Assessing New Zealand's spider (Araneae) fauna, using DNA barcoding

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Abstract

The ability to accurately monitor biological diversity is the foundation to most ecological research. However, the morphological identification of spiders, as with many arthropod taxa, is often complicated by phenotypic plasticity and sexual dimorphism. New Zealand's spider fauna is highly endemic (97% of known species), consisting of an estimated 2,000 species. Given such high diversity, it is critical to seek cost-effective measures of assessing species diversity and distributions, and for the identification of cryptic unidentified taxa.

The aim of my research was to determine whether the described spider fauna can be effectively delineated from unidentified taxa using DNA barcoding. Overall, I establish a molecular inventory for 100 described species and 71 additional unidentified species of spiders found in New Zealand. Using barcoding I determined there to be 59 native described species and classify 66 of the unidentified species as native, leaving 45 species which were recognised as having international distributions by taxonomic descriptions and/or from comparative DNA sequences from the Barcode of Life Datasystem (www.boldsystems.org). The repository of species presented here represent native species pertaining to 26 families and cosmopolitan species from 17 families of which species from the Araneidae, Linyphiidae and Theridiidae families are the most diversly represented in this study. This foundational inventory was used to assess the presence of spider species around the marginal habitats of 5 Waikato study lakes (Puketi, Rotoiti, Kohahuake, Waiwhakareke and Koraha), in relation to community assemblage variation across lakes, habitat (shoreline vs. pasture) and sampling method.

The combined morphological and molecular approach to identification used here has demonstrated community composition assessments of spiders are viable. Furthermore, the study of these community assemblages revealed a greater diversity of habitat specialists among shoreline samples from lakes Kohahuake, Koraha and Waiwhakareke where greater vegetative heterogeneity along the shoreline provides a

greater range of niches for spiders to occupy. This compiled species inventory indicates that New Zealand's diverse Araneae fauna has been infiltrated by species with international distributions. Further, not all species are easily recognisable due to dissimilarities in their morphological appearance, or because they remain to be genetically identified, and therefore the extent of non-native species infiltration into New Zealand ecosystems is unclear. This lack of knowledge highlights an important area for future biosurveillance work. Lake ecosystems were selected for this study because a variety of spider species prey upon adult aquatic invertebrates as they emerge from the aquatic realm and into the terrestrial.

Methods which provide complementary data for ranking lake ecosystems is a priority for biodiversity management in the Waikato region due to habitat fragmentation primarily associated with deforestation and subsequent conversion to pastoral habitat. Land use practices affect the distribution and dispersal of many native species and connected expanses of pasture provide opportunities for exotic species to infiltrate established food-webs associated with ecosystems of significant natural character. In conclusion, this molecular inventory provides a key foundation for a COI barcode library for New Zealand's most commonly-encountered spiders and validates the ability of this identification method to discern between male, female and juvenile specimen from described and unrecognised species with native or international distributions.

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Chapter 1

General introduction.

1.1 Spiders

Spiders (order Araneae) are prominent predators of terrestrial biological communities from the coast to the snowlines of mountains. They form the most ubiquitous group of predaceous terrestrial organisms in the animal kingdom (Riechert & Lockley, 1984), and with more than 45,000 species are recognised worldwide as the most abundant predator group, providing model systems for studies of food webs, sociality, mating, and sexual dimorphism (Riechert & Lockley, 1984; Wise, 1994). The structure of spider assemblages is influenced by topography and plant architecture (Gomez, Lohmiller & Joern, 2016; Pearson, 2009; Smith, Emien & Pearson, 2016), and the broad variety of predation techniques supported within habitat assemblages are primarily determined by prey diversity and abundance (Forster & Forster, 1999; Reay & Norton, 2002). Spiders also play key roles in controlling herbivorous invertebrate damage to plant seeds and seedlings, and in diverse assemblages spiders help maintain the integrity of vegetative communities by eliminating intruders which typically arrive in small numbers relative to the number of resident predator species (Burger et al. 2001). This is one way in which intact native spider communities aid their habitats to resist invasive/pest species establishment. However, when stable food webs are disrupted by anthropogenic activities such as land conversion, burn-off, stock grazing, water pollution, and introduced mammalian predators, the biotic assemblages they support can be compromised (Jonsson et al. 2010; Gibson, Hambler & Brown, 1992; Ryndock et al. 2012).

Like many other groups of invertebrates, spiders have received little attention from taxonomists in recent years and the threat classification of New Zealand's spiders was last assessed in 2012 (Sirvid et al. 2012). At that time, 538 of the 1,134 presently described species in New Zealand were recognised as "data deficient/poor", three species as "nationally critical" and one as "nationally endangered". In New Zealand the described species represent 236 genera and 57 recognised families, and of these approximately 97% are considered native (Paquin, Vink & Duperre, 2010; Forster & Forster 1999; Sirvid et al. 2012). Approximately 36 species of spider have been introduced by anthropogenic vectors, and a further 30

species are suspected to be natural introductions from elsewhere in Australasia (Paquin, Vink & Duperre, 2010).

Despite recognition as a highly diverse group, the diversity of New Zealand's spiders remains understudied and many taxa are in need of taxonomic attention. For example, members of New Zealand's Tetragnathidae and Thomisidae families are predicted to have greater diversity than presently described (Paquin, Vink & Duperre, 2010). This lack of knowledge is primarily due to a lack of taxonomists who appreciate the appearance, behaviours and questionably venomous nature of this diverse taxa. Internationally, work on this arthropod order is regularly constrained by challenges to morphological identification (Paquin, Vink & Duperre, 2010; Pugh, 2004; Cardoso et al. 2003a; Barrett & Hebert, 2005).

1.2 Biosecurity and biocontrol

Biocontrol species are ones that track populations of the target prey, controlling them at low densities (Basnet & Mukhopadhyay, 2014; Huffaker, Messenger & DeBach. 1971; Kumar & Mukhopadhyay, 2014; Liu et al. 2015), and biosecurity is a management practice concerned with the infiltration of species into existing faunal communities. Historically, intentional introductions of international predator and parasitoid invertebrates have been explored to combat prey species considered pests. Since spiders feed almost exclusively on insects as generalist predators, they have the potential to reduce insect prey densities. However, information on multispecies predator-prey dynamics is generally lacking in the literature primarily because the modelling of community systems in this way is very complex due to the challenges associated with providing identifications for the different stages in the lifecycle of undescribed and described species.

An example of the potential value of spiders as biocontrol agents is provided by the cosmopolitan *Tenuiphantes tenuis* Blackwall 1852 (family Linyphiidae) which is recognised for the role it plays as a biocontrol agent

in agricultural systems, consuming invasive insect pests (Clark, Gerard & Melsop, 2004). This species has a reported distribution across Caucasus, central Asia, Europe, Indonesia, northern Africa, Turkey and is considered introduced to USA, Chile, Argentina and New Zealand (World Spider Catalog, 2017). PCR gut analyses by Vink et al. (2013) revealed that *T. tenuis* is a potentially significant predator of the Argentine stem weevil (*Listronotus bonariensis* Kuschel 1955), a major pest in New Zealand ryegrass pastures (Barker, Pottinger & Addison, 1989; Patrick, 1994). Dense *T. tenuis* populations could potentially remove 3.9 *L. bonariensis* m^{-2} per day (~94 m^{-2} individuals). Peak *L. bonariensis* densities in Canterbury paddocks range from 100 to 400 adults m^{-2} during February (Goldson, Proffitt & Baird, 1998), which is also when *T. tenuis* numbers peak (Vink et al. 2004), hence during this time *T. tenuis* have a measurable effect on *L. bonariensis* populations. Based on their calculations, using minimum spider densities and consumption rates, the New Zealand population of spiders have been estimated to consume ~140,000 tonnes of insects and other arthropods every year (Vink, 2013).

1.3 Population fragmentation

Fringe habitats (ecotones), between ecosystems, support diverse spider and invertebrate prey communities (Krell et al. 2014; Paetzold et al. 2011; Ulrich et al. 2010). In particular, the aquatic/terrestrial boundary provides a stark ecotone which supports diverse spider and invertebrate prey communities (Burdon & Harding, 2008; Krell et al. 2014; Paetzold et al. 2011). In many instances, communities of invertebrates are under intense pressure from competition with introduced species which rapidly disperse from pasture to adjacent ecosystems through ecotone boundaries (Baldissera, Grande & Fontoura, 2003; Patrick 1994). With less than 1% of dense mixed podocarp forest left in the central North Island, it is likely that endemic spider species adapted to native forest habitats have very likely undergone a change in geographic range resulting from a loss of suitable habitat, including change to ecotones, and metapopulation dynamics (Jamieson, Wallis & Briskie, 2006).

Isolated populations of cursorily-dispersing ground-dwelling spiders, such as New Zealand's heaviest spider the endemic *Porrhothele antipodianus* (Walckenaer, 1837), live within fragmented habitats such as forest and wetland fragments, ecological islands and conservation reserves (Noss, 1987; Scott, 1987). These fragmented or isolated communities largely rely on self-sustaining populations, and therefore they and their associated habitats have a high value in terms of conservation and susceptility to infiltration. This is especially so where these same habitats harbour numerous other endemic species and/or unique assemblages (Almany et al. 2009).

In addition, coastal sand-dune habitat degradation and loss are the primary drivers of sub-population extinction of New Zealand's endemic katipo (*Latrodectus katipo* Powell 1871) spider (Costall & Death, 2010; Hann, 2016; Patrick, 2002). Population re-establishment to sand-dune restoration sites is inhibited by competition for space by the false katipo spider (*Steatoda capensis* Hann 1990) which is native to South Africa and competes with *L. katipo* for space within the same habitats. *Latrodectus katipo* is considered iconic, vulnerable to harm, and in serious decline. The second species with this same protection status, the Nelson Cave Spider (*Spelungula cavernicola* Forster 1987), is considered "Range restricted" and is threatened by habitat fragmentation limiting the diversity of their gene pool, however, the populations are considered stable under the New Zealand Threat Classification System (Molloy et al. 2002; Sirvid et al. 2012).

The vulnerability of fragmented populations is exacerbated by edge effects on organisms, including changes in micro-climate, nutrient cycling, dispersal rates, predation and resource competition pressure over time (Baldissera, Grande & Fontoura, 2003; Cobbold & Supp, 2012). Species extinctions are often attributed to non-genetic factors, but genetic factors such as gene flow between sub-populations and inbreeding depression can increase the extinction probability of a population or species (Frankham, 1995).

1.4 Response to habitat restoration

A goal in restoring networks of connected habitat is to maximise protection of biotic diversity and system functionality, so it is logical to use the most diverse biotic elements as indicators of restoration success and when establishing conservation values (Kremen et al. 1993; Midega et al. 2008). In terrestrial ecosystems, most spiders disperse either by air (ballooning) or moving overland (cursorily) (Forster & Forster 1999; Paquin, Vink & Duperre, 2010; Pugh, 2004). Due to the ability of many spiders to disperse by ballooning (aeronauts), they can be highly mobile and rapidly settle in suitable habitats, especially in sites of recent disturbance (Buchholz, 2010; Malumbres-Olarte et al. 2014). For this reason, ballooning species have been recommended as indicators of successional rehabilitation in British wetland ecosystems (Scott et al. 2006) and reclaimed limestone quarries (Wheater, Cullen & Bell, 2000). In support of the utility of spiders for ecological monitoring, Haase & Balkenhol (2015) showed that spider communities provided a long-term indicator of ecological condition of endangered peat and bog ecosystems in Saxony, Germany.

Mounting evidence shows aquatic-terrestrial interfaces are hotspots for spider diversity, suggesting that spider communities may have utility as indicators of the success of riparian vegetation restoration initiatives near freshwater habitats where condition is regularly assessed by monitoring the aquatic arthropod diversity. Riparian terrestrial community assemblages are among the most endangered worldwide because they are under chronic pressure from agricultural land use, infrastructure development, and invasion by pest animals and plants (Myers et al. 2013; Ricciardi & Rasmussen, 1999; Revenga & Kura, 2003).

Riparian vegetation can stabilise banks, contribute coarse particulate organic matter and provide habitats which support diverse invertebrate communities (Burdon & Harding, 2008). Spiders are important predators in riparian areas as their abundance is linked to that of their prey (Krell et al. 2014). Between terrestrial and freshwater boundaries, specialist predatory taxa, such as *Tetragnatha nitens* (Audouin 1826) and *Dolomedes*

aquaticus (Goyen 1888; Williams, 1979), prey upon larval and adult aquatic insects whose abundance can fluctuate seasonally, declining with distance from the water edge (Collier & Smith, 1998; Henschel, Mahsberg & Stumpf, 2001). When riparian vegetation is used to construct dispersal corridors between fragmented freshwater habitats, ground-dwelling species may disperse beyond the bounds of existing populations (Ferretti & Gonzalez, 2014; Pedersen & Loeschcke, 2001; Petillon et al. 2012), and provide an indication of connectivity restoration success.

Generalist aeronaut species are recognised as early successional species and could provide an indication of post-disturbance re-establishment and successional recruitment (Malumbres-Olarte et al. 2014). Research of native cursorial spider taxa as surrogate-indicators of terrestrial and aquatic arthropod emigration to new habitat localities is a previously unexplored opportunity in New Zealand. As spiders are predators of insects and other arthropods, their assemblages require the support of diverse food webs (Gibson, Hambler & Brown, 1992; Henschel, Mahsberg & Stumpf, 2001). Hence, established predator communities may indicate the presence of available prey species. Further, spider communities inhabiting urban or agro-ecosystems could provide a disturbed baseline for examining the transition and distribution of biotic communities along a reference to degraded scale.

1.5 Biodiversity inventory

Inventory and monitoring are two essential and interrelated activities necessary for conservation and planning. They differ in their objectives and hence in the types of indicators useful to each activity. Inventory programmes document the spatial distribution of populations, species, communities and ecosystems. In terms of conservation planning, inventories provide information which can be used to: (1) select and design reserves, (2) strengthen the case for habitat conservation by documenting the distribution of threatened or endangered species, and (3) provide the basis for selecting indicator species or assemblages for ecological monitoring and determining biodiversity restoration success

(Usher, 1986; Noss, 1987; Scott et al. 1987; McKenzie et al. 1989). To aptly represent the biological diversity and ecological complexity present within a region, an inventory of the biotic community should include a number of high-order species with different ecological functions, habitat and niche specialisations, and their known distributions.

Threatened habitats with significant natural character support diverse biotic communities which are increasingly vulnerable to pressures from catchment intensification and competition from invasive plants, invertebrates and other animals. In the North Island of New Zealand, where few large natural areas remain to be protected, an in-depth species inventory of fauna would provide a critical aid in the determination of biodiversity condition and restoration success around natural and novel ecosystems. Spider species that are able to persist in small habitat patches are potential future umbrella species for the protection and management of terrestrial arthropod communities in remnant natural areas (Main, 1987; Murphy, Freas & Weisset, 1990).

Studies conducted in Portuguese natural protected areas assessed indicator taxa of spider community richness richness and their application to conservation (Cardoso et al. 2003a; Cardoso et al. 2003b). The research aimed to gather information in order to prioritise conservation areas based on the complementarity of research site communities. They determined that no single family was a good surrogate of total diversity. However, one group of the two families was determined to be an efficient and reliable indicator for ranking sites according to spider taxa richness, and for prioritising sites for conservation management. The authors placed considerable emphasis on using alternative approaches to predict species richness in order to overcome the enormous amount of time and money required for taxonomists to compile complete inventories. They concluded that there is a need for indicators of diversity because species determination of all specimens based solely on traditional morphological identification methods was not possible.

1.6 Molecular identification of spiders

The heightened interest in restoration of biodiversity presents contemporary ecological researchers with an impetus to assess biological communities and the dynamic food webs required to support indigenous invertebrate assemblages. The challenge of identifying each species by traditional morphological means that is often difficult to identify the complete range of species found within a community. Molecular identification provides one option to remove the complications of confounding morphoplasticity, maturity and sexual dimorphism in the process of identifying described and cryptic species.

Research conducted by Barrett & Hebert (2005) on North American spider taxa demonstrated that nucleotide sequence diversity, in a standard segment of the mitochondrial gene coding for Cytochrome *c* Oxidase I (*COI*), is highly effective in discriminating spider species. Their study compiled a *COI* profile containing 168 spider species, correctly assigning 100% of the analysed specimens to molecular operational taxonomic units (MOTU's) representative of individual species. This study of North American Araneae supports the use of *COI* barcoding as a rapid and accurate identification tool for the assessment of spider diversity (Hebert et al. 2003; Robinson et al. 2009).

Molecular identification enables scientists to approach biodiversity research from a species richness perspective as complete species-level assemblage information is more effective than higher taxa surrogacy of diversity (Cardoso, 2003a; Pederson & Loeschcke, 2001). Such data could help prioritise the allocation of labour effort required throughout the network of conservation sites in New Zealand. As a tool for conservation, molecular phylogenetic analyses enable users and researchers to compare patterns of gene flow and the molecular variability (genetic divergence) exhibited by species across broad spatial scales. Using DNA barcoding, it is now possible to discern previously recognised species' barcodes from a worldwide database (www.boldsystems.org).

As a tool for monitoring restored or degraded habitat and biotic community changes, DNA barcoding is a rapid and relatively cheap process, capable of resolving undescribed species, and discerning species richness and distributions associated with sampling locations across broad geographic ranges. Applied to monitoring, DNA barcoding can be used to discern shifts in community composition and indigenous species dominance through time. In terms of biodiversity restoration, barcoding could be used to direct management effort to habitats and ecosystems where rare and endangered species persist. For instance, *L. katipo*, represented by two morpho-variants, black and red, are commonly confused with the invasive competitor *Steatoda capensis* due to a similarity in their appearance (Patrick, 2002). As an approach to biodiversity restoration, the identification of whole community assemblages to species-level could provide information which benefits the determination of priority habitat sites that, when protected, best represent the taxa present within an area.

There are a variety of potential applications for using DNA barcoding to identify spiders, including: (i) delineation of described and unidentified native and cosmopolitan species inhabiting marginal vegetation; (ii) increased comparability and rigour of sampling methods for biodiversity inventory and monitoring studies; and (iii) further understanding of biogeographic patterns of spider species distribution and New Zealand's endemic fauna.

1.7 Objectives of thesis

There is little in the way of comprehensive research available from New Zealand that assesses whole communities of spider species and their distributions across lake-side ecosystems, with species-level resolution. Such research can be made difficult by an inability to identify male and juvenile specimens to species level, leading to their exclusion from community analysis studies. A better understanding of the relationships within and between biotic communities and ecosystem features is required.

The Waikato region has more than 100 lakes, many of which are considered particularly vulnerable to shifts in water quality. Demand for productive land close to water has meant that most of these lakes are now much smaller and less vegetated than they were in the early 1900s. Once broadly inter-connected by wetlands and lowland forest, lake ecosystems have been reduced to fragmented remnants separated by broad expanses of pasture and agricultural fields. Hence there is a recognised need to conserve and restore functional aspects of freshwater ecosystems, and to this end "best management practices" are being implemented around many of the Waikato region's freshwater lakes (Dean-Speirs et al. 2014; Waikato Regional Policy Statement, 2016; Waikato Regional Council, 2016;).

The following general objectives were developed for this thesis:

1. Establish a molecular reference inventory for identifying native and introduced species from a sub-set of 98 described New Zealand spider taxa using DNA barcoding (Chapter 2).
2. Use DNA barcoding to detect described and unidentified native and cosmopolitan species associated with spider communities inhabiting the shoreline and adjacent pasture habitats of five study lakes (Chapter 3).

DNA barcoding, implemented in this thesis, provided an opportunity to identify males, females and juvenile specimens to levels previously unattainable using solely traditional morphological identification methods. The inclusion of this supporting molecular data provides an additional dimension for taxonomic studies of dispersal and distribution of this largely endemic group of arthropods. Additionally, this research supports a developing awareness of terrestrial/aquatic linkages at regional and national scales and helps resolve taxa interpretations of native and cosmopolitan species which can often be misleading and inconclusive. Three pasture-dominated lake catchments were selected from northern Waikato to compare with a restoration site (Lake Waiwhakareke) and a lake which best

represents reference conditions in the Waikato region (Lake Koraha) to examine whether pasture habitat was dominated by cosmopolitan species, and whether greater riparian heterogeneity provided by native vegetation architecture around lake margins supports greater endemic species richness in the associated spider community.

1.8 Outline of thesis

This thesis comprises four chapters with two main chapters set out in the style of individual manuscripts for submission to scientific journals. As such there is some repetition in parts of this thesis, especially within the introduction sections. This chapter sets out the objectives of the thesis and provides an introduction to the detection and monitoring of the ecological roles and relevance spiders have in terrestrial ecosystems and aquatic-terrestrial interfaces, discussing factors which may affect their community structure. Chapter 2 presents a molecular study of New Zealand's highly endemic and diverse spider fauna from available museum collections and field sampling to augment a DNA barcode library available to future researchers. Chapter 3 details an applied study which investigates the species richness and community composition of spider assemblages inhabiting the shoreline and pasture habitats of contrasting freshwater lakes in the Waikato. As part of that work, I evaluated the effectiveness of two methods for sampling spider communities. The final discussion chapter summarises the main findings from Chapters 2 and 3, and discusses possible future applications of this molecular identification method. Raw data and species tables and presented as appendices. The work presented is part of an on-going collaboration between the Canterbury Museum, the University of Waikato and the Waikato Regional Council.

Chapter 2

Identifying New Zealand's spiders using DNA barcoding.

2.1 Abstract

I analysed a 550-bp region of the COI gene, taken from 98 morphologically identified species (n = 601 COI sequences) sourced from museum collections. A further 173 sequences, pertaining to 40 morpho-species were also analysed to determine the ability of the reference sequences to identify unknown specimens. COI sequences successfully delineated each of the identified species, placing them into highly similar (≥98%) clusters where Barcode Index Numbers (BINs) were assigned as a surrogate for Molecular Operational Taxonomic Units (MOTUs). From the combined inventory there were 35 described species and 4 unidentified species that corresponded to overseas records and with cosmopolitan distributions. The mean intraspecific sequence variation for native species was 0.41% (minimum 0%, maximum 2.18%) compared to 0.28% (minimum 0%, maximum 2.03%) for most cosmopolitan species. However, two cosmopolitan species had maximum divergence thresholds which exceeded these values; *Dysdera crocata* (C. L Koch, 1838) and *Steatoda grossa* (C. L Koch, 1838; 3.14% and 5.12% maximum divergence respectively). Six of the native species are currently only known from adult female specimens. However, DNA barcoding was used to provide positive identifications for each based on juvenile and adult male specimens from the morpho-species collection. This study has commenced a reference library for native and introduced spiders in New Zealand. Ultimately, these data can be used as a means of identifying and monitoring all endemic and introduced species within New Zealand.

2.2 Introduction

There is growing concern about impoverishment at multiple levels of biological organisation in ecosystem food webs, highlighting a need for cost-effective and accurate identification approaches that enable biodiversity outcomes of restoration objectives to be assessed (Wise, 1994; Topping & Lovei, 1997; Burger et al. 2001). Interpretations based solely upon morphological characteristics can be time-consuming, require specialist taxonomic expertise, and in some cases provide misleading and inconclusive results. The heightened interest in the losses of biodiversity in natural ecosystems requires the accurate assessment of biotic community composition. DNA-based approaches to identification can provide insights into phylogeny and biogeography, improving identifications of species and subsequent recognition in descriptions of invertebrate community composition (Heden, 2001; Hebert et al. 2003; Sweeney et al. 2011; Stein et al. 2013).

The need to accurately assess biological communities is particularly true in New Zealand where there are high levels of endemism in diverse, but partially unresolved arthropod taxa, such as beetles (Coleoptera; Ewers & Didham, 2004), springtails (Collembola; Greenslade, 2015) and spiders (Araneae; Forster & Forster, 1999; Paquin, Vink & Duperre, 2010; Lamont et al. 2017). Partially unresolved, spider phylogeny will benefit from the application of DNA barcoding as it provides support to morphological identifications by alleviating the challenges associated with refined levels of identification of both described species and morphologically-cryptic specimens. This is a significant advantage because it provides a method to accurately and rapidly assess, with species-level refinement, the composition of communities inhabiting different ecosystems.

Spiders are synonymous with terrestrial ecosystems worldwide, represented by greater than 47,000 described species (www.wscnmbe.ch/). New Zealand's spider fauna is highly endemic with approximately 2,000 species nationwide. Of these, only 1,126 native species have been formally described and there are approximately 70 further species that are known introductions from overseas (Forster &

Forster, 1999; Paquin, Vink & Duperre, 2010). Traditionally, species-level identifications are only possible from adult specimens, often requiring thorough analyses of genital morphology, imposing a selection bias towards identifiable adult specimens. Recent genetic polymorphisms have been identified in some species, adding a new layer of complexity to taxonomic decisions (Huber & Gonzalez, 2001; Jocque, 2002).

Due to the intensity of sexual dimorphism exhibited by many spider species, a separate range of taxonomic criteria is often required to identify females and males. Further, morphological diagnostic traits applied to single sex identification of many native New Zealand species has limited many species identifications to female only (Paquin, Vink & Duperre, 2010). The frequency of sexual dimorphism across a range of spider taxa is problematic in terms of cladistics and systematics, and the overwhelming reliance on any singular piece of morphology for the determination of species boundaries has imposed a bias against the discovery of intra-specific polymorphisms of phenotypic expression. Because of these limitations, a more widely-accessible identification tool would provide a major advance in the study of this diverse group by ecologists, resource managers, and the science community at large.

The focus of this study was determining whether the mitochondrial Cyctocrome *c* oxidase subunit I (COI) gene could be used to effectively discriminate between, and inventory, biological identifications of New Zealand's 86 most common spider species, as published in the Photographic Guide to Spiders of New Zealand (Vink & McQuillan, 2015). The research presented in this chapter contributes to the International Barcode of Life Datasystem (BOLD; www.boldsystems.org) by analysing 774 COI sequences from New Zealand spiders. This collection included specimens assigned morphologically-recognised species names, provided from the Canterbury Museum, augmented by field collections of cryptic taxa from a diverse range of locations in the Bay of Plenty, Canterbury, Northland and Waikato regions. The analyses consisted of a 550 base pair (bp) region of the COI gene taken from professionally identified museum specimen at the Canterbury Museum, and morphologically cryptic

specimens collected by individuals in other locations. Cryptic specimens were only coarsely identified, either to genus or family level, and included unrecognised male and juvenile specimens from a range of species.

The resulting COI barcode inventory was used to reliably place specimen sequences into corresponding Molecular Operational Taxonomic Units (MOTUs) which were assigned Barcode Index Numbers (BINs) in BOLD. BINs were assigned regardless of sexual dimorphism, maturity or morphological plasticity exhibited by individual specimens and form the foundation of the barcode library which supports this method of cataloging New Zealand's most commonly-encountered spider fauna. Furthermore, comparing barcodes with other BIN barcodes provides a means of identifying the infiltration of invasive species into existing communities. Furthermore, the study provides a means to assess the distribution of endemic species within established invertebrate communities inhabiting a range of ecosystems.

2.3 Methods and materials

2.3.1 Specimen acquisition and morphological identification

Spider specimens were analysed from two sources, the Canterbury Museum (n = 137) and collections made by The University of Waikato (n = 636). These collections incorporated species-level identified specimens collected from the Auckland, Bay of Plenty, Canterbury, central North Island, Hawke's Bay, Nelson/Tasman, Northland, Otago, Southland, Waikato and West Coast regions of New Zealand (Figure 2.1). Specimens were stored in 100% ethanol before examination with a dissection stereo-microscope and subsequent removal of tissue for genetic analysis.

Figure 2.1: Sample collection sites across New Zealand's North Island and South Island pertaining to museum collection specimens and material collected by the University of Waikato during this study.

2.3.2 Genetic analysis

In most instances, a tarsal segment from the 4th leg from each specimen was removed under microscopic magnification, and placed in a single well on a 96-well plate for genetic analysis at the Canadian Centre for DNA Barcoding, University of Guelph, Canada. All photographs, collection information, primer combinations and sequence data pertaining to each specimen have been uploaded to BOLD, housed in the project Spiders of New Zealand (NZSPI).

