diversity of permanent ectosymbionts. Variation in feather
structure has enabled habitat partitioning of the host's body by ectosymbionts and allows co-
occurrence of multiple species on a single host individual (Dubinin, 1947a; Dabert and Mironov,
1999). There are 2 broad groups of permanent ectosymbionts infesting birds: chewing lice
(Phthiraptera) and mites (many families of Astigmata and Prostigmata). Mites associated with
the integument of birds include those found on the skin (dermicoles), on the surface of the
feathers (plumicoles), and inside feathers (syringicoles) (Walter and Proctor, 2013).
Syringicolous mites live and reproduce inside the hollow quill (= calamus) of the feather and
include several families from the Astigmata (e.g., Ascouracaridae, Dermoglyphidae) and one
from the Prostigmata (Syringophilidae). Syringophilid mites are known from 95 families of birds
(Glowska et al., 2015a); however, generalizations about the ecology and biology of
syringophilids are typically based on a single well-studied species, *Syringophiloidus minor*
(Berlese) infesting House Sparrows, *Passer domesticus* (Linnaeus) (Skoracki et al., 2016).
Syringophilid mites are thought to enter through the superior umbilicus of a developing feather
before it becomes plugged (Casto, 1974a). Once the plug is formed, mites exit either by
dislodging the plug and crawling through the umbilicus or by constructing a channel through the
cortex and pith of the feather (Casto, 1974a). Inside the quill, mites feed on fluids in tissues
adjacent to the quill by piercing the quill wall with their long stylet-like chelicerae (Kethley,
1971). Mated female mites disperse to newly forming feathers and once inside the quill start to

produce eggs, one at a time. Syringophilids are haplodiploid, meaning that unfertilized eggs develop into males and fertilized eggs become females. The first egg laid by a foundress *S. minor* is unfertilized and develops into a male and all subsequent eggs develop into diploid females, which results in a highly female-biased sex ratio (Kethley, 1971). Kethley, (1971) observed that the majority of House Sparrow quills were invaded by a single founding female, but two or more foundresses were not uncommon, which would provide the opportunity for outcrossing by offspring produced within the quill.

Quill wall thickness and quill volume are thought to determine which quills can be inhabited by syringophilids (Casto, 1975). Mites can only survive in quills with walls thin enough for their chelicerae to reach the tissue on the outside of the quill. At the same time, the volume of the quill must be large enough to house multiple generations until the host's next molt. Therefore, the distribution pattern of quill mites among a host's feathers should reflect habitability of particular feathers, which is unlikely to be uniform across a bird's plumage. However, infestation parameters of quill mites are rarely reported, and when they are, they usually refer to the bird as a whole, and not per feather type or per individual feather location (Skoracki et al., 2001a, 2017). This is most likely because unlike mites that dwell on the surface of feathers, quill mites provide few outward signs of their presence and are not easily detectable by human examiners. The only way to definitively detect quill mites is to remove the feather from the host and examine the calamus using a dissecting microscope. Therefore, it is hard to conduct quill mite studies on live hosts.

Syringophilid species within the subfamily Syringophilinae are known to inhabit the quills of wing feathers (primaries, secondaries, tertials, and wing coverts), tail feathers (rectrices), and occasionally body feathers (Glowska et al., 2015a). In a previous study, the

syringophiline *Betasyringophiloidus seiuri* (Clark) was used as a biological marker for population structure of its host, the Ovenbird *Seiurus aurocapilla* (Linnaeus) (Haché et al., 2017). In that study, mites were obtained by removing 2 tail feathers from each live bird. The authors examined 875 Ovenbirds and reported *B. seiuri* from 45 of them (prevalence of 5.1%). However, *B. seiuri* are not exclusively found in tail feathers; Bochkov and Galloway, (2001) observed *B. seiuri* in primary wing feathers of Ovenbirds but did not record prevalence. Reported prevalences of other syringophiline species with passerine hosts range from 5.2% - 15.5% (Skoracki et al., 2010). This raises the question of whether the prevalence of 5.1% observed by Haché et al. (2017) is at the very low end of this range because these authors did not inspect flight feathers of the wings as well.

In this study I examined the distribution of *B. seiuri* in the flight feathers (primaries and secondaries of the wing and rectrices of the tail) of 21 dead Ovenbirds from Canada. The distributions of mites both between and within feather types were compared to determine which feathers are most often infested and which feathers contain the greatest number of mites. When determining the intensity of mites in each quill, the contents of the entire quill were examined, which gave me the opportunity to assess absolute and relative abundance of male, female and juvenile *B. seiuri* to augment the currently scarce data on population structure of syringophilids. We also measured quill wall thickness and quill volume of the flight feathers from 3 additional Ovenbirds. All else being equal, I would expect quills with larger volumes to contain more mites than quills with smaller volumes. However, if those larger quills also have thicker quill walls this may exclude them as suitable habitats, as the mites' chelicerae may not be able to fully puncture the quill wall. To see how quill wall thickness compares to chelicerae length, I measured the chelicera of larval and adult female mites from different flight feather locations.

4.2 Materials and Methods

Dead Ovenbirds were salvaged from a wildlife rehabilitation centre in Winnipeg, Manitoba, Canada (2014-2015), and from window-strike kills in central Alberta, Canada (2014-2015) and Toronto, Ontario, Canada (2016). Ovenbirds were frozen as soon as possible after being euthanized or discovered dead. To assess whether quill mites leave the quill after the host's death, I washed the entire body of each Ovenbird using the methods of Grossi et al., (2014). No quill mites were found in any of the washings. Each primary, secondary and rectrix feather (see Figure 4.1 for individual feather locations) was examined for quill mites by splitting open the calamus longitudinally under a dissecting microscope (Leica MEB126, Leica Microsystems Inc., Concord, Canada). As each bird had 12 tail feathers and 36 wing feathers (18 per wing), this was a total of 1,008 flight feathers from the 21 birds. I acknowledge that *B. seiuri* may also occupy body feathers, but exhaustively assessing their presence in the many thousands of body feathers of our specimens would have been too time-consuming. All mites found were stored in 95% ethanol until they were slide mounted. Prior to slide-mounting, mites were cleared in lactic acid (Fisher Scientific, Fair Lawn, New Jersey) for 24 hr, and then mounted in commercially available phenol-free polyvinyl alcohol medium (product #6371NS1, BioQuip Products, Rancho Dominguez, California). Slides were cured for 4 days at ca. 40 C on a slide-warmer and then examined using Differential Interference Contrast optics on a Leica DMLB compound microscope (Leica Microsystems Inc., Concord, Canada). All slide-mounted mites were identified to juvenile (larvae and nymphs) or to adult stage and sexed (for adults). Adults were identified to species using primary literature (see Results). Vouchers are deposited in the University of Alberta E. H. Strickland Entomological Museum (accession numbers UASM80566, UASM80567, UASM80568 and UASM80569).

To assess whether mites found in wing feathers were genetically the same species as those that were found in tail feathers by Haché et al. (2017), 4 quills containing mites were selected at random for molecular work. Following the methods described by Haché et al. (2017) a fragment of the cytochrome oxidase subunit I (*COI*) gene was amplified using the primers Aseq01F and Aseq03R (Glowska et al., 2014) and sequenced at the Molecular Biology Service Unit of the University of Alberta. Sequences were compared to those in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). Mite exoskeletons were recovered from DNA extractions and slide mounted using the same methods that were described above, without the clearing step as the extraction process had already cleared the mites.

Prevalence and mean intensities were calculated with Quantitative Parasitology 3.0 (Rózsa et al., 2000). Prevalence is defined as the number of hosts, or body regions of the host (feather locations in this case), infested by a parasite divided by the number of hosts/regions examined. Mean intensity is the average number of mites in infected hosts, or in infested regions of the host (hence excluding counts of zero mites).

To measure the length of the chelicerae, I took photographs of slide mounted mites with clearly visible chelicerae using a Leica MC170 HD camera (Leica Microsystems, Inc., Concord, Canada) that was mounted onto the compound microscope. These pictures were then imported into the image-processing program ImageJ (Schneider and Rasband, 2012), which we used to measure the chelicerae. One chelicera was measured from 10 adult female and 10 larvae infesting each feather location from Primary 7 to Secondary 7. Adult females and larvae were selected because they were the largest and smallest life stages, respectively, and hence should show the full range in chelicera size in this species. Due to low intensities, in particular feather

locations, Primary 8 only had photographs of 3 adult females and 1 larva and Secondary 8 had, 10 adult females and 1 larva taken. No photographs of mites were taken from Primary 9, Secondary 9 or from any of the rectrices.

While measuring the chelicerae I noticed that when they were extended, a portion of each chelicera always remained inside the gnathosoma of the mite; therefore, the whole length of the chelicera could not be used to pierce the quill wall. When I came across a mite with its chelicera extended, the extended portion of the chelicera was also measured in addition to the full length. The majority of mites did not have their chelicerae extended but in order to estimate the possible extended chelicerae length, I found the female and larva that had the greatest proportion of their chelicerae extended. For females this was 60.6% of the full chelicera length and for larvae 75.9%. I then calculated this proportion from the full length of all the chelicerae measured and considered this the 'maximum extended length'.

