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Monitoring spider communities of Waiuku dune lakes using DNA barcoding

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Monitoring spider communities of dune lakes using DNA barcoding

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Table of Contents

Executive summary

The University of Waikato was contracted by Waikato Regional Council to provide an assessment of the spider (Araneae) community inhabitants in the marginal vegetation of three Waiuku dune lakes, Parkinson, Puketi and Rotoiti.

In terms of conservation, restoration, and rehabilitation management prioritisation, there is a concern about impoverishment at multiple levels of biological organisation in freshwater ecosystem food webs. There is a need for economical approaches to accurately assess the biological diversity outcomes of restoration objectives. The identification of invertebrate taxa at species-level traditionally relies on morphological characteristics. This form of classification is often an inconclusive process as phenotypic plasticity and sexual dimorphism severely complicate taxonomic designations.

This project compared mitochondrial Cytochrome Oxidase subunit I gene nucleotide sequences to discriminate between species. Of the 190 spider (Araneae) specimen sent for DNA sequencing as part of this study, 172 were successfully barcoded. From these it was determined that there were 37 species present between the three lake sites, 16 of which have international distributions (INDI species). There were 17 shared species present at all lakes, including 8 INDI species.

Biogeographic taxa interpretations of spiders are often misleading and inconclusive, however, molecular data provides an additional dimension for the taxonomic study of New Zealand's largely endemic spider taxa. It is concluded that DNA barcoding is an effective method of alleviating species identifications in the marginal vegetation of lakes. This project enhances the inventory and understanding of spider diversity and population distributions around Waikato lake margins. The community assemblages of these pasture dominated catchment lakes share a range of spider species in common, including cosmopolitan pasture generalists and freshwater specialists worthy of further biogeographic investigation.

There are several potential applications for using DNA barcoding to identify spiders in the Waikato region, including: (i) identification of indigenous spiders in marginal vegetation as a restoration effectiveness monitoring tool; (ii) increase comparability and rigour of biodiversity inventory and monitoring studies; and (iii) contribute to further understanding of biogeographic patterns of spider species distribution.

1 Introduction

Catchment modification and land-use intensification alters habitat dynamics around freshwater margins, often resulting in the degradation and decline of terrestrial and freshwater biodiversity. Habitat and ecosystem conservation and restoration are inherently interlinked with biological diversity (biodiversity), an issue which involves identifying and maintaining, or improving indigenous species diversity and endangered species protection (Wildlife Act 1953; Resource Management Act – section 6 1991; Waikato Regional Policy Statement - section 11.1 2016). In terms of conservation, restoration, and rehabilitation management prioritisation, there is a concern about impoverishment at multiple levels of biological organisation in ecosystem food webs.

Spiders (Araneae) are the most prolific predators of terrestrial and aquatic insects in terrestrial ecosystems. They provide an important ecosystem function as they have a key role in the control of invertebrates and the protection of vegetation in stable food-webs. New Zealand spiders are highly endemic. Although experts have only formally described 1136 species, conservative estimates of more than 2,000 species nationwide have been documented (Paquin & Vink 2010). Endemic/indigenous species dominance is a priority in native habitats, however, many internationally distributed (INDI) species have broadly established across the North Island and entrenched themselves within existing food-webs.

The identification of invertebrate taxa at species-level traditionally relies on morphological characteristics. This form of classification is often an inconclusive process as phenotypic plasticity and sexual dimorphism severely complicate taxonomic designations (Buchholz, 2010; Ewers et al., 2002; New, 1999; Topping & Lovei 1997). This research project compares mitochondrial Cytochrome Oxidase subunit I (COI) gene nucleotide sequences (DNA Barcoding) to discriminate between species previously identified by professional Arachnologist. Traditionally, invertebrate morphological identification relies primarily on mature, single-sex specimen (females) for description (Paquin & Vink 2010; Forster & Forster 1999). Morphologically identifying individual specimen accurately to species-level is a challenging and time consuming process, requiring specialist knowledge. It is for this reason that DNA barcoding in conjunction with morphological identifications is a potentially more efficient method of establishing molecular operational taxonomic units (species) which are used to determine species-level community assemblages.

