# Biodiversity, evolution and microbiome of the New Zealand Psylloidea (Hemiptera: Sternorrhyncha)

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A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Francesco Martoni

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He mihi tēnei ki te whenua o Aotearoa.

Na te hōnore i te hikoitia i nga huanui, me te akona i nga taonga.

To the Land of New Zealand.

Because it was an honour to walk its tracks and study its treasures.

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Psyllids, also known as jumping plant lice, belong to the superfamily Psylloidea (Hemiptera: Sternorrhyncha) and globally are divided into almost 4000 described species. Psyllids are phloem-feeders, with a number of species considered economic pests including through the vectoring of phloem-restricted plant pathogens, and others used as biological control agents against invasive plants. In recent years some economically important plant diseases have emphasised the role of highly polyphagous psyllids in pathogen epidemiology, for example of *Candidatus* Liberibacter spp. vectored by *Bactericera cockerelli* causing zebra chip disease, and by *Diaphorina citri* causing Huanglongbing disease. This has generated substantial interest in the biology of such psyllids. However, it has also highlighted the lack of information on other psyllid species, many of which are poorly studied and are difficult to identify or completely undescribed. This can confound accurate species diagnosis for both ecological and biosecurity applications, and undermines any understanding of their potential role in maintaining disease-causing bacteria in the environment.

The psyllid fauna of New Zealand provides a cross section of the superfamily Psylloidea, with species representatives in the families Aphalaridae, Calophyidae, Homotomidae, Liviidae, Psyllidae and Triozidae, including pests and bio control agents. However, despite almost 100 known species there, information about the endemic fauna, which has representatives across three families, is scarce and many taxa still await description. Documented knowledge on New Zealand psyllids is now outdated as a result of new taxonomic classifications and new arrivals. Furthermore, the recent introduction of *B. cockerelli* and the spread of the zebra chip disease raised a number of questions on the role of other psyllid species in its horizontal transmission and also presence of any other pathogens that might already exist.

This study aimed to understand which psyllid species are present in New Zealand and their evolutionary relationships, and to develop the first information on the composition of their natural, internal bacterial community. This will not only enable new psyllid species arrivals to be recognised, but also allow interrelationships across psyllid taxa, their microflora and host plants to be understood. In turn, hypotheses as to the potential for native psyllids to also transmit introduced pathogens can be advanced.

Field-collected specimens from almost 600 locations around New Zealand, Australia and United States of America were used to generate (a) an up-to-date list of the New Zealand Psylloidea, based on a morphological-molecular integrative taxonomy concept; (b) a phylogenetic analysis of the psyllid collection using sequences of cytochrome oxidase subunit 1 [COI] DNA barcode region plus partial 18S ribosomal DNA, and including a region of elongation factor 1-alpha (EF-1α) for a species subset; and (c) a partial 16S metabarcode next generation sequencing (MiSeq, Illumina) bacterial inventory.

Morphological and genetic analysis, together with distribution and host plant associations, resulted in the identification of 90 different taxa of psyllids in New Zealand; this was in addition to another 30 species known to be present in this region but not collected. The collection included one newly introduced species from Australia and 20 novel undescribed native species including a number of morphologically cryptic taxa. The phylogenetic study performed on these species revealed an evolutionary structure that was congruent with the current taxonomy. Furthermore, the position of the genus *Atmetocranium* was clarified and re-attributed to the family Aphalaridae, confirming an original placement. The presence of likely six ancestral arrivals (for the psyllids included in this work) has been proposed together with the different evolutionary strategies that led to the present psyllid fauna of New Zealand. These include a number of host switches for the species of the genus *Trioza,* that likely happened when the insect colonized the host plant, and a relatively more strict psyllid-plant association for the genera *Ctenarytaina* and *Psylla.*

Subsequent partial 16S metabarcode analysis of 220 individual psyllids from 65 species across the six New Zealand families confirmed the universal presence of the primary symbiont *Candidatus* Carsonella rudii; this included some unexpected species-level variation (>4% divergence) according to the operational taxonomic units (OTUs) defined by the VSEARCH pipeline. A prevalence of symbionts belonging to the family Enterobacteriaceae was also revealed, but species-level assignment was not possible with the partial 16S r DNA region used. Nevertheless, the Mantel and partial Mantel tests confirmed that, the microbial composition is highly correlated (almost 40%) to the genetic distance between insects after accounting for the host plant variation. On the other

hand, inverting the variables, host plant associations are responsible for just 15% of the microbial composition after accounting for the psyllid genetic distance. These observations are consistent with the idea that the psyllid microbial composition is mostly influenced by the psyllids species and not the plant. Furthermore, potential coevolution between psyllids and some secondary symbionts is proposed. The pathogen-containing bacterial genera *Liberibacter* and *Phytoplasma* were detected with BLAST indications from the 16S sequences as to species previously not recorded in New Zealand.

The range of curated specimens and the molecular framework generated here supplies a substantial resource for further taxonomic and ecological enquirey. This work provides a valuable dataset enabling comparisons between both species native to New Zealand and between these and other psyllid taxa from all over the world. In turn this provides fundamental taxonomic and biodiversity information that subsequently can be exploited as outcomes for plant health bio-protection and biosecurity.

**Keywords: psyllid, cryptic species, biodiversity, evolution, phylogeny, COI, 18S, species radiation, host plant, host switch, New Zealand, Australia, bacteria, symbionts, 16S metabarcoding.**

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The past 44 months have been a unique experience that completely changed my life on multiple levels, from the formative and working ones, to the deepest personal aspects. This PhD not only contributed to shape my future career, but also the person I am. For these reasons, the number of people I would need to acknowledge and thank is almost infinite and no thesis would allow enough space. However, I found some words that perfectly fit with my feelings:

*"I blame all of you.*
*Writing this thesis has been an exercise in sustained suffering. The casual readers may, perhaps, exempt themselves from excessive guilt, but for those of you who have played the larger role in prolonging my agonies with your encouragement and support, well…*
*…you know who you are and you owe me."*
(*Dispensational modernism*, B. M. Pietsch)

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# Table of Contents

# List of Acronyms and symbols:

AMNZ: Auckland Museum

BLAST: Basic Local Alignment Search Tool

BOLD: Barcode of Life Datasystem

bp: Base Pairs

CABI: Centre for Agriculture and Biosciences International

CAD: Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, Dihydroorotase

CMNZ: Canterbury Museum

COI: Cytochrome oxidase subunit 1 gene

CTAB: Cetyl Trimethylammonium Bromide

*Cyt b*: Cytocrhome b

DNA: Deoxyribonucleic acid

DOC: Department of Conservation

EPPO: European and Mediterranean Plant Protection Organization

FHDB: Forest Health Database

GPS: Global Positioning System

GTR: Generalised Time-Reversible (model)

h: Hours

K2P: Kimura two Parameters

LUNZ: Lincoln University Entomology Research Collection

M: Molar

MCMC: Markov Chain Monte Carlo

Min: Minute(s)

ML: Maximum Likelihood

mL: Millilitre

MONZ: Museum of New Zealand

MPI: Ministry for Primary Industry

NCBI: National Center for Biotechnology Information

NGS: Next Generation Sequencing

NJ: Neighbour Joining

NSW: New South Wales

NUMTS: Nuclear mitochondrial pseudogenes

NZAC: New Zealand Arthropod Collection

NZGL: New Zealand Genomic Limited

OTU: Operational Taxonomic Unit

°C: Degree Celsius

PC2: Physical Containment facility of level two

PCR: Polymerase Chain Reaction

PFR: Plant and Food Research

QIIME: Quantitative Insights Into Microbial Ecology

RNA: Ribonucleic acid

rpm: Rounds per Minute

SA: South Australia

S: Second(s)

Sp.: Species (singular)

Spp. Species (plural)

TPP: Tomato Potato Psyllid

URL: Uniform Resource Locator

USA: United States of America

VIC: Victoria (Australia)

Wg: Wingless gene

ZC: Zebra Chip Disease

µL: Microlitre

µm: Micrometer

µM: Micromolar

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# Chapter 1

# General introduction

## 1.1 Context

In the last 120 years, almost 4000 species of psyllids (Hemiptera: Sternorrhyncha: Psylloidea) have been described worldwide (Burckhardt and Ouvrard 2012, Ouvrard 2017). During this time, a reasonable understanding has been developed of the psyllid species in Australasia, including New Zealand, through the work of a number of entomologists [e.g. (Dale 1985)]. Currently, 73 described psyllid species are reported as present in New Zealand (Macfarlane et al. 2012, Martoni et al. 2016) together with another 26 taxa as yet undescribed (Dale 1985, Macfarlane et al. 2012).

A small number of higly polyphagous psyllids are associated with crop damage (McClean and Oberholzer 1965, Capoor et al. 1967, Martinez and Wallace 1967), with an economic impact significant enough to have earned them the category of major quarantine pests (EPPO/CABI 1997). The discovery that some species are also the vectors of high risk bacterial plant pathogens (e.g. *Diaphorina citri* and *Bactericera cockerelli*) has also raised further questions around psyllid diversity, their evolution and their interactions with hosts and plant pathogens (Martoni et al. 2016). While information on these species has increased over recent years, especially at the population level [e.g. (Liu et al. 2006, Swisher et al. 2013, Swisher et al. 2014)], almost nothing is understood of the other lesser known species which are quite often endemic to a small region with a specific host range.

Psyllid taxonomy has largely been driven through morphological assessment, which is limiting for these small insects with often cryptic species characters (Dale 1985). A more complete understanding of these insects might be possible using an integrative approach that includes molecular phylogenetic analysis alongside the traditional morphological analyses. In fact, while molecular techniques are now being applied to psyllid taxonomy [e.g. (Percy 2017)] and identification [e.g. (Taylor 2016)], published data for only two such integrative approaches exist but both are taxonomically fairly restricted (Taylor 2016, Percy 2017). Yet, in general, their use for phylogenetic delineation of psyllids is still very poor, including for a recent major taxonomic revision that has been introduced (Burckhardt and Ouvrard 2012). Indeed, DNA sequence analyses, which have clarified relationships among many other insect groups [e.g. (Miller et al. 2016)] have been applied to psyllids in only a very preliminarly way [e.g. (Ouvrard and Burckhardt 2008)], with most efforts to develop genetic information focused on the high risk pest species [e.g. (Xiong et al. 2017)].

The recently recorded microbial/pathogen-psyllid associations suggest that an understanding of these relationships may provide critical information for assessing the risk of psyllids, arrivals/introductions. This is especially pertinent given they have been repeatedly introduced as biocontrol agents [e.g. *Arytainilla spartiophila* to control Scotch Broom, (Syrett et al. 1999)]. Yet, the scant knowledge on the relationships between psyllid species and pathogens is limited to just a few associations such as between the pyllids *D. citri, B.cockerelli* and *Trioza erytreae* with different species of the plant pathogen *Liberibacter* (Jagoueix et al. 1994, Teixeira et al. 2005). Today, more sophisticated molecular techniques are available to enable microbial associations to be explored in more detail in psyllids [e.g. (Hall et al. 2016)]. Consequently, it may be possible to discover how psyllid communities in an area contribute to local pathogen diversity, persistence and spread, and how host-plant associations evolve.

In countries with significant agricultural economies and a heightened sense of biosecurity, such as New Zealand, reliance on a morphology-based summary of psyllid diversity restricts the ability to understand critical aspects of their plant-microbe interactions. This in turn compromises attempts to associate disease agents with plant symptoms. In this study, the intent was to use a more integrative approach to understand New Zealand psyllids, including a phylogenetic basis upon which pathogen associations with specific psyllid species can be evaluated.

## 1.2 Psyllids


**Figure 1.1:** Habitus illustrations of different groups of Hemiptera (not to the same scale) A. *Trialeurode vaporariorum* (Westwood) (Aleyrodidae). B. *Bemisia argentifolii* Bellows & Perring (Aleyrodidae). C. *Aleurodicus dugesii* Cockerell (Aleyrodidae). D. *Adelges cooleyi* (Gillette) (Adelgidae). E. *Aphis nerii* Fonscolombe (Aphididae). F. *Pseudococcus longispinus* (Targioni-Tozzetti) (Pseudococcidae). G. *Aspidaspis arctostaphyli* Cockerell & Robbins (Diaspididae).H. *Russelliana solanicola* Tuthill (Psyllidae). I. *Sphenorhina melanoptera* (Germar) (Cercopidae). J. *Prosapia bicincta* (Say) (Cercopidae). K. Cicadidae. L. *Cladonota* sp. (Membracidae). M. *Heteronotus* sp. (Membracidae). N. *Ferrariana trivittata* [Signoret] (Cicadellidae). O. *Platygonia spatulata* [Signoret] (Cicadellidae). P. *Proconia* sp. (Cicadellidae). Reproduced with permission (Forero 2008).

### 1.2.1 Higer Systematics

#### Order Hemiptera

Psyllids, also known as jumping plant lice, belong to the order Hemiptera, suborder Sternorrhyncha superfamily Psylloidea. The name Hemiptera is derived from two ancient Greek words, ἡμι- (hemi; "half") and πτερόν (pteron; "wing"), and reflects the characteristic forewing structure of the insects, which is partly hardened at the base and partly membranous. The order Hemiptera was first recognized by Linné in the *Systema Naturae* of 1758 (Linné 1758). Nowadays, it is considered a monophyletic group of insects (Hennig 1969, Carver et al. 1991), which can be recognized by the peculiar structure of the mouthparts: the mandibles and maxillary laciniae are modified into concentric stylets, the mandibular enclosing the maxillary ones forming the food and salivary channels, and the multi segmented sheet-like labium covering the mandibular and maxillary stylets.

#### Suborder Sternorrhyncha

The basal grouping within the Hemiptera is the monophyletic suborder Sternorrhyncha (Figure 1.2). This includes four superfamilies: Psylloidea, Aleyrodoidea, Aphidoidea, and Coccoidea [e.g., (Schlee 1969, Carver et al. 1991, Von Dohlen and Moran 1995, Bourgoin and Campbell 2002)]. Insects belonging to the Sternorrhyncha are characterized by several features including: 1. absence of vannus and vannal folds in the hind wing (CSIRO 1991); 2. base of labium in posterior position (opisthognathous) (CSIRO 1991, Ax 1999); 3. two tarsal segments (Ax 1999); 4. radius, media, and cubitus fused basally (Ax 1999).

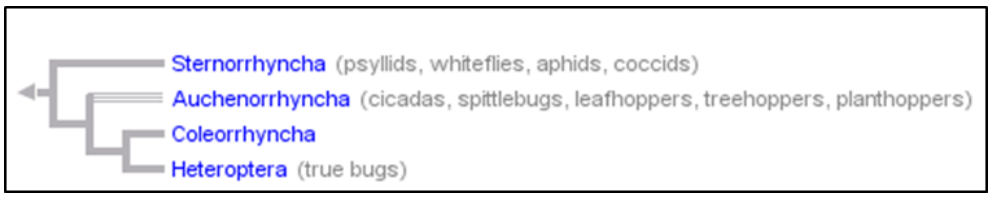


**Figure 1.2:** A representative tree showing the phylogenetic grouping within the Hemiptera. The tree was obtained from the "tree of life web project", and is based on a number of works (Schuh 1979, Carver et al. 1991, Wheeler et al. 1993, Von Dohlen and Moran 1995).

Goodchild (1966), suggested that, based on the morphology of the alimentary tract, the Aphidoidea should be considered the sister group of Coccoidea + (Psylloidea + Aleyrodoidea). Schlee, basing his study on external morphology and male genitalia, considered two sister groups within Sternorrhyncha: the "Psylliformes" (Psylloidea + Aleyrodoidea), and the "Aphidiformes" (Aphidoidea + Coccoidea) (Schlee 1969). Interestingly, in some other analyses, Psylloidea is considered the sister group to the rest of Sternorrhyncha [e.g. (Campbell et al. 1994, Campbell et al. 1995)], therefore in separate position compared to the other superfamilies. However, despite these competing

hypotheses, no modern comprehensive phylogenetic analysis is available for the group [although a complete review is present in (Schlee 1969)].

## Superfamily Psylloidea

The first systematic treatment of psyllids was proposed by Löw (Löw 1879) who divided them into four subfamilies: Liviinae, Aphalarinae (which also included the genera *Rhinocola* Löw, 1879 and *Psyllopsis* Löw, 1879), Psyllinae (includng the genus *Psylla* Geoffroy, 1762), and Triozinae (including the genus *Trioza* Foerster, 1848) (Geoffroy 1762, Foerster 1848). The subfamilies Livillinae (for the genus *Creiis* Scott 1882) and Prionocnemidae were later added by Scott (Scott 1882), but were subsequently proven invalid and disestablished. Edwards also tried to raise the four original subfamilies to family status (Edwards 1896), but this was never formally accepted. A fifth subfamily, Spondiliaspinae, was formerly erected by Schwarz (Schwarz 1898), mainly to account for the Australian genus *Spondyliaspis* Signoret, 1879 (Signoret 1879). Similarly, Enderlein erected a sixth subfamily Ciriacreminae to group some of the tropical genera together (*Carsidara* Walker, 1869; *Tyora* Walker, 1869 and *Ciriacremum* Enderlein, 1910) (Walker 1869, Enderlein 1910).

Aulman (1913) also divided the Psylloidea into six subfamilies: Liviinae, Aphalarinae, Psyllinae, Triozinae, Ciriacreminae, and Spondyliaspidinae (Aulmann 1913). Crawford (1914) then added information on the psyllids of North and South America and made some changes to the taxonomy accordingly, listing Liviinae (including *Rhinocola* and *Aphalara* Foerster, 1848 in two different tribes), Pauropsyllinae, Carsidarinae, Ciriacreminae, Psyllinae (including *Psyllopsis* and *Euphalerus* Schwarz, 1904) and Triozinae (Schwarz 1904, Crawford 1913, Crawford 1914). This taxonomic structure was once again modified by Heslop-Harrison (1960), who gave a key to the six subfamilies of Aphalarinae, Ciriacreminae, Liviinae, Triozinae, Psyllinae and Spondyliaspinae (which he had redefined in 1954) (Heslop-Harrison 1954, 1960).

Vondracek eventually raised the six subfamilies to family status: Aphalaridae, Carsidaridae, Liviidae, Psyllidae, Spondyliaspidae (later corrected to Spondiliaspididae) and Triozidae (Vondráček 1957). In this list, the subfamily Anomalopsyllinae was part of the Spondyliaspidae. Klimaszewski subsequently transferred this subfamily to the Aphalaridae (Klimaszewski 1964), before Loginova revised the subfamily Arytaininae (in the family Psyllidae) and listed the genera *Acizzia* Heslop-Harrison, 1961 (1977) and *Psylla* (1978) (Loginova 1977, 1978).

The ongoing reclassifications of the psyllids were based primarily on the morphology of adults and to a lesser degree on the features of the nymphs. They were also founded mostly upon species from the Northern Hemisphere. As a consequence, significant advances in the understanding of psyllid taxonomy were made when White and Hodkinson reviewed species from the Southern

Hemisphere. Not only did they study psyllid nymphs to create a new system of classification, but they also hypothesized that psyllids had a Gondwanan origin (White 1970, White and Hodkinson 1980, Hodkinson and White 1981, White and Hodkinson 1982, White and Hodkinson 1985). White and Hodkinson's classification (1980) retained the Aphalaridae as a more primitive group adding the tribe Ctenarytainini and eliminating the family Spondyliaspidae. Moreover, they added three families, the Calophyidae, Homotomidae, and Pacopteronidae (White and Hodkinson 1980), bringing the total to eight. The classification proposed by White and Hodkinson (1985) was based on a cladistic and phenetic study of the larval and adult morphological characters from a comprehensive sampling of specimens from around the world (White and Hodkinson 1985). Indeed, the work described in detail the history of psyllid classification, providing the most complete document ever published of the known world fauna of psyllids.

Specific taxa and geographic subsets of White and Hodkinson's classification have been modified and expanded over the last 30 years [e.g. (Hollis 1985, Burckhardt 1987, Hollis 1987, Burckhardt 1991, Burckhardt and Basset 2000, Li 2011)] and, with the advent of molecular techniques, many different groupings have been confirmed at the family and genus level (Ouvrard et al. 2000, Thao et al. 2001, Percy 2003b, Ouvrard and Burckhardt 2008). The current classification of the world Psylloidea was presented by Burckhardt and Ouvrard (2012) and it confirmed six of the eight families as defined by White and Hodkinson (White and Hodkinson 1985, Burckhardt and Ouvrard 2012). Only the positions of the families Aphalaridae and Spondyliaspididae were modified; each was identified as polyphyletic, a trait that was supported by other works (Burckhardt 1987, 1991). The similarities in the classifications presented by Burckhardt and Ouvrard (2012) with the work of White and Hodkinson (1985) made the proposed structure not only convincing, but also retained the same nomenclature of the previous classifications. This allowed a more robust continuity with the past, that was not present in other recent taxonomical reviews, such as the one undertaken by Li (Li 2011).

As a result of all the taxonomic studies, the superfamily Psylloidea presently includes eight families: Aphalaridae, Liviidae, Calophyidae, Homotomidae, Psyllidae, Triozidae, Pacopteronidae and Carsidaridae (Figure 1.3). This classification comprises more than 3850 described species (Burckhardt and Ouvrard 2012, Ouvrard 2017) distributed worldwide.

A detailed morphological description of the Psylloidea is given in Appendix A.


**Figure 1.3:** Examples of insects belonging to the eight families of psyllids: Aphalaridae (a), Liviidae (b), Calophyidae (c), Triozidae (d), Psyllidae (e), Homotomidae (f), Carsidaridae (g) and Phacopteronidae (h). Photos reproduced with permission of the authors [a-b, G. Kunz; c, G. Seljak; d-f D. Ouvrard; g, G. McCormack; h, J. Botz].

### 1.2.2 Biology

#### Life Cycle

The hemimetabolous psyllid life cycle starts with eggs that are laid, singly or in clusters, on host plants at or in proximity to potential feeding sites for the larvae (nymphs). Nymphs are largely sedentary. They usually are free living, but can be gall-inducing or live under a scale or a lerp (Hollis 2004). After the eggs hatch, five larval instars precede development into the adult life stage (Figure 1.4). After full moult, the adults copulate and disperse. While females tend to mate only once within a few hours of emergence, males can mate several times. They usually wait a few days after emergence before mating (Hollis 2004). Diapause can occur at any stage during the life cycle and, depending on the climate, there can be from one to several overlapping generations per year (Hollis 2004).

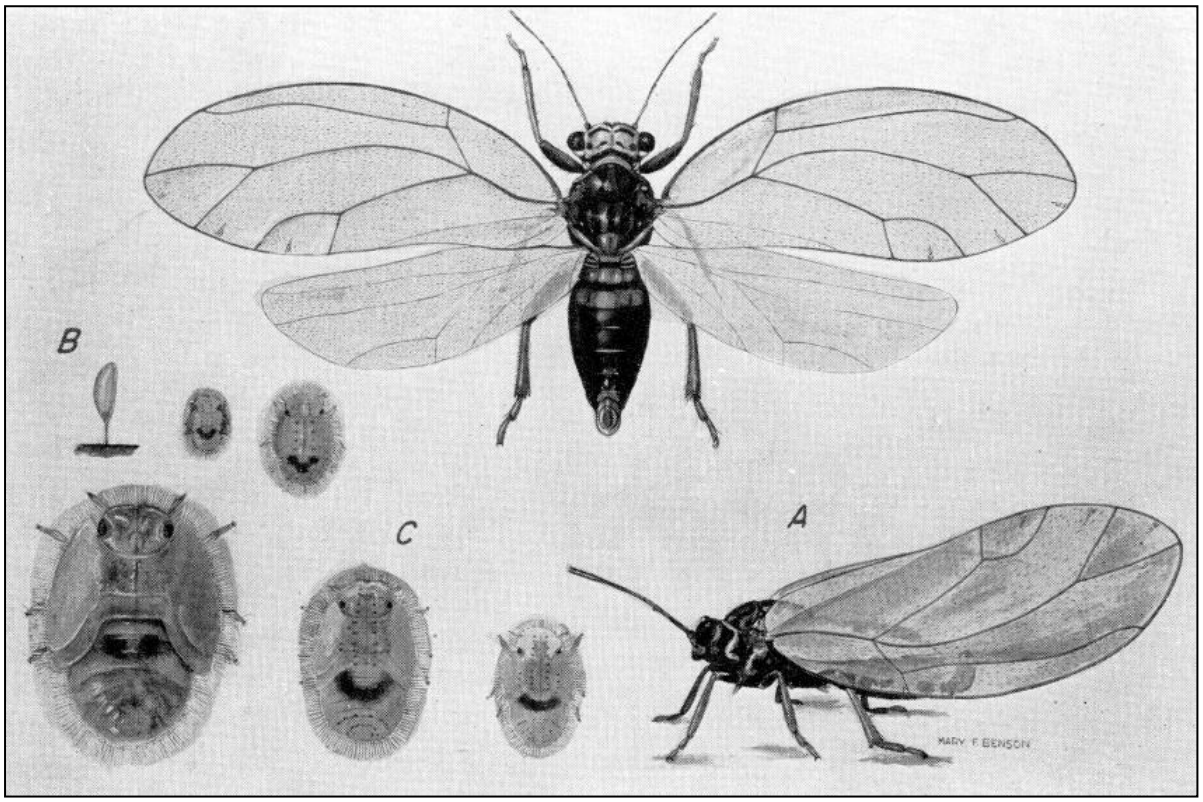


**Figure 1.4:** Life stages of the tomato potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), showing the adult (A), egg (B) and nymphs (C) (Wallis 1955). Reproduced with permission.

Reproduction is typically bisexual with heterogametic males and is therefore relatively straightforward when compared with the parthenogenetic life cycles of other hemipterans such as aphids or coccoids. However, there is evidence of facultative parthenogenetic reproduction in *Cacopsylla myrtilli* [Wagner 1947] (Wagner 1947, Nokkala et al. 2008, Nokkala et al. 2013). Similarly, Hodkinson (1978) reported facultative parthenogenesis in some Alaskan psyllids, including

*Cacopsylla rara* [Tuthill 1944] (Tuthill 1944, Hodkinson 1978). Hodkinson suggested that *"there is strong circumstantial evidence that certain psyllids may be facultatively parthenogenetic under more severe climatic regimes, and as one moves into alpine/tundra regions, such as Alaska, the incidence of parthenogenesis increases."* (Hodkinson 1978). Anecdotally, Moore reported that some Australian species of the genus *Glycaspis* have seasonally induced parthenogenetic phases since he could not find males or found them in much lower numbers (Moore 1970). The majority (75%) of psyllid species have free living nymphs, with 25% being gall inducing and lerp forming. This latter life style is predominant in Australian psyllids (Hollis 2004). Adults tend to have a 1:1 sex ratio, but males are inclined to emerge earlier while females live longer. Hollis (2004) reported a male karyotype number of 2n=25 for 95% of the species studied, the exceptions being the Australian Spondyliaspidinae (varying from 2n=7 to 2n=11) and *Ctenarytaina eucalypti* (2n=21) (Hollis 2004).

Eggs are characterized in psyllids as subovoid in shape, broader basally, and usually with a basal or ventro-basal pedicel. The pedicel is inserted into subepidermal tissue at the oviposition site allowing water to be supplied to the egg from the host plant tissue (Hollis 2004). In *Arytainilla spartiophila*, eggs are laid into incisions and covered with a protective layer made of wax (Watmough 1968). The oviposition site may vary considerably depending on the different species. For example, *Ctenarytaina* spp. and *Acizzia* spp. tend to lay eggs between auxiliary buds and young leaf pedicels, between unfurled leaflets, in leaf axils of terminal shoots and on flower buds and developing fruits in order to protect the free-living nymphs after they hatch (Hollis 2004).

The flight of adult psyllids is limited to short distances, but longer distances can be travelled on prevailing wind systems, the direction of which strongly influence the direction of their dispersal (Hollis 2004, Yen et al. 2014). In the absence of wind, psyllids tend to jump and fly for a maximum of 3 metres from their host plant (Moore 1961). The adult is the main dispersive stage, although if disturbed the free-living nymphs can disperse more than their gall-forming counterparts.

Nymphs and adult psyllids feed on the soluble contents of the vascular tissues of their host plants. Since phloem tissue is the preferred feeding site, the mouth parts include paired maxillary and mandibular stylets that form a food channel up which plant sap is sucked. As the stylets move through the plant tissue, a tubular feeding track or salivary sheath is secreted encasing the stylets within the tissue.

**Host Plant**

The relationships occurring between psyllids and their host plants have been widely studied (Hodkinson 1974, 1984, Percy et al. 2004, Hodkinson 2009, Burckhardt et al. 2014). Psyllids tend to be extremely specific in their host choice (Burckhardt et al. 2014). In fact, closely related psyllid species typically occur on closely related host plants (Brown and Hodkinson 1988) to the point that a psyllid species narrows down its host range to a single host plant genus (Eastop 1973, Hodkinson 1974). The concept of a host plant can, however, be misrepresented. Following the definition of Hodkinson (2009), Burckhardt and colleagues (Burckhardt et al. 2014) defined a host plant as:

*"..a plant on which psyllids can feed, generate offspring and on which the nymphs can develop up to adulthood."*

This clarifies that all those plants on which psyllid nymphs are not capable of fully developing into adults are not considered host plants. Nonetheless, the presence of psyllids on plants other than their host plants is common (Hodkinson 2009). This led Burckhardt and colleagues to distinguish and define the other plants on which psyllids can be found as:

***Overwintering*** or ***Shelter plants*****:** plants on which adult psyllids overwinter and on which they may feed.

***Food Plants*****:** plants on which adult psyllids feed, but do not breed and do not spend an extended period of time (e.g. diapause or winter season).

***Casual Plants*****:** plants on which adult psyllids land actively or passively, and on which adults may probe but do not feed.

### 1.2.3 Molecular studies on psyllids

Molecular studies on psyllid systematics and evolution are not extensive and have yet to add substantial value to the more traditional measures of biodiversity and ecological relationships. The limited molecular studies on psyllids conducted to date have taken three main approaches: mitochondrial DNA barcoding [e.g. (Taylor et al. 2016, Percy 2017)], a limited number of higher level phylogenetic studies [e.g.(Percy 2003b, Ouvrard et al. 2015),] and microbiome comparison [e.g. (Thao et al. 2000b, Thao et al. 2000a, Hall et al. 2016)]. DNA barcoding *sensu stricto* (Hebert et al. 2003) has proven an effective tool for the identification and distinction of psyllid species (Taylor et al. 2016, Percy 2017). This group of insects can be difficult to distinguish morphologically, and poor understanding of its diversity has been compounded by incomplete characterisation of the immature life stages. Comparisons of the mitochondrial COI barcode gene regions in psyllids,

however, have proven sufficient to resolve different species, attribute them to their respective genera (Percy 2017) and to delimit population haplotype variation within a species (Swisher et al. 2012, Swisher et al. 2013, Swisher et al. 2014).

While barcoding studies have shown the potential of this technique to identify and diagnose some psyllids, sufficient nucleotide sequences are not available for comparison across the Psylloidea, with only five genera and 26 species identified on the BOLD database to date (Ratnasingham and Hebert 2007) (Figure 1.5). Moreover, the complete mitochondrial genomes of only five psyllid species have been sequenced: *Bactericera cockerelli, Diaphorina citri, Pachypsylla venusta, Cacopsylla coccinea* and *Paratrioza sinica* (Thao et al. 2004, Que et al. 2016, Wu et al. 2016, Zhang et al. 2016), allowing only a small number of comparisons.

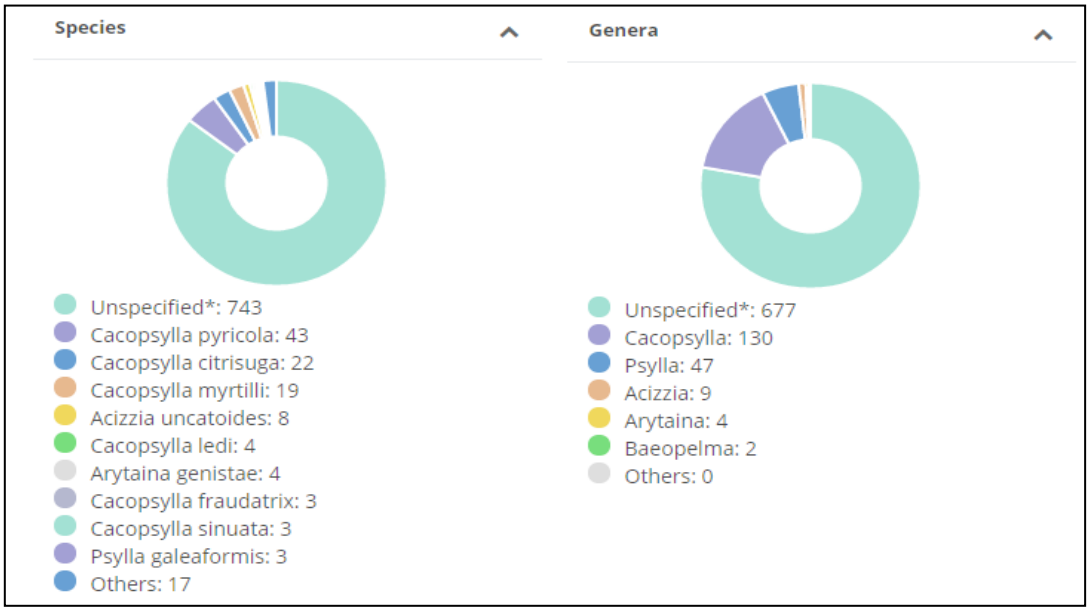


**Figure 1.5:** Psyllids represented by COI nucleotide sequences in the BOLD System dataset (http://www.boldsystems.org/index.php/Public_SearchTerms) as of August 2017.

Phylogenetic studies of psyllids have answered questions about psyllid systematics and evolution, including instances of supposed co-evolution between insect and plant (Percy 2003b). These studies were based largely on closely related taxa, however, so did not include higher level phylogenetic analysis. In order to study the psyllid phylogeny more broadly, slower evolving molecular markers were required (Lin and Danforth 2004). With this in mind, the ribosomal 18S gene was tested, and appeared to be useful to understand the relationships between psyllid families (Ouvrard and Burckhardt 2008). Unfortunately, this marker was used mostly in studies on the wider Hemipteran subgroups (Sorensen et al. 1995), and not on specific plant pathogen vector species (Yvon et al. 2009).

## 1.3 Microbial associations in phloem feeding hemipterans

As a result of advances in molecular biology, an increasing focus of research has been to understand how insects vector pathogens and interact with their microbiome more generally.

All animals, including humans, have a plethora of associated bacteria (Buchner 1965, Douglas 2010, Human Microbiome Project 2012, Findley et al. 2013). The diversity of this microbiota is broadly influenced by animal phylogeny, showing a greater diversity in vertebrates than in invertebrates. It is also dependent on the host tissue, with higher diversity in the gut than in the cells (McFall-Ngai 2007, Bright and Bulgheresi 2010). Age, sex, physiological conditions, genotype and environmental circumstances are also involved in the biodiversity of the animal microbiome (Turnbaugh et al. 2009, Claesson et al. 2012, Wernegreen 2012, Franzenburg et al. 2013, Hildebrand et al. 2013).

Insects are among the animals that clearly show an adaptive advantage due to their obligate symbiosis with bacteria. In fact, this symbiosis allowed them to reach novel niches by improving their metabolism, altering their mating systems and changing the reproductive compatibility of different populations (Moran 2007).

### 1.3.1 Structure of the Microbiome

Sap-feeding insects are a prominent example of animals who have developed a strong association with their microbiome, since they evolved a partnership with a wide variety of bacterial symbionts (Buchner 1965) that provided the insects with essential nutrients otherwise lacking in their diet. The only animals feeding on nutrient-deficient plant sap for the entirety of their life cycle are the insects of the order Hemiptera (Douglas 2003, 2006). This specialized diet (Baumann 2005, Moran et al. 2008) resulted in the absence of important nutrients that need to be provided by other means, such as the symbiosis with bacteria.

Most such insects have a primary endosymbiont such as *Candidatus* Portiera aleyrodidarum in the whitefly *Bemisia tabaci* (Thao and Baumann 2004, Jiang et al. 2012), *Buchnera aphidicola* in aphids (Thao et al. 2000a) or *Candidatus* Sulcia muelleri in spittlebugs (McCutcheon and Moran 2010). Primary endosymbionts are characterized by living in specifically evolved organelles (bacteriocytes) that form an aggregate (bacteriome) within the insect body cavity (Thao et al. 2000a). In all studied cases for bacteriome-associated symbionts in sap-feeding insects, the relationship is mutually obligate: the host and its symbionts are completely dependent on each other to survive (McCutcheon and Moran 2010). They are transmitted vertically to host progeny and almost invariably show large-scale reductions in genome size. Symbiosis leads to redundancy

between host and symbionts, driving extraordinary losses of genes and thus genome compaction in a wide number of bacterial and eukaryotic systems (Keeling and Corradi 2011).

In addition to primary symbionts, sap sucking insects are colonized by a range of secondary bacterial symbionts (Skaljac et al. 2013). For example, whitefly populations from around the world have been reported to harbour secondary symbionts including *Hamiltonella, Arsenophonus, Cardinium, Wolbachia, Rickettsia, Fritschea* and *Hemipteriphilus* (Nirgianaki et al. 2003, Baumann 2005, Gottlieb et al. 2006, Li et al. 2007, Jing et al. 2014). Next generation amplicon sequencing techniques are now allowing rapid and more comprehensive analyses of microbial populations in insects (Yoccoz 2012). However, recent screening of sap-sucking insects revealed an unexpectedly low bacterial diversity (3-7 operational taxonomic units) per insect (Jing et al. 2014).

The ecological roles of secondary symbionts remain largely unknown (Werren et al. 2008, Feldhaar and Gross 2009, Kikuchi et al. 2012). *Hamiltonella* has been shown to confer resistance against parasitoids in the pea aphid *Acyrthosiphon pisum* [(Oliver et al. 2003, Ferrari et al. 2004, Brumin et al. 2011)] and to increase the ability of *B. tabaci* to be an efficient virus vector (Gottlieb et al. 2010). *Rickettsia* in *B. tabaci* has been shown to confer resistance to heat stress (Brumin et al. 2011), to increase its susceptibility to chemical insecticides (Kontsedalov et al. 2008), and to provide general fitness benefits (Himler et al. 2011). Several of the bacteria such as *Wolbachia, Rickettsia, Arsenophonus* and *Cardinium* have all been implicated in manipulation of their host's reproduction (Gherna et al. 1991, Zchori-Fein and Perlman 2004, Dale and Moran 2006, Werren et al. 2008). In particular *Wolbachia* has attracted considerable interest because of its extensive penetration of different insect lineages and profound effects on host phenotype (Werren et al. 2008). There is no concordance between the phylogeny of *Wolbachia* and its hosts, indicative of extensive lateral movement between host species (Werren et al. 2008).

### 1.3.2 The microbiome of psyllids

The psyllid primary symbiont, *Candidatus* Carsonella rudii, has been widely studied since it was discovered and morphologically described in the bacteriocyte of 18 psyllids (Profft 1937). The presence of this symbiont was confirmed also in the Pear psyllid, *Psylla piricola* Foerster (Chang and Musgrave 1969) and *Anomoneura mori* Schwartz (Waku and Endo 1987). *Candidatus* Carsonella rudii was finally named when Thao and colleagues confirmed, by molecular methods, its coevolution with psyllids as a primary endosymbiont (Figure 1.6) (Thao et al. 2000a). The following year, *C.* Carsonella rudii was confirmed to be vertically transmitted (Thao et al. 2001). This was also confirmed in a recent work (Hall et al. 2016). In 2006, *Candidatus* Carsonella rudii's complete genome was published for the first time and, with a size of less than 160 Kb compared to all other cases of

genome reduction then recorded at about 400 Kb, it was the smallest ever discovered (Nakabachi et al. 2006). A study of this genome highlighted genome reduction resulting from the bacterium's symbiosis with psyllids, to the point that it was proposed as a step towards the degeneration of the primary endosymbiont and its transformation into a new subcellular entity (or organelle) (Tamames et al. 2007).

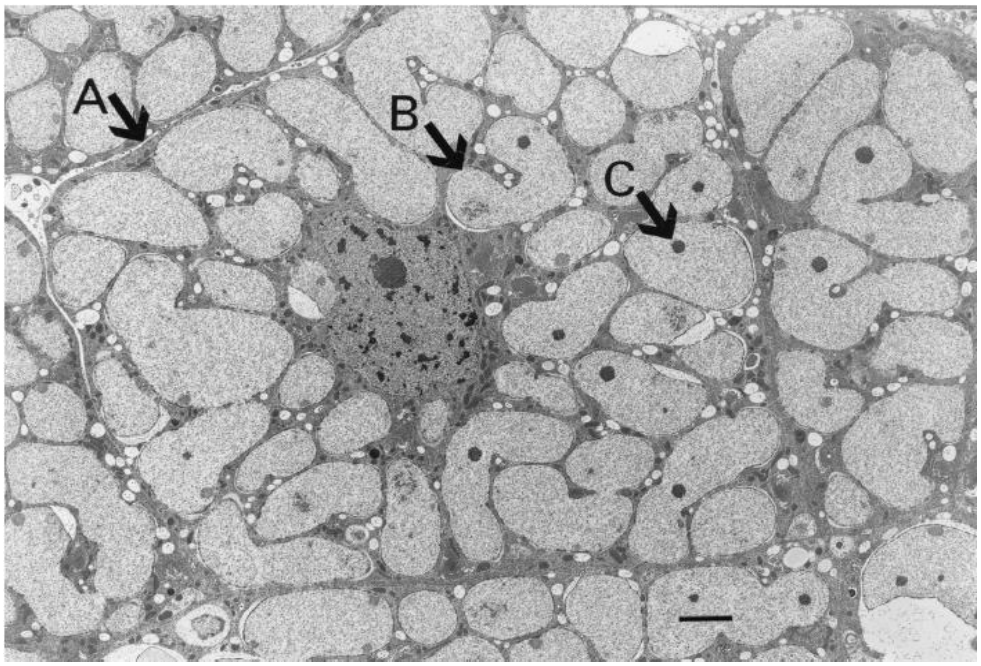


**Figure 1.6:** A transmission electron micrograph of a bacteriocyte from *Pachipsylla venusta*. The picture shows a bacteriocyte (A) containing endosymbionts (B). C is an unidentified electron-dense aggregate. The bar is 2 µm. The picture is reproduced with permission by the American Society for Microbiology (Thao et al. 2000a).

### Secondary Symbionts

Excluding the bacteria that are of economic interest (see next section), the secondary symbionts of psyllids are in general poorly studied. Enterobacteriaceae including *Arsenophonus*, *Sodalis* and *Blochmannia* have all been reported by Thao and colleagues as S-symbionts of psyllids (Thao et al. 2000b). While a number of Enterobacteriaceae were not defined to a species or genus level, some other were well known P-symbionts of other insects [e.g. *Blochmannia* is the P-symbiont of ants, (Schroder et al. 1996, Sauer et al. 2000)]. A 1998 study on the psyllid *Anomoneura mori*, highlighted the presence of a S-symbiont belonging to the γ subdivision of the Proteobacteria (Fukatsu and Nikoh 1998). The same symbiont was recorded also by Thao and colleagues (Thao et al. 2000b).

A study on *Ctenarytaina eucalypti* showed that genes necessary for arginine and tryptophan biosynthesis are provided by a second bacterial endosymbiont from Enterobacteriaceae (Sloan and Moran 2012) that plays an important role in complementing amino acid biosynthesis pathways in

*Carsonella* (Sloan and Moran 2012). The genomes of those secondary symbionts showed signatures of long-term vertical transmission (accelerated rates of sequence evolution, absence of large repeats and mobile genetic elements, genomes reduced in size) (Sloan and Moran 2012). Previous studies have shown multiple independent origins of secondary symbionts in psyllids (Sloan and Moran 2012). In other psyllids, enterobacterial secondary symbionts are absent and thus unavailable to complement pathways missing from *Carsonella*. By comparing the genomes of the hackberry petiole gall psyllid *Pachypsylla venusta* to that of a mealybug, it emerged that these pathways were provided by transfers of bacterial genes to the insect host (Sloan et al. 2014). Although gene loss is seen to be an active process in the *Carsonella* genome, most of the genes transferred to the insect hosts were apparently sourced from secondary symbionts (Sloan and Moran 2012). Overall, psyllids can be seen to have a dynamic relationship with their primary symbiont and a range of current or past secondary symbionts. Other mechanisms, such as changes in host diet may also be at play (Sloan and Moran 2012).

The first phylogenetic comparison between the primary and secondary symbionts of psyllids was made by Thao and colleagues (Thao et al. 2000b, Thao et al. 2000a) and then recently built upon by Hall et al. [Figure 1.7a, (Hall et al. 2016)]. Both studies suggested multiple infections of psyllids with ancestors of the S-endosymbionts through horizontal gene transfer, and that the P-symbiont, which coevolved with its psyllid host, was vertically transmitted [Figure 1.7b, (Hall et al. 2016)]. This highlighted the different strategies and behaviours of P- and S-symbionts in accordance with the work of Aksoy and colleagues on *Sodalis glossinidius* (S-endosymbiont) from different species of tsetse flies: the bacterium was virtually identical, indicating multiple infections or horizontal transmission of the same organism (Aksoy et al. 1997). Interestingly though, (Hall et al. 2016) suggested that some of the S-symbionts were obligate and not facultative, proposing the idea of at least one obligate P- and S-symbiont for each species.

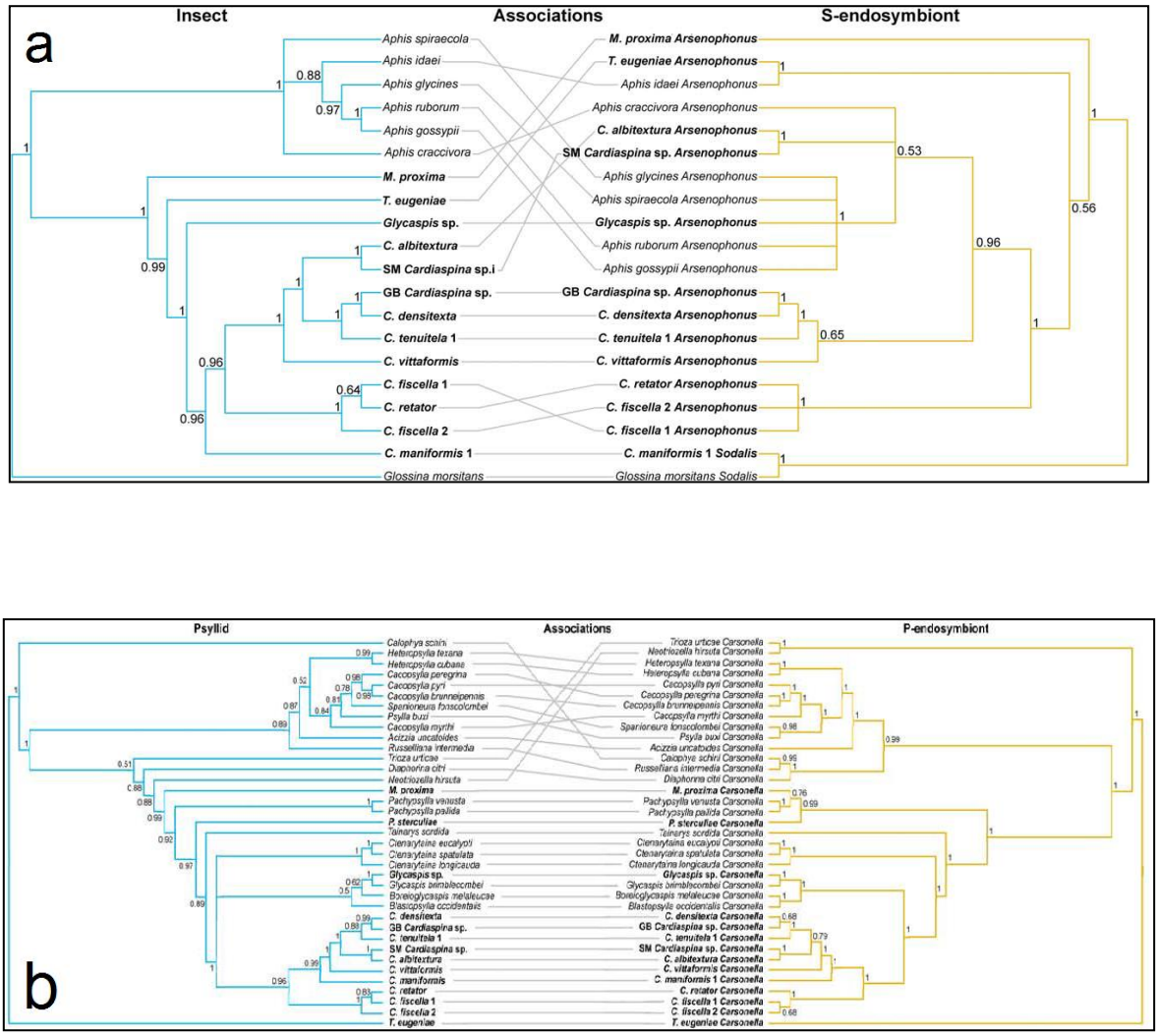


**Figure 1.7:** Reported here for a group of psyllids are (a) the associations between the insects and their S-symbionts, showing non matching molecular phylogenies as a result of horizontal gene transfer and multiple infection, and (b) the associations between psyllids and their P-symbiont showing matching molecular phylogeny suggesting a single ancestral infection and vertical transmission. The pictures are reproduced with permission by the journal (Hall et al. 2016).

### 1.3.3 The role of bacteria in the psyllids-host plants interactions

#### Bacterial mediated associations

The importance of some bacteria within the insect is related to their relationship with the insect's host plants (Figure 1.8). The role of endosymbionts in the insect-plant relationship has been widely studied in aphids. For example, Tsuchida and colleagues (2011) demonstrated that the injection of a symbiont from a clover-adapted pea aphid allowed another aphid species (that normally couldn't feed on clover) to use this host plant (Tsuchida et al. 2011). This discovery led to the hypothesis of a symbiont-mediated process for the acquisition of novel host plants by insects (Tsuchida et al. 2011). Host plants are known to be a route for horizontal transfer of mutualistic microorganisms into their host insects. Therefore, insect mutualists may be more important "hidden

players" in insect-plant interactions than is currently realized (Frago et al. 2012). In fact, if horizontal transmission is influenced by plant species, and since symbionts influence insect fitness, this three-way interaction may affect the host plant selection process of the insect. This could lead insects to specialize on different food plants (Frago et al. 2012). While natural selection acts on the insects and their vertically transmitted symbionts in the same way (what has a positive effect on one does so on the other), horizontal transmission through the plant implies that their evolutionary interest may differ, with important ramifications for the biology of the symbiosis (Frago et al. 2012).

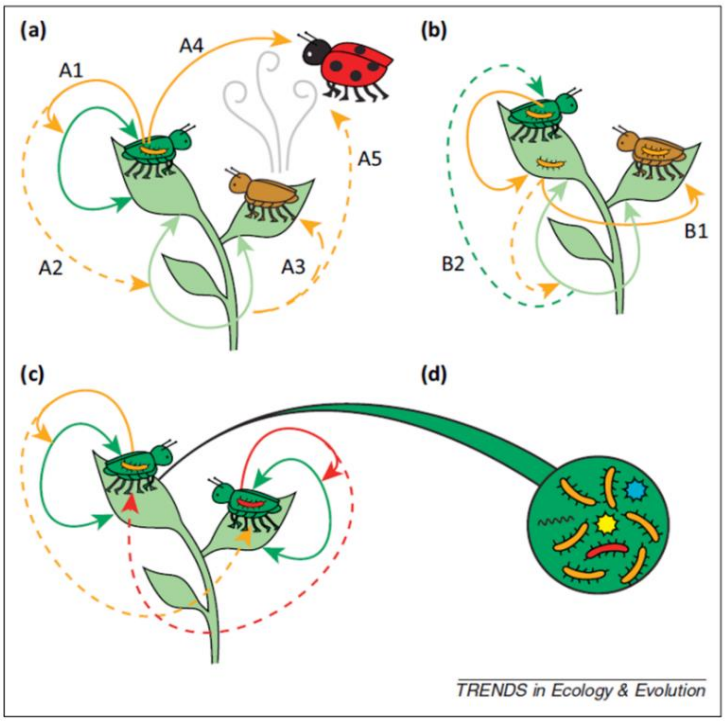


**Figure 1.8:** Insect symbionts (represented by an insect carrying a bacterium) influence insect–plant interactions through direct interactions (solid lines) as well as through indirect plant-mediated interactions (dashed lines). Yellow lines represent symbiont-mediated interactions, deep green lines represent insect–plant interactions, and pale green lines represent changes in plant state or physiology. (a) Insect symbionts can directly influence host plant use in herbivorous insects (A1), but also indirectly through changes to plant state or physiology (A2). Such changes can affect other insects sharing the same host plant (A3). Insect symbionts can directly affect the host's interactions with natural enemies (A4), but also indirectly through changes in plant physiology and the emission of herbivore-induced plant volatiles (A5). (b) Insect symbionts can colonize plants, which is a likely route for horizontal transmission (B1). Similarly, plant pathogens can be vectored by insects and this may evolve into mutualism if the insect benefits from a diseased host plant (B2). (c) Different insect symbionts can differentially affect insect host plant use and ultimately modulate interactions between insects. (d) Communities of insect symbionts, including bacteria, fungi, and viruses, are found in both insects and plants, where they can engage in complex interactions. The picture is reproduced with permission by the journal (Frago et al. 2012).

## Psyllid vectored plant pathogens

While the above paragraph illustrates the positive role of some bacteria in the psyllid-plant interaction, psyllids can also transmit plant pathogens.

The first report linking psyllids to plant disease was dated 1965, and identified the African citrus psyllid *Trioza erytreae* as responsible for the citrus greening disease in South Africa (McClean and Oberholzer 1965). More importantly, that first report stated that transmission of the disease was caused by the alphaproteobacterium *Candidatus* Liberibacter. In subsequent years, *Ca.* Liberibacter species were also reported in India, associated with the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Capoor et al. 1967) and with *Bactericera cockerelli*, the tomato potato psyllid (TPP) (Munyaneza et al. 2007). In New Zealand and North America, the highly invasive and damaging *B. cockerelli* acts as a vector of *Ca.* Liberibacter solanacearum that is recognized as the causal agent of the zebra chip disease (ZC) of potatoes. More recently, it has also been confirmed in Europe as a significant pest for carrots (Alvarado et al. 2012, Munyaneza et al. 2014) (Figure 1.9).

**Figure 1.9:** Symptoms of zebra chip disease in potatoes. Reproduced with permission of the authors (M. Paget & D. Gibson, Plant & Food Research).

*Liberibacters* are gram negative mostly unculturable bacteria belonging to the alphaproteobacteria group and are almost exclusively associated with psyllids as vectors. It is unclear if all *Liberibacters* are true plant pathogens or whether they are in fact insect endosymbionts that are an emergent group of pathogens (Raddadi et al. 2011). Recently, a new *Liberibacter* species has been associated with *Acizzia solanicola* in Australia but no pathogenicity has been recorded (Morris et al. 2017). While *Liberibacter* europaeus was found in European pear and Scotch broom in New Zealand, it was proposed to be non-pathogenic to the former (Raddadi et al. 2011). In a similar situation, the bacterial genus *Arsenophonus* that is mostly composed of insect symbionts, is now known to contain a number of plant pathogens (Duron et al. 2014).

Other species of psyllids act as vectors for damaging *Phytoplasma* species. *Phytoplasmas* are nonculturable degenerate gram-positive prokaryotes that cause more than 700 diseases in plant species (Weintraub and Beanland 2006). Insect vectors of phytoplasmas are primarily leafhoppers and planthoppers, as well as psyllids. In Europe and North America, some *Cacopsylla* species vector the *Phytoplasma* species *Candidatus* Phytoplasma pyri, *Ca.* Phytoplasma mali and *Ca*. Phytoplasma prunorum. The psyllid *Bactericera trigonica* is reported to transmit a *Phytoplasma* to carrots (Weintraub and Beanland 2006).

Today, several psyllids and their associated *Ca.* Liberibacter species are considered major threats to a variety of crops grown globally. Indeed, the EPPO/CABI dataset lists three psyllid species as major pests due to the *Ca.* Liberibacter species they vector (EPPO/CABI 1997). *Bactericera cockerelli* and *Diaphorina citri* are reported in the EPPO A1 list, as unwanted species not present in the Mediterranean area, while *Trioza erytreae* (vector of *Ca.* Liberibacter africanus) is reported in the A2 list as a species present but under management (EPPO/CABI 1997).

## 1.4 The New Zealand Psylloidea

In 1879, Maskell published the first record of a psyllid in New Zealand (Maskell 1879) and in the years that followed reported the presence of additional species (Maskell 1880, 1890, 1894). It wasn't until 1932, however, that a wider list of New Zealand psyllid species was published by Ferris and Klyver (Ferris and Klyver 1932), which included 25 proposed species. This list remained the standard reference until Tuthill published an updated list of psyllids in New Zealand in 1952 (Tuthill 1952).

Dale's list (1985) is the most comprehensive documentation of the psyllids of New Zealand (Chapter 2) and includes the biology, morphology, distribution and systematics of these insects. Dale recognized 81 species, including 24 species and three genera that were reported for the first time. Descriptions were provided for all the taxa recognized, and a key to the identification of each species was included. Unfortunately, many of the newly identified taxa were not formally named, with only one subsequently described as *Blastopsylla occidentalis* (Taylor 1987).

In the 25 years after Dale's work, other psyllid species arrived in New Zealand, including those that were introduced intentionally. For example, *Arytainilla spartiophila* was introduced from Europe as a biological agent for control of the weed *Cytisus scoparius*, the common or Scotch broom (Syrett et al. 1999). Some of the arrivals were summarized in the New Zealand Inventory of Biodiversity (Macfarlane et al. 2010), including the important crop pest *Bactericera cockerelli*, the tomato potato psyllid (TPP), whose distribution in New Zealand was described the year before the publication of the inventory (Teulon et al. 2009). Additional arrivals included the psyllids *Calophya*

*schini* (Calophyidae) on *Schinus molle* and *Mycopsylla fici* (Homotomidae) on *Ficus macrophylla* (Macfarlane et al. 2010). The arrival of these two species, probably mediated by importation of their ornamental host plants, resulted in the addition of two new psyllid families to the fauna of New Zealand. Taylor and Kent reported the arrival of *Acizzia solanicola* in 2013 (Taylor and Kent 2013).

In 2012, the new classification of the Psylloidea proposed by Burckhardt and Ouvrard also contributed to outdating the information on the New Zealand psyllids due to taxonomic reclassifications that changed some of the names previously used (Burckhardt and Ouvrard 2012). At this point, knowledge about the New Zealand Psylloidea was not only scattered and partial, but completely out of date, reporting imprecise names and nomenclature. Moreover, the lack of a genetic database that included New Zealand psyllids made morphological differentiation a challenging means for the identification of these insects. Then, since discovery of the pathogen *Ca.* Liberibacter solanacearum there in 2008 (Liefting 2009), virtually all study on psyllids has focused on TPP [(Teulon et al. 2009)]. This has only served to maintain the knowledge gap that exists for the New Zealand psyllid fauna in general, and particularly in terms of their contribution to maintaining horizontally transferable pathogenic bacteria in the environment.

## 1.5 Aims, hypotheses and scope of the research

### 1.5.1 Aims

Given the recent identification of new psyllids in New Zealand and the discovery that psyllids can vector plant pathogens, the overall aims of this thesis were three.

The **first aim** was to develop a better identification method and inventory for the New Zealand psyllids. An integrative taxonomic approach was applied to include molecular techniques in addition to the known information on morphology and host plant association.

The **second aim** of this study was to clarify the phylogenetic and evolutionary relationships between the different psyllid species present in New Zealand and, subsequently, to compare this information with the data available on the host plants in order to understand how psyllid-plant host associations evolved there. Understanding the processes that led to the present psyllid fauna could inform the risks associated with new arrivals and incursions.

The results obtained in the first two aims would lead to the **third aim**: to analyse the bacteria comprising the psyllid's microbiome and attempt to determine the relationships between symbiotic and non-symbiotic bacteria carried by these hemipterans.

Overall, his study aimed to combine the information obtained from the psyllid phylogenetic study and the analysis of their microflora to determine if bacterial associations were following the same evolutionary path and potentially brought about by the insect's radiation. This might enable correlations between pathogen transmission and vector genetic distance to be made for future risk assessment.

### 1.5.2 Objectives

In order to fulfil these Aims and test the Hypotheses, an updated understanding of psyllid biodiversity was required as well as information on the bacterial profile for each species or population. It was anticipated that a comparison of this data with that associated with their host plants would also aid in understanding the evolution of these insects. At this point it was prudent to define host plants as "all those categories defined by Burkhardt and colleagues where feeding or probing on the plant is known to occur" (Burckhardt et al. 2014). Thus, a series of objectives were developed and two hypotheses generated to be tested to fulfil the aim of the thesis:

**Objective 1:** Generate an accurate list of the psyllid species in New Zealand, as well as their geographic distribution and their hosts using existing literature and Entomological collections in New Zealand and Australia (for the species in common between the two countries) (Chapter 2).

**Objective 2:** Based on the distribution of the psyllids and/or their hosts identified in Objective 1, perform new field collections to update information on the current New Zealand biodiversity of these insects. At the same time, it was considered that the new specimens could be used to extract DNA and proceed with a more comprehensive assessment of biodiversity supported by DNA barcoding, morphological analysis, distribution and host plant association (Chapter 3).

**Objective 3.** Undertake a phylogenetic study of the New Zealand psyllid taxa to dissect their evolutionary relationships in more detail. This, in particular with regard to geographic distribution and host plant association, would allow a better understanding of the relationships between the different psyllid species and their relationships with the New Zealand landscape (e.g. native/adventive; number of arrivals) (Chapter 4).

**Objective 4:** Once psyllid biodiversity, systematics and host groupings were established, define the microbiome of a representative group of the psyllids to assess whether predictable relationships between the psyllid taxa and/or their host plants could be discerned (Chapter 5). This objective would include the possible record of plant pathogens in the New Zealand Psylloidea.

### 1.5.3 These objectives would lead to the questioning of two main hyportheses

**HYPOTHESIS 1:** A species concept approach based on integrative taxonomy, using a molecular analysis of the psyllids together with their morphology, distribution and host plant association, would reveal greater psyllid biodiversity in New Zealand than reported by studies based solely on morphology. Moreover, these additional taxa would contribute to a better understanding of the origin and evolution of the New Zealand Psylloidea.

**HYPOTHESIS 2:** Psyllid microbiomes show discernible species-species composition patterns. These would also show a stronger association with one of the following characters: geographical distribution, insect phylogeny or host plant association. Therefore, the microbiomes of newly arrived exotic psyllid species would be able to be evaluated in terms of this knowledge.

# Chapter 2

# Checklist of the New Zealand Psylloidea, 1985-2014

## 2.1 Context

The work presented in this chapter has been published in Zootaxa as "An Annotated checklist of the psyllids of New Zealand (Hemiptera: Psylloidea)" (http://doi.org/10.11646/zootaxa.4144.4.6) (Martoni et al. 2016). It describes the current knowledge of psyllids in New Zealand at the outset of this thesis, bringing together information from disparate sources using the latest taxonomic classifications for this group of insects (Burckhardt and Ouvrard 2012).

The text has been reformatted for the thesis.

## 2.2 Introduction

The first recorded psyllids from New Zealand were reported by Maskell (Maskell 1879, 1880, 1890, 1894), who described the adult stages from *Psylla acaciae*, *Rhinocola eucalypti* (both native to Australia), *Powellia doryphora, R. fuchsiae, Trioza panacis* and *T. pellucida* as well as *Powellia vitreoradiata* from immatures. A decade later, Marriner described *Trioza alexina* (Marriner 1903). Surprisingly, Hutton's Index Fauna Novae Zealandiae (Hutton 1904) listed only four species (*R. eucalypti, R. fuchsiae, T. panacis* and *T. pellucida*) despite these earlier findings, which forced Kirkaldy to comment on the omission of species from the Index (Kirkaldy 1906). Myers (1922) continued to list only five psyllid species in a review of Hemiptera from New Zealand (Myers 1922). Not until Ferris and Klyver (1932) and Tuthill (1952) revised the list of psyllids in New Zealand was a higher diversty of psyllids recognised: six genera consisting of a total of 25, and 51 species were described respectively by Ferris and Klyver (1932) and Tuthill (1952). Dumbleton (1964, 1967) recorded two further introduced *Psyllopsis* species on ash and described *T. dentiforceps* (Dumbleton 1964, 1967). These psyllid data for New Zealand were also summarised in two checklists of the New Zealand insects (Wise 1977, Spiller and Wise 1982).

A significant increase in knowledge of New Zealand's psyllids resulted from a detailed field survey and taxonomic study conducted by Dale (Dale 1985). She identified 81 species, including 24 newly proposed, and three new genera. Descriptions were provided for the proposed new taxa, although they were not formally named by Dale. One of them, *Blastopsylla occidentalis*, was formally described by Taylor (Taylor 1987). The New Zealand Inventory of Biodiversity (Henderson et al. 2010) subsequently reported 95 species of psyllid, of which 26 were undescribed; although Taylor

described one of these as *Casuarinicola australis* (Taylor et al. 2010). The New Zealand Inventory of Biodiversity included the introduced pest *Bactericera cockerelli* and the intentionally introduced *Arytainilla spartiophila* from Europe. *Arytainilla spartiophila* was introduced as a biological control agent against the weed *Cytisus scoparius*, the common or Scotch broom (Syrett et al. 2007). Finally, new introductions were reported by Taylor and Kent (2013: *Acizzia solanicola*) and Thorpe (NatureWatchNZ 2016: *Mycopsylla* sp.) (Taylor and Kent 2013). The occurrence of the Australian species *Phellopsylla formicosa* was reported for the first time in a publication of the Ministry for Primary Industries (MPI 2015).

This chapter focuses on the development of an updated psyllid checklist, consolidating the historic information on the New Zealand psyllid fauna from disparate sources using the latest methods for taxonomic classification. The development of the checklist was driven by the need to understand what species occur in New Zealand as a result of the arrival of *B. cockerelli* (Teulon et al. 2009), which vectors the plant pathogen *Candidatus* Liberibacter solanacearum. This pathogen has many solanaceous plant hosts, but its most notable economic consequence is the cause of the Zebra chip disease in potatoes (Liefting et al. 2009). Another recent arrival is the pest species *Acizzia solanicola*, which causes 'psyllid yellows' in eggplants, *Solanum melongena* (Solanaceae) (Kent and Taylor 2010, Taylor and Kent 2013). The detection of these insects demonstrated the ongoing vulnerability of New Zealand to new invasions.

## 2.3 Materials and Methods: entomological collections and databases.

The checklist contains all original (primary) records of psyllids from New Zealand. Those mentioned in secondary sources, such as checklists or the website Psyl'list (Ouvrard 2017), were not automatically repeated. Species are listed alphabetically using the classification of Burckhardt and Ouvrard (Burckhardt and Ouvrard 2012).

Comprehensive geographic distribution information was developed, drawing from the literature, in particular the work of Dale (1985), the five main entomological collections of New Zealand, the Forest Health Database (FHDB) and the website http://naturewatch.org.nz. The entomological collections were those from the following institutions: the New Zealand Arthropod Collection (NZAC; containing the specimens collected and identified by Dale), the Lincoln University Entomology Research Collection (LUNZ), the Canterbury Museum (CMNZ), the Museum of New Zealand (MONZ) and the Auckland Museum (AMNZ). The FHDB included more than a thousand records, several hundred of which were identified to species. From the NatureWatchNZ website only observations marked as 'quality grade research' were considered; these comprised pictures, GPS coordinates, information about the host plant and the name of the identifier. Distributions across

New Zealand were described using the regional labels of Crosby et al. (Crosby et al. 1998)(Figure 2.1).

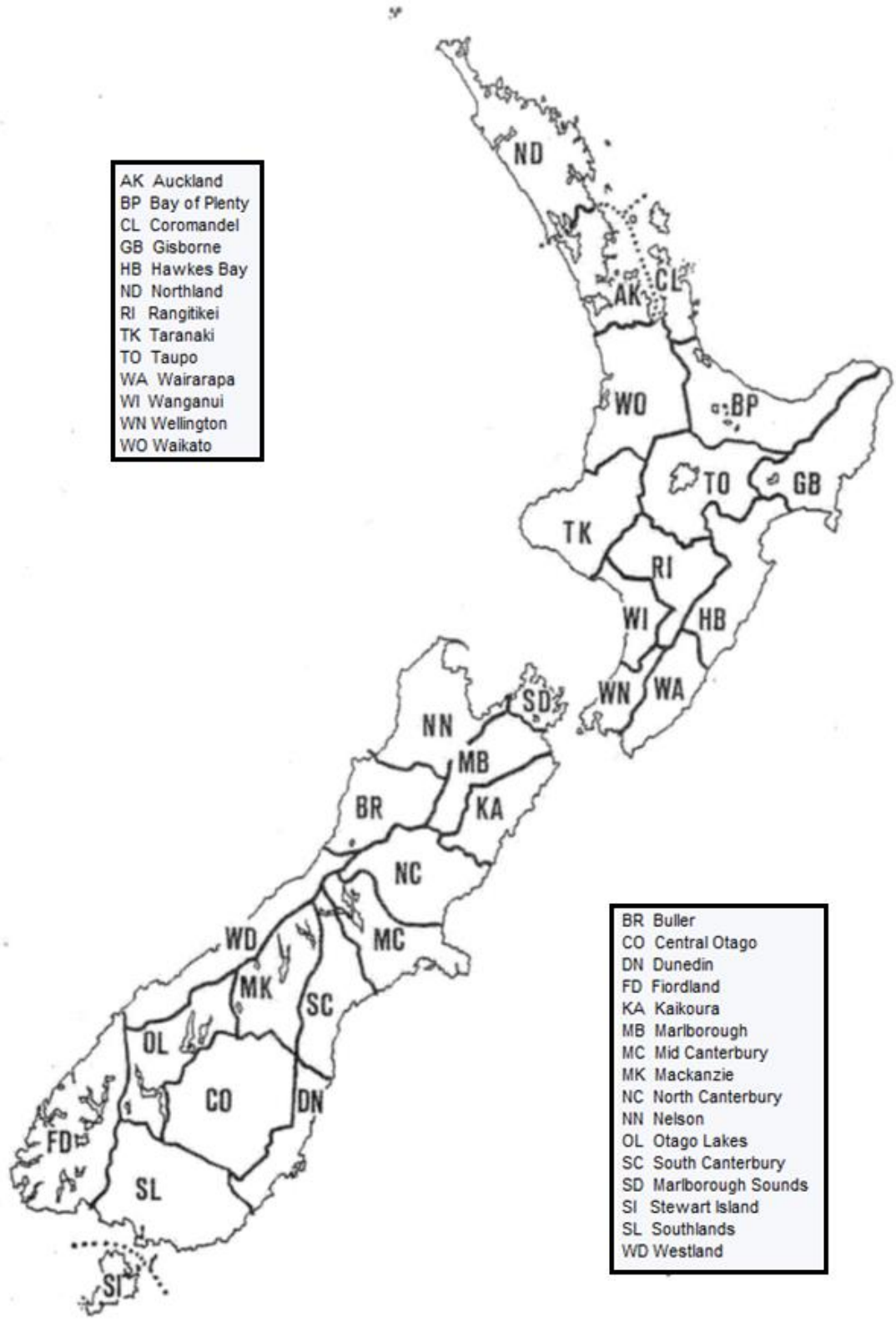


**Figure 2.1:** Map of New Zealand with regional subdivisions used in the checklist (modified from Crosby et al. 1998).

Additional information on the host plants and natural enemies (parasitoids, predators) of each psyllid species was given exclusively for records from New Zealand (Table 2.1). The nomenclature for host plants was defined by Burckhardt and colleagues (Burckhardt et al. 2014), and followed The Plant List (The Plant List 2016). For host families, the Angiosperm Phylogeny Website (Angiosperm Phylogeny Website 2016) was used.

Further information on the general distribution and host plants can be found in Psyl'list (Ouvrard 2017).

## 2.4 Results: the checklist

A summary of the psyllid families and genera with their associated host plants is provided in Table 2.1. Details of the species and their distributions are reported in the following checklist.

**Table 2.1:** New Zealand psyllid families, subfamilies, genera (with number of species) and host plant families (genera).

| Psyllid family | Psyllid subfamily | Psyllid genus (# species) | Host plant family (genus) |
|---|---|---|---|
| Aphalaridae | Rhinocolinae | *Anomalopsylla* (3) | Asteraceae (*Olearia*), Rutaceae (*Geijera*) |
| Aphalaridae | Spondyliaspidinae | *Anoeconeossa* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Blastopsylla* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Cardiaspina* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Creiis* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Cryptoneossa* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Ctenarytaina* (10) | Myrtaceae (*Eucalyptus*, *Kunzea*, *Leptospermum*, *Lophostemon*), Onagraceae (*Fuchsia)*, Rutaceae (*Boronia*) |
| Aphalaridae | Spondyliaspidinae | *Eucalyptolyma* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Glycaspis* (1) | Myrtaceae (*Eucalyptus*) |
| Aphalaridae | Spondyliaspidinae | *Phellopsylla* (1) | Myrtaceae (*Eucalyptus*) |
| Calophyidae | Atmetocraniinae | *Atmetocranium* (1) | Cunoniaceae (*Weinmannia*) |
| Calophyidae | Calophyinae | *Calophya* (1) | Anacardiaceae (*Schinus*) |
| Liviidae | Euphyllurinae | "*Gyropsylla*" (1) | unknown |
| Liviidae | Euphyllurinae | *Psyllopsis* (2) | Oleaceae (*Fraxinus*) |
| Homotomidae | Macrohomotominae | *Mycopsylla* (2) | Moraceae (*Ficus*) |
| Psyllidae | Acizziinae | *Acizzia* (11) | Fabaceae (*Acacia*, *Albizia*), Proteaceae (*Grevillea*, *Hakea*), Sapindaceae (*Dodonaea*), Solanaceae (*Brugmansia*, *Physalis*, *Solanum*) |
| Psyllidae | Psyllinae | *Arytainilla* (1) | Fabaceae (*Cytisus*) |
| Psyllidae | Psyllinae | *Baeopelma* (1) | Betulaceae (*Alnus*) |
| Psyllidae | Psyllinae | '*Psylla*' (2) | Fabaceae (*Carmichaelia*, *Sophora*) |
| Triozidae | | *Bactericera* (1) | polyphagous, mostly Solanaceae |
| Triozidae | | *Casuarinicola* (1) | Casuarinaceae (*Casuarina*) |

| | | |
|---|---|---|
| Triozidae | *Trioza* (52) | Alseuomiaceae (*Alseuosmia*), Araliaceae (*Pseudopanax*, *Schefflera*), Asteraceae (*Brachyglottis*, *Cassinia*, *Celmisia*, *Cotula*, *Leptinella*, *Olearia*), Elaeocarpaceae (*Aristotelia*, *Elaeocarpus*), Ericaceae (*Dracophyllum*), Malvaceae (*Plagianthus*), Primulaceae (*Myrsine*), Myrtaceae (*Acca*, *Acmena*, *Metrosideros*, *Syzygium*), Pittosporaceae (*Pittosporum*), Podocarpaceae (*Halocarpus*), Polygonaceae (*Muehlenbeckia*), Rhamnaceae (*Discaria*), Plantaginaceae (*Hebe*) |
| Triozidae | Gen. Dale (1985) (1) | Apiaceae (*Anisotome*) |
| Triozidae | Gen. Henderson *et al.* (2010) (1) | Casuarinaceae (*Casuarina*) |

**Family Aphalaridae**

**Subfamily Rhinocolinae**

***Anomalopsylla insignita*** Tuthill, 1952

Distribution: New Zealand: AK, BR, NC, SL, WN (Dale 1985), MC (LUNZ), NN (Tuthill 1952, Dale 1985).

Host plants: *Olearia albida, O. avicenniifolia, O. nummulariifolia, O. paniculata* (Asteraceae).

***Anomalopsylla* sp.**

Distribution: New Zealand: AK (Dale 1985, as *Anomalopsylla* n. sp. 'Pollen Island').

Host plants: *Olearia solandri* (Asteraceae).

***Anomalopsylla* sp.**

Distribution: New Zealand: SD (Dale 1985, as *Anomalopsylla* n. sp. 'Port Underwood').

Host plants: *Olearia solandri* (Asteraceae).

Comments: This species is listed as a threatened species in New Zealand (Stringer et al. 2012).

**Subfamily Spondyliaspidinae**

***Anoeconeossa communis*** Taylor, 1987 (Taylor 1987).

Distribution: Australia; introduced into New Zealand (Henderson et al. 2010).

Host plants: *Eucalyptus* sp. (Myrtaceae).

Natural enemies: *Psyllaephagus richardhenryi* (Hymenoptera: Encyrtidae) (Macfarlane et al. 2010).

***Blastopsylla occidentalis*** (Taylor, 1985)

Distribution: Australia; introduced into Africa, North and South America, Asia, Europe and New Zealand: AK [(Taylor 1985); Dale 1985, as 'genus C' n. sp.], BP, ND, NN (Dale 1985, as 'genus C' n. sp.).

Host plants: *Eucalyptus leucoxylon, E. maideni, E. nicholii, E. viridis* (Myrtaceae).

***Cardiaspina fiscella*** Taylor, 1962 (Taylor 1962)

Distribution: Australia; introduced into New Zealand: (Henderson et al. 2010); AK, BP, CL, GB, HB, ND, RI, TK, WA, WI, WN, WO (AMNZ, MONZ, FHDB).

Host plants: *Eucalyptus* sp. (Myrtaceae).

Natural enemies: *Coccidoctonus gemitus, Psyllaephagus gemitus* (Hymenoptera: Encyrtidae) (Macfarlane et al. 2010).

***Creiis lituratus*** (Froggatt 1900)

Distribution: Australia; introduced into New Zealand: (Henderson et al. 2010, as *Creiis liturata* [sic]); AK (AMNZ, FHDB), BP, CL, GB, ND, WI, WO (FHDB).

Host plants: *Eucalyptus* sp. (Myrtaceae). In Australia on *Eucalyptus robusta* (Hollis 2004).

***Cryptoneossa triangula*** Taylor, 1990 (Taylor 1990)

Distribution: Australia; introduced into USA and New Zealand: (Henderson et al. 2010); AK,

BP, HB, ND (AMNZ, FHDB, MONZ).

Host plants: *Eucalyptus citriodora, E. maculata* (Myrtaceae).

***Ctenarytaina clavata*** Ferris & Klyver, 1932

Distribution: New Zealand: (Tuthill 1952), AK, BP, BR, NC, ND, NN, TO, WI (Dale 1985), WN

(Ferris and Klyver 1932, Dale 1985); MC (LUNZ).

Host plants: *Leptospermum scoparium* (Myrtaceae).

Comments: Tuthill (1952) 'found this minute species to be present on both *Leptospermum scoparium* and *L. ericoides* [=*Kunzea ericoides*] at many localities throughout New Zealand'. Judging from the host plants he collected from, Tuthill's (1952) record is a mix of *C. clavata* and *C. pollicaris* (Dale 1985).

***Ctenarytaina eucalypti*** (Maskell 1890)

Distribution: Australia; introduced into Africa, America (North and South), Asia, Europe and New

Zealand: (Maskell 1890; (Myers 1922, Clark 1938); Clark 1938, all as *Rhinocola eucalypti*;

(Miller 1971)), BP, MC (Tuthill 1952), WI (Tuthill 1952; Dale 1985); AK, BR, CL, DN, GB,

HB, MB, NC, ND, NN, SC, SL, TK, TO, WD, WO, WN (FHDB, LUNZ).

Host plants: *Eucalyptus globulus* (Myrtaceae). In Australia on several *Eucalyptus* spp. (Hollis 2004).

Natural enemies: *Psyllaephagus pilosus* Noyes, 1988 (Hymenoptera: Encyrtidae) (Macfarlane et al.

2010).

***Ctenarytaina fuchsiae*** (Maskell 1890)

Distribution: New Zealand: [(Maskell 1890), as *Rhinocola fuchsiae*; Tuthill 1952], AK (Dale 1985), MC

(Ferris & Klyver 1932), NN, TK, TO, WD (Dale 1985), WN (Ferris & Klyver 1932; Dale

1985); RI, SI (CMNZ, LUNZ).

Host plants: *Fuchsia excorticata* (Onagraceae).

***Ctenarytaina longicauda*** (Taylor 1987)

Distribution: Australia; introduced into the USA and New Zealand: (Henderson et al. 2010), AK (AMNZ, FHDB, LUNZ).

Host plants: *Lophostemon confertus* (Myrtaceae). In Australia on *Lophostemon suaveolens* (Hollis 2004).

***Ctenarytaina pollicaris*** (Ferris & Klyver, 1932)

Distribution: New Zealand: AK, BP, BR, MB (Dale 1985), MC (Tuthill 1952; Dale 1985), ND, NN (Dale 1985), WI (Ferris & Klyver 1932; Tuthill 1952; Dale 1985), WN (Ferris & Klyver 1932; Dale 1985).

Host plants: *Kunzea ericoides* (Myrtaceae).

***Ctenarytaina spatulata*** Taylor, 1997 (Taylor 1997)

Distribution: Australia; introduced into America (North and South), Europe and New Zealand: (Henderson et al. 2010), South Island (Taylor 1997), MC (Bullians 2015); AK, BP, DN, FD, HB, NC, ND, RI, SI, SL, WN (FHDB, LUNZ).

Host plants: *Eucalyptus* sp. (Myrtaceae). In Australia on several *Eucalyptus* spp. (Hollis 2004).

***Ctenarytaina thysanura*** Ferris & Klyver, 1932

Distribution: Australia and New Zealand: (Tuthill 1952), AK, WI (Dale 1985), DN (introduced from Australia: Melbourne, Ferris & Klyver 1932; Dale 1985); SC, TO, WN (FHDB, LUNZ).

Host plants: *Boronia heterophylla*, *B. megastigma* (Rutaceae).

***Ctenarytaina* sp.**

Distribution: New Zealand: AK, BP, ND, NN, WN (Dale 1985, as *Ctenarytaina* n. sp. 'cutaway').

Host plants: *Kunzea ericoides* (Myrtaceae).

Comments: When Dale (1985) described this species, she listed *Leptospermum ericoides* as the only host plant. However, a revision of the *Leptospermum* genus had already been made (Thompson 1983) with *L. ericoides* transferred to *Kunzea* as *K. ericoides,* therefore it is now listed here as such. Henderson and colleagues listed two *Ctenarytaina* spp. both with *Leptospermum* as host plant, not specifying the species; this probably referred to Dale's work but without updating the plant classification (Henderson et al. 2010).

***Ctenarytaina* sp.**

Distribution: New Zealand: BR, MB, MC, NN, WN (Dale 1985, as *Ctenarytaina* n. sp. 'short').

Host plants: *Kunzea ericoides* (Myrtaceae).

Comments: When Dale (1985) described this species, she listed *Leptospermum* ericoides as the only host plant. However, a revision of the *Leptospermum* genus had already been made (Thompson 1983) with *L. ericoides* transferred to *Kunzea* as *K. ericoides* therefore it is now listed here as such. Henderson and colleagues listed two *Ctenarytaina* spp. both with Leptospermum as host plant, not specifying the species; this probably referred to Dale's work but without updating the plant classification (Henderson et al. 2010).

***Ctenarytaina* sp.**

Distribution: Australia; introduced into New Zealand: (Henderson et al. 2010).

Host plants: *Syzygium* sp. (Myrtaceae).

***Eucalyptolyma maideni*** Froggatt, 1901

Distribution: Australia; introduced into USA and New Zealand: (Henderson et al. 2010), AK, BP, MC (AMNZ, FHDB).

Host plants: *Eucalyptus* sp. (Myrtaceae). In Australia on several *Eucalyptus* spp. (Hollis 2004).

***Glycaspis granulata*** (Froggatt 1901)

Distribution: Australia; introduced into New Zealand: (Henderson et al. 2010), AK (LUNZ; AMNZ; FHDB), BP, CL (AMNZ; FHDB), GB, HB, KA, ND, NN, TK, WA, WI, WN, WO (FHDB).

Host plants: *Eucalyptus* sp. (Myrtaceae). In Australia on several *Eucalyptus* spp. (Hollis 2004).

***Phellopsylla formicosa*** (Froggatt 1900)

Distribution: Australia: introduced into New Zealand: AK (AMNZ).

Host plants: *Eucalyptus saligna* (Myrtaceae). In Australia on *Eucalyptus piperita* (Hollis 2004).