The calamus of the quill was opened lengthwise to avoid damaging the mites inside; however, while doing this the quill was destroyed and not useable for taking quill measurements. Instead, I used 3 dead Ovenbirds that had not been examined for quill mites for quill measurements. I removed the flight feathers (primaries, secondaries and rectrices from both sides) from these 3 birds. To calculate the volume of the quill, each flight feather was scanned with an HP Scanjet 7400 scanner (Hewlett-Packard, Palo Alto, California) at high resolution (1200 dpi). The scans were then imported into ImageJ. The length of the quill and diameter of the quill where the barbules started and diameter of the quill tip were measured (Appendix 4.1). These measurements were then used to calculate the volume of a truncated cone. Average quill wall thickness (see next paragraph) was subtracted from the radius for each feather prior to calculating volume.

To assess quill wall thickness, the quill was separated from the rest of the feather using a pair of fine scissors, immediately below where the barbules started. The quill was then stuck upright in mounting putty (LePage Fun-Tak®, Henkel Canada Corporation, Mississauga Canada) and examined under a dissecting microscope. Using an ocular micrometer, I measured the thickest and thinnest spots in the quill wall. The quill was then cut right before the tip and the thickest and thinnest spots in the quill wall were measured (Appendix 4.1). These 4 measurements were then averaged to get the average quill wall thickness for a single feather. In addition, the 2 thinnest and 2 thickest measurements were averaged to get the average thinnest and thickest measurements per feather.

To see if there was a correlation between quill volume and the number of mites found in each feather location, Kendall rank correlation coefficients were calculated using R (R Core Team, 2018). The variables compared were average quill volume and average number of mites (including zero counts), per feather location.

4.3 Results

I examined a total of 21 Ovenbirds for quill mites: 9 from Winnipeg, 7 from central Alberta and 5 from Toronto. Three birds were infested from each location (n = 9, prevalence = 42.9%, 23.3 – 64.6, 95% CI). I extracted and slide-mounted 8,674 quill mites from the flight feathers of these 9 birds. All adult mites were morphologically identified as *Betasyringophiloidus seiuri* as illustrated in Bochkov and Galloway (2001) (see Skoracki et al., [2016], for comments on taxonomy of this species) (Appendix 4.2). This identification was also supported genetically. Of the 4 calami that had their mites sequenced, 1 from Winnipeg produced a useable sequence. After ambiguous calls were trimmed from the ends of the sequence, a 386 base pair segment remained (GenBank accession number: MN006956). When BLAST-searched,

the top 38 hits were *Betasyringophiloidus seiuri* from Haché et al. (2017), all with an E value of 0.0 and identities between 99 – 95%.

The prevalence of *B. seiuri* in the flight feathers of the wings was 38.1% (19.7 – 59.7, 95% CI, 8 of 21 birds). The prevalence per feather location (see Figure 4.1), ranged from 0 – 31.4% (n = 42: 21 birds with 1 left and 1 right feather per location) (Figure 4.2A and Appendix 4.3). Primaries 2 and 1, and Secondaries 1, 2 and 5 all had prevalences greater than 20% (Figure 4.2A). Wing feathers that never contained mites were Primary 9 and Secondary 9 (Figure 4.2A, Appendix 4.4). Quill mites were found in the tail feathers of 2 birds (prevalence = 9.5%, 1.7 – 30.5, 95% CI). One Ovenbird had only a single tail feather infested (Rectrix 6), which contained 1 juvenile mite. The other Ovenbird also had a single tail feather infested (Rectrix 1) containing 17 female and 12 juvenile mites; this bird also had quill mites infesting its wing feathers.

When each wing feather location was assessed separately, the mean mite intensity per feather location ranged from 2 (for Primary 8) to 135.6 (for Primary 7) (Figure 2b). The greatest number of *B. seiuri* in a single quill was 311 mites in a Primary 1 (50 females, 9 males and 272 juveniles, Figure 3.3). The mean intensity of adult mites per quill over all feather locations was 28.4 (23.7 – 33.7, 95% CI). The sex ratio was highly female biased, with only 11.6% of adult mites being male (Figure 4.3). There were only 2 individual feathers in which male mites outnumbered female mites: in 1 quill there was 1 male, 0 females, and 3 juveniles and in the other there were 2 males, 1 female, and 7 juveniles. Female reproductive output was estimated from quills that contained only 1 female and 1 male (n = 15 feathers), with the assumption that all the juveniles within such a quill were the offspring of a single female and that the adult male mite within the quill was also her offspring. Juveniles per female ranged from 0 – 7, therefore a single female *B. seiuri* can produce at least 8 eggs.

Average quill volume and quill wall thickness differed among wing feather locations (Figure 4.2C, 4.2D, respectively). Both showed a general trend of decreasing from the more distal feathers to those located closer to the body. Exceptions were Primaries 9 and 8, which had smaller volumes than those following them. In contrast, volume and quill wall thickness of the rectrices were consistent across all feathers. Rectrices had quill walls 1.7 times thicker than feathers of similar volume from the wings.

The full chelicera length of adult females was longer than that of larvae (Figure 4.4A). The full chelicera length of both life stages was greater than the thickest part of the wall of any of the quills. The extended portions of the female and larval chelicerae were more similar in length than the full chelicera (Figure 4.4B). When the extended portion of the chelicera is compared to the quill wall, the chelicerae are not longer than the thickest portion of the quill wall for all feather locations. For Primaries 5 to 8, the extended chelicerae of both females and larvae were shorter than the thickest part of the quill wall, and for Primary 4 only the females' extended chelicera was shorter. Extended portions of female and larval chelicerae were longer than the thinnest portion of the quill wall for all feather locations (Figure 4.4B).

There was a strong positive correlation between quill volume and mean number of mites at each feather location (τ_b= 0.645, p < 0.05) (Figure 4.5). Figure 4.5 shows that there are 2 outliers, Primary 8 and 9, both of which have mean numbers of mites that are lower than would be expected based on their volumes. These feather locations also have the thickest quill walls (Figures 4.4A, 4.4B).

4.4 Discussion

The quill mite *Betasyringophiloidus seiuri* was not uniformly distributed among the flight feathers of the 21 Ovenbirds I examined. Although I found quill mites in both the tail and wing

feathers, the tail feathers had a prevalence of only 9.5% compared to 38.1% for the wings. Haché et al. (2017) reported a similarly low prevalence (5.1%) of *B. seiuri* in the tail feathers from a much larger sample size of Ovenbirds (n = 875 birds). Skoracki et al., (2010) examined 8 species of passerines in Poland for quill mites by dissecting 1 primary, 1 secondary, 1 greater covert, 1 tail feather, and 10 – 20 breast feathers from each host. Nine quill mite species within the subfamily Syringophilinae were found; however, none were found in tail feathers and the majority of species resided in the secondaries. Skoracki et al. (2010) also found one species of syringophilid in body feathers. I did not examine any body feathers and suggest that future studies should also inspect a subsample of Ovenbird body feathers for quill mites.

Quill volume and quill wall thickness may influence which feather locations are inhabited by *B. seiuri*. I found a positive correlation between quill volume and mean number of mites for each feather location (Figure 4.5), with the exception of Primaries 8 and 9 which had smaller mean numbers then one would expect based on their volumes. This is most likely due to these feathers having the thickest quill walls, which in their thickest parts exceeded the length of the mites' extended chelicerae (Figure 4.4B). Although the thinnest portion of the quill wall for Primary 9 was the same thickness as the thickest portion of the 'preferred' feathers located in the middle of the wing, the area of Primary 9 in which the chelicerae can pierce is limited to a small portion of the quill wall, which could make colonization of this feather location difficult. Similarly, when the extended length of the chelicerae is compared to quill wall thickness, the thickest portions of Primaries 5 to 8 are greater than the chelicera lengths of females and larva, and Primary 4 is greater than the chelicera length of females. If a female were to enter one of these quills and did not find a location where her chelicera could fully pierce the quill wall to feed, she may die before being able to reproduce. Currently we do not know how long a female

90

can survive without feeding, or how readily quill mites move to new areas of the quill when they are unable to pierce the wall of their current location.

Kethley (1971) found a similar pattern for House Sparrows infested with *S. minor*. The chelicerae of *S. minor* are long enough to pierce at least the thinnest part of the quill wall of all the primaries. But Primaries 7, 8, and 9 were always either empty or contained a dead female or a dead female and larvae. Casto (1974b) observed that the chelicerae of *S. minor* protrude as far as 30-45 μm into the tissue surrounding the quill wall. Thus, in addition to needing to pierce through the quill wall, the length that the chelicerae need to protrude to successfully uptake liquid from the tissue around the quill could further limit the area mites can feed from.

Quill wall thickness probably acts as a strong selection pressure that influences diversification of quill mites. Glowska et al., (2013) compared the *COI* and *D2* region of *28S* of *Torotrogla merulae* Skoracki, Dabert and Ehrnsberger from two hosts species (the Eurasian Blackbird *Turdus merula* Linnaeus and the Song Thrush *Turdus philomelos* Brehm) with that of *Torotrogla rubeculi* Skoracki from the European Robin *Erithacus rubecula* (Linnaeus). They found that *T. merulae* was paraphyletic, with *T. rubeculi* forming a clade nested within *T. merulae*. The molecular data also indicated that the *T. rubeculi* clade originated from mites inhabiting Eurasian Blackbirds and showed no evidence of reverse gene flow from *T. rubeculi* to *T. merulae*. The main morphological difference between these mites is the size and shape of the hypostomal medial protuberance, length of the movable digit of the chelicerae and length of the stylophore, with *T. rubeculi* having smaller structures than *T. merulae*. This corresponds with their host's size: Eurasian Robins are smaller with quill walls that range from 37 – 46 μm in thickness compared to Eurasian Blackbirds which are larger with quill walls that range from 58 – 81 μm (Glowska et al., 2013). The smaller feeding structures that evolved in *T. rubeculi* may

prevent them from re-colonizing the thicker-quilled Blackbirds, which may be why there is no back-crossing of mites from Robins to Blackbirds.