A 658 base pair fragment of the mitochondrial COI gene was amplified using standard CCDB protocols (Ivanova, deWaard & Hebert, 2006) for 1 to 37 individuals per species. Genomic DNA was extracted via the AcroPrep™ PALL Glass Fibre plate method (Ivanova, deWaard & Hebert, 2006) and a 658 bp fragment of the mitochondrial COI gene was amplified following standard CCDB protocols (see Ivanova, deWaard & Hebert, 2006) using the universal forward primer cocktail C_LepFolF (LepF1: 5'-ATTCAACCAATCATA AAGATATTGG-3'; LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3') and the reverse primer cocktail C_LepFolR (LepR1: 5'-TAAACTTCTGGATGTCCAAAAA ATCA-3'; HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994; Hebert et al. 2004; Ivanova, deWaard & Hebert, 2006). BINs were assigned in BOLD (Ratnasingham & Hebert, 2013) and used as a measure of MOTU's.

2.3.3 Data analysis

All sequences were deposited in the NZSPI project and cross-referenced to GenBank. COI sequences were aligned using the multi-alignment software MUSCLE (Edgar, 2004), and subsequently pruned to 550 bp using Geneious R10 (v10.1.2). Mega 4.0 (Tamura et al. 2007) was used to determine genetic divergence for specimen barcodes and to create a Neighbour-Joining tree of Kimura-2-parameter distances for the Araneae order. Sequences were filtered to exclude <500 bp sequences, and any records flagged as misidentifications or contaminated. A key step in the analysis of large DNA sequence datasets is the clustering of sequences based on their similarity, which form the basis for subsequent biodiversity

analyses. Clustering reduces the complexity of the data and limits the effects of PCR and sequencing errors on biodiversity estimates, as sequences with a modest number of errors are grouped together and treated as single MOTU (Nei & Kumar, 2000). COI sequences from this study were grouped together based on a sequence similarity guideline of 98%. The subsequent taxon identification tree functionally allows for the visualisation of phylogenetic trees from selected sequences using the Neighbour-Joining algorithm.

Non-parametric bootstrap analysis (Felsenstein, 1985) was implemented with 1000 pseudo-replicates to assess support for nodes in the tree. All bootstrap values <50% were removed. Mega (4.0) was also used to create pairwise distance matrices to calculate intraspecific and interspecific divergences. Pairwise distances were used to compare the similarity of the New Zealand spider fauna with all publicly available sequences on BOLD. A species-identification threshold of 98% similarity was used to cluster specimen sequences and assign BINs (Barrett & Hebert, 2005) in BOLD (Ratnasingham & Hebert, 2013), which were used as a measure of MOTU's.

Although not a definitive threshold, as intra-specific or genus pairwise similarity can vary depending on the taxon, this study tested a 2% divergence threshold for the assignment of BINs to determine whether this is indicative of species delineation thresholds for morphologically-described taxa. In instances where intra-specific divergence difference exceeded the tested threshold, a distance summary analysis was conducted in BOLD using the Kimura 2 Parameter distance model (Kimura, 1980) and aligning sequences using BOLD Aligner (Amino Acid Base HMM). The distance summary reports the sequence divergence between barcode sequences at the species, genus and family levels, contrasting the distribution of within-species divergence to between-species divergence.

2.4 Results

2.4.1 DNA barcode profile

This study examined 774 COI barcodes of sufficient length (>600 bp) to make a comparative examination of a 550 bp region of the COI gene from identified and morphologically cryptic taxa. The subsequent sequence inventory comprised 601 specimens identified as members of 98 described species (refer to Appendix A1), 68 of which were represented by multiple sequences, and an additional 173 specimen barcodes corresponded to 40 separate unidentified MOTUs presently identified at genus-level only (refert to Appendix A2). This compilation of species and genus-level identifications represents 31 Araneae families in total (Figure 2.2). The corresponding Neighbour-Joining profile contains 136 terminal nodes, each representing MOTUs (Figure 2.3).

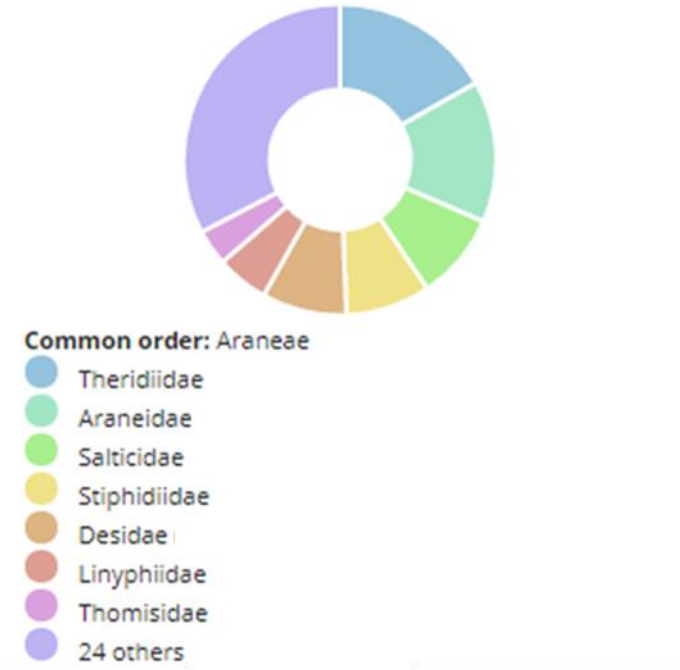
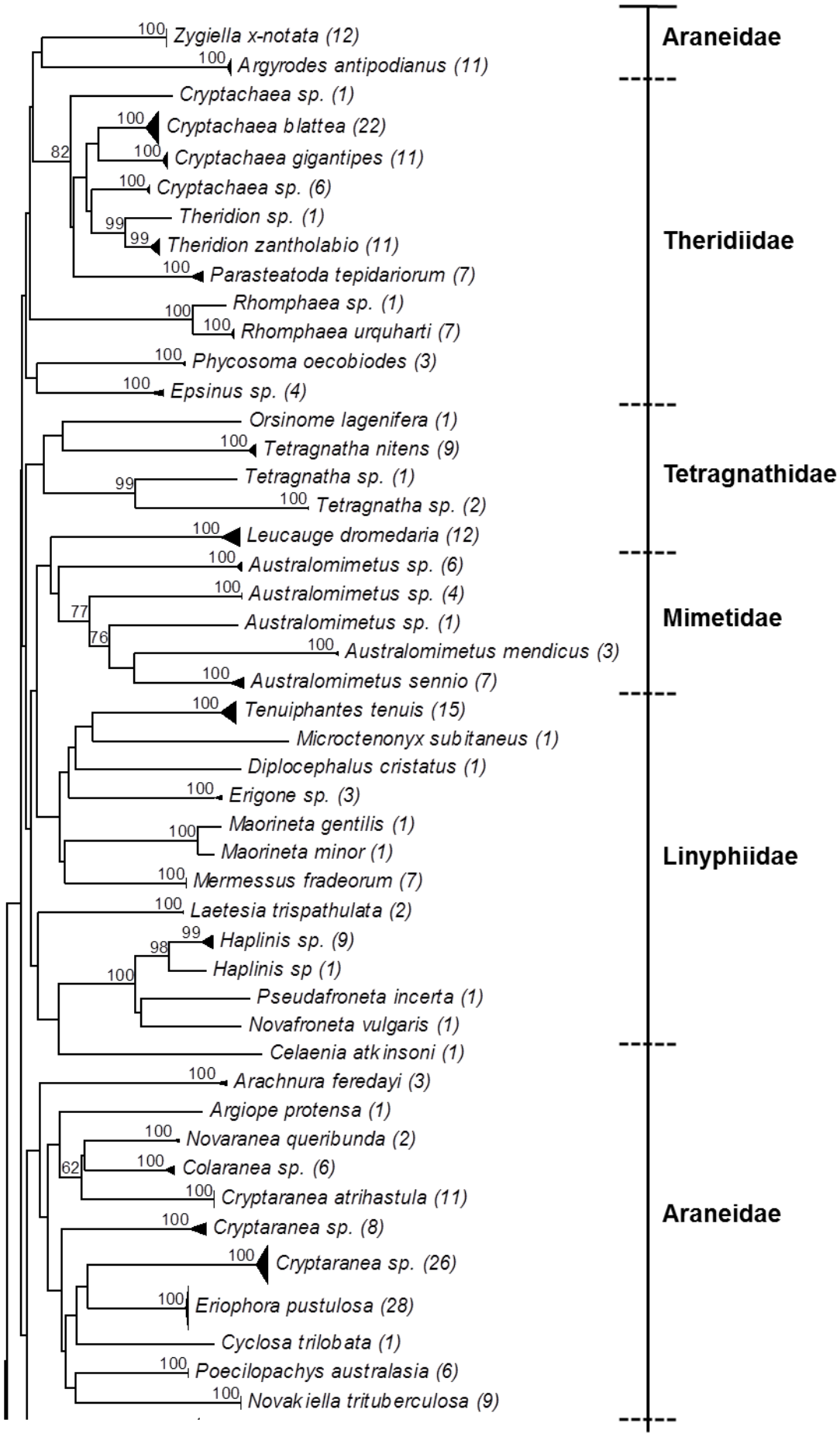
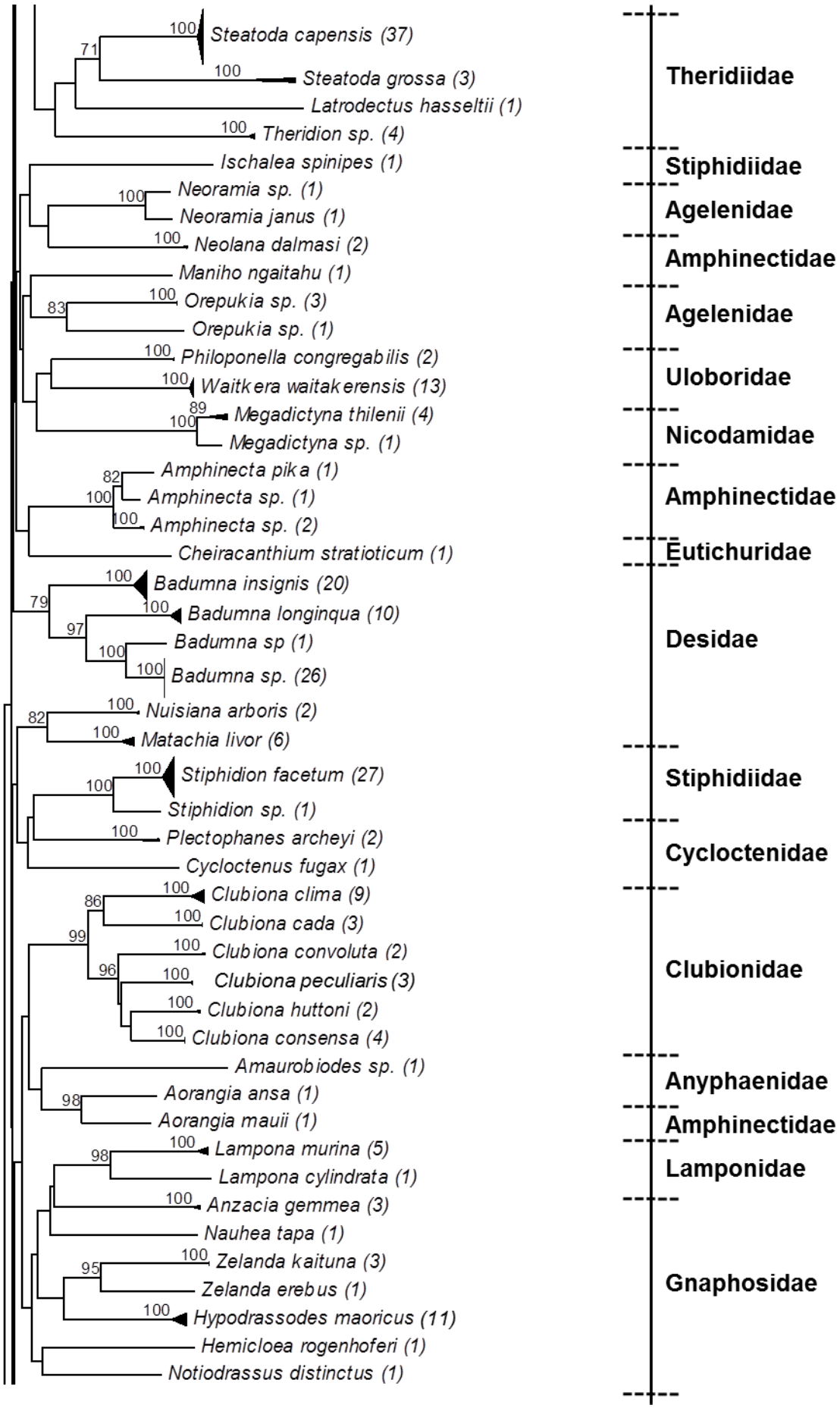


Figure 2.2: The relative proportion of specimens from each family in this study of New Zealand's spiders, analysed using BOLD (www.boldsystems.org/).

Using DNA barcoding as a tool for identification and recognition, I coupled males and females of the same species from 54 of the 68 described MOTUs represented by multiple sequences in this study. Therefore, of all nodes on the corresponding Neighbour-joining tree (Figure 2.3), 40% were represented by multiple sequences representing both males and females. Interestingly, undescribed male specimens belonging to 6 native species

were positively identified with species-level refinement for the first time in this study, using these methods: *Plectophanes archeyi* (Forster, 1964), *Uliodon albopunctatus* (L. Koch, 1873), *Sidymella benhami* (Hogg, 1990), *Sidymella angularis* (Urquhart, 1885), *Theridion zantholabio* (Urquhart, 1886), *Paradictyna ilamia* (Forster, 1970).


Zygiella x-notata (12)
Argyrodes antipodianus (11)
Cryptachaea sp. (1)
Cryptachaea blattea (22)
Cryptachaea gigantipes (11)
Cryptachaea sp. (6)
Theridion sp. (1)
Theridion zantholabio (11)
Parasteatoda tepidariorum (7)
Rhomphaea sp. (1)
Rhomphaea urquharti (7)
Phycosoma oecobiodes (3)
Epsinus sp. (4)
Orsinome lagenifera (1)
Tetragnatha nitens (9)
Tetragnatha sp. (1)
Tetragnatha sp. (2)
Leucauge dromedaria (12)
Australomimetus sp. (6)
Australomimetus sp. (4)
Australomimetus sp. (1)
Australomimetus mendicus (3)
Australomimetus sennio (7)
Tenuiphantes tenuis (15)
Microctenonyx subitaneus (1)
Diplocephalus cristatus (1)
Erigone sp. (3)
Maorineta gentilis (1)
Maorineta minor (1)
Mermessus fradeorum (7)
Laetesia trispathulata (2)
Haplinis sp. (9)
Haplinis sp (1)
Pseudafroneta incerta (1)
Novafroneta vulgaris (1)
Celaenia atkinsoni (1)
Arachnura feredayi (3)
Argiope protensa (1)
Novaranea queribunda (2)
Colaranea sp. (6)
Cryptaranea atrihastula (11)
Cryptaranea sp. (8)
Cryptaranea sp. (26)
Eriophora pustulosa (28)
Cyclosa trilobata (1)
Poecilopachys australasia (6)
Novakiella trituberculosa (9)
Araneidae
Theridiidae
Tetragnathidae
Mimetidae
Linyphiidae
Araneidae


Steatoda capensis (37)
Steatoda grossa (3)
Latrodectus hasseltii (1)
Theridion sp. (4)
Theridiidae
Ischalea spinipes (1)
Stiphidiidae
Neoramia sp. (1)
Neoramia janus (1)
Agelenidae
Neolana dalmasi (2)
Amphinectidae
Maniho ngaitahu (1)
Orepukia sp. (3)
Orepukia sp. (1)
Agelenidae
Philoponella congregabilis (2)
Waitkera waitakerensis (13)
Uloboridae
Megadictyna thilenii (4)
Megadictyna sp. (1)
Nicodamidae
Amphinecta pika (1)
Amphinecta sp. (1)
Amphinecta sp. (2)
Amphinectidae
Cheiracanthium stratioticum (1)
Eutichuridae
Badumna insignis (20)
Badumna longinqua (10)
Badumna sp (1)
Badumna sp. (26)
Desidae
Nuisiana arboris (2)
Matachia livor (6)
Stiphidion facetum (27)
Stiphidion sp. (1)
Stiphidiidae
Plectophanes archeyi (2)
Cycloctenus fugax (1)
Cycloctenidae
Clubiona clima (9)
Clubiona cada (3)
Clubiona convoluta (2)
Clubiona peculiaris (3)
Clubiona huttoni (2)
Clubiona consensa (4)
Clubionidae
Amaurobiodes sp. (1)
Anyphaenidae
Aorangia ansa (1)
Aorangia mauii (1)
Amphinectidae
Lampona murina (5)
Lampona cylindrata (1)
Lamponidae
Anzacia gemmea (3)
Nauhea tapa (1)
Zelanda kaituna (3)
Zelanda erebus (1)
Hypodrassodes maoricus (11)
Gnaphosidae
Hemicloea rogenhoferi (1)
Notiodrassus distinctus (1)

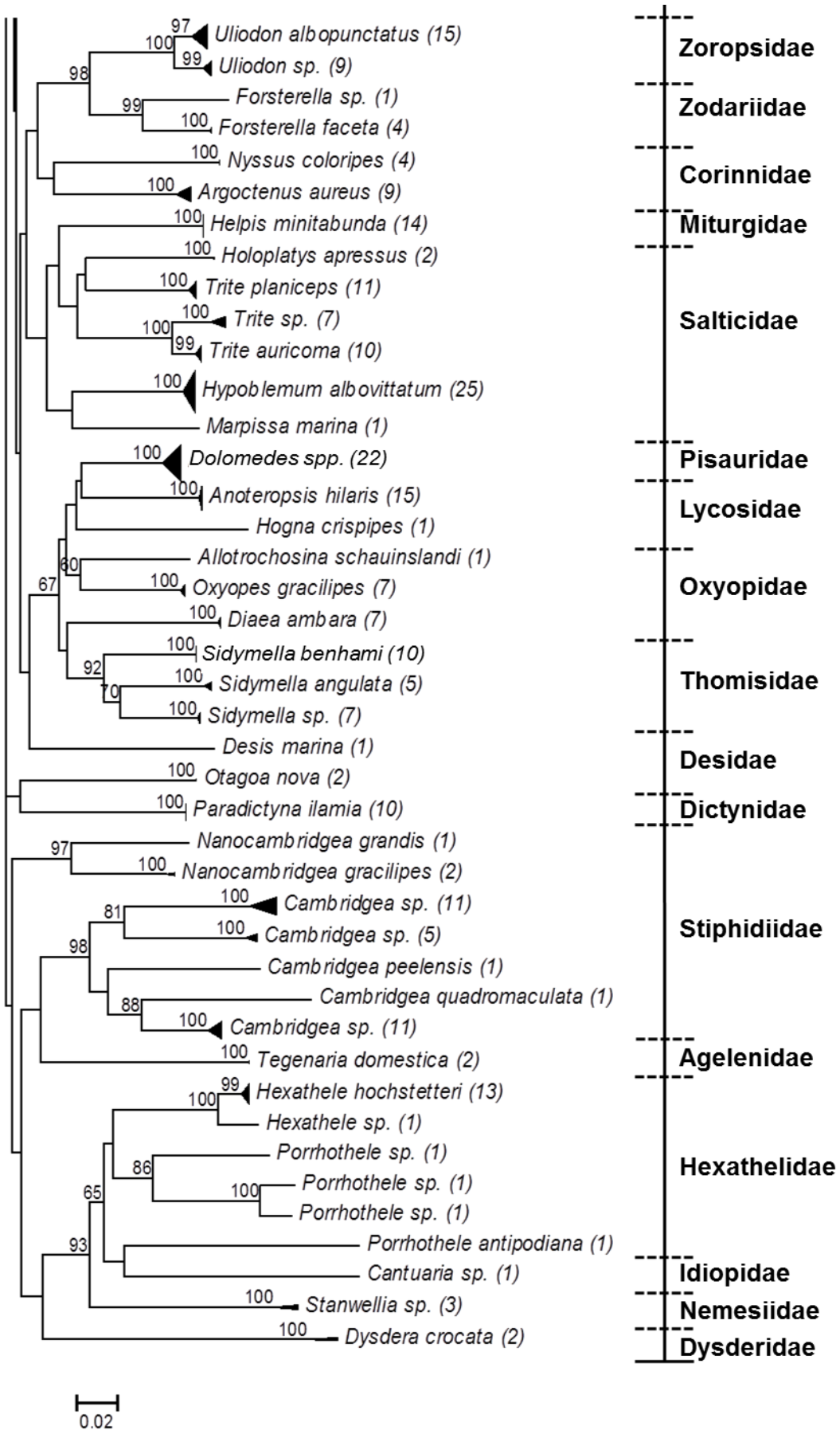


Figure 2.3: Neighbour-joining analysis grouping mitochondrial Cyctocrome c oxidase subunit I (COI) gene haplotype diversity with morphology-based names applied. An identity threshold of 98% was accepted to cluster Molecular

Operational Taxonomic Units (MOTUs) into species groupings. For each taxon, the vertical height of the triangle is proportional to number of specimen sequences and the horizontal length represents maximum genetic diversity within the branch. Values in parentheses indicate the number of individual barcodes within the taxon. Italicised names are binomial nomenclature for genus and species designations. Family names are recorded in bold to the right of the profile.

Two sequences identified as *Philoponella congregabilis* (Rainbow, 1916) were collected in the Canterbury region of the South Island. DNA barcoding provided a positive match for these two identified specimens to two *P*. *congregabilis* sequences collected in Canberra, Australian Capital Territory, Australia. This species is considered a native of Australia (Downes, 1995) and has previously not been recognised as established in New Zealand.

Analyses of the BIN inventory revealed an instance where multiple morphological species' designations did not adhere to the minimum 2% species' delineation threshold for identification. In this study, the *Dolomedes* genus is represented by 3 morphologically-identified native species, *D. minor* (L. Koch, 1876): *D. aquaticus* (Goyen, 1888), and *D. dondalei* (Vink & Dupérré, 2010) pertaining to 3, 4, and 15 barcodes, respectively (Table 2.1). Distance summary analysis revealed an average intraspecific sequence divergence of 0.39% and a minimum interspecific sequence divergence of 0.92% between the three taxa. This low level of within-genus COI divergence did not provide a clear differentiation between the three species in this instance.

Table 2.1: The distribution of *Dolomedes* spp. CO1 sequence divergence at two taxonomic levels using a minimum 2% species divergence threshold. SE = standard error.

Label	n	Taxa	Comparisons	Min. distance (%)	Mean distance (%)	Max. distance (%)
Within Species	22	3	114	0	0.39	1.24
Within Genus	22	1	117	0.92	1.5	2.28

Overall, intraspecific sequence divergence was low (mean distance = 0.31%), in comparison to other arthropod taxa such as Chironomidae (mean = 2.3%), Trichoptera (mean = 0.7%; Hogg et al. 2009) and Ephemeroptera (mean = 1%; Ball et al. 2005). However, in contrast to the average, the cosmopolitan species *Steatoda grossa* (C. L. Koch 1838) is represented by 3 sequences in this study which form a monophyletic clade with *Steatoda capensis* in the Neighbour-Joining analysis. Each of the three study sequences were assigned a different BIN in BOLD as distance summary results report that the sequence divergence of these 3 COI sequences in this study exhibits a high degree of intraspecific genetic divergence (mean = 3.82%, maximum = 5.12%). However, when each sequence is individually compared with all available sequences on BOLD, they indicate a 100% match to other specimen sequences identified as *Steatoda grossa* from Bulgaria, the United States of America and Canada. In contrast, the monophyletic genus *Clubiona*, represented in this study by 6 described species, shows a relatively low level of intraspecific barcode sequence variation (mean = 0.75%, maximum = 1.40 %) compared with between-species divergence (range = 8.78% to 10.41%). These considerable differences in the corresponding BIN sequences provided positive species delineations.

The maximum intraspecific sequence divergence of 5 species exceeded the tested 2% threshold for species delineations (Table 2.2). Of these, only *Cambridgea* sp. is a native to New Zealand, whereas *Leucauge dromedaria* (Thorell, 1881) is native to Australia and the other three species are considered introduced to New Zealand, having cosmopolitan distributions across multiple continents (Paquin, Vink & Duperre, 2010). By comparison, the average distance between each respective species and their nearest species counterpart exceeds 3%, and in the case of both *Cambridgea*. sp. and *L*. *dromedaria* the average distance to their respective nearest neighbours exceeds the compiled average distance in this study.

Table 2.2: Species whose maximum intraspecific COI sequence divergence exceeds 2%. NZSPI = number of sequences represented in this study; BOLD = the number of sequences available for comparison using the Barcode of Life Datasystem. Overall spp. total represents all species from this study with ≥ 2

barcodes available for comparison. Average distance refers to genetic dissimilarity to nearest neighbour.

Species	n NZSPI	n BOLD	Mean distance (%)	Maximum distance (%)	Average distance (%)
Argyrodes antipodianus (O. Pickard-Cambridge, 1880)	11	523	0.12	2.74	3.08
Cambridgea sp.	11	11	0.94	2.16	10.37
Cryptachaea blattea (Urquhart, 1886)	22	49	0.92	2.54	3.17
Leucauge dromedaria (Thorell, 1881)	12	34	1.41	3.41	12.84
Tenuiphantes tenuis (Blackwall, 1852)	15	114	0.6	2.91	4.49
Overall spp. total	**717**	**2123**	**0.50**	**1.06**	**6.53**

By testing a 2% COI divergence threshold, to group sequences for species identification, all of the specimens examined could be distinguished from one-another and were most closely associated with specimens of the same morphospecies and BIN. This threshold reflects a lower level of intraspecific sequence variation exhibited within species than between closely-related species (Barrett & Hebert, 2005; DeSalle, Egan & Siddall, 2005). Exceedences of this threshold were identified in described species with recognised international distributions (Table 2.3). Nevertheless, extensive research of species metapopulations may reveal some recently-diverged species with comparatively low levels of sequence divergence. In a review of intraspecific COI sequence divergence, Hebert et al. (2003) found that over 98% of different animal species possess greater than 2% COI divergence. Although no single divergence threshold will enable the delineation of all species, sequence divergence values greater than 2% were typically indicative of different species in this study. These results support the conclusion that a 2% threshold value can be used as an initial guideline for assessing species richness and community composition where refined morphological analysis is not feasible.

Table 2.3: Described species with recognised international distributions represented by COI specimen sequences in this study. BOLD BINs refer to the corresponding species index references associated with clusters of COI sequences matching species identifications (www.boldsystems.org). Mean BIN distances compare intraspecific dissimilarity between sequences. Average distance to nearest neighbour indicates the dissimilarity to the nearest neighbouring species. * indicates a species not presently recognised as an established component of New Zealand's spider fauna (www.wsc.nmbe.ch/; (Paquin, Vink & Duperre, 2010).

