CIRCULATION OF PATHOGENIC SPIROCHETES IN THE GENUS *BORRELIA* WITHIN TICKS AND SEABIRDS IN BREEDING COLONIES OF NEWFOUNDLAND AND LABRADOR

by © Hannah Jarvis Munro

A Thesis submitted

to the School of Graduate Studies in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

Department of Biology

Memorial University of Newfoundland

May 2018

St. John’s, Newfoundland and Labrador

ABSTRACT

Birds are the reservoir hosts of *Borrelia garinii*, the primary causative agent of neurological Lyme disease. In 1991 it was also discovered in the seabird tick, *Ixodes uriae*, in a seabird colony in Sweden, and subsequently has been found in seabird ticks globally. In 2005, the bacterium was found in seabird colonies in Newfoundland and Labrador (NL); representing its first documentation in the western Atlantic and North America. In this thesis, aspects of enzootic *B. garinii* transmission cycles were studied at five seabird colonies in NL. First, seasonality of *I. uriae* ticks in seabird colonies observed from 2011 to 2015 was elucidated using qualitative model-based statistics. All instars were found throughout the June-August study period, although larvae had one peak in June, and adults had two peaks (in June and August). Tick numbers varied across sites, year, and with climate. Second, *Borrelia* transmission cycles were explored by polymerase chain reaction (PCR) to assess *Borrelia* spp. infection prevalence in the ticks and by serological methods to assess evidence of infection in seabirds. Of the ticks, 7.5% were PCR-positive for *B. garinii*, and 78.8% of seabirds were sero-positive, indicating that *B. garinii* transmission cycles are occurring in the colonies studied. Five *I. uriae* from two seabird colonies were positive for the Asian strain of the Lyme disease-causing species, *B. bavariensis*, which has previously only been described from the terrestrial realm associated with rodent reservoirs. The complete microbiome of ticks from two seabird colonies was also explored, which was consistent with PCR-based estimates of *Borrelia* spp. prevalence and identified infections with *Coxiella* and *Ehrlichia* spp., which may also be tick-borne. Third, the phylogenetic relationships of *B. garinii* found in

the study samples, with *B. garinii* from elsewhere in the world, were explored using concatenated multi-locus sequence typing (MLST) gene sequences. This revealed close relationships between *B. garinii* in Eurasia and seabird colonies in NL. These results add to our knowledge of all levels of this complex, under-studied system, and help to inform us on how seabirds facilitate the global dispersion of *B. garinii* and other *Borrelia* species.

ACKNOWLEDGEMENTS

I would like to acknowledge the contributions of numerous individuals who provided assistance with sample collection, sample organization and data management. I would like to thank Alex Bond, Donald Pirie-Hay, Amy-Lee Kouwenberg, Paul Regular, Michelle Fitzsimmons, Natasha Walzthoni, Laura Tranquilla McFarland, Sheena Roul, Rafael Teixeira, Esther London, Robyn Butt, Holly Hogan and Ludmila Ferreira Gouveia. Pierre Ryan, Sabina Wilhelm (Canadian Wildlife Service), Dave Fifield, and Greg Robertson (Environment Canada) who were crucial for providing access to seabird colonies, aid in capture of birds, and assisting in sample collection. Further, I would like to thank the members of the Lang Lab, particularly Marta Canuti for her never ending support with PCR and other molecular techniques, as well as support in phylogenetic analysis. I would also like to thank Samir Mechai and Joost Verhoeven for support in analysis of MLST and microbiome data.

I would like to thank Robbin Lindsay and Antonia Dibernardo from the National Microbiology Lab for support in setting up and designing screening protocols. They were always happy to answer any of my questions about PCR and confirming my results. Invaluable support was provided by Thierry Boulinier and Karen McCoy at Université Montpellier and their student, Amandine Gamble, by performing serological analysis of seabird blood samples.

Finally, I would like to thank my supervisory committee: Andrew Lang, Hugh Whitney, Greg Robertson and Nick Ogden for all their encouragement, support and patience throughout this project.

Support for this work was provided by the Government of Newfoundland and Labrador (Department of Natural Resources), Environment Canada, and the Public Health Agency of Canada (PHAC). Research in the Lang lab is supported by funding from Natural Sciences and Engineering Research Council (NSERC). I was supported by funding from PHAC and Government of Newfoundland and Labrador (Department of Natural Resources) and graduate student fellowships from the Memorial University of Newfoundland School of Graduate Studies. Fieldwork in Labrador was supported by a Northern Scientific Training Program grant (Government of Canada, Department of Indian and Northern Affairs).

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aBayes	Bayesian-like transformation of approximate likelihood ratio test (1)
23S rRNA	23S rRNA component of the 50S subunit of the bacterial ribosome
AF	Adult female
AIC	Akaike information criterion (2)
AM	Adult male
ATPU	Atlantic Puffin, *Fratercula arctica*
BAPS	Bayesian analysis of population structure
BLKI	Black-legged Kittiwake, *Rissa tridactyla*
CI	Confidence interval
clpA	Gene, ATP-dependent Clp protease ATP-binding subunit ClpA
clpX	Gene, ATP-dependent Clp protease ATP-binding subunit ClpX
COMU	Common Murre, *Uria aalge*
DLVs	Double-locus variants
GAM	General additive model
GANN	Gannet Islands
GLM	Generalized linear model
GREAT	Great Island
GULL	Gull Island
HERG	Herring Gull, *Larus smithsonianus*
IGS	Intergenic spacer
JSD	Jensen-Shannon Divergence
L	Larva
LFOGO	Little Fogo Islands
MDS	Multidimensional scaling
MLST	Multilocus sequence typing
N	Nymph
NCBI	National Centre for Biotechnology

nifS	Gene, cysteine desulfurase
NL	Newfoundland and Labrador
nPCR	Nested polymerase chain reaction
Osp	Outer surface protein
PCR	Polymerase chain reaction
pepX	Gene, Xaa-Pro dipeptidyl-peptidase
pyrG	Gene, CTP synthase
qPCR	Quantitative polymerase chain reaction
RAZO	Razorbill, *Alca torda*
recG	Gene, ATP-dependent DNA helicase RecG
RFCO	Red-faced Cormorant, *Phalacrocorax urile*
rplB	Gene, 50S ribosomal protein L2
s.l.	*sensu lato*
s.s.	*sensu stricto*
SLVs	Single-locus variants
ST	Sequence type
TBMU	Thick-billed Murre, *Uria lomvia*
TLVs	Triple-locus variants
uvrA	Gene, UvrABC system protein A

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CHAPTER 1: INTRODUCTION AND OVERVIEW

1.1. Introduction

1.1.1. General introduction

Zoonotic diseases are caused by pathogens passed from other species of animals to humans (3). These pathogens require non-human hosts known as reservoir species. Within populations of these reservoir species the pathogen continuously cycles among individuals. Humans are often accidental hosts and are a dead end for the pathogen, as subsequent human-to-human transmission does not occur (e.g., West Nile Virus; 4).

Zoonotic diseases pose an important human health risk. Of the 1700 infectious diseases recognized by the World Health Organization (WHO), 58% are caused by zoonotic pathogens. Within emerging diseases (those that are expanding in range or newly discovered to infect humans) the percentage of zoonotic diseases is higher at 73% (3, 5, 6). Zoonotic diseases are believed to be the greatest disease burden on humankind, with one billion human infections and millions of human deaths every year worldwide (7).

Some zoonotic diseases result from pathogen transmission from the non-human host directly to the human, with little or no transmission occurring between humans (e.g., rabies). Alternatively, some diseases are readily transmitted among humans once introduced from the animal reservoir (e.g., severe acute respiratory syndrome, SARS). Zoonotic diseases that are readily transmitted among humans may be contained to a small geographic area with disease-management measures (e.g., most previous outbreaks of

Ebola virus) or spread on a global level and require international cooperation to avoid a pandemic (e.g., influenza A virus). Many diseases can be classified as having a zoonotic origin (8), but some pathogens can evolve to no longer require a non-human animal reservoir for maintenance of the disease. In such cases, a pathogen may no longer be considered zoonotic but still require a vector for transmission and remain vector-borne (e.g., malaria; 9). In most cases, zoonotic diseases cannot be maintained in the human population and require reintroductions from the reservoir species (6).

Emerging diseases are those that have recently (often defined as the last 60 years; 10) been found within humans (e.g., SARS), or are dramatically increasing their geographic range (e.g., Lyme disease). These changes may have resulted from evolution of a new strain (e.g., the H5N1 avian influenza A virus; 11) or increased pathogenicity (e.g., Sin Nombre hantavirus; 12). It can also include pathogens that are re-emerging after a period of low prevalence (e.g., Ebola virus; 13).

1.1.1. Migratory species

Migratory species provide unique opportunities to examine the movement of pathogens that are implicated in zoonotic diseases. This includes seabirds (, many of which undertake long-distance migrations (14, 15). The migratory paths of these birds vary greatly, from those that move between northern and southern regions, over great distances, such as the Arctic Tern (*Sterna paradisaea*) (16), to those that move east to west across the oceans, such as Thick-billed Murres (*Uria lomvia*) (15) and Atlantic Puffins (*Fratercula arctica*) (17). These movements have been implicated in movement of influenza A (18, 19) and other viruses (20). Seabirds are also infested by ectoparasites

such as ticks (Ixodida), feather mites (Astigmata), and lice (Amblycera, Ischnocera) (21-23) that can serve as vectors for pathogen transmission. Seabirds are the primary hosts for the tick, *Ixodes uriae*, which is found associated with seabirds and their colonies around the world. These ticks are known vectors of the spirochete bacterial pathogen *Borrelia garinii*, and they transmit this bacterium throughout seabird colonies. *Borrelia garinii* is a member of the *B. burgdorferi* sensu lato species complex, with 3 members commonly, and 4 occasionally, causing Lyme disease in humans (24). While *B. burgdorferi* sensu stricto is the primary causative agent of Lyme disease in North America, *B. garinii* and *B. afzelii* are the causative agents throughout Eurasia, affecting humans, cats, and dogs. The circulation of *B. garinii* within seabird colonies is unique in that there is predominantly only a single tick species, *I. uriae*, involved in transmission with a single type of host. These ticks will often feed on the same host species at each life stage. This contrasts with *I. scapularis* transmission of *B. burgdorferi* s.s., where the nymphal and larval ticks feed on mice and other small rodents (Rodentia) but the adult ticks feed on deer (Cervidae) (25), and the bacterium can be transmitted to all the different hosts.

1.1.2. The system for my thesis

1.1.2.1. The seabird tick, Ixodes uriae

Ixodes uriae, the common seabird tick, has a circumpolar distribution in both the Northern and Southern Hemispheres found throughout the Afrotropical, Australasian, Nearctic, Neotropical and Palearctic regions (23) and is known to parasitize over 50 species of colonial seabirds (23, 26). In the Northern Hemisphere they infest alcids, such as Common Murre (*Uria aalge*), and other cliff-nesting seabirds, such as the Black-legged Kittiwake (*Rissa tridactyla*) (22, 23). They can have negative effects on host

survival and breeding success (27-29) and can carry various potential human pathogens (e.g., *Borrelia garinii* and Great Island virus; 22, 23, 30-32). Though these pathogenic agents are known to cause illness in humans little is known about their pathogenicity in seabirds. Pathogenic in this thesis is meant as pertaining to human illness and not seabird or wildlife.

Although *I. uriae* are found on land, they are intimately linked to the marine ecosystem. This is because they feed on seabirds, by definition marine species of birds that spend their entire lives at sea except for the few months during the breeding season (33, 34). Seabirds derive all nutrients from marine sources such as fish and zooplankton (33, 34). The ticks' off-host environment is very harsh with high salinity, low or no vegetation, and often within tens of meters of the ocean's edge. This means that we can consider them ecologically as marine.

The seabird tick is considered a 'three host' tick, with three active life stages: larva, nymph, and a sexually dimorphic adult stage (35). At each life stage, they require a single blood meal from a suitable host, except for adult males, which do not feed. Each blood meal is taken from a single host over a 4 to 8 day period (36) with the length of attachment varying based on life stage (35). After feeding, ticks of all life stages fall off the host and remain within the host burrow, or crawl under nearby rocks or into dark crevices to molt to the next life stage. In the case of female adults, they subsequently mate, oviposit and die. Because the summer season is relatively short, ranging from May to early August, and the availability of seabird hosts can be limited to when they are on

land, the tick life cycle for a single tick may take multiple years (from 2 to 8) to be completed (35). Given the limited amount of time spent attached to a host, most of the tick life cycle is spent in crevices and dark cool spaces around breeding seabird sites.

Seabirds can breed in high densities, such as the Common Murre that breeds at densities of up to 40 birds/m^2 (37), and therefore represent a large potential host population for these parasites to exploit. Seabirds also have high levels of inter-annual site fidelity, such as Atlantic Puffins that generally return to the same burrow year after year (38) and Common Murres that generally return to the same location on a cliff edge (39). In addition to their site fidelity, these seabirds are also highly synchronous in breeding attempts and are seasonally predictable for their presence on the colonies (40). These characteristics are important to ticks, which have limited mobility, only moving meters not kilometers on their own, and require a high probability of finding suitable hosts.

I. uriae is considered as being solely a seabird parasite, but there are reports of them also parasitizing deer mice (*Peromyscus maniculatus*) that are resident on the Gannet Islands in Canada (41), although other studies have not found *I. uriae* on mammals residing in close proximity to seabirds (42).

1.1.2.2. Borrelia *species*

Borrelia burgdorferi s.l. was initially detected in *I. uriae* feeding on Razorbills (*Alca torda*) in a colony on islands off the Swedish coast in 1991 (31). It was later determined to be *B. garinii* and subsequently found in seabird colonies in both the Northern and Southern Hemispheres (30). *Borrelia garinii* has now been recorded in

seabird colonies along the coasts of Europe, Antarctica, and in a few colonies in North America (43-47).

The prevalence of *B. garinii* in *I. uriae* varies widely, both among colonies and across years. The prevalence of *B. garinii* within ticks when found, has been documented to be as low as 8% in some colonies (30) and as high as 63% in others (44). There is also variation between years at the same colony (48). Prevalence may also vary spatially within colonies as tick spatial distribution is not homogeneous, even within single-species colonies (47). The reasons for these variations remain unexplored, but the likely major determinants of prevalence of *B. garinii* within seabird colonies should relate to the densities of either their hosts or vectors.

1.1.2.3. Seabirds

B. garinii is known to cycle between its vector, *I. uriae*, and its vertebrate hosts, seabirds. This bacterium is a spirochete that is transmitted from the mid-gut of ticks to the interstitial fluid around the site of entry, the tick bite (25). Bacterial cells are found within the blood (49) but only during the early weeks of infection (50), making screening blood samples problematic. The bacteria do not travel to the gastro-intestinal or respiratory tracts, ruling out the use of oral or cloacal swabs, although some past studies attempted using such techniques (51, 52). Clinical symtoms in seabirds have never been described.

Seabirds make excellent hosts as they have high site fidelity (37, 53, 54) and are relatively long-lived, with life-spans of over 20 years (37, 39, 54). This predictable return of birds from year to year means that ticks are guaranteed a suitable host in consecutive years, and therefore they do not need to quest for hosts to the degree observed in other

species of *Ixodes* such as *I. scapularis* or *I. ricinus*. Questing is the host-seeking behaviour commonly characterized by crawling up stems of grass or perching on the edges of leaves with front legs extended, ready to climb onto a passing potential host. This is also a valuable trait for transmission of the bacterium, as there is always a new cohort of susceptible hosts in the young that hatch, which are often heavily parasitized by *I. uriae* (27, 55). Young seabirds will spend multiple years prospecting before their first breeding attempt and will visit multiple colonies during this period (17), leading to opportunities for the bacterium to be spread geographically. Indeed, these birds show a high level of inter-annual persistence of anti-*Borrelia* antibodies, supporting high seroprevalence within populations (56) as indivdiuals retain their sero-positive status for long periods of time.

Seabird colonies often contain different species, each with differing ecological characteristics. It is important to note that seabird species are not all equally suited for hosting *I. uriae*, which is thought to most frequently associate with Common and Thick-billed Murre. Their nesting areas are often bare cliffs, with little or no vegetation and high exposure to the elements. There are few spaces for ticks to over-winter so they cluster into the limited spaces that exist. The advantages of this type of habitat for ticks are the high density of birds and the assured return of hosts between years. However, ticks are faced with poor overwintering conditions in these locations, and need to burrow under rocks and into small crevices. Many seabird colonies do not have accessible crevices or earthen habitat for ticks to over-winter in as they can be very rocky with little to no vegetation. Similar conditions are found within the breeding habitats of Black-

legged Kittiwakes and many penguin species, which also host *I. uriae*. Atlantic Puffins are also known to have high *I. uriae* infestation rates. These birds lay their eggs within earthen burrows. Though puffin burrows can be only tens of centimetres apart, they do not have the same high densities as with breeding murres. However, these earthen burrows do provide ticks with greater protection from the elements, both during the breeding season and throughout the winter. These ecological variations among seabirds may help to explain why some species are more heavily parasitized, and thus have a higher risk of *B. garinii* infection.

1.1.3. Location of study

The seabird colonies within Newfoundland and Labrador are important as they are the largest and most diverse in Eastern North America, and are tightly connected to colonies in Canadian Eastern Arctic and sub-Arctic through seabird feeding patterns and migration. Patterns observed in these colonies will drive what occurs elsewhere in the Western Atlantic and Arctic seabird colonies. Many of the birds using these colonies migrate long distances during the non-breeding season and interact with birds that breed elsewhere. Therefore, it is important to study *B. garinii* within these colonies to understand how the Canadian birds fit within the global situation and to evaluate the potential for transmission among regions. For example, it is possible that this bacterium is being transmitted across the seabird colonies of the Arctic because it has also been documented in the far east of Russia and the western Aleutian Islands (30, 45) though transmission has yet to be confirmed.

B. garinii has only previously been documented in two breeding colonies in North America, both within the province of Newfoundland and Labrador, and had never been found in the terrestrial realm in North America. The first colony, the Gannet Islands, an archipelago with thousands of seabirds nesting on them, is located 30 km off the coast of Labrador near the community of Cartwright. Although ticks were collected in 1995, no *Borrelia*-positive samples were found at that time (30). However, a broader collection of ticks from many habitats was screened in 2006, and 17% of the ticks were positive for the bacterium (48). The second location is south of St. John's, in the Witless Bay Ecological Reserve. At this location, 20% of ticks collected in 2005 were positive and 7.2% of those collected in 2006 were positive (43, 48).

The colonies discussed above represent only two of nearly 60 seabird colonies within the Canadian North Atlantic region (33). However, they represent two key locations, with the Gannet Islands located in Labrador and closer to Iceland and Europe, where *I. uriae* and *B. garinii* are also present. The more southern location, off the eastern coast of Newfoundland, includes the largest breeding colony of Atlantic Puffins in North America and represents a potential link to the colonies further south in Maine and Nova Scotia, where *B. garinii* has yet to be documented (43).

1.2. Thesis overview

Within the Northwest Atlantic, *B. garinii* has only been documented in two seabird colonies. With the large number of colonies spanning a vast geographical area in this region, it is important to first identify the prevalence of this bacterium within colonies that both span this geographical range and also the spectrum of seabird species.

Ixodes uriae is known to have host preference and specialization, meaning that seabird host species needs to be considered alongside geographical scale. The seasonal variation in population size of *I. uriae* plays an important role in transmission of *B. garinii*, and this variation must be documented to understand how it relates to the seasonal breeding cycles of its host seabirds.

It is important to understand how the marine cycle of *Borrelia* transmission links with the terrestrial cycle, where the greatest risk of human impact exists. It is believed that the population structure of *B. garinii* is highly complex as a result of the various avian and vector species involved in circulation in both the marine and terrestrial systems (46). Indeed, the lack of population genetic structure between the marine and terrestrial systems and genetic overlap suggest that mixing is occuring (46), and it has been suggested that the marine cycle may play an important role in generation of new strains that can be introduced to the terrestrial realm within Eurasia (46).

In this thesis, I explore the *I. uriae*-*Borrelia*-seabird system of Newfoundland and Labrador. The major goals of my research were to examine the seasonality of *I. uriae* within these colonies, document the prevalence of *Borrelia* spp. in *I. uriae*, and examine the genetic relationships of *Borrelia* from these colonies to *Borrelia* around the world. In Chapter 2 I examine the numbers of *I. uriae* collected across multiple summers and how this was related to colony, associated hosts, seasonality, and climatic factors. A subset of these ticks was screened for *Borrelia*; I analyze the prevalence within these colonies in Chapter 3, integrating a serological analysis of seabirds from these colonies to link

Borrelia prevalence to host infection rates. These ticks also carry bacteria other than *Borrelia*, so total bacterial assemblages (microbiomes) were analyzed in relationship to the presence or absence of *Borrelia* and seabird colony of origin. Unexpectedly, while looking for *B. garinii* in the ticks, I found that some ticks were in fact infected with a different species, *B. bavariensis*, which had previously only been found in terrestrial settings in Eurasia; this is covered in Chapter 4. Finally, I report on phylogenetic analyses of *B. garinii* from different colonies in Chapter 5, employing multi-locus sequence typing (MLST) according to a scheme not previously applied to *B. garinii* from *I. uriae*, to understand the relationship between bacteria in Newfoundland and Labrador and those from throughout Eurasia, within both the marine and terrestrial realms.

1.3. Co-authorship statement

I drafted the entire thesis myself and received feedback from multiple people including my committee and future co-authors for papers that will originate from my thesis. I performed the analyses in each chapter after consulting with my committee and co-authors, with the exception of serological analysis which was performed at Université Montpellier by Amandine Gamble and goeBURST which was performed by Sambir Mechai. Figures for the microbiome analysis were created with aid from Joost Verhoeven. Supplemental tables and figures pertaining to serological analysis were created by Amandine Gamble from Université Montpellier. Samir Mechai created goeBURST networks and I modified output from this program to create the figure.

Chapter 4 is a version of a co-authored manuscript that has been accepted for publication. I wrote the original draft of the manuscript, which was then modified in response to co-authors' comments.

Manuscript associated with work presented in this thesis:

Chapter 4 Munro H. J., Ogden, N. H., Lindsay, L. R., Robertson, G. J., Whitney, H., & Lang, A. S. (2017). Evidence for *Borrelia bavariensis* infections of *Ixodes uriae* within seabird colonies of the North Atlantic Ocean. **Applied and Environmental Microbiology** 83:e01087-17

CHAPTER 2: SEASONALITY OF *IXODES URIAE* IN SEABIRD COLONIES OF NEWFOUNDLAND AND LABRADOR

2.1. Abstract

Ixodes uriae, the common seabird tick, has a circumpolar distribution, feeds exclusively on seabirds, and is a known vector of *Borrelia garinii*. It is important to study the seasonality of this species to understand the tick's life-history strategies and the ecology of the diseases that it transmits. I examined the seasonal patterns of abundance of *I. uriae* at five seabird colonies in Newfoundland and Labrador between 2011 and 2015, during the months of June, July, and early August, exploring the effects of different seabird species compositions in the colonies. Using model-based statistical techniques, I documented seasonal variation similar to that observed in previous studies and at other colonies. The number of larvae collected peaked in mid to late June before declining for the rest of the season. Nymphs increased in numbers, with more collected in August than June. Adults had two peaks, one in mid June, before larva, and another starting in late July into early August. The number of ticks collected varied between years, with host species, and was related to variation in precipitation. The proportion of male to female adult ticks varied across location and year, and was correlated with precipitation from the previous season. In all cases precipitation departures from average related to more ticks collected. At each life stage, the proportion of engorged to flat (non-engorged) ticks varied with host species, and in nymphs and adult females it varied among years. Characterizing these patterns of variation is important so that we can understand the life cycle, population dynamics, and phenology of *I. uriae*, a vector of *Borrelia* and other pathogens.

2.2. Introduction

The common seabird tick, *Ixodes uriae*, has a circumpolar distribution in both the Northern and Southern Hemispheres, including the Afrotropical, Australasian, Nearctic, Neotropical and Palearctic regions and is known to parasitize over 50 species of colonial seabirds (23, 26). In the Northern Hemisphere they commonly infest alcids, such as the Common Murre (*Uria aalge*), and other cliff-nesting seabirds, such as the Black-legged Kittiwake (*Rissa tridactyla*; 22, 23). They can have negative effects on the survival and breeding success of multiple avian species (27-29). Although this species only occasionally bites people, it may be infected with potential human pathogens, such as *Borrelia garinii* and Great Island Virus (*Orbivirus*), and thus can act as vectors of human pathogens (22, 23, 30-32).

Seabird ticks require a blood meal at all three life stages: larva, nymph and female adult (35), and only adult males do not engorge on hosts. Each blood meal is taken from a single host over a 4 to 8 day period (36), with length of time the tick is attached varying with life stage (35). After feeding, ticks of all life stages fall off the host and remain within the host burrow or crawl under nearby rocks or into crevices to molt to the next life stage. In the case of female adults, they oviposit and die (35). There is evidence of mating occurring either before or after feeding of females (35). Where the summer season is short, ranging from May to early August, and the availability of seabird hosts is limited to when they are on land, it may take from 2 to 8 years for seabird ticks to complete the life cycle (35). Because the duration of attachment is relatively short, a matter of days,

most of the tick life cycle is spent in crevices and other dark cool places, usually in or around seabird breeding sites.

Seabird ticks are reliant on the regular and predictable presence of their seabird hosts and a suitable habitat within the colonies for overwintering. Seabirds can breed in high densities; for example, the Common Murre breeds at densities of up to 40 birds/m^2 (37), and therefore can represent a large potential host population for these parasites to exploit. Seabirds also have high levels of inter-annual site fidelity; for example, Atlantic Puffins (*Fratercula arctica*) return to the same burrow year after year (38), and murres return to the same location on cliff edges (39). In addition to their site fidelity, these seabirds are also highly synchronous in breeding and seasonally predictable in their presence on the colonies (40). Site fidelity and high population density within colonies undoubtedly increases the probability of ticks finding suitable hosts.

A previous study documented seasonal increases in adult female and nymphal seabird ticks in mid July (57), which is thought to correspond to the peak chick-rearing period of the Atlantic Puffin and other primary host species. The seasonal population dynamics of *I. uriae* ticks are known to fluctuate among years, likely influenced by annual climatic variation (57), leading to annual variation in tick abundance and the timing of peak activity for the different life stages. Climate influences the breeding behaviour and success of the ticks' primary hosts but also can directly affect *I. uriae* as they are sensitive to desiccation (58) and their activity and development are influenced by temperature (59). When collected directly from birds, the timing of peak numbers of *I.*

uriae can also differ among different host species (57). Studies on seasonality of *I. uriae* in Newfoundland have focused on a single seabird colony and most often on ticks associated with Atlantic Puffins (35, 57). Past work has also used a qualitative approach to analyzing seasonality without more detailed statistical quantitative analysis of seasonal variation.

Understanding the seasonality of *I. uriae* ticks, and how seasonality may vary among colonies, host species and years, is essential for understanding the dynamics of transmission of tick-borne pathogens in seabird colonies (31, 60), as well as how tick abundance/feeding and tick-borne pathogens impact survival and breeding of infested seabirds (27, 61, 62). In this study, I examined the seasonal population dynamics of *I. uriae* at five seabird colonies throughout Newfoundland and Labrador. I sampled within the colonies over several years and explored effects of seabird species compositions on tick abundance and seasonality. I also sampled from colonies that have been studied previously, allowing comparisons over larger time scales.

2.3. Methods

2.3.1. Ethics

Access to the Witless Bay, Gannet Islands, and Cape St. Mary's Ecological Reserves was obtained through permits from the Parks and Natural Areas Division of the Newfoundland and Labrador Department of Environment and Conservation. This work was carried out under the guidelines specified by the Canadian Council on Animal Care with approved protocols 11-01-AL, 12-01-AL, 13-01-AL, and 14-01-AL from the Memorial University Institutional Animal Care Committee.

2.3.2. Tick collection

Ticks were collected from five seabird colonies in Newfoundland and Labrador: Cape St. Mary's, the Gannet Islands, Little Fogo Islands, Gull Island, and Great Island (Figure 2.1, Appendix 1, Supplementary Figure 1). All trips coincided with the breeding season of the seabirds on these islands (Appendix 1, Supplementary Table 1 and 2).

Ticks were collected from the substrate around breeding birds' habitats by a single searcher. *Ixodes uriae* do not exhibit questing behaviour like other ticks, but can be found crawling on the substrate around their hosts' burrows and nesting sites, a questing-like behaviour. They were found under rocks near breeding Common Murre, Thick-billed Murre (*U. lomvia*), and Razorbill (*Alca torda*). I searched the environment by looking with the naked eye under rocks, finding ticks on surfaces between rocks and among the limited organic material. The breeding cliffs used by murres and razorbills are a hostile environment with very little soil or organic material, and ticks find refuge from the elements in crevices under rocks in which organic material collects, referred to as hibernacula. I am confident of the identification of suspected hosts for these environmentally recovered ticks because Common Murres nest in dense colonies with Razorbills found along the edges. In the case of the Gannet Islands where Common Murres and Thick-billed Murres are interspersed in colonies, ticks were not collected from these breeding sites. Ticks were also found crawling on researchers' clothes within Atlantic Puffin colonies after investigating nesting burrows. These burrows are solely used by Atlantic Puffins with occasional American Herring Gull (*Larus smithsonianus*) and Great Black-backed Gull (*L. marinus*) nests interspersed, and were assigned as

having puffin hosts. In a few cases, the exact hosts were unknown because ticks were found in a location where multiple species were breeding.

A single search of a murre/razorbill colony ranged from 30 to 60 minutes based on accessibility, and time spent within these colonies was limited to minimize disturbance. Roughly 3 m^2 of the murre colonies were searched each time. The same sites were visited in different years and multiple times throughout the season. The time spent in puffin colonies was longer, with each search ranging from 1 to 3 hours. This longer period was needed because breeding density of Atlantic Puffins is much lower than Common Murres and more time was required to search the individual burrows, and other data were also being collected while in the colony, which resulted in large variation in the length of time during sampling periods for puffins. These Atlantic Puffin breeding habitats ranged between 10-20 m^2.

All ticks were collected into dry sterile vials either individually or as a large collection when more than 10 ticks were found and transported to the laboratory or frozen on the day of collection. Tick species identifications were confirmed using keys (63-65).

2.3.3. Statistical Analysis

2.3.3.1. Variation in counts

All statistics were performed with R 3.1.2 (R Core Team 2015). Seasonality data from larvae, nymphs and adults were analyzed separately to account for different factors influencing seasonal patterns and variation in detection probability between stages. A two-step approach was used to explore the population dynamics of *I. uriae*. First, I explored variation among years, host species (or most likely ones), and site/colony as

sources of variation in tick abundance using a generalized linear model (GLM) with a negative binomial distribution. In these models 'host species' captures variation resulting from host but also any bias resulting from differential sampling techniques between species as a result of habitat. A series of biologically plausible models (limited to those that ask meaningful questions) accounting for multiple combinations of variables was developed and a 'best' model was chosen, being the one that explained the most variation with the fewest variables as determined by minimizing Akaike information criterion (AIC) (2). To explore how yearly variations may result from variations in climatic variables, precipitation anomaly data for the summer months were incorporated into each model, with year removed. Precipitation was used because seabird ticks are hydrophilic and very sensitive to variability in humidity (58). Ticks are highly sensitive to low precipitation with *I. uriae* having the risk of desiccating (58) but with high precipitation, the environment can become oversaturated, resulting in puddles and streams that are also unfavourable for the ticks. I did not include variables in respect to winter as ticks are only active during the summer months, spending the winter in the more moderated environments of their hibernacula. Temperature was not considered, as these ticks are either found attached to birds or under rocks and I did not have temperature readings for either of these environments across time. Precipitation anomaly was calculated using historical data from Environment Canada weather stations (http://www.climate.weatheroffice.gc.ca) within the closest proximity of each colony (Appendix 1, Supplementary Table 3). The absolute value of the difference between the 30-year average (1981-2010) and the previous June, July, and August total precipitation,

and the May of the year in question were included in the model selection. The results of analysis of variance (ANOVA) are reported from the top model to determine the significance of the factors in the model. Post-hoc tests were performed using the Tukey test to assess differences among levels within factors. Second, the selected model was then incorporated into a general additive model (GAM; 66) in which Julian day was explored as a variable smoother. The output from the GAM describes the amount of variation explained by the different model explanatory variables, the number of curves in the smoother, and statistics related to the curve of the smoother. All means are reported with 95% confidence intervals (CI).

2.3.3.2. Proportion of female adults

Adult males and females of *I. uriae* differ morphologically. Eveleigh and Threlfall (35) examined the seasonal variation in the adult sex ratio as an indicator of shifts in the population dynamics and behaviour of ticks, with males being rare in early June but they outnumber females in July and August. Sex ratio is important as both males and females are required for mating. To examine the variation in the ratio of females to males, the same methods as described above were applied using AIC to determine the top model and GAM to describe variation across seasons. I looked at the proportion of female adult ticks in the sample, as a measure of the sex ratio. Tick sex was included as the dependent variable, with a binomial distribution. This allowed me to determine whether the proportion of females in the sample differed based on year, host, or location, and the four precipitation anomaly variables. Using a GAM, I described how the proportion varies across the season.

2.3.3.3. Proportion of engorged ticks

The proportion of engorged ticks at each life stage was examined. This provides information regarding when each life stage is feeding and any variation related to year and host. I considered all ticks as either engorged or non-engorged. Very few partially engorged ticks were observed due to the sampling technique, so all partially engorged ticks were considered engorged. To examine variation in the proportion of engorged ticks between years, locations, and associated hosts, a GLM with binomial distribution was used, with engorged or non-engorged as the dependent variable; and year, host, and location as the predictor variables. Model selection, as described above, was utilized with the top model included in a GAM to describe seasonal variation.

2.4. Results

A total of 1876 *I. uriae* were collected from the five colonies over the five breeding seasons (Appendix 1, Supplementary Table 2): 433 larvae, 880 nymphs, and 563 adults (287 females and 276 males). Both engorged and unfed ticks were found at each life stage; larvae, 427 engorged, 6 unfed; nymphs, 513 engorged, 367 unfed; female adults, 109 engorged, 178 unfed.

2.4.1. Larval counts

Using AIC, the model that described the most variation in the number of larval ticks collected with the least number of variables contained year and tick host (Table 2.1), but none of the precipitation anomaly variables. The number of larval ticks collected differed significantly across years ($\chi^2 = 33.5$, $p < 0.001$, Table 2.2) and hosts ($\chi^2 = 11.9$, $p < 0.01$, Table 2.2). In 2015, more larval ticks were collected on average than in 2013 (Tukey, $p < 0.01$) or 2014 (Tukey, $p < 0.01$) and more ticks were collected in 2012 than

2014 (Tukey, $p < 0.01$). More larval ticks were collected from Razorbills than Atlantic Puffins (Tukey, $p < 0.01$). When examining seasonality, the GAM explained 85.8% of the variation, which accounts for variation in year and host as well as seasonal variation. The smoother was not significant, with variation being explained by other variables (edf = 2.3, $\chi 2 = 7.46$, $p = 0.149$, Figure 2.2).

2.4.2. Nymphal counts

Before accounting for precipitation variation, the model that explained the most variation in numbers of nymphs collected contained year, location, and host species (Table 2.1). When including precipitation variables, the top model contained the previous August precipitation anomaly as well as location and host species (Table 2.1). The number of nymphs collected varied significantly among hosts ($\chi 2 = 18.092$, $p < 0.001$, Table 2.2), colony ($\chi 2 = 13.106$, $p < 0.001$, Table 2.2), and with the previous August precipitation anomaly ($\chi 2 = 19.697$, $p < 0.001$, Figure 2.3). There were more nymphs collected from Great Island (Tukey, $p < 0.01$) and Gull Island (Tukey, $p < 0.01$) than Little Fogo Islands. There were more ticks collected from areas around Common Murre nest areas than those of Atlantic Puffins (Tukey, $p < 0.01$). There were also more nymphs collected in years where the previous August's precipitation differed more from the 30-year average, meaning that years with greater rainfall or lesser rainfall had more ticks collected. The GAM showed a linear increase in the numbers of nymphs collected over the breeding season (edf = 1.0, $\chi 2 = 3.777$, $p = 0.05$, Figure 2.2) with the entire model explaining 70.7% of the variation.

2.4.3. Adult counts

The model that explained the most variation in adult ticks collected, before accounting for variation in precipitation, contains year, host, and colony (Table 2.1), but when including precipitation anomaly variables, year was no longer in the top model and the May precipitation anomaly variable was included in the model. The number of adult ticks collected differed between hosts ($\chi2 = 15.0$, $p < 0.01$, Table 2.2), colony ($\chi2 = 19.0$, $p < 0.01$, Table 2.2), and May precipitation anomaly ($\chi2 = 11.4$, $p < 0.01$, Figure 2.4). More adult ticks were collected on the Gannet Islands (Tukey, $p = 0.01$), Great Island (Tukey, $p < 0.01$), Gull Island (Tukey, $p = 0.02$), and Little Fogo Islands (Tukey, $p = 0.02$) than at Cape St. Mary's. There were more adults collected from areas around where Common Murres were breeding compared to Atlantic Puffins (Tukey, $p < 0.01$). The number of adult ticks collected increased as the May precipitation anomaly increased relative to the 30-year average (Figure 2.4), meaning drier and wetter than average years resulted in more ticks being collected. The variation in the number of adults across the season was almost significant, with the GAM, including all variables explaining 67.3% of the variation (edf = 3.6, $\chi2 = 9.6$, $p = 0.06$) with a peak in late June followed by a decline until mid July and then a general increase late in the summer (Figure 2.2).

2.4.4. Proportion of adult females

The top model that explained the most variation in the proportion of female ticks before accounting for precipitation variation contained colony, host, and year (Table 2.1). When accounting for precipitation variables, year was no longer in the top model and the June anomaly variable was found in the top model. Sex ratio varied between location ($\chi2 = 64.25$, $p < 0.01$) and host ($\chi2 = 83.20$, $p < 0.01$), June's precipitation anomaly only had

a weak effect ($\chi2 = 3.18$, $p = 0.07$, Figure 2.5). Sex ratio differed between ticks collected on Gull Island (30.4% CI 25.1-35.3%) vs. Great Island (60.0 CI 52.1-67.9%, Tukey, $p < 0.01$) and around Common Murres (37.2% CI 32.8-41.7%) and Atlantic Puffins (98.9% CI 96.8-100%, Tukey, $p = 0.01$). There was also seasonal variation, with the GAM explaining 33.1% of the variation seen (edf = 8.6, $\chi2 = 36.07$, $p < 0.001$) with disproportionately more females found in early June and early August, and males outnumbering females in mid June and early July (Figure 2.6).

2.4.5. Proportion of engorged larvae

The proportion of engorged larvae collected was explained by host (Table 2.1), with the proportion varying significantly ($\chi2 = 35.5$, $p < 0.001$, Table 2.3). The proportion of engorged ticks was greater when collected near Razorbills than Atlantic Puffins (Tukey, $p < 0.01$) or Common Murres (Tukey, $p < 0.01$). There was also a larger proportion of engorged ticks collected around Common Murres compared to Atlantic Puffins (Tukey, $p < 0.01$). The GAM did not detect any seasonal variation in the proportion of engorged larvae.

2.4.6. Proportion of engorged nymphs

Year and tick host had the lowest AIC score when examining the proportion of engorged nymphs (Table 2.1). Both host ($\chi2 = 112$, $p < 0.001$, Table 2.3) and year ($\chi2 = 145$, $p < 0.001$, Table 2.3) were significantly related to the proportion of engorged ticks. The proportion of engorged ticks was larger in 2012 (Tukey, $p = 0.03$) and 2013 (Tukey, $p < 0.001$) than 2014. The proportion of engorged ticks was greater when collected near

Common Murres than Atlantic Puffins (Tukey, $p < 0.01$). The GAM did not detect any seasonal variation in the proportion of engorged nymphs.