In this study, *B. seiuri* had a strongly female-biased sex ratio with only 11.6% of adult mites being male. Female biases have also been reported in *S. minor* from House Sparrows and *Syringophiloidus hirundinis* Skoracki, Møller and Tryjanowski from the Barn Swallow, *Hirundo rustica* (Linnaeus) (Kethley, 1971; Skoracki et al., 2003). Female mites are likely the dispersal stage for all syringophilids (Kethley, 1971; Casto, 1975b); therefore, a foundress that produces a high number of daughters increases the chance of new feathers being colonized by her offspring. However, natural selection may not be the only reason for high proportion of female quill mites. *Wolbachia* is a genus of symbiotic intracellular bacteria that is found in a wide range of arthropods. Three supergroups of *Wolbachia* have been discovered in 5 species of quill mites (Glowska et al., 2015b). *Wolbachia* has been reported to cause male killing, feminization and parthenogenesis induction in several groups of insects, all of which result in female-biased sex ratios (Charlat et al., 2003). It is possible, though yet untested, that *Wolbachia* has a role in female-biased sex ratios in syringophilids.

Based on our observations, *Betasyringophiloidus seiuri* is not uniformly distributed within the flight feathers of Ovenbirds and prefers particular wing feathers over tail feathers. With regard to tail feathers, even though the quill walls are thin enough for the mites to pierce they are rarely occupied, possibly due to their relatively small volume. There was a correlation between volume and mean number of mites per quill, but quill wall thickness appears to limit which quills can become successfully colonized by *B. seiuri*.
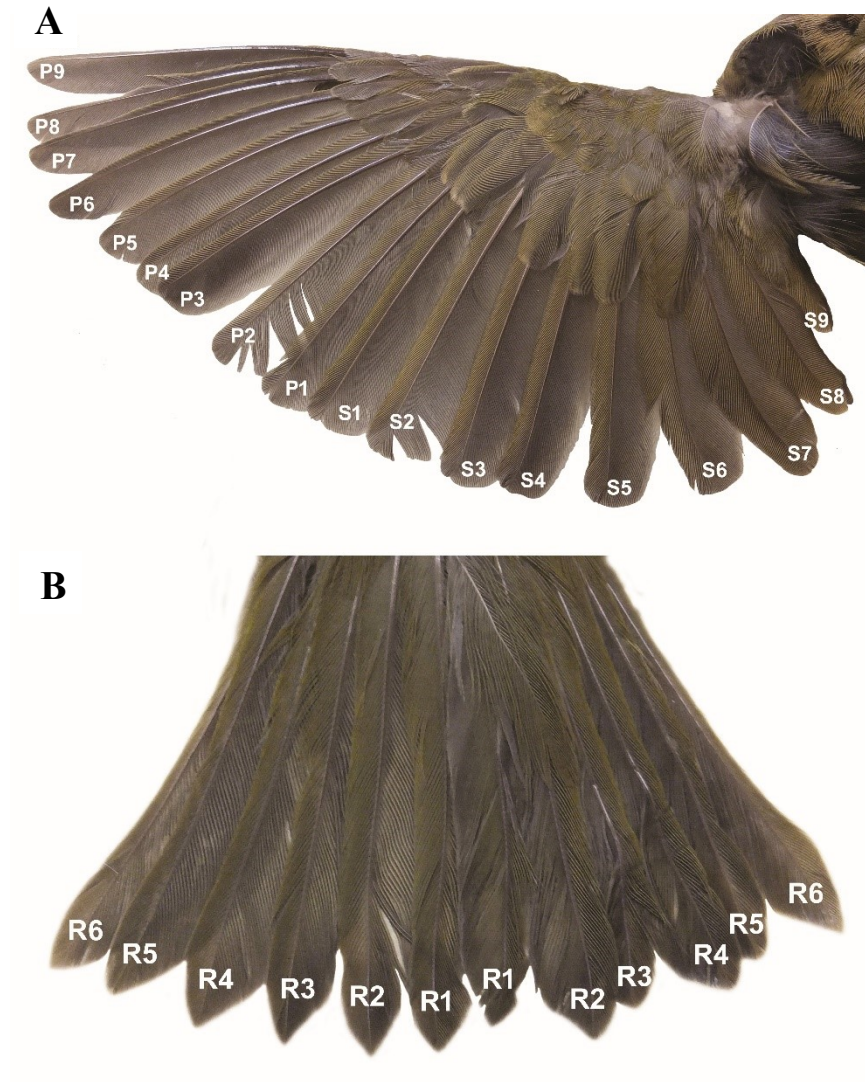
Figure 4.1: (A) The left wing of *Seiurus aurocapilla* showing codes for flight feather locations. P - primary, S – secondary. (B) The tail of *S. aurocapilla* showing codes for flight feather locations. R – rectrix.