Identification	Distribution	BOLD BIN(s)	BIN mean distance	Average distance to nearest neighbour
Tegenaria domestica Clerk, 1757	Australia, China, Europe, Japan, New Zealand, North America, South America	AAF1312	0.05	12.84
Arachnura feredayi L. Koch, 1872	Australia, New Zealand	ACM2091	1.02	12.2
Argiope protensa L. Koch, 1872	Australia, New Caledonia, New Guinea, New Zealand	ACR1958	-	2.51
Argyrodes antipodianus O. Pickard-Cambridge, 1880	Australia, New Caledonia, New Zealand	ACM2161	0.12	3.08
Celaenia atkinsoni O. Pickard-Cambridge, 1880	Australia, New Zealand	ADD4033	0.41	3.30
Cyclosa trilobata Urquhart, 1885	Australia, New Zealand	ACM2466	0.08	2.41
Eriophora pustulosa Walckenaer, 1841	Australia, New Zealand	AAV4783	0.08	9.63
Novakiella trituberculosa Roewer, 1942	Australia, New Zealand	ACR1174	0	3.53
Poecilopachys australasia Griffith & Pidgeon, 1933	Australia, Samoa	ACM2770	0.14	8.99
Zygiella x-notata Clerk, 1757	Argentina, Caucasus, Chile, China, Europe, Japan, North America, Reunion Is., Turkey, Uruguay	AAJ9891	0.05	4.72
Nyssus coloripes Walckenaer, 1805	Australia, New Zealand	AAZ4223	0.8	8..44
Badumna insignis L. Koch, 1872	Australia, Japan, New Zealand	ACI6073	1.22	7.87
Badumna longinqua L. Koch, 1867	Australia, Japan, Mexico, New Zealand, United States of America, Uruguay	AAW2980	0.47	7.32
Desis marina Hector, 1877	New Caledonia, New Zealand	ADD1311	-	12.77
Dysdera crocata C. L. Koch, 1838	Australia, Brazil, Chile, Europe, Hawaii, New Zealand	AAE8008	0.26	1.39
Cheiracanthium stratioticum L. Koch, 1873	Australia, New Zealand	ADD2397	-	7.38
Anzacia gemmea Dalmas, 1917	Australia, New Zealand	ACT2059	0.64	7.32
Hemicloea rogenhoferi L. Koch, 1875	Australia, New Zealand	ACM2437	0.39	9.85
Lampona cylindrata L. Koch, 1866	Australia, New Zealand	ACM1683	1.52	8.83
Lampona murina L. Koch, 1873	Australia, New Zealand	ACO6095	0	1.01
Diplocephalus cristatus Blackwall, 1833	Cosmopolitan: Europe, Falkland Is., Kazakhstan, New Zealand, North America, Russia, Siberia	AAL2095	0.14	2.45
Mermessus fradoerum Berland, 1932	Azores, China, New Zealand, North America, Saudi Arabia, South Africa	AAH3496	0.15	6.46
Microctenonyx subitaneus O' Packard-Cambridge, 1875	Australia, Europe, Kyrgstan, New Zealand, North Africa, South Africa, United States of America	ACA4997	1.54	13.11
Tenuiphantes tenuis Blackwall, 1852	Argentina, Caucasus, Central Asia, Chile, Europe, Macronesia, New Zealand, United States of America	AAG9172	0.6	4.49
Hogna crispipes L. Koch, 1877	Australia, New Guinea, New	ACK2896	0.62	2.73

	Zealand, Polynesia			
Oxyopes gracilipes White, 1849	Australia, New Zealand	ACR1894	0.43	1.95
Helpis minitaunda L. Koch, 1880	Australia, New Guinea, New Zealand	AAX1573	0.09	8.08
Hypoblemum albovittatum Keyserling, 1882	Australia, New Zealand	AAY3385	0.49	4.52
Stiphidion facetum Simon, 1902	Australia, New Zealand	AAJ4144	0.26	5.3
Leucauge dromedaria Thorell, 1881	Australia, New Zealand	AAG8513	1.41	12.84
Tetragnatha nitens Audouin, 1826	Asia, Canary Is., Europe, Egypt, Maderia, Madagascar, Pacific Islands, New Zealand, North America, South America	AAD3791	0.61	12.04
Cryptachaea blattea Urquhart, 1886	Africa, Azores, Australia, Chile, Europe, Hawaii, New Zealand, United States of America	ACH6516	0.92	3.17
Cryptachaea gigantipes Keyserling, 1890	Australia, Norfolk Is., New Zealand	AAV1555	0.4	4.76
Latrodectus hasseltii Thorell, 1870	Australia, Southeast Asia, India, New Zealand	AAB0102	0.24	2.9
Parasteatoda tepidariorum C. L. Koch, 1841	Canada, China, Europe, Japan, Hawaii, New Zealand, Seychelles, South America	AAC0175	1.21	6.26
Steatoda capensis Hann, 1990	Lesotho, South Africa, St Helena, New Zealand	AAY6263	0.23	2.25
Steatoda grossa C. L. Koch, 1838	Algeria, Chile, China, Ecuador, Europe, Hawaii, Korea, Japan, Macronesia, New Zealand, North America, Peru	ACS5951 AAL5865 ACE3320	0.01	1.45
Sidymella benhami Hogg, 1910	Australia, New Zealand	ACL9074	0.14	8.25
*Philoponella congregabilis** Rainbow, 1916	Australia	AAZ4225	0.29	8.51

2.5 Discussion

This study sought to determine the suitability of COI barcoding for identifying spider taxa by examining the sequence divergence across a broad range of species. The mitochondrial COI sequences incorporated in this study infer 136 species assignments, and the study represents one of New Zealand's first molecular assessments of species richness across a broad range of Araneae taxa. Using a combination of morphological characteristics and COI sequence data, the 774 specimens incorporated into this study were sorted into corresponding BINs, using a 2% intra-specific similarity threshold to group sequences. I tested the ability of the 2% divergence threshold to discriminate between morphologically-described taxa, determining that 6 species failed to adhere strictly to this value. Regardless of the exceedance of this threshold, 100% of the respective sequences grouped most closely with other specimens of the same morphological identification. The majority of BINs were identified (98) with species-level refinement with the remaining 40 cryptic BINs being identified to genus-level.

Embedded within this study are species representing 31 of New Zealand's 57 spider families (Paquin, Vink & Duperre, 2010). Furthermore, the species richness incorporated in this study represents approximately 12% of the total diversity of described spider species found in New Zealand (Paquin, Vink & Duperre, 2010). However, traditional morphological methods of identifying spiders are often inadequate for establishing species-level identifications, species richness, and community composition of whole communities represented by adult and juvenile specimens (Lamont et al. 2017; Bowie et al. 2014; Malumbres-Olarte et al. 2013; Vink et al. 2004; Alley et al. 2001; Topping & Lovei, 1997). The challenges of identification are primarily associated with the diverse, sexually-dimorphic characters and intense morphoplasticity featured amongst this diverse arthropod taxa (Lamont et al. 2017; Lester et al. 2014).

Almost all of New Zealand's endemic spiders are strictly associated with native forest habitat and other ecosystems with significant natural vegetative character (Lamont et al. 2017). Contrastingly, species with international distributions are almost exclusively restricted to disturbed habitats and agricultural areas, reinforcing the need for the preservation of significant natural areas (Mallis & Hurd, 2005; Brockerhoff et al. 2010; Malumbres-Olarte et al. 2014). Of the described species in this study, forty are classed as cosmopolitan species as they have recognised international distributions (www.wsc.nmbe.ch). Of these, 17 are recognised as having Australasian distributions as they are common to both Australia and New Zealand. Further, *Philoponella congregabilis* (Rainbow, 1916) was identified in New Zealand for the first time using DNA barcodes, positively matching specimen sequences from Canberra, Australia.

Molecular systems provide insights which address the problem of species identification, speeding up the routine diagnosis of species (Tautz et al. 2003; Hedin, 2001). Mitochondrial COI sequences have rapidly become the marker-of-choice for identifications of chordates and invertebrates (Hebert et al. 2003). Although mitochondrial DNA sequences have been

implemented for phylogenetic research internationally (Hogg et al. 2009; Robinson et al. 2009; Tanzler et al. 2012), the use of COI for species identification has, to date, been limited to studies and descriptions of our most common spider species (Vink & McQuillan, 2015; Vink et al. 2009). Research conducted on North American spider taxa (Barrett & Hebert, 2005) and Austrian spider communities (Raso et al. 2014) has demonstrated that nucleotide sequence diversity in a standard segment of the mitochondrial gene coding for COI is highly effective at discriminating between spider species associated with BINs. These studies correctly assigned 100% of their subsequently analysed specimen barcodes to the appropriate species. The results of this study are in line with their findings and support the use of COI barcodes as a method of positively identifying juvenile and adult specimen across broad spatial and temporal scales.

Chapter 3

An assessment of spider communities inhabiting shoreline and pasture habitats of Waikato lakes using DNA barcoding.

3.1 Abstract

This research assessed the species assemblages of spiders inhabiting pasture and shoreline habitats, among 5 lakes representing differing degrees of ecological character. Spiders are the primary predators of aquatic invertebrates during the terrestrial phase of their lifecycles, hence lake ecosystems provide a diverse range of prey for predatory spider communities inhabiting the shoreline. I analysed a 500-bp region of the Mitochondrial COI gene, taken from successfully barcoded morphospecies (75 of 79; n = 337 COI sequences) collected from pasture and shoreline transects at each lake. This inventory of species consists of 38 described and 41 unidentified species obtained from habitat surrounding these study lakees. Of these, 18 described species and 6 unidentified species possitvely matched overseas records from the Barcode of Life Datasystem and were determined to have cosmopolitan distributions. Pasture habitats supported similar compositions of common cosmopolitan species, whereas lake catchments with significant native vegetative character, providing habitat heterogeneity, supported diverse community assemblages dominated by native species. The results from this study establish a foundation for further assessments of this diverse predatory taxon, and provides complimentary data to the reference library in Chapter 2, contributing 31 new BINs representing undescribed/unknown species and 2 BINs representing morphologically described species to the BOLD COI repository.

3.2 Introduction

Investigating and developing an understanding of spider community composition, within and between ecosystems, addresses a fundamental element of ecological research and provides insights into species distribution, dispersal, and spatial and temporal variation associated with fragmented terrestrial habitats. A basic understanding of species presence allows more complex ecological questions to be developed. In addition, measures of community diversity are inherently valuable as indicators of ecological wellbeing (Eaton, 2001), and for evaluating vegetation restoration success in terrestrial ecosystems (Pearce & Venier, 2006). New Zealand has a diverse and highly endemic spider fauna with approximately 2,000 species, however, many of these remain to be formally described. Given such high diversity, it is critical to seek rapid and accurate methods for discriminating between native and non-native species, and for characterising the spider communities occurring in habitats with significant natural character and ecological value.

Morphological identification and classification can be an inconclusive process as maturity, phenotypic plasticity and sexual dimorphism can severely complicate taxonomic designations (Mallis & Hurd, 2005; Paquin, Vink & Duperre, 2010). DNA-based analyses of sequence diversity in small segments of DNA have been developed for those instances where morphology-based identification proves problematic (Tautz et al. 2003; Sarkar & Trizna, 2011; Taberlet et al. 2012; Tanzler et al. 2012). In particular, diversity in the amino acid sequences coded by the 5' section of the cytochrome oxidase *c* subunit I (COI) mitochondrial gene has been widely used to place arthropod taxa, such as weevils (*Trigonopterus*; Tanzler et al. 2012), spiders (Araneae; Robinson et al. 2009; Vink et al. 2009; Raso et al. 2014), Ephemeroptera, Plecoptera and Trichoptera (Hogg et al. 2009; Sweeney et al. 2011; Beet, 2016) into higher taxonomic categories.

A key aim of this study was to provide insights into the use of spider communities as indicators of lake and riparian zone condition. This study measured the diversity of spider communities and determined whether

there were differences in community composition that reflected riparian habitat type among lakes in catchments with different land uses in the Waikato region, North Island, New Zealand. Lake habitats were selected because the emergence of aquatic insects from the aquatic-terrestrial interface, can provide a trophic subsidy to predatory arachnids (Burdon & Harding, 2008), and may therefore support more diverse communities compared to pasture habitats more distant from lake shores. DNA barcodes of the COI mitochondrial gene were used to place specimens into Molecular Operational Taxonomic Units (MOTUs) affiliated with individual species (see Chapter 2), regardless of sexual dimorphism, maturity or morphological plasticity.

The resulting molecular inventory was used to ask 3 specific research questions: 1: Are there detectable differences between the community compositions of species across the 5 lakes representing different degrees of ecological state and catchment condition? 2: Is there a significant difference in the native and cosmopolitan spider community composition collected from shoreline and pasture habitat transects? 3: How do manual searching and substrate suction collection methods compare when assessing species richness along shoreline sampling sites? Three lakes had pasture-dominated catchments (Kohahuake, Puketi, Rotoiti) and were compared to a lake with a predominantly native forest catchment (Lake Koraha), and a lake transitioning from a pasture-dominated catchment to one replanted with native riparian vegetation (Lake Waiwhakareke).

This research provides insights into the diversity of cosmopolitan versus native spider communities in disturbed and exposed pasture habitats. The results of this study have contributed to the COI library for New Zealand's most commonly-encountered spiders, providing a means of identifying endemic and introduced species across broad geographical areas.

3.3 Methods

3.3.1 Study sites

Three dune lakes with pasture-dominated catchments, Puketi, Rotoiti and Kohahuake, were selected because they provided good examples of multi-generational property ownership pre-dating forest vegetation clearance, with low-intensity land-use for stock grazing. Stock exclusion fencing had been completed at these lakes in the 6 months prior to the commencement of this study, and the inner shoreline habitats were scheduled to undergo an intensive riparian planting management programme within the following 6 months. The results of my sampling therefore provide valuable baseline information on spider communities prior to riparian planting.

Lake Puketi is the largest of the study lakes (area 6.4 ha); all others have a total surface area <2 ha (Table 3.1). Like lakes Kohahuake and Rotoiti, Puketi is a northern coastal dune lake. Lake Waiwhakareke was selected because it is subject to both agricultural and urban influences, and has undergone considerable riparian re-vegetation along the shoreline habitat (0-5 m from the water's edge) and in the wider catchment. This peat lake represents a lake catchment that is transitioning from pasture-dominated to predominantly native riparian vegetation cover. Lake Waiwhakareke is the shallowest of the study lakes (maximum depth 3 m) with a comparably low Secchi depth of 1.03 m. The total nitrogen (1.37 mgL^{-1}), total phosphorous (0.12 mgL^{-1}) and chlorophyll *a* (0.05 mgL^{-1}) were all highest in this lake (Table 3.1). For reference, a karst lake with significant natural vegetative character around the shoreline and in the catchment (Koraha) was also sampled. The locations and physicochemical characteristics of each lake are shown in Figure 3.1 and Table 3.1. Detailed site photos and locations of sampling transects are shown in Figures 3.2-3.6.

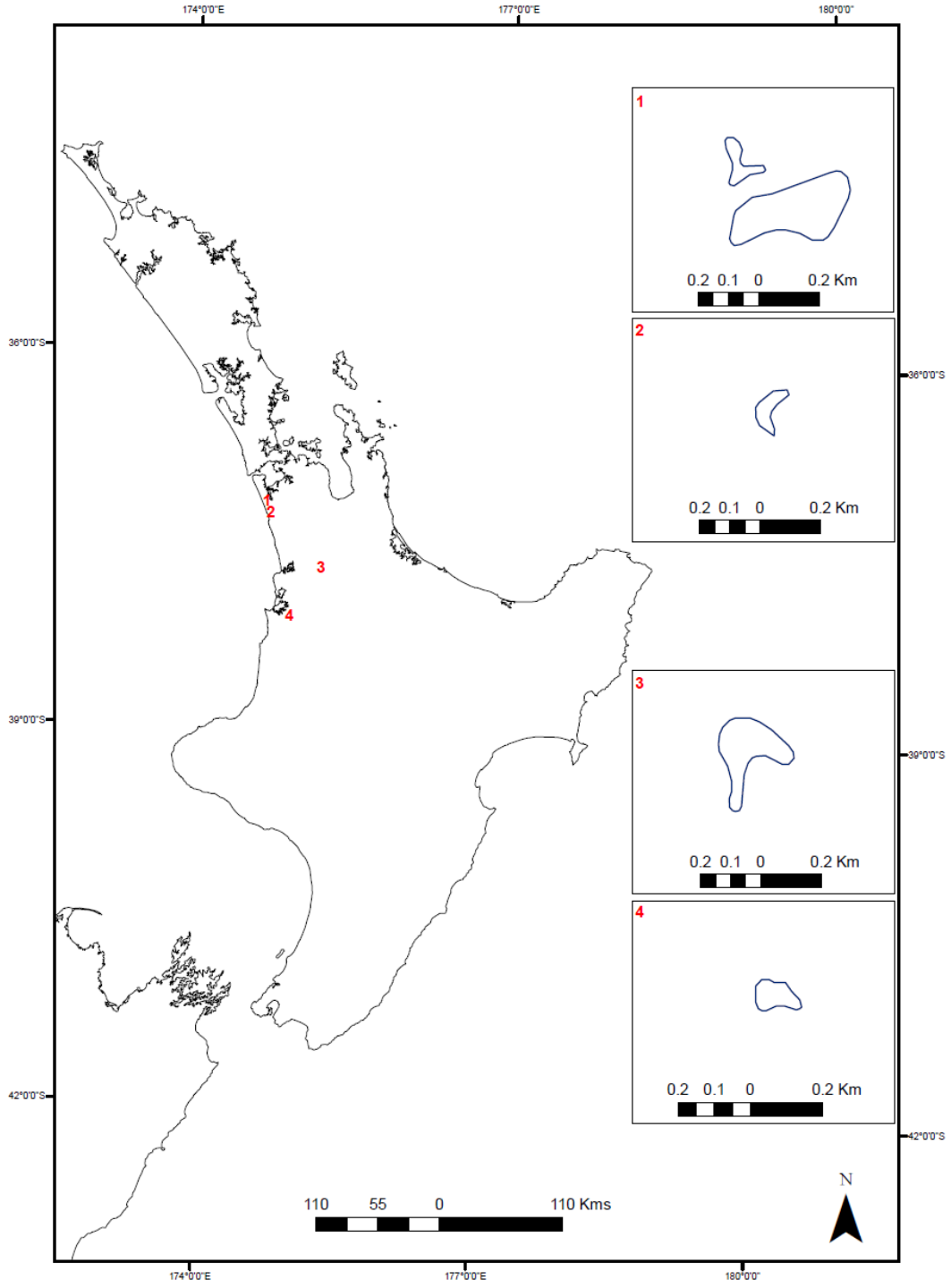


Figure 3.1: A map indicating the locations of the five lake sampling sites in the Waikato region, North Island, New Zealand. Inset images (1) Lakes Rotoiti (top) and Lake Puketi (bottom), (2) Lake Kohahuake (3) Lake Waiwhakareke (4) Lake Koraha.

Table 3.1: Summary of basic characteristics of the study lakes (Dean-Spiers et al. 2014). ND = no data.

	Kohahuake	Koraha	Puketi	Rotoiti	Waiwhakareke
Longitude	174.6845	174.9217	174.6753	174.6748	175.1332
Latitude	-37.3148	-38.1634	-37.2796	-37.2780	-37.4613
Lake area (ha)	1.9	0.8	6.4	1.2	3
Max. depth (m)	7.1	7.0	6.5	6.6	3.0
Catchment area (ha)	108	177	114	62	66
Dominant vegetation cover	Pasture	Native podocarp forest	Pasture	Pasture	Native re-forestation
Total nitrogen (mg L^{-1})	0.86*	0.74**	0.42*	0.28*	1.37***
Total phosphorus (mg L^{-1})	0.06*	0.06**	0.01*	0.03*	0.12***
Chlorophyll *a* (mg L^{-1})	0.01*	0.01**	<0.01*	0.02*	0.05***
Secchi depth (m)	1.4*	ND	5.7*	1.5*	1.03***

***single samples collected or measurements taken as part of the Data Deficient Lakes Survey (WRC, 2015)**

****samples collected by Waikato Regional Council in March 2015**

*****samples collected by Waikato Regional Council in March 2016**

, **,water quality samples analysed by Hill Laboratories, Hamilton**

Lake Puketi

Lake Puketi (Figure 3.1, inset 1; Figure 3.2) is one of a small group of northern coastal dune lakes. The surrounding land is predominantly pasture with 1% of the catchment land cover in native riparian vegetation. Lake Puketi is located in close proximity to Lake Rotoiti (see below); direct stock exclusion from the lake was instigated using fencing in 2016.

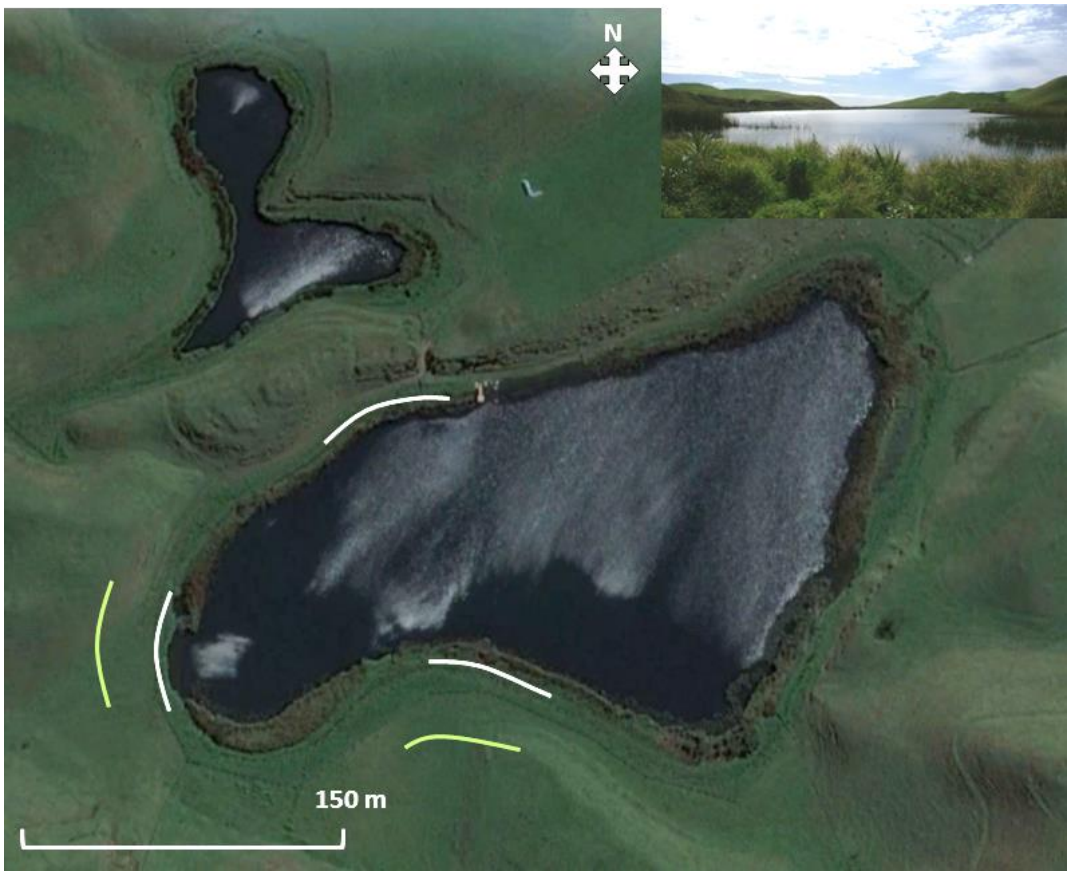


Figure 3.2: Lake Puketi. White lines depict the shoreline sampling transects. Green lines represent approximations of pasture suction transects. The inset photo faces east and was taken during a preliminary site inspection on 31st March 2016.

Lake Rotoiti

Lake Rotoiti is a coastal dune lake located near Lake Puketi (Figure 3.1, inset image 1; Figure 3.3). The surrounding catchment is predominantly pasture with no observable native vegetation cover above 50 cm in height. Stock exclusion fencing was implemented in 2016 around the lake margin (c.1200 m). Because there is insufficient information to rank the lake for its biodiversity values, it is a priority to monitor and assess its values and management requirements as a novel ecosystem (Waikato Regional Council, 2016).

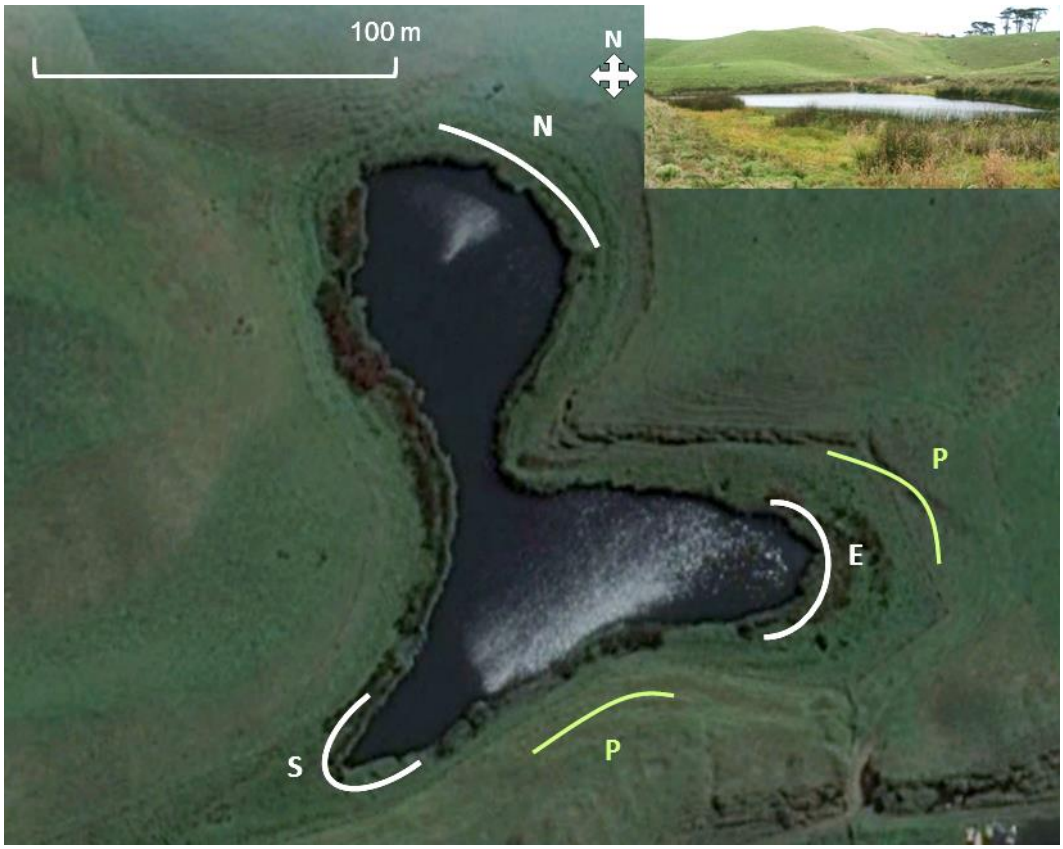


Figure 3.3: Lake Rotoiti. White lines depict the shoreline sampling transects. Green lines represent approximations of pasture suction transects. The inset photo faces west and was taken during a preliminary site inspection on the 31st March 2016.

Lake Kohahuake

Lake Kohahuake (Figure 3.1, inset 3; Figure 3.4) is a small lake (1.9 ha) in a predominantly pastoral catchment (1% native riparian vegetation cover), with an area of wetland on one arm. The lake margin (c. 950 m) is not fully fenced but no livestock have access to graze around its margins. A 30-m stretch of the south-eastern shoreline includes an area of native riparian plants, including mature flaxes (*Phorium tenax*) and cabbage trees (*Cordyline australis*).

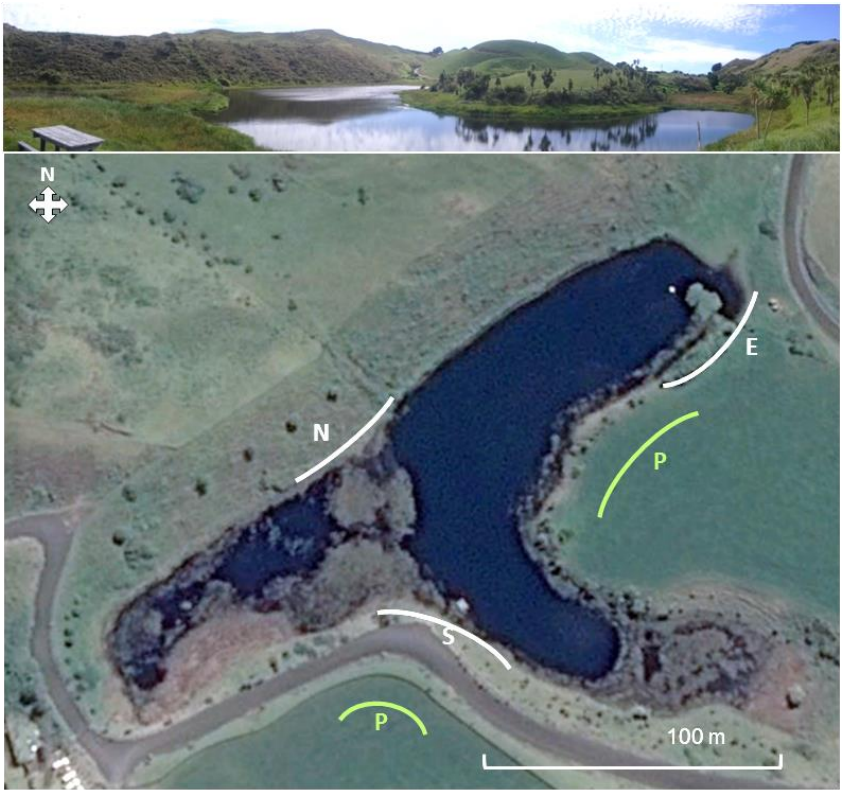


Figure 3.4: Lake Kohahuake. White lines depict the shoreline sampling transects. Green lines represent approximations of pasture suction transect locations. The inset photo faces northeast and was taken during a preliminary site inspection 31st March 2016.