2.4.7. Proportion of engorged adult females

The fully saturated model with year, host and location had the lowest AIC score when examining the proportion of engorged adult females (Table 2.1). Colony ($\chi2 = 40.7$, $p < 0.001$, Table 2.3), tick host ($\chi2 = 44.3$, $p < 0.001$, Table 2.3) and year of collection ($\chi2 = 15.7$, $p = 0.003$, Table 2.3) were all significantly related to the proportion of engorged female ticks. The proportion of engorged ticks was higher on Gull Island than Great Island (Tukey, $p < 0.01$) or the Gannet Islands (Tukey, $p < 0.01$). The proportion of engorged ticks was higher when collected near Common Murres (Tukey, $p < 0.01$) and Razorbills (Tukey, $p < 0.01$) compared to Atlantic Puffins. The proportion of engorged ticks was higher in 2012 and 2013 (Tukey, $p < 0.001$). When examining seasonality, the GAM explained 53% of variation including day, host, year, and colony. There was some seasonal variation but this pattern was weak (edf = 1.9, $\chi2 = 9.17$, $p = 0.06$, Figure 2.7).

2.5. Discussion

The number of *I. uriae* collected at seabird colonies throughout Newfoundland and Labrador showed variation among years of collection and seabird host types, and temporally within years at all three life stages. The seasonal patterns differed slightly between the three life stages, but were similar to those documented in past studies with one peak in June and increases late in the season (35, 57). The number of ticks was

related to variation in precipitation between years for two life stages, consistent with the ticks' sensitivity to desiccation (58).

2.5.1. Variation between years

Variation in numbers of *I. uriae* has been noted by others (57), and may be caused in part by inter-annual variation in climate as ticks are sensitive to both precipitation and temperature. *Ixodes uriae* is considered hydrophilic, having a high body-water content, low dehydration tolerance limit, and a high water-loss rate (58). Higher than average precipitation can also have negative effects, leading to oversaturation of seabird breeding sites and pooling of water. The climatic conditions varied over the five years in which ticks were collected, with an unseasonably cold wet summer in 2011 and a drier than average summer in 2012. Variations in host populations could also contribute to inter-annual variation. There was an almost total breeding failure of Common Murres on Great Island in 2014 and a large die-off of Atlantic Puffin chicks on Gull Island in 2011. Past reports have documented shifts in *I. uriae* collection numbers between species in years of seabird breeding failure (35). However, in my study, seasonal differences in total adult and nymphal tick counts were likely driven primarily by inter-annual variations in precipitation, as the effect of year dropped out of the top model when inter-annual variation in precipitation was accounted for. Year still had an important effect, but precipitation accounts for more of the variation.

For nymphs and adults, the number of ticks collected was correlated with previous precipitation. The number of nymphs was related to the August anomaly of the previous summer. This represents the time when the ticks were likely molting from larvae

to nymphs; the larval stage is most sensitive to desiccation (58). For adults, the number of individuals collected is correlated with the May anomaly in precipitation of the year of collection. Although nymphs are not as sensitive to desiccation as larvae, they still must seek appropriate habitat to maintain crucial water balance (58).

2.5.2. Host density

Variation in tick numbers collected from hosts can be explained by breeding density and habitat type, which also contributes to collection efficiency. Common Murre breeding densities can be almost 40-fold higher than those of Atlantic Puffin, with murres nesting at an average density of 28 birds/m^2 and occupied puffin burrows at 0.66 burrow/m^2 (54, 68). The breeding Razorbills that were relevant for this study were found near Common Murres and in similarly high-density groups. The environment around the nesting habitat of Common Murres is also conducive to high tick densities, where they can congregate under rocks to avoid desiccation. The cool, damp environment under rocks likely allows them to maintain water balance. The nature of the murre breeding areas means that ticks from multiple birds can be found under a single rock, whereas ticks found within a puffin burrow represent those from a single breeding pair and their single chick. Past studies have documented a preference of *I. uriae* for Common Murre and Atlantic Puffin as hosts over other sympatric species (35). In years when fewer Common Murres are present due to breeding failure, ticks are known to shift towards Black-legged Kittiwake, which often breed on cliffs interspersed with murres, while in the case of years with low Atlantic Puffin numbers, Herring Gulls are more heavily infested (35). As

success at engorgement is also influenced by host age (35), switching host species likely has a cost in terms of feeding success.

Past studies have reported a bias towards adult male ticks over adult females when the ticks are collected from the environment (35). This pattern is supported by my results from Great Island, but not on Gull Island where I collected more female ticks than males. The proportion of male to female adult ticks was related to the associated host. This is likely the result of different habitat types resulting in varying sampling efficiency for Atlantic Puffin compared to Common Murres. When sampling in puffin nesting habitat, I would preferentially select ticks exhibiting a questing-like behaviour, as the ticks would be found crawling around the burrows. Alternatively, when sampling in murre nesting habitat, I collected ticks that were exhibiting a questing-like behaviour and those within hibernacula. Past studies have not recorded questing male ticks (57), resulting in more females being collected when samples were biased towards questing individuals. This would also result in more unfed individuals being collected from Atlantic Puffin areas, as only unfed ticks exhibit the questing-like behaviour. Ticks collected from around Common Murre and Razorbill breeding habitat were more likely to be engorged as it is easier to find their hibernacula in these areas, and both fed and unfed ticks can be found in these hibernacula.

2.5.3. Colony

Related to the above points, the different seabird colonies had different numbers of ticks at the nymph and adult life stages, as well as proportion of engorged adult female ticks, possibly because the habitat varies among colonies. The colonies involved are also

spread over a large geographic area, with Cape St. Mary's located 775 km from the most northern colony, the Gannet Islands. These sites have different climates, seabird compositions and densities, and geological characteristics. At both Great and Gull Islands, large numbers of larvae and adults were collected, likely because these islands have large colonies of Common Murres. In contrast, most of the ticks collected at Little Fogo Islands and the Gannet Islands came from Atlantic Puffins. The proportion of engorged ticks is also driven by host density, with more engorged ticks being found around Common Murres but I did not search the Common Murre areas on the Gannet Islands as the field season was cut short when a polar bear (*Ursus maritimus*) arrived and camp was evacuated. Low numbers of ticks were collected from Cape St. Mary's, but this is mostly due to poor sampling access and steep cliffs.

2.5.4. Seasonal patterns

The seasonal patterns of ticks collected differed for the three life stages but followed a general pattern of a peak in the early season for nymphs, a peak late in the summer for larvae, and peaks in both early season and late in the summer for adults (Figure 2.2). Past study has documented that the period of activity of *I. uriae* coincides with the breeding season for seabirds in the Northern Hemisphere (55) as these seabirds only come ashore for a few weeks before the breeding season (37). On Gull Island, Common Murres lay eggs between 18-26 May (69), with chicks hatching approximately 32 days later (33) and then fledging approximately 20 days after that (69), which can range from 3 to 24 July. The other breeding seabirds on the Island have a similar breeding phenology. Past work on Gull Island, which was my primary study site,

documented low levels of host-seeking ticks collected before mid June and no ticks collected after the first week of August (57). In my study ticks were only collected during the breeding season, with no attempts to collect outside of this time period made because ticks retreat deep into the organic matter or rock crevices to over-winter and moult.

I found that larval tick abundance tended to peak in late June and early July before declining over the remainder of the season. This pattern was observed in ticks collected several decades ago on Gull Island, when larvae were abundant throughout June but increased in the last week of June and rose to a peak during the first week of July (35, 57). The peak of ticks collected off Common Murres and Black-legged Kittiwakes on the Isle of May, Scotland was earlier, with a peak in mid May for those found on murres and a week later for those found on kittiwakes (55). I found engorged larvae starting in mid June. On the Isle of May, the peak in percentage of engorged ticks was observed in June, with all ticks collected in that month being engorged (55). Due to the warming effect of the Gulf Stream, seabirds in the United Kingdom breed earlier than those along the Newfoundland coast (38, 69, 70). Eveleigh and Threlfall (35) did not record engorged larvae until the middle of July in Newfoundland, and started to observe newly molted nymphs at the end of August. This may be the result of inter-annual variation, sampling technique or micro-habitat dynamics.

The study by Eveleigh and Threlfall (35) occurred on Gull Island within the same general areas I sampled, but the exact sampling sites were not replicated, and the habitat has changed on the island over the last forty years due to erosion and other factors.

During this time-period the composition of seabirds has also changed, with slopes used for Atlantic Puffin research being abandoned as burrows collapse or become inaccessible and Common Murre colonies expand. Population changes in these birds have been noted at both colonies and wintering areas (68, 71).

I observed a general increase in number of nymphs over the entire season, which does not match with what has been previously observed. Muzaffar and Jones (57) found a peak in the numbers of questing nymphs in mid July in both years sampled on Gull Island, although with peaks of smaller magnitude before and after the highest peak in 2004. A bimodal pattern was also observed on the Isle of May, where peaks occurred in mid-May and early July for murres and early June and mid July for kittiwakes (55). My data show a general increase across the entire season when location, year, and seasonal precipitation are accounted for. In the 1970s, Eveleigh and Threlfall (35) found that nymphs were the dominant stage found when searching the environment. Like Eveleigh and Threlfall (35), I found engorged ticks for most of the sampling period, from early July until mid August. Other studies on Gull Island have documented the season for tick activity as ranging from as early as 18 May to early August (57) with the first date of observed tick activity varying by up to a month between years (57). Barton et al. (36) saw a peak in the percentage of engorged nymphs in May, whereas I did not observe a peak but a high level all season long.

The abundance of adult ticks reached a peak in mid June before declining with an increase late in the season starting in mid July. This peak in adults, which could occur in

either late-June or early-July, was also observed by Eveleigh and Threlfall (35) and for *I. uriae* collected off birds and host-seeking ticks in the 2000s (57). Past work found that the number of males declined until August, after which their numbers remained constant for the entire month, with no females observed in August, and newly molted males observed around mid August (35). My data show the number of all adults increasing again in mid July, but there are not enough data to determine when the peak declines. It should be noted that host-seeking behaviour of female adults ends in early August (57) and therefore the means of collection employed can play a role in the observed seasonal patterns.

All tick life stages exhibit variation across the season. There is an increase in ticks collected late in the season but this is the result of finding hibernacula and not increased activity. Unlike most other tick species, it is not possible for *I. uriae* to pass through multiple life stages in one season. The availability of seabird hosts ranges from the beginning of egg-laying (mid May for both Common Murre and Atlantic Puffin), through brooding when ticks can feed on the adult seabirds incubating their eggs or attending their brood. This period is on average 52 days for Common Murre (69), and 92 days for Atlantic Puffin (70). Ticks can then feed on the chicks, which stay within their nests for approximately 20 days for Common Murre and approximately 53 days for Atlantic Puffin before they fledge. At each life stage, ticks need to take a blood meal, which can range from 5 to 8 days long, depending on the life stage (57). They then must molt or, in the case of a female adult, mate and oviposit eggs. These processes are all dependent on temperature. On Gull Island, the soil temperature averages 9.4°C during the breeding

season (35). At this temperature, molting from larva to nymph takes 79-105 days, and from nymph to adult 71-145 days (35, 72). Oviposition can take 56-110 days (35, 72). In other geographic locations and at different temperatures these processes can occur faster. The process of oviposition is highly temperature-dependent: at 20°C oviposition occurred after 7-14 days, but at 15°C it can take 14-22 days (73). Once laid, these eggs take 157-196 days to hatch at 3°C (35) and 84-129 days at warmer temperatures (74). The molting period for engorged larvae has been documented as short as 50 days and nymphal molting as short as 55 days (73). If ticks can only accomplish feeding and a single molt in one summer, it would take at least three breeding seasons for *I. uriae* to complete an entire life cycle within the seabird colonies of Newfoundland and Labrador. I propose that the double peak in ticks collected from the environment during the seabird breeding season is the result of some individuals within cohorts of ticks having enough time to molt at the end of the summer, while others do not and must molt in the spring, based on when they can achieve a complete blood meal (Figure 2.2). This dynamic of cohorts being non-synchronous may lead to differential survival and also possibly protects the population from severe events that might negatively affect only portions of the population and not all cohorts.

This study expanded our knowledge of seasonality of *I. uriae* by bringing data from multiple colonies and years together. However, the conclusions are limited by the sampling techniques used, which were not standardized across seabird species, and this is further complicated by different colonies having differing levels of accessibility. *Ixodes uriae* reside in places that are very difficult to access, do not display a consistent host-

seeking behaviour, and appear to not be attracted by carbon dioxide traps. Though it is possible to look at ticks attached to both adult and juvenile seabirds, this requires highly invasive methods and provides different information about timing than what is observed from studies like mine, where ticks are collected from the substrate around the nesting seabirds.

The peaks in numbers of *I. uriae* collected in seabird colonies within Newfoundland and Labrador are highly synchronous. Year and precipitation play roles in determining the abundance of ticks as these parameters influence the ticks' behaviour and survival as well as host behaviour. These ticks are highly reliant on their hosts, requiring a blood meal at each life stage. Host species and density affect the number of ticks collected from the environment. My data confirm seasonal patterns observed previously on Gull Island in Witless Bay, but they also expand these patterns to other colonies within Newfoundland and Labrador, more broadly illustrating the seasonal dynamics of *I. uriae* populations. These patterns of variation are important so that we can understand the life cycle, population dynamics, and phenology of *I. uriae*, a vector of *Borrelia* and other pathogens.

Table 2.1. Models for model selection and AIC values.

Model[a]	**AIC**	**Log Likelihood**	**N**	**ΔAIC**
Larvae: count				
Year+Host	128.441	-57.221	7	0
Year+Host+MayAnom	130.025	-57.0126	8	1.584
Year+Loc+Host	130.568	-55.284	10	2.127
Year	133.737	-61.869	5	5.296
Year+Loc	136.786	-60.393	8	8.345
Host+MayAnom	141.441	-65.721	5	13
Host	141.658	-66.829	4	13.217
Host+PJuneAnom	143.062	-66.531	5	14.621
Loc+Host	143.101	-64.551	7	14.66
Host+PJulyAnom	143.62	-66.8107	5	15.179
Host+PAugAnom	144.172	-67.086	5	15.731
Loc	145.008	-67.504	5	16.567
Larvae: percent engorged				
Host	40.733	-17.367	3	0
Year+Host	41.562	-14.781	6	0.829
Loc+Host	46.683	-17.342	6	5.95
Year+Loc+Host	47.541	-14.77	9	6.808
Year+Loc	59.206	-22.603	7	18.473
Loc	60.725	-26.362	4	19.992
Year	62.748	-27.374	4	22.015
Nymphs: count				
Loc+Host+PAugAnom	225.475	-105.737	7	0
Year+Loc+Host	230.438	-105.219	10	4.963
Year+Host	232.323	-108.162	8	6.848
Loc+Host+PJuneAnom	237.544	-111.772	7	12.069
Loc+Host+MayAnom	239.21	-112.605	7	13.735
Loc+Host	239.245	-113.623	6	13.77
Loc+Host+PJulyAnom	240.868	-113.434	7	15.393
Year+Loc	240.988	-111.494	9	15.513
Host	249.734	-120.867	4	24.259
Year+Loc+Host+MayAnom	250.2598	-105.218	10	24.7848
Year	250.526	-119.263	6	25.051
Loc	251.49	-120.745	5	26.015
Nymphs: percent engorged				
Year+Host	954.114	-470.057	7	0
Year+Loc	971.135	-476.568	9	17.021

Model[a]	AIC	Log Likelihood	N	ΔAIC
Loc	1049.098	-519.549	5	94.984
Loc+Host	1049.303	-517.651	7	95.189
Year	1062.363	-526.182	5	108.249
Host	1091.561	-542.781	3	137.447
Year+Loc+Host	22078.72	-11029.36	10	21124.606
Adults: count				
Loc+Host+MayAnom	301.352	-141.675	9	0
Year+Loc+Host	302.723	-139.362	12	1.371
Year+Host	303.273	-142.636	9	1.921
Loc+Host+PAugAnom	306.178	-144.089	9	4.826
Year+Loc+Host+MayAnom	306.178	-139	13	4.826
Year	306.268	-147.134	6	4.916
Year+Loc	308.227	-144.114	10	6.875
Loc+Host+PJulyAnom	308.631	-145.316	9	7.279
Loc+Host	309.736	-146.868	8	8.384
Loc+Host+PJuneAnom	309.928	-145.964	9	8.576
Host	312.212	-151.106	5	10.86
Loc	321.528	-154.764	6	20.176
Adult females: percent engorged				
Year+Loc+Host	212.309	-94.154	12	0
Loc+Host	220.059	-102.029	8	7.75
Year+Host	245.068	-114.534	8	32.759
Year+Loc	250.649	-116.325	9	38.34
Loc	271.063	-130.532	5	58.754
Host	284.229	-138.114	4	71.92
Year	337.255	-163.627	5	124.946
Adults: Sex ratio				
Loc+Host+PJuneAnom	542.676	-263.338	8	0
Year+Loc+Host	543.219	-261.6095	10	0.543
Loc+Host+PJulyAnom	543.742	-263.871	8	1.066
Loc+Host	543.852	-264.926	7	1.176
Loc+Host+MayAnom	544.306	-264.153	8	1.63
Loc+Host+PAugAnom	544.752	-264.376	8	2.076
Year+ Loc+Host+JuneAnom	545.219	-261.609	11	2.543
Year+Loc	588.438	-287.219	7	45.762
Year+Host	595.172	-290.586	7	52.496
Host	599.211	-295.605	4	56.535
Loc	620.836	-306.417	4	78.16

Model[a]	AIC	Log Likelihood	N	ΔAIC
Year	698.639	-345.319	4	155.963

[a]Loc: Location (colony) of collection; Host: host that tick is associated with; Year: year collected; PJuneAnom: precipitation anomaly for June of year previous to year of collection; PJulyAnom: precipitation anomaly for July of year previous to year of collection; PAugAnom: precipitation anomaly for August of year previous to year of collection; MayAnom: precipitation anomaly for May of year of collection

Table 2.2 Summary of the average numbers of each life stage of tick collected based on year, colony, and host.

	Larvae	**Nymphs**	**Adults**
		Year	
2011	**NA**[ab]	3 (3-3) 1	3.7 (1-22) 12
2012	**37.5 (6-69) 2**[b]	3.7 (2-7) 6	2.3 (1-9) 12
2013	**16.6 (1-68) 10 *** [c]	5.9 (1-18) 13	11.4 (1-46) 14
2014	**2.2 (1-3) 4 ****	55.3 (1-491) 13	36.2 (1-164) 9
2015	**183 (183-183) 1 */****	58 (58-58) 1	21 (21-21) 1
		Colony	
Gull Island	27.6 (1-183) 12	**14.3 (1-77) 22 ***	**11.8 (1-164) 28 ***
Great Island	2 (2-2) 1	**165.3 (2-491) 3 ****	**70 (9-131) 2 ****
Cape St. Mary's	NA	**NA**	**2 (1-3) 4 */**/***/******
Little Fogo Islands	1 (1-1) 1	**8.2 (1-59) 8 */****	**16 (16-16) 1 *****
Gannet Islands	35 (2-68) 2	**2 (2-2) 1**	**6.6 (1-26) 13 ******
		Host	
Atlantic Puffin	**1 (1-1) 2 ***	**4.9 (1-59) 16 ***	**4.4 (1-26) 24 ***
Common Murre	**25.9 (2-183) 14**	**47.1 (1-491) 17 ***	**23.8 (1-164) 19 ***
Razorbill	**68 (68-68) 1 ***	**2.0 (2-2) 1**	**12 (12-12) 1**
Unknown	**NA**	**NA**	**2 (1-3) 4**

[a]Groups in bold found within models with highest support

[b]Average ticks collected (range of ticks collected) number of times ticks collected; NA, none collected

[c]*/**/***/****comparisons that are significantly different within group, Tukey $p<0.05$

Table 2.3 Summary of percent engorged individuals at each life stage of tick collected based on year, colony, and host.

	Larvae	**Nymphs**	**Female Adults**
	Year		
2011	NA	**100% (100-100%)**	**8.3% (0-19.4%)**
		1[ab]	**36**
2012	97.3% (93.3-100%)	**85.7% (71-100%)**	**14.2% (0-35.7%)**
	75	**21 *** [c]	**14 ***
2013	98.2% (95.8-100.0%)	**93.5 (87.0-98.7%)**	**41.1% (30.6-51.8%)**
	166	**77 ****	**85 ***
2014	88.9% (66.7-100%)	**50.1% (46.5-53.8%)**	**36.5% (28.3-44.7%)**
	9	**720 */****	**134**
2015	100% (100-100%)	**100% (100-100%)**	**100% (100-100%)**
	183	**58**	**17**
	Colony		
Gull Island	98.6% (97.2-99.7%)	73.8% (68.8-73.8%)	**74.0% (65.3-81.7%)**
	361	314	**104 */****
Great Island	100% (100-100%)	55.7% (51.2-60.0%)	**27.3% (17.8-36.9%)**
	2	494	**84 ****/*****
Cape St. Mary's	NA	NA	**0% (0-0%)**
			5
Little Fogo Islands	0% (0-0%)	100% (100-100%)	**0% (0-0%)**
	1	2	**16**
Gannet Islands	100% (100-100%)	0% (0-0%)	**7.7% (2.6-14.3%)**
	69	66	**77 */*****
	Host		
Atlantic Puffin	**0% (0-0%)**	**5.3% (1.3-10.6%)**	**3.8% (0.9-7.7%)**
	3	**75 ***	**104 */****
Common Murre	**99% (98-100%)**	**63% (59.6-66.2%)**	**57.5% (50-64.9%)**
	362	**800 ***	**174 ***
Razorbill	**100% (100-100%)**	**100% (100-100%)**	**80% (40-100%)**
	68	**2**	**5 ****
Unknown	**NA**	**NA**	**0% (0-0%)**
			5

[a]Groups in bold found within models with highest support

[b]Average ticks collected (range of ticks collected) number of times ticks collected:NA, none collected

[c]*/**/***/****comparisons that are significantly different within group, Tukey $p<0.05$

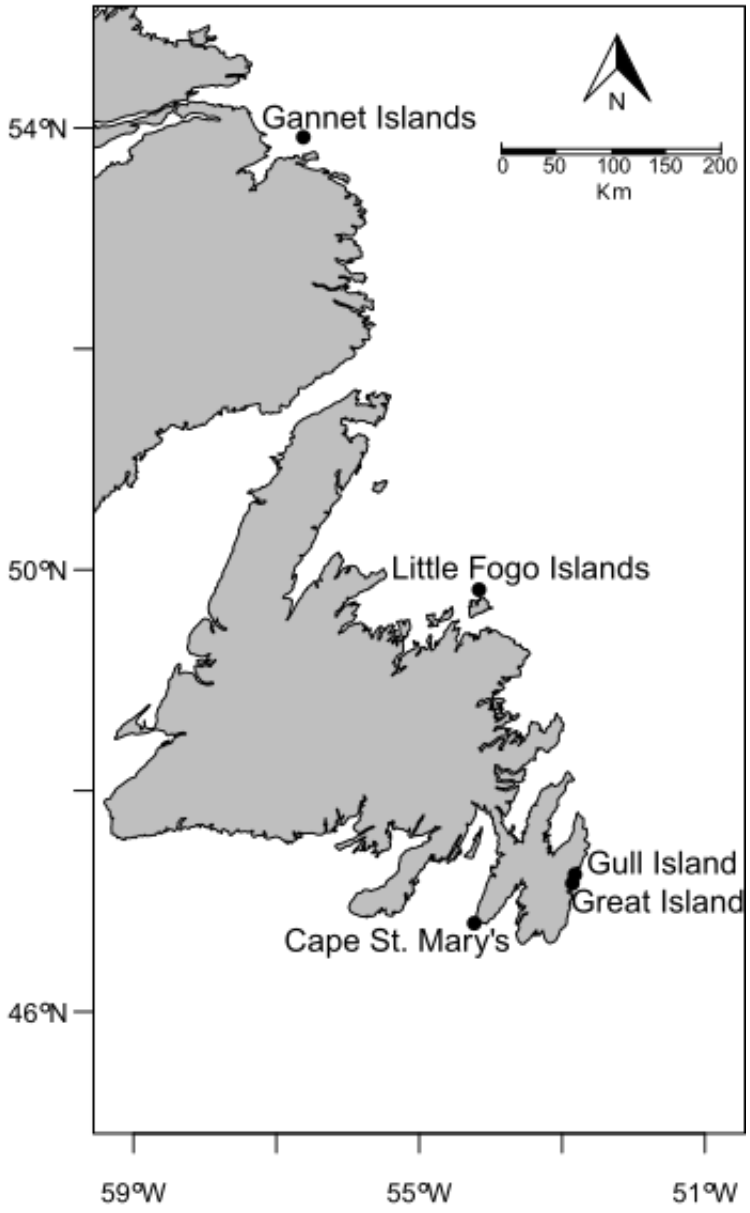

Figure 2.1 Seabird ticks (*Ixodes uriae*) were collected from five seabird colonies in Newfoundland and Labrador during the breeding season (June-August) in 2011-2015.

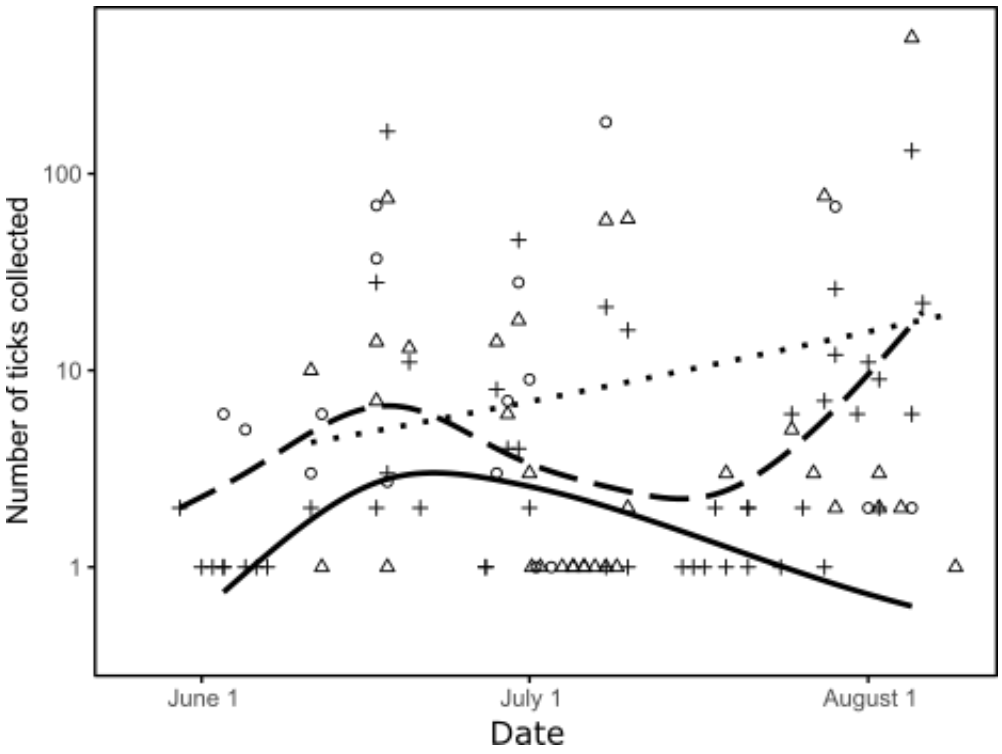

Figure 2.2 Seasonal variation in the number of ticks collected. Life stages are differentiated by symbols: nymphs, dots; larvae, triangles; and adults, crosses. General additive model for each life stage is fitted to the data: larvae, solid line; nymphs, dotted line; and adult females, dashed line.

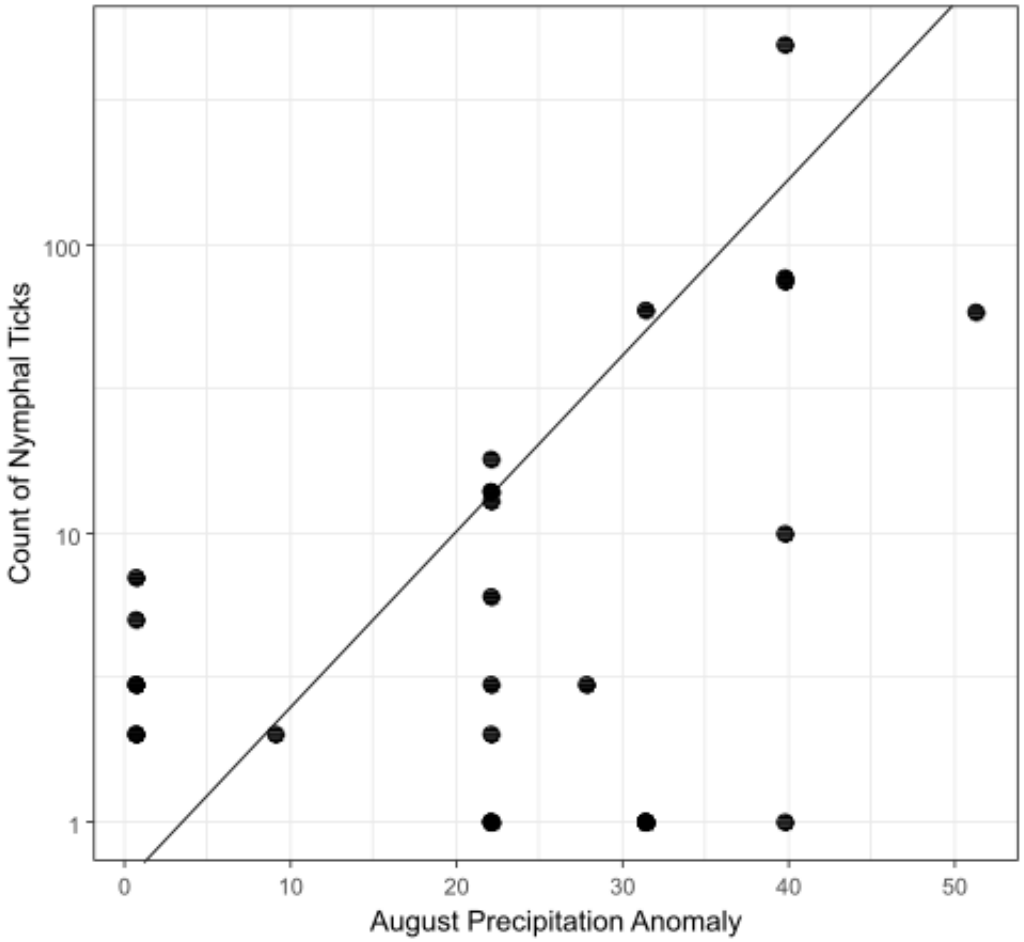

Figure 2.3 Variation in nymphs collected with August precipitation anomaly (variation from 30-year monthly precipitation average). The count of nymphal ticks is represented on a log scale to allow for better representation of data points. Line of fit based on GLM parameters fitted through data.

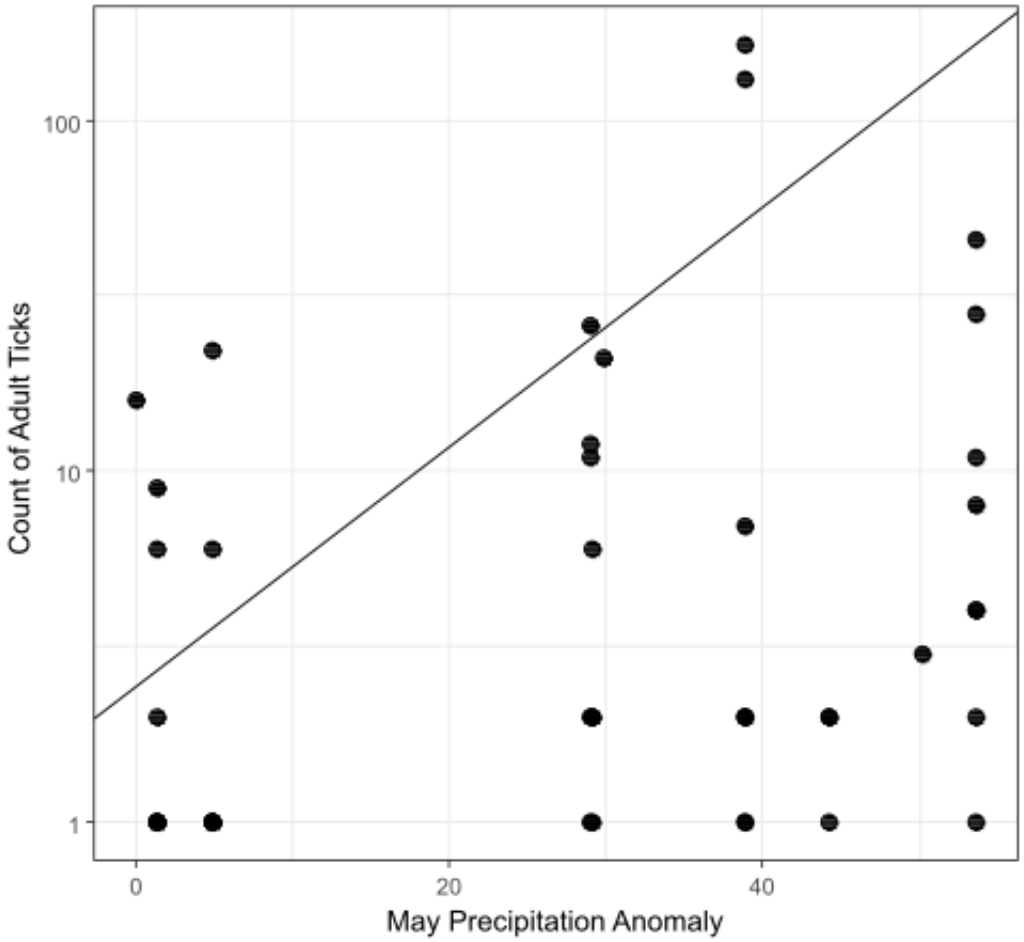

Figure 2.4 Variation in adult ticks collected with May precipitation anomaly (variation from 30-year monthly precipitation average). The count of adult ticks is represented on a log scale to allow for better representation of data points. Line of fit based on GLM parameters fitted through data.

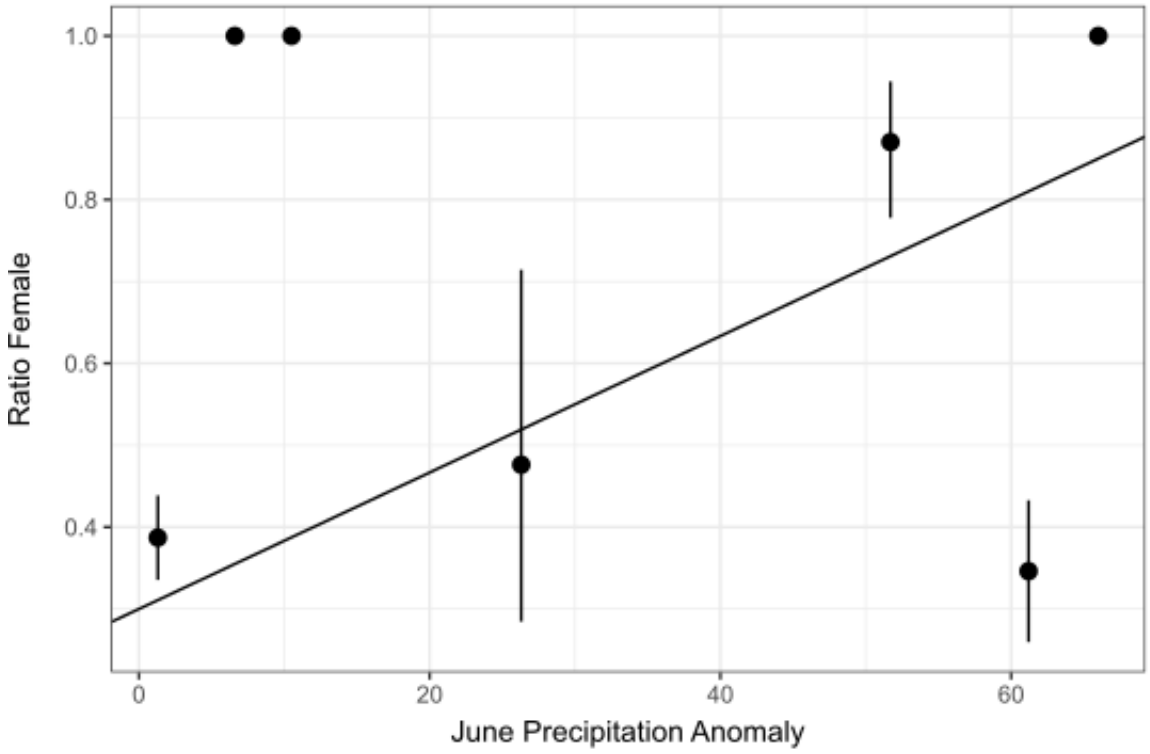

Figure 2.5 Variation in the proportion of female ticks collected with June precipitation anomaly (variation from 30-year monthly precipitation average). All points are depicted with 95% confidence intervals. Line of fit based on GLM parameters fitted through data.

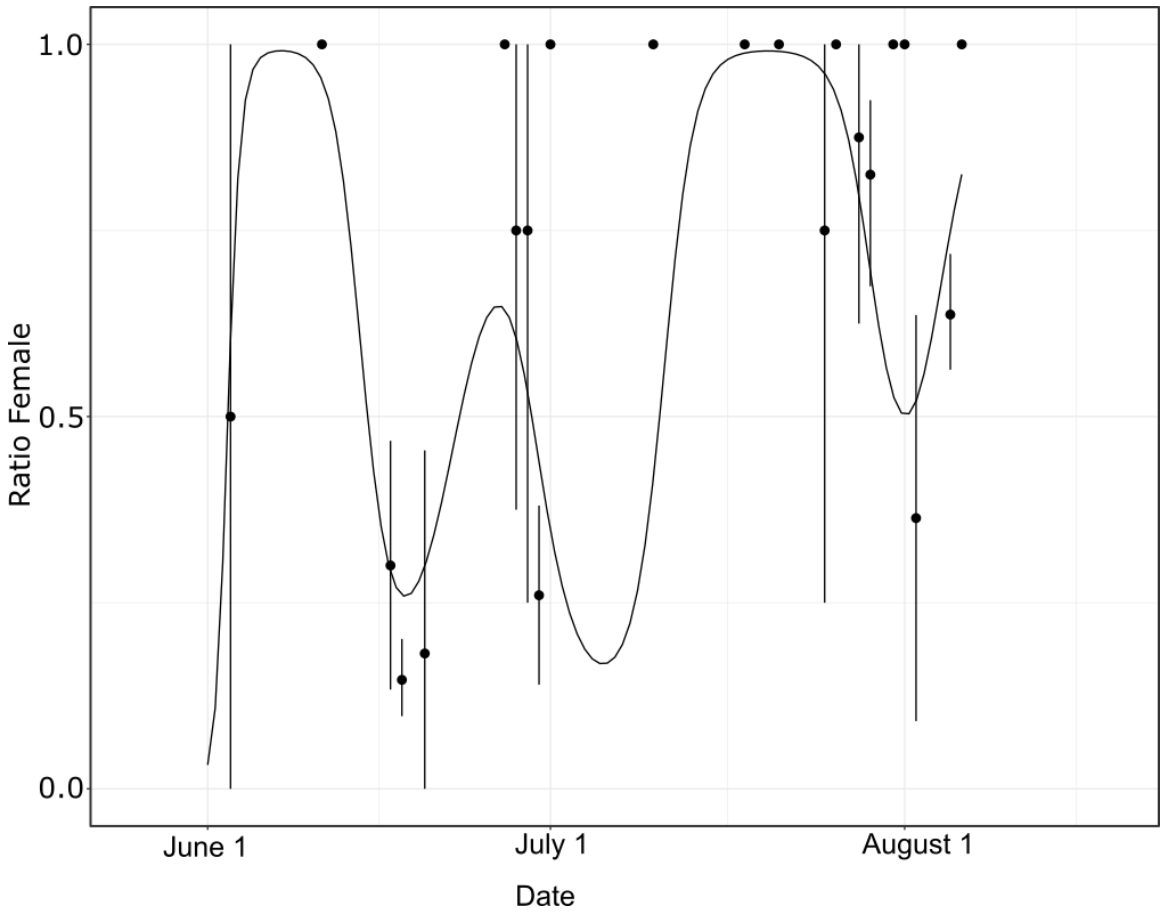

Figure 2.6 Seasonal changes in the proportion of female adult ticks. Lines above and below points represent 95% confidence intervals. General additive model is fitted through data in a solid black line.