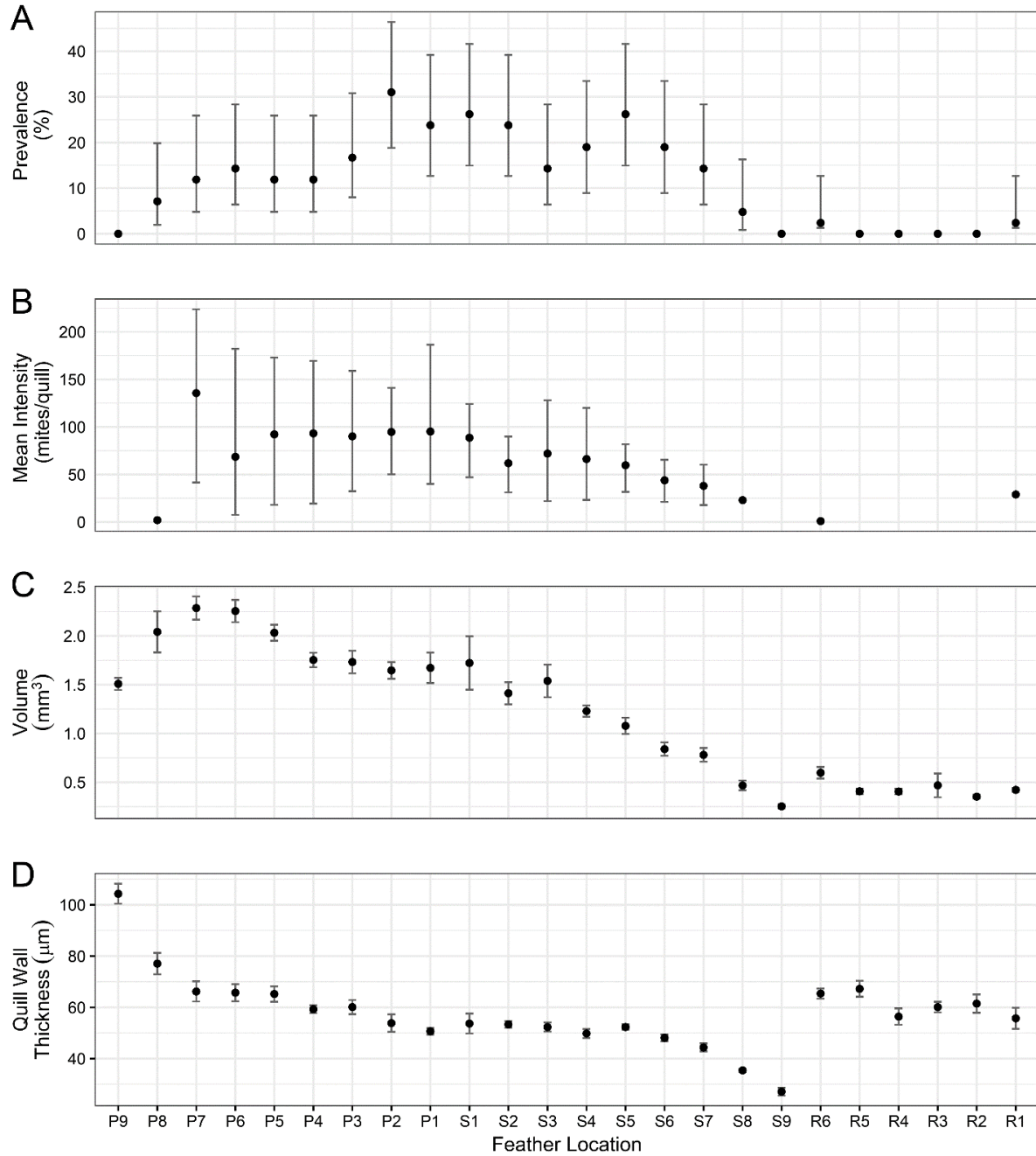
Figure 4.2: (A) Prevalence and (B) mean intensity of *Betasyringophiloidus seiuri* infesting *Seiurus aurocapilla* (feathers of left and right side combined, n = 42: 21 birds x 2 feathers per location). (C) Average quill volume and (D) average quill wall thickness for each feather location (feathers of left and right side combined, n = 6: 3 birds x 2 feathers per location). P – primary, S – secondary, R – rectrix. Error bars indicate 95% confidence intervals.
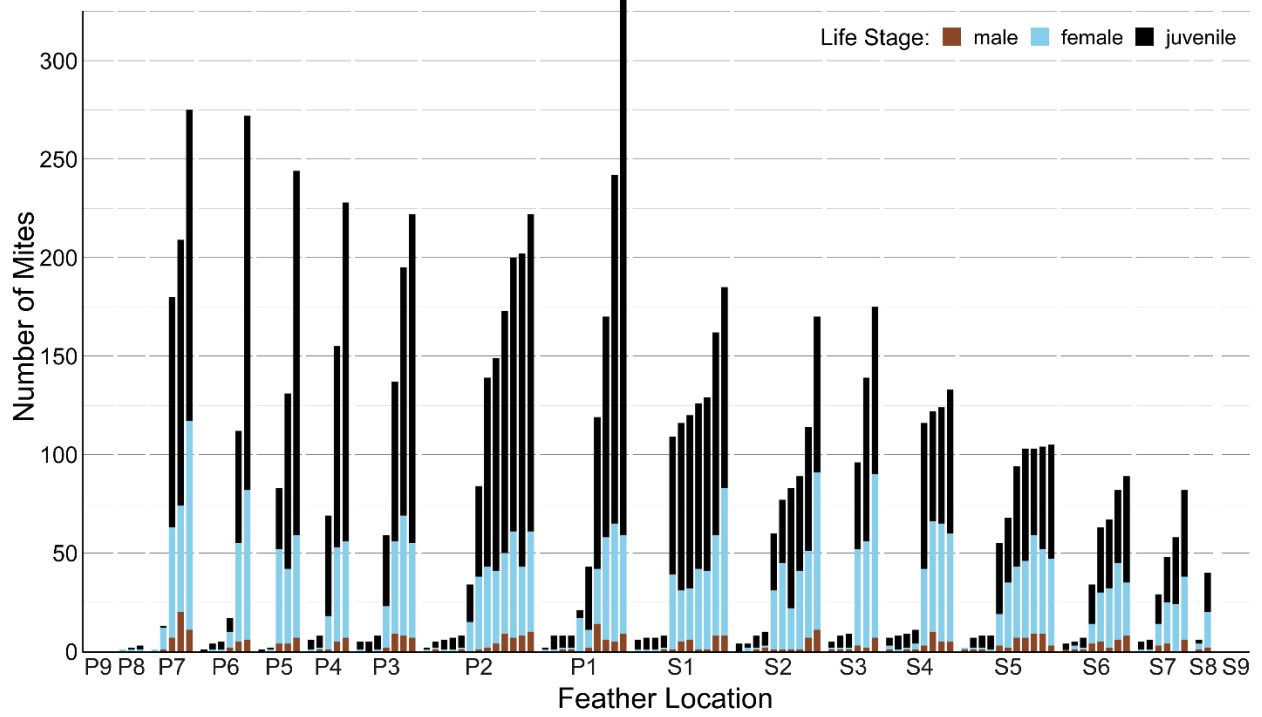
Figure 4.3: Counts of *Betasyringophiloidus seiuri* within all infested wing feathers from *Seiurus aurocapilla* (n = 8 birds). P – primary, S – secondary.
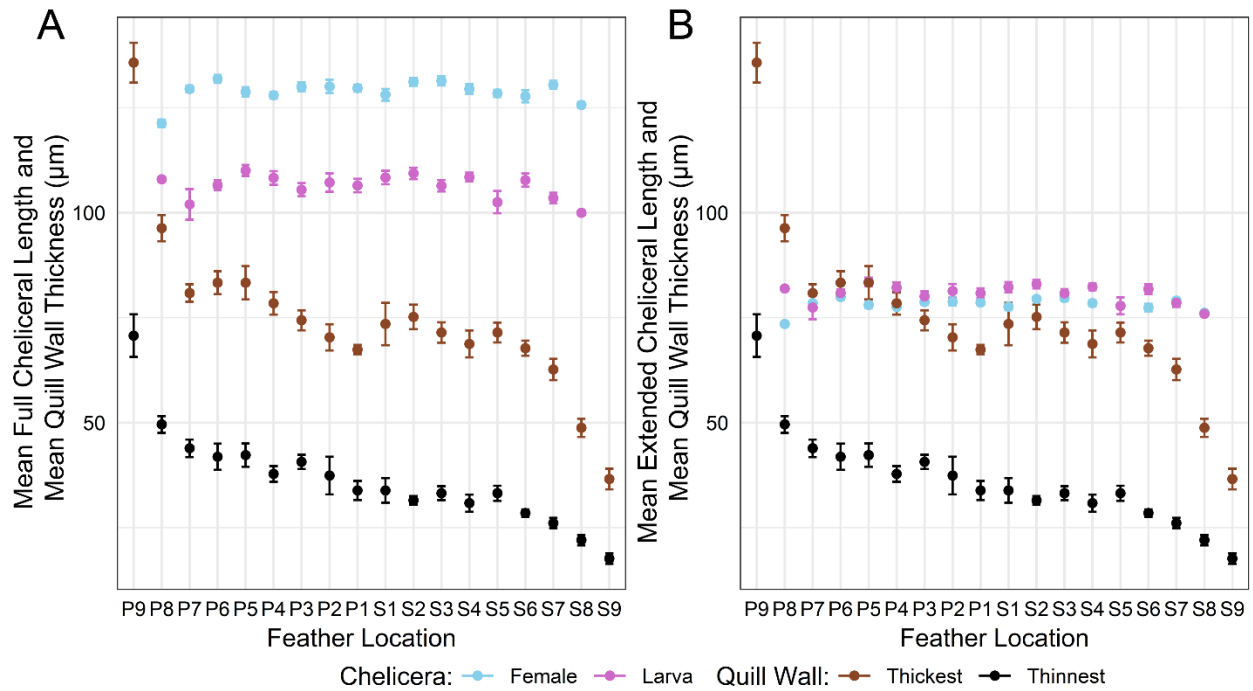
Figure 4.4: The upper two series show (A) full chelicera length and (B) extended chelicera length of female and larval *Betasyringophiloidus seiuri* infesting *Seiurus aurocapilla* (n = 10 mites per feather location except for P8, with 3 adult females and 1 larva, and S8, with 10 adult females and 1 larva). The lower 2 series show quill walls at the thickest and thinnest spots measured (n = 6, 3 birds x 2 feathers per location). P – primary, S – secondary. Error bars indicate 95% confidence intervals.
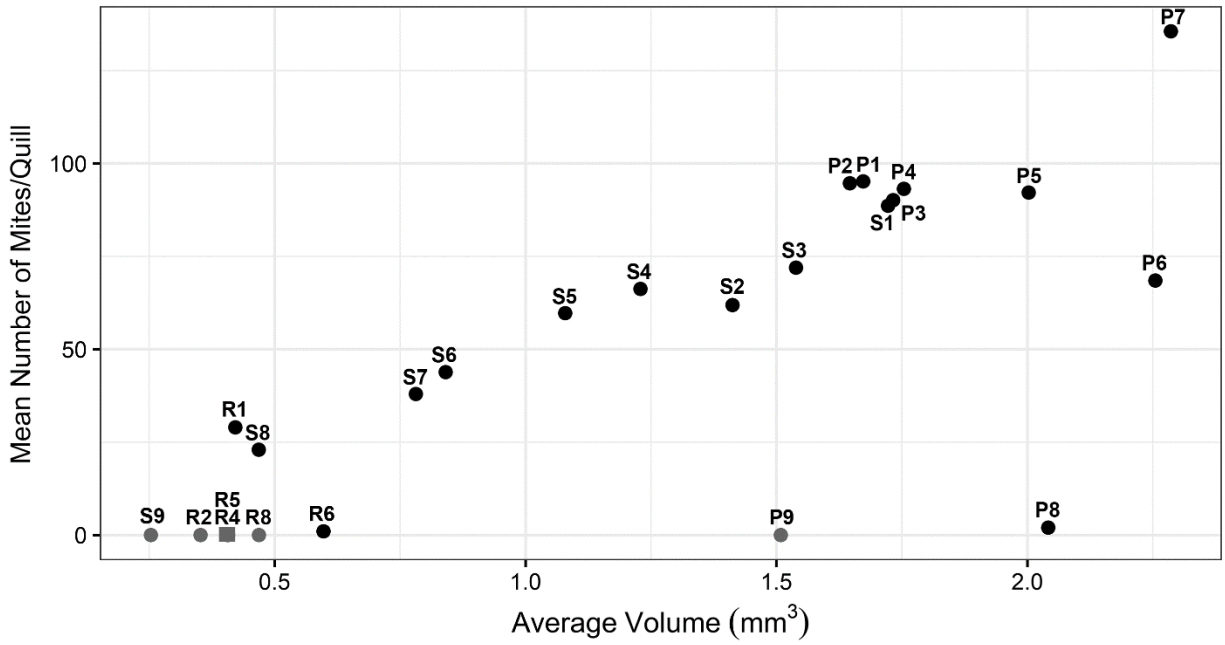
Figure 4.5: Relationship between average volume of *Seiurus aurocapilla* feathers in different locations (see Fig. 1 for location codes) and mean number of *Betasyringophiloidus seiuri* per feather location. Grey points indicate feather locations that contained no mites and therefore had their mean intensities set to zero. The square symbol indicates two points that are on top of each other. Feather locations are placed beside points. P – primary, S – secondary, R – rectrix.