Lake Koraha

Lake Koraha (Figure 3.1 inset 4; Figure 3.3) is a relatively small lake (<1 ha) that is a rare example of an intact, lowland karst lake ecosystem with nationally significant natural character (Waikato Regional Council, 2016). It has predominantly native mixed podocarp-broadleaf vegetation cover in the catchment (68%) and is completely surrounded by native mixed podocarp forest (Figure 3.1, inset image B). However, the lake remains in close proximity to large expanses of pasture habitat.

Figure 3.5: Lake Koraha. White lines depict the shoreline sampling transects. The green line represents an approximation of the single pasture suction transect location. The inset photo faces southeast and was taken during the afternoon of the sampling event, 18th December 2016.

Lake Waiwhakareke

The central feature of the Waiwhakarke Natural Heritage Park is Lake Waiwhakareke, a relatively small peat lake in a predominantly urban/pastoral catchment, located on the margin of the Te Rapa peat bog, Hamilton City (Figure 3.1, inset D; Figure 3.5). The land is now subject to retirement and an ecological restoration programme of native re-vegetation, including cane rush (*Sporadanthus ferrugineus*), harakeke (*Phormium* spp.) kahikatea (*Dacrycarpus dacrydiodes*), kauri (*Agathus australis*), matai (*Prumnopitys taxifolia*), pukatea (*Laurelia novae-zelandia*), raupo (*Typha* spp.), totara (*Podocarpus totara*) and wire rush (*Sporadanthus similis*), covers approximately 75% of the catchment. This programme involves planting and pest control of an area including the lake and its wetland margins.

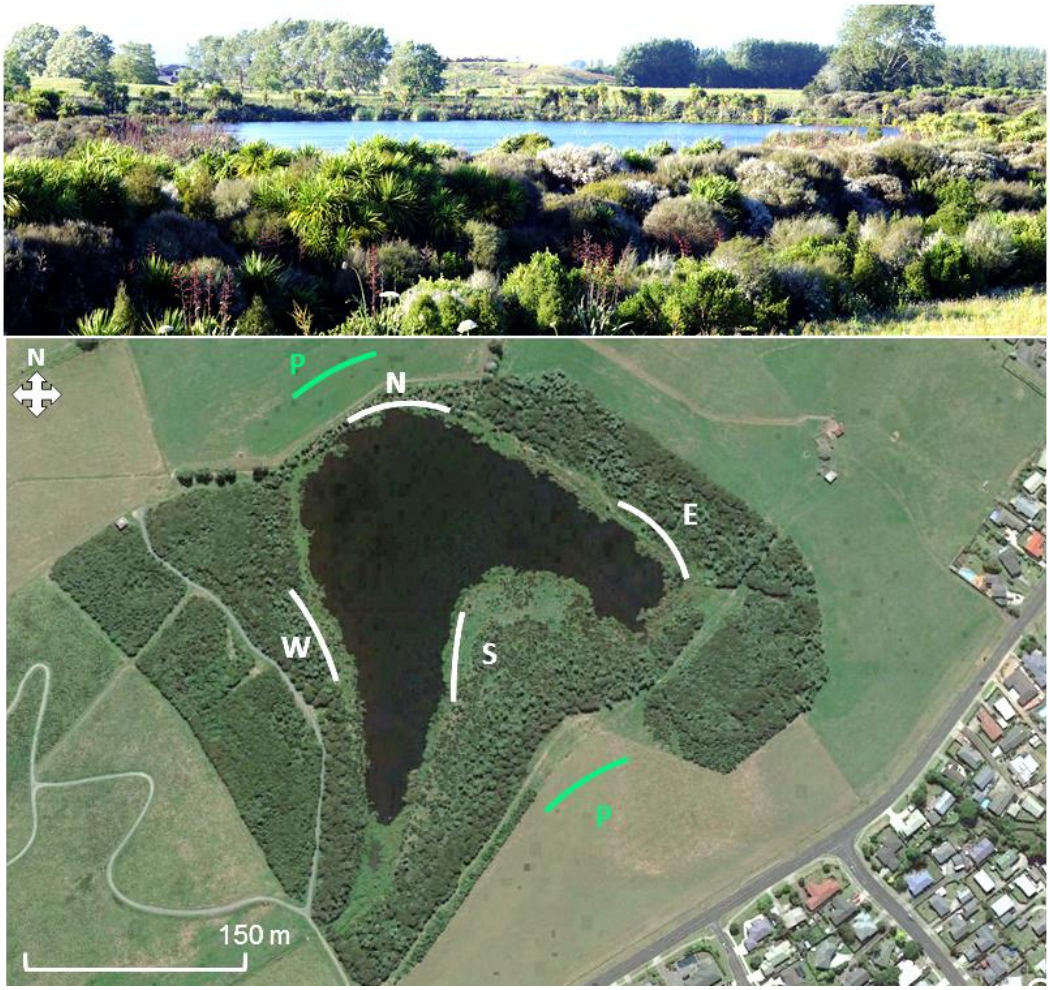


Figure 3.6: Lake Waiwhakareke. White lines depict the shoreline sampling transects. Green lines represent approximations of pasture suction transects. The inset photo faces north and was taken during a site inspection visit on the 15th December 2016.

3.3.2 Collection methods

In order to obtain representative community samples from each lake, sampling locations were standardised to 50-metre length transects on the northern, eastern, southern and western shorelines of each lake (see Figures 3.2-3.6). I aimed for 3-4 shoreline transects and 1-2 pasture transects, depending on the site. Pasture locations were selected with a preference for opposing shores where possible and ran parallel to the shore, at a proximity of 50 metres. However, the eastern shoreline habitat of Lake Puketi, and the western shorelines of lakes Rotoiti and Kohahuake were saturated above ground level, and this prevented samples from being safely obtained in those locations, so fewer but longer transects were collected to maintain consistent sampling effort amongst lakes. Sampling at lakes Kohahuake, Puketi and Rotoiti was conducted during the first week of April, 2016. However, the early onset of winter-like conditions prevented further sampling at these sites. Lakes Koraha and Waiwhakareke were sampled in December 2016 following the onset of summer.

Specimens were collected using two methods. Manual collection involved two researchers visually searching and collecting specimens for a total period of 30 minutes along each 50-m shoreline transect up to 2 metres from the water's edge. Specimens were collected from vegetation, between 30 cm and 200 cm above ground level. The second method involved suctioning with a 33cc Troy-Built (model TB310QS) gas handheld leaf/air blower vacuum with an installed intake filter to collect incoming material. Both methods were used to collect specimens from shoreline transects to enable a comparison of methods, while only suctions sampling was used on pasture transects as vegetation was below 30 cm in height. Suction samples were obtained over a band of 2-5 m width for a total period of 1 minute at each sampling transect. During this process the intake filter was emptied and reset at halfway. All spiders collected using these methods were stored in 100% ethanol.

3.3.3 COI sequences

A tarsal segment, preferably from the 4th leg, was removed from each specimen under magnification and placed in a single well on a 96-well plate for genetic analysis at the Canadian Centre for DNA Barcoding (CCDB), University of Guelph, Canada. The remainder of the specimen was preserved in 100% ethanol. All photographs, collection information, primer combinations and sequence data pertaining to each specimen were uploaded to Barcode of Life Datasystems (BOLD; www.boldsystems.org), housed in the project New Zealand Spiders (NZSPI).

At the CCDB, a 658 base pair fragment of the mitochondrial COI gene was amplified from the tarsal segment using standard protocols (Ivanova, deWaard & Hebert, 2006) for 1 to 22 individuals per species, using the universal forward primer cocktail C_LepFolF (LepF1: 5'-ATTCAACCAATCATAAAGATATTGG-3'; LCO1490:5'GGTCAACAAATCATAAAGATATTGG-3'), and the reverse primer cocktail C_LepFolR (LepR1:5'-TAAACTTCTGGATGTCCAAAAAATCA-3'; HCO2198:5-'TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994; Hebert et al. 2004; Ivanova, deWaard & Hebert, 2006). Barcode Index Numbers (BINs) were assigned in BOLD (Ratnasingham & Hebert, 2013) and used as a measure of MOTUs, representing individual species.

Key steps in the analysis of large DNA sequence datasets are (i) the clustering of sequences based on their similarity, and (ii) providing an index reference for each sequence cluster (BIN), which forms the basis for subsequent biodiversity analyses. Clustering reduces the complexity of the data and limits the effects of PCR and sequencing errors on biodiversity estimates, as sequences with a modest number of errors would be grouped together and treated as a MOTU. A genetic species identifying threshold of 98% similarity has been previously demonstrated for spider species clustering internationally using the COI gene (Barrett & Hebert, 2005; Vink et al. 2009), and was used as a species delineation guideline for the purposes of this study.

COI sequences were aligned using Muscle (Edgar, 2004) and the alignment was subsequently pruned to 550 base pairs in Geneious R10 v10.1.2 (Drummond et al. 2010; Kearse et al. 2012). Neighbour-joining analyses using MEGA v5.05 examined relationships among taxa. Non-parametric bootstrap analysis (Felsenstein, 1985) was implemented with 1,000 pseudo-replicates to assess support for nodes in the tree and all bootstrap values <70% were removed. We measured the success of the classification by determining whether each test sequence grouped most closely with other representatives of the same species. For the purpose of this study, all MOTUs were categorised as either those with recognised international distributions (referred to hereafter as cosmopolitan), or described and unidentified native species.

3.3.4 Statistical analysis

Non-metric multidimensional scaling (nMDS) and permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) were used to detect patterns in taxa presence-absence for native and cosmopolitan spiders between different lakes, habitats (shoreline vs. pasture), and the two collection methods (suction vs. manual collections). For analysis of community composition in respect to collection methodology, only shoreline manual and suction samples were used as both methods were used at all shoreline transects. Shoreline suctions were compared with pasture suction samples to assess the effects of habitat type, while shoreline and suction data were combined for comparisons among lakes. For the purpose of these analyses, transects of each lake were analysed individually to account for spatial heterogeneity of spider communities within lake locations. nMDS was performed on a dissimilarity matrix based on the Gower distance calculated on presence/absence data. nMDS is an unconstrained ordination method based on the dissimilarities among samples as defined by the distance matrix using a stress value as a measure of the goodness of the ordination fit relative to the dissimilarity matrix. Stress values were low enough (≤0.15, see Results) to adequately represent patterns in two dimensional ordination space (McCune, Grace & Dean, 2002).

PERMANOVA was applied to the distance matrix underlying the nMDS ordinations. PERMANOVA is a non-parametric permutation test to examine *a priori* hypotheses under the null hypothesis of no differences between groups (Anderson, 2001). PERMANOVA provides F-ratios that are analogous to Fisher's F-ratio in multivariate analysis of variance (Anderson, 2001). For the PERMANOVA, I used permutation of residuals under a reduced model with 999 permutations and a Type III sums of squares method. nMDS and PERMANOVA were performed using the PRIMER 7 statistical software package with the PERMANOVA+ add-on package (Primer-E Ltd, Plymouth, U.K., version 7.0). Where main effects were significant, Monte Carlo post-hoc tests were used to determine pairwise differences.

The Shapiro-Wilk statistic was used to determine whether the species richness of native and cosmopolitan groups collected along transects were normally distributed. As untransformed and log transformed data failed this test, a Kruskal-Wallis non-parametric one-way analysis of variance was conducted using Systat v. 13.00.05 to test for differences among lakes. Where a significant effect of lake on shoreline species richness was detected, the Dwass-Steel-Critchlow-Flinger test for all pairwise comparisons was conducted to determine any significant difference between lakes. Wilcoxon rank sum tests were conducted to compare average species richness by sampling method and habitat type.

3.4 Results

3.4.1 Barcoding

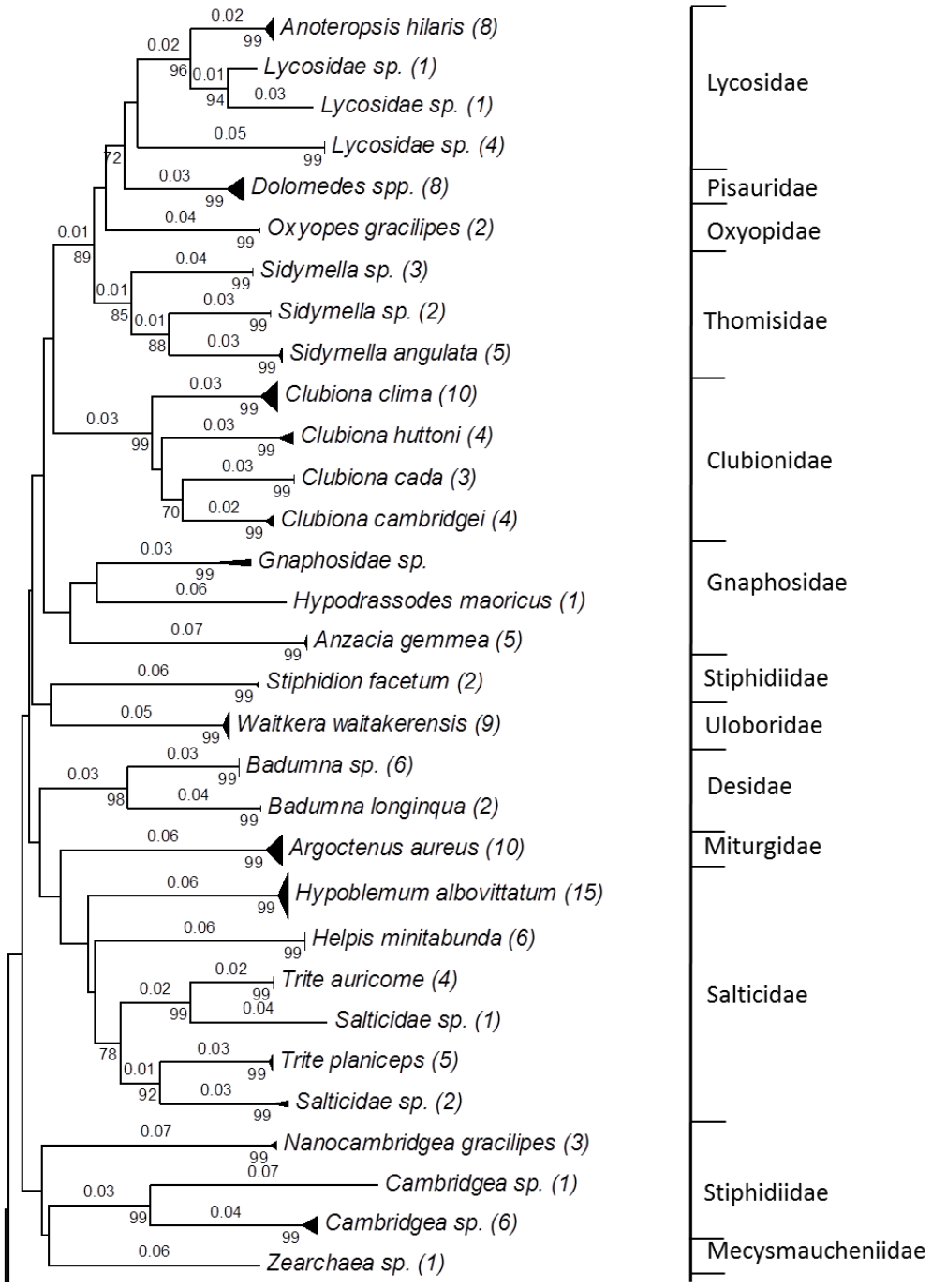
A total of 337 barcodes were of sufficient length to make a comparative examination of a 550bp region of the COI gene. The sequencing DNA barcoding failure rate (11%) experienced during this study was within the typical bounds of other barcoding studies (Hogg & Hebert, 2004; Barrett & Hebert, 2005; Hogg et al. 2010; Duggan et al. 2012). Instead of randomly selecting 1 sequence to represent each MOTU, all 337 sequences of sufficient length were included by collapsing subtrees with ≤2% (≤ 0.02) sequence divergence (Barrett & Hebert, 2005). Subtrees were collapsed

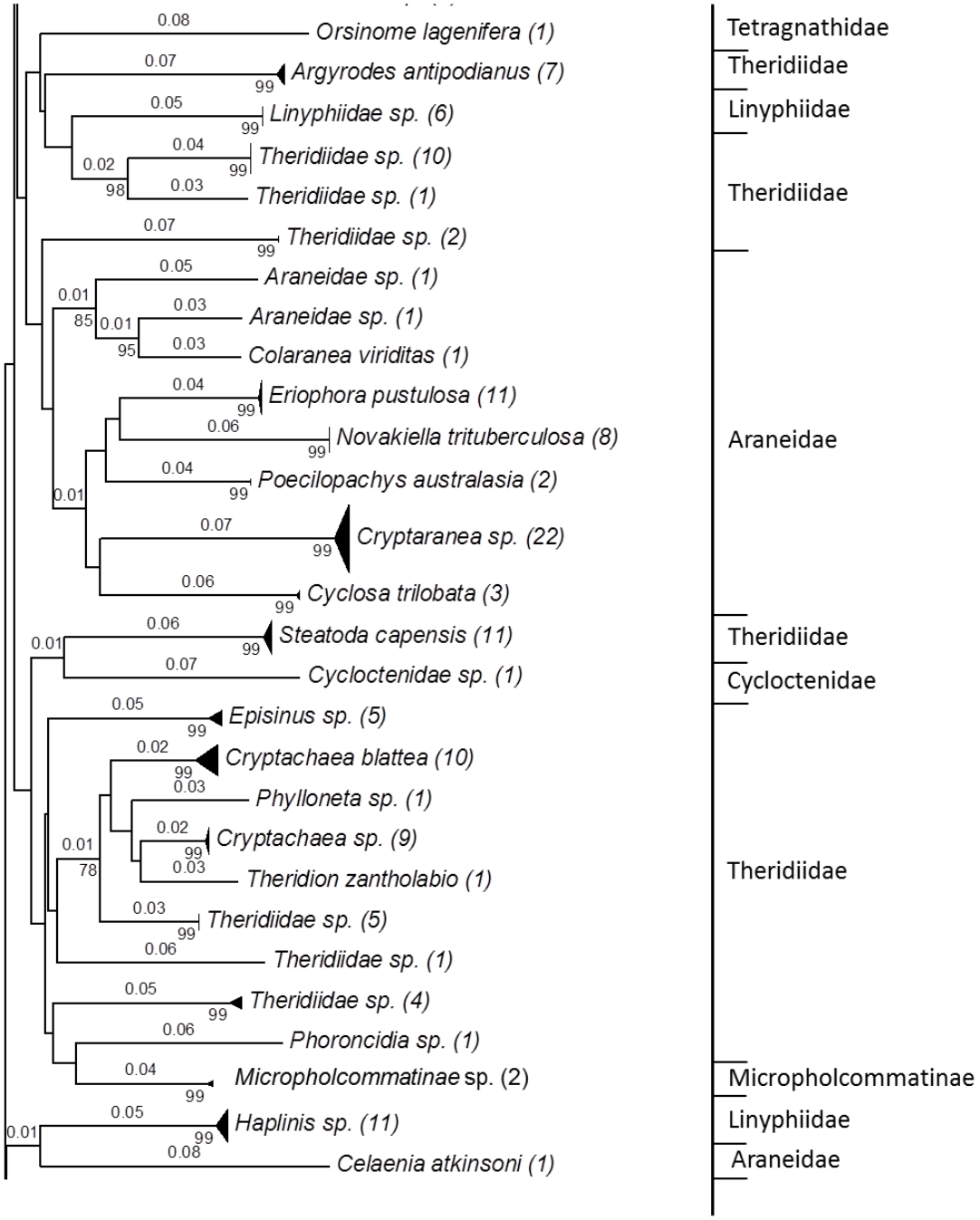
because the fewer taxa that are included in a tree the more challenging it can be to place newly-added taxa into the correct group (Zwicki & Hillis, 2002).

The resulting Neighbour-Joining profile contains 75 terminal nodes (Figure 3.7). Each of these nodes corresponds to a separate MOTU, revealing the presence of 38 described species, represented by 194 individual specimen barcodes. A further 143 COI sequences correspond to an additional 41 morphospecies whose identification was considered cryptic and lacking formal identification beyond family or genus level. Nine specimens belonging to a single, morphologically-identified species (*Tetragnatha* sp.) failed to successfully barcode; 2 of these 9 were successfully barcoded but both sequences were flagged as contaminated by the presence of an endosymbiotic proteobacteria of the order Rickettsiales.

I tested the ability of this profile to recognise and discriminate cryptic species from described species by including multiple sequences for as many MOTUs as possible in the analysis. The Neighbour-joining profile was subsequently used as a classification tool by re-running and incorporating into the analysis a further 143 sequences pertaining to the 41 confounding species, each with its own unique BIN (Figure 3.7). Four morphospecies that failed to barcode have been included in the community composition and species distribution analyses as they represent different morphospecies and make up the total of 79 species in this study: (1) *Tetragnatha* sp., commonly observed and collected from the marginal habitat of the northern dune lakes and Lake Waiwhakareke; (2) *Diaea ambara* (Urquhart, 1885), a native ambush predator spider collected from riparian vegetation near the shoreline of Lake Waiwhakareke; (3) A single cryptic *Hexathele* sp. specimen collected in a suction sample obtained from Lake Koraha's shoreline (members of the *Hexathele* genus are ground-dwelling predators associated with forested habitats); and (4) Two *Rhomphaea urquharti* (Bryant, 1933), an endemic predator of other spiders, collected from the shoreline of Lake Waiwhakareke.

A 2% species divergence threshold for identification and delineation of species revealed that 100% of sequences grouped most closely with other representatives from the same MOTU, supporting this divergence threshold in this instance (Barrett & Hebert, 2005). I tested the ability of the Neighbour-joining profile to recognise and discriminate between confounding specimens from all life stages and both sexes, from both the recognised and cryptic species, and determined that 20 of the MOTUs in this study were represented by a single specimen barcode (singletons). Of these singletons, 5 were identified in this survey by male specimens, 6 by female specimens and 9 by juvenile specimens only. Of all MOTU represented by multiple barcode sequences in this study, 35 were identified by specimens pertaining to both sexes, 9 to male specimens and only 12 to female specimens (traditional morphological identification methods can often only be used to identify mature females, due to the lack of description of males associated with many native species. Interestingly, 14 of the singletons (70%) were recorded solely around the margins of Lake Koraha.


Anoteropsis hilaris (8)
Lycosidae sp. (1)
Lycosidae sp. (1)
Lycosidae sp. (4)
Dolomedes spp. (8)
Oxyopes gracilipes (2)
Sidymella sp. (3)
Sidymella sp. (2)
Sidymella angulata (5)
Clubiona clima (10)
Clubiona huttoni (4)
Clubiona cada (3)
Clubiona cambridgei (4)
Gnaphosidae sp.
Hypodrassodes maoricus (1)
Anzacia gemmea (5)
Stiphidion facetum (2)
Waitkera waitakerensis (9)
Badumna sp. (6)
Badumna longinqua (2)
Argoctenus aureus (10)
Hypoblemum albovittatum (15)
Helpis minitabunda (6)
Trite auricome (4)
Salticidae sp. (1)
Trite planiceps (5)
Salticidae sp. (2)
Nanocambridgea gracilipes (3)
Cambridgea sp. (1)
Cambridgea sp. (6)
Zearchaea sp. (1)
Lycosidae
Pisauridae
Oxyopidae
Thomisidae
Clubionidae
Gnaphosidae
Stiphidiidae
Uloboridae
Desidae
Miturgidae
Salticidae
Stiphidiidae
Mecysmaucheniidae


Orsinome lagenifera (1)
Argyrodes antipodianus (7)
Linyphiidae sp. (6)
Theridiidae sp. (10)
Theridiidae sp. (1)
Theridiidae sp. (2)
Araneidae sp. (1)
Araneidae sp. (1)
Colaranea viriditas (1)
Eriophora pustulosa (11)
Novakiella trituberculosa (8)
Poecilopachys australasia (2)
Cryptaranea sp. (22)
Cyclosa trilobata (3)
Steatoda capensis (11)
Cycloctenidae sp. (1)
Episinus sp. (5)
Cryptachaea blattea (10)
Phylloneta sp. (1)
Cryptachaea sp. (9)
Theridion zantholabio (1)
Theridiidae sp. (5)
Theridiidae sp. (1)
Theridiidae sp. (4)
Phoroncidia sp. (1)
Micropholcommatinae sp. (2)
Haplinis sp. (11)
Celaenia atkinsoni (1)
Tetragnathidae
Theridiidae
Linyphiidae
Theridiidae
Araneidae
Theridiidae
Cycloctenidae
Theridiidae
Micropholcommatinae
Linyphiidae
Araneidae

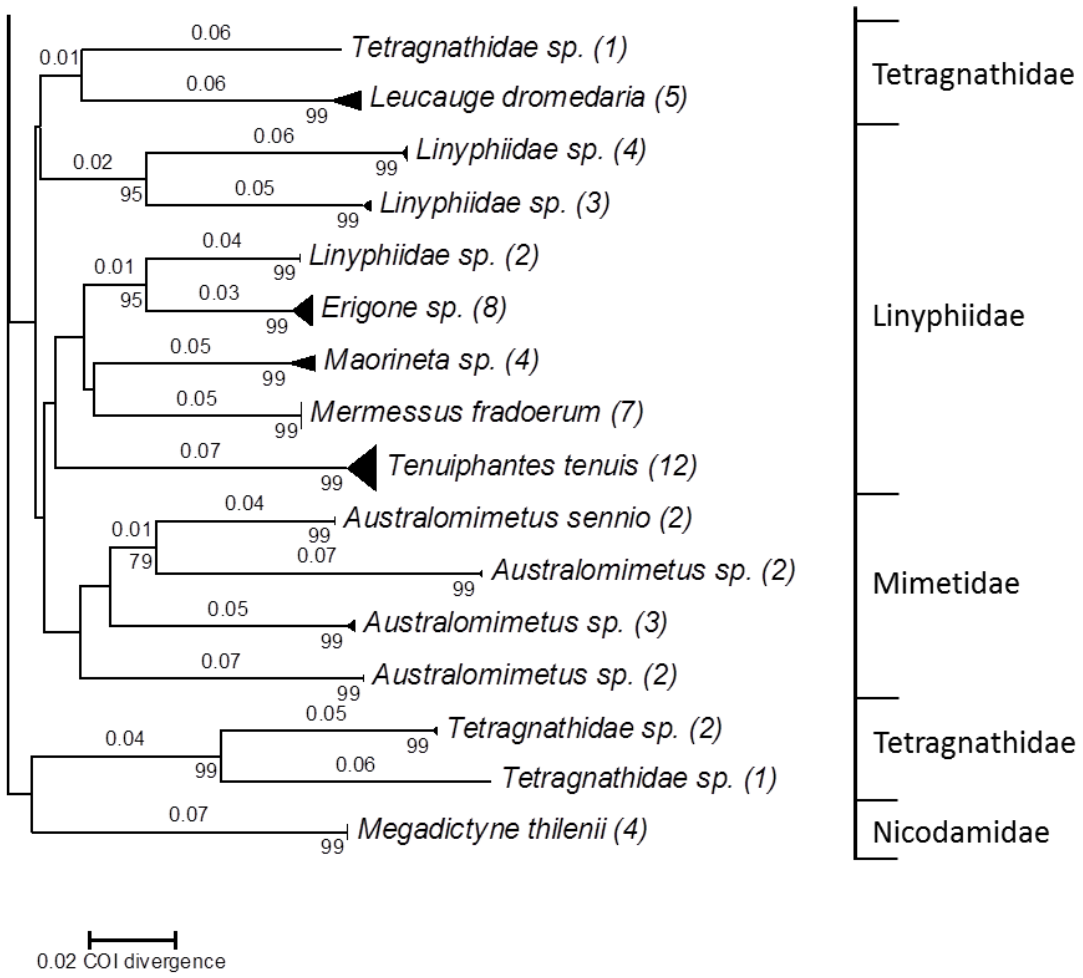


Figure 3.2: Neighbour-joining analysis grouping COI haplotype diversity with morphology-based names applied. An identity threshold of 98% is accepted to cluster COI sequence species groupings. For each taxon the vertical height of the triangle is proportional to number of specimen sequences and the horizontal length represents maximum genetic diversity within the branch. Numbers below branches indicate nonparametric bootstrap support (>50%) from 1000 pseudo-replicates. Numbers above branches indicate COI divergence between nodes. Values in parentheses indicate the number of individuals with identical sequences. Italicised names are binomial nomenclature for genus and species designations. Non-italicised names indicate cryptic species unidentified beyond family resolution. Family names are recorded to the right of the profile.