2 Materials and Methods

2.1 Lake sites

Three lakes were chosen for the spider survey in this study, located in the Waiuku area, northern Waikato: Lakes Puketi, Rotoiti and Parkinson. Sampling of the spider community occurred after nightfall at Lakes Puketi and Rotoiti on the 1st April 2016, between 7.30pm-11.30pm. Parkinson Lake was sampled on the 15th April, 2016 between 7.30pm – 9.30pm. There was no significant rainfall in the area three days prior to either sampling occasion and the Meteorological Service forecast conditions were similarly fine on both nights. Outside temperatures were moderate ranging through 18-9°C. Lake Puketi's eastern and Lake Rotoiti's western shorelines were saturated at surface-level within 5m of the lake. Lake Parkinson's south-eastern shoreline featured an inaccessible steep bank. It was determined that these were unsuitable areas for sampling.

2.2 Spider collection methods

Juvenile and adult spiders of both sexes were collected from the marginal habitat of the three dune lakes, Puketi, Rotoiti and Parkinson. Daytime photographs were taken during a preliminary health and safety induction day (refer Figures 1, 2 and 3). At each lake, spiders were suctioned from the substrate using a modified 27cc Stihl compact vacuum shredder with a fine gauze intake filter installed. Suctioning involved holding the device intake adjacent to the ground surface, to the side of the body facing the Lake, reducing sampling bias caused by the disturbance of footfall by the researcher. Each suction was undertaken at a steady walking pace along a 50 m transect running parallel to the lake shore, for a duration of 60 seconds (refer Figures 1-3). This process was repeated along three shorelines within the stock exclusion fence of each lake, within 2-5 m of the waterline. Two additional sets of suction samples were taken amongst the surrounding pasture, 20 m beyond the stock exclusion fence. Furthermore, hand collection methods manually captured specimen from between 0.3 m to 2 m above ground level. This process was undertaken by two individuals simultaneously, for a period of 30 minutes.

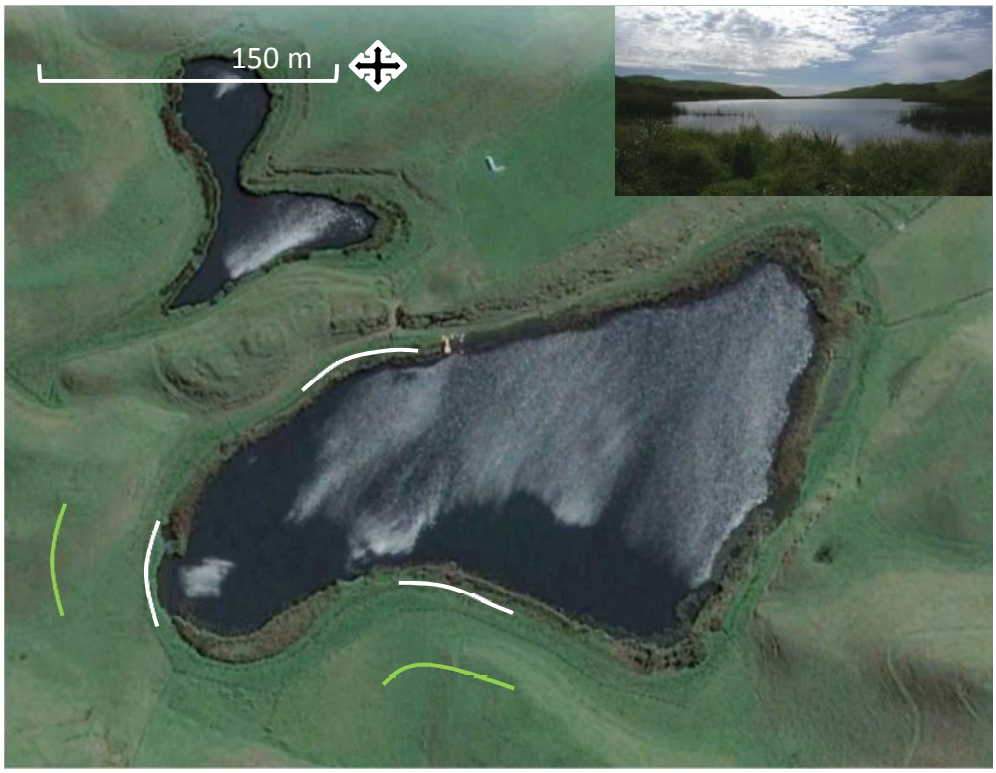

Figure 1: GoogleEarth photograph of Lake Puketi. White lines depict the shore-side suction sampling transects; green lines represent pasture suction transects. Inset daytime photo of Lake Puketi faces east, taken during a preliminary site visit 31st March 2016 at 11:45am.