Comments: The record of this species was reported on the published checklist based on samples present in the NZAC, Auckland. However, a personal communication of S. Thorpe, "kindly" highlighted the presence of an MPI publication that had reported it previously (MPI 2015).

**Family Calophyidae**

**Subfamily Atmetocraniinae**

***Atmetocranium myersi*** (Ferris & Klyver, 1932)

Distribution: Australia and New Zealand: BP (Dale 1985; Tuthill 1952), FD, NN (Dale 1985), SI (Tuthill 1952; Dale 1985), SL (Tuthill 1952), TO (Tuthill 1952; Dale 1985), WN (Ferris & Klyver 1932, as *Pauropsylla myersi*; Dale 1985); BR (CMNZ), CL (AMNZ), WD (FHDB).

Host plants: *Weinmannia racemosa* (Cunoniaceae).

**Subfamily Calophyinae**

***Calophya schini*** Tuthill, 1959

Distribution: Bolivia, Peru; introduced into Argentina, Chile, Colombia, Africa, North America, Europe and New Zealand: AK (Burckhardt and Basset 2000), MC (Anonymous 2011); BP, HB, MB, ND, NN, WA, WI, WN (FHDB).

Host plants: *Schinus molle* (Anacardiaceae).

**Family Liviidae**

**Subfamily Euphyllurinae**

***'Gyropsylla' zealandica*** (Ferris & Klyver, 1932)

Distribution: New Zealand: FD, NC (Ferris & Klyver 1932, as *Metaphalara zealandica*; Dale 1985, as 'Genus A' *zealandica*), NN, SI, SL, WD (Dale 1985, as 'Genus A' *zealandica*).

Host plants: Unknown.

Comments: When describing *Metaphalara zealandica*, Ferris & Klyver (1932) pointed out its 'doubtful position' taxonomically, but did not provide reasons for including it in the New World genus *Metaphalara*. Tuthill (1952) cited the species under *Gyropsylla*, a senior synonym of *Metaphalara*. After examining the type species of *Gyropsylla*, Dale (1985) concluded that it was not congeneric with *G. zealandica*, which is related to *Psyllopsis*, and instead represented an undescribed genus in the 'Diaphorininae' (= Diaphorinini sensu Burckhardt & Ouvrard 2012). This classification was followed by subsequent authors (Burckhardt 1986, 1987, Brown and Hodkinson 1988).

***Psyllopsis fraxini*** (Linné 1758)

Distribution: Asia, Europe; introduced into North America, Australia and New Zealand: AK, SL (Dale 1985), MC (Dumbleton 1964; Dale 1985); SC (FHDB).

Host plants: *Fraxinus excelsior* (Oleaceae). In the Palaearctic on several *Fraxinus* spp. (Ossiannilsson, 1992).

Natural enemies: *Ausejanus albisignatus* (Knight 1938) (Hemiptera: Miridae) (Macfarlane et al. 2010).

***Psyllopsis fraxinicola*** (Foerster 1848)

Distribution: North Africa, Asia, Europe; introduced into North and South America, Australia and New Zealand: MC (Dumbleton 1964; Dale 1985), WI (Dale 1985); CO, SC, TK, WN, WO (FHDB).

Host plants: *Fraxinus excelsior* (Oleaceae). In the Palaearctic on several *Fraxinus* spp. (Ossiannilsson, 1992).

Natural enemies: *Ausejanus albisignatus* (Knight 1938) (Hemiptera: Miridae) (Macfarlane et al. 2010).

**Family Homotomidae**

**Subfamily Macrohomotominae**

***Mycopsylla fici*** (Tryon 1895)

Distribution: Australia, New Guinea; introduced into New Zealand: (Henderson et al. 2010); AK (AMNZ, LUNZ).

Host plants: *Ficus macrophylla* (Moraceae).

***Mycopsylla* sp.**

Distribution: probably Australia; introduced into New Zealand: AK.

Host plants: *Ficus rubiginosa* (Moraceae).

Comments: From the photographs given by Thorpe (NatureWatch.nz 2016) this appears to be an undescribed species probably introduced from Australia along with its host.

**Family Psyllidae**

**Subfamily Acizziinae**

***Acizzia acaciae*** (Maskell 1894)

Distribution: Australia; introduced into New Zealand: AK (Ferris & Klyver 1932, as *Psylliae* [sic] *acaciae*), BP (Tuthill 1952, as *Psylla (Acizzia) acaciae*; Dale 1985), MB, ND, SC, WI (Dale

1985), NN (Tuthill 1952, as *Psylla (Acizzia) acaciae*), WN (Maskell 1894, as *Psylla acaciae*; Ferris & Klyver 1932, as *Psylliae [sic] acaciae*); BR, CL, GB, HB, MC, RI, SD, TO (FHDB).

Host plants: *Acacia melanoxylon* (Fabaceae).

Natural enemies: *Adalia bipuncatata* (Linnaeus, 1758), *Cleobora mellyi* (Mulsant 1850), *Halmus chalybeus* (Boisduval 1835), *Harmonia conformis* (Boisduval 1835), *Drepanacra binocula* (Newman 1838) (Coleoptera: Coccinellidae) (Macfarlane et al. 2010).

***Acizzia acaciaebaileyanae*** (Froggatt 1901)

Distribution: Australia, Philippines, introduced in South Africa, Europe, USA and New Zealand: AK (Dale 1985), MC (Ferris & Klyver 1932, as *Psyllia uncata*; Tuthill 1952, as *Psylla (Acizzia) acaciae-baileyanae* [sic]; Dale 1985), WI, (Tuthill 1952, as *Psylla (Acizzia) acaciae-baileyanae* [sic]; Dale 1985); NN, TO, WN, WO (FHDB).

Host plants: *Acacia baileyana*, *A. podalyriifolia* (Fabaceae).

Natural enemies: *Psyllaephagus acaciae* Noyes, 1988 (Hymenoptera: Encyrtidae); *Cleobora mellyi* (Mulsant 1850) (Coleoptera: Coccinellidae) (Macfarlane et al. 2010).

***Acizzia albizziae*** (Ferris & Klyver, 1932)

Distribution: Australia; introduced into New Zealand: (Tuthill 1952, as *Psylla (Acizzia) albizziae*), MC (Ferris & Klyver 1932, as *Psyllia albizziae*; Dale 1985), NN, SD, WI (Dale 1985).

Host plants: *Acacia dealbata, A. decurrens, A. mearnsii* (Fabaceae). Ferris & Klyver (1932) reported adults and immatures from *Albizia lophantha* (Fabaceae) but neither Tuthill (1952) nor Dale (1985) found any material on this species making this record doubtful.

Natural enemies: *Drepanacra binocula* (Newman 1838) (Neuroptera: Hemerobiidae) (Macfarlane et al. 2010).

***Acizzia conspicua*** Tuthill, 1952

Distribution: Australia; introduced into New Zealand: AK, HB, NN, WI (Dale 1985), ND (Tuthill 1952, as *Psylla (Acizzia) conspicua*; Dale 1985); GB, TO (FHDB).

Host plants: *Acacia longifolia* (Fabaceae). Tuthill (1952) listed *A. melanoxylon* as host, but Dale (1985) never found it on that plant. Hollis (2004) also listed for Australia *A. dealbata* and *A. melanoxylon.*

***Acizzia dodonaeae*** Tuthill, 1952

Distribution: Australia; introduced into New Zealand: AK, NC, ND, WN (Dale 1985), BP (Tuthill 1952, as *Psylla (Acizzia) dodonaeae*; Dale 1985), NN (Tuthill 1952, as *Psylla (Acizzia) dodonaeae*); HB, MC, SL, TK (FHDB).

Host plants: *Dodonaea viscosa* (Sapindaceae).

***Acizzia exquisita*** Tuthill, 1952

Distribution: Australia; introduced into New Zealand: AK (Tuthill 1952, as *Psylla (Acizzia) exquisita*; Dale 1985), ND, WI (Dale 1985).

Host plants: *Acacia decurrens* (Fabaceae). Hollis (2004) listed for SE Australia *A. melanoxylon* and *A. obliquinervia.*

***Acizzia hakeae*** Tuthill, 1952

Distribution: Presumably Australia but as yet undocumented (Percy et al. 2012); introduced into USA (California) and New Zealand: AK, ND (Tuthill 1952, as *Psylla (Acizzia) hakeae*; Dale 1985), BP (Tuthill 1952, as *Psylla (Acizzia) hakeae*); GB, HB, MC, NN, SD, TK, WA, WI, WN (FHDB).

Host plants: *Hakea acicularis* (Proteaceae). In Australia possibly on *Hakea* spp. (see remarks below)

although the native host plant preferences are unknown; in California recorded from *Grevillea* and *Hakea* spp. (Percy et al. 2012).

Comments: Tuthill (1952) suggested that the species is 'apparently introduced from Australia' and mentioned that 'Keith L. Taylor of the Division of Entomology [CSIRO], Australia, has taken a closely related species from *Hakea dactyloides* in New South Wales.'

***Acizzia jucunda*** Tuthill, 1952

Distribution: Australia and New Zealand: AK (Tuthill 1952, as *Psylla (Acizzia) jucunda*; Dale 1985), MC, NN, RI, SC, WO (Dale 1985); BP, ND, SL, WI (FHDB).

Host plants: *Acacia baileyana, A. dealbata, A. decurrens, A. mearnsii* (Fabaceae).

***Acizzia solanicola*** Kent & Taylor, 2010

Distribution: Australia; introduced into New Zealand: AK (Kent & Taylor 2010).

Host plants: *Brugmansia* sp., *Physalis peruviana*, *Solanum mauritianum*, *S. melongea*, *S. petrophilum* (Solanaceae).

***Acizzia uncatoides*** (Ferris & Klyver, 1932)

Distribution: Australia; introduced in Chile, Colombia, Europe, Guadeloupe, Mexico, USA and New Zealand: AK, HB, WD (Dale 1985), BP, ND, TK, WI (Tuthill 1952, as *Psylla (Acizzia) uncatoides*; Dale 1985), NN (Ferris & Klyver 1932, as *Psyllia uncatoides*; Dale 1985); TO, WO (FHDB).

Host plants: *Acacia* and *Albizia* spp. (Fabaceae).

Natural enemies: *Adalia bipuncatata* (Linnaeus, 1758), *Cleobora mellyi* (Mulsant 1850), *Halmus chalybeus* (Boisduval 1835) and *Harmonia conformis* (Boisduval. 1835) (Coleoptera: Coccinellidae); *Drepanacra binocula* (Newman 1838) (Neuroptera: Hemerobiidae) (Macfarlane et al. 2012).

***Acizzia* sp.**

Distribution: Probably Australia although it has not been reported there yet; introduced into New Zealand: AK (Dale 1985, as n. sp. "Waitakere").

Host plants: *Acacia mearnsii* (Fabaceae).

**Subfamily Psyllinae**

***Arytainilla spartiophila*** (Foerster 1848)

Distribution: Europe; introduced as bio-control agent into Australia, USA and New Zealand: BP, CO, DN, FD, HB, KA, MC, MK, NC, NN, RI, SC, SL, WA, WI, WO (Syrett et al. 2007); TO (FHDB).

Host plants: *Cytisus scoparius* (Fabaceae).

***Baeopelma foersteri*** (Flor 1861)

Distribution: Europe, Northern Africa, Middle East; introduced into New Zealand: AK, WI (Dale 1985, as *Psylla foersteri*).

Host plants: *Alnus glutinosa*, *A. incana* (Betulaceae).

***"Psylla" apicalis*** (Ferris & Klyver, 1932)

Distribution: New Zealand: AK, BR, CL, NN, WD (Dale 1985, as *Euphalerus apicalis*), FD, ND (Tuthill 1952), MC (Ferris & Klyver 1932, as *Psyllia apicalis*; Dale 1985).

Host plants: *Sophora microphylla, S. prostrata, S. tetraptera* (Fabaceae).

Comments: Tuthill (1952) stated that the species resembles *Euphalerus nidifex* Schwartz in appearance, but left it in *Psylla* until a more adequate concept of Neotropical *Euphaerus* becomes available. He also suggested that the Oriental and Pacific species referred to as *Euphalerus* depart widely from the type species *E. nidifex*. Dale (1985) pointed out important differences of the immatures of *P. apicalis* and *P. carmichaeliae* to those of *E. nidifex*. However, based on the resemblence of adults to *E. nidifex* and two Japanese species referred to as *Euphalerus*, she tranferred the two New Zealand species to *Euphalerus*. Hollis & Martin (1997), when redefining *Euphalerus* to include only New World species, confirmed Tuthill's (1952) suggestion that Asian

species referred to as *Euphalerus* are not congeneric with the type species. The last instar immatures of the two New Zealand species possess 8-segmented antennae, marginal setae on the caudal plate, a ventrally positioned anus with a unilayered circumanal ring and lack additional porefields on the caudal plate. These characters place the two species in the Psyllinae, but outside *Psylla* and probably in a new genus. While awaiting a revision of the species they are left in *Psylla*.

***"Psylla" carmichaeliae*** Tuthill, 1952

*Psylla carmichaeliae indistincta* Tuthill, 1952; Dale 1985: 196.

Distribution: New Zealand: AK, CL, MC, ND, NN, TK, TO, SL (Dale 1985, as *Euphalerus carmichaeliae*), CO (Tuthill 1952; Dale 1985, as *Euphalerus carmichaeliae*), MB, WD (Tuthill 1952, as *Psylla carmicaheliae indistincta*; Dale 1985, as *Euphalerus carmichaeliae*), OL (Tuthill 1952); WN (FHDB).

Host plants: *Carmichaelia* spp. (Fabaceae).

Comments: Tuthill (1952) erected *Psylla carmichaeliae indistincta* for populations from Fox Glacier and Rai Valley, but Dale (1985) showed that these lie within the morphological range of the nominal species and synonymised the two. Henderson et al. (2010) listed the two taxa separately. '*Psylla* aff. *carmichaeliae'* has been listed as a threatened species in New Zealand (Stringer et al. 2012). Its host plant, *Carmichaelia torulosa*, is nationally endangered.

**Family Triozidae**

***Bactericera cockerelli*** (Šulc 1909)

Distribution: USA, Canada, Mexico; introduced into New Zealand: AK, BP, HB, MC, ND, WO, CL, GB, TK, TO, WI, WN, NN, NC, SC, DN (Teulon et al. 2009).

Host plants: Polyphagous, but mostly on species of Solanaceae including *Capsicum*, *Lycium* and *Solanum*.

Comments: A pest of potatoes, tomatoes, capsicum and aubergine (Solanaceae). Sporadic but sometimes devastating outbreaks are known in greenhouses and potato growing areas of Arizona,

California, Colorado, New Mexico, Texas, and also New Zealand since 2006. Heavy infestations of immatures cause symptoms known as 'psyllid yellows'. Importantly *B. cockerelli* is vector of the bacterium *Candidatus* Liberibacter solanacearum, the causal agent of the "zebra chips" disease. Listed in the New Zealand national register of pests (Biosecurity New Zealand 2016).

***Casuarinicola australis*** Taylor, 2010

Distribution: Australia; introduced into New Zealand: (Henderson et al. 2010, as Gen. sp. indet. *Casuarina*), AK (Thorpe 2013); ND (LUNZ).

Host plants: *Casuarina cristata, C. cunninghamiana, C. equisetifolia, C. glauca, C. obesa, C. pauper* (Casuarinaceae).

***Trioza acuta*** (Ferris & Klyver, 1932)

Distribution: New Zealand: MC, NN (Dale 1985), SD (Tuthill 1952; Dale 1985), WN (Ferris & Klyver, 1932, as *Powellia acuta*; Dale 1985).

Host plants: *Ozothamnus leptophyllus* (Asteraceae).

***Trioza adventicia*** Tuthill, 1952

Distribution: Probably Australia; likely to be introduced into New Zealand: AK (Dale 1985), NN (Tuthill 1952; Dale 1985); BP, CL, GB, HB, MC, WI, WN (FHDB, LUNZ).

Host plants: *Angophora floribunda, Syzygium smithii* (Myrtaceae).

Comments: The description of *T. adventicia* is extremely similar to that of *T. eugeniae* Froggatt and these may be synonymised in the future (Percy 2017).

***Trioza alseuosmiae*** Tuthill, 1952

Distribution: New Zealand: BP (Tuthill 1952; Dale 1985), TO, WO (Dale 1985).

Host plants: *Alseuosmia macrophylla* (Alseuosmiaceae).

***Trioza australis*** Tuthill, 1952

Distribution: New Zealand: SI (Tuthill 1952; Dale 1985).

Host plants: *Brachyglottis rotundifolia* (Asteraceae).

***Trioza bifida*** (Ferris & Klyver, 1932)

Distribution: New Zealand: AK, BR, MK, SL (Dale 1985), DN (Ferris & Klyver 1932, *Powellia bifida*; Dale 1985), NC (Ferris & Klyver 1932, *Powellia bifida*; Tuhill 1952; Dale 1985), NN, SI, WD (Tuthill 1952; Dale 1985), OL (Tuthill 1952); CL (FHDB).

Host plants: *Olearia albida, O. avicenniaefolia, O. moschata, O. paniculata* (Asteraceae).

***Trioza colorata*** (Ferris & Klyver, 1932)

Distribution: New Zealand: MC (Dale 1985), TO, NN (Tuthill 1952; Dale 1985), NC (Ferris & Klyver 1932, as *Powellia colorata*; Tuthill 1952; Dale 1985).

Host plants: *Halocarpus bidwillii, H. biformis* (Podocarpaceae).

***Trioza compressa*** Tuthill, 1952

Distribution: New Zealand: FD, NC, WD (Tuthill 1952), NN, SI, SL (Tuthill 1952; Dale 1985), OL, TO (Dale 1985).

Host plants: *Olearia arborescens* (Asteraceae). Tuthill (1952) listed as *O. rani* as host but Dale (1985) questioned this record.

***Trioza crinita*** Tuthill, 1952

Distribution: New Zealand: FD, SL (Tuthill 1952; Dale 1985), OL, NC, NN, TK, TO (Dale 1985), WD (Tuthill 1952).

Host plants: *Olearia arborescens, O. ilicifolia, O. macrodonta* (Asteraceae).

***Trioza curta*** (Ferris & Klyver, 1932)

Distribution: New Zealand: AK (Tuthill 1952; Dale 1985), ND (Ferris & Klyver 1932, as *Powellia curta*; Dale 1985), HB, NN, TK, WN (Dale 1985), WD (Ferris & Klyver 1932, as *Powellia curta*);

BR, CL, TO, (Dale 1985); DN, WI (FHDB).

Host plants: *Metrosideros excelsa, M. robusta, M. umbellata, Syzygium maire* (Myrtaceae).

***Trioza dacrydii*** Tuthill, 1952

Distribution: New Zealand: HB, NN (Tuthill 1952; Dale 1985), NC, TO (Tuthill 1952; Dale 1985).

Host plants: *Halocarpus bidwillii, H. biformis* (Podocarpaceae).

***Trioza decurvata*** (Ferris & Klyver, 1932)

Distribution: New Zealand: AK, MC (Ferris & Klyver 1932, as *Powellia decurvata*; Dale 1985), NN, TK, WD (Dale 1985), TO (Tuthill 1952; Dale 1985), WN (Ferris & Klyver 1932, as *Powellia decurvata*; Dale 1985).

Host plants: *Dracophyllum longifolium* (Ericaceae).

***Trioza dentiforceps*** Dumbleton, 1967

Distribution: New Zealand: CH (Dumbleton 1967, Dale 1985).

Host plants: *Olearia traversii* (Asteraceae).

***Trioza discariae*** Tuthill, 1952

Distribution: New Zealand: (Maskell 1879, as *Powellia vitreoradiata* p. p.; Maskell 1890, as *Trioza pellucida* p. p.), NN, OL (Tuthill 1952; Dale 1985), CO, MB, MC/NC, MK, SC, SL (Dale 1985).

Host plants: *Discaria toumatou* (Rhamnaceae).

***Trioza doryphora*** (Maskell 1880)

Distribution: New Zealand: (Maskell 1880, as *Powellia doryphora*), FD, SL, TK, TO (Tuthill 1952; Dale 1985), NC, WD (Dale 1985); DN (FHDB).

Host plants: *Olearia ilicifolia* (Asteraceae).

***Trioza emarginata*** (Ferris & Klyver, 1932)

Distribution: New Zealand: BR, NN, OL, WD (Dale 1985), NC, WN (Ferris & Klyver 1932, as *Powellia emarginata*; Dale 1985), TK (Dale 1985, as *Trioza emarginata* and as "unidentified nymphs from *Coprosma* spp."), TO (Tuthill 1952; Dale 1985, as "unidentified nymphs from *Coprosma* spp.").

Host plants: *Coprosma foetidissima, C. lucida* (Rubiaceae).

Comments: Dale (1985) suggested that the host plant of *Trioza emarginata* is unknown. She also mentioned and described immatures of an unidentified species from *Coprosma*. In Henderson (2010), the host of *Trioza emarginata* is listed as *Coprosma*.

***Trioza equalis*** (Ferris & Klyver, 1932)

Distribution: New Zealand: NC (Ferris & Klyver 1932, as *Powellia equalis*; Dale 1985).

Host plants: Unknown.

***Trioza falcata*** (Ferris & Klyver, 1932)

Distribution: New Zealand: BR, CO, MK, NC, NN, TK (Dale 1985), DN, TO (Ferris & Klyver 1932, as *Powellia falcata*; Dale 1985), SL (Tuthill 1952; Dale 1985), OL, SC, SI (Tuthill 1952); WD (FHDB).

Host plants: *Aristotelia fructicosa, A. serrata* (Elaeocarpaceae).

***Trioza fasciata*** (Ferris & Klyver, 1932)

Distribution: New Zealand: BP, BR, ND, CL, NN, SD, TK (Dale 1985), TO (Ferris & Klyver 1932, as *Powellia fasciata*), WI (Ferris & Klyver 1932, as *Powellia fasciata*; Dale 1985), WN (Tuthill 1952); AK (AMNZ, FHDB, LUNZ).

Host plants: *Muehlenbeckia australis, M. complexa* (Polygonaceae).

***Trioza flavida*** Tuthill, 1952

Distribution: New Zealand: NN (Tuthill 1952; Dale 1985).

Host plants: *Olearia lacunosa* (Asteraceae).

***Trioza gourlayi*** Tuthill, 1952

Distribution: New Zealand: OL (Tuthill 1952; Dale 1985).

Host plants: Perhaps *Olearia lacunosa* (Asteraceae).

***Trioza hebicola*** Tuthill, 1952

Distribution: New Zealand: TO (Tuthill 1952; Dale 1985); SC (FHDB).

Host plants: *Hebe salicifolia, H. stricta* (Plantaginaceae).

***Trioza irregularis*** (Ferris & Klyver, 1932)

Distribution: New Zealand: (Tuthill 1952), AK (Tuthill 1952), BP, SL, TO (Dale 1985), MC, WN (Ferris & Klyver 1932, as *Powellia irregularis*; Dale 1985), SI (Tuthill 1952; Dale 1985).

Host plants: *Neopanax arboreus, N. colensoi, N. laetus, Raukaua anomalus, R. edgerleyi, R. simplex* (Araliaceae).

Natural enemies: *Adelencyrtoides variabilis* Noyes, 1988 (Hymenoptera: Encyrtidae) (Macfarlane et al. 2010).

***Trioza latiforceps*** Tuthill, 1952

Distribution: New Zealand: NN (Tuthill 1952; Dale 1985).

Host plants: *Olearia lacunosa* (Asteraceae).

***Trioza obfusca*** (Ferris & Klyver, 1932)

Distribution: New Zealand: WN (Ferris & Klyver 1932, as *Powellia obfusca*; Dale 1985).

Host plants: *Hebe* sp. (Plantaginaceae).

***Trioza obscura*** Tuthill, 1952

Distribution: New Zealand: OL, (Tuthill 1952), NN, TO (Tuthill 1952; Dale 1985), NC, TK, WN (Dale 1985).

Host plants: *Hebe angustifolia, H. coarctata, H. odora, H. stricta* (Plantaginaceae).

***Trioza panacis*** Maskell, 1890

Distribution: New Zealand: (Maskell 1890; Ferris & Klyver 1932, as *Powellia panicis*; Tuthill 1952), AK, BR (Dale 1985), FD (Tuthill 1952); BP, DN, MC, NN, SC, TK, WN, WO (FHDB, MONZ).

Host plants: *Neopanax arboreus, Pseudopanax crassifolius, P. ferox, P. lessonii* (Araliaceae).

***Trioza parvipennis*** Tuthill, 1952

Distribution: New Zealand: FD (Dale 1985), NN (Tuthill 1952; Dale 1985).

Host plants: *Brachyglottis adamsii, B. revoluta* (Asteraceae).

***Trioza schefflericola*** Tuthill, 1952

Distribution: New Zealand: AK, BP (Tuthill 1952; Dale 1985), BR, CL, ND, SL, TO (Dale 1985); DN, WO, WN (FHDB).

Host plants: *Schefflera digitata* (Araliaceae).

***Trioza scobina*** Tuthill, 1952

Distribution: New Zealand: NN (Tuthill 1952; Dale 1985), WD (Dale 1985).

Host plants: *Olearia lacunosa*, also possibly *O. colensoi* (Asteraceae).

***Trioza styligera*** (Ferris & Klyver, 1932)

Distribution: New Zealand: FD, NC, TK (Dale 1985), WN (Ferris & Klyver 1932, as *Powellia styligera*; Dale 1985), perhaps TO (Ttuthill 1952).

Host plants: Unknown, perhaps *Brachyglottis buchananii* (Asteraceae) (Dale 1985).

***Trioza subacuta*** (Ferris & Klyver, 1932)

Distribution: New Zealand: AK, BP, NN (Tuthill 1952; Dale 1985), ND, TO, WI (Tuthill 1952), SD, TK, WO (Dale 1985), WN (Ferris & Klyver 1932, as *Powellia subacuta*; Tuthill 1952; Dale 1985).

Host plants: *Brachyglottis repanda* (Asteraceae).

***Trioza subvexa*** Tuthill, 1952

Distribution: New Zealand: AK, BR, MK (Dale 1985), NC, NN, WD (Tuthill 1952; Dale 1985).

Host plants: *Olearia avicenniaefolia* (Asteraceae).

***Trioza vitreoradiata*** (Maskell 1879)

Distribution: Introduced into France, Ireland, UK; New Zealand: (Maskell 1879, 1880, p. p., as *Powellia vitreoradiata*; Maskell 1890, as *Trioza pellucida*; Marriner 1903, as *Trioza alexis*), AK, WN (Ferris & Klyver 1952, as *Powellia vitreoradiata*; Tuthill 1952; Dale 1985), BR, CL, ND, NN, SD, WI (Dale 1985), MC (Nelson 2012), ND (Tuthill 1952; Dale 1985), SI (Tuthill 1952).

Host plants: *Pittosporum colensoi, P. crassifolium, P. ellipticum, P. eugenioides, P. tenuifolium, P. tobira, P. undulatum* and rarely *Hymenosporum flavum* (Pittosporaceae); *Feijoa sellowiana* (Myrtaceae) as host needs confirmation. Can also complete its life cycle on *Citrus paradisi* (Rutaceae) with high population noted nearby on *Pittosporum* shrubs (Nelson 2012).

Natural enemies: *Halmus chalybeus, Drepanacra binocular, Boriomyia maorica, Micromus tasmaniae* (Henderson et al. 2010).

***Trioza* sp.**

Distribution: New Zealand: AK, CL, WN, WO (Dale 1985, as *Trioza* n. sp. 'Brenda May').

Host plants: *Olearia furfuracea, O. rani* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: CH (Dale 1985, as *Trioza* n. sp. 'Chathams').

Host plants: *Leptinella featherstonii* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: NN (Dale 1985, as *Trioza* n. sp. 'Flora Hut').

Host plants: *Olearia lacunosa* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: SL, WN (Dale 1985, as *Trioza* n. sp. 'Fortrose').

Host plants: *Elaeocarpus hookerianus*, possibly also *E. dentatus* (Elaeocarpaceae).

***Trioza* sp.**

Distribution: New Zealand: FD, MC, NN, OL, SL, TK (Dale 1985, as *Trioza* n. sp. 'Hut Creek').

Host plants: *Hebe odora, H. subalpina* (Plantaginaceae).

***Trioza* sp.**

Distribution: New Zealand: CO (Dale 1985, as *Trioza* n. sp. 'Hyde Rock').

Host plants: *Celmisia brevifolia* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: MK, NC, TK (Dale 1985, as *Trioza* n. sp. 'Kea Point').

Host plants: *Brachyglottis buchananii, B. elaeagnifolia* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: CO (Dale 1985, as *Trioza* n. sp. 'Logan Burn').

Host plant: Unknown, possibly *Celmisia* sp. (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: TO, WI (Dale 1985, as *Trioza* n. sp. 'Massey').

Host plants: *Olearia solandri, O. virgata* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: BR (Dale 1985, as *Trioza* n. sp. 'Mt Dewar').

Host plants: Unknown.

***Trioza* sp.**

Distribution: New Zealand: OL (Dale 1985, as *Trioza* n. sp. 'Niger Mt').

Host plants: Unknown.

***Trioza* sp.**

Distribution: New Zealand: CO (Dale 1985, as *Trioza* n. sp. 'Old Man Range').

Host plants: *Celmisia haastii* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: CL, ND (Dale 1985, as *Trioza* n. sp. 'Omahuta').

Host plants: *Brachyglottis kirkii* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: MC, NN, SL (Dale 1985, as *Trioza* n. sp. 'Price's Valley').

Host plants: *Plagianthus betulinus* (Malvaceae).

***Trioza* sp.**

Distribution: New Zealand: SI, SN (Dale 1985, as *Trioza* n. sp. 'Snares I').

Host plants: Probably *Olearia colensoi, O. lyallii* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: SN (Dale 1985, as *Trioza* n. sp. 'Snares II').

Host plants: probably *Brachyglottis stewartiae* (Asteraceae).

***Trioza* sp.**

Distribution: New Zealand: TO (Dale 1985, as *Trioza* n. sp. 'Taranaki Falls').

Host plants: *Rapanea divaricata* (Primulaceae).

***Trioza* sp.**

Distribution: New Zealand: CO, FD, MB, OL (Dale 1985, as *Trioza* n. sp. 'Wards Pass').

Host plants: Unknown, possibly *Celmisia sessiliflora* (Asteraceae) (Dale 1985).

***Trioza* sp.**

Distribution: New Zealand: FD (Dale 1985, as *Trioza* n. sp. 'Wilmot Pass').

Host plants: *Olearia crosby-smithiana* (Asteraceae).

**Gen. sp.**

Distribution: New Zealand: AU, CA (Dale 1985, as n. gen., n. sp. 'Campbell Island').

Host plants: *Anisotome antipoda* (Apiaceae).

Comments: A species with highly modified forewings but otherwise similar to New Zealand triozids (Dale 1985).

**Gen. sp.**

Distribution: Australia (Gary Taylor 2015, pers. comm); introduced into New Zealand: (Henderson 2010, as Gen. sp. indet. *Casuarina*); AK, ND (LUNZ).

Host plants: *Casuarina* sp. (Casuarinaceae).

## 2.5 Discussion

In this check list, a total of 99 species of Psylloidea were listed as occurring in New Zealand. These species included 25 described by Dale (1985) that were not formally named, as well as two listed by Henderson *et al.* (2010) and one by Thorpe (2016) that had neither been described nor named.

The psyllid fauna of New Zealand had two major features. Firstly, there were a large number of endemic Triozidae, currently referred to the possibly artificial genus *Trioza*. The 52 species of *Trioza* present in New Zealand, especially if compared to only 10 amongst the more numerous and diverse psyllid fauna in Australia (Ouvrard 2017), suggests a radiation of the triozids involving a series of host switches along their evolutionary history there. The genera *Anomalopsylla* and *Ctenarytaina* also show species native to Australia, but *Trioza* is peculiar for having radiated far more in New Zealand. Drawing conclusions based solely on the host plant associations would result in the hypothesis seeing the majority of the *Trioza species* being the descendants of a single Australian ancestor associated with Asteraceae. In fact, the number of *Trioza* species associated with Asteraceae was very elevated (16). Upon arrival, it was likely that the psyllid immediately switched onto other hosts. A second, though much smaller group, may have descended from an Australian or Oceanian ancestor that was associated with Myrtaceae (Burckhardt, unpublished information). This could be explained by the association of two *Trioza* species with plants belonging to this family: *T. curta* and *T. adventicia.* Futhermore, this would support the possibility that *T. adventicia* is a synonym of *T. eugeniae,* and therefore not native to New Zealand.

Secondly, there are many introduced species of psyllids in New Zealand. Indeed, of the 35 species considered introduced, 29 are native to Australia, four to the Palaearctic region and two to the Americas. The preponderant flow of species from Australia to New Zealand probably resulted from the close social and political-economic relationships, which have increased over the last centuries (Withers 2001). For example, the importation of Australian *Acacia* (Fabaceae) and *Eucalyptus* (Myrtaceae) plant species for forestry and as ornamentals may have aided the establishment of their associated psyllid populations in New Zealand. These species make up the

majority of the introduced species in the checklist. In addition to this possible pathway, the Plant Biosecurity CRC report "Understanding the significance of natural pathways into Australia and New Zealand" (Yen et al. 2014) suggests that aerial dispersal from Australia to New Zealand is very possible because of the prevailing wind patterns. This theoretical means of spread was confirmed for many insect groups such as the Lepidoptera (Yen et al. 2014) New arrivals from Australia would not be unexpected considering the high numbers of psyllid species occurring there: over 350 are currently reported (Hollis 2004, Ouvrard 2017) and 446 estimated (Yen 2002). Thus, an important aspect of preventing or managing future invasions of psyllids will be the continued and accurate identification of psyllid species and their host plants in both Australia and New Zealand (Goldson et al. 2010). This will contribute in immediately identifying new pathways between New Zealand and Australia.

Establishment of this up to date list of the New Zealand Psylloidea is a fundamental step towards a better understanding of their biodiversity and a valuable foundation for further studies, such as the species delimitation presented in Chapter 3. In particular, by summarizing the extent of undescribed species as recognized by Dale (1985) but not formally named, the extent of the taxonomic revision that is needed is easier to appreciate. Formal descriptions of the undescribed species are planned in the context of a volume on psyllids in the Fauna of New Zealand series (Dale, personal communication), as are publications to resolve the taxonomy based on morphological (Dale, in preparation) and molecular data (see chapter 4). In addition, while the native fauna of New Zealand has previously been treated in detail concerning biology and biogeography (Dale 1985), this list will help to establish more accurate distribution, host plant and natural enemy data. This will be particularly important to those psyllids of relevance to conservation, such as *Anomalopsylla* "Port underwood" and "*Psylla" carmichaeliae* whose host plants are threatened (Stringer et al. 2012). For New Zealand, this list will also be very useful in preparedness for distinguishing new potentially invasive pest species that may arrive and in the surveillance for associated psyllid-vectored plant pathogens. Accurate records of the New Zealand species is also central to understanding the ecology and physiology of psyllid-microbial associations, which may in turn be important in the context of susceptibility of plants to disease (Chuche et al. 2016).

## 2.6 Conclusion

To date, the work of Dale (1985), who recognized 81 species, has provided the most complete work on psyllid diversity in New Zealand. Thirty years on, however, the checklist described here reports a total of 99 species of Psylloidea as recorded in New Zealand. This checklist includes 25 species that were not formally named by Dale (1985), as well as two species listed by Henderson et al. (2010) and one by Thorpe (2016) that were neither described nor named. New arrivals have also been added. Consolidation of information on psyllids in this updated checklist provides the basis for a supplementary survey of the psyllid fauna in New Zealand. That, together with an integrated morphological/molecular/host plant/geographic assessment, will enable a much more robust and contemporary appraisal of species presence (Chapter 3). Further phylogenetic study, including that information will then permit the hypotheses expounded here, regarding ancestral arrivals, species radiations and host switching, to be empirically tested (Chapter 4).

# Chapter 3

# The psyllids of New Zealand: a contemporary checklist from new collections and integrative taxonomy

## 3.1 Introduction

In Chapter 2, consolidation of the historical data on psyllid species present in New Zealand generated a psyllid checklist that could be used as a basis for future studies of this insect group. Over the last 30 years, there have been large increases in anthropogenically-related global movements of insect species. This has raised the probability that additional adventive psyllid species have established in New Zealand since the most recent surveys of these insects, which were conducted some 30 years or more ago. Modern methods for taxonomic evaluation, which incorporate molecular genetic analysis with morphology and other diverse information such as host plant associations and geography, are being utilised in an integrative or iterative manner to improve diversity and taxonomic assessments (Yeates et al 2011). The power of molecular methods to redefine psyllid diversity has been demonstrated already, but only for a narrow range of psyllid species collected elsewhere in the world (Taylor 2016, Percy 2017). Thus, a new survey of the psyllid populations in New Zealand, using tools to study genetic variation alongside differences in morphology and host plant associations, seemed prudent to ensure the latest information was available on psylld diversity. These methods could also provide new information on the diversity of New Zealand psyllid populations by resolving cryptic species.

Of the molecular tools available for studying insect diversity, COI barcoding has become widely used for determining species separation (Hebert et al. 2003). Indeed, today, our understanding of this mitochondrial gene and its possible applications, have improved sufficiently to use this gene to study a broad spectrum of insect groups. Although limitations are also being appreciated, such as the presence of nuclear mitochondrial pseudogenes (NUMTS) (Song et al. 2008) may lead to overestimates of the number of species in some insect groups, whilst others have such low variation in their COI nucleotide sequences that the numbers of species are underestimated [e.g. Lepidoptera (Burns et al. 2007)]. This has led to modification sometimes of the conventional intra- and interspecific threshold (3% COI nucleotide variation) suggested by Hebert and colleagues (Hebert et al. 2003) depending on the organism studied (Hubert and Hanner 2015).

An updated checklist could also be facilitated by further assessment of insect samples collected in the last 30 years. Unfortunately, existing dry and alcohol preserved samples would add little to this effort, being too few in the various entomological collections in New Zealand and of a

quality not suited to molecular analysis because of their age and the percentage of alcohol preservative used. Fresh insect samples are therefore desirable as well as being essential for any subsequent analysis of the microbiomes associated with psyllids to meet the overarching goal of this study towards a better understanding of host plant/vector/pathogen relationships in this group of insects. Thus, any new survey of psyllid diversity in New Zealand was undertaken here and collection of fresh material made available for molecular analysis.

### 3.1.1 Aims and Objectives

The aim of this chapter was to utilise the psyllid Checklist developed in Chapter 2 to design and undertake a field collection of psyllids that would then be used for an integrative assessment of current diversity. Collection would be based in the first instance on the known geographic and host range of genera from all six families of psyllids recorded as present in New Zealand, with a goal to collect as many different populations as possible (see 3.2.1 below for definition of a populations). Following COI DNA barcode analysis of the collection, the "unified species concept" suggested by De Queiroz was adopted for making species-level decisions (De Queiroz 2007). The "unified species" concept considers the importance of morphology, ecology and distribution data, in addition to genetic information in delimiting species (De Queiroz 2007). More specifically, this was based on integration by congruence of the multiple characters (Padial et al. 2010). This concept, while only one of many [e.g. (Padial et al. 2010, Schlick-Steiner et al. 2010 and references therein)], works for the premise that an aggregate of characters is more valuable than any single one of them. This idea has historical origins. Darwin, for example, wrote the following lines in his book "The origin of species"

> *"The value indeed of an aggregate of characters is very evident in natural history. Hence, as has often been remarked, a species may depart from its allies in several characters, both of high physiological importance and of almost universal prevalence, and yet leave us in no doubt where it should be ranked. Hence, also, it has been found, that a classification founded on any single character, however important that may be, has always failed; for no part of the organisation is universally constant."*

(Darwin 1859)

The objectives to develop a revised psyllid checklist were:

**Objective 1:** To locate and identify host plants across New Zealand and undertake psyllid collection accordingly.

**Objective 2:** To identify psyllids to species (as far as possible), based on existing morphological keys and on known associated host plant information.

**Objective 3:** To prepare insect voucher specimens for submission to an entomological collection.

**Objective 4:** To undertake molecular analysis of the specimens by COI barcode sequencing, to support their species delimitation using the "unified species concept".

## 3.2 Materials and Methods

### 3.2.1 Host plant identification

The most efficient approach to locating specific psyllid species is to look for their host plants (Hollis 2004), which can vary because of differences in host specificity [e.g. (Hodkinson 2009, Burckhardt et al. 2014)]. Putitive host plants were located using the information collected in Chapter 2. Tentative identification of the plants was made in the field. Leaf samples were also collected and preserved (both in ethanol and dry mounted) for subsequent morphological confirmation using multiple keys (Breitwieser et al. 2010, Angiosperm Phylogeny Website 2016, The Plant List 2016). Difficult identifications were referred to expert botanists at the Landcare Research Herbarium (Lincoln, Canterbury). The identity of only one plant remained unknown using these approaches, so DNA analysis of plant tissue was used for a positive identification (using the same approach adopted for the insects, see below).

Given that the New Zealand Psylloidea have extremely wide ranging host plants, from small weedy annual bushes to perennial conifers (Chapter 2), plants not known as hosts were also periodically checked for the presence of psyllids. Discoveries were mindful of the host plant definition provided in Chapter 1, especially the need to consider evidence of feeding or presence of nymphs.

Populations of psyllids were initially defined as the psyllids collected from a single plant. This was straightforward if the plant was isolated from other individuals of the same species (by many kilometres), such as in alpine and subalpine habitats. However, it was more complex if psyllids were found on contiguous plants and were difficult to verify in-field as of the same species. Consequently, a population was defined as insects of the same species collected from a single plant or from a group of contiguous plants of the same species. Populations were confirmed by morphological and/or genetic analysis, retrospectively.

### 3.2.2 Psyllid collection

Specimens were collected from more than 500 locations across New Zealand including Stewart Island (Crosby et al. 1998). In addition, psyllids were collected from 102 locations in Southern and Eastern Australia. An extemporaneous collection of psyllids was performed in the United States of America [USA] for three species to be used as comparisons (Appendix B).

Collection permits from the New Zealand Department of Conservation (DOC) to Lincoln University enabled collections from the whole South Island, whilst another released to the author of this thesis also enabled sampling in the Tongariro National Park and other forests in the North Island such as the Pureora Forest. Psyllids from Australia and the USA were imported into New Zealand in high grade ethanol according to Section 6.1 of the "Import health standard for the importation into New Zealand of nonviable animal specimens from all countries" published by the Ministry for Primary Industry (MPI) (available at http://www.mpi.govt.nz/dmsdocument/1840-preserved-animal-specimens-from-all-countries-import-health-standard). Field collections were made from September 2014 to June 2017 using the method of Hollis (2004). This involved the beating and/or brushing of the branches of the plants with a fine-mesh net and the collection of the psyllids from it using an entomological aspirator. This was most useful for collecting from tall trees with branches otherwise difficult to reach. A modification of this method was also used in which a tray was used instead of a net. Since psyllids are not good fliers, collection was easier made from the tray than the net, which is sometimes difficult to carry and/or to operate in remote areas.

A portable and light weight kit was used for collections as illustrated in Figure 3.1.

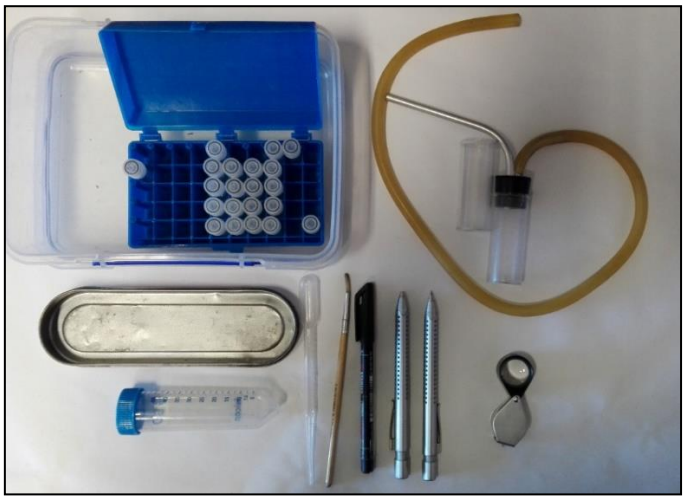

**Figure 3.1:** Psyllid field collection kit. A transparent Sistema box containing a Bioquip entomological aspirator with two spare vials, an Eppendorf container box with 64 x 2 mL screw-cap Eppendorf vials filled with ethanol, pencil, pen, permanent marker, drop counter, 20x hand lens and a fine painter brush in a pencil case, an additional 50 mL falcon tube filled with ethanol and two A4 sheets of paper.

Individual psyllids were collected from the tray or net with an entomological aspirator (Bioquip model 1135A) and immediately killed, counted and placed into an ethanol-containing vial using the paint brush. Metadata, including date of collection, GPS coordinates, name of the location, plant species from which the samples were collected and name of the collector, were written on a paper label and placed inside the vials. The same information was written on the outside of the vials and in

a notebook for backup. The vials in the box were refrigerated at -4° C where possible or in a chill box during transportation.

### 3.2.3 Morphological analysis

Initial identification was made using a binocular microscope to examine fundamental characters such as wing shape, wing vein pattern, and patterns or shape of the terminalia. Accordingly, different species collected from the same tree were separated and an identification number assigned to each as a different record. Each population (as defined above) was assigned a specific serial number.

A dichotomous key for the identification of the New Zealand psyllids (Dale 1985) was successfully used to identify the majority of species. However, many forms were not reported there. In those cases, other keys were used for identification [e.g. (Hollis 2004)] as determined by host plant and its likely origin. In other cases, multiple forms of psyllids belonging to a particular genus (e.g. *Trioza*), based on wing morphology and collection from specific host plants (e.g. *Olearia*), lead to inconclusive identification. In those circumstances higher resolution microscope inspection (dry and slide mounted) and molecular methods were used to support identification.

For microscopic analysis of psyllids, insects were dry mounted by removing excess ethanol using absorbent paper and gluing them to small cardboard triangles. The insect preparations were then pinned on nº1 entomological pins (Bohemia) above the label containing the relevant metadata. Microscope slide-mounted specimens were prepared when morphological characters were either too small to be unambiguous (e.g. for the *Trioza*) or not visible on the exterior of the insect. In these cases, the insects were cleared by covering in 10% potassium hydroxide (KOH) for 3 to 4 h, or until the specimen was sufficiently clear to see through, but not completely transparent. They were washed in a mix of 20% acetic acid, 50% ethanol and 30% water to neutralize the oxide, then with a step-wise series of 5 min ethanol washes of 70%, 80%, 90% and 100%. The insect was then dissected into head, thorax and abdomen. Each part was placed on a drop of Euparal mounting medium on a microscope slide and then covered with a cover slip without creating air bubbles. To show both the dorsal and the ventral part of the thorax, this was cut open on the lateral side and dorsal and ventral parts were positioned near each other. Legs and wings were cut off the thorax and positioned immediately adjacent to be sure they could be seen clearly, and they would not overlap with each other. A label containing the metadata was attached to the slide. Slides were then kept for at least 48 h on a slide drier at 40 °C, until the mounting medium was dry. Remaining specimens were retained as ethanol-preserved populations by moving all insects from each 2 mL Eppendorf into a

labelled glass vial containing 99% ethanol. Labels, including the metadata, were added to the vials and the vials were stored at -20 °C.

### 3.2.4 Molecular analysis

For all specimens used for molecular identification, photographs were taken as vouchers. Photographs were taken using a Nikon DS-Ri2 camera connected to a Nikon SMZ25 microscope. Pictures were the result of stacking images using the software Nikon NIS-Elements D v4.5. The magnification of each picture depended upon the dimension of the insects (e.g. Figure 3.2).

**Figure 3.2:** Stacked image of *Ctenarytaina pollicaris.* The scale bar measures 100 µm.

In 17 cases, photographs were also uploaded on the NatureWatchNZ website database, http://naturewatch.org.nz/observations/f_martoni, together with GPS coordinate and host plant identification. This enabled confirmation of the species identification by other members of the science community and, at the same time, provided a resource to improve public awareness of the biodiversity of New Zealand's fauna.

For molecular analysis, individual specimens were distinguished from other specimens of the same population by assigning a letter from "a" to "z". Therefore, the samples 116a and 116b were two different insects, both belonging to the same species, collected from population 116. DNA extractions from individual specimens were performed using a modification of an existing protocol

(Doyle and Doyle 1987). Essentially, whole insects were ground with a micro pestle in 100 µL 2x CTAB buffer (cetyl trimethylammonium bromide), a cationic detergent, and then incubated at 50°C overnight. A volume of 100 µL of chloroform:IAA (Isoamyl alcohol) (29:1) was added, and the digests were vortexed and centrifuged at 13,000 rpm for 10 min. The supernatant was removed to a clean tube, 2.5 volumes of 100% ethanol were added, and then the mix was gently inverted. Each sample was incubated at -20°C for 1 h or overnight, then centrifuged at 13,000 rpm for 20 min. The supernatant was discarded avoiding the DNA pellet, 300 µL 72% ethanol was added, and the solution was gently inverted to mix. The DNA-containing solution was centrifuged at 13000 rpm for 5 min, the ethanol removed and the pellet air dried (~15-20 min). The pellet was re-suspended in 20-30 µL of PCR grade water.

The DNA barcode region (Hebert et al. 2003) of subunit 1 of the COI (Douglas et al. 2006) gene was amplified by polymerase chain reaction (PCR). PCR primer C1-J1709 (Simon et al. 2006) was paired with HCO2198 (Folmer et al. 1994) to generate an amplicon of 403 bp, as prior experiments had shown that the LCO1490 primer (Folmer et al. 1994) was poor for psyllid DNA amplification (Gary Taylor, personal communication). PCR was performed using the KAPA3G plant PCR Kit (Kapa Biosystems, Massachusetts, USA). In each 20 µL reaction, 10 µL 1x PCR buffer, 1 µL each primer (10 µM), 0.2 µL Taq polymerase and 1 µL DNA template was added. Thermal cycling conditions were an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min; followed by a final extension of 7 min.

PCR products were Sanger sequenced in both directions with the COI PCR primers described above and the Big Dye Terminator v 3.1, from the Cycle Sequencing Kit (Applied Biosystems; Foster City, California, United States) on an ABI 3130xl Genetic Analyzer. Sequencing reads were assembled using MEGA version 6 (Tamura et al. 2013) to generate a consensus sequence for each sample and also to align all sample sequences. The Kimura-2-parameter [K2P] model (Kimura 1980) with a bootstrap of 10,000 replicates was used for phylogenetic analysis by Neighbour Joining [NJ] and Maximum likelihood [ML] algorithms. Genetic distances between taxa were visualised in a ML tree.

### 3.2.5 Species identifications

Specimens were allocated to described species based on congruence of at least three of four factors: morphology, plant association and geographic location according to described species using mainly the keys of Dale (1985) and Hollis (2004), and molecular DNA barcode divergence. For the latter, divergence of >3% between taxa was considered supportive of species-level differences, as this

value has been previously used for psyllids (Percy 2003b, Taylor et al. 2016, Wonglersak et al. 2017). As a result, a divergence <3% was considered intra-specific variation (Hebert et al. 2003).

## 3.3 Results

### 3.3.1 Field collection of psyllids

Appendix B lists all populations collected on the North (174) and South (314) Islands of New Zealand, Stewart Island (22) (Table B.1) and Australia (South Australia, Queensland, Victoria, New South Wales and Norfolk Islands) (102) (Table B.2). No collections were made on other New Zealand islands except Waiheke Island. Figure 3.3 illustrates how some areas were more thoroughly sampled. Differential sampling occurred because urban areas tended to include both native and introduced plants, growing both as wild plants and cultivated plants. The populations considered in this study greatly varied in size between a few insects and hundreds of them.

Of the New Zealand populations, 320 were collected from native plant species and 190 from introduced species (Appendix B). In general, the collections confirmed the psyllid/host plant associations reported in the literature (Table 3.1, host plants reported in black). Exceptions included the species *Trioza gourlayi* and *T. subvexa*, which were collected from *Olearia virgata* and *O. avicenniifolia* (Asteraceae), respectively (Table 3.1, host plant reported in blue), and the new 'proposed' species for which there were no prior host plant records (e.g. *Trioza acuta* B). In the cases of *T. gourlayi* and *T. subvexa*, populations were largely represented by a high number of adults, although immature stages were also collected for a few populations. Thus, while *Olearia virgata* and *Olearia avicenniifolia* could not be confirmed as hosts *per se* (Chapter 1.2.2), the high numbers of individuals found on these plants indicates they are hosts rather than casual associations. In contrast, the plant names reported in red in Table 3.1 represent plants on which only a few adults were found, suggesting these finds are probably a result of incidental movements of the insects (perhaps via wind).

The species collected both in New Zealand and Australia were found on the same hosts in both countries. This could not be confirmed for the ten specimens from Norfolk Island (Australia) since they were provided from sticky traps (Grant Smith and Jessica Vereijssen, Plant and Food Research, Lincoln, New Zealand). On the other hand, the species collected in the USA are not present in New Zealand and were added to the analysis as a comparison.

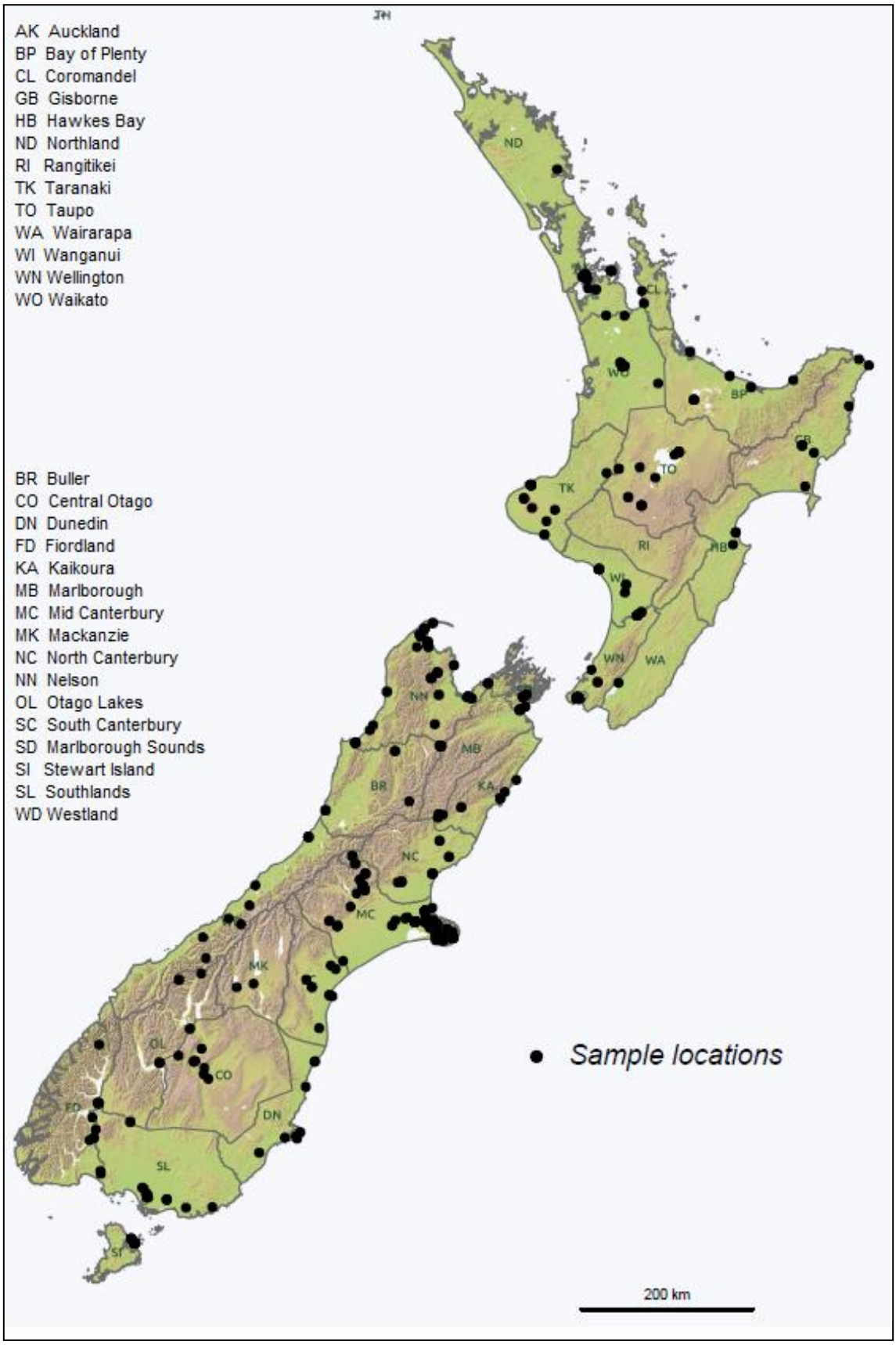


**Figure 3.3:** The distribution of all 510 populations collected across the different regions of New Zealand.

### 3.3.2 Development of a reference collection

The samples collected were divided into three collections to facilitate the identification process and to preserve morphological reference samples. The resulting EtOH collection was the most complete of these and comprised representatives of all species across a total of 488 populations from the North and South islands, and 22 from Stewart Island. In addition, the collection included 92 populations collected in Australia and three from the USA. The number of insects preserved in this collection, even after the DNA analysis was performed and selection of some individuals for slide and dry mounted collection, was estimated at around 4000-5000 insects. This collection is preserved in a -20 °C freezer (Figure 3.4a) at Lincoln University. The dry mounted collection included 200 insects from 70 populations and 66 species (Figure 3.4b). The collection of insects held on microscope slides included 94 insects belonging to 49 populations and 40 species. Insects held on microscope slides were prepared as in Figure 3.4c.

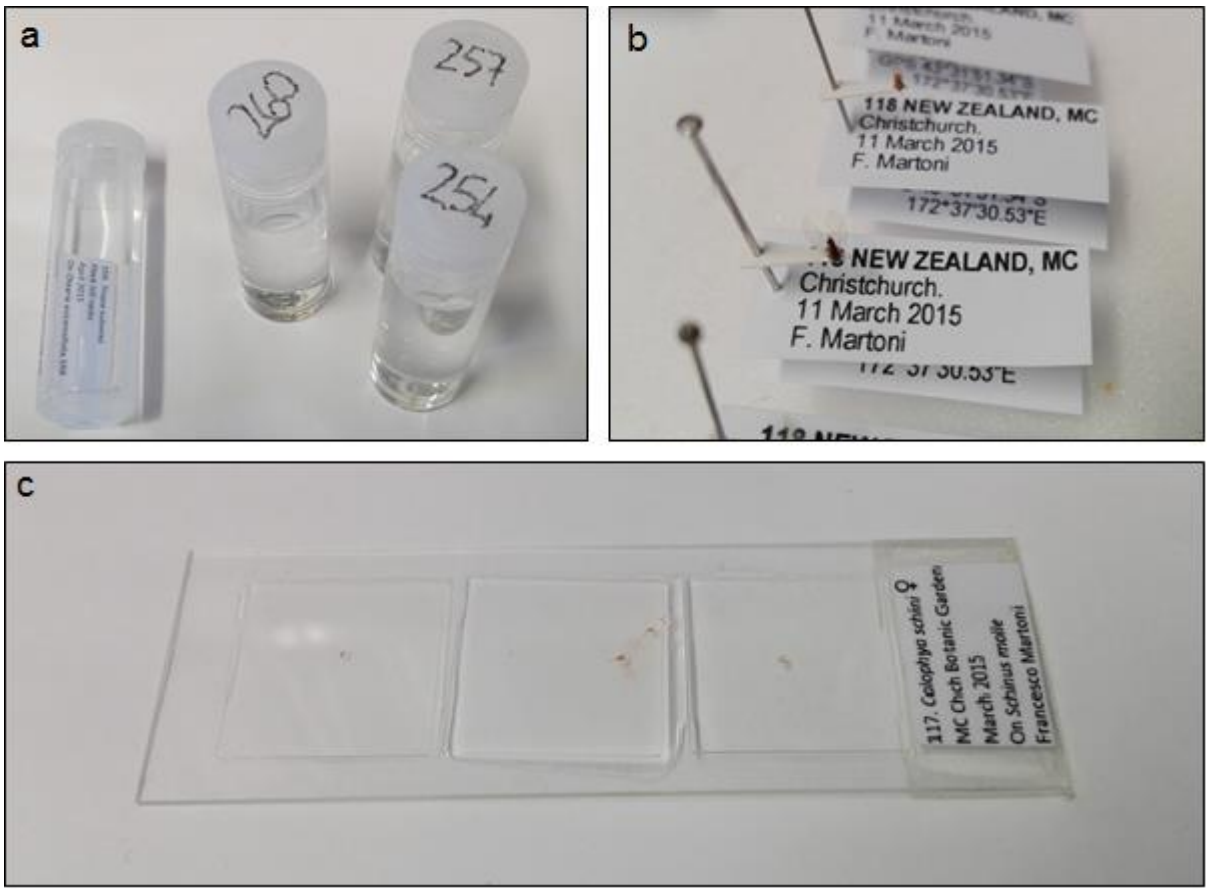


**Figure 3.4:** Examples of specimens in (a) the EtOH collection, (b) the dry mounted collection, and (c) the microscope slide collection.

### 3.3.3 Species identification

Species identification was relatively straight forward for specimens that were morphologically consistent with the existing New Zealand and Australian keys (Dale 1985, Hollis 2004). These species were identified through a combination of morphological characters from dry and slide mounted preparations (Figure 3.4, Appendix A) and host plant associations (Table 3.1). For other, however, a process to delimit them as putative species was devised according to different combinations of geographical (distribution), ecological (host plant association), morphological and genetic [COI] data as appropriate. This process is discussed in Section 3.4.1.

Species delimitation using the the "unified species" concept did not always lead to species identification. In fact, many of the newly reported species appeared to belong to a complex because of subtle differences around a species that had been previously described (e.g. *Psylla carmichaeliae, Ctenarytaina clavata* and *C. pollicaris* in Table 3.1). This meant that while the species delimitation was possible, and a number of taxa could be separated both morphologically and based on COI divergences indicative of species-level differences, it was not always possible to tell which of these taxa were consistent with the originally defined taxa in the literature (i.e. to name them). As it was beyond the scope of this work to describe new species, specimens belonging to complexes were defined as distinct, without trying to determine which of them corresponded to the described species. After initial attempts to identify specimens based on morphology and host associations, DNA was extracted from 465 samples representing 346 populations of psyllids. From the 465 DNA samples, a COI gene fragment was amplified and sequenced from 443 (Appendix C.1). These 443 represented all species in Table 3.1, except for nine specimens [*Acizzia solanicola* (seven specimens) and *Atmetocranium myersi* (two specimens)], from which a COI fragment could not be amplified. Fortunately, these specimens had been unambiguously identified using morphology and host information.

In addition to the 443 COI sequences isolated in this work, Gary Taylor (The University of Adelaide) provided another five COI sequences belonging to four individuals of *Trioza tricornuta* and one individual of an undescribed Australian *Trioza* species to be used as a comparison. In order to have an outgroup, a sequence of *Acyrthosiphon pisum* was obtained from GenBank (KR579669). Therefore, a total of 449 sequences of COI were generated in this study and are presented as a ML phylogenetic tree in Appendix C.

The COI sequences indicated populations clusters allocated 88 anticipated species, based on the 3% threshold for divergence (Appendix C). Pulling the COI data together with the morphological and

host data for *Atmetocranium myersi* and *Acizzia solanicola* identified a total of 90 species belonging to six families and 21 genera in New Zealand.

**Table 3.**1: New Zealand psyllid species defined by morphology, plant association, and population COI sequence divergence >3% divergence. Of the 90 taxa identified, 57 were described species, 21 newly recorded taxa (*), 10 non-described but previously known taxa (ʏ) and two taxa not identified to the species level (?). New Zealand locations are based on (Crosby et al. 1998); Australian locations (**in bold**) are New South Wales (NSW), South Australia (SA), Victoria [VIC]. The number of populations from each region is reported in brackets. The host plants are differentiated between previously known (in black), possible new host plants (in blue) and uncertain associations (in red).

| | **Species** | **Location** | **Host plant** |
|---|---|---|---|
| | **Family Psyllidae** | | |
| 1 | *Acizzia acaciaebaileyanae* | MC [2], **SA [1]** | *Acacia baileyana* |
| 2 | *Acizzia acaciae* | AK [1], WO [1], MC [2], SL [1], WN [1] | *Acacia melanoxylon* |
| 3 | *Acizzia albizziae* | MB [1], NN [2], MC [5], WD [2], TK [1], **NSW [1]** | *Acacia* sp. |
| 4 | *Acizzia conspicua* | WI [1] | *Acacia* sp. |
| 5 | *Acizzia dodonaeae* | KA [1], NN (4), TO [1], WN [1], DN [1] | *Dodonaea viscosa* |
| 6 | *Acizzia exquisita* | GB [1] | *Acacia* sp. |
| 7 | *Acizzia hakae* | MC [1], NN [1], SC [1], WN [1] | *Acacia* sp., *Grevillea* sp. |
| 8 | *Acizzia jucunda* | MB [1], NN [1], GB [1] | *Acacia* sp. |
| 9 | *Acizzia solanicola* | AK [1] | *Solanum melongena* |
| 10 | *Acizzia* sp.* | MC [1], **NSW [1]** | *Acacia baileyana* |
| 11 | *Acizzia uncatoides* | MC [4], CO [2], OL [1], BR [1], TK [1], WI [1], NN [6] | *Acacia* sp. |
| 12 | *Acizzia* "Waitakere"ʏ | GB [1], WN [1] | *Acacia* sp. |
| 13 | *Arytainilla spartiophila* | MC [3], OL [1] | *Cytisus scoparius* |
| 14 | *Baeopelma foersteri* | SL [1], SC [1] | *Alnus glutinosa* |
| 15 | *Psylla apicalis* A | DN [1], SL [1], FD [1], MC [2], OL [1] | *Sophora microphylla* |
| 16 | *Psylla apicalis* B* | DN [2], CO [2], OL [1], WD [1], BR [1], NN [3], MC [1], SL [1] | *Sophora microphylla* |
| 17 | *Psylla carmichaeliae* A | MC [4] | *Carmichaelia australis* |
| 18 | *Psylla carmichaeliae* B* | TK [1] | *Carmichaelia* sp. |
| 19 | *Psylla carmichaeliae* C* | WD [2], NC [1] | *Carmichaelia* sp. |
| 20 | *Psylla carmichaeliae* D* | CO [1] | *Carmichaelia compacta* |
| 21 | *Psylla carmichaeliae* E* | CO [2], OL [1] | *Carmichaelia petri* |
| | **Family Calophyidae** | | |
| 22 | *Calophya schini* | HB [1], MC [2] | *Schinus molle* |
| | **Family Homotomidae** | | |
| 23 | *Mycopsylla fici* | AK [2], **NSW [2]** | *Ficus macrophylla* |
| | **Family Liviidae** | | |
| 24 | *Psyllopsis fraxini* | SL [1], SC [1], BP [1] | *Fraxinus excelsior* |
| 25 | *Psyllopsis fraxinicola* | MC [2], FD [1], **NSW [1]** | *Fraxinus excelsior* |
| | **Family Aphalaridae** | | |
| 26 | *Anoeconeossa communis* | WO [1] | *Eucalyptus* sp. |
| 27 | *Anomalopsylla insignita* | MC [3] | *Olearia paniculata* |

| | | | |
|---|---|---|---|
| 28 | *Anomalopsylla* "Pollen island"ɣ | MC [1] | *Olearia odorata* |
| 29 | *Atmetocranium myersi* | MC [1] | *Weinmannia racemosa* |
| 30 | *Blastopsylla occidentalis* | AK [1], WO [2] | *Eucalyptus* sp. |
| 31 | *Cardiaspina fiscella* | WO [1], WI [1] | *Eucalyptus* sp. |
| 32 | *Creiis lituratus* | WO [1] | *Eucalyptus* sp. |
| 33 | *Cryptoneossa triangula* | WI [1], **SA [1]** | *Eucalyptus* sp. |
| 34 | *Ctenarytaina clavata* A | MC [1], NN [1], NC [1], MK [1] | *Kunzea ericoides* |
| 35 | *Ctenarytaina clavata* B* | MB [1] | *Kunzea ericoides* |
| 36 | *Ctenarytaina clavata* C* | NN [1] | *Leptospermum scoparius* |
| 37 | *Ctenarytaina clavata*. D* | TO [2], WN [1] | *Kunzea ericoides* |
| 38 | *Ctenarytaina eucalypti* | MC [2], NC [3], WA [1], SL [3], GB [1], TO [2], SC [1], DN [1], FD [2], SI [2], **VIC [1], SA [1]** | *Eucalyptus globulus* |
| 39 | *Ctenarytaina fuchsiae* A | MC [4], FD [2], SC [1], NC [1], WD [5], NN [5], SI [7] | *Fuchsia excorticata* |
| 40 | *Ctenarytaina fuchsiae* B* | KA [3] | *Fuchsia excorticata* |
| 41 | *Ctenarytaina fuchsiae* C* | TO [1] | *Fuchsia excorticata* |
| 42 | *Ctenarytaina longicauda* | AK [2] | *Lophostemon confertus* |
| 43 | *Ctenarytaina pollicaris* | MC [2] | *Leptospermum scoparium* |
| 44 | *Ctenarytaina pollicaris* B* | MC [1], NN [3] | *Leptospermum scoparium* |
| 45 | *Ctenarytaina* "Short"ɣ | MC [5], NC [1], NN [1] | *Leptospermum scoparium* |
| 46 | *Ctenarytaina* sp. A* | NN [1] | *Olearia paniculata* |
| 47 | *Ctenarytaina spatulata* | NC [1], MC [2], SC [1], FD [3], SL [1], AK [1], WO [2], WI [2], SI [1], TO [1] | *Eucalyptus nicholii* |
| 48 | *Ctenarytaina* sp. B* | SI [2] | *Kunzea ericoides* |
| 49 | *Ctenarytaina* sp. C* | BP [1], WO [1] | *Kunzea ericoides* |
| 50 | *Ctenarytaina* sp. D*(266) | NN [1] | *Kunzea ericoides* |
| 51 | *Ctenarytaina* sp. E* (314) | WO [1] | *Kunzea ericoides* |
| 52 | *Ctenarytaina thysanura* | SC [1] | *Eucalyptus* sp. |
| 53 | *Ctenarytaina* sp. unknownɣ | AK [2], WN [1] | *Syzygium* sp. |
| 54 | *Eucalyptolyma maideni* | **SA [1]** | *Eucalyptus* sp. |
| 55 | *Glycaspis granulata* | AK [1], WO [1], WI [1] | *Eucalyptus* sp. |
| | **Family Triozidae** | | |
| 56 | *Bactericera cockerelli* | AK [1], BR [1], MC [1] | *Solanum tuberosum* |
| 57 | *Casuarinicola australis* | ND [1], QLD [1] | *Casuarina* sp. |
| 58 | *Trioza acuta* A | MC [3], MB [1] | *Ozothamnus leptophyllus* |
| 59 | *Trioza acuta* B* | | *Ozothamnus leptophyllus* |
| 60 | *Trioza eugeniae (T. adventicia)* | MC [1], HB [1], **SA [2]** | *Syzygium smithii* |
| 61 | *Trioza bifida* | MC [6], NC [1], MB [1], SI [2], DN [1] | *Pseudowintera* sp., *Olearia* sp., *Hebe* sp. |
| 62 | *Trioza* "Brenda May"ɣ | SL [1], FD [1] | *Olearia ilicifolia* |
| 63 | *Trioza colorata* | MC [1], NC [1] | *Halocarpus bidwillii* |
| 64 | *Trioza compressa* | NN [4] | *Olearia*, *Fuchsia* |
| 65 | *Trioza curta* | NN [1] | *Metrosideros* |
| 66 | *Trioza dacrydii* | NN [1] | *Halocarpus bidwillii* |
| 67 | *Trioza decurvata* | MB [1], TO [1], NN [1] | *Dracophyllum* sp. |
| 68 | *Trioza discariae* | MC [2], NC [1] | *Discaria toumatou* |
| 69 | *Trioza doryphora* | MC [4] | *Olearia ilicifolia*, *Coprosma* sp. |

| | | | |
|---|---|---|---|
| 70 | *Trioza emarginata* | NC [1] | *Coprosma* sp. |
| 71 | *Trioza falcata* A | MC [1], NC [3] | *Aristotelia fruticosa* |
| 72 | *Trioza falcata* B* (480) | NN [1] | *Aristotellia fruticosa* |
| 73 | *Trioza fasciata* | AK [1], NN [1] | *Muehlenbeckia complexa* |
| 74 | *Trioza* "Fortrose"ɣ (471) | NN [1] | *Elaeocarpus hookerianus* |
| 75 | *Trioza gourlayi* | NN [1] | *Olearia virgata* |
| 76 | *Trioza hebicola* | NN [1] | *Hebe* sp. |
| 77 | *Trioza irregularis* | MC [8], NN [2] | *Pseudopanax arboreus, Schefflera digitata.* |
| 78 | *Trioza* "Massey"ɣ | MC [1], NN [1] | *Olearia* sp. |
| 79 | *Trioza obscura* | MB [1], NN [1] | *Hebe* sp. |
| 80 | *Trioza* "Omahuta"ɣ (472) | NN [3] | *Metrosideros umbrellata, Brachygliottis repanda* |
| 81 | *Trioza panacis* | MC [2], WN [1] | *Pseudopanax crassifolius* |
| 82 | *Trioza* "Price's valley"ɣ | MC [2] | *Plagianthus regius* |
| 83 | *Trioza* sp. A* (410, 468, 469) | NN [3] | *Pittosporum divaricatum* |
| 84 | *Trioza* sp. B? (412) | NN [1] | *Olearia arborescens* |
| 85 | *Trioza* sp. C* (47) | MC [1] | *Pseudopanax edgerleyi* |
| 86 | *Trioza* sp. D? (442) | MC [1] | *Olearia virgata* |
| 87 | *Trioza subacuta* | MC [2] | *Olearia avicennifolia* |
| 88 | *Trioza subvexa* | NN [4] | *Olearia avicennifolia* |
| 89 | *Trioza vitreoradiata* | SL [3], FD [1], CL [1], MC [3], NN [2], GB [1], TK [2], MB [2], AK [1] | *Pittosporum crassifolium, Pittosporum* spp. |
| 90 | Triozid sp.ɣ | AK [2], CL [1], **NSW [1]** | *Casuarina* sp. |

### 3.3.4 *Trioza adventicia* and *T. eugenieae* are one species?

Specimens of *Trioza adventicia* were collected from two locations in New Zealand, while two different populations of *T. eugeniae* were collected from South Australia by Gary Taylor. Both the host plant associations and the morphology contributed to their respective species identification; *T. adventicia* collected in New Zealand from *Syzygium smithii* presented the typical morphological feature of three tibial spurs on the posterior leg, while *T. eugeniae* collected from Australia both from *Syzygium smithii* and *Acmena*, had only two tibial spurs. The COI sequences for all the specimens defined as *T. adventicia* (three sequences) or *T. eugeniae* (ten sequences) revealed a nucleotide identity greater than 99%. A second morphological assessment conducted as a result of COI data, discovered that there were a different number of spurs, not only within the same population, but also between the right and left leg of the same individual (Figure 3.5). This indicated that the number of spurs was not a robust species-defining character for these two species. With parallel host and DNA information, there was also no evidence to separate them as species beyond the fact that they were collected in different countries.

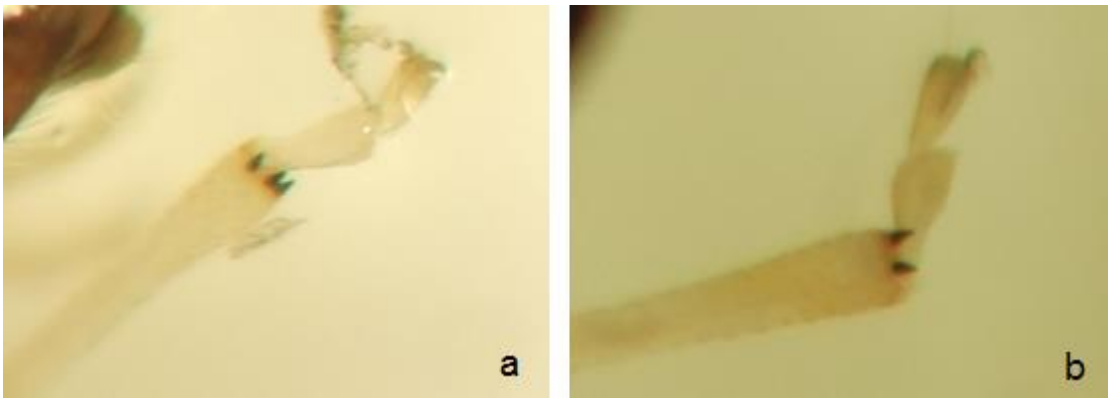


**Figure 3.5:** Posterior legs of the same individual of *T. adventicia* collected in New Zealand: a leg is showing three spurs (a) while the other only two (b).

## 3.4 Discussion

### 3.4.1 Unified species concept and species delimitation

The species concept, as proposed by De Queiroz (2007), overcomes the issue of having many alternative and sometimes conflicting concepts trying to define a species (De Queiroz 2007). Other concepts rely on defining criteria, which are largely beyond the scope of this study, such as reproductive isolation or ecological divergence. Instead, species are retained as "s*eparately evolving metapopulation lineages*", and all other defining criteria are considered "*contingent properties: properties that species may or may not acquire during the course of their existence. In other words, lineages do not have to be phenetically distinguishable, diagnosable, monophyletic, intrinsically reproductively isolated, ecologically divergent, or anything else to be considered species. They only have to be evolving separately from other lineages.*" Consequently, this concept accommodates integrative taxonomy as a method for species delimitation (Padial et al. 2010), which considers combinations of characters, such as geographical, ecological, morphological, genetic and reproductive aspects that contribute to species delimitation. The idea of integration by congruence delimitates a species when at least two of its characters support a variation from another given species (Padial et al. 2010).

In this study, up to four different characters were chosen to delimit species. However, since in some instances not all the characters could be obtained for the same taxa (e.g. no COI could be isolated from *A. myersi*), species delimitation was assessed only when at least three of these were congruent. These characters were geographical (distribution), ecological (host plant association), morphological and genetic [COI] (Figure 3.6). The most common process for species delimitation, **host plant - morphology – DNA** (black line, Figure 3.6), started with the identification of plant

species known to host particular psyllid species. Morphological characters of the anticipated species were examined and their presence confirmed or rejected, and finally, if the COI sequences delimitated the species from all others, then the identification as described in the keys was accepted.

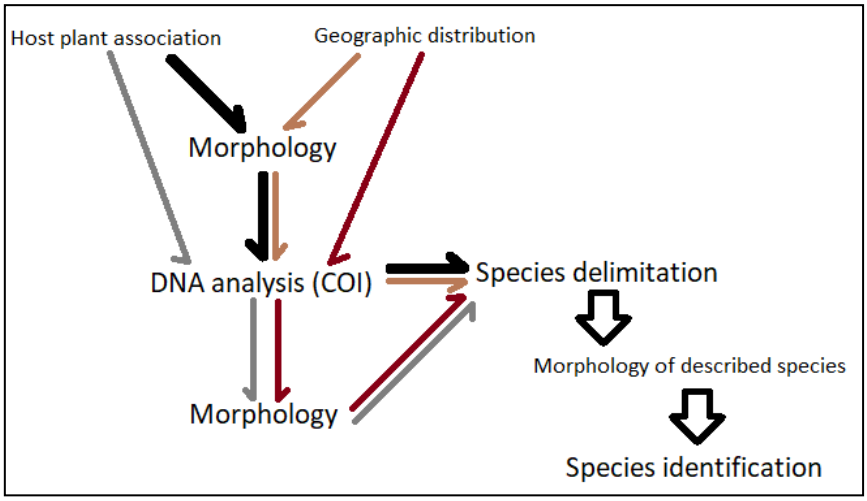


Figure 3.6: Pathways to species identification. At least three separate characters were required for species delimitation, with the four coloured arrows (black, grey, brown, red) representative of the different pathways taken to achieve this for different species (see text for examples).

For other specimens, where the morphology of the insects collected from different plant species did not appear different, but where the expectation was to find a particular species on a particular host plant species, the **host plant – DNA – morphology** delimitation pathway (grey line, Figure 3.6) was followed. This was necessary for psyllids on *Carmichaelia* spp., where COI sequence divergence (between 7% and 17%) confirmed that separate populations required further morphological analysis, which subsequently confirmed the presence of different characters (e.g. wing patterns) in different populations. Where psyllids showed morphological differences when collected from the same host plant species at different locations, the **geography – morphology – DNA** (brown, Figure 3.6) pathway was followed. This was the case of psyllids collected in New Zealand and Australia on *Acacia baileyana*. In some cases, the morphology immediately suggested species delimitation (e.g. male parameres), which was then confirmed by DNA. In fact, while a single species had previously been described on *Acacia baileyana* (*Acizzia acaciaebaileyanae*). This process highlighted the presence of a second psyllid species, present both in New Zealand and in Australia, where only a single species had previously been described on *Acacia baileyana* (*Acizzia acaciaebaileyanae*).

In a single case, *Trioza eugeniae* from Australia, and *T. adventicia* psyllids from New Zealand collected from the same host plant (*Syzygium smithii*) were thought to belong to these two previously described species. However, using **geography – DNA – morphology (red, Figure 3.6)**, DNA analysis showed a lower-than-expected variation which led to deeper morphological analysis. This highlighted that previously recorded morphological characters were not consistent enough to delimitate two separate species. For this reason, a synonymisation appears to be required (Chapter 3.3.4).

### 3.4.2 Identification of New Zealand psyllids

Using a combination of COI DNA sequences (Appendix C.1), bioinformatics analysis, morphological characters and host plant associations, 90 psyllid species were identified within the largest field collection in New Zealand undertaken in the last 30 years. Morphological features together with reports of host plants were used to link these taxa with previously described New Zealand psyllids (Maskell 1890, Ferris and Klyver 1932, Tuthill 1952, Dale 1985). In most cases, matching the COI defined taxa to the recognised New Zealand psyllid taxa was straightforward; usually a single psyllid species was found on a host plant and these insects were morphologically consistent with reports of psyllids from that host species. There were also a few cases of psyllids found on plants not previously regarded as hosts, in terms of supporting a complete life cycle (Chapter 1.2.2). For the most part, these appeared to be casual host associations (Burckhardt et al. 2014), with low psyllid numbers in contrast to the higher numbers on adjacent plants of the expected hosts; these psyllids were not usually collected. Other cases of atypical host associations were more difficult to reconcile when a high number of psyllids were present on an isolated plant. A conspicuous example was *T. bifida*, which was collected in substantial numbers from unexpected plant hosts of the genera *Hebe* and *Pseudowintera* (Table 3.1) in addition to the expected *Olearia* species (Chapter 2). These may be examples of food plants on which adult psyllids can feed but not breed (Burckhardt et al. 2014), and confirms what Tuthill described as “a very active species” (Tuthill 1952).

While the findings here will contribute to a better understanding of New Zealand psyllid biology, observation of any one psyllid species was insufficiently detailed to draw definitive conclusions about their ecology. Nymphs were found only infrequently, so it was difficult to confirm “true” host associations, which are defined by life cycle completion on a host plant species (Burckhardt et al. 2014). Earlier researchers have established host relationships for many New Zealand psyllids by securing nymphs and rearing them to adulthood (Tuthill 1952).

Despite the undoubted value of morphological descriptions for New Zealand psyllids (Ferris and Klyver 1932, Tuthill 1952, Dale 1985), in a number of cases the initial taxonomic assignment to species was incorrect. These were revealed by the COI sequence phylogeny (Appendix C) and led to morphological reassessment of other individuals for the same population to confirm. For example, having found *T. subacuta* on *Olearia* instead of *Brachyglottis* initially led to misidentification as *T. subvexa*. Similarly, collecting psyllids from *Schefflera digitata* initially led to the assumption they belonged to the species *T. schefflericola.*

### 3.4.3 New diversity revealed in New Zealand psyllids

Within the 90 taxa found in this field collection, only 57 had been previously described. Another 10 taxa, even if not described, were previously known to be present in New Zealand (Dale 1985, Macfarlane et al. 2010). In addition to these 67 species, COI sequencing revealed some diversity among the psyllids found on New Zealand native plant hosts that clustered phylogenetically separate from those expected. Together with the identification of morphological variations, this led to the identification of a total of 21 new taxa, while another two taxa of the genus *Trioza* could not be identified to the species level (Table 3.1).