Chapter 5 - General Discussion and Synthesis

5.1 Thesis Conclusions

My goals in this thesis research were the following: to explore the diversity of ectosymbionts of Rock Pigeons in Canada and assess whether there was evidence of geographic structuring or effects of local climate on arthropod assemblages (Chapter 2); to use genetic methods to test for evidence of population structure in Rock Pigeons and two of their louse species (Chapter 3); to determine how (and potentially why) quill mites partition the plumage of their host Ovenbirds (Chapter 4). Of the ectosymbiotic arthropods known from Rock Pigeons around the world, I found 13 of them from samples from pigeons in Canada. This leaves 14 other species absent from Canada, two of which are known to occur in the U.S.A. The U.S.A. includes many more climatically mild regions than Canada, yet doesn't have that much greater an ectosymbiont diversity, suggesting that at a large geographic scale, many ectosymbiont species "missed the boat" when their hosts were taken to North America. I found that within Canada, ectosymbionts did not have a homogenous distribution; Halifax and Vancouver were the only localities to have all 13 ectosymbiont species present, and only six ectosymbionts were found in all seven sampling locations. Two of these five were skin mites (*H. gallowayi, O. hallae*), two were nasal mite (*T. melloi, T. columbae*) and two were chewing lice (*Colu. columba* and *Camp. compar*). To explain the differences in assemblage, I examined local environmental factors (Chapter 2) as well as host population structure (Chapter 3). Local meteorological variables alone accounted for ~10% of the variation seen in both the louse and mite assemblages, and the population genetic structure of Rock Pigeons had surprising low levels of genetic differentiation.

The statistical results of Chapter 2 left about 80% of the variation in the ectosymbiont assemblages unexplained. There are many potential reasons for the poor explanatory powers of

my models. The most obvious is that in looking only at local climatic variables I missed some environmental variables that affect pigeon ectosymbionts. Diet of their hosts might affect the quality of their feathers, skin, blood and uropygial oil as food for lice, skin mites, nasal mites and feather-dwelling mites. For example, pigeon flocks that feed on spilled grain along railways may ingest the insecticides used to treat grain that is being stored or transported (https://www.grainscanada.gc.ca/en/grain-quality/manage/control-grain-insect-pests/insecticides.html). Some of these chemicals may persist in the tissues of the host and negatively affect their arthropod ectosymbionts. Phenology of the ectosymbiont undoubtedly influences abundance on the host and also how easy it is to detect using my simple methods of washing birds. It has been shown for both mites and lice that there are annual fluctuations in intensities of symbiont loads, usually with peak intensities correlating with either with the hosts breeding season or when chicks are hatching (Hamstra and Badyaev, 2009; Galloway and Lamb, 2015). Unfortunately, due to salvaging Rock Pigeons from rehabilitation centres I had no control on when pigeons were collected. If I were to do this survey again, I would sample the same number of pigeons every month for a year from every sampling location, and therefore would be able to compare seasonal variation between sampling locations.

There are two potential explanations for the weak geographical structuring of Rock Pigeon populations over a span of >4000 km, both of which could be operating simultaneously. First, even though Rock Pigeons are non-migratory they may not be sedentary, and some movement between urban centres may take place. Second, pigeon fanciers in Canada and the adjacent U.S.A. may occasionally lose pigeons they have imported from Europe, which may then mingle with and homogenize the gene pools of Canadian pigeons. Depending on how successful insecticidal and acaricidal treatments are for imported birds, the pigeon-fancy trade could also

introduce mites and lice from Europe that would have similar effects on the genetic diversity of pigeon ectosymbionts in Canada.

But even though the pigeons I sequenced had low levels of genetic differentiation, there was still some geographical structuring as I found evidence for three genetic clusters: cluster 1 for individuals from Halifax, cluster 2 individuals from Vancouver and cluster 3 for those from all other sampling locations. These clusters

Like its host, the body louse *Camapanulotes compar* indicated that Halifax lice clustered separately from lice from other locations. However, the wing louse *Columbicola columbae* showed no genetic structure based on sampling location. Halifax experienced a catastrophic event on December 6, 1917 in which two ships collided setting off a munitions explosion, now known as the Halifax Explosion (Cuthbertson, 2017). The explosion destroyed the port and flattened everything else for almost 1 km around it. Perhaps this event killed a sizable portion of the Halifax pigeon population, causing a bottleneck; this would explain why Halifax has the highest inbreeding coefficient of the locations sampled. Why this would result in a bottleneck for the body lice but not the wing lice is unclear, unless phoretic transfer of wing lice by hippoboscid flies resulted in more wing lice 'immigrating' to Halifax birds after the explosion. One difficulty in comparing pigeon and louse genetic data is that CO1 was used to examine the genetic structure for the lice; ideally SNPs would have been used, as they were with the pigeons. In retrospect I believe that sampling methods, freezing and thawing hosts possibly multiple times if they were shipped, as well as washing hosts was not kind to louse DNA. Lice should have been removed directly from euthanized host and preserved.

My sampling of quill mites from the flight feathers of Ovenbirds showed that wing feathers have a higher probability of being infested than tail feathers and that mites residing in

feathers located in the middle of the wing. There was also a positive correlation between average quill volume and abundance of mites/quill, and mites appeared to be limited to which feather they can successfully reproduce in by wall thickness. In my study the measurements for the quill parameters were taken from three additional Ovenbirds that were not examined for mites, because quills were damaged when mites were removed. Ideally the quill measurements would have come from the same individuals, perhaps by measuring the quill parameters from one wing and sampling quill mites from the other.

5.2 Future Directions

One of the most interesting discoveries when it comes to the ectosymbiont diversity of Rock Pigeons is the presence of the vane-dwelling feather mite *Pterophagus columbae*, which has never previously been reported in North America, and was found here only on the east and west coasts and nowhere in between. This opens up several questions: did these mite populations come from the same introduction and just gradually died out in the middle of Canada, or (which seems more likely) from separate ones? Is this species only found along the coasts in its native range in the Old World? The next step would be comparing the genetics of these two populations to each other and to *P. columbae* from Old World locations to try and determine where these mites could have come from. It would also be interesting to review ectosymbiont surveys associated with other terrestrial host taxa to see if the trend of higher ectosymbiont diversity can be found in coastal regions.

Based on my study of *B. seiuri* in Ovenbirds, the ability of a quill mite to survive and produce offspring appears to be highly linked to quill parameters. To test this further it would be interesting to artificially infest birds with quill mites that are sourced from related bird species both smaller and larger than the one being infested, with the prediction that only mites from

101

larger birds will survive. One could also try this in a host bird with a great deal of body size variation, such as breeds of the domestic chicken, which hosts at least two species of syringophilid (Skoracki et al., 2001b). If mites do survive the host switch it would also be interesting to raise them over multiple generation to see if their chelicerae change in either form or length in response to the quill structure of their new host.

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Appendices

Appendix 2.1: Collection information for *Columba livia.*

Location	Number of Hosts	Sampling years	Latitude	Longitude	Rehabilitation Centre
British Columbia					
Vancouver	30	March 2013 – November 2014	49.28273	-123.121	Wildlife Rescue Association of BC
Alberta					
Calgary	27	January 2015 – July 2015	51.04862	-114.071	Calgary Wildlife Rehabilitation Society
Strathmore	1	January 2015	51.05011	-113.38523	
Airdrie	1	July 2015	51.291668	-114.014442	
Okotoks	1	July 2015	50.72549	-113.975	
Edmonton	23	August 2015 – December 2015 & April 2017	44.66521	-63.5677	Northern Alberta Wildlife Rehabilitation and Rescue
Saskatchewan					
Saskatoon	14	2016	52.13321	-106.67	Living Sky Wildlife Rehabilitation
Manitoba					
Winnipeg	30	April 2015 – November 2015	49.89514	-97.1384	Wildlife Haven, Prairie & Wildlife Rehabilitation Centre
Southern Ontario					
Belleville	2	January 2017	44.16276	-77.3832	Sandy Pines Wildlife Centre
Kingston	3	January 2017	44.23117	-76.486	
Toronto	6	May 2017	43.65323	-79.3832	Fatal Light Awareness Program
Nova Scotia					
Halifax	20	August 2015 – October 2016	44.64876	-63.5752	Hope for Wildlife & Cobequid Wildlife Rehabilitation Centre
Greenwood	1	January 2016	44.97172	-64.9341	
Sackville	1	January 2016	45.8979	-64.3683	
Truro	2	June 2016 – July 2016	45.36577	-63.2869	