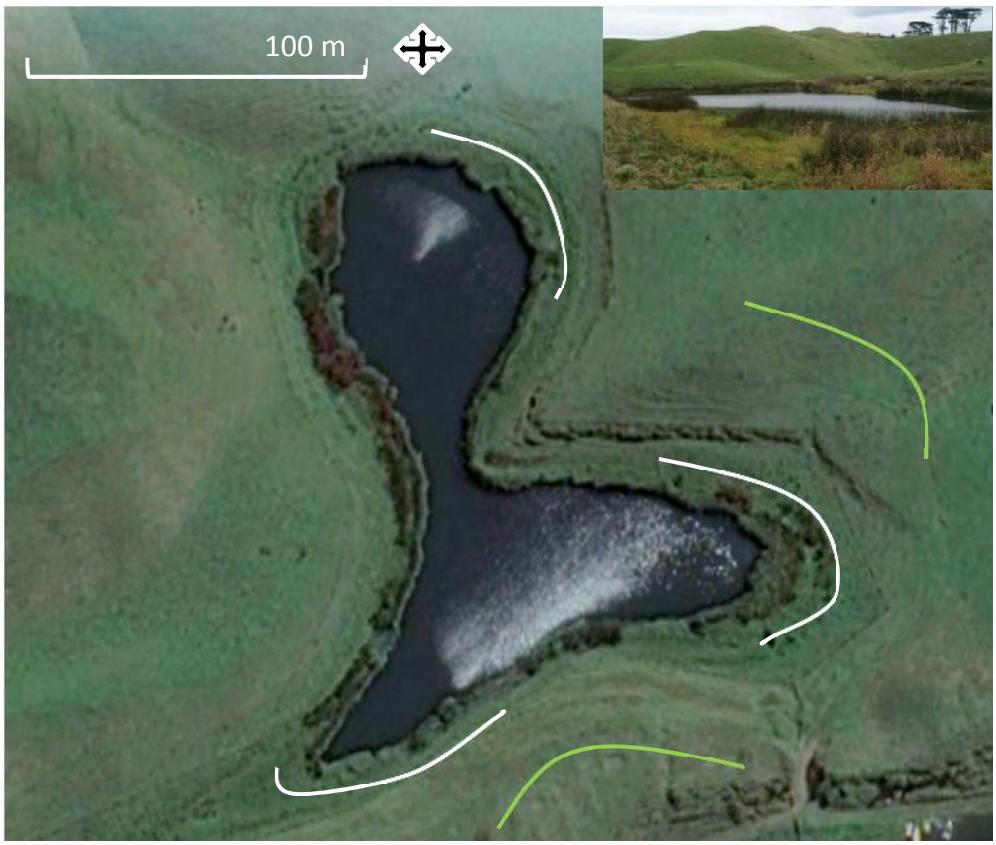

Figure 2: GoogleEarth photograph of Lake Rotoiti. Inset photo faces west, taken during a preliminary site visit 31st March 2016 at 11:00am.

Figure 3: Aerial photograph of Lake Parkinson. Inset photo faces northeast, taken during a preliminary site visit 31st March 2016 at 12:30pm.

Specimens were euthanized by immersion in 100% ethanol. A tarsal segment of the 4th leg was removed from each specimen under microscopic 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 and archived as part of the University of Waikato New Zealand Spiders (NZSPI) project collection.

2.3 Sequences

Genomic DNA was extracted via the AcroPrep™ PALL Glass Fibre plate method (Ivanova et al. 2006). A 658 base pair fragment of the mitochondrial COI gene was amplified using standard CCDB protocols (see Ivanova et al. 2006) 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 et al. 2006). All photographs, collection information, primer combinations and sequence data were uploaded to Barcode of Life Datasystems (www.boldsystems.org) and housed in the project New Zealand Spiders (NZSPI), within the Waikato Lake Spiders (WAISPI) dataset.