3.4.2 Biodiversity patterns

The spider communities associated with the 5 study lakes were comprised of 5 species-rich families, each represented by 6 or more species in this study: Araneidae, Linyphiidae, Salticidae, Tetragnathidae and Theridiidae (refer Figure 3.8). Of these, the Araneidae, Linyphiidae and Theridiidae families were represented by a diverse array of described species (refer to Appendix C for photographic examples). The distribution of these families amongst the lakes illustrates that cosmopolitan species of the Theridiidae

and Araneidae family were most common at the pasture-dominated lakes: Kohahuake, Puketi and Rotoiti. However, no cosmopolitan members of the Theridiidae family were recorded at Lake Kohahuake. Regardless, Theridiidae was the most species-diverse family in this study, represented by 10 native and 5 cosmopolitan species across the sampling sites. Notably, Lake Koraha supported a diverse range of native species from all 5 families and supported the greatest numbers of native species from all lakes. Of these families, 4 are considered generalist predators, using webs as their primary method to capture prey (Araneidae, Linyphiidae, Theridiidae, Tetragnathidae), whereas members of the Salticidae family, commonly termed 'jumping spiders', are mobile predators who stalk and pounce upon their prey in ambush.

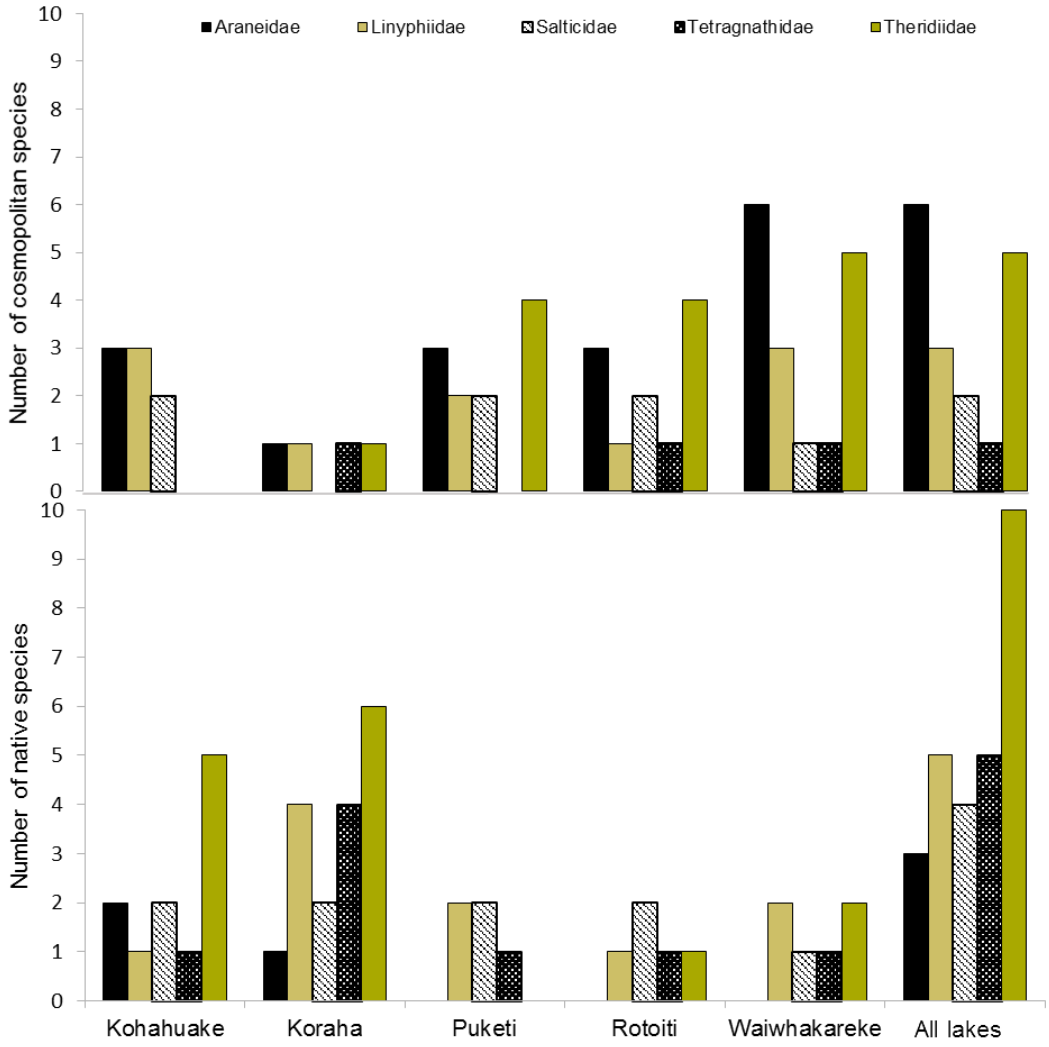


Figure 3.3: The distribution of combined native (bottom) and cosmopolitan (top) species across the 5 study lakes from the 5 most species-diverse families (≥6 species) represented in this study. All lakes indicates the total number of native or cosmopolitan species represented by each family.

Samples from all 5 lakes shared 2 native species (*Haplinis* sp. and *Dolomedes minor* (L.Koch, 1876) and 1 cosmopolitan species (*Eriophora pustulosa* Walckenaer, 1841), whereas lakes Kohahuake, Puketi, Rotoiti and Waiwhakareke shared 3 native species (*Anoteropsis hilaris* L. Koch, 1877; *Clubiona clima* Forster, 1979; *Trite planiceps* Simon, 1899) and 7 cosmopolitan species (*Argyrodes antipodianus* O.Pickard-Cambridge, 1880; *Cryptachaea* sp., *Cryptaranea* sp., *Helpis minitabunda* L. Koch, 1880; *Mermessus fradoerum* Berland, 1932; *Novakiella trituberculosa* Roewer, 1942; *Steatoda capensis* Hann, 1990) (refer to Appendices B1-5). Lake Koraha had the greatest level of diversity, supporting 36 native and 5 cosmopolitan species. Of these 5 cosmopolitan species, 2 were collected only from the shoreline habitat surrounding the lake (*Leucauge dromedaria* Thorell, 1881; *E. pustulosa*) and the other 3 were collected from the adjacent pasture habitat (*Anzacia gemmea* Dalmas, 1917; Theridiidae sp; *Tenuiphantes tenuis* Blackwall, 1852). By comparison, Lake Rotoiti supported the lowest diversity with 8 native and 15 cosmopolitan species in the community. Lakes Puketi, Rotoiti, Kohahuake and Waiwhakareke supported similar proportions of cosmopolitan species in their respective catchments when compared to Lake Koraha: Lake Puketi 56%, Lake Rotoiti 65%, Lake Kohahuake 60%, Waiwhakareke 63%, Koraha 12%.

In order to test for disparities in community composition of spiders collected at each shoreline transect, species obtained using manual collections and shoreline suctions were combined and the mean number of species present at each shoreline site was determined. The mean species richness collected at each shoreline site highlights a similar richness of cosmopolitan species at lakes Kohahuake, Puketi, Rotoiti and Waiwhakareke (Figure 3.9). A non-parametric Kruskal-Wallis test revealed no significant effect of lake on shoreline richness of cosmopolitan species (H = 9.06; P = 0.06). However, there was a significant effect of lake on shoreline native species richness (H = 10.18, P = 0.04) although Dwass-Steel-Critchlow-Flinger tests did not reveal any pairwise difference between lakes.

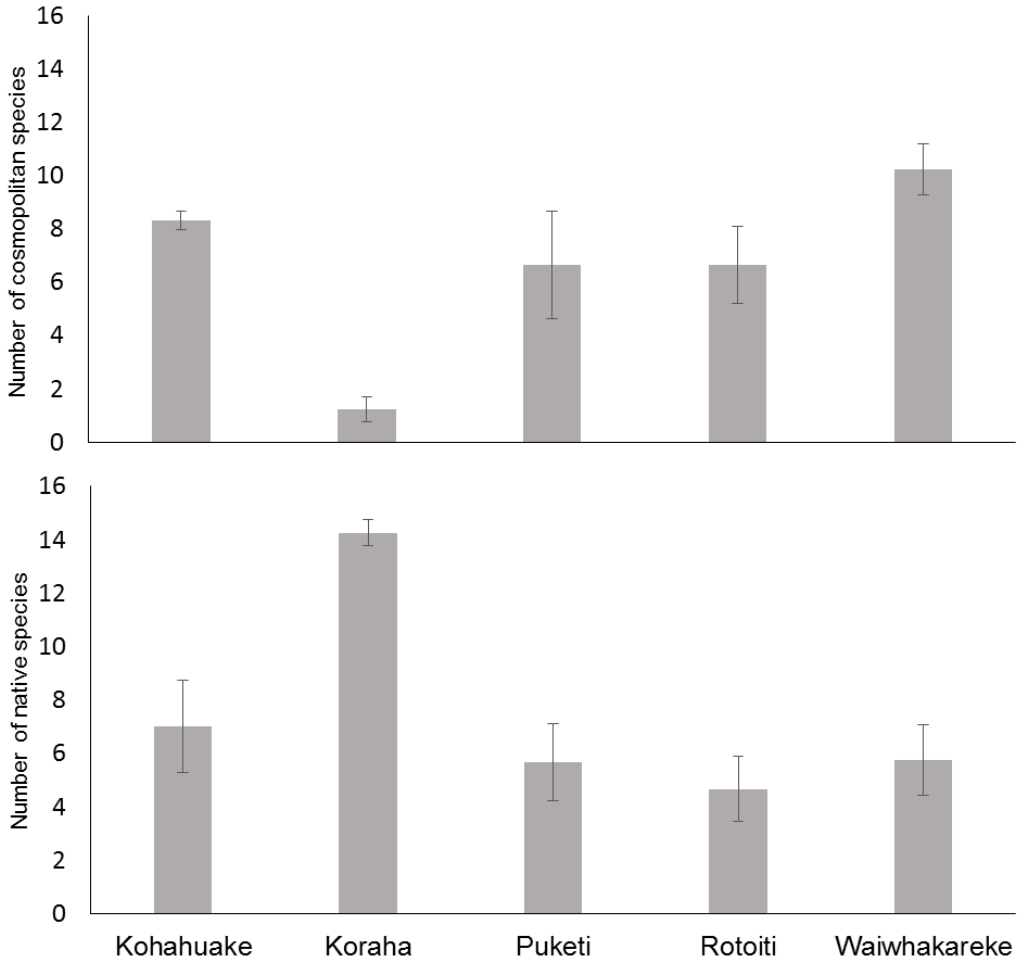


Figure 3.4: Mean (±SE) number of native (bottom) and cosmopolitan (top) species collected across the shoreline sampling sites of each lake (suction and manual sampling combined).

A comparison of native versus cosmopolitan species richness obtained using suction samples was made among lake pasture transects and their nearest respective shoreline transects. The mean number of native and cosmopolitan species were compiled separately, and together, in order to compare the representative communities collected between these two habitat types. The result of this comparison shows that there were more species found in shoreline habitats than pasture, and the composition of shoreline communities was comprised of a similar number of native and cosmopolitan species (Figure 3.10). Data normality was apparent between native and cosmopolitan species, however, it was not confirmed when native and cosmopolitan species were combined. A Wilcoxon Signed-Rank test was then performed and showed significant difference is spider community richness and inferred higher species richness in shoreline

habitat when native and cosmopolitan taxa were combined (Z = -2.08; P = 0.01)

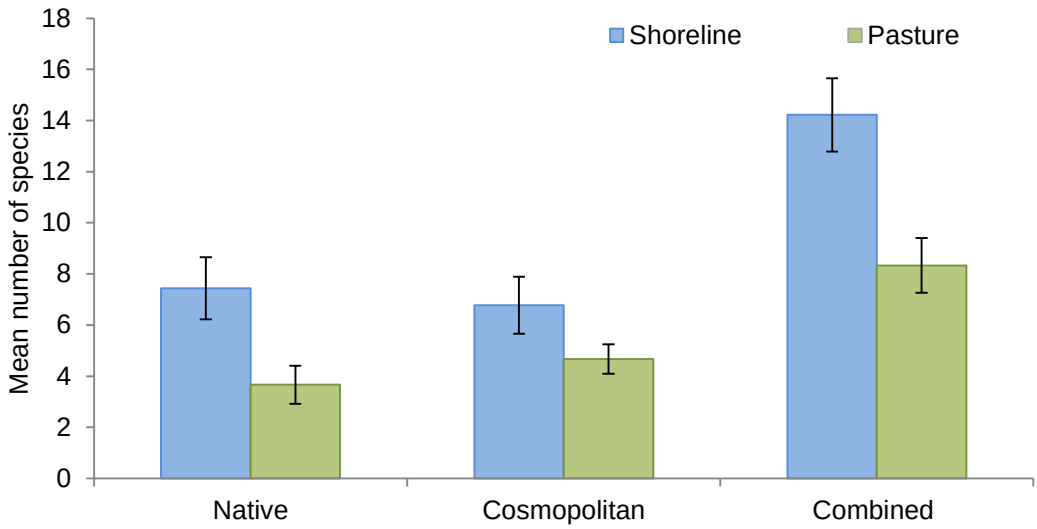


Figure 3.5: Mean (±1SE) species richness collected from suction samples obtained from pasture transects and their nearest adjacent shoreline transects, comparing native, cosmopolitan and combined (native + cosmopolitan) species.

A comparison between suction and manual sampling methods, used to obtain samples in shoreline habitats, was made to determine whether resulting native and cosmopolitan species richness was comparable between methods (Figure 3.11). The mean number of native and cosmopolitan species were compiled separately, and together, in order to compare the efficacy of these methods. The result of this comparison highlights that manual collections were more effective, yielding greater species richness than suction samples. The Shapiro-Wilk statistics revealed that data normality could not be confirmed for manual collections of native species and suction collections of cosmopolitan species. Therefore a Wilcoxon Signed-Rank test was conducted and confirmed that manual collections yielded significantly more native species (Z = 2.171, P = 0.03), cosmopolitan species (Z = 2.04, P = 0.04) and combined species richness (Z = 2.59, P = 0.01) than suction samples from shoreline habitats.

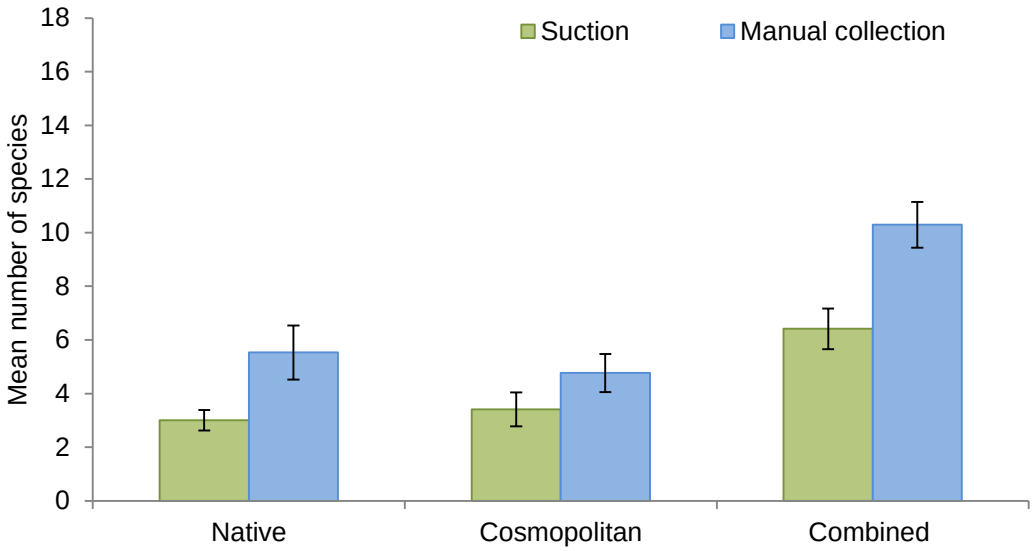


Figure 3.6: Mean (±1SE) number of species collected among shoreline transects using suction and manual collection methods, compared to combined native and cosmopolitan species richness.

3.4.3 Habitat associations and rare species

Species inhabiting solely shoreline habitat across all the study lakes were considered shoreline specialists for the purposes of this study, whereas species inhabiting solely pasture habitat across all lakes were considered pasture specialists. Furthermore, species observed inhabiting both shoreline and pasture habitat across multiple lake locations were considered generalists and species collected from single transects were considered rare (Figure 3.12). Lake Koraha's pasture community was comprised of 7 native and 3 cosmopolitan species. Of these, 3 native species (*Tetragnatha* sp., Theridiidae sp., *Maorineta* sp.) were sampled solely in the adjacent pasture habitat at Lake Koraha, and *Maorineta* sp. was also recorded around Lake Koraha's shoreline habitat. Two of the native species found in Lake Koraha's pasture habitat were common to all the study lakes: *Haplinis* sp. and *Dolomedes minor* (L. Koch, 1876).

Lake Waiwhakareke's shoreline community supported 6 'rare' native species, 4 of which had comparable specimen sequences in their respective BINs which were collected from forested habitat in the Waikato region, while the other two species represent newly founded BINs in BOLD (www.boldsystems.org; Ratnasingham & Hebert, 2007; Ratnasingham & Hebert, 2013). At Lake Waiwhakareke, five "rare"

cosmopolitan species were collected from shoreline habitat: *Celaenia atkinsoni* (O. Packard-Cambridge, 1880), *Poecilopachys australasia* (Griffith & Pidgeon, 1833), *Stiphidion facetum* (Simon, 1902), *Badumna longinqua* (L. Koch, 1867), and *Cyclosa trilobata* (Urquhart, 1885). Although classed as rare for the purposes of this study, additional sequences pertaining to these five MOTUs were available for comparison in BOLD.

Contrastingly, pasture transects supported relatively few species and were inhabited by cosmopolitan pasture specialists and generalist species found across multiple lakes in this study. In total 27 species were recorded in adjacent pasture habitat, 15 of which were considered cosmopolitan.

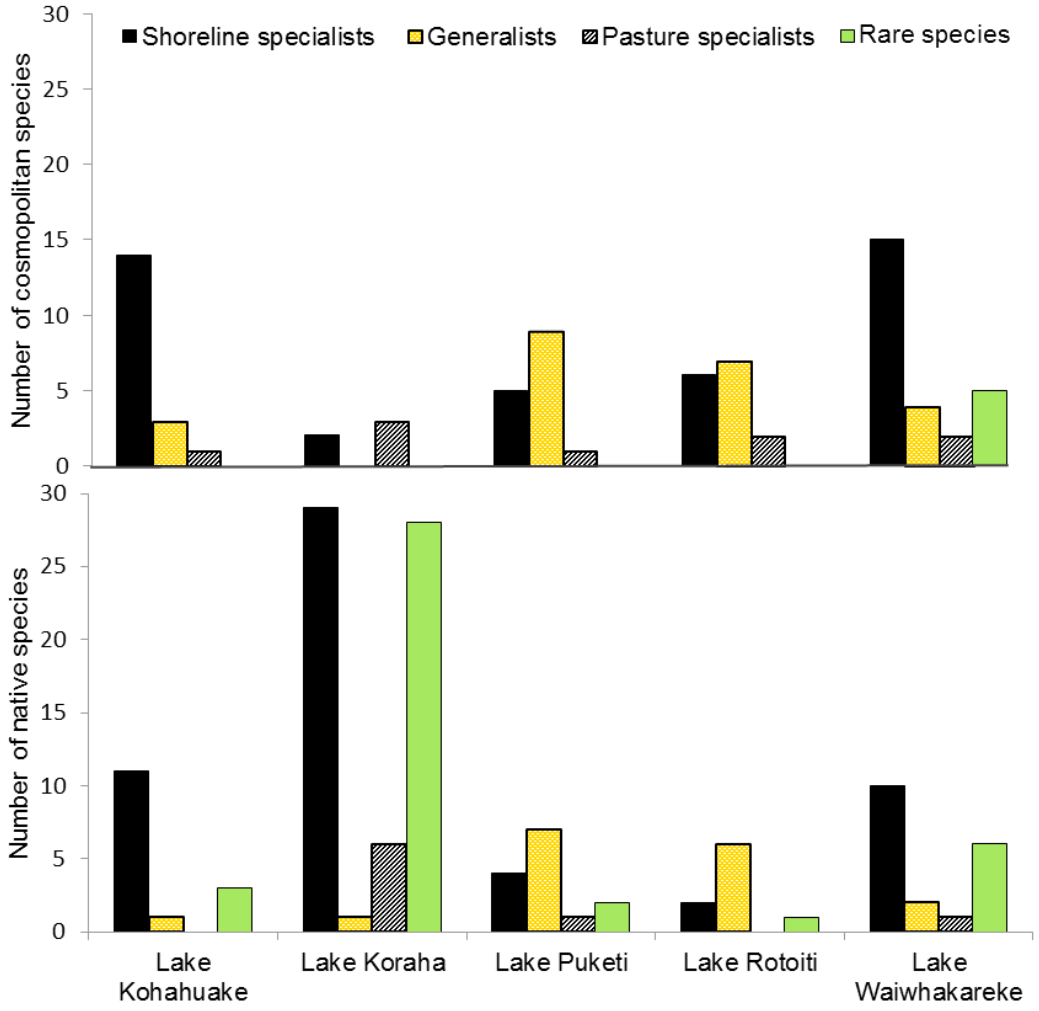


Figure 3.7: The habitat affinity of native (bottom) and cosmopolitan (top) species captured at each lake. Shoreline and pasture habitat specialists were found solely in one habitat class, while generalist species were found in both habitats across the 5 study lakes. “Rare” species are those found at only one transect.

3.4.4 Community structure

3.4.4.1 Comparison among lakes

The distributions of cosmopolitan and native spider species collected from shoreline transects and pasture transects, using suction sampling, were compared among lakes in separate two-dimensional ordination analyses (Figures 3.13-3.14). The relatively low stress values of the nMDS plots indicate that the ordinations provided a good representation of the relationships amongst the lake sites in two-dimensional ordination space. In the cosmopolitan species plot (Figure 3.13), the dissociation of Lake Waiwhakareke from the corresponding pasture site and from other lake sites was apparent, while Lake Koroha sites all grouped close together irrespective of habitat type. Heterogeneity of species composition was evident amongst shoreline sites of other lakes where individual transects were widely separated (e.g., Lake Kohahuake) (Figures 3.13-3.14).

The results from PERMANOVA, examining the differences in cosmopolitan species composition among lakes for suction and manual collections combined, support the finding that species composition differed between lakes (Table 3.2A; P <0.01). Lakes Waiwhakareke and Koraha differed significantly from the spider assemblages of lakes Rotoiti and Puketi, however, there were no significant differences between these lakes and the cosmopolitan spider community associated with Lake Kohahuake, which similarly did not show a significant difference from the species compositions associated with lakes Puketi and Rotoiti (Table 3.2B).

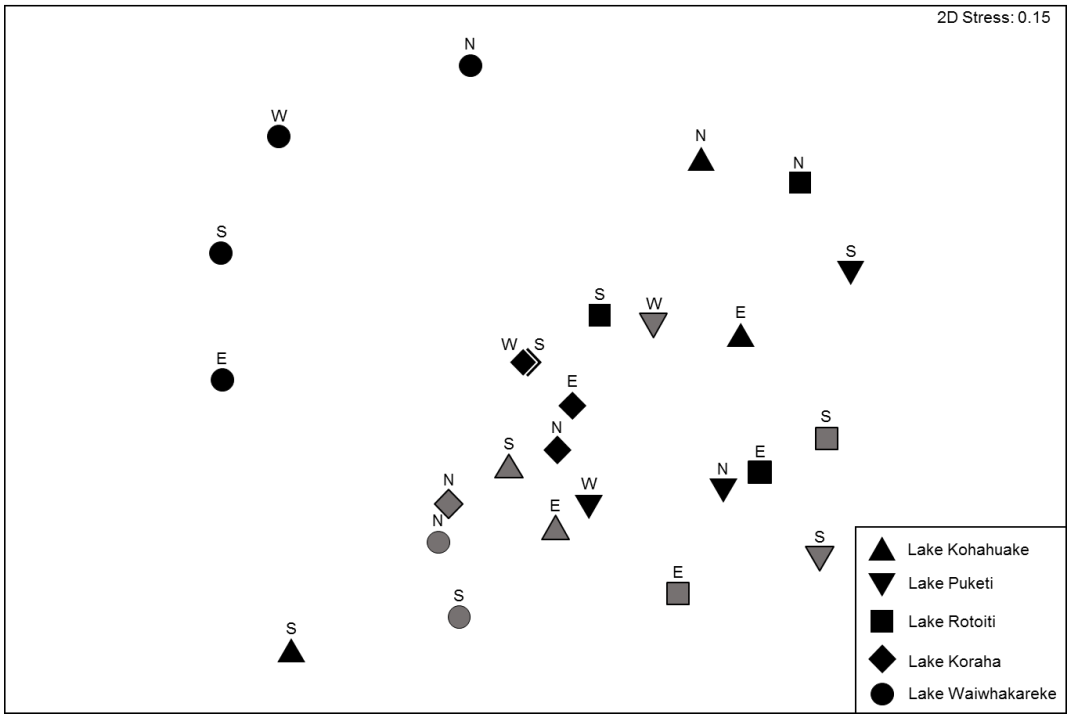


Figure 3.8: Non-metric multidimensional scaling ordination plot of similarity between cosmopolitan spider communities based on species' presence-absence for each shoreline sampling site (Black symbols; N, E, S, W) and the corresponding pasture sites (Grey symbols) from lakes Kohahuake, Koraha, Puketi, Rotoiti and Waiwhakareke.

Table 3.2: Summary of PERMANOVA examining differences in cosmopolitan spider species composition across the study lakes. Significant *P*-values are in bold ($P \leq 0.05$).

A. Global test

Factor	DF	SS	Square root estimates of components of variation	Pseudo-*F*	*P*-value (Monte Carlo)
Lake	4	5674.30	1418.60	2.74	**0.01**
Residual	21	10855.00	516.90		
Total	25	16529.00			

B. Pair-wise comparison

Group	*t*-value	*P*-value (Monte Carlo)	Average % dissimilarity between lakes
Kohahuake vs. Puketi	0.93	0.52	32.64
Kohahuake vs. Rotoiti	1.52	0.11	36.48
Kohahuake vs. Koraha	1.16	0.28	28.00
Kohahuake vs. Waiwhakareke	1.21	0.22	39.60
Puketi vs. Rotoiti	0.92	0.54	30.56
Puketi vs. Koraha	2.38	**<0.01**	30.56
Puketi vs. Waiwhakareke	2.07	**<0.01**	44.67
Rotoiti vs. Koraha	2.44	**<0.01**	29.92
Rotoiti vs. Waiwhakareke	2.07	**0.01**	43.67
Koraha vs. Waiwhakareke	2.02	**0.05**	34.27

In the two-dimensional native species ordination plot (Figure 3.14) the dissociation of Lake Koraha with the other lakes was clear, although samples from the respective pasture transects more closely resembled the communities associated with other lakes sites. Further, the shoreline sites of Lake Koraha showed considerable heterogeneity amongst their respective spider communities, especially the eastern shoreline.

The results from PERMANOVA, examining the differences in native spider species composition across the lake communities, support the finding that the native spider assemblage, inclusive of pasture species, differed significantly between the study lakes (P <0.01) (Table 3.3A). Pair-wise analyses determined that lakes with more vegetated shorelines, Koraha

and Waiwhakareke, were comprised of native species compositions that differ significantly from the other lakes and from each other (Table 3.3B).