Appendix 2.2: Months *Columba livia* were collected.
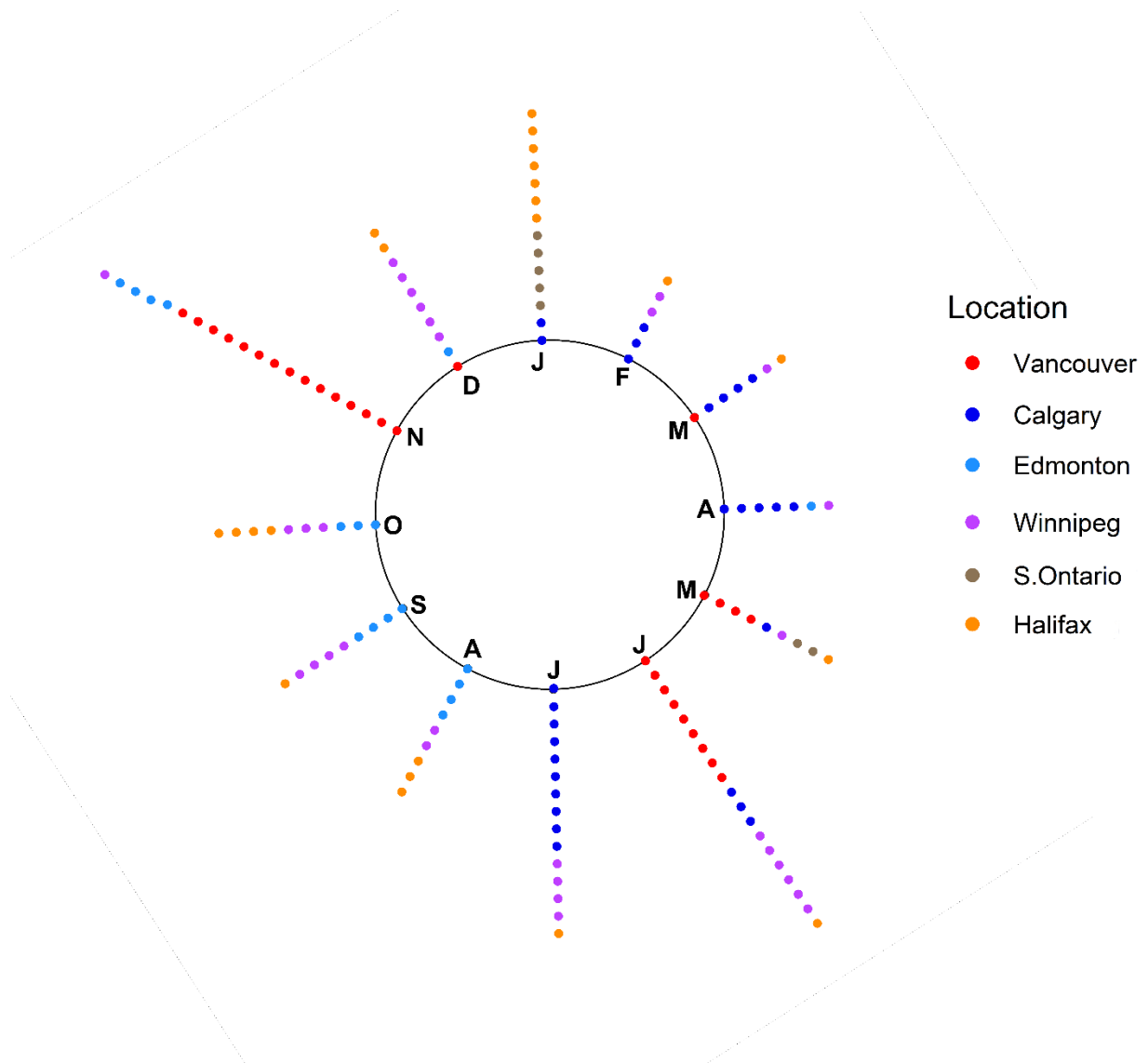
Appendix 2.3: Ectosymbionts collected from *Columba livia*, with collection data in parentheses:

(A) *Falculifer rostratus* (5 Sept. 2014; Vancouver, British Columbia) ; (B) *Pterophagus columbae* (17 Oct. 2016; Halifax, Nova Scotia); (C) *Diplaegidia columbae* (12 Sept. 2014; Vancouver, British Columbia); (D) *Harpyrhynchoides gallowayi* (11 July 2015; Calgary, Alberta); (E) *Harpyrhynchoides columbae* (17 Oct. 2016; Halifax, Nova Scotia); (F) *Ornithocheyletia hallae* (7 Feb. 2015, Winnipeg, Manitoba); (G) *Tinaminyssus melloi* (7 Sept. 2014; Vancouver, British Columbia); (H) *Tinaminyssus columbae* (2006;Vancouver, British Columbia); (I) *Columbicola columbae* (8 Sept. 2014, Vancouver, British Columbia); (J) *Coloceras tovornikae* (3 May 2016, Winnipeg, Manitoba); (K) *Campanulotes compar* (29 April 2015, Winnipeg, Manitoba); (L) *Hohorstiella lata* (7 Sept. 2014, Vancouver, British Columbia); (M) *Bonomiella columbae* (17 Oct. 2016, Halifax, Nova Scotia).
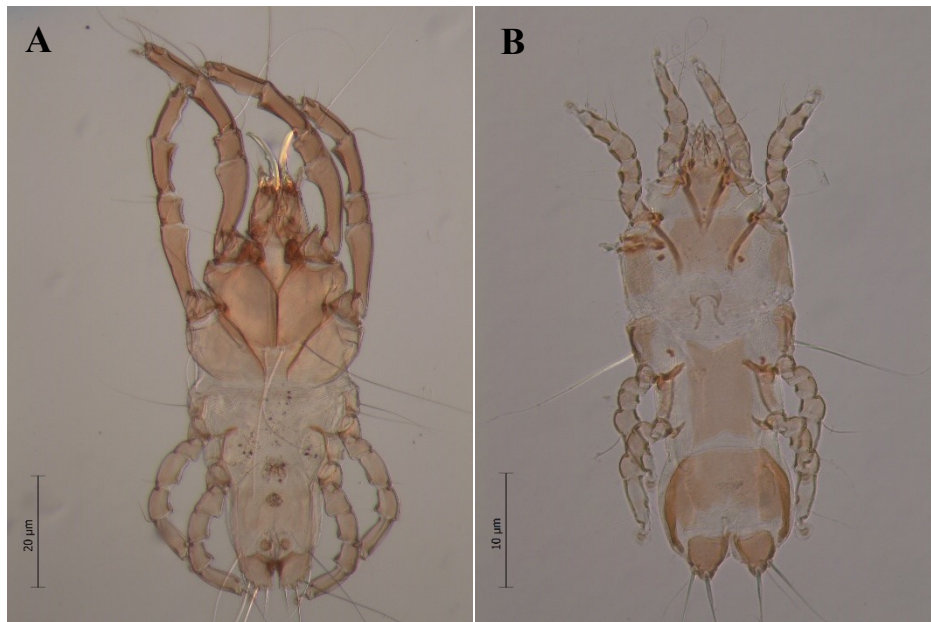
Appendix 2.3: Continued.

Appendix 3.1: Canadian Food inspection Agency (CFIA) importation records of Rock Pigeons between 2016-2018. Data received by A. Grossi on 29/I/2020.

Country of origin	Province of Destination	Date of Import (year-month-day)	Number of Birds
Netherlands	Ontario	16-03-11	91
		16-05-20	56
		16-05-24	28
		16-12-28	163
United States	Alberta	16-09-16	15
	British Colombia	16-01-05	856
		16-01-12	776
		16-01-19	857
		16-01-26	868
		16-02-02	772
		16-02-09	772
		16-02-16	820
		16-02-17	2,000
		16-03-01	1,000
		16-03-08	868
		16-03-15	784
		16-03-22	1,958
		16-03-29	1,055
		16-04-05	1,158
		16-04-12	1,086
		16-04-19	1,045
		16-04-20	42
		16-04-26	1,191
		16-05-03	1,012
		16-05-10	1,319
		16-05-17	1,206
		16-05-24	960
		16-05-27	10
		16-05-31	1,102
		16-06-07	990
		16-06-14	1,136
		16-06-21	1,258
		16-06-28	1,332
		16-07-05	853
		16-07-12	977
		16-07-19	1,319
		16-07-26	999
		16-08-02	3,266
		16-08-09	894
		16-08-16	961
		16-08-23	1,110
		16-08-30	891
		16-09-06	1,182

Appendix 3.1: Continued.

		16-09-13	1,298
		16-09-27	730
		16-10-04	688
		16-10-11	1,051
		16-10-12	44
		16-10-18	698
		16-10-25	1,050
		16-11-01	730
		16-11-04	110
		16-11-08	978
		16-11-15	990
		16-11-22	566
		16-11-29	954
		16-11-25	3
		16-12-06	750
		16-12-13	774
		16-12-20	750
		16-12-28	762
	Manitoba	16-06-30	2
		16-12-11	80
	Ontario	16-01-08	62
		16-01-20	61
		16-03-18	180
		16-03-29	73
		16-03-31	104
		16-04-04	20
		16-04-13	84
		16-04-22	122
		16-05-02	32
		16-05-13	79
		16-05-27	54
		16-06-01	14
		16-06-10	47
		16-06-22	10
		16-07-15	35
		16-08-04	7
		16-09-16	33
		16-10-07	45
		16-10-27	23
		16-12-22	12
		16-12-13	76
	Quebec	16-01-21	12
		16-02-23	95
Netherlands	Ontario	17-04-21	35
		17-05-10	88
		17-05-26	65
		17-10-13	136
		17-12-12	179
United States	Alberta	17-08-28	20
	British Colombia	17-01-03	594

Appendix 3.1: Continued.