Sequences were aligned using Muscle (Edgar, 2004) and the alignment was subsequently pruned to 500 bp. Nucleotide sequence divergence was calculated using Jukes-cantor. Neighbour-joining analysis was conducted in MEGA v5.05 (Tamura et al. 2011). This setting included 1000 bootstrap replicates with Tamura-Nei used as the model of evolution for the neighbour-joining analysis. Tamura-Nei was used as it accepts unequal base frequencies and multiple substitution types (Simon et al. 2006). All the other settings were set to default in MEGA. The Neighbour-

joining method is computationally efficient and has a record of producing trees that are at least as good as those generated by alternative methods (Nei and Kumar 2000). Barcode Index Numbers (BINs) were assigned in BOLD (Ratnasingham and Hebert 2013) and used as a measure of Molecular Operational Taxonomic Units (MOTU's). Pairwise distance matrices were used to test the similarity of the Waiuku lake spider fauna with all publicly available spider sequences on BOLD.

2.4 *COI* Araneae species profile

A key step in the analysis of large DNA sequence datasets is the clustering of sequences based on their similarity. The derived clusters form the basis for subsequent biodiversity analyses. Clustering reduces the complexity of the data. It 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 MOTU. An identifying threshold of 98 % similarity is accepted for spider species clustering internationally (Barrett & Hebert, 2005), although it is not a definitive threshold as intra-specific or genus pairwise similarity can vary depending on the taxon.

3 Results and Discussion

3.1 COI sequence analysis

A total of 190 spider specimen were collected and sent for DNA sequencing. 172 specimen were successfully barcoded. Of these, 167 barcodes were of sufficient length to make a comparative examination of a 500bp region of the COI gene (Figure 4). The sequencing failure rate experienced during this study was well within the typical bounds of other barcoding studies. Instead of randomly selecting one sequence to represent each of the species, all of the 167 sequences were included by collapsing subtrees with less than 2 % sequence divergence. The Neighbour-joining profile contains 36 terminal nodes which each corresponded to a separate MOTU. Eight specimens belonging to a single morphologically identified species of the Tetragnathidae family failed to successfully barcode and were subsequently excluded from the phylogenetic analysis. Of these eight specimens sent for sequencing, two were successfully barcoded. However, both of these sequences were flagged as contaminated sequences belonging to an endosymbiotic proteobacteria of the order Rickettsiales and were thus not considered further in this study.

3.2 Phylogenetic analysis

Comparative COI analysis revealed that 100% of sequences grouped most closely with representatives from the same MOTU in the profile. Each branch of the corresponding Neighbour-joining phylogenetic tree represents the group of sequences which correspond to that species assignment (Figure 4). The fewer taxa included in a tree, the more difficult it is to place newly added taxa into the correct family group. For this reason it is important to include as many sequences as possible.