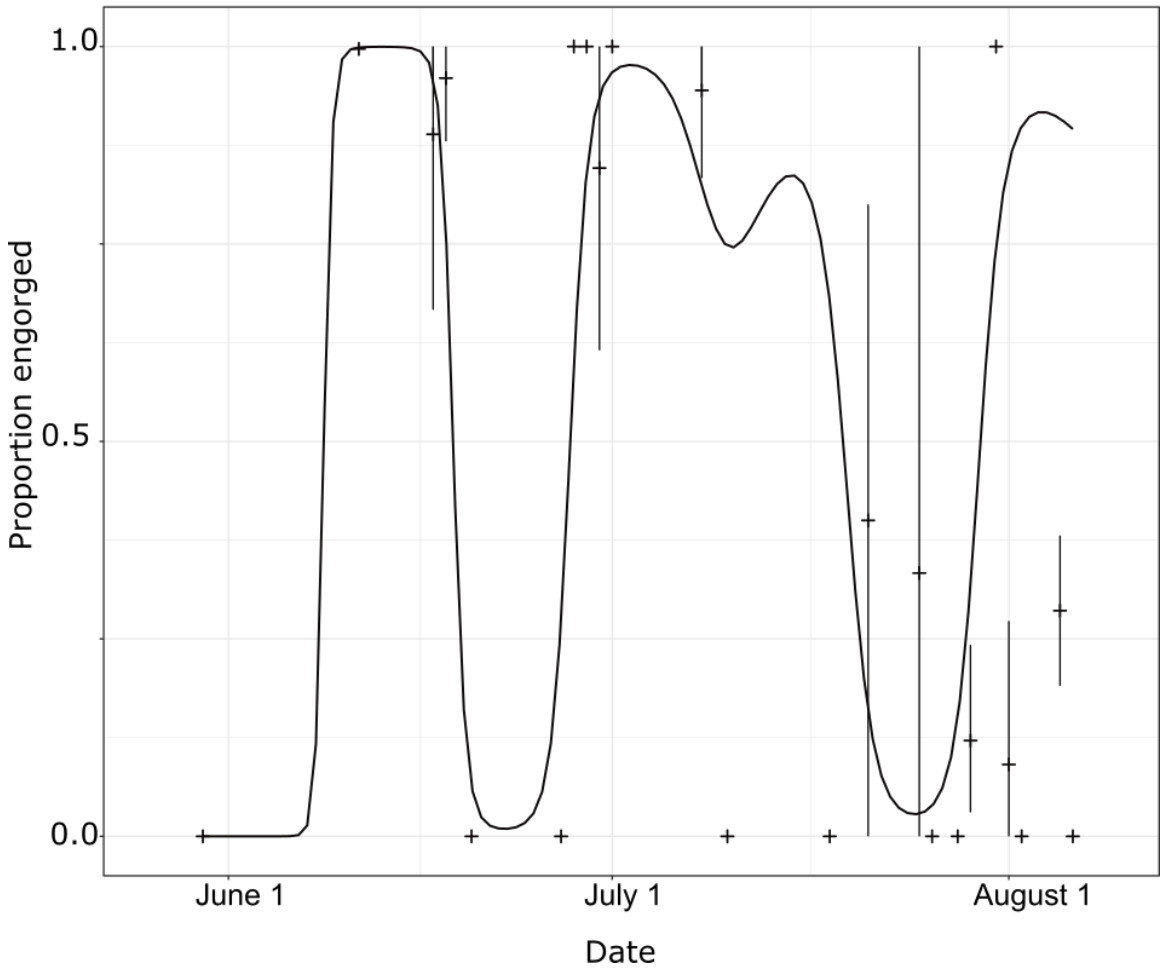

Figure 2.7 Proportion of engorged adult females tick across the season. Lines above and below points represent 95% confidence intervals. General additive model is fitted through data in a dashed line.

CHAPTER 3: *IXODES URIAE*-ASSOCIATED BACTERIA WITHIN SEABIRD COLONIES OF NEWFOUNDLAND AND LABRADOR

3.1. Abstract

Borrelia garinii has been documented in the seabird tick, *Ixodes uriae,* in seabird colonies around the world. In eastern North America, it has only been documented in two seabird colonies in Newfoundland and Labrador, Canada. The seroprevalence of *Borrelia* within seabirds, hosts of the seabird tick, and by extension an important host to *Borrelia,* is not known in Newfoundland and Labrador. These ticks are also known to carry viruses, but characterization of their total bacterial microbiome and investigation if they carry other pathogenic bacteria has yet to be performed. I collected *I. uriae* from five seabird colonies in Newfoundland and Labrador over four summers and screened them for *B. garinii*. *Borrelia garinii* was found in all five seabird colonies, demonstrating that the bacterium is widespread in Newfoundland and Labrador. The prevalence of the bacterium in ticks varied significantly across years. I also collected blood samples from seabirds from two colonies for serological analysis of anti-*Borrelia* antibodies to determine exposure rates of seabirds to the bacterium. Seroprevalence for the bacterium was high in all seabird species sampled in both colonies. I characterized the complete bacterial microbiome from a subset of the ticks from two colonies, including ticks infected and not infected by *B. garinii*, by high-throughput sequencing of the 16S rDNA hyper-variable regions V6/V7/V8. The microbiome analysis showed that *B. garinii* can constitute a large proportion of the bacterial community in positive individuals. The microbiome of these ticks appears to be site specific, with correlation between the presence of *B. garinii* and

other species that are present. This work indicates that *I. uriae* is an important vector of *B. garinii* and other potentially zoonotic bacteria within seabird colonies, and thus provides an enlarged understanding of the global distribution of *B. garinii*.

3.2. Introduction

Lyme disease is caused by infection with bacteria within the species complex *Borrelia burgdorferi* sensu lato (s.l.), primarily *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, and *B. afzelii* (75, 76). The geographical ranges of these bacteria vary, with *B. burgdorferi* s.s. found in North America and Europe, and both *B. garinii* and *B. afzelii* widely distributed in Eurasia. Transmission of *B. burgdorferi* (s.l.) is primarily by ticks within the *Ixodes ricinus* complex, which feed on mammalian and avian reservoir hosts in the woodland habitats of the ticks (77-79). Migratory birds are believed to play an important role in dispersal of *B. burgdorferi* s.s. and *B. garinii* due to their ability to move ticks long distances, and because migration can produce recrudescence of infections of the birds (80-82). Small European mammals, rather than birds, are the reservoir hosts for *B. afzelii* (76, 83).

In addition to the typical transmission cycles in woodland habitats, *B. garinii* is transmitted by *Ixodes. uriae*, a tick not within the *I. ricinus* complex, and exclusively associated with seabirds (30, 31, 45). *Borrelia burgdorferi* s.l. was initially detected in *I. uriae* feeding on Razorbills (*Alca torda*) on islands off the Swedish coast (31). It was subsequently identified as *B. garinii* and is now known from seabird colonies in both the Northern and Southern Hemispheres (30). Seabirds are competent reservoirs and there is a risk to humans of being infected by the bacterium via *I. uriae* when working in or visiting seabird colonies (42).

Ixodes uriae has been recorded on more than 50 different colonial seabird species in both hemispheres (26). Despite its broad geographic and host range, it appears that local populations are structured by host species (26, 84-86).

The prevalence of *B. garinii* infection in *I. uriae* varies widely among colonies and years. For example, the prevalence of *B. garinii* has been documented to be as low as 8% (30) and as high as 63% (44) in different colonies. There is also variation between years in single colonies (48). Prevalence may also vary spatially within colonies, even single-species colonies (47). The reasons for these variations remain largely unexplored, but the likeliest are spatio-temporal variations in the densities of recently infected seabirds, which likely transmit *B. garinii* to ticks most efficiently (87).

Newfoundland and Labrador has several globally significant large seabird breeding colonies, and *B. garinii* has previously been documented in two of them. One colony is on the Gannet Islands, located 30 km off the coast of Labrador near the community of Cartwright. Ticks were first collected from this location in 1995 but no *B. garinii*-positive samples were found (30). In 2006, a larger collection of ticks from numerous habitats representing multiple seabird species was analysed, and 17% were positive for the bacterium when screened using immunofluorescence microscopy (48). Another large colony is in the Witless Bay Ecological Reserve in southeastern Newfoundland, approximately 40 km south of the city of St. John's. In 2005, 41% of adult and 3.4% of nymphal ticks collected at this site were positive for *B. garinii* (43), but only 7.2% of all ticks collected in 2006 were positive (48).

The main method of detecting evidence of *Borrelia* infection in wild birds is detection of anti-*Borrelia* antibodies (42, 47, 88-90). Over large geographic scales, the exposure to *Borrelia* can vary both spatially and among species (89). However, in infected individuals high levels of antibodies persist over years (91), likely due to persistent infection (87).

In addition to *B. garinii*, ticks are also known to carry a variety of other pathogenic bacteria. The introduction of high-throughput sequencing has allowed for the rapid characterization of entire microbiomes of many organisms (92, 93). The microbiomes of several tick species have been characterized in recent years (94), with detection of pathogenic bacteria being a major driver of these efforts (95). In addition, there has been some work to examine the relationship between bacterial community structure and the presence of pathogenic species (96). It has been shown that the overall microbiomes of arthropod vectors can influence the vectors' competence or ability to transmit a pathogen (97). On a broader scale, there are questions about how these bacterial communities are controlled and determined. It is also important to understand the relationship between the bacterial community structures and other aspects related to the ticks, such as sampling locations and the ticks' hosts (98). The total microbiome of *I. uriae* has not been investigated, but previous studies have found other potential human pathogens, including *Rickettsiella* spp. (99) and *Coxiella burnetii* (100).

In this study, I documented the prevalence of *B. garinii* at several seabird colonies in the province of Newfoundland and Labrador in four successive years. These colonies

are diverse with respect to their seabird populations and spanned a large geographic range of 300 to 500 kilometers. In addition, I characterized the microbiomes of ticks from two seabird colonies, on Gull and Great Island both within the Witless Bay Ecological Area. I identified putative pathogenic bacteria present in the ticks and investigated the relationship of the bacterial community to the *B. garinii* infection status and location of tick collection. In addition, the seroprevalence of *Borrelia* antibodies in the associated seabird populations was documented.

3.3. Methods

3.3.1. Ethics

Birds were captured and banded under Environment Canada banding permit 10559. This work was carried out under the guidelines specified by the Canadian Council on Animal Care with approved protocols 11-01-AL, 12-01-AL, 13-01-AL, and 14-01-AL from the Memorial University Institutional Animal Care Committee. Lab work was approved under biosafety permit S-103 from the Memorial University Biosafety Committee. Access to the Witless Bay, Gannet Islands, and Cape St. Mary's Ecological Reserves was through permits from the Parks and Natural Areas Division of the Newfoundland and Labrador Department of Environment and Conservation.

3.3.2. Sample collection

Ticks were collected from five seabird colonies in Newfoundland and Labrador (Figure 3.1): 1) Cape St. Mary's, located on the southwest corner of the Avalon Peninsula and containing breeding Northern Gannet (*Morus bassanus*), Black-legged Kittiwake (*Rissa tridactyla*), and Common Murre (*Uria aalge*); 2) the Gannet Islands, an archipelago of six islands that features both Common and Thick-billed Murre (*U.*

lomvia), Atlantic Puffin (*Fratercula arctica*), Black-legged Kittiwake, and the highest density of breeding Razorbill in North America; 3) Little Fogo Islands, an archipelago with breeding Atlantic Puffin and Leach's Storm-petrel (*Oceanodroma leucorhoa*); 4) Gull Island, a colony with breeding Herring Gull (*Larus smithsonianus*), Great Black-backed Gull (*L. marinus*), Atlantic Puffin, Common Murre, and Razorbill; and 5) Great Island, located 8 km south of Gull Island and hosting a similar assemblage of species.

Ticks were primarily collected from the environment around nesting bird habitats. These ticks were found under rocks near breeding Common and Thick-billed Murres, and Razorbills, and it was assumed that the hosts of the ticks collected were the species of the closest nesting species. Some ticks were also collected directly off adult and pre-fledging Atlantic Puffins, Common Murres, Thick-billed Murres, Razorbills, Black-legged Kittiwakes, and Herring Gulls. Ticks were also collected when found crawling on the clothing of researchers, which was most frequent after working in Atlantic Puffin breeding areas.

3.3.3. Borrelia *prevalence within ticks*

All ticks were visually examined to confirm that they were *I. uriae*. Stage and engorgement were noted. All ticks collected in 2013 and 2014 were surface sterilized by washing with a 10% hydrogen peroxide solution, then rinsed with sterile water three times. All adult and engorged nymphs were bifurcated and half of the tick kept in storage at -80°C. DNA was extracted from the other half using the DNeasy extraction kit (Qiagen). Negative extraction controls were included in each batch of 7-23 DNA extraction reactions. Extracted DNA was the template for qPCR targeting the *Borrelia*

23S rDNA as previously described (101). Extractions controls were screened for tick *Borrelia* to test for contamination during the process. Each qPCR assay contained three negative controls and three *B. garinii* positive controls at standard dilutions of (10^2, 10^4, and 10^6). All samples were screened twice to confirm their positive or negative status. Samples were considered positive if they had a CT value of less than 40 on two or more assays. In cases where conflicting results were obtained qPCR was repeated a third time.

3.3.4. Serology of seabirds

Blood samples of up to 2 mL were collected from breeding adult seabirds at two colonies: Gull Island and the Gannet Islands, during either the incubation or nestling period. Common Murres, Thick-billed Murres, and Razorbills were captured on nesting ledges using noose poles. Atlantic Puffins were captured from within nesting burrows. Herring Gulls were captured using noose carpets at feeding sites or using cannon nets near nests. Black-legged Kittiwakes were captured from nests on cliffs using noose poles. Blood samples were collected from the brachial vein of birds. These samples were centrifuged to separate red blood cells from serum, and the serum was then removed divided into alloquots of 200-500µl and kept at -20°C until analysis.

Sera were analyzed using Borrelia + VIsE IgG ELISA kit (IBL International), which was initially designed for screening of human serum for *B. garinii*, *B. afzelii*, and *B. burgdorferi* s.s., but adapted for screening seabird serum by switching the anti-human conjugate with an anti-chicken conjugate (A9046 Anti-Chicken IgY-Peroxidase, Sigma Aldrich) diluted at 1:750 with the supplied diluent buffer (56). Sixteen of the 150 samples were replicated on separate plates (Appendix 2, Supplementary Figure 1). To determine

cut-off values, I used the method described in Garnier et al (102) to determine the best fit to the data between one normal distribution (all samples considered negative or positive) or a mixture of two normal distributions (mixture of negative and positive samples; Appendix 2, Supplementary Figures 2 and 3). Due to the small sample size in some groups, the normal distribution representing negative samples had a high variance, and the cut-offs were fixed relative to the positive sample distribution. Different cut-off values were used for the two main groups of species, the Alcidae and Laridae (Appendix 2, Supplementary Table 1, Supplementary Figures 4 and 5).

3.3.5. Statistical analysis

All statistics on prevalence were performed using R 3.1.2 (R core team). Prevalence of *B. garinii* within *I. uriae* according to engorgement/life stage, likely host species, seabird colony, year, and month was compared using a generalized linear model (GLM) with a binomial distribution. Groups with less than ten individuals were excluded from the GLM for analysis (e.g., only six ticks were collected from Cape St. Mary's so these were excluded from the location analysis). Seroprevalence was compared across species and seabird colonies using a GLM with a binomial distribution. All serum samples were collected in 2013, and over a short period of time, so no temporal variables were considered.

3.3.6. Microbiome analyses

A subset of ticks (n = 19) collected in 2013 and 2014 from Great and Gull Islands was used to identify their associated bacteria (microbiome). These samples were selected due to high DNA concentrations and higher success for down scream PCR analysis. The

same DNA extracts used for screening for *Borrelia* were used. DNA extractions from soil samples, collected from around tick collection areas on each island, were also analyzed to provide reference environmental communities. DNA was extracted from soil using the Puregene Gram Positive Bacteria DNA extraction kit (Qiagen). Two hundred mg of soil were placed in 1 mL of sterile viral transport medium and the mixture vortexed for one minute. The medium was passed through a 0.45-µm filter to remove particulates and the resulting filtrate centrifuged for 15 minutes at 6000 x *g*. The resulting pellet was treated as bacterial cells and DNA extraction completed according to the manufacturer's recommendations. DNA concentrations were determined using a NanoDrop 1000 (Thermo Scientific) and samples submitted to the Integrated Microbiome Resource at the Center for Comparative Genomics and Evolutionary Bioinformatics (Dalhousie University, Halifax, Canada) for Illumina MiSeq 2x300-bp paired-end sequencing of the V6/V7/V8 region of the 16S rRNA gene using using universal bacterial primers B969F and B1406R (103, 104).

Sequence analysis was performed using the in-house developed SPONS2 (Streamlined Processor Of Next-gen Sequences, version 2) pipeline (105) with modifications (106). The pipeline performs a quality control, removes primers and adapters, checks read quality and length, merges paired reads, and creates operational taxonomic units (OTUs) with taxonomy assigned using the SILVA SSU reference database and taxonomy (release 128; 107, 108). Operational taxonomic units (OTUs) with taxonomic assignments other than bacteria were removed from the dataset. Data were resolved to level of genus for all analyses.

The diversity in each sample was analysed using count data, taxonomic classifications and dataset phylogeny using the 'phyloseq' package in R (109). The dataset was filtered to remove potentially spurious OTUs (those with less than 10 reads across the entire dataset). Rarefaction curves were produced for richness indices "Observed" (number of OTUs) and "Simpson" (110), measuring 10 replicates at the sequence depths of 1, 10, 1000, 2000, 5000, and 10000. The richness of each sample was analysed using the number of observed OTUs, Simpson's diversity index (D = sum p_i^2 where p_i is the proportional abundance of species) to estimate the total species richness (110), and the inverse of the Simpson's index (1/D) to estimate evenness. Unpaired t-tests were used to compare samples based on colony, *Borrelia* status, and tick sex. Ordination analysis was performed on reduced (retained OTUs with > 10 reads in at least two samples) and log-transformed datasets for each sample, using the Jensen-Shannon Divergence (JSD) as a distance matrix ordinated using multidimensional scaling (MDS) in 'phyloseq'. A hierarchical cluster analysis of the JSD data with ward.D2 as the agglomerate method was performed (106). The differential abundance of taxa was tested using the R package DESeq2 (111) with the parametric Wald significance test. In this analysis, I looked at the differential abundance of taxa at the genus level of agglomerated datasets, where the abundance variance for OTUs for samples from Gull and Great Island, regarding OTUs with an adjusted p-value < 0.05 and a minimum log2-fold increase, were considered as significantly differentially abundant. Bacterial assemblages from soil samples were used to identify OTUs only found in ticks and over-represented in

tick samples. To examine the relationship between reads classified as *Borrelia* and CT values from qPCR screening, a linear model was performed using R.

3.4. Results

3.4.1. Prevalence

A total of 865 *I. uriae* from the five seabird colonies were tested for the presence of *B. garinii*. Sixty-five ticks were positive, for an overall prevalence of 7.5±1.8% (±95% confidence intervals). Only 7 of these ticks were collected off birds with each coming from a separate individual bird, thus representing individual samples. The repeatability of the CT values for samples that tested positive was 3.6% the variance among all positive CT values. Positive ticks were found on all colonies surveyed (Table 3.1). This confirmed the presence of *B. garinii* in three new colonies: Cape St. Mary's, Little Fogo Islands and Great Island. The differences in prevalence among colonies was not significant ($p = 0.64$; Table 3.1). Prevalence differed significantly among tick stages ($\chi^2 = 27.4$, $p < 0.001$; Figure 3.2A). The highest prevalence was found in engorged female adults (16.5±7.0%) and the lowest in engorged larvae (2.4±2.1%). Prevalence of *B. garinii* did not vary according to hosts but the difference was only marginally non-significant ($\chi^2 = 9.92$ $p = 0.08$; Figure 3.2B). Prevalence varied significantly among years ($\chi^2 = 8.7$, $p = 0.003$; Figure 3.2C) but not month (Figure 3.2D).

3.4.2. Serology

Serological results were highly correlated between the replicate plates ($r^2 = 0.966$). Seropositive individuals were found for all seabird species, at all colonies, with all groups having > 50% prevalence (Table 3.2). Seroprevalence varied significantly among species ($\chi^2 = 18.5$, $p = 0.002$, Table 3.2) but not colonies. The highest

seroprevalence was recorded in Herring Gulls (100 ± 0%) and lowest in Razorbills (60±40%) though no between species comparisons were significant (Tukey, $p > 0.05$).

3.4.3. Microbiome analyses

The microbiomes of 19 ticks were analyzed (Table 3.3, Figure 3.3). In total, 513 unique OTUs (at the genus level) were identified in the ticks. The number of OTUs per tick ranged from 73 to 211 and only one genus was found in all 19 tick samples (*Paracoccus*). There were 109 OTUs that were found in both ticks and soil samples (Appendix 2, Supplementary Table 2). There were 67 OTUs found in more than 50% of tick samples but not in soil (Appendix 2, Supplementary Table 3). These OTUs represented on average 58% of the reads in each tick sample (range: 6-89%). Simpson's Diversity Index also varied widely among ticks (0.228 to 0.973), and Simpson's Evenness Index ranged from 37.3 to 1.30 (Table 3.3, Figure 3.4). OTUs from the phylum Proteobacteria dominated the microbiomes (50.6±10.2%, mean relative abundance ± 95% CI) with smaller contributions from the phyla Bacteroidetes (16.7±7.7%), Actinobacteria (13.2±7.8%), Spirochaetes (10.8±11.8%), and Firmicutes (4.9±3.0%). The remaining 1.7% of the OTUs belonged to a variety of other phyla, each contributing < 1% of the total relative sequence abundance (Figure 3.4). The dominant OTUs were highly variable among individual ticks, with 21 genera found at a relative abundance > 1% (Figure 3.3).

There were no significant differences for richness, diversity, or community structure between *Borrelia* qPCR-positive and -negative ticks (status originating from qPCR analysis described above) or microbiome-positive and -negative ticks (status originating from presence or absence of *Borrelia* reads in 16S rDNA analysis) (Figure

3.4, Table 3.3). There were no significant differences in diversity measures between the two sites, but the measures of richness did differ significantly ($\chi 2 = 7.8$, $p = 0.002$, Figure 3.4), with Great Island having higher inverse Simpson scores than Gull Island, and community structure did differ when looking at ordination MDS on JSD and distances plotted on a hierarchical clustering graph (Figure 3.5). Thirty-four genera across six phyla were over-represented or under-represented based on site (Figure 3.6). In samples from Great Island, genera from the phyla Actinobacteria, Chlamydiae, Planctomycetes, and Proteobacteria were over-represented, whereas genera from Bacteroidetes and Firmicutes were over-represented on Gull Island.

Nine of the 19 ticks for which microbiome data were obtained were positive for *B. garinii* based on qPCR screening and these tick samples all contained sequences that were classified as *Borrelia* in the microbiome data. Comparison of the CT values from qPCR-positives to the percentage of reads mapping to *Borrelia* in the microbiome data showed a strong correlation ($r^2 = -0.78$, $p < 0.001$), with low CT values being associated with high *Borrelia* sequence counts (Figure 3.7). There was one tick that had an average CT value of 35 but did not have any *Borrelia* reads.

Of note, evidence of infections by *Coxiella* and *Ehrlichia*, which can be tick-borne human pathogens, was detected in four and nine ticks, respectively. Co-infections by multiple potential pathogens were identified in 8 of 19 ticks. Two ticks contained both *Coxiella* and *Ehrichia* and six contained *Borrelia* and *Ehrichia*. There were no co-infections of *Borrelia* and *Coxiella* found.

3.5. Discussion

In this study, I showed that *B. garinii* transmission cycles within seabird colonies in Newfoundland and Labrador are efficiently maintained between *I. uriae* ticks and nesting seabirds. I documented *B. garinii* for the first time at three seabird colonies within the province (Little Fogo Islands, Great Island, and Cape St. Mary's), representing an expansion of the known range for this species of bacteria in the western North Atlantic Ocean. Within these colonies, all seabird species sampled showed evidence of current or past *Borrelia* infection, suggesting that the bacterium has a diverse range of seabird host species that contribute to its transmission cycle.

3.5.1. Known range expansion of B. garinii

Combined with results of previous studies (43, 48), my results suggest that *B. garinii* has been present in some seabird colonies of Newfoundland and Labrador for at least decades. The first study of *B. garinii* in the region was conducted in the 1990s when ticks were collected on the Gannet Islands, Labrador (30). No ticks were *B. garinii*-positive, although only four were tested. In 2006 the bacterium was identified within that colony, detected in 9.8% of 132 ticks tested (3% in nymphs and 18% in adults) (48), rates that are comparable to those in my study.

Similarly, for Gull Island, Newfoundland, where initial screening found no evidence of the bacterium, with 91 ticks screened in 2001-2002 (112). Subsequent research there in 2005 and 2006 confirmed the presence of the bacterium (43) at prevalences of 7.4-20.4% (48). Great Island, located south of Gull Island and ticks from this location had not previously been screened, and my data therefore represent the first

confirmed circulation of *B. garinii* in ticks on this island. The presence of *B. garinii* within the seabird colonies at Gull and Great Islands in the Witless Bay Ecological Reserve suggests that the bacterium is likely also present in other colonies within the Reserve, and in nearby colonies.

Attempts to survey Cape St. Mary's for *I. uriae* and *B. garinii* have been made in the past. *Ixodes uriae* are commonly found associated with breeding Common Murres (e.g., 44, 113, 114) and there are records for Northern Gannets (115), but *B. garinii* could not be confirmed due to samples being compromised prior to screening (48). Therefore, my results represent the first confirmation of *B. garinii* within this mainland seabird colony. Ticks are difficult to collect from this location due to steep cliffs, so my sample numbers are low. The site is notable in that it is the furthest south that *B. garinii* has been found in North America, as colonies to the south, in the Gulf of Maine have so far been negative (43). With my recent findings, it may warrant a renewed effort to screen *I. uriae* in seabird colonies further south, which have similar breeding seabird species such as Atlantic Puffins and Razorbills, both of which are known to carry *I. uriae* infected by *B. garinii* from this thesis and previous work (45).

The Little Fogo Islands are located near the northeastern coast of Newfoundland and are not geographically close to either Gull Island or the Gannet Islands, which have previous records of *B. garinii*. These islands primarily host breeding Atlantic Puffin and Leach's Storm-petrels, with the outer rockier island also hosting Razorbills, Common Murres, and Black Guillemots (*Cepphus grylle*). The ticks collected in this study all

originated from Atlantic Puffin breeding slopes. The presence of *B. garinii* here demonstrates that this bacterium is likely found throughout the majority of seabird colonies in the province, from Labrador in the north through northeastern Newfoundland to the southeast and the southern Avalon region.

3.5.2. Transmission cycles

The prevalence of *B. garinii* increases over *I. uriae* life stages, and is consistent with: 1) little or no transovarial transmission of *B. garinii* (116-118); 2) acquisition of *B. garinii* from hosts at each feeding stage; and 3) transstadial maintenance of infections acquired by immature ticks through one or more moults (119), resulting in increasing prevalence from nymphs to adults. The proportion of engorged larvae that was infected were similar to the proportion of questing nymphs that were positive, and the proportion of engorged nymphs that was infected was comparable to the proportion of questing adults that were positive (Figure 3.2). This suggests that *B. garinii* is efficiently transmitted transstadially in *I. uriae*.

There was variation among likely seabird hosts in the prevalence of infection in the ticks. The variation may also be confounded by the relationship of prevalence to tick age, but due to uneven sample numbers among species and variation in tick-age composition, this pattern was not tested. It should be noted that I collected 30 ticks from Thick-billed Murres and found no *B. garinii*, while > 90% of birds of this species were seropositive. Those ticks came from a single geographic location and were predominantly engorged nymphs. It is possible that all of those ticks were parasitizing a small number of birds, none of which carried *Borrelia*, and engorged adults are the most likely to carry the

bacterium. The seroprevalence data represent a much broader sampling of individual birds, but also inform us on exposure over a wider time period. Overall, the infection of ticks associated with Thick-billed Murres in Newfoundland and Labrador warrants further investigation as *B. garinii* has been found in ticks collected off this species in Sweden at a relatively high prevalence of 13.5% compared to 7.5% I documented (31).

In all seabird species surveyed, at all locations, I observed high seroprevalence, indicating that a high proportion of these birds are exposed to infection with *B. garinii* or *B. bavariensis*, which is also present on some of these islands (120). This is consistent with the relatively high prevalence in the ticks and supports the conclusion that the seabird-*I. uriae* transmission cycles of *B. garinii* on the sites studied are very efficient. Seroprevalence differed between species, although what this means in terms of inter-species differences in infection status, infectivity for ticks, development of acquired immunity, elimination of infections, as well as inter-species differences in test performance, remains to be studied. Herring Gulls, which had never been surveyed previously, had a 100% seroprevalence, although I note there was a small sample size for this species. This may be an important observation, however, as Herring Gulls commonly nest in habitats near Atlantic Puffin and other burrow-nesting species. High seroprevalence values suggest that this species is frequently bitten by ticks, so they may play an important role in dispersing ticks within the colonies and bacteria across large distances (18).

3.5.3. Tick microbiomes

The most commonly identified bacteria within *I. uriae* belonged to the phylum Proteobacteria (e.g. in the genera *Psychrobacter* and *Providencia*) with the remaining representing the phyla Bacteroidetes (e.g. *Flavobacterium* and *Chryseobacterium*), Actinobacteria (e.g. *Mycobacterium*), Spirochaetes (e.g. *Borrelia*) and Firmicutes (e.g. *Brevibacillus*). There was no overall significant difference between the microbiomes of ticks with *B. garinii* compared to those without, and those with *B. garinii* did test positive in my real-time PCR screening. There was a strong relationship between the relative abundance of *Borrelia* reads in microbiome analysis and CT values in the qPCR assay, further validating my original screening protocol.

Proteobacteria represent one of the largest phyla of bacteria in terms of species richness. In past microbiome analyses of ticks (*Amblyomma americanum* and *I. affinis*; 98,121) they often make up a large proportion of the microbiome and appear to be the predominant phylum across *Ixodes* species (94). The phylum is diverse, and ticks have been found to contain everything from potentially pathogenic representatives (e.g. *Ehrlichia* and *Coxiella*; 122) to those common in the environment (e.g. *Psychrobacter*; 123) and those observed in association with insects, such as *Providencia* that has been found associated with fruit flies (124).

The phylum Bacteroidetes contains aerobic and anaerobic bacteria that are common in the environment and also associated with the gut and skin of animals. The most dominant genera from this phylum were *Flavobacterium*, which is commonly found within the soil, and *Chryseobacterium*, also common in the environment but which has

also been associated with various microbiomes from mammals and reptiles (125, 126). A study of the microbiome of *Ixodes persulcatus* also found *Chryseobacterium* within the microbiome of both fed and unfed ticks and *Flavobacterium* was found within the same unfed ticks (127).

Actinobacteria and Firmicutes are both common phyla detected within tick microbiomes (94) and were found within the *I. uriae* microbiome. Actinobacteria are primarily soil bacteria but there are also some pathogenic members, such as *Mycobacterium* that was detected in my ticks. Firmicutes are found in a variety of environments including soil (e.g. *Brevibacillus*) but some genera that I detected also contain pathogens (e.g. *Bacillus*, *Clostridium*, *Staphylococcus* and *Streptococcus*).

The only genus observed in my analysis from the phylum Spirochaetes was *Borrelia*. The marker gene used for the microbiome analysis is not sensitive enough to identify species within this genus, but it is likely that all were *B. garinii* as this is the dominant *Borrelia* species in *I. uriae*, but I cannot conclusively determine species. The proportion of *Borrelia* sequences varied greatly, from more than 80% to less than 1% relative abundance and was strongly correlated with the qPCR screening results. This demonstrates that the relative abundance can vary greatly and this may play a role in transmission of the pathogen to vertebrate hosts when feeding. The qPCR screening protocol can detect *Borrelia* even when the relative abundance is extremely low, with the lowest abundance detected in our analysis being 0.007%. It has been suggested that tick microbial communities are affected by the presence of various pathogens, including

Borrelia (96), but the variation in communities among ticks was not related to *Borrelia* status in this study. In addition, other potentially pathogenic bacteria were widespread among the 19 ticks sampled. The high variability in the microbiomes of *I. uriae* means that large sample sizes will be required to disentangle the correlative relationships for the microbial community structure. Such a broad range of diversity is found in many arthropods (98).

Microbial community composition did appear to be related to geographic location in which ticks are collected. This can be observed in both the difference in inverse Simpson's Index, a measure of community evenness (Figure 3.4), which was higher in samples from Great Island than Gull Island, as well as ordination analysis (Figure 3.5), with 34 genera in six phyla that are over-represented in one colony over the other (Figure 3.6). This was also observed in *I. affinis* and *I. scapularis* in the eastern United States (98) and likely can be explained by differing source populations for the bacteria from the environment of the tick. These ticks spend most of their life cycle within the soil or environment and the habitat utilized by all life stages is similar as there are few suitable overwintering environments within many of these seabird colonies, especially the harsh cliff edges where Common Murres breed. The ticks analyzed here all originated from these harsh cliffs and were found in large mixed-stage groupings under rocks, which is also their primary overwintering site.

B. garinii is widespread within the seabird colonies of Newfoundland and Labrador, being found in every colony sampled and every species of seabird studied

showing evidence of exposure. The lack of variation between colonies for both seroprevalence and prevalence of the bacterium in ticks indicates the exposure and transmission processes are similar in all colonies surveyed. Seroprevalence varies between seabird species despite the fact that prevalence of the bacterium in ticks associated with the seabirds does not vary, an indication that both measures are linked to differing biological processes. Seroprevalence provides a biological overview of total exposure, whereas prevalence of the bacterium in ticks provides a single 'snap-shot' of a single moment and this rate is highly sensitive to factors such as year and life stage structure of the ticks collected. I documented an increase in *B. garinii* prevalence in each successive tick life stage, reflecting the bacterium being passed on between stages and potentially picked up with each feeding. The detection of other potentially pathogenic bacteria within these colonies also brings to question what other important bacteria might be found within these hosts and this ecosystem.

Table 3.1. Screening results for the presence of *Borrelia* in ticks collected from five seabird colonies throughout Newfoundland and Labrador.

Island/Colony	**Number of ticks collected**	**Number of ticks positive (Prevalence)**
Cape St. Mary's	9	1 (11.1%)
Gannet Islands	164	11 (6.7%)
Great Island	176	10 (5.7%)
Gull Island	436	37 (8.8%)
Little Fogo Islands	80	6 (7.5%)

Table 3.2 Seroprevalence for *Borrelia* in seabirds in Newfoundland and Labrador.

	Gannet Islands		Gull Island		Total	
Species[a]	**Positive[a]**	**N[b]**	**Positive[a]**	**N[b]**	**Positive[a]**	**N[b]**
Atlantic Puffin	93%	14			93%	14
Common Murre	75%	4	65%	55	66%	59
Razorbill	60%	5			60%	5
Thick-billed Murre	95%	20			95%	20
Black-legged Kittiwake			79%	39	79%	39
Herring Gull			100%	13	100%	13

[a]Percentage of samples that were above the cut-off value
[b]Number of samples tested

Table 3.3 Summary of results from Illumina 16S rDNA sequencing.

Tick ID	Location[a]	Sex[b]	qPCR *Borrelia*[c]	Number of reads[d]	OTUs[e]	Simpson's Diversity	Microbiome *Borrelia*[f]
NL13 156	Gull	M	+	13491	201	0.83	+
NL13 159	Gull	F	-	5365	77	0.63	-
NL13 221	Gull	M	+	4033	111	0.88	-
NL13 244	Gull	F	-	4957	126	0.81	-
NL13 266	Gull	M	-	21551	153	0.84	-
NL13 268	Gull	M	-	10122	126	0.84	-
NL13 276	Gull	M	-	17157	143	0.45	-
NL13 277	Gull	F	-	16398	90	0.40	-
NL13 278	Gull	F	+	6738	86	0.91	+
NL13 279	Gull	F	+	6964	77	0.83	+
NL14 515	Great	F	-	3657	132	0.96	-
NL14 553	Great	F	-	14892	221	0.97	-
NL14 669	Great	F	+	1044	75	0.90	+
NL14 673	Great	M	-	4231	145	0.92	-
NL14 698	Great	M	+	9733	96	0.23	+
NL14 1000	Great	F	+	18251	136	0.30	+
NL14 1060	Great	F	+	8144	181	0.97	+
NL14 1127	Great	F	-	12744	195	0.89	-
NL14 1134	Great	F	+	8800	126	0.93	+

[a]Gull, Gull Island; Great, Great Island
[b]F, engorged adult female; M, adult male
[c]qPCR screening results for *B. garinii*: +, positive; -, negative
[d]Number of reads used in the 16S analysis after data filtering steps
[e]Count of unique genera found in each sample
[g] *Borrelia* reads found in the microbiome analysis: +, reads mapping to *Borrelia*; -, no read mapping to *Borrelia*

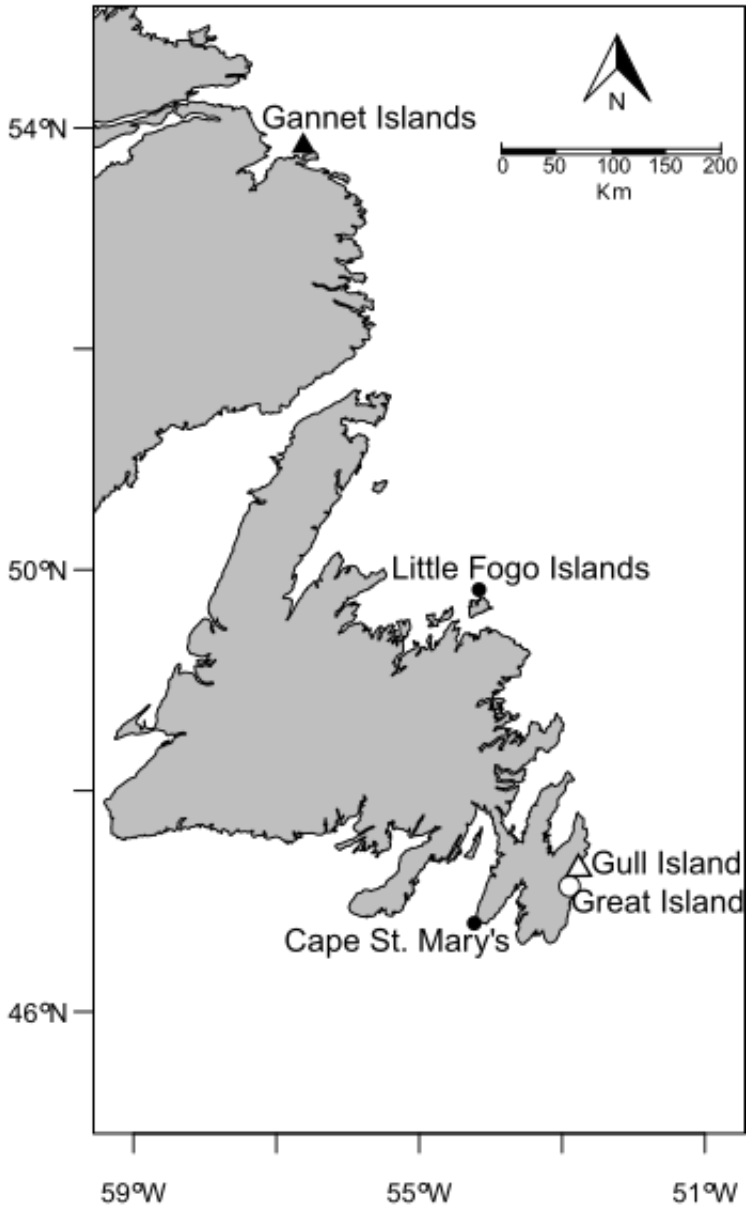

Figure 3.1 Seabird ticks (*Ixodes uriae*) were collected from five seabird colonies (circles and triangles) in Newfoundland and Labrador during the breeding season (June-August) in 2011-2015. Blood samples were collected from seabirds from two seabird colonies (triangles) and tick microbiome analysis at two seabird colonies (open symbols).