		17-01-10	586
		17-01-24	634
		17-01-17	474
		17-01-31	366
		17-02-07	426
		17-04-03	8
		17-04-13	41
		17-06-16	14
		17-11-03	26
		17-11-03	16
		17-11-03	93
		17-10-03	32
		17-12-11	44
	Ontario	17-01-17	51
		17-01-06	6
		17-01-27	43
		17-02-07	41
		17-03-21	64
		17-03-23	44
		17-04-07	180
		17-04-18	158
		17-05-03	87
		17-05-18	42
		17-02-14	70
		17-06-12	103
		17-06-28	230
		17-08-04	99
		17-08-14	1
		17-08-24	192
		17-10-13	82
		17-10-12	29
		17-11-01	61
		17-12-04	59
		17-12-06	16
		17-12-15	31
	Quebec	17-03-01	4
Netherlands	Ontario	18-03-21	83
		18-07-27	91
United States	British Colombia	18-01-22	36
		18-01-25	20
		18-02-15	32
		18-06-08	10
		18-10-19	43
		18-11-02	138
		18-12-10	100
	Ontario	18-01-23	99
		18-02-02	35
		18-02-13	31
		18-02-26	13
		18-03-19	74

Appendix 3.1: Continued.

Date	Value
18-03-29	27
18-04-03	103
18-04-13	65
18-04-17	243
18-04-26	58
18-05-01	119
18-05-03	13
18-05-25	216
18-06-08	198
18-06-15	138
18-06-29	45
18-07-03	81
18-07-27	309
18-08-23	51
18-09-24	82
18-10-04	17
18-10-24	7
18-11-05	14
18-11-07	95
18-11-23	24
18-12-14	3
18-12-31	76

Appendix 3.2:Evanno table output generated in STRUCTURE HARVESTER from *Columba*

livia SNP data.

K	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Δ K
1	-291585.4800	1.2264	NA	NA	NA
2	-288936.3300	2.5478	2649.150000	1976.740000	775.865270
3	-288263.9200	58.1253	672.410000	93.250000	1.604294
4	-287684.7600	908.8784	579.160000	228.890000	0.251838
5	-286876.7100	389.6897	808.050000	503.230000	1.291361
6	-286571.8900	676.5441	304.820000	3864.720000	5.712443
7	-290131.7900	6428.2204	-3559.900000	NA	NA

Appendix 3.3: Structure plot for *Columba livia* of probabilities of assignment of individuals to different genetic clusters for K=2 to 7, based on analysis of SNP using STRUCTURE.
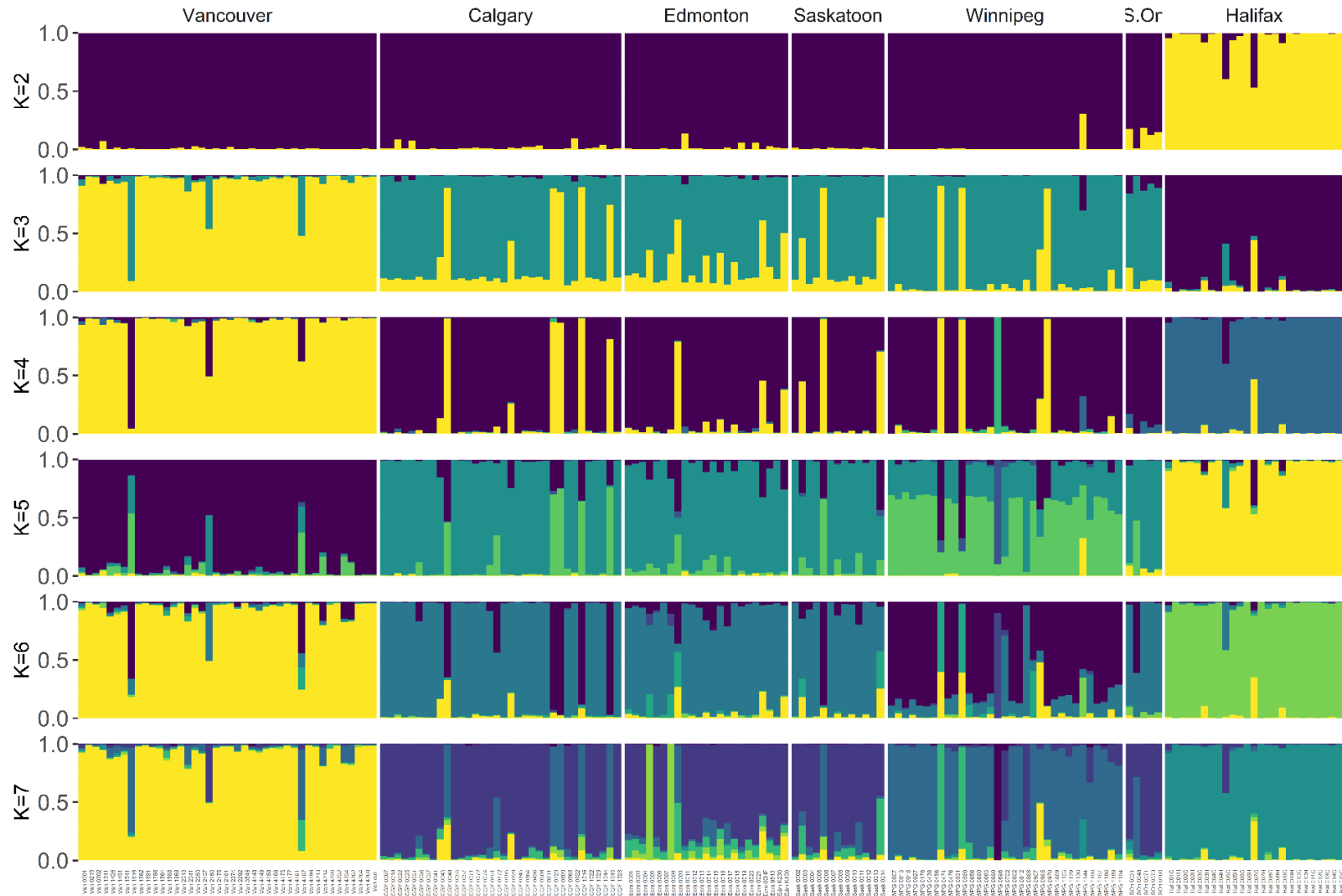
Appendix 3.4: Evanno table output generated in STRUCTURE HARVESTER from Columba

livia (sampled from everywhere but Halifax) SNP data.

| K	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	-249960	1.1468	NA	NA	NA
2	-249243	4.8532	716.96	283.12	58.33649
3	-248243	172.817	1000.08	570.28	3.299907
4	-247813	97.7631	429.8	801.91	8.202583
5	-248185	1385.022	-372.11	1586.56	1.145513
6	-246971	198.2463	1214.45	NA	NA

