Microbiota of an Invasive Wasp *Vespula vulgaris* and Hymenopteran relatives

Interpreting the microbiome

By

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"It always seems impossible until it's done"

Nelson Mandela

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ABSTRACT

Invasive species represent a critical threat to ecosystems and ecological communities, causing changes through overwhelming predation as well as competing with native species for resources. Understanding the mechanisms behind invasive success is essential for understanding why they invade and the consequences of their invasions. Furthermore, invasive species, like all macroscopic organisms, harbour symbiotic and pathogenic microbes that constitute their microbiomes, which could explain invasive success.

The complex ecological interaction networks within the microbiome can have a positive or negative impact on host abundance and dominance. These interactions may be significant for invasive species, where microbial influences acting on an exotic host can potentially drive the ecological success of an invasive population to the detriment of recipient communities. This thesis explores the microbiota of one of the most globally invasive species, the common wasp *Vespula vulgaris*, with the overall aim to investigate and characterise the microbiome of *V. vulgaris*, using metagenomics, bioinformatics and molecular techniques.

The initial comparative microbiota study focused on three distinct life stages (larvae, worker and queen), from two ranges. This analysis revealed a core bacteriome community present in *V. vulgaris*. There was evidence of higher microbial diversity in wasp larvae compared with workers and queens. The Queen (gyne) microbiome revealed a more specific microbiome with absences of certain microbiota found in larvae and workers from the same nest, indicating a more distinctive microbiome. Interestingly, analysis of life stages between ranges showed significant dissimilarity in microbiomes, with microbiota losses, and acquisitions in the introduced New Zealand range.

Using the same techniques, the microbiota of *V. vulgaris* and four hymenopteran hosts (*Apis mellifera*, *Bombus terrestris*, *Vespula germanica* and *Linepithema humile*), were comparatively analysed. The analysis investigated both shared microbiota and host specific microbiota. This analysis indicated the polyphagous *V. vulgaris* as having a diverse

microbiome varying between nests and sites, indicating less specific microbiota in comparison to other hymenopteran hosts in this study.

Vespid wasp colonies are known to occasionally crash in the presence of diseases; however, there is a lack of reliable evidence indicating pathogenic micro-organisms play an essential role in wasp colony dynamics. Incorporating knowledge gained in previous analyses, the next aim was to investigate *V. vulgaris* nests symptomatic of an infectious agent to discover the cause of pathology. Through molecular techniques, such as Illumina RNA-Seq, PCR and Sanger sequencing, the potential cause of infection and decline of diseased nests was examined. The metatranscriptomic comparison of diseased and healthy larvae highlighted five putative infectious agents. The bacteria *Moellerella wisconsensis*, *Moku virus*, *Kashmir Bee Virus*, *Aspergillus* and the microsporidian *Vavraia culicis floridensis* found in infected larvae, potentially causing pathology in the host. The first known instance of *Moku virus*, and potentially *V. culicis floridensis* and *M. wisconsensis* was documented as potential pathogens of *V. vulgaris* present in New Zealand. To test for potential virulence of these putative infectious agents, an infection study was carried out. *Vespula vulgaris* nests and larvae were orally infected in the lab using homogenised infected larvae. Subsequently, test and control larvae were sampled to conduct and quantify a time series analysis of infection using RT-qPCR using designed primers.

This dissertation provided the first insight into the microbiome of *V. vulgaris* in the native and introduced range providing a baseline for further research. This analysis and the subsequent microbiota identified may play a role in wasp population dynamics, giving a better understanding of the observed thriving *V. vulgaris* population dynamics in New Zealand.

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STATEMENT OF AUTHORSHIP

I Oliver Quinn (OQ), hereby confirm that this thesis is my own work and that all sources quoted, paraphrased or otherwise referred to have been acknowledged in the references. I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university. Any parts of this thesis that resulted from the contribution of others are detailed below or in the acknowledgements.

Chapters 2, 3 and 4 were written as independent manuscripts intended for publication, however not yet submitted for publication. I am the primary author of each of these papers, with my supervisors, Prof Phillip Lester and Dr Monica Gruber (MG), as co-authors. For all the chapters, Professor Philip J. Lester (PJL) financed the fieldwork and provided advice throughout the project, contributing to the editing of all the thesis chapters. Dr Monica Gruber provided advice and contributed to the editing of all the manuscripts as well as statistical and bioinformatic knowledge and advice. Dr Tom Wenseleers (TW) organised and provided wasp samples from Belgium used in chapters 2 and 3. Dr Bob Brown (BB) provided samples for chapter 4. In all instances, designed studies, fieldwork sampling and drafted manuscripts were carried out after consultation with my supervisors providing advice throughout, contributing to study designs, data analyses and the editing of the manuscripts. The contributions of other co-authors to each chapter are outlined below, indicated by author initials.

Chapter 2 author contributions: PJL and MG conceived, OQ designed the study and conducted fieldwork sampling, and laboratory work. OQ and MG conducted bioinformatics analysis. PJL and MG provided advice on the Bioinformatic and statistical analyses. OQ drafted manuscript and PJL and MG helped edit final draft.

Chapter 3 author contributions: PJL and OQ conceived, OQ designed the study and conducted fieldwork sampling, laboratory work and bioinformatics analysis. PJL and MG provided advice on the Bioinformatic and statistical analyses. OQ drafted manuscript and PJL and MG helped edit final draft.

Chapter 4 author contributions: PJJ and OQ conceived BB provided field samples, OQ designed the study and conducted laboratory and bioinformatics analysis. MG and OQ conducted statistical analyses. OQ drafted the manuscript. and PJJ and MG helped edit final draft.

Appendix 7.2 author contributions: Phillip J Lester, Jana Dobelmann and Kevin Loope conceived. Oliver Quinn and Jana Dobelmann conducted fieldwork sampling. OQ provided samples and nest analyses (nest measurements, area, worker and queen cell counts). Monica Gruber and Oliver Quinn ran bioinformatics analyses. Oliver Quinn completed extractions and designed primers. Erin Wilson-Rankin, Kevin Loope and Jana Dobelmann conducted statistical analyses. Jana Donelmann and Phillip Lester wrote the manuscript with contributions from all authors.

Appendix 7.3 author contributions: Monica Gruber and Phillip Lester conceived and designed the study. Meghan Cooling, James Baty, Oliver Quinn, Anna Friedlander and Jessica Russell collected the data. Monica Gruber, Phillip Lester, James Baty, Meghan.Cooling, and AnnaFriedlander analysed the data. Monica Gruber and Phillip Lester wrote the manuscript with contributions from all authors.

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1. GENERAL INTRODUCTION

1.1 INVASIVE SPECIES

Invasive species represent a critical threat to ecosystems and ecological communities. The establishment of invasive species can transform environments, alter ecosystem services, and radically shift community structure (D'Antonio & Vitousek, 1992; Mack et al., 2000). Invasive alien species (IAS) are defined as non-native species introduced to new ranges via human actions and natural dispersion, a subset of which can threaten ecosystems, causing ecological and economic harm (Convention on Biological Diversity, 2008; Lockwood et al., 2007).

Anthropogenic insect invasions occur indirectly as stowaways or directly to a new range to fulfil an environmental function (Nentwing, 2007). This enables propagules of alien species to reach new habitats where they may become invasive (Lockwood et al., 2007). With human-mediated transportation, alien species can reach every spot on earth within a relatively short time (Nentwing, 2007). This continual accidental or deliberate global redistribution of invasive species by humans is widely recognised and can cause a detrimental effect to native species and biodiversity in the invaded range (Mack et al., 2000; Pimentel et al., 2001; Williamson, 1996; Rüdiger Wittenberg & Cock, 2001). Invaders have the potential to displace native species through predation and competition, (Christian, 2001; Lockwood et al., 2007; O'Dowd, Green, & Lake, 2003), as well as having significant economic impacts in their new regions (Lockwood et al. 2009). Invasion research focuses on the principle that introduced species have a high probability of causing a deleterious impact on native species (Simberloff, Souza, Nuñez, Barrios-Garcia, & Bunn, 2012). However, most introduced species that arrive in a new range do not become invasive, failing to establish and many that do, become innocuous members of their new community (Lockwood et al. 2009). These species facilitate diversity by coexisting with native species (Rodriguez, 2006) and adapt to their new ecosystems without having any noticeable impact.

Consequently, this poses the question, what facilitates one species to be successfully invasive over another? Previous studies have focused on contrasting differences found between native and introduced range populations. This comparison enables inferences to be made based on ecological and life history changes that possibly contribute to the invasive success

of a species (Elton 1958; Hierro et al. 2005; Suarez and Tsutsui 2008; Sanders and Suarez 2011). The few species that impact profoundly on ecology and biodiversity in their new range tend to have few or no competition or predation in their new habitat and consequently increase exponentially in abundance (Mack et al., 2000). This ecological impact can consist of any significant change in an ecological pattern or process, with the potential to change the ecosystem, disassembling communities around them (Sanders, Gotelli, Heller, & Gordon, 2003). Pimentel et al. (2005) outlined the environmental and economic costs associated with invasive species in the United States, reporting 42% of endangered species were primarily threatened, due to invasive species. These invaders can also have substantial economic impacts on agricultural productivity and trigger immense costs in control measures, costing up to US\$137 billion per annum (Pimentel, Lach, Zuniga, & Morrison, 2000).

New Zealand is particularly vulnerable to biological invasions due to its unique island ecosystem biota and strong agricultural, forestry and fishing sectors. Many early documented introductions occurred, with 33 species of introduced birds and 32 species of introduced mammals (Wodzicki & Wright, 1984). Recently, an ambitious goal to eradicate New Zealand of the most damaging introduced predators (such as rats, possums and stoat), by 2050, has been implemented. These species are of concern as they threaten native species, the economy and primary sector (Department of Conservation, 2017).

Insect invasions are also of concern in New Zealand with entomological communities continually changing with the introduction and extinction of insect species (Lester, Brown, et al., 2014). It is estimated that New Zealand Hymenoptera consists, of 2,000–3,000 species of wasp and bee, most of which are native (Craig et al., 2000), with new species still being discovered (Lester, Brown, et al., 2014). The impact of most hymenopterans accidentally or intentionally introduced to New Zealand has not been quantified (Lester, Brown, et al., 2014). However, social wasps such as *V. vulgaris* have demonstrated their adverse effect on the New Zealand environment (Brockerhoff et al., 2010). The invasive vespid wasp *Vespula vulgaris* and *Vespula germanica* form a fraction of the total number of species, but they are of most concern to the New Zealand economy, wellbeing and environment (J.R. Beggs, 2001). In recent years, New Zealand's pre-border approach to new organism introductions has tightened restricting the flow of invasive species by providing policies and legislation that

effectively inhibit the introduction of new potentially invasive non-native species (E. Brenton-Rule, Frankel, & Lester, 2016).

1.2 STUDY ORGANISM – *VESPULA VULGARIS* AND ITS NEGATIVE IMPACT

The common wasp *V. vulgaris* is part of the cosmopolitan vespine family encompassing 60 known species, native in the Northern Hemisphere throughout the Holarctic Region and the Oriental tropics (Archer, 2012; Greene, Ross, & Matthews, 1991). Introduced accidentally by humans in many non-native areas, this species has become highly adept at colonising new ranges such as the Southern Hemisphere, in New Zealand, Australia and, more recently, Argentina (J.R. Beggs et al., 2011; Lester, Brown, et al., 2014). A recent genetic study demonstrated that common wasps in the invaded New Zealand range, are likely to have originated from western Europe, with New Zealand populations exhibiting high genetic similarity to those from Belgium and the United Kingdom (Lester, Gruber, et al., 2014).

Since occurring sporadically in New Zealand since 1921 (B. Donovan, 1984), populations of *V. vulgaris* have established as a significant pest (Macintyre & Hellstrom, 2015). Recognised as a voracious predator, they now represent one of the most significant invertebrate threats to New Zealand native fauna (Lester, Brown, et al., 2014). The presence of *V. vulgaris* on the '100 of the World's worst invasive alien species' list demonstrates this threat (Lowe, Browne, Boudjelas, & De Poorter, 2000), and is now abundant in New Zealand beech forests (Beggs et al. 2011). The high abundance of common wasps is mainly due to large quantities of honeydew. Native scale insects (Coelostomidiidae) produce this honeydew, while feeding on the sap of native beech trees (*Fuscospora* spp.). This honeydew then fuels the wasp population, by providing a rich carbohydrate food resource (Beggs 2001, Gardner-Gee & Beggs 2013). These honeydew beech forests cover over 1 million Ha⁻¹ in the South Island of New Zealand, where common wasps are recorded in extremely high densities (Beggs 2001). In some cases, wasps are recorded exceeding 370 wasp's m⁻² of tree trunk, with nest densities of 34 ha⁻¹ (Moller et al. 1991; Beggs et al. 1998).

This insatiable appetite for honeydew as well as polyphagous predation on native insects has many consequences, outcompeting native species for food and resources. This knock on effect changes the ecology and biodiversity of New Zealand environments (Austin & Dowton,

2000; J.R. Beggs & Wilson, 1991; Fordham, 1991; Richard J Harris, 1991; Moller & Tilley, 1989; Toft & Rees, 1998). During peak wasp densities, certain native species abundances are reported to be decimated. Native caterpillars are attacked with few surviving to adulthood (Jacqueline R Beggs & Rees, 1999), as with orb web spiders with few surviving to the end of a wasp season (Toft & Rees, 1998). Wasps can also out-compete native kaka for honeydew resource (J.R. Beggs & Wilson, 1991) as well as attacking native bird chicks (Thomas & Moller, 1990), with indications that a variety of birds have decreased in abundance in New Zealand beech forests due to wasps (Elliott, Wilson, Taylor, & Beggs, 2010). This ravenous appetite for honeydew has also meant that common wasps have altered the natural nutrient cycling in the beech forest ecosystem, by removing honeydew and reducing the flow of carbon-rich resource to microorganisms in the phyllosphere and the soil (J.R. Beggs, 2001).

Interestingly, the high abundance of common wasps in New Zealand is not seen annually in the native range where nest abundance can fluctuate substantially. Years of high abundance are followed by years of scarcity, worker abundance, nest densities and queen productivity varying by two orders of magnitude between successive years in England (Archer, 1981, 1985, 2001). In New Zealand, the observed population fluctuation of *V. vulgaris* seems less severe than that observed in England (Barlow, Beggs, & Barron, 2002). This endogenous density-dependence, which in addition to exogenous factors such as climate can promote years of high and low wasp abundances in England (Archer, 1981, 1985). In New Zealand, the milder population fluctuation may be linked to several factors, including food availability and the high or low abundance of natural enemies such as pathogens and parasites (Lester et al., 2015). Recently a study demonstrated that density dependence is overwhelmingly crucial in predicting wasp densities in both the native and invaded ranges. The same study highlighted that spring weather conditions in both ranges could play a significant role in wasp densities, impacting wasp colony initiation and early development (Lester, Haywood, Archer, & Shortall, 2017).

The economic and social impacts of invasive wasps were also highlighted in a recent study, where vespid wasps were estimated to cost New Zealand's economy more than \$130 million per annum, with the most significant economic impacts on farming, beekeeping, horticulture and forestry workers (Macintyre & Hellstrom, 2015). The report also found wasps cost pastoral farming more than \$60m a year by disrupting bee pollination, reducing the amount

of clover in pastures and increasing fertiliser costs (Macintyre & Hellstrom, 2015). Vespids were also highlighted as a significant pest of the beekeeping industry in New Zealand. The extraction of honey can be delayed due to the aggressive nature of these wasps, and their presence causes direct financial loss by robbing hives of honey and killing bees (Plunkett, Moller, Hamilton, Clapperton, & Thomas, 1989), while also competing with honey bees for food resources (Moller & Tilley, 1989). Common wasps can also have an impact on human health, inflicting wasp stings (Dymock, Forgie, & Ameratunga, 1994) and in some cases cause death (Low & Stables, 2006).

1.3 LIFE HISTORY

Similar to other eusocial insects, *V. vulgaris* are characterised by overlapping adult worker generations, cohabiting and cooperating in brood care, as well as the reproductive division of labour (Wilson, 1971; Wilson & Holldobler, 2005). Colonies have an annual life cycle with alternation of solitary and social phases, although in the invaded New Zealand range some northern colonies can overwinter (Leathwick & Godfrey, 1996; Plunkett et al., 1989). By mid-winter, a typical *V. vulgaris* colony is represented by one hibernating, fertilised queen (Edwards 1980), sheltering in a dry cavity such as a tree hollow or underneath bark. When temperatures increase in spring, the queen will emerge to feed and find a suitable nest site, usually subterranean (Spradbery 1973). Nest construction involves the construction of a paper nest envelope and hexagonal brood cells using masticated wood pulp obtained from chewed rotting wooden fibres mixed with saliva, giving nests their characteristic mottled light brown coloured envelopes (Leathwick, 1997; J. Philip Spradbery, 1973). Following oviposition by the queen, workers will begin to emerge one month later and start provisioning the nest, allowing the queen to dedicate her time to oviposition (Matsuura & Yamane, 1990). Over the course of spring and summer, nests will increase in size and by autumn reproductive adults are produced (J. Philip Spradbery, 1973). Mating occurs between gynes and drones, with gynes mating with multiple partners in a bid to increase colony fitness (Dobelmann et al., 2017; Loope, Chien, & Juhl, 2014). Once mated, queens will find shelter before winter hibernation and emerge in the spring to start a new cycle.

1.4 ABUNDANCE AND ECOLOGICAL FACTORS CONTRIBUTING TO THE INVASION SUCCESS OF *VESPULA VULGARIS*

Insects are exceptionally well suited to become invaders, being small they can easily translocate unnoticed by human vectors (i.e. car, ship, airplane). Insect food demands are quite small ensuring they survive long distances in varying modes of transport (Speight, Hunter, & Watt, 2008). Social wasps have proven to be highly adept at invading new habitats, with excellent dispersal abilities, and high reproductive rates, attaining high abundance numbers, dominating the new ecological community (Moller, 1996; John H. Werren et al., 2010). Their broad diets and habitat ranges, effective predator defences, and superior competitive abilities enhances their ability to establish and spread (Moller, 1996). These invasive strategies together with eusociality (division of labour and task differentiation) among colony members, is considered to be a significant cause of success in Hymenoptera (Hölldobler & Wilson, 1990), allowing for rapid adaptation to changing environmental conditions in new ranges.

1.5 MICROBIOTA OF INSECT HOSTS

The microbiome of *V. vulgaris* has not been comprehensively studied as it has in other hymenopteran species, such as *Apis mellifera* and *Bombus terrestris* (Cariveau, Elijah Powell, Koch, Winfree, & Moran, 2014; J. A. Chandler, Lang, Bhatnagar, Eisen, & Kopp, 2011; Engel, Martinson, & Moran, 2012; Hamdi et al., 2011; Ishak et al., 2011; J. Li et al., 2012; Runckel et al., 2011; Sabree & Moran, 2014; David R. Tarpy, Mattila, & Newton, 2015). Invasive species such as the common wasp can bring an array of microorganisms, including pathogens or EID's (emerging infectious diseases) (Woolhouse, Haydon, & Antia, 2005). For this reason, microbial community diversity of social insects has grown in interest in recent years. Research has highlighted critical functional relationships and how the microbiome is highly intertwined with the insect host and essential for insect development (Hamdi et al., 2011; Lanan, Rodrigues, Agellon, Jansma, & Wheeler, 2016; N. a. Moran, 2015; Weiss & Aksoy, 2011). Vespids wasps can harbour many protozoa, fungal, bacterial and viral parasites (Bailey & Ball, 1991). These resident microorganisms can enhance insect fitness by contributing to reproduction and nutrition, primarily by providing essential amino acids, B vitamins and, for fungal partners, sterols (Bordenstein, Marshall, Fry, Kim, & Wernegreen, 2006; Angela E.

Douglas, 2015; Gil, Latorre, & Moya, 2004; Warnecke et al., 2007). Some microorganisms have demonstrated a protective role against microbial pathogens and parasitoids in their insect hosts by synthesizing specific toxins or modifying the insect immune system (Azambuja, Garcia, & Ratcliffe, 2005; Dillon & Dillon, 2004; Angela E. Douglas, 2015; Eleftherianos, Atri, Accetta, & Castillo, 2013; Engel et al., 2012; Teixeira et al., 2008; Vásquez et al., 2012). Gut bacterial communities such as the Enterobacteriaceae community in the Mediterranean fruit fly can impact host longevity by preventing the establishment of pathogenic bacteria (Behar, Yuval, & Jurkevitch, 2008), suggesting the elimination of a protective microbial species could have deleterious effects on the host.

As well as the host-pathogen relationships, recent research has also focused on the role endosymbionts such as *Wolbachia* have on the host, emphasising the extent microbes have integrated with insects (Dillon & Dillon, 2004). *Wolbachia* has been successfully used with mosquitoes to block the virus causing Dengue fever in humans, which involved inoculating or trans-infecting mosquitoes with strains of the *Wolbachia*, inhibiting viruses (A. A. Hoffmann et al., 2011). This approach could be used elsewhere with a greater understanding of disease dynamics and microbial relationships (Emerson & Gillespie, 2008). Therefore, the exploitation of an individual host's microbiota could result in practical applications such as a biocontrol for invasive species or for the development of probiotic defences in pollinating insects (Elena Crotti et al., 2012).

Much of this research has focused on the microbiota of economically beneficial social Hymenoptera and their relationship with pathogens, such as the central role pathogens may play in the colony collapse of honey bees (Cornman et al., 2012). This focus on pathogens may not tell the full story. Researchers studying the human microbiome have found that nearly everyone routinely carries pathogens and microorganisms known to cause illnesses (Huttenhower et al., 2012). In healthy individuals, however, pathogens cause no disease; coexisting with their host and the rest of the human microbiome (Huttenhower et al., 2012). The interaction between a host and its microbiota can range from mutualism through commensalism to that of a pathogenic interaction in a holobiont relationship. These intimate functional interactions support the hologenome theory of evolution, where microorganisms and their hosts can form a single unit in the context of evolution (McFall-Ngai, 2008; Zilber-Rosenberg & Rosenberg, 2008). Therefore, an in-depth understanding of an organism must

also include the analysis of its core microbiome and associated microbes. This awareness may lead to clues of how to solve and understand the causes of problems such as CCD (colony collapse disorder), preventing the decline of honey bees or perhaps a bio-control in invasive wasps. Investigating these microbial relationships and networks will show why some pathogens turn deadly and under what conditions. Human microbiome research has also shown that metabolic capabilities encoded in the genes of these microbial communities can be more important than the species of microbes providing them (Huttenhower et al., 2012). This microbial framework can be individual to a species with some studies demonstrating significant microbial differences found among species in addition to microbiota having differing functioning roles within each species (Behar et al., 2008; Dillon & Dillon, 2004; Hongoh, 2010; Robinson, Schloss, Ramos, Raffa, & Handelsman, 2010; C. N. A. Wong, Ng, & Douglas, 2011). For example, in termites, the microbiota is more complex compared with fruit flies. Termites harbour up to several hundred species of gut microbes (Hongoh, 2010), while *Drosophila melanogaster* has less than ten (C. N. A. Wong et al., 2011). In a literature review of social wasp pathogens Rose et al. (Rose, Harris, & Glare, 1999) detailed 50 fungal, 12 bacterial, five to seven nematodes, four protozoans and two viral potential pathogens from wasps in the genera *Vespula*, *Vespa*, and *Dolichovespula* in both native and invaded ranges.

It is also essential to not only understand the microbiome of the focal organism but also look at the interaction between other hymenopterans in the pollinating guild which could lead to clues of microbial spillover, symbiotic and pathogenic. Commercially bred bumble bees used to pollinate greenhouses were shown to be reservoirs of several pollinator parasites including *Apicystis bombi* from the phyla Apicomplexa and two microsporidians *Crithidia bombi* and *Nosema bombi*. When bumble bees escaped their closed greenhouse environment, they were shown to interact and infect wild bee populations that are typically disease and parasite free, thus providing evidence for spillover hypothesis (Graystock et al., 2013). The spillover hypothesis states that parasite infection rates within one species results in transmission to another species and results in a higher or more consistent infection of a second species (Strauss, White, & Boots, 2012). This horizontal transfer of pathogens and mutualistic microbes between species may occur through feeding on the same food source, for example, nectar (Aizenberg-Gershtein, Izhaki, & Halpern, 2013) and honey through hive robbing (Fries & Camazine, 2001). This interspecies pathogen transfer may have implications when

investigating potential biocontrols within a closely related group, making host specificity key to finding a biological control.

1.6 IMPORTANCE OF BIOLOGICAL CONTROL

The term “biocontrol” refers to a natural phenomenon, a field of study, or an applied pest management technique with the action of parasites, predators and pathogens in maintaining another organism’s density at a lower average than would occur in their absence (Van den Bosch, Messenger, & Gutierrez, 1982). Classical biocontrol deals with the control of non-native pests through the introduction of natural enemies from the pest’s native range whereas conservation biocontrol aims to control pests through enhancement of natural enemies native to the location (Lockwood et al., 2007). Conservation control is regarded safest although not always as effective as classical control. The history of biocontrol has demonstrated that the use of a non-native species to control an invasive species has the danger of potentially introducing an even worse invader to control an already present invasive species (Mack et al., 2000; Simberloff, Stiling, & Peter, 1996). Some of the most infamous examples of classical biocontrol gone wrong include the introduction of the mongoose (*Herpestes auropunctatus*) in the West Indies to control rats (Simberloff et al., 1996), the introduction of the cane toad (*Bufo marinus*) in Australia and Hawaii to control beetles in sugar cane (Boland, 2004), and the introduction of the rosy wolf snail (*Euglandina rosea*) to control the giant African snail (*Achatina fulica*) on tropical islands worldwide (Hadfield, Miller, & Carwile, 1993). With each introduced species there was an adverse effect on native species and each introduction now requiring control measures of their own.

Pathogenic microorganisms including bacteria, fungi and viruses have also been used as biocontrol agents, killing or debilitating their host. Various microbial insect diseases occur naturally, but may also be used as biological pesticides (D. Chandler, Davidson, Grant, Greaves, & Tatchell, 2008). The most widely applied species of bacteria *Bacillus thuringiensis*, used for biological control, of Lepidopteran, Coleopteran and Dipteran insect pests. Typically sachets of dried bacterial spores are used when mixed with water and sprayed onto vulnerable plants such as brassicas and fruit trees (Kumar & Bambawale, 2002). The highly specific bacterium *Paenibacillus popilliae* is also used as a biocontrol of the Japanese beetle *Popillia japonica* killing the larvae by causing milky spore disease, (Grady, MacDonald, Liu,

Richman, & Yuan, 2016). Entomopathogenic fungi have also been used in the control of up to 14 species of aphid (Mahr, Whitaker, & Ridgway, 2008), as well as the mass-produced *Beauveria bassiana* used to manage a wide variety of insect pests including whiteflies, thrips, aphids and weevils (Latifian, Rad, Amani, & Rahkhodaei, 2013). Virus as a biocontrol has mainly focused on the Baculoviruses which are often specific to individual insect host species and demonstrated a effective biological pest control. For example, the *Lymantria dispar* multicapsid nuclear polyhedrosis virus is used as a biological control agent in a spray over large areas of forest in North America, where larvae of the gypsy moth cause severe defoliation. The virus kills the moth larvae they have consumed and die shortly after, with the disintegrating cadavers leaving virus particles on the foliage to infect other larvae (Rohrmann, 2013).

Previous research and attempts at the control of *Vespula* wasps in New Zealand have focused on biological control, baiting and pheromone lures (J.R. Beggs, Rees, Toft, Dennis, & Barlow, 2008; R J Harris & Etheridge, 2001; Lester et al., 2013). A biological control programme was instigated in 1980 to control vespine wasps in New Zealand using the introduced parasitoid *Sphexophaga vesparum* (B. J. Donovan, 1989; B. J. Donovan & Read, 1987). Unfortunately, no evidence was found that the wasp population density had declined as a result of the *Sphexophaga* parasitoid release, with authors concluding that the parasitoid is unlikely to have had any significant effect on wasp populations as a biocontrol agent (J.R. Beggs et al., 2008).

Reviews on wasp research and control methods in New Zealand to date point to a lack of research on pathogens as biocontrol (Akre, 1991; Lester, Brown, et al., 2014; Rose et al., 1999). In these reviews pathogens such as entomopathogenic fungi, bacteria, viruses, protozoa, and nematodes have been suggested to have potential as biological control agents for wasp colonies. Several promising candidates were explored (Glare et al. 1996; Harris et al. 2000; Brownbridge et al. 2009), with research culminating in the examination of two fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, mixed into non-toxic protein bait (Brownbridge et al. 2009). Field trials showed reduced wasp numbers in some situations (R. Toft, pers. comm.), but these pathogen-baits did not have the same fast-acting effect as toxic baits. Therefore to date, no suitable candidate has been identified for *V. vulgaris* biocontrol in New Zealand (J.R. Beggs et al., 2008; Glare, Harris, & Donovan, 1996; Richard J Harris,

Harcourt, Glare, Rose, & Nelson, 2000; Lester et al., 2015; Thomas & Moller, 1990). Recent studies suggest that *V. vulgaris* suffer from a variety of pathogens in their home range (Evison et al., 2012). These native wasp pathogens could have the potential to induce population crashes in social insects (S. A. Cameron et al., 2011). However, to date no confirmatory evidence exists of pathogenic micro-organisms playing a crucial role in wasp colony dynamics (Spradberry 1973). The hygienic behaviour of colonies could play a significant role in the prevention of pathogen establishment. Therefore, colony hygiene would need to be overcome for pathogens to spread in the colony (Richard J Harris et al., 2000). The issue of pathogen specificity would similarly need to be investigated. Many pathogens previously examined are generalists and therefore not specific to wasps. These broad-spectrum pathogens could potentially be a spillover risk which could influence economically valuable honey bees and bumble bees.

Once a viable pathogen specific to the host target has been isolated and proven to infect the host, developing efficient methods of pathogen dispersal widely into the environment would also need to be a consideration due to large areas of inaccessible terrain in New Zealand.

1.7 INVASIVE SPECIES SUCCESS/ENEMY RELEASE HYPOTHESIS (ERH)

The enemy release hypothesis proposes that invasion success may be facilitated by a release from natural enemies such as herbivores, predators and parasites, potentially leading to a rapid increase in distribution and abundance of the invasive species (Keane & Crawley, 2002; Torchin, Lafferty, & Dobson, 2003). Evidence supporting the enemy release hypothesis has previously been found in studies of plant–herbivore interactions (AA Agrawal, Kotanen, & Mitchell, 2005; Anurag a. Agrawal & Kotanen, 2003; Colautti, Ricciardi, Grigorovich, & MacIsaac, 2004; H. Liu & Stiling, 2006) but the evidence from animal–parasite systems is less apparent (Georgiev, Sánchez, Vasileva, Nikolov, & Green, 2007; MacNeil et al., 2003). In a recent publication, microbial communities in wasp populations in the native (Belgium and England) and invaded range (Argentina and New Zealand) were compared using mass spectrometry proteomic methods (Lester et al., 2015). However, no evidence of enemy release was discovered, as the number of microbial taxa was similar in both the introduced and native range. Nevertheless, some evidence of distinctiveness in the microbial communities was observed between countries, with observed pathogens similar to a variety

of taxa found in honey bees (Lester et al., 2015). The increase in research into microbiome comparisons and relationships, and the advancement of more refined molecular techniques, and downstream bioinformatics analysis allows us to test the enemy release hypothesis further. With these tools, we can develop an ecological network analysis to characterise microbial communities' specific to life stages within a nest or across populations and ranges.

1.8 MOLECULAR APPROACHES TO UNRAVEL A COMPLEX MICROBIAL COMMUNITY

We are only beginning to understand the extent of microbial associations in ecology. Reliably assessing diversity is a key challenge, and next-generation sequencing approaches are facilitating this endeavour (K. E. Anderson et al., 2012). When studying the microbiome of an organism, researchers sometimes fail to include the whole microbiome, in addition to prokaryotes, viruses and microbial eukaryotes are also important. Viruses alone are estimated to be the largest reservoir of unexplored genetic diversity on earth (Suttle, 2007). Deep sequencing techniques such as Illumina 16S, 18S and RNA-Seq, has in recent years proven to be a powerful tool in the analysis of microbial communities and has led to the development of metagenomics and metatranscriptomics studies of various microbiomes. The term metagenomics is used to describe the recovery of information from metagenomes via the creation of shotgun sequence libraries (Pallen, 2014). Culture-independent nucleic acid approaches include analysis of whole genomes or selected genes such as 16S and 18S rRNA (ribosomal RNA) for prokaryotes and eukaryotes, respectively (Ahmad, Ahmad, & Pichtel, 2011). Metatranscriptomics refers to the total content of gene transcripts (RNA copies of genes) in a community (a unique entity at a specific moment of sampling) varying with time and environmental changes. This expression profile in a community allows the dynamics of gene expression patterns over time or environmental parameters to be realised, improving our understanding of the structure and function and adaptive mechanisms in complex communities (Parro & Paz, 2014). Whole-transcriptome analysis with total RNA sequencing captures a broad range of gene expression changes in that moment of time and enables the detection of novel transcripts in both coding and non-coding RNA species (Izard, 2015).

Recent efforts based on these technologies have uncovered novel and unexpected taxa associated with honey bees (Cornman et al., 2012; Runckel et al., 2011; Singh et al., 2010) providing estimates of healthy microbial communities versus those of diseased colonies. In

addition to the tried and tested 16S and 18S sequencing, RNA-Seq allows analyses of viral entities and gene expression are proving to be increasingly important in the microbiome characterisation and function of insects (Boncristiani et al., 2012; Ikeda, Yamada, Hamajima, & Kobayashi, 2013; Johansson et al., 2013; S. Liu, Vijayendran, & Bonning, 2011; Mokili, Rohwer, & Dutilh, 2012). Data-mining from transcriptomic, genomic and metagenomic studies may allow us to address the exact ecological role of these viruses. Viral metagenomic analysis of environmental samples suggests that the field of virology has explored less than 1% of the extant viral diversity. In the last decade alone, NGS has permitted the discovery of many viruses in a wide range of samples (Mokili et al., 2012). From three mosquito samples, it was demonstrated that a broad survey of viral diversity could be achieved (Ng et al., 2011). Most viral sequences found were novel, suggesting that the viral community in mosquitoes, as well as the animal and plant hosts they feed on, are highly diverse and mostly uncharacterized.

The analysis of the copious data generated by high-throughput sequencing is the most challenging aspect of metagenomics. An inherent difficulty in assigning taxonomic designations to viral sequences is the issue with not having universally homologous nucleic acid components present in all viruses that may be used to build phylogenetic trees (Fawaz, Raut, Mishra, & Kambla, 2014). An estimation of species richness and diversity of microbial eukaryotes and their relationship with prokaryotic organisms is also of high priority (Medinger et al., 2010), giving another dimension to these relationships within the host. Therefore, efficient simultaneous amplification of 16S and 18S rDNA sequences in addition to viral data from a given sample is critical to forming a complete microbial network analysis (Y. Wang, Tian, Gao, Bougouffa, & Qian, 2014). NGS techniques allow for screening the microbiome for microbiota as well as predicting the functional processes these microbiotas provide in the community and host based on previous research. This screening then permits a more targeted approach, using significant target candidates found in new high throughput techniques, such as Microarrays and TaqMan® Array Cards. These methods are ideal for screening biomarkers such as specific microbiota and immune genes, allowing for a complete pathogen, symbiont and immune gene network interaction.

1.9 IMMUNITY

The advances in molecular biology over the last few years, especially with the advent of full genome sequencing, offer previously unforeseen solutions to disease and human health (D. Butler, 2010). We have also seen the field of ecological immunology ('eco-immunology') emerge, addressing the complexity of immune responses across different ecologies, environments, species and in the evolutionary context. Focusing on differences in resistance to parasites, ecological immunology (Brunner, Schmid-Hempel, & Barribeau, 2014), seeks to understand the variation in immune function across individuals, determining fitness consequences of such interactions (Norris, 2000; Rolff, 2003; P. Schmid-Hempel, 2003).

The genomes of eusocial insects, including several ants and the honey bee, *Apis mellifera*, have a reduced complement of immune genes relative to solitary insects (Evans et al., 2006; C. D. Smith et al., 2011; C. R. Smith et al., 2011). For example, the genome of the honey bee contains approximately 66% fewer innate immune genes than found in Dipterans and 10–20% fewer immune genes than the solitary Jewel wasp, *Nasonia vitripennis* (Evans et al., 2006; John H. Werren et al., 2010). This reduced number of immune genes in honey bees is reported to be the trade-off related to the antimicrobial properties of substances such as propolis and honey found in eusocial insects. An Australian study also found that the effectiveness of these antimicrobial compounds produced by a *Polistes* wasp colony increased the genetic diversity, with the trade-off of decreasing colony size (Hoggard, Wilson, Beattie, & Stow, 2013). Recent studies have demonstrated that social insects foraging on antimicrobial plant compounds can also play a key role in "social immunity" behaviour (Erler, Denner, Bobiş, Forsgren, & Moritz, 2014; Manson, Otterstatter, & Thomson, 2010). Therefore in social hymenopterans, social immunity using antimicrobial compounds as well as the innate immune system are essential and work together to fight off pathogenic invaders.

The innate immune system of arthropods consists of two parts; the humoral and the cellular response. In general, the cellular immune system is composed of the combined action of circulating cells in the haemolymph, reactions of phagocytosis, encapsulation and melanisation. The humoral immune response involves the production of substances with antimicrobial activity (Erler, Popp, & Lattorff, 2011). These immune genes are silent in the absence of an immune challenge caused by pathogens which may be specific to the host (J. A. Hoffmann, 1995). Metatranscriptomics analysis of differential immune gene expression

gives an insight into the different immune reactions and biological processes (Harshman & James, 1998) that can occur in hymenopterans. Deciphering these evolutionary patterns of immune defence genes will help to understand the evolutionary dynamics between hosts and pathogens (Viljakainen, 2015), giving an insight into the diverse mechanisms insects employ for defence. It may also contribute to the development of new insecticides, and a biocontrol for insect pests (Brucker, Funkhouser, Setia, Pauly, & Bordenstein, 2012).

Immune gene expression can vary with parasite exposure, among colonies and populations of social insects (Brunner et al., 2014). Brunner et al. (2014) showed how colonies of the bumble bee *Bombus terrestris* can vary in their susceptibility to the trypanosome gut parasite *Crithidia bombi*, reducing colony fitness. They assayed the expression of 28 immunologically important genes in foraging workers showing differences among colonies in their immune gene expression profiles providing clues to inter-colony variation in susceptibility to this parasite (Brunner et al., 2014). Nurse honey bees (*Apis mellifera*) exposed to synthetic acaricides also showed varying immune-related gene expression (Garrido et al., 2013). This exposure to acaricides indicated that the use of acaricides in honey bee husbandry could cause varying degrees of immune reaction, relaying fears among the beekeeper community that acaricides used for mite control may cause harmful effects on honeybee physiology. These examples show that an in-depth investigation into the immune response of insects can demonstrate the effect that an invader or chemical can have on the host. Therefore, the immune expression resulting from a chemical or pathogen effect on the host immune system can aid in finding significant biocontrol candidates, which can overcome natural host immunity, as well as decipher the microbiota that improves fitness.

1.10 THESIS AIMS

This thesis, investigated the microbiome of *Vespula vulgaris* in three distinct life stages from two ranges, the native European and invaded New Zealand range. The initial aim was to characterise microbiota found that may enhance invasion success as well as pathogenic microbiota that may be deleterious to these wasps. Microbiome community data analysed from *V. vulgaris* was also compared with four hymenopteran species by examining the specificity of microbiota found. Diseased and healthy *V. vulgaris* nests was also investigated, comparing both in a metatranscriptomic study. This metatranscriptomic analysis was used to elucidate the cause of infection as well as attempting to infect wasp nests in a time-series infection study using target candidates revealed in the transcriptomic analysis. By using molecular-based techniques, the results from this research provide new incidence records of the microbiome of *V. vulgaris* and additional microbiome data in related hymenopterans, as well as testing the effect candidate microbial targets have on *V. vulgaris* hosts.

Chapter 2 characterises the microbial community, including the core microbiome of *V. vulgaris* in three distinct life stages (larvae, workers and queens (gynes)). This comparative analysis investigated the possibility that there are distinctions found between each life stages, as well as a preliminary examination of the differences found between ranges. These differences include the acquisition of microbiota as well as loss of enemies from the native to the invaded ranges.

Chapter 3 examines comparative microbial community diversity identified in five hymenopteran hosts (*Vespula vulgaris*, *Apis mellifera*, *Bombus terrestris*, *Vespula germanica* and *Linepithema humile*). Preliminary evidence of host microbial specificity was also investigated. It was hypothesised that there would be greater microbiota diversity in generalist feeders, vespid wasps (*V. vulgaris* and *V. germanica*) and Argentine ant (*L. humile*) compared with honey bee and bumble bee pollinators *A. mellifera* and *B. terrestris*. These analyses give insight into the microbial interactions of hymenopteran species in a community. This interpretation of the microbial diversity among pest and beneficial hymenopterans adds to hymenopteran microbial knowledge, highlighting the specificity of microbiota crucial in the management and control of invasive pests such as *V. vulgaris*, *V. germanica* and *L. humile* while protecting beneficial pollinators such as honey bees and bumble bees.

Chapter 4 investigates diseased *V. vulgaris* nests. In a comparative metatranscriptome analysis of infected larvae and healthy larvae, the aim was to decipher pathogen candidates unique to the diseased nest. Five potential pathogens were identified of which four were investigated in a time series infection study. Larval samples were then analysed in RT-qPCR analysis using designed primers, to test potential pathogen loads and viral immune gene expression, highlighting potential pathogenic effects on the host.

Chapter 5 summarises and synthesises the main findings of this thesis, outlining constraints and future research to further strengthen our knowledge of the invasive success of *V. vulgaris*.

2 CHARACTERISATION OF THE CORE AND COMMUNITY MICROBIOME OF THE COMMON WASP (*VESPULA VULGARIS*)

2.1 ABSTRACT

The critical impact that a microbiome has on host fitness makes this interaction increasingly important as we evaluate invasive species. These intertwined relationships can have positive or negative effects on the host, causing a shift in microbial balance and may affect host fitness. These microbial influences acting at the individual host or the colony level can potentially drive the success of a population.

For the first time, the microbiota of an invasive wasp *Vespula vulgaris* is investigated in native Europe and invaded New Zealand range. This wasp can have detrimental effects on invaded ranges, causing ecosystem changes through predation of native invertebrates causing mass mortality events as well as competing with native species for resources. The goal of this study was to test for microbiota differences in three distinct life stages across two ranges, native and invaded, using 16s and 18S amplicon sequencing together with Illumina RNAseq. The bacterial, microbial eukaryote and viral communities of larvae, workers and queens were compared in both ranges. In addition, the core microbiome of *V. vulgaris* is investigated and described as microbiota characterised present in 100% of microbial communities, including the core differences between life stages. The results demonstrated a change in community composition between life stages of wasps within the same nest. Larvae had greater bacterial, microbial eukaryote and viral sequence diversity compared with that of workers and queens. Remarkably, queens were largely free of known microbial eukaryotes and completely free of known RNA viruses. Evidence of a microbial shift was also highlighted, occurring in the invaded New Zealand range potentially increasing the fitness of this invasive wasp. This microbial shift demonstrates the loss of taxa from the native Belgian range to the invaded New Zealand range, releasing the host from possible enemies (*Providencia*, *Serratia*, *Rickettsiella*, *Diplorickettsia*, *Microbacterium*, *Rhopalosiphum padi virus* (RhPV), *Aphid lethal paralysis virus* (ALPV), and the protozoan *Mattesia*). As well as the potential loss of enemies seen in the

invaded New Zealand range samples, potential microbiota acquisition was also observed. *Apis mellifera* and *Bombus* spp. endosymbionts (*Snodgrassella alvi*, *Gilliamella apicola*, *Saccharibacter*, *Commensalibacter*, *Bifidobacterium* and *Bombella*) as well as *Kashmir Bee Virus* and the protozoan *CCA5*, were not observed in the native Belgian samples. These results demonstrate that the microbiome changes between distinct life stages, and also provides preliminary data proposing microbial differences between the native and invaded ranges.

2.2 INTRODUCTION

The continual accidental or deliberate global redistribution of species by humans can cause a detrimental effect on native species in new ranges (Mack et al., 2000), disrupting natural ecosystems (Williamson, 1996). Introduced species can bring an array of microorganisms, including pathogens or emerging infectious diseases (EID's) (Woolhouse et al., 2005). Social insects have proven to be highly adept at invading new habitats and are capable of causing social, economic and environmental damage (Moller, 1996; John H. Werren et al., 2010). These ecologically significant social insects harbour protozoan, fungal, bacterial and viral parasites (Bailey & Ball, 1991; Rose et al., 1999) and can be significant drivers of global environmental change. Invasive vespid wasps have demonstrated how successful they can be in an introduced range, negatively impacting on native species and ecosystem functioning, as well as having significant economic impacts (Lockwood et al., 2007; Vitousek & D'Antonio, 1997).

Vespula vulgaris is a voracious predator, listed in '100 of the world's worst invasive alien species' (Lowe et al., 2000), representing one of the most significant invertebrate threats to New Zealand native fauna (Lester, Brown, et al., 2014). Having arrived from its native range of Eurasia (Archer, 2012), it has now become well established in Argentina, Australia and New Zealand (J.R. Beggs et al., 2011). In New Zealand, measures of wasp nests abundance show less dramatic fluctuations (mean density of 12.0 nests ha⁻¹ and varying by a twofold range) (Barlow et al., 2002) compared with those in the native European range. In England, wasps showed a more cyclical 7-year cycle (varying by a 42 fold range) (Archer, 1985), although this study measured individual wasp abundance (malaise study) and not nest abundance. This variation in abundance may be related to several factors including food availability and the absence of natural enemies such as pathogens and parasites (Lester et al., 2015).

Research suggests that *V. vulgaris* hosts a variety of pathogens in its home range (Evison et al., 2012). These pathogens could have the potential to induce population crashes as in other social insects (S. A. Cameron et al., 2011). Studies have also highlighted critical functional symbiotic relationships with the microbiota of insects highly intertwined with the host and essential for insect development (Hamdi et al., 2011; Lanan et al., 2016; N. a. Moran, 2015; Weiss & Aksoy, 2011). To our knowledge

the microbiome (bacterial, microbial eukaryotes and virus communities) of *V. vulgaris* has not been as comprehensively studied as other hymenopteran species (Cariveau et al., 2014; J. A. Chandler et al., 2011; Engel et al., 2012; Hamdi et al., 2011; Ishak et al., 2011; J. Li et al., 2012; Runckel et al., 2011; Sabree & Moran, 2014; David R. Tarpy et al., 2015). Previous social insect research has demonstrated that the microbiome can change between life stages (Benjamino & Graf, 2016; Kapheim et al., 2015; David R. Tarpy et al., 2015; Yun et al., 2014) and microbial diversity can change due to the environment, habitat and diet (Ishak et al., 2011; Meriweather, Matthews, Rio, & Baucom, 2013; Villegas & Pimenta, 2014; Yun et al., 2014). These microbiome differences can take place among life stages despite their social nature, sharing resources through the process of trophallaxis, giving rise to a social gut (Oster, G.F. & Wilson, 1978). Consequently, a broad assessment and characterisation of the microbiome of *V. vulgaris* will enable correlation between life stage microbial communities from individual *V. vulgaris* nests and across populations and ranges. Thus it was hypothesised based on previous social insect research, that the microbiome of a *V. vulgaris* colony (nest) would vary between life stages due to the social role of each distinct caste (larva, worker and queen (gyne)), based on nutrition and environmental exposure. It was also hypothesised that microbial bottlenecks may have occurred with loss of microbiota found in the native range and possible acquisition of microbiota in the new range. Using culture-independent sequence-based metagenomics, prokaryote, microbial eukaryote and viral communities were analysed to characterise the microbiome diversity and evaluate the core microbiota across six *V. vulgaris* nests, focusing on the life stages, and life stages differences between ranges.

2.3 MATERIALS AND METHODS

This examination of the microbial community of three common wasp life stages was replicated in their native and invaded range. This replication enabled a robust design to test if there are differences in microbial communities between life-stages within nests, and a preliminary comparison of differences in microbial communities between native and introduced range.

2.3.1 *V. vulgaris* Collections

Nest were sampled from three separate sites within the native (Belgium) and invaded (New Zealand) ranges. Sampling sites were chosen, with a minimum distance of 20 km between sites (**Table 2-1**). All nests were sampled in the autumn season of both ranges in 2014. Autumn sampling was chosen as the reproductive forms have emerged at this time allowing sampling of three life stages larvae, workers and gynes (newly emerged queens), i.e. a total of 18 samples (nine in each range). In Belgium, samples were taken in the area surrounding the city of Leuven in September/October. In New Zealand, sampling sites were selected in the Nelson/Tasman district and sampled in March/April (**Table 2-1**).

Figure 2-1 Map illustrating the native Belgian and invaded New Zealand ranges of *Vespula vulgaris*. Areas of sampling marked in red boxes with three sites from Belgium around the city of Leuven, representing the native range and three sites from New Zealand, around the Nelson/Tasman district, representing the invaded range. Sites are marked with red dots on the map. (For coordinates see Table 2-1).

Table 2-1 Nest collection site coordinates in the native Belgian range and invaded New Zealand range

Range	Nest	Site	Longitude	Latitude
Native Belgium	N1	Wijgmaal, Leuven	N 50.5539	E 4.4143
	N2	Hasselt, Hasselt	N 50.5523	E 5.2017
	N3	Wezemaal, Leuven	N 50.5720	E 4.4444
Invaded New Zealand	N1	Six Mile Reserve	S -41.7685	E 172.9572
	N2	Tin Line Reserve	S -41.2809	E 173.5087
	N3	Braeburn Track	S -41.7954	E 172.5156

All nests sampled were from subterranean extractions, where whole nests were taken alive and placed in 20L buckets. The entire live wasp nests were then placed in a -20°C freezer for approximately 1-2 hours to slow wasp metabolism and impede wasp movement and attack, therefore sampling wasps alive. Sampling was undertaken aseptically using different petri dishes between nests and disinfecting the bench area, forceps and scissors with ethanol between each sample collection. Collected samples of larvae, workers and queens (five individuals per vial) were cut up and placed in RNeasy® (Sigma-Aldrich Co. LLC) in separate DNA/RNA free vials according to manufacturer instructions and stored at -80°C until homogenisation and nucleic acid extraction. Each sample consisted of five representative individuals from each life stage (larval, worker and queen). At the time of sampling, five random individuals from each colony life stage was deemed an appropriate number to characterise the colony microbiome. Individuals were not surface cleaned, enabling characterisation of both endosymbiotic and exosymbiotic microbiota in addition to eukaryotes such as mites. Also, *V. vulgaris* wasps prefer a subterranean habitat, excavating and ingesting soil and decaying wood for nest expansion. This lifestyle would ensure both internal and external sample environmental contamination. Therefore, surface cleaning the individual samples prior to pooling would not have made a crucial difference in the overall microbiota characterised.

2.3.2 DNA Extractions and Library Preparation

All samples were homogenised aseptically in a laminar flow hood using a Qiagen TissueRupter® before extractions were conducted. Purification of total DNA from

pooled samples of five whole insects was carried out using DNeasy Blood & Tissue Kit using the supplementary protocol:

‘DY14 (www.qiagen.com/literature/protocols/DNeasyTissue.aspx)’.

The purity of DNA samples was verified using the absorption ratio (260/280 nm) determined by NanoDrop 1000 and samples were run on 1.5% agarose gel to ensure product banding. DNA samples were sent for library preparation and de novo 16S and 18S Illumina MiSeq sequencing at New Zealand Genomics Ltd (NZGL). 16S ribosomal RNA gene amplicons were prepared following the Illumina 16S Metagenomic Sequencing Library Preparation Protocol (Illumina, 2013). The V3-V4 region of the 16S rRNA gene was amplified using Nextera adapter primers and dual-index barcodes to the amplicon target (Illumina, 2013). Specific primer pair (**Table 2-2**) sequences were used to create a single paired-end amplicon for the V3-V4 region of approximately 300 bp.

18S ribosomal RNA gene amplicons were prepared according to the protocol of the Earth Microbiome Project (J Gregory Caporaso et al., 2012). The V9 region (200 bp) of the eukaryotic 18S rRNA gene was amplified for each sample with 1391f and EukBr primers (Amaral-Zettler, McCliment, Ducklow, & Huse, 2009) (**Table 2-2**). PCR DNA products were pooled 80% for the 18S libraries to allow for swamping of host genomic DNA and 20% for the 16S and sequenced on a MiSeq run. Adapters and barcodes were removed and read quality trimmed using the SolexaQA package (Cox, Peterson, & Biggs, 2010) and reads were quality checked using FASTQC (Andrews, 2010).

Table 2-2 Primers used in 16S & 18S amplicon sequencing

16S Primers	
16S Primer F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
16S Primer R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC (Klindworth et al., 2013)
18S Primers	
Illumina_Euk_1391f	AATGATACGGCGACCACCGAGATCTACACTATCGCCGTTCCGGTACACACCGCCCGTC
Illumina_EukBr	CAAGCAGAAGACGGCATACGAGATAGTCAGTCAGCAGATCCTTCTGCAGGTTACACTAC (Klindworth et al., 2013)

2.3.3 RNA Extractions and Library Preparation

The RNA-Seq approach was used for the detection of RNA viruses. Total RNA was extracted using the PureLink®RNA Mini Kit. Total RNA quantity and integrity was assessed on an Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Nano Chip kit according to the manufacturer's instructions. Total RNA was sent for sequencing on Illumina HiSeq platform (2 x 100 bp paired-end), by NZGL. Total RNA samples were used to make RNA-Seq libraries using the "TruSeq RNAseq Sample Prep kit" according to the manufacturer's instructions.

2.3.4 Prokaryote Bioinformatics Pipeline

Microbial prokaryote diversity was primarily analysed using QIIME version 1.9.1 (J G Caporaso et al., 2010). First, the 16S read sequences primers and adaptors were removed. Paired-end reads were assembled to reconstruct the complete nucleotide sequence using PANDAsq, v 2.8 (Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012). PANDAsq quality filters sequences and corrects sequencing errors to obtain a higher quality sequence yield. Of the 37,351 16S input reads, 31,902 sequences were retained. Following preliminary data quality checking, libraries were split and Operational Taxonomic Units (OTUs), were picked and clustered to known taxa based on 97% sequence identity using UCLUST (Edgar, 2010) closed-reference OTU picking. The longest sequence in a cluster was used as the representative sequence for that OTU. Final OTUs were taxonomically classified and finally aligned to the SILVA 128 database (last modified 06-Feb-2017)(Quast et al., 2013; Yilmaz et al., 2014) using the PyNASt alignment tool (J. Gregory Caporaso et al., 2010). Chimaeras were excluded using ChimeraSlayer (B. J. Haas et al., 2011) along with singleton OTUs. A phylogenetic tree was constructed from the aligned, representative sequence set using FastTree (Price, Dehal, & Arkin, 2010) and filtered using normalised reads. The iTOL website (Letunic & Bork, 2011) was used to visualise bacterial taxa.

2.3.5 Microbial Eukaryote Bioinformatics Pipeline

Microbial eukaryote diversity was analysed using the SILVAngs v 1.3 pipeline (Quast et al., 2013). 18S rRNA read sequences were pre-processed by removing primers and adaptors and assembled using PANDAsq, v 2.8 package (Masella et al., 2012). Quality filtering of 164,750 18S input reads resulted in 163,173 output sequences. Reads were

aligned using the SILVA Incremental Aligner (SINA v1.2.10 for ARB SVN (revision 21008) (Pruesse, Peplies, & Glöckner, 2012) against the SILVA SSU rRNA SEED database (January 2016) and quality controlled (Quast et al., 2013). Reads, either shorter than 50 aligned nucleotides, with more than 2% of ambiguities, or with 2% of homopolymers, were excluded from further processing as recommended by SILVAngs (Quast et al., 2013). Putative contamination and artefact reads with low alignment quality (50 alignment identity, 40 alignment score reported by SINA), were also identified and excluded from downstream analysis. Following quality control, identical reads were clustered into OTUs, on a per sample basis, with the reference read of each OTU classified. De-replication and clustering was achieved using cd-hit-est v 3.1.2 (W. Li & Godzik, 2006). The classification was performed by a local nucleotide BLASTn (version 2.2.30+) search against the non-redundant (nr) version of the SILVA SSU Ref dataset (release 123; <http://www.arb-silva.de>) with standard settings (Camacho et al., 2009). The classification of each OTU reference read was mapped onto all reads that were assigned to the respective OTU. Reads without any BLAST hits or reads with weak BLAST hits, where the function $((\% \text{ sequence identity} + \% \text{ alignment coverage})/2)$ did not exceed 93, were not classified, as per the recommended settings assigned by SILVAngs. These reads were then assigned to the meta group "No Relative" in the SILVAngs fingerprint and Krona charts (Ondov, Bergman, & Phillippy, 2011).