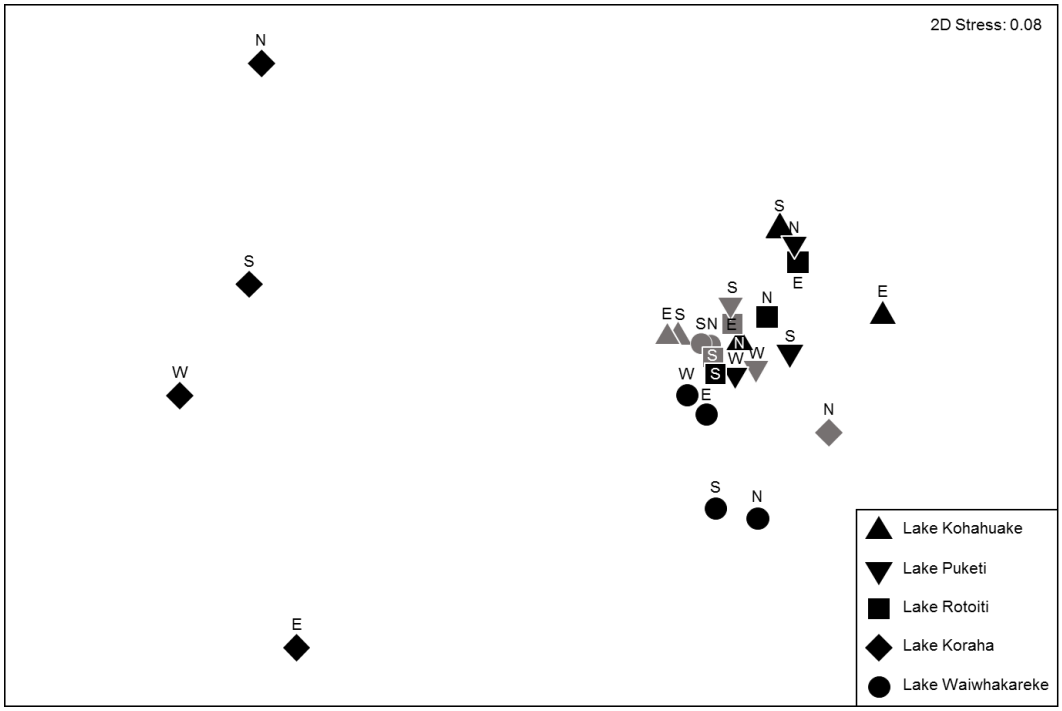


Figure 3.9: Non-metric multidimensional scaling ordination plot of similarity between native spider communities based on species' presence-absence for each shoreline sampling site (Black symbols; N, E, S, W) and the corresponding pasture sites (grey symbols) from lakes Kohahuake, Koraha, Puketi, Rotoiti and Waiwhakareke.

Table 3.3: Summary of PERMANOVA examining differences in native spider species composition between the study lakes. Significant *P*-values are in bold ($P \leq 0.05$).

A. Global test

Factor	DF	SS	Square root estimates of components of variation	Pseudo-*F*	*P*-value (Monte Carlo)
Lake	4	2765.90	691.48	4.79	**<0.01**
Residual	21	3029.80	144.28		
Total	25	5795.80			

B. Pair-wise comparison

Group	*t*-value	*P*-value (Monte Carlo)	Average dissimilarity between lakes
Kohahuake vs. Puketi	0.85	0.55	11.26
Kohahuake vs. Rotoiti	0.60	0.73	9.41
Kohahuake vs. Koraha	2.28	**0.02**	31.49
Kohahuake vs. Waiwhakareke	2.12	**0.03**	14.14
Puketi vs. Rotoiti	0.63	0.70	8.23
Puketi vs. Koraha	2.30	**<0.01**	31.20
Puketi vs. Waiwhakareke	2.28	**0.01**	13.64
Rotoiti vs. Koraha	2.37	**0.01**	30.96
Rotoiti vs. Waiwhakareke	2.33	**0.01**	10.74
Koraha vs. Waiwhakareke	2.37	**0.01**	30.43

3.4.4.2 Comparison among habitats

Native and cosmopolitan species presence was compiled for suction samples taken from either shoreline or pasture habitat transects, and the similarity of these community assemblages was assessed (Figure 3.15). Clustering of shoreline communities was most evident at Lake Koraha, and Lake Waiwhakareke where the shoreline samples differ significantly from other lakes. Samples obtained from the shoreline habitat of the northern dune lakes shows no clear dissimilarity among sites and a strong resemblance to pasture assemblages. In contrast, the pasture

assemblages sampled from Lake Koraha and from the western pasture site at Lake Puketi are quite dissimilar to all other assemblages.

The results from PERMANOVA support the finding that suction community composition sometimes varied significantly between pasture and shoreline habitats (*P* <0.01) (Table 3.4A). Pair-wise analyses confirmed that Lake Koraha showed significant dissimilarity and Lake Waiwhakareke indicated a strong probability of dissimilarity between shoreline and pasture community compositions, while lakes Kohahuake, Puketi and Rotoiti indicated no significant difference between these two habitats using this method of collection (Table 3.4B). Further pair-wise analyses between lakes determined there to be significant differences between communities sampled from shoreline habitats (Table 3.4C), but no significant differences were detected between pasture habitats (Table 3.4D) when comparisons were made between all 5 lakes.

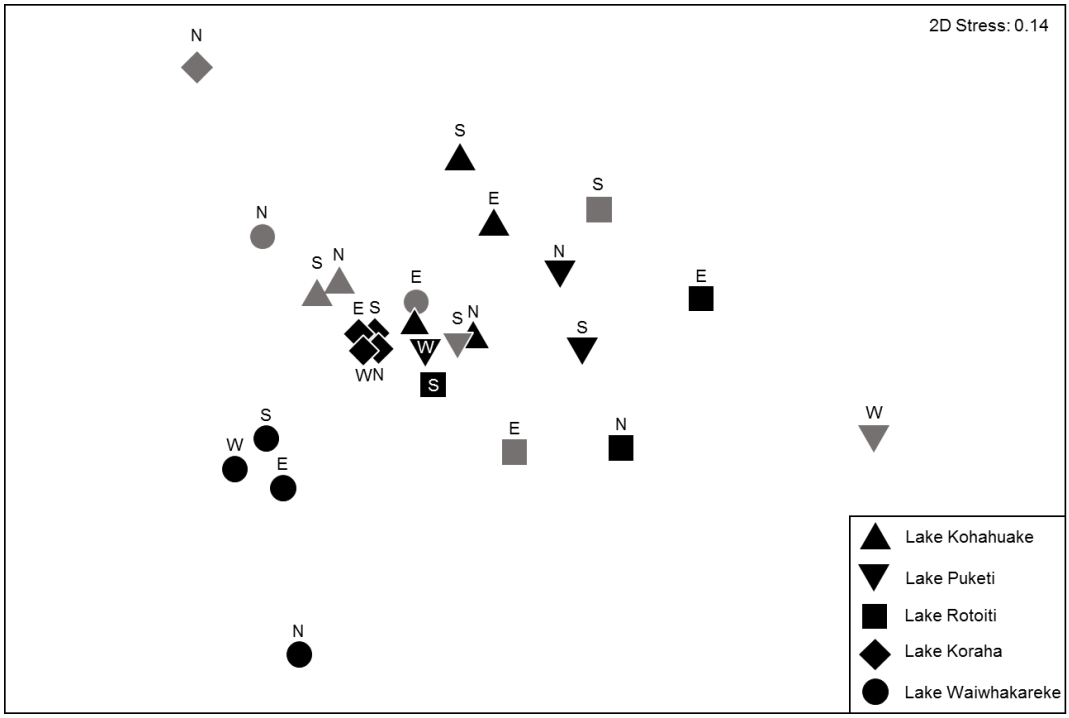


Figure 3.10: Non-metric multidimensional scaling ordination using presence/absence data of combined native and cosmopolitan spider community assemblages compiled from samples collected using the aforementioned suctioning method from shoreline (black) and pasture (grey) habitat transects.

Table 3.4: Summary of PERMANOVA examining differences in community compositions compiled from pasture and shoreline transect samples using suction sampling. Pair-wise comparisons were made between pasture (P) and shoreline (S) habitat by lake and between all lakes. Significant *P*-values are in bold ($P \leq 0.05$).

A. Global test

Factor	DF	SS	Square root estimates of components of variation	Pseudo-*F*	*P*-value (Monte Carlo)
Habitat	9	5082.80	564.75	3.31	**<0.01**
Residual	16	2728.10	170.51		
Total	25	7810.90			

B. Pair-wise comparison

Lake: Shoreline vs. pasture	*t*-value	*P*-value (Monte Carlo)	Average % dissimilarity between habitats
Kohahuake	1.72	0.14	75.97
Koraha	5.75	**<0.01**	70.93
Puketi	2.28	0.09	65.12
Rotoiti	1.10	0.33	72.87
Waiwhakareke	1.93	**0.05**	76.74

C. Pair-wise comparison

Shoreline habitat vs. lake			
Kohahuake vs. Koraha	2.75	**0.01**	20.83
Kohahuake vs. Puketi	1.31	0.23	18.94
Kohahuake vs. Rotoiti	1.79	0.06	24.24
Kohahuake vs. Waiwhakareke	2.66	**0.01**	28.03
Koraha vs. Puketi	2.68	**0.02**	20.08
Koraha vs. Rotoiti	3.05	**0.01**	23.86
Koraha vs. Waiwhakareke	3.69	**<0.01**	19.38
Puketi vs. Rotoiti	0.90	0.48	17.44
Puketi vs. Waiwhakareke	2.87	**<0.01**	29.93
Rotoiti vs. Waiwhakareke	2.90	**<0.01**	31.44

D. Pair-wise comparison

Pasture habitat vs. lake			
Kohahuake vs. Koraha	4.04	0.39	31.82
Kohahuake vs. Puketi	1.08	0.39	25.00
Kohahuake vs. Rotoiti	1.10	0.41	22.73
Kohahuake vs. Waiwhakareke	1.30	0.31	12.5
Koraha vs. Puketi	1.13	0.42	38.64
Koraha vs. Rotoiti	1.15	0.42	32.92
Koraha vs. Waiwhakareke	1.84	0.25	19.32
Puketi vs.Waiwhakareke	0.97	0.44	25.00
Rotoiti vs. Waiwhakareke	0.93	0.50	21.59

3.4.4.3 Comparison among methods

Two sampling methods were used along the shorelines to obtain samples, suction sampling and manual collection. Native and cosmopolitan species richness was compiled and the comparative similarity assessed (Figure 3.16). The subsequent ordination depicts a strong similarity among sites with species compositions obtained using suction samples. There was a strong dissimilarity between hand-collected manual samples from Lake Koraha, and to a lesser degree Lake Waiwhakareke, compared to other lake sites which more closely resembled communities associated with suction samples.

The results from PERMANOVA, comparing the differences in combined native and cosmopolitan community composition, support the finding that manual collections resulted in community compositions which differed significantly from communities obtained from suction samples in shoreline habitats (P <0.01) (Table 3.5A). Pair-wise analyses determined that these methods resulted in significantly different communities across all study lakes (Table 3.5B).

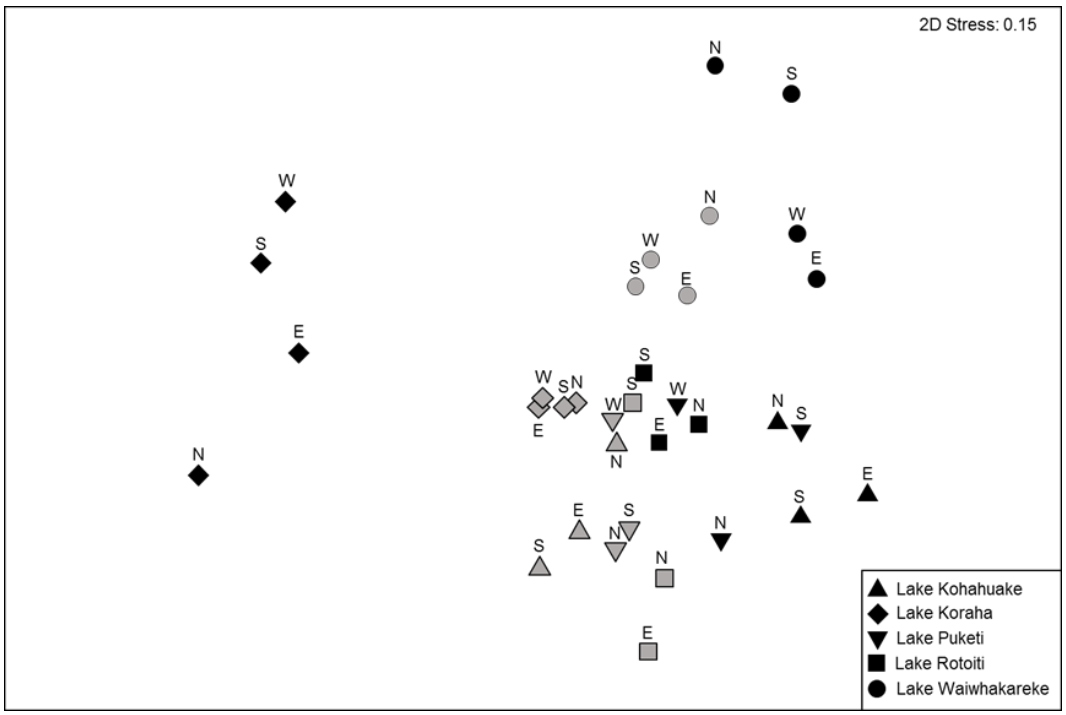


Figure 3.11: Non-metric multidimensional scaling ordination comparison of native and cosmopolitan spider communities collected using manual collection samples (black) and suctions (grey) along the shorelines of each study lake.

Table 3.5: Summary of PERMANOVA examining differences in compiled native and cosmopolitan community assemblages obtained using manual collection and suction samples from the shoreline transects of each lake. Significant *P*-values are in bold ($P \leq 0.05$).

A. Global test

Factor	DF	SS	Square root estimates of components of variation	Pseudo-*F*	*P*-value (Monte Carlo)
Method	9	4581.30	509.03	7.03	**<0.01**
Residual	24	1737.80	72.41		
Total	33	6319.10			

B. Pair-wise comparison

Lake: manual vs. suction	*t*-value	*P*-value (Monte Carlo)	Average % dissimilarity between methods
Kohahuake	2.39	**0.02**	19.39
Koraha	2.61	**<0.01**	20.72
Puketi	2.10	**0.05**	15.06
Rotoiti	2.19	**0.05**	13.01
Waiwhakareke	1.88	**0.03**	16.45

3.5 Discussion

3.5.1 Molecular inventory

Because traditional methods of identification are often inadequate for accurately establishing identifications at species-level of highly diverse groups, such as New Zealand's beetle and spider faunas (New, 1999; Lester et al. 2014; Lamont et al. 2017), DNA barcoding of the COI gene was applied to support the delineation of described and cryptic species. A combination of morphological characteristics and COI barcodes, from expertly-identified native and cosmopolitan species, were used to sort specimens from this study into one of 75 species' assignments, represented by MOTUs and associated with individual BINs. A further 4 morphogroups were included in the analyses of community composition as they were positively delineated from these MOTUs but failed to produce successful barcodes. Of the MOTUs, 38 were positively identified to

species and 18 to genus. The remaining 19 MOTUs were unidentified beyond family level or higher and require further taxonomic investigation. Furthermore, a comparison with all available sequences on BOLD and GENBANK verified that twelve of the singleton MOTUs featured in this study have been observed for the first time at Lake Koraha, highlighting the need for further taxonomic investigation of comparative habitats in the region (Ratnasingham & Hebert, 2007; Sarker & Trizna, 2011; Ratnasingham & Hebert, 2013).

The application of DNA barcoding to this study effectively delineated identifications of individual specimens, from both sexes irrespective of maturity, from greater than 113,000 Araneae barcode sequences worldwide, using BOLD systems (Ratnasingham & Hebert, 2007; Ratnasingham & Hebert, 2013). The sequence variation in the barcoded region of the COI gene was effective for subsequent identification of individuals from native New Zealand spiders, and a range of internationally-distributed cosmopolitan species. The application of COI sequences enabled identification of cryptic spiderlings and males to be associated with their respective adult female forms, thus improving the ability of ecological studies to discern identifications in future, regardless of morphological variability. High intra-specific COI divergence may reflect cryptic species clades, whereas low divergence between species reaffirms the need for more representative specimens, broader biogeographic coverage, and indeed a comprehensive coverage of all recognised species to ascertain whether any species cannot be separated through barcode analysis.

Intraspecific sequence divergence was low in most instances (mean distance = 0.36%), in comparison to other arthropod taxa such as Chironomidae (mean = 2.3%), Trichoptera (mean = 0.7%; Hogg et al. 2009) and Ephemeroptera (mean = 1%; Ball et al. 2005). However, the cosmopolitan species *Leucauge dromedaria* (Thorell, 1881), represented by 34 COI records on BOLD, revealed a comparatively high degree of intraspecific genetic divergence (mean = 1.41%, maximum = 3.41%) amongst this study's species repository. In contrast, the monophyletic

genus *Clubiona*, represented in this study by 4 described species, showed a relatively low level of intraspecific barcode sequence variation (mean = 0.75%, maximum = 1.40%) compared with between-species divergence (range = 8.78% to 10.41%). A high level of interspecific sequence divergence in this genus contrasts with comparably low sequence divergence exhibited by each of the individual species. These considerable differences in the corresponding BIN sequences provided positive species delineations.

During this study, a male specimen of the native species *T. zantholabio* was collected solely along the northern shoreline of Lake Koraha. In respect to morphological identification, the male of this species presently remains undescribed (www.wsc.nbe.ch; World Spider Catalog, 2017). Hence, the specimen from my study was considered morphologically cryptic and without a confirmed identification, prior to barcoding. BOLD was used to compare the *T. zantholabio* COI barcode from this study with approximately 113,000 other Araneae specimens worldwide, producing a positive identification. Although represented by a singleton barcode from this study, the *T. zantholabio* COI barcode from Lake Koraha positively matches (≤ 2% COI sequence distance; mean: 0.42%, maximum: 1.08%) thirteen other sequences registered in BOLD, including other identified males and females in the Waikato, Northland and Canterbury regions of New Zealand. Five other morphologically-cryptic male specimens, 4 native (*Anoteropsis hilaris* L. Koch, 1877, *Nanocambridgea gracillipes* Forster & Wilton 1973, *Rhomphaea urquharti* Bryant 1933*, Trite planiceps* Simon 1899) and 1 cosmopolitan (*Badumna longinqua* L. Koch 1867), were also resolved solely by their COI sequences from this study.

3.5.2 Species distributions and richness patterns

Samples of the spider communities associated with pasture and shoreline transects of lakes Kohahuake, Puketi and Rotoiti, which have little (<1%) native vegetation in their catchments, revealed widespread occurrence of species common to each of the northern dune lakes, collectively representing 54% of all species identified in this study (25 cosmopolitan and 18 native species). Overall, the majority of shoreline specialists and

habitat generalist species found around more than two study lakes belong to the Araneidae and Theridiidae families. These diverse families are represented by species the world over and predominantly use silk to produce sticky webs to catch flying invertebrates (Forster & Forster, 1999). Their presence around the shorelines of these lakes is likely a consequence of the available insect prey emerging across the aquatic-terrestrial interface (Henschel, Mahsberg & Stumpf, 2001).

Species belonging to the Araneidae and Theridiidae have the ability to rapidly disperse by ballooning on air currents and encounter few physical obstructions when dispersing over relatively uniform pasture habitat. Both families were represented by a single cosmopolitan species in the shoreline habitat of Lake Koraha, however, native web-building species belonging to the Tetragnathidae, Theridiidae and Linyphiidae families were represented in each of its shoreline communities also. These native species were observed to have greatest diversity along the shoreline habitats, where there is an abundance of aquatic invertebrates emerging from the water into the terrestrial realm during the aerial phases of their lifecycles (Graham, Buddle & Spence, 2003; Haase & Balkenhol, 2015).

Not all cosmopolitan species show an affinity for disturbed habitats (Malumbres-Olarte et al. 2014). In this study there was a strong association between cosmopolitan species in pasture and the adjacent habitats of the northern dune lakes. One cosmopolitan species in particular was recorded around the margins of all 5 study lakes, New Zealand's most common orbweb spider *Eriophora pustulosa* (Walckenaer, 1841), an Australian immigrant considered to have ballooned to New Zealand (Derraik et al. 2010). In this study both male and female *E. pustulosa* were frequently observed at all lakes. The silvery vagabond spider, *Anzacia gemmea* (Dalmas, 1917), is another cosmopolitan species recognised as established in both New Zealand and Australia (Paquin, Vink & Duperre, 2010) and found at all 5 lakes in the present study. Both males and females of this species were collected from shoreline and pasture habitats of lakes Kohahuake, Puketi, Rotoiti and Waiwhakareke, but unlike *E. pustulosa*, were only associated with the pasture habitat

beyond the outskirts of the forest nearest to Lake Koraha. *A. gemmea* is broadly distributed in New Zealand and is capable of dispersal through pasture habitat (Topping & Lovei, 1997). Considered a generalist species in this study due to their presence in pasture and shoreline habitats across multiple study lakes, this internationally-distributed species could represent a potential infiltration threat to undisturbed lakes like Lake Koraha. Nevertheless, in this instance, the mature vegetative architecture surrounding lakes Koraha and Waiwhakareke support diverse communities of native spiders.

Two native species, *Dolomedes minor* (L. Koch, 1876) and *Haplinis* sp., were recorded in the shoreline and pasture habitats of lakes Kahuhuake, Puketi, Rotoiti and Waiwhakareke, however, both species were only recorded in nearby pasture habitat to Lake Koraha. Both these species appear to be habitat generalists based on the definition used in this study, and this finding is supported by a study from Clark, Gerard & Mellsop (2004) in which spider diversity in the Waikato region was assessed following cultivation of pasture habitat, recording the presence of a *Dolomedes* sp. in both uncultivated and cultivated pasture habitats. These findings support the classification of *D. minor* from this study as a habitat generalist with a tolerance for pasture habitat in both undisturbed modified pastoral habitat.

Overall, species richness was lowest among the northern dune lakes and highest at lakes Koraha and Waiwhakareke. A comparison of species richness obtained from suction samples from pasture and shoreline habitat revealed greater diversity in shoreline habitat. Manual sampling produced more species rich samples than suction samples along shoreline transects and indicated that the greatest species richness was at Lake Koraha. However, suction samples were more effective than manual collections for collecting cosmopolitan species in shoreline habitats.

The species richness of cosmopolitan spiders obtained using both sampling methods indicated no significant differences between the northern dune lakes, or between lakes Waiwhakareke, Koraha and

Kohahuake. With comparison to Lake Koraha, the native species richness at Lake Waiwhakareke was more similar to lakes Rotoiti, Puketi and Waiwhakareke, however, there were 5 native species in common between lakes Koraha and Waiwhakareke. Species richness observed in pasture was comparatively low compared to the shoreline habitats and consisted of a similar proportion of native and cosmopolitan species, few of which were only found exclusively in pasture habitat. Pasture habitat exhibits considerable vegetative homogeneity and this low-lying vegetation benefits aerial invading cosmopolitan species which can disperse via wind currents and infiltrate communities in recently disturbed grasslands (Vink et al. 2004; Malumbres-Olarte et al. 2013; Malumbres-Olarte et al. 2014).

The difference in shoreline architecture between lakes, and the native vegetation restoration at Lake Waiwhakareke, provides one plausible explanation for the observed differences between these communities as habitat heterogeneity promotes niche availability which in-turn supports greater diversity of spider assemblages (Mallis & Hurd, 2005; Buccholz, 2010; Malumbres-Olarte et al. 2013). Overall, the combination of the two sampling methods used in this study resulted in 79 MOTU which represented 21 identified families and 44 genera (refer to Appendix B1-B5). Although this study obtained samples from each lake on only one occasion, the combined results from the two sampling methods provide a comparable species richness to that obtained using longer term collection methods, such as pitfall traps. Lamont et al. (2017) assessed the community composition of ground-dwelling specimens, collected between 1998 and 2015 from pitfall traps in native broadleaf-podocarp forest fragments in northern Hawke's Bay, New Zealand. The compiled species inventory from three forest fragment sampling sites provided a total species richness of 55 species from 31 families. By comparison, my samples from the mature vegetation architecture in the shoreline habitats of lakes Koraha and Waiwhakareke, collected using manual and suction methods, yielded 41 and 34 species respectively, from 14 familieis compared to 30, 27 and 23 species from 15 families in the pasture dominated lakes (Kohahuake, Puketi and Rotoiti respecitvely).

3.5.3 Community composition

This study reveals that the shoreline habitats of the 3 northern dune lakes support comparatively similar community assemblages of both native and cosmopolitan species in shoreline and pasture habitat, by comparison to lakes Koraha and Waiwhakareke where communities showed considerable heterogeneity between sampling transects. Lake Kohahuake showed no discernible dissimilarity in cosmopolitan species composition inhabiting shoreline habitat compared to any of the other lakes, however, the adjacent pasture habitat showed greater resemblance to samples obtained from pasture habitat at lakes Waiwhakareke and Koraha, than to lakes Puketi and Rotoiti which are in closer spatial proximity. This is significant because, in most respects, the native and cosmopolitan spider communities inhabiting the shorelines of the three northern dune lakes show no discernible dissimilarity, which may infer that spatial proximity could be a factor in the structuring of these communities. The community composition of native spiders at lakes Puketi, Rotoiti and Kohahuake showed no significant dissimilarity, and resembled to the species compositions inhabiting the pasture communities from all 5 lakes. Shoreline community assemblages of the four more developed lakes have been infiltrated by a range of cosmopolitan species (Gibson, Hambler & Brown, 1992; Oxbrough et al. 2004). In contrast, Lake Koraha's shoreline community was dominated by rare native species sampled only from transects surrounding the lake, and showed significant dissimilarity to the composition of species collected from the nearest pasture transect. Additional samples obtained from forest transects 50 m from the shore would have provided a beneficial comparison to test where differences were related to habitat type or distances from the shoreline.

A study by Gomez, Lohmiller & Joern (2016) highlighted the importance of vegetation structure to the assembly of aerial web-building spider communities, observing higher abundances of orb-building spiders in areas with added structural complexity compared to tall-grass prairie habitat. In this study the most diverse community assemblage was sampled from Lake Koraha whose community composition was dominated by native and rare species. The most diversely represented families

among the shoreline habitat included Tetragnathidae, Theridiidae and Linyphiidae species which build webs for prey capture and require the complexity of the associated vegetation architecture to support their webs.

The analyses of the native species community from Lake Waiwhakareke discerned significant dissimilarities in the shoreline community to those from the other study lakes. Dense vegetation surrounding the shorelines of lakes Waiwhakareke, where an extensive replanting programme has been underway, and Koraha provide greater habitat heterogeneity and vegetative structure than those of the northern dune lakes, and this heterogeneity may increase niche diversity leading to greater spider diversity and altered community composition.

3.6 Conclusion

The combined morphological and molecular approach to identification used here has demonstrated community composition assessments of spiders are viable. Therefore, future research, investigating a range of wetland and forested habitats in the Waikato region and beyond, is encouraged so that we may come to fully appreciate both the diversity and true distributions of the species recorded in this study. Such research would then contribute to developing spiders as biological indicators of lake and riparian shoreline condition. As predators in the riparian zone, spiders have an important role in the regulation of aquatic and terrestrial invertebrate populations and further investigation of predator-prey relationships from members of this predatory taxa may reveal and infer diversity and condition of prey levels in ecosystem food webs. In relatively stable but isolated lake ecosystems this reliance upon aquatic prey may potentially limit their distribution to shoreline habitats as mature native vegetation architecture provides habitat heterogeneity along shoreline margins.

The Waikato region contains a number of natural areas that provide habitats for native plant and animal species. Due to habitat fragmentation, primarily associated with deforestation and drainage across the lowland

areas of the region, and subsequent conversion to pastoral habitat, the distribution and dispersal of many native species has been affected. Lake ecosystems were selected for this study because methods for ranking lake ecosystems are a priority for biodiversity management in the Waikato region. Where significant native vegetation was absent in the catchment (<1%), or where shoreline habitat was reflective of surrounding pastoral habitat, the relative proportion of cosmopolitan to native species was higher indicating a decline in native biodiversity. Lake Waiwhakareke has undergone considerable replanting in the catchment since 2004. In comparison to lakes Puketi, Rotoiti and Kohahuake, Lake Waiwhakareke provides greater niche heterogeneity, and subsequently supports a greater diversity of common and rare species. Lake Koraha provided comparative reference-like conditions and the results of this study indicate that the largest community assemblage was supported in the shoreline habitat, and was dominated by native species.