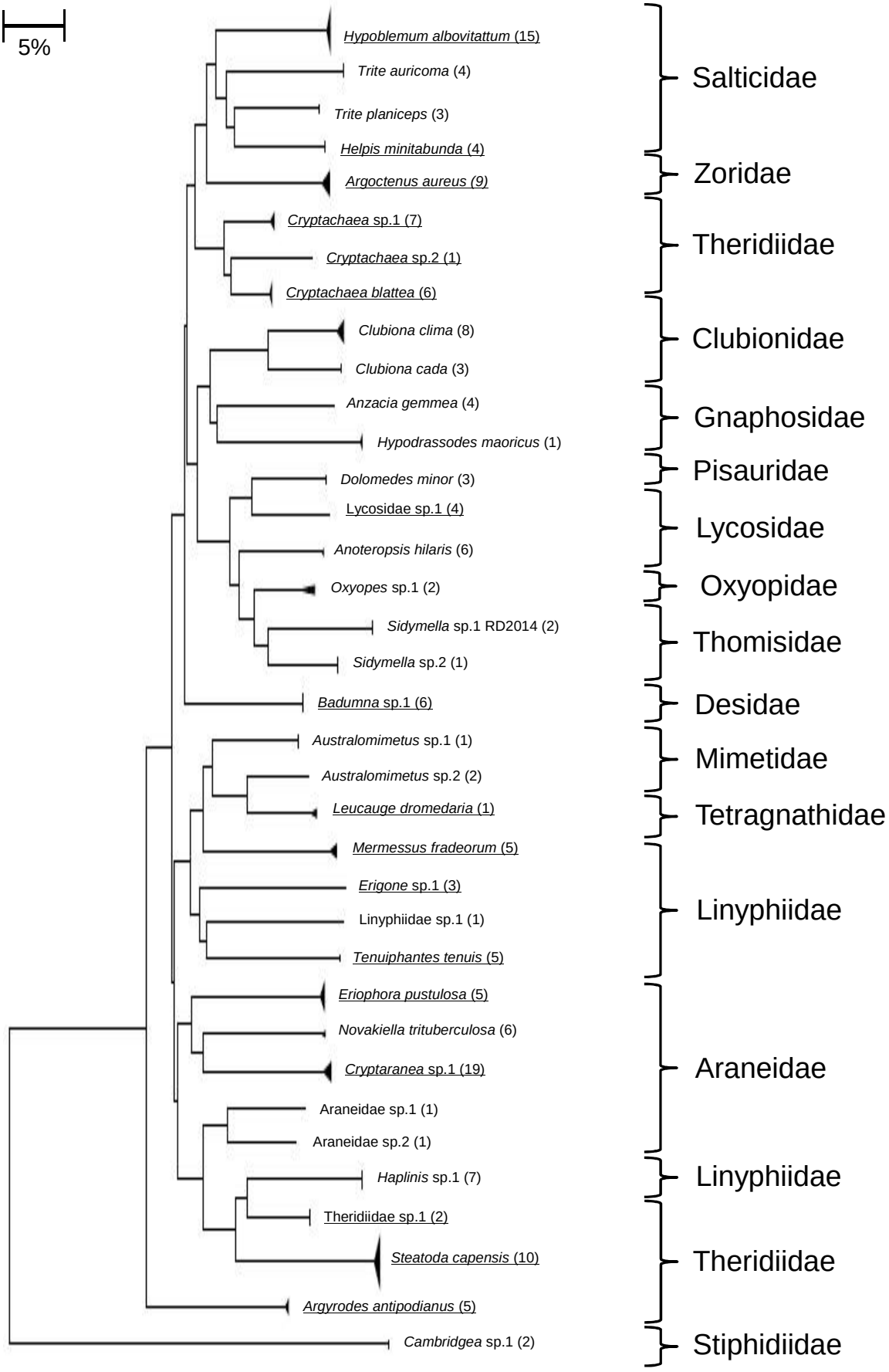

Figure 4: A Neighbour-joining tree of the 36 species assignments recorded at the Waiuku dune lakes, using an identity threshold of 98% to cluster species. Italicised names are binomial nomenclature for species designations. Names that are not italicised indicate an un-recognised species of that Araneae family. The number of individuals with identical sequences is indicated in parentheses next to the taxonomic name. Underlined names indicate internationally distributed species.

3.3 Species distributions

Of the 37 taxonomic assignments in this study, 16 are recognised to have international distributions (i.e. INDI species). Eight INDI species were recorded at all lakes: *Argoctenus aureus* (Australia), *Argyrodes antipodianus* (Australia), *Cryptaranea* sp.1 (South Africa, Australia), *Eriophora pustulosa* (Australia), *Hypoblemum albovittatum* (Australia), Lycosidae sp.1 (Australia), *Mermessus fradoerum* (European), *Steatoda capensis* (South Africa), refer Appendix A. An additional nine species with national distributions (NADI) were observed at every lake: *Anoteropsis hilaris*, *Anzacia gemmea*, *Clubiona clima*, *Dolomedes minor*, *Haplinis* sp.1, *Novakiella* sp.1, *Tetragnatha* sp.1, *Trite auricoma*, *Trite planiceps*, refer Appendix B. A Venn diagram is used to depict the distributions of NAD and INDI species amongst these lakes (Figure 5).