Seven new *Psylla* taxa were resolved: two on kowhai (*Sophora microphylla*) and five on native broom (*Carmichaelia* spp.). Only *P. apicalis* on kowhai and *P. carmichaeliae* on *Carmichaelia* had previously been recognised (Dale 1985). Interestingly, once the genetic difference (7-8% COI divergence depending on populations) between the kowhai psyllid taxa became apparent, considerable corroborating morphological differences were observed between the two linages; these included overall dimensions, colour and head shape. In at least four locations in the Central Otago region, both the species were collected from the same individual kowhai plants, suggesting a sympatric distribution.

In contrast, the five *Psylla* taxa from native brooms showed a much broader range of COI variation from 7% to 17%. Each of the five taxa was collected from different *Carmichaelia* host species: three were identified to the species level using the existing morphological keys (Heenan 1995, 1996), while the two others, which were morphologically distinct from those and each other, could not be identified according to the existing keys (Table 3.1). There were no occurrences of more than one of these species on the same *Carmichaelia* host species or on the same individual plant. Moreover, two of the *Carmichaelia* species each hosting a different psyllid species (*Psylla carmichaeliae* D or E) were growing within 10 km of each other in Cromwell (Central Otago, CO, Table 3.1). This suggested that the genetic variation between these psyllid species is not to be ascribed to geographical distance but to the different host plants. Two possible evolutionary

scenarios could arise from this. Either the common psyllid ancestor of these species could have colonised an ancestral *Carmichaelia* plant species which then evolved into the two present species; or the ancestral psyllid in the past could be hosted by both the *Carmichaelia* host plants in the past, and have subsequently coevolved with each of them as two separated psyllid species. In light of these findings, it is possible that there is a host-specific association of *Psylla* taxa with different *Carmichaelia* species. The sampling of *Carmichaelia* in this study was insufficiently extensive in terms of either species diversity or geography to answer this question. After checking for the presence of insects on eight different *Carmichaelia* species, collections were only able to be made from five of the 17 *Carmichaelia* species recorded in New Zealand (Heenan 1995, 1996), and from only 11 locations (10 in South Island and one in North Island). Interestingly, Tuthill (1952) reported the presence of *P. carmichaeliae indistincta*, which he described as a subspecies of *P. carmichaeliae* as part of what he considered an "*inseparable complex*" (Tuthill 1952). An undescribed *Psylla* aff. *carmichaeliae* collected from *C. torulosa* has also been listed amongst the endangered hemipterans of New Zealand (Stringer et al. 2012). This study now provides a useful start towards the resolution of Tuthill's inseparable complex of *Carmichaelia* psyllids, but a more extensive sampling of from *Carmichaelia* spp. will be needed to complete this.

High COI sequence diversity was also found amongst the New Zealand *Ctenarytaina*. A pronounced example was in *Ctenarytaina fuchsiae* from *Fuchsia excorticata*. Sampling of *Fuchsia* in 22 locations across widespread sites in the South and Stewart Islands revealed a single, broadly distributed lineage of *C. fuchsiae* (COI divergence at 2%). The exception to this pattern was a substantially divergent lineage (12% COI divergent from the other lineage) restricted to plants growing within a few kilometres of one another on the Kaikoura coastline (*C. fuchsiae* sp. B, Table 3.1). A single *C. fuchsiae* population collected in the North Island was also distantly related (22% COI variation) to both South Island taxa (*C. fuchsiae* sp. C, Table 3.1). Interestingly, the northern part of the North Island (above 39°S) is known to generally show higher levels of biodiversity compared to both the southern part of the North Island and the South Island (Buckley et al. 2015). This diversity, which can be both intra- and inter-specific, leads to the expectation of a much greater variation in the North Island psyllid biodiversity. Potentially there may be more divergent *C. fuchsiae* lineages in North Island and the Kaikoura population may represent a recent colonisation from the north, especially given that the insects are found immediately alongside the main arterial route from North to South. On the other hand, it is puzzling that such small and apparently mobile insect does not have a more homogenous distribution across the country, especially when considering that some of these insects are known to use wind currents to cover distances of hundreds of kilometres (Yen et al.

2014). For these reasons, the basis for the apparently restricted distribution of the Kaikoura *C. fuchsiae* is unclear and puzzling.

The highest diversity was recorded among the *Ctenarytaina* psyllids from the tea trees mānuka (*Leptospermum scoparium*) and kānuka (*Kunzea ericoides*). In fact, clusters of psyllids were observed to be consistent with those previously recorded of *C. clavata* (four COI lineages) and *C. pollicaris* (six COI lineages). The *C. pollicaris* cluster included *Ctenarytaina* "short" (Dale 1985), despite the morphology and colour of these taxa being distinctive (black with long female terminalia versus yellow with short female terminalia, respectively). These two taxa were found together more than once on the same individual plant, but only in the South Island. Identification of the mānuka/kānuka *Ctenarytaina* species was very difficult based on morphology alone. One of the few observed differences was a darker brownish colouration in the body of "*Ctenarytaina clavata* D" compared with other lineages of *C. clavata* that tended towards a dark orange. *Ctenarytaina clavata* D was also collected only from the North Island (in multiple locations from Wellington up to the Tongariro). Interestingly, the three populations of *Ctenarytaina clavata* D are the only instance, in this study, where a divergence of 3% between the population 334 and the other two (335 and 402) has been considered intra-specific variation and not inter-specific (Figure 3.7). However, morphological similarity, the immediate proximity of the populations 334 and 335, and the same host plant species have been considered factors important enough to overlook the 3% COI variation.

Dale (1985) recognised that her accounts of four *Ctenarytaina* species on mānuka and kānuka was not a complete record of psyllids from these plants species and, further, that additional observed variation might be due to geographical isolation and/or hybridizations of *Ctenarytaina clavata* and *C. pollicaris* (Dale 1985). In this study, the first case possibly corroborating Dale's hypothesis was *Ctenarytaina* sp. "B", which was collected from mānuka in Stewart Island, but which fell in an intermediate position between the "*clavata*" and "*pollicaris*" COI clusters. The distribution of samples in this study does lend some support to the idea that these species are geographically isolated; only *Ctenarytaina pollicaris* "B" was found in both islands (Figure 3.7). The species *Ctenarytaina* "cut away" described by Dale (1985) was not collected since the exact locations reported by her were not reached during the field collection (Dale 1985).

*Ctenarytaina* psyllids tend to be abundant in native shrub land environments where their host plants are common. Their small size makes them prone to movement by wind and rain, so they are often found on a range of plants which may not be their true hosts. Thus, lineages of *Ctenarytaina* on plants other than mānuka/kānuka could easily be dismissed as casual host associations during field collections. More extensive surveying coupled with COI barcoding may well

reveal further *Ctenarytaina* diversity on native New Zealand plants. Accordingly, one new *Ctenarytaina* taxa detected in this study, *Ctenarytaina* sp. "A", was genetically distinct (12-17%) from the mānuka and kānuka psyllids and was instead collected from *Olearia*, a host not previously known to harbour psyllids from this genus. In Figure 3.7 it can be seen how this taxon clearly separates both from the "*C. clavata* complex" and from the "*C. pollicaris* – short complex". Evidently this is an important finding, possibly representing a marked host switch with New Zealand *Ctenarytaina*. Despite having found only a single population, more than 10 individuals were collected from the same plant together with nymphs. Repeat sampling from this location and confirmation of true host association by monitoring the progress of nymphs is warranted. A *Ctenarytaina* sp. collected from *Syzygium* is marked here as "Unknown" (Table 3.1). Although not having access to any reference samples for morphological comparison, this species may be the *Ctenarytaina* sp. previously reported by Macfarlane and colleagues from the same host plant (Macfarlane et al. 2010). With these new taxa proposed here, a total of 21 *Ctenarytaina* species can be considered present in New Zealand, making *Ctenarytaina* the second most numerous genus in the country. This result is even more interesting considering to date the Australian *Ctenarytaina* fauna is composed of only seven species (Ouvrard 2017).

Of the 52 species of ***Trioza*** that were previously recognised as present in New Zealand (Chapter 2), 26 have been collected and analysed here (Table 3.1). Of the remaining 26 species, 12 have been described while the other 14 have only been reported by Dale (1985) and are yet to be described. Amongst the reasons why these species could not be collected is their distribution in either isolated locations (e.g. *T. dentiforceps* in the Chatham Islands, *T. australis* in Stewart Island) or in narrow areas (e.g. *T. scobina* and *T. latiforceps,* both around Nelson). Another reason is the very strong morphological similarities between these species. This, together with the fact that many species share the same host plant genera (e.g. *Brachyglottis* and *Olearia* host a total of 11 species) makes identification in the field almost impossible; collection from each plant in order not to miss possible species thus becomes very important albeit time-consuming. Compounding this, the host plant of six of these species is unknown: *T. equalis, T. styligera, T.* "Wards Pass", *T.* "Mt. Dewar", *T.* "Niger Mt." and *T.* "Logan Burn" (Chapter 2). On the other hand, for the purpose of this study, 26 described species belonging to the genus *Trioza* (plus an additional six undescribed taxa) can be considered a success and, noting their widespread distribution and high number of associated host plant families, was sufficient for both phylogenetic and evolutionary analysis (Chapter 4).

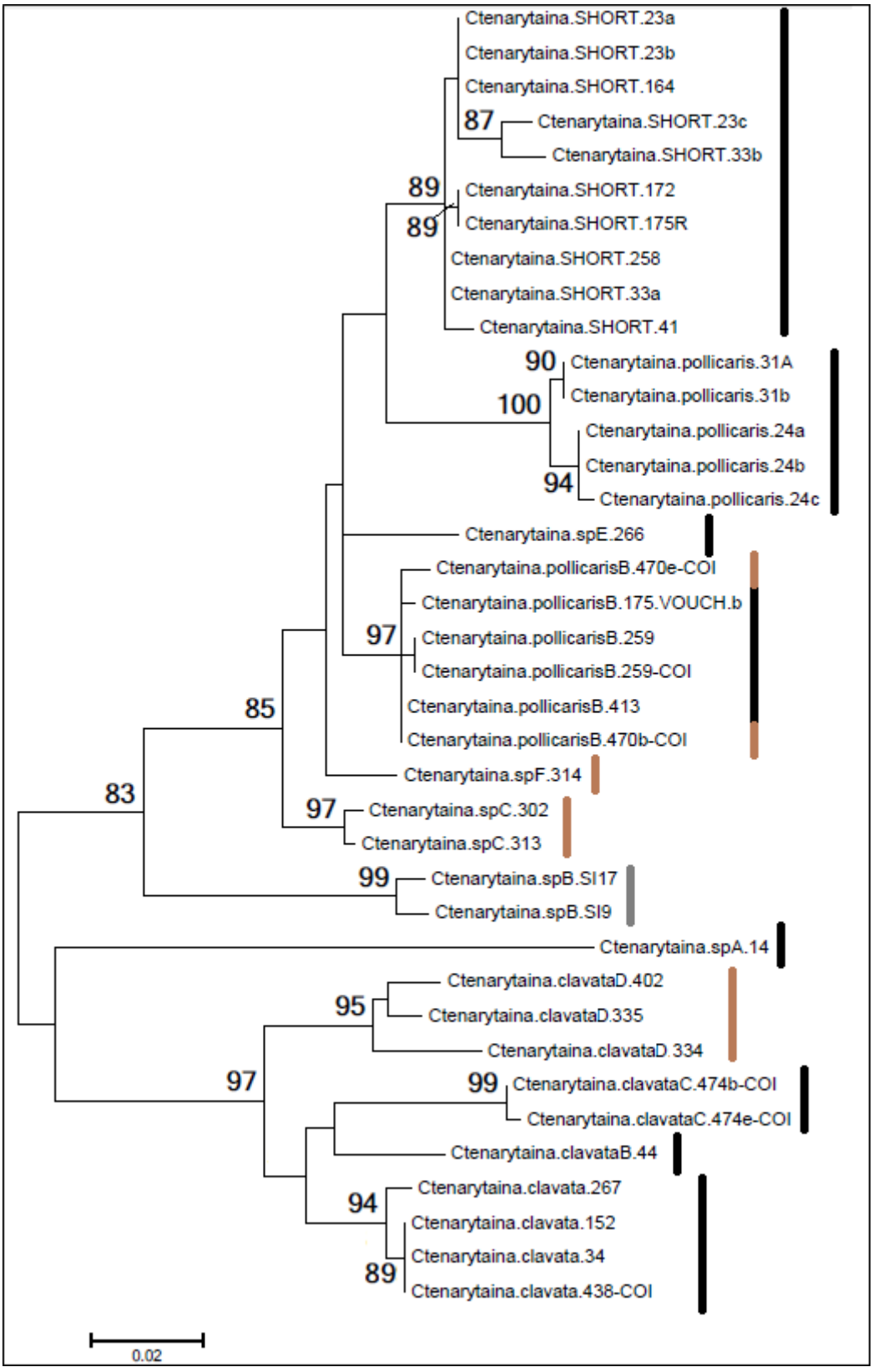


**Figure 3.7:** COI tree (ML, 1000 replicates, bootstrap <60 not shown) including all the different *Ctenarytaina* samples collected for the kānuka-mānuka complex and *C.* sp. A. The tree is associated to the insects' distribution across the country: North Island (brown), South Island (black) and Stewart Island (grey).

However, also within the genus *Trioza*, six lineages were detected which did not reconcile with any of the prior records from New Zealand. In two of these cases, both morphological and COI data suggested a strong similarity with two other described New Zealand species, *T. acuta* found associated with *Ozothamnus leptophyllus* and *T. falcata* with *Aristotelia fruticosa*. These species have been respectively reported here as *T. acuta* B (lineage 1) and *T. falcata* B (lineage 2) (Table 3.1). While the observed morphological similarity was less pronounced in these two *Trioza* species compared with the cryptic similarity between species within the *Psylla* and *Ctenarytaina*, this was restricted to only two samples from each of these taxa and collection over limited geographical ranges. Indeed, the two *T. acuta* specimens were collected only 10 km apart in inland Canterbury.

Two of the other new taxa each appeared to represent species associated with new host plant species within the same plant genus. Both were morphologically distinguishable (e.g. variation in the terminalia, Dale 1985) and genetically distinct from other *Trioza* species present on these host genera. The first was a new *Trioza* taxa (lineage 3) collected from *Pittosporum divaricartum* (sp. A, Table 3.1); the closest relative to this psyllid, based on COI similarity, was *T. vitreoradiata*, which is widespread and highly abundant on a range of *Pittosporum* species but has not been reported from *P. divaricartum* (Dale 1985).

A second *Trioza* (lineage 4) (sp. C, Table 3.1), from *Pseudopanax edgerlii*, was distinct from other known psyllids on *Pseudopanax*. In fact, the only three species expected to be found on similar plants were *Trioza irregularis,* on *P. arboreus* (Ferris and Klyver 1932), *T. panacis* on *P. crassifolius* (Maskell 1890) and *T. schefflericola,* on *Schefflera digitata* (Tuthill 1952). In general, however, delineation of psyllid species within the "*Pseudopanax-Schefflera* host plant group" was poor, from both morphological and molecular assessments. For example, within the samples collected, *Trioza* samples from *Schefflera digitata* (Araliaceae) had COI sequences less than 2% different to *Trioza irregularis* from *Pseudopanax arboreus*. This could suggest within-species variation rather than two separate species. In fact, *Trioza schefflericola* (Tuthill 1952, Dale 1985) has been described from *S. digitata* but the validity of this species has been doubted by Tuthill himself, who affirmed that many of the differences between the two species are simply "*slight differences of degree*" (Tuthill 1952).

The differentiation of these *Trioza* species from *Pseudopanax* and *Schefflera* will require more extensive sampling and possibly finer scale molecular markers to provide more robust evidence of their distinction or otherwise. In fact, based on evidence from other taxa, the COI barcode region does not always reflect species differences that have been achieved and accepted by other means (Burns et al. 2007). Therefore, this could indicate unsuitability of this gene region for

delineation of these species, and that other gene regions need to be surveyed together with biological clues to come to any conclusion.

The final two novel *Trioza* species [sp. B (lineage 5) and sp. D (lineage 6), Table 3.1) were collected from *Olearia*. This is consistent with the majority of New Zealand *Trioza* being reported as hosted on *Olearia* (Dale 1985), many of which were not among the nine species collected here. These two putatively unidentified *Trioza* may actually be known species for which the morphological evidence by comparison to reference samples was lacking due to current inaccessibility to many prior collections. Understanding the degree of diversity in this cluster will be aided by DNA barcoding since morphological differences between these *Trioza* are less well defined than for other species. Of note, in this study no *Trioza* psyllids were found on native *Celmisia*, despite the reported presence of species on these plants (Dale 1985).

Even among psyllids hosted by adventive plant species, a new ***Acizzia*** species was identified. Sampling revealed two distinct taxa on *Acacia baileyana* with COI sequence variation of 22% between each other and clear morphological differences (e.g. male parameres longer in the new species compared to *A. acaciaebaileyanae*). The psyllid *Acizzia acaciaebaileyanae* has been described from *Acacia baileyana* [as *Psyllia uncata* (Ferris and Klyver 1932)] in both New Zealand and Australia, but a second *Acizzia* lineage on this host has never been described. This appears to be another example of a native Australian psyllid which is described in New Zealand before being identified among the Australian psyllid fauna, as it happened for *A. hakeae* (Chapter 2.2.3).

Many biogeographical studies have shown close relationships between the flora and insect fauna of New Zealand and Australia [e.g. (Buckley et al. 2015)], with winds acting as an underlying driver of insect dispersal to New Zealand (Yen et al. 2014). The movement of psyllids from Australia appears consistent with these studies, but it is not known how many introductions have occurred and if they were facilitated by wind alone or by other means such as increased trade and tourism between the two countries (Withers 2001). The lack of knowledge surrounding the relationship between the New Zealand and Australian psyllid fauna is exemplified by the fact several species presumed adventive have been described in New Zealand before they were recorded in Australia. Moreover, similarities between the New Zealand psyllid fauna and the fauna of countries in South America or of New Caledonia have been previously drawn (Dale 1985), placing in doubt the concept that New Zealand's psyllid diversity is primarily the consequence of the geographical and political relationship between New Zealand and Australia.

### 3.4.4 Overestimation of psyllid biodiversity

Interestingly, the depth of survey here to include collections from the southern states of Australia, uncovered a potential synonymisation. *Trioza eugeniae* was described in Australia in 1901 by Froggatt. He collected this psyllid from *Acmena* and *Syzygium* in both New South Wales and Victoria (Froggatt 1901). A few years later, *Trioza adventicia* was described by Tuthill in New Zealand (Tuthill 1952), and in the present study accordingly collected from *Syzygium*. However, in describing the insects collected as a new species, Tuthill reported the similarity with the Australian samples of *T. eugeniae* specifying that the main difference was the number of tibial spurs: three in *T. eugeniae* and only two in *T. adventicia* (Tuthill 1952). This single morphological character, together with the geographic distribution, maintained the two taxa as separate for almost 100 years.

The genetic comparison of the COI sequences here, on the other hand, immediately highlighted a significant (99%) similarity between the samples from the two countries. This is well within the level of population variation for the majority of species here. As a result, a deeper analysis of the morphology has been undertaken, including additional populations from different locations in Australia, New Zealand and USA (Percy 2017). Consequently, the number of spurs on the posterior tibia were revealed to be a very variable character. In fact, individuals from the same population could show both the presence of two and three spurs. Interesting cases where the same individual was showing a different number of spurs between the right and the left leg were also reported.

Once that the number of spurs as a diagnostic character was discarded, and with the addition of the COI genetic similarity data, there was no obvious morphological, molecular or biological data separating these two 'species' and a synonymisation was proposed. The synonymisation process was performed following the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999) which states that the first species described would attribute the name to the synonymised species. Therefore, the name *Trioza eugeniae* has been retained and is now the only species belonging to this genus to be considered as adventive and not native to New Zealand. At the time this thesis is being completed, a synonymisation manuscript is being prepared (see Chapter 6.3).

## 3.5 Conclusion

Sampling is key as to the conclusions that can be made about biodiversity. Clearly this will have been a factor here, with some of the species that were not found and additional ones that were. With modern methods to enhance the biodiversity information from collections, however, there is inevitably going to be an ongoing increase in discoveries. Thus, despite field collections being

more focused and exhaustive in the South Island, this study has vastly increased the known diversity and distribution of the New Zealand Psylloidea. Compared to the total of 99 species considered at the outset to be present in New Zealand, 90 were identified here. Of these, 57 had previously been formally described and a further eight species informally reported by Dale (Dale 1985). Two further species, a triozid from *Casuarina* and a *Ctenarytaina* from *Syzygium* were reported recently as new arrivals in New Zealand, but not as yet formally described (Macfarlane et al. 2010). This constitutes 67 taxa already known to be present in New Zealand and another two *Trioza* species that may be included in Dale's list but for which comparison with historic collections was not possible. Therefore, out of the 99 previously reported species (Chapter 2), 69 are included here while another 21 species are reported for the first time. In conclusion, the New Zealand fauna of the Psylloidea counts at least 120 different taxa. Moreover, this number is expected to rise with a more exhaustive survey focused in the North Island, were insect diversity is expected to be higher (Buckley et al. 2015), and on plants of the genera *Fuchsia*, *Carmichaelia*, *Leptospermum*, *Kunzea* and *Olearia* that recorded a high psyllid biodiversity in the South Island.

The depth of species surveyed here together with their associated host, distribution, morphological and molecular data provides a solid platform for the subsequent phylogenetic (Chapter 4) and microbial communities (Chapters 5) analyses. However, it is also important to recognise that the same specimens collected, identified and carefully preserved and recorded through this study will make a valuable contribution to the study of the New Zealand Psylloidea generally beyond this work, including by others.

Finally, it is important to highlight the practicality of the results obtained here for the COI amplifications. With the only exception of two taxa, the generic primers and the PCR cycle adopted here always resulted in single bright bands, providing clean DNA sequence. This makes COI a useful locus for ongoing delimitation of taxa and as a molecular component of an integrative taxonomy concept. However, the clearly inadequate support for many of the deeper nodes in the COI gene tree (Appendix C.1) reminds us that caution must be placed in interpreting this marker alone for taxonomically broad phylogenetic purposes. Despite this could be considered a limitation within the data, this marker was not intended for that purpose here. Rather the interest here was only in the tips of the branches that denote taxonomic distinctiveness (Boykin et al 2012) to assist with species delimitation. Phylogenetic relationships using this data were only considered when paired with additional markers to complement slower evolutionary time frames of the deeper nodes (Chapter 4).

# Chapter 4

# Molecular phylogeny and evolution of the New Zealand Psylloidea

## 4.1 Introduction

Studies on psyllids, addressing questions such as their introduction, radiation and coevolution with plant hosts or pathogens, require a robust phylogenetic framework from which to draw findings. Unfortunately, few phylogenetic studies of psyllids have been performed to date, and none included the New Zealand fauna. The earliest studies, using morphological characters alone, investigated the taxonomy and phylogeny of the genus *Paurocephala* (Mifsud and Burckhardt 2002) and the phylogenetic significance of the wing base structure in Sternorrhyncha (Ouvrard and Burckhardt 2008). More recent phylogenetic studies of the Psylloidea employed molecular genetic techniques, which enabled the resolution of taxa that were historically difficult to differentiate using morphology alone. Indeed, using COI DNA sequences, the taxonomy of more than 35 psyllid species that had previously been assigned to the genus *Trioza* were attributed to the genus *Pariaconus* (Percy 2017) and delimitation of a number of Australian species was confirmed (Taylor et al. 2016).

Previously, COI DNA barcode sequences (Hebert et al. 2003) were used to generate psyllid phylogenies because they were convenient to use (Taylor et al. 2016, Percy 2017). Nevertheless, a comprehensive DNA-based phylogenetic framework for worldwide populations of the superfamily Psylloidea or for New Zealand species remains elusive. Moreover, for deeper, family level phylogenies, COI tends to reach saturation and is not informative especially at the third codon positions (Lopez et al. 1999, Philippe and Forterre 1999). Thus, to appreciate the evolutionary relationships amongst the New Zealand Psylloidea (Chapters 2 and 3), from the family to the genus level, a multi-gene phylogeny was required.

Overall, when compared to single-gene data, the use of multiple genes is associated with lower stochastic errors (Phillips et al. 2004, Delsuc et al. 2005, Holland et al. 2006), which result in more consistent phylogenetic signals [e.g. (Jermiin et al. 2005)] and allow discrimination between competing tree topologies (Strimmer and Rambaut 2002, Shi et al. 2005). A range of evolutionary rates are also accommodated by multi-gene phylogenies, which enable respective separation times to be considered (Lin and Danforth 2004) whilst providing robust nodal support for species tree inferences that cannot be achieved using single-gene trees (Kjer et al. 2016).

COI is best complemented in multi-gene phylogenetic analyses with more slowly evolving gene regions. With this in mind, Wilson (2010) identified a set of markers, defined as “priority genes” of great phylogenetic value. The priority genes, Elongation Factor-1 alpha (EF-1α), wingless

(*wg*), 18S rDNA and Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase and Dihydroorotase (CAD), were used together with the DNA barcode region to successfully resolve macrolepidopteran species phylogenies (Wilson 2010). Coincidentally, a similar set of genes was used to construct the phylogeny of Australian psyllid species within the Aphalaridae in relation to their microbial associations (Hall et al. 2016), although *Cytochrome b* (*cytb)* was used instead of 18S. Prior to that, studies associating psyllids with their primary and secondary symbionts did not use a molecular phylogeny of the insects, rather systematic relationships were inferred by morphology alone (Thao et al. 2000b, Thao et al. 2000a, Thao et al. 2001). In other phylogenetic studies, the evolution and host plant association of legume-feeding species on the Canary Islands was examined using the mitochondrial small subunit rRNA (12S) and cytochrome oxidase I/II regions (Percy and Cronk 2002, Percy 2003b, Percy et al. 2004). Taylor used the ribosomal 18S gene with COI for his phylogenetic approach to the Australian psyllids at a species level (Taylor, personal communication), following the method previously employed for taxonomic purposes elsewhere (Ouvrard and Burckhardt 2008).

Considering that many of the New Zealand psyllids analysed in this thesis were common to Australia, for continuity with the studies above, the 18S gene was chosen to be used in addition to the previously used COI (Chapter 3), to obtain a first phylogeny of the New Zealand psyllids. In addition to the 18S gene, EF-1α was considered as it has been employed extensively in other phylogenetic studies to evaluate insect taxonomic placement (Cho et al. 1995). This was despite evidence in several instances of paralogous copies (Danforth and Ji 1998) and an intron/exon structure in the Hexapoda that might lead to misinterpretation due to incorrect alignment of sequences. In contrast, no introns were reported across nine Australian psyllid species and four genera (Hall et al. 2016), nor during recent work on aphids pairing EF-1α with COI (Durak et al. 2014) or COII (Yang et al. 2010).

It was anticipated that the phylogeny obtained in this thesis would endorse the identities of the species of psyllids in New Zealand defined in Chapter 3 using the integrative 'species concept' approach. More importantly, it was also expected that the phylogeny would provide supporting information on the relationships of these taxa. For example, to clarify some long standing taxonomic questions regarding the position of the genera *Atmetocranium* and *Anomalopsylla*, which have been debated for more than 70 years (Ferris and Klyver 1932, Tuthill 1952, Heslop-Harrison 1960, Bekker-Migdisova 1973, Dale 1985, Burckhardt and Ouvrard 2012); *Atmetocranium* is currently (provisionally) assigned to the family Calophyidae (Burckhardt and Ouvrard 2012). Finally, psyllid phylogenetic work has previously enabled hypotheses on the processes behind the evolution of these insects to be examined, such as those in the Canary Islands and Madeira (Percy 2003b, Percy et al. 2004), and the Iberian Peninsula, Morocco and Macaronesia (Percy 2002). Therefore, this

study was expected to reveal the interactions of the New Zealand psyllids with host plant as well as the role of biogeography. Towards the latter, since the previous study on the evolution of the New Zealand psyllids was conducted (Dale 1985), novel information and a wide number of studies have improved our knowledge of the biogeography of New Zealand (Goldberg et al. 2008, Buckley et al. 2015). Therefore, together with the phylogeny, this information, was expected to clarify if the New Zealand psyllids have their closest relative(s) in Australia or in other land masses [e.g. Pacific islands, as for other terrestrial animals such as snails (Goldberg et al. 2008)].

Part of the work presented in this chapter has been published in the Journal of Economic Entomology as "Elongation Factor-1α accurately reconstructs relationships amongst psyllid families (Hemiptera: Psylloidea), with possible diagnostic implications" DOI 10.1093/jee/tox261. (Martoni et al. 2017). The text has been reformatted for the thesis.

### 4.1.1 Aim and Objectives

The main aim of this Chapter was to build a comprehensive multigene phylogeny of the New Zealand and Australian psyllids to achieve a better understanding of their evolution. A greater understanding of the evolution of the psyllids might include knowledge of the number of arrivals, the species radiation of different endemic psyllid groups, and their host switches. A robust phylogeny would also provide a robust platform for an assessment of their microbial associations and the role they play in the psyllid-host plant relationship (Chapter 5).

**The specific objectives to develop the phylogeny are:**

**Objective 1:** To generate DNA sequences for at least two markers to combine with the previously generated COI DNA barcode sequences for each New Zealand psyllid species (Chapter 3).

**Objective 2:** To infer a phylogenetic framework for the New Zealand Psylloidea based on Bayesian inference and use branch length and node support to finalise placement and delimitation of species, including the potentially new species indicated by previous DNA barcode and morphological assessment (Chapter 3).

**Objective 3:** To pair the phylogenetic information with ecological information on the host plant association in order to address: i) how many psyllid arrivals have occurred in New Zealand, ii) the mechanisms associated with the evolution of different families, genera or species once established in New Zealand.

## 4.2 Materials and methods

### 4.2.1 PCR amplification of phylogenetic markers

The DNA extracted from individual adult psyllids (Chapter 3) was used as template for PCR amplifications targeting the 18S, EF-1α, CAD and *wg* gene fragments. Each reaction was performed in a total volume of 20 µL using the KAPA3G plant PCR Kit (Kapa Biosystems, Massachusetts, USA) and included 10 µL of 1x PCR buffer, 1 µL for each of the two primers (10 µM) (see below for primers sequences), 0.5 U of Taq polymerase and 1 µL of DNA template (usually between 100 and 200 ng/µL).

#### Ribosomal 18S gene

A 544-bp 18S PCR product was amplified from the DNA of 179 specimens using the primers 18S_F [CTGGTTGATCCTGCCAGAGT (Ouvrard et al. 2000)] and 18S_Rmod, (ACCAGACTTGCCCTCCAAT); the latter was modified in this study from the primer 18S_R (Ouvrard et al. 2000), using the software Primer3 v. 0.4.0 (available at http://bioinfo.ut.ee/primer3-0.4.0/ ). All 90 distinct taxa identified according to the morphological, COI and host plant association criteria (Chapter 3) were represented among the 179 specimens, with at least two specimens from each taxa used where possible. Thermal cycling conditions for amplification of the partial 18S rRNA gene were: an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min, followed by a final extension of 7 min. After confirming amplification of the appropriate fragment in each reaction by gel (agarose 1%) electrophoresis, the amplicons were sequenced directly using the Sanger method (Bio-Protection Research Centre, Lincoln University, New Zealand), with the PCR primers used for the initial amplification.

#### Nuclear Elongation Factor 1 alpha (EF-1α)

A 240 bp Elongation Factor-1 α PCR amplicon was produced initially according to the method of Hall et al. (2016) using the primers PSEF1aF (CAGTACCTGTTGGTCGTGTTGAGAC) and PsEF1aR (ACGACGRTCACAYTTTTCTTTGATC), specifically designed for that study. However, the PCR cycle was then modified to improve amplification success using an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min. After confirming the quality of the product by electrophoresis, the amplicons were sequenced directly.

**Other phylogenetic markers tested**

In order to test additional genes as psyllid phylogenetic markers, two nuclear genes, CAD (primers: PsCADF, CGTATGGTAGATGAAAGTGT and PsCADR, AATTTGTTTGWGCAGGATAYTCTGC) and *wg* (primers: PswgF, ACATGYTGGATGAGAYTACCA and PswgR, TCTTGTGTTCTATAACCACGCCCAC), were tested according to the protocols used on Australian species (Hall et al. 2016).

### 4.2.2 DNA sequence and phylogenetic analysis

DNA sequences were quality-checked by manually scanning electropherograms, before edited/trimmed reads were assembled into consensus DNA sequences for each gene using MEGA version 6 (Tamura et al. 2013). When analysing EF-1α, exon sequences were identified by alignment with intron-less psyllid sequences and then compared to the reference sequence of *Drosophila melanogaster* (Hovemann et al. 1988) in order to assess the intron position.
Alignment of the COI (456 sequences, Chapter 3), 18S and EF-1α DNA sequences from each specimen were performed using MEGA version 6 (Tamura et al. 2013). Neighbour Joining [NJ] and Maximum likelihood [ML] phylogenetic trees were then constructed for each gene using the Kimura-2-parameter [K2P] model with a bootstrap of 10,000 replicates (Appendix C).

Multigene trees were developed using the Bayesian inference in BEAST v2.4.6, with the Markov Chain Monte Carlo (MCMC) method (Drummond et al. 2012) and 1 billion replicates used for both the two-gene species tree (COI, 18S) and a three-gene species tree (COI, 18S and EF-1α). The Generalised time-reversible [GTR] model was used and the different datasets were not concatenated into a single sequence so that different evolutionary rates could be selected for mitochondrial [COI] and nuclear genes [18S – EF-1α]. The software Tracer v1.6 (Rambaut et al. 2004) was used for visualization and diagnostics of the MCMC output, while Tree Annotator (Drummond et al. 2012) was used to summarize the information in a sample of trees produced by BEAST and to set a 10% burn in based on the information visualized with Tracer. Multi-locus phylogenetic trees were drawn using FigTree v1.4.3 (Rambaut 2016).

### 4.2.3 Host plant phylogeny

Information on the phylogenetic relationships of the New Zealand plants have been obtained from the Landcare Research database, Phylogeny of New Zealand Plants, available online (http://plantphylogeny.landcareresearch.co.nz/WebForms/Home.aspx) (Wagstaff et al. 2004). All the psyllid-host plant association reported here follow the host plant definition reported in Chapter 1 (Burckhardt et al. 2014) and are either obtained from the literature [e.g. (Ferris and Klyver 1932,

Tuthill 1952, Dale 1985)] or, for the newly reported taxa, from the host psyllids were located on Table 3.1 (Chapter 3).

## 4.3 Results

### 4.3.1 Multi-gene phylogenetic trees confirm established species and reveal taxonomic misclassifications plus cryptic diversity

In addition to the 443 COI sequences generated in Chapter 3 (Accession numbers MF197452-56; MF197458-72; MG132221-630), ribosomal 18S DNA sequences (Accession numbers MG195288-460) were obtained from a total of 173 specimens, representing 89 of the 90 previously delimited taxa (Table 3.1). This included specimens of *Acizzia solanicola*, for which the COI fragment could not be amplified, but did not include *Anomalopsylla* "Pollen island" for which the partial 18S rRNA gene could not be amplified. The phylogenetic tree constructed using the COI sequences clearly separated the taxa at the tips of the branches for species (Appendix C.1), but failed to resolve genera and families satisfactorily (bootstraps were under 50%). In contrast, the 18S tree resolved taxa at the family level, but support for nodes at a species level were very poor (bootstrap values under 10 %) (Appendix C.2).

A two-gene phylogenetic tree was inferred from a combination of the COI and 18S DNA sequences, which resulted in a tree with strong support for resolution of species, genera and families (Figure 4.1). For the most part, psyllids fell within their expected families (Burckhardt and Ouvrard 2012). Thus the Psyllidae comprises of the genera *Psylla, Acizzia, Baeopelma,* and *Arytainilla*; the Triozidae includes the genera *Bactericera, Trioza, Casuarinicola* and the Triozid genus on *Casuarina*; the Homotomidae and Calophyidae comprise of the genera *Mycopsylla* and *Calophya,* respectively, although only single species were present to represent these two families (Figure 4.1). The family Aphalaridae includes the genera *Ctenarytaina, Blastopsylla, Cardiaspina, Cryptoneossa, Anoeconessa, Creiis, Glycaspis,* and *Eucalyptolyma.* Moreover, the accepted subfamily separations (Burckhardt and Ouvrard 2012) were resolved within the Psyllidae, separating the Acizzinae (genus *Acizzia*) from the Psyllinae (genera *Psylla, Arytainilla* and *Baeopelma*). On the other hand the separation between Spondyliaspidinae and Rhinocolinae, within the Aphalaridae, was not resolved due to the position of the genus *Anomalopsylla* (Rhinocolinae) (Figure 4.1). In contrast to all other genera, *Anomalopsylla* and *Atmetocranium* did not conform to previous morphological classifications that had placed them in the family Aphalaridae and Calophyidae, respectively (Burckhardt and Ouvrard 2012). *Atmetocranium* clustered here within the family Aphalaridae, branching earlier than the genus *Ctenarytaina,* while *Anomalopsylla* fell outside this family, in a deep

position in the phylogeny, with no clear affinity for any other psyllid group, and appearing the most archaic genus present in New Zealand.

The *Ctenarytaina* and *Psylla* species formed monophyletic clades (Figure 4.1). The most closely related psyllids to the New Zealand *Ctenarytaina* were *Ctenarytaina* spp. from Australia. The earliest branch within the New Zealand *Ctenarytaina* was between *Fuchsia* and Myrtaceae hosted species. However, the single *Ctenarytaina* taxa collected from *Olearia* (*C.* sp. A), Asteraceae, branched between the *Fuchsia* and the Myrtaceae groups. New Zealand *Psylla* species did not have close relatives in the phylogeny from the same genus. The closest genera were *Arytainilla* and *Baeopelma,* from the same subfamily. No *Psylla* species from outside of New Zealand were included.

All but one of the *Trioza* species known to be endemic to NZ were clustered in a single clade (Figure 4.1). While the closest non New Zealand relatives to this clade were two *Bactericera* species that formed a monophyletic lineage. The endemic *T. curta* from Myrtaceae in New Zealand formed a monophyletic association with *T. eugeniae* which is an Australian psyllid that has recently colonised New Zealand. Beside *T. eugeniae,* all of the Australian triozids were substantially more distant to this main New Zealand clade. A large number (11 species) of the New Zealand *Trioza* from *Olearia* (Asteraceae) formed a closely related cluster. Other species of *Trioza* from Asteraceae, *T. "*Omahuta" from *Brachyglottis* and *T. acuta* from *Ozothamnus* also clustered within this clade. The two New Zealand *Trioza, T. colorata* and *T. dacrydii,* from podocarp hosts proved to be sister taxa in the phylogeny. Furthermore, the genus *Bactericera* (Triozidae), while clustering outside the New Zealand endemic species of *Trioza,* was still branching between these and the Australian *Trioza* species (plus *T. curta*).

Irrespective of the 18S gene showing smaller genetic variation, a very high posterior probability value is reported here for the morphologically cryptic species of the genera *Psylla* (between 0.9 and 1) and *Trioza* (value 1 for both *T. acuta* B and *T. falcata* B) (Figure 4.1). Variable results have been recorded for the species of the genus *Ctenarytaina,* with *C. fuchsiae* A, B and C all showing a posterior probability value of 1, together with *C.* sp. A and B., and *C. clavata* D. On the other hand, lower posterior probability values are reported for the remaining *Ctenarytaina* species and are discussed below.

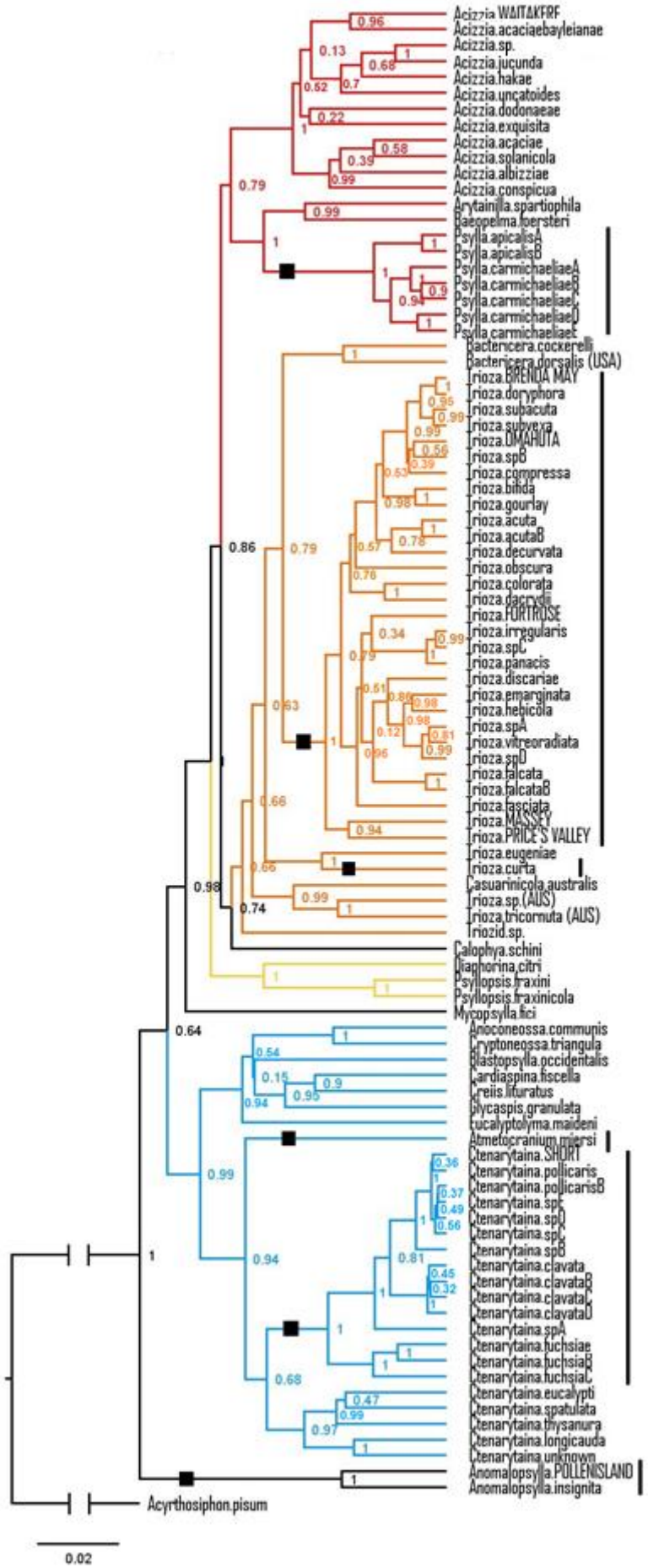
Acizzia WAITAKERE
Acizzia.acaciaebayleianae
Acizzia.sp.
Acizzia.jucunda
Acizzia.hakae
Acizzia.uncatoides
Acizzia.dodonaeae
Acizzia.exquisita
Acizzia.acaciae
Acizzia.solanicola
Acizzia.albizziae
Acizzia.conspicua
Arytainilla.spartiophila
Baeopelma.foersteri
Psylla.apicalisA
Psylla.apicalisB
Psylla.carmichaeliaeA
Psylla.carmichaeliaeB
Psylla.carmichaeliaeC
Psylla.carmichaeliaeD
Psylla.carmichaeliaeE
Bactericera.cockerelli
Bactericera.dorsalis (USA)
Trioza.BRENDA MAY
Trioza.dryophora
Trioza.subacuta
Trioza.subvexa
Trioza.OMAHUTA
Trioza.spB
Trioza.compressa
Trioza.bihda
Trioza.gourlay
Trioza.acuta
Trioza.acutaB
Trioza.decurvata
Trioza.obscura
Trioza.colorata
Trioza.dacrydii
Trioza.FORTROSE
Trioza.irregularis
Trioza.spC
Trioza.panacis
Trioza.discariae
Trioza.emarginata
Trioza.hebicola
Trioza.spA
Trioza.vitreoradiata
Trioza.spD
Trioza.falcata
Trioza.falcataB
Trioza.fasciata
Trioza.MASSEY
Trioza.PRICE'S VALLEY
Trioza.eugeniae
Trioza.curta
Casuarinicola.australis
Trioza.sp.(AUS)
Trioza.tricornuta (AUS)
Triozid.sp.
Calophya.schini
Diaphorina.citri
Psyllopsis.fraxini
Psyllopsis.fraxinicola
Mycopsylla.fici
Anoconeossa.communis
Cryptoneossa.triangula
Blastopsylla.occidentalis
Cardiaspina.fiscella
Creiis.lituratus
Glycaspis.granulata
Eucalyptolyma.maideni
Atmetocranium.miersi
Ctenarytaina.SHORT
Ctenarytaina.pollicaris
Ctenarytaina.pollicarisB
Ctenarytaina.spE
Ctenarytaina.spD
Ctenarytaina.spC
Ctenarytaina.spB
Ctenarytaina.clavata
Ctenarytaina.clavataB
Ctenarytaina.clavataC
Ctenarytaina.clavataD
Ctenarytaina.spA
Ctenarytaina.fuchsiae
Ctenarytaina.fuchsiaB
Ctenarytaina.fuchsiaC
Ctenarytaina.eucalypti
Ctenarytaina.spatulata
Ctenarytaina.thysanura
Ctenarytaina.longicauda
Ctenarytaina.unknown
Anomalopsylla.POLLENISLAND
Anomalopsylla.insignita
Acyrthosiphon.pisum
0.02

**Figure 4.1:** A Bayesian phylogenetic tree inferred from an alignment of the partial COI and 18S DNA sequences of the New Zealand psyllids. The tree was constructed using the Bayesian Inference in the phylogenetic software BEAST v2.4.6 (Drummond et al. 2012). Different colours were used for each family: Psyllidae (red), Triozidae (orange), Calophyidae (*Calophya schini;* white), Liviidae (Yellow), Homotomidae (*Mycopsylla fici,* white) and Aphalaridae (blue). Nucleotide sequences from *Acyrthosiphon pisum* (Hemiptera), the pea aphid, were used as an out group. The black squares on six of the tree branches indicate the separate ancestral arrivals inferred from the tree, while the black line on the right of the tree marks the New Zealand endemic species. An enlarged version of the tree is available in Appendix C.

Additional nuclear genes were tested for their suitability as markers for use in the multi-gene phylogeny. Of the markers tested, *wg* and CAD produced no PCR amplicons irrespective of attempts to optimise PCR cycling conditions. In contrast, a partial EF-1α PCR product was amplified, although the size of the PCR amplicon varied indicative of the presence of introns in some psyllid families (Figure 4.2a). PCR amplicons of EF-1α from *Atmetocranium myersi* and for all species of the Aphalaridae had no introns (Red arrow, Figure 4.2b), while PCR from two individuals of the family Calophyidae produced two products, both of a different size from the expected ~300bp amplicon in Aphalaridae (Blue arrow, Figure 4.2b; numbers 25-26, Figure 4.2a). The second band recorded for the samples 1 and 2 in Figure 4.2a was sequenced and confirmed to be a contaminant and thus discarded.

The isolation of a partial EF-1α DNA sequences was attempted from a subset of specimens including all the genera studied in this thesis. Only a subset of 17 genera and 21 psyllid species (Accession numbers KY983256-72; KY983275-77) produced amplicons suitable to construct a phylogeny based on the three genes EF-1α, 18S, COI (Figure 4.3a). This tree provided important information on the positions of the genus *Anomalopsylla* and the species *Atmetocranium myersi,* especially when compared to the two-genes tree (COI+18S, Figure 4.3b) obtained from the same dataset. The number of samples analysed here included ten genera belonging to the family Aphalaridae, providing a set of additional information on this group. As described previously, the COI-18S tree placed the species *Atmetocranium myersi* within the family Aphalaridae while the genus *Anomalopsylla* clustered outside of this family. These placements were in disagreement with the taxonomic classification of these psyllids using morphological data (Burckhardt and Ouvrard 2012), which placed *Atmetocranium* in the family Calophyidae and the *Anomalopsylla* in the Aphalaridae. The addition of nucleotide sequences for EF-1α, generated a tree confirming the newly recorded position of *Atmetocranium* while the position of *Anomalopsylla* was consistent with that inferred by morphological taxonomy. In fact, the position of *Anomalopsylla* in this tree, within the Aphalaridae but separated from the other genera, is consistent with the most recent subfamily

classification (Burckhardt and Ouvrard 2012), separating Rhinocolinae (*Anomalopsylla*) from Spondyliaspidinae (all the other genera included in this work).

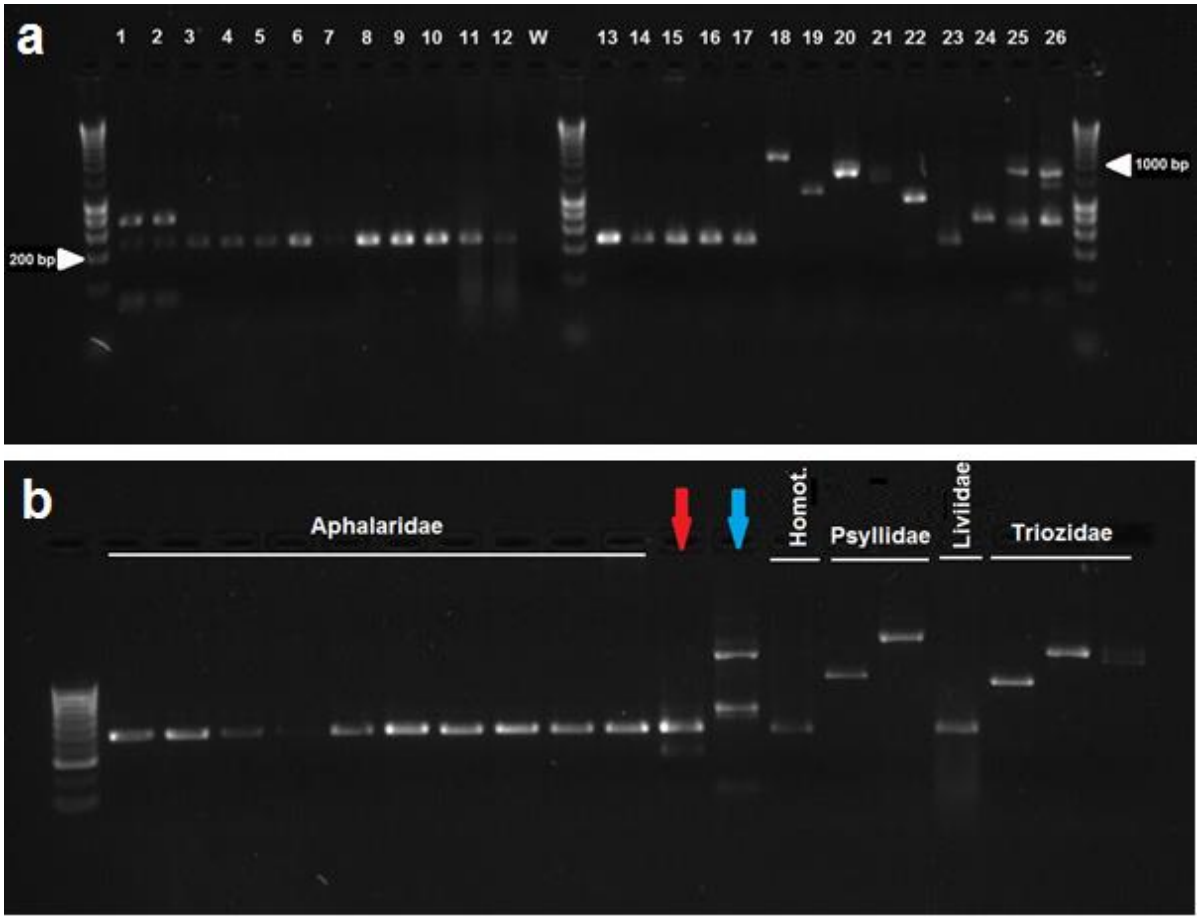


**Figure 4.2:** Electrophoresis gels (1% agarose) showing separation of EF-1α PCR products amplified from DNA of various psyllids. Fragment size was estimated by comparison to the Hyperladder I molecular weight standard (Bioline Reagents, London, UK). (a) A gel showing the presence of single DNA bands, indicative of an EF-1α fragment without introns, from specimens belonging to genera of the family Aphalaridae (1-15), Liviidae (23) and Homotomidae (16-17). Larger fragments indicative of the presence of introns were detected in amplicons from the families Psyllidae (18-19), Triozidae (20-22) and Calophyidae (25-26). (b) *Atmetocranium myersi* (red arrow) and *Calophya schini* (blue arrow), that were presumed to belong to the same family, show a clear variation in fragment size, with *Atmetocranium* showing the same size as Aphalaridae, Homotomidae and Liviidae.

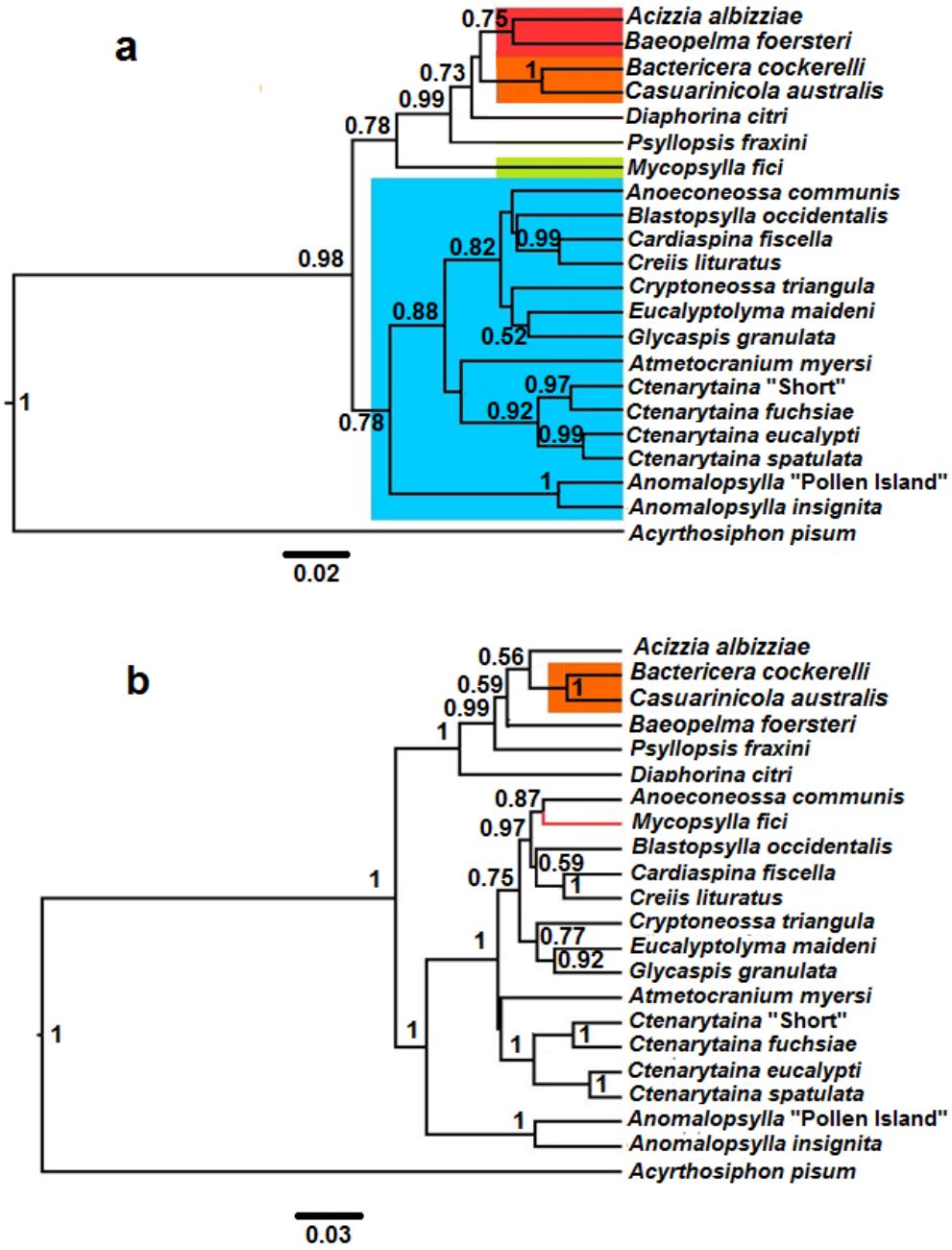


**Figure 4.3:** A phylogenetic Bayesian inference tree highlighting the position of the taxa *Anomalopsylla* and *Atmetocranium* within a group of New Zealand psyllids using a COI and 18S (b), and adding EF-1α (a). The trees were constructed using BEAUti and the BEAST (Drummond et al. 2012). Psyllidae (red), Triozidae (orange), Homotomidae (green) and Aphalaridae (blue). Posterior probability values were provided at the nodes. The aphid *Acyrthosiphon pisum* was used as the outgroup. The scale bars show genetic distances of 0.03 (a) and 0.02 (b). The taxon *M. fici* is reported in red in Figure a, to highlight that the position reported invalidates the cluster of the family Aphalaridae, which is appropriately delimited in Figure b.

### 4.3.2 Mapping psyllid species to their host plant phylogenies

Adding the new species recorded in Chapter 3 to those previously reported in the checklist, a total of 120 species are recorded in New Zealand. Of these, 84 species, belonging to six genera and three families are considered endemic to New Zealand. This totals 55 species of native *Trioza* (Figure 4.4, red) distributed amongst 13 host plant families, 16 species of *Ctenarytaina* (Figure 4.4, green) associated with three host plant families, while *Psylla* (Figure 4.4, blue), *Anomalopsylla* (Figure 4.4, yellow), *Gyropsylla*, *Atmetocranium* and the Genus "B" (Figure 4.4, orange) are all restricted to a single host plant family. Based on the results in Figure 4.1, the radiations of the different genera do not appear to be linked to a longer time since their arrival to New Zealand. In fact, the genera *Atmetocranium* and *Anomalopsylla* appear to branch earlier than the monophyletic group of *Trioza.* Furthermore, the genus *Ctenarytaina* seems to branch at the same depth of the phylogenetic tree as the genus *Trioza.* Nonetheless, *Trioza* is the genus showing the greater radiation in species and, from the sampling made possible here, it appears to be associated with the highest number of host plant families.

Using the results obtained from the inferred two-gene phylogenetic tree (Figure 4.1), the New Zealand native psyllid taxa collected during this study (Chapter 3) were associated with those previously reported to live in New Zealand from the literature (Chapter 2). This analysis included seven genera and 84 species of the New Zealand endemic Psylloidea (Figure 4.4).

Similar previous works associating psyllids to their host plants were usually based on a morphological identification of the psyllids [e.g. (Ouvrard et al. 2015)]. This led to doubt over the authenticity of the genus *Trioza,* which has been considered a "catch-all" genus; treated as "*an artificial receptacle for species not showing any particular morphological modifications*" (Burckhardt and Ouvrard 2012). However, the data obtained here suggest otherwise for the radiation of this genus in New Zealand; more robust delineation of taxa consistent with species-level separations, made possible here with the addition of genetic data, indicates that this is not due to *Trioza* being a "catch-all" genus and it is not linked to the speciation time of the genus. Therefore, the association of this genus with such a large number of host plant families and genera may be investigated further with the phylogenetic information obtained in this chapter.

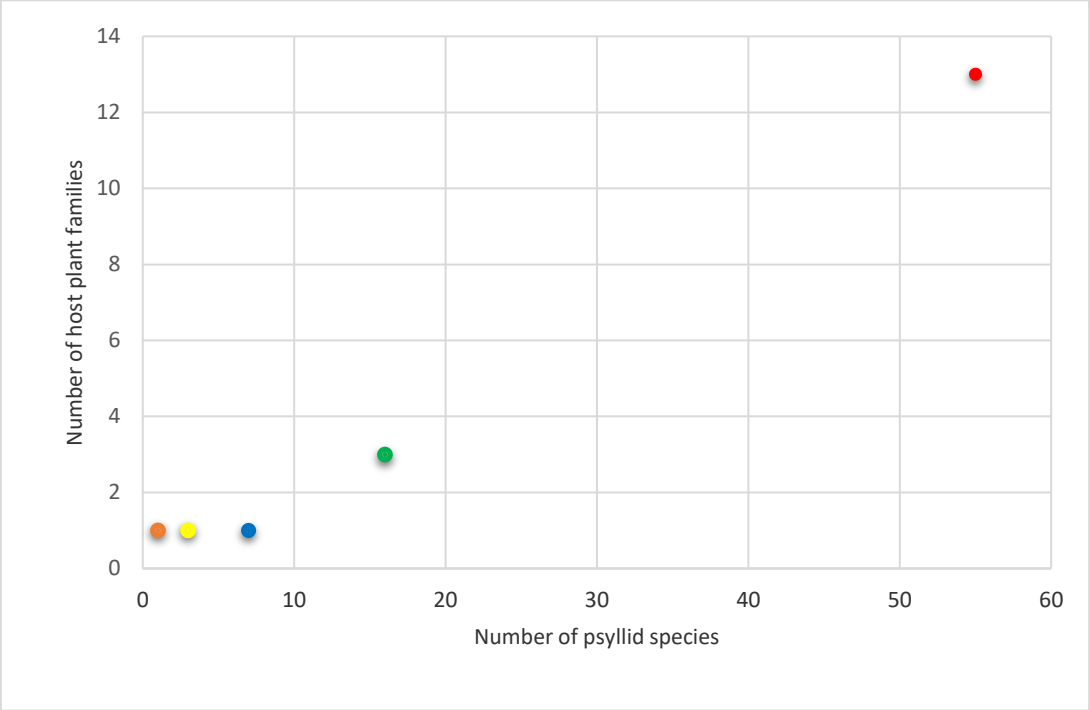


**Figure 4.4:** The relationship between the number of all known New Zealand native psyllid genera and the number of host plant families. The genus *Trioza* [55 species] is represented in red, *Ctenarytaina* [16] in green, *Psylla* [7] in blue, *Anomalopsylla* [3] in yellow and *Atmetocranium* [1], *Gyropsylla* [1] and Genus "B" [1] are overlapping in orange. This highlights how the genus *Trioza* was able to colonize a higher number of host plants compared to the other native genera.

Using the results obtained from the inferred two-gene phylogeny (Figure 4.1), the New Zealand native psyllid taxa (Chapter 3) were associated with host plant information obtained both from published records (Chapter 2) and from those plants on which they were collected here. This included five genera and 56 species of the New Zealand endemic Psylloidea and enabled a more detailed understanding of endemic psyllid-host plant associations in New Zealand when overlaid with the information on plant host (Figure 4.5).

In particular, the comparison confirmed that although the New Zealand Psylloidea have a broad host range overall (including the family Podocarpaceae), only two plant families, Myrtaceae and Asteraceae, are hosts to multiple psyllid genera. Furthermore, the genus *Olearia,* within the Asteraceae, is host to psyllids belonging to two families and three genera: *Trioza, Ctenarytaina* and *Anomalopsylla.*

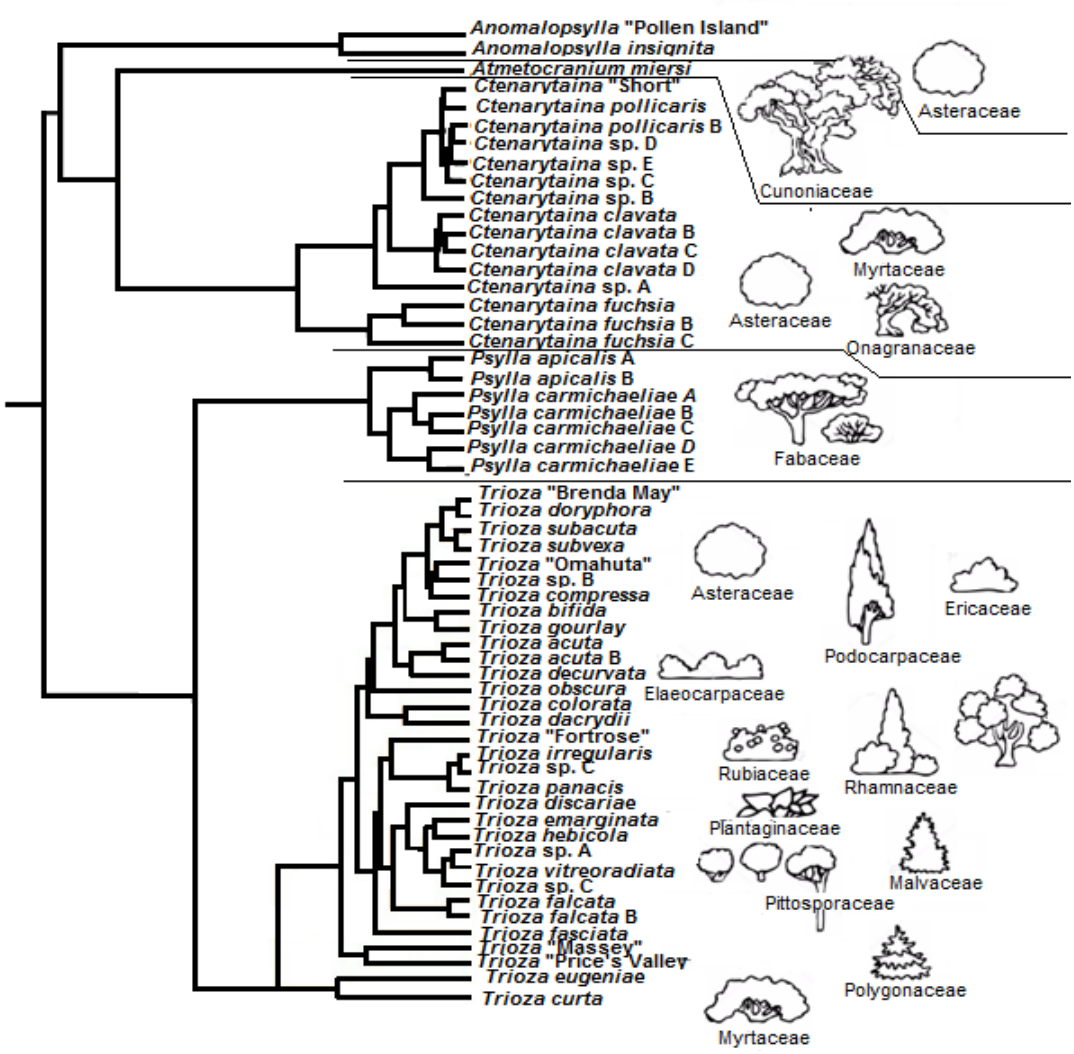


**Figure 4.5:** A graphical representation showing the association of host plant families [on the right] with the phylogenetic tree inferred from the partial COI and 18S DNA sequences of New Zealand native Psylloidea collected in this study [on the left]. Refer to Figure 4.1 for genetic distances. The lines on the right separate the five psyllid genera analysed.

Focusing on the most speciose and broadest host-range group of the New Zealand *Trioza*, this two-gene phylogeny indicates almost all of the New Zealand *Trioza* have come from one introduction (Figure 4.1 and 4.6). This confirms that the adoption of phylogenetically distinct host plants has occurred during the radiation process in New Zealand (Figure 4.6). For example, the closest relatives of *T. decurvata* (Ericaceae) are all found on Asteraceae. Moreover, the presence of two species (*T. colorata* and *T. dacrydii*) associated with Podocarpaceae, the only known psyllids on Gymnosperm hosts, highlights a host switch that was followed by speciation to produce two species that can now be found on the same individual host plants (in blue in Figure 4.6).

Moreover, branching basally to the other New Zealand *Trioza,* is recorded a first lineage composed of *T.* "Massey" and *T.* "Price's Valley", hosted respectively by Asteraceae and Malvaceae

(Figure 4.6). Within the remaining 28 *Trioza* spp., a cluster of twelve species was revealed, of which eleven were hosted on Asteraceae (made up of three plant genera, in grey in Figure 4.6). A single species in this cluster was found on *Dracophyllum* (Ericaceae, in bordeaux in Figure 4.6).

Another three species associated with Araliaceae (*T. irregularis, T. panacis* and *T.* sp. C) clustered together (in light blue in Figure 4.6). The remaining 11 *Trioza* species studied here, had a great diversity of hosts, across eight plant families. The presence of these separate groups shows how, even within the same genus, psyllid-host plant associations can be heterogeneous, indicating that some lineages are more prone to switch hosts while others are limited to a single host plant family. In general, however, related psyllid species often have related hosts. This is the case of 11 of the Asteraceae-feeding species, the three species feeding on Araliaceae, the two species feeding on Pittosporaceae and the two species feeding on Podocarpaceae.

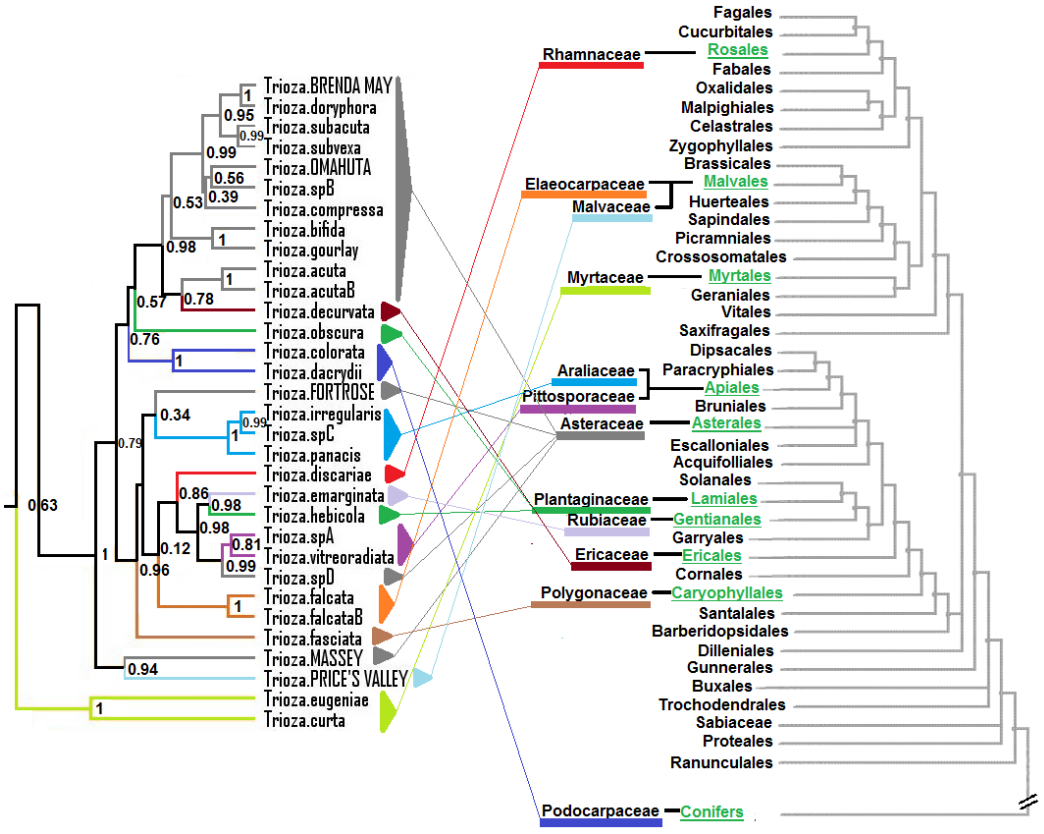


**Figure 4.6:** Association between the phylogeny of the New Zealand native *Trioza* species recorded in this study (COI-18S Bayesian Inference tree from Figure 4.1, left) and the phylogeny of their host plant orders and families (Landcare Research database, right). The 32 species of *Trioza* are hosted by 12 different host plant families and 10 plant orders (in green).

## 4.4 Discussion

### 4.4.1 A multi-gene phylogeny for New Zealand psyllids was difficult to construct using existing genetic markers

Phylogenetic analyses using multiple genetic markers enhance inferences of relationships between taxa. Within the Hemiptera, for example, the estimation of the divergence time of the Heteroptera, was obtained by combining the nucleotide sequences of four genes (Li et al. 2012). Thus, to examine psyllid evolution in New Zealand in more detail several genetic markers were evaluated for their compatibility with the COI DNA sequence data generated in Chapter 3. Unfortunately, neither *wg* nor CAD could be amplified from DNA of specimens despite using published primers and PCR cycles (Hall et al. 2016). It is not quite clear how results using same primers and PCR cycles could be obtained in previous works (Hall et al. 2016), especially considering that one of the species analysed was the same (*Cardiaspina fiscella*). A possible explanation could be the use of a different polymerase enzyme. While multiple PCR cycle regimes were trialled in this study, none produced measurable amplicons, so any future work to include these loci would require the design and optimisation of new primers and/or the adoption of a different polymerase.

A partial sequence of the EF-1α gene was amplified using DNA from a selection of specimens and contributed to the results obtained from the other two genetic markers (discussed below). The generic utility of this region was undermined by likely priming-site sequence variation, however, which led to a lack of amplification from some specimens as well as the presence of introns in some species tested. These issues suggested that a different region of the EF-1α should be targeted, which might exclude intron sequences. However, the presence of introns has been used as a novel diagnostic marker elsewhere [e.g. (Simon et al. 2010)] and, should the need be apparent, could be considered as a rapid non-sequencing diagnostic for some psyllid species as indicated here.

The partial 18S gene was amplified from DNA of 89 of the 90 psyllid taxa tested. This almost ubiquitous amplification was consistent with the results of Wilson (2010) on Lepidoptera, demonstrating the suitability of this DNA region as a genetic marker for evolutionary studies of psyllids. The placement of taxa in the resulting Bayesian inference tree constructed using the partial 18S DNA and COI gene sequences were consistent with the species delimitations using the integrative taxonomy approach (COI, morphology, and host plant) described in Chapter 3. Most importantly, the support for the deeper branches within the trees was enhanced relative to the COI gene tree (which had greatest bootstrap support at the tips of the branches), demonstrating that the 18S marker was highly complementary to the COI gene and enabled a more robust assessment of higher level taxonomic relationships among psyllids.

### 4.4.2 A two-gene phylogeny confirmed previous taxonomic placements of the New Zealand psyllids

Considering the taxonomy of psyllids has been limited by cryptic and complex morphology and host range [(Burckhardt and Ouvrard 2012); Chapter 3], the genetic data produced here proved invaluable in confirming previous taxonomic placements of New Zealand psyllids. The COI-18S phylogenetic tree, although comprising data from only two gene regions, clearly distinguished all six psyllid families in New Zealand consistent with the most recent taxonomic classification of Psylloidea (Burckhardt and Ouvrard 2012). For example, *Diaphorina citri* clustered within the family Liviidae together with species of the genus *Psyllopsis*, rather than previous proposals that placed it in the family Psyllidae (Gullan and Martin 2003). The COI-18S-based phylogenetic analysis also confirmed the placement of the species identified in Chapter 3 using the integrative taxonomy approach. For example, within the genus *Trioza,* both *T. falcata* A and B, and *T. acuta* A and B, were separated with a nodal support of 1. Similarly, the *Trioza* species A, C and D had good support [between 0.81 and 0.99]. The only exception appeared to be *T.* sp. B, that had a lower nodal support [0.56] but a longer branch.

Within the genus *Ctenarytaina,* nodal support for the species proposed was more variable. The three species comprising the *C. fuchsiae* complex were separated with the maximum posterior probability, whereas some of the species of the *C. clavata* complex were supported by probabilities lower than 0.50. The variation in both nodal support and branch length for these taxa was expected as the mānuka and kānuka groups were clearly the two complexes showing the highest cryptic variation in Chapter 3. However, the combination of morphology (Chapter 3 and Appendix A), distribution (Chapter 3) and COI variation (Chapter 3 and Appendix C) suggested that an ongoing radiation was the source of the cryptic variation in these taxa.

Of the genera that did not cluster as expected in the phylogeny, the *Bactericera* branched outside the endemic New Zealand *Trioza* species (as expected), but between these and the *Trioza* species endemic to Australia. If New Zealand and Australian *Trioza* are actually species belonging to the same genus, *Bactericera* clearly cannot branch within another genus. Therefore, this apparently incorrect clustering might be explained by the low number of representative species and samples of the genus *Bactericera* included in the analysis and/or the very long branches that resulted in the low Bayesian inference values for the nodes separating these taxa.

However, another possible explanation could be that the long-lasting hypothesis that the genus *Trioza* is actually a “catch-all” genus and that not all the species currently ascribed to it belong to the same genus is true.

### *Anomalopsylla* and *Atmetocranium*

The position of the genera *Anomalopsylla* and *Atmetocranium* in the phylogeny were also unexpected, based on the latest classification of the Psylloidea (Burckhardt and Ouvrard 2012).

**The genus *Anomalopsylla*** is composed by small psyllids presenting a number of morphological peculiarities such as crossveins in the forewing and absence of merachantus (Tuthill 1952). The genus, erected in 1952 by Tuthill, is putatively composed of five species; the only described species is *A. insignita* Tuthill, 1952, there are then two undescribed New Zealand species on *Olearia* (Dale 1985) and two undescribed Australian species on *Geijera* (Hollis 2004). Tuthill tentatively put this genus together with the South American genus *Tainarys* Brèthes, but also suggested a possible future erection of a new subfamily (Tuthill 1952). Later, Vondráček (Vondráček 1963) erected the subfamily Anomalopsyllinae within the Spondyliaspididae for the genera *Anomalopsylla, Tainarys* and *Phytolyma; Apsylla* was then added to the subfamily ten years later (Bekker-Migdisova 1973). The genus *Anomalopsylla* has most recently been placed in the subfamily Rhinocolinae within the Aphalaridae (Burckhardt and Ouvrard 2012). In this study, the two species clustered together, but were separated by significant genetic distance. Furthermore, the COI+18S tree clustered *Anomalopsylla* outside of the Aphalaridae with no obvious affinity for any other psyllid families (posterior probability = 1) consistent with the significant morphological differences associated with the genus (Tuthill 1952, Dale 1985).

To better resolve the positions of *Anomalopsylla*, EF-1α was amplified. In conjunction with the COI and 18S DNA sequences, EF-1α sequences generated a tree that supported the placement of *Anomalopsylla* within the Aphalaridae. While aware of the limitations of the present dataset, the anomalous morphology that gives the name to the genus *Anomalopsylla*, together with the analysis conducted here, suggested there is good reason to question the position of this genus in existing taxonomic classifications. This may even be stretched to the future erection of a new family. More genes plus additional species of Rhinocolinae and other subfamilies, such as the Aphalarinae (e.g. species of the Australian genus *Aphalara*) will be crucial to confirm the hypothesis either way.

***Atmetocranium myersi,*** a gall forming psyllid hosted by *Weinmannia racemosa*, has "*highly autapomorphic morphology which makes it difficult to relate to other psylloid groups*" (Mifsud and Burckhardt 2002), such as the surprisingly long ultimate rostral segment of the antennae (Heslop-Harrison 1960). The only species within the New Zealand endemic *Atmetocranium* genus was provisionally placed in the family Calophyidae, mostly because of its distinctive metatibia, which have an internal comb of apical spurs, and the segmented symmetric larval antenna (Burckhardt and Ouvrard 2012). However, Calophyidae is represented by at least 118 taxa and 11 genera (Ouvrard

2017), but it has no other representatives native to New Zealand. In the two-gene phylogeny generated here, *A. myersi* clustered separately from the only other calophid species, *Calophya schini* Tuthill. Instead, *A. myersi* clustered with the Aphalaridae, falling in the middle of the clade. Indeed, an affiliation between *Atmetocranium* and the family Aphalaridae was proposed previously based on wing morphology (Klimaszewski 1964), and the tribe Atmetocranini was erected in 1973 within this (modern concept of) family (Bekker-Migdisova 1973).

The position of *Atmetocranium myersi* within the Aphalaridae, while in contrast to the present taxonomic classification, was confirmed by the results of both the COI-18S and the COI-18S-EF-1α phylogenetic trees. Its position within the Aphalaridae appears less controversial and well supported than that of *Anomalopsylla*. In particular, the results suggest that the earlier placement of *Atmetocranium* in Aphalaridae (Dale 1985) was correct. In contrast, while the data also supported the idea that the New Zealand Aphalaridae comprised two subfamilies [the Spondyliaspidinae (consisting of *Ctenarytaina* and all the Australian genera) and the Rhinocolinae (*Anomalopsylla*)], *Atmetocranium* did not appear to belong to either. Thus, an analysis of a wider pool of species and genera as well as taxa within the three subfamilies of the Aphalaridae not represented in this data, would be necessary to determine if *Atmetocranium* should be assigned to any of the existing groups or if the present subfamily (Atmetocraniinae) should be maintained and moved within the Aphalaridae.

In summary, the use of multiple genes to construct the phylogeny of the New Zealand psyllids enabled many taxonomic classifications and relationships to be confirmed or proposed. The phylogenetic and taxonomic information obtained here form a fundamental tool that can be used for psyllids evolutionary analyses and future microbiome works.

### 4.4.3 Phylogenetic suport for multiple arrivals of ancestral psyllids into New Zealand

Previous work on the origin and evolution of the New Zealand psyllids (Dale 1985) discussed skeptically the possibility of a psyllid dispersal into this country. The presence of common genera (both of psyllids and their host plants) in New Zealand, New Caledonia, Australia and South America was regarded as a possible explanation for a Gondwanan origin for these insects (Dale 1985). This interpretation was probably influenced by the view that the New Zealand native biota was primarily a product of long-standing geographical isolation resulting from the Gondwanan split (~80 Mya) (Goldberg et al. 2008). If New Zealand had been isolated since 80 Mya, however, it would be expected that an ancient biota with high diversity would have evolved, and would show complex co-evolutionary associations, endemicity at deeper taxonomic/phylogenetic levels and a more complete

faunal composition (Goldberg et al. 2008). Instead, New Zealand's fauna is more similar to that of other Pacific Islands (Quammen 1996, Gibbs 2006). This is consistent with the 'Oligocene Drowning' hypothesis of Cooper and Cooper (1995), which states that by the late Oligocene New Zealand's land area had decreased to approximately 15% of its current land area (Fleming 1979, Cooper and Cooper 1995, Landis et al. 2008, Scott et al. 2014). This reduction in land area caused a bottleneck in vertebrate lineages that can be observed in the post-Oligocene radiations of some vertebrate groups (Cooper and Cooper 1995, Bunce et al. 2009).

In this study, the COI-18S-based phylogeny separated adventive and endemic species at various nodes in the tree (e.g. within the genus *Ctenarytaina*), suggesting that New Zealand's psyllid population was a result of at least six independent arrivals. The time of these arrivals was not discussed here, mostly because of the limited information on the evolutionary history of psyllids (discussed further in Chapter 6).

### Arrivals of *Anomalopsylla* and *Atmetocranium*

The position in the phylogenetic tree of *Anomalopsylla* and *Atmetocranium* confirmed that these genera arrived separately into New Zealand and that they were distinct genera. In fact, when considering the position of *Anomalopsylla* (in the two-gene tree), its separation from any other family suggested a very ancient origin. This led to the hypothesis that *Anomalopsylla* was a relic from Gondwanan times, before New Zealand separated from the other land masses (Australia, ~80 Mya and New Caledonia ~55 Mya). This hypothesis would explain both the genetic distance and the anomalous morphology of the genus, although testing of this hypothesis would require future dating of the arrivals (as discussed in Chapter 6).

### Arrivals of *Ctenarytaina* and *Psylla*

The *Ctenarytaina* and *Psylla* genera hosted by native New Zealand plants were monophyletic. Therefore, in each of these cases, a single colonisation event was followed by species radiation in New Zealand.

Within the native *Ctenarytaina* clade, the earliest branch separated the species hosted by *Fuchsia excorticata* (Onagraceae) from the species on *Olearia* (Asteraceae) (sp. A) and Myrtaceae (the mānuka-kānuka complex). Considering all of the Australian *Ctenarytaina* species were hosted by plants in the family Myrtaceae (Ouvrard 2017) and all of the adventive *Ctenarytaina* psyllids in New Zealand were Australian species (Martoni et al. 2016), the ancestral New Zealand *Ctenarytaina* probably arrived by wind from Australia and subsequently diverged onto different host(s), such as Onagraceae. Such an event would suggest a major host switch at the base of the New Zealand radiation of this clade, jumping from the Australian Myrtaceae onto the New Zealand Onagraceae and even further, onto Asteraceae.