The findings presented in this study suggest that these inventories of spider assemblages collected using these two sampling methods provide an effective comparison of a fraction of the community composition found across pasture and shoreline habitats from multiple ecosystems. Further, DNA Barcoding provides an effective method for discriminating between communities of identified morphospecies and morphologically-cryptic specimens. Overall the results of this study suggest that refined assessments of spider biodiversity may be achieved by the careful use of DNA barcoding. In order to develop a more comprehensive understanding of spider biodiversity further investigations of other lakes, vegetation types and strata will be required, and is strongly recommended, as too often the limitations of funding and time restrict comprehensive follow-through surveys.

Chapter 4

General discussion

In this thesis, I used DNA barcoding to (i) supplement the identification of morphologically-identified museum specimens and morphologically cryptic spiders (Araneae) collected from around New Zealand (Chapter 2), and (ii) compare native and cosmopolitan spider communities collected from pasture and shoreline habitats of contrasting Waikato lakes, using manual and suction collection methods (Chapter 3).

Spiders (Araneae) have received relatively little attention in New Zealand, and this is exacerbated by instinctive fears and a general dislike for their appearance, behaviour and the perceived venomous reputation of some species. However, as a megadiverse fauna dominated by indigenous predatory species, spiders can act as natural biological control agents in both unmodified and agricultural ecosystems (Vink & Kean, 2013; Malumbres-Olarte et al. 2014; Bowie et al. 2014; Haase & Balkenhol, 2015). Additionally, terrestrial arthropods such as spiders provide a key food resource for native and non-native invertivores (Wise, 1994; Nakano & Murakami, 2000; Sanders, Platner & Oecologia, 2007; Benjamin, Fausch & Baxter, 2011). However, terrestrial invertebrates represent the most data deficient taxonomic group in New Zealand (Department of Conservation, 2001; Department of Conservation, 2002) and only two species of New Zealand spiders have been issued full protection status (Wildlife Act, 1953 – section 7 amendment, 2010).

I selected lake ecosystems with contrasting riparian vegetation for this comparative study of spider community composition because the abundance and diversity of spiders in shoreline riparian areas is linked to the structure of vegetative communities (Petillon et al. 2012; Smith, Emien & Pearson, 2016), and to the abundance and diversity of prey including emerging aquatic insects (Wise, 1994; Topping & Lovei, 1997; Revenga & Kura, 2003). In the Waikato region 18 out of the 71 shallow lakes have been defined as ‘Data deficient’ meaning that there is insufficient information known about them to enable an effective classification for prioritising management effects and interventions (Dean-Speirs et al. 2014). Moreover, national legislation identifies freshwater systems as a matter of national importance, and the protection of these systems on

private land is a priority (Section 6c Resource Management Act, 1991; Waikato Regional Policy Statement, 2016).

4.1 Applications of barcoding

Chapter 2 catalogues 774 individual spider specimens, provided by Canterbury Museum or collected as uncatalogued specimens by this researcher and others. A comparison of the mitochondrial COI gene from these specimens was made, effectively discriminating each into 1 of 98 described species or 40 unidentified MOTUs resolved to genus-level. DNA barcodes successfully clustered sequences by comparative similarity and enabled all sequences pertaining to described species to be compared to those already available on the BOLD datasystem (Ratnasingham & Hebert, 2007; Ratnasingham & Hebert, 2013). By testing a 2% COI divergence threshold, to group sequences for species identification, all of the specimen sequences examined could be distinguished and were most closely associated with other specimens of the same morphospecies. This threshold reflects an upper level of intraspecific sequence variation exhibited within species than between closely-related species (Barrett & Hebert, 2005; DeSalle, Egan & Siddall, 2005). Nevertheless, extensive research of species metapopulations may reveal some recently-diverged species with comparatively low levels of sequence divergence. In a review of intraspecific COI sequence divergence, Hebert et al. (2003) found that over 98% of different animal species possess greater than 2% COI divergence. Although no single divergence threshold will enable the delineation of all species, sequence divergence values greater than 2% were typically indicative of different species in this study. The results contained in Chapter 2 and Chapter 3 support the conclusion that a 2% threshold value can be used as an initial guideline for assessing species richness and community composition of spiders where refined morphological analysis is not feasible. The resulting MOTUs featured in Chapter 2 and Chapter 3 have comparatively distinct COI sequences lodged in BOLD which can now be used in the future to provide positive molecular identifications that support morphological assignments, thereby

alleviating previously confounding morphological identifications of adult and juvenile specimens.

Internationally, work on the arthropod order Araneae is often constrained by challenges to morphological identification across broad geographic ranges (Kremen et al. 1993; New, 1999; Heden, 2001; Mallis & Hurd, 2005) and the identification of exotic species infiltration is challenged by our knowledge of their appearance, lifecycles and dispersal capability. Populations of the protected New Zealand spider *Latrodectus katipo* (Powell, 1871) have been monitored in recent years and are determined to be in competition for resources and habitat with a pervasive exotic competitor, *Steatoda capensis* (Hann, 1990), which has been observed in comparatively high abundance at sites of historical *L. katipo* populations (Hann, 1990; Costall & Death, 2010). Comparisons made using BOLD can reveal incursions and the infiltration of cosmopolitan species into terrestrial food-webs dominated by indigenous species, and can be used to discern changes in community composition and indigenous/exotic dominance through time. In the present studies, DNA barcoding provided a positive identification for *Philoponella congregabilis* (Rainbow, 1916; Distribution: Australia; World Spider Catalog, 2017), a described species which previously has not been formally described as part of the New Zealand fauna (www.wsc.nmbe.ch; Paquin, Vink & Duperre, 2010).

Although the combined species inventory of the studies in this thesis indicates that New Zealand's Araneae fauna has been infiltrated by a host of species with international distributions, not all have been morphologically or genetically described, and therefore the extent of their infiltration into New Zealand ecosystems is unclear. This lack of knowledge highlights an important area for future biosurveillance work. COI barcodes obtained in my study and available internationally will assist biosecurity agencies to rapidly identify the presence of little-known non-native species which have infiltrated New Zealand's spider fauna. Barcoding also provides information to conservation managers on the distribution of rare species, the composition of spider communities, and the biodiversity of the spider fauna generally. For example, Vink et al.

(2009) redescribed a cosmopolitan species of the family Theridiidae, *Cryptachaea blattea* (Urquhart, 1886) based on morphological evidence, COI DNA sequences and notes on its distribution. Similarly, Raso et al. (2014) used the COI gene as a marker for the description of juvenile and adult specimens pertaining to the Linyphiidae and Theridiidae families occupying alpine glacier habitats in Fiordland, where morphologically-confounding juvenile specimens were commonly collected.

My study of spider communities around lake margins enhanced the BOLD COI database of New Zealand's spiders with sequences from 38 described species and 41 unidentified MOTU. In addition, this study reported two previously unknown species in New Zealand whose morphological descriptions include international distributions, to the BOLD datasystem for the first time: *Argiope protensa* (L. Koch, 1872) and *Desis marina* (Hector, 1877). These distributions are now expanded to include New Zealand from Australia/New Caledonia/New Guinea and New Caledonia, respectively.

Furthermore, barcoding a diverse range of morphologically-identified specimens to species-level, from a range of locations, may assist in determining the extent of intra-specific divergence in relation to biogeographic interpretations of dispersal and distribution over evolutionary timescales (Cowie & Holland, 2008; Jansen, Savolainen & Vepsalainen, 2010; Mantooth & Riddle, 2011). On a shorter timescale, understanding spatial variations in COI sequence divergence across species provides insights into the dispersal and connectivity of fragmented populations. A study by Fernandez & Giribet (2014) explored the genetic diversity and population structure, phylogeography and diversification patterns of *Aoraki denticulata* a widespread mite harvestmen endemic to the South Island of New Zealand. Their results showed a high geographic structure and low genetic connectivity among modern populations, but coalescence methods estimated a large number of cryptic species. Hence, they propose that such methods may overestimate species in which genetic divergence is unusually large. The information compiled in this chapter provides a complementary baseline for future research of New

Zealand's diverse spider fauna, and important information for biosecurity applications and the management of spider faunas in significant natural areas.

The universality of COI-based identification is dependent on establishing a foundational reference sequence library for New Zealand's spider fauna from taxonomically-confirmed individuals. Achieving this goal requires the collaboration and cooperation of taxonomists, research institutions and government agencies. By matching DNA barcodes with morphological identifications, future identifications can be made concisely from whole or partial specimens, regardless of their maturity, sex or life stage. Although species-level identification for undescribed morphospecies is not always possible, by barcoding specimens and comparing their respective COI sequences to national and international records it is possible to delineate these as different BINs (Ratnasingham & Hebert, 2013). Moreover, for all currently undescribed or cryptic species included in both chapters of this study, a Barcode Index Number (BIN) has been assigned by BOLD (Refer to Appendix A) to allow for similar sequences to be grouped together. Hereafter, any future specimens or formal identifications can be attributed to the original specimens. Further, for species that have not yet been barcoded, the reference index can be continually updated as new specimens are obtained and identifications are revised. Although the species profiles obtained in this study represent a relatively small proportion of the total species found across New Zealand, it represents an important step in developing a nationwide library of barcodes for native and cosmopolitan species.

4.2 Spider collection methods for assessing richness and community composition

Although museum collections can be used to efficiently generate relatively complete species lists when focused on an individual site over time, they rarely gather quantitative data on relative abundances due to the sampling complexity required to obtain representative samples (Coddington et al. 1991). Passive survey methods which trap specimens can reliably sample

the presence and absence of individuals in a community over short time periods, and specimens can be processed in a fraction of the time necessary to handle equivalent numbers of vertebrate specimens (Kremen et. 1993). Pitfall traps are commonly used in studies of spiders, although the validity of their use has been questioned (Turnbull, 1973; Uetz & Unzicker, 1976). Other methods such as quadrat sampling, suctions and manual collections are also used although their efficacy varies between taxa, habitats and trap density (Vlijm & Kessler-Geschiere, 1967; Oliver & Beattie, 1996; Ward, New & Yen, 2001).

As there have been criticisms regarding the accuracy of collection methods for obtaining representative samples of arthropod taxa (Curtis, 1978), my study around lake ecosystems focused on making a rapid assessment of community assemblages by trialling a dual approach in shoreline habitats where spider diversity was expected to be highest. Due to access, logistical and financial constraints, it was only possible to visit each lake on a single occasion, as is often the case for large-scale biodiversity surveys. For this reason I used two collection methods that required single site visits rather than attempting to assess the density of individual species with long-standing traps, such as pitfall traps (e.g. Lamont et al. 2017). I used suction sampling to obtain ground-dwelling spiders and manual collections to collect species inhabiting vegetation from 30 cm – 2 m in height. The results demonstrated that manual collection was more effective overall in terms of the number of species detected, collecting a greater number of both native and cosmopolitan species along shoreline habitat transects compared to suction samples.

4.3 Use of spiders as indicators of riparian condition

In Chapter 3, I used DNA barcoding to assess the comparative community composition of native and cosmopolitan species obtained from shoreline and pasture habitat surrounding five lakes (Kohahuake, Koraha, Puketi, Rotoiti, Waiwhakareke) with contrasting riparian and catchment vegetation. DNA barcoding was implemented as a method of molecular

identification because, for reasons noted earlier, morphological identification of highly-diverse taxa such as spiders can be challenging.

Spider assemblages are influenced by localised environmental conditions (Paetzold et al. 2011). Habitat heterogeneity and riparian vegetation cover are important environmental factors in invertebrate community structuring (Ulrich et al. 2010) as vegetation heterogeneity influences the complexity of above-ground architecture and subsequently reduces desiccation resulting from direct exposure of sunlight at ground-level. Therefore, in broad expanses of pasture habitat, lake margins provide habitat niches which support species rich communities within the more complex vegetative structure. Lake shorelines surrounded by indigenous forests support spider communities consisting of predominantly native species. When stable food-webs, such as those inhabiting the shoreline vegetation of lakes with significant natural vegetative character are disrupted, community assemblages are subjected to pressure from population fragmentation and competition from cosmopolitan species which can disperse rapidly by ballooning over or dispersing through pasture from adjacent modified systems (Gibson, Hambler & Brown, 1992; Miyashita, Shinkai & Chida, 1998; Haase & Balkenhol, 2015). A recent study by Watts & Thornburrow (2016) highlighted that the pasture-dominated lake catchments of lakes Areare and Ruatuna, Waikato region, support invertebrate assemblages which have been infiltrated by non-native species, including cosmopolitan spider species which were previously discussed in Chapter 3.

My study infers that, although pasture habitat supports some native species, more cosmopolitan species are resident in pasture habitat, having dispersed across the open landscape to establish within shoreline habitats. This dispersal has resulted in the development of novel community assemblages dominated by cosmopolitan and generalist native species along the shores of the more modified lakes Kohahuake, Puketi, Rotoiti and Waiwhakareke. The native riparian and catchment vegetation surrounding Lake Koraha may have provided some resilience from invasion by cosmopolitan species which were present in pasture habitat

nearby, highlighting the importance of maintaining significant natural vegetative character for the conservation of predominantly native biodiversity. With sufficient re-vegetation of the riparian zone, such as that of Lake Waiwhakareke, mature vegetation provides structural complexity and shoreline heterogeneity. Hence, lake ecosystems surrounded by mature vegetation provide complex shoreline ecotones which support greater diversity by providing habitat which encourages recruitment from nearby habitats, such as forest fragments, and supports the transition of community composition towards native-species dominance. My results therefore provide initial support for the use of spider communitites as a biological indicator of riparian restoration success.

4.4 Recommendations

Investigation of spider community assemblages inhabiting freshwater margins is essential if we are to accurately determine the distribution of native specialist species and internationally-distributed, cosmopolitan generalists. Because maturity, morphoplasticity and environmental influences cause considerable challenges for morphological identifications of invertebrates, I recommend DNA barcoding as an economical approach to spider community analysis as it can be used to effectively identify and delineate native and exotic/cosmopolitan taxa, regardless of biogeographic differences, and without the considerable amount of specialist knowledge and associated cost often required to monitor and provide comprehensive identifications of whole communities. Nevertheless, I suggest that a DNA identification approach serves to complement taxonomic identifications, rather than render traditional identification obsolete.

My studies provide a foundation for the development of a uniform, practical molecular method of spider identification. The data contained herein will complement future research of freshwater-terrestrial linkages and inform the development of tools for habitat and lake ecological quality monitoring. Studying the linkages between these ecosystems is recommended and crucial to our understanding of how environmental

impacts on one ecosystem can propagate to adjacent ecosystems (Knight et al. 2005; Paetzold et al. 2011). Environmental pollutants which bioaccumulate within the tissues of aquatic organisms may be transferred to the adjacent riparian zone within emergent insect prey. In this way pollutants from the aquatic realm may result in the loss of biodiversity of emergent insect prey and cause a trophic cascade which affects adjacent shoreline riparian communities by reducing food resource availability (Krell et al. 2014). Research which investigates the use of spiders as indicators of aquatic habitat quality and fod-web functionality may provide measurable value to studies of vegetative habitat quality and invertebrate biodiversity, biosecurity and conservation around freshwater margins.

In the near-future, with the development of a comprehensive barcode library for invertebrates, large-scale collection programmes will have the capacity to deliver large numbers of specimens for analyses (Fisher 1999; Janzen, 2004). Current analytical and database platforms have the scaling capacity needed to create a nationwide bio-identification system. Future advances in DNA sequencing and computational technologies will likely see the development of portable devices that will both gather barcode sequences in minutes and make comparisons with the barcode reference library to generate identifications. Access to such a rapid method of identification promises important benefits to both science's and society's comprehension of native biodiversity, and should be supported as it provides a crucial integration of interests between scholarly researchers and citizen science.

Overall, the two studies featured within this thesis support a recommendation for further applications using DNA barcoding as a tool for positively discriminating between species and discerning community assemblages with species-level refinement. Furthermore, the single greatest advantage it provides for research of arthropods and invertebrate taxa is the ability to remove the complications of confounding morphoplasticity, maturity and sexual dimorphism in the process of identification. Invertebrate taxonomists recognise these key difficulties of providing morphological identification and will benefit from such tools that

may rapidly and reliably distinguish species, regardless of morphoplasticity. Because DNA barcoding provides a cost-effective solution to specimen identification I recommend its application to future assessments of community composition and the monitoring of populations of native species over large spatial scales, and for the assessment of distribution, dispersal, recruitment, and incursions by exotic species.

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Appendices

Appendix A

Table A1: The combined inventory of identified species from Chapter 2 (NZ) and Chapter 3 (WAI), including the Barcode of Life Datasystem Barcode Index Number and known distributions (www.boldsystems.org; Ratnasingham & Hebert, 2013). * indicates a species with an International distribution. 1 = present.

NZ	WAI	BIN #	Family	Identification	Distribution
1		ACM3089	Agelenidae	*Neoramia janus* (Bryant, 1935)	Native
1		ADD1534	Agelenidae	*Orepukia grisea* (Forster & Wilton, 1973)	Native
1		AAF1312	Agelenidae	**Tegenaria domestica* (Clerk, 1757)	Australia, China, Europe, Japan, New Zealand, North America, South America
1		ADD1935	Amphinectidae	*Amphinecta pika* (Forster & Wilton, 1973)	Native
1		ADD3458	Amphinectidae	*Aorangia ansa* (Forster & Wilton, 1973)	Native
1		ADD3459	Amphinectidae	*Aorangia mauii* (Forster & Wilton, 1973)	Native
1		ADD3319	Amphinectidae	*Maniho ngaitahu* (Forster & Wilton, 1973)	Native
1		ACR1678	Amphinectidae	*Neolana dalmasi* (Marples, 1959)	Native
1		ACM2091	Araneidae	**Arachnura feredayi* (L. Koch, 1872)	Australia, New Zealand
1		ACR1958	Araneidae	**Argiope protensa* (L. Koch, 1872)	Australia, New Caledonia, New Guinea, New Zealand
1	1	ADD4033	Araneidae	**Celaenia atkinsoni* (O. Pickard-Cambridge, 1880)	Australia, New Zealand
1	1	ACM2883	Araneidae	*Colaranea viriditas* (Urquhart, 1887)	Native
1		ACM2178	Araneidae	*Cryptaranea atrihastula* (Urquhart, 1891)	Native
1	1	ACM2466	Araneidae	**Cyclosa trilobata* (Urquhart, 1885)	Australia, New Zealand
1	1	AAV4783	Araneidae	**Eriophora pustulosa* (Walckenaer, 1841)	Australia, New Zealand
1	1	ACR1174	Araneidae	**Novakiella trituberculosa* (Roewer, 1942)	Australia, New Zealand
1		ACR1630	Araneidae	*Novaranea queribunda* (Keyserling, 1887)	Native
1	1	ACM2770	Araneidae	**Poecilopachys australasia* (Griffith & Pidgeon, 1833)	Australia, Samoa
1		AAJ9891	Araneidae	**Zygiella x-notata* (Clerk, 1757)	Argentina, Caucasus, Chile, China, Europe, Japan, North America, Reunion Is., Turkey, Uruguay
1	1	ACB4433	Clubionidae	*Clubiona cada* (Forster, 1979)	Native
	1	ACB6408	Clubionidae	*Clubiona cambridgei* (L. Koch, 1873)	Native

1	1	ACB4381	Clubionidae	*Clubiona clima* (Forster, 1979)	Native
1		ACB4383	Clubionidae	*Clubiona consensa* (Forster, 1979)	Native
1		ACB4382	Clubionidae	*Clubiona convoluta* (Forster, 1979)	Native
1	1	ACB4128	Clubionidae	*Clubiona huttoni* (Forster, 1979)	Native
1		ACH4737	Clubionidae	*Clubiona peculiaris* (L. Koch, 1873)	Native
1		AAZ4223	Corinnidae	**Nyssus coloripes* (Walckenaer, 1805)	Australia, New Zealand
1		ADD1747	Cycloctenidae	*Cycloctenus fugax* (Goyen, 1890)	Native
1		ADD0756	Cycloctenidae	*Plectophanes archeyi* (Forster, 1964)	Native
1		ACI6073	Desidae	**Badumna insignis* (L. Koch, 1872)	Australia, Japan, New Zealand
1	1	AAW2980	Desidae	**Badumna longinqua* (L. Koch, 1867)	Australia, Japan, Mexico, New Zealand, United States of America, Uruguay
1		ADD1311	Desidae	**Desis marina* (Hector, 1877)	New Caledonia, New Zealand
1		ACM2512	Desidae	*Matachia livor* (Urquhart, 1893)	Native
1		ACQ7870	Desidae	*Nuisiana arboris* (Marples, 1959)	Native
1		ADD3342	Desidae	*Otagoa nova* (Forster, 1970)	Native
1		ACM2605	Dictynidae	*Paradictyna ilamia* (Forster, 1970)	Native
1		AAE8008	Dysderidae	**Dysdera crocata* (C. L. Koch, 1838)	Australia, Brazil, Chile, Europe, Hawaii, New Zealand
1	1	ADD2397	Eutichurudae	**Cheiracanthium stratioticum* (L. Koch, 1873)	Australia, New Zealand
1	1	ACT2059	Gnaphosidae	**Anzacia gemmea* (Dalmas, 1917)	Australia, New Zealand
1		ACM2437	Gnaphosidae	**Hemicloea rogenhoferi* (L. Koch, 1875)	Australia, New Zealand
1	1	ACM2602	Gnaphosidae	*Hypodrassodes maoricus* (Dalmas, 1917)	Native
1		ADD3566	Gnaphosidae	*Nauhea tapa* (Forster, 1979)	Native
1		ACR1197	Gnaphosidae	*Notiodrassus distinctus* (Bryant, 1935)	Native
1		ADD3146	Gnaphosidae	*Zelanda erebus* (L. Koch, 1873)	Native
1		ADD3755	Gnaphosidae	*Zelanda kaituna* (Forster, 1979)	Native
1		ACM2090	Hexathelidae	*Hexathele hochstetteri* (Ausserer, 1871)	Native
1		ACR9280	Hexathelidae	*Porrhothele antipodiana* (Walckenaer, 1837)	Native
1		ACM1683	Lamponidae	**Lampona cylindrata* (L. Koch, 1866)	Australia, New Zealand
1		ACO6095	Lamponidae	**Lampona murina* (L. Koch, 1873)	Australia, New Zealand
1		AAL2095	Linyphiidae	**Diplocephalus cristatus* (Blackwall, 1833)	Europe, Falkland Is., Kazakhstan, New Zealand, North America, Russia, Siberia
1		ADD2199	Linyphiidae	*Laetesia trispathulata* (Urquhart, 1886)	Native

1		ADD3957	Linyphiidae	*Maorineta gentilis* (Millidge, 1988)	Native
1		ACT2414	Linyphiidae	*Maorineta minor* (Millidge, 1988)	Native
1		AAH3496	Linyphiidae	**Mermessus fradoerum* (Berland, 1932)	Azores, China, New Zealand, North America, Saudi Arabia, South Africa
1		ACT0195	Linyphiidae	*Novafroneta vulgaris* (Blest, 1979)	Native
1		ADD2538	Linyphiidae	*Pseudafroneta incerta* (Bryant, 1935)	Native
1	1	AAG9172	Linyphiidae	**Tenuiphantes tenuis* (Blackwall, 1852)	Argentina, Caucasus, Central Asia, Chile, Europe, Macronesia, New Zealand, United States of America
1		ACR1897	Lycosidae	**Allotrochosina schaunislandi* (Simon, 1899)	New Zealand (Incl. Chatham Is.)
1	1	ACM2356	Lycosidae	*Anoteropsis hilaris* (L. Koch, 1877)	Native (New Zealand incl. Stewart Is. & Auckland Is.)
1		ACK2896	Lycosidae	**Hogna crispipes* (L. Koch, 1877)	Australia, New Guinea, New Zealand, Polynesia
	1	ADF5912	Mecysmaucheniidae	*Zearchaea clypeata* (Wilton, 1946)	Native
1		ACR9465	Mimetidae	*Australomimetus mendicus* (O. Pickard-Cambridge, 1880)	Native
1	1	ACM2338	Mimetidae	*Australomimetus sennio* (Urquhart, 1891)	Native
1	1	ACM2529	Miturgidae	*Argoctenus aureus* (Hogg, 1911)	Native
1	1	ACT2644	Nicodamidae	*Megadictyna thilenii* (Dahl, 1906)	Native
1	1	ACR1894	Oxyopidae	**Oxyopes gracilipes* (White, 1849	Australia, New Zealand
1		ACB4869	Pisauridae	*Dolomedes aquaticus* (Goyen, 1888)	Native
1		ACB4869	Pisauridae	*Dolomedes dondalei* (Vink & Dupérré, 2010)	Native
1	1	ACB4869	Pisauridae	*Dolomedes minor* (L. Koch, 1876)	Native
1	1	AAX1573	Salticidae	**Helpis minitabunda* (L. Koch, 1880)	Australia, New Guinea, New Zealand
1		ACR9300	Salticidae	*Holoplatys apressus* (Powell, 1873)	Native
1	1	AAY3385	Salticidae	**Hypoblemum albovittatum* (Keyserling, 1882)	Australia, New Zealand
1		ACR1662	Salticidae	*Marpissa marina* (Goyen, 1892)	Native
1	1	ACM2517	Salticidae	*Trite auricoma* (Urquhart, 1886)	Native
1	1	ACH9488	Salticidae	*Trite planiceps* (Simon, 1899)	Native
1		ACR1877	Stiphidiidae	*Cambridgea peelensis* (Blest & Vink, 2000)	Native
1		ACR1962	Stiphidiidae	*Cambridgea quadromaculata* (Blest & Taylor, 1995)	Native
1		ACR9239	Stiphidiidae	*Ischalea spinipes* (L. Koch, 1872)	Native
1	1	ACO6316	Stiphidiidae	*Nanocambridgea gracilipes* (Forster & Wilton, 1973)	Native

1		ADD1450	Stiphidiidae	*Nanocambridgea grandis* (Forster & Wilton, 1973)	Native
1	1	AAJ4144	Stiphidiidae	**Stiphidion facetum* (Simon, 1902)	Australia, New Zealand
1	1	AAG8513	Tetragnathidae	**Leucauge dromedaria* (Thorell, 1881)	Australia, New Zealand
1	1	ACO4994	Tetragnathidae	*Orsinome lagenifera* (Urquhart, 1888)	Native
1		AAD3791	Tetragnathidae	**Tetragnatha nitens* (Audouin, 1826)	Asia, Canary Is., Europe, Egypt, Maderia, Madagascar, Pacific Islands, New Zealand, North America, South America
1	1	ACM2161	Theridiidae	**Argyrodes antipodianus* (O. Pickard-Cambridge, 1880)	Australia, New Caledonia, New Zealand
1	1	ACH6516	Theridiidae	**Cryptachaea blattea* (Urquhart, 1886)	Africa, Azores, Australia, Chile, Europe, Hawaii, New Zealand, United States of America
1		AAV1555	Theridiidae	**Cryptachaea gigantipes* (Keyserling, 1890)	Australia, Norfolk Is., New Zealand
1		AAB0102	Theridiidae	**Latrodectus hasseltii* (Thorell, 1870)	Australia, Asia, India, New Zealand
1		AAC0175	Theridiidae	**Parasteatoda tepidariorum* (C. L. Koch, 1841)	Canada, China, Europe, Japan, Hawaii, New Zealand, Seychelles, South America
1		ACP4074	Theridiidae	*Phycosoma oecobiodes* (O' Packard-Cambridge, 1880)	Native
1	1	ACO4876	Theridiidae	*Rhomphaea urquharti* (Bryant, 1933)	Native
1	1	AAY6263	Theridiidae	**Steatoda capensis* (Hann, 1990)	Lesotho, South Africa, St Helena, New Zealand
1		ACS5951 ACE3320 AAL5865	Theridiidae	**Steatoda grossa* (C. L. Koch, 1838)	Algeria, Chile, China, Ecuador, Europe, Hawaii, Korea, Japan, Macronesia, North America, Peru,
1	1	ACM2485	Theridiidae	*Theridion zantholabio* (Urquhart, 1886)	Native
1	1	AAZ0408	Thomisidae	*Diaea ambara* (Urquhart, 1885)	Native
1	1	ACM2752	Thomisidae	*Sidymella angulata* (Urquhart, 1885)	Native
1		ACL9074	Thomisidae	**Sidymella benhami* (Hogg, 1910)	Australia, New Zealand
1		AAZ4225	Uloboridae	**Philoponella congregabilis* (Rainbow, 1916)	Australia
1	1	ACM2627	Uloboridae	*Waitkera waitakerensis* (Chamberlain, 1946)	Native
1		ACM2115	Zodariidae	*Forsterella faceta* (Jocque, 1991)	Native
1		ACM3066	Zoropsidae	*Uliodon albopunctatus* (L. Koch, 1873)	Native

Table A2: The combined species inventory of MOTU from Chapter 2 (NZ) and Chapter 3 (WAI) which have not been assigned species-level identifications, including the Barcode of Life Datasystem Barcode Index Number (BOLD BIN) and known distributions (www.boldsystems.org; Ratnasingham & Hebert, 2013). * indicates a species with an International distribution. 1 = present.