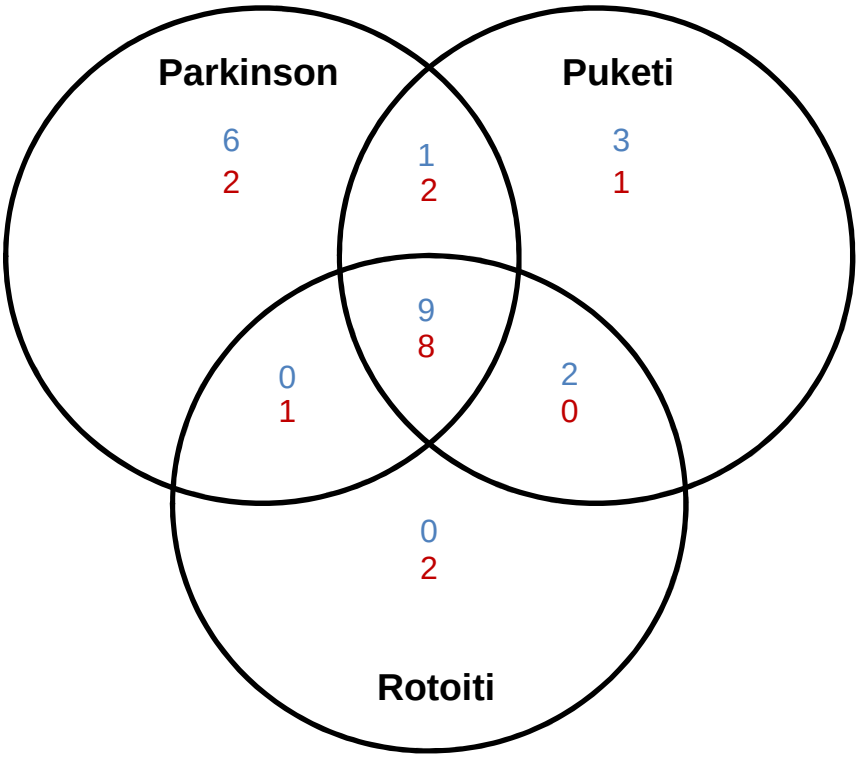

Figure 5: Venn diagram depicting the distribution of NADI (blue numbers) and INDI (red numbers) species between lakes Puketi, Rotoiti and Parkinson.

The spider community composition of the three lakes was distributed amongst fifteen Araneae families (refer Appendix C). The broadest families represented were Theridiidae (6 spp.) and Linyphiidae (5 spp.). Of these, 100% of the Theridiidae and 60% of the Linyphiidae species observed in these systems are INDI species. These two 'spaceweb' spider families are generally strongly associated with pasture habitats (Forster & Forster, 1999). Twelve species were recorded in the surrounding pasture habitat, 7 INDI and 5 NADI species. Further, eleven of these species were recorded within the stock exclusion zone surrounding each lake. Only Linyphiidae sp.1 was recorded exclusively, in the surrounding pasture at Lake Puketi. This species was not observed within close proximity to the shoreline.

Biogeographic taxa interpretations of Araneae species are often misleading and inconclusive. The inclusion of supporting molecular data provides an additional dimension for taxonomic study of New Zealand's largely endemic Araneae taxa (DeSalle et al. 2005). This study concludes that DNA barcoding is an effective method of alleviating species identifications while enhancing the inventory and understanding of spider diversity and distribution around Waikato lake margins.

The community composition of these pasture dominated lake catchments share a range of spider species in common, including pasture generalists and lakeshore specialists. Biogeographic patterns of spider species distributions at larger spatial scales (e.g. regional scale) along riparian habitats are, however, still poorly documented. As such, further investigation of degraded, relic, novel and restored lake ecosystem communities is warranted for the development of a spider indicator of ecosystem susceptibility to invasion. Because spiders have potential to integrate several aspects of biodiversity, including habitat quality and structure and food availability, such an indicator may also provide the foundation for developing restoration effectiveness indicators for riparian planting studies that aim to improve indigenous biodiversity.

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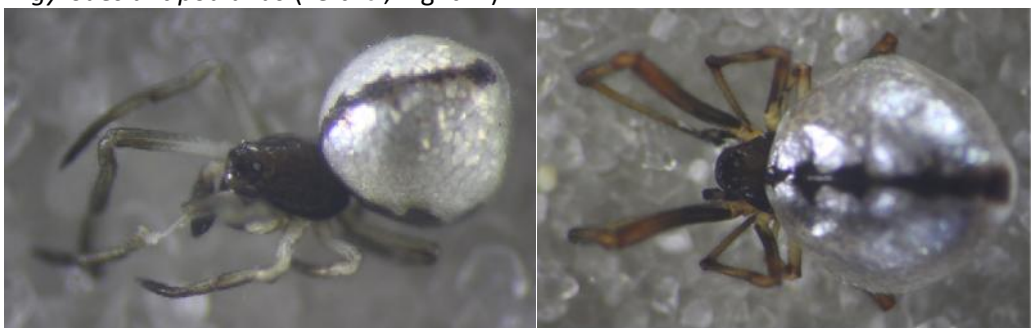
Appendix A

Examples laboratory specimen photos of eight INDI species recorded at all lakes.