In contrast to the evolution of the *Ctenarytaina* in New Zealand, radiation from the ancestral *Psylla* appears to have occurred only recently. Furthermore, while *Ctenarytaina* arrived from across the Tasman Sea, no *Psylla* species have been detected in Australia (Hollis 2004, Ouvrard 2017). Instead, they are widely distributed throughout the remainder of the world, with the closest location to New Zealand being Fiji [*P. compta* Crawford, 1919 (Ouvrard 2017)]. This distribution of *Psylla* suggested an alternative origin for native *Psylla*, although a comparison of New Zealand *Psylla* with those from other countries (such as Fiji) would be required in future to confirm this theory. Unfortunately, no additional information can be extrapolated by the host plant association of the Fijian psyllid, since this psyllid's host plant is still unknown (Ouvrard 2017). Future phylogenetic studies including sequences for *P. compta* and other Fabaceae-hosted species from other countries could clarify the origin of the New Zealand *Psylla* species and, therefore, their evolution. Overall, combining the host plant associations and molecular data of the *Psylla* species led to two main considerations. Firstly, that an allopatric speciation, such as that proposed for the neotropical and often polyphagous species within the genus *Russelliana* (Psyllidae) (Serbina and Burckhardt 2017) could be excluded. In fact, different species co-occur in close proximity but hosted by separate plants. At the same time, however, a process of co-speciation (Figure 4.7) or co-evolution between psyllids and broom (Fabaceae) was unlikely given the ancient radiation of the New Zealand native broom dating back to the Tertiary (Wagstaff et al. 1999). Ultimately, the data collected here is not sufficient to formulate a solid hypothesis, but further analyses including a wider number of *Psylla* species may enable a better understanding.

### Phylogenetics revealed two introductions of ancestral *Trioza*

The division of endemic *Trioza* into two clades within the COI-18S-based phylogenetic tree was indicative of two arrivals of ancestral triozids into New Zealand, one leading to *Trioza curta* and the second leading to all other endemic species. *Trioza curta* is the only native New Zealand triozid hosted on *Metrosideros*, which belongs to the family Myrtaceae. Interestingly, *T. curta* showed a robust affiliation with *T. eugeniae*, an Australian species that recently colonised New Zealand and is hosted by *Syzygium* (Myrtaceae) (See Chapter 3). This affiliation would suggest perhaps a common ancestor on Myrtaceae that switched host upon arrival into New Zealand or the existence of an Australian triozid on *Metrosideros*. But there may be an alternative explanation given there are no *Trioza* known to be associated with *Metrosideros* in Australia, and in fact *Metrosideros* does not occur on the Australian continent. Instead, *Metrosideros*-feeding psyllids and their hosts are abundant in the Hawaiian Islands (Percy 2017) while the host plant is also present elsewhere in the Pacific (Percy et al. 2008).

Moreover, a large number of *Metrosideros*-feeding Triozidae have recently been reclassified as belonging to a new genus, *Pariaconus* (Percy 2017). A tentative comparison of the COI sequences

from *Pariaconus* [*P. gracilis* (KY293755.1 and KY293756.1)*, P. proboscideus* (KY294097.1 and KY294095.1)*, P. wyvernus* (KY294136.1) and *P. hina* (KY293816.1)] and the COI sequences of triozids from New Zealand and Australia showed a lower COI genetic distance (19%-22%) between the main clade of the New Zealand *Trioza* and *Pariaconus* species than between the New Zealand *Trioza* and the Australian species (between 23% and 24%); with *T. curta* and *T. eugeniae* in average 20% distant from *Pariaconus* and 25% distant from the Australian *Trioza*. Together, these data suggest there may be a Pacific origin for the ancestor of both *T. eugeniae* and *T. curta* than the COI-18S-based phylogenetic tree would initially imply.

The COI-18S-based tree revealed that the main clade of New Zealand *Trioza* were most closely related to the *Bactericera* species, originally from South America (Ouvrard 2017), rather than to the three Australian *Trioza* species included in the analysis. This unexpected result may have occurred as an artefact of the long phylogenetic branches and the limited selection of *Bactericera* taxa in the dataset. Indeed, it is possible that other Australian species not sampled, such as *T. oleariae* Froggatt 1903, would be closer relatives to the New Zealand species and would contribute to separating them from *Bactericera*. Supporting this explanation is the fact that *T. oleariae* is hosted by a similar plant (*Olearia*, Asteraceae) to the New Zealand *Trioza* in this clade (e.g. *T. doryphora*), and is also present in Tasmania, which is closer to New Zealand than the remainder of Australia (Ouvrard 2017). Nevertheless, the data may also imply an alternative origin for the main group of NZ *Trioza*. This hypothesis could be explored further by a phylogenetic study of *Trioza*, *Bactericera* and *Pariaconus* from the Americas, the Pacific Rim and perhaps elsewhere in the world as well as Australian triozids on *Olearia*. At this stage, little nucleotide sequence information is available for these genera, with no 18S sequence available on GenBank for species of the genus *Pariaconus.*

In summary, the phylogenetic analysis performed in this chapter enabled the detection of six arrivals of psyllids. Detection of these arrivals, relative to the large number of species there, led to the conclusion that New Zealand's psyllid diversity was not the result of a large number of introductions, but was a consequence of the radiation/evolution of relatively few ancestors. More intensive sampling of global populations will be required to establish their origin.

### 4.4.4 Species radiation within the genus *Trioza* reveals multiple evolutionary lineages and host plant associations

The Triozidae is an extremely diverse family globally, which includes 70 genera and at least 1000 species (Ouvrard 2017). Of these, the *Trioza* genus is the most numerous, with 423 species worldwide (Ouvrard 2017). This genus shows an unusually large range of associations with different plant genera, with a recent study associating 346 psyllid species on 154 plant genera in 59 plant families (Ouvrard et al. 2015). The present genus *Trioza* might include many groups and species that require review and reclassification (Burckhardt, personal communication), and the potentially polyphyletic nature of *Trioza* may distort the actual breadth of host-plant associations in a single genus (Ouvrard et al. 2015).

The new molecular phylogeny presented here now enables this debate to be better informed. Importantly it confirms that the main group of the New Zealand *Trioza* (except *T. curta*) is genetically monophyletic. Given the wide array of plants currently host to New Zealand *Trioza*, this suggests that the *Trioza* have undergone host switching many times. As an extreme example, the two closely related species *Trioza colorata* and *T. dacrydii* are found on *Halocarpus bidwillii,* a conifer belonging to the family Podocarpaceae. A gymnosperm host is rare in the Psylloidea involving only four psyllid species in two genera on three plant genera in two families of gymnosperms (Ouvrard et al. 2015, Ouvrard 2017). However, within the monophyletic group of the New Zealand *Trioza, T.* "Massey" and *T.* "Price's Valley" (respectively on Asteraceae and Malvaceae) are clearly separated from all the other species. Supporting this result, *T.* "Price's Valley" is a specialised gall-former on *Plagianthus* (Malvaceae), described by Dale as "quite isolated from all other New Zealand species" (Dale 1985). Similarly, *T.* "Massey" has been reported to be an isolated species showing likenesses to *T. dentiforceps,* another *Olearia*-feeding species described from the Chatham Islands (Dale 1985). Unfortunately, *T. dentiforceps* could not be included in this study, but Dale's observations suggest that comparisons with this taxon may also enable an understanding of the origin of *T.* "Massey".

The other 28 endemic *Trioza* included in this study were split in two additional groups. One lineage includes the species *T.* "Brenda May", *T. doryphora, T. subacuta, T. subvexa, T.* "Omahuta", *T.* sp. B, *T. compressa, T. bifida, T. gourlayi, T. acuta* and *T. acuta* B (all from Asteraceae); *T. decurvata* (Ericaceae); *T. obscura* (Plantaginaceae); *T. colorata* and *T. dacrydii* (Podocarpaceae). This lineage shows 11 species associated with Asteraceae and another four species associated with three different host plant families. Of these, *T. decurvata* (from *Dracophyllum*) was described by Dale (1985) as "not being closely related to any other group". The results obtained here, however, suggest a recent separation from the Asteraceae-feeding *Trioza*. When assessing the morphology of *T. obscura*, Dale hypothesised a shared ancestor between this psyllid and *T. colorata* based on the

wing form (Dale 1985). The results obtained here are consistent with this hypothesis, suggesting that both *T. colorata* and *T. dacrydii* (hosted by Podocarpaceae) are the closest relatives of *T. obscura.* The remaining 13 *Trioza* species, associated with eight plant families, include two groups observed by Dale: those feeding on *Pittosporum, Aristotelia* and *Hebe,* and the group on Araliaceae (*Pseudopanax).*

Consequently, one hypothesis might be that the ancestral *Trioza* was associated with plants similar to those associated with the *T.* "Price's Valley"/*T.* "Massey" lineage, of the families Asteraceae or Malvaceae. In fact, this ancestral psyllid was either polyphagous or extremely prone to adaptation and invasion/colonization of new host plants. In evolutionary times, this resulted in a high number of host switches and a radiation on multiple plant families. Therefore, an improved capability to adapt to different plants could well be the key driving factor of speciation in *Trioza*. This is supported by saltationary host switching events being identified as one of the key factors for the distribution of psyllid host plants (Ouvrard et al. 2015). Despite host switching being recorded also in the aphids of the genus *Cinara* (Durak et al. 2014); the ancestors of the New Zealand triozids may have been more adaptive, compared to the ancestors of the less speciose and more host-restricted *Ctenarytaina* and *Psylla*.

The host plant associations of this New Zealand monophyletic group may raise additional doubts on the origin of the genus *Trioza,* which was previously hypothesised to be Australian based on the large number of species present on Asteraceae (Dale 1985). However, the Asteraceae-feeding psyllids were shown here to be mostly a more recent adaptation than an ancestral association, especially if considering the 11 species clustering together. Nonetheless, *T.* "Massey" (Asteraceae-feeding) clusters together with *T.* "Price's Valley", in the earliest branching lineage. This species, together with the morphologically similar *T. dentiforceps* (Dale 1985), may be key to our future understanding of origin of Asteraceae-feeding psyllids of New Zealand. In fact, the presence of *T. dentiforceps* has been confirmed so far only on the Chatham Island, a small archipelago East of New Zealand. If a future DNA analysis of this species confirms it to branch together with *T.* "Massey", at a basal position, this may lead to the hypothesis of a psyllid westward colonization of New Zealand from other Pacific Islands. Nonetheless, T. "Price's Valley", which appears to be the most ancestral species together with T. "Massey", is hosted by *Plagianthus*, a Malvaceae, that is present also in Australia, leaving open the hypothesis that these ancestral psyllids are indeed of Australian origins.

The high incidence of host switches can be explained by a predisposition of the ancestral *Trioza* in colonizing multiple host plants and subsequent isolation due to geography or climatic events, which acted as promoter of this radiation process. In fact, geographical characteristics of New Zealand territory are considered to have played a key role in insect evolution (Buckley et al. 2015). Therefore, as for many insect groups, variation in habitat (e.g. Alpine, Subalpine and Coastal)

and the geological history (e.g. Volcanic eruptions and fragmentation in smaller islands) (Buckley et al. 2015) may have contributed to the first geographical isolation (both literally and figuratively) of the ancestral psyllid populations and the first step of a subsequent relationship between the psyllids and their host plants.

### 4.4.5 The different evolutionary histories of *Ctenarytaina* and *Psylla*: an example of phylogenetic tracking.

*Ctenarytaina* and *Psylla* host plant associations are similar to many others within the Psylloidea, involving just one or a few host plant families (Burckhardt et al. 2014); the seven *Psylla* species are only found on two plant genera in the Fabaceae while the 15 native *Ctenarytaina* species are associated with four host plant genera amongst three host plant families (Onagraceae, Myrtaceae and Asteraceae). The results presented here confirmed that both the endemic *Psylla* and the endemic *Ctenarytaina* are monophyletic and the result of a single ancestral arrival each.

Of the *Psylla* species*, P. carmichaeliae* has been collected from five different plant species of *Carmichaelia*, the native broom. Elsewhere, analysis of psyllids feeding on other broom species from the Canary Islands and Madeira (Percy 2002, 2003a) suggested that they may be highly effective at tracking the phylogenetic diversification of a particular host-plant group, but that there was unlikely to be extensive contemporaneous co-speciation with their hosts (Percy et al. 2004).
Similarly, phylogenetic tracking (Figure 4.7) is a potential evolutionary process for *Psylla* spp. associated with *Carmichaelia* spp. in New Zealand.
This was described as:

"*a pattern in which speciation events in one lineage mirror speciation events in another lineage. Usually assumed that one lineage speciates first and is followed by speciation in the other*" (Althoff et al. 2014).

This is consistent here with the COI species genetic distances, being between 7% and 17% denoting a clear but recent separation, and the *Psylla* spp. not appearing to share the same plant species which, nevertheless, have a close evolutionary proximity. Thus, it is possible that the speciation and diversification events of the *Psylla* species would have followed the radiation of *Carmichaelia,* and consistent with the famous statement made by Hodkinson (1984) referring to host plants not as "*islands in evolutionary time*" but as "*the rafts transporting the insects down the river of evolutionary time*".

Similarly, in the Canary Islands, the structure of metapopulations of psyllids and the variation and fragmentation of their leguminous host plants is today considered to be an example of sequential speciation of these insects following the host plant speciation (Percy et al. 2004) and not a

coevolution as initially hypothesised (Percy 2003b). In fact, the hypothesis of detecting coevolution at a microscale has been strongly debated [(Suchan and Alvarez 2015) and references therein], with the alternative hypotheses of phylogenetic tracking (Althoff et al. 2014) or sequential evolution (Jermy 1976) preferentially supported. Therefore, when comparing the phylogenies of insects and plants, phylogenetic patterns can only determine whether partner fidelity or host switches are associated with diversification and speciation (Suchan and Alvarez 2015).

Potential evolutionary mechanisms for the New Zealand endemic *Ctenarytaina* are not as easily narrowed down as they are for the *Psylla*. *Ctenarytaina* occur on four host plant genera (*Fuchsia, Kunzea, Leptospermum* and *Olearia*), with multiple species on a single host plant genus (and a single species, "sp. A", on *Olearia*). Phylogenetic analysis here indicates three closely related "species-clusters" comprising *Ctenarytaina clavata*, *C. pollicaris* and *C. fuchsiae.* This may be attributed to similarly close genetic variability amongst host plants that is being unravelled only in recent times. For example, the genus *Kunzea* was recently revised with a reclassification of kānuka into this genus (and mānuka remaining in the *Leptospermum*) (de Lange 2014). There, molecular genetic analysis indicated the presence of possible cryptic species within the genus *Kunzea* (de Lange 2014). Similarly, while mānuka is still considered a single species, many variants and subspecies have been recorded and identified in the last century (Stephens et al. 2005). Certainly, the very low genetic divergence of the mānuka and kānuka *Ctenarytaina* species could suggest speciation at an early stage is underway, similarly to the speciation process occurred between the *Psylla* species.

An alternative explanation for the *Ctenarytaina* radiation on kānuka and mānuka is geographic isolation. The distribution reported in chapter 3 shows how these different taxa (except *Ctenarytaina pollicaris* sp. B) have been found in distinct areas. Therefore, in the absence of additional field collections, the hypothesis advanced here is that speciation was caused by geographic isolation.

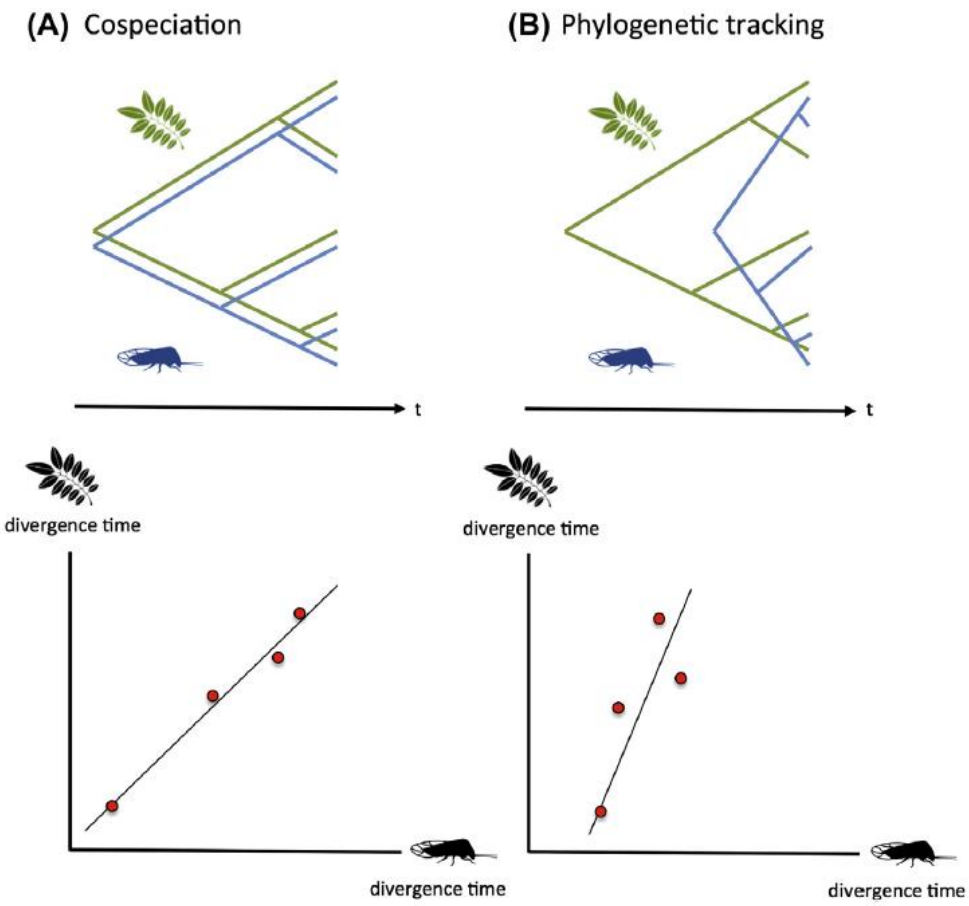


**Figure 4.7:** Relative evolutionary patterns and rates expected under a strict cospeciation pattern (A) or resulting from phylogenetic tracking (B) (Kergoat et al. 2017). Reproduced with publisher's permission.

## 4.5 Conclusion

In conclusion, the molecular phylogeny developed here indicates that different New Zealand endemic psyllid lineages have radiated through multiple evolutionary pathways. Some genera include just one or few species that appear to have remained in their archaic morphology (e.g. *Anomalopsylla* and *Atmetocranium*), while other more prolific genera radiated and dispersed resulting in a variety of morphologies. This ability to radiate has contributed to the utilisation of different host plants. Following at least six separate arrivals, some lineages have radiated on a multitude of host plant families (e.g. one lineage of *Trioza*), while others have a marked preference for closely related host plants. The geology and ecological landscape of New Zealand may have impacted on these different evolutionary strategies in psyllids, as they have for many other endemic insects (Buckley et al. 2015), including by acting on the distribution of their host plants. Finally, a

more adaptive behaviour/life history of the ancestral insects may have played a key role in the pronounced radiation and host plant diversification of *Trioza* in New Zealand.

What appears certain is the predisposition to adaptation to new host and invasiveness of the first ancestor of the New Zealand *Trioza.* This work confirmed for the first time the monophyly of this genus in New Zealand, except *T. curta* which is proposed as having a separate ancestral arrival, and highlighted host switch as a fundamental evolutionary trait that contributed to the success of *Trioza.* Moreover, other genera within New Zealand showed a different evolutionary approach to host plant association (e.g. *Psylla* and *Ctenarytaina*). However, the presence of a group of 11 *Trioza* species associated with the plant family Asteraceae has led to hypothesize that this predisposition for adaptation on multiple host plants may have been lost during the radiation of this smaller lineage feeding solely on Asteraceae.

The capability of colonizing and feeding from multiple plants is a trait that has important implications not only on the fitness of the psyllid, but also in plant pathogens transmission. In fact, psyllids with multiple host plants, such as *B. cockerelli,* can also spread plant pathogens within a larger range of plants (Butler and Trumble 2012). Therefore, a better understanding of the possible adaptive mechanisms behind this "invasiveness trait" could result in better chances to control psyllid pest and their vectored plant pathogens.

One such adaptive trait is linkage to endosymbiotic bacteria that may have a role in suppressing insect-related host plant defences (Hansen and Moran 2014) (Chapter 5).

# Chapter 5

# Microflora composition of the New Zealand Psylloidea

## 5.1 Introduction

Chapter 4 highlighted that six ancestral psyllid arrivals formed the New Zealand psyllid fauna of today. Three of these arrivals generated radiations of the more prolific New Zealand endemic genera *Ctenarytaina, Psylla* and *Trioza* and each of them shows different evolutionary strategies. In particular, the genus *Trioza* appeared to be more prone to colonization of different host plants, showing a high number of host switches that indicate a possible mechanism for the successful radiation of this psyllid group in New Zealand. The different insect-plant associations appeared to be unrelated to the geographical distribution, given the same psyllid species could be found on the same plant species at different places in the country. However, host plant was clearly correlated with the insect's genetics, with each psyllid species associating with a specific plant species.

The association between phloem feeding hemipterans and their host plant is at least partially dependant on the role of specific bacterial endosymbionts, which provide nutrients to the insects [reviewed in (Douglas 2016)]. Recent research even suggests that symbiosis is a major driver of insect diversification, as it provides the insect with new physiological capabilities that allow niche expansion, which is a first step towards adaptive radiation (Vavre and Kremer 2014). Variation in the microbiome has also been linked to insect phenotypic traits associated with diversification and speciation. For example, direct evidence was generated by the experimental transfer of symbionts from the pest stink bug, *Megacopta punctatissima*, to the non-pest stinkbug, *Megacopta cribaria,* enabling a niche shift of the latter to use soybean and pea as hosts (Hosokawa et al. 2007). Moreover, a rapid adaptation of some insects to a new host plant has been linked to modifications of the microbiome. In the case of the western corn rootworm, *Diabrotica virgifera*, this enabled the pest to feed on what was originally a non-host and effectively made it resistant to the crop rotation component of an integrated pest management scheme (Chu et al. 2013). These recent studies have led to the hypothesis that the switch by insects to novel host plants may be symbiont-mediated (Tsuchida et al. 2011, Frago et al. 2012). Given these findings, it was hypothesised here that one of the factors influencing psyllid-host plant associations, and in particular the capacity of the insect to colonise new host plants, might be its microbiome.

In psyllids, factors such as host plant species-specificity (Brown and Hodkinson 1988) has been linked to bacterial symbionts that enable the insect to feed on a specific plant (Hansen and Moran

2014). Similarly, S-symbionts in aphids were proposed to facilitate or restrict the use of certain host plants (Hansen and Moran 2014). In particular, the facultative symbiont *Regiella insecticola* enhances reproduction of infected pea aphids specifically on clover, thereby broadening the suitable food plant range of an insect that is usually limited to vetch (Tsuchida et al. 2011). Hansen and Moran (2014) reported that the role of S-symbionts may depend on their location within the insect host (intracellular, gut, and environmental) and the fidelity of their association with the host. Given these findings, a better understanding of what can influence bacterial biodiversity would provide a greater appreciation of how insects switch host plants and potentially why some groups of psyllids are more invasive than others (Bennett 2013). So far, the microflora of animals has been widely associated with factors such as their diet [e.g. (Ley et al. 2008, David et al. 2014)], but correlation with the host phylogeny has also been recorded (Ley et al. 2008). Furthermore, association of the insect host's genome with its microbial composition are starting to be demonstrated [e.g. (Brucker and Bordenstein 2012, Brooks et al. 2016, Davenport 2016)].

The first studies on the psyllid microbiome reported that it is relatively conserved, consisting of a single vertically transmitted P-symbiont, *Candidatus* Carsonella rudii, and a pool of both vertically and horizontally transmitted S-symbionts (Thao et al. 2000b, Thao et al. 2000a, Thao et al. 2001). The Enterobacteriaceae family (Gammaproteobacteria) forms the major group of S-symbionts (Hall et al. 2016; Chapter 1). Studies of the association between psyllids and their symbionts have been driven largely by the need to understand the plant pathogens vectored by these insects, and in particular for insect-pathogen pairings of economic concern [e.g. (Saha et al. 2012)]. This has restricted our understanding of the general principles surrounding the microbiome and its impact on psyllid evolution. New Zealand currently has only one pest species that is known to vector a pathogen, the tomato potato psyllid (TPP), *Bactericera cockerelli* Sulc (Teulon et al. 2009), which vectors the bacterium *Candidatus* Liberibacter solanacearum (Lso). New Zealand is, however, considered at high risk of invasion by other high risk species, particularly the citrus pest *Diaphorina citri* Kuwayama, which transmits *Ca*. L. asiaticus (Aurambout et al. 2009) and the potato pest, *Russelliana solanicola* Tuthill (Syfert et al. 2017), the putative vector of an uncharacterised virus (Tenorio et al. 2003).

The *Liberibacter* genus has come under increasing scrutiny since several species were linked to plant disease (Haapalainen 2014). In addition to Lso, other *Liberibacter* have been detected in New Zealand, but have not yet been associated with disease. These include *Ca.* L. europaeus (Thompson et al. 2013), vectored by *Arytainilla spartiophila*, and a new species of *Liberibacter*, temporarily named '*Ca.* L. ctenarytainae', from *Ctenarytaina fuchsiae* (Smith and Thompson 2017, personal communication). *Candidatus* L. europaeus' sp. nov. was previously shown to be transmitted by the psyllid *Cacopsylla pyri*, and appears to behave as an endophyte on pear (Raddadi et al. 2011).

In Australia, another new *Liberibacter* species has been associated with the eggplant psyllid *Acizzia solanicola* (Morris et al. 2017). This psyllid is also present in New Zealand (Kent and Taylor 2010, Taylor and Kent 2013), but the bacterium has not yet been reported. Interestingly, in *D. citri*, a protein interaction network at the psyllid–microbe interface, involving the bacteria *Carsonella*, *Wolbachia* and *Proftella,* has been shown to interfere with the psyllids ability to transmit *Ca.* Liberibacter asiaticus (Ramsey et al. 2017). These discoveries highlight the importance of understanding the psyllid microbiome in more detail. Indeed, even a more general study of psyllid microbiomes might provide useful information on the bacterial biodiversity in these insects, from which psyllid-bacteria associations could be inferred. The value of this approach was demonstrated in a similar study on the American pikas [small mammals, (Kohl et al. 2017)], and by reports that the plant-microbe-insect interaction (PMI) may enhance or interact with pathogen spread when new plants or new insects are added to the PMI (Bennett 2013). Such studies have led to the hypothesis that the acquisition of novel host plants by insects is a symbiont-mediated process [(Vavre and Kremer 2014) and references reported earlier].

Any study focused on PMI interactions involving psyllids would require a solid phylogenetic base enabling the relationships between the insects to be understood, as well as a dataset describing the bacterial diversity associated with the different psyllid groups. This would not be limited to the information available on the P- and S-symbionts. In fact, in addition to the symbiotic bacteria that provide the amino acids lacking in the phloem-based diet of psyllids (Thao et al. 2001), other bacteria enable other insects such as different aphid species to share the same host plant (Tsuchida et al. 2011) and provide mutualistic roles that could lead insects to specialize on different food plants (Frago et al. 2012) (Chapter 1).

In this context, the concept of “phylosymbiosis”, proposed elsewhere to describe the pattern of parallels between microbial communities and their insect host (Brucker and Bordenstein 2012, Brooks et al. 2016), might be considered. Phylosymbiosis does not presume that a certain microbial composition is constantly stable or only vertically transmitted. Rather, it considers it as an eco-evolutionary pattern where evolutionary changes in the insect host are associated with ecological changes in the microbiome (Brooks et al. 2016). In light of this, the psyllid phylogeny associated with the insects’ host plants (Chapter 4) can be tested as to the evolutionary changes (e.g. host switches) that may be associated with the psyllid microbial composition. So far, when testing for this host-microbiome relationship in insects, phylosymbiosis has been recorded in ants (Sanders et al. 2014), but not in flies (Wong et al. 2013) nor termites (Dietrich et al. 2014).

The molecular taxonomic analysis in the current study (Chapters 3 and 4) revealed new details of psyllid diversity in New Zealand and provided a phylogenetic scaffold illustrating

evolutionary relationships between the insects and between the insects and their host plants. This supported the hypothesis that a large proportion of the New Zealand psyllid fauna are probably derived from a limited number of transoceanic dispersals followed by radiations onto new hosts plants (Chapter 4). As a consequence, the taxa in New Zealand may provide an ideal model system for understanding how the microbiota influences or is influenced when a psyllid enters a new environment and feeds on different hosts. In particular, this may improve the understanding of the relationships between microbial composition and insect genetic variation. Furthermore, this would enable a better understanding of the roles of psyllids in native and agricultural systems in Australasia. This is of much interest because of the region's high psyllid diversity as well as the recent introduction of pest species into both countries. From a New Zealand perspective, recent introductions of Australian psyllid species into New Zealand are widely documented, with genera such as *Creiis, Eucalyptolyma, Cryptoneossa, Anoeconeossa* and *Glycaspis* arriving in the last 30 years (Dale 1985, Henderson et al. 2010). Furthermore, many established species of psyllids in New Zealand are hosted by Australian eucalypt or wattle plants that were not present in New Zealand prior to European colonisation events. For these reasons, 37 psyllid species on these hosts in New Zealand are considered to be adventive species (Chapters 2 and 3). Therefore, adding information on the microbiome of these insects may result a useful tool for biosecurity.

Utilising the phylogenetic dataset developed in Chapter 4, the microbiomes of New Zealand psyllid species are considered here for their potential link to the insects' phylogeny. Moreover, the microbial composition is tested for association with the ecological characters of psyllid geographical distribution and host plant association. Simultaneously, the use of MiSeq Illumina technology allowed the psyllid species to be screened for bacterial insect and plant pathogens in order to improve the knowledge of the psyllid species in New Zealand that may be vectors.

### 5.1.1 Aim and hypotheses

The **overarching aim** of this chapter was to develop fundamental microbiome data needed to enable molecular, ecological, and/or evolutionary aspects of the insect-plant-microbial community interactions to be better understood. Ultimately, this was with a view to postulating areas for further research that could address how such tri-trophic relationships may influence the pest status of some psyllid species. Traits that influence pest status might be their invasiveness, their capacity to vector pathogenic microbes, or their inhibition of plant pathogenic bacteria. With this in mind, any plant and insect pathogens together with putative unculturable bacteria with associations with plant disease were recorded as well as those probably involved in symbiosis.

This study links the psyllid microbiota composition from 65 psyllid species to the psyllid phylogeny, and accordingly their associated host plants (Chapter 4), and distribution (Chapter 3) to test the following **hypotheses:**

**Hypothesis 1:** At least two of these tri-trophic components (insect, host plant, bacteria) are strongly correlated.

**Hypothesis 2:** The insect-bacteria associations can be understood in light of the "phylosymbiosis" theory (Brucker and Bordenstein 2012, Brooks et al. 2016) i.e. that congruence between host evolutionary history and microbial communities will be apparent.

**Hypothesis 3:** If phylosymbiosis is inferred as the major driver of bacterial composition, then host plant specificity does not seem to be driven by symbionts as proposed by Hansen and Moran (2014).

The **objecives** to enable this are to:

**Objective 1:** Produce the first evaluation of New Zealand psyllid symbiotic bacterial composition using 16S metabarcoding.

**Objective 2:** Use the detected bacterial community to test for correlations between the psyllid microbiome and i) the insect phylogeny, ii) their host plant association and iii) geographical distribution. This would enable hypotheses to be developed as to whether the psyllid microbial composition is mostly correlated to the nature or distribution of the insect's host plant (e.g. across New Zealand or between New Zealand and Australia), or entirely driven by evolution of the psyllids (phylogenetically-associated). In this context, if a psyllid's microbial community shows higher similarity to communities of the same psyllid species than to those from different host species, phylosymbiosis will be confirmed (Brooks et al. 2016).

**Objective 3:** Scan the psyllid microbial community for presence of potential pathogens, which may include unculturable bacteria.

## 5.2 Materials and Methods

### 5.2.1 Molecular analyses

The V3 and V4 regions of the bacterial 16S ribosomal RNA gene were amplified from whole insect genomic DNA prepared as described previously (Chapter 3.2.4) from a total of 220 insects (Table D.1, Appendix D), encompassing 65 species across 178 populations. DNA extractions, amplification and purification were performed in a Physical Containment (PC2) facility in order to minimize the risk of

environmental contamination. Sixteen of the 200 individuals were sequenced twice (as technical replicates), in order to confirm the consistency of the results (Table 5.1).The use of the 16S_F and 16S_R primers (Klindworth et al. 2013), modified with Illumina adapters, followed the Illumina Demonstrated Protocol v. 15044223 Rev. B (available at https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html). PCR amplification was performed using an initial denaturation at 95°C for 3 min, followed by 25 cycles of 95°C denaturation for 30 s, 55°C annealing for 30 s and 72°C elongation for 30 s. A final 72°C elongation was performed for 5 min.

PCR products were purified using the Agencourt® AMPure® XP kit (Beckman Coulter, Brea, California, United States). The concentrations of PCR products were measured using a NanoDrop 1000 (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and samples at concentrations between 10 ng/µL and 50 ng/µL were sequenced using the Illumina high throughput sequencing platform at New Zealand Genomics Limited (NZGL). Control samples with no DNA were amplified in every PCR run, these were then checked on 1% agarose gel electrophoretic runs and resulted in the absence of DNA.

### 5.2.2 Metabarcode data analysis

Nucleotide sequences were analysed with VSEARCH (Rognes et al. 2016) using the software R v3.0.2 (R Core Team 2013) on a computer using 20 GB of RAM and running the Ubuntu operative system. The VSEARCH pipeline can be found in the Appendix E.2. Initially, forward and reverse sequencing reads were assembled and primer sequences were removed to produce a consensus sequence for each bacterial amplicon. The quality control (QC) was performed using the VSEARCH pipeline by tail trimming and primers removal; sequences shorter than 150-bp were then removed together with chimeric sequences and reads appearing only a single time (singletons). Operational taxonomic units (OTUs) were produced by clustering sequences with greater than 97% identity using the Usearch algorithm implemented in VSEARCH [Usearch v9 v9.0.2132; (Edgar 2010)] on a i86linux32 computer with 4.0 GB RAM (16.3 GB total) and 8 cores. An identity to the lowest possible taxonomic level was given to each amplicon by sequence comparison of the 16S OTUs against the Greengenes database (http://greengenes.secondgenome.com) using the Basic Local Alignment Search Tool (BLAST) algorithm implemented in the VSEARCH pipeline. The representative sequence chosen for the blast was the centroid of an OTU, as in the default UPARSE manual, available at https://www.drive5.com/usearch/manual/uparseotu_algo.html.

In order to confirm the results obtain using VSEARCH, two additional pipelines were run using the software Quantitative Insights Into Microbial Ecology: QIIME and QIIME2 (Caporaso et al. 2010). The default options where generally used, as reported on the scripts section of the QIIME

website (http://qiime.org/scripts/), and the scripts can be found in the Appendix E.1 and E.3. Of particular note, the RDP Classifier (Wang et al. 2007) was used to assign taxonomy instead of BLAST. The addition of these pipelines was not aimed to generate a comparative study of separate workflows, instead the aim was to confirm the presence/absence of the taxa reported using VSEARCH.

When using QIIME2, the pipeline was run on the complete dataset from a Jupyter notebook, as reported in Appendix E.3. While the QIIME pipeline blasted the OTUs against the Greengenes database (DeSantis et al. 2006), QIIME2 used the SILVA ribosomal RNA database [https://www.arb-silva.de/ ; (Quast et al. 2013)]. To enable comparison of the OTUs obtained using the different pipelines and databases, alignments of DNA sequences were generated using MEGA6. These alignments were then used to construct a 16S gene tree using the ML algorithm (1000 replicates, bootstrap <50% not shown). This specifically focused on the primary symbiont and on the bacterial family Enterobacteriaceae due to the high number of sequences recorded for that group (see below). In addition to the Enterobacteriaceae sequences from QIIME, VSEARCH and QIIME2, multiple sequences from the same QIIME OTUs (using the cut-off of 3% divergence) were added to the alignment to test if the multiple VSEARCH OTUs were actually similar to each other and to the QIIME sequences.

### 5.2.3 Statistical analyses of metabarcode data

The following analyses and tests have been performed using R v3.0.2 (R Core Team 2013) on a computer using 16 GB of RAM and running the Windows operative system. The scripts for the analyses are presented in Appendix E.4.

#### Sample replicates

To assess the reliability of both the technology and the data analysis performed on the metabarcoding dataset, DNA extracted from 16 samples (Table 5.2) was used to amplify the partial 16S rDNA sequences in duplicate PCRs, and the resulting amplicons sequenced in separate runs. Since species richness increases with sample size, and differences in richness actually may be caused by differences in sample size (Hurlbert 1971). The comparison of the two replicates required a rarefaction (Sanders 1968), whereby adjustment is made to the larger dataset to make it directly comparable to the smaller; in effect the number of observations for an OTU in the larger dataset was reduced to what would be expected as equivalent in the smaller dataset. Therefore, the number of observed OTUs was rarefied using the R package Vegan (v 2.3-5). Comparison of the rarefied number of OTUs between replicates was then tested using a generalized linear model (GLM) in R package "stats" (v 3.2.2), based on the Poisson error distribution. To visualize information on these pairwise

similarities, non-metric multidimensional scaling (nMDS) ordination plots was used. This was generated using the package "ggplot2" (v. 2.1.0) to interpret multivariate distance between sample replicates as a treatment factor.

### Alpha diversity

Alpha diversity is the diversity of organisms in one environment (Whittaker 1960). Therefore, every sample has a value which may measure richness (such as the OTU count) or evenness (distribution of different bacteria). Alpha diversity was calculated with R using both the number of reads and the OTUs. A rarefaction to the smaller number of reads recorded was required in order to compare all the different species independently from the number of samples analysed per species.

### Beta diversity

Beta diversity is described as the difference in diversities across environments or samples (Whittaker 1960, 1972). Beta diversity measures pairwise sample dissimilarity among sample units (Anderson et al. 2011). In this work, presence/absence of OTUs between each pair of samples was compared by calculating un-weighted UniFrac metric (Navas-Molina et al. 2013). The UniFrac metric was used since it is considered to be most useful in revealing biologically meaningful patterns (Navas-Molina et al. 2013). The unweighted UniFrac was preferred to the weighted UniFrac since it considers taxon abundance in terms of absence/presence of the OTUs, whereas the weighted UniFrac is sensitive to the bias from DNA extraction efficiency and PCR amplification (Lozupone et al. 2007, Navas-Molina et al. 2013). The Beta diversity test was performed on all the samples but, for spatial reasons, a graphic representation was developed using Microsoft Excel 2013 (Microsoft - Redmond, Washington, USA) only for the species of the genus *Acizzia.*

### ADONIS distance matrix analysis

ADONIS is a function for the analysis and partitioning sums of squares using semi-metric and metric distance matrices, based on a nested nonparametric (permutational) multivariate analysis of variance. This function is directly analogous to MANOVA (Multivariate ANalysis Of VAriance) (McArdle and Anderson 2001). The ADONIS approach was used here to test the correlation between the microbial composition and psyllid taxonomy, with the percentage of correlation explained by the $R^2$ (effect size), as well as a p-value representing statistical significance.

### Three-way intersect and Mantel test of the distance matrices

The Mantel test measures the correlation between two matrices (Mantel 1967, Manly 1985, 1997). In this study, Mantel test was used to test the correlation between the psyllid microfloral biodiversity and i) the psyllids genetic distance, ii) the host plant evolutionary distance (in million years), and iii) the geographical distances between psyllid specimens. To perform the Mantel and

Partial Mantel tests, four matrices were designed for the following characters: bacterial community, psyllid genetic distance, plant genetic distance and geographic distance. While the matrix of the bacterial communities was created using R, from the results of the VSEARCH pipeline (Appendix E.2), the psyllid genetic distance matrix was created using MEGA6 (Tamura et al. 2013) and the host plant evolutionary distance matrix using Phylocom V4.2 (Webb et al. 2008). In order to obtain the geographical distance matrix, the GPS coordinates of the Appendix B were converted to a spatial distance between two locations (script in the Appendix E.2). Both Mantel and Partial Mantel tests were performed using the scripts in R (Appendix E.4).

**Bacterial and psyllid data subset analyses**

To trace any correlations to specific bacterial groups (Section 5.3), the overall dataset was subdivided according to the following characteristics. Firstly, the overall number of bacteria recorded was subdivided into “rare” and “common” bacteria, where "rare" was defined as those species that, when present, are in a lower-than-mean abundance (Figure 5.10). This definition is independent of being frequently present which would be biased by those samples where a single individual per species was considered. A second subset included only the psyllid species collected more than five times. This may include multiple insects from the same populations. This reduced the dataset to a total of 12 species.

### 5.2.4 Plant pathogen-specific PCR test

A *Liberibacter*-specific PCR was performed on a sample of *A. acaciae* (sample 123) and on one of *C. spatulata* (sample 143), while the *Phytoplasma*-specific PCR was performed on a sample of *T. irregularis* (sample 4) after identifying the DNA sequences for these pathogens amongst the OTUs from these samples. The *Liberibacter* positive samples were amplified using the three primers (OA2, Lib16SF, Lib16R) and PCR cycle reported by Beard and colleagues (Beard et al. 2013). A *Phytoplasma* DNA fragment was amplified from *T. irregularis* with the P1/P7 primer pair (Deng and Hiruki 1991) (Schneider et al. 1995). The PCR started with denaturation at 95°C for 3 min, followed by 40 cycles of 95°C denaturation for 20 s, 52°C annealing for 20 s [as suggested in (Lorenz et al. 1995)] and 72°C elongation for one min. A final 72°C elongation was performed for 5 min. PCR amplicons were cloned into a TOPO® TA Cloning kit (Thermo Fisher Scientific; Waltham, Massachusetts, USA) following the manufacturer instructions, and the nucleotide sequence of the insert was sequenced by the Sanger sequencing Unit (Bio-Protection Research Center, Lincoln University) using the same primers used for the pathogen-specific PCRs.

## 5.3 Results

The data output obtained from sequencing 220 individual psyllids across 65 species comprised 23,832,596 total reads. This data included the reads from 16 specimens that were sequenced twice as technical replicates to later assess fidelity of the bacterial diversity captured for a total of 236 samples. Using VSEARCH, 9,245,588 (38.79%) of the total reads were able to be merged as complimentary forward and reverse sequences. The quality control (QC) discarded a first subset of sequences: 1922 forward tails Q <= 2 trimmed (0.01%), 26741 reverse tails Q <= 2 trimmed (0.11%), 15798 forward too short (< 64) after tail trimming (0.07%) and 8903 reverse too short (< 64) after tail trimming (0.04%). The remaining could either not be merged because of too many (>10) polymorphisms (13,759,826; 57.74%) or were unable to be aligned at all (802,481; 3.37%). Following a quality filtering process, chimeras were discarded leaving 8,833,277 sequences, and an additional 103,896 sequences shorter than 150bp were also removed.

### 5.3.1 Evaluation of the 16S rDNA sequence pipelines used to generate OTUs

A total of 8,729,381 consensus sequences were considered suitable for analysis with VESEARCH. Of these, 3,461,033 reads represented unique sequences, from which 1,454 OTUs were detected using Usearch v9.0.2132 (from the VSEARCH pipeline, Appendix E.2). A total of 333 out of the 1,454 OTUs generated no hit to the sequences held on the Greengenes 16S database. These sequences were discarded, leaving a total of 1,121 OTUs represented by 6,504,262 sequences. The QIIME pipeline generated 651 OTUs that could be taxonomically assigned using the Greengenes dataset. Similarly, the QIIME2 pipeline, using the SILVA database, could identify 367 OTUs.

Table 5.1 was generated selecting only the OTUs of the VSEARCH pipeline that recorded more than 10,000 reads each (arbitrary cut-off). This resulted in the top 34 most recorded OTUs for each pipeline illustrated in Table 5.1. From the VESEARCH output, and consistent with expectations [see (Hall et al. 2016)], the most common bacterial family in psyllids was the Enterobacteriaceae. This family was recorded for 24 of the first 34 OTUs, and was represented by a total of 2,881,708 sequences. Within these OTUs, six had closest identity to *Sodalis*-like OTUs in the database. In contrast, only five of the top 34 QIIME1 results were Enterobacteriaceae (including a single *Sodalis* OTU) while only 12 were recorded by QIIME2 (including a single *Sodalis* OTU).

When considering this variation in Enterobacteriaceae OTUs, it was discovered that the first nine bacteria recorded with QIIME and QIIME2 covered 76.4% and 79.2% of the total count of reads, respectively, whereas the first nine OTUs identified by VSEARCH covered only 17.5%. This highlighted a tendency of QIIME and QIIME2 to cluster higher numbers of sequences together in the

same OTU, resulting in an underestimation of OTU diversity (Table 5.1, Figure 5.1). In fact, despite using the same threshold for the OTU picking (set at 97% similarity), and blasting the results on the same database (Greengenes for QIIME and VSEARCH), results from the different pipelines gave different taxonomic assignments for sequences with very close percentage identity (Figure 5.1).

For example, sequences belonging to the same two Enterobacteriaceae OTUs identified by QIIME appear to cluster distant from each other and close to some of the OTUs identified with VSEARCH (Figure 5.1). These QIIME sequences have a genetic distance >3%, therefore they may suggest that multiple OTUs have been merged in the same one. This was not specified in any of the scripts used from the QIIME website (http://qiime.org/scripts/), and no information could be found on this scenario which may be a default setting of the command lines adopted.

Beside the Enterobacteriaceae, high read counts were recorded using all the pipelines for two bacterial OTUs with identity to *Wolbachia* and *Pseudomonas.* More than 1 million reads (1,010,642) were identified as *Wolbachia* (Rickettsiaceae) using the VSEARCH pipeline, while the *Pseudomonas* genus was the eleventh most recorded OTU, with almost 90,000 reads (89,966) across the samples tested (Figure 5.1). A comparison of the nucleotide sequences for the *Wolbachia* and *Pseudomonas* OTUs defined by the three software packages generated a ML tree (Figure 5.1) that showed these partial 16S rDNA sequences were more similar than the Enterobacteriaceae OTUs identified by the same software packages.

In addition to the 34 OTUs reported in Table 5.1, VSEARCH also identified all the OTUs recorded using the two additional pipelines.

**Table 5.1:** The most recorded OTUs (listed from highest to lowest) using QIIME, VSEARCH and QIIME2. OTUs were classified to either the family or genus level. OTUs belonging to the Enterobacteriaceae are reported in green. The total number of OTUs recorded by each pipeline is reported in parentheses.

| | **QIIME1 (651)** | **VSEARCH (1121)** | **QIIME2 (367)** |
|---|---|---|---|
| **1** | Enterobacteriaceae | *Wolbachia* | *Wolbachia* |
| **2** | Enterobacteriaceae | Enterobacteriaceae | *Baumannia* |
| **3** | *Wolbachia* | Enterobacteriaceae | *Blochmannia* |
| **4** | *Carsonella* | Enterobacteriaceae | *Schneideria* |
| **5** | *Sodalis* | *Sodalis* | *Buchnera* |
| **6** | *Pseudomonas* | *Sodalis* | uncultured bacterium |
| **7** | *Blochmannia* | Enterobacteriaceae | *Candidatus* Curculioniphilus |
| **8** | *Schneideria* | Enterobacteriaceae | uncultured bacterium |
| **9** | *Acidovorax* | Enterobacteriaceae | *Arsenophonus* |
| **10** | *Agrobacterium* | Enterobacteriaceae | *Carsonella* |
| **11** | *Liberibacter* | *Pseudomonas* | *Pseudomonas* |
| **12** | *Acinetobacter* | *Sodalis* | *Aquabacterium* |
| **13** | *Janthinnobacterium* | Enterobacteriaceae | *Sodalis* |
| **14** | Acetobacteraceae | *Sodalis* | *Riesia* |
| **15** | *Rickettsiella* | *Sodalis* | *Rhizobium* |
| **16** | Oxalobacteraceae | *Sodalis* | *Flavobacterium* |
| **17** | Caulobacteraceae | Enterobacteriaceae | *Afipia* |
| **18** | Rhodospirillaceae | Enterobacteriaceae | uncultured bacterium |
| **19** | Rhodocyclaceae | Enterobacteriaceae | *Erwinia* |
| **20** | Sphingobacteriales | Flavobacteriaceae | *Reyranella* |
| **21** | Phycispaerales | Enterobacteriaceae | *Sulfuritalea* |
| **22** | Bradyrhizobiaceae | *Agrobacterium* | *Staphylococcus* |
| **23** | Sphingomonas | Enterobacteriaceae | Enterobacteriaceae |
| **24** | Streptomyces | Enterobacteriaceae | uncultured bacterium |
| **25** | Cardinium | Cyanobacteria | *Acinetobacter* |
| **26** | Pedobacter | Enterobacteriaceae | *Sediminibacterium* |
| **27** | Sediminibacterium | Enterobacteriaceae | *Brenneria* |
| **28** | Staphylococcus | Cyanobacteria | *Brevundimonas* |
| **29** | Streptococcus | Halomonadaceae | *Escherichia-Shigella* |
| **30** | Rhizobiales | Enterobacteriaceae | *Sphingomonas* |
| **31** | Xanthomonadaceae | Enterobacteriaceae | *Asaia* |
| **32** | Phytoplasma | Halomonadaceae | *Acidovorax* |
| **33** | Corynebacterium | Stapylococcaceae | *Janthinobacterium* |
| **34** | Rhodococcus | Bacteroidetes | *Cardinium* |

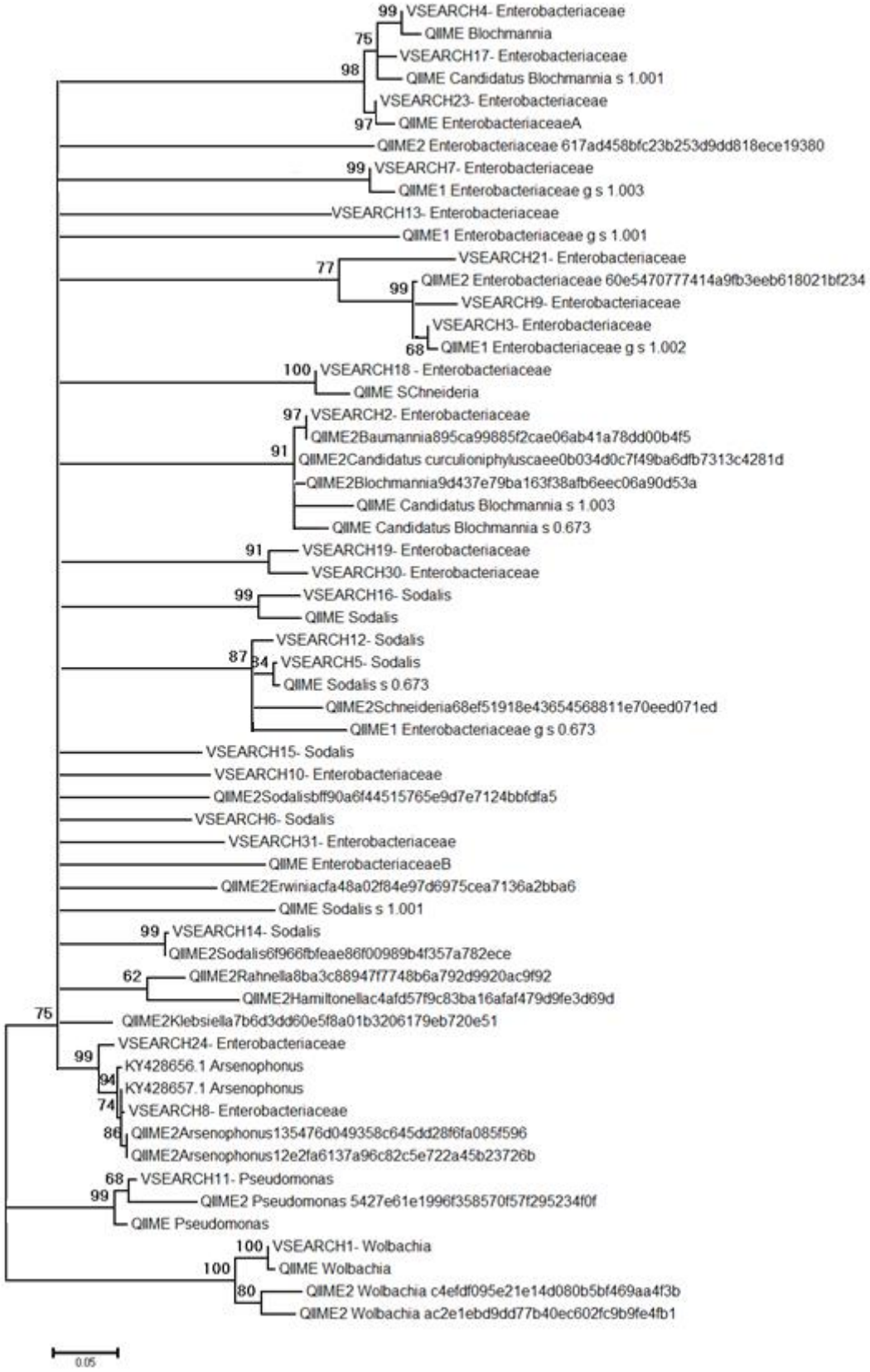


**Figure 5.1:** A partial 16S gene tree (Maximum Likelihood, 1000 replicates) including the main OTUs recorded using QIIME, VSEARCH and QIIME2. Multiple sequences were included for the QIIME OTUs and two sequences of *Arsenophonus* have been added from GenBank as an additional comparison. Bootstrap values lower than 50% are not shown and branches are collapsed.

### 5.3.2 Psyllid species-associated bacterial sequences and within-sample OTU reliability

The comparative level of partial 16S rDNA sequences (post quality control with VSEARCH) that were generated for the 65 psyllid species tested is summarised in Table 5.2. The average number of reads per individual for each species ranged from ~6000 to ~51,000, with the large majority (87.5%) producing >10,000 DNA reads (arbitrary cut-off). For the few insect specimens that produced <10,000 DNA reads, such as a specimen of *Ctenarytaina pollicaris* B and one of *Ctenarytaina thysanura*, the diversity may be under-represented in comparative assessments. On the other hand, for three *Ctenarytaina* species, *C. eucalypti, C. fuchsiae* and *C. spatulata*, more than 10 populations were included in the analysis (Table 5.2) in order to assess the inter-population heterogeneity of the microbial composition.

**Table 5.2:** The average number of partial 16S rDNA metabarcode sequence reads obtained for each of 65 psyllid species comprising single or multiple geographic populations. Of 220 individuals 16 were sequenced twice as replicates to assess consistency of the results.

| Ref Nº | Species | Nº Ind | Nº reps | Nº Pops | Av. Nº seq reads | Reads interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Min | Max |
| 1 | *Acizzia acaciaebaileyanae* | 1 | | 1 | 39960 | | |
| **2** | *Acizzia acaciae* | 4 | | 4 | 17982.5 | 13469 | 27672 |
| **3** | *Acizzia albizziae* | 6 | 1 | 5 | 24545.5 | 9709 | 47369 |
| **4** | *Acizzia dodonaeae* | 8 | | 6 | 39676 | 16968 | 63076 |
| **5** | *Acizzia hakeae* | 3 | | 3 | 9869 | 8398 | 10956 |
| **6** | *Acizzia jucunda* | 4 | 1 | 2 | 34671 | 9545 | 63987 |
| **7** | *Acizzia solanicola* | 2 | | 1 | 9997.5 | 4689 | 15306 |
| **8** | *Acizzia* sp. A | 3 | 1 | 1 | 16516 | 803 | 37310 |
| **9** | *Acizzia uncatoides* | 6 | | 5 | 9517 | 476 | 16239 |
| **10** | *Anoeconeossa* sp. | 1 | | 1 | 13711 | | |
| **11** | *Anomalopsylla* POLL ISL. | 3 | 1 | 1 | 27962.5 | 23343 | 37184 |
| **12** | *Arytainilla spartiophila* | 2 | | 2 | 10391 | 9416 | 11366 |
| **13** | *Bactericera cockerelli* | 1 | | 1 | 20290 | | |
| **14** | *Baeopelma foersteri* | 2 | | 2 | 18589 | 12294 | 24884 |
| **15** | *Blastopsylla occidentalis* | 2 | | 2 | 27286 | 23641 | 30931 |
| **16** | *Calophya schini* | 2 | | 1 | 22356.5 | 12668 | 32045 |
| **17** | *Casuarinicola australis* | 2 | | 2 | 21473.5 | 4706 | 38241 |
| **18** | *Cryptoneossa* sp. | 1 | | 1 | 14554 | | |
| **19** | *Ctenarytaina clavata* | 2 | | 2 | 36618.5 | 32108 | 41129 |
| **20** | *Ctenarytaina clavata* B | 1 | | 1 | 24826 | | |
| **21** | *Ctenarytaina eucalypti* | 23 | | 15 | 13084 | 687 | 27207 |
| **22** | *Ctenarytaina fuchsiae* | 18 | | 15 | 23665 | 1279 | 79234 |
| **23** | *Ctenarytaina fuchsiae* B | 7 | | 1 | 19607 | 10246 | 28906 |

| Ref Nº | Species | Nº Ind | Nº reps | Nº Pops | Av. Nº seq reads | Reads interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Min | Max |
| **24** | *Ctenarytaina longicauda* | 3 | | 2 | 13985 | 3550 | 25461 |
| **25** | *Ctenarytaina pollicaris* | 5 | 5 | 2 | 32802.5 | 4773 | 64846 |
| **26** | *Ctenarytaina pollicaris* B | 1 | | 1 | 7637 | | |
| **27** | *Ctenarytaina* SHORT | 9 | 5 | 6 | 13813 | 3132 | 39329 |
| **28** | *Ctenarytaina* sp. A | 1 | | 1 | 33784 | | |
| **29** | *Ctenarytaina* sp. B | 2 | | 2 | 19045 | 18263 | 19827 |
| **30** | *Ctenarytaina spatulata* | 11 | | 11 | 21169 | 10471 | 34942 |
| **31** | *Ctenarytaina thysanura* | 1 | | 1 | 5927 | | |
| **32** | *Ctenarytaina* unknown | 1 | | 1 | 11024 | | |
| **33** | *Eucalyptolyma maideni* | 1 | | 1 | 15675 | | |
| **34** | *Glycaspis granulata* | 1 | | 1 | 18064 | | |
| **35** | *Mycopsylla fici* | 3 | | 2 | 45125 | 38481 | 48970 |
| **36** | *Psylla apicalis* A | 4 | | 4 | 15513 | 8536 | 21755 |
| **37** | *Psylla apicalis* B | 4 | | 4 | 16178 | 746 | 30865 |
| **38** | *Psylla carmichaeliae* A | 3 | | 2 | 38325 | 36910 | 39342 |
| **39** | *Psylla carmichaeliae* C | 2 | | 2 | 48956 | 31826 | 66086 |
| **40** | *Psylla carmichaeliae* E | 3 | | 3 | 50848 | 23635 | 93753 |
| **41** | *Psyllopsis fraxini* | 3 | | 3 | 19445 | 11534 | 33671 |
| **42** | *Psyllopsis fraxinicola* | 4 | | 4 | 20492 | 9827 | 41926 |
| **43** | *Trioza acuta* A | 2 | | 2 | 7775.5 | 590 | 14961 |
| **44** | *Trioza acuta* B | 1 | | 1 | 17515 | | |
| **45** | *Trioza eugeniae* | 1 | | 1 | 14852 | | |
| **46** | *Trioza bifida* | 5 | 2 | 4 | 21806 | 4545 | 49218 |
| **47** | *Trioza* BRENDA MAY | 1 | | 1 | 82411 | | |
| **48** | *Trioza colorata* | 4 | | 2 | 8351 | 2476 | 12110 |
| **49** | *Trioza decurvata* | 2 | | 2 | 21458 | 9100 | 33816 |
| **50** | *Trioza discariae* | 3 | | 2 | 31084 | 8667 | 53590 |
| **51** | *Trioza doryphora* | 4 | | 4 | 19772 | 15072 | 25325 |
| **52** | *Trioza emarginata* | 1 | | 1 | 33407 | | |
| **53** | *Trioza falcata* | 4 | | 4 | 17440 | 9979 | 28914 |
| **54** | *Trioza fasciata* | 2 | | 2 | 42540 | 11935 | 73145 |
| **55** | *Trioza hebicola* | 1 | | 1 | 28041 | | |
| **56** | *Trioza irregularis* | 3 | | 3 | 21419 | 3204 | 33966 |
| **57** | *Trioza* MASSEY | 1 | | 1 | 16677 | | |
| **58** | *Trioza obscura* | 1 | | 1 | 8577 | | |
| **59** | *Trioza* OMAHUTA | 1 | | 1 | 26891 | | |
| **60** | *Trioza panacis* | 1 | | 1 | 15982 | | |
| **61** | *Trioza* PRICE'S VALLEY | 1 | | 1 | 38151 | | |
| **62** | *Trioza* sp. C | 1 | | 1 | 16267 | | |
| **63** | *Trioza subacuta* | 2 | | 2 | 22206 | 19955 | 24457 |
| **64** | *Trioza vitreoradiata* | 9 | | 9 | 26953 | 6946 | 45812 |
| **65** | Triozid sp. | 3 | | 3 | 23134 | 15346 | 36798 |

To assess the reliability of the OTU taxon calling made for the psyllid species through the VSEARCH pipeline, DNA extracted from 16 samples, comprising five each of *Ctenarytaina pollicaris* and *C.* "Short", plus two samples of *Trioza bifida,* and one each for *Acizzia jucunda, A. albizziae, A.* sp. A and *Anomalopsylla* "Pollen Island" was used to amplify the partial 16S rDNA sequences in duplicate PCRs, and the resulting amplicons were sequenced in separate runs (Table 5.2, Figure 5.2). The number of OTUs observed with VSEARCH was rarefied based on the smaller number of reads recorded in the 32 samples in order to allow a comparison between the replicate 1 and 2 (Figure 5.2a). The variation in the rarefied number of OTUs between replicate 1 and 2 was between 0.45 (sample 31a/A) and 16.8 (sample 24b/B) (Figure 5.2b). However, univariate analysis of the rarefied species (GLM) demonstrated that species richness was not significantly different between the sample replicates (P = 0.104993). Visualization of the sample replicates divided by species by nMDS (Jaccard similarity) shows that variation in the number of rarefied OTUs appears to be in the limit of inter-population species variability. In fact, the MDS plot shows a clear separation between all the species analysed here, except for both the replicates of a single specimen of *C.* "Short" (Figure 5.2c). The univariate analysis performed on the rarefied species therefore confirmed that the data from independent metabarcoding runs were relatively consistent. Thus, the data was further analysed to determine the microbiomes of psyllids and any potential relationships to phylogeny, host range or geographical distribution.

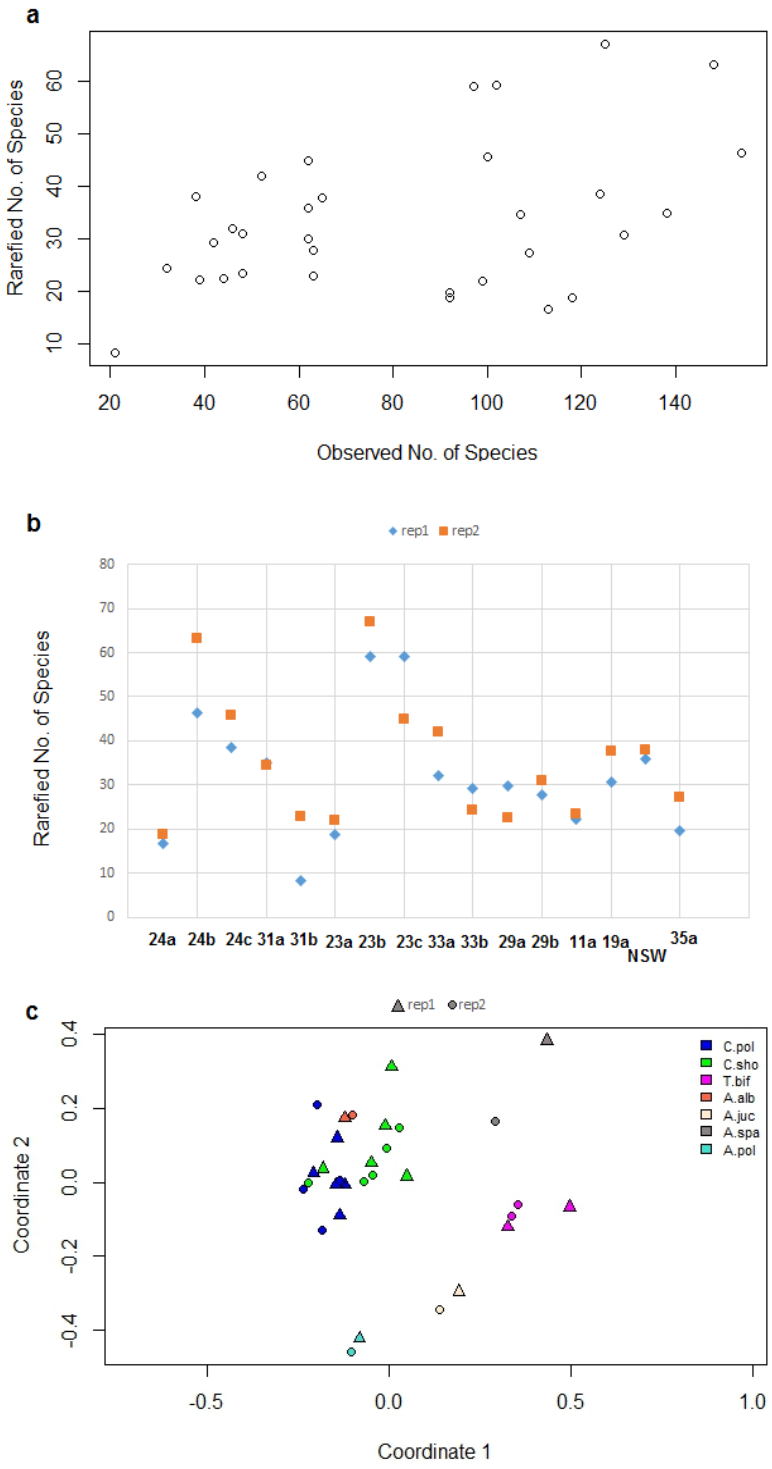


**Figure 5.2:** The number of 16S OTUs ("species" in the Figure) observed using VSEARCH for the 16 samples that have been replicated is rarefied based on the smaller number of reads recorded **(a).** The rarefied number of OTUs observed allowed to compare the two separate replicates for each of the 16 samples analysed **(b)**. An MDS plot illustrates the relationships between the different psyllid species based on the rarefied OTUs and the two replicates **(c)**. This shows clear separation between the different species except for a single sample of *C.* "short" clustering with *C. pollicaris* consistently in both its replicates.

### 5.3.3 Bacterial diversity and composition

Using the VSEARCH OTU results, which were considered more conservative relative to the QIIME outputs, the diversity of the Greengenes OTU matches was assessed with respect to the bacterial taxa associated with each of the psyllid species. With the diversity revealed, albeit to mixed taxonomic levels, detection of any specific correlations or anomalies that may be apparent was also considered. In some cases, further confirmation was sought by individual BLASTs to NCBI's GenBank. A broad overview of the main bacterial groups across all the psyllid species analysed is presented in Table 5.3, each group of which is considered in more detail below.

**Table 5.3:** Presence of the most recorded bacteria is reported for each psyllid species, comprising a number of individuals (**Indiv**) and populations (**pop**). This include P-symbiont (***Carsonella***), S-symbiont (Enterobacteriaceae, **Ent.**), *Wolbachia* (***Wol.***) and *Pseudomonas* (***Pseu.***). For the P-symbiont, the different OTUs recorded are reported, in agreement with Figure 5.3, including the three OTUs that resulted identical to others, reported in brackets **(=)**. For the Enterobacteriaceae, the number of different OTUs is reported; with different OTUs reported in brackets for the species that showed different Enterobacteriaceae between New Zealand and Australia. The presence of *Wolbachia* and *Pseudomonas* is marked with X. The presence of putative insect and plant pathogens is also reported as: **Ri**=*Rickettsiella*, **My**=*Mycoplasma*, **Lib**=*Liberibacter*, **Card**=*Cardinium*, **Ham**=*Hamiltonella*, **Phlo**=*Phlomobacter*, **Rh**=*Rhabdoclamydia*, **Phyto**=*Phytoplasma*.

| **Species** | **Indiv/ pop** | ***Carsonella* OTUs** | **Ent. nº** | ***Wol.*** | ***Pseu.*** | **Pathogens** | |
|---|---|---|---|---|---|---|---|
| | | | | | | **Insect** | **Plant** |
| **Aphalaridae** | | | | | | | |
| *Anoeconeossa* sp. | 1/1 | 149 | 1 | | | | |
| *Anomalopsylla* POLLEN ISLAND | 3/1 | 128(=178) | 2 | | | Card, Ham | |
| *Blastopsylla occidentalis* | 2/2 | 103 | 4 | | | | |
| *Cryptoneossa* sp. | 1/1 | 203 | 1 | | | | |
| *Ctenarytaina clavata* | 2/2 | 543; 943 | 1 | X | X | My | My |
| *Ctenarytaina clavata* B | 1/1 | 543; 943 | 1 | X | X | | |
| *Ctenarytaina eucalypti* | 23/15 | 142 | 3 | | X | My | Phlo, My |
| *Ctenarytaina fuchsiae* | 18/15 | 71 | 4 | X | | My | Lib, Rh, My |
| *Ctenarytaina fuchsiae* B | 7/1 | 71 | 1 | X | | My | Lib, Rh, My |
| *Ctenarytaina longicauda* | 3/2 | 142 | 1 | | | Ri, My | My |
| *Ctenarytaina pollicaris* | 5/2 | 543; 943 | 1 | X | | My | My |
| *Ctenarytaina pollicaris* B | 1/1 | 543; 943 | 1 | X | | My | My |
| *Ctenarytaina* SHORT | 9/6 | 543; 943 | 1 | X | X | My | Rh, My |
| *Ctenarytaina* sp. A | 1/1 | 71 | 1 | | | My | My |
| *Ctenarytaina* sp. B | 2/2 | 71 | 1 | X | | My | Phyto, My |

| Species | Indiv/ pop | *Carsonella* OTUs | Ent. nº | *Wol.* | *Pseu.* | Pathogens | |
|---|---|---|---|---|---|---|---|
| | | | | | | Insect | Plant |
| *Ctenarytaina spatulata* | 11/11 | 69 | 3 | X | X | | Lib |
| *Ctenarytaina thysanura* | 1/1 | 69 | 1 | | | My | My |
| *Ctenarytaina* unknown | 1/1 | 142 | 1 | | | My | My |
| *Eucalyptolyma maideni* | 1/1 | 158 | 1 | | | | |
| *Glycaspis granulata* | 1/1 | 293 | 1 | | | Ri | |
| **Calophyidae** | | | | | | | |
| *Calophya schini* | 2/1 | 190 | 1 | | | | |
| **Homotomidae** | | | | | | | |
| *Mycopsylla fici* | 3/2 | 247(=304) | 1 | X | | | |
| **Liviidae** | | | | | | | |
| *Psyllopsis fraxini* | 3/3 | 243 | 1 | X | | | |
| *Psyllopsis fraxinicola* | 4/4 | 146 | 1 | X | | | Rh |
| **Psyllidae** | | | | | | | |
| *Acizzia acaciaebaileyanae* | 1/1 | 677 | 1 | | | Ri | |
| *Acizzia acaciae* | 4/4 | 85 | 1 | | X | My | Lib, My |
| *Acizzia albizziae* | 6/5 | 91 | 1 | X | X | | |
| *Acizzia dodonaeae* | 8/6 | 119 | 2 | X | X | | |
| *Acizzia hakeae* | 3/3 | 677; 588; 101 | 1 | | | My | My |
| *Acizzia jucunda* | 5/2 | 677; 588; 85 | 2 | X | | My | My |
| *Acizzia solanicola* | 2/1 | 189 | 2 | | | My | My |
| *Acizzia* sp. A | 3/3 | 101 | 4 | | | | |
| *Acizzia uncatoides* | 6/5 | 101 | 1(+1) | X | | My | My |
| *Arytainilla spartiophila* | 2/2 | 113; 1780 | 1 | | | Ri | |
| *Baeopelma foersteri* | 2/2 | 171 | 2 | | | | |
| *Psylla apicalis* A | 4/4 | 195 | 3 | X | | | |
| *Psylla apicalis* B | 4/4 | 195 | 3 | | | | |
| *Psylla carmichaeliae* A | 3/2 | 108; 895 | 1 | X | | | |
| *Psylla carmichaeliae* C | 2/2 | 108; 895 | 1 | X | | | |
| *Psylla carmichaeliae* E | 3/3 | 108 | 1 | | | | Rh |
| **Triozidae** | | | | | | | |
| *Bactericera cockerelli* | 1/1 | 471 | 1 | X | | | |
| *Casuarinicola australis* | 2/2 | 210 (=230) | 2(+1) | | | My | My |
| *Trioza acuta* | 2/2 | 262 (=274) | 2 | | | | |
| *Trioza acuta* B | 1/1 | 246 | 2 | X | | | |
| *Trioza bifida* | 5/4 | 173 | 2 | | | My | My |
| *Trioza* BRENDA MAY | 1/1 | 173; 412; 470 | 4 | X | | | |
| *Trioza colorata* | 3/2 | 161 | 6 | X | | My | My |
| *Trioza decurvata* | 2/2 | 173 | 1 | X | X | | |
| *Trioza discariae* | 3/2 | 412 | 6 | X | | | |
| *Trioza doryphora* | 4/4 | 173; 968 | 2 | | | | |
| *Trioza emarginata* | 1/1 | 470 | 2 | X | | | |

| Species | Indiv/ pop | *Carsonella* OTUs | Ent. nº | *Wol.* | *Pseu.* | Pathogens | |
|---|---|---|---|---|---|---|---|
| | | | | | | Insect | Plant |
| *Trioza eugeniae* | 1/1 | 113 | 1 | | | My | My |
| *Trioza falcata* | 4/4 | 60; 161; 173 | 6 | X | | | Phlomo |
| *Trioza fasciata* | 2/2 | 60 | 2 | X | | My | My |
| *Trioza hebicola* | 1/1 | 173 | 1 | X | | | |
| *Trioza irregularis* | 3/3 | 38 | 1 | | X | My | Phyto, My |
| *Trioza* MASSEY | 1/1 | 38; 60 | 2 | | | | |
| *Trioza obscura* | 1/1 | 173 | 1 | X | | | |
| *Trioza* OMAHUTA | 1/1 | 173 | 4 | X | X | | |
| *Trioza panacis* | 1/1 | 38 | 1 | | | My | My |
| *Trioza* PRICE'S VALLEY | 1/1 | 963 | 1 | X | | | |
| *Trioza* sp. C | 1/1 | 38 | 1 | X | | | |
| *Trioza subacuta* | 2/2 | 173; 968 | 4 | X | | | |
| *Trioza vitreoradiata* | 9/9 | 38; 60; 173; 161; 968; 412; 470 | 2 | X | X | | Phyto |
| Triozid sp. | 4/4 | 152;240 | 2 | X | | | Phlo |

#### *5.3.3..1 Diversity of the primary symbiont: Candidatus Carsonella rudii.*

Using VSEARCH, *Ca.* Carsonella rudii, was never identified to the species level. However, a total of 50 OTUs equivalent to 3.7% of the sequences (239,523 reads) across all six families known in New Zealand were identified as Halomonadaceae, the family to which *Carsonella* belongs. When blasted against the nucleotide NCBI database website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) all were identified as *Ca.* Carsonella rudii but with considerable variation (Figure 5.3) that amounted to a 95% to 100% range in sequence similarity to the top blast results.

An alignment of the 50 Halomonadaceae OTUs revealed 100% similarity for three of them, bringing the total to 47 different OTUs. The same alignment (Figure 5.3) showed that the different OTUs matched the psyllid taxonomy at the family and genus level, with different psyllid genera and families recording different *Carsonella* OTUs.

Some species such as *T. bifida, T. emarginata,* and *T.* "Omahuta", recorded a single *Carsonella* OTU. However, for others within the same genus different OTUs are shared between multiple species. Moreover, some species such as *Trioza vitreoradiata* recording up to seven different *Carsonella* sequences (Table 5.3). This unexpected record strongly contrasts with the hypothesis of a single infection of the P-symbiont and the subsequent coevolution with the psyllid host (Thao et al. 2000a, Hall et al. 2016). However, comparing different OTUs recorded within the same sample revealed that many were divergent by only 2% or 3%, suggesting this list may be an

overestimation in the biodiversity. For example, OTUs 108 and 895 found in both *Psylla carmichaeliae* "A" and "C"; OTUs 943, 543 and 71 found in various species of native *Ctenarytaina*; and the OTUs 38, 60, 161, 173 and 470 recorded in the genus *Trioza* show variation amongst them of 2% - 3% suggesting that they could be consolidated as only three OTUs instead of 10. Conversely, OTUs 262 from *T. acuta* and 246 from *T. acuta* "B" showed variation of 4% between each other and 4% to 7% compared to the Trioza-containing OTUs above. Also OTU 963 from *T.* "Price's Valley" was >4% different from any other sequence, except for a 97% similarity with OTU 173.

Therefore, even after this partial reduction in OTU count, different *Carsonella* sequences could be found within the same insect species. For example, *T.* "Brenda May" showed the co-occurrence within the same specimen of the 4% divergent OTUs 968 and 412. Then *T. vitreoradiata* not only reported the same two OTUs as in *T.* "Brenda May", but also OTU 161 showing a 4% variation compared to OTUs 60, 412 and 968.

#### *5.3.3..2 Inventory of secondary symbionts: Enterobacteriaceae*

The bacteria that were recorded with the greatest number of reads and of OTUs from the Greengenes database, independently of the pipeline used, belong to the family Enterobacteriaceae. While the QIIME1 pipeline identified only five OTUs belonging to this family, VSEARCH isolated 106, of which 24 appear among the 34 most recorded OTUs (Table 5.1). Of these 106 Enterobacteriaceae OTUs, 28 recorded less than 300 reads and were therefore not considered further, while the other 78 were aligned to generate a 16S gene tree to assess the genetic distance between them (Figure 5.4). The genetic distances between the bacterial sequences do not match the psyllid taxonomic subdivision at a species level, with the same OTU often present in different species and families (green dots, Figure 5.4) or with multiple OTUs in the same individual insect (e.g. *T. falcata*, red dots in Figure 5.4). The average genetic distance between these OTUs was 11%, with the greatest of 26%. This, and the fact that some psyllid species recorded multiple OTUs (Table 5.3), suggested the presence of multiple S-symbionts even within the same insect. When comparing the different Enterobacteriaceae OTUs with sequences in NCBI, the closest results were between 92% and 94% similarity to "*Sodalis*-like sequences" and between 99%-100% similar to other S-symbiont sequences isolated from other psyllids (such as *C. schini* and *M. fici*). This high level of variation had also been reflected within the same QIIME1 OTU, showing sequences more similar to *Sodalis* and other more similar to *Arsenophonus* being clustered together (Figure 5.1). Some OTUs for this family could also be taxonomically resolved to genus, to include *Sodalis*-like, *Blochmannia*-like, *Arsenophonus*-like and *Schneideria*-like. In addition to these more defined clusters in Figure 5.1, many other sequences could not be identified any more specifically than to the family-level.

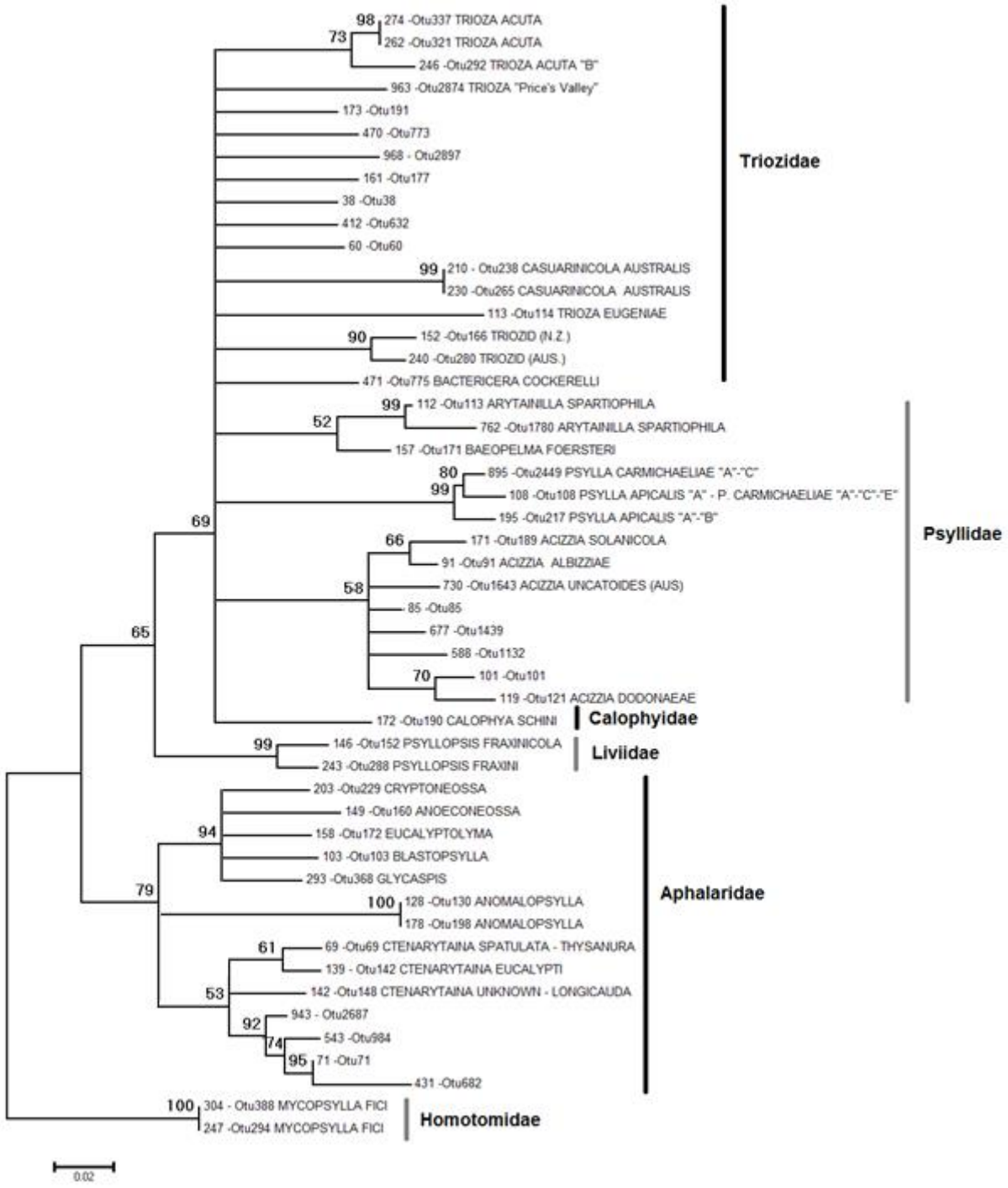


**Figure 5.3:** 16S gene tree (Maximum Likelihood, 10000 replicates, branches collapsed when bootstrap <50%) of *Carsonella* sequences generated by VSEARCH amongst all the samples analysed across all New Zealand psyllid families. For each OTU, the first number identifies the OTU post quality control, while the "Otu-number" is the number originally assigned by VSEARCH. The bacterial OTU tree matches the psyllid taxonomic subdivision at a species level (species names are reported on the right, unless the same OTU was recorded in multiple species, as per the genus *Trioza,* in which case no name is reported).