2.3.6 Viral Bioinformatics Pipeline

De novo transcript reconstruction was undertaken using the Trinity platform (Grabherr et al., 2011) for *de novo* transcriptome assembly from RNA-Seq data in non-model organisms. The transcriptome assembled from the trimmed reads was performed with the software Trinity Release v2.0.6 2015-03-03 (Grabherr et al., 2011). FastQC 0.11.3 (Andrews, 2010) sequences were adapter and quality trimmed at a Phred score of Q20 (probability of 0.01) to capture most sequences. SolexaQA 3.0 was then used to carry out a further quality analysis using DynamicTrim and LengthSort in combination to remove poor quality bases and reads from high throughput sequence data (Cox et al., 2010). Transcriptome *de novo* assembly of normalised reads was performed with Trinity v 2.0.6 (Grabherr et al., 2011). Reads were then normalised using Trinity's (v 2.0.6) In silico read normalisation (B. J. Haas et al., 2013).

The Trinity assembled contigs were submitted to the National Centre for Biotechnology Information (NCBI) nt database with an e-value of 0.001. Searches were run at the Victoria University of Wellington Science Faculty High-Performance Computing Facility's BLAST v 2.2.30 installation (Zhang, Schwartz, Wagner, & Miller, 2000). A custom Perl script was used to summarise the data and append taxonomic assignment to individual BLAST results (Gruber et al., 2017). This process allowed filtering of taxa and extraction of viral targets from each FASTA file. Extracted viral targets were searched with BLASTx against the nr database with an e-value of 0.0001, overhang value of 0.25, and score edge value of 0.05 (Camacho et al., 2010). BLAST nr assigned targets were visualised to MEGAN v.6.6.3 using the default settings with the weighted LCA algorithm of 75% (Huson et al., 2016). Weighted LCA reduces the need to process each read in isolation and improves the specificity of taxonomic assignment (Huson et al., 2016).

To confirm identity of viral candidates a variety of bioinformatics techniques were used. VirusFinder2 (Q. Wang, Jia, & Zhao, 2015) was used to complement the BLAST and MEGAN analysis, further identifying viruses. This process uses raw sequences reads before assembly to search against the virus database, included with the RINS (Rapid identification of non-human sequences) package (Bhaduri, Qu, Lee, Ungewickell, & Khavari, 2012). The RINS virus.fa database contains viruses of all known classes (32,012 in total) (Bhaduri et al., 2012). Briefly, using BLAST+, an index of the virus database was created, and the raw files were run against this database. The *Polistes dominula* genome (Weiner et al., 2013) was used to carry out read subtraction, which extracts all viral reads excluding host sequences. The *P. dominula* genome was the closest annotated genome to *V. vulgaris* available at the time of analysis. As MEGAN & Virusfinder2 use contrasting approaches, this made it difficult to compare viral abundances between the two methods. Instead, presence/absence of virus between groups was used in further analyses. Virus candidates were omitted if both MEGAN and Virusfinder2 analysis did not represent them. The remaining viruses were discounted if they had less than 97% identity and not investigated further. Subsequent viral candidates were confirmed following RT-PCR analysis using designed primers

(**Table 2-3**), and cDNA sent for Sanger sequencing at Massey Genome Service, Massey University, Palmerston North.

To detect potential novel viral pathogen sequences in the assemblies, we used the Trinity assembled contigs to query the National Center for Biotechnology Information (NCBI) GenBank databases using BLAST (Zhang et al., 2000). Blastx searches were used to identify putative viral proteins from pre-processed Blastn searches of the NCBI Genbank nt databases. Blastn contigs were searched with a threshold e-value of 0.001, percent identity of 90, best_hit_overhang of 0.25 and best_hit_score_edge of 0.05. The blastx results were visualised in MEGAN 5.10.720. To confirm these as virus candidates coding sequences of novel viral origin were identified by visualising the contigs of blastx results in Blast2GO (Conesa et al., 2005) and translating these contigs using ExPASy (Bioinformatics Resource Portal, Swiss Institute of Bioinformatics, <http://web.expasy.org/>) to determine the appropriate reading frame.

Table 2-3 RT-PCR Primers used to confirm virus presence.

Primer	Forward	Reverse
KBV	TGATGGTGATTGTGGAGCCC	TCGACTCCCGGATAACCTGT
DWV	AGTGCAGGCAAAACCAGAGA	GAACCGCGCCATTGCATAAA
RhPV	AACGGGTCACCGTCAATACC	GATCCACCAGACACTTGGCA
ALPV	ATATGGTGTTGTTGCCCCGT	TGCGATAGGCATAGCAGTGG

2.4 STATISTICAL ANALYSES

Richness (OTU abundance) and diversity (Shannon's Log e) metrics were assessed using square root transformed raw data (882 taxa assigned by Silva 128 (Quast et al., 2013)). The DIVERSE function in Primer 6 (Clarke & Warwick, 2001) was applied and richness and diversity metrics visualised using the R package ggplot2 (Wickham, 2009).

A one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the richness and diversity means of three life stages (larvae (n=6), workers (n=6) and queens (n=6)). The same ANOVA analysis was used to determine if there were any statistically significant differences for richness and diversity between life stages between ranges (New Zealand (NZ) larvae (n=3) and Belgium (BE) larvae (n=3), (NZ workers (n=3) and BE workers (n=3), (NZ queens (n=3) and BE queens (n=3)) and overall between ranges (New Zealand (n=9) and Belgium (n=9)). Where ANOVA *p*-values were statistically significant ($p \leq 0.05$), post hoc two tailed *t* tests were performed to determine which specific groups significantly differed from each other.

All subsequent 16S data were normalised following filtering of OTUs with less than 100 reads reducing negative value outputs for less abundant OTUs. Due to the lower number of 18S OTUs, filtering of OTUs less than 100 were not required before normalisation of the 18S dataset. The R package metagenomeSeq's CSS (cumulative sum scaling) (Paulson, 2014) was used to carry out the normalisation on the 16S and 18S datasets (McMurdie & Holmes, 2014). The dissimilarity between bacterial and microbial eukaryote communities was assessed using Bray-Curtis dissimilarity matrix and tested for significance between each life stage, range and life stage within each range using PERMANOVA in Primer-E (Clarke & Warwick, 2001). To partition for variance and assess significance in each community pairwise tests were run over 999 permutations. Each group (life stages, ranges and life stages within ranges) were evaluated separately as fixed effects, with type III partial sum of squares and Monte Carlo sampling permuted over unrestricted raw data. To visualise significant clustering patterns of bacterial and microbial eukaryote communities, three methods were used, group average clustering, non-metric multidimensional scaling (nMDS) and a canonical analysis of principal coordinates (CAP) analysis all performed in Primer-E

(Clarke & Warwick, 2001). Group-averaging clustering measured the mean (unweighted) distance apart of each sample, averaging over all between-group pairs (Clarke & Warwick, 2001). The relationship between individual microbiomes was then assessed using unconstrained ordination nMDS plots measuring ecological distance preserving rank orders of relative distances among data placing data in relation to each other based on dissimilarities among the samples (Clarke & Ainsworth, 1993). Each nMDS plot was run with 1000 random restarts, avoiding local minima using Kruskal stress formula 1 with minimum stress of 0.01 (nMDS ordination ≤ 0.1 stress is acceptable, while values equal to or ≤ 0.05 indicate a good fit) (Clarke & Warwick, 2001). Distinctness of each group was then measured using a CAP analysis. Unlike the nMDS analysis the CAP analysis is constrained, analysing the data based on permutation showing the effect of the interaction between factors in the community (Marti Anderson & Willis, 2003; MJ Anderson, Gorley, & Clarke KR, 2008).

The total sample core microbiota, as well as each life stage core, was identified using QIIME 1.9.1 (J G Caporaso et al., 2010). The core was determined by calculating OTU abundance in the core at intervals (from 0% to 100% of samples). Taxa consistently present in 100% of pooled samples were considered to represent the core microbiome of *V. vulgaris*. The contribution of each prokaryote and microbial eukaryote with dissimilarity differences among each life stage and range life stage was assessed using a similarity percentage analysis (SIMPER) analysis (Clarke & Warwick, 2001) on normalised data. SIMPER dissimilarity was verified using log-likelihood G-tests, carried out in QIIME 1.9.1, extrapolating statistical significance ($p < 0.05$) between taxa abundance in the different sample groups (J G Caporaso et al., 2010). The p -values from G-test analysis were corrected for multiple comparisons using the Bonferroni procedure within QIIME.

2.5 RESULTS

2.5.1 Core Microbiome

In this study, the core microbiota was defined as OTUs with a 97% identity level that was present in 100% of the samples analysed. Across all eighteen samples, the data showed that the core microbiota from three life stages consisted of 29 OTUs representing 23 unique taxa identified to the lowest taxonomic level (denoted by c = class, o = order, f = family, g = genus) (**Table 2-4**). One third of the total core was represented by the bacterial class Spartobacteria (34.3%), consisting of the family DA101 soil group (18.7%) and genus *Candidatus Xiphinematobacter* (15.6%). The class Alphaproteobacteria (31.1%) was also well represented, consisting of the order Rhizobiales. Bacteria from the classes Ktedonobacteria, Phycisphaerae, Thermoleophilia, Actinobacteria, Gammaproteobacteria, Solibacteres and Blastocatellia accounted for the remainder of the total core microbiome of *V. vulgaris*. The same 100% core analysis was repeated on each life stage, to investigate specificity of core microbiota in each life stage (**Table 2-5**). Life stage queens had the most core taxa (41 taxa), followed by larvae (38 taxa) and workers (30 taxa). There was greater specificity of core taxa seen in the queen (11) and larvae (10) life stages, with worker core microbiota the least (3) (**Figure 2-2**).

Table 2-4 The core prokaryote taxa and relative mean prokaryote abundances identified in three *V. vulgaris* wasp life stages. Taxonomic levels Phylum and Class are provided in addition to the genus. Where genus is not defined in italics, the lowest taxonomic level is denoted in brackets: class (c), order (o) and family (f).

Phylum	Class	Lowest Taxon	Average Abundance (%)
Acidobacteria	Blastocatellia	{ <i>RB41</i>	0.8
	Solibacteres	{ <i>C. solibacter</i>	1.1
	Acidobacteria	{ Subgroup 1 (f)	1.4
		{ Subgroup 2 (c)	1.7
Actinobacteria	Acidimicrobiia	{ Acidimicrobiales (o)	1.2
	Actinobacteria	{ <i>Corynebacterium 1</i>	1.2
		{ <i>Acidothermus</i>	2.7
	Thermoleophilia	{ Gaiellales (o)	4.5
		{ Solirubrobacterales (o)	0.9
Planctomycetes	Phycisphaerae	{ Tepidisphaeraceae (f)	5.4
Verrucomicrobia	Spartobacteria	{ DA101 soil group (f)	18.7
		{ <i>Candidatus</i>	
		{ <i>Xiphinematobacter</i>	15.6
Proteobacteria	Gammaproteobacteria	{ <i>Pseudomonas</i>	1.4
		{ <i>Bradyrhizobium</i>	0.8
		{ <i>Roseiarcus</i>	1.6
	Alphaproteobacteria	{ <i>Variibacter</i>	4.1
		{ <i>Nitrobacter</i>	18.9
		{ <i>Pedomicrobium</i>	1.5
		{ Bradyrhizobiaceae (f)	0.5
		{ <i>Rhizobium</i>	1.2
		{ Xanthobacteraceae (f)	2.4
	Ktedonobacteria	{ HSB0F53-F07 (f)	10.2
		{ JG30a-KF-32 (f)	2.2

Table 2-5 Prokaryote taxa unique to each life stage core microbiome and the total relative mean abundance of each taxa contributing to the core found in each life stage. Taxonomic levels Phylum and Class are provided in addition to genus. Where the genus is not defined in italics, the lowest taxonomic level is denoted in brackets: class (c), order (o) and family (f).

Life Stage	Phylum	Class	Lowest Taxa	Core Contribution (%)
Larvae	Firmicutes	Bacilli	<i>Enterococcus</i>	16.31
	Bacteroidetes	Sphingobacteriia	<i>Flavisolibacter</i>	0.89
	Planctomycetes	Planctomycetacia	<i>Pir4 lineage</i>	0.86
	Proteobacteria	Alphaproteobacteria	JG37-AG-20 (f)	0.54
	Proteobacteria	Alphaproteobacteria	<i>Brevundimonas</i>	0.48
	Actinobacteria	Actinobacteria	Dermacoccaceae (f)	0.46
	Actinobacteria	Thermoleophilia	TM146 (f)	0.39
	Proteobacteria	Deltaproteobacteria	<i>Haliangium</i>	0.37
	Planctomycetes	Planctomycetacia	<i>Isosphaera</i>	0.34
	Proteobacteria	Deltaproteobacteria	<i>H16</i>	0.30
Worker	Acidobacteria	Acidobacteria	<i>Candidatus Koribacter</i>	1.17
	Actinobacteria	Actinobacteria	<i>Micromonospora</i>	0.28
	Actinobacteria	Actinobacteria	<i>Actinoplanes</i>	0.24
Queen	Actinobacteria	Actinobacteria	<i>Mycobacterium</i>	0.54
	Bacteroidetes	Flavobacteriia	<i>Flavobacterium</i>	0.55
	Chloroflexi	Ktedonobacteria	1959-1 (F)	0.55
	Planctomycetes	Planctomycetacia	Planctomycetaceae (f)	1.07
	Proteobacteria	Alphaproteobacteria	<i>Tardiphaga</i>	0.65
	Proteobacteria	Alphaproteobacteria	<i>Rhodoplanes</i>	0.29
	Proteobacteria	Alphaproteobacteria	<i>Rhizomicrobium</i>	0.35
	Proteobacteria	Alphaproteobacteria	<i>Sphingomonas</i>	0.29
	Proteobacteria	Betaproteobacteria	Nitrosomonadaceae (f)	0.41
	Proteobacteria	Betaproteobacteria	SC-I-84 (o)	0.98
	Proteobacteria	Gammaproteobacteria	Xanthomonadales (o)	0.46

Figure 2-2 Relative abundances (%) of each core prokaryote class identified in *V. vulgaris* samples. The total core (all life stages) 100% of samples are provided in addition to relative core class abundances observed in each life stage, larval, worker and queen. Colours placed in the legend defines class core abundances

2.5.2 Prokaryote Community Distinctions

QIIME analysis returned 574,248 non-singleton reads from 18 pooled samples (5 individuals per sample), six larvae, six workers and six queen samples. A mean of 31,902 reads $\pm$ 45,271 standard deviation was identified from the 18 samples. A total of 736 bacterial 16SrRNA OTU sequences were assigned at the 97% sequence similarity level and 375 taxa classified to genus.

The dissimilarity of life stage prokaryote communities within each range was illustrated with average linkage clustering (**Figure 2-3A**) and nMDS plot (**Figure 2-3B**). All three native larvae, workers and queens except one queen clustered together with less than 30% dissimilarity. Wasps in the invaded range showed an opposite pattern with no life stages clustering together suggesting variable communities. This pattern is supported by the two-dimensional nMDS plots (2D Stress 0.09) which indicates greater clustering of native range life stages compared with the invaded range. Native larvae and workers displayed an overlapping trend in communities with separation from the native queen communities. Life stages in the New Zealand range overlapped. Larvae and queen sample communities were tightly clustered compared with the invaded worker sample communities, which displayed more variability. CAP analysis (**Figure 2-3C**) confirmed prokaryote community distinctiveness in the native range life stages, while the invaded range life stages showed little distinction with a high degree of overlap between life stage communities. PERMANOVA pairwise comparisons (**Table 2-6**) of OTU variance revealed between life stages dissimilarity was not statistically significant ($p > 0.05$). However, the overall pairwise comparison between the two ranges was significantly different. Pairwise comparisons between life stages in ranges showed considerable dissimilarity in larvae between ranges and New Zealand queens and Belgian workers. Belgian larvae and workers were also significantly dissimilar to Belgian queens, whereas Belgian larvae and workers were not significantly dissimilar to one another.

Figure 2-3 Figures of prokaryote community dissimilarity (A-D). Illustrating clustering distances and diversity of individually normalised microbiomes in the host *V. vulgaris* life stage within each range. Larvae (green triangle), workers (blue triangle) and queens (cyan square). The sampling range is annotated with BE=Belgian, NZ=New Zealand. Graphs A-C are based on Bray-Curtis dissimilarity of bacterial OTU frequencies. (A) Group average cluster dendrogram from average-linkage clustering of bacterial OTU frequency. (B) Non-metric multi-dimensional (nMDS) scaling plot of bacterial OTU frequency, Stress value: 0.09 (≤ 0.1 is acceptable). (C) Canonical analysis of principal coordinates (CAP), illustrating distinctness of bacterial communities. (D) The richness and Shannon's diversity metrics calculated on total square root transformed dataset. The standard error shows the variance of each range life stage (n=3).

Table 2-6 PERMANOVA pairwise tests comparing dissimilarity between each *V. vulgaris* life stage, range and life stage within each range. The t statistics along with the Monte Carlo corrected *p*-value are provided. Shaded values indicate statistically significant results.

PERMANOVA Pairwise test		
Life Stages		
	t	<i>p</i> (MC)
Larvae, Workers	1.118	0.289
Larvae, Queens	1.002	0.422
Workers, Queens	1.265	0.181
Range		
	t	<i>p</i> (MC)
Belgium, New Zealand	2.036	0.006
Life Stages in Range		
	t	<i>p</i> (MC)
New Zealand Larvae, Belgian Larvae	1.839	0.035
New Zealand Workers, Belgian Workers	1.477	0.141
New Zealand Queens, Belgian Queens	1.447	0.178
Belgian Larvae , Belgian Workers	1.636	0.098
Belgian Larvae, Belgian Queens	2.328	0.033
Belgian Workers, Belgian Queens	2.803	0.014
New Zealand Larvae, New Zealand Workers	0.806	0.591
New Zealand Larvae, New Zealand Queens	0.815	0.586
New Zealand Workers, New Zealand Queens	0.396	0.895

P values have been corrected denoted *P* (MC) = Monte Carlo test corrected, t=test statistic

An ANOVA was performed on the richness and Shannon diversity metrics for all groups including life stages, range and life stages within each range. The ANOVA, demonstrated no significant ($p < 0.05$) difference between life stages and life stages within ranges, however between ranges, the Shannon's diversity metric was significantly different (ANOVA $F(1, 16) = 6.25$ $p = 0.02$).

Mean richness and diversity metrics (**Figure 2-3D**) revealed higher richness (mean= 256.2 ± 19.1 SE) and diversity (5.0 ± 0.2) in larvae compared with workers (richness 245.5 ± 16.8 ; diversity 4.7 ± 0.4) and queens (richness 215.3 ± 12.6 ; diversity 4.8 ± 0.3). Native Belgian larvae (richness 292.0 ± 29.0 ; diversity 5.1 ± 0.1), when compared with New Zealand larvae (richness 220.3 ± 19.1 ; diversity 4.8 ± 0.2), had higher richness and diversity means. There was a similar trend in richness and diversity when comparing worker samples from Belgium (richness 260.3 ± 18.8 ; diversity 5.0 ± 0.2) and New Zealand (richness 230.7 ± 16.8 ; diversity 4.5 ± 0.4). Belgian queen richness (richness 197.7 ± 13.2 ; diversity 5.0 ± 0.1) was lower than that of New Zealand queens (richness 233.0 ± 12.6 ; diversity 4.6 ± 0.3). However the community diversity was again

highest in the native range (Graph D). Overall samples from Belgium (richness 250 ± 13.2 ; diversity 5.0 ± 0.1) exhibited higher mean richness and diversity than their New Zealand counterparts (richness 228 ± 12.6 ; diversity 4.6 ± 0.3).

2.5.3 Characterisation of Prokaryotic Communities

Of the 375 OTUs, 98 OTUs exceeded the threshold of 100 similar OTUs required prior to normalisation. Based on the mean of OTU reads across all 18 samples, eleven phyla were identified, of which nine represented 99.2% of the community. The top five phyla that represented 81.4% of the total community were made up of 37.1% Proteobacteria, 12.8% Verrucomicrobia, 11.7% Firmicutes, 11.8% Actinobacteria, and 7.8% Acidobacteria. The iTOL phylogenetic tree (**Figure 2-4**) illustrated similarities as well as some differences between life stages in each range. Of the 75 taxa represented in the microbiome, larvae hosted 71 taxa, workers 72 taxa and queens 61 taxa.

Of the taxa represented across the life stages, four taxa were not present in the larval life stage, three Gammaproteobacteria (*Erwinia*, *Acinetobacter* and *Orbus*) and the Alphaproteobacteria (*Mesorhizobium*). Absent taxa in the worker life stage included Gammaproteobacteria (*Diplorickettsia*), Alphaproteobacteria (*Asaia*), and a Chlamydiae (*Candidatus Rhabdochlamydia*). Taxa seen in larval and worker life stages but absent in the queen life stage included, Actinobacteria (*Microbacterium*), Alphaproteobacteria (*Asaia*), Bacilli (*Leuconostoc*, *Vagococcus*, PeH08 (f) and Streptococcaceae (f)), Chlamydiae (*C. Rhabdochlamydia*) and seven Gammaproteobacteria (*Rickettsiella*, *Diplorickettsia*, *Providencia*, *Pantoea*, *Erwinia*, *Acinetobacter*, *Orbus*). Genera unique to larvae included *Diplorickettsia*, *Candidatus Rhabdochlamydia* and *Asaia*. Genera unique to the worker life stage included *Erwinia*, *Acinetobacter* and *Orbus*. No unique taxa were presented in queens.

There was more significant variation in taxa incidence when comparing microbiomes between life stages in each range. The following constitutes taxa presence or absence in both high abundance and low abundance microbiomes: Native Belgian larvae hosted 60 bacterial taxa, and invaded larvae hosted 57 taxa. This comparison revealed *Diplorickettsia*, *Asaia*, *Pantoea*, *Raoultella*, *Serratia*, *Providencia*, *Vagococcus*, PeH08, *Lactobacillus*, Streptococcaceae (f), *Lactococcus*, *Leuconostoc* and Leuconostocaceae (f) were present in the native range but absent from the invaded range. Taxa present in the invaded range not seen in the native range included *Apibacter*, *Bifidobacterium*, *Spiroplasma*, *Bacillus*, *Commensalibacter*, *Bombella*, *Saccharibacter*, *Arsenophonus*, Orbaceae (f) and Orbaceae (buff-tailed Bumble bee). Native workers hosted 59 taxa of which, *Providencia*, *Vagococcus*, Leuconostocaceae (f), *Raoultella*, PeH08 (f), *Leuconostoc*, *Orbus*, and *Bacillus* were not present in the invaded range. In

comparison, invaded workers had 61 taxa, with the taxa *Arsenophonus*, *Orbaceae* (*Buff-tailed BB*), *Bombella*, *Apibacter*, *Wolbachia*, *Gilliamella*, *Commensalibacter*, *Spiroplasma*, *Snodgrassella*, *Bifidobacterium*, *Orbaceae* (f), and *Saccharibacter* not seen in the native range. Native queens had 48 taxa, with *Convivina*, *Serratia*, *Raoultella*, *Leuconostocaceae* (f), *Lactococcus* and *Bacillus* taxa not seen in the invaded range. Invaded queens accounted for presences of *Wolbachia*, *Gilliamella*, *Orbaceae* (*Buff-tailed BB*), *Snodgrassella*, *Bifidobacterium*, *Saccharibacter*, *Commensalibacter*, *Bombella*, *Arsenophonus*, *Apibacter*, *Spiroplasma* and *Enterobacter* not seen in the native range. Relative mean class bacterial abundances of life stages within each range was also calculated (**Figure 2-5**), identifying the most abundant class in each group and all taxa identified to the lowest taxonomic level, contributing higher than 1% to that class.

Figure 2-4 Phylogeny of prokaryote taxa identified from *V. vulgaris* to the genus level where possible. This rooted maximum likelihood phylogeny reveals the specificity of microbiota in host life stage in each range. Colours in the inner circle represent the phyla that bacterial taxa are from based on OTUs assigned in SILVA (see Phylum legend). The first two outer concentric circles from the inside out displays the bacteria found in larvae from each range. The middle two concentric circles presentsys bacteria found in workers from each range. The outermost circles display bacterial diversity found in queens from each range. Where taxonomy was not assigned to the genus level, the lowest taxonomic level was assigned and denoted in brackets, i.e. (f) = family, (o) = order and (c) = class.

Relative Bacterial Class presence in each Range Life Stage

Figure 2-5 Relative (high > 100 OTUs) abundance bacteria found in *V. vulgaris*, sampled from three life stages (larvae, worker and queen) across two ranges (Belgium and New Zealand). Colours, annotated in the legend represent bacterial classes. Each class is represented by all taxa >1% contribution to that class. All taxa are identified to the genus level where possible, if not the lowest taxonomic level was assigned and denoted in brackets, i.e. (f) = family, (o) = order and (c) = class.

SIMPER analysis revealed that 50% of dissimilarity between larvae and workers was attributed to six class of bacteria, 12 of which were identified to the genus taxonomic level (**Table 2-7**). Larvae had higher mean abundances of *Enterococcus*, *Variibacter*, *Rickettsiella* and *Convivina*, whereas workers had higher mean abundances of *Lactobacillus*, *Wolbachia*, *Fructobacillus*, *Gilliamella*, *Pseudomonas*, *Serratia* and *Providencia*. Larvae and queens had (**Table 2-8**) 50% dissimilarity, made up of 16 taxa from seven taxonomic classes, 11 of which were identified to the genus level. Compared with queens, larvae had a higher mean abundance of *Wolbachia*, *Enterococcus*, *Fructobacillus*, *Rickettsiella*, *Convivina* and *Microbacterium*, whereas queens had higher abundances of *Lactobacillus*, *Variibacter*, *Saccharibacter*, *Orbaceae* (*buff-tailed bumble bee*) and *Gilliamella*. Workers, compared with queens had a higher mean abundance of *Lactobacillus*, *Wolbachia*, *Enterococcus*, *Fructobacillus*, *Serratia*, *Providencia*, *Pseudomonas*, *Convivina* and *Commensalibacter* and queens having a higher abundance of *Variibacter* (**Table 2-9**).

When comparing life stages between ranges, Belgian larvae had an abundance of *Enterococcus*, *Fructobacillus*, *Rickettsiella*, *Lactobacillus*, *Leuconoctoc*, *Convivina*, *Serratia*, *Diplorickettsia* and *Variibacter*, compared to New Zealand larvae, which had higher mean abundance of *Wolbachia*, *Spiroplasma* and *Variibacter* (**Table 2-10**). Worker comparisons between the ranges showed higher mean abundances of *Enterococcus*, *Fructobacillus*, *Providencia*, *Serratia*, *Convivina*, *Enterobacter* and *Pseudomonas* in the Belgian range. New Zealand workers had higher mean abundances of *Lactobacillus*, *Wolbachia*, *Gilliamella* and *Commensalibacter* compared with workers in the native range (**Table 2-11**). Queen comparisons between both ranges also revealed a higher abundance of *Lactobacillus*, *Wolbachia*, *Gilliamella* and *Orbaceae* (*buff-tailed bumble bee*) in the New Zealand range. Belgian queens had greater mean abundances of Bacilli such as *Enterococcus* and *Fructobacillus* in Belgian queens as seen in Belgian larvae and workers (**Table 2-12**). The overall dissimilarity between ranges revealed higher abundances of Bacilli (*Enterococcus*, *Fructobacillus*, *Convivina* and Gammaproteobacteria (Enterobacteriaceae (f), *Rickettsiella*, *Serratia* and *Providencia*), whereas the Invaded range had higher abundances of *Lactobacillus*, *Wolbachia* the Orbaceae family including *Gilliamella* (**Table 2-13**). These group differences were also assessed for biological significance using log-likelihood ratio G-tests to compare

OTU frequencies between life stages, ranges and life stage differences within ranges (**Table 2-14**).

Log-likelihood G-test analyses confirmed significant OTUs highlighted in the SIMPER analysis (Bonferroni corrected p values < 0.05). No significant OTUs were identified between life stage and range comparisons. However, there were significant ($p < 0.05$) taxa differences found in life stages between the ranges, represented by 17 taxa. The class Bacilli (*Convivina*, *Lactobacillus*, *Leuconostoc*, *Fructobacillus* *Vagococcus* and *Lactococcus*) and Gammaproteobacteria (Enterobacteriaceae (f), *Providencia*, *Pantoea*, *Rickettsiella* and *Serratia*) were significantly higher in the native Belgian life stages compared with New Zealand life stages. Belgian larvae and workers hosted considerably higher abundances of Bacilli compared with Belgian queens, and Belgian workers had higher abundances of Gammaproteobacteria compared with larvae and queens. In the invaded New Zealand range, all life stages had significantly higher abundances of the family Orbaceae and genera *Gilliamella* and *Snodgrassella* compared with their native counterparts. These taxa found in invaded wasps are typically associated with *A. mellifera* as core endosymbionts (Kwong & Moran, 2013).

Table 2-7 SIMPER results showing bacterial class and lowest taxonomic identity, which contributed to approximately 50% of the dissimilarity between larval and worker mean bacterial abundances. Where the genus has not been defined, the family (f) was used instead.

Class	Taxon	Mean Abundance Larvae	Mean Abundance Workers	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Bacilli	<i>Lactobacillus</i>	1.22	4.83	2.38	0.69	6.35	6.35
Alphaproteobacteria	<i>Wolbachia</i>	2.60	2.73	2.28	0.66	6.08	12.42
Bacilli	<i>Enterococcus</i>	4.32	2.89	1.91	1.22	5.1	17.53
Gammaproteobacteria	Enterobacteriaceae (f)	0.52	3.87	1.89	1.45	5.03	22.56
Bacilli	<i>Fructobacillus</i>	2.44	2.57	1.44	1.21	3.84	26.39
Spartobacteria	DA101 Soil Group (f)	9.86	8.33	1.17	1.34	3.12	29.51
Phycisphaerae	Tepidisphaeraceae (f)	6.18	4.83	0.93	1.68	2.47	31.99
Alphaproteobacteria	<i>Variibacter</i>	5.27	3.58	0.92	1.47	2.45	34.43
Gammaproteobacteria	<i>Rickettsiella</i>	1.89	0.77	0.87	1.12	2.31	36.75
Gammaproteobacteria	<i>Gilliamella</i>	0.67	1.18	0.79	0.72	2.11	38.86
Alphaproteobacteria	Acetobacteraceae (f)	2.56	1.87	0.79	1.38	2.10	40.96
Gammaproteobacteria	<i>Pseudomonas</i>	1.26	2.55	0.75	1.45	2.00	42.95
Gammaproteobacteria	<i>Serratia</i>	0.84	1.47	0.73	1.20	1.96	44.91
Gammaproteobacteria	<i>Providencia</i>	0.55	1.38	0.73	1.01	1.95	46.86
Mollicutes	<i>Spiroplasma</i>	0.79	0.82	0.64	0.76	1.72	48.58
Bacilli	<i>Convivina</i>	1.26	1.04	0.63	1.29	1.67	50.25

Table 2-8 SIMPER results showing bacterial class and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between larval and queen mean bacterial abundances. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family c=class).

Class	Taxon	Mean Abundance Larvae	Mean Abundance Queens	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Bacilli	<i>Lactobacillus</i>	1.22	3.80	2.15	0.64	6.74	6.74
Alphaproteobacteria	<i>Wolbachia</i>	2.60	2.36	2.11	0.65	6.61	13.35
Bacilli	<i>Enterococcus</i>	4.32	1.49	1.82	1.13	5.72	19.07
Bacilli	<i>Fructobacillus</i>	2.44	1.24	1.28	1.08	4.02	23.09
46 Spartobacteria	DA101 Soil Group (f)	9.86	10.86	1.14	1.53	3.59	26.68
Gammaproteobacteria	<i>Gilliamella</i>	0.67	1.71	1.00	0.84	3.14	29.82
Gammaproteobacteria	<i>Rickettsiella</i>	1.89	0.00	0.97	1.01	3.06	32.87
Phycisphaerae	Tepidisphaeraceae (f)	6.18	6.63	0.83	1.66	2.59	35.47
Bacilli	<i>Convivina</i>	1.26	0.89	0.71	1.27	2.22	37.69
Alphaproteobacteria	Acetobacteraceae (f)	2.56	2.33	0.71	1.41	2.22	39.92
Alphaproteobacteria	<i>Variibacter</i>	5.27	5.58	0.68	1.60	2.13	42.04
Thermoleophilia	Gaiellales (c)	3.36	4.19	0.68	1.70	2.12	44.16
Gammaproteobacteria	Orbaceae (f)	0.67	1.09	0.64	0.99	2.02	46.19
Actinobacteria	<i>Microbacterium</i>	1.05	0.00	0.54	1.74	1.69	47.87
Alphaproteobacteria	<i>Saccharibacter</i>	0.52	0.67	0.50	0.77	1.57	49.44
Gammaproteobacteria	<i>Orbaceae (buff-tailed BB)</i>	0.51	0.73	0.48	0.90	1.52	50.96

Table 2-9 SIMPER results showing bacterial class and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between worker and queen mean bacterial abundances. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family c=class).

Class	Taxon	Mean Abundance Workers	Mean Abundance Queens	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Bacilli	<i>Lactobacillus</i>	4.83	3.80	3.24	0.81	8.20	8.20
Alphaproteobacteria	<i>Wolbachia</i>	2.73	2.36	2.20	0.62	5.57	13.77
Gammaproteobacteria	Enterobacteriaceae (f)	3.87	0.35	1.91	1.42	4.83	18.61
Spartobacteria	DA101 Soil Group (f)	8.33	10.86	1.69	1.40	4.28	22.89
Bacilli	<i>Enterococcus</i>	2.89	1.49	1.45	1.06	3.68	26.57
Bacilli	<i>Fructobacillus</i>	2.57	1.24	1.27	1.16	3.21	29.78
Phycisphaerae	Tepidisphaeraceae (f)	4.83	6.63	1.20	1.52	3.04	32.81
Gammaproteobacteria	<i>Gilliamella</i>	1.18	1.71	1.17	0.83	2.97	35.78
Alphaproteobacteria	<i>Variibacter</i>	3.58	5.58	1.10	1.90	2.79	38.57
Gammaproteobacteria	<i>Serratia</i>	1.47	0.23	0.74	1.02	1.87	40.44
Thermoleophilia	Gaiellales (c)	3.22	4.19	0.72	1.82	1.83	42.27
Gammaproteobacteria	<i>Providencia</i>	1.38	0.00	0.70	0.81	1.78	44.05
Gammaproteobacteria	<i>Pseudomonas</i>	2.55	1.61	0.66	1.40	1.66	45.71
Bacilli	<i>Convivina</i>	1.04	0.89	0.65	1.11	1.64	47.35
Alphaproteobacteria	Acetobacteraceae (f)	1.87	2.33	0.63	1.51	1.59	48.94
Alphaproteobacteria	<i>Commensalibacter</i>	0.85	0.59	0.63	0.69	1.59	50.53

Table 2-10 SIMPER results showing bacterial class and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between native Belgian larvae and invaded New Zealand larvae mean bacterial abundances. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family).

Class	Taxon	Mean Abundance Belgian Larvae	Mean Abundance New Zealand Larvae	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Alphaproteobacteria	<i>Wolbachia</i>	0.46	4.74	2.52	0.74	7.11	7.11
Bacilli	<i>Enterococcus</i>	6.65	1.99	2.40	1.32	6.77	13.88
Bacilli	<i>Fructobacillus</i>	4.69	0.19	2.32	2.13	6.55	20.43
Gammaproteobacteria	<i>Rickettsiella</i>	3.42	0.36	1.59	2.01	4.47	24.90
Bacilli	<i>Lactobacillus</i>	2.44	0.00	1.26	1.90	3.55	28.45
Bacilli	<i>Leuconostoc</i>	1.81	0.00	0.93	8.55	2.64	31.09
Spartobacteria	DA101 Soil Group (f)	8.97	10.74	0.91	3.85	2.58	33.67
Alphaproteobacteria	Acetobacteraceae (f)	2.03	3.09	0.88	1.29	2.49	36.16
Gammaproteobacteria	<i>Serratia</i>	1.68	0.00	0.87	3.46	2.45	38.60
Bacilli	<i>Convivina</i>	2.04	0.49	0.87	1.85	2.44	41.05
Mollicutes	<i>Spiroplasma</i>	0.00	1.57	0.81	0.92	2.28	43.33
Phycisphaerae	Tepidisphaeraceae (f)	5.42	6.94	0.79	5.24	2.22	45.55
Alphaproteobacteria	<i>Variibacter</i>	4.60	5.94	0.74	1.35	2.09	47.64
Gammaproteobacteria	<i>Diplorickettsia</i>	1.40	0.00	0.72	1.61	2.03	49.67

Table 2-11 SIMPER results showing bacterial class and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between native Belgian workers and invaded New Zealand workers mean bacterial abundances. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family).

Class	Taxon	Mean Abundance Belgian Workers	Mean Abundance New Zealand Workers	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Bacilli	<i>Lactobacillus</i>	2.12	7.54	3.69	0.87	8.21	8.21
Alphaproteobacteria	<i>Wolbachia</i>	0.00	5.47	2.75	0.67	6.12	14.33
Gammaproteobacteria	Enterobacteriaceae (f)	6.28	1.46	2.48	2.06	5.51	19.83
Bacilli	<i>Enterococcus</i>	5.10	0.68	2.26	1.31	5.02	24.85
Bacilli	<i>Fructobacillus</i>	4.67	0.48	2.14	2.22	4.76	29.61
Gammaproteobacteria	<i>Providencia</i>	2.76	0.00	1.41	1.89	3.14	32.75
Spartobacteria	DA101 Soil Group (f)	7.94	8.73	1.40	1.84	3.10	35.85
Gammaproteobacteria	<i>Gilliamella</i>	0.00	2.37	1.24	0.80	2.77	38.62
Gammaproteobacteria	<i>Serratia</i>	2.41	0.54	1.08	1.51	2.41	41.03
Bacilli	<i>Convivina</i>	2.09	0.00	1.07	8.16	2.38	43.41
Phycisphaerae	Tepidisphaeraceae (f)	4.01	5.65	0.99	1.60	2.19	45.60
Alphaproteobacteria	<i>Commensalibacter</i>	0.00	1.70	0.89	0.84	1.98	47.58
Gammaproteobacteria	<i>Enterobacter</i>	1.98	0.29	0.88	1.47	1.95	49.53
Gammaproteobacteria	<i>Pseudomonas</i>	3.16	1.95	0.83	1.60	1.85	51.38

Table 2-12 SIMPER results showing bacterial class and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between native Belgian queens and invaded New Zealand queens mean bacterial abundances. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family).

Class	Taxon	Mean Abundance Belgian Queens	Mean Abundance New Zealand Queens	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Bacilli	<i>Lactobacillus</i>	0.35	7.25	3.65	0.80	10.83	10.83
Alphaproteobacteria	<i>Wolbachia</i>	0.00	4.72	2.41	0.67	7.14	17.97
Gammaproteobacteria	<i>Gilliamella</i>	0.00	3.42	1.75	1.15	5.19	23.16
Phycisphaerae	Tepidisphaeraceae (f)	8.09	5.17	1.49	2.60	4.43	27.58
Spartobacteria	DA101 Soil Group (f)	12.22	9.49	1.43	1.06	4.25	31.83
Bacilli	<i>Enterococcus</i>	2.54	0.44	1.22	1.31	3.61	35.44
Bacilli	<i>Fructobacillus</i>	2.25	0.22	1.11	1.17	3.29	38.74
Bacilli	<i>Convivina</i>	1.78	0.00	0.91	1.21	2.69	41.43
Gammaproteobacteria	Orbaceae (f)	0.30	1.87	0.91	1.49	2.68	44.11
Alphaproteobacteria	<i>Variibacter</i>	6.38	4.78	0.82	1.41	2.43	46.54
Gammaproteobacteria	<i>Orbaceae (buff-tailed BB)</i>	0.00	1.46	0.75	1.21	2.22	48.76
Solibacteres	<i>Candidatus Solibacter</i>	3.22	1.80	0.73	1.42	2.16	50.92

Table 2-13 SIMPER results showing bacterial class and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between native Belgian queens and invaded New Zealand queens mean bacterial abundances. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family).

Class	Taxon	Mean Abundance Belgium	Mean Abundance New Zealand	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Bacilli	<i>Lactobacillus</i>	1.64	4.93	2.72	0.75	7.00	7.00
Alphaproteobacteria	<i>Wolbachia</i>	0.15	4.98	2.56	0.72	6.58	13.58
Bacilli	<i>Enterococcus</i>	4.76	1.04	2.07	1.30	5.32	18.90
Bacilli	<i>Fructobacillus</i>	3.87	0.30	1.87	1.71	4.80	23.70
Gammaproteobacteria	Enterobacteriaceae (f)	2.60	0.56	1.34	1.02	3.45	27.14
Spartobacteria	DA101 Soil Group (f)	9.71	9.66	1.28	1.41	3.29	30.44
Gammaproteobacteria	<i>Gilliamella</i>	0.05	2.32	1.20	0.91	3.08	33.51
Bacilli	<i>Convivina</i>	1.97	0.16	0.95	1.93	2.45	35.96
Phycisphaerae	Tepidisphaeraceae (f)	5.84	5.92	0.94	1.60	2.42	38.38
Alphaproteobacteria	<i>Variibacter</i>	4.75	4.87	0.79	1.43	2.04	40.41
Gammaproteobacteria	<i>Rickettsiella</i>	1.56	0.22	0.77	0.95	1.98	42.40
Alphaproteobacteria	Acetobacteraceae (f)	1.79	2.72	0.76	1.51	1.96	44.35
Gammaproteobacteria	<i>Serratia</i>	1.51	0.18	0.75	1.20	1.93	46.29
Gammaproteobacteria	Orbaceae (f)	0.10	1.47	0.74	1.17	1.91	48.19
Gammaproteobacteria	<i>Providencia</i>	1.29	0.00	0.66	0.90	1.70	49.89
Alphaproteobacteria	<i>Saccharibacter</i>	0.00	1.18	0.61	0.98	1.56	51.45

Table 2-14 Group Significance (log likelihood) bacterial taxa with a significantly different mean number of OTU reads between range life stages. Where the genus has not been defined, the next closest taxonomic level was assigned (denoted f=family).

Class	Taxon	Belgian Larvae	Belgian Worker	Belgian Queen	New Zealand Larvae	New Zealand Worker	New Zealand Queen	^a P value
Bacilli	<i>Convivina</i>	10.43	11.07	7.05	1.94	0.00	0.00	<0.01
Bacilli	<i>Lactobacillus</i>	8.45	8.48	1.39	0.00	0.00	0.00	<0.01
Bacilli	<i>Leuconostoc</i>	9.25	4.56	0.00	0.00	0.00	0.00	<0.01
Gammaproteobacteria	Enterobacteriaceae (f)	0.00	10.49	0.00	0.00	0.00	0.00	<0.01
Gammaproteobacteria	Enterobacteriaceae (f)	3.04	11.95	1.90	0.00	3.02	0.00	<0.05
Gammaproteobacteria	<i>Providencia</i>	0.94	8.70	0.00	0.00	0.00	0.00	<0.05
Gammaproteobacteria	Orbaceae (f)	0.00	0.00	1.01	5.54	6.94	9.13	<0.05
Gammaproteobacteria	<i>Gilliamella</i>	0.82	0.00	0.00	4.91	6.75	8.67	<0.05
Gammaproteobacteria	<i>Pantoea</i>	2.27	8.98	0.00	0.00	3.59	0.00	<0.05
Gammaproteobacteria	<i>Rickettsiella</i>	8.46	4.30	0.00	0.00	1.31	0.00	<0.05
Gammaproteobacteria	<i>Rickettsiella</i>	8.94	2.41	0.00	1.43	0.00	0.00	<0.05
Gammaproteobacteria	<i>Serratia</i>	6.86	6.38	1.80	0.00	0.00	0.00	<0.05
Bacilli	<i>Fructobacillus</i>	7.37	7.60	1.61	0.00	0.00	0.00	<0.05
Bacilli	<i>Fructobacillus</i>	12.15	11.89	7.32	0.82	2.71	0.90	<0.05
Bacilli	<i>Vagococcus</i>	4.97	7.27	0.00	0.00	0.00	0.00	<0.05
Bacilli	<i>Lactococcus</i>	3.23	10.81	1.08	0.00	2.17	0.00	<0.05
Betaproteobacteria	<i>Snodgrassella</i>	0.00	0.00	0.00	1.60	7.15	7.12	<0.05

^a *p* values have been corrected for multiple comparisons using the Bonferroni procedure

2.5.4 Microbial Eukaryote Community Distinctions

This section, similar to the previous bacterial section, compares and characterises the microbial eukaryote community of *V. vulgaris* providing distinctions between microbial eukaryote communities.

From 18 samples, 18S analysis retrieved 2,937,114 sequences, of which 28,391 (< 1.0%) low-quality reads were rejected. The remaining sequences were a mix of mainly OTUs of host origin, bacterial and microbial eukaryotes. Using the fingerprint function in SILVAngs (Quast et al., 2013), host (Hymenoptera sequences) and bacterial sequences were excluded (92.1% (2,704,175)) revealing the microbial eukaryote community. Further filtering was performed to eliminate plant and mammalian taxonomic assignments resulting in the identification of 80 microbial eukaryotic taxa. A PERMANOVA pairwise test revealed no significant difference in the microbial eukaryote community among three life stages, life stages within each range, and range ($p < 0.05$).

Known microbial eukaryotes were greatly reduced in queens with two samples (one from each range) observed to have no known microbial eukaryote presence. These samples were excluded from subsequent clustering analysis to preventing clustering of communities with taxa and those without, allowing us to see clustering of communities with taxa. Group average clustering revealed a low clustering of life stages apart from three larval samples from New Zealand and one from Belgium, which was indicative of the high abundance of microbial eukaryotes found in larvae compared with workers and queens. (Figure 2-6A). The two-dimensional nMDS plot (stress value of 0.12) supported what was seen in the group average cluster. There was a greater overlap of larvae and workers, indicating a separation from the queen community which had fewer microbial eukaryotes. Group average cluster and the nMDS plot confirmed no separation between the native and invaded range for the microbial eukaryote community (Figure 2-6B). The CAP analysis illustrates a distinction between the life stage communities (Figure 2-6C) which is potentially attributed to high variation in microbial eukaryote richness between life stages.

ANOVA analysis revealed the richness of taxa present was significantly different among life stages ($F(2, 15) = 4.61$ $p = 0.027$), with no significant difference in microbial eukaryotes richness between each range ($F(1, 16) = 1.85$ $p = 0.192$) and life stages within each range ($F(5, 12) = 2.71$ $p = 0.072$). Shannon's diversity metrics between ranges also showed no

significant dissimilarity ($F(1, 16) = 1.81$, $p = 0.197$), whereas diversity dissimilarity among life stages was significantly dissimilar ($F(2, 12) = 3.65$, $p = 0.007$) as was each range life stage ($F(5, 12) = 4.61$, $p = 0.031$). Post-hoc two tailed pairwise testing revealed that the richness of larval and queen communities were significantly dissimilar ($p = 0.019$) as was diversity dissimilarity in larvae and queens ($p = 0.007$). Post-hoc two tailed pairwise testing revealed no significant dissimilarity in diversity between life stages within each range.

Richness and diversity graphs (**Figure 2-6D**) illustrate that on average larvae had the highest microbial eukaryote richness (Belgian larvae $10.6 \text{ SE} \pm 4.33$, New Zealand larvae $20.7 \text{ SE} \pm 7.35$) and diversity (Belgian larvae $1.9 \text{ SE} \pm 0.44$, New Zealand larvae $2.35 \text{ SE} \pm 0.46$). Larvae from the New Zealand range showed greater variability with double the incidence of microbial eukaryote OTUs compared with that of the native Belgian range. Worker richness and (Belgian workers $7.0 \text{ SE} \pm 3.00$, New Zealand workers $8.3 \text{ SE} \pm 0.67$) and diversity (Belgian worker $1.6 \text{ SE} \pm 0.32$, New Zealand worker $1.9 \text{ SE} \pm 0.06$) was more variable in the native range. In comparison to the other life stages, queens had the lowest microbial eukaryote richness (Belgian queen $1.0 \text{ SE} \pm 0.58$, New Zealand queen $5.7 \text{ SE} \pm 3.71$) and diversity (Belgian queen $0.2 \text{ SE} \pm 0.2$, New Zealand queen $1.1 \text{ SE} \pm 0.63$), with low richness and diversity in the native Belgian range. Overall between ranges, richness and diversity of microbial eukaryotes were highest in New Zealand, with life stages showing similar trends in both ranges. However, it must be noted that there was considerable variation seen in both richness and diversity throughout microbial eukaryote communities.

Figure 2-6 Figures of microbial eukaryote communities (A-D), illustrating individual microbial eukaryote microbiomes in *V. vulgaris*. Larvae (green triangle), worker (blue triangle) and queens (cyan square). Range samples sites are annotated with BE=Belgium NZ=New Zealand. Graphs A-C based on a Bray-Curtis dissimilarity analysis. (A) Group average cluster dendrogram from average-linkage clustering of bacterial OTU frequency. (B) Non-metric multi-dimensional (nMDS) scaling plot of microbial eukaryote OTU frequency. Stress value 0.12 (C) Canonical analyses of principal coordinates (CAP) of microbial eukaryote OTU frequencies, illustrating distinctness of microbial eukaryote communities. (D) The richness and Shannon's diversity metrics. The standard error shows the variance experienced in each range life stage (n=3).

Three major microbial eukaryote groups were identified in the microbiome of *V. vulgaris*: fungal, protozoan and nematode. Overall, the life stage fungal OTUs dominated microbial eukaryote communities (larvae mean $1.75 \text{ SE} \pm 0.38$; worker 1.24 ± 0.08 and queen 1.03 ± 0.39). Protozoans (larvae 0.69 ± 0.40 ; worker 0.58 ± 0.16 and queen 0.10 ± 0.10) were observed in all life stages from the invaded New Zealand range compared with fungal (larvae 1.38 ± 0.39 ; worker 1.13 ± 0.21 and queen 0.2 ± 0.20) and protozoan (larvae 0.36 ± 0.23) abundances in the native Belgian range. Nematodes were identified in one New Zealand larval sample. Life stages within ranges were also assessed separately (**Figure 2-7**) using the mean (log) of absolute abundances of fungi, protozoan and nematodes and an ANOVA was run to assess significance between life stages in each range. This analysis demonstrated no significant difference ($p < 0.05$) in the mean abundances of fungi, protozoan and nematodes between life stages, life stages in each range and ranges.

Figure 2-7 Absolute mean (log) abundances of microbial eukaryotes found in each life stage, denoted by a dot. Variance between samples is indicated by standard error bars. Fungi are denoted red, Protozoan blue and nematode green.

2.5.5 Characterisation of Microbial Eukaryote Communities

The microbial eukaryote community was characterised, with the majority of sequences aligned to the fungal sub-kingdom Dikarya with phyla Ascomycota (55.8%) and Basidiomycota (29.2%) making up 85% of the microbial eukaryote community. The community also contained Miozoa (6.7%), Zygomycota (4.1%), Apicomplexa (2.4%), Chlorophyta (1.3%), Euglenozoa (0.9%), Cercozoa (0.7%), Nematode (0.7%) Ciliophora (0.4%), Chytridiomycota (0.4%), and Nematomorpha (0.4%). From these phyla and subphyla, 78 microbial taxa were identified (**Supplementary Table 1**). The fungal orders Pezizomycotina, Agaricomycotina, Saccharomycotina and Ustilaginomycotina made up 80% of the community, with protozoans (8.0%), nematode (0.7%) and other fungal orders (6.3%) making up the remainder. A total of 532 phylum/subphylum OTU reads were calculated, 372 of which were found in larvae, 90 in workers and 70 in queens (**Table 2-15**). On average across all samples the top 11 genera that made up 50% of the microbial eukaryote community were *Debaryomyces* (10.9%), *Cryptococcus* (5.01%), *Conidiobolus* (4.87%), *Gregarina* (4.57%), *Bionectria* (4.42%), *Hygrocybe* (4.42%), *Penicillium* (4.13%), *Sympodiomyces* (3.83%), *Lachancea* (2.95%), *Metschnikowia* (2.80%) and *Priceomyces* (2.51%). Presence and absence of microbial eukaryotes were again visualised using an iTOL phylogenetic tree (**Figure 2-8**). This analysis emphasises the greater richness of microbial eukaryotes in larva, particularly in the invaded New Zealand range. It also highlights the lack of microbial eukaryotes seen in queens compared with the other life stages. However this absence of microbial eukaryotes is more pronounced in Belgian queens.

Table 2-15 Accumulated absolute OTU abundances of microbial eukaryote for each Phylum/Subphylum in *V. vulgaris* life stages and total taxa reads found.

Kingdom	Phylum/Subphylum	Larvae	Worker	Queen	Total taxa
Fungi	Pezizomycotina	106	39	49	194
Fungi	Agaricomycotina	83	10	11	104
Fungi	Saccharomycotina	67	17	8	92
Eukaryota	Apicomplexa	38	14	2	54
Fungi	Ustilaginomycotina	35	6	0	41
Fungi	Pucciniomycotina	18	1	0	19
Fungi	Taphrinomycotina	12	3	0	15
Fungi	Microsporidia	6	0	0	6
Eukaryota	Nematomorpha	5	0	0	5
Fungi	Chytridiomycota	2	0	0	2

Figure 2-8 Phylogeny of microbial eukaryotes identified from *V. vulgaris* to genus where possible. This rooted maximum likelihood phylogeny reveals specificity of microbial eukaryotes in each range life stage. The inner circle colours illustrate the microbial eukaryote phyla, based on the OTUs assigned in SILVA (see Phylum legend). The first two outer concentric circles from the inside out displays the microbial eukaryotes identified in larvae from each range. The middle two concentric circles presents the microbial eukaryotes identified in workers from each range. The outermost circles display microbial eukaryote identified in queens from each range. Where taxonomy was not assigned to the genus level, the lowest taxonomic level was assigned and denoted in brackets, i.e. (f) = family, (o) = order and (c) = class.

To determine which OTUs contributed most to community abundance composition a similarity percentage (SIMPER) analysis was performed and log likelihood G-tests were used to support the significance of taxa in each community.

SIMPER analysis revealed that 50% of dissimilarity between larvae and workers (**Table 2-16**) attributed nine classes of microbial eukaryote, with larvae having higher mean abundances of the fungal taxa *Cryptococcus*, *Sympodiomyces*, *Dothideales*, *Glarea*, *Debaryomyces*, *Metschnikowia*, *Candida* and *Saccharomycetales* compared with workers. Workers had higher mean abundances of the taxa *Conidiobolus*, *Hygrocybe*, *Cladosporium*, *Priceomyces* and *Penicilliosis* in addition to the protozoan CCA5. Larvae also had higher mean abundances of *Cryptococcus* *Dothideales* *Sympodiomyces* *Glarea* *Metschnikowia* *Candida* *Debaryomyces* and the protozoan CCA5, compared with queen OTUs which had higher mean abundances of *Penicilliosis* *Priceomyces* *Thelebolales* and *Talaromyces* (**Table 2-17**). In the SIMPER analysis between workers and queens (**Table 2-18**), *Penicilliosis* was also in high abundance in queens, and workers had higher mean abundances of fungal taxa *Conidiobolus*, *Priceomyces*, *Hygrocybe* *Cladosporium* and the protozoan CCA5. The microbial eukaryote comparison between life stages between ranges showed presence of the fungal taxa *Cryptococcus*, *Sympodiomyces*, *Dothideales*, *Priceomyces*, in Belgian larvae, not seen in the invaded New Zealand range. In New Zealand, there was the presence of the fungal taxa *Glarea*, *Saccharomycetales*, *Kazachstania*, *Hygrocybe* and *Pucciniales* as well as the Protozoan CCA5 (**Table 2-19**).

Similarly, in New Zealand, workers hosted *Priceomyces* *Penicilliosis* *Talaromyces* and *Debaryomyces* not seen in the native Belgian range. In Belgian workers, there were higher abundances of the Entomopathogen *Conidiobolus* as well as the fungal taxa *Cladosporium* and *Hygrocybe* (**Table 2-20**). In the SIMPER comparison only four fungal taxa made up the dissimilarity in queens between the two ranges, *Priceomyces* was present in the native range and not observed in the invaded ranges, whereas *Thelebolales* and *Talaromyces* were present in the invaded range and absent from the native range (**Table 2-21**). Overall, between-range dissimilarity (**Table 2-22**) was attributed to higher mean abundances of *Conidiobolus*, *Sympodiomyces* *Cladosporium* and *Cryptococcus* in the native Belgian range and *Thelebolales* (o), *Talaromyces*, *Debaryomyces* and the protozoan CCA5 in the invaded New Zealand range.

Log-likelihood ratio G tests revealed that there was no significant ($p < 0.05$) dissimilarity between life stage microbial eukaryote taxa, however, four fungal taxa and one protozoan was significantly dissimilar among life stages between ranges (**Table 2-23**). This significant dissimilarity in abundance was attributed to larvae between the ranges, with higher mean abundances of the fungal taxa *Debaryomyces*, *Bionectria* and *Hygrocybe* as well as the protozoan CCA5, seen in larvae from the invaded range compared with larvae from the native range. In the native range, *Cryptococcus* was the only significantly dissimilar taxa observed larvae, with higher mean abundances when compared with the invaded range.

Table 2-16 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between larvae and workers. Where the genus is not defined, the next closest taxonomic level was assigned (denoted o=order).