Argoctenus aureus (Left: ♀; Right: close-up of fangs, chelicerae and eyes)

Argyrodes antipodianus (Left: ♂; Right: ♀)



Cryptaranea sp.1 (Left: ♀; Middle: Juvenile; Right: Juvenile)

Eriophora pustulosa (Left: ♂; Right: ♀)

Hypoblemum albovittatum (Left & Middle: Juvenile ♀; Right: ♂)

Lycosidae sp.1 (Left: ♂; Right: ♀)

Mermessus fradoerum (Left & Middle: ♂; Right: ♀)

Steatoda capensis.(Left: ♂; Middle & Right: ♀)

Appendix B

Laboratory specimen photos of nine NADI species observed at every lake.

Anoteropsis hilaris (Left: ♀; Middle: ♂; Right: Juvenile)

Anzacia gemmea (Left: ♂; Middle & Right: ♀)

Clubiona clima (Left: ♀; Middle: Juvenile; Right ♀)

Dolomedes minor(Left: ♂; Middle: ♀; Right: Juvenile)

Haplinis sp.1 (Left & Right: ♀)

Novakiella sp.1 (Left: ♂; Middle & Right: ♀)

Tetragnatha sp.1 (Left & Middle: ♀; Right: Juvenile)

Trite auricoma (Left: ♀; Middle & Right: Juvenile)

Trite planiceps.(Left: ♂; Middle & Right: Juvenile)

Appendix C

Table 1: A presence/absence table of species distributions across the three study lakes. Identifications highlighted in bold indicate INDI species.

			Parkinson		Puketi		Rotoiti	
Family	**Genus**	**Species**	Shoreline vegetation	Pasture	Shoreline vegetation	Pasture	Shoreline vegetation	Pasture
Araneidae	Araneidae	sp.1	✔					
	Araneidae	sp.2	✔					
	Cryptaranea	**sp.1**	✔		✔		✔	
	Eriophora	***pustulosa***	✔		✔		✔	
	Novakiella	*trituberculosa*	✔		✔		✔	
Clubionidae	*Clubiona*	*cada*	✔					
	Clubiona	*clima*	✔	✔	✔		✔	
Desidae	*Badumna*	sp.1	✔		✔			
Gnaphosidae	*Anzacia*	sp.1	✔		✔		✔	
	Hypodrassodes	*maoricus*			✔		✔	
Linyphiidae	***Erigone***	**sp.1**	✔	✔		✔		
	Haplinis	sp.1	✔		✔		✔	✔
	Linyphiidae	sp.1				✔		
	Mermessus	***fradoerum***		✔	✔	✔		✔
	Tenuiphantes	***tenuis***	✔		✔			
Lycosidae	*Anoteropsis*	*hilaris*	✔	✔	✔	✔		✔
	Lycosidae sp.1	**sp.1**	✔		✔	✔	✔	
Mimetidae	*Australomimetus*	sp.1	✔					
	Australomimetus	sp.2			✔			
	Argoctenus	***aureus***	✔			✔	✔	✔
Oxyopidae	*Oxyopes*	sp.1				✔	✔	
Pisauridae	*Dolomedes*	*Minor*	✔	✔	✔	✔	✔	✔
Salticidae	***Helpis***	***minitabunda***	✔	✔				
	Hypoblemum	***albovittatum***	✔		✔		✔	✔
	Trite	*auricoma*	✔		✔		✔	
	Trite	*planiceps*	✔		✔		✔	
Stiphidiidae	*Cambridgea*	sp.1			✔			
Tetragnathidae	***Leucauge***	***dromedaria***					✔	
	Tetragnathidae	sp.1	✔		✔		✔	
Theridiidae	***Argyrodes***	***antipodianus***	✔		✔		✔	
	Cryptachaea	**sp.1**					✔	
	Cryptachaea	**sp.2**			✔			
	Cryptachaea	***blattea***	✔		✔			
	Steatoda	***capensis***	✔		✔		✔	
	Theridiidae	**sp.1**	✔					✔
Thomisidae	*Sidymella*	*sp. 1 JR2014*	✔					
	Sidymella	sp.2	✔					
	Species diversity (INDI/NADI)		14/15		13/14		11/11	