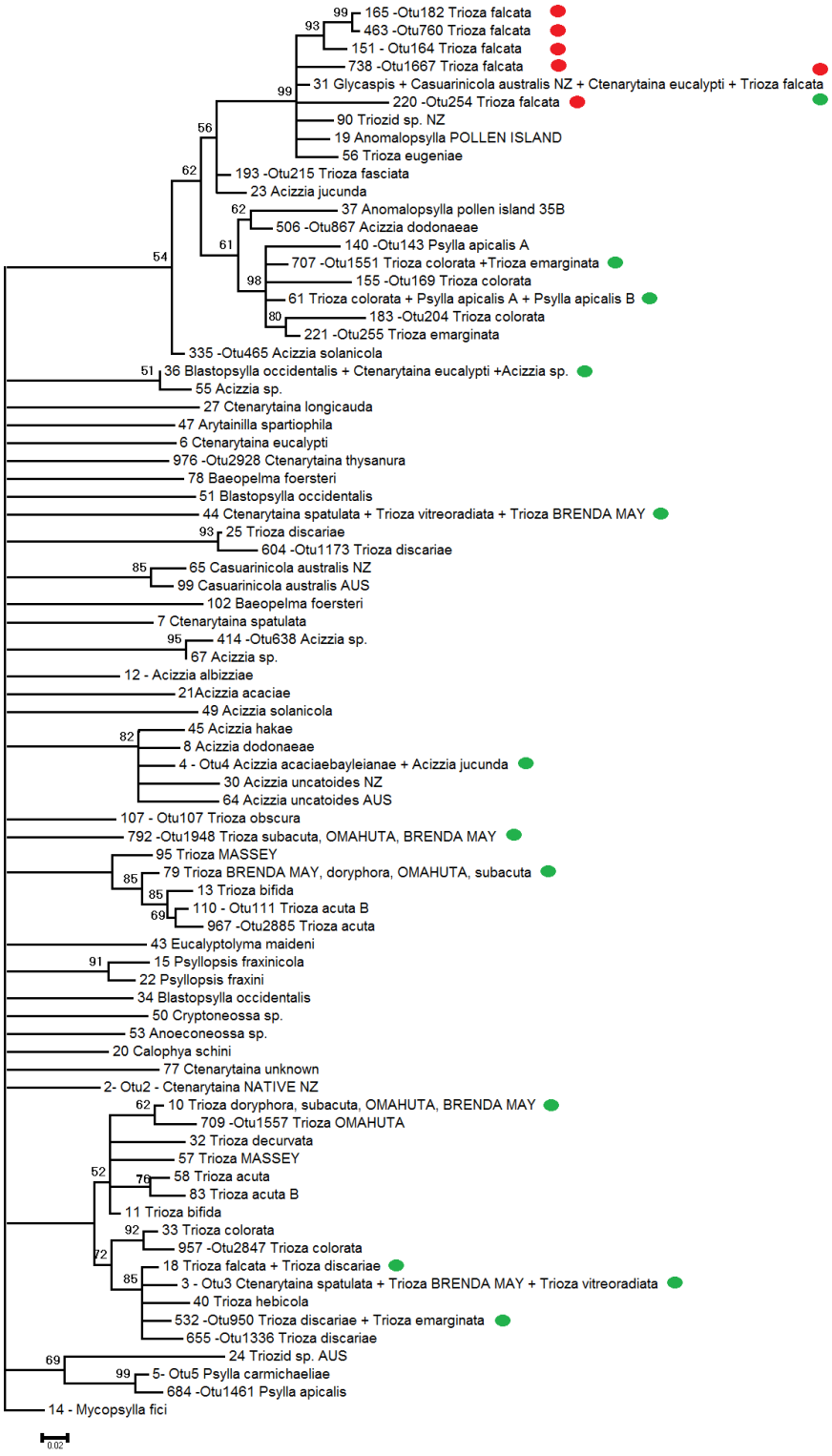


**Figure 5.4:** 16S gene tree (Maximum Likelihood, 10000 replicates, branches collapsed when bootstrap <50%) of Enterobacteriaceae sequences generated by VSEARCH from all the New Zealand psyllid taxa in this study. For each OTU, the first number identifies the OTU post quality control, while the "Otu-number" is the number originally assigned by VSEARCH. Red dots highlight the presence of multiple OTUs in the same individual insect, while green dots report the same OTU across different families.

#### *5.3.3..3 Inventory of additional bacteria*

### Insect pathogens and sex modifying bacteria

In addition to the P- and S-symbionts a number of other culturable and unculturable (*Candidatus*) bacteria were recorded by all pipelines, and as illustrated by the QIIME and QIIME2 records in Table 5.2. A brief inventory is provided here:

***Wolbachia*** was the most abundant bacterium after the Enterobacteriaceae. *Wolbachia* (Alphaproteobacterium) was recorded in 135 insects belonging to ten of the 17 psyllid genera across all psyllid families.

***Rickettsiella*** is a Gammaproteobacterium and known insect pathogen. Here it was recorded in five insects at a maximum compositional level of 41.2%: *Acizzia acaciaebaileyanae, Arytainilla spartiophila, Ctenarytaina longicauda* (both the samples analysed) and *Glycaspis granulata*. All these psyllid species are adventive to New Zealand.

***Mycoplasma*** was recorded from 42 samples consisting of eight *Trioza,* 28 *Ctenarytaina,* one *Casuarinicola* and five *Acizzia.* The seven samples recording the highest levels of *Mycoplasma* were all *Ctenarytaina* species.

***Candidatus* Rhabdochlamydia** was recorded in six psyllids comprising four *Ctenarytaina* spp., one *Psylla carmichaeliae* "C" and one *Psyllopsis fraxinicola.*

***Candidatus* Hamiltonella** was recorded only in a single sample of the four *Anomalopsylla* "Pollen Island", with 5,200 reads, enough to suggest it was not contamination.

***Candidatus* Cardinium** was recorded in all four *A.* "Pollen Island" samples analysed with an average number of reads of 1,500.

### Other high level bacteria

An additional three OTUs covered 356,926 reads across the dataset, equal to the 5.5% of the total reads. All are known to occur in insects:

***Acidovorax*** (Comamonadaceae, Betaproteobacteria) was recorded in a total of 216 samples with the average abundance of 1.39%. It was not recorded in the genera *Bactericera, Baeopelma* and *Mycopsylla*, and in the species *Trioza emarginata.*

***Pseudomonas*** (Gammaproteobacteria) was detected in 135 psyllids from seven genera. This OTU made up 7.72% of all sequences from the *Acizzia*, compared to 2.83% from other genera combined.

***Agrobacterium*** was recorded in 73 specimens, usually at very low levels (<1%) with the exception of nine samples of *Ctenarytaina* where it occurred at much higher levels of 18.2% - 42.7%).

## Plant pathogens

A number of bacterial genera identified as plant pathogens or possible plant pathogens due to previous report present in the literature. While pathogenicity was not assessed in this study, the presence of these bacteria was considered of interest and therefore reported here.

***Candidatus*** **Liberibacter** was isolated in a single OTU using QIIME1 recorded in seven samples consisting of four *Ctenarytaina fuchsiae* and one sample of each *Ctenarytaina fuchsiae* B, *Ctenarytaina spatulata* and *Acizzia acacia.* The single OTU identified here as *Liberibacter* included different sequences showing a high genetic variation (>3%). These same sequences have been identified only as *Candidatus* species of the family Rhizobiaceae using VSEARCH, but in three separate OTUs. However, using the specific *Liberibacter* primers, three different sequences could be isolated:

- ***Candidatus*** **Liberibacter,** possibly **ctenarytainae,** isolated from *C. fuchsiae* and recently described (Smith and Thompson 2017, personal communication). The five samples of *C. fuchsiae* were not analysed further with specific PCR despite recording *Liberibacter*. In fact, these samples reported the presence of a single *Liberibacter* DNA sequence that, in other geographically close population had previously been identified as *C.* Liberibacter ctenarytainae.

- ***Candidatus*** **Liberibacter brunswickensis** was recorded in the single OTU from *A. acacia,* with a 99% similarity to this bacterium recorded from *Acizzia solanicola* in Australia (Morris et al. 2017)

- **Undescribed *Liberibacter*** OTU from *C. spatulata* showed 99% similarity with a sequence previously recorded in the Hamilton area from a single potato in 2008 (Liefting 2017, personal communication).

***Candidatus*** **Phytoplasma 'pseudopanacis'** from a single OTU was recorded in all three samples of *Trioza irregularis* to a maximum compositional level of 32.5%, and at comparatively low levels in one sample each of the nine *T. vitreoradiata,* five *T. bifida,* three *Anomalopsylla "*Pollen Island" and one individual of *Ctenarytaina* sp. B. This is a new *Phytoplasma* species detected in 2011 associated with dieback of *Pseudopanax* and *Pittosporum* species in New Zealand (Liefting 2017, personal communication).

***Candidatus Phlomobacter*** from a single OTU was recorded in six insects: two *Trioza falcata,* three *Ctenarytaina eucalypti* and one undescribed species from *Casuarina.*

### 5.3.4 Comparison of New Zealand and Australian microbial communities

In order to assess whether microbiome composition was influenced by geographic region, samples of the Australian native *Ctenarytaina eucalypti* on *Eucalyptus globulus* and *Acizzia uncatoides* on *Acacia* sp. collected in both New Zealand and Australia were compared. All samples were run in the same sequencing plate to avoid possible bias due to the sequencing run.

In Figure 5.5, two New Zealand populations (both from the central area of the South Island) of *C. eucalypti* are compared to two Australian populations (Adelaide and Melbourne). Of note, the presence of the orange Enterobacteriaceae OTU 31 appears limited to the New Zealand samples with those from Australia showing higher levels of a different Enterobacteriaceae (OTU 36, grey), a Bradyrhizobiaceae (green) and a Flavobacteriaceae (dark blue) OTUs. The genetic distance between the Enterobacteriaceae OTUs 31 and 36 is 11%. However, intra-population variation, comprising single insects from the same individual plant, is also observed. For example, Enterobacteriaceae (orange) OTU 31 is absent in sample 39C compared to 39A and 39B and in 42C compared to 42A and 42B, plus absence of the Enterobacteriaceae (grey) OTU 36 in the samples SA2B and SA2C compared to SA2A. Therefore, while this small study suggests differences in microbial composition of well separated geographic populations may occur, multiple within-population sampling is key and more in-depth analysis by quantitative PCR is needed to understand if this variation is statistically significant or quantitatively biased by the PCR amplification process.

Any qualitative bias that may be associated with the universal PCR priming used here was considered unlikely as any sequence variation within these single VSEARCH OTUs was <3%.

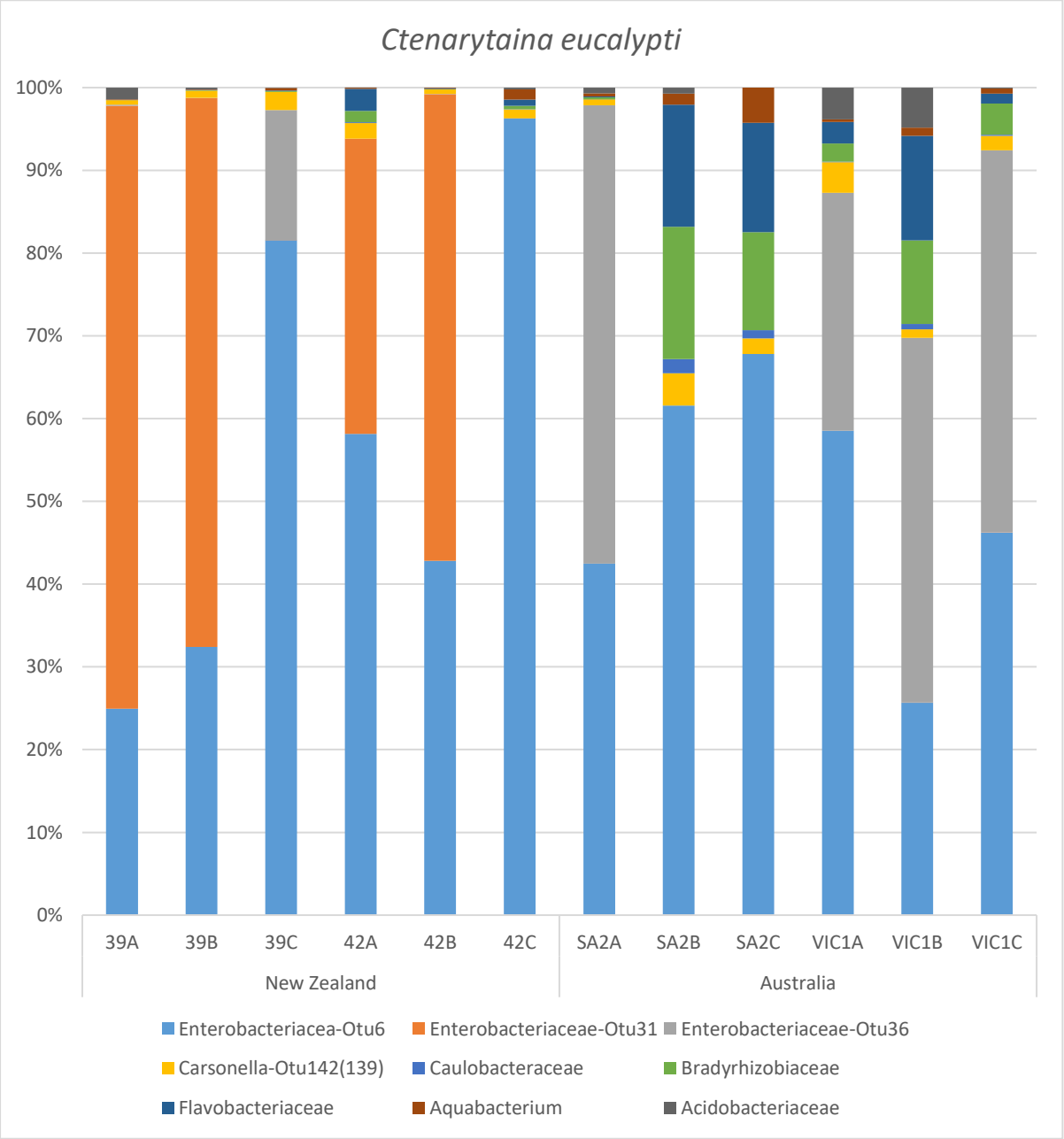


**Figure 5.5:** Microbial composition of 12 *C. eucalypti* samples belonging to two New Zealand populations (three insects each for individual plant populations 39 and 42, from the Canterbury region) and two Australian populations (three individuals each for populations VIC1 and SA2, respectively from Melbourne, Victoria and Adelaide, South Australia). The presence of multiple Enterobacteriaceae OTUs is reported across all the samples (OTU 6, light blue), only in the New Zealand specimens (OTU 31, orange), or at higher levels, but not exclusively, in the Australian samples (OTU 36, grey). The bacteria reported were selected for being present with more than 100 reads across the 12 samples.

Similarly, in Figure 5.6, *Acizzia uncatoides* was collected from *Acacia* sp. in Australia (Melbourne, Victoria) and New Zealand (four populations, 65, 75, 178, 181). Different Enterobacteriaceae OTUs were reported in the two countries, with a *Sodalis*-like OTU for the New Zealand samples (OTU 30) and a *Brenneria*-like OTU for the Australian ones (OTU 64). These two Enterobacteriaceae OTUs show a genetic distance of 19%. Moreover, higher levels of *Wolbachia* and *Pseudomonas* are reported for the Australian species. Here, while the presence of *Wolbachia* and *Pseudomonas* was recorded in both the countries (despite being at different levels), the presence of different Enterobacteriaceae OTUs was strictly associated with each country. However, this limited dataset can only highlight possible OTUs to target in future studies and, in light of the *C. eucalypti* results above, would need to be supplemented with multiple insects from the same individual plants.

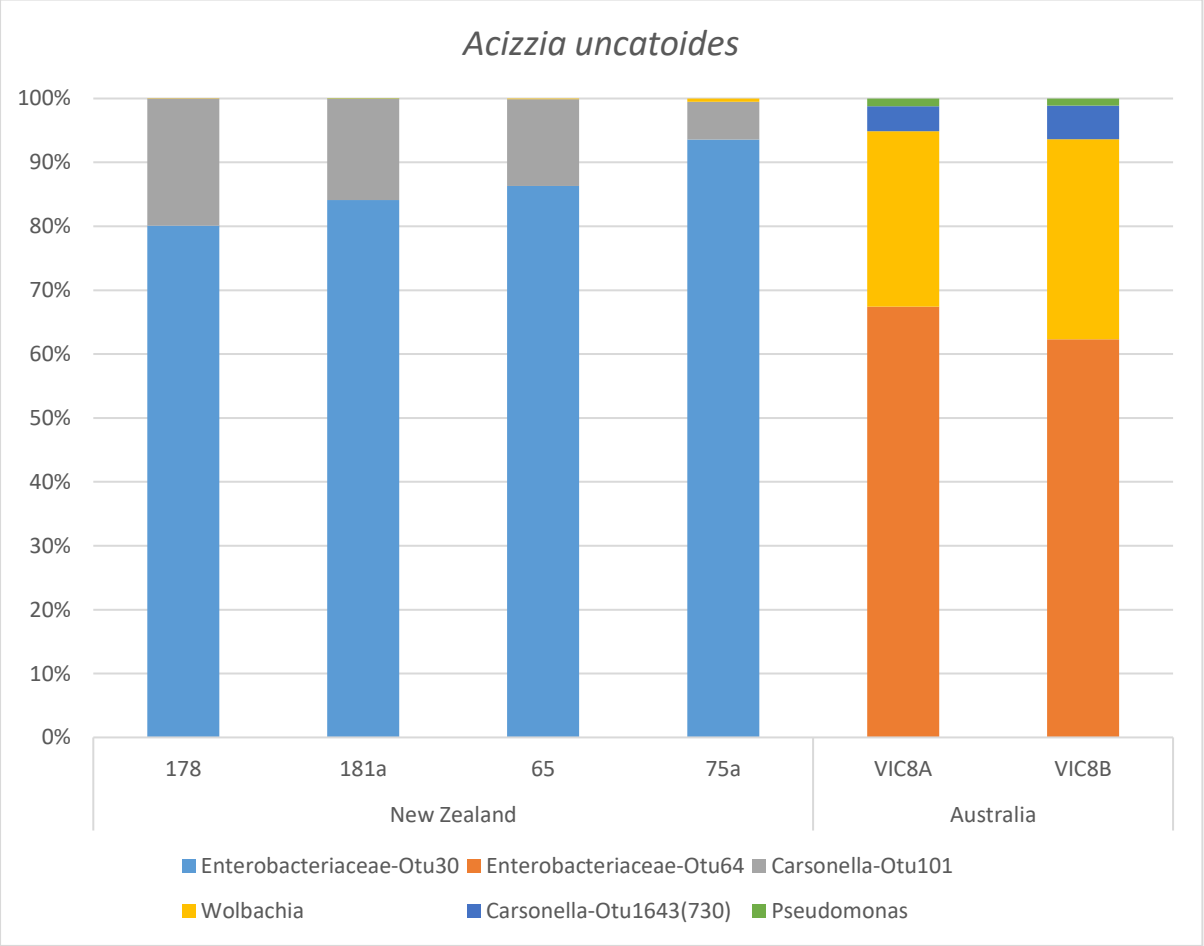


**Figure 5.6:** Microbial composition of six samples of *A. uncatoides* belonging to four New Zealand populations (across the South Island) and one Australian population (Melbourne, Victoria). The presence of different Enterobacteriaceae is reported, with OTU 30 (light blue) for the New Zealand specimens and OTU 64 (orange) for the Australian ones. Moreover, higher levels of *Wolbachia* (yellow) and *Pseudomonas* (green) are reported for the Australian species. The bacteria reported were selected for being present with more than 100 reads across the six samples.

### 5.3.5 Comparison of different psyllid species from the same individual plant

Variation in the microbiome composition of different psyllid species feeding on the same individual plant was considered in order to assess if there were any microbiome-host plant specific relationships. Two analyses were performed on samples belonging to two psyllid species of the *Fraxinus*-feeding genus *Psyllopsis*, *P. fraxinicola* and *P. fraxini,* and on two species belonging to the *Sophora*-feeding genus *Psylla, P. apicalis* A and *P. apicalis* B.

For *Psyllopsis,* different P-symbiont OTUs and different Enterobacteriaceae OTUs are consistently recorded between the two psyllid species (Table 5.4). Conversely, for *Psylla* the same P-symbiont OTU and two Enterobacteriaceae OTUs are present in both species, although three additional Enterobacteriaceae OTUs (5, 143, 208) appear associated with psyllid species (Table 5.5).

Of the other bacteria, *Wolbachia* appeared to be present at consistent levels in the *Psyllopsis fraxini* and *Psylla apicalis* B populations, but vary markedly in the *Psyllopsis fraxinicola* and *Psylla apicalis* A populations. Otherwise there were no stark presence/absence or highly variable levels apparent between either of the pairs for the other OTUs (Table 5.4 and 5.5); the Sphingomonadaceae and Comamonadaceae OTUs appear missing from *Psyllopsis fraxini* population 93-94 (Table 5.4), but as these OTUs are at low to very low levels in the other populations and species, this could as easily be an artefact.

**Table 5.4:** Bacterial reads detected in individuals of *Psyllopsis fraxini* and *P. fraxinicola* collected from the same individual plant in three separate locations (populations 93-94; 106-107; 112-113). The table lists the seven most recorded OTUs

| **Species** | ***P. fraxinicola*** | ***P. fraxini*** | ***P. fraxinicola*** | ***P. fraxini*** | ***P. fraxinicola*** | ***P. fraxini*** |
|---|---|---|---|---|---|---|
| **Psyllid plant population** | **93 -94** | | **106-107** | | **112-113** | |
| **Bacterial OTUs** | | | | | | |
| ***Carsonella*-Otu288(243)** | 891 | 0 | 199 | 0 | 285 | 0 |
| ***Carsonella*-Otu152(146)** | 0 | 18 | 0 | 26 | 0 | 206 |
| **Enterobacteriaceae-Otu22** | 17885 | 0 | 15187 | 1 | 9170 | 1 |
| **Enterobacteriaceae-Otu15** | 0 | 7760 | 1 | 10362 | 0 | 22775 |
| ***Wolbachia*** | 2 | 3484 | 25600 | 2315 | 1 | 9763 |
| **Sphingomonadaceae** | 54 | 0 | 1 | 14 | 24 | 1 |
| **Comamonadaceae** | 80 | 0 | 2 | 21 | 44 | 0 |

**Table 5.5:** Bacterial sequence read levels detected in individuals of *Psylla apicalis* A and *P. apicalis* B were collected from the same individual plant in two separate occasions (populations 200 and 201). The Table lists the 10 most recorded OTUs.

| Species | *P. apicalis* A | *P. apicalis* B | *P. apicalis* A | *P. apicalis* B |
|---|---|---|---|---|
| **Psyllid plant population** | **200** | | **201** | |
| **Bacterial OTUs** | | | | |
| ***Carsonella*-Otu207(195)** | 222 | 718 | 14 | 305 |
| **Enterobacteriaceae-Otu1461** | 11006 | 21936 | 6348 | 8618 |
| **Enterobacteriaceae-Otu61** | 2404 | 5095 | 3291 | 1532 |
| **Enterobacteriaceae-Otu143** | 341 | 0 | 513 | 0 |
| **Enterobacteriaceae-Otu5** | 6 | 1840 | 2 | 733 |
| **Enterobacteriaceae-Otu208** | 0 | 534 | 0 | 103 |
| ***Wolbachia*** | 4 | 2 | 11473 | 1 |
| ***Pseudomonas*** | 7 | 31 | 10 | 42 |
| **Sphingomonadaceae** | 13 | 62 | 16 | 43 |
| **Comamonadaceae** | 10 | 72 | 16 | 52 |

### 5.3.6 Microbial diversity assessment.

#### Alpha diversity test

Alpha diversity is the diversity of organisms in one environment (Whittaker 1960), and the environment considered in this study is the insect. However, diversity of the microbial composition is a function of the sequencing depth, which is extremely variable where extremes of low sequencing depth may still capture high diversity or high sequencing depth may still result in low apparent diversity (Figure 5.7). In fact, a variation can be clearly observed with samples recording a number of reads even higher than 80,000 (for more than 100 bacterial OTUs) while others have just a few thousands reads. Diversity has also been demonstrated here to be a function of the individual PCR characteristics (5.3.2, Figure 5.2). For this reason, the number of OTUs recorded and the alpha diversity generated could be biased by the number of specimens analysed for each species. Consequently, the sample with the smallest number of reads has been used to rarefy the plot in Figure 5.8. While aware of the limitations of rarefaction (McMurdie and Holmes 2014), this allowed the alpha diversity to be compared between the different psyllid species as if every sample had the same number of sequences. The rarefied test shows how the microbiome diversity compares amongst species within the same genus and family (Figure 5.8).

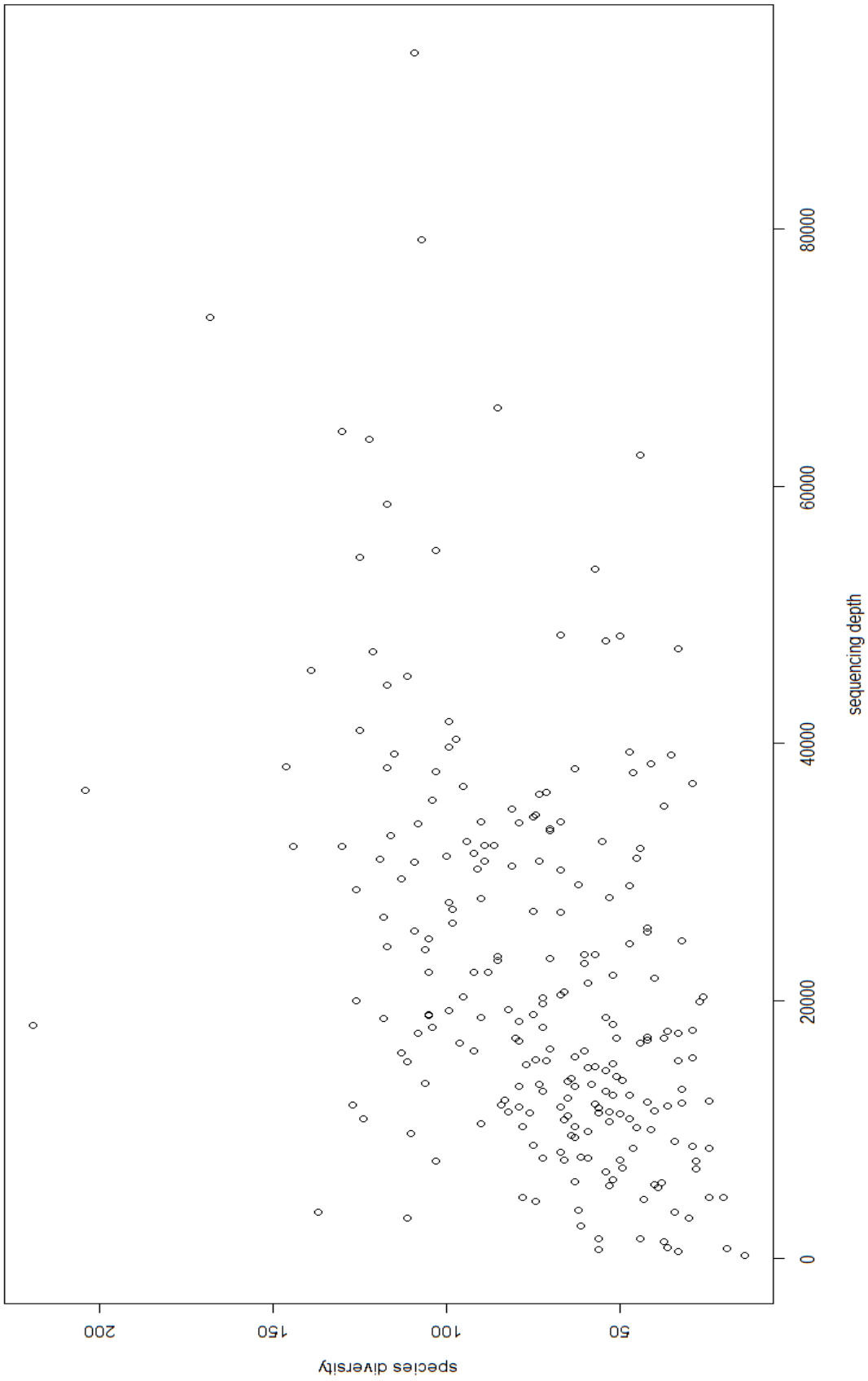


**Figure 5.7:** Bacterial diversity (OTUs) in function of the sequencing depth (Number of reads). Each point is a single psyllid specimen analysed in this work.

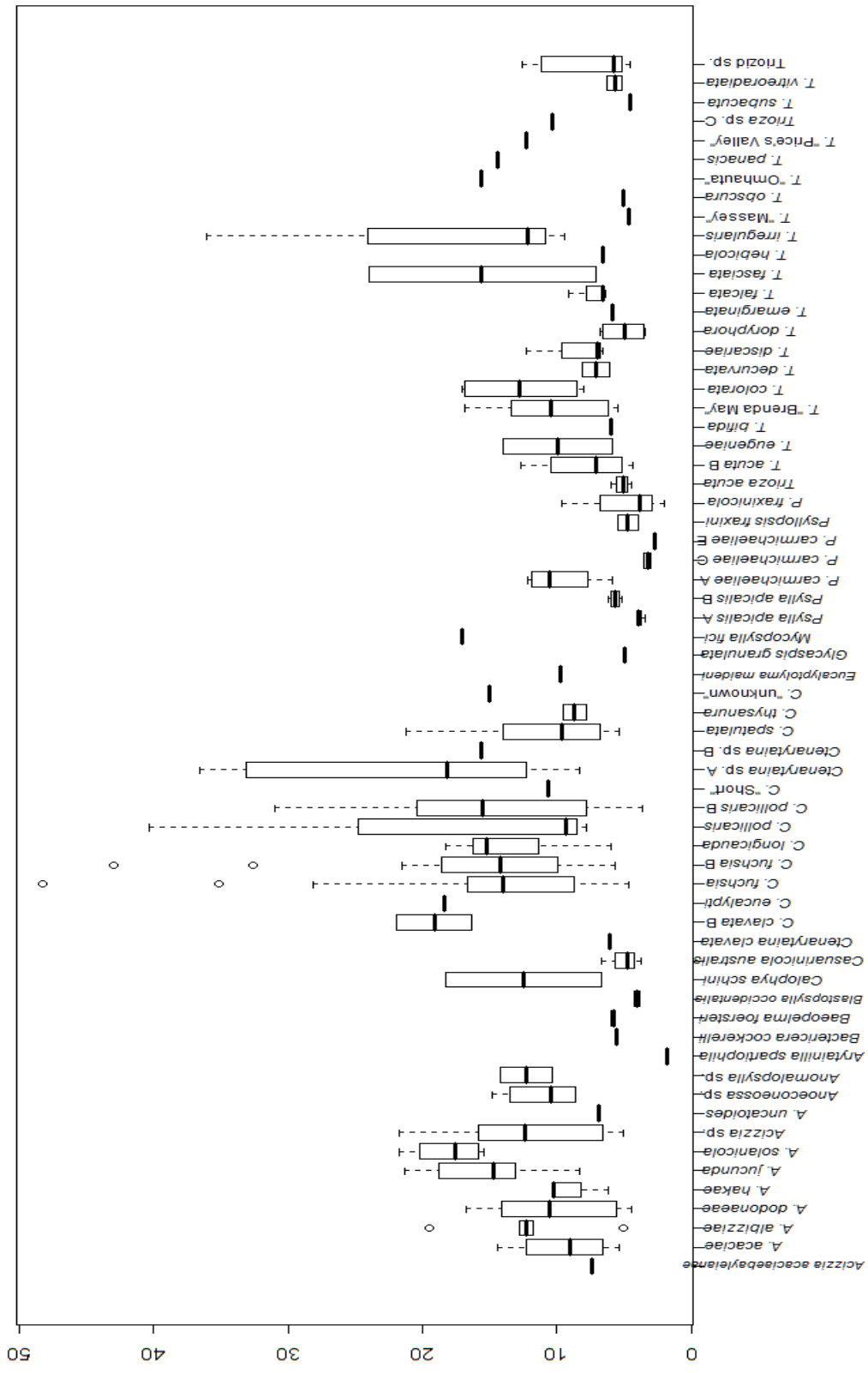


**Figure 5.8:** Rarefied alpha diversity box plot for each psyllid species set at 216 OTUs per sample. Number of OTUs per species range from n=2-48. The columns are the variation in OTU recorded among the samples of each species, the bars are the mean, while the whiskers are the errors.

**Beta diversity test**

Additional tests, such as Beta diversity, are required to determine if the variation recorded with the Alpha diversity test is purely random or can be otherwise associated with other factors.

A beta diversity test using the unweighted UniFrac measure was performed on all the species. From the complete dataset, the genus *Acizzia* has been selected in order to illustrate the patterns between individuals belonging to the same species (Figure 5.9). As expected a lower beta diversity values between individuals belonging to the same species is apparent in the heatmap (in yellow, between 60% and 80%) compared to specimens belonging to different species (in blue, between 80% and 100%). This result was observed across all the samples analysed but, for reasons of space, only *Acizzia* was reported graphically.

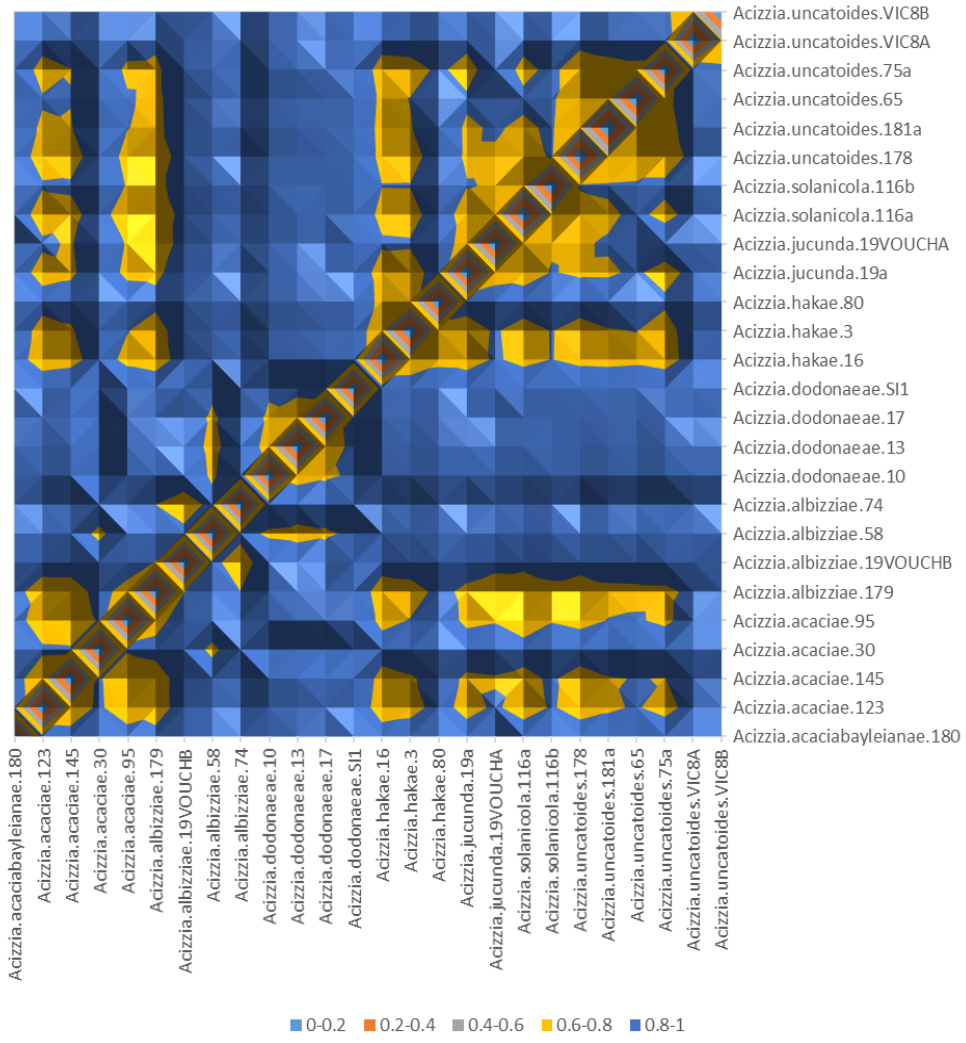


**Figure 5.9:** Heatmap of Beta diversity for *Acizzia*. A lower beta diversity corresponds to a higher similarity in microflora composition between samples. The regions in yellow represent the most similar microbiomes (60% - 80% similarity). The scale shows the colours attributed to the different Beta diversity ranges.

### 5.3.7 Statistical evaluation of geographic and genetic influences on microbial diversity

In order to test how microbiome differences were related to insect phylogeny and insect-host plant associations, a number of analyses were undertaken. The outputs of the different analyses are summarised in Table 5.6 and detailed below.

**ADONIS approach**

The ADONIS approach was performed only on the species with more than two samples present in the dataset for greater robustness. The p-value of 0.001 indicates that at an alpha of 0.05, the grouping of bacteria by psyllid taxonomy is statistically significant. The $R^2$ value 0.6332 indicated that approximately 63.3% of the microflora community groupings can be ascribed to the insect species (Table 5.6). However, this method did not consider the psyllids phylogenetic distance, but simply their subdivision in taxa. Therefore, while confirming that different taxa had different microbial communities, this could not confirm if this pattern was due to insect phylogenetic distance or host plant similarities.

**Mantel Test and partial Mantel test.**

The Mantel test was used to compare the relationship between the psyllid microbiota diversity with i) geographical distances, ii) psyllids phylogenetic distance, and iii) host plant evolutionary distance (in million years).

With the Mantel test the geographic distance accounted for 12.8% of the bacterial biodiversity (Table 5.6). On the other hand, the Mantel test of insect phylogenetic distance accounted for 39.23% of the bacterial community composition (Table 5.6).

Consistent with these results, partial Mantel tests combining these factors, showed that after accounting for the psyllid phylogenetic variation, the geographic variation accounted for 10.7% of the microbial composition (Table 5.6). Similarly, 38.6% of the microbial composition could be ascribed to the psyllid phylogenetic distance after accounting for geographic distance (Table 5.6).

At this stage, a fourth matrix was created for two additional partial Mantel tests to include the genetic distance between the host plants. Firstly, plant genetic distance could account for 15% of the microbial variation after taking into consideration the psyllid phylogenetic distances. Secondly, after considering the host plant genetic distance, the insect phylogeny accounted for 35% of the microbial composition (Table 5.6).

**Table 5.6:** List of analyses performed. The table shows the objects of the analysis (two for Mantel and Adonis, three for Partial Mantel), the $R^2$ value and the P value. The analyses were performed on the full dataset, the "rare species dataset" and the "common species dataset". Highly significant values are reported in yellow, on the right.

| Analysis performed | First object | Second object | Third object | R2 | Pr(>F) | Significance |
|---|---|---|---|---|---|---|
| **FULL DATASET** | | | | | | |
| **ADONIS approach** | | | | | | |
| | **Microbial Community** | Psyllids species | | 0.6332 | 0.001 | Highly signigicant |
| **MANTEL test** | | | | | | |
| | **Microbial Community** | Geographic Distance | | 0.1281 | 0.001 | Significant |
| | **Microbial Community** | Psyllids phylogenetic distance | | 0.3923 | 0.001 | Highly Significant |
| **PARTIAL MANTEL test** | | | | | | |
| | **Microbial Community** | Geographic Distance | Psyllids phylogenetic distance | 0.1071 | 0.001 | Significant |
| | **Microbial Community** | Psyllids phylogenetic distance | Geographic Distance | 0.3856 | 0.001 | Highly Significant |
| | **Microbial Community** | Plants Genetic Distance | Psyllids phylogenetic distance | 0.1509 | 0.001 | Significant |
| | **Microbial Community** | Psyllids phylogenetic distance | Plants Genetic Distance | 0.3535 | 0.001 | Highly Significant |
| | | | | | | |
| **RARE SPECIES DATASET** | | | | | | |
| **PARTIAL MANTEL test** | | | | | | |
| | **Microbial Community** | Psyllids phylogenetic distance | Geographic Distance | 0.033 | 0.063 | Not Significant |
| | | | | | | |
| **COMMON SPECIES DATASET** | | | | | | |
| **PARTIAL MANTEL test** | | | | | | |
| | **Microbial Community** | Psyllids phylogenetic distance | Geographic Distance | 0.3858 | 0.001 | Highly Significant |

To understand if the association between microbial communities and insect phylogenetic distance could be ascribed to a particular group of bacteria, statistical tests on different subsets of the insect microbiomes were performed.

**(a) Rare and common bacteria species:** Since the dataset included psyllid species represented by a single sample, the concept of "rarity" for a bacterium could have been biased by the fact it was found in an under-represented psyllid species. Therefore, "rare" was here defined as those bacterial OTUs that, when present, are in a lower-than-mean abundance (Figure 5.10).

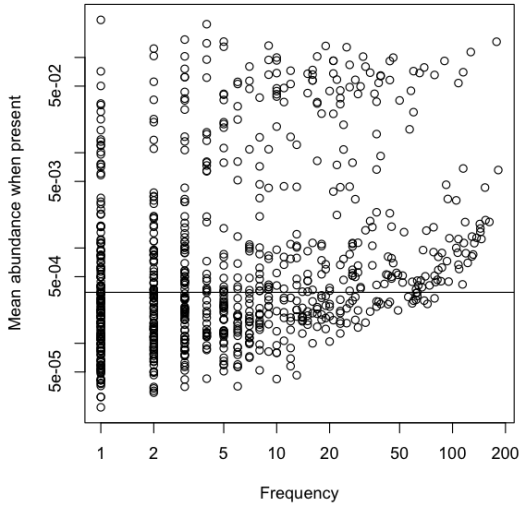


**Figure 5.10:** OTUs frequency compare with their mean abundance when present. This graph resulted in the subdivision of the dataset between "rare" and "abundant" species at the line representing the average mean abundance.

A partial Mantel test found that, after accounting for the geographical distance, the correlation between the composition of rare bacterial species and the insect phylogenetic distance, was not statistically significant (Table 5.6). By contrast, a further partial Mantel test found that 37.5% of the composition of the common bacterial species showed was accounted for by the insects' phylogenetic distance, after accounting or the geographic distance. This was statistically significant (Table 5.6).

**(b) Highly sampled psyllid species:** This data subset consisted of species that were sampled more than five times; *Acizzia albizziae, A. dodonaeae, A. jucunda, A. uncatoides, Ctenarytaina eucalypti, C. fuchsiae, C. fuchsiae* B, *C.* short, *C. pollicaris*, *C. spatulata, Trioza bifida* and *T. vitreoradiata.* A quantitative comparison of the microbial diversity associated with these species is represented in a multi-dimensional scaling (MDS) plot in Figure 5.11. The most obvious difference is that the number of microbial taxa in *T. vitreoradiata* appeared to be less than in all other species. This observation was confirmed by a subsequent Alpha diversity test performed on the same 12 species using all bacterial species (Figure 5.12A) and abundant bacterial species (Figure 5.12B). Here, low diversity was shown for *A. dodonaeae*, which contrasted with the MDS plot (Figure 5.11). For *T. vitreoradiata* the low level of microbial diversity was associated to a high level of Enterobacteriaceae (Figure 5.13). In fact, for the nine individuals analysed, the two Enterobacteriaceae OTUs (with both QIIME and VSEARCH analyses) accounted for more than 70% of the total reads (Figure 5.13). The remainder were mostly accounted for by multiple *Carsonella* OTUs (Table 5.3).

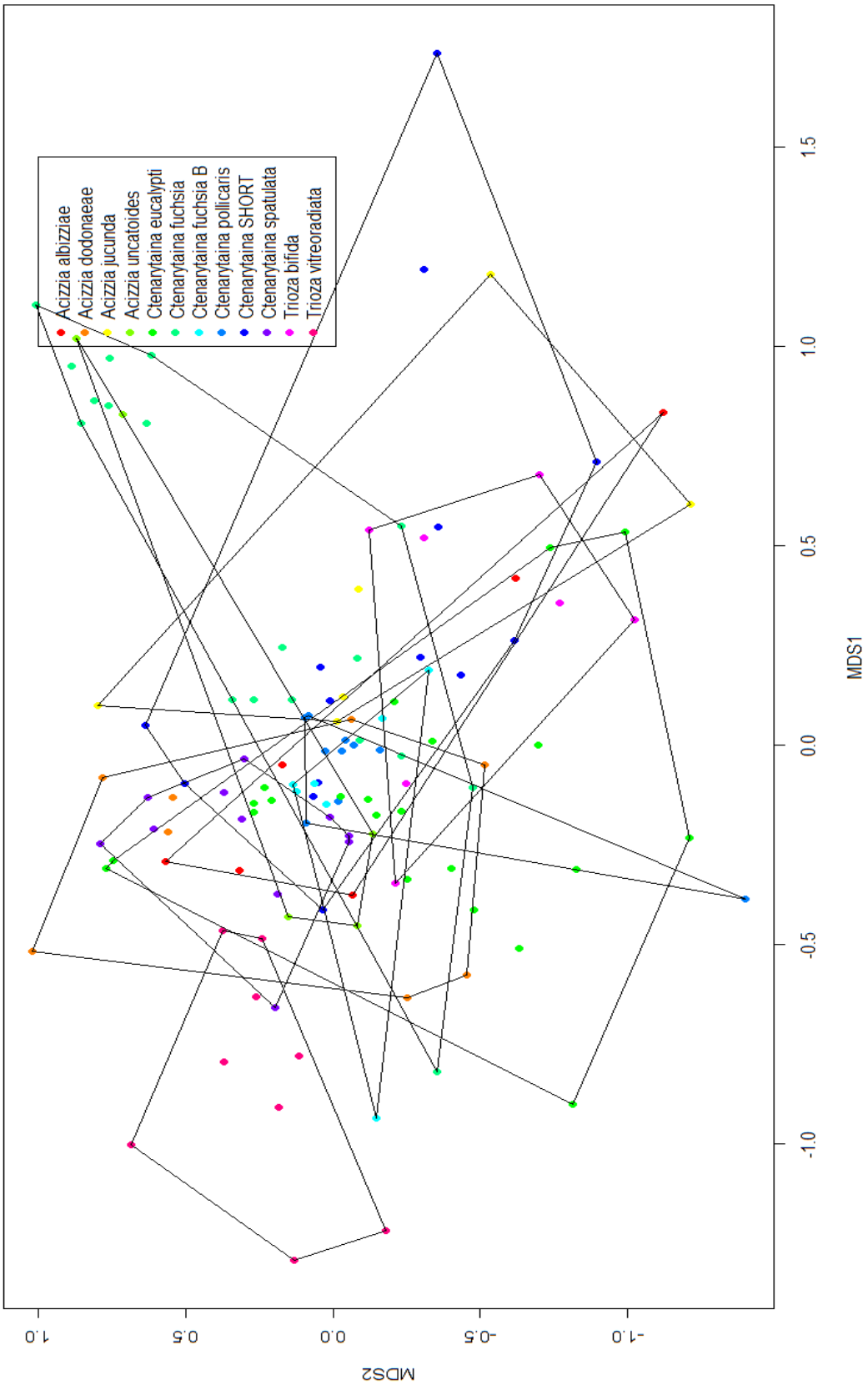


**Figure 5.11:** The MDS plot shows the Beta diversity test of microbial composition of different individuals belonging to the most collected species. Each point is an individual insect.

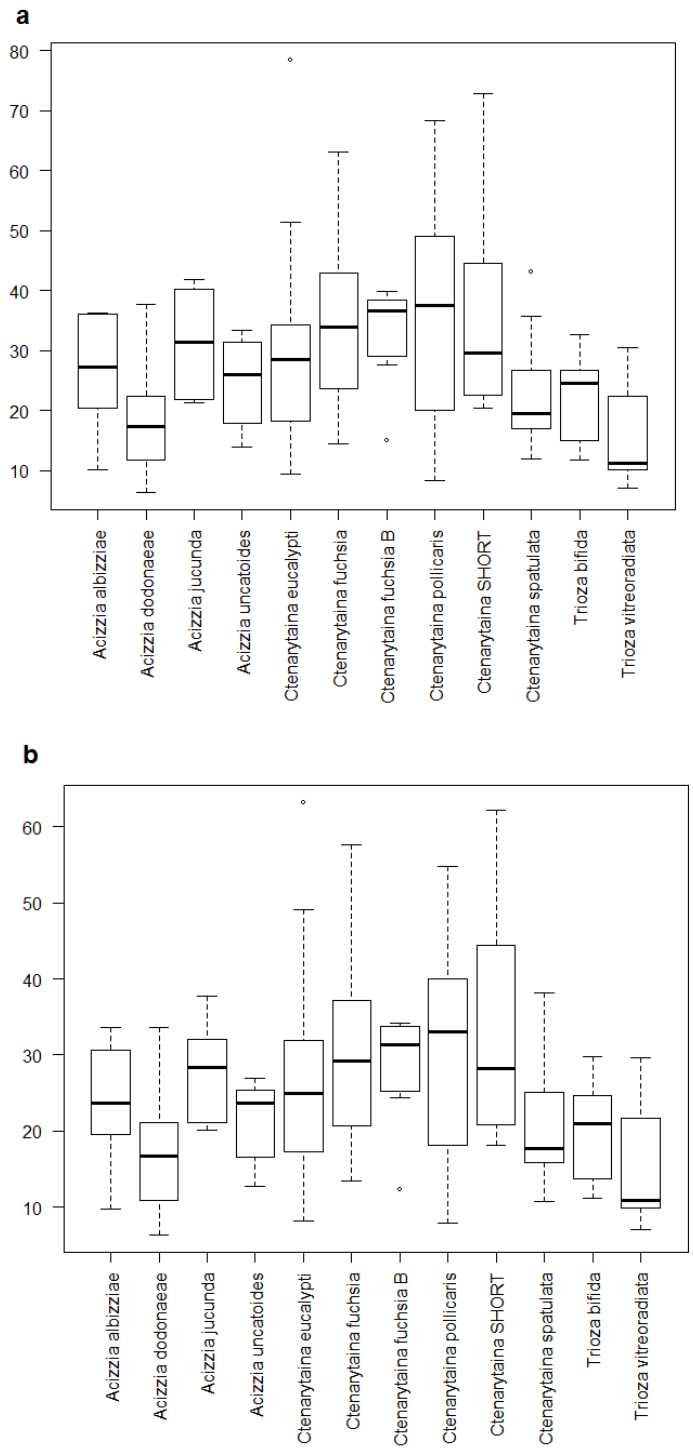


**Figure 5.12:** Alpha diversity analysis box plot performed on the 12 most collected species using both all the bacteria (A) and only the most abundant bacteria (B) as defined *per* Figure 5.10.

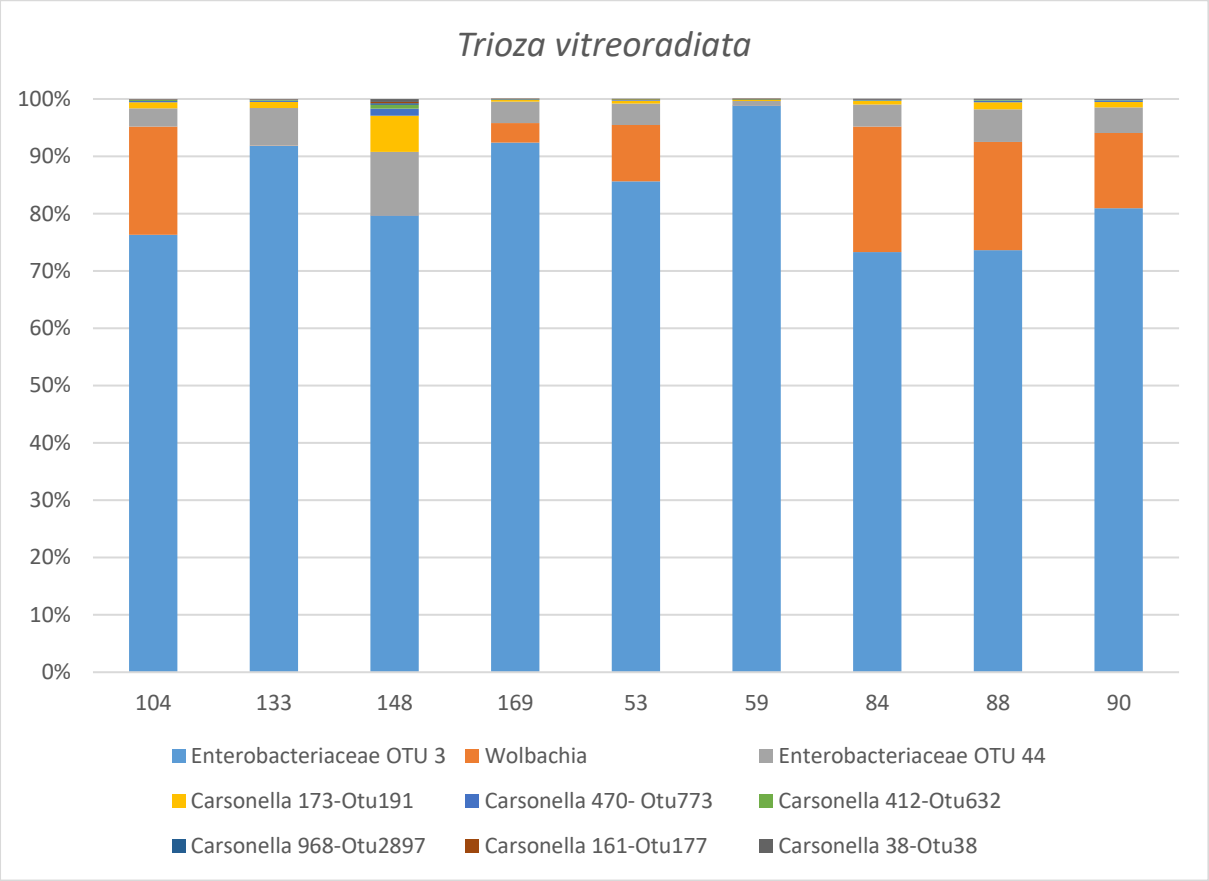


**Figure 5.13:** Histogram showing the nine most present bacteria comprising the microbiomes of nine individuals of *T. vitreoradiata.* High levels of Enterobacteriaceae (in blue and grey) and a number of P-symbiont *Carsonella* OTUs are apparent.

## 5.4 Discussion

### 5.4.1 Consistency of the results and comparison of the different pipelines

#### PCR bias

Variation both in the number of reads and in the number of OTUs was apparent when multiple samples belonging to the same population were compared. This occurred for the number of reads for each OTU as well as in the microbial composition (presence/absence of certain bacteria) in a few instances (e.g. Figures 5.2 and 5.5). At least in part, this may be due to natural variation as the number of reads for each OTU was never the same for samples from the same population either. However, it is also potentially linked to the numerous opportunities for PCR bias known to occur in metabarcode sequencing. Such bias can have a quantitative influence (Krehenwinkel et al. 2017), and may explain the variation observed between separate PCR amplifications for the same individual insect. Consequently, the analysis performed here was based on a presence/absence basis as opposed to considering that a bacterium was "more present" than others, or that a S-symbiont was "dominant" based solely on the number of reads obtained, as has been inferred elsewhere (Morrow et al. 2017)]; although those authors note in the same paper that the risk of PCR bias, for example due to primer mismatch, can be very high for some of the taxa analysed. Quantitative statements, after assessing presence/absence of bacteria with NGS, can instead only be made through taxon-specific real time PCR (Zhang and Fang 2006). Moreover, here rarefaction of the observed OTUs has always been performed in order to avoid bias based on the sequencing depth (number of reads recorded). In fact, the number of sample analysed can bias the OTUs richness recorded as well.

#### Challenges of the taxonomic assignment

Using VSEARCH, the 16S V3-V4 rRNA region used in this study can provide genus-level identification for some taxa, but here was usually confined to a family-specific identification. This is unavoidable due to the highly conserved nature of the 16S rRNA gene that is often insufficient to distinguish evolutionary relationships at a species level (Powell et al. 2016). However, the pipelines QIIME and QIIME2 generally showed a taxonomy assignment at a genus level based merely on the top BLAST hit for each OTU. Consequently, some identifications are quite tenuous; for example, some identified by comparison to GenBank through QIIME as *Sodalis*, had actually only 93% similarity to other *Sodalis* sequences. In addition, the high volume of reads for the two QIIME OTUs identified as "Enterobacteriaceae" compared to the long list of VSEARCH OTUs composed of lower numbers of sequences could indicate that OTUs with the same taxonomical identification may have merged into a single OTU. While this was not specified in any of the scripts used from the QIIME website (http://qiime.org/scripts/), and no information could be found on this scenario, it may be a default setting of the command lines adopted. This would explain i) why the volume of reads in just

two QIIME “Enterobacteriaceae” OTUs is much more elevated as compared to any VESEARCH OTUs and, most importantly, ii) why DNA sequences within the same OTU can show >3% variation, as shown in Figure 5.1.

The accuracy of taxonomic assignments that generate the microbial composition is associated with two main issues. Firstly, underestimation/overestimation of the number of OTUs (as above) may influence interpretation of presence/absence, as revealed elsewhere with QIIME resulting in a very high number (56% - 88%) of false positive genus assignments (Edgar 2017). While that reflected an overestimation in the number of OTUs, and consequent exaggeration of between-sample diversity, the opposite effect was observed with QIIME in the current study where clusters of many Enterobacteriaceae sequences within the same OTU effectively underestimate the total number of bacterial taxa. On the other hand, VSEARCH appeared to slightly overestimate the diversity of bacteria in some instances. For example, three pairs of identical sequences for the Halomonadaceae rendered the initial 50 OTUs to only 47. However, despite this slight discrepancy, this pipeline allowed the different OTUs for both the P- and for S-symbionts to be compared, which would have not been possible using QIIME or QIIME2 since many species-specific P- and S-symbionts OTUs were amalgamated into a few.

Secondly poor curation of the gene sequence databases used is a potential variable (Pible et al. 2014), although these are generally considered highly accurate (Keseler et al. 2014). Deep critiquing of individual sequence assignments was beyond the scope of the current study, but any assignment claiming to distinguish bacterial genera could be better informed by, for example, examination of shared SNPs or phylogenetic analyses of sequences within each OTU.

While the discrepancies above were apparent, comparison of separate pipelines was not the main aim of this study. The use of VSEARCH was a practical tool to investigate the microbial composition of the New Zealand psyllids within the limited number of analyses that were required for this study. QIIME and QIIME2 were trialled as a means to verify the VSEARCH diversity and abundance results. Even so, the same biological conclusions were arrived at, consistent with similar more specific comparative pipeline studies [e.g. (Allali et al. 2017)].

### 5.4.2 The internal microbial diversity of New Zealand psyllids

The microbial dataset generated and analysed here was that for 236 psyllids, belonging to 65 species, 18 genera and six families. This is a significant advance on that of previous such studies that either focused on a smaller taxonomic range of insects, such as the Australian genus *Cardiaspina* (Hall et al. 2016), or used different techniques that generated smaller numbers of sequences (Thao et al. 2000b, Spaulding and von Dohlen 2001). The microbial biodiversity associated with the New

Zealand psyllids revealed both symbionts and pathogens. The following discussion considers the inventory of each of these categories depending on the bacterial role in relation to the insect hosts.

**Primary symbiont: *Candidatus* Carsonella rudii**

Initial analysis performed with VSEARCH did not report a single *Carsonella* OTU. However, direct comparison to sequences in GenBank of 47 OTUs originally identified as Halomonadaceae, matched sequences of *Carsonella rudii.* Given the obligate status of this species in psyllids has been confirmed elsewhere (Thao et al. 2000a, Thao et al. 2001, Hall et al. 2016) and *C. rudii* was recorded in all the samples here, its role as primary symbiont was assumed. Also, alignment of the 47 different OTUs and the construction of a Maximum Likelihood tree obtained suggests that the radiation of this bacterium generally matched the psyllid phylogeny (Figure 5.3). This supports the long-lasting *Carsonella*-psyllids coevolution hypothesis, possibly originated from a single, ancestral infection (Thao et al. 2000a, Thao et al. 2001, Hall et al. 2016).

However, the record of multiple *Carsonella* OTUs (with up to 6% variation) within some native *Trioza, Psylla* and *Ctenarytaina* species was unexpected. There were no obvious technical reasons for this to highlight an anomaly and no literature found to indicate other instances of multiple P-symbiont haplotypes within a single insect. The composition was also consistent within individual insects of the same species; all had the complete OTU set. Opportunity for a psyllid to gather multiple haplotypes is unclear, given the bacterium's vertical transmission within the specialised bacteriocyte cell (Baumann 2005). Also, its close, co-evolved reciprocal symbiosis does not lend it to this scenario, which instead should be a single OTU of *Carsonella* in each psyllid species. With the 6% 16S sequence variation observed here for *Ca.* Carsonella rudii, sequencing of additional genes is required to confirm the presence of multiple haplotypes in the New Zealand native *Trioza* species. This bacterium may also be useful as an additional integrative taxonomy marker for psyllid species delimitation as has been proposed for Australian *Cardiaspina* species (Hall et al. 2016).

If multiple haplotypes in a single insect were to be confirmed, the traditional view of a strict co-evolutionary history between psyllids and their P-symbiont may need to be reconsidered. Possibly a hypothesis of multiple ancestral infections of *Carsonella* and/or the horizontal transmission of this bacterium could be proposed. In keeping with this is the fact that different *Trioza,* such as the *Pseudopanax*-hosted species *T. panacis, T.* sp. C and *T. irregularis* show the same *Carsonella* OTU (OTU 38); this may either be related to their more recent species separation, and/or combined with the limited species specificity of the 16S gene.

**S-symbionts: Enterobacteriaceae**

All analyses reported the family Enterobacteriaceae as the most abundant (Table 5.1). While the QIIME and QIIME2 pipelines clustered the reads in a few OTUs to separate them as specific genera (e.g. *Sodalis*-like, *Blochmannia*-like, *Arsenophonus*-like and *Schneideria*-like), the VSEARCH pipeline identified them as merely 78 distinct Enterobacteriaceae OTUs, only some of which identified as *Sodalis*. When compared to sequences on GenBank, some of the *Sodalis* OTU showed a 91%-94% similarity with *Sodalis* sequences, such as OTU 102 (94% similarity) or OTUs 45, 79, 32 (93%). However, the other Enterobacteriaceae OTUs showed different similarities to other sequences belonging to this family. For example, some were closely associated with the genus *Arsenophonus,* such as OTU 5 (92% similarity), OTU 19 (97%), OTU 165 (95%) and OTU 31 (99%). However, they also show a distribution across the different psyllid species which is sometimes limited to a single OTU per taxa. For example, OTU 37 (100% identical to *Hamiltonella*) present only in *Anomalopsylla.* This highlights a strict psyllid-Enterobacteriaceae relationship. This is in agreement with recent work suggesting coevolution between psyllids and S-symbionts, and may indicate an obligate instead of the anticipated facultative symbiosis (Hall et al. 2016).

Nonetheless, the psyllid relationships with their S-symbionts appear to be less strict than that with the P-symbionts, with a number of taxa recording multiple S-symbiont OTUs with greater than 12% genetic variation. Moreover, the same OTU was recorded in insects belonging to different families, such as OTU 3 retrieved from both *Trioza vitreoradiata* (Triozidae) and *Ctenarytaina spatulata* (Aphalaridae), which is consistent with horizontal transmission. Importantly, these two species are native to different countries and have likely only been together in New Zealand for the last 150/200 years (Chapter 2). This suggests that the S-symbiont acquisition in one or other of these species must have happened in recent times. Again, this supports the concept of a dual nature for psyllid S-symbionts, suggesting they could be both vertically and horizontally transmitted as hypothesised elsewhere (Hall et al. 2016).

The role of other Enterobacteriaceae genera, such as *Arsenophonus*, *Sodalis, Schneideria* and *Blochmannia,* as a S-symbiont of insects has been widely reported for other insects, such as Glossinidae flies (Diptera) (Aksoy et al. 1997), lygaeid stinkbugs (Matsuura et al. 2012), carpenters ants (Schroder et al. 1996, Sauer et al. 2000) and a weevil (Heddi et al. 1998). This may explain why, with the exception of *Schneideria*, all Enterobacteriaceae have been previously assumed as S-symbionts of psyllids as well [e.g. (Thao et al. 2000b, Hall et al. 2016)]. However, the results obtained here suggest that some Enterobacteriaceae S-symbionts of psyllids may be a separate group, with a strong history of coevolution with the Psylloidea. The ~90% sequence similarity with the closer sequences on the database reported here unfortunately does not enable these bacteria to be clearly

assigned to any specific genera, for which further analyses using multiple markers will be needed. Such an approach has been taken for a S-symbiont phylogeny, but was restricted to only 16 psyllid species (Hall et al. 2016).

## Non-symbiotic bacteria

Contrary to the Enterobacteriaceae OTUs, where groups of highly divergent sequences were sometimes clustered together, other OTUs for the non-symbiotic bacteria (considered below) were able to be identified to the genus level, using both QIIME and VSEARCH. All the sequences that clustered in the same OTU were at least 99% similar, showing that these bacteria show almost no variation across the different psyllid species. This may suggest that there is no history of co-evolution, and these bacteria are more related to the environment rather than symbionts of psyllids. Therefore, within the bounds of any potential PCR bias, the wide number of bacteria recorded here is likely to represent the "core microbiome" of the New Zealand psyllids. This checklist will allow future comparison of the microbial diversity between different species, as has been achieved with psyllids in Australia (Hall et al. 2016, Morrow et al. 2017) and other animals, such as the American pikas (Kohl et al. 2017).

In general, no pattern could be recorded that associated the presence/absence of a certain bacterium with the presence/absence of others. However, a number of observations discussed below were made based on i) bacteria present across a higher number of taxa, ii) bacteria strictly associated with a limited number of psyllid species, and iii) pant pathogens known to be associated with psyllids.

### *5.4.2..1 The most recorded individual OTUs: Wolbachia, Pseudomonas, Acidovorax and Mycoplasma*

***Wolbachia*** was the most abundant bacterium here (present in 135 samples across seven genera and five families), and with very high levels in some individual insects (>70% of the total number of reads). In *Diaphorina citri*, *Wolbachia* infection density has been associated with inter-population genotype variation of the bacterium (Chu et al. 2016). However, its occurrence across the broad taxonomic range of infected psyllids in the current study is extremely variable, even including within populations. Moreover, the presence of *Wolbachia* was on occasion recorded together with plant pathogens (such as *Liberibacter*). No apparent variation could be noticed in the composition of microflora of individuals reporting *Wolbachia.* However, the presence of an almost identical OTU of the same *Wolbachia* bacterium (~99%) across different genera and families suggest that this bacterium must be mostly horizontally transmitted; this is consistent with multiple infections (as opposed to the single one of the P-symbiont). Therefore, despite a long standing belief that *Wolbachia* is maternally inherited (Stouthamer et al. 1999), this does not appear consistent with

that in psyllids. Common horizontal transfer of *Wolbachia* has been demonstrated in other insects, including white flies (Li et al. 2017) and butterflies and moth (Ahmed et al. 2016). In psyllids, vectored transmission by parasitoids has been postulated (Morrow et al. 2014, Morrow et al. 2015), although the broad range of psyllids infected by *Wolbachia* in this study include species with no known record of associated parasitoid species.

The interest revolving around this ubiquitous endosymbiotic bacterium of arthropods is mostly due to its diverse repertoire of host reproductive manipulations, such as cytoplasmic-incompatibility (Stouthamer et al. 1999, Duron et al. 2008). Although it is also known to alter insect responsiveness to host plant volatiles or conspecific insects (Koukou et al. 2006, Peng et al. 2008), insect super cooling capacity (Maes et al. 2012), and immune response to pathogens (Frentiu et al. 2010, Wong et al. 2011). In a recent work, *Wolbachia* infections were present in all *D. citri* samples with one sequence type with a broader distribution than the others; therefore it was suggested that it could be used as an alternative strategy to control *D. citri* (Guidolin and Consoli 2013). Based on the broad range of taxa found infected in this study, this bacterium is not target-specific and therefore would not be useful in any pest management effort in New Zealand.

***Pseudomonas*** (Pseudomonadaceae) was recorded in 135 psyllids. This genus has previously been recorded in psyllids (Hail et al. 2012). In particular, Hail and colleagues reported it in *B. cockerelli,* together with *Rhizobium, Gordonia, Mycobacterium, Wolbachia* and *Xanthomonas.* However, no information is available about its role in the psyllids microbiome

***Acidovorax*** (Comamonadaceae) was found in a very high number of samples (214) despite being usually at low levels, as has been recorded for *D. citri* (Saha et al 2012). However, since the semi-quantitative aspect of the metabarcoding analysis used here could be a limiting factor, further analyses with more specific PCR primers would be needed to assess the presence and amount of this bacterium. In New Zealand, this genus includes only *A. cattleyae* and *A. delafieldii* (The Landcare's New Zealand Fungi database - http://nzfungi2.landcareresearch.co.nz). However, other species are widely known for being plant pathogens of crops (Fegan 2007). Therefore, further analyses and screening of this bacterium, including an identification at a species level, would be useful.

***Mycoplasma*** (Mycoplasmataceae) was recorded from 42 samples; in eight *Trioza*, 28 *Ctenarytaina*, one *Casuarinicola* and five *Acizzia*. No specificity with any particular taxon was apparent, which is in contrast to the majority of >125 *Mycoplasma* species known in animals that each infects only one type of animal to generate infections called mycoplasmoses (Nicolet 1996). *Mycoplasmas* are also well known plant pathogens (Hampton 1972), usually transmitted by leafhoppers and psyllids (Garnier et al. 2001). No pathology was obvious in the insects or plants

analysed here, but further biological analysis would need to determine if presence of the bacterium is associated with higher mortality rates.

#### *5.4.2..2 Species-specific OTUs: Cardinium and Rickettsiella*

***Candidatus* Cardinium** (Bacteroidetes) was recorded only in the three individuals of *Anomalopsylla* "Pollen Island". Similar to *Wolbachia, Cardinium* is widespread and associated with various reproductive manipulations in arthropods (Zchori-Fein and Perlman 2004). This includes cytoplasmic-incompatibility (Stouthamer et al. 1999, Duron et al. 2008) and, through infected males mating with healthy females, death of the embryo has been speculated to lead to rapid speciation (Werren 1997). This could be consistent with the genetic distinctiveness of the species analysed here (Figure 4.1 and 4.3), although this could also be influenced by the relative geographic isolation of this single population. A wider genetic analysis of *Anomalopsylla,* possibly including *A. insignita,* may enable a better understanding of the role of this bacterium, but its presence here in a single species (but in all its samples) suggests a strong specificity with this psyllid taxon.

***Rickettsiella*** (Gammaproteobacteria) is an arthropod-pathogenic bacterium and was recorded in only five individuals, all amongst the adventive species *Acizzia acaciaebaileyanae, Arytainilla spartiophila, Ctenarytaina longicauda* and *Glycaspis granulata*. *Rickettsiella* has been previously recorded in psyllids from various genera, including *Psylla* (Spaulding and von Dohlen 2001). Much research has focused on *Rickettsiella*'s biologically diverse functions in arthropods (Duron et al. 2016), from colour changes (Tsuchida et al. 2010) and fungi resistance (Lukasik et al. 2013) in aphids, to arthropod pathogenicity and death in crustacean (Cordaux et al. 2007).

#### *5.4.2..3 Plant pathogens unculturable bacteria*

***Candidatus* Liberibacter** was recorded here in seven samples: four *Ctenarytaina fuchsiae*, one *Ctenarytaina fuchsiae* B, one *Ctenarytaina spatulata* and one *Acizzia acacia.*

- The *Liberibacter* sequence from *Ctenarytaina spatulata* showed 99% similarity with another undescribed species of *Liberibacter* previously recorded in the Hamilton area in 2008 from a potato. This led to a biosecurity incursion response although no other samples tested positive (Liefting 2017, personal communication). Coincidentally, the sample of *Ctenarytaina spatulata* was collected in Hamilton, from an *Eucalyptus* tree.
- The *Liberibacter* sequence from *Acizzia acacia* was 99% identical to the novel '*Candidatus* Liberibacter brunswickensis' reported very recently from *Acizzia solanicola* as a first discovery of *Liberibacter* species in Australia (Morris et al. 2017). This is also now a first report for New Zealand, as well as recording a new psyllid host and new host plant (*Acacia melanoxylon*). When considering the origin of these bacterial species, *A. acaciae* and its plant host *Acacia melanoxylon* are both Australian native. This suggests that the presence of

this new *Liberibacter* in New Zealand may be recorded on the same plant in Australia too, and thus with a wider distribution than reported originally (Morris et al. 2017).

- The *Liberibacter* sequences isolated from both *C. fuchsiae* and *C. fuchsiae* "B", if confirmed to be '*Candidatus* Liberibacter ctenarytainae' would record the first report for this new *Liberibacter* in *C. fuchsiae* B.

*Liberibacter* species have been known for a long time to infect economic pests such as *Bactericera cockerelli* (Triozidae) (Munyaneza et al. 2007), *Trioza erytreae* (Triozidae) (McClean and Oberholzer 1965) and *Diaphorina citri* (Liviidae) (Capoor et al. 1967). However, adding the more recently described species of *Liberibacter brunswickensis* and *Liberibacter 'ctenarytainae',* for which no plant symptoms were obvious, indicates that not all species in this genus are pathogenic. Therefore species specific analysis is recommended when genus-level "*Liberibacter*-positive" results are detected.

***Candidatus* Phytoplasma** was recorded in all the three samples of *Trioza irregularis,* in a sample of *T. vitreoradiata,* and in a sample of *Ctenarytaina* sp. B. This sequence is not new to New Zealand as it matches '*Candidatus* Phytoplasma pseudopanacis', an unpublished *Phytoplasma* species detected by MPI in 2011 (Liefting Personal communication) that is associated with dieback of *Pseudopanax* and *Pittosporum* species. Consistent with this work, *T. irregularis* and *T. vitreoradiata* are hosted respectively by *Pseudopanax* and *Pittosporum* suggesting that this bacterium is indeed pathogenic. However, no observation was made at the time of field collection that could confirm this. Psyllids belonging to the genus *Cacopsylla* have been previously reported to vector *Phytoplasma pyri* in Austria (Lethmayer et al. 2011). Similarly, an undescribed species of *Phytoplasma* has been recently reported from Australia (Hall et al. 2016).

A ***Candidatus* Phlomobacter** OTU was recorded at low levels (0.10% - 0.30%) in six samples: two *Trioza falcata*, three *Ctenarytaina eucalypti* and one undescribed species of triozid from *Casuarina*. This plant-pathogenic genus of poorly characterised bacteria includes the species *Candidatus* Phlomobacter fragariae, which has been associated with the strawberry marginal chlorosis (Zreik et al. 1998, Danet et al. 2003) and the low sugar content syndrome of sugar beet (Salar et al. 2010). While no data suggesting pathogenicity of this bacterium was obtained here, the record of a bacterium belonging to this genus may be of possible interest in a biosecurity context and warrant additional genus-specific analyses to confirm.

#### *5.4.2..4 Other bacteria recorded*

With the intent of providing a cross section of the core microbiome of the New Zealand psyllids, other symbiotic bacteria that have been associated elsewhere with other insects include *Acinetobacter* (Minard et al. 2013), *Janthinobacter* (Zhang et al. 2011) and an Oxalobacteraceae (Staudacher et al. 2016). However, due to the lower amount of reads linked to these species, their symbiotic role may be debated. Previous work that has reported some of these bacteria in psyllids includes ten bacteria in *Diaphorina citri,* comprising *Acinetobacter*, *Staphylococcus*, *Janthinobacter* and an Oxalobacteraceae (Marutani-Hert et al. 2011). Similarly, *Acinetobacter* was detected in *Bactericera cockerelli* (Nachappa et al. 2011).

### 5.4.3 The microbial composition is influenced by the psyllid phylogeny.

After confirming that the microbial composition of psyllids was not randomly distributed (Alpha and Beta diversity) and associating this variation with psyllid taxa (ADONIS approach accounting for the 60% of the variation in the microbial composition), the microbial composition was shown to be more similar in closely related psyllid species. Thus, considering the P-symbiont and pool of S-symbionts, the phylogeny of the insect would be expected to be the predominant driver of microbiota structure. In fact, studies elsewhere focusing both on the P-symbiont (Thao et al. 2000a, Spaulding and von Dohlen 2001, Thao et al. 2001, Hall et al. 2016) and on the S-symbionts (Thao et al. 2000b, Hall et al. 2016, Morrow et al. 2017), showed different degrees of association between psyllids and their symbionts. This includes recent studies confirming degrees of vertical transmission for S-symbionts (Hall et al. 2016). Moreover this signal of a strong association of the "group of common OTUs" with the insect phylogeny is in accordance with the recently defined insects-bacteria relationship of "phylosymbiosis" (Brooks et al. 2016). In fact, not only the P-symbiont, but also the group of S-symbionts recorded here appears to be a component of the psyllids microflora composition strongly connected with the insects evolution.

Geography may also influence distribution of the P- and S-symbionts as indicated here with the same psyllid species collected in New Zealand compared to Australia showing different P- and S-symbiont composition. This has similarly been recorded for *D. citri,* which shows genetic variation in its P-symbionts between populations in Asia and in the United States (Wang et al. 2017). However, a Mantel test in this study showed relatively little signal associated with the geographical distribution of the species collected in New Zealand. Possibly, a more extensive sampling across a wider area could report bio-geographic associations as those recently presented for the nettle-psyllid, *Trioza urticae,* in Europe (Wonglersak et al. 2017).

Distribution of the non-symbiotic bacteria across the insect hosts showed no apparent pattern of association with the different psyllid taxa. Neither was a connection apparent between the presence/absence of some bacteria and the presence/absence of others. This included plant pathogens which also did not show a particular positive or negative link to any other bacteria as far as they were able to be taxonomically resolved here.

Given the specificity of psyllids to their plant hosts, close association of the microbes to the species of psyllid may also suggest that microbial composition could depend on the psyllid host plants. However, the Mantel and Partial Mantel tests confirmed that, while the microbial composition is highly correlated (almost 40%) to the genetic distance between insects after accounting for the host plant variation, inverting the variables does not support plants as a driver; the host plant associations are responsible for just 15% of the microbial composition after accounting for the psyllid genetic distance. Similarly, the different psyllid species collected from the same individual plants, but which gave different Enterobacteriaceae OTUs, also showed different levels of *Wolbachia.* These observations are consistent with the idea that the psyllid microbial composition is influenced by the psyllid species and not the plant.

Together, the analyses here support acceptance of **Hypothesis 1:** that at least two of the insect-plant-microbe interactions are strongly correlated, and being insect and microbe. In turn, this suggests that evolutionary changes in the insect associate with changes in the microbiome. Therefore, these results also support **Hypothesis 2:** that the insect-bacteria associations can be understood in light of the "phylosymbiosis" theory shown by congruence between the psyllids evolutionary history and the associated microbial communities. Phylosymbiosis has already been demonstrated not only under laboratory settings (Brooks et al. 2016) but also in the field, both at an intraspecific level [for the American pika, (Kohl et al. 2017)] and for the identification of cryptic species [for mosquitoes, (Minard et al. 2017)]. Accordingly, there is acceptance of **Hypothesis 3:** that, as phylosymbiosis is inferred as the major driver of bacterial composition, then host plant specificity of the psyllids is not driven by symbionts as was proposed by Hansen and Moran (2014). Furthermore, the current study showed at least two instances where closely related species feeding on the same host plant show different symbiotic bacteria. With a reduced, 12 species dataset, the psyllid *T. vitreoradiata* clearly separated from the other most collected psyllids, showing a very low diversity in the microbial composition. This appear to be associated here with a very high presence of the S-symbiont Enterobacteriaceae and a high presence of the P-symbiont. Moreover, *T. vitreoradiata* is known for its ecological association with several host plant species of the genus *Pittosporum,* while the other species of the reduced dataset are all found on a single host plant species. This result may indicate a role of the primary and secondary symbionts in actively allowing

the psyllid to feed from multiple plants, as demonstrated for many other insects and animals [e.g. (Hosokawa et al. 2007, Chu et al. 2013, Vavre and Kremer 2014)]. Moreover, the lower bacterial diversity in *T. vitreoradiata* microbiome composition could be due to competition between P- and S-symbionts against other bacteria, as suggested elsewhere for symbionts actively cooperating for their animal host survival and protecting it from other infections (Haine 2008, Vautrin and Vavre 2009).

## 5.5 Conclusion

The results obtained here highlighted that the multitrophic relationships between psyllids, plants and bacteria in the New Zealand region that contribute to a cross section of the present knowledge on this subject [(Tamborindeguy et al. 2017) and references therein]. Presence of the anticipated P- and S- symbionts has been confirmed, as have psyllid-vectored plant pathogens in some samples and that which belong to the bacterial genera widely reported elsewhere (e.g. *Liberibacter*). Furthermore, the record of newly reported bacterial species probably endemic to New Zealand (e.g. the *Phytoplasma* in *T. irregularis* or the *Liberibacter* in *C. fuchsiae*), together with the recently reported species from Australia (Morris et al. 2017) may suggest that the known psyllid-plant pathogen association has deeper ramifications for psyllid radiations generally. A better understanding of this may avoid the risk of introducing psyllids that may act as vectors in ecosystems where new interactions may occur, such as in the case of *D. citri* in Oman and Brazil (Queiroz et al. 2016). Furthermore, better informed decisions can also be made before releasing psyllids as bio-control agents, avoiding the unwitting release of potential plant pathogens as well, as it happened in New Zealand with *A. spartiophila* and *Ca.* L. europaeus (Nelson et al. 2013, Nelson 2016). At the very least, this study has produced a valuable tool for the comparison of the New Zealand psyllids-bacteria-plants ecosystem with those of other regions of the world.

# Chapter 6

# General discussion

## 6.1 Summary and hypotheses tested

This study aimed to investigate the diversity and evolutionary relationships of the New Zealand psyllid fauna so that accurate associations with their host plants and internal microflora could be elucidated. The intent ultimately was to develop the fundamental knowledge required to understand the epidemiology of current plant pathogens vectored by these insects, as well as the risk to New Zealand from any new psyllid pests and plant-pathogens as may arrive.

Central to achieving this, a contemporary and comprehensive list of psyllid species and their host plants in New Zealand was compiled. Based on a checklist derived here from the literature and from entomological collections, field collections were undertaken to target specific locations and taxa for phylogenetic and microbial analysis. Integrating morphological, plant host and newly generated COI barcode data for this collection resulted in the addition of more than 20 species not previously recorded present in New Zealand. This included proposal of 21 novel species for which formal descriptions will be necessary. Thus, the total number of psyllid taxa as we know it today has increased from 99 to 120.

Building on this information through generation of additional 18S and partial EF-1α data, a Bayesian Inference phylogenetic analysis was able to establish evolutionary relationships between psyllid species. This confirmed and, in the case of the genus *Atmetocranium,* helped to clarify their taxonomic status. Monophyly of the genera was also confirmed for those where multiple species were available. Important clues as to six ancestral arrivals to New Zealand also became apparent to provide context as to the speciation that has occurred here since. Together this has facilitated acceptance of the thesis **Hypothesis 1**: *there is greater psyllid biodiversity in New Zealand than reported by previous studies based solely on morphology, and this has led to a better understanding of the origin and evolution of the New Zealand Psylloidea*.

Finally, use of next generation metabarcode technologies enabled the first assessment of New Zealand psyllid microbiomes. Within the bounds of the bioinformatics tools and reference databases available today, an inventory of the most prevalent taxa was documented. This included confirmation of the anticipated *Candidatus* Carsonella rudii as primary symbiont and various taxa within the Enterobacteriaceae as secondary symbionts. Linking these symbiotic OTUs to the psyllid

species revealed a clear phylogenetic correlation. Thus, although relatively cursory in terms of the level of bacterial taxonomic resolution possible, this has enabled acceptance of thesis **Hypothesis 2**: that the p*syllid microbiomes show discernible species-species composition patterns and that a stronger association with geography, insect phylogeny or host plant association would be apparent. Therefore, the microbiomes of newly arrived exotic psyllid species would be comparable to previously studied species.*

## 6.2 Hypothesis generation and future research

This thesis provides an up-to-date understanding of the psyllid composition in New Zealand and a novel evaluation of their microbiome that has enabled present knowledge gaps to be better defined. These gaps, with respect to both fundamental science and biosecurity-related application, have led to the formation of further hypotheses and areas of future research to test them.

### 6.2.1 The challenge of accurate psyllid biodiversity assessment.

Obstacles at the outset for generation of the underpinning species checklist were that many species are not represented in the various entomological collections and much of the previous work remains unpublished. This was compounded by reliance on several of the more recently discovered species being only tag-named but not formally described. While designed to indicate an entity that may be a separate species, a tag-name is an informal name that exists outside of the International Codes of Nomenclature (Leschen et al. 2009). Consequently, assignment of specimens to these taxa is not always robust. Unfortunately this is not uncommon, with a "taxonomic impediment" that leaves a largely unknown insect fauna; of approximately 20,000 species known to be present in New Zealand, 10,000 remain to be described (Leschen et al. 2009).

With the need to delimit the taxa collected here to the species level, a molecular genetic framework was developed. This capitalised on the utility of the COI DNA barcode region to discriminate taxa in an integrative taxonomy context (Padial and de la Riva 2007), as has been achieved elsewhere for other insects [e.g. (Brunetti et al. 2017, Cruaud et al. 2017)], including psyllids [e.g. (Taylor et al. 2016, Percy 2017)]. In using this to match morphologically identified specimens to species, some of the new taxa reported here were morphologically similar to existing described or tag-named species. Certainly, the genetic clusters helped to overcome any ambiguities resulting from the lack of reference specimens in the national collections. Perhaps inevitably with new field collections, this study added to the number of tag-named species. Specifically, 20 native new species distributed in three of the 21 genera (*Ctenarytaina, Psylla* and *Trioza*) analysed are proposed, supported by both COI barcode and retrospective morphological examination. Further empirical multivariate evidence of their delimitation will be useful, however, perhaps with additional

genes or based on a biological species concept using behaviour, cytogenetics or chemistry (Schlick-Steiner et al. 2010). Nevertheless, for the purposes here, and despite potential limitations such as the presence of pseudogenes (Song et al. 2008, Dasmahapatra et al. 2010), DNA barcodes were highly valuable species characters. On the one hand, the COI barcode and morphology were congruent for the large majority of cases, clustering individuals and populations as either the same as or distinct from existing species. On the other hand, absence of genetic variation between *Trioza adventicia* and *T. eugeniae* was instrumental in supporting the lack of other distinctions and, therefore, supporting also the proposed synonymisation.