Class	Taxon	Mean Abundance Larvae	Mean Abundance Workers	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Entomophthorales	<i>Conidiobolus</i>	0.61	2.88	4.88	1.15	5.45	5.45
Agaricomycetes	<i>Hygrocybe</i>	0.00	2.44	4.55	1.22	5.07	10.52
Tremellomycetes	<i>Cryptococcus</i>	2.28	0.00	4.34	0.78	4.85	15.37
Conoidasida	CCA5	1.28	2.05	3.89	1.04	4.34	19.71
Dothideomycetes	<i>Cladosporium</i>	0.35	1.89	3.52	0.70	3.92	23.64
Exobasidiomycetes	<i>Sympodiomyces</i>	1.71	0.68	3.47	0.87	3.87	27.51
Dothideomycetes	Dothideales (o)	1.55	0.39	3.45	0.64	3.85	31.36
Saccharomycetes	<i>Priceomyces</i>	0.72	1.68	3.34	0.78	3.73	35.08
Leotiomycetes	<i>Glarea</i>	1.46	0.00	2.85	0.52	3.18	38.27
Saccharomycetes	<i>Debaryomyces</i>	1.17	0.96	2.85	0.73	3.18	41.45
Saccharomycetes	<i>Metschnikowia</i>	1.43	0.74	2.46	1.07	2.75	44.20
Eurotiomycetes	<i>Penicillioptis</i>	0.42	1.21	2.31	0.78	2.57	46.77
Saccharomycetes	<i>Candida</i>	1.28	0.00	2.30	1.01	2.56	49.33
Saccharomycetes	Saccharomycetales (o)	0.87	0.64	2.17	0.79	2.42	51.75

Table 2-17 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between larvae and queens. Where the genus is not defined, the next closest taxonomic level was assigned (denoted o=order).

Class	Taxon	Mean Abundance Larvae	Mean Abundance Queens	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Tremellomycetes	<i>Cryptococcus</i>	2.28	0.00	6.04	0.69	6.24	6.24
Saccharomycetes	<i>Priceomyces</i>	0.72	1.67	5.18	0.55	5.35	11.59
Eurotiomycetes	<i>Penicillioptis</i>	0.42	2.29	4.99	0.69	5.15	16.74
Dothideomycetes	Dothideales (o)	1.55	0.00	4.74	0.53	4.90	21.64
Exobasidiomycetes	<i>Sympodidomycopsis</i>	1.71	0.00	4.65	0.73	4.80	26.44
Leotiomycetes	Thelebolales (o)	0.00	1.67	4.11	0.42	4.25	30.68
Leotiomycetes	<i>Glarea</i>	1.46	0.00	3.92	0.49	4.05	34.73
Saccharomycetes	<i>Metschnikowia</i>	1.43	0.56	3.44	0.98	3.56	38.29
Saccharomycetes	<i>Candida</i>	1.28	0.00	3.05	0.92	3.14	41.43
Eurotiomycetes	<i>Talaromyces</i>	0.23	1.39	2.90	0.57	2.99	44.42
Conoidasida	CCA5	1.28	0.00	2.73	0.67	2.81	47.23
Saccharomycetes	<i>Debaryomyces</i>	1.17	0.47	2.62	0.73	2.71	49.94
Eurotiomycetes	Eurotiales (o)	0.23	0.83	2.15	0.50	2.22	52.16

Table 2-18 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between queens and workers.

Class	Taxon	Mean Abundance Queens	Mean Abundance Workers	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Entomophthorales	<i>Conidiobolus</i>	0.00	2.88	8.16	1.10	8.69	8.69
Saccharomycetes	<i>Priceomyces</i>	1.67	1.68	7.65	0.75	8.14	16.82
Agaricomycetes	<i>Hygrocybe</i>	0.00	2.44	7.25	1.15	7.72	24.54
Eurotiomycetes	<i>Penicilliosis</i>	2.29	1.21	6.63	0.85	7.05	31.59
Dothideomycetes	<i>Cladosporium</i>	0.00	1.89	5.58	0.63	5.93	37.53
Conoidasida	<i>CCA5</i>	0.00	2.05	5.32	0.83	5.66	43.18
Eurotiomycetes	<i>Talaromyces</i>	1.39	1.13	4.8	0.84	5.11	48.29

Table 2-19 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between Belgian larvae and New Zealand larvae. Where the genus is not defined, the next closest taxonomic level was assigned (denoted o=order).

Class	Taxon	Mean Abundance Belgian Larvae	Mean Abundance New Zealand Larvae	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Tremellomycetes	<i>Cryptococcus</i>	3.64	0.92	5.64	1.09	6.17	6.17
Exobasidiomycetes	<i>Sympodiomyces</i>	3.41	0.00	5.49	1.90	6.02	12.19
Leotiomyces	<i>Glarea</i>	0.00	2.92	5.44	0.80	5.95	18.14
Dothideomycetes	Dothideales (o)	3.10	0.00	5.35	0.96	5.86	24.00
Conoidasida	CCA5	0.00	2.56	4.01	1.28	4.39	28.39
Saccharomycetes	Saccharomycetales (o)	0.00	1.75	3.03	1.16	3.31	31.70
Saccharomycetes	<i>Debaryomyces</i>	0.59	1.74	2.80	0.87	3.07	34.77
Saccharomycetes	<i>Kazachstania</i>	0.00	1.36	2.71	0.65	2.97	37.74
Saccharomycetes	<i>Metschnikowia</i>	1.02	1.84	2.68	1.22	2.93	40.67
Agaricomycetes	<i>Hygrocybe</i>	0.00	1.64	2.27	1.02	2.48	43.15
Saccharomycetes	<i>Candida</i>	1.15	1.42	2.19	0.98	2.39	45.55
Saccharomycetes	<i>Priceomyces</i>	1.44	0.00	2.12	0.65	2.32	47.87
Pucciniomycetes	Pucciniales (o)	0.00	1.35	1.83	1.27	2.00	49.87

Table 2-20 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between Belgian workers and New Zealand workers.

Class	Taxon	Mean Abundance Belgian Workers	Mean Abundance New Zealand Workers	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Conoidasida	<i>CCA5</i>	0.00	4.10	8.15	2.35	9.39	9.39
Entomophthorales	<i>Conidiobolus</i>	4.95	0.81	8.06	2.65	9.28	18.67
Saccharomycetes	<i>Priceomyces</i>	0.00	3.36	6.73	1.28	7.75	26.42
Dothideomycetes	<i>Cladosporium</i>	3.14	0.64	6.06	0.96	6.98	33.40
Eurotiomycetes	<i>Penicillium</i>	0.00	2.42	4.73	1.24	5.44	38.84
Eurotiomycetes	<i>Talaromyces</i>	0.00	2.27	4.43	1.27	5.10	43.94
Agaricomycetes	<i>Hygrocybe</i>	2.75	2.13	4.02	1.19	4.63	48.57
Saccharomycetes	<i>Debaryomyces</i>	0.00	1.92	3.79	0.66	4.36	52.94

Table 2-21 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between Belgian queens and New Zealand queens. Where the genus is not defined, the next closest taxonomic level was assigned (denoted o=order).

Class	Taxon	Mean Abundance Belgian Queens	Mean Abundance New Zealand Queens	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Leotiomyces	Thelebolales (o)	0.00	3.33	21.36	0.60	21.90	21.90
Saccharomyces	<i>Priceomyces</i>	3.33	0.00	12.24	0.63	12.54	34.44
Eurotiomyces	<i>Penicilliosis</i>	2.89	1.69	11.09	0.82	11.36	45.80
Eurotiomyces	<i>Talaromyces</i>	0.00	2.78	10.75	0.79	11.02	56.82

Table 2-22 SIMPER results showing mean microbial eukaryote class abundances and lowest taxonomy where possible which contributed to approximately 50% of the dissimilarity between Belgian and New Zealand samples. Where the genus is not defined, the next closest taxonomic level was assigned (denoted o=order).

Class	Taxon	Mean Abundance Belgium	Mean Abundance New Zealand	Mean Dissimilarity	Dissimilarity Standard Deviation	% Cumulative	% Cumulative contribution
Saccharomycetes	<i>Priceomyces</i>	1.59	1.12	5.52	0.59	5.89	5.89
Eurotiomycetes	<i>Penicilliopsis</i>	1.24	1.37	4.85	0.68	5.18	11.07
Conoidasida	<i>CCA5</i>	0.00	2.22	4.70	0.92	5.02	16.09
Leotiomycetes	Thelebolales (o)	0.00	1.11	4.50	0.30	4.80	20.89
Eurotiomycetes	<i>Talaromyces</i>	0.00	1.84	4.47	0.67	4.77	25.66
Entomophthorales	<i>Conidiobolus</i>	1.75	0.57	3.87	0.83	4.13	29.79
Saccharomycetes	<i>Debaryomyces</i>	0.20	1.54	3.15	0.67	3.36	33.15
Agaricomycetes	<i>Hygrocybe</i>	0.92	0.71	2.96	0.69	3.16	36.31
Exobasidiomycetes	<i>Sympodiomyces</i>	1.59	0.00	2.95	0.75	3.15	39.46
Dothideomycetes	<i>Cladosporium</i>	1.28	0.21	2.78	0.55	2.97	42.43
Saccharomycetes	<i>Metschnikowia</i>	0.60	1.22	2.76	0.91	2.94	45.37
Tremellomycetes	<i>Cryptococcus</i>	1.21	0.31	2.72	0.54	2.90	48.28
Dothideomycetes	Dothideales (o)	1.30	0.00	2.60	0.53	2.78	51.05

Table 2-23 Group Significance (log likelihood) microbial eukaryote taxa with significantly different mean number of reads between range life stages. Mean reads are based on normalised values

Class	Taxon	Belgian Larvae	Belgian Worker	Belgian Queen	New Zealand Larvae	New Zealand Worker	New Zealand Queen	^a P value
Sordariomycetes	<i>Bionectria</i>	0.00	0.00	0.00	10.00	0.00	0.00	<0.01
Agaricomycetes	<i>Hygrocybe</i>	0.00	0.00	0.00	9.67	0.00	0.33	<0.01
Conoidasida	<i>CCA5</i>	0.00	0.00	0.00	8.33	4.00	0.00	<0.01
Tremellomycetes	<i>Cryptococcus</i>	8.67	0.00	0.00	2.67	0.00	0.00	<0.01

^a *p* values corrected for multiple comparisons using the Bonferroni procedure

2.5.6 Characterisation of Viral Communities

From the 18 RNA samples, a total of 4,446,727 total reads were assembled, of which 445,932 were unique contigs. The mean sequence read quality was 35.7 with an average of 94% sequences at Q30 quality for all libraries contigs were subjected to BLASTx analyses against the NCBI GenBank nonredundant (nr) protein database. Following assignment, 91.8% of RNA reads were eukaryote (host genomic RNA), 6.2% was not assigned, 1.9% bacteria and 0.1% viruses. A total of 271 viral contigs were identified of which 17 were assigned in MEGAN as viruses. Virusfinder2 identified 13 viruses. As a result of quantitative variability between each method, it was decided to focus the investigation on presence and absence of contigs that occurred using both methods, as well as excluding plant viruses and contigs that did not identify to the 97% alignment threshold. This filtering, culminated in four viral taxa investigated further, *Kashmir bee virus (KBV)*, *Deformed wing virus (DWV)*, *Rhopalosiphum padi virus (RhPV)*, and *Aphid lethal paralysis virus (ALPV)* (**Table 2-24**). RT-PCR and subsequent Sanger sequencing confirmed the presence of *KBV*, *DWV* and *ALPV* but not *RhPV* which was identified in the transcriptome analysis.

Eight putative viral sequences were also aligned to proteins and confirmed as viral candidates. From the aligned sequences, one matched to *DWV* (Capsid protein) and two to *ALPV* (Capsid Protein and RNA polymerase) viruses. Five putative contigs were identified as potential novel viruses based on their similarity to known virus coding proteins (**Table 2-25**). Putative viral proteins identified matched to *Ribgrass mosaic virus* (RNA replicase), *Invertebrate iridescent virus 6* (274L protein), and two of the contigs matched to *Himetobi P virus*, (capsid protein and RNA polymerase). A single viral contig aligned to *Polisties canadensis* (RNA binding protein), possibly indicating it as non-viral.

Table 2-24 Presence/Absence of Viruses in *V. vulgaris* using two bioinformatic methods MEGAN and Virusfinder2. Viruses identified using both methods confirm the presence of putative viruses.

Virus	Belgium		New Zealand	
	MEGAN	Virusfinder2	MEGAN	Virusfinder 2
<i>Rhopalosiphum padi virus</i> (RhPV)	L, W	L, W	-	-
<i>Kashmir bee virus</i> (KBV)	-	-	L, W	L, W
<i>Deformed Wing Virus</i> (DWV)	L, W	L, W	L	L
<i>Aphid Lethal paralysis virus</i> (ALPV)	L, W	W	-	-

Table 2-25 Putative virus candidates identified by Blastn and Blastx and visualised in Blast2GO

	Belgian Larvae	Belgian Workers	Belgian Queens	New Zealand Larvae	New Zealand Workers	New Zealand Queens	Blastn matched to:	Blastx matched to:
71	0	0	0	3	0	0	DWV (100/100)	Capsid protein VP1, DWV, BAM15917.1 (100/100)
	25	0	0	0	0	0	<i>Armadillidium vulgare iridescent virus</i> (33/79)	IIV 6, 274L, NP_149737.1 (39/84)
	72	59	0	0	0	0	<i>Cripavirus</i> NB-1/2011/HUN, (85/82)	Capsid protein, partial Himetobi P virus, AIY53984.1 (97/47)
	1	7	0	0	0	0	ALPV isolate ALPV-An, genome (98/88)	Capsid protein precursor ALPV, AMH41169.1 (73/92)
	94	0	0	0	0	0	<i>Ribgrass mosaic virus</i> strain RMV Actinidia-AC isolate M2/A4, genome (99/94)	RNA replicase RMV, ACV13194.1 (99/98)
	537	222	86	110	166	80	PREDICTED: <i>P. canadensis</i> RNA-binding protein MEX3B (LOC106785898), mRNA(81/87)	RNA-binding protein, MEX3B, XP_014602207.1
	1	5	0	0	0	0	ALPV isolate ALPV-An, genome (100/89)	RNA-dependent RNA polymerase ALPV AIJ00043.1 (99/99)
	1	17	0	0	0	0	<i>Cripavirus</i> NB-1/2011/HUN, complete genome (93/77)	RNA-dependent RNA polymerase Himetobi P virus AIY53983.1 (92/59)

2.6 DISCUSSION

This study is the first to comprehensively examine and characterise the microbial community of *V. vulgaris* using next-generation sequencing. It is a starting point to elucidate the presence and possible function of microbial taxa associated with this invasive wasp. It must also be noted that microbial function can change from host to host and new microbial species and strains are continually discovered. As this study reports on the homology to sequences in microbial databases such as SILVA, RINS and BLAST, it can only infer identity and function in *V. vulgaris* based on what research in other insects and microbial environments.

Various types of biological interactions occur between microbiota and their hosts, which can be beneficial, harmful or neutral. Beneficial microbiota protect the host and provide essential nutrients and energy to the host (Mackie, Aminov, White, & McSweeney, 2000; Simpson, 2002). The difference in the microbial community within an individual is, therefore, due in part to host diet and habitat (Colman, Toolson, & Takacs-Vesbach, 2012) and is likely a controlling factor for microbial diversity in *V. vulgaris* due to the predatory and opportunistic omnivorous nature of *V. vulgaris* diet. The inconstancy of the *V. vulgaris* microbiome found across two ranges in this study further supports diet and the environment as potentially shaping the microbiome structure resulting in more diverse microbiome. A total of 736 OTUs were identified in *V. vulgaris*, highlighting a highly diverse prokaryote microbiome compared with honey bees (397 OTUs) Corbiculate bees (199 OTUs) and bumble bees (373 OTUs), although comparable with termites (726 OTUs) (Köhler, 2011; Kwong, Medina, et al., 2017; N. A. Moran, Hansen, Powell, & Sabree, 2012; Newbold et al., 2015). In the mammalian gut, the diversity of bacteria increases according to a host's diet, from carnivores to omnivores to herbivores (Ley et al., 2008). Aphids host *Acinetobacter*, *Staphylococcus* and *Buchnera* in their gut when they fed on honeydew melon (A. E. Douglas, 1998; Leroy et al., 2011) and western flower thrips obtain *Erwinia* when they feed on cucumber leaves (de Vries, Jacobs, Sabelis, Menken, & Breeuwer, 2004). Whereas the genera *Acetobacter*, *Gluconacetobacter*, *Gluconobacter*, *Saccharibacter* and *Commensalibacter* are found in nectivorous insects (E. Crotti et al., 2010). The relationship between host and microbiota may be transmitted in two ways. Vertical transmission to the offspring from the parent and incapable of growing in the environment outside of the host (Hosokawa, Kikuchi, Nikoh, Shimada, & Fukatsu, 2006; Sachs,

Essenberg, & Turcotte, 2011) and horizontally as short term residents in the host, showing little or no host species specificity and may be able to grow in the environment outside of the host (Gordon, 2001; Y. Kikuchi, Hosokawa, & Fukatsu, 2007; Sachs et al., 2011). Therefore a consistent vertical transmission would see a subset or core microbiota promoted by high rates of transmission between conspecific hosts (including parent-to-offspring and among kin), and by extended residence time within individual hosts (A. C.-N. Wong, Chaston, & Douglas, 2013). In this study, the core microbiota were identified and a comparative analysis of microbiota between life stages and ranges revealed possible specificity of particular taxa in life stages, in addition to the flexibility of *V. vulgaris* microbiome in the native and introduced range potentially aiding in their invasive success.

2.6.1 Core Microbiome

The successful spread of *V. vulgaris* in new environments suggests that a change in microbiome composition as seen in this study from native to invaded ranges does not negatively affect the wasp. However, the perpetuation of a core set of bacteria in *V. vulgaris* may have been fundamental for survival and subsequent spread in a new range. In this study the core microbiota of *V. vulgaris* is defined as the taxa present in 100% of all samples across life stages and two ranges. A total of 23 taxa were identified as core representing 30.5% of the relative community. Twelve bacterial genera were identified from four phyla, Acidobacteria (*Candidatus Solibacter* and RB41) Actinobacteria (*Corynebacterium 1* and *Acidothermus*), Verrucomicrobia (*Candidatus Xiphinematobacter*), Proteobacteria (*Pseudomonas*, *Bradyrhizobium*, *Roseiarcus*, *Variibacter*, *Nitrobacter*, *Pedomicrobium* and *Rhizobium*). There were no microbial eukaryotes or viruses in the core microbial community. The class Spartobacteria (DA101 soil group and *Candidatus. Xiphinematobacter*) and Alphaproteobacteria, attributed mainly to the order Rhizobiales, dominated the core. These bacteria are known soil bacteria and symbionts of plants, aiding in nitrogen fixation at the root level (Daniel, 2005; Sangwan, Kovac, Davis, Sait, & Janssen, 2005; Spain, Krumholz, & Elshahed, 2009; Urich et al., 2008; Young, 1996), possessing complex enzymatic nitrogenase machinery (LeBauer & Treseder, 2008). *Nitrobacter* was the most abundant Alphaproteobacteria genus in *V. vulgaris*, typically involved in oxidising ammonia into nitrate (Sepers, 1981). These nitrogen-fixing bacteria have also been associated as insect gut endosymbionts in other insects (Vandekerckhove, Willems, Gillis, & Coomans, 2000),

indicating a potential beneficial relationship with *V. vulgaris*, playing a role in the nitrogen uptake in the host as seen in other insects (Russell et al., 2009). The abundance of nitrogen-fixing endosymbiotic prokaryotes residing in *V. vulgaris* and other insects may be mutualistic and essential in many terrestrial ecosystems similar to the well-known legume–rhizobia mutualism. This acquisition of nitrogen from food and supplementation via the assimilation of atmospheric nitrogen may be a primary function of endosymbionts in insects (Ulyshen, 2015).

The phylum Actinobacteria was also represented in the core, and are known to be beneficial in insects, producing antibiotics for microbial defence in ants, beetles and wasps against harmful microorganisms (Kaltenpoth, 2009). The Gammaproteobacteria *Pseudomonas* are typically linked as pathogenic, although this genus has also been reported to produce antimicrobial compounds which are active against various plant pathogens (D. Haas & Défago, 2005; Ramette et al., 2011). Another study demonstrated that the presence of *Pseudomonas* isolates in the nematode *Caenorhabditis elegans* produce an anti-fungal effect in vitro contributing to the worm's defence against fungal pathogens (Dirksen et al., 2016).

Differences in the core microbiome between life stages were attributed mostly to the larval life stage where there was an increase in the number of bacterial taxa, with the addition of three unique phyla, Firmicutes, Planctomycetes and Bacteroidetes and seven genera associated with the larval core. The most abundant phylum in the larval core, Firmicutes was represented by the genus *Enterococcus*, accounting for 16.3% of the larval core. *Enterococcus* has been identified as a common genus in insect species that feed on nectar and succulent plant parts, and are considered commensals of the gastrointestinal tract of insects (Martin & Mundt, 1972). The phylum Planctomycetes has also been associated with soil-feeding termites (*Cubitermes spp.*), colonising their alkaline hindgut in high densities and potentially involved in the digestion of both decaying wood and humus (Andert, Marten, Brandl, & Brune, 2010; Ji & Brune, 2001; Köhler, Stingl, Meuser, & Brune, 2008). A similar symbiotic relationship may also be the case with *V. vulgaris* using these bacteria to aid in the breakdown of decaying wood used as a nest-building tool by masticating and stretching the wood pulp to turn it into a paper construction material (Matsuura & Yamane, 1990; J. Philip Spradbery, 1973).

Worker core analysis revealed the addition of one Acidobacteria genus *Candidatus Koribacter*, a known soil nitrogen fixing bacterium (Ward et al., 2009) and two Actinobacteria (*Micromonospora* and *Actinoplanes*). A recent study investigated novel antimicrobial producing Actinobacteria, identified and isolated *Micromonospora* and *Actinoplanes* from insect nest material of *Polistes dominulus*. Subsequent challenge studies with bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, and *Bacillus subtilis* revealed 60% of Actinobacteria isolates inhibited the growth of these bacteria (Madden, Grassetti, Soriano, & Starks, 2013), providing evidence for the protective role Actinobacteria play in social wasp nests.

Queen core analyses revealed eleven taxa unique to queens, of which six identified to genus (*Mycobacterium*, *Flavobacterium*, *Tardiphaga*, *Rhodoplanes*, *Rhizomicrobium* and *Sphingomonas*). *Flavobacterium* is a known intracellular symbiont of insects and has been linked, to reproductive manipulations resulting in female-biased sex-ratios (Duron, Wilkes, & Hurst, 2010; G. D. D. Hurst & Frost, 2015). These manipulations are caused by parthenogenesis, male-killing or the feminisation of genetic males, increasing the number of infected females in host populations, thereby enhancing maternal symbiont transmission (Narita, Kageyama, Nomura, & Fukatsu, 2007; Stouthamer, Breeuwer, Luck, & Werren, 1993). The Betaproteobacteria order SC-I-84 closely matched to the genus *Burkholderia* and is identified as important mutualists in honey bees and bumble bees.

2.6.2 Microbiome

There was no significant dissimilarity in prokaryote and microbial eukaryote communities between the three life stages. However, there was considerable dissimilarity between ranges, and between life stages within those ranges, indicating potential acquisition and loss of microbiota. This variability between the ranges makes it difficult to infer a definitive microbiota representative of *V. vulgaris* and suggests a more general microbiota. Nevertheless, distinctions between life stages of *V. vulgaris* were mainly observed when comparing the queens' microbiota with that of larvae and workers. There was also significant dissimilarity in larval prokaryote communities between each range and between larvae and workers in comparison to queens in the native Belgian range. Microbial eukaryote community dissimilarity also revealed significant differences in richness and diversity of taxa between larvae and queens. Known viruses also occurred in larvae and workers across both ranges but

not in Queens. These differences potentially highlight a more specific microbiome with fewer pathogenic species in queens than in larvae and workers.

Lactic Acid Bacteria (LAB) taxa were present in all life stages in both ranges. However, there was more significant presence and abundances of LAB genera observed in the native range, compared with the invaded range. In the New Zealand range *Enterococcus* was the only LAB genus found in New Zealand larvae, and the genus *Lactobacillus* occurred in higher mean abundances in New Zealand workers and queens. Most enterococci bacteria are known commensals of the gastrointestinal tract of many insects, with some members able to fix nitrogen, thus contributing to a supplemental nitrogen source (Behar, Yuval, & Jurkevitch, 2005; Morales-Jiménez, Zúñiga, Villa-Tanaca, & Hernández-Rodríguez, 2009). These bacteria are mutualistic in *A. mellifera*, with *Enterococcus* and *Lactobacillus* protecting their hosts from microbial pathogens (Vásquez et al., 2012) and associated predictors of honey bee colony health (Budge et al., 2016). However, *Enterococcus faecalis* and *Enterococcus faecium* have been known to become pathogenic when they infect sites outside of the gut (Yuen & Ausubel, 2014). The genus *Fructobacillus* and its by products are reported to promote the growth of core members of the gut microbiome (Rokop, Horton, & Newton, 2015) and *Convivina* a newly assigned genus to the LAB group has been reported as a mutualist in the bumblebee gut (Praet et al., 2015). One study demonstrated that abundance of LAB in diseased honey bees was significantly lower than in healthy bees, providing additional evidence, LAB bacteria confer health benefits in bumble bees and honey bees (Cox-Foster et al., 2007).

Assuming the native range provided the more typical and most natural *V. vulgaris* microbiome, a change in microbiota was observed indicative of microbiota acquisition in the invaded range. LAB bacteria, Bacilli and Alphaproteobacteria found in the native range appear to be largely replaced in the invaded range life stages with an abundance of honey bee and bumble bee associated endosymbiotic bacteria (Kwong & Moran, 2013). These acquired endosymbionts are from the class Gammaproteobacteria (Orbaceae (f), *Gilliamella* and *Bombella*), Alphaproteobacteria (*Commensalibacter*, Acetobacteraceae (f), *Saccharibacter*) and Betaproteobacteria (*Snodgrassella*). This acquisition may suggest these bacteria are essential associates of *V. vulgaris* in the invaded range as they are in honey bee and bumble bee health (Cariveau et al., 2014; E. Crotti et al., 2010; Engel et al., 2012; Kwong & Moran, 2013; J. Li et al., 2015; Martinson et al., 2011; N. A. Moran & Kwong, 2016). Recent studies

provide evidence that these gut bacteria offer adult bumble bees and honey bees protection against parasitic eukaryotes (H. Koch & Schmid-Hempel, 2011). Higher *Crithidia* incidence in individuals coincided with low presence of *Snodgrassella* and *Gilliamella*, *Lactobacillus* and *Commensalibacter* consistent with a protective role (Cariveau et al., 2014; Jan Hubert et al., 2017). A recent Nature publication, highlighted the effect of *Snodgrassella alvi* on viral infections, inferring that the presence of *Snodgrassella alvi* may cut the number of viruses in a hive by as much as half, by initiating a systemic immune response (Katsnelson, 2015). In honeybees, *Gilliamella apicola* has also been confirmed to degrade pectin which encompasses pollen grains, facilitating the perforation and loosening of the primary cell wall of pollen, possibly aiding in digestion (Engel et al., 2012). Endosymbionts in many social insects can upgrade the quality of the diet of their host by utilising nonessential constituents and transforming them into compounds that are essential for the host (Cook & Davidson, 2006; Feldhaar et al., 2007).

Lactic acid bacteria (LAB) such as *Lactobacillus* and *Bifidobacterium* are also associates of honey and bumble bees, and were present in *V. vulgaris* workers and queens but not in larvae. These endosymbionts are also known to inhibit pathogens, and have been used for centuries in food preservation to prevent microbial spoilage (Maki, 2004) as well as coevolving with hymenopteran hosts (McFrederick et al., 2013). This potential acquisition of microbiota associated with *Apis mellifera* and *Bombus* spp. may indicate close interactions between wasps, honey bees and bumble bees in New Zealand where horizontal transmission is occurring with the potential spill-over or spill-back of microbiota between bees and other social insects (Lester et al., 2015). It could also be the case that microbiota are horizontally transmitted on flowers. A study on corbiculate bees suggested *Gilliamella*, transmits horizontally outside of the nest more frequently than *Snodgrassella* with transmission occurring on flowers as well as through direct contact with other bee species (H. Koch, Abrol, Li, & Schmid-Hempel, 2013). This environmental transmission has previously been demonstrated with the transfer of the trypanosomatid *C. bombi* to bumble bees on shared flowers (Durrer & Schmid-Hempel, 1994) and RNA viruses in hymenopterans including bees (Singh et al., 2010). The active horizontal transfer of microbiota may explain the acquisition of honey bee and bumble bee endosymbionts discovered in *V. vulgaris*. This transfer of microbiota likely occurs where individuals are in most contact, via hive robbing and predation

of honey bees and bumble bees (Clapperton, Alspach, Moller, & Matheson, 1989; Fries & Camazine, 2001). This acquisition may benefit *V. vulgaris* in the New Zealand range, making them fitter by providing a larder of food and beneficial endosymbionts. Subsequently, *V. vulgaris* may have unfavourable effects on honey bees by hive robbing and as a source of disease spill-over from wasp to bee as well as predation of workers. To confirm this hypothesis, additional sampling of *V. vulgaris* nests is needed along with molecular methods to ascertain the degree of acquisition in the New Zealand range.

The genera *Wolbachia* and *Arsenophonus* were found to co-occur in all life stages in one invaded range nest, whereas low abundances of *Wolbachia* occurred in larvae in the native range. Previous studies have also highlighted this co-occurrence (Duron et al., 2008; Qu et al., 2013; Sebastien, Gruber, & Lester, 2012; Yañez, Gauthier, Chantawannakul, & Neumann, 2016). Strains of *Wolbachia* most likely protect their host against several vectored RNA viruses (Teixeira et al., 2008) and are regarded as part of host immunity (Zindel, Gottlieb, & Aebi, 2011). At the same time, *Wolbachia* strains have also been implicated as parasitic/pathogenic, manipulating reproduction of their host to ensure their vertical transmission via the death of male eggs (Dyer & Jaenike, 2005) and feminisation of the host (Azzouna, Greve, & Martin, 2004; Oliver & Moran, 2009). *Arsenophonus*, like *Wolbachia*, is an intracellular bacteria found mainly in arthropods (Nováková, Hypša, & Moran, 2009), and like *Wolbachia*, *Arsenophonus* can modify the sex-ratio of its hosts via male-killing (Gherna et al., 1991). Transmission of *Arsenophonus* is typically maternal, although horizontal transmission can occur (Thao & Baumann, 2004). A recent study highlighted *Arsenophonus* as an indicator of poor health in honey bee colonies and potentially implicated in Colony Collapse Disorder (CCD) in the United States (Budge et al., 2016; Cornman et al., 2012). More recently *Arsenophonus* spp. were found in *Varroa destructor*, implying this mite may play a role as a vector in the horizontal transmission of *Arsenophonus* spp. between honey bees at both individual and colony level (J. Hubert et al., 2015).

The apparent microbial alteration from native to invaded range also came with observed losses of microbial genera. These range differences suggest a possible release from enemies. The Enemy Release Hypothesis (ERH) may explain the lack of specific native microbial taxa not seen in the invaded range. This hypothesis predicts that an exotic species introduced to a new region will become invasive because key natural enemies are absent (Keane & Crawley,

2002). Lester et al. (2015) investigated ERH in *V. vulgaris* and found no evidence of enemy release using proteomic and PCR methods (Lester et al., 2015). The results in this study, using different methods, indicate that there are differences in the microbial communities between the native and introduced range, with potential losses having occurred along the invasion pathway from Europe to New Zealand. In the native range, life stages hosted Gammaproteobacteria (*Rickettsiella*, *Diplorickettsia*, *Serratia* *Enterobacter*, *Pantoea*, *Erwinia* and *Providencia*) and Bacilli (*Fructobacillus*, *Convivina*, *Leuconostoc*, *Lactococcus*, *Vagococcus*) not seen in the invaded range. The bacterium *Vagococcus* has previously been isolated from the digestive tract of *Vespula vulgaris* (Killer et al., 2014), but the ecological importance of *Vagococci* has not been extensively studied. Previous research suggests some strains to be potential pathogens (Ruiz-Zarzuela, de Bias, Gironés, Ghittino, & MúAzquiz, 2005) and others indicating a probiotic function (Román et al., 2012). The Gammaproteobacteria *Diplorickettsia* has previously been isolated from *Ixodes ricinus* ticks, and the species *Diplorickettsia massiliensis* has been confirmed as a human pathogen in tick-borne infections (Mathew et al., 2012; Subramanian et al., 2012).

Members of the genus *Rickettsiella* have also been described as pathogens of diverse invertebrates (Bouchon, Cordaux, & Pierre, 2012) although in the pea aphid (*Acyrtosiphon pisum*), *Rickettsiella* exhibited either weakly beneficial or nearly neutral effects on host fitness, although *Rickettsiella* and *Hamiltonella* coinfections had adverse effects on the host. Interestingly *Rickettsiella* in the pea aphid altered its hosts' body colour from red to green (Tsuchida, Koga, Fujiwara, & Fukatsu, 2014). The genus *Providencia* is a bacterial pathogen of many organisms, including humans and insects. One species, *Providencia sneebia* is suggested to avoid recognition of the immune system in *Drosophila melanogaster*. The bacterial genus *Serratia* has been isolated from various insects hosts (Cruden & Markovetz, 1987; Feldhaar & Gross, 2009; Hamdi et al., 2011; Villegas & Pimenta, 2014). One species *Serratia marcescens* is known to be a pathogen of insects (Lauzon, Bussert, Sjogren, & Prokopy, 2003), including *A. mellifera*, and has also been identified as a potential pathogen of *V. vulgaris* (Rose et al., 1999).

The enterobacterium genus *Pantoea* is diverse, and has been isolated from many environments. *Pantoea*, has known capabilities for degrading and utilising different types of plant materials and therefore considered endosymbiotic (Walterson & Stavrinos, 2015).

Candidatus Rhabdochlamydia was found in native larvae and in low abundances in the invaded range and has previously been found in yellow crazy ants *A. gracilipes* (Cooling, 2015) and the cockroach *Blatta orientalis*. In *B. orientalis* it was shown to be an intracellular bacterial pathogen, causing swelling of the abdomen and infecting ovaries and fat bodies (Corsaro et al., 2007). There is a lack of knowledge on the pathogenicity of individual microbiota in *V. vulgaris*. However there is potential for ERH to contribute to the high abundance of wasps in New Zealand. ERH could explain improved fitness in the new range but is one part of a complex network. Knowledge of these networks and their complexity is critical for distinguishing mechanisms that may facilitate invasiveness and is essential for mediating the impacts of exotic species on biodiversity (Allendorf & Lundquist, 2003; Hatcher, Dick, & Dunn, 2006).

In the invaded range, there was more significant richness and diversity of microbial eukaryotes, with an increase in fungal, protozoan and nematode species. Fungal OTUs dominated the microbial eukaryote community with 88% of the 18S sequences from the two fungal phyla Ascomycota and Basidiomycota. Protozoan taxa accounted for 8% of the microbial eukaryote community. Fungi are likely to play a part in digestion as is seen in guts of insects that feed on wood or detritus (Engel & Moran, 2013).

G tests revealed *Debaryomyces*, *Bionectria*, *Hygrocybe* and *Cryptococcus* as well as the protozoan CCA5 as significant microbial eukaryote taxa in the New Zealand range. There was also a significant presence of the fungal class *Saccharomycetes*, known yeast including the genus *Debaryomyces*. The presence of yeasts in insects has been demonstrated to provide nutrient supplements in the host. Yeasts provided nitrogen to the scale insect *Pseudococcus citri* (Koch 1954), and the genus *Debaryomyces* is associated with the biosynthesis of B vitamins in xylophagous insects (Gusteleva, 1975).

The protozoan *Mattesia* identified in the native range is reported to cause yellow-head disease in populations of the red imported fire ant (Pereira, Williams, Becnel, & Oi, 2002). In New Zealand CCA5 a protozoan was identified, occurring also in arctic environments although the possible role in insect microbial communities remains unexplored (Stoeck et al., 2007). SIMPER analysis also highlighted the fungal entomopathogen *Conidiobolus* and the genus *Cryptococcus* found in both ranges but in higher abundances in the Belgian range. Infection

with *Conidiobolus coronatus* in *Galleria mellonella* was shown to increase phagocytic activity suggesting this species is pathogenic (Kędra, Boguś, Kędra, & Boguś, 2006).

There was a definite distinction of known virus found in larvae and worker that were absent in queen samples. The viral landscape was also different with the native Belgian samples predominantly hosting *RhPV*, and *ALPV* while the invaded New Zealand samples hosting the bee-related virus *KBV*, with both ranges having instances of *DWV*. All viruses were members of the family Dicistroviridae. There was a higher quantified viral presence using Virusfinder2 compared with the MEGAN analysis, likely due to the differences in analysis approaches. The MEGAN analysis is based on BLAST results of searches on GenBank databases using assembled contigs and a stricter lowest common ancestor algorithm (Huson, Auch, Qi, & Schuster, 2007), while Virusfinder2 uses unassembled paired-end reads which are aligned to the RINS (Bhaduri et al., 2012) database.

Discovery of putative viruses aligned *ALPV* to partial genes involved in replication (RNA polymerase), as well as *DWV* and *ALPV* both aligning to capsid proteins. Five other putative contigs were identified as potential novel viruses based on their similarity to known virus coding proteins and will need to be further investigated. *KBV*, *ALPV* and *DWV* were all confirmed viruses in *V. vulgaris* using PCR analysis with designed primers. The virus *RhPV* was not confirmed and needs further investigation.

To date, the effect these viruses have on *V. vulgaris* is unknown. In this study active replication of viruses was not tested. Therefore, the viruses present in samples may not be parasites but merely part of nutritional intake from the environment, however, the abundance of *RhPV* OTUs in the Belgian range may indicate infectivity. *RhPV*, an aphid virus (Gildow & D'Arcy, 1990), may suggest this aphid is predated by *V. vulgaris* in the native range. *KBV* infects many types of bees including *Apis mellifera* (J. R. de Miranda, 2004) with the virus affecting both brood and adult bees, causing death within days of exposure (Berenyi, Bakonyi, Derakhshifar, Koglberger, & Nowotny, 2006). A recent study confirmed the presence and replication of *KBV* in New Zealand *V. vulgaris* colonies and observed its high prevalence in wasps sampled (Dobelman et al., 2017). *DWV* is another RNA virus known to commonly infect *Apis mellifera* via the vector *Varroa*, however it has also been documented in *B. terrestris*, indicating it is not specific to just honey bees as seen in *V. vulgaris* (Möckel, Gisder, & Genersch, 2011).

The presence of *DWV* and *KBV* in invaded range wasps may indicate spill-over of honey bee viruses via the same route that *V. vulgaris* acquired beneficial honey bee endosymbionts. In the native range, *ALPV* and *RhPV* viruses are both associated with aphids and were not seen in the invaded range samples. This may indicate a loss of enemies, or potentially an alteration of diet in the new range. There may be fewer aphids or less of a need for wasps to consume aphids in an environment full of resources such as honeydew from the beech forests (*Fuscospora* spp.), or the ample supply of hives to rob and predate in the thriving New Zealand honey industry.

The absence of many Bacilli, Gammaproteobacteria, microbial eukaryotes and known viruses in the queen life stage samples was dramatic in comparison to larvae. This potential segregation of microbiota may suggest a specific queen food source, a complex immune system or possibly increased nest hygiene in queen combs inferring methods of protection that potentially select symbionts and exclude non-symbionts (Dillon & Dillon, 2004). This overall change or segregation of specific microbiota in life stages is interesting due to the social nature of vespids with food resources shared through the process of trophallaxis (The transfer of food from adult wasp to larvae for digestion, once digested the larva feeds back food to adult wasps as part of a salivary gland secretion)(Matsuura & Yamane, 1990) facilitating a social stomach (David R. Tarpy et al., 2015).

One study demonstrated how the microbiota of honey bee queens could be altered and maintained using a distinct diet to produce a different microbiome. Honey bee workers, using their hypopharyngeal glands, produce royal jelly known for its antiseptic properties which they continually feed to mature queens, resulting in a distinctive queen microbiome (David R. Tarpy et al., 2015). Similarly, trophallactic salivary regurgitations produced by *V. vulgaris* larvae has been demonstrated to contain antibiotic properties that significantly inhibit the growth of microbiota such as *Bacillus* sp. found in the brood chamber of *V. vulgaris*, which limits disease and reduces the incidence of epidemic outbreaks within the colony (Gambino, 1993; Harcourt, 2002). Survival of larvae of the solitary digger wasp *Philanthus triangulum* is greatly reduced when *Streptomyces* bacteria are removed from brood cells. The adult female smears this bacterium with antennal secretions containing antibiotic-producing *Streptomyces* spp. which the larvae then transfer to the surface of the cocoon for protection against fungal infestation (Kaltenpoth, Göttler, Herzner, & Strohm,

2005). Resident gut flora in the queen life stage may also play a protective role (Dillon & Dillon, 2004), producing antimicrobial substances themselves (Forsgren, Olofsson, Vásquez, & Fries, 2010; Vásquez et al., 2012) or through the symbiotic and competitive exclusion of newly invading bacteria (Dillon, Vennard, Buckling, & Charnley, 2005).

The relationship between host microbiota and immunity is also an important factor where the immune system is primed by specific taxa to invoke an immune response. There is evidence of immune priming and pathogen resistance in queens of the ants *Lasius niger* and *Formica selysi*. Mated queens have higher pathogen resistance than virgin queens when challenged with the entomopathogenic fungus *Beauveria bassiana*, suggesting up regulation of the immune system when triggered by mating (Gálvez & Chapuisat, 2014). Immune priming also reported in *A. mellifera* and *Bombus* spp., indicates non-pathogenic gut flora induces immune responses regulating microbiota and benefitting host health. (Cisarovsky, Koch, & Schmid-Hempel, 2012; Kwong, Mancenido, & Moran, 2017; Sadd & Schmid-Hempel, 2006).

2.7 CONCLUSION

This study provides the characterised core and general microbial community of three *V. vulgaris* life stages and made preliminary comparative analyses of the communities of an invasive wasp from two geographical ranges. A greater presence of LAB taxa and *RhPV* in the native range and the potential acquisition of honey bee and bumble bee endosymbionts, as well as KBV in the invaded range, suggests a scenario where the microbiome community of an introduced wasp is positively altered, potentially increasing wasp fitness, leading to its success in the invaded range. It also suggests an overlap in pollinator communities in the invaded range, where spillover of the microbiota is occurring, and diet, as well as the environment, is the main precursor of a generalist horizontally transmissible *V. vulgaris* microbiome. A combination of fastidious nest maintenance together with hygienic behaviour and an abundance of trophallactic exchanges as well as an efficient immune system may aid in developing a beneficial microbiome in *V. vulgaris*, however to date it is still unclear what most of these mechanisms are and the protective functions they may have in *V. vulgaris*.

This study was comprehensive, although low in sample size, therefore further analysis is required to confirm communities and to clarify the ecological function of the phylotypes

found in this study. Additional investigation is also needed to determine whether queen wasp immune expression is a regulating factor in the queen microbiome, excluding specific microbiota.

3 A CROSS TAXON ANALYSIS OF HYMENOPTERA MICROBIOME DIVERSITY

3.1 ABSTRACT

Economically beneficial hymenopterans such as *Apis mellifera* and *Bombus* spp. are associated with an abundance of microbiota, both symbiotic and pathogenic. However, the specificity of microbiota among related hymenopterans remains elusive. Here comparative investigations unravel the microbial diversity of five hymenopteran species (*Apis mellifera*, *Bombus terrestris*, *Vespula vulgaris*, *Vespula germanica* and *Linepithema humile*). Microbiome characterisation of each sample was investigated using Illumina Next Generation Sequencing (NGS) techniques to detect bacterial, microbial eukaryote and viral taxa. Overall microbial communities were not significantly different between hymenopteran hosts, although dissimilarities were noted. *B. terrestris* had an abundance of *Wolbachia*, *Segniliparus*, *Leptomonas* and the mite *Aleuroglyphus ovatus*, whereas *V. germanica* and *A. mellifera* had greater abundances of *Spiroplasma*. Sixteen viral phylotypes were detected, of which seven identified in *A. mellifera*. Overall there was a lack of specificity observed in the *V. vulgaris* microbiome in comparison to their hymenopteran relatives. This lack of specificity in *V. vulgaris*, potentially demonstrates an interaction between host species in the movement of microbiota and parasites, through shared use of flowers by multiple pollinator species and hive robbing.

3.2 INTRODUCTION

Insects play vital ecosystem roles as pollinators, nutrient recyclers and vectors of symbiotic as well as pathogenic microbes. Increasingly, the evidence demonstrates that insects have close associations with microbial communities which can have a positive or negative effect on insect fitness depending on their abundance and impact. Therefore hosts with beneficial microbes are more likely to succeed in competition with hosts that have fewer such microbes. This beneficial microbiota must be able to withstand the selective filters imposed by host environments, such as antimicrobial defences (Zeng, Wu, Sukumaran, & Rodrigo, 2017). Understanding the host microbial community balance, specificity and how the microbiome affects the fitness of its host may inform how best to manage beneficial insects and control invasive insects.

Social Hymenoptera are ecologically as well as economically important because they play important roles in terrestrial ecosystem function, such as pollinating, predating and scavenging (Wilson, 1971). This array of hymenopteran lifestyles and feeding strategies dictates how hymenopteran species interact with the microbial environment. The supply of colony provisions can vary from dead arthropods (in many wasps and ants) to pollen and nectar (in most bees) and fungal crops in some ant species (Mueller et al. 2005). Therefore the range of diet and environmental lifestyle factors can influence host microbial communities (Colman et al., 2012). Consequently, an understanding of host microbiome specificity is important to identify crucial microbiota and pathogen effects on host fitness.

Many entomopathogens are widely distributed in the environment and can be readily isolated from soil, water, plant surfaces, air, excretions and from bodies of infected insect hosts (Federici & Maddox, 1996). Studies have documented symbioses and pathology of certain insect species and their microbial community (Brodeur, 2012; Cariveau et al., 2014; Dale & Moran, 2006; Harvey et al., 2016; N. a. Moran, 2015). However the specificity of the microbial community to the host is less well known and the degree of microbial distribution of microbiota amongst related host insect species (Jones, Sanchez, & Fierer, 2013).

Introduced host insect species can play a significant role in the environment whether purposely placed to carry out a function such as honey bees and bumble bees, or like

V. vulgaris, mistakenly introduced to the detriment of the newly invaded environment. Microbiota, co-introduced by introduced species in a new range, can be transmitted by spillover between host species. A spillover corresponds to the transmission of infections from one species to another species (Colla, Otterstatter, Gegear, & Thomson, 2006). Host species investigated in this study have been known to share similar habitat resources, with the increased potential of microbiota spillover and spillback through predation, hive robbing and shared floral resources.

Hive robbing is known to occur between honey bee colonies (Engel et al., 2012). Also, bumble bee and wasp species (*V. vulgaris* and *V. germanica*) are known to frequently rob hive resources (Clapperton et al., 1989; Fries & Camazine, 2001; Meeus, Brown, de Graaf, & Smagghe, 2011). Recently a study also highlighted the effect of the Argentine ant (*Linepithema humile*) on honey bee colonies, contributing to honey bee hive collapse through the combined effects of predation, hive robbing, and disease spillover (E. C. Brenton-Rule, Baty, Russell, Saunders, & Lester, 2017). Additionally, the spillover and spillback in the environment can occur through shared floral resources increasing the transmission potential for microbiota between hosts (Manley, Boots, & Wilfert, 2015; Ruiz-González & Brown, 2006; Singh et al., 2010). However, the spillover and spillback transmission hypothesis between hosts is not conclusive in the wild as it is difficult to measure spillover from one host to another unless conducted in a controlled environment. Therefore an initial understanding of individual host species' microbiota and comparative analyses of inter-host species is the first stage of elucidating specificity and shared microbiota between hosts.

Here the microbiome of five hymenopteran species was examined, introduced to New Zealand, two beneficial pollinators (*Apis mellifera* and *Bombus terrestris*) and three invasive species (*Vespula vulgaris*, *Vespula germanica* and *Linepithema humile*). This study aimed to conduct a comparative analysis of microbial community diversity as well as a preliminary analysis of host-microbial specificity. It was hypothesised that there would be greater microbiota diversity in generalist feeders, vespid wasps (*V. vulgaris* and *V. germanica*) and Argentine ants (*L. humile*) compared with the honey bee and bumble bee pollinators *A. mellifera* and *B. terrestris*.

3.3 MATERIALS AND METHODS

3.3.1 Sample Collections

Workers of five hymenopteran species were sampled for microbiome examination (*A. mellifera*, *B. terrestris*, *V. germanica*, *L. humile* and replicated samples (Chapter 1) of *V. vulgaris* from Europe and New Zealand). All workers were sampled during the autumn (March-May) of 2014, except European *V. vulgaris*, which were sampled in the autumn (September-October) of 2014. A total of 14 pooled samples were collected, two pooled (5 individuals) *A. mellifera* samples from two separate hives. Three pooled (5 individuals) *B. terrestris* samples from three sites around the Wellington region. Two pooled *L. humile* samples, one consisting of a northern population (Auckland: 150 (50 x 3) individuals pooled from three sites), and the other a southern population (120 ants (20 x 6) from six sites: Paraparaumu, Picton, New Plymouth, Christchurch, Petone, Gisborne). One *V. germanica* (1 nest) and six *V. vulgaris* (Chapter 1). See **Figure 3-1** for a map of sampling sites and

Table 3-1 for sampling site coordinates in addition to sample number and number of pooled individuals per sample. At the time of sampling, a comparison of three host species microbiomes was initially the aim (*V. vulgaris*, *A. mellifera* and *B. terrestris*). However, due to competing interests of another project, Illumina costs and the number of samples we could place on illumina lanes, it was decided to add in two pooled samples of *L. humile* from various geographical areas around New Zealand and one sample of *V. germanica* which completed the last illumina lane. This ad hoc approach caused disparity of samples species and geographical sample location. A more balanced sampling approach may have allowed a more in-depth investigation into host microbiota differences and potentially a comparison of host taxon vs location as a determinant of microbiome community.

To retain the integrity of the DNA and RNA, vespid samples and *L. humile* samples were collected in RNeasy Lysis Solution (QIAGEN Inc., Austin, USA), whereas *A. mellifera* and *B. terrestris* were preserved in a -80°C freezer. Both DNA and RNA extractions were conducted on all sample pools post homogenisation using a QIAGEN TissueRupter® (QIAGEN, Tokyo, Japan). Samples were placed in a suitably sized tube (with lysis buffer and β mercaptoethanol), depending on insect size (15 mL tube for *B. terrestris* and two mL microcentrifuge tubes for other smaller insects). The homogenising disruption time was kept as short as possible (< 30 seconds) to avoid shearing of DNA and RNA.

Figure 3-1 Maps of host insect species sampling sites. Hymenoptera host and sample sites are denoted by a coloured point and a number as seen in legend. Full details of sites sampled including GPS coordinates and number of pooled individuals per sample presented in **Table 3-1**.

Table 3-1 Details of sampling sites including geographical coordinates and number of pooled individuals per sample. BE = Belgium; NZ = New Zealand

Host Species	Sample Number	Sample locations	Latitude	Longitude	Number of pooled worker individuals per sample
1 <i>V. vulgaris</i>	6	Tin Line, Nelson Lakes (NZ)	-41.28	173.50	5
		Braeburn, Nelson Lakes (NZ)	-41.79	172.51	5
		Six Mile, Nelson Lakes (NZ)	-41.76	172.95	5
		Wijgmaal, Leuven (BE)	4.70	50.93	5
		Hasselt, Hasselt (BE)	5.20	50.55	5
		Wezemaal, Leuven (BE)	4.75	50.94	5
2 <i>A. mellifera</i>	2	Wellington (Victoria University) (NZ)	41.29	174.76	5
		Wellington (Victoria University) (NZ)	41.29	174.76	5
3 <i>B. terrestris</i>	3	Wellington (NZ)	-41.28	174.76	5
		Upper Hutt (Kaitoke) (NZ)	-41.04	175.17	5
		Porirua (Plimmerton) (NZ)	-41.08	174.86	5
4 <i>V. germanica</i>	1	Palmerston North (NZ)	-40.35	175.60	5
5 <i>L. humile</i>	1	<u>Northern Sample (n):</u>	-36.84	174.763	50 ants from each of three sites =150 pooled ants.
		Auckland (3 sites) (NZ)	-36.84	174.763	
			-36.84	174.763	
	1	<u>Southern Sample (s): (six sites)</u>			20 ants from each of six sites=120 pooled ants
		Paraparaumu (NZ)	-40.91	175.00	
		Picton (NZ)	-41.29	174.00	
		New Plymouth (NZ)	-39.05	174.07	
		Christchurch (NZ)	-43.53	172.63	
		Petone (NZ)	-41.22	174.88	
		Gisborne (NZ)	-38.66	178.01	

3.3.2 DNA Extractions and Library Preparation

Purification of total DNA from pooled samples was carried out using DNeasy Blood & Tissue Kit, using protocol DY14 (www.qiagen.com/literature/protocols/DNeasyTissue.aspx). The purity of DNA samples was determined using the absorption ratio (260/280 nm) determined by NanoDrop 1000 and samples were run on 1.5% agarose gel to ensure DNA was not fragmented. DNA samples were sent for library preparation and de novo 16S and 18S Illumina MiSeq sequencing at New Zealand Genomics Ltd (NZGL). 16S ribosomal RNA gene amplicons were prepared following the Illumina 16S Metagenomic Sequencing Library Preparation Protocol (Illumina, 2013). The V3-V4 region of the 16S rRNA gene was amplified using Nextera adapter primers and dual-index barcodes to the amplicon target (Illumina, 2013). Specific primer pair (**Table 3-2**) sequences were used to create a single paired-end amplicon for the V3-V4 region of approximately 300 bp.

18S ribosomal RNA gene amplicons were prepared according to the protocol of the Earth Microbiome Project (J Gregory Caporaso et al., 2012). The V9 region (200 bp) of the eukaryotic 18S rRNA gene was amplified for each sample with 1391f and EukBr primers (Amaral-Zettler et al., 2009) (**Table 3-2**). PCR products were pooled 80% for the 18S libraries and 20% for the 16S libraries and sequenced on a MiSeq run. At the time of sampling the 18S (80%) + 16S (20%) method was advised by the Illumina sequencing provider to allow for swamping of 18S host genomic DNA. Adapters and barcodes were removed and read trimmed using the SolexaQA package (Cox et al., 2010) and quality checked using FASTQC (Andrews, 2010).

3.3.3 Prokaryote Bioinformatics Pipeline

Briefly, microbial prokaryote diversity was analysed using QIIME version 1.9.1 (J G Caporaso et al., 2010), following paired-end assembly using PANDAseq, v 2.8 (Masella et al., 2012) as outlined in detail in Chapter 2. Of the 37,351 16S input reads, 31,902 sequences (85%) were retained.

3.3.4 Microbial Eukaryote Bioinformatics Pipeline

Briefly, microbial eukaryote diversity was analysed using the SILVAngs v 1.3 pipeline (Quast et al., 2013) following paired end assembly using PANDAseq, v 2.8 (Masella et al., 2012) as

outlined in detail in Chapter 2. Quality filtering of 164,750 18S input reads resulted in 163,173 output sequences.

Table 3-2 Table is outlining forward and reverse primers used in NGS Miseq 16S & 18S amplicon sequencing.

16S Primers		Product Size	rRNA Region
16S Primer F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG	300bp	V3-V4
16S Primer R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC (Klindworth et al., 2013)		
18S Primers			
Illumina_Euk_1391f	AATGATACGGCGACCACCGAGATCTACACTATCGCCGTTCCGGTACACACCGCCCGTC	200bp	V9
Illumina_EukBr	CAAGCAGAAGACGGCATACGAGATAGTCAGTCAGCAGATCCTTCTGCAGGTTCACCTAC (Klindworth et al., 2013)		

3.3.5 RNA Extractions and Library Preparation

The RNA-Seq approach was used for detection of RNA viruses. Total RNA was extracted using the PureLink®RNA Mini Kit® (Thermo Fisher Scientific Inc., Waltham, USA) according to the manufacturers' instructions after homogenisation with a QIAGEN Tissuerrupter (QIAGEN, Tokyo, Japan). Total RNA quantity and integrity was assessed on an Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Nano Chip kit according to the manufacturer's instructions. High-quality RNA was sent for sequencing on Illumina HiSeq platform (2 x 100 bp paired-end), carried out by NZGL. Total RNA samples were then used to make RNA-Seq libraries using the "TruSeq RNaseq Sample Prep kit" according to the manufacturer's instructions.

3.3.6 Viral Bioinformatics Pipeline

Briefly, *de novo* transcript reconstruction was undertaken using Trinity platform read normalisation, with software Trinity Release v2.0.6 2015-03-03 (Grabherr et al., 2011). Novel viral sequences in the assemblies were detected using the Trinity assembled contigs to query the National Center for Biotechnology Information (NCBI). A custom Perl script was used to summarise the BLASTn data and append taxonomic assignment to individual BLAST results (Gruber et al., 2017). The resulting BLASTn candidates were searched against the BLASTx database with an e-value of 0.0001 (Camacho et al., 2010). BLAST nr assigned targets were visualised to MEGAN v.6.6.3 using the default settings with the weighted LCA algorithm of 75% (Huson et al., 2016). To complement the BLAST and MEGAN analyses, VirusFinder2 was used to further identify viral candidates (Q. Wang et al., 2015). As MEGAN and Virusfinder2 use contrasting approaches that make it inappropriate to compare abundance between the two methods, I used presence/absence of virus for further analysis. A more detailed version of the viral pipeline is outlined in Chapter 2.