NZ	WAI	BOLD BIN	Family	Identification	Distribution
1		ACR1332	Agelenidae	*Neoramia* sp.	Native
1		ADD1192	Agelenidae	*Orepukia* sp.	Native
1		ACM2868	Amphinectidae	*Amphinecta* sp.	Native
1		ACR1724	Amphinectidae	*Amphinecta* sp.	Native
1		ADD1635	Anyphaenidae	*Amaurobiodes* sp.	Native
	1	ACT1361	Araneae	Araneae sp.	Native
	1	ACM1251	Araneidae	Araneidae sp.	Native
	1	ACS6036	Araneidae	Araneidae sp.	Native
1	1	AAV4782	Araneidae	**Cryptaranea* sp.	Australia, French Guinea, New Zealand
1		ACR1067	Araneidae	*Cryptaranea* sp.	Native
	1	ADF8039	Cycloctenidae	Cycloctenidae sp.	Native
1	1	AAZ4228	Desidae	**Badumna* sp.	Australia, New Zealand
1		ACM2334	Desidae	*Badumna* sp.	Native
	1	ACP7194	Gnaphosidae	Gnaphosidae sp.	Native
1		ACM0913	Hexathelidae	*Cantuaria* sp.	Native
1		ADD1673	Hexathelidae	*Hexathele.* sp.	Native
1	1	Morpho species	Hexathelidae	*Hexathele* sp.	Native
1		ACM2587	Hexathelidae	*Porrhothele* sp.	Native
1		ACT1088	Hexathelidae	*Porrhothele* sp.	Native
1		ACT2255	Hexathelidae	*Porrhothele* sp.	Native
1		ACM2575	Hexathelidae	*Stanwellia* sp.	Native
1	1	AAU4599	Linyphiidae	**Erigone* sp.	Australia, New Zealand
1		ACT1820	Linyphiidae	*Haplinis* sp.	Native
	1	ACT1820	Linyphiidae	*Haplinis* sp.	Native
1		ADD1832	Linyphiidae	*Haplinis* sp.	Native
	1	ACT4867	Linyphiidae	Linyphiidae sp.	Native
	1	ADC7344	Linyphiidae	Linyphiidae sp.	Native
	1	ADF7135	Linyphiidae	Linyphiidae sp.	Native
	1	ADF5767	Linyphiidae	*Maorineta* sp.	Native
	1	AAZ4173	Lycosidae	Lycosidae sp.	Native
	1	ACV4355	Lycosidae	Lycosidae sp.	Native
	1	ADF6025	Lycosidae	Lycosidae sp.	Native
	1	ADF7866	Micropholcommatidae	*Micropholcommatinae* sp.	Native
1	1	ACM2514	Mimetidae	*Australomimetus* sp.	Native
1	1	ACR9464	Mimetidae	*Australomimetus* sp.	Native
1		ACS9720	Mimetidae	*Australomimetus* sp.	Native
	1	ADF6963	Mimetidae	*Australomimetus* sp.	Native
1		ACR1661	Nicodamidae	*Megadictyna* sp.	Native
	1	ADF7565	Salticidae	Salticidae sp.	Native
	1	ADF8357	Salticidae	Salticidae sp.	Native
1		ACR9118	Salticiidae	*Trite* sp.	Native
1	1	ACM2679	Stiphidiidae	*Cambridgea* sp.	Native
	1	ACR1091	Stiphidiidae	*Cambridgea* sp.	Native
1		ACM2186	Stiphidiidae	*Cambridgea* sp.	Native
1		ACR1091	Stiphidiidae	*Cambridgea* sp.	Native
1		ACS4527	Stiphidiidae	*Cambridgea* sp.	Native
1		ADD3159	Stiphidiidae	*Stiphidion* sp.	Native
1		ACR9344	Tetragnathidae	*Tetragnatha* sp.	Native
1		ACV4521	Tetragnathidae	*Tetragnatha* sp.	Native
1	1	Morpho species	Tetragnathidae	*Tetragnatha* sp.	Native

	1	ACT1641	Tetragnathidae	Tetragnathidae sp.	Native
	1	ADF8038	Tetragnathidae	Tetragnathidae sp.	Native
	1	ADF8478	Tetragnathidae	Tetragnathidae sp.	Native
1	1	AAC6431	Theridiidae	**Cryptachaea* sp.	Canada, Chile, Portugal, USA New Zealand
1	1	ACM2683	Theridiidae	*Episinus* sp.	Native
	1	ADF7225	Theridiidae	*Phoroncidia* sp.	Native
	1	ADC7324	Theridiidae	*Phylloneta* sp.	Native
1		ACO5290	Theridiidae	*Rhomphaea* sp.	Native
	1	AAV1730	Theridiidae	Theridiidae sp.	Native
	1	ACO5154	Theridiidae	Theridiidae sp.	Native
	1	ACR1395	Theridiidae	Theridiidae sp.	Native
	1	ACS4745	Theridiidae	Theridiidae sp.	Native
	1	ACS5727	Theridiidae	Theridiidae sp.	Native
	1	ADF8007	Theridiidae	Theridiidae sp.	Native
1		ACM2603	Theridiidae	*Theridion* sp.	Native
1		ACM2604	Theridiidae	*Theridion* sp.	Native
1	1	ACO4995	Thomisidae	*Sidymella* sp.	Native
1		ACT5104	Zodariidae	*Forsterella* sp.	Native
1		ACO5908	Zoropsidae	*Uliodon* sp.	Native

Appendix B

Table B1: Lake Kohahuake species compilation, including capture method and Barcode of Life Barcode Index Number (BOLD BIN). * indicates a species with an international distribution. 1 = present.

Family	Identification	Manual collection	Shore suction	Pasture suction	BOLD BIN
Araneidae	Araneidae sp.	1			ACM1251
Araneidae	Araneidae sp.	1			ACS6036
Araneidae	**Cryptaranea* sp.	1			AAV4782
Araneidae	**Eriophora pustulosa* (Walckenaer, 1841)	1			AAV4783
Araneidae	**Novakiella trituberculosa* (Roewer, 1942)		1		ACR1174
Clubionidae	*Clubiona cada* (Forster, 1979)		1		ACB4433
Clubionidae	*Clubiona clima* (Forster, 1979)		1	1	ACB4381
Desidae	**Badumna* sp.	1			AAZ4228
Gnaphosidae	**Anzacia gemmea* (Dalmas, 1917)	1			ACT2059
Linyphiidae	**Erigone* sp.			1	AAU4599
Linyphiidae	*Haplinis* sp.		1		ACT1820
Linyphiidae	**Mermessus fradoerum* (Berland, 1932)			1	AAH3496
Linyphiidae	**Tenuiphantes tenuis* (Blackwall, 1852)	1	1		AAG9172
Lycosidae	*Anoteropsis hilaris* (L. Koch, 1877)		1	1	ACM2356
Lycosidae	*Lycosidae sp.		1		AAZ4173
Mimetidae	*Australomimetus* sp.	1			ACM2514
Miturgidae	*Argoctenus aureus* (Hogg, 1911)		1		ACM2529
Pisauridae	*Dolomedes minor* (L. Koch, 1876)		1		ACB4869
Salticidae	**Helpis minitabunda* (L. Koch, 1880)	1		1	AAX1573
Salticidae	**Hypoblemum albovittatum* (Keyserling, 1882)		1		AAY3385
Salticidae	*Trite auricoma* (Urquhart, 1886)	1	1		ACM2517
Salticidae	*Trite planiceps* (Simon, 1899)	1			ACH9488
Tetragnathidae	*Tetragnatha* sp.	1			Morpho species
Theridiidae	**Argyrodes antipodianus* (O. Pickard-Cambridge, 1880)	1			ACM2161
Theridiidae	**Cryptachaea blattea* (Urquhart, 1886)	1			ACH6516
Theridiidae	**Cryptachaea* sp.				AAC6431
Theridiidae	**Steatoda capensis* (Hann, 1990)	1			AAY6263

Theridiidae	*Theridiidae sp.		1		AAV1730
Thomisidae	**Sidymella benhami* (Hogg, 1910)	1			ACL9074
Thomisidae	*Sidymella* sp.		1		ACO4995

Table B2: Lake Koraha species compilation, including capture method and Barcode of Life Barcode Index Number (BOLD BIN). * indicates a species with an international distribution. 1 = present.

Family	Identification	Manual collection	Shore suction	Pasture suction	BOLD BIN
Araneae	Araneae sp.	1			ACT1361
Araneidae	*Colaranea viriditas* (Urquhart, 1887)	1			ACM2883
Araneidae	**Eriophora pustulosa* (Walckenaer, 1841)	1			AAV4783
Clubionidae	*Clubiona huttoni* (Forster, 1979)	1			ACB4128
Cycloctenidae	Cycloctenidae sp.		1		ADF8039
Gnaphosidae	**Anzacia gemmea* (Dalmas, 1917)			1	ACT2059
Hexathelidae	*Hexathele* sp.	1			Morpho Species
Linyphiidae	*Haplinis* sp.			1	ACT1820
Linyphiidae	Linyphiidae sp.			1	ADC7344
Linyphiidae	Linyphiidae sp.	1	1		ADF7135
Linyphiidae	*Maorineta* sp.	1		1	ADF5767
Linyphiidae	**Tenuiphantes tenuis* (Blackwall, 1852)			1	AAG9172
Lycosidae	Lycosidae sp.		1		ACV4355
Lycosidae	Lycosidae sp.		1		ADF6025
Mecysmaucheniidae	*Zearchaea clypeata* (Wilton, 1946)	1			ADF5912
Micropholcommatidae	*Micropholcommatinae* sp.	1			ADF7866
Mimetidae	*Australomimetus sennio* (Urquhart, 1891)	1			ACM2338
Mimetidae	*Australomimetus* sp.	1			ADF6963
Mimetidae	*Australomimetus* sp.			1	ACR9464
Nicodamidae	*Megadictyna thilenii* (Dahl, 1906)	1			ACT2644
Pisauridae	*Dolomedes minor* (L. Koch, 1876)			1	ACB4869
Salticidae	Salticidae sp.		1		ADF8357
Salticidae	Salticidae sp.	1			ADF7565
Stiphidiidae	*Cambridgea* sp.	1			ACR1091
Stiphidiidae	*Cambridgea* sp.	1			ACM2679
Stiphidiidae	*Nanocambridgea gracilipes* (Forster & Wilton, 1973)	1			ACO6316
Tetragnathidae	**Leucauge dromedaria* (Thorell, 1881)	1			AAG8513
Tetragnathidae	*Orsinome lagenifera* (Urquhart, 1888)	1			ACO4994
Tetragnathidae	Tetragnathidae sp.		1		ADE8478
Tetragnathidae	Tetragnathidae sp.			1	ACT1641
Tetragnathidae	Tetragnathidae sp.	1			ADF8038
Theridiidae	*Phoroncidia* sp.	1			ADF7225
Theridiidae	*Theridiidae sp.			1	AAV1730
Theridiidae	Theridiidae sp.	1			ACS4745
Theridiidae	Theridiidae sp.			1	ADF8007
Theridiidae	Theridiidae sp.	1			ACR1395

Theridiidae	Theridiidae sp.	1			ACS5727
Theridiidae	*Theridion zantholabio* (Urquhart, 1886)	1			ACM2485
Thomisidae	*Sidymella angulata* (Urquhart, 1885)	1			ACM2752
Thomisidae	*Sidymella* sp.	1			ACO4995
Uloboridae	*Waitkera waitakerensis* (Chamberlain, 1946)	1			ACM2627

Table B3: Lake Puketi species compilation, including capture method and Barcode of Life Barcode Index Number (BOLD BIN). * indicates a species with an international distribution. 1 = present.

Family	Identification	Manual collection	Shore suction	Pasture suction	BOLD BIN
Araneidae	**Cryptaranea* sp.	1	1	1	ACR1174
Araneidae	**Eriophora pustulosa* (Walckenaer, 1841)	1		1	AAV4783
Araneidae	**Novakiella trituberculosa* (Roewer, 1942)		1		AAV4782
Clubionidae	*Clubiona clima* (Forster, 1979)	1		1	ACB4381
Desidae	**Badumna* sp.	1			AAZ4228
Gnaphosidae	*Hypodrassodes maoricus* (Dalmas, 1917)	1			ACM2602
Linyphiidae	*Haplinis* sp.		1	1	ADC7344
Linyphiidae	Linyphiidae sp.			1	ACT1820
Linyphiidae	**Mermessus fradoerum* (Berland, 1932)		1	1	AAH3496
Linyphiidae	**Tenuiphantes tenuis* (Blackwall, 1852)		1		AAG9172
Lycosidae	*Anoteropsis hilaris* (L. Koch, 1877)		1	1	AAZ4173
Lycosidae	*Lycosidae sp.		1	1	ACM2356
Mimetidae	*Australomimetus* sp.		1		ACR9464
Miturgidae	*Argoctenus aureus* (Hogg, 1911)		1	1	ACM2529
Oxyopidae	**Oxyopes gracilipes* (White, 1849)		1	1	ACR1894
Pisauridae	*Dolomedes minor* (L. Koch, 1876)	1		1	ACB4869
Salticidae	**Helpis minitabunda* (L. Koch, 1880)	1			AAX1573
Salticidae	**Hypoblemum albovittatum* (Keyserling, 1882)	1	1	1	AAY3385
Salticidae	*Trite auricoma* (Urquhart, 1886)		1		ACM2517
Salticidae	*Trite planiceps* (Simon, 1899)	1		1	ACH9488
Stiphidiidae	*Cambridgea* sp.	1		1	ACM2679
Tetragnathidae	*Tetragnatha* sp.	1		1	Morpho Species
Theridiidae	**Argyrodes antipodianus* (O. Pickard-Cambridge, 1880)	1			ACM2161
Theridiidae	**Cryptachaea blattea* (Urquhart, 1886)	1		1	ACH6516
Theridiidae	**Cryptachaea* sp.		1	1	AAC6431
Theridiidae	**Steatoda capensis* (Hann, 1990)	1		1	AAY6263
Theridiidae	*Phylloneta* sp.	1			ADC7324

Table B4: Lake Rotoiti species compilation, including capture method and Barcode of Life Barcode Index Number (BOLD BIN). * indicates a species with an international distribution. 1 = present.

Family	Identification	Manual collection	Shore suction	Pasture suction	BOLD BIN
Araneidae	**Cryptaranea* sp.	1	1	1	AAV4782
Araneidae	**Eriophora pustulosa* (Walckenaer, 1841)	1		1	AAV4783
Araneidae	**Novakiella trituberculosa* (Roewer, 1942)		1		ACR1174
Clubionidae	*Clubiona clima* (Forster, 1979)		1	1	ACB4381
Gnaphosidae	**Anzacia gemmea* (Dalmas, 1917)		1		ACT2059
Linyphiidae	*Haplinis* sp.		1	1	ACT1820
Linyphiidae	**Mermessus fradoerum* (Berland, 1932)			1	AAH3496
Lycosidae	*Anoteropsis hilaris* (L. Koch, 1877)		1	1	ACM2356
Lycosidae	*Lycosidae sp.		1	1	AAZ4173
Miturgidae	*Argoctenus aureus* (Hogg, 1911)		1	1	ACM2529
Oxyopidae	**Oxyopes gracilipes* (White, 1849		1		ACR1894
Pisauridae	*Dolomedes minor* (L. Koch, 1876)	1		1	ACB4869
Salticidae	**Helpis minitabunda* (L. Koch, 1880)	1			AAX1573
Salticidae	**Hypoblemum albovittatum* (Keyserling, 1882)	1	1	1	AAY3385
Salticidae	*Trite auricoma* (Urquhart, 1886)		1		ACM2517
Salticidae	*Trite planiceps* (Simon, 1899)		1	1	ACH9488
Tetragnathidae	**Leucauge dromedaria* (Thorell, 1881)	1			AAG8513
Tetragnathidae	*Tetragnatha* sp.	1		1	Morpho Species
Theridiidae	**Argyrodes antipodianus* (O. Pickard-Cambridge, 1880)	1			ACM2161
Theridiidae	**Cryptachaea* sp.		1	1	AAC6431
Theridiidae	*Episinus* sp.		1		ACM2683
Theridiidae	**Steatoda capensis* (Hann, 1990)	1	1	1	AAY6263
Theridiidae	*Theridiidae sp.			1	AAV1730

Table B5: Lake Waiwhakareke species compilation, including capture method and Barcode of Life Barcode Index Number (BOLD BIN). * indicates a species with an international distribution. 1 = present.

Family	Identification	Manual collection	Shore suction	Pasture suction	BOLD BIN
Araneidae	**Celaenia atkinsoni* (O. Pickard-Cambridge, 1880)	1			ADD4033
Araneidae	**Cryptaranea* sp.	1			AAV4782
Araneidae	**Cyclosa trilobata* (Urquhart, 1885)	1			ACM2466
Araneidae	**Eriophora pustulosa* (Walckenaer, 1841)	1			AAV4783
Araneidae	**Novakiella trituberculosa* (Roewer, 1942)	1	1		ACR1174
Araneidae	**Poecilopachys australasia* (Griffith & Pidgeon, 1833)	1			ACM2770
Clubionidae	*Clubiona cambridgei* (L. Koch, 1873)	1	1		ACB6408
Clubionidae	*Clubiona clima* (Forster, 1979)			1	ACB4381
Clubionidae	*Clubiona huttoni* (Forster, 1979)	1			ACB4128
Desidae	**Badumna longinqua* (L. Koch, 1867)	1			AAW2980
Gnaphosidae	**Anzacia gemmea* (Dalmas, 1917)			1	ACT2059
Gnaphosidae	Gnaphosidae sp.	1			ACP7194
Linyphiidae	**Erigone* sp.	1	1	1	AAU4599
Linyphiidae	*Haplinis* sp.		1	1	ACT1820
Linyphiidae	Linyphiidae sp.		1		ACT4867
Linyphiidae	**Mermessus fradoerum* (Berland, 1932)			1	AAH3496
Linyphiidae	**Tenuiphantes tenuis* (Blackwall, 1852)		1	1	AAG9172
Lycosidae	*Anoteropsis hilaris* (L. Koch, 1877)		1	1	ACM2356
Mimetidae	*Australomimetus* sp.		1		ACM2514
Pisauridae	*Dolomedes minor* (L. Koch, 1876)	1	1	1	ACB4869
Salticidae	**Helpis minitabunda* (L. Koch, 1880)	1			AAX1573
Salticidae	*Trite planiceps* (Simon, 1899)	1	1		ACH9488
Stiphidiidae	**Stiphidion facetum* (Simon, 1902)	1			AAJ4144
Tetragnathidae	**Leucauge dromedaria* (Thorell, 1881)	1	1		AAG8513
Tetragnathidae	Tetragnathidae sp.		1		ADF8478
Theridiidae	**Argyrodes antipodianus* (O. Pickard-Cambridge, 1880)	1			ACM2161
Theridiidae	**Cryptachaea blattea* (Urquhart, 1886)	1	1		ACH6516
Theridiidae	**Cryptachaea* sp.	1	1		AAC6431
Theridiidae	*Rhomphaea urquharti* (Bryant, 1933)	1			Morpho Species
Theridiidae	**Steatoda capensis* (Hann, 1990)		1		AAY6263
Theridiidae	*Theridiidae sp.		1	1	AAV1730
Theridiidae	Theridiidae sp.	1			ACO5154
Thomisidae	*Diaea ambara* (Urquhart, 1885)	1			Morpho Species
Thomisidae	**Sidymella benhami* (Hogg, 1910)	1	1		ACL9074

Appendix C

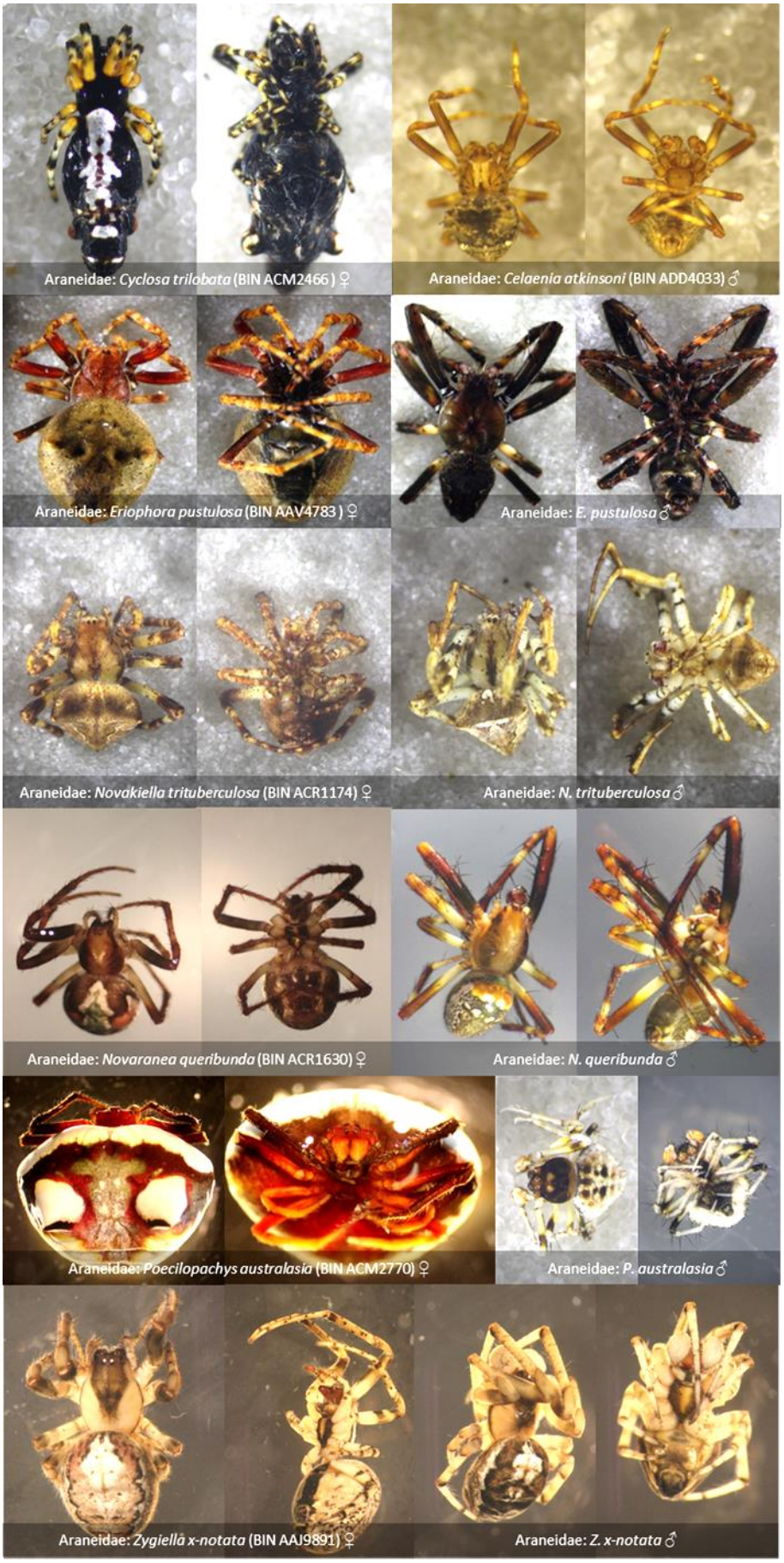

Figure C2: Examples of identified cosmopolitan species from the Araneidae family.

Figure C1: Examples of identified native species from the Araneidae family.

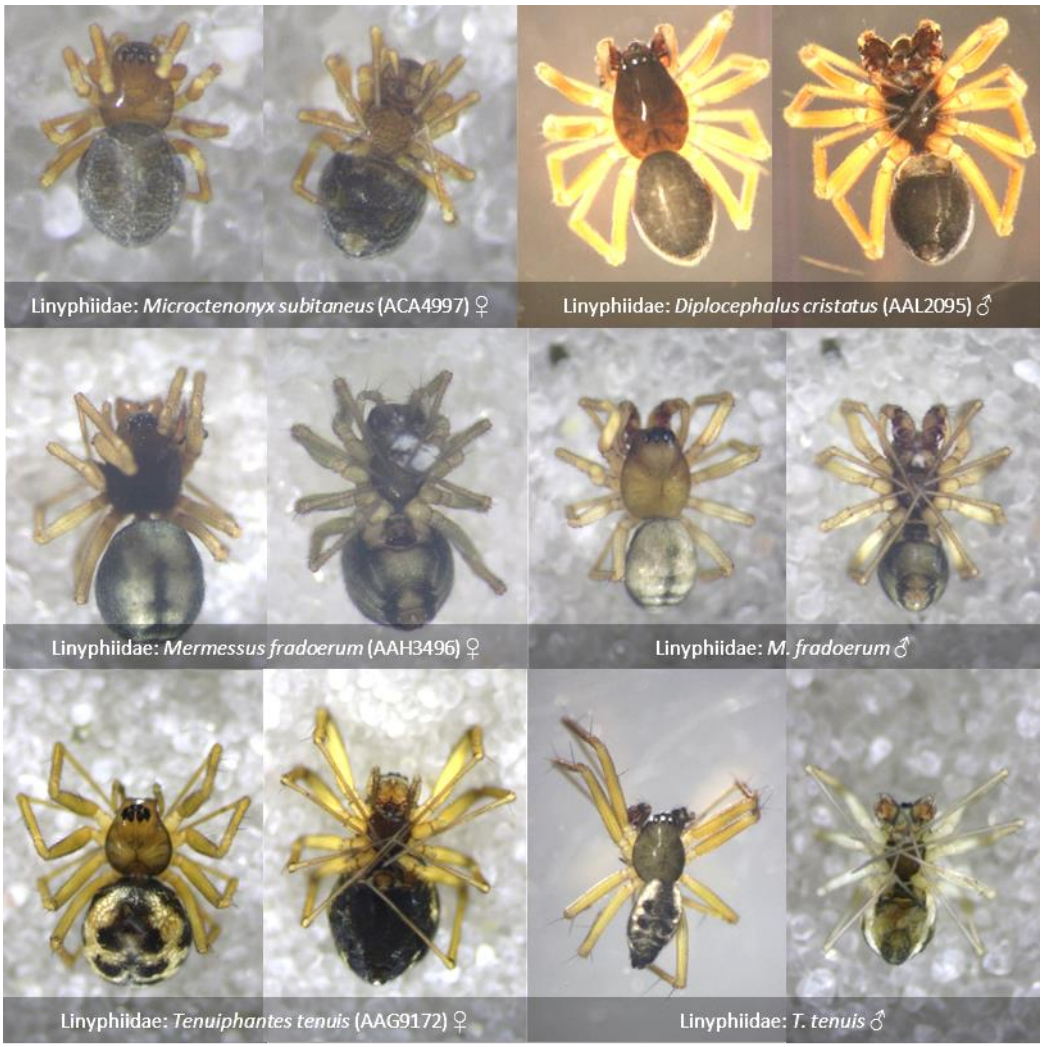

Figure C4: Examples of identified cosmopolitan species from the Linyphiidae family

Figure C4: Examples of identified native species from the Linyphiidae family.

Figure C5: Examples of identified native species from the Theridiidae family

Figure C6a: Examples of identified cosmopolitan species from the Theridiidae family

Figure C6b: Examples of identified cosmopolitan species from the Theridiidae family