Updating the New Zealand Psylloidea here supports the growing recognition of DNA barcodes as a major contributor to sustainable practices in taxonomy (Hubert and Hanner 2015). It also highlights the importance of generating a DNA database of voucher sequences from morphological described, curated specimens (Song et al. 2008, Dasmahapatra et al. 2010, Astrin et al. 2013). This is an output of this thesis to facilitate the efficient identification of species especially those with cryptic morphology.

### 6.2.2 Origin of psyllid species diversity in New Zealand

The foundation of psyllid diversity in New Zealand may be the result of a combination of ancestral arrivals and subsequent species radiation within the country. Understanding this, and thus the evolutionary strategies adopted by the different families and genera, would be useful for many reasons. First of all, a better understanding of the origin of psyllids could inform the modelling of future routes or risks of invasion by pest species (Syfert et al. 2017). Moreover, the geographic origin of psyllids may help in understanding the ancestral psyllid-host plant associations, which could be useful to retrospectively understand the risks for future plant colonisations, possibly based on climatic similarities as it has been demonstrated elsewhere (Syfert et al. 2017). A much more complete taxonomic dataset, including that from potential ancestral sources, is necessary to enable these in the future. Nevertheless, based on the phylogenetic range here, a number of hypotheses can be generated that may form the basis for such future work.

#### Dating psyllid arrivals to New Zealand

Associating the phylogeny here with a molecular clock may allow the arrival times of different psyllid groups to be determined. Consequently, knowing the time of ancestral arrivals could contribute to answers as to their geographic origin. This especially considering that New Zealand was part of Gondwana and some archaic lineage could have originated from that time. For example, are some of the lineages, such as *Anomalopsylla*, relics of the super continent land mass of Gondwanan times, or

are they modern-day dispersers? Unfortunately, calibration methods based on fossils, geological events or mutation rates (Hipsley and Muller 2014) are not easily accessible here.

**Fossils** belonging to the family Psylloidea or its ancestors are scarce. The oldest crown group psyllid appear in Baltic amber during the Eocene (Klimaszewski 1996). While recent studies on fossils preserved in Mexican amber suggest that the Miocene fauna was quite similar to the contemporary one (Drohojowska et al. 2016). The superfamily Psylloidea, however, may well have had representatives from the late Jurassic (Mesozoic). In fact, specimens dated back to that period have been assigned to the extinct families Liadopsyllidae and Malmopsyllidae (Bekker-Migdisova 1985). However, a fossil to confirm the split time between psyllids and other Sternorrhyncha such as aphids, or between families within the Psylloidea, is still missing.

In absence of a fossil a **geological event** such as Zealandia's separation from Gondwanaland 83 Mya (Goldberg et al. 2008) or from New Caledonia 55 Mya (Schellart et al. 2009) has been considered elsewhere [see (Goldberg et al. 2008)]. However, an arc of volcanoes between New Zealand and New Caledonia along the Three Kings ridge may have provided a path between the two land masses (Schellart et al. 2009). We know that after separation from Gondwanaland starting ~80 Mya, the continent of Zealandia gradually submerged beneath the sea, and that modern New Zealand is primarily the product of tectonic activity initiated ∼25 Ma [e.g. (Campbell and Hutching 2007)]. It is not known how much land persisted, probably fragmented in a number of smaller islands; however, extreme reduction of the landmasses is likely to have caused biological bottlenecks (Cooper and Cooper 1995). Land connectivity, however, may not have been instrumental in ancestral arrivals as small winged insects such as psyllids are known to be easily windblown (Burckhardt et al. 2014). This could account for more recent movement amongst land masses, with wind dispersal from Australia still considered as one of the most probable means of arrival (Yen et al. 2014). Future research in this area may instead find that the use of the **substitution rate** of mitochondrial DNA is the best option for determining evolution of psyllids in New Zealand. A specific substitution rate has not been applied before to this group. The closest has been for the *Metrosideros*-hosted psyllids of Hawaii where the psyllid arrival on the islands was estimated according to arrival time of their hosts. Such data could provide an approximate substitution rate that could be then compared with the some of the most recent estimates for mitochondrial DNA substitution rates [e.g. (Brower 1994, Papadopoulou et al. 2010)]. However, the limitation of any given substitution rate, due for example to rate variation among lineages and over time, must be considered and accounted for at all times (Ho and Lo 2013).

The phylogeny obtained in this study may also generate some hypotheses as to arrivals vs radiation. For example, the presence of species native both to New Zealand and Australia, such as *Ctenarytaina,* may suggest a recent, post Gondwanan, split of this genus between the two countries. In fact, the position of the crown speciation of the New Zealand native species in the phylogenetic tree appears to be at the same depth of other genera, such as *Trioza.* On the other hand, *Anomalopsylla* and *Atmetocranium* appear to have diverged much earlier than *Ctenarytaina*. The study of these two genera would particularly benefit from a molecular clock to support a pre- or post- Gondwanan split. However, while the hypothesis of a Gondwanan origin for *Atmetocranium* and *Anomalopsylla* might explain the very distinct morphology of these psyllids, a pre-Gondwanan origin may be unlikely based on a recent compilation of plant and animal phylogenetic analyses revealed that only 10% of those could be dated back to the splitting of Zealandia from Gondwana (Wallis and Trewick 2009).

**The origin of the ancestral psyllids that colonized New Zealand: dispersal and radiation in the Pacific region.**

The phylogenetic information obtained here on the current New Zealand fauna can also contribute to a better understanding of the origin and pathways that led to the arrival of ancestral psyllids to New Zealand. Similarly, comparisons between the New Zealand psyllids and those present in other Pacific Islands may cast some light on the present distribution of psyllids in the Pacific region. These analyses, together with the most recent information on the geological history of New Zealand, could then enable new hypothesis on the psyllids origin to be formulated. For example, thirty years ago the dispersal of psyllids was considered unlikely: "*it cannot be assumed that they did* [disperse] *just because they are small and have wings*" (Dale 1985). Today, on the other hand, the presence of psyllids on recently emerged Pacific islands such as the Hawaiian Islands [estimated origin around 28 Mya (McDougall and Swanson 1972)] suggests that dispersal can be the only reason Hawaii is home to more than a 100 psyllid taxa (Ouvrard 2017). In fact, the arrival of the triozid genus *Pariaconus* in Hawaii has been dated after the arrival of its host, *Metrosideros,* about 3.9-6.3 Mya (Percy et al. 2008, Percy 2017). Therefore, the fact that the Hawaiian Islands emerged from the sea leaves no doubt on the present psyllid fauna must have originated via dispersal.

The mechanism and pathway of this dispersal, however, remain uncertain. Recent studies confirm that insect wind dispersal is feasible, at least between Australia and New Zealand [e.g. (Yen et al. 2014)]. Similarly, evidence of the trans-oceanic dispersal of plants has been known for a long time (Davis 1950, Gillespie et al. 2012); this might explain the arrival of *Metrosideros* to Hawaii probably from Australia (Tarran et al. 2016, Tarran et al. 2017) and not from New Zealand as previously thought (Percy et al. 2008). Oceanic drift of host plant material is in fact well known [e.g.

(Winkworth et al. 2002, Gillespie et al. 2012, Percy 2017)] and may have directly connected Australia or New Zealand with Hawaii. Alternatively, a psyllid wind-mediated dispersal may have been facilitated by an "Oceanic pathway", with Pacific Islands as stepping stones to accommodate the large distances. This would support the hypothesis of an initial plant radiation followed by a psyllid colonization of the plant as suggested for Hawaiian Islands (Percy et al. 2008, Percy 2017). Phylogeographic evidence of this would require inclusion of the triozid faunas of other Pacific Islands, including Australia (and Tasmania), New Caledonia, Fiji, Vanuatu, up to the Marshall Islands. Observation of genetic variation correlated to inter-island proximity may consequently suggest an establishment "pathway" between them.

As a first step in the comparison between the New Zealand psyllid species and those present in other countries, COI sequences available from previous work were able to be included; unfortunately, a complete set of 18S sequences were not available. This cursory comparison of the COI barcode sequences between New Zealand's most basal triozid species of *T. curta*, plus the Australian triozids species analysed here (except *T. eugeniae*) and Hawaiian triozids (Percy 2017) suggests that the New Zealand species are more closely related to the Hawaiian than the Australian species. This is in contrast to the hypothesis of a pathway between Pacific Islands originating from Australia, where it might be anticipated that more closely located islands, such as New Zealand and Australia, would have more closely related species. Moreover, while insect wind dispersal has been confirmed between New Zealand and Australia (Yen et al. 2014), this would be less realistic for the more distant New Zealand and Hawaii islands, especially considering that the southern hemisphere trade winds are predominately from the south east (https://en.wikipedia.org/wiki/Prevailing_winds). Thus, the development of a different hypothesis may be required to account for psyllid dispersal in the Pacific.

Psyllid biological habit was also considered as a possible facilitator of oceanic dispersal. In fact, considering the gall-forming guild of psyllids, it appears plausible that psyllids encased in their galls at the nymphal stage may be dispersed via oceanic drift of their host plants. Obviously, in order to confirm this hypothesis, ecological experiments on the survival rate of psyllid nymphs exposed to salt water while within their galls would be useful. If confirmed, this theory would be consistent with the idea of ancestral species arrivals potentially being gall-formers as is indicated by the phylogenetic positions of those present in New Zealand: *T. curta* and *T. eugeniae* being basal to the New Zealand triozids, *T.* "Price's Valley" basal to the monophyletic group of the New Zealand *Trioza*, and the Aphalarid *Atmetocranium myersi* basal to the New Zealand Aphalaridae. In keeping with this, the gall-forming habit may be an ancestral feature, as has been considered for the triozid genus *Pariaconus* (Percy 2017), which enabled dispersal in the Pacific region. The galling habit, known to be

common in the present day for Asian and Hawaiian psyllids (Crawford 1918), could be a residual characteristic of their ancestors. For example, more than 50% of the triozid species of Taiwan and Japan are reported to consist of gall-formers (Yukawa and Masuda 1996, Percy et al. 2015).

### 6.2.3 Improved understanding of the plant-microbe-insect relationships

Plant-microbe-insect (PMI) interactions are of increasing interest in the context of invasive species (Bennett 2013), especially for phloem-feeders such as psyllids which also vector plant pathogens (Tamborindeguy et al. 2017). Towards this the present work developed the first estimation of the bacterial associations for New Zealand native psyllids, focusing in particular on P- and S-symbionts. Furthermore, the record of plant pathogens damaging a number of host plants, highlighted how these may influence the psyllids-host plants relationship consequently.

#### Predicted and unpredicted associations between psyllids and bacterial symbionts

Observations here of the P-symbiont generally supported the well-developed theory of a single, ancestral infection of *C.* Carsonella rudii that has been vertically transmitted through the radiation of the superfamily Psylloidea [e.g. (Baumann et al. 2000, Thao et al. 2000a, Thao et al. 2001, Hall et al. 2016)]. This was reinforced by congruence of the partial 16S sequences of *Carsonella* here with the psyllid phylogeny, as has been empirically demonstrated elsewhere (Thao et al. 2001, Hall et al. 2016). Additionally, for multiple taxa of recently diverged psyllid species (e.g. within the genera *Trioza, Psylla* and *Ctenarytaina*) the presence of the same *Carsonella* OTU can be explained by 16S being a relatively slowly evolving gene with little genetic variation at the species level. Conversely, the presence of multiple *Carsonella* OTUs in the same individual insect that are also divergent by 4%-6% cannot be explained by a single ancestral infection. Confirmation of this requires specific targeting of *Carsonella* by PCR and sequencing of multiple genes [see for example (Hall et al. 2016)] to rule out any technological error. However, if multiple *Carsonella* OTUs within the same psyllid species were confirmed, and simultaneously the same OTU in other closely related psyllids, this may suggest that, similarly to S-symbionts (Thao et al. 2000b, Hall et al. 2016), the P-symbiont may also be horizontally transmitted. This would raise a number of questions as to the possible different routes for horizontal transmission, such as those recorded for *Wolbachia* including host plants, parasitoids and mating strategies (Vavre et al. 1999, Moran and Dunbar 2006, Sintupachee et al. 2006).

Moreover, this work recorded at least one Enterobacteriaceae OTU in each of the samples analysed. This may suggest that the theoretical separation between the Primary and Secondary roles of symbionts may not be as strict and generalised as it appears, with the P-symbiont being part of an obligate symbiosis and S-symbiont being facultative (Baumann 2005). Moreover, in agreement with

the most recent work describing the insect-bacteria relationship as a phylosymbiosis [e.g. (Brucker and Bordenstein 2012, Brooks et al. 2016)], the work here indicates that the insect phylogeny is a factor influencing the distribution of some of the S-symbionts, with separate OTUs recorded in distinct genera. Could therefore the association between the insect and what was considered a "secondary" symbiont be more important and long-term than previously expected? Indeed, other recent studies have reported the incidence of vertical transmission of some S-symbionts (Hall et al. 2016). While the current study cannot demonstrate vertical transmission, it suggests that within the limits of metabarcode technology, the relationship between insect and S-symbiont is extremely species-specific, with each psyllid species ubiquitously showing association patterns with one or a few Enterobacteriaceae. Other *Carsonella*-specific studies have confirmed a clear vertical transmission and presence of this bacterium in all psyllids (Thao et al. 2000a, Thao et al. 2001, Hall et al. 2016), with the isolation of the Enterobacteriaceae group more generalised and often limited by the presence of different genera and taxonomic attributions that were not always clear (Thao et al. 2000b, Hall et al. 2016). Targeted sequencing to understand how many different taxa are present, if they are present in all the psyllids groups, and how they relate to each other, would be necessary before pursuing an understanding of their role in psyllid fitness, polyphagy or vectoring of plant pathogens.

### The association between psyllids and plant pathogenic bacteria in biosecurity

Progress to understand the interactions between economically important plant pathogens, such as those belonging to *Ca.* Phytoplasma and *Ca.* Liberibacter species, and their vectors has been undermined in the past by the inability to cultivate them on laboratory media (Trivedi et al. 2016). This has been overcome to a degree using high-throughput DNA sequencing technologies for a better understanding of both microbes and their hosts (Mitter et al. 2013). However, these techniques are still not used much in the insect-pathogen-biocontrol agent area, despite knowing that any kind of association between pathogens and hosts in the natural environment is influenced by the plant and/or insects bacterial community (Trivedi et al. 2016). For example, the concentration of *Ca.* Liberibacter asiaticus in *D. citri* was found to have a strong negative relationship with an endosymbiont residing in the syncytium of the mycetocyte (Fagen et al. 2012). Improving on this observation will require well-defined experiments based on evolutionary and ecological theory to recognise and understand these interactions, as well as on availability of appropriate and well curated bacterial species reference sequence data.

Capitalising on the modern accessibility to next generation sequencing metabarcode technology, the current study recorded at least five bacterial species from the pathogen-containing genera *Liberibacter* and *Phytoplasma* that, depending on further research, could be of interest to

New Zealand's biosecurity. Unfortunately, while the generic partial 16S primers and a single PCR, allowed automated screening of a single individual for many bacterial organisms, from environmental to symbiotic and pathogenic, non-specificity of the primers for a generally conserved gene do not often allow identification to the species level. To the genus level, nevertheless, it serves to raise awareness of potential biosecurity risk. At the very least this can focus follow-up analyses to define a detection as pathogenic or innocuous, as was undertaken for the recently described non-pathogenic *Liberibacter* brunswickensis (Morris et al. 2017).

The present trend for this technology to become cheaper and quicker, and data analyses to become more precise and accurate and with standardised pipelines will undoubtedly encourage wider adoption of 16S metabarcoding. This is likely to provide a "step change" for biosecurity screening and risk assessment (Hodgetts et al. 2016) and better enable non-native communities to be recognised in an invasion process (Comtet et al. 2015). Where completely new or never-recorded-before species are detected, more specific screening would clearly be needed. But this could not be achieved without a study such as the one here to convert fundamental and local biodiversity assessment into outcomes for biosecurity (Holdaway et al. 2017).

What a wonderful time to be a scientist.

## 6.3 Concluding remarks

A better understanding of the New Zealand psyllid fauna, its evolution and its microbial associations has been developed herein. The data itself will enhance prioritisation for future research. For example to explore the variable psyllid-*Carsonella* associations, the nature, variation and distribution of the psyllids S-symbionts, and the geographic origin of the ancestral psyllids as a prelude to understanding plant-host colonisation processes in New Zealand. A number of outputs towards these are outlined here.

**Outputs**

Firstly, this work resulted in the collection, identification and preservation of psyllid samples from more than 600 locations in New Zealand, Australia and USA. These curated samples in three separate collections, preserved in EtOH, dry mounted or in microscope slides at Lincoln Entomological Research Museum (LUNZ), will be made available for future studies.

Secondly, the submission to GenBank of more than 600 DNA sequences for COI, 18S and EF-1α will improve identification of New Zealand psyllids for non-expert psyllid systematists, diagnosticians and ecologists, as well as for improved global psyllid delineations and phylogenies.

Thirdly the depth of microbial data developed provides an initial, but substantial platform for future research on local and comparative biodiversity assessment, trophic and ecological interactions, and biosecurity. Finally, the methods as well as the results presented here will encourage improvements for less encumbered adoption of the technology, both taxonomic and metabarcoding, and for this multidisciplinary approach to be valuable for a variety of different organisms in order to answer similar biological questions.

This thesis has so far also resulted in two articles published in international journals:

**Martoni F.**, Burckhardt D. & Armstrong K. (2016) An annotated checklist of the psyllids of New Zealand (Hemiptera: Psylloidea). *Zootaxa*, 4144 (4): 556-574 doi:10.11646/zootaxa.4144.4.6.

**Martoni F.**, Bulman S.R., Pitman A. & Armstrong K. (2017) Elongation Factor-1α Accurately Reconstructs Relationships Amongst Psyllid Families (Hemiptera: Psylloidea), with Possible Diagnostic Implications. *Journal of Economic Entomology,* 110(6): 2618-2622 doi: 10.1093/jee/tox261

A further five manuscripts are in preparation at the time of this thesis completion under the following topics:

- The synonymisation of *Trioza adventicia* and *T. eugeniae,* with morphological and genetic data collected from New Zealand, Australia and USA (Taylor and Martoni, in preparation).
- Morphological description and distribution of new psyllid species in New Zealand confirmed by COI barcoding (Chapters 3 and 4; Appendix A).
- A phylogeny of the psyllids of New Zealand and proposal for a new taxonomic attribution for the genus *Atmetocranium* (Chapter 4). Possible additional works may allow to clarify the position of *Anomalopsylla,* too.
- Microbiome analysis of the New Zealand psyllids and its relationship with psyllid phylogeny and host plants. This includes the statistical analyses performed (ADONIS, Mantel, partial Mantel). (Chapter 5).
- The use of metabarcoding of psyllids as a surveillance tool for New Zealand biosecurity, with a specific focus on the *Liberibacter* and *Phytoplasma* species, including the first record of *L. brunswickensis* outside of Australia and in a different species than previously reported (Chapter 5).

# Appendix A

# Morphology of the Psylloidea

General morphology of the Psylloidea is reported here in order to provide a better understanding of the chapters where morphological characters are discussed. While the description of new psyllid species was not an objective of this thesis, the presence of cryptic species, similar to New Zealand endemic described taxa, were detected through the molecular analysis of the COI gene. The more thorough comparison of morphological characters required in those cases is presented below (A.2).

## A.1 Morphology of the superfamily Psylloidea

The following describes the psyllid morphology observed in this study for the New Zealand species. While the overarching aim of this Appendix is that of providing the reader with basic notions in order to better appreciate the morphological comments, a more general and more detailed accounts can be found in other, more specific publications [e.g. (Crawford 1914, Weber 1929, Lal 1934, Heslop-Harrison 1951, Vondráček 1957, Matsuda 1970, Hodkinson and White 1979, Dale 1985)].

### Adults morphology

#### Head and thorax

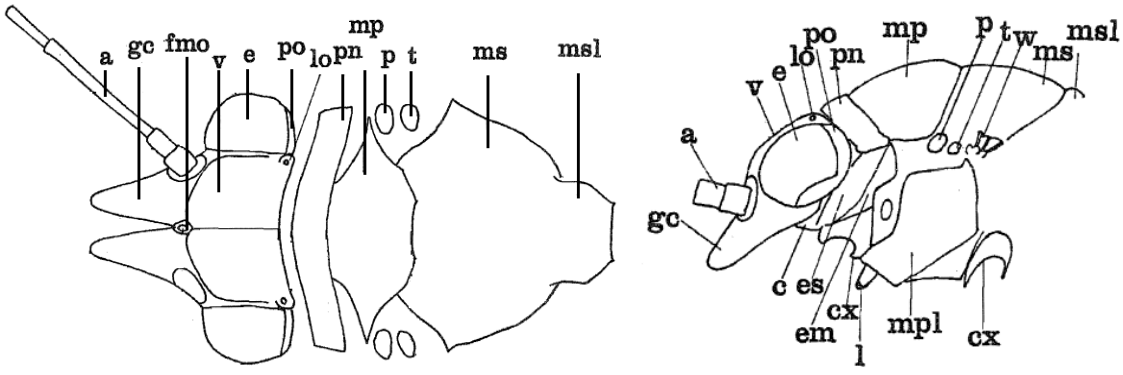


**Figure A.1:** Psyllid head and thorax in dorsal view (left, modified and adapted from Hodkinson and White 1979) and lateral view (right, Hodkinson and White 1979). a=antenna, b=clypeus, ex=coxa, e=eye, em=epimeron, es=episternum, fmo=frons bearing median ocellus, gc=genal cones, l=labium, lo=lateral ocellus, mp=mesothoracic praescutum, mpl=mesothoracic pleurites, ms=mesothoracic scutum, msl=mesothoracic scutellum, p=paryptera, pn=pronotum, po=post-orbital ridge, t=tegula, v=vertex, w=forewing. *Published under "Creative Commons Attribution – Non Commercial – ShareAlike 2.0 UK"*

The general structure of the adult psyllid head and thorax is shown in the figure above; size, shape and proportions of the heads and its parts can all be useful taxonomic characters (Hodkinson and White 1979). For example, the head and pronotum may be strongly deflexed or not. The head can also be wider than the thorax. The vertex is broad and generally flat in almost all the New Zealand species, usually showing a depression in each posterior half. The length of the vertex is variable and a useful diagnostic feature (Dale 1985). In the genus *Ctenarytaina*, a pair of small lobes is present anteriorly between the eye and the antennal socket and is defined as the ante-occipital lobe. The psyllid head nearly always has three ocelli, with two on the lateral caudal angles of the vertex and a median ocellus on the frons. The compound eyes, usually large and hemispherical, may sometimes be elongate and recessive. The part of the head behind the eye is referred to as "postocular region" (Tuthill 1952). The genae can be quite developed into a pair of anteriorly directed processes known as the genal cones, meeting across the frons and isolating the median ocellus (Figure A.1). This is quite noticeable in the families Triozidae, or in some genera belonging to the Aphalaridae, such as *Glycaspis*. These processes may also be scarcely developed (Figure 4a), with a large frons visible on the under-surface of the head, such as the native *Ctenarytaina* species. Their varying shapes are another useful diagnostic aid, as well as their position relative to the plane of the vertex and to each other. Some genera have one or two stout setae set subapically on each process while other have numerous finer setae. The function of the genal processes is unknown, but the presence of the long setae suggests that they are sensory. Dale observed that live insects do not seem to explore the substrate with these structures, but they occasionally use the antennae instead. Possibly, the genal processes and their setae aid the positioning of the head when feeding (Dale 1985). The antennae are 10-segmented. The basal pair of segments form a stout pedicel and the remainder a slender flagellum, with 9 and 10 often shorter or broader and usually darker. Segments 4, 6, 8 and 9 have often rhinaria in most species but additional sensoria are sometimes present in Anomalopsylla. Segment 10 always has two large setae, with minute, round sensory structures near their bases. The antennae arise from large round sockets which may be placed laterally on the head, taking up most of the genae in side view or higher, above the eye. The antennal sockets may be separate from the eye or form a small extension towards it, or meet it in a straight boundary.

The thoracic features of taxonomic interest are illustrated in Figure A.1. The pronotum is usually broadly transverse. The position of the propleurites relative to the pronotum and the relative size and shape of the prothoracic epimeron and episternum are generally good taxonomic characters (Hodkinson and White 1979). The relative size and shape of the meta-thoracic sclerites is comparatively uniform throughout the Psylloidea.

**Wings**

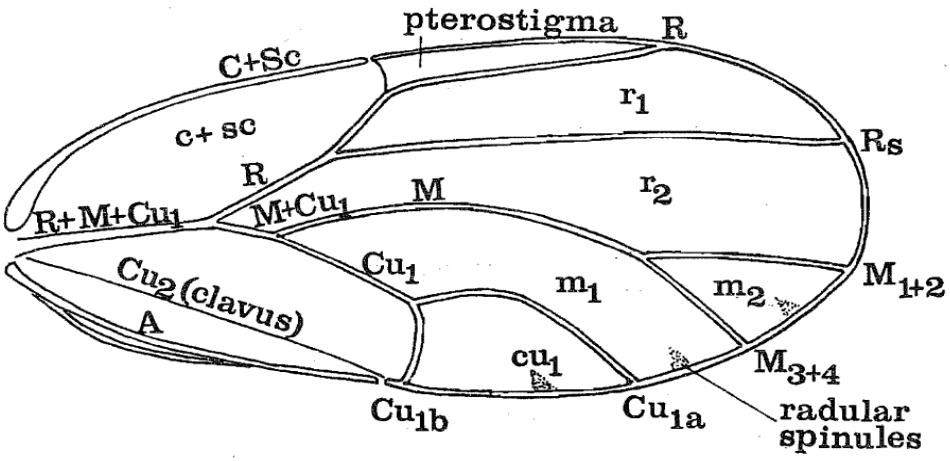


**Figure A.2:** Psyllid forewing showing the names of veins and cells (Hodkinson and White 1979).


While the hindwings are small, thin, and membranous, therefore of little taxonomical use, the forewings are a fundamental taxonomic character. The forewings are variable in shape, colours and venations. The single basal vein R+M+Cu divides either into two (R and MCu; in Aphalaridae and Psyllidae) or into three (R, M, Cu; in Triozidae). In Aphalaridae, the R and MCu are equal, while in Psyllidae R is clearly longer than MCu. Other important forewing characters include shape, texture and pattern, relative length and shape of veins, relative shape and size of cells, the presence or absence of a costal break and the point at which vein Cu2 meets the hind margin (Hodkinson and White 1979).

The pro- and mesothoracic legs are simple and of little taxonomic significance (Hodkinson and White 1979). The meta-thoracic limb is strongly modified for jumping, with the meta-coxal enlarged and bearing a characteristic caudally directed process, the meracanthus. This process can be very large, small or completely absent (*Anomalopsylla* and *Atmetocranium*). The function of the meracanthus is still unknown (Dale 1985).

The number of thick, black saltatorial spines at the apex of the metatibia varies from three in some *Trioza* spp. to a maximum of ten in *Psyllopsis*. While the saltatorial spines on the metatarsus varies from a maximum of two in *Psylla* spp. to none in *Trioza* spp.

**Abdomen and terminalia.**

The abdomen of psyllids is divided into 11 segments, with 8 segments in the anterior part and the reminder modified to form the terminalia (Heslop-Harrison 1951). The male has a large subgenital plate which contains a bipartite aedeagus and bears a pair of parameres (Figure A.3). These are showing great diversity of forms and are the single most useful diagnostic characters in the male (Dale 1985). The proctiger is formed from the tenth and eleventh abdominal segments, either separately or fused, with the anus opening at the apex.

The female terminalia consist of two large outer structures, a dorsal proctiger bearing the anus and a ventral subgenital plate, with palps closing part of the lateral opening. The inner ovipositor has paired dorsal and ventral valves. The anus is surrounded by a double ring of wax-secreting pores.

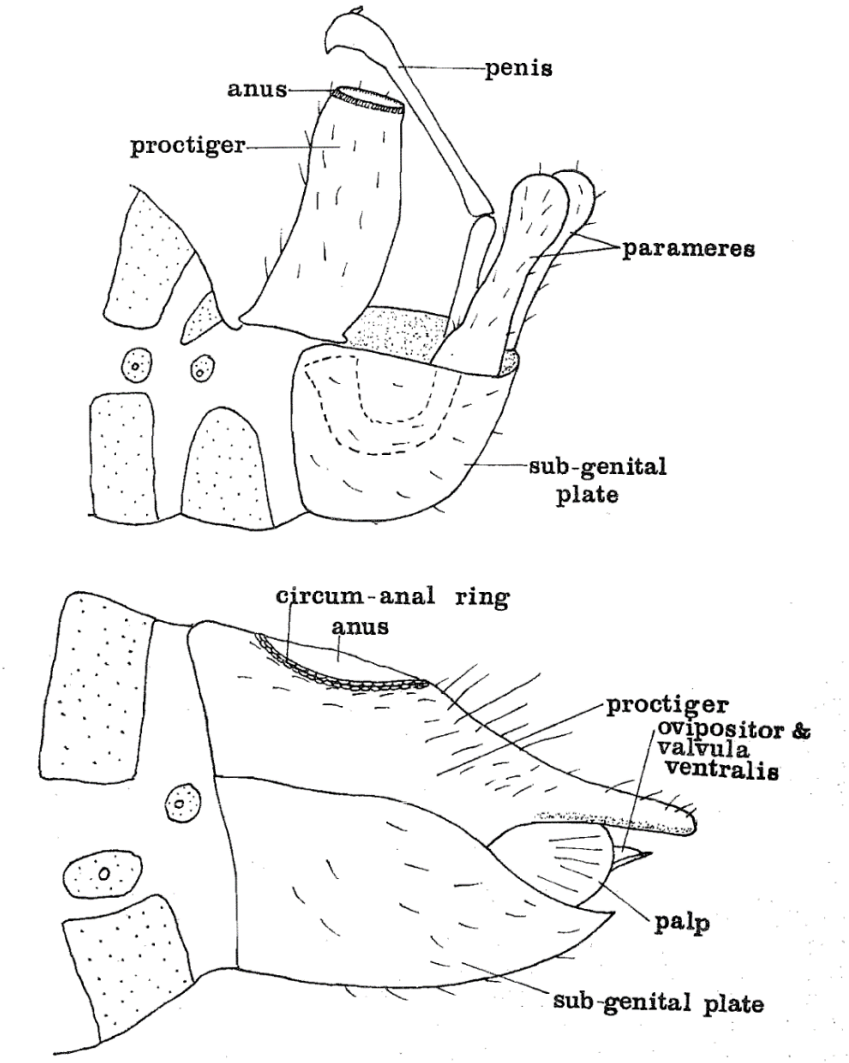


**Figure A.3:** Psyllid genitalia in lateral view: male (above) and female (below) (Hodkinson and White 1979). *Published under "Creative Commons Attribution – Non Commercial – ShareAlike 2.0 UK".*

**Nymph morphology**

All the New Zealand psyllid species have a life cycle composed by 5 nymph instars (Dale 1985). Of these, the last one is recognizable by the presence of a distinct tarsal segment. This 5th instar is usually used in taxonomical classifications (White and Hodkinson 1982, White and Hodkinson 1985). The body is covered on setae of several types and forms, all important taxonomically. These are: simple, capitate, clavate, lanceolate and sectasetae. The form of the nymphs in all groups is strongly influenced by its environment, and particularly by the need to avoid dessication (Dale 1985).

Nymphal psyllids are dorso-ventrally flattened, a condition reaching its maximum expression in the Triozidae (White and Hodkinson 1982). The head and anterior part of the thorax are covered by a pair of large sclerites which are considered to be formed by fusion of the vertex and part of the pronotum. These are separated in Psyllidae and Aphalaridae but fused in Triozidae. The antennae vary in length, with a maximum of 8-9 division in the New Zealand Psylloidea (Dale 1985) and up to ten in other psyllids (White and Hodkinson 1982).

The mouth parts are ventral, with prominent clypeus and labium extending back to the meso-thorax. The legs are usually simple, with an incipient second tarsal division often marked by a row of simple setae on the tibio-tarsus. The tarsus has a pair of apical claws. The abdomen extends posteriorly from the caudal edge of the attachment of the hindwing-pad. The anus is placed ventrally and is surrounded by an anal pore field consisting of a double row of pores. Additional pore fields are present in *Ctenarytaina eucalypti* and *Anomalopsylla insignita* (Dale 1985).

The nymphs form copious amount of honeydew which is coated with wax from the circum-anal pores and disposed of as solid granules or streamers. Wax is also produced as dusty powder on the body, or in tubes (Dale 1985).

## A.2 Preliminary morphological study of the New Zealand cryptic species

The presence of some of the morphological variations recorded for the newly reported taxa of Chapter 3 is reported. The intention is to demonstrate that morphological variations are present even in those taxa showing low genetic variation, namely the genera *Ctenarytaina* (mānuka and kānuka complex) and *Psylla* (both kowhai and *Carmichaelia* complexes). The characters presented here have been found consistent in individuals amongst populations of the same taxa.

Figure A.4 shows the wings of ten *Ctenarytaina* species. Variation can be observed in the general shape of the wing, with a species showing a particularly elongated wing (A.4J, *C.* sp. E) and

another (A.4G, *C.* sp. B) showing a rounder shape. Other characters that show clear variation are the cell m1+2, the shape of the vein A and the angle between the veins Cu and Cu1b.

In Figure A.5, the variation between the wings of different *Psylla* species ranges from variation in the colours (including the presence of brown and dark brown bands) to different shapes. The variation in the vein Cu1a and the consequent shape of the cell cu1, for example, immediately highlight the differences between the species *P. apicalis* A (A.5A) and B (A.5B).

The Figures A.6, A.7 and A.8 focus on the psyllids terminalia. Figure A.6 shows the male terminalia belonging to six *Ctenarytaina* spp. where the shape of parameres and proctiger together with the number and distribution of setae on the parameres will allow a very detailed description for these species.

The male terminalia of the *Psylla* species in Figure A.7 are more homogeneous. However, when focusing on the shape of the anal opening, this is more pronounced in the species *P. carmichaeliae* A (A.7C) and B (A.7D) than D (A.7F) and E (A.7G); with *P. carmichaeliae* C (A.7E) showing an intermediate shape. Similarly, the shape of the parameres highlights different forms of the terminal parts.

The *Ctenarytaina* female terminalia in Figure A.8 show that while the male terminalia can be more divergent, length of the female terminalia (A.8C, *C. clavata* C) and the general shape and length of the subgenital plate can help in the species identification.

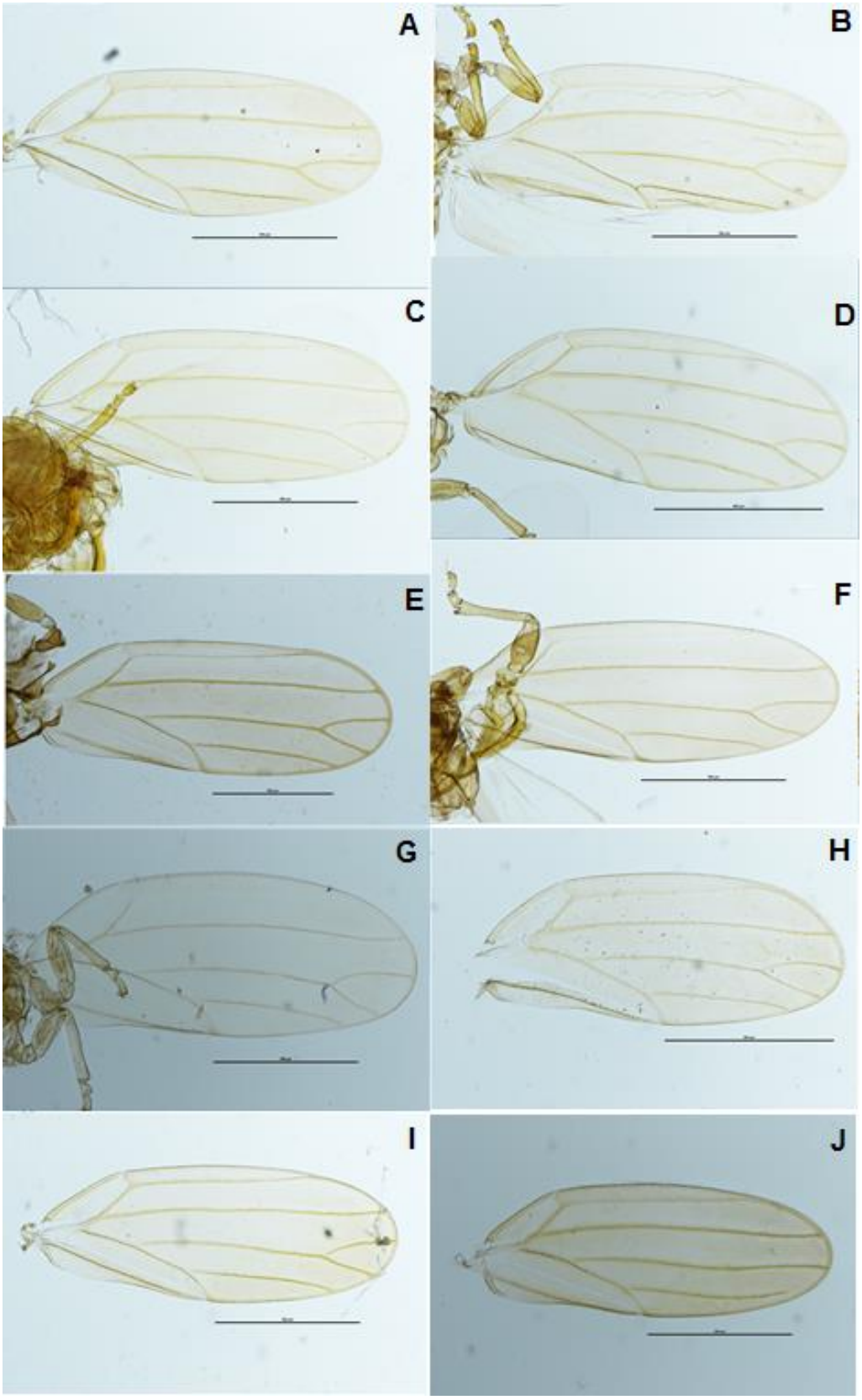


**Figure A.4:** Wings of *Ctenarytaina* species. A= *C. clavata*; B= *C. clavata* B; C= *C. clavata* C; D= *C. clavata* D; E= *C. pollicaris;* F= *C. pollicaris* B; G= *C.* sp. B; H= *C.* sp. C; I= *C.* sp. D; J= *C.* sp. E.

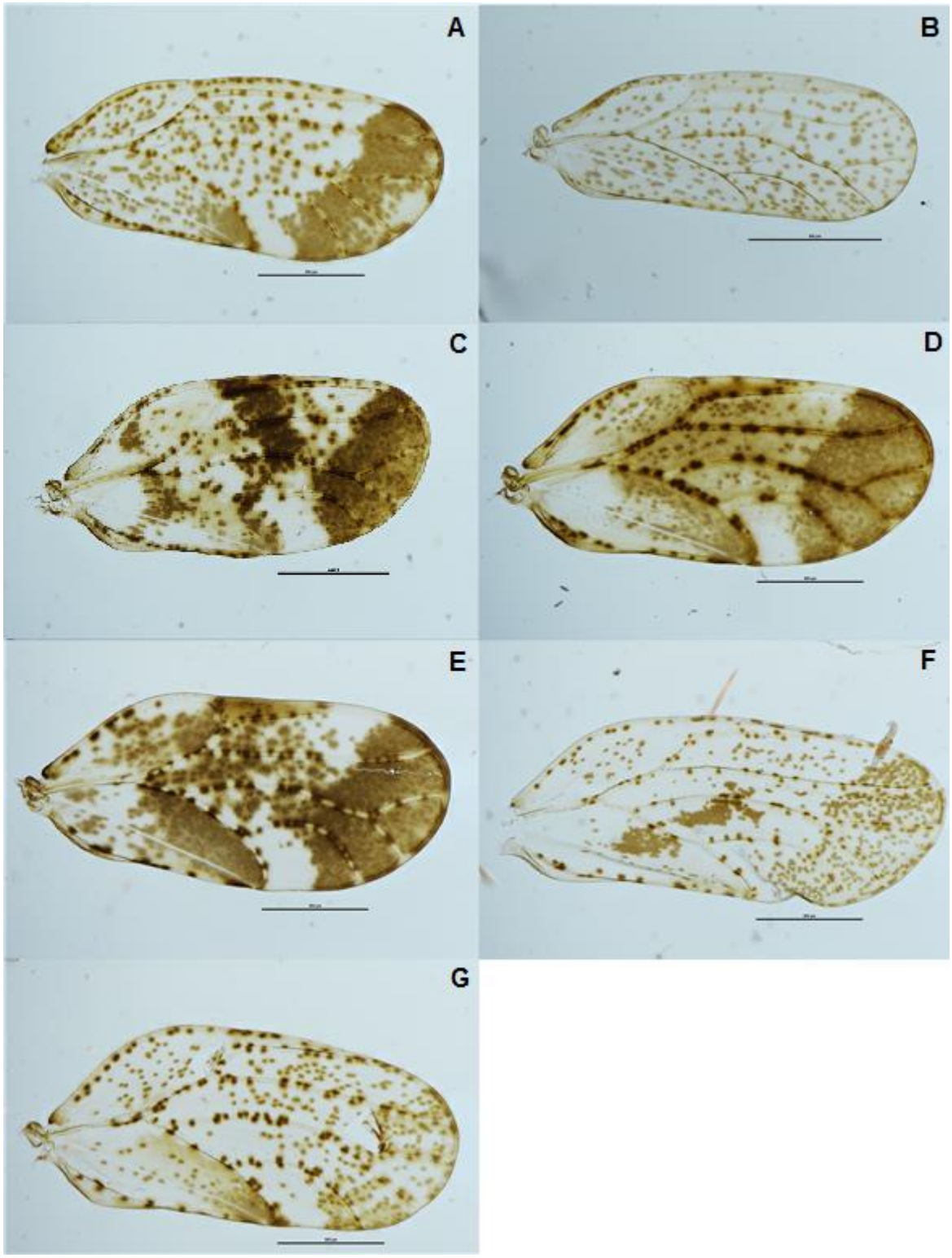


**Figure A.5:** Wings of *Psylla* species. A= *P. apicalis* A; B= *P. apicalis* B; C= *P. carmichaeliae* A; D= *P. carmichaeliae* B; E= *P. carmichaeliae* C; F= *P. carmichaeliae* D; G= *P. carmichaeliae* E.

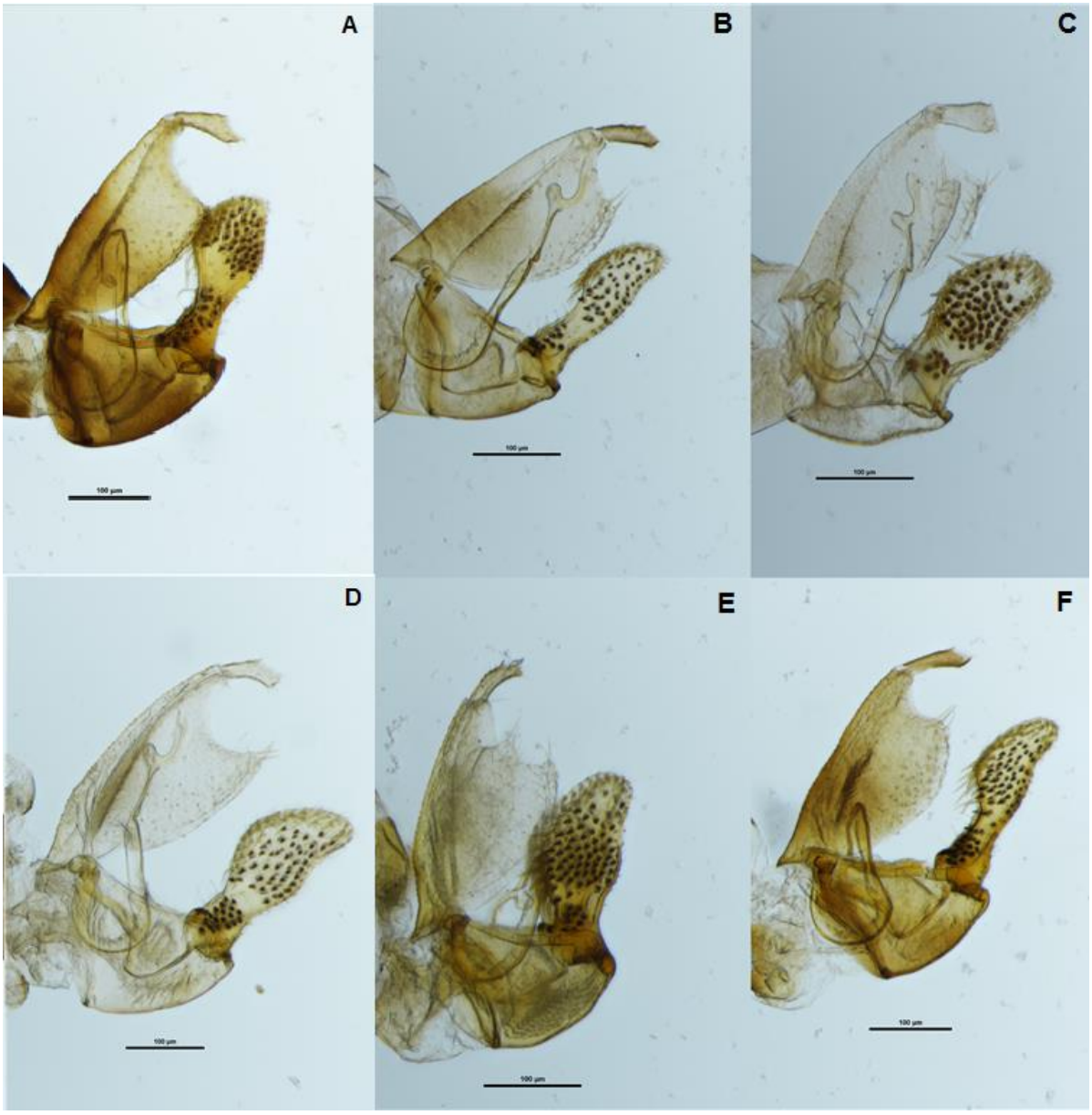


**Figure A.6:** Male terminalia of *Ctenarytaina* species. A= *C. pollicaris;* B= *C. pollicaris* B; C= *C. "*Short"; D= *C.* "sp. B"; E= *C.* "sp. C"; F= *C.* "sp. D".

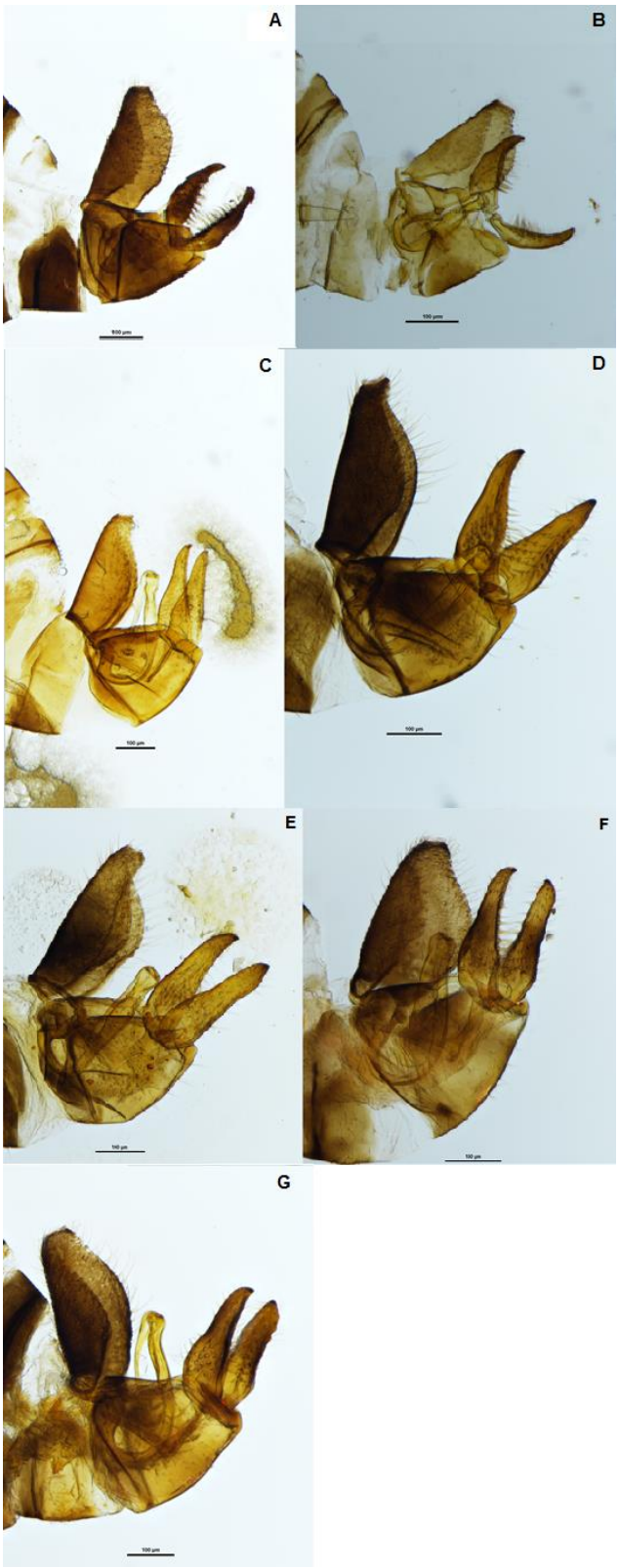


**Figure A.7:** Male terminalia of *Psylla* species. A= *P. apicalis* A; B= *P. apicalis* B; C= *P. carmichaeliae* A; D= *P. carmichaeliae* B; E= *P. carmichaeliae* C; F= *P. carmichaeliae* D; G= *P. carmichaeliae* E.

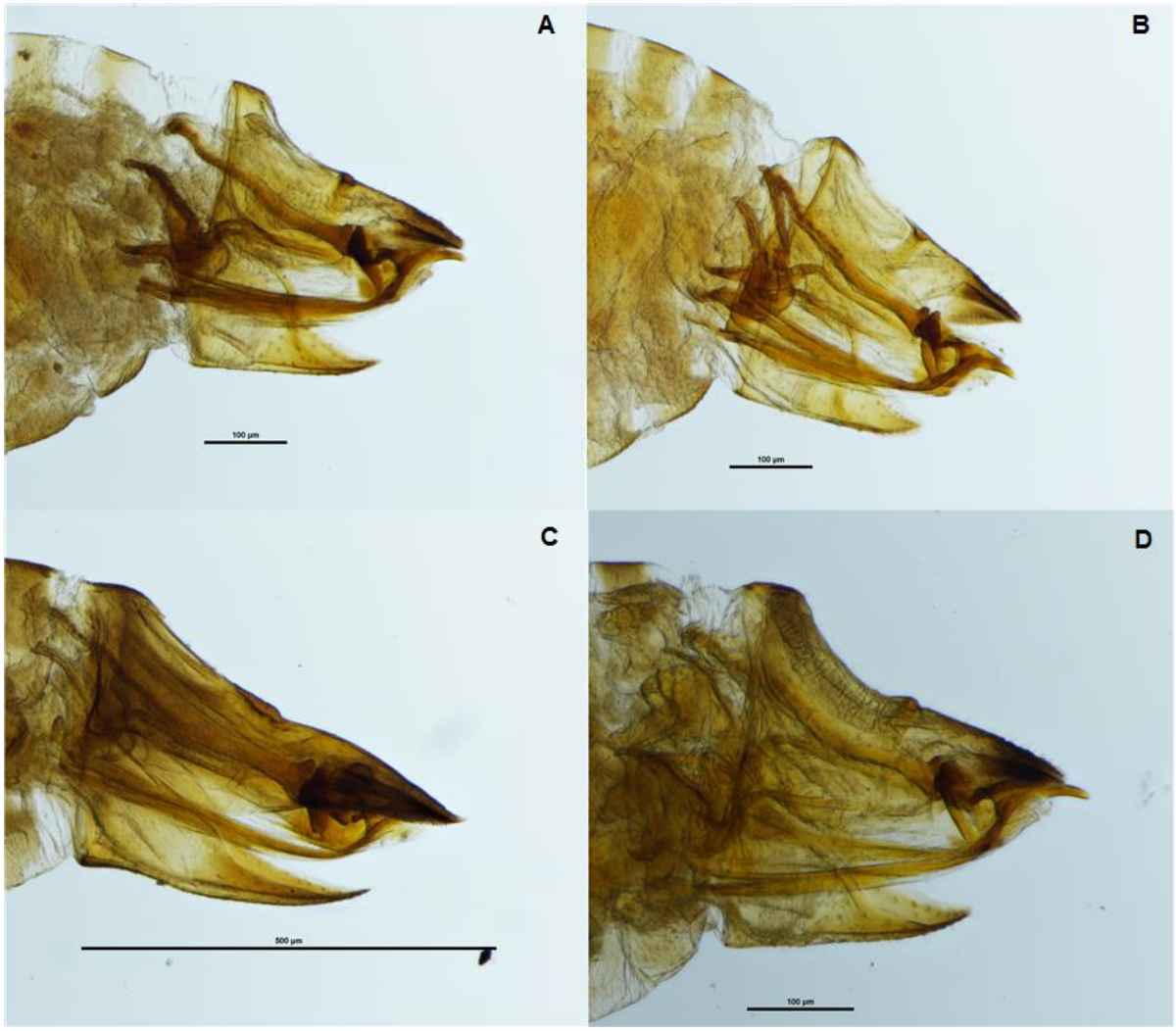


**Figure A.8:** Female terminalia of *Ctenarytaina* species. A= *C. clavata;* B= *C. clavata* B; C= *C. clavata* C; D= *C. clavata* D.

# Appendix B

# Field Collections

## B.1 New Zealand and Stewart Island

List of all the populations collected in New Zealand.

A population was defined (see chapter 3.2.1) as insects of the same species collected from a single plant or from a group of contiguous plants of the same species. Populations were confirmed by morphological and/or genetic analysis, retrospectively.

All the samples collected, including microscope slides, dry mounted and ethanol preserved have been deposited at the Lincoln University Entomological Collection (LUNZ), Lincoln, New Zealand.

**Table A.1:** Table includes the ID number, the collection date, the collector(s)' name(s), the GPS coordinates, the host plant name, the psyllid species and the samples DNA was extracted from.

| ID | Date | Collect | Latitude | Longitude | Crosby | Location | ***Plant*** | ***Species*** | DNA |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20/08/2014 | FMSRB | -43.5053 | 172.3525 | MC | West Melton | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | |
| 2 | 20/08/2014 | FMSRB | -43.4647 | 172.2258 | MC | Courtenay Dom. | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | a |
| 3 | 20/08/2014 | FMSRB | -43.4647 | 172.2258 | MC | Courtenay Dom. | *Grevillea* | *Acizzia hakeae* | a |
| 4 | 20/08/2014 | FMSRB | -43.2936 | 171.9266 | MC | Kowhai bush | *Pseudopanax* sp. | *Trioza irregularis* | a |
| 5 | 20/08/2014 | FMSRB | -43.301 | 171.7497 | MC | Porters Pass | *Ozothamnus* sp. | *Trioza acuta* | a |
| 5b | 20/08/2014 | FMSRB | -43.301 | 171.7497 | MC | Porters Pass | Unknown | *Trioza acuta* | a |
| 6 | 20/08/2014 | FMSRB | -43.3396 | 171.6324 | MC | Lake Coleridge | | *Trioza* sp. | |
| 7 | 20/08/2014 | FM | -43.6398 | 172.4752 | MC | Lincoln - PFR | *Sophora microphylla* | *Psylla apicalis* A | a,b |
| 8a | 18/09/2014 | FM | -42.9645 | 172.9678 | NC | Greta valley | *Eucalyptus* | *Acizzia* | |
| 8b | 18/09/2014 | FM | -42.9645 | 172.9678 | NC | Greta valley | *Eucalyptus* | *C. spatulata* | a |
| 9 | 19/09/2014 | FM | -42.2849 | 173.7609 | KA | Kaikoura | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a-c |
| 10 | 19/09/2014 | FM | -42.1555 | 173.924 | KA | Clarence | *Dodonaea viscosa* | *Acizzia dodonaeae* | a |
| 11 | 19/09/2014 | FM | -41.4182 | 173.9648 | MB | Tuamarina | *Acacia* | *Acizzia albizziae* | a-c |
| 11b | 19/09/2014 | FM | -41.4182 | 173.9648 | MB | Tuamarina | Same | *Acizzia jucunda* | b |
| 11c | 19/09/2014 | FM | -41.4182 | 173.9648 | MB | Tuamarina | Same | *Acizzia hakeae* | |
| 12 | 19/09/2014 | FM | -41.2911 | 173.2345 | NN | Nelson | *Dodonaea* | *Acizzia dodonaeae* | a,b |
| 13 | 21/09/2014 | FM | -41.2911 | 173.2345 | NN | Nelson | *Eucalyptus* | *Acizzia dodonaeae* | a |
| 14 | 21/09/2014 | FM | -41.2911 | 173.2345 | NN | Nelson | *Olearia paniculata* | *Ctenarytaina* sp. A | a |
| 15 | 21/09/2014 | FM | -41.2911 | 173.2345 | NN | Nelson | *Eucalyptus* | MIX | |
| 16 | 22/09/2014 | FM | -40.9596 | 173.0371 | NN | Abel Tasman | Hakae | *Acizzia hakeae* | a |
| 17 | 22/09/2014 | FM | -40.9596 | 173.0371 | NN | Abel Tasman | Hakae | *Acizzia dodonaeae* | a |
| 18 | 23/09/2014 | FM | -40.7635 | 172.683 | NN | Takaka | *Dodonaea viscosa* | *Acizzia dodonaeae* | a,b |
| 19 | 24/09/2014 | FM | -41.2688 | 172.8268 | NN | Woodstock | *Acacia* | *Acizzia jucunda* | a-c |
| 19b | 24/09/2014 | FM | -41.2688 | 172.8268 | NN | Woodstock | *Acacia* | *Acizzia albizziae* | a,b |
| 20 | 24/09/2014 | FM | -41.5772 | 172.7683 | NN | Atapo | *Olearia paniculata* | MIX | |
| 21 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Little River | *Acacia melanoxylon* | *Acizzia acaciae* | a,b |
| 22 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Little River | *Carmichaelia australis* | *Psylla carmichaeliae* A | a,b |
| 23 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Kunzea ericoides* | *Ctenarytaina* SHORT | a-c |
| 24 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Kunzea ericoides* | *Ctenarytaina pollicaris* | a-c |
| 25 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Bossu Rd | *Dracophyllum* | *Trioza bifida* | |
| 26 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Carmichaelia* sp. | *Psylla carmichaeliae* A | a,b |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 27 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Carmichaelia* sp. | *Psylla carmichaeliae* A | a,b |
| 28 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Fuchsia excorticata* | *C. spatulata* | a |
| 29 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Pseudowintera colorata* | *Trioza bifida* | a,b |
| 30 | 09/14/2014 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Pseudowintera colorata* | *Acizzia acaciae* | a |
| 31 | 09/14/2014 | SRB | -43.8355 | 172.7766 | MC | Te Oka | *Kunzea ericoides* | *Ctenarytaina pollicaris* | a,b |
| 32 | 09/14/2014 | SRB | -43.8355 | 172.7766 | MC | Te Oka | *Kunzea ericoides* | *Ctenarytaina clavata* | |
| 33 | 09/14/2014 | SRB | -43.6762 | 171.345 | MC | Mt Somers | *Leptospermum scoparium* | *Ctenarytaina* SHORT | a,b |
| 34 | 09/14/2014 | SRB | -43.6762 | 171.345 | MC | Mt Somers | *Leptospermum scoparium* | *Ctenarytaina clavata* | a |
| 35 | 09/14/2014 | SRB | -43.6762 | 171.345 | MC | Mt Somers | *Olearia odorata* | *A.*"POLLEN ISLAND" | a-c |
| 36 | 09/14/2014 | SRB | -43.6762 | 171.345 | MC | Mt Somers | *Leptospermum scoparium* | *Ctenarytaina clavata* | |
| 37 | 09/14/2014 | SRB | -43.6217 | 171.2306 | MC | Mt Barossa | Bush lawyer *(Rubus cissoides)* | *Trioza* | |
| 38 | 09/14/2014 | SRB | -43.6217 | 171.2306 | MC | Mt Barossa | *Discaria tomatou* | *Trioza discariae* | a,b |
| SI1 | 10/14/2014 | SRB | -45.0907 | 170.9662 | DN | Oamaru | *Dodonnea viscosa* Akeake | *Acizzia dodonaeae* | a |
| SI2 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Stewart Island | *Pseudopanax* sp. | *Trioza* | |
| SI3 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Dracophyllum* (Inaka) | *Trioza bifida* | a,b |
| SI4 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Hebe* | *Trioza bifida* | |
| SI5 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Olearia arborescens* | *Trioza bifida* | |
| SI6 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Weinmannia racemosa* | *Trioza* (only females) | |
| SI7 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| SI7b | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Fuchsia excorticata* | *Trioza bifida* | |
| SI8 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Eucalyptus* | *C. spatulata* | a |
| SI8b | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | | *Trioza* | |
| SI8c | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | | *Ctenarytaina* | |
| SI9 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Leptospermum scoparius* | *Ctenarytaina* sp. B | a |
| SI10 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Horseshoe Pt | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| SI11 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Horseshoe Pt | *Brachyglottis rotundifolia* | *Ctenarytaina* | |
| SI12 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Pittosporum* | *Trioza bifida* | a |
| SI13 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Aristotelia serrata* | *Ctenarytaina fuchsiae* | a,b |
| SI14 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Oban, SI | *Myrsine australis* | *Trioza bifida* | |
| SI15 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Aakers Pt, SI | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| SI16 | 10/14/2014 | SRB | -46.903 | 168.1278 | SI | Aakers Pt, SI | Small leaved shrub | *Ctenarytaina fuchsiae* | a |
| 39 | 23/10/2014 | FMSRB | -43.1405 | 172.7298 | NC | Amberley | *Eucalyptus* | *Ctenarytaina eucalypti* | a-c |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 23/10/2014 | FMSRB | -43.1405 | 172.7298 | NC | Amberley | | *Trioza discariae* | a |
| 41 | 23/10/2014 | FMSRB | -42.7967 | 172.8331 | NC | Culverden | | *Ctenarytaina* SHORT | a |
| 42 | 23/10/2014 | FMSRB | -42.7967 | 172.8331 | NC | Culverden | *Eucalyptus* | *Ctenarytaina eucalypti* | a-c |
| 43 | 23/10/2014 | FMSRB | -43.1405 | 172.7298 | NC | Amberley | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 44 | 23/10/2014 | FMSRB | -42.5196 | 172.8219 | MB | Jacks Pass | *Leptospermum scoparium* | *Ctenarytaina clavata* B | a |
| 45 | 23/10/2014 | FMSRB | -42.5196 | 172.8219 | MB | St James | *Unknown* | *Trioza acuta* | a |
| 46 | 27/10/2014 | SRB | -43.0292 | 171.6184 | NC | Bealey Spur | *Halocarpus* | *Trioza colorata* | a,b |
| 47 | 27/10/2014 | SRB | -43.0292 | 171.6184 | NC | Bealey Spur | *Pseudopanax edgerlii* | *Trioza* sp. C | a,b |
| 48 | 27/10/2014 | SRB | -43.2566 | 171.7222 | NC | Castle Hill | *Coprosma* | *Trioza emarginata* | a |
| 49 | 27/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | a |
| 50 | 23/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Halocarpus* | *Trioza colorata* | a,b |
| 51 | 27/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 52 | 27/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Fraxinus excelsior* | *C. spatulata* | a |
| 53 | 30/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Pittosporum eugenoides* | *Trioza vitreoradiata* | a |
| 54 | 30/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Pittosporum eugenoides* | *Trioza vitreoradiata* | a |
| 55 | 30/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Sophora microphylla* | *Psylla apicalis B* | a |
| 56 | 30/11/2014 | FM | -43.64 | 172.4743 | MC | Lincoln | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 57 | 9/11/2014 | SRB | -43.7031 | 172.6918 | MC | Kaituna Reserve | *Plagianthus* | *Trioza* PRICE S VALLEY | a,b |
| 58 | 23/01/2015 | SRB | -40.7353 | 172.6781 | NN | Parapara | *Acacia* (Wattle) | *Acizzia albizziae* | a |
| 59 | 23/01/2015 | SRB | -40.7353 | 172.6781 | NN | Parapara | *Pittosporum tobira* | *Trioza vitreoradiata* | a |
| 60 | 23/01/2015 | SRB | -40.7353 | 172.6781 | NN | Parapara | *Grevillea* | *Acizzia* | |
| 61 | 27/01/2015 | SRB | -40.6573 | 172.5779 | NN | Mt Stevens | *Dracophyllum* sp. | *Trioza decurvata* | a |
| 62 | 27/01/2015 | SRB | -40.6573 | 172.5779 | NN | Mt Stevens | *Metrosideros umbellata* | *Trioza* OMAHUTA | a |
| 63 | 27/01/2015 | SRB | -40.6573 | 172.5779 | NN | Mt Stevens | *Hebe* sp. | *Trioza hebicola* | a,b |
| 64 | 29/01/2015 | SRB | -40.5184 | 172.741 | NN | Farewell Spit | *Muehlenbeckia complexa* | *Trioza fasciata* | a |
| 65 | 30/01/2015 | SRB | -40.7148 | 172.6754 | NN | Milnthorpe | *Acacia* (Wattle) | *Acizzia uncatoides* | a |
| 66 | 30/01/2015 | SRB | -40.7148 | 172.6754 | NN | Milnthorpe | *Acacia* (Blackwood) | *Acizzia uncatoides* | a |
| 67 | 30/01/2015 | SRB | -40.7148 | 172.6754 | NN | Milnthorpe | *Acacia* (Wattle) | *Acizzia uncatoides* | a |
| 68 | 8/01/2015 | SRB | -42.4453 | 173.1424 | MB | Mt Lyford | *Hebe* sp. | *Trioza obscura* pop. D | a |
| 69 | 8/01/2015 | SRB | -42.4453 | 173.1424 | MB | Mt Lyford | *Dracophyllum* sp. | *Trioza decurvata* | a |
| 70 | 3/02/2015 | SRB | -36.8989 | 174.785 | AK | Auckland | *Solanum tuberosum* | *Bactericera cockerelli* | a,b |
| 71 | 6/02/2015 | FM | -43.6311 | 169.9471 | MK | Copland Track | *Olearia* sp. | *Trioza bifida* | |

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| 72 | 16/02/2015 | FM | -43.64 | 172.4743 | MC | Lincoln | *Pseudopanax crassifolius* | *Trioza panacis* | a,b |
| 73 | 17/02/2015 | SRB | -43.5778 | 172.6282 | MC | Christchurch | *Albizia* sp. | *Acizzia uncatoides* | a |
| 73 B | 17/02/2015 | SRB | -43.5778 | 172.6282 | MC | Christchurch | *Albizia* sp. | *Acizzia albizziae* | a |
| 74 | 17/02/2015 | SRB | -43.5778 | 172.6282 | MC | Christchurch | *Acacia* (Wattle) | *Acizzia albizziae* | a |
| 75 | 20/02/2015 | FM | -43.64 | 172.4743 | MC | Lincoln | *A. longifolium* | *Acizzia uncatoides* | a,b |
| 76 | 23/02/2015 | FM | -43.6279 | 172.1913 | MC | Dunsandel | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 77 | 23/02/2015 | FM | -44.0448 | 171.4188 | MC | Ealing | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 78 | 23/02/2015 | FM | -44.1278 | 171.3084 | SC | Orari | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 79 | 23/02/2015 | FM | -44.3992 | 171.2112 | SC | Timaru | *Eucalyptus nicholii* | *C. spatulata* | a |
| 80 | 23/02/2015 | FM | -44.3992 | 171.2112 | SC | Timaru | *Grevillea* | *Acizzia hakeae* | a |
| 81 | 23/02/2015 | FM | -44.3992 | 171.2112 | SC | Timaru | *Boronia* | *C. thysanura* | a |
| 82 | 24/02/2015 | FM | -45.8751 | 170.489 | DN | Dunedin | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 83 | 24/02/2015 | FM | -46.0234 | 170.0928 | DN | Waihola | *Pittosporum* | *Trioza vitreoradiata* | |
| 84 | 25/02/2015 | FM | -46.468 | 168.6583 | SL | The lignite Pit | *Pittosporum* | *Trioza vitreoradiata* | a |
| 85 | 25/02/2015 | FM | -46.4685 | 168.6586 | SL | The lignite Pit | *Pittosporum* | *Trioza vitreoradiata* | |
| 86 | 25/02/2015 | FM | -46.4677 | 168.6607 | SL | The lignite Pit | *Pittosporum* | *Trioza bifida* | |
| 87 | 25/02/2015 | FM | -46.4246 | 168.3605 | SL | Invercargill | *Alnus* | *Baeopelma foersteri* | a |
| 88 | 25/02/2015 | FM | -46.4246 | 168.3605 | SL | Invercargill | Same tree | *Trioza vitreoradiata* | a |
| 89 | 26/02/2015 | FM | -46.4246 | 168.3605 | SL | Invercargill | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 90 | 26/02/2015 | FM | -46.5642 | 168.9447 | SL | Tokanui | *Hoheria sextylosa* | *Trioza vitreoradiata* | a |
| 91 | 26/02/2015 | FM | -46.5722 | 169.3467 | SL | | *Olearia ilicifolia* | *Trioza* BRENDA MAY | a |
| 92 | 26/02/2015 | FM | -46.3707 | 168.3595 | SL | Invercargill | *Pittosporum* | *Trioza vitreoradiata* | |
| 93 | 26/02/2015 | FM | -46.3707 | 168.3595 | SL | Invercargill | *Fraxinus* | *Psyllopsis fraxinicola* | |
| 94 | 26/02/2015 | FM | -46.3707 | 168.3595 | SL | Invercargill | Same tree | *Psyllopsis fraxini* | a |
| 95 | 26/02/2015 | FM | -46.3707 | 168.3595 | SL | Invercargill | *Acacia melanoxylon* | *Acizzia acaciae* | a |
| 96 | 27/02/2015 | FM | -46.3707 | 168.3595 | SL | Invercargill | *Fraxinus* | *Psyllopsis fraxinicola* | |
| 97 | 27/02/2015 | FM | -46.3707 | 168.3595 | SL | Invercargill | Same tree | *Psyllopsis fraxini* | |
| 98 | 27/02/2015 | FM | -46.3299 | 168.2958 | SL | Wallacetown | *Pittosporum* | *Trioza vitreoradiata* | |
| 99 | 27/02/2015 | FM | -46.1572 | 167.6855 | FD | Tuatapere | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | a |
| 100 | 27/02/2015 | FM | -46.129 | 167.68 | FD | Tuatapere | *Pittosporum* | *Trioza vitreoradiata* | |
| 101 | 28/02/2015 | FM | -45.7985 | 167.5467 | FD | Fiordland | *Eucalyptus* | *C. spatulata* | a |
| 102 | 28/02/2015 | FM | -45.7784 | 167.6153 | FD | Monowai | *Eucalyptus* | *C. spatulata* | a |

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| 103 | 28/02/2015 | FM | -45.7784 | 167.6153 | FD | Monowai | *Pittosporum* | *Trioza vitreoradiata* | |
| 104 | 2/03/2015 | FM | -45.5648 | 167.6107 | FD | Manapouri | *Pittosporum* | *Trioza vitreoradiata* | a |
| 105 | 2/03/2015 | FM | -45.4247 | 167.7189 | FD | Te Anau | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 106 | 2/03/2015 | FM | -45.4247 | 167.7189 | FD | Te Anau | *Fraxinus* | *Psyllopsis fraxinicola* | a |
| 107 | 2/03/2015 | FM | -45.4247 | 167.7189 | FD | Te Anau | Same tree | *Psyllopsis fraxini* | |
| 108 | 2/03/2015 | FM | -45.4247 | 167.7189 | FD | Te Anau | Same tree | *C. spatulata* | a |
| 109 | 2/03/2015 | FM | -45.637 | 168.1752 | SL | Mossburn | *Eucalyptus* | *C. spatulata* | a |
| 110 | 3/03/2015 | FM | -44.2569 | 170.0993 | SC | Twizel | *Alnus* | *Baeopelma foersteri* | a |
| 111 | 4/03/2015 | FM | -44.2294 | 170.8734 | SC | Albury | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 112 | 4/03/2015 | FM | -44.3098 | 170.9528 | SC | Cave | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 113 | 4/03/2015 | FM | -44.3098 | 170.9528 | SC | Cave | Same tree | *Psyllopsis fraxini* | a |
| 114 | 26/02/2015 | FM | -46.4246 | 168.3605 | SL | Invercargill | *Eucalyptus* same as 89 | *C. spatulata* | |
| 115 | 2/03/2015 | FM | -45.637 | 168.1752 | SL | Mossburn | *Eucalyptus* same as 109 | *Ctenarytaina eucalypti* | a |
| 116 | 6/03/2015 | SRB | -36.8989 | 174.785 | AK | Auckland | *Solanum tuberosum* | *Acizzia solanicola* | a-e |
| 117 | 11/03/2015 | FM | -43.531 | 172.6194 | MC | Christchurch | *Schinus molle* | *Calophya schini* | a,b |
| 118 | 11/03/2015 | FM | -43.531 | 172.6194 | MC | Christchurch | *Acmena floribunda (Syzygium)* | *Trioza adventicia* | a,b |
| 119 | 11/03/2015 | FM | -43.531 | 172.6194 | MC | Christchurch | *Olearia* | *Trioza* "Massey" | a,b |
| 120 | 10/03/2015 | SRB | -43.6325 | 172.6248 | MC | Sign of Bellbird | *Olearia avicenniifolia* | *Trioza subacuta* | a,b |
| 121 | 10/03/2015 | SRB | -43.6325 | 172.6248 | MC | same | Same tree | *Trioza* sp. | |
| 122 | 22/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Ficus macrophylla* | *Mycopsylla fici* | a |
| 123 | 22/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Acacia* | *Acizzia acaciae* | a |
| 124 | 22/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Lophostemon* | *C. longicauda* | a |
| 125 | 23/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Casuarina* | *Casuarinicola* sp. | a |
| 126 | 23/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Casuarina* | *Casuarinicola* sp. | |
| 127 | 23/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Syzygium smithii* | *Ctenarytaina* unknown | a,b |
| 128 | 23/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Eucalyptus* | *B. occidentalis* | |
| 129 | 23/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | Same tree | mix | |
| 130 | 24/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Casuarina* | Triozid sp. | |
| 131 | 25/03/2015 | FM | -36.8989 | 174.785 | AK | Manukau | *Lophostemon* | *C. longicauda* | a,b |
| 132 | 25/03/2015 | FM | -36.8989 | 174.785 | AK | Manukau | *Muehlenbackia auxiliaris x complexa* | *Trioza fasciata* | a |

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| 133 | 25/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Near Olearia* | *Trioza vitreoradiata* | a |
| 134 | 26/03/2015 | FM | -36.8989 | 174.785 | AK | Auckland | *Ficus macrophylla* | *Mycopsylla fici* | a,b |
| 135 | 26/03/2015 | FM | -36.8107 | 175.098 | AK | Waiheke Island | *Eucalyptus* | *Glycaspis granulata* | a,b |
| 136 | 26/03/2015 | FM | -36.8107 | 175.098 | AK | Waiheke Island | Same tree | *C. spatulata* | a |
| 137 | 26/03/2015 | FM | -36.8107 | 175.098 | AK | Waiheke Island | Same tree | *B. occidentalis* | a |
| 138 | 26/03/2015 | FM | -36.8107 | 175.098 | AK | Waiheke Island | Same tree | mix | |
| 139 | 26/03/2015 | FM | -36.8107 | 175.098 | AK | Waiheke Island | *Casuarina* | *Casuarinicola* sp. | a |
| 140 | 28/03/2015 | FM | -37.2789 | 175.047 | AK | Mercer | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 141 | 28/03/2015 | FM | -37.7685 | 175.2474 | WO | Hamilton | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 142 | 28/03/2015 | FM | -37.7685 | 175.2474 | WO | Hamilton | *Eucalyptus* | *B. occidentalis* | a |
| 143 | 28/03/2015 | FM | -37.7685 | 175.2474 | WO | Hamilton | Same tree | *C. spatulata* | a |
| 144 | 28/03/2015 | FM | -37.7685 | 175.2474 | WO | Hamilton | *Casuarina* | *Casuarinicola* sp. | |
| 145 | 28/03/2015 | FM | -37.7685 | 175.2474 | WO | Hamilton | Same tree | *Acizzia acaciae* | a |
| 146 | 28/03/2015 | FM | -37.7685 | 175.2474 | WO | Hamilton | Same tree | *Trioza* | |
| 147 | 29/03/2015 | FM | -37.141 | 175.5414 | CL | Thames | *Casuarina* | *Casuarinicola* sp. | a |
| 148 | 29/03/2015 | FM | -37.0136 | 175.5103 | CL | Ruamahunga | | *Trioza vitreoradiata* | a |
| 149 | 30/03/2015 | FM | -37.2772 | 175.2906 | WO | Mangatarata | *Acacia* | *Acizzia acaciae* | |
| 150 | 31/03/2015 | FM | -35.7566 | 174.3774 | ND | Whangarei | *Casuarina* | *Casuarinicola australis* | a,b |
| 151 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | Hawdon | *Aristotelia fruticosa* | *Trioza falcata* | a |
| 152 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | Castle Hill | *Leptospermum scoparius* | *Ctenarytaina clavata* | a |
| 153 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | Sudden Valley | *Olearia avicenniifolia* | *Trioza* MIX | |
| 154 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | Hawdon river | *Aristotelia fruticosa* | *Trioza falcata* | a |
| 155 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | same | Same plant | *Trioza* sp. | |
| 156 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | Castle Hill | *Aristotelia fruticosa* | *Trioza falcata* | |
| 157 | 4/04/2015 | SRB | -43.2566 | 171.7222 | NC | same | Same plant | *Trioza falcata* | a |
| 158 | 5/04/2015 | SRB | -43.1341 | 171.7636 | MC | Flock hill | *Aristotelia fruticosa* | *Trioza falcata* | a |
| 159 | 5/04/2015 | SRB | -43.1341 | 171.7636 | MC | Flock hill | *Olearia avicenniifolia* | *Trioza subacuta* | a,b |
| 160 | 5/04/2015 | SRB | -43.1341 | 171.7636 | MC | Flock hill | *Ozothamnus leptophyllus* | *Trioza acuta* B | a |
| 161 | 12/04/2015 | SRB | -43.8113 | 173.0285 | MC | Hinewai | *Coprosma* sp. | *Trioza doryphora* | a |
| 162 | 12/04/2015 | SRB | -43.8113 | 173.0285 | MC | Hinewai | *Olearia ilicifolia* | *Trioza doryphora* | a |
| 163 | 12/04/2015 | SRB | -43.8113 | 173.0285 | MC | Hinewai | *Hebe* sp. | *Trioza* | |
| 164 | 29/03/2015 | SRB | -43.7769 | 172.7893 | MC | Dan's Little River | *Kunzea ericoides* | *Ctenarytaina* SHORT | a |

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| 164B | 29/03/2015 | SRB | -43.7769 | 172.7893 | MC | Same location | Same plant | *B. occidentalis* | |
| 165 | 27/02/2015 | SRB | -43.64 | 172.4743 | MC | Lincoln | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 166 | 16/04/2015 | FMSRB | -43.7212 | 172.9373 | MC | Pigeon Bay | *Pseudopanax* | *Trioza irregularis* | a |
| 167 | 16/04/2015 | FMSRB | -43.7212 | 172.9373 | MC | Same location | *Same plant* | *Trioza irregularis* | |
| 168 | 16/04/2015 | FMSRB | -43.7212 | 172.9373 | MC | Same location | *Melicytus ramiflorus* | *Trioza irregularis* | a |
| 169 | 16/04/2015 | FMSRB | -43.7521 | 173.0157 | MC | Otepatotu Res. | *Pittosporum* | *Trioza vitreoradiata* | a |
| 170 | 16/04/2015 | FMSRB | -43.7521 | 173.0157 | MC | Same location | *Olearia ilicifolia* | *Trioza doryphora* | a |
| 171 | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Hinewai Reserve | *Coprosma* | | |
| 172 | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Hinewai Reserve | *Round leaves plant* | *Ctenarytaina* SHORT | a |
| 173 | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Hinewai Reserve | *Olearia ilicifolia* | *Trioza doryphora* | a |
| 174 | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Hinewai Reserve | *Olearia fragmantissima* | *Trioza bifida* | a |
| 175 | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Hinewai Reserve | *Kunzea ericoides* | *Ctenarytaina* SHORT | a |
| 175B | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Same location | Same plant | *C. pollicaris* B | a |
| 176 | 16/04/2015 | FMSRB | -43.8113 | 173.0285 | MC | Hinewai Reserve | *Hebe* | *Trioza bifida* | |
| 177 | 16/04/2015 | FMSRB | -43.8067 | 172.9696 | MC | Akaroa | *Acacia* | *Acizzia* | |
| 178 | 16/04/2015 | FMSRB | -43.8067 | 172.9696 | MC | Akaroa | *Brachyglottis repanda* | *Acizzia uncatoides* | a |
| 179 | 17/04/2015 | FM | -43.64 | 172.4743 | MC | Lincoln | *Acacia* | *Acizzia albizziae* | a |
| 180 | 17/07/2015 | FM | -43.64 | 172.4743 | MC | Lincoln | *Acacia baileyana* | *Acizzia* sp. | a,b |
| 181 | 17/07/2015 | FM | -43.64 | 172.4743 | MC | Lincoln | *Acacia* | *Acizzia uncatoides* | a,b |
| 182 | | SRB | -44.0892 | 171.2379 | MC | Geraldine | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a,b |
| 183 | | SRB | -43.8225 | 172.7862 | MC | Te Oka | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a,b |
| 184 | | SRB | -43.6325 | 172.6248 | MC | Sign of Bellbird | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 185 | 1/09/2015 | FM | -43.64 | 172.4743 | MC | Lincoln | *Sophora microphylla* | *Psylla apicalis* | |
| 186 | 1/09/2015 | SDJB | -43.531 | 172.6194 | MC | Christchurch | *Melicytus ramiflorus* | | |
| 187 | 1/09/2015 | SDJB | -43.531 | 172.6194 | MC | Christchurch | *Sophora microphylla* | *Psylla apicalis* | a |
| 188 | 1/09/2015 | SDJB | -43.64 | 172.4743 | MC | Lincoln | *Pittosporum eugenioides* | | |
| 189 | 8/10/2015 | SRB | -45.864 | 170.658 | DN | Hoopers Inlet Rd | *Eucalyptus* | *Ctenarytaina eucalypti* | |
| 190 | 8/10/2015 | SRB | -45.8897 | 170.6719 | DN | Sandymount pt. | *Olearia arborescens* | *Trioza bifida* | a |
| 191 | 7/10/2015 | SRB | -45.8245 | 170.7239 | DN | Wickliffe Bay | *Olearia avicenniifolia* (likely) | *Trioza subacuta* | |
| 192 | 13/10/2015 | SRB | -37 | 174.8023 | AK | Butterfly Creek | *Pittosporum tobira* | *Trioza bifida* | |
| 193 | | SRB | -43.7031 | 172.6918 | MC | Kaituna Valley | *Acacia* | *Acizzia* | |
| 194 | 25/10/2015 | FM | -44.8104 | 167.7832 | FD | Milford Track | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |

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| 195 | 2/11/2015 | FM | -43.5968 | 172.3636 | MC | Christchurch | *Cytisus scoparius* | *Arytainilla spartiophila* | a |
| 196 | 2/11/2015 | FM | -43.5968 | 172.3636 | MC | Christchurch | *Acacia* | *Acizzia albizziae* | a |
| 197 | 2/11/2015 | FM | -43.5968 | 172.3636 | MC | Same location | *Acacia* | *Acizzia albizziae* | |
| 198 | 2/11/2015 | FM | -43.5968 | 172.3636 | MC | Christchurch | *Cytisus scoparius* | *Arytainilla spartiophila* | |
| 199 | 2/11/2015 | FM | -43.5968 | 172.3636 | MC | Same location | *Acacia* | *Acizzia albizziae* | a |
| 200 | 2/11/2015 | FM | -45.087 | 170.9755 | DN | Oamaru gardens | *Sophora microphylla* | *Psylla apicalis* A and B | a |
| SI 17 | 5/11/2015 | FM | -46.8553 | 168.0804 | SI | Rakiura Track | *Pseudowintera colorata* | *Ctenarytaina* sp. B | a |
| SI 18 | 6/11/2015 | FM | -46.8553 | 168.0804 | SI | Rakiura Track | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| SI 19 | 6/11/2015 | FM | -46.8553 | 168.0804 | SI | Rakiura Track | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| SI 20 | 6/11/2015 | FM | -46.8553 | 168.0804 | SI | Oban | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| SI 21 | 6/11/2015 | FM | -46.8553 | 168.0804 | SI | Oban | *Carmichaelia* (windblown) | *Trioza* | |
| SI 22 | 7/11/2015 | FM | -46.8553 | 168.0804 | SI | Oban | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 201 | 8/11/2015 | FM | -46.4246 | 168.3605 | SL | Invercargill | *Sophora microphylla* | *Psylla apicalis* A and B | a |
| 202 | 8/11/2015 | FM | -46.3422 | 168.322 | SL | Wallacetown | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 203 | 8/11/2015 | FM | -45.6929 | 167.653 | FD | Whare Creek | *Cytisus scoparius* | *Arytainilla spartiophila* | |
| 204 | 8/11/2015 | FM | -45.4223 | 167.7229 | FD | Te Anau - DOC | *Sophora microphylla* | *Psylla apicalis* A | a |
| 205 | 8/11/2015 | FM | -45.4223 | 167.7229 | FD | Te Anau - DOC | *Cytisus scoparius* | *Arytainilla spartiophila* | |
| 206 | 9/11/2015 | FM | -45.0368 | 168.6606 | OL | Queenstwon | *Sophora microphylla* | *Psylla apicalis* A | a |
| 207 | 9/11/2015 | FM | -45.0368 | 168.6606 | OL | Queenstwon | *Cytisus scoparius* | *Arytainilla spartiophila* | a |
| 208 | 9/11/2015 | FM | -45.0368 | 168.6606 | OL | Queenstwon | *Sophora microphylla* | *Psylla apicalis* | |
| 209 | 9/11/2015 | FM | -45.036 | 169.1927 | CO | Cromwel | *Sophora microphylla* | *Psylla apicalis* B | a |
| 210 | 9/11/2015 | FM | -45.0432 | 169.172 | CO | Cromwel | *Acacia* | *Acizzia uncatoides* | a |
| 211 | 9/11/2015 | FM | -45.0432 | 169.172 | CO | Cromwel | *Acacia* | *Acizzia uncatoides* | a |
| 212 | 9/11/2015 | FM | -44.9125 | 169.2948 | CO | Cromwel -DOC | *Carmichaelia* | *Psylla carmichaeliae* E | a |
| 213 | 9/11/2015 | FM | -45.0432 | 169.172 | CO | Cromwell | *Carmichaelia petri* | *Psylla carmichaeliae* E | a |
| 214 | 9/11/2015 | FM | -45.1155 | 169.3237 | CO | Clyde | *Carmichaelia compacta* | *Psylla carmichaeliae* D | a |
| 215 | 9/11/2015 | FM | -45.231 | 169.3741 | CO | Alexandra | *Sophora microphylla* | *Psylla apicalis* B | a |
| 216 | 9/11/2015 | FM | -45.1786 | 169.3094 | CO | Clyde | *Cytisus scoparius* | *Arytainilla spartiophila* | |
| 217 | 10/11/2015 | FM | -44.9747 | 168.9485 | OL | Crown Range Rd | *Carmichaelia* | *Psylla carmichaeliae* E | a |
| 218 | 10/11/2015 | FM | -44.6979 | 169.136 | OL | Wanaka | *Sophora microphylla* | *Psylla apicalis* B | a |
| 219 | 10/11/2015 | FM | -44.6979 | 169.136 | OL | Wanaka | *Acacia* | *Acizzia uncatoides* | a |
| 220 | 10/11/2015 | FM | -44.1274 | 169.3387 | WD | Haast Pass | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |

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|---|---|---|---|---|---|---|---|---|---|
| 221 | 10/11/2015 | FM | -43.9709 | 169.4166 | WD | Haast Pass | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 222 | 10/11/2015 | FM | -43.751 | 169.3871 | WD | Haast Highway | *Carmichaelia* | *Psylla carmichaeliae C* | a |
| 223 | 10/11/2015 | FM | -43.5652 | 169.7703 | WD | Haast Highway | *Carmichaelia* | *Psylla carmichaeliae C* | a |
| 224 | 11/11/2015 | FM | -43.4362 | 170.0784 | WD | Fox Glacier | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 225 | 11/11/2015 | FM | -43.2275 | 170.1741 | WD | Okarito | *Acacia* (Wattle) | *Acizzia albizziae* | a |
| 226 | 11/11/2015 | FM | -42.7397 | 170.956 | WD | Hokitika | *Sophora microphylla* | *Psylla apicalis B* | a |
| 227 | 11/11/2015 | FM | -42.7397 | 170.956 | WD | Hokitika | *Acacia* (Wattle) | *Acizzia albizziae* | a |
| 228 | 11/11/2015 | FM | -42.7397 | 170.956 | WD | Hokitika | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 229 | 11/11/2015 | FM | -42.7397 | 170.956 | WD | Hokitika | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 230 | 11/11/2015 | FM | -42.4645 | 171.2088 | BR | Greymouth, | *Sophora microphylla* | *Psylla apicalis B* | a |
| 231 | 11/11/2015 | FM | -42.4645 | 171.2088 | BR | Greymouth | plant sample 43 | *Trioza* | |
| 232 | 11/11/2015 | FM | -42.4645 | 171.2088 | BR | Greymouth | *Acacia* | *Acizzia uncatoides* | a |
| 233 | 12/11/2015 | FM | -41.7624 | 171.6506 | NN | Westport | *Acacia* (Wattle) | *Acizzia uncatoides* | a |
| 234 | 12/11/2015 | FM | -41.7624 | 171.6506 | NN | Westport | *Sophora microphylla* | *Psylla apicalis B* | a |
| 235 | 12/11/2015 | FM | -41.7624 | 171.6506 | NN | Westport | *Acacia* | *Trioza vitreoradiata* | a |
| 236 | 12/11/2015 | FM | -41.7624 | 171.6506 | NN | Westport | *Acacia* | *Psylla apicalis B* | a |
| 237 | 12/11/2015 | FM | -41.5822 | 171.9024 | NN | Karamea | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 238 | 12/11/2015 | FM | -41.2334 | 172.1009 | NN | Karamea | *Sophora microphylla* | *Psylla apicalis B* | a |
| 239 | 12/11/2015 | FM | -41.2334 | 172.1009 | NN | Karamea | *Acacia* (Wattle) | *Acizzia uncatoides* | a |
| 240 | 12/11/2015 | FM | -41.6337 | 171.8532 | NN | Granity | *Acacia* (Wattle) | *Acizzia uncatoides* | a |
| 241 | 15/11/2015 | SRB | -43.7799 | 172.7738 | MC | Port Levy | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | |
| 242 | 17/10/2015 | SDJB | -43.4987 | 172.7252 | MC | New Brighton | *Dodonaea viscosa* | | |
| 243 | 23/10/2015 | SDJB | -43.64 | 172.4743 | MC | Lincoln | *Pittosporum* | | |
| 244 | 4/12/2015 | SRB | -45.3486 | 170.8239 | DN | Moeraki | *Sophora microphylla* | *Psylla apicalis B* | a |
| 245 | 6/12/2015 | SRB | -44.7407 | 171.0455 | SC | Waimate | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 246 | 2/01/2016 | SRB | -43.1957 | 171.6831 | MC | Hogs back | *Hebe prob Matagouri* | *A. spartiophyla* | a |
| 247 | 2/01/2016 | SRB | -43.1957 | 171.6831 | MC | same | *Same plant* | *Trioza discariae* | a |
| 248 | 4/01/2016 | SRB | -42.9495 | 171.5815 | NC | Kelly Saddle | *Pseudopanax/Coprosma/*Celery pine but under Kamhai. | *Atmetocranium myersi* | a,b |
| 249 | 4/01/2016 | SRB | -42.9495 | 171.5815 | NC | Kelly Saddle | *Carmichaelia* sp. | *Psylla carmichaeliae C* | a |
| 250 | 7/01/2016 | SRB | -41.1471 | 173.5134 | NN | Whangamoa | *Fuchsia excorticata* | *Trioza compressa* | a |
| 251 | 7/01/2016 | SRB | -41.1471 | 173.5134 | NN | same | Same plant | *Ctenarytaina fuchsia* | a |

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|---|---|---|---|---|---|---|---|---|---|
| 252 | 7/01/2016 | SRB | -41.1471 | 173.5134 | NN | same | Same plant | *Trioza irregularis* | a |
| 254 | 16/01/2016 | SRB | -41.8008 | 172.8379 | NN | St Arnaud | *Olearia* | *Trioza subvexa* | a |
| 255 | 16/01/2016 | SRB | -41.8008 | 172.8379 | NN | same | Same plant | *Trioza* | |
| 256 | 17/01/2016 | SRB | -40.7635 | 172.683 | NN | Takaka | *Fuchsia excorticata* | *Ctenarytaina fuchsia* | |
| 257 | 17/01/2016 | SRB | -40.7635 | 172.683 | NN | Takaka hill top | *Olearia* | *Trioza subvexa* | a |
| 258 | 24/01/2016 | SRB | -40.7681 | 172.5252 | NN | 15 mile creek | *Kunzea ericoides* | *Ctenarytaina* SHORT | a |
| 259 | 24/01/2016 | SRB | -41.0933 | 172.7215 | NN | Powerstation | *Coprosma* (thin leaved) | *C. pollicaris* B | a,b |
| 260 | 24/01/2016 | SRB | -41.0933 | 172.7215 | NN | Powerstation | *Olearia rani var colorata* | *Trioza compressa* | a |
| 261 | 24/01/2016 | SRB | -41.0933 | 172.7215 | NN | Powerstation | *Schefflera digitata* | *Trioza irregularis* | a |
| 262 | 24/01/2016 | SRB | -41.0933 | 172.7215 | NN | Powerstation | *Fuchsia excorticata* | *Ctenarytaina* | |
| 263 | 24/01/2016 | SRB | -41.0933 | 172.7215 | NN | Powerstation | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 264 | 26/01/2016 | SRB | -40.577 | 172.6285 | NN | Wanganui inlet | *Kunzea ericoides* | *Ctenarytaina* sp. | |
| 265 | 28/01/2016 | SRB | -40.5163 | 172.75 | NN | Farewell spit | *Kunzea ericoides* | *Ctenarytaina* sp. | |
| 266 | 29/01/2016 | SRB | -40.577 | 172.6285 | NN | Druggans dam | *Kunzea ericoides* | *Ctenarytaina* sp. E | a |
| 267 | 29/01/2016 | SRB | -40.577 | 172.6285 | NN | Druggans dam | *Leptospermum scoparius* | *Ctenarytaina clavata* | a |
| 268 | 29/01/2016 | SRB | -40.577 | 172.6285 | NN | Druggans dam | *Kunzea ericoides* | *Ctenarytaina* sp. | |
| 269 | 30/01/2016 | SRB | -40.7635 | 172.683 | NN | Takaka hill | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a,b |
| 270 | 30/01/2016 | SRB | -41.8551 | 172.2073 | NN | Shenandoah hill | *Fuchsia excorticata* | *Bactericera cockerelli* | a |
| 271 | 30/01/2016 | SRB | -41.8551 | 172.2073 | NN | Same | *Same plant* | *Ctenarytaina fuchsiae* | a |
| 272 | 30/01/2016 | SRB | -42.3836 | 172.4017 | NC | Lewis Pass | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 273 | 24/01/2016 | FM | -42.9495 | 171.5815 | NC | Arthur Pass | wind blown | *Trioza bifida* | a |
| 274 | 2/02/2016 | FM | -43.64 | 172.4743 | MC | Lincoln | Lance wood | *Trioza panacis* | |
| 275 | 6/02/2016 | FM | -44.1825 | 169.0088 | FD | Gillespie track | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | a |
| 276 | 6/02/2016 | FM | -44.1825 | 169.0088 | FD | Gillespie track | *Olearia* | *Trioza BRENDA MAY* | a |
| 277 | 9/02/2016 | FM | -43.1405 | 172.7298 | NC | Amberley | *Acacia* | *Acizzia uncatoides* | |
| 278 | 10/02/2016 | FM | -41.4281 | 173.9595 | MB | Blenheim | *Acacia* | *Acizzia albizziae* | |
| 279 | 10/02/2016 | FM | -41.4281 | 173.9595 | MB | same car park | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | |
| 280 | 10/02/2016 | FM | -41.263 | 174.0533 | MB | North of Picton | *Acacia* | *Acizzia* | |
| 281 | 10/02/2016 | FM | -41.263 | 174.0533 | MB | same spot | *Pseudowintera colorata* | *Trioza bifida* | a |
| 282 | 10/02/2016 | FM | -41.2877 | 174.0059 | MB | Picton marina | *Brachyglottis* | *Trioza vitreoradiata* | a |
| 283 | 10/02/2016 | FM | -41.2877 | 174.0059 | MB | Picton marina | *Brachyglottis* | *Trioza vitreoradiata* | a |
| 284 | 10/02/2016 | FM | -41.2877 | 174.0059 | MB | Picton marina | *Kunzea ericoides* | *Ctenarytaina clavata?* | |

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|---|---|---|---|---|---|---|---|---|---|
| 285 | 10/02/2016 | FM | -41.2877 | 174.0059 | MB | Picton marina | *Same plant* | *Ctenarytaina pollicaris* | |
| 286 | 11/02/2016 | FM | -41.1183 | 175.0433 | WN | Upper hutt | *Pittosporum* | *Trioza vitreoradiata* | |
| 287 | 11/02/2016 | FM | -41.1183 | 175.0433 | WN | same | *windblown* | *Acizzia albizziae* | |
| 288 | 11/02/2016 | FM | -41.1191 | 175.3341 | WA | Featherstone | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | a |
| 289 | 11/02/2016 | FM | -39.6329 | 176.8476 | HB | Hastings Park | *Syzygium* | *Trioza adventicia* | a |
| 290 | 12/02/2016 | FM | -39.505 | 176.8762 | HB | Napier | windblown | *Acizzia albizziae* | |
| 291 | 12/02/2016 | FM | -39.505 | 176.8762 | HB | Napier | *Schinus molle* | *Calophya schini* | a |
| 292 | 12/02/2016 | FM | -38.9902 | 177.7871 | GB | Gisborne | *Dodonaea viscosa* | *Acizzia dodonaeae* | |
| 293 | 13/02/2016 | FM | -38.5627 | 177.7183 | GB | Gisborne | *Acacia robusta* | *Acizzia uncatoides* | |
| 294 | 13/02/2016 | FM | -38.5627 | 177.7183 | GB | Gisborne | *Acacia* (Wattle) | *Acizzia jucunda* | a |
| 295 | 13/02/2016 | FM | -38.5627 | 177.7183 | GB | Gisborne | *Acacia (*Wattle*)* | *Acizzia exquisita* | a,b |
| 296 | 13/02/2016 | FM | -38.5627 | 177.7183 | GB | Same location | *Same plant* | *Acizzia albizziae* | |
| 297 | 13/02/2016 | FM | -38.6318 | 177.8823 | GB | Gisborne | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | a |
| 298 | 13/02/2016 | FM | -38.125 | 178.3155 | GB | East Cape | *Acacia (*Wattle*)* | *Acizzia uncatoides* | |
| 299 | 14/02/2016 | FM | -37.6311 | 178.4116 | GB | East Cape | *Acacia (*Wattle*)* | *Acizzia* WAITAKERE | a |
| 300 | 14/02/2016 | FM | -37.6889 | 178.5482 | GB | East Cape | *Pittosporum* | *Trioza vitreoradiata* | a |
| 301 | 14/02/2016 | FM | -37.8856 | 177.5571 | BP | Maraenui | *Acacia (*Wattle*)* | *Acizzia uncatoides* | |
| 302 | 14/02/2016 | FM | -37.98 | 176.9981 | BP | Whakatane | *Kunzea ericoides* | *Ctenarytaina* sp. C | a |
| 303 | 15/02/2016 | FM | -37.98 | 176.9981 | BP | Whakatane | *Acacia (*Wattle*)* | *Acizzia uncatoides* | |
| 304 | 15/02/2016 | FM | -37.8711 | 176.7089 | BP | Matata | Plant sample 26 | *Cardiaspina fiscella* | |
| 305 | 15/02/2016 | FM | -37.8711 | 176.7089 | BP | Same location | Same plant | *Trioza* | |
| 306 | 15/02/2016 | FM | -37.6333 | 176.1708 | BP | Mt Manganui | *Eucalyptus* | *Ctenarytaina eucalypti* | |
| 307 | 15/02/2016 | FM | -37.6333 | 176.1708 | BP | Same location | Same plant | *2 Trioza 1 Ctenarytaina* | |
| 308 | 15/02/2016 | FM | -38.1336 | 176.2442 | BP | Rotorua | *Eucalyptus* | *B. occidentalis* | |
| 309 | 15/02/2016 | FM | -38.1336 | 176.2442 | BP | Rotorua | *Fraxinus excelsior* | *Psyllopsis fraxini* | a |
| 310 | 15/02/2016 | FM | -38.1336 | 176.2442 | BP | Same location | Same plant | MIX | |
| 311 | 15/02/2016 | FM | -38.1336 | 176.2442 | BP | Rotorua | *Pittosporum* | *Trioza vitreoradiata* | |
| 312 | 16/02/2016 | FM | -37.9754 | 175.7593 | WO | Tirau | *Acacia* | *Acizzia uncatoides* | |
| 313 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Hamilton | *Kunzea ericoides* | *Ctenarytaina* sp. C | a |
| 314 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *Ctenarytaina* sp. F | a |
| 315 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Hamilton | *Eucalyptus* | *B. occidentalis* | a |
| 316 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Hamilton | *Eucalyptus* | *B. occidentalis* | |

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| 317 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *Ctenarytaina* | |
| 318 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Hamilton | *Eucalyptus* | *Glycaspis granulata* | a |
| 319 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *Creiis lituratus* | a |
| 320 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *Cardiaspina fiscella* | a |
| 321 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *C. occidentalis* | |
| 322 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *D. spatulata* | a |
| 323 | 16/02/2016 | FM | -37.7714 | 175.2495 | WO | Same location | Same plant | *A. communis* | a |
| 324 | 16/02/2016 | FM | -38.717 | 176.0101 | TO | Taupo | *Eucalyptus* | *Ctenarytaina eucalypti* | |
| 325 | 16/02/2016 | FM | -38.717 | 176.0101 | TO | Same location | Same plant | *Trioza* sp. | |
| 326 | 17/02/2016 | FM | -38.717 | 176.0101 | TO | Taupo | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 327 | 17/02/2016 | FM | -38.717 | 176.0101 | TO | Taupo | *Eucalyptus* | *B. occidentalis* | |
| 328 | 17/02/2016 | FM | -38.717 | 176.0101 | TO | Same location | Same plant | *C. spatulata* | a |
| 329 | 17/02/2016 | FM | -38.717 | 176.0101 | TO | Taupo | *Acacia* | *Acizzia dodonaeae* | a |
| 330 | 19/02/2016 | FM | -39.1745 | 175.4004 | TO | National Park | *Kunzea ericoides* | *Ctenarytaina clavata* | |
| 331 | 19/02/2016 | FM | -39.2575 | 175.5841 | TO | Tongariro | Mix, windblown | *Trioza decurvata* | a |
| 332 | 19/02/2016 | FM | -39.2575 | 175.5841 | TO | Same location | Same plant | *Ctenarytaina clavata* | |
| 333 | 19/02/2016 | FM | -39.2575 | 175.5841 | TO | Tongario | *Kunzea ericoides* | *Ctenarytaina clavata* | |
| 334 | 20/02/2016 | FM | -39.2575 | 175.5841 | TO | Tongariro | *Kunzea ericoides* | *Ctenarytaina* sp. D | a |
| 335 | 21/02/2016 | FM | -39.2575 | 175.5841 | TO | Tongariro | *Kunzea ericoides* | *Ctenarytaina* sp. D | a |
| 336 | 21/02/2016 | FM | -39.1745 | 175.4004 | TO | Nat. Park Village | *Eucalyptus* | *Ctenarytaina eucalypti* | a |
| 337 | 22/02/2016 | FM | -38.963 | 175.7618 | TO | Tokaanu | *Eucalyptus* | *Ctenarytaina eucalypti* | |
| 338 | 22/02/2016 | FM | -38.881 | 175.2634 | TO | Taumarunui | *Pittosporum* | *Trioza vitreoradiata* | |
| 339 | 22/02/2016 | FM | -38.881 | 175.2634 | TO | Taumarunui | *Pittosporum* | *Trioza vitreoradiata* | |
| 340 | 22/02/2016 | FM | -38.9279 | 175.1011 | TK | Forgotten World | *Acacia* (Wattle) | *Acizzia albizziae* | a |
| 341 | 22/02/2016 | FM | -39.3234 | 174.4096 | TK | Toko | *Pittosporum* | *Trioza vitreoradiata* | |
| 342 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | New Plymouth | *Dodonaea viscosa* | *Acizzia dodonaeae* | |
| 343 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | Same location | *Pittosporum crassifolius* | *Trioza vitreoradiata* | a |
| 344 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | Same location | *Acacia melanoxylon* | *Acizzia acaciae* | |
| 345 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | Same location | *Syzygium* | *Ctenarytaina* | |
| 346 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | Same location | Same plant | *Acizzia* | |
| 347 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | Same location | *Pseudopanax* | *Trioza vitreoradiata* | a |

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| 348 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | New Plymouth | *Acacia* | *Acizzia uncatoides* | |
| 349 | 23/02/2016 | FM | -39.2091 | 173.9866 | TK | Taranaki | *Carmichaelia* | *Psylla carmichaeliae B* | a |
| 350 | 23/02/2016 | FM | -39.0651 | 174.08 | TK | New Plymouth | *Pittosporum crassifolius* | *Trioza* | |
| 351 | 24/02/2016 | FM | -39.2091 | 173.9866 | TK | Taranaki | *Pittosporum* | *Trioza* | |
| 352 | 24/02/2016 | FM | -39.2091 | 173.9866 | TK | Taranaki | *Acacia* | *Acizzia acaciae* | |
| 353 | 24/02/2016 | FM | -39.2091 | 173.9866 | TK | Same location | *Pittosporum* | *Trioza vitreoradiata* | |
| 354 | 24/02/2016 | FM | -39.4439 | 174.2973 | TK | Eltham | *Acacia* (Wattle) | *Acizzia uncatoides* | a |
| 355 | 24/02/2016 | FM | -39.5856 | 174.2716 | TK | Hawera | *Pittosporum crassifolius* | *Trioza vitreoradiata* | |
| 356 | 24/02/2016 | FM | -39.5856 | 174.2716 | TK | Same location | *Dodonaea viscosa* | *Acizzia dodonaeae* | |
| 357 | 25/02/2016 | FM | -39.9362 | 175.026 | WI | Wanganui | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | |
| 358 | 25/02/2016 | FM | -39.9362 | 175.026 | WI | Same location | *Pittosporum crassifolius* | *Trioza vitreoradiata* | |
| 359 | 25/02/2016 | FM | -39.9362 | 175.026 | WI | Same location | *Acacia melanoxylon* | *Acizzia acaciae* | |
| 360 | 25/02/2016 | FM | -39.9362 | 175.026 | WI | Same location | *Eucalyptus* | *Cryptoneossa triangula* | a,b |
| 361 | 25/02/2016 | FM | -39.9362 | 175.026 | WI | Same location | Lemonwood | *Trioza* | |
| 362 | 25/02/2016 | FM | -39.9362 | 175.026 | WI | Same location | *Eucalyptus* | *Ctenarytaina spatulata* | a |
| 363 | 25/02/2016 | FM | -40.0904 | 175.4029 | WI | Marton | *Eucalyptus* | *Glycaspis* | a |
| 364 | 25/02/2016 | FM | -40.0904 | 175.4029 | WI | Same location | *Same plant* | *Cardiaspina fiscella* | a |
| 365 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Palmerston Nth | *Acacia* (Wattle) | *Acizzia uncatoides* | |
| 366 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | Same plant | *Acizzia* | |
| 367 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | *Pittosporum eugenioides* | *Trioza vitreoradiata* | |
| 368 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | *Eucalyptus* | *Ctenarytaina spatulata* | |
| 369 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | Same plant | *Trioza* sp. | |
| 370 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | *Eucalyptus* | *Ctenarytaina spatulata* | a |
| 371 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | Same plant | *B. occidentalis* | |
| 372 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | *Pittosporum crassifolius* | *Trioza vitreoradiata* | |
| 373 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Palmerston Nth | *Acacia* | *Acizzia conspicua* | a,b |
| 374 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | *Same plant* | *Acizzia uncatoides* | a |
| 375 | 26/02/2016 | FM | -40.41 | 175.5692 | WI | Same location | *Same plant* | *Acizzia acaciae* | |

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|---|---|---|---|---|---|---|---|---|---|
| 376 | 26/02/2016 | FM | -40.174 | 175.3898 | WI | Bulls | *Eucalyptus* | *A. occidentalis* | |
| 377 | 27/02/2016 | FM | -40.9895 | 174.9518 | WI | Paekakariki | *Acacia (Wattle)* | *Acizzia* (hairy nymphs) | |
| 378 | 27/02/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Kunzea ericoides wind blown* | | |
| 379 | 27/02/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Dodonaeae viscosa* | *Acizzia dodonaeae* | |
| 380 | 27/02/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Same plant* | MIX (2 *Ctenarytaina*) | |
| 381 | 27/02/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pittosporum eugenioides* | *Trioza vitreoradiata* | |
| 382 | 27/02/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Kunzea ericoides* | *Ctenarytaina clavata* | |
| 383 | 28/02/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Pseudopanax crassifolius* | *Trioza panacis* | a |
| 384 | 28/02/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Dodonaeae viscosa* | *Acizzia dodonaeae* | |
| 385 | 28/02/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pittosporum eugenioides* | *Trioza vitreoradiata* | |
| 386 | 28/02/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Same plant* | *Ctenarytaina* unknown | a |
| 387 | 29/02/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Eucalyptus* | *Ctenarytaina spatulata* | |
| 388 | 29/02/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Acacia* | *Acizzia acaciae* | |
| 389 | 29/02/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pittosporum crassifolius* | *Trioza vitreoradiata* | |
| 390 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | |
| 391 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Acacia* | *Acizzia hakeae* | a |
| 392 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Same plant* | *Ctenarytaina* | |
| 393 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | MIX | *Acizzia, Ctenarytaina* | |
| 394 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Dodonaeae viscosa* | *Acizzia dodonaeae* | |
| 395 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Acacia* (Wattle) | *Acizzia* WAITAKERE | a |
| 396 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Acacia melanoxylon* | *Acizzia acaciae* | a |
| 397 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pittosporum crassifolius* | *Trioza vitreoradiata* | |
| 398 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Kunzea ericoides* | *Ctenarytaina* | |
| 399 | 1/03/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Pittosporum* | *Trioza vitreoradiata* | |
| 400 | 2/03/2016 | FM | -41.2829 | 174.7664 | WN | Wellington | *Pittosporum eugenioides* | *Trioza vitreoradiata* | |
| 401 | 2/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Dodonaea viscosa* | *Acizzia dodonaeae* | a |
| 402 | 2/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Kunzea ericoides* | *Ctenarytaina* sp. D | a |
| 403 | 2/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pittosporum* | *Trioza vitreoradiata* | |
| 404 | 2/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pseudopanax crassifolius* | *Trioza vitreoradiata (* | |
| 405 | 2/03/2016 | FM | -41.2829 | 174.7664 | WN | Same location | *Pittosporum* | *Trioza vitreoradiata* | |

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|---|---|---|---|---|---|---|---|---|---|
| 406 | 9/03/2016 | FM | -43.531 | 172.6194 | MC | Christchurch | *Eucalyptus* | *Ctenarytaina spatulata* | |
| 407 | 9/03/2016 | FM | -43.531 | 172.6194 | MC | Same location | *Schinus molle* | *Calophya schini* | a |
| 408 | 9/03/2016 | FM | -43.531 | 172.6194 | MC | Same location | *Fraxinus excelsior* | *Psyllopsis fraxinicola* | a |
| 409 | 8/02/2016 | SRB | -42.5246 | 172.8811 | MC | Montgomery res. | *Schefflera digitata* | *Trioza irregularis* | a |
| 410 | 27/03/2016 | SRB | -41.8067 | 172.8564 | NN | St Arnaud | *Pittosporum divaricartum* | *Trioza* sp. *A* | a |
| 411 | 27/03/2016 | SRB | -41.8067 | 172.8564 | NN | St Arnaud | *Olearia avicenniifolia* | *Trioza bifida* | |
| 412 | 28/03/2016 | SRB | -41.8067 | 172.8564 | NN | St Arnaud | *Olearia arborescens* | *Trioza* sp. *B* | a |
| 413 | 28/03/2016 | SRB | -41.8067 | 172.8564 | NN | same | *Same plant* | *Ctenarytaina pollicaris* B | a |
| 414 | 29/03/2016 | SRB | -41.8067 | 172.8564 | NN | St Arnaud | *Halocarpus* | *Trioza colorata* | a |
| 415 | 29/03/2016 | SRB | -41.8067 | 172.8564 | NN | Same | Same plant | *Trioza dacrydii* | a,b |
| 416 | 26/04/2016 | SRB | -43.7799 | 172.7738 | MC | Little River | | *Bactericera cockerelli* | a |
| 417 | 14/05/2012 | SRB | -41.3877 | 174.0379 | MB | Rarangi | *Hebe salicifolia* | *Trioza bifida* | |
| 418 | 14/05/2012 | SRB | -41.3877 | 174.0379 | MB | Rarangi | *Olearia* | *Trioza bifida* | |
| 419 | 21/02/2014 | SRB | -36.8989 | 174.785 | AK | Auckland | *Syzygium* | *Ctenarytaina* unknown | a |
| 420 | 28/10/2013 | SRB | -43.7799 | 172.7738 | MC | Banks Peninsula | *Plagianthus* | *Trioza Price's valley* | a |
| 421 | 28/10/2013 | SRB | -43.301 | 171.7497 | MC | Porters Pass | *Ozothamnus* | *Trioza acuta* | a |
| 422 | 22/11/2012 | SRB | -42.5518 | 172.8102 | MB | Hanmer | *Olearia* | *Trioza* sp. | |
| 423 | 22/11/2012 | SRB | -42.5518 | 172.8102 | MB | Hanmer | *Discaria toumatou* | *Trioza* sp. | |
| 424 | 10/12/2015 | | -43.7799 | 172.7738 | MC | Little River | *Acacia baileyana* | *Acizzia* sp. NEW | a-c |
| 425 | 20/12/2015 | | -43.7799 | 172.7738 | NN | Spring grove | *Acacia baileyana* | *A. acaciaebaileyanae* | a-c |
| 426 | 6/10/2016 | SRB | -42.3521 | 173.6969 | KA | Kaikoura | *Fuchsia excorticata* | *C. fuchsiae* sp. B | a,b |
| 427 | 6/10/2016 | SRB | -42.3521 | 173.6969 | KA | Kaikoura | *Fuchsia excorticata* | *C. fuchsiae* sp. B | a,b |
| 428 | 6/10/2016 | SRB | -42.3521 | 173.6969 | KA | Kaikoura | *Fuchsia excorticata* | *C. fuchsiae* sp. B | a,b |
| 429 | 10/09/2016 | FM | -43.1405 | 172.7298 | NC | Amberley | *Sophora microphylla* | *Psylla apicalis* | |
| 430 | 13/10/2016 | FM | -43.64 | 172.4743 | MC | Lincoln | *Sophora microphylla* | *Psylla apicalis* | |
| 431 | 13/10/2016 | FM | -43.64 | 172.4743 | MC | Lincoln | *Sophora microphylla* | *Psylla apicalis* | |
| 432 | 16/10/2016 | FM | -43.531 | 172.6194 | MC | Christchurch | *Acacia* (Wattle) | *2 Acizzia* spp. | |
| 433 | 13/10/2016 | FM | -43.64 | 172.4743 | MC | Lincoln | *Sophora microphylla* | *Psylla apicalis* | |

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|---|---|---|---|---|---|---|---|---|---|
| 434 | 1/10/2016 | FM | -43.531 | 172.6194 | MC | Christchurch | *Sophora microphylla* | *Psylla apicalis* | |
| 435 | 3/12/2016 | FM | -43.2291 | 172.2292 | MC | Ashley Gorge | *Pseudopanax arboreus* | *Trioza irregularis* | a |
| 436 | 3/12/2016 | FM | -43.2243 | 172.2825 | MC | Mt. Thomas | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | |
| 437 | 22/07/2016 | FM | -43.64 | 172.4743 | MC | Lincoln | *Pseudopanax* | *Trioza panacis* | a |
| 438 | 3/09/2016 | FM | -44.2865 | 169.8505 | MK | Lake Ohau | *Kunzea ericoides* | *Ctenarytaina clavata* | a |
| 439 | 16/07/2016 | FM | -43.6261 | 172.7395 | MC | Diamond harbour | *Acacia* (Wattle) | *Acizzia* sp. | |
| 440 | 23/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Plagianthus* | *Anomalopsylla insignita* | a |
| 442 | 24/10/2016 | SRB | -43.2243 | 172.2825 | MC | Glentui | *Olearia virgata* | *Trioza* sp. D | a,b |
| 443 | 24/10/2016 | SRB | -43.2243 | 172.2825 | MC | Glentui | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | |
| 444 | 24/10/2016 | SRB | -43.2243 | 172.2825 | MC | Glentui | *Pseudopanax* | *Trioza irregularis* | a |
| 445 | 16/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Pseudopanax* | *Trioza irregularis* | a |
| 446 | 16/10/2016 | SRB | -43.7031 | 172.6918 | MC | Same location | Same plant | *Ctenarytaina* sp. | |
| 447 | 30/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Hebe/Fuchsia* | *Trioza bifida* | a |
| 448 | 30/10/2016 | SRB | -43.7031 | 172.6918 | MC | Same location | *Hebe* | *Trioza bifida* | a |
| 449 | 30/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Dracophyllum, 2 Coprosma* | *Trioza bifida* | a |
| 450 | 30/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Carmichaelia* (small) | *Psylla carmichaeliae* A | a |
| 451 | 30/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Olearia paniculata* | *Anomalopsylla insignita* | a |
| 452 | 30/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Pseudopanax* | *Trioza* sp. | |
| 453 | 28/10/2016 | SRB | -43.7031 | 172.6918 | MC | Banks Peninsula | *Pseudopanax* | *Trioza irregularis* | a |
| 454 | 28/10/2016 | SRB | -43.7031 | 172.6918 | MC | Same location | *Olearia avicenniifolia* | *Trioza bifida* | a |
| 455 "A" | 28/10/2016 | SRB | -43.7031 | 172.6918 | MC | Same location | *Olearia paniculata* | *Arytainilla spartiophila* | a |
| 455 "B" | 28/10/2016 | same | -43.7031 | 172.6918 | MC | Same location | *Olearia paniculata* | *Anomalopsylla insignita* | a,b |
| 456 | 1/12/2016 | SRB | -41.2911 | 173.2345 | NN | Nelson | *Brachyglottis repanda* | *Trioza "OMAHUTA"* | a |
| 457 | 1/12/2016 | SRB | -41.2911 | 173.2345 | NN | Same location | *Olearia virgata* | *Trioza gourlayi* | a,b |
| 458 | 1/12/2016 | SRB | -41.2911 | 173.2345 | NN | Same location | *Olearia avicenniifolia* | *Trioza subvexa* | a |
| 459 | 17/12/2016 | SDJB | -43.301 | 171.7497 | MC | Porters Pass | *Hebe odorosa* | *Arytainilla spartiophila* | |
| 460 | 17/12/2016 | SDJB | -43.301 | 171.7497 | MC | Porters Pass | *Ozothamnus* | *A. spartiophila* | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *Trioza falcata* | |
| 461 | 17/12/2016 | SDJB | -43.301 | 171.7497 | MC | Porters Pass | *Discaria toumatou* | *Trioza discariae* | |
| 462 | 17/12/2016 | SDJB | -43.301 | 171.7497 | MC | Porters Pass | Unknown | *Trioza* | |
| 463 | 17/12/2016 | SDJB | -43.301 | 171.7497 | MC | Porters Pass | *Cytisus scoparium* | *Arytainilla spartiophila* | |
| 464 | 19/12/2016 | SDJB | -43.7028 | 172.7514 | MC | Banks peninsula | *Fuchsia excorticata* | *Ctenarytaina fuchsiae* | |
| 465 | 28/12/2016 | FM | -38.8599 | 175.5464 | TO | Taupo | *Fuchsia excorticata* | *C. fuchsiae* sp. C | a-e |
| 466 | 4/01/2017 | FM | -44.4112 | 171.2516 | SC | Timaru | *Eucalyptus nicholii* | *C. spatulata* | |
| 467 | 28/01/2017 | SDJB | -43.4782 | 171.5413 | MC | Mt Hutt | *Plagianthus* | *Trioza decurvata* | |
| 468 | 14/01/2017 | SRB | -41.2911 | 173.2345 | NN | Nelson | *Pittosporum divaricartum* | *Trioza* sp. A | a,b |
| 469 | 14/01/2017 | SRB | -41.2911 | 173.2345 | NN | Same location | *Phyllocladus* | *Trioza* sp. A | a |
| 470 | 15/01/2017 | SRB | -41.2911 | 173.2345 | NN | Nelson | *Coprosma* | *C. pollicaris* B | a,b |
| 471 | 15/01/2017 | SRB | -41.2911 | 173.2345 | NN | Same location | *Elaeocarpus* | *Trioza FORTROSE* | a,b |
| 472 | 17/01/2017 | SRB | -40.7442 | 172.6809 | NN | Golden Bay | *Brachyglottis repanda* | *Trioza* OMAHUTA | a |
| 473 | 20/01/2017 | SRB | -40.7442 | 172.6809 | NN | Golden Bay | *Olearia rani* | *Trioza compressa* | a,b |
| 474 | 23/01/2017 | SRB | -40.6367 | 172.5681 | NN | Golden Bay | *Leptospermum scoparius* | *Ctenarytaina clavata* C | a,b |
| 475 | 23/01/2017 | SRB | -40.6367 | 172.5681 | NN | Same location | Unknown | *Trioza compressa* | a,b |
| 476 | 23/01/2017 | SRB | -40.6367 | 172.5681 | NN | Golden Bay | *Metrosideros robusta* | *Trioza curta* | a,b |
| 477 | 24/01/2017 | SRB | -41.037 | 172.8075 | NN | Golden Bay | *Olearia avicenniifolia* | *Trioza subvexa* | a,b |
| 478 | 24/01/2017 | SRB | -41.037 | 172.8075 | NN | Same plant | *Hebe* | *Trioza obscura* | a,b |
| 479 | 24/01/2017 | SRB | -41.037 | 172.8075 | NN | Golden Bay | *Olearia odorata* | *Trioza* MASSEY | a,b |
| 480 | 24/01/2017 | SRB | -41.037 | 172.8075 | NN | Same plant | *Aristotelia fruticosa* | *Trioza falcata* B | a |

## B.2 Australia and USA

The following Table includes the samples collected in Australia and United States of America.

The collectors' names are:

**Francesco Martoni** = FM

**Gary Taylor**, The University of Adelaide, Adelaide – South Australia = GT

**Alan Yen**, La Trobe University, Melbourne - Victoria = AY

**Mark Blecket**, AgriBio, La Trobe University, Melbourne - Victoria = MB

**Isabel Valenzuela**, AgriBio, La Trobe University, Melbourne - Victoria = IV

**Peter Gillespie**, Orange Institute of Agriculture, Orange – New South Wales =PG

**Susan Halbert**, The Florida State Arthropods Collection, Gainesville, Florida =SH

**Table A.2:** Australian and American field collections. Table includes the ID number, the collection date, the collector(s)' name(s), the GPS coordinates, the host plant name, the psyllid species and the samples DNA was extracted from.

| ID | Date | Collector | Latitude | Longitude | Location | Plant | Species | DNA |
|---|---|---|---|---|---|---|---|---|
| SA1 | 04/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Eucalyptus* sp. | *Anoeconeossa communis* | |
| SA2 | 04/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Eucalyptus globulus* | *Ctenarytaina eucalypti* | a-c |
| SA3 | 04/11/2014 | FM & GT | -35.0604 | 138.8373 | Mt. Barker | *Casuarina* sp. | *Casuarinicola australis* | |
| SA4 | 04/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Eucalyptus* sp. | *Cryptoneossa triangula* | |
| SA5 | 04/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Eucalyptus* sp. | *Eucalyptolyma maideni* | a-c |
| SA6 | 06/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Eucalyptus* sp. | *Cryptoneossa triangula* | |
| SA7 | 06/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Casuarina* sp. | *Casuarinicola* | |
| SA8 | 06/11/2014 | FM & GT | -34.9165 | 138.6044 | Adelaide | *Casuarina* sp. | Triozid sp. | |
| SA9 | 06/11/2014 | FM & GT | -35.0604 | 138.8373 | Mt. Barker | *Acacia baileyana* | *Acizzia* sp. | |
| VIC1 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | *Eucalyptus* sp. | *Ctenarytaina eucalypti* | a-c |
| VIC2 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | *Ctenarytaina bipartita* | |
| VIC3 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | *Acacia* sp. | *Aacanthocnema dobsoni* | |
| VIC4 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | *Eucalyptus* sp. | *Anoeconeossa bundoorensis* | |
| VIC5 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC6 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC7 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC8 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC9 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC10 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC11 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC12 | 12/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |
| VIC13 | 13/11/2014 | FM & AY | -37.7180 | 145.0531 | Melbourne | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| VIC14 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC15 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC16 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC17 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC18 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC19 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC20 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC21 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC22 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC23 | 13/11/2014 | FM & AY | | | Melbourne | | | |
| VIC24 | 23/10/2016 | FM | -37.4334 | 143.9084 | Melbourne- Creswick Hotel | *Acacia* | | |
| VIC25 | 23/10/2016 | FM | -37.4334 | 143.9084 | Melbourne- Creswick Hotel | *Acacia melanoxylon* | | |
| VIC26 | 26/10/2016 | FM | -37.4334 | 143.9084 | Melbourne- Creswick | *Acacia* (Wattle) | | |
| VIC27 | 01/11/2016 | FM | -37.7214 | 145.0483 | La Trobe University Campus | *Solanum* | | |
| VIC28 | 01/11/2016 | FM | -37.7214 | 145.0483 | La Trobe University Campus | *Casuarina* | | |
| VIC29 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Acacia* | | |
| VIC30 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Casuarina* | | |
| VIC31 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Solanum* | *Acizzia solanicola* | |
| VIC32 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Acacia* (Wattle) | | |
| VIC33 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Solanum* | *Acizzia solanicola* | |
| VIC34 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Acacia* wattle | | |
| VIC35 | 18/11/2016 | FM, MB, IV | -37.7214 | 145.0483 | Melbourne | *Eucalyptus* | | |
| VIC36 | 26/11/2016 | FM | -37.8045 | 144.9733 | Melbourne Museum park | *Ficus macrophylla* | | |

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|---|---|---|---|---|---|---|---|---|
| VIC37 | 20/11/2016 | FM | -37.8028 | 144.9631 | Swanston Street, Ridge Hotel | *Ficus macrophylla* | | |
| QUE1 | 13/08/2015 | FM | -26.6351 | 153.0940 | Twin Waters | *Casuarina* | *Casuarinicola* | |
| QUE2 | 13/08/2015 | FM | -26.6351 | 153.0940 | Twin Waters | *Eucalyptus* | | |
| QUE3 | 13/08/2015 | FM | -26.6351 | 153.0940 | Twin Waters | *Eucalyptus* | | |
| QUE4 | 13/08/2015 | FM | -26.6351 | 153.0940 | Twin Waters | *Acacia* | | |
| QUE5 | 29/09/2015 | FM | -16.8411 | 145.7136 | Cairns | *Ficus* | | |
| QUE6 | 29/09/2015 | FM | -16.8411 | 145.7136 | Cairns | *Acacia* | | |
| QUE7 | 01/10/2015 | FM | -16.4863 | 145.4630 | Port Douglas | *Eucalyptus* | | |
| QUE8 | 01/10/2015 | FM | -16.4863 | 145.4630 | Port Douglas | *Eucalyptus* | | |
| NSW1 | 01/10/2015 | FM | | | Kingsvale | *Acacia baileyana* | *Acizzia acaciaebaileyanae* | |
| NSW2 | 05/04/2016 | FM | -33.2857 | 149.1080 | Orange- Mc Lachlan St. | *Casuarina* | *Trioza* sp. | |
| NSW3 | 05/04/2016 | FM | -33.2808 | 149.1039 | Orange- Behind library | *Eucalyptus* | MIX (*Glycaspis*) | |
| NSW4 | 05/04/2016 | FM | -33.2808 | 149.1039 | Orange- Behind library | *Eucalyptus* | MIX | |
| NSW5 | 06/04/2016 | FM | -33.3225 | 149.0861 | Orange Agriculture Institute | *Eucalyptus* | *Ctenarytaina* | |
| NSW6 | 06/04/2016 | FM | -33.3104 | 149.0957 | Orange- Forest Rd. | *Eucalyptus* | 1 insect | |
| NSW7 | 06/04/2016 | FM | -33.3104 | 149.0957 | Orange- Forest Rd. | *Acacia* | *Acizzia* | |
| NSW8 | 06/04/2016 | FM | -33.2841 | 149.1036 | Orange- Peisley Rd. | *Eucalyptus* | *Glycaspis* (+1 insect) | |
| NSW9 | 07/04/2016 | FM, PG | | | Orange- The pinnacles | *Eucalyptus* | 1 insect | |
| NSW10 | 07/04/2016 | FM, PG | -33.34339 | 148.9826 | Orange- Mt. Canobolas | *Acacia* sp. | *Acizzia* | |
| NSW10A | 07/04/2016 | FM, PG | -33.34339 | 148.9826 | Same location | Same plant | unknown | |
| NSW11 | 07/04/2016 | FM, PG | -33.34339 | 148.9826 | Orange- Mt. Canobolas | *Acacia* sp. | *Acizzia* | |
| NSW12 | 07/04/2016 | FM, PG | -33.34339 | 148.9826 | Orange- Mt. Canobolas | *Acacia* (Wattle) | 1 insect | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| NSW13 | 07/04/2016 | FM, PG | -33.34339 | 148.9826 | Orange- Mt. Canobolas | *Eucalyptus* sp. | 1 insect | |
| NSW14 | 07/04/2016 | FM | -33.2567 | 149.0970 | Orange- Botanic Gardens | *Casuarina* sp. | *Casuarinicola* | |
| NSW15 | 07/04/2016 | FM | -33.2567 | 149.0970 | Orange- Botanic Gardens | *Eucalyptus* sp. | *Glycaspis* | |
| NSW16 | 08/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder Park | *Fraxinus* sp. | *Psyllopsis fraxinicola* | |
| NSW17 | 08/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder Park | *Casuarina* sp. | *Casuarinicola* | |
| NSW18 | 08/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder Park | *Eucalyptus* sp. | *Glycaspis* | |
| NSW19 | 08/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder Park | *Casuarina* sp. | *Trioza* sp. | |
| NSW20 | 08/04/2016 | FM | | | Orange- Creek crossing | *Acacia* (Wattle) | Unknown | |
| NSW21 | 08/04/2016 | FM | | | Orange- Creek crossing | *Casuarina* sp. | *Casuarinicola* | |
| NSW22 | 08/04/2016 | FM | | | Orange- Creek crossing | *Casuarina + Eucalyptus* | *Trioza* sp. + *Glycaspis* | |
| NSW23 | 08/04/2016 | FM | | | Orange- Creek crossing | Eucalyptus MIX | | |
| NSW24 | 09/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder park | *Casuarina* sp. | *Trioza* sp. | |
| NSW25 | 09/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder Park | *Acacia* sp. | *Acizzia* | |
| NSW26 | 09/04/2016 | FM | -33.28884 | 149.0965 | Orange- Moulder Park | *Acacia* sp. | *Acizzia* | |
| NSW27 | 13/04/2016 | FM | -33.8727 | 151.2117 | Sydney- Hyde Park | *Ficus macrophylla* | *Mycopsylla fici* | |
| NSW28 | 13/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Eucalyptus* sp. | Only nymphs | |
| NSW29 | 13/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Lophostemon* (under *Ficus*) | *Mycopsylla fici* | |
| NSW30 | 13/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Eucalyptus* sp. | Only nymphs | |
| NSW31 | 13/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Eucalyptus* sp. | Only nymphs | |
| NSW32 | 14/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Ficus macrophylla* | *Mycopsylla fici* | |
| NSW33 | 14/04/2016 | FM | -33.8782 | 151.1947 | Sydney- Wentworth Park | *Eucalyptus* sp. | Only nymphs | |
| NSW34 | 14/04/2016 | FM | -33.8782 | 151.1947 | Sydney-Wentworth Park | *Eucalyptus* sp. | *Glycaspis* | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| NSW35 | 15/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Eucalyptus* + *Acacia* (Wattle) | *Glycaspis + Acizzia* | |
| NSW36 | 15/04/2016 | FM | -33.8655 | 151.2189 | Sydney- Botanic Gardens | *Acacia* (Wattle) | *Acizzia* | |
| NSW37 | 18/04/2016 | FM | -33.8752 | 151.2102 | Sydney-West Sydney Univ. | *Ficus macrophylla* | *Mycopsylla fici* | |
| NSW38 | 18/04/2016 | FM | -33.8655 | 151.2189 | Sydney-Botanic Gardens | *Eucalyptus* sp. | | |
| N5 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cryptoneossa triangula* | |
| N6 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cardiaspina fiscella* | |
| N7 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Blastopsylla occidentalis* | |
| N10 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Blastopsylla occidentalis* | |
| N17 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cryptoneossa triangula* | |
| N22 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cryptoneossa triangula* | |
| N27 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cryptoneossa triangula* | |
| N31 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Blastopsylla occidentalis* | |
| N33 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cryptoneossa triangula* | |
| N34 | 2015 | G. Smith | | | Norfolk islands | Sticky Traps | *Cryptoneossa triangula* | |
| USA1 | 04/10/2016 | FM, SH | 29.6336 | -82.3701 | Gainesville – Florida State Collection of Arthropods | Lemon | *Diaphorina citri* | a-c |
| USA2 | 04/10/2016 | FM, SH | 29.6336 | -82.3701 | Gainesville – Florida State Collection of Arthropods | Lemon | *Diaphorina citri* | |
| USA3 | 04/10/2016 | FM, SH | 29.6336 | -82.3701 | Gainesville – Florida State Collection of Arthropods | | *Russelliana solanicola* | |

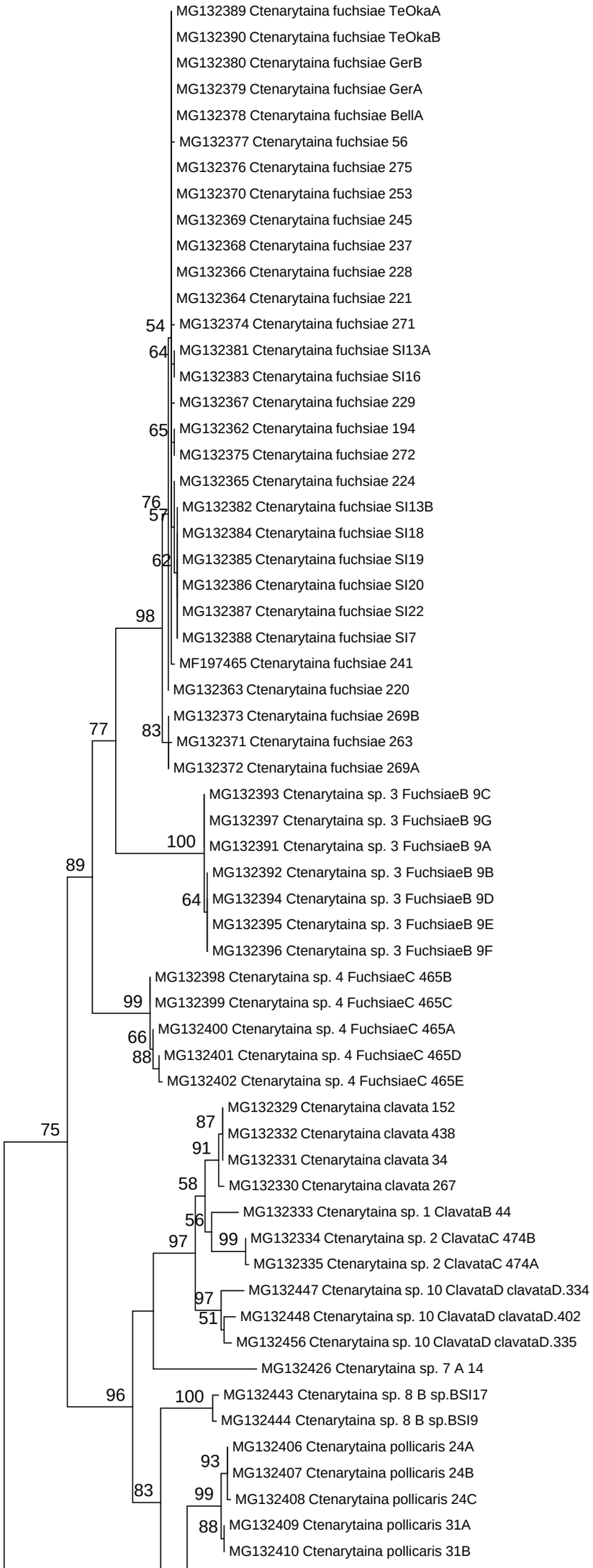
# Appendix C

# Gene trees and phylogenetic tree

**Figure A.9:** COI gene tree. ML 10,000 replicates performed using MEGA6. Bootstrap <50% are not shown. Accession numbers are reported at the tip labels.

**Figure A.10:** 18S gene tree. ML 10,000 replicates performed using MEGA6. Bootstrap <50% are not shown. Accession numbers are reported at the tip labels.

**Figure A.11:** COI-18S phylogenetic tree. Bayesian inference 1 billion replicates obtained using BEAST.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MG132390 Ctenarytaina fuchsiae TeOkaB
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MG132379 Ctenarytaina fuchsiae GerA
MG132378 Ctenarytaina fuchsiae BellA
MG132377 Ctenarytaina fuchsiae 56
MG132376 Ctenarytaina fuchsiae 275
MG132370 Ctenarytaina fuchsiae 253
MG132369 Ctenarytaina fuchsiae 245
MG132368 Ctenarytaina fuchsiae 237
MG132366 Ctenarytaina fuchsiae 228
MG132364 Ctenarytaina fuchsiae 221
54
MG132374 Ctenarytaina fuchsiae 271
64
MG132381 Ctenarytaina fuchsiae SI13A
MG132383 Ctenarytaina fuchsiae SI16
MG132367 Ctenarytaina fuchsiae 229
65
MG132362 Ctenarytaina fuchsiae 194
MG132375 Ctenarytaina fuchsiae 272
MG132365 Ctenarytaina fuchsiae 224
76
57
MG132382 Ctenarytaina fuchsiae SI13B
MG132384 Ctenarytaina fuchsiae SI18
62
MG132385 Ctenarytaina fuchsiae SI19
MG132386 Ctenarytaina fuchsiae SI20
98
MG132387 Ctenarytaina fuchsiae SI22
MG132388 Ctenarytaina fuchsiae SI7
MF197465 Ctenarytaina fuchsiae 241
MG132363 Ctenarytaina fuchsiae 220
MG132373 Ctenarytaina fuchsiae 269B
77
83
MG132371 Ctenarytaina fuchsiae 263
MG132372 Ctenarytaina fuchsiae 269A
MG132393 Ctenarytaina sp. 3 FuchsiaeB 9C
MG132397 Ctenarytaina sp. 3 FuchsiaeB 9G
100
MG132391 Ctenarytaina sp. 3 FuchsiaeB 9A
89
MG132392 Ctenarytaina sp. 3 FuchsiaeB 9B
64
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MG132395 Ctenarytaina sp. 3 FuchsiaeB 9E
MG132396 Ctenarytaina sp. 3 FuchsiaeB 9F
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99
MG132399 Ctenarytaina sp. 4 FuchsiaeC 465C
66
MG132400 Ctenarytaina sp. 4 FuchsiaeC 465A
88
MG132401 Ctenarytaina sp. 4 FuchsiaeC 465D
MG132402 Ctenarytaina sp. 4 FuchsiaeC 465E
MG132329 Ctenarytaina clavata 152
87
75
MG132332 Ctenarytaina clavata 438
91
MG132331 Ctenarytaina clavata 34
58
MG132330 Ctenarytaina clavata 267
56
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97
99
MG132334 Ctenarytaina sp. 2 ClavataC 474B
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97
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51
MG132448 Ctenarytaina sp. 10 ClavataD clavataD.402
MG132456 Ctenarytaina sp. 10 ClavataD clavataD.335
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96
100
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MG132444 Ctenarytaina sp. 8 B sp.BSI9
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MG132407 Ctenarytaina pollicaris 24B
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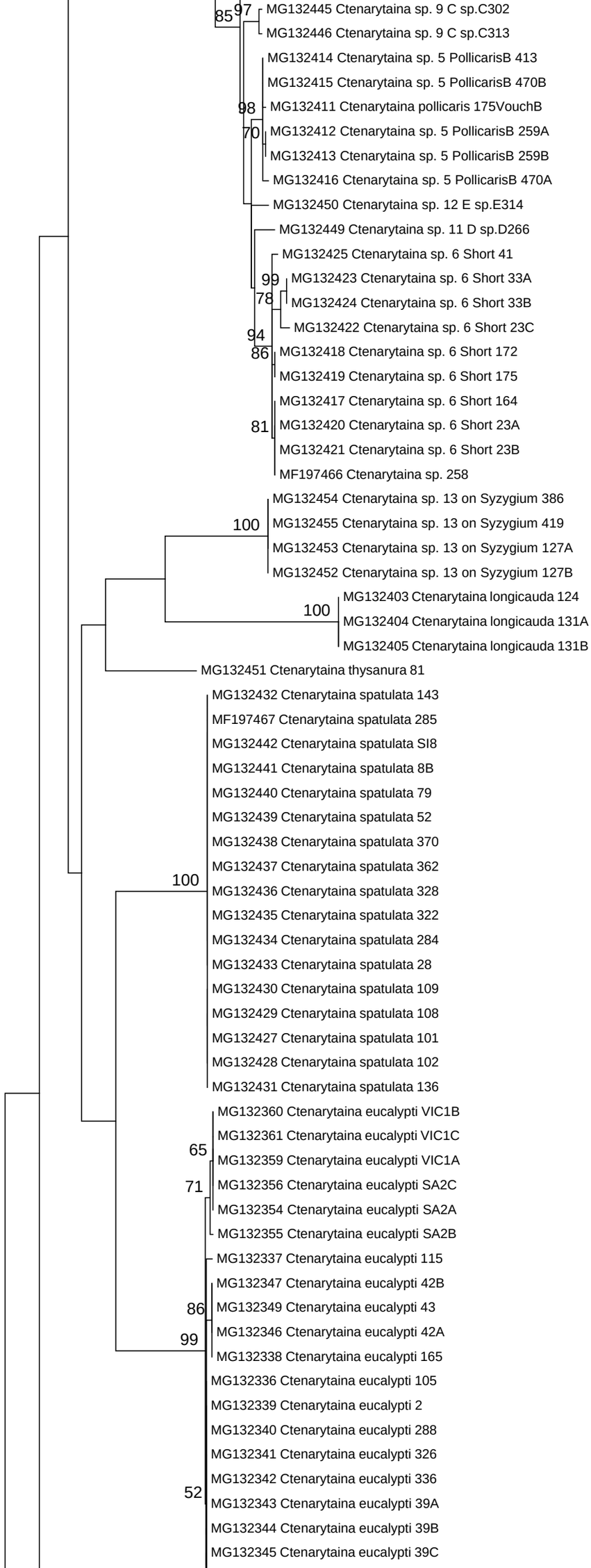
MG132410 Ctenarytaina pollicaris 31B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MG132415 Ctenarytaina sp. 5 PollicarisB 470B
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70
MG132412 Ctenarytaina sp. 5 PollicarisB 259A
MG132413 Ctenarytaina sp. 5 PollicarisB 259B
MG132416 Ctenarytaina sp. 5 PollicarisB 470A
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MG132449 Ctenarytaina sp. 11 D sp.D266
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99
MG132423 Ctenarytaina sp. 6 Short 33A
78
MG132424 Ctenarytaina sp. 6 Short 33B
MG132422 Ctenarytaina sp. 6 Short 23C
94
86
MG132418 Ctenarytaina sp. 6 Short 172
MG132419 Ctenarytaina sp. 6 Short 175
MG132417 Ctenarytaina sp. 6 Short 164
81
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MG132421 Ctenarytaina sp. 6 Short 23B
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100
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MG132451 Ctenarytaina thysanura 81
MG132432 Ctenarytaina spatulata 143
MF197467 Ctenarytaina spatulata 285
MG132442 Ctenarytaina spatulata SI8
MG132441 Ctenarytaina spatulata 8B
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100
MG132436 Ctenarytaina spatulata 328
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MG132427 Ctenarytaina spatulata 101
MG132428 Ctenarytaina spatulata 102
MG132431 Ctenarytaina spatulata 136
MG132360 Ctenarytaina eucalypti VIC1B
MG132361 Ctenarytaina eucalypti VIC1C
65
MG132359 Ctenarytaina eucalypti VIC1A
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MG132356 Ctenarytaina eucalypti SA2C
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MG132349 Ctenarytaina eucalypti 43
99
MG132346 Ctenarytaina eucalypti 42A
MG132338 Ctenarytaina eucalypti 165
MG132336 Ctenarytaina eucalypti 105
MG132339 Ctenarytaina eucalypti 2
MG132340 Ctenarytaina eucalypti 288
MG132341 Ctenarytaina eucalypti 326
MG132342 Ctenarytaina eucalypti 336
52
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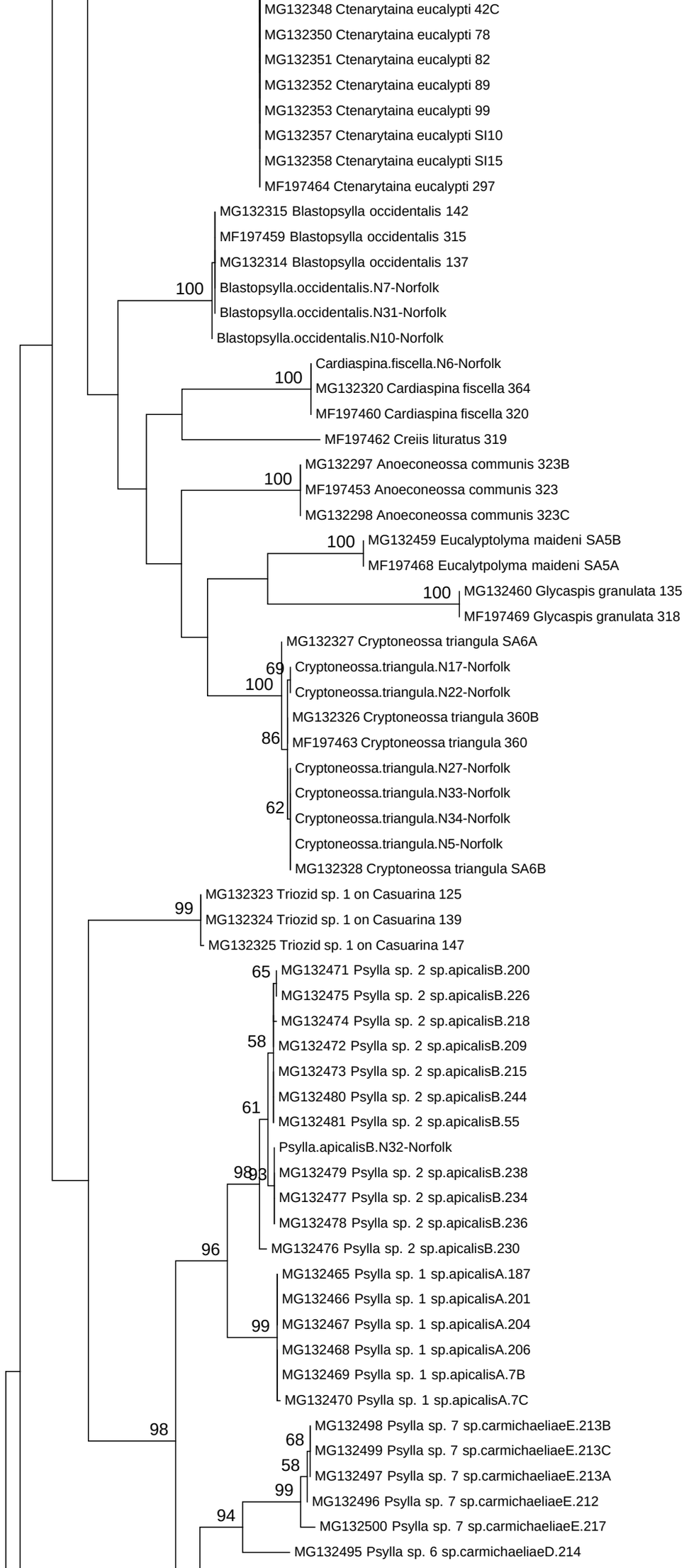
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MG132353 Ctenarytaina eucalypti 99
MG132357 Ctenarytaina eucalypti SI10
MG132358 Ctenarytaina eucalypti SI15
MF197464 Ctenarytaina eucalypti 297
MG132315 Blastopsylla occidentalis 142
MF197459 Blastopsylla occidentalis 315
MG132314 Blastopsylla occidentalis 137
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Blastopsylla.occidentalis.N10-Norfolk
Cardiaspina.fiscella.N6-Norfolk
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MF197462 Creiis lituratus 319
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MG132328 Cryptoneossa triangula SA6B
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MG132325 Triozid sp. 1 on Casuarina 147
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99
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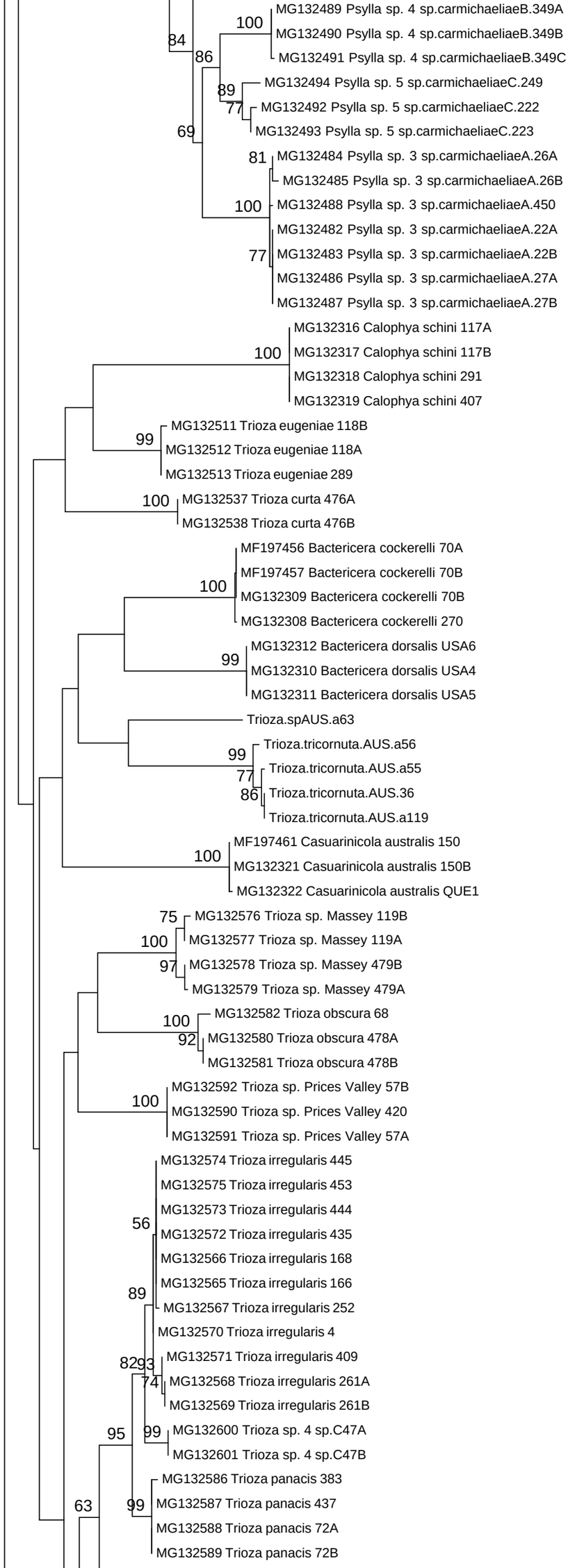
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MG132538 Trioza curta 476B
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MG132309 Bactericera cockerelli 70B
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MG132580 Trioza obscura 478A
MG132581 Trioza obscura 478B
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MG132591 Trioza sp. Prices Valley 57A
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MG132601 Trioza sp. 4 sp.C47B
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MG132587 Trioza panacis 437
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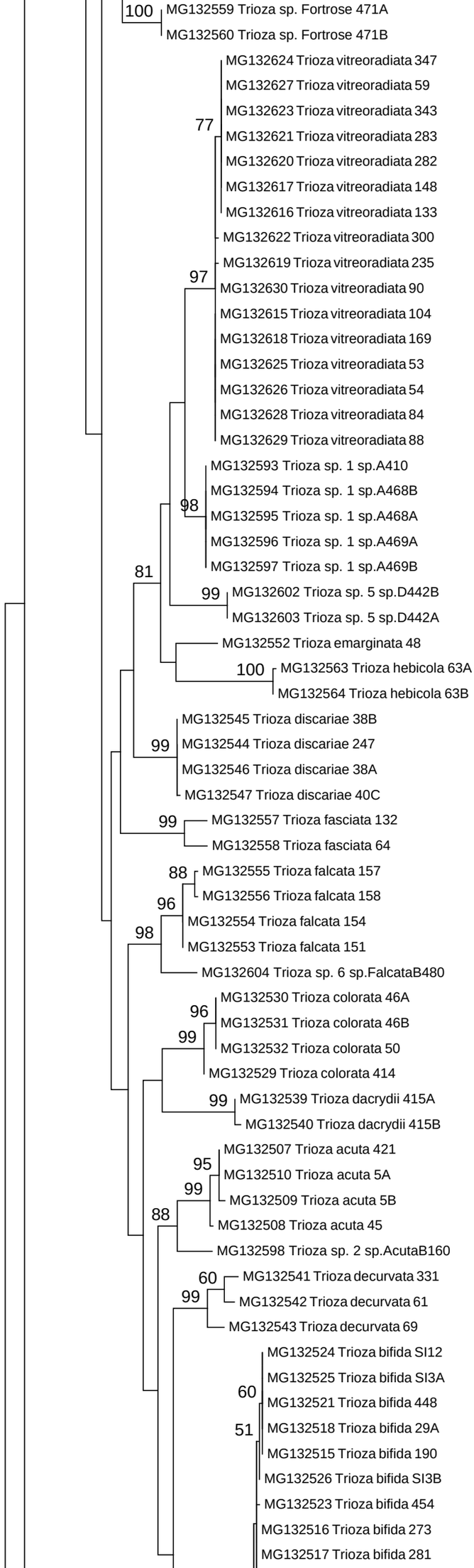
MG132589 Trioza panacis 72B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77
MG132621 Trioza vitreoradiata 283
MG132620 Trioza vitreoradiata 282
MG132617 Trioza vitreoradiata 148
MG132616 Trioza vitreoradiata 133
MG132622 Trioza vitreoradiata 300
MG132619 Trioza vitreoradiata 235
97
MG132630 Trioza vitreoradiata 90
MG132615 Trioza vitreoradiata 104
MG132618 Trioza vitreoradiata 169
MG132625 Trioza vitreoradiata 53
MG132626 Trioza vitreoradiata 54
MG132628 Trioza vitreoradiata 84
MG132629 Trioza vitreoradiata 88
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MG132594 Trioza sp. 1 sp.A468B
98
MG132595 Trioza sp. 1 sp.A468A
MG132596 Trioza sp. 1 sp.A469A
MG132597 Trioza sp. 1 sp.A469B
81
99
MG132602 Trioza sp. 5 sp.D442B
MG132603 Trioza sp. 5 sp.D442A
MG132552 Trioza emarginata 48
100
MG132563 Trioza hebicola 63A
MG132564 Trioza hebicola 63B
MG132545 Trioza discariae 38B
99
MG132544 Trioza discariae 247
MG132546 Trioza discariae 38A
MG132547 Trioza discariae 40C
99
MG132557 Trioza fasciata 132
MG132558 Trioza fasciata 64
88
MG132555 Trioza falcata 157
MG132556 Trioza falcata 158
96
MG132554 Trioza falcata 154
98
MG132553 Trioza falcata 151
MG132604 Trioza sp. 6 sp.FalcataB480
MG132530 Trioza colorata 46A
96
MG132531 Trioza colorata 46B
99
MG132532 Trioza colorata 50
MG132529 Trioza colorata 414
99
MG132539 Trioza dacrydii 415A
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MG132543 Trioza decurvata 69
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MG132518 Trioza bifida 29A
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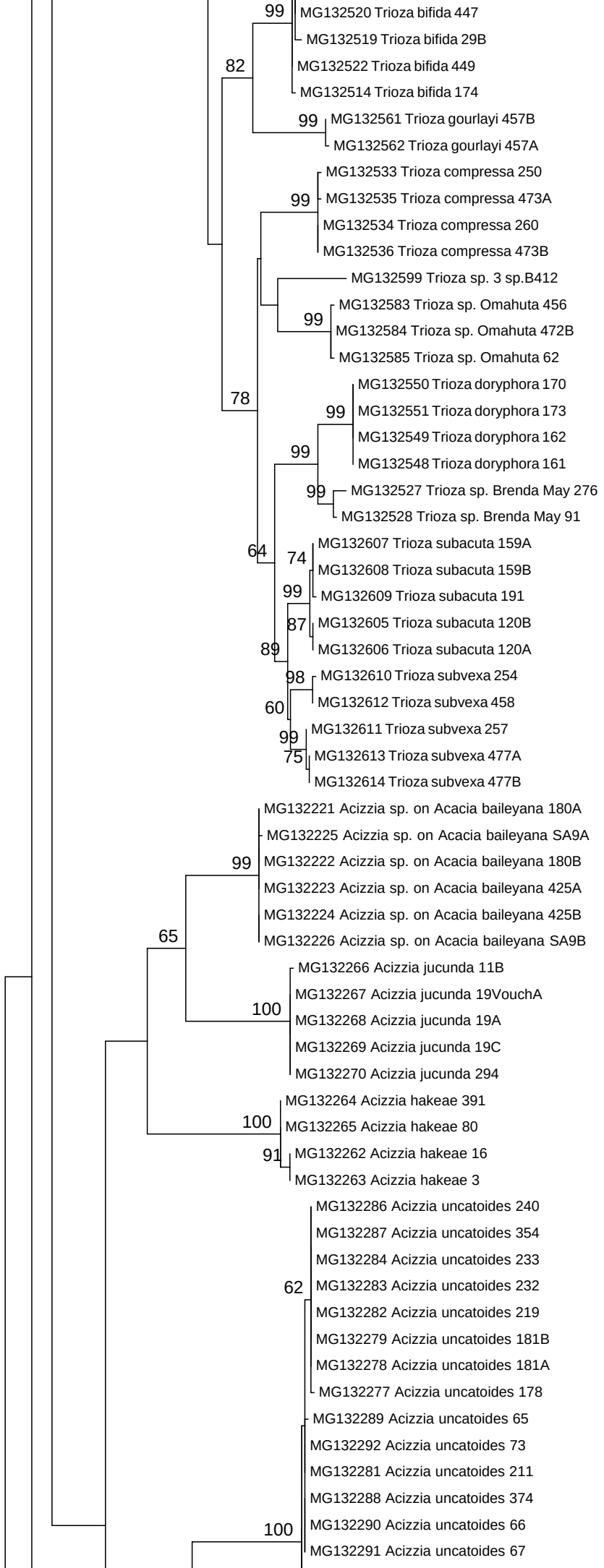
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MG132562 Trioza gourlayi 457A
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MG132528 Trioza sp. Brenda May 91
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MG132609 Trioza subacuta 191
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MG132605 Trioza subacuta 120B
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MG132267 Acizzia jucunda 19VouchA
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MG132268 Acizzia jucunda 19A
MG132269 Acizzia jucunda 19C
MG132270 Acizzia jucunda 294
MG132264 Acizzia hakeae 391
100
MG132265 Acizzia hakeae 80
91
MG132262 Acizzia hakeae 16
MG132263 Acizzia hakeae 3
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MG132287 Acizzia uncatoides 354
MG132284 Acizzia uncatoides 233
62
MG132283 Acizzia uncatoides 232
MG132282 Acizzia uncatoides 219
MG132279 Acizzia uncatoides 181B
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MG132277 Acizzia uncatoides 178
MG132289 Acizzia uncatoides 65
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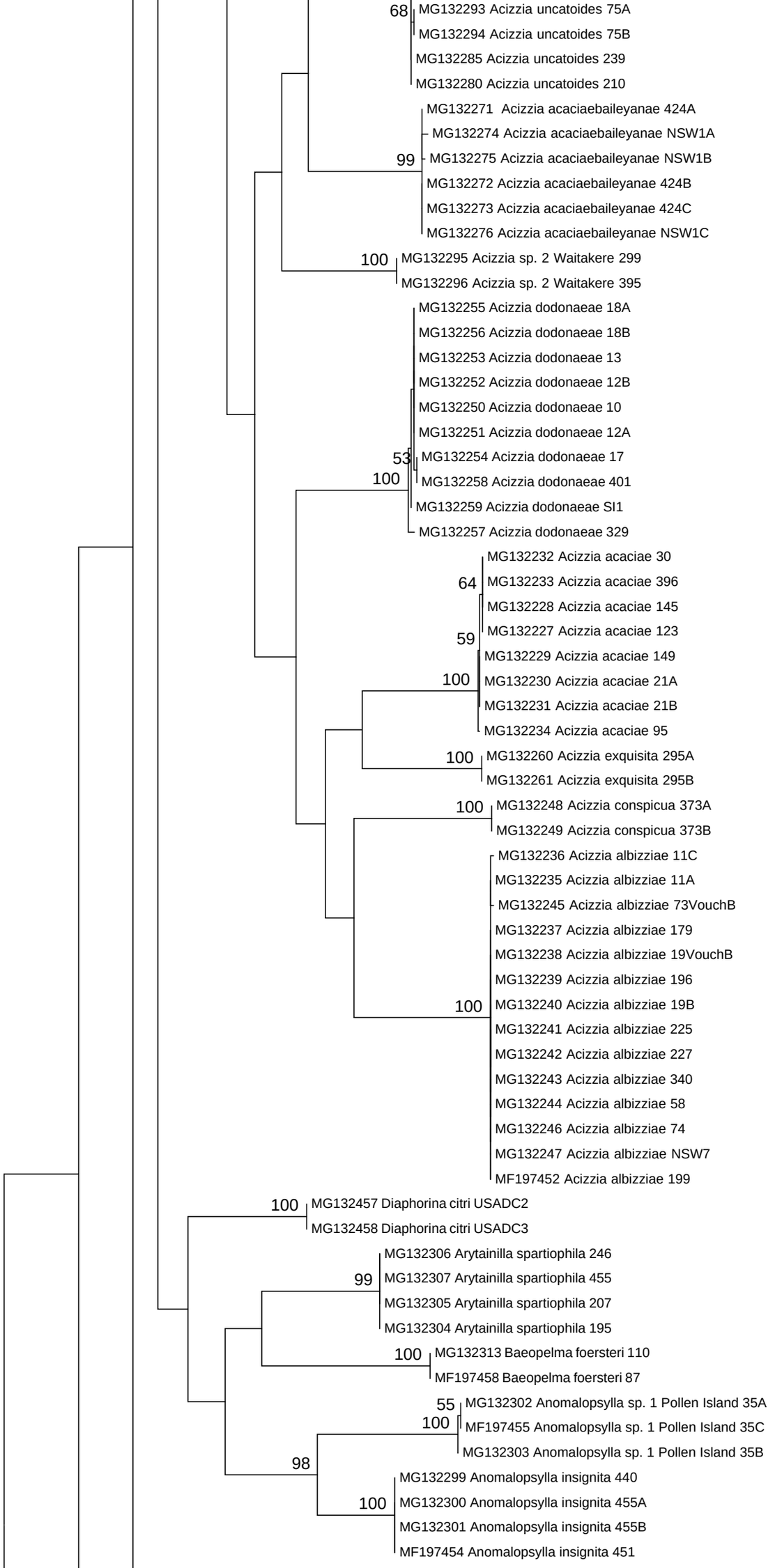
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MG132296 Acizzia sp. 2 Waitakere 395
MG132255 Acizzia dodonaeae 18A
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MG132253 Acizzia dodonaeae 13
MG132252 Acizzia dodonaeae 12B
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MG132231 Acizzia acaciae 21B
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MG132261 Acizzia exquisita 295B
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MG132249 Acizzia conspicua 373B
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MG132235 Acizzia albizziae 11A
MG132245 Acizzia albizziae 73VouchB
MG132237 Acizzia albizziae 179
MG132238 Acizzia albizziae 19VouchB
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MG132242 Acizzia albizziae 227
MG132243 Acizzia albizziae 340
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MG132246 Acizzia albizziae 74
MG132247 Acizzia albizziae NSW7
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MG132458 Diaphorina citri USADC3
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MF197458 Baeopelma foersteri 87
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MF197455 Anomalopsylla sp. 1 Pollen Island 35C
MG132303 Anomalopsylla sp. 1 Pollen Island 35B
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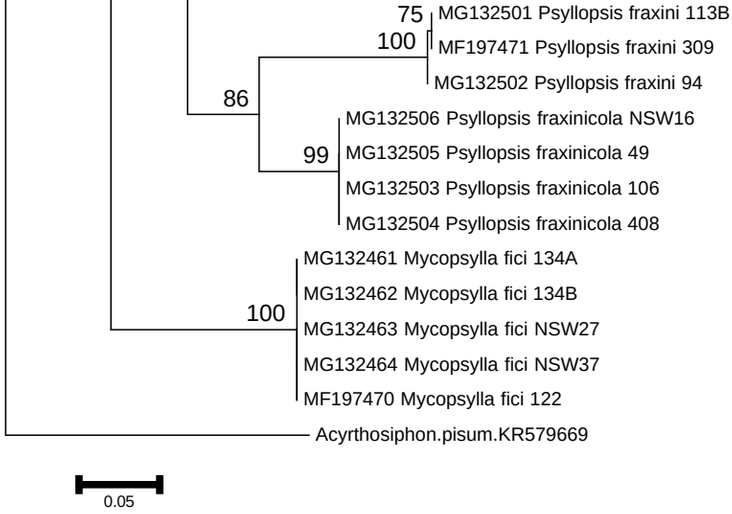
MG132301 Anomalopsylla insignita 455B
MF197454 Anomalopsylla insignita 451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MG132505 Psyllopsis fraxinicola 49
MG132503 Psyllopsis fraxinicola 106
MG132504 Psyllopsis fraxinicola 408
MG132461 Mycopsylla fici 134A
MG132462 Mycopsylla fici 134B
MG132463 Mycopsylla fici NSW27
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MF197470 Mycopsylla fici 122
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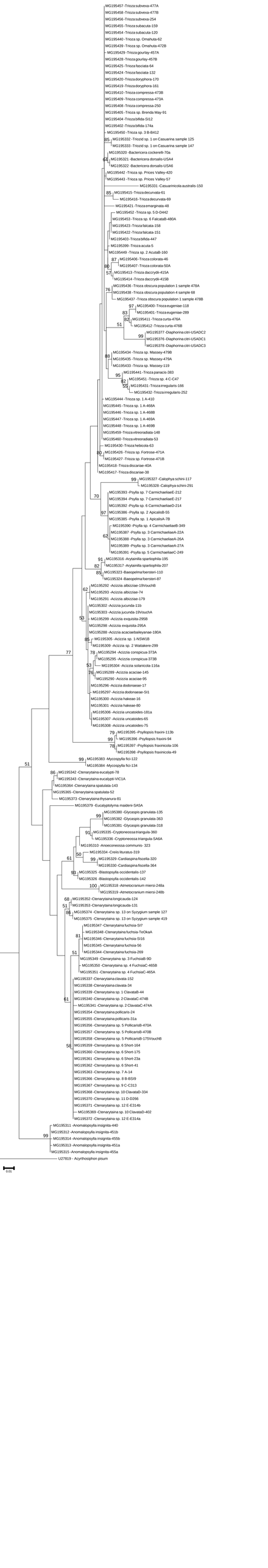
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MG195428 -Trioza.gourlay-457B
MG195425 -Trioza.fasciata-64
MG195424 -Trioza.fasciata-132
MG195420 -Trioza.doryphoria-170
MG195419 -Trioza.doryphoria-161
MG195410 -Trioza.compressa-473B
MG195409 -Trioza.compressa-473A
MG195408 -Trioza.compressa-250
MG195405 -Trioza sp. Brenda May-91
MG195404 -Trioza.bifida-S112
MG195402 -Trioza.bifida-174a
MG195450 -Trioza sp. 3 B-B412
MG195332 -Triozid sp. 1 on Casuarina sample 125
MG195333 -Triozid sp. 1 on Casuarina sample 147
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MG195321 -Bactericera dorsalis-USA4
MG195322 -Bactericera dorsalis-USA6
MG195442 -Trioza sp. Prices Valley-420
MG195443 -Trioza sp. Prices Valley-57
MG195331 -Casuarinicola australis-150
MG195415 -Trioza decurvata-61
MG195416 -Trioza decurvata-69
MG195421 -Trioza emarginata-48
MG195452 -Trioza sp. 5.0-D442
MG195453 -Trioza sp. 6 FalcataB-480A
MG195423 -Trioza falcata-158
MG195422 -Trioza falcata-151
MG195403 -Trioza bifida-447
MG195399 -Trioza acuta-5
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MG195407 -Trioza colorata-50A
MG195413 -Trioza diacrydii-415A
MG195414 -Trioza diacrydii-415B
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MG195438 -Trioza obscura population 4 sample 68
MG195437 -Trioza obscura population 1 sample 478B
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MG195401 -Trioza eugeniae-289
MG195411 -Trioza curta-476A
MG195412 -Trioza curta-476B
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MG195376 -Diaphorina citri-USADC1
MG195378 -Diaphorina citri-USADC3
MG195434 -Trioza sp. Massey-479B
MG195435 -Trioza sp. Massey-479A
MG195433 -Trioza sp. Massey-119
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MG195451 -Trioza sp. 4 C-C47
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MG195432 -Trioza irregularis-252
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MG195446 -Trioza sp. 1 A-468B
MG195447 -Trioza sp. 1 A-469A
MG195448 -Trioza sp. 1 A-469B
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MG195427 -Trioza sp. Fortrose-471B
MG195418 -Trioza discariae-40A
MG195417 -Trioza discariae-38
MG195327 -Calophya schini-117
MG195328 -Calophya schini-291
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MG195394 -Psylla sp. 7 CarmichaeliaeE-217
MG195392 -Psylla sp. 6 CarmichaeliaeD-214
MG195386 -Psylla sp. 2 ApicalisB-55
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MG195390 -Psylla sp. 4 CarmichaeliaeB-349
MG195387 -Psylla sp. 3 CarmichaeliaeA-22A
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MG195389 -Psylla sp. 3 CarmichaeliaeA-27A
MG195391 -Psylla sp. 5 CarmichaeliaeC-249
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MG195317 -Arytainilla spartophila-207
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MG195291 -Acizzia albizziae-179
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MG195303 -Acizzia jucunda-19VouchA
MG195299 -Acizzia exquisita-295B
MG195298 -Acizzia exquisita-295A
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MG195305 -Acizzia sp. 1-NSW1B
MG195309 -Acizzia sp. 2 Wattakere-299
MG195294 -Acizzia conspicua-373A
MG195295 -Acizzia conspicua-373B
MG195304 -Acizzia solanicola-116a
MG195289 -Acizzia acaciae-145
MG195290 -Acizzia acaciae-95
MG195296 -Acizzia dodonaeae-17
MG195297 -Acizzia dodonaeae-St1
MG195300 -Acizzia hakeae-16
MG195301 -Acizzia hakeae-80
MG195306 -Acizzia uncatoides-181a
MG195307 -Acizzia uncatoides-65
MG195308 -Acizzia uncatoides-75
MG195395 -Psyllopsis fraxini-113b
MG195396 -Psyllopsis fraxini-94
MG195397 -Psyllopsis fraxinicola-106
MG195398 -Psyllopsis fraxinicola-49
MG195383 -Mycopsylla fici-122
MG195384 -Mycopsylla fici-134
MG195342 -Ctenarytaina eucalypti-78
MG195343 -Ctenarytaina eucalypti-VIC1A
MG195364 -Ctenarytaina spatulata-143
MG195365 -Ctenarytaina spatulata-52
MG195373 -Ctenarytaina thysanura-81
MG195379 -Eucalyptolyma maideni-SASA
MG195380 -Glycaspis granulata-135
MG195382 -Glycaspis granulata-363
MG195381 -Glycaspis granulata-318
MG195335 -Cryptoneossa triangula-360
MG195336 -Cryptoneossa triangula-SA6A
MG195310 -Anoeconeossa communis- 323
MG195334 -Creiis lituratus-319
MG195329 -Cardiaspina fiscella-320
MG195330 -Cardiaspina fiscella-364
MG195325 -Blastopsylla occidentalis-137
MG195326 -Blastopsylla occidentalis-142
MG195318 -Ametrocraniurn miersi-248a
MG195319 -Ametrocraniurn miersi-248b
MG195352 -Ctenarytaina longicauda-124
MG195353 -Ctenarytaina longicauda-131
MG195374 -Ctenarytaina sp. 13 on Syzygium sample 127
MG195375 -Ctenarytaina sp. 13 on Syzygium sample 419
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MG195348 -Ctenarytaina fuchsia-TeOkaA
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MG195351 -Ctenarytaina sp. 4 FuchsiaC-465A
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MG195338 -Ctenarytaina clavata-34
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MG195357 -Ctenarytaina sp. 5 PollicarisB-470B
MG195358 -Ctenarytaina sp. 5 PollicarisB-175VouchB
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MG195360 -Ctenarytaina sp. 6 Short-175
MG195361 -Ctenarytaina sp. 6 Short-23a
MG195362 -Ctenarytaina sp. 6 Short-41
MG195363 -Ctenarytaina sp. 7 A-14
MG195366 -Ctenarytaina sp. 8 B-BSI9
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MG195368 -Ctenarytaina sp. 10 ClavataD-334
MG195370 -Ctenarytaina sp. 11 D-D266
MG195371 -Ctenarytaina sp. 12 E-E314b
MG195369 -Ctenarytaina sp. 10 ClavataD-402
MG195372 -Ctenarytaina sp. 12 E-E314a
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MG195312 -Anomalopsylla insignita-451b
MG195314 -Anomalopsylla insignita-455b
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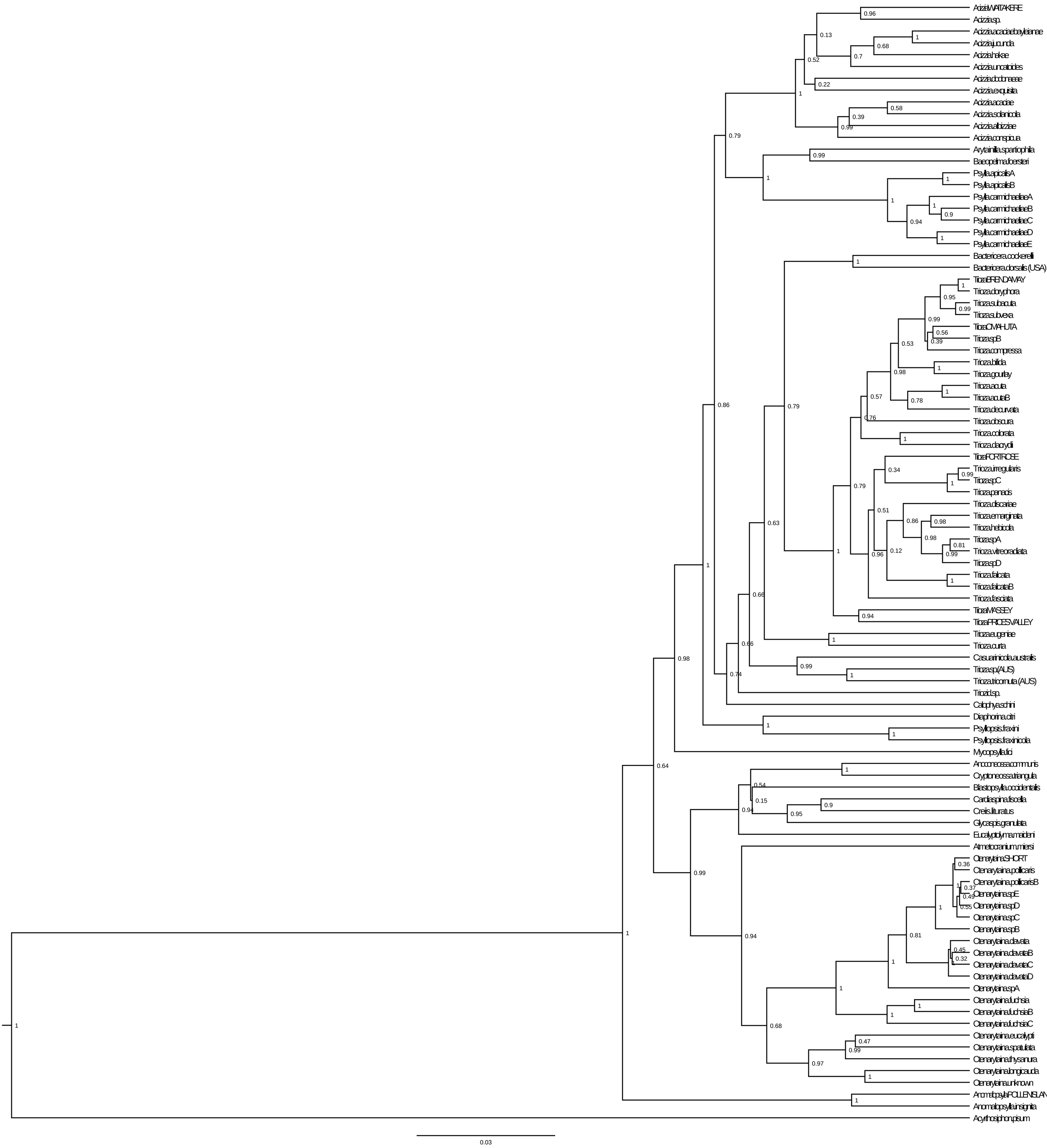
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Acizia exquisita
Acizia acaciae
Acizia solanicola
Acizia albiziae
Acizia conspicua
Arytainilla spartiophila
Baeopelma foersteri
Psylla apicalisA
Psylla apicalisB
Psylla carmichaeliaeA
Psylla carmichaeliaeB
Psylla carmichaeliaeC
Psylla carmichaeliaeD
Psylla carmichaeliaeE
Bactericera cockerelli
Bactericera dorsalis (USA)
TriozaBRENDAMAY
Trioza dryophora
Trioza subacuta
Trioza subvexa
TriozaOMAHUTA
Trioza spB
Trioza compressa
Trioza bifida
Trioza gourlay
Trioza acuta
Trioza acutaB
Trioza decurvata
Trioza obscura
Trioza colorata
Trioza dacrydii
TriozaFORTROSE
Trioza irregularis
Trioza spC
Trioza panacis
Trioza discariae
Trioza emarginata
Trioza hebicola
Trioza spA
Trioza vitreoradiata
Trioza spD
Trioza falcata
Trioza falcataB
Trioza fasciata
TriozaMASSEY
TriozaPRICES VALLEY
Trioza eugeniae
Trioza curta
Casuariniola australis
Trioza sp(AUS)
Trioza tricornuta (AUS)
Triozid sp.
Calophya schini
Diaphorina citri
Psyllopsis fraxini
Psyllopsis fraxinicola
Mycopsylla fici
Anoconeossa communis
Cyptoneossa triangula
Blastopsylla occidentalis
Cardaspina fiscella
Creiis lituratus
Glycaspis granulata
Eucalyptolyma maideni
Atmetocranium miersi
CtenarytainaSHORT
Ctenarytaina poaicaris
Ctenarytaina poaicarisB
Ctenarytaina spE
Ctenarytaina spD
Ctenarytaina spC
Ctenarytaina spB
Ctenarytaina clavata
Ctenarytaina clavataB
Ctenarytaina clavataC
Ctenarytaina clavataD
Ctenarytaina spA
Ctenarytaina fuchsia
Ctenarytaina fuchsiaB
Ctenarytaina fuchsiaC
Ctenarytaina eucalypti
Ctenarytaina spatulata
Ctenarytaina thysanura
Ctenarytaina longicauda
Ctenarytaina unknown
AnomalopsylaPOLLENSLAN
Anomalopsylla insignita
Acythosiphon pisum
0.03