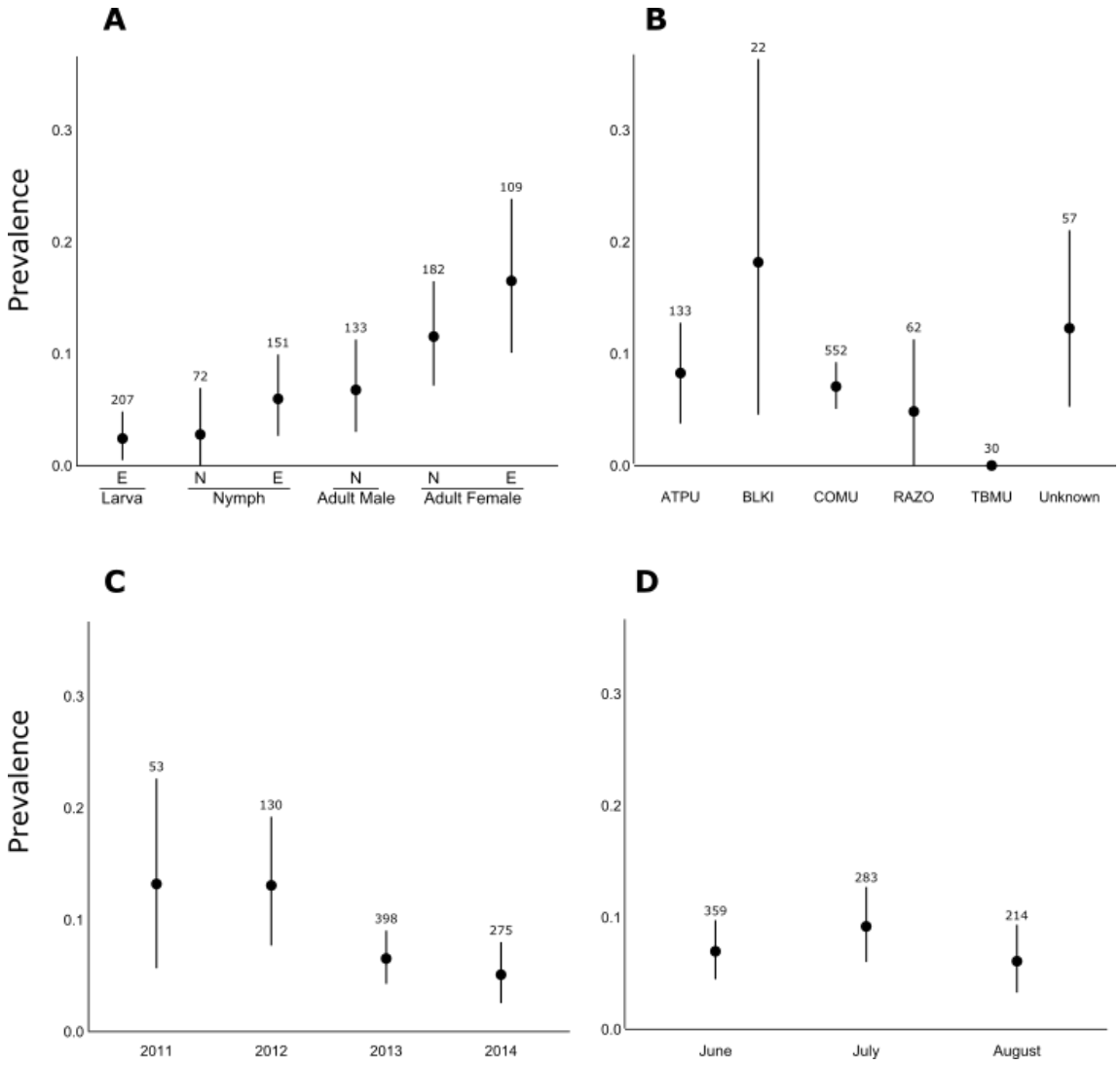

Figure 3.2. Prevalence of *Borrelia garinii* in *Ixodes uriae* from seabird colonies in Newfoundland and Labrador. Data are presented according to stage and engorgement level of ticks (A), seabird species (B), year (C), and month of tick collection (D). The middle dots represent prevalence, and the lines show the 95% confidence intervals. The number above the lines are the number of samples in each category. E, engorged tick; N, not engorged tick; ATPU, Atlantic Puffin; BLKI, Black-legged Kittiwake; COMU, Common Murre; RAZO, Razorbill; TBMU, Thick-billed Murre; Unknown, unknown tick host.

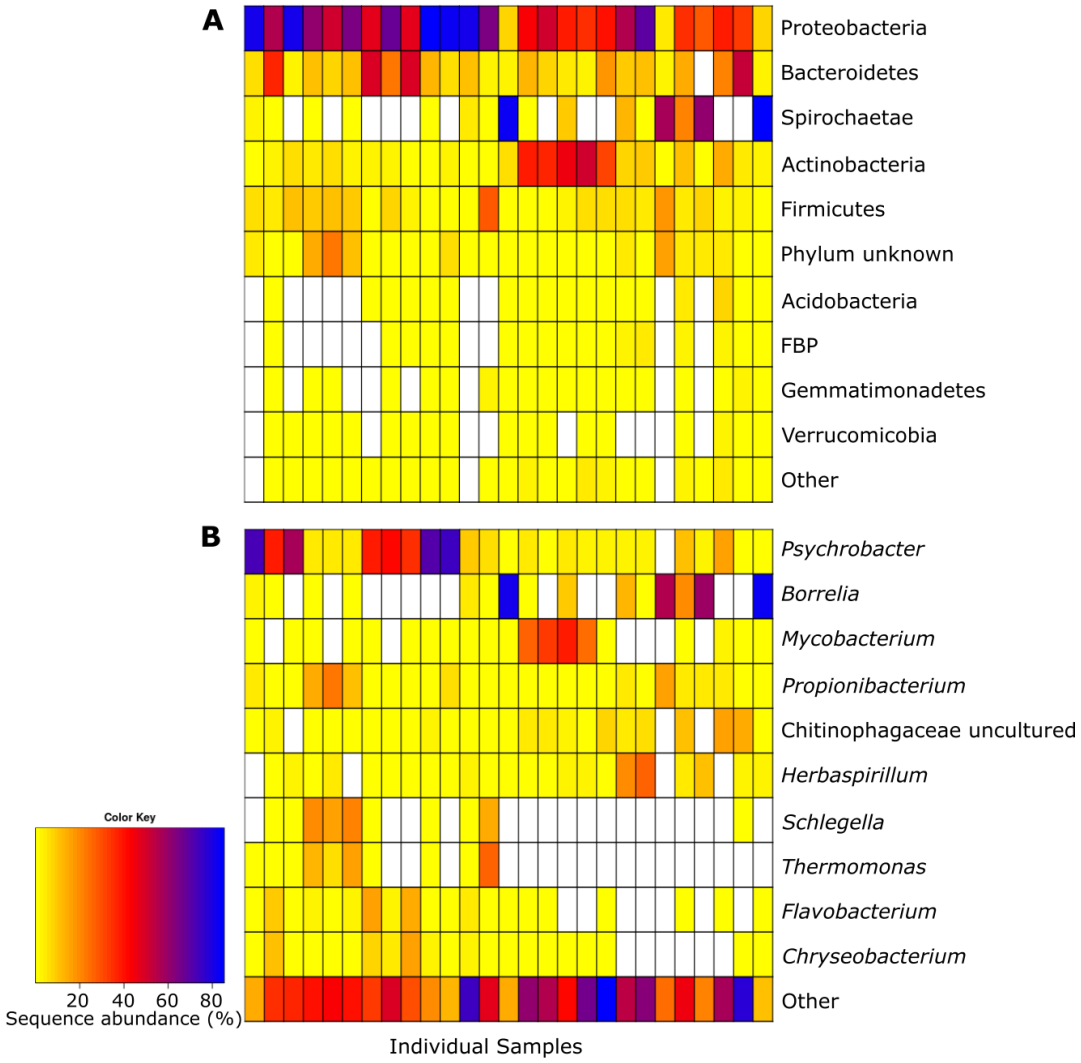

Figure 3.3 Dominant bacteria found within ticks. The heatmaps show the top 10 phyla (A) and genera (B) found in the 19 individual tick samples analyzed, based on numbers of sequence reads mapping to these taxa. "Other" represents all taxa that were not represented in the top 10.

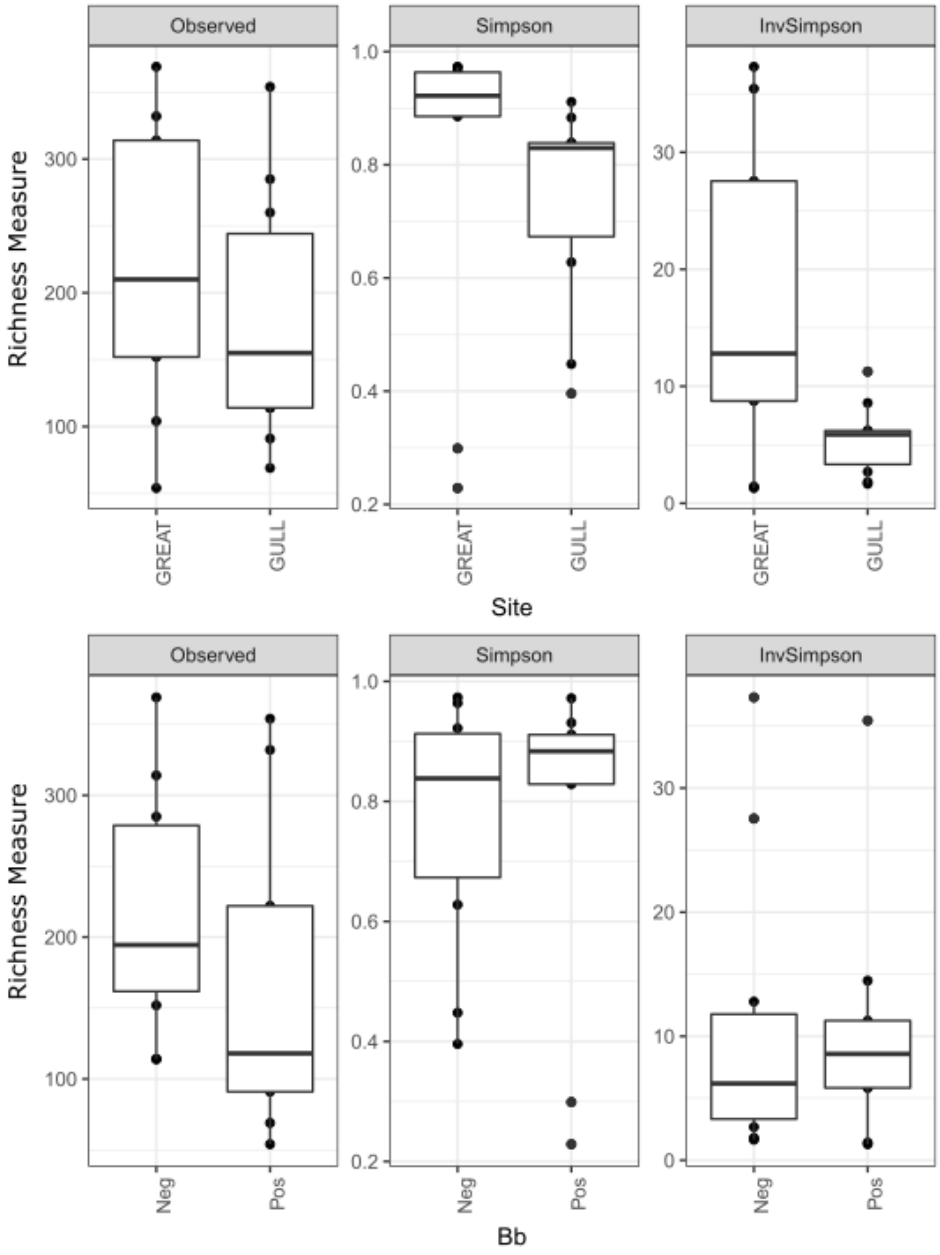

Figure 3.4 Microbiome diversity within ticks. Comparisons of richness between sites (top) and according to qPCR *Borrelia* screening results (Bb) (bottom), Neg, negative for qPCR screening of *Borrelia*; Pos, positive for screening of *Borrelia*. The total number of observed OTUs (Observed), Simpson Diversity Index (Simpson), and Inverse Simpson (InvSimpson) were compared in each case. Center lines represent the mean, with upper and lower bounds of the boxes indicating upper and lower quantiles, lines representing ranges excluding outliers, and dots are data points outside of the upper and lower quantiles.

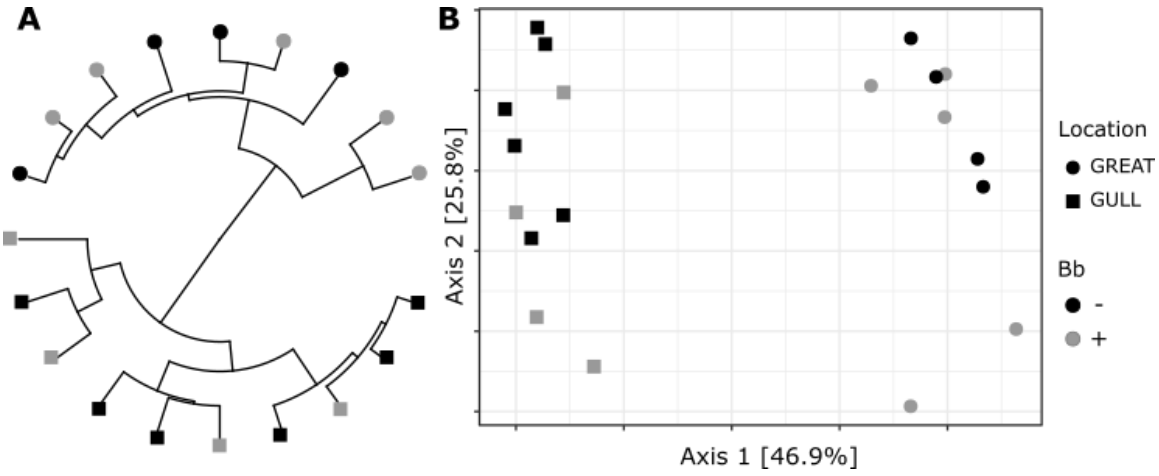

Figure 3.5 Between sample diversity measures in samples based on colony and *Borrelia* infection status. Ordination analysis MDS on Jensen-Shannon divergence (A) and distances plotted on a hierarchical clustering graph (B). Stress r^2=0.999. Locations are differentiated by shape and *Borrelia* qPCR status by colour: GREAT, Great Island, circle; GULL, Gull Island, square; black, negative in qPCR for *Borrelia*; grey, positive in qPCR for *Borrelia*.

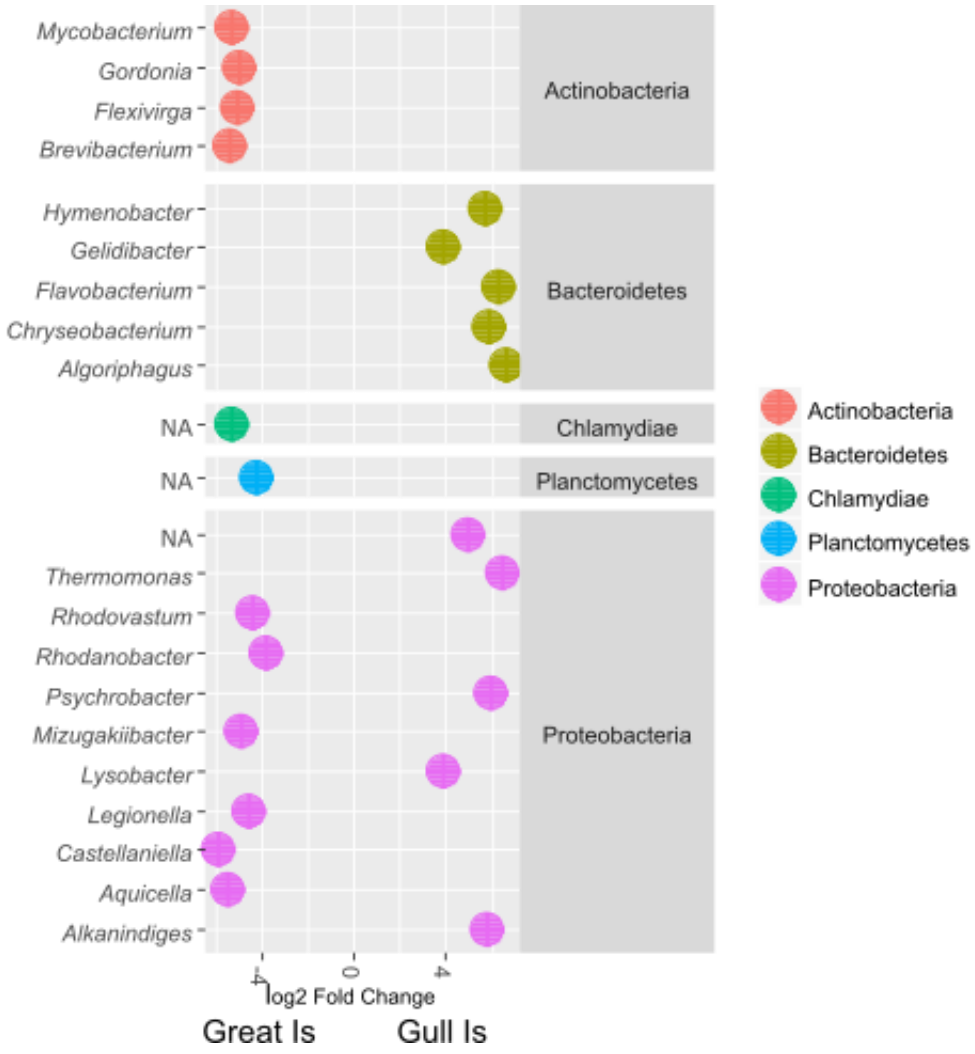

Figure 3.6 Genera over-represented in samples based on colony of collection. Genera that differ by more than log2-fold change between Great Island and Gull Island are shown. Genera labelled NA could not be placed in a known genus and so represent OTUs only identified to level of family.

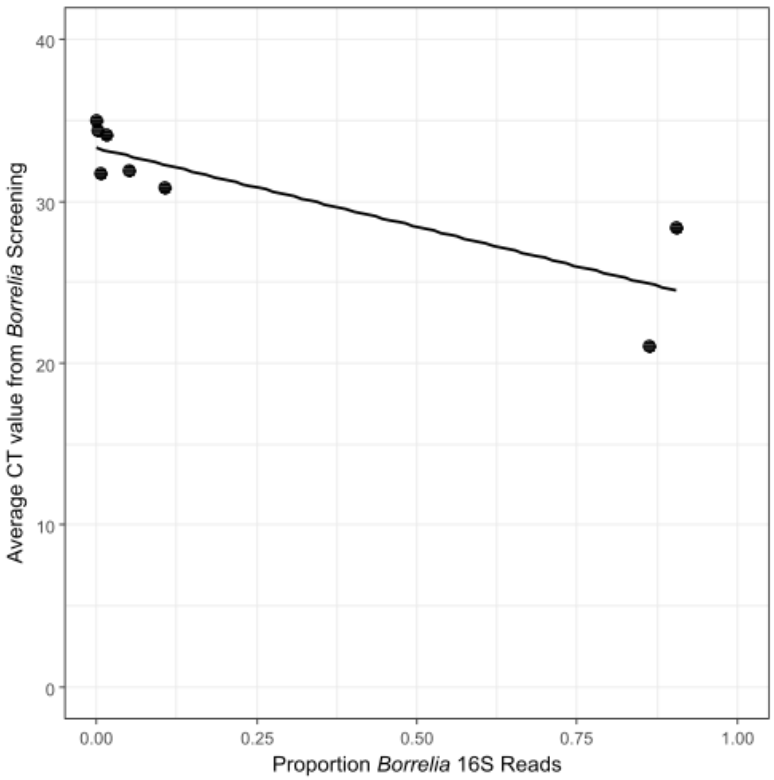

Figure 3.7 Relationship between the proportion of reads mapping to *Borrelia* in the microbiome analysis and CT values from qPCR assays. The data are averaged from two qPCR screening assays; $r^2 = -0.78$, $p < 0.001$, linear regression.

CHAPTER 4- EVIDENCE FOR *BORRELIA BAVARIENSIS* INFECTIONS OF *IXODES URIAE* WITHIN SEABIRD COLONIES OF THE NORTH ATLANTIC OCEAN

(Based on the manuscript published in Applied and Environmental Microbiology with the same title)

4.1. Abstract

The first report of members of the spirochete genus *Borrelia* in the seabird tick, *Ixodes uriae*, and seabird colonies occurred during the early 1990s. Since then, *Borrelia* spp. have been detected in these ticks and seabird colonies around the world. To-date, the primary species detected has been *B. garinii*, with rare occurrences of *B. burgdorferi* sensu stricto and *B. lusitaniae.* During our research on *Borrelia* and *I. uriae* in seabird colonies of Newfoundland and Labrador, Canada, we have identified *B. bavariensis* in *I. uriae*. To our knowledge, *B. bavariensis* has previously only been found in the Eurasian tick species, *I. persulcatus* and *I. ricinus,* and it was believed to be a rodent-specific *Borrelia* ecotype. We found *B. bavariensis* within *I. uriae* from three seabird colonies, over three calendar years. We also reanalyzed *B. garinii* sequences collected from *I. uriae* from Eurasian seabird colonies, and determined that sequences from two Russian seabird colonies likely also represent *B. bavariensis*. The Canadian *B. bavariensis* sequences from *I. uriae* analysed in this study cluster with previously described sequences from Asia. Overall, this is an important discovery that illustrates and expands the range of hosts and vectors for *B. bavariensis* and it raises questions regarding the possible mechanisms of pathogen dispersal from Asia to North America.

4.1.1. Importance

To our knowledge this is the first documentation of *B. bavariensis* outside Eurasia. Additionally, the bacterium was found in a marine ecosystem involving the seabird tick, *I. uriae,* and its associated seabird hosts. This indicates the epizootiology of *B. bavariensis* transmission is much different from what was previously described, with this species previously believed to be a rodent-specific ecotype, and indicates that this pathogen is established, or establishing, much more widely.

4.2. Introduction

Lyme disease in humans and some other mammals is caused by infection with bacteria of the species complex *Borrelia burgdorferi* sensu lato (s.l.), which currently comprises 18 recognized and three proposed species. Ten new species have been recognized between 2010 and 2015 (128). Many of these are broadly dispersed geographically, with some being found in both the Northern and Southern Hemispheres and/or in both North America and Eurasia. These bacteria are maintained in natural transmission cycles between ticks of the genus *Ixodes* and vertebrate reservoir hosts (76, 128).

Species of the *B. burgdorferi* s.l. complex have, in the past, been delineated using DNA-DNA hybridization or sequencing of the *rrs* locus (75, 129), but multi-locus sequence typing (MLST) has been used to examine their taxonomy (130) and delineate species (131, 132) more recently. Using this technique, it was proposed that the unique subgroup *Borrelia garinii* OspA type 4 was a distinct species, named *B. bavariensis* (133). This proposal was supported by the fact that this subtype is predominantly detected in rodents (Rodentia) (134) whereas other subtypes of *B. garinii* are typically associated only with birds (135, 136). Further study has demonstrated that *B. bavariensis* is widely distributed throughout parts of Europe and Asia (137, 138), and strains of *B. bavariensis* can be separated into two clades representing the two geographic regions (139).

Members of *Borrelia* were first detected in seabird ticks, *Ixodes uriae,* feeding on Razorbill (*Alca torda*) within a colony on islands off the Swedish coast (31). The species was eventually identified as *B. garinii*, which has subsequently been found in seabird colonies throughout Europe, Antarctica, and in two colonies in North America (43-47).

Since this discovery, *B. burgdorferi* sensu stricto and *B. lusitaniae* have also been reported in a few seabird colonies in the eastern North Atlantic (44). In the western North Atlantic, *B. garinii* has only been confirmed in two seabird colonies in Newfoundland and Labrador, Canada (43, 48).

Ixodes uriae utilizes a wide range of seabird hosts and has been recorded on more than 50 different colony-nesting seabird species (26). Although these ticks clearly have a preference for avian species, there are rare reports of them infesting mammals. For example, on the Gannet Islands in Newfoundland and Labrador, resident deer mice (*Peromyscus maniculatus*) have been observed to be infested with *I. uriae* ticks (41).

During a study to characterize the epizootiology of *B. garinii* associated with *I. uriae* and seabirds in Newfoundland and Labrador, we have documented *B. bavariensis* outside Eurasia. This demonstrates this bacterium has a much broader geographic range than previously recognized. As this species had not previously been identified as being associated with avian hosts or *I. uriae*, this discovery also indicates that *B. bavariensis* may have a broader range of vectors and vertebrate hosts than previously recognized.

4.3. Results

4.3.1. Identification of Borrelia bavariensis *in seabird ticks in Atlantic Canada*

A total of 67 ticks (40 adult females, 10 adult males, 12 nymphs and 5 larvae), collected from five seabird colonies in Newfoundland and Labrador (11 from the Gannet Islands, 11 from Great Island, 38 from Gull Island, 1 from Cape St. Mary's and 6 from Little Fogo Island; Figure 4.1) and associated with 5 seabird hosts (12 from Atlantic Puffins (*Fratercula arctica*), 4 from Black-legged Kittiwake (*Rissa tridactyla*), 40 from

Common Murre (*Uria aalge*), 1 from American Herring Gull (*Larus smithsonianus*), 3 from Razorbill (*Alca torda*), and 6 off-host ticks collected from areas where the host was unknown), showed evidence of infection with *Borrelia* based on PCR targeting 23S rDNA. MLST analysis identified *B. bavariensis* in five of these ticks (Table 4.1). For the remaining 62 ticks, MLST loci could not be successfully PCR-amplified from 23, three showed mixed peaks and ambiguous base calls in the sequencing chromatogram data and were excluded, and 36 contained *B. garinii*. Identification as *B. bavariensis* was based on high similarity with alleles in the MLST database, although none of the *B. bavariensis* alleles identified in samples from our study was 100% identical to an allele in the database (Table 4.1). Therefore, all alleles from our samples were new to the MLST database. The five *I. uriae* ticks infected with *B. bavariensis* were collected on three islands (one from Gull Island, one from Great Island, and three from the Gannet Islands) and from the burrows or nesting sites of two seabird hosts (two from Common Murre and four from Atlantic Puffin). The infected ticks were collected in 2011 (n = 3), 2013 (n = 1), and 2014 (n = 1) and all were adult females, with three fully engorged and the remaining two appeared unfed.

4.3.2. Re-evaluation of B. bavariensis *sequences from seabird ticks reported in the literature*

We evaluated the possibility that some sequences previously defined as *B. garinii* in the literature could in fact be *B. bavariensis*. The only study in which sequences matching the MLST scheme were obtained was that of Gomez-Diaz et al. (2011), in which sequences were obtained from samples from both the North Atlantic and North Pacific, and from a range of seabird species (30, 43-45, 140). Among the 42 samples

from this paper that were identified as *B. garinii*, two (T2937 and T3221) had alleles that aligned most closely with previously described *B. bavariensis* sequences (Table 4.1). These sequences originated from *I. uriae* found on a Tufted Puffin (*F. cirrhata*) and a Red-faced Cormorant (*Phalacrocorax urile*) from two seabird colonies in Russia (on the Commander Islands and the Kuril Islands). One other sequence from the Kuril Islands and four sequences from the Commander Islands represented *B. garinii*. The remaining 36 samples from colonies in the Northeast Atlantic were all *B. garinii* based on MLST.

It was not possible, using a phylogenetic approach, to definitively determine if other non-MLST published sequences previously identified as *B. garinii* might also be *B. bavariensis* because the identity to reference *B. bavariensis* sequences was not high enough. However, the *fla* sequence from one tick (T1674) from an Atlantic Puffin in Iceland (44) clustered with *B. bavariensis* sequences in a phylogenetic tree, was 100% identical to sequences from two other seabird-origin samples that had been identified as *B. bavariensis* using MLST (141), and shared 98.7% identity with BgVir, a *B. bavariensis* strain from a rodent (142) (Figure 4.2). It is possible that other samples represent unrecognized instances of *B. bavariensis* detections and MLST approaches would be useful for further confirmation (Appendix 3, Supplementary Figures 1-8).

4.3.3. Phylogeographic relationships of B. bavariensis *sequences*

Samples identified as *B. bavariensis* based on the MLST database were analyzed with previously available sequences to determine if they belonged to the Asian or European clade (139). All *B. bavariensis* sequences from this study fell within the clade of *B. bavariensis* reference sequences from Asia (Figure 4.3A). Similarly, all previously reported sequences from seabirds or their ticks in the Russian Commander and Kuril

Islands colonies belonged to this clade (Figure 4.3B). This same pattern was observed in the individual gene trees (Appendix 3, Supplementary Figures 9-16). In addition, the Newfoundland and Labrador sequences were not dispersed among the other sequences but formed their own group (Figure 4.3A).

4.4. Discussion

We detected *B. bavariensis* in *I. uriae* individuals collected from seabird colonies in Newfoundland and Labrador, Canada. To our knowledge this represents the first time this bacterial species has been identified outside of Eurasia and in the marine realm. Our samples came from *I. uriae* ticks collected in three distinct seabird colonies with *B. bavariensis* representing 9% of the *Borrelia*-positive ticks collected at these sites. Our analyses place these new Canadian sequences in the diverse Asian *B. bavariensis* clade, not in the European clade. In addition to samples collected in this study, several *Borrelia* sequences previously detected in *I. uriae* from seabird colonies in Russia also likely represent *B. bavariensis*. Therefore, in addition to its previously known transmission in rodent-tick cycles, *B. bavariensis* also appears to be transmitted between *I. uriae* and a variety of seabirds in both Eurasia and North America.

Borrelia bavariensis is thought to be a rodent-transmitted ecotype (143, 144) and play a key role in Lyme borreliosis in regions such as Mongolia (138). Currently there are 170 *B. bavariensis* occurrences described in the MLST database, with 24 from Europe and 146 from Asia and representing two sequence types in Europe and 53 in Asia. Within Europe, *B. bavariensis* PBi-like strains predominate and are primarily transmitted by *I. ricinus*, whereas in Asia, *B. bavariensis* NT29-like strains predominate and are

transmitted by *I. persulcatus* (138). The lower diversity of PBi-like strains found in Europe compared to the NT29-like Asian strains suggests an Asian origin for *B. bavariensis* (137, 138). In regions where *B. bavariensis* occurs, other species of *Borrelia*, such as *B. afzelii* and *B. garinii*, are frequently found in the same tick species (138).

MLST was successfully used to differentiate between *B. bavariensis* and *B. garinii* (133) and this remains the best tool to discriminate among *Borrelia* species. The 5S-23S intergenic spacer region, which has been used to distinguish *Borrelia* species (145), does not provide enough resolution to separate *B. bavariensis* and *B. garinii* (138) and has more recently been shown to not accurately represent the evolution within the genus (146). By reviewing previously published *Borrelia* sequences from seabird ticks and comparing them to known *B. bavariensis* sequences, we found that some targets previously used in *Borrelia* research, including *fla* and 16S rDNA, are not suitable for differentiating between these two *Borrelia* species.

Our *B. bavariensis*-positive samples originated from three separate seabird colonies and different years. Three of the infected ticks were collected from the Gannet Island cluster in Labrador in two different years. The ticks that contained this bacterium were associated with two seabird species, Atlantic Puffin and Common Murre. This occurrence of *B. bavariensis* suggests that the bacterium might not have a high degree of host specialization on the islands in this location. The seabird colony on the Gannet Islands is unique in that it is one of the few locations where *I. uriae* has been reported to infest mammals. In 2006, Baggs et al. collected 36 deer mice from the islands and found one *I. uriae* individual on each of 10 infested mice (41). These islands are remote, located

20 km from the mainland, and little is known about the resident mouse populations, with no records of mice on the islands prior to 1978 and no known estimates of population sizes. No ticks, or other samples from mice, from these islands have been screened for *Borrelia* prior to our research, and our finding of *B. bavariensis* warrants future efforts to study the possibility of the epizootiology of this transmission cycle involving mice in more detail.

The other two *B. bavariensis*-positive ticks were from Gull Island and Great Island, which are in the Witless Bay Ecological Reserve off the east coast of Newfoundland's Avalon Peninsula and 775 km southeast of the Gannet Islands. These islands are 7 km apart and neither island has a permanent population of land mammals.

Based on our results, it is not possible to distinguish whether our *B. bavariensis* detections represent multiple independent introductions or if there is endemic transmission in the seabird colonies sampled. However, the multi-site, multi-host and multi-year detection of *B. bavariensis* better supports endemic transmission of the bacterium. Seabirds that breed in North America stage and winter together in the North Atlantic with those that breed in Eurasia (14, 15) and some even move between the continents (18). These interactions and movements have been proposed to facilitate the movement of other pathogens across the North Atlantic (18) and might also be involved in movement of *Borrelia*. Introduction via infected ticks would be less likely as their attached feeding period is short relative to the duration of such migration (35) and also does not generally coincide with the timing of seabird migration. Unfortunately, neither the exact origin nor the timing of arrival of the *B. bavariensis* we detected in the western

North Atlantic can be determined based on the limited data currently available for this species.

Most *Borrelia* sequences from *I. uriae* in the literature are described as *B. garinii* (30, 43-45, 140) with the rare case of *B. burgdorferi* sensu stricto and/or *B. lusitaniae* (44). According to our MLST re-analysis, two of these detections are likely *B. bavariensis*, and a third instance might represent *B. bavariensis* based on phylogenetic analyses. This third sequence came from an Atlantic Puffin on Skrudur, Iceland, and had 100% identity to *B. bavariensis* sequences from Russian seabird colonies and high identity to the *B. bavariensis* BgVir strain. The two samples identified by MLST originated from the Commander Islands and Kuril Islands in Russia. These comprise numerous islands, spanning large areas and containing varied ecosystems.

Identification of sequences from other studies as definitively representing *B. bavariensis* was difficult as phylogenetic analyses using some markers do not provide enough resolution to distinguish *B. bavariensis* from *B. garinii*. Though we suggest that the identified samples are most likely *B. bavariensis*, confirmation by MLST would be more definitive.

In conclusion, we document for the first time, to our knowledge, the occurrence of *B. bavariensis* within *I. uriae* in North America. This is important as it would represent the first identification of *B. bavariensis* outside of the terrestrial realm and beyond Eurasia. Although further work is clearly required, the association of this species of *Borrelia* with seabirds and seabird ticks also suggests that it is not solely a rodent-associated ecotype. All *B. bavariensis* sequences from *I. uriae* identified to-date belong

to the Asian clade. It also remains to be investigated if there is some specialized adaptation within this "Asian" clade for seabird hosts in comparison to the European clade.

4.5. Methods

4.5.1. Ethics

Birds were captured and banded under Environment Canada banding permit 10559. This work was carried out under the guidelines specified by the Canadian Council on Animal Care with approved protocols 11-01-AL, 12-01-AL, 13-01-AL, and 14-01-AL from the Memorial University Institutional Animal Care Committee. Lab work was approved under biosafety permit S-103 from the Memorial University Biosafety Committee. Access to the Witless Bay, Gannet Islands, and Cape St. Mary's Ecological Reserves was through permits from the Parks and Natural Areas Division of the Newfoundland and Labrador Department of Environment and Conservation.

4.5.2. **Borrelia** *screening and species identification from Newfoundland and Labrador*

Ixodes uriae were collected from five seabird colonies in Newfoundland and Labrador, Canada, during a survey designed to estimate the prevalence of *B. garinii* within colonies in this region. Ticks were collected from Gull Island, Great Island, Cape St. Mary's, Little Fogo Islands and Gannet Islands (Figure 4.1). Ticks were removed directly off birds and collected from the environment around breeding sites. The seabirds in these colonies nest in dense aggregations dominated by single species. Ticks found within burrows of breeding Atlantic Puffins or under rocks where Razorbills or Common Murres were breeding were assumed to have fed on those respective species. In case of

doubt, the host was categorized as unknown. Ticks were identified using the key of Durden and Keirans (63). Ticks were stored at -20°C or -80°C prior to processing. DNA was extracted from ticks using the Qiagen DNeasy Kit as per the manufacturer's instructions. Extracts were determined to contain *Borrelia* DNA using quantitative polymerase chain reaction (qPCR) targeting a conserved portion of the 23S rDNA (101). Positive DNA extracts were subsequently used for PCR amplification of genes used in previous *B. garinii* MLST (141). PCR was performed according to published protocols (141) using GoTaq (Promega). All PCR products were sequenced in both directions at The Center for Applied Genomics (Toronto, Ontario). The forward and reverse sequences were aligned and visually examined for ambiguities; possibilities of mixed infections were noted based on occurrence of multiple peaks, and data from ticks with suspected mixed infections were not included in analyses. Sequences were compared using the MLST database (http://pubmlst.org/borrelia/). The closest matching allele was noted, and, because not all genes of the MLST scheme could be amplified from some samples, those closest to *B. bavariensis* (Appendix 3, Supplementary Table 1) at one or more locus were considered to represent that species.

All sequences were deposited in the NCBI GenBank database with the accession numbers KY985327-KY985349.

4.5.3. Literature search and species re-identifications

Sequences from other studies on *Borrelia* spp. in *I. uriae* were assembled from the NCBI GenBank database and previous publications (Appendix 3, Supplementary Table 2). Sequences from alleles or MLST sequence types that were already found in the MLST database were simply ascribed those allele or sequence type numbers. The

remaining sequences were analyzed by constructing phylogenetic trees, as described below, to determine if sequences fell into clades associated with *B. garinii* or *B. bavariensis*. Reference sequences for *B. garinii* and *B. bavariensis* were included, and trees were rooted with sequences from *B. burgdorferi* s.s. (Appendix 3, Supplementary Table 2). Because all sequences used in the phylogenetic analyses were not available for all reference strains, different reference strains were used in different analyses.

Phylogenetic analyses

For phylogenetic analyses, sequences for each target were aligned using MUSCLE (147) along with reference sequences that included *B. bavariensis*, *B. garinii* and *B. burgdorferi* s.s. (Appendix 3, Supplementary Table 2). The *B. bavariensis* reference sequences were the same as used in a previous publication (139) and represent the diversity found throughout Asia and Europe. Model selection was performed using JModelTest (148, 149) and maximum likelihood trees were produced using PhyML (150). Two trees of concatenated sequences were produced using a partitioned data approach with different models applied to each partition/gene: one with all eight MLST loci for the samples from this study, and the other with only two loci that allowed analysis of the samples from the MLST database (45). Branch support was calculated using a Bayesian-like transformation of approximate likelihood ratio test (aBayes) (1). Rooting of trees with *B. afzelii* in place of *B. burgdorferi* did not change the tree topologies.

Table 4.1 Samples collected in this study and from the literature identified as *B. bavariensis* based on comparisons to MLST alleles (from http://pubmlst.org/borrelia/).

Sample identifier	**Location**	**Host[a]**	**Tick age[b]**	**Percent identities to most similar MLST database alleles**							
				***clpA*, 56**	***clpX*, 64**	***nifS*, 66**	***pepX*, 76**	***pyrG*, 71**	***recG*, 119**	***rplB*, 125**	***uvrA*, 42**
NL11 025B	Gull Island	ATPU	AF, E		99.5					99.4	
NL11 061	Gannet Islands	ATPU	AF, N	98.6	99.5	99.3	99.4	98.3	99.2	99.4	99.3
NL11 152A	Gannet Islands	ATPU	AF, N	98.6	99.5	99.3	99.4	98.3	99.2	99.4	99.3
NL13 478	Gannet Islands	COMU	AF, E			99.3		98.3			99.3
NL14 1134	Great Island	COMU	AF, E				99.4	98.3		99.0[c]	
T2937	Russia	TUPU	NA					98.7	97.7		
T3221	Russia	RFCO	NA					98.7	97.7		

[a] ATPU, Atlantic Puffin; COMU, Common Murre; TUPU, Tufted Puffin; RFCO, Red-faced Cormorant

[b] AF, Adult female; E, engorged; N, not engorged; NA, not available.

[c] Most similar allele is 110, not 125.