3.4 STATISTICAL ANALYSES

Venn diagrams were constructed to represent the number of 16S OTUs shared or not shared among the five hymenopteran species using the website Bioinformatics & Evolutionary Genomics (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). In these analyses, both high abundance prokaryote OTUs (>100 OTUs) and low abundance OTUs (<100s) were assessed prior to normalisation.

Richness (OTU abundance) and diversity (Shannon's Log e) metrics were assessed using square root transformed raw data (882 taxa assigned by Silva 128 (Quast et al., 2013)). The DIVERSE function in Primer 6 (Clarke & Warwick, 2001) was applied and richness and diversity metrics visualised using the R package ggplot2 (Wickham, 2009).

A one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the richness and diversity means of five hymenopteran hosts (*V. vulgaris* (n = 6), *A. mellifera* (n = 2), *B. terrestris* (n = 3), *V. germanica* (n=1) and *L. humile* (n = 2)). Where ANOVA *p*-values were statistically significant ($p \leq 0.05$), post hoc two tailed t tests were performed to determine which specific groups significantly differed from each other.

The dissimilarity between bacterial and microbial eukaryote and viral communities was assessed following the same methods in Chapter 2. Briefly, communities were assessed using Bray-Curtis dissimilarity matrix and tested for significance between each life stage, range and life stage within each range using PERMANOVA in Primer-E (Clarke & Warwick, 2001). To partition for variance and assess significance in each community pairwise tests were run over 999 permutations. Each group (life stages, ranges and life stages within ranges) were evaluated separately as fixed effects, with type III partial sum of squares and Monte Carlo sampling permuted over unrestricted raw data. To visualise clustering patterns between sample communities. To visualise significant clustering patterns of bacterial and microbial eukaryote communities, three methods were used, group average clustering, non-metric multidimensional scaling (nMDS) and a canonical analysis of principal coordinates (CAP) analysis all performed in Primer-E (Clarke & Warwick, 2001).

The contribution of each prokaryote and microbial eukaryote with dissimilarity differences among each life stage and range life stage was assessed using a similarity percentage analysis (SIMPER) analysis (Clarke & Warwick, 2001) on normalised data. SIMPER dissimilarity was verified using log-likelihood G-tests, carried out in QIIME 1.9.1, extrapolating statistical significance ($p < 0.05$) between taxa abundance in the different sample groups (J G Caporaso et al., 2010). The *p*-values from G-test analysis were corrected for multiple comparisons using the Bonferroni procedure within QIIME.

3.5 RESULTS

The microbiomes of the five hymenopterans were determined by Illumina deep sequencing of microbial SSU rRNA gene amplicons generated from pooled DNA and RNA extractions. Characterisation of the prokaryote, microbial eukaryote and viral communities (97% alignment identity) from 14 samples resulting from five host species, (*Vespula vulgaris* (n=6), *Apis mellifera* (n=2), *Bombus terrestris* (n=3), *Vespula germanica* (n=1) and *Linepithema humile* (n=2)) was carried out (**Table 3-1**). In total, 714,955 16S reads (mean sequence reads 55,688 SE $\pm$ 21,974), 2,502,384 (178,741 $\pm$ 26,332) 18S reads and 700,331,362 (50,023,669 $\pm$ 2,694,209) rRNA sequence reads were generated from the 14 samples. The overall prokaryote community was represented by 659 bacterial (> 97% Identity) OTUs after filtering of singletons. Following normalisation and filtering OTU's greater than 100 reads, 61 high abundance OTUs remained. Post filtering the microbial eukaryote community was represented (>97% Identity) by 74 taxa. Sixteen viral OTUs (>97% Identity) represented the viral community, identified using MEGAN and Virusfinder2. It was hypothesised that the overall microbial richness and diversity associated with hosts would be higher in vespids wasps and *L. humile* reflecting their polyphagous diet compared with their more specific pollen feeding counterparts *A. mellifera* and *B. terrestris*.

3.5.1 Prokaryote Community Distinctions

A pre-normalisation analysis was performed on absolute raw prokaryote community counts. This revealed, a higher abundance trend in prokaryote OTUs in both the high (>100 OTUs) and low abundance (<100 OTUs) groups in the *V. vulgaris* prokaryote community (**Figure 3-2**). However, post-normalisation there was no significant differences among hymenopteran host prokaryote communities, following ANOVA analysis and post hoc two-tailed t-tests for richness and diversity metrics. The richness and Shannon diversity metric trends revealed *V. vulgaris* and *L. humile* workers had high mean richness compared with the other hymenopteran hosts. Community richness in *B. terrestris* was the lowest. Prokaryote diversity was highest in *A. mellifera* and lowest in *B. terrestris* and *V. germanica* (**Table 3-3; Figure 3-3D**).

distinct in comparison with *V. germanica*, *A. mellifera* and *L. humile* communities. PERMANOVA pairwise comparisons revealed that *B. terrestris* prokaryote community was significantly different ($p(\text{MC}) < 0.05$) to all other hymenopteran communities. However, no significant differences were found among other host communities.

Table 3-3 Table is detailing mean richness and Shannon diversity metrics observed in five hymenopteran prokaryote communities. Standard errors are provided for each metric mean. Sample number is also provided (N). Richness and diversity metrics were analysed using the PRIMER-e software. Richness ($p = 0.314$) Diversity ($p = 0.2139$).

Species	N	Richness Mean	Standard Error	Shannon Diversity Mean	Standard Error
<i>Vespula vulgaris</i>	6	221	14.87	4.64	0.30
<i>Apis mellifera</i>	2	204	6.53	5.11	0.00
<i>Bombus terrestris</i>	3	188	10.39	4.03	0.38
<i>Vespula germanica</i>	1	202	N/A	4.14	N/A
<i>Linepithema humile</i>	2	225	11.84	4.94	0.15

Table 3-4 Table is detailing results of PERMANOVA pairwise comparison analysis of prokaryote community dissimilarity between five hymenopteran hosts. Results provided include the t statistic and PERMANOVA p -value with unique permutations. For all pairwise comparisons, p -values were corrected for multiple comparisons using Monte Carlo corrections. * denotes significant Monte Carlo corrected p -values.

Hymenoptera Host Pair-Wise tests	t-statistic	Permanova p -value	Unique permutations	p -value Monte Carlo Corrected
<i>V. vulgaris</i> , <i>V. germanica</i>	0.76	0.58	7.00	0.64
<i>V. vulgaris</i> , <i>A. mellifera</i>	0.97	0.47	28.00	0.44
<i>V. vulgaris</i> , <i>B. terrestris</i>	1.84	0.07	84.00	0.05*
<i>V. vulgaris</i> , <i>L. humile</i>	1.18	0.37	28.00	0.27
<i>V. germanica</i> , <i>A. mellifera</i>	0.91	0.68	3.00	0.55
<i>V. germanica</i> , <i>B. terrestris</i>	3.38	0.25	4.00	0.03*
<i>V. germanica</i> , <i>L. humile</i>	3.09	0.35	3.00	0.16
<i>A. mellifera</i> , <i>B. terrestris</i>	2.77	0.11	10.00	0.03
<i>A. mellifera</i> , <i>L. humile</i>	1.63	0.35	3.00	0.19
<i>B. terrestris</i> , <i>L. humile</i>	4.19	0.12	10.00	0.01*

Figure 3-3 High abundance normalised prokaryote community structure of five hymenopter species, *V. vulgaris* (VV) (green triangle)(* = Belgian samples), *A. mellifera* (AM) (red diamond), *B. terrestris* (BT) (blue diamond), *V. germanica* (VG) (cyan square) and *L. humile* (pink circle)(n=nothern sample; s=southern sample) based on a Bray-Curtis dissimilarity (A-C), richness and diversity (D). (A) Group average cluster dendrogram, illustrating dissimilarity clustering distances of individual normalised bacterial communities. (B) Non-metric multi-dimensional (nMDS) scaling plot illustrating dissimilarity distances of individual normalised bacterial communities. Stress value: 0.03 (≤ 0.1 is acceptable). (C) Canonical analysis of principal coordinates (CAP) illustrating individual normalised bacterial community distinctness. (D) Mean richness and Shannon's diversity metrics calculated on the non-normalised dataset. The standard error shows the variance of bacterial community richness and diversity between each species samples, note *V. germanica* n=1. The colour of each bar corresponds to each hymenopter species in legend.

3.5.2 Characterisation of Prokaryote Communities

The prokaryote community across all hymenopteran species (mean relative abundance) was dominated by ten phyla, represented by Proteobacteria (36.8%), Verrucomicrobia (14.4%), Firmicutes (10.8%), Chloroflexi (9.0%), Planctomycetes (7.7%), Actinobacteria (7.6%), Acidobacteria (7.3%), Tenericutes (4.9%), Cyanobacteria (1.3%) and Bacteroidetes (0.1%). Of the phyla represented across all host communities, Proteobacteria was the most abundant, comprising of the classes Alphaproteobacteria (24.8%), Gammaproteobacteria (10.6%) and Betaproteobacteria (1.4%). The phylum Chloroflexi was represented by two classes Ktedonobacteria (8.6%) and KD4-96 (1.8%). The remaining phyla, Verrucomicrobia, Firmicutes, Planctomycetes and Tenericutes were represented by one bacterial class each, Spartobacteria (14.4%), Bacilli (10.8%), Phycisphaerae (7.7%) and Mollicutes (4.9%) respectively. These classes together represented 85% of the total host communities observed.

The iTOL phylogenetic tree illustrated similarities as well differences between hymenopteran host prokaryote communities. Of the 51 taxa represented across all hymenopteran hosts, *V. vulgaris* hosted 50 taxa, *A. mellifera* 34 taxa, *B. terrestris* 30 taxa, *V. germanica* 28 taxa and *L. humile* 25 taxa (**Figure 3-4**). From the taxa presented, 15 were common to *V. vulgaris*. Of these, ten genera were identified: *Erwinia*, *Vagococcus*, *Bifidobacterium*, *Snodgrassella*, *Acinetobacter*, *Saccharibacter*, *Corynebacterium*, *Apibacter*, *Orbus* and *Mesorhizobium*. The genus *Segniliparus* was the only other unique taxon attributed to the host *B. terrestris*. Relative mean class bacterial abundance of hymenopteran hosts were also calculated, identifying the most abundant class in each group and all taxa identified to the lowest taxonomic level, contributing greater than 1% to that class (**Figure 3-5**).

The most evident differences in taxa between hosts was observed in *B. terrestris*, revealing a high mean abundance of Alphaproteobacteria attributed to the genus *Wolbachia*. There was also a reduced relative abundance of Bacilli observed in *B. terrestris*, compared with other hosts. The hymenopteran hosts *Apis mellifera* and *V. germanica* had an abundance of Mollicutes attributed to the genus *Spiroplasma*. Compared with other hosts, *V. vulgaris* had a greater richness of taxa from the class Gammaproteobacteria and Bacilli.

Figure 3-4 iTol phylogenetic tree of 16S rRNA high abundant OTUs (>100 reads). The phylogeny of bacterial taxa from five Hymenopteran species identified to the genus level where possible. This rooted maximum likelihood phylogeny reveals each host's prokaryote community presence, absence and specificity of taxa. Colours within the inner circle illustrate the phylum that the bacterial taxa are in, based on the OTUs assigned in SILVA (see Phylum legend). The first three outer concentric circles from the inside out, display the bacteria found in each hymenopteran host (*V. vulgaris* = green, *A. mellifera* = red, *B. terrestris* = blue, *V. germanica* = cyan and *L. humile* = pink). Where taxonomy was not assigned to the genus level, the lowest taxonomic level was assigned and denoted in brackets, i.e. (f) = family, (o) = order, (c) = class and (p) = phylum. Host colours listed in legend correspond to colours used in subsequent figures.

Figure 3-5 Figure illustrating mean prokaryote class taxonomic abundances (>100 OTUs) observed in each hymenopteran host. Colours, annotated in the legend represent bacterial classes. Each coloured class block is represented by all taxa contributing >1% to that class. All taxa are identified to the genus level where possible, if not the lowest taxonomic level was assigned and denoted in brackets, i.e. (f) = family, (o) = order and (c) = class.

Differences in prokaryote communities were assessed using SIMPER analyses revealing dominant taxa contributing to each host-to-host dissimilarity (**Table 3-5-Table 3-14**). To confirm significance ($p < 0.05$) of dominant taxa, log likelihood G-tests were also performed (**Table 3-15**).

SIMPER analysis revealed higher mean abundances of *Spiroplasma* found in *V. germanica*, which accounted for 16-19.5% of the dissimilarity compared with all other hymenopteran hosts. Three *Spiroplasma* OTUs significantly attributed to this dissimilarity. There was also higher mean abundance of the genus *Spiroplasma* found in *A. mellifera* accounting for 6.9-11.5 % of the dissimilarity seen in other hosts. The genus *Lactobacillus* also contributed to dissimilarity with higher observed mean abundances seen in *V. vulgaris* and *L. humile* compared with other hosts. The host *L. humile* alone reveals the highest mean abundance of *Lactobacillus* accounting for 10-19% dissimilarity compared with other hosts. Log-likelihood analysis revealed two *Lactobacillus* OTUs significantly attributed to this dissimilarity. Greater mean abundances of the family Enterobacteriaceae were observed in both *V. vulgaris* and *V. germanica* accounting for 4.6-8.8% dissimilarity seen in other hosts. One Enterobacteriaceae OTU each was identified as significantly higher in *V. vulgaris* and *V. germanica*. The Enterobacteriaceae genus *Pantoea agglomerans* further attributed to the significant dissimilarity seen between *V. germanica* and other hosts with 4.3-6.9% of the dissimilarity attributed to one significantly dissimilar OTU. Overall compared with other hosts, SIMPER analysis revealed *V. vulgaris* and *V. germanica* had higher abundances of the class Bacilli and Gammaproteobacteria.

Interestingly, *B. terrestris* harboured the most distinctive community attributed to three taxa, the family Acetobacteraceae and genera *Wolbachia* and *Segniliparus*. The genus *Wolbachia* compared to all other hosts accounted for 26-30% of the dissimilarity, with five OTUs attributing to this significant difference. The genus *Segniliparus* only detected in *B. terrestris*, accounted for 3-5% of the dissimilarity with other hosts, attributed to one significant OTU. The family Acetobacteraceae accounted for 5-8% of the dissimilarity seen with other hosts, with one OTU significantly different.

Table 3-5 SIMPER analysis comparing *V. vulgaris* and *V. germanica*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f = family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. vulgaris</i>	Mean Abundance <i>V. germanica</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Mollicutes	<i>Spiroplasma</i>	1.43	15.27	6.92	4.59	16.95	16.95
Bacilli	<i>Lactobacillus</i>	6.33	0.00	3.16	0.70	7.75	24.69
Alphaproteobacteria	<i>Wolbachia</i>	4.36	2.93	3.16	0.76	7.73	32.42
Bacilli	<i>Fructobacillus</i>	3.55	4.53	1.71	3.32	4.20	36.62
Gammaproteobacteria	Enterobacteriaceae (f)	4.50	5.28	1.43	1.40	3.50	40.12
Gammaproteobacteria	<i>Pantoea</i>	1.66	4.51	1.43	2.47	3.49	43.61
Gammaproteobacteria	<i>Pseudomonas</i>	3.51	1.45	1.13	1.44	2.76	46.37
Bacilli	<i>Enterococcus</i>	2.31	1.58	1.10	1.16	2.70	49.07
Gammaproteobacteria	<i>Providencia</i>	1.93	0.00	0.96	0.75	2.36	51.43
Gammaproteobacteria	<i>Serratia</i>	2.05	1.28	0.91	1.32	2.22	53.65

Table 3-6 SIMPER analysis comparing *V. vulgaris* and *A. mellifera*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f = family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. vulgaris</i>	Mean Abundance <i>A. mellifera</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Alphaproteobacteria	<i>Wolbachia</i>	4.36	2.73	3.09	0.77	7.43	7.43
Bacilli	<i>Lactobacillus</i>	6.33	0.98	2.94	0.71	7.08	14.51
Mollicutes	<i>Spiroplasma</i>	1.43	6.38	2.88	1.54	6.94	21.44
Gammaproteobacteria	Enterobacteriaceae (f)	4.50	1.27	2.04	1.54	4.90	26.34
Bacilli	<i>Fructobacillus</i>	3.55	4.34	1.68	3.22	4.05	30.39
Phycisphaerae	Tepidisphaeraceae (f)	4.17	7.29	1.56	1.73	3.76	34.15
Spartobacteria	DA101 soil group (f)	5.40	8.04	1.39	1.39	3.34	37.49
Bacilli	<i>Enterococcus</i>	2.31	1.52	1.25	1.20	3.01	40.50
Gammaproteobacteria	<i>Providencia</i>	1.93	0.84	0.99	1.01	2.38	42.88
Gammaproteobacteria	<i>Gilliamella</i>	1.50	1.29	0.98	0.90	2.35	45.23
Gammaproteobacteria	<i>Serratia</i>	2.05	0.63	0.97	1.08	2.32	47.55
Gammaproteobacteria	<i>Pseudomonas</i>	3.51	2.45	0.85	1.47	2.05	49.60
Gammaproteobacteria	<i>Enterobacter</i>	1.58	0.00	0.79	0.94	1.90	51.49

Table 3-7 SIMPER analysis comparing *V. vulgaris* and *B. terrestris*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f = family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. vulgaris</i>	Mean Abundance <i>B. terrestris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Alphaproteobacteria	<i>Wolbachia</i>	4.36	25.26	10.66	2.32	21.97	21.97
Bacilli	<i>Lactobacillus</i>	6.33	0.00	3.16	0.74	6.52	28.49
Alphaproteobacteria	Acetobacteraceae (f)	0.84	5.68	2.49	2.08	5.13	33.62
Gammaproteobacteria	Enterobacteriaceae (f)	4.50	0.00	2.25	1.32	4.63	38.25
Actinobacteria	<i>Segniliparus</i>	0.00	3.54	1.77	7.63	3.65	41.9
Bacilli	<i>Fructobacillus</i>	3.55	0.47	1.67	1.01	3.45	45.35
Bacilli	<i>Enterococcus</i>	2.31	0.25	1.11	0.83	2.29	47.64
Betaproteobacteria	<i>Delftia</i>	0.68	2.79	1.05	2.34	2.17	49.81
Gammaproteobacteria	<i>Serratia</i>	2.05	0.00	1.02	0.96	2.11	51.92

Table 3-8 SIMPER analysis comparing *V. vulgaris* and *L. humile*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f = family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. vulgaris</i>	Mean Abundance <i>L. humile</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Bacilli	<i>Lactobacillus</i>	6.33	11.24	4.63	2.49	10.37	10.37
Gammaproteobacteria	Enterobacteriaceae (f)	4.50	0.00	2.25	1.30	5.03	15.40
Alphaproteobacteria	<i>Wolbachia</i>	4.36	0.00	2.18	0.43	4.88	20.28
Spartobacteria	DA101 soil group (f)	5.40	9.03	1.81	2.17	4.06	24.34
Bacilli	<i>Fructobacillus</i>	3.55	0.58	1.62	0.97	3.63	27.97
Phycisphaerae	Tepidisphaeraceae (f)	4.17	7.30	1.57	1.90	3.50	31.48
Bacilli	<i>Enterococcus</i>	2.31	0.62	1.10	0.88	2.46	33.94
Alphaproteobacteria	<i>Variibacter</i>	3.07	5.23	1.08	2.10	2.42	36.36
Gammaproteobacteria	<i>Serratia</i>	2.05	0.00	1.02	0.95	2.29	40.97
Gammaproteobacteria	<i>Providencia</i>	1.93	0.00	0.96	0.78	2.16	43.13
Bacilli	<i>Lactococcus</i>	1.76	0.00	0.88	1.24	1.97	45.10
Alphaproteobacteria	<i>Nitrobacter</i>	2.49	4.10	0.80	3.06	1.80	46.90
Gammaproteobacteria	<i>Pseudomonas</i>	3.51	3.74	0.80	1.48	1.79	48.69
Gammaproteobacteria	<i>Enterobacter</i>	1.58	0.00	0.79	0.94	1.77	50.45

Table 3-9 SIMPER analysis comparing *V. germanica* and *A. mellifera*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f = family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. germanica</i>	Mean Abundance <i>A. mellifera</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Mollicutes	<i>Spiroplasma</i>	15.27	6.38	4.44	1.68	19.53	19.53
Gammaproteobacteria	Enterobacteriaceae (f)	5.28	1.27	2.01	2.24	8.82	28.35
Gammaproteobacteria	<i>Pantoea</i>	4.51	1.35	1.58	1.65	6.94	35.29
Phycisphaerae	Tepidisphaeraceae (f)	5.51	7.29	0.89	1.22	3.92	39.21
Alphaproteobacteria	Rhodobiaceae (f)	0.00	1.67	0.83	1.71	3.66	42.87
Bacilli	<i>Enterococcus</i>	1.58	1.52	0.76	17.12	3.34	46.21
Gammaproteobacteria	<i>Enterobacter</i>	1.45	0.00	0.73	0.32	3.20	49.40
Spartobacteria	DA101 soil group (f)	6.69	8.04	0.70	0.73	3.06	52.47

Table 3-10 SIMPER analysis comparing *V. germanica* and *B. terrestris*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f=family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. germanica</i>	Mean Abundance <i>B. terrestris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Alphaproteobacteria	<i>Wolbachia</i>	2.93	25.26	11.17	11.43	26.38	26.38
Mollicutes	<i>Spiroplasma</i>	15.27	1.31	6.98	51.83	16.48	42.86
Alphaproteobacteria	Acetobacteraceae (f)	0.00	5.68	2.84	2.55	6.71	49.57
Gammaproteobacteria	Enterobacteriaceae (f)	5.28	0.00	2.64	6.30	6.24	55.81

Table 3-11 SIMPER analysis comparing *A. mellifera* and *B. terrestris*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f=family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>A. mellifera</i>	Mean Abundance <i>B. terrestris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Alphaproteobacteria	<i>Wolbachia</i>	2.73	25.26	11.27	12.55	29.93	29.93
Alphaproteobacteria	Acetobacteraceae (f)	0.00	5.68	2.84	2.85	7.54	37.47
Mollicutes	<i>Spiroplasma</i>	6.38	1.31	2.53	1.23	6.73	44.20
Bacilli	<i>Fructobacillus</i>	4.34	0.47	1.94	5.19	5.14	49.35
Actinobacteria	<i>Segniliparus</i>	0.00	3.54	1.77	7.16	4.70	54.05

Table 3-12 SIMPER analysis comparing *V. germanica* and *L. humile*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f=family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>V. germanica</i>	Mean Abundance <i>L. humile</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Mollicutes	<i>Spiroplasma</i>	15.27	0.00	7.63	8.23	19.22	19.22
Bacilli	<i>Lactobacillus</i>	0.00	11.24	5.62	3.35	14.15	33.37
Gammaproteobacteria	Enterobacteriaceae (f)	5.28	0.00	2.64	6.25	6.65	40.01
Bacilli	<i>Fructobacillus</i>	4.53	0.58	1.97	4.79	4.97	44.98
Gammaproteobacteria	<i>Pantoea</i>	4.51	1.07	1.72	2.27	4.32	49.30
Alphaproteobacteria	<i>Wolbachia</i>	2.93	0.00	1.46	1.20	3.69	52.99

Table 3-13 SIMPER analysis comparing *A. mellifera* and *L. humile*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f=family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>A. mellifera</i>	Mean Abundance <i>L. humile</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Bacilli	<i>Lactobacillus</i>	0.98	11.24	5.13	3.46	18.54	18.54
Mollicutes	<i>Spiroplasma</i>	6.38	0.00	3.19	1.48	11.53	30.08
Bacilli	<i>Fructobacillus</i>	4.34	0.58	1.88	5.40	6.80	36.87
Alphaproteobacteria	<i>Wolbachia</i>	2.73	0.00	1.36	6.32	4.93	41.80
Bacilli	<i>Lactococcus</i>	1.62	0.00	0.81	2.18	2.94	48.48
Bacilli	<i>Enterococcus</i>	1.52	0.62	0.76	1.21	2.74	51.22

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Table 3-14 SIMPER analysis comparing *B. terrestris* and *L. humile*. Results representing class and lowest taxonomy of prokaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f=family). Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Class	Taxa	Mean Abundance <i>B. terrestris</i>	Mean Abundance <i>L. humile</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
Alphaproteobacteria	<i>Wolbachia</i>	25.26	0.00	12.63	14.45	30.24	30.24
Bacilli	<i>Lactobacillus</i>	0.00	11.24	5.62	4.32	13.45	43.69
Alphaproteobacteria	Acetobacteraceae (f)	5.68	0.00	2.84	2.85	6.80	50.49

Table 3-15 Results of G-tests group significance (log likelihood). Prokaryote taxa identified as significantly different between hymenopteran host communities. Mean abundance of each significant OTU taxa determined at the class level and lowest taxonomic level. Where the genus is not defined, the next closest taxonomic level was assigned (denoted f=family). The test statistic in addition to the Bonferroni corrected p value is provided showing significance among hymenopteran hosts.

Class	Taxon	<i>B. terrestris</i> mean	<i>L. humile</i> mean	<i>A. mellifera</i> mean	<i>V. vulgaris</i> mean	<i>V. germanica</i> mean	Test Statistic	Bonferroni corrected p value
Alphaproteobacteria	Acetobacteraceae (f)	13.70	0.00	0.00	1.60	0.00	36.63	0.00002
Mollicutes	<i>Spiroplasma</i>	1.90	0.00	7.90	2.10	17.60	33.29	0.00011
Mollicutes	<i>Spiroplasma</i>	2.20	0.00	7.60	1.60	17.20	32.91	0.00013
Actinobacteria	<i>Segniliparus</i>	11.00	0.00	0.00	0.00	0.00	32.50	0.00016
Bacilli	<i>Lactobacillus</i>	0.00	13.00	1.60	0.50	0.00	32.20	0.00018
Mollicutes	<i>Spiroplasma</i>	0.00	0.00	4.00	0.90	13.40	31.27	0.00028
Alphaproteobacteria	<i>Wolbachia</i>	13.50	0.00	1.60	2.20	0.00	30.57	0.00039
Bacilli	<i>Lactobacillus</i>	0.00	13.60	1.60	4.20	0.00	30.48	0.00041
Alphaproteobacteria	<i>Wolbachia</i>	13.50	0.00	0.00	2.30	3.10	29.38	0.00069
Alphaproteobacteria	<i>Wolbachia</i>	18.30	0.00	6.40	3.10	6.10	28.63	0.00098
Gammaproteobacteria	Enterobacteriaceae (f)	0.00	0.00	4.00	3.90	13.60	28.61	0.00098
Alphaproteobacteria	<i>Wolbachia</i>	10.30	0.00	0.00	1.70	0.00	26.56	0.00257
Alphaproteobacteria	<i>Wolbachia</i>	8.90	0.00	0.00	1.50	0.00	22.60	0.01597
Gammaproteobacteria	<i>Pantoea</i>	0.00	2.90	4.30	6.20	14.30	21.72	0.02392
Gammaproteobacteria	Enterobacteriaceae (f)	0.00	0.00	0.00	7.50	0.00	21.34	0.02850

3.5.3 Microbial Eukaryote Community Distinctions

As in Chapter 1, the microbial eukaryote community profile was sparse in comparison to the prokaryote community. The mean richness of taxa was highest in *A. mellifera* (mean 12.5 ± 4.49), *L. humile* (12.5 ± 1.22), *V. germanica* (11 ± 0) *B. terrestris* (8.3 ± 1.85) and lowest in *V. vulgaris* (7.8 ± 2.17). Shannon diversity revealed *L. humile* (mean 2.3 ± 0.21), *V. germanica* (2.27 ± 0) and *A. mellifera* (2.25 ± 0.32) had comparable mean diversity in comparison to the lower observed microbial eukaryote diversity in *V. vulgaris* (1.9 ± 0.27) and *B. terrestris* (1.9 ± 0.22). An ANOVA revealed no significant difference in richness and diversity between the number of OTUs detected in each host community ($p < 0.05$) (**Figure 3-6D**).

The group average cluster dendrogram illustrated clustering of host-microbial eukaryote communities. There was a high degree of dissimilarity found within each host sample community, potentially due to the sparse microbial eukaryote abundance (**Figure 3-6A**). In *V. vulgaris* samples alone there was up to 80 % dissimilarity observed between samples. However there was dissimilarity between hymenopteran host communities, with greater than 90% dissimilarity between *V. vulgaris* and the other host microbial eukaryote communities. *B. terrestris* communities clustered with *A. mellifera* although there was up to 80% dissimilarity seen between hosts. Microbial eukaryote communities in *L. humile* and *V. germanica* clustered together although with 90% dissimilarity observed.

Host microbial eukaryote community clustering seen in the group average cluster dendrogram, was further confirmed with the nMDS plot (**Figure 3-6B**). The nMDS showed a loose cluster of host sample communities although again demonstrating a degree of separation between host communities. Microbial eukaryote communities observed in *B. terrestris* clustered closest together, indicating a similarly shared community. Weak clustering was observed in *V. vulgaris*, *A. mellifera* and *L. humile* which potentially indicating higher variation seen among host samples. A CAP analysis was performed, illustrating microbial community distinction found between host sample communities (**Figure 3-6C**). The CAP analysis identified that each host's sample communities clustered together, in addition to host communities clustering separately from one another indicating distinction between host microbial eukaryote communities. CAP analysis revealed *A. mellifera* *B. terrestris* and *V. germanica* communities, although distinct,

clustered near one another, in comparison *V. vulgaris* and *L. humile* microbial eukaryotes clustered further away from other hymenopteran hosts. This clustering of microbial eukaryote communities was owing to the overall mean abundances of five taxonomic groups, fungi, Acari (mite), nematode, protozoan and trypanosome (**Figure 3-7**).

3.5.4 Characterisation of Microbial Eukaryote Host Communities

The iTOL phylogenetic tree illustrated similarities as well differences between hymenopteran host microbial eukaryote communities. Of the 69 microbial eukaryote taxa represented across all hymenopteran hosts, *V. vulgaris* hosted 30 taxa, *A. mellifera* 20 taxa, *L. humile* 17 taxa, *B. terrestris* 14 taxa, and *V. germanica* 12 taxa (**Figure 3-8**). Fungi were found present in all hymenopteran hosts, with *L. humile*, *B. terrestris* and *V. germanica* resulting in the highest mean abundances of fungi observed. Mites were present in both *A. mellifera* and *B. terrestris*; although higher mean abundances of mites were observed in *B. terrestris*. Nematode incidence was found in low abundances in *L. humile* and *B. terrestris*. Protozoan taxa were found present in all hymenopteran hosts except *V. germanica*, with highest incidences seen in *L. humile*. Trypanosome taxa were absent from both vespid wasps *V. vulgaris* and *V. germanica* but present in *B. terrestris* and *L. humile* and observed in high numbers in one the *A. mellifera* sample accounting for the high variability seen (**Figure 3-7**). SIMPER analyses **Table 3-16** to **Table 3-25** identified dominant taxa between each hymenopteran host community, and Log likelihood G-tests (**Table 3-26**) identified significantly abundant OTUs attributing to dominance in the microbial eukaryote community. SIMPER analysis revealed the hymenopteran host *B. terrestris* displayed most dissimilarity with all other hosts. This contrast in community dissimilarity was attributed to the community dominance of four taxa *Leptomonas*, *Aleurogylphus Ovatus*, *Metschnikowia* and *Candida* (**Table 3-17**, **Table 3-18**, **Table 3-21** and **Table 3-24**). These four taxa had significantly higher mean abundances in *B. terrestris* compared with the other hymenopteran hosts (**Table 3-26**). The genera *Leptomonas* and *Aleurogylphus* alone accounted for up to 40% of the community dissimilarity with other hosts communities. Dominance of microbial eukaryotes in the *A. mellifera* community was attributed to the fungal taxa *Kazachstania*, *Penicillioopsis*, *Hamigera* and *Kirkomyces* as well as the trypanosome *Crithidia* (**Table 3-16**, **Table 3-17**, **Table 3-19** and **Table 3-22**). *Crithidia* alone accounted for up to 10% of the dissimilarity seen in other hosts. Log-likelihood

G-tests confirmed *Kazachstania*, *Hamigera*, *Penicilliopsis* and *Crithidia* were significantly dominant in *A. mellifera* in comparison to other hymenopteran hosts (**Table 3-26**). The community dominance in *L. humile* was attributed to the taxa *Allomyces*, *Adinetida*, *Rhabditida* and *Blastocladales* in contrast to other hosts (**Table 3-16**, **Table 3-18** and **Table 3-23**). The fungus *Allomyces* alone accounted for up to 15% of the dissimilarity seen in other hosts. In the SIMPER analysis of *L. humile* and *V. vulgaris*, *L. humile* had greater abundances of *Acrospermum*, *Mecoptera*, *Taphrina* and *Pleosporales* (**Table 3-20**). Of these taxa, only two were significantly more abundant in *L. humile* in comparison to other hymenopteran hosts, *Allomyces* and *Acrospermum* (**Table 3-26**). The taxa *Capnodiales*, *Hamigera*, *Pleosporales*, *Exobasidiales* and *Meyerozyma*, were dominant in *V. germanica* (**Table 3-22**, **Table 2-23**, **Table 3-24** and **Table 3-25**) and significantly dissimilar to the other hymenopteran host's (**Table 3-26**). *Aspergillus* was detected in both *V. germanica* and *V. vulgaris*, but in considerably higher abundances in *V. germanica* (**Table 3-26**), where it accounted for 15% of the dissimilarity in the SIMPER analysis. There were no significantly abundant taxa attributed to *V. vulgaris*. However the SIMPER analysis revealed higher mean abundances of the fungal genera *Conidiobolus* and *Athelia* in *V. vulgaris* in comparison to *L. humile* and *A. mellifera* (**Table 3-19** and **Table 3-20**).

Figure 3-6 Figures of microbial eukaryote host community based on a Bray-Curtis dissimilarity (A-C), richness and diversity (D) in five hymenopteran species, *V. vulgaris* (VV) (green triangle; * = Belgian samples), *A. mellifera* (AM) (red diamond), and *B. terrestris* (BT) (blue diamond), *V. germanica* (VG) (cyan square) and *L. humile* (pink circle) (n = northern sample; s = southern sample). (A) Group average cluster dendrogram, illustrating dissimilarity clustering distances of individual microbial eukaryote communities. (B) Non-metric multi-dimensional (nMDS) scaling plot illustrating dissimilarity distances of individual microbial eukaryote communities. Stress value: $0.07 \leq 0.1$ is acceptable). (C) Canonical analysis of principal coordinates (CAP) illustrating individual microbial eukaryote community distinctness. (D) Mean richness and Shannon's diversity metrics of microbial eukaryote communities, including the standard error. Note *V. germanica* $n=1$, therefore, there is no mean result or standard error. The colour of each bar corresponds to each hymenopteran species.

Figure 3-7 Figures of mean abundances of microbial eukaryote OTUs (log transformed) found in each host species. Mean abundance of organism group is denoted by the point, with error bars illustrating variance in host samples for each organism group. Organism groups present in each hymenopteran host sample indicated above by hymenopteran host name. Note the host *V. germanica* is represented by one sample (no error bars). In this one sample only fungi was detected.

Figure 3-8 iTol phylogenetic tree of 18S rRNA high abundant OTUs (>100 reads). Phylogeny of microbial eukaryote taxa identified from five hymenopteran species to the genus level where possible. This rooted maximum likelihood phylogeny reveals each host's microbial eukaryote community presence, absence and specificity of taxa. Colours within the inner circle illustrate the phylum that the bacterial taxa are in, based on the OTUs assigned in SILVA (see Phylum legend). The first three outer concentric circles from the inside out displays the bacteria found in each hymenopteran host (*V. vulgaris* = green, *A. mellifera* = red, *B. terrestris* = blue, *V. germanica* = cyan and *L. humile* = pink). Where taxonomy was not assigned to the genus level, the lowest taxonomic level was assigned and denoted in brackets, i.e. (f) = family, (o) = order, (c) = class and (p) = phylum. Host colours listed in legend correspond to colours used in subsequent figures.

Table 3-16 SIMPER analysis comparing *A. mellifera* and *L. humile*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>A .mellifera</i>	Mean Abundance <i>L. humile</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Allomyces</i>	0.00	3.25	11.07	0.99	11.07	11.07
<i>Kazachstania</i>	2.09	0.00	7.76	1.87	7.76	18.83
<i>Crithidia</i>	3.18	0.00	7.45	0.87	7.45	26.28
<i>Penicillliopsis</i>	1.35	0.00	4.48	5.23	4.48	30.76
<i>Blastocladales</i>	0.00	1.15	3.88	0.79	3.88	34.64
<i>Adinetida</i>	0.00	0.97	3.28	0.79	3.28	37.92
<i>Hamigera</i>	0.69	0.00	3.27	0.86	3.27	41.19
<i>Kirkomyces</i>	0.55	0.00	2.59	0.86	2.59	43.78
<i>Candida</i>	1.10	0.00	2.57	0.87	2.57	46.35
<i>Rhabditida</i>	0.00	0.69	2.56	0.78	2.56	48.91

Table 3-17 SIMPER analysis comparing *A. mellifera* and *B. terrestris*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>A. mellifera</i>	Mean Abundance <i>B. terrestris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Leptomonas</i>	0.55	6.67	15.67	2.65	18.87	18.87
<i>Aleuroglyphus</i>	0.35	5.65	13.69	2.33	16.49	35.36
<i>Crithidia</i>	3.18	0.00	5.97	0.91	7.19	42.55
<i>Metschnikowia</i>	0.69	2.78	5.58	1.62	6.72	49.27
<i>Candida</i>	1.10	2.84	5.43	1.05	6.55	55.82

Table 3-18 SIMPER analysis comparing *L. humile* and *B. terrestris*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>L. humile</i>	Mean Abundance <i>B. terrestris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Leptomonas</i>	0.00	6.67	16.76	13.88	17.08	17.08
<i>Aleuroglyphus</i>	0.00	5.65	14.38	4.36	14.66	31.74
<i>Allomyces</i>	3.25	0.00	8.02	1.16	8.17	39.91
<i>Candida</i>	0.00	2.84	7.10	1.94	7.24	47.15
<i>Metschnikowia</i>	0.00	2.78	6.91	7.41	7.05	54.20

Table 3-19 SIMPER analysis comparing *A. mellifera* and *V. vulgaris*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>A. mellifera</i>	Mean Abundance <i>V. vulgaris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Kazachstania</i>	2.09	0.00	11.90	1.45	12.38	12.38
<i>Crithidia</i>	3.18	0.00	9.07	0.94	9.44	21.81
<i>Penicillioptosis</i>	1.35	0.18	5.80	1.60	6.03	27.84
<i>Hamigera</i>	0.69	0.00	5.40	0.88	5.62	33.46
<i>Kirkomyces</i>	0.55	0.00	4.28	0.88	4.45	37.92
<i>Conidiobolus</i>	0.00	0.88	4.06	0.95	4.22	42.14
<i>Meyerozyma</i>	0.90	0.23	3.49	0.76	3.63	45.77
<i>Athelia</i>	0.00	0.66	3.45	1.03	3.59	49.36
<i>Candida</i>	1.10	0.00	3.13	0.94	3.26	52.62

Table 3-20 SIMPER analysis comparing *L. humile* and *V. vulgaris*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>L. humile</i>	Mean Abundance <i>V. vulgaris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Allomyces</i>	3.25	0.00	14.73	1.20	15.03	15.03
<i>Blastocladales</i>	1.15	0.00	5.15	0.93	5.26	20.29
<i>Adinetida</i>	0.97	0.00	4.35	0.93	4.44	24.74
<i>Conidiobolus</i>	0.00	0.88	3.73	1.11	3.81	28.54
<i>Neuroptera</i>	0.90	0.12	3.61	2.05	3.68	32.23
<i>Rhabditida</i>	0.69	0.00	3.47	0.92	3.55	35.77
<i>Pleosporales</i>	0.69	0.18	3.45	0.94	3.52	39.30
<i>Acrospermum</i>	0.69	0.00	3.29	4.82	3.36	42.65
<i>Mecoptera</i>	0.69	0.00	3.29	4.82	3.36	46.01
<i>Athelia</i>	0.00	0.66	3.13	1.30	3.19	49.20
<i>Taphrina</i>	0.55	0.00	2.75	0.92	2.81	52.01

Table 3-21 SIMPER analysis comparing *B. terrestris* and *V. vulgaris*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>B. terrestris</i>	Mean Abundance <i>V. vulgaris</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Leptomonas</i>	6.67	0.00	20.83	6.31	21.35	21.35
<i>Aleuroglyphus</i>	5.65	0.00	17.94	3.67	18.39	39.74
<i>Candida</i>	2.84	0.00	8.81	1.99	9.03	48.77
<i>Metschnikowia</i>	2.78	0.30	7.79	3.21	7.98	56.76

Table 3-22 SIMPER analysis comparing *A. mellifera* and *V. germanica*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>A. mellifera</i>	Mean Abundance <i>V. germanica</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Aspergillus</i>	0.00	3.26	11.17	2.15	12.60	12.60
<i>Kazachstania</i>	2.09	0.00	7.52	1.56	8.49	21.09
<i>Crithidia</i>	3.18	0.00	7.32	0.71	8.26	29.35
<i>Capnodiales</i>	0.00	2.08	7.13	2.15	8.04	37.40
<i>Pleosporales</i>	0.00	1.39	4.75	2.15	5.36	42.76
<i>Penicillioptis</i>	1.35	0.00	4.35	4.73	4.91	47.67
<i>Candida</i>	1.10	1.10	3.77	2.15	4.25	51.92

Table 3-23 SIMPER analysis comparing *L. humile* and *V. germanica*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>L. humile</i>	Mean Abundance <i>V. germanica</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Aspergillus</i>	0.00	3.26	10.94	18.26	12.30	12.30
<i>Allomyces</i>	3.25	0.00	10.57	0.92	11.89	24.19
<i>Capnodiales</i>	0.55	2.08	5.06	2.18	5.70	29.88
<i>Hamigera</i>	0.00	1.39	4.65	18.26	5.23	35.12
<i>Blastocladales</i>	1.15	0.00	3.71	0.71	4.18	39.29
<i>Candida</i>	0.00	1.10	3.69	18.26	4.15	43.44
<i>Exobasidiales</i>	0.00	1.10	3.69	18.26	4.15	47.59
<i>Meyerozyma</i>	0.00	1.10	3.69	18.26	4.15	51.74

Table 3-24 SIMPER analysis comparing *B. terrestris* and *V. germanica*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>B. terrestris</i>	Mean Abundance <i>V. germanica</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Leptomonas</i>	6.67	0.00	16.45	14.20	18.03	18.03
<i>Aleuroglyphus</i>	5.65	0.00	14.11	3.97	15.47	33.5
<i>Aspergillus</i>	0.23	3.26	7.43	17.80	8.15	41.65
<i>Metschnikowia</i>	2.78	0.00	6.78	6.74	7.44	49.08
<i>Acarus</i>	2.3	0.00	5.68	14.69	6.23	55.31

Table 3-25 SIMPER analysis comparing *V. vulgaris* and *V. germanica*. Results representing class and lowest taxonomy of microbial eukaryote mean abundances. Taxa presented in table contribute approximately 50% of the dissimilarity between the hymenopteran hosts. Mean abundance of each taxon is presented in each host and the mean taxa dissimilarity between hosts. Dissimilarity standard deviation shows variance of means. Dissimilarity (%) and cumulative dissimilarity (%) represents dominant taxa contributing to the dissimilarity between host communities.

Taxa	Mean Abundance <i>V. vulgaris</i>	Mean Abundance <i>V. germanica</i>	Mean Dissimilarity	Dissimilarity Standard Deviation	% Dissimilarity Contribution	% Cumulative Contribution
<i>Aspergillus</i>	0.18	3.26	14.29	3.46	14.96	14.96
<i>Capnodiales</i>	0.00	2.08	9.50	5.00	9.95	24.91
<i>Hamigera</i>	0.00	1.39	6.33	5.00	6.63	31.54
<i>Pleosporales</i>	0.18	1.39	5.73	2.28	6.00	37.54
<i>Candida</i>	0.00	1.10	5.02	5.00	5.26	42.80
<i>Exobasidiales</i>	0.00	1.10	5.02	5.00	5.26	48.05
<i>Chaetothyriales</i>	0.00	1.10	5.02	5.00	5.26	53.31

Table 3-26 Results of G-tests group significance (log likelihood). Microbial eukaryote taxa identified as significantly different between hymenopteran host communities. Mean (log transformed) abundance of each significant OTU taxa defined at the class level and lowest taxonomic level. The Bonferroni corrected *p* value is provided showing significance among hymenopteran hosts.

Significant Taxa	<i>B. terrestris</i> Mean Abundance	<i>L. humile</i> Mean Abundance	<i>A. mellifera</i> Mean Abundance	<i>V. vulgaris</i> Mean Abundance	<i>V. germanica</i> Mean Abundance	Bonferroni Corrected <i>p</i> -value
<i>Leptomonas</i>	15.34	0.00	3.33	0.00	0.00	2.66E-06
<i>Aleuroglyphus</i>	13.86	0.00	2.84	0.00	0.00	1.85E-05
<i>Allomyces</i>	0.00	11.41	0.00	0.00	0.00	6.22E-05
<i>Kazachstania</i>	5.32	0.00	9.47	0.00	0.00	0.0018
<i>Aspergillus</i>	2.33	0.00	0.00	1.12	11.03	0.0044
<i>Candida</i>	9.65	0.00	4.32	0.00	7.39	0.0101
<i>Acarus</i>	8.89	0.00	2.84	0.00	0.00	0.0103
<i>Hamigera</i>	0.00	0.00	4.62	0.00	7.97	0.0136
<i>Crithidia</i>	0.00	0.00	7.41	0.00	0.00	0.0235
<i>Exobasidiales</i>	0.00	0.00	0.00	0.00	7.39	0.0242
<i>Chaetothyriales</i>	0.00	0.00	0.00	0.00	7.39	0.0242
<i>Penicillioptis</i>	0.00	0.00	8.15	1.25	0.00	0.0274
<i>Acrosporum</i>	0.00	7.23	0.00	0.00	0.00	0.0305
<i>Metschnikowia</i>	9.63	0.00	3.62	2.21	0.00	0.0338
<i>Capnodiales</i>	1.85	3.90	0.00	0.00	9.19	0.0440

3.5.5 Virus Results

MEGAN and Virusfinder2 analyses of 14 RNA samples revealed a total of 16 viruses (**Table 3-27**). Total viral count data using both MEGAN and Virusfinder2 can be accessed and observed in the Supplementary section (Supplementary Table 2). Of the viruses identified, four were assigned by both analyses, *Rhopalosiphum padi virus* (*RhPV*), *Kashmir bee virus* (*KBV*), *Black Queen Cell Virus* (*BQCV*), and *Prunus Necrotic Ringspot Virus* (*PNRV*). Overall a higher abundance of viral phylotypes was found in *A. mellifera* (7). Five virus phylotypes were identified in *V. vulgaris*, *V. germanica* and *B. terrestris* viral communities, while *L. humile* hosted four viral phylotypes. Previous analyses (Chapter 2), found *RhPV* present only in European *V. vulgaris* samples and *KBV* in New Zealand samples, however here all *V. vulgaris* samples are treated as one composite sample. Interestingly *RhPV* and *KBV* were also found in *L. humile* samples. Positive sequence matches for *DWV* were discovered using Virusfinder2 and found only in *A. mellifera* and *V. germanica* samples, with no positives in the MEGAN analysis (**Table 3-27**). The viral bacteriophage *Enterobacteria phage DE3* was identified only in *B. terrestris* samples. The MEGAN analysis also revealed positive viral matches not seen in Virusfinder2. The virus *ALPV* and *Enterobacteria phage VK* were detected in *V. vulgaris* viral communities, while *Kakugo Virus* was found in *V. vulgaris*, *A. mellifera* and *V. germanica*. The viral phylotype *Arabis mosaic virus* was found present in *A. mellifera*, *B. terrestris* and *V. germanica*. There were also incidences of virus species unique to one host. *Linepithema humile Virus 1* was found in *L. humile*, *Raphanus latent virus* in *B. terrestris*, *Sacbrood virus* in *V. germanica* and *A. mellifera* harboured *Lake Sinai Virus 1* and *Filamentous Virus* (**Table 3-27**).

Table 3-27 Presence and absence of viral phylotypes found using MEGAN and Virusfinder2. Due to the differing results in the number of virus reads detected using each method; results are displayed as presence and absence of virus. A ✓ is indicated for both analysis where virus presence was identified and a – where no virus was detected. See **Appendix A-1 Supplementary Table 2** for viral counts using both methods.

	<i>Vespula vulgaris</i>		<i>Apis mellifera</i>		<i>Bombus terrestris</i>		<i>Vespula germanica</i>		<i>Linepithema humile</i>	
Virus	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2
<i>Rhopalosiphum padi virus (RhPV)</i>	✓	✓	-	-	-	-	-	-	✓	✓
<i>Kashmir bee virus (KBV)</i>	✓	✓	-	-	-	-	-	-	✓	-
<i>Deformed Wing Virus (DWV)</i>	-	-	-	✓	-	-	-	✓	-	-
<i>Aphid Lethal paralysis virus (ALPV)</i>	✓	-	-	-	-	-	-	-	-	-
<i>Black Queen Cell Virus (BQCV)</i>	-	-	✓	-	✓	✓	✓	-	✓	-
<i>Enterobacteria phage DE3</i>	-	-	-	-	-	✓	-	-	-	-
<i>Prunus Necrotic Ringspot Virus</i>	-	-	✓	✓	-	-	-	-	-	-
<i>Linepithema humile Virus 1</i>	-	-	-	-	-	-	-	-	✓	-
<i>Arabid Mosaic Virus</i>	-	-	✓	-	✓	-	✓	-	-	-
<i>Lake Sinai Virus 1</i>	-	-	✓	-	-	-	-	-	-	-
<i>Raphanus latent Virus</i>	-	-	-	-	✓	-	-	-	-	-
<i>Sacbrood Virus</i>	-	-	-	-	-	-	✓	-	-	-
<i>Enterobacteria phage VK</i>	✓	-	-	-	-	-	-	-	-	-
<i>Kakugo Virus</i>	✓	-	✓	-	-	-	✓	-	-	-
<i>A. mellifera Filamentous Virus</i>	-	-	✓	-	-	-	-	-	-	-

3.6 DISCUSSION

In this comparative microbiome study, the microbial community diversity was characterised, focusing on the prokaryote, microbial eukaryote and viral diversity seen in five hymenopteran hosts (*V. vulgaris*, *A. mellifera*, *B. terrestris*, *V. germanica* and *L. humile*). These hosts were introduced into the New Zealand environment both intentionally as species widely considered as beneficial pollinators (*A. mellifera* and *Bombus terrestris*) and accidentally (*V. vulgaris*, *V. germanica* and *L. humile*), causing ecological and economic harm. It was hypothesised that there would be greater microbiota diversity in generalist feeders, vespid wasps (*V. vulgaris* and *V. germanica*) and Argentine ants (*L. humile*) compared with honey bee and bumble bee pollinators *A. mellifera* and *B. terrestris*. While it was noted *V. vulgaris* had greater prokaryote OTU reads, overall, microbial richness and diversity was not significantly higher in omnivorous vespid wasp samples and *L. humile* compared with palynivores *A. mellifera* and *B. terrestris*. This increased diversity seen in vespid wasp may have been due to sample disparity between hosts, in addition to low sample numbers.

In similar comparative studies, diet as a controlling factor of microbiome diversity was found to be significant (K. E. Anderson et al., 2013; Yun et al., 2014). In the mammalian gut, diversity of bacteria increases according to host diet, from carnivores to omnivores to herbivores (Ley et al., 2008). In Insects, the richness of microbial species is reported to be highest in xylophagous insects, while bees and wasps harboured the least diverse bacterial communities (Colman et al., 2012). The capacity of insects to take on certain nutritional lifestyles is in association with microorganisms. This symbiosis promotes the acquisition and processing of food, providing the insect with supplementary nutrients (A E Douglas, 2009). For example, gut bacteria in termites and aphids produce compounds essential in the digestion of detritus, phloem, sap, wood, and xylem, allowing the host to assimilate nutrients (Engel et al., 2012; N. A. Moran, McCutcheon, & Nakabachi, 2008; Tartar et al., 2009). The bean bug, *Riptortus pedestris*, must acquire the symbiont *Burkholderia* orally, if unsuccessful in acquisition it can display stunted growth (Y. Kikuchi & Yumoto, 2013).

On average this study reported 35.2 OTUs per sample (mean 35.2 SE $\pm$ 0.17), which is high in comparison to another study reporting low bacterial diversity, with fewer than eight OTU

phylotypes in each insect sample (Jones et al., 2013). However, another study reported 84.3 OTUs per sample (Yun et al., 2014). The predominant phyla adding to the diversity across host hymenopterans examined, were Proteobacteria (39.9%) Verrucomicrobia (13.5%) and Firmicutes (12.5%), accounting for 65.9% of the total sequences aligned. In similar studies, Proteobacteria and Firmicutes were also predominant making up 85% of the cross taxon community (Yun et al., 2014). Another study sampling 81 insect gut samples, comprised 29.94% Proteobacteria and 56.98% Firmicutes of sequences (Colman et al., 2012) highlighting variation in community composition between studies. However, this study was not based on insect guts and instead on whole insects accounting for the possible contamination of soil bacteria as observed with the phyla Verrucomicrobia.

3.6.1 Similarities in Host Species

While no significant dissimilarity was found among host microbial communities, these comparative analyses revealed shared microbiota among hosts as well as observed differences. All hymenopteran hosts in this study shared similar relative abundances of nineteen prokaryote taxa from six phyla indicating that among hosts there is a shared or possible core microbiota. The dominance of these six phyla across five hymenopteran species, suggests these phyla comprise ecological attributes allowing them to reside in the host (Jones et al., 2013). Proteobacteria were the most prevalent shared phyla across all hosts with seven genera belonging to the order Rhizobiales and the Gammaproteobacteria genus *Pseudomonas*. Also common taxa seen across hosts included Acidobacteria, Actinobacteria (*Acidothermus* and *Actinoplanes*), Ktedonobacteria and the family Tepidisphaeraceae. These trends in shared microbiota have also been observed in other studies of insect-associated microbial communities and infer mutualistic importance in the evolution of insects (Jones et al., 2013; N. A. Moran et al., 2012).

The shared abundance of nitrogen-fixing bacteria, suggests importance in hymenopteran hosts as seen in other insects, such as termites, stag beetles and fruit flies (Behar et al., 2005; Breznak, Brill, Mertins, & Coppel, 1973; Kuranouchi et al., 2006; Ohkuma, Noda, & Kudo, 1999; Potrikus & Breznak, 1977). Nitrogen-fixing bacteria are suggested to enable insects to subsist on low nitrogen diets (Nardi, Mackie, & Dawson, 2002). However, the nutritional significance of nitrogen-fixing bacteria in insects remains uncertain (A E Douglas, 2009). In all hymenopteran hosts Lactic Acid Bacteria (LAB) were also prevalent,

with each sample hosting three or more genera. However nine LAB genera were found in *V. vulgaris*. The genera *Fructobacillus* and *Enterococcus* were detected in all samples. The genus *Lactobacillus* was not found in *B. terrestris* or *V. germanica* but found in relatively high abundance in *L. humile*. This high abundance of *Lactobacillus* is in agreement with a recent publication reporting on bacterial diversity along *L. humile* invasion pathway to New Zealand (Lester, Sébastien, Suarez, Barbieri, & Gruber, 2017). However, here I found no presence of *Gluconobacter*, *Ralstonia* or *Achromobacter* in *L. humile* as found in Lester et al. (2017b). Lactic Acid Bacteria are mutualistic in *A. mellifera*, with *Enterococcus* and *Lactobacillus* inferring pathogen defence (Forsgren et al., 2010; Vásquez et al., 2012). Acetic Acid Bacteria found in *V. vulgaris*, *B. terrestris* and *A. mellifera* are suggested to be involved in the regulation of innate immune system homeostasis in *Drosophila* (Roh et al., 2008). A study demonstrated, colonies of *A. mellifera* infested with high loads of *Varroa* also have a higher relative abundance of several taxa including *Commensalibacter* indicating a change in the microbial community as a result of an invading eukaryote parasite. (Jan Hubert et al., 2016).

The presence of *Candida* and *Metschnikowia* and *Kazachstania* predominantly in both *B. terrestris* and *A. mellifera* may indicate a shared function. Yeasts commonly associated with *A. mellifera*, stingless bees and solitary bees (Rosa et al., 2003), produce and release enzymes that enhance, protect and preserve pollen. They also have a putative role as producers of antimycotic substances that safeguard hives from disease (Gilliam, 1997; Rosa et al., 2003). *Bombus terrestris* may also benefit from the presence of yeast on flowers. In a symbiotic relationship, yeasts improve flower recognition and nutritional enhancement of nectar for pollinators while enhancing dispersal abilities through pollinators in addition to overwintering in *B. terrestris* (Brysch-Herzberg, 2004).

The metatranscriptomic analysis detected 16 viral phylotypes, with *A. mellifera* hosting seven viruses, identified in previous studies (Chen, Evans, & Feldlaufer, 2006; J. R. de Miranda, 2004; Joachim R. de Miranda et al., 2013; McMahon et al., 2015; Ravoet et al., 2014). The results demonstrate that RNA viruses are shared among hymenopteran populations. Consequently, as found in other studies, known honey bee viruses are not restricted to the honey bee, but may be prevalent in other hymenopterans (Dobelmann et al., 2017; Evison et al., 2012; Levitt et al., 2013; McMahon et al., 2015). Interestingly,

KBV and *RhPV* were only detected in *V. vulgaris* and *L. humile* samples. Although not identified in the *A. mellifera* samples, *KBV* infects many types of bees including *Apis mellifera* (Joachim R. de Miranda et al., 2013), infecting both brood and adult bees, causing death within days of exposure (Berenyi et al., 2006). A recent study, found a 100% *KBV* infection rate in six different nests of *V. vulgaris* (Dobelmann et al., 2017), indicating that this is a widespread virus in *V. vulgaris* communities. The most cosmopolitan virus in this study was *BQCV*, identified in *A. mellifera*, *B. terrestris*, *V. germanica* and *L. humile*. *BQCV* is known to be widespread in bee populations, generally exhibiting low titre levels (McMahon et al., 2015). Typically associated with aphids, *RhPV* (Moon, Domier, McCoppin, D'Arcy, & Jin, 1998) is another common intestinal Dicistrovirus. Using the plant vascular system as a passive reservoir, *RhPV* transmits horizontally between aphids (Gildow & D'Arcy, 1988, 1990), where they primarily infect aphid gut tissues (Bonning, 2009). Relatively little research has been done on the impact of *RhPV* infection on hosts outside of aphids. However, *RhPV* has been found to decrease longevity and fecundity of the aphid host (Ban, Ahmed, Ninkovic, Delp, & Glinwood, 2008; Gildow & D'Arcy, 1990). *Deformed wing virus* is another Dicistrovirus known to commonly infect *Apis mellifera* via the vector *Varroa* (Möckel et al., 2011). *RhPV* has also been documented in other bee species, including *B. terrestris*, indicating it has a broader host specificity than previously anticipated (Levitt et al., 2013) as seen in this study with *V. vulgaris* larvae (Chapter 1) and *V. germanica*.

It must be noted that there were instances where the two viral alignment tools used in this analysis, MEGAN and Virusfinder 2 conflicted in reporting presence and absence of some viral phylotypes. In addition to varying amounts of quantified virus reported (**Table 2-24**), there was a greater quantified viral presence using Virusfinder2 compared with the MEGAN analysis, likely to be attributed to the difference in analysis approaches. The MEGAN analysis is based on BLAST results of searches on GenBank databases using assembled contigs and a stricter lowest common ancestor algorithm (Huson et al., 2007), while Virusfinder2 uses unassembled paired-end reads which are aligned to the RINS (Bhaduri et al., 2012) database.

3.6.2 Distinctions in Host Specificity

In addition to many similarities seen among hymenopteran host microbial communities, the analysis also highlighted host-specificity patterns of dominant members of the microbiome community. This distinctness was initially revealed through CAP analysis and observed in both prokaryote and microbial eukaryote communities illustrating the variation in microbiota among hosts. This variation was attributed to a few significant taxa. Most notable was the contribution of significant taxa to the dissimilarity seen in *B. terrestris* compared to the other hymenopteran hosts. *Bombus terrestris* had a considerable abundance of the prokaryotes *Wolbachia*, *Segniliparus*, *Leptomonas* and the family Acetobacteraceae in addition to the microbial eukaryotes *Aleuroglyphus*, *Acarus* and *Metschnikowia*. Compared to *B. terrestris* the specificity in microbiota decreased in the other hymenopteran hosts. However, both *A. mellifera* and *V. germanica* had significantly higher abundances of *Spiroplasma*. The vespid wasps *V. vulgaris* and *V. germanica* had significant abundances of the genus *Pantoea* and *V. germanica* alone had significant abundances of the family Enterobacteriaceae, in addition to the fungal genera *Aspergillus* and *Capnoidiales*. Three microbial eukaryotes were highlighted as significant in *A. mellifera*, *Kazachstania*, *Penicillioptosis*, *Crithidia* and *L. humile* had significant amounts of *Lactobacillus*, *Allomyces*, and *Acrospermum*. Species specific microbiome community membership suggests these species are non-random members but relevant to host biology and evolution (Newton 2013). For example, herbivory in ants *Cephalotes* and *Procrystocerus*, is correlated with the presence of Rhizobiales, expanding the ability of the host to survive on a plant-based diet (Kautz et al., 2013; Russell et al., 2009).

Members of the genus *Wolbachia* infect many members of the Arthropoda (Hilgenboecker, Hammerstein, Schlattmann, Telschow, & Werren, 2008; J H Werren & Windsor, 2000). However, studies report varying measures of *Wolbachia* prevalence in members of Arthropoda 17 to 35%, (Yoshitomo Kikuchi & Fukatsu, 2003; J. H. Werren, Windsor, & Guo, 1995). In more recent studies up to 40% (Zug & Hammerstein, 2012) and 54.4% *Wolbachia* prevalence was reported (Yun et al., 2014). A previous study also confirmed the presence of *Wolbachia* in pollinators as found in this study (Evison et al., 2012). The absence of *Wolbachia* in the *L. humile* samples is also in agreement with other studies. *Wolbachia* infections in *L. humile* have been reported throughout their native Argentinian range,

however, *Wolbachia* has been documented in just one introduced population in Hawaii (Reuter, 2003; Tsutsui, Kauppinen, Oyafuso, & Grosberg, 2003). A recent study reported *Wolbachia* in addition to *Rickettsia*, as being lost in the *L. humile* invasion process from Argentina to New Zealand, further confirming the absence of *Wolbachia* in this study (Lester, Sébastien, et al., 2017). The endosymbionts *Wolbachia* and *Spiroplasma* are some of the most commonly found facultative bacteria in insects (Eleftherianos et al., 2013). *Wolbachia* is found as an intracellular symbiont and *Spiroplasma* are associated both endocellular and extracellularly (Ahn et al., 2012; Eleftherianos et al., 2013). In *Drosophila*, ladybird beetles and butterflies files, *Spiroplasma* has been known to cause female-biased sex ratios of their host insects caused by selective culling of male offspring at the embryogenesis stage (Haselkorn, 2010; Regassa, 2006). While *Spiroplasma* in *Drosophila hydei* has been implicated in the protection of the host against parasitoid wasps (Xie, Tiner, Vilchez, & Mateos, 2011; Xie, Vilchez, & Mateos, 2010). In some cases *Wolbachia* manipulates insect host reproductive systems by inducing parthenogenesis, male-killing, feminisation and most commonly, cytoplasmic incompatibility (Bourtzis, Pettigrew, & O'Neill, 2000; Evison et al., 2012; Merçot & Poinso, 2009; Saridaki & Bourtzis, 2010; John H. Werren, Baldo, & Clark, 2008). Strains of *Wolbachia* have also been associated with aiding pathogenic resistance in their hosts, ranging from antiviral immune effects to general parasitic resistance in insects (Eleftherianos et al., 2013; Glaser & Meola, 2010; Hedges, Brownlie, O'Neill, & Johnson, 2008; Jupatanakul, Sim, & Dimopoulos, 2014; Kambris et al., 2010; Osborne, Iturbe-Ormaetxe, Brownlie, O'Neill, & Johnson, 2012; Osborne, Leong, O'Neill, & Johnson, 2009). One study demonstrated the stimulation of an immune system response in fruit flies by *Wolbachia* inhibiting viral infection (Hedges et al., 2008).

In *B. terrestris* samples, a significant abundance of the genus *Segniliparus* was detected, a recently identified new genus of Actinobacteria (W. R. Butler, 2005). Recently *Segniliparus* has been identified in *Ixodes ricinus* ticks (Tveten & Sjøstad, 2011), however, this is the first report of the genus *Segniliparus* infecting *B. terrestris*. Although little is known of the function of *Segniliparus*, the Actinobacteria class have been found to defend insects against harmful microorganisms by producing antibiotics (Bode, 2009; Kaltenpoth, 2009;

Kaltenpoth & Engl, 2014; Salem, Florez, Gerardo, & Kaltenpoth, 2015; Seipke et al., 2012; Visser, Nobre, Currie, Aanen, & Poulsen, 2012).

The mite *Aleuroglyphus ovatus* was also identified in *B. terrestris* and *Acarus* mites in both *A. mellifera* and *B. terrestris*. Mites (Acari) are common parasites of social pollinators (Eickwort, 1990; Goka, Okabe, Yoneda, & Niwa, 2001; Michael Christopher Otterstatter & Whidden, 2004; H. H. Schwarz & Huck, 1997), the most commonly known to be the *Varroa* mite (*Varroa destructor*). This mite attacks honey bees, weakening the bee by sucking its fat bodies and in the process transmitting RNA viruses such as Deformed wing virus (DWV) to the host (Guzmán-Novoa et al., 2010). *Aleuroglyphus ovatus* is known to have a worldwide distribution, a common pest of various stored products (Xia, Luo, Zou, & Zhu, 2009; Xin, J.L., Shen, 1964), but little is known of its effects on social insects. The trypanosome *Leptomonas* identified in *B. terrestris* has previously been identified in honey bees (Dornhaus & Chittka, 1999) but is highly similar to *Crithidia mellificae* with substantial genetic divergence among isolates (Cornman et al., 2012; R. Schmid-Hempel & Tognazzo, 2010). Little is known about the function of *Leptomonas*, possibly due to its low pathogenicity (Ruiz-González & Brown, 2006), but similarly to these results, other studies have found it to be highly prevalent among workers and drones (Bailey & Ball, 1991; Morse, 1978). *Crithidia* was found in significant abundance in one *A. mellifera* sample and is linked with winter mortality of *A. mellifera* colonies along with the microsporidian parasite *Nosema ceranae* (Ravoet et al., 2013). The high relative abundance of *Allomyces*, a genus of chytrid fungus, in one *L. humile* sample is likely to be environmental as there is little evidence of *Allomyces* in ant studies or other insects and currently no functional information is known. The fungal genus *Aspergillus* identified in both *V. germanica* and *V. vulgaris* was found in significantly higher abundances. Although the species of *Aspergillus* is not known, pathogenic *Aspergillus flavus* has previously been isolated from vespid wasps. These isolates inoculated onto *V. germanica* and *V. vulgaris* larvae demonstrated natural pathogenicity, with most larvae never emerging post infection (Glare et al., 1996). This effect on larval emergence may indicate that *Aspergillus* is a common pathogen of *Vespula* wasps in New Zealand.

There were also incidences of viral specificity seen in hymenopteran samples. Presence of *Linepithema humile* Virus 1 (LHUV1) in *L. humile* confirms this recently identified virus as

L. humile specific (Gruber et al., 2017; Sébastien et al., 2015). In *V. vulgaris* ALPV was found, although it is also known to occur in *A. mellifera* (Chen et al., 2011; Joachim R. de Miranda et al., 2013; Runckel et al., 2011). The *Aapis mellifera filamentous virus* was also identified in *A. mellifera*, although to date it is not known if this virus is pathogenic as it has no physical symptoms (Joachim R. de Miranda et al., 2013).

3.6.3 Transmission

Research into the microbiome diversity in bees has investigated the social context of microbiome transmission. Microbiota assemblages both harmful or helpful can be transmitted vertically (mother to offspring) or horizontally (environment) (G. D. D. Hurst & Frost, 2015; Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007). Due to the pollinating nature of hosts in this study, with the exception of *L. humile*, horizontal transmission of microbiota may occur on shared resources such as flowers. Several studies have identified flowers as microbial transmission routes. One study identified the transfer of *Lactobacilli* from flowers to sweat bees (McFrederick et al., 2012). The trypanosomatid *C. bombi*, an intestinal parasite of bumble bees, in addition to RNA viruses have also been demonstrated to be horizontally transmitted from flowers to hymenopterans (Durrer & Schmid-Hempel, 1994; Singh et al., 2010). At the host level the frequency, population density and degree of interspecific contact in the environment may play a role in cross-species transmission (Levitt et al., 2013). Several plant viruses were also detected including *Prunus Necrotic Ringspot Virus*, *Arabidopsis Mosaic Virus*, and *Raphanus latent Virus*. Previous research on plant viruses indicates *A. mellifera* and possibly other pollinators play a role in the transmission of plant-borne viruses, by moving pollen from plant to plant (Bristow & Martin, 1999; Childress, 1987; Singh et al., 2010). This transfer of virus means pollinators may be reservoirs of plant viruses but may also become infected with a new virus introduced to the environment by another species, mediated by plant pollen (Singh, 2014). Infectious diseases can also arise through association with a host reservoir, where a change in the community through the arrival of a novel microbe may cause a latent opportunistic microbe to become pathogenic (McMahon et al., 2015).

The act of robbing food stores in some hymenopterans, such as *Vespula* wasps and bumble bees has the potential to make the transmission or vectoring of parasites between taxa relatively frequent (Evison et al., 2012). In one study the presence of infective *DWV*

particles in honey indicates the potential spillover of *DWV* from *A. mellifera* to *B. terrestris* and *Vespula* wasps (Singh et al., 2010). In this study *DWV* was seen in high abundance in *A. mellifera* and presence of *DWV* in *V. germanica* may indicate possible spillover. In addition to their varied diet, the interaction of hosts on flowers and hive robbing may explain the lack of specificity and variability seen in *V. vulgaris* samples. This lack of a characteristic microbiota in vespid wasps indicates their non-dependence on gut flora for nutrition due to their wide and varied diet, similar to cockroaches (Cruden & Markovetz, 1987; Evison et al., 2012; Reeson, Jankovic, Kasper, Rogers, & Austin, 2003). Microbial community variation may also be attributed to variation in habitat, with *Vespula* wasps typically foraging within a few hundred metres of their nest (Edwards 1980), which could promote the development of alternative bacterial communities via alternate diets (Evison et al., 2012). Variability in diet may also explain microbiota variability between *V. vulgaris* nests and ranges, as seen from the native to the invaded range.

In addition to the transmission of microbiota, the host system must be receptive. For example, the insect immune system, through the production of antimicrobial peptides and reactive oxygen species may directly control introduced microbiota such as pathogens (Vallet-Gely, Lemaitre, & Boccard, 2008). The variety in gut morphology factors found in insects, such as pH and oxygen availability may also hinder certain microbiota, while being a haven for others (Dillon & Dillon, 2004; Engel & Moran, 2013). The resident gut flora is also important and may play an important protective role (Azambuja et al., 2005; Dillon & Dillon, 2004), producing antimicrobial substances (Forsgren et al., 2010), or through competitive exclusion of newly invading microbiota (Dillon et al., 2005).

The identification of mites in this study also adds to the complexity of transmission routes in Hymenoptera especially so for *B. terrestris* and *A. mellifera*. Mites have been demonstrated to act as vectors of microbiota particularly RNA viruses such as *DWV*. Suggesting mites such as *Varroa* are a significant cause of infection in pollinators (Fürst, McMahon, Osborne, Paxton, & Brown, 2014; Genersch & Aubert, 2010). In one study it was suggested the tracheal mite *Locustacarus buchneri* might alter the foraging behaviour of the bumble bee *Bombus impatiens*, influencing interactions between plants and pollinators, as well as the reproductive output of bumble bee colonies (Michael C. Otterstatter, Gegear, Colla, & Thomson, 2005).

3.6.4 Conclusion

This study presents a thorough investigation into the microbiota seen in each sample, characterising prokaryote, microbial eukaryote and viral associates observed in each hymenopteran host. Providing insights into the relationships between close hymenopteran species in a shared environment, and instances of certain microbiota not previously identified in these hosts.

The five hymenopteran hosts analysed in this study displayed a range of microbiota indicating high community diversity. These results show that microbiota among hymenopteran hosts as well as between host samples can vary, potentially indicate there is a constant exchanging of microbiota between individuals, hosts, diet and environment. However, this study would also have benefitted from additional replication of samples and less disparity between host samples. Despite sample disparity, some microbial distinctions were observed, indicating specificity with possible relevance to host biology. Further investigations are needed to evaluate these significant taxa.

4 A METATRANSCRIPTOMIC ANALYSIS OF DISEASED AND HEALTHY WASP LARVAE AND TIME SERIES INFECTION ANALYSIS

4.1 ABSTRACT

The interactions between hosts and pathogens are complex relationships in which the success of each organism depends on its ability to overcome the other. This chapter, investigates potential host-pathogen relationships in the invasive wasp *Vespula vulgaris*. Using Next Generation Sequencing (NGS) and subsequent metatranscriptomic analysis the transcriptome of *V. vulgaris* larvae was characterised from nests symptomatic of an unknown disease and apparent healthy larvae. From both healthy and diseased larvae, 27 known phylotypes were identified, of which five candidates selected as potential pathogens and four observed in diseased samples alone (*Moellerella wisconsensis*, *Aspergillus*, *Vavraia. culicis floridensis* and *Moku Virus*). *Kashmir Bee Virus* was found to be present in both healthy and diseased larvae. To my knowledge this is the first time *M. wisconsensis*, *V. culicis floridensis* and *Moku Virus* has been identified in *V. vulgaris*.

To test for prevalence of selected pathogens, putative diseased and healthy larvae were homogenised and orally fed to test and control *V. vulgaris* nests, in addition to test and control larvae, in a time series infection study. PCR and RT-qPCR molecular techniques were used to assess presence and virulence of pathogens, together with the immune gene Dicer testing immune gene expression of potential viral infections over time. PCR and subsequent Sanger sequencing using designed primers, confirmed the identity of *M. wisconsensis*, *Aspergillus*, *KBV* and *Moku Virus*. In the RT-qPCR analysis, there was a significant correlation between *KBV* and Dicer expression in *V. vulgaris*. *M. wisconsensis* and *Aspergillus* sp. were significantly correlated with *KBV* suggesting possible secondary or coinfections. Detailed knowledge of infection prevalence in vespine wasps is lacking. This study highlights potential new pathogens in *V. vulgaris* that may be important drivers of population decline leading to a natural biocontrol for invasive wasps.

4.2 INTRODUCTION

The increased potential of microbial control of insect pests over the past few decades has mostly been the result of the discovery and development of new species and strains of entomopathogens (Stock, Kaya, & Lacey, 2009). These entomopathogens cause disease through the effects of infection, parasitism and toxemia and may not always be apparent in the host through gross pathology (Stock et al., 2009). The presence of opportunistic /saprophytic microbes can cause issues when identifying the causal agent of insect disease, in many cases; these microbes will develop rapidly in sick/dead insects and will overgrow the true disease agent. Therefore, the most abundant microbe affiliated with an insect may not be the causal agent (Boucias & Pendland, 1998). These issues in the identification of disease-causing agents may be clarified following Koch's postulates for a more conclusive diagnosis (R. Koch, 1893).

Koch's Postulates

- 1) Identify observed disease symptoms.
- 2) Isolation and culture of the disease agent.
- 3) Use a cultured agent to cause diseased state, when administered to susceptible hosts.
- 4) Re-isolation of an infectious agent from inoculated hosts

However, all these criteria may not always fit, due to the inability of specific microorganisms to be cultured. Therefore in some cases an in vivo approach may be used (Stock et al., 2009). Mode of transmission of an infectious agent is also critical, when and how it is passed from one host to another, in addition to the lifecycle of a disease in a biological system. Advances in molecular approaches in the identification of non-cultivable microorganisms as well their quantification add another dimension to fulfilling some if not all of Koch's postulates (Cohen, 2017).

Vespid wasps are a significant pest throughout global temperate regions (Edwards, 1980; J. Philip Spradbery, 1973) and continue to invade new ranges causing social, economic and ecological damage (J.R. Beggs et al., 2011; Crosland, 1991; Lester et al., 2013; Masciocchi & Corley, 2013; Matthews, Goodisman, Austin, & Bashford, 2000; J. Spradbery & Maywald, 1992; Thomas & Moller, 1990). Even though vespid wasps are known pollinators, they are also predatory and opportunists. They cause extensive mortalities in native species and

continue to be an on-going biosecurity concern in New Zealand and around the world, resulting in a focused effort to develop control strategies (J.R. Beggs et al., 2011).

Much research has been undertaken to find biocontrol candidates that could efficiently be used to reduce, or ideally eradicate, this proficient invader in new ranges (J.R. Beggs et al., 2008; Fallis, 2013; Glare et al., 1996; R J Harris & Etheridge, 2001; Richard J Harris et al., 2000; Merino, France, & Gerding, 2007; Rose et al., 1999). From this research, many pathogens and parasites have been identified, however to date no effective biocontrol has been successful, and vespid wasps continue to proliferate in newly acquired ranges such as New Zealand.

The interaction of hosts and pathogens are complex relationships in which the success of each organism depends on its ability to overcome the other. Consequently, hosts have evolved surveillance and defence mechanisms to detect and eliminate invading microorganisms, whereas pathogens use sophisticated strategies to counteract such responses to enable host exploitation and overcome immune resistance (Liehl, Blight, Vodovar, Boccard, & Lemaitre, 2006). Therefore, there is considerable interest in the potential impact pathogens could have on the dynamics of insects and in particular social insects (Anderson & May, 1981; Payne, 1982; Bowers & Begon, 1991; Fuxa, 1991; Sait et al., 1994b; Briggs & Godfray, 1996; Boots & Norman, 2000; Briggs et al., 2000). These advances in invertebrate pathology and dynamic population models show that host populations can be profoundly influenced by their pathogens (Boots, 2004).

Vespid wasp colonies are known to occasionally crash in the presence of diseases (J. Philip Spradbery, 1973). However, there is a lack of substantial evidence indicating pathogenic micro-organisms play an essential role in wasp colony dynamics (Spradberry 1973). Pathogenicity may also succumb to social immunity. The evolution of social immunity in social insects results from the cooperation of the individual group members to combat the increased risk of disease transmission that arises from sociality and group living (Cremer, Armitage, & Schmid-Hempel, 2007). Examples of social immunity include social fever in honey bees, fastidious nest hygiene, grooming, social exclusion, antimicrobial secretions and salivary regurgitations (Cremer et al., 2007; Gambino, 1993; Harcourt, 2002; Starks, Blackie, & Seeley, 2000). Therefore, for a pathogen to be successful as a biocontrol, it must overcome social immunity and be virulent enough to invade a host.

Wasp nests that are symptomatic of an infectious agent are consequently of interest in deciphering the cause and virulence of infection, highlighting possible biocontrols (Valles et al., 2012). Applying co-occurrence comparative microbiota analyses can also provide valuable information for characterising the biogeography, functional distribution or ecological interactions of microbes at the community scale (Williams, Howe, & Hofmockel, 2014). The goal of this study was to identify potential candidate pathogens that may have caused pathology in common wasp (*V. vulgaris*) nests, and experimentally induce a time series infection study in *V. vulgaris* nests and larvae. Diseased *V. vulgaris* nests were found in a separate study investigating the effects of a mite on *V. vulgaris* nests (Brown, 2013) in the Canterbury region of New Zealand (**Figure 4-1**). Larvae from these nests were sampled to detect the pathogen causing the demise of the nests, with the hope of identifying a potential natural biocontrol for further study via molecular methods.

Figure 4-1 This figure shows a worker *V. vulgaris* wasp, burdened by mites. The mites attach to wasps with their mouthparts and were located in areas difficult for the wasps to groom. Wing deformation was apparent in infected wasps, and heavily infected wasp colonies collapsed (Photo: Dr Bob Brown).

It was noted at the time that the unknown pathogen was particularly virulent, causing infections in other close-by nests, and therefore a possible candidate for further investigation and possible infection study (Brown, 2013). These nests showed signs of deterioration with larvae presenting abnormal opaque discolouration beneath the cuticle, which was suspected as a protozoan infection, resulting in hypertrophied tissues (Stock et al., 2009) (**Figure 4-2**)

Figure 4-2 Photos A and B: *V. vulgaris* larvae presenting abnormal opaque discolouration beneath the cuticle, indicating a possible infection, resulting in hypertrophied tissues. Photo C: Apparent healthy larvae for visual comparison.

This study aimed to identify the infectious agent causing pathology in the host using Illumina RNA-Seq NGS, and Polymerase Chain Reaction (PCR). Another aim was to test the infectivity and virulence of pathogen candidates by orally infecting apparent healthy larvae and whole *V. vulgaris* nest colonies in the lab to test prevalence and virulence on the host.

Here I (i) add additional knowledge of the microbial community that may affect *V. vulgaris* health, by comparing diseased and healthy larvae from two separate nests (NGS metatranscriptome analysis) and confirming candidates (PCR and Sanger sequencing); (ii) Initiate a time series infection study, infecting larvae and nests with acquired diseased larvae symptomatic of an unknown pathogen; and (iii) quantify infection of potential pathogen candidates through RT-qPCR as well as the immune response expressed in the immune gene candidate Dicer.

4.3 MATERIAL AND METHODS

4.3.1 Sample Collection

Six live diseased and six live apparent healthy *V. vulgaris* larvae were sampled aseptically into sterile DNA/RNA free vials from two separate nests (April 2016) from the Lincoln area in Canterbury. Larvae were transported live to Victoria University of Wellington. On arrival, live larvae were investigated for apparent signs of disease. Diseased larvae presented opaque tissues beneath the cuticle, whereas there were no similar observations noted for healthy larvae. Three larvae from each nest were snap frozen at -80°C for subsequent RNA analysis. At the time of sampling, the cause of infection and virulence was unknown. Therefore, before freezing and the possible suppression of the unknown pathogen virulence, fresh larval samples were used immediately in an infection study.

4.3.2 RNA Extractions and Illumina Library Preparation

RNA-Seq was undertaken to investigate candidates for the cause of the infection, through a metatranscriptomic comparison of healthy and diseased larvae. Total RNA was aseptically extracted in a laminar flow hood, from three diseased and three healthy larvae which had been snap frozen at the time of collection (-80°C). RNA was extracted by bead-beating (BeadBeater 16, Biospec Products, USA) samples in GENEzol reagent (Geneaid, Taiwan) with 5% β -mercaptoethanol, followed by chloroform and isopropanol purification. RNA integrity was confirmed and quantified with an RNA 6000 Nano chip on the Agilent 2100 Bioanalyser (Agilent Technologies Co. Ltd., Diegem, Belgium), according to the manufacturer's instructions. Extracted RNA samples were then sent for Illumina RNA-seq. RNA samples, (test and control) were sequenced as 125 bp paired-end barcoded Truseq libraries (Macrogen, South Korea), using Illumina Hiseq. Post-processing included quality control, excluding bases with quality scores $Q > 30$ and trimming of adapters. Illumina RNA-Seq data was assembled using Trinity v 2.0.6 using read normalisation (B. J. Haas et al., 2013).

4.3.3 Bioinformatics Analysis

To detect potential novel pathogen sequences in the assemblies, metatranscriptomic contigs from both diseased and healthy larvae were analysed. To elucidate the possible cause of infection sequences aligned (e-value of 0.001) to a binary non-redundant (nr) protein database (Zhang et al., 2000) using DIAMOND for faster processing times (Buchfink,

Xie, & Huson, 2015). Taxonomic identity was assigned in MEGAN6 community edition (Version 6.8.18) (Huson et al., 2016). The meganize function in MEGAN6 assigned taxonomy based on 97% identity to accession files provided (May 2017). The resulting meganized DIAMOND files were visualised and compared in MEGAN6 (Huson et al., 2016). BLASTx protein alignments were used to identify putative viral, microbial eukaryote and bacterial proteins. Known candidate pathogens were selected if they were present in the infected larvae only. These aligned candidate sequences were confirmed by extracting sequences from the assembly file and blasted in the Genbank NCBI database (Camacho et al., 2010).

4.3.4 Infection Study Nests

For the infection study, six subterranean *V. vulgaris* nests were extracted from several closely spaced sites within a 5 km radius in the Wainuiomata area of Lower Hutt, Wellington. Each live nest was transferred to buckets in the field and cooled in a -20°C freezer for an hour to disable stinging workers, but not kill them. From each nest, two combs were removed containing fourth and fifth instar *V. vulgaris* larvae for infection. One wasp comb was designated control while the other was used for subsequent larval infection. All combs were placed in storage containers with ventilation in a separate incubator kept at 27°C. Combs were glued to the lid of each storage container (**Figure 4-3**), so that when placed back on the box, combs faced downwards (**Figure 4-4**), mimicking the orientation in the natural environment. The remaining wasp nests were then transferred and sealed into a predesigned and fabricated wooden box (**Figure 4-5**). Three nests each were designated for pathogen infection and three used as control nests. Each nest was placed on a wire mesh hammock for suspension, to allow nest repair and continued growth. A foraging box area was attached to the nest box by a 1.5m pipe, where wasps were allowed to leave the nest box to forage. The foraging arena was where food was located and enabled monitoring of the wasp nest individuals for the duration of the experiment. All nests were acclimated for two days in a 27°C environmentally controlled room, where they were fed a 50:50 water and honey solution daily in the foraging arena.

Figure 4-3 Figure is displaying nest combs with a scale bar. Food was administered daily using a 10ul pipette, feeding each larva individually. Nest combs are glued to the lids of a storage container and then placed on storage container box in the downwards position, mimicking comb orientation in the nest.

Figure 4-4 Figure is showing glued nest combs to the lid of a storage container. All combs were orientated downwards, so when placed back on the box, combs mimicked the natural orientation in the nest.

Figure 4-5 Wooden nest box connected to foraging arena. Once two combs were retrieved the remaining nests were transferred and sealed into a predesigned and fabricated wooden box, three nests designated test and three control. Each nest was placed on a wire mesh hammock so as suspended to allow nest repair and continued growth. A 1.5m pipe connected the wooden nest box to a foraging arena, where wasps travelled to back and forth, as seen in the picture.

4.3.5 Nest and larvae Infection Study

On the day of infection (Day 3), infected and apparently uninfected larvae were administered to the test and control wasp nests respectively (three test and three control). Wasp combs designated test and control were also orally administered infected and control larvae. All larvae were homogenised aseptically in a laminar flow hood using a Qiagen Tissuerrupter® before being administered to larvae and nests. The test (infected) and control (healthy) larvae diet were separately homogenised, three pooled infected larvae (2.5g) and three pooled healthy larvae (2.5g). Homogenisation of both infected and healthy larvae took place in 50:50 honey (6mL) and water (6mL) to make up the test and control diet respectively. Nests were fed directly in the foraging arena while a subset of each diet was individually administered orally (5µL) to each larva in both test and control

combs by pipette. This measurement of 5ul was based on larvae reportedly, regurgitate up to 5 ul of saliva in a single trophallactic exchange (Harcourt, 2002).

Post-infection day all nests and larvae were fed daily 50:50 water and honey with homogenised healthy larvae as a proteinaceous source for the remainder of the experiment. Sampling of three larvae from control and infected combs, as well as three workers from each control and infected nests, began two days before infection and was carried out for the duration of the experiment (Day 12). Subsequently, test and control larval samples were then used in an RT-qPCR time series analysis of pathogen expression in conjunction with one immune gene, *Dicer*. *Dicer* expression was used to test the immune gene expression in relation to *KBV* and *Moku* viral load temporally.

4.3.6 Larvae Infection Study

RNA Sample Extractions

Total RNA was extracted from test and control larvae sampled from test and control combs at each time point. RNA was extracted by bead-beating (BeadBeater 16, Biospec Products, USA) samples in GENEzol reagent (Geneaid, Taiwan) with 5% β -mercaptoethanol, followed by chloroform and isopropanol purification. Concentrations of RNA were quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA).

PCR

PCR primers were designed in Primer BLAST (Ye et al., 2012) and used to initially confirm candidate targets (**Table 4-1**). PCR reactions were run using REDTaq[®] DNA polymerase (SIGMA-ALDRICH[®]), 2 μ L cDNA/reaction and designed primers. Following optimisation, all PCR reactions were run on a PCR thermocycler (Biometra, Germany) (Stage 1: 95°C, 2 mins; Stage 2: 40 cycles of 95°C, 20s; 60°C, 20s; 72°C, 30s Stage 3 72°C, 5 mins). Subsequent PCR products where clear bands were achieved were treated with ExoSAP-IT (USB) and sequenced by Massey Genome Service, Palmerston North, New Zealand. PCR product sequences were checked and aligned using MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Based on positive sequence alignment on Genbank, qPCR designed primers designed for this experiment (**Table 4-1**) were used to test the prevalence of four potential pathogen targets and one viral immune gene. This time series analysis comparison included both test and control larvae.

Quantitative Reverse Transcription PCR (RT-qPCR)

First strand cDNA synthesis was activated using SuperScript IV (Invitrogen/Thermo Fisher Scientific, Waltham, USA), on 1 µg of total RNA per sample, as per manufacturer instructions and run on a PCR thermocycler (Biometra, Germany). Next cDNA representing the time series of test and control samples were analysed in duplicate by RT-qPCR using PowerUp™ SYBR® Green Master Mix reagent (Applied Biosystems/Thermo Fisher Scientific, USA), 1 µL cDNA/reaction and designed primers (**Table 4-1**).

Dicer was chosen as an immune gene based on the presence of two RNA viruses *Moku* and *KBV*. Endoribonuclease dicer proteins recognise double-stranded RNA and processed into siRNA, guiding a specific anti-viral immune response (Aliyari & Ding, 2009; Brutscher, Daughenbaugh, & Flenniken, 2015). The Dicer Immune gene sequence was selected from the *Polistes canadensis* and *P. dominula* genomes, and orthologs identified using a BLAST search on a common wasp draft transcriptome assembly. BLAST searches were run against the Victoria University of Wellington's Science Faculty High-Performance Computing (Sci Fac HPC) Facility's BLAST ver. 2.2.25 installation. Two reference gene orthologs were also identified in this search: eukaryotic translation initiation factor 3 subunit C (eIF3- S8) and Proteasome 54kD subunit (Pros54). These genes were previously shown to be stably expressed in the honey bee (R. C. Cameron, Duncan, & Dearden, 2013; Vannette, Mohamed, & Johnson, 2015) and were included to normalise gene expression, as recommended by MIQE guidelines (Bustin et al., 2009) (**Table 4-1**). At the time of testing no other immune genes were used to test the pathogen candidates *Aspergillus* and *M. wisconsensis* due to time constraints, however, additional testing would benefit further analysis.

All qPCR reactions were run on QuantStudio 7 (Applied Biosystems/Thermo Fisher Scientific, USA) using fast cycling conditions and fluorescence detection during the elongation step (Stage 1: 95°C, 30s; Stage 2: 40 cycles of 95°C, 5s; 60°C, 15s; 72°C, 20s). Quantification cycle (Cq) values were used to calculate candidate microbial loads and Dicer expression. To account for variation in sample quality, an upper cycle threshold (Cq) of 35 was set for expression levels, above which values were excluded from quantitative analysis.

4.3.7 Statistical Analyses

To assess differences in pathogen prevalence and *Dicer* expression in larvae from the control and treatment groups after inoculation, we used a one-way ANCOVA in R (R Core Team, 2016). We included time since infection as a covariate. The response variables were log-transformed. We used the package PerformanceAnalytics (Peterson and Carl, 2015) to produce Pearson correlation statistics and plots to examine correlations between pathogens and the immune gene *Dicer* expression. To illustrate the the percentage of nest survival over the duration of the study (days), Kaplan–Meier survival curves were constructed in R (R Foundation for Statistical Computing, 2016).

Table 4-1 Table is detailing oligonucleotide primers designed and used in this study for PCR and real-time quantitative PCR. Reference genes *Pros54* and *eIF3-S8* and the immune gene *Dicer* were selected from the *Polistes canadensis* and *P. dominula* genomes, and orthologs identified using a BLAST search on a common wasp draft transcriptome assembly. Candidate pathogen primers for *KBV*, *Moku virus*, *A. fumigatus* and *M. wisconsensis* were designed using sequences extracted from the metatranscriptomic assembly in this study. All primers were designed using PRIMER Blast

Target	Forward	Reverse	Product Length
Reference Gene: Pros54 (RT-qPCR)	TGGTGAAATTAGCAAAACGACTCA	GAACAGTGACGAGGTGGCTA	150
Reference Gene: eIF3-S8 (RT-qPCR)	AGAAGAGTATGGCTGATGGTGA	TCACTCTCTGAATCGCTACCC	70
<i>Kashmir Bee Virus</i> (PCR)	TGATGGTGATTGTGGAGCCC	TCGACTCCCGGATAACCTGT	690
<i>Kashmir Bee Virus</i> (PCR & RT-qPCR)	ACCAGGAAGTATTCCCATGGTAAG	TGGAGCTATGGTTCCGTTTCCAG	79
<i>Moku Virus</i> (PCR)	ATCTGAACTGCCACCGTCAG	TGCTGCTGCCTCATTAAACCA	744
<i>Moku Virus</i> (PCR & RT-qPCR)	TGGTGCGATAGCTAAGCCTG	GAACCCAGAATGGTGCTGGA	134
<i>Aspergillus fumigatus</i> (PCR & RT-qPCR)	CTAACTTTCGTTCCCTGATTAATG	CTTGGATTGCTGAAGACTAAC	76
<i>Moellerella wisconsensis</i> (PCR & RT-qPCR)	TACCGACAGAAGAAGCACCG	CTTAATCAACCGCCTGCGTG	112
<i>Dicer</i> (PCR & RT-qPCR)	CAACTCCGAGATAGGTGACAGAAG	AGAAGAGGGTCCGCATCCTT	73

4.4 RESULTS

4.4.1 Metatranscriptome Study

We performed metatranscriptomic comparative analyses using RNA-seq to elucidate the cause of *V. vulgaris* larvae symptomatic of an unknown infection. The metatranscriptomic comparative analysis identified matches to 27 known microbiota phylotypes in both healthy and diseased communities (**Figure 4-6**). Differences in the comparison analysis highlighted five potential pathogen candidates, the bacterium *Moellerella wisconsensis*, the fungus *Aspergillus*, the microsporidian *Vavraia cucilis subsp floridensis* and two RNA dicistroviruses, *Kashmir Bee Virus (KBV)* and *Moku Virus*. The first four of these candidates were identified in the infected larvae alone, with KBV identified in both disease and healthy wasps. Sequences were extracted, and primers were designed to investigate further and confirm the identity of these five candidates (**Table 4-1**). The metatranscriptome analysis also highlighted the genus *Penicillium* found only in diseased larvae of which eleven species were identified. In general, *Penicillium* fungi are considered non-pathogenic (Gilliam, 1997), therefore, this genus was not investigated further. Taxa present in healthy larvae and absent from infected larvae consisted of *Burkholderiales*, *Escherichia coli*, *Acinetobacter baumannii*, *Mycobacterium abscessus subsp. Abscessus*, *Cutibacterium acnes*, *Streptococcus*, *Debaryomyces*, *Basidiomycota* and *Metschnikowia bicuspidata var. bicuspidate* (**Figure 4-6**).

Figure 4-6 Phylogenetic tree comparison of diseased and healthy larvae microbiota constructed in MEGAN. This visual representation is of the metatranscriptome sequence reads that aligned at 97% identity to known sequences in NCBI nt and nr databases. These alignments to taxa represented here are the most likely candidates. However confirmation is always needed. Lowest taxa identified are displayed with the number of read sequences aligned healthy larvae (green) and diseased (pink). Bilateria sequences include all host sequences, the majority of which aligned to *P. dominula* and *P. canadensis*. These genomes are the most closely related annotated wasp genomes to *V. vulgaris*.

4.4.2 Molecular Analysis

To analyse pathogen load, primers were designed for *M. wisconsensis*, *A. flavus*, *V. culicis floridensis*, *KBV* and *Moku Virus*. Primers were initially designed for RT-qPCR analysis. Therefore, products were short. These products were sent for Sanger sequencing and confirmed on Genbank (**Supplementary Table 3**). Viral candidates *Moku Virus* and *KBV* were further confirmed using long-range primers, resulting in more extended confirmatory products and again confirmed on Genbank (**Supplementary Table 4**). Analysis of nucleotide sequences confirmed the identity of all candidates apart from *V. culicis floridensis* which was based on alignment to a *V. culicis floridensis* hypothetical mRNA protein (XM_008075167.1). Therefore, this target was not investigated further. The candidate sequence *M. wisconsensis* also aligned to *Hafnia* sp. (98% identity) and *Hafnia alvei* (97% identity), which is a related bacterium. Given the limited knowledge of bacteria and pathogens in these wasps, we are not confident that the bacterium identified is actually *Moellerella wisconsensis* or that the fungus is *Aspergillus fumigatus*. Typically, *A. fumigatus* is described as a pathogen of mammals that does not cause mortality to insects after injection (St. Leger, Screen, & Shams-Pirzadeh, 2000). Similarly, *M. wisconsensis* is a known human pathogen (Hickman-Brenner, Huntley-Carter, & Saitoh, 1984). Consequently, from this point forward I refer to each as *Moellerella* sp. and *Aspergillus* sp., respectively.

4.4.3 Experimental Infection of Wasp Nests

Whole wasp nests were fed either homogenised wasp larvae presenting infection symptoms (treatment), or the same weight of homogenized larvae from an apparently healthy nest (control). Worker and queen samples were sampled at each time point. However, molecular analysis was not conducted in this study.

A Cox Proportional Hazards model indicated a significant difference in the survivorship between the control and treated nests ($\beta = -0.913 \pm 0.283$ SE; $\exp(\beta) = 0.401$; $p = 0.001$) (**Figure 4-7**). Two of the three control nests survived the entire duration of the study, while the third untreated nest died 6 days after treatment. This nest was relatively small and produced only males (which do not forage or feed themselves), likely indicating that the queen had died or was not producing viable diploid workers. Colony death would be expected if only males are produced because they do not forage or contribute to the colony maintenance. All the nests in the infection treatment initially appeared large and healthy at the beginning of the study.

These treated nests infected with diseased larvae died after 4, 7 and 19 days. We were, unfortunately, unable to monitor for disease in the nests over the duration of the experiment because such sampling would require substantial disturbance in the form of anesthetising the entire colony, and then breaking it apart to sample larvae.

Figure 4-7 Kaplan–Meier survival curves showing the percentage of nest survival over the duration of the study (days). Green indicating control nests that were inoculated with homogenized wasp larvae from a separate and apparently healthy nest, and red indicating nests that feed homogenized larvae from a separate wasp nest showing signs of disease. Shaded areas represent the 95% confidence intervals, $n = 3$ nests per treatment. A Cox's proportional hazards model indicated a significant difference between the treatments ($\beta = -0.913 \pm 0.283$ SE; $\exp(\beta) = 0.401$; $p = 0.001$).

4.4.4 Experimental Infection of Wasp Larvae

Following the infection study of treated and control larvae and subsequent RT-qPCR of sampled larvae, pathogen prevalence was quantified and Dicer immune expression over time. GLMM analysis determined that prevalence of the four target pathogens and Dicer expression did not significantly increase following time of infection (**Figure 4-8**; **Table 4-2**). Surprisingly, all larvae showed expression of all bacteria and viruses that we examined, though with considerable variability over time. The expression of KBV, for example, seemed to fluctuate substantially from low levels of infection on day 5, to much higher levels on day 6, then back again to a low infection on day 7 (**Figure 4-8i**). Moku virus and *Moellerella* sp. showed a similar

pattern (**Figure 4-8**). KBV typically was present in an order of magnitude higher levels than Moku virus.

Because there were no significant differences in expression values between treatments, we combined the data to analyse for correlations between pathogens and the immune gene Dicer. Four of the 10 correlation analyses showed significant positive correlations ($p < 0.05$). These included KBV and Moku virus, KBV and *Aspergillus* sp., and KBV and the immune gene Dicer (**Figure 4-9**). The Pearson's correlation coefficient r between KBV and Moku virus was 0.46 ($n = 59$; $p < 0.001$), though when all 0 values were removed so that relationship was examined only when both viruses were present, this value increased to 0.75 ($n = 41$; $p < 0.001$). Similarly, the correlation coefficient between KBV and *Aspergillus* sp. increased from 0.26 ($n = 59$; $p < 0.05$) to 0.69 ($n = 44$; $p < 0.001$) when analysing data only when both species were detected.

Table 4-2 Results of one-way ANCOVA analyses comparing *Moellerella wisconsensis*, *Aspergillus fumigatus*, Kashmir Bee Virus and Moku virus prevalence, and Dicer expression between treatment and control larvae. Interactions were not significant and are not reported.

Target		Estimate ($\pm$ SE)	t Statistic	p-value
<i>Kashmir Bee Virus</i> (KBV)	Treatment	-0.270 (1.093)	-0.246	0.806
	Time	-0.051 (0.112)	-0.454	0.652
<i>Moku virus</i>	Treatment	-0.028 (0.096)	-0.294	0.770
	Time	0.203 (0.983)	0.207	0.836
<i>Moellerella</i> sp.	Treatment	0.185 (0.637)	0.290	0.773
	Time	0.089 (0.065)	1.380	0.173
<i>Aspergillus</i> sp.	Treatment	-0.035 (0.954)	-0.037	0.971
	Time	0.084 (0.098)	0.865	0.390
Dicer expression	Treatment	0.013 (0.702)	0.019	0.985
	Time	0.023 (0.072)	0.322	0.748

Figure 4-8 The mean prevalence expression of *Kashmir Bee Virus* (i), *Moku virus* (ii), *Moellerella* sp. (iii), *Aspergillus* sp. (iv) and *Dicer* (v) in *V. vulgaris* larvae relative to the reference genes *Pros54* and *eIF3-S8*. Mean relative expression was achieved averaging the target RT-qPCR cq values from larvae sampled from four separate nest combs at each time point. The inoculation of homogenised diseased larvae or homogenised healthy larvae for the control, on day 3 is signified by the black arrow. Three larvae were sampled at each time point from control and test combs extracted from four *V. vulgaris* nests. (vi) shows larvae being fed via a pipette.

Figure 4-9 Correlations between the four potential pathogens and the immune gene Dicer, quantified using RT-qPCR in larval wasps. The frequency distribution of each is shown in the central diagonal. The scatterplot is shown with a loess fit in red and on the corresponding side for each pairing is the Pearson correlation coefficient r . Statistical significance is denoted by: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Values for all viruses and bacteria were $\log(x+1)$ transformed prior to analysis.

4.5 DISCUSSION

Vespula vulgaris is known to host a range of pathogens (Dobelmann et al., 2017; Evison et al., 2012; Glare et al., 1996; Harcourt, 2002; Richard J Harris et al., 2000; Lester et al., 2015; Rose et al., 1999). This study aimed to investigate the causative agent of diseased *V. vulgaris* nests isolated from the south island of New Zealand.

The experimental infection of entire wasp colonies in the laboratory resulted in the complete mortality of initially healthy nests after 4, 7 and 19 days. This experiment demonstrated that the pathogens responsible are infectious and can kill both adult and juvenile wasps. Few researchers have experimentally infected wasps with pathogens, though the published work that exists supports our observed mortality timelines. Two other studies infecting wasp workers with *Aspergillus* have found disease symptoms within 24 h, substantially reduced pupation rates, or adult mortality in as short a period as 2 days (Glare et al., 1996; Harcourt, 2002). Other bacterial pathogens such as *Beauveria bassiana* and *Metarhizium anisopliae* can also kill workers in less than 10 days (Harcourt, 2002; Merino et al., 2007). We would have liked to have additional experimental nests and to have been able to sample the larvae within the nests during the experiment. Nevertheless these experiments demonstrated that the pathogen involved is infectious, present in larvae, and is pathogenic to all wasp life stages.

Through a metatranscriptomic approach, four potential pathogens candidates were identified, using MEGAN analysis. Candidate pathogens were successfully sequenced and aligned to *KBV*, *Moku Virus*, *Aspergillus* sp. and *M. wisconsensis*. The candidate *M. wisconsensis* also aligned to *Hafnia* sp. (98% identity) and *Hafnia alvei* (97% identity), which are closely related to each other in addition to *Providencia* species (Hickman-Brenner et al., 1984). *Moellerella wisconsensis* known to be pathogenic in humans and other vertebrates including sharks (Hickman-Brenner et al., 1984) but is not known from invertebrates. However, *Hafnia alvei* has been found to occur in codling moth *Cydia pomonella*, *B. terrestris* and *A. mellifera*, where it has been highlighted as an opportunistic environmental bacteria (Kwong & Moran, 2013; J. Li et al., 2015; Zimmermann, Huger, & Kleespies, 2013). Therefore, it is likely that the MEGAN analysis misidentified *M. wisconsensis* due to the close relation with *Hafnia* sp.

Moku virus was recently discovered and described from wasps (*Vespula pensylvanica*), Honeybees (*Apis mellifera*) and mites (*Varroa destructor*) on the Big Island of Hawai'i (Mordecai et al., 2016). It is unknown what effects *Moku virus* has on their hosts. However, it was suggested that *Moku virus* has the potential to be highly virulent in *A. mellifera*, as is the closely related *Slow bee paralysis virus* (Mordecai et al., 2016). This is the first known incidence of *Moku virus* in *V. vulgaris* found in New Zealand.

KBV was also identified in the MEGAN analysis as present in both healthy and infected larvae. KBV has been identified in a number of social insects and seems to be a commonly found pathogen in *V. vulgaris*, honey bees and even invasive ants in New Zealand (Dobelmann et al., 2017; Gruber et al., 2017; Lester et al., 2015; Mondet, de Miranda, Kretzschmar, Le Conte, & Mercer, 2014). The significant correlation between KBV prevalence and subsequent *Dicer* expression found in this study agrees with findings suggesting KBV levels influence a viral immune response and can influence wasp fitness (Dobelmann et al., 2017). KBV has also been described as part of a pathosphere implicated in honey bee colony collapse disorder (R. S. Schwarz, Huang, & Evans, 2015) and can be extremely virulent when in high titres (Joachim R. de Miranda, Cordon, & Budge, 2010). We found that KBV was present in wasp larvae at titres often an order of magnitude higher than *Moku virus*. However, it is still unclear as to the pathogenicity of KBV in these *V. vulgaris* samples, as it may potentially be latent virus (Shen, Cui, Ostiguy, & Cox-Foster, 2005).

The fungal pathogen *Aspergillus* sp., has previously been identified as a pathogen of vespine wasps (Glare et al., 1996) and are also part of the known pathosphere of honeybees (Schwarz et al., 2015). *Aspergillus* are generalistic and opportunistic pathogens that often require wounds or weakened hosts (St Leger et al., 2000). *Aspergillus* are known to release mycotoxins and cause the bee larval disease stonebrood (Schwarz et al., 2015). The disease symptoms are similar to those we observed in infected wasp larvae. In New Zealand, the collapse and death of wasp nests has been associated with the mite *Pneumolaelaps niutirani* (Brown, 2013; Fan, Zhang, Brown, France, & Bennett, 2016). This genus of mites is a known detritivore. They have, however, also been observed feeding on secretions around the mouths of wasp larvae. *Aspergillus* are known symbionts of detritivorous mites that may contribute to mite micronutrient diet deficits (Hay, Hart, & Douglas, 1993). It is possible that the mite transmits *Aspergillus* during this feeding, though any *Aspergillus*

infection may still be secondary and opportunistic after wounding by other pathogen(s). The correlations we observed between pathogens including KBV and *Aspergillus* may support an opportunistic infection by this pathogen. The metatranscriptome analysis also highlighted the microsporidian *Vavraia culicis*. This pathogen can be induced by oral feeding and has a very broad host range that includes shrimp, beetles and butterflies (Andreadis, 2007) . Attempts have been made at using *V. culicis* for biological control of mosquitos. The pathogen can reduce adult survival, increase adult female mosquito age-dependent mortality, and reduce female fecundity (Sy et al., 2014) Our primers designed for *V. culicis*, and published primers, were unsuccessful in amplifying desired sequences in the PCR and RT-qPCR analysis. However, MEGAN analysis identified this candidate only in diseased larvae, suggesting it is still a potential pathogen causing infection.

The isolation and experimental infection of larvae was designed to enable a close examination of pathogen dynamics over time. The experiment required the removal of combs, positioning them in containers, and the daily artificially feeding the larvae. No contact with adult wasp workers was allowed. The results showed no clear differences in pathogen abundance between larvae that were fed a homogenate of diseased or healthy larvae from other nests. The absence of workers may have resulted in unhygienic conditions for larvae. Post feeding, on a number of occasions, both control and test larvae were observed on the bottom of the storage container having abandoned their comb cells and died. This unnatural environment may have stressed larvae incurring increases in opportunistic pathogens as seen in this study in addition to the two day periodicity seen in pathogen prevalence (**Figure 4-8**). Typically worker wasps carry out domestic duties including the distribution of food to larvae, nest cleaning and clearing of comb cells (Spradbery, 1973) . Therefore, maintaining larvae in a lab situation may need to include workers to ensure unnatural surroundings are not the cause of stress and disease, and mimic a more natural response. It is also conceivable that infected larvae were suffering from the inoculum received in this study and may have been presenting disease symptoms from a pathogen not tested by us. Larvae dropping from their cells is a symptom of disease (Spradbery, 1973).

4.6 CONCLUSION

It is clear from this study, that an array of pathogens are present in *V. vulgaris*. Some of these pathogens appear to be initiating an immune response within the wasps, and may even result in entire nest mortality. Primary infection on average can generate more than one secondary infection by weakening the host, allowing coinfections of pathogens capable of invading the hymenopteran hosts (Michael C. Otterstatter & Thomson, 2006). These wasps have a 'pathosphere' (R. S. Schwarz et al., 2015) that includes pathogens found in many other organisms. Some may be symbiotic in nature while others are potential pathogens that can decimate a nest. This study highlights candidate pathogens for further investigation. Isolation of pathogens in culture and additional metatranscriptome analyses would allow a more controlled and quantifiable investigation, confirming prevalence and virulence of identified pathogens.

5 GENERAL DISCUSSION

Insects are by far the most diverse and abundant clade of metazoans (Basset et al., 2012; Labandeira & Sepkoski, 1993; Stork, 1988). They are also exceptional invaders with outstanding dispersal abilities, as seen in vespine wasps. *Vespula vulgaris* dispersal is facilitated in part to their broad diets, effective predator defences and superior competitive abilities in addition to high reproductive rates, dominating the novel ecological community (Moller, 1996; John H. Werren et al., 2010). However, successful biological invasions involve complex interactions between the invading species and the physical and biological characteristics of the recipient environment (Hayes & Barry 2008). The microbiome is another factor that potentially increases the chance of invasion success. The term microbiota refers to the microbial community that stably or transiently colonises the insect epithelium, organs and their intracellular compartments. These communities include varied taxa from bacteria to viruses, yeasts and protists (Villegas 2014).

Many studies have focused on the ecological impacts that *V. vulgaris* have imposed in their introduced ranges. The primary goal of this research was to study aspects of the microbial diversity pertaining to these wasps and their invasion success. This research aimed to develop an understanding of the processes and interactions between wasps and their microbiota as well as the broader hymenopteran community. To my knowledge, this is the first examination of the bacterial, microbial eukaryote and viral microbiota present in the common wasp, *V. vulgaris*. This body of work has been achieved through a detailed investigation of the microbiota using NGS/molecular and bioinformatic techniques.

The overall aims were to document and investigate *V. vulgaris* microbiota between life stages and ranges to elucidate possible reasons for the invasive success of *V. vulgaris*. Evidence was also sought of microbiota specificity in *V. vulgaris* when compared to the microbiome of four introduced hymenopterans. In addition, the final aim was to elucidate the cause of pathology in putatively infectious larvae sampled from an observed diseased nest and investigate the transmissibility and prevalence of identified putative pathogens in a time series infection study.

5.1 RESEARCH SUMMARY

Chapter 2 provides the first descriptive account of the microbiome in *V. vulgaris*. This analysis tested the hypothesis that there would be differences observed between microbial communities between life stages (larvae, worker and queen (gyne)). There was no significant difference in community dissimilarity between life stages. However, larvae were observed to host greater bacterial, microbial eukaryote and viral sequence diversity compared with that of workers and queens. Further examinations of life stages in each range, revealed significant differences observed between life stages in the native range. These differences, seen in the dissimilarity between the prokaryote community of larvae and queens. Surprisingly, queens were free of many Bacilli, Gammaproteobacteria and mostly free of known microbial eukaryotes and completely free of known RNA viruses. The core microbiome at the life stage level also revealed queens had the highest number of core taxa, in comparison to workers and larvae, with eleven unique taxa found in Queens. A core microbiome was also presented a representative of all life stages, highlighting microbiota that is potentially important for *V. vulgaris* host survival. This analysis emphasised the possible importance of nitrogen-fixing bacteria from the order Rhizobiales, in addition to Actinobacteria and the genus *Pseudomonas*, known beneficial bacteria producing antibiotics for microbial defence.

It was also hypothesised that there would be a change in diversity from the native to the invaded range. This was perhaps the most interesting result of Chapter 2. Comparative range analysis revealed significant dissimilarity in the prokaryote community, attributed to both losses and gains in microbiota from the native Belgian to invaded New Zealand range (although I note that with more sampling, more and different taxa would have been observed in both the native and introduced range). This microbial shift observed abundances of Bacilli, and Gammaproteobacteria in the native range, replaced by new instances and increased abundances of *A. mellifera* and *B. terrestris* endosymbionts in the invaded range. The diversity of Bacilli also changed with more diversity seen in the native range compared to *Enterococcus* and *Lactobacillus* in the invaded range. The viral analysis also indicated a wide prevalence of RNA viruses in *V. vulgaris*. However there were distinct differences between the ranges, with Belgian wasps exhibiting a high prevalence of *RhPV* not seen in the invaded range and high prevalence of *KBV* not seen in the native range.

Chapter 3, characterised microbiomes of wasps, bees and ants to elucidate microbiome similarities as well as differences within five hymenopteran hosts. It was hypothesised that there would be greater richness and diversity of microbiota in omnivorous hosts such as *V. vulgaris*, *V. germanica* and *L. humile* in comparison to palynivorous species *A. mellifera* and *B. terrestris*. No significant community dissimilarity was observed among host microbial communities, suggesting a shared microbiota. All hymenopteran hosts shared similar relative abundances of nineteen prokaryote taxa from six phyla. This indicated a cross taxon host core microbiota. Some of the observed similarities included an abundance of nitrogen-fixing bacteria, and Lactic Acid Bacteria (LAB). No shared microbial eukaryotes were observed among all the hymenopteran hosts. However, *B. terrestris* and *A. mellifera* both had incidences of *Leptomonas*, *Acari*, *Candida*, and *Kazachstania* not seen in other hosts. There were also significant differences in abundances observed with specific microbes among hosts. *Linepithema humile* had an abundance of *Lactobacillus* the only LAB bacteria found. The genera *Spiroplasma* and *Wolbachia* were found in all hosts except *L. humile*. However, *Spiroplasma* was in significant higher abundances in *A. mellifera* and *V. germanica* and *Wolbachia* in higher abundances in *B. terrestris*. An array of virus phylotypes were also documented, with known *A. mellifera* viruses identified in other hymenopteran hosts, highlighting these viruses are not restricted to the honey bee. The most host diverse virus, BQCV was found in all hosts except for *L. humile*. The BQCV has been observed in *L. humile* in our other work (Gruber et al. 2017; see appendix 7.3) and I note that additional sampling would likely have resulted in the discovery of this and more microbial taxa, especially for species like *V. germanica* which had only one sample in this analysis.

In Chapter 4, the aim was to identify the potential causative agent of diseased *V. vulgaris* nests. Following a metatranscriptomic analysis of putatively infected and healthy larvae, five potential pathogen candidates were highlighted (*M. wisconsensis*, *Aspergillus sp.*, *Vavraia culicis floridensis*, *KBV* and *Moku Virus*). To my knowledge this is the first time *M. wisconsensis*, *V. culicis floridensis* and *Moku Virus* have been identified in *V. vulgaris*. Interestingly *Moku virus* was not found in previous analyses (Chapter 2 and 3), which may have been down to the timing of the analysis and deposition of Moku virus sequences in Genbank (2016). Following this analysis, primers were designed to amplify sequences and

confirm through Sanger sequencing. Four pathogen targets were successfully sequenced, *KBV*, *Moku virus*, *Aspergillus* sp. and *M. wisconsensis*. The second aim of Chapter 4 was to test these four candidate pathogen targets for prevalence and virulence. *V. vulgaris* nests in addition to larvae were orally administered homogenised infected larvae. Time series sampling of inoculated larvae pre and post infection was undertaken daily until the end of the experiment. RT-qPCR was then used to assess the presence and quantify pathogen loads, together with the immune gene Dicer testing immune gene expression of potential viral infections over time.

Results highlighted the presence of candidate pathogens before inoculation. However, there was a significant positive correlation between *KBV* and Dicer in infected larvae. Also, both control and test larvae had significant negative correlation between *Moku virus* and the immune gene Dicer. The pathogen candidates *M. wisconsensis* and *Aspergillus* sp. were significantly correlated with *KBV* suggesting possible secondary or coinfections. This study presents incidences of potential new pathogens in *V. vulgaris* that may be relevant drivers of population decline.

5.2 SYNTHESIS

Populations of *V. vulgaris* have increased in their invaded ranges in comparison to the native Eurasian range (Archer, 1981, 1985, 2001; Barlow et al., 2002; J.R. Beggs et al., 2011; Burne, Haywood, & Lester, 2014; Crosland, 1991; Lester, Gruber, et al., 2014; Matthews et al., 2000; J. Spradbery & Maywald, 1992). Within their native range, population densities of this wasp fluctuate dramatically, with queen productivity varying by a factor of 100 between nests and years (Archer, 1981). Exceptional years of high abundance in the native range are frequently followed by years of scarcity (Archer, 1985). This variation of abundance is not observed in the invasive range, including New Zealand. New Zealand nest densities can fluctuate annually by only a two to three-fold difference (Archer, 1985; Barlow et al., 2002).

The niche hypothesis may explain why some introduced species reach higher densities than in their native range. Factors such as more abundant or higher quality resources, a milder climate and a more suitable physical environment may lead to an increase in invasive

species populations (MacLeod, Newson, Blackwell, & Duncan, 2009). A recent statistical study analysed *V. vulgaris* population dynamics in England and New Zealand. This study demonstrated that the primary determining factor of wasp abundances was wasp abundance from the previous year as well as increases in rainfall in spring, summer and winter associated with reduced wasp abundances (Lester, Haywood, et al., 2017).

However, *V. vulgaris* is known to host a range of pathogens (Dobelmann et al., 2017; Evison et al., 2012; Glare et al., 1996; Harcourt, 2002; Richard J Harris et al., 2000; Lester et al., 2015; Rose et al., 1999). The Enemy Release Hypothesis (ERH) predicts that an exotic species introduced to a new region will become invasive because key natural enemies are absent (Colautti et al., 2004; Heger & Jeschke, 2014; Keane & Crawley, 2002). Studies have reported the lack of evidence for this hypothesis in *V. vulgaris* populations, demonstrating no evidence of microbiota differences between the native and introduced range (Lester et al., 2015; Lester, Gruber, et al., 2014).

Is there evidence that the microbiome of *V. vulgaris* can positively increase fitness?

This is the first study to characterise the microbiome of *V. vulgaris*, documenting microbial diversity in three distinct life stages. Life stage diversity across two ranges was not significant. However, the dissimilarity between the prokaryote community in larvae and queens from the native range highlights that there is a potential trend exhibiting a distinct queen microbiome. The lack of abundance of microbial eukaryotes, as well as the absence of known viral phylotypes in both ranges further, confirms queen microbiome distinction. This absence of a variety of viral, microbial eukaryotes and bacterial microbiota in the queen life stage, which is seen in the larvae and worker life stages, potentially highlights a more specific microbiome. This overall change or segregation of specific microbiota in life stages is interesting due to the social nature of vespids with food resources shared through the process of trophallaxis. It seems highly likely that newly produced queen wasps within a nest would have been exposed to many of the viruses and bacterial taxa observed in workers, though these did not infect the new queens. This may in part be due to an alternative food source or possibly increased nest hygiene in queen combs, inferring methods of protection that potentially select symbionts and exclude non-symbionts.

Few hymenopteran studies are focusing on the microbiome community of Queens. One extensive study demonstrated the gut microbiome of *A. mellifera* queens did not reflect those of workers who tended them, lacking many bacteria considered vital to workers (David R. Tarpy et al., 2015). Specific Alphaproteobacteria from the family Acetobacteraceae dominate the mature queen, also, low abundances of core honey bee bacteria are also present in the mature queen microbiome (*Lactobacillus*, *Bifidobacterium*, *Snodgrassella* and other Alphaproteobacteria from the family Acetobacteraceae) (David R. Tarpy et al., 2015). Similar bacteria are seen in *V. vulgaris*, such as Acetobacteraceae in addition to *Gilliamella*, *Snodgrassella*, *Saccharibacter* and *Bifidobacterium* in the invaded range. However, these bacteria were not exclusive to the queen life stage. What's interesting is the associated Acetobacteraceae in *A. mellifera*, are known to be harboured by the worker hypopharyngeal glands. These glands produce royal jelly known for its antiseptic properties, which is continually fed to queens by attending workers maintaining this microbiota in *A. mellifera* queens (Elango, Hunt, Goodisman, & Yi, 2009; Mattila, Rios, Walker-Sperling, Roeselers, & Newton, 2012). *V. vulgaris* workers are known to produce a strong antibiotic substance in their saliva potentially produced from the hypopharyngeal gland as in honey bees. It has been demonstrated, that *V. vulgaris* saliva can inhibit the growth of *Bacillus* spp. and *Serratia* spp. (Harcourt, 2002). This inhibition of microbiota may indicate a similar process in wasps as in honey bees. Where workers feed queens/gynes a distinct diet produced from their hypopharyngeal glands. This potent antimicrobial cocktail may be capable of restricting the growth of specific microbes such as microbial eukaryote and viral phylotypes not seen in queens in this study. This lack of viruses and microbial eukaryotes seen in queen wasps may indicate a reduction in potential pathogens and in turn positively increase fitness of gynes and founder colonies. Previously published work (**Appendix 7.2**) also demonstrated that polyandry, viral load and expression of an immune gene are significant predictors of variation in nest size. Wasp colonies that had higher genetic diversity tended to be significantly larger and produce more queens. This study also highlighted the effect KBV can have on the immune system of a wasp demonstrating that *V. vulgaris* can suffer from pathogens effects, expending immune resources, in the control of pathogens such as KBV (Dobelmann et al., 2017).

Is there evidence that the microbiome of *V. vulgaris* has changed from the native to the invaded range?

Invasive species typically present a lower genetic diversity in their invasive range than in their home range, compounded by propagule pressure causing genetic bottlenecks and low genetic diversity (Dlugosch & Parker, 2008; Puillandre et al., 2008). Since occurring sporadically in New Zealand since 1921 (B. Donovan, 1984), populations of *V. vulgaris* have established in high numbers. Six haplotypes have been identified in the New Zealand range, confirming multiple incursions from Europe with similar haplotypes (Lester, Gruber, et al., 2014). In addition to genetic diversity, microbial diversity may also decrease along the invasion pathway. As a consequence of serial bottlenecks, invasive species may lose microbial symbionts including pathogenic taxa (the enemy release hypothesis) (Lester, Sébastien, et al., 2017). The enemy release hypothesis states that invasive species can become abundant due to the absence of co-evolved natural enemies such as pathogens and parasites (Keane & Crawley, 2002; Torchin et al., 2003). In Chapter 2, the preliminary analysis of the microbiome between the native Belgian range and invaded New Zealand range highlights a shift in the microbiome. This shift identified a number of significant prokaryotes, in addition to microbial eukaryote and viral taxa present in the native Belgian range not seen in the Invaded New Zealand range. This loss of taxa may indicate a release from enemies and increase in fitness in the invaded range. These losses of microbiota have not affected *V. vulgaris* in their invasive success, and it is likely the retention of a core microbiome as defined in Chapter 2 may contribute to *V. vulgaris* fitness. However, it is unclear if these bacterial taxa lost from the native range are pathogens of this wasp. In addition to losses, there was the acquisition of microbiota in the invaded range. Prokaryotes such as LAB, Bacilli and Alphaproteobacteria genera found in the native range appear to be replaced in the invaded range life stages with honey bee and bumble bee associated core endosymbionts. This acquisition may indicate these endosymbiotic bacteria are now important associates of *V. vulgaris* in the invaded range as they are in honey bee and bumble bee health (Cariveau et al., 2014; E. Crotti et al., 2010; Engel et al., 2012; Kwong & Moran, 2013; J. Li et al., 2015; Martinson et al., 2011; N. A. Moran & Kwong, 2016). This provides evidence that the purported ‘honey bee endosymbionts’ may not be

restricted to the genus *Apis* or *Bombus* and may facilitate *V. vulgaris* in their invasive success.

This preliminary evidence of microbial change is likely due to the social relationship between *V. vulgaris*, and *A. mellifera* observed in New Zealand where microbiota may be transmitted horizontally from honey bee to wasp. This transmission may occur via shared resources such as flowers and robbing of food resources resulting in the spillover of microbiota (Colman et al., 2012; Santo Domingo et al., 1998; Yun et al., 2014). This potential spillover may also explain the abundance of KBV seen in New Zealand *V. vulgaris* wasps (Chapter 2 & (Dobelmann et al., 2017)). In New Zealand, honey production is on the increase, due to strong market demand for mānuka honey driving the increase in hive numbers, increasing by 21% in the last year alone (New Zealand Ministry for Primary Industries, 2016). This report also highlighted the concern beekeepers have in keeping bees in heavily wasp infected areas. This increase in honey production may mean increased future competition between honey bees and vespids wasps. These higher densities of both honey bee and *V. vulgaris* wasps may mean greater incidences of pathogen spillover. However, more evidence is needed to confirm potential transmission of microbiota, including increased sample size.

Is the microbiome of *V. vulgaris* similar to microbiomes of related hymenopterans?

Throughout nature, hosts provide niche microhabitats with each with distinct abiotic environments, which shape the structure and functioning of microbial communities. Despite these different factors influencing microbial community structures, microbiota between communities is connected to each other and share microbial diversity (Berg, Krause, & Mendes, 2015). The hologenome theory of evolution, states that this microbial diversity about a host can be viewed as a superorganism, where microorganisms play a crucial role in the evolution of the host (Rosenberg & Zilber-Rosenberg, 2013).

Most insects have dozens of microbial species in their guts, while the more complex mammalian guts may contain thousands, increasing in diversity in herbivores (Dillon & Dillon, 2004; Ley et al., 2008). Certain insects have distinct gut structures which create microhabitats for microbiota. These structures such as midgut sacs and tubular outgrowths called ceca or crypts can then host specific bacteria (Y. Kikuchi, Hosokawa, & Fukatsu,

2008), and others with less complicated guts may regulate microbiota through pH and oxygen gradients (Brune, Emerson, & Breznak, 1995). These differences in gut morphology and conditions can cause specificity in the microbiota. However, there are cross-kingdom similarities seen in microbiome ecology as is observed in interspecies insect systems (Berg et al., 2015).

In this study (Chapter 3), although no significant dissimilarity was found among host microbial communities, these comparative analyses revealed shared microbiota among hosts as well as well observed differences. The similarity across hosts, of nineteen prokaryote taxa from six phyla, indicates that among hosts there is a shared or possible core microbiota. Maintaining a core set of functional diversity is likely through vertical transmission allowing persistence and evolution through the generations (Berg et al., 2015). Proteobacteria were the most prevalent shared phyla across all hosts with seven genera belonging to the order Rhizobiales and the Gammaproteobacteria genus *Pseudomonas*. Nitrogen-fixing bacteria are common across insect species and similarly in plant roots provides a nitrogen source in insects (Behar et al., 2005; Desai & Brune, 2012). In particular, this commonality of Rhizobiales *Pseudomonas* and *Streptomyces* in roots and gut systems have been demonstrated to have similar functions (Ramirez-Puebla et al., 2013). In addition, less abundant shared taxa observed across hosts included Acidobacteria, Actinobacteria (*Acidothrmus* and *Actinoplanes*), Ktedonobacteria and the family Tepidisphaeraceae. These trends in shared microbiota have also been observed in other studies of insect-associated microbial communities and infer mutualistic importance in the evolution of insects (Jones et al., 2013; N. A. Moran et al., 2012).

All hymenopteran hosts were observed to host Lactic Acid Bacteria (LAB), with each hymenopteran hosting three or more genera. However nine LAB genera were found in *V. vulgaris*. LAB in insects are modulators of insect health (Vásquez et al., 2012). Known in honey bees and bumble bees in particular (Praet et al., 2015; Rokop et al., 2015) as an endosymbiont providing antimicrobial properties for pathogen defence (Forsgren et al., 2010; Vásquez et al., 2012).

The microbiome of *V. vulgaris* was variable among samples, and overall it was difficult to infer similarity to other hosts. Based on the wide-ranging microbiota seen in *V. vulgaris*, similarities of microbiota were seen in each hymenopteran host, highlighting the lack of

characteristic microbiota in *V. vulgaris*, suggesting a non-dependence on gut flora for nutrition due to their extensive and varied diet, as observed in other studies (Cruden & Markovetz, 1987; Evison et al., 2012; Reeson et al., 2003). This variation seen in *V. vulgaris* samples may be attributed to variation in habitat, with *Vespula* wasps typically foraging within a few hundred metres of their nest (Edwards 1980). As three nests were from two differing ranges, this may also contribute to the lack of characteristic microbiota and variation in samples.

Is there specificity seen in the microbiome of *V. vulgaris* and related hymenopterans?

In the past, host microbiome research has focused on the specificity of host pathogens in search of biological controls (Federici & Maddox, 1996). While pathogen specificity is an important avenue of investigation, endosymbiont specificity is also paramount in elucidating microbial functioning and the roles these microbiotas have in the host (Bignell, 2016; E. Crotti et al., 2010; Elena Crotti et al., 2013; Feldhaar, 2011; Hughes, Pierce, & Boomsma, 2008; Salem, Kreutzer, Sudakaran, & Kaltenpoth, 2013).

There was a lack of dissimilarity between host hymenopteran communities seen in this study (Chapter 3), however there were incidences of specific microbiota observed in hosts. The *V. vulgaris* microbiome community consisted of 13 prokaryotic, 20 microbial eukaryotes and two viral taxa, illustrating the most diversity unique to one host. However, this result is based on counts and may be a result of sample disparity and low replication of samples in other hymenopteran hosts. Specificity was also observed in the other hymenopteran hosts. *Linepithema humile* had no specific prokaryotes, but harboured 14 additional microbial eukaryotes not seen in the other hosts, in addition to *Linepithema humile Virus 1*. *Vespula germanica* also had no specific prokaryotes but hosted five additional microbial eukaryotes and *Sacbrood virus*. One prokaryote was unique to *B. terrestris*, the genus *Segniliparus* in addition to four microbial eukaryotes and *Raphanus latent Virus*. Viral specificity was seen in the *A. mellifera* microbiome, hosting three viral phylotypes *Prunus Necrotic Ringspot Virus*, *Lake Sinai Virus 1* and *A. mellifera Filamentous Virus* in addition to seven microbial eukaryotes.

The relative abundance of taxa may also demonstrate specificity, potentially indicating an important function in the host. For example, all hymenopteran hosts hosted *Wolbachia*

and *Spiroplasma* with the exception of *L. humile*. However, the significant relative abundance of *Wolbachia* seen in *B. terrestris*, and *Spiroplasma* in *A. mellifera* and *V. germanica* was notable. All three *B. terrestris* samples exhibited similar relative abundances of *Wolbachia* (23-27%), each taken from separate sites.

Wolbachia and *Spiroplasma* are known to infect many insects (Anbutsu & Fukatsu, 2011; Hilgenboecker et al., 2008; J. H. Werren et al., 1995; J H Werren & Windsor, 2000; Zug & Hammerstein, 2015). Both of these genera also have varying strains, displaying varying functions. However, both *Spiroplasma* and *Wolbachia* have been implicated in host protection in addition to host reproductive system manipulations, potentially inferring the same functions in these hymenopterans (Bourtzis et al., 2000; Eleftherianos et al., 2013; Glaser & Meola, 2010; Haselkorn, 2010; Hedges et al., 2008; Jupatanakul et al., 2014; Kambris et al., 2010; Merçot & Poinot, 2009; Osborne et al., 2009; Regassa, 2006; John H. Werren et al., 2008; Xie et al., 2011, 2010).

Mites were specific to *A. mellifera* and *B. terrestris* microbial eukaryote communities. However mites can invade multiple host species (Eyer, Chen, Schäfer, Pettis, & Neumann, 2009). Mites such as the genus *Varroa* have been found to have a profound effect on honey bees. One study demonstrated the morphological deformities these mites have on individual colony members (Bowen-Walker & Gunn, 2001). Pathogens can also be horizontally transmitted from mite to host. This is particularly true for honey bee viruses including the widespread DWV (Ribière, Ball, & Aubert, n.d.). In this study *A. mellifera* hosted the highest diversity of viruses, indicating a potential correlation with mite presence and the potential spillover of infections from mites to bees. *Bombus terrestris* also had two mite taxa present. However, the diversity of virus was reduced in comparison to *A. mellifera*. Also only one bee pathogen was present (BQCV) in addition to two plant virus, *Arabis Mosaic Virus* and *Raphanus latent virus*. The reduction in viral phylotypes may correlate to the increase in *Wolbachia* seen in *B. terrestris*, which may infer protection against horizontal transmission of the virus from mite to the host bumble bee.

Elucidating the specificity of microbiomes of related hymenopteran hosts both invading and beneficial hymenopterans broadens our knowledge of microbiota and possible transmission routes of pathogens This allows for better management of invaders and protection of beneficial pollinators.

Potential pathogens of *V. vulgaris*

Through bioinformatic analyses (Chapter 2 & 4) potential pathogens were highlighted and in some cases investigated further. Research suggests that *V. vulgaris* host a variety of pathogens having the potential to induce population crashes (S. A. Cameron et al., 2011; Evison et al., 2012; Richard J Harris et al., 2000; Rose et al., 1999). In Chapter 2, a shift in the microbiota from the native to the invaded range was observed. With this change, there was a loss seen in the invaded range, ERH (Heger & Jeschke, 2014; Roy, Lawson Handley, Schönrogge, Poland, & Purse, 2011) potentially explaining increased fitness in the invaded range. Native range genera from the class Gammaproteobacteria (*Rickettsiella*, *Diplorickettsia*, *Serratia*, *Enterobacter*, *Pantoea*, *Erwinia* and *Providencia*) and Bacilli (*Fructobacillus*, *Convivina*, *Leuconostoc*, *Lactococcus*, *Vagococcus*) were not seen in the invaded range.

It is unclear if these genera have pathogen qualities in *V. vulgaris*. However some of these have been observed as pathogenic in previous studies. The genus *Diplorickettsia* has previously been isolated from *Ixodes ricinus* ticks, and the species *Diplorickettsia massiliensis* has been confirmed as a human pathogen in tick-borne infections (Mathew et al., 2012; Subramanian et al., 2012). Members of the genus *Rickettsiella*, *Providencia*, *Serratia*, have also been described as pathogens of diverse invertebrates (Bouchon et al., 2012; Cruden & Markovetz, 1987; Feldhaar & Gross, 2009; Hamdi et al., 2011; Juneja & Lazzaro, 2009; Lauzon et al., 2003; Villegas & Pimenta, 2014). *Candidatus Rhabdochlamydia* was found in native larvae, however in low abundances. This genus has previously been found in yellow crazy ants *A. gracilipes* (Cooling, 2015) and the cockroach *Blatta orientalis*, where it was shown to be an intracellular bacterial pathogen, causing swelling of the abdomen and infecting ovaries and fat bodies (Corsaro et al., 2007). The protozoan *Mattesia* was identified in the native range and is reported to cause Yellow-head disease in populations of the red imported fire ant (Pereira et al., 2002). In the New Zealand range, another protozoan was identified CCA5 previously identified in arctic environments although the possible role in microbial communities remains unexplored (Stoeck et al., 2007). The entomopathogen *Conidiobolus* was found in both ranges but in higher abundances in the Belgian range. Infection with *Conidiobolus coronatus* in *Galleria*

mellonella was shown to increase phagocytic activity suggesting this species is pathogenic (Kędra et al., 2006).

In Chapter 2 there was a clear distinction of known virus found in larvae and workers that were absent in queen samples. The viral landscape was also different with the native Belgian samples predominantly hosting *RhPV*, and *ALPV* while the invaded New Zealand samples hosting the bee-related virus *KBV*, with both ranges, with *DWV*. To date, the effect these viruses have on *V. vulgaris* is unknown, and testing for active replication in *V. vulgaris* would eliminate the presence of this virus as a product of nutritional intake alone. A recent study confirmed the presence and replication of *KBV* in New Zealand *V. vulgaris* colonies and observed its high prevalence in wasps sampled (Dobelmann et al., 2017). The presence of *DWV* and *KBV* in the invaded range wasps may indicate the spill-over of these predominantly honey bee viruses potentially from the same route *V. vulgaris* acquired beneficial honey bee endosymbionts.

In Chapter 4, *V. vulgaris* nests symptomatic of an unknown pathogen was investigated. In this analysis 27 known phylotypes were identified, of which five candidates were selected as potential pathogens, *Moellerella wisconsensis*, *Aspergillus*, *Vavraia culicis floridensis*, *KBV* and *Moku Virus*). PCR and subsequent Sanger sequencing using designed primers, confirmed the identity of *M. wisconsensis*, *Aspergillus*, *KBV* and *Moku Virus*.

KBV and *Aspergillus* have previously been identified as potential pathogens in *V. vulgaris* (Dobelmann et al., 2017; Glare et al., 1996; Richard J Harris et al., 2000; Lester et al., 2015). However, this is the first time *M. wisconsensis*, *V. culicis floridensis* and *Moku Virus* have been identified in *V. vulgaris* wasps in New Zealand. *Moellerella wisconsensis* is a newly identified species and is known to be pathogenic in humans (Hickman-Brenner et al., 1984; Quevedo, Martín, & Velasco, 2006), however, there is no evidence that it is a pathogen of insects. Blasted *M. wisconsensis* Sanger sequences also matched to *Hafnia alvei*, with a 97% identity, which is a more likely match as it is a known opportunistic pathogen of insects (Kwong & Moran, 2016; J. Li et al., 2015; Zimmermann et al., 2013). *Moku virus* has been identified infecting *Vespula pensylvanica*, *A. mellifera* and *Varroa* mites in the same area of Hawaii, indicating it is highly virulent, although there is no evidence to date of its pathogenicity (Mordecai et al., 2016).

In Chapter 4 the metatranscriptome analysis indicated the presence of *Vavraia culicis subsp floridensis* in diseased *V. vulgaris* larvae. Unfortunately, primers designed and tested for this microsporidian were unsuccessful in amplifying desired sequences in the PCR and RT-qPCR analyses. The lack of amplification in *V. culicis floridensis* might be due to primers being designed based on alignment to a hypothetical mRNA protein and needs to be investigated further. Hypothetical proteins are uncharacterised or unknown proteins (Sivashankari, S Shanmughavel, 2006). However, it may be a potential pathogen for *V. vulgaris* as it is of *Culex* spp. mosquitoes, producing larval mortality (Andreadis, 2007). *Vavraia culicis* has been investigated as a biocontrol for mosquitos; spore infection reduced mosquito longevity and fecundity. However infection did not persist in the Mosquito population (Anthony et al., 1978; Reynolds, 1972; Vavra & Becnel, 2007). Although unsuccessful in this instance it is anticipated that *V. culicis* can potentially be used as a control agent of malaria by shortening the lifespan of female *Anopheles gambiae* mosquitoes prior to malaria transmission to humans (Lorenz & Koella, 2011).

Though, there was an association between the presence of five potential parasites in *V. vulgaris*, RT-qPCR analysis was inconclusive in identifying the cause of infection in these diseased nests. There was no distinct pattern in 'time since infection' and expression of pathogen loads. The potential presence of these pathogens prior to infection suggest there may be an alternative pathogen that has not been identified in this study causing the disease. Therefore further investigation is needed.

5.3 CONSTRAINTS AND FUTURE RESEARCH

The characterisation of the *V. vulgaris* microbiome provides new knowledge of microbial fitness effects in an invasive host. However, this study would benefit from a robust network analysis of these wasps in the native and invaded ranges, identifying mechanisms that facilitate invasiveness.

The overall aim of this thesis was to carry out a network analysis, with a large number of samples in both the native and invaded range, to confirm patterns in microbial taxa observed here. However, due to time constraints, this investigation was unable to be completed before submission. A substantial sample set was collect but not analysed in this

dissertation. Over three seasons (2013-2016) 89 nests were extracted from the native Belgian and invaded New Zealand range, totalling 228 samples of larvae, workers and queens. Each nest was quantified for the area in addition to worker and queen cell number using Image J software (Schindelin, Rueden, Hiner, & Eliceiri, 2015). The nests were collected in autumn when the new queen wasps were produced. The productivity of queens in each nest was used as an estimate for nest fitness. The goal was to directly relate microbial networks to the fitness of wasps in the native and introduced range. This data has been collected but was unable to be included here.

A subset of these nest measurements, in addition to samples and designed primers, were used in a co-authored paper “Fitness in invasive social wasps: the role of variation in viral load, immune response and paternity in predicting nest size and reproductive output”, published in *Oikos* (**Appendix 7.2**). This study indicated that immunity and pathogens could play a role in the relative fitness of these wasp populations.

Fat analysis data was also collected in the New Zealand range following Richards and Packer (1994) methods (Richards & Packer, 1994). Fat in insects is an indicator of general health and linked to immunity (Fellous & Lazzaro, 2011). In this analysis, I measured fat in workers, queens and drones from one season in the invaded range

As part of this thesis and network analysis of native and invaded range *V. vulgaris* samples, an RT-qPCR microarray plate was developed using designed primers and probes to target 48 microbial candidates (prokaryote, microbial eukaryote and viruses) in addition to immune genes (spanning the insect immune gene pathways). These targets previously identified in the metagenomics analysis (Chapter 2, 3 & 4). This target identification allowed for high throughput testing and quantification of the 228 *V. vulgaris* samples. These targeted microbial and immune gene results from the native and invaded range together with nest data, and fat analysis data will be analysed as part of a network analysis. This network analysis (Deng et al., 2012) will test the hypothesis that a complex interplay between a diversity of pathogens, parasites, symbionts determines the abundance and dominance of invasive species.

Sampling was a considerable constraint in this study. Initially, when the study was conceived, sampling was planned for two native range sites (England and Belgium), and

two invaded range sites (New Zealand and Argentina). The abundance of wasp populations in New Zealand and Argentina allowed for easy sampling. However in the native range especially England it was difficult to find sites due to bad wasp years (low abundances). In both native ranges, there was reliance on calls from the public to notify locations of nests, usually in people's gardens and homes, which were scarce. Therefore it was decided to focus on Belgium alone for representation of the native range.

In the invaded Argentina I had hoped to collect a subsequent set of samples to potentially add weight to the 'enemy release hypothesis' argument, that through the invasion pathway, *V. vulgaris* lost enemies leading to increased fitness in the invaded ranges. However, Mother Nature had other ideas. After successfully collecting a quota of samples in Argentina (Patagonia), I was unfortunately caught up in a volcanic eruption close to my sampling sites. This meant all my flights were cancelled and I had to endure a 30+ hour bus trip with luggage in addition to a 40kg dry shipper filled with wasp samples and liquid N₂, to make a connecting flight in Santiago Chile. To complete my journey I had to cross the Argentina/Chile border. Here I was detained by border police and questioned for hours due to the frozen contents of the dry shipper. This ended in my ejection from the border back into Argentina. Sadly after much effort to save my samples, I had to abandon the dry shipper in Mendoza to a random friend of a friend 'Diego' who would send samples ASAP to our lab in NZ. This enabled me to make my connecting flight in Chile. Six weeks later after a round trip around the Americas the samples arrived in New Zealand, however, they were putrefied and unusable, resulting in one native (Belgian) and one introduced (New Zealand) range (**Figure 5-1**).

Figure 5-1 Argentina sampling trip: escaping the ash cloud. Covered in ash: the dry shipper had seen better days. My carefully collected samples from Argentina were subsequently destroyed.

Sample replication in Chapter 2 was also low. However this study was a preliminary look at characterising the microbiota in both ranges, with the aim of elucidating targets for the Microarray. Chapter 3 analysis would also have benefitted from equal replication of samples, reducing the disparity in samples used. At the time of Illumina sequencing, I used a local provider NZGL. However there were many issues with the provider sequencing the samples and due to a substantial delay (9 months), I ended up with the resulting number of samples detailed in Chapter 3.

In the first collection season, samples were collected in *RNA Later*. It was noticed that these samples once extracted had a high carryover of contaminating salt in the extract which needed to be purified further to prevent complications in the downstream analysis. Based on the salt contamination of samples in the first sampling season, it was decided to collect all subsequent samples and snap freeze in liquid N_2 , thus improving the DNA and RNA extraction process. I would recommend in any future studies that samples are collected directly into liquid nitrogen.

Over the course of this thesis, bioinformatics was a significant theme behind much of the analyses. The primary challenge was the steep learning curve and understanding how to handle the massive datasets, as well as deciphering patterns in microbial abundance. Generally, when studies talk about the microbiome, they are talking about prokaryotes. Here I conducted an in-depth study of three datasets prokaryote, microbial eukaryote and viral. Another constraint in bioinformatics was the initial method of microbial classification. Iterations of databases such as Greengenes, (McDonald et al., 2012) recommend by QIIME, resulted in many analyses, lost time and effort. My analysis started with greengenes however due to it not being updated since 2013; I found it mostly outdated. During the analysis I noticed many taxa not identified to a low level and opted to redo all my analyses again using SILVA (Quast et al., 2013) databases which proved more fruitful. A lot of the significant taxa not identified in Greengenes were now identified using SILVA. An example of this was the identification of honey bee endosymbionts in Chapter 2. Greengenes identified two taxa as Neiserraceae and Pasturellaceae. However SILVA was successful in identifying these taxa to the genus level *Snodgrassella* and *Gilliamella*. This reclassification of taxa, using alternate databases enabled a more rounded understanding of trends and patterns seen in microbiome communities. Bioinformatics is moving at a fast pace, and it pays to have the most up to date reference databases. For example in chapter 4 analysis, I was able to identify *Moku virus* as it was recently submitted to Genbank, it remains to be seen will my other samples from Chapter 2, and 3 also have the presence of *Moku virus*. From these analyses I recommend having the most up to date sequence databases prior to alignment of sequences, keeping your dataset relevant.

Classification of sequence alignments also highlights the occasional uncertainty observed in bioinformatic methods. Most studies are completed at the 97% identity which is not always confirmatory. For example the alignment of sequences to the bacteria *M. wisconsensis* in the metatranscriptome analysis, when blasted also aligned to *Hafnia* sp. Therefore, other significant microbial taxa (Chapter 2 and 3), would benefit further confirmatory analyses. It would be beneficial to have further confirmatory analyses on these samples, as we did for Gruber et al. (2017).

Time was a limiting factor in Chapter 4. Due to receiving samples late in the season, many quick decisions had to be made, locating nests, acclimatising nests, and inoculating with an

unknown pathogen. In hindsight this chapter may have benefitted more from a more concise order of events, following Koch's postulates more closely. Failure to identify the pathogen prior to infection meant I was unable to quantify an effective inoculating dose. The unknown pathogen and lack of dose information may have limited the comparisons that could be drawn between test and control treatments. Individuality in samples may also have played a part in the variability in prevalence and lack of infection trend seen in this study. Identification of the pathogen before infection may have allowed for the isolation and growing of the pathogen in vitro. This study would also have benefitted from using more immune genes to quantify immune gene expression of bacterial (*M. wisconsensis*) and fungal (*Aspergillus*) pathogen loads. For the RT-qPCR microarray I did design primers and probes for immune genes, with confirmatory testing, but these failed on the designed microarray.

5.4 CONCLUSION

The microbiomes, including bacteria, fungi, and viruses, live within and upon all organisms and have become a growing area of research. With the advances of new technologies, it is now possible to entangle complex microbial communities found across animal kingdoms (Bahrndorff, Alemu, Alemneh, & Lund Nielsen, 2016). The bioinformatics and molecular techniques used over the course of this thesis was successful in identifying and characterising the microbiome of *V. vulgaris*. This characterisation provided a baseline of microbiota associated with *V. vulgaris*, highlighting potential candidate symbionts and pathogens for further investigation. It also allows for a more targeted approach, improving *V. vulgaris* comparative microbiome research. Therefore, broadening our understanding of the interactions among insects, endosymbionts and their environments.

6 REFERENCES

- Agrawal, A. a., & Kotanen, P. M. (2003). Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters*, 6(8), 712–715. doi:10.1046/j.1461-0248.2003.00498.x
- Agrawal, A., Kotanen, P. M., & Mitchell, C. E. (2005). Enemy release? An experiment with congeneric plant pairs and diverse above and below ground enemies. *Ecology*, 86(11), 2979–2989.
- Ahmad, I., Ahmad, F., & Pichtel, J. (2011). *Microbes and Microbial Technology*. (I. Ahmad, F. Ahmad, & J. Pichtel, Eds.), Springer. New York, NY: Springer New York. doi:10.1007/978-1-4419-7931-5
- Ahn, J.-H., Hong, I.-P., Bok, J.-I. B., Kim, B.-Y., Song, J., & Weon, H.-Y. (2012). Pyrosequencing analysis of the bacterial communities in the guts of honey bees *Apis cerana* and *Apis mellifera* in Korea. *Journal of Microbiology*, 50(5), 735–745. doi:10.1007/s12275-012-2188-0
- Aizenberg-Gershtein, Y., Izhaki, I., & Halpern, M. (2013). Do Honeybees Shape the Bacterial Community Composition in Floral Nectar? *PLoS ONE*, 8(7). doi:10.1371/journal.pone.0067556
- Akre, R. D. (1991). Wasp research: strengths, weaknesses, and future directions. *New Zealand Journal of Zoology*, 18(2), 223–227. doi:10.1080/03014223.1991.10757970
- Aliyari, R., & Ding, S. W. (2009). RNA-based viral immunity initiated by the Dicer family of host immune receptors. *Immunological Reviews*, 227(1), 176–188. doi:10.1111/j.1600-065X.2008.00722.x
- Allendorf, F., & Lundquist, L. (2003). Introduction: population biology, evolution, and control of invasive species. *Conservation Biology*, 17(1), 24–30. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1046/j.1523-1739.2003.02365.x/full>
- Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable

- Regions of Small-Subunit Ribosomal RNA Genes. *PLoS ONE*, 4(7), e6372. doi:10.1371/journal.pone.0006372
- Anbutsu, H., & Fukatsu, T. (2011). *Spiroplasma* as a model insect endosymbiont. *Environmental Microbiology Reports*, 3(2), 144–153. doi:10.1111/j.1758-2229.2010.00240.x
- Anderson, K. E., Russell, J. A., Moreau, C. S., Kautz, S., Sullam, K. E., Hu, Y., ... Wheeler, D. E. (2012). Highly similar microbial communities are shared among related and trophically similar ant species. *Molecular Ecology*, 21(9), 2282–96. doi:10.1111/j.1365-294X.2011.05464.x
- Anderson, K. E., Sheehan, T. H., Mott, B. M., Maes, P., Snyder, L., Schwan, M. R., ... Corby-Harris, V. (2013). Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). *PloS One*, 8(12), e83125. doi:10.1371/journal.pone.0083125
- Anderson, M., Gorley, R., & Clarke KR. (2008). Permanova+ for Primer: Guide to Software and Statistical Methods. Plymouth, UK: PRIMER-E.
- Anderson, M., & Willis, T. (2003). Canonical Analysis of Principal Coordinates : A Useful Method of Constrained Ordination for Ecology. *Ecology*, 84(2), 511–525. doi:10.1890/0012-9658(2003)084
- Andert, J., Marten, A., Brandl, R., & Brune, A. (2010). Inter- and intraspecific comparison of the bacterial assemblages in the hindgut of humivorous scarab beetle larvae (*Pachnoda* spp.). *FEMS Microbiology Ecology*, 74(2), 439–449. doi:10.1111/j.1574-6941.2010.00950.x
- Andreadis, T. G. (2007). Microsporidian Parasites of Mosquitoes. *Journal of the American Mosquito Control Association*, 23(sp2), 3–29. doi:10.2987/8756-971X(2007)23[3:MPOM]2.0.CO;2
- Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Anthony, D. W., Savage, K. E., Hazard, E. I., Avery, S. W., Boston, M. D., & Oldacre, S. W.

- (1978). Field tests with *Nosema algerae* Vavra and Undeen (Microsporida: Nosematidae) against *Anopheles albimanus* Wiedmann in Panama. *Entomological Society of America*.
- Archer, M. E. (1981). Successful and unsuccessful development of colonies of *Vespula vulgaris* (Linn.) (Hymenoptera: Vespidae). *Ecological Entomology*, 6(1), 1–10. Retrieved from <http://dx.doi.org/10.1111/j.1365-2311.1981.tb00966.x>
- Archer, M. E. (1985). Population dynamics of the social wasps *Vespula vulgaris* and *Vespula germanica* in England. *Journal of Animal Ecology*, 54(2), 473–485. Retrieved from <http://www.cabdirect.org/abstracts/19850526028.html>
- Archer, M. E. (2001). Changes in abundance of *Vespula germanica* and *V. vulgaris* in England. *Ecological Entomology*, 26(1), 1–7. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2311.2001.00296.x/full>
- Archer, M. E. (2012). *Vespine Wasps of the World: Behaviour, Ecology & Taxonomy of the Vespinae*. SRI Scientific Press:2012.
- Austin, A., & Dowton, M. (2000). *The Hymenoptera: Evolution, Biodiversity, and Biological Control*. CSIRO Publishing.
- Azambuja, P., Garcia, E. S., & Ratcliffe, N. a. (2005). Gut microbiota and parasite transmission by insect vectors. *Trends in Parasitology*, 21(12), 568–572. doi:10.1016/j.pt.2005.09.011
- Azzouna, A., Greve, P., & Martin, G. (2004). Sexual differentiation traits in functional males with female genital apertures (σ^{fga}) in the woodlice *Armadillidium vulgare* Latr. (Isopoda, Crustacea). *General and Comparative Endocrinology*, 138(1), 42–49. doi:10.1016/j.ygcen.2004.05.003
- Bahrndorff, S., Alemu, T., Alemneh, T., & Lund Nielsen, J. (2016). The Microbiome of Animals: Implications for Conservation Biology. *International Journal of Genomics*, 2016(January), 1–7. doi:10.1155/2016/5304028
- Bailey, L., & Ball, B. V. V. (Brenda V. . (1991). *Honey Bee Pathology* (2nd ed.). London: Academic Press.

- Ban, L., Ahmed, E., Ninkovic, V., Delp, G., & Glinwood, R. (2008). Infection with an insect virus affects olfactory behaviour and interactions with host plant and natural enemies in an aphid. *Entomologia Experimentalis et Applicata*, 127(2), 108–117. doi:10.1111/j.1570-7458.2008.00675.x
- Barlow, N. D., Beggs, J. R., & Barron, M. C. (2002). Dynamics of common wasps in New Zealand beech forests: a model with density dependence and weather. *Journal of Animal Ecology*, 71(4), 663–671. doi:10.1046/j.1365-2656.2002.00630.x
- Basset, Y., Cizek, L., Cuenoud, P., Didham, R. K., Guilhaumon, F., Missa, O., ... Laponche, M. (2012). Arthropod Diversity in a Tropical Forest. *Science*, 338(6113), 1481–1484. doi:10.1126/science.1226727
- Beggs, J. R. (2001). The ecological consequences of social wasps (*Vespula* spp.) invading an ecosystem that has an abundant carbohydrate resource. *Biological Conservation*, 99(1), 17–28. doi:10.1016/S0006-3207(00)00185-3
- Beggs, J. R., Brockerhoff, E. G., Corley, J. C., Kenis, M., Masciocchi, M., Muller, F., ... Villemant, C. (2011). Ecological effects and management of invasive alien Vespidae. *BioControl*, 56(4), 505–526. doi:10.1007/s10526-011-9389-z
- Beggs, J. R., & Rees, J. S. (1999). Restructuring of Lepidoptera communities by introduced *Vespula* wasps in a New Zealand beech forest. *Oecologia*, 119(4), 565–571.
- Beggs, J. R., Rees, J. S., Toft, R. J., Dennis, T. E., & Barlow, N. D. (2008). Evaluating the impact of a biological control parasitoid on invasive *Vespula* wasps in a natural forest ecosystem. *Biological Control*, 44(3), 399–407. doi:10.1016/j.biocontrol.2007.10.016
- Beggs, J. R., & Wilson, P. (1991). The kaka *Nestor meridionalis*, a New Zealand parrot endangered by introduced wasps and mammals. *Biological Conservation*, 56, 23–38. Retrieved from <http://www.sciencedirect.com/science/article/pii/0006320791900860>
- Behar, A., Yuval, B., & Jurkevitch, E. (2005). Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly *Ceratitis capitata*. *Molecular Ecology*, 14(9), 2637–2643. doi:10.1111/j.1365-294X.2005.02615.x

- Behar, A., Yuval, B., & Jurkevitch, E. (2008). Gut bacterial communities in the Mediterranean fruit fly (*Ceratitis capitata*) and their impact on host longevity. *Journal of Insect Physiology*, 54(9), 1377–1383. doi:10.1016/j.jinsphys.2008.07.011
- Benjamino, J., & Graf, J. (2016). Characterization of the Core and Caste-Specific Microbiota in the Termite, *Reticulitermes flavipes*. *Frontiers in Microbiology*, 7(February), 1–14. doi:10.3389/fmicb.2016.00171
- Berenyi, O., Bakonyi, T., Derakhshifar, I., Koglberger, H., & Nowotny, N. (2006). Occurrence of Six Honeybee Viruses in Diseased Austrian Apiaries. *Applied and Environmental Microbiology*, 72(4), 2414–2420. doi:10.1128/AEM.72.4.2414-2420.2006
- Berg, G., Krause, R., & Mendes, R. (2015). Cross-kingdom similarities in microbiome ecology and biocontrol of pathogens. *Frontiers in Microbiology*, 6(NOV), 1–5. doi:10.3389/fmicb.2015.01311
- Bhaduri, A., Qu, K., Lee, C. S., Ungewickell, A., & Khavari, P. A. (2012). Rapid identification of non-human sequences in high-throughput sequencing datasets. *Bioinformatics*, 28(8), 1174–1175. doi:10.1093/bioinformatics/bts100
- Bignell, D. E. (2016). The Role of Symbionts in the Evolution of Termites and Their Rise to Ecological Dominance in the Tropics. In C. J. Hurst (Ed.), *The Mechanistic Benefits of Microbial Symbionts, Advances in Environmental Microbiology 2* (pp. 121–172). doi:10.1007/978-3-319-28068-4_6
- Bode, H. B. (2009). Insects: True Pioneers in Anti-Infective Therapy and What We Can Learn from Them. *Angewandte Chemie International Edition*, 48(35), 6394–6396. doi:10.1002/anie.200902152
- Boland, C. R. J. R. J. (2004). Introduced cane toads *Bufo marinus* are active nest predators and competitors of rainbow bee-eaters *Merops ornatus*: observational and experimental evidence. *Biological Conservation*, 120(1), 53–62. doi:10.1016/j.biocon.2004.01.025
- Boncristiani, H., Underwood, R., Schwarz, R. S., Evans, J. D., Pettis, J. S., & VanEngelsdorp, D. (2012). Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. *Journal of Insect Physiology*, 58(5), 613–620.

doi:10.1016/j.jinsphys.2011.12.011

- Bonning, B. C. (2009). The Dicistroviridae: An emerging family of invertebrate viruses. *Virologica Sinica*, 24(5), 415–427. doi:10.1007/s12250-009-3044-1
- Boots, M. (2004). Modelling insect diseases as functional predators. *Physiological Entomology*, 29(3), 237–239. doi:10.1111/j.0307-6962.2004.00403.x
- Bordenstein, S. R., Marshall, M. L., Fry, A. J., Kim, U., & Wernegreen, J. J. (2006). The tripartite associations between bacteriophage, *Wolbachia*, and arthropods. *PLoS Pathogens*, 2(5), 384–393. doi:10.1371/journal.ppat.0020043
- Bouchon, D., Cordaux, R., & Pierre, G. (2012). *Rickettsiella*, intracellular pathogens of Arthropods. In *Manipulative Tenants Bacteria Associated with Arthropods* (pp. 127–148). Boca Raton, FL: CRC Press.
- Boucias, D. G., & Pendland, J. C. (1998). *Principles of Insect Pathology*. Boston, MA: Springer US. doi:10.1007/978-1-4615-4915-4
- Bourtzis, K., Pettigrew, M. M., & O'Neill, S. L. (2000). *Wolbachia* neither induces nor suppresses transcripts encoding antimicrobial peptides. *Insect Molecular Biology*, 9(6), 635–639. doi:10.1046/j.1365-2583.2000.00224.x
- Bowen-Walker, P. L., & Gunn, A. (2001). The effect of the ectoparasitic mite, *Varroa destructor* on adult worker honeybee (*Apis mellifera*) emergence weights, water, protein, carbohydrate, and lipid levels. *Entomologia Experimentalis et Applicata*, 101(3), 207–217. doi:10.1046/j.1570-7458.2001.00905.x
- Brenton-Rule, E. C., Baty, J. W., Russell, J. F. E. J., Saunders, M., & Lester, P. J. (2017). Honey bee hive collapse driven by a combination of an invasive ant and viral pathogens. *Unpublished*.
- Brenton-Rule, E., Frankel, S., & Lester, P. (2016). Improving Management of Invasive Species approach to pre- and post border pests. *Policy Quarterly*, 12(1), 17–25.
- Breznak, J. A., Brill, W. J., Mertins, J. W., & Coppel, H. C. (1973). Nitrogen Fixation in Termites. *Nature*, 244(5418), 577–580. doi:10.1038/244577a0
- Bristow, P. R., & Martin, R. R. (1999). Transmission and the role of honeybees in field spread

- of blueberry shock ilarvirus, a pollen-borne virus of highbush blueberry. *Phytopathology*, 89(2), 124–130. doi:10.1094/PHYTO.1999.89.2.124
- Brockerhoff, E., Barratt, B., Beggs, J., Fagan, L., Kay, M., Phillips, C., & Vink, C. (2010). Impacts of exotic invertebrates on New Zealand's indigenous species and ecosystems. *New Zealand Journal of Ecology*, 34(1), 158–174.
- Brodeur, J. (2012). Host specificity in biological control: insights from opportunistic pathogens. *Evolutionary Applications*, 5(5), 470–480. doi:10.1111/j.1752-4571.2012.00273.x
- Brown, R. L. (2013). *Chemical ecology of invasive social wasps in New Zealand*. The University of Auckland.
- Brucker, R. M., Funkhouser, L. J., Setia, S., Pauly, R., & Bordenstein, S. R. (2012). Insect Innate Immunity Database (IIID): an annotation tool for identifying immune genes in insect genomes. *PloS One*, 7(9), e45125. doi:10.1371/journal.pone.0045125
- Brune, A., Emerson, D., & Breznak, J. A. (1995). The Termite Gut Microflora as an Oxygen Sink: Microelectrode Determination of Oxygen and pH Gradients in Guts of Lower and Higher Termites. *Applied and Environmental Microbiology*.
- Brunner, F. S., Schmid-Hempel, P., & Barribeau, S. M. (2014). Protein-poor diet reduces host-specific immune gene expression in *Bombus terrestris*. *Proceedings of the Royal Society B: Biological Sciences*, 281(1786), 20140128–20140128. doi:10.1098/rspb.2014.0128
- Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2015). Antiviral defense mechanisms in honey bees. *Current Opinion in Insect Science*, 10(Ccd), 71–82. doi:10.1016/j.cois.2015.04.016
- Brysch-Herzberg, M. (2004). Ecology of yeasts in plant–bumblebee mutualism in Central Europe. *FEMS Microbiology Ecology*, 50(2), 87–100. doi:10.1016/j.femsec.2004.06.003
- Buchfink, B., Xie, C., & Huson, D. H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, 12(1), 59–60. doi:10.1038/nmeth.3176

- Budge, G. E., Adams, I., Thwaites, R., Pietravalle, S., Drew, G. C., Hurst, G. D. D., ... Brown, M. (2016). Identifying bacterial predictors of honey bee health. *Journal of Invertebrate Pathology*, 141, 41–44. doi:10.1016/j.jip.2016.11.003
- Burne, A. R., Haywood, J., & Lester, P. J. (2014). Density-dependent effects of an invasive wasp on the morphology of an endemic New Zealand ant. *Biological Invasions*, 17(1), 327–335. doi:10.1007/s10530-014-0731-0
- Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., ... Wittwer, C. T. (2009). The MIQE guidelines: Minimum Information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611–622.
- Butler, D. (2010). Human genome at ten: Science after the sequence. *Nature*, 465(7301), 1000–1001. doi:10.1038/4651000a
- Butler, W. R. (2005). Novel mycolic acid-containing bacteria in the family Segniliparaceae fam. nov., including the genus *Segniliparus* gen. nov., with descriptions of *Segniliparus rotundus* sp. nov. and *Segniliparus rugosus* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 55(4), 1615–1624. doi:10.1099/ijs.0.63465-0
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST plus: architecture and applications. *BMC Bioinformatics*, 10(421), 1. doi:Artn 421\nDoi 10.1186/1471-2105-10-421
- Camacho, C., Madden, T. L., Coulouris, G., Avagyan, V., Ma, N., Tao, T., & Agarwala, R. (2010). NCBI BLAST user manual, 1–30. Retrieved from papers://560067a4-156f-4d6d-b6cc-2f64c208798b/Paper/p7513
- Cameron, R. C., Duncan, E. J., & Dearden, P. K. (2013). Stable reference genes for the measurement of transcript abundance during larval caste development in the honeybee. *Apidologie*, 44(4), 357–366. doi:10.1007/s13592-012-0187-0
- Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*, 108(2), 662–667. doi:10.1073/pnas.1014743108

- Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., & Knight, R. (2010). PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics*, 26(2), 266–267. doi:10.1093/bioinformatics/btp636
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... Knight, R. (2010). QIIME allows analysis of high- throughput community sequencing data. *Nature Publishing Group*, 7(5), 335–336. doi:10.1038/nmeth0510-335
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6(8), 1621–1624. doi:10.1038/ismej.2012.8
- Cariveau, D. P., Elijah Powell, J., Koch, H., Winfree, R., & Moran, N. A. (2014). Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (*Bombus*). *The ISME Journal*, 8(12), 1–11. doi:10.1038/ismej.2014.68
- Chandler, D., Davidson, G., Grant, W. P., Greaves, J., & Tatchell, G. M. (2008). Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. *Trends in Food Science & Technology*, 19(5), 275–283. doi:10.1016/j.tifs.2007.12.009
- Chandler, J. A., Lang, J. M., Bhatnagar, S., Eisen, J. A., & Kopp, A. (2011). Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. *PLoS Genetics*, 7(9), e1002272. doi:10.1371/journal.pgen.1002272
- Chen, Y., Evans, J. D., & Feldlaufer, M. F. (2006). Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 92(3), 152–9. doi:10.1016/j.jip.2006.03.010
- Chen, Y., Zhao, Y., Hammond, J., Hsu, H. T., Evans, J. D., & Feldlaufer, M. F. (2011). Multiple virus infections in the honey bee and genome divergence of honey bee viruses. *Journal of Invertebrate Pathology*, 87(2–3), 84–93. doi:10.1016/j.jip.2004.07.005
- Childress, A. M. (1987). Bee-Mediated Transmission of *Blueberry Leaf Mottle Virus* Via Infected Pollen in Highbush Blueberry. *Phytopathology*, 77(2), 167. doi:10.1094/Phyto-77-167

- Christian, C. E. (2001). Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature*, 413(6856), 635–639. doi:10.1038/35098093
- Cisarovsky, G., Koch, H., & Schmid-Hempel, P. (2012). A field study on the influence of food and immune priming on a bumblebee-gut parasite system. *Oecologia*, 170(3), 877–884. doi:10.1007/s00442-012-2333-9
- Clapperton, B. K., Alspach, P. a., Moller, H., & Matheson, A. G. (1989). The impact of common and German wasps (Hymenoptera: Vespidae) on the New Zealand beekeeping industry. *New Zealand Journal of Zoology*, 16(3), 325–332. doi:10.1080/03014223.1989.10422897
- Clarke, K. R., & Ainsworth, M. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, 92(3), 205–219. doi:10.3354/meps092205
- Clarke, K. R., & Warwick, R. M. (2001). Change in marine communities. An approach to statistical analysis and interpretation, 2nd Edition. *Natural Environment Research Council*. doi:1
- Cohen, J. (2017). The Evolution of Koch's Postulates. In *Infectious Diseases* (p. 1–3.e1). Elsevier. doi:10.1016/B978-0-7020-6285-8.00001-0
- Colautti, R. I., Ricciardi, A., Grigorovich, I. a., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7(8), 721–733. doi:10.1111/j.1461-0248.2004.00616.x
- Colla, S. R., Otterstatter, M. C., Gegear, R. J., & Thomson, J. D. (2006). Plight of the bumble bee: Pathogen spillover from commercial to wild populations. *Biological Conservation*, 129(4), 461–467. doi:10.1016/j.biocon.2005.11.013
- Colman, D. R., Toolson, E. C., & Takacs-Vesbach, C. D. (2012). Do diet and taxonomy influence insect gut bacterial communities? *Molecular Ecology*, 21(20), 5124–5137. doi:10.1111/j.1365-294X.2012.05752.x
- Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M., & Robles, M. (2005).

- Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, 21(18), 3674–3676. doi:10.1093/bioinformatics/bti610
- Convention on Biological Diversity. (2008). *Alien Species that Threaten Ecosystems, Habitats or Species. Convention on Biological Diversity (CBD) United Nations Review* (Vol. 2).
- Cook, S. C., & Davidson, D. W. (2006). Nutritional and functional biology of exudate-feeding ants. *Entomologia Experimentalis et Applicata*, 118(1), 1–10. doi:10.1111/j.1570-7458.2006.00374.x
- Cooling, M. (2015). *Population dynamics and pathogens of the invasive yellow crazy ant (Anoplolepis gracilipes) in Arnhem*. Retrieved from <http://hdl.handle.net/10063/5032>
- Cornman, R. S., Tarpy, D. R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J. S., ... Evans, J. D. (2012). Pathogen webs in collapsing honey bee colonies. *PloS One*, 7(8), e43562. doi:10.1371/journal.pone.0043562
- Corsaro, D., Thomas, V., Goy, G., Venditti, D., Radek, R., & Greub, G. (2007). “*Candidatus Rhabdochlamydia crassificans*”, an intracellular bacterial pathogen of the cockroach *Blatta orientalis* (Insecta: Blattodea). *Systematic and Applied Microbiology*, 30(3), 221–228. doi:10.1016/j.syapm.2006.06.001
- Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., ... Lipkin, W. I. (2007). A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder. *Science*, 318(5848), 283–287. doi:10.1126/science.1146498
- Cox, M. P., Peterson, D. A., & Biggs, P. J. (2010). SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. *BMC Bioinformatics*, 11(1), 485. doi:10.1186/1471-2105-11-485
- Craig, J., Anderson, S., Clout, M., Creese, B., Mitchell, N., Ogden, J., ... Ussher, G. (2000). Conservation Issues in New Zealand. *Annual Review of Ecology and Systematics*. doi:10.1146/annurev.ecolsys.31.1.61

- Cremer, S., Armitage, S. A. O., & Schmid-Hempel, P. (2007). Social Immunity. *Current Biology*, 17(16), R693–R702. doi:10.1016/j.cub.2007.06.008
- Crosland, M. W. J. (1991). The spread of the social wasp, *Vespula germanica*, in Australia. *New Zealand Journal of Zoology*, 18(4), 375–387. doi:10.1080/03014223.1991.10422843
- Crotti, E., Balloi, A., Hamdi, C., Sansonno, L., Marzorati, M., Gonella, E., ... Daffonchio, D. (2012). Microbial symbionts: a resource for the management of insect-related problems. *Microbial Biotechnology*, 5(3), 307–317. doi:10.1111/j.1751-7915.2011.00312.x
- Crotti, E., Rizzi, A., Chouaia, B., Ricci, I., Favia, G., Alma, A., ... Daffonchio, D. (2010). Acetic Acid Bacteria, Newly Emerging Symbionts of Insects. *Applied and Environmental Microbiology*, 76(21), 6963–6970. doi:10.1128/AEM.01336-10
- Crotti, E., Sansonno, L., Prosdocimi, E. M., Vacchini, V., Hamdi, C., Cherif, A., ... Balloi, A. (2013). Microbial symbionts of honeybees: a promising tool to improve honeybee health. *New Biotechnology*, 30(6), 716–722. doi:10.1016/j.nbt.2013.05.004
- Crozier, R. H., & Fjerdingstad, E. J. (2001). Polyandry in social Hymenoptera disunity in diversity? *Annales Zoologici Fennici*, 38(3), 267–285.
- Cruden, D. L., & Markovetz, A. J. (1987). Microbial Ecology of the Cockroach Gut. *Annual Review of Microbiology*, 41(1), 617–643. doi:10.1146/annurev.mi.41.100187.003153
- D'Antonio, C. M., & Vitousek, P. M. (1992). Biological Invasions by Exotic Grasses, the Grass/Fire Cycle, and Global Change. *Annual Review of Ecology and Systematics*, 23(1), 63–87. doi:10.1146/annurev.es.23.110192.000431
- Dale, C., & Moran, N. A. (2006). Molecular interactions between bacterial symbionts and their hosts. *Cell*, 126(3), 453–65. doi:10.1016/j.cell.2006.07.014
- Dangel, A. W., Shen, L., Mendoza, A. R., Wu, L. chu, & Yu, C. Y. (1995). Human helicase gene SKI2W in the HLA class III region exhibits striking structural similarities to the yeast antiviral gene SKI2 and to the human gene KIAA0052: Emergence of a new gene family. *Nucleic Acids Research*, 23(12), 2120–2126. doi:10.1093/nar/23.12.2120

- Daniel, R. (2005). The metagenomics of soil. *Nature Reviews. Microbiology*, 3(6), 470–8. doi:10.1038/nrmicro1160
- de Miranda, J. R. (2004). Complete nucleotide sequence of *Kashmir bee virus* and comparison with *acute bee paralysis virus*. *Journal of General Virology*, 85(8), 2263–2270. doi:10.1099/vir.0.79990-0
- de Miranda, J. R., Bailey, L., Ball, B. V, Blanchard, P., Budge, G. E., Chejanovsky, N., ... van der Steen, J. J. M. (2013). Standard methods for virus research in *Apis mellifera*. *Journal of Apicultural Research*, 52(4), 1–56. doi:10.3896/IBRA.1.52.4.22
- de Miranda, J. R., Cordoni, G., & Budge, G. (2010). The *Acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus* complex. *Journal of Invertebrate Pathology*, 103(SUPPL. 1). doi:10.1016/j.jip.2009.06.014
- de Vries, E. J., Jacobs, G., Sabelis, M. W., Menken, S. B. J., & Breeuwer, J. A. J. (2004). Diet-dependent effects of gut bacteria on their insect host: the symbiosis of *Erwinia* sp. and western flower thrips. *Proceedings of the Royal Society B: Biological Sciences*, 271(1553), 2171–2178. doi:10.1098/rspb.2004.2817
- Deng, Y., Jiang, Y.-H., Yang, Y., He, Z., Luo, F., & Zhou, J. (2012). Molecular ecological network analyses. *BMC Bioinformatics*, 13, 113. doi:10.1186/1471-2105-13-113
- Department of Conservation. (2017). Predator free 2050. Retrieved from <http://www.doc.govt.nz/predator-free-2050>
- Desai, M. S., & Brune, A. (2012). Bacteroidales ectosymbionts of gut flagellates shape the nitrogen-fixing community in dry-wood termites. *The ISME Journal*, 6(7), 1302–1313. doi:10.1038/ismej.2011.194
- Dillon, R. J., & Dillon, V. M. (2004). The Gut Bacteria of Insects: Nonpathogenic Interactions. *Annual Review of Entomology*, 49(1), 71–92. doi:10.1146/annurev.ento.49.061802.123416
- Dillon, R. J., Vennard, C. T., Buckling, A., & Charnley, A. K. (2005). Diversity of locust gut bacteria protects against pathogen invasion. *Ecology Letters*, 8(12), 1291–1298. doi:10.1111/j.1461-0248.2005.00828.x

- Ding, S.-W. (2010). RNA-based antiviral immunity. *Nature Reviews. Immunology*, 10(9), 632–44. doi:10.1038/nri2824
- Dirksen, P., Marsh, S. A., Braker, I., Heitland, N., Wagner, S., Nakad, R., ... Schulenburg, H. (2016). The native microbiome of the nematode *Caenorhabditis elegans*: gateway to a new host-microbiome model. *BMC Biology*, 14(1), 38. doi:10.1186/s12915-016-0258-1
- Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, 17(1), 431–449. doi:10.1111/j.1365-294X.2007.03538.x
- Dobelmann, J., Loope, K. J., Wilson-Rankin, E., Quinn, O., Baty, J. W., Gruber, M. A. M., & Lester, P. J. (2017). Fitness in invasive social wasps: the role of variation in viral load, immune response and paternity in predicting nest size and reproductive output. *Oikos*, 126(8), 1208–1218. doi:10.1111/oik.04117
- Donovan, B. (1984). Occurrence of the common wasp, *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) in New Zealand. *New Zealand Journal of Zoology*, 11(4), 417–427. doi:10.1080/03014223.1984.10428256
- Donovan, B. J. (1989). Potential enemies of the introduced wasp parasitoid *Sphecophaga vesparum* (Hymenoptera: Ichneumonidae) in New Zealand. *New Zealand Journal of Zoology*, 16(3), 365–367. doi:10.1080/03014223.1989.10422901
- Donovan, B. J., & Read, P. E. C. (1987). Attempted biological control of social wasps, *Vespula* spp., (Hymenoptera: Vespidae) with *Sphecophaga vesparum* (Curtis) (Hymenoptera : Ichneumonidae) in New Zealand. *New Zealand Journal of Zoology*, 14(3), 329–335. doi:10.1080/03014223.1987.10423002
- Dornhaus, A., & Chittka, L. (1999). Biology and Physiology of the Lower Trypanosomatidae. *Nature*, 401(6748), 38–38. doi:10.1038/43372
- Douglas, A. E. (1998). Nutritional Interactions in Insect-Microbial Symbioses: Aphids and Their Symbiotic Bacteria *Buchnera*. *Annual Review of Entomology*, 43(1), 17–37. doi:10.1146/annurev.ento.43.1.17

- Douglas, A. E. (2009). The microbial dimension in insect nutritional ecology. *Functional Ecology*, 23(1), 38–47. doi:10.1111/j.1365-2435.2008.01442.x
- Douglas, A. E. (2015). Multiorganismal Insects: Diversity and Function of Resident Microorganisms. *Annual Review of Entomology*, 60(1), 17–34. doi:10.1146/annurev-ento-010814-020822
- Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstadter, J., & Hurst, G. D. (2008). The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biology*, 6(1), 27. doi:10.1186/1741-7007-6-27
- Duron, O., Wilkes, T. E., & Hurst, G. D. D. (2010). Interspecific transmission of a male-killing bacterium on an ecological timescale. *Ecology Letters*, 13(9), 1139–1148. doi:10.1111/j.1461-0248.2010.01502.x
- Durrer, S., & Schmid-Hempel, P. (1994). Shared Use of Flowers Leads to Horizontal Pathogen Transmission. *Proceedings of the Royal Society B: Biological Sciences*, 258(1353), 299–302. doi:10.1098/rspb.1994.0176
- Dyer, K. A., & Jaenike, J. (2005). Evolutionary Dynamics of a Spatially Structured Host-Parasite Association: *Drosophila innubila* and Male-Killing *Wolbachia*. *Evolution*, 59(7), 1518. doi:10.1554/04-666
- Dymock, J. J., Forgie, S. A., & Ameratunga, R. (1994). A survey of wasp sting injuries in urban Auckland from December to April in 1991/2 and 1992/3. *The New Zealand Medical Journal*, 107(971), 32–3. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8302500>
- Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460–2461. doi:10.1093/bioinformatics/btq461
- Edwards, R. (1980). *Social wasps - Their biology and control*. East Grinstead: The Rentokill Library.
- Eickwort, G. C. (1990). Associations of Mites with Social Insects. *Annual Review Entomology*, 35, 469–88.
- Elango, N., Hunt, B. G., Goodisman, M. A. D., & Yi, S. V. (2009). DNA methylation is

- widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. *Proceedings of the National Academy of Sciences*, 106(27), 11206–11211. doi:10.1073/pnas.0900301106
- Eleftherianos, I., Atri, J., Accetta, J., & Castillo, J. C. (2013). Endosymbiotic bacteria in insects: guardians of the immune system? *Frontiers in Physiology*, 4(March), 1–10. doi:10.3389/fphys.2013.00046
- Elliott, G. P., Wilson, P. R., Taylor, R. H., & Beggs, J. R. (2010). Declines in common, widespread native birds in a mature temperate forest. *Biological Conservation*, 143(9), 2119–2126. doi:10.1016/j.biocon.2010.05.022
- Emerson, B. C., & Gillespie, R. G. (2008). Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology & Evolution*, 23(11), 619–30. doi:10.1016/j.tree.2008.07.005
- Engel, P., Martinson, V. G., & Moran, N. A. (2012). Functional diversity within the simple gut microbiota of the honey bee. doi:10.1073/pnas.1202970109/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1202970109
- Engel, P., & Moran, N. A. (2013). The gut microbiota of insects - Diversity in structure and function. *FEMS Microbiology Reviews*, 37(5), 699–735. doi:10.1111/1574-6976.12025
- Erler, S., Denner, A., Bobiş, O., Forsgren, E., & Moritz, R. F. A. (2014). Diversity of honey stores and their impact on pathogenic bacteria of the honeybee, *Apis mellifera*. *Ecology and Evolution*. doi:10.1002/ece3.1252
- Erler, S., Popp, M., & Lattorff, H. M. G. (2011). Dynamics of immune system gene expression upon bacterial challenge and wounding in a social insect (*Bombus terrestris*). *PloS One*, 6(3), e18126. doi:10.1371/journal.pone.0018126
- Evans, J. D. J., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J.-L. L., Jiang, H., ... Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology*, 15(5), 645–656. doi:10.1111/j.1365-2583.2006.00682.x
- Evans, J. D. J., & Schwarz, R. S. (2011). Bees brought to their knees: microbes affecting honey bee health. *Trends in Microbiology*, 19(12), 614–20.

doi:10.1016/j.tim.2011.09.003

- Evison, S. E. F., Roberts, K. E., Laurenson, L., Pietravalle, S., Hui, J., Biesmeijer, J. C., ... Hughes, W. O. H. (2012). Pervasiveness of parasites in pollinators. *PloS One*, 7(1), e30641. doi:10.1371/journal.pone.0030641
- Eyer, M., Chen, Y. P., Schäfer, M. O., Pettis, J., & Neumann, P. (2009). Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. *Apidologie*, 40(4), 419–428. doi:10.1051/apido:2008051
- Fallis, A. . (2013). Options for biological control of vespula wasps in New Zealand. *Journal of Chemical Information and Modeling*, 53(9), 1689–1699. doi:10.1017/CBO9781107415324.004
- Fan, Q.-H., Zhang, Z.-Q., Brown, R., France, S., & Bennett, S. (2016). New Zealand *Pneumolaelaps* (Acari: Laelapidae): description of a new species, key to species and notes on biology. *Systematic and Applied Acarology*, 21(1), 119. doi:10.11158/saa.21.1.8
- Fawaz, M., Raut, A. A., Mishra, A., & Kambla, N. (2014). Viral Metagenomics : A Review, 2(4), 81–89.
- Federici, B. A., & Maddox, J. V. (1996). Host specificity in microbe-insect interactions - Insect control by bacterial, fungal, and viral pathogens. *Bioscience*, 46(6), 410–421.
- Feldhaar, H. (2011). Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology*, 36(5), 533–543. doi:10.1111/j.1365-2311.2011.01318.x
- Feldhaar, H., & Gross, R. (2009). Insects as hosts for mutualistic bacteria. *International Journal of Medical Microbiology : IJMM*, 299(1), 1–8. doi:10.1016/j.ijmm.2008.05.010
- Feldhaar, H., Straka, J., Krischke, M., Berthold, K., Stoll, S., Mueller, M. J., & Gross, R. (2007). Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC Biology*, 5(1), 48. doi:10.1186/1741-7007-5-48
- Fellous, S., & Lazzaro, B. P. (2011). Potential for evolutionary coupling and decoupling of larval and adult immune gene expression. *Molecular Ecology*, 20(7), 1558–1567.

doi:10.1111/j.1365-294X.2011.05006.x

Fordham, R. A. (1991). Vespulid wasps at the upper forest margin in Tongariro National Park—a threat to the native biota? *New Zealand Journal of Zoology*, 18(2), 151–153. doi:10.1080/03014223.1991.10757961

Forsgren, E., Olofsson, T. C., Vásquez, A., & Fries, I. (2010). Novel lactic acid bacteria inhibiting *Paenibacillus* larvae in honey bee larvae. *Apidologie*, 41(1), 99–108. doi:10.1051/apido/2009065

Foster, K. R., & Ratnieks, F. L. (2001). Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proceedings. Biological Sciences / The Royal Society*, 268(1463), 169–174. doi:10.1098/rspb.2000.1346

Fries, I., & Camazine, S. M. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie*, 32(3), 199–214. doi:10.1051/apido:2001122

Fürst, M. a., McMahon, D. P., Osborne, J. L., Paxton, R. J., & Brown, M. J. F. (2014). Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature*, 506(7488), 364–6. doi:10.1038/nature12977

Gálvez, D., & Chapuisat, M. (2014). Immune priming and pathogen resistance in ant queens. *Ecology and Evolution*, 4(10), 1761–1767. doi:10.1002/ece3.1070

Gambino, P. V. (1993). Antibiotic activity of larval saliva of *Vespula* wasps. *Journal of Invertebrate Pa- Thology*, (61), 110.

Garrido, P. M., Antúnez, K., Martín, M., Porrini, M. P., Zunino, P., & Eguaras, M. J. (2013). Immune-related gene expression in nurse honey bees (*Apis mellifera*) exposed to synthetic acaricides. *Journal of Insect Physiology*, 59(1), 113–9. doi:10.1016/j.jinsphys.2012.10.019

Genersch, E., & Aubert, M. (2010). Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). *Veterinary Research*, 41(6), 54. doi:10.1051/vetres/2010027

Georgiev, B. B., Sánchez, M. I., Vasileva, G. P., Nikolov, P. N., & Green, a J. (2007). Cestode parasitism in invasive and native brine shrimps (*Artemia* spp.) as a possible factor

- promoting the rapid invasion of *A. franciscana* in the Mediterranean region. *Parasitology Research*, 101(6), 1647–55. doi:10.1007/s00436-007-0708-3
- Ghera, R. L., Werren, J. H., Weisburg, W., Cote, R., Woese, C. R., Mandelco, L., & Brenner, D. J. (1991). NOTES: *Arsenophonus nasoniae* gen. nov., sp. nov., the Causative Agent of the Son-Killer Trait in the Parasitic Wasp *Nasonia vitripennis*. *International Journal of Systematic Bacteriology*, 41(4), 563–565. doi:10.1099/00207713-41-4-563
- Gil, R., Latorre, A., & Moya, A. (2004). Bacterial endosymbionts of insects: insights from comparative genomics. *Environmental Microbiology*, 6(11), 1109–1122. doi:10.1111/j.1462-2920.2004.00691.x
- Gildow, F. E., & D’Arcy, C. J. (1988). Barley and oats as reservoirs for an aphid virus and the influence on barley yellow dwarf virus transmission. *Phytopathology*, 78, 811–816.
- Gildow, F. E., & D’Arcy, C. J. (1990). Cytopathology and experimental host range of *Rhopalosiphum padi virus*, a small isometric RNA virus infecting cereal grain aphids. *Journal of Invertebrate Pathology*, 55(2), 245–257. doi:10.1016/0022-2011(90)90060-J
- Gilliam, M. (1997). Identification and roles of non-pathogenic microflora associated with honey bees. *FEMS Microbiology Letters*, 155(1), 1–10. doi:10.1016/S0378-1097(97)00337-6
- Glare, T. R., Harris, R. J., & Donovan, B. J. (1996). *Aspergillus flavus* as a pathogen of wasps, *Vespula* spp., in New Zealand. *New Zealand Journal of Zoology*, 23(4), 339–344. doi:10.1080/03014223.1996.9518093
- Glaser, R. L., & Meola, M. A. (2010). The Native *Wolbachia* Endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* Increase Host Resistance to West Nile Virus Infection. *PLoS ONE*, 5(8), e11977. doi:10.1371/journal.pone.0011977
- Goka, K., Okabe, K., Yoneda, M., & Niwa, S. (2001). Bumblebee commercialization will cause worldwide migration of parasitic mites. *Molecular Ecology*, 10(8), 2095–2099. doi:10.1046/j.0962-1083.2001.01323.x
- Goodisman, M. a D. M. a D., Kovacs, J. L. J., & Hoffman, E. E. a. E. (2007). The significance

- of multiple mating in the social wasp *Vespula maculifrons*. *Evolution*, 61(9), 2260–2267. doi:10.1111/j.1558-5646.2007.00175.x
- Gordon, D. M. (2001). Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. *Microbiology*, 147(5), 1079–1085. doi:10.1099/00221287-147-5-1079
- Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. a, Amit, I., ... Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, 29(7), 644–652. doi:10.1038/nbt.1883
- Grady, E. N., MacDonald, J., Liu, L., Richman, A., & Yuan, Z.-C. (2016). Current knowledge and perspectives of *Paenibacillus*: a review. *Microbial Cell Factories*, 15(1), 203. doi:10.1186/s12934-016-0603-7
- Graystock, P., Yates, K., Evison, S. E. F., Darvill, B., Goulson, D., & Hughes, W. O. H. (2013). The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *Journal of Applied Ecology*. Retrieved from <http://doi.wiley.com/10.1111/1365-2664.12134>
- Greene, A., Ross, G. &, & Matthews, R. W. (1991). *Dolichovespula* and *Vespula*. In *The Social Biology of Wasps* (pp. 263–305). Ithaca, New York: Cornell University Press.
- Gruber, M. A. M., Cooling, M., Baty, J. W., Buckley, K., Friedlander, A., Quinn, O., ... Lester, P. J. (2017). Single-stranded RNA viruses infecting the invasive Argentine ant, *Linepithema humile*. *Scientific Reports*, 7(1), 3304. doi:10.1038/s41598-017-03508-z
- Gusteleva, L. (1975). Biosynthesis of group B vitamins by yeasts--symbionts of xylophagous insects. *Mikrobiologiia*.
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443–450. doi:10.1051/apido/2009076
- Haas, B. J., Gevers, D., Earl, A. M., Feldgarden, M. M., Ward, D. V., Giannoukos, G., ... Birren, B. W. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-

- pyrosequenced PCR amplicons. *Genome Research*, 21(3), 494–504. doi:10.1101/gr.112730.110
- Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M. G., Blood, P. D., Bowden, J., ... Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, 8(8), 1494–512. doi:10.1038/nprot.2013.084
- Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3(4), 307–319. doi:10.1038/nrmicro1129
- Hadfield, M. G., Miller, S. E., & Carwile, A. H. (1993). The Decimation of Endemic Hawai'ian Tree Snails by Alien Predators. *American Zoologist*, 33(6), 610–622. doi:10.1093/icb/33.6.610
- Hamdi, C., Balloi, A., Essanaa, J., Crotti, E., Gonella, E., Raddadi, N., ... Cherif, A. (2011). Gut microbiome dysbiosis and honeybee health. *Journal of Applied Entomology*, 135(7), 524–533. doi:10.1111/j.1439-0418.2010.01609.x
- Harcourt, S. J. (2002). *Disease mitigation and pathogenic control in German and common wasps, Vespula germanica and V. vulgaris (Hymenoptera : Vespidae)*. Lincoln University. Retrieved from <https://hdl.handle.net/10182/1790>
- Harris, R. J. (1991). Diet of the wasps *Vespula vulgaris* and *V. germanica* in honeydew beech forest of the South Island, New Zealand. *New Zealand Journal of Zoology*, 18(2), 159–169. doi:10.1080/03014223.1991.10757963
- Harris, R. J., & Etheridge, N. D. (2001). Comparison of baits containing fipronil and sulfluramid for the control of *Vespula* wasps. *New Zealand Journal of Zoology*, 28(1), 39–48. doi:10.1080/03014223.2001.9518255
- Harris, R. J., Harcourt, S. J., Glare, T. R., Rose, E. A. F., & Nelson, T. J. (2000). Susceptibility of *Vespula vulgaris* (Hymenoptera: Vespidae) to Generalist Entomopathogenic Fungi and Their Potential for Wasp Control. *Journal of Invertebrate Pathology*, 75(4), 251–258. doi:10.1006/jipa.2000.4928

- Harris, R. J., Moller, H., & Winterbourn, M. J. (1994). Competition for honeydew between two social wasps in South Island beech forests , New Zealand. *Insectes Sociaux*, 39(1994), 379–394.
- Harshman, L. G., & James, A. a. (1998). Differential Gene Expression in Insects: Transcriptional Control. *Annual Review of Entomology*, 43(1), 671–700. doi:10.1146/annurev.ento.43.1.671
- Harvey, J. J., Chester, S., Burke, S. A., Ansbrosio, M., Aden, T., Gose, R., ... Mawle, A. C. (2016). Comparative analytical evaluation of the respiratory TaqMan Array Card with real-time PCR and commercial multi-pathogen assays. *Journal of Virological Methods*, 228, 151–157. doi:10.1016/j.jviromet.2015.11.020
- Haselkorn, T. S. (2010). The *Spiroplasma* heritable bacterial endosymbiont of *Drosophila*. *Fly*, 4(1), 80–87. doi:10.4161/fly.4.1.10883
- Hatcher, M. J., Dick, J. T. a, & Dunn, A. M. (2006). How parasites affect interactions between competitors and predators. *Ecology Letters*, 9(11), 1253–71. doi:10.1111/j.1461-0248.2006.00964.x
- Hawley, D. M., & Altizer, S. M. (2011). Disease ecology meets ecological immunology: Understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology*, 25(1), 48–60. doi:10.1111/j.1365-2435.2010.01753.x
- Hay, D. B., Hart, B. J., & Douglas, A. E. (1993). Effects of the fungus *Aspergillus penicillioides* on the house dust mite *Dermatophagoides pteronyssinus*: an experimental re-evaluation. *Medical and Veterinary Entomology*, 7(3), 271–274. doi:10.1111/j.1365-2915.1993.tb00687.x
- Hedges, L. M., Brownlie, J. C., O'Neill, S. L., & Johnson, K. N. (2008). *Wolbachia* and Virus Protection in Insects. *Science*, 322(5902), 702–702. doi:10.1126/science.1162418
- Heger, T., & Jeschke, J. M. (2014). The enemy release hypothesis as a hierarchy of hypotheses. *Oikos*, (November 2013), no-no. doi:10.1111/j.1600-0706.2013.01263.x
- Hickman-Brenner, F. W., Huntley-Carter, G. P., & Saitoh, Y. (1984). *Moellerella*

- wisconsensis*, a new genus and species of Enterobacteriaceae found in human stool specimens. *Journal of Clinical Microbiology*, 19(4), 460–463.
- Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H. (2008). How many species are infected with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiology Letters*, 281(2), 215–220. doi:10.1111/j.1574-6968.2008.01110.x
- Hoffmann, A. A., Montgomery, B. L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P. H., Muzzi, F., ... O'Neill, S. L. (2011). Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature*, 476(7361), 454–457. doi:10.1038/nature10356
- Hoffmann, J. A. (1995). Innate immunity of insects. *Current Opinion in Immunology*, 7(1), 4–10. doi:10.1016/0952-7915(95)80022-0
- Hoggard, S. J., Wilson, P. D., Beattie, A. J., & Stow, A. J. (2013). The Effectiveness of Antimicrobial Defenses Declines With Increasing Group Size and Genetic Similarity. *Annals of the Entomological Society of America*, 106(1), 53–58. doi:10.1603/AN11187
- Hölldobler, B., & Wilson, E. (1990). *The Ants*. Springer Berlin Heidelberg.
- Hongoh, Y. (2010). Diversity and genomes of uncultured microbial symbionts in the termite gut. *Bioscience, Biotechnology, and Biochemistry*, 74(6), 1145–1151. doi:10.1271/bbb.100094
- Hosokawa, T., Kikuchi, Y., Nikoh, N., Shimada, M., & Fukatsu, T. (2006). Strict Host-Symbiont Cospeciation and Reductive Genome Evolution in Insect Gut Bacteria. *PLoS Biology*, 4(10), e337. doi:10.1371/journal.pbio.0040337
- Hubert, J., Bicianova, M., Ledvinka, O., Kamler, M., Lester, P. J., Nesvorna, M., ... Erban, T. (2017). Changes in the Bacteriome of Honey Bees Associated with the Parasite *Varroa destructor*, and Pathogens *Nosema* and *Lotmaria passim*. *Microbial Ecology*, 73(3), 685–698. doi:10.1007/s00248-016-0869-7
- Hubert, J., Erban, T., Kamler, M., Kopecky, J., Nesvorna, M., Hejdankova, S., ... Zurek, L. (2015). Bacteria detected in the honeybee parasitic mite *Varroa destructor* collected from beehive winter debris. *Journal of Applied Microbiology*, 119(3), 640–654.

doi:10.1111/jam.12899

- Hubert, J., Kamler, M., Nesvorna, M., Ledvinka, O., Kopecky, J., & Erban, T. (2016). Comparison of *Varroa destructor* and Worker Honeybee Microbiota Within Hives Indicates Shared Bacteria. *Microbial Ecology*, 72(2), 448–459. doi:10.1007/s00248-016-0776-y
- Hughes, D. P., Pierce, N. E., & Boomsma, J. J. (2008). Social insect symbionts: evolution in homeostatic fortresses. *Trends in Ecology & Evolution*, 23(12), 672–7. doi:10.1016/j.tree.2008.07.011
- Hurst, G. D. D., & Frost, C. L. (2015). Reproductive Parasitism: Maternally Inherited Symbionts in a Biparental World. *Cold Spring Harbor Perspectives in Biology*, 7(5), a017699. doi:10.1101/cshperspect.a017699
- Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Research*, 17(3), 377–386. doi:10.1101/gr.5969107
- Huson, D. H., Beier, S., Flade, I., Górská, A., El-Hadidi, M., Mitra, S., ... Tappu, R. (2016). MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. *PLOS Computational Biology*, 12(6), e1004957. doi:10.1371/journal.pcbi.1004957
- Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., ... White, O. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, 486(7402), 207–214. doi:10.1038/nature11234
- Ikeda, M., Yamada, H., Hamajima, R., & Kobayashi, M. (2013). Baculovirus genes modulating intracellular innate antiviral immunity of lepidopteran insect cells. *Virology*, 435(1), 1–13. doi:10.1016/j.virol.2012.10.016
- Illumina. (2013). 16S Metagenomic Sequencing Library Preparation. *Illumina.com*, (B), 1–28. Retrieved from http://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf
- Ishak, H. D., Miller, J. L., Sen, R., Dowd, S. E., Meyer, E., & Mueller, U. G. (2011).

- Microbiomes of ant castes implicate new microbial roles in the fungus-growing ant *Trachymyrmex septentrionalis*. *Scientific Reports*, 1(1), 20–22. doi:10.1038/srep00204
- Izard, J. (2015). *Steps in Metagenomics: Let's Avoid Garbage in and Garbage Out. Metagenomics for Microbiology*. Elsevier Inc. doi:10.1016/B978-0-12-410472-3/00001-4
- Ji, R., & Brune, A. (2001). Transformation and mineralization of 14 C-labeled cellulose, peptidoglycan, and protein by the soil-feeding termite *Cubitermes orthognathus*. *Biology and Fertility of Soils*, 33(2), 166–174. doi:10.1007/s003740000310
- Johansson, H., Dhaygude, K., Lindström, S., Helanterä, H., Sundström, L., & Trontti, K. (2013). A metatranscriptomic approach to the identification of microbiota associated with the ant *Formica exsecta*. *PLoS ONE*, 8(11), 1–11. doi:10.1371/journal.pone.0079777
- Jones, R. T., Sanchez, L. G., & Fierer, N. (2013). A cross-taxon analysis of insect-associated bacterial diversity. *PLoS One*, 8(4), e61218. doi:10.1371/journal.pone.0061218
- Juneja, P., & Lazzaro, B. P. (2009). *Providencia sneebia* sp. nov. and *Providencia burhodogranariae* sp. nov., isolated from wild *Drosophila melanogaster*. *International Journal of Systematic and Evolutionary Microbiology*, 59(5), 1108–1111. doi:10.1099/ijs.0.000117-0
- Jupatanakul, N., Sim, S., & Dimopoulos, G. (2014). The Insect Microbiome Modulates Vector Competence for Arboviruses. *Viruses*, 6(11), 4294–4313. doi:10.3390/v6114294
- Kaltenpoth, M. (2009). Actinobacteria as mutualists: general healthcare for insects? *Trends in Microbiology*, 17(12), 529–535. doi:10.1016/j.tim.2009.09.006
- Kaltenpoth, M., & Engl, T. (2014). Defensive microbial symbionts in Hymenoptera. *Functional Ecology*, 28(2), 315–327. doi:10.1111/1365-2435.12089
- Kaltenpoth, M., Göttler, W., Herzner, G., & Strohm, E. (2005). Symbiotic Bacteria Protect Wasp Larvae from Fungal Infestation. *Current Biology*, 15(5), 475–479. doi:10.1016/j.cub.2004.12.084

- Kambris, Z., Blagborough, A. M., Pinto, S. B., Blagrove, M. S. C., Godfray, H. C. J., Sinden, R. E., & Sinkins, S. P. (2010). *Wolbachia* stimulates immune gene expression and inhibits plasmodium development in *Anopheles gambiae*. *PLoS Pathogens*, 6(10), e1001143. doi:10.1371/journal.ppat.1001143
- Kapheim, K. M., Rao, V. D., Yeoman, C. J., Wilson, B. A., White, B. A., Goldenfeld, N., & Robinson, G. E. (2015). Caste-Specific Differences in Hindgut Microbial Communities of Honey Bees (*Apis mellifera*). *PLOS ONE*, 10(4), e0123911. doi:10.1371/journal.pone.0123911
- Katsnelson, A. (2015). Microbiome: The puzzle in a bee's gut. *Nature*, 521(7552), S56–S56. doi:10.1038/521S56a
- Kautz, S., Rubin, B. E. R., Russell, J. A., Moreau, C. S., Moreau, C. S., & Moreau, C. S. (2013). Surveying the microbiome of ants: Comparing 454 pyrosequencing with traditional methods to uncover bacterial diversity. *Applied and Environmental Microbiology*, 79(2), 525–534. doi:10.1128/AEM.03107-12
- Keane, R., & Crawley, M. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17(4), 164–170. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0169534702024990>
- Kędra, E., Boguś, M. I., Kędra, E., & Boguś, M. I. (2006). The influence of *Conidiobolus coronatus* on phagocytic activity of insect hemocytes. *Journal of Invertebrate Pathology*, 91(1), 50–52. doi:10.1016/j.jip.2005.06.013
- Kikuchi, Y., & Fukatsu, T. (2003). Diversity of *Wolbachia* Endosymbionts in Heteropteran Bugs. *Applied and Environmental Microbiology*, 69(10), 6082–6090. doi:10.1128/AEM.69.10.6082-6090.2003
- Kikuchi, Y., Hosokawa, T., & Fukatsu, T. (2007). Insect-Microbe Mutualism without Vertical Transmission: a Stinkbug Acquires a Beneficial Gut Symbiont from the Environment Every Generation. *Applied and Environmental Microbiology*, 73(13), 4308–4316. doi:10.1128/AEM.00067-07
- Kikuchi, Y., Hosokawa, Y., & Fukatsu, T. (2008). Diversity of bacterial symbiosis in stinkbugs. *Microbial Ecology Research Trends*.

- Kikuchi, Y., & Yumoto, I. (2013). Efficient Colonization of the Bean Bug *Riptortus pedestris* by an Environmentally Transmitted *Burkholderia* Symbiont. *Applied and Environmental Microbiology*, 79(6), 2088–2091. doi:10.1128/AEM.03299-12
- Killer, J., Vec, P., Sedlacek, I., Cernohlavkova, J., Benada, O., Hroncova, Z., ... Kofronova, O. (2014). *Vagococcus entomophilus* sp. nov., from the digestive tract of a wasp (*Vespula vulgaris*). *International Journal of Systematic and Evolutionary Microbiology*, 64(Pt 3), 731–737. doi:10.1099/ijs.0.054940-0
- Kingsolver, M. B., Huang, Z., & Hardy, R. W. (2013). Insect antiviral innate immunity: Pathways, effectors, and connections. *Journal of Molecular Biology*, 425(24), 4921–4936. doi:10.1016/j.jmb.2013.10.006
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41(1). doi:10.1093/nar/gks808
- Koch, H., Abrol, D. P., Li, J., & Schmid-Hempel, P. (2013). Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees. *Molecular Ecology*, 22(7), 2028–2044. doi:10.1111/mec.12209
- Koch, H., & Schmid-Hempel, P. (2011). Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences of the United States of America*, 108(48), 19288–19292. doi:10.1073/pnas.1110474108
- Koch, R. (1893). Ueber den augenblicklichen Stand der bakteriologischen Choleradiagnose. *Zeitschrift Für Hygiene Und Infektionskrankheiten*, 14(1), 319–338. doi:10.1007/BF02284324
- Köhler, T. (2011). *Diversity and evolutionary patterns in the bacterial gut microbiota of termites and cockroaches*.
- Köhler, T., Stingl, U., Meuser, K., & Brune, A. (2008). Novel lineages of Planctomycetes densely colonize the alkaline gut of soil-feeding termites (*Cubitermes* spp.). *Environmental Microbiology*, 10(5), 1260–1270. doi:10.1111/j.1462-

- Kumar, P. A., & Bambawale, O. M. (2002). Insecticidal Proteins of *Bacillus Thuringiensis* and Their Application in Agriculture. In *Advances in Microbial Toxin Research and Its Biotechnological Exploitation* (pp. 259–280). Boston, MA: Springer US. doi:10.1007/978-1-4757-4439-2_16
- Kuranouchi, T., Nakamura, T., Shimamura, S., Kojima, H., Goka, K., Okabe, K., & Mochizuki, A. (2006). Nitrogen fixation in the stag beetle, *Dorcus (Macrodercus) rectus (Motschulsky) (Col., Lucanidae)*. *Journal of Applied Entomology*, 130(9–10), 471–472. doi:10.1111/j.1439-0418.2006.01080.x
- Kwong, W. K., Mancenido, A. L., & Moran, N. A. (2017). Immune system stimulation by the native gut microbiota of honey bees. *Royal Society Open Science*, 4(2), 170003. doi:10.1098/rsos.170003
- Kwong, W. K., Medina, L. A., Koch, H., Sing, K.-W., Soh, E. J. Y., Ascher, J. S., ... Moran, N. A. (2017). Dynamic microbiome evolution in social bees. *Science Advances*, 3(3), e1600513. doi:10.1126/sciadv.1600513
- Kwong, W. K., & Moran, N. A. (2013). Cultivation and characterization of the gut symbionts of honey bees and bumble bees: description of *Snodgrassella alvi* gen. nov., sp. nov., a member of the family Neisseriaceae of the Betaproteobacteria, and *Gilliamella apicola* gen. nov., sp. *International Journal of Systematic and Evolutionary Microbiology*, 63(Pt 6), 2008–2018. doi:10.1099/ijs.0.044875-0
- Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. *Nature Reviews Microbiology*, 14(6), 374–384. doi:10.1038/nrmicro.2016.43
- Labandeira, C., & Sepkoski, J. (1993). Insect diversity in the fossil record. *Science*, 261(5119), 310–315. doi:10.1126/science.11536548
- Lanan, M. C., Rodrigues, P. A. P., Agellon, A., Jansma, P., & Wheeler, D. E. (2016). A bacterial filter protects and structures the gut microbiome of an insect. *The ISME Journal*, 10(8), 1–11. doi:10.1038/ismej.2015.264
- Latifian, M., Rad, B., Amani, M., & Rahkhodaei, E. (2013). Mass production of

- entomopathogenic fungi *Beauveria bassiana* (Balsamo) by using agricultural products based on liquid-solid diphasic method for date palm. *International Journal of Agriculture and Crop Sciences*, 5(19), 2337–2341. Retrieved from <http://ijagcs.com/wp-content/uploads/2013/08/2337-2341.pdf>
- Lauzon, C. R., Bussert, T. G., Sjogren, R. E., & Prokopy, R. J. (2003). *Serratia marcescens* as a bacterial pathogen of *Rhagoletis pomonella* flies (Diptera: Tephritidae). *European Journal of Entomology*, 100(1), 87–92. doi:10.14411/eje.2003.017
- Leathwick, D. M. (1997). Growth and development of queen colonies of *Vespula germanica* and *V. vulgaris*. *New Zealand Journal of Zoology*, 24(1), 17–23. doi:10.1080/03014223.1997.9518102
- Leathwick, D. M., & Godfrey, P. L. (1996). Overwintering colonies of the common wasp (*Vespula vulgaris*) in Palmerston North, New Zealand. *New Zealand Journal of Zoology*, 23(4), 355–358. doi:10.1080/03014223.1996.9518095
- LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems is Globally Distributed. *Ecology*, 89(2), 371–379. doi:10.1890/06-2057.1
- Lemaitre, B., & Hoffmann, J. a. (2007). The host defense of *Drosophila melanogaster*. *Annual Review of Immunology*, 25, 697–743. doi:10.1146/annurev.immunol.25.022106.141615
- Leroy, P. D., Sabri, A., Heuskin, S., Thonart, P., Lognay, G., Verheggen, F. J., ... Haubruge, E. (2011). Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. *Nature Communications*, 2, 348. doi:10.1038/ncomms1347
- Lester, P. J., Beggs, J. R., Brown, R. L., Edwards, E. D., Groenteman, R., Toft, R. J., ... Ward, D. F. (2013). The outlook for control of New Zealand ' s most abundant , widespread and damaging invertebrate pests: social wasps, 70(4).
- Lester, P. J., Bosch, P. J., Gruber, M. A. M., Kapp, E. A., Peng, L., Brenton-Rule, E. C., ... Wenseleers, T. (2015). No Evidence of Enemy Release in Pathogen and Microbial Communities of Common Wasps (*Vespula vulgaris*) in Their Native and Introduced Range. *PLOS ONE*, 10(3), e0121358. doi:10.1371/journal.pone.0121358

- Lester, P. J., Brown, S. D. J., Edwards, E. D., Holwell, G. I., Pawson, S. M., Ward, D. F., & Watts, C. H. (2014). Critical issues facing New Zealand entomology. *New Zealand Entomologist*, 37(1), 1–13. doi:10.1080/00779962.2014.861789
- Lester, P. J., Gruber, M. A. M., Brenton-Rule, E. C., Archer, M. E., Corley, J. C., Dvořák, L., ... Van Oystaeyen, A. (2014). Determining the origin of invasions and demonstrating a lack of enemy release from microsporidian pathogens in common wasps (*Vespula vulgaris*). *Diversity and Distributions*, 20(8), 964–974. doi:10.1111/ddi.12223
- Lester, P. J., Haywood, J., Archer, M. E., & Shortall, C. R. (2017). The long-term population dynamics of common wasps in their native and invaded range. *Journal of Animal Ecology*, 86(2), 337–347. doi:10.1111/1365-2656.12622
- Lester, P. J., Sébastien, A., Suarez, A. V., Barbieri, R. F., & Gruber, M. A. M. (2017). Symbiotic bacterial communities in ants are modified by invasion pathway bottlenecks and alter host behavior. *Ecology*, 98(3), 861–874. doi:10.1002/ecy.1714
- Letunic, I., & Bork, P. (2011). Interactive Tree of Life v2: Online annotation and display of phylogenetic trees made easy. *Nucleic Acids Research*, 39(SUPPL. 2), 1–4. doi:10.1093/nar/gkr201
- Levitt, A. L., Singh, R., Cox-Foster, D. L., Rajotte, E., Hoover, K., Ostiguy, N., & Holmes, E. C. (2013). Cross-species transmission of honey bee viruses in associated arthropods. *Virus Research*, 176(1–2), 232–240. doi:10.1016/j.virusres.2013.06.013
- Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., ... Gordon, J. I. (2008). Evolution of Mammals and Their Gut Microbes. *Science*, 320(5883), 1647–1651. doi:10.1126/science.1155725
- Li, J., Chen, W., Wu, J., Peng, W., An, J., Schmid-Hempel, P., & Schmid-Hempel, R. (2012). Diversity of *Nosema* associated with bumblebees (*Bombus* spp.) from China. *International Journal for Parasitology*, 42(1), 49–61. doi:10.1016/j.ijpara.2011.10.005
- Li, J., Powell, J. E., Guo, J., Evans, J. D., Wu, J., Williams, P., ... Zhang, Z. (2015). Two gut community enterotypes recur in diverse bumblebee species. *Current Biology*, 25(15), R652–R653. doi:10.1016/j.cub.2015.06.031

- Li, W., & Godzik, A. (2006). Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics*, 22(13), 1658–1659. doi:10.1093/bioinformatics/btl158
- Liehl, P., Blight, M., Vodovar, N., Boccard, F., & Lemaitre, B. (2006). Prevalence of Local Immune Response against Oral Infection in a *Drosophila/Pseudomonas* Infection Model. *PLoS Pathogens*, 2(6), e56. doi:10.1371/journal.ppat.0020056
- Liu, H., & Stiling, P. (2006). Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, 8(7), 1535–1545. doi:10.1007/s10530-005-5845-y
- Liu, S., Vijayendran, D., & Bonning, B. C. (2011). Next generation sequencing technologies for insect virus discovery. *Viruses*, 3(10), 1849–69. doi:10.3390/v3101849
- Lockwood, J. L., Hoopes, M. F., Marchetti, M. P., Marchetti, Michael, P., Martha, F. H., Michael, P. M., ... Marchetti, Michael, P. (2007). *Invasion Ecology. Chemistry & ...* Retrieved from <http://onlinelibrary.wiley.com/doi/10.1002/cbdv.200490137/abstract>
- Loope, K. J., Chien, C., & Juhl, M. (2014). Colony size is linked to paternity frequency and paternity skew in yellowjacket wasps and hornets. *BMC Evolutionary Biology*, 14(1), 277. doi:10.1186/s12862-014-0277-x
- Lorenz, L. M., & Koella, J. C. (2011). The microsporidian parasite *Vavraia culicis* as a potential late life-acting control agent of malaria. *Evolutionary Applications*, 4(6), 783–790. doi:10.1111/j.1752-4571.2011.00199.x
- Low, I., & Stables, S. (2006). Anaphylactic deaths in Auckland, New Zealand: a review of coronial autopsies from 1985 to 2005. *Pathology*, 38(4), 328–332. doi:10.1080/00313020600820831
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of the world's worst invasive alien species: a selection from the global invasive species database. *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database. Auckland,, New Zealand: Invasive Species Specialist Group.*, 12. doi:10.1614/WT-04-126.1

- Macintyre, P., & Hellstrom, J. (2015). *An evaluation of the costs of pest wasps (Vespula species) in New Zealand*.
- Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic Invasions: Causes, Epidemiology, Global Consequences, and Control. *Ecological Applications*, 10(3), 689. doi:10.2307/2641039
- Mackie, R. I., Aminov, R. I., White, B. A., & McSweeney, C. S. (2000). Molecular ecology and diversity in gut microbial ecosystems. In *Ruminant physiology: digestion, metabolism, growth and reproduction* (pp. 61–77). Wallingford: CABI. doi:10.1079/9780851994635.0061
- MacLeod, C. J., Newson, S. E., Blackwell, G., & Duncan, R. P. (2009). Enhanced niche opportunities: can they explain the success of New Zealand’s introduced bird species? *Diversity and Distributions*, 15(1), 41–49. doi:10.1111/j.1472-4642.2008.00498.x
- MacNeil, C., Dick, J. T. a, Hatcher, M. J., Terry, R. S., Smith, J. E., & Dunn, A. M. (2003). Parasite-mediated predation between native and invasive amphipods. *Proceedings. Biological Sciences / The Royal Society*, 270(1521), 1309–14. doi:10.1098/rspb.2003.2358
- Madden, A. A., Grassetti, A., Soriano, J.-A. N., & Starks, P. T. (2013). Actinomycetes with Antimicrobial Activity Isolated from Paper Wasp (Hymenoptera: Vespidae: Polistinae) Nests. *Environmental Entomology*, 42(4), 703–710. doi:10.1603/EN12159
- Mahr, D., Whitaker, P., & Ridgway, N. (2008). *Biological control of insects and mites: an introduction to beneficial natural enemies and their use in pest management. North central regional publication (USA)*. Retrieved from <http://agris.fao.org/agris-search/search.do?recordID=US9536388>
- Maki, M. (2004). Lactic Acid Bacteria in Vegetable Fermentations. In *Lactic Acid Bacteria*. CRC Press. doi:10.1201/9780824752033.ch14
- Manley, R., Boots, M., & Wilfert, L. (2015). Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. *Journal of Applied Ecology*, 52(2). doi:10.1111/1365-2664.12385

- Manson, J. S., Otterstatter, M. C., & Thomson, J. D. (2010). Consumption of a nectar alkaloid reduces pathogen load in bumble bees. *Oecologia*, 162(1), 81–9. doi:10.1007/s00442-009-1431-9
- Martin, J. D., & Mundt, J. O. (1972). Enterococci in insects. *Applied Microbiology*, 24(4), 575–80. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/4628796> <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC380616>
- Martinson, V. G., Danforth, B. N., Minckley, R. L., Rueppell, O., Tingek, S., & Moran, N. A. (2011). A simple and distinctive microbiota associated with honey bees and bumble bees. *Molecular Ecology*, 20(3), 619–628. doi:10.1111/j.1365-294X.2010.04959.x
- Masciocchi, M., & Corley, J. C. (2013). Distribution, dispersal and spread of the invasive social wasp (*Vespula germanica*) in Argentina. *Austral Ecology*, 38(2), 162–168. doi:10.1111/j.1442-9993.2012.02388.x
- Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G., & Neufeld, J. D. (2012). PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics*, 13(1), 1–7. doi:10.1186/1471-2105-13-31
- Mathew, M. J., Subramanian, G., Nguyen, T.-T., Robert, C., Mediannikov, O., Fournier, P.-E., & Raoult, D. (2012). Genome Sequence of *Diplorickettsia massiliensis*, an Emerging *Ixodes ricinus* associated Human Pathogen. *Journal of Bacteriology*, 194(12), 3287–3287. doi:10.1128/JB.00448-12
- Matsuura, M., & Yamane, S. (1990). *Biology of the Vespine Wasps*. Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-642-75230-8
- Matthews, R. W., Goodisman, M. A., Austin, A. D., & Bashford, R. (2000). The introduced English wasp *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) newly recorded invading native forests in Tasmania. *Australian Journal of Entomology*, 39(3), 177–179. doi:10.1046/j.1440-6055.2000.00173.x
- Mattila, H. R., Rios, D., Walker-Sperling, V. E., Roeselers, G., & Newton, I. L. G. (2012). Characterization of the Active Microbiotas Associated with Honey Bees Reveals Healthier and Broader Communities when Colonies are Genetically Diverse. *PLoS ONE*,

7(3), e32962. doi:10.1371/journal.pone.0032962

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., ... Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *Isme J*, 6(3), 610–618. doi:10.1038/ismej.2011.139

McFall-Ngai, M. (2008). Are biologists in “future shock”? Symbiosis integrates biology across domains. *Nature Reviews Microbiology*, 6(10), 789–792. doi:10.1038/nrmicro1982

McFrederick, Q. S., Cannone, J. J., Gutell, R. R., Kellner, K., Plowes, R. M., & Mueller, U. G. (2013). Specificity between Lactobacilli and Hymenopteran Hosts Is the Exception Rather than the Rule. *Applied and Environmental Microbiology*, 79(6), 1803–1812. doi:10.1128/AEM.03681-12

McFrederick, Q. S., Wcislo, W. T., Taylor, D. R., Ishak, H. D., Dowd, S. E., & Mueller, U. G. (2012). Environment or kin: whence do bees obtain acidophilic bacteria? *Molecular Ecology*, 21(7), 1754–1768. doi:10.1111/j.1365-294X.2012.05496.x

McMahon, D. P., Fürst, M. a., Caspar, J., Theodorou, P., Brown, M. J. F., & Paxton, R. J. (2015). A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. *Journal of Animal Ecology*, 84(3), 615–624. doi:10.1111/1365-2656.12345

McMenamin, A. J., & Genersch, E. (2015). Honey bee colony losses and associated viruses. *Current Opinion in Insect Science*, 8, 121–129. doi:10.1016/j.cois.2015.01.015

McMurdie, P. J., & Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Computational Biology*, 10(4), e1003531. doi:10.1371/journal.pcbi.1003531

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology*, 19(SUPPL. 1), 32–40. doi:10.1111/j.1365-294X.2009.04478.x

- Meeus, I., Brown, M. J. F., de Graaf, D. C., & Smagghe, G. (2011). Effects of invasive parasites on bumble bee declines. *Conservation Biology : The Journal of the Society for Conservation Biology*, 25(4), 662–71. doi:10.1111/j.1523-1739.2011.01707.x
- Merçot, H., & Poinso, D. (2009). Infection by *Wolbachia*: from passengers to residents. *Comptes Rendus Biologies*, 332(2–3), 284–297. doi:10.1016/j.crv.2008.09.010
- Merino, L., France, A., & Gerding, M. (2007). Selection of Native Fungi Strains Pathogenic to *Vespula germanica* (Hymenoptera: Vespidae). *Agricultura Técnica*, 67(4), 335–342. doi:10.4067/S0365-28072007000400001
- Meriweather, M., Matthews, S., Rio, R., & Baucom, R. S. (2013). A 454 Survey Reveals the Community Composition and Core Microbiome of the Common Bed Bug (*Cimex lectularius*) across an Urban Landscape. *PLoS ONE*, 8(4), e61465. doi:10.1371/journal.pone.0061465
- Möckel, N., Gisder, S., & Genersch, E. (2011). Horizontal transmission of *Deformed wing virus*: pathological consequences in adult bees (*Apis mellifera*) depend on the transmission route. *The Journal of General Virology*, 92(Pt 2), 370–7. doi:10.1099/vir.0.025940-0
- Mokili, J. L., Rohwer, F., & Dutilh, B. E. (2012). Metagenomics and future perspectives in virus discovery. *Current Opinion in Virology*, 2(1), 63–77. doi:10.1016/j.coviro.2011.12.004
- Moller, H. (1990). Wasps kill nestling birds. *Notornis*, 37(Grant 1959), 76–77.
- Moller, H. (1996). Lessons for invasion theory from social insects. *Biological Conservation*, 3207(96). Retrieved from <http://www.sciencedirect.com/science/article/pii/0006320796000225>
- Moller, H., & Tilley, J. A. V. (1989). Beech honeydew: Seasonal variation and use by wasps, honey bees, and other insects. *New Zealand Journal of Zoology*, 16(3), 289–302. doi:10.1080/03014223.1989.10422894
- Moller, H., Tilley, J. A. V., Thomas, B. W., & Gaze, P. D. (1991). Effect of introduced social wasps on the standing crop of honeydew in New Zealand beech forests. *New Zealand*

Journal of Zoology, 18(2), 171–179. doi:10.1080/03014223.1991.10757964

- Mondet, F., de Miranda, J. R., Kretzschmar, A., Le Conte, Y., & Mercer, A. R. (2014). On the Front Line: Quantitative Virus Dynamics in Honeybee (*Apis mellifera* L.) Colonies along a New Expansion Front of the Parasite *Varroa destructor*. *PLoS Pathogens*, 10(8), e1004323. doi:10.1371/journal.ppat.1004323
- Moon, J. S., Domier, L. L., McCoppin, N. K., D'Arcy, C. J., & Jin, H. (1998). Nucleotide Sequence Analysis Shows that *Rhopalosiphum padi* Virus Is a Member of a Novel Group of Insect-Infecting RNA Viruses. *Virology*, 243(1), 54–65. doi:10.1006/viro.1998.9043
- Morales-Jiménez, J., Zúñiga, G., Villa-Tanaca, L., & Hernández-Rodríguez, C. (2009). Bacterial Community and Nitrogen Fixation in the Red Turpentine Beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae). *Microbial Ecology*, 58(4), 879–891. doi:10.1007/s00248-009-9548-2
- Moran, N. a. (2015). Genomics of the honey bee microbiome. *Current Opinion in Insect Science*, 10(10), 22–28. doi:10.1016/j.cois.2015.04.003
- Moran, N. A., Hansen, A. K., Powell, J. E., & Sabree, Z. L. (2012). Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. *PloS One*, 7(4), e36393. doi:10.1371/journal.pone.0036393
- Moran, N. A., & Kwong, W. K. (2016). *Apibacter adventoris* gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from honey bees. *International Journal of Systematic and Evolutionary Microbiology*, 66(3), 1323–1329. doi:10.1099/ijsem.0.000882
- Moran, N. A., McCutcheon, J. P., & Nakabachi, A. (2008). Genomics and Evolution of Heritable Bacterial Symbionts. *Annual Review of Genetics*, 42(1), 165–190. doi:10.1146/annurev.genet.41.110306.130119
- Mordecai, G. J., Brettell, L. E., Pachori, P., Villalobos, E. M., Martin, S. J., Jones, I. M., & Schroeder, D. C. (2016). Moku virus; a new Iflavirus found in wasps, honey bees and *Varroa*. *Scientific Reports*, 6(1), 34983. doi:10.1038/srep34983

- Morse, R. . A. . (1978). *Honey Bee Pests, Predators and Diseases*. Ithaca, New York: Cornell University Press.
- Nardi, J. B., Mackie, R. I., & Dawson, J. O. (2002). Could microbial symbionts of arthropod guts contribute significantly to nitrogen fixation in terrestrial ecosystems? *Journal of Insect Physiology*, 48(8), 751–763. doi:10.1016/S0022-1910(02)00105-1
- Narita, S., Kageyama, D., Nomura, M., & Fukatsu, T. (2007). Unexpected Mechanism of Symbiont-Induced Reversal of Insect Sex: Feminizing *Wolbachia* Continuously Acts on the Butterfly *Eurema hecabe* during Larval Development. *Applied and Environmental Microbiology*, 73(13), 4332–4341. doi:10.1128/AEM.00145-07
- Nentwing, W. (2007). *Biological Invasions*. (W. Nentwig, Ed.) (Vol. 193). Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-540-36920-2
- New Zealand Ministry for Primary Industries. (2016). *Ministry for primary industries apiculture monitoring programme*.
- Newbold, L. K., Oliver, A. E., Cuthbertson, L., Walkington, S. E., Gweon, H. S., Heard, M. S., & van der Gast, C. J. (2015). Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota. *Environmental Microbiology Reports*, 7(4), 634–641. doi:10.1111/1758-2229.12299
- Ng, T. F. F., Willner, D. L., Lim, Y. W., Schmieder, R., Chau, B., Nilsson, C., ... Breitbart, M. (2011). Broad Surveys of DNA Viral Diversity Obtained through Viral Metagenomics of Mosquitoes. *PLoS ONE*, 6(6), e20579. doi:10.1371/journal.pone.0020579
- Niu, J., Meeus, I., Cappelle, K., Piot, N., & Smagghe, G. (2014). The immune response of the small interfering RNA pathway in the defense against bee viruses. *Current Opinion in Insect Science*, 6, 22–27. doi:10.1016/j.cois.2014.09.014
- Norris, K. (2000). Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology*, 11(1), 19–26. doi:10.1093/beheco/11.1.19
- Nováková, E., Hypša, V., & Moran, N. A. (2009). Arsenophonus, an emerging clade of intracellular symbionts with a broad host distribution. *BMC Microbiology*, 9(1), 143. doi:10.1186/1471-2180-9-143

- O'Dowd, D. J., Green, P. T., & Lake, P. S. (2003). Invasional “meltdown” on an oceanic island. *Ecology Letters*, 6(9), 812–817. doi:10.1046/j.1461-0248.2003.00512.x
- Ohkuma, M., Noda, S., & Kudo, T. (1999). Phylogenetic Diversity of Nitrogen Fixation Genes in the Symbiotic Microbial Community in the Gut of Diverse Termites. *Applied and Environmental Microbiology*, 65(11).
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. (2007). The vegan package. *Community Ecology Package*, 10.
- Oliver, K., & Moran, N. (2009). *Defensive Mutualism in Microbial Symbiosis*. (J. White & M. Torres, Eds.), *Defensive Mutualism in Microbial Symbiosis* (Vol. 20090677). CRC Press. doi:10.1201/9781420069327
- Ondov, B. D., Bergman, N. H., & Phillippy, A. M. (2011). Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics*, 12(1), 385. doi:10.1186/1471-2105-12-385
- Osborne, S. E., Iturbe-Ormaetxe, I., Brownlie, J. C., O'Neill, S. L., & Johnson, K. N. (2012). Antiviral protection and the importance of *Wolbachia* density and: Tissue tropism in *Drosophila simulans*. *Applied and Environmental Microbiology*, 78(19), 6922–6929. doi:10.1128/AEM.01727-12
- Osborne, S. E., Leong, Y. S., O'Neill, S. L., & Johnson, K. N. (2009). Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathogens*, 5(11). doi:10.1371/journal.ppat.1000656
- Oster, G.F. & Wilson, E. O. (1978). *Caste and Ecology in the Social Insects*. Princeton, NJ: Princeton University Press.
- Otterstatter, M. C., Gegear, R. J., Colla, S. R., & Thomson, J. D. (2005). Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behavioral Ecology and Sociobiology*, 58(4), 383–389. doi:10.1007/s00265-005-0945-3
- Otterstatter, M. C., & Thomson, J. D. (2006). Within-host dynamics of an intestinal pathogen of bumble bees. *Parasitology*, 133(6), 749.

doi:10.1017/S003118200600120X

- Otterstatter, M. C., & Whidden, T. L. (2004). Patterns of parasitism by tracheal mites (*Locustacarus buchneri*) in natural bumble bee populations. *Apidologie*, 35(4), 351–357. doi:10.1051/apido:2004024
- Pallen, M. J. (2014). Diagnostic metagenomics: potential applications to bacterial, viral and parasitic infections. *Parasitology*, 1–7. doi:10.1017/S0031182014000134
- Parro, V., & Paz, M. M. (2014). Metatranscriptome. In *Encyclopedia of Astrobiology* (pp. 1–3). Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-642-27833-4_5171-1
- Paulson, J. (2014). metagenomeSeq: Statistical analysis for sparse high-throughput sequencing. *Bioconductor.Jp*, 1–20. Retrieved from <http://bioconductor.jp/packages/2.14/bioc/vignettes/metagenomeSeq/inst/doc/metagenomeSeq.pdf>
- Pereira, R. M., Williams, D. F., Becnel, J. J., & Oi, D. H. (2002). Yellow-head disease caused by a newly discovered *Mattesia* sp. in populations of the red imported fire ant, *Solenopsis invicta*. *Journal of Invertebrate Pathology*, 81(1), 45–48. doi:10.1016/S0022-2011(02)00116-7
- Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and economic costs associated with nonindigenous species in the United States. *BioScience*, 50(1), 53–65.
- Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., ... Tsomondo, T. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, 84(1), 1–20. doi:10.1016/S0167-8809(00)00178-X
- Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52(3), 273–288. doi:10.1016/j.ecolecon.2004.10.002
- Plunkett, G. M., Moller, H., Hamilton, C., Clapperton, B. K., & Thomas, C. D. (1989). Overwintering colonies of German (*Vespula germanica*) and common wasps (*Vespula*

- vulgaris*) (Hymenoptera: Vespidae) in New Zealand. *New Zealand Journal of Zoology*, 16(3), 345–353. doi:10.1080/03014223.1989.10422899
- Potrikus, C. J., & Breznak, J. A. (1977). Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites. *Applied and Environmental Microbiology*, 33(2).
- Praet, J., Meeus, I., Cnockaert, M., Houf, K., Smagghe, G., & Vandamme, P. (2015). Novel lactic acid bacteria isolated from the bumble bee gut: *Convivina intestini* gen. nov., sp. nov., *Lactobacillus bombicola* sp. nov., and *Weissella bombi* sp. nov. *Antonie van Leeuwenhoek*, 107(5), 1337–1349. doi:10.1007/s10482-015-0429-z
- Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 - Approximately maximum-likelihood trees for large alignments. *PLoS ONE*, 5(3). doi:10.1371/journal.pone.0009490
- Pruesse, E., Peplies, J., & Glöckner, F. O. (2012). SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics*, 28(14), 1823–1829. doi:10.1093/bioinformatics/bts252
- Puillandre, N., Dupas, S., Dangles, O., Zeddari, J.-L., Capdevielle-Dulac, C., Barbin, K., ... Silvain, J.-F. (2008). Genetic bottleneck in invasive species: the potato tuber moth adds to the list. *Biological Invasions*, 10(3), 319–333. doi:10.1007/s10530-007-9132-y
- Qu, L.-Y., Lou, Y.-H., Fan, H.-W., Ye, Y.-X., Huang, H.-J., Hu, M.-Q., ... Zhang, C.-X. (2013). Two endosymbiotic bacteria, *Wolbachia* and *Arsenophonus*, in the brown planthopper *Nilaparvata lugens*. *Symbiosis*, 61(1), 47–53. doi:10.1007/s13199-013-0256-9
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590–D596. doi:10.1093/nar/gks1219
- Quevedo, S. M., Martín, M. D., & Velasco, A. C. (2006). *Moellerella wisconsensis*: A hidden enteric pathogen? *Clinical Microbiology Newsletter*, 28(18), 142–143. doi:10.1016/j.clinmicnews.2006.09.002
- R Foundation for Statistical Computing. (2016). R Core Team. Vienna, Austria. Retrieved from <https://www.r-project.org>

- Ramette, A., Frapolli, M., Saux, M. F.-L., Gruffaz, C., Meyer, J.-M., Défago, G., ... Moënne-Loccoz, Y. (2011). *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Systematic and Applied Microbiology*, 34(3), 180–188. doi:10.1016/j.syapm.2010.10.005
- Ramirez-Puebla, S. T., Servin-Garciduenas, L. E., Jimenez-Marin, B., Bolanos, L. M., Rosenblueth, M., Martinez, J., ... Martinez-Romero, E. (2013). Gut and Root Microbiota Commonalities. *Applied and Environmental Microbiology*, 79(1), 2–9. doi:10.1128/AEM.02553-12
- Ravoet, J., De Smet, L., Meeus, I., Smagghe, G., Wenseleers, T., & de Graaf, D. C. (2014). Widespread occurrence of honey bee pathogens in solitary bees. *Journal of Invertebrate Pathology*, 122, 55–58. doi:10.1016/j.jip.2014.08.007
- Ravoet, J., Maharramov, J., Meeus, I., De Smet, L., Wenseleers, T., Smagghe, G., & de Graaf, D. C. (2013). Comprehensive Bee Pathogen Screening in Belgium Reveals *Crithidia mellificae* as a New Contributory Factor to Winter Mortality. *PLoS ONE*, 8(8), e72443. doi:10.1371/journal.pone.0072443
- Reeson, A. F., Jankovic, T., Kasper, M. L., Rogers, S., & Austin, A. D. (2003). Application of 16S rDNA-DGGE to examine the microbial ecology associated with a social wasp *Vespula germanica*. *Insect Molecular Biology*, 12(1), 85–91. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12542639>
- Regassa, L. B. (2006). *Spiroplasmas*: evolutionary relationships and biodiversity. *Frontiers in Bioscience*, 11(1), 2983. doi:10.2741/2027
- Reuter, M. (2003). High Levels of Multiple *Wolbachia* Infection and Recombination in the Ant *Formica exsecta*. *Molecular Biology and Evolution*, 20(5), 748–753. doi:10.1093/molbev/msg082
- Reynolds, D. (1972). Experimental introduction of a microsporidian into a wild population of *Culex pipiens fatigans*. *Bulletin of the World Health Organization*.
- Ribire, M., Ball, B., & Aubert, M. (n.d.). Natural history and geographical distribution of honey bee viruses. *Virology and the Honey Bee*, 15–84.

- Richards, M. H., & Packer, L. (1994). Trophic aspects of caste determination in *Halictus ligatus*, a primitively eusocial sweat bee. *Behavioral Ecology and Sociobiology*, 34(6), 385–391. doi:10.1007/BF00167329
- Robinson, C. J., Schloss, P., Ramos, Y., Raffa, K. F., & Handelsman, J. (2010). Robustness of the bacterial community in the cabbage white butterfly larval midgut. *Microbial Ecology*, 59(2), 199–211. doi:10.1007/s00248-009-9595-8
- Rodriguez, L. F. (2006). Can Invasive Species Facilitate Native Species? Evidence of How, When, and Why These Impacts Occur. *Biological Invasions*, 8(4), 927–939. doi:10.1007/s10530-005-5103-3
- Roh, S. W., Nam, Y.-D. Y. Do, Chang, H. W. H.-W., Kim, K. H. K.-H., Kim, M. S. M.-S., Ryu, J.-H. J. H., ... Bae, J.-W. J. W. (2008). Phylogenetic Characterization of Two Novel Commensal Bacteria Involved with Innate Immune Homeostasis in *Drosophila melanogaster*. *Applied and Environmental Microbiology*, 74(20), 6171–6177. doi:10.1128/AEM.00301-08
- Rohrmann, G. F. (2013). *Baculovirus Molecular Biology* (3rd editio). Bethesda (MD): National Center for Biotechnology Information (US). doi:NBK114593
- Rokop, Z. P., Horton, M. A., & Newton, I. L. G. (2015). Interactions between cooccurring lactic acid bacteria in honey bee hives. *Applied and Environmental Microbiology*, 81(20), 7261–7270. doi:10.1128/AEM.01259-15
- Rolff, J. (2003). Invertebrate Ecological Immunology. *Science*, 301(5632), 472–475. doi:10.1126/science.1080623
- Román, L., Real, F., Sorroza, L., Padilla, D., Acosta, B., Grasso, V., ... Acosta, F. (2012). The in vitro effect of probiotic *Vagococcus fluvialis* on the innate immune parameters of *Sparus aurata* and *Dicentrarchus labrax*. *Fish & Shellfish Immunology*, 33(5), 1071–1075. doi:10.1016/j.fsi.2012.06.028
- Rosa, C., Lachance, M., Silva, J., Teixeira, A., Marni, M., Antonini, Y., & Martins, R. (2003). Yeast communities associated with stingless bees. *FEMS Yeast Research*, 4(3), 271–275. doi:10.1016/S1567-1356(03)00173-9

- Rose, E. A. ., Harris, R. J., & Glare, T. R. (1999). Possible pathogens of social wasps (Hymenoptera: Vespidae) and their potential as biological control agents. *New Zealand Journal of Zoology*, 26(3), 179–190. doi:10.1080/03014223.1999.9518188
- Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, 5(5), 355–362. doi:10.1038/nrmicro1635
- Rosenberg, E., & Zilber-Rosenberg, I. (2013). *The hologenome concept: Human, animal and plant microbiota. The Hologenome Concept: Human, Animal and Plant Microbiota*. Cham: Springer International Publishing. doi:10.1007/978-3-319-04241-1
- Roy, H. E., Lawson Handley, L.-J., Schönrogge, K., Poland, R. L., & Purse, B. V. (2011). Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? *BioControl*, 56(4), 451–468. doi:10.1007/s10526-011-9349-7
- Ruiz-González, M. X., & Brown, M. J. F. (2006). Honey bee and bumblebee trypanosomatids: specificity and potential for transmission. *Ecological Entomology*, 31(6), 616–622. doi:10.1111/j.1365-2311.2006.00823.x
- Ruiz-Zarzuela, I., de Bias, I., Gironés, O., Ghittino, C., & MúAzquiz, J. L. (2005). Isolation of *Vagococcus salmoninarum* in Rainbow Trout, *Oncorhynchus mykiss* (Walbaum), Broodstocks: Characterization of the Pathogen. *Veterinary Research Communications*, 29(7), 553–562. doi:10.1007/s11259-005-2493-8
- Runckel, C., Flenniken, M. L. M., Engel, J. J. C., Ruby, J. G., Ganem, D., Andino, R., & DeRisi, J. L. (2011). Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, *Nosema*, and *Crithidia*. *PloS One*, 6(6), e20656. doi:10.1371/journal.pone.0020656
- Russell, J. a, Moreau, C. S., Goldman-Huertas, B., Fujiwara, M., Lohman, D. J., & Pierce, N. E. (2009). Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proceedings of the National Academy of Sciences*, 106(50), 21236–21241. doi:10.1073/pnas.0907926106
- Sabree, Z. L., & Moran, N. A. (2014). Host-specific assemblages typify gut microbial communities of related insect species. *SpringerPlus*, 3(1), 138. doi:10.1186/2193-

- Sachs, J. L., Essenberg, C. J., & Turcotte, M. M. (2011). New paradigms for the evolution of beneficial infections. *Trends in Ecology & Evolution*, 26(4), 202–209. doi:10.1016/j.tree.2011.01.010
- Sadd, B. M., & Schmid-Hempel, P. (2006). Insect Immunity Shows Specificity in Protection upon Secondary Pathogen Exposure. *Current Biology*, 16(12), 1206–1210. doi:10.1016/j.cub.2006.04.047
- Salem, H., Florez, L., Gerardo, N., & Kaltenpoth, M. (2015). An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. *Proceedings of the Royal Society B: Biological Sciences*, 282(1804), 20142957–20142957. doi:10.1098/rspb.2014.2957
- Salem, H., Kreutzer, E., Sudakaran, S., & Kaltenpoth, M. (2013). Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). *Environmental Microbiology*, 15(7), 1956–1968. doi:10.1111/1462-2920.12001
- Sanders, N. J., Gotelli, N. J., Heller, N. E., & Gordon, D. M. (2003). Community disassembly by an invasive species. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 2474–2477. doi:full free text available at www.pnas.org
- Sangwan, P., Kovac, S., Davis, K. E. R., Sait, M., & Janssen, P. H. (2005). Detection and Cultivation of Soil Verrucomicrobia. *Applied and Environmental Microbiology*, 71(12), 8402–8410. doi:10.1128/AEM.71.12.8402-8410.2005
- Santo Domingo, J. W., Kaufman, M. G., Klug, M. J., Holben, W. E., Harris, D., & Tiedje, J. M. (1998). Influence of diet on the structure and function of the bacterial hindgut community of crickets. *Molecular Ecology*, 7(6), 761–767. doi:10.1046/j.1365-294x.1998.00390.x
- Saridaki, A., & Bourtzis, K. (2010). *Wolbachia*: more than just a bug in insects genitals. *Current Opinion in Microbiology*, 13(1), 67–72. doi:10.1016/j.mib.2009.11.005
- Schindelin, J., Rueden, C. T., Hiner, M. C., & Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction and*

Development, in press, DOI: 10.1002/mrd.22489. doi:10.1002/mrd.22489

- Schmid-Hempel, P. (1994). Infection and Colony Variability in Social Insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 346(1317), 313–321. doi:10.1098/rstb.1994.0148
- Schmid-Hempel, P. (2003). Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society B: Biological Sciences*, 270(1513), 357–366. doi:10.1098/rspb.2002.2265
- Schmid-Hempel, R., & Tognazzo, M. (2010). Molecular Divergence Defines Two Distinct Lineages of *Crithidia bombi* (Trypanosomatidae), Parasites of Bumblebees. *Journal of Eukaryotic Microbiology*, 57(4), 337–345. doi:10.1111/j.1550-7408.2010.00480.x
- Schwarz, H. H., & Huck, K. (1997). Phoretic mites use flowers to transfer between foraging bumblebees. *Insectes Sociaux*, 44(4), 303–310. doi:10.1007/s000400050051
- Schwarz, R. S., Huang, Q., & Evans, J. D. (2015). Hologenome theory and the honey bee pathosphere. *Current Opinion in Insect Science*, 10, 1–7. doi:10.1016/j.cois.2015.04.006
- Sebastien, A., Gruber, M. A. M., & Lester, P. J. (2012). Prevalence and genetic diversity of three bacterial endosymbionts (*Wolbachia*, *Arsenophonus*, and Rhizobiales) associated with the invasive yellow crazy ant (*Anoplolepis gracilipes*). *Insectes Sociaux*, 59(1), 33–40. doi:10.1007/s00040-011-0184-8
- Sébastien, A., Lester, P. J., Hall, R. J., Wang, J., Moore, N. E., & Gruber, M. A. M. (2015). Invasive ants carry novel viruses in their new range and form reservoirs for a honeybee pathogen. *Biology Letters*, 11(9), 20150610. doi:10.1098/rsbl.2015.0610
- Seeley, T. D., & Tarpy, D. R. (2007). Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society B: Biological Sciences*, 274(1606), 67–72. doi:10.1098/rspb.2006.3702
- Seipke, R. F., Barke, J., Ruiz-Gonzalez, M. X., Orivel, J., Yu, D. W., & Hutchings, M. I. (2012). Fungus-growing *Allomerus* ants are associated with antibiotic-producing Actinobacteria. *Journal of Microbiology*, 101(2), 443–447. doi:10.1007/s10482-011-

- Sepers, A. B. J. (1981). Diversity of ammonifying bacteria. *Hydrobiologia*, 83(2), 343–350. doi:10.1007/BF00008285
- Shen, M., Cui, L., Ostiguy, N., & Cox-Foster, D. (2005). Intricate transmission routes and interactions between picorna-like viruses (*Kashmir bee virus* and *Sacbrood virus*) with the honeybee host and the parasitic *Varroa* mite. *Journal of General Virology*, 86(8), 2281–2289. doi:10.1099/vir.0.80824-0
- Sherman, P. W., Seeley, T. D., & Reeve, H. K. (1988). Parasites, Pathogens, and Polyandry in social Hymenoptera. *The American Naturalist*, 131(4), 602–610.
- Simberloff, D., Souza, L., Nuñez, M. A., Barrios-Garcia, M. N., & Bunn, W. (2012). The natives are restless, but not often and mostly when disturbed. *Ecology*, 93(3), 598–607. doi:10.1890/11-1232.1
- Simberloff, D., Stiling, P., & Peter, S. (1996). How Risky is Biological Control? *Ecology*, 77(7), 1965–1974. doi:10.2307/2265693
- Simpson, J. M. (2002). Comparative Microbial Diversity in the Gastrointestinal Tracts of Food Animal Species. *Integrative and Comparative Biology*, 42(2), 327–331. doi:10.1093/icb/42.2.327
- Singh, R. (2014). Ecology and epidemiology of RNA viruses in hymenopteran pollinators, (May).
- Singh, R., Levitt, A. L., Rajotte, E. G., Holmes, E. C., Ostiguy, N., VanEngelsdorp, D., ... Cox-Foster, D. (2010). RNA viruses in hymenopteran pollinators: evidence of inter-Taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. *PloS One*, 5(12), e14357. doi:10.1371/journal.pone.0014357
- Sivashankari, S Shanmughavel, P. (2006). Functional annotation of hypothetical proteins – A review. *Bioinformation*, 1(8), 335–338.
- Smith, C. D., Zimin, A., Holt, C., Abouheif, E., Benton, R., Cash, E., ... Tsutsui, N. D. (2011). Draft genome of the globally widespread and invasive Argentine ant (*Linepithema humile*). *Proceedings of the National Academy of Sciences of the United States of*

- America*, 108(14), 5673–8. doi:10.1073/pnas.1008617108
- Smith, C. R., Smith, C. D., Robertson, H. M., Helmkampf, M., Zimin, A., Yandell, M., ... Gadau, J. (2011). Draft genome of the red harvester ant *Pogonomyrmex barbatus*. *Proceedings of the National Academy of Sciences of the United States of America*, 108(14), 5667–72. doi:10.1073/pnas.1007901108
- Spain, A. M., Krumholz, L. R., & Elshahed, M. S. (2009). Abundance, composition, diversity and novelty of soil Proteobacteria. *The ISME Journal*, 3(8), 992–1000. doi:10.1038/ismej.2009.43
- Speight, M. R., Hunter, M. D., & Watt, A. D. (2008). *Ecology of Insects: Concepts and Applications*. Chichester: Wiley-Blackwell.
- Spradbery, J., & Maywald, G. (1992). The Distribution of the European or German Wasp, *Vespula germanica* (F) (Hymenoptera, Vespidae), in Australia - Past, Present and Future. *Australian Journal of Zoology*, 40(5), 495. doi:10.1071/ZO9920495
- Spradbery, J. P. (1971). Seasonal Changes in the Population Structure of Wasp Colonies (Hymenoptera : Vespidae). *Journal of Animal Ecology*, 40(2), 501–523.
- Spradbery, J. P. (1973). *Wasps: An account of the biology and natural history of social and solitary wasps*.
- St. Leger, R. J., Screen, S. E., & Shams-Pirzadeh, B. (2000). Lack of Host Specialization in *Aspergillus flavus*. *Applied and Environmental Microbiology*, 66(1), 320–324. doi:10.1128/AEM.66.1.320-324.2000
- Starks, P. T., Blackie, C. A., & Seeley, T. D. (2000). Fever in honeybee colonies. *Die Naturwissenschaften*, 87(5), 229–31. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10883439>
- Stock, S. P., Kaya, H. K., & Lacey, L. A. (2009). Field Manual of Techniques in Invertebrate Pathology, Second Edition. *Journal of Economic Entomology*, 102(4), 1726–1726. doi:10.1603/029.102.0444
- Stoeck, T., Kasper, J., Bunge, J., Leslin, C., Ilyin, V., & Epstein, S. (2007). Protistan diversity in the Arctic: A case of paleoclimate shaping modern biodiversity? *PLoS ONE*, 2(8).

doi:10.1371/journal.pone.0000728

- Stork, N. E. (1988). Insect diversity: fact, fiction and speculation. *Biol.J.Linn.Soc.*, 35(January), 321–337.
- Stouthamer, R., Breeuwer, J. A. J., Luck, R. F., & Werren, J. H. (1993). Molecular identification of microorganisms associated with parthenogenesis. *Nature*, 361(6407), 66–68. doi:10.1038/361066a0
- Strauss, A., White, A., & Boots, M. (2012). Invading with biological weapons: the importance of disease-mediated invasions. *Functional Ecology*, 26(6), 1249–1261. doi:10.1111/1365-2435.12011
- Subramanian, G., Mediannikov, O., Angelakis, E., Socolovschi, C., Kaplanski, G., Martzolf, L., & Raoult, D. (2012). *Diplorickettsia massiliensis* as a human pathogen. *European Journal of Clinical Microbiology & Infectious Diseases*, 31(3), 365–369. doi:10.1007/s10096-011-1318-7
- Suttle, C. A. (2007). Marine viruses-major players in the global ecosystem. *Nature Reviews. Microbiology*, 5(10), 801–12. doi:10.1038/nrmicro1750
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. doi:10.1093/molbev/mst197
- Tarpy, D. R. (2003). Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc. R. Soc. Lond. B*, 270(August), 99–103. doi:10.1098/rspb.2002.2199
- Tarpy, D. R., Mattila, H. R., & Newton, I. L. G. G. (2015). Development of the Honey Bee Gut Microbiome throughout the Queen-Rearing Process. *Applied and Environmental Microbiology*, 81(9), 3182–3191. doi:10.1128/AEM.00307-15
- Tarpy, D. R., & Seeley, T. D. (2006). Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. *Naturwissenschaften*, 93(4), 195–199. doi:10.1007/s00114-006-0091-4
- Tartar, A., Wheeler, M. M., Zhou, X., Coy, M. R., Boucias, D. G., & Scharf, M. E. (2009).

- Parallel metatranscriptome analyses of host and symbiont gene expression in the gut of the termite *Reticulitermes flavipes*. *Biotechnology for Biofuels*, 2(1), 25. doi:10.1186/1754-6834-2-25
- Teixeira, L. L., Ferreira, A., Ashburner, M., Ferreira, Á., Ashburner, M., Ferreira, A., & Ashburner, M. (2008). The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biology*, 6(12), 2753–2763. doi:10.1371/journal.pbio.1000002
- Thao, M. L., & Baumann, P. (2004). Evidence for Multiple Acquisition of *Arsenophonus* by Whitefly Species (Sternorrhyncha: Aleyrodidae). *Current Microbiology*, 48(2), 140–144. doi:10.1007/s00284-003-4157-7
- Thomas, C. D., & Moller, H. (1990). The prevalence of introduced *Vespula vulgaris* wasps in a New Zealand beech forest community. *New Zealand Journal ...*, 63–72. Retrieved from http://newzealandecology.org/nzje/free_issues/NZJEcol13_63.pdf
- Toft, R. J., & Rees, J. S. (1998). Reducing predation of orb-web spiders by controlling common wasps (*Vespula vulgaris*) in a New Zealand beech forest. *Ecological Entomology*, 23(1), 90–95. doi:10.1046/j.1365-2311.1998.00100.x
- Torchin, M. E., Lafferty, K., & Dobson, A. P. (2003). Introduced species and their missing parasites. *Nature*, 421(February), 628–630. doi:10.1038/nature01346.1.
- Tsuchida, T., Koga, R., Fujiwara, A., & Fukatsu, T. (2014). Phenotypic Effect of “*Candidatus Rickettsiella viridis*,” a Facultative Symbiont of the Pea Aphid (*Acyrtosiphon pisum*), and Its Interaction with a Coexisting Symbiont. *Applied and Environmental Microbiology*, 80(2), 525–533. doi:10.1128/AEM.03049-13
- Tsutsui, N. D., Kauppinen, S. N., Oyafuso, A. F., & Grosberg, R. K. (2003). The distribution and evolutionary history of *Wolbachia* infection in native and introduced populations of the invasive argentine ant (*Linepithema humile*). *Molecular Ecology*, 12(11), 3057–3068. doi:10.1046/j.1365-294X.2003.01979.x
- Tveten, A.-K., & Sjøstad, K. K. (2011). Identification of Bacteria Infecting *Ixodes ricinus* Ticks by 16S rDNA Amplification and Denaturing Gradient Gel Electrophoresis. *Vector-Borne and Zoonotic Diseases*, 11(10), 1329–1334. doi:10.1089/vbz.2011.0657

- Ulyshen, M. D. (2015). Insect-mediated nitrogen dynamics in decomposing wood. *Ecological Entomology*, 40(S1), 97–112. doi:10.1111/een.12176
- Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., & Schuster, S. C. (2008). Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. *PLoS ONE*, 3(6), e2527. doi:10.1371/journal.pone.0002527
- Vallet-Gely, I., Lemaitre, B., & Boccard, F. (2008). Bacterial strategies to overcome insect defences. *Nat Rev Microbiol*, 6(4), 302–313. doi:10.1038/nrmicro1870
- van Baalen, M., & Beekman, M. (2006). The costs and benefits of genetic heterogeneity in resistance against parasites in social insects. *The American Naturalist*, 167(4), 568–577. doi:10.1086/501169
- Van den Bosch, R., Messenger, P. S., & Gutierrez, A. P. (1982). *An Introduction to Biological Control*. New York, NY.: Plenum Press.
- Vandekerckhove, T., Willems, A., Gillis, M., & Coomans, A. (2000). Occurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in *Xiphinema americanum*-group species (Nematoda, Longidoridae). *International Journal of Systematic and Evolutionary Microbiology*, 50(6), 2197–2205. doi:10.1099/00207713-50-6-2197
- Vannette, R. L., Mohamed, A., & Johnson, B. R. (2015). Forager bees (*Apis mellifera*) highly express immune and detoxification genes in tissues associated with nectar processing. *Scientific Reports*, 5(October), 16224. doi:10.1038/srep16224
- Vásquez, A., Forsgren, E., Fries, I., Paxton, R. J., Flaberg, E., Szekely, L., & Olofsson, T. C. (2012). Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PloS One*, 7(3), e33188. doi:10.1371/journal.pone.0033188
- Vavra, J., & Becnel, J. J. (2007). *Vavraia culicis* (Weiser, 1947) Weiser, 1977 revisited: cytological characterisation of a *Vavraia culicis*-like microsporidium isolated from mosquitoes in Florida and the establishment of *Vavraia culicis floridensis* subsp. n. *Folia Parasitologica*, 54(4), 259–271. doi:10.14411/fp.2007.034
- Viljakainen, L. (2015). Evolutionary genetics of insect innate immunity. *Briefings in*

Functional Genomics, 14(6), 407–412. doi:10.1093/bfpg/elv002

- Villegas, L. M., & Pimenta, P. F. P. (2014). Metagenomics, paratransgenesis and the *Anopheles* microbiome: a portrait of the geographical distribution of the anopheline microbiota based on a meta-analysis of reported taxa. *Memórias Do Instituto Oswaldo Cruz*, 109(5), 672–684. doi:10.1590/0074-0276140194
- Visser, A. A., Nobre, T., Currie, C. R., Aanen, D. K., & Poulsen, M. (2012). Exploring the Potential for Actinobacteria as Defensive Symbionts in Fungus-Growing Termites. *Microbial Ecology*, 63(4), 975–985. doi:10.1007/s00248-011-9987-4
- Vitousek, P. M., & D’Antonio, C. M. (1997). Introduced Species: A Significant Component of Human-caused Global Change. *New Zealand Ecological Society*, 21, 1–16. Retrieved from http://ns.yubawatershed.org/sites/default/files/null/landuse_vitouseketal_1997_inv_spp_prj.pdf
- Walterson, A. M., & Stavriniades, J. (2015). *Pantoea*: insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiology Reviews*, 39(6), 968–984. doi:10.1093/femsre/fuv027
- Wang, Q., Jia, P., & Zhao, Z. (2015). VERSE: a novel approach to detect virus integration in host genomes through reference genome customization. *Genome Medicine*, 7(1), 2. doi:10.1186/s13073-015-0126-6
- Wang, Y., Tian, R. M., Gao, Z. M., Bougouffa, S., & Qian, P.-Y. (2014). Optimal eukaryotic 18S and universal 16S/18S ribosomal RNA primers and their application in a study of symbiosis. *PloS One*, 9(3), e90053. doi:10.1371/journal.pone.0090053
- Ward, N. L., Challacombe, J. F., Janssen, P. H., Henrissat, B., Coutinho, P. M., Wu, M., ... Kuske, C. R. (2009). Three Genomes from the Phylum Acidobacteria Provide Insight into the Lifestyles of These Microorganisms in Soils. *Applied and Environmental Microbiology*, 75(7), 2046–2056. doi:10.1128/AEM.02294-08
- Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T. H., Stege, J. T., ... Leadbetter, J. R. (2007). Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*, 450(7169), 560–565.

doi:10.1038/nature06269

Weiner, S. a, Galbraith, D. a., Adams, D. C., Valenzuela, N., Noll, F. B., Grozinger, C. M., & Toth, A. L. (2013). A survey of DNA methylation across social insect species, life stages, and castes reveals abundant and caste-associated methylation in a primitively social wasp. *Die Naturwissenschaften*, 100(8), 795–9. doi:10.1007/s00114-013-1064-z

Weiss, B., & Aksoy, S. (2011). Microbiome influences on insect host vector competence. *Trends Parasitol.*, 27(11), 514–522. doi:10.1016/j.pt.2011.05.001

Werren, J. H., Baldo, L., & Clark, M. E. (2008). *Wolbachia*: Master Manipulators of Invertebrate Biology. *Nature Rev Microbiol*, 6(10), 741–751. doi:10.1038/nrmicro1969

Werren, J. H., Richards, S., Desjardins, C. a, Niehuis, O., Gadau, J., Colbourne, J. K., ... Gibbs, R. a. (2010). Functional and Evolutionary Insights from the Genomes of Three Parasitoid *Nasonia* Species. *Science*, 327(5963), 343–348. doi:10.1126/science.1178028

Werren, J. H., Windsor, D., & Guo, L. (1995). Distribution of *Wolbachia* among Neotropical Arthropods. *Proceedings of the Royal Society B: Biological Sciences*, 262(1364), 197–204. doi:10.1098/rspb.1995.0196

Werren, J. H., & Windsor, D. M. (2000). *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proceedings of the Royal Society B: Biological Sciences*, 267(1450), 1277–1285. doi:10.1098/rspb.2000.1139

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.

Williams, R. J., Howe, A., & Hofmockel, K. S. (2014). Demonstrating microbial co-occurrence pattern analyses within and between ecosystems. *Frontiers in Microbiology*, 5(July), 1–10. doi:10.3389/fmicb.2014.00358

Williamson, M. (1996). *Biological invasions*. Chapman & Hall London.

Wilson, E. O. (1971). *The Insect Societies*. New York: Cornell University Press.

Wilson, E. O., & Holldobler, B. (2005). Eusociality: Origin and consequences. *Proceedings of*

- the *National Academy of Sciences*, 102(38), 13367–13371.
doi:10.1073/pnas.0505858102
- Wittenberg, R., & Cock, M. J. W. (2001). *Invasive alien species: a toolkit of best prevention and management practices*. (R. Wittenberg & M. J. W. Cock, Eds.), *Global Invasive species Programme*. Wallingford: CABI. doi:10.1079/9780851995694.0000
- Wodzicki, K., & Wright, S. (1984). Introduced Birds and Mammals in New Zealand and their effect on the Environment. *Tuatara: Journal of the Biological Society*, 27(2), 78–102.
- Wong, A. C.-N., Chaston, J. M., & Douglas, A. E. (2013). The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. *The ISME Journal*, 7(10), 1922–32. doi:10.1038/ismej.2013.86
- Wong, C. N. A., Ng, P., & Douglas, A. E. (2011). Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environmental Microbiology*, 13(7), 1889–1900. doi:10.1111/j.1462-2920.2011.02511.x
- Woolhouse, M. E. J., Haydon, D. T., & Antia, R. (2005). Emerging pathogens: the epidemiology and evolution of species jumps. *Trends in Ecology & Evolution*, 20(5), 238–244. doi:10.1016/j.tree.2005.02.009
- Xia, B., Luo, D., Zou, Z., & Zhu, Z. (2009). Effect of temperature on the life cycle of *Aleuroglyphus ovatus* (Acari: Acaridae) at four constant temperatures. *Journal of Stored Products Research*, 45(3), 190–194. doi:10.1016/j.jspr.2009.02.001
- Xie, J., Tiner, B., Vilchez, I., & Mateos, M. (2011). Effect of the *Drosophila* endosymbiont *Spiroplasma* on parasitoid wasp development and on the reproductive fitness of wasp-attacked fly survivors. *Evolutionary Ecology*, 25(5), 1065–1079. doi:10.1007/s10682-010-9453-7
- Xie, J., Vilchez, I., & Mateos, M. (2010). Spiroplasma Bacteria Enhance Survival of *Drosophila hydei* Attacked by the Parasitic Wasp *Leptopilina heterotoma*. *PLoS ONE*, 5(8), e12149. doi:10.1371/journal.pone.0012149
- Xin, J.L., Shen, Z. P. (1964). *Study on life history of Aleuroglyphus ovatus (Acari: Acaridae)*. (A. E. Sinica, Ed.).

- Yañez, O., Gauthier, L., Chantawannakul, P., & Neumann, P. (2016). Endosymbiotic bacteria in honey bees: *Arsenophonus* spp. are not transmitted transovarially. *FEMS Microbiology Letters*, 363(14), fnw147. doi:10.1093/femsle/fnw147
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, 13(1), 134. doi:10.1186/1471-2105-13-134
- Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., ... Glöckner, F. O. (2014). The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids Research*, 42(D1), D643–D648. doi:10.1093/nar/gkt1209
- Young, J. P. W. (1996). Phylogeny and taxonomy of rhizobia. *Plant and Soil*, 186(1), 45–52. doi:10.1007/BF00035054
- Yuen, G. J., & Ausubel, F. M. (2014). *Enterococcus* infection biology: Lessons from invertebrate host models. *Journal of Microbiology*, 52(3), 200–210. doi:10.1007/s12275-014-4011-6
- Yun, J.-H., Roh, S. W., Whon, T. W., Jung, M.-J., Kim, M.-S., Park, D.-S., ... Bae, J.-W. (2014). Insect Gut Bacterial Diversity Determined by Environmental Habitat, Diet, Developmental Stage, and Phylogeny of Host. *Applied and Environmental Microbiology*, 80(17), 5254–5264. doi:10.1128/AEM.01226-14
- Zeng, Q., Wu, S., Sukumaran, J., & Rodrigo, A. (2017). Models of microbiome evolution incorporating host and microbial selection. *Microbiome*, 5(1), 127. doi:10.1186/s40168-017-0343-x
- Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A Greedy Algorithm for Aligning DNA Sequences. *Journal of Computational Biology*, 7(1–2), 203–214. doi:10.1089/10665270050081478
- Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, 32(5), 723–735. doi:10.1111/j.1574-6976.2008.00123.x
- Zimmermann, G., Huger, A. M., & Kleespies, R. G. (2013). Occurrence and prevalence of

insect pathogens in populations of the codling moth, *Cydia pomonella* L.: A long-term diagnostic survey. *Insects*, 4(3), 425–446. doi:10.3390/insects4030425

Zindel, R., Gottlieb, Y., & Aebi, A. (2011). Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *Journal of Applied Ecology*, 48(4), 864–872. doi:10.1111/j.1365-2664.2011.01984.x

Zug, R., & Hammerstein, P. (2012). Still a Host of Hosts for *Wolbachia*: Analysis of Recent Data Suggests That 40% of Terrestrial Arthropod Species Are Infected. *PLoS ONE*, 7(6), e38544. doi:10.1371/journal.pone.0038544

Zug, R., & Hammerstein, P. (2015). Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. *Biological Reviews*, 90(1), 89–111. doi:10.1111/brv.12098

7 APPENDIX

7.1 SUPPLEMENTARY DATA

Supplementary Table 1 Absolute values of microbial eukaryote taxa found in each range life stage, seen in *Vespula vulgaris*. Taxon described includes phylum and class and assigned to the genus level where possible. If the genus was not assigned the next lowest taxonomy was assigned (denoted (o) =order, (f) =family, (c) =class). The table is continued on next page

Phylum	Class	Lowest Taxon	Belgian Larvae	Belgian Workers	Belgian Queens	New Zealand Larvae	New Zealand Workers	New Zealand Queens
Basidiomycota	Tremellomycetes	<i>Cryptococcus</i>	26	0	0	8	0	0
Basidiomycota	Agaricomycetes	<i>Hygrocybe</i>	0	0	0	29	0	1
Basidiomycota	Exobasidiomycetes	<i>Sympodiomyces</i>	20	6	0	0	0	0
Basidiomycota	Tremellomycetes	<i>Dioszegia</i>	13	0	0	0	0	0
Basidiomycota	Pucciniomycetes	Pucciniales (o)	0	0	0	12	0	0
Basidiomycota	Exobasidiomycetes	<i>Exobasidium</i>	0	0	0	10	0	0
Basidiomycota	Agaricomycetes	<i>Hypsizygus</i>	0	0	0	8	0	0
Basidiomycota	Tremellomycetes	Tremellales (o)	6	2	0	0	0	0
Basidiomycota	Agaricomycetes	<i>Athelia</i>	0	3	0	0	4	0
Basidiomycota	Agaricomycetes	<i>Lactarius</i>	0	0	0	2	0	3
Basidiomycota	Agaricomycetes	<i>Athelia</i>	0	0	0	3	0	1
Basidiomycota	Agaricomycetes	<i>Suillus</i>	0	0	0	0	0	4
Basidiomycota	Agaricomycetes	<i>Aleurodiscus</i>	0	0	0	4	0	0
Basidiomycota	Agaricomycetes	<i>Repetobasidium</i>	0	0	0	3	0	0
Basidiomycota	Agaricomycetes	<i>Lentinus</i>	0	0	0	3	0	0
Basidiomycota	Tremellomycetes	<i>Derxomyces</i>	3	0	0	0	0	0
Basidiomycota	Microbotryomycetes	<i>Sporobolomyces</i>	1	1	0	1	0	0
Basidiomycota	Ustilaginomycetes	<i>Ustilago</i>	3	0	0	0	0	0
Basidiomycota	Agaricomycetes	<i>Mycocalia</i>	0	0	0	2	0	0
Basidiomycota	Agaricomycetes	<i>Tulasnella</i>	0	0	0	2	0	0
Basidiomycota	Agaricomycetes	<i>Resinicium</i>	0	0	0	2	0	0
Basidiomycota	Agaricomycetes	Hydnodontaceae (f)	0	2	0	0	0	0

Supplementary Table 1 continued

Phylum	Class	Lowest Taxon	Belgian Larvae	Belgian Workers	Belgian Queens	New Zealand Larvae	New Zealand Workers	New Zealand Queens
Basidiomycota	Tremellomycetes	<i>Sebacina</i>	0	0	0	0	0	2
Basidiomycota	Tremellomycetes	Tremellales (o)	0	0	0	2	0	0
Basidiomycota	Tremellomycetes	<i>Trichosporon</i>	0	0	0	1	1	0
Basidiomycota	Microbotryomycetes	<i>Sporidiobolus</i>	2	0	0	0	0	0
Basidiomycota	Pucciniomycetes	Pucciniales (o)	0	0	0	2	0	0
Basidiomycota	Exobasidiomycetes	<i>Microstroma</i>	2	0	0	0	0	0
Chytridiomycota	Chytridiomycetes	Chytridiomycetes (c)	0	0	0	2	0	0
Mucoromycotina	Mucoromycotina	<i>Mucor</i>	0	2	0	0	0	0
Zygomycota	Entomophthorales (o)	<i>Conidiobolus</i>	1	15	0	16	1	0
Ascomycota	Saccharomycetes	<i>Debaryomyces</i>	1	0	0	59	9	5
Ascomycota	Sordariomycetes	<i>Bionectria</i>	0	0	0	30	0	0
Ascomycota	Eurotiomycetes	<i>Penicillium</i>	2	0	3	0	7	16
Ascomycota	Saccharomycetes	<i>Lachancea</i>	4	0	0	16	0	0
Ascomycota	Saccharomycetes	<i>Metschnikowia</i>	3	2	0	6	1	7
Ascomycota	Saccharomycetes	<i>Saccharomycetales</i> (o)	0	0	0	13	4	0
Ascomycota	Saccharomycetes	<i>Priceomyces</i>	6	0	1	0	10	0
Ascomycota	Dothideomycetes	Dothideales (o)	12	2	0	0	0	0
Ascomycota	Eurotiomycetes	<i>Paracoccidioides</i>	0	0	0	0	0	14
Ascomycota	Eurotiomycetes	<i>Talaromyces</i>	0	0	0	2	6	5
Ascomycota	Leotiomycetes	Helotiales (o)	8	0	0	2	0	3
Ascomycota	Taphrinomycetes	<i>Protomyces</i>	7	2	0	3	1	0
Ascomycota	Dothideomycetes	<i>Cladosporium</i>	5	6	0	0	1	0
Ascomycota	Leotiomycetes	<i>Glarea</i>	0	0	0	9	0	0
Ascomycota	Eurotiomycetes	Chaetothyriales (o)	0	0	0	8	0	0
Ascomycota	Eurotiomycetes	Eurotiales (o)	0	0	1	4	3	0
Ascomycota	Leotiomycetes	<i>Meliniomyces</i>	0	0	0	6	0	0
Ascomycota	Saccharomycetes	<i>Candida</i>	3	0	0	3	0	0

Supplementary Table 1 continued

Phylum	Class	Lowest Taxon	Belgian Larvae	Belgian Workers	Belgian Queens	New Zealand Larvae	New Zealand Workers	New Zealand Queens
Ascomycota	Eurotiomycetes	<i>Exophiala</i>	0	0	0	4	0	0
Ascomycota	Eurotiomycetes	<i>Aspergillus</i>	0	2	0	0	0	2
Ascomycota	Saccharomycetes	<i>Cyberlindnera</i>	4	0	0	0	0	0
Ascomycota	Saccharomycetes	<i>Pichia</i>	4	0	0	0	0	0
Ascomycota	Dothideomycetes	<i>Peltaster</i>	0	1	0	1	1	0
Ascomycota	Leotiomycetes	Leotiomycetes (c)	0	0	0	3	0	0
Ascomycota	Leotiomycetes	<i>Neobulgaria</i>	0	0	0	0	1	2
Ascomycota	Leotiomycetes	<i>Botryotinia</i>	3	0	0	0	0	0
Ascomycota	Leotiomycetes	<i>Collophora</i>	0	0	0	3	0	0
Ascomycota	Leotiomycetes	Thelebolales (o)	0	0	0	0	0	3
Ascomycota	Saccharomycetes	<i>Hanseniaspora</i>	3	0	0	0	0	0
Ascomycota	Dothideomycetes	<i>Pleosporales</i> (o)	0	2	0	1	1	0
Ascomycota	Dothideomycetes	<i>Fodinomyces</i>	0	0	0	2	0	0
Ascomycota	Eurotiomycetes	<i>Penicillium</i>	0	0	0	1	1	0
Ascomycota	Incertae Sedis	<i>Symbiotaphrina</i>	0	2	0	0	0	0
Ascomycota	Lecanoromycetes	<i>Acarospora</i>	0	0	0	0	0	2
Ascomycota	Leotiomycetes	<i>Oidiodendron</i>	0	0	0	0	2	0
Ascomycota	Pezizomycetes	<i>Urnula</i>	0	2	0	0	0	0
Ascomycota	Saccharomycetes	<i>Kazachstania</i>	0	0	0	2	0	0

Supplementary Table 1 continued

Phylum	Class	Lowest Taxon	Belgian Larvae	Belgian Workers	Belgian Queens	New Zealand Larvae	New Zealand Workers	New Zealand Queens
Protists								
Apicomplexa	Conoidasida	<i>CCA5</i>	0	0	0	25	12	0
Apicomplexa	Gregarinasina	<i>Ascogregarina</i>	0	0	0	0	0	2
Apicomplexa	Neogregarinorida	<i>Mattesia</i>	3	1	0	0	0	0
Intramacronucleata	Conthreep	<i>Dexiotricha</i>	2	0	0	0	0	0
Euglenozoa	Kinetoplastea	<i>Crithidia</i>	3	0	0	2	1	0
Microsporidia	Incertae Sedis	Berwaldia (f)	0	0	0	6	0	0
Cercomonas	Cercozoa	Cercomonas (o)	0	0	0	3	0	0
Nematoda								
Nematoda	Chromadorea	<i>Tylenchida</i>	0	0	0	3	0	0
Nematomorpha	Gordioda	<i>Chordodea</i>	0	0	0	2	0	0

Supplementary Table 2 Viral counts using two analysis methods MEGAN and Virusfinder2. Both methods also had varying results of virus presence and absence, indicating further confirmatory work. A ✓ is indicated for both analysis where virus presence was detected and a – where no virus was detected.

Virus	Vespula vulgaris		Apis mellifera		Bombus terrestris		Vespula germanica		Line humile	
	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2	MEGAN	Virusfinder 2
<i>Rhopalosiphum padi virus</i> (RhPV)	16	13899	-	-	-	-	-	-	14	20
<i>Kashmir bee virus</i> (KBV)	9	107	-	-	-	-	-	-	9	-
<i>Deformed Wing Virus</i> (DWV)	-	-	-	5292564	-	-	-	10	-	-
<i>Aphid Lethal paralysis virus</i> (ALPV)	5	-	-	-	-	-	-	-	-	-
<i>Black Queen Cell Virus</i> (BQCV)	-	-	6	-	3	10	1	-	4	-
Enterobacteria phage DE3	-	-	-	-	-	17	-	-	-	-
Prunus Necrotic Ringspot Virus	-	-	7	455	-	-	-	-	-	-
Linepithema humile Virus 1	-	-	-	-	-	-	-	-	4	-
Arabis Mosaic Virus	-	-	20	-	3	-	6	-	-	-
Lake Sinai Virus 1	-	-	3	-	-	-	-	-	-	-
Raphanus latent Virus	-	-	-	-	4	-	-	-	-	-
Sacbrood Virus	-	-	-	-	-	-	1	-	-	-
Enterobacteria phage VK	1	-	-	-	-	-	-	-	-	-
Kakugo Virus	1	-	3	-	-	-	1	-	-	-
Apis mellifera Filamentous Virus	-	-	1	-	-	-	-	-	-	-

Supplementary Table 3 Forward and reverse primers used to sequence short products for PCR and RT-qPCR. Sanger sequenced products were aligned to taxa in Genbank and the resulting percentage identity and product length is provided, along with the sequence and closest taxa match.

Candidate	Forward Primer	Reverse Primer	Identity	Product Length	Sequence Match
Moku Virus	TGGTGCGATAGCT AAGCCTG	GAACCCAGAATGGT GCTGGA	95%	134	Moku Virus isolate Big Island, complete genome KU645789.1 GGGGGAGGCTTGATTTGGTTAGATGTAGCTGGAAGTGTGACAAAGTTTTAGGAG ATATAAATTGCGATAATCCACCAGATACTCGTCCAGCACCATTCTGGGTTCA
<i>Moellerella wisconsensis</i>	TACCGACAGAAGA AGCACCG	CTTAATCAACCGCC TGCGTG	98%	112	Hafnia sp. CB1(2010) 16S ribosomal RNA gene, partial sequence GU944485.1 ATACGGGGGAGGGGGTGGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCAC GCAGGCRGTTGATTAAGA
			97%	112	Moellerella wisconsensis strain NTS31501793 16S ribosomal RNA gene, partial sequence GenBank: KR339013.1 ATACGGGGGAGGGGGTGGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCAC GCAGGCRGTTGATTAAGA
Dicer	CAACTCCGAGATA GGTGACAGAAG	AGAAGAGGGTCCG CATCCTT	93%	36	PREDICTED: Polistes canadensis endoribonuclease Dicer (LOC106783690), mRNA GenBank: XM_014742462.1 TTATATTATTGTGGCACCAAAAAGTCCATCAGCAGA
<i>Aspergillus fumigatus</i>	CTAACTTTCGTTCC CTGATTAATG	CTTGATTTGCTGA AGACTAAC	93%	76	Aspergillus sp. 18003 18S ribosomal RNA gene, partial sequence GenBank: EU710836.1 TTGGCCAACGTATGTTTTATTATCAGGGAACGAAAGTTAGA
Kashmir bee virus	ACCAGGAAGTATT CCCATGGTAAG	TGGAGCTATGGTTC CGTTCAG	100%	79	Kashmir bee virus isolate 950406b-6 structural protein gene, partial cds Genbank: AF263732.1 TAAAAAATTTTATGGACCTGAATCATCACAACGGAACCATAGCTCCAA

Supplementary Table 4 Forward and reverse primers used in PCR to sequence long products for confirmatory identification of *Kashmir Bee virus* and *Moku virus*. PCR and RT-qPCR. Sanger sequenced products were aligned to taxa in Genbank and the resulting percentage identity and product length is provided, along with the sequence and closets taxa match.

Moku Virus	Forward Primer ATCTGAACTGCCACCGTCAG	Reverse Primer TGCTGCTGCCTCATTAACCA	Identity: 89%	Sanger product length: 744
Moku virus isolate Big Island, complete genome GenBank: KU645789.1 GAAATAGAAATCTGCTTTTAATTGAACTTGTTAGACGTGAGAAAGAATTAATTTTCGAAATAGGATGATTGCGGCATATGCTTTAGACCCGATTATACTCAATTGGATGAATTAAATTATG TCCACGGATCTACCTTACCCACTACTACATTACATGAACTATTCATCCACCAGCGGAATGAAGCTCGACGTATTCTATATGCAGCACCGCCCTCTGTCTGAAGAGGAGGAAGACCCGTATTTG TATAATATTTTACAACGATTCCGATACTTATGGGAACCACTGAAACACCACAACCTGAAATGGATGAACCATCATGTAGCTGGGAGTTTCGATCCTACACGAATTGTTAGTAACAGTATGTT CAGAGAGAGAGAAACGAATAATGCAAGTATTGTTGGGTGATCGAGTTAAGATGGATAGAGGTGCTCTTCTGAAGTTGGTTTCGGGTGGGATAGATGTTACAGAGGATGACATAAGAGGTT TATCATAGATCCACATTCGAAAAGGTTTATTCGAAAAGCTGGTTCAACTGACGGTATCTGGTCTAGGTTTTTGTGTTTTGAATATAATTCTATGTGGCCGATGATTTAACAGATGG ATGTCAAAAACGATGTTTATGACTTCGTCGCATACGGAGGAGAATATAACACATATGAGATTTTACCTCTTGGGAAGATGAACTGGACTGATTGTTAAGATCTTTAGGTATATTGGTTAA GAGCAGCAGCTAATGT				
Kashmir Bee Virus	Forward Primer TGATGGTGATTGTGGAGCCC	Reverse Primer TCGACTCCCGGATAACCTGT	Identity: 97%	Sanger product length: 690
Kashmir bee virus, complete genome GenBank :AY275710.1 TACATGTTGCTGGTGCAGCATGKGGTCGAGCTTATGCAGAATCTATAACCCAGAAAGATTTGGAGCGTACATTTAGTAAAATAGATGTTACAATGCAAATCCAATTGGATTTAGATTCTTC ATTAGATTTTTCTAGAATCGAACCACAAAATCCCTACTGGAGTTGAATTTGGACCGGAAGCTCTCACTTTTTGTGATCTTCCAGCCCTTAAGATGATACCAGTAGGACGACTTCCCGAACCCCT ATTTGAACCAGGCAAAACAGATATTAGGCCTTCCTTAGTGCATGGAATGATTTCTGATATTAAGACTAAACCAGCTTACTTACGTAATGTAAGAGTAGATGGCGAAGTCRTAAATATGAAA CACAGAAATTTAATGAAATGTGCTATGGATACACCTCATATCGACAAGGACATGATCGAAGAAGCATATCAATTGACTAAATCTGTTTGGCTAAAAGGTATGCGAGATGAATTGAAAAAG GTTCTCACTTATGAAGAAGCCATTTGTGGCTCTGAAGTAAGTGAATACATTTCTCAATTAACAGGAGTAGTTCCCCAGGATATCCTTGGATAAAGGATAGAGTGAAAGGAAGTAAAGGAA AACAAGGTTGGTTTGGCACTGATGGAGAATTTATTCTCAATGAAAATGTGCAATTAGCCGTACAACAACGACTACAAGCAGCTC				

7.2 FITNESS IN INVASIVE SOCIAL WASPS: THE ROLE OF VARIATION IN VIRAL LOAD, IMMUNE RESPONSE, AND PATERNITY IN PREDICTING NEST SIZE AND REPRODUCTIVE OUTPUT

SYNTHESISING ECOLOGY

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Key words: invasive, social wasp, *Vespula vulgaris*, fitness, polyandry, *Kashmir Bee Virus*, nest size, immune response, *Dicer*, *Superkiller*

Abstract

Within any one habitat, the relative fitness of organisms in a population can vary substantially. Social insects like the common wasp are among the most successful invasive animals, but show enormous variation in nest size and other fitness-related traits. Some of this variation may be caused by pathogens such as viruses that can have serious consequences in social insects, which range from reduced productivity to colony death. Both individual immune responses and colony-level traits such as genetic diversity are likely to influence effects of pathogen infections on colony fitness. Here we investigate how infections with *Kashmir Bee Virus* (KBV), immune response and intracolony genetic diversity (due to queen polyandry) affect nest size in the invasive common wasp, *Vespula vulgaris*. We show that KBV is highly prevalent in wasps and expression of antiviral immune genes is significantly increased with higher viral loads across individuals. Patriline membership within a nest did not influence KBV susceptibility or immune response. A permutational MANCOVA revealed that polyandry, viral load, and expression of the immune gene *Dicer* were significant predictors of variation in nest size. High intracolony genetic diversity due to polyandry has previously been hypothesized to improve colony-level resistance to parasites and pathogens. Consistent with this hypothesis, we observed genetically diverse colonies to be significantly larger and to produce more queens, although this effect was not driven by the pathogen we investigated. Invasive wasps clearly suffer from pathogens and expend resources, as indicated here by elevated immune gene expression, toward reducing pathogen-impact on colony fitness.

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7.3 SINGLE-STRANDED RNA VIRUSES INFECTING THE INVASIVE ARGENTINE ANT, *LINEPITHEMA HUMILE*

SCIENTIFIC REPORTS

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Social insects host a diversity of viruses. We examined New Zealand populations of the globally widely distributed invasive Argentine ant (*Linepithema humile*) for RNA viruses. We used metatranscriptomic analysis, which identified six potential novel viruses in the *Dicistroviridae* family. Of these, three contigs were confirmed by Sanger sequencing as *Linepithema humile virus-1* (LHUV-1), a novel strain of *Kashmir bee virus* (KBV) and *Black queen cell virus* (BQCV), while the others were chimeric or misassembled sequences. We extended the known sequence of LHUV-1 to confirm its placement in the *Dicistroviridae* and categorised its relationship to closest relatives, which were all viruses infecting Hymenoptera. We examined further for known viruses by mapping our metatranscriptomic sequences to all viral genomes, and confirmed KBV, BQCV, LHUV-1 and *Deformed wing virus* (DWV) presence using qRT-PCR. Viral replication was confirmed for DWV, KBV and LHUV-1. Viral titers in ants were higher in the presence of honey bee hives.

Argentine ants appear to host a range of 'honey bee' pathogens in addition to a virus currently described only from this invasive ant. The role of these viruses in the population dynamics of the ant remain to be determined, but offer potential targets for biocontrol approaches.

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