Appendix 3.5: Structure plot for Columba livia of probabilities of assignment of individuals to different genetic clusters for K=2 to 7, based on analysis of SNP using STRUCTURE.
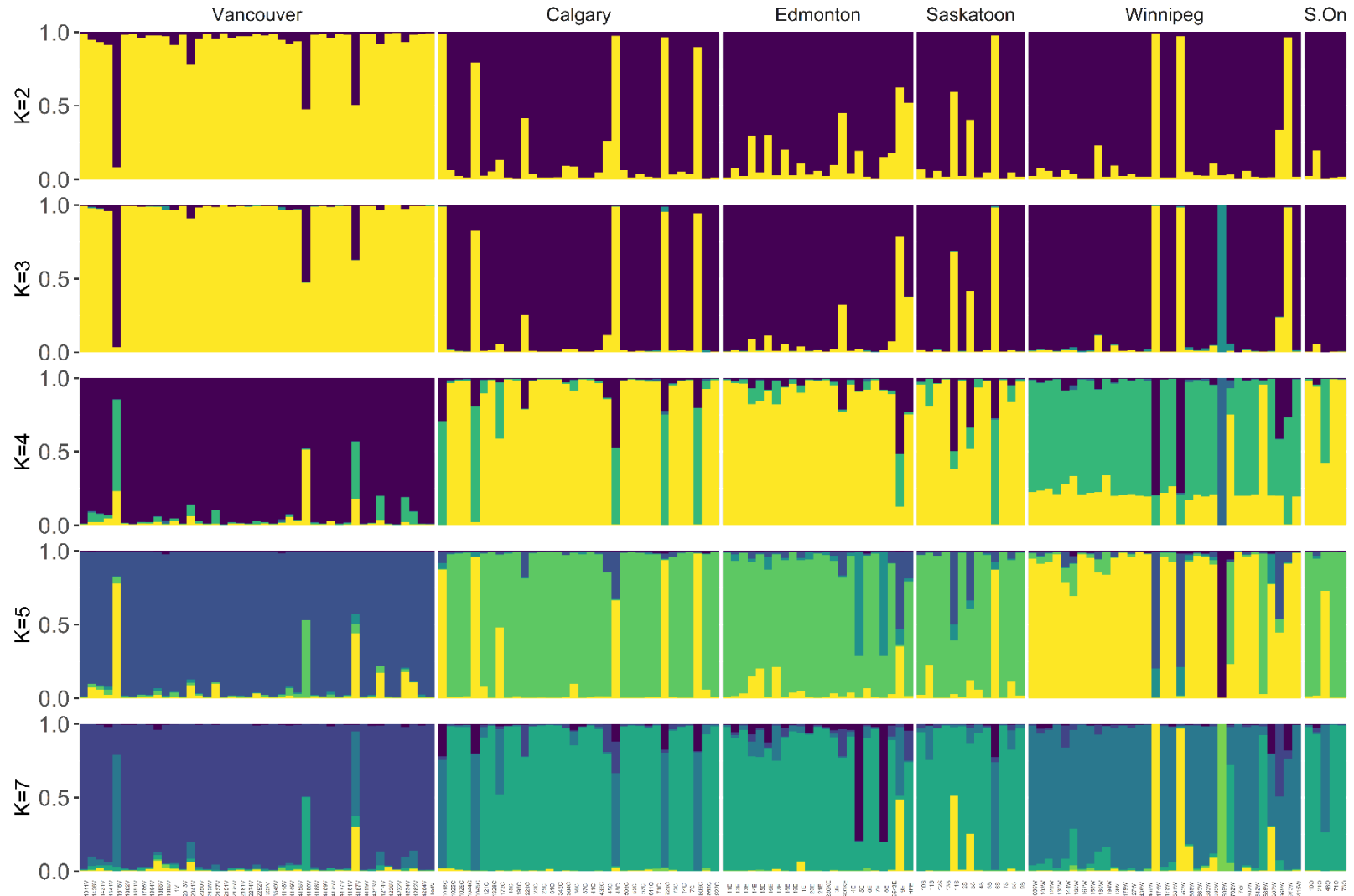
Appendix 4.1: (A) Red lines indicate where measurements were taken to calculate the volume of a truncated cone. (B) Red lines indicate where the feather was cut to take measurements of quill wall thickness. (C) Cross section of quill embedded upright in mounting putty. Red lines indicate where measurements were taken at the thickest and thinnest spots in the quill wall.
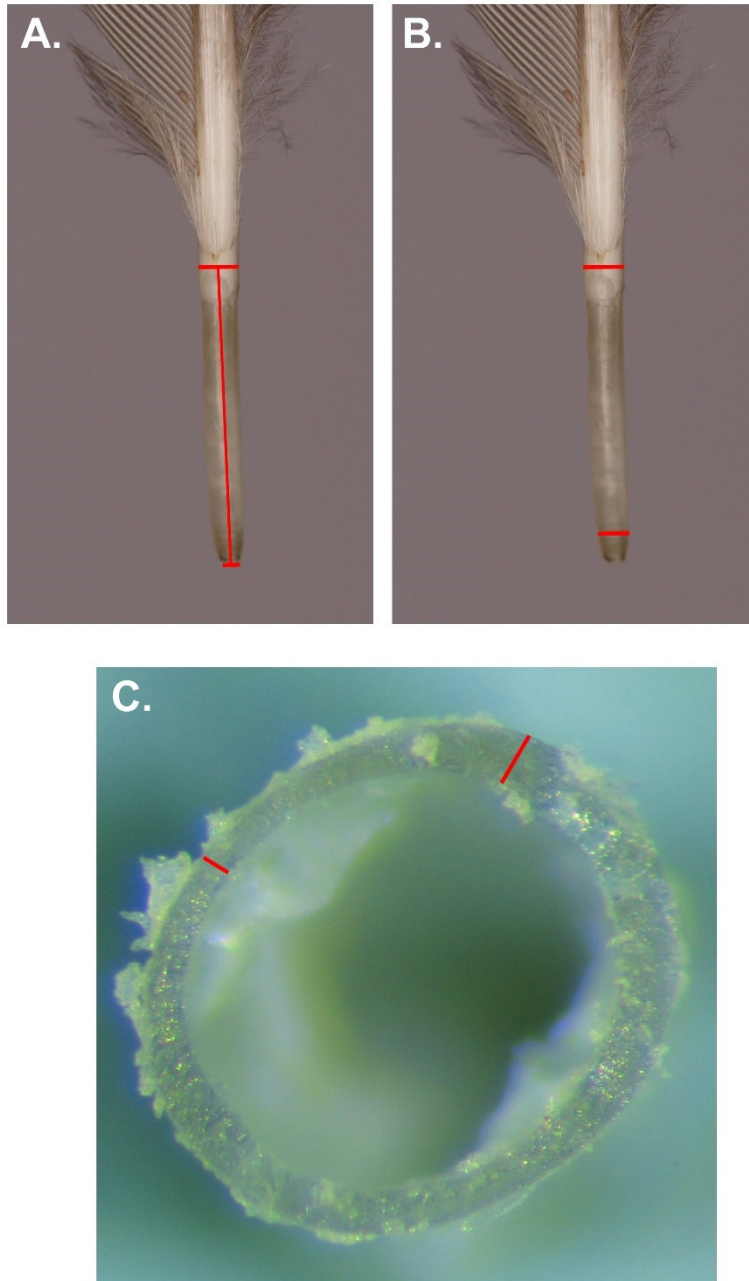
Appendix 4.2: *Betasyringophiloidus seiuri.* (A) larva, (B) nymph, (C) adult female, (D) adult

male. Scale: 100 μm.
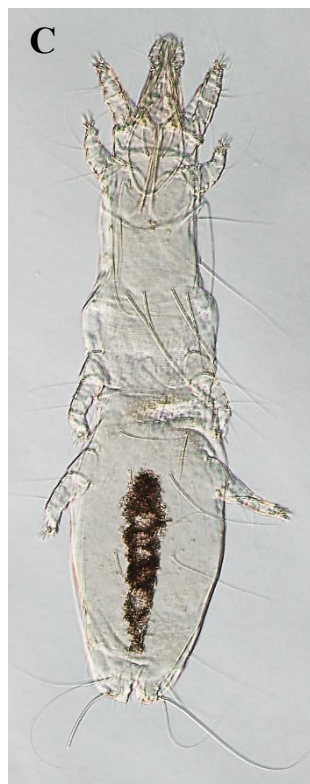
Appendix 4.3: Prevalence (P) and mean intensity (MI) of *Betasyringophiloidus seiuri* infesting *Seiurus aurocapilla* (feathers of left and right side combined, n = 42: 21 birds x 2 feathers per location). P – primary, S – secondary, R – rectrix, 95% confidence intervals are in parentheses.

Feather	P (%) ± 95% CI	MI ± 95% CI
P9	0	-
P8	7.1 (2.0 - 19.9)	2 (1.0 - 2.3)
P7	11.9 (4.8 - 25.9)	135.60 (41.6 - 223.8)
P6	14.3 (6.4 - 28.4)	68.50 (7.5 - 182.2)
P5	11.9 (4.8 - 25.9)	92.2 (18.0 - 173.0)
P4	11.9 (4.8 - 25.9)	93.20 (19.4 - 169.4)
P3	16.7 (8.0 - 30.8)	90.14 (32.4 - 159.0)
P2	31.42 (18.8 - 46.4)	94.69 (50.1 - 141.2)
P1	23.8 (12.7 - 39.2)	95.20(40.1 - 186.4)
S1	26.2 (14.9 - 41.6)	88.64 (47.1 - 124.1)
S2	23.8 (12.7 - 39.2)	61.90 (31.1 - 98.9)
S3	14.3 (6.4 - 28.4)	72 (22.0 - 128.0)
S4	19.0 (8.9 - 33.5)	66.25 (23.2 - 120.0)
S5	26.2 (14.9 - 41.6)	59.73 (31.7 - 81.7)
S6	19.0 (8.9 - 33.5)	43.88 (21.1 - 65.5)
S7	14.3 (6.4 -28.4)	38.00 (17.8 - 60.3)
S8	4.8 (0.86 - 16.3)	23.00 (*)
S9	0	-
R6	2.4 (1.3 - 12.7)	29 (*)
R5	0	-
R4	0	-
R3	0	-
R2	0	-
R1	2.4 (1.3 - 12.7)	1 (*)

Appendix 4.4: Infestation of individual feathers of *Seiurus aurocapilla* by *Betasyringophiloidus seiuri* for the 9 of 21 birds that had mites. Black squares = mites present; white squares = mites absent, R – right side, L – left side.

	Bird #:	1		2		3		4		5		6		7		8		9	
	Side:	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Wing Feathers	Primary 9																		
	Primary 8	■	■	■															
	Primary 7					■	■			■		■				■			
	Primary 6			■	■	■				■	■					■			
	Primary 5			■		■			■	■	■					■			
	Primary 4		■		■	■		■					■						
	Primary 3	■			■		■	■					■	■					
	Primary 2	■	■	■	■	■	■	■	■		■		■	■					
	Primary 1	■	■	■		■	■	■		■		■	■						
	Secondary 1	■	■	■	■	■	■	■		■	■	■							
	Secondary 2	■	■	■		■	■	■	■		■	■							
	Secondary 3	■	■		■	■	■												
	Secondary 4	■	■	■	■	■	■				■								
	Secondary 5	■	■	■	■	■		■	■		■	■							
	Secondary 6	■	■		■		■		■	■		■							
	Secondary 7	■		■	■	■		■		■									
	Secondary 8	■						■											
	Secondary 9																		
Tail Feathers	Rectrix 6																■		
	Rectrix 5																		
	Rectrix 4																		
	Rectrix 3																		
	Rectrix 2																		
	Rectrix 1					■													