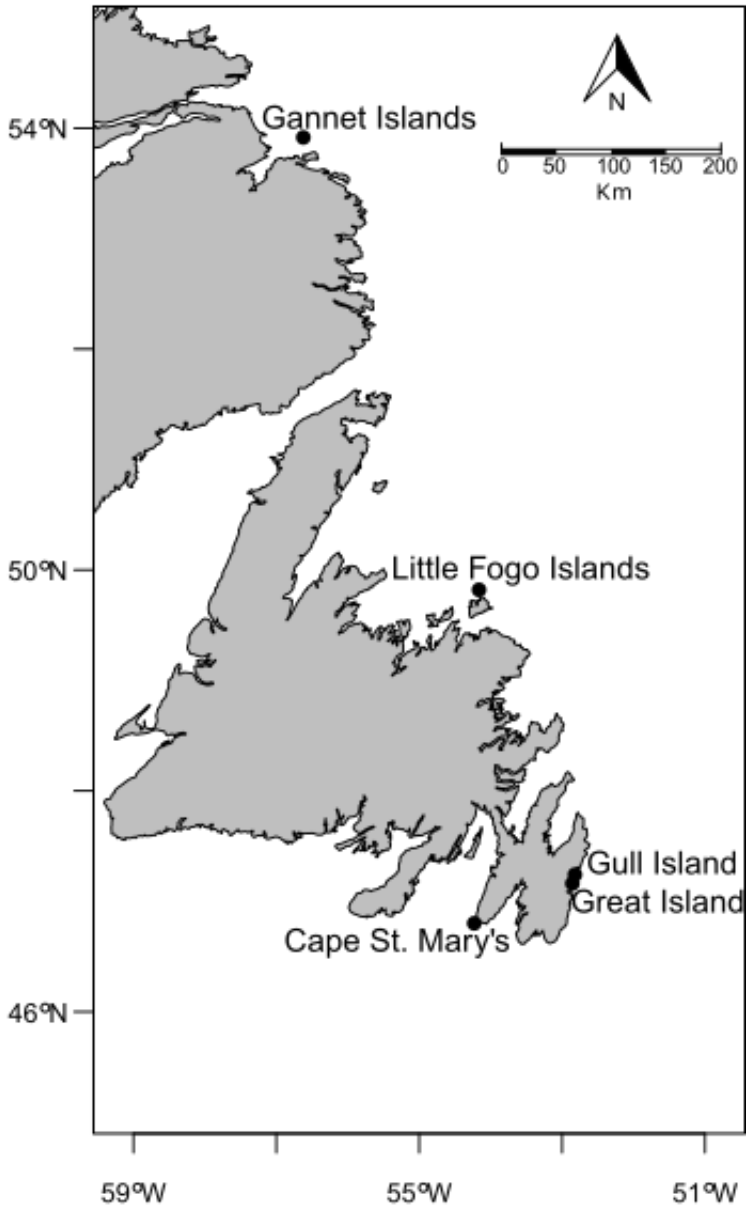

Figure 4.1 Map of Newfoundland and Labrador showing the seabird colonies where ticks were collected.

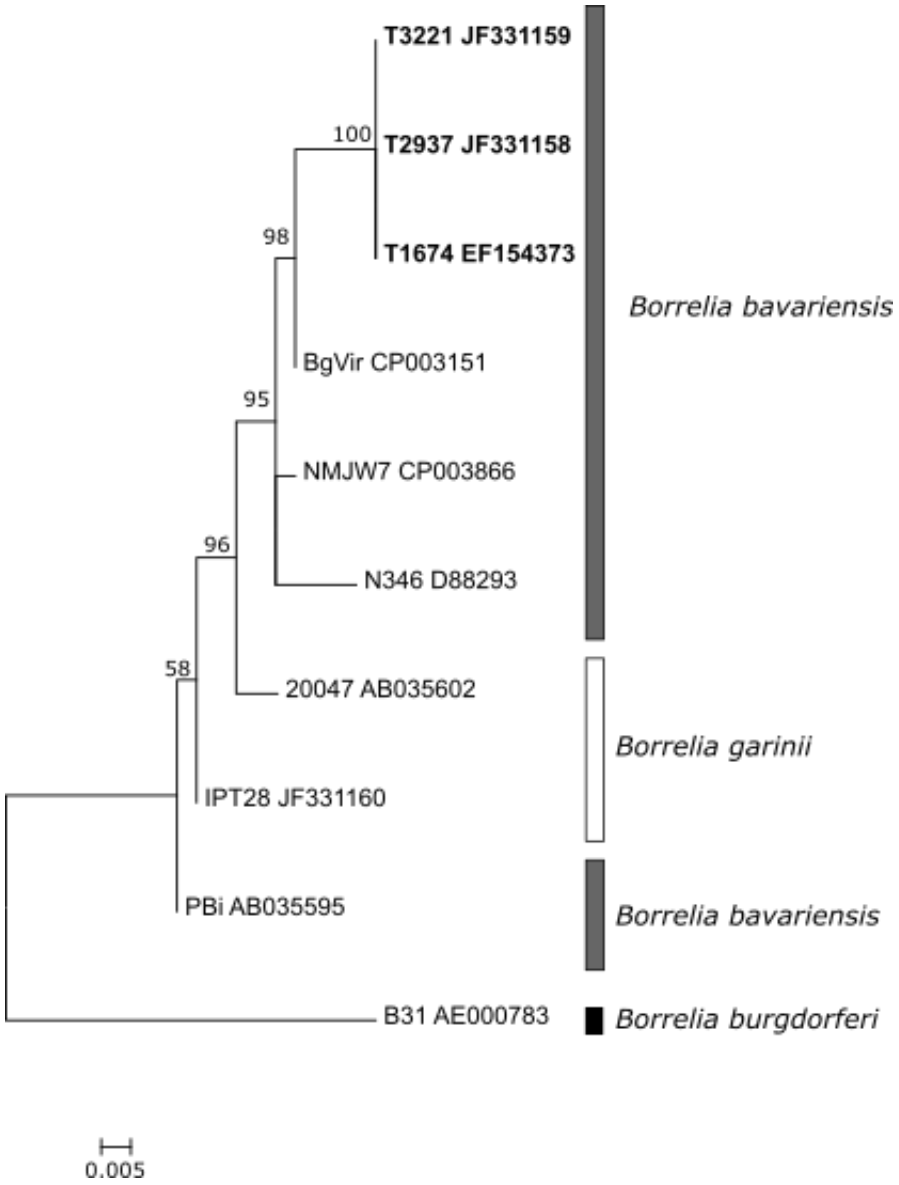

Figure 4.2 Phylogenetic analysis of *fla* sequences to identify possible instances of *B. bavariensis* detection. The maximum likelihood phylogeny was constructed using PhyML and *fla* sequences representing *B. bavariensis* (grey bar) and *B. garinii* (white bar). Sequences that originated from seabirds are in bold. Accession numbers of the sequences from the NCBI database are included in the branch labels. *Borrelia burgdorferi* (black bar) was used as the outgroup. Numbers at branch nodes represent bootstrap support based on aBayes and the scale bar indicates substitutions per site.

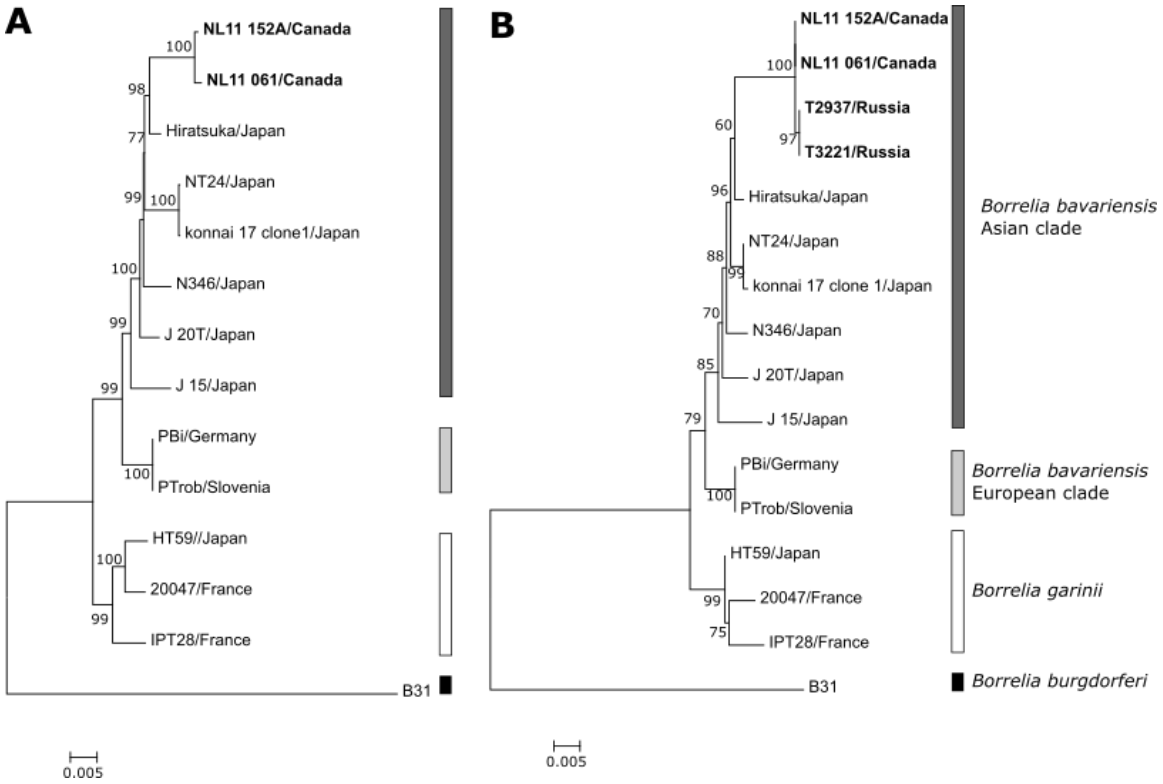

Figure 4.3 Phylogenetic analyses of *B. bavariensis* sequences identified in *I. uriae* from seabirds. (A) Analysis of eight concatenated MLST genes: *clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB*, and *uvrA*. (B) Analysis of two concatenated MLST genes: *pyrG* and *recG*. The maximum likelihood phylogenies were constructed using PhyML with sequences representing European (light grey bar) and Asian (dark grey bar) *B. bavariensis*, *B. garinii* (white bar), and *B. burgdorferi* (black) as the outgroup. Sequences that originated from seabirds are in bold. Numbers at branch nodes represent support based on aBayes and the scale bars indicate substitutions per site.

CHAPTER 5: POPULATION STRUCTURE OF *BORRELIA GARINII* AMONG THE SEABIRD COLONIES OF NEWFOUNDLAND AND LABRADOR

5.1. Abstract

Borrelia garinii in seabird colonies has been studied since the early 1990s. Research on the population structure of this bacterium in seabird colonies in the Northeastern Atlantic Ocean, in Iceland and Norway, has revealed admixture between this marine population and the terrestrial realm. I studied *B. garinii* population structure among seabird colonies in the Northwestern Atlantic Ocean, in Newfoundland and Labrador, Canada. I applied multi-locus sequence typing (MLST) to *B. garinii* found in the seabird tick, *Ixodes uriae*, and expand the known diversity of *B. garinii* by discovering new MLST sequence types and alleles. Diverse *B. garinii* sequences were found in Newfoundland and Labrador colonies, and these samples showed close relationships to those from Asia and Europe, including clinical human samples. Through the analysis of MLST genes used in previous work, I documented clustering of Newfoundland and Labrador samples with those from Iceland and Norway. My results highlight the genetic complexity of *B. garinii* circulating in seabird colonies, and close connections among seabird colonies in the North Atlantic Ocean and those in Eurasia.

5.2. Introduction

Borrelia burgdorferi sensu lato (s.l.) is a bacterial species complex that includes the causative agents of Lyme disease, one of the most common vector-borne diseases in the Northern Hemisphere. Lyme disease is a multisystem illness, and the causative bacteria are transmitted by ticks of the genus *Ixodes*. In North America, *B. burgdorferi* sensu stricto (s.s.) is the only species known to be associated with Lyme disease in humans to date, though several other species in the complex have been isolated from hard ticks (128, 151-153). The main tick vectors transmitting *B. burgdorferi* to humans in North America are *Ixodes scapularis* (in eastern, central, and southern regions) and *I. pacificus* (in western, particularly Pacific-coastal areas). In Eurasia, *B. afzelii*, *B. garinii*, *B. burgdorferi* and other *Borrelia* spp. are associated with Lyme disease in humans (75, 137, 154). The vectors are *Ixodes ricinus* in western Europe and *I. persulcatus* in eastern Eurasia. The three bacterial species have different reservoir hosts, with *B. afzelii* associated with rodents (Rodentia), *B. garinii* with birds (Aves), and *B. burgdorferi* s.s. a generalist found in both birds and rodents (83).

Natural transmission cycles of these bacteria and the risk for humans of contracting Lyme disease generally occur in woodland habitats in which ticks can survive during non-parasitic periods of their life cycle, and where the mammal and avian hosts of the ticks and bacteria are found (25). However, *B. garinii* was first reported in *Ixodes uriae* feeding on Razorbills (*Alca torda*) in the early 1990s in a seabird colony off the coast of Sweden (31). This led to investigations of seabird colonies worldwide, and the bacterium has now been found in both Northern and Southern Hemispheres (30).

Seabirds act as competent vertebrate reservoirs, and humans that come into contact with infected *I. uriae* can become infected (42). Since the 1990s there have been efforts to document the distribution and prevalence of this bacterium in seabird colonies (43-45), and to examine bacterial movement using population genetics (45, 46, 90).

The marine cycle of *B. garinii* involving seabirds is complex, occurring over a wide geographic range with many possible vertebrate hosts but a single vector species (i.e., *I. uriae*). These factors provide multiple opportunities for genetic diversification of the bacterium and therefore this provides an interesting system to examine population genetics on multiple geographic scales. There is evidence of transhemispheric *B. garinii* exchange, with identical *fla* gene sequences being found in both the Northern and Southern Hemispheres (30). At the same time, there appears to be genetic structure between the Atlantic and Pacific Ocean basins in the Northern Hemisphere, based on a multilocus analysis approach (45). However, within these ocean basins, evolution is clonal and little structure is apparent (45). Compared to the classical terrestrial realm, there is more genetic diversity in the marine/seabird *B. garinii* system (46). Recombination analysis has demonstrated admixture between the terrestrial and marine genetic pools (45) and it is therefore important to study both the seabird and other terrestrial *B. garinii* cycles.

The genome of *B. burgdorferi* s.l. consists of a linear chromosome that carries the genes for cell maintenance and replication, and a large number of linear and circular plasmids that encode most of the outer surface proteins (Osp), which are associated with

interactions with hosts and vectors (155). Previously, DNA-DNA hybridization and 23S-5S intergenic spacer (IGS) sequences were used to delineate *Borrelia* species (75, 129). Attempts to classify strains have also utilized 16S-23S IGS sequences (145, 156) and the plasmid-encoded *ospA* and *ospC* genes (157, 158). Recently, multilocus sequence typing (MLST) approaches, using the sequences of housekeeping genes, have been increasingly used (141, 156, 159, 160) as these allow for analysis at multiple genetic levels, from delineation of species (133) to population structure (159).

Past research has documented *B. garinii* at seabird colonies and has shown genetic evidence for linkage between strains found in terrestrial and seabird colony environments (45, 161) but samples from the North American seabird colonies have never been included. Past work also used a different suite of genes including both housekeeping genes and those located on plasmids (45), with the MLST scheme applied to multiple *Borrelia* species and strains world-wide having never been applied to this system. I characterized the population structure of *B. garinii* in seabird colonies in Newfoundland and Labrador, Canada, and, to my knowledge, this represents the first sequence-based study from the western Atlantic Ocean. Using the MLST scheme (141), I examined population structure among these colonies and how this relates to *B. garinii* found in Asia and Europe. Utilizing loci found in two previous MLST-based *Borrelia* studies (141, 162), I looked at relationships among *B. garinii* from both sides of the North Atlantic and those found within the North Pacific.

5.3. Methods

5.3.1. Ethics

Birds were captured and banded under Environment Canada banding permit 10559. This work was carried out under the guidelines specified by the Canadian Council on Animal Care with approved protocols 11-01-AL, 12-01-AL, 13-01-AL, and 14-01-AL from the Memorial University Institutional Animal Care Committee. Lab work was approved under biosafety permit S-103 from the Memorial University Biosafety Committee. Access to the Witless Bay, Gannet Islands, and Cape St. Mary's Ecological Reserves was through permits from the Parks and Natural Areas Division of the Newfoundland and Labrador Department of Environment and Conservation.

5.3.2. **Ixodes uriae** *collection and* **Borrelia** *screening*

From 2011 to 2014, *I. uriae* was collected from five seabird colonies in Newfoundland and Labrador, Canada (Figure 5.1). Ticks were collected from various breeding seabird habitats within these colonies representing a range of seabird hosts at each colony and every tick stage: larva, nymph, and adult (Table 5.1). Ticks were collected directly off birds or from the nesting habitat. Birds were captured for a range of research projects and long-term bird-banding programs. Birds were searched around their feet and on their heads where ticks are commonly found (67). Ticks were collected from the environment around the burrows of breeding Atlantic Puffins (*Fratercula arctica*), under the rocks and in crevices along the nesting ledges used by Common Murres (*Uria aalge*) or Razorbills (*Alca torda*), and within the nests of Black-legged Kittiwakes (*Rissa tridactyla*). This was done by visual inspection and lifting of rocks and other debris as

well as directly off the clothing of researchers. Ticks were identified using taxonomic keys (63, 64) and stored at -20°C or -80°C until processed.

DNA was extracted using the DNeasy Kit (Qiagen). Samples were identified as *Borrelia*-positive using quantitative polymerase chain reaction (qPCR) targeting a conserved portion of the 23S rDNA (101). Positive samples were subsequently used for PCR amplification of genes (*clpA, clpX, nifS, pepX, pyrG, recG, rplB,* and *uvrA*; Appendix 4, Supplementary Table 1) used in previous *B. garinii* MLST (141). Not all genes could be amplified from some samples and were excluded from further analysis. This could have been the result of low DNA yields or suboptimal PCR conditions with potential inhibitors from DNA extracts. PCR amplifications were performed according to published protocols (141) using GoTaq (Promega). All PCR products were sequenced in both directions using Sanger sequencing technology at The Center for Applied Genomics (Toronto, Ontario). Using Geneious 8.0.3 (163), sequences were visually examined for ambiguities, primer sequences were removed, forward and reverse sequences were aligned, and consensus sequences trimmed to the lengths of reference sequences. The possibility of mixed infections, indicated by mixed peaks, were noted and data from such samples were not included in analyses. No attempt was made to separate individual sequences by cloning or any other method as it would be difficult to match alleles for mixed infections.

All sequences were deposited in the NCBI GenBank database with the accession numbers MF536145-MF536294 and added to the pubMLST database (http://pubmlst.org/borrelia/).

5.3.3. MLST analysis

Sequences from this study were compared using the pubMLST database functions for sequence query (http://pubmlst.org/borrelia/), with each allele being ascribed a number corresponding to an existing identical allele, or a new number in the case that the allele was new to the database. In this case a submission to the pubMLST database was applied to get a new ID numbers for the new alleles and new sequence types (STs). Based on allelic profiles of 8 housekeeping genes, each sample was assigned an existing or new (for sequences with new combinations of alleles or novel alleles) ST number (141, 164). To investigate the role of sampling effort on ST and allele diversity, species accumulation curves were calculated in R. The diversities of STs at the four geographic locations and according to the different host species were examined. Due to uneven sample distribution and small sample sizes for some geographic locations and host species, diversities were compared between Common Murres and other seabird species, and Gull Island and other locations.

5.3.4. Phylogenetic analysis

To investigate phylogenetic relationships among the 12 *B. garinii* STs from this study, I compared them to all 118 *B. garinii* STs found within the pubMLST database, all of which originated from Eurasia. Sequences were aligned using MUSCLE (147). Model selection was performed using JModelTest (148, 149) for each locus and maximum

likelihood trees were produced using PhyML for the eight concatenated loci (150). Branch support was calculated using a Bayesian-like transformation of the approximate likelihood ratio test (aBayes) (1). The number of sequences visualized in trees was limited to closely related sequences for easier viewing and trees were rooted with *B. burgdorferi* s.s. as it is known to be basal to *B. garinii* (165). Eight individual trees, one for each of the MLST loci, were also produced using the same methods.

To examine the relationships among samples collected in this study and those collected by Gomez-Diaz et al. (45), representing seabird-derived sequences from the eastern Atlantic, sequences were analyzed as described above with the *recG* and *pyrG* loci.

5.3.5. Population structure analysis

Using sequence data from the 12 samples from this study, two different pairwise F_{ST} analyses were performed in R using the 'hierfstat' package (166) to determine the population structure based on colony of sample collection and seabird host. Genetic distance was computed using F_{ST} as described (167). To determine the significance of the F_{ST} value, 10,000 bootstraps were performed and the level of significance was altered from $p < 0.05$ by Bonferroni correction to a $p < 0.01$ to account for multiple pairwise comparisons. These analyses allowed me to determine the genetic distance between populations based on colonies and seabird hosts.

To identify clonal clustering of my samples in relationship to all *B. garinii* STs, related clusters of MLST STs were 'classified' into clonal complexes using eBURST v3 (http://eburst.mlst.net/) (168) and goeBURST v 1.2.1 (169) and then uploaded into the

Phyloviz v2 program (170). This analysis was performed with all *B. garinii* STs in the pubMLST database as of April 2017. These programs are designed for use with MLST data and cluster STs using algorithms on a set of hierarchical rules related to the number of single-locus variants (SLVs), double-locus variants (DLVs; eBURST), and triple-locus variants (TLVs; goeBURST). eBURST uses local optimization and is based on a simple model of clonal expansion and divergence, whereas goeBURST allows for global optimization and the identification of the founder ST among the set of STs, and an extended set of tiebreak rules, which leads to improved graphic representation of clonal complexes relating to the ancestral links among ST components. This analysis provides a global perspective of relationships of new STs and previously described STs, showing founders for the populations and closely related samples based on clonal complexes, as opposed to a phylogeny. Nevertheless, clonal complexes from the MLST analysis and clades on the phylogenetic trees are often concordant (160, 171, 172).

The community structure of the different STs found within Newfoundland and Labrador was computed with Bayesian Analysis of Population Structure (BAPS) version 6.0 (173), using clustering with a linked locus module and codon model. Mixture analysis was performed with K values from 1 to 12, and optimal partitions were identified by maximum log marginal likelihood value. The analysis was repeated with all *B. garinii* STs in the pubMLST database to identify STs from across Eurasia that clustered with STs found in Newfoundland and Labrador, with K values from 2 to 20. This provided an understanding of community structure of the samples from this study and how they fit

together on a local scale, as well as on a larger global scale, and it allowed for clonal complexes to be classified into clusters using BAPS.

5.4. Results

5.4.1. ST identification

Twenty samples of *B. garinii* from *I. uriae* in Newfoundland and Labrador were used in this study (Table 5.1) and characterized by complete MLST analysis. These samples originated from four seabird colonies and represented four seabird hosts. They were not picked at random but instead represent samples that had high *Borrelia* DNA concentrations. This resulted in identification of 12 STs, 10 of which were novel (new STs were assigned the numbers 684 and 686-694). The novel STs contained 26 new alleles and 18 alleles that could be identified within the pubMLST database (Appendix 4). Only two previously identified STs were found: ST244 and ST575.

In species accumulation curves, a method to determine saturation of sampling for both STs and alleles, saturation was not reached for STs but allelic diversity did start to plateau (Appendix 4).

The 12 STs identified in this study were distributed across four seabird colonies and from four seabird hosts (Table 5.2). The two previously described STs were found on Gull Island and Little Fogo Islands, associated with ticks collected on or near Common Murres and Atlantic Puffins, respectively. Novel STs (not previously described in the MLST database) were found at all colonies and associated with all seabird species investigated. The diversity (the number of STs per sample at a location or in seabird host) did not differ between Gull Island and other locations. The diversity also did not differ

between Common Murre and other seabird hosts. Of the 12 STs identified, unique STs (those not found at another location or associated with another host) were found at each location except for Little Fogo Islands, and were associated with both Common Murres other seabird hosts (Table 5.2, Appendix 4, Supplementary Figure 1). The proportion of samples carrying unique STs did not differ between Gull Island and other locations ($p = 1$), or between Common Murre and other seabird hosts ($p = 0.65$). Relationships between ST diversity based on geographic location and host were not independent, with most of the samples from Gull Island originating from Common Murres (10 of 13) whereas at other locations the distribution was more even (3 of 7).

5.4.2. Phylogenetic relationships

The sequences found in my study were phylogenetically diverse, with two lone sequences (ST692 and ST690) and three multi-sample clades (Figure 5.2). Two of these clades contained reference sequences from Europe and, in both cases, some reference sequences had 100% identity to one or more of my samples. The third clade contained no reference sequences with 100% identity to my sequences, and the closest reference sequence in this instance was ST304, at 99.3% nucleic acid identity. This sequence was found in *I. ricinus* from Scotland, and it differed at every locus in the overall ST. The two lone sequences (ST692 and ST690) did not cluster closely with any reference sequences. The first sequence, ST692 was basal to the clade containing ST244, sharing 99.8% nucleic acid identity and differing at every locus with respect to this closest ST. The other, ST690 was basal to a clade of sequences from Europe, and over the eight genes it

shared a nucleic acid identity of 99.4% to the nearest ST, but no two alleles were 100% identical.

Previous research examining *B. garinii* in *I. uriae* analyzed two loci that overlap with my MLST scheme: *pyrG* and *recG*, with 42 sequences available for each locus (45). All but eight of these samples clustered in clades with the *B. garinii* found in my study (Figure 5.3). Thirteen samples had 100% nucleic acid identity to ST244, ST691, and ST684 and fell in the same clade as ST694. One other sample was basal to this clade, with 99.68% nucleic acid identity to ST244. Six samples fell into the same clade as ST692, with one sharing 99.92% nucleic acid identity and five identical sequences that shared 99.84% nucleic acid identity. Two samples shared 99.94% nucleic acid identity and 99.92% identity with, and falling in the same clade as, ST690. Seven samples were in the same clade as ST575 and ST686, with one having 99.92% and five having 100% nucleic identity with these STs, and the final sample basal to the clade and 99.52% identical to these STs. Six samples were in the same clade and shared 100% nucleic acid identity with ST689, ST688, and ST693. Five of the samples had higher identities to STs from outside of this study. One shared 100% nucleic acid identity with ST89, two had 99.94% identity with each other and 99.14% identity with ST269, ST312, and ST364 (even though these three STs do not share 100% identity), and the final two had 97.51% nucleic acid identity to ST614 and are most likely not *B. garinii* but instead *B. bavariensis* (120).

5.4.3. Population genetic structure

Pairwise F_{ST} values (Table 5.3) indicated genetic differentiation and population structuring of ticks among colonies and tick host species. STs from Little Fogo Islands showed the highest values for genetic differentiation from Great Island ($F_{ST} = 0.733$, $p < 0.01$) and lower from Gull Island ($F_{ST} = 0.228$, $p < 0.01$). Gannet Islands showed significant differentiation from Gull Island STs ($F_{ST} = 0.049$, $p < 0.01$) but lesser than between Little Fogo Islands and those in Witless Bay (Gull and Great Islands). Atlantic Puffin STs showed differentiation from all other species, with the largest value for genetic differentiation being from Razorbills ($F_{ST} = 0.695$, $p < 0.01$) and the least with Common Murres ($F_{ST} = 0.248$, $p < 0.01$). The genetic differentiation was greater on the whole between tick host species than for that of colonies.

The eBURST analysis with all 130 *B. garinii* STs revealed that the samples clustered into 21 CCs (SLV) and 63 singletons, with two founders, ST244 and ST84. When examining the 12 STs found in this study, they cluster into four clonal complexes when either SLVs or both DLVs and SLVs were included, including three singletons (Figure 5.4B and 5.4C). The inferred founder, ST244, previously identified in 12 tick and human samples from Germany, Russia, and the UK, was also indicated for the clonal complex.

The BAPS analysis best supported the existence of five subpopulations (Figure 5.4B and Figure 5.4C) for which the log marginal likelihood value was the highest. These subpopulations showed some level of geographic structuring, with all STs from Gannet Islands clustering together with two STs found Gull Island. There were two

subpopulations with exclusively Gull Island samples, and a single ST found on Great Island formed a solo subpopulation. The final subpopulation contained STs found on Gull Island, Great Island, and Little Fogo Islands. These subpopulations showed some level of tick-host structuring, with three subpopulations clustering STs only found associated with Common Murres. The remaining two subpopulations contained STs found associated with multiple tick hosts.

The BAPS analysis using all *B. garinii* STs supported the existence of 3 subpopulations, with one containing my samples from Newfoundland and Labrador (Figure 5.4A). This population consisted of samples from across Eurasia and showed no geographic structure. These samples also originated from a range of sources including various species of ticks and ticks collected from humans.

5.5. Discussion

In this study, *B. garinii* found in *I. uriae* were analysed by MLST, determining the STs associated with this understudied system. My results expand our knowledge of *B. garinii* globally and I discovered 10 novel STs and many new alleles at all eight MLST loci, representing an increase in the known genetic diversity of this species. I determined that there is population structure in *B. garinii*, at both regional and global scales. At the local scale, sequences show evidence of genetic clustering by both seabird colonies and/or seabird hosts. Sequences found in Newfoundland and Labrador do not all cluster together and show similarity to those found in human clinical samples and terrestrial ticks from Europe. This may suggest multiple introductions and a close connection to the

terrestrial realm of *B. garinii* cycling (45). Sequences found in Newfoundland and Labrador also show a high similarity to previously collected sequences from the eastern Atlantic (44).

5.5.1. B. garinii *genotype diversity*

Although multiple species of *Borrelia* have been found circulating in the *I. uriae*-seabird system, *B. garinii* remains the dominant species (30, 42, 161, 162). Two of the STs I found in Newfoundland and Labrador are identical to previously identified STs originating in Europe. One of these STs has a wide geographic range, with six samples in the pubMLST database from the UK, Germany, and Russia, and also collected from diverse sources (human cerebrospinal fluid, *I. persulcatus*, and *I. ricinus*). This ST was in a single tick collected from Gull Island and was associated with Common Murres. The other previously described ST has only been observed in Germany, from bacteria isolated from a human skin sample collected in 1994.

The high level of sequence identity for samples from North America and European seabird colonies indicates a link for *B. garinii* across the North Atlantic Ocean. Possible scenarios for movement of the bacterium include transport in infected ticks or in infected birds. The movement of ticks on seabirds is unlikely as the period of attachment is between 4 and 8 days (35, 36), while many of these seabirds would take many days to cross the Atlantic Ocean and do not visit land along the way (14, 15). Seabirds generally leave their colonies at the end of the breeding season, spend most of the year out at sea feeding with no need to come to land before the subsequent breeding season. Therefore, it is more likely that the bacterium is moved between colonies in infected birds, suggesting

that the birds remain persistently infected as is often the case for mammalian hosts (174) and perhaps some woodland birds (175). These birds have high site fidelity but are known to prospect for new breeding locations as young adults, resulting in movement among colonies (15, 17).

In past studies of *B. garinii* in seabirds and *I. uriae*, high genetic diversity has been documented (44-46) and I also observed this. Twelve STs are present in the 20 samples analyzed, along with many unique alleles. Such high diversity is also seen in Europe (176). In contrast, a much lower diversity is observed in *B. burgdorferi* s.s. in North America, with 111 STs identified in 564 samples, although diversity of *B. burgdorferi* s.s does differ among geographic regions (177). *Borrelia garinii* is known to be one of the most heterogeneous of the *Borrelia* species, having both high genetic and antigenic diversity (178). It is likely that the diversity found in my study is only a small subset of what actually exists in these seabird colonies and more novel STs are likely to be found with further sampling.

My study advances knowledge of the phylogeographic relationship of marine-associated *B. garinii* with respect to sequences collected throughout Eurasia, for both terrestrial and clinical samples, as well as those collected from *I. uriae* in seabird colonies in the eastern Atlantic. I used multiple methods to examine the same question, using the phylogenetic, goeBURST, and BAPS analyses, and assessment of the origin of inferred founders of clonal complexes, allowing me to have stronger certainty in my conclusions. Previous work documented structure among *B. garinii* collected from seabird colonies in

the Atlantic and Pacific Oceans (45). In addition, admixture between the terrestrial realm and seabirds in the North Atlantic has been documented (45). My phylogenetic results indicate five clades of Newfoundland and Labrador sequences, two of which are represented by single samples. These five clades are dispersed throughout the total *B. garinii* tree and some of my sequences show close relationships with those from throughout Eurasia. This indicates multiple introductions of sequences from Europe into the marine realm and mixing between these eco-zones (30, 45, 46, 179). These samples from Newfoundland and Labrador, when examined within clonal complexes and stucture on a species levels, cluster into the same clonal complex and the same subpopulation. Highly clonal bacterial species are known to have populations that display clusters of closely related genotypes or clonal complexes. These complexes can be globally distributed and stable over time (164).

My samples show a high level of identity with previously identified sequences of *B. garinii* within *I. uriae* from the eastern North Atlantic. Many of these have 100% sequence identity, indicating recent shared ancestors. All samples from my study cluster phylogenetically with sequences from Gomez-Diaz et al. (45, 120), indicating a close connection between *B. garinii* on both sides of the Atlantic; the bacterium is most likely being moved by migrating seabirds. Rodents do occur in and around some of these colonies, but few cases of *I. uriae* infesting mammals are known (41, 179), and they likely do not play an important role in transmission of *B. garinii*. Seabirds are known to migrate long distances, moving across the Atlantic between breeding and wintering locations (14, 15). Seabirds from both the eastern and western Atlantic spend their winter

months together feeding on common food resources and intermingling (14, 15), and these interactions have been proposed to play a role in movement of other avian-transported pathogens across the Atlantic (18, 20).

5.5.2. **B. garinii** *population structure at a regional level*

At a local level, my data show that high *B. garinii* diversity exists in the Northwest Atlantic seabird colonies, with several independent and divergent clonal groups, similar to what is found within eastern Atlantic seabird colonies (45). The distribution of these genotypes are somewhat heterogeneous. One cluster of STs originated solely from Common Murres on Gull Island in both 2012 and 2013. The other two clusters both comprise more than one sample and originate from two or more colonies and two or more seabird host species. It should also be noted that one cluster consists of STs originating exclusively from hosts other than Common Murre.

A basic diversity analysis looking at STs per sample did not show a relationship between ST diversity and geographic location or tick host. The lack of a relationship indicates that there are no processes limiting STs at either the geographic or host level. Despite this observation, I note that my results are limited and an increase in the number of samples collected and analyzed would help to strengthen these conclusions. This same observation can be made about the diversity of novel STs at a geographic and host level.

Even though phylogenetic and BAPS analysis did not highlight a strong geographic or tick host-based structure, F_{ST} analysis, which examined the genetic distance between populations, revealed some structure among populations. There was significant genetic differentation between samples collected at Little Fogo Islands vs.

Great Island and Gull Island, and Gannet Islands vs. Gull Island. These three comparisons represent large geographic distances with Little Fogo Islands 300 km northwest from both the Great and Gull Islands, and the Gannet Islands, located on the Labrador coast,500 km northwest of Gull Island. This pattern is likely driven by *I. uriae* population structure, which has been observed between colonies in Iceland and Norway (113, 180, 181). Vector-borne microbes co-occur with their hosts and vectors, and the population genetic structure of these hosts and vectors is expected to have a strong driving force on pathogen population structure (182, 183). Lack of genetic distance between Gull and Great Islands is not suprising as they are very close to each other, share similar seabird species, and would have the easiest opportunities for exchanges of birds, ticks and bacteria.

At the tick-host level, genetic differentiation exists between STs found associated with Atlantic Puffins vs. Black-legged Kittiwakes, Common Murres, and Razorbills. Atlantic Puffins use different breeding habitats, nesting in earthen burrows along grassy slopes (54, 70), whereas the other three species are found along rocky ledges on cliff edges (37, 69, 184), or talus slopes (185). Therefore, the differences among bird species can be attributed to *I. uriae* having a population structure based on their hosts on a local geographic level (186, 187) and this could further drive the large geographic patterns seen. Population subdividisions, like those seen among seabird species, may act as barriers to gene flow for these bacteria and other pathogens (i.e. multiple niche polymorphism; 188).

The colonies sampled here were seperated by hundreds of kilometers and they also differ in their host makeups. Little Fogo Islands is a large Atlantic Puffin colony with no other seabird species, while the others are large multi-species colonies and contain large numbers of Common Murres. This will lead to different host conditions on the different islands and varying levels of connectivity for ticks to disperse the bacterium.

My work has demonstrated that there is a greater diversity within *B. garinii* than previously recognized. It also expands our knowledge of where *B. garinii* is found geographically and connects STs found in Europe to those found in seabirds in the western North Atlantic. The use of multiple techniques to examine population structure gives us improved insight into the connections between *B. garinii* in the seabird colonies of the western Atlantic and those found throughout Asia and Europe, revealing a tight connection for seabird samples that are closely related or identical to samples from terrestrial environments. This diversity and the close associations between marine- and terrestrial-derived *B. garinii* are important and fit with what has been observed previously (45).

Overall, this study greatly increases the knowledge of *B. garinii* on a global scale. Previous research utilizing MLST has focused on the terrestrial realm and asking questions about reservoir species (144, 189) and prevalence in clinical settings (190). Application of MLST to *B. garinii* collected from *I. uriae* in the marine realm has allowed me to document the close connection between *B. garinii* found in the seabird colonies off Newfoundland and Labrador to those found within clinical samples and

terrestrial ticks of Eurasia. It also illustrates the close connection to previous sequences from *B. garinii* from the eastern Atlantic seabird colonies in Iceland and Norway. These close connections highlight a complicated system with movement on large geographic scales that must be linked to the diverse and broad migration patterns of seabirds, songbirds, and other hosts within this complicated host-pathogen complex, with admixture and movement of the bacteria occurring (45).

Table 5.1. Summary of ticks analyzed by location, host, and life stage.

Location	Host[a]							Total
	ATPU	BLKI	COMU				RAZO	
Life stage[b]	AF	AF	L	N	AF	AM	AF	
Gannet Is.	(1)	-	-	-	-	-	(2)	**3**
Gull Is.	2	1	(3)	(3)	(4)	-	-	**13**
Great Is.	-	-	-	(1)	(1)	1	-	**3**
Little Fogo Is.	1	-	-	-	-	-	-	**1**
Total	**4**	**1**	**13**				**2**	**20**

[a]ATPU, Atlantic Puffin; BLKI, Black-legged Kittiwake; COMU, Common Murre; RAZO, Razorbill

[b]L, larva; N, nymph; AF, adult female; AM, adult male; numbers in brackets represent engorged ticks

Table 5.2 Diversity of MLST sequences types (STs) by locations and tick hosts.

	Number of samples	Number (new) of STs		Number (proportion) of samples carrying unique STs
Location				
Gull Is.	13	9 (2)	0.69	6 (0.46)
Other	7	6 (1)	0.86	3 (0.43)
Great Is.	3	2	0.67	1 (0.33)
Little Fogo Is.	1	1 (1)	1	0
Gannet Is.	3	3	1	2 (0.67)
Host				
Common Murre	12	9 (2)	0.67	7 (0.58)
Other	8	5 (1)	0.63	3 (0.38)
Atlantic Puffin	5	4 (1)	0.8	1 (0.20)
Black-legged Kittiwake	1	1	1	0
Razorbill	2	2	1	1 (0.50)

Table 5.3 Matrix of pairwise F_{ST} values of the MLST sequence types (STs) for different colonies and hosts, with 99% CI.

Location	Gannet Islands	Great Island	Gull Island
Great Island	0 (0-0)		
Gull Island	**0.05[a]** **(0.01-0.08)**	0 (0-0)	
Little Fogo Islands	0.18 (0-0.32)	**0.73** **(0.32-0.17)**	**0.23** **(0.07-0.33)**
Host	**Atlantic Puffin**	**Black-legged Kittiwake**	**Common Murre**
Black-legged Kittiwake	**0.49** **(0.28-0.65)**		
Common Murre	**0.25** **(0.11-0.35)**	0.01 (0-0.05)	
Razorbill	**0.70** **(0.28-1)**	0 (0-0)	0 (0-0)

[a]Bold denotes significant comparisons

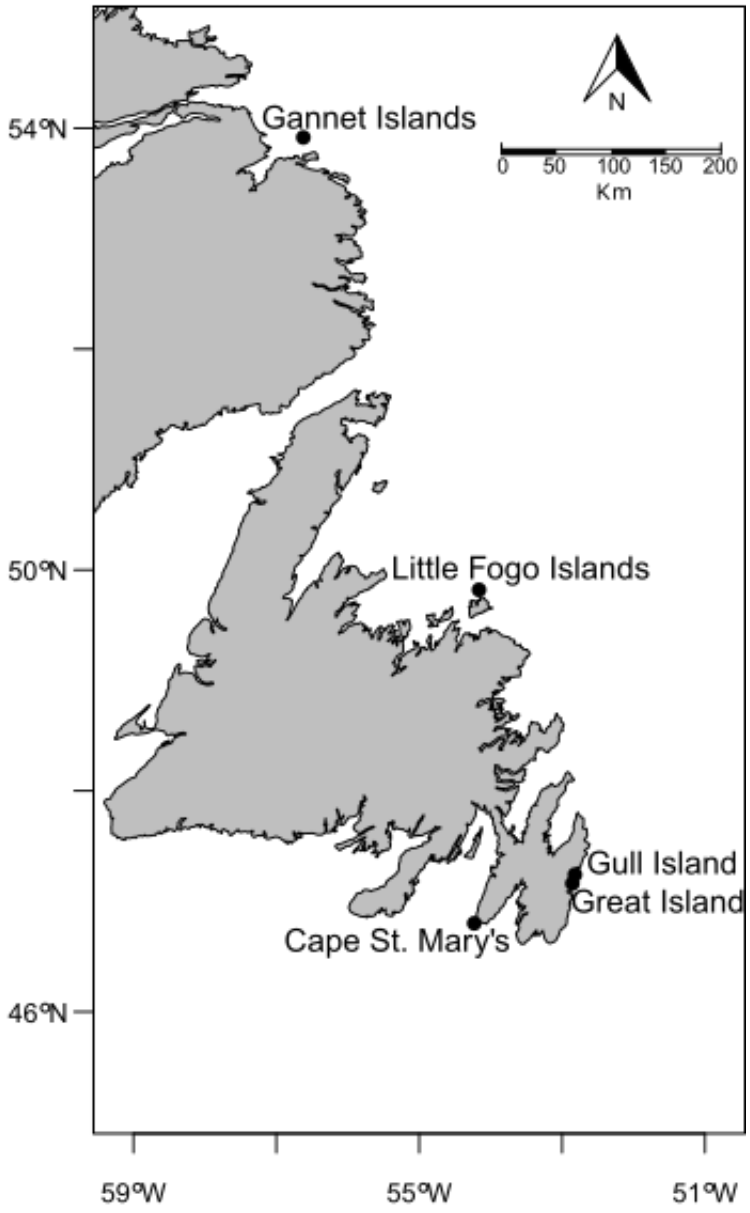

Figure 5.1 Seabird ticks (*Ixodes uriae*) were collected from five seabird colonies in Newfoundland and Labrador during the breeding season (June-August) in 2011-2015.

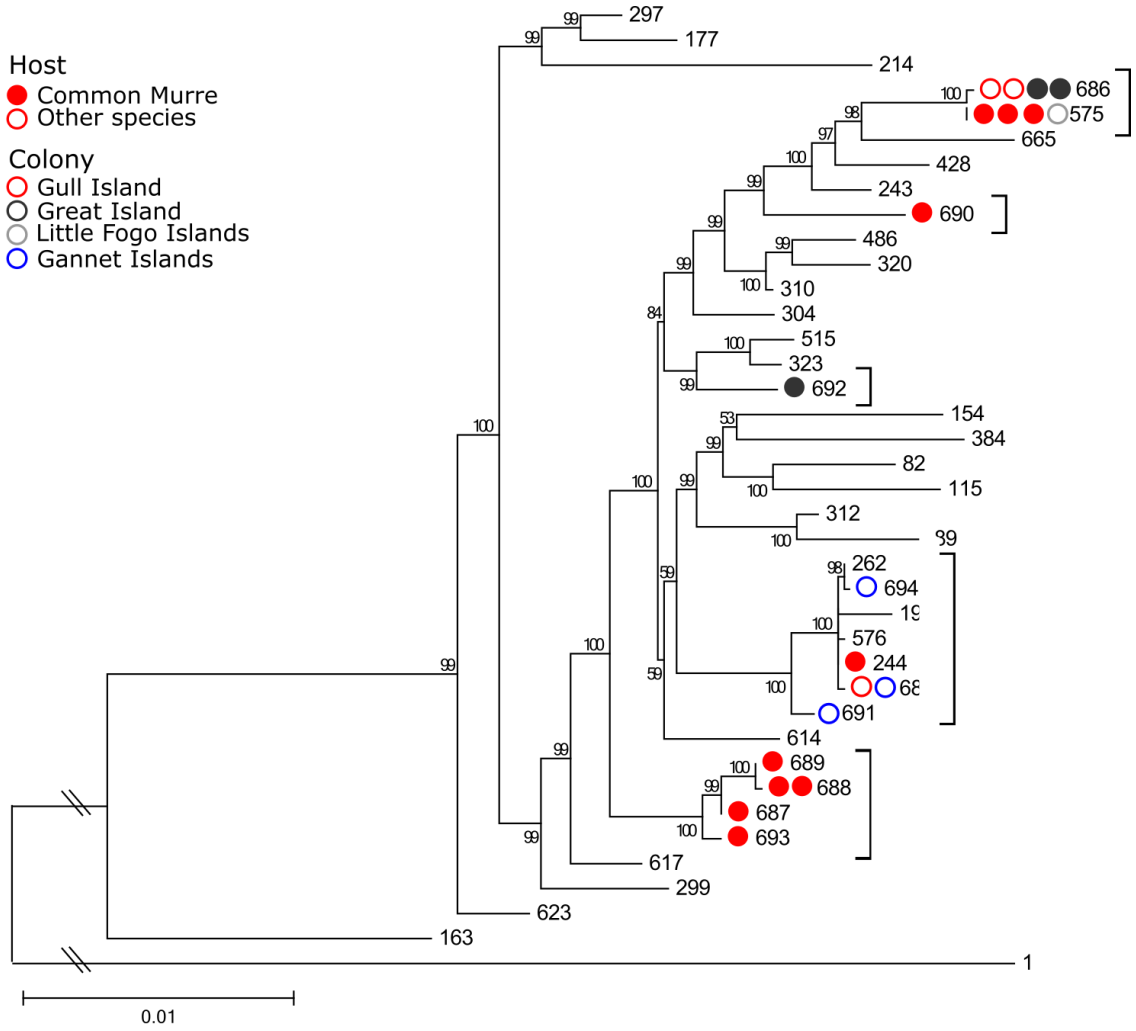

Figure 5.2 Phylogenetic analysis of *B. garinii* sequences in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML for eight concatenated MLST genes (*clpA, clpX, nifS, pepX, pyrG, recG, rplB, and uvrA*). Labels are sequence types (STs) from the pubMLST database. Samples from this study are denoted with dots. *Borrelia burgdorferi* was used as the outgroup, labeled as "1". Numbers at branch nodes represent support based on aBayes and the scale bar represents the number of substitutions per site. Clades with samples from Newfoundland are noted with brackets.

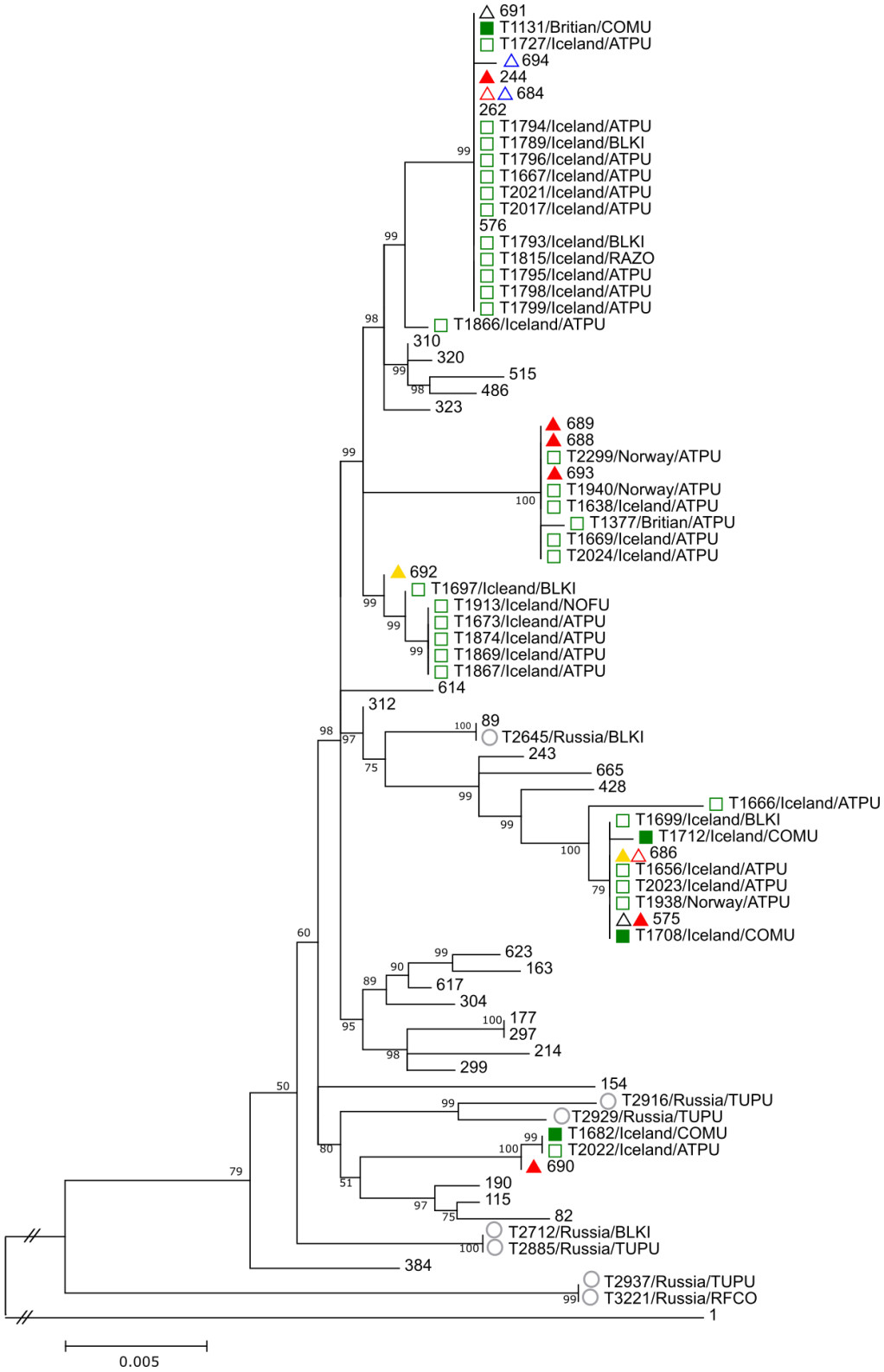
691
T1131/Britian/COMU
T1727/Iceland/ATPU
694
244
684
262
T1794/Iceland/ATPU
T1789/Iceland/BLKI
T1796/Iceland/ATPU
T1667/Iceland/ATPU
T2021/Iceland/ATPU
T2017/Iceland/ATPU
576
T1793/Iceland/BLKI
T1815/Iceland/RAZO
T1795/Iceland/ATPU
T1798/Iceland/ATPU
T1799/Iceland/ATPU
T1866/Iceland/ATPU
310
320
515
486
323
689
688
T2299/Norway/ATPU
693
T1940/Norway/ATPU
T1638/Iceland/ATPU
T1377/Britian/ATPU
T1669/Iceland/ATPU
T2024/Iceland/ATPU
692
T1697/Icleand/BLKI
T1913/Iceland/NOFU
T1673/Icleand/ATPU
T1874/Iceland/ATPU
T1869/Iceland/ATPU
T1867/Iceland/ATPU
614
312
89
T2645/Russia/BLKI
243
665
428
T1666/Iceland/ATPU
T1699/Iceland/BLKI
T1712/Iceland/COMU
686
T1656/Iceland/ATPU
T2023/Iceland/ATPU
T1938/Norway/ATPU
575
T1708/Iceland/COMU
623
163
617
304
177
297
214
299
154
T2916/Russia/TUPU
T2929/Russia/TUPU
T1682/Iceland/COMU
T2022/Iceland/ATPU
690
190
115
82
T2712/Russia/BLKI
T2885/Russia/TUPU
384
T2937/Russia/TUPU
T3221/Russia/RFCO
1
0.005

Figure 5.3 Phylogenetic analysis of *B. garinii* sequences. The maximum likelihood phylogeny was constructed using PhyML for two concatenated MLST genes (*pyrG* and *recG*). Triangles indicate sequences originating from the western Atlantic (this study) and the colour of the triangle represents the seabird colony: blue, Gannet Islands; red, Gull Island; black, Little Fogo Islands; yellow, Great Island. Green squares indicate samples from the eastern Atlantic and gray circles those from the Pacific (45). Closed symbols indicate samples from Common Murres with open symbols from other species. *Borrelia burgdorferi* was used as the outgroup, labelled as "1". Numbers at branch nodes represent support based on aBayes and the scale bar represents substitutions per site.

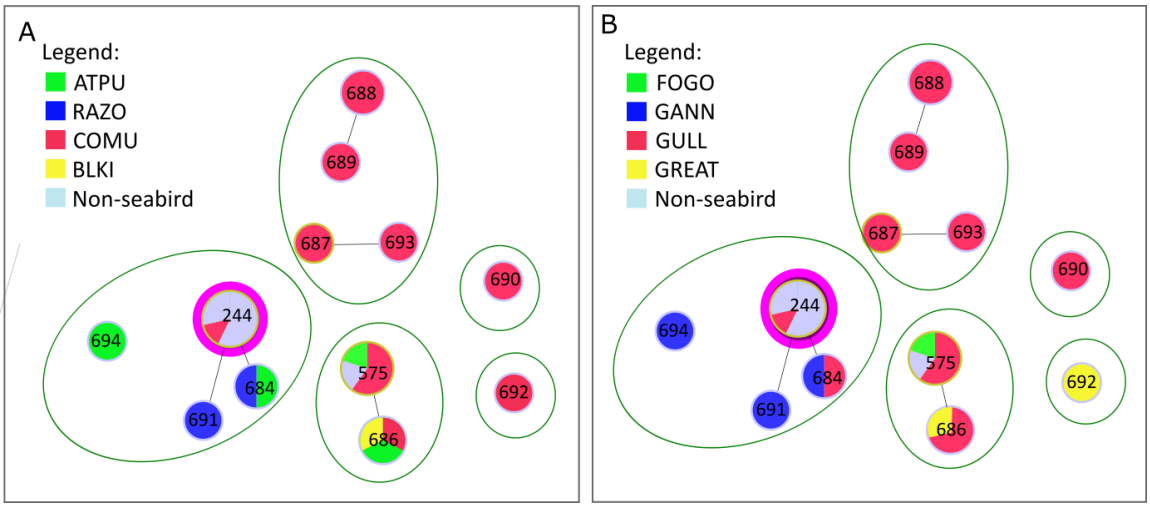

Figure 5.4 goeBURST network of 12 sequences types (STs) of *B. garinii* from this study. STs are highlighted by seabird hosts (A) and colonies of collection (C). Green circles denote BAPS clusters from BAPS analysis. The lines denote connections within clonal complexes. Sizes of the circles are proportional to number of samples in the STs. Inferred founder STs with > 60% bootstrap support are highlighted in pink. ATPU, Atlantic Puffin; RAZO, Razobill; COMU, Common Murre; BLKI, Black-legged Kittiwake; FOGO, Little Fogo Island; GANN, Gannet Islands; GULL, Gull Island; and GREAT, Great Island.

CHAPTER 6: SUMMARY

6.1. Zoonotic diseases

Research on zoonotic and emerging diseases has been steadily increasing since the mid 1900s. This has led to an increasing number of publications on these diseases, providing an increased understanding of how fast they can appear and move. Zoonotic diseases pose a great human threat, potentially leading to pandemics (e.g., influenza A virus H1N1 in 2009; 191). Zoonotic diseases can be caused by a wide range of pathogens: bacteria, viruses, fungi, protozoans or prions.

The way that zoonotic pathogens are transmitted between hosts, and to humans, can vary widely as well. Pathogens introduced to the host's system via inhalation of aerosol particles are often passed rapidly between hosts, and can cause large scale outbreaks (e.g., influenza A virus; 11). Those passed via bites or direct contact with bodily fluids are often easily traceable, as the point of contact between the human and non-human host can often be isolated (e.g., rabies virus; 192). Vector-borne diseases are those that are transmitted from one host to another via an arthropod vector (e.g., West Nile virus transmitted by mosquitos) (4). These diseases can be complex in transmission as the ecology of pathogen, vector, and host determines the prevalence of the disease.

The movement of zoonotic diseases can be complicated, involving the reservoir host and occasionally humans. Birds are often implicated in the movement of these diseases around the world, as observed for influenza A virus (4, 11). They also can play a role in vector-borne zoonotic disease as seen in *Borrelia burgdorferi* sensu lato (189,

193) and as discussed in this thesis. This includes songbirds migrating north-south across large landmasses (194) and seabirds which may migrate east-west across oceans (14, 15) and thereby connect continents otherwise considered isolated.

The ecologies of the vector and host also play important roles in determining the dynamics of vector-borne disease systems. For transmission of the pathogen, there must be a suitable number of hosts and vectors, and below a threshold density the pathogen cannot be efficiently transmitted within a population (195). Many factors can affect the population dynamics of both vectors and hosts. Vectors often constrain the pathogen population due to their short life cycles relative to that of their hosts. For example, the tick *Ixodes uriae* lives for two to eight years (35) whereas its seabird hosts, murres and puffins, can live for 20 years (37, 38, 69, 70). This can result in the pathogen-host abundances cycling between years, much like the pattern observed in predator-prey cycles.

6.2. Summary of findings

As part of my thesis work, I examined the seasonality of the seabird tick, *I. uriae*, and its age structure at four seabird colonies throughout Newfoundland and Labrador (Chapter 2). The number of *I. uriae* collected at these seabird colonies showed variation among years of collection, according to seabird host types present, and temporally within years at all three tick life stages. The seasonal patterns differ slightly between the three life stages, but are similar to those documented in past studies with one peak in June and increases late in the season (35, 57). The number of ticks is related to variation in precipitation between years for two life stages, consistent with the ticks' sensitivity to

desiccation (58). Previous studies have looked at the seasonal changes in *I. uriae* numbers. Seasonal increases in adult female and nymphal ticks in mid-July (57) is correlated with the peak chick-rearing period of the Atlantic Puffin (*Fratercula arctica*) and other primary host species. The seasonal dynamics of tick age structure are known to fluctuate among years, likely as a result of climatic changes (57), leading to different peak numbers of ticks and shifts in the timing of peaks. Climate influences the breeding behaviour and success of the ticks' primary hosts but also can directly affect *I. uriae* as they are sensitive to desiccation (58) and their activity and development are influenced by temperature (59). When collected directly from birds, the seasonality of *I. uriae* is also related to factors related to the associated hosts, with peaks of tick burdens differing among host species (57). Studies on seasonality of *I. uriae* in Newfoundland have focused on a single colony and looked primarily at ticks associated with Atlantic Puffins. Past work has also used a qualitative approach to analyzing seasonality without more detailed statistical quantitative analysis of seasonal variation. This demonstrates how environmental factors influence the number of ticks and age structure of ticks within the environment, which then has effects on the potential for disease transmission.

The application of statistical techniques to describe seasonality of tick numbers can be used for improved comparisons in future studies and will provide a better understanding of the environmental correlations. These data may play an important role as climate change leads to shifts in timing and breeding success of seabirds as well as alters the environmental conditions that are important to these ticks, as observed with respect to precipitation amounts and tick seasonality.

In another portion of my thesis work, I documented the prevalence of *B. garinii* in seabird colonies throughout the province of Newfoundland and Labrador, at several colonies and over four years (Chapter 3). Prior to my work, *Borrelia garinii* has only been observed in two colonies in North America (43). I documented *B. garinii* at three new seabird colonies within the province (Little Fogo Islands, Great Island, and Cape St. Mary's), representing an expansion of the known range for this species of bacteria along the western North Atlantic. These colonies span a wide geographical range. Documenting *B. garinii* at Cape St. Mary's is an important finding because this is the first location where this bacterium has been found on the mainland island of Newfoundland and close to humans in North America. This site has thousands of visitors each year that walk along the grassy cliffs to observe the breeding Northern Gannets (*Morus bassanus*). There are also sheep and other mammals that might allow for interactions between the marine and terrestrial cycles of *Borrelia* transmission. The colonies studied are also diverse with respect to their breeding species, with Little Fogo Islands being dominated by Atlantic Puffins and Cape St. Mary's having no Atlantic Puffins but dominated by Northern Gannets. The other colonies are more multi-species, with Common Murres (*Uria aalge*), Atlantic Puffins, Razorbills (*Alca torda*), and Black-legged Kittiwakes (*Rissa tridactyla*) found on Gull Island, Great Island, and the Gannet Islands.

In addition to studying the prevalence of the bacterium *B. garinii* within *I. uriae,* I determined the seroprevalence for *Borrelia* within the seabird hosts. Within the studied colonies, all seabird species surveyed showed evidence of previous exposure to *Borrelia,* demonstrating these bacteria are transmitted to a diverse collection of hosts. I also

identified other putative pathogenic bacteria present in the ticks and investigated the relationship of the tick bacterial communities to the *Borrelia* spp. infection status and location of tick collection.

During my study of the epizootiology of *B. garinii* associated with *I. uriae* and seabirds in Newfoundland and Labrador, I also found another species of *Borrelia*, *B. bavariensis*, in these ticks (Chapter 4). This appears to be the first record of this species that has been found outside Eurasia and in the marine realm. Most *Borrelia* sequences from *I. uriae* are described as *B. garinii* (30, 43-45, 140) with the rare case of *B. burgdorferi* sensu stricto and/or *B. lusitaniae* (44) being detected. My *B. bavariensis*-positive *I. uriae* were collected at three distinct seabird colonies and *B. bavariensis* actually represents 9% of my *Borrelia* detections at these sites. My analyses place these new Canadian sequences in the diverse Asian *B. bavariensis* clade, not in the European clade. This is surprising giving the relative proximities of these three locations and indicates that there are close links between *Borrelia* in seabird colonies and the terrestrial *B. bavariensis*-*I. ricinus*-rodent system in Eurasia, which is geographically far away and appears to have no direct ecological link. I also found that several *Borrelia* sequences previously reported from *I. uriae* from seabird colonies in Russia likely represent *B. bavariensis* and not *B. garinii*. Therefore, in addition to its previously known transmission in rodent-tick cycles, *B. bavariensis* appears to be transmitted between *I. uriae* and a variety of seabirds in both Eurasia and North America.

The marine cycle of *B. garinii* is complicated, covering a wide geographic range, many possible vertebrate hosts, but only a single vector species is currently known. These factors provide multiple opportunities for genetic diversification of the bacterium and this provides an interesting system to examine population genetics on multiple scales. There is evidence of transhemispheric movement of the bacterium, with detection of identical marker gene sequences in both the Northern and Southern Hemispheres (30). At the same time, there appears to be genetic differences between the Atlantic and Pacific Ocean basins in the Northern Hemisphere, based on a multilocus analysis approach (45). However, within these ocean basins, evolution is clonal and little structure is apparent (45). Compared to the classical terrestrial realm of *B. garinii* circulation, there is more genetic diversity in the *B. garinii*-*I. uriae*-seabird system (46). Recombination analysis has demonstrated admixture between the two genetic pools (45) and it is therefore important to study both the seabird and other terrestrial *B. garinii* cycles.

I examined the population structure of *B. garinii* from *I. uriae* within Newfoundland and Labrador seabird colonies and evaluated how this structure relates to what is found throughout Asia and Europe. This was done using a MLST scheme applied to multiple *Borrelia* species and with data from throughout Eurasia and North America (141, 146, 196), and represents the first time that MLST has been applied in its entirety to this system. I added 10 novel sequence types (STs) and multiple new allele sequences at all eight MLST loci to the *Borrelia* database. This represents an increase in the known genetic diversity for this species and thereby expands our knowledge of global *B. garinii* diversity. I determined that there is population structure in *B. garinii,* at both local and

global scales. At the local scale, sequences show evidence of genetic clustering by both seabird colonies and seabird hosts. Sequences found in Newfoundland and Labrador do not all cluster together but instead show similarity to those found in clinical samples and terrestrial tick species in Europe. This may suggest multiple introductions and a close connection to the terrestrial realm of *B. garinii* cycling (45). The Newfoundland and Labrador sequences also show a high similarity to those previously collected from the eastern Atlantic (44).

This *B. garinii-I. uriae*-seabird system remains relatively under-studied, with most previous work focusing on the eastern Atlantic within colonies in Norway and Iceland (44, 88, 161, 197-199). Though there has been work outside of this geographic area, including Russia (45), Newfoundland (43), and Antarctica (47), few studies have taken a comprehensive approach incorporating information from terrestrial Eurasia alongside marine samples. My research used MLST, which allowed for this more comprehensive approach to be taken. The highly dynamic nature of the *B. garinii-I. uriae*-seabird system makes it important to conduct multi-year and even multi-decade studies. It is also important to integrate ecological knowledge about seabirds, as they are likely one of the key drivers to the geographic patterns observed in *B. garinii* and other *Borrelia* species observed in these seabird colonies. The close connection between *B. garinii* in the marine realm and clinical samples from Europe exemplifies the importance of these seabirds to the movement of zoonotic diseases worldwide and the relevance in studying these seemingly isolated systems when considering diseases of human importance.

6.3. Future work

Even though my research adds to our knowledge about this complicated *B. garinii-I. uriae*-seabird system we still have more work to do. My research found a previously undocumented *Borrelia* species in the marine realm, raising questions of what other unexpected species are circulating between *I. uriae* and seabirds. Studies documenting the prevalence of *Borrelia* spp. in seabird colonies throughout the North Atlantic would provide important information on the geographic distributions of these bacteria and contribute to a better understanding of the complex patterns of vector-borne zoonotic diseases. In Atlantic Canada there are over 50 seabird colonies with breeding auks (33), the main hosts for *I. uriae*. Even with my research, only five of these colonies have been surveyed. That leaves the majority where little or no information is known about the presence of *Borrelia* spp. or *I. uriae*. These colonies include those at the limits of the breeding ranges of auks, which can provide information about the latitudinal constraints on *I. uriae* and therefore *Borrelia* spp. associated with the marine realm. It also includes colonies that have closer connections with human populations, such as Machias Seal Island in the Gulf of Maine and Bonaventure Island in Québec, which have frequent tourist visitors.

Though work has focused on the seasonality of ticks within these colonies, more is known about their terrestrial counterparts, *I. scapularis* and *I. ricinus*. *Ixodes uriae* has a very different ecology than these tick species, with their hosts being marine and only coming to land for very short periods, thereby limiting when attachment and feeding are possible. The compositional diversity on these seabird colonies and effects that latitude

and ocean currents have on the breeding biology of seabirds and suitability of tick over-wintering environments make each colony unique and, without a broad understanding of what affects the seasonality of ticks, it is not possible to understand the variation of risk of *B. garinii* infection within these colonies. In conjunction, it is also important to look at the seasonality of *B. garinii* risk from ticks themselves as this will help to understand the important parts of the seabird ecology. It will also inform us on when risk is highest for researchers and other visitors to colonies. This seasonal information could also provide information on whether *B. garinii* has the risk of entering the terrestrial realm in North America in *I. scapularis* or another North American tick species.

Understanding the geographic connections between bacteria in the genus *Borrelia* is very relevant for understanding the movement and ecology of other zoonotic diseases that are transported by seabirds. The population genetics of *B. garinii* and how the marine and terrestrial realms interact is obviously complicated, and the two realms are clearly not distinct. It is important that we sample the bacterial population to saturation to get a better understanding of the total diversity that exists. This would lead to a better understanding of how tightly the two realms are connected and the level of gene flow between them. It also would highlight the importance of seabirds to the dissemination of clinically relevant strains. With many seabird colonies being unexplored with respect to *B. garinii*, it brings into question how these colonies are interconnected. Additionally, how all seabird colonies fit into the *B. garinii* structure we observed, with Eurasia and seabird colonies of the eastern North Atlantic tightly connected through seabird migration. Ultimately, we still need to ask how seabirds facilitate the global transmission of *B. garinii*.

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APPENDICES

Appendix 1: Supplementary material for Chapter 2

Supplementary Table 1. Summary of months and years of tick collections at five seabird colonies in Newfoundland and Labrador.

	Year and month[a] of field work:															
	2011			2012							2013			2014		2015
Colony	**Ju**	**Jl**	**A**	**Ju**	**Jl**	**A**	**Ju**	**Jl**	**A**	**Ju**	**Jl**	**A**	**Ju**	**Jl**	**A**	
Cape St. Mary's				X[b]	X		X									
Gannet Islands			X					X	X							
Great Island						X						X				
Gull Island	X	X		X	X	X	X	X	X	X	X			X		
Little Fogo Islands											X					

[a]Month: Jn, June; Jl, July; A, August

[b]X: ticks were collected during this time period

Supplementary Table 2. Summary of number of ticks collected at colony each day broken down by life stage and from environment around breeding seabirds.

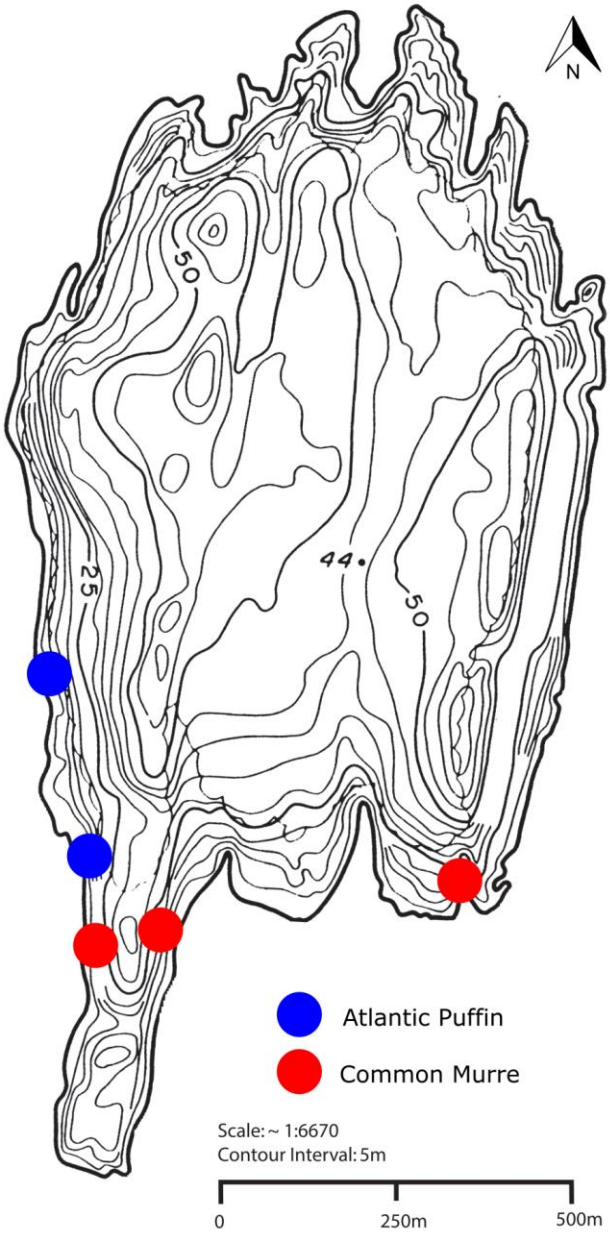
Year	Date	Number of ticks from environment[a]			
		COMU[b]	RAZO	ATPU	Unknown
Cape St. Mary's (2012, 3 days; 2013, 1 day)					
2012	May 29				0,0,2
	June 20				0,0,2
	July 20				0,0,1
2013	June 18				0,0,3
Gannet Islands (2011, 3 days; 2013, 4 days)					
2011	August 1			0,0,5	
	August 5			0,0,6	
	August 6			0,0,22	
2013	July 28			0,0,1	
	July 29		68,2,12	0,0,26	
	August 1			0,0,11	
	August 2			0,0,2	
Great Island (2012, 1 day; 2014, 1 day)					
2012	August 1	0,3,9			
2014	August 5	2,494,140			
Gull Island (2011, 5 days; 2012, 9 days; 2013, 12 days; 2014, 7 days; 2015, 1 day)					
2011	July 17			0,0,1	
	July 21			0,0,2	
	July 26			0,0,2	
	July 27			0,3,0	
	July 31			0,0,6	
2012	June 2	6,0,1			
	June 16	69,7,2			
	June 26	0,0,1		0,0,1	
	July 7			0,0,1	
	July 18	0,3,1			
	July 23			0,0,1	
	July 24	0,5,6			
	August 3	0,2,0			
2013	June 12	6,1,0			
	June 17	37,14,28			
	June 20	0,13,11			
	June 28	3,14,8			

Year	Date	Number of ticks from environment[a]			
		COMU[b]	RAZO	ATPU	Unknown
	June 29	7,6,4			
	June 30	28,18,46		0,0,4	
	July 1	9,3,2			
	July 2			1,1,0	
	July 5			0,1,0	
	July 6			0,1,0	
	July 10			0,2,1	
	August 9			0,1,0	
2014	June 11	3,10,2			
	June 18	3,75,164		0,1,0	
	July 15			0,0,1	
	July 16			0,0,1	
	July 18			0,0,2	
	July 21	0,0,2			
	July 28	0,77,7			
2015	July 8	183,58,21			
		Little Fogo Islands (2014, 9 days)			
	July 2			0,1,0	
	July 3			1,0,0	
	July 4			0,1,0	
	July 5			0,1,0	
	July 6			0,1,0	
	July 7			0,1,0	
	July 8			0,1,0	
	July 9			0,59,16	

[a]Collected from habitat around breeding Common Murre (COMU), Razorbill (RAZO), Atlantic Puffin (ATPU), and unknown species.
[b]Broken down by larva, nymph, and adult

Supplementary Table 3. Origin of climatic data used for each colony.

Colony	Monthly weather station	Distance to colony	30-year average weather station	Distance to colony
Gull Is. 47.26228°N, 52.7738°W	St John's West Climate 47.51 N 52.78 W	27.55 km	Same	27.55 km
Great Is. 47.18205°N, 52.8093°W	St John's West Climate 47.51 N 52.78 W	36.53 km	Same	36.53 km
Cape St. Mary's 46.82149°N, 54.1837°W	St. Mary's 46.91 N 53.56 W	48.53 km	Same	48.53 km
Little Fogo Is. 49.84098°N, 54.1168°W	Twillingate 49.68 N 54.8 W	52.38 km	Musgrave Harbour 49.45 N 53.98 W	44.59 km
Gannet Is. 53.9364°N, 56.5758°W	Cartwright 53.71 N 57.04 W	39.62 km	Same	39.62 km



Supplementary Figure 1. Map of Gull Island showing locations where ticks from the breeding habitats around Common Murres (red) and Atlantic Puffins (blue) were collected. Ticks from Great Island were collected from the southern-most point. All ticks from Cape St. Mary's were collected from the western portion of the colony located on the Island of Newfoundland. In Little Fogo Islands ticks were collected from Bakeapple and Hennessey Islands. Ticks from the Gannet Islands were collected on islands known as GC1 and GC4.

Appendix 2: Supplementary material for Chapter 3

Positive/negative cut-off values used in serological analysis

I used the method described in Garnier et al (102) to determine the best fit to the data between one normal distribution (all samples considered negative or positive) or a mixture of two normal distributions (mixture of negative and positive samples). In this method, if the two-normal distribution is estimated as the best fit, the cut-offs are calculated relative to the negative sample distribution as:

$cut_{99} = mean_{neg} + 3 * sd_{neg}$

(samples are considered negative with 99 % confidence interval)

$cut_{95} = mean_{neg} + 2 * sd_{neg}$

(samples are considered negative with 95 % confidence interval)

Samples with $cut_{95} \leq OD < cut_{99}$ were considered ambiguous

Due to the difficulties in determining the cut-offs (only a few samples per supposed serological state per species), species were grouped as following:

Alcidae: Atlantic Puffin, Razorbill, Common Murre, and Thick-billed Murre (Supplementary Figures 1 and 2)

Laridae: Black-legged Kittiwake and Herring Gull (Supplementary Figures 3 and 4)

Supplementary Table 1. Summary of cut-off values used in serological study.

Group	Negative	Ambiguous	Positive	Comment
Alcidae	< 2.25	≥ 2.25 < 2.54	≥ 2.54	Relative to the positive samples distribution
Laridae	< 0.71	≥ 0.71 < 0.72	≥ 0.72	Relative to the negative samples distribution

Supplementary Table 1. Genera found in soil samples and at least 50% of the tick samples.

Rank2[a]	**Rank3**	**Rank4**	**Rank5**	**Rank6**	**Average**[b]	**Count**[c]
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae (Subgroup1)	uncultured	0.001043	10
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae (Subgroup1)	*Granulicella*	0.004803	11
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Leucobacter*	0.00143	12
Actinobacteria	Actinobacteria	Corynebacteriales	Dietziaceae	*Dietzia*	0.011712	17
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	0.001411	14
Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	*Mycobacterium*	0.076283	17
Actinobacteria	Actinobacteria	NA[d]	NA	NA	0.002787	14
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	NA	0.003393	16
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	*Microbacterium*	0.004239	14
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	*Nocardioides*	0.002036	15
Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	*Marmoricola*	0.001269	11
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter*	0.003624	15
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Gelidibacter*	0.016904	17
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	0.034174	16
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Aequorivita*	0.018094	17
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NA	0.006564	15
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Chryseobacterium*	0.028641	18
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	KD3-93	NA	0.052702	6
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Ferruginibacter*	0.002764	13
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	uncultured	0.032551	18
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	*Erysipelothrix*	0.00255	13
Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Atopostipes*	0.003978	14
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured	0.002795	11
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ottowia*	0.002198	12

Rank2[a]	Rank3	Rank4	Rank5	Rank6	Average[b]	Count[c]
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	0.000503	12
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	0.004425	19
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	NA	0.005984	14
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Mizugakiibacter*	0.036442	12
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Rhodanobacter*	0.023238	17
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Lysobacter*	0.001597	16
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0.00967	15
Proteobacteria	NA	NA	NA	NA	0.218007	19
Proteobacteria	Gammaproteobacteria	NA	NA	NA	0.004846	15
Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Oceanisphaera*	0.012199	16
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA	0.01043	10
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	0.002389	12
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	0.000958	14
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	0.000548	10
Saccharibacteria	NA	NA	NA	NA	0.000862	10
TM6	NA	NA	NA	NA	0.003422	11

[a]Rank represents the taxonomy classification assigned using the SILVA SSU database with Rank6 representing genus.
[b]The average number of reads within samples containing reads belonging to a specific genus.
[c]The number of samples containing at least one read belonging to a specific genus.
[d]NA, sequence could not be classified below this rank.

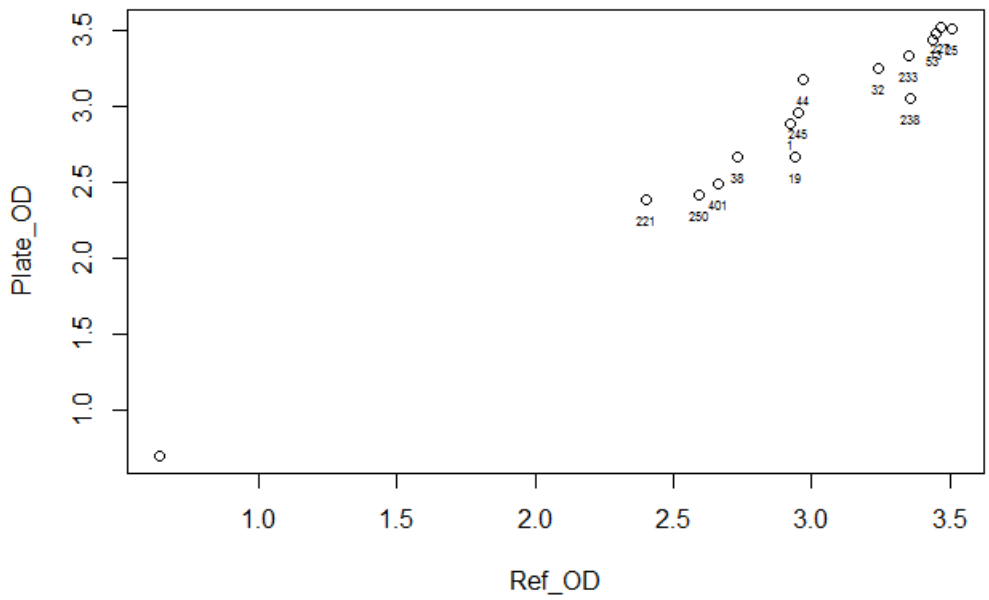
Supplementary Table 2. Genera found only in ticks, and found in at least 50% of samples.

Rank2[a]	Rank3	Rank4	Rank5	Rank6	Average[b]	Count[c]
Actinobacteria	Actinobacteria	Micrococcales	Brevibacteriaceae	*Brevibacterium*	0.016	15
Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	*Brachybacterium*	0.001	11
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Paeniglutamicibacter*	0.002	12
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Arthrobacter*	0.004	16
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriales	*Tomitella*	0.003	13
Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	*Gordonia*	0.011	13
Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	*Rhodococcus*	0.002	13
Chloroflexi	Thermomicrobia	JG30-KF-CM45	NA	NA	0.001	12
FBP	NA	NA	NA	NA	0.003	14
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	*Brevibacillus*	0.047	6
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Anoxybacillus*	0.010	6
Firmicutes	Bacilli	Bacillales	Bacillaceae	NA	0.004	8
Firmicutes	Bacilli	Bacillales	Planococcaceae	*Sporosarcina*	0.005	18
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	0.002	10
Firmicutes	NA	NA	NA	NA	0.001	12
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	0.006	5
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Catellicoccus*	0.000	12
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	*Paenibacillus*	0.004	5
Firmicutes	Clostridia	Clostridiales	FamilyXI	*Gottschalkia*	0.006	17
Firmicutes	Clostridia	Clostridiales	FamilyXI	*Peptoniphilus*	0.005	1
Firmicutes	Clostridia	Clostridiales	FamilyXI	*Tissierella*	0.002	14
Firmicutes	Clostridia	Clostridiales	FamilyXI	*Anaerococcus*	0.005	3
Acidobacteria	Blastocatellia	Blastocatellales	Blastocatellaceae (Subgroup4)	*Blastocatella*	0.001	10
Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	*Deinococcus*	0.000	11

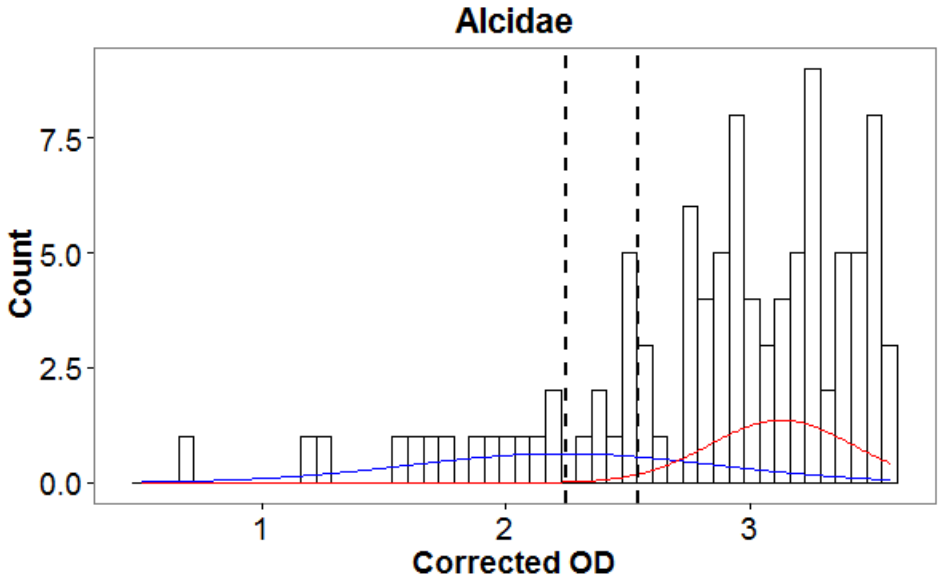
Rank2[a]	Rank3	Rank4	Rank5	Rank6	Average[b]	Count[c]
Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	*Nitrosococcus*	0.003	10
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Simplicispira*	0.004	16
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Delftia*	0.001	11
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Schlegelella*	0.045	8
Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Pusillimonas*	0.002	15
Proteobacteria	Betaproteobacteria	Hydrogenophilales	Hydrogenophilaceae	Uncultured	0.031	4
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	0.012	19
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia-Paraburkholderia*	0.003	16
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	0.060	7
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Psychrobacter*	0.016	13
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Alkanindiges*	0.004	12
Proteobacteria	Gammaproteobacteria	Xanthomonadales	JTB255	NA	0.011	2
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Providencia*	0.178	3
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	*Gemmatimonas*	0.003	11
Proteobacteria	Alphaproteobacteria	Rhizobiales	OCS116clade	NA	0.007	1
Proteobacteria	Alphaproteobacteria	Sphingomonadales	NA	NA	0.007	2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	*Erythrobacter*	0.007	1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Phyllobacterium*	0.003	18
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Rhodovastum*	0.003	11
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria	Unknown Family	Uncultured	0.008	1
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pontibaca*	0.011	11
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Roseovarius*	0.014	15
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Uncultured	0.001	13
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Rhodobacter*	0.003	15
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	0.004	11
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Paracoccus*	0.016	19

Rank2[a]	Rank3	Rank4	Rank5	Rank6	Average[b]	Count[c]
Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*	0.024	9
Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.227	9
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Persicitalea*	0.003	10
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Hymenobacter*	0.001	11
Bacteroidetes	Cytophagia	Cytophagales	Cyclobacteriaceae	*Algoriphagus*	0.005	5
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Marixanthomonas*	0.007	1
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Gilvibacter*	0.004	2
Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	0.000	10
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	0.001	10
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Moheibacter*	0.002	11
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Taibaiella*	0.006	16
BRC1	NA	NA	NA	NA	0.001	10
Verrucomicrobia	Spartobacteria	Chthoniobacterales	NA	NA	0.014	1
Actinobacteria	Actinobacteria	Micrococcales	Dermacoccaceae	*Flexivirga*	0.004	9
Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	*Janibacter*	0.001	11
Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Tessaracoccus*	0.002	15

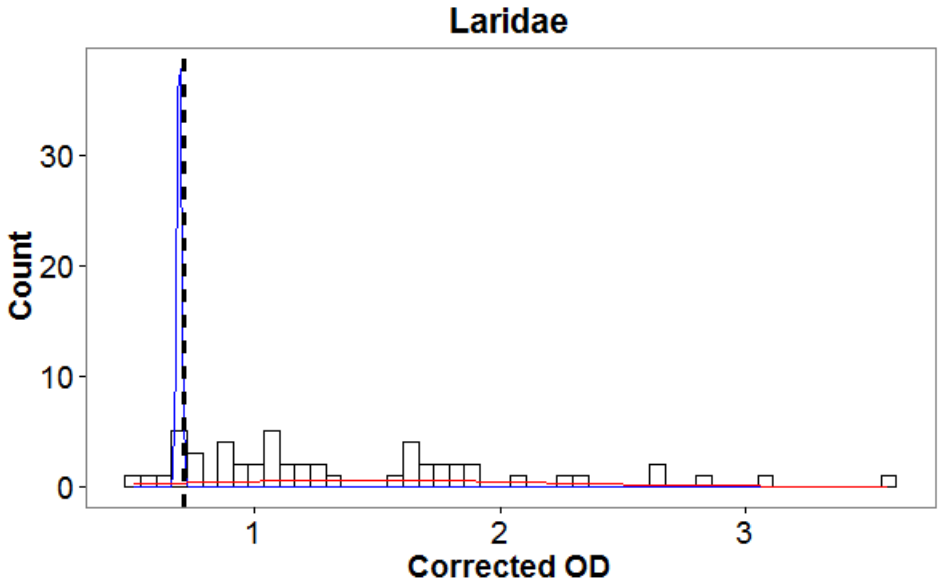
[a]Rank represents the taxonomy classification assigned using the SILVA SSU database with Rank6 representing genus.
[b]The average number of reads within samples containing reads belonging to a specific genus.
[c]The number of samples containing at least one read belonging to a specific genus.
[d]NA, sequence could not be classified below this rank.



Supplementary Figure 1. Repeatability of serological results between two replicate plates. X-axis represents plate 1 optical density readings and y-axis represents plate 2 optical density readings. Linear regression of plate 2 on plate 1 for 16 replicated samples ($y = 1.18e^{-3} + 0.985 * x$, $r^2 = 0.966$)



Supplementary Figure 2. Distribution of Alcidae samples over corrected optical densities from the ELISA. The red line represents the distribution of positive samples and the blue line represents the distribution of a mixture of positive and negative samples. The dotted lines represent the two cut-off values with samples left of the first dotted line being negative, those to the right of the second line being positive, and those in the middle being ambiguous.



Supplementary Figure 3. Distribution of Laridae samples over corrected optical densities from the ELISA. The red line represents the distribution of positive samples and the blue line represents the distribution of a mixture of positive and negative samples. The dotted lines represent the two cut-off values with samples left of the first dotted line being negative, those to the right of the second line being positive, and those in the middle being ambiguous.

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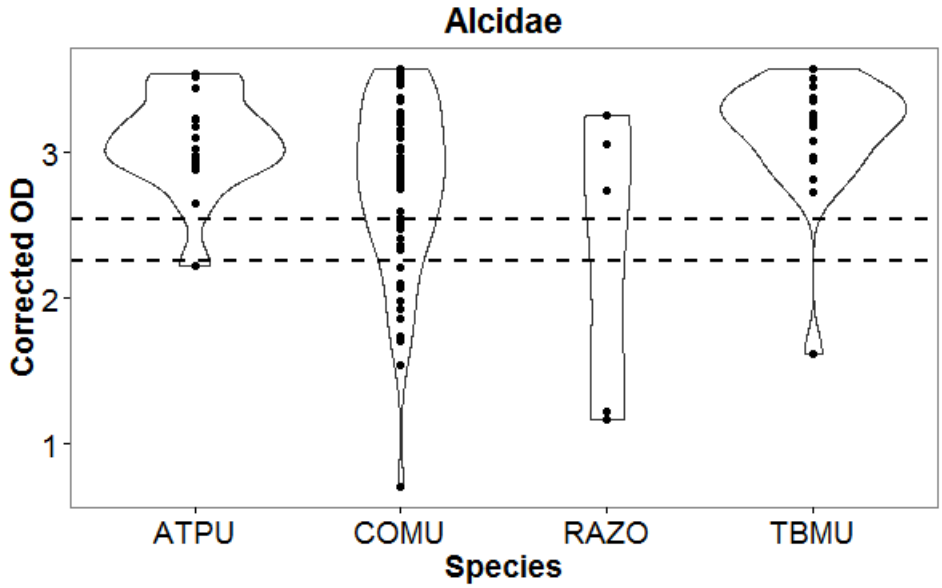
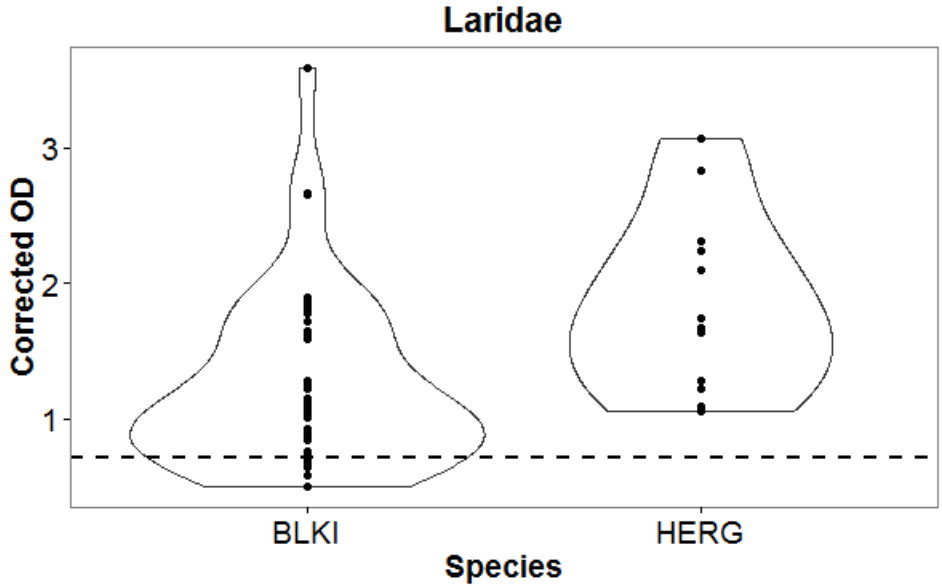


Supplementary Figure 4. Distribution of samples for each Alcidae species with respect to the corrected optical densities from the ELISA. The dotted lines represent the two cut-off values with samples below the first dotted line being negative, those above the second line being positive, and those in the middle being ambiguous. ATPU, Atlantic Puffin; COMU, Common Murre; RAZO, Razorbill; and TBMU, Thick-billed Murre.



Supplementary Figure 5. Distribution of samples for the two Laridae species with respect to the corrected optical densities from the ELISA. The dotted lines represent the two cut-off values with samples below the first dotted line being negative, those above the second line being positive, and those in the middle being ambiguous. BLKI, Black-legged Kittiwake; and HERG, Herring Gull.

Appendix 3: Supplementary material for Chapter 4

Supplementary Table 1. Alleles associated with *B. bavariensis* within the MLST database (as of November 2016 from http://pubmlst.org/borrelia/) that were used to determine the closest matches for MLST gene sequences in this study.

Allele number							
clpA	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***
41	26	27	37	28	35	26	32
69	53	28	62	58	57	49	53
56	42	43	51	47	46	38	42
70	54	41	63	59	58	50	54
72	55	53	64	60	59	52	55
73	47	54	56	52	60	43	47
60	45	55	54	50	49	41	45
62	63	44	76	71	51	64	67
85	64	46	77	72	70	65	68
86	65	62	69	73	72	66	69
87	67	52	79	75	48	68	71
90	68	64	80	76	115	40	44
59	44	65	53	49	119	115	111
57	92	66	135	128	127	108	112
153	99	110	130	129	128	110	113
144	108	105	131	130	129	111	114
145	103	106	132	131	130	112	115
146	104	107	134	132	132	113	116
147	105	109	146	133	143	114	117
148	106	121	167	135	146	125	119
154	109	145	168	143	116	128	130
150	107	158	184	146	149	153	135
166	127	159		152	173	166	139
168	128			155	176	167	146
197	137			174	184		163
207	136			192	199		169
208	153						182
	161						183
	162						184
	175						

Supplementary Table 2. Accession numbers for all literature and reference sequences used in this study.

Designation	Location	Source	Tick host	Accession number(s)
05-F35P	Canada	*I. uriae*	Unknown	DQ463373
Far01	Faroe Is	*I. uriae*	*Fr. arctica*	AJ009749, AY737710, AY330929, DQ986206
Far02	Faroe Is	*I. uriae*	*Fr. arctica*	AJ009750, AF080262, AF080264, AY330930
Far03	Faroe Is	Bird blood	*Fr. arctica*	AJ009752, AF080261, AF080263
Far04	Faroe Is	Bird blood	*Fr. arctica*	AJ009753
Fis01	Iceland	*I. uriae*	*Fr. arctica*	AJ009751, AY330931
Var1	Norway	*I. uriae*	*U. aalge*	EF190482
Var2	Norway	*I. uriae*	*U. aalge*	EF190483
Var3	Norway	*I. uriae*	*U. aalge*	EF190484
Var4	Norway	*I. uriae*	*U. aalge*	EF190485
T1755	Norway	*I. uriae*	*U. aalge*	EF154357
T1715	Iceland	*I. uriae*	*R. tridactyla*	EF154358
T1885	Iceland	*I. uriae*	*R. tridactyla*	EF154359
T1720	Iceland	*I. uriae*	*U. aalge*	EF154361
T1751	Iceland	*I. uriae*	*R. tridactyla*	EF154362
T1750	Iceland	*I. uriae*	*R. tridactyla*	EF154363
T1723	Iceland	*I. uriae*	*U. aalge*	EF154364
T1783	Iceland	*I. uriae*	*R. tridactyla*	EF154365
T1870	Iceland	*I. uriae*	*Fr. arctica*	EF154366
T1760	Iceland	*I. uriae*	*U. aalge*	EF154367
T1742	Iceland	*I. uriae*	*U. aalge*	EF154368
T1739	Iceland	*I. uriae*	*U. aalge*	EF154369
T1781	Iceland	*I. uriae*	*R. tridactyla*	EF154370
T1638	Iceland	*I. uriae*	*Fr. arctica*	EF154371
T1788	Iceland	*I. uriae*	*R. tridactyla*	EF154372
T1674	Iceland	*I. uriae*	*Fr. arctica*	EF154373
T1828	Iceland	*I. uriae*	*U. aalge*	EF154374
T1708	Iceland	*I. uriae*	*U. aalge*	EF154375
T703	Norway	*I. uriae*	*U. aalge*	EF154376
T1849	Iceland	*I. uriae*	*Fu. glacialis*	EF154377
T1890	Iceland	*I. uriae*	*R. tridactyla*	EF154378
T1734	Iceland	*I. uriae*	*U. aalge*	EF154379
T704	Norway	*I. uriae*	*U. aalge*	EF154381
T1793	Iceland	*I. uriae*	*Fr. Arctica*	EF154382
T1823	Iceland	*I. uriae*	*A. torda*	EF154383
T1845	Iceland	*I. uriae*	*Fu. glacialis*	EF154384
T1697	Iceland	*I. uriae*	*R. tridactyla*	EF154385

Designation	Location	Source	Tick host	Accession number(s)
T1904	Iceland	*I. uriae*	*Fr. arctica*	EF154387
T1888	Iceland	*I. uriae*	*R. tridactyla*	EF154388
T1862	Iceland	*I. uriae*	*Fr. arctica*	EF154389
T1604	Iceland	*I. uriae*	*R. tridactyla*	EF154390
T1878	Iceland	*I. uriae*	*Fr. arctica*	EU567327
T693	Norway	*I. uriae*	*U. aalge*	EU567328
T1740	Iceland	*I. uriae*	*U. aalge*	EU567329
T1131	United Kingdom	*I. uriae*	*U. aalge*	JF331014, JF331066, JF331118, JF330910, JF331274, JF331326, JF331378, JF331222, JF330962, JF331170
T1377	United Kingdom	*I. uriae*	*Fr. arctica*	JF331015, JF331067, JF331119, JF330911, JF331275, JF331327, JF331379, JF331223, JF330963, JF331171
T1638	Iceland	*I. uriae*	*Fr. arctica*	JF331016, JF331068, JF331120, JF330912, JF331276, JF331328, JF331380, JF331224, JF330964, JF331172
T1656	Iceland	*I. uriae*	*Fr. arctica*	JF331017, JF331069, JF331121, JF330913, JF331277, JF331329, JF331381, JF331225, JF330965, JF331173
T1666	Iceland	*I. uriae*	*Fr. arctica*	JF331018, JF331070, JF331122, JF330914, JF331278, JF331330, JF331382, JF331226, JF330966, JF331174
T1667	Iceland	*I. uriae*	*Fr. arctica*	JF331019, JF331071, JF331123, JF330915, JF331279, JF331331, JF331383, JF331227, JF330967, JF331175
T1669	Iceland	*I. uriae*	*Fr. arctica*	JF331020, JF331072, JF331124, JF330916, JF331280, JF331332, JF331384, JF331228, JF330968, JF331176
T1673	Iceland	*I. uriae*	*Fr. arctica*	JF331021, JF331073,

Designation	Location	Source	Tick host	Accession number(s)
				JF331125, JF330917, JF331281, JF331333, JF331385, JF331229, JF330969, JF331177
T1682	Iceland	*I. uriae*	*U. aalge*	JF331022, JF331074, JF331126, JF330918, JF331282, JF331334, JF331386, JF331230, JF330970, JF331178
T1697	Iceland	*I. uriae*	*R. tridactyla*	JF331023, JF331075, JF331127, JF330919, JF331283, JF331335, JF331387, JF331231, JF330971, JF331179
T1699	Iceland	*I. uriae*	*R. tridactyla*	JF331024, JF331076, JF331128, JF330920, JF331284, JF331336, JF331388, JF331232, JF330972, JF331180
T1708	Iceland	*I. uriae*	*U. aalge*	JF331025, JF331077, JF331129, JF330921, JF331285, JF331337, JF331389, JF331233, JF330973, JF331181
T1712	Iceland	*I. uriae*	*U. aalge*	JF331026, JF331078, JF331130, JF330922, JF331286, JF331338, JF331390, JF331234, JF330974, JF331182
T1727	Iceland	*I. uriae*	*Fr. arctica*	JF331027, JF331079, JF331131, JF330923, JF331287, JF331339, JF331391, JF331235, JF330975, JF331183
T1789	Iceland	*I. uriae*	*R. tridactyla*	JF331028, JF331080, JF331132, JF330924, JF331288, JF331340, JF331392, JF331236, JF330976, JF331184
T1793	Iceland	*I. uriae*	*R. tridactyla*	JF331029, JF331081, JF331133, JF330925, JF331289, JF331341, JF331393, JF331237,

Designation	Location	Source	Tick host	Accession number(s)
				JF330977, JF331185
T1794	Iceland	*I. uriae*	*Fr. arctica*	JF331030, JF331082, JF331134, JF330926, JF331290, JF331342, JF331394, JF331238, JF330978, JF331186
T1795	Iceland	*I. uriae*	*Fr. arctica*	JF331031, JF331083, JF331135, JF330927, JF331291, JF331343, JF331395, JF331239, JF330979, JF331187
T1796	Iceland	*I. uriae*	*Fr. arctica*	JF331032, JF331084, JF331136, JF330928, JF331292, JF331344, JF331396, JF331240, JF330980, JF331188
T1798	Iceland	*I. uriae*	*Fr. arctica*	JF331033, JF331085, JF331137, JF330929, JF331293, JF331345, JF331397, JF331241, JF330981, JF331189
T1799	Iceland	*I. uriae*	*Fr. arctica*	JF331034, JF331086, JF331138, JF330930, JF331294, JF331346, JF331398, JF331242, JF330982, JF331190
T1815	Iceland	*I. uriae*	*A. torda*	JF331035, JF331087, JF331139, JF330931, JF331295, JF331347, JF331399, JF331243, JF330983, JF331191
T1866	Iceland	*I. uriae*	*Fr. arctica*	JF331036, JF331088, JF331140, JF330932, JF331296, JF331348, JF331400, JF331244, JF330984, JF331192
T1867	Iceland	*I. uriae*	*Fr. arctica*	JF331037, JF331089, JF331141, JF330933, JF331297, JF331349, JF331401, JF331245, JF330985, JF331193
T1869	Iceland	*I. uriae*	*Fr. arctica*	JF331038, JF331090, JF331142, JF330934,

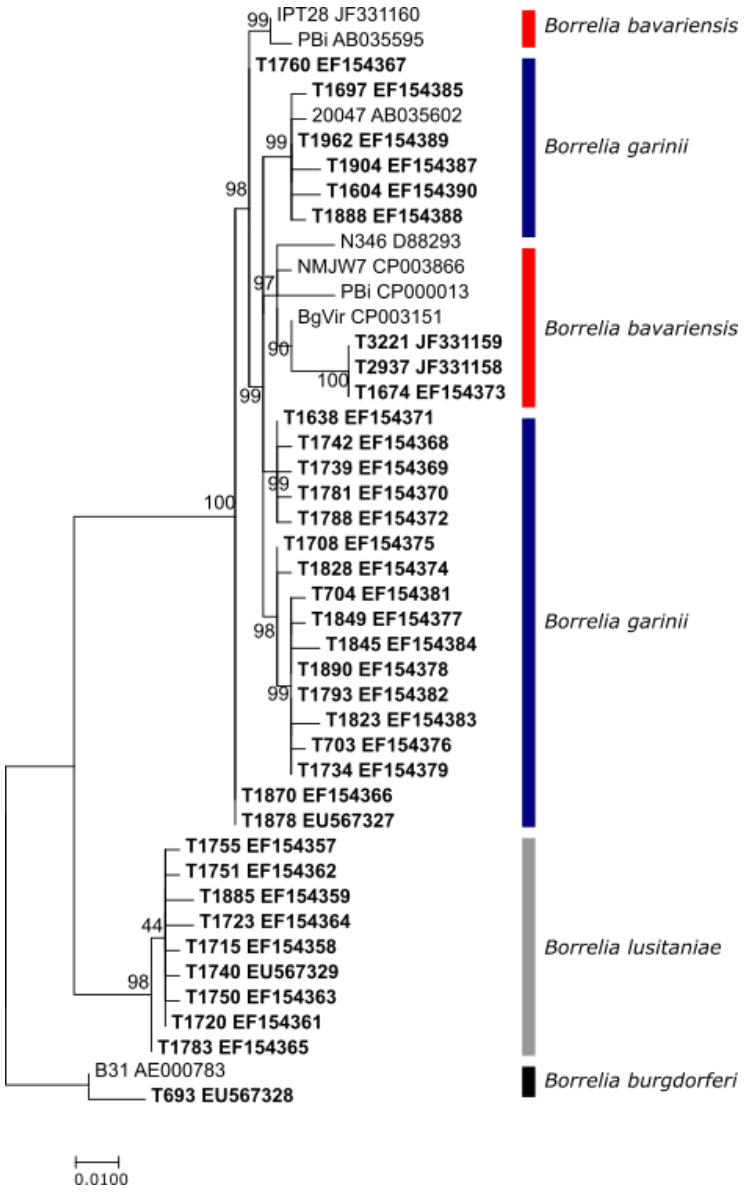
Designation	Location	Source	Tick host	Accession number(s)
				JF331298, JF331350, JF331402, JF331246, JF330986, JF331194
T1874	Iceland	*I. uriae*	*Fr. arctica*	JF331039, JF331091, JF331143, JF330935, JF331299, JF331351, JF331403, JF331247, JF330987, JF331195
T1913	Iceland	*I. uriae*	*Fu. glacialis*	JF331040, JF331092, JF331144, JF330936, JF331300, JF331352, JF331404, JF331248, JF330988, JF331196
T1938	Norway	*I. uriae*	*Fr. arctica*	JF331041, JF331093, JF331145, JF330937, JF331301, JF331353, JF331405, JF331249, JF330989, JF331197
T1940	Norway	*I. uriae*	*Fr. arctica*	JF331042, JF331094, JF331146, JF330938, JF331302, JF331354, JF331406, JF331250, JF330990, JF331198
T2017	Iceland	*I. uriae*	*Fr. arctica*	JF331043, JF331095, JF331147, JF330939, JF331303, JF331355, JF331407, JF331251, JF330991, JF331199
T2021	Iceland	*I. uriae*	*Fr. arctica*	JF331044, JF331096, JF331148, JF330940, JF331304, JF331356, JF331408, JF331252, JF330992, JF331200
T2022	Iceland	*I. uriae*	*Fr. arctica*	JF331045, JF331097, JF331149, JF330941, JF331305, JF331357, JF331409, JF331253, JF330993, JF331201
T2023	Iceland	*I. uriae*	*Fr. arctica*	JF331046, JF331098, JF331150, JF330942, JF331306, JF331358, JF331410, JF331254, JF330994, JF331202

Designation	Location	Source	Tick host	Accession number(s)
T2024	Iceland	*I. uriae*	*Fr. arctica*	JF331047, JF331099, JF331151, JF330943, JF331307, JF331359, JF331411, JF331255, JF330995, JF331203
T2299	Norway	*I. uriae*	*Fr. arctica*	JF331048, JF331100, JF331152, JF330944, JF331308, JF331360, JF331412, JF331256, JF330996, JF331204
T2645	Russia	*I. uriae*	*R. tridactyla*	JF331049, JF331101, JF331153, JF330945, JF331309, JF331361, JF331413, JF331257, JF330997, JF331205
T2712	Russia	*I. uriae*	*R. tridactyla*	JF331050, JF331102, JF331154, JF330946, JF331310, JF331362, JF331414, JF331258, JF330998, JF331206
T2885	Russia	*I. uriae*	*Fr. cirrhata*	JF331051, JF331103, JF331155, JF330947, JF331311, JF331363, JF331415, JF331259, JF330999, JF331207
T2916	Russia	*I. uriae*	*Fr. cirrhata*	JF331052, JF331104, JF331156, JF330948, JF331312, JF331364, JF331416, JF331260, JF331000, JF331208
T2929	Russia	*I. uriae*	*Fr. cirrhata*	JF331053, JF331105, JF331157, JF330949, JF331313, JF331365, JF331417, JF331261, JF331001, JF331209
T2937	Russia	*I. uriae*	*Fr. cirrhata*	JF331054, JF331106, JF331158, JF330950, JF331314, JF331366, JF331418, JF331262, JF331002, JF331210
T3221	Russia	*I. uriae*	*P. urile*	JF331055, JF331107, JF331159, JF330951, JF331315, JF331367,

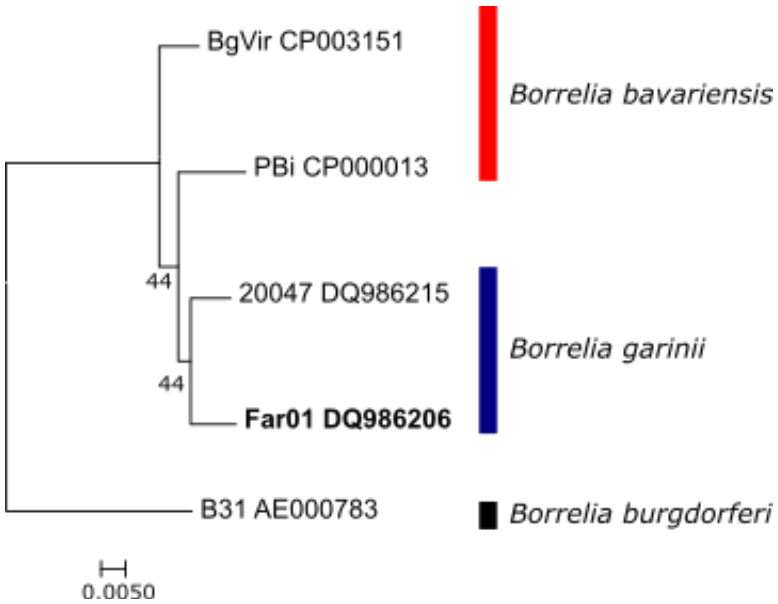
Designation	Location	Source	Tick host	Accession number(s)
				JF331419, JF331263, JF331003, JF331211

Supplementary Table 3. Details and identification information for reference sequences used in this study (from https://pubmlst.org/borrelia/).

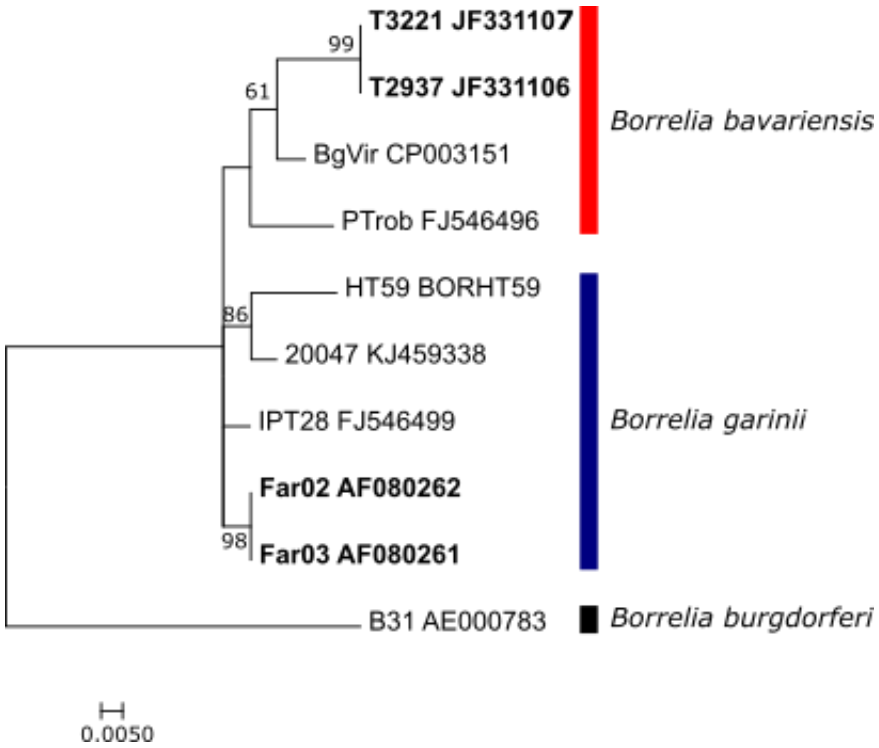
Designation	**Location**	**Source**	**MLST reference number(s) and/or accession number(s)**
Hiratsuka	Japan	Human	MLST isolate 1116
NT24	Japan	*I. persulcatus*	MLST isolate 1087
Konnai 17 clone 1	Japan	*Ixodes* sp.	MLST isolate 1135
N346	Japan	*I. persulcatus*	MLST isolate 1082; D88293, D88294
J 20T	Japan	Human	MLST isolate 1122
J 15	Japan	Human	MLST isolate 1118
PBi	Germany	Human	MLST isolate 155; X85199, CP000013, CP000013, AB035595, CP000013, FJ546560, CP000013, CP000013
Ptrob	Slovenia	Human	MLST isolate 158; X83554
HT59	Japan	*I. persulcatus*	MLST isolate 1081
20047	France	*I. ricinus*	MLST isolate 153; D67018, AY836549, DQ986215, AB035602, AY737708, D49498, DQ986215
IPT28	France	*I. ricinus*	MLST isolate 160; JF331160
B31	USA	*I. scapularis*	MLST isolate 1; AE000785, AE000785, AE000785, AE000785, AE000785, AE000785
NMJW7	China	*I. persulcatus*	CP003866, CP003866, CP003866, CP003866,
BgVir	Russia	*I. persulcatus*	CP003151, CP003151, CP003151, CP003151, CP003151
BA B12	Norway	Rodent	FJ750349
PBr	Denmark	Human	ABJV02000003



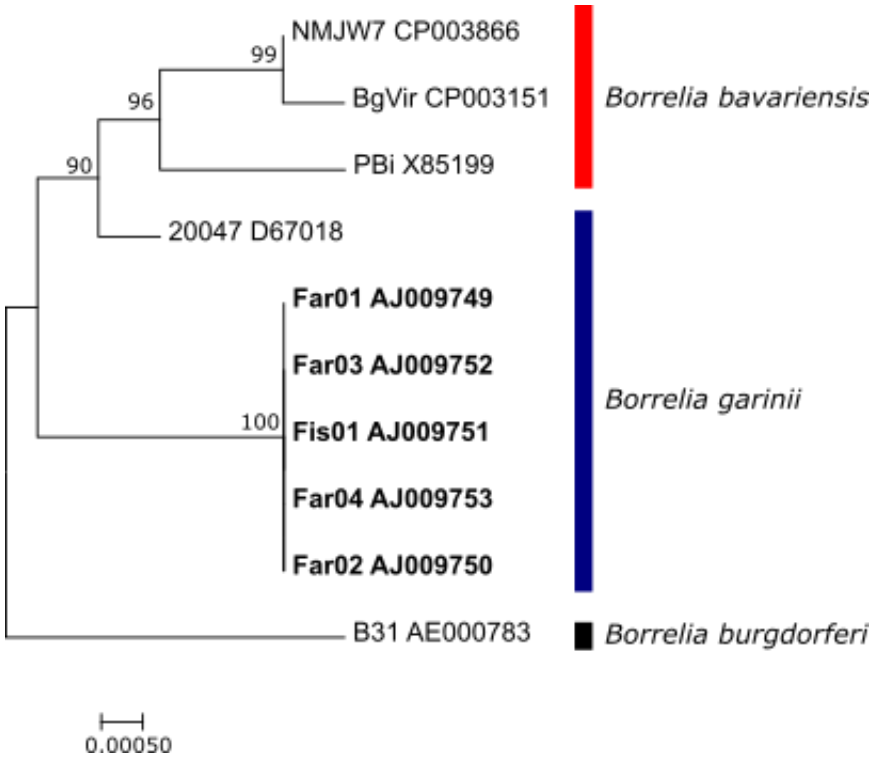
Supplementary Figure 1. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML with *fla* sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. NCBI sequences originating from seabirds are in bold and accession numbers of sequences from NCBI are included in branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



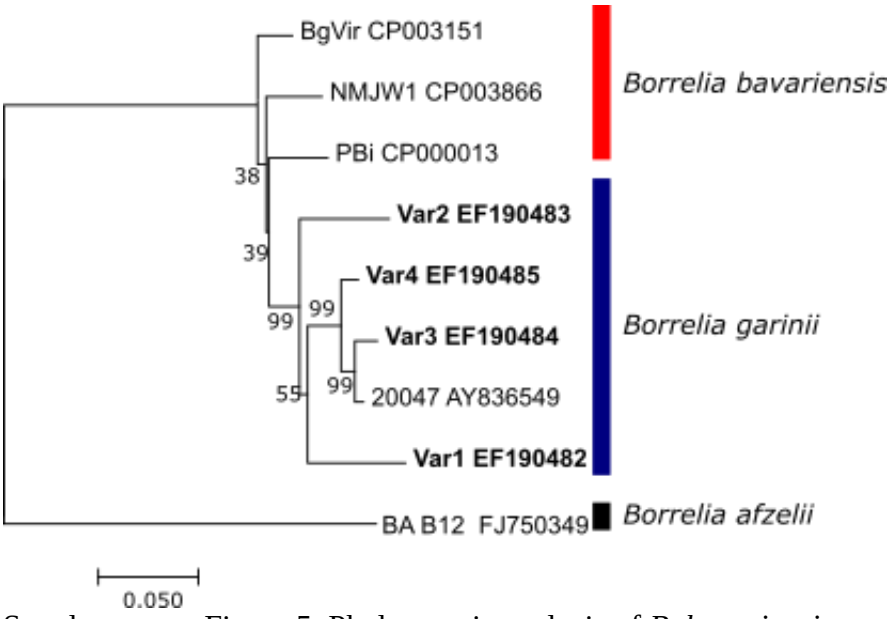
Supplementary Figure 2. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML with *trxA* sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. The NCBI sequence originating from a seabird is in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



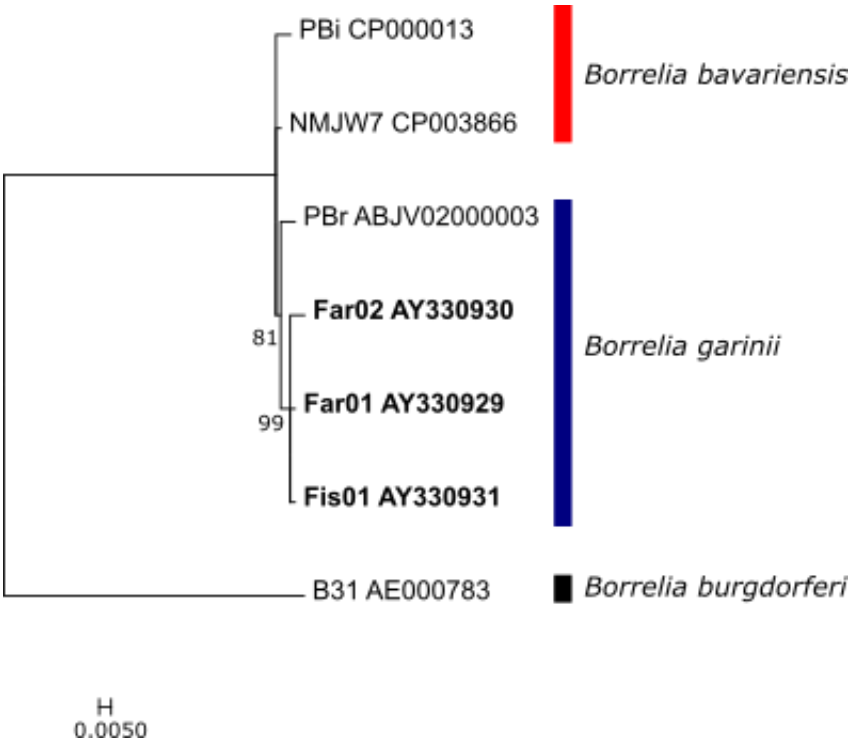
Supplementary Figure 3. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and 5S-23S intergenic spacer region sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. NCBI sequences originating from seabirds are in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



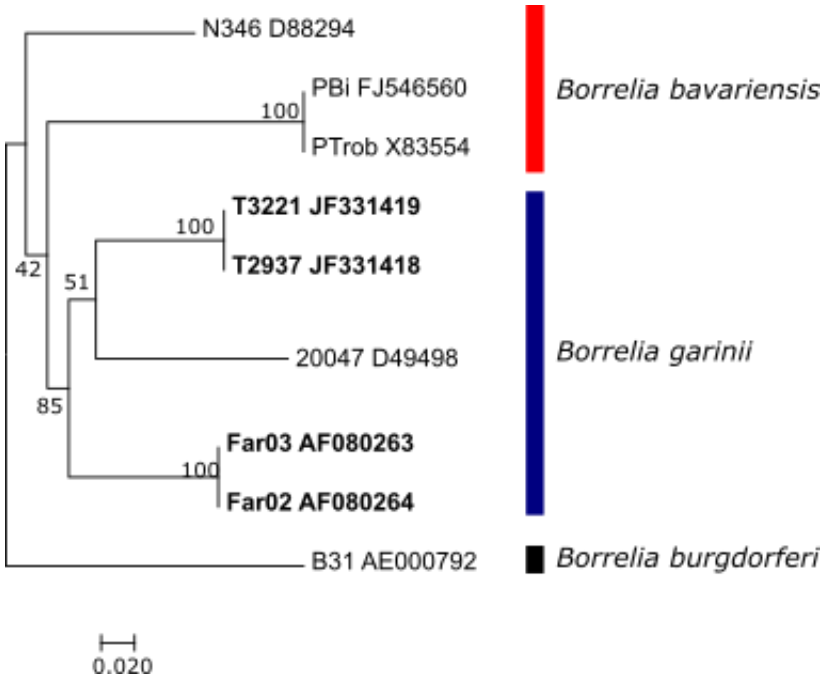
Supplementary Figure 4. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and 16S rDNA sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. NCBI sequences originating from seabirds are in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



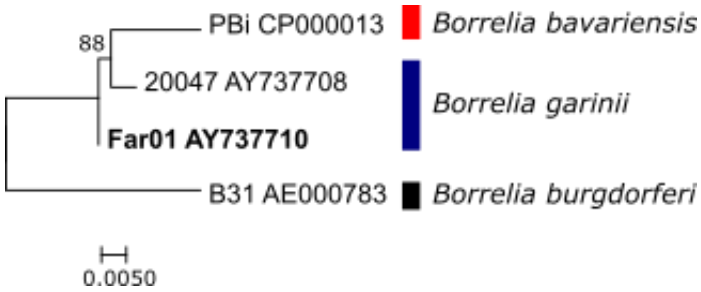
Supplementary Figure 5. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and 16S-23S intergenic spacer region sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia afzelii* (black) was used as the outgroup. NCBI sequences originating from seabirds are in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



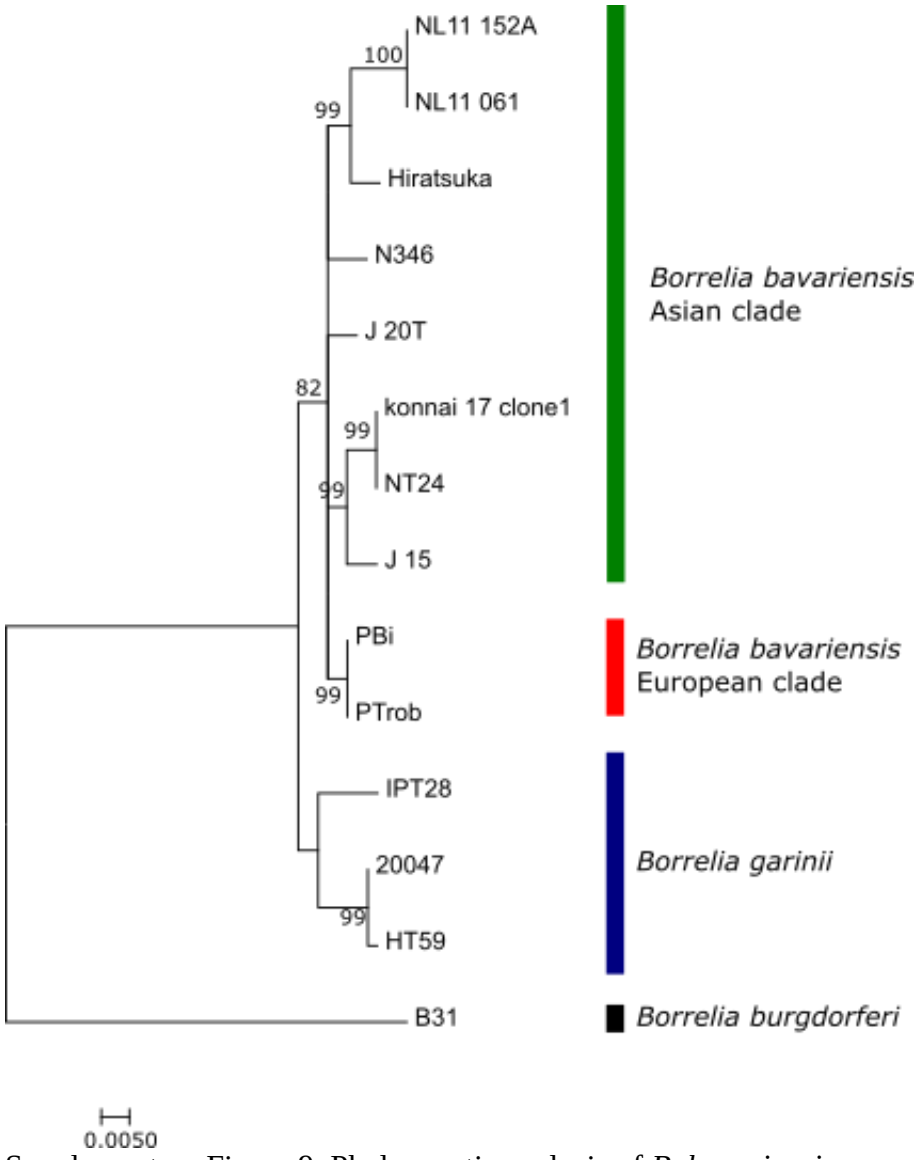
Supplementary Figure 6. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and *p13* sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. NCBI sequences originating from seabirds are in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



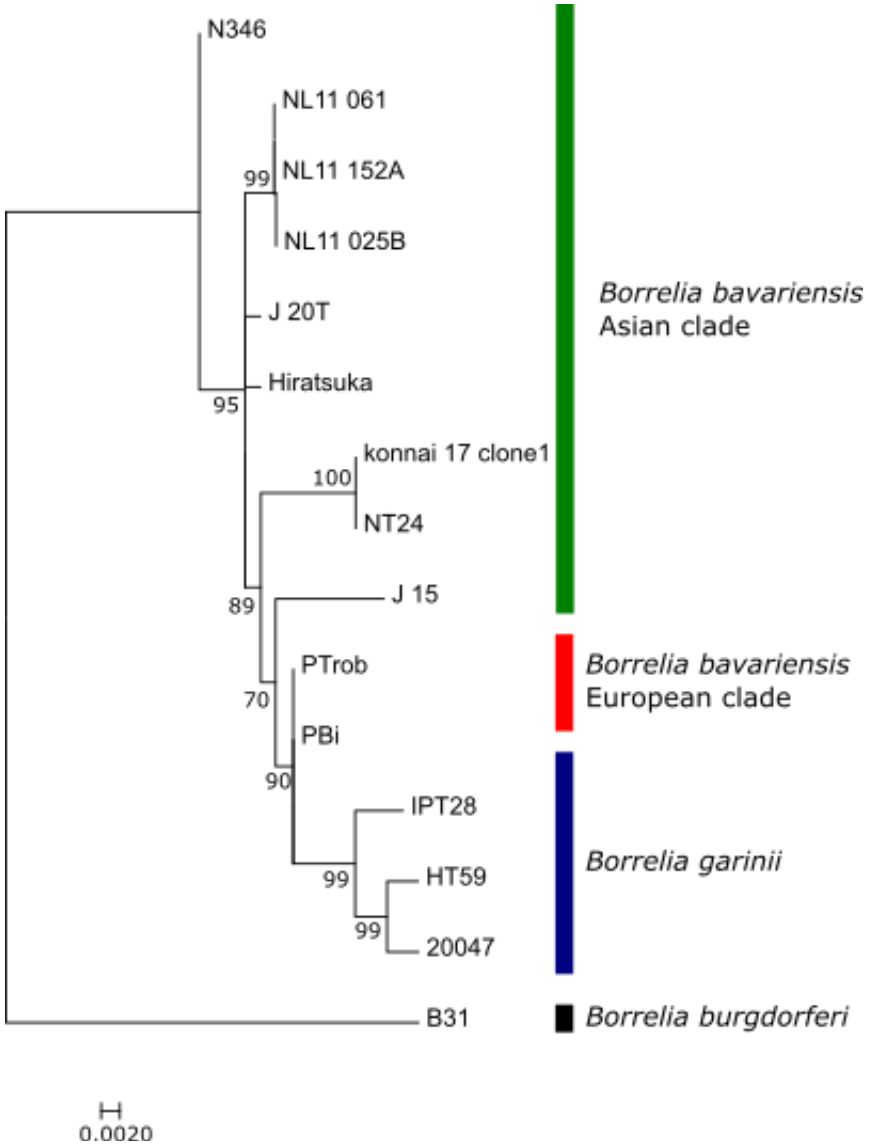
Supplementary Figure 7. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and *ospC* sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. NCBI sequences originating from seabirds are in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



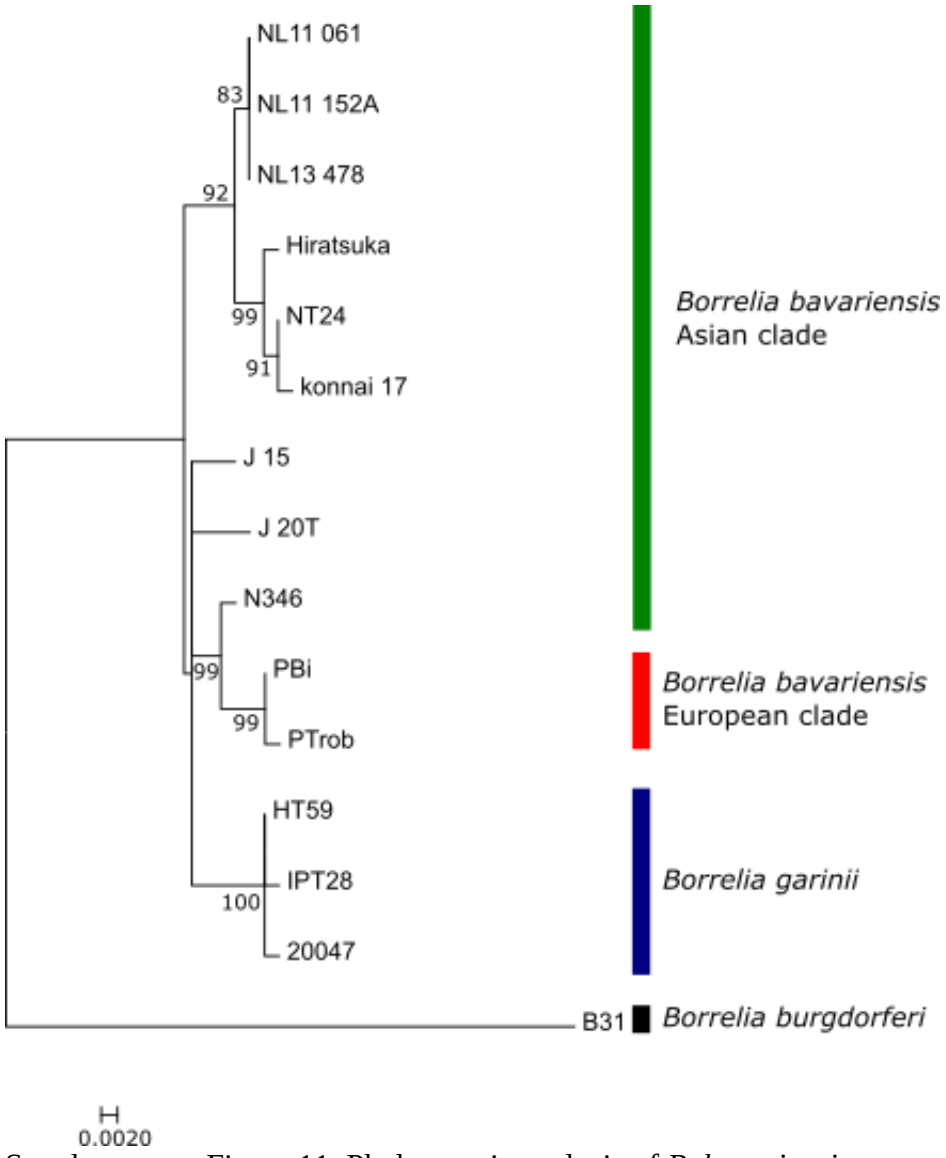
Supplementary Figure 8. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and *rplL* sequences representing *B. bavariensis* (red bar) and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. The NCBI sequence originating from a seabird is in bold and accession numbers of all sequences from NCBI are included in the branch labels. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



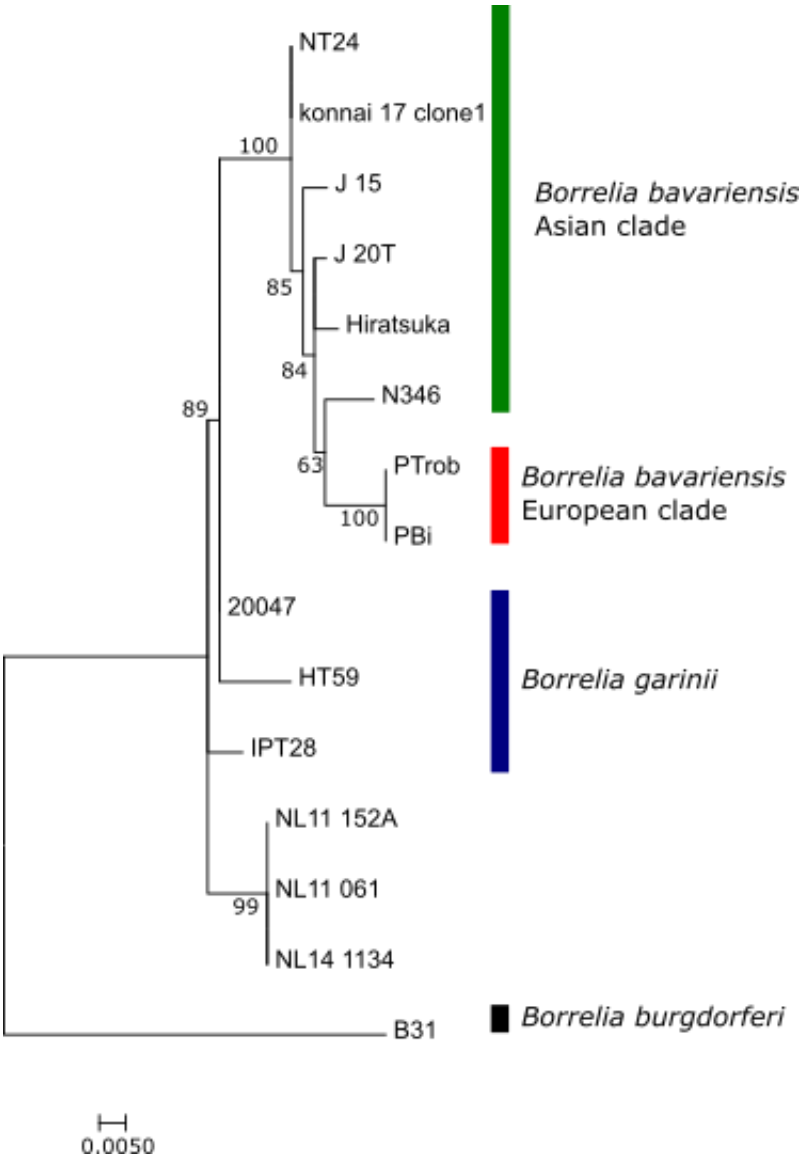
Supplementary Figure 9. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and *clpA* MLST sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



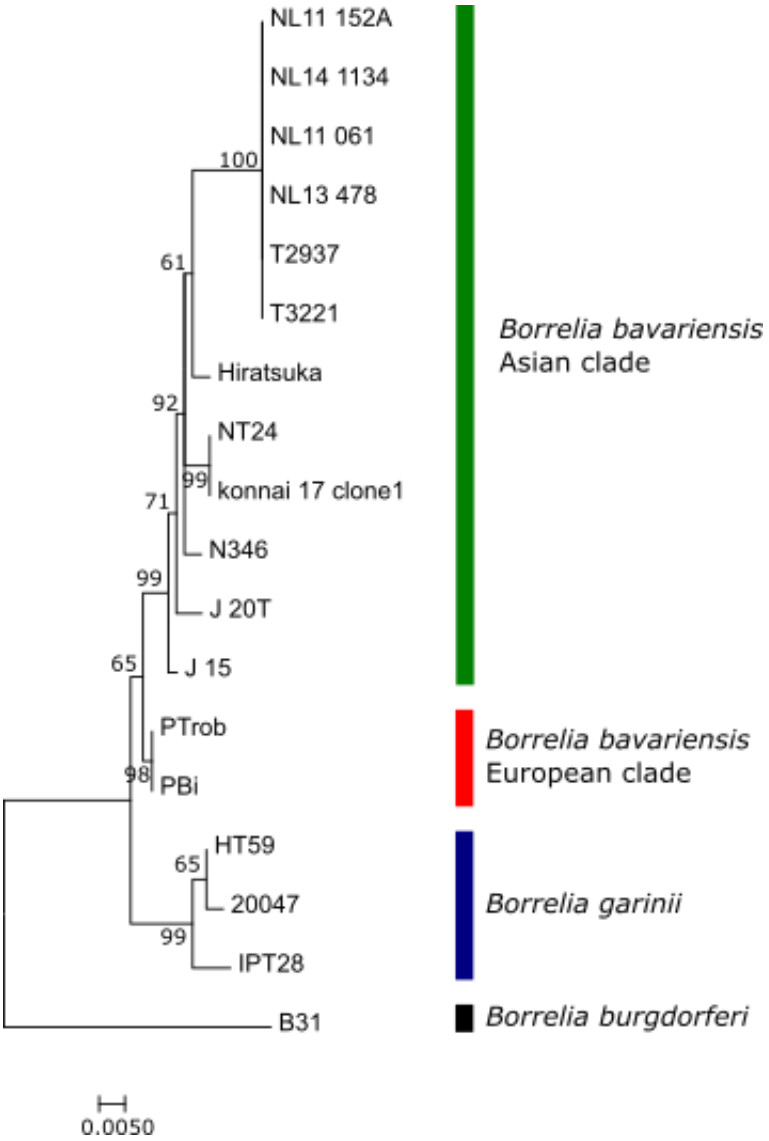
Supplementary Figure 10. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and MLST *clpX* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



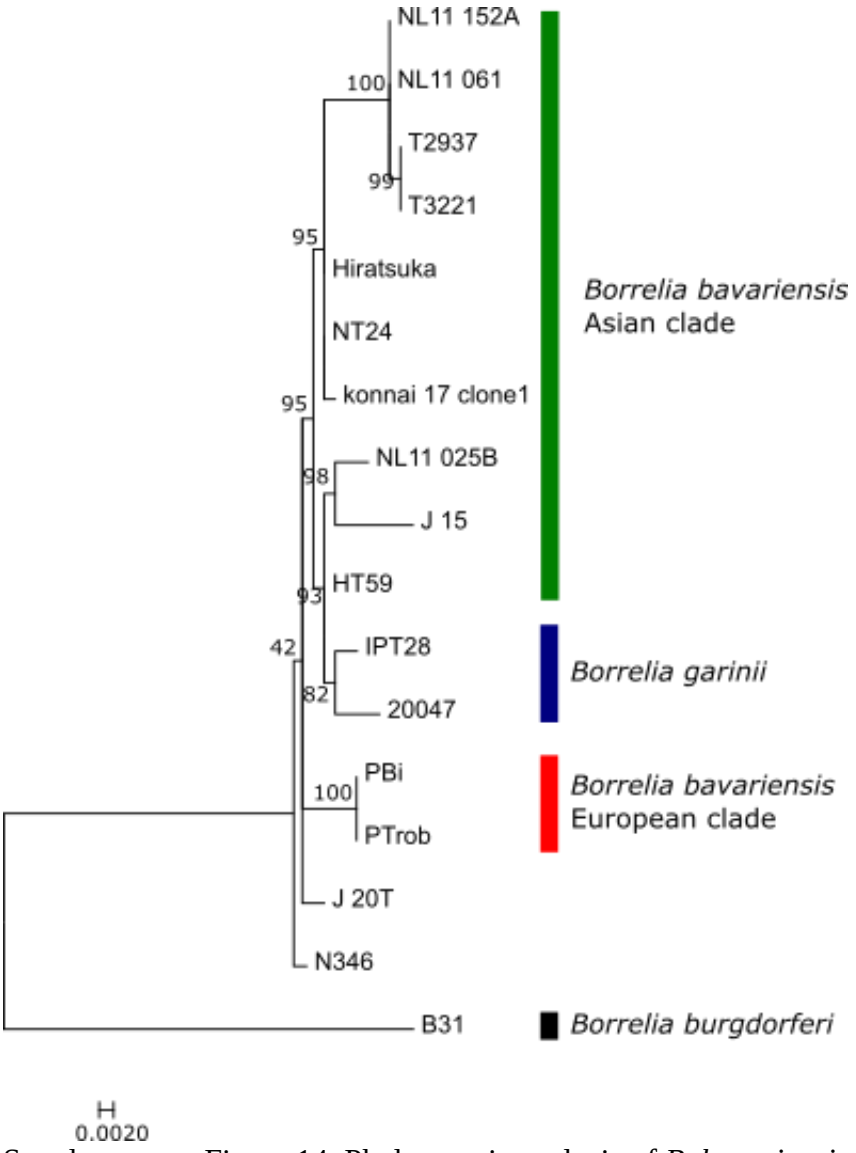
Supplementary Figure 11. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and MLST *nifS* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



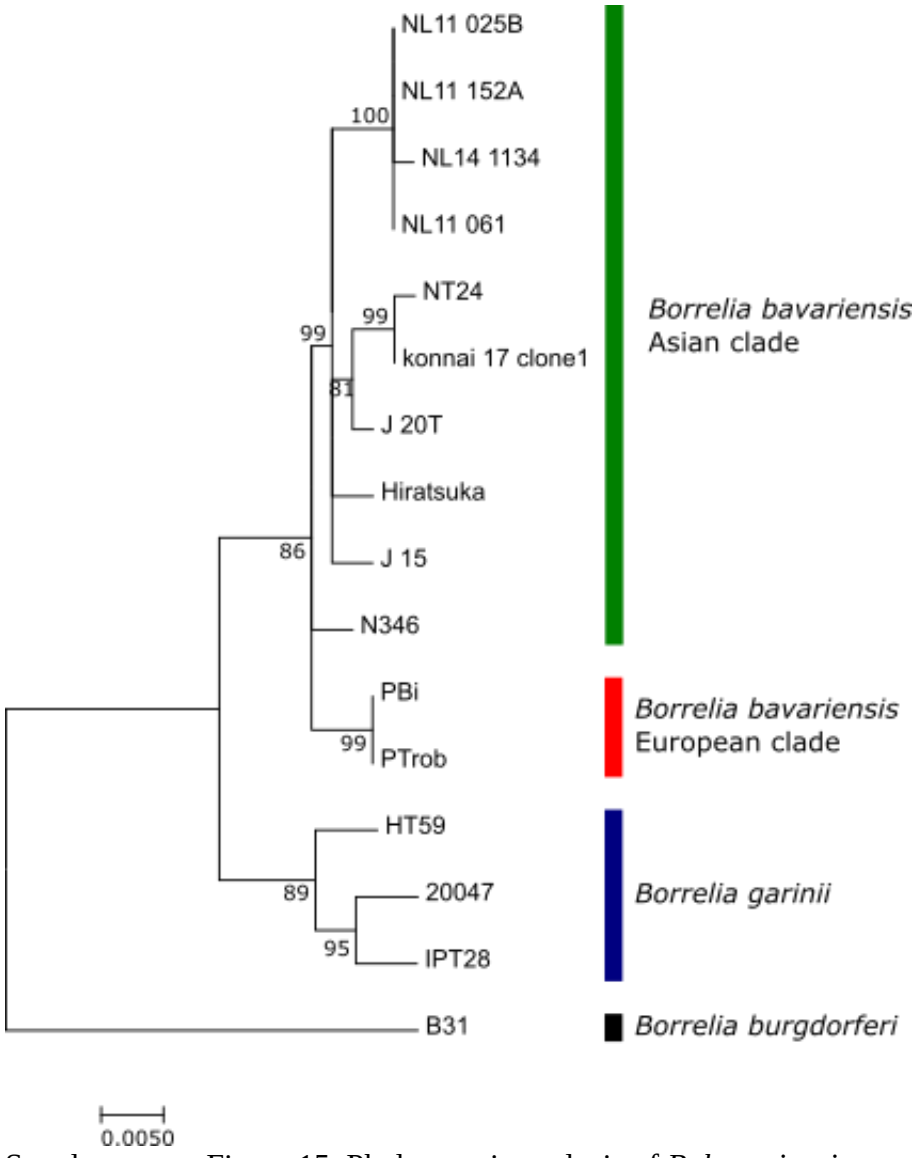
Supplementary Figure 12. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and MLST *pepX* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



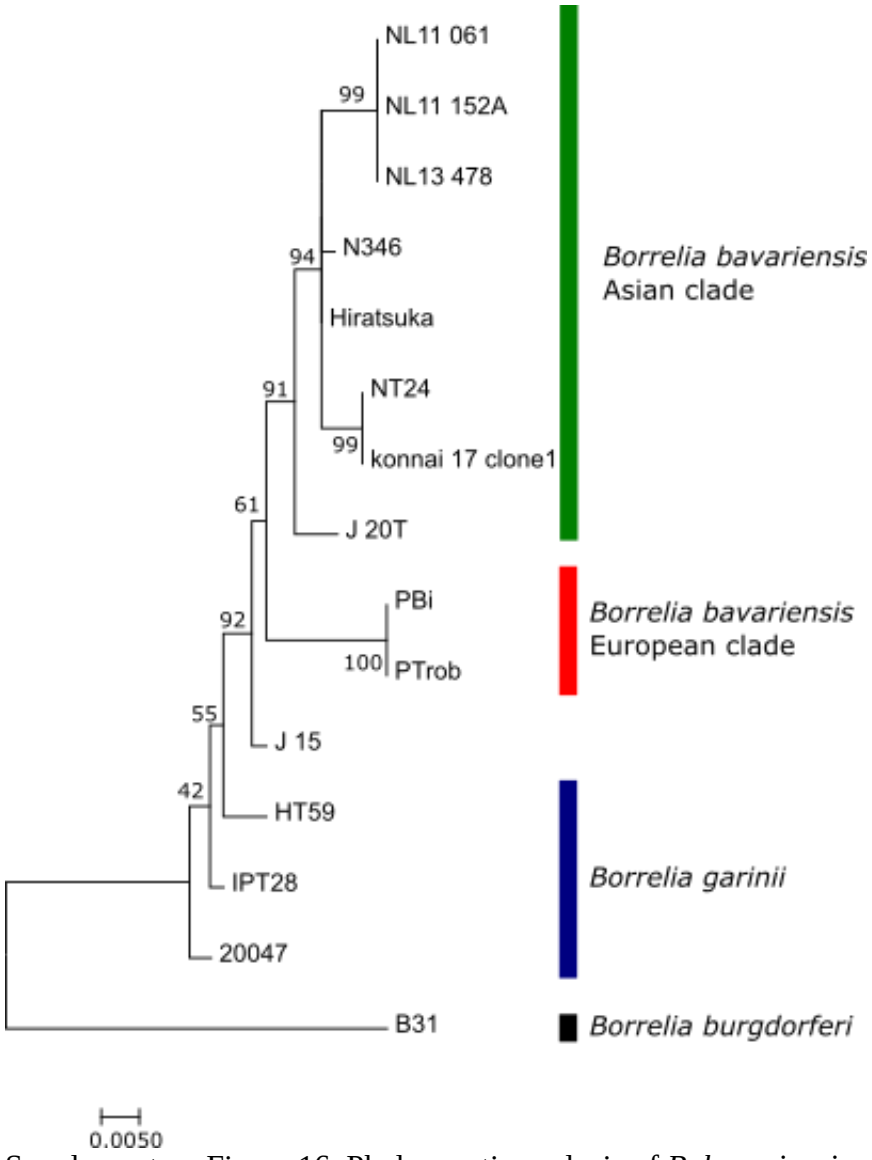
Supplementary Figure 13. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and MLST *pyrG* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



Supplementary Figure 14. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and MLST *recG* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



Supplementary Figure 15. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. The maximum likelihood phylogeny was constructed using PhyML and MLST *rplB* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.



Supplementary Figure 16. Phylogenetic analysis of *B. bavariensis* sequences identified in *I. uriae* from seabirds. Maximum likelihood phylogeny was constructed using PhyML and MLST *uvrA* sequences representing European (red bar) and Asian (green bar) *B. bavariensis* and *B. garinii* (blue bar). *Borrelia burgdorferi* (black) was used as the outgroup. Numbers at branch nodes represent support based on aBayes and the scale bar indicates substitutions per site.

Appendix 4: Supplementary material for Chapter 5

Supplementary Table 1. PCR primers and conditions used in nested PCR to amplify MLST housekeeping genes.

Gene	Primer	Sequence	Annealing temp	Product size
clpA	clpAF1237	AAAGATAGATTTCTTCCAGAC	55°C to 48°C[a]	981
	clpAR2218	GAATTTCATCTATTAAAAGCTTTC		
nPCR[b]	clpAF1258	AAAGCTTTTGATATTTTAGATG	50°C	849
	clpAR2104	CAAAAAAAACATCAAATTTTCTATCTC		
clpX	clpXF391	GCTGCAGAGATGAATGTGCC	55°C to 48°C	882
	clpXR1273	GATTGATTTCATATAACTCTTTTG		
nPCR	clpXF403	AATGTGCCATTTGCAATAGC	50°C	721
	clpXR1124	TTAAGAAGACCCTCTAAAATAG		
nifS	nifF1	ATGGATTTCAAACAAATAAAAAG	55°C to 48°C	1049
	nifR1049	GATATTATTGAATTTCTTTTAAG		
nPCR	nifF1[c]	ATGGATTTCAAACAAATAAAAAG	50°C	719
	nifR719	GTTGGAGCAAGCATTTTATG		
pepX	pepXF362	ACAGAGACTTAAGCTTAGCAG	55°C to 48°C	810
	pepXR1172	GTTCCAATGTCAATAGTTTC		
nPCR	pepXF449	TTATTCCAAACCTTGCAATCC	50°C	666
	pepXR1115	TGTGCCTGAAGGAACATTTG		
pyrG	pyrF391	GATTGCAAGTTCTGAGAATA	55°C to 48°C	799
	pyrR1190	CAAACATTACGAGCAAATTC		
nPCR	pyrF448	GATATGGAAAATATTTTATTTATTG	50°C	706
	pyrR1154	AAACCAAGACAAATTCCAAG		

Gene	Primer	Sequence	Annealing temp	Product size
recG	recF890	CCCTTGTTGCCTTGCTTTC	55°C	804
	recR1694	GAAAGTCCAAAACGCTCAG		
nPCR	recF917	CTTTAATTGAAGCTGGATATC	55°C	741
	recR1658	CAAGTTGCATTTGGACAATC		
rplB	rplF2	TGGGTATTAAGACTTATAAGC	55°C to 48°C	758
	rplR760	GCTGTCCCCAAGGAGACA		
nPCR	rplF40	CGCTATAAGACGACTTTATC	50°C	720
	rplR760[c]	GCTGTCCCCAAGGAGACA		
uvrA	uvrF1408	GAAATTTTAAAGGAAATTAAAAGTAG	55°C to 48°C	903
	uvrR2318	CAAGGAACAAAAACATCTGG		
nPCR	uvrF1434	GCTTAAATTTTTAATTGATGTTGG	50°C	677
	uvrR2111	CCTATTGGTTTTTGATTTATTTG		

[a]Touchdown, annealing temperatures starting from 55°C and decreasing 1°C each cycle

[b]nPCR, nested PCR with product from initial PCR used as template for nPCR

[c]semi-nested, with same primer used in initial PCR and nPCR

Supplementary Table 2. MLST profile for all samples from study

Sample	Year	Host[b]	Colony[c]	Allele number[a]								ST (MLST)
				clpA	*clpX*	*nifS*	*pepX*	*pyrG*	*recG*	*rplB*	*uvrA*	
NL11 021	2011	ATPU	GULL	**223**	34	34	44	37	42	33	39	**684**
NL12 114	2012	ATPU	GULL	**225**	147	134	160	34	170	144	161	**686**
NL12 300N	2012	COMU	GULL	48	34	34	44	37	42	33	39	244
NL12 312D	2012	COMU	GULL	185	147	134	160	34	170	144	161	575
NL12 314	2012	COMU	GULL	185	147	134	160	34	170	144	161	575
NL12 344	2012	COMU	GREAT	**225**	147	134	160	34	170	144	161	**686**
NL12 330	2012	BLKI	GULL	**225**	147	134	160	34	170	144	161	**686**
NL12 334C	2012	COMU	GULL	**226**	28	34	**198**	**207**	**215**	**180**	**200**	**687**
NL12 340F	2012	COMU	GULL	**226**	28	34	**198**	**207**	**215**	**180**	**201**	**693**
NL12 341A	2012	COMU	GREAT	**225**	147	134	160	34	170	144	161	**686**
NL13 029	2013	COMU	GULL	**227**	**189**	**172**	**199**	**207**	**215**	**180**	**200**	**688**
NL13 053	2013	COMU	GULL	**227**	**189**	**172**	**199**	**207**	**215**	**180**	**200**	**688**
NL13 080	2013	COMU	GULL	**226**	**189**	**172**	**199**	**207**	**215**	**180**	**200**	**689**
NL13 127	2013	COMU	GULL	185	147	134	160	34	170	144	161	575
NL13 245	2013	COMU	GULL	**228**	**190**	**173**	**200**	**208**	**216**	**181**	**201**	**690**
NL13 440	2013	ATPU	GANN	116	34	34	44	37	**218**	33	39	**694**
NL13 533	2013	RAZO	GANN	**223**	34	34	44	37	42	33	39	**684**
NL13 534	2013	RAZO	GANN	48	147	34	44	37	42	33	39	**691**
NL14 370	2014	ATPU	LFOGO	185	147	134	160	34	170	144	161	575
NL14 1000	2014	COMU	GREAT	**228**	74	34	**201**	209	217	**182**	36	**692**

[a]Allele numbers in bold represent novel alleles

[b]ATPU, Atlantic Puffin; BLKI, Black-legged Kittiwake; COMU, Common Murre; RAZO, Razorbill

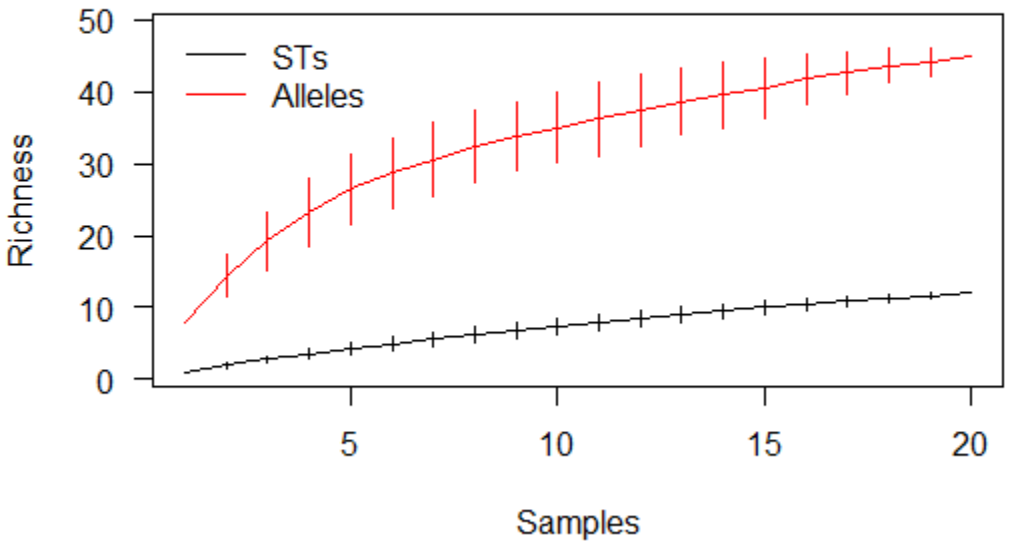
[c]GANN, Gannet Islands; GREAT, Great Island; GULL, Gull Island; LFOGO, Little Fogo Islands

Supplementary Table 3. Summary of samples, STs, and BAPS clustering results.

Isolate	Year	Host[a]	Colony[b]	Tick stage[c]	ST (MLST)	Clade	Closest neighbor	BAPS cluster
NL11-021	2011	ATPU	GULL	AFn	684	C1	244 (99.98%)	L1
NL12-114	2012	ATPU	GULL	AFn	686	C4	575 (99.98%)	L4
NL12-300N	2012	COMU	GULL	Le	244	C1	244 (100%)	L1
NL12-312D	2012	COMU	GULL	Le	575	C4	575 (100%)	L4
NL12-314	2012	COMU	GULL	AFe	575	C4	575 (100%)	L4
NL12-330	2012	BLKI	GULL	AFn	686	C4	575 (99.98%)	L4
NL12-334C	2012	COMU	GULL	AFe	687	C5	304[b] (99.25%)	L3
NL12-340F	2012	COMU	GULL	Ne	693	C5	304 (99.25%)	L3
NL12-341A	2012	COMU	GREAT	AM	686	C4	575 (99.98%)	L4
NL13-029	2013	COMU	GULL	Ne	688	C5	304[d] (99.17%)	L3
NL13-053	2013	COMU	GULL	Ne	688	C5	304[b] (99.17%)	L3
NL13-080	2013	COMU	GULL	AFe	689	C5	304[d] (99.18%)	L3
NL13-127	2013	COMU	GULL	AFe	575	C4	575 (100%)	L4
NL13-245	2013	COMU	GULL	Le	690	C3	310[e] (99.33%)	L5
NL13-440	2013	ATPU	GANN	AFn	694	C1	262 (99.96%)	L1
NL13-533	2013	RAZO	GANN	AFe	684	C1	244 (99.98%)	L1
NL13-534	2013	RAZO	GANN	AFe	691	C1	244[e] (99.98%)	L1
NL14-1000	2014	COMU	GREAT	AFe	692	C2	323[e] (99.48%)	L2
NL14-370	2014	ATPU	LFOGO	AFn	575	C4	575 (100%)	L4

[a]ATPU, Atlantic Puffin; BLKI, Black-legged Kittiwake; COMU, Common Murre; RAZO, Razorbill

[b]GANN, Gannet Islands; GREAT, Great Island; GULL, Gull Island; LFOGO, Little Fogo Islands
[c]AF, Adult female; AM, adult male; L, larva; N, nymph; e, engorged; n, flat
[d]Samples form own clade, reference sequence in next closest clade
[e]NL sequence basal to reference



Supplementary Figure 1. Species accumulation curves, depicting the increase in richness of STs (black) and alleles (red) as increase sampling effort is added to the analysis. Vertical bars represent two standard deviations from the mean.


0.0010

Supplementary Figure 2. Maximum likelihood tree, constructed with PhyML, of all 8 MLST genes from all *B. garinii* STs within the pubMLST database (as of March 2017) and 20 sequences from this study. Branches with triangles represent sequences from this study. The tree was rooted with *B. burgdorferi*, but the length of the root branch is not proportional. Numbers at nodes represent support based on aBayes, and the scale bar represents substitutions per site.