# Appendix D

# Microflora analysis samples

## D.1 Psyllids samples used for the NGS analysis.

**Table A.3:** The table lists the samples used for the NGS analysis, their host plants and the GPS coordinate of collection site. The S-ID column lists the ID code used only in this analysis to distinguish different specimens analysed multiple times.

| | S-ID | Species | Host Plant | Location |
|---|---|---|---|---|
| 180_S144 | S144 | *Acizzia acaciaebaileyanae* | *Acacia baileyana* | 43°38'03.50"S 172°29'12.80"E |
| 123_S103 | S103 | *Acizzia acaciae* | *Acacia melanoxylon* | 36°51'01.51"S 174°46'04.29"E |
| 145_S118 | S118 | *Acizzia acaciae* | *Acacia melanoxylon* | 37°46'14.54"S 175°15'02.77"E |
| 30_S30 | S30 | *Acizzia acaciae* | *Acacia melanoxylon* | 43°42'51.72"S 172°47'00.86"E |
| 95_S81 | S81 | *Acizzia acaciae* | *Acacia melanoxylon* | 46°24'08.13"S 168°21'39.78"E |
| 11A | S89 | *Acizzia albizziae* | *Acacia* sp. | 41°25'05.84"S 173°57'53.38"E |
| 11B | S90 | *Acizzia albizziae* | *Acacia* sp. | 41°25'05.84"S 173°57'53.38"E |
| 179_S143 | S143 | *Acizzia albizziae* | *Acacia* sp. | 43°38'05.70"S 172°29'24.20"E |
| 19VOUCHB_S18 | S18 | *Acizzia albizziae* | *Acacia* sp. | 41°15'27.81"S 172°49'21.79"E |
| 58_S55 | S55 | *Acizzia albizziae* | *Acacia* sp. | 40°43'36.60"S 172°41'19.40"E |
| 74_S66 | S66 | *Acizzia albizziae* | *Acacia* sp. | 43°34'15.50"S 172°37'26.70"E |
| 19B | S62 | *Acizzia albizziae* | *Acacia* sp. | 41°15'27.81"S 172°49'21.79"E |
| 10_S10 | S10 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 42°09'20.09"S 173°55'26.43"E |
| 12A | S85 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 41°17'28.02"S 173°14'04.21"E |
| 12B dil | S86 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 41°17'28.02"S 173°14'04.21"E |
| 13_S12 | S12 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 41°18'20.94"S 173°17'03.14"E |
| 17_S15 | S15 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 40°56'53.20"S 173°02'31.40"E |
| SI1_S178 | S178 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 46°54'01.20"S 168°07'15.90"E |
| 18A dil | S87 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 40°47'06.88"S 172°43'35.24"E |
| 18B | S88 | *Acizzia dodonaeae* | *Dodonaea viscosa* | 40°47'06.88"S 172°43'35.24"E |
| 16_S14 | S14 | *Acizzia hakeae* | *Hakea acicularis* | 40°56'53.20"S 173°02'31.40"E |

| | | | | |
|---|---|---|---|---|
| 3_S2 | S2 | *Acizzia hakeae* | *Hakea acicularis* | 43°27'29.28"S 172°12'23,28"E |
| 80_S70 | S70 | *Acizzia hakeae* | *Grevillea* sp. | 44°24'32.20"S 171°15'11.20"E |
| 11_S11 | S11 | *Acizzia jucunda* | *Acacia* sp. | 41°25'05.84"S 173°57'53.38"E |
| 19A dil | S61 | *Acizzia jucunda* | *Acacia* sp. | 41°15'27.81"S 172°49'21.79"E |
| 19a_S16 | S16 | *Acizzia jucunda* | *Acacia* sp. | 41°15'27.81"S 172°49'21.79"E |
| 19C | S63 | *Acizzia jucunda* | *Acacia* sp. | 41°15'27.81"S 172°49'21.79"E |
| 19VOUCHA_S17 | S17 | *Acizzia jucunda* | *Acacia* sp. | 41°15'27.81"S 172°49'21.79"E |
| 116a_S95 | S95 | *Acizzia solanicola* | *Solanum tuberosum* | 36°53'58.80"S 174°46'55.90"E |
| 116b_S96 | S96 | *Acizzia solanicola* | *Solanum tuberosum* | 36°53'58.80"S 174°46'55.90"E |
| NSW1A | S64 | *Acizzia* sp. | *Acacia baileyana* | 33°49'59.30"S 150°32'55.60"E |
| NSW1B | S65 | *Acizzia* sp. | *Acacia baileyana* | 33°49'59.30"S 150°32'55.60"E |
| NSW1C | S66 | *Acizzia* sp. | *Acacia baileyana* | 33°49'59.30"S 150°32'55.60"E |
| NSWab_S172 | S172 | *Acizzia* sp. | *Acacia baileyana* | 33°49'59.30"S 150°32'55.60"E |
| 178_S142 | S142 | *Acizzia uncatoides* | *Acacia* sp. | 43°48'04.20"S 172°58'33.10"E |
| 181a_S145 | S145 | *Acizzia uncatoides* | *Acacia* sp. | 43°38'37.80"S 172°28'07.80"E |
| 65_S61 | S61 | *Acizzia uncatoides* | *Acacia* sp. | 40°42'53.20"S 172°40'49.90"E |
| 75a_S67 | S67 | *Acizzia uncatoides* | *Acacia* sp. | 43°38'25.90"S 172°28'31.20"E |
| VIC8A_S189 | S189 | *Acizzia uncatoides* | *Acacia* sp. | 37°49'43.60"S 144°58'33.10"E |
| VIC8B_S190 | S190 | *Acizzia uncatoides* | *Acacia* sp. | 37°49'43.60"S 144°58'33.10"E |
| SA1B_S173 | S173 | *Anoeconeossa* sp. | *Eucalyptus* sp. | 34°57'33.20"S 138°40'45.30"E |
| 35A dil | S82 | *Anomalopsylla* POLLEN ISLAND | *Olearia odorata* | 43°37'51.90"S 171°18'02.48"E |
| 35a_S36 | S36 | *Anomalopsylla* POLLEN ISLAND | *Olearia odorata* | 43°37'51.90"S 171°18'02.48"E |
| 35B | S83 | *Anomalopsylla* POLLEN ISLAND | *Olearia odorata* | 43°37'51.90"S 171°18'02.48"E |
| 35C dil | S84 | *Anomalopsylla* POLLEN ISLAND | *Olearia odorata* | 43°37'51.90"S 171°18'02.48"E |
| 195_S148 | S148 | *Arytainilla spartiophila* | *Cytisus scoparius* | 43°35'48.53"S 172°21'49.17"E |
| 207_S154 | S154 | *Arytainilla spartiophila* | *Cytisus scoparius* | 45°02'12.80"S 168°39'38.30"E |
| 70a_S64 | S64 | *Bactericera cockerelli* | *Solanum tuberosum* | 43°38'25.90"S 172°28'31.20"E |
| 110_S91 | S91 | *Baeopelma foersteri* | *Alnus glutinosa* | 44°15'24.97"S 170°05'57.58"E |
| 87_S74 | S74 | *Baeopelma foersteri* | *Alnus glutinosa* | 46°25'28.87"S 168°21'38.03"E |
| 91_S167 | S167 | blank | ///// | |

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| 137_S115 | S115 | *Blastopsylla occidentalis* | *Eucalyptus* sp. | 36°46'54.53"S 175°00'33.32"E |
| 142_S116 | S116 | *Blastopsylla occidentalis* | *Eucalyptus* sp. | 37°47'58.62"S 175°16'19.41"E |
| 117a_S97 | S97 | *Calophya schini* | *Schinus molle* | 43°31'52.23"S 172°37'32.10"E |
| 117b_S98 | S98 | *Calophya schini* | *Schinus molle* | 43°31'52.23"S 172°37'32.10"E |
| 150b_S121 | S121 | *Casuarinicola australis* | *Casuarina* sp. | 35°45'23.84"S 174°22'38.88"E |
| SA3A_S174 | S174 | *Casuarinicola australis* | *Casuarina* sp. | 34°57'33.20"S 138°40'45.30"E |
| 125_S105 | S105 | Triozid sp. | *Casuarina* sp. | 36°54'10.60"S 174°46'47.00"E |
| 147_S119 | S119 | Triozid sp. | *Casuarina* sp. | 37°13'20.84"S 175°37'44.54"E |
| SA8A_S177 | S177 | Triozid sp. | *Casuarina* sp. | 34°57'33.20"S 138°40'45.30"E |
| SA6A_S176 | S176 | *Cryptoneossa* sp. | *Eucalyptus* sp. | 34°57'33.20"S 138°40'45.30"E |
| 152_S123 | S123 | *Ctenarytaina clavata* | *Kunzea ericoides* | 43°13'13.20"S 171°43'08.80"E |
| 34a_S35 | S35 | *Ctenarytaina clavata* | *Kunzea ericoides* | 43°37'51.90"S 171°18'02.48"E |
| 44_S42 | S42 | *Ctenarytaina clavata* | *Kunzea ericoides* | 42°31'06.10"S 172°49'17.10"E |
| 105_S86 | S86 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 45°25'29.05"S 167°43'08.34"E |
| 115_S94 | S94 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 45°38'13.11"S 168°10'30.77"E |
| 165_S132 | S132 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 43°38'25.10"S 172°28'31.50"E |
| 2_S1 | S1 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 43°27'29.28"S 172°12'23.28"E |
| 39A dil | S49 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 43°09'36.90"S 172°43'51.70"E |
| 39B | S50 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 43°09'36.90"S 172°43'51.70"E |
| 39C dil | S51 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 43°09'36.90"S 172°43'51.70"E |
| 42A | S52 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 42°46'24.70"S 172°50'54.00"E |
| 42B dil | S53 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 42°46'24.70"S 172°50'54.00"E |
| 42C dil | S54 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 42°46'24.70"S 172°50'54.00"E |
| 43_S41 | S41 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 43°08'58.00"S 172°43'46.40"E |
| 78_S68 | S68 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 44°07'40.19"S 171°18'30.33"E |
| 82_S72 | S72 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 45°52'41.60"S 170°29'21.30"E |
| 89_S76 | S76 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 46°26'14.28"S 168°22'35.47"E |
| 99_S82 | S82 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 46°09'26.21"S 167°41'08.11"E |
| SA2A dil | S55 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 34°57'33.20"S 138°40'45.30"E |
| SA2B | S56 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 34°57'33.20"S |

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| | | | | 138°40'45.30"E |
| SA2C | S57 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 34°57'33.20"S 138°40'45.30"E |
| SI10_S179 | S179 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 46°54'01.20"S 168°07'15.90"E |
| SI15_S181 | S181 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 46°54'01.20"S 168°07'15.90"E |
| VIC1A | S58 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 37°49'43.60"S 144°58'33.10"E |
| VIC1B | S59 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 37°49'43.60"S 144°58'33.10"E |
| VIC1C | S60 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 37°49'43.60"S 144°58'33.10"E |
| VIC1C_S188 | S188 | *Ctenarytaina eucalypti* | *Eucalyptus globulus* | 37°49'43.60"S 144°58'33.10"E |
| 194_S147 | S147 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 44°40'19.30"S 167°53'32.50"E |
| 220_S159 | S159 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 44°07'38.90"S 169°20'19.60"E |
| 221_S160 | S160 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 43°58'15.40"S 169°25'00.10"E |
| 224_S163 | S163 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 43°26'10.50"S 170°04'42.50"E |
| 229_S164 | S164 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 42°43'28.70"S 170°59'07.80"E |
| 237_S165 | S165 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 41°30'14.40"S 171°57'05.50"E |
| 238_S166 | S166 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 41°15'29.40"S 172°07'00.80"E |
| 56_S53 | S53 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 43°38'23.40"S 172°28'31.30"E |
| geraldineA_S168 | S168 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 44°05'44.00"S 171°14'42.20"E |
| geraldineB_S169 | S169 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 44°05'44.00"S 171°14'42.20"E |
| SI13B_S192 | S192 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SI16_S182 | S182 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SI22_S184 | S184 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SI7_S185 | S185 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SignOfBellbird_S26 | S26 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 43°37'58.40"S 172°37'36.00"E |
| TeOkaA_S170 | S170 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 43°49'37.10"S 172°43'06.00"E |
| TeOkaB_S171 | S171 | *Ctenarytaina fuchsiae* | *Fuchsia excorticata* | 43°49'37.10"S 172°43'06.00"E |
| SI13A | S70 | *Ctenarytaina fuchsiae A* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SI13B | S71 | *Ctenarytaina fuchsiae A* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| 9A dil | S67 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |
| 9B | S68 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |
| 9C | S69 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |

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| 9d_S6 | S6 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |
| 9e_S7 | S7 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |
| 9f_S8 | S8 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |
| 9g_S9 | S9 | *Ctenarytaina fuchsiae B* | *Fuchsia excorticata* | 42°17'05.99"S 173°45'39.53"E |
| 124_S104 | S104 | *Ctenarytaina longicauda* | *Lophostemon confertus* | 36°51'41.38"S 174°46'32.21"E |
| 131a_S107 | S107 | *Ctenarytaina longicauda* | *Lophostemon confertus* | 37°00'28.60"S 174°54'22.20"E |
| 131b_S108 | S108 | *Ctenarytaina longicauda* | *Lophostemon confertus* | 37°00'28.60"S 174°54'22.20"E |
| 175VOUCHA_S141 | S141 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°48'35.70"S 173°01'27.50"E |
| 24A dil | S72 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°39'14.80"S 172°48'44.20"E |
| 24a_S22 | S22 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°39'14.80"S 172°48'44.20"E |
| 24B dil | S73 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°39'14.80"S 172°48'44.20"E |
| 24b_S23 | S23 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°39'14.80"S 172°48'44.20"E |
| 24C | S74 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°39'14.80"S 172°48'44.20"E |
| 24c_S24 | S24 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°39'14.80"S 172°48'44.20"E |
| 31A dil | S75 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°49'08.40"S 172°46'43.10"E |
| 31a_S31 | S31 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°49'08.40"S 172°46'43.10"E |
| 31B dil | S76 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°49'08.40"S 172°46'43.10"E |
| 31b_S32 | S32 | *Ctenarytaina pollicaris* | *Leptospermum scoparius* | 43°49'08.40"S 172°46'43.10"E |
| 164_S131 | S131 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°46'30.60"S 172°46'40.40"E |
| 172_S137 | S137 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°48'35.70"S 173°01'27.50"E |
| 175_S140 | S140 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°48'35.70"S 173°01'27.50"E |
| 23A dil | S77 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°39'14.80"S 172°48'44.20"E |
| 23a_S19 | S19 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°39'14.80"S 172°48'44.20"E |
| 23B | S78 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°39'14.80"S 172°48'44.20"E |
| 23b_S20 | S20 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°39'14.80"S 172°48'44.20"E |
| 23C | S79 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°39'14.80"S 172°48'44.20"E |
| 23c_S21 | S21 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°39'14.80"S 172°48'44.20"E |
| 33A | S80 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°37'51.90"S 171°18'02.48"E |
| 33a_S33 | S33 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°37'51.90"S 171°18'02.48"E |
| 33B | S81 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°37'51.90"S |

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| | | | | 171°18'02.48"E |
| 33b_S34 | S34 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 43°37'51.90"S 171°18'02.48"E |
| 41_S40 | S40 | *Ctenarytaina* SHORT | *Kunzea ericoides* | 42°46'24.70"S 172°50'54.00"E |
| 14_S13 | S13 | *Ctenarytaina* sp.14 | *Olearia paniculata* | 41°18'20.94"S 173°17'03.14"E |
| SI17_S183 | S183 | *Ctenarytaina* sp.SI9 | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SI9_S187 | S187 | *Ctenarytaina* sp.SI9 | *Leptospermum scoparius* | 46°54'01.20"S 168°07'15.90"E |
| 101_S83 | S83 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 45°47'54.90"S 167°32'48.30"E |
| 102_S84 | S84 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 45°46'42.34"S 167°36'55.42"E |
| 108_S89 | S89 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 45°24'57.69"S 167°42'38.62"E |
| 109_S90 | S90 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 45°38'13.11"S 168°10'30.77"E |
| 136_S114 | S114 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 36°46'54.53"S 175°00'33.32"E |
| 143_S117 | S117 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 37°47'58.62"S 175°16'19.41"E |
| 28_S27 | S27 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 43°39'14.80"S 172°48'44.20"E |
| 52_S49 | s49 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 43°38'23.40"S 172°28'31.30"E |
| 79_S69 | S69 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 44°24'32.20"S 171°15'11.20"E |
| 8b_S5 | S5 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 42°57'52.31"S 172°58'04.14"E |
| SI8_S186 | S186 | *Ctenarytaina spatulata* | *Eucalyptus* sp. | 46°54'01.20"S 168°07'15.90"E |
| 81_S71 | S71 | *Ctenarytaina thysanura* | *Eucalyptus* sp. | 44°24'32.20"S 171°15'11.20"E |
| 127a_S106 | S106 | *Ctenarytaina unknown* | *Eucalyptus* sp. | 36°52'26.30"S 174°43'45.50"E |
| SA5A_S175 | S175 | *Eucalyptolyma maideni* | *Eucalyptus* sp. | 34°57'33.20"S 138°40'45.30"E |
| 135a_S113 | S113 | *Glycaspis granulata* | *Eucalyptus* sp. | 36°46'54.53"S 175°00'33.32"E |
| mockA_S51 | S51 | MOCK | | |
| 122_S102 | S102 | *Mycopsylla fici* | *Ficus macrophylla* | 36°51'01.51"S 174°46'04.29"E |
| 134a_S111 | S111 | *Mycopsylla fici* | *Ficus macrophylla* | 36°51'20.20"S 174°45'43.70"E |
| 134b_S112 | S112 | *Mycopsylla fici* | *Ficus macrophylla* | 36°51'20.20"S 174°45'43.70"E |
| MockEven_S193 | S193 | ND | | |
| MockStaggered_S194 | S194 | ND | | |
| 187_S146 | S146 | *Psylla apicalis* A | *Sophora microphylla* | 43°31'22.44"S 172°35'07.44"E |
| 200big_S149 | S149 | *Psylla apicalis* A | *Sophora microphylla* | 45°05'13.47"S 170°58'31.85"E |
| 201big_S151 | S151 | *Psylla apicalis* A | *Sophora microphylla* | 46°24'57.20"S 168°21'45.70"E |
| 206_S153 | S153 | *Psylla apicalis* A | *Sophora microphylla* | 45°02'12.80"S 168°39'38.30"E |

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| 200small_S150 | S150 | *Psylla apicalis* B | *Sophora microphylla* | 45°05'13.47"S 170°58'31.85"E |
| 201small_S152 | S152 | *Psylla apicalis* B | *Sophora microphylla* | 46°24'57.20"S 168°21'45.70"E |
| 209_S155 | S155 | *Psylla apicalis* B | *Sophora microphylla* | 45°02'09.70"S 169°11'33.80"E |
| 55_S52 | S52 | *Psylla apicalis* B | *Sophora microphylla* | 43°38'23.40"S 172°28'31.30"E |
| 22A | S91 | *Psylla carmichaeliae* A | *Carmichaelia* sp. | 43°46'42.50"S 172°47'10.90"E |
| 22B dil | S92 | *Psylla carmichaeliae* A | *Carmichaelia* sp. | 43°46'42.50"S 172°47'10.90"E |
| 26_S25 | S25 | *Psylla carmichaeliae* A | *Carmichaelia* sp. | 43°39'14.80"S 172°48'44.20"E |
| 222_S161 | S161 | *Psylla carmichaeliae* C | *Carmichaelia* sp. | 43°45'03.70"S 169°23'13.60"E |
| 223_S162 | S162 | *Psylla carmichaeliae* C | *Carmichaelia* sp. | 43°33'54.80"S 169°46'13.30"E |
| 212_S156 | S156 | *Psylla carmichaeliae* E | *Carmichaelia petri* | 44°57'19.90"S 169°15'51.90"E |
| 213_S157 | S157 | *Psylla carmichaeliae* E | *Carmichaelia petri* | 45°02'03.10"S 169°11'59.10"E |
| 217_S158 | S158 | *Psylla carmichaeliae* E | *Carmichaelia petri* | 44°58'29.00"S 168°56'54.80"E |
| 107_S88 | S88 | *Psyllopsis fraxini* | *Fraxinus excelsior* | 45°24'57.69"S 167°42'38.62"E |
| 113_S93 | S93 | *Psyllopsis fraxini* | *Fraxinus excelsior* | 44°18'35.41"S 170°57'10.41"E |
| 94_S80 | S80 | *Psyllopsis fraxini* | *Fraxinus excelsior* | 46°24'07.73"S 168°21'23.71"E |
| 106_S87 | S87 | *Psyllopsis fraxinicola* | *Fraxinus excelsior* | 45°24'57.69"S 167°42'38.62"E |
| 112_S92 | S92 | *Psyllopsis fraxinicola* | *Fraxinus excelsior* | 44°18'35.41"S 170°57'10.41"E |
| 49_S47 | S47 | *Psyllopsis fraxinicola* | *Fraxinus excelsior* | 43°38'23.40"S 172°28'31.30"E |
| 93_S79 | S79 | *Psyllopsis fraxinicola* | *Fraxinus excelsior* | 46°24'07.73"S 168°21'23.71"E |
| 45_S43 | S43 | *Trioza acuta* | /// | 42°31'02.20"S 172°48'58.70"E |
| 5b_S4 | S4 | *Trioza acuta* | *Ozothamnus* sp. | 43°17'48.63"S 171°44'13.02"E |
| 118a_S99 | S99 | *Trioza adventicia* | *Syzygium smithii* | 43°31'51.34"S 172°37'30.53"E |
| 174_S139 | S139 | *Trioza bifida* | *Olearia fragmentissima* | 43°48'35.70"S 173°01'27.50"E |
| 29A | S95 | *Trioza bifida* | *Pseudowintera colorata* | 43°42'51.72"S 172°47'00.86"E |
| 29a_S28 | S28 | *Trioza bifida* | *Pseudowintera colorata* | 43°42'51.72"S 172°47'00.86"E |
| 29B | S96 | *Trioza bifida* | *Pseudowintera colorata* | 43°42'51.72"S 172°47'00.86"E |
| 29b_S29 | S29 | *Trioza bifida* | *Pseudowintera colorata* | 43°42'51.72"S 172°47'00.86"E |
| SI12_S180 | S180 | *Trioza bifida* | *Fuchsia excorticata* | 46°54'01.20"S 168°07'15.90"E |
| SI3B_S191 | S191 | *Trioza bifida* | *Dracophyllum* sp. | 46°54'01.20"S 168°07'15.90"E |
| 91_S78 | S78 | *Trioza* BRENDA MAY | *Olearia ilicifolia* | 46°34'20.08"S |

| | | | | |
|---|---|---|---|---|
| | | | | 169°20'48.30"E |
| 46A dil | S93 | *Trioza colorata* | *Halocarpus* sp. | 43°01'45.90"S 171°37'46.70"E |
| 46a_S44 | S44 | *Trioza colorata* | *Halocarpus* sp. | 43°01'45.90"S 171°37'46.70"E |
| 46B | S94 | *Trioza colorata* | *Halocarpus* sp. | 43°01'45.90"S 171°37'46.70"E |
| 50a_S48 | S48 | *Trioza colorata* | *Halocarpus* sp. | 43°38'23.40"S 172°28'31.30"E |
| 61_S57 | S57 | *Trioza decurvata* | *Dracophyllum* sp. | 43°17'48.63"S 171°44'13.02"E |
| 69_S63 | S63 | *Trioza decurvata* | *Dracophyllum* sp. | 42°33'56.10"S 173°11'19.70"E |
| 38a_S37 | S37 | *Trioza discariae* | *Discaria toumatou* | 43°38'07.60"S 171°13'34.10"E |
| 38b_S38 | S38 | *Trioza discariae* | *Discaria toumatou* | 43°38'07.60"S 171°13'34.10"E |
| 40_S39 | S39 | *Trioza discariae* | *Discaria toumatou* | 43°09'36.90"S 172°43'51.70"E |
| 161_S129 | S129 | *Trioza doryphora* | *Olearia ilicifolia* | 43°48'35.70"S 173°01'27.50"E |
| 162_S130 | S130 | *Trioza doryphora* | *Olearia ilicifolia* | 43°48'35.70"S 173°01'27.50"E |
| 170_S136 | S136 | *Trioza doryphora* | *Olearia ilicifolia* | 43°45'07.68"S 173°00'56.54"E |
| 173_S138 | S138 | *Trioza doryphora* | *Olearia ilicifolia* | 43°48'35.70"S 173°01'27.50"E |
| 48_S46 | S46 | *Trioza emarginata* | *Coprosma* sp. | 43°12'23.00"S 171°43'21.10"E |
| 151_S122 | S122 | *Trioza falcata* | *Aristotelia fruticosa* | 42°59'48.30"S 171°44'23.60"E |
| 154_S124 | S124 | *Trioza falcata* | *Aristotelia fruticosa* | 42°59'48.30"S 171°44'23.60"E |
| 157_S125 | S125 | *Trioza falcata* | *Aristotelia fruticosa* | 43°13'13.20"S 171°43'08.80"E |
| 158_S126 | S126 | *Trioza falcata* | *Aristotelia fruticosa* | 43°07'56.20"S 171°46'01.10"E |
| 132_S109 | S109 | *Trioza fasciata* | *Muehlenbeckia auxiliaris* | 37°00'28.60"S 174°54'22.20"E |
| 64_S60 | S60 | *Trioza fasciata* | *Muehlenbeckia complexa* | 40°30'51.60"S 172°45'17.20"E |
| 63a_S59 | S59 | *Trioza hebicola* | *Hebe* sp. | 43°17'48.63"S 171°44'13.02"E |
| 166_S133 | S133 | *Trioza irregularis* | *Pseudopanax arboreus* | 43°43'16.56"S 172°56'14.46"E |
| 168_S134 | S134 | *Trioza irregularis* | *Pseudopanax arboreus* | 43°43'16.56"S 172°56'14.46"E |
| 4_S3 | S3 | *Trioza irregularis* | *Pseudopanax arboreus* | 43°17'27.61"S 171°55'30.99"E |
| 119a_S100 | S100 | *Trioza* MASSEY | *Olearia* sp. | 43°31'49.10"S 172°37'15.20"E |
| 68_S62 | S62 | *Trioza obscura* | *Hebe* sp. | 42°33'56.10"S 173°11'19.70"E |
| 72_S65 | S65 | *Trioza panacis* | *Pseudopanax crassifolius* | 43°38'25.90"S 172°28'31.20"E |
| 57b_S54 | S54 | *Trioza* PRICE'S VALLEY | *Plagianthus* sp. | 37°45'54.80"S 176°24'01.10"E |
| 47_S45 | S45 | *Trioza* sp. D | *Pseudopanax edgerlii* | 43°01'45.90"S 171°37'46.70"E |

| | | | | |
|---|---|---|---|---|
| 160_S128 | S128 | *Trioza* sp. B | *Ozothamnus* sp. | 43°07'56.20"S 171°46'01.10"E |
| 62_S58 | S58 | *Trioza* sp. 62 | *Metrosideros umbellata* | 43°17'48.63"S 171°44'13.02"E |
| 120a_S101 | S101 | *Trioza subacuta* | *Olearia avicennifolia* | 43°37'57.40"S 172°37'29.60"E |
| 159a_S127 | S127 | *Trioza subacuta* | *Olearia avicennifolia* | 43°07'56.20"S 171°46'01.10"E |
| 104_S85 | S85 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 45°33'53.50"S 167°36'38.66"E |
| 133_S110 | S110 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 36°50'48.80"S 174°45'17.50"E |
| 148_S120 | S120 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 37°00'49.13"S 175°30'37.32"E |
| 169_S135 | S135 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 43°45'07.68"S 173°00'56.54"E |
| 53_S50 | S50 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 43°38'23.40"S 172°28'31.30"E |
| 59_S56 | S56 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 40°43'36.60"S 172°41'19.40"E |
| 84_S73 | S73 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 46°28'05.04"S 168°39'30.15"E |
| 88_S75 | S75 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 46°25'28.87"S 168°21'38.03"E |
| 90_S77 | S77 | *Trioza vitreoradiata* | *Pittosporum crassifolius* | 46°33'51.23"S 168°56'41.04"E |

# Appendix E

# Next Generation Sequencing Pipelines

This appendix presents the three pipelines used in this thesis for the analysis of the NGS dataset (Appendix C). The reader may find that some of the scripts could be resolved with shorter/different commands. However, the purpose of this appendix is just to report the analysis as it happened when it was run, with no intention on suggesting what the best command is.

The line of codes are reported here highlighted in grey while the symbol "#" or the absence of highlighting mark a comment or a text.

## E.1 QIIME

This first pipeline was run in an Oracle VM Virtual Box running a Linux environment on a Windows computer with 16 GB of RAM. This was done following the instructions on the QIIME website (http://qiime.org/index.html) (Caporaso et al. 2010).

### FLASH: paired ends joining

Examples showed for the first 5 samples:

```
/home/qiime/FLASH-1.2.11/flash -o Ctenarytaina.eucalypti.2 -M 450
/home/qiime/Desktop/Shared_Folder/NGS-2016/2_S1_L001_R1_001.fastq
/home/qiime/Desktop/Shared_Folder/NGS-2016/2_S1_L001_R2_001.fastq
/home/qiime/FLASH-1.2.11/flash -o Acizzia.hakae.3 -M 450
/home/qiime/Desktop/Shared_Folder/NGS-2016/3_S2_L001_R1_001.fastq
/home/qiime/Desktop/Shared_Folder/NGS-2016/3_S2_L001_R2_001.fastq
/home/qiime/FLASH-1.2.11/flash -o Trioza.irregularis.4 -M 450
/home/qiime/Desktop/Shared_Folder/NGS-2016/4_S3_L001_R1_001.fastq
/home/qiime/Desktop/Shared_Folder/NGS-2016/4_S3_L001_R2_001.fastq
/home/qiime/FLASH-1.2.11/flash -o Trioza.acuta.5b -M 450
/home/qiime/Desktop/Shared_Folder/NGS-2016/5b_S4_L001_R1_001.fastq
/home/qiime/Desktop/Shared_Folder/NGS-2016/5b_S4_L001_R2_001.fastq
/home/qiime/FLASH-1.2.11/flash -o Ctenarytaina.spatulata.8b -M 450
/home/qiime/Desktop/Shared_Folder/NGS-2016/8b_S5_L001_R1_001.fastq
/home/qiime/Desktop/Shared_Folder/NGS-2016/8b_S5_L001_R2_001.fastq
```

### Split Libraries

```
split_libraries_fastq.py -i
Acizzia.acaciabayleianae.180.extendedFrags.fastq,Acizzia.acaciae.30.
extendedFrags.fastq,Acizzia.acaciae.95.extendedFrags.fastq,Acizzia.a
caciae.123.extendedFrags.fastq,Acizzia.acaciae.145.extendedFrags.fas
tq,Acizzia.albizziae.11A.extendedFrags.fastq,Acizzia.albizziae.11B.e
xtendedFrags.fastq,Acizzia.albizziae.19VOUCHB.extendedFrags.fastq,Ac
izzia.albizziae.58.extendedFrags.fastq,Acizzia.albizziae.74.extended
```

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eae.10.extendedFrags.fastq,Acizzia.dodonaeae.12A.extendedFrags.fastq
,Acizzia.dodonaeae.12B.extendedFrags.fastq,Acizzia.dodonaeae.13.exte
ndedFrags.fastq,Acizzia.dodonaeae.17.extendedFrags.fastq,Acizzia.dod
onaeae.18A.extendedFrags.fastq,Acizzia.dodonaeae.18B.extendedFrags.f
astq,Acizzia.dodonaeae.SI1.extendedFrags.fastq,Acizzia.hakae.3.exten
dedFrags.fastq,Acizzia.hakae.16.extendedFrags.fastq,Acizzia.hakae.80
.extendedFrags.fastq,Acizzia.jucunda.19a.extendedFrags.fastq,Acizzia
.jucunda.19VOUCHA.extendedFrags.fastq,Acizzia.solanicola.116a.extend
edFrags.fastq,Acizzia.solanicola.116b.extendedFrags.fastq,Acizzia.sp
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.fastq,Acizzia.uncatoides.75a.extendedFrags.fastq,Acizzia.uncatoides
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lla.spartiophila.195.extendedFrags.fastq,Arytainilla.spartiophila.20
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,Baeopelma.foersteri.87.extendedFrags.fastq,Baeopelma.foersteri.110.
extendedFrags.fastq,Blastopsylla.occidentalis.137.extendedFrags.fast
q,Blastopsylla.occidentalis.142.extendedFrags.fastq,Calophyia.schini
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alis.SA3A.extendedFrags.fastq,Casuarinicola.sp.125.extendedFrags.fas
tq,Casuarinicola.sp.147.extendedFrags.fastq,Casuarinicola.sp.SA6A.ex
tendedFrags.fastq,Casuarinicola.sp.SA8A.extendedFrags.fastq,Ctenaryt
aina.clavata.34a.extendedFrags.fastq,Ctenarytaina.clavata.44.extende
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aina.fuchsia.geraldineB.extendedFrags.fastq,Ctenarytaina.fuchsia.SI7
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sylla.carmichaeliaeA.22B.extendedFrags.fastq,Psylla.carmichaeliaeA.2
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Psylla.carmichaeliaeB.213.extendedFrags.fastq,Psylla.carmichaeliaeB.
217.extendedFrags.fastq,Psylla.carmichaeliaeC.222.extendedFrags.fast

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oza.colorata.46a.extendedFrags.fastq,Trioza.colorata.46A.extendedFra
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reoradiata.90.extendedFrags.fastq,Trioza.vitreoradiata.104.extendedF
rags.fastq,Trioza.vitreoradiata.133.extendedFrags.fastq,Trioza.vitre
oradiata.148.extendedFrags.fastq,Trioza.vitreoradiata.169.extendedFr
ags.fastq\
--sample_id
Acizzia.acaciabayleianae.180,Acizzia.acaciae.30,Acizzia.acaciae.95,A
cizzia.acaciae.123,Acizzia.acaciae.145,Acizzia.albizziae.11A,Acizzia
.albizziae.11B,Acizzia.albizziae.19VOUCHB,Acizzia.albizziae.58,Acizz
ia.albizziae.74,Acizzia.albizziae.179,Acizzia.dodonaeae.10,Acizzia.d
odonaeae.12A,Acizzia.dodonaeae.12B,Acizzia.dodonaeae.13,Acizzia.dodo
naeae.17,Acizzia.dodonaeae.18A,Acizzia.dodonaeae.18B,Acizzia.dodonae
ae.SI1,Acizzia.hakae.3,Acizzia.hakae.16,Acizzia.hakae.80,Acizzia.juc
unda.19a,Acizzia.jucunda.19VOUCHA,Acizzia.solanicola.116a,Acizzia.so
lanicola.116b,Acizzia.sp.NSW1A,Acizzia.sp.NSW1B,Acizzia.sp.NSW1C,Aci
zzia.uncatoides.65,Acizzia.uncatoides.75a,Acizzia.uncatoides.178,Aci
zzia.uncatoides.181a,Acizzia.uncatoides.VIC8A,Acizzia.uncatoides.VIC
8B,Anoconeossa.sp.SA1B,Anomalopsylla.POLLENISLAND.35a,Anomalopsylla.
POLLENISLAND.35A,Anomalopsylla.POLLENISLAND.35B,Anomalopsylla.POLLEN
ISLAND.35C,Arytainilla.spartiophila.195,Arytainilla.spartiophila.207
,Bactericera.cockerelli.70a,Baeopelma.foersteri.87,Baeopelma.foerste
ri.110,Blastopsylla.occidentalis.137,Blastopsylla.occidentalis.142,C
alophyia.schini.117a,Calophyia.schini.117b,Casuarinicola.australis.1
50b,Casuarinicola.australis.SA3A,Casuarinicola.sp.125,Casuarinicola.
```

sp.147,Casuarinicola.sp.SA6A,Casuarinicola.sp.SA8A,Ctenarytaina.clavata.34a,Ctenarytaina.clavata.44,Ctenarytaina.clavata.152,Ctenarytaina.eucalypti.2,Acizzia.jucunda.19A,Acizzia.albizziae.19B,Acizzia.jucunda.19C,Ctenarytaina.eucalypti.39A,Ctenarytaina.eucalypti.39B,Ctenarytaina.eucalypti.39C,Ctenarytaina.eucalypti.42A,Ctenarytaina.eucalypti.42B,Ctenarytaina.eucalypti.42C,Ctenarytaina.eucalypti.43,Ctenarytaina.eucalypti.78,Ctenarytaina.eucalypti.82,Ctenarytaina.eucalypti.89,Ctenarytaina.eucalypti.99,Ctenarytaina.eucalypti.105,Ctenarytaina.eucalypti.115,Ctenarytaina.eucalypti.165,Ctenarytaina.eucalypti.SA2A,Ctenarytaina.eucalypti.SA2B,Ctenarytaina.eucalypti.SA2C,Ctenarytaina.eucalypti.SI10,Ctenarytaina.eucalypti.SI15,Ctenarytaina.eucalypti.VIC1A,Ctenarytaina.eucalypti.VIC1B,Ctenarytaina.eucalypti.VIC1C,Ctenarytaina.fuchsia.56,Ctenarytaina.fuchsia.194,Ctenarytaina.fuchsia.220,Ctenarytaina.fuchsia.221,Ctenarytaina.fuchsia.224,Ctenarytaina.fuchsia.229,Ctenarytaina.fuchsia.237,Ctenarytaina.fuchsia.238,Ctenarytaina.fuchsia.geraldineA,Ctenarytaina.fuchsia.geraldineB,Ctenarytaina.fuchsia.SI7,Ctenarytaina.fuchsia.SI13A,Ctenarytaina.fuchsia.SI13B,Ctenarytaina.fuchsia.SI16,Ctenarytaina.fuchsia.SI22,Ctenarytaina.fuchsia.SignOfBellbird,Ctenarytaina.fuchsia.TeOkaA,Ctenarytaina.fuchsia.TeOkaB,Ctenarytaina.fuchsiaB.9A,Ctenarytaina.fuchsiaB.9B,Ctenarytaina.fuchsiaB.9C,Ctenarytaina.longicauda.124,Ctenarytaina.longicauda.131a,Ctenarytaina.longicauda.131b,Ctenarytaina.pollicaris.24a,Ctenarytaina.pollicaris.24A,Ctenarytaina.pollicaris.24b,Ctenarytaina.pollicaris.24B,Ctenarytaina.pollicaris.24c,Ctenarytaina.pollicaris.24C,Ctenarytaina.pollicaris.31a,Ctenarytaina.pollicaris.31A,Ctenarytaina.pollicaris.31b,Ctenarytaina.pollicaris.31B,Ctenarytaina.pollicaris.175VOUCHA,Ctenarytaina.SHORT.23a,Ctenarytaina.SHORT.23A,Ctenarytaina.SHORT.23b,Ctenarytaina.SHORT.23B,Ctenarytaina.SHORT.23c,Ctenarytaina.SHORT.23C,Ctenarytaina.SHORT.33a,Ctenarytaina.SHORT.33A,Ctenarytaina.SHORT.33b,Ctenarytaina.SHORT.33B,Ctenarytaina.SHORT.41,Ctenarytaina.SHORT.164,Ctenarytaina.SHORT.172,Ctenarytaina.SHORT.175,Ctenarytaina.sp14.14,Ctenarytaina.spatulata.8b,Ctenarytaina.spatulata.28,Ctenarytaina.spatulata.52,Ctenarytaina.spatulata.79,Ctenarytaina.spatulata.101,Ctenarytaina.spatulata.102,Ctenarytaina.spatulata.108,Ctenarytaina.spatulata.109,Ctenarytaina.spatulata.136,Ctenarytaina.spatulata.143,Ctenarytaina.spatulata.SI8,Ctenarytaina.sp.fuchsia.9d,Ctenarytaina.sp.fuchsia.9e,Ctenarytaina.sp.fuchsia.9f,Ctenarytaina.sp.fuchsia.9g,Ctenarytaina.spSI9.SI9,Ctenarytaina.spSI9.SI17,Ctenarytaina.thysanura.81,Ctenarytaina.unknown.127a,Eucalyptolima.maideni.SA5A,Glycaspis.granulata.135a,Mock.sequence91,Mock.sequence.A,Mock.sequence.B,Mock.sequence.C,Mycopsylla.fici.122,Mycopsylla.fici.134a,Mycopsylla.fici.134b,Psylla.apicalisBIG.187,Psylla.apicalisBIG.200big,Psylla.apicalisBIG.201big,Psylla.apicalisBIG.206,Psylla.apicalisSMALL.55,Psylla.apicalisSMALL.200small,Psylla.apicalisSMALL.201small,Psylla.apicalisSMALL.209,Psylla.carmichaeliaeA.26,Psylla.carmichaeliaeB.212,Psylla.carmichaeliaeB.213,Psylla.carmichaeliaeB.217,Psylla.carmichaeliaeC.222,Psylla.carmichaeliaeC.223,Psyllopsis.fraxini.94,Psyllopsis.fraxini.107,Psyllopsis.fraxini.113,Psyllopsis.fraxinicola.49,Psyllopsis.fraxinicola.93,Psyllopsis.fraxinicola.106,Psyllopsis.fraxinicola.112,Trioza.acuta.5b,Trioza.acuta.45,Trioza.adventicia.118a,Trioza.bifida.29a,Trioza.bifida.29A,Trioza.bifida.29b,Trioza.bifida.29B,Trioza.bifida.174,Trioza.bifida.SI3B,Trioza.bifida.SI12,Trioza.colorata.46a,Trioza.colorata.46A,Trioza.colorata.46B,Trioza.colorata.50a,Trioza.compressa.47,Trioza.decurvata.69,Trioza.discariae.38a,Trioza.discariae.38b,Trioza.discariae.40,Trioza.doryphora.161,Trioza.doryphora.162,Trioza.doryphora.170,Trioza.doryphora.173,Trioza.emarginata.48,Trioza.fa

```
lcata.151,Trioza.falcata.154,Trioza.falcata.157,Trioza.falcata.158,T
rioza.fasciata.64,Trioza.fasciata.132,Trioza.hebicola.63a,Trioza.irr
egularis.4,Trioza.irregularis.166,Trioza.irregularis.168,Trioza.MASS
EY.119a,Trioza.panacis.72,Trioza.PRICESVALLEY.57b,Trioza.sp62.62,Tri
oza.sp68.68,Trioza.sp91.91,Trioza.sp160.160,Trioza.subacuta.120a,Tri
oza.subacuta.159,Trioza.vitreoradiata.53,Trioza.vitreoradiata.59,Tri
oza.vitreoradiata.84,Trioza.vitreoradiata.88,Trioza.vitreoradiata.90
,Trioza.vitreoradiata.104,Trioza.vitreoradiata.133,Trioza.vitreoradi
ata.148,Trioza.vitreoradiata.169 -o slout_not_multiplexed_q20B/ -m
/home/qiime/Desktop/Shared_Folder/MappingFile_mock.txt -q 20 --
barcode_type 'not-barcoded'
```

**Identify chimeric sequences**

```
identify_chimeric_seqs.py -i seqs.fna --
chimera_detection_method=usearch61 --output_fp=usearch_output --
suppress_usearch61_ref
```

**Filter out chimeric sequences**

```
filter_fasta.py --input_fasta_fp=seqs.fna --
output_fasta_fp=seqs_chimeras_filtered.fna --
seq_id_fp=usearch_output/non_chimeras.txt
```

**De novo picking OTUs**

```
pick_de_novo_otus.py --input_fp=seqs_chimeras_filtered.fna --
output_dir=de_novo_OTU –force
```

## Assign taxonomy

```
assign_taxonomy.py -i  otus_trunk_fun.fa -m rdp -o 12-rdp_assigned_taxonomy/
```

**Summarize samples**

```
biom summarize-table -i de_novo_OTU/otu_table.biom -o
de_novo_OTU/otu_table_summary.txt
```

**Summarize taxa**

```
summarize_taxa_through_plots.py -o taxa_summary -i
de_novo_OTU/otu_table.biom
```

## E.2 VSEARCH

The VSEARCH pipeline was run on a Linux computer with 16 gb of RAM. All the following scripts were run on the software R.
Requirements to run the pipeline are the installation of the software Vsearch, Usearch, BLAST and cutadapt.

### Load Libraries

```
source("~/GoogleDrive/R_DNA_tools/FASTA_tools.txt")
library(ShortRead)
library("parallel")
library("foreach")
library("doParallel")
library(ShortRead)
library(vegan)
```

### Load Libraries

```
for file in *; do [ -f "$file" ] && ( mv "$file" "$(echo $file | sed
-e 's/_001//g')" ); done
cd ~/BigData/Martoni_Psyllid_2017/samples
for file in *; do [ -f "$file" ] && ( mv "$file" "$(echo $file | sed
-e 's/_/-/g')" ); done
for file in *; do [ -f "$file" ] && ( mv "$file" "$(echo $file | sed
-e 's/-L/_L/g')" ); done
for file in *; do [ -f "$file" ] && ( mv "$file" "$(echo $file | sed
-e 's/.S/-S/g')" ); done
for file in *; do [ -f "$file" ] && ( mv "$file" "$(echo $file | sed
-e 's/1-R/1_R/g')" ); done
```

### Run Vparse

```
runVparse("/Volumes/Big_Data/Martoni_Psyllid_2017/samples/",
"CCTACGGGNGGCWGCAG","GACTACHVGGGTATCTAATCC", minseqlength = 150)
dir <- "/Volumes/Big_Data/Martoni_Psyllid_2017/samples/"
joinedReads <- readFastq(paste(dir, "merged_reads.fq", sep=""))
uniquesfa <- readFAST(paste(dir, "uniques.fa", sep=""))
OtusFasta <- readFAST(paste(dir, "otus.fa", sep=""))
database <- "~/BLAST/db/current_GREENGENES_gg16S_unaligned.fasta"

psyllidBacteriaOtus <- runBlastOnOtus(dir, OtusFasta, database,
cores=NULL)
uniquesMatches <- match(as.character(sread(joinedReads)),
uniquesfa$sequences)
seqData <- data.frame(ID = as.character(id(joinedReads)), uniqueSeq
= uniquesfa$descs[uniquesMatches])
OtuMatching <- read.table(paste(dir, "sequence_match_to_Otus.txt",
sep=""))
Bacteria <-
readFAST("~/GoogleDrive/NGBA_GoogleDrive/Reference_libraries/current
_GREENGENES_gg16S_unaligned.fasta")
```

**Update bacterial data**

```
data.frame(OTU = sapply(bacteriaDescList, function(x) x[1]), GB =
sapply(bacteriaDescList, function(x) x[2]))
bacteriaDescList <- strsplit(Bacteria$descs, " ")
remove(list="Bacteria")
bacteriaData <- cbind(OTU = sapply(bacteriaDescList, function(x)
x[1]),
              genBank = sapply(bacteriaDescList, function(x) x[2]),
              kingdom = sapply(bacteriaDescList, function(x)
gsub("k__", "", x[grep("k__", x)])),
              phylum = sapply(bacteriaDescList, function(x)
gsub("p__", "", x[grep("p__", x)])),
              class = sapply(bacteriaDescList, function(x)
gsub("c__", "", x[grep("c__", x)])),
              order = sapply(bacteriaDescList, function(x)
gsub("o__", "", x[grep("o__", x)])),
              family = sapply(bacteriaDescList, function(x)
gsub("f__", "", x[grep("f__", x)])),
              genus = sapply(bacteriaDescList, function(x)
gsub("g__", "", x[grep("g__", x)])),
              species = sapply(bacteriaDescList, function(x)
gsub("s__", "", x[grep("s__", x)])))
bacteriaData <- apply(bacteriaData, 2, function(x) gsub(";", "", x))
bacteriaData <- apply(bacteriaData, 2, function(x)
gsub("character(0)", NA, x))
bacteriaData <- as.data.frame(bacteriaData)
psyllidBacteriaOtus$nearestKingdom <-
bacteriaData$kingdom[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$nearestPhylum <-
bacteriaData$phylum[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$nearestClass <-
bacteriaData$class[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$nearestOrder <-
bacteriaData$order[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$nearestFamily <-
bacteriaData$family[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$nearestGenus <-
bacteriaData$genus[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$nearestSpecies <-
bacteriaData$species[match(psyllidBacteriaOtus$otuMatch,
bacteriaData$OTU)]
psyllidBacteriaOtus$sequence <-
OtusFasta$sequences[match(psyllidBacteriaOtus$otu, OtusFasta$desc)]
seqData$otu <- OtuMatching$V2[match(seqData$ID, OtuMatching$V1)]
seqData$cleanID <- sub("\\.[0-9]+","",seqData$ID)
bacteriaSeqData  <- seqData
bacteriaResM <-
table(bacteriaSeqData$cleanID[!is.na(bacteriaSeqData$otu)],
bacteriaSeqData$otu[!is.na(bacteriaSeqData$otu)])
```

```
save(file=paste("~/GoogleDrive/Martoni_Feb_2017_Analysis/Psyllid_NGS
_", Sys.Date(), "_small", sep=""), list=c("psyllidBacteriaOtus",
"bacteriaSeqData", "bacteriaResM"))
save(file=paste("Psyllid_NGS_", Sys.Date(), "_messy", sep=""),
list=ls())
```

## E.3 QIIME2

This workflow uses the command-line interface of QIIME2 and it was run from a jupyter notebook. The pipeline was written by Dan Jones (Plant and Food Research, Auckland) and modified to adapt the present dataset.

### Preliminary operations

**Set user, project and Input:**

```
USER=cflfxm
PROJECT=jupyter4
INPUT=/workspace/cflfxm/Martoni_Psyllids/samples
```

**Set directories**

```
WORKING=/workspace/$USER/$PROJECT
RAW=$WORKING/000.raw
FASTQC=$WORKING/010.fastqc
MULTIQC=$WORKING/020.multiqc
IMPORT=$WORKING/030.import
FILTER=$WORKING/040.filter
OTU=$WORKING/050.otu
PHYLOGENY=$WORKING/060.phylogeny
DIVERSITY=$WORKING/070.diversity
TAXONOMY=$WORKING/080.taxonomy
DIFF=$WORKING/090.differential_abundance
TMPDIR=$WORKING/tmp; \
LOGS=$WORKING/log
mkdir -p $LOGS;
mkdir -p $TMPDIR;
export TMPDIR=$TMPDIR;
mkdir -p $WORKING
mkdir -p $RAW
```

```
mkdir -p $FASTQC
mkdir -p $MULTIQC
mkdir -p $IMPORT
mkdir -p $FILTER
mkdir -p $OTU
mkdir -p $PHYLOGENY
mkdir -p $DIVERSITY
mkdir -p $TAXONOMY
mkdir -p $DIFF
```

### Prepare raw data

The data has been demultiplexed and the raw data will be used from the "RAW" directory after ensuring the files are read/writeable.

```
ln -s $INPUT/*.fastq.gz $RAW;
ls -s $RAW
```

### Quality check of raw data

FastQC and MultiQC were run on the raw data to assess the quality. Assessments of quality are important for downstream steps where trimming parameters are decided.

```
module load FastQC;
?
fastqc \
$RAW/*.fastq.gz \
-o $FASTQC;
?
module unload FastQC;
```

Run MultiQC on the outputs of FastQC, so that a combined report can be viewed (rather than an individual FastQC report for each file).

```
module unload qiime2;
module load MultiQC;
?
multiqc \
$FASTQC \
-o $MULTIQC;
?
```

```
module unload MultiQC;
```

### Prepare sample metadata file and fastq manifest file

Two text files are required for further analysis:

**A sample metadata file** that includes (at a minimum) the name and a text description of the sample. The sample metadata file also includes barcodes and linker sequences, but if the files have already been demultiplexed, the barcode and linker sequence columns can be left blank. The sample metadata file also includes any metadata (any data that can be associated with a sample).

**A FASTQ manifest file** that details the full file path of all the FASTQ files This file must contain full paths to the files, and a description of whether they are forward or reverse reads (since this data is paired-end).

**Prepare the fastq manifest file**

```
# Remove existing fastq manifest files to avoid confusion
?
rm -f $WORKING/fastq_manifest.csv
# Write the header line
echo "sample-id,absolute-filepath,direction" > $WORKING/fastq_manifest.csv
# Define a list of the forward and reverse reads
FORWARDREADS=$(ls $RAW|grep R1|tr "\n" " ");
REVERSEREADS=$(ls $RAW|grep R2|tr "\n" " ")
# Use the defined list to grab a sample name (we use the start of the FASTQ filename before the first underscore),
# the location and the direction, and append this to the existing manifest file
for READ in $FORWARDREADS
do
SAMPLENUM=$(echo $READ| awk 'BEGIN { FS = "_" } ; { print $1"_"$2 }')
echo $SAMPLENUM,$RAW/$READ,forward >> $WORKING/fastq_manifest.csv
done

for READ in $REVERSEREADS
do
SAMPLENUM=$(echo $READ| awk 'BEGIN { FS = "_" } ; { print $1"_"$2 }')
```

```
echo $SAMPLENUM,$RAW/$READ,reverse >> $WORKING/fastq_manifest.csv
done

# View the FASTQ manifest file to make sure it looks correct
cat $WORKING/fastq_manifest.csv
```

The FASTQ manifest file is a .csv (comma separated value) file with three columns (the sample Id, the absolute filepath, and the direction of the read (forward or reverse). All "forward" files must be matched by a "reverse" file.

**Prepare a sample metadata file**

```
# Remove existing fastq manifest files to avoid confusion
rm -f $WORKING/sample_metadata.tsv
# Write the header line
echo -e "#SampleID\tBarcodeSequence\tLinkerPrimerSequence\tMethods\tDescription" >>
$WORKING/sample_metadata.tsv
for READ in $FORWARDREADS
do
SAMPLENUM=$(echo $READ| awk 'BEGIN { FS = "_" } ; { print $1"_"$2 }')
echo -e "$SAMPLENUM\t\t\t$SAMPLENUM" >> $WORKING/sample_metadata.tsv
done
# Check the file
cat $WORKING/sample_metadata.tsv
```

**Import your FASTQ files (with appropriate metadata) into a QIIME2 .qza file**

```
echo "backend: Agg" > ~/.config/matplotlib/matplotlibrc
module unload qiime2;
module load qiime2
TMPDIR=$WORKING/tmp;
export TMPDIR=$TMPDIR;
module load qiime2;
qiime tools import \
 --type SampleData[PairedEndSequencesWithQuality] \
 --input-path $WORKING/fastq_manifest.csv \
 --output-path $IMPORT/Paired_end_demux.qza \
```

```
 --source-format PairedEndFastqManifestPhred33;
module unload qiime2
# Run demux summarise to produce viewing file
module load qiime2;
qiime demux summarize \
 --i-data $IMPORT/Paired_end_demux.qza \
 --o-visualization $IMPORT/Paired_end_demux.qzv;
module unload qiime2
```

**Filtering and Chimera removal with DADA2**

Dada2 denoise-paired was used to remove artefacts in paired-end Illumina data. DADA2 is a pipeline for detecting and correcting (where possible) Illumina amplicon sequence data. As implemented in the q2-dada2 plugin, this quality control process will additionally filter any phiX reads (commonly present in marker gene Illumina sequence data) that are identified in the sequencing data, and will filter chimeric sequences.

Note that --p-trim-left-f \ --p-trim-left-r was set to 7 (which will trim off the first 7 bases) and --p-trunc-len-f \ --p-trunc-len-r to 245 (which will truncate the ~250bp reads to 245).

This is the longest step in the process, taking ~60 minutes (approx). Here the --verbose switch was set to show more detailed output on what's happening.

```
COMMAND="module load qiime2; \
qiime dada2 denoise-paired \
 --i-demultiplexed-seqs $IMPORT/Paired_end_demux.qza \
 --p-trim-left-f 7 \
 --p-trim-left-r 7 \
 --p-trunc-len-f 245 \
 --p-trunc-len-r 245 \
 --verbose \
 --o-representative-sequences $FILTER/rep-seqs-dada2.qza \
 --o-table $FILTER/table-dada2.qza; \
module unload qiime2;"
?
bsub -J Filter_Chimera -o $LOGS/%J_Filter_Chimera.out -e $LOGS/%J_Filter_Chimera.err
"$COMMAND"
```

```
bpeek -f 572142
```

### Examine features and sequences

DADA2 produces tables of sequences and "features" (which are groups of similar sequences that may be from the same species or taxonomic unit). A feature-table summarise and feature-table tabulate-seqs was used to produce .qzv files that could then be used to explore the sequences and features.

After the quality filtering step completes, the resulting data can be explored. This can be done using the following two commands, which will create visual summaries of the data. The feature-table summarize command provides information on how many sequences are associated with each sample and with each feature, histograms of those distributions, and some related summary statistics. The feature-table tabulate-seqs command will provide a mapping of feature IDs to sequences, and provide links to easily BLAST each sequence against the NCBI nt database.

```
module load qiime2;
qiime feature-table tabulate-seqs \
 --i-data $FILTER/rep-seqs-dada2.qza \
 --o-visualization $OTU/rep-seqs.qzv \
 --verbose;
module unload qiime2
```

**Generate a phylogeny of the sequences**

Phylogenetic diversity (PD) measures the diversity of species in a sample, and is expressed as the number of tree units which are found in a sample. For this pipeline was used Faith (1992)'s measure of PD. The phylogeny show "representative" sequences from each feature (or OTU), so all features from all samples are included.

# First, to do a multiple sequence alignment of all representative sequences

```
module load qiime2;
qiime alignment mafft \
 --i-sequences $FILTER/rep-seqs-dada2.qza \
 --o-alignment $PHYLOGENY/aligned-rep-seqs.qza;
module unload qiime2
```

# Next, to mask (or filter) the alignment to remove positions that are highly variable.These positions are generally considered to add noise to a resulting phylogenetic tree.

```
module load qiime2;
qiime alignment mask \
 --i-alignment $PHYLOGENY/aligned-rep-seqs.qza \
 --o-masked-alignment $PHYLOGENY/masked-aligned-rep-seqs.qza;
module unload qiime2

# Now, to generate a tree using FastTree.
module load qiime2;
qiime phylogeny fasttree \
 --i-alignment $PHYLOGENY/masked-aligned-rep-seqs.qza \
 --o-tree $PHYLOGENY/unrooted-tree.qza;
module unload qiime2

# The FastTree program creates an unrooted tree, so a midpoint rooting is applied to place the root of the tree at the midpoint of the longest tip-to-tip distance in the unrooted tree.
module load qiime2;
qiime phylogeny midpoint-root \
 --i-tree $PHYLOGENY/unrooted-tree.qza \
 --o-rooted-tree $PHYLOGENY/rooted-tree.qza;
module unload qiime2

# Export the tree to a "newick" format file in the PHYLOGENY directory
module load qiime2;
qiime tools export \
$PHYLOGENY/rooted-tree.qza --output-dir $PHYLOGENY;
module unload qiime2
```

**Alpha and Beta diversity**

```
# Remove existing files in $DIVERSITY to avoid confusion.
rm -f $DIVERSITY/*.qzv
# Remove existing files in $DIVERSITY to avoid confusion.
rm -f $DIVERSITY/*.qza
# Since this program fails if the output directory already exists, any previous directory is removed.
rmdir $DIVERSITY;
```

```
module load qiime2;
qiime diversity core-metrics \
 --i-phylogeny $PHYLOGENY/rooted-tree.qza \
 --i-table $FILTER/table-dada2.qza \
 --p-sampling-depth 100 \
 --output-dir $DIVERSITY;
module unload qiime2

module load qiime2;
qiime diversity alpha-group-significance \
 --i-alpha-diversity $DIVERSITY/faith_pd_vector.qza \
 --m-metadata-file $WORKING/sample_metadata.tsv \
 --o-visualization $DIVERSITY/faith-pd-group-significance.qzv;
module unload qiime2;

module load qiime2;
qiime diversity alpha-group-significance \
 --i-alpha-diversity $DIVERSITY/evenness_vector.qza \
 --m-metadata-file $WORKING/sample_metadata.tsv \
 --o-visualization $DIVERSITY/evenness-group-significance.qzv;
module unload qiime2;
# Cannot run this without a relevant metadata category.
for METADATACATEGORY in Species Host_Plant Location
do
module load qiime2;
qiime diversity beta-group-significance \
 --i-distance-matrix $DIVERSITY/unweighted_unifrac_distance_matrix.qza \
 --m-metadata-file $WORKING/sample_metadata.tsv \
 --m-metadata-category ${METADATACATEGORY} \
 --o-visualization $DIVERSITY/${METADATACATEGORY}_unweighted-unifrac-significance.qzv;
module unload qiime2;

module load qiime2;
qiime emperor plot \
```

```
 --i-pcoa $DIVERSITY/unweighted_unifrac_pcoa_results.qza \
 --m-metadata-file $WORKING/sample_metadata.tsv \
 --o-visualization $DIVERSITY/unweighted-unifrac-emperor.qzv;
module unload qiime2;

module load qiime2;
qiime emperor plot \
 --i-pcoa $DIVERSITY/bray_curtis_pcoa_results.qza \
 --m-metadata-file $WORKING/sample_metadata.tsv \
 --o-visualization $DIVERSITY/bray-curtis-emperor.qzv;
module unload qiime2;
```

**Taxonomic classification**

```
#Obtain SILVA classifier data.
wget -O "$WORKING/silva-119-99-nb-classifier.qza" "https://data.qiime2.org/2017.7/common/silva-119-99-nb-classifier.qza"
# Classify features according to the supplied taxonomy: SILVA.
for i in 0 0.1 0.3 0.5 0.7
do
COMMAND="module load qiime2; \
export TMPDIR=$TMPDIR; \
qiime feature-classifier classify-sklearn \
 --i-classifier $WORKING/silva-119-99-nb-classifier.qza \
 --i-reads $FILTER/rep-seqs-dada2.qza \
 --p-confidence $i \
 --verbose \
 --o-classification $TAXONOMY/taxonomy_silva${i}.qza; \
qiime metadata tabulate \
 --m-input-file $TAXONOMY/taxonomy_silva${i}.qza \
 --o-visualization $TAXONOMY/taxonomy_silva${i}.qzv; \
qiime taxa barplot \
 --i-table $FILTER/table-dada2.qza \
 --i-taxonomy $TAXONOMY/taxonomy_silva${i}.qza \
 --m-metadata-file $WORKING/sample_metadata.tsv \
 --o-visualization $TAXONOMY/taxa-bar-plots_silva${i}.qzv; \
```

```
module unload qiime2"

IN=$WORKING/002.dada2;
OUT=$WORKING/008.differential_abundance_testing;
LOG=$WORKING/008.differential_abundance_testing/log;
mkdir -p $OUT
mkdir -p $LOG

module load qiime2;
qiime gneiss add-pseudocount \
  --i-table $FILTER/table-dada2.qza \
  --p-pseudocount 1 \
  --o-composition-table $DIFF/composition.qza;
module unload qiime2

module load qiime2;
qiime gneiss correlation-clustering \
  --i-table $DIFF/composition.qza \
  --o-clustering $DIFF/hierarchy.qza;
module unload qiime2

module load qiime2;
qiime gneiss ilr-transform \
  --i-table $DIFF/composition.qza \
  --i-tree $DIFF/hierarchy.qza \
  --o-balances $DIFF/balances.qza;
module unload qiime2

for METADATACATEGORY in Species Host_Plant Location
do
module load qiime2;
qiime gneiss ols-regression \
  --p-formula "${METADATACATEGORY}" \
  --i-table $DIFF/balances.qza \
```

```
  --i-tree $DIFF/hierarchy.qza \
  --m-metadata-file $WORKING/sample_metadata.tsv \
  --o-visualization $DIFF/${METADATACATEGORY}_regression_summary.qzv;
module unload qiime2;
done

for METADATACATEGORY in Species Host_Plant Location
do
module load qiime2;
qiime gneiss dendrogram-heatmap \
  --i-table $DIFF/composition.qza \
  --i-tree $DIFF/hierarchy.qza \
  --m-metadata-file $WORKING/sample_metadata.tsv \
  --m-metadata-category ${METADATACATEGORY}\
  --p-color-map seismic \
  --o-visualization $DIFF/${METADATACATEGORY}_heatmap.qzv;
module unload qiime2;
done

for METADATACATEGORY in Species Host_Plant Location
do
module load qiime2;
qiime gneiss balance-taxonomy \
  --i-balances $DIFF/balances.qza \
  --i-tree $DIFF/hierarchy.qza \
  --i-taxonomy $TAXONOMY/taxonomy.qza \
  --p-taxa-level 2 \
  --p-balance-name 'y0' \
  --m-metadata-file $WORKING/sample_metadata.tsv \
  --m-metadata-category ${METADATACATEGORY} \
  --o-visualization $DIFF/${METADATACATEGORY}_taxa_summary.qzv;
module unload qiime2;
done
```

## E.4 DATA ANALYSIS

In this section are reported the ecological analyses performed on the result obtained from the Vsearch pipeline (D.2).

### Load libraries

```
library(sp)
library(vegan)
convert<-function(coord){
```

**Bespoke function to convert a degree minute second coordinate into a fully numeric one, assuming D is used for degree sign**

```
t1 <- strsplit(coord, "D")
d <- as.numeric(unlist(lapply(t1, "[", 1)))
min <- as.numeric(unlist(sapply(strsplit(unlist(lapply(t1, "[",
2)),"'"), "[", 1)))
sec <- as.numeric(substr(unlist(sapply(strsplit(unlist(lapply(t1,
"[", 2)),"'"), "[", 2)),1,5))
return(d+min/60+sec/(60*60))
}
```

**Get the spatial distance between samples:**

```
envData <- read.csv("~/Google
Drive/Martoni_Feb_2017_Analysis/Psyllids_data.csv",stringsAsFactors=
FALSE)
```

**Convert the locations into S and E**

```
envData$S <- convert(unlist(lapply(strsplit(envData$Location," "),
"[", 1)))
envData$E <-  convert(unlist(lapply(strsplit(envData$Location," "),
"[", 2)))
```

**Create a spatial distance between all samples**

```
spatDist <- spDists(cbind(envData$E,envData$S)[!is.na(envData$E),],
longlat=TRUE)
rownames(spatDist) <- colnames(spatDist) <-
envData$ID[!is.na(envData$E)]
```

**Get phylogenetic distance:**

```
phyloDist <- read.csv("~/Google
Drive/Martoni_Feb_2017_Analysis/Matrix1_phyloDist_Martoni.csv",
header=FALSE, stringsAsFactors=FALSE)
phyloSnum <- unlist(sapply(strsplit(phyloDist[,1], "_"),function(x)
paste(x[1],x[2],sep="_")))
phyloDistM <- as.matrix(phyloDist[,-1],
dimnames=list(phyloSnum,phyloSnum))
colnames(phyloDistM) <- rownames(phyloDistM) <- phyloSnum
```

**Given two distance matrices, need to pair rows based on matching names.**

```
intersectNames <- intersect(colnames(spatDist),colnames(phyloDistM))
```

```
phyloDistD <- as.dist(phyloDistM[match(intersectNames,
rownames(phyloDistM)),match(intersectNames, colnames(phyloDistM))])
spatDistD <- as.dist(spatDist[match(intersectNames,
rownames(spatDist)),match(intersectNames, colnames(spatDist))])
plot(phyloDistD~c(spatDistD+1),log="x")
```

**Add the molecular data from a previously saved file**

```
load("~/Google Drive/Martoni_Feb_2017_Analysis/Psyllid_NGS_2017-03-
08_small")
psyllidBacteriaOtus$length <- as.numeric(psyllidBacteriaOtus$length
)
psyllidBacteriaOtus$identity <-
as.numeric(psyllidBacteriaOtus$identity )
rownames(communityM) <- gsub("-","_",rownames(communityM))
communityM <- communityM[rownames(communityM) %in% phyloSnum,]
plot(rowSums(communityM),log="y",main="Sequencing depth
variability")
mds <- metaMDS(communityM, trymax=100)
```

#fails to converge with 100 tries.

**Try with 3 axes:**

```
mds <- metaMDS(communityM, trymax=100, k=3)
```

#still fails to converge.

**Try with only samples > 500 sequence and only OTUs found in > 1 psyllid**

```
mds <-
metaMDS(communityM[rowSums(communityM)>500,colSums(communityM>0)>1],
trymax=100)
plot(mds$points, cex=0)
text(mds$points,rownames(communityM[rowSums(communityM)>500,]),cex=0
.5)
quartz(height=6,width=11) ##Use X11 on PC?
par(mfrow=c(1,2))
```

**Rank abundance graph:**

```
plot(sort(colSums(communityM),decreasing=TRUE),log="y")
```

**Frequency**

```
plot(sort(colSums(communityM>0),decreasing=TRUE),log="y")
```

**what are most frequent:**

```
head(sort(colSums(communityM>0),decreasing=TRUE),20)
```

**get names: (This just takes the line above and uses match to find it in the OTU data)**

```
psyllidBacteriaOtus[match(names(head(sort(colSums(communityM>0),decr
easing=TRUE),20)), psyllidBacteriaOtus$otu),]
communityD <-
as.matrix(vegdist(communityM[rowSums(communityM)>500,colSums(communi
tyM>0)>1]))
intersectNames <-
intersect(colnames(communityD),colnames(phyloDistM))
phyloDistD <- as.dist(phyloDistM[match(intersectNames,
rownames(phyloDistM)),match(intersectNames, colnames(phyloDistM))])
communityDD <- as.dist(communityD[match(intersectNames,
rownames(communityD)),match(intersectNames, colnames(communityD))])
plot(communityDD~phyloDistD)
mantel(communityDD, phyloDistD)
intersectNames <- intersect(colnames(communityD),colnames(spatDist))
spatDistD <- as.dist(spatDist[match(intersectNames,
rownames(spatDist)),match(intersectNames, colnames(spatDist))])
```

```
communityDD <- as.dist(communityD[match(intersectNames,
rownames(communityD)),match(intersectNames, colnames(communityD))])
plot(communityDD~c(spatDistD+1), log="x")
mantel(communityDD, spatDistD)
```

### Three way intersect and mantel tests

```
intersectNames <-
intersect(intersect(colnames(communityD),colnames(spatDist)),colname
s(phyloDistM))
spatDistD <- as.dist(spatDist[match(intersectNames,
rownames(spatDist)),match(intersectNames, colnames(spatDist))])
communityDD <- as.dist(communityD[match(intersectNames,
rownames(communityD)),match(intersectNames, colnames(communityD))])
phyloDistD <- as.dist(phyloDistM[match(intersectNames,
rownames(phyloDistM)),match(intersectNames, colnames(phyloDistM))])
mantel.partial(communityDD, spatDistD, phyloDistD)
mantel.partial(communityDD, log(spatDistD+1), phyloDistD) ##Log
distance not better
mantel.partial(communityDD, phyloDistD, spatDistD)
```

### Adonis approach

**Only look at species with > 2 records**

```
speciesFreq <-
table(envData$Species[match(rownames(communityD),envData$ID)])
subCommunity <-
communityM[rowSums(communityM)>500,colSums(communityM>0)>1]
freqSppSamples <- envData$ID[envData$Species %in%
names(speciesFreq)[speciesFreq>2]]
subCommunity <- subCommunity[rownames(subCommunity) %in%
freqSppSamples,]
speciesSubCom <- envData$Species[match(rownames(subCommunity),
envData$ID)]
adonis(subCommunity~as.factor(speciesSubCom))
```

**Clustering:**

```
plot(hclust(
vegdist(communityM[rowSums(communityM)>500,colSums(communityM>0)>0])
))
```

**Add plant phylogenetic distance**

```
test <- read.table("/Volumes/NO NAME/00PLANTS/NGSplantcorrect.txt",
header=TRUE, sep="\t")
test <- test[,-1]
rownames(test) <- colnames(test)
speciesList <- unlist(sapply(strsplit(rownames(test), "\\."), "[",
1))
for(i in ncol(test))
    {
    test[speciesList == speciesList[i],i] <- 